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# XXI International Workshop on CLL 2025

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ABSTRACTS

# XXI International Workshop on CLL 2025

## Abstract ID: 1409

**Title:** Factors influencing physician's choice of fixed-duration ibrutinib + venetoclax or continuous ibrutinib monotherapy in the treatment of patients with previously untreated CLL: physician questionnaire results from the prospective phase 2 TAILOR study

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**Background:** Ibrutinib monotherapy or in combination with venetoclax (I+V) has dramatically changed the treatment landscape for patients with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), providing overall survival comparable with that of an age-matched general population. Ten-year follow-up data from the RESONATE-2 study showed robust long-term efficacy of continuous ibrutinib monotherapy in previously untreated CLL/SLL. Fixed-duration (FD) I+V demonstrated clinical benefit with deep and durable responses in the GLOW and CAPTIVATE studies. Considering disease heterogeneity and other factors that influence treatment decisions, an unmet need exists to better understand which factors influence physician's treatment choice.

**Objective:** The ongoing prospective phase 2 TAILOR study assesses the efficacy and safety of FD I+V and continuous ibrutinib monotherapy using proactively reduced dose design and reactive dose adjustment per label in patients with previously untreated CLL (NCT05963074). A questionnaire was designed to capture the decision-making factors influencing either FD I+V or continuous ibrutinib monotherapy.

**Methods:** TAILOR is an open-label, prospective, phase 2, 2-regimen, 4-cohort interventional study. After physician's choice of regimen, patients with previously untreated CLL/SLL were randomized 1:1 between 2 cohorts per arm: FD I+V (n = 80 each cohort) or continuous ibrutinib monotherapy (n = 80 each cohort). A physician questionnaire was used to determine factors that influence treatment decisions. These parameters were age, general risk factors, baseline comorbidities, anticipated superior efficacy, avoidance of anti-CD20 antibodies, advantage in route of administration, overall health status, overall treatment costs, safety profile of regimen, social circumstances, patient preference, and quality of life. Responses were categorized as: (1) strongly disagree; (2) disagree; (3) neither agree nor disagree; (4) agree; and (5) strongly agree. Responses with both agree/strongly agree and disagree/strongly disagree were combined for this analysis.

**Results:** As of the clinical cutoff date on 14 March 2025, 240 patients from United States, Canada, France, Poland, Czechia, Hungary, Italy, and Spain have been enrolled and received treatment with either FD I+V or continuous ibrutinib monotherapy, and physician questionnaires were completed for 236 of these patients (FD I+V, n = 166; continuous ibrutinib monotherapy, n = 70). As of 10 April 2025, 63 of 64 investigators who have screened or enrolled patients have completed the physician treatment questionnaire. Among patients who received FD I+V, 43.5% were aged ≥65 years, 65.3% were male, 68.2% were from Europe and 31.8% from North America, 11.2% had TP53/del17p mutations, and 48.9% had unmutated immunoglobulin heavy chain variable (uIGHV) genes. The five most common factors that influenced physician treatment decisions included advantage in the route of administration (88.0%), quality of life over standard care (83.7%), patient preference (81.9%), age (80.1%), and anticipated superior efficacy (80.1%). Factors that were less likely to influence physician treatment decisions included overall treatment costs (37.3%), avoidance of anti-CD20 antibodies (38.0%), and social circumstances (46.4%). When asked, 63.9% of physicians preferred FD I+V. Among patients who received continuous ibrutinib monotherapy, 78.6% were aged ≥65 years, 55.1% were male, 71.4% were from Europe and 28.6% from North America, 17.1% had TP53/del17p mutations, and 64.3% had uIGHV. The five most common factors that influenced physician treatment decisions included advantage in the route of administration (84.3%), quality of life over standard care (77.1%), general risk factors

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(74.3%), safety of regimen (72.9%), and patient preference (72.9%). Avoidance of anti-CD20 antibody treatment (62.9%) was also shown to influence physician treatment decisions, while treatment costs were less likely to impact decision making (35.7%).

**Conclusions:** Data from this prospective study provide important perspectives from physicians regarding the choice of FD I+V or continuous ibrutinib monotherapy in the first-line treatment of patients with CLL. These data show that advantage in administration route (an all-oral regimen), quality of life over standard care, and patient preference were the key priorities that influenced physician treatment decision with both FD I+V and continuous ibrutinib monotherapy. Avoidance of anti-CD20 antibody treatment was an important consideration in the selection of continuous ibrutinib monotherapy but less so for FD I+V. These results can help further inform and facilitate improved shared decision-making approaches between physicians and patients when selecting a frontline treatment option to treat CLL/SLL.

**Keywords:** Ibrutinib-venetoclax (TAILOR); physician-questionnaire; treatment-decision

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## Abstract ID: 1504

### **Title:** What measure of treatment effect should be preferred? A critical review of 20 randomized clinical trials of targeted therapies in chronic lymphocytic leukemia

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**Purpose:** In hematology, the treatment effect of a randomized clinical trial (RCT) is often measured on right-censored outcomes, using the hazard ratio (HR) to quantify the effect size, with HR< 0.8 as being of clinical interest. However, it is difficult to translate an HR into clinical benefit because it does not tell us the amount of improvement the intervention achieved for the population. In contrast, absolute measures, such as difference in mean or median survival times, or difference in estimated survival rates have been considered more meaningful. However, median wastes information by focusing on the center to the exclusion of other information. In contrast, a difference in mean times is easily interpreted as a mean difference between groups within the observation window. The so-called restricted mean survival time (RMST) - defined as the area under the survival function up to a time horizon, which can be interpreted as the life expectancy within the time interval - has therefore been promoted to quantify survival benefits particularly for oncology drugs. More recently, statistical approaches based on generalized pairwise comparisons have been proposed. The restricted net survival benefit (rNB) of at least m months is defined as the probability that a randomly chosen patient in the experimental arm survives m months longer than a randomly chosen patient chosen in the control arm minus the probability of the opposite situation. Furthermore, little is known about the differences in these absolute measures of treatment effect that are used to quantify the benefit of a new treatment. We hypothesized that the effect size depends on the measure of effect. Therefore, we aimed to compare and illustrate, using real RCT data, the differences in reported differences in medians versus mean gains in survival time, using difference in survival rates or net survival benefit, focusing on the evaluation of targeted therapies in patients with CLL hypothesizing that absolute measures of effect might provide a more meaningful benefit.

**Materials and methods:** We selected all articles in the English medical literature that reported the results of RCTs in patients with CLL to evaluate targeted therapies, unless curves of progression-free survival (PFS), event-free survival (EFS), or overall survival (OS) were not plotted. Once those curves were digitized, we calculated HR and absolute measures of treatment effects: differences in survival rates and in mean survival times, both measured at the median or maximal follow-up, and the net survival benefit by 6 and 12 months.

**Results:** A total of 20 articles were selected evaluating 4 targeted therapies, 12 targeting 1st line patients, 7 in relapsed/refractory (RR) patients, and 1 in both. A total of 9,379 patients were included, with a median sample size per trial of 455 [160–926], and no evidence of difference across lines ($p = 0.38$), comprising 50 comparisons of experimental arms with the control arm on PFS (n = 24), EFS (n = 1), or OS (n = 25). The proportional hazards assumption was violated in 17 (34%) comparisons, mostly evaluating BCL2-inhibitors. All effect measures were roughly consistent in their ranking of effects, except for the mean survival benefit. For HR <0.8, 78% of comparisons showed a benefit of at least 2.5 months and 53% showed a benefit of 6 months; however, all these comparisons agreed on a net survival benefit by 6 months, although the median proportion of patients who benefited from this was 30% (interquartile range, 12 to 42%).

**Conclusion:** There are many tools available to assess the potential benefit of a new treatment or therapeutic strategy in randomized phase III trials. We suggest that in the future, not only HRs, but also absolute measures of treatment effect should be reported more systematically and used to create benefit 'scores,' which are currently constructed in a rather rudimentary way and do not really enlighten clinicians or regulators.

**Keywords:** Randomized trial; evaluation; hazard ratio

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## Abstract ID: 1502

### **Title:** Sequencing single novel agent-based therapy for patients with CLL: a retrospective study of patients treated with covalent Bruton tyrosine kinase inhibitor (cBTKi) followed by B-cell lymphoma 2 inhibitor (BCL2i) versus BCL2i followed by cBTKi

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**Introduction:** Novel agents, including covalent Bruton tyrosine kinase inhibitors (cBTKi) and B-cell lymphoma 2 inhibitors (BCL2i) as monotherapies or in combination with anti-CD20 monoclonal antibodies (CD20mAb), are effective for the treatment of chronic lymphocytic leukemia (CLL) in both the frontline (1L) and relapsed/refractory (R/R) settings. Sequential single novel agent-based therapies allow for durable disease control in many patients with CLL, though whether cBTKi followed by BCL2i (cBTKi->BCL2i) or BCL2i followed by cBTKi (BCL2i->cBTKi) leads to a longer duration of disease control remains unknown.

**Methods:** In this single-center retrospective study, we examined patients seen at Mayo Clinic for CLL between 1/1/2012 and 4/1/2025 who had received cBTKi- or BCL2i-based therapy during their disease course. We then identified patients who received sequential single novel agent-based therapies (monotherapy or in combination with CD20mAb; the first novel agent in the sequence could be used in either the 1L or R/R setting) to establish two cohorts: cBTKi->BCL2i and BCL2i->cBTKi. Patients were excluded if they had received (1) a CLL-directed novel agent prior to the sequence of interest, (2) novel agent-based treatment under study for Richter transformation, or (3) any other CLL-directed therapy between the treatments under study. We examined baseline clinical and disease characteristics at the time of initiation of the first novel agent, as well as prior treatment history. Duration of each single novel agent therapy and reasons for therapy discontinuation were recorded. For patients who discontinued therapy due to intolerance or completion of planned therapy and subsequently received treatment within the same class of novel agent, the entire duration of treatment and treatment-free observation was included in duration of therapy. This method allowed for capturing the entire duration of disease control from each novel agent mechanism. The Kaplan-Meier method was used to estimate time to next mechanism of therapy or death (TTNMD) and overall survival (OS). Cox models were used for outcome analysis. TTNMD[cBTKi] was defined as time from initiation of cBTKi therapy to initiation of the BCL2i, alternate CLL-directed therapy following BCL2i->cBTKi, or death, whichever occurred first. TTNMD[BCL2i] was defined as time from initiation of BCL2i therapy to initiation of cBTKi, alternate CLL-directed therapy following cBTKi->BCL2i, or death, whichever occurred first. TTNMD[cBTKi+BCL2i] encompassed TTNMD for the combination of both agents, regardless of sequence. The primary objective was to compare TTNMD[cBTKi+BCL2i] between the cohorts to determine which treatment sequence allowed for more durable disease control.

**Results:** 149 patients received cBTKi->BCL2i, and 19 received BCL2i->cBTKi. At initiation of the first novel agent, median age was 65 years (range 58–73), 73% were male, Rai stage was III/IV in 45%, 82% (102/124) had unmutated IGHV, and 28% (34/123) had TP53 aberration; characteristics were not significantly different between the cohorts, though patients who received cBTKi->BCL2i tended to be younger (median age 64 vs 70 years, $p=0.051$) and had a lower proportion of TP53 aberration (25% vs 46%, $p=0.16$). The majority received the first novel agent in the R/R setting (63% of cBTKi->BCL2i and 74% of BCL2i->cBTKi); 36% received the first novel agent in the sequence in the 1L setting (37% cBTKi->BCL2i and 26% BCL2i->cBTKi). Fifty-two percent received chemoimmunotherapy prior to the novel agent-based sequences (53% cBTKi->BCL2i and 42% BCL2i->cBTKi). Median TTNMD[cBTKi+BCL2i] was 107 months for cBTKi->BCL2i and 95 months for BCL2i->cBTKi ($p=0.18$). There was no significant difference in TTNMD[cBTKi+BCL2i] for patients treated with cBTKi->BCL2i vs BCL2i->cBTKi (HR 1.61, 95% CI 0.54, 4.81) after adjusting for TP53 aberration, prior chemoimmunotherapy exposure, and age. TP53 aberration was associated with inferior TTNMD[cBTKi+BCL2i] regardless of treatment sequence (HR 3.15, $p=0.0006$). For those who received cBTKi->BCL2i, the median TTNMD[cBTKi] was 39.5 months, and median TTNMD[BCL2i] was 44.5 months. For those who received BCL2i->cBTKi, median TTNMD[cBTKi] was 22.4 months, and median TTNMD[BCL2i] was not reached. OS at 60 months was estimated at87% for cBTKi->BCL2i and 75% for BCL2i->cBTKi ($p=0.09$).

**Conclusion:** For patients who received sequential novel agents, either cBTKi->BCL2i or BCL2i->cBTKi, the overall duration of disease control achieved with these two classes of agents was not significantly different regardless of sequence. Future multicenter collaborations may allow for greater power to detect differences between these sequences. These data support basing treatment decisions on patient preference and comorbidities for patients with CLL when selecting between cBTKi and BCL2i as the first novel agent-based therapy.

**Keywords:** Chronic lymphocytic leukemia; novel agents; sequencing

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## Abstract ID: 1703

### **Title:** Targeting immunoglobulin deficiency in CLL: a retrospective evaluation of IgM/IgA-enriched IVIg use in a small cohort of patients from Argentina

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Infectious complications represent a major cause of morbidity and mortality in CLL due to both, intrinsic immune dysfunction and detrimental effects of anti-leukemic therapies on the

patient´s immune system. Hypogammaglobulinemia is the most common immune defect. Its prevalence ranges between 20 and 70% depending on the cohort analyzed, with a decrease of all immunoglobulin (Ig) isotypes usually starting with IgM, then IgG and IgA (Freeman, Crassini et al. 2013, Parikh, Leis et al. 2015, Andersen, Vojdeman et al. 2016, Ishdorj, Streu et al. 2019). The administration of immunoglobulin replacement therapy (IgRT) is not recommended as routine prophylaxis, and is only reserved for patients with severe recurrent infections in the presence of hypogammaglobulinemia (Hallek, Cheson et al. 2018). Remarkably, Ig preparations used in CLL contain more than 95% IgG and as a result, IgA and IgM deficiencies persist unaddressed. An analysis of the factors associated with infections in CLL patients showed a stronger association between major infections and combined Ig deficiency rather than isolated IgG deficiency (Visentin, Compagno et al. 2015), thus an enriched Ig preparation may offer enhanced benefits. Moreover, we have previously reported that in contrast to standard IgRT, IgA- and IgM-enriched IVIg (IVIg-GAM) preparation showed a significantly lower inhibitory effect on T cells, a feature highlighted as a potential benefit of this particular preparation with the potential to preserve T cell function when administered in vivo (Khan, Molica et al. 2022). This study evaluates the impact of IVIg-GAM on infection rates and antimicrobial use in a small cohort of CLL patients. We have conducted a retrospective analysis on six CLL patients who initiated IVIg-GAM (Pentaglobin) between 2018 and 2022. Patients were followed for 24 months. IVIg-GAM was administered at 400 ml every 25–28 days. All patients met local criteria for IgRT due to hypogammaglobulinemia (IgG <400 mg/dl) and/or recurrent infections (≥3 per year). Data on infection episodes, antimicrobial use, and Ig levels were collected. All patients exhibited low baseline levels of IgG, IgA, and IgM. Median baseline immunoglobulin levels were: IgG 419 mg/dl, IgA 25.5 mg/dl, and IgM 9 mg/dl. IVIg-GAM treatment led to a sustained increase in all Ig isotypes over 24 months, with median levels reaching: IgG 591 mg/dl, IgA 48 mg/dl, and IgM 23 mg/dl ($p < 0.05$. Friedman test, Dunńs multiple comparisons test 6 and 24 months vs pre-IgRT). Prior to IVIg-GAM, patients experienced a mean of 3 infections/year and required antimicrobial therapy 2.8 times/year, with at least one severe infection requiring hospitalization. The most common severe infection was pneumonia. During IVIg-GAM treatment, no patient required hospitalization for infection, and both minor infections and antimicrobial use significantly decreased ($p < 0.05$. Two-way ANOVA, Sidak's multiple comparisons test). During the COVID-19 pandemic, five patients were on IVIg-GAM; two contracted mild SARS-CoV-2 infections, while the remaining three remained asymptomatic despite lacking vaccine-induced immunity. Regarding CLL treatment, five patients required therapy due to disease progression. Three received ibrutinib as first-line therapy, achieving complete hematological response. Two patients obtained partial response, one of whom had long-standing disease and multiple relapses. Notably, one patient experienced spontaneous disease control without requiring anti-leukemic therapy following IVIg-GAM initiation. The IVIg-GAM was well-tolerated, with mild adverse events (rash and transient hypotension) observed in some patients during initial infusions. Premedication with paracetamol, dexamethasone, and diphenhydramine was administered to mitigate infusion reactions. To our knowledge, this study is the first to evaluate infection events, antimicrobial use, and Ig levels in CLL patients receiving IVIg-GAM. Our findings show that IVIg-GAM was safe and effective in reducing infection rates and antimicrobial use in CLL patients with hypogammaglobulinemia. Larger studies are needed to confirm these findings. An updated cohort follow up will be presented at the meeting.

**Keywords:** Infections; hypogammaglobulinemia; IVIg-replacement-therapy

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1307

### **Title:** Developing a novel lipid-based imaging tool for the surveillance of chronic lymphocytic leukemia

**Authors:** Olivia Burling[a], Bryone Kuss[b], Shane Hickey[c], Oliver Best[a] and Lauren Thurgood[a]

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**Background:** The reliance of B-lymphocytes on non-glucose-based metabolic pathways in chronic lymphocytic leukemia (Rozovski et al., Mol Cancer Res. 2016) largely explains why [18F] fluoro-2-deoxyglucose positron emission tomography (FDG-PET) provides limited diagnostic value in patients who have not undergone Richter's transformation (Nabih et al., ARNM. 2024). We have previously shown that B-CLL cells preferentially sequester large amounts of palmitic acid (C16:0) over glucose and actively downregulate pathways involved in glucose uptake and utilization, thereby highlighting a clear metabolic preference for lipids. We hypothesize a lipid-based imaging tool would be more informative for CLL diagnosis and surveillance than the current FDG-PET tracer. Four derivatives of palmitic acid were synthesized and evaluated in vitro to investigate the efficacy of a novel lipid-based imaging tool in CLL.

**Methods:** Four derivatives of palmitic acid were prepared following multi-step syntheses, including two fluorescently tagged and two fluorinated lipids as methyl ester and carboxylic acid derivatives. Uptake of the fluorescent lipids was quantified using flow cytometry in CLL (OSU-CLL, HG-3) and healthy (FH-9) B-lymphocyte lines and other lipid-avid tissue including murine skeletal muscle (C2C12). Cell lines were treated with up to 50 µM of each probe for <48 hours and uptake was quantified by measuring mean fluorescence intensity. Cell proliferation was assessed concurrently by staining with CellTrace Far Red™ prior to incubation with the lipid. Viability at 50 µM after 48 hours was assessed using DilC1(5). Uptake of the fluorinated lipids was assessed by incubating the cell lines listed above with up to 50 µM of lipid for 24 hours. Lipid extracts were isolated using Folch extraction and were derivatized prior to analysis using gas chromatography mass spectrometry (GCMS).

**Results:** Flow cytometry analysis confirmed uptake of the fluorescent lipid probes was up to 5-fold higher in CLL cells compared to healthy B-lymphocytes. Uptake of the methyl ester was consistently higher compared to the carboxylic acid variant over 48 hours for the CLL cell lines. No significant change in proliferation was observed over 48 hours in the CLL cell lines; proliferation was slightly decreased in the healthy B-lymphocytes for both fluorescent lipids at concentrations above 5 µM. However, viability remained consistent over 48 hours for all cell lines at all lipid

concentrations. Uptake of the fluorescent lipids in skeletal muscle cells was 2-fold lower compared to CLL cell lines but was approximately 1.5-fold higher than healthy B-lymphocytes. Early GCMS results have shown high uptake of the fluorinated lipids in CLL cell lines after 24 hours. Samples of healthy B-lymphocytes and skeletal muscle are being prepared at the time of application.

**Discussion:** Flow cytometry data revealed the CLL cell lines had significantly higher uptake of the fluorescent lipids compared to the skeletal muscle and healthy B-lymphocytes. These results are consistent with previously published data (Thurgood et al, Exp. Hematol. 2022) and could suggest the lipid PET tracer would be primarily sequestered by B-CLL cells in vivo, followed by minimal uptake into skeletal muscle. The CLL cells showed preference for the methyl ester derivative over the carboxylic acid, possibly due to the structure of the methyl ester being more amenable to transport and storage in the cell (Havel, Am J Physiol. 1987; Czech et al, Diabetologia. 2013). Cell proliferation did not change significantly over 48 hours, suggesting the lipids are not driving CLL cell growth. Microscopy experiments currently being optimized will help elucidate the fate of the lipids inside the cell (e.g. metabolism, storage). Early GCMS results corroborate the flow cytometry data, demonstrating high uptake of the lipid probes in the CLL cell lines, however, uptake is yet to be confirmed in healthy B-lymphocytes and skeletal muscle.

**Conclusions:** We hypothesized that a lipid PET tracer would be more informative for CLL diagnosis and surveillance than the FDG-PET tracer. Flow cytometry (and GCMS) data has shown high uptake of novel lipid probes in CLL cell lines, with minimal uptake in healthy B-lymphocytes and skeletal muscle. Additionally, this project has established a pipeline for the development of novel lipid PET tracers that could be applied to other lipid-avid malignancies.

**Keywords:** Leukemia; lipid; imaging

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**Please indicate the name of the funding organization:** Flinders Foundation

## Abstract ID: 1505

### **Title:** A meta-analysis investigating response rates of continuous Bruton tyrosine kinase inhibitor monotherapies in the treatment of b-cell lymphomas

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**Introduction:** Bruton tyrosine kinase inhibitor (BTKi) monotherapy has led to improved patient outcomes in B-cell lymphomas (BCLs), including chronic lymphocytic leukemia (CLL), Waldenström macroglobulinemia (WM), marginal zone lymphoma (MZL), and mantle cell lymphoma (MCL). This meta-analysis aimed to compare response rates associated with BTKi monotherapy across BCLs in treatment-naive (TN) and/or relapsed and refractory (R/R) stages.

**Methods:** Trials reporting complete response (CR) rates or objective response rates (ORRs) for zanubrutinib, acalabrutinib, or ibrutinib monotherapies in patients with one of the BCLs were identified by systematic literature review. Response rates at similar follow-up time points (maximum 12-month difference) and longest available follow-up time points were pooled across all applicable studies. Odds ratios (ORs) comparing CR and ORR of zanubrutinib with those of the other 2 BTKis were calculated within each BCL indication and then meta-analyzed across all indications, using random effects models.

**Results:** The meta-analysis found zanubrutinib to be associated with statistically significant improvements in investigator-assessed CR and ORR vs acalabrutinib and ibrutinib across BCLs, using data with similar duration of follow-up. The pooled estimates of ORs (95% CI) for CR were 1.80 (1.03–3.13) for zanubrutinib vs acalabrutinib and 2.85 (1.16–7.04) for zanubrutinib vs ibrutinib. In R/R MCL, zanubrutinib demonstrated statistically superior efficacy over both acalabrutinib and ibrutinib for CR, with ORs (95% CI) of 3.33 (1.91–5.81) and 9.53 (5.45–16.66), respectively. In R/R MZL, zanubrutinib showed superior efficacy over ibrutinib for CR, with an OR of 3.32 (95% CI 1.28–8.61). The pooled estimates of ORs (95% CI) for ORR were 1.59 (1.0003–2.53) for zanubrutinib vs acalabrutinib and 2.25 (1.40–3.61) for zanubrutinib vs ibrutinib. In TN CLL, zanubrutinib demonstrated statistically superior ORR (95% CI) over acalabrutinib (4.33 [1.68–11.15]) and ibrutinib (5.47 [2.47–12.12]). In R/R MCL and R/R MZL, zanubrutinib showed superior ORR over ibrutinib, with ORs (95% CI) of 2.23 (1.21–4.12), and 2.39 (1.18–4.85), respectively.

**Conclusions:** Zanubrutinib demonstrated significantly higher CR rates and ORRs compared to acalabrutinib and ibrutinib across BCLs. Findings are consistent with results of existing trials, which report higher rates of overall and complete response with zanubrutinib relative to other BTKis in certain BCLs.

**Keywords:** Meta-analysis; Bruton tyrosine kinase inhibitor; B-cell lymphoma

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** BeOne Medicines Ltd

## Abstract ID: 1410

### **Title:** Impact of testing for genetic markers on treatment selection and clinical outcomes among patients with chronic lymphocytic leukemia

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**Background:** Genetic markers like del17p, TP53 mutation (TP53m), and immunoglobulin heavy variable chain mutation (IGHVm) are important for chronic lymphocytic leukemia (CLL) prognosis. Del17p, TP53m, and unmutated IGHV are associated with early disease progression, particularly among

patients receiving chemoimmunotherapy (CIT) and ibrutinib. Knowledge is limited on the impact of genetic testing on front-line (1L) treatment choices and clinical outcomes in CLL.
**Methods:** This retrospective cohort study utilized the US electronic health record-derived de-identified Flatiron Health Research Database. Eligible patients included adults with CLL diagnosis who started 1L treatment January 1, 2020–30 November 2024. Primary exposures included evidence of testing any time before 1L initiation, regardless of testing status. Tests included FISH for del17p, DNA sequencing for TP53m, and IGHV. Descriptive statistics were summarized by each test and by combining patients with del17p and TP53m. Primary exposures consisted of combined del17p/TP53 and IGHV tests, including patients who received tests before 1L as the reference group. Outcomes included real-world time to next treatment or death (rwTTNT) and OS (rwOS) from 1L initiation. Landmark treatment and survival probabilities were estimated using Kaplan-Meier method. HRs and 95% CIs were estimated using Cox proportional hazard models, adjusting for age, sex, race/ethnicity, insurance, comorbidity, socioeconomic status, practice type, Rai stage, year of 1L initiation, and Eastern Cooperative Oncology Group (ECOG) performance status (PS). Stratified analyses were performed by 1L treatment, including CIT, ibrutinib, and National Comprehensive Cancer Network® (NCCN) guideline–preferred novel therapies (NTs; acalabrutinib, zanubrutinib, venetoclax-based therapies). In exploratory analyses, the proportion of patients with 1L treatment by testing result was summarized.
**Results:** Of the 5,481 patients included, the majority received FISH before 1L treatment (81.9%) and only 26.5% were tested for TP53m. About half of patients were tested for IGHV before treatment (51.9%). Compared with patients not tested before treatment, tested patients were younger (median age: 71 vs 75 [del17p/TP53], 70 vs 73 [IGHV]), more likely to be male (65% vs 61% [TP53], 64% vs 59% [IGHV]), non-Hispanic White (74% vs 72% [del17p/TP53 and IGHV]), and had ECOG PS 0 (42% vs 33% [del17p/TP53], 44% vs 37% [IGHV]). Patients tested by FISH were more likely to be treated at community practices (80% vs 75%) and patients with TP53 tests were more likely to be treated at academic practices (23% vs 20%). Patients tested for IGHV status were more likely to have commercial insurance (31% vs 26%) and less likely to have Medicare (45% vs 50%). Survival outcomes were worse among patients not tested before treatment versus those tested. Median rwTTNT was 30.0 months for patients without del17p/TP53 testing (95% CI 26.5–36.4) versus 41.7 months (95% CI 39.1–44.1) for those tested (HR 1.10; 95% CI 0.99–1.23). Patients without IGHV testing had lower median rwTTNT of 34.0 months (95% CI 31.1–37.8) than 45.3 months (95% CI 41.6–49.3) for those tested (adjusted HR 1.12; 95% CI 1.03–1.22). Median rwOS was not reached (NR) for any group. After adjustment, patients without IGHV or del17p/TP53 testing had 27% and 26% higher hazards of death than those with IGHV testing (HR 1.27; 95% CI 1.12–1.44) and del17p/TP53 testing (HR 1.26; 95% CI 1.09–1.47), respectively. Additionally, patients who were not tested before treatment were less likely to receive NCCN guideline–preferred NTs (del17p/TP53: 35% vs 48%; IGHV: 41% vs 50%) and more likely to receive CIT (del17p/TP53: 28% vs 22%; IGHV: 26% vs 20%). Further stratified by test results, patients who were del17p/TP53m-positive were less likely to receive 1L CIT (15% vs 23%) or 1L venetoclax + obinutuzumab (9% vs 14%) than those without del17p/TP53m. Among all patients, median (95% CI) rwTTNT was longest for those who received NCCN guideline–preferred NTs (60.2 months; 55.9-NR), followed by ibrutinib (38.7 months; 34.9–42.3) and CIT (9.9 months; 8.4–12.1). In the stratified analysis, among patients who received 1L NTs, landmark probabilities of staying on current treatment or death were numerically lower in patients without than with IGHV testing (12 months: 82% vs 85%; 24 months: 71% vs 76%) or del17p/TP53 testing (81% vs 84%; 70% vs 74%).
**Conclusion:** This large, real-world study suggests patients who received pre-treatment prognostic genetic marker testing for CLL were more likely to have better clinical outcomes, further emphasizing the need for testing before 1L treatment as a likely proxy for optimal care management in CLL.

**Keywords:** Biomarker testing; treatment patterns; survival outcomes

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**Please indicate the name of the funding organization:** BeOne Medicines Ltd.

## Abstract ID: 1506

**Title:** Bexobrutideg (NX-5948), a novel Bruton's tyrosine kinase (BTK) degrader, demonstrates rapid and durable clinical responses in relapsed/refractory CLL: updated findings from an ongoing phase 1a study

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**Background:** Although BTK inhibition has proven to be a successful treatment modality in CLL, the emergence of BTK inhibitor (BTKi) resistance mutations and identification of kinase-independent signaling via the scaffolding function of BTK underscores the need for alternative approaches to target the totality of BTK protein functions. Bexobrutideg is a novel, orally administered small molecule degrader that induces removal of wild-type and mutant forms of BTK through ubiquitination by the cereblon E3 ligase complex

and subsequent proteasomal degradation. We report updated findings from a Phase 1a trial of bexobrutideg in patients with relapsed/refractory (R/R) CLL.

**Methods:** NX-5948-301 is a Phase 1, first-in-human trial of bexobrutideg in R/R B-cell malignancies, including CLL and NHL/WM, in parallel 3+3 dose-escalation (1a) and dose-expansion (1b) cohorts. Key eligibility criteria include ≥2 prior therapy lines that included a BTKi and ECOG PS 0–1. Primary objectives are evaluation of safety/tolerability and identification of a recommended Phase 2 dose. Key secondary objectives include characterization of the PK/PD profile and assessment of preliminary efficacy according to iwCLL criteria.

**Results:** As of the 2 January 2025 cutoff date, the Phase 1a cohort was fully enrolled including 48 patients with CLL/SLL who were treated at 6 dose levels p.o. daily: 50 mg (n = 3), 100 mg (n = 5), 200 mg (n = 9), 300 mg (n = 8), 450 mg (n = 7), 600 mg (n = 16). Median age was 68.5 (range 35–88) years; 66.7% were male; there was a median of 4 (range 2–12) prior lines of therapy, including: cBTKi (97.9%), ncBTKi (27.1%), BCL2i (83.3%), BTKi + BCL2i (81.3%), chemo/chemoimmunotherapy (72.9%), PI3Ki (29.2%), and CAR-T (6.3%). In 47 patients with genetic testing available at baseline, mutations identified were: TP53 (44.7%), BTK (38.3%), PLCG2 (14.9%), and BCL2 (12.8%). Bexobrutideg was well tolerated across all doses in the Phase 1a CLL cohort (n = 48), consistent with the overall study safety population. The median duration of treatment was 7.0 (range 0.2–23.8) months. There were no DLTs; 1 patient discontinued due to a TEAE. The most common TEAEs were: purpura/contusion (41.7%, no Gr ≥3); fatigue (31.3%, no Gr ≥3); neutropenia (29.2%, 22.9% Gr ≥3); rash (29.2%, 2.1% Gr ≥3, 2.1% SAE), diarrhea (29.2%; 4.2% Gr ≥3), and petechiae (25.0%, no Gr ≥3). One Grade 5 event (pulmonary embolism, considered unrelated to bexobrutideg) occurred in a patient with a history of atrial fibrillation. No systemic fungal infections or new onset atrial fibrillation were observed. In 47 response-evaluable patients with CLL from the Phase 1a cohort, the ORR was 80.9% (all PR). Responses were rapid with a median time to first response of 1.9 (range 1.6–11.1) months. Median duration of response has not been reached. Thirteen patients have reached >12 months on study, and 5 patients >18 months. Durable responses were seen regardless of prior treatments, mutations in TP53, PLCG2, BCL2, and BTK (including kinase active and kinase dead), or CNS involvement.

**Conclusions:** Bexobrutideg was well tolerated in patients with R/R CLL, including those with longer duration of treatment and higher doses. In this fully enrolled Phase 1a cohort, bexobrutideg showed rapid and durable responses independent of prior treatment or high-risk features such as double refractory status and unfavorable genetic profile. Phase 1b dose expansion (200 and 600 mg cohorts) is underway with plans to initiate pivotal trials in 2025.

**Keywords:** Bexobrutideg; NX-5948; BTK degrader

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## Abstract ID: 1704

**Title:** Feasibility of an individualized physical activity intervention in CLL patients receiving ibrutinib +/-venetoclax: preliminary results from the QOLIBRI study

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Physical activity (PA) has proven benefits on health-related quality of life (HRQoL), fatigue, and psychological wellbeing in individuals with chronic diseases and solid tumors (Delrieu, 2020; Cheville, 2013). However, evidence in heme cancers, including chronic lymphocytic leukemia (CLL), remains scarce due to limited sample sizes (Knips, 2019). In CLL, only one pilot study combining high-intensity interval and resistance training in 15 patients reported a clinically meaningful HRQoL improvement (Artese, 2022). The World Health Organization recommends that adults engage in at least 150 to 300 minutes of moderate-intensity PA, or 75 to 150 minutes of vigorous-intensity PA, or an equivalent combination of both throughout the week, to achieve substantial health benefits. However, these guidelines were based on questionnaires and did not include goals for step counts. Recent studies using activity trackers suggest that even lower doses of PA are associated with improved HRQoL (Lee IM, 2025). The primary objective of the QOLIBRI study is to assess the impact of an individualized physical activity intervention (IPAI), supported by an activity tracker (connected watch) on HRQoL in patients with CLL undergoing treatment with Ibrutinib (I). Indeed, it is essential to preserve the quality of life for patients due to the chronic nature of this disease. The aim of this interim analysis is to evaluate the feasibility of the intervention, focusing on acceptability, adherence, compliance, and participant retention (ibrutinib until PD or ibrutinib + venetoclax [I+V] fixed duration). We report here on the feasibility phase (N = 29) of the trial. Methods QOLIBRI (ID-RCB: 2023-A01563-42, NCT06299540) is a prospective, randomized, open-label, multicenter low-interventional study in France. Eligible patients initiating BTKi therapy (I alone or I+V) for CLL (1L or R/R setting) are randomized 1:1 to an IPAI or standard of care (SoC) arm. All participants are equipped with an activity tracker for 12 months. The IPAI group follows a 6-month structured, remote, predefined, individualized program combining walking goals and weekly remote APA sessions supervised by an APA trainer through monthly videoconferences. The intervention is structured into two phases: a four-month supervised period led by the trainer, followed by a two-month unsupervised period designed to promote patient autonomy. Following the 6-month IPAI program, the patients undergo an additional 6-month follow-up period where they keep wearing the activity tracker and continue to complete PROs. In the SoC arm, the patients continue their PA according to their lifestyle and recommendations from the medical team. The primary endpoint is HRQoL at 4.5 months (FACT-G questionnaire). Secondary outcomes and evaluation

tools include fatigue (FACIT), anxiety and depression (HAD), sleep quality (PSQI, and activity tracker), physical fitness (six-minute walk test [6MWT]), PA (quantified by step counts from the activity tracker), and tolerance (treatment-related AE). A total of 180 patients are expected to have 80% power to detect statistically significant changes in FACT-G scores between the study arms. A feasibility assessment is conducted 12 months after study initiation on approximately 30 patients to evaluate acceptability (percentage of patients consenting to participate), adherence (percentage of patients from IPAI arm who were actually initiating the IPAI program), compliance (percentage of adherent patients who correctly follow the planned IPAI program), and retention (percentage continuing the IPAI and connected watch use across the different study timepoints). Results In the feasibility phase, 29 patients with CLL were enrolled across seven sites, with 13 randomly assigned to the IPAI arm and 16 to the SoC arm. The cohort was predominantly male (62.1%) with a mean age of 65.3 years (range 49–77 years), 57.1% initiated I alone and 42.9% I+V. All patients in the IPAI arm (100%) started the intervention and completed their initial session with the APA trainer. Additionally, 100% of IPAI patients and 93.8% of SoC patients (15 out of 16) began wearing the activity tracker. The complete feasibility assessment is ongoing, and study expansion to all sites will be considered if adherence, compliance, and retention rates exceed 70% during this phase. Discussion These preliminary results indicate high adherence to both the IPAI program and activity tracker usage. Complete feasibility results and preliminary efficacy data will be presented during the congress.

**Keywords:** Chronic lymphocytic leukemia; adapted physical activity; ibrutinib

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1103

### **Title:** Identification and multi-omic characterization of a novel molecular subtype of CLL based on EBF1 expression levels

**Authors:** Víctor Jiménez-Martínez[a], Judith Mateos-Jaimez[a], Marta Kulis[a], Vicente Chapaprieta[a], Martí Duran-Ferrer[a], Anna Vidal-Crespo[a], Alba Maiques-Diaz[a], Ferran Nadeu[a], Laura Llaó-Cid[a], Irene Lopez-Oreja[a], Estella Matutes[a], Armando López-Guillermo[a], Elias Campo[a], Raúl F. Pérez[a] and José Ignacio Martin-Subero[a]

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**Background:** Chronic lymphocytic leukemia (CLL) is a lymphoid neoplasm with considerable molecular and clinical diversity. A common transcriptional hallmark of CLL is the aberrant loss of expression of the B-cell transcription factor Early B cell factor 1 (EBF1), involved in early B-cell commitment and maturation, while illegitimate expression of T-cell related genes such as Lymphoid enhancer-binding factor 1 (LEF1) and Cytotoxic T-lymphocyte associated protein 4 (CTLA4) is typically observed. Nonetheless, a small number of CLL cases display high levels of EBF1 expression. In this scenario, whether the aberrant expression of these genes is interconnected and whether the retention of EBF1 expression may shape the molecular configuration of these cases is currently unknown.

**Aims:** We aimed to dissect the differential molecular characteristics of those CLL cases that maintain EBF1 expression and explore the functional consequences of this retention, as well as its relationship with cell of origin and B-cell identity.

**Methods:** We gathered diverse multi-omic datasets across a variety of CLL cases, including genomic features (N=560), transcriptomic (N=591), proteomic (N=72), and epigenomic profiling (i.e. ATAC-seq, N=104; H3K27ac ChIP-seq, N=102; DNA methylation 450k arrays, N=253). We first used the RNA-seq data to define EBF1+ CLLs and then performed integrative computational analyses for a deep characterization of their molecular profiles. We performed Multi-Omics Factor Analysis (MOFA), differential analyses with limma, gene set enrichment analyses, and epigenomic Locus Overlap Analysis (LOLA). Furthermore, we profiled a cohort of selected EBF1+, EBF1-, and healthy control samples (N=28) using 10X Chromium Single Cell Multiome (ATAC+Gene Expression), which was processed with Seurat and used to infer regulome networks.

**Results:** First, we observed a dichotomic distribution of EBF1 levels across patients, with 52/591 (8.8%) of CLL cases classified as EBF1+ based on elevated expression. These EBF1+ CLLs were significantly enriched in mutated IGHV status, trisomy 12, and class switch status (FDR <0.05). EBF1 positivity did not show strong links to clinical outcome, although its simultaneous presence with trisomy 12 was associated with worse overall survival. Unsupervised MOFA revealed that an EBF1-associated factor was the second most relevant, after IGHV status, in explaining the molecular landscape of CLL. Importantly, this integration revealed that EBF1 and trisomy 12 display independent molecular patterns. We defined a specific EBF1 expression signature (N=51 genes), which was further validated at the proteomics level. As compared to EBF1- CLL, EBF1+ cases showed higher expression of genes involved in B cell receptor (BCR) signaling (e.g. CD22, CD79B, CD20) and lower expression levels of typical CLL markers, particularly T-cell factors (i.e. LEF1 and CTLA4). The EBF1 signature could also be detected at the single-cell level, and we also observed gradients of the EBF1 program within CLL cases. A comprehensive epigenomic analysis indicated that EBF1+ CLL maintained a molecular profile more similar to mature B cells compared to EBF1- cases. First, differential ATAC and H3K27ac chromatin peaks grouped EBF1+ CLLs closer to mature B cell samples. In fact, EBF1+ CLL displayed lower levels of typical CLL de novo active/accessible chromatin regions ($p$ value <0.05). Secondly, in the EBF1+ subgroup epigenomic alterations were enriched in B-cell associated enhancers, whilst EBF1- changes were linked to T-cell enhancers (FDR <0.05). The quantification of accessibility in the single-cell data also revealed a closer epigenetic profile between EBF1+ and normal B cells. However, harnessing the DNA methylation data to explore divergences in cell of origin showcased that both EBF1+ and EBF1- CLL exhibited similar patterns in terms of B-cell maturation imprints.

**Conclusion:** Our results show that EBF1 expression may define a biological subtype of CLL with markedly distinct features, characterized by a higher molecular resemblance to mature B cells than EBF1- CLLs, including a lack of expression of T-cell genes and higher expression of BCR signaling related genes. Our findings imply that EBF1 downregulation might be a primary event in most CLLs and a major non-genetic driver contributing to the acquisition of canonical CLL expression features. Given their markedly different molecular

features, further studies are required to determine whether EBF1+ and EBF1- CLLs are associated with specific therapeutic vulnerabilities.

**Keywords:** EBF1; multi-omic profiling; B-cell identity

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**Please indicate the name of the funding organization:** European Research Council (ERC); Spanish Ministry of Science, Innovation and Universities

## Abstract ID: 1403

### **Title:** Factors influencing the regimen choice for first-line therapy in chronic lymphocytic leukemia: the FIRST-CLL study

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**Background:** Treatment options for patients with chronic lymphocytic leukemia (CLL) have significantly increased over the years, with chemoimmunotherapy-free regimens now available from first-line therapy (2024 CLL ESMO guidelines). Selecting the optimal treatment requires careful consideration of multiple factors, including biological risk, side effect profiles, concomitant medications, patient fitness, and individual preferences. In the absence of mature head-to-head comparisons, this decision-making process remains complex.
**Aims:** The FIRST-CLL study describes the allocation of first-line treatments for CLL in a real-world Italian cohort and explores the relative weight of several parameters – such as clinical-biological characteristics, logistical factors, and patient preference - in the regimen selection.
**Patients and methods:** We collected data from consecutive CLL patients who started first-line treatment from February 2024 to February 2025 in 9 Italian centers outside of clinical trials. Patients' characteristics before treatment start - including clinical and prognostic features, age, fitness status and comorbidities, concomitant therapies and availability of caregivers - were assessed. For each case, the treating physician was asked to score from 1 to 5 the importance of different variables in the treatment choice (i.e. prognostic features, patient's age and comorbidities, tumor lysis syndrome (TLS) risk, lymph nodes size, concomitant treatments, route and timing of administration, and patient's preference).
**Results:** A total of 133 patients were enrolled. Among them, 56 (42%) received a continuous Bruton tyrosine kinase inhibitor (BTKi) (zanubrutinib, n= 31; acalabrutinib, n= 24; ibrutinib n = 1), 46 (35%) ibrutinib plus venetoclax (IV) and 31 (23%) venetoclax plus obinutuzumab (VO). IV was the most prescribed regimen in patients <70 years old (60%) and the least used in patients ≥70 years old (12%), who were mostly treated with continuous BTKi (59%) or VO (24%). In patients ≥80 years old (n = 34), continuous BTKi were largely preferred (overall, 84%; acalabrutinib, n = 16; zanubrutinib, n = 14). When caregiver support was lacking (n = 22), patients mostly received continuous BTKi (55%), while VO was generally avoided (18%). Among patients with TP53 disruption (n = 11), the majority received zanubrutinib (73%), a small group acalabrutinib (18%), and one patient the IV regimen. Patients carrying unmutated IGHV status were preferentially treated with a BTKi-containing regimen (continuous BTKi, 54%; IV, 30%; VO, 16%). As expected, in patients with a history of cardiac arrhythmias (n = 14), the treatment not including a BTKi (VO) was favored in 50% of cases. Interestingly, a history of heart failure or a recent major bleeding did not preclude BTKi use, although numbers were limited (overall, n = 5; zanubrutinib, n = 2; acalabrutinb, n = 2; IV, n = 1). Among patients on antiplatelet therapy (n = 26), VO and acalabrutinib resulted the preferential choices (VO, n = 9; acalabrutinib, n = 9; zanubrutinib, n = 5; IV, n = 3). We then analyzed the impact of each parameter on treatment selection, according to treating physicians, measured with the 1-to-5 scale. Overall, prognostic features, routes and timing of administration, patient's preference, age and comorbidities emerged as main determinants in therapeutic decision-making (mean score: 2.61, 3.2, 3.3, 2.86, 3.36 and 2.44, respectively), while TLS risk and lymph node size did not significantly affect physicians' choices (mean score: 1.77 and 1.88, respectively). When choosing a continuous BTKi, comorbidities and patient's age resulted key factors (mean score: 2.96 and 3.67, respectively), while for IV and VO the fixed-duration schedule was among the primary drivers (mean score: 4.8 and 4.35, respectively). Regarding fixed-duration regimens, in the IV cohort patient's preference and route of administration resulted as additional influential factors (mean score: 3.06 and 3.67, respectively), while patient's age was of importance for the VO choice (mean score: 3.68). Interestingly, when a continuous BTKi regimen was selected, TP53 disruption and other prognostic

factors (e.g. IGHV status) had a different relevance for distinct drugs: for zanubrutinib mean scores were 2.13 and 2.55, respectively, whereas for acalabrutinib were 1.67 and 2.04, respectively. Conversely, patients' age and comorbidities emerged as more important in the selection of acalabrutinib (mean score: 3.67 and 2.96, respectively) compared to zanubrutinib (mean score: 2.97 and 2.16, respectively).
**Conclusions:** In the absence of comparative trial data, regimen selection in treatment-naïve CLL patients remains challenging. With the limit of subjective judgment of individual physicians, our real-world findings highlight how a range of clinical and logistical factors influence therapeutic decisions in the current Italian scenario for first-line CLL treatment.

**Keywords:** CLL; front-line; therapy

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**Please indicate the name of the funding organization:**

## Abstract ID: 1404

### **Title:** A phase 2 study with Tafasitamab and Zanubrutinib in newly diagnosed chronic lymphocytic leukemia/small lymphocytic lymphoma—TaZa CLL study

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**Background:** Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia in the Western countries and is characterized by the clonal expansion of monoclonal CD5+ and CD19+ B lymphocytes. Despite the advances of the clinical outcomes with the Bruton tyrosine kinase inhibitors (BTKi) and the BCL2 inhibitors (BCL2i), relapses and acquisition of resistance remain a clinical challenge. Therefore, novel therapies in the front-line setting are urgently needed to prolong the survival of the CLL patients and delay the development of progression. CD19 is a B-cell surface antigen expressed in CLL cells which makes it an attractive target for drug development. Tafasitamab is a humanized, FC-enhanced anti-CD19 monoclonal antibody, which has demonstrated favorable response rates and toxicity profile when combined with venetoclax or idelalisib in relapsed and refractory CLL (R/R CLL) (Staber PB et al, ASH 2019). We hypothesized that a time-limited doublet combination of tafasitamab with Zanubrutinib (TaZa) could achieve high efficacy with good tolerability. Herein, we report for first time, the safety and initial preliminary results of TaZa in previously untreated CLL/SLL.
**Methods:** This is an ongoing multi-center, phase II investigator-initiated trial with safety lead-in of TaZa in treatment-naïve CLL patients (NCT05718869). Eligibility: patients with CLL requiring treatment per iwCLL criteria with ECOG ≤2, creatinine clearance ≥30 ml/min, absolute neutrophil count ≥1000/mm$^{3,}$ and platelet count ≥75,000/mm$^3$. The primary endpoint is to evaluate the safety/tolerability of TaZa in the safety lead-in (6–12 patients) and to assess the complete response in the phase II according to iwCLL 2018 criteria (Hallek M et al, Blood 2018). Patients receive tafasitamab – 12 mg/kg on Cycle 1 Day 1 (C1D1) and C1D4, then weekly in C1-3, every other week in C4-6, and monthly during C7-24. Zanu 160 mg (DL 1) or 80 mg (DL -1) is given orally twice daily. Each cycle is 28 days. All patients continue for at least 12 cycles in the absence of toxicities. Patients who achieve undetectable minimal residual disease (uMRD) status (by clonoSEQ) in the peripheral blood and bone marrow after 12 or 24 cycles of combination therapy will stop both agents. Patients who remain with detectable MRD after 24 cycles may continue treatment with single-agent Zanu for up to a total of 5 years. The study follows Simon's minimax two-stage design with a total sample size of 25 patients based on a projected CR rate ≥30% vs 10% (H0), type I error alpha 9.5% and a power 90%.
**Results:** The date for the data cut of this interim analysis was 5 June 2025. Twenty-one patients were enrolled and all were evaluable for safety, with a median follow-up of 14.9 months (range 0.9–23), while 17 patients were evaluable for response. Median age was 65 years (range 41–80); 81% were male; Rai stage 0–1 (29%), 2 (33%), and 3–4 (38%). Baseline prognostic characteristics: unmutated IGVH in 12 pts (57%), TP53 aberrancy in 5 pts (24%), and complex karyotype in 9 pts (43%). The overall response rate was 94% with a CR rate of 24%. The 18-month PFS was 94% (95% CI 67%–99%). The most frequent any grade AEs have been infusion related reaction (76%), neutropenia (52%), and bruising (48%). The most frequent G3-4 AEs have been neutropenia (29%), infusion related reaction (19%), and hypertension (10%). One patient developed atrial fibrillation. Treatment was discontinued in 2 patients as a result of grade 5 toxicities: infection (pulmonary and brain aspergillus) and end-stage liver disease (due to pre-existing alcoholic liver cirrhosis).
**Conclusions:** Our preliminary results suggest that the combination of tafasitamab and Zanubrutinib leads to high overall response rate, including patients with unfavorable prognostic factors. TaZa is well tolerated with no unexpected toxicity. Updated response data with additional patients and correlative MRD studies will be presented in the meeting.

**Keywords:** CLL; zanubrutinib; tafasitamab

**Please indicate how this research was funded:** This study is funded by Incyte

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## Abstract ID: 1903

### **Title:** Hematopoietic progenitor kinase-1 (HPK-1) inhibition improves the in vitro efficacy of bispecific antibodies in CLL

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**Background:** Chronic lymphocytic leukemia (CLL) is marked by T-cell dysfunction, which results in immune suppression and inadequate antitumor immune responses. Hematopoietic progenitor kinase-1 (HPK-1) acts as a negative regulator of T-cell activity, and its inhibition has shown promise in improving T-cell functionality. While cellular therapies have

proven effective in various lymphomas, initial CLL studies have been less successful, largely due to the disease's immunosuppressive characteristics.
**Aims:** This study aimed to explore the effectiveness of HPK-1 inhibition combined with CD20XCD3 bispecific antibodies as a treatment strategy for CLL.
**Methods:** Peripheral blood mononuclear cells (PBMCs) from CLL patients were treated with selective HPK-1 inhibitors, such as HY-138568 or BGB15025, and subsequently activated with anti-CD3/CD28 antibodies. Flow cytometry was used to assess the expression of activation markers, CD69 and CD25. The secretion of interferon-gamma (IFN-γ) and granzyme B from the treated cells was measured using an ELISA assay. The impact of the bispecific antibody glofitamab on T-cell morphology, immune synapse formation, and granzyme B induction was analyzed through confocal microscopy, while cytotoxic effects on CLL cells were evaluated using the Incucyte assay. In this assay, PBMCs were negatively selected to isolate B cells (CLL cells) and T cells, with B cells labeled using BioTracker CSFE dye. The isolated T cells underwent a 2-hour pretreatment with HPK-1 inhibitors before co-culturing with CLL cells at a 4:1 effector to target (E:T) ratio, using glofitamab or an isotype control, with Cytotox red dye to detect cell death.
**Results:** PBMCs from CLL patients treated with HPK-1 inhibitors and activated with anti-CD3/CD28 beads showed a significant increase in CD69 and CD25 surface expression on CD8+ T cells, with no significant change in CD4+ T cells. Additionally, HPK-1 inhibitor pretreatment led to a notable increase in IFN-γ and granzyme B release in response to CD3/CD28 activation. The incubation of CLL patient PBMCs with CD20xCD3 bispecific antibodies, including mosunetuzumab, epcoritamab, and glofitamab, resulted in increased IFNγ secretion, particularly with glofitamab. This effect was enhanced when combined with HPK-1 inhibitors. Glofitamab treatment also induced morphological changes in T-cells, indicating activation and cytotoxicity, characterized by increased CD8+ T-cell size, granzyme B release, and the formation of CLLCD8+ T-cell clusters, with HPK-1 inhibitors further promoting these clusters. The combined treatment of glofitamab and HPK-1 inhibition significantly enhanced granzyme B release and anti-CLL T-cell cytotoxicity, with a considerable number of dead CLL cells observed in the clusters.
**Summary:** This study highlights the promising potential of integrating HPK-1 inhibitors with bispecific antibodies to boost antitumor T-cell cytotoxicity in patient-derived CLL samples. Our findings may pave the way for the development of innovative combination therapies that utilize HPK-1 inhibition to enhance cellular therapies in CLL

**Keywords:** Bispecific; B-CLL; HPK-1

**Please indicate how this research was funded:** Internal funding

**Please indicate the name of the funding organization:** Tel Aviv Sourasky Medical center

## Abstract ID: 1214

### **Title:** CD49d promotes T cell senescence in chronic lymphocytic leukemia

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While CD49d (α4 integrin) is an established prognostic marker in chronic lymphocytic leukemia (CLL) and is associated with aggressive disease, its impact on T-cell biology remains poorly understood. Compared to healthy donors, CLL patients exhibited significantly elevated CD49d expression in both CD4+ and CD8+ T cells ($p < 0.001$) as detected by flow cytometry, which was also confirmed by the single-cell RNA sequencing (scRNA-seq) ($p < 0.001$). Differentially expressed genes in CD49d+T (both CD8+ and CD4+ T cells) vs CD49d- T cells identified in CLL patients were enriched in cellular senescence pathways, while this phenomenon absents in healthy individuals. Functional validation demonstrated that CD49d+T cells displayed elevated senescence-associated markers (e.g. IFN-γ, granzyme B) and a shift toward memory phenotypes, correlating with immunosuppressive signatures. This discovery suggests that targeting CD49d-dependent senescence pathways may reverse T-cell dysfunction in CLL immunotherapy.

**Keywords:** Chronic lymphocytic leukemia; T cells; senescence

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 2002

### **Title:** Improving information provision to enhance chronic lymphocytic leukaemia (CLL) care in resource-limited countries

**Authors:** Peter Haggert[a], Comfort Maluleke[b], John Seymour[c], Catherine Moura[d], Vandana Gupta[e], Tetiana Perekhrestenko[f], Paulo Miranda[g], Bahija Gouimi[h] and Yasmine Hassan[g]

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**Background:** In 2024, we convened a group of healthcare professionals (HCPs) and patient advocacy groups (PAGs) with expert knowledge in CLL to identify unmet needs for people living with the disease. We developed a charter summarizing the key principles people living with CLL expect from their care experience. It comprises six principles surrounding improving diagnosis, information provision, shared decision-making (SDM), access to care, psychosocial support, and survivorship models. These needs are particularly pronounced in resource-limited countries (RLCs) (CLLAN 2023), where the disease burden is increasing (AU Rehman 2024).
**Aims:** We outline recommendations to improve information provision for people living with CLL, particularly in RLCs. We

propose that improving information provision is increasingly important as SDM becomes the norm in clinical practice (VM Montori 2022). This is especially true in CLL, a disease involving complex care and treatment decisions (EC Kranzler 2021). Yet, a global survey found that 41% of patients reported having inadequate information to make well-informed treatment decisions (C Tam 2023).
**Methods:** The charter's development was iterative, with input from 29 HCPs and PAGs. AstraZeneca initiated and funded the charter following a roundtable with experts at the European Hematology Association 2024 Congress. The roundtable identified several unmet needs and recommended principles to address care gaps. These principles were further refined by a roundtable at the 2024 CLL Horizons conference. They were expanded through a structured literature review and two rounds of offline feedback.
**Results:** Information provision is poor for people living with CLL in RLCs (CLLAN 2023). People living with CLL cannot be expected to make important care and treatment decisions without having access to adequate information about their disease. Ensuring increased participation in SDM in RLCs is essential. Research shows that the process can lead to significant patient benefits, cost savings, and better resource allocation through improved health outcomes (VM Montori 2022). Therefore, improving information provision is also a cost-effective measure that RLCs should prioritise while navigating an increasingly challenging fiscal environment (E Warner 2025). Below are some recommendations from the CLL charter to improve information provision for people living with CLL: People living with CLL must be able to access information specific to their disease in the local language. CLL is a complex disease, and people living with CLL often have different needs from those with other types of cancer. These needs include deciding whether to undergo active surveillance (Blood cancer UK) and dealing with the impact of frequent infections due to being immunocompromised (International COVID-19 Blood Cancer Coalition). However, research shows that people living with CLL in RLCs are less likely to be able to access tailored information about their disease (CLLAN 2023). CLL information must be tailored to meet specific literacy and cultural needs in RLCs. There is often low functional literacy in these settings, so many people can struggle to read and understand basic health information (P Murthy 2009). These countries are very diverse (E Gören 2013), which can often mean significant cultural differences and specificities directly affecting how health information is received, understood, and acted upon (SJ Shaw 2009). Tailoring information to different literary and cultural needs can help ensure more inclusive communication (US Centers for Disease Control and Prevention 2024), promoting trust and increasing engagement (World Health Organization 2017). Efforts should also be made to improve literacy and health literacy levels. Ensure that people living with CLL receive information directly from their HCP at an appropriate time. Who delivers the information and when also matters. A global survey showed that people living with CLL prefer receiving information directly from their HCP when they are not in a state of shock (C Tam 2023; D Howell 2024). However, not everyone living with CLL has the opportunity to have this dialogue (D Howell 2024).
**Conclusion:** We, as HCPs and PAGs from across the globe, have developed a charter that outlines six principles to improve care for people living with CLL worldwide. Ensuring people living with CLL can access high-quality information about their disease, tailored to their needs and care pathway, is a valued starting point for delivering meaningful care improvements.

**Keywords:** CLL; charter; education

**Please indicate how this research was funded:** AstraZeneca initiated and funded the charter following a roundtable with experts at the European Hematology Association 2024 Congress. This abstract was organized and funded by AstraZeneca.

**Please indicate the name of the funding organization:** AstraZeneca

## Abstract ID: 1411

### **Title:** Outcomes, inequities, and access to front-line preferred therapies for chronic lymphocytic leukemia in the United States: a real-world evidence study

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**Background:** The treatment landscape for chronic lymphocytic leukemia (CLL) has evolved over the past decade, shifting from chemoimmunotherapy (CIT) and first-generation Bruton tyrosine kinase (BTK) inhibitors (eg, ibrutinib) to next-generation BTK and B-cell lymphoma 2 (BCL2) inhibitors. While these novel therapies (NTs) are now National Comprehensive Cancer Network® (NCCN) guideline–preferred, evidence supporting their real-world effectiveness and equitable use remains limited. Prior research suggests that socioeconomic and racial/ethnic disparities may impact cancer treatment access and outcomes. We evaluated real-world clinical outcomes and inequities with front-line (1L) NT utilization among patients with CLL in the US.
**Methods:** This retrospective cohort study utilized the US-based, electronic health record-derived deidentified Flatiron Health Research Database, linked to neighborhood (US Census track or block group) data from multiple sources. Eligible patients included adults with CLL who started 1L treatment between 1 January 2019 and 31 July 2024. Outcomes included real-world overall survival (rwOS) and time-to-next treatment or death (rwTTNT), assessed using Kaplan-Meier estimates. Patients were grouped by 1L therapy: CIT, ibrutinib, or NCCN guideline–preferred NTs (acalabrutinib, zanubrutinib, and BCL2-based regimens). Trends in treatment utilization by race/ethnicity, insurance, practice type, and 18 area-level social determinants of health factors were assessed. Association between race/ethnicity and 1L treatment was assessed using multinomial logistic regression, adjusting for age, sex, year of 1L initiation, IGHV, and del17p/TP53 status.
**Results:** Of the 4,452 patients included in the study, 3,717 were White (83%), 371 were Black (8%), 209 were Hispanic (5%), and 155 were Other race (4%; includes Asian, American Indian/Alaska Native, Native Hawaiian/Other Pacific Islander, and people reporting multiple races). Overall, 37% of patients received an NCCN guideline–preferred NT (eg, acalabrutinib, zanubrutinib, BCL2-based regimens). Among preferred NTs,

BTK inhibitor monotherapy was used the most often (60%). At a median follow-up of 28.5 months, median rwOS was not reached (NR). Survival probabilities tended to be highest with NCCN guideline–preferred NTs at 6, 12, and 18 months (97%, 94%, and 91%, respectively), followed by ibrutinib (96%, 92%, and 89%) and CIT (94%, 89%, and 86%). Median rwTTNT was NR in patients receiving a preferred NT; the median rwTTNT in patients receiving ibrutinib was 40 months (95% CI 37–45) and in those receiving CIT was 13 months (95% CI 11–15). The receipt of preferred NTs was highest among White (38%), followed by Other (35%), Black (34%), and Hispanic (26%) patients. More patients receiving preferred NTs were treated at academic centers (23% vs 15% ibrutinib), while more patients receiving ibrutinib were treated at community practices (73% preferred NTs vs 81% ibrutinib). In general, compared with patients living in census tracts with the lowest social deprivation, those residing in areas with the highest social deprivation appeared less likely to receive preferred NTs. Among those residing in areas with the highest socioeconomic status (SES) index, 40% received preferred NTs versus only 32% of patients residing in areas with the lowest SES index. In total, 26% and 27% of patients residing in predominantly Black or Hispanic neighborhoods, respectively, received a preferred NT versus 39% of patients residing in predominantly White neighborhoods. Higher NT use also seemed to be associated with residence in areas with the highest levels of internet access (39% vs 32% in areas with lowest levels of internet access), vehicle ownership (39% vs 33% in areas with lowest vehicle ownership), and health insurance coverage (41% vs 34% in areas with least health insurance coverage). Compared with White patients, Hispanic patients were more likely to receive CIT (adjusted odds ratio [aOR] 2.04; 95% CI 1.98–2.09) and ibrutinib (aOR 1.69; 95% CI 1.65–1.73) than preferred NTs. Black patients were also more likely to receive ibrutinib than preferred NTs (aOR 1.38; 95% CI 1.20–1.59) compared with White patients.
**Conclusion:** Receipt of 1L NCCN guideline–preferred NTs was associated with better real-world survival and treatment durability compared with ibrutinib and CIT among patients with CLL. We observed lower use of preferred NTs among Black and Hispanic patients, and among patients residing in neighborhoods with higher social deprivation. These patterns highlight potential inequities in the adoption of guideline-recommended treatments, underscoring the need for research to better understand barriers leading to these inequities and tailored interventions to promote equitable treatment access.

**Keywords:** Racial disparities; front-line treatment patterns; outcomes

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## Abstract ID: 1705

**Title:** Inequalities in the diagnosis and survival of 30,071 people consecutively diagnosed with chronic lymphocytic leukaemia in England between 2014 and 2021: preliminary analysis from the UNCOVER study group

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**Background-aim:** Population-based datasets are essential for understanding epidemiology and evaluating the impact of changes in therapeutic paradigms for conditions such as chronic lymphocytic leukemia (CLL). UNCOVER is a blood cancer health data research programme that utilises routinely collected NHS data provided by the National Disease Registration Service (NDRS). The data is a quality-assured collation from multiple sources and includes unselected patients diagnosed with hematological malignancies in England from 2014 to 2021, along with patient-level survival and treatment data. The present study used this data to profile the recent epidemiology of CL L in England.
**Methods and Cohort Description:** Information was available on 30,071 patients aged ≥18 who were diagnosed with CLL from January 2014 to December 2021 with follow-up until July 2023. Diagnoses made by death certificate only were excluded. Incidence rate ratios (IRR) were calculated using Poisson regression to examine associations between incidence and baseline characteristics. Cox proportional hazards regression was applied to assess associations between baseline characteristics and overall survival (OS). Fine-Gray (F-G) competing risk regression was utilized to estimate sub-distribution hazard ratios (sHR) for mortality from blood cancer vs other causes. Net survival was calculated using the Pohar-Perme estimator. Models included gender, age at diagnosis (categorized), ethnicity, government region, indices of multiple deprivation (IMD) quintiles, and year of diagnosis. The Cox and F-G models also included the Charlson comorbidity index (CCI).
**Incidence analysis:** The age-standardized incidence of CLL peaked at 8.12 per 100,000 population in 2015 and dropped to 5.94 per 100,000 in 2020. Males accounted for 62% of cases, median age at diagnosis of 72 years (IQR: 65–80). Females were 49% less likely to develop CLL compared to males [IRR =0.51; 95% CI: 0.50, 0.52]. Predominant ethnicity was white (87%), and most (68%) had a CCI of 0. Among years with data available for route of diagnosis, most (54%) were referred via primary care, while a minority (10.6%) were identified via emergency presentation . Incidence rates for the least deprived areas were lower than for the most deprived areas with an IRR of 0.94 (95% CI: 0.91, 0.98). Compared to 2014, the rates of CLL diagnoses were lowest in 2020 [IRR =0.72; 95% CI: 0.69, 0.76] and 2021 [IRR =0.70; 95% CI: 0.67, 0.74] coinciding with the COVID pandemic. All examined factors (age, gender, ethnicity, region, deprivation quintile, and year of diagnosis were independently associated ($p < 0.01$) with CLL incidence in multivariable analysis. Analysis by government region showed lower incidence outside Greater London, with the Southeast accounting for the lowest rate [IRR =0.70; 95% CI: 0.67, 0.74].

**Survival analysis:** Median follow-up time was 49.1 months. 37.5% of patients died with OS rates at 1, 3, and 5 years of 88%, 76%, and 65%, respectively, while the corresponding net survival rates were 92%, 85%, and 79%, suggesting that patients become increasingly susceptible to non-CLL causes of mortality as they survive longer. Cox regression showed that the risk of all-cause mortality was 19% lower in females compared to males [HR =0.81; 95% CI: 0.78, 0.84] and 31% lower among individuals from the least deprived areas compared to their counterparts in the most deprived neighbourhoods [HR =0.69; 95% CI: 0.65, 0.74] . The hazard ratio for all-cause mortality was highest for patients diagnosed in 2020 [HR =1.21; 95% CI: 1.12, 1.32] compared to 2014. The F-G model for competing risk of survival showed year of diagnosis, age, ethnicity, region, and CCI were independently associated with OS and competing risk survival, with no significant increase in the sHR when compared to the Cox model, suggesting a low CLL-related mortality in this cohort.
**Conclusions:** UNCOVER has provided the largest European study of CLL epidemiology and demonstrated to date, with clear associations with demographic, socioeconomic, and clinical variables. Incidence was higher and survival shorter for males, older individuals, and those from more deprived areas. The COVID-19 pandemic was associated with fewer diagnoses and increased mortality. Most patients were diagnosed early, with low CLL-specific mortality suggesting that competing survival risks play a significant role in long-term outcomes. Further analysis of the data including timing and type of treatment is underway and will provide further insight into the correlations observed in this initial analysis.

**Keywords:** Population-based; real world; CLL; inequalities; incidence; net survival

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** Cancer Research UK, Grant Ref. 23010

## Abstract ID: 1412

### **Title:** Real-world utilization of novel therapies and overall survival among U.S. medicare beneficiaries initiating frontline chronic lymphocytic leukemia treatment

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**Introduction:** Over the last decade, the treatment landscape for chronic lymphocytic leukemia (CLL) has shifted significantly with the introduction of novel therapies such as treat-to-progression Bruton's tyrosine kinase inhibitors (BTKis) and fixed-duration BCL-2 inhibitors (BCL-2is). Though BTKis and BCL-2is (venetoclax) have largely supplanted traditional chemotherapy in treatment guideline recommendations, little is known about utilization of these novel therapies and how it varies by age, race/ethnicity, and income. Furthermore, overall survival and associated factors in the real-world setting are poorly understood. Real-world evidence is especially lacking in patients covered under Medicare, a federal insurance program covering most older adults and representing the largest payer for CLL-related care in the U.S.
**Aim:** To examine access to novel therapies, overall survival, and associated factors in a national sample of fee-for-service U.S. Medicare beneficiaries initiating CLL treatment in the frontline setting.
**Methods:** This retrospective cohort study used 2016–2023 100% Medicare fee-for-service claims, identifying beneficiaries age ≥65 years initiating treatment for frontline CLL between 1 June 2019 and 31 December 2022. The index date was the date of the first prescription fill or infusion. Novel therapies included BTKis (ibrutinib-, acalabrutinib-, or zanubrutinib-based regimens) and BCL-2is (venetoclax-based regimens); all other CLL treatments (anti-CD20 monotherapy, traditional chemotherapy, etc.) were classified as non-novel. Novelty of CLL treatment received (BTKi/BCL-2i [novel] vs. other [non-novel]) was examined in the overall sample and by key subgroups defined by age (65–69, 70–74, 75–79, 80+), race/ethnicity (White, Black, Hispanic, Other), and Part D low-income subsidy (LIS) status (LIS duals, LIS non-duals, and non-LIS). Among novel treatment users, we further examined treatment type by duration (treat-to-progression BTKis vs. fixed-duration BCL-2i), overall and by key subgroups. Kaplan-Meier curves for overall survival [OS] were generated in the overall sample, by CLL treatment received, and by key subgroups of interest. Binomial logistic regressions and Cox regressions were used to examine the association of key sociodemographic factors with novel treatment utilization and OS, respectively, while controlling for other patient demographic, clinical, and insurance plan characteristics.
**Results:** The final sample contained 10,949 patients treated for CLL in the frontline setting. In the overall sample, 68.1% of the patients received novel treatments (n = 7,459); 13.7% of all patients received fixed-duration venetoclax (n = 1,503), 54.5% received BTKis (n = 5,956), whereas the remaining (31.8%) received other non-novel therapies. Descriptive results showed differences in novel treatment utilization by age and income status, but no differences were observed by race/ethnicity. Regression results confirmed these findings. For example, relative to patients aged 65–69 years, patients aged 75–79 years were less likely to receive novel therapies (odds ratio [OR]: 0.82, 95% confidence interval [CI] 0.69–0.98, $p = 0.030$). No statistically significant differences in receipt of novel therapies or fixed-duration treatment were observed by race/ethnicity. Compared to non-LIS beneficiaries, LIS duals were more likely to receive novel therapy (OR: 1.32, 95% CI 1.09–1.60, $p = 0.005$). Kaplan-Meier curves showed longer OS for novel vs. non-novel treatment users. Furthermore, venetoclax was associated with higher OS than BTKis. Specifically, the 1-year and 3-year survival rates, respectively, were highest for patients receiving venetoclax (90% and 77%), followed by BTKis (85% and 67%) and other therapies (81% and 62%). Cox regressions confirmed that relative to venetoclax use, BTKi (hazard ratio [HR]: 1.49, 95% CI 1.32–1.68, $p < 0.001$) and other CLL therapies (HR: 1.65, 95% CI 1.46–1.87, $p < 0.001$) use were associated with poorer OS. In the overall sample, relative to patients aged 65–69 years, OS was worse in patients aged 75–79 years (HR: 1.55, 95% CI 1.30–1.84, $p < 0.001$) and aged 80+ years (HR: 2.66, 95% CI 2.25–3.15, $p < 0.001$). No statistically significant differences in OS were observed by race/ethnicity in the overall sample. Compared to non-LIS beneficiaries, OS was poorer for both LIS dual (HR: 1.15, 95% CI 1.00–1.33, $p = 0.049$) and LIS non-dual (HR: 1.34, 95% CI 1.14–1.58, $p < 0.001$) beneficiaries.

**Conclusion:** This national real-world study of Medicare beneficiaries initiating frontline CLL therapy found that novel treatment, particularly the BCL-2i venetoclax, was associated with longer overall survival. Given these differences in survival, consideration of novel agent use, especially venetoclax combinations, is warranted in older CLL patients. Our findings highlight the importance of understanding the reasons for and addressing potential access barriers to novel therapies among Medicare beneficiaries with CLL in the U.S.

**Keywords:** CLL; venetoclax; survival/overall survival

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## Abstract ID: 1215

### **Title:** Expression and signalling patterns of CD180/MD1 and CD150 in chronic lymphocytic leukaemia (CLL) cells and MEC 1 cell line

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**Introduction:** We have previously shown that, differently from normal peripheral blood (PB) and tonsillar B cells, expression of the CD180 toll-like receptor is highly heterogeneous on PB and lymph node chronic lymphocytic leukemia (CLL) cells [1–3]. The MD-1 satellite molecule, essential for CD180 expression on the cell surface, could play a role in this heterogeneity [3]. Importantly, we have shown that CD180 could hold prognostic value, since its high expression is associated with early-stage disease, M-CLL, and superior overall survival [1,3]. The ability of CD180 to signal via pro-apoptotic p38MAPK pathway and re-wire IgM-mediated AKT survival signalling to a p38MAPK pathway [4], explains the positive association between CD180 expression and favourable prognosis. Similarly, higher expression of CD150, a member of the signalling lymphocyte activation molecule family (SLAMF), that is co-expressed with CD180 on CLL cells, has been associated with enhanced survival [5].
We therefore aimed to characterise the cell surface (CS) and intracellular (IC) expression of CD180 and MD-1, as well as the surface expression of CD150, in primary CLL cells and the CLL-derived MEC-1 cell line. Additionally, we assessed the correlation between these expression patterns with intracellular signalling.
**Methods:** Peripheral blood mononuclear cells were isolated from 26 untreated CLL patients (clinic 'Aversi,' care of Dr Datikashvili-David, upon informed consent) using density gradient and immunophenotyped with APC-Cy7-conjugated anti-CD180 (clone G28-8, BD), PE-conjugated polyclonal anti-MD-1, and APC-conjugated anti-CD150 (clone SLAM.4, Abcam). CD19+ cells were identified using anti-CD19 FITC (BD). For intracellular expression, cells were stained with anti-CD19 FITC and anti-CD150 APC, fixed and permeabilized, and stained for anti-CD180 or anti-MD-1. Eleven CD180+MD1+ CLL samples were stimulated with anti-CD180 and anti-CD150 for 30 minutes at 37 °C, followed by staining with PE-conjugated anti-pAKT or APC-conjugated anti-p-p38MAPK (BD). Flow cytometry analysis was conducted on a NovoCyte 2060 (ACEA Biosciences). The same procedures were performed on the MEC1 cell line. MEC1 cells were synchronized by serum starvation and analyzed at 0, 24, 48, and 72 hours.
**Results:** CD180 and MD-1 expression in CLL samples was assessed as cell surface (CS) or intracellular (IC)+CS. As expected, permeabilization significantly increased the percentages of CD180-positive IC+CS across all samples ($p=0.01$), even in those with low CS levels, thus indicating a substantial intracellular CD180, regardless of surface expression. A similar trend was observed for MD-1 ($p=0.06$). With the applied threshold of 60% to define positivity for csCD150 and csCD180, the levels of csMD-1-postive and cs+icMD-1-positive cells were increased in csCD180-positive (43.3±11.0% vs 10.7±6.3%; $p=0.001$), but also in csCD150-postive (49.3±11.0% vs 17.6±5.7%; $p=0.001$) cases. Stimulation with anti-CD180 revealed distinct signalling subsets: AKT-signalers (AKT-S), p38MAPK-signalers (p38MAPK-S), non-signalers (NS), and dual AKT/p38MAPK signalers (DS). Upon anti-CD180 stimulation, CD180+CD150- CLL cells demonstrated a significant increase in p-AKT (unstimulated 2.4±1.6%, stimulated 23.2±6.7%; $p=0.003$), whereas p-p38MAPK remained low. In contrast, CD180+CD150+ CLL cells exhibited no AKT response, but showed slightly enhanced p38MAPK activation (unstimulated 3.6±1.4%, stimulated 7.8±1.8%, $p=0.05$). Like CD180, anti-CD150 stimulated CLL cells appeared to signal via preferential AKT-S, p38MAPK-S, DS, or NS patterns. In MEC-1 cells, csCD180 expression peaked at 24 and 48 hours before returning to baseline at 72 hours, while cs+icCD180 peaked at 48 hours and remained elevated. cs+icMD-1 peaked at 24 hours, consistently exceeding surface expression. CD150 expression remained high. MEC-1 cells did not show appreciable AKT or p38MAPK phosphorylation upon CD180 ligation. However, at 48 hours, anti-CD150 significantly reduced expression of p-p38MAPK (unstimulated 55.0±7.5%, stimulated 7.8±1.5%; $p=0.002$) and slightly increased p-AKT (unstimulated 3.9±0.7%, stimulated 7.5±1.7%; $p=0.05$). Similarly, combined CD150 and CD180 ligation resulted in reduced p-p38MAPK (unstimulated 55.0±7.5%, stimulated 10.9±4.9%; $p=0.02$) and increased p-AKT (unstimulated 3.9±0.9%, stimulated 23.2±19.0%; $p=0.003$).
**Conclusion:** Downregulation of MD-1 is associated with reduced CD180 surface expression, potentially weakening CD180-mediated signalling in CLL. CD150 appears to regulate CD180-induced AKT activation and also functions as an independent signalling receptor. Time-dependent expression changes observed in MEC1 cells highlight the importance of cellular context and timing in receptor-driven signalling. Overall, the CD180/MD-1/CD150 axis plays a key role in CLL pathophysiology.

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**Keywords:** CD180; MD-1; CD150

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## Abstract ID: 1507

**Title:** Allogeneic stem cell transplantation is effective after pathway inhibitor treatments in CLL – a retrospective study on behalf of the chronic malignancies working party of EBMT

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**Introduction:** The life expectancy of CLL patients refractory to both BTK- and BCL2-inhibitors (BTKi and BCL2i) is dismal, even when non-covalent BTKi become available. We investigated if double-exposed patients may benefit from alloSCT.

**Methods:** From the EBMT Registry CLL patients were identified who had been exposed to both BTKi and BCL2i before alloSCT, excluding those with Richter's transformation. The probabilities of Graft-versus-Host and Relapse-free survival (GRFS), Progression-free survival (PFS), and Overall Survival (OS) were calculated using the Kaplan-Meier method. Cumulative Incidence of Relapse and nonrelapse mortality (NRM) were analyzed as competing risks.

**Results:** We identified 127 patients, with a median age of 60 years (range 31–70). 78% had received chemotherapy before treatment with a BTKi and BCL2i, and 22 had also been treated with a PI3K-inhibitor (PI3Ki). Most patients (76%) were transplanted right after BCL2i treatment and only six after a PI3Ki. 76% were transplanted with responsive disease. The median follow-up of patients alive was 35 months. There were 105 double-exposed patients, with a median age of 59 years. Their 3-year GRFS, PFS, and OS were 30%, 48%, and 56%, and 3-year Relapse Incidence and NRM were 30% and 21% respectively. For the 22 triple-exposed patients (median age 63 years), 3-year GRFS, PFS, and OS were 20%, 36%, and 56%, and 3-year Relapse Incidence and NRM were 34% and 30% respectively. Most NRM and relapse events occurred within 6 months after the transplantation. Three-year PFS for double- or triple exposed patients with responsive disease at transplant was 51% and 41%. Double-exposed patients with refractory disease at transplant had a 3-year PFS of 42%, while only one of the six triple-exposed refractory patients survived without progression. There were 38 patients with known del(17p) and/or TP53 mutation, of whom 80% had been treated with chemotherapy before starting on a BTKi, BCL2i, and, in six, on a PI3Ki. For 76% the last therapy before alloSCT was a BCL2i. 3-year GRFS, PFS, and OS were 25%, 42% and 49%, and 3-year NRM and Relapse rate 22% and 35% respectively.

**Conclusions:** The median survival for double-exposed patients who subsequently underwent alloSCT exceeded 3 years, with a 3-year OS of 56%. When relapse occurred, most cases were observed within 6 months after alloSCT. Non-relapse mortality (NRM) appears comparable to rates reported for CLL patients prior to the BTKi and BCL2i era (van Gelder et al., BMT 2017), where it was influenced by age. These results are favorable compared to those achieved with treatment using a non-covalent BTKi in double-exposed CLL patients. Finally, the optimal strategy remains unclear: whether to begin with a non-covalent BTKi before proceeding to alloSCT, or to perform alloSCT first and reserve non-covalent BTKi for use in relapsed patients.

**Keywords:** Relapsed/refractory; allogeneic stem cell transplantation; TP53 anbormalities

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## Abstract ID: 1508

**Title:** Measurable residual disease in chronic lymphocytic leukemia (CLL) in clinical practice: a single-center report over the years 2018–2024

**Authors:** Jana Mihályová[a], Kamila Kutějová[b], Ingrid Kováčová[a], Jana Zuchnická[a], Klára Lančová[a], Michaela Horňáková[a], Václav Lipka[a], Viktor Kubala[b], Tomáš Jelínek[c] and Roman Hájek[c]

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**Introduction:** The European Medicines Agency has approved the assessment of measurable residual disease (MRD) as a surrogate marker of treatment response in patients with chronic lymphocytic leukemia (CLL) treated within clinical trials. In routine clinical practice, MRD monitoring is not systematically implemented. The clinical relevance of MRD-guided therapy, particularly in modern treatment regimens, remains uncertain. In the following analysis, we present an overview of MRD assessment in CLL patients treated at a single hemato-oncology center between 2018 and 2024.

**Methods:** Measurable residual disease was evaluated in peripheral blood (PB) using 8-color flow cytometry (CD81/CD43/CD79b/CD22/CD5/CD19/CD20/CD3) (Hallek et al., 2018; Rawstron et al., 2016) with he median limit of detection (LOD) in PB 0.0036% (range: 0.0003%–0.0088%). MRD testing was performed in patients with an absolute lymphocyte count below $5\times10^9$/L who had achieved at least a partial remission. The initial analysis was carried out no earlier than 3 months after the start of treatment, with follow-up monitoring at 3–6 month intervals. In a limited subset of patients with undetectable MRD (MRDneg) confirmed in PB on two consecutive occasions, bone marrow (BM) samples were analyzed to verify MRD status in the BM compartment.

**Results:** Between 2018 and 2024, MRD in PB was monitored in 157 patients, with a total of 450 measurements performed. Of these, 83 were treated in the first-line setting (median follow-up of 32.2 months) and 77 received second-line or later therapy (median of 1 prior therapy, median follow-up of 38.0 months). Out of 83 newly diagnosed (ND) patients, 47% (39/83) achieved MRDneg and 53% (44/83) had detectable MRD (MRDpos). The most commonly used regimen was chemoimmunotherapy (n = 49), venetoclax-based regimen (Ven) (n = 31), and Bruton's tyrosine kinase inhibitors (BTKi) (n = 3). Baseline characteristics were comparable between the groups, except for a higher frequency of trisomy 12 in the MRDpos cohort. Venetoclax-based regimen was more frequently used in MRDneg patients (51.3% vs 25%), whereas chemoimmunotherapy (48.7% vs 68.2%) predominated in MRDpos cases ($p = 0.013$). Among MRDneg patients, the longest median PFS was observed in those treated with Ven regimens (not reached vs. 57 months for Ven vs. chemoimmunotherapy). Median PFS in MRDpos NDCLL patients was longer in Ven cohort (not reached vs. 32 months Ven vs. chemoimmunotherapy). Of the 77 relapsed/refractory (RR) CLL patients, 44.2% (34/77) achieved MRDneg in PB and 55.8% (43/77) remained MRDpos. The majority received venetoclax (n = 34) or BTKi (n = 29), while a minority were treated with chemoimmunotherapy (n = 14). Baseline patient characteristics were similar across the groups except that Ven were more commonly used in the MRDneg cohort (73.5% vs. 21%), while BTKi were more frequently administered in the MRDpos group (60.5% vs. 8.8%) ($p < 0.001$). When analyzed by treatment regimen, the longest median PFS was observed in Ven cohort (not reached vs. 30 months for Ven vs. chemoimmunotherapy). Median PFS was also not reached in MRDneg patients on BTKi, but this subgroup was represented only by three individuals. In the MRDpos cohort, patients receiving BTKi demonstrated the longest median PFS (60 vs 33 vs 20 months in BTKi vs Ven vs chemoimmunotherapy). A limited subset of patients underwent BM aspiration for MRD assessment, with MRD neg confirmed in 83.3% (10/12) of first-line and 85.7% (6/7) of relapsed cases.

**Conclusion:** In our primary analysis, venetoclax-based regimens, followed by chemoimmunotherapy, were the most effective in achieving MRDneg in both NDCLL and RRCLL. During the current follow-up period, the median PFS was not reached in MRDneg patients treated with Ven in either setting. Median PFS observed in MRDneg patients treated with chemoiimunotherapy was longer in NDCLL (57 and 30 months in NDCLL and RRCLL, respectively). Among MRDpos RRCLL patients, the longest PFS was seen with BTKi, possibly reflecting the impact of continuous therapy, although this finding may also be influenced by differences in cohort size. Expanding number of assessed patients and adopting a more sensitive MRD threshold (i.e. $<10^{-5}$) may improve the ability to stratify patients by PFS benefit and potentially guide treatment decisions in real-world settings. A subanalysis by TP53 aberrations and IGHV mutation status and the deep of remission (complete vs partial) will be presented at the conference.

**Keywords:** Leukemia; measurable residual disease; 8-color flow cytometry

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** Department of Hematooncology, University hospital Ostrava

## Abstract ID: 1413

**Title:** A fixed-duration, chemo-sparing approach in CLL: 8-year results of the phase 2 ICLL-07 FILO trial

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In previously untreated, medically fit, patients with chronic lymphocytic leukemia, research is focused on developing fixed-duration strategies to improve long-term outcomes whilst sparing patients from serious toxicities. The ICLL-07 trial evaluated a fixed-duration (15-month) immunochemotherapy approach with a 9 months obinutuzumab-ibrutinib (O+I) induction. Patients (n=10) in complete remission (CR) with low bone marrow (BM) measurable residual disease (MRD <0.01%) continued ibrutinib alone at 420 mg/day for 6 additional months (I arm). All other patients (n=118) received up to four cycles of fludarabine-cyclophosphamide-obinutuzumab consolidation together with ibrutinib (I-FCG arm). Primary analysis at month 16 showed that 62.2% (84/135) of all patients enrolled achieved CR with BM MRD <0.01% (Michallet AS, Lancet hematol 2019; Blood 2021). Peripheral blood (PB) MRD (low-level [i.e. detectable but <0.01%] or undetectable at a threshold of $10^{-4}$), in evaluable patients, was still 92.5% (74/80) at month 40 and 80.6% (50/62) at month 64. No difference in PB MRD status was apparent according to the IGHV mutational status (Michallet AS, Blood advances 2023). Here follow up with a median of 97 months (Inter quartile range [IQR] 93–102.5) is reported. Progression-free and overall survival rates are at 87 % and 93%, respectively. At last follow up, 92/128 (72%) patients are alive in CR, 8 in PR, 1 in stable disease. Fourteen (11%) patients are progressing, 4 of them receiving treatment (rituximab venetoclax, n=3, or ibrutinib+venetoclax, n=1). Twelve patients died, including 2 from cardiac toxicity during treatment with O+I attributable to I (M6 and M7). The 10 other deaths are distributed among 4 infections (3 bacterial pneumonias at M38, M55, M50, and 1 COVID 19 at M49), 1 histiocytic sarcoma (M47), 1 myelodysplasia (MDS; M56) and 1 acute myeloid leukemia (AML; M68). Three deaths were due to progression (n=1, M103) or transformation in Richter large B-cel lymphoma (n=1; M54) or Hodgkin lymphoma (n=1; M23). No second cancer developed in 88% of the patients (104/118). With a median time between treatment initiation and development of a second disease of 72 months (IQR 10.28–97.77), nine patients developed, respectively, prostate cancer (n=2), kidney cancer (n=1), Merkel cell carcinoma (n=1), choroidal melanoma (n=1), hepatocellular carcinoma (n=1), skin cancer (n=1), MDS (n=1) or AML (n=1). In long-term infectious follow-up, no serious infections (grade 3–4) were observed in 84% (99/118) of the patients, while 10 (8%) developed a grade 3 infection (2 SARS-CoV2, 1 viral gastroenteritis, 2 respiratory syncytial virus, 3 bacterial pneumonias, 1 bronchitis, 1 shingles). These long-term results show excellent survival rates (close to those of a healthy population in the same age range) in patients with first-line CLL regardless of IGHV mutation status, with no increased risk of infection or second cancer.

**Keywords:** CLL; OS; long term follow up

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## Abstract ID: 1803

**Title:** Real-world treatment outcomes among patients with high-risk CLL/SLL receiving venetoclax-based therapy in 1L or 2L: an updated international study

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**Introduction:** Clinical trials have demonstrated deep responses and extended survival with venetoclax-based therapy (VEN) in patients with high-risk (i.e. unmutated IGHV [uIGHV] and/or del[17p]/TP53) CLL/SLL). However, real-world data on clinical outcomes for patients with high-risk CLL/SLL are still limited or constrained by short follow-up durations.

**Objectives:** This study evaluated clinical outcomes in patients who initiated VEN in first-line (1L) or second-line (2L) settings as mono- or combination therapy and had high-risk CLL/SLL.

**Methods:** Data on adult patients with CLL/SLL from 25 sites of the CLL Collaborative Study of Real-World Evidence (CORE), a global, retrospective, observational study were used. Patients included had del(17p)/TP53 and/or uIGHV prior to initiation of VEN in 1L or 2L. Clinical outcomes assessed were overall response rate (ORR; proportion of patients with a documented physician-defined complete/partial response), progression-free survival (PFS; time from VEN initiation to disease progression/death or end of follow-up), time to next treatment/death (TTNT-D; time from VEN initiation to subsequent treatment/death or end of follow-up), and overall survival (OS; time from VEN initiation to death or end of follow-up). Outcomes were reported by high-risk feature (non-mutually exclusive: del[17p]/TP53; uIGHV; uIGHV-only (i.e. without confirmed del[17p/TP53]); both uIGHV+del[17p]/TP53), line of therapy (1L, 2L), including further by high-risk feature, and VEN regimen (monotherapy [VMono]; combination [VCombo]).

Kaplan-Meier methodology was used to estimate 24-month rates.

**Results:** Of 2,696 patients in CORE, 317 patients (11.8%) with high-risk CLL/SLL initiated VEN and were included (1L: 206 [65.0%]; 2L: 111 [35.0%]). Among the 317 patients (median age: 64.9 years), 87 (27.4%) had del(17p)/TP53 (1L: 39 patients; 2L: 48 patients), 272 (85.8%) had uIGHV (1L: 191 patients; 2L: 81 patients), 221 (69.7%) had uIGHV-only (1L: 159 patients; 2L: 62 patients), and 42 (13.2%) had both uIGHV + del(17p)/TP53 (1L: 24 patients; 2L: 18 patients). VMono was initiated by 45 (14.2%) patients, while 272 (85.8%) received VCombo (mostly with obinutuzumab: 199 [73.2%]). Among patients who initiated VEN in 2L, common 1L therapy included ibrutinib- (26/48 [54.2%]), CT/CIT- (9 [18.8%]), and rituximab-based therapy (4 [8.3%]). Median follow-up was 24.0 (IQR: 11.1, 37.8) months (median VEN duration of 11.1 [IQR: 8.2, 13.2] and 13.6 [IQR: 5.7, 24.6] months in 1L and 2L, respectively). In 87 patients with del(17p)/TP53, ORR was 87.9% with 24-month rates of 77.6% for PFS, of 74.5% for TTNT-D, and of 87.5% for OS. In 272 patients with uIGHV, ORR was 90.1% with 24-month rates of 89.4% for PFS, of 88.3% for TTNT-D, and of 92.6% for OS. In 221 patients with uIGHV-only, ORR was 91.9% with 24-month rates of 91.5% for PFS, of 91.3% for TTNT-D, and of 93.0% for OS. In 42 patients with both uIGHV + del(17p)/TP53, ORR was 90.9% with 24-month rates of 81.0% for PFS, of 78.8% for TTNT-D, and of 89.1% for OS. In 1L, ORR was 92.0% (del(17p)/TP53: 89.7%, uIGHV: 92.8% among 142 patients, uIGHV-only: 94.5% among 120 patients, uIGHV + del(17p)/TP53: 94.7% among 18 patients) with 24-month rates of 90.8% for PFS (del(17p)/TP53: 84.0%, uIGHV: 91.8%, uIGHV-only: 92.6%, uIGHV + del(17p)/TP53: 88.1%), of 90.9% for TTNT-D (del(17p)/TP53: 85.5%, uIGHV: 92.5%, uIGHV-only: 93.5%, uIGHV + del(17p)/TP53: 95.5%), and of 94.4% for OS (del(17p)/TP53: 94.6%, uIGHV: 94.5%, uIGHV-only: 94.2%, uIGHV + del(17p)/TP53: 95.5%). In 2L, ORR was 84.3% (del(17p)/TP53: 86.5%, uIGHV: 83.3%, uIGHV-only: 84.4%, uIGHV + del(17p)/TP53: 85.7%) with 24-month rates of 80.6% for PFS (del(17p)/TP53: 72.9%, uIGHV: 83.5%, uIGHV-only: 88.3%, uIGHV + del(17p)/TP53: 72.6%), of 75.9% for TTNT-D (del(17p)/TP53: 66.8%, uIGHV: 78.0%, uIGHV-only: 85.3%, uIGHV + del(17p)/TP53: 59.4%), and of 86.3% for OS (del(17p)/TP53: 82.4%, uIGHV: 87.8%, uIGHV-only: 89.6%, uIGHV + del(17p)/TP53: 81.7%). In patients who initiated VMono, ORR was 72.7% with 24-month rates of 75.7% for PFS, of 68.1% for TTNT-D, and of 83.7% for OS. In patients who initiated VCombo, ORR was 92.0% with 24-month rates of 89.2% for PFS, of 88.9% for TTNT-D, and 93.0% for OS.

**Conclusion:** This is one of the largest studies to date on VEN use in high-risk CLL/SLL, particularly for those with both uIGHV + del[17p]/TP53. These results, which build on previous findings from clinical trials and real-world data, demonstrate the clinical effectiveness of VEN in 1L/2L for treatment of patients across high-risk features (i.e. uIGHV and/or del[17p]/TP53). Future research should continue to investigate the use of VEN in treating patients with high-risk CLL/SLL.

**Keywords:** Treatment effectiveness; high-risk; BCL2i

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# Abstract ID: 1104

## Title: A multi-gene expression-based prognostic model for risk stratification in chronic lymphocytic leukemia (CLL)

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**Introduction:** Aberrant gene expression impacts disease biology and correlates with patient outcomes in CLL, but expression-based prognostic models have had limited clinical applicability to date. We aimed to develop a composite gene signature-based risk stratification model using transcriptomic data.

**Methods:** Transcriptomic profiles of 405 CLL patients with available samples from the first-line phase 3 CLL14 trial (Ven-Obi vs. Clb-Obi) were used to train a prognostic model for progression-free survival (PFS). Cox proportional hazards and cross-validated LASSO models were applied subsequently to highly variable genes (HVGs) to identify those associated with PFS. A risk score was derived by applying nonzero model coefficients to normalized expression values. Risk status was assessed using a multivariable Cox model adjusted for genetic markers. The model was subsequently validated in cohorts from the MURANO relapse trial (BR vs. Ven-Rit; n = 204) and the Broad (cllmap.org; n = 568) dataset.

**Results:** A total of 107 of 251 HVGs were significantly associated with PFS ($p < 0.01$). An eight-gene (BCAT1, LHFPL6, C10orf10, ZNF471, PLD1, DNAH14, KLK4, and IL15) derived risk score stratified patients into high-risk (n = 251) and low-risk (n = 154) classes (A,B). Risk status correlated with PFS (hazard ratio [HR] = 3.12 [2.05–4.75], $p < 0.001$), independent of IGHV mutation and del17p/TP53 alterations (C). Both high-risk IGHV-mutated and unmutated patients had shorter PFS

compared to their respective low-risk counterparts (HR =3.68 [2.16–6.29], $p<0.001$ for IGHV-mutated; HR =0.403 [0.22–0.73], $p=0.002$ for IGHV-unmutated, comparing low- vs. high-risk). Among del17p/TP53 subgroups, low-risk patients with or without del17p/TP53 exhibited longer PFS than high-risk patients (HR =0.10 [0.023–0.44], $p=0.002$ for mutated; HR =3.02 [2.20–4.13], $p<0.001$ for wild-type, comparing high- vs. low-risk). C-statistics of 0.62 and 0.63 from the two validation cohorts supported the model's prognostic performance.
**Conclusions:** The multi-gene prognostic model reproducibly identifies clinically relevant subgroups with distinct prognoses, facilitating refined risk stratification in both frontline and relapsed CLL.

**Keywords:** Leukemia; relapse; biomarker

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1216

### **Title:** Biological features of B-CLL and residual immunity at treatment initiation determine immunity on BTKi therapy

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**Background:** Impaired immunity on continuous BTKi therapy is associated with substantial infection related morbidity/mortality and poor response to vaccination in patients with CLL. The recently published UK IMPROVE Randomized control trial (ISRCTN14197181) investigated vaccine immunity for patients taking BTKi and reported heterogeneity in patient humoral immunity. An appreciation of the biological determinants of poor immunity during covalent BTKi therapy and an improved understanding of why such variability exists amongst patients, is now needed.
**Aims:** To determine the biological features of CLL and humoral immunity that influence infection risk and vaccine responsiveness during covalent BTKi therapy.
**Methods:** Using participant samples from IMPROVE, immunophenotyping of peripheral blood B cells was undertaken in vaccine responders versus non-responders to identify CLL and non-CLL B cell phenotype, followed by detection and phenotypic analysis of antigen-specific B cells amongst responders. Calcium flux analysis following IgM stimulation, along with mRNA sequencing of detectable B cells in antibody responders versus non-responders, was next investigated. An assessment of pre-treatment bone marrow samples by immunohistochemistry was also performed. Finally, serial immunophenotypic analysis of peripheral blood samples from therapy initiation to >1 year on BTKi were used to corroborate findings and the association with infection admission risk assessed.
**Results:** Of 99 patients with controlled CLL, following >1 year of BTKi therapy (mean age 70.5, SD 8.8), 32 participants (32%) demonstrated no SARS-CoV-2 Ab response despite a median of 5 (IQR 5–6) SARS-COV-2 vaccine doses. No difference in antibody titre was observed between: first or subsequent therapy line, acalabrutinib or ibrutinib therapy. Importantly, the number of non-CLL CD19+ B cells differed between responders (8.23 cells/μl (IQR 4.4–20.9)) and non-responders (3.4 cells/μl (IQR 1.4–5.2); $p=0.02$), with the greatest differences observed in IgM+IgD+, CD27- naïve cells (responders:1.09 (IQR 0.3–3.1) Vs non-responders: 0.01(IQR 0–0.06) cells/μl; $p<0.0001$). Amongst antibody responders, B cell klickmers revealed a class switched IgG antigen-specific response and an intact calcium flux following BCR stimulation amongst non-CLL naïve B cells, despite continuous covalent BTK inhibition. Detectable CLL cells were evident in both responders and non-responders and no difference in total B cell number was observed ($p=0.55$). Non-responders strongly associated with a C2 transcription factor profile (p adj: 3.3e-35), whilst IGHV (unmutated Vs mutated) status did not. Immunohistochemistry and spatial transcriptomics demonstrated a heterogeneous degree of marrow infiltration by CLL at therapy initiation, contributing to the variability in normal B and plasma cell numbers. Serial analysis of peripheral immunophenotyping found the presence of normal B cells pretreatment impacts the B cell immune repertoire >1 year post therapy.
**Summary/conclusion:** Current IWCLL criteria for treatment initiation leads to heterogeneity in patient immunity, with heavy marrow infiltration associated with the poor prognostic C2 transcriptional profile of CLL and impaired immunity. The depletion of naïve and memory B cells in pre-treatment work up, provides a tool for identifying those at risk of poor antigen-specific Ab responses during long term BTKi and may highlight individuals where antimicrobial prophylaxis may be beneficial.

**Keywords:** Immunity; leukaemia; BTKi

**Please indicate how this research was funded:** Trial funding and Clinician Scientist funding for the laboratory work.

**Please indicate the name of the funding organization:** UKRI (UK Research and Innovation), BCUK (Blood Cancer UK) and NIHR (National Institute for Health Research).

## Abstract ID: 1414

### **Title:** Final analysis of fixed-duration ibrutinib + venetoclax for chronic lymphocytic leukemia / small lymphocytic lymphoma in the phase 2 CAPTIVATE study

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**Background:** First-line ibrutinib (Ibr) + venetoclax (Ven) treatment for chronic lymphocytic leukemia/ small lymphocytic lymphoma (CLL/SLL) was tested in the phase 2 CAPTIVATE study, including minimal residual disease (MRD)–guided randomized discontinuation (MRD cohort) and Fixed Duration (FD) cohorts. We report final analysis results for patients treated with FD Ibr+Ven in the FD cohort and MRD cohort placebo arm.

**Methods:** Patients aged ≤70 years with previously untreated CLL/SLL received 3 cycles of Ibr, then 12 cycles of Ibr+Ven (Ibr, 420 mg/day orally; Ven, 5-week ramp up to 400 mg/day orally), up to 13 cycles in the MRD cohort placebo arm. On-study retreatment included single-agent Ibr; FD cohort patients with progressive disease (PD) > 2 years after end of treatment (EOT) could be retreated with FD Ibr+Ven.

**Results:** A total of 202 patients completed FD Ibr+Ven (FD cohort, n = 159; MRD cohort placebo arm, n = 43). With a median follow-up of 68.9 months (range, 0.8–83.9), 5.5-year progression-free survival (PFS) and overall survival (OS) rates (95% CI) were 66% (58–72) and 97% (93–99), respectively. 5.5-year PFS rates (95% CI) in patients without and with del(17p)/mutated TP53 were 70% (62–76) and 36% (17–55), respectively. In patients with unmutated IGHV, 5.5-year PFS was 55% (45–64): 63% (49–74) in patients without, and 44% (28–60) in patients with, concomitant del(17p)/mutated TP53/ complex karyotype. The corresponding rates for patients with mutated IGHV were 79% (68–87), 85% (71–93), and 62% (34–81). Undetectable MRD (uMRD4; < 10–4 by flow cytometry) was achieved in peripheral blood (PB) in 54% of patients at C7 and 69% at EOT, and in bone marrow in 69% of patients at EOT. 5.5-year PFS rates (95% CI) were higher in patients with uMRD4 in PB at EOT (75% [67–82]) vs those with MRD (47% [33–59]). 64 patients had PD after completion of FD Ibr+Ven. 5.5-year freedom from next-line treatment was 73% (95% CI 66–79). Of 40 patients with available samples at PD to date, 1 had an acquired subclonal mutation in BCL2 of unclear significance (A113G, VAF 8.3%); none had acquired resistance-associated mutations in BTK or PLCG2. 36 patients initiated retreatment with Ibr (n = 25) or Ibr+Ven (n = 11). With 28.4 months median follow-up on Ibr retreatment (range, 3.7–59.1), overall response rate (ORR) was 76% (best response: 1 complete response [CR]; 1 nodular partial response [PR]; 17 PR; 4 stable disease [SD]; 1 PD [Richter transformation]; 1 no assessment); 2-year PFS and OS rates from the start of retreatment were 91% and 96%, respectively. With 15.2 months median follow-up on Ibr+Ven retreatment (range, 7.4–29.3), ORR was 82% (best response: 1 CR; 8 PR; 2 SD); 1-year PFS and OS rates from the start of retreatment were both 100%. Second malignancies occurred in 24 patients across the entire study period, including 12 initial treatment and 4 retreatment treatment emergent adverse events.

**Conclusions:** Ibr+Ven is an all-oral, once-daily, chemotherapy-free FD regimen for first-line treatment of CLL/SLL that continues to provide durable PFS and OS with long-term follow-up, including in patients with high-risk genomic features. Ibr-based retreatment provided durable responses in patients needing subsequent therapy after completion of FD Ibr+Ven.

**Keywords:** CLL/SLL; ibrutinib + venetoclax; fixed-duration treatment

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## Abstract ID: 1509

### **Title:** Nemtabrutinib in relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma: Cohort J of the phase 2 BELLWAVE-003 study

**Authors:** Thomas Kipps[a], Farrukh Awan[b], Barbara Eichhorst[c], Yair Herishanu[d], Wojciech Jurczak[e], David Lavie[f], Nicolas Martinez-Calle[g], Andras Masszi[h], Carolyn Owen[i], Christian Poulsen[j], Christof Schneider[k], Yan Xu[l,m], Jing Yang[n], Mohammed Farooqui[n] and Jaimal Kothari[o]

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**Background:** Treatment options are limited for patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) who have relapsed or refractory disease after failing to respond to both Bruton tyrosine kinase (BTK) inhibitors and B-cell lymphoma 2 (BCL2) inhibitors. Nemtabrutinib is a potent, noncovalent, reversible BTK inhibitor with a unique kinase profile that targets and inhibits BTK and other kinases involved in the B-cell receptor pathway. The multicenter, open-label, single-arm, phase 2 BELLWAVE-003 study (NCT04728893) is being conducted to assess nemtabrutinib at the recommended phase 2 dose (RP2D) in participants with relapsed or refractory CLL/SLL, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, Richter transformation, and Waldenström macroglobulinemia. Cohort J specifically focuses on evaluating nemtabrutinib in participants with CLL/SLL who have experienced relapsed or refractory disease to both BTK inhibitor and BCL2 inhibitor treatment.

**Methods:** Participants eligible for cohort J must be aged ≥18 years with CLL/SLL relapsed or refractory to previous treatment with both a BTK inhibitor (covalent or irreversible) and a BCL2 inhibitor, and have an Eastern Cooperative Oncology Group performance status of 0 to 2. The use of noncovalent or reversible BTK inhibitors is permitted if the disease is relapsed or refractory to such therapy. Participants must have received and not responded to, been intolerant to, or been deemed by their treating physician to be a poor candidate for or ineligible for treatment with phosphoinositide 3-kinase inhibitor per local (institution) guidelines. Exclusion criteria include prior exposure to nemtabrutinib, systemic therapy with a monoclonal antibody within 5 half-lives or 4 weeks before allocation, active central nervous system disease, and clinically significant gastrointestinal abnormalities that might alter absorption. The BELLWAVE-003 study includes a dose escalation and confirmation phase (part 1) to establish the RP2D, followed by a cohort expansion phase (part 2). Part 1 evaluated nemtabrutinib in 6 to 20 participants with relapsed or refractory CLL/SLL after at least 2 prior lines of therapy, establishing the RP2D as 65 mg once daily. In part 2, approximately 460 participants will be enrolled across 9 expansion cohorts with approximately 40 participants in cohort J. Treatment will continue until unacceptable toxicity, disease progression, or participant withdrawal. Adverse events will be monitored and graded using National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0, and hematologic toxicities in participants with CLL will be assessed using International Workshop on Chronic Lymphocytic Leukemia (iwCLL) 2018 criteria. Imaging (CT/MRI and/or PET) will be performed every 12 weeks or as needed. The primary end point for cohort J is objective response rate per iwCLL 2018 criteria by independent central review. Additional end points include overall survival, safety and tolerability, and duration of response and progression-free survival per iwCLL 2018 criteria by independent central review. Recruitment is ongoing, making this the first clinical trial with a dedicated cohort to evaluate noncovalent BTK inhibitors in participants whose disease has not responded to both a BTK inhibitor and BCL2 inhibitor. ©2025 American Society of Clinical Oncology, Inc. Reused with permission. This abstract was accepted and previously presented at the 2025 ASCO Annual Meeting. All rights reserved.

**Keywords:** Chronic lymphocytic leukemia/small lymphocytic lymphoma; relapsed or refractory; nemtabrutinib

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## Abstract ID: 1415

**Title:** Health-related quality of life in patients with previously untreated chronic lymphocytic leukaemia treated with ibrutinib and rituximab or fludarabine, cyclophosphamide and rituximab: patient-reported outcomes from the multicentre, open-label, randomised, phase 3 FLAIR trial

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**Background:** The FLAIR trial showed significantly improved progression-free survival (PFS) for ibrutinib and rituximab (IR) when compared with fludarabine, cyclophosphamide and rituximab (FCR) in people with previously untreated chronic lymphocytic leukemia (CLL). This analysis is of the patient-reported outcomes (PROs), a predefined secondary end point in FLAIR.

**Methods:** This study is an interim analysis of FLAIR, a phase three, open label, randomized, controlled trial in persons with previously untreated CLL conducted at 101 UK National Health Service (NHS) hospitals. Eligible patients were between 18 and 75 years of age with a World Health Organisation (WHO) performance status (PS) of two or less and disease status requiring treatment according to iwCLL criteria. Those with more than 20% of CLL cells with d17p were excluded. Participants had to be fit to receive FCR and have adequate renal function. Participants were randomly assigned (1:1) using minimization with a random element in a web-based system. Stratification was by Binet stage, age, sex, and centre. Allocation was to either IR (ibrutinib 420 mg/day orally for 6 years, until minimal residual disease stopping rules were reached, until toxicity requiring cessation or until disease progression, whichever occurred first with rituximab: 375/500 mg/m$^2$ IV on day 1 of cycle 1/2–6 of a 28-day cycle) or FCR (fludarabine 24 mg/m$^2$/day orally on day 1–5, cyclophosphamide 150 mg/m$^2$/day orally on day 1–5 and rituximab: as for FCR). The primary end point was PFS. Participants completed the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life C30 Questionnaire (QLQ-C30), Chronic Lymphocytic Leukemia Module (QLQ-CLL16), Euroqol 5 dimensions (EQ-5D-3L), and EQ-5D visual analogue scale (VAS) at baseline and pre-determined follow-up timepoints. PROs included function and symptom scales which were analyzed on an intention to treat basis using repeated measures multi-level regression models. FLAIR is ISRCTN and clinicaltrialsregister.eu registered (ISRCTN01844152 and 2013-001944-76).

**Findings:** 651 of 771 (84.4%) FLAIR participants completed the baseline PROs. Compliance was good up to 48 months. Characteristics of the PRO population were similar to all FLAIR participants. Median age was 63 years, 27% were female and the majority identified their ethnicity as white. Observed trajectories in quality of life (QOL) were similar between participants treated with IR and FCR for the EQ-5D, EORTC-QLQ-C30, and EORTC-QLQ-CLL16. Several scales showed worse function or symptoms at the end of FCR treatment, but such scores recovered to ultimately be equivalent, or better than those for IR at 48 months. A comparison between baseline and 48-month timepoints showed that higher proportions of FCR-treated participants experienced improvement for the EQ-Utility Index, EQ-VAS, cognitive functioning, fatigue, diarrhoea, treatment side effects, disease effects, and infections scales. The estimated mean differences from repeated measures multi-level modeling were not statistically significant for the EQ-5D Utility Score or EQ-VAS. Physical functioning, role functioning, and social functioning

were statistically significant in favour of IR. In contrast emotional function measures favoured FCR. Symptoms of diarrhoea were lower in the FCR group, whilst fatigue and dyspnoea were higher. Disease effects were lower in those allocated FCR, but CLL-related fatigue, infection, and social problems were lower in those allocated IR. The observed differences in all symptom scales did not exceed the between-group minimally important difference for improvement.

**Interpretation:** Although moderate differences between the two treatment arms were seen in HR-QoL up to 4 years post-randomization, generally PROs are similar between the two treatment groups. These findings suggest that up to 48 months the delivery of continuous therapy with IR is associated with similar HR-QoL as compared to FCR. However, the impact of the late effects of both therapies may become only apparent with prolonged follow-up. A planned future QOL analysis of FCR, IR, ibrutinib, and ibrutinib combined with venetoclax will shed further light on the impacts of these therapies. In future, the updated CLL module EORTC QLQ-CLL17 may enable detection of differences in HR-QoL, specifically in relation to the benefits in contemporary targeted treatments for CLL patients.

**Keywords:** Leukaemia; therapy; quality of life

**Please indicate how this research was funded:** Primary financial support was from Cancer Research UK [ C18027/A15790 ]. Unrestricted educational grants from Janssen, Pharmacyclics and AbbVie supported trial coordination and laboratory studies. Study drug (ibrutinib) was provided by Janssen. This work was also supported by Core Clinical Trials Unit Infrastructure from CRUK [C7852/A25447].

**Please indicate the name of the funding organization:** Cancer Reseach UK Johnson and Johnson

## Abstract ID: 1510

### **Title:** BELLWAVE-010: an open-label, phase 3 study of nemtabrutinib plus venetoclax in relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma

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**Background:** Venetoclax plus rituximab is the standard therapy for relapsed or refractory (R/R) chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). However, patients with genetic high-risk disease may experience shorter durations of response. Nemtabrutinib, a reversible, noncovalent, competitive inhibitor of wild type and C481-mutant Bruton tyrosine kinase (BTK), showed manageable safety and durable antitumor activity regardless of C481 mutation status in the BELLWAVE-001 study of participants with R/R CLL/SLL. In the randomized, active-controlled, open-label, phase 3 BELLWAVE-010 study (NCT05947851), safety and efficacy of nemtabrutinib plus venetoclax will be compared with venetoclax plus rituximab as second-line or later treatment for participants with R/R CLL/SLL.

**Methods:** Eligible participants are aged ≥18 years with active R/R CLL/SLL following at least 1 previous therapy per International Workshop on Chronic Lymphocytic Leukemia (iwCLL) 2018 criteria and have an Eastern Cooperative Oncology Group performance status of 0 to 2. Exclusion criteria include participants diagnosed with Richter transformation and those who received prior treatment with a noncovalent BTK inhibitor therapy or previous systemic anticancer therapy at least 4 weeks before study intervention. The study consists of two phases. Phase 1 (n≈30) is a nonrandomized dose-escalation and confirmation phase to determine safety, tolerability, and recommended dose of nemtabrutinib plus venetoclax using a modified toxicity probability interval design. Nemtabrutinib will be administered at two doses (45 mg escalating to 65 mg by mouth once daily) for 4 weeks, then nemtabrutinib plus venetoclax (20–400 mg by mouth once daily escalating over 28 days). Phase 2 (n≈690) is a parallel-group, randomized phase to compare safety and efficacy of nemtabrutinib plus venetoclax with venetoclax plus rituximab. Randomly assigned participants (1:1) will receive nemtabrutinib for 4 weeks then nemtabrutinib plus venetoclax (dosing consistent with part 1) or venetoclax (20–200 mg) for 4 weeks then venetoclax (400 mg) plus rituximab (375 mg/m$^2$ intravenously at week 6, then 500 mg/m$^2$ every 28 days during weeks 10–26). Treatment will be administered for ~2 years until any discontinuation criteria are met. Randomization will be stratified by BTK C481 mutation status (detected vs not detected), risk (high vs low), and geographic region (United States/Canada vs Europe vs rest of the world). Responses will be evaluated every 12 weeks until week 97, then every 24 weeks. Adverse events will be graded per National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0. Hematologic toxicity will be graded using iwCLL 2018 criteria. The primary end points for part 1 are safety and tolerability. The primary end point for part 2 is progression-free survival per iwCLL 2018 criteria by blinded independent central review. The secondary end points for part 2 include undetectable minimal residual disease in bone marrow at month 14 (assessed by central laboratory), objective response rate and duration of response per iwCLL 2018 criteria, overall survival, and safety and tolerability. Recruitment is ongoing. ©2024 American Society of Clinical Oncology, Inc. Reused with permission. This abstract was accepted and previously presented at the 2024 ASCO Annual Meeting. All rights reserved.

**Keywords:** Chronic lymphocytic leukemia/small lymphocytic lymphoma; relapsed or refractory; nemtabrutinib

**Please indicate how this research was funded:** Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

**Please indicate the name of the funding organization:** Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

## Abstract ID: 1511

### **Title:** Nemtabrutinib in untreated chronic lymphocytic leukemia/small lymphocytic lymphoma: phase 3 BELLWAVE-011 study

**Authors:** Tamar Tadmor[a], Toby A. Eyre[b], Ohad Benjamini[c], Arvind Chaudhry[d], Juan Shen[e], Siyang Leng[e], Mohammed Farooqui[e] and David Lavie[f]

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**Background:** Bruton tyrosine kinase (BTK) inhibitors have contributed greatly to the advancement of treatment for chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). Nonetheless, resistance to irreversible inhibitors such as ibrutinib can develop, often because of a BTK C481 mutation. Nemtabrutinib, a reversible, noncovalent, competitive inhibitor that targets wild type and C481S-mutated BTK, has shown promising antitumor activity in participants with relapsed or refractory CLL/SLL in the phase 1/2 BELLWAVE-001 study. The randomized, active-controlled, parallel, multisite, open-label, ongoing BELLWAVE-011 study (NCT06136559) is being conducted to compare efficacy and safety of nemtabrutinib with investigator's choice of ibrutinib or acalabrutinib in participants with untreated CLL/SLL.
**Methods:** Eligible participants are aged ≥18 years with confirmed CLL/SLL and active disease per International Workshop on Chronic Lymphocytic Leukemia (iwCLL) 2018 criteria, which include at least 1 of the following: progressive marrow failure; progressive lymphocytosis; massive, progressive, or symptomatic splenomegaly or lymphadenopathy; symptomatic or functional extranodal involvement; autoimmune complications, including anemia or thrombocytopenia refractory to corticosteroids; and disease-related symptoms such as unintentional weight loss, significant fatigue, fever, or night sweats. Participants must be treatment naive and have an Eastern Cooperative Oncology Group performance status of 0 to 2. Participants with Richter transformation, central nervous system involvement by CLL/SLL, and second malignancy or severe bleeding disorder are excluded. Participants will be randomly assigned 1:1 to receive nemtabrutinib 65 mg once daily by mouth or investigator's choice of ibrutinib 420 mg once daily by mouth or acalabrutinib 100 mg twice daily by mouth. Treatment will continue in both arms until unacceptable toxicity, disease progression, withdrawal, or other discontinuation criteria are met. Treatment response assessment, including imaging, physical examination, constitutional symptoms, hematologic evaluation, and bone marrow biopsy, will be completed every 12 weeks up to week 97 and every 24 weeks thereafter. Adverse events will be monitored throughout the trial and for 30 days (90 days for serious adverse events) after treatment and graded per National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0, and iwCLL 2018 criteria for hematologic toxicity. The primary end points are objective response rate and progression-free survival per iwCLL 2018 criteria by blinded independent central review. The secondary end points are overall survival, duration of response per iwCLL 2018 criteria by blinded independent central review, and safety and tolerability. Recruitment is ongoing. ©2024 American Society of Clinical Oncology, Inc. Reused with permission. This abstract was accepted and previously presented at the 2024 ASCO Annual Meeting. All rights reserved.

**Keywords:** Chronic lymphocytic leukemia/small lymphocytic lymphoma; treatment naive; nemtabrutinib

**Please indicate how this research was funded:** Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

**Please indicate the name of the funding organization:** Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

## Abstract ID: 1512

**Title:** Pirtobrutinib after a covalent BTK inhibitor in pretreated chronic lymphocytic leukemia: a phase 2 dose optimization trial in progress

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**Background:** Covalent Bruton tyrosine kinase inhibitors (cBTKi) have significantly improved the outcomes for patients with chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). However, resistance and intolerance to these agents is common, limiting the duration of treatment, and resulting in poorer outcomes after cBTKi failure. Pirtobrutinib, a highly selective, non-covalent BTKi, demonstrated clinically meaningful efficacy and a favorable safety profile amongst patients who had received a prior cBTKi in the pivotal 1/2 BRUIN study (LOXO-BTK-18001, NCT03740529), leading to FDA accelerated approval for adults with CLL/SLL after at least two prior lines of therapy including a BTK inhibitor and a BCL-2 inhibitor. Pirtobrutinib exhibited efficacy across a wide range of doses (25–300 mg daily), with no dose-limiting toxicities. The efficacy and safety were confirmed in a randomized phase 3 study (BRUIN CLL-321, NCT04666038) in patients with relapsed/refractory CLL/SLL. However, most data reflect later-line use, and relatively few patients received doses below the recommended dose of 200 mg. This has prompted further evaluation of lower doses to determine if there is an improved safety profile without compromising efficacy.
**Aims:** This ongoing phase 2, open-label, randomized study (NCT06588478) evaluates the efficacy and safety of 3 dose levels of pirtobrutinib in a less heavily pretreated post-cBTKi CLL/SLL population that better reflects current treatment patterns.
**Methods:** Approximately 249 patients will be randomly allocated across three different dose groups and stratified by del(17p) status (present/absent) and 1 vs 2–3 lines of prior treatment. Eligible patients require therapy per iwCLL 2018 criteria and key exclusion criteria include central nervous system involvement by CLL/SLL, prior Richter transformation, prior treatment with BTK degrader or non-covalent BTKi, or history of hematopoietic stem cell transplant or chimeric antigen receptor T-cell therapy within 90 days prior to randomization. Patients will receive pirtobrutinib continuously in this study until discontinuation criteria are met, including if patients experience disease progression, withdraw consent, receive subsequent anticancer therapy, the investigator deems it necessary for safety reasons, or criteria are met for the end of the study. The primary endpoint is comparison of investigator assessed overall response rate (ORR) per iwCLL 2018 criteria. ORR is defined as the proportion of participants who achieve the best overall response of complete remission, complete remission with incomplete hematologic recovery, nodular partial response, or partial response at or before the initiation of subsequent anticancer therapy. Secondary endpoints include investigator-assessed duration of response and safety endpoints.
**Results:** This study is a Trial in Progress. The results will be presented at a later date.
**Conclusions:** This study is a Trial in Progress. The conclusions will be presented at a later date.

**Keywords:** BTK inhibitor; chronic lymphocytic leukemia; small lymphocytic lymphoma

**Please indicate how this research was funded:** Eli Lilly and Company

**Please indicate the name of the funding organization:** Eli Lilly and Company

## Abstract ID: 1408

**Title:** Optimizing time-limited therapy in unfit CLL/SLL patients: orelabrutinib, bendamustine, and obinutuzumab combination (OBG) regimen

**Authors:** Xutao Guo[a], Yunxia Zhang[a], Xiaolei Wei[a], Yongqiang Wei[a], Haohao Lei[a], Ning Su[b] and Ru Feng[a]

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**Background:** BTK inhibitors (BTKi) are recommended for long-term therapy in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) but face challenges including limited depth of response, high dropout rates, and acquired mutations, hindering optimal survival benefits. In fit patients, exploration of time-limited therapy models such as fixed-duration or MRD-guided therapies has shown promising results, highlighting the need for similar investigations in unfit patients.

**Methods:** We explored a time-limited therapy model combining orelabrutinib, bendamustine, and obinutuzumab (OBG) in unfit CLL/SLL patients. Orelabrutinib's superior efficacy and safety profile were chosen based on its selective BTK inhibition and minimal impact on ITK kinase, preserving obinutuzumab's ADCC effect. Obinutuzumab's enhanced ADCC and ADCP capabilities were leveraged alongside bendamustine's cytotoxicity. The primary endpoints include the rate of complete response (CR) combined with undetectable minimal residual disease (uMRD) at the end of seven cycles (CR/CRi and uMRD, with patients achieving incomplete CR categorized as CRi). Secondary endpoints encompass CR/CRu and uMRD rates at the end of seven cycles (CRu defined as splenic longest diameter ≤16 cm with other criteria meeting CR/CRi standards), uMRD rate (detected via NGS at a level of $10^{-6}$), progression-free survival (PFS), and overall survival (OS).

**Results:** As of July 2024, eight patients have been enrolled in our study. The data showed a 100% complete response rate after six cycles, with peripheral blood and bone marrow uMRD rates of 100% and 87.5%, respectively. CR with uMRD was achieved in 87.5% of cases, indicating potential clinical benefits. Safety analysis revealed that among the eight patients evaluated, thrombocytopenia and neutropenia were observed in seven patients, with adverse events ≥ Grade 3 occurring in 25% of cases, all of which were reversible. No incidences of bleeding or atrial fibrillation were reported. One patient experienced Grade 3 pulmonary infection, which has since resolved.

**Conclusion:** The OBG regimen demonstrates efficacy as a promising time-limited therapy in unfit CLL/SLL patients, achieving high rates of complete response (CR) combined with undetectable minimal residual disease (uMRD). Safety analysis indicates that the OBG regimen is well-tolerated, with manageable adverse events such as reversible thrombocytopenia and neutropenia observed in a subset of patients. These findings support further investigation of the OBG regimen to optimize treatment outcomes in this patient population.

**Keywords:** CLL; orelabrutinib; obinutuzumab

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1416

**Title:** CLL18 / MOIRAI: an international phase-III trial aiming to establish measurement of individual residual disease for adjustment of treatment duration to improve outcomes in first-line treatment of CLL/SLL

**Authors:** Paula Cramer[a], Ellinor Görgen[b], Léa Mazot[b], Miriam Schüler-Aparicio[b], Sandra Robrecht[b], Christina Paulitschek[b], Aline Zey[b], Antonia Albrecht[b], Julia Blau[b], Laura Jung[b], Sophia Reidel[b], Othman Al-Sawaf[b], Francesc Bosch[c], Caspar da Cunha-Bang[d], Michael Doubek[e,f,g], Emadoldin Feyzi[h], Anna-Maria Fink[b], Paolo Ghia[i], Michael Gregor[j], Romain Guieze[k], Krzysztof Jamroziak[l], Ann Janssens[m], Arnon Kater[n], Sabina Kersting[o], Petra Langerbeins[p], Mark-David Levin[q], Vesa Lindström[r], Mattias Mattsson[s], Carsten U. Niemann[t], Anne Quinquenel[u], Matthias Ritgen[v], Lydia Scarfò[i], Philip Staber[v], Stephan Stilgenbauer[w], Tamar Tadmor[x], Patrick Thornton[y], Eugen Tausch[z], Loic Ysebaert[aa], Kirsten Fischer[b], Barbara Eichhorst[bb] and Michael Hallek[cc]

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**Background:** The two major options for the first-line therapy of chronic lymphocytic leukemia (CLL) are a continuous BTK inhibitor treatment given as long as possible for disease control or a venetoclax-based fixedduration treatment, which usually leads to deep responses and a treatment-free interval of several years. There is an increased use of fixed-duration venetoclax-based regimens, such as venetoclax plus obinutuzumab (12 cycles) or venetoclax plus ibrutinib or acalabrutinib with/without obinutuzumab. While these combination therapies are more intense compared to monotherapies, the treatment-free interval carries advantages in terms of quality of life, safety, and costs.
**Aims:** To evaluate if a more individualized approach is beneficial in the context of time-limited therapy, we have designed this pan-European, academic phase III trial with three arms - two evaluating a fixed-duration regimen and one evaluating a treatment duration based on measurable residual disease (MRD), using a treatment duration tailored to the depth of remission.
**Methods:** The standard arm A is the established combination of venetoclax and obinutuzumab (Ven-Obi, 12 cycles with a duration of 28 days, Obi only during cycles 1–6), while arm B evaluates venetoclax plus pirtobrutinib (Ven-Pirto) for 15 cycles (3 cycles pirtobrutinib alone, then 12 cycles combined with venetoclax). In arm C, Ven-Pirto will be administered for at least 15 and up to 36 cycles (as long as there is a deepening of response) until achievement of undetectable MRD (uMRD). To facilitate the transfer to clinical routine, MRD is measured in peripheral blood, by multi-colour flow cytometry and with a cut-off of $10^{-4}$. Two MRD assessments with an interval of 12 weeks both documenting uMRD are needed to allow for a treatment discontinuation and treatment will be continued for an additional 12 weeks after the second uMRD result as a consolidation. 813 patients with previously untreated CLL or SLL irrespective of age, comorbidities, or CLL risk-factors will be recruited 1:1:1 to the three arms (271 each) with a stratification according to TP53 deletion and/or mutation, IGHV mutational status, type of disease (CLL vs. SLL), and age. The primary endpoint is the investigator-assessed progression-free survival (PFS). The trial is designed to show both superiority of MRD-guided Ven-Pirto over Ven-Obi and over fixed duration Ven-Pirto. Secondary endpoints include iwCLL response, MRD, overall survival and safety parameters.
**Results:** At the time of submission of this abstract, the CLL18 / MOIRAI protocol was approved by the competent authorities of 13 of the 16 participating countries and recruitment was about to start. The estimated recruitment time of the 813 patients in approximately 160 sites in 16 countries is 20 months. Approximately 41 months after start of recruitment, a sufficient number of PFS events shall be reached for the primary endpoint analysis.
**Summary/conclusion:** The CLL18/MOIRAI trial will address the question whether an individualized, MRD-guided first-line treatment with pirtobrutinib and venetoclax improves the outcome of patients with CLL/SLL compared to fixed duration venetoclax with either obinutuzumab or pirtobrutinib.

**Keywords:** Firstline; MRD-guided

**Please indicate how this research was funded:** academic IIT trial with research support by the pharmaceutical companies

**Please indicate the name of the funding organization:** Lilly, AbbVie and Roche

## Abstract ID: 2003

### **Title:** Evaluating gaps and opportunities in chronic lymphocytic leukemia treatment access: institutional capacity assessments in 18 LMICs

**Authors:** Andrielle Yost[a], Alicia Annamalay[b], Colin Forsyth[b], Ines Garcia Gonzalez[b], Mei Ching Ong[b], Catherina Scheepers[c], Viji Venkatesh[d], Anna Schuh[e] and Pat Garcia-Gonzalez[a]

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**Introduction:** Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults, accounting for approximately one third of new leukemia cases worldwide (Yao Y, et al., 2022). In low- and middle-income countries (LMICs), cancer incidence and mortality are rapidly increasing, yet access to targeted therapies and key diagnostic tools remains limited. To address this inequity, The Max Foundation (Max) in partnership with local health care providers (HCPs), global key opinion leaders from the International Workshop on CLL (iwCLL), and a biotechnology donor, launched a treatment access program to provide zanubrutinib, a second-generation BTK inhibitor (BTKi), at no cost to patients. To inform program implementation, a comprehensive institutional capacity assessment was conducted across potential partner institutions. The goal was to evaluate readiness to diagnose and treat CLL, identify resource gaps, and inform program planning.
**Methods:** A standardized capacity assessment tool was distributed to partner institutions in eligible countries. The tool collected quantitative and qualitative data across several domains, including annual CLL patient volume, clinical staffing, availability of diagnostic and prognostic services, access to CLL treatments, and presence of local cancer advocacy organizations. Preliminary analysis explored correlations between site capacity indicators and reported annual CLL patient volume using a Spearman's correlation. Descriptive analysis identified trends, capacity, and key gaps in readiness.
**Results:** Between 2023 and 2025, 21 institutions across 18 countries completed the capacity assessment. The CLL treatment access program was implemented in three institutions in Armenia, Ethiopia, and Nepal, reaching 248

patients with access to zanubrutinib. These three institutions collectively reported seeing 680 patients annually. Across all 21 institutions, the total reported annual CLL patient volume was 1,779 (range 5–600) and the number of hematologists per institution ranged from 0–22 (average 6). A positive, though not statistically significant, correlation was observed between number of hematologists and annual patient volume (rs =0.425, $p=0.055$). All 21 institutions reported on-site availability of complete blood count with differential, and 18 institutions reported on-site peripheral smear capabilities. However, access to flow cytometry, the gold standard diagnostic for CLL diagnosis, varied widely; 8 institutions (38%) reported on-site capabilities, 10 institutions (48%) relied on off-site or out-of-country services, and 3 institutions (14%) reported no access to flow cytometry. All 21 sites reported the availability of at least one chemotherapy treatment, with 11 having access to monoclonal antibodies for combination regimens. Only three sites reported access to targeted treatments like BTKis, but the cost of these therapies made them unaffordable for most patients. A significant correlation was found between the total number of available medications and patient volume (rs =0.450, $p=0.04$). Only one institution reported awareness of blood cancer patient advocacy groups, and no CLL-specific advocacy groups were reported.

**Discussion:** Overall, the findings indicate a strong landscape of readiness. Most institutions demonstrated strong clinical staffing and access to chemotherapy but lacked reliable access to advanced diagnostics and affordable targeted therapies. Despite these gaps, 85% of institutions reported sufficient infrastructure and staffing to begin treating patients immediately if medicines were made available. Program launch and implementation in Armenia, Ethiopia, and Nepal reached operational capacity within 12 months. In response, efforts have focused on maximizing support within the current institutions while preparing for future expansion. Expanding access will require sustained support from donor companies, as well as continued assessment and strengthening of institutional capacity in new settings.

**Keywords:** LMIC; treatment access; Bruton's tyrosine kinase inhibitor (BTKi)

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1513

**Title:** Comparative assessment of MRD detection and immunological effects in relapsed CLL patients treated with rituximab-venetoclax in real world: insights from the 'dedalus' protocol

**Authors:** Maria D' Amato, Claudia Baratè, Clara Bono, Gaspare Tancredi, Francesca Guerrini, Edoardo Benedetti and Sara Galimberti

University of Pisa, Pisa, Toscana, Italy;

**Introduction:** The fixed-duration treatment Rituximab (R)-Venetoclax (Ven) has revolutionized the treatment of chronic lymphocytic leukemia (CLL), achieving a 7-year overall survival rate of 69.6%, allowing for measurable residual disease (MRD) eradication. Sensitive techniques are vital for accurate MRD evaluation and response assessment.

**Aim:** The 'Dedalus' protocol evaluates MRD using flow cytometry (MFC) and next-generation sequencing (NGS) in relapsed/refractory CLL patients treated with fixed-duration therapy R-Ven, correlating MRD status with clinical and immunological outcomes.

**Materials and methods:** Twenty-two CLL patients were analyzed using ERIC-compliant flow cytometry and the Lymphotrack NGS panel to detect IgH clonality with a sensitivity of $10^{-5}$. Additionally, advanced ultrasound techniques were employed to assess response. Immunological profiles and MRD were analyzed at 1, 6, 12, and 18 months or upon treatment discontinuation.

**Results/outputs:** Among the 22 patients, 20 achieved complete response (CR), with MRD undetectable in 15 patients at the first checkpoint by both methods. Two patients remained in partial response (PR): in these cases, NGS outperformed MFC, detecting residual IgH clonality (3–45 clonal cell equivalents/$10^6$ cells) when flow was negative. MRD detection by NGS mirrored clinical outcomes better than flow. The detection limits were $10^{-4}$ for MFC and up to $10^{-5}$ for NGS in 15% of tests. Immunologically, Ven induced significant and sustained depletion of B cells and a reduction in CD4+ T cells, altering the CD4/CD8 ratio in 25% of cases.

**Conclusions:** The study underscores the efficacy of R-Ven and highlights the superior accuracy of NGS over MFC in MRD detection. While MFC remains cost-effective and widely used, NGS provides deeper insights into residual disease. Future studies may explore digital PCR for IgH rearrangements as a non-invasive MRD biomarker, alongside investigations into Ven's immunomodulatory effects in larger cohorts.

**Keywords:** Leukemia relapsed/refractory venetoclax

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1514

**Title:** Updated results from the phase 1 study of sonrotoclax (BGB-11417), a novel B-cell lymphoma 2 inhibitor, in combination with zanubrutinib for relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma demonstrate deep and durable responses

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**Background:** Despite recent therapeutic advances, most treated patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) experience disease relapse, necessitating further treatment with novel agents. B-cell lymphoma 2 (BCL2) inhibition is an established CLL/SLL therapeutic strategy, and adding Bruton tyrosine kinase (BTK) inhibition may be synergistic. Sonrotoclax (BGB-11417), a next-generation BCL2 inhibitor, is a more selective and pharmacologically potent inhibitor of BCL2 than venetoclax, with a shorter half-life and no drug accumulation. Zanubrutinib, a next-generation BTK inhibitor, is highly effective in CLL, including in patients with high-risk disease features, and has shown superior progression-free survival (PFS) with fewer cardiac adverse events (AEs) vs ibrutinib in a randomized study in patients with relapsed/refractory (R/R) CLL/SLL.
**Aims:** To report updated safety and efficacy data for sonrotoclax + zanubrutinib in patients with R/R CLL/SLL in the ongoing BGB-11417-101 (NCT04277637) study.
**Methods:** Patients with R/R CLL/SLL received zanubrutinib (320 mg once daily [QD] or 160 mg twice daily) 8–12 weeks before starting sonrotoclax (40, 80, 160, 320, or 640 mg QD) with ramp-up to the target dose to prevent tumor lysis syndrome (TLS). Patients who previously progressed while on a BTK inhibitor were excluded from this cohort. Patients were treated until disease progression or unacceptable toxicity. The primary endpoint was safety per CTCAE v5.0; overall response rate (ORR) per iwCLL 2018 criteria and undetectable measurable residual disease (uMRD) in blood by standardized ERIC flow cytometry every 24 weeks (uMRD4) were secondary and exploratory endpoints, respectively.
**Results:** As of 6 December 2024, 47 patients with R/R CLL/SLL were enrolled and had received combination treatment (sonrotoclax doses: 40 mg, n = 4; 80 mg, n = 9; 160 mg, n = 6; 320 mg, n = 22; 640 mg, n = 6). Median age was 65 (range, 36–76) years; 26.2% of tested patients (11/42) had del(17p) and 73.2% (30/41) had unmutated IGHV. Median number of prior treatments was 1 (range, 1–3); 7 patients had a BTK inhibitor as their last prior therapy. Median follow-up was 29.4 (range, 10.2–45.8) months. No dose-limiting toxicities occurred; sonrotoclax maximum tolerated dose was not reached with doses up to 640 mg. Dose expansion was completed with a recommended phase 2 dose of 320 mg. The most common any-grade treatment-emergent AE (TEAE) was COVID-19 (n = 17; 36.2%). Neutropenia was the most common grade ≥3 TEAE (n = 13; 27.7%; no febrile neutropenia). No cases of TLS occurred. Four patients (8.5%) discontinued treatment due to TEAEs (myelodysplastic syndromes, meningococcal sepsis, plasma cell myeloma, and intracranial hemorrhage [discontinued zanubrutinib only]; n = 1 each). No TEAEs led to death. In 46 response-evaluable patients, ORR was 95.7% (n = 44; 2 patients [40 and 80 mg] had stable disease); complete response (CR) rate was 50.0% (320 mg, n = 10 [47.6%]; 640 mg, n = 3 [50.0%]). Median time to CR was 10.2 (range, 5.3–42.4) months. Of 7 response-evaluable patients with prior BTK inhibitor treatment, 6 achieved partial response (n = 5) or CR (n = 1). Of 45 MRD-evaluable patients, 36 (80.0%) achieved uMRD4, with evidence of responses deepening over time. All patients treated with sonrotoclax 160, 320, or 640 mg + zanubrutinib who reached week 96 (n = 14) achieved uMRD4, indicating deep and durable responses. One patient converted from uMRD to MRD4 + 6 months after elective treatment discontinuation and still remains in CR. With a median study follow-up of 29.4 months, only 2 PFS events occurred (40 mg, n = 1; 320 mg, n = 1) and the 24-month PFS rate was 94.5%.
**Conclusion:** Sonrotoclax + zanubrutinib combination treatment demonstrated a tolerable safety profile across all dose levels tested. Antitumor activity of this combination is encouraging, with a 95.7% ORR, deep responses, and uMRD observed in patients with R/R CLL/SLL, including those previously treated with a BTK inhibitor.

**Keywords:** Relapsed/refractory CLL; sonrotoclax; zanubrutinib

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## Abstract ID: 1217

### **Title:** IGHV repertoire in Israeli CLL patients reveals significant low frequency of stereotyped subsets – a single center retrospective study

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**Background:** The immunoglobulin heavy-chain variable region (IGHV) gene status is a key biomarker in chronic lymphocytic leukemia (CLL), with mutated IGHV (M-IGHV) generally indicating a more indolent course than unmutated IGHV (UM-IGHV). In addition to mutational status, 30–40% of CLL cases in international cohorts can be classified into stereotyped subsets based on similarities in IGHV gene sequences. These subsets—particularly #1, #2, and #4—are associated with specific IGHV genes and distinct clinical features. However, data on IGHV usage and subset distribution among Israeli CLL patients remain limited.
**Aims:** To characterize the IGHV gene repertoire and prevalence of B-cell receptor (BcR) stereotyped subsets in an Israeli CLL cohort, and compare findings with global data.
**Methods:** This single-center retrospective study included all CLL patients with known IGHV mutational and subset status performed at Tel Aviv Sourasky Medical Center (TASMC) between 2020 and 2025. All IGHV sequencing was performed via next-generation sequencing (NGS) in an ERIC-accredited laboratory. Sequences with ≥98% germline homology were defined as UM-IGHV; those with <98% as M-IGHV. Stereotyped subset assignment was performed using the ARResT/AssignSubsets platform. Data analysis was conducted using SPSS v29.

**Results:** A total of 375 CLL patients included, 193 (51.5%) were M-IGHV and 182 (48.5%) were UM-IGHV. IGHV4-34 was the most common gene (54 patients, 14.4%), with a predominance of mutated cases (41 M vs.13 UM). IGHV1-69 was the second most frequent (43 patients, 11.5%), predominantly unmutated (41 UM vs. 2 M). Other frequently used genes included IGHV3-23 (6.4%), IGHV3-7 (6.1%), IGHV3-30 (5.6%), and IGHV1-2 (4.5%). Stereotyped BcR subsets were identified in 36 patients (9.6%), of whom 21 (58.3%) were UM-IGHV. The most prevalent subsets were #4 (n = 9; all M-IGHV4-34) and #1 (n = 7; all UM). Subsets #201 and #3 were each detected in three patients; #201 cases were all M-IGHV, while #3 cases were UM-IGHV. Subsets #16 (M), #6 (UM), and #99 (UM) were each found in two patients. Eight additional subsets were found in single cases, including #2 (M), and #5, #8, #31, #59, #64B, #7H, and #202 (all UM). Among IGHV4-34 carriers, 14/54 (25.9%) were assigned to subsets (mainly #4 and #201). Among IGHV1-69 carriers, 6/41 (14.6%) were assigned (mainly #3 and #6). Other IGHV genes showed minimal subset clustering. Compared to the large multinational cohort reported by Agathangelidis et al. (Blood, 2021), in which 41% of patients were classified into stereotyped subsets and major subsets comprised 13.5%, our cohort showed a markedly lower overall prevalence (9.6%) and reduced representation of major subsets. IGHV1-69 was the most common gene in the international dataset and displayed extensive connectivity to subsets #1, #3, #5, and #6. While IGHV1-69 also appeared frequently in our cohort, only a minority of cases (14.6%) were subset-assigned. Conversely, IGHV4-34, though less common globally, was frequently used in our cohort and maintained a strong association with subset #4; Despite comparable IGHV usage patterns, the subset distribution was considerably sparser than in international datasets, particularly for commonly stereotyped IGHV genes such as IGHV3-23, IGHV3-30, and IGHV3-7.
**Conclusion:** The prevalence of stereotyped BcR subsets in Israeli CLL patients was significantly lower than in global series (~10% vs. ~30–41%). This divergence may reflect underlying population-specific genetic factors, including the diverse IGHV haplotype composition among Jewish ethnic subgroups. Environmental or antigenic exposures may also contribute, potentially altering B-cell repertoire dynamics. While standardized tools were used, underrepresentation of rare or uncharacterized subsets may further account for the reduced detection rate. These findings underscore the importance of population-specific immunogenetic profiling and highlight the limitations of applying universal prognostic models to genetically diverse populations. Future studies should focus on high-resolution immunogenetic mapping and outcome correlations to define the clinical relevance of stereotypy in Israeli and other underrepresented cohorts.

**Keywords:** CLL; IGHV; Israeli patients

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## Abstract ID: 2004

**Title:** Clinicopathological features and treatment outcomes of chronic lymphocytic leukemia in Pakistan: a real-world analysis

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**Background:** There is paucity of data from low-middle income countries on demographics, clinical presentation and outcomes of Chronic Lymphocytic Leukemia (CLL). This study investigates the clinical, cytogenetic, and molecular characteristics of CLL patients in Pakistan and their correlation with treatment response and survival.
**Methods:** This is a retrospective multicenter cohort analysis of CLL patients treated at various public or private sector hospitals between 2011 and 2024. Data on demographics, clinical features, cytogenetics, molecular mutations, treatment modalities, and survival were extracted. Kaplan-Meier analysis was used to estimate overall survival (OS) and disease-free survival (DFS). Univariate and multivariate Cox regression models evaluated prognostic variables including age, ECOG status, cytogenetics and treatment type.
**Results:** Thus far, 486 CLL patients across six centers in Pakistan have been analyzed. Median age at diagnosis was 65 years (range 32–89) and 61% were male. Comorbidities were present in 48%, and 19% had ECOG ≥2. Common presenting symptoms included lymphadenopathy (68%) and fatigue (41%). Majority (49.3%) patients presented in Binet Stage A. Of the current study cohort, 48.4% were government sponsored, 45.1% paid out of pocket, while 6.5% received treatment on charity/donations. Among all patients, 80% had cytogenetics done. Cytogenetic abnormalities included del(17p) in 13%, trisomy 12 in 18%, del(13q) in 22%. Testing for IgHV mutation was done in only 23 (4.76%) patients and out of these 19 (82.6%) had unmutated IgHV. The median time from diagnosis to treatment was 144 (14–1289) days. Most common first-line therapies included FCR (32%) and BR (21%), while targeted therapies (BTKi/BCL2i) were used in only 14%. Overall response rate (ORR) to first-line therapy was 67%, with complete remission in 24%. Median OS was 42 months (IQR 27–65), and median DFS was 30 months. On univariate analysis, del(17p) (HR 2.14, 95% CI: 1.31–3.48), age >65 (HR 1.82), ECOG ≥2 (HR 1.91), and unmutated IgHV (HR 1.75) were associated with inferior OS. On multivariate analysis, del(17p) (adjusted HR 2.09, $p = 0.001$) and age >65 (adjusted HR 1.68, $p = 0.02$) remained independent predictors of poor survival. Use of novel agents was associated with improved OS as compared to chemotherapy alone (HR 0.56, $p = 0.03$).
**Conclusions:** Single center studies have reported that CLL has an overall prevalence of 0.9% and accounts for approximately 9.7% of leukemia cases in Pakistan (Dodhy M, et al. Ann Pak Inst Med Sci 2011; Zeeshan R, et al. Asian Pac J Cancer Prev 2015; 16(4):1587-90. doi: 10.7314/apjcp.2015.16.4.1587). However, the true incidence is likely underreported due to the lack of a national CLL registry in Pakistan. Furthermore, immunophenotyping and FISH testing

is available at 5–6 reference labs in the country, while IgHV mutation is available at only one center in northern Pakistan, making the uniform diagnosis and prognostication of this disease potentially difficult. In the current study, data was pooled in a web-based database from various treatment centers in some of the largest cities of the country. In this first multicenter analysis of CLL in Pakistan, it appears that median age at diagnosis of 65 years is slightly younger compared to global data. While IgHV mutation analysis was not universally done, of the 23 patients tested, an unusually high rate (82.6%) of poor risk unmutated IgHV was detected. There is significant underutilization of novel agents in Pakistan because availability of ibrutinib and venetoclax is limited to a small number of patients due to high cost and lack of insurance coverage. Newer covalent and non-covalent BTKi options are not available in Pakistan thus far. Currently, Pakistan is the 5th most populous country in the world. Improved access to subsidized novel targeted therapies can enhance survival outcomes in this resource-constrained country. Fixed duration treatments will be more cost effective overall.

Additional patient numbers and data will be presented at the conference.

**Keywords:** Chronic lymphocytic leukemia; Pakistan; low-middle income

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## Abstract ID: 1105

### **Title:** Defining signalotypes in chronic lymphocytic leukemia and applying machine learning to better characterize biological and clinical subgroups

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**Introduction:** The mutational landscape of chronic lymphocytic leukemia (CLL) is characterized by a broad spectrum of recurrent genetic alterations, which can generally be mapped onto a limited set of key signaling pathways. However, the genetic programming within CLL cells alone is insufficient explain their survival and proliferation capabilities. Instead, interactions with the microenvironment are critical drivers of disease initiation, progression, and maintenance. Therefore, gaining a comprehensive understanding of the complex interplay between genetic abnormalities and microenvironmental signals is crucial. Such insight holds promise for improving prognostic accuracy and tailoring therapeutic strategies. In this context, signaling patterns derived from RNA expression analyses represent a promising integrative approach, as they likely capture the combined influence of both intrinsic genetic factors and extrinsic microenvironmental cues. Despite this potential, the individual variability in CLL cellular responsiveness at the signaling level has not been extensively explored to date.

**Methods:** To address this gap, we utilized a large publicly available dataset generated by Knisbacher et al., employing a custom-built bioinformatics pipeline with rigorous quality control measures to identify differential signaling patterns across CLL samples. This approach enabled us to define so-called 'signalotypes,' distinct signaling phenotypes characterized by unique pathway activation profiles. Subsequent analyses linked these signalotypes to clinical annotations within the dataset. Furthermore, we applied advanced machine learning algorithms to capture the molecular signatures underlying each signalotype, allowing us to accurately classify fully annotated CLL samples from our own biobank into the established signalotype clusters. Building on this classification, we conducted extensive in vitro toxicity screens using these biobank samples. We tested a broad range of compounds in solo culture conditions and selected subsets of substances under more complex coculture setups, simulating microenvironmental interactions.

**Results:** Our bioinformatics analysis of RNA-seq from 661 chemo-naïve CLL patients from the large public dataset yielded a robust definition of 11 signalotypes, supported by multiple layers of validation. These groups exhibited marked differences in the activation status of signaling pathways and metabolic activity, highlighting underlying biological heterogeneity. Notably, the clusters demonstrated substantial divergence in clinical outcomes, including time to first treatment and overall survival, suggesting clinically relevant biological distinctions. Moreover, established molecular markers such as IgHV mutation status, cytogenetic abnormalities, and mutations in recognized CLL driver genes were non-randomly distributed among the signalotypes, further reinforcing the presence of biologically distinct subgroups. We trained an ensemble of machine learning models to successfully learn the molecular patterns of each signalotype, enabling the mapping of 101 individual patient samples from our biobank to their corresponding clusters with high confidence. Leveraging this classification, we subjected patient samples to a high-throughput drug screening platform using 117 substances. The results revealed considerable heterogeneity in drug response profiles across different signalotypes. Interestingly, while many investigational compounds showed variable efficacy depending on the cluster, established CLL therapeutics like Venetoclax displayed relatively more uniform activity across clusters. This finding suggests that our screening approach can effectively differentiate between broadly effective agents and those with more selective activity tied to specific biological subgroups. More detailed analysis of 20 substances in coculture settings validated the high heterogeneity between signalotypes.

**Conclusions:** Our study demonstrates that signaling patterns derived from RNA sequencing data can be used to stratify CLL patients into subgroups with distinct biological characteristics, clinical trajectories, and treatment responses. Machine learning techniques enable the extraction and recognition of core molecular features defining each signalotype, allowing accurate patient classification based on these signatures, also allowing to extract a minimum number of features that are essential to define the molecular core of a specific signalotype. By integrating these insights with functional drug screening, we uncover substantial variability in therapeutic susceptibility across CLL subgroups, which could inform more personalized treatment approaches. Continued refinement and validation of this strategy hold significant promise for enhancing the understanding of CLL biology and may enable improved clinical management of

CLL by facilitating more precise prognostication and targeted therapy selection in the future.

**Keywords:** Transcriptomics; signaling patterns; drug screening

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## Abstract ID: 1003

### **Title:** A phenotypically distinct population of CLL cells with lower CD20 expression is present within abdominal subcutaneous adipose tissue of patients with CLL

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There is increasing evidence that adipocytes play an active role in the microenvironment of many cancers, including leukemias. High body mass index (BMI) is a major contributor for CLL-related mortality (Yao et al., Biomed Eng, 2022) and obesity is associated with poorer response to first-line therapy and decreased progression-free survival (Furstenau et al., Leuk, 2020). It is unknown however if CLL cells accumulate within abdominal subcutaneous adipose tissue (AT) or indeed whether CLL cells interact with adipocytes within that microenvironment. We examined whether clonal cells were present within the AT stromal vascular fraction (SVF) of patients with CLL, and whether interaction between adipocytes and CLL cells in vitro affected CLL cell survival and migration. Paired abdominal subcutaneous AT biopsies and peripheral blood (PB) samples were obtained from patients with stable, treatment naïve CLL, Binet stages A and B (n = 15 [male =11, female =4]). An automated hematology analyser was used to enumerate leukocyte subsets in blood and AT, and leukocyte subsets were immunophenotyped by flow cytometry. Blood contamination of AT samples was excluded via monitoring of erythrocyte counts. CD5 + CD19+ kappa/lambda restricted B-CLL cells were identified in subcutaneous AT of all 15 participants with untreated CLL (range $0.230 \times 10^6$–$14.903 \times 10^6$ CLL cells per gram AT, median $0.133 \times 106$). CLL cells isolated from AT demonstrated lower expression of CD20 ($p < 0.001$) and CD5 ($p < 0.005$) compared with those in PB, but no significant difference was seen in CXCR4 expression. In patients with bi-modal CD38 expression a significantly higher proportion of CLL cells isolated from AT expressed CD38 (n = 8, $p = 0.0319$), a marker associated with enhanced migration, compared to PB. No difference was seen in the percentage expressing sIgM or CD49d. There was a trend toward the number of CLL cells (per gram AT) present in adipose SVF being positively associated with the number of CLL cells per µl present in PB, however this did not reach statistical significance (Pearson r = 0.5228, $p = 0.0551$). The number of macrophages per gram AT was positively correlated with body fat percentage, as assessed by DEXA scan, (Pearson r = 0.7929, $p = 0.0062$) in keeping with data from healthy participant studies. However, no correlation was found between AT CLL counts and either body fat percentage or number of macrophages per gram AT. We undertook in vitro studies to investigate the effect of adipocyte and CLL cell interaction on CLL survival and migration. Primary CLL cells isolated from PB were cultured alone and in the presence of mature human subcutaneous adipocytes (HAd). Culture of primary CLL cells (n = 20) in the presence of HAd significantly increased the percentage viability of CLL cells at 48 hours compared to those cultured in media alone ($p < 0.0001$). We assessed the impact of HAd on the migratory capacity of CLL cells using a Transwell migration assay (n = 6). The presence of adipocyte conditioned media (ACM) increased migration of primary CLL cells across a 3 µm pore membrane ($p < 0.05$) at 3 hours. ELISA analysis of the ACM demonstrated the presence of SDF1-α, the primary chemokine responsible for migration of CLL cells in vivo. To investigate whether SDF1-α contributed to increased CLL cell migration in the presence of ACM, we incubated CLL cells with the CXCR4 antagonist AMD3100 (5 or 10 µg/ml) prior to the Transwell assay (n = 6). AMD3100 did not significantly reduce the migration of CLL cells in response to ACM, suggesting that chemotaxis associated with HAd may additionally involve pathways other than CXCR4/SDF1-α. Our study demonstrates that a phenotypically distinct population of clonal B cells with lower CD20 expression is present within subcutaneous AT of patients with treatment naïve CLL. We show that the presence of subcutaneous HAd cells increases the survival of primary CLL cells in vitro, and that primary CLL cells migrate toward ACM in vitro. Collectively, our findings raise the possibility that AT provides an advantageous microenvironment niche for CLL cells in vivo, with further studies needed to establish whether this contributes to treatment resistance and relapse.

**Keywords:** CLL; adipocytes

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## Abstract ID: 1515

### **Title:** Re-treatment with venetoclax-obinutuzumab after time-limited, MRD-guided venetoclax-obinutuzumab: final analysis of 7 patients with chronic lymphatic leukemia (CLL) treated in the CLL2-BAG trial of the GCLLSG

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**Introduction:** While the armamentarium for the treatment of chronic lymphatic leukemia (CLL) has increased and improved, the best treatment sequence remains unclear. Specifically, there is a debate whether patients who relapse after time-limited venetoclax-based therapy should undergo re-treatment or switch to a different mechanism of action, namely BTK inhibition. The CLL2-BAG trial is one of the first phase-II trials which evaluated the now well-established combination of Venetoclax-Obinutuzumab (Ven-Obi) with an individualized measurable residual disease (MRD) guided treatment duration in an all-comer population irrespective of prior treatments, fitness and CLL risk factors (Cramer et al, Lancet Oncol. 2018). During the conduct of the trial, the option of a re-treatment with Ven-Obi was included in the trial and offered to patients with a progression in need of treatment. This is the first analysis of all seven patients with two consecutive treatment lines with Ven-Obi in phase-II CLL2-BAG trial.
**Methods:** Between May 2015, and Jan 2016, a total of 66 patients were enrolled in the CLL2-BAG trial and received Ven-Obi in an induction phase (8 cycles with 28 days each) and maintenance phase (up to 8 cycles with 84 days) depending on individual response and MRD in peripheral blood (PB). Two option-al cycles of bendamustine debulking could be administered in patients with high tumor load before Ven-Obi. In case of progression with need of treatment as per iwCLL criteria ≥6 months after end of Ven-Obi treatment, a re-treatment with Ven-Obi could be administered in the clinical trial.
**Results:** Of the seven patients who later received a re-treatment, six had a high/very high CLL-IPI, four had a del(17p)/TP53 mutation, two had a complex karyotype and all had an unmutated IGHV at base-line. Four patients had no prior treatment and three had received up to four prior therapies before first Ven-Obi administration. Four of the seven patients received bendamustine debulking, the median dura-tion of the first Ven-Obi treatment was 16 months (range 12–27). Six patients discontinued mainte-nance treatment due to undetectable MRD (uMRD), while one continued for the full 8 maintenance cycles because uMRD was achieved in PB but not in bone marrow. At end of maintenance treatment, 6 of 7 patients responded with uMRD in peripheral blood. Median progression-free survival (PFS) was 57 months and median time to next treatment was 68 months. Median duration of re-treatment with Ven-Obi was 21 months (range 11–30). Three patients discontin-ued due to adverse events (infective exacerbation of bronchiectasis, immune thrombocytopenia and mycoplasma pneumonia), one due to progressive disease, and three due to a deep remission with uMRD. All patients responded to induction treatment and uMRD in PB was achieved in three patients, but one patient without uMRD progressed during maintenance. To date, in total five patients pro-gressed after Ven-Obi re-treatment and two patients are still in remission. The median PFS after start of Ven-Obi re-treatment is 33 months.
**Conclusion:** Initial treatment and re-treatment with Ven-Obi was efficacious in this cohort of seven patients: even if uMRD rates and PFS are lower than observed in the initial Ven-Obi treatment, re-treatment led to deep remissions with uMRD, especially in the two patients who are still in remission. Further information regarding this strategy of first exhausting Ven-Obi before switching to BTK-I is generated by the recruiting ReVenG trial, evaluating Ven-Obi re-treatment after fixed duration Ven-Obi.

**Keywords:** CLL; venetoclax; obinutuzumab

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1302

### **Title:** Pevonedistat enhances the cytotoxic effect of rohinitib on B cells of chronic lymphocytic leukemia

**Authors:** Jose Luis Castaño[a], Víctor Arenas[a], Daniela Vallejo[b], Juan José Domínguez-García[c], Lucrecia Yáñez[d] and Carlos Pipaón[a]

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**Introduction:** It has long been known that there are discrepancies between the mRNA and protein levels of certain genes in CLL cells. Recently, overexpression of the translation initiation factor eIF4A1 has been described in CLL and other tumors, and how its inhibition blocks the dependence of these tumors on the translation of oncogenes. On the other hand, CLL cells show an elevation of ubiquitin-like post-translational modifications (UBL-PTM), among which the role of NEDDylation in its pathogenesis has been highlighted. NEDDylation modulates the homeostasis of a wide range of proteins, as it is necessary for their degradation by the ubiquitin-proteasome system. However, the direct NEDDylation of other proteins does not affect their stability but does affect their function. An inhibitor of NEDDylation, Pevonedistat, has shown a cytotoxic effect on CLL cells, and results from a clinical study in combination with Ibrutinib have recently been published.
**Objective:** The aim of this work is to study the effect of the combined inhibition of RNA translation and NEDDylation on CLL and analyze its mechanism to provide information on the molecular causes of this pathology.
**Methods:** We analyzed the cytotoxicity of a translation inhibitor (Rohinitib) and a NEDDylation inhibitor (Pevonedistat) on B cells from CLL patients treated ex vivo, using analysis of 7-AAD staining by flow cytometry, as well as by oxidation of tetrazolium salts (XTT). We conducted an analysis of the changes in gene expression produced by RNAseq and the accumulation of key proteins by western blot.

**Results:** Both Pevonedistat and Rohinitib separately induced a significant increase in the death of B cells from a cohort of CLL patients treated ex vivo. However, the combined treatment with both drugs enhanced their effect synergistically, peaking 80% after 72 hours. The analysis of the cell cycle of CLL B cells under the different treatments showed an increase in the number of events in the sub-G0 peak, suggesting activation of the apoptosis machinery. The induction of cell death by this combined treatment was much lower in B lymphocytes obtained from healthy donors. Furthermore, this synergy does not occur in multiple myeloma cell lines. Moreover, this effect appears to be specific to direct NEDDylation, as the same synergy is not observed with the ubiquitination inhibitor, TAK-243. To decipher the mechanism of this synergistic effect, we carried out a series of molecular determinations. First, we studied the possible interrelation between the elevated level of UBL-PTM and the increase in translation in B-CLL cells. A massive screening in patient samples showed that multiple translation factors exhibit an altered UBL-PTM modification pattern compared to that of circulating B lymphocytes from healthy donors. Treatment with Pevonedistat did not produce changes in the accumulation of eIF4A1 or its natural inhibitor, PDCD4. On the contrary, treatment with Rohinitib reduced the accumulation of two members of the NEDDylation pathway. Most interestingly, Rohinitib also affected the accumulation of the kinases BTK, SYK, LYN and PLCγ2, suggesting a role of RNA translation in the constitutive BCR signaling in CLL. Finally, we conducted a study of the variations in the transcriptome of five CLL patients in response to treatment with Rohinitib and Pevonedistat. Our data showed a cooperative effect of these drugs in the inhibition of BCL2 and the activation of TP53, suggesting their mediation in the synergistic induction of apoptosis.
**Conclusion:** Our data demonstrate how the combined inhibition of two distinctive characteristics of CLL cells (their elevated RNA translation and ubiquitin-like post-translational modifications) induces a high and specific cell death of B-CLL cells treated ex vivo, suggesting its potential therapeutical use. In vivo experiments are currently ongoing.

**Keywords:** NEDDylation; RNA translation; ex-vivo

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## Abstract ID: 1306

### **Title:** Distinctive features in NF-kB regulation favor a synergistic action of pevonedistat and laduviglusib on B cells of chronic lymphocytic leukemia

**Authors:** Víctor Arenas[a], Jose Luis Castaño[a], Daniela Vallejo[b], Juan José Domínguez-García[c], Lucrecia Yáñez[d] and Carlos Pipaón[a]

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**Introduction:** The causes of chronic lymphocytic leukemia (CLL) remain largely unknown to this day. Characteristically, B cells in CLL exhibit prolonged survival, partly due to the activation of survival signaling pathways, such as NF-kB. This pathway is activated by extracellular signals and intercellular contacts. Additionally, other signaling pathways can modulate NF-kB-dependent transcription. GSK-3β, for example, which accumulates in the nucleus of CLL B cells, can phosphorylate the p65 subunit of NF-kB, turning it into a transcriptional repressor. However, it has been reported that its inhibition induces cell death in CLL B cells. To prevent continued signaling in healthy cells, the NF-kB pathway activates the transcription of its own inhibitor, IkBα, whose stability mediated by the ubiquitin-proteasome system is the central mechanism by which the pathway is regulated. Because of this, several groups have explored a possible way to inhibit this pathway through post-translational modifications like ubiquitination. Thus, it was found that NF-kB is a key target of a NEDD8-activating enzyme (NAE) inhibitor, related to the ubiquitin signaling cascade, Pevonedistat.
**Objective:** The aim of this study is to evaluate the possible synergies of Pevonedistat with other agents targeting NF-kB, in order to understand the regulation of this survival pathway in CLL and explore new therapeutic strategies for its treatment.
**Methods:** Using western blot and qPCR, along with cell viability assays, including flow cytometry with 7AAD staining and XTT, we evaluated the cytotoxic effects of treatments with Pevonedistat and Laduviglusib.
**Results:** We studied various mechanisms that modulate NF-kB signaling in B-CLL cells. We found a discrepancy between the mRNA and protein levels of IkBα. Our observations revealed a translational control of its expression not previously described in these cells. The high translation of IkBα mRNA would contribute to the tumor B lymphocytes in CLL not inducing the NF-kB pathway in response to GSK-3β inhibition, which does occur in other peripheral blood cells from these patients.
Given this, we decided to study the effect of the combined inhibition of GSK-3β and ubiquitin-like post-translational modifications, both elevated in CLL cells. We observed a synergistic effect between the specific GSK-3β inhibitor, Laduviglusib, and the NEDD8-activating enzyme inhibitor, Pevonedistat, on B cells from CLL patients treated ex vivo. Since the action on the NF-kB pathway alone did not seem to explain the cytotoxic effect of this combination, we conducted a study of the variations in the transcriptome of tumor B cells from CLL in response to treatment. The results indicate that the combined treatment enhances the reduction of BCL2 mRNA levels, suggesting an alternative pathway for the inhibition of BCL-2 in CLL. This finding could have therapeutic implications for the treatment of refractory cases of CLL, as it is capable of enhancing the action of Venetoclax at low concentrations. Additionally, we also observed a significant decrease in the expression of BIRC3 mRNA, an important anti-apoptotic factor, but whose mutation in CLL paradoxically is associated with a worse prognosis.
**Conclusions:** In this work, we report a novel translational regulation of IkBα in CLL B cells that explains the lack of correlation between the levels of its mRNA and protein and provides a rational basis for the use of GSK-3β inhibitors in these cells. We found a synergy in the ex vivo combinational treatment with Laduviglusib and Pevonedistat. Finally, we provide evidence of a collaborative inhibition of BCL-2 expression by this combined treatment, which offers new mechanistic support for its cytotoxic action.

**Keywords:** NEDDylation; GSK-3ß; RNA translation

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## Abstract ID: 1004

**Title:** Ubiquitin-like post-translational modifications may modulate the pathogenesis of chronic lymphocytic leukemia mediated by RRAS2

**Authors:** Daniela Vallejo[a], Jose Luis Castaño[b], Víctor Arenas[b], Juan José Domínguez-García[c], Lucrecia Yáñez[d] and Carlos Pipaón[b]

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**Introduction:** Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in adults. It is characterized by the accumulation of mature B lymphocytes in the blood, bone marrow, and lymphoid tissues. The overexpression of the RRAS2 gene, present in 82% of patients, has been described as the most likely molecular cause of the disease. Although the transformation by RRAS2 has been traditionally associated with genetic mutations, recent studies indicate that its overexpression without mutations is sufficient to induce CLL. On the other hand, two other molecular mechanisms, i.e. the elevation of the ubiquitin-like post-translational modifications (UBL-PTM) and the increment in RNA translation of certain oncogenes, have demonstrated a high prevalence and relevance in the maintenance of the tumoral phenotype of CLL cells.
**Objective:** Our objective is to shed light on the role of the aberrant UBL-PTM of RRAS2 found in B-CLL cells on the pathogenesis mediated by RRAS2, in search for new therapeutic targets.
**Methods:** We have cloned the ORF of RRAS2 and generated mutants of the lysines aberrantly modified in CLL. Preliminary transfection experiments have been performed in HEK293T cells. Transcription of the RRAS2 gene was also analyzed by RT-qPCR and luciferase assays. Then, MEC-1 cell line and ex-vivo experiments on B lymphocytes from patients are utilized to try to extrapolate the data.
**Results:** RRAS2 exhibit several lysines overmodified in CLL, being K177 the most sensitive to NEDDylation inhibition with Pevonedistat, but not to the ubiquitin inhibitor TAK243. As Pevonedistat has shown therapeutic value in CLL, we wondered whether the NEDDylation at K177 could be potentiating the pathogenetic potential of RRAS2. We first analyzed the stability of RRAS2 wild type or its K177R mutant when transfected into HEK293T cells. While wt RRAS2 protein accumulation decreased in response to the NEDDylation inhibitor Pevonedistat, K177R mutant was unaffected or even exhibited a slight stabilization. A similar response was observed when NEDDylation was impaired by genetic means, including RNA interference constructs against NEDD8 or the NEDD8 activation enzyme NAE1. On the contrary, an increase in overall NEDDylation in HEK293T cell induced by deletion of the de-NEDDylation enzyme SENP8 induced the accumulation of RRAS2. In concordance with a protective function of NEDDylation against ubiquitin-proteasome degradation, the NEDD8-degrader enzyme NUB1 induced a reduction in RRAS2 protein accumulation, while the ubiquitin activator enzyme (UBA) inhibitor TAK243 stabilized the protein. In the CLL cell line MEC-1, RRAS2 protein decrease after Pevonedistat treatment came along with a down regulation of its mRNA., suggesting a possible additional regulation at the transcriptional level. Functionally, our data demonstrated a higher sensitivity of RRAS2-overexpressing MEC-1 cells to specific combinational treatments like Ibrutinib + Pevonedistat or Rohinitib + Pevonedistat, just as CLL cells do. Interestingly, K177R mutation of RRAS2 hindered such sensitivity. MEC-1-RRAS2 cells exhibit higher mRNA expression of BCL2 and EIF4A1 as well as an increase in general RNA transcription. In addition, our preliminary data in HeLa cells show how Pevonedistat induces a redistribution of RRAS2 from the membrane to a more cytoplasmic location.
**Conclusion:** Our work suggests a regulatory function of NEDDylation over RRAS2 CLL pathogenesis at different upstream and downstream levels.

**Keywords:** NEDDylation; RNA translation; proteostasis

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## Abstract ID: 1417

**Title:** Acalabrutinib monotherapy versus acalabrutinib plus obinutuzumab combination therapy in treatment-naïve and relapsed/refractory chronic lymphocytic leukemia: a 'real-world' study of efficacy and safety

**Authors:** Ken Zou[a], Rudy Mrad[a], Heather Wolfe[a] and Farrukh Awan[b]

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**Background:** At present, there is no single consensus treatment regimen for CLL in either the treatment-naïve (TN) or relapsed/refractory (R/R) settings. For TN patients, common options currently include Bruton tyrosine kinase inhibitors (BTKi) (i.e. acalabrutinib, ibrutinib, zanubrutinib), which are used as monotherapy or in combination therapy with anti-CD20 monoclonal antibodies (rituximab or obinutuzumab), and/or BCL-2 inhibitors (venetoclax) in combination with anti-CD20 antibody. The phase III ELEVATE-TN study demonstrated significant improvement in progression-free survival (PFS) for TN CLL/SLL patients who received acalabrutinib +/- obinutuzumab versus chemoimmunotherapy (obinutuzumab-chlorambucil). However, the study was not powered to assess the differences between acalabrutinib monotherapy and acalabrutinib-obinutuzumab combination therapy. Combination therapy is anecdotally thought to provide deeper responses at the expense of increased toxicity, although both randomized trial and retrospective 'real-life' data remain limited. This is a retrospective study using real-world data to evaluate the differences in efficacy and safety between BTKi and anti-CD20 antibody combination therapy (acalabrutinib + obinutuzumab (AO)) and BTKi monotherapy (acalabrutinib (AM)) in both TN and R/R settings.
**Methods:** We conducted an IRB-approved, retrospective review of adult CLL/SLL patients managed at our tertiary academic medical center between 2018 and 2024, and who received treatment with acalabrutinib +/- obinutuzumab. 114 patients were assessed in four groups – AO-TN (n = 47), AM-TN (n = 15), AO-RR (n = 22), and AM-RR (n = 30). Data was collected on patient demographics, baseline disease characteristics, treatment patterns, adverse events (AEs), and outcomes. Statistical analysis was limited due to small sample sizes.
**Results:** Treatment-Naive Cohorts: Baseline demographics and disease characteristics were similar between AO-TN and AM-TN for median age (65 vs 60 years), ECOG performance

status ≤1 (97.9% vs 100%), and high-risk genetic features, including chromosome 17p13.1 deletion (27.7% vs 26.7%), unmutated IGHV (63.8% vs 60.0%), and TP53 mutation (23.4% vs 33.3%). Gender distribution was more balanced for AO-TN (46.8% female, 53.2% male) than AM-TN (26.7% female, 73.3% male). The most common treatment indication in both groups was progressive marrow failure (57.4% vs 60.0%). AEs were greater for AO-TN vs AM-TN, including the proportion of patients experiencing CTCAE grade ≥3 events (68.1% vs. 33.3%), cardiac events (27.7% vs 0%), bleeding (44.7% vs 26.7%), infections (68.1% vs 46.7%), and infusion-related reactions (12.8% vs 0%). With a median follow-up of 28.5 months, patients who received combination AO in the front-line setting had improved outcomes including improved overall survival (OS) (97.9% vs 73.3%), PFS (89.4% vs 72.7%), and rates of clinical complete response +/- complete marrow recovery (CR/CRi) (73.9% vs 13.3%), including higher rates of minimal residual disease negativity by flow cytometry (MRD(-)) (26.0% vs 0%). Relapsed/Refractory Cohorts: Baseline demographics and disease characteristics were also similar between AO-RR and AM-RR for median age (57 vs 64 years), gender distribution (31.8% vs 30.0% female, 68.2% vs 70.0% male), ECOG performance status ≤1 (86.4% vs 76.7%), and high-risk genetic features, including chromosome 17p13.1 deletion (31.8% vs 20.0%), unmutated IGHV (54.5% vs 63.3%), and TP53 mutation (31.8% vs 20.0%). The most common treatment indication in both groups was progressive marrow failure (36.4% vs 50.0%). Rates of AEs were comparable, albeit slightly greater for AO-RR vs AM-RR, including the proportion of patients experiencing CTCAE grade ≥3 events (77.3% vs. 63.3%), cardiac events (22.7% vs 20.0%), bleeding (31.8% vs 20.0%), infections (68.2% vs 60.0%), and infusion-related reactions (4.5% vs 0%). With a median follow-up of 38.4 months, patients who received combination AO in the relapsed setting had improved outcomes, including improved OS (81.8% vs 60.0%), PFS (80.0% vs 46.7%), CR/CRi rates (59.0% vs 30.0%), and higher rates of MRD(-) (27.0% vs 0%).

**Conclusions:** Our results demonstrate that in both TN and R/R patients, AO was associated with an increased risk for AEs compared to AM. Nonetheless, combination therapy may offer a significant improvement across multiple outcome measures (i.e. OS, PFS, rates of CR/CRi and MRD(-)), which in some patients may outweigh the increased safety risks. In patients followed at specialized CLL treatment centers with robust coordination of care, AEs may be more rapidly triaged and managed, lending further support for the use of acalabrutinib + obinutuzumab combination therapy over acalabrutinib monotherapy alone. Larger sample sizes are needed to better understand the optimal patient population in which combination therapy would be preferred.

**Keywords:** BTKi combination therapy

**Please indicate how this research was funded:**

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## Abstract ID: 1407

### **Title:** The impact of body mass index on overall survival in chronic lymphocytic leukemia

**Authors:** Noomi Vainer[a], Henrik Hjalgrim[b,c,d,e], Klaus Rostgaard[f], Carsten U. Niemann[g] and Emelie C. Rotbain[f]

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**Background:** Comorbidities are common in patients with chronic lymphocytic leukemia (CLL) due to the typical high age at diagnosis. Cardiometabolic conditions such as hypertension and type 2 diabetes (T2D), among the most common comorbidities in patients with CLL (prevalence 48% and 12% respectively), are associated with lower overall survival. Being overweight/obese is closely intertwined with the development of cardiometabolic disease. Little is known about whether the impact of the comorbidities is due to the presence of the diseases or inherent in the risk factors associated with being overweight.

**Aim:** To assess the impact of body mass index (BMI) on treatment and survival outcomes for patients with CLL from time of CLL treatment.

**Methods:** The DALY-CARE data source holds data from the Danish National Clinical Quality Database for CLL, in which we identified patients registered from 2008 to 2023 receiving care in the Capital Region and the Zealand Region of Denmark and treated for CLL after May 2016. Patients were followed from first-line treatment until death or end of follow-up, which was 31st of December 2023. Event analysis following first line treatment were conducted using Kaplan-Meier estimates and Cox's proportional hazard model adjusted for age at first-line CLL treatment, sex, treatment year and presence of TP53 mutation/aberration or deletion 17p.

**Results:** We identified 566 patients of whom 499 patients with information on BMI available at time of first line treatment (88%) were included in further analysis; 70% were male and the median age at treatment initiation was 68 years (IQR 61–75). Univariable Cox regression showed better event-free survival (EFS) and overall survival (OS) for overweight patients with CLL compared to normal weight patients with CLL (hazard ratio (HR): 0.66, 95% confidence interval (CI) [0.46;0.95] and HR: 0.63, 95% CI [0.42;0.96], respectively). No difference in risk was found for EFS or OS in obese ($p = 0.27$ and $p = 0.26$) compared to normal BMI patients. Female obese and overweight patients had better survival compared to their male comparators within BMI groups, while no difference between sex was seen for those with normal BMI (Figure 1). For patients receiving targeted treatment, no difference was seen between BMI groups for EFS ($p = 0.74$) or OS ($p = 0.62$) in univariate analysis. In multivariable analysis adjusted for sex, age at treatment, treatment year and presence of TP53 mutation/aberration or deletion 17p, overweight patients had decreased EFS (HR: 0.73 95% CI [0.51;1.05]) and OS (HR: 0.75 95% CI [0.50;1.14]) compared with normal weight patients, although not statistically significant. No difference was seen in multivariable analysis between obese and normal weight patients for either EFS ($p = 0.97$) or OS ($p = 0.97$). In multivariable analysis, obese patients had an increased OS (HR: 1.33 95% CI [0.72;2.46]) and EFS (HR: 1.54 95% CI [0.94;2.55]), although not statistically significant. We did not have enough events in the underweight BMI group to report any meaningful risk for this group. To secure simple interpretation of results, underweight people were therefore excluded from the analyses.

**Conclusion and further analysis:** These preliminary findings on the association between BMI and survival from first-line

treatment in CLL, suggest that obesity may not be a risk factor in CLL, and that being overweight might even be protective. This highlights the importance of nutritional optimization in patients treated for CLL. To further inform management of CLL, these results will be followed up by assessing patterns of toxicity in fixed-dosage chemoimmunotherapy and targeted treatment, comparing real-world data with a clinical trial cohort while also including genetic risk for increased BMI.

**Keywords:** Chronic lymphocytic leukemia; BMI; survival

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## Abstract ID: 1418

### **Title:** A refined continuous individualized risk index (CIRI2-CLL) for accurately predicting outcomes after limited-duration CLL therapy

**Authors:** Can Zhang[a], Othman Al-Sawaf[b], Mohammad Esfahani[c], Sandra Robrecht[b], Eugen Tausch[d], Anke Schilhabel[e], Matthias Ritgen[f], Christof Schneider[d], Martin Peifer[g], Arnon Kater[h], John Seymour[i], Yanwen Jiang[j], Barbara Eichhorst[k], Stephan Stilgenbauer[d], Michael Hallek[l], Ash Alizadeh[m], David Kurtz[n] and Kirsten Fischer[b]

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**Introduction:** Measurable residual disease (MRD) status after limited-duration treatment for patients (pts) with CLL is a strong prognostic factor for survival times, but most prognostic indices such as CLL-IPI rely solely on pre-treatment features. To incorporate dynamic MRD data in prognostic models across diverse cancer types including CLL, the continuous individualized risk index was previously proposed (CIRI, Kurtz et al. 2019). CIRI dynamically determines outcome probabilities for individual pts by integrating diverse factors over time, including treatment modality and serial MRD measurements on and post-treatment. Since developed in the context of chemoimmunotherapy we here propose a refined version of CIRI, CIRI2-CLL, in the context of fixed-duration targeted therapy, which includes MRD status on-treatment (interim), at end-of-treatment (EoT) and in addition at 12-month post-EoT (FU12).

**Methods:** We inferred model parameters from patient level data of the CLL8/10/11 (FC [±rituximab (R)], BR, Clb [±R/Obi], n = 614) and MURANO (venetoclax (Ven)-R vs BR, n = 299) trials. For previously established factors, including CLL-IPI (age, IGHV, TP53del/mut, Binet/Rai and ß2M), therapy effect, and MRD status (interim and EoT), we used the updated development set from CLL8/10/11. For the therapeutic effect of Ven and MRD status at FU12, we used the MURANO trial data. To capture the nonuniform change in PFS following therapy coinciding with end of fixed-duration therapy, we utilized piecewise proportional hazard modeling with a single change-point at 18 months in a modified Bayesian model. We then applied the refined model CIRI2-CLL to an independent validation set from the CLL14 study (Ven-Obi vs Clb-Obi, n = 381) to predict progression-free survival (PFS) and overall survival (OS). Model calibration, stratification and performance were assessed and compared to individual indices.

**Results:** We first applied CLL-IPI (previously established for chemoimmunotherapy) to CLL14 pts treated with Ven-Obi, and observed significantly shorter PFS ($p < 0.001$) in comparing pts with very high vs low risk. Outcomes of pts with very high or high ($p = 0.11$), high or intermediate CLL-IPI ($p = 0.41$) were similar. We tested whether CIRI2-CLL improves upon CLL-IPI by comparing their prognostic values in the full CLL14 validation set. CIRI2-CLL demonstrated good calibration with <5% difference between observed and predicted event probabilities. The performance was significantly improved compared to CLL-IPI as shown by 21–31% improvements in the C-statistic from 1–5 years, including superior prediction to single-factor indices, such as MRD. Performance was maintained when limiting to the Ven-Obi treated pts only. When stratifying pts by CIRI2-CLL into low (17pts), intermediate (140 pts) and high-risk (24 pts) groups on the basis of optimal separation of PFS, the corresponding 3-year PFS rates from most recent landmark were 100%, 70% and 11%. Furthermore, CIRI2-CLL allows for patient stratification according to 3-quantiles (3-year PFS rate from most recent landmark 89.4%, 68.8% and 37.1% respectively in Ven-Obi treated pts, 3-year PFS rate from most recent landmark 65.1%, 23.0% and 5.9% respectively in Clb-Obi treated pts). Nine (19.6%) and 7 (15.6%) pts with intermediate CLL-IPI risk had high CIRI2-CLL risk in the Ven-Obi cohort and Clb-Obi cohort, respectively. Nineteen (18.8%) pts in Ven-Obi and 17 (17.5%) pts in Clb-Obi with high CLL-IPI risk had low CIRI2-CLL risk. In the Ven-Obi cohort, 50 (45.5%) of the pts with undetectable ($<10^{-4}$) MRD at FU12 had intermediate CIRI2-CLL risk. In the Clb-Obi cohort, 30 (33.7%) of the pts with detectable MRD at FU12 had low CIRI2-CLL risk. For OS, stratification of risk by CIRI2-CLL significantly improved compared to any individual index as seen by a C-statistic between 0.77 and 0.99 at all timepoints, with good calibration (≤ 5% difference from 1–5 years between observed and predicted event probabilities in Ven-Obi treated pts) and clear risk stratification into low, intermediate and high CIRI2-CLL OS risk (3-year OS rate from most recent landmark 92.7%, 58.1% and 0% respectively in Ven-Obi treated pts).

**Conclusions:** Our results validate CIRI2-CLL in the context of limited-duration CLL therapy, and suggest adaptability with emerging additional longitudinal outcome data. Upon risk stratification, pts with increased risk for disease progression after Ven-Obi can be reliably identified, providing benefits for clinical routine care and important implications for future risk-adapted CLL trials.

**Keywords:** Leukemia; dynamic prognostic index including measurable residual disease; fixed-duration targeted therapy

**Please indicate how this research was funded:**

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## Abstract ID: 1005

### **Title:** hnRNPU regulates alternative splicing of Cav1.2 calcium channel: implications in ibrutinib-associated ventricular arrhythmia

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**Aims:** Ibrutinib, a Bruton tyrosine kinase inhibitor widely used in the treatment of chronic lymphocytic leukemia (CLL), has been associated with an increased risk of ventricular arrhythmia (VA). Alternative splicing (AS) of CaV1.2 calcium channel has been implicated in cardiac arrhythmias. This study aims to elucidate how ibrutinib alters CaV1.2 splicing and promotes VA susceptibility.
**Methods:** We conducted a retrospective analysis of clinical data from CLL patients treated with ibrutinib for ≥3 months at The First Affiliated Hospital of Nanjing Medical University between January and December 2023, focusing on electrocardiogram (ECG) parameters. To explore potential cardiotoxic mechanisms, 8-week-old male C57BL/6 mice were administered ibrutinib or vehicle at 25 mg/kg/day for 4 consecutive weeks via intragastric gavage. Cardiac function and morphology were assessed by echocardiography and Masson's staining, respectively. Electrophysiological studies assessed VA inducibility in vivo. Neonatal rat ventricular myocytes (NRVMs) were used to examine action potential duration (APD), CaV1.2 channel function, and intracellular $Ca^{2+}$ concentration ([$Ca^{2+}$]i) via whole-cell patch clamp and Fluo-4 AM fluorescence imaging. Bulk RNA sequencing, RT-PCR, and RNA immunoprecipitation (RIP) were employed to analyze AS events and protein-RNA interactions.
**Results:** 76 CLL patients meeting the inclusion criteria were analyzed, and statistical analysis demonstrated that ~13% of these patients developed cardiac arrhythmias including VA after 3 months of continuous ibrutinib therapy. In mice, 4 weeks ibrutinib administration increases the susceptibility to ventricular arrhythmias to 30%. Bulk RNA sequencing revealed that ibrutinib significantly altered calcium signaling pathway and induced AS of Cacna1c. Indeed, ibrutinib increased the expression of the CaV1.2 alternative exon 9* (CaV1.2E9*) without affecting alternative exon 33. In NRVMs, ibrutinib hyperpolarized the current-voltage curve of cardiac CaV1.2 channels, prolonged APD, and raised K+-triggered [Ca2+]i. By using transcriptomics analysis, the splicing factor hnRNPU was found to be upregulated by ibrutinib treatment. RIP assay confirmed the direct binding of hnRNPU to CaV1.2 pre-mRNA. Knockdown of hnRNPU significantly reduced CaV1.2E9* expression, reversed the hyperpolarization trend of the CaV1.2 channel currents, and mitigated APD prolongation induced by ibrutinib.
**Conclusion:** Our study identifies a novel arrhythmogenic mechanism in which ibrutinib upregulates hnRNPU, promoting pathogenic CaV1.2E9* splicing. This aberrant splicing alters channel gating, disrupts calcium homeostasis, and ultimately increases susceptibility to VA.

**Keywords:** Alternative splicing; Cav1.2 calcium channel; hnRNPU; Ibrutinib; ventricular arrhythmia

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## Abstract ID: 1006

### **Title:** Apoliprotein E induces ferroptosis in chronic lymphocytic leukaemia cells

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Chronic lymphocytic leukemia (CLL) is characterized by a heterogeneous clinical course, ranging from patients with indolent disease to others with progressive disease that may ultimately evolve into the Richter syndrome (RS). Understanding the molecular mechanisms driving CLL heterogeneity and identifying specific vulnerabilities in CLL cells represent promising strategies to develop targeted therapies with higher efficacy, particularly for aggressive CLL and RS. Here, we identified a novel regulatory mechanism active in CLL cells that involves Apolipoprotein E (ApoE). ApoE is an abundant serum protein primarily known for its role in lipid transport and homeostasis. Beyond its canonical functions, ApoE has been implicated in signal transduction and in the pathogenesis of inflammatory and neurological disorders, including Alzheimer's disease and atherosclerosis (Huang YWA, et al. Cell, 2017). Our results demonstrate that recombinant ApoE reduces CLL cell viability and proliferation triggered by CD40L stimulation. Functional analyses revealed that ApoE induces lipid peroxidation in proliferating CLL cells. This ultimately leads to ferroptosis, a type of non-apoptotic cell death characterized by the accumulation of toxic lipid peroxides damaging cellular membranes and organelles. Notably, we also found that ApoE is a copper-binding protein and that its cytotoxicity is further promoted by elevated intracellular copper levels. Interestingly, our findings indicate that aggressive CLL cells are less sensitive to ApoE inhibition, while resistance to ApoE can be possible in RS cells. Nevertheless, both aggressive and RS cells remain susceptible to ferroptosis-inducing drugs. In conclusion, this study uncovers a previously unrecognized role for ApoE in inducing ferroptosis in CLL cells. Importantly, although ApoE toxicity is less pronounced in aggressive disease forms, leukemic B cells from these patients can still be effectively targeted using ferroptosis-inducing agents. These findings offer new insights

into the molecular mechanisms underlying CLL and suggest potential avenues for the development of targeted therapies.

**Keywords:** CLL; cancer vulnerabilities; ferroptosis

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**Please indicate the name of the funding organization:** AIRC, Italian Ministry of University and Research

## Abstract ID: 1106

**Title:** Genomic characterization of chronic lymphocytic leukemia patients with TP53 alterations from a spanish real-world cohort treated with targeted agents

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**Introduction:** TP53 alterations [17p13 deletions (TP53del) and TP53 mutations (TP53mut)] are adverse prognostic factors in chronic lymphocytic leukemia (CLL). Double-hit cases (TP53del+mut or multiple TP53mut) have a particularly poor prognosis (Brieghel et al, 2021). Complex karyotype (CK, ≥3 alterations) predicts worse outcome in chemoimmunotherapy (CIT)-treated patients. Low/intermediate CK (3–4 alt.) worsens evolution only in TP53-altered cases, while high CK (≥5 alt.) confers an aggressive outcome independently of other risk factors (Baliakas et al, 2019). The impact of CK in targeted therapy is still under analysis. Besides, although CK associates with TP53 alterations, the link between TP53-alteration type (single hit or double hit) and genomic complexity remains unclear. In recent years, optical genome mapping (OGM) has emerged as a new cytogenomic method that allows the detection of both balanced and unbalanced abnormalities with high resolution with promising results for CK assessment in CLL.

**Objectives:** Whitin the context of the COMPLEX project, which aims to assess the impact of CK on the outcome of CLL patients treated with targeted therapies in a real-life study in Spain, the main aims of this study were: (1) To characterize the genomic profile of TP53-altered patients and (2) To describe numerical/structural alterations in the TP53 genomic region and genomic complexity in a subgroup of cases using OGM.

**Patients and methods:** We included 355 CLL patients treated with iBTK and/or iBCL2 ± anti-CD20 from Nov 2021 to April 2025, with available data on FISH (13q,12, 11q, 17p), IGHV, TP53 mutations, and CK before treatment. Among them, 83 TP53-altered patients were selected: TP53 alteration type (del

and/or mut) and its association with IGHV status and presence of CK were characterized. Peripheral blood samples from 65 of the TP53-altered patients were analyzed by OGM to detect numerical/structural alterations in 17p13 and to assess genomic complexity (GC). GC by OGM was defined as the presence of ≥5 alterations, including those ≥5 Mb, rearrangements, CLL-related abnormalities, and smaller alterations (≥200 Kb) that were related with other events.
**Results:** TP53-altered patients represented 23% of the cohort (83/355). TP53 alterations were more frequent in patients treated in second and subsequent lines (37%, 32/87) vs. first line (19%, 51/268) ($p < 0.001$). Prior CIT exposure did not significantly impact TP53 alteration rates (34% [23/68] vs. 47% no-CIT [9/19], $p = 0.279$). Among 83 TP53-altered patients, 37 (45%) were double-hit (30 TP53del + mut, 7 multiple TP53mut), while 46 (55%) were single-hit (25 TP53mut, 21 TP53del). Median FISH-positive nuclei in the 51 patients carrying TP53 deletion was 45% [7–98%]. TP53 mutations had a median VAF of 24%, with 10/62 (16%) patients showing VAF <5% [1–4.7%]. CK was found in 40 (48%) patients, including 31 (37%) high CK. High CK was more frequent in double-hit TP53 patients (50% vs. 27%, $p = 0.018$), but unmutated IGHV frequency showed no differences between groups (71% vs. 60%, $p = 0.326$). Among the 65 cases analyzed by OGM [28 TP53del + mut, 20 TP53mut, 13 TP53del and 4 multiple TP53mut], OGM detected 33/41 (80%) TP53 deletions previously identified by FISH. Discrepancies were due to infiltrations below OGM sensitivity (20%). Deletions at 17p13 showed a median size of 17 Mb (480 Kb-25.1 Mb), and 8/33 (24%) were associated with structural alterations: a simple translocation [t(2;17)(q37.3;p11.2)] (n = 1), a complex three-way translocation [t(3;13;17)(p21.31;q14.2;p13.1)] (n = 1), and a concomitant 17p loss/17q gain compatible with isochromosome 17q (n = 6). OGM detected GC in 43/65 (66%), with 34/43 (79%) also CK by conventional cytogenetics. GC was more frequent in double-hit than in single-hit TP53 groups (81% vs. 51%, $p = 0.011$). OGM identified chromoanagenesis in 22/65 (34%) cases, mostly in double-hit TP53 (77%, 17/22). Chromothripsis was observed in 20 cases; one also had chromoanasynthesis, and three showed chromoplexy. Moreover, in two cases, chromoplexy represented the only chromoanagenesis event.
**Conclusion:** (1) The COMPLEX cohort is enriched in TP53-altered patients, mainly TP53del + mut, who showed higher frequencies of high CK and chromoanagenesis events. (2) Deletions in 17p13 are often large and generally not associated with other 17p structural alterations, though mostly found in a complex genomic context. (3) Although OGM is a robust technique that allows the identification and characterization of 17p13 deletions, FISH should be maintained to detect minor deleted clones.
**Acknowledgments:** 2021SGR25, PI21/00022, PI24/00229, FEHH, ACIF/2021/169, CIBEFP/2022/35.

**Keywords:** Complex karyotype; TP53-alterations; targeted therapy; OGM; chromoanagenesis

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## Abstract ID: 1904

**Title:** Development and implementation of a novel flow cytometry CD19-agnostic MRD assay in CLL, including in patients treated with anti-CD19 antibody tafasitamab

**Authors:** Lorinda Soma[a], Winston Lee[a], Olga Danilova[a], Jessica Hughes[a], Parastou Tizro[a], Matthew Mei[b], Alexey Danilov[c]* and Joo Song[a]*

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**Introduction:** Measurable residual disease assessment (MRD) in chronic lymphocytic leukemia (CLL) has prognostic significance and has served as an endpoint in clinical trials. While not routinely used in clinical practice, MRD may guide therapy in the future. MRD can be quantified by flow cytometry (FC) and by next-generation sequencing, with each method having its own pitfalls. With additional treatments being introduced in CLL, we aimed to develop a FC-based MRD assay that could quantify CLL in the setting of both anti-CD19 and anti-CD20 therapies. As part of the development, we included samples from patients treated with anti-CD19 antibody tafasitamab on a clinical trial (combined with zanabrutinib; NCT05718869).
**Methods:** We analyzed 61 samples in two cohorts:

1. Development cohort:
   a. 31 CLL cases (29 peripheral **blood** and 2 bone marrow)
      i. 13 patients on active targeted antibody therapy: 11 on tafasitamab (anti-CD19), 1 on obinutuzimab and 1 on rituximab.
      ii. 9 patients on Bruton Tyrosine Kinase (BTK) inhibitor or BCL2 inhibitor therapy.
      iii. 8 patients on surveillance.
   b. 10 normal peripheral bloods
2. Implementation cohort: 20 CLL cases (16 peripheral blood and 4 bone marrow)
   a. 3 patients on tafasitamab
   b. 13 patients on BTK inhibitor and/or BCL2 inhibitor
   c. 4 patients on surveillance (1 received liso cel CART 4 months prior)

The FC assay consisted of a two-tube panel. Tube 1: CD5, CD19, CD20, CD23, CD38, CD45, CD200, BAFFR, surface kappa and surface lambda. Tube 2: CD5, CD19, CD20, CD43, CD45, CD79b, CD81, ROR1, and exclusion markers CD3, CD13, CD16, and CD64. One million events were collected in each tube on a Navios cytometer with the percentage of abnormal B cell based on total white blood cells. Kaluza software was used for sample assessment using WBC as the denominator for enumeration of the abnormal population. When CD19 and/or CD20 were not ideal for gating the abnormal B cell population (e.g. only subset dim or negative), the following were used: BAFF-R (predominantly), CD23 and CD200 (tube 1) and ROR1 (tube 2). Samples were deemed MRD undetectable (MRDu) at $<10^{-4}$ CLL cells of total WBC count. Immunophenotype and enumeration were compared to a reference FC standard (University of Washington Medical Center, Seattle WA, USA) in 20 cases during the development phase.
**Results:** Dilutional studies demonstrated a limit of sensitivity of 0.004% of total WBCs, with 25 events forming a population

in 1,000,000 events collected. In both the development and implementation cohorts, CLL cells were detectable (in both tubes analyzed) including patients treated with anti-CD19 or anti-CD20 antibodies. No abnormal cells were detected in healthy control samples. Twenty CLL cases were run in tandem with the reference standard for development and demonstrated 100% concordance for enumeration and immunophenotype. Of 31 CLL patients included in the development cohort, 8 cases (26%) were MRDu ($<10^{-4}$) and the remaining 23 were MRD positive. Among MRD positive cases, 19 had CLL at $>10^{-2}$, 2 at $>10^{-3}$ but $<10^{-2}$ and 2 at $>10^{-4}$ but $<10^{-3}$. Of the 20 CLL cases in the implementation cohort, 25% were MRDu. In MRD positive cases, 8 had CLL at $>10^{-2}$, 4 at $>10^{-3}$ but $<10^{-2}$ and 4 at $>10^{-4}$ but $<10^{-3}$. Of the 14 tafasitimab cases, 1 was MRDu and 13 were MRD positive, 9 had CLL at $>10^{-2}$, 3 at $>10^{-3}$ but $<10^{-2}$ and 1 at $>10^{-4}$ but $<10^{-3}$.

**Discussion:** The novel two tube assay was able to detect CLL MRD levels at the current standard of $10^{-4}$. Incorporation of BAFF-R (alternative B cell marker) and ROR1 (often positive in CLL) are useful gating strategies in CD19 and/or CD20 dim to negative cases, helping to assess patients treated with CD19-targeted therapies such as tafasitamab, bi-specific antibodies or CAR-T. Additionally, exclusion gates removing T cells, NK cells, granulocytes and monocytes can be used to decrease interference from populations that may confound the interpretation.

**Keywords:** MRD; tafasitamab; CD19

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## Abstract ID: 1309

### **Title:** Effects of pirtobrutinib, a non-covalent BTK inhibitor, on T-cell function in chronic lymphocytic leukemia (CLL)

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Covalent Bruton tyrosine kinase inhibitors (cBTKi), e.g. ibrutinib, transformed the treatment paradigm in CLL and Mantle Cell Lymphoma (MCL). cBTKi demonstrate direct anti-neoplastic effects. Additionally, cBTKi's have clear immunomodulatory effects which are more prominent with ibrutinib compared with selective cBTKi's acalabrutinib and zanubrutinib. However, resistance is inevitable, and in CLL is mainly driven by BTK mutations in the C481 residue. Patients who fail treatment with cBTKi have limited therapeutic options. Pirtobrutinib is a highly selective, reversible, non-covalent BTKi, that has been FDA approved for treatment of patients with CLL and MCL who have previously received BTKi. However, the effects of pirtobrutinib on T-cell function are largely unknown. In our work we focused on studying the effects of pirtobrutinib in the T-cell compartment in CLL patients. First, we demonstrated that pirtobrutinib abrogated B-cell receptor signaling in primary CLL cells and induced CLL cell apoptosis both off stroma and in CD40L expressing stromal conditions, which mimic the lymph node microenvironment. Pirtobrutinib treatment of mice inoculated with ibrutinib-resistant patient-derived MCL xenografts slowed expansion of MCL cells and extended survival by 1.5 weeks compare to ibrutinib and by 2.5 weeks compared with vehicle control. Next, we measured the effects of pirtobrutinib on T-cell apoptosis, cell cycle and activation. CD3+ T-cells were isolated with DynabeadsTM FlowCompTM (ThermoFisher) from peripheral blood of CLL patients and stimulated up to 72 hours with αCD3/CD28 in the presence or absence of pirtobrutinib. Ibrutinib was used as control. Flow cytometry analysis showed that pirtobrutinib did not induce apoptosis or altered cell cycle progression in CD3+ T-cells. We observed downregulation of T-cell activation markers (CD69, CD25, and CD38) by ibrutinib, but not by pirtobrutinib which thus did not affect T-cell activation. Neither BTKi affected PD-1 expression in T cells. To further evaluate effects of pirtobrutinib with conducted RNA-Seq analysis of sorted naïve T-cells from healthy donors treated with the drug for 24 hours. Pirtobrutinib treatment led to ~80 significantly differentially expressed genes, compared with ~330 for ibrutinib. MYC and E2F2 targets, oxidative phosphorylation, unfolded protein response and DNA repair were the most significantly downregulated pathways following pirtobrutinib treatment. Then, we used sorted naïve CLL-derived T-cells for in vitro polarization assays. Under Th1 polarization conditions, pirtobrutinib significantly upregulated secretion of IFN-γ and IL-2, to a greater extent than ibrutinib. Meanwhile, neither drug altered secretion of IL-4 and GATA3 under Th2 polarization conditions. Pirtobrutinib significantly reduced differentiation of Tregs in polarized conditions, albeit to a lesser degree compared with ibrutinib. We next evaluated formation of immunological synapse. T-cells and CLL cells were treated with drugs for 24 hours, with T-cells simultaneously activated with αCD3/CD28, combined at a 1:1 ratio and analyzed by confocal microscopy. Interestingly, pirtobrutinib but not ibrutinib significantly enhanced immunological synapse formation. This increase correlated with upregulation of CD54 (ICAM-1) expression in both T-cell (T-cell activation and antigen uptake) and B-cell (cell-cell adhesion) populations, which may account for enhanced synapse formation. Next, we used SU-DHL4, Raji and murine A20 cell lines to conduct in vitro cytotoxicity experiments. Consistent with synapse data, pirtobrutinib but not ibrutinib enhanced T-cell mediated killing of neoplastic B-cells, accompanied by a significant increase of Granzyme B, Perforin and TNF-α secretion. In conclusion, pirtobrutinib exerted anti-tumor effects in vitro and in vivo. Pirtobrutinib promoted Th1 polarization and reduced Treg differentiation, thus partially rescuing the CLL immunosuppressive phenotype, without compromising T-cell activation and expansion. Pirtobrutinib facilitated immunologic synapse formation and enhanced T-cell cytotoxicity. Our results justify continued investigation of pirtobrutinib for treatment of lymphoid malignancies.

**Keywords:** Pirtobrutinib; BTK inhibitor; T-cell

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## Abstract ID: 1706

### **Title:** A qualitative study to explore the patient experience of continuous covalent Bruton tyrosine kinase inhibitors in chronic lymphocytic leukemia: study methodology

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**Background:** Bruton tyrosine kinase (BTK) inhibitors, including first-generation (ibrutinib) and next-generation (zanubrutinib and acalabrutinib), are novel therapies that have greatly improved treatment outcomes in patients with chronic lymphocytic leukemia (CLL). While side effects of BTK inhibitors are documented in their respective clinical trials, there is limited data on the impact of these side effects on patient lives. Furthermore, the relevance and importance of these side effects may be viewed differently by clinicians and patients, impacting the clinician and patient experience. There is also limited research on the extent to which treatment-related side effects impact the lives of care partners, who support a patient's journey in CLL.

**Aims:** The aim of this study is to explore the experiences and relative importance of side effects associated with the use of continuous covalent monotherapy BTK inhibitors from the perspectives of patients, care partners, and clinicians. The study will also examine the impact of side effects on the daily activities of individuals with CLL and their care partners and the experience of treating CLL with continuous covalent monotherapy BTK inhibitors from the clinician perspective. Exploratory objectives include assessing the similarities and differences in the patient experience with different covalent BTK inhibitors.

**Methods:** This is a non-interventional, qualitative interview study. Participants include individuals diagnosed with CLL currently taking a continuous covalent BTK inhibitor as a monotherapy for ≥6 months, care partners of such individuals, and clinicians with experience treating patients with CLL with continuous BTK inhibitors. Participants are being recruited across the UK, Germany, France, Italy, Ireland and Spain and are identified via a specialist recruitment agency; eligibility is confirmed via a screening questionnaire. Upon confirmation of eligibility, participants first complete an online background questionnaire followed by a 60-minute qualitative interview conducted via teleconference in the relevant participant language. Interviews follow a semi-structured interview guide and include open-ended questions to explore experiences with continuous BTK inhibitors, including side effects and related impacts on patients and care partners. Participants are also asked about the most and least impactful side effects and any experience they may have had with fixed duration therapies. The target sample for this study is 60 patients, 15 care partners, and 15 clinicians. The best practice in qualitative research is to keep collecting data until data saturation is reached (the point at which no new insights are obtained from conducting further interviews). The data collected during interviews will be analyzed using a combination of both content (summary of responses to specific interview questions) and thematic (identifying, analyzing and reporting themes) analyses. This study was reviewed and approved by the WIRB-Copernicus Group independent review board (tracking number: 20244460).

**Results:** Recruitment is ongoing and nearly complete with all clinicians, most care partners and half the patients recruited. The results of this study will be summarized by concept relating to covalent BTK inhibitor side effects, related impacts on patients and care partners, and the relative importance of different side effects to patients, clinicians, and care partners. Descriptions will be illustrated with participant quotes.

**Conclusion:** Qualitative research can provide rich insight into the patient, care partner, and clinician experience. This study will provide valuable insights into the patient experience of side effects associated with covalent BTK inhibitors and potentially identify discrepancies in how these side effects are viewed by patients and clinicians. Ultimately, these findings will empower patients to know what to expect from their treatment and facilitate shared decision-making between patients and clinicians on the best treatment options.

**Keywords:** Bruton tyrosine kinase inhibitors; CLL; patient experience

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**Please indicate the name of the funding organization:** BeOne Medicines Ltd

## Abstract ID: 1516

### **Title:** Real-world outcomes of idelalisib in relapsed/refractory CLL including high-risk patients and post-BTKi/BCL2 therapy: a single-center experience from 2016 to 2025

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**Background:** The therapeutic landscape of chronic lymphocytic leukemia (CLL) has significantly evolved with the development of targeted therapies. However, treatment options for patients with relapsed/refractory (R/R) CLL after BTK and BCL2 inhibitor failure remain limited. Idelalisib, a PI3Kδ inhibitor, represents a potential option in this setting, though its use is often limited by toxicity. We present a real-world retrospective analysis of idelalisib in R/R CLL patients.

**Methods and characteristics:** We retrospectively reviewed 46 patients with R/R CLL treated with idelalisib (± rituximab) between 2016 and early 2025 at the Hemato-Oncology Clinic, University Hospital Olomouc. Median age was 71 years, range: 44–84. IGHV mutation status was unmutated in 83% (33 of 40 tested). TP53 mutation and/or del(17p) was detected in 37% (17/46). Most patients were heavily pretreated, with a median of 3 prior therapy lines.

**Results:** The median duration of treatment with idelalisib among all treated patients was 17.6 months (range: 0.5–60 months) with ORR 77%. . In patients with del(17p)/TP53 mutation, the median treatment duration was 16.3 months

(range: 1–55 months). Treatment was discontinued in 33 patients: 18 due to progressive disease (PD), including 4 cases of Richter's transformation; median duration of treatment in this group was 16.7 months (range: 1–48 months). 13 due to adverse events (AEs), with a treatment duration of 15.3 months (range: 1–24 months). 1 patient discontinued due to cancer duplicity, and 1 due to complete remission (CR) and pneumonia. In a subgroup of 10 patients who had previously received BTKi or BCL2i therapy (7 of whom were double-refractory), the overall response rate (ORR) was 50%, and the median treatment duration was 15 months (range: 1–16). Among 15 patients treated with venetoclax following idelalisib failure, the ORR was 60%. Adverse events (AEs) occurred in 46% of patients (21 out of 46). Colitis was observed in 26% (12 patients), infections in 9% (5 patients), hepatopathy in 6% (3 patients) and pneumonitis in 2% (1 patient). Fatal adverse events was observed in 2 pts: 1 patient died from COVID-19 and 1 patient died from hepatitis with subsequent multiple organ dysfunction syndrome (MODS).

**Conclusions:** In this single-center real-world cohort, idelalisib demonstrated clinical activity in patients with relapsed/refractory CLL, including those with high-risk genetic features and prior exposure to targeted therapies. While efficacy was modest in heavily pretreated patients, selected individuals derived clear benefit. Toxicity remains a significant limitation and underscores the need for careful monitoring. These results support a role for idelalisib in selected high-risk patients and highlight the unmet need for better-tolerated salvage options in R/R CLL.

**Keywords:** R/R CLL; BTKi/BCL2i refractory; idelalisib

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## Abstract ID: 1419

### **Title:** SEQUOIA 5-year follow-up in arm C: front-line zanubrutinib monotherapy in patients with del(17p) and treatment-naive chronic lymphocytic leukemia/small lymphocytic lymphoma

**Authors:** Constantine S. Tam[a], Paolo Ghia[b], Mazyar Shadman[c], Talha Munir[d], Stephen Opat[e], Patricia Walker[f], Masa Lasica[g], Ian Flinn[h], Tian Tian[i], Stephanie Agresti[i], Jamie Hirata[i] and Jennifer Brown[j]

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**Background:** Zanubrutinib is a next-generation Bruton tyrosine kinase inhibitor that is approved for five indications, including chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Initial results from the SEQUOIA study (NCT03336333), at a median follow-up of 26.2 months, demonstrated superior progression-free survival (PFS) by independent review with zanubrutinib vs bendamustine + rituximab (arms A and B) in patients with treatment-naive (TN) CLL/SLL without del(17p) as well as high overall response rate (ORR) and PFS benefit in patients with del(17p) (arm C). Additionally, the 5-year follow-up in arm A demonstrated durable PFS benefit, with estimated 54- and 60-month PFS rates of 80% and 76%, respectively. Here we report updated results in SEQUOIA arm C, in patients with del(17p), after approximately 5 years of follow-up (data cutoff: 30 April 2024).

**Methods:** Arm C is a nonrandomized cohort of SEQUOIA patients with del(17p) that received zanubrutinib monotherapy. Investigator-assessed PFS, overall survival (OS), ORR, and safety/tolerability were evaluated. Adverse events were recorded until disease progression or start of next-line therapy.

**Results:** Between February 2018 and March 2019, 111 TN patients with del(17p) were enrolled to receive zanubrutinib. The median age was 71 years (range, 42–87), 79 (71%) were male, 67 (60%) were IGHV unmutated, and 47 (42%) had both del(17p) and TP53 mutation. At a median follow-up of 65.8 months (range, 5–75), median PFS was not reached. The estimated 60-month PFS rate was 72.2% (62.4%–79.8%), or 73.0% (63.3%–80.6%) when adjusted for COVID-19. Median OS was also not reached. The estimated 60-month OS rate was 85.1% (76.9%–90.6%), or 87.0% (79.0%–92.1%) when adjusted for COVID-19. The ORR was 97.3%, and the complete response/complete response with incomplete hematologic recovery rate was 18.2%. Zanubrutinib treatment was ongoing in 62.2% of patients. The most common causes for treatment discontinuation were adverse events and progressive disease (in 17.1% and 15.3%, respectively). Key adverse events of interest (AEI) included any-grade infection (82%), bleeding (60%), neutropenia (19%), hypertension (18%), anemia (9%), thrombocytopenia (8%), and atrial fibrillation/flutter (7%). Grade ≥3 AEI included infection (33%), neutropenia (16%), hypertension (8%), bleeding (6%), atrial fibrillation/flutter (5%), and thrombocytopenia (2%).

**Conclusions:** With this 5-year follow-up in SEQUOIA, the efficacy of zanubrutinib in TN higher-risk patients with del(17p) was maintained and patients continue to demonstrate PFS benefits consistent with the randomized cohort of patients without del(17p) (arm A). Additionally, with longer-term follow-up, no new safety signals were identified. This update, in the largest cohort of uniformly treated patients with del(17p), suggests that zanubrutinib remains a valuable frontline treatment option for patients with or without del(17p) CLL/SLL. Updated data from a more recent data cut-off will be available for the presentation.

**Keywords:** Zanubrutinib; CLL; treatment-naive

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** BeOne Medicines Ltd

## Abstract ID: 1420

### **Title:** Combination of zanubrutinib + venetoclax for treatment-naive chronic lymphocytic leukemia/small lymphocytic lymphoma: results in SEQUOIA arm D

**Authors:** Mazyar Shadman[a], Talha Munir[b], Shuo Ma[c], Masa Lasica[d], Monica Tani[e], Tadeusz Robak[f,g], Ian Flinn[h], Jennifer Brown[i], Paolo Ghia[j], Emmanuelle Ferrant[k], Constantine S. Tam[l], Wojciech Janowski[m], Wojciech Jurczak[n], Linlin Xu[o], Tian Tian[p], Stephanie Agresti[p], Jamie Hirata[p] and Alessandra Tedeschi[q]

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**Background:** Zanubrutinib monotherapy demonstrated superior progression-free survival (PFS) compared with bendamustine + rituximab in patients without del(17p) at 26.2-month follow-up and sustained PFS benefit at 5-year follow-up. In a single-arm cohort, zanubrutinib monotherapy was also shown to be effective in patients with del(17p). Several chronic lymphocytic leukemia (CLL) studies have demonstrated promising efficacy with the combination of B-cell lymphoma 2 + Bruton tyrosine kinase inhibitors; however, patients with del(17p)/TP53 mutation comprised a small percentage of or were excluded from study populations. Here, we present results from SEQUOIA (NCT03336333) arm D with zanubrutinib + venetoclax in patients with or without del(17p) and/or TP53 mutation.

**Methods:** Arm D is a nonrandomized cohort of the SEQUOIA study in patients aged ≥65 years (or 18–64 years with comorbidities). Patients received zanubrutinib (160 mg twice daily) + venetoclax (ramp-up to 400 mg once daily) from cycle 4 to cycle 28, followed by continuous zanubrutinib monotherapy until progressive disease, unacceptable toxicity, or meeting undetectable minimal residual disease (uMRD)–guided early zanubrutinib or venetoclax stopping rules (complete response/complete response with incomplete hematologic recovery rate [CR/CRi] and uMRD [$< 1 \times 10^{-4}$ by flow cytometry] in peripheral blood and bone marrow on two consecutive tests ≥12 weeks apart). Efficacy responses were assessed by investigator every 3 cycles until cycle 28, then every 6 cycles with peripheral blood minimal residual disease assessment.

**Results:** Between November 2019–July 2022, 114 patients were enrolled: 66 (58%) with del(17p) and/or TP53 mutation, 47 (41%) without del(17p) and TP53 mutation, and 1 with missing TP53 results. In all patients, median age was 67 years (range, 26–87), 64 (56%) were male, 86 (75%) had unmutated IGHV, and 47 (41%) had complex karyotype (≥3 abnormalities). As of 16 September 2024, the median study follow up was 31 months; 85 (75%) patients remained on zanubrutinib treatment and all patients had discontinued or completed venetoclax treatment. The most common reasons for early discontinuation were reaching the uMRD-guided early stopping rules (zanubrutinib: 7%; venetoclax: 7%), adverse events (zanubrutinib: 8%; venetoclax: 6%), and progressive disease (zanubrutinib: 5%; venetoclax: 4%). Six patients died (5 due to non–treatment-related adverse events and 1 due to progressive disease). In the total population, the 24-month PFS rate was 92%. For patients with del(17p) and/or TP53 mutation, the 24-month PFS rate was 94% and for patients without del(17p) and TP53 mutation, 24-month PFS rate was 89%. The best peripheral blood uMRD rate was 59% in the total population and 59% and 60% in patients with del(17p) and/or TP53 mutation and without del(17p) and TP53 mutation, respectively. Median time to first peripheral blood uMRD was 19 months (range 3–47) in patients with del(17p) and/or TP53 mutation and 11 months (6–25) in patients del(17p) and TP53 mutation. There were 112 patients with at least one evaluable efficacy assessment. For evaluable patients, the overall response rate and CR/CRi rate was 99% and 49%, in the total population, 100% and 48% in patients with del(17p) and/or TP53 mutation, and 98% and 50% in patients without del(17p) and TP53 mutation. The most common any-grade treatment-emergent adverse events (TEAEs) in all patients were COVID-19 (54%), diarrhea (41%) contusion (32%), and nausea (30%). The most common grade ≥3 TEAEs were neutropenia (17%), hypertension (10%), diarrhea (6%), and neutrophil count decreased (6%).

**Conclusions:** SEQUOIA arm D data demonstrate promising efficacy and tolerability of zanubrutinib + venetoclax combination treatment in treatment-naive CLL/SLL, regardless of del(17p) and/or TP53 mutational status. Best peripheral blood uMRD was also similar regardless of mutational status. The safety profile of zanubrutinib + venetoclax was consistent with results of prior zanubrutinib studies, and no new safety signals were identified. These data suggest that zanubrutinib plus venetoclax is a promising treatment option for patients with treatment-naive CLL/SLL, regardless of the presence of del(17p) and/or TP53 mutations. Updated data from a more recent data cut-off will be available for the presentation.

**Keywords:** Zanubrutinib; Venetoclax; CLL

**Please indicate how this research was funded:**

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## Abstract ID: 1707

**Title:** Evaluating CLL/SLL clinical trials: a participation-to-prevalence analysis using institutional and SEER data from 2012–2024

**Authors:** Anna Oh[a], Justin Abuel[b], Briana Williams[c], Alfonso Molina[b], Michael Conte[c], Gabriel Mannis[b], William Shomali[b], Michaela Liedtke[b], Jason Gotlib[d], Beverly Mitchell[b], Tait Shanafelt[d] and Bita Fakhri[e]

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**Introduction:** Chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) are related B-cell malignancies that represent the most common form of adult leukemia. Although clinical trials are crucial in advancing novel therapeutic options, unequal participation based on sociodemographic factors can limit the generalizability of trial findings to a broader patient population. This project assessed the demographic characteristics of CLL/SLL clinical trial participants at a large National Cancer Institute (NCI)-designated Cancer Center in Northern California (2012–2024) and compared these characteristics with national and regional disease prevalence estimates from the NCI's Surveillance, Epidemiology, and End Results (SEER) database.
**Methods:** We conducted a retrospective analysis of all interventional trials involving adults aged 18 years and older with CLL/SLL at the Stanford Cancer Institute from 2012 to 2024, utilizing institutional databases. Demographic data, including age, sex, race/ethnicity, preferred language, insurance status, and residential ZIP code, were extracted from electronic medical records. The trials were cross-referenced with ClinicalTrials.gov and categorized based on study phase, intervention class (including BTK inhibitors, PI3K inhibitors, BCL2 inhibitors, and cell-based therapies), and primary outcomes. To assess the representation of trial participants, we calculated participation-to-prevalence ratios (PPR) by comparing the demographics of trial participants with the prevalence data from the SEER 21 database, both nationally and within the nine-county Bay Area, which serves as our institution's catchment area. A PPR of 1.0 indicates proportional representation, a PPR of less than 1.0 indicates underrepresentation, and a PPR greater than 1.0 indicates overrepresentation. Descriptive statistics were employed to summarize patient and trial characteristics, providing a comprehensive overview of the demographic landscape of CLL/SLL clinical trial participants in relation to the broader population affected by these malignancies.
**Results:** A total of 212 unique patients enrolled in 30 different clinical trials for CLL/SLL between 2012–2024, contributing to 280 trial participations. Median age at first enrollment was 66.6 years (range: 21.5–87.9); 64% identified as male. The racial/ethnic distribution included: 5% non-Hispanic Asian, 2% non-Hispanic Black, 2% Hispanic, 7% non-Hispanic other/multiple groups, 2% unknown, and 82% non-Hispanic White; 98% reported English as their primary language. Insurance coverage was primarily Medicare (54%), private insurance (22%), and managed care (20%). Most trial participants (54%) resided in the Bay Area, with median driving distance of 80.5 kilometers (range: 3.2–4,829.7). Of the 212 participants, 68 (32%) enrolled in multiple trials. The highest number of trial enrollments observed was two individuals who participated in four trials. The 30 trials represented a range of therapeutic strategies. Trials between 2012–2014 involved first-generation BTK, BCL2, or PI3K inhibitors, either as monotherapy or combined with immunotherapy. From 2015–2019, trials evaluated second-generation BTK inhibitors and additional BTK and BCL2 inhibitor combinations. Since 2020, studies have focused on reversible BTK inhibitors, BTK degraders, and cell-based therapies. Eighteen trials (60%) targeted relapsed/refractory disease, while 11 (37%) targeted treatment-naïve populations. The most common endpoints were progression-free survival (47%), safety or tolerability (30%) and overall response rate (27%). Compared with national SEER prevalence data, observed trial enrollment was not reflective of CLL/SLL prevalence in most sex and racial/ethnic groups. The largest gaps in enrollment were among non-Hispanic Black and Hispanic females (both PPR =0.06, proportions not reported due to n< 5). Non-Hispanic White females were also enrolled at a lower rate (31% vs. 83%, PPR =0.37) compared to non-Hispanic White males (52% vs. 84%, PPR =0.61). Non-Hispanic Asian males (3% vs. 2%, PPR =1.17) and Asian females (2% vs. 3%, PPR =0.96) were the only groups with participation rates similar to population prevalence. Regionally, all groups showed lower representation, with the lowest PPRs among Hispanic females (0.06), non-Hispanic Black females (0.09), non-Hispanic Asian females (0.24), and Hispanic males (0.26).
**Conclusion:** Despite increased clinical trial activity in CLL/SLL, our institution's clinical trial participants did not consistently reflect the sex and racial/ethnic profile of national and regional CLL/SLL populations. PPRs ranged from 0.06 to 1.17, with representation differing across sex and racial/ethnic categories relative to disease prevalence. These findings underscore a persistent gap in equitable representation and suggest that systemic barriers continue to limit access to trials for diverse populations, even within high-volume, research-intensive cancer centers. Future efforts should prioritize targeted outreach within institutional catchment areas, inclusive trial design, and strategies to address logistical and sociocultural barriers to enrollment, thereby enhancing clinical trial equity and generalizability of results.

**Keywords:** CLL/SLL; clinical trial enrollment; patient representation

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## Abstract ID: 1421

### **Title:** Safety and efficacy of zanubrutinib in a subgroup of older patients (≥75 years) with treatment-naive chronic lymphocytic leukemia/small lymphocytic lymphoma from the SEQUOIA study

**Authors:** Alessandra Tedeschi[a], Anders Österborg[b,c], Talha Munir[d], Patricia Walker[e], Tadeusz Robak[f,g], Hanna Ciepluch[h], Richard Greil[i,j,k], Tian Tian[l], Marcus Lefebure[m], Jamie Hirata[l] and Constantine S. Tam[n]

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**Introduction:** Chronic lymphocytic leukemia (CLL) commonly affects the elderly, with a median age of diagnosis of 72 years (Eichhorst B, et al. Ann Oncol 2015). In these patients,

comorbidities and suboptimal performance status may limit the efficacy of newer therapeutic interventions. In the Phase 3 SEQUOIA study (NCT03336333), zanubrutinib, a highly potent and selective next-generation Bruton tyrosine kinase inhibitor, demonstrated a superior safety and efficacy profile compared with bendamustine + rituximab (BR) in treatment-naïve patients with CLL/small lymphocytic lymphoma (SLL). Furthermore, patients with del(17p) treated with zanubrutinib have achieved high overall response rates (ORR) and progression-free survival (PFS). This subgroup analysis aimed to assess the safety and efficacy of zanubrutinib vs BR in older (≥75 years) patients.

**Methods:** SEQUOIA enrolled treatment-naive patients with CLL/SLL. Cohort 1 included patients without del(17p) randomized 1:1 to receive zanubrutinib or BR; Cohort 2 included patients with del(17p) assigned to receive zanubrutinib. Patients included in this analysis were restricted by age ≥75 years (elderly).

**Results:** As of 30 April 2024, the median study follow-up for patients aged ≥75 years was 62.1 (range, 1.4–74.1) months for Cohort 1 and 66.5 (range, 5.0–73.3) months in Cohort 2. In Cohort 1, 120 patients were aged ≥75 years, 56 patients received BR and 64 patients received zanubrutinib; median age was 78 (range, 75–87) years. Cohort 2 had 30 patients aged ≥75 years. In Cohort 1 patients treated with zanubrutinib, the ORR was 97%, with a complete response/complete response with incomplete hematopoietic recovery (CR/CRi) rate of 19% (vs 86% and 30%, respectively, with BR). Estimated PFS rates (95% CI) at 54 and 60 months were 77% (65%–86%) and 69% (55%–80%), respectively, with zanubrutinib and 49% (34%–62%) and 44% (28%–59%) with BR. When adjusted for COVID-19, zanubrutinib PFS rates were 79% (66%–87%) and 71% (56%–81%), and BR rates were 49% (34%–62%) and 44% (28%–59%). Estimated OS rates (95% CI) at 54 and 60 months were 86% (74%–92%) and 82% (70%–90%), respectively, with zanubrutinib and 79% (64%–88%) and 76% (62%–86%) with BR. When adjusted for COVID-19, zanubrutinib OS rates were 87% (76%–93%) and 84% (72%–91%), and BR rates were 80% (66%–89%) and 78% (64%–87%). In Cohort 2, the ORR was 93% with a CR/CRi rate of 20% with zanubrutinib. The 54- and 60-month PFS rates (95% CI) were 73% (53%–86%) and 69% (49%–83%), respectively, with and without adjustment for COVID-19; the 54- and 60-month OS rates were 80% (60%–90%) and 76% (56%–88%). In Cohort 1, grade ≥3 adverse events (AEs) were observed in 77% with zanubrutinib and 89% with BR; serious AEs (SAEs) occurred in 64% and 65% of patients, respectively. In Cohort 2, the rates of grade ≥3 AEs and SAEs were 67%, and 63%, respectively. Key AEs of special interest (AESI, any grade/grade ≥3) in Cohort 1 patients treated with zanubrutinib included atrial fibrillation/flutter (11%/2%), bleeding (59%/11%), and hypertension (34%/19%); with BR, rates of these AESI were 4%/0%, 4%/0%, and 6%/2%, respectively. AESI (any grade/grade ≥3) in Cohort 2 patients treated with zanubrutinib included atrial fibrillation/flutter (13%/10%), bleeding (67%/3%), and hypertension (20%/7%). AEs led to death in 9% and 13% of patients treated with zanubrutinib in Cohorts 1 and 2, respectively, and 17% of patients treated with BR in Cohort 1. In Cohorts 1 and 2, 64% and 57% remained on zanubrutinib at data cutoff, respectively. The most frequent reason for treatment discontinuation in patients across cohorts was AEs (Cohort 1: zanubrutinib, 25% and BR, 20%; Cohort 2: 27%).

**Conclusion:** In this subgroup analysis, the efficacy of zanubrutinib was largely comparable for elderly patients with and without disease harboring del(17p). In elderly patients without del(17p), zanubrutinib demonstrated PFS benefit over BR. The PFS and response rates reported in this analysis are comparable to published data in the intention-to-treat population, demonstrating that treatment benefit with zanubrutinib is not restricted to a younger population. With the understanding that older patients typically have more comorbidities and experience more AEs, the safety profile of zanubrutinib in those aged ≥75 years was consistent with previously published data. These data continue to support zanubrutinib as a valuable treatment option for all-comers in the first-line CLL/SLL setting, including the elderly population with or without disease bearing del(17p).

**Keywords:** Zanubrutinib; CLL; elderly

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## Abstract ID: 1218

### **Title:** CD11c: a promising indicator for zanubrutinib efficacy evaluation in high-risk CLL

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Chronic lymphocytic leukemia (CLL) patients with high-risk features, including del(17p), TP53 mutations, or unmutated IGHV, respond differently to treatment, highlighting the urgent need for effective predictive biomarkers. We investigated the association between CD11c expression and the efficacy of Bruton's tyrosine kinase inhibitor (BTKi) therapy in high-risk CLL patients. Mean fluorescence intensity (MFI) of CD11c expression was assessed by multiparameter flow cytometry in pretreatment bone marrow samples from 78 CLL patients with high-risk cytogenetic features (62% with del(17p)/TP53 mutations, and 71% with unmutated IGHV). Patients were stratified into CD11c-high and CD11c-low expression groups based on the median MFI. All patients received zanubrutinib treatment, with a median follow-up of 28 months. CD11c-low group had a significantly higher overall response rate (ORR) compared to CD11c-high group (84.6% vs. 56.4%, $p = 0.008$), as well as a superior 24-month progression-free survival (PFS) rate (79.5% vs. 51.3%, $p = 0.003$). Multivariate analysis confirmed that CD11c expression was an independent predictor for PFS (hazard ratio [HR] = 0.38, 95% confidence interval [CI]: 0.19–0.76, $p = 0.006$), regardless of TP53 status. Mechanistic studies revealed that high CD11c expression was significantly associated with downregulation of B-cell receptor (BCR) signaling-related genes, such as SYK and PLCγ2 ($p < 0.05$). This is the first study to define CD11c as a predictive biomarker for BTKi treatment efficacy in high-risk CLL patients, providing important evidence for precision treatment decisions in this refractory population.

**Keywords:** CLL; CD11c; BTKi

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1517

**Title:** Central nervous system involvement in chronic lymphocytic leukemia: a real-world analysis

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**Introduction:** Secondary central nervous system (CNS) involvement is a rare occurrence in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Our understanding of this rare complication, and how to approach treatment for these patients, is largely drawn from case reports. Herein, we evaluated the incidence of CNS involvement in CLL/SLL, and we evaluated real-world evidence regarding treatment for this rare complication.

**Methods:** This retrospective observational study used an IRB-approved lymphoid malignancy registry. Eligible patients had CLL/SLL and were cared for at Massachusetts General Hospital (MGH). Patients were identified through retrospective chart review of CLL/SLL patients in the lymphoid malignancy registry. Cumulative incidence of CNS involvement was calculated from CLL/SLL diagnosis to CNS diagnosis and estimated using the Kaplan-Meier method (censored when last known alive without CNS involvement). CNS response was defined as resolution of associated CNS disease symptoms for at least 3 months and, as applicable, reduction/resolution by CSF cytology and/or MRI. Analyses were performed using R version 4.4.2.

**Results:** Between 1 January 15–22 November 2024, among 715 consecutive CLL/SLL patients, with a median follow-up of 6.8 years, the cumulative incidence of CNS involvement was 1.1% (95% CI 0.3–1.9) at 5 years and 1.7% (95% CI 0.2–3.1) at 15 years. Of 9 (1.3%) patients with CNS disease, the median time from initial diagnosis of CLL/SLL to diagnosis of CNS involvement was 13.8 months (range, 0–224.1), all 9 patients had cerebrospinal fluid (CSF) cytology reviewed by an MGH hematopathologist confirming increased number of clonal B cells in the CSF without significant red cell contamination and diagnostic of leptomeningeal involvement by CLL/SLL, and all 9 patients had neurologic findings (n = 8; cranial nerve deficits [n = 3], motor deficits [n = 2], seizure [n = 1], papilledema [n = 1], and altered mental status [n = 1]) and/or MRI evidence of leptomeningeal involvement (n = 6). The median age was 76.5 years (range, 64.1–78.2), the median absolute lymphocyte count was 10,200/μL (range, 220–126,630/μL), 33.3% (n = 3) had TP53 mutation or 17p deletion, 44.4% (n = 4) had unmutated IGHV, and 33.3% (n = 3) had 3 or more karyotypic abnormalities. The median number of therapies prior to secondary CNS diagnosis was 0 (range, 0–5), including 7 treatment-naïve and 2 previously treated. One of the patients had systemic Richter's transformation (diffuse large B-cell lymphoma) but only CLL/SLL was demonstrated in the CNS with exhaustive CNS evaluation (this patient was included as a case of CNS disease but excluded from treatment results below). The median number of therapies for CNS involvement was 2 (range, 1–4) and the median follow-up calculated from CNS diagnosis was 6.1 years (range, 0.1–8.3). Among 7 cBTKi-naïve patients, 4 received HD-MTX: 1 MTX-refractory in an ongoing response after 62.5 mo on cBTKi (acalabrutinib); 3 responded to HD-MTX followed by maintenance cBTKi (2 systemic-only PD at 78.6 [zanubrutinib] and 10.5 mo [zanubrutinib]; 1 CNS/systemic PD at 48.8 mo on cBTKi [ibrutinib]); 3 received upfront cBTKi: 2 responded (ongoing at 50.4 [ibrutinib] and 8.5 mo [zanubrutinib]); 1 was not evaluable for efficacy evaluation (discontinued cBTKi [acalabrutinib] within 2 weeks due to concurrent candidemia and septic shock, and opted against further therapy). One additional patient had CLL refractory to cBTKi and venetoclax at the time of CNS diagnosis, and this patient received lisocabtagene maraleucel with uMRD6 PR at 1 mo, with response ongoing at 3 mo. An additional patient with MTX/cBTKi-refractory leptomeningeal disease achieved durable CNS response with venetoclax, which was ongoing at 43.5 mo, at which point they had systemic-only PD and initiated pirtobrutinib with ongoing CNS/systemic response at 5.2 mo on pirtobrutinib.

**Conclusion:** The cumulative incidence of secondary CNS involvement in patients with CLL/SLL cared for at an academic cancer center was 1.1% at 5 and 10 years, and 1.7% at 15 years from diagnosis, respectively. We observed two approaches for initial treatment of cBTKi-naïve patients with CLL/SLL with CNS disease – (1) upfront cBTKi or (2) HD-MTX followed by maintenance cBTKi. While the optimal approach has not been defined, upfront use of a cBTKi achieved rapid and durable CNS responses, and cBTKi monotherapy is generally our preferred approach in this setting. We also observed CNS responses in patients receiving venetoclax or lisocabtagene maraleucel, suggesting a role for these agents in cBTKi-refractory or intolerant patients.

**Keywords:** Chronic lymphocytic leukemia; central nervous system; leptomeningeal

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** MGH Lymphoma Translational Research and Biobanking Collaborative supported by the Scott Nathan and Laura DeBonis Fund for Clinical Research

## Abstract ID: 1107

**Title:** Dissecting clonal hematopoiesis in the myeloid compartment of chronic lymphocytic leukemia and Richter transformation

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**Background:** Clonal hematopoiesis (CH) is the clonal expansion of a hematopoietic stem cell in otherwise healthy individuals, due to somatic driver mutations in genes mainly implicated in myeloid neoplasms. The clinical and biological significance of CH has not been investigated in the myeloid compartment of chronic lymphocytic leukemia (CLL).

**Methods:** Genomic DNA was extracted from granulocytes of CLL patients referring at our institution at the time of diagnosis. Multicenter cohorts of CLL patients with available granulocytes before and after fixed-duration venetoclax-based therapy and BTK inhibitors were analyzed. Samples were investigated by targeted next-generation sequencing (NGS), employing a custom panel of genes (N = 28) recurrently mutated in CH. Cell sorting, single cell (sc) DNA and scRNA sequencing were also performed in cases provided with viable cells.

**Results:** A total of 488 consecutive CLL have been included in the present study. Overall, at least 1 CH mutation was identified in 231 (47.3%) patients. The most frequent mutations identified on granulocytes affected DNMT3A in 129 (26.4%) patients, followed by TET2 in 74 (15.2%), ASXL1 in 15 (3.1%), TP53 in 13 (2.7%), and PPM1D in 11 (2.3%). To evaluate the distribution of CH mutations across different peripheral blood cell compartments, 33 CH + patients who never developed a Richter transformation (RT) were sorted by FACS and the CLL compartment (CD19+/CD5+ cells), the T-cell compartment (CD3+ cells) and the myelomonocytic compartment (CD14+ cells) were independently sequenced. Overall, the mutational analysis of CH in sorted cells showed that CH mutations are not present in the CLL compartment but specifically localize in the myelomonocytic compartment. In addition, DNMT3A mutations in 12 patients and TET2 mutations in 1 patient were also detected in the T-cell compartment, confirming the possible lympho-monocyte skewage of some CH variants. This analysis also allowed to evaluate the distribution of TP53 variants in different cell compartments. In 5 patients, TP53 mutations were found exclusively in the myelomonocytic compartment (i.e. CD14+ cells), therefore representing CH-related, and not CLL-related, TP53 variants. After a median FU of 12.3 years, the presence of at least one CH mutation significantly associated with shorter OS (10.8 years vs 13.6 years, $p = 0.023$). In particular, TET2 mutations retained an independent association with shorter OS (HR 1.53, $p = 0.01$) when adjusted for age, IGHV and TP53 status. The presence of CH also predisposed to a higher risk of second hematological malignancies, that developed in 9 CH + patients and in only 1 CH- patient, accounting for a 20-year risk of developing a second hematological malignancy of 30.9% for CH + patients compared to 1.2% for CH- patients ($p = 0.0004$). By analyzing 30 patients treated with venetoclax-based therapy, CH predicted higher risk of grade >3 neutropenia ($p = 0.004$). In patients receiving continuous BTKi (N = 73), CH SF3B1 mutations (HR 9.75, $p = 0.003$) associated with an increased risk of atrial fibrillation when adjusted for age and other cardiovascular comorbidities. scRNA sequencing of 7 CH + compared to 6 CH- CLL (matched for FISH, IGHV and TP53 status) revealed that CH influences the transcriptomic profiles of both B and T cells. CH + patients displayed cell pathways indicative of heightened T-cell activation and a more pro-inflammatory profile, corroborated also by increased serum levels of CCL3 and CCL4. T-cells of CH + patients exhibit a less exhausted phenotype, as documented by a lower expression of TOX, the master regulator of T-cell exhaustion induction. By evaluating RT, ASXL1 mutations associated with a higher risk of RT (HR 6.80, $p = 0.01$) also when adjusted for the TP53 and NOTCH1 status. Interestingly, in 2 patients who subsequently developed RT, scDNA sequencing identified CH mutations also in CLL cells.

**Conclusions:** CH in CLL harbors potential clinical relevance for both CLL and RT, while also influencing the T-cell immune compartment.

**Keywords:** Clonal hematopoiesis; Richter Transformation

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## Abstract ID: 1518

**Title:** Ultrasound assessment of chronic lymphocytic patients during BTK treatment: comparison of dimensional response vs overall ultrasound assessment in general practice. Preliminary results of a prospective study

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**Introduction:** IWCC and ESMO guidelines suggest abdominal ultrasound (US) and superficial lymphnodes (SLN) palpation to assess patients with chronic lymphocytic leukemia (CLL) in general practice (GP). Computed tomography (CT) is generally not indicated out of a clinical trial. The CLL expert Tuscany panel included SLN ultrasound (SLUS) in general practice (doi10.1007/s10238-023-01244-5). SLN US assessment in CLL patients has been previously published and CLL SLN US-characteristics have been described (doi: 10.3390/jcm11113206). Ultrasound over palpation has resulted to be more accurate to define the number and the characteristics of CLL SLN (doi: 10.3390/jcm11113206).
**Materials and methods:** We performed N = 320 US examinations. Sixty patients were prospectively enrolled in the study (IRB approved): Ibrutinib (N = 20 patients), Acalabrutinib (N = 20 patients), and Zanubrutinib (N = 20 patients). Over 800 SLN were assessed with US. Median follow up was 18 months (range: 9 to 24 months). We compared BTKi response based on SLN dimensions, as suggested by the IWCC and ESMO guidelines, vs an overall SLN US-characteristics evaluation as previously described (doi: 10.3390/jcm11113206).
**Results:** Best SLN response was obtained at a median of 9, 9, and 6 months for patients treated with Ibrutinib, Acalabrutinib, and Zanubrutinib, respectively. SLN response based on SLN dimensions was complete remission (CR) in N = 24 patients (40%); partial remission (PR) in N = 15 patients (25%); stable disease (SD) in N = 16 patients (27%); and progressive disease (PD) in N = 5 patients (8%). US-characteristics evaluation assessment of SLN response was CR in N= 30 patients (50 %); PR in N = 24 patients (48%); SD in N = 2 patients (3%); and PD in N = 4 patients (7%). There was a statically significant difference between patients obtaining SD and a strong trend for patients obtaining PR using SLN dimensions vs US-characteristics evaluation ($p = 0.0002$, and $p = 0.009$, respectively). No statistically significant difference was found in patients in CR or PD ($p = 0.272$, and $p = 0.835$, respectively).
**Conclusions:** SLN US analysis of SLN characteristics allows to better define CLL patients' response to BTKi in respect to only SLN dimensions assessment with a potential impact on treatment decisions. These preliminary results suggest a role of US of SLN to help defining response to BTK inhibitors in general practice.

**Keywords:** Lymphnodes; ultrasound sonography; BTKi

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**Please indicate the name of the funding organization:** NA

## Abstract ID: 1519

**Title:** Clinical and pharmacoeconomic impact of ultrasound assessment of patients with chronic lymphocytic leukemia treated with BTK inhibitors (BTKi)

**Authors:** Edoardo Benedetti[a], Maria Costanza Caparello[a], Benedetto Bruno[b,c], Sara Galimberti[a], Emilia Bramanti[d], Riccardo Morganti[a] and Enrico Capochiani[e]
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**Introduction:** IWCC and ESMO guidelines suggest abdominal ultrasound (US) and superficial lymphnodes (SLN) palpation to assess CLL patients in general practice (GP). The CLL expert Tuscany panel included SLN US in general practice (doi10.1007/s10238-023-01244-5). SLN US assessment in CLL patients has been previously published and CLL SLN US-characteristics have been described (doi: 10.3390/jcm11113206). According to IWCC and ESMO guidelines, progressive lymphadenopathy or splenomegaly are a sign of active disease. O'Brien et al. reported that lymphnodes dimensions have an impact on response to Ibrutinib (doi 10.1182/blood-2017-10-810044).
**Materials and methods:** We present here a procedure model in which the US methodology previously published (doi10.1007/s10238-023-01244-5; doi: 10.3390/jcm11113206) was applied to determine the potential clinical benefit and the pharmacoeconomic impact, of treatment decision in multiple clinical scenarios. According to the IWCC and ESMO guidelines we defined positive a correct CLL treatment at the correct timing, or negative when the timing of the treatment is incorrect. We applied the US vs palpation of SLN assessment previously described (doi: 10.3390/jcm11113206) to different clinical scenarios of CLL patients who might initiate a BTKi treatment (scenario 1) or are on a BTKi treatment (scenario 2). Theoretical model. Scenario 1: patients who have not yet initiated a BTKi treatment. Scenario 1a: if patients are treated too early there is not a clinical benefit and the pharmacoeconomic impact is negative. Scenario 1b: if patients are treated too late with BTKi there is not a clinical benefit and the pharmacoeconomic impact is negative. In this scenario the treatment response and duration is expected to be sub-optimal, with an expected earlier second line treatment. Scenario 2 patients have initiated a BTKi treatment. Scenario 2a: If patients are responsive with a complete or partial remission the clinical benefit is positive and the pharmacoeconomic impact is positive. Scenario 2b: if patients' response to BTKi is erroneously considered in progressive disease, with a switch to a second line treatment, the clinical benefit is negative and the pharmacoeconomic impact is negative.
**Conclusions:** US was previously shown to be more accurate over palpation to identify CLL SLN (doi: 10.3390/jcm11113206). Both the timing of initial BTKi treatment and the correct assessment of response to a BTKi is crucial for a positive clinical benefit and a positive pharmacoeconomic impact. Ultrasound sonography of SLN is a useful tool in general practice to assess CLL patients and help to reach a clinical benefit with a positive pharmacoeconomic impact.

**Keywords:** Ultrasound sonography; lymphnodes; pharmacoeconomic impact; BTKi

**Please indicate how this research was funded:** No funds required

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## Abstract ID: 1207

**Title:** In vitro investigation of the phenotype of CLL cells during trafficking and homing in a 3D-printed

bioreactor

**Authors:** Margherita Pauri[a], Riccardo Pinos[a], Federica Barbaglio[a], Giulia Maria Di Gravina[b], Lydia Scarfò[c], Emanuela Sant'antonio[d], Paolo Ghia[c], Michele Conti[e] and Cristina Scielzo[a]

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**Background:** The trafficking of malignant B cells between peripheral blood, bone marrow, and lymph node is pivotal for Chronic Lymphocytic Leukemia (CLL) cell survival, proliferation, and resistance to therapies. However, the mechanisms governing intra and extravasation from lymphoid tissues remain poorly understood, one reason being the absence of models able to capture these dynamic interactions in vitro. We propose a system to study leukemic cells trafficking using an in-house designed and 3D printed bioreactor (Vesselbox) which perfuses a 3D bioprinted vascularized scaffold.

**Methods:** The scaffold includes an external layer, mimicking the lymphoid tissue microenvironment and composed of Human Lymphatic Fibroblasts (HLF) and an inner layer, vascularized by Human Umbilical Vein Endothelial Cells (HUVEC) and Bone Marrow – Mesenchymal Stem Cells (BM-MSC). First, the external layer is bioprinted followed by the casting the inner layer. The scaffold is allocated in the VesselBox for selective perfusion. A peristaltic pump enables controlled medium and cells recirculation. MEC1-GFP (CLL cell line) and primary CLL cells have been circulated in the system and immunophenotype analysis has been performed after 1, 3, and 7 days of circulation to assess changes in markers expression in presence or absence of the 3D bioprinted microenvironment or drugs.

**Results:** After biofabrication, the scaffolds are cultured in static condition for 14 days to allow cell organization and vascular lumen formation. Immunofluorescence confirmed the expression of endothelial markers (CD31, Ve-Cadherin, VWF), revealing a vascularized network. In the external layer, cells appear elongated and homogenously distributed. Considering the positive computational simulations on oxygen diffusion, pressure distribution, and shear stress, the scaffolds were perfused in the VesselBox. We used MEC1 cells to set up all the culture settings. Primary CLL cells (n = 3) were added to circulating medium and their extravasation and immunophenotype were monitored through confocal microscopy and flow cytometry at 1, 3, and 7 days of recirculation. We observed that cells can extravasate in the scaffold and a CXCR4high/CD5high population emerges (24%) when cells circulate in the presence of the microenvironment. This subset is associated with tissue homing (Burger JA et al., Br J Haematol., 2007) and has been described as highly proliferative (Friedman et al., Hemasphere, 2024). Consistent with these results, CD23 expression increased when cells circulate in the presence of the microenvironment (67%) compared to the empty bioreactor (52%), potentially indicating cell activation and growth (Lampert I A et al., Hum Pathol., 1999). Moreover, CD62L + cells increase with the microenvironment (38%) compared to its absence (2%) suggesting a tissue-homing phenotype, as CD62L has a role in the extravasation of CLL cells (Lafouresse F et al., Blood, 2015). CXCR4 and CD62L increase was also confirmed by RT-PCR, showing coherent results. We are currently validating the system for drug testing. In this setting, CLL cells are 3D bioprinted in the external layer together with HLF. Then, treatment with BTK-inhibitors (i.e. ibrutinib) or BCL2-antagonists is performed by adding the drug to the circulating medium meaning that the drugs have to pass the endothelial lumen to reach CLL cells. So far, we confirmed that the drugs can pass the barrier and preliminary results show that ibrutinib mobilizes cells from the microenvironment as expected toward the circulation, leading to a reduction of the number of cells in the scaffold. Additionally, cells in the untreated scaffolds form aggregates and clusters, which are reduced in number and dimension by ibrutinib treatment. Finally, ibrutinib leads to a downregulation of CXCR4, mimicking what occurs in vivo (Chen SS, et al., Leukemia, 2016).

**Conclusion:** We demonstrated that the VesselBox device enables the investigation of CLL cells dissemination ex vivo, supporting the study CLL dynamic interactions with the microenvironment, their extravasation through the endothelial barrier and the colonization of the tissue-specific microenvironment in real-time. These features make the device suitable for elucidating the cross-talk between CLL cells and the endothelium also in comparison with healthy B cells. Additionally, the VesselBox provides a controlled platform to investigate the function and relevance of specific cell subsets, such as CXCR4high/CD5high which dynamic regulation in vivo remains unclear. We are exploiting the system to characterize CLL phenotype during the response to therapies. Moreover, this set-up is adaptable for other applications by customizing both microenvironment and the endothelial barrier, potentially enabling the study of other malignancies.

**Keywords:** CLL trafficking; vascularized microenvironment; ex vivo modelling

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**Please indicate the name of the funding organization:** EHA. AIRC.

## Abstract ID: 1520

**Title:** Unveiling the journey of chronic lymphocytic leukemia patients in Portugal: insights into the Rituximab-Venetoclax protocol

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**Introduction and methods:** Rituximab-Venetoclax (RV) has become the standard of care for relapsed/refractory Chronic Lymphocytic Leukemia (CLL), following its demonstrated superiority over chemoimmunotherapy (CIT) in the phase III MURANO trial. This has led to the approval of this combination in Europe in 2018 for patients with previously treated CLL. Robust population-level data reflecting routine clinical practice is lacking. Therefore, on behalf of the Portuguese Lymphoma Group (GPL), we conducted a multicenter retrospective analysis across 12 Portuguese centers, including patients treated with RV from 2019 to 2023, with a data cut off on 30 April 2025. The primary endpoints were progression-free survival (PFS) and overall survival (OS). As secondary endpoints we report time to next treatment or death (TTNTD), response rates, feasibility and safety outcomes, assessed per iwCLL criteria.

**Results:** A total of 156 patients was included, with a median age of 70 years (IQR: 62.3–75); 61.5% were male and 93.9% had ECOG ≤1. Unfavorable genetic features were observed in a substantial subset, with mutated TP53 and/or del(17p) in 30.1%. Most patients had previously received CIT (85.3%) and 30.1% had already been exposed to BTK inhibitors (BTKi). Indications for treatment initiation included bone marrow failure (48.4%), lymphocytosis (49.0%), lymphadenopathy (54.2%), and B symptoms (23.1%). Ninety-two patients (58.9%) underwent venetoclax ramp-up entirely in the outpatient setting, with a median of 6 appointments during this period. Although tumor lysis syndrome (TLS) risk estimates were intermediate or high in 71% of the patients, laboratory and clinical TLS were observed in only 12.3% and 3.2%, respectively. At the time of analysis, 20 patients remained on treatment, while 70 had completed the 24-month protocol. Permanent early discontinuation occurred in 66 pts due to disease progression (30.3%), toxicity (33.3%), death (30.3%) or other causes (7.5%). Hematologic toxicity was the most frequently observed adverse event (AE), with grade ≥3 cytopenias in 59% of patients: neutropenia (51.6%), thrombocytopenia (20.5%), and anemia (10.9%). Febrile neutropenia was reported in 16.7%, and recurrent infections in 17.9%, most of which occurred during or following the venetoclax monotherapy phase. Grade ≥3 gastrointestinal toxicity was observed in 5.1%. Temporary venetoclax interruptions (≥14 days) were required in 28.2% of patients, predominantly due to AE. The overall response rate was 86.5%, with 68.6% achieving complete responses. For those who completed the 24-month treatment protocol (n = 70), response was assessed in 60 patients with 86.7% presenting complete response. Disease progression (PD) was documented in 26.3%, 9 of them with Richter's transformation upon PD. With a median follow-up of 28.5 months, the median OS was not reached, while median PFS and TTNTD were 44.6 and 45.5 months, respectively. No baseline clinical or biological features, including known high-risk factors, were found to significantly impact response rates or PFS. OS was impacted by prior exposure to BKTi (46.4 months vs NR; $p = 0.015$). The subgroup analysis of these patients (of which 57% presented del17p/TP53) also revealed significantly inferior TTNTD (34.7 vs 47.8 months; $p = 0.005$), with no difference on PFS (33.6 vs 47.9 months; $p = 0.081$). Deaths were registered in 26.9% - 39% due to PD, 19.5% due to toxicity, 19.5% due to causes non CLL or treatment related and 19.5% due to unknown causes.

**Conclusions:** In this real-world cohort, the RV regimen yielded high overall and complete response rates, with no apparent impact of established high-risk features on response or PFS. Although prior BTKi exposure did not affect response rates, it was associated with inferior OS, underscoring the need for further investigation into optimal sequencing strategies for this subgroup. Additionally, our findings support the feasibility of outpatient venetoclax ramp-up, with no compromise in safety or efficacy.

**Keywords:** CLL; relapsed/refractory

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**Please indicate the name of the funding organization:**

## Abstract ID: 1422

### **Title:** Risk of acute kidney injury and cytopenias during treatment of chronic lymphocytic leukemia in a nationwide real-world cohort

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**Introduction:** Adverse events (AEs) from treatment of chronic lymphocytic leukemia (CLL) affects quality of life, treatment adherence, and survival. Although targeted therapies have improved prognosis, AEs such as cytopenias and renal impairment remain common. Most evidence on AE risk stems from clinical trials, which often exclude older, frailer and more comorbid patients making findings unrepresentative of routine care populations. We present real-world data on predictors of specific AEs from a nationwide cohort study using comprehensive Danish register data, aiming to support a more personalized and safer approach to CLL treatment.

**Methods:** We conducted a nationwide cohort study using the Danish Lymphoid Cancer Research (DALY-CARE) data resource, which integrates national clinical registries,

administrative databases, and electronic health records. Patients diagnosed with CLL or small lymphocytic lymphoma (SLL) receiving first-line treatment registered in the Danish National CLL Registry (DCLLR) or the Danish National Lymphoma Registry (LYFO) were included. AEs were assessed for 6 months from treatment initiation using Common Terminology Criteria for Adverse Events system version 5. Included AEs were grade ≥3 anemia, thrombocytopenia, leukopenia (neutropenia and lymphopenia), and grade ≥2 acute kidney injury (AKI), using increased creatinine levels as a proxy. Absolute values for laboratory measurements were included without reference to baseline values. Laboratory data were retrieved from the Clinical Laboratory Information System Database. Descriptive statistics were used to characterize the cohort. Cumulative risk of AEs was estimated using Kaplan-Meier curves, censoring at death (133 patients) or at 180 days of follow-up. Associations between patient characteristics and AEs were analyzed using Cox proportional hazards models.

**Results:** We included 2,646 patients diagnosed with CLL (n = 2,355) or SLL (n = 291) between 2005 and 2023, who initiated first-line treatment. 2,531 patients had data on AEs. Median age was 69 years (range 22–98), and 65% were male. Median time from diagnosis to treatment was 1.1 years. The most common first-line treatment was bendamustine-based (25%), followed by chlorambucil-based (24%), fludarabine-based (17%), CD20 antibody monotherapy (13%) (likely for AIHA/ITP), venetoclax (8%), BTK-inhibitor (6%), and ibrutinib-venetoclax (1%). Other treatments were used in 7%, and 3% had unknown treatment types. Within 6 months of treatment initiation, 52% of females and 57% of males experienced at least on AE. AE occurrence varied by age: < 60 years 54%, 60–69 years 58%, 70–79 years 57%, and ≥80 years 49%. The estimated cumulative risk of AE was highest for patients treated with bendamustine (76%) and venetoclax (72%) and lowest with BTK inhibitors (40%). The most common AEs were lymphopenia (44%), neutropenia (42%), and leukopenia (31%), while anemia (20%), thrombocytopenia (15%), and AKI (14%) were less frequent. In univariable Cox regression analyses, older age was associated with increased risk of anemia, with hazard ratios of 1.51 (95% CI: 1.05–2.19) for patients aged 60–69 years, 1.61 [1.13–2.30] for 70–79 years, and 1.87 [1.29–2.73] for ≥80 years compared with patients <60 years. Risk of AKI also rose with age (2.71 [1.42–5.17] for 60–69 years, 4.15 [2.24–7.70] for 70–79 years, and 7.82 [4.21–14.55] for ≥80 years). Conversely, age ≥80 years was associated with reduced risk of lymphopenia (0.47 [0.37–0.61]), neutropenia (0.62 [0.49–0.79]), and leukopenia (0.43 [0.32–0.58]). Males had a significantly higher risk of thrombocytopenia (1.35 [1.05–1.73]) and AKI (1.29 [1.00–1.67]) compared with females. No significant sex differences were found for other AEs. Patients treated with venetoclax alone or in combination with BTK-inhibitors had a higher risk of neutropenia (2.72 [1.84–4.01]), lymphopenia (9.57 [3.85–23.80]) and leukopenia (5.73 [2.73–12.03]) compared to those receiving BTK-inhibitors alone. No significant differences were observed for anemia ($p = 0.92$), thrombocytopenia ($p = 0.20$), or AKI ($p = 0.06$).

**Conclusion:** Over half of CLL and SLL patients in routine care experienced an AE within 6 months of first-line treatment. AE risk varied by treatment type, age, and sex. Males had higher risks of thrombocytopenia and AKI. AEs were most frequent with bendamustine or venetoclax, and least frequent with BTK inhibitors and chlorambucil. These findings highlight the importance of patient characteristics when selecting and managing CLL treatment. Future analyses will include cardiovascular events and infections to provide a more comprehensive understanding of age- and treatment-related AE patterns.

**Keywords:** CLL; adverse events; first-line treatment

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## Abstract ID: 1521

### **Title:** Real-world efficacy and safety of venetoclax +/- rituximab in >75-years old patients or with cardiovascular comorbidities and R/R chronic lymphocytic leukemia: interim results of the VERONE study

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**Background:** The efficacy and safety of venetoclax (ven) monotherapy or in combination with anti-CD20 antibodies have been demonstrated in several clinical trials in 1L and R/R in chronic lymphocytic leukemia (CLL). Yet, because elderly/highly comorbid patients are often underrepresented in registration studies, we need real-world cohorts to investigate whether specific sub-groups do benefit from ven-based regimens. BTKis are not considered a reliable option in elderly patients, especially with cardiac comorbidities, but we lack data supporting the safe use of venetoclax.

**Aims:** This French observational ongoing study (VERONE) aims to evaluate the efficacy and safety of ven monotherapy or in combination with rituximab (VenR), in R/R patients from routine clinical practice. This analysis focuses on treatment outcomes in <75 and ≥75-year-old relapsed refractory (R/R) CLL patients in a real-world setting.

**Methods:** VERONE is a prospective non-interventional study conducted in 367 adult R/R CLL patients having started Ven+/-R with included in 64 French centers with a planned follow-up period of 48 months.

**Results:** Between March 2018 and June 2022, 356 patients (including 236 Ven; 104 VenR and 16 non-rituximab combinations) met selection criteria (analysis population). The characteristics of the Ven and VenR patients (n = 340) were, respectively: median age 74.0 and 73.0 years; male gender: 69.1% and 65.4%, ECOG ≥2 15.6% and 14.8%, TP53 and/or del(17)p in 40.6% (86/212) and 41.1% (39/95), median prior lines of therapy 2 and 1, prior treatment with ibrutinib in 58.5% and 41.3% of patients. With a median follow-up of 33.6 and 36.6 months, median [95% CI] PFS was 33.3 [29.7–40.6] months for Ven and 34.8 [33.2-NR] months for VenR cohorts, respectively. Among Ven cohort,111 (47%) patients were ≥75-year-old, 165 (69.9%) had ≥2 prior lines of

treatment, 59 (28.8%) had atrial fibrillation (AF) or heart failure, 81 (39.5%) had hypertension (HT) and 139 (81.3%) had ≥5 concomitant medications. ORR [95% CI] was 93.3% [86.21;96.91] for patients <75 years and 92.9 [85.28;96.69] for patients ≥75 years. Median PFS were 31.1 months [28.02;40.64] for patients <75 years and 34.1 months [29.73 -.] for patients ≥75 years (log-rank $p=0.14$). In evaluable patients with HT (n=64) and AF or heart failure (n=46), median PFS was 39.8 months and 42.3 months [29.60;], respectively. Among VenR cohort, 40 (38.5%) patients were ≥75 years old and 46 (44.3%) had ≥2 prior lines of treatment. 21 patients (22.3%) had AF or heart failure, 44 (46.8%) had HT and 85 (91.4%) had ≥5 concomitant medications. ORR [95% CI] was 94.4% [84.89;98.09] for patients <75 years and 92.3% [79.68;97.35] for patients ≥75 years. The median PFS was not reached for patients <75 years and 33.2 months [24.34;.] for patients ≥75-year-old (log-rank, $p=0.06$). In evaluable patients with HT (n=41) and AF or heart failure (n=20), median PFS was 34.1 months and not reached, respectively. In safety population, 218 Ven patients (91.6%) and 104 VenR patients (100%) completed the ramp-up period; 187 (78.6%) and 95 (91.3%) reached the recommended daily dose of 400 mg at the time of analysis, respectively. Overall, 91.6% and 96.2% of patients experienced adverse events (AEs) in Ven and VenR cohorts, respectively (SAE =52.9% and 53.8% of patients (including 3 fatal events all in Ven patients: deterioration of the general status, ascites and hepatic failure, death with no precision)). Over the ramp-up period, 8.8% of Ven patients and 9.6% of VenR patients experienced tumor lysis syndrome which led to VEN discontinuation in 1.8% and 1.1% of patients. No new safety signals have been identified.

**Conclusion:** Our real-world data confirm the efficacy of VEN regimens in <75 and ≥75-years old non-selected pre-treated CLL patients. Ven-based regimens seem a good option for pts >75 years based on our analyses, with very similar PFS rates with or without RTX. In addition, our data emphasized that Ven-based regimens are effective and safe in patients with cardiac comorbidities, otherwise deemed ineligible to BTKi.

**Keywords:** CLL; relapsed/refractory; venetoclax; rituximab; elderly

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## Abstract ID: 1108

**Title:** CLL10000: robust classification of 1172 ATM variants employing a five-factor score derived from matched non-tumor controls

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Mutations in ATM, an essential gene for DNA damage repair, are common in CLL but difficult to assess. Due to a dispersion across a large gene (63 exons, coding region >9,000 bp) without hotspots and mostly missense mutations it is difficult to distinguish mutations from rare, non-pathogenic congenital variants, and thus to investigate their role in the pathogenesis and prognostic significance of chronic lymphocytic leukemia (CLL). To assess the incidence and co-factors of ATM mutations we screened 2,829 untreated patients from five different CLL trials via Next Generation Sequencing (NGS). In total, we identified 1,172 variants, of which 607 were unique, in 877 patients. Of these, 34.8% were truncating, 60.5% were nonsynonymous single-nucleotide variants (SNVs), and 4.8% were non-frameshift insertions or deletions. Variant pathogenicity assessment employed standardized frameworks, including guidelines from the Association for Molecular Pathology (AMP) (Li et al., 2017) and the ClinGen/CGC/VICC consortium (Horak et al., 2022), to enable systematic evaluation. However, applying these gene-agnostic scores, a significant subset of variants – approximately 70% by AMP and 30% by ClinGen/CGC/VICC - were classified as variants of uncertain significance (VUS). Based on the hypothesis that pathogenic ATM driver variants in CLL are tumor-specific, i.e. somatically acquired, and therefore absent from the germline, we sequenced matched non-tumor samples from 203 patients with ATM variants confirming 212 of the variants found in them as somatic/pathogenic and 87 as germline/benign. Factors associated with a somatic origin were amino acid position (AA >1898 corresponding to FAT/KD/FATC domain), mutation type (truncating), VAF adjusted for del(11q) (either at <45% or 55–90%), gnomAD allele frequency (< 1e-5), and ClinVar annotation (not listed or at least 1 classification as pathogenic); each showed strong concordance with their respective odds ratios (OR 5.6, 11.3, 12.4, 28.4, and 386.7), whereas listing of variants in the COSMIC database was not associated with somatic status. Therefore, we developed a weighted five-factor score based on the respective lnOR, ranging from 0 to 16. Variants scoring above the threshold of 7 were classified as somatic/pathogenic, while those below were considered germline/benign; importantly, the score was specifically designed to exclude uncertain significance (VUS) statuses due to their limited utility for statistical analysis in large cohorts. Our score classified 284 of 299 variants (95.0%) in concordance with their somatic status as determined by comparison to germline. Overall, 15 variants (5.0%) were discordant with germline-matching results. However, since some variants that classified as somatic but were found in the germline have been previously reported in ataxia-telangiectasia (G1003T, G5932T), this finding may indicate a rare heterozygous A-T carrier status rather than a misclassification. Compared to the AMP guidelines (applied to our 299 variants: 27.4% concordance, 1.0% misclassification, 71.6% uncertain) and the ClinGen/CGC/VICC guidelines (64.9% concordance, 5.4%

misclassification, 29.8% uncertain), our scoring system achieved a higher concordance rate (95%) and more accurately distinguished somatic from germline variants while minimizing misclassifications. When applied to all 1,172 ATM mutations, 42.7% were classified as somatic/pathogenic by our score, including 38.8% nonsense, 56.9% missense, and 4.4% non-frameshift indel mutations. Among the 434 patients with somatic ATM mutations according to our scoring system, 73.7% exhibited an unmutated immunoglobulin heavy-chain variable (IGHV) status, 46.3% had a deletion at 11q22 [del(11q22)], and only 1.4% presented with a deletion at 17p13 [del(17p13)]. In contrast, among the 443 patients carrying exclusively ATM variants that were classified as germline/benign, 56.0% displayed an unmutated IGHV status, 15.6% harbored del(11q22), and 3.4% exhibited del(17p13). We herein present a scoring system that reliably identifies pathogenic ATM variants and may facilitate the investigation of their prognostic impact in CLL across large cohorts. Independent validation will be performed in an additional patient cohort with matched non-tumor controls. In parallel, efforts will be made to complete the genetic landscape of ATM mutations and to assess the co-occurrence of other relevant markers. Associations with specific clinical and molecular factors will also be investigated.

**Keywords:** CLL; ATM; biomarker; genetics

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## Abstract ID: 1212

### **Title:** High-dimensional profiling of CLL and T cell interactions during early phase of disease using a 42-color full-spectrum cytometry panel

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**Background:** CLL cells behave as regulatory B cells, altering the functionality and proportion of immune subsets, especially T cells (Mékinian et al., 2023). T lymphocytes from CLL patients exhibit dysregulated expression of activation molecules and increased expression of exhaustion markers (Riches et al., 2013). Previous evidence from our group suggests that immune dysfunction, particularly T cell exhaustion, is increased at disease progression (Jiménez et al., 2021). Therefore, we hypothesized that progressive immune dysfunction during the first months after diagnosis can identify patients at higher risk of early progression. To assess that, we longitudinally analyzed patient samples at diagnosis and 6 months later to correlate immune dysfunction with progression risk.

**Methodology:** Cryopreserved peripheral blood mononuclear cells were obtained from 34 CLL patients at diagnosis and 6 months later. Patients were classified as progressors or non-progressors. After thawing, 1x107 cells were stained for the detection of surface and intracellular markers using an in-house developed 42-color full-spectrum cytometry panel. Samples were acquired on a 5-laser Cytek Aurora cytometer and analyzed with OMIQ software.

**Results:** After a median follow-up of 99 months, the median time to progression was 79 months for progressors. The proportion of main immune populations (B, T, and NK cells) remained stable at 6 months compared to diagnosis, except for a decrease in monocytes, regardless of patient progression ($p=0.0069$). Similarly, the expression of chemokine receptors (CXCR3, CXCR4, CXCR5, CCR6, CCR7) and poor prognosis markers (CD38, CD49d, Ki67, ZAP-70) in CLL cells remained constant. The frequency of naïve CD4+ T cells was significantly reduced at 6 months from diagnosis ($p=0.0075$). In addition, CD4+ T cells showed higher levels of TIGIT ($p=0.0151$) and CD244 ($p=0.0163$) over time. On the contrary, the proportion of CD8+ subsets and the expression of activation and exhaustion markers were unaltered throughout the evaluated period, as well as for NKT cells. When comparing timepoints, an increased CD4:CD8 ratio was observed in non-progressors over time ($p=0.0488$), while it remained constant for progressors. CD4+ T cell subsets frequency and the expression of activation and exhaustion markers within the CD4+ compartment was also stable regardless of disease status. The proportion of CD8+ T cell subsets was unaltered in non-progressors, while in patients who progressed increased effector memory CD8+ T cells were observed ($p=0.0137$). Additionally, CD8+ T cells from non-progressors showed lower levels of CD244 at 6 months compared to diagnosis ($p=0.0156$). However, the expression of activation and exhaustion markers in the CD8+ population remained constant in progressors. The frequency of NKT cells and the expression of activation and exhaustion markers within this compartment did not fluctuate over time across patient groups. To capture T-cell heterogeneity, we followed an unsupervised approach involving dimensionality reduction (UMAP), FlowSOM clustering, annotation, and statistical analysis of resulting clusters using edgeR for differential abundance testing. A total of 28 metaclusters (mc) were identified in CD4+, CD8+ and NKT cells. We observed stable cluster sizes over time within the aforementioned cell populations in all patient samples. At diagnosis, we detected an increased size of mc16 (FDR =0.0191) in CD8+ T cells from patients who did not progress compared to patients that progressed. This metacluster is enriched in terminally differentiated effector memory cells expressing CD127, CD244, CD49d and CD95, indicating a cytotoxic, partially exhausted but with a potentially active phenotype. Additionally, NKT cells from progressors showed an increased abundance of mc24 (FDR =0.0434) at diagnosis. In this case, mc24 is enriched in unconventional CD8+ terminally differentiated effector memory cells expressing DNAM-1, TIGIT, CD244, CD49d and CD95. This suggests chronic activation alongside functional exhaustion, as indicated by TIGIT and CD244 co-expression. Comparing samples obtained at 6 months, CD8+ T cells exhibited higher counts of mc14 (FDR =0.0136), and mc16 (FDR =0.0112) in

non-progressors, as observed at diagnosis for mc16. The detected mc14 increase in size in patients that did not progress is characterized by the expression of Ki67, HLA-DR, DNAM-1, CD49d and CD95, indicating highly activated and potentially cytotoxic proliferative cells. Finally, no significant clusters were observed in NKT cells at 6 months. Considering the phenotype of significant metaclusters, those over-represented in progressors reflect an exhausted profile, whereas in non-progressors, they exhibit activation features. However, ongoing analyses are required to elucidate potential immune changes occurring early after CLL diagnosis that may help predict disease progression.

**Keywords:** Tumor microenvironment; T-cell exhaustion; full-spectrum cytometry

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## Abstract ID: 1522

### **Title:** MAVRiC: a phase 2 study of mutation-guided finite-duration acalabrutinib plus venetoclax for relapse after frontline finite-duration covalent Bruton tyrosine kinase inhibitor plus venetoclax–based combination therapy in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma

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**Introduction:** Fixed-duration treatment with a covalent Bruton tyrosine kinase inhibitor (cBTKi) and a B-cell lymphoma 2 inhibitor (BCL-2i) is now a first-line (1L) treatment option for patients with chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). CLL is a persistent disease for which a definitive cure remains elusive. Despite advancements in novel combination targeted therapies, a significant proportion of patients experience disease progression following fixed-duration frontline treatment, necessitating subsequent lines of therapy or retreatment. Furthermore, as seen in prior studies of cBTKi plus BCL-2i, patients with unmutated IGHV were more likely to achieve undetectable minimal residual disease (uMRD) compared with those with mutated IGHV yet were also more likely to relapse sooner. Whether longer treatment duration for patients with higher genetic-risk CLL may lead to longer remissions remains unclear. More prospective data in a clinical trial setting are needed to better understand whether patients can be effectively retreated with finite-duration (total 14 cycles or 24 cycles) cBTKi + BCL-2i at relapse, as the only published data are derived from a small cohort of patients. Fixed-duration (total 14 cycles) combination therapy with the highly selective cBTKi acalabrutinib (A) plus V ± obinutuzumab (O) was found to improve progression-free survival (PFS) compared with chemoimmunotherapy in the phase 3 AMPLIFY trial in patients with TN CLL (Brown JR, et al. NEJM. 2025). The efficacy of AV-based retreatment in patients with CLL who relapse after BTKi + BCL-2i 1L therapy has not been evaluated previously.

**Methods:** This phase 2, open-label, multicenter, single-arm, global study (MAVRiC: Mutation-guided finite-duration AV for Relapse in CLL/SLL) aims to assess the efficacy and safety of IGHV- and TP53-mutation risk–guided, finite-duration AV after prior fixed-duration BTKi + BCL-2i treatment. Eligible patients are aged ≥18 y with a diagnosis of CLL/SLL who require treatment according to International Workshop on CLL (iwCLL) 2018 criteria, had prior 1L treatment with finite cBTKi + BCL-2i ± O, and had a partial response or better maintained for ≥2 y after treatment completion with subsequent relapse. Key exclusion criteria include clinically significant cardiovascular disease within 6 months of enrollment, active central nervous system involvement, Richter transformation, active significant infection, and creatinine clearance of <30 ml/min according to the Cockroft-Gault equation or serum creatinine >2 times the upper limit of normal. Patients will receive AV (A 100 mg oral twice daily [BID] and V 400 mg oral once daily [QD]) in 28-day cycles (C); lead-in A 100 mg BID will be given in C1–2, and V dosing will begin in C3 with the label-prescribed 5-week ramp-up dosing up to 400 mg QD. IGHV and TP53 mutational status will then guide AV duration. Cohort 1 will include patients with lower-risk CLL/SLL (those with mutated IGHV and no TP53 aberrations) treated with AV for C3–14; cohort 2 will include patients with higher-risk CLL/SLL (those with unmutated IGHV and/or TP53 aberrations) treated with AV for C3–24. The primary endpoint is overall response rate per iwCLL 2018 criteria after 12 cycles of combination AV. Key secondary endpoints include PFS, duration of response, event-free survival, time to next treatment, overall survival, safety/tolerability (including tumor lysis syndrome), and rate of undetectable minimal residual disease (< $10^{-5}$) in peripheral blood at 3 months after end of treatment with clonoSEQ. Patients will be followed for 5 years from C1 day 1 until death, withdrawal of consent, or end of study. Global enrollment is planned for 80 patients to begin in 2025 at 25 sites.

**Keywords:** Chronic lymphocytic leukemia/small lymphocytic lymphoma; Bruton tyrosine kinase inhibitor; retreatment

**Please indicate how this research was funded:** Study funded by AstraZeneca

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## Abstract ID: 1423

### **Title:** MRD-driven venetoclax and rituximab combination in previously untreated chronic lymphocytic leukemia: results of the prospective, multicenter, phase II trial of the Polish Adult Leukemia Group (VERITA PALG-CLL5)

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**Introduction:** Fixed-duration regimens combining a selective BCL-2 inhibitor, venetoclax, with an anti-CD20 antibody (rituximab or obinutuzumab) are the mainstay of current management of chronic lymphocytic leukemia (CLL). However, it is unknown whether the same duration of treatment is optimal for all patients. Clinical trials have shown that achieving undetectable measurable residual disease (MRD) with venetoclax-based regimens is associated with superior long-term outcomes in CLL. It can be therefore hypothesized that adjusting the duration of therapy to MRD status may provide an optimal balance between treatment activity and toxicity.

**Methods:** This prospective, multicenter, phase II study aimed to assess the efficacy and safety of MRD-driven combination of venetoclax and rituximab (VR) in treatment-naïve CLL. Following the standard venetoclax ramp-up phase, patients received venetoclax 400 mg orally once daily, combined with rituximab 375 mg/m$^2$ during the first infusion, then 500 mg/m$^2$ intravenously every 4 weeks during Cycles 1–6, and every 8 weeks thereafter. Treatment duration was determined based on depth of response, with VR administered for 12, 18, or up to 24 cycles. For patients who achieved a complete remission (CR) with bone marrow MRD $<10^{-4}$ (MRD-) by flow cytometry at the response assessments at Cycle 12 or Cycle 18, therapy was discontinued. The primary objective of the study was to demonstrate that this strategy leads to a 35% CR MRD- rate. Here, we present the results of a preplanned interim analysis performed after all study participants had responses assessed at Cycle 12.

**Results:** Between February 2022 and May 2023, all planned 103 patients were recruited in 10 Polish Adult Leukemia Group centers. The patients' median age was 66 years (range 38–85), and 56 (54%) of them were male. Del17p or TP53 mutation were present in 13 (13%) patients. Unmutated IGHV was found in 53 (51%) patients, mutated IGHV in 41 (40%), while IGHV status was undetermined in 9 patients. Tumor lysis syndrome (TLS) risk was high in 38% and medium in 51% of patients. The median study follow-up time was 27.3 months (1.2–37.1). Response to treatment was assessed in 101 patients, as two were withdrawn early (ramp-up and Cycle 1) due to complications (prolonged COVID-19 and autoimmune hemolytic anemia (AIHA) with sepsis) and followed only for survival. The study met its primary endpoint with CR with bone marrow MRD- achieved by 60 (58%) study participants, while three patients continued the therapy. At the assessment at Cycle 12, the overall response rate (ORR) was 98%, including 45 (44%) CR MRD-, 4 (4%) CR MRD+, 39 (39%) partial remission (PR) MRD-, 11 (11%) PR MRD+, 1 (1%) stable disease and 1 (1%) progressive disease. Notably, 9 (17.6%) patients who continued therapy to Cycle 18, and additional 6 (16.2%) patients who continued therapy to Cycle 24, achieved CR MRD- response. During the follow-up period, three patients experienced disease progression. Four deaths were recorded, all in patients maintaining CR MRD- response (reason of death: stroke, other primary malignancy, suicide, and one unknown). The estimated 24-month progression-free survival (PFS) was 95.9% (95% CI 92.1–99.9%), and overall survival (OS) was 98.0% (95% CI 95.3–100.0%). Treatment was generally well tolerated. Hematological toxicity was the most prominent, with neutropenia of all grades (G) comprising 38% of adverse events (AEs). Grade 3/4 AEs were noted in 72 (69.9%) patients including G3/4 neutropenia observed in 66 (64.1%) study participants. However, G 3/4 infections were reported only in 7 (6.8%) patients. Other AEs of interest included 5 cases of laboratory TLS, 3 AIHA, and 2 second primary malignancies.

**Conclusions:** The results of the VERITA PALG-CLL5 study indicate high efficacy of MRD-driven venetoclax plus rituximab combination in treatment-naïve CLL. Extending treatment beyond 12 cycles in patients with suboptimal response was safe and led to the achievement of CR MRD- response in a significant proportion of patients.

**Keywords:** Chronic lymphocytic leukemia; venetoclax; measurable residual disease

**Please indicate how this research was funded:** The study has received financial support from pharmaceutical company.

**Please indicate the name of the funding organization:** AbbVie Inc.

## Abstract ID: 1424

**Title:** Safety analysis of fixed-duration acalabrutinib-venetoclax combinations vs chemoimmunotherapy: a post hoc analysis from the phase 3 AMPLIFY trial

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**Introduction:** The phase 3 AMPLIFY trial evaluated fixed-duration acalabrutinib + venetoclax (AV) or AV + obinutuzumab (AVO) versus fludarabine + cyclophosphamide + rituximab/bendamustine + rituximab (FCR/BR) in fit patients with treatment-naive (TN) chronic lymphocytic leukemia (CLL), where AV and AVO demonstrated prolonged progression-free survival versus FCR/BR and manageable safety profiles (Brown et al. NEJM. 2025). This post hoc analysis from AMPLIFY assesses the safety and tolerability profiles of fixed-duration AV, AVO, and FCR/BR.

**Methods:** AMPLIFY (ACE-CL-311; NCT03836261) is an ongoing, randomized, open-label, phase 3 trial in patients with TN CLL aged ≥18 years with ECOG performance status ≤2 and without del(17p) or TP53 mutation. Patients were randomized 1:1:1 to receive AV (acalabrutinib, cycles [C] 1–14; venetoclax, C3–14), AVO (AV as described; obinutuzumab, C2 − 7), or investigator's choice of chemoimmunotherapy (FCR/BR, C1 − 6). Exposure-adjusted event rates (EAERs; events/100 person-months) were calculated for treatment-emergent adverse events (AEs) of clinical interest (ECI), including cardiac events, neutropenia, hypertension, infections, and tumor lysis syndrome (TLS). Cumulative incidences of cardiac events and infections were analyzed by Kaplan-Meier analysis (descriptive *p* values). TLS risk at baseline and C3 (post-debulking/pre-venetoclax) was evaluated (high risk = lymph node [LN] ≥ 10 cm, or absolute lymphocyte count [ALC] ≥ 25 × 109/L and LN ≥5 cm; medium risk = LN ≥5–< 10 cm or ALC ≥25 × 109/L; low risk = LN< 5 cm and ALC <25 × 109/L). Time and cause of early treatment discontinuation or death (combination treatment or any treatment in the regimen) were analyzed; patients who completed treatment were censored.

**Results:** 867 patients were randomized and 834 received ≥1 dose of study drug (AV, n = 291; AVO, n = 284; FCR/BR, n = 259) and were included in this analysis. Median (range) duration of acalabrutinib exposure was 12.9 months (1–18) in AV and 12.9 months (0–18) in AVO; treatment exposure was 5.6 months (1–11) in FCR/BR. Incidence of any-grade ECIs overall was similar with AV (76.3%) versus FCR/BR (71.4%; $p = 0.229$) and higher with AVO (85.2%; $p < 0.0001$). However, considering exposure duration, EAERs were lower with AV (25.3) and AVO (36.1) versus FCR/BR (57.8). Similarly, cardiac event incidence was higher with AV (9.3%) and AVO (12.0%) versus FCR/BR (3.5%; $p \leq 0.006$, both comparisons), whereas EAERs were similar across arms (0.83, 1.11, and 0.86, respectively); any-grade atrial fibrillation, ventricular tachyarrhythmias, and hypertension EAERs were similar across arms (AV, 0.05, 0.05, 0.30; AVO, 0.16, 0.08, 0.32; FCR/BR, 0.14, 0, 0.57, respectively). Any-grade neutropenia EAERs were lower for AV (5.4) and AVO (8.0) versus FCR/BR (17.2). Any-grade infection incidences were higher with AV (50.9%) and AVO (53.9%) versus FCR/BR (31.7%; $p < 0.0001$, both comparisons), whereas EAERs were similar across arms (6.47, 8.38, and 8.77, respectively). Cumulative infection incidence was similar for AV and AVO versus FCR/BR (HR 0.75, 95% CI 0.56–1.01 and HR 0.84, 95% CI 0.63–1.12, respectively). Infection incidences of any grade (grade ≥3) by pathogen in the AV, AVO, and FCR/BR arms were 8.6% (1.0%), 8.1% (1.4%), and 3.1% (1.2%) (bacterial); 28.2% (8.2%), 29.6% (16.5%), and 10.0% (4.2%) (viral); 2.4% (0.3%), 4.6% (1.4%), and 4.2% (0%) (fungal); and 27.5% (3.8%), 31.3% (6.7%), and 20.5% (4.6%) (unspecified), respectively. Any confirmed/suspected COVID-19 AE was reported in 64 (22.0%) patients in AV, 69 (24.3%) in AVO, and 10 (3.9%) in FCR/BR. Discontinuation of combination treatment or death occurred in 8.4% (AV), 5.1% (AVO), and 18.6% (FCR/BR); time-to-event (discontinuation or death) analysis favored AV and AVO versus FCR/BR (HR 0.07, 95% CI 0.03–0.15; $p < 0.0001$; HR 0.16, 95% CI 0.07–0.30; $p < 0.0001$, respectively). AEs were the most common reason for any treatment discontinuation (AV, 7.0%; AVO, 19.6%; FCR/BR, 10.5%), mainly related to COVID-19 for AV and AVO, and cytopenias for FCR/BR. Among patients with high TLS risk at baseline (AV, n = 93; AVO, n = 75; FCR/BR, n = 86), most had medium (60.2%, 21.3%, 8.1%) or low (19.4%, 61.3%, 77.9%) TLS risk at C3. Thus, over 90% at high risk at baseline transitioned to low/medium risk by C3. TLS observed in AV (n = 1; during venetoclax ramp-up) and AVO (n = 1; during acalabrutinib lead-in) was limited to laboratory TLS only.

**Conclusion:** This safety analysis from AMPLIFY showed no new safety signals with fixed-duration AV and AVO. Infection incidence was similar to previous reports and cardiac event rates were similar in the AV and AVO arms versus FCR/BR. Over 90% of patients at high TLS risk at baseline transitioned to low/medium risk with initial tumor debulking with 2 cycles of acalabrutinib lead-in.

**Keywords:** Chronic lymphocytic leukemia; safety; fixed duration

**Please indicate how this research was funded:** Study funded by AstraZeneca

**Please indicate the name of the funding organization:** AstraZeneca

## Abstract ID: 1401

### **Title:** Risk of second primary malignancies in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) receiving first-line therapy: a real-world study

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**Background:** Patients with CLL/SLL are at increased risk of developing second primary malignancies (SPM) due to underlying immune dysfunction, individual risk, and the

effects of treatment. Covalent Bruton tyrosine kinase inhibitors (cBTKi) have become widely used in the first-line setting for treatment of CLL/SLL. Therefore, it is important to understand how first-line treatment strategies influence the incidence of SPM. Our study aimed to evaluate the real-world incidence of SPM in patients treated with BTKi or chemo-immunotherapy (CIT) and to compare these rates with those in patients managed with observation.

**Methods:** Between January 2019 and December 2024, patients with newly diagnosed CLL/SLL were identified using the Symphony Health Solutions database. Patients were categorized into three cohorts based on clinical management: (1) patients receiving first-line treatment with cBTKi monotherapy (ibrutinib, acalabrutinib, or zanubrutinib); (2) patients receiving first-line treatment with CIT; and (3) patients managed with observation. The index date was defined as the date of treatment initiation or date of diagnosis for the observation group. Outcomes included the 24-month incidence of SPM (including non-melanoma skin cancer (NMSC)) based on ICD-10-CM diagnosis codes. Baseline demographics, including age, gender, and race/ethnicity were compared across cohorts.

**Results:** A total of 112,966 patients were included in this analysis. 15,565 patients received cBTKi as initial therapy; 15,463 patients received CIT as initial therapy; and 81,938 patients remained on observation. Mean age across cohorts was 68–69 years; approximately 55–59% were male. The 24-month incidence of SPM was 12.0% in patients treated with cBTKi, 14.9% in patients treated with CIT, and 13.7% in patients that remained on observation. The incidence of solid tumors excluding NMSC was 7.7% (cBTKi cohort), 10.5% (CIT cohort), and 9.8% (observation cohort). The incidence of skin malignancies was reviewed and NMSC were observed in 5.0% of patients treated with cBTKi, 5.3% of patients treated with CIT, and 4.8% of patients that remained on observation, the incidence of melanoma was in 0.6% in the cBTKi cohort, 0.7% in the CIT cohort, and 0.7% in the observation cohort. Compared to patients that remained on observation, SPM were less frequent with cBTKi (OR =0.86, 95% CI: 0.82–0.91, $p<0.001$) and more frequent with CIT (OR =1.10, 95% CI: 1.05–1.16, $p<0.001$).

**Conclusions:** This analysis of real-world patients highlights a higher incidence of SPM in patients treated with CIT compared to patients of observation or those treated with cBTKi. No differences in the incidence of cutaneous malignancies including NSMC and melanoma were observed between patients treated with CIT and patients on observation and between patients treated with BTKi and patients on observation. Although the observation time of 24 months is short and a longer follow-up is planned, these findings emphasize the importance of ongoing cancer surveillance in patients with CLL/SLL and highlight the need for prospective studies to further explore the relationship between treatment modality and SPM.

**Keywords:** Chemo-immunotherapy; cBTKi; second primary malignancies

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** BeOne Medicines Ltd

## Abstract ID: 1425

**Title:** IGLV3-21R110 as a prognostic marker for early stage CLL patients – clinical implications in patients treated with BTK inhibitors and diagnostic challenges

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**Introduction:** IGLV3-21R110 is a point mutation in light chain allele IGLV3-21*01 or IGLV3-21*04 that enables autonomous B cell receptor (BCR) signalling in chronic lymphocytic leukemia (CLL). It has been associated with shorter time to first treatment and shorter overall survival in various CLL cohorts. Although various methods exist for detecting IGLV3-21R110, their relative performance and concordance remain unclear. Furthermore, it is not yet known whether BTK inhibitors (BTKi) can effectively inhibit BCR signalling in IGLV3-21R110 positive CLL, and whether this mutation influences treatment outcomes.

**Methods:** The CLL12 trial compared ibrutinib vs. placebo in previously untreated Binet A CLL patients with intermediate to very high risk, while low risk patients were assigned to watch and wait (w&w). We analyzed all samples from this trial (n = 515) using targeted next-generation sequencing (tNGS). Ambiguous results (n = 5) were clarified by flow cytometry. All samples with IGLV3-21 rearrangement identified by tNGS, as well as 42 IGLV3-21R110-negative samples were reanalyzed by Sanger sequencing of cDNA and multiplex IGLV3-21R110-specific PCR (msPCR). Selected BCRs carrying either wild-type IGLV3-21G110 or mutated IGLV3-21R110 light chains were expressed in murine triple knock-out (TKO) B cells lacking Rag2, λ5, and SLP-65, but expressing inducible ERT2-SLP65 (EST) fusion protein. Cell-autonomous and antigen-stimulated BCR signalling were assessed in these TKO-EST cells by flow cytometric measurement of Ca2+ flux, as well as survival upon ibrutinib treatment in a concentration-dependent manner.

**Results:** Using tNGS, we identified 24 of 515 CLL samples as IGLV3-21R110-positive. All were confirmed by msPCR, and 23 also by Sanger sequencing. The false-negative result was due to a point mutation in the binding site for the Sanger forward sequencing primer. Two additional discrepancies

were observed: One patient expressed the IGLV3-21*03 allele without the G110R mutation (confirmed by tNGS and Sanger) but showed elevated normalized intensity of the non-specific 140-bp band in msPCR, meeting the formal threshold for IGLV3-21R110 detection (Syrykh et al., Blood Advances, 2023). However, as the 240-bp amplicon's forward primer is specific for IGLV3-21*01 and IGLV3-21*04 alleles only, the sample was classified as IGLV3-21G110. A third discrepant sample was identified as IGLV3-21R110 by both msPCR and Sanger, while tNGS detected the G110R mutation in only 5 out of 1,424 reads. A similar pattern was observed in a second sample and the patient was classified as IGLV3-21R110. In total, 34 patients had productive IGLV3-21 rearrangements: 5/152 (3.3%) in the w&w, 15/181 (8.3%) in the placebo and 14/182 (7.7%) in the ibrutinib group. Among these, 25 were IGLV3-21R110-positive (3/5, 12/15, and 10/14, respectively). IGLV3-21R110-positive patients had an intermediate IGHV mutational load and all expressed the IGLV3-21*04 allele. Among the 9 patients expressing IGLV3-21 chains without G110R, 3 used the IGLV3-21*04 allele, 4 IGLV3-21*02 and 2 IGLV3-21*03. All 5 cases using IGLJ1, but none of the 6 with IGLJ2, had the G110R mutation. Nine of the 25 IGLV3-21R110-positive cases (36%) carried stereotyped IGH genes and belonged to subset #2. Other frequently used heavy chains were IGHV3-23 and IGHV3-48. None of the IGLV3-21R110 cases had del(17p13), whereas SF3B1 mutations were more frequent in this group (32.0% vs. 6.1%, $p < 0.001$). CLL patients with IGLV3-21R110 had shorter EFS in all study arms: w&w (HR 17.03, 95% CI 4.99–58.13, $p < 0.001$), placebo (HR 2.31, 95% CI 1.16–4.6, $p = 0.017$), and ibrutinib (HR 2.99, 95% CI 1.17–7.65, $p = 0.023$). In multivariable analysis including other prognostic factors associated with EFS in univariate analyses (treatment with ibrutinib, serum β2-microglobulin, IGHV status, del(17p), del(11q) and +(12)), IGLV3-21R110 emerged as an independent prognostic factor of EFS (HR 3.18, 95% CI 1.73–5.83, $p < 0.001$), in addition to U-IGHV, del(17p13) and treatment with ibrutinib. Preliminary functional experiments using the TKO-EST system demonstrated that BCRs carrying the IGLV3-21R110 mutation require higher concentrations of ibrutinib to achieve effective inhibition of cell-autonomous BCR signalling and loss of cell viability, compared to their non-autonomous counterparts.

**Conclusions:** IGLV3-21R110 was identified as an independent prognostic factor for shorter EFS in early stage CLL and was associated with reduced efficacy of ibrutinib, both clinically and in vitro, as consequence of activated autonomous signalling. The emergence of IGLV3-21R110 as an important prognostic factor in CLL underscores the need for reliable detection, especially as its presence might also influence therapeutic decisions. Our comparison demonstrated overall concordance between the different sequencing and PCR-based methods, but also possible limitations with each of them.

**Keywords:** IGLV3-21R110; biomarker; BTK inhibitors

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1523

**Title:** SOUNDTRACK-E: a phase 1/2 open-label multicenter study to evaluate the safety/efficacy of AZD0486 as monotherapy or in combination with acalabrutinib in patients with relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma

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**Introduction:** AZD0486 is an IgG4 fully human CD19xCD3 bispecific T-cell engager that binds CD3 with low affinity to potentially reduce cytokine release upon T-cell activation while preserving effective T-cell cytotoxicity against malignant B cells. In a first-in-human phase 1 trial (NCT04594642), AZD0486 was active and well tolerated in patients with relapsed/refractory (R/R) follicular lymphoma or R/R diffuse large B-cell lymphoma (Gaballa S, et al. Blood. 2024;144:868; Hou JZ, et al. Blood. 2024;144:341). This study assesses fixed-duration subcutaneous (SC) AZD0486 as monotherapy or in combination with acalabrutinib in patients with R/R chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). This study is the first to evaluate AZD0486 in CLL/SLL and the first to evaluate SC AZD0486.

**Methods:** SOUNDTRACK-E (NCT06564038) is a phase 1/2 dose-escalation, global, multicenter trial of AZD0486 with 3 substudies, 1 of which is evaluating patients with R/R CLL/SLL. The study is recruiting patients aged ≥18 years with Eastern Cooperative Oncology Group performance status 0–2 and a histologically confirmed diagnosis. Patients with clinically significant central nervous system events (eg, seizure, stroke) or cardiovascular disease are excluded. The CLL/SLL substudy evaluates SC AZD0486 in patients with R/R CLL/SLL and includes a monotherapy cohort, in which patients with ≥2 prior lines of therapy (including Bruton tyrosine kinase inhibitor and BCL-2 inhibitor exposure) are eligible, and a combination therapy cohort that receives SC AZD0486 and acalabrutinib, in which patients with ≥1 prior line of therapy are eligible. In each cohort, SC AZD0486 is administered via a double step-up dosing schedule in cycle 1; the target dose is given every 2 weeks. Patients in the combination therapy cohort receive acalabrutinib 100 mg orally twice daily beginning at cycle 2. Dose escalation decisions will be based on a modified probability interval (mTPI-2) design. Approximately 46 patients for each cohort will be recruited. Primary objectives are to assess safety and tolerability, and to determine the recommended phase 2 dose for AZD0486 as monotherapy and in combination with acalabrutinib in patients with R/R CLL/SLL. Secondary objectives include efficacy endpoints, pharmacokinetics, and immunogenicity.

**Results:** Enrollment opened in October 2024 and is ongoing.

**Conclusion:** SOUNDTRACK-E will evaluate the safety and efficacy of AZD0486 as monotherapy or in combination with acalabrutinib for patients with CLL/SLL. This study is the first to evaluate SC AZD0486, and the first to evaluate AZD0486 in CLL/SLL.

**Keywords:** CLL/SLL; bispecific antibodies; relapsed/refractory

**Please indicate how this research was funded:** Study funded by AstraZeneca.

**Please indicate the name of the funding organization:** AstraZeneca

## Abstract ID: 1426

**Title:** Efficacy and safety of zanubrutinib in a fit subgroup of patients with treatment-naive chronic lymphocytic leukemia: post hoc analyses from the SEQUOIA study

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**Background:** Zanubrutinib, a highly potent and selective next-generation Bruton tyrosine kinase (BTK) inhibitor, is approved for chronic lymphocytic leukemia (CLL). SEQUOIA (NCT03336333) is a phase 3 study comparing zanubrutinib with bendamustine-rituximab (BR) in patients with treatment-naive CLL/small lymphocytic lymphoma (SLL) without del(17p) who were considered unsuitable for treatment with fludarabine, cyclophosphamide, and rituximab by age ≥65 years and/or comorbidities (Tam CS, et al. Lancet Oncology 2022;23(8):1031–1043). Outcomes among 'fit' patients in this study who may be candidates for more intensive fixed-duration combination treatments were not previously examined.

**Objective:** To determine the efficacy and safety of zanubrutinib in a 'fit' subgroup of patients without TP53-aberrations enrolled in SEQUOIA Cohort 1.

**Methods:** In this post hoc analysis, patients with SLL, del(17p), TP53 mutation (or missing), baseline creatinine clearance <50 ml/min (or missing), and Cumulative Illness Rating Scale >6 were removed. The remaining patients were analyzed as the 'fit' subgroup. Progression-free survival (PFS) estimates were determined using Kaplan–Meier methods.

**Results:** Of 479 patients enrolled in Cohort 1, 252 patients (53%; zanubrutinib, n = 123; BR n = 129) met the fit criteria. Median follow-up (range) was 43.9 (0.0–56.7) months. Median age was 71 (35–87) years, with 92.7% and 94.6% aged ≥65 years in zanubrutinib vs BR, respectively. The PFS estimates for zanubrutinib vs BR were 89.2% (95% CI, 82.1–93.6) vs 57.9% (95% CI, 48.2–66.5), respectively, at 36 months and 87.1% (95% CI, 79.5–92.1) vs 50.0% (95% CI, 39.8–59.4), respectively, at 42 months. Investigator-assessed overall response rates with zanubrutinib vs BR were 97.6% vs 88.4%. With median safety follow-up time of 44.0 months for zanubrutinib and 39.2 months for BR, the incidence rates adjusted for follow-up time for key events of interest (EOI) of any grade per 100 person-months were atrial fibrillation/flutter (0.16 vs 0.10), hypertension (0.50 vs 0.40), hemorrhage (2.04 vs 0.36), major hemorrhage (0.12 vs 0.07), neutropenia (0.54 vs 3.77), infections (4.01 vs 4.25), and second malignancies (0.46 vs 0.48); the adjusted rates for ≥ Grade 3 EOI were atrial fibrillation/flutter (0.06 vs 0.05), hypertension (0.28 vs 0.20), neutropenia (0.41 vs 2.99), infections (0.64 vs 0.87), and second malignancies (0.14 vs 0.20).

**Conclusions:** These data demonstrate that zanubrutinib is effective and safe in fit patients. The estimated 36-month PFS outcomes appear favorable compared to those reported in contemporary studies enriched for younger, fit patients. These results support continuous zanubrutinib monotherapy as an effective treatment option for all patients, including fit patients who might otherwise be considered for more intensive fixed-duration combination regimens.

**Keywords:** Zanubrutinib; Bruton tyrosine kinase inhibitor; CLL/SLL

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## Abstract ID: 1109

**Title:** Integrating clinical and genomic factors to predict time to first treatment in chronic lymphocytic leukemia

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**Introduction:** Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in adults, marked by significant clinical heterogeneity. Most patients are diagnosed in early, asymptomatic stages, where the standard approach is active surveillance until disease progression warrants treatment. In this context, time to first treatment (TTFT) is a key parameter for assessing disease evolution. Various clinical and biological factors have been previously identified and associated with a shorter TTFT. These factors include components of the IPS-E prognostic scale, such as lymphocytosis, the presence of adenopathy's, and the IGHV mutational status. Other established risk factors are elevated beta-2 microglobulin levels, advanced disease stages according to the Rai classification I-IV and Binet classification B-C, and age over 65 years. Furthermore, the advent of Next-Generation Sequencing (NGS) has facilitated the identification of specific genetic alterations with established prognostic value. Studies, including those proposed by Mansouri et al. (Leukemia, 2023), have correlated these genetic alterations with a decrease in TTFT. Given this background, the primary objective of the study described in the sources was to identify the most frequent clinical variables and genetic alterations that influence TTFT, aiming to deepen the understanding of their prognostic value.

**Methods:** The study was designed as a multicenter, retrospective, and observational analysis. It involved the analysis of clinical and genomic data from patients diagnosed with CLL between 2009 and 2024. The data were collected from two hospitals in Spain: Hospital Universitario de Gran Canaria Dr. Negrín and Hospital Universitario de Canarias (Tenerife). Inclusion criteria stipulated that all patients included must not have required initial treatment at the time

of diagnosis, adhering strictly to the criteria defined by the International Workshop on Chronic Lymphocytic Leukemia (IWGCLL). Genetic testing was performed at diagnosis using NGS with the SOPHiA Genetics v3 panel for CLL, restricted to pathogenic or likely pathogenic variants with a Variant Allele Frequency (VAF) ≥ 10%. Statistical analysis included univariate chi-square tests and multivariate Cox regression. Variables with $p \leq 0.2$ in univariate analysis were included in the multivariate models. Survival curves were generated using Kaplan-Meier and compared with the Log-rank test.

**Results:** The study analyzed 70 CLL patients, of whom 8 (11.4%) required treatment during follow-up. The cohort was predominantly male (62.9%) and over 65 years old (57%). Frequent clinical features included lymphocytosis >15 x$10^9$/L (31.4%), palpable lymphadenopathy (31.3%), and β2-microglobulin >2.5 mg/L (43.1%). The most common genetic alterations were unmutated IGHV (28.3%) and trisomy 12 (18.2%), while TP53 (8.6%), NOTCH1 (11.4%), and SF3B1 (10%) mutations appeared less frequently. Due to sample size limitations, a 'high-risk genetic variable' was created for TTFT univariate analysis ($p \leq 0.25$), grouping mutations in SF3B1, ATM, and NFKBIE. Notably, TP53 mutations did not show a significant association with TTFT in this univariate setting. The survival analysis demonstrated a statistically significant difference in TTFT based on the presence of these high-risk mutations(62 months vs 198 months ($p < 0.001$). In the multivariate analysis, the unmutated IGHV status was consistently and significantly associated with a worse prognosis regarding TTFT ($p < 0.05$) and was associated with high hazard ratios (HR). Furthermore, patients with any mutation in the high-risk genes – and especially SF3B1 – presented a higher risk of progression. This increased risk was observed independently of IGHV status, thereby reinforcing their value as adverse prognostic markers. The observed absence of association for TP53, this finding could potentially be attributed to the limited sample size of the cohort7. Additionally, the study methodology only considered mutations in the TP53 gene and did not include the detection. Furthermore, later studies, such as that by Malcikova et al. (Leukemia 2024), have suggested that the impact of TP53 mutations on TTFT might be restricted primarily to cases with mutated IGHV.

**Conclusions:** In conclusion, the study successfully identified a set of high-risk genetic mutations – specifically SF3B1– that are independently associated with a shorter TTFT. While the established IPS-E prognostic scale does not currently incorporate these identified genetic alterations, the findings from this study strongly suggest the potential utility of developing predictive models that integrate genetic information alongside traditional clinical factors for more accurate risk stratification8. However, to robustly validate this proposal and confirm these results in broader populations, larger studies with a greater sample size will be necessary.

**Keywords:** Leukemia; time to first treatment; high-risk genes

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## Abstract ID: 1110

**Title:** Clinical profiles of chronic lymphocytic leukemia patients with SF3B1 mutations: a single-institution study

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**Introduction:** SF3B1 plays a crucial role in RNA splicing catalysis by facilitating the removal of introns from precursor messenger RNAs (mRNA). Next generation sequencing (NGS) studies have identified an incidence of approximately 6–18% for SF3B1 mutations in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). This range is attributed to the observation that SF3B1 mutations are primarily subclonal genetic alterations, typically arising later in the course of disease. In CLL, SF3B1 mutations are associated with more aggressive disease. Given its rarity, comprehensive studies to further characterize the clinical features associated with SF3B1 mutations are needed to enhance our understanding of its role in CLL/SLL.

**Methods:** The Stanford Tumor Actionable Mutation Panel for Hematopoietic and Lymphoid Neoplasms (HemeSTMAP) is a targeted NGS test that identifies single nucleotide variants (SNVs), short insertion-deletions, and selected fusions in 164 genes commonly altered in hematologic neoplasms. The workflow involves acoustic shearing of genomic DNA, efficient library preparation, and targeted enrichment using custom-designed capture oligonucleotides. Genes are selected for inclusion based on their relevance as actionable targets for current and emerging anti-cancer therapies, their prognostic significance, and mutational rate. The panel achieves a minimum analytic detection limit of 5% for SNVs and insertion-deletion variants, with pooled libraries sequenced on an Illumina sequencing platform. We conducted a retrospective analysis of all Stanford HemeSTAMP assays performed in individuals with CLL/SLL since 2020. Patients with likely pathogenic SF3B1 mutations were identified, and their clinical characteristics were collected from electronic medical records. Descriptive statistics are calculated to characterize the patient cohort. To test for potential associations, Wilcoxon rank sum test, Pearson Chi-squared test, and Fisher's exact test are calculated.

**Results:** Among 507 samples, 32 SF3B1 mutations were identified, representing a prevalence of 6.3%. The median time from diagnosis to NGS collection was 33 months (range 1–227.5). Of the 32 patients, 20 (62.5%) were male, median age was 60.5 years (range 34–82) and the majority were White (84.4%). The specimen type for HemeSTAMP collection included peripheral blood in 17 patients (53.1%), bone marrow in 13 (40.6%), formalin-fixed paraffin-embedded (FFPE) in one (3.1%), and one sample from an outside source (3.1%). At diagnosis, 16 patients (50%) were Rai stage II-IV, and 17 (53.1%) were classified as Binet stage B-C. Regarding FISH abnormalities, 18 (58.1%) had del(13q), 9 (29%) had del(11q); no cases of trisomy 12 were detected. Further, though there were no cases of del(17p), 7 patients (21.9%) had a TP53 mutation. Additionally, 21 patients (77.8%) had unmutated IGHV, with an additional two patients IGHV3-21, which is associated with adverse prognostic implications. The median CLL-international prognostic index score (CLL-IPI) was 2.5 (0–7). For the 15 patients with NGS data available within 2 years of diagnosis, median time to initiation of therapy was 5.9 months (range 0.7–31.7). The median number of lines of therapy from CLL/SLL diagnosis was 1 (range 0–8). Treatments included: chemoimmunotherapy (18.8%), covalent BTK inhibitors (62.5%), BCL2 inhibitors

(40.6%), PI3K inhibitors (3.1%), non-covalent BTK inhibitors (9.4%), CD19-directed CAR-T therapy (3.1%), a bi-specific T-cell engager (BiTE) on a clinical trial (9.4%), and a BTK degrader on a clinical trial (3.1%). Consistent with other reports, the most common SF3B1 mutation variant was SF3B1 K700E (N = 14, 45.2%) followed by G742D (9.7%), H662Q (9.7%), H662D (6.5%), and R625C (6.5%). The median variant allele frequency (VAF) was 0.11 (range 0.01–0.48). The most frequently observed co-occurring mutations included ATM (19.4%), NOTCH1/NOTCH2 (16%), BRAF/MAP2K1 (12.9%), NFKBIE (12.9%), XPO1 (9.7%), and DDX3X (6.5%).

**Conclusion:** Our analysis of 507 CLL/SLL patients identified that 6.3% carried a likely pathogenic SF3B1 mutation. The SF3B1 K700E variant was the most prevalent, identified in 45.2% of these patients. Those with SF3B1 mutations tended to exhibit more aggressive disease characteristics at presentation, as indicated by higher Rai/Binet stage, unmutated IGHV status, del(11q) on FISH, and co-occurring mutations in ATM, and NOTCH1/NOTCH2. Longitudinal studies that integrate data from multiple institutions are essential for gaining deeper insights into the timing of SF3B1 emergence throughout the disease progression. Such studies will enhance our understanding of its associations with therapeutic responses and survival, particularly in the context of targeted therapies.

**Keywords:** Chronic lymphocytic leukemia; SF3B1; RNA splicing

**Please indicate how this research was funded:**

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## Abstract ID: 1708

**Title:** Chronic obstructive pulmonary disease and previous pneumonia have an impact on infectious complications in patients with chronic lymphocytic leukemia treated with acalabrutinib and zanubrutinib: a multicentre SEIFEM study

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Infections are a major source of morbidity and mortality in patients with Chronic Lymphocytic Leukemia (CLL). The development of targeted agents decreased this rate but patients, often elderly, with comorbidities, heavily treated, experienced serious infectious complications. The aim of our study is to describe in a real-world setting the infectious complications in patients with CLL treated with second generation Bruton's Tyrosine Kinase inhibitors (BTKis) Acalabrutinib (Acala) and Zanubrutinib (Zanu) in routine clinical practice, and to identify additional factors of infectious risk. This retrospective multicenter analysis was conducted on 460 CLL patients: 324 treated with Acala and 136 with Zanu. Patients of the second group were older, more frequently had TP53mut, pulmonary and renal impairment, more advanced Rai stage and more often already treated. We recorded 164 events of infections during treatment with Acala, occurring in 110 patients (34%) with an occurrence rate of 0.51. The total number of G3-G4 infections was 47 in 41 patients (12.7%) with an occurrence rate of 0.14. We recorded 71 events of infections during treatment with Zanu, occurring in 49 patients (36%) with an occurrence rate of 0.52. The total number of G3-G4 infections was 15 in 13 patients (9.6%) with an occurrence rate of 0.11. We registered a trend toward an earlier median time to infection with Zanu (4.5 months vs 8.2 months, $p = 0.068$) and a significant median time to infections G3-G4 with Zanu (3.1 months vs 7.8 months, $p = 0.025$). Risk factors for infection all grade in Acala were age at treatment, diabetes, chronic obstructive pulmonary disease (COPD), CrCl< 70, CIRS, previous lines of treatments, infections and/or pneumonia in the previous 12 months; in Zanu were diabetes, Rai stage, infections and/or pneumonia in the previous 12 months. If considering only G3-G4 infections, COPD and pneumonia in the previous 12 months were the unique significant variable for Acala ($p < 0.001$), pneumonia in the previous 12 months for Zanu ($p < 0.001$). Stratifying patients according to COPD and previous infections in the last 12 months we obtained 3 groups, significantly different in terms of risk for infection ($p < 0.001$). The cumulative incidence of infection was 89.4% in the first year if patients showed both characteristics. We recorded a 5-years overall survival OS of 71.2% per Acala with 35 deaths, of whom 16 due to infections. For Zanu a 5-years OS of 80.0% with 9 deaths, of whom 4 due to infections. Acala was withdrawn

for infections in 38 patients (34.5%), 7 of them were definitive: mainly pneumonia (6 cases) and sepsis (1 cases); 6 patients in this subgroup died for these infections in BTKi. Zanu was withdrawn for infections in 12 patients (24.5%), 1 of them was definitive due to pneumonia. This large study showed a mild rate of infections, most of grade 1–2. We found risk factors for G3-G4 infections COPD and previous pneumonia, confirming a role of these risk factors already described with venetoclax associated to anti-CD20 antibody in previous analysis from SEIFEM (Autore et al., AJH 2024). According to our analysis, we could consider selecting patients on BTKis therapy with specific risk factors for infection such as COPD and previous pneumonia, to be better and more closely monitored.

**Keywords:** LLC; acalabrutinib; zanubrutinib; infections; COPD; pneumonia

**Please indicate how this research was funded:** none

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## Abstract ID: 1427

### **Title:** A prospective multicenter study of FCR alternating with ibrutinib in treatment-naive patients with chronic lymphocytic leukemia (CLL)

**Authors:** Tingyu Wang[a,b], Yuting Yan[a,b], Zengjun Li[c], Hui Wang[c], Ying Sun[d], Xiantao Liu[d], Rui Lv[a,b], Wenjie Xiong[a,b], Gang An[a,b], Wei Liu[a,b], Yan Xu[a,b], Shuhui Deng[a,b], Qi Wang[a,b], Chenxing Du[a,b], Dehui Zou[a,b], Yaozhong Zhao[a,b], Lugui Qiu[a,b] and Shuhua Yi[a,b]

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Background BTK inhibitors, such as ibrutinib, have propelled chronic lymphocytic leukemia (CLL) into the era of targeted therapy. Nevertheless, continuous treatment with these agents not only leads to a progressive increase in resistance but also escalates treatment costs. On the other hand, the FCR regimen, consisting of fludarabine, cyclophosphamide, and rituximab, holds curative potential for CLL. However, its clinical application is significantly hampered by severe toxicity and long-term risks. To address these challenges, we developed the HAMBURGER regimen, a novel time-limited, alternating treatment approach combining ibrutinib with three cycles of FCR. This regimen aims to achieve deep remissions in CLL patients while enhancing tolerability and establishing a fixed treatment duration. Methods This multicenter, single-arm, phase II clinical trial (NCT03980002) enrolled 50 previously untreated CLL patients aged between 18 and 65 years. The treatment protocol involved the induction therapy: one cycle (lasting 1 month) of FCR, followed by 3 months of ibrutinib monotherapy at a dosage of 420 mg per day, then two alternating cycles of FCR and ibrutinib were given and the maintenance therapy with ibrutinib monotherapy after induction. After the completion of induction therapy, the treatment discontinuation strategy was based on the achievement of complete remission (CR) with undetectable minimal residual disease (uMRD) and TP53 status: patients without TP53 deletion or mutation could discontinue treatment; those with TP53 deletion or mutation continued maintenance therapy for 6 months before stopping; all other patients continued single-agent ibrutinib maintenance until they achieved CR with uMRD, followed by an additional 6 months of treatment. The primary endpoint of this study was the achievement of CR after induction therapy. Secondary endpoints included overall response rate (ORR), uMRD rate, progression-free survival (PFS), overall survival (OS), and safety assessment. Results Among the 50 enrolled patients (with a median age of 57 years and 72% being male), 47 completed the induction therapy. The overall response rate (ORR) was 97.9% (46/47), with 66.0% (31/47) of patients achieving CR. Notably, 38.3% (18/47) of patients achieved CR with undetectable MRD in both bone marrow and peripheral blood. The bone marrow uMRD rate after induction therapy was 42.6% (20/47). Patients with immunoglobulin heavy chain variable region (IGHV)-mutated status demonstrated a higher bone marrow uMRD rate (53.8% vs. 22.2%, $p = 0.036$). Among the 4 patients with TP53 abnormalities, 3 achieved partial remission (PR) and 1 achieved CR with uMRD after induction therapy, and 2 patients achieved CR during the maintenance therapy phase. With a median follow-up of 55 months, the 5-year PFS and OS rates were 86.7% and 88.7%, respectively. In IGHV-unmutated patients, the 5-year PFS and OS were both 94.4%, compared with 80.8% and 84.1% in IGHV-mutated patients (PFS: $p = 0.666$; OS: $p = 0.561$). For patients with TP53 abnormalities, the 5-year PFS and OS were 75.0%, which were lower than those in patients without TP53 abnormalities (PFS 87.3%, OS 89.5%), although the differences did not reach statistical significance (PFS: $p = 0.527$; OS: $p = 0.211$). Among the 28 patients who received ibrutinib maintenance therapy, five patients transitioned from PR to CR. Fourteen patients discontinued ibrutinib, including nine who did so voluntarily, and only one patient experienced relapse and successfully resumed ibrutinib treatment. Safety was evaluated in all 50 enrolled patients. Hematologic toxicities were common, with leukopenia observed in 24 (48.0%) patients, thrombocytopenia in 20 (40.0%), and lymphocytopenia in 27 (54.0%). Grade 3/4 neutropenia occurred in 26% of patients. Non-hematologic adverse events (AEs) were predominantly Grade 1–2, including nausea (56%) and infusion reactions (36%). No therapy-related myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) cases were reported, and ibrutinib dosage reduction was required in only two patients due to tolerable toxicities. Conclusion The HAMBURGER regimen showed remarkable efficacy, inducing durable remissions with manageable toxicity. The high CR rate of 66% and CR-uMRD rate of 38% are particularly impressive, especially considering the fixed treatment duration. This innovative approach presents a cost-effective and patient-centric alternative to continuous BTKi therapy, especially in healthcare settings with limited access to newer treatment agents. The MRD-guided treatment discontinuation strategy further enhances the long-term benefits and practicality of this regimen. These findings support the potential of the HAMBURGER regimen as a front-line treatment option for appropriately selected CLL patients, including those with high-risk features.

**Keywords:** CLL; time limited regimen; uMRD

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## Abstract ID: 1428

**Title:** A prospective, single-center, single-arm clinical trial of zanubrutinib monotherapy and ZFCG combined therapy was given sequentially for the treatment of primary symptomatic CLL/SLL (stop trial)

**Authors:** Tingyu Wang[a,b], Yuting Yan[a,b], Ying Yu[a,b], Wenjie Xiong[a,b], Rui Lv[a,b], Tengteng Yu[a,b], Wei Liu[a,b], Yan Xu[a,b], Weiwei Sui[a,b], Wenyang Huang[a,b], Shuaishuai Zhang[a,b], Qi Wang[a,b], Lugui Qiu[a,b] and Shuhua Yi[a,b]

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**Background:** Although Bruton tyrosine kinase (BTK) inhibitor monotherapy yields an overall response rate (ORR) of 80–90% and prolongs survival in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), its complete response (CR) rate remains suboptimal (< 10%). Even after 7–8 years of continuous treatment, the CR rate only improves to around 30%. More critically, achieving undetectable minimal residual disease (uMRD) is infrequent, compelling patients to undergo long-term BTK inhibitor therapy. To address these limitations, our trial investigated a time-limited combination immunochemotherapy strategy in treatment-naïve CLL patients, aiming to deepen remission, achieve uMRD, and enable treatment cessation to enhance quality of life.
**Methods:** Eligible pts were treatment-naïve CLL/SLL patients aged ≤65 years. The regimen consisted of zanubrutinib (Z) monotherapy (160 mg twice daily) for 12 cycles, followed by four cycles of combination therapy with fludarabine (F, 25 mg/m$^2$ IV D1–3 cycles 13–16), cyclophosphamide (C, 250 mg/m$^2$ IV D1–3 cycles 13–16), and obinutuzumab (G, 1,000 mg IV D0,7,14 cycle 13; 1,000 mg IV D0 cycles 14–16) (ZFCG regimen). After cycle 16, response and MRD status in peripheral blood (PB) and bone marrow (BM) were assessed by four-color flow cytometry. Patients achieving CR/CRi with uMRD in both PB and BM could stop treatment; others could choose to continue or stop zanubrutinib monotherapy. (NCT05287984).
**Results:** As of 1 April 2025, 59 pts initiated therapy. Thirty pts completed combination therapy; 3 withdrew (1 due to COVID-19 impact, 2 due to disease progression: one in zanubrutinib monotherapy phase, the other post the ZFCG phase). Baseline characteristics: median age 58 years (range 33–65); 67.8% male. High-risk and very-high-risk CLL-IPI scores were present in 20.3% and 3.4% of pts, respectively. IGHV was unmutated in 38.3%. Cytogenetic abnormalities included TP53 deletion (del) (1.7%), ATM del (15.3%), RB1 del (22.0%), and trisomy 12 (20.3%). TP53 mutation was detected by NGS in 3.4%. All 59 patients received zanubrutinib monotherapy, with 41 progressing to the ZFCG phase. Among the 30 patients who completed 4 cycles of ZFCG and underwent efficacy assessment, after 12 cycles of zanubrutinib monotherapy alone, the CR rate was 13.3% (4/30), and only 1 patient (3.3%) achieved CR with uMRD in both PB and BM. After 2 cycles of ZFCG, 18 patients (60%) achieved both CR and CR with PB + BM uMRD. Upon completion of 4 cycles of ZFCG, the proportion of patients achieving CR and CR with PB + BM uMRD increased to 76.7% (23/30) and 73.3% (22/30), respectively. Additionally, 100% of patients (30/30) achieved PB uMRD, and 96.7% (29/30) achieved BM uMRD. All 30 patients met the criteria for treatment cessation after completing 16 cycles of therapy. With a maximum treatment-free interval of 20 months, 29 patients maintained CR with uMRD without treatment. However, 1 patient experienced disease progression, characterized by the reappearance of a pulmonary mass noted at diagnosis, 7 months post-cessation, although the BM remained in CR with uMRD at recurrence. During the ZFCG phase, the most common grade ≥3 adverse events (AEs) were thrombocytopenia (32.5%), neutropenia (27.5%), and leukopenia (25%). Grade ≥3 non-hematological AEs included lung infection (15%) and febrile neutropenia (5%).
**Conclusion:** The ZFCG regimen demonstrated high rates of CR with uMRD in treatment-naïve CLL patients after four cycles of combination therapy, accompanied by excellent uMRD rates in PB and BM. All patients who completed 16 cycles of therapy fulfilled the criteria for treatment cessation. With a median follow-up of nearly 1 year (max 20 months) post-treatment, 29 out of 30 patients maintained continuous CR with uMRD. However, longer follow-up is required to determine the durability of treatment-free remission. These preliminary findings from the Stop Trial indicate that ZFCG is an effective, time-limited treatment option for treatment-naïve CLL patients.

**Keywords:** Zanubrutinib; chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL); combined therapy

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**Please indicate the name of the funding organization:** National Nature Science Foundation of China (82200215, 82170193, 82170194, 82370197), the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (2022-I2M-1-022) and Beijing Xisike Clinical Oncology Research Foundation (Y-2024AZ(BTK)ZD--0074)

## Abstract ID: 1007

**Title:** Clonality assessment in multiclonal chronic lymphocytic leukemia using Sanger and next-generation sequencing approaches

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Chronic lymphocytic leukemia (CLL) is typically characterized by the presence of a dominant B-cell clone with a unique immunoglobulin heavy-chain (IGH) gene rearrangement. However, a subset of patients exhibits multiple clonal IGH rearrangements, reflecting underlying multiclonality. Understanding the clonal architecture and its evolution over time is essential for insights into disease biology and progression. In a cohort of 3,167 CLL patients, routine diagnostic assessment of IGHV mutational status was performed using cDNA and Sanger sequencing. Among these, 249 patients (8%) exhibited multiple clonal productive IGH gene rearrangements. Specifically, we identified 224 biclonal, 23 triclonal, and 2 tetraclonal CLL cases. In 173/249 cases, the co-detected clones were concordantly mutated (115) or unmutated (58). The remaining 76 cases showed discordant mutational status among the clones. IGHV mutational status was examined repeatedly over time in 100/249 multiclonal CLL patients, with a median interval of 3.4 years between the first and last examinations. Over time, one or more clones disappeared in 53 patients, while in 21 patients a new clone emerged later (17/21 patients were monoclonal at diagnosis). In 5 cases, the original dominant clone was replaced by another.

To get deeper insight into the genomic clonal composition of multiclonal CLL cases, we analyzed tumor DNA from a representative cohort of 87 such cases (35% of the multiclonal cohort) with capture-based NGS panel LYNX. This panel allows for detection of markers associated with the most common lymphoid malignancies, including antigen receptor rearrangements. In parallel, we compared detected clonotypes with results from amplicon IGH NGS. The median number of fragments covering complete IGH rearrangements in the LYNX panel was 248, while the median number of usable reads in IGH NGS was 1.7 million. The most abundant clonotype expected was detected in all 87 cases using both methods. Using LYNX, a second expected clone was confidently detected in 53 out of 87 (61%) cases, and in an additional 10 cases below the confident detection threshold (defined as ≥3 fragments and >1% of clonal productive IGH rearrangements). IGH NGS identified the expected second clone in 53/87 (61%) cases at ≥1% of productive IGH rearrangements, and in other 22 cases at levels below 1%. Using either method, we identified additional clonal rearrangements (>1%) that were missed by Sanger sequencing—11 cases using LYNX and 17 using IGH NGS. Notably, in cases where the Sanger-identified clones had concordant IGHV mutational status, all newly identified rearrangements also matched in mutational status: 11 in LYNX and 12 in IGH NGS.

We also performed amplicon IGH NGS analysis on a cohort of 62 consecutive CLL cases seemingly monoclonal by Sanger IGH analysis. In 60 out of 62 cases, the expected rearrangement was the dominant clone. The remaining two cases exhibited a highly polyclonal pattern. In 6 of the 60 cases, an additional clone representing more than 1% of productive IGH rearrangements was detected. In 5 of these 6 cases, the newly identified clone had a concordant IGHV mutational status with the concurrent dominant clone. Notably, three of the additional clonal rearrangements had a relative size >25%, and all three belonged to the same IGHV family as the respective dominant rearangements.

Our results provided a coherent clonality assessment in the analyzed multiclonal CLL cases, with notable prevalence of concordant IGHV mutational status among co-detected clones.

**Keywords:** Clonality; IGH rearrangement; NGS

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The Ministry of Education, Youth and Sports, Czech Republic: NICR LX22NPO5102 - Funded by the European Union - Next Generation EU
The Ministry of Education, Youth and Sports, Czech Republic: e-INFRA CZ project (ID:90254)

## Abstract ID: 1901

### **Title:** Similarities and differences in immune modulation by acalabrutinib and zanubrutinib in chronic lymphocytic leukemia

**Authors:** Maria Andersson[a,b], Olina Lind[a], Kia Heimersson[a], Anna Berglof[c], Teresa del Peso Santos[d,e], Lucía Peña-Pérez[d,e], Qing Wang[c], Tom Mulder[a,b], Rula Zain[f,g,h], Robert Månsson[i], Richard Rosenquist[d,j], C. I. Edvard Smith[f,k], Anders Österborg[a,b] and Marzia Palma[a,b]

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**Background:** The covalent Bruton tyrosine kinase inhibitors acalabrutinib and zanubrutinib are preferred as single-agent treatment in clinical practice for patients with chronic lymphocytic leukemia (CLL) due to their high efficacy and improved safety profiles compared to the first-in-class

ibrutinib. Despite enhanced kinase selectivity, their long-term immunomodulatory effects remain incompletely characterized. We conducted a longitudinal study to compare the biological effects of acalabrutinib and zanubrutinib on CLL tumor cell fractions, immune cell composition and plasma protein biomarkers.

**Methods:** Peripheral blood samples from 34 CLL patients treated with either acalabrutinib (n = 18) or zanubrutinib (n = 16) were collected at multiple time points over a 24-month (mo) period. Immune monitoring included flow cytometry and proximity extension assay (Olink) to assess immune cell subsets and 92 inflammatory plasma proteins. Tumor cell subpopulations were classified into proliferative (PF, CD5^bright CXCR4^dim) and resting (RF, CD5^dim CXCR4^bright) fractions. ddPCR was performed to detect BTK hotspot mutations. Clinical outcomes were recorded, and age- and sex-matched healthy donors (HD) served as controls.

**Results:** B cell (CD19+) counts declined as expected during the treatment in both cohorts, with a significant reduction vs baseline occurring earlier in the acalabrutinib cohort (2 mo) compared to the zanubrutinib cohort (6 mo). Analysis of tumor fractions revealed a reduction in the PF population during early treatment, followed by a plateau or slight increase at later time points. Two patients in the zanubrutinib group had high PF at the 2-year follow-up and within a month both had progressive disease. RF showed an initial rise but decreased by month 24 in the acalabrutinib cohort. At baseline both cohorts exhibited elevated $CD4^+$ and $CD8^+$ T cell counts, more pronounced in the zanubrutinib group. Both subsets decreased significantly in both cohorts at mo 3 in the acalabrutinib cohort and mo 4–6 in the zanubrutinib cohort. While $CD4^+$ T cells reached HD levels, $CD8^+$ T cells remained elevated at the final 2-year follow-up. T cell memory subsets distribution, initially skewed toward effector subsets, shifted toward restoration, however, naïve $CD4^+$ T cells declined below HD levels by 2 years. Th1 cells decreased significantly during treatment in both cohorts; Th2 and Th17 cell changes were less marked. Regulatory T cells (Tregs) declined early in both cohorts and normalized. Exhausted T cells ($PD\text{-}1^+/TIGIT^+$) decreased during therapy but remained partially elevated compared to HD. Most T-cell subsets declined and normalized faster during treatment with acalabrutinib compared to zanubrutinib, which paralleled with a faster reduction of the circulating tumor burden (i.e. CD19+ cells).

Of the 92 plasma biomarkers assessed, 17 changed significantly in both cohorts—mostly decreasing—including CLL-associated markers CCL3, CCL4, CD5, and CD6. Acalabrutinib uniquely altered 15 biomarkers, including a transient increase in MMP1 and AXIN1 at 1 month. Zanubrutinib selectively modulated 11 markers, including upregulation of the monocyte chemoattractant proteins MCP2 and, MCP3.

**Conclusion:** Acalabrutinib and zanubrutinib both induce substantial immune modulation and reductions in tumor-associated biomarkers in CLL. Despite similarities in immune cell kinetics, distinct plasma protein profiles were observed with the two drugs suggesting differences in their off-target effects.

**Keywords:** cBTKi; T cells; biomarkers

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**Please indicate the name of the funding organization:**

# Abstract ID: 1804

## **Title:** BRD4 maintains the surface phenotype of chronic lymphocytic leukemia cells

**Authors:** Athina Georgiou[a], Friedman Daniel[b,c,d], Ka Ka Yu[a], Drshika Mehtani[a], Piers Patten[a], Eric So[a] and Robbert Hoogeboom[a]

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Global changes in the epigenetic landscape are a hallmark of cancer, including chronic lymphocytic leukemia (CLL). The epigenetic regulator Bromo domain-containing protein 4 (BRD4) is overexpressed in CLL and controls super enhancer regions that regulate the expression of multiple genes and pathways involved in the development and progression of CLL (e.g. B-cell receptor signalling), emphasizing the importance of BRD4 in CLL pathology (Ozer et al, Cancer Discov. 2018). In other cell types, BRD4 has also been implicated in differentiation and maintaining cell identity. However, the effect of BRD4 inhibition on the differentiation of CLL cells has not been explored yet.

To study the effect of BRD4 inhibition on differentiation of CLL cells, we exposed CLL cells to the BRD4 inhibitor JQ1 and analyzed the surface phenotype by flow cytometry. In the CLL cell lines MEC-1 and PCL-12, we observed a 47% and 60% reduction CD19 expression after 72 hours of 1 µM JQ1, respectively. In murine CLL cells derived from Eu-TCL1-transgenic mice (n = 4), 48 hours of BRD4 inhibition with 0.5 µM JQ1 in vitro reduced CD19 expression by 35% and in primary human CLL cells (n = 7), 48 hours of 0.5 µM JQ1 treatment in vitro reduced CD19 expression by 25%. In addition, we found CD5 expression reduced by 40% in human primary CLL cells (n = 7) and 25% in murine primary CLL cells (n = 4), demonstrating that BRD4 maintains the phenotype of CLL cells. Of note, CD5 levels on T cells remained stable, indicating that this effect is specific for B cells.

In vivo, CLL cells maintain an activated memory B cell phenotype with limited differentiation into plasma cells. To investigate the effect of BRD4 inhibition on terminal differentiation of CLL cells, we cultured primary CLL cells on CD40L-transfected fibroblasts, in the presence of IL-4 and IL-21, conditions that promote terminal differentiation of healthy donor B cells but not primary CLL cells. After 5 days of stimulation, when CLL cells are proliferating, the CLL cells were treated with JQ1 (0.5 µM) or the BTK inhibitor Ibrutinib (1 µM) for a further 6 days. As expected, treatment with JQ1 had no effect on the viability of CLL cells, whilst proliferation of CLL cells was significantly suppressed in all patients tested (n = 16). In contrast, ibrutinib, a standard-of-care first-line treatment for CLL, had a minimal effect on proliferation in these culture conditions. Interestingly, BRD4 inhibition, but not BTK inhibition, induced a 10% increase in cell size and a 50% increase in granularity as determined by flow cytometry and strikingly reduced the surface levels of CD5 and CD19. Increased size and granularity and reduced CD19 expression could be indicative of terminal differentiation into plasma cells. To investigate potential differentiation into plasma cells, we analyzed surface CD138 expression by flow cytometry

and found a 2-fold increase in JQ1-treated CLL cells but not DMSO or ibrutinib treated cells (n = 12). In addition, May-Grunewald Giemsa staining revealed eccentric nuclei and conspicuous perinuclear hofs, features of plasmacytic differentiation, in BRD4 inhibited cells.
In conclusion, our findings demonstrate that inhibition of BRD4 modulates the phenotype of CLL cells by reducing CD19 and CD5 and inducing features of plasma cells, opening new avenues to develop differentiation therapy for CLL and other B cell malignancies.

**Keywords:** Leukemia; differentiation; phenotype

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Blood Cancer UK (BCUK)

## Abstract ID: 1111

**Title:** Functional analysis of in-frame variants in TP53 gene identified in adult leukemias: a study of the TP53 network of European Research Initiative on CLL

**Authors:** Sarka Pavlova[a], Johana Gombíková[b], Silvia Petrezsélyová[c], Libor Macůrek[c], Marcela Zenatova[b], Marie Zádrapová[b], Blanca Ferrer-Lores[e], Alicia Serrano Alcala[f], Eugen Tausch[g], Stephan Stilgenbauer[g], Fanny Baran-Marszak[h], Gregory Lazarian[h], Eva Zapletalová[b], Jana Kopecna[b], Miguel Alcoceba[i], Ana Balanzategui[i], Lisa Bonello[j,k], Ivana Ježíšková[b], Imrich Hikkel[l], Martina Sopkovičová[l], Silvia Zibellini[m], Marzia Varettoni[m], Audrey Bidet[n], Helena Podgornik[o,p], Sandra Sucurovic[o], Riccardo Moia[q], Monica Lopez-Guerra[r,s,t], Mark Catherwood[u], Šárka Pospíšilová[b,v,w] and Jitka Malcikova[a]

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**Background and Aims:** The prognostic and predictive impact of TP53 variants in leukemia has led to their inclusion in diagnostic and treatment guidelines, and increased the need for rapid and reliable laboratory analysis, interpretation, and reporting. Within its activities, European Research Initiative on CLL (ERIC) collects data on TP53 variants identified during routine assessment in CLL with the aim to create CLL-specific TP53 variant database, and addresses challenges faced by diagnostic laboratories. While most somatic TP53 variants can be interpreted using data from large-scale functional studies, data on the impact of deletions and insertions not disturbing the reading frame remain limited (Kotler et al. 2018, PMID: 29979965; Funk et al., PMID: 39774325), and classification often relies on expert judgment. Here, we approached ERIC laboratories that had identified in-frame TP53 variants and collected samples to analyze their transactivation capacity.
**Methods:** cDNA prepared from primary leukemic samples was used as an input material. The yeast-based Functional Analysis of Separated Alleles in Yeast (FASAY; Flaman et al. 1995, PMID: 7732013) enabled fast functional readout of individual p53 variants; variants causing loss of ability to activate ADE2 reporter gene transcription led to the accumulation of red intermediate in yeast colonies. The presence of the specific variants in yeast colonies was confirmed by Sanger sequencing. Selected variants were further tested in RPE1 TP53-knockout human cells expressing doxycyclin-inducible p53 variants, with functional assessment via nutlin-3 stabilization and measurement of endogenous MDM2 and p21 proteins and BAX mRNA levels.
**Results:** Sixty cDNA samples containing in-frame TP53 variants previously detected by routine gene sequencing in chronic lymphocytic leukemia (CLL; 88%) or other hematological malignancies were collected from 13 ERIC centers. The set included 54 unique variants (5 variants detected more than once). The most frequent were deletions (N = 30; 1–13 amino acids), followed by delins (N = 9), duplications (N = 8; 1–9 amino acids), and insertions (N = 7; 1–10 amino acids). The variants were mainly located in DNA-binding domain (DBD; N = 51; affected codons 106 to 291) or oligomerization domain (OD; N = 3; codons 336 to 350). In FASAY, 51 variants produced red colonies, indicating a complete loss of function (LOF). Two variants located at the distal margin of the DNA-binding domain (DBD; p.Glu287_Asn288delinsAsp and p.Thr284_Arg290dup), and one in the oligomerization domain (p.Gly334_Arg335dup) yielded pink colonies, suggesting partial activity, in contrast to white colonies carrying wild-type TP53. Testing in human cells yielded concordant results for all four selected variants that exhibited complete LOF in the yeast system.

Among the variants with a partially functional phenotype in yeast, the oligomerization domain variant showed complete LOF in mammalian cells, whereas the two variants at the DBD boundary retained over 70% of wild-type p53 activity. Notably, in the original patient samples, the p.Glu287_Asn288delinsAsp variant co-occurred with a LOF missense variant in a compound heterozygous state, and the p.Thr284_Arg290dup variant was detected at variant allele frequency of only 1.9%.
**Conclusion:** All 49 in-frame TP53 variants located within the core region of the DNA-binding domain resulted in a loss of transactivation function, while the variants downstream of codon 287 displayed heterogeneous phenotypes depending on testing system and localization, with variants located in OD being predominantly non-functional. Our findings support the interpretation that in-frame TP53 variants within the core DBD region are likely pathogenic when identified in tumor samples. In contrast, interpretation of in-frame variants located outside this region requires integration of multiple data sources, including functional studies, published literature, and curated variant databases. Supported by MH CZ - DRO (FNBr, 65269705), NW24-03-00114, MUNI/A/1685/2024; the project National Institute for Cancer Research (MEYS, Programme EXCELES, ID Project No. LX22NPO5102) – co-funded by the European Union - Next Generation EU.

**Keywords:** In-frame; TP53; mutation

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## Abstract ID: 1702

### **Title:** Immune cytopenia with chronic lymphocytic leukemia: a real-world clinical characteristic analysis

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**Objective:** To analyze the clinical characteristics and treatment of chronic lymphocytic leukemia (CLL) patients with autoimmune cytopenia.
**Methods:** A retrospective analysis was conducted on 39 CLL patients with autoimmune cytopenia treated at The First Affiliated Hospital of Nanjing Medical University between July 2011 and September 2024. Clinical features and treatment strategies were evaluated.
**Results:** Among the 39 CLL patients, 26 (67%) had autoimmune hemolytic anemia (AIHA), 12 (31%) had immune thrombocytopenia (ITP), and 1 (3%) had Evans syndrome. The cohort included 23 males (59%) and 16 females (41%), with a median age of 61 years (range: 43–87) at CLL diagnosis. According to the CLL-International Prognostic Index (CLL-IPI), 3 patients (8%) were low-risk, 11 (28%) intermediate-risk, 15 (38%) high-risk, and 10 (26%) very high-risk. Monoclonal proteins were detected by immunofixation electrophoresis in 7 patients (18%). Six patients (15%) had concurrent autoimmune diseases (e.g. systemic lupus erythematosus, dermatomyositis, psoriasis). Twenty-five patients (64%) were with mutated IGHV and 14 (36%) were with unmutated IGHV. Genetic abnormalities included TP53 aberrations in 9 (23%), NOTCH1 mutations in 4 (10%), and SF3B1 mutations in 5 (13%). Regarding disease progression: 22 patients (56%) were diagnosed with CLL and cytopenia simultaneously; 3 (8%) were diagnosed with ITP first, with CLL diagnosed 1, 4, and 10 years later; 13 (33%) were diagnosed with CLL first—8 developed cytopenia during watch-and-wait (median time from CLL diagnosis to ITP-directed therapy: 71.5 months; 7 AIHA, 1 ITP), while 4 developed cytopenia post-CLL treatment (2 AIHA, 2 ITP). With a median follow-up of 49 months (range: 8–166), 28 patients (72%) initiated CLL-directed therapy.
**Conclusion:** CLL patients with autoimmune cytopenia exhibit significant clinical heterogeneity and variable sequences of diagnosis. Suboptimal response to initial cytopenia-directed therapy in some patients necessitates timely initiation of CLL-targeted treatment for disease control.

**Keywords:** Autoimmune cytopenia; autoimmune hemolytic anemia; immune thrombocytopenia; treatment

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1429

### **Title:** Orelabrutinib, fludarabine, cyclophosphamide and obinutuzumab (OFCG) for first-line treatment of chronic lymphocytic leukemia: a multicenter, investigator-initiated phase II trial (cwCLL-001 trial)

**Authors:** Huayuan Zhu[a], Yi Miao[a], Ming Liu[a], Shuchao Qin[b], Fei Li[c], Jin Zhang[d], Hongling Peng[e], Chunyan Ji[f], Luomengjia Dai[a], Ziyuan Zhou[a], Chongyang Ding[g], Zhen Wang[h], Yeqin Sha[a], Tong-lu Qiu[a], Hanning Tang[i], Hui Jin[i], Lei Fan[i], Wei Xu[j], Jianyong Li[k] and Yi Xia[a]

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**Introduction:** Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) are incurable B-cell malignancy with a heterogeneous clinical course despite advancements in therapies including targeted agents. Ibrutinib combined with fludarabine, cyclophosphamide, and rituximab or obinutuzumab (iFCR or iFCG) in patients with CLL/SLL as first-line therapy demonstrated remarkable efficacy. Orelabrutinib is a novel and highly selective second generation irreversible BTK inhibitor with remarkably less off-target inhibition. This study evaluated the efficacy and safety of the orelabrutinib, fludarabine, cyclophosphamide, and obinutuzumab regimen as the first-line treatment for patients with CLL/SLL without restriction by TP53 aberrations[del(17p) and/or TP53 mutation] and IGHV status.
**Methods:** This is a multicenter, open-label, non-randomized phase II study for previously untreated patients with CLL/SLL. Eligible patients were 18–65 years old with ECOG performance status 0–1. Patients received single-agent oral orelabrutinib during a 7-day lead-in period, followed by OFCG regimen for three 28-day cycles. Patients who achieved BM-uMRD4 (undetectable MRD $<10^{-4}$) by FCM after 3 cycles of OFCG therapy would receive 3 cycles of OG therapy, and other patients would receive additional 3 cycles of OFCG therapy. After six cycles of therapy, patients who achieved BM-uMRD4 would receive 6 cycles of orelabrutinib monotherapy, and other patients would receive 6 cycles of OG therapy. After 12 cycles of therapy, patients who achieved BM-uMRD4 would discontinue all therapy, and others would receive 12 cycles of orelabrutinib monotherapy. The primary endpoint was the rate of BM-uMRD4 after 6 cycles by FCM. Exploratory endpoints included the rates of PB-uMRD6 (undetectable MRD $<10^{-6}$) and BM-uMRD6 by NGS.
**Results:** Twenty-five patients with CLL were enrolled from 30 May 2022, to 30 June 2023. At the data cut-off (31 December 2024), 2 patients voluntarily discontinued the study after receiving 2 cycles of OFCG. 23 patients completed 3 cycles of OFCG therapy and 13 of them achieved BM-uMRD4. After 6 cycles of treatment, 95.2% (20/21) of patients achieved PB-uMRD4 and 86.4% (19/22) achieved BM-uMRD4 by FCM. 22.7% (5/22) of patients achieved CR/CRi with BM-uMRD4 after 3 cycles of treatment, 59.1% (13/22) after 6 cycles of treatment and 72.7% (16/22) after 12 cycles of treatment. 61.9% (13/21) of patients achieved PB-uMRD6 and 57.1% (12/21) of patients achieved BM-uMRD6 after 6 cycles of treatment and changed to 79.0% (15/19) and 55.0% (11/20) after 12 cycles of treatment. The median follow-up time was 26.8 months (95% CI: 24.9–28.7). The median PFS and OS were not reached with a 2-year PFS rate of 96% (95% CI: 89%–100%) and a 2-year OS rate of 96% (95% CI: 89%–100%). Compared with patients with unmutated IGHV, patients with mutated IGHV are more likely to achieve PB-uMRD4 (OR =0, 95% CI: 0–0.4671; $p=0.0167$) and BM-uMRD4 (OR =0, 95% CI: 0–0.2268; $p=0.0016$) at C4D0. Significant differences in BM-uMRD6 at C7D0 (OR =0, 95% CI: 0–0.22; $p=0.0011$) and C13D0 (OR =0.06, 95% CI: 0.01–0.61; $p=0.02$) were also observed between IGHV mutated and unmutated patients. Most AEs were mild and manageable with dose interruptions or supportive care during the 12 treatment cycles. The most common grade 1–2 AEs were anemia (25/25, 100.0%) and nausea/vomiting (18/25, 72.0%). Neutropenia (20/25, 80.0%) and thrombocytopenia (15/25, 60.0%) were the most common grade 3–4 AEs.
**Conclusions:** The OFCG regimen showed promising efficacy and a favorable safety profile as a first-line treatment for previously untreated patients with CLL/SLL, which indicated that this time-limited treatment regimen would provide a new choice for patients, especially those with mutated IGHV. Given these data, a larger randomized controlled trial is needed in the future to validate these findings.

**Keywords:** CLL/SLL; combination therapy; clinical trials

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1524

**Title:** Patient-reported outcomes (PRO) among patients with previously treated chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) receiving pirtobrutinib: final analysis from the BRUIN phase 1/2 study

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**Background:** Pirtobrutinib is a highly selective, non-covalent Bruton tyrosine kinase inhibitor (BTKi) and is approved in the US for the treatment of patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (hereafter, CLL) after prior systemic therapy that includes a BTKi. BRUIN (NCT03740529) was an open-label, multi-center phase 1/2 study that investigated the safety and efficacy of pirtobrutinib for the treatment of B-cell malignancies, including CLL. The final patient-reported outcome (PRO) endpoint analyses are presented here from patients enrolled in the BRUIN study with relapsed or refractory (R/R) CLL treated with at least two prior regimens, including a covalent BTKi (cBTKi).

**Methods:** Patients in the BRUIN study completed PRO assessments at each in-person clinic visit using paper forms. Patient-reported physical functioning (PF) and Global Health Status/Quality of Life (GHS/QoL) were measured using the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire Core-30 (QLQ-C30); CLL/SLL-related symptoms and fatigue were assessed using 13 and 6 EORTC Item Library (IL) items, respectively. These were scored from 0–100, where higher scores represent improved PF and GHS/QoL and where higher scores reflect worse CLL/SLL-related symptoms and fatigue. Time to worsening was evaluated using the Kaplan-Meier method for PF, GHS/QoL, CLL/SLL-related symptoms, and fatigue. Events were defined as the first observation of worsening that was sustained through at least one subsequent assessment or patient death, whichever occurred first. Worsening in each PRO endpoint was defined based on pre-specified clinically meaningful within patient change (MWPC) thresholds. Patients without events were censored at their last observed study clinic visit. Pre-specified clinically MWPC thresholds in each PRO score were also used to describe the proportion of patients who improved, remained stable, or worsened based on their individual change from baseline (Cycle 1 Day 1) through Cycle 30 (beyond which fewer patients consistently recorded PRO data). The data cutoff date was 27 January 2025.

**Results:** There were 263 patients with CLL included in the final analysis with a median (interquartile range) follow-up of 46.5 months (35.5–54.7) from study enrollment. Median age at enrollment was 68 years (62.0–74.0), and the majority were male (68.1%). The completion rate of all PRO measures was 82% at baseline. The baseline mean (± standard deviation) score was 80.8 (±19.5) for PF, 61.6 (±23.2) for GHS/QoL, 24.7 (±18.4) for CLL/SLL-related symptoms, and 33.4 (±24.7) for fatigue. Median time to worsening was not reached for any of the PRO endpoints due to the lack of observed worsening or death events among patients treated with pirtobrutinib. The censoring rates for the time to worsening analyses were high (i.e. 76.9% for PF, 78.1% for GHS/QoL, 78.8% for CLL/SLL-related symptoms, and 73% for fatigue). Most patients reported stable or clinically improved PROs at each of the post-baseline visits through Cycle 30. The proportion of patients who improved or remained stable from baseline through Cycle 30 ranged from 73.2% to 94.1% for PF, 80.4% to 92.0% for GHS/QoL, 86.3% to 94.7% for CLL/SLL-related symptoms, and 78.7% to 91.1% for fatigue.

**Conclusion:** Consistent with the known favorable safety and efficacy profile of pirtobrutinib, the final analysis of the BRUIN Phase 1/2 trial demonstrated that each PRO endpoint evaluated remained consistently stable or improved over time in more than 70% of patients with R/R CLL/SLL. Data are also limited in that completion of PRO instruments declined over time, limiting the ability to evaluate outcomes beyond Cycle 30. While this is a single arm trial, the data demonstrate that pirtobrutinib provided meaningful benefit to patients during treatment.

**Keywords:** Patient-reported outcomes; relapsed/refractory; pirtobrutinib

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**Please indicate the name of the funding organization:** Loxo Oncology Inc., a wholly owned subsidiary of Eli Lilly and Company

## Abstract ID: 1709

### **Title:** Impact of dose intensity of ibrutinib on clinical outcomes of CLL patients: analysis of the EVIdeNCE study

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**Background:** Despite the introduction of second-generation BTK inhibitors (BTKis), ibrutinib remains central to chronic lymphocytic leukemia (CLL) management. However, dose reductions due to adverse events may compromise efficacy, underscoring the importance of sustained adherence.
**Aim:** This study investigates factors associated with ibrutinib dose reduction and assesses the impact of adherence to the full prescribed dose on progression-free (PFS) and overall survival (OS) in patients with CLL treated in routine clinical practice. Particular focus is given to adherence during the first 90 days, a period critical to long-term outcomes.
**Methods:** We analyzed data from the EVIdeNCE study (NCT03720561), a multicenter, observational, prospective, cohort study conducted at 39 hematological institutions in Italy between November 2018 and October 2019. The study enrolled consecutive adult patients with a confirmed diagnosis of CLL who started treatment with ibrutinib in either first-line or more advanced line as part of routine clinical practice.
Adherence was calculated for each patient, as the ratio between the average daily dose received during the specified timeframe (i.e. total cumulative dose divided by the number of days) and the full prescribed daily dose of 420 mg.
A 100% adherence to treatment indicates that patients received the full daily dose throughout the evaluated period. Treatment interruptions or discontinuations were accounted for by assigning a dose of 0 mg per day for the corresponding days.
For the purpose of the analysis, ibrutinib adherence was assessed at 30, 60, and 90 days of treatment.
Finally, we analyzed the associations between adherence and clinical outcomes (PFS and OS) using both unadjusted and multivariable models. Models were adjusted for patient and disease characteristics.
**Results:** Data from 275 CLL patients receiving ibrutinib either as a first-line (37%) or subsequent line (67%) treatment were analyzed. Among them, 82.2% received the full dose of ibrutinib (420 mg), while 17.8% required dose reductions (26 patients at 280 mg and 23 at 140 mg). Dose adjustments were equally distributed among patients who received ibrutinib as first or subsequent line of therapy ($p = 0.956$).
Patients in the reduced-dose cohort exhibited significantly higher median CIRS scores (5 vs. 3, $p = 0.022$) and poorer ECOG PS ($p < 0.001$) compared to those who maintained the full dose. While age differences approached significance ($p = 0.076$), no significant differences were observed in CIRS subdomains or specific comorbidities, including cardiovascular diseases ($p = 0.262$).
Adherence to the full prescribed dose declined over time—73.8% at 30 days, 70.3% at 60 days, and 65.7% at 90 days—yet approximately 80% of patients maintained ≥80% adherence throughout.
In the unadjusted analyses, initial dose reduction did not significantly affect OS (HR: 1.19, 95% CI: 0.52–2.39) or PFS (HR: 1.23, 95% CI: 0.63–2.39). In contrast, maintaining full treatment adherence (100%) at 90 days was significantly associated with improved PFS (HR: 1.90, 95% CI: 1.09–3.33), particularly among patients with high-risk disease features (TP53 mutations or deletion 17p, advanced Rai stage; PFS: HR 2.07, 95% CI: 1.17–3.70).
However, this association was no longer observed after adjusting for patient-related factors, including advanced age and high comorbidity burden (OS: HR 1.47, 95% CI: 0.67–3.24; PFS: HR 1.23, 95% CI: 0.65–2.31).
**Conclusions:** In this analysis of the EVIDENCE trial, maintaining 100% adherence to the prescribed ibrutinib dose at 90 days was significantly associated with improved PFS in patients with high-risk CLL. Conversely, among elderly or unfit patients, an adherence rate of at least 80% appeared sufficient to achieve comparable clinical outcomes. These findings underscore the importance of individualized adherence strategies—highlighting the critical role of strict adherence in high-risk patients, while lower adherence thresholds may remain appropriate for select patient subgroups.

**Keywords:** Chronic lymphocytic leukemia; ibrutinib

**Please indicate how this research was funded:** Company sponsored study

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## Abstract ID: 1219

### **Title:** The immunogenicity landscape of CLL and its association with patient outcome

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Although the molecular landscape of CLL has been thoroughly explored, its immunogenicity landscape, vital for

designing and predicting response to immunotherapies, remains largely uncharted. Additionally, despite its indolent nature, the clinical trajectory of CLL patients is diverse, with survival periods varying extensively. One significant factor contributing to this variation could be the intrinsic immunogenicity features of CLL, which are yet to be systematically assessed.
We, therefore, aimed to harness the vast multiomic data within the CLL-map cohort (n = 1,148) to reveal a compendium of predicted neoantigens across CLL patients, deduce each patient's immunogenicity status, and associate these immunogenomic attributes with patient outcome.
In further detail, we investigated four aspects of CLL immunogenicity and immunoediting and their impact on patient survival, using the CLL-map dataset. First, CLL neoantigens from diverse sources were predicted, leveraging both point mutation data and non-canonical sources (e.g. gene fusions, aberrant splicing and endogenous retrovirus expression). Second, we quantified intrinsic immunogenicity features across CLL patients by examining baseline antigen presentation capacity and costimulatory/coinhibitory potential. Third, we searched for sources of immunoediting as a source of immune evasion. Last, we evaluated the effects of neoantigen load, immunoediting and immunogenicity on CLL patient outcomes.
Our results demonstrate that delineating CLL's immunogenic landscape can unveil tentative mechanisms of immune dysregulation and evasion in CLL, potentially paving the way to the design of effective immuno-therapeutic interventions. Given the current limited success of immunotherapy in CLL, a deeper profiling of CLL's intrinsic immunogenic features may identify the subset of patients that can benefit from immunotherapy and improve patient selection for clinical trials. Finally, the computational tools and insights gained do not only shed light on this blood malignancy but can also provide a foundation for similar analyses in other malignancies.

**Keywords:** CLL; immunogenicity; survival

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**Please indicate the name of the funding organization:** American Society of Hematology

Israel Science Foundation

## Abstract ID: 1430

### **Title:** Assessing measurable residual disease (MRD) by cell-free DNA (cfDNA): results from a real-world series

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**Introduction:** Measurable residual disease (MRD) correlates with outcome in a variety of hematological malignancies, including chronic lymphocytic leukemia (CLL). The techniques most frequently employed to detect MRD are flow cytometry (FC) and molecular approaches including the detection of Ig rearrangement by high-throughput sequencing (HTS) or real-time quantitative PCR (RQ-PCR). While cell-free DNA (cfDNA) is widely used in lymphomas, in CLL there is little information on whether cfDNA could be more representative of the tumor burden than other methods. Against this background, we aimed to define the role of cfDNA, tentatively compared to other techniques, to assess MRD in patients with CLL.
**Methods:** This is a non-randomized, prospective, and observational study in which patients with CLL that required therapy (either treatment-naïve or relapsed/refractory) according to iwCLL guidelines.
Peripheral blood samples were prospectively collected before treatment initiation, at the time of response, and every 3 months thereafter up to 2 years. Bone marrow samples were collected at the time of response whenever possible.
MRD was assessed by multiparameter FC (by 6-colour, CD19, CD43, CD20, CD81, CD79b, and CD5) and clonoSEQ®. For the detection of the rearranged IGHV sequence, genomic DNA was extracted from PMN cells and sent to Adaptive alongside with frozen plasma samples. The cut-off used for MRD positivity by HTS was above $10^{-6}$ (LOD) and $10^{-4}$ in flow MRD samples.
**Results:** Between 2020 and 2024, 55 patients with CLL who required therapy were enrolled. Here we show the preliminary data from the first 49 patients. Ten patients were excluded due to progressive disease (n = 7), premature dead (n = 2), and screening failure (n = 1). Among responders (n = 39), median age was 69 (range 42–86) and 41% were male. Twenty patients were treated with BTKi (19 ibrutinib, 1 acalabrutinib), 15 with venetoclax-based regimens (4 with obinutuzumab, 4 with rituximab, 3 with ibrutinib, and 4 in monotherapy), and 5 with FCR; 28 were treatment-naïve and 11 relapsed/refractory. Twenty patients (51.3%) achieved complete response and 19 (48.7%) partial response. At the first post-treatment evaluation, 9/26 (34.6%) achieved undetectable (uMRD) by FC, 10/21 (47.6%) by cfDNA and 9/26 (34.6%) by gDNA. MRD follow-up was available for 26 patients. The median number of MRD determinations was 2 (range 1–5). Both cfDNA (56 samples from 22 patients) and gDNA (68 samples from 26 patients) results from peripheral blood samples were moderately correlated with FC (r = 0.48, $p < 0.01$; r = 0.62, $p < 0.0001$), independent of type of therapy. In addition, the correlation between cfDNA and gDNA determinations was very high (r = 0.92, $p < 0.0001$).
When comparing cfDNA vs FC, the sensitivity and specificity were 68.6% and 76.2%, and for gDNA 79.1% and 60%, respectively. The positive and negative predictive values for cfDNA were 82.8% and 59.3%, and for gDNA they were 77.3% and 62.5%, respectively. There were 16/56 (28.6%) discordant results in cfDNA and 19/68 (28%) in gDNA vs FC. We further explored the assessment of MRD using cfDNA in patients who had detectable lymph nodes by CT scan vs those who did not. The cfDNA was positive in 9/12 (75%) patients with lymph nodes vs 3/10 (30%) without them [OR 6.31 (95% CI 0.80–68.43, $p = 0.08$)]. Of note, in this setting cfDNA did not show an increased sensitivity compared to either gDNA or FC MRD detection.

**Conclusions:** In summary, these results show that cfDNA HTS did not increase sensitivity or specificity over genomic DNA nor multiparametric FC MRD assessment including patients with lymph nodes. Collectively, there was a strong correlation between cfDNA and gDNA, suggesting that cfDNA MRD monitoring in CLL is feasible.

**Keywords:** cfDNA; MRD; clonoSEQ

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## Abstract ID: 1213

### **Title:** Characterizing the tumor microenvironment in CLL: the role of NK cells and prognostic gene markers

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**Background:** Natural killer (NK) cells are critical regulators within the tumor immune microenvironment (TME) of chronic lymphocytic leukemia (CLL), yet their systematic profiling for clinical prediction remains unexplored. This study establishes an NK cell-centric framework to predict patient outcomes and therapeutic responses.
**Methods:** Using scRNA-seq data from 52,784 CLL cells, we identified 265 NK cell marker genes (NKGs) through Seurat-based clustering (resolution =0.3) and CellMarker annotation. Prognostic NKGs were refined via multi-split survival analysis (ICGC cohort, n = 487) and consensus clustering. Differential expression analysis (|logFC|>1, adj.P.Val< 0.01) and functional enrichment (GO/KEGG) delineated NK subtypes. A five-gene prognostic signature was constructed through 500-replicate LASSO-COX regression and stepwise selection. Immune landscapes were characterized via ESTIMATE scores, TCR repertoire analysis, and immunophenoscore.
**Results:** Consensus clustering stratified CLL patients into two NK subtypes: Subtype 1 demonstrated superior overall survival (OS, $p < 0.001$), enriched IGHV mutations (OR =2.1, $p = 0.007$), and heightened immune cytolytic activity, while Subtype 2 exhibited advanced Rai stage predominance (68% vs 32%, $p = 0.002$) and TP53/ATM mutational enrichment (q< 0.01). The five-gene NKscore robustly discriminated high- risk and low-risk groups (median cutoff), with high-risk patients showing significantly worse OS (HR =3.21, 95% CI 2.18–4.73, $p < 0.001$), progression-free survival (HR =2.67, $p < 0.001$), and disease-specific survival (HR =4.02, $p < 0.001$), corroborated by 5-year time-dependent ROC analysis (AUC =0.84). Mechanistic profiling revealed high-NKscore tumors harbored an immunosuppressed microenvironment characterized by depleted TCR clonality (Shannon index: 5.2 vs 8.1, $p = 0.008$), elevated T-cell exhaustion markers, reduced ESTIMATE ImmuneScore (-412 vs +285, $p < 0.001$), and TGF-β pathway activation (FDR< 0.01), collectively conferring resistance to PD-1/CTLA-4 inhibitors (Immunophenoscore: 0.32 vs 0.68, $p = 0.004$) and ibrutinib (IC50: 1.8 μM vs 0.7 μM, $p = 0.02$). Conversely, low-NKscore tumors displayed inflamed microenvironments with Th1/Th17 polarization, CXCL9/10 upregulation (>5-fold), and enhanced chemokine signaling. Integration of NKscore into a clinical nomogram significantly improved 5-year survival prediction (C-index: 0.79 vs 0.68, $p = 0.003$).
**Conclusion:** We pioneer an NK cell-originated prognostic system that quantifies TME immunosuppression and predicts immunotherapy efficacy in CLL. This work positions NK cells as master regulators of tumor evolution and provides a clinically actionable tool for risk stratification and personalized therapy selection.

**Keywords:** Chronic lymphocytic leukemia; NK cell; prognosis

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## Abstract ID: 1905

### **Title:** Academic CD19-directed CAR-T ARI-0001 (varnimcabtagene autoleucel) for patients with relapsed/refractory chronic lymphocytic leukemia and Richter's transformation

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**Introduction:** Patients with CLL refractory to both BTK and BCL2 inhibitors show poor outcomes with no standard treatment available besides clinical trials. CD19-directed CAR-T can achieve complete responses even in those with high-risk genomics (i.e. TP53 aberrations). Still, few patients attain long-term remissions with currently approved products (median PFS around 12 months), and their availability worldwide is scarce. Additionally, those presenting with Richter's transformation (RT) have no CAR-T products approved for.

We report the results of our academic CD19-directed CAR-T product (varnimcabtagene autoleucel, or ARI-0001) in patients with R/R CLL or RT.

**Methods:** This study included subjects with CLL or RT treated with ARI-0001 in the CART19-BE-01 trial (NCT03144583) and in the subsequent compassionate use program.

ARI-0001 is an autologous, CD19-directed, second-generation 4-1BB costimulated CAR-T product fully developed at Hospital Clínic (Barcelona, Spain) currently approved in Spain for the treatment of R/R B-cell acute lymphoblastic leukemia. Patients were infused in single or fractionated dosing (10%, 30%, and 60%) with 0.1–1.0 or 0.5–5.0 × 10e6 ARI-0001 cells/kg (CLL or RT, respectively), after lymphodepleting chemotherapy with fludarabine 30 mg/m$^2$/day and cyclophosphamide 300 mg/m$^2$/day on days -6 to -4.

**Results:** We included 29 patients treated with ARI-0001 between 2017 and 2024, 13 with CLL and 16 with RT. The median age was 60 years (range 29–78), 11 (38%) were female. Patients had received a median of 3 prior lines (range 2–7). Virtually all had been previously exposed to ibrutinib (97%) and 20 (69%) to venetoclax. Most cases with RT (94%) had received prior R-CHOP as frontline therapy. Five RT individuals had also undergone previous allogeneic stem cell transplantation. The population was comprised of mostly high-risk patients, including 19 (90%) with unmutated IGHV, 21 (78%) with TP53 aberrations, and 11 (50%) with complex karyotype. The CLL cohort included 7 patients (54%) with Binet C stage; 11 had a CLL-IPI of ≥4 (85%). Regarding the RT cohort, 9 patients (58%) were stage IV and 11 (69%) showed elevated LDH values. Five cases were clonally related, the others were unknown.

Bridging therapy was employed in all patients but one (one RT): in CLL usually BTKi (30.4%) or venetoclax with (22.7%) or without ibrutinib (30.8%), while in RT chemotherapy regimens with (20.1%) or without ibrutinib (20.1%). All but two (1 CLL and 1 RT) received ARI-0001 in a fractionated manner.

The median vein-to-vein time was 35 days (29–50); ARI-0001 was manufactured in a median of 8 days (IQR 7–8). Most patients received the full target dose of ARI-0001 (62%). Although 90% of the patients developed cytokine release syndrome (CRS) only 3.4% were grade 3 or higher. Nineteen (66%) patients received tocilizumab, and 9 (31%) steroids for their CRS. There were only two cases (6.9%) of neurotoxicity (ICANS), all of them grade ≤2, treated with steroids. Median time to CRS and ICANS onset was 1 (IQR 1–3) and 7 days (IQR 1–13), respectively.

The overall response rates (ORR) and complete response rates (CR) in the entire cohort were 86.2% and 75.9%, respectively; in the CLL cohort, the ORR (CR) was 84.6% (84.6%), and in Richter's 87.6% (68.8%). With a median follow-up of 1.3 years, the median duration of response, progression-free survival (PFS), and overall survival (OS) were not reached for either CLL or RT patients. The estimated 1- and 4-year PFS were 80% and 60%, respectively. There were no differences in outcomes with respect to the ARI-0001 dose received. The median duration of B-cell aplasia was not reached, and no difference was seen between CLL and RT. It was achieved in 93% of patients, in a median of 14 (IQR 7–20) days. CAR-T expansion peak occurred at day 15 (IQR 13–35). The median AUC (0–30 days) for complete responders was significantly higher (28.79 vs 6.59, $p = 0.03$), indicating that non-responders showed lower var-cel expansion, with no differences between CLL and RT. In fact, patients who did not reach CR exhibited inferior outcomes than those who did (mOS 4.7 months, $p < 0.0001$). The median duration of circulating CAR T-cells was 3 years.

**Conclusions:** ARI-0001 achieves a high proportion of complete responses and long-term CAR-T persistence in patients with R/R CLL and RT, with a very favorable safety profile in this high-risk population with great tumor burden, altogether accompanied by a negligible ICANS incidence.

**Keywords:** CAR-T; ARI-0001; var-cel

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**Please indicate the name of the funding organization:**

## Abstract ID: 1431

### **Title:** The role of minimal residual disease in chronic lymphocytic leukemia

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**Introduction:** Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) are incurable B-cell malignancy with a heterogeneous clinical course especially those with high risk factors. Ibrutinib combined with fludarabine, cyclophosphamide, and rituximab (iFCR) as a time-limited regimen in treatment naïve patients with CLL/SLL demonstrated remarkable efficacy. MRD (Measurable Residual Disease) was considered as a surrogate endpoint of PFS. This study aimed to explore the application of MRD in efficacy assessment, prognostic stratification and discuss the clinical factors influencing the achievement of undetectable MRD (uMRD) under the time-limited therapies based on BTK inhibitors combined with FCR in CLL/SLL.

**Methods:** This study retrospectively enrolled 40 patients treated with Bruton tyrosine kinase inhibitor (BTKi) in combination with FCR regardless of TP53 aberrations and IGHV status. A 0–7-day lead-in period with single-agent ibrutinib (420 mg daily) was feasibly allowed, followed by intravenous rituximab, fludarabine and cyclophosphamide, every 28-day cycle, up to a maximum of 6 cycles according

to the response and tolerance of patients. Ibrutinib was given continuously after combination therapy and discontinued after patients achieved sustaining uMRD ($<10^{-4}$). uMRD was defined as less than one CLL cell per 10,000 leukocytes by FCM. [Miao Y, Sha Y, Xia Y, et al. Blood Cancer J. 2023;13(1):121. doi:10.1038/s41408-023-00890-y]. MRD recurrence was defined as two consecutive values of ≥0.01% after achieving uMRD. The primary outcome was the rate of PB-uMRD and BM-uMRD. MRD results in peripheral blood (PB) and bone marrow (BM) at different timepoints detected by Flow Cytometry (FCM) with a threshold of $10^{-4}$ and Next Generation Sequence (NGS) with a threshold of $10^{-6}$ during treatment and after discontinuing drugs were collected. Correlation between MRD and response was analyzed. PB-MRD was monitored by FCM during long-term follow-up and the influencing factors of uMRD ($<10^{-4}$) were investigated.
**Results:** This study retrospectively enrolled 40 patients treated with Bruton tyrosine kinase inhibitor (BTKi) in combination with FC and rituximab (R) between March 2019 and April 2025. The Median follow-up time was 51.3 months (95% CI: 45.5–57.1) and median PFS and median OS were not reached. After 3 cycles of treatment, 52.6% (20/38) and 38.7% (12/31) patients attained PB-uMRD ($<10^{-4}$) and BM-uMRD ($<10^{-4}$) by FCM, and improved to 63.9% (23/36) and 55.9% (19/34) at the end of combination therapy. Patients who achieved PB-uMRD ($p=0.033$) by FCM at the end of combination therapy were more likely to achieve CR/CRi. PB-MRD was monitored by FCM during long-term follow-up. Significant differences in unmutated IGHV ($p=0.007$), 17p deletion ($p=0.014$), 11q deletion ($p=0.014$), TP53 mutation ($p=0.012$) and NOTCH1 mutation ($p=0.042$) were observed between patients with disease progression and patients with sustaining uMRD ($<10^{-4}$). Compared with patients with sustaining uMRD ($<10^{-4}$), patients with relapsed MRD were enriched with 11q deletion ($p=0.036$). Among patients who achieved uMRD ($<10^{-4}$), the duration of uMRD ($<10^{-4}$) was shorter in patients with unmutated IGHV ($p=0.045$) and/or NOTCH1 mutation ($p=0.039$).
**Conclusions:** In this time-limited regimen which combined BTKi with FCR, MRD status at the end of combination therapy could serve as a complementary prognostic factor in CLL/SLL. uMRD at the end of combination therapy indicated a better response. The long-term dynamic monitoring of PB-MRD by FCM could assist in distinguishing patients who are at high risk of disease progression, which may be applied in clinical practice.

**Keywords:** CLL/SLL; MRD; combination therapy

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## Abstract ID: 1525

**Title:** Updated efficacy and safety of the Bruton tyrosine kinase degrader BGB-16673 in patients with relapsed or refractory chronic lymphocytic leukemia/small lymphocytic lymphoma: results from the ongoing phase 1 CaDAnCe-101 study

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**Background:** Bruton tyrosine kinase (BTK) inhibitors are effective treatments for chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), but intolerance and/or acquired resistance due to BTK mutations can emerge. BGB-16673 is a protein degrader that blocks BTK signalling by tagging BTK for degradation through the cell's proteasome pathway.
**Aims:** To describe updated phase 1 results for patients with relapsed/refractory (R/R) CLL/SLL in CaDAnCe-101 (BGB 16673-101; NCT05006716), a phase 1/2 open-label study.
**Methods:** Eligible patients must have confirmed R/R CLL/SLL (≥2 prior therapies), an Eastern Cooperative Oncology Group performance status of 0–2, and adequate organ function. In the US/EU/Australia, patients must have previously received a covalent BTK inhibitor (cBTKi). BGB-16673 was dosed once daily orally. Primary objectives were to assess safety/tolerability (CTCAE v5.0; iwCLL hematologic toxicity criteria) and establish the maximum tolerated dose and recommended dose for expansion. A secondary objective was to assess overall response rate (ORR) (iwCLL 2018 criteria with partial response with lymphocytosis [PR-L] modification; 2014 Lugano criteria for SLL).
**Results:** As of 17 December 2024, 66 patients with CLL/SLL were enrolled and treated (50 mg, n = 1; 100 mg, n = 22; 200 mg, n = 16; 350 mg, n = 15; 500 mg, n = 12). Median age was 70 (range 47 91) years; the median number of prior therapies was 4 (range, 2–10), including prior cBTKis (n = 61; 92.4%), BCL2 inhibitors (BCL2is; n = 54; 81.8%), and noncovalent BTKis (ncBTKis; n = 14;21.2%). In total, 65.2% (43/66) of patients had del(17p) and/or TP53 mutation and 79.6% (39/49) had unmutated IGHV. Median follow-up was 13.1 (range, 0.3–29.9) months. Overall, 92.4% of patients had any-grade treatment-emergent adverse events (TEAEs; grade ≥3, 51.5%); those in ≥30% of patients were fatigue (36.4%; grade ≥3, 1.5%) and contusion/bruising (30.3%; no grade ≥3). Grade ≥3 TEAEs in ≥10% of patients were neutropenia/neutrophil count decreased (21.2%) and pneumonia (12.1%). Atrial fibrillation (grade 1 in the context of bacterial pneumonia) and febrile neutropenia (in the context of COVID-19 pneumonia and norovirus diarrhea) occurred in 1 pt (1.5%) each. Hypertension (n = 2, both grade 3) and major hemorrhage occurred in 2 patients each (3.0%, grade 1 subarachnoid hemorrhage resolved; grade 3 subdural hemorrhage outcome unknown). Six patients (9.1%) had a TEAE leading to dose reduction. Four patients had TEAEs that led to death (pneumonia in the context of disease progression, septic shock, bronchopulmonary

and cerebral aspergillosis, and acute respiratory failure; n = 1 each); no deaths were deemed related to BGB-16673. In 66 response-evaluable patients, ORR (PR-L or better) was 80.3% (n = 53), and complete response (CR)/CR with incomplete recovery (CRi) rate was 3.0% (n = 2). At 200 mg, ORR was 93.8% (15/16), including 1 CR. Median time to first response was 2.8 (range, 2.0–10.9) months. Thirty-three patients (50.0%) remained on treatment for ≥12 months; 38 patients had ongoing responses. Responses deepened over time: of 19 patients with initial PR-L, 10 transitioned to partial response (PR) and 1 to CR; of 15 patients with initial stable disease, 1 transitioned to PR-L, 5 to PR, and 1 to CRi. Responses were seen at the lowest dose (50 mg, 1/1); in patients previously treated with a cBTKi (49/61; 80.3%) or ncBTKi (10/14; 71.4%) and with double- (cBTKi and BCL2i; 36/41; 87.8%) and triple-exposure (cBTKi, BCL2i, ncBTKi; 9/12; 75.0%); and in patients with (17/24; 70.8%) and without (33/39; 84.6%) BTK mutations, and with del(17p) and/or TP53 mutation (33/43; 76.7%). Median progression-free survival was not reached.

**Summary/conclusion:** Data from this ongoing study demonstrate that the novel BTK degrader BGB-16673 has a tolerable safety profile and shows robust and deepening responses in patients with heavily pretreated R/R CLL/SLL, including those with prior BTKi treatment and BTKi mutations.

**Keywords:** Chronic lymphocytic leukemia; dose escalation; phase 1

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## Abstract ID: 1906

### **Title:** Fibroblast mediated modulation of bispecific antibody treatment in chronic lymphocytic leukemia

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**Introduction:** Chronic lymphocytic leukemia (CLL) survival and progression are critically supported by the tumor microenvironment (TME) (Burger and Gribben, 2014). Fibroblasts, as key TME components at homing sites, can acquire a CLL-induced cancer associated fibroblast (CAF) phenotype that promotes disease progression and therapy resistance (vom Stein et al, 2023). Due to their immunosuppressive properties, fibroblasts may affect the efficacy of immunotherapies such as bispecific antibodies (BsAb), which are generally less effective in CLL compared to other B cell malignancies (Apollonio et al., 2023; Falchi et al., 2023). While CAFs have been extensively studied in solid tumors, their role in modulating immune responses in CLL is poorly understood. This study investigates how stromal fibroblasts influence BsAb efficacy in CLL and whether stromal modulation enhances immunotherapy responsiveness.

**Methods:** In vitro coculture assays were established using stromal cell lines and peripheral blood mononuclear cells (PBMC) from healthy donors and CLL patients. CD3/CD20 BsAbs and stromal modulators were applied, and effects on CLL cell viability and T cell phenotype including activation and exhaustion markers were assessed via flow cytometry. Additionally, an ex vivo coculture system using patient-derived lymph node fibroblasts and autologous CLL cells was developed.

**Results:** Our data demonstrates that various stromal cells support the viability of leukemic cells, consistent with previous reports. CLL PBMCs cocultured with stromal cells exhibited an enhanced response to BsAb treatment, whereas CLL PBMCs cultured without stromal support showed limited sensitivity. This establishes stromal coculture systems as a valuable model to study BsAb efficacy in primary CLL cells. The strongest effects were observed in autologous cocultures using patient-derived primary fibroblasts. Furthermore, coculture with stromal cells led to increased expression of T cell activation markers, indicating enhanced T cell cytotoxic activity and alterations in the composition of $CD8^+$ and $CD4^+$ T cell subsets. Notably, BsAb treatment induced a strong reduction in CD8 T cell frequencies, independent of the culture condition. Interestingly, stromal TGFβ receptor inhibition promoted CLL cell survival in the absence of BsAb, yet in combination with BsAbs, it increased cytotoxicity, implying that modulation of stroma cells affects BsAb efficiency.

**Conclusion:** This study indicates the potential of targeting the tumormicroenvironment in CLL, specifically stromal cells, to enhance immunotherapy efficacy.

**Keywords:** Bispecific antibodies; cancer associated fibroblasts; tumormicroenvironment

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## Abstract ID: 1008

### **Title:** Clinical characteristics and outcome of chronic lymphocytic leukemia patients with renal involvement in the era of target drugs

**Authors:** Ling Gao[a,b], Jiaojiao Zhang[c], Xiaoqian Liu[d], Lixia Sheng[e], Tong-lu Qiu[a], Wei Zhang[b], Minjie Yu[a], Luomengjia Dai[a], Ziyuan Zhou[a], Lei Fan[f], Yi Xia[a], Jianyong Li[g] and Huayuan Zhu[a]

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**Background:** Chronic lymphocytic leukemia (CLL) is a mature B lymphocyte clonal proliferative tumor, and some patients may involve the kidneys. This study reports on the clinical characteristics, pathological types, treatment and outcomes in the new drug era of CLL patients with renal dysfunction and pathological diagnosis of concomitant kidney disease in multiple centers in China.
**Methods:** From January 2017 to October 2024, 13 patients with CLL who underwent renal biopsy in four centers in China and were pathologically confirmed as having renal lesions were included. The indications for renal biopsy included hematuria, proteinuria, renal insufficiency, or nephrotic syndrome, and all other secondary causes leading to chronic kidney disease were excluded. Clinical data such as complete blood count, biochemical profile, urinalysis, 24-hour urine protein, peripheral blood flow cytometry, IGHV mutation, chromosomal analysis, and genetic mutations were collected. Pathological markers of the kidneys, including CD20, CD3, CD5, CD10, CD23, LEF-1, and CyclinD1, were also analyzed. The clinical characteristics, pathological features of the kidneys, and treatment outcomes of these patients were evaluated.
**Results:** Thirteen patients with CLL/SLL with renal involvement were included, nine were CLL and four were SLL, with a median age of 58 years (range 40–72 years). Proteinuria, hematuria, renal dysfunction, and nephrotic syndrome were found in 92.3%, 38.5%, 50%, and 7.7%, respectively. The median time from diagnosis to renal biopsy was 40.7 months (range 0.2–159 months), 12 patients were treatment naïve before renal biopsy and the other had undergone alkylating agents and chemotherapy previously. Interstitial infiltration by tumor cell, membranoproliferative glomerulonephritis, and membranous nephropathy were identified in 38.5% (5/13), 23.1% (3/13), and 15.4% (2/13) of patients, respectively. Ninety percent (10/11) of patients were IGHV mutated and 62.5% (5/8) was low risk stratified by CLL-IPI. Meanwhile, 23.1% (3/13) were with monoclonal immunoglobulin and 15.4% (2/13) had cryoglobulinemia. All patients exclude one received target drugs-based regimen after biopsy, including six patients with BTK inhibitors (BTKi) plus CD20 monoclonal antibody, two patients with BTKi monotherapy, one with CD20 monotherapy antibody, one with BTKi plus Fludarabine, cyclophosphamide, obinutuzumab, another with BTKi plus Bendamustine, obinutuzumab, and the other with venetoclax plus obinutuzumab. As of 15 May 2025, the median follow-up was 18.5 months (range 8–31 months), with 11 patients were available for efficacy evaluation. According to iwCLL criteria for CLL and Lugano criteria for SLL, best overall response rate was 100% and complete remission was 67%. As for the improvement for renal according to KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases, best overall response rate was 100% and complete remission was 33.2%. Four patients achieved CR in both CLL and kidney efficacy assessments. The median time to PR in the renal efficacy assessment was 6.03 months (range 3.1–13.3 months); The median time to achieve PR in the efficacy evaluation of CLL/SLL was 3.47 months (range 2.52–6.07 months), and the median time to achieve CR was 6.27 months(4.03–14.9) months. The median PFS was not reached.
**Conclusion:** Majority of CLL patients with renal involvement were with IGHV mutated status and often with favorable prognosis according to CLL-IPI low risk. The most renal pathological type was primarily interstitial infiltration nephritis and membranoproliferative glomerulonephritis. The outcome of CLL and improvement of renal abnormalities were satisfied under treatment of new drugs.

**Keywords:** Chronic lymphocytic leukemia; renal involvement; targeted drugs

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

# Abstract ID: 1112

## **Title:** Artificial intelligence–driven clustering of peripheral blood cell morphology reveals disease heterogeneity and prognostic relevance in CLL

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**Background:** Real-time monitoring is essential for the management of chronic lymphocytic leukemia (CLL) patients. Peripheral blood analysis offers advantages of affordability, convenience, and suitability for longitudinal disease tracking. However, current blood film evaluation methods lack automation and are subject to observer bias, affecting reproducibility and clinical translation. Moreover, CLL exhibits complex morphological and biological heterogeneity, with subtle cellular changes potentially heralding disease progression or transformation. To address these unmet needs, we developed an AI-driven system for objective, high-throughput evaluation of peripheral blood cell morphology in CLL.
**Methods:** Peripheral blood cell morphology were collected from blood films using whole-slide imaging at high resolution. Regions of interest (ROI) were automatically selected using a ResNet-18 based deep neural network classifier, and lymphocyte segmentation and feature extraction were performed via a VGG16 convolutional neural network. Based on this AI-driven system, we calculated the distribution of lymphocyte cell areas and analyzed different patterns of each patient and cell. Both unsupervised clustering and manual pattern recognition were performed based on cell size distribution curves and distinct morphological cell types. Then we systematically correlated morphological clusters and patient cluster types with key molecular markers, laboratory parameters, survival data. A multiple instance learning (MIL) model was built using aggregated cell features to predict clinical and molecular risk profiling.
**Results:** Building on our previous work, we conducted a large-scale retrospective study of 260 CLL patients at our center, extracting morphological features from over 1.5 million peripheral blood cells using a high-throughput, AI-driven imaging pipeline. Cell size areas analysis revealed patient-specific lymphocyte distribution patterns and peak types, reflecting underlying morphological heterogeneity. Clustering based on cell size distribution patterns

successfully stratified patients into groups (e.g. unimodal-narrow, unimodal-broad, and multimodal). Notably, patients with broad/multimodal peaks pattern showed significantly shorter overall survival (OS) ($p = 0.04$) and were enriched for high-risk molecular features, including TP53 mutation and complex karyotype, indicating worse prognosis linked to underlying morphological diversity. For each patient, the proportion of different cell categories was quantified, and unsupervised mass clustering at single-cell level defined distinct morphological cell types, resulting in five robust patient clusters. This patient-level composition of morphology-based cell clusters was strongly correlated with key molecular risk factors and could inform individualized patient monitoring. Furthermore, we developed a MIL model built on aggregated cell features to clinical and molecular features. This MIL model achieved an AUC of 0.83 for the prediction of TP53 mutation and 0.68 for complex karyotype, highlighting the translational potential of cell morphology as a noninvasive biomarker for integrated risk prediction in CLL.

**Conclusion:** Our large-scale, AI-driven morphologic analysis reveals not only striking inter-patient heterogeneity in CLL blood cell phenotype but also robust associations between cell morphology, molecular features, and clinical outcomes. This approach offers a scalable avenue for routine, objective, and high-throughput risk monitoring in CLL and provides a template for future multi-center validation and real-world clinical integration.

**Keywords:** Artificial intelligence; peripheral blood cell morphology; chronic lymphocytic leukemia

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## Abstract ID: 1805

### **Title:** Targeting the p53/xCT/GSH axis with PRIMA-1Met combined with sulfasalazine shows therapeutic potential in chronic lymphocytic leukemia

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Chronic lymphocytic Leukemia (CLL) is a highly heterogeneous form of leukemia with a heterogeneous clinical course ranging from a rapid disease progression to decades of survival without the need for treatment. At diagnosis CLL is characterized by complex karyotype aberrations, the most frequent being partial deletions at 13q (~55%), 11q (~15%), 17p (~8%), gain of chromosome 12 (~15%), and by gene mutations at different genes including TP53, SF3B1, BIRC3, NOTCH1, and ATM. The TP53 tumour suppressor gene encodes a tetrameric transcription factor that regulates a multitude of key pathways, including cell cycle control, DNA damage response, apoptosis, senescence, DNA repair, cell migration, autophagy, and energy metabolism. Mutations in the TP53 gene represent a significant hallmark of the disease, as they can significantly impact the success of treatment protocols. A low incidence of TP53 mutations, ranging from 5% to 7%, is found at diagnosis, while it increases to approximately 40% in refractory CLL. Thus, the deletion at chromosome 17p13.1 (del17p) and/or TP53 mutations are considered the most important predictive biomarkers in CLL. Nowadays, new treatments are available for CLL patients carrying TP53 aberrations, such as ibrutinib and idelalisib, which inhibit the signalling pathway initiated by the B-cell antigen receptor, or venetoclax, which facilitates cell apoptosis. However, with these new drugs, TP53 alterations may be associated with therapy resistance. In recent years, several molecules that can target mutant TP53 for reactivation have been isolated. PRIMA-1Met/APR-246 is the most clinically advanced mutant p53-targeting agent, which has been shown to reactivate wild-type p53 apoptotic functions. Furthermore, PRIMA-1Met can deplete the glutathione (GSH) reservoir, thereby leading to an increased production of reactive oxygen species (ROS). The GSH level can also be diminished by Sulfasalazine (SAS), an anti-inflammatory drug belonging to the class of sulphonamides. This drug inhibits the xC- transporter, a key component of the xC- system that imports cystine, a dimer of cysteine, the limiting amino acid for GSH synthesis. Since the inhibition of the xC- system by SAS leads to decreased levels of GSH, the association of SAS and PRIMA-1Met could further increase the intracellular ROS and promote cell death. In this work, we investigate the p53/xCT/GSH axis as a potential therapeutic target for CLL. For this purpose, we used two CLL cell lines expressing wild-type or mutant p53 proteins (OSU and MEC-1 cells, respectively) and performed combined treatments with PRIMA-1Met and SAS. Through MTT and Annexin/PI assays, we found that this drug combination may sensitise CLL cells to cell death. In addition, PRIMA-1Met and SAS depleted the intracellular amount of GSH and augmented the level of ROS, particularly in MEC-1 cells carrying mutant p53. The modulation of metabolic markers, such as glutathione reductase (GR) and glutathione peroxidase (GPx), as indicators of glutathione deficit, NADPH oxidase as a marker of reactive oxygen species (ROS) production, and malondialdehyde (MDA) as a marker of lipid peroxidation, was investigated. We found that SAS impacted GR and GPx levels, while PRIMA-1Met determined an increase in NADPH oxidase. Interestingly, a remarkable increase in MDA was observed in both cell lines following co-treatment with PRIMA-1Met and SAS. Also, we demonstrated that PRIMA-1Met and SAS synergistically decrease survival in CLL cells, regardless of their p53 status. The same studies have also been performed in CLL primary

cells carrying wild-type or mutated p53. Preliminary data and evidence will be presented and discussed.

**Keywords:** CLL; p53; PRIMA-1Met

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## Abstract ID: 1301

### **Title:** Selective NIK inhibition overcomes micro-environment-mediated resistance and synergizes with venetoclax in CLL

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**Background:** Aberrant NF-κB signalling plays a critical role in chronic lymphocytic leukemia (CLL), driven by both recurrent mutations in NF-κB-regulating genes (Cuní et al. 2004, Baliakas et al. 2015, Mansouri et al. 2015) the protective lymph node (LN) microenvironment (Herishanu et al. 2011). Elevated NF-κB activity is associated with disease aggressiveness (Cuní et al. 2004, Hewamana et al. 2008) and resistance to therapies such as ibrutinib and venetoclax (Jayappa et al. 2017). This resistance is partially mediated by the upregulation of anti-apoptotic Bcl-2 family members in the LN niche (Smit et al. 2006). We investigated the therapeutic potential of targeting NF-κB-inducing kinase (NIK), a central regulator of non-canonical NF-κB signalling. NIK expression is upregulated as CLL cells migrate into the LN, where they acquire therapy resistance (Jayappa et al. 2017). Since NIK is normally constitutively active only in pathological contexts (Thu et al. 2011), it presents a promising tumour-selective target. We hypothesised that NIK inhibition would preferentially affect LN-resident CLL cells reliant on non-canonical NF-κB signalling. We evaluated three selective NIK inhibitors (CW15337, Amgen16, and B022) in MEC-1 cells and primary CLL samples, cultured with CD40L-expressing fibroblasts to mimic the LN microenvironment. Non-transfected fibroblasts (NTLs) served as controls.

**Results:** All three NIK inhibitors induced dose-dependent reductions in nuclear p52 levels, confirming on-target inhibition of non-canonical NF-κB signalling. In parallel, they significantly impaired MEC-1 cell migration toward CXCL12 ($p<0.001$) and induced a concentration-dependent G1 cell cycle arrest ($p=0.001$). Importantly, the compounds also reduced p52 activation and triggered dose-dependent cytotoxicity in both MEC-1 cells and 25 primary CLL samples cultured on CD40L-expressing fibroblasts. Despite CD40L-mediated enhancement of both canonical and non-canonical NF-κB signalling, NIK inhibition consistently overcame this cytoprotective effect. LC50 values were significantly lower in CD40L co-cultures compared to non-transfected fibroblasts (NTLs) for Amgen16 ($p=0.01$) and B022 ($p=0.01$), and comparable for CW15337 ($p=0.9$). Sensitivity to NIK inhibition was correlated with basal non-canonical NF-κB activity, as measured by nuclear p52 ($p=0.006$; $r^2=0.46$ for B022). CD40L stimulation increased protein levels of the anti-apoptotic proteins Bcl-xL ($p<0.0001$) and Mcl-1 ($p=0.0003$), but not Bcl-2 ($p=0.8$), and upregulated transcription of BCL2L1 and BCL2A1. Treatment with NIK inhibitors reversed these effects, leading to dose-dependent downregulation of Bcl-xL ($p=0.001$) and Mcl-1 ($p<0.001$), with no significant effect on Bcl-2 expression ($p=0.45$). At the transcript level, NIK inhibition suppressed BCL2A1 and BCL2L1 expression in a dose-dependent manner, without affecting MCL1 or BCL2. Consistent with prior reports (Vogler et al. 2009, Haselager et al. 2020), CD40L co-culture induced in vitro resistance to venetoclax (ABT-199), which was effectively reversed by co-treatment with NIK inhibitors. Combination therapy yielded strong synergy, with mean synergy scores of 21.0, 23.9, and 32.3 for Amgen16, B022, and CW15337, respectively ($p<0.0001$).

**Conclusions:** NIK inhibition effectively neutralises the cytoprotective effects of the LN microenvironment, likely through suppression of CD40L-induced anti-apoptotic signals and NF-κB pathway activation. NIK inhibitors target CLL cells in protective niches and inhibit their migration, suggesting utility in eliminating LN-resident disease and preventing niche re-entry. Furthermore, NIK inhibition resensitises CLL cells to venetoclax, supporting a rationale for combination strategies.

**Future work:** Given that NF-κB signalling is a key driver of Richter's transformation (Rohan et al. 2024) and that the lymph node microenvironment is a critical component of the disease biology, NIK represents a compelling therapeutic target. We now plan to evaluate the efficacy of NIK inhibition in this high-risk context using both in vitro and in vivo models.

**Keywords:** NF-κB-inducing kinase; tumour microenvironment; drug resistance

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## Abstract ID: 1202

### **Title:** Advanced 3D culture system revealed the dynamic regulation of podoplanin in CLL cells within the microenvironment

**Authors:** Dafne Barozzi[a], Federica Barbaglio[b], Daniela Belloni[b], Davide Ribezzi[c], Silvia Farè[d], Riccardo Pinos[b], Marta Sampietro[b], Margherita Pauri[b], Elisa Albi[e], Francesca Martini[f], Paolo Ghia[g] and Cristina Scielzo[b]

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**Introduction:** Bioengineering three-dimensional (3D) lymphoid tissues represent a rapidly expanding area in oncohematology, offering strong translational potential for modeling cancer cells-tumor microenvironment (TME) interactions. Due to the challenges in optimizing models that can incorporate the TME and capture the dynamicity of blood cancers, this research field is still poorly explored [Barozzi, D. et al; 2023]. In this study we exploited our lymphoid-like 3D in vitro dynamic model to investigate the physio-pathological mechanisms of Chronic Lymphocytic Leukemia (CLL). The TME plays a central role in CLL progression, relapse and therapy resistance [Caligaris-Cappio, F. et al; 2014], driving the development of targeted therapies to disrupt related key crosstalk pathways. This underscores the need for efficient in vitro models reflecting tissue-specific contexts.
Our system allowed us to reproduce both bone marrow (BM) and lymph node (LN) microenvironments, recreating the journey of CLL cells disseminating through the circulation. We were able to investigate various aspects of the leukemia-TME crosstalk, observing the expression of podoplanin (PDPN) on the neoplastic clone and not only by the stromal cells as expected. PDPN is known in various biological contexts, particularly as central regulator of fibroblastic reticular cells and endothelium within the LN. Interestingly its expression in B lymphocytes and CLL cells is not well-established, yet it is reported its overexpression in inflammation contexts and as cancerassociated factor in various tumor microenvironments such as in the vasculature of CLL/SLL TME, if compared with the benign control. [Ruan, J. et al; 2006].
**Methods:** The 3D system consists of a peristaltic pump which allows the circulation of CLL cells inside and outside two different bioreactors containing lymphoid-like 3D constructs. The tissue-like samples were generated seeding human mesenchymal stromal cells (for the BM model) and human lymphatic fibroblasts together with endothelial cells (for the LN model) on a biocompatible porous scaffold. The first was developed inside a bioreactor under dynamic tangential flow at a perfusion rate of 100 µl/min, while the LN underwent a bottom-to-top sigmoidal flow at a prefusion rate of 200 µl/min. Leukemic cells were added to the recirculation after 15 days of culture reflecting the diagnostic threshold for the total leukocyte count: 5×106 cells/ml. Recirculating cells were withdrawn at two different timepoints (24 and 72 hours after the circulation start point) and key functional markers were analyzed through flow cytometry, gene expression analysis and protein quantification. The 3D scaffolds were processed for confocal imaging and histochemistry analysis, together with gene and protein expression analysis.
**Results:** We successfully optimized a perfused 3D in vitro model mimicking both BM and LN tissues, demonstrating its ability in sustaining CLL cell viability while replicating critical interactions with the TME [Barozzi, D. et al. accepted for publication]. Using both a CLL cell line (MEC1) and primary patient-derived cells, we confirmed that leukemic cells can infiltrate and populate both 3D BM and LN microenvironments, showing time-dependent changes in key functional markers compared to 2D cultures. In response to biochemical and mechanical stimuli (e.g. shear stress and compression), leukemic cells modulated the expression of key functional markers, involved in homing and dissemination (e.g. CXCR4), adhesion-related proteins involved in retention of CLL cells inside the tissues (e.g. CD44/hyaluronic acid), and in transendothelial migration (e.g. VLA-4/fibronectin and VCAM-1). While assessing spatial distribution and organization of CLL cells inside the 3D constructs, we interestingly noted the expression of PDPN in both MEC1 and primary cells. We are now screening patients' cells (n = 24 so far), observing by flow cytometry a highly heterogeneous inter-patient expression of PDPN in the neoplastic clone - ranging from 4% to 90% of PDPN + cells - contrarily to a more homogeneous expression observed in healthy B cells from different donors, remaining around 70%. Preliminary data using our 3D dynamic system, revealed a significant increase in PDPN + CLL cell in circulation in presence of the LN microenvironment but not in the BM, respectively if compared to their basal levels.
**Conclusion:** We generated a reliable 3D in vitro model to reproduce the CLL-TME crosstalk. In particular, we observed the dynamic expression of PDPN in primary CLL cells from different donors. The regulation of this microenvironment-dependent protein could possibly be associated with disease progression, transformation, or microenvironmental interactions. A deep analysis is ongoing also correlating with the clinical outcome and response to therapies.

**Keywords:** 3D; microenvironment; perfusion; bioengineering; biomaterial; lymphoid tissues; leukemia

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## Abstract ID: 1220

**Title:** Chronic lymphocytic leukemia and renal cell carcinoma: a case series of twelve patients

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**Background:** Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults, with a median age at diagnosis of 72 years (SEER Program, 2019). CLL patients (pts) have an increased risk of second malignancies due to multiple synergistic factors: genetic, environmental, treatment-induced and immunomodulatory effects (Wiernik, anno non specificato; Kyasa et al., 2004). Renal cell carcinoma (RCC) has an annual incidence of 14.9 per 100,000 people, with a median age at diagnosis of 65 years (Howlander et al., 2019). In 6–9% of cases, it is associated with germline mutations in genes linked to cancer predisposition (Wu et al., 2019). Despite nephrectomy, 30% of RCC pts develop metastatic disease, requiring systemic therapy and carrying a high mortality risk (Hsieh et al., 2017). No association on the epidemiology and genetics of the two diseases has been reported so far.
**Results:** Out of 740 CLL pts followed at our center in the last 25 years, we found 12 pts (1.62%) with a diagnosis of RCC (Table 1). Eleven (92%) were male. The median age at diagnosis was 61 (43–73) years for CLL, and 61 (42–73) for RCC. In 3 pts (25%), RCC was diagnosed prior to CLL; in 6 pts (50%), the malignancies were detected concurrently; finally, 3 pts (25%) developed RCC after CLL. Our pts did not show other malignancies and family history revealed no tumours clustering. Nine pts (75%) had RCC at early stage (I, II) and received surgical resection. After an average follow-up of 6.5 years since RCC diagnosis, 9/12 (75%) pts are still alive.
**Discussion:** Currently, this is the largest single-center series of pts with CLL and RCC. An increased incidence of RCC in pts with NHL has been reported (Anderson et al., 1998), but its concomitance with CLL remains a sporadic finding (Serefhanoglu et al., 2010; Dutcher & Wiernik, 2015; Nishikubo et al., 1996). The young age at diagnosis for both malignancies might suggest a genetic predisposition. Del(13q), a common lesion in CLL, was present in 7 pts, but its specific role is unclear. In our cohort, almost all RCC diagnoses occurred before or concurrently with CLL, excluding CLL treatment toxicity as a cause. Six pts were smokers, an established risk factor for RCC (RR 1.5–2.0) (Huang et al., 2022). Regular medical check-ups and abdominal ultrasounds, routinely performed by our CLL pts, likely increased the incidental finding of renal lesions (Hsieh et al., 2017). Early detection may explain why our pts were successfully treated with surgery, without the need for systemic therapies.
**Conclusion:** Our study suggests a potential link between CLL and RCC, warranting further study. Frequent CLL follow-up may aid early detection of other cancers, improving quality of life and survival of our pts.

**Keywords:** Chronic lymphocytic leukemia; second malignancies; renal cell carcinoma

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## Abstract ID: 1604

### **Title:** The CDK2/9 inhibitor fadraciclib in Richter transformation

**Authors:** Yuling Chen[a], Ping Xiong[a], William G. Wierda[a], William Plunkett[a] and Rong Chen[a]

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Richter transformation (RT)—the progression of chronic lymphocytic leukemia (CLL) into an aggressive large-cell lymphoma—remains a significant therapeutic challenge. This transformation is frequently driven by genetic alterations such as MYC activation, TP53 abnormalities, NOTCH1 mutations, and CDKN2A loss, which collectively promote uncontrolled proliferation and evasion of apoptosis. Among these, Myc activation—detected in approximately 70% of RT cases—plays a central role in RT pathogenesis. As a transcription factor, Myc exerts broad effects on cell survival, proliferation, adhesion, and metabolism, making it a compelling therapeutic target in RT. Fadraciclib (CYC065) is a second-generation cyclin-dependent kinase (CDK) inhibitor with selectivity for both CDK2 and CDK9. We previously demonstrated that fadraciclib induces apoptosis in primary CLL cells by inhibiting CDK9-mediated transcription, leading to downregulation of the short-lived anti-apoptotic protein Mcl-1. Similar to Mcl-1, Myc is a rapidly turned-over oncoprotein at both the mRNA and protein levels, making it an attractive target for CDK9 inhibition. Combined with the essential role of CDK2 in cell cycle progression, we hypothesized that fadraciclib could simultaneously target key survival and proliferative pathways in RT through coordinated inhibition of Mcl-1, Myc, and CDK2. Our findings support this hypothesis through three key observations: First, CDK9 inhibition was evident in the RT cell line HPRT1 and in MYC-amplified lymphoma cell lines (Raji, Ramos, REC-1, and SU-DHL-2), as demonstrated by reduced phosphorylation of RNA polymerase II (RNA Pol II), indicative of decreased transcriptional activity. This was accompanied by marked reductions in both mRNA and protein levels of Mcl-1 and Myc, confirmed by real-time RT-PCR and immunoblotting. CDK2 inhibition was also observed, as shown by decreased phosphorylation of its downstream targets, RB and NPM. Notably, siRNA-mediated knockdown of Mcl-1 induced apoptosis in HPRT1 cells within 3 hours, underscoring the cells' critical dependence on Mcl-1 for survival. Second, fadraciclib effectively induced apoptosis in these cell lines. In HPRT1 cells, the $IC_{50}$ for apoptosis was 0.3 μM at 24 hours—substantially lower than the average $IC_{50}$ in primary CLL cells (0.8 μM). In the MYC-amplified lymphoma cell lines, $IC_{50}$ values for apoptosis ranged from 0.8 to 1.3 μM. Third, fadraciclib suppressed cell proliferation. In HPRT1 cells, the $IC_{50}$ for growth inhibition was 0.25 μM, as measured by CellTiter-Glo analysis. In the lymphoma lines, $IC_{50}$ values ranged from 0.5 to 0.7 μM. CFSE tracking confirmed reduced cell division and Click-iT EdU incorporation assays showed decreased S-phase entry and cell cycle arrest at the G1 phase, consistent with CDK2's role in G1/S transition. In summary, our results demonstrate that fadraciclib is active in RT and Myc-driven lymphoma cell lines by targeting both survival and proliferation pathways, supporting its further clinical evaluation in patients with Richter transformation.

**Keywords:** Fadraciclib; Richter transformation; CDK

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## Abstract ID: 1432

### **Title:** A phase Ib study evaluating the safety and efficacy of tafasitamab, acalabrutinib, and obinutuzumab (TAO) in previously untreated chronic lymphocytic leukemia and small lymphocytic lymphoma

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**Background:** Fixed-duration frontline regimens for chronic lymphocytic leukemia and small lymphocytic lymphoma (CLL/SLL) are needed. Irrespective of the treatment used, achieving undetectable minimal residual disease (uMRD) has been shown to improve outcomes. Acalabrutinib and obinutuzumab are commonly used for CLL treatment. Tafasitamab, an Fc-modified anti-CD19 immunotherapy approved in combination with lenalidomide for relapsed/refractory diffuse large B-cell lymphoma, has demonstrated clinical activity in CLL. The TAO triplet aims to (1) synergistically target multiple pathways, (2) reduce drug exposure to limit resistance and toxicity, (3) allow for future BTK inhibitor-based re-treatment and/or use of venetoclax.

**Methods:** Eligibility criteria include: adults ≥18 years, with untreated CLL/SLL requiring treatment per iwCLL criteria, at least one measurable disease site, and adequate organ function. The co-primary endpoints of the study include safety and efficacy. The DLT observation period includes Cycle 2 Day 1 through Cycle 6. Heme and non-heme toxicities are graded using iwCLL and CTCAE v. 5.0 respectively. Efficacy is defined as the proportion of patients who achieve peripheral blood uMRD ($<10^{-4}$, Clonoseq® Adaptive Biosciences, Seattle, WA) at any time during the study. MRD timepoints include: month 3, 6, 9, or 12, and 2–3 months after the last dose of any study drug in responding patients. Additional MRD testing is performed every 3 months up to year 2 post treatment. Secondary objectives include iwCLL response criteria, progression freesurvival (PFS), and overall survival (OS). Exploratory objectives include potential association of response to baseline cytogenetics, mutations, and IGHV status. Quantification and characterization of T cells is also performed. Eligible patients are treated with acalabrutinib (100 mg PO BID) for a total of 12 cycles (cycles are 28 days). Obinutuzumab is administered on day 1 at 100 mg, day 2 at 900 mg, and then 1,000 mg on days 8, 15 for cycle 1, and on day 1 of cycle 2–6. Tafasitamab 12 mg/kg is administered weekly (Days 1, 8, 15, and 22) for cycle 2–4. One additional dose is administered on Day 4 of Cycle 2 only. Tafasitamab is administered biweekly in cycles 5–7. Dose reduction is allowed for acalabrutinib. Delays (up to 28 days) are allowed for acalabrutinib, tafasitamab, and obinutuzumab. Participants are followed for 1 year of treatment and 2 years thereafter. The study aims to enroll 23 efficacyevaluable patients. The TAO regimen will be considered promising if uMRD rates reach 94% with type I error 0.05 and 80% power.

**Results:** Between October 2023 and May 2025, 14 treatment-nave CLL/SLL patients (median age 66; 10 males, 4 females) were consented (13 enrolled, 1 currently in screening). Of the enrolled patients, 12 had Rai stage 0–2 disease and one had stage 3. The patient in screening has stage 4 disease. One patient harbors del17p, a TP53 mutation, and complex karyotype. One other patient has a complex karyotype. Seven (50%) have an unmutated IGHV and a CLL-IPI score of 3 or greater. Currently, six patients have completed all treatment; four have completed TO and continue acalabrutinib, and 3 continue the TAO combination. Median time on treatment is 11 months (range 2–19). All but one subject is evaluable for MRD. To date, uMRD ($10^{-4}$) in peripheral blood has been achieved in 6 of 13 (46%) evaluable patients, including 4 of 6 (67%) who have completed the 12-month regimen. uMRD at 10–6 has been seen in 4 patients including 3 of 5 (60%) who have completed treatment. The overall response rate in evaluable patients is 100%. Four of four (100%) patients who have completed treatment have attained CR. Nine treatment-related or potentially treatment-related toxicities have been observed including one DLT (grade 3 febrile neutropenia during cycle 3). The most common AE was neutropenia; grade 3, n = 4; grade 4, n = 3. No grade 3 infections were seen. Additional toxicities (≥ grade 3) included one case of grade 3 thrombocytopenia.

**Future directions:** Enrollment continues to reach 23 efficacy-evaluable patients. Ongoing follow-up will further characterize the primary endpoints as well as durability of uMRD responses. Exploratory analyses are ongoing. Updated results will be presented at the meeting. The trial is registered at clinicaltrials.gov (NCTNCT05943496).

**Keywords:** Treatment naive chronic lymphocytic leukemia MRD

**Please indicate how this research was funded:** Investigator Initiated Trial with funding from sponsor

**Please indicate the name of the funding organization:** Incyte

## Abstract ID: 1907

### Title: Autoimmune cytopenias in patients with chronic lymphocytic leukemia receiving acalabrutinib treatment

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**Background:** Among autoimmune complications, autoimmune cytopenias (AIC) involve 5–9% of patients affected by chronic lymphocytic leukemia (CLL), (Vitale C. et al, Cancers 2020). To date, the effects of acalabrutinib - a second-generation, covalent inhibitor of the BTK - on preexisting and treatment emergent AIC have not been fully investigated. In a few case reports, acalabrutinib has been successfully administered for the treatment of patients with immune thrombocytopenia (ITP) or pure red cell aplasia (PRCA), (Allsup D. et al. Leuk Lymphoma 2023; Fresa A. et al. Ther Adv in Hematology 2024). A phase II study evaluating the role of acalabrutinib in relapsed refractory autoimmune hemolytic anemia (AIHA) associated with CLL is currently ongoing.
**Aims:** The aim of this study is to analyze the characteristics and outcome of preexisting AIC and the incidence, features and outcome of treatment-emergent AIC in patients with CLL receiving acalabrutinib monotherapy.
**Methods:** we retrospectively collected clinical and biological data from consecutive patients diagnosed with CLL and treated with acalabrutinib in 13 Italian centers.
**Results:** we collected data from 285 patients with CLL (male/female ratio: 1.6) receiving acalabrutinib treatment. The median age at treatment initiation was 73 years (range: 44–93), and the majority of patients (77%) were treatment-naïve. In our cohort, 192 of 271 patients (71%) harbored unmutated IGHV, 36/279 (13%) had TP53 mutations, and 37/271 (14%) carried del(17p). The median follow-up for the entire cohort was 13 months. A history of AIC was present in 20 patients (7%), including 11/20 (55%) with AIHA, 8/20 (40%) with ITP, and 1/20 (5%) with PRCA. Nearly all patients with pre-existing AIC (95%) had received AIC-directed therapy, predominantly corticosteroids (37%) or corticosteroids combined with rituximab (26%). At the initiation of acalabrutinib treatment, AIC were active in 40%, controlled in 35%, and resolved in 20% of the patients. Following treatment with acalabrutinib, AIC resolved in 55% of cases and improved in an additional 5%. In the entire cohort, 4 patients (1.5%) developed treatment-emergent AIC, including 2 cases of ITP, 1 of AIHA, and 1 of autoimmune granulocytopenia (AIG). Of the ITP cases, one achieved resolution with corticosteroids, while the second one required sequential therapy with corticosteroids, rituximab, intravenous immunoglobulin but failed to achieve control of the autoimmune complication thus requiring a temporary interruption of acalabrutinib. The patient with AIHA remained stable and did not require additional AIC-directed therapy. The case of AIG resolved with administration of granulocyte colony-stimulating factors (G-CSF). In terms of CLL outcome, patients with or without AIC – considering both preexisting and treatment-emergent AIC - did not differ significantly in terms of progression-free survival and overall survival.
**Conclusions:** Our retrospective analysis indicates that preexisting AIC can be effectively managed in the majority of patients with CLL treated with acalabrutinib. Similarly, treatment-emergent AIC were observed infrequently and were generally manageable. Notably, the presence of AIC did not negatively impact treatment outcomes in patients receiving acalabrutinib.

**Keywords:** Autoimmune cytopenias; chronic lymphocytic leukemia; acalabrutinib

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1402

**Title:** Cytogenetic instability and therapeutic pressure in fixed vs continuous regimens for chronic lymphocytic leukemia after first line treatment: preliminary data from an italian multicenter experience

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**Background:** Novel targeted therapies have transformed the treatment landscape of chronic lymphocytic leukemia (CLL),

with Bruton tyrosine kinase inhibitors (BTKi) and BCL2 inhibitors showing remarkable efficacy. However, their long-term impact on genomic evolution - particularly regarding cytogenetic complexity and clonal dynamics - remains poorly understood. Kittai et al. retrospectively analyzed CLL patients treated with ibrutinib and found that cytogenetic evolution independently predicted poor outcomes at disease progression (PD). Among 75 patients, 56% showed an increase in chromosomal abnormalities, and 80% displayed karyotypic evolution at PD. However, the cohort's heterogeneous treatment history limited therapy-specific interpretation. In contrast, Fürstenau et al. evaluated venetoclax-based regimens and observed relative cytogenetic stability, though the small sample size (n = 20) precludes firm conclusions. Together, these studies highlight both the clinical relevance and the current gaps in understanding cytogenetic evolution under targeted therapies in CLL.

**Aim:** To compare the frequency and dynamics of cytogenetic evolution in treatment naïve patients receiving continuous BTKi therapy versus those receiving fixed-duration Ven-based regimens.

**Methods:** We conducted a retrospective, multicenter cohort study involving patients with CLL who underwent treatment with targeted therapies from 2015 to 2024. Not all participating centers have yet completed data submission, and analyses presented here reflect an interim dataset. An initial cohort of 834 patients who had undergone cytogenetic analysis was screened. In the final analysis we included only treatment naïve patients who received a targeted agent and of whom conventional karyotyping data were available both before treatment initiation and at the time of PD. Complex karyotype (CK) was defined by the presence of at least 3 chromosomal alterations in the neoplastic clone (low-CK =3–4 alterations, high-CK =5 or more alterations). Cytogenetic evolution was defined as either an increase in the number of chromosomal abnormalities or the acquisition of a new clone at PD.

**Results:** Among relapsed patients paired cytogenetic analysis at baseline and at relapse was successfully performed in 36 patients. Median time to progression was 40 months (range 7–88) and median time to next treatment was 46 months (range 10–114). Sixteen patients received fixed-duration venetoclax-based therapy (BTKi + Ven n = 11, Ven + obinutuzumab n = 5) and 20 received continuous BTKi (ibrutinib n = 17, acalabrutinib n = 3). At baseline, median age was 63 (range 44–78), 23 (64%) were males, 81% (n = 29) had unmutated IGHV, 14% (n = 5) had TP53 mutations 27% (n = 10) had del(17p), and 33% (n = 12) complex karyotype (low-CK n = 10, high-CK n = 2). No differences in terms of genetic alterations were observed among the two groups ($p > 0.05$). Cytogenetic evolution was significantly more frequent in the continuous group (52.6%) compared to the fixed-duration group (32%, $p = 0.047$). The median number of cytogenetic abnormalities increased in the continuous group from 2 (range: 0–5) before treatment to 4 (range: 0–8) at progression, while it remained stable in the fixed-duration group (median 2 pre and post). The difference in alterations between pre- and post-treatment was significantly higher in the continuous group ($p = 0.028$). Four patients with non-CK evolved to low-CK while 2 non-CK and 5 low-CK patients became high-CK. 18 out of 24 non-CK patients and 4 out of 12 CK patients remain stable at PD. Importantly, the presence of TP53 mutations, del(17p), and baseline complex karyotype were all significantly associated with cytogenetic evolution. TP53 mutations were observed in 31.2% of evolving cases versus 0% in non-evolving cases ($p = 0.013$), while del(17p) was present in 47.1% of evolving patients compared to only 10.5% in the non-evolving group ($p = 0.025$). Complex karyotype at baseline was detected in 52.9% of evolving cases versus 15.8% of non-evolving patients ($p = 0.033$).

**Conclusions:** These preliminary findings suggest that both the type of targeted therapy and the presence of specific baseline genetic features may influence the likelihood of cytogenetic evolution during the clinical course of CLL. A greater cytogenetic instability was observed in patients receiving continuous treatment, while fixed-duration regimens appeared less frequently associated with such changes. These data raise the hypothesis that prolonged therapeutic exposure may contribute to clonal dynamics and genomic evolution, particularly in patients with pre-existing genetic instability. This underscores the potential relevance of integrating cytogenetic profiling into longitudinal disease monitoring strategies, especially in those patients with a high-risk genetic disease.

**Keywords:** CLL; clonal-evolution; karyotyping

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

# Abstract ID: 1806

## **Title:** Dual mTORC1/2 inhibition by Torin 2 induces cytotoxic and cytostatic effects in in vitro models of chronic lymphocytic leukaemia

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**Background:** Targeted therapies have transformed the management of chronic lymphocytic leukemia (CLL), yet relapse and drug resistance remain prevalent. The PI3K/AKT/mTOR axis is constitutively active in CLL cells and supports their survival within the tumour microenvironment (TME). While PI3K inhibitors have shown promise, their long-term use is hampered by toxicity and resistance. mTOR, a key downstream effector, exists as two complexes: mTORC1 and mTORC2. Previous studies have shown that mTORC1-specific inhibitors lacked efficacy in CLL, suggesting dual inhibition of both mTOR complexes may be more effective. Torin 2 is a potent inhibitor of both mTORC 1 and 2 and has demonstrated efficacy in various cancers. This study evaluated the cytotoxic and cytostatic potential of Torin 2 in in vitro CLL models.

**Methods:** We conducted a large, high-throughput screen of 397 compounds, including a range of mTORC1, mTORC2 and dual mTOR inhibitors. CLL cell lines (HG3 and OSU-CLL) were treated with 10 μM of the compounds for 48 hours and their viability assessed using the CellTiter-Glo luminescent assay (n = 6 technical replicates per condition). For detailed characterization of lead compounds, dose- and time-response studies were conducted using 0.001–100 μM Torin 2 over 48 and 72 hours. Proliferation was assessed by CellTrace Violet dye dilution. Data were normalized to baseline mean fluorescence intensity (MFI), with statistical analysis performed using a one-way ANOVA with Dunnett's correction. Western blotting was used to evaluate the phosphorylation status of mTOR downstream targets (p70S6K at Thr389 for mTORC1; AKT at Ser473 and Thr308 for mTORC2) following Torin 2 treatment for 1, 6, or 24 hours. To elucidate the mechanism of action, cytotoxicity assays were performed with or without the apoptosis inhibitor z-VAD-FMK and the

autophagy inhibitor Bafilomycin A1. Finally, primary CLL cells (n = 4 at 48 hours; n = 3 at 72 hours) were treated with Torin 2 while in co-culture with stromal cell monolayers to mimic the TME.

**Results:** Data from the high throughput screen determined that Torin 2 was significantly more effective at reducing CLL cell viability than the PI3K, AKT, mTORC1-selective and alternate pan-mTOR inhibitors. Torin 2 reduced cell viability below 50% in both the HG3 and OSU-CLL lines ($p < 0.01$, Welch's t-test). Independent experiments confirmed that low micromolar concentrations (≤5 μM) were sufficient to induce significant time- and dose-dependent cytotoxicity on both lines. Proliferation assays also revealed potent cytostatic effects of Torin 2 in both CLL cell lines, with significant growth arrest observed following treatment with low nanomolar concentrations. Western blot analyses confirmed inhibition of mTORC1 by ablation of p70S6K phosphorylation and demonstrated suppression of mTORC2 through reduced phosphorylation of AKT at Ser473 and Thr308. Treatment with z-VAD-FMK or Bafilomycin A1 partially inhibited Torin 2-induced cell death, suggesting that both apoptosis and autophagy may be involved in mediating the cytotoxic effects of the drug. Torin 2 also significantly reduced the viability of primary CLL cells cultured with stromal cells (mean decrease >30%, $p < 0.05$, Welch's one-tailed t-test), confirming efficacy of the drug under in vitro conditions that mimic the TME.

**Discussion:** The findings of this study highlight Torin 2 as a potent inhibitor of CLL cell viability and proliferation. Unlike earlier generation mTORC1-specific agents, Torin 2 targets both mTOR complexes, which may be the underlying reason for the enhanced anti-leukemic activity observed. Mechanistically, the dual cytotoxic and cytostatic effects of Torin 2 appear to involve apoptosis and autophagy, consistent with the role of mTOR in cellular metabolism and survival. The ability of Torin 2 to overcome the pro-survival effects of the interaction between CLL and stromal cells is particularly encouraging, as apoptosis resistance conferred by the TME is a major clinical challenge in CLL. These results suggest further pre-clinical studies of Torin 2 are warranted, both as a single agent and in combination with other targeted agents.

**Conclusion:** Torin 2 has significant anti-leukemic effects in vitro by blocking signalling via both mTORC1 and mTORC2. The cytostatic and cytotoxic activity of the agent, even under in vitro conditions that mimic the TME, provide a strong rationale for further preclinical development of Torin 2 as a therapeutic strategy for patients with CLL.

**Keywords:** mTOR inhibitor; Torin 2; autophagy

**Please indicate how this research was funded:** Both internal and external grant organisations.

**Please indicate the name of the funding organization:** Flinders Foundation and The Beat Cancer Project which was funded by Cancer Council South Australia and the South Australian Government.

## Abstract ID: 2005

### **Title:** Expression of TP53 deletion and clinical presentations of Nigerians with chronic lymphocytic leukaemia: a preliminary report

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**Background:** A fluorescence in-situ hybridization (FISH) laboratory was established due to inequity in the prognostication of chronic lymphocytic leukemia (CLL) in Nigeria, and to determine the prevalence of TP53 deletion in these patients, its relationship to the clinical features at enrolment and response to chemotherapy.

**Method:** Sixteen patients with CLL from four centres were investigated over a period of 16 months. Clinical data were collected using forms on REDcap database, blood samples were obtained for full blood count (FBC) and FISH analysis using standard operating procedures. Data was analyzed using descriptive and inferential statistics; $p < 0.05$ defined the level of significance.

**Results:** The majority (68.7%) of patients were under the age of 65 years (range - 49 to 80 years). Males were not significantly more than females (M: F ratio of 1.67: 1 ($p = 0.317$), but females were significantly older than males [74.67 (±4.27) versus 64.50 (±10.6); $p = 0.044$]. Half (8) of the patients enrolled were treatment naïve, and the majority (87.5%) were symptomatic in Binet Stage C. Two (12.5%) patients had TP53 deletion, and two subclones of nuclei were identified in one of them. The common presenting clinical features were pallor in 11 (68.7%); splenomegaly in 11 (68.8%); lymph node enlargement in 9 (56.2%); and fatigue in 8 (50.0%). Two (12.5%) patients had WHO performance status of 0, while 8 (50%) had a score of 1; and 6 (37.5%) had a score of 2. Males had a significantly higher performance status score than females ($p = 0.024$). Binet Stage A was seen in 2 (12.5) patients; while 14 (87.5%) patients were in Binet Stage C of whom eight (57.1%) had fatigue, six (37.5%) had unplanned weight loss, and four (28.6%) had fever. The white cell count ranged from 5,600/μL to 164,200/μL. Anemia (Hb less than 10g/dl) occurred in 9/16 (56.2%) and thrombocytopenia occurred in four patients (25%). There were no significant differences in the hematology profile between patients with or without TP53 deletion and between males and females. The 14 (87.5%) patients who were treated received chemotherapy as none could afford immunotherapy. The median overall survival at 38 months after commencing the study was short (32 ± 10.7 months), and it was not influenced by the Binet Stage at diagnosis, previous history of treatment, type of chemotherapy, or TP53 deletion status.

**Conclusion:** This study has established the first FISH laboratory in Nigeria and has shown that one in eight patients with CLL has TP53 deletion. The clinical features of this small cohort of patients with CLL are similar to those previously reported in the region, but the relationship with TP53 status is being reported for the first time in Nigeria. This study shows that the response to chemotherapy was independent of TP53 deletion status probably because other prognostic biomarkers, such as unmutated IGHV gene and TP53 mutation(s), that were not investigated, might have been present. The availability of TP53 deletion test would reduce the time spent and resources wasted on giving patients chemotherapy, as first-line therapy, thus reducing the inequity that is evident, and improving the quality of life and overall survival of these patients.

**Keywords:** Chronic lymphocytic leukaemia (CLL); TP53 deletion; fluorescence in-situ hybridisation (FISH); prognostication

**Please indicate how this research was funded:** An National Research Fund (NRF) grant through the university.

**Please indicate the name of the funding organization:** Tertiary Education Trust Fund (TETFund), Nigeria.

## Abstract ID: 1113

### **Title:** Stereotyped BCR subset #8 in Chinese CLL: molecular genetics, clinical behaviour, and prognosis

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**Objective:** To analyze the molecular genetic characteristics, clinical features, and survival outcomes of Chinese chronic lymphocytic leukemia (CLL) patients belonging to stereotyped B-cell receptor (BCR) subset #8 (including its satellite subset #8b; collectively referred to as subset #8), and to further explore the clinical utility of identifying subset #8 patients in evaluating disease prognosis and guiding treatment decisions.
**Methods:** This study included CLL patients with available immunoglobulin heavy variable (IGHV) gene sequencing data from two centers: the First Affiliated Hospital of Nanjing Medical University and Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences. Patients belonging to subset #8, subset #1 (including its satellite subset #99, collectively referred to as subset #1), and subset #4 were identified. Clinical and molecular genetic data were retrospectively collected and analyzed, including β2-microglobulin (β2-MG), lactate dehydrogenase (LDH), fluorescence in situ hybridization (FISH) results, karyotype (using CpG-oligonucleotide and interleukin-2 stimulation), and next-generation sequencing (NGS) results. Statistical and survival analyses were conducted to summarize the distinct molecular genetic features and clinical characteristics of subset #8 patients, as well as their prognostic differences compared with non-subset #8 patients. The probability of progression-free survival (PFS) and overall survival (OS) was estimated by using Kaplan-Meier method and the differences between the survival curves of different groups of patients were assessed by using the log-rank test.
**Result:** This retrospective study included 1,824 CLL patients from two centers, with a median follow-up of 53 months. Among these patients, 1,083 patients (59.4%) were categorized as mutated CLL(M-CLL) and 741 patients (40.6%) as unmutated CLL(U-CLL). A total of 87 patients (4.8%) belonged to subset #8, representing a significantly higher proportion than previously reported in Western studies (4.8% vs. 2.9%, $p < 0.001$). Compared to non-subset #8 patients, patients of subset #8 exhibited higher levels of β2-microglobulin (β2-MG) (4.67 mg/L vs. 3.81 mg/L, $p = 0.004$) and lactate dehydrogenase (LDH) (416.7 U/L vs. 231.9 U/L, $p < 0.001$). Genetically, subset #8 showed a higher incidence of del(11q) (27.8% vs. 12.7%, $p = 0.001$), trisomy 12 (+12) (48.4% vs. 18.2%, $p < 0.001$), and complex karyotype (52.9% vs. 36.5%, $p = 0.006$), but a lower incidence of del(13q) (7.4% vs. 30.6%, $p < 0.001$). Clinically, subset #8 patients demonstrated a significantly higher proportion of patients requiring treatment indications (95.0% vs. 70.8%, $p < 0.001$). Both the median time to first treatment (TTFT) (14.4 months vs. 37.9 months, $p < 0.001$) and median overall survival (OS) (120.8 months vs. 173.2 months, $p < 0.001$) were significantly shorter compared to non-subset #8 patients. These findings indicate that subset #8 patients generally follow an aggressive disease course with inferior overall survival requiring early therapeutic intervention. Patients belonging to subset #8, subset #1, and subset #4 were stratified based on whether Bruton's tyrosine kinase inhibitor (BTKi) were included in their first-line treatment. BTKi-containing regimens improved survival and prolonged time to next treatment (TTNT) in subset #8, but not in subset #1. Analysis of NGS results of paired pre-treatment and post-treatment suggested that BTKi-based first-line treatment reduced clonal evolution risk in subset #8 compared to chemoimmunotherapy (CIT), though BTKi resistance mutations remained a concern.
**Conclusion:** Subset #8 patients exhibit distinct molecular genetic features and clinical manifestations. Subset #8 is characterized by a high frequency of molecular genetic abnormalities indicative of poor prognosis, demonstrates an aggressive clinical course requiring early treatment after diagnosis. For subset #8 patients, first-line therapy containing BTKi may improve clinical outcome. Early IGHV sequencing and identification of subset #8 in clinical practice facilitates prognostic assessment and aids in selecting treatment strategies.

**Keywords:** Chronic lymphocytic leukemia; immunoglobulin heavy variable region; stereotyped BCR

**Please indicate how this research was funded:**

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## Abstract ID: 1009

### **Title:** Epigenetic and transcriptional single-cell profiling reveals CLL-like states in low-count MBL

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Chronic lymphocytic leukemia (CLL) is preceded by low-count (LC-) and high-count monoclonal B cell lymphocytosis (HC-MBL). As the precise order of events underlying initial clonal B cell expansion and subsequent leukemic transformation - including epigenetic and transcriptomic reprogramming – have not been fully characterized, progression from MBL to CLL remains difficult to anticipate clinically. Resolving CLL-initiating events would facilitate the prediction of progression from MBL to overt leukemia and inform the discovery of novel therapeutic strategies. Here, we performed a single-cell multi-omics characterization of MBL and CLL at the transcriptional, epigenetic, and proteomic levels. We performed lineage tracing with natural genetic barcode analysis of B cell receptor (BCR) sequences, mitochondrial DNA (mtDNA) mutations, and copy number variations (CNV), and further augmented this analysis through longitudinal tracking of MBL/CLL subclones to provide a comprehensive map of phenotypic and genetic evolution from LC- via HC-MBL to CLL. The study cohort comprises 34 CD19+ enriched peripheral blood mononuclear cell (PBMC) samples from cases of LC-MBL (n=6), HC-MBL (n=7), and CLL (n=11), including paired HC-MBL and CLL specimens of 10 individuals (median follow-up time: 1,926 days, range: 916–2,859 days). We obtained 301,853 high-quality single-cell transcriptome (RNA-seq) and BCR profiles, along with 168,533 single-cell chromatin accessibility (ATAC-seq) and mtDNA mutational profiles. Based on a healthy PBMC reference and marker gene expression, we categorized cells into the major immune subsets of B cells, T cells, NK cells, and monocytes. We annotated the B cell compartment as physiologic or malignant based on BCR clonotype dominance (scRNA-seq) or distinct chromatin accessibility profiles of MBL/CLL (scATAC-seq). While physiologic B cells exhibited conserved epigenetic profiles, MBL/CLL cells formed distinct, case-specific gene expression and chromatin accessibility clusters. Within this variability, MBL/CLL cells consistently exhibited altered activity of transcription factor binding motifs in comparison to physiologic B cells, such as EBF1, SMARCC1, AP-1, and BACH. These changes were already detectable at the stage of LC-MBL and persisted throughout the MBL/CLL transition. They were mirrored at the transcriptomic level, which showed evidence for the acquisition of a CLL-like phenotype starting at the LC-MBL stage. This CLL-like transcriptomic state reflected loss of B cell identity, inflammation, and dysregulation of cell survival due to altered expression of genes such as CD5, FCER2, MME, EBF1, IL6, and MS4A1. Together, epigenetic and transcriptional reprogramming is an early event in MBL initiation, and core changes remain stable throughout the progression to CLL. Given the presence of CLL-like epigenetic and transcriptional states in LC-MBL, we explored its immunophenotypic and genetic features. BCR repertoire analysis demonstrated that LC-MBL retained only a small residual polyclonal B cell compartment (median: 25% polyclonal; range: 3–95%), which was consistent with the low percentage of CD19+ CD5- non-MBL cells on flow cytometry (median: 21%; range: 0.3–91%). Further, epigenetically defined LC-MBL cells exhibited a CLL-like surface immunophenotype, characterized by the overexpression of markers such as CD5 and CD200 or down-regulation of CD49d, Integrin β7, CD73, and CD35. Notably, in 2 of 6 LC-MBL cases, we found large-scale chromosomal alterations, such as trisomy 12. Together, molecular hallmarks of CLL emerge in LC-MBL at the genetic and surface marker level. Finally, we investigated the relationship between epigenetic and transcriptional stability during the MBL/CLL transition and clonal evolution. While most MBL/CLL populations were characterized by a monoclonal BCR, we reasoned that mtDNA mutation analysis would further resolve (sub)clonal hierarchies with high resolution. Indeed, we found a median of 33 subclones per MBL/CLL (range: 4–90). The majority of HC-MBL subclones (median: 6 per case; 73% of all clones) were stable during progression to CLL. More granular analysis of heteroplasmy distributions – quantified via variant allele frequency of mtDNA mutations – further demonstrated subclonal persistence and stability. These results indicate that HC-MBL/CLL progression is predominantly driven by disease proliferation rather than the emergence or selection of novel clones. In summary, we find that epigenetic and transcriptomic transformation toward a CLL-like phenotype occurs already in LC-MBL and remains stable throughout the HC-MBL/CLL transition. This supports a model in which core CLL-like states are acquired early and together with patient-specific cell states drive leukemogenesis. LC-MBL is a biologically heterogeneous and thus far under-characterized premalignant condition that warrants deeper investigation to uncover biomarkers and potential therapeutic targets for the prevention of progression to CLL.

**Keywords:** Leukemic transformation; single-cell multi-omics; clonal expansion

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## Abstract ID: 1807

### **Title:** Therapeutical potential of NRF2 inhibition by brusatol in chronic lymphocytic leukemia and in combination with current therapy

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**Introduction:** Chronic lymphocytic leukemia (CLL) is characterized by proliferation and accumulation of monoclonal B lymphocytes, of which pathophysiology involves chromosomal abnormalities, somatic mutations, and

dysregulated signalling pathways, including B-cell receptor (BCR) and BCL2-mediated apoptosis resistance. Oxidative stress (OS) plays a crucial role in CLL development, progression, and resistance to therapy. Nuclear factor erythroid 2-related factor 2 (NRF2), a major oxidative stress regulator, is deregulated in CLL cells. Current treatment strategies targeting BCR and BCL2 are associated with prolonged progression free survival. However, resistance mechanisms, particularly mutations in Bruton tyrosine kinase (BTK) and in BCL2, pose significant therapeutic challenges. Novel strategies, such as NRF2 modulation with Brusatol (BRU), an enhancer of ubiquitination and degradation of NRF2, may offer alternative therapeutic approaches. This study evaluates the impact of NRF2 pathway inhibition in CLL patient's cells using Brusatol in monotherapy and in combination with conventional therapy, namely ibrutinib and/or venetoclax.

**Methods:** For this proposal, peripheral blood mononuclear cells from 27 CLL patients (pts) were isolated by ficol gradient and cultured in the absence (controls, CTL) as well as with different concentrations of BRU (5, 10, 25, 50, 100 nM), Venetoclax, a BCL-2 inhibitor (VEN 2.5 uM) and Ibrutinib, a BTK inhibitor (iBTK) (IBR 10 uM). The cells were also exposed to BRU in association with VEN or IBR or both. Cell viability was assessed using the Fluorometric Microculture Cytotoxicity Assay (FMCA) at 24 hours and 48 hours. Cell death was assessed vy flow cytometry at 24 hours the using Annexin V assay. Statistical analysis was performed using GraphPad Prism software, version 9.5 Windows (GraphPad Software, USA).

**Results:** We included 27 pts, 74% male, mean age at diagnosis of 69.1 years. At the time of analysis, 63% (n = 17) of pts were Binet A, 3.7% (n = 1) Binet B and 33.3% (n = 9) Binet C. Eight pts had immunoglobulin heavy chain variable region (IGHV) unmutated (in 10 pts analyzed), 3 pts harboured del17p (in 12) and 2 pts were TP53 mutated (in 14). We observed that, in monotherapy, BRU induced a reduction in cell survival in a dose, time, and Binet stage dependent manner. IC50 at 48 hours was 115 nM. We also observed a bigger therapeutic effect with lower drug dose in CLL cells harbouring either a TP53 mutation, del17p, and/or a unmutated IGHV when compared to those who did not have these molecular characteristics, as in first cases the IC50 at 48 hours is lower, about 83 nM, compared with those we didńt have these characteristics (359 nM). Further, we observed that BRU alone induced CLL cell death mainly by apoptosis ($p < 0.001$). Moreover, when BRU were used in therapeutic combination, dual and triple therapies were not statistically superior to BRU in monotherapy ($p \geq 0.05$), at least in the FMCA analysis, whereas in the cell death analysis, these combinations significantly increased apoptosis ($p < 0.001$). However, they also increased apoptosis in normal cells, raising concerns about toxicity.

**Conclusion:** Our results suggest an effect of NRF2 inhibition on CLL cell survival, highlighting BRU as a potential therapeutic armamentum in CLL pts, potentially in those who have contraindication or resistance to BTKi or VEN. These findings support NRF2 as a novel therapeutic target in CLL, however further investigation into combination strategies and its role for overcoming treatment resistance are needed.

**Keywords:** Brusatol; ibrutinib; venetoclax

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

# Abstract ID: 1114

## **Title:** Clonal shifts of TP53 mutations in patients treated with BTK and BCL2 inhibitors: analysis of accompanying genomic changes

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**Background:** The selective advantage of TP53-deficient clones is unambiguous in patients receiving chemoimmunotherapy (CIT), where minor TP53-mutated subclones frequently undergo expansion. In contrast, the diminishing of the TP53-deficient cell population is extremely rare in the CIT context. Drugs inhibiting BcR signaling or BCL2 are efficient even in patients with disrupted p53 pathway. Although TP53 remains a marker of aggressive disease, its aberrations confer less selective advantage under these treatments. Still, clonal shifts involving TP53 mutations have been occasionally observed. The extent to which additional genomic alterations contribute to such shifts, and which genomic lesions might outcompete TP53-dysfunctional clones, is not yet fully understood.

**Objectives:** We aimed to investigate mechanisms underlying pronounced clonal shifts of TP53-mutated clones during treatment with BTK (BTKi) or BCL2 inhibitors (BCL2i), with the focus on accompanying genomic changes that may drive or counteract TP53 clonal dynamics.

**Methods:** In a single-center cohort of 171 patients with CLL treated with targeted agents, TP53 mutations were repeatedly tested using NGS on purified B-cells with a limit of detection of 0.3% variant allele frequency (VAF). Most patients previously received chemo(immuno)therapy (96%; range 1–10 prior lines, median 2). TP53 mutations were detected in 108 patients (63%). Serial samples from selected cases with marked TP53 clonal shifts underwent genomic profiling using the NGS panel LYNX (PMID: 34082072) covering 72 lymphoma-associated genes and genome-wide chromosomal aberrations, and/or whole-exome sequencing (WES) combined with genomic arrays. Single-cell DNA sequencing (Tapestri CLL panel) was carried out in one representative case.

**Results:** Three main patterns of clonal evolution, i.e. diminishing, increase, and exchange of TP53-mut clone, were identified in 18 patients. The first group included four cases in which the TP53 mutated population diminished from a baseline VAF >5% (BTKi n = 2; BCL2i n = 2). In two out of three cases with available detailed analysis, the outcompeting wt-TP53 population carried alterations previously associated

with CLL progression and treatment resistance, i.e. highly complex karyotype with CDKN2A/B biallelic loss in one case, and BCL2 mutation the other. The cause of TP53-mut clone regression in the third case (VAF decrease from 28% to 1%) remained unexplained.
The second group involved eight patients with a significant increase of TP53 mutation (VAF_timepoint2 – VAF_timepoint1 > 20%; BTKi: n = 6; BCL2i: n = 2). In three cases with detailed analysis, one showed an acquired BTK resistance mutation within the expanding TP53-mutant clone, while two others developed complex genomic alterations: chromothripsis of chromosome arm 1q and a highly complex karyotype with del(17p13), respectively.
The third group comprised six cases (BTKi: n = 3; BCL2i: n = 3) with inter-clonal TP53 competition and replacement of the dominating TP53-mutant clone. The clonal exchange in three out of four patients analyzed in detail was accompanied by the emergence of two predominant BTK mutations, several subclonal mutations in BCL2, and a highly complex karyotype with del(17p13), respectively. No additional changes were identified in the fourth case. In a representative case from this group, single-cell DNA sequencing revealed convergent evolution of resistance driven by the emergence of four independent BTK Cys481Ser-mutated clones, collectively accounting for 99% of CLL cells (c.1442_1443delinsCT, and three clones carrying c.1442G > C). Each of these mutations was acquired within a clone carrying different TP53 mutation. Further clonal evolution led to the acquisition of mutation in the SPEN gene, transcriptional repressor of NOTCH1 target genes, likely contributing to the dominance of one TP53/BTK-mutant clone representing 70% of CLL cells.
**Conclusion:** Pronounced clonal shifts of TP53 mutations during treatment with BTK or BCL2 inhibitors are infrequent, even in this heavily pretreated cohort. In contrast to CIT, a significant reduction of TP53-mutant population can occur under targeted therapy but is typically associated with expansion of aggressive TP53 wild-type clones that acquired resistance-associated genomic alterations. In cases of TP53-mut clonal expansion or competition, we frequently observed the emergence of complex karyotype with del(17p13), and/or resistance mutations in BTK or BCL2. Single-cell analysis confirmed that targeted therapies exert strong selective pressure, promoting convergent evolution of resistance-associated mutations independently across multiple clones.

**Keywords:** TP53; clonal evolution; leukemia

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## Abstract ID: 1310

### **Title:** Evaluation of two novel cyclin dependent kinase 9 inhibitors for the treatment of myeloid cell leukaemia 1 (MCL1) high chronic lymphocytic leukaemia

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**Background:** Despite the introduction of BTK and BCL2 targeted drugs, chronic lymphocytic leukemia (CLL) remains largely incurable, and patients who become double refractory to these inhibitors have limited options representing an emerging clinical challenge. Clinical responses are highly variable in CLL highlighting the need to predict which patients will respond to which drugs. BCL-XL and MCL1, members of the BCL2 family of anti-apoptotic proteins, play a crucial role in CLL cell survival and their compensatory upregulation in the lymphoid microenvironment is known to confer resistance to the BCL2 inhibitor venetoclax (Thijssen et al, 2022). Dual inhibition of BCL2 and MCL1/BCL-XL could be a promising therapeutic strategy to overcome venetoclax resistance in CLL. Direct MCL1 inhibitors have shown promising preclinical results in CLL (Wei et al, 2020), but these results have failed to translate to the clinic due to the labile nature of MCL1 protein and concerns over cardiotoxicity (Wang et al, 2013). However, MCL1 protein levels can also be decreased indirectly by inhibiting transcriptional cyclin dependent kinases (CDKs). CDK9 promotes transcription elongation via RNA Polymerase II phosphorylation which rapidly increases the abundance of short-lived proteins, including MCL1 (Shapiro, 2006). The CDK9 inhibitor VIP152 has been shown to significantly reduce disease burden and improve overall survival in a murine CLL model (Sher et al, 2022), providing a rationale for the use of CDK9 inhibitors in CLL. We evaluated two novel selective CDK9 inhibitors, AU4-53 and AU11-8, for the treatment of CLL and hypothesized that patients with high MCL1 expression will be particularly responsive and that patients with high BCL2 and MCL1 expression may benefit from combination therapy with venetoclax.
**Methods:** Peripheral blood expression of BCL2, BCL-XL, and MCL1 was measured in primary CLL cells by multi-colour flow cytometry. To mimic interactions of CLL cells in the lymphoid microenvironment where they are known to upregulate BCL-XL and MCL1 and show marked resistance to venetoclax, primary CLL cells were stimulated using; anti-IgM-coated dynabeads to activate the B cell receptor (BCR), ± 1 µM ODN 2006 to activate Toll-like receptor 9 (TLR9), or co-cultured with NIH3T3 fibroblasts expressing human CD40L or untransfected cells (NTLs). CD40L mimics the stimulation CLL cells receive from activated T-cells in the lymph nodes. CLL cell viability was measured ± CD40L co-culture by flow cytometry using Annexin V/7-AAD staining. Subsequently, MCL1 protein expression and synergy between AU4-53/AU11-8 and venetoclax was assessed under these cytoprotective conditions.
**Results:** We observed heterogeneous basal expression of BCL2, BCL-XL and MCL1 in primary CLL cells (n = 25). Stimulation of primary CLL cells through the BCR, TLR9, and CD40 receptor to mimic the lymphoid niche induced small average fold-increases in BCL2 (BCR 1.10, TLR9 1.39, CD40 1.26, n = 18), but larger average fold-increases in BCL-XL (BCR; 1.45, TLR9; 2.25, CD40; 3.15, n = 18) and MCL1 (BCR; 4.96, TLR9; 4.23, CD40; 3.35, n = 18). Despite this, MCL1 expression remained extremely heterogeneous, and CLL cells which upregulated this protein did so consistently within all three in vitro stimulation models. From this we have identified

patients whose CLL cells appear strongly dependent on MCL1 in the lymphoid microenvironment and are potentially more likely to be venetoclax resistant. In all six primary CLL samples tested, the CDK9 inhibitors AU4-53 and AU11-8 effectively induced apoptosis of MCL1 high CLL cells on the CD40L co-culture, indicating their potential to overcome the cytoprotective lymphoid microenvironment. CLL cells most sensitive to AU11-8 had greater MCL1 expression ($p = 0.02$, r=-0.94), implicating MCL1 as a potential biomarker of sensitivity to this inhibitor. Both AU4-53 and AU11-8 caused a dose-dependent reduction in MCL1 protein expression on the CD40L co-culture in all three primary CLL samples tested so far, and preliminary data suggests that both inhibitors may show potential to synergise with venetoclax under these cytoprotective conditions.

**Conclusion:** The novel selective CDK9 inhibitors AU4-53 and AU11-8 show in vitro efficacy in CLL, implicating targeted inhibition of this kinase as an exciting therapeutic strategy for the treatment of MCL1 high CLL.

**Keywords:** MCL1; CDK9; drug resistance

**Please indicate how this research was funded:** Paul Stanforth PhD Scholarship and Brighton and Sussex Medical School

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## Abstract ID: 1010

### **Title:** Toll-like receptor 9 signalling is a potential tumour escape mechanism following B-cell receptor targeted treatments in subsets of patients with chronic lymphocytic leukaemia

**Authors:** Emma Kennedy[a], Lauren Stott[a], Iona Ashworth[a], Rosalynd Johnston[b], John Jones[c], Eleni Ladikou[c], Valter Gattei[d], Antonella Zucchetto[d], Fabio Simoes[a], Simon Mitchell[a], Chris Pepper[a] and Andrea Pepper[a]

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**Background:** Current B-cell receptor (BCR)-targeted therapies promote CLL cell egress from secondary lymphoid tissues yet often fail to achieve a complete systemic response. Acquired resistance to BTK inhibitors (BTKi) cannot be solely attributed to acquired BTK mutations (Bonfiglio et al., 2023), implicating the involvement of other signaling pathways. Toll-like receptor 9 (TLR9) is an intracellular pattern recognition receptor, which recognizes unmethylated CpG motifs in bacterial/viral/mitochondrial DNA. Since unmethylated DNA levels are up to 28-fold higher in CLL patient plasma relative to healthy controls (Kennedy et al., 2021), we investigated TLR9-signaling as a potential mechanism of resistance to BTKi therapy. We explored the effects of TLR9-activation upon primary CLL cell migration and identified subsets of patient samples with a heightened capacity for TLR9 signaling.

**Methods:** Primary PBMCs were stimulated overnight ± ODN 2006 (TLR9 agonist) ± ibrutinib (BTKi) ± ODN INH-18 (TLR9 antagonist) and transferred into transwell migration chambers to migrate against a CXCL12-gradient. At 4 hours, migrated PBMCs were stained for CD5/CD19/CD3 and counted volumetrically by flow cytometry.

**Results/Conclusions:** ODN 2006 induced heterogenous migratory changes in a cohort of 89 patient samples (ranging from 3.7 to 0.09-fold), which showed no correlation with the protein expression levels of either TLR9 or CXCR4 (CXCL12-receptor). We performed RNA sequencing on samples at either end of the migratory response spectrum to look for differences in the expression levels of downstream signaling components. We compared Responders (R) (i.e. migration increased >1.2-fold) vs Reverse Responders (RR) (i.e. migration decreased >0.8-fold) and found no transcriptional differences in TLR9, MYD88, IRAK4, IRAK1, IRAK2, TRAF6, or CXCR4. We then wanted to assess whether TLR9 signaling was inducible following ODN 2006-stimulation in both groups. At 24 hours post-stimulation, protein expression of IRAK1 was strongly degraded in R and RR alike, therefore confirming signal inducibility. IRAK4 expression however was significantly higher in R vs RR samples, suggesting an increased capacity for TLR-signaling in R samples. Following stimulation with ODN 2006, our RNA sequencing data identified significant upregulation of the NFκB-target genes: NFKBIA, NFKB2, and TNFAIP3 (which code for IκBα, P100, and A20, respectively) in both R and RR samples. Importantly, P100 was confirmed to upregulate at the protein level, and this upregulation correlated with the migratory response to ODN 2006; this suggested that the strength of TLR9-induced NFκB-signaling may influence migratory outcomes. We hypothesized that TLR9-induced signaling may differ between patient samples due to heterogenous levels of basal BCR-signaling. Using in-silico modeling, we simulated TLR9 activation with low/high levels of basal BCR activation. TLR9 activation induced IκBα degradation and p65:p50 dimerization only when basal signaling was low; simulated cells with high basal signaling remained unresponsive. In keeping with these predictions, laboratory experiments showed RR samples to have significantly higher basal p-BTK and significantly lower IκBα, relative to R samples; this is indicative of higher basal BCR-NFκB activation in RR vs R samples. Additionally, RR samples were associated with markers of aggressive disease (i.e. U-CLL, CD49d/CD38 positivity), whilst R samples were associated with markers of indolent disease (i.e. M-CLL, CD49d/CD38 negativity); since poor prognosis CLL is known to signal strongly through the BCR signaling pathway (Stevenson et al., 2011), we hypothesized that aggressive and indolent CLL may be driven by distinct signaling pathways. We then investigated the effects of BTK/TLR9-repression on migratory outcomes by stimulating samples with ODN 2006 ± ibrutinib ± ODN INH-18. In R samples, ODN 2006 induced an increase in CLL migration in both the absence and presence of ibrutinib; a combination of ibrutinib + ODN INH-18 resulted in a significant reduction in ODN 2006-stimulated migration (relative to ibrutinib-alone). In RR samples, ODN 2006 no longer induced a reduction in CLL migration in the presence of ibrutinib, and some samples instead showed an increase in CLL migration; this was indicative of a heightened responsiveness to TLR9 activation when BCR-signaling was repressed. Ibrutinib + ODN INH-18 induced a reduction in ODN 2006-stimulated migration in 5/8 RR samples. We therefore hypothesized that TLR9 may promote BTKi resistance in subsets of CLL patients by promoting migration in a BTK-independent manner. Early analyses of clinical response data suggest that TLR9 may be implicated in the depth of treatment responses to ibrutinib monotherapy in patients with poor prognosis CLL.

**Keywords:** Toll-like receptor 9; migration; ibrutinib

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## Abstract ID: 1221

**Title:** The integrin adaptor kindlin-3 regulates chronic lymphocytic leukemia development and progression

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Chronic lymphocytic leukemia (CLL) is a CD5+CD19+ B cell malignancy of the elderly. Tumor proliferation is driven by interactions of CLL cells with the microenvironment in secondary lymphoid organs. Integrins, stabilized by adaptor proteins such as kindlin-3, mediate these interactions and carry out functions such as modulation of B cell receptor clustering and antigen-triggered signaling. Previous research in the lab showed that B cell specific deletion of kindlin-3 in mice (hereafter referred to as K3ΔB) promotes antigen-independent B cell activation and differentiation, and hyperactivates CXCR5 signaling. Here, we investigated the role of kindlin-3 in development and progression of CLL. We generated a B cell specific kindlin-3 knockout mouse model of CLL-like disease, by crossing K3ΔB mice with the Eμ-TCL1-tg mouse model of CLL (here on referred to as TCL1-K3ΔB). Early time point analysis revealed that kindlin-3 deficiency almost completely abolished tumor onset in the peritoneal cavity. Blood tumor loads were significantly lesser in TCL1-K3ΔB mice through development, and end-point analysis showed that tumors were almost completely abrogated in the spleen and bone marrow, compared to TCL1-tg littermates. Using an inducible kindlin-3 knockout TCL1-tg model and transplantation experiments, we observed that kindlin-3 deficiency, in the short-term, leads to altered CLL cell localization in the spleen. Long-term engraftment experiments revealed that kindlin-3 deficient tumor cells displayed significantly lower levels of the negative prognostic markers CD49d (the alpha subunit of the integrin VLA-4) as well as CXCR4. Importantly, in these mice, an induced knockout of kindlin-3 led to reduction in blood tumor loads and arrested tumor progression. In summary, our research unveils an important role of kindlin-3 in CLL pathogenesis, playing an indispensable role in CLL onset as well as progression.

**Keywords:** Chronic lymphocytic leukemia; integrin adaptor; tumor microenvironment

**Please indicate how this research was funded:**

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## Abstract ID: 1710

**Title:** A nomogram for survival prediction based on socioeconomic factors in patients with chronic lymphocytic leukemia: an analysis of SEER database

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**Introduction:** Socioeconomic factors has been demonstrated to impact the outcomes of several malignancies. The prognostic role of socioeconomic factors in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) has not been systematically investigated.

**Methods:** We identified 67,238 patients diagnosed with CLL/SLL from the Surveillance, Epidemiology, and End Results database. In addition to survival data, the following variables were collected: age, gender, race, year of diagnosis, subsequent malignancies (defined by total number of in situ/malignant tumors for patient), marital status, median household income, rural-urban difference (defined by rural-urban continuum code). The patients were randomly divided into the training (n = 47,066) and validation (n = 20,172) cohorts in a 7:3 fashion. The univariate and multivariate Cox regression analyses were performed to screen the predictors for overall survival (OS). The nomograms were developed and validated by the C-index, calibration curve, receiver operating characteristic curve, and decision curve analysis.

**Results:** The median age was 69 years, and the male/female ratio was approximately 1.5:1. Most patients were married (42,310, 62.9%), followed by widowed (10,666, 15.9%), single (8380, 12.5%), and divorced (5882, 8.7%). There was no difference in baseline characteristics between the training and validation cohorts. In the training cohort, compared to married patients, single (harzard ratio [HR] = 1.15, $p < 0.001$), divorced (HR =1.17, $p < 0.001$), and widowed (HR =2.33, $p < 0.001$) patients had worse OS. In multivariate analysis, older age, male gender, diagnosis in 2000–2013, presence of subsequent malignancies, unmarried marital status, lower income (< $75,000), and rural residence predicted worse OS in the training cohort. A nomogram for OS based on marital status, age, gender, race, year of diagnosis, subsequent malignancies, median household income, and rural-urban difference was constructed. The C-indexes of the nomogram for OS were 0.720 (95% confidence interval [CI]: 0.717–0.724) and 0.721 (95% CI: 0.715–0.727) in the training and validation

cohorts, respectively. In the training cohort, the area under the curve (AUC) of ROC curves predicting 5- and 10-year OS were 0.750 and 0.759, respectively. In the validation cohort. The AUC of ROC curves predicting 5- and 10-year OS were 0.747 and 0.753, respectively. Calibration curves showed good concordance between the predicted 5- and 10-year OS with the actual survival in both the training and validation cohorts. Decision curve analysis (DCA) demonstrated considerable clinical net benefits for the 5- and 10-year survival prediction.

**Conclusions:** We constructed a clinically feasible nomogram containing socioeconomic factors that perform well in predicting the survival of patients with CLL/SLL. Socioeconomic factors need to be taken into consideration when making clinical decisions for patients with CLL/SLL.

**Keywords:** Chronic lymphocytic leukemia; socioeconomic factors; survival

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## Abstract ID: 1711

### **Title:** A 3 week pause versus continued BTKi during vaccination (IMPROVE): a randomised, open-label, superiority trial

**Authors:** Helen Parry[a], Jonathan Cook[b], Neil Phillips[c], Georgina Talbot[c], Abhishek Abhishek[d], Lelia Duley[e], Marie Hodges[a], Thomas Roberts[b], Vicki Barber[b], Anne Francis[b], Robbert Hoogeboom[f], Brian Willett[g], Sam Scott[g], Nilima Parry-Jones[h], Toby A. Eyre[i], Claire Hutchinson[j], Farooq Wandroo[k], Shankara Paneesha[l], Duncan Murray[m], Stephen Jenkins[n], Paul Moss[a], Earnest Heartin[o], Nicolas Martinez-Calle[d] and Piers Patten[f]

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CLL is the commonest leukemia and associated with profound immunosuppression. Bruton Tyrosine Kinase inhibitors (BTKi) have revolutionized CLL management, however therapy impairs vaccine-induced immunity and serious infection is observed in around 20%. Pausing therapy for a short duration has been postulated to improve vaccine immunity but has never been tested in a randomized setting. We evaluated if a three-week pause of BTKi treatment improved spike protein (S) receptor binding domain (RBD) immunity to SARS-CoV-2 vaccination whilst maintaining disease control. We performed an open-label, 2-arm parallel group, randomized trial in secondary-care hematology clinics in 11 UK hospitals. Patients aged 18 and over, diagnosed with CLL and currently taking BTKi therapy for at least 12 months were eligible. Participants were randomized (1:1 stratified by BTKi therapy line) to pause BTKi for 3 weeks, starting 6 days before their COVID-19 vaccine booster date or continue therapy as usual. The primary outcome measure was anti-spike-RBD-specific antibody titre 3 weeks post-vaccination. Secondary outcomes included antibody titre at 12 weeks, neutralization to variants of concern and cellular responses measured by IFNγ. Trial registration: ISRCTN 14197181. Between 10 October 2022 and 6 August 2023, 99 individuals were randomized to pause (n = 50) or continue (n = 49) their BTKi therapy. Median prior doses of COVID-19 vaccinations at enrolment was 5. At three-weeks post vaccine, the geometric mean (SD) RBD antibody level was 218.8 (122.9) and 153.4 (103.2) U/ml in the continue and pause arms, respectively, with geometric mean ratio (95% CI) 1.104 (0.565–2.158), $p = 0.772$, mixed-effects model and no difference in seroconversion between the 2 arms observed. Therapy interruption did not improve antibody neutralization or cellular IFNγ response to ancestral B.1 or variants of concern (VOCs) at 3 weeks post vaccine either. No intervention related serious adverse events were reported although self reported lymphadenopathy was more common in the pause arm than those that continued on therapy. Further supportive scientific work studying the heterogeneous responses observed in either arm, is now in progress. This study provides no evidence that pausing BTKi around the time of vaccination is beneficial for immunity and should not be recommended in clinical practice. Funding NIHR:151892.

**Keywords:** Vaccination; BTKi; antibody

**Please indicate how this research was funded:** NIHR EME trial

**Please indicate the name of the funding organization:** NIHR

## Abstract ID: 1011

### **Title:** A novel preclinical mouse model of chronic lymphocytic leukemia driven by BCOR loss in B cells

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**Background:** Chronic lymphocytic leukemia (CLL) is a hematological malignancy characterized by the accumulation of CD19+CD5+ cells that are resistant to apoptosis due to genetic lesions and microenvironmental stimuli. Despite recent advances in treatment, CLL remains incurable, underscoring the need to better understand the pathways supporting leukemic cell survival and to identify novel therapeutic targets. Within the genetic diversity of CLL, disruptive mutations of the BCL6 co-repressor (BCOR) occur in up to 2% of cases and

are considered disease-driving events. BCOR mutations often co-occur with unmutated IGHV, trisomy 12, and NOTCH1 mutations (Sportoletti et al., Blood 2021). BCOR is a known tumor suppressor in B cells, and its loss enhances NOTCH1 transactivation. In the TCL1 mouse model, we recently demonstrated that B-cell-specific BCOR inactivation promotes Richter Transformation (Rompietti et al., Leukemia 2025). However, no mouse model has yet demonstrated whether BCOR loss in B cells alone is sufficient to initiate CLL, highlighting a gap in our understanding.
**Aims:** To develop and characterize a novel mouse model with B-cell-specific BCOR deletion and evaluate its ability to spontaneously develop a CLL-like disease with aging.
**Methods:** We generated a conditional knockout mouse model by crossing Bcorflox/flox female and Bcorflox/y male mice with CD19-Cre + transgenic mice to achieve Bcor deletion specifically in CD19+ B cells (Hereafter, homozygous Bcorflox/flox;Cre + and hemizygous Bcorflox/Y;Cre + will be referred as CD19Bcor−/−). Mice were longitudinally monitored, and peripheral blood (PB), bone marrow (BM), and spleen were systematically analyzed through complete blood counts, multiparametric flow cytometry, histopathological examination, and immunophenotyping. To assess clonality, CD19 + CD5+ splenic B cells were sorted and analyzed for immunoglobulin heavy chain (IGH) gene rearrangements. To evaluate disease transplantability, 107 splenic cells from aged CD19Bcor-/- mice were injected intravenously into NSG recipient mice.
**Results:** At 18 months, CD19Bcor-/- mice exhibited B-cell lymphopenia with decreased follicular (Fo) and increased marginal zone (MZ) B cells, and reduced mature recirculating BM B cells, but no overt lymphoproliferative disease. With extended follow-up of 28 months, CD19Bcor-/- mice showed reduced survival (median survival of 762.5 days; range from 510 to 821 days) compared to wild type (WT) mice (undefined) as control (Bcorflox/flox;Cre- and Bcorflox/Y;Cre − indicated as WT). In CD19Bcor-/- mice, blood count analysis revealed elevated white blood cell levels compared to WT controls (12,910 ± 995.8 N = 17 vs. 9162 ± 1204 N = 14 cells/μL) during the 22 to 28-month follow-up period. Flow cytometric analysis of PB, spleen, and BM revealed a marked expansion of CD19 + CD5+ cells in CD19Bcor-/- mice compared to WT controls (538.1 ± 351.3 N = 17 vs 63.41 ± 16.76 N = 14 cells/μL in PB; 16.35 × 106 ± 4.728 N = 9 cells vs. 3.237 × 106 ± 0.9909 N = 5 cells in spleen; 1.913 × 106 ± 0.7439 N = 9 cells vs. 0.3721 × 106 ± 0.08494 N = 5 cells in BM, respectively). Histological analysis of CD19Bcor-/- moribund mice showed a multiple organ infiltration (including lung, liver, spleen, and kidney) of a lymphoid cell population co-expressing CD19 and CD5. To have a proof of clonality of the lymphoproliferations highlighted by flow cytometry, we conducted an analysis of IGHV rearrangements in splenic sorted CD19 + CD5+ cells, revealing monoclonality with a perfect match of 100% to the IGHV germline sequences. Immunophenotyping and molecular data were consistent with the diagnosis of a lymphoproliferative disorder, mimicking low-grade CLL. The CLL cells derived from the spleen were also transplantable into secondary NSG recipients, which developed CLL exhibiting phenotypic characteristics similar to those of the primary tumor. Adoptive transfer experiments of CD19Bcor-/- cells was performed to induce a disease with accelerated kinetics. Following the first round of transplantation, 38.55 ± 12.30 (N = 4) CD19 + CD5+ cells/μL were detected in peripheral blood after 60 days. In contrast, the second round resulted in a marked increase, with 532.3 ± 192.8 (N = 4) CD19 + CD5+ cells/μL observed after 50 days. These cells, similar to the primary tumor, were capable of infiltrating peripheral organs such as the spleen and liver.
**Conclusions:** These findings establish CD19Bcor$^{-/-}$ mice as a novel preclinical model that recapitulates key features of human low-grade CLL. Our data demonstrate that BCOR loss in B cells is sufficient to drive a chronic lymphoproliferative disorder with CLL-like features, providing a unique platform to dissect the molecular consequences of BCOR inactivation and to explore therapeutic vulnerabilities in CLL.

**Keywords:** CLL; BCOR; mouse model

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## Abstract ID: 1012

### **Title:** Evidence for a pre-switched population and unmutated immunoglobulin receptors in IgG-expressing CLL stereotyped subset #4

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**Background:** Chronic lymphocytic leukemia (CLL) subset #4 represents the largest stereotyped subset with somatically hypermutated clonotypic B cell receptor immunoglobulin (BcR IG) and a prototype for indolent disease. The BcR IG is encoded by the IGHV4-34/IGKV2-30 gene pair, which is rare in the normal repertoire. Moreover, it is IgG-switched, which is noteworthy considering the low frequency of IgG clones in CLL (5-8%). Subset #4 BcR IG are capable of specific homotypic interactions, which induce cellular activation. These interactions appear to be structurally mediated by elements of the gamma constant domain, providing a plausible mechanistic explanation for the obligatory IgG isotype expression observed in this subset. Subset #4 BcR IGs have been shown to be intraclonally diversified, alluding to prolonged interactions with antigen(s), albeit the available evidence is limited due to the low-throughput methodologies employed in the respective studies.
**Aims:** We aimed to characterized in-depth the subclonal architecture of CLL subset #4 by integrating high-throughput immunogenetic and transcriptomic analyses toward gaining insights into the evolutionary trajectories and clonal dynamics of this biologically distinctive CLL variant.
**Methods:** We undertook bulk next-generation sequencing (NGS) of IG gene rearrangements in longitudinal samples from 6 patients from subset #4 and 6 patients who also expressed IGHV4-34 yet did not belong to subset #4. For

precise tracking of isotype expression, leader primers specific for IGHV4-34 and 3′ primers specific to the mu or gamma constant domains were used. IG NGS was also performed in fluorescence-activated cell sorted (FACS) IgG$^+$ and IgM$^+$ B cells from subset #4 cases, combined with RNA sequencing to explore subpopulation-specific transcriptional programs.
**Results:** Subset #4 cases exhibited significantly ($p<0.05$) higher intraclonal diversity versus non-subset cases, with distinct subclonal architecture. Specifically, 4 cases were dominated by a single clonotype, while the remaining 2 displayed co-existence of ≥2 significantly expanded clonotypes at comparable frequencies. Regarding the light chains, 5 cases harbored a single dominant clonotype, whereas the remaining case exhibited multiple expanded clonotypes. In contrast, all non-subset #4 cases displayed a single dominant clonotype for both IG heavy and light chains. Longitudinal analysis documented significantly ($p<0.05$) greater subclonal drift in subset #4 versus non-subset #4 cases, as evidenced by great shifts in the size of expanded clonotypes, particularly for the heavy chains. Furthermore, precise SHM targeting was observed in subset #4, evidenced by recurrent amino acid substitutions at certain positions of the variable domain. Prominent examples concerned: (i) G-to-D at VH CDR1 position 28, detected in all cases at either the clonal (n=4) or subclonal level (n=2); N-to-D at VK FR3 position 66, detected in all cases at either clonal (n=4) or subclonal level (n=2). Rather unexpectedly, all subset #4 cases harbored IgG transcripts that lacked any SHM, albeit at extremely low frequencies (0.0003–0.03%). Furthermore, in 3 out of 6 patients, sorted IgM$^+$ cells contained clonally-related mu transcripts that were unmutated, also at low frequencies (0.06–1.3%). RNA sequencing revealed that IgM$^+$ and IgG$^+$ cells exhibited distinct transcriptional signatures. In particular, IgM$^+$ cells showed upregulated genes involved in metabolic processes, ribosome biogenesis, oxidative phosphorylation, and protein synthesis, compared to IgG$^+$ cells, indicative of a metabolically active and potentially more proliferative phenotype. In contrast, IgG$^+$ cells displayed a transcriptional signature characterized by reduced metabolic activity and cell proliferation, consistent with a functionally anergic state, in keeping with previous reports documenting an anergic state for subset #4 IgG cells.
**Summary/Conclusion:** Our findings reveal extensive intraclonal dynamics of the malignant clone in CLL subset #4, underlying a particularly complex architecture of the BcR IG gene repertoire. Moreover, they indicate that isotype switching to IgG may precede SHM in CLL subset #4, while also documenting the existence of pre-switched clonally-related cells, at least in a fraction of cases, whose precise biological role requires further investigation.

**Keywords:** Chronic lymphocytic leukemia; B cell receptor; stereotyped subset 4

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**Please indicate the name of the funding organization:** Institute of Applied Biosciences, Centre for Research and Technology Hellas

## Abstract ID: 1712

### **Title:** Other cancers in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma

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**Introduction:** Patients diagnosed with chronic lymphocytic leukemia or small lymphocytic lymphoma (CLL/SLL) exhibit an elevated risk of developing other cancers (OC) relative to the general population. Historically, chemoimmunotherapy (CIT) represented the cornerstone of CLL/SLL management. A systematic review of clinical trials of CIT for CLL that included 28 studies reported 352/3297 (12.6%) patients developed OC (Csanadi 2022). The introduction of targeted agents beginning in the mid-2010's, including Bruton tyrosine kinase inhibitors (BTKi) and BCL2 inhibitors, has shifted the standard of care toward these novel therapies. In this study, we determined the risk of OC in newly diagnosed CLL/SLL patients cared for at the US Department of Veteran Affairs (VA) and Mayo Clinic in Rochester, MN.
**Methods:** We identified patients with newly diagnosed CLL/SLL after 1 January 2016 using the VA Central Cancer Registry and the Mayo Clinic CLL Database. The baseline clinical characteristics, CLL treatments, and the occurrence of OC were abstracted by electronic health record (EHR) review. Receipt of CLL directed therapy was categorized into: (a) CIT only; (b) targeted therapy only; (c) CIT and targeted therapy; and (d) other (including monotherapy with monoclonal antibody therapy, etc). The occurrence of OC was modeled using CLL treatment as a time-dependent variable. Prior and concurrent OC diagnosed within 3 months of CLL/SLL were excluded from analyses, to reduce ascertainment bias.
**Results:** We identified 6346 patients with CLL/SLL who met inclusion criteria. Median follow up was 3.8 years (interquartile range [IQR] 2.1–5.9). The median age at diagnosis of CLL/SLL was 71 years (range, 23 - 102) and most patients were white (81%) and male (92%). Although many patients had missing values, among those with available information, IGHV genes were unmutated in 673 (51%), CLL FISH panel showed del17 in 186 (7.3%), del11q in 329 (13%), trisomy 12 in 578 (23%), and del13q in 1249 patients (50%). A total of 2201 patients received CLL directed therapy during follow-up: 285 (12.9%) received CIT only, 1680 (76.3%) received targeted therapy only, 95 (4.3%) received CIT and targeted therapy, and 141 (6.4%) monoclonal antibody therapy. Among patients treated with targeted therapies, 931 (52%) received ibrutinib,357 (20%) received acalabrutinib, 157 (9%) received zanubrutinib, and 258 (15%) received venetoclax. Other treatments included combination of a covalent BTKi (cBTKi) and BCL2i in 28 (2%) patients, among others. Five hundred seventy patients were diagnosed with OC during the study period. Types of OC observed included neoplasms of the prostate (n=127, 22.3%), lung (n=117, 20.5%), hematologic malignancies (n=90, 15.8%), melanoma (n=66, 11.6%), gastrointestinal tract (n=56, 9.8%), kidney and bladder (n=44, 7.7%), head and neck (n=19, 3.3%), and other/unknown (n=51, 8.9%).

Estimated risk of occurrence of OC was 4.3% at 24 months and 9.7% at 60 months after a diagnosis of CLL/SLL. Multivariable analyses demonstrated that older age (HR: 1.011, 95% CI: 1.003–1.018), male sex (HR: 1.7, 95% CI: 1.1–2.5), and receipt of CLL directed therapy (HR: 1.4, 95% CI: 1.2–2.7) were associated with an increased risk of OC. When considering different types of CLL directed treatments, those who received both CIT and targeted therapies had the highest risk of development of OC (HR: 1.7, 95% CI: 1.3–2.1), compared to those who received CIT only (HR: 1.5, 95% CI: 1.1–2.1) and those who received targeted treatments alone (HR: 1.26, 95% CI: 1.03–1.56).

**Conclusion:** In this large dataset of newly diagnosed patients with CLL/SLL in the past decade, approximately 10% patients developed OC at 5 years. Receipt of CLL directed therapy was associated with a 40% increased risk of development of OC compared to those patients who did not receive therapy. The risk was highest among patients who received CIT + targeted therapy compared to those who received CIT only or those who received targeted therapy alone.

**Keywords:** Secondary neoplasms; therapy-related cancer; survivorship

**Please indicate how this research was funded:**

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## Abstract ID: 1713

### **Title:** An assessment of NK and T cell functional immunity in response to vaccination whilst taking acalabrutinib or ibrutinib monotherapy

**Authors:** Aiysha Khan[a], Marie Hodges[a], Jared Darcy[a], Paul Moss[a], Becky Sinclair[a], Thomas Roberts[b], Piers Patten[c], Jianmin Zuo[a] and Helen Parry[a]

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**Background:** Humoral immunity to vaccination is known to be impaired on continuous BTKi therapy. In comparison, less is known regarding cellular responses to vaccination. Cellular immunity has been reported to improve during covalent BTKi therapy, evidenced through enhanced antigen specific responses to latent herpes viruses and the improved generation of CAR-T cells amongst patients previously treated with BTKi. Several mechanisms have been postulated to underpin this improvement. An appreciation of the contribution of NK and T cell antigen-specific immunity and a comparison of functional responses elicited by the different covalent BTKi drugs remains unknown.

**Aims:**

- To assess and compare antigen-specific cellular responses amongst patients taking either ibrutinib or acalabrutinib 3 weeks following vaccination and compare with humoral responses.
- To investigate the relative contribution of NK and T cell responses to 2 different pools of overlapping peptides from SARS-CoV-2 spike glycoprotein (S1 and S2) following vaccination.
- To assess the phenotypic and transcriptional differences between 'strong' and 'poor' cellular responders.
- To identify whether NK cells can respond to bioinformatically predicted individual peptides independent of T cell stimulation.

**Methods:** Fresh whole blood samples from participants of the IMPROVE trial (ISRCTN 14197181) were obtained 3 weeks following vaccination, stimulated with SARS-COV-2 peptides overnight and IFNγ production assessed using QuantiFERON ELISA (Qiagen 626715). Matched frozen PBMC samples were subsequently defrosted, rested and stimulated for 4 hours with 2 different peptide pools consisting of 315 peptides (15 mers with 11aa overlap) covering the spike glycoprotein (JPT peptides) and IFNγ production measured by ELISPOT. Using correlation analysis of these 2 functional assays, the top and bottom 17% of functional responders were identified. Using 10 colour flow cytometry panels, the surface phenotype and transcription factor profiles of CD3+ T cells and NK cells were assessed and compared amongst participants exhibiting the greatest and poorest cellular responses. To phenotype antigen-specific responses, PBMC samples were next rested overnight and subsequently stimulated with either S1 or S2 peptide pools for 4 hours before measuring IFNγ, TNFα and IL2 by flow cytometry alongside NK and T cell phenotypic markers.

**Results:** Functional T cell responses were observed in 93% of participants (86/92) after a median of 5 doses of SARS-CoV-2 vaccination. Only 2 participants (2%) lacked either an antibody or cellular response to vaccination. Cellular responses were increased following vaccination ($p = 0.0005$) compared to pre-vaccine samples, with no difference observed between those taking acalabrutinib (n = 43) and ibrutinib (n = 49) therapy. Despite this, amongst ibrutinib treated patients, a higher proportion of Th1 CD4+ T cells were observed compared to those taking acalabrutinib (ibrutinib 34.5% vs 16.1%; $p = 0.0007$). The reverse was noted, with higher proportions of Th2 CD4+ T cells observed amongst acalabrutinib patients. Fresh and frozen correlative analysis of Spike peptide responses showed a good correlation r = 0.589 $p = <0.0001$. A comparison of the best and worst cellular responders found no difference in the percentage of CD3+, CD4+ or CD8+ T cells. Amongst CD8+ T cells, expression of the transcription factor Tbet, was significantly higher in the best responders (median 68.53%) compared to those with poorer cellular responses (median 45.65%) ($p = 0.0045$). No difference in NK % or phenotypic expression of CD16+/ CD56+ was noted between the best and poorest responders. Amongst best cellular responders, the dominant peptide response was observed amongst CD8+ T cells (rather than CD4+ T or NK cells) and was predominantly combined IFNγ and TNFα production in response to the RBD (receptor binding domain) containing S1 peptide pool. In contrast, amongst poorer responders, NK responses dominated compared to CD4 or CD8+ T cell responses, with TNFα secretion observed in response to the S2 peptide pool. Work is now ongoing to establish if NK responses observed are dependent on T cell stimulation or peptide specific and results will be presented at IWCLL.

**Conclusion:** In conclusion, regardless of which covalent BTKi is taken, an improved cellular response to SARS-CoV-2 is observed following vaccination and will provide important protection for patients, particularly where antibody response are lacking. CD8+ T cell responses to S1 peptide pool provide the strongest cellular responses and are associated with increased Tbet expression. In comparison NK responses may compensate for some patients with poorer CD3+ T cell

responses and work is now ongoing to identify if this is T cell independent.

**Keywords:** Cellular; immunity; BTKi

**Please indicate how this research was funded:** Laboratory research grant and trial funding.

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## Abstract ID: 1115

### **Title:** Clonal evolutionary dynamics shaped by genetic profiles in chronic lymphocytic leukemia: a new era in disease monitoring and therapy resistance

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**Introduction:** Prognostic stratification in Chronic Lymphocytic Leukemia (CLL) relies on the CLL-IPI index, which incorporates clinical, cytogenetic, and biological markers, notably IGHV mutational status and TP53 alterations. Over the past decade, next-generation sequencing (NGS) has revealed key genomic, epigenomic, and transcriptomic features of CLL, identifying alterations linked to disease onset, progression, and therapy resistance. These advances have supported the development of refined prognostic tools and targeted therapies. However, CLL remains a paradigm of cancer evolution: despite effective treatments, clonal progression and relapse are common. Clonal evolution post-immunochemotherapy (ICT) is typical, and resistance to targeted agents like Ibrutinib (IB) arises through tumor adaptation. The prognostic and predictive significance of clinical and clonal parameters in guiding therapy remains unclear. This study aims to evaluate the impact of these variables on clonal evolution in CLL.

**Patients and methods:** A retrospective observational study was conducted, including 96 untreated patients diagnosed with CLL/CLL-like (MBL) between 1999–2024. Key clinical and biological disease characteristics were recorded, along with dates of progression, treatment initiation, and response. Mutational status of TP53, SF3B1, BIRC3, NOTCH1, MYD88, XPO1, EGR2, POT1, NFKBIE, ATM, and FBXW7 was assessed in baseline samples and 302 follow-up samples using four independents targeted NGS panels based on Illumina paired-end short-read (150×2) sequencing technology. The IBR-R panel was designed and validated using Illumina short-read NGS for detecting resistance-associated mutations in BTK and PLCγ2 in patients treated with IB.

**Results:** In the baseline cohort, missense mutations were predominant in SF3B1, TP53, XPO1, and ATM, while NOTCH1 and BIRC3 mutations were primarily frameshift. Clonal and subclonal variants (VAF <10%) shared similar profiles, except in ATM, MYD88, and NOTCH1, where only clonal variants were identified. Convergent evolution was observed in 13 patients across TP53, SF3B1, ATM, XPO1, and BIRC3. Among 57 patients treated with ICT, longitudinal analysis was feasible in 52 cases (91%) with 205 sequential samples. Mutations (n = 11; range 1–3) with clonal VAFs were detected in 8/19 patients in remission (CR: 16; PR: 3); most mutations (91%) became undetectable within a median of 13 months. Non-responders showed greater baseline molecular heterogeneity (median 2 [0–5] mutations; 33% more mutations at relapse, $p = 0.045$). Three major clonal evolution patterns were identified in paired samples: (1) Cytogenetic lesions remained stable over time (excluding del(17p13)); (2) Emergence of TP53, SF3B1 mutations, and del(17p13) was associated with ICT resistance ($p < 0.031$, $p < 0.003$, $p < 0.05$, respectively), with most TP53 mutations subclonal (62%) and acquired (85%), and SF3B1 mutations mostly clonal (62%) and partially acquired (54%); (3) Other gene mutations showed variable VAF dynamics. In the IB-treated cohort (n = 39), 88% achieved response; 8% progressed with Richter transformation. Median follow-up was 38 months; median time to relapse was 34.6 months. Clonal composition was tracked over time (mean 26.9 months). Clonal trajectories were tracked across serial samples using the VAF% and cancer cell fraction (CCF) of all identified variants. An initial CCF rise at 3 months was followed by a 70.9% decrease by month 9. CCF reduction stabilized beyond 12 months (mean reduction: 83–95% at 24–48 months). When assessing the absolute rate of CCF change over time (dCCF/dt), showed exponential deceleration during the first year, then plateaued. BTK mutations (5%) co-occurred exclusively with TP53 alterations (isolated or del(17p13)). All BTK-mutated cases exhibited clonal expansion, with no observed convergent evolution or correlation with treatment history. While TP53/del(17p13) clones showed mixed dynamics, BTK clones consistently expanded (increasing CCF). Other driver genes displayed heterogeneity and varied across patients. In this patients, molecular progression preceded clinical relapse. Although BTK mutations were significantly associated with relapse ($p = 0.036$), and TP53 mutations showed a trend ($p = 0.093$), BTK status did not significantly impact progression-free survival (PFS) or overall survival (OS) in the studied cohort.

**Conclusions:** The results underscore the value of early genetic profiling via NGS, enabling the tracking of allelic fractions to assess clonal evolution over time. This reveals potential links between early clonal dynamics and unfavorable treatment outcomes: (1) Early genetic profiling by NGS in CLL provides valuable insight into clonal evolution and treatment response. Mutations in TP53, SF3B1, and del(17p13) are strongly associated with resistance to immunochemotherapy, while SF3B1 and NOTCH1 contribute to clonal expansion under Ibrutinib. (2) Early detection of BTK resistance mutations reinforces the role of molecular monitoring in anticipating relapse.

**Keywords:** Clonal evolution; next-generation sequencing (NGS); molecular monitoring

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** Lymphoproliferative Syndrome Group, INCLIVA Biomedical Research Institute. Hematology Service, Valencia University Clinical Hospital

## Abstract ID: 1116

### **Title:** IGHV next-generation sequencing in CLL: integrating mutational status and clonotype tracking for MRD assessment

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**Introduction:** Measurable residual disease (MRD) is a key biomarker for assessing treatment response and prognosis in chronic lymphocytic leukemia (CLL). Flow cytometry (FC), the standard MRD method, offers rapid immunophenotyping with sensitivity around $10^{-4}$ and established prognostic value, especially for undetectable MRD (uMRD). However, its limited sensitivity and inability to track clonal heterogeneity restrict its use. Next-generation sequencing (NGS) of the immunoglobulin heavy chain variable region (IGHV) advances MRD detection by providing higher sensitivity (~$10^{-6}$) without patient-specific primers. It also allows simultaneous determination of IGHV mutational status and detailed longitudinal clonal tracking within a polyclonal background. This study evaluates IGHV analysis by NGS for mutational status and dynamic MRD monitoring to improve prognostic accuracy and treatment guidance in CLL.

**Patients and methods:** A retrospective observational study included 58 untreated patients diagnosed with CLL/CLL-like monoclonal B-cell lymphocytosis (MBL) from 1999 to 2024. IGHV mutational status was determined by Sanger sequencing and compared to an automated method using shorter reads (Illumina 2×150 bp), and the subsequent analysis with in-house pipeline (https://github.com/afuentri/B347 MyRepCLL). Clonotype monitoring by NGS was performed on 127 sequential samples from 23 patients treated with chemoimmunotherapy (CIT; 40%) and 35 patients treated with ibrutinib (60%). Flow cytometry was conducted on peripheral blood after erythrocyte lysis using the ERIC 8-color panel following EuroFlow guidelines. Data acquisition was performed on a FACSCanto II (BD) and analyzed with Infinicyt™ (Cytognos). MRD was expressed as the fraction of CLL cells among nucleated cells, with negativity defined as $<1\times10^{-4}$.

**Results:** The mean number of sequences analyzed per patient was comparable between groups—112,885 for CIT (range: 1,471–1,427,745) and 118,308 for ibrutinib (range: 1,000–585,659). The average dominant clonotype count was higher in CIT-treated patients (104,823; range: 0–1,427,019) than in those receiving ibrutinib (48,288; range: 0–488,194). V-gene mapping efficiency was lower in CIT (mean: 25.85%) compared to ibrutinib (mean: 80.46%). In the CIT subgroup, the primary IGVH rearrangement was undetectable in 34% of samples (19/56). Among these, 100% (19/19) of patients achieved complete remission (CR). In contrast, 81.3% (13/16) of patients with an NGS IGVH ratio >10% relapsed ($p<0.001$). In 5% of cases (3/56), the clonotype read ratio was <1%, while in 29% (16/56) it remained >18%. Among ibrutinib-treated patients, the initial clonotype was undetectable in only 3% of samples (2/80), both from the same individual. After excluding 28 samples (35%) with persistent lymphocytosis, 62% (50/80) of the remaining samples showed an NGS IGVH ratio >14%, with a mean ALC of $2.95\times10^{9}$/L (range: 0.90–4.98). A separate comparative analysis of MRD detection by NGS and flow cytometry (FC) was conducted in 42 CIT-treated patients (67 follow-up samples). At baseline, 81% had a single dominant clonotype, while 19% had dual IGVH rearrangements. The median number of sequences per sample was 148,381 (range: 1,897–1,845,191), with a mean dominant clonotype count of 111,219 and median V-gene mapping of 48.68%. NGS demonstrated >90% concordance with FC at the <0.01% (<$10^{-4}$) threshold, with two discordant cases in which NGS detected low-level MRD (0.4% and 0.9%) not identified by FC. Both cases converted to MRD-negative in subsequent samples. In samples with MRD between <$10^{-4}$ and $10^{-4}$, polyclonal patterns were observed (mean maximum inter-rearrangement difference: −1.25×). In dual-rearranged cases, MRD was undetectable by FC in 11 of 16 samples. NGS showed full concordance (11/11) when tracking only the productive clonotype; non-productive rearrangements yielded detectable MRD down to 0.020%.

**Conclusions:** NGS-based MRD monitoring offers high sensitivity and strong clinical correlation in CLL. Undetectable clonotypes post-chemoimmunotherapy were associated with complete remission, while persistent clonotypes predicted relapse. In ibrutinib-treated patients, clonotypes often remained detectable, reflecting distinct clonal dynamics. NGS showed high concordance with flow cytometry, particularly when tracking productive rearrangements. These results support NGS as a reliable tool for MRD assessment and risk stratification in CLL.

**Keywords:** Next-generation sequencing (NGS); immunoglobulin heavy chain (IGHV); minimal residual disease

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** Lymphoproliferative Syndrome Group, INCLIVA Biomedical Research Institute, Hematology Service, Valencia University Clinical Hospital.

## Abstract ID: 1526

**Title:** Ibrutinib and acalabrutinib in the treatment of relapsed/refractory chronic lymphocytic leukemia: a real-world analysis from the Czech Chronic Lymphocytic Leukemia Patients Registry

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**Introduction:** Real-world evidence (RWE) analyses of unselected patient populations offer valuable insights into the effectiveness and safety of Bruton tyrosine kinase inhibitors (BTKi) in relapsed/refractory chronic lymphocytic leukemia (R/R CLL), supplementing data from randomized clinical trials.

**Methods:** We conducted a retrospective analysis using data from the Czech CLL Patients Registry (CLLEAR). Adult R/R CLL patients treated with either ibrutinib or acalabrutinib at one of 11 Czech hematology centers between December 2015 and June 2024 were included. All patients met the International Workshop on CLL (iwCLL) diagnostic criteria and had provided informed consent. The analysis endpoints included overall response rate (ORR), time to next treatment (TTNT), overall survival (OS), and adverse event (AE) incidence. Treatment response was evaluated per iwCLL 2008 criteria. TTNT was defined as the time from BTKi initiation to either subsequent treatment or death. TTNT and OS were estimated using the Kaplan-Meier method. AEs were graded using CTCAE v5.0, with a focus on atrial fibrillation, hypertension, bleeding, and infections. The impact of molecular risk factors (17p deletion and/or TP53 mutation, IGHV mutational status), clinical risk factors (bulky disease; Cumulative International Rating Scale, CIRS >6), and treatment-related toxicity on TTNT and OS was also evaluated.

**Results:** A total of 679 R/R CLL patients treated with ibrutinib (n = 556) or acalabrutinib (n = 123) were analyzed. Median age at BTKi initiation was 69.9 years for ibrutinib and 72.6 years for acalabrutinib; males predominated in both groups (66.2% vs. 68.3%). High comorbidity burden (CIRS >6) was observed in over half the cohort (54.6% vs. 60.4%), and the majority had advanced Rai stage III/IV disease (53.4% vs. 52.6%). The median number of prior treatment lines was 2 in both cohorts. Adverse molecular risk features were common: unmutated IGHV was present in 85.8% (ibrutinib) and 81.3% (acalabrutinib), while 17p deletion and/or TP53 mutations were seen in 56.3% and 44.7%, respectively. Isolated TP53 mutation was detected only in 11.7% (ibrutinib) and 6.5% (acalabrutinib) patients. Median follow-up durations were 28.1 months (ibrutinib) and 9.6 months (acalabrutinib). Treatment duration medians were 20.9 and 7.9 months, respectively. The ORR was 89% (ibrutinib) and 86.4% (acalabrutinib), with complete responses in 17.1% and 5.7%, respectively. Overall, 60.7% discontinued BTKi treatment (65.3% ibrutinib, 39.8% acalabrutinib). Treatment discontinuation due to AEs occurred in 16.5% (ibrutinib) and 6.5% (acalabrutinib) patients; progression accounted for 17.6% (ibrutinib) and 10.6% (acalabrutinib). Dose reductions were needed in 44.6% (ibrutinib) and 28.8% (acalabrutinib) patients. The estimated median TTNT was 51.6 months (ibrutinib, 95% CI: 48.2–65.8) and 60.1 months (acalabrutinib, 95% CI: 33.3–NA). Among all BTKi-treated patients, TP53 mutation and/or 17p deletion significantly reduced TTNT (e.g. 53.4 vs. 43.8 months for 17p deletion, $p = 0.007$). The estimated median OS in the ibrutinib cohort was 51.4 months (95% CI: 45.4–62.5). Molecular risk factors did not significantly affect OS; however, high comorbidity burden (CIRS >6) had a negative impact (36.7 vs. 74.2 months), without significantly affecting TTNT. Infections were the most common AEs: 42.3% (ibrutinib) and 31.1% (acalabrutinib), with grade ≥3 infections in 24.0% and 17%, respectively. Atrial fibrillation was reported in 13.8% (ibrutinib) and 5.1% (acalabrutinib), with a median onset time of 10.2 months (range: 0.4–51.1) post-treatment initiation in the ibrutinib group. Bleeding events occurred in 13.8% of patients, but only 1.9% were grade ≥3. None of the AEs showed a statistically significant impact on TTNT or OS.

**Conclusion:** Our real-world data confirm the clinical efficacy and manageable safety profile of BTKi in R/R CLL. The incidence and spectrum of cardiovascular toxicities, particularly atrial fibrillation, were consistent with findings from randomized clinical trials. Minor bleeding events appear underreported, likely due to limited clinical impact or under recognition by clinicians. The shorter follow-up period in the acalabrutinib cohort reflects its later approval and adoption in the Czech Republic, which limits longitudinal comparisons.

**Keywords:** Relapsed/refractory; ibrutinib; acalabrutinib

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## Abstract ID: 1013

### **Title:** The S100A9/EMMPRIN axis is involved in chronic lymphocytic leukemia progression and offers novel therapeutic opportunities

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**Introduction:** Chronic Lymphocytic Leukemia (CLL) is characterized by the clonal expansion of B cells in the peripheral blood, bone marrow (BM), and secondary

lymphoid organs (SLOs), with variable proliferation rates observed in classical proliferative centers (PCs). Several studies have shown that poor prognosis in CLL is associated with increased proliferation, primarily driven by interactions between the leukemic clone and the tumor microenvironment (TME). A major challenge in CLL is the development of therapies capable of blocking these interactions to overcome treatment resistance. To address this, our laboratory previously characterized the proteomic profile of serum-derived exosomes released by CLL cells from poor-prognosis patients. Among the most abundant cargo proteins, we identified the pro-inflammatory molecule S100A9, which is aberrantly expressed in leukemic cells from these patients. Exosomes carrying S100A9 activate the NF-κB pathway in CLL cells from patients with active disease. We also found that EMMPRIN (Extracellular Matrix Metalloproteinase Inducer), a key receptor for dimeric S100A9, is overexpressed in clinically progressive cases compared to indolent forms. Our results further demonstrated that the S100A9–EMMPRIN interaction activates not only NF-κB but also the PI3K/AKT and MAPK/ERK pathways, promoting tumor cell proliferation and survival. These findings suggest that the S100A9/EMMPRIN axis is a critical driver of CLL progression and represents a potential therapeutic target. Previous studies have shown that CLL cells infiltrate BM and SLOs by degrading the basement membrane through MMP2 and MMP9 (Angeles García-Pardo and Redondo-Muñoz 2021). BTK inhibitors (BTKi) disrupt TME interactions, promoting egress of CLL cells from PCs to peripheral blood and preventing re-entry, thereby inducing tumor cell death (Lu, Wang et al. 2021). BTKi also alter chemokine expression, including CCL3 and CCL4, which mediate cell homing (Ponader, Chen et al. 2012). Given that EMMPRIN is a classical inducer of MMP2/9, we investigated whether S100A9/EMMPRIN pathway activation influences CLL cell migration in response to BTKi. Working Hypothesis: The S100A9/EMMPRIN axis is a novel pathway involved in CLL progression, which is blocked by BTKi in treatment-responsive patients. However, its behavior in non-responder or refractory patients remains unknown.

**Goals:**

1. To demonstrate that the S100A9/EMMPRIN axis is a new putative therapeutic target in CLL.
2. To explore molecular mechanisms underlying resistance to BTKi in CLL with focus on S100A9/EMMPRIN axis.

**Methodology:** CLL samples were obtained with informed consent and Ethics Committee approval. PBMCs before and after treatment were collected and stored in the GURU-LLC Biobank (project GURU-01). In-vitro activation experiments with S100A9 and inhibition of BTK pathway were performed using PBMCs from untreated CLL patients. Ex-vivo studies with PBMC from treated CLL patients were also conducted.

**Results:** Our results show that S100A9 stimulation promotes survival, proliferation, and angiogenic capacity of CLL cells. After in-vitro S100A9 stimulation, primary CLL cells exhibit increased expression of EMMPRIN and its transcription factor SP1 (specificity protein 1). In addition, proliferating subsets (CXCR4dim/CD5high, or IgM/IgG) obtained after treatment show higher EMMPRIN expression than quiescent cells. These data suggest that the proliferative fitness of these subsets is enhanced, as evidenced by increased S100A9/EMMPRIN activity. Moreover, CLL cells activating this axis express higher levels of MMP2, MMP9, CCL3, and CCL4, all key molecules involved in migration and leukemic progression. Interestingly, in vitro treatment with ibrutinib (1 μM, 24 hours) downregulates this pro-angiogenic profile. SP1 and EMMPRIN upregulation, and their subsequent reduction after ibrutinib treatment, suggest that the S100A9/EMMPRIN axis is a TME-triggered program involved in disease progression. Consistent with this, CLL cells from patients treated with ibrutinib show decreased SP1 and EMMPRIN levels post-treatment, along with reduced MMP2, MMP9, and CCL3/4 mRNA expression. Together, these findings suggest that inhibition of BTK pathway also blocks the S100A9/EMMPRIN axis by suppressing SP1 and EMMPRIN expression, thereby hindering MMP2/9 and CCL3/4 induction, which in turn prevent lymph node re-entry, and promoting leukemic cell death in periphery.

**Conclusions:** Our data indicate that the S100A9/EMMPRIN axis significantly contributes to CLL cell proliferation, survival, and migration. Understanding how this pathway interacts with targeted therapies such as BTKi may reveal new insights into resistance mechanisms and guide therapeutic strategies.

**Keywords:** S100A9/EMMPRIN; BTKi; migration

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## Abstract ID: 1211

### **Title:** Unraveling a poor-prognosis CLL subgroup: from IGHV bias and AID activation to altered T-cell profiles

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Chronic lymphocytic leukemia (CLL) cell proliferation and survival are supported by tumor microenvironment (TME) interactions, antigen/autoantigen (Ag/autoAg) stimulation, and/or autonomous signalling, among others (Kipps et al., 2017). B-cells are typically activated in the germinal center upon B-cell receptor (BCR)-Ag stimulation. B-cells interact with T-follicular helper cells, inducing upregulation of activation-induced cytidine deaminase (AID), a mutagenic enzyme essential to initiate somatic hypermutation and class-switch recombination. AID preferentially mutates Ig genes (VDJ or pre-switched regions); however, uncontrolled expression of this enzyme could result in 'off-target' mutations in proto/oncogenes, originating cancer development and/or tumor progression (Okazaki et al., J Exp Med, 2003; Morande and Yan et al., Blood, 2021). In CLL, AID expression by CLL

cells with a 'naïve' phenotype (CD5+/IgM+/IgD+) in unmutated patients (U-CLL) with clinical poor outcome (Oppezzo et al., Blood, 2003), as well as its role during disease progression, remain open questions. Despite significant progress in prognostic tools, accurately predicting disease progression remains challenging in many CLL patients. To address this issue, in previous work by Souto, Landoni et al. (abstract #1144, iwCLL_2023), we studied AID expression in the peripheral blood of U-CLL patients and analyzed its association with immunoglobulin heavy chain gene (IGHV) status/usage and time to first treatment (TTFT). This analysis led to the identification of a novel CLL subgroup, Subgroup_1 (Sb_1), characterized by high AID expression, specific IGHV rearrangements (1-69, 1-02, 3-30, and 4-39), and shorter TTFT. The second subgroup (Sb_2) is characterized by expression of the same IGHV rearrangements but low levels of AID and longer TTFT. Our central hypothesis is that the poor clinical outcome observed in Sb_1 corresponds to an activated tumour microenvironment (aTME) that enhances AID expression through continuous stimulation by an unknown antigen or autoantigen. This is supported by the biased usage of BCR variable regions in these patients and by previous WES data showing canonical AID off-target mutations in key leukemic drivers (Morande and Yan et al., Blood, 2021). We believe that some of these AID mutations confer a greater tumour 'fitness' of CLL cells which explain at least in part, the poorest clinical outcome of this subgroup. To better characterize the TME in these two subgroups and considering the relevance of the T-cell compartment in CLL evolution, we analyzed the phenotypic profile of the T-cells in Sb_1 and Sb_2. To this, PBMCs were collected from patients with informed consent and Ethics Committee approval and stored in the GURU-LLC Biobank at the Institut Pasteur de Montevideo. Flow cytometry was used to characterize both the tumor clone and T-cell populations. Our results revealed a significantly higher percentage of exhausted-like T cells in Sb_1 compared to Sb_2. This was evidenced by increased CD3+/CD8+/PD1+ cells (mean difference: 7.1; means 8.29 vs. 1.13; $p = 0.0264$, n = 18) and CD3+/CD8+/TOX + cells (mean difference: 5.98; means 7.75 vs. 1.77; $p = 0.0445$, n = 18). For CD4+ T-helper cells, subsets were defined by CXCR3 and CCR6 expression: Th1-like (CXCR3+ CCR6−), Th2-like (CXCR3− CCR6−), Th17-like (CXCR3− CCR6+), and CXCR3+/CCR6 + (Th0 or Tregs). Interestingly, Sb_1 showed a decrease in Th17-like cells (mean difference: 3.65; means 8.11 vs. 11.77; $p = 0.0535$) and a significantly reduced Th2/Th1 ratio (mean difference: 3.13; means 1.54 vs. 4.68; $p = 0.0370$). Persistent antigen stimulation in CLL contributes to T-cell dysfunction and exhaustion, correlating with poor outcomes (Jimenez et al., Biomarker Research, 2021). Our finding that Sb_1 is enriched in exhausted-like CD8+ T cells suggest a more immunosuppressive TME in this subgroup, supporting our hypothesis. Although the role of T-helper cells in CLL remains debated, Th1/IFN-γ+ cells may promote disease progression by increasing CD38 expression and inhibiting apoptosis in leukemic cells (Bürgler et al., JOI, 2015; Roessner et al., BJH, 2020). The lower Th2/Th1 ratio in Sb_1 may reflect increased IFN-γ activity, potentially contributing to early progression. Despite it is an interesting observation in the context of the earlier progression of Sb_1, more studies will be performed in this line. Altogether, these results suggest that Sb_1, representing 36% of U-CLL cases, is characterized by a distinct T-cell profile associated with an adverse TME. Further studies are needed to explore the contributions of IGHV rearrangements, AID expression, and T-cell dysfunction to CLL progression and therapy resistance.

**Keywords:** CLL; AID; T-cells

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# Abstract ID: 1527

## **Title:** Ibrutinib can be effective even at the lowest dose after 10 years of treatment

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**Introduction:** After the first approval of ibrutinib in 2013 for the treatment of of chronic lymphocytic leukemia (CLL), it has been evident that Bruton tyrosine kinase inhibitors will change the treatment paradigm in CLL. Five-year follow-up studies of ibrutinib have shown impressive results with a 92% progression-free survival (PFS) and overall survival (OS) when used as first line and 43% PFS, 57% OS when used in relapsed-refractory CLL . The discontinuation rates were more than 30% at 5 years due to disease progression and more than 20 % due to ibrutinib intolerance. With a median follow-up of almost 10 years, a median PFS for majority of patients is more than 85 months, but lower in relapsed/refractory patients (up to 49 months). In case of intolerance or adverse event, ibrutinib dose can be reduced and several studies have shown that patients who reduced the dose remained on ibrutinib longer compared with those who did not have the dose reduced.

**Case presentation:** A 52-year old male has been diagnosed with CLL in 2005. At the time, he was asymptomatic, RAI 1, Binet B, CLL IPI low risk so he was monitored without treatment. In 3 years he started to develop gradual symptoms, including intermittent fever, increasing fatigue, loss of appetite, and weight loss. On exam, he was found to have increasing lymphadenopathy and lymphocytosis. FISH was repeated and didn't show any abnormalities. After discussion of treatment options, he received 3 cycles of fludarabine, cyclophosphamide (FC) and prednisone with complete regression of lymphadenopathy and lymphocytosis but prolonged neutropenia. After 2 years, he progressed and FC with Rituximab was administered for 3 cycles. Due to persistent lymphadenopathy, the treatment continued with cyclophosphamide, vincristine, prednisone and rituximab (COP-R) for 3 cycles but with partial response. In 2 years he progressed again and received additional 3 cycles of R-COEP, but the disease was refractory to treatment. In May 2015. the patient started treatment with ibrutinib in a compassionate use program. At start of treatment he was Rai stage IV with bulky lymphadenopathy. Due to increased liver enzymes, hepatitis B serological testing was done and showed parameters for immunity following infection, while HBV-DNA was negative. Because HBsAg positivity was considered as an exclusion criterion in ibrutinib clinical trials, no recommendation was provided in guidelines for the management of past HBV infection. The patient started antiviral prophylaxis and initial ibrutinib dose of 140 mg daily. After 6 months of ibrutinib treatment, his lymphadenopathy resolved for more than 50%. After 12 months of treatment, the patient achieved complete remission and has continued with daily dose of 140 mg

ibrutinib for the next 10 years . Ibrutinib has been well tolerated without significant adverse events and great quality of life.

**Conclusion:** The first dose reduction of ibrutinib in R/R CLL patients in real-world settings was often reported during the first year of treatment. Dose modifications were mainly attributed to toxicity, but the patients who had at least a dose reduction had a similar PFS than patients with no dose reduction, implying the fact that ibrutinib can still be administrated in case of AEs while keeping CLL under control. This case report demonstrates the efficacy of the lowest ibrutinib dose from the beginning and continuous treatment for more than 10 years in relapsed / refractory CLL patient with minimal adverse effects. Previous HBV infection should not be a contraindication for treating CLL patients with ibrutinib or other signal inhibitors, but regular follow up and antiviral prophylaxis are needed when there is a risk of reactivation.

**Keywords:** CLL relapsed/refractory; hepatitis B; ibrutinib

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## Abstract ID: 1223

### **Title:** Depletion of normal B-cells, defined as <20 cells/μL by high-sensitivity analysis, identifies early-stage CLL and MBL patients at increased risk of severe infection and early mortality

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Background Chronic Lymphocytic Leukaemia (CLL) and CLL-type Monoclonal B-cell Lymphocytosis (MBL), are characterized by progressive immune dysfunction, contributing to significant infectious morbidity. Although stage A CLL is associated with increased mortality, early treatment has not been shown to extend overall survival (OS), potentially due to treatment-induced immunosuppression in immunocompetent patients. Humoral immune impairment is now usually reversed in patients receiving time-limited treatment for progressive disease. Identifying patients at increased risk of severe infection at an early stage could help develop strategies to improve outcomes for patients on active monitoring. Although hypogammaglobulinemia is a recognized predictor of infection-related issues, the specific impact of normal B-cell depletion on immune function and mortality risk remains less well defined. Aims To determine whether the depletion of normal B-cells is associated with increased infectious morbidity and reduced survival in stage A CLL and CLL-type MBL. Methods This retrospective study analyzed patients with stage A CLL (n = 753) or CLL-type MBL patients (n = 377) under active monitoring within a regional diagnostic service (HMDS). Neoplastic and normal B-cells were assessed at 6–12 monthly intervals using ≥6-color flow cytometry that is sufficiently sensitive to measure residual normal B-cells at the level of 20/μL or better. Serum immunoglobulin levels were also tracked. Severe infections were identified from ICD10-coded hospital statistics in a cohort of 575 stage A CLL registered with the Haematological Malignancy Research Network (HMRN) which collects national outcome data. Survival was measured from the time of first normal B-cell assessment. Results In the CLL cohort, baseline normal B-cell counts were >50/μL (normal) in 237/753 (31%, 101M/136F, median age 71.8 years); 20–50/μL (partial depletion) in 353/753 (47%, 208M/145F, median age 72.9 years); and <20/μL (depleted, based on assay detection limit) in 163/753 (21%, 107M/56F; median age 74.5 years). Patients with normal B-cell counts ≥20/μL at diagnosis exhibited a median OS of 10.4 years, significantly longer than the 7.6 years observed in those with <20/μL normal B-cells ($p < 0.001$). Progressive normal B-cell depletion occurred over time: median time to depletion (< 20/μL) was 3.0 years for CLL patients with normal B-cell levels between 20–50/μL at first assessment, and 10.4 years for those starting with ≥50/μL normal B-cells. At 3 years post-diagnosis, CLL patients with ≥20/μL normal B-cells showed equivalent survival to the age- and sex-matched general population, whereas those with depletion (< 20/μL) had a 14% lower net survival. The severe/hospitalized infection rate in the 2 years following diagnosis was nearly double in the patients with normal B-cell depletion (< 20/μL: 17% vs. ≥20/μL: 9.8%; Chi-square $p = 0.033$). Treatment for progressive disease was initiated in similar proportions regardless of baseline normal B-cell count (< 20/μL: 9.1% vs. ≥20/μL: 6.2%; Chi-square $p = 0.17$). In the CLL-type MBL cohort, normal B-cell counts were ≥20/μL in 339/377 (90%) and this group demonstrated survival equivalent to the age- and sex-matched general population, while 38/377 (10%) had <20/μL normal B-cells at first assessment and showed a trend toward poorer survival (age-adjusted HR 1.57; 95% CI 0.98–2.52; $p = 0.063$). Normal B-cell depletion correlated with decreased serum IgG levels in both cohorts. Patients with a severe infection in the 3 years prior to diagnosis, and/or presenting with low normal B-cell counts at diagnosis experienced the worst survival outcomes. Summary/Conclusion Normal B-cells are not usually enumerated in patients presenting with CLL because it requires an MRD-style approach that is not necessary for routine diagnosis. In this analysis we have demonstrated that the depletion of total normal B-cells below 20/μL effectively identifies a subset of stage A CLL and CLL-type MBL patients at substantially increased risk of severe infections and early mortality. The rate of B-cell depletion is highly variable, suggesting heterogeneous immune trajectories, with some patients maintaining immune stability for over a decade while others experience rapid attrition within a few years. We are performing survival analysis with normal B-cell depletion as a time-dependent covariate and investigating the impact of naïve vs. memory B-cell depletion. However, the current results indicate that the residual normal B-cell level is a key parameter for identifying individuals who may benefit from earlier interventions to mitigate infection-related morbidity and mortality.

**Keywords:** Immune impairment; normal B-cells; survival

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## Abstract ID: 1433

**Title:** Ibrutinib plus venetoclax with measurable residual disease-guided duration of treatment is superior to both ibrutinib monotherapy and fludarabine-cyclophosphamide-rituximab for previously untreated chronic lymphocytic leukaemia: report of the phase III UK NCRI FLAIR study

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**Introduction:** Ibrutinib (I) and venetoclax (V) improve CLL survival outcomes compared to chemoimmunotherapy. Mathematical disease modeling and Phase II studies favour defining treatment duration according to individual patient (pt) sensitivity to I+V combination. We hypothesized that I monotherapy or I+V where duration of therapy is guided by measurable residual disease (MRD) is more effective than FCR in CLL and that treatment duration personalized using MRD response would optimize outcomes.

**Methods:** FLAIR (ISRCTN01844152) is an ongoing, phase III, multicentre, randomized, controlled, open, parallel group trial for pts with untreated CLL. We report a planned analysis of I+V vs I vs FCR. Peripheral blood (PB) MRD at a threshold of <0.01% was assessed at 12 months and then 6 monthly. Time to undetectable MRD (uMRD) in the I+V and I monotherapy arms was calculated as date of therapy initiation to date of initial uMRD in PB, giving a treatment duration between 2 and 6 years. The primary endpoint for I+V vs I was uMRD in bone marrow (BM) up to 2 years. A powered secondary endpoint for I+V vs I vs FCR was progression-free survival (PFS), and other secondary endpoints were overall survival (OS), iwCLL response, MRD and safety.

**Results:** 786 patients (pts) were randomized to FCR (n=263), I (n=263) or I+V (n=260) from 96 UK Centres from 20 July 2017 to 24 March 2021 with data-lock on 4 November 2024. 71.1% were male, median age was 62 years, 42% were Binet Stage C, IGHV unmutated 49.6%, 33% IGHV mutated and 6% Subset 2. Hierarchical FISH were 16.7% 11q del, 16.7% trisomy 12, 23.5% normal and 37.5% 13q del. At 2 years, 299 participants were uMRD in BM: 127/263 in FCR (48.3), 0/263 in I only (0%) and 172/260 in I+V (66.2%) arms ($p<0.0001$). At a median of 62.2 months, there were 189 PFS events: 112 FCR, 59 I and 18 I+V. Hazard ratio (HR) for PFS for I+V vs I was 0.29 (95% CI: [0.17, 0.49]; $p<0.001$). HR for PFS for I vs FCR was 0.44 (95% CI: [0.32, 0.60]; $p<0.001$). HR for PFS for I+V vs FCR was 0.13 (95% CI: [0.08, 0.21]; $p<0.001$). 5-year estimated PFS rates for FCR, I and I+V were 58.1%, 79.0%, 93.9% respectively. There were 76 deaths: 39 FCR, 26 I and 11 I+V. OS was significantly better for I+V vs. I (HR 0.41; 95% CI: [0.20,0.83]; $p=0.013$) with a trend toward improved OS for I vs. FCR (HR 0.64 (95% CI: [0.39, 1.05]; $p=0.07$). 5-year estimated OS rates for FCR, I and I+V were 88.2%, 91.3% and 96.2%. At 9 months, uMRD in BM was higher in FCR arm as compared to I and I+V arm: 40.7% vs 0% vs 33.1%. However, with continued I+V more pts became uMRD in BM and PB. MRD stopping rules resulted in 115 participants stopping at 2 years, 25 participants stopping at 3 years and 13 participants stopping at 4 years. A higher proportion achieved an ORR for I and I+V; ORR 83.7% for FCR, 94.7% for I and 96.2% for I+V. 657 SAEs were reported in 390 (51.6%) pts (129 FCR vs 130 I vs 131 I+V). SAEs by organ class for FCR vs I vs I+V were infections in 18.8% vs 25.4% vs 23.7%; hematological in 31% vs 4.2% vs 5.8%; and cardiac in 0.4% vs 10.4% vs 10.9% respectively. 15 pts had sudden or cardiac deaths: 4 FCR, 8 I and 3 I+V. The incidence of other cancers per 100 pt-years was greater for FCR than I and I+V; 5.5 vs 3.2 vs 2.8.

**Conclusion:** I+V significantly improved uMRD, PFS and OS rates compared to I monotherapy and FCR in untreated CLL. FLAIR is the first trial confirming the superiority of MRD-guided I+V over I monotherapy and further substantiates the use of risk-adapted approach in untreated CLL patients to optimise outcomes.

**Keywords:** Leukaemia; MRD; personalized

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**Please indicate the name of the funding organization:** Cancer Research UK, Johnson and Johnson, Pharmacyclics, AbbVie.

## Abstract ID: 1224

**Title:** A fraction of lymph node CLL B cells exhibits high expression of T-bet

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**Introduction:** T-bet has been shown to suppress proliferation of chronic lymphocytic leukemia (CLL) cells and expression of T-bet in peripheral blood (PB) CLL cells has been associated with increased patient survival (Roessner et al., Blood, 2024). Paradoxically, T-bet induction is driven by NF-κB activity, a known driver of CLL B cell proliferation in lymphoid tissue. We therefore set out to ascertain levels of expression of T-bet in different CLL disease compartments, and to examine how T-bet expression may relate to microenvironmental stimuli and CLL B cell proliferation.

**Method:** We assessed T-bet expression in CLL cell lines, a murine CLL model, and primary human CLL cells derived from PB and Lymph Nodes (LN) using qPCR, flow cytometry and multi-parameter immunofluorescence confocal microscopy, followed by investigation of the morphology and the proliferative state of T-bet high CLL B cells in primary tumour material, using conventional and imaging flow cytometry. The phenotype of T-bet high CLL subset was further characterized using a mass cytometry (CyTOF) panel containing antibodies for functional markers. Additionally, we explored mechanisms of T-bet induction by mimicking the tumour microenvironment (TME) in vitro.

**Result:** T-bet expression was measured in CLL and non-Hodgkin lymphoma cell lines. Elevated T-bet expression was observed in CLL cell lines (MEC-1: 3.2-fold; PCL-12: 1.8-fold), alongside activated B-cell DLBCL lines (OCI-Ly3: 4.1-fold; HBL-1: 2.0-fold), and the MYD88 L265P-mutated Burkitt's lymphoma line Bjab (1.6-fold), normalized to germinal centre DLBCL lines (OCI-Ly1; DHL-4) and Burkitt lymphoma (Ramos) controls, where T-bet was undetectable. Quantitative PCR analysis confirmed TBX21 mRNA upregulation in CLL cell lines, consistent with T-bet protein expression trends. T-bet was also detected in PB, LN, and spleen cells derived from the Eμ-TCL1-tg mouse model for CLL. In contrast, primary human PB CLL B cells almost exclusively exhibited intermediate T-bet expression. In cryopreserved PBMCs from CLL patients, the CD5+CD19+ CLL B cell subset displayed significantly higher T-bet protein levels relative to fluorescence minus one (FMO) control (Mean ΔgMFI= 52.72; $p=0.004$), with obvious interpatient heterogeneity ($p<0.0001$; n=11). A small subset of PB CLL B cell (median frequency: 1.49%; range 0.02%–8.27%) exhibited T-bet protein levels equivalent to T-bet positive PB-derived CLL T cells (internal positive control), though their frequency remained significantly lower than T-bet high T cells ($p=0.0008$; n=15). However, we identified a fraction of highly expressing T-bet CLL B cells (T-bet HI) in both formalin-fixed paraffin-embedded (FFPE) LN samples (range: 2.1–4.5%; n=8), and LN-derived fine needle aspirates (range: 1.55–26.6%; n=4). In 3 of 4 LN samples, T-bet HI frequencies were increased compared to paired PB samples (Median fold increase: 4.7). T-bet HI cells exhibited a larger size and enrichment in the G2 phase of the cell cycle. Mass cytometry data indicated that the T-bet HI cells co-expressed higher levels of Ki67 and BCL2, and lower levels of homing receptors (CXCR5, CXCR4). In vitro stimulation experiments demonstrated that the TLR9-ligand CpG and IFN-γ synergistically induced T-bet expression, with TLR9 signalling upregulating the IFN-γ receptor. Furthermore, induction of proliferation in vitro using CD40L+IL21 or IFN-γ+CpG gave rise to T-bet HI cells.

**Conclusion:** These findings indicated that levels of T-bet expression in CLL are variable and dynamic. Expression was low in blood while increased expression was observed in cells at sites of known tumour proliferation. Further, we demonstrated that T-bet can be induced in PB CLL cells under conditions that mimic the TME, potentially via TLR9-driven enhancement of IFN-γ signalling. The overall relationship between the level of T-bet expression, cellular proliferation and patient outcomes may therefore be more complex than to date explained, meriting future studies.

**Keywords:** T-bet; CLL; tumour-microenvironment

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1117

### **Title:** The clinical significance of specific SF3B1 mutation variants in Chinese patients with chronic lymphocytic leukemia

**Authors:** Yi Miao[a], Chun Qiao[b], Huimin Jin[c], Lei Fan[c] and Jianyong Li[d]

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**Introduction:** In chronic lymphocytic leukemia (CLL), SF3B1 mutations, identified in approximately 10–15% of cases, are recognized as potential driver mutations. SF3B1 mutations have been reported to predict worse outcomes in patients with CLL. While the majority of SF3B1-mutated CLL cases harbored SF3B1 K700E, the clinical significance of other SF3B1 variants remain less well-studied. This study aimed to investigate the clinical significance of specific SF3B1 mutation variants in Chineses patients with CLL.

**Methods:** A total of 531 cases of CLL from the First Affiliated Hospital of Nanjing Medical University (Jiangsu Province Hospital) were included in this study. Clinical data were collected. Targeted sequencing was performed on fresh bone marrow aspirate or peripheral blood samples with sufficient tumor cells. Genomic DNA (gDNA) was used to generate sequencing libraries using the MultipSeq Custom Panel (Rightongene Biotechnology Co. Ltd., Shanghai, China) following the manufacturer's protocols. Sequencing was performed on the MiSeq/Novaseq (Illumina, United States) platform. Overall survival (OS) was defined as time from diagnosis to death from any cause or last follow-up. Time to the fisrt treatment (TTFT) was defined as time from diagnosis to the first treatment or last known untreated date.

**Results:** Among 531 CLL patients, 45 (8.5%) patients were SF3B1 mutated. No significant differences in clinical parameters, including age, sex, hemoglobin levels, PLT counts, β-2-microglobulin, lactate dehydrogenase, IGHV status, Rai/Binet stage, and CLL-IPI risk stratification, were found between the SF3B1-mutated and SF3B1-unmutated

groups (all $p > 0.05$). The five most frequently mutated genes in the entire cohort were ATM (13.9%), TP53 (13.2%), NOTCH1 (11.1%), SF3B1 (8.5%), and MYD88 (7.5%). In the SF3B1-mutated CLL patients, the most common co-mutated genes were ATM (11/45, 24.4%), TP53 (11/45, 24.4%), NOTCH1 (8/45, 17.8%), and KMT2D (4/45, 8.9%). Compared to the SF3B1-unmutated group, SF3B1-mutated patients had higher frequencies of ATM, PTPRD, XPO1, and TP53 mutations ($p = 0.042$, $p = 0.045$, $p = 0.024$, and $p = 0.034$, respectively) but were less likely to have MYD88 mutations ($p = 0.039$). SF3B1 mutation variants analysis revealed 19 different mutations, with K700E (19/45, 42.2%), I704F (4/45, 8.9%), E622D (3/45, 6.7%), and I704N (3/45, 6.7%) being the most frequent. No significant differences in variant-allele frequencies were observed across these variants. OS did not significantly differ between SF3B1-mutated and SF3B1-unmutated patients ($p = 0.757$). SF3B1-mutated CLL patients had a shorter TTFT compared to SF3B1-unmutated patients (median TTFT: 1.2 vs. 8.5 months, $p = 0.012$). OS in SF3B1-unmutated patients was significantly longer than in those with SF3B1 I704F mutation ($p < 0.001$) but was not significantly different from those with SF3B1 K700E ($p > 0.05$). Patients with SF3B1 K700E had significantly shorter TTFT as compared with SF3B1-unmutated patients ($p = 0.025$). In multivariate analysis, SF3B1 K700E was also an independent risk factor for TTFT ($p = 0.027$).

**Conclusion:** SF3B1 K700E is most common SF3B1 variant in Chinese patients with CLL. Specific SF3B1 variants should be incorporated into prognostic stratification and therapeutic decision-making for patients with CLL.

**Keywords:** Chronic lymphocytic leukemia; SF3B1; variants

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## Abstract ID: 1118

**Title:** Optical genome mapping identifies a high number of chromoanagenesis events in chronic lymphocytic leukemia patients with altered TP53

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**Introduction:** Chromoanagenesis phenomena (CP), including chromothripsis (Cth), chromoplexy (Cpl), and chromoanasynthesis (Cas), are characterized by extensive chromosomal rearrangements arising from a single catastrophic event. CP has been identified in chronic lymphocytic leukemia, being Cth the most frequently reported. Although Cth incidence is low (1–3% in unselected cohorts), it has been associated with adverse prognostic factors, including TP53 alterations, high genomic complexity, and poor clinical outcomes. Conventional cytogenetic techniques routinely used in CLL, such as CBA and FISH, lack the resolution required to detect CP. Recently, optical genome mapping (OGM) has emerged as a powerful tool capable of providing a detailed overview of these complex

genomic profiles. OGM can detect the copy number abnormalities (CNV) related to CP but also the related intra- and inter-chromosomal rearrangements. Finally, limited data is available regarding the mechanisms underlying CP, as well as their clinical impact in the current CLL therapeutic era.
**Objective:** To characterize chromoanagenesis phenomena in a cohort of CLL patients with TP53 alterations using OGM.
**Patients and methods:** We included 70 TP53 altered CLL patients (N=37 double hit TP53del+mut or TP53multiple mut and N=33 single hit TP53del or TP53mut) before receiving treatment with iBTK and/or iBCL2 (± anti-CD20) (May 2022-Dec 2024) from 24 Spanish hospitals. For OGM analysis, samples (25 peripheral blood and 45 PBMC) were processed using the Saphyr System and analyses were conducted using the Rare Variant Analysis pipeline from Bionano Access 1.8.2 (Bionano Genomics). Genomic Complexity (GC) by OGM was defined as ≥5 alterations, including those ≥5 Mb in size, and/or rearrangements, CLL-related abnormalities (irrespective of size), and smaller variants (200 Kb–5 Mb) associated with other events. As for chromoanagenesis phenomena, Cth was defined as ≥7 oscillating switches between two or three copy number states in one or a few chromosomes and associated rearrangements; Cpl as ≥4 chained rearrangements between at least 2 chromosomes accompanied or not with CNV at junctions; and Cas as presence of chromosomal duplications and triplications locally clustered on one single or few chromosomes, associated with various other types of structural rearrangements.
**Results:** From the global cohort, OGM identified CP in 36% (25/70), being most frequently identified in patients with double hit TP53 (20/25, 80%) vs single hit TP53 (5/25, 20%, $p<0.001$). Of them, 84% (21/25) showed a CK (≥3 abn). Cth was the most common event, detected in 22 patients. Cpl and Cas were observed in six and one patient, respectively. Concomitant CP were identified in four patients, showing Cth accompanied by Cpl (n=3) or Cas (n=1). Regarding Cth, a total of 35 events were identified, as 36% (8/22) of patients showed events in two to four chromosomes. As expected, losses were more frequently detected than gains (18 only losses and 17 gains+losses). CNV were associated with inter and intrachromosomal translocations in 31/35 events while four showed only intrachromosomal rearrangements. The most affected chromosome was the 13 (n=5) always showing loss of 13q14 (MIR-15/MIR-16A), and in four cases also involving (RB1) and in three cases 13q12 (LATS2). Other recurrent regions included gains at 2p16 (REL) and 8q24 (MYC), and losses at 2q36 (PAX3), 3p25 (FANCD2, VHL), 3p21 (SETD2, BAP1), 7q31 (POT1), 9q21 (GNAQ, CDKN2A), and 9q22 (FANCC). Concerning Cpl cases, the median number of chromosomes involved was 3 (range: 2–5), with a median of 4 breakpoints (range: 4–6). Chromosome regions implicated in Cpl were heterogeneous, being the only recurrent region of rearrangement 13q14, observed in two events, involving DLEU2 and TRIM13. Strikingly, rearranged regions affected different regulatory genes, including COL5A1, GPR107, STK24, THAP9, and PIGL among others, many of them previously described to have a role in tumor development and progression in other types of cancer. All Cpl events showed non-recurrent losses and one also displayed alternating gains at breakpoints. As for the only case with Cas, it was identified in chromosome 8 affecting 8q22.2q24.3 with a gain of MYC (3 copies).
**Conclusions:** (1)CLL patients with TP53 alterations are enriched in chromoanagenesis events, especially those with TP53del+mut;(2)Chromothripsis is more frequent than chromoplexy and chromoanagenesis, and all events involve genomic regions with a potential tumoral role;(3)Further investigation of chromoanagenesis events in larger cohorts with extended follow-ups is needed to determine their biological significance and prognostic impact.

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**Keywords:** Chromoanagenesis; OGM; genomic complexity; TP53

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## Abstract ID: 1014

### **Title:** Highlighting a crucial role for EGR2 mutations in CLL pathogenesis and BTKi resistance

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**Background:** Chronic lymphocytic leukemia (CLL) is a B-cell malignancy characterized by a tissue involvement of both primary and secondary lymphoid organs, as a result of a complex intertwining between microenvironmental stimuli and genetic defects. We previously reported recurrent mutations in the EGR2 gene in 8% of advanced-stage patients with CLL, that associated with distinctive biological and clinical features of poor prognosis. EGR2 encodes for a transcription factor, activated in B cells through ERK phosphorylation upon BcR stimulation. Clinical data suggest that EGR2 mutations are associated with a rapidly progressive disease course characterized by shorter time to first treatment and overall survival. Interestingly, we also reported EGR2 mutations in patients becoming refractory to continuous BTK inhibitors, namely ibrutinib, particularly in association with BTK mutations. However, the precise role of mutated EGR2 in the leukemogenic process and in the occurrence of resistance to BTKi remains poorly explored, with recent evidence indicating an impact on the transcription of EGR2 target genes. Here, we are providing initial support to the hypothesis that EGR2 mutations may cause a gain of function of the transcription factor activity of the gene, thereby modulating relevant intracellular pathways that contribute to CLL aggressiveness, dissemination and drug resistance.
**Aims:** We aim at understanding the functional role of EGR2 mutations in CLL development and progression.
**Methods:** We engineered the MEC-1 CLL cell line using CRISPR/Cas9 technology to generate EGR2-mutated cells, selectively harboring each of the three recurrent missense mutations found in patients: E356K, H384N, and D411H. We analyzed the effect of EGR2 mutations by studying in vitro cell proliferation, transwell migration in the presence or absence of chemokines, expression of EGR2 target genes, and sensitivity to the BTK inhibitor Ibrutinib.
**Results:** We obtained three distinct EGR2-mutated clones for each missense mutation, along with control clones. All cell lines exhibited comparable expression of EGR2 both at mRNA and protein levels, as well as similar proliferation rates. Notably, EGR2-mutated cell lines demonstrated an increased migration capacity in the presence of SDF-1 and CCL19, chemotactic stimuli crucial for homing of malignant cells into bone marrow and lymph nodes. Gene expression analysis revealed that the mutated clones exhibited a

differential expression of known EGR2 targets compared to controls, with certain genes (e.g. EGR1 and FOS) upregulated and others (e.g. GADD45B and DTX1) downregulated. Furthermore, EGR2-mutated cell lines had elevated levels of cleaved NOTCH1 protein, indicating a potential interaction between the EGR2 and NOTCH1 pathways, the latter known driver of the disease. Lastly, in line with our previous observation in refractory patients, the EGR2-mutated cell lines demonstrated reduced sensitivity to apoptosis induced by the BTK inhibitor Ibrutinib, in contrast to the control clones.

**Conclusion:** Our model, consisting of EGR2-mutated cells, shows an enhanced migratory capacity and altered expression of EGR2 target genes in the presence of each of the 3 missense gene mutations. In particular, we were able to demonstrate a cross-talk with the NOTCH-1 pathway leading to its activation, a well-known mechanism acting in the pathogenesis of the disease, being frequently altered in patients with CLL and associated with poor outcome. As originally reported by us in patients refractory to Ibrutinib, also in the mutated cell lines, we were able to confirm the association of EGR2 mutations and BTKi resistance in CLL; further exploiting this model may help shed light on potentially druggable molecules and/or pathways that will overcome or prevent resistance.

**Keywords:** Leukemia; gene mutation; BcR signaling

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## Abstract ID: 1434

### **Title:** STATIC TRIAL (stopping therapy to avoid treatment-resistance in CLL): a randomised phase III trial comparing intermittent with continuous treatment strategies in CLL

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Trial Design: STATIC is designed with multiple pathways, the 'Randomization Pathway' and the 'Clinical Need Cohort' - which route a participant enters will be determined by their eligibility. Randomization Pathway: A prospective, national, multicentre, open-label, randomized, controlled, 2-arm, parallel-group, non-inferiority, phase III trial to assess whether patients with CLL on long-term treatment with a BTK inhibitor (including ibrutinib or acalabrutinib) have similar disease control with an intermittent treatment strategy (experimental arm) compared with standard continuous treatment (control arm). Clinical Need Cohort: A prospective, national, multicentre, open-label, single-arm, non-randomized cohort to assess the safety and overall survival of patients with CLL receiving long-term continuous treatment with ibrutinib. Aims and Objectives: The primary aim was to assess whether patients with CLL on long-term treatment with BTK inhibitor (including ibrutinib or acalabrutinib) have similar disease control with an intermittent treatment strategy, known as the 'Pausing BTKi' arm compared with standard continuous treatment, the 'Continuous BTKi' arm. The primary objective is to assess non-inferiority (NI) of the intermittent treatment strategy in terms of time to treatment strategy failure, defined as the first documented instance of active disease (as defined by the 2018 iwCLL criteria) that does not respond to treatment, or death. The secondary objectives are to assess Overall survival, toxicity and tolerability, cost effectiveness, Quality of life, Summative treatment-free interval, Response to retreatment in intermittent treatment arm, Time to next treatment for CLL, Response to next treatment for CLL, Rate of resistance mutations and Evolution of resistant sub-clones. Hypothesis: We hypothesise that an intermittent treatment strategy using BTKi including ibrutinib or acalabrutinib, will reduce treatment-emergent resistance and thus be at least non-inferior to continuous treatment with regards to time to treatment strategy failure whilst reducing resource impact for the NHS and improving quality of life. Number of participants: 800 participants will be randomized into the randomization trial over a 6-year period with ~45% being front-line CLL participants receiving treatment with ibrutinib as their first-line and only line of treatment and ~55% being patients receiving ibrutinib or acalabrutinib off-trial (via NHS access) for previously treated for CLL. An additional cohort of ~30 patients from the ibrutinib-based treatment arms of FLAIR and IcICLLe will be enrolled to a non-randomized Clinical Need Cohort to receive continuous ibrutinib. Current Recruitment: Currently STATIC trial is open to recruitment in UK running from University of Leeds. The trial has been amended recently to include the utilization of acalabrutinib in relapsed refractory cohort. As of 29.5.2025, 168 participants have been randomized, and 12 participants are treated on the clinical need cohort.

**Keywords:** Front-line; relapsed/refractory; BTK inhibitor

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## Abstract ID: 1808

### **Title:** Setting a 3D model to study chronic lymphocytic leukemia

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**Background:** The tumour microenvironment plays a crucial role in promoting survival and proliferation of malignant cells. Traditional 2D culture does not allow for recreating this level of complexity; thus, 3D systems are becoming promising tools for recreating more physiological cell conditions. While 3D cultures of solid tumours are more commonly set up, hematological cancers are poorly characterized in this sense. This project aims to create a 3D model to investigate how the tumour microenvironment of Chronic Lymphocytic Leukemia (CLL) cells may influence their response to specific drugs used in cancer therapy.

**Methods:** VitroGel Hydrogel® Matrix, a ready-to-use, animal-origin-free functional hydrogel, was used as a scaffold to test different culture conditions of MEC-1 human CLL cell lines. Cell viability was measured through trypan blue exclusion, flow cytometry and annexin V-propidium iodide staining. Western blot analysis was used to detect the levels of different proteins involved in the apoptotic pathway (i.e. Bcl-2, PARP), and qPCR was performed to determine the expression of genes related to apoptosis, migration, and proliferation pathways.

**Results:** We first plated MEC-1 cells in 3D and 2D conditions, and observed higher viability in 3D after 28 days of culture compared to 2D cells. Despite the high mortality rate in 3D conditions during the first week of culture, 3D cells showed good recovery over time. On the other hand, 2D culture appears to facilitate better survival initially, whereas viability begins to decrease after 7 days of culture. The anti-apoptotic trend was confirmed in 3D conditions by Western blot analysis: in 3D cells, we observed an increase in Bcl-2 and PARP (but not the cleaved form) after 21 and 28 days of culture. This suggests a general antiapoptotic behaviour. We also investigated autophagy, and we detected an increase in p62 and a decrease in LC3-II in 3D conditions at 21 and 28 days of culture. 2D cells exhibited a general opposite trend, which is consistent with the decreased cell viability observed after 3 weeks of culture. We then decided to treat cells with PRIMA-1Met, a p53-refolding and pro-oxidant molecule, to investigate whether the presence of the scaffold can increase cell resistance even after treatment. We observed no differences in cell viability between control and treated cells in 3D cultures, while in 2D conditions the molecule kills the cells. The qPCR analysis highlights an increase in Bcl-2 levels following rising concentrations of PRIMA-1Met (from 5 µM to 40 µM) in 3D conditions and the opposite trend was observed in 2D cultures. We also evaluated the expression of genes related to cytoskeleton remodeling and migration, but these aspects still further evaluation.

**Conclusions:** These preliminary results indicate that the gel seems to provide protection to CLL cells both in normal conditions and after treatment with PRIMA-1Met. This is probably related to the cell-scaffold interaction, which recreates a more physiological environment than 2D cultures. Based on these results, we will exploit this model to test new combinations of drugs already used in clinical practice to treat CLL. We will also apply this model to study primary CLL cells, aiming to enhance cell survival and assess the actual effects of various drugs under more physiological conditions.

**Keywords:** Leukemia; 3D

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## Abstract ID: 1435

**Title:** Absence of lymphocytosis after starting treatment with cBTKi is a rare phenomenon except for trisomy 12 and CD49d positive CLL

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**Background:** Covalent Bruton tyrosine kinase inhibitors (cBTKi) are a class of drugs that have revolutionized Chronic Lymphocytic Leukemia (CLL) treatment. They target Bruton tyrosine kinase (BTK), an enzyme stimulated by B cell receptor (BCR) activation in B lymphocytes, able to trigger pathways involved in cell surviving, proliferation and migration. BTK inhibition in neoplastic lymphocytes results in their demarginalization from lymph nodes to the peripheral blood. This phenomenon of cell redistribution was widely studied until to define a standard kinetics of lymphocytosis during therapy with cBTKi: an initial further increase of the pre-existing lymphocytosis occurs during the first month of therapy, followed by a gradual reduction over time, sometimes until to the normalization of the absolute lymphocyte count (ALC).
**Methods:** We studied the kinetics of lymphocytosis in patients with CLL treated in I line with Ibrutinib, Acalabrutinib and Zanubrutinib. We enrolled 346 patients from 16 Italian centers, evaluating for each one the median ALC at baseline and at well-defined time-points. Then we carried out a descriptive statistics study.
**Results:** Analyzing our data, we found an irregular behavior in 51/346 patients: the 15% of patients didn't show an increase of baseline ALC. Since the beginning they underwent an immediate reduction of circulating lymphocytes. Considering the biological features, the 70.6% of this group of patients was affected by an IGHV unmutated CLL, while only the 27.4% had a IGHV mutated disease. Instead, analyzing the cohort according to the cytogenetic profile, we found that the 44.9% had a CLL with trisomy 12, the 18.4% had a del17p, the 24.5% a del11q, the 34.7% a del13q, the 12.2% a normal karyotype. Only for 24/51 patients we had data about CD49d (VLA-4 integrin alfa subunit) expression before starting treatment. Among these, 15 patients had a CLL CD49d+, 4 a disease CD49d- bimodal and 5 a CLL CD49d-. So, the 79% (19/24) patients had at least a neoplastic lymphocytes' subpopulation expressing CD49d.
**Discussion:** Among patients who didn't show a further rise of lymphocytosis after starting therapy with cBTKi, we didn't find any significative difference according to drug employed (Ibrutinib, Acalabrutinib or Zanubrutinib) and IGHV mutational status. However, we observed a more rapid lymphocytosis decrease in trisomy 12 CLL patients. This data was statistically significative on days 15–30–60 and confirmed, even in this setting, what is already known from literature (Thompson et al. 2014). All patients (100%) with trisomy 12 showed a complete or partial CD49d expression. Considering the other patients (not trisomy 12), CD49d was expressed in the 65% and was negative in the 35%, and those patients with a CD49d+/CD49d- bim CLL showed a faster decrease in ALC, statistically significative on days 15–30–90, than those ones with a CD49d- CLL. In conclusion, our data suggest that the adhesion molecule CD49d seems to be the main 'no lymphocytosis' predictive factor in patients on treatment with cBTKi.

**Keywords:** Chronic lymphocytic leukemia; covalent BTKi; lymphocytosis

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## Abstract ID: 1304

### **Title:** Linking cytokine signaling and metabolism in chronic lymphocytic leukemia: the translational relevance of the IL-12 family

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Chronic Lymphocytic Leukemia (CLL) is a malignancy of mature B cells, characterized by the clonal accumulation of $CD5^+$ CD19+ lymphocytes in blood, bone marrow, and lymphoid tissues (Chiorazzi et al, N Engl J Med, 2005). Despite the significant clinical advances brought by targeted therapies, such as B-cell receptor (BCR) and BCL2 inhibitors, the disease remains incurable and relapse is common (Hallek et al, Lancet, 2018; Hallek et al, Blood, 2018). It is now well established that the CLL microenvironment plays a pivotal role in sustaining leukemic cell survival, contributing to drug resistance through both direct contact with accessory cells and cytokine-mediated signaling. Among these, IL-23 has emerged as a key player. Leukemic cells can express a functional IL-23 receptor (IL-23R) complex and produce IL-23 upon activation, creating an autocrine/paracrine loop that promotes clonal selection and proliferation, independent of BCR signaling (Cutrona et al, Sci Transl Med., 2018). This mechanism may contribute to the persistence of minimal residual disease under targeted treatment. Given the shared structural features of IL-12 family cytokines (IL-12, IL-23, and IL-35) and the overlapping components of their receptor subunits, we hypothesized that these cytokines could differentially influence CLL cell behavior, particularly at the metabolic level, potentially uncovering novel vulnerabilities. To investigate this, B cells from CLL patients and age-matched healthy donors were stimulated in vitro with a TLR9 agonist (CpG-ODN), mimicking a BCR-independent microenvironmental activation. Expression of IL-12 family receptor chains (IL-12Rβ1, IL-12Rβ2, IL-23R, gp130, and WSX-1) was evaluated by flow cytometry. We then examined the metabolic consequences of stimulation with IL-12, IL-23, and IL-35 by assessing mitochondrial respiration, glycolysis, and ATP production through oximetric, luminometric, and spectrophotometric assays. All analyses were performed under

protocols that were ethically approved (CER-Liguria-ID 10115) and in accordance with the Declaration of Helsinki, with informed consent obtained from all participants. Our results show that all receptor chains for IL-12 family cytokines can be induced in both CLL and normal B cells following TLR9 stimulation, indicating potential responsiveness to IL-12, IL-23, and IL-35. Metabolically, only IL23 maintained or enhanced mitochondrial function in CLL cells, supporting oxidative phosphorylation and ATP-linked respiration. In contrast, exposure to IL-12 and IL-35 led to a decline in mitochondrial efficiency and a shift toward glycolysis, suggestive of metabolic stress or adaptation. Notably, these cytokine effects were more pronounced in leukemic cells than in their normal counterparts, indicating disease-specific metabolic vulnerabilities. These findings reveal that structurally related cytokines can exert functionally opposite effects on CLL cells' metabolism and survival. While IL-23 promotes energy-efficient metabolism and favors leukemic persistence, IL-12 and IL-35 impair mitochondrial function, potentially sensitizing cells to metabolic stress or additional therapeutic interventions. The differential impact of IL-12 family members on leukemic metabolism may help explain the incomplete responses observed with current targeted therapies and opens new avenues for therapeutic modulation of the microenvironment. Inhibiting the IL-23/IL-23R axis or harnessing the anti-survival effects of IL-12 and IL-35 may represent promising strategies to overcome resistance and improve long-term disease control in CLL.

**Keywords:** Chronic lymphocytic leukemia; IL-12 family; microenvironment; mitochondrial metabolism

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**Please indicate the name of the funding organization:** Italian Ministry of Health

## Abstract ID: 1714

**Title:** Trends and predictors of engagement in preventive health measures in patients with chronic lymphocytic leukemia: a prospective analysis of vaccinations and cancer screening practices

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**Background:** Patients with CLL are at increased risk of infections and other cancers due to both disease-related immune dysfunction and treatment-related immunosuppression. While vaccination, routine cancer screening, and primary care engagement are well-established for the general population, their uptake in patients with CLL remains incompletely characterized, especially in the post-COVID-19 pandemic era.

**Methods:** We conducted a prospective, survey-based analysis of preventive health behaviors in patients with CLL from 2020 to 2025 to assess and improve adherence to preventive health recommendations. Each year, during in-person clinic visits, we administered a standardized health maintenance questionnaire that collected information on immunization history (influenza, pneumococcal, shingles, COVID-19), primary care visits, and age-appropriate cancer screenings (mammogram, Pap smear, PSA, colonoscopy, dermatologic skin exam). Due to COVID-19-related clinic disruptions, we paused the survey administration in 2021. To evaluate longitudinal adherence, we calculated cumulative uptake of influenza and COVID-19 vaccines among patients who completed multiple surveys. We used multivariable logistic regression to examine associations with demographic variables, adjusting for age, sex, race, ethnicity, and survey year. We reported odds ratios (OR) with 95% confidence intervals (CI).

**Results:** A total of 1,083 patients with CLL completed at least one survey. Yearly influenza vaccination rates declined from 92% in 2020 to 67% in 2025 (OR =0.18, 95% CI: 0.02–1.51, $p=0.027$). Pneumococcal vaccine uptake similarly dropped from 58% in 2020 to 33% in 2025 (OR =0.37, 95% CI: 0.09–1.39, $p=0.037$). Completion of at least one shingles vaccination was 20% in 2020, peaked at 55% in 2021 and declined to 34% by 2025. COVID-19 vaccination was recorded starting in 2021, was highest in 2022 at 80 % but fell to 48% in 2025 (OR 2025 vs 2022 = 0.23, 95% CI: 0.09–0.58, $p=1.9\times10^{-5}$). Additionally, we identified older age as a consistent predictor of higher vaccine uptake across all vaccine categories, with a linear increase in odds per year of age. Sex, race, and ethnicity were not significant in multivariable models. Among patients with serial surveys, cumulative uptake of ≥2 doses were 81% for influenza and 74% for COVID-19 serving as indicators of sustained preventive engagement. We evaluated sex and age-appropriate cancer screening rates in our patients. Mammography rates fell from 75% in 2020 to 50%, in 2023, then rebounded to 86% by 2025 (2025 vs. 2023 OR =6.07, 95% CI: 1.04–35.4, $p=0.0049$). Colonoscopy adherence declined from 76% in 2020 to 47% in 2023 (OR =0.03, 95% CI: 0.07–1.12, $p=0.01$), and then fully recovered to 100% by 2025 among a limited number of patients surveyed so far. Pap smear rates improved modestly from 27% in 2020 to 42% in 2025 (2025 vs. 2020: OR =1.94, 95% CI: 0.19–18.9, $p=0.42$). Prostate cancer screening rate followed a similar trend and increased from 29% in 2020 to 45% in 2025 (OR =1.99, 95% CI: 0.38–10.5, $p=0.25$). Ethnic disparities were noted in prostate cancer screenings: non-Hispanic patients had higher screening uptake (OR =1.9, 95% CI: 1.1–3.4, $p=0.023$). Racial disparities were also present—compared to White patients, Black patients had significantly lower screening odds (OR =0.07, 95% CI: 0.029–0.184, $p<0.0001$), as did Asian patients (OR =0.12, 95% CI: 0.041–0.337, $p<0.0001$). Skin cancer screening occurring at least once a year by a dermatologist was reported by 67% of patients and increased with age (OR/year =1.035, 95% CI: 1.025–1.046, $p=2.6\times10^{-11}$). Primary care visit rates remained consistently high throughout the study period, with an overall engagement rate of 87%.

**Conclusions:** In this large, prospective CLL cohort, we observed a post-2022 decline in vaccination uptake across all vaccine types, with older age emerging as a consistent positive predictor. Serial survey participation was associated with higher cumulative adherence to influenza and COVID-19 vaccination. Cancer screening rates similarly declined during the pandemic but demonstrated partial recovery by 2025, particularly for mammography and colonoscopy. While primary care engagement remained high, racial and ethnic disparities persisted in specific preventive domains. These findings underscore the importance of sustained, targeted interventions to optimize preventive care in patients with CLL.

**Keywords:** Vaccination; cancer screening; quality improvement

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1605

### **Title:** Phase 2 study of the combination of liso-cabtagene maraleucel, nivolumab, and ibrutinib in Richter's transformation - NCT05672173

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Rationale: Richter's transformation (RT) is an aggressive transformation of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) typically to large B cell lymphoma (LBCL) that is difficult to treat and has a poor prognosis. The goal of treatment is to achieve a complete remission (CR) and proceed to consolidative allogeneic hematopoietic cell transplantation (alloHCT). However, outcomes with standard chemoimmunotherapy are dismal, with median OS <1 year (long-term survival <20%) and CR rates ranging from 5–38%, most of which are transient. Many patients who achieve durable CR are ineligible for alloHCT due to age, comorbidities and/or lack of a suitable donor. Retrospective analyses have shown that chimeric antigen receptor (CAR) T cell monotherapy does not typically yield durable results in RT. Thus, there is an urgent, unmet medical need for well-tolerated, potent therapies for RT. The combination of the BTK inhibitor ibrutinib and the PD-1 checkpoint inhibitor nivolumab has shown activity in RT, with an overall response rate (ORR) of 43% and CR rate of 35%. Most responses to PD-1 inhibition were transient, although potentially could allow bridging to alloHCT. The use of ibrutinib and nivolumab as bridging and maintenance therapy may enhance responses to CAR T cell therapy since CAR T cell products that have PD-1 expression and are skewed toward terminally-differentiated effector T cells are associated with inferior efficacy, suggesting that PD-1 inhibition may enhance CAR T cell efficacy. The safety and approval of lisocabtagene maraleucel (liso-cel) in relapsed/refractory DLBCL as well as CLL makes it the ideal commercial CAR T cell product available in RT where CLL may also be present. We hypothesized that the addition of ibrutinib and nivolumab to CAR T cells in patients with RT may enhance responses to CAR T cell therapy. Therefore, we proposed studying the combination of ibrutinib, nivolumab and liso-cel in patients with relapsed/refractory RT with a curative intent. Study Design: This is a phase 2 study with a planned sample size of 20 participants who receive all 3 agents on the study: ibrutinib, nivolumab, and liso-cel. Enrollment is open at 2 sites - City of Hope National Medical Center (CA) and Memorial Sloan Kettering Medical Center (NY). The primary objective is to evaluate the CR rate after cycle 3 following liso-cel in combination with nivolumab and ibrutinib to treat patients with RT. Safety lead-in of 6 patients will be to assess the unacceptable toxicity (UT) rate within the first 28 days during cycle 1 following liso-cel infusion. Main Inclusion Criteria: Age 18 years or older; patients with histologically confirmed RT with radiographically measurable lymphadenopathy (≥1.5 cm) or measurable extra-nodal disease; relapsed / refractory following therapy for RT, or not eligible for alloHCT due to comorbidities or age; eligible to receive liso-cel and ibrutinib per package inserts; prior BTKi is allowed; adequate organ function and counts. Main Exclusion Criteria: Patients with de novo RT; patients who previously received PD1 or PD-L1 inhibitors. Intervention description: Following consent, patients will be started on daily ibrutinib, or will continue taking ibrutinib if they were already taking it at the time of study entry, and commence nivolumab, 240 mg IV every 2 weeks. Patients will receive a minimum of 2 doses of nivolumab prior to apheresis. Patients will undergo apheresis for liso-cel manufacturing at least 2 weeks after the last dose of nivolumab (anticipated to be 4 weeks after commencing study therapy) and nivolumab will be resumed immediately following apheresis. Ibrutinib will continue throughout the induction period (study treatment prior to apheresis through lymphodepletion), as well as during lymphodepletion with fludarabine/cyclophosphamide and liso-cel infusion (cycle 1), and the maintenance period (cycles 2–12). Nivolumab will be stopped through day 28 post liso-cel and then restarted at 240 mg on days 1 and 15 of cycle 2 followed by 480 mg nivolumab on day 1 of cycles 3–12. Disease response assessments (PET/CT, peripheral blood and bone marrow biopsy) will be at the end of induction and cycles 1, 3, 6 and 12. While UT monitoring in the safety lead-in will be from the start of liso-cel infusion through day 28 post liso-cel, patients will be closely monitored for toxicity throughout the study with stopping rules to ensure safety.

**Keywords:** Richter's transformation; CAR T cell therapy; nivolumab

**Please indicate how this research was funded:** Funding and nivolumab provided by Bristol Myers Squibb.

**Please indicate the name of the funding organization:** Bristol Myers Squibb.

## Abstract ID: 1406

### **Title:** Venetoclax and obinutuzumab in first-line treatment of unfit patients with CLL – real-life data analysis of the Polish Adult Leukemia Group

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**Background:** Venetoclax combined with obinutuzumab (VG) is regarded as the standard of care for patients with previously untreated chronic lymphocytic leukemia (CLL) and comorbid conditions, based on the results of the CLL14 study. Here, we present data on real-world patients treated with this combination at the centers of the Polish Adult Leukemia Group (PALG). The study aimed to analyze the efficacy and toxicity of VG in the first-line setting in CLL patients with comorbid conditions treated outside clinical trials.

**Material and methods:** This was a retrospective analysis of adult patients with untreated CLL and comorbid conditions defined as a Cumulative Illness Rating Scale (CIRS) total score of >6 or a creatinine clearance (CrCl) < 70 ml/minute treated within CLL Therapeutic Programme of the Polish National Health Fund.

**Results:** A total of 220 CLL patients treated with first-line VG were included. The median age at VG commencement was 70 years (range: 45–86), and 134/220 (60.9%) were male. The median time between diagnosis and treatment initiation was 1.6 years (range: 0–19). The median ECOG was 1 (range: 0–3). The median CIRS score was 8 (range: 0–30), and 101 (45.9%) patients had CrCL< 70 ml/min. TP53 gene aberration (del17p and/or TP53 mutation) was detected in 19 (9.9%) patients out of 191 tested individuals. According to Rai, CLL stage was ≥3 in 111/220 (50.4%) patients. Tumor lysis syndrome (TLS) risk was intermediate in 102/203 (50.2%) and high in 69/203 (34%). Median time of observation was 26 months (95% CI: 24.5–27.9). Six (2.7%) patients were continuing treatment at the time of analysis, and 214/220 (97.3%) completed the therapy. In that group, 169/214 (79%) patients completed treatment as per protocol. The main reasons for treatment discontinuation were adverse events (AEs), most commonly liver toxicity, hematological toxicity, second primary malignancy (SPM), and infections. Clinical TLS was observed in 11/203 (5.4%) patients, while laboratory TLS was seen in 41/203 (20.2%) patients. The overall response rate according to iwCLL 2018 Response Criteria for evaluable patients was 97%. Estimated 24-month progression-free survival (PFS) was 88.9% (95% CI: 84.4–93.6), while estimated 24-month overall survival (OS) was 92.6% (95% CI: 89.1%–96.3%). Harboring del17p/TP53 gene mutation impacted neither PFS ($p = 0.525$; HR: 1.487, 95% CI: 0.438–5.054) nor OS ($p = 0.156$; HR: 2.521, 95% CI: 0.703–9.044). Alike, no differences were observed when comparing patients with comorbidities (CIRS >6 vs. CIRS< 6) and estimates for OS ($p = 0.274$; HR: 3.078, 95% CI: 0.411–23.059) and PFS ($p = 0.142$; HR: 4.469, 95 %CI: 0.606–32.941) did not significantly differ. Hematological toxicity of any grade was the most predominant, with 83.2% of patients developing neutropenia, 57.3% thrombocytopenia, and 54.5% anemia. The other most frequent AEs were SARS-CoV-2 infection (11.8%), febrile neutropenia (10%), obinutuzumab infusion-related reactions (9.5%), elevated liver enzyme activity (8.2%), pneumonia (8.6%), and upper respiratory tract infection (7.7%). Autoimmune phenomena occurred in 11 (5%) patients, comprising 9 autoimmune hemolytic anemias and 6 immune thrombocytopenias. There were 5 SPM in the analyzed cohort. Nineteen (8.6%) patients died. The leading causes of death were infections (5 patients), CLL progression (3 patients) and SPM (2 patients).

**Conclusions:** VG combination was effective and well-tolerated in patients with de novo CLL and comorbidities under real-world conditions. In our short observation TP53 aberrations do not seem to impact significantly the early treatment outcomes, though extended follow-up is mandatory.

**Keywords:** Chronic lymphocytic leukemia; comorbidities; venetoclax

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1715

**Title:** Real-world outcomes following pemivibart (Pemgarda™) for COVID-19 prophylaxis in immunocompromised patients with hematologic malignancies

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**Background:** Patients with hematologic malignancies are at significantly higher risk for severe COVID-19 due to disease-related and treatment-induced immunosuppression. Despite widespread vaccine availability, many patients fail to mount effective immune responses. On 22 March 2024, the FDA issued Emergency Use Authorization for pemivibart (Pemgarda™), a long-acting monoclonal antibody for pre-exposure prophylaxis in individuals with moderate-to-severe immunocompromise. While initial approval was based on laboratory and pharmacokinetic data, real-world evidence on Pemivibart's safety and effectiveness in high-risk populations remains limited. This study evaluates adverse reactions and breakthrough infections following Pemivibart use in immunocompromised patients with hematologic malignancies.
**Methods:** We analyzed 112 adult patients with lymphoid malignancies who received Pemivibart between March 2024 and May 2025. Clinical data included age, sex, vaccine history, adverse reactions, and incidence of COVID-19 infection after Pemivibart. COVID-19 infection was confirmed through antigen or PCR testing. Descriptive statistics summarized the cohort.
**Results:** All patients had lymphoid malignancies (CLL, n = 69 (62% of the cohort)) at various disease and treatment course points. The mean age was 69.6 years; 48.2% were female. The average number of prior COVID-19 vaccine doses was four, and 34 patients (30.4%) received a second Pemivibart dose. Adverse reactions occurred in 6.3% (7/112), including grade 3 anaphylaxis in one (0.9%) patient that resolved with supportive care, and grade 2 infusion-related reactions in two (1.8%) patients, all of which responded well to supportive care. Three patients (2.7%) developed COVID-19 after Pemivibart administration. Of these, two required hospitalizations: one had no history of vaccination or prior pre-exposure prophylaxis; the other had received two vaccine doses without prior pre-exposure prophylaxis. The third patient, who had three vaccine doses and prior pre-exposure prophylaxis, experienced mild symptoms and recovered with supportive care. No COVID-19-related deaths were reported.
**Conclusion:** Pemivibart was well-tolerated in immunocompromised patients with lymphoid malignancies, with a low incidence of adverse reactions and breakthrough infections. Hospitalizations occurred only in those with minimal prior immunologic protection, highlighting the continued importance of layered COVID-19 prevention strategies. These real-world findings support using Pemivibart as an adjunct to vaccination in high-risk populations and underscore the need for pre-exposure prophylaxis, especially in patients with a compromised ability to generate adequate vaccine-mediated immunity. Detailed data will be provided at the conference.

**Keywords:** Pemivibart; COVID-19; lymphoid malignancies; pre-exposure prophylaxis; immunocompromised

**Please indicate how this research was funded:** Not funded

**Please indicate the name of the funding organization:**

## Abstract ID: 1908

**Title:** Number needed to treat analyses of zanubrutinib vs acalabrutinib in relapsed/refractory chronic lymphocytic leukemia

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**Introduction:** Bruton tyrosine kinase inhibitors (BTKis), including zanubrutinib and acalabrutinib, have become a standard of care in chronic lymphocytic leukemia (CLL). However, there is a lack of head-to-head comparative trial data. In the phase 3 ALPINE study (NCT03734016), zanubrutinib showed favorable progression free survival (PFS) compared to ibrutinib in the treatment of relapsed/refractory (R/R) CLL. In the ASCEND study (NCT02970318), acalabrutinib showed improved PFS vs rituximab-idelalisib/bendamustine in R/R CLL. A previously published matching-adjusted indirect comparison (MAIC) reported significantly improved PFS and complete response rates for zanubrutinib compared to acalabrutinib (Shadman 2024). This study aimed to compare zanubrutinib versus acalabrutinib in R/R CLL by calculating the number needed to treat (NNT) to avoid one progression or death and associated incremental costs.
**Methods:** A health-economic model was developed to estimate the number of R/R CLL patients needed to be treated to avoid one progression or death from the US payer perspective. Clinical efficacy data from a MAIC of ALPINE and ASCEND trials were extracted. Final analysis results of 24-month PFS (85.5% for zanubrutinib and 75.1% for acalabrutinib) were used for the base-case analysis in the model. Costs associate with direct treatment (2025 wholesale acquisition costs), adverse event management, healthcare resource utilization, and subsequent treatment were considered in the model. The NNT, incremental cost per treated patient, and incremental cost per additional patient with progression or death were estimated. Deterministic sensitivity analyses were conducted to assess parameter uncertainties and explore key model drivers. Sensitivity analyses were conducted to test the impact of alternative PFS rates from the unadjusted population of the MAIC.
**Results:** The base-case results from the NNT model showed that for every 10 patients treated with zanubrutinib instead of acalabrutinib, one event of progression or death would be avoided. The total costs per patient treated with zanubrutinib and acalabrutinib are $436,186 and $443,521, respectively, with a cost savings of $7,335 associated with using zanubrutinib in a 24-month timeframe. Drug costs and PFS have the greatest impact on the incremental cost per patient. A sensitivity analysis was conducted using PFS from an unadjusted population from the MAIC analysis. The NNT result changed from 10 to 14 and is associated with cost savings of $5,152 per zanubrutinib-treated patient in a 24-month time frame. Applying the base-case model result to a hypothetical scenario of a clinical practice of 100 patients treated with zanubrutinib versus acalabrutinib suggests that approximately 10 patients will avoid disease progression events or death in 24 months, and the practice would realize a savings of $733,500.
**Conclusions:** The NNT model analysis demonstrates that using zanubrutinib to treat R/R CLL patients, compared to acalabrutinib, is associated with more favorable clinical and economic outcomes in the US. Notably, zanubrutinib demonstrated a NNT of 10 to avoid one disease progression or death, along with an estimated cost saving of $7,335 per patient in the US.

**Keywords:** CLL; NNT; zanubrutinib

**Please indicate how this research was funded:** BeOne Medicines Ltd

**Please indicate the name of the funding organization:** BeOne Medicines Ltd

## Abstract ID: 1716

### **Title:** Incidence of second primary malignancies in chronic lymphocytic leukemia

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**Introduction:** Chronic lymphocytic leukemia (CLL) involves a broad spectrum of genetic mutations and is treated with multiple therapeutic approaches. This disorder may be associated with second primary malignancies (SPM), including solid tumors (ST) and other hematologic neoplasms (HN), potentially due to therapy side effect and genetic predisposition.
**Aim:** This study aimed to evaluate the incidence and and long-term prognostic value of SPM in CLL patients.
**Method:** We conducted a retrospective analysis at the Hematology Department of the University of Szeged including consecutive CLL patients from January 2012 to January 2025. The study assessed the prevalence of SPM, the influence of therapy and genetic background on SPM development, and the long-term prognostic significance of SPM.
**Results:** CLL was confirmed in 515 patients and therapy was initiated in 268 cases. Among the treated 268 patients (164 male, the average age at diagnosis was 66 ± 10 years) SPM was diagnosed in 84 (31%) cases, from which 66 (25%) was ST and 19 (7%) was HN.
In the treated patient cohort the following therapies was administreted: Rituximab, Cyclophosphamide, Vincristine, Prednisolon (R-CVP) in 51 (19%) patients, Rituximab, Fludarabine, Cytoxan (R-FC) in 26 (10%), Rituximab, Bendamustine in 68 (25%), Rituximab, Chlorambucil in 90 (34%), Venetoclax in 41 (15%), Bruton tyrosine kinase-inhibitor (BTK-inhibitor) in 104 (39%), obinutuzumab in 49 (18%). Non of these therapies had significant influence upon SPM developement. A non-significant tendency toward higher SPM was noticed in case of R-CVP (OR 1.71, 95% CI:0.91–3.21, $p = 0.095$) and R-FC (OR 2.02, 95% CI:0.89–4.59, $p = 0.092$), while obinutuzumab exhibited a protective effect (OR 0.50, 95% CI:0.23–1.06, $p = 0.072$, 15.1%). In 91 patients with known IGHV status (30 positive, 61 negative) the presence of mutation did not influenced the SPM developement (OR 0.95, 95% CI:0.38–2.36, $p = 0.923$). Further genetic results are still in progress. The mean follow-up time was 6.5 ± 4.8 years. The presence of SPM did not influenced the long-term survival in the treated CLL subgroup (aHR 0.7, 95% CI:0.56–1.13, $p = 0.210$).
**Conclusion:** In our cohort, 31% CLL patients were diagnosed with SPM, emphasizing the importance of regular screening. The therapy and genetic status were not significant risk factors for SPM development. Interestingly, SPM did not significantly worsen survival outcomes in the studied population.

**Keywords:** CLL; SPM; incidence

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**Please indicate the name of the funding organization:** It has been supported, by the Hematology Department, University of Szeged, Hungary

## Abstract ID: 1528

### **Title:** Estimating the cardiac deaths associated with treating chronic lymphocytic leukemia with ibrutinib versus zanubrutinib in the United States

**Authors:** Talha Munir[a], Rhys Williams[b], David Campbell[c], Swetha Challagulla[b], Qianhong Fu[b], Mei Xue[b], Keri Yang[b] and Andrew Lipsy[d]

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**Introduction:** Bruton tyrosine kinase inhibitors zanubrutinib and ibrutinib have demonstrated efficacy in the first-line (1L) chronic lymphocytic leukemia (CLL) setting in SEQUOIA (NCT03336333) and RESONATE-2 (NCT01722487) trials, respectively, while the efficacy and safety of zanubrutinib and ibrutinib were compared in patients receiving second-line or later (2L+) treatment in the phase 3 ALPINE trial (NCT03734016). However, there are limited real-world head-to-head comparative data on cardiac deaths of patients being treated with zanubrutinib and ibrutinib. The objective of this study was to conduct a modeling analysis to estimate the number of cardiac deaths in the US that could be avoided by using zanubrutinib instead of ibrutinib for 2L + CLL treatment based on published head-to-head data from ALPINE. Additionally, an exploratory analysis was conducted to investigate the number of cardiac deaths that could be avoided with 1L use of zanubrutinib vs ibrutinib.
**Methods:** A number needed to harm (NNH) modeling analysis was conducted to assess how many patients would need to be treated with ibrutinib vs zanubrutinib to be associated with an incremental cardiac death in the 1L and 2L + settings. Rates of cardiac deaths for zanubrutinib and ibrutinib in 2L + CLL were extracted from the ALPINE study (0% for zanubrutinib vs 1.852% for ibrutinib) and SEQUOIA (0% for zanubrutinib) and RESONATE-2 (2.941% for ibrutinib). NNH was calculated as the reciprocal of the absolute cardiac death risk increase for ibrutinib when compared with zanubrutinib (1/ [cardiac death risk in ibrutinib – cardiac death risk in zanubrutinib]). The number of CLL patients receiving ibrutinib in the real-world setting was estimated from a retrospective analysis of the US Symphony Integrated Dataverse database from 2020 to 2024. To estimate the potential total number of avoidable deaths over a 5-year period, NNH for incremental cardiac death in 2L + and 1L treatment of CLL was calculated by applying the risks of cardiac death data from ALPINE (2L+) and RESONATE-2 and SEQUOIA (1L) to the number of real-world CLL patients receiving ibrutinib.
**Results:** The model used published trial data and real-world CLL patient numbers for NNH analysis. A total of 10,884 CLL patients treated with ibrutinib were identified in the Symphony database, with 6,465 patients in 1L setting, and 4,419 in 2L + setting. The NNH model estimated that in

2L+setting up to 61 cardiac deaths may be avoided over a 5-year period in the US if 2L+CLL patients were treated with zanubrutinib instead of ibrutinib. Additionally, for every 54 patients treated with ibrutinib instead of zanubrutinib for R/R CLL, one additional cardiac death occurred over 5 years. Exploratory NNH analysis of the 1L setting suggested that up to 84 deaths may be avoided a 5-year period if CLL patients were treated with zanubrutinib instead of ibrutinib. Over this same period, for every 34 patients treated with ibrutinib instead of zanubrutinib one additional cardiac death occurred.

**Conclusion:** This model suggests that by switching from ibrutinib to zanubrutinib for CLL treatment, an estimated 61 and 84 cardiac deaths may potentially be prevented over a 5-year period in 2L+and 1L settings, respectively. Results from the study should be interpreted based on model assumptions and data input. Further real-world studies are needed to evaluate the broader impacts beyond cardiac death from treatment with ibrutinib over zanubrutinib.

**Keywords:** CLL; BTKi; cardiac deaths

**Please indicate how this research was funded:** BeOne Medicines Ltd.

**Please indicate the name of the funding organization:** BeOne Medicines Ltd.

## Abstract ID: 1909

**Title:** A network meta-analysis (NMA) of efficacy of zanubrutinib versus fixed-duration acalabrutinib plus venetoclax in treatment-naïve (TN) chronic lymphocytic leukemia (CLL)

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**Background:** While the efficacy of zanubrutinib (ZANU) has been evaluated in the phase 3 SEQUOIA trial (NCT03336333), and the combination regimen of fixed duration acalabrutinib plus venetoclax (AV) has been reported in recent interim analysis of the phase 3 AMPLIFY trial (NCT03836261), the efficacy of these oral regimens has not been directly compared in head-to-head trials. Thus, a NMA was conducted to estimate the relative efficacy in low-risk TN CLL patients.

**Methods:** A systematic literature review was conducted to identify phase 3 randomized controlled trials including low-risk CLL patients to be included in the NMA. Low-risk populations were defined based on the pre-specified trial definitions, including patients without del(17p) or TP53 mutations. Bendamustine plus rituximab (BR) and fludarabine plus cyclophosphamide and rituximab (FCR)/BR were assumed to be treated as common control arms in the network. Bayesian NMA framework was used to estimate hazard ratios (HRs) with 95% credible intervals (CrIs). Outcomes analyzed included progression-free survival (PFS) in low-risk patients and subgroup analysis by IGHV mutation status. Given the timing of the included trials in relation to the COVID-19 pandemic, PFS data were analyzed with and without adjustment for COVID-19 related deaths.

**Results:** The NMA demonstrated a favorable PFS of ZANU over AV, HR for PFS (95% CrI) = 0.41 (0.25, 0.67). The 36-month PFS rate for ZANU was 85.6% versus 76.5% for AV. Results were consistent with COVID-19 adjustment, HR for PFS =0.28 (0.16, 0.49). Subgroup analysis examining IGHV mutation status demonstrated that the HR for PFS (95% CrI) of ZANU versus AV in low-risk IGHV unmutated and mutated patients were 0.30 (0.16, 0.57) and 0.49 (0.21, 1.13), respectively.

**Conclusions:** This NMA found a statistically significant improvement in PFS for ZANU over AV for patients with low-risk TN CLL. The observed efficacy differences should be interpreted under the limitation and assumptions of NMA, with further analysis upon trial data maturation.

**Keywords:** CLL; zanubrutinib; BTKi

**Please indicate how this research was funded:** BeOne Medicines Ltd.

**Please indicate the name of the funding organization:** BeOne Medicines Ltd.

## Abstract ID: 1910

**Title:** Comparative efficacy of zanubrutinib (ZANU) versus fixed-duration acalabrutinib plus venetoclax (AV) for first-line treatment of chronic lymphocytic leukemia (CLL): a matching-adjusted indirect comparison (MAIC)

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**Background:** In treatment-naive (TN) CLL, the efficacy of continuous ZANU has been investigated in the phase 3 SEQUOIA trial (NCT03336333) and efficacy of fixed duration combination regimen AV has been reported in the interim analysis of phase 3 AMPLIFY trial (NCT03836261). In the absence of head-to-head clinical trials, an anchored MAIC was conducted to investigate the comparative efficacy of ZANU and AV in low-risk TN CLL patients (without del(17p) or TP53 mutation).

**Methods:** This MAIC was conducted using datasets with similar median follow-ups (SEQUOIA, 40.52 months; AMPLIFY, 41 months). With the assumption of bendamustine plus rituximab (BR) and fludarabine plus cyclophosphamide and rituximab (FCR)/BR treated as common control arms, SEQUOIA and AMPLIFY can be linked through FCR/BR and the comparison of ZANU and AV were conducted in an anchored MAIC. Specifically, individual patient data of low-risk ZANU patients in SEQUOIA were re-weighted to match the key population characteristics of AMPLIFY and adjusted for age, sex, ECOG PS, disease stage, del(11q) and IGHV mutation status. Reconstructed individual patient data for AMPLIFY were generated from digitized Kaplan-Meier (KM) curves of progression-free survival (PFS). Sensitivity analyses were conducted in unanchored MAIC without the common control arm assumption, as well as in model scenarios of different matching variables.

**Results:** Relative PFS for ZANU vs AV in the unadjusted population were 0.42 (95% CI: 0.25–0.68; $p < 0.0001$). After matching adjustment, the effective sample size was 125.6 for SEQUOIA. PFS was superior for ZANU (HR for PFS =0.23 [95% CI: 0.12–0.48]; $p < 0.0001$). The 36-month PFS rate for ZANU

was 85.6% before matching and 88.5% after matching, compared with 76.5% for AV. Sensitivity analyses confirmed consistent results.
**Conclusions:** This MAIC examined the relative efficacy of ZANU versus AV and suggested a significant PFS advantage for ZANU over AV regimen. Results should be interpreted with considerations of MAIC model assumptions. Future analyses upon trial data maturation are warranted.

**Keywords:** CLL; zanubrutinib; BTKi

**Please indicate how this research was funded:** BeOne Medicines Ltd.

**Please indicate the name of the funding organization:** BeOne Medicines Ltd.

## Abstract ID: 1911

### **Title:** Prospective patient preference study for chronic lymphocytic leukemia (CLL) treatment attributes impacting patient shared-decision making

**Authors:** Sikander Ailawadhi[a], Swetha Challagulla[b], Dominic Pilon[c], Todor Totev[d], Yan Meng[e], Lilian Diaz[f], Zhuo Chen[d] and Keri Yang[b]

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**Background:** Treatments for CLL differ in efficacy, safety, treatment duration and monitoring needs, all of which can impact overall treatment experience and outcomes. To better understand patient preferences for various treatment attributes, a comprehensive quantitative analysis was conducted.
**Methods:** A patient survey with a discrete choice experiment (DCE) design was conducted from December 2024 to February 2025 among adults (≥18 years) from the United States with confirmed diagnosis of CLL, recruited through online patient panels, physician referrals, and support groups. Treatment attributes were selected based on results of a targeted literature review and clinical inputs. Patients responded to DCE questions on attributes related to efficacy (PFS), safety (impacts of diarrhea, headache, atrial fibrillation, hypertension and tumor lysis syndrome [TLS]/kidney dysfunction on quality of life [QoL]) and treatment convenience (treatment duration: continuous vs fixed duration with monitoring/hospitalization requirements). A conditional logistic regression model was used to calculate the relative importance of each attribute. Results: A total of 199 patients with CLL completed the survey (median age: 60 years; 91% White; 46% female; 66% with a bachelor's degree or above; 46% employed; 54% commercially insured; 86% suburban/urban residence; 49% diagnosed ≥5 years ago). While 30% of patients had received ≥3 lines of therapy, 23% of all patients were treatment-naïve, 25% received 1 line of prior therapy, and 23% received 2 lines of prior therapy. Most patients (88%) reported having experienced ≥1 AE from treatment previously, with the most common AEs being headache (53%), fatigue (53%), diarrhea (44%), and nausea and/or vomiting (34%). Based on DCE preference results, patients favored treatments with longer PFS and less impact of headache, atrial fibrillation and TLS/kidney dysfunction on QoL ($p < 0.001$). Impact of diarrhea and hypertension on QoL and treatment convenience did not have a statistically significant influence on treatment preferences. The top 3 treatment attributes with the highest relative importance to patients were PFS (30%), impact of headache on QoL (26%) and impact of atrial fibrillation on QoL (24%), followed by impact of TLS/kidney dysfunction (10%) on QoL, treatment convenience (5%), and impact of diarrhea (4%) and hypertension (1%) on QoL.
**Conclusions:** This patient preference survey showed that efficacy measured by PFS, the impact of headache, and impact of atrial fibrillation on QoL, were the most important attributes of treatment for patients with CLL. To support patient-centered care, shared decision-making in CLL treatment selection should incorporate a comprehensive discussion on AEs in addition to efficacy endpoints, as patients may prioritize treatments with less impact of AEs on their QoL. Future studies should assess the impact of shared decision-making on treatment adherence and outcomes.

**Keywords:** CLL; patient preference; impact of adverse events

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**Please indicate the name of the funding organization:** BeOne Medicines Ltd.

## Abstract ID: 2001

### **Title:** Comparative analysis of patients' characteristics, treatment, and survival outcomes in CLL from China and the US

**Authors:** Yuting Yan[a,b], Yin Liu[c], Ying Yu[a,b], Wenjie Xiong[a,b], Tingyu Wang[a,b], Lugui Qiu[a,b], Jifang Zhou[c] and Shuhua Yi[a,b]

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**Background:** Chronic lymphocytic leukemia (CLL) is considerably more common in Americans compared with Asians. The bases for these differences and implications for therapy outcomes are controversial and mostly unknown.
**Methods:** We compared baseline co-variates, therapies and outcomes from two databases, Flatiron Health database in the United States (N = 15,786) and Tianjin CAMS database from China (N = 2,996).
**Results:** Chinese subjects had younger age at diagnosis, more advanced Rai stage and an increased prevalence of lymphadenoma, thrombocytopenia and increased β2-microglobulin. Americans had higher rates of unmutated IGHV, TP53 deletion and cytogenetic abnormalities. These differences persisted after adjusting for age, Rai stage and IGHV mutation state. There were also substantial differences in therapy patterns between the cohorts. Median survival in Chinese was 9.7 versus 7.5 years in Americans ($p < 0.001$). In sub-group analyses Chinese CLL had better 5-year survivals

with chemotherapy (69% [95% CI, 66, 72%] versus 49% [47, 52%]; $p<0.001$), immune therapies (67% [63, 72%] versus 65% [64, 66%]; $p=0.041$) and targeted therapies (85% [81, 88%] versus 65% [64, 67%]; $p<0.001$). These advantages were pronounced among older patients and those with early stage, mutated IGHV and without TP53 deletion.
**Conclusion:** This cross-sectional study identifies significant clinical and treatment outcome disparities in CLL between Eastern and Western populations, attributed to distinct genetic and molecular profiles.

**Keywords:** Chronic lymphocytic leukaemia; China; comparative analysis

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1436

**Title:** Real-world treatment utilization patterns, discontinuation and healthcare resource utilization of first-line (1L) Bruton tyrosine kinase inhibitor (BTKi) therapy in chronic lymphocytic leukemia (CLL): age-related disparity

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**Background:** CLL tends to affect older individuals, and older patients often endure greater health disparities and treatment burdens that may impact their access to healthcare resources, adherence to treatment regimens and treatment outcomes (UN, Health Inequalities in Old Age). This study aimed to assess treatment utilization patterns, discontinuation, and healthcare resource utilization (HCRU) of 1L use of BTKi in CLL, focusing on older patients >65 years (yrs).
**Methods:** A retrospective observational study using the Symphony Integrated Dataverse was conducted to identify adult CLL patients initiating a 1L BTKi (zanubrutinib [ZANU], acalabrutinib [ACA] or ibrutinib [IBR]) treatment regimen from February 2022 and September 2024 (index period). Patients were required to have ≥30 days of continuous enrollment in the pre- and post-index periods. Patients initiating a BTKi were further categorized by age at index date. Patient sociodemographic, clinical characteristics, and outcomes were reported in overall and older (>65 yrs) CLL patients by each BTKi group. Treatment discontinuation rate was examined at 60, 90, 120, 150, 180, and 360 days, and by median time to discontinuation (TTD), estimated using the Kaplan-Meier method, censoring patients at the end of follow-up. HCRU (outpatient, inpatient, and other services) was evaluated during time on treatment and over the follow-up period and reported as per-patient-per-year (PPPY).
**Results:** A total of 6,846 patients initiated a 1L BTKi (ZANU: n=1,853; ACA: 3,519; IBR: 1,474) for CLL during the index period. Patients receiving ZANU were older (mean age: 70.9 yrs; 78% >65 yrs) than those receiving ACA (69.4 yrs; 71% >65 yrs) and IBR (69.6 yrs; 73% >65 yrs). Payer type and geography were significantly different across each BTKi group ($p<0.001$). There were no significant differences across the BTKis regarding baseline comorbidities. ZANU patients had the longest estimated median TTD overall (ZANU: 18.9 months [m], ACA: 17.8 m, IBR: 14.5 m; $p<0.001$) and in patients >65 yrs (ZANU: 17.2 m, ACA: 15.2 m, IBR: 12.6 m; $p<0.001$). ZANU patients also had the lowest estimated discontinuation rates at 60-, 90-, 120-, 150-, 180-, and 360-days post BTKi initiation in the overall and >65 yrs groups. There were significant differences across the BTKi group for inpatient visits PPPY in all patients and >65 yrs with ZANU having the lowest visits of the BTKis (ZANU: 2.6, ACA: 3.6, IBR: 4.6; $p=0.029$ and ZANU: 2.5, ACA: 4.3, IBR: 5.3; $p=0.004$, respectively).
**Conclusion:** This real-world study demonstrated that CLL patients treated with ZANU had longer TTD, lower discontinuation rates, and less HCRU than ACA and IBR across all patients and specifically in older patients >65 yrs. Further studies on long-term and clinical outcomes are warranted.

**Keywords:** CLL; BTKi; age disparity

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**Please indicate the name of the funding organization:** BeOne Medicines Ltd.

## Abstract ID: 1208

**Title:** Selectively targeting foreign antigen to surface membrane IgD leads to enhanced T-cell activation in patients with CLL and normal people

**Authors:** Martina Cardillo[a], Matthew Friedman[b], Anita Ng[a], Randall Davis[c], XiaoJie Yan[a], Ashna Shah[b], Michael Ryan[a], Peter Gregersen[a], Steven Allen[d], Jonathan Kolitz[d], Kanti Rai[d], Paolo Ghia[e] and Nicholas Chiorazzi[f]

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T-cell activation via T-cell receptor (TCR) signaling is less robust in patients with chronic lymphocytic leukemia (CLL) than in healthy controls (HC), contributing to the immune deficiency characteristic of CLL. In HCs, activated B cells are potent antigen-presenting cells (APCs), initiating T-cell responses through the presentation of processed antigenic peptides via MHC class II. However, in CLL, this process, particularly immune synapse formation, is defective. Most CLL cells and unswitched normal B cells express surface membrane IgM (smIgM) and IgD (smIgD), which share identical antigen-binding sites, and each isotype participates in antigen uptake. However, whether they do so equivalently and activate T cells equally have not been directly tested. Therefore, we evaluated if selectively targeting a foreign antigen to smIgM or smIgD affected antigen internalization, processing, and T-cell activation in HCs and people with CLL. We engineered two murine monoclonal antibodies (mAbs) that are specific for human IgM or IgD and have very similar affinity constants for the respective antigens to express human IgG4 Fc domains (to avoid Fc receptor binding) and either included or excluded the receptor-binding domain (RBD) of the SARS-CoV-2 spike

protein to the Fc domain: r-αIgM, r-αIgM-RBD, r-αIgD, and r-αIgD-RBD. The CXCR4DimCD5Bright CLL subset, also known as the proliferative fraction (PF), which is enriched for antigen presentation molecules, was the target of antigen delivery, uptake, and presentation. T-cell activation was measured by flow cytometry based on the enhanced expression of CD69, CD134, CD137, and the presence of intracellular IFNγ and IL-4. Confocal microscopy using the r-αIgM and r-αIgD conjugated with AF488 or AF647 or with pHrodo-labeled mAbs (Deep Red for IgM, Green for IgD) was used to track internalization and compartment deposition. Despite higher smIgM than smIgD expression on PF cells ($p = 0.0141$), internalization at 37 °C showed significantly greater intracellular IgD than IgM ($p = 0.0402$), a pattern also observed in normal B cells ($p = 0.0302$). While treatment with r-αIgM or r-αIgD alone had no impact, delivery of RBD via r-αIgD-RBD, but not via r-αIgM-RBD, resulted in significantly enhanced T-cell proliferation and activation (CD8+ $p = 0.0445$; CD8 + CD134+ $p = 0.0385$). Numerous activation markers were significantly upregulated with r-αIgD-RBD versus r-αIgD alone, and also when directly compared to r-αIgM-RBD (e.g. CD4 + CD69+ $p = 0.0012$; CD4 + CD134+ $p = 0.0014$; CD8 + CD137+ $p = 0.0004$; CD8 + IL-4+ $p = 0.0012$). To validate these findings in a physiologic setting, we tested B cells from 19 healthy donors using the same antibody panel. In contrast to the CLL setting, both r-αIgM-RBD and r-αIgD-RBD stimulated T-cell responses, although r-αIgD-RBD consistently elicited stronger T-cell activation than r-αIgM-RBD across multiple readouts. Hence, even for normal B cells, smIgD mediates a more immunostimulatory outcome. To ensure that this effect was specific to the PF and not generalizable across all CLL cells, we tested the resting fraction (RF, CXCR4BrightCD5Dim) in 10 patients. In contrast to the consistent PF data, the RF results were heterogeneous. While some patients showed responsiveness to r-αIgM-RBD, overall T-cell activation was less consistent and not as robust as with the PF. These findings are consistent with antigen-presenting capacity varying with the activation state of B cells and PF cells being more effective for modeling immunostimulatory mechanisms. To further explore the mechanistic basis for these differential responses, we evaluated HLA-DR expression, an essential component of antigen presentation, in 20 CLL samples (10 mutated [M-CLL] and 10 unmutated [U-CLL]). After 1-hour incubation with the r-αIgM-RBD or r-αIgD-RBD, HLA-DR expression was significantly higher in the PF compared to the RF and was significantly enhanced only by r-αIgD-RBD. Notably, the highest levels of surface HLA-DR expression were observed in U-CLL samples, suggesting that these cells may have a greater intrinsic capacity for antigen presentation. Finally, we used confocal microscopy to identify the intracellular locations of IgM and IgD after surface membrane engagement. Remarkably, this revealed that smIgM and smIgD arrive in distinct intracellular compartments. Collectively, these findings suggest that smIgD elicits more effective antigen processing and presentation, resulting in greater activation of foreign antigen-specific T cells in both CLL and healthy individuals. This distinct smIgD-mediated pathway may offer a novel strategy to enhance immune responses to foreign and tumor-associated antigens, particularly in immunodeficient states like CLL.

**Keywords:** Leukemia; BCR; IgD

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:** CLL Global Research Foundation

# Abstract ID: 1529

**Title:** Prevalence of BTK, PLCG2, and BCL2 mutations in CLL patients in Croatia - KROHEM study

**Authors:** Branimir Gizdic[a], Ivona Aric Zrna[a], Mia Nizetic Govic[a], Petra Veic[a], Barbara Dreta[b], Inga Mandac-Smoljanovic[c], Sandra Basic-Kinda[b], Renata Babok-Flegaric[d], Hana Matijaca[e], Dragana Grohovac[f], Martina Marjanović[g], Jasminka Sincic-Petricevic[h], Ena Soric[a], Marija Ivic[a], Vlatko Pejša[a], Igor Aurer[i] and Ozren Jaksic[j]

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**Introduction:** Targeted therapies in Chronic Lymphocytic Leukemia (CLL), including Bruton tyrosine kinase inhibitors (BTKi) and Bcl-2 antagonists (Bcl2a), have led to significant improvements in outcomes for both treatment-naive (TN) and relapsed/refractory (RR) patients. These therapies are now standard options for almost all CLL patients (ESMO, KROHEM). However, high-risk molecular features of the disease, such as TP53 aberrations (del17p and/or TP53 mutations), are still linked to inferior outcomes and guide therapeutic decisions. Testing for these features (by FISH and sequencing) before each line of therapy is a standard recommendation. In relapsed/refractory patients previously treated with BTKi and/or BCL2a, resistance mutations in BTK and PLCG2 and/or BCL2 have been described, respectively. However, testing for these mutations is still not part of routine practice.

**Methods:** Since September 2023, all CLL patients in Croatia whose samples are tested for TP53 mutation are also tested by NGS (with sensitivity 1%) using a customized panel that includes BTK, PLCG2, and BCL2 genes along with TP53 (exon2-11). To evaluate the prevalence of BTK, PLCG2, and BCL2 mutations among TP53 mutation-tested patients and their relation to treatment status, treatment data for CLL patients tested from 8 centers were retrospectively collected, while all mutation analyses were performed in one laboratory. Routine practice is to test all patients for TP53 mutation before each treatment line unless del17p was detected by FISH, or the reason for treatment change is adverse events.

**Results:** The analysis included 135 patients with CLL, for whom data were available. There were 85 males and 50 females, with a median age of 69 years (range 41–90 years). There were 93 treatment-naive patients and 41 previously treated patients (relapsed/refractory). Based on epidemiologic estimates of the number of 1st line and 2nd + lines of CLL treated in Croatia, around 1/3 of all patients treated were included in this study. Among 41 previously treated patients, 28 had been exposed to BTKi (20 ibrutinib, 8 acalabrutinib, 2 zanubrutinib, 1 pirtobrutinib) and 13 had been exposed to venetoclax-based treatment (12 monotherapy, 3 time-limited regimens), with several patients having received multiple BTKi and/or Bcl2a-based treatments. TP53 aberrations (TP53

mutation and/or del17p) were found in 15/94 TN and 15/41 RR patients. In TN patients, no BTK and BCL2 mutations were detected, while one TN patient had a pathogenic PLCG2 mutation (subsequently treated with a venetoclax-based regimen). In RR patients, BTK mutations were detected in 10/28 BTKi-treated patients, PLCG2 mutations were detected in 4/28 BTKi-treated patients (combined 14/28), and BCL2 mutations were found in 3/13 venetoclax-treated patients.
**Conclusions:** BTK, PLCG2, and BCL2 resistance mutations are found in a significant proportion of individuals who relapsed after or were refractory to targeted agents. Although routine testing for these mutations is not currently recommended, they may provide additional information that can be considered in treatment decisions. This could become more relevant with the increasing number of drugs targeting selected pathways and different resistance patterns. Additionally, due to intraclonal heterogeneity, combination treatment strategies may be considered in patients with resistance mutations in multiple genes. Further studies are needed to establish the role of BTK, PLCG2, and BCL2 mutation testing in routine settings.

**Keywords:** Leukemia; relapsed/refractory; biomarker

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## Abstract ID: 1437

**Title:** Effect of targeted treatment on IGHV subset #2 and #8 patients in CLL: comparison of MRD defined Ibrutinib plus Venetoclax treatment to the Ibrutinib and FCR arms of the FLAIR trial

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**Introduction:** Determination of the immunoglobulin heavy variable (IGHV) gene somatic hypermutation (SHM) status remains an important and independent prognostic marker in CLL. Additionally, in approximately one third of CLL the B-cell receptor (BcR) immunoglobulins (IG) display highly homologous variable heavy complementarity-determining region 3 (VH CDR3), and can be assigned to distinct subsets, with virtually identical or stereotyped BcR IG. Of the major subsets described, CLL subset #2 is the largest stereotyped subset (Agathangelidis et al, Blood. 2012). Present in CLL using the IGHV3-21 gene, this subset is characterized by poor clinical outcome, irrespective of SHM status (Baliakas et al, Blood. 2015). Stereotyped CLL subset #8, defined by the use of the IGHV4-39 gene, is associated with clinically aggressive disease and high risk of Richter's transformation (RT) (Rossi et al, Hematol Oncol. 2009). Compared to chemoimmunotherapy, numerous trials using targeted treatment strategies with Ibrutinib (I) an irreversible Btk inhibitor, and Venetoclax (V), a Bcl-2 inhibitor have shown improved outcomes in CLL. FLAIR has reported improved Progression-Free Survival (PFS) and Overall Survival with MRD defined I+V compared to Fludarabine, Cyclophosphamide and Rituximab (FCR) in CLL (Munir et al, N Engl J Med. 2024). However, little is known regarding the synergistic effect of I+V on the response of subset #2 and #8 patients. In this study we have investigated the 5-year PFS of CLL subset #2 patients comparing MRD defined I+V to the Ibrutinib (I alone and IR) and FCR arms of FLAIR. We also report on RT in FLAIR including CLL subset #8 patients.
**Methods:** FLAIR (ISRCTN01844152) is an ongoing phase III, multicentre, randomized, controlled, open-label trial in previously untreated CLL patients, comparing IR with FCR. It was subsequently adapted in July 2017 to compare I+V and I alone with FCR. Somatic hypermutation status was determined by PCR amplification of IGHV-IGHD-IGHJ gene rearrangements using IGHV leader/FR1 primers. Bidirectional Sanger sequencing was analyzed using IMGT/V-Quest and the ARResT/AssignSubsets tool.
**Results:** Of the 1,172 patients included in this study, 79 patients were assigned to CLL subset #2 (6.7%). Of these 16 were treated with I+V, 49 with ibrutinib based therapies (I alone and IR arms combined) and 14 with FCR, with a median follow up of 58 months (63, 51 and 56 months, respectively). Using Log-rank test, a significant difference in PFS was observed between subset #2 patients treated with I&IR and I+V compared to FCR ($p=0.003$ and 0.002 respectively) but not between I+V and I&IR ($p=0.1873$). The 5 years PFS for subset #2 patients was 100% for I+V, 88.9% for I&IR and 52.7% for FCR. Using Cox's proportional hazards, a significant difference in PFS was also present between I&IR and I+V compared to FCR for non-subset #2 patients (HR:0.41 $p<0.001$ and HR:0.13 $p<0.001$ respectively). Additionally, the difference in PFS between I+V and I&IR was also significant (HR:0.32 $p<0.001$). The 5 years PFS for non-subset #2 patients was 93.5% for I+V, 80.2% for I&IR and 58.5% for FCR. Overall, there was no significant difference in PFS between non-subset #2 patients and subset#2 patients ($p=0.3$). Overall, 12/1,172 patients (1%) on FLAIR were reported to have undergone RT (I+V n=2; I&IR n=6; FCR n=4). However, none of these patients were stereotyped CLL subset #8.
**Conclusion:** Flair trial has shown that targeted treatment is highly effective at mitigating the poor outcome previously associated with CLL subset #2 patients, with exceptional responses seen following MRD defined I+V treatment. To date none of the CLL subset #8 patients on FLAIR have undergone RT.

**Keywords:** FLAIR; IGHV; Subset#2

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## Abstract ID: 1225

**Title:** Screening people with persistent absolute lymphocytosis favors the identification of high-count monoclonal B-cell lymphocytosis

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**Background:** Monoclonal B-cell lymphocytosis (MBL) is a common finding in the aging population, with a reported average prevalence of 15% in individuals between age 40 to 100 years. MBL is divided to 2 categories, lcMBL (< 500 cells/μl), which represent ~95% of cases, and hcMBL (500–4,999 cells/μl), representing ~5% of cases. Since people with hcMBL progress to CLL at rate of ~5% per year, identifying those people with hcMBL who will progress to CLL is very important clinically reasons and studying this process could allow pre emptive, preventive therapy. Although random population-based studies provide valuable information about MBL prevalence, developing a screening approach that would favor finding people with hcMBL would be more valuable for studying the natural history and understanding the pathobiology of this disorder. Here we show that focusing on individuals with persistent absolute lymphocytosis (PAL) offers a targeted strategy for identifying hcMBL, thereby allowing studies to improving our understanding of this pre-leukemic condition.

**Methods:** This study investigated the prevalence and characteristics of MBL within a well-defined cohort of patients with PAL. We define PAL as an absolute lymphocyte count (ALC) consistently above 3.3 x 10^9/L for at least 90 days. All participants underwent multiparameter flow cytometry analysis using a comprehensive panel of antibodies targeting B-, T-, and myeloid-cell markers.

**Results:** Of the 608 PAL patients screened, 14% (85) had a clonal B-cell expansion, indicative of MBL. Detailed flow cytometry analysis of 71 MBL cases allowed us to determine the distribution of MBL subtypes: 66% (47) CLL-like MBL (CD5 + CD20Dim), 5.4% (4) atypical MBL (CD5 + CD20+), and 20.3% (15) non-CLL-like MBL (CD5-CD20+). These frequencies are consistent with those previously described. Seven percent (5) of the clonal B-cell expansions did not fall into any of these categories. Although the prevalence of CLL-like MBL aligns with that reported in the general population ≥40 years old, the ratio of hcMBL to lcMBL is strikingly different, i.e. 64% vs 36% in our series compared to 5% vs 95% in the reported random analysis ($p < 0.0001$), highlighting the effectiveness of the PAL approach for enriching the identification of clinically relevant MBL cases. The median age for the PAL cohort excluding the MBL cases was 60.6 years (range 38–100), while the median age for the MBL group was 70.3 years (range 38–97; $p < 0.0001$). Within the MBL group, the median age for hcMBL cases was 71 years (range 55–97), and for lcMBL cases was 66.5 years (range 49–87) (not significant). Moreover immunoglobulin (IG) light (L) chain analysis revealed a predominance of Igκ L chains in lcMBL (14 κ, 3 λ) and a Igλ L chain bias in hcMBL (11 κ, 17 λ, and 2 with no detectable L chain restriction). Analysis of the cellular immune microenvironment revealed significant differences between healthy controls (HC), PAL individuals without clonal expansions, and the MBL subgroups. All PAL exhibited significantly higher absolute T-cell counts compared to HC. However, people with hcMBL had significantly lower T-cell counts than both PAL and lcMBL patients ($p < 0.05$). This pattern was mirrored in the CD4+ T-cells, while CD8+ T cells showed no significant differences with the MBL cases and HC. Interestingly, monocyte counts were significantly higher in MBL cases compared to HC, with a more pronounced elevation observed in the hcMBL subgroup.

**Discussion:** Our findings demonstrate a significantly higher prevalence of hcMBL among people with PAL than estimated for the general population. In addition, the observed differences in age, Ig L chain use, and T and monocyte numbers between lcMBL and hcMBL underscore the distinct clinical and biological characteristics of these subgroups. Moreover, the elevated T-cell and monocyte counts observed in hcMBL might reflect an ongoing immune response to the expanding clonal B-cell population or evidence for an immature, precursor population bearing oncogenic mutations. Thus, screening people with PAL is an effective strategy to identify clinically relevant, hcMBL cases, to allow an understanding of the natural history of this condition and define the molecular and immunologic processes that influence its conversion to CLL.

**Keywords:** Lymphocytosis; high-count monoclonal B-cell lymphocytosis; microenvironment

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## Abstract ID: 1226

### **Title:** Ibrutinib enhances the bias of T-cell responses towards staphylococcal superantigens sustaining inflammation in chronic lymphocytic leukaemia

**Authors:** Fisal Tantoush[a], David Allsup[b], Leigh Naylor-Adamson[c], Frank Voncken[c] and Stefano Caserta[c]

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**Introduction:** Chronic lymphocytic leukemia (CLL) is an uncurable hematological malignancy, associated with significant infection morbidity. Bruton's tyrosine-kinase inhibitors (e.g. ibrutinib) have improved disease outcomes, but severe infections and poor immunization responses afflict patients. Recently, carriage of the endemic Staphylococcus aureus (SA) was associated with lymphocytosis and decreased survival in CLL patients. We then hypothesized that exposure to staphylococcal superantigens (SAgs), known to promote hyper-inflammatory responses, impairs immunity and increases severe infection risk in CLL patients.

**Methods:** We evaluated the reactivity of T-cells and CLL cells to SA SAgs, in cultures derived from ibrutinib-treated and untreated CLL patients. We used multiparametric flow cytometry to study the responses to SA SAgs in T and B/tumour cells derived from patients.

**Results:** We found that ibrutinib-treated patients had less naive CD8+ T-cells ($p = 0.0348$), more checkpoint receptor (TIM-3) expression in memory T cells ($p < 0.0001$), and lower IFNγ/cytokine responses in patient T cells ($p \leq 0.0298$). In vitro,

exposure to SA SAg further increased the accumulation of memory T cells with an exhaustion-phenotype, preferentially in cultures derived from ibrutinib-treated patients ($p \leq 0.035$). Nevertheless, staphylococcal SAgs could not induce regulatory T cells from CLL patients inasmuch as healthy donors ($p \leq 0.0461$) and this was associated with accumulation of inflammatory T cells. Significantly, SAg-exposure enhanced inflammatory activation of CLL tumour cells, which acquired CD38, CD40, CD86, while downregulating CD27 ($p \leq 0.005$), even in cultures from ibrutinib-treated CLL patients.
**Conclusion:** We suggest that environmental SAg-exposure promotes the accumulation of pseudo-exhausted T cells, which induce/sustain tumour cell activation, not counteracted by ibrutinib. Our study critically helps understand the chronic inflammatory milieu in CLL patients, with implications for infection morbidity, disease etiology and future interventions.

**Keywords:** Tumour microenvironment; staphylococcal toxin; leukemia

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## Abstract ID: 1227

### **Title:** Clinical and biological impact of plasma cytokine levels in newly diagnosed chronic lymphocytic leukemia

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**Background:** Concentrations of specific cytokines/chemokines are consistently associated with patient prognosis in different cancer types (Lippitz, Lancet Oncol. 2013). In the context of chronic lymphocytic leukemia (CLL), the impact of baseline cytokine levels on biological features and clinical outcomes remains poorly understood. Previous findings have suggested a prognostic role for MIP-1α in predicting unfavorable clinical and biological characteristics (Sivina et al., Blood. 2011). However, little is known about the role of other cytokines/chemokines in CLL.
**Methods:** Plasma from newly diagnosed CLL patients was analyzed for cytokine/chemokine levels using the Bio-Plex Pro Human Cytokine 27-plex assay (BioRad). Associations with baseline clinical and biological features were assessed via Mann-Whitney U test and Spearman correlation. Survival analyses were performed through the Kaplan-Meier and log-rank tests. Different optimal cut-offs for overall survival (OS), probability of Richter transformation (RT) and second malignancies risk analyses were defined using maximally selected rank statistics (maxstat package). For each clinical outcome, patients with cytokine/chemokine concentrations above the optimal cutoff for every specific cytokine/chemokine were marked as 'high,' while those below were marked as 'low.' $p$ values were adjusted using the Benjamini-Hochberg correction.
**Results:** A total of 244 patients with newly diagnosed CLL were included. Median age at diagnosis was 68.9 years; 73 patients (29.9%) had unmutated immunoglobulin heavy chain variable region (IGHV) genes, and 16 (6.6%) had TP53 disruption. After a median follow-up of 13.4 years, the median OS was 15.7 years. By correlating cytokines/chemokines with patient baseline clinical and molecular features, elevated MIP-1α levels were significantly associated with unmutated IGHV genes ($p < 0.0001$), greater lymph node diameter ($p < 0.0001$), and advanced Rai and Binet stages (both $p < 0.0001$). Higher IP-10 levels significantly associated with advanced Rai ($p = 0.02$) and Binet ($p = 0.01$) stages. High levels of G-CSF correlated with unmutated IGHV ($p = 0.049$), and elevated GM-CSF levels were associated with del(17p) ($p = 0.03$). Increased MCP-1 was linked to lower platelet count ($p < 0.0001$). At diagnosis, multiple cytokines/chemokines with levels exceeding the optimal cut-offs for predicting OS, namely MIP-1α ($p = 0.001$), IP-10 ($p = 0.003$), G-CSF ($p = 0.011$), PDGF-BB ($p = 0.023$), IL-4 ($p = 0.023$), GM-CSF ($p = 0.025$), IL-5 ($p = 0.025$), IL-6 ($p = 0.027$), and IL-2 ($p = 0.027$) were significantly associated with shorter survival. In multivariate analysis (adjusted for age, IGHV, and TP53 status), high levels of IL-4, using a cut-off of 5.78 pg/ml, independently associated with shorter OS (HR 2.2, $p = 0.008$). Pathway gene enrichment analysis revealed that cytokines/chemokines predicting shorter OS are involved in key molecular pathways regulating cell proliferation, survival, and apoptosis. In particular, G-CSF, GM-CSF, IL-2, IL-4, IL-5, IL-6, and PDGF-BB clustered in the JAK-STAT signaling pathway (fold enrichment 109.9, $p = 2.23E-12$), whereas G-CSF, IL-2, IL-4, IL-6, and PDGF-BB clustered in the PI3K-Akt pathway (fold enrichment 35.9, $p = 9.17E-07$). Regarding RT, high levels of GM-CSF ($p < 0.0001$), MIP1-α ($p = 0.034$), IL-4 ($p = 0.034$), and IL-10 ($p = 0.034$) were significantly associated with increased risk of RT in univariate analysis. GM-CSF, with a cutoff of 0.86 pg/ml, independently predicted RT in multivariate analysis (HR 9.32, $p = 0.009$). Moreover, among patients who developed a second malignancy after CLL diagnosis, high levels of MIP-1β ($p = 0.0001$), RANTES ($p = 0.023$), and MIP-1α ($p = 0.023$), were linked to shorter time of cancer occurrence. MIP-1β, using a cutoff of 255.36 pg/ml, retained an independent prognostic value for shorter time to second cancer in multivariate analysis also including prior chemo-immunotherapy exposure for CLL (HR 4.64, $p = 0.002$).
**Conclusions:** Plasma levels of immunomodulatory cytokines/chemokines are associated with unfavorable clinical and biological features at the time of CLL diagnosis. Moreover, higher concentrations of immunomodulatory cytokines/chemokines correlate with lower OS, higher risk of RT and second malignancies. If confirmed also in the context of patients treated with BTK inhibitors and BCL2 inhibitors, cytokine/chemokine levels might integrate current prognostic biomarkers in CLL.

**Keywords:** Chronic lymphocytic leukemia; cytokines; biomarker

**Please indicate how this research was funded:**

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## Abstract ID: 1015

**Title:** Monoclonal gammopathy defines a distinct clinical in chronic lymphocytic leukemia across therapeutic eras

**Authors:** Yuting Yan[a,b], Bolin Yuan[c], Shuhua Yi[a,b], Liang Wang[d], Tonglu Qiu[e], Guoqiang Liu[d], Weihao Chen[a], Tingyu Wang[a,b], Ying Yu[a,b], Wenjie Xiong[a,b], Gang An[a], Dehui Zou[a,b] and Jianyong Li[e]

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Monoclonal gammopathy (MG) in chronic lymphocytic leukemia (CLL) portends heterogeneous outcomes, yet its molecular drivers and therapeutic implications remain undefined. In this retrospective analysis of 2,075 CLL patients (1999–2024), MG was detected in 18.47% cases, with IgM (8.18%), IgG (8.09%), light-chain (1.14%) and IgA (1.06%) subtypes demonstrating divergent clinicogenomic profiles. Patients with IgA-MG were older at diagnosis, whereas those with IgG-MG had younger age and a higher frequency of mutated IGHV. In contrast, IgM-MG was associated with unmutated IGHV, elevated LDH and β2-microglobulin, and higher frequencies of TP53 aberrations and enrichment of MYD88, BIRC3, DDX3X mutations. IgG-MG was associated with shorter time to first treatment (TTFT) only, whereas IgM-MG correlated with significantly inferior TTFT, progression-free survival (PFS), and overall survival (OS). Subgroup analyses revealed that the adverse prognostic impact of MG was pronounced in IGHV-mutated CLL but attenuated in unmutated cases. Prognostic discrimination by the CLL-IPI remained robust regardless of MG status. Notably, patients with IgM-MG did not experience significant survival benefit from targeted therapy compared with conventional regimens. These findings demonstrate that MG subtypes, particularly IgM-MG, define biologically and clinically distinct subsets of CLL. Given the limited efficacy of BTK inhibitors in IgM-MG, immunofixation-based MG profiling may inform risk-adapted treatment strategies and personalized therapy selection.

**Keywords:** Chronic lymphocytic leukemia; immunoglobulin; monoclonal gammopathy; target therapy; outcome

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## Abstract ID: 2006

**Title:** Understanding the unmet needs in diagnostics and therapeutics of chronic lymphocytic leukemia in Korea

**Authors:** Eun sang Yu[a], Soyean Kwon[b], Sung-Soo Park[c], Youngil Koh[b], Ja Min Byun[b], Ki-Seong Eom[c] and Chul Won Choi[a]

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**Background:** Since the FDA approval of Bruton's tyrosine kinase inhibitor (BTK inhibitor) ibrutinib in 2012, the treatment landscape for chronic lymphocytic leukemia (CLL) and small lymphocytic leukemia (SLL) has undergone transformative changes. However, the disease remains incurable, posing a long-term challenge. In Korea, the younger median age at diagnosis and higher annual percentage change (4.17% in Korea vs. 0.68% in United States during 1999 to 2010) suggest a growing prevalence and healthcare burden. This study aims to provide updated epidemiologic data to guide care planning and support innovative therapeutic approaches.

**Methods:** This multicenter retrospective study included 519 patients diagnosed with CLL/SLL between 2006 and 2024 across three major Korean institutions. Their medical records were reviewed and analyzed for demographics, baseline disease characteristics, details of treatment, treatment outcomes, and survival. When available, results from cytogenetic and molecular genetics data were collected. Treatment regimens were classified as follows: immunochemotherapy (including fludarabine- cyclophosphamide-rituximab and obinutuzumab-chlorambucil), chemotherapy (which is consisted of only traditional cytotoxic chemotherapy drugs, such as chlorambucil or fludarabine), BTK inhibitors and BCL-2 inhibitor.

**Results:** The median follow-up period was 45.3 months. Of the 519 patients (median age at diagnosis 62 years;61% male), cytogenetic data, including FISH and karyotyping, were available for nearly half (55.9 - 57.5%) of the study population. TP53 mutations were detected in 7.1% (18/253), and unmutated IGHV was found in 93.1% (136/146) of patients. International Prognostic Index for Chronic Lymphocytic Leukemia (CLL-IPI) scores could be calculated for 40.9% of the cohort, among whom 15.9% and 16.7% were classified as low- and intermediate-risk, respectively, while high-risk and very high-risk categories comprised 7.3% and 0.7% of patients. In a cohort of 267 patients who received first-line treatment, 68.9% (n = 184/267) underwent immunochemotherapy, 19.4% (n = 52/267) received cytotoxic chemotherapy, and 11.2% (n = 30/267) were treated with BTK inhibitor. BTK inhibitor use has steadily increased since 2016, following ibrutinib's approval for CLL/SLL treatment in Korea. Subgroup analyses of showed that higher CLL-IPI scores correlated with poorer overall and progression-free survival. Compared to the low-risk group, the high- and very high-risk group had a significantly increased risk of death (HR 5.16, 95% CI 1.71–15.56, $p = 0.004$) and progression (HR 7.49, 95% CI 2.48–22.64, $p = 0.0003$). Regarding treatment modality, individuals receiving BTK inhibitor demonstrated more favorable outcomes compared to those treated with immunochemotherapy. The 36-month Overall survival (OS) and Progression-free survival (PFS) rate were 96.0% vs 91.3% and 91.8% vs 79.7%, respectively, favoring the BTK inhibitor-based therapy.

**Discussion:** Although patients with CLL/SLL in Korea are diagnosed at a younger age than those in Western countries,

first-line treatment outcomes are not notably superior. This may reflect differences in therapeutic approaches, with immunochemotherapy remains the predominant regimen in Korea, whereas BTK inhibitors are more commonly used in Western populations (11.2% in Korea vs 45.0% in United states (Clin Lymphoma Myeloma Leuk. 2024;24(9):e301–e313.) and 31.2% in Spain (Clin Lymphoma Myeloma Leuk. 2021;21(12):e985–e999.). While acknowledging the inherent limitations of cross-national comparisons—including disparities in healthcare infrastructure and treatment accessibility—similar trends were observed within our cohort. Patients aged >= 65 years received BTK inhibitor more frequently than younger patients (12.8%, 15/117 vs 10.0%, 15/150) and were less often treated with immunochemotherapy (64.9%, 76/117 vs 72.0%, 108/150). Despite their older age and likely comorbidity burden, overall response rates were comparable between these two groups (Overall response rate [ORR] 82.9% [Complete response (CR) 51; Partial response (PR) 46] vs 88.6% [CR 93; PR 40]. Although these findings are subject to potential confounding, they support the feasibility and potential clinical benefit of broader BTK inhibitor use in Korea, where access remains limited by reimbursement policies. Additionally, despite the demonstrated prognostic value of the CLL-IPI, its use remains limited in Korea due to the low rate of genetic testing. Beyond CLL-IPI, comprehensive genetic testing plays a critical role in modern CLL/SLL management. The integration of molecular and response-based biomarkers is redefining not only risk stratification, but also response assessment and treatment decision-making, including considerations for therapy discontinuation. Optimizing CLL/SLL management thus requires not only broadening access to effective agents but also implementing precision medicine tools to match treatment intensity with disease risk and response.

**Keywords:** Chronic lymphocytic leukemia; small lymphocytic leukemia; real-world data

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## Abstract ID: 1001

**Title:** IgM monoclonal gammopathy arises directly from MBL/CLL cells: evidence from proteomic analyses

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**Background:** Chronic Lymphocytic Leukemia (CLL) is the most frequent leukemia in adults and is typically preceded by a pre-malignant condition called Monoclonal B cell Lymphocytosis (MBL), which is detected in up to 10% of otherwise healthy individuals. Monoclonal gammopathy of undetermined significance (MGUS) of the IgM isotype is also a common pre-neoplastic condition concerning healthy individuals, also requiring regular clinical follow-up. MGUS can be detected before, after or simultaneously with a MBL/CLL diagnosis. Using innovative Ig proteomics methods in our study, we aimed at exploring whether the clonal CLL B cells could be responsible for the production of the serum IgM monoclonal component (MC), thus being directly responsible for the development of IgM MGUS, or if the latter should be considered a manifestation of another concomitant disease, given the increased risk of other neoplasias in patients with MBL/CLL.

**Methods:** A series of 263 patients diagnosed with either MBL (91) or CLL (172), of whom 97 also had a circulating IgM monoclonal component, were analyzed for the expression of clonal Immunoglobulin heavy and light chains (Ig HC and LC, respectively). As a first step, RNA was isolated from the monoclonal B cells, those obtained from peripheral blood of patients and cryopreserved in the dedicated Dana-Farber Cancer Institute (DFCI) biobank. Using synthesized cDNA, PCR amplification was performed with specific IG gene primers, followed by bidirectional Sanger sequencing. Furthermore, sera from the same patients were analyzed with two different proteomic techniques: (i) Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry (MS) to achieve a precise quantification of the LC's mass-to-charge ratio (m/z), allowing the validation of Ig molecular mass results calculated from Sanger sequences; and, (ii) high resolution quantitative proteomic sequencing using an EvoSep One Liquid Chromatography system coupled to a timsTOF Pro 2 mass spectrometer (Bruker) to identify the most abundant serum proteins and compare the obtained protein Ig HC and LC sequences against the respective Sanger sequences in each individual patient.

**Results:** In a cohort of 3,127 patients (followed up at DFCI) with serum protein electrophoresis (SPEP) results, 658 (21%) cases presented with a serum MC (either IgM, IgG or IgA). For this study, we selected 263 patients, including 97 cases with IgM MC (85 CLL and 12 MBL), for the thorough characterization of the paraprotein. First, we compared the data from the Ig LC expressed by the leukemic cells, as obtained by Sanger sequencing and flow cytometry with the SPEP and MALDI-TOF MS data for the LC associated with the serum monoclonal protein. Interestingly, the serum Ig LC mass measured by MALDI-TOF MS matched the calculated mass from the expressed clonal Ig in 65% (51/78) of the MBL/CLL cases. We then utilized the high-resolution quantitative MS technique, which allows for the exact identification of the variable domain of the Ig HC and/or LC. Comparisons led to the identification of a high level of identity between the serum Ig protein sequences and the Ig expressed by the leukemic cells in a total of 64/89 cases (72%). In particular, we were able to identify a match for the HC and LC variable domains in 47/89

(53%) and 52/85 cases (61%), respectively. No match was identified in the remaining cases, due to low levels of the circulating paraprotein and/or the lack of MS results not being able to identify a discrete abundant protein. These unmatched cases are being studied in detail and are subject to further evaluation with more sensitive approaches in order to get definitive results that will be presented.

**Conclusions:** For the first time, to the best of our knowledge, we here provide robust evidence that, in the majority (~3 out of 4) of MBL/CLL cases with a concomitant IgM paraprotein, the clonal B cells are responsible for the production and release of the circulating IgM MC. This subset of IgM-producing cases closely resembles what occurs in lymphoplasmacytic lymphoma/Waldenström disease and raises a number of questions on the biological and immunological nature of the cells underlying the Ig production. In particular, these data could suggest a different path of B cell maturation and transformation in a significant fraction of CLL/MBL that warrants further research.

**Keywords:** Leukemia; monoclonal gammopathy; immunoglobulins

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**Please indicate the name of the funding organization:** Center for Early Detection and Interception of Blood Cancers (CEDI-BC) grant 2023–24, Dana-Farber Cancer Institute

## Abstract ID: 1016

### **Title:** Integrating phosphoproteomic analysis and innovative 3D cell culture systems to dissect the role of extracellular stiffness in CLL pathogenesis

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**Background:** CLL is a dynamic disease recirculating between different anatomical sites in which a wide range of mechanical forces act in an orchestrated manner on malignant B lymphocytes. It is well established that cells can sense physical stimuli and convert them into biochemical signals through mechanotransduction. However, mechanobiology has been primarily explored in solid tumors, and its relevance in hematological malignancies remains poorly understood, likely due to the lack of physiologically relevant experimental models (Sampietro, Cellani et al. 2025). A deeper understanding of the implications of physical forces in the pathogenesis of CLL could pave the way for innovative strategies to control the disease. To address this gap, our research aims to investigate how mechanical stimuli influence CLL cell behavior and drug sensitivity, using both 2D and 3D culture systems, with the latter generated via 3D bioprinting. We previously demonstrated that 3D-bioprinted CLL cells can survive up to 28 days without additional stimuli, with associated changes in their gene expression profile (Sbrana et al. 2021).

**Materials and methods:** CLL cells were cultured in 2D on stiffness-tuned polydimethylsiloxane (PDMS)-coated plates or 3D-bioprinted in polymer-based hydrogels: CELLINK Laminink 411 (commercial) and 3A1M ink (in-house; composed of methylcellulose, alginate, and gelatin). Phenotypic responses to stiffness variation were evaluated by kinome profiling (PamGene International B.V.), western blot, and immunofluorescence. Drug response was assessed by treating both 2D-cultured and 3D-bioprinted cells with fludarabine or the BCL2 inhibitor venetoclax for 24 or 72 hours, with or without 7 days of 3D culture adaptation. Cell viability was measured by Alamar Blue assay; gene expression was analyzed by RNA-Seq and RT-qPCR.

**Results:** RNA-Seq performed on 3D-bioprinted CLL cells compared to 2D (n = 6) revealed the modulation of markers involved in homing, adhesion, and cytoskeletal remodeling (e.g. CXCR5, PTK2, TLN1) potentially supporting CLL cells long survival. For this reason, we used this model to test the response to drugs and observed an increased resistance of 3D-bioprinted CLL cells to both chemotherapy (n = 7) and targeted therapy (n = 3) compared to 2D counterparts. These findings led us to reason about the mechanical characteristics of the 3D model, thus, we performed a dynamic mechanical analysis on the scaffold, showing that the latter exhibits a stiffness between 5–8 kPa, similar to the one present in lymphoid organs (Qin et al. 2020). Therefore, we hypothesized that physical cues may influence CLL cells behavior through a mechanotransduction mechanism. To test this hypothesis, we cultured CLL cells in a controlled 2D culture system using PDMS-coated plates with tunable stiffness (from 0,2 to 64 kPa). Cells proved to be viable in all the conditions and, after optimizing coating and timing of adhesion, we selected three stiffness values (0.5, 2, and 8 kPa) showing stronger variation in selected marker expression for further investigation. Following 1 hour of CLL cells adhesion to the substrate, functional kinome profiling revealed an enhanced activity of kinases involved in the ERK, PI3K/AKT, and mTOR pathways at higher stiffness (8 kPa), with concurrent upregulation of the VLA-4 antigen, suggesting upstream activation triggered by a physical signal. In parallel, we observed modulation of kinases in the Ephrin family (EPHA2 and EPHB2), a group of receptor tyrosine kinases known to interact with an intricate network of pathways affecting cancer cells' growth, migration, and invasiveness (Pasquale et al. 2010). Further experiments are ongoing to validate these pathways and to confirm their role in response to mechanical stimulation and therapy in both primary CLL cells and healthy B lymphocytes. Alongside, we are optimizing the bioprinting of hydrogels with tunable rigidities to translate findings from 2D to 3D contexts and perform drug testing in controlled stiffness settings.

**Conclusions:** We demonstrate that the adaptation of CLL cells to new culture settings reflects a different response to therapies and that this may be due to mechanical stimuli from the microenvironment. Functional analysis showed that stiffness influences the behaviour of CLL cells, and the pathways involved in this response are currently under investigation to confirm their prospect as mechanosensitive mechanisms, dissecting their potential involvement in CLL pathogenesis. These findings support the generation of more complex in vitro models, including the microenvironment and controlled mechanical settings, for a deeper and more reliable investigation of the mechanisms that drive CLL onset, progression, and relapse in vivo.

**Keywords:** Leukemia; 3D models; mechanobiology

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# Abstract ID: 1017

## **Title:** Long non-coding RNA LINC00173 regulates BCR signaling propensity and mRNA translation via ribonucleoprotein granules in CLL cells

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**Background:** Short non-coding RNAs, namely microRNAs, are well known to influence CLL biology, but the role of long non-coding RNAs (lncRNAs) remains unclear. This is largely due to lncRNAs' complex mechanisms of action depending on interaction partners (DNA/RNA/proteins).

**Results:** To screen for lncRNAs involved in microenvironmental interactions, we identified lncRNAs differentially expressed in sorted intraclonal CLL subpopulations recently activated in immune niches vs. resting cells (CXCR4dimCD5bright vs. CXCR4brightCD5dim, n = 10 pairs) and in CLL cells obtained before and during ~1–3 months of BCR-inhibitor treatment (ibrutinib n = 5; idelalisib n = 10). Using this strategy, we identified 7 intergenic lncRNAs with changed expression after CLL activation and BCR inhibition. Based on manual inspection of gene annotation, intergenic LINC00173 was selected for further studies, also considering its association with prognosis of multiple tumors (May-Hau et al.,2022). LINC00173 was induced (6.7-fold) in CLL samples by BCR activation in vitro (bead-bound anti-IgM, n = 9, 72 hours; $p = 0.003$), and this was blocked by ibrutinib or idelalisib treatment. To test its function, we generated MEC1 cells with LINC00173 knockdown (KD) using CRISPR-interference (3 sgRNAs vs. control sgRNA). We hypothesized that LINC00173 might regulate BCR signal propensity and/or CLL activation. Indeed, LINC00173 KD enhanced the MEC1 cells' responsiveness to BCR crosslinking (anti-IgM, Ca2+ flux assay, n = 5, $p < 0.001$), and the magnitude was comparable to manipulating proteins forming BCR signalosome (SYK, GAB1). The analysis of CLL transcriptomes (ICGC-CLL) revealed significantly higher BCR activity (gene signatures score) in CLL samples with lower vs. higher LINC00173 levels (n = 57 vs. 57, $p = 0.04$). Additionally, CLL patients with lower LINC00173 expression had shorter OS in our CLL cohort (n = 100 [chemoimmunotherapy era]; $p = 0.04$, median OS: 9.7 vs. 16.8 years), and in ICGC-CLL dataset (n = 172). Interestingly, CLL cells (n = 18) exhibit ~3-fold lower LINC00173 levels than peripheral blood B cells from healthy donors (n = 5; $p = 0.01$). Altogether, this demonstrates that LINC00173 is induced by BCR and also limits BCR signaling propensity. To further dissect the LINC00173 functions, we conducted mRNA profiling in the MEC1 KD/control cells, revealing differential expression of 470 mRNAs (n = 3, fold-change ≥1.5; Padj< 0.05). This included repression of 'negative regulation of lymphocyte activation' in KD cells (NES=-0.42; Padj =0.01), such as PTEN and PTPN22 phosphatases. Moreover, this also revealed the upregulation of 87 mitochondrial genes (NES =2.6; Padj =1.5e$^{-}$10). However, in contrast to transcriptomic data, MEC1 KD cells had reduced ($p < 0.05$) basal mitochondrial respiration (-25%) and maximal respiration capacity (-40%; n = 6, Seahorse assay). These results suggest a compensatory transcriptional response to a functionally deficient metabolic capacity. To understand this, we performed an RNA pulldown (20 probes binding LINC00173) followed by mass spectrometry, which revealed 23 proteins interacting with LINC00173 (n = 3, $p < 0.05$). Notably, this included 5 proteins known to interact and form ribonucleoprotein (RNP) granules involved in mRNA translation control (G3BP2, MOV10, RALY, IGF2BP3, EIF4E), but no proteins annotated as BCR regulators. In line with this, MEC1 KD cells had a higher amount of RNP granules (~3-fold, $p < 0.001$) when exposed to stress conditions (0.5 mM sodium arsenite) and ~65% reduction in de novo protein synthesis in comparison to control cells (SunSET assay; n = 4, $p < 0.001$). Collectively, this suggests that LINC00173 induction by BCR allows for increased protein synthesis via modulating RNPs formation. Next, we investigated LINC00173's impact on normal B cell fate. We have generated a germline knockout (KO) of mouse LINC00173 ortholog (Gm30604). Gm30604-/- homozygous mice are viable, fertile, and macroscopically normal. The 12-week-old Gm30604-/- mice contained ~20% less B cell numbers in the bone marrow (-24% proB, -13% PreB, -32% immature, -31% recirculating B cells) and 9% less splenic B cells compared to wild-type mice ($p < 0.01$, n = 10 KO vs. 10 WT). Intriguingly, Gm30604-/- mice had an expansion of peritoneal B1b cells (42% increase, $p = 0.01$). This is in line with increased BCR signaling in B1 cells and decreased metabolic/protein synthesis capacity or elimination of BCR-overreactive bone marrow B cells. The KO mice phenotype is under investigation, including the evaluation of any B cell malignancy development with aging.

**Conclusion:** We have identified the first lncRNA that regulates BCR signaling propensity and impacts protein production capacity after CLL activation. We generated a novel knockout mouse model revealing the impact of

LINC00173 on normal B cell production in the bone marrow and B1 cell expansion in the peritoneum.

**Keywords:** BCR; lncRNA; RNP-granules

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## Abstract ID: 1018

### **Title:** Unraveling the NOTCH1/MSI2/c-MYC signaling pathway reveals a novel vulnerability in chronic lymphocytic leukemia progression

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Gene expression regulation is vital for cellular homeostasis, and its dysregulation is associated with various types of cancers (Rosselló-Tortella, et al., 2020). At the post-transcriptional level, RNA-binding proteins are crucial in controlling gene expression by regulating the temporal, spatial, and functional dynamics of messenger RNAs (mRNA) (Pereira, et al., 2017). Among these proteins, the oncoprotein Musashi2 (MSI2) plays a significant role in these regulatory processes. MSI2 regulates protein translation by binding to consensus sequences of target mRNAs. Interestingly, elevated MSI2 levels have been reported in various cancers, including chronic lymphocytic leukemia (CLL). Our group has identified that MSI2 promotes cell survival and tumor growth of CLL patient cells (Palacios, et al., Leukemia 2021). Later, we proposed a novel regulatory mechanism for MSI2, where the activation of the NOTCH1 signaling pathway induces MSI2 expression through the downregulation of a negative regulator of MSI2, Kruppel-like factor 4 (KLF4). Moreover, the reduction of MSI2 in NOTCH1 independent activated CLL cells results in an up-regulation of proteins associated with cell migration (Querol, et al., iwCLL 2023). Together, we propose that, in an activated tumor microenvironment, NOTCH1 signaling pathway induces MSI2 expression, and that increased MSI2 levels influence cell migration, likely retaining cells within proliferative niches where they receive survival signals and contribute to disease progression. Despite advances in MSI2 research, it is still unknown to which mRNAs MSI2 binds and regulates in B-lymphocytes from CLL cells. Interestingly, in CLL, NOTCH1 signaling induces the transcription of the oncogene c-MYC (Fabbri et al., 2017), a transcription factor that regulates proliferation, apoptosis, differentiation, and metabolism. In addition, in acute myeloid leukemia the MSI2 regulatory pathway has been linked to c-MYC (Minuesa, et al., Nature 2019) and in hepatocellular carcinoma MSI2 has been shown to bind to c-MYC mRNA (Yeh et al., 2023). Based on these, we aimed to determine whether MSI2 regulates c-MYC translation in tumor clones of CLL patients. To answer this, we first determined MSI2/c-MYC protein expression levels in 10 B-CLL samples. Results showed a positive correlation between MSI2/c-MYC expression ($r = 0.47$; $p = 0.029$), where patients with poor outcomes show higher levels of both proteins. For further investigation, B-cells from 13 CLL patients were treated in-vitro with either the MSI2 inhibitor Ro082750 (5 μM) or vehicle for 24 hours, and c-MYC protein levels were assessed by flow cytometry. The results showed that blocking MSI2 function reduced c-MYC protein levels in 12 of 13 samples ($p = 0.0042$). Additionally, we examined the effect of the MSI2 inhibitor on c-MYC expression in TCL1 mice. Animals treated with the inhibitor showed a less number of tumoral cells and a reduction of c-MYC protein levels in CD19+CD5+ cells, further supporting the role of MSI2 in regulating c-MYC. Together, these findings support the interaction between MSI2 and c-MYC in CLL. To document that MSI2 directly binds to c-MYC mRNA, we performed RNA immunoprecipitation on primary cells from 5 CLL samples using an anti-MSI2 antibody and the anti-isotype IgG as negative control. After retro-transcription, c-MYC was amplified by PCR, confirming that MSI2 regulates c-MYC translation by binding to its mRNA in CLL samples. Moreover, because NOTCH1 activates the expression of survival genes, including the transcription of c-MYC, we wonder whether blocking NOTCH1 and MSI2 signaling could enhance c-MYC reduction. To address this, CLL samples ($n = 13$) were treated with both NOTCH1 (DAPT, gamma-secretase inhibitor) and MSI2 (Ro082750) inhibitors. The results showed that blocking NOTCH1 and MSI2 signaling led to a greater reduction of c-MYC levels than a single inhibitor in CLL cells ($p \leq 0.0001$), suggesting a potential strategy for reducing tumor cell viability. In this work, we propose that the NOTCH1/MSI2/C-MYC signaling axis contributes to the proliferation of the leukemic B cells from CLL patients. NOTCH1 induces MSI2 expression by downregulating KLF4 and promotes the transcription of c-MYC. In addition, we observed that MSI2 binds to c-MYC mRNA and positively regulates its translation. Our results provide insights into the regulation of c-MYC in B-cells from CLL patients and suggests that disrupting the NOTCH1/MSI2/c-MYC axis could offer a different targeted therapeutic strategy for certain patients. Confirmatory studies are crucial, and may open new avenues for highly targeted CLL therapies.

**Keywords:** Notch1; Musashi2; c-MYC

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## Abstract ID: 1019

**Title:** Immunological landscape of CLL development and response to early BTK inhibition in del(13q14) mice

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**Background:** The deletion of 13q14 (del(13q14)) is the most prevalent genetic alteration in Chronic Lymphocytic Leukemia (CLL), affecting approximately 55% of patients and associated with favorable prognosis. Deletion of the minimal deleted region (MDR) within del(13q14), encompassing the DLEU2 gene and the miR-15a/16-1 cluster, leads to CLL development in around 40% of mice by 15–18 months (Klein et al., 2010). In patients, clinical progression is marked by increasing T-cell exhaustion and dysfunction. BTK inhibition can reduce immunosuppression and restore T-cell function. Based on these observations, we hypothesized that immune surveillance initially restrains CLL in del(13q14) mice, but progressive immune dysfunction allows disease emergence. We further postulated that early BTK inhibition could modulate immune– CLL interactions, preventing immune escape and overt disease. Our objectives were to elucidate the immune mechanisms driving CLL progression through longitudinal immune profiling, and to assess how early therapeutic intervention with acalabrutinib alters CLL development and immune trajectories in del(13q14) mice.
**Methods:** Two murine cohorts were established: one with 62 del(13q14) mice and 10 wild-type (WT) controls, with monthly blood sampling from 12 months of age to endpoint; and another with 61 del(13q14) mice randomized to acalabrutinib (n = 28, 25 mg/kg daily) or vehicle (n = 33) from 9 months of age (prior to CLL onset), with sampling beginning at 8 months. All samples were analyzed by spectral flow cytometry to monitor immune dynamics. Longitudinal changes in CLL and immune subsets (T, NK, monocytes) were assessed using linear mixed models. Generalized Bayesian trajectory modeling (GBTM) clustered mice by immune trajectory for each population. CLL was defined as >10% $CD5^+B220$^low among $CD19^+$ cells.
**Results:** Untreated del(13q14) mice had shorter survival than WT (median: 20 vs. 25 months, $p = 0.01$). CLL developed in 42% of del(13q14) mice (median onset: 18 months), with leukemic burden increasing over time: 4.5% at 12 months, 11.9% at 20 months, and 27% at 25 months. Although overall T, NK, and monocyte proportions were similar between WT and del(13q14) mice, those developing CLL showed greater increases in regulatory T cells (Tregs; $CD4^+CD25^+$ and $CD4^+CD39^+$), immunosuppressive $CD4^+IL33R^+$ cells, T follicular helper cells (Tfh; $CD4^+CXCR5^+$), and exhausted $CD4^+PD1^+$ T cells compared to non-diseased del(13q14) mice. A more pronounced decrease in $CD4^+$ naïve T cells, along with an increase in effector memory (EM) $CD4^+$ T cells, were also observed in CLL-developing mice from the start of the follow-up period. Similarly, $CD8^+CD244^+$ and $CD8^+PD1^+$ T cells increased in these mice, with early loss of naïve $CD8^+$ T cells and EM expansion. GBTM identified two major immune trajectory clusters per variable. Over 70% of CLL-developing mice followed trajectories with increasing Tregs and a shift from naïve to EM CD8+ T cells (–55% naïve, +20% memory) by endpoint. Acalabrutinib did not significantly affect overall survival but reduced CLL incidence (6% vs. 28% in vehicle-treated mice). Mean CLL cell frequencies remained lower with acalabrutinib: 2% vs. 6% at 12 months; 2% vs. 11% at 19 months; and 3% vs. 13% at 24 months. Mice receiving vehicle showed significantly higher increases in Tregs, Tfh, $IL33R^+$, and $PD1^+$ $CD4^+$ T cells ($p < 0.05$ in all populations during >50% of follow-up). They also exhibited a greater decline in $CD4^+$ naïve T cells, with marked expansion of central memory (CM) and especially EM T cells ($p < 0.01$ from months 12–26). No major differences in $CD8^+CD244^+$ and $CD8^+CD160^+$ T cells were observed between groups, but $CD8^+PD1^+$ levels were significantly higher in vehicle-treated mice from months 17–22. $CD8^+$ naïve T cells declined more rapidly in vehicle-treated mice ($p < 0.05$ from months 11–26), with a significant increase in $CD8^+$ memory cells. Consistently, GBTM showed that 57% of vehicle-treated mice followed trajectories with increasing regulatory/exhausted T cells, ~50% reduction in naïve $CD8^+$, and +20% increase in EM $CD8^+$ cells by endpoint. In contrast, >70% of acalabrutinib-treated mice showed only modest increases in these subsets, and a limited decline in naïve $CD8^+$ cells (–15%).
**Conclusion:** Progressive immune dysfunction facilitates CLL development in del(13q14) mice. Early acalabrutinib treatment prevents CLL in most mice, delays onset in others, and modifies the immune landscape. Although early BTK inhibition has not yet improved OS in patients diagnosed with CLL, our findings suggest that early immunomodulatory strategies could prevent or delay MBL/CLL progression.

**Keywords:** Immune landscape; mouse models; BTK inhibition

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## Abstract ID: 1020

**Title:** KRAS^G13D-dependent PI3K/AKT signaling regulates fatty acid oxidation in chronic lymphocytic leukemia

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**Background:** Chronic lymphocytic leukemia (CLL) is a biologically heterogeneous malignancy characterized by altered metabolic dependencies, including an increased reliance on fatty acid oxidation (FAO). While the oncogenic role of KRAS mutations is well-established in solid tumors,

their functional impact in CLL remains unclear. The G13D mutation in KRAS (KRAS^G13D) is particularly understudied in hematologic malignancies. The present study aimed to elucidate the functional consequences of KRAS^G13D in CLL pathogenesis, focusing on its regulation of fatty acid metabolism via the PI3K/AKT/mTOR pathway, and to assess the therapeutic efficacy of co-targeting KRAS and PI3K.
**Methods:** KRAS mutation patterns were analyzed in 3,205 CLL patients who underwent whole-genome or next-generation. Functional studies were performed by transfecting CLL cell lines (MEC1, EHEB) and primary CLL cells with KRAS^WT or KRAS^G13D plasmids. Cell proliferation, FAO, and mitochondrial respiration were evaluated with functional assays. Molecular mechanisms were investigated using transcriptomic profiling, qRT-PCR, Western blotting, and bioinformatics. A direct protein interaction was validated through molecular docking and co-immunoprecipitation. Drug sensitivity to KRAS (BI-2865) and PI3K (MIPS-21335, idelalisib) inhibitors was tested to assess combinatorial treatment strategies.
**Results:** Among 3,205 CLL patients, 119 (3.71%) harbored KRAS mutations. Notably, KRAS^G13D accounted for 29.13% of all KRAS-mutant cases, making it the most prevalent variant. Functional assays demonstrated that KRAS^G13D significantly enhanced proliferation in MEC1, EHEB, and primary CLL cells compared to KRAS^WT controls ($p < 0.05$). KRAS^G13D-expressing cells also exhibited increased sensitivity to the pan-KRAS inhibitor BI-2865, with dose-dependent suppression of proliferation. Transcriptomic and biochemical analyses revealed that KRAS^G13D upregulated key fatty acid oxidation genes, including CPT1A, CPT2, and ACOX1, while increasing cellular oxygen consumption and FAO activity ($p < 0.01$). Mechanistically, KRAS^G13D induced significant upregulation of the class II PI3K gene PIK3C2A, with concurrent activation of AKT and mTOR signaling components at both total and phosphorylated protein levels. Bioinformatic enrichment highlighted strong associations with lipid metabolism and PI3K binding pathways. Molecular docking and Co-IP studies confirmed a direct interaction between KRAS^G13D and PI3K-C2α, establishing a mechanistic link to downstream PI3K/AKT/mTOR pathway activation. Pharmacologic inhibition of KRAS^G13D suppressed expression of PI3K-C2α, *p*-AKT, and *p*-mTOR, corroborating its central role in signaling activation. KRAS^G13D mutant cells exhibited relative resistance to PI3K inhibitors MIPS-21335 and idelalisib. However, co-treatment with BI-2865 restored sensitivity to PI3K inhibition, producing a synergistic anti-proliferative effect that overcame resistance in mutant cells. This therapeutic synergy was consistent across both CLL cell lines and primary patient samples.
**Conclusions:** The KRAS^G13D mutation represents a functionally significant driver in a subset of CLL patients, promoting disease progression through enhanced fatty acid oxidation and activation of the PI3K/AKT/mTOR axis. Our findings establish a direct mechanistic link wherein KRAS^G13D binds and upregulates PI3K-C2α, thereby connecting oncogenic signaling with metabolic reprogramming. The present study provides a compelling rationale for the co-inhibition of KRAS and PI3K as a precision therapy to overcome drug resistance in patients with KRAS^G13D-mutant CLL.

**Keywords:** Chronic lymphocytic leukemia; KRAS mutation; PI3K/AKT signaling pathway; fatty acid metabolism

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## Abstract ID: 1021

**Title:** Normal B cells in MBL show abnormal transcriptomic and subset distributions compared to healthy donors: evidence for follicular maturation in IGHV-mutated MBL and extrafollicular maturation in IGHV-unmutated MBL

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**Background:** People with CLL and monoclonal B-cell lymphocytosis (MBL), the precursor to CLL, exhibit immune dysfunction. Since in MBL there are more normal B cells (NBC), it provides an opportunity to study B-cell defects.
**Aims:** Compare the transcriptomes of NBC from healthy donors (NBC-HD) with those from MBL patients (NBC-MBL), including $CD5^-$ and $CD5^+$ subsets of IGHV-mutated (M-MBL) and unmutated (U-MBL) patients; Analyze B-cell subset distribution in the NBC of MBL and HD.
**Methods:** PBMCs were collected from 18 high-count MBL (hcMBL) patients (12 M-MBL, 6 U-MBL) and 13 HD, and 99 FACS-purified fractions were obtained: 21 MBL clones ($CD20LowCD5^+Ig\kappa^+/Ig\lambda^+$), 36 NBC-MBL (30 $CD20BrightCD5^-$; 6 $CD20BrightCD5^+$), and 42 NBC-HD (25 $CD20BrightCD5^-$; 17 $CD20BrightCD5^+$). RNA from these was sequenced using SMART-Seq v4 Ultra-Low-Input and HiSeq platform. DESeq2 was used to analyze RNA-seq data. Differentially expressed genes (DEGs) were defined by Padj< 0.05 and $|FC| \geq 1.5$. PCA was used to assess transcriptome variance, and IPA to identify enriched pathways. Protein expression of DEGs was validated by flow cytometry in 6 hcMBL and 8 HD. For dissection of B-cell subset types, PBMCs from 14 hcMBL (10 M-MBL and 4 U-MBL) and 9 HD were studied. Data were acquired on a FACSymphony.
**Results:** PCA of all B-cell fractions distinctly separated NBC-HD, NBC-MBL, and MBL clones. Within NBC-MBL, $CD5^+$ NBC and NBC-U-MBL clustered closer to MBL clones. Comparing $CD5^-$ NBC-M-MBL to NBC-HD revealed 8,380 DEGs. IPA predicted inhibition of 95% of Summary Graph pathways (20/21),

including B-cell activation and proliferation. Canonical pathway analysis indicated upregulation (Z-score ≥2) of mismatch repair (MMR) and base-excision repair (BER), typically found in NBC undergoing follicular responses when CSR and SHM occur. $CD5^-$ NBC-U-MBL vs. NBC-HD showed 12,793 DEGs, a larger difference, consistent with PCA showing NBC-U-MBL farther from NBC-HD. IPA predicted activation of 88% of Summary Graph pathways (23/26); these included Cell cycle progression and Migration. Inflammation-related Canonical pathways such as Pathogen-induced cytokine storm, IL-8, Chemokine, and IFNG signaling, were all exclusively upregulated in NBC-U-MBL, indicating augmented inflammation specific to this group. Comparing NBC-U-MBL and NBC-M-MBL showed the fewest differences among comparisons (1,316 DEGs), consistent with both groups being NBC-MBL. Nevertheless, IPA identified six significantly upregulated (Z-score ≥2) Canonical pathways in NBC-U-MBL: Cytokine storm, NET signaling, S-phase, Cyclophilin signaling, RIPK1-mediated necrosis, and Fibrin clot dissolution. Note that NETosis can occur in B cells (Bukhari 2023). These pathways converge in the immune response, connecting pathogen sensing, inflammation (cytokine storm, NETs), cell proliferation (S phase), stress signaling (cyclophilin), and inflammatory cell death (RIPK1). This exclusive activation in NBC-U-MBL could foster rapid, uncontrolled inflammation that typically drives extrafollicular B-cell responses. Flow cytometry analyses of selected proteins validated significant overexpression of 10 DEG measurements. Phenotypically, NBC-MBL had reduced naïve B cells ($IgM^+IgD^+CD27^-$) and increased $CD27^+$ memory B cells compared to NBC-HD (32.8% vs. 76%, $p<0.0001$; 34% vs. 9.1%, $p=0.0003$). ABC ($T\text{-}bet^+CD11c^+CD21^-$) and DN ($IgD^-CD27^-$) B cells were also elevated among NBC-MBL (0.9% vs. 0.2%, $p=0.0492$; 11.1% vs. 4.7%, $p=0.0001$). Subgroup analysis by IGHV mutation status revealed distinct memory B-cell profiles. NBC-M-MBL uniquely showed increased DN1 cells ($IgD^-CD27^-CD38^+CD21^+CD24^+$, 3.3% vs. 1.2%, $p=0.0459$), which are precursors to switched memory B cells, and expanded switched memory B cells ($IgM^-IgD^-CD27^+$; 26.4% vs. 5%, $p=0.0076$). On the contrary, only NBC-U-MBL displayed increased percentages of unswitched memory B cells compared to NBC-HD ($IgM^{++}IgDLowCD27^+CD21^+CD24^+$; 8.6% vs. 1.7%, $p=0.0182$). NBC-U-MBL also exhibited higher percentages of DN2/3 cells ($IgD^-CD27^-CD21^-$) compared to NBC-HD (6% vs. 0.7%, $p=0.0028$) and NBC-M-MBL (6% vs. 2.1%, $p=0.0240$). DN2 and DN3 cells are precursors to antibody-secreting cells.

**Conclusions:** NBC-MBL exhibit transcriptomes that lie between NBC-HD and MBL clones, suggesting a global B-cell defect potentially shaped by genetic and/or environmental factors. This defect affects both $CD5^+$ and $CD5^-$ NBC-MBL and is present in M-MBL and U-MBL. Follicular maturation signatures (BER and MMR) and increased follicular B-cell subsets (switched memory and DN1 cells) characterize $CD5^-$ NBC-M-MBL. $CD5^-$ NBC-U-MBL exhibit inflammatory pathway activation and extrafollicular unswitched and DN2/3 memory subsets. These results align with IPA Summary Graph pathways showing inhibition in NBC-M-MBL, and activation in NBC-U-MBL, supporting a low/controlled inflammation in M-MBL (typical for follicular responses), and high/uncontrolled inflammation in U-MBL (typical in extrafollicular responses). BCR repertoire analysis to verify follicular versus extrafollicular maturation is ongoing.

**Acknowledgments:** GB was supported by the Lymphoma Research Foundation.

**Keywords:** Monoclonal B-cell lymphocytosis (MBL); normal B cells; immune deficiency

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**Please indicate the name of the funding organization:**

# Abstract ID: 1022

## **Title:** Unravelling the clinical and biological features of accelerated chronic lymphocytic leukemia

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**Introduction:** Accelerated chronic lymphocytic leukemia (aCLL) is a rare and understudied form of chronic lymphocytic leukemia (CLL) with aggressive histological features, considered to fall biologically between typical CLL and Richter's transformation (RT). We conducted a multicenter retrospective study to shed light on the clinical and biological characteristics of aCLL in relation to CLL and RT.

**Methods:** We enrolled patients with a histologically proven diagnosis of aCLL according to the criteria published by Giné et. al in 2010. Clinical and biological information were extracted from the medical records and pathology reports across participating institutions. Two cohorts of patients with histologically proven diagnoses of CLL and RT treated at the University Hospital of Padua were enrolled as control groups in order to compare them with aCLL patients. Moreover, we performed NGS analysis using a 108-gene panel (Sophia lymphoma solution by NextSeq) and scRNA-seq (at least 10.000 nuclei/sample, 150 bases per end by NovaSeq) on FFPE lymph-node tissues from 12 patients (n = 7 aCLL, n = 2 RT, n = 3 CLL) and 16 patients (n = 10 aCLL, n = 3 RT, n = 3 CLL) respectively. For time-to-event outcomes, overall survival (OS) was calculated as the time from aCLL diagnosis until death or last follow-up, and time-to-next treatment from the time at which the first therapy for aCLL was started until the next line of therapy or death.

**Results:** Among 244 patients enrolled, 73 (30%) had aCLL, 16 (7%) RT, 155 (63%) CLL. Median age was 68 years (range: 39–88), 94 patients (39%) were female and 150 (61%) were male, with no significant differences in terms of age or sex distribution between the three groups. Among aCLL patients, 20 (27%) were diagnosed de-novo with aCLL, while 53 (73%) evolved from CLL after a median time of 79 months (range: 1.4–267). Compared to CLL, aCLL patients more frequently presented extranodal involvement (27% vs. 8%; $p < 0.001$), lymphadenopathies >5cm (41% vs 5%; $p < 0.001$), constitutional symptoms (26% vs 10%; $p = 0.002$), and had a higher CLL-IPI scores (median 5 vs 4; $p = 0.02$). A total of 77 (32%) patients also had PET/CT data available, with aCLL displaying a higher SUVmax than CLL (median 7.3 vs. 4.0; $p < 0.001$), but still lower than in RT (median 15.4; $p = 0.01$). Similarly, Ki-67 expression was higher in aCLL than CLL (median 14% vs. 6%; $p < 0.001$) but much lower than in RT (70%; $p < 0.001$). Both aCLL and RT were enriched in patients with unmutated IGHV status (81% and 86% respectively vs. 54%; $p < 0.001$), and aCLL also had a significantly higher frequency of TP53 disruption (del17p and/or TP53 mutations) (36% vs. 20%; $p = 0.01$), and a higher median number of karyotype abnormalities (3 vs. 1; $p = 0.001$) compared to CLL. There were instead no differences between the three groups regarding the frequency of del(11q), del(13q) and trisomy 12. RT patients had the higher rate of NOTCH1 mutations (44% vs. 31% in aCLL and 12% in CLL; $p = 0.03$). NGS analysis of aCLL lymph-node tissue revealed recurrent mutations in TP53, BRAF, and ITPKB genes (each one present in 16% of the analyzed patients), but also in CIITA, IRF4, SF3B1, NRAS, PLCG1, and PTPRD. scRNA-seq data demonstrated that aCLL samples were enriched in cells expressing a proliferative transcriptional signature, likely corresponding to histologic proliferation centers. The OS of patients with aCLL was significantly shorter than CLL patients (5-year OS 64% vs. 74%; $p = 0.03$) but longer than those with RT (median 7.5 months; $p < 0.001$). Among aCLL patients, 48 (66%) were treated with targeted agents (n = 28 BTKi, n = 11 venetoclax, n = 7 ibrutinib-venetoclax, n = 1 BTK-degraders, n = 1 idelalisib), 13 (18%) received chemoimmunotherapy (CIT), while 12 (16%) required no treatment. Patients with aCLL receiving first line treatment with targeted agents experienced longer TTNT compared with those receiving CIT (median NR vs. 47 months; $p = 0.02$).

**Conclusion:** This multicenter study highlights aCLL as a clinically and biologically distinct, aggressive entity with features intermediate between CLL and RT. aCLL is enriched in adverse molecular alterations and proliferative subclones, contributing to its distinct histology and inferior outcomes relative to CLL. Our findings also suggest that targeted therapies may provide superior disease control compared with CIT in aCLL, warranting further prospective validation.

**Keywords:** Accelerated chronic lymphocytic leukemia; singe-cell RNA sequencing; NGS

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## Abstract ID: 1023

### Title: Clinical and biological impact of MYC overexpression in chronic lymphocytic leukemia with and without TP53 disruption

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**Introduction:** The activation of the MYC pathway as well as loss-of-function alterations in TP53 are well-established drivers associated with poor prognosis in chronic lymphocytic leukemia (CLL) and with higher risk of Richter Transformation (RT). Despite their individually key roles, the biological consequences of the concurrence of MYC overexpression and TP53 dysfunction in CLL and how both alterations could contribute to RT remain poorly understood. In this study, we investigated MYC activation in the presence or absence of TP53 alterations and analyzed their clinical and biological impact by the integration of transcriptomic analyses and functional assays in patient CLL samples and in genetically engineered CLL cell models.

**Methods:** We analyzed 566 CLL patients from the CLLmap database with clinical and transcriptomic data. The patients were grouped according to MYC expression using the MaxStat algorithm, and the presence or absence of TP53 alterations (deletion and/or mutation). The prognostic impact of these alterations was assessed in terms of overall survival (OS) and time to first treatment (TFT). Moreover, differentially expressed genes (DEG) and functional enrichment analysis (GSEA) were performed.

We used CRISPR/Cas9-SAM genome editing, with three different sgRNA to create biological replicates, in order to generate cellular models with MYC overexpression (MYC-OE) in two CLL cell lines: MEC1, which harbors TP53 alterations (TP53-affected), and PGA, which harbors wild-type TP53 (TP53-WT). MYC-OE was validated by western blot, immunofluorescence and qPCR. Transcriptomic analysis by RNA-seq was conducted to evaluate the impact of MYC overexpression in both cell lines, followed by DEG and GSEA analyses. Biological mechanisms were further assessed by western blot, qPCR and cellular proliferation assays.

**Results:** In the group of patients with TP5-WT, higher MYC expression exhibited a poorer prognosis in terms of both OS and TFT ($p = 0.006$; $p = 0.018$, respectively). In addition, CLL patients with TP53-affected/MYC-OE were not shown shorter

OS or TFT. Furthermore, CLL patients with TP53-WT/MYC-OE exhibit OS and TFT similar to CLL cases with TP53-affected. Biologically, MYC overexpression induced deregulation of MYC targets in both cell lines as we observed in patients and further validated by qPCR (including CCNE1, MCL1 or NCL) in cellular models. Moreover, other pathways were deregulated such as the G2/M checkpoint and NFKB and NOTCH signalings. Consistently, both MYC-OE cell lines (MEC1-TP53-affected/MYC-OE and PGA-TP53-WT/MYC-OE) showed increased cellular proliferation compared to their respective WT, being more significant within TP53-WT background). In addition, we also revealed differences in the expression and activation of Akt, mTOR, and ERK, which could be in line with the altered biological pathways detected by RNA-seq and the prognosis of patients. Interestingly, MEC1-TP53-affected/MYC-OE, revealed enrichment of DNA repair pathway that might be linked to the genomic instability associated with RT.

**Conclusions:** CLL patients with TP53-WT and MYC-OE exhibited a worse prognosis than those with low MYC expression, but with a similar disease course to patients with TP53 affected. This could be related to significant transcriptomic changes observed both in patients and in MYC-OE cellular models, involving altered biological pathways and genes that was associated with increased proliferation.

**Keywords:** CLL; CRISPR; MYC

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## Abstract ID: 1024

### **Title:** ROR1-signaling enhances survival and drug-resistance of del(17p)/TP53-mutated CLL by mitigating oxidative stress via activation of NRF2

**Authors:** Emanuela Ghia[a], Can Huang[a], Michael Choi[a], George Widhopf II[a] and Thomas Kipps[b]

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Deletion of chromosome 17p (del[17p]), a high-risk genomic aberration in chronic lymphocytic leukemia (CLL), results in loss of one TP53 allele, with somatic mutations in the retained allele occurring in >80% of cases. This dual TP53 inactivation drives genomic instability, associates strongly with complex karyotype (CK; ≥3 chromosomal abnormalities), and confers increased risk for resistance to targeted therapies such as Bruton's tyrosine kinase inhibitors (BTKis). Analysis of pretreatment CLL cells from 493 untreated patients revealed del(17p) and/or CK are significantly enriched in cases with high ROR1 expression (ROR1Hi; $\chi^2$ $p<0.01$). ROR1, an oncofetal receptor for Wnt5a, activates pro-survival pathways (ERK1/2, NF-κB, NRF2) and correlates with inferior outcomes in CLL. To investigate ROR1's role in del(17p) CLL, we employed MEC1 cells—a CLL-derived line harboring monoallelic del(17p) and a frameshift TP53 mutation (Q317fs*29)—which express Wnt5a but lack ROR1. Using CRISPR-Cas9, we generated WNT5A knockout (MEC1Wnt5a−/−), TP53 knockout (MEC1TP53−/−), and SQSTM1 (p62) knockout (MEC1p62−/−) variants, then transduced these lines with ROR1 to create MEC1-ROR1, MEC1Wnt5a−/−-ROR1, MEC1TP53−/−-ROR1, and MEC1p62−/−-ROR1. In MEC1-ROR1 cells, ROR1 signaling upregulated NRF2 target genes (FDR q=0.07–0.002) and cell-cycle control genes (FDR q < 0.001), including CCNA1 (cyclin A1), which regulates G1/S and G2/M transitions and DNA double-strand break repair. Compared to controls (MEC1, MEC1Wnt5a−/−-ROR1), MEC1-ROR1 cells exhibited elevated NQO1 ($p=0.005$–$0.001$), mutant p53 protein ($p=0.001$–$0.004$), p62 ($p=0.004$–$0.002$) and cyclin A1 ($p=0.003$–$0.001$). ROR1 expression also enhanced resistance to eprenetapopt (APR-246), a compound that depletes glutathione and elevates reactive oxygen species (ROS), such effect is abolished in p62-deficient cells, confirming p62's role in NRF2 activation. While TP53 knockout increased APR-246 resistance in MEC1 and MEC1Wnt5a−/−-ROR1, this effect was absent in MEC1-ROR1, suggesting ROR1 signaling compensates for TP53 loss. In primary ROR1+ del(17p) CLL cells, Wnt5a increased NQO1, cyclin A1, and mutant p53 ($p\leq0.02$), effects blocked by zilovertamab, a humanized anti-ROR1 antibody. These findings were recapitulated in vivo, where zilovertamab treatment suppressed NRF2 target and cell-cycle genes in patient CLL cells (FDR q < 0.001 at day 28 vs. baseline). Mechanistically, ROR1 signaling stabilizes mutant p53 via NRF2-driven NQO1, a chaperone that prevents proteasomal degradation of conformationally unstable p53 mutants. Cyclin A1, transcriptionally activated by both wild-type and mutant p53, facilitates cell-cycle progression and DNA repair, enabling del(17p) CLL cells to tolerate genomic stress. This axis creates a survival niche: NRF2 activation mitigates ROS (e.g. via glutathione synthesis), while cyclin A1 promotes replication despite DNA damage. Consequently, ROR1Hi del(17p) CLL cells evade APR-246's oxidative stress–inducing effects, a vulnerability restored by ROR1 inhibition. Clinically, this pathway explains the enrichment of del(17p)/CK in ROR1Hi cohorts and their propensity for BTKi resistance, as persistent cycling and stress adaptation foster clonal evolution. Therapeutic targeting of ROR1 disrupts this survival network. Zilovertamab, by blocking Wnt5a-ROR1 interactions, suppresses NRF2 activity, reduces NQO1/cyclin A1, and destabilizes mutant p53, sensitizing cells to ROS-inducing agents like APR-246. In ROR1Hi del(17p) CLL cells, zilovertamab reversed Wnt5a-mediated resistance ($p=0.0003$), while in vivo data showed reduced NRF2/cell-cycle pathway activation in treated patients. These results position ROR1 as a linchpin in del(17p) CLL biology, linking oncogenic signaling (TP53 mutation, CK) to therapeutic resistance. By preserving mutant p53 and enabling proliferation under genotoxic stress, ROR1Hi cells evade apoptosis and acquire mutations that drive relapse. In conclusion, ROR1 signaling sustains del(17p) CLL through a NRF2-NQO1-cyclin A1 axis that stabilizes mutant p53, promotes cell cycling, and confers oxidative stress resistance. This pathway underpins the association between ROR1Hi expression, CK, and poor outcomes, while zilovertamab emerges as a strategy to counteract these mechanisms. Combining ROR1 inhibition with BTKis or ROS-inducing therapies (e.g. APR-246) could mitigate resistance in high-risk CLL, offering a dual-pronged approach to target both survival signaling and genomic instability.

**Keywords:** Chronic lymphocytic leukemia; ROR1; NRF2; p53; deletion 17p

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## Abstract ID: 1101

**Title:** An immuno-(epi)genomic classification of chronic lymphocytic leukemia refines outcome prediction

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Immunogenetic features, such as IGHV and IGLV3-21R110 (R110) mutational status, and B-cell receptor (BcR) stereotypy, along with epigenetic features, such as the 3 epitypes (naïve-like, n-CLL; intermediate, i-CLL; and memory-like, m-CLL), contribute to the pathogenesis of chronic lymphocytic leukemia (CLL). However, the interplay between them remains largely unexplored, primarily due to the scarcity of matched data. Here, we report a comprehensive analysis of a cohort of 995 patients enriched in cases harboring the R110 mutation (n = 99) and the most frequent stereotyped subsets (#1–8, n = 180). We systematically profiled their DNA methylome (Illumina arrays, n = 995), chromatin landscape (H3K27ac-ChIP-seq, n = 99; ATAC-seq, n = 101), transcriptome (RNA-seq, n = 285) and correlated with time to first treatment (TTFT) and overall survival (OS) (n = 885) from diagnosis. Unsupervised analyses revealed that both subset and non-subset cases exhibited a similar DNA methylome, suggesting that stereotypy alone is not a major source of methylation variability. Nonetheless, poor-prognostic subset #2 and non-stereotyped cases carrying the R110 clustered together, indicating that the R110 mutation rather than subset #2 assignment was the molecular feature separating the i-CLL cases into 2 subgroups. Remarkably, i-CLL-R110 cases demonstrated a significantly higher ($p = 0.006$) proliferative history measured by the epiCMIT score. Furthermore, i-CLL-R110 cases showed 316 differentially methylated CpGs, of which 154 were hypomethylated and located in regulatory regions (H3K27ac and ATAC-seq peaks). Next, we used these 316 CpGs to develop (n = 631/995) and validate (n = 364/995) an epigenetic classifier that led to the classification of all CLL patients into two immuno-(epi) genomic clusters, named IEC1 and IEC2. IEC1 cases predominantly included IGHV-unmutated (U-CLL) and related U-CLL subsets as well as n-CLL and R110-mutated cases. Conversely, IEC2 cases included mainly IGHV-mutated (M-CLL), subset #4, m-CLL and i-CLL lacking the R110 mutation. However, we observed around 10% (84/884) of discordant cases compared to IGHV subgrouping, with 4% of all U-CLL (14/401) classified as IEC2, and 14% of all M-CLL (70/483) classified as IEC1. Considering both IGHV and R110 mutational status, the discordant cases were reduced to 3% (20/794, 5 M-CLL as IEC1, and 15 U-CLL as IEC2). Subsequently, we performed a multi-omic analysis comparing both the newly defined clusters or IGHV subgroups. The clusters showed 5,011 differentially methylated CpGs, of which 476 displayed patterns independent of IGHV or epitype subgroups. Of these, 230 CpGs were hypomethylated and strongly enriched in regulatory regions, potentially contributing to the pathogenesis of IEC1 cases. In fact, we identified associations with TFAP2A, E2F, SP1 and MAZ transcription factor (TF) family members. From the chromatin perspective, we found 4,423 and 1,051 regions with differential chromatin accessibility (ATAC-seq) and activity (H3K27ac-ChIP-seq), respectively. Differential chromatin regions were predominantly related to higher accessibility/ activation in IEC1 and targeted genes related to CLL pathogenesis such as MSI2 or TP63, and others less explored like GFI1 or TFAP2A. At the transcriptomic level, 923 genes were differentially expressed between clusters, with the majority overexpressed in IEC1 cases and overlapping with chromatin profiling results. IEC1 cases were enriched in gene signatures related to metabolism, cell cycle, NF-KB and WNT signaling, along with TF regulomes associated with these pathways, including SP1, NF-KB, TP53, TFAP2A, MYC or EGR1. Importantly, integrating all data types uncovered ~20% more differential features compared to IGHV subgroups, with discordant cases resembling cases of their cluster assignement and not their immunogenetic classification. Clinically, IEC1

cases were associated with a markedly shorter TTFT and OS, regardless of their immunogenetic and epitype subgroups, whereas C2 cases were independently associated with a longer TTFT ($p$ = 4e-89) and OS ($p$ = 4e-36). Akaike information criterion (AIC) demonstrated that clusters showed a better model performance than IGHV and R110 mutational status (for TTFT, 5,035 vs 5,069 and for OS, 2,892 vs 2,918, respectively), which became even more significant when including also epitypes and the epiCMIT (TTFT =5,001 and OS =2,880). Collectively, our DNA methylation analyses identify two novel immuno-(epi)genetic clusters, reflecting mainly IGHV and R110 mutational status together with epitype subgroups. We also identify discordant patients exhibiting molecular and clinical features that align more closely with their cluster assignment than with their immunogenetic status. These clusters, together with epitypes and the epiCMIT proliferative history score appear to refine the prognostic power of IGHV and R110 mutational status, and can be detected with a single DNA methylation assay.

**Keywords:** Immunogenomics; epigenetics; clinics

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## Abstract ID: 1102

### **Title:** Unravelling prognostic significance: long-read nanopore sequencing of TP53 and IGHV in chronic lymphocytic leukaemia patients in tanzania

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Chronic lymphocytic leukemia (CLL) displays substantial clinical heterogeneity, necessitating molecular biomarkers for accurate risk stratification and therapeutic guidance. Mutations in the TP53 gene and the somatic hypermutation status of the immunoglobulin heavy chain variable region (IGHV) are among the most reliable prognostic markers (Zenz et al., 2010; Malcikova et al., 2018). However, resource-constrained settings often lack access to advanced molecular diagnostics. This study evaluates the feasibility and utility of Oxford Nanopore long-read sequencing to detect TP53 mutations and IGHV rearrangements in Tanzanian CLL patients, addressing an urgent need for region-specific genomic insight. Thirty-one treatment-naïve CLL patients were recruited from Muhimbili National Hospital in Tanzania. Genomic DNA was extracted from peripheral blood. TP53 exons 1–11 and the IGHV locus were PCR-amplified using barcoded primers. Sequencing was performed on a MinION Mk1C platform. A bioinformatics pipeline (Minimap2, Samtools, VarScan, Sniffles, Annovar) was employed for alignment, variant calling, and annotation (Li, 2018; Koboldt et al., 2012). IgHV sequences were aligned and analyzed per European Research Initiative on CLL (ERIC) recommendations (Stamatopoulos et al., 2007). We detected 201 TP53 variants, 74% of which were intronic. After filtering, seven nonsynonymous SNVs were identified in the coding region, including four pathogenic variants: p.Arg249Gly (variant allele frequency [VAF] 67%), p.Arg273Cys (VAF 38%), p.Arg306* (VAF 10%), and p.Gly244Val (VAF 13.5%). The p.Arg249Gly variant was confirmed using an Illumina Myeloid panel with a concordant VAF of 56%. Although p.Arg273Cys was not validated by the panel, its detection at subclonal frequency highlights the capacity of long-read sequencing to identify minor leukemic populations, which may drive clonal evolution (De Luca et al., 2022). IgHV status revealed 10 patients with unmutated sequences (U-CLL) and 2 with mutated sequences (M-CLL), reflecting a predominance of high-risk disease. Frequent IgHV gene usage included IGHV1-69, IGHV3-21, and IGHV3-23, consistent with patterns reported in other populations (Forconi & Potter, 2004). This study demonstrates the feasibility of long-read sequencing for comprehensive genomic profiling in a low-resource setting. The pathogenic TP53 variants identified occur in the DNA-binding domain, a hotspot region associated with aggressive clinical behaviour and resistance to chemoimmunotherapy (Kwok et al., 2021). The presence of both clonal (VAF >20%) and subclonal (VAF <20%) mutations illustrates the complex clonal architecture of CLL. While clonal variants such as p.Arg249Gly represent dominant disease-driving mutations, subclonal variants like p.Arg306* may serve as reservoirs for relapse post-treatment (Bomben et al., 2021). Clinically, patients with TP53 mutations exhibited features of advanced disease, including lymphadenopathy and leucocytosis, consistent with high-risk CLL. Despite this, most patients lacked access to targeted agents like Bruton's tyrosine kinase (BTK) inhibitors or BCL2 antagonists, limiting the impact of molecular diagnostics on treatment decisions. These findings emphasise the importance of not only introducing molecular testing but also ensuring access to genomics-guided therapies (Hallek et al., 2018). This is the first study to apply Oxford Nanopore long-read sequencing to profile TP53 mutations and IgHV status in Tanzanian CLL patients. The technology demonstrated robust performance in identifying both clonal and subclonal mutations, along with accurate IgHV classification. This integrated approach is scalable and cost-effective, offering a promising solution for improving precision diagnostics in resource-limited environments. The findings advocate for broader implementation of long-read sequencing in Sub-Saharan Africa to support equity in cancer genomics and guide clinical decision-making.

**Keywords:** Chronic lymphocytic leukaemia; TP53 mutations; long-read sequencing

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**Please indicate the name of the funding organization:** International Workshop on Chronic Lymphocytic Leukaemia (iwCLL)

## Abstract ID: 1119

### **Title:** MYD88 mutations in chronic lymphocytic leukemia exhibit distinct biological behaviors and prognostic implications

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**Background:** MYD88 has been identified as a pivotal driver gene in hematologic B-cell malignancies. While MYD88 mutations are rare in western CLL populations (0.5%–3%), they are significantly more common in Asian cohorts, including China, Korea, India and Singapore, with an incidence of 10–28%. These mutations potentially correspond to a distinct CLL subtype with distinctive biological behaviors and survival outcomes. This study aims to delineate the clinical profiles and prognostic implications of MYD88-mutated (MYD88MUT) CLL patients under various treatment backgrounds.

**Methods:** We analyzed clinical data from 1,081 CLL patients diagnosed between 2000 and 2024, who underwent MYD88 detection via next-generation sequencing or Sanger sequencing. We gathered information on laboratory indicators, IGHV status, genetic traits, treatment regimens, and survival rates.

**Results:** In our cohort, we identified MYD88 mutations in 139 patients (12.86%). Specifically, 58 (41.7%) harboring the L265P variant, 49 cases (35.3%) with V217F, 15 (10.8%) with S219C, and 11 (7.2%) with M232T. MYD88MUT CLL showed a notable male predominance ($p<0.001$) and a higher prevalence of IGHV mutations (95.0% vs. 60.6%, $p<0.001$) compared to MYD88 wild-type (MYD88WT) patients. These patients also exhibited a higher presence of monoclonal gammopathy and marked absence of adverse cytogenetic aberrations, such as Del(17p) (1.6% vs. 11.9%, $p<0.001$) and Del(11q) (1.6% vs. 12%, $p<0.001$), with a reduced incidence of complex karyotypes (11.0% vs. 26.6%, $p<0.001$). Similar proportions of atypical CLL with a Matutes score of 3 were observed between MYD88MUT and MYD88WT patients (10.1% vs. 8.0%, $p=0.398$). MYD88MUT CLL showed a reduced FMC7 negativity rate and a significantly diminished CD38 positivity. IGH VH3-7 and VH3-74 were prevalent in V217F and S219C mutations, whereas VH3-23 and VH4-34 were dominant in the MYD88 L265P variant. MYD88MUT CLL demonstrated superior clinical outcomes, including extended time to first treatment (TTFT), progression-free survival (PFS), and overall survival (OS) (m-TTFT 43 vs. 19 months, $p<0.001$; m-PFS 132 vs. 49 months, $p<0.001$; m-OS not reached vs. 135 months, $p=0.001$). Even within the IGHV-mutated subpopulation, MYD88MUT CLL showed significantly improved outcomes compared to MYD88WT CLL (m-TTFT 47 vs. 32 months, $p=0.040$; m-PFS 132 vs. 72 months, $p<0.001$, m-OS not reached vs. 155 months, $p=0.019$). MYD88MUT patients with IGHV-unmutated status demonstrated poorer outcomes, mirroring the outcomes of MYD88WT patients with unmutated IGHV. Patients were categorized into three treatment groups based on their first-line therapy: target therapy, immunochemotherapy, and chemotherapy. Subgroup analysis based on initial treatment showed that MYD88MUT patients experienced significantly enhanced PFS across all treatment modalities compared to non-mutated counterparts. In the MYD88WT cohort, patients undergoing target therapy had superior PFS compared to immunochemotherapy (72 vs. 60 months, $p=0.007$) and chemotherapy groups (72 vs. 35 months, $p<0.001$); and experienced a favorable OS compared to chemotherapy groups (not reached vs. 115 months $p=0.003$). However, in the MYD88MUT CLL group, all three treatment groups resulted in comparable PFS and OS, with impressive long-term outcomes across all groups (5-year PFS exceeding 90%, 5-year OS surpassing 80%, and 10-year OS above 70% in all three groups). The survival benefit of targeted therapy was not evident in MYD88MUT CLL. Among MYD88-mutated patients, there were no significant differences in PFS and OS based on the mutation sites. Those harboring the L265P mutation experienced a notably TTFT compared to non-L265P mutated patients (35 months vs. 67 months, $p=0.003$). However, there was no statistically significant difference in TTFT when comparing L265P patients to wild-type patients (35 months vs. 19 months, $p=0.350$). Additionally, no significant differences in PFS and overall survival OS were observed between patients receiving BTK inhibitors (BTKi) and those not receiving BTKi within the L265P cohort.

**Conclusion:** CLL patients with MYD88 mutations exhibited distinct biological characteristics, and had excellent survival outcomes, irrespective of the treatment modality.

**Keywords:** CLL; MYD88 mutation; biological behaviors; prognostic

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## Abstract ID: 1120

**Title:** Serum LC-MS based untargeted metabolomics machine learning for distinguishing asymptomatic and symptomatic CLL/SLL and predicting TTFT

**Authors:** Huayuan Zhu[a], Luomengjia Dai[a], Qian Wang[b], Ziyuan Zhou[a], Siqi Qian[a], Xiao Lu[a], Tong-lu Qiu[a], Yeqin Sha[a], Yi Miao[a], Shuchao Qin[c], Yi Xia[a], Lei Fan[d], Wei Xu[c], Juncheng Dai[b] and Jianyong Li[e]

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**Aims:** Metabolic reprogramming is a hallmark of cancer and may play a pivotal role in driving disease progression. However, large-scale studies focused on serum metabolite profiles across different stages of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) remain scarce. Therefore, this study aims to employ untargeted metabolomics to depict the metabolic reprogramming landscape and explore differential metabolites associated with advanced disease and develop a model to distinguish asymptomatic and symptomatic CLL/SLL and further investigate their prognostic potential for predicting time to first treatment (TTFT).

**Methods:** Between October 2020 and November 2024, patients newly diagnosed with CLL/SLL in Jiangsu Province Hospital with available serum samples were enrolled in the cohort (N = 183) and were divided into asymptomatic group (N = 70) and symptomatic group (N = 113) according to iwCLL2018 criteria. All serum samples were collected prior to any treatment for LC-MS based untargeted metabolomics analysis. Baseline clinical and biologic characteristics of two groups were compared using Chi-square test or Fisher 's exact test. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to visualize metabolic differences between asymptomatic group and symptomatic group. Variable impact projection (VIP) values of metabolites were obtained from OPLS-DA model, and the fold changes (FC) and *p* values of metabolites were obtained with MetaboAnalyst 6.0 based on the intensities of the spectrum. Initially, metabolites with VIP >1, FC >1.25 or <0.8 and *p* values <0.05 were selected as differential metabolites. Differential metabolites enrichment analysis was conducted based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A diagnostic model for distinguishing symptomatic from asymptomatic CLL/SLL patients was built using a random forest algorithm with LASSO feature selection. The participants were randomly stratified sampling into discovery dataset (n = 128) and test dataset (n = 55). The ROC curve was utilized to evaluate the performance of model. In asymptomatic group, we further used LASSO-Cox regression to predict time to first treatment.

**Results:** Baseline clinical and biological characteristics including age, BMI, del(17p), TP53 mutation, etc. were balanced between two groups except for higher proportion of male patients (67.26% vs 51.43%, $p = 0.033$), elevated B2MG level (65.14% vs 13.24%, $p < 0.001$), IGHV unmutated status (82.35% vs. 51.38%, $p < 0.001$) and SF3B1 mutation (14.00% vs 1.82%, $p = 0.014$) was found in symptomatic group. Serum untargeted metabolomics based on LC-MS totally identified 1,157 metabolites. OPLS-DA model distinguished asymptomatic patients from symptomatic patients, indicating the metabolic remodeling in advanced stage. Hydrogen phosphate and Sphingosine 1-phosphate were top two ranked metabolites with highest VIP values and may play a key in disease progression. Then by screening with VIP >1, FC >1.25 or <0.8 and *p* values <0.05, 51 metabolites were initially identified as differential metabolites and 37 metabolites were upregulated while 17 metabolites were downregulated in advanced stage. Further KEGG pathway analysis revealed significant dysregulation in pyrimidine metabolism and arginine biosynthesis between two groups. Hydrogen-phosphate, Riboflavine 2′,3′,4′,5′-tetrabutanoate, Sphingosine 1-phosphate, N-Undecanoylglycine, and beta-D-Glucopyranosyl-11-hydroxyjasmonic acid were incorporated to construct the 5-metabolite diagnostic model, the ROC-AUC is 0.93 (95% confidence interval (CI), 0.86–1.00, sensitivity: 0.90, specificity: 0.97) in test dataset. Among asymptomatic CLL/SLL patients, with a median follow-up of 385 days (range, 95 days-1632 days), totally 29 patients met the iwCLL2018 criteria for treatment initiation. Based on LASSO-Cox regression, an 11-metabolite signature dominated by amino acid (e.g. Threonine, Palmitoylglycine, Glutaminylproline, L-beta-aspartyl-L-leucine, ect.) and phospholipid (e.g. LysoPE, C24:1 Cer, ect.) derivatives further stratified asymptomatic patients into distinct progression risk groups.

**Conclusion:** Herein, we reported a large-scale study investigating serum metabolic profiles in different CLL/SLL stage and revealed metabolic reprograming in advanced disease and innovatively developed machine learning based diagnostic and prognosis models by serum metabolites combination.

**Keywords:** Leukemia; biomarker; metabolic reprogramming

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## Abstract ID: 1121

### **Title:** High-risk molecular features eclipse genomic complexity in predicting CLL patient outcomes; insights from the UK CLL4, ARCTIC and ADMIRE trials

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**Introduction:** High genomic complexity (HGC) is associated with poor prognosis in chronic lymphocytic leukemia (CLL) treated with (immuno)chemotherapy and targeted agents. However, whether HGC independently contributes to outcome, in the context from emerging biomarkers—such as telomere attrition and methylation-based cell-of-origin subtypes—remains unclear.

**Methods:** Consequently, we profiled copy number alterations (CNA) in 495 treatment-naïve CLL patients from three randomized (immuno)chemotherapy trials (CLL4, ADMIRE, ARCTIC), incorporating FISH, CD38, ZAP70, IGHV mutational status, telomere length (TL, n = 495), targeted sequencing of nine recurrently mutated genes (n = 454), and methylation subtypes (m-CLL, i-CLL, n-CLL, n = 446).

**Results:** Telomere lengths varied from 1.13 to 10.23 kb (median 3.21 kb), categorized as short (TL-S, 39%), intermediate (TL-I, 25%), or long (TL-L, 36%). (Epi)genomic profiling revealed n-CLL (49%), i-CLL (32%), and m-CLL (19%) subgroups, with 426 somatic mutations identified, in SF3B1 (22%), NOTCH1 (15%), ATM (11%), TP53 (9%), XPO1 (8%), BIRC3 (6%), MYD88 (4%), SAMHD1 (4%) and MED12 (3%).

CNAs were detected in 452 patients (mean 2.62), including 65 aneuploidies. Recurrent CNAs included del(17p) (4.4%), del(11q) (18%), del(13q) (47.3%), and trisomy 12 (12.5%), with additional recurrent lesions on 2p, 4p, 6q, 8, and 14. Based on total CNA count, patients were stratified into low (LGC, 0–2 CNAs; n = 334), intermediate (IGC, 3–4 CNAs; n = 97), or high (HGC, ≥5 CNAs; n = 64) genomic complexity. HGC was significantly enriched in U-CLL (81%, $p < 0.001$) and associated with higher CNA counts (mean: 2.86 vs. 2.01 in M-CLL, $p < 0.001$). TL varied by GC group, with TL-S more frequent in HGC (61%) and TL-L enriched in LGC (41%, $p < 0.05$). TL inversely correlated with CNA burden as a continual variable (Kendall's $\tau = -0.147$, $p < 0.001$). Although not significantly associated with methylation subtype overall, 62% of HGC patients were classified as n-CLL. Trisomy 12 was more prevalent in LGC (16.5%) versus IGC (5.2%) and HGC (3.1%, $p < 0.01$), and frequently co-occurred with NOTCH1 mutations. Class II deletions of 13q (>2 Mb) were more common in HGC (45%) and IGC (49%, $p < 0.05$). Deletion of 11q and ATM mutations were enriched in HGC (6.3%) and IGC (9.3%, $p < 0.001$). Whilst BIRC3 deletions (n = 63), which always co-occurred with ATM loss, were enriched in IGC (23%) and HGC (18.7%, $p < 0.001$), biallelic BIRC3 inactivation showed no clear GC association. TP53 dysfunction (mutation and/or deletion, n = 51) was strongly associated with HGC (36%, $p < 0.001$). Tumour mutation burden (TMB) correlated with CNA count ($\tau = 0.26$, $p = 0.042$), and HGC patients harboured more mutations per sample (mean HGC:1.08 vs. LGC:0.83, $p < 0.05$). NOTCH1 mutations were more frequent in LGC, while SF3B1 mutations were distributed across GC groups, with 70% found in LGC. Due to differing treatment regimens and follow-up durations, survival analyses were performed separately for ARC/ADM and CLL4 cohorts. In both, HGC was significantly associated with lower 5-year survival rates (ARC/ADM: 29% vs. 51%; CLL4: 42% vs. 61%; $p < 0.01$). Univariate Cox models confirmed HGC as a significant predictor of shorter progression-free (PFS) (ARC/ADM: HR =1.97, $p = 0.002$; CLL4: HR =1.49, $p = 0.04$) and overall survival (OS) (ARC/ADM: HR =2.13, $p = 0.015$; CLL4: HR =1.59, $p = 0.018$). Other adverse prognostic indicators included TP53 dysfunction, n-CLL, TL-S/I, U-CLL, del(11q), and tumour mutational burden (TMB, >3 mutations). Using multivariate Cox analysis to account for the confounding presence of HGC with multiple other poor-risk biomarkers, we demonstrated that HGC did not independently predict PFS in either cohort. Instead, independent PFS predictors included TP53 dysfunction (ARC/ADM: HR =3.59; CLL4: HR =2.68), U-CLL (ARC/ADM: HR =2.04; CLL4: HR =1.94), and TL-S (ARC/ADM: HR =1.92; CLL4: HR =1.52). For OS, HGC remained independently significant only in the CLL4 model (HR =1.61, $p = 0.02$), alongside TP53 dysfunction, TL-S, trisomy 12, U-CLL, SF3B1 mutations, and age. As our data suggests HGC may be a surrogate for other high-risk features, we looked further at the biomarker profile of 64 HGC patients, showing 23 had TP53 dysfunction and 36/41 TP53 wild-type patients harboured at least one high-risk feature (TL-S: n = 18; U-CLL: n = 26; n-CLL: n = 17), leaving only five (8%) without an identifiable adverse marker.

**Conclusions:** HGC is linked to poor CLL outcomes but may primarily reflect underlying biological risk, driven by impaired cell cycle control, telomere attrition, and genomic instability, without consistent independent prognostic value. Its association with adverse features—telomere length, immunogenetics, and epigenetic subtypes—suggests these biomarkers may improve predictive accuracy with or without GC. Further studies should validate these findings in cohorts treated with targeted agents.

**Keywords:** CLL; complexity; genomics

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## Abstract ID: 1122

### **Title:** Integrative epi(genome) analysis of CD49d bimodal expression reveals epigenetically driven phenotypic transitions in CLL

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CD49d (integrin α4), a key mediator of cell adhesion and tissue homing in chronic lymphocytic leukemia (CLL), is one of the most powerful independent prognostic markers. While most CLL cases exhibit homogeneous CD49d expression, ~20% display a distinctive bimodal pattern, with coexisting CD49d + and CD49d- subpopulations deriving from the same malignant clone. Clinically, these patients experience outcomes similar to CD49d + cases, with the CD49d + fraction preferentially expanding over time, especially after therapy, exhibiting greater proliferative potential, enrichment in the bone marrow, and increased representation within the CXCR4dim/CD5bright proliferative compartment, supporting a dynamic contribution to disease progression. However, the mechanisms driving the dynamic modulation of CD49d expression, whether through clonal evolution, epigenetic regulation, or both, remain unclear. To address this, we performed a multi-omic analysis on untreated CLL cases with either homogeneous (10 CD49d+, 10 CD49d–) or bimodal CD49d expression (n = 8). RNA-seq of homogeneous CD49d+ (n = 9) and CD49d– (n = 8) cases identified 443 differentially expressed genes (257 upregulated in CD49d+), with gene set enrichment pointing to proliferative programs. Principal component analysis confirmed distinct clustering. Accordingly, CD49d + and CD49d– fractions from bimodal cases displayed distinct transcriptional profiles. In particular, CD49d + fractions aligned more closely with proliferative signatures, including those of CXCR4dim/CD5bright cells, while CD49d– fractions

resembled resting CXCR4high/CD5dim profiles. Single-cell RNA-seq on bimodal CLL (n = 7) further clarified this heterogeneity, revealing both distinct and shared transcriptional features between CD49d + and CD49d- cells, supporting the presence of reversible expression states. To investigate whether such states were genetically encoded, we conducted whole-genome sequencing (WGS) on FACS-sorted CD49d + and CD49d- fractions from 8 bimodal cases sampled at 2–3 timepoints (mean interval: 3.3 years). A median of 4,815 heterozygous SNVs and 61 driver mutations in 22 genes were identified. Phylogenetic reconstruction revealed branching architectures shared between CD49d + and CD49d- compartments, with no evidence for clone-specific segregation, and transitions predominantly occurred from CD49d- to CD49d + states over time. These findings were further validated at single-cell resolution using the Tapestri platform (Mission Bio) on ~5,000–10,000 cells per sample (CD49d pre-sorted) from 7 WGS-characterized samples. A WGS-driven custom panel targeting 50–60 mutations per case enabled high-resolution clonal reconstruction. Single-cell genotyping confirmed that CD49d + and CD49d- cells shared common mutational profiles, reinforcing the model of phenotypic modulation within genetically stable clones. To quantify the contribution of phenotypic reprogramming to clonal evolution, we developed a mathematical model integrating clonal structure, cellular plasticity, and population genetics. Applying this model to WGS-derived somatic mutations, we estimated patient-specific parameters that predicted the proliferation rates of CD49d + cells and the frequency of CD49d expression switching. Results indicate that the interplay between proliferation and phenotypic reversibility is central to shaping the clonal trajectories observed in bimodal CLL. Epigenetic profiling provided insights into CD49d regulation. DNA methylation analysis of the ITGA4 promoter revealed significantly lower methylation levels in CD49d + cells compared to CD49d– cells in cases with homogeneous expression (n = 20), consistent with a transcriptionally permissive state. In bimodal cases (n = 8), sorted fractions confirmed reduced methylation in CD49d + cells, though with inter-patient variability, suggesting that hypomethylation contributes to, but not fully explains, CD49d expression dynamics. To further explore chromatin regulation, we performed ATAC-seq on sorted fractions from 10 homogeneous and 7 bimodal cases. CD49d + fractions consistently displayed increased chromatin accessibility in 14 upstream regions from the ITGA4 locus spanning ~150 kb. These regions overlap with putative enhancer elements altogether suggesting an ITGA4-specific super enhancer. These accessible regions were enriched for binding motifs of transcription factors implicated in B-cell development and activation, including CTCF, EBF1, IRF4, and RUNX3, suggestive of coordinated transcriptional and epigenetic network governing ITGA4 expression. Importantly, these chromatin features were preserved across both homogeneous and bimodal CD49d + samples, indicating a shared regulatory architecture, although dynamic transitions in gene expression appear restricted to bimodal cases. Our data show that the over time increase of the CD49d + fraction in CD49d bimodal CLL reflects a dynamic, epigenetically regulated transcriptional state rather than the emergence of genetically distinct subclones. The longitudinal expansion of CD49d + cells is driven by both their proliferative advantage and a permissive chromatin landscape at the ITGA4 locus. These findings underscore how the interplay between clonal dynamics and epigenetic modulation contributes to phenotypic heterogeneity, positioning CD49d bimodal CLL as a model for studying non-genetic drivers of tumor evolution and resistance.

**Keywords:** CD49d expression; clonal dynamics; epigenetic regulation

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## Abstract ID: 1201

### **Title:** IFNγ/IL21-JAK-STAT1 signaling axis drives T-cell–mediated CLL proliferation and venetoclax resistance

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**Background:** Multiple microenvironmental factors have been implicated in the induction of CLL cell proliferation in lymph nodes. However, it remains unclear which signals are most essential and how they are (patho)physiologically combined to stimulate CLL cells into proliferation.
**Results:** To decipher the changes in the CLL microenvironment, we performed transcriptomic profiling (n = 10 pairs) of resting (CXCR4brightCD5dim) and proliferative (CXCR4dimCD5bright) intraclonal CLL subpopulation that recently exited lymph nodes (LN). Subsequent gene expression analysis (decoupleR, IPA, GSEA) revealed the JAK-STAT as the most prominently activated pathway in CXCR4dimCD5bright cells, and specifically, the STAT1 transcription factor as most likely driving gene expression differences between CXCR4/CD5 subpopulations. The JAK-STAT1 signaling enrichment was confirmed in a re-analysis of RNA-seq from paired LN vs. peripheral blood samples (n = 29 pairs, Sun et al., 2023). Notably, higher STAT1 mRNA levels are strongly associated with a shorter time to first treatment and overall survival in 2 CLL cohorts (n = 106, in-house cohort; n = 191, EGAS00001001746; both chemoimmunotherapy-era). Next, to identify STAT1 activator(s), we stimulated primary CLL cells (n = 4) with a panel of 16 microenvironmental signals (anti-IgM, CD40L, CpG, CXCL12, IFNα/β/γ, IL2/4/6/10/21/27, LPS, TNFα, VEGF). IFNγ and IL21 emerged as the only inducers of STAT1 phosphorylation (Y701, S727), and IL21 also activated STAT3 in a phospho-protein analysis, but not other proteins (ELISA detecting 44 phospho-proteins). Indeed, in silico analysis (IPA) identified IFNγ and IL21 as receptor

ligands most significantly influencing gene expression differences in LN-resident and CXCR4dimCD5bright CLL cells. We next observed that T-cells from CLL patients (CD3/28-activated) produce IFNγ (312 ng/ml) and IL21 (1 ng/ml), induce CLL proliferation, and long-term (6-day) STAT1 phosphorylation (STAT3 phosphorylated transitionally). The expression of IL21 in CLL niches has been documented (Pascutti et al., 2013), but the presence of IFNγ in CLL LNs seems counterintuitive considering the relatively immunologically 'cold' microenvironment. We determined that IFNγ is clearly present in CLL LNs (n = 8, immunohistochemistry), and CD8+ T-cells are its major source (scRNA-seq re-analysis, Sun et al., 2023). IFNγ stimulation (10 ng/ml) not only significantly enhanced CLL cell viability (42% vs. 86%, n = 10, $p < 0.001$), but also increased CLL cell proliferation (64% vs. 76% proliferation, n = 10, $p = 0.02$) in combination with a cytokine cocktail (CD40L, IL4, IL21, anti-IgM, CpG). IL21 also increased proliferation in CLL cells co-cultured with HS5-CD40L-IL4 stromal cells (10% vs. 49%, $p < 0.05$, n = 16; model by Hoferkova et al., 2024) while inhibition of JAK-STAT1 signaling by JAK-inhibitors (ruxolitinib or AZD1480) reduced proliferation by ~80% in the HS5-CD40L-IL4-IL21 co-cultures. Moreover, STAT1 knockout in primary CLL cells (Cas9-gRNA transfection) impaired proliferation induced by HS5-CD40L-IL4-IL21 co-culture (n = 6; $p < 0.05$). In addition to STAT1 phosphorylation being triggered by IFNγ and IL21, we noted that its levels are controlled post-transcriptionally by miR-150. miR-150 overexpression (lentiviral) repressed STAT1 mRNA and protein levels in CLL-derived HG-3 and OSU-CLL cells (n > 3) and in primary CLL cells transfected with synthetic miR-150 (n = 5; $p < 0.05$). Indeed, miR-150 is downregulated, and STAT1 levels are upregulated in LN-derived CLL cells (1.9- and 1.5-fold, respectively, n = 10, $p < 0.001$). We further hypothesized that the IFNγ/IL21-JAK-STAT1 axis might promote venetoclax resistance driven by CLL-T cell interactions. Supporting this, re-analysis of transcriptomes from samples obtained before venetoclax therapy and at relapse (n = 7, Ghia et al., 2022) revealed significant IFNγ-pathway activation (GSEA, $p = 0.018$), while IL21 signaling was not activated. In line with this, IFNγ (50 ng/ml) induced MCL1 and BCLxL upregulation in CLL cells (~2.4- and 2.0-fold, respectively; $p < 0.05$). IFNγ treatment completely rescued CLL cell apoptosis induced by 10 nM venetoclax (viability 56% vs. 100%, n = 3, $p = 0.024$). Importantly, JAK inhibition with ruxolitinib or AZD1480 (1 μM) reversed venetoclax resistance induced by IFNγ, or conditioned media from activated T-cells, or by HS5-CD40L–IL4–IL21 co-culture (n > 3, $p < 0.05$).

**Conclusions:** We reveal for the first time that IFNγ and IL21 are the key factors driving CLL cell proliferation and survival in lymph nodes. Both activate STAT1, which we identified as responsible for most gene expression changes in LN-resident CLL cells and their ability to proliferate. Additionally, IFNγ mediates robust venetoclax resistance in CLL, which can be effectively reversed by JAK inhibitors (ruxolitinib or AZD1480), providing a rationale for their combination.

**Keywords:** Microenvironment; STAT1; venetoclax-resistance

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# Abstract ID: 1203

## **Title:** SF3B1 K700E mutation promotes SAT1-mediated metabolic reprogramming and suppresses T-cell immune response in chronic lymphocytic leukemia

**Authors:** Hao Guo[a], Yongyi Yuan[a], Siming Zheng[b] and Keshu Zhou[a]

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**Background:** Chronic lymphocytic leukemia (CLL) is an uncurable hematologic malignancy marked by metabolic reprogramming and immune evasion. SF3B1 mutations, present in 10–15% CLL, are associated with poor prognosis due to their disruption of RNA splicing. While SF3B1 mutations impact numerous cellular processes, their effects on tumor metabolism and immune modulation remain incompletely understood. SAT1 (Spermidine/spermine N1-acetyltransferase 1) plays a crucial role in polyamine metabolism, which is essential for cell growth and survival. This study investigates how SF3B1 K700E mutation regulates SAT1 expression, leading to metabolic reprogramming and immune modulation in CLL.

**Methods and results:** To explore the effects of SF3B1 K700E mutation on metabolic gene expression, we conducted RNA profiling on MEC-1 cells expressing SF3B1 K700E and primary CLL samples from SF3B1-mutated patients. Integrated RNA expression and splicing analysis revealed SAT1 upregulation in SF3B1-mutated samples, with exon 4 skipping and increased mRNA stability. We then used LC-MS/MS-based targeted metabolomics to assess the impact of SAT1 overexpression (SAT1-OE) on polyamine metabolism. The results revealed a significant increase in N1-acetylspermidine levels in SAT1-OE CLL cells, consistent with upregulation in SF3B1-mutated cells compared to wild-type (WT) controls. Additionally, untargeted metabolomics analysis identified glutamine metabolism and oxidative phosphorylation (OXPHOS) metabolism as significantly enriched in SAT1-OE CLL cells, suggesting a shift in metabolic pathways that support cell survival and proliferation. In functional assays, SAT1 overexpression in MEC-1 and JVM-3 cells, as well as primary CLL cells, enhanced cell proliferation and clonogenic potential, while SAT1 silencing suppressed these effects. We next performed in vivo studies using NSG mice to evaluate SAT1's impact on leukmia infiltration and progression. SAT1 overexpression in CLL cells led to increased leukemia burden, with significantly larger spleens and livers. In contrast, SAT1 knockdown decreased tumor growth and infiltration. In co-transplantation model with CD8+ T-cells, SAT1 overexpression increased leukemic infiltration and induced immune suppression, evidenced by decreased T-cell number and reduced IFN-γ production. In contrast, SAT1 knockdown reduced leukemia burden, restored CD8+ T-cell activation, indicating improved immune responses. Further, we performed ChIP-seq combined with RNA-seq to examine the role of SAT1 in epigenetic regulation. We identified H3K27 acetylation enrichment at the IL18BP promoter, which facilitated IL18BP transactivation in SAT1-overexpressing MEC-1 cells. IL18BP levels were significantly higher in the culture supernatants of SAT1-OE CLL cells, confirming its role

in immune suppression within the tumor microenvironment (TME). Interestingly, SAT1 was found to non-canonically acetylate H3K27 domains in multiple mitosis-regulating genes in ovarian cancer (Zheng et al., Nature Communications 2025). This finding suggests that SAT1-mediated regulation of the IL18-IL18BP axis may serve as a conserved oncogenic mechanism that supports immune evasion in CLL.

**Conclusion:** This study provides evidence for a dual mechanism by which SAT1 regulates polyamine metabolism and immune suppression in SF3B1-mutated CLL. SAT1 enhances polyamine catabolism and supports leukemia survival through metabolic reprogramming. Additionally, SAT1 probably acetylates H3K27 at the IL18BP promoter, increasing IL18BP expression and inhibiting CD8+ T-cell activation, leading to immune evasion. These findings suggest that targeting SAT1 could provide a therapeutic strategy to enhance T-cell-mediated immunity and improve outcomes in SF3B1-mutated CLL.

**Keywords:** SF3B1 mutation; SAT1; chronic lymphocytic leukemia

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## Abstract ID: 1204

### **Title:** EGR2 hotspot mutations drive BTKi resistance in chronic lymphocytic leukemia via CD27-CD70 interaction-mediated T-cell exhaustion

**Authors:** Huayuan Zhu[a], Dan Yang[a], Xiao Lu[a], Yeqin Sha[a], Luomengjia Dai[a], Ziyuan Zhou[a], Yi Miao[a], Yi Xia[a], Hui Jin[b] and Jianyong Li[c]

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**Objectives:** EGR2 (Early Growth Response Factor 2), a zinc-finger transcription factor critical for cellular differentiation and apoptosis, has been implicated as a recurrently mutated gene in chronic lymphocytic leukemia (CLL). However, the prevalence and prognostic relevance of EGR2 mutation in Chinese CLL patients remain unknown. Therefore, we systematically characterized EGR2 mutational profiles and their correlation with clinical outcomes in a Chinese CLL cohort. Furthermore, we explored the molecular mechanisms driven by EGR2 using single-cell RNA sequencing (scRNA-seq) technology to elucidate its role in CLL.

**Methods:** We retrospectively investigated CLL patients initially diagnosed and evaluated by next-generation sequencing (NGS) at Jiangsu Provincial People's Hospital between January 2015 and January 2025. Comprehensive analyses were performed on clinical parameters, molecular characteristics, genomic mutation profiles, and therapeutic outcomes. Lentiviral vector infection was used to construct MEC-1 cell lines overexpressing EGR2 wild-type and EGR2 p.E356K hotspot mutant, and their proliferation was evaluated with CCK-8 assays. And we implemented scRNA-seq using peripheral blood mononuclear cells (PBMCs) from three treatment-naïve and three relapsed/refractory (R/R) patients resistanted to Bruton tyrosine kinase inhibitor (BTKi) including EGR2 wild-type and p.E356K mutant subtypes alongside six healthy controls for multi-dimensional comparative analysis. ChIP-seq to explore the binding sites of EGR2 in the CLL MEC-1 cell line.

**Results:** 297 patients with newly diagnosed CLL meeting the inclusion criteria were analyzed. EGR2 mutations were detected in 9.8% of cases (29/297), with predominant variants located at p.E356K (75.6%, 22/29), p.H384 (13.8%, 4/29), and p.D411 (10.3%, 3/29). The median variant allele frequency (VAF) was 47%. Compared to wild-type counterparts, patients with EGR2 mutations exhibited: younger diagnosis age (50 vs 57 years, $p<0.001$), advanced Binet staging (Stage C: 52.4% vs 25.2%, $p=0.016$), higher prevalence of monoclonal gammopathy (52.4% vs 18.0%, $p<0.001$), increased co-occurrence of del(11q) (51.9% vs 15.4%, $p<0.001$), ATM mutations (55.2% vs 19.0%, $p<0.001$), and complex karyotype (CK) (31.0% vs 13.6%, $p=0.029$), significantly reduced del(13q) incidence (16.6% vs 45.0%, $p=0.008$). Furthermore, EGR2-mutated cases predominantly showed unmutated immunoglobulin variable heavy chain (IGHV) status (93.1% vs 44.1%, $p<0.001$), the most frequent IGHV family was IGHV1 (37.9%, 11/29), enrichment in B-cell receptor (BCR) stereotyped subsets #1 (16.7%, 4/24) and #8 (16.7%, 4/24). Among 113 patients receiving continuous BTKi monotherapy, the EGR2-mutated cohort (n = 13) demonstrated significantly shorter median progression-free survival (PFS) (30.1 vs 84.1 months; HR =5.69, $p<0.001$) and elevated Richter transformation risk (23.1% vs 1.0%, $p<0.001$) compared to wild-type patients (n = 100). Furthermore, in five patients with EGR2 p.E356K hotspot mutations who developed resistance to BTKi monotherapy, longitudinal VAF monitoring showed no significant variations between baseline and post-resistance timepoints. Clonal evolutionary tracking revealed persistent dominance of the EGR2 p.E356K mutation as a major clone (VAF >45%) in two zanubrutinib treated patients, maintaining clonal prevalence both at initial diagnosis and upon acquired resistance. Furthermore, at the cellular level, the proliferation rate of EGR2 p.E356K-overexpressing MEC-1 cells was significantly higher than that of EGR2 wild-type-overexpressing MEC-1 cells ($p<0.01$). scRNA-seq analysis further revealed that treatment-naïve patients with EGR2 p.E356K mutation had significantly higher peripheral blood T-cell proportions than EGR2 wild-type patients (13.24% vs 3.64%). Further T-cell subset analysis showed that EGR2 p.E356K-mutated patients had more exhausted T-cell infiltration than wild-type patients both pre-treatment (20.54% vs 10.53%) and after developing resistance to BTKi (31.02% vs 18.93%). CellPhoneDB analysis identified enhanced CD27-CD70 ligand-receptor interactions in mutated leukemic cells interacting with T lymphocytes, which further intensified upon BTKi resistance development. Differential gene expression analysis and Gene Set Enrichment Analysis (GSEA) identified NF-κB pathway hyperactivation in resistant patients. In the CLL MEC-1 cell line, ChIP-seq analysis showed 32.83% of binding peaks reside in promoter regions. EGR2 was found to interact with RelA (p65) binding motifs in these peaks, indicating its potential role in activating the NF-κB pathway via direct RelA binding.

**Conclusions:** In CLL, EGR2 hotspot mutations constitute an independent biomarker of poor prognosis and the EGR2 p.E356K mutation likely promotes disease progression and resistance to BTKi by enhancing CD27-CD70-mediated expansion of CD8+T exhausted cells and activating the NF-κB pathway.

**Keywords:** Chronic lymphocytic leukemia; EGR2; Bruton tyrosine kinase inhibitor

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## Abstract ID: 1205

### **Title:** Investigating the eEF2K/eEF2 axis as a potential target in CLL following nelfinavir treatment

**Authors:** Jiatian Li[a], Jodie Hay[a], Karen Dunn[a], Martin Bushell[a] and Alison Michie[a]

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Appropriate regulation of protein synthesis is critical for cell fate decisions: eEF2K regulates cell survival through its phosphorylation of eEF2 T56 (p-eEF2 T56) to negatively regulate eEF2 and subsequently reduce translation elongation. Nelfinavir (NFR), a safe, oral drug that was originally developed as an HIV-1 aspartyl protease inhibitor, can exert anti-cancer effects by inducing cell cycle arrest, apoptosis and inhibiting protein synthesis. This has resulted in the inclusion of NFR in clinical trials for myeloma, prostate, and cervical cancer. NFR can also activate eEF2K, leading to an inhibition of eEF2-mediated protein translation. Our previous studies demonstrated that treatment of chronic lymphocytic leukemia (CLL) cells with the mTORC1 inhibitor rapamycin, resulted in eEF2 inhibition (increased p-eEF2 T56) via elevated eEF2K activity, leading to reduced MCL1 expression. We aim to investigate whether the eEF2K/eEF2 pathway can be exploited as a therapeutic target in CLL, through the combination of NFR with first-line CLL treatments, thus providing novel treatment options. We first stratified CLL patient samples for the expression/activation status of eEF2K/eEF2 and demonstrated that CLL samples exhibited higher total eEF2 protein expression and lower p-eEF2 T56 levels compared to healthy B cells. This indicates that CLL cells possess enhanced translation elongation activity. Generating shRNA-knockdown (KD) models in the CLL cell line MEC1, sh-eEF2K KD cells exhibited reduced p-eEF2 T56 levels and increased proliferation, suggesting that eEF2K inhibition promotes cell proliferation. In an in vitro CLL mouse model derived from wild type (WT) or eEF2K D273A/D273A (catalytically-reduced eEF2K; eEF2K-KI) bone marrow, we compared eEF2 activation status in control-B cells vs. WT-CLL model and WT-CLL vs. eEF2K-KI-CLL cells. The WT-CLL model, compared with control-B cells, and the eEF2K-KI-CLL, compared with WT-CLL, both showed lower p-eEF2 T56 levels compared with their respective controls, consistent with findings in primary CLL cells. Furthermore, analysis of proliferation in vitro using cell counts and cell trace violet assays demonstrated that eEF2K-KI-CLL cells proliferated faster than WT-CLL cells. Transplantation of WT-CLL or eEF2K-KI-CLL cells into immunodeficient (NSG) mice revealed that eEF2K-KI-CLL transplanted mice developed disease earlier in vivo and showed a significantly reduced survival compared to the WT-CLL transplanted mice. These data identify the eEF2K/eEF2 signalling axis as a potential target in CLL. To address whether NFR has the ability to regulate the eEF2K/eEF2 signalling axis in our CLL models, cells were treated with clinically achievable doses of NFR. We noted that NFR promoted apoptosis in both MEC1 and primary CLL cells and increased p-eEF2T56 levels, suggesting that it activated eEF2K. Combining NFR with the BTK inhibitor ibrutinib enhanced the anti-proliferative activity of NFR in MEC1 cells. Of note, both sh-eEF2K- and sh-eEF2-MEC1 KD were found to be more resistant to NFR-mediated apoptosis and proliferation, indicating that each of these proteins plays a role in promoting NFR-sensitive cell fate decisions. In the WT-CLL mouse model, NFR significantly inhibited cell proliferation in vitro, while this trend was attenuated in eEF2K-KI-CLL cells, further strengthening the central role of eEF2K in the mechanism of action of NFR. In summary, our data demonstrate that NFR inhibits CLL cell proliferation and survival through the activation of eEF2K signaling, supporting the eEF2K/eEF2 axis as a potential therapeutic target and highlighting NFR as a novel treatment for CLL.

**Keywords:** Translation elongation; eEF2K/eEF2; nelfinavir

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## Abstract ID: 1206

### **Title:** Subversion of ERK-MAPK-mediated mRNA translation elongation as a therapeutic strategy in CLL

**Authors:** Jodie Hay[a], Karen Dunn[a], Barbara Cadden[b], Colin Nixon[b], Jiatian Li[a], Andrew Clear[c], John Gribben[c], Alison McCaig[d] and Alison Michie[a]

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Ibrutinib, a selective and irreversible inhibitor of the non-receptor tyrosine kinase BTK, is responsible for improving survival rates of poor prognostic CLL patients. However, BTK inhibitors are not suitable for all patients, with some being unresponsive from the outset, or developing resistance mutations after initial treatment responses, ultimately leading to relapse. To develop new treatment strategies, we must first gain a deeper understanding of pathways involved in CLL pathogenesis. Our previous studies highlighted the importance of mRNA translation elongation as a driver of disease progression, identifying inactivation of eEF2 activity as a novel therapeutic target for blocking CLL progression. eEF2 activity can be mediated by a number of post-translational modifications, most notably through phosphorylation by eEF2K. eEF2K inhibition, through phosphorylation at residue Ser366 by p90RSK and p70S6 kinase or by SAPK4/p38delta at Ser359, can promote protein synthesis. This occurs in part through the reduction in eEF2T56 phosphorylation, thereby increasing eEF2 activity

and promoting translation elongation. Analysis of CLL patient-derived lymph node (LN) biopsies (n = 27) revealed that higher p-eEF2KS366 was associated with significantly less time to treatment, indicative of higher protein synthesis being associated with poorer clinical outcome. Elevated p-eEF2KS366 further correlated with increased eEF2 expression and higher phosphorylation of ribosomal protein S6, a marker of protein translation. These data support our hypothesis that increased protein synthesis can drive CLL tumorigenesis and identify eEF2K as a potential therapeutic target Analysis also highlighted that activated ERK-MAPK (p-p42/p44 ERK-MAPKT202/Y204) significantly correlated with faster relapse time, indicative of a more aggressive disease. Active ERK-MAPK also significantly aligned with high expression of eEF2 and was associated with eEF2K inactivation (p-eEF2KS366), indicative of active protein synthesis. Together these data support rationale for therapeutically targeting ERK-MAPK-mediated protein synthesis. To determine the effects of targeting ERK-MAPK signalling, we combined AZ6197, a novel ERK1/2 inhibitor, alone and in combination with ibrutinib (ERKi IBR). In the proliferating primary CLL-NTL-CD40L/IL21 co-culture model ERKi IBR had anti-tumour activity, by significantly reducing protein synthesis, cell proliferation and cell survival. The efficacy of ERK-MAPK inhibition was also tested in our poor prognostic CLL mouse model, which is generated by retrovirally transducing murine HSPCs with a catalytically inactive PKCα construct (PKCα-KR) producing CLL-like cells with elevated BCR signalling. In in vitro studies using CLL-like cells ERKi IBR also induced tumour suppressive effects by reducing protein synthesis and cell proliferation compared to control or single treatment arms. To specifically investigate the role of the eEF2K/eEF2 signalling axis in the generation of CLL in our mouse model we used the HSPCs derived from eEF2K with reduced catalytic activity (Eef2kD273A/D273A) or constitutively active eEF2 (Eef2T56A/T56A) mice to generate CLL-like cells. In this way we established that both models proliferated faster and had increased protein synthesis compared to wild-type cells. In wild-type cells, treatment with ERKi IBR induced a block in proliferation and reduced protein synthesis compared to control conditions. However, these tumorigenic effects were only partially attenuated in Eef2T56A/T56A and Eef2kD273A/D273A derived CLL-like cells with ERKi IBR, suggesting that the eEF2/eEF2K signalling is a key mechanistic target of ERKi IBR. Collectively, these studies indicate that reduced eEF2K activity is associated with poor outcome in CLL, through the promotion of protein synthesis. These analyses also identify ERK-MAPK as a novel target for modulating protein synthesis in CLL. Through in vitro CLL modeling, and in vivo studies, we have demonstrated that targeting both ERK and BTK signalling simultaneously, to therapeutically block protein synthesis via eEF2 signalling, is an attractive clinical strategy.

**Keywords:** Protein synthesis; therapeutic; ERK/MAPK

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## Abstract ID: 1209

**Title:** Early effects of venetoclax monotherapy on immune cells in patients with chronic lymphocytic leukemia

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**Background:** Chronic lymphocytic leukemia (CLL) is characterized by a dysregulated immune system contributing to high risk of infections, low vaccination response and treatment failure to cellular therapies. Defects in the apoptosis pathway are also seen, which are mediated by an increased expression of the B-cell lymphoma-2 (BCL-2) prosurvival protein. The first selective small molecule inhibitor of BCL-2, venetoclax, targets this dysregulated pathway and has significantly improved the prognosis in patients with CLL. Treatment with venetoclax induces a rapid decrease in leukemic cells and apoptosis has been observed within 4–8 hours after drug exposure. However, the modulation induced by venetoclax monotherapy on the immune environment during early treatment has not yet been investigated. In the present study, we aimed to analyze the changes in the different populations of immune cells during the first 4 weeks of venetoclax monotherapy in patients with CLL.

**Method:** Peripheral blood (PB) samples were collected from 10 patients with CLL at the Dept. of Hematology, Karolinska University Hospital, before start of treatment and at 4–6 hours, 24 hours and after 4 weeks of treatment. The starting dose was 20 mg, ramping up weekly to the final dose of 400 mg if tolerated. Six patients had relapsed refractory (R/R) disease and 4 patients were treatment-naïve. Eight healthy individuals were included as controls. PB cells were analyzed by flow-cytometry.

**Results:** At week 4, 7/10 patients had reached the full dose of 400 mg. The absolute number of CD19+ cells decreased significantly already at 4–6 hours compared to baseline, and at week 4 levels were no longer significantly higher than those of healthy controls. At baseline, the numbers of CD4+ T cells were in the range of healthy controls and decreased to significantly lower levels at week 4. CD8+ T cells were significantly higher at baseline and normalized at week 4. Compared to baseline, both CD4+ and CD8+ T cells decreased significantly at the 4-week timepoint ($p \leq 0.05$). For the T-helper subsets, Th1 and Th2 cell numbers were not elevated at baseline. Th1 cells showed a significant decrease at week 4 ($p = 0.05$), and Th2 cells did not change during treatment. Th17 and T regulatory cells (T-regs) were higher compared to controls at early timepoints (but not at baseline) and decreased significantly at week 4 ($p = 0.05$). NK cells were within normal range at baseline and did not change throughout treatment ($p = 0.05$). The number of CD4+ and CD8+ cells co-expressing the exhaustion markers T cell immunoreceptor with Ig and ITIM domains (TIGIT) and programmed cell death protein 1 (PD-1) were elevated at baseline, and both populations decreased significantly and normalized at week 4 ($p \leq 0.05$). A significant reduction ($p \leq 0.05$) was observed for all memory T cell populations at week 4 (CD4+ & CD8+ naïve cells, central memory CD4+ & CD8+ cells TCM, effector memory CD4+ & CD8+ cells TEM and T cells re-expressing CD45RA, CD8+ TEMRA), except for CD4+ TEMRA, which did not decrease compared to baseline. Most memory T cells were not elevated at baseline, except for CD8+ TCM and CD8+ TEM, while CD4+ naïve cells were lower than in healthy controls at baseline.

**Conclusion:** Investigating how targeted treatment modulates the immune system is important since infections are still a

major complication in patients with CLL. Our analysis of venetoclax monotherapy reveals early changes in several immune cell populations. We observed a significant decrease in CLL cells already at 4–6 hours, and showed that major changes in T cells are induced primarily at the 4-week timepoint. All T cell populations decreased with a few exceptions, and cells initially elevated such as CD8+ T cells, CD8+ TEM as well as T cells expressing markers of exhaustion were normalized at week 4. Altogether, we showed here that venetoclax reduced immune cells including immunosuppressive features in T cells, possibly allowing for immune restoration following the reduction of leukemic cells. Indirect effects might contribute to the changes observed, where depletion of CLL cells might affect the number of normal lymphocytes. Olink analysis is ongoing to reveal very early changes in the proteomic pattern.

**Keywords:** Venetoclax; early; immunomodulation

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1210

### **Title:** Imaging mass cytometry reveals distinct cellular architectures in proliferative and non-proliferative regions of the chronic lymphocytic leukaemia lymph node tumour microenvironment

**Authors:** Paul Buckley[a], Stella Bouziana[a], Timothy Woo[a], Hanna Mohamed[a], Aamina Ahmed[a], Julie Chan[a], Liron Barnea Slonim[a], Cynthia Bishop[b] and Piers Patten[b]

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**Background:** The tumour microenvironment (TME) of chronic lymphocytic leukemia (CLL) lymph nodes (LN) plays a critical role in disease biology, but its spatial organization at the single cell level remains incompletely understood. Proliferation centres (PCs) in CLL LN, histologically defined zones enriched for larger, activated tumour cells, are considered drivers of disease progression. We hypothesized that PCs are spatially organized hubs of tumour–immune–stromal interaction that can be systematically mapped using multiplex imaging, revealing cellular phenotypes and interaction patterns not discernible by conventional histology.
**Aim:** To characterise the spatial architecture and immune microenvironment of CLL LNs using Imaging Mass Cytometry (IMC), with a particular focus on proliferative versus non-proliferative tumour regions.
**Methods:** We optimized a 31-plex panel to identify tumour, immune, and stromal cells. IMC was applied to 15 regions of interest from 6 LN biopsies of untreated CLL patients. Samples were stained with metal isotype-tagged primary antibodies and imaged by Hyperion mass cytometer. The 'steinbock' pipeline permitted raw image pre-processing, via which DeepCell Mesmer was used for deep-learning based automated cell segmentation. In total, 314,668 cells were segmented from 15 images. We employed consensus clustering to phenotype cells based on marker expression. Post-phenotyping, cellular neighbourhood (CN) and spatial contexts were computed using imcRtools. Subsequently, we analyzed (i) the cellular composition within neighbourhoods and (ii) cellular neighbourhood presence (%) across samples. We focused on regions enriched in Ki67 + CD20 + CD79a + cells as a surrogate for PCs, reflecting their histological definition as zones enriched for larger, proliferating tumour cells.
**Results:** We found a heterogenous abundance of cell populations across CLL LNs with clustering identifying a range of key immune and stromal subsets. B cell presence ranged from 31% to 52% (mean 38%). The second most common population identified was CD4+ T cells. Subsequently, we characterized 15 distinct CNs, each with diverse cellular composition. Presence of CNs across CLL samples was variable. We identified 7 distinct B cell rich regions, with e.g. CN10 almost exclusively comprising B cells. Ki67 + B cells were present across multiple tissue regions/CNs, but predominated in CN9. CN9 was also enriched with phenotypically diverse B cell subsets and a wider array of immune cells, including CD8/CD4 T subsets, Tregs, and CD11c + cells. There was considerable variation in CN9 presence across CLL samples (mean 4.6%, range 0.5–12.3%) which were small and scattered across samples. Intriguingly, we noted strong CD45RA + CD20+ expression throughout these tumours, finding that CD45RA + B cells typically were distinct from Ki67+ B cells. We next focused on the proliferative versus non-proliferative areas. A highly common feature across CLL samples were aggregates with a core strongly enriched for CD20++ Ki67-CD45RA + cells (CN10) and a lower immune infiltrate compared to the proliferative regions. CN15 surrounded the core and comprised B cells with numerous CD8T, DCs, CD68+/CD16+ cells. Spatial context analysis revealed the most common interfacing neighborhoods across these CLL LNs involved CN10 and CN15, together forming distinct structured regions. Across samples, CN10-15 interfacing cells represented a mean ~14.17% of cells ranging from 0.91% to 37.01%. DBSCAN was used to delineate these distinct, non-proliferative CN10-15 structures. The circularity of these regions were highly variable. On average there were ~22 structures per image (sd = 14.77, range 2–49). The mean area of these structures was 5,680 $um^2$ (sd = 19,579 $um^2$). Indeed, the presence, size and shape of these non-proliferative regions varied drastically across CLL tumours.
**Conclusion:** IMC revealed that Ki67+ vs. Ki67- tumour regions are spatially segregated and biologically distinct. These compartments differ in B cell phenotypes and surrounding immune milieu, forming functionally unique micro-environments. This spatial heterogeneity highlights the complexity of the CLL TME and offers critical insights into disease biology. Further, it provides a foundation for developing novel prognostic tools and therapeutic strategies, particularly those aimed at targeting or modulating the tissue microenvironment in CLL.

**Keywords:** Tumour-microenvironment; CLL; spatial

**Please indicate how this research was funded:** CARP Fellowship

**Please indicate the name of the funding organization:** MRC

## Abstract ID: 1303

### **Title:** Targeting lysosomes in the chronic lymphocytic leukemia microenvironment

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**Background:** Chronic lymphocytic leukemia (CLL) is the most prevalent hematological malignancy and is characterized by the accumulation of monoclonal B cells in the blood, bone marrow, lymph nodes, and spleen (Hallek et al., 2018). Although treatment outcomes have been improved using targeted therapies such as BTK and BCL-2 inhibitors, cures are not achieved, and therapy-resistant disease is frequently observed upon relapse (Lewis et al., 2024). It has been determined that a key driver of drug resistance is the protective microenvironment, which shields CLL cells from apoptosis (Kurtova et al., 2009). Lysosomes have been identified as emerging therapeutic targets in cancer due to their roles in nutrient recycling, reactive oxygen species (ROS) regulation, and secretory activity through extracellular vesicles (EVs) (Eriksson & Öllinger, 2024; Tang et al., 2020). Lysosome-disrupting agents lead to apoptosis in cancer cells, including CLL cells (Manivannan et al., 2025). Our research identified siramesine as a lysosome-disrupting agent that selectively kills CLL cells by inducing lysosomal membrane permeabilization (LMP), autophagy inhibition, and ROS accumulation (Manivannan et al., 2024). However, since siramesine is not FDA-approved, we repurposed the FDA-approved antipsychotic drug pimozide (Meyer et al., 2017; Nelson et al., 2011). This study aims to answer whether pimozide can act as an effective lysosome-disrupting agent in CLL and whether it can overcome the protective effects of the microenvironment by inducing lysosomal membrane permeabilization.

**Methods:** Primary CLL cells were treated with pimozide, and apoptotic cell death was measured using Annexin V/7-AAD staining through a flow cytometer. LMP was assessed via Lysotracker Red staining and confirmed by the cytosolic release of cathepsin D. Intracellular ROS production was detected using DHE, and mitochondrial ROS was detected using MitoSox staining. Autophagy was evaluated through LC3 and LAMP1 co-localization using immunofluorescence microscopy. To mimic the protective effects of the bone marrow microenvironment, we stimulated CLL cells with IL-4/CD40L or co-cultured them with HS-5 stromal cells. Additionally, we analyzed the impact of pimozide on intracellular survival pathways, including STAT3, STAT5, and MCL-1 expression, using Western blotting. Importantly, the cytotoxic effects of these treatments were assessed on non-malignant immune cells and relapsed patient samples using flow cytometry.

**Results:** Pimozide monotherapy induced pronounced lysosomal membrane permeabilization (LMP), demonstrated by the loss of Lysotracker Red signal and redistribution of cathepsin D into the cytosol. It also impaired the co-localization of autophagosomes with lysosomes, effectively blocking autophagy, a key cell survival mechanism. This lysosomal disruption was associated with elevated levels of intracellular reactive oxygen species (ROS) and mitochondrial ROS. Notably, when combined with venetoclax, a Bcl-2 inhibitor, pimozide markedly enhanced ROS production and mitochondrial superoxide generation, resulting in synergistic induction of apoptotic cell death. Importantly, this combinatorial effect persisted even in the presence of microenvironmental protection conferred by CD40L and IL-4. Pimozide also demonstrated efficacy in primary CLL samples derived from relapsed patients, highlighting its potential in treatment-resistant disease. Importantly, pimozide exhibited selective cytotoxicity toward CLL cells, with minimal impact on normal B cells from healthy donors, as well as $CD3^+$ and $CD8^+$ T cells. Mechanistically, pimozide inhibited the phosphorylation of STAT5 and STAT3, key mediators of cytokine-driven survival pathways in CLL, even in the presence of IL-4 stimulation. Additionally, pimozide downregulated the anti-apoptotic protein MCL-1, which is commonly upregulated in venetoclax-resistant CLL, further supporting its potential role in overcoming therapeutic resistance.

**Conclusion:** The lysosomotropic agent pimozide sensitizes CLL cells to venetoclax by targeting lysosomal integrity, inducing ROS, and inhibiting survival signaling. Pimozide represents a novel treatment strategy for patients with drug-resistant CLL because it induces apoptosis and inhibits cell survival.

**Keywords:** Lysosome targeted therapy; drug repurposing; bone marrow microenvironment

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**Please indicate the name of the funding organization:** University Hospital Foundation

## Abstract ID: 1305

**Title:** $CLECL1^+$ proliferative CLL cells remodel the lymph node microenvironment through fibroblast reprogramming, Th2 polarization, and M2 macrophage induction to promote immune evasion and disease progression

**Authors:** Shih-Shih Chen[a], Susan Buhl[b], Antonio Ferreira[c], YuJu Chen[a], Kelly Wong[a], Samantha Stoneking[a], Angelika Jiang[a], Anita Ng[a], Martina Cardillo[a], Gonzalo Blanco[a], XiaoJie Yan[a], Jon Aster[c], Matthew Scharff[b] and Nicholas Chiorazzi[d]

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Secondary lymphoid organs (SLOs) are architecturally organized tissues where stromal cells, T cells, and accessory immune cells coordinate effective immune responses. However, in the context of tumor infiltration, these non-malignant components undergo profound reprogramming. The structural integrity of SLOs becomes disrupted, facilitating pathological expansion and the emergence of an immunosuppressive tumor microenvironment (TME). This cancer-reprogrammed niche is typically characterized by the accumulation of exhausted T cells and elevated levels of tumor-promoting growth factors, ultimately supporting malignant cell survival and immune evasion. Elucidating the mechanisms that drive TME remodeling is essential for developing strategies to restore immune function in tumor-draining lymphoid tissues. Proliferation centers (PCs) in chronic lymphocytic leukemia (CLL) are specialized micro-anatomical niches within lymphoid tissues where CLL cells receive critical survival and proliferative signals from the microenvironment. These centers play a key role in disease progression by fostering CLL cell expansion, immune evasion, and resistance to therapy. Recently, multiplex fluorescence confocal microscopy of FFPE lymph node (LN) samples from 13 CLL patients revealed a disrupted stromal network characterized by CCL19highCCL21low LN fibroblasts

interacting with the proliferative fraction (PF) of CLL cells within PCs. Single-cell RNA sequencing (scRNA-seq) of sorted CD45$^+$ and CD45$^-$ cells from these LNs further demonstrated ligand-receptor-mediated crosstalk between CCL19$^+$ stromal cells and cycling CLL cells co-expressing CCR7 and CLECL1. CLECL1 (C-type lectin-like protein 1) is a T-cell costimulatory molecule that induces IL-4 production. Consistent with scRNA-seq findings, co-culture of CLECL1$^+$ PF cells or recombinant CLECL1 (rCLECL1) with monocytes promoted the differentiation of CD206$^+$CD80$^-$ macrophages, indicative of an M2-biased immunosuppressive phenotype. Furthermore, rCLECL1 engagement in T cells induced Th2 differentiation and expansion, marked by Th2-associated cytokines (IL-4, IL-5, IL-9, IL-13), transcription factors involved in naïve T cells (TN) to-Th2 conversion (GATA3, MAF, JAK1), and cytokines associated with other Th subsets (IL-2, IFN-γ, IL-12, IL-17, IL-21). We further found that CLECL1$^+$ but not CLECL1$^-$ CLL cells are enriched for CCR7, the receptor for CCL19. CLECL1$^+$ CLL B cells promote nurse-like cell (NLC) formation and secrete lymphotoxin (LTαβ), which stimulates LN stromal cells to produce CXCL13, CXCL12, and CCL19. Treating healthy human LN fibroblasts (HLFs) with lymphotoxin similarly induced CCL19 production, suggesting that CLECL1$^+$ CLL cells may directly reprogram LN fibroblasts. Moreover, exposure of HLFs to rCLECL1 or co-culture with activated CLECL1$^+$ CLL cells induced morphological changes in fibroblasts, including cell stretching, pulling, and gap formation—alterations that may facilitate malignant B cell infiltration. Supporting this, patient-derived xenograft (PDX) models involving flank injections of Matrigel mixed with CLL-conditioned HLFs and autologous CLL B cells demonstrated expansion of CLECL1$^+$ B cells alongside reprogrammed fibroblasts, characterized by loss of podoplanin (PDPN) and increased secretion of CCL19 and CXCL13. Depletion of CLECL1$^+$ B cells in the standard CLL-PDX model resulted in reduced Th2 T cell frequency and impaired CLL B cell growth. To evaluate CLECL1 as a therapeutic target, we generated IgG monoclonal antibodies (mAbs) reactive to the external domain of human CLECL1 using mice immunized with a KLH-conjugated peptide. These mAbs recognize CLECL1 by ELISA and flow cytometry and effectively block the expansion of Th2 cells and NLCs. Importantly, they induce antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) in vitro and inhibit the growth of MEC1 in NSG mice. Furthermore, anti-CLECL1 mAbs also block Th2 T cells and CLL cell growth in PDX models. These findings highlight the therapeutic potential of CLECL1 targeting in CLL. Together, these findings establish CLECL1$^+$ CLL cells as central architects of the tumor-supportive microenvironment within lymphoid tissues. Through their ability to engage T cells, monocytes, and fibroblasts, CLECL1$^+$ cells orchestrate a complex immunosuppressive and pro-proliferative niche that facilitates disease progression. Targeting CLECL1 disrupts these pathogenic interactions, making it a promising therapeutic strategy to dismantle the CLL-associated TME and restore anti-tumor immunity. These insights provide a strong rationale for further preclinical and clinical development of CLECL1-directed therapies in CLL and other B-cell leukemias and lymphomas.

**Keywords:** Tumor microenvironment; anti-CLECL1 antibody; preclinical mouse model

**Please indicate how this research was funded:** AWSM foundation and SAA foundation.

**Please indicate the name of the funding organization:** AWSM foundation and SAA foundation.

# Abstract ID: 1311

## **Title:** Targeted degradation of NF-κB RelA/p65 by PBD-based PROTACs as a novel therapeutic strategy in chronic lymphocytic leukaemia

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Dysregulation of NF-κB signalling is a hallmark of chronic lymphocytic leukemia (CLL), promoting disease progression, microenvironment-mediated resistance, and treatment failure. However, direct pharmacological inhibition of canonical NF-κB components has been limited by significant on-target toxicities in healthy tissues. To address this, we developed a novel class of pyrrolobenzodiazepine (PBD)-based proteolysis-targeting chimeras (PROTACs) designed to selectively degrade RelA/p65, a critical NF-κB subunit in CLL. PROTACs induce degradation rather than inhibition by co-opting the ubiquitin-proteasome system to eliminate a protein of interest (POI). Through bifunctional design, PROTACs simultaneously bind the POI and recruit an E3 ubiquitin ligase, leading to POI ubiquitination and subsequent proteasomal degradation. This mechanism allows for selective targeting of non-enzymatic, 'undruggable' proteins such as transcription factors, while reducing the need for sustained high drug concentrations. Our lead compound, JP-163-16, was synthesised by conjugating a C8-linked PBD warhead to a cereblon-binding ligand via a five-carbon linker. This design abrogated the DNA-binding activity of the PBD, shifting its mode of action from genotoxicity to targeted protein degradation. JP-163-16 demonstrated potent cytotoxicity in MEC-1 CLL cells (mean $LC_{50}$ = 0.14 μM) and 20 primary CLL samples (mean $LC_{50}$ = 0.23 μM), while exhibiting significantly lower toxicity in normal B and T lymphocytes (mean $LC_{50}$ = 19.1 μM (83-fold) and 36.4 μM (158-fold), respectively), indicating a favourable therapeutic index. Mechanistic evaluation showed that JP-163-16 induced proteasome-dependent degradation of RelA/p65. Furthermore, cereblon-dependent E3 ligase recruitment was confirmed as PROTAC activity was significantly reduced (5-fold) in cereblon-deficient, lenalidomide-resistant RPMI-8226 cells. Importantly, selective degradation of RelA/p65 provides a targeted approach to suppress NF-κB signalling in CLL cells, while sparing normal tissues and avoiding the systemic toxicity observed with previous NF-κB inhibitors. To our knowledge, JP-163-16 represents the first PROTAC capable of selectively degrading a single NF-κB subunit. These findings establish a proof-of-concept for subunit-specific NF-κB targeting and support further development of this strategy as a novel, precision-based therapy for RelA/p65-driven malignancies such as CLL.

**Keywords:** PROTAC; NF-kB RelA/p65

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**Please indicate the name of the funding organization:** N/A

## Abstract ID: 1312

**Title:** Zelebrudomide (NX-2127) is a cereblon-modulating BTK degrader that reverses immune dysfunction and modifies both T-cell and CLL-cell transcriptional signatures in patients with CLL

**Authors:** Tiana Huynh[a], Sonia Rodriguez-Rodriguez[b], Carly Roleder[b], Andrew Chen[b], Vi Lam[b], Alexandra Muir[b], Geoffrey Shouse[c], Ernestine Lee[d], May Tan[d], Sarah Whelan[d] and Alexey Danilov[e]

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**Introduction:** Targeted protein degradation has emerged as a promising strategy to overcome resistance to Bruton tyrosine kinase inhibitor (BTKi) therapy in chronic lymphocytic leukemia (CLL). Zelebrudomide and bexobrutideg are clinical stage degraders that target BTK for degradation through recruitment of the cereblon (CRBN) E3 ubiquitin ligase complex. Zelebrudomide is uniquely engineered to also maintain CRBN immunomodulatory activity, enabling a dual mechanism of action. While BTKis enhance T cell function in CLL, the impact of BTK and IKZF1/3 degradation on the immune microenvironment and malignant CLL cells remains poorly understood. To elucidate the BTK-centric role in CLL and contrast that with the enhanced immunomodulation of zelebrudomide, we evaluated zelebrudomide and bexobrutideg in preclinical models and in patients with CLL.
**Methods:** Peripheral blood mononuclear cells (PBMCs) were isolated from patients with CLL. CLL cells were obtained via CD3+ exclusion. T cells were purified using Dynabeads, stimulated with αCD3/CD28, and analyzed by flow cytometry. For Th1/Th2/Treg-polarization assays and bulk RNA-seq analysis, FACS-sorted naïve T cells were used. Lenalidomide and ibrutinib were used as controls. For in vivo studies, NSG mice were co-engrafted with OCI-LY19-Luc-GFP cells on day 0 and healthy donor PBMCs on day 3, followed by daily oral treatment with zelebrudomide (90 mg/kg), bexobrutideg (30 mg/kg), or vehicle starting on day 5 for up to 3 weeks. Tumor burden was monitored via bioluminescence imaging (BLI). PBMCs from patients treated with zelebrudomide (NCT04830137) were collected at baseline, C1D8, and C2D1 and analyzed by bulk and single-cell RNA-sequencing (scRNA-seq).
**Results:** Both zelebrudomide and bexobrutideg induced BTK degradation in primary CLL cells. Zelebrudomide, but not bexobrutideg, induced degradation of IKZF1/3 in CLL and T cells, confirming its CRBN immunomodulatory activity. RNA-seq of naïve T cells from CLL patients treated with zelebrudomide revealed a strong enrichment of inflammatory response genes (e.g. IL15, CCR7, CXCL10, CXCL11, and ICAM1) after 3 and 24 hours of treatment. In contrast, bexobrutideg did not induce the inflammatory gene signature. In CD40L-expressing stromal co-cultures, neither degrader (nor ibrutinib or lenalidomide) induced apoptosis in CLL cells. However, under IL-21-driven proliferative conditions, zelebrudomide and lenalidomide, but not bexobrutideg or ibrutinib, induced S phase arrest while increasing G0/G1 entry, thereby significantly reducing CLL cell proliferation. While BTK degraders did not change expression of the activation markers CD69, CD25 and PD1, zelebrudimide, but not bexobrutideg caused upregulation of HLA-DR and CD38 in CD4+/CD8+ T cells, promoted IFN-γ and IL-2 under Th1 polarizing conditions, and reduced Treg differentiation, without affecting Th2 associated markers (IL-4, GATA3). Furthermore, zelebrudomide enhanced immunological synapse formation similar to lenalidomide (E:T ratio 1:2, assessed by confocal microscopy), accompanied by upregulation of CD54/ICAM-1 on both T cells and CLL cells. In cytotoxicity assays, zelebrudomide increased granzyme B secretion and enhanced CD8+ T cell-mediated killing of OCI-LY19 cells (E:T ratio 1:10). In a PBMC humanized mouse model engrafted with OCI-LY19 cells, zelebrudomide and bexobrutideg significantly prolonged survival in vivo. Analysis of tumor-infiltrating lymphocytes and BLI is ongoing. We next evaluated transcriptional correlates of clinical response in zelebrudomide-treated CLL patients. Bulk RNA-seq at baseline showed that responders had lower expression of Th17 and Th2 pathway genes compared to non-responders. By C2D1, scRNA-seq analysis showed that responder CD4+ T cells exhibited increased activation and oxidative phosphorylation, while CD8+ T cells demonstrated reduced exhaustion. At baseline, CLL cells from responders displayed upregulation of DNA damage response and MAPK/RTK signaling pathways, along with downregulation of mitochondrial quality control, protein-folding, and antigen presentation pathways. By C1D8, responder CLL cells selectively downregulated multiple heat shock protein genes (e.g. DNAJB1, HSPH1, HSPD1, HSPE1), a pattern not observed in non-responders.
**Conclusion:** Zelebrudomide and bexobrutideg induce rapid BTK degradation in primary CLL cells. Through CRBN engagement, zelebrudomide induces anti-proliferative activity in primary CLL cells. Furthermore, zelebrudomide uniquely restores Th1 polarization, suppresses Tregs, and enhances cytotoxic immune function in vitro, while reducing tumor burden in vivo. Distinct transcriptional programs in both immune and CLL cells differentiated zelebrudomide clinical responders from non-responders. These findings highlight zelebrudomide's dual antitumor and immunomodulatory mechanisms and support its continued development in CLL and other lymphoid malignancies.

**Keywords:** CLL; targeted protein degradation; degrader

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1438

**Title:** Central nervous system involvement in chronic lymphocytic leukemia: an international retrospective study by ERIC, the European Research Initiative on CLL

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**Background:** CNS involvement (CNSi) of chronic lymphocytic leukemia (CLL) is a rare condition with no consensus on diagnosis and limited evidence for the management and outcome.

**Methods:** This is an international, multicenter, retrospective study conducted by the European Research Initiative on CLL (ERIC). The study defined CNSi of CLL by: (1) detection of CLL cells in the cerebrospinal fluid (CSF) or confirmation of CLL infiltration of the CNS based on a tissue biopsy, (2) clinical or radiographic evidence of neurologic disease, and (3) the absence of other explanations for the neurologic findings. We defined complete remission (CR) of CNS disease by resolution of clinical symptoms with normalization of CSF and neuro-imaging findings. Partial response (PR) was defined by improvement of clinical symptoms with normalization of CSF and at least a 50% decrease of involved sites in neuro-imaging. For the CLL-CNSi prevalence calculation, we restricted the analysis to data from the Danish Lymphoid Cancer Research (DALY-CARE) data resource.

**Results:** A total of 48 patients from 26 centers in 15 countries met all three diagnostic criteria of CLL-CNSi. Median age at diagnosis of CNSi was 64 years. Most patients were males (73%), had Binet stage A at CLL diagnosis (61%), and had untreated CLL at the time of CNSi (63%). Motor impairment was the most common symptom (38%) followed by visual impairment (32%). Overall, 4,421 patients in DALY-CARE had medical notes from hematologists in the electronic health records covering half of the Danish population. Seven patients were treated for CLL-CNSi, while 6 of them met the pre-defined diagnostic criteria for CLL-CNSi. Thus, the estimated population-based prevalence of CLL-CNSi in Denmark was 0.14% (6/4,421). Of 48 patients in our cohort, 47 received CNSi-directed treatment. Over 80% of patients received a single line of CNSi-directed treatment (38/47, 80.9%). Smaller proportions of patients received two (6/47, 12.7%) to three or more lines of treatment (3/47, 6.4%). Intrathecal chemotherapy was administered in 20/47 (42.6%) patients, and 3/47 (6.4%) proceeded to allogeneic stem cell transplantation. In the first line setting, Bruton tyrosine kinase inhibitors (BTKis) given as monotherapy or in combination with an anti-CD20 antibody, were most commonly used (18/47, 38.3%), followed by fludarabine, cyclophosphamide, and rituximab (FCR; 7/47, 14.9%), venetoclax plus anti-CD20 antibody (4/47, 8.5%), and other chemoimmunotherapy (CIT) regimens typically used in CNS lymphoma (4/47, 8.5%). In the second-line setting, BTKi-based treatment with ibrutinib (3/9, 33.3%) and CNS lymphoma regimens (3/9, 33.3%) were most commonly used. Rates of overall response (ORR) and CR for the CNS disease to any CNSi-directed treatment were 82.9% (34/41) and 70.7% (29/41), respectively. Patients treated with BTKis (monotherapy

or in combination with other treatment modalities) had the highest ORR (17/17, 100%) and CR (15/17, 88.2%), followed by CIT (ORR: 9/11, 81.8%; CRR: 8/11, 72.7%) and CNS lymphoma protocols (ORR: 2/3, 66.7%; CRR: 2/3, 66.7%). Patients who achieved a CR had significantly longer TTNT-D and OS (median not reached for both TTNT-D and OS) than those without a CR (median TTNT-D 3.8 months, median OS 14.5 months). Patients who received CNSi-directed treatment as their first-ever treatment for CLL (treatment-naïve CNSi) had a more favorable outcome than those who had been previously treated for CLL and presented with CNSi (relapsed/refractory CNSi). Median TTNT-D and OS were not reached for the treatment-naïve CNSi, and were 23 and 100 months, respectively, for the relapsed/refractory CNSi. Finally, patients treated with BTKis for CLL-CNSi had a longer TTNT-D compared with those treated with CIT including CNS lymphoma regimens (5-year TTNT-D 94.1% [95% CI =83.6%–100%] vs. 64.2% [95% CI =28.7%–100%]); OS did not differ statistically between the two groups. We next performed a univariable analysis to identify risk factors associated with inferior OS in patients with confirmed CNSi. Older age, not achieving CR after the first CNS-directed treatment, and the number of treatment lines for CLL before CNS-directed treatments were associated with worse OS in the univariable analysis. In the multivariable analysis, age and CR status after the first CNS-directed treatment correlated with OS, although only age reached statistical significance.

**Conclusions:** CLL-CNSi is a rare condition that can affect treatment-naïve patients or those with low-burden disease including MBL. Our study reinforces the importance of targeted agents in CLL, including patients presenting with neurologic symptoms.

**Keywords:** CLL-CNSi; BTKi

**Please indicate how this research was funded:**

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## Abstract ID: 1439

**Title:** Ten-year follow-up of a phase II study of ibrutinib in relapsed/ refractory chronic lymphocytic leukemia (CLL)/ small lymphocytic lymphoma (SLL)

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Ibrutinib, the first-in-class covalent Bruton tyrosine kinase (BTK) inhibitor, has revolutionized the treatment landscape for CLL/SLL since its approval over a decade ago. Here we report long-term outcomes of ibrutinib in an investigator-initiated trial established prior to its regulatory approval. (NCT01589302). OSU11133 is a phase II, single-center, open-label, non-randomized trial of ibrutinib monotherapy in patients with relapsed CLL/SLL. Patients were enrolled into two arms depending on presence vs absence of del (17p) by FISH. Patients were eligible if they were ≥18 years with a confirmed diagnosis of CLL/SLL, required treatment per the iwCLL guidelines, and had received ≥1 prior therapy. Ibrutinib was given daily at a dose of 420 mg until disease progression or unacceptable toxicity. The primary endpoint was 2-year progression-free survival (PFS). Secondary endpoints included 6-month overall response rate (ORR), overall survival (OS), PFS, and the incidence of adverse events. The Kaplan-Meier method was used to assess PFS and OS. Fine-Gray model treating death without Richter transformation (RT) as the competing risk was used for the univariable analysis. Cox regression models were used to evaluate PFS and OS. A total of 152 patients were enrolled between May 2012 and July 2014 and received treatment on study, with a median follow-up of 11.7 years. Median age was 65 (range 26–91), 44 (29%) were females, and 147 (97%) were White. Seventy-six patients had del(17p) identified by FISH or karyotype, and 76 did not have del(17p). Among 147 patients with available immunoglobulin variable region heavy chain (IGHV) mutational status, 115 (78%) patients had unmutated IGHV and 32 (22%) had mutated IGHV. Del(11q) was identified in 40 patients including 13 patients in the del(17p) arm. The median number of prior therapies was 3 (range 1–9). The 2-year PFS rate was 71.6% (95% CI 63.7–78.1) and the median PFS was 43.2 months (95% CI 38–55.5). Among patients with and without del(17p), median PFS was 38.6 (95% CI 27.6–43.2) and 61.6 months (95% CI 42.1–85.5), respectively. Multivariable models demonstrated that del(17p), higher number of prior therapies, high lactate dehydrogenase (LDH) at baseline, and IGHV unmutated status were associated with shorter PFS. The median OS was 79.5 months (95% CI 55.8–93.9). Median OS for patients with and without del(17p) was 49.7 months (95% CI 40.8–68.6) and 115.9 months (95% CI 80.2- not reached), respectively. Multivariable models demonstrated del(17p), number of prior therapy lines, and higher LDH at baseline to be associated with shorter OS, while higher hemoglobin at baseline was associated with improved OS. When including partial response with lymphocytosis, the ORR was 81.7%, regardless of del(17p) status. Twenty-six patients developed RT, which occurred more frequently in patients with del(17p) (19 patients, 25%) than in patients without del(17p) (7 patients, 9%), hazard ratio 2.88 (95% CI 1.2–6.9). The 6-month, 12-month, and 24-month cumulative incidences of RT were 2.6%, 5.9%, and 7.9%, respectively. Univariable models demonstrated del(17p), female sex, and higher LDH at baseline to be associated with higher risk of RT. Out of 93 patients tested routinely for BTK mutations during treatment on the study, BTK mutations were detected in 53 (57%) patients: 27 (out of 48) with del(17p) and 26 (out of 45) without del(17p). PLCG2 mutations were detected in 8 patients: 7 with del(17p) and 1 without del (17p). Four patients remain on treatment on trial, with a median of 12 years of treatment. Grade 3 or higher treatment emergent adverse events occurring in >20% of patients included neutropenia (53.9%), hypertension (30.9%), thrombocytopenia (28.9%), pneumonia (26.3%), and anemia (25.7%). Atrial fibrillation occurred in 9% of patients. This report presents long term outcomes of ibrutinib therapy in patients with relapsed/refractory CLL in a trial opened after initial efficacy signals, but prior to ibrutinib's regulatory approval. While newer generation BTK inhibitors have demonstrated more favorable toxicity profiles, ibrutinib remains an effective therapy that results in durable responses and that continues to be used across the world. The higher incidence of RT observed is likely related to the higher number of patients

with adverse risk characteristics and prior chemotherapy treatments. We emphasize the importance of investigator-initiated trials that are inclusive of high risk disease populations, and that provide life-saving therapies and valuable insight into long-term outcomes and safety.

**Keywords:** CLL; relapsed/ refractory; ibrutinib

**Please indicate how this research was funded:** Investigator initiated trial

**Please indicate the name of the funding organization:**

## Abstract ID: 1440

**Title:** Transcriptional reprogramming and survival co-dependencies of chronic lymphocytic leukemia resistant to venetoclax

**Authors:** Deepa Sampath[a], Daisy Diaz Rohena[a], Yu Zhou[a], Janani Ravikrishnan[b], Jyotsana Singh[a], Andrew Mitchell[b], Chaomei Liu[a], John Sanchez[a], Charmelle Williams[a], Trisha Wathan[a], Bailey Slawin[a], Kevin Bowman[a], Yaxia Yuan[c], Peiyi Zhang[d], Wanyi Hu[d], Julio Monteil[a], Guangrong Zheng[d], William G. Wierda[a], Nitin Jain[a], Daohong Zhou[c] and Jennifer Woyach[e]

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**Background:** High-risk chronic lymphocytic leukemia (CLL) that progresses on both Bruton's tyrosine kinase (BTK) inhibitors and venetoclax, particularly in the context of dysfunctional TP53, lacks effective treatment options. In a cohort of 15 dual-relapsed CLL, whole exome sequencing revealed that BCL2 mutations emerge in minor subclones at relapse, while TP53, BTK, and PLCG2 mutations dominate the relapsed population.

**Results:** Using single-cell RNA sequencing on paired pre-treatment and relapse samples, we observed significant transcriptional reprogramming impacting CLL survival. Among CLL cells (ROR1+/MS4A1+/CD19+/CD5+), we identified two minor relapse-specific subclusters (5 and 6) and three major clusters (0, 1, 2) present at both timepoints. Relapse-enriched clusters co-expressed BCL2, MCL1, and gained the MCL1-binding pro-apoptotic factor PMAIP1/NOXA, indicating a shift in apoptotic interactomes. We also noted expansion of a pre-existing BCL2-low cluster (cluster 1), which gained NF-κB signaling, upregulated BCL2A1/BFL-1 and MCL1, and expressed high levels of p53 targets CDKN1A/p21 and GADD45A. This cluster expanded in patients with mutant TP53 at relapse (e.g. R282W, S215I), but not in wild-type or mutation-lost cases, suggesting mutant TP53 may drive this phenotype. The most striking case involved R282W-mutant CLL expanding post-treatment cessation, suggesting intrinsic resistance features. Meanwhile, most relapsed cells fell into clusters 0 and 2, which downregulated BCL2 and upregulated BCL2L1/BCL-XL. Drug sensitivity profiling showed primary CLL cells progressed on venetoclax failed to activate pore-forming proteins BAK/BAX (< 30% activation), despite maintaining BAK1 and BAX transcripts, and gaining BCL2L11/BIM, implying blockade of the apoptotic machinery. BH3 profiling confirmed functional co-dependence on BCL2 and alternative anti-apoptotic proteins, especially BCL-XL, as XXA1_Y4eK, a BCL-XL–specific BH3 peptide, triggered the strongest cytochrome C release. To target this vulnerability, we tested WH25244, a novel BCL2/BCL-XL-targeting PROTAC, in 8 venetoclax-relapsed CLL samples. WH25244 is a bifunctional molecule derived from navitoclax that recruits VHL E3 ligases to BCL2 and BCL-XL proteins, resulting in degradation via the ubiquitin-proteasome system. The requirement of VHL for its activity allows WH25244 to target BCL2 / BCL-XL dependent cancer cells while sparing platelets from toxicity (BCL-XL dependent but VHL low). WH25244 effectively induced cytochrome C release and BAK/BAX activation ($R2 = 0.7035$, $p = 0.0007$). The highest sensitivity was observed in samples harboring BCL2 mutations (V156D, R107_110dup, G101V, F104S) and co-occurring TP53, BTK, and PLCG2 mutations. One highly sensitive sample had relapse-acquired BCL2L1 and BCR pathway genes (PLCG2, PRKCB, PRKCA). WH25244 was validated in BCL-XL–dependent leukemia cell lines and in BCL2-mutant CRISPR knock-in CLL models (G101V, F104L, A113G, R107_110dup), where it degraded ~50% mutant BCL2 at 100 nM and 72 hr of treatment and induced more cell death than venetoclax. Structural modeling showed WH25244 forms stable ternary complexes with mutant BCL2 and VHL. Inactivation of the VHL E3 ligase ligand on WH25244 hampered its anti-leukemic effect and abrogated BCL-XL/BCL2 degradation. Importantly, WH25244 avoids platelet toxicity linked to BCL-XL inhibition, as platelets express minimal VHL. Last, we studied the effect of WH25244 against normal lymphocyte subsets from healthy donors. Apoptosis was detected at 18 hours of treatment by Annexin V / TMRM staining of CD19+ B cells, CD3+ T cells, and CD56+ NK cells via flow cytometry. Like venetoclax, WH25244 induced apoptosis of normal B cells in the nanomolar range. Toxicity to T cells remained low, and 100 nM WH25244 caused 1.7-fold more toxicity to NK cells than venetoclax.

**Summary/conclusion:** These findings support WH25244 as a promising agent to overcome venetoclax resistance in dual BCL2/BTK inhibitor–relapsed CLL, by targeting resistant BCL2 variants and compensatory anti-apoptotic programs.

**Keywords:** CLL; dual refractory CLL; transcriptional reprogramming

**Please indicate how this research was funded:** Peer reviewed grant funding.

**Please indicate the name of the funding organization:** CLL Global Research Foundation, University Of Texas MD Anderson CLL Moonshot, Leukemia Lymphoma Society - Translational research project.

## Abstract ID: 1441

**Title:** A functional prognostic model predicts progression free survival in patients with relapsed chronic lymphocytic leukemia treated with ibrutinib + venetoclax

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**Background:** Targeted therapies have made a breakthrough in treatment of chronic lymphocytic leukemia (CLL), but many patients develop resistance, experience side effects, or relapse during therapy. Since traditional biomarkers for chemoimmunotherapy have failed to predict clinical outcomes on time-limited combination targeted therapies, new clinicobiological features are warranted. HO141/VISION (NCT03226301) is a randomized phase II trial that evaluates the efficacy of minimal residual disease (MRD) guided combination treatment with the Bcl-2 inhibitor (Bcl-2i) venetoclax and the BTK inhibitor (BTKi) ibrutinib in relapsed/refractory (R/R) patients with CLL.

**Aims:**

i. Identify associations between functional, genetic, and clinical characteristics in CLL
ii. Define biomarkers that predict progression free survival (PFS) on the HO141/VISION trial

**Methods:** Peripheral blood mononuclear cells (PBMCs) were collected from patients with CLL (n = 177) enrolled in the HO141/VISION trial prior to start of treatment. The samples were subjected to functional analyses: immunophenotyping (6 surface markers for B-, T- and NK-cell subpopulations) and (phospho)protein profiling (31 markers) by flow cytometry, and drug sensitivity screening (95 single agents, 87 combinations) using the CellTiter-Glo luminescent cell viability assay. Association analyses between functional, genetic (IGHV mutational status, TP53 aberrations, karyotypes), and clinical (Binet stage, MRD, PFS) features were investigated using the Wilcoxon rank sum test. SHAP values were calculated from random forest to understand the directionality and importance of each feature in predicting ex vivo drug sensitivity. To identify a biomarker predictive of PFS (20 events), the patient samples were divided into a training set (n = 80) and a test set (n = 77) stratified on IGHV and TP53 mutational status, as well as an independent validation set (n = 20). The (phospho)proteins detected by flow cytometry were paired and used as binary features. Univariate Cox regression and permutation testing in random survival forest identified the most predictive features using 10-cross validation in the training set ($p < 0.1$), and the overlapping features from the two selection methods were utilized as a multi-modal signature to predict PFS with various machine learning models.

**Results:** Genetic subgroups of CLL showed distinct ex vivo drug sensitivities. We confirmed that TP53 aberrant CLL cells are less sensitive to chemotherapy and nutlin 3a relative to TP53 wild-type CLL cells. We identified a trend toward higher sensitivity to BTKi and PI3Ki single agents or combinations in CLL cells from high-risk (TP53 aberrant, IGHV unmutated) patients, and to Bcl-2i/combinations in CLL cells from low-risk (IGHV mutated) patients. We found that phosphorylation of PLCγ2 (pY759), which is downstream of BTK, was significantly higher in CLL cells from low-risk patients than in CLL cells from high-risk patients. This may explain the reduced ex vivo sensitivity to BTKi in this patient group. Association studies of functional, genetic, and clinical features showed that (phospho)protein profiles had the highest predictive power on ex vivo drug sensitivity. We used both functional and genetic features to predict patient-specific PFS. Our machine learning model selected only functional features as most predictive of PFS, ex vivo sensitivity to vandetanib (VEGFR/EGFR inhibitor), ruxolitinib (JAK1/2 inhibitor) + venetoclax (Bcl-2i), and acalabrutinib (BTKi) + ZSTK474 (PI3Ki); relative expression/phosphorylation level of Bcl-2|BTK (pY223) and p90RSK (pS380)|PLCγ2 (pY759); and a high proportion of CD8+ T cells. These findings demonstrate that patterns of ex vivo drug sensitivity profiles can predict response to a given but different treatment. The resulting functional prognostic model (fPM) demonstrated increased and robust performance compared to the CLL international prognostic index (CLL-IPI) and other reported scores.

**Summary/conclusion:**

i. Genetic subgroups of CLL show distinct ex vivo drug sensitivities, and protein profiles have the highest predictive power on ex vivo drug sensitivity

Functional features predict PFS in patients with R/R CLL treated with ibrutinib + venetoclax, warranting clinical validation also for frontline treatment of CLL.

**Keywords:** Biomarker; targeted therapy; outcome prediction

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1442

**Title:** NGS-based MRD assessment refines prognostication after venetoclax-based first-line combinations for chronic lymphocytic leukemia: a prospective analysis of the phase 3 GAIA/CLL13 trial

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**Introduction:** The GAIA/CLL13 trial showed superior progression-free survival (PFS) for venetoclax-obinutuzumab (GV) and GV + ibrutinib (GIV) compared to chemoimmunotherapy (CIT) and venetoclax-rituximab (RV) in fit treatment-naive patients with CLL without TP53 aberrations. Here, we investigate the prognostic value of high sensitivity measurable residual disease (MRD) assessment by next-generation sequencing (NGS) of immunoglobulin gene rearrangements.

**Methods:** MRD in peripheral blood (PB) at month 9 (MO9) and MO15 was analyzed by the amplicon-based EuroClonality NGS assay, reaching a sensitivity of $10^{-6}$, MRD in bone marrow (BM) at final restaging (2 months after the last intake of venetoclax) was assessed by four-colour flow cytometry (Flow). Landmark PFS and overall survival (OS) from MO15 were analyzed according to different MRD groups (uMRD4, $<10^{-4}$; uMRD6, $< 10^{-6}$). In the GIV arm, treatment with ibrutinib could be continued if a patient had MRD $\geq 10^{-4}$ at MO12/15.

**Results:** In total, 926 patients were randomized (CIT: 229, RV: 237, GV: 229, GIV: 231). Median follow-up was 63.8 months. NGS-based MRD data was available for 799 patients (86%) at MO9 and 816 patients (88%) at MO15. UMRD6 rates in PB at MO15 were 23% in the CIT arm, 24% with RV, 60% with GV, and 66% with GIV. In a landmark analysis, patients with MRD $\geq 10^{-6}$ by NGS in PB at MO15 had significantly shorter PFS than patients with uMRD6 across treatment groups (4-year landmark PFS rates, CIT: 36% vs. 89%, HR 9.89 [95% CI 4.01–24.41], $p < 0.001$; RV: 49% vs. 84%, HR 3.97 [2.20–7.18], $p < 0.001$; GV: 56% vs. 76%, proportional hazards assumption invalid $p < 0.001$; GIV: 73% vs. 86%, HR 2.25 [1.27–3.99], $p = 0.005$). In patients with PB uMRD6 at MO15, achievement of a complete response – as opposed to a partial response – was not associated with longer PFS in any of the treatment arms (uMRD6 and PR vs uMRD6 and CR, CIT: HR 1.51 [95% CI 0.25–9.05]; RV: HR 1.00 [0.33–3.07]; GV: HR 1.03 [0.53–1.98]; GIV: HR 1.03 [0.44–2.40]). Only 12 of 399 patients (3%) with uMRD6 in PB at MO15 had detectable MRD $\geq 10^{-4}$ by Flow in BM (CIT, n = 4; RV, n = 3; GV, n = 4; GIV, n = 1). In addition to PB MRD-positive patients ($\geq 10^{-4}$), those who achieved uMRD below the conventional cut-off of $10^{-4}$ in PB at MO15 but still had low levels of detectable MRD ($\geq 10^{-6}$ and $<10^{-4}$) by NGS also had shorter PFS than patients achieving uMRD6 in the CIT, RV and GIV arms while the difference in the GV arm was not significant (4-year landmark PFS rates, $\geq 10^{-4}$ vs $\geq 10^{-6}$ and $<10^{-4}$ vs uMRD6, CIT: 14% vs 60% vs 89%, $p < 0.0001$; RV: 23% vs 71% vs 84%, $p < 0.0001$; GV: 0% vs 68% vs 76%, $p < 0.0001$; GIV: 64% vs 75% vs 86%, $p = 0.002$). Finally, MRD $\geq 10^{-6}$ in PB by NGS at MO15 was not associated with significantly shorter OS in all treatment arms compared with uMRD6 (CIT: 90% vs. 98%, $p = 0.06$; RV: 96% vs. 100%, $p = 0.08$; GV: 95% vs. 96%, $p = 0.67$; GIV: 96% vs. 96%, $p = 0.84$).

**Conclusions:** The amplicon-based EuroClonality NGS assay reliably identifies a group of patients with uMRD6 who show exceptionally long PFS after venetoclax-based first-line treatment and chemoimmunotherapy. Post-treatment NGS-based MRD assessment further refines prognostication and reliably groups patients into MRD categories (uMRD6 vs $\geq 10^{-6}$ and $<10^{-4}$ vs $\geq 10^{-4}$) with significantly different PFS outcomes. Given the lack of additional prognostic value of clinical responses in patients with uMRD6 and the high concordance of uMRD6 in PB and uMRD4 in BM, the assessment of bone marrow might be omitted when NGS-based MRD is available.

**Keywords:** CLL; MRD; venetoclax

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## Abstract ID: 1501

**Title:** Efficacy and tolerability of venetoclax plus rituximab in relapsed or refractory chronic lymphocytic leukemia – real-world analysis of polish adult leukemia study group

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**Background:** The prognosis of patients with relapsed/refractory chronic lymphocytic leukemia (RR-CLL) has improved following the introduction of venetoclax into clinical practice. In the MURANO trial, RR-CLL patients treated with venetoclax and rituximab (VEN-R) experienced significantly longer progression-free survival (PFS) and overall survival (OS) compared to those treated with bendamustine and rituximab (BR). Since then, this regimen has become firmly established in the standard therapeutic landscape for RR-CLL and is widely used in clinical practice.

**Aims:** To assess the clinical efficacy and safety profile of VEN-R treatment in patients with RR-CLL outside of clinical trials.

**Methods:** We conducted a retrospective analysis of RR-CLL patients treated with VEN-R at hematology centers affiliated with the Polish Adult Leukemia Study Group (PALG) between 2019 and 2024.

**Results:** Clinical data of 363 RR-CLL patients treated with VEN-R were collected. The median age at initiation of VEN-R therapy was 69 years (range 32–91 years), and 215 patients (59.2%) were male. The median Cumulative Illness Rating Scale (CIRS) was 6 (range 0 - 19) and the median Eastern Cooperative Oncology Group (ECOG) performance status was 1 (range 0 - 3) with 57 (57/358, 15.9%) patients with ECOG status 2 or higher. Patients had received a median of 2 (range 1–10) prior lines of therapy, whereas 151 patients (41.6%) had relapsed after first-line treatment. 52 (52/363, 14.3%) recipients had previously received BTKi or 12 (12/362, 3.3%) PI3Ki-based therapies. Among evaluable patients, 48 of 331 (14.5%) had 17p deletion, whereas TP53 mutation was identified in 33 of 172 patients (19.2%). The median follow-up was 22.8 months. Response was assessed according to iwCLL 2018 criteria. The overall response rate (ORR) was 97.1% with 121 of 312 patients (38.8%) achieving complete remission (CR), 182 (58.3%) partial remission (PR), and 1 patient (0.3%) showing disease progression at the best response. Among patients with TP53 mutation, CR and PR were observed in 30 (90.9%), SD in 2 (6.1%), and the response was not assessed in one patient. Among patients with 17p deletion, 39 (81.3%) achieved CR or PR, 3 (6.3%) achieved SD, and the response was not assessed in six patients. In the whole cohort the median PFS and the median OS were not reached. The estimated 3-year PFS rate was 62.1% (95% CI 54.9–70.2), and the 3-year OS rate was 69.0% (95% CI 62.3–76.4). Six cases of Richter transformation (1.8%) were diagnosed during the follow-up period. There were 72 deaths recorded during observation: 24 (33.3%) due to infection and 11 (15.3%) due to disease progression. At last follow-up, 85 (23.5%) patients completed their scheduled chemotherapy regimen, therapy was discontinued in 106 (29.4%) cases, while 170 patients (47.1%) remained on treatment. Reasons for therapy discontinuation included mainly patient's death (n = 36, 34%) and treatment-related cytopenias (n = 17, 16.0%). The following adverse events of VEN-R treatment were reported during the study: all grade neutropenia during dose escalation (223/331, 67.4%, with grade 3/4 in 104/331 patients, 31.4%) and after dose escalation period (276/345, 80%, with grade 3/4 in 139/345, 40.3% of all neutropenia). Other adverse effects observed independently of treatment phase were: anemia (grade 3/4 in 38/328, 11.6%), thrombocytopenia (grade 3/4 in 57/328, 17.4%), pneumonia (66/351, 18.8%), neutropenic fever (14/351, 4.0%), diarrhea (15/349, 4.3%), autoimmune hemolytic anemia (8/346, 2.3%) and immune thrombocytopenic purpura (8/344, 2.3%) and in one case exacerbation of heart failure was observed.

**Summary/conclusion:** In this retrospective analysis, the outcomes of treatment with the VEN-R regimen in a real-life setting were slightly worse as compared to the results of the MURANO trial, probably due to the inclusion of a higher number of patients with advanced ECOG and more heavily pretreated, including BTK inhibitors.

**Keywords:** Venetoclax; relapsed/refractory CLL; real-world data

**Please indicate how this research was funded:**

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# Abstract ID: 1503

**Title:** Analysis of resistance-related mutations in patients with chronic lymphocytic leukemia treated with BTK or BCL2 inhibitors suggests different underlying resistance mechanisms and subclonal dynamics

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**Introduction:** Bruton tyrosine kinase (BTK) and B-cell lymphoma 2 (BCL2) inhibitors have revolutionized the treatment of chronic lymphocytic leukemia (CLL). However, acquired resistance remains a major clinical challenge. BTK and PLCG2 mutations are the primary drivers of resistance to BTK inhibitors (BTKi), while BCL2 mutations are associated with resistance to BCL2 inhibitors (BCL2i). Understanding the clonal evolution driven by these mutations is essential for optimizing subsequent therapeutic choices.

**Objectives:** To characterize the mutational landscape of CLL patients who discontinued BTKi and/or BCL2i due to progression, transformation, or toxicity. Specific aims included: (i) evaluating the frequency and distribution of BTK and PLCG2 mutations in patients progressing on BTKi; (ii) identifying BCL2 mutations and their impact on venetoclax resistance; (iii) analyzing the relationship between duration of treatment (DOT) and mutation emergence; and (iv) exploring the persistence of mutated clones post-progression and their impact on following therapies.

**Methods:** We included 114 samples from 101 CLL patients treated with BTKi (84 ibrutinib, 5 acalabrutinib, 1 zanubrutinib) and/or BCL2i (24 venetoclax). A total of 90 patients discontinued BTKi (63 for progression, 9 for Richter's transformation [RT], and 18 for toxicity/other causes), and 24 discontinued venetoclax (16 for progression, 5 for RT, and 3 for toxicity). Eleven patients who progressed on ibrutinib and subsequently on venetoclax had both samples sequenced. All cases were analyzed using a custom next-generation sequencing (NGS) panel with a limit of detection of 0.5% in an Illumina MiniSeq System.

**Results:** Among the patients that progressed under BTKi, we found BTK/PLCG2 mutations in 39/63 patients (61.9%). Moreover, BTK/PLCG2 mutations were underrepresented in RT patients in comparison to patients that progressed (22.2% vs. 61.9%, $p < 0.05$). Mutations in BTK/PLCG2 were totally absent in toxicity cases. Progression under BTKi displayed a bimodal distribution, distinguishing late progressors (LP, DOT >24 months) from early progressors (EP, DOT< 24 months) (median DOT 57.0 vs. 10.0 months, $p < 0.001$). LP patients showed a markedly higher frequency of BTK/PLCG2 mutations compared to EP patients (81.8% vs. 11.1%; $p < 0.001$). Similarly, they were more likely to appear in late-transformed patients with a higher DOT in BTK/PLCG2 mutated cases (median DOT 36.8 vs. 7.2 months, $p = 0.056$). NGS revealed 72 BTK-mutated clones, predominantly affecting cysteine 481 (n = 68; 94.4%), with the C481S mutation as the most frequent (n = 55; 76.4%). Additionally, resistance-associated mutations against non-covalent BTKi were identified in 7% of cases (L528W: 4.2%; T474I: 2.8%). In the case of PLCG2-mutated cases, we found 24 clones, whose mutations were mainly within the SH2 domain (41.7%). Among the 16 patients that progressed after venetoclax, 37.5% (n = 6) presented BCL2 mutations. We identified 16 clones, being the most prevalent mutation G101V (n = 4; 25.0%). No significant differences were observed in the frequency of mutations between RT and non-RT cases (37.5% vs. 20.0%; $p = 0.62$) or between LP and EP (33.3% vs. 37.5%; $p = 1.0$). Additionally, we analyzed four patients with mutations in BTK, PLCG2, or BCL2 across two distinct anatomical compartments. Two of these cases had developed Richter's transformation (RT) after ibrutinib or venetoclax treatment, whereas the others had experienced nodal progression following ibrutinib therapy. Notably, differences in the infiltration of the resistant clone were

observed across compartments in all four cases, with the disparity being particularly pronounced in RT, where the resistant clone showed a marked increase in nodal versus non-nodal samples. This finding highlights the importance of studying the compartment with the highest tumor burden, when possible. For the 11 patients who progressed on BTKi and then on venetoclax, seven of them carried, at least, one BTK mutated clone prior to venetoclax treatment. BTK-mutated clones persisted in three cases (42.9%), with those exhibiting a higher variant allele frequency (normalized VAF >40%) more likely to remain as dominant clones.

**Conclusions:** Our study highlights the complexity of resistance mechanisms in CLL, particularly with the emergence of different resistance pathways in BTKi-treated patients. Cross-resistance to next-generation non-covalent BTKi may arise under ibrutinib treatment. The tumor burden of BTK-mutated clones prior to venetoclax initiation appears to influence their persistence post-treatment, potentially affecting the efficacy of future therapies. These findings underscore the need for precise molecular monitoring and tailored therapeutic strategies to overcome resistance in CLL.

**Keywords:** Resistance; relapsed/refractory; sequencing

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## Abstract ID: 1601

### **Title:** Central nervous system involvement in patients with richter transformation

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**Introduction:** Central nervous system (CNS) involvement in patients with Richter transformation (RT) of chronic lymphocytic leukemia (CLL) is not well studied. This study aimed to describe clinical characteristics, treatment patterns, outcomes, and prognostic factors of CNS involvement in patients with RT seen at Mayo Clinic.

**Methods:** Patients with RT (diffuse large B-cell lymphoma [DLBCL] or high-grade B-cell lymphoma [HGBCL]) diagnosed between January 2005 and August 2024 who had CNS involvement were identified from the Mayo Clinic CLL Database and electronic health record. Clinical characteristics, treatment and follow-up data were abstracted. Overall survival (OS) was defined as time from CNS involvement detection to death from any cause and analyzed using the Kaplan-Meier method.

**Results:** A total of 36 patients with RT with documented CNS involvement were included: 24 with CNS biopsy confirmation and 12 with MRI evidence consistent with CNS involvement of biopsy-confirmed systemic RT. The median age at CLL diagnosis was 63 years (range 34–78), and 21 (58%) patients were males. IGHV was unmutated in 8 of 15 (53.3%) patients with data available. CLL FISH results prior to or at RT diagnosis were available for 26 patients: 9 (34.6%) with del(17p), 6 (23.1%) with trisomy 12, 7 (26.9%) normal, and 4 (15.4%) with del(13q). Four of 11 (36.4%) patients had TP53 somatic mutation prior to or at RT diagnosis. The median time between CLL and RT diagnoses was 1.4 years (0–23.1). Prior to RT, 12 of 36 patients had received treatment for CLL (median lines of therapy 2 [range 1–5]), 5 with chemoimmunotherapy (CIT) alone, 5 with CIT and novel agents, and 2 with novel agents only. The histology of RT was DLBCL in 33 patients and HGBCL in 3, including 2 MYC/BCL2 double-hit by FISH and 1 NOS. Thirteen (61.9%) had GCB subtype and 8 (38.1%) had non-GCB subtype by Hans algorithm (15 unknown). Ten (27.8%) had MYC/BCL2 double expression by IHC. Eight of 17 (47.1%) had PD-1 expression by IHC. Twenty of 28 (71.4%) exhibited clonal relatedness between CLL and RT. Twenty-two patients had CNS involvement at RT diagnosis (15 with isolated CNS involvement and 7 with concurrent systemic and CNS involvement); the remaining 14 had received a median of 1 line of therapy (range 1–5) for RT before developing CNS involvement (4 with isolated CNS involvement and 10 with concurrent systemic and CNS involvement), with a median time from RT diagnosis to CNS involvement of 11.1 months (range 1–41.6). Predominant CNS involvement sites included parenchymal (n = 24, 66.7%), leptomeningeal (n = 9, 25.0%), and ocular or cranial/spinal root (n = 3, 8.3%). The first treatment for CNS involvement included high-dose methotrexate-based (n = 25), other regimens (n = 4), none (n = 5) and unknown (n = 2). The median OS following CNS involvement detection for all patients was 13.0 months (95% CI 1.4–24.6). Patients with isolated CNS involvement (n = 19) had significantly better OS compared to those with concurrent CNS and systemic disease (n = 17) (median OS 34.7 vs. 3.1 months; $p < 0.001$; Figure 1A). Similarly, patients who presented with CNS involvement at RT diagnosis (n = 22) had longer OS than those who developed CNS involvement at RT progression (n = 14) (median OS 18.4 vs. 3.6 months; $p = 0.023$; Figure 1B). CLL treatment-naïve patients (n = 24) had significantly longer OS compared to those with previous CLL treatment (n = 12) (median OS 32.0 vs. 1.7 months; $p < 0.001$; Figure 1C). Patients with clonally unrelated RT (n = 8) showed a trend toward better OS than those with clonally related RT (n = 20) (median OS 61.6 vs. 5.7 months; $p = 0.056$; Figure 1D). Moreover, patients without TP53 alterations (n = 12) showed longer OS compared to those with TP53 alterations (n = 13) (median OS 17.8 vs 3.2 months; $p = 0.019$).

**Conclusions:** In this cohort of patients with RT who had CNS involvement, we found that CNS involvement at RT progression, concurrent CNS and systemic involvement, treatment exposure for CLL prior to RT, clonal relatedness between CLL and RT, and TP53 alterations were associated with poor OS after CNS involvement. Multicenter studies are needed to better define the incidence, clinical characteristics,

molecular profile, optimal therapeutic strategies, and prognosis for CNS involvement in patients with RT, particularly in the era of novel agents.

**Keywords:** Chronic lymphocytic leukemia (CLL); central nervous system (CNS); Richter Transformation (RT)

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## Abstract ID: 1602

### **Title:** Venetoclax in combination with chemotherapy in Richter syndrome patients: a real-life experience

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**Background:** The prognosis of Richter Transformation (RT) with standard chemoimmunotherapy (CIT) remains poor. A previous study with Venetoclax (V) in combination with R-DA-EPOCH in 26 RT pts demonstrated an ORR of 62% with a median PFS and OS of 10.1 and 19.6 months, respectively. Other subsequent experiences demonstrated the feasibility and safety of V based regimens in RT (Davids MS et al. Venetoclax plus dose-adjusted R-EPOCH for Richter syndrome. Blood. 2022 Feb 3;139(5):686-689).
**Aims:** This is a retrospective, multicenter study aimed to assess the efficacy and safety of the off-label use of V plus CIT in the treatment of RT.
**Methods:** All previously untreated RT pts, who received at least one cycle of V in combination with CIT, were included. We collected data about CLL, type of RT treatment. Response assessments were evaluated according to the Lugano 2014 criteria.
**Results:** 20 RT pts from 9 Italian centers, treated with V-based regimen from October 2018 to July 2024, were included. Median age was 63.5 years (33–73), with 75% of males. 11/17 pts were IGHV unmutated and 8/18 patients carried a mutation for Tp53 and/or a del17p. The median number of prior treatments for CLL was 2 (0–4) wich included: CIT in 10, BTKi in 10, V based-treatment in 4; 5 pts were previously untreated. The median time from CLL diagnosis to RT was 6.5 years (0–17). The clonal relationship was available and demonstrated in 16 pts. V was associated with R-DA-EPOCH, R-CHOP, and BFM in 10, 9 and 1 pts, respectively. Pts received a median number of 4 cycles (1–6). After at least 2 cycles, for the evaluable patients, ORR was 70% with 8 CR, 6 PR and 1 SD; 3 pts had a PD and 1 patient died after a cycle of R-DA-EPOCH for multiorgan failure. At the end of treatment ORR was 55% with 10 CR (50%), 1 PR (5%). 7 pts received allo-HSCT consolidation; 1 of them after a salvage treatment due to PD. At a median follow up of 11.5 months (1–78), 10 pts (including 5 who received HSCT) are alive in CR; median PFS and OS were not reached. All pts experienced grade 3–4 hematological toxicities: neutropenia (100%), anemia (50%) and thrombocytopenia (25%). Non-hematological toxicities included: febrile neutropenia (15%), covid19 (25%), other infections (30%), nausea (10%), thrombotic/hemorrhagic events (10%).
**Conclusions:** data from this real-life experience are in line with previous studies confirming the feasibility and the activity of V based combination in younger fit RT pts, where the high rate of initial response may bridge a significant proportion to allo-HSCT. New ongoing studies with V combinations are ongoing and the comparison with this real-life data may be worthwhile to better understand their therapeutic impact.

**Keywords:** Venetoclax; Richter Syndrome; allogeneic hematopoietic stem cell transplantation

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1603

### **Title:** Single-cell transcriptome analysis reveals molecular mechanism of stem-like cells in Richter transformation

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**Aims:** Richter Transformation (RT) is a heterogeneous lymphoma defined as the histologic progression of chronic lymphocytic leukemia (CLL) into an aggressive subtype, associated with extremely poor prognosis. Although several

molecular mechanism researches have been reported in RT, the key drivers remain incompletely understood. This study aims to construct the differentiation trajectory of RT cells and to identify key mechanisms driving RT by single-cell RNA sequencing (scRNA-seq).

**Methods:** scRNA-seq were performed for 12 lymph node tissue samples from 7 clonally related RT-DLBCL, 2 CLL, and 3 normal controls (NC) using the GEXSCOPE® platform (Singleron, China). Raw sequencing data were preprocessed using Cell Ranger. Quality control, dimension reduction, clustering, and cell type annotation were performed via Scanpy. Evaluation of tumor cell chromosomal copy number variations (CNVs) and genomic alterations was conducted using inferCNV. AUCell was used to score every single cell within HALLMARK pathways and metabolism-related pathways, and their correlation with CNV scores was analyzed. CytoTRACE2 was applied to predict cellular developmental potential and identify stem-like tumor cell subpopulations. Monocle2 was utilized to perform pseudotime analysis of tumor cells to evaluate the developmental trajectory of RT cells. Differential expression and enrichment analyses were conducted to identify key genes associated with tumor stemness.

**Results:** We obtained scRNA-seq data, comprising 80,253 cells, and we annotated the cells into five major clusters, B cells, T/NK cells, myeloid cells, plasma cells, and stromal cells. Based on tissue type, B cells were further classified into normal B cells, CLL cells, and RT cells. In RT, a remarkedly higher proportion of myeloid cells was observed compared to CLL and NC, suggesting a potentially important role of myeloid cells in RT. InferCNV analysis further revealed that CLL and RT cells exhibited higher CNV scores compared to normal B cells, with RT cells showing the most complex alterations. This supported both the accuracy of tumor cell annotation and the complex genomic landscape of RT. Furthermore, RT cells exhibited more activity in multiple hallmark pathways, including E2F targets, G2M checkpoint, MYC targets, NOTCH signaling, PI3K/AKT/mTOR signaling, and apoptosis, and were positively correlated with CNV scores. In addition, RT cells exhibited upregulation in metabolic activity, particularly oxidative phosphorylation, glycolysis, and TCA cycle, suggesting that metabolic reprogramming may promote proliferation and resistance to apoptosis in RT. CytoTRACE2 identified a subpopulation with high differentiation potential and stemness in RT cells, and pseudotime analysis revealed a developmental trajectory from low-differentiation/high-stemness to high-differentiation/low-stemness states in RT cells. A total of nine RT cell subclusters were defined along this trajectory. Based on stemness scores, the developmental starting point was confirmed as state 8, and genes highly expressed in this subcluster were enriched in pathways such as MYC signaling, cell cycle, oxidative phosphorylation, and mTORC1 signaling. Notably, we found BIRC5 (survivin) were significantly overexpressed in stem-like RT cells, suggesting that BIRC5 may represent novel molecular drivers in the pathogenesis and progression of RT.

**Conclusions:** In this study, we constructed a single-cell atlas of RT lymph node samples using scRNA-seq and identified a stem-like cell subcluster as the origin of cellular differentiation and proliferation in RT. The characterization of stemness-associated genes and metabolic reprogramming provides novel insights into the molecular drivers and targeted therapeutic strategies of RT.

**Keywords:** Richter transformation; single-cell transcriptome; molecular mechanism

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

# Abstract ID: 1606

## Title: Epcoritamab monotherapy in patients with first-line Richter's transformation: 2-year follow-up results from the phase 1b/2 EPCORE CLL-1 clinical trial

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**Background:** Richter's Transformation (RT) is a rare, but often fatal, evolution of chronic lymphocytic leukemia (CLL) into an aggressive lymphoma, in most cases diffuse large B-cell lymphoma (DLBCL). RT occurs in ~2%–10% of patients. Outcomes are particularly poor in patients previously treated for CLL, with median overall survival (OS) as short as 12.3 months compared to 59.6 months in treatment-naïve (1L) cases (Kittai AS, et al. Blood Cancer J. 2025;15:23). There is no established standard of care for RT, which highlights a critical unmet need for more effective therapies. Epcoritamab is a subcutaneous CD3×CD20 bispecific antibody approved for relapsed or refractory (R/R) follicular lymphoma and DLBCL after ≥2 lines of therapy (LOTs). In the ongoing EPCORE CLL-1 trial (NCT04623541), an early timepoint evaluation showed promising efficacy and manageable safety in patients with RT and ≤2 prior LOTs (Kater AP, et al. HemaSphere. 2024;8[suppl 1]. Abstract S163). Here, we report updated efficacy and safety results of epcoritamab monotherapy from the EPCORE CLL-1 trial, with a focus on 1L RT.

**Methods:** Adults with biopsy-proven transformation to CD20+ DLBCL, a history of CLL or small lymphocytic lymphoma, and with no prior treatment for RT (including patients who were ineligible for or refused chemotherapy) received epcoritamab 48 mg in 28-day cycles (C; once weekly [QW], C1–3; Q2W, C4–9; Q4W, C ≥ 10) until progression or unacceptable toxicity. Step-up dosing and corticosteroid prophylaxis were used in C1 to minimize cytokine release syndrome (CRS). Primary endpoint was overall response rate (ORR) assessed by PET-CT (Lugano criteria). Additional endpoints included complete response (CR) rate, progression-free survival (PFS), OS, duration of response (DOR), and safety. Safety data are

presented for the entire cohort of patients with RT, regardless of receipt of prior therapy (N = 42).

**Results:** As of 21 March 2025, 21 of 42 patients with RT received epcoritamab monotherapy as 1L treatment for RT (median follow-up, 23.9 months [range, 0.5+ to 39.9]). Median age was 67 years (range, 50–79); 52% had TP53 aberrations, 90% had Ann Arbor stage III/IV, and 95% had received prior CLL-directed therapy prior to transformation. In patients with 1L RT, ORR was 57% (95% CI, 34–78) and CR rate was 52% (11/21). Responses were durable; median DOR (range, 1.5 to 37.6+ months) and duration of CR (range, 2.8+ to 37.6+ months) were not reached. At 24 months, ~52% of responders and ~56% of complete responders remained in response. Median PFS and OS were 8.5 months (range, 0.03+ to 38.7+) and 27.5 months (range, 0.5 to 39.9+; 9/21 events) in patients with 1L RT, respectively. At 15 months, ~67% of patients were alive. Pharmacodynamic data will be presented. In the broader population of patients with RT regardless of prior treatment status (N = 42), safety findings were consistent with prior epcoritamab experience across disease states. Common treatment-emergent adverse events (TEAEs) included CRS (86%), anemia (50%), diarrhea (36%), neutropenia (33%), thrombocytopenia (31%), and fatigue (31%). Most CRS events occurred after the first full dose and were low grade (79% grade 1–2; 7% grade 3), resolving in 97% of patients within a median of 3 days (range, 1–14). Immune effector cell-associated neurotoxicity syndrome (ICANS) occurred in 12% of patients (all grade 1–2) and clinical tumor lysis syndrome (CTLS) in 5%. No events of CRS, ICANS, or CTLS led to treatment discontinuation. Four patients had ≥1 TEAE leading to treatment discontinuation (general physical health deterioration, sepsis, increased hepatic enzymes, and interstitial lung disease [n = 1 each]) and 3 had fatal TEAEs (general physical health deterioration, sepsis, and cerebrovascular accident [n = 1 each]). No new safety signals were observed.

**Conclusions:** Epcoritamab monotherapy induced high response rates with prolonged survival in patients with 1L RT, of whom 95% had prior CLL-directed therapy, a population with historically poor outcomes. Epcoritamab demonstrated a manageable safety profile that was consistent with that described for other disease states, with predominantly low-grade CRS that resolved, and no new safety concerns. These results underscore the clinical potential of epcoritamab in RT and support its ongoing evaluation in combination with other treatment modalities for non-Hodgkin lymphoma, including RT. The ongoing EPCORE-CLL-1 trial continues to explore efficacy and safety of epcoritamab in combination with lenalidomide or R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) in patients with RT.

**Keywords:** Richter's transformation; bispecific antibodies; first-line therapy

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**Please indicate the name of the funding organization:** Genmab A/S and AbbVie

## Abstract ID: 1607

### **Title:** MGA loss drives cristae remodeling and redox adaptation via MICOS and PRDX2 in Richters transformation

**Authors:** Prajish Iyer[a], Brian Jiang[a], Girish Venkataraman[b], Joo Song[c], Wing C Chan[d], Tanya Siddiqi[e], Steven Rosen[d], Alexey Danilov[f], Antje Gohla[g] and Lili Wang[a]

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Despite the advent of targeted therapies in chronic lymphocytic leukemia (CLL), Richter's Transformation (RT), the aggressive progression of CLL to diffuse large B-cell lymphoma, remains a major therapeutic challenge. RT is marked by rapid proliferation, metabolic rewiring, and resistance to therapy, accompanied by increased glucose uptake and mitochondrial oxidative phosphorylation (OXPHOS), resulting in oxidative stress. MYC, a key regulator of glucose metabolism and OXPHOS, is dysregulated in approximately 70% of RT cases, with ~30% of patients harboring mutations in its negative regulator, MGA (Max gene-associated). Given the frequency of MGA loss in RT and its role in restraining MYC-driven metabolism, we hypothesize that MGA deletion increases oxidative stress and promotes mitochondrial remodeling to support the high metabolic demands of RT cells. To dissect the role of MGA in driving RT, we recently developed a B-cell-restricted RT murine model by in vitro CRISPR-Cas9 editing of LSK progenitors from Cd19-Cre-Cas9 del(13q)-Sf3b1 (Sf3b1-K700E) mice using sgRNAs against Mga. This RT murine model recapitulated human RT features, including large cell size, high MYC and Ki67 expression, high cellular reactive oxygen species (ROS), and mitochondrial DNA copy number compared to CLL and normal B cells. Transmission electron microscopy (TEM) analysis revealed increased mitochondrial cristae (folded inner membrane of mitochondria) width, area, and abnormal shapes in RT cells. Transcriptomic analysis of murine CLL-RT B cells revealed significant ($p < 0.01$) upregulation (log2 FC (fold change) > 1.5) of oxidative stress response genes (PRDX1, 2, 4 and TNXRD2- Peroxiredoxin and Thioredoxin family of antioxidant genes) and MICOS (mitochondrial contact site and cristae organizing system- MIC25, MIC19, MIC26) complex genes in RT compared to CLL, suggesting that mitochondrial and redox adaptations are central to RT biology. Given these observations, we focused on the mitochondrial contact site and cristae organizing system (MICOS) complex, which maintains inner membrane architecture and cristae junction integrity. Disruption of MICOS alters cristae morphology, impairs mitochondrial compartmentalization, and compromises OXPHOS efficiency. Immunoblot analysis revealed significant upregulation of multiple MICOS complex components-MIC60, MIC25 (CHCHD6), MIC19, and MIC27 (APOOL), in murine RT samples compared to CLL and normal B cells. Among these components, APOOL was one of the significantly upregulated MICOS protein in murine RT and human RT-PDX samples (n = 3) and MGA KO HG3 cell lines. This coordinated increase in cristae-organizing proteins suggests that RT cells may undergo active mitochondrial remodeling to sustain elevated OXPHOS under metabolic stress. To assess the function of APOOL, we generated APOOL KO human B cell lines (NALM6, HG3) via CRISPR-Cas9. APOOL KO decreased OXPHOS, reduced cell growth rates, and total NADH in NALM6 and HG3 cell lines and was associated with increased expression of MIC25 and MIC19, decreased phosphorylation of 4E-BP1 (mTOR signaling), and reduced mitochondrial membrane

potential. TEM analysis revealed disrupted mitochondrial ultrastructure, characterized by fragmented cristae, reduced cristae density, and altered inner membrane organization, consistent with impaired MICOS function and mitochondrial instability. To further elucidate the response to oxidative stress, we profiled the peroxiredoxin (PRDX) family members, which detoxify ROS and protect mitochondrial function. First, PRDX1,2 was upregulated in human DLBCL tumors compared to normal GTEX (genotype-tissue expression) samples in the TCGA data by gene-expression profiling interactive analysis (GEPIA). Further, PRDX1 and PRDX2 were upregulated in murine RT and human RT PDX samples (n =3) compared to CLL (n = 3) and normal B (n = 4) cells by immunoblot analysis. As cMYC can upregulate PRDX2 expression by binding to the promoter but not PRDX1, we focused on functional studies with PRDX2. PRDX2 KO in human B cell lines (Nalm6 and HG3 cells expressing Cas9) led to reduced cell growth, decreased glucose uptake, suppressed mTOR signaling (p4EBP1), and impaired OCR (oxygen consumption rate), highlighting PRDX2 as a critical regulator of redox balance and metabolic fitness in RT cells. Our findings reveal a coordinated mitochondrial adaptation program in RT, wherein the upregulation of MICOS complex proteins and PRDX2 reflects a survival strategy that preserves mitochondrial fitness under metabolic stress. Targeting cristae integrity and redox homeostasis may offer novel therapeutic opportunities for treating metabolically rewired, MYC-driven refractory RT.

**Keywords:** Mitochondrial cristae; Richters transformation; metabolism

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## Abstract ID: 1701

**Title:** Development and evaluation of a selection-algorithm optimised ensemble model for predicting infection risk in CLL using structured and unstructured electronic patient record data

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**Rationale:** Infection (including bacterial, viral and opportunistic infection) remains a major cause of morbidity and mortality in patients with chronic lymphocytic leukemia (CLL). Events such as the COVID-19 pandemic further underscored the vulnerability of this population and the need for improved infection risk stratification. However, quantifying individual infection risk remains challenging due to the fragmented nature of clinical data and the absence of predictive tools that integrate real world complexity.

**Objectives:** To develop a clinically applicable machine learning model that predicts 2-year infection risk in patients with CLL, using routinely collected structured and unstructured electronic patient records (EPRs). The goal was to support early identification of high-risk individuals and enable proactive infection prevention strategies in routine CLL care.

**Methods:** Using the CogStack natural language processing (NLP) platform, 1,896 CLL patients (2003 - 2023) were identified at King's College Hospital. MedCAT was fine-tuned in two stages: (1) to detect CLL-related concepts (10,386 SNOMED terms) and (2) to identify infections (13,783 terms), producing high-fidelity annotations from 200,290 unstructured documents. Structured variables and NLP-derived features were processed via the pat2vec module. A selection algorithm (SA) optimized ensemble configurations from 2,000 base learners including tree-based, boosting, and neural models to predict 2-year infection risk, using varied hyperparameters and ensemble weighting strategies.

**Results:** MedCAT yielded F1 scores of 0.829 (for CLL-related concepts) and 0.831 (for infection-related terms), enabling over 3 million high-fidelity annotations from unstructured clinical text. The final ensemble model, trained to predict 2-year infection risk in CLL patients, demonstrated strong predictive performance (AUC: 0.859, F1: 0.904, Accuracy: 0.872), indicating a high ability to correctly identify patients likely to develop infections. Three independent optimization runs confirmed the model's robustness (AUC range: 0.793–0.859). Integrating NLP-derived features significantly improved predictive accuracy compared to structured data alone, highlighting the value of leveraging free-text EPR information in forecasting infection risk.

**Conclusion:** Scalable tools for identifying CLL patients at heightened risk of infection are essential for enabling timely, preventive interventions. This study demonstrates the feasibility of combining structured and unstructured EPR data to build robust, clinically relevant risk models. The SA-optimized ensemble enriched with domain-specific NLP features, offers a data-driven solution for use in routine care and shows promise for broader application across immunocompromised populations. Future work will benchmark this approach against transformer-based NLP systems and evaluate time-dependent modeling strategies.

**Keywords:** Machine learning; natural language processing; infection risk prediction

**Please indicate how this research was funded:** A mixture of pharmaceutical funding and charity funding.

**Please indicate the name of the funding organization:**

## Abstract ID: 1717

**Title:** MVA-based GEO-CM04S1 vaccine results in improved cellular immune response in patients with chronic lymphocytic leukemia (CLL) compared with mRNA-based vaccine: initial results of a phase II randomized study

**Authors:** Alexey Danilov[a], Steven Rosen[b], Sandra Thomas[c], Dongyun Yang[c], Tanya Siddiqi[d], Sandra Ortega-Francisco[c], Maria Aquino[c], Flavia Chiuppesi[c], Miguel Gutierrez[c], Jing Li[c], Jada Mack-Onyeike[c], Jacqueline Miller[c], Yifei Zhou[c], Tony Le[c], Jennifer Johnson[c], Corinna La Rosa[c], Qiao Zhou[c], Teodora Kaltcheva[c], Angela Patterson[c],

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Patients (pts) with CLL have impaired immune responses to vaccination and are at increased risk for infections, including SARS-CoV-2. Pt's inability to mount effective antibody responses to vaccinations is caused by both disease-related immune suppression and treatments which negatively impact B cell function. Hence, a vaccine which could induce a strong cellular immune response warrants investigation in CLL. GEO-CM04S1 is a Modified Vaccinia Ankara-based vaccine which expresses both Spike (S) and Nucleocapsid (N) SARS-CoV-2 antigens, the former known to have high variability. Aim: To evaluate immune response following administration of GEO-CM04S1 vaccine in CLL pts. We conducted a 1:1 randomized observer-blinded phase 2 trial in pts with CLL (NCT05672355). Pts received two doses of either Pfizer/BioNTech mRNA (with XBB.1.5 S sequence) (Ctrl) or GEO-CM04S1 (Exp; expressing the Wuhan S and N) vaccine on day (d) 1 and d84 of the study. Primary endpoint was T cell immune response at d56 post-injection of either vaccine. Secondary endpoints included safety, the duration/magnitude of T cell response, Th1/Th2 polarization, S- and N-specific IgG levels and neutralizing antibodies levels against SARS-CoV-2 variants up to d365. Simon 2-stage design was used to suspend accrual in an arm after interim analysis if T cell immune response results in that arm was disappointing. We enrolled 31 pts (16 on Exp and 15 on Ctrl arm) who received a median of 1 prior therapy (range, 1–8); 52% were men. Two pts (1 per arm) withdrew from the study before receipt of first dose. All pts exhibited moderate baseline serum S-specific IgG levels, with no significant change post-vaccine. Pts form both arms mounted a significant increase in RBD-specific IgG response on d28; and on d84 in the Ctrl arm. Predictably, only the Exp arm showed a 2–4-fold increase in the level of N-specific IgG post-vaccination. Neutralizing antibodies against the ancestral strain Wuhan (D614G) and Omicron variants (BA.1 and XBB.1.5) were present in both arms and reached a statistically significant increase at d28 for Omicron BA.1. When analyzing the primary endpoint, only the GEO-CM04S1 vaccinated pts generated statistically significant S- and N specific IFN-γ secretion in stimulated T-cells at d56. Neither group had a significant increase in M (membrane)-specific IFN-γ secretion. We observed a significant increase of S-specific IL-4 secretion at d112 in the Ctrl arm and on d28 and d180 in Exp arm. Neither arm had a significant increase in N-specific IL-4 secretion. Expression of activation-induced markers (AIM) by CD4+ T cells (OX40/CD137) was significantly increased only in Exp arm: S-specific on d84 and d180 and N-specific on d84. Meanwhile, AIM on CD8+ T cells (CD69/CD137) were seen only in S-specific CD8+ T cells on d84 and d180 and on d56 and d84 in the Exp and Ctrl arms, respectively. Both vaccines were well tolerated. No grade ≥3 events occurred. The most common Grade 1 adverse events were injection site reaction (n = 19), fatigue (n = 12) and cough (n = 10). CLL pts who received GEO-CM04S1 COVID19 vaccine generated a more robust T cell response compared with mRNA vaccine recipients. With failure of the mRNA vaccine to meet the primary endpoint, enrollment is now restricted to the Exp arm. While the mRNA vaccine stimulated a more pronounced and sustained humoral immune response, GEO-CM04S1 induced a robust cellular immune response, and thus may be a more effective option for preventing COVID-19 in CLL.

**Keywords:** Covid-19; vaccine; T-cell

**Please indicate how this research was funded:**

**Please indicate the name of the funding organization:**

## Abstract ID: 1801

### **Title:** Targeting BTKi-resistant CLL using the dual irreversible/reversible 4th generation BTK inhibitor rocbrutinib

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**Background:** Treatment of chronic lymphocytic leukemia (CLL) has been transformed by targeted therapies including inhibitors of Bruton's tyrosine kinase (BTKi). Currently three covalent BTKi (cBTKi) are approved for CLL, but most patients eventually relapse, commonly through acquisition of the C481S BTK mutation (Woyach et al., 2014). Non-covalent BTKi (ncBTKi), retain activity against C481 mutations but are susceptible to secondary BTK mutations (T474I, L528W, M427R, and V416L), which can also confer resistance to covalent BTKi (Wang et al., 2022). Regardless of these mutations, BCR signaling remains intact, suggesting that inhibition of the BCR pathway retains its therapeutic importance. Agents capable of targeting and inhibiting both wild-type (WT) and mutant BTK (C481S, T474I, and L528W) are ideal for treating CLL as they would retain BTK inhibition and prevent clinical relapse through the most common resistance mechanisms. Rocbrutinib is a novel selective 4th generation BTKi with an active warhead capable of binding WT and mutant BTK.

**Methods:** Validation of covalent binding was performed by X-Ray co-crystallography. To confirm binding of rocbrutinib to WT and mutant BTK, we performed a NanoBRET assay against WT, C481S, T474I, and L528W BTK. To further measure the binding affinity of rocbrutinib and the currently available BTKi toward full-length WT and mutant BTK in HEK293T cells, we utilized a fluorescent probe cellular binding assay (FPCBA) (Yin et al., 2024). Using genetically modified TMD8 cells that

express only C481S, T474I, or L528W mutated BTK, we determined the effect of rocbrutinib on cell-viability and BCR signaling. Primary CLL B-cells were isolated and treated with rocbrutinib for all experiments. BCR signaling alterations were assessed via immunoblot to observe changes in target protein phosphorylation. Changes in NFκB signaling were interrogated by RT-qPCR. In vivo studies were performed using Eμ-TCL1 and Eμ-MTCP1 mouse engraftment models.

**Results:** A crystal structure of BTK in complex with rocbrutinib at 1.7 Å resolution showed rocbrutinib binds BTK in the ATP-binding site, forms a covalent bond to the C481 residue, and forms hydrogen bonds to M477, D539, and K430. In a NanoBRET assay, rocbrutinib demonstrated low nanomolar binding affinity toward the WT, C481S, T474I, and L528W BTK proteoforms with IC50 values of 4.7, 49.7, 13.1, and 80.4 nM, respectively. This assay also showed rocbrutinib exhibits a long half-life when bound to WT and mutant BTK. Using FPCBA, rocbrutinib was the only BTKi—among ibrutinib, acalabrutinib, zanubrutinib, nemtabrutinib, and pirtobrutinib—to retain nanomolar potency against WT BTK as well as clinically relevant resistance mutations, including C481S, V416L, M437R, and L528W. In TMD8 cells, rocbrutinib maintained nanomolar GI50 toward WT, C481S, T474I, and L528W BTK. Rocbrutinib also inhibited BCR signaling regardless of BTK mutation status demonstrated by reducing production of CCL3 (WT: 98.8%, $p<0.0001$; C481S: 99.1%, $p<0.0001$; T474I: 98.9%, $p<0.0001$; L528W: 99.6%, $p<0.0001$) and CCL4 (WT: 97.3%, $p<0.0001$; C481S: 96.3%, $p<0.0001$; T474I: 97.3%, $p<0.0001$; L528W: 99.3%, $p<0.0001$) as well as inhibition BTK, PLCγ2, ERK, and AKT activation. Following two-hour drugging, 500 nM rocbrutinib inhibited BCR signaling in primary CLL B-cells, demonstrated by reduced phosphorylation of BTK (98.9%, $p<0.0001$), PLCγ2 (85.6%, $p<0.0001$), ERK (59.3%, $p=0.0029$), and AKT (68.9%, $p=0.0014$). In patient samples, mRNA expression of NFκB pathway targets BCL2, BCL2L1, MCL1, BCL2A1, MYC, and CD40 were significantly decreased after 48-hours of treatment with 500 nM rocbrutinib. Furthermore, we observed retained inhibition of BCR signaling in primary CLL B-cells harboring C481S via immunoblotting and cytokine production. Finally, to evaluate the in vivo efficacy of rocbrutinib, we evaluated its therapeutic impact in both the Eμ-TCL1 and Eμ-MTCP1 mouse models. We treated mice daily via oral gavage and found that 50 mg/kg of rocbrutinib significantly improved survival in the Eμ-TCL1 model when compared to vehicle (median 51 vs 44 days; $p=0.0018$) or ibrutinib at the same dose (median 51 vs 45 days; $p=0.0098$). Similarly, in the Eμ-MTCP1 model, rocbrutinib (50 mg/kg) markedly improved survival relative to vehicle (median 122 vs 62 days; $p<0.0001$) or ibrutinib at the same dose (median 122 vs 96 days; $p=0.0162$).

**Conclusion:** Collectively, our findings demonstrate rocbrutinib is a potent and selective inhibitor of BTK with activity even in the presence of mutations that mediate resistance to cBTKi and ncBTKi. These data support the continued investigation of rocbrutinib, which is currently being studied in the phase 1 setting of CLL and NHL (NCT04775745 and NCT04993690).

**Keywords:** CLL; relapsed/refractory; BTK

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# Abstract ID: 1802

## **Title:** Targeting SF3B1 disrupts chronic lymphocytic leukemia survival via PFKFB-mediated glycolytic dependency

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**Introduction:** Chronic lymphocytic leukemia (CLL), the most frequent type of leukemia in Western countries, continues to demand novel therapeutic strategies tailored to the subset of patients with rapid progression or relapsed/refractory (R/R) disease. While splicing factor SF3B1 mutations occur in 10–15% of CLL cases, the pathological role of the more prevalent wild-type SF3B1 remains poorly defined. Emerging evidence implicates aberrant RNA splicing in cancer metabolic reprogramming, yet the mechanistic connection between SF3B1-mediated splicing regulation and the metabolic pathways that support CLL cell survival remains unexplored. This study investigates the therapeutic potential of targeting wild-type SF3B1 using the splicing modulator h3b-8800, with a focus on its metabolic consequences in CLL.

**Methods:** CLL cell lines (MEC-1, JVM3) and primary CD19+ CLL cells from treatment-naïve patients were utilized. Lentiviral shRNA knockdown of SF3B1 in MEC-1 was validated by qRT-PCR. CLL cells were treated with the preclinical SF3B1 modulator h3b-8800 at a gradient of 0–10 μM for 48 hours, and cell viability was assessed using the CCK-8 assay to determine IC50 values. RNA sequencing (RNA-seq) was performed to analyze differential splicing events and gene expression following total RNA extraction. Key metabolic genes were selected and further validated using qRT-PCR and Western blot. Metabolic phenotypes were evaluated using glucose/lactateassay kits.

**Results:** SF3B1 knockdown significantly inhibited MEC-1 cell growth. h3b-8800 demonstrated potent cytotoxicity across models: IC50 values for 48 hours were 31.1 nM for primary cells, 32.55 nM for MEC-1, and 68.85 nM for JVM3. Transcriptomic analysis identified an SF3B1 splicing-dependent inverse expression pattern of PFKFB1 (a glycolysis suppressor) and PFKFB3 (a glycolysis activator) upon h3b-8800 treatment. These splicing shifts translated to reciprocal protein expression patterns: PFKFB1 decreased by 78.6% while PFKFB3 increased 186%. Western blot analysis further confirmed the upregulation of PFKFB3 protein expression. Metabolic assays demonstrated that h3b-8800 treatment resulted in a concurrent reduction in glucose uptake (FC =0.793, 20.7% decrease for 50 μM; FC =0.673, 32.7% decrease for 100 μM) and lactate secretion (FC =0.565, 43.5% decrease for 50 μM; FC =0.509, 49.1% decrease for 100 μM).

**Conclusions:** Our findings establish wild-type SF3B1 as a critical metabolic regulator in CLL through PFKFB-mediated glycolytic control. The splicing modulator h3b-8800 exerts potent anti-leukemic effects by rewiring the PFKFB1/PFKFB3 balance. INotably, our study connects oncogenic spliceosomal dysregulation with metabolic vulnerabilities in CLL, positioning SF3B1-targeting as a promising therapeutic strategy regardless of mutation status. Ongoing investigations

are focused on in-depth mechanistic elucidation and efficacy evaluation of the combination of h3b-8800 with first-line targeted therapies.

**Keywords:** Chronic lymphocytic leukemia (CLL); SF3B1; metabolic reprogramming

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## Abstract ID: 1902

### **Title:** Impact of treatment with ibrutinib or venetoclax on CAR-T cell generation in patients with chronic lymphocytic leukemia. Interim analysis of the GIMEMA CLL2020 study

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**Background:** The therapeutic effectiveness of CAR-T cells in chronic lymphocytic leukemia (CLL) remains suboptimal, primarily due to dysfunctions in patient-derived T cells. The immunological factors affecting the functionality of CAR-T cells generated from CLL patients, as well as the impact of targeted agents used to treat CLL on T-cell fitness, has yet to be fully elucidated.

**Aims:** This project aims at assessing in CLL patients the impact of treatment with targeted agents (i.e. ibrutinib or venetoclax) on T cells, and on the manufacturing efficiency, the phenotype and the functional properties of the CAR-T cells generated.

**Methods:** Analyses were performed on 15 CLL patients enrolled in the GIMEMA CLL2020 trial (NCT04640909). Samples were collected before the start of treatment and after 6 and 12 months of therapy with ibrutinib (n = 7) or venetoclax (n = 8). The T-cell phenotype was evaluated by flow cytometry. Anti-CD19-4-1BB CAR-T cells were produced by lentiviral transduction, and tested for generation efficiency, immunophenotype and in vitro anti-tumor activity.

**Results:** CAR-T cells were successfully generated from peripheral blood mononuclear cells (PBMC) in 13 out of 15 patients (87%) at pre-treatment and at the 6-month timepoint, and in all 11 patients (100%) with available 12-month samples. Transduction efficiency remained consistent across the analyzed timepoints. Specifically, in the venetoclax-treated cohort, the mean CAR expression on T cells was 47% at pre-treatment, 41% at 6 months and 57% at 12 months, whereas in the ibrutinib-treated cohort, the corresponding values were 47%, 42% and 64%, respectively. Moreover, no significant impact of therapy was noted on the cell yield at the end of the manufacturing process in both cohorts. Immunophenotypic analysis revealed a preferential enrichment of $CD4^+$ T lymphocytes in the final CAR-T cell product compared to the starting T-cell source - a trend observed consistently across all timepoints and treatment groups. Interestingly, in the cohort of patients treated with venetoclax, CAR-T cells generated after 12 months of treatment demonstrated a more favorable distribution of the differentiation subsets - with a predominance of less mature subpopulations in both the CD4+ and CD8+ compartments - compared to the pre-treatment timepoint. We also evaluated the expression of inhibitory immune checkpoint molecules on T cells before and after CAR-T cell manufacturing. After 6 months of treatment with targeted agents - both ibrutinib and venetoclax - we observed a significant decrease in the percentage of $TIM\text{-}3^+$ $CD8^+$ T cells in the pre-manufacturing T cells. Despite this positive immunomodulatory effect, TIM-3 expression was again upregulated during the generation process, across both $CD4^+$ and $CD8^+$ CAR-T cells and irrespective of the treatment regimen received. During treatment with targeted agents, we also observed a decreasing trend in PD-1 expression across CD4+ and CD8+ T-cell populations, which was detectable - though not significantly - in both the initial T-cell source and in the final CAR-T cell products. Notably, CAR-T cells produced after 12 months of treatment with venetoclax showed a meaningfully reduced expression of intracellular CTLA-4 compared to pre-treatment levels. In terms of antitumor functionality, the generated CAR-T cells were able to proliferate and effectively induce target cell lysis when co-cultured with a $CD19^+$ tumor target (i.e. MEC1 cell line). The magnitude of this response was dependent on the effector-to-target ratio. Despite the more favorable profile in terms of exhaustion markers expression observed after 6 and even more after 12 months of treatment with targeted agents - especially venetoclax - no significant differences were observed in the killing or proliferative ability of CAR-T cells compared to pre-treatment levels.

**Conclusions:** CAR-T cells can be effectively generated from CLL patients but display immunological features of dysfunction. From patients receiving 12-month venetoclax therapy we could generate CAR-T cells with a more favorable differentiation profile and partial restoration of exhaustion marker expression. Enrollment has now been completed, and assessments from an additional cohort of 8 patients, covering all timepoints, are currently underway. Conclusive results, including an evaluation of functional changes observed in CAR-T cells generated at different stages of targeted treatment, will be available at the time of data presentation.

**Keywords:** CAR-T cells; targeted therapies; immunomodulation

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# Abstract ID: 1912

**Title:** A tandem CD19/ROR1 CAR-NK to overcome apoptotic resistance in chronic lymphocytic leukemia

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**Introduction:** Although autologous CD19-directed CAR-T cell therapy has demonstrated favorable efficacy in several B cell malignancies, this approach has been less efficacious and more toxic in patients with CLL, likely due to the T-cell dysfunction that is a hallmark of CLL pathophysiology. CAR natural killer (CAR-NK) cell therapy may offer several advantages over CAR-T in CLL such as a favorable toxicity profile, potential for off-the-shelf allogeneic sourcing, and less susceptibility to the immunosuppressive effects of the disease. Indeed, a recent study found that CD19-directed CAR-NK showed efficacy without neurotoxicity or graft-versus-host disease (Marin, D. et al., Nature Medicine, 2024). Yet, a potential challenge with CAR-NK therapy in CLL is that tumor cells may develop compensatory pro-survival signaling which inhibits CAR-NK cell-mediated apoptosis. Receptor tyrosine kinase-like orphan receptor (ROR1) is ubiquitously expressed on CLL cells, and signaling via its ligand Wnt5a has been associated with both ibrutinib and venetoclax resistance (Yu, J. et al., Oncotarget, 2018; Ghia, E. et al., Leukemia, 2022). However, its connection to cell therapy resistance remains incompletely understood, although apoptotic resistance has been hypothesized to reduce cell therapy efficacy (Pourzia, A. L., Cell Death & Disease, 2023). Here, we studied Wnt5a-induced ROR1 signaling as an underlying CAR-NK resistance mechanism in CLL and leveraged our findings to develop a novel CAR-NK product designed to overcome apoptotic resistance in CLL.

**Methods:** ROR1 expression on peripheral blood-derived primary CLL patient samples was measured via flow cytometry. CLL cells were incubated with 200 ng/ml of recombinant Wnt5a and were subsequently assessed for their apoptotic priming via BH3 profiling, a functional assay that measures the proximity of tumor cells to the apoptotic threshold and their dependencies on specific anti-apoptotic proteins. Resistance to NK-mediated killing was evaluated by treating CLL cells with 1 ug/ml Wnt5a and exposing them to either CD19-CAR-NK or untransduced primary NK cells, with Annexin V and propidium iodide (PI) staining used to assess CLL cell apoptosis. The ROR1-inhibiting monoclonal antibody (mAb) zilovertamab was used to investigate attenuation of Wnt5a/ROR1-associated survival. Tandem, second-generation anti-CD19/ROR1 (Dual) CAR constructs were designed by joining an anti-CD19 scFv with an anti-ROR1 scFv via a (G4S)4 linker, followed by a 4-1BB costimulatory domain and a CD3Z signaling domain. CAR constructs were transduced into Jurkat and peripheral blood-derived primary NK cells via an optimized baboon lentivirus protocol. Effector cell activation and cytotoxicity against primary CLL samples, a CD19+ CLL-derived cell line MEC-1, and the ROR1+ NCCIT cell line were evaluated via flow cytometry-based readouts of NK activation marker expression and Annexin/PI staining.

**Results:** We first confirmed expression of ROR1 across primary CLL cells (ΔMFI [ROR1 – isotype]: 346-2199) and demonstrated that treatment of these samples with Wnt5a notably decreased overall apoptotic priming (BIM 0.1 uM) from baseline by between 11–18% reduction at 4 hours and 14–42% reduction at 24 hours. More pronounced reductions were associated with higher ROR1 expression. Correspondingly, high-ROR1+ CLL cells incubated with Wnt5a exhibited up to 20% reduction in cell death compared to untreated conditions when co-cultured with either CD19 CAR-NK or untransduced primary NK cells. Wnt5a-treated CLL cells incubated with zilovertamab showed no significant difference in viability compared to untreated cells when co-cultured with untransduced NK cells, indicating inhibition of Wnt5a/ROR1-mediated survival signaling. Zilovertamab treatment increased apoptotic priming in Wnt5a-treated CLL cells. Dual CAR-Jurkat cells achieved high expression of the early activation marker CD69 against both CD19+ MEC1 and ROR1+ NCCIT cell lines, whereas single-directed CD19-CAR-Jurkat and ROR1-CAR-Jurkat cells exhibited minimal activation against nonspecific targets comparable to untransduced Jurkat cells. Dual CAR-NK cells achieved increased CD69 expression compared to single-directed or untransduced NK cells against high- and low-ROR1+ CLL samples (n = 6) at 16 hours. Furthermore, the Dual CAR-NK had comparable cytotoxicity to CD19 CAR-NK against CLL cells and greater cytotoxicity than ROR1 CAR-NK and untransduced NK at 16 hours.

**Conclusion:** We identified Wnt5a-induced ROR1 signaling as a key pathway reducing CLL cell priming for apoptosis and enhancing their survival against NK cells, which was reversible by anti-ROR1 mAb zilovertamab. We subsequently developed a novel tandem CD19/ROR1 CAR-NK product which may potentially overcome ROR1-associated cell-mediated killing resistance and subvert single-antigen escape. Our early data suggest such a construct has favorable activation and cytotoxicity profiles, warranting further study of this approach to treat CLL.

**Keywords:** CAR-NK; ROR1; resistance

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