HEMATOLOGIA
Número Educacional

5th LAG – CCL 2024

Latin American Congress on Chronic Lymphocytic Leukemia

21 Y 22 DE NOVIEMBRE
CONVENTION & EXHIBITION CENTER
PUNTA DEL ESTE | URUGUAY

Volumen 28 • Número Extraordinario
NOVIEMBRE 2024

Sociedad Argentina
de Hematología

HEMATOLOGÍA

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5th Latin American Congress on Chronic Lymphocytic Leukemia

CONTENIDO

Palabras de bienvenida

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Estimadas Autoridades de la Revista de la
Sociedad Argentina de Hematología

Es para mí un honor como Presidente de la 5ª. Edición del Congreso del Grupo Latino Americano de Leucemia Linfocítica Crónica (LAG.CLL) a realizarse en Punta del Este durante los días 21 y 22 de noviembre 2024, poder incluir en vuestra prestigiosa revista los resúmenes de los trabajos científicos recibidos del área biológica y clínica de la especialidad.

Es un evento que se realiza en conjunto con el IWCLL que contará con la presencia de distinguidos panelistas internacionales, pero con una marcada presencia de especialistas de la región Latino Americana, donde se volcarán las actualizaciones del conocimiento biológico, su traslación hacia la clínica y las consideraciones de manejo terapéutico.

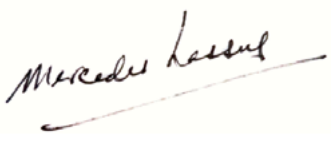
Se abordarán los avances terapéuticos con los nuevos agentes, sin desconocer las dificultades de disponibilidad y accesibilidad que transitan nuestros países para lograr los mejores tratamientos oportunos con la mayor cobertura de equidad para los pacientes portadores de LLC.
No obstante, los trabajos presentados reflejan un alto nivel de investigación clínica y biológica que traducen un trabajo denodado de los y las colegas a pesar de las dificultades, que merece un muy alto reconocimiento y agradecimiento.

Hemos considerado que una de las formas de extender la información vertida en el evento, es publicando todos sus resúmenes en una revista de alta difusión como la vuestra.

En nombre del Comité Organizador y Comité Científico, agradecemos haber aceptado la propuesta de compartir este rico material con todos sus suscriptores.

Saluda atentamente

Dr. Raul Gabus
Presidente Comité Organizador
5TH. LAG.CLL 2024 URUGUAY



Dra. Mercedes Lassus
Presidenta Comité Científico
5TH. LAG.CLL 2024 URUGUAY

5th Latin American Congress on Chronic Lymphocytic Leukemia
Noviembre 2024

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Rodrigo Santacruz

Co-organizers iwCLL
Nicholas Chiorazzi

EXTENDED PROGRAM

Thursday 21 November — **Auditorium 2**

07:30 - 08:30 **Registration**

08:30 - 08:45 **Welcome and introduction**
Dr. Guillermo Dighiero (Uruguay), Dr. Raúl Gabus (Uruguay), Dr. Thomas Kipps (USA)

08:45 - 09:45 **Plenary Lectures - Session 1**
Chairmen: Dr. Guillermo Dighiero (Uruguay), Dr. Raúl Gabus (Uruguay)

08:45 - 09:15 **Plenary Lecture: CLL today: accomplishments, main active research areas, key pending issues**
Dr. Thomas Kipps (USA)

09:15 - 09:45 **Plenary Lecture: Origin and CLL clone evolution / IGHV and prognosis**
Dr. Nicholas Chiorazzi (USA)

09:45 - 10:15 **Coffee break**

10:15 - 11:15 **Plenary Lectures - Session 2**
Chairmen: Dr. Eliana Abdelhay (Brazil), Dr. Gimena Dos Santos (Uruguay), Dr. Romina Gamberale (Argentina)

10:15 - 10:45 **Plenary Lecture: Contribution of the microenvironment in the progression of chronic lymphocytic leukemia and its implications in therapy**
Dr. Jan Burger (USA)

10:45 - 11:15 **Plenary Lecture: Immunotherapy in chronic lymphocytic leukemia, where we are, biological and clinical update**
Dr. Martina Seiffert (Germany)

11:15 - 12:30 **Ongoing Biology Research in the region (1)**
Chairmen: Dr. Jan Burger (USA), Dr. Nicholas Chiorazzi (USA) , Dr. Martina Seiffert (Germany)

MICROENVIRONMENT AND PRE-CLINICAL STUDIES OF NEW PUTATIVE TARGETS FOR THERAPY

11:15 - 11:20 **Role of G Protein coupled receptor KINASE 2 (GRK2) in the migration and activation of T Cells from chronic lymphocytic**
Dra. María Chiara Cassarino (Argentina)

> THERAPY **11:20 - 11:25** **Defects in the frequency and effector function of KIR+ and NKG2A+ virtual memory CD8+ T cells in patients with Chronic Lymphocytic Leukemia**
Dra. María Cecilia Rodríguez Galán (Argentina)

11:25 - 11:30 **Venetoclax resistance induced by autologous T cells**
Dra. Valeria Sarapura (Argentina)

11:30 - 11:35 **Impacto clínico de la disrupción de la microbiota oral en pacientes con LLC.**
Dra. Fernanda Azevedo (Brazil)

11:35 - 11:45 Discussion

MUTATIONAL LANDSCAPE OF CLL DURING DISEASE PROGRESSION

11:45 - 11:50 **Machine learning-based characterization of immunoglobulin structures in aggressive CLL.** Dr. Diego Álvarez (Chile)

11:50 - 11:55 **Mutational profile as genomic markers of prognosis of chronic lymphocytic leukemia in brazilian patients** Dra. María Paula dos Santos (Brazil)

11:55 - 12:00 **A subgroup of patients with unmutated IgHV 1-69; 3-30; 1-02; 4-39 and high expression of activation-induced cytidine deaminase**
Dr Jorge Souto (Uruguay)

12:00 - 12:10 Discussion

12:30 - 12:45 **Break**

12:45 - 14:00 **Symposium Beigene**
Personalization of Therapy in CLL: New Paradigms

12:45 - 12:55 **Introduction Scientific Sesión**
Chairmen: Dr. Carolina Mahuad (Argentina)

12:55 - 13:25 New challenges in the treatment of CLL - paradigm changes
Dr. Raúl Cordoba Mascuñano (Spain)

13:25 - 13:50 "Round Table"
Dr. Carolina Mahuad (Argentina)
Dr. Raúl Cordoba Mascuñano (Spain)
Dr. Andrés Danielle (Argentina)
Dr. Maximiliano Smietniasky (Argentina)

13:50 - 14:00 **Q&A**

Thursday 21 November **Auditorium 2**

14:00 - 15:30 **Plenary Lectures - Session 3**
Chairmen: Dr. Juan Dupont (Argentina), Dr. Cecilia Guillermo (Uruguay)

14:00 - 14:30 **Front line therapy in CLL**
Dr. Miguel Pavlovsky (Argentina)

14:30 - 15:00 **Optimization of time-limited treatment strategies, use of MRD**
Dr. Othman Al Sawaf (Germany)

15:00 - 15:30 **Toxicities with current therapies**
Dr. Francesc Bosch (Spain)

15:30 - 16:00 **Coffee break**

16:00 - 17:00 **Session**
Clinical trials and tumor registries in LLC in 3 Latin American countries
Chairmen: Dr Celso Arrais (Brazil), Dr. Pablo Muxi (Uruguay), Dr. Ana Inés Landoni (Uruguay)

16:00 - 16:15 **CLL: The Argentinian experience**
Dr. Miguel Pavlovsky (Argentina)

16:15 - 16:30 **CLL: The Brasilian experience**
Dr. Nelson Hamerschlack (Brazil)

16:30 - 16:45 **CLL: The Uruguayan experience**
Dr. Mercedes Lassus (Uruguay)

16:45 - 17:00 **Discussion**

17:00 - 17:30 **Session**
Tumor registries
Dr. Celso Arrais (Brazil), Dr. Andres Addiego (Uruguay)

Thursday 21 November **Auditorium 1**

17:30 - 19:30 **Poster session/Wine and Cheese evening**
Chairmen: Dr. Jan Burger (USA), Dr. Carolina Oliver (Uruguay),
Dr. Pablo Oppezzo (Uruguay), Dr. Martina Seiffert (Germany)

EXTENDED PROGRAM

Friday 22 November — **Auditorium 2**

08:30 - 09:30 **Plenary Lectures - Session 4**
Chairmen: Dr. Fernando Bezares (Argentina), Dr. Carlos Chiattone (Brazil)

- **08:30 - 09:00** **Beyond the first line. The conundrum of therapy selection in the era of new targeted therapies**
  Dr. Othman Al Sawaf (Germany)
- **09:00 - 09:30** **Overcoming resistance. New therapies and therapeutic approaches**
  Dr. Thomas Kipps (USA)

09:30 - 10:00 **Coffee break**

10:00 - 11:00 **Session**
Molecular biology in CLL prognosis and therapy resistance
Chairmen: Dr. Mirta Giordano (Argentina), Dr. Victoria Irigoin (Uruguay), Dr. Florencia Palacios (Uruguay)

- **10:00 - 10:30** **Recent molecular findings, importance for prognostic evaluation**
  Dr. Francesc Bosch (Spain)
- **10:30 - 11:00** **Resistance mechanisms during clone evolution after treatment**
  Dr. Jan Burger (USA)

11:00 - 12:15 **Session: Focus in CLL. Questions and answers with clinical cases and experts panelists.**
Discussion of Clinical Cases
Dr. Othman Al Sawaf (Germany), Dr. Francesc Bosch (Spain)
Presenters: Dr Virginia Lema (Uruguay), Dr Victoria Remedi (Uruguay), Dr Sabrina Ranero (Uruguay), Dr Victoria Irigoin (Uruguay)

12:15 - 12:30 **Group Photo**

12:30 - 13:30 **Symposium Abbvie**
Making smart decisions in CLL treatment
Dr. Othman Al Sawaf (Germany)

13:30 - 14:45 **Ongoing Biology Research in the region (2)**
Chairmen Dr Jan Burger (USA) - Dr Nicholas Chiorazzi (USA) - Dr Martina Seiffert (Germany)

13:30 - 13:40 Dr Romina Gamberale (Argentina)

13:40 - 13:50 Dr Mercedes Borge (Argentina)

13:50 - 14:00 Dr Pablo Morande (Argentina)

14:00 - 14:10 Dr Eliana Abdelhay (Brazil)

14:10 - 14:20 Dr Marcelo Navarrete (Chile)

14:20 - 14:30 Dr Florencia Palacios (Uruguay)

14:30 - 14:40 Dr Florencia Palacios (Uruguay)

14:45 - 15:00 **Break**

15:00 - 16:00 **Symposium AstraZeneca**
Trends in CLL: Navigating the present, shaping the future
Dr. Francesc Bosch (Spain)

16:00 - 16:30 **Coffee break**

EXTENDED PROGRAM

Friday 22 November — Auditorium 2

16:30 - 17:00 **Oral presentation of selected posters**
Chairmen: Dr. Pablo Muxi (Uruguay), Dr. Mercedes Lassus (Uruguay), Dr. Florencia Palacios (Uruguay)

17:00 - 17:15 **Ceremony**
Catovsky Dighiero Medal Ceremony.
Announcement of the 6th edition of the LAG-CLL meeting

17:15 - 17:45 **Closing Remarks**
Dr. Guillermo Dighiero (Uruguay), Dr. Raúl Gabus (Uruguay), Dr. Thomas Kipps (USA)

Friday 22 November — Punta Ballena

07:30 - 08:30 **Career Development in Fundamental Biology Research (1)**
Dr. Mercedes Borge (Argentina), Dr. Nicholas Chiorazzi (USA), Dr. Florencia Palacios (Uruguay), Dr. Martina Seiffert (Germany)

Friday 22 November — José Ignacio

07:30 - 08:30 **Clinical Issues (2)**
Dr. Jan Burger (USA), Dr. Ana Inés Landoni (Uruguay), Dr. Carolina Oliver (Uruguay), Dr. Victoria Remedi (Uruguay)

Clinical Issues (3)
Dr. Francesc Bosch (Spain), Dr. Victoria Irigoin (Uruguay), Dr. Rodrigo Santacruz (Paraguay)

Clinical Issues (4)
Dr. Othman Al Sawaf (Germany), Dr. Fernando Bezares (Argentina), Dr. Virginia Lema (Uruguay)

Clinical Issues (5)
Dr. Celso Arrais (Brazil), Dr. Miguel Pavlovsky (Argentina), Dr. Sabrina Ranero (Uruguay)

Presentaciones orales

SESIÓN VIERNES 22 DE NOVIEMBRE 16:30 – 17:00 HS.

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Evolving Treatment Patterns and Biomarker Utilization for Chronic Lymphocytic Leukemia in Latin America: A Multinational Real-World Study

Carolina Oliver[1]; Luis Villela[2]; Victoria Irigoin[3]; Macarena Roa[4]; Sofia Rivarola[5]; Lorena Cardozo[6]; María Alejandra Torres[7]; Denisse Castro[8]; Fabiola Valvert[9]; María Camila Martínez[10]; Virginia Lema[11]; Ana Ines Landoni[12]; Sabrina Ranero[13]; Victoria Remedi[12]; Alana Von Glasenapp[6]; *María Orlova*[4]; *Nancy Cristaldo*[4]; José Alvarez[15]; Fernando Perez-Jacobo[16]; Arianna Robles[17]; Melanie Otañez[18]; Camila Peña[4]; Sally Rose Paredes[8]; German Stemelin[5]; Henry Idrobo[19]; Brady Beltran[8]; Jorge Castillo[20]; Luis Malpica[21]

[1] CASMU. Hospital Britanico. [2] Escuela de Medicina Del Tecnológico de Monterrey. Servicio de hematología, Centro Medico Dr. Ignacio Chavez del ISSSTESON, Hermosillo. [3] COSEM, CASMU. [4] Hospital Del Salvador, Santiago. [5] Hospital Británico, Buenos Aires. [6] Hospital Central del Instituto de Previsión Social, Asuncion. [7] Universidad Central de Venezuela. Clinica Santa Sofia, Caracas. [8] Hospital Edgardo Rebagliati Martins. [9] Instituto de Cancerología de Guatemala INCAN. [10] Universidad Tecnológica de Pereira. [11] Hospital Central de las Fueras Armadas. [12] Hospital Maciel. [13] Hospital de Clínicas. [14] Hospital Italiano de Buenos Aires. [15] Centro Médico Nacional 20 Noviembre, Mexico City. [16] Hospital Central Norte PEMEX. [17] Universidad de Guadalajara. [18] Hospital General del Estado de Sonora. [19] Universidad Tecnológica de Pereira. Liga Colombiana contra el cáncer. Hospital Universitario San Jorge. [20] Dana Farber Cancer Institute, Boston. [21] The University of Texas MD Anderson Cancer Center, Houston. - Uruguay

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Introduction: Chronic Lymphocytic Leukemia (CLL) is the most common leukemia in adults in Western countries, with an incidence of 4.8–5 per 100,000 in the US and Europe, and significantly lower in Asia (0.48 per 100,000). Latin America (LATAM) presents a complex and variable landscape for CLL incidence, with some countries like Uruguay and Argentina exhibiting rates similar to Europe, while others (e.g. Mexico, Peru, Chile) reporting lower incidences. The region's ethnic, cultural, and economic heterogeneity leads to disparities in access to diagnostic and prognostic tools, as well as therapeutic options, especially with the increasing use of targeted agents. In 2022, the GELL-CLL cohort presented initial data from 459 patients across six countries. Here, we provide an updated analysis, expanding the cohort and further exploring treatment patterns and biomarker usage. **Objective:** To describe the epidemiology, access to biomarkers, and treatment patterns in CLL patients across Latin America. **Methodology:** We conducted a retrospective cohort study of CLL patients aged ≥18 years, diagnosed and treated between 2010-2024, or diagnosed since 2000 and treated from 2010 onwards, from centers participating in the GELL-CLL registry. **Results:** A total of 958 patients from nine countries (Argentina, Chile, Colombia, Guatemala, Mexico, Paraguay, Peru, Uruguay, Venezuela) were included, with 860 eligible for analysis. Of these, 66% were treated in private institutions. The median age was 68 years (30–94), with 40.3% female. Racial distribution included 91.2% White, 0.9% African-ancestry, 0.4% Indigenous, 7.4% mixed-race, and 0.1% Asian. At diagnosis, 91.3% had an ECOG performance status of 0–1, with 73.6% Rai 0 (I: 12.4%, II: 7.5%, III: 3.7%, IV: 2.8%). Key prognostic factors revealed that 13.5% were CD38 positive, and elevated B2-microglobulin was found in 51.1%. IgVH mutational status was studied in 30.6% of patients, with 53% mutated and 47% unmutated. At diagnosis, 77.2% of patients were under observation, with 51% requiring treatment after a median of 9 months. Of those treated, only 34% underwent cytogenetic or FISH analysis prior to therapy. Del17p was identified in 3.7% of patients, while P53 mutations were found in 7.2%. First-line treatments included chemo-immunotherapy (56.2%), chemotherapy (28%), BTK inhibitors (11.7%), and Venetoclax-based regimens (4.2%). Remarkably, 85.8% of patients receiving chemotherapy had no prior cytogenetic or FISH testing. Differences in biomarker testing between countries were stark, ranging from 0% in Venezuela to 50% in Colombia. With a median follow-up of 57 months (0–362), overall survival (OS) differed significantly by treatment era: OS was 116 months for 2010-2014, 127 months for 2015-2019, and was not reached for 2020-2024 (p=0.006). Four-year OS rates were 65% for chemotherapy, 81% for chemo-immunotherapy, 85% for BTK inhibitors, and 90% for Venetoclax-based regimens. **Conclusions:** This real-world analysis highlights significant disparities in CLL management across LATAM. Despite recent shifts toward targeted therapies, many patients still lack access to essential prognostic testing, potentially leading to suboptimal treatment choices. Efforts to improve biomarker availability and targeted therapy access are crucial for enhancing outcomes across the region. Expanding this cohort will further elucidate regional variations and support initiatives to address treatment inequities.

Figure 1. Treatment patterns according to different time periods

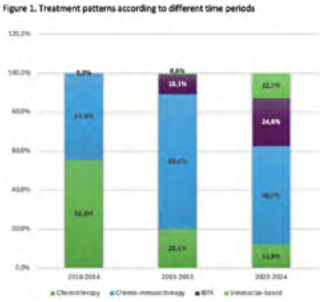
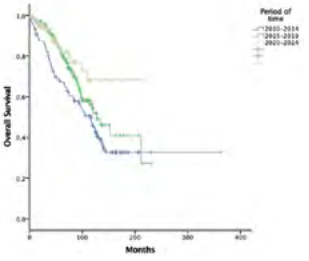


Figure 1. Overall Survival according to time period



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Risk assessment of second malignancies in patients with Chronic Lymphocytic Leukemia

Ana Inés Landoni[1]; María Victoria Remedi[1]; Carolina Oliver[2]; Victoria Irigoin[3]; Virginia Lema[4]; Sabrina Ranero[5]; Gabriel Borelli[1]; Pablo Muxi[6]; Maria Gabriela De Galvez[7]; Rita Uria[8]; Mercedes Lassus[9]; Pablo Oppezzo[10]; Raul Gabus[11]

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[4] *Hospital Militar.*
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[6] *Hospital Britanico.*
[7] *CASMU/COSEM.*
[8] *Instituto de Italia.*
[9] *Grupo uruguayo LLC.*
[10] *Instituto Pasteur.*
[11] *Hospital Maciel.*

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Introduction: Patients with chronic lymphocytic leukemia (CLL) have an increased risk of developing second malignancies compared to the general population. **Objectives:** 1) to evaluate the frequency of second malignancies in CLL patients from a Uruguayan cohort; 2) to study the relationship between these cancers and the clinical and biological characteristics of CLL; and 3) to determine if there is a relationship between the type of treatment (target therapy, FCR or FC) and the frequency of the second malignancies. **Method:** This is a retrospective national multicenter study conducted by GURU-LLC (Uruguayan Group of CLL, "Grupo Uruguayo de Leucemia Linfoide Crónica") with 521 patients diagnosed with CLL between 1998 – 2024. **Results:** The median age of this population is 70 years (range 36-87 years), 69% male and 31% female. Distribution by Binet A, B and C stages was 66%, 18% and 12% respectively (4% without data), and Rai stages 0 to IV were 45%, 24%, 13%, 8% and 5% respectively (5% without data). Eighteen percent of individuals (n=94) were diagnosed with a second malignancy. Second neoplasms were in 85% solid tumors (n=80) and 15% hematologic malignancies (n=14). Among solid tumors, frequencies were as follows: skin (34%), prostate (16%), colon (14%), breast (11%), kidney (8%), lung (8%) and other tumors 10%. Hematologic malignancies were Richter transformation (RT), essential thrombocythemia and acute myeloblastic leukemia (AML) (79%, 14% and 7% respectively). Six percent of patients with second malignancies associated more than one neoplasm. The appearance of second malignancies occurred during the course of CLL in 56%, in 5% were present at diagnosis and in 18% they preceded the diagnosis of CLL (20% without data). Forty-five percent of the study population received treatment for their CLL, 71% with alkylating agents, and 3.7% of them developed AML. Median overall survival (OS) of CLL patients with or without second neoplasms were 88 and 138 months respectively (p=0.03). OS at 60 months was 55% for CLL with second neoplasms and 71% for individuals without neoplasm. Population with and without a second neoplasm are comparable with regard to the risk of their CLL, whether or not they received treatment, and the type of treatment received. Second malignancy was the cause of death in 36% of individuals. **Conclusions:** Our results highlight the presence of second malignancies in patients with CLL. Similar to previously published data in Caucasian cohorts (Shen et al., 2021; van der Straten et al., 2023), these findings demonstrate an increased frequency of second malignancies, which negatively impacts the overall survival (OS) of CLL patients. These results emphasize the importance of regular surveillance and early diagnosis of secondary neoplasms in order to improve long-term outcomes in CLL patients.

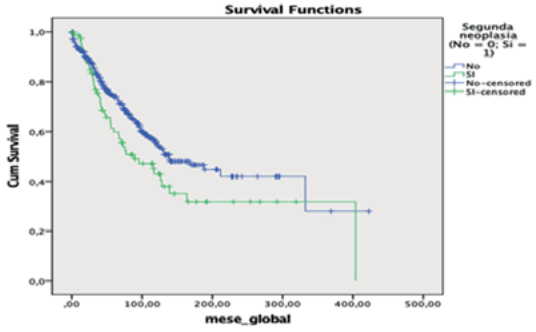

Figure 1: Median overall survival (OS) of CLL patients with or without second neoplasms were 88 and 138 months respectively (p=0.03).

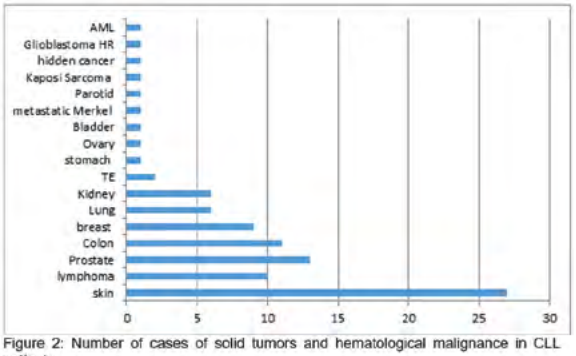

Figure 2: Number of cases of solid tumors and hematological malignance in CLL patients.

20 Natural and vaccine-induced antibodies to Streptococcus pneumoniae (Spn) in the Eμ-TCL1 adoptive transfer mouse model of CLL. Lower levels of antibodies are associated with increased susceptibility to Spn pulmonary infection.

Ana Colado[1]; María Chiara Cassarino[1]; Valeria Sarapura Martinez[1]; Martin Bertini[2]; Fernando Bezares[2]; Mónica Vermeulen[1]; Pablo Morande[1]; Romina Gamberale[1]; Mercedes Borge[1]; Mirta Giordano[1]

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Introduction
CLL patients are at increased risk for infections caused by Streptococcus pneumoniae (Spn). Both antigen induced- and natural-anti-Spn antibodies represent a key component of the anti-pneumococcal immune response. These antibodies play important roles during the early immune response to Spn by effector mechanism such as opsonophagocytosis and reduction of bacterial adherence. While hipogammaglobulinemia is commonly observed in patients, the amount and relevance of anti-Spn antibodies in CLL have not been addressed in depth.

Objectives
To determine the level of natural and vaccine-induced anti-Spn antibodies and their role in protection during Spn-pulmonary infection in the Eμ-TCL1 mouse model of CLL.

Methods
For murine adoptive transfer (AT)-model of CLL, C57BL/6 mice (8-10 weeks old) were intraperitoneally (ip) injected with 20 x 106 leukemic cells obtained from spleens of Eμ-TCL1 mice (C57BL/6 background). To evaluate the response to vaccination, control (age-matched C57BL/6) and leukemic mice (> 60% of CD5+ CD19+ cells in peripheral blood) were ip injected with Prevnar 13 vaccine or adyuvant, and blood samples were collected 1 and 3 weeks thereafter. Plasma levels of total and anti-Spn specific IgG and IgM were determinate by ELISA. Neutrophil phagocytosis of FITC-Spn was evaluated by flow cytometry (FC). For Spn-infection experiments, groups of control and leukemic mice were intranasally infected with a total of 2 x 106 CFU of Spn serotype 3. For survival experiments, mice were daily controlled for 11 days. In other set of experiments mice were euthanized 24 h after infection and the bronchoalveolar lavage fluid (BALF) was obtained to determine: bacterial load by serial dilution in Columbia blood agar plates, cell number and phenotype by FC and cytokines by ELISA. Statistical analysis was performed with Prism v8 (GraphPad).

Results
AT-TCL1 mice had lower levels of total IgG and similar levels of total IgM compared to control animals ($n=10$, $p<0.05$). Interestingly, AT-TCL1 mice had lower levels of natural anti-Spn IgM, that decreased as leukemic burden increased ($n=10$, $p<0.05$). The lower levels of anti-Spn IgM in AT-TCL1 serum was accompanied with a lower capacity to induce Spn-phagocytosis by control neutrophils ($n=8$, $p<0.05$). Regarding the susceptibility of the AT-TCL1 mice to infection, we found an increased mortality rate compared to Spn-infected control ($n=8$, $p<0.05$). At 24 h after infection, the BALF of AT-TCL1 mice showed a higher bacterial burden than control mice ($n=12$, $p<0.05$) and similar levels of neutrophils and classical inflammatory parameters as total protein concentration, TNF-α, IL-1β and CXCL1. Finally, we observed a poor response of anti-Spn IgM and IgG to vaccination in AT-TCL1 mice compared to adjuvant-injected mice ($n=8$, $p<0.05$).

Conclusion
Leukemic burden in AT-TCL1 mice was associated with a decrease of natural IgM anti-Spn levels, resulting in diminished opsonophagocytosis of the bacteria in vitro and a higher mortality rate and bacterial burden in vivo. As occurs with CLL patients, leukemic mice showed a very low response to conjugated vaccine suggesting a weak protection against Spn infection. This work adds new insight into the immune defects that predispose to Spn infection in CLL.

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Inflammasome and Chronic Lymphocytic Leukemia: the quest towards a new therapeutic target

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Although an inflammatory tumour microenvironment (TME) is known to be involved in the initiation and progression of CLL, the role of the inflammasome, the multiprotein complex that activates caspase-1 and drives maturation of proinflammatory cytokine IL-1b is not fully characterized in this disease. Transmembrane protein 176A/B (TMEM176 A/B) channels are important regulators of the NLRP3 inflammasome (Hill M, 2020). Our previous work demonstrate that TMEM176A expression is increased in primary cells isolated from active versus indolent disease and healthy donors.

Aims: To characterize the role of the inflammasome during CLL progression and to investigate whether pharmacological inhibition of the inflammasome regulator TMEM176A enhances antitumoral responses *in-vitro* and *in-vivo*. Primary CLL cells were obtained after informed consent and stored in the Uruguayan Group of CLL Biobank. Cell culture, flow cytometry, PCR and Western blot analysis were performed. TCL-1 mice housed at *Institut Pasteur de Montevideo* were used for *in-vivo* experiments. Ethics Committee approval was granted for human and animal studies.

Results: Increased TMEM176A expression correlates with impaired inflammasome activation in active disease as lower percentages of mature caspase-1 were observed by flow cytometry and lower Gasdermin (another surrogate for inflammasome activation) by Western blot. Furthermore, inhibition of TMEM176A with Boritinib (a pharmacologic inhibitor of TMEM176A/B proteins -Segovia and Russo, 2019-) *in-vitro* triggered caspase-1-dependent cell death suggesting pharmacologic modulation of the inflammasome is feasible as a therapeutic option in active CLL.

Since TMEM176A is overexpressed in CLL cells with active disease, we stimulated leukemic cells *in-vitro* with CD40L+IL-4, a classical activator of B cells that has been described as a key signaling pathway in active disease (Granziero et al., 2001). Our results demonstrate that activation with CD40L+IL-4 *in-vitro* increases TME-M176A expression. The mainstay treatment of CLL includes BCL-2 and BTK inhibitors. To assess the effect of Boritinib combined with Ibrutinib while maintaining an "activated microenvironment" *in-vitro*, primary CLL cells were stimulated with CD40L+IL-4 and treated with these drugs, either alone or in combination. Our findings show that the combination of Ibrutinib and Boritinib induces inflammasome-dependent cell death in over 90% of treated patients.

To determine whether the combination of Ibrutinib plus Boritinib, would also lead to increased anti-leukemic responses *in-vivo*, we performed adoptive transfer of splenocytes from Eμ-TCL1 mice. Monotherapy with Ibrutinib (78 d) and Boritinib (163 d) increased overall survival compared to mice assigned to vehicles (69 d), $p = 0.0088$ and 0.0044, respectively. Median survival was not reached in the combination group, $p < 0.0001$. Mice receiving ibrutinib plus boritinib reached day 170 of the experiment looking healthy with no ruffled fur, lethargy or hair loss.

In this work we propose a new axis of progression in CLL associated with inflammasome activation. Our results suggest that TME-derived signals might be responsible for the upregulation of TMEM176A in active CLL, contributing to disease progression by impairing inflammasome activation and halting cell death. The combination of Boritinib plus ibrutinib enhances cell death, improves survival of treated mice and sets the ground for the clinical evaluation of the inflammasome as a potential target in CLL.

Clinical and translational studies

POSTER SESSION

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Mutational profile as genomic markers of prognosis of Chronic Lymphocytic Leukemia in brazilian patients

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Introduction: Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by clonal proliferation of mature CD5+, CD23+, CD19+, and CD20+ B cells in the bone marrow and peripheral blood. It is the most prevalent leukemia in Western adults. CLL can remain asymptomatic for several years, but in some cases, symptoms can manifest rapidly, leading to disease progression. Genomic markers, such as the mutational status of the immunoglobulin heavy chain variable region (*IGHV*) in neoplastic B cells, are critical for prognosis. Patients with mutated *IGHV* (CLL-M) typically have a more indolent course and better outcomes, while those with unmutated *IGHV* (CLL-UM) often experience aggressive disease progression and poorer prognosis. Additionally, mutations in important genes regarding genomic stability like *TP53, SF3B1, NOTCH1, MYD88, BIRC3*, and *ATM* genes have been associated with CLL prognosis and disease evolution. Objectives: This study aims to correlate these genetic markers with CLL progression in patients from a Brazilian hospital.

Methodology: This study analyzed 44 CLL patients from a Brazilian hospital, whose DNA underwent IGHV gene sequencing to determine mutational status, and next-generation sequencing (NGS) for a panel of 6 genes (*TP53, SF3B1, NOTCH1, MYD88, BIRC3*, and *ATM* genes) to detect pathogenic variants (Approved by ethics committee, CEP n°6.193.523). The findings were then correlated with CLL evolution and prognosis.

Results: This cohort consisted of 41% mutated and 59% unmutated patients based on *IGHV* status. Notably, all patients in the indolent phase of the disease had mutated *IGHV*. Additionally, consistent with existing literature, 72% of patients undergoing treatment had unmutated IGHV status. No significant differences were observed between these groups regarding interval between diagnosis and treatment initiation (IBDTI). Data provided by NGS analyses presented mutations of uncertain significance in *NOTCH1* (n =2), *TP53* (n=1), *ATM* (n=2), and *MYD88* (n=1). Pathogenic mutations were found in *TP53* (n=4), *ATM* (n=2), *MYD88* (n=2), *SF3B1* (n=1) and *BIRC3* (n=1). CLL-M patients exhibited pathogenic mutations in *TP53, ATM*, and *MYD88*, while CLL-UM patients presented pathogenic mutations in *TP53, ATM, SF3B1*, and *BIRC3*. No pathogenic mutations were identified in the group of patients in the indolent phase of the disease. The group of patients undergoing treatment showed the highest number of pathogenic variants across different genes (*TP53, ATM, MYD88, SF3B1, BIRC3*) (Figure 1a). Interestingly, patients with shortest IBDTI presented pathogenic mutations in *TP53* and *ATM*, followed by the group of patients with disease transformation (*TP53* and *ATM*) (Figure 1b).

Conclusions: The results demonstrated that the analysis of *IGHV* mutational status and the mutational profile of the six genes studied is a prognostic factor for CLL. Patients with a better prognosis do not present gene mutations and have an LLC-M phenotype. Mutations in the *TP53* gene appear to lead to a more rapid disease progression. This panel can be instrumental in determining prognosis and assisting in clinical decision-making regarding the management of patients with CLL.

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Frequency and Survival Analysis of del(17p) TP53 and del(11q) ATM in Brazilian Patients with Chronic Lymphocytic Leukemia

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Introduction: Chronic lymphocytic leukemia (CLL) is characterized by a heterogeneous clinical course. Chromosomal abnormalities in CLL have an important role for the follow-up of disease evolution, to guide treatment and monitoring the response. Fluorescence *in situ* hybridization (FISH) is an indispensable tool for CLL patients due the low mitotic index in conventional cytogenetics. The presence of molecular cytogenetic alterations such as del(17p) and del(11q) confer an unfavorable prognostic outcome for CLL patients. This unfavorable prognosis is associated with the loss of one allele of *TP53* in del(17p) and *ATM* in del(11q), tumor supressor genes that have a fundamental role in apoptosis, cell cycle arrest, and DNA repair. However, few studies have been performed comparing the impact of clone size between del(17p) and del(11q) on survival of the patients with CLL.

Objectives: The aims of this study was analyse the frequency of del(17p) and del(11q) by FISH in a Brazilian cohort of CLL patients, and the percentage of positive cells presenting del(17p) and del(11q) (the clone size) comparing the impact of these molecular cytogenetic alterations on survival.

Methodology: Clinical and FISH studies were performed on 158 patients with CLL between 2010-2023. Median age was 65 years old and there were 63% of males. FISH analysis were performed on peripheral blood samples using the probes: TP53/CEP17 (spectrum Orange *TP53*/ spectrum green CEP17); ATM/CEP11 (spectrum Orange ATM/spectrum green CEP11); TP53(spectrum Orange)/ ATM spectrum green), Vysis, USA. The slides preparation was done according to manufacturer protocols. Survival analysis was performed using Kaplan Meier curves and Log-rank tests.

Results: The FISH analysis showed del(17p) monoallelic loss of TP53 in 37% of the total CLL patients, being 42% from this positive FISH cases with >20% of cells presenting del(17p). Twenty-one (13%) patients showed positive FISH for del(11q). These cases showed >20% of cells with del(11q). The presence of more than 20% del(17p) positive cells was related to a worse overall survival (OS) ($p<0.0001$), comparing with <20% del(17p) positive cells and negative del(17p) cells. No difference in survival was observed between negative FISH patients and those with less than 20% del(17p) cells. Comparing the impact of del(17p) and del(11q) on survival, the clone with more of 20% of cells presenting del(17p) presented a worse OS than that with del(11q) ($p<0.0001$).

Conclusions: The frequency of positive FISH for del(17q) (37%) was higher than positive FISH for del(11q) (13%) in Brazilian CLL patients. Patients presenting del(17p) in more than 20% of cells had a worse prognosis with reduced survival comparing with those patients with del(11q). Our results suggest the prognostic relevance of del(17p) clone size with monoallelic loss of TP53 detected by FISH in CLL patients.

23 A subgroup of patients with unmutated IgHV 1-69; 3-30; 1-02; 4-39 and high expression of activation-induced cytidine deaminase require earlier treatment

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Chronic lymphocytic leukemia (CLL) is characterized by its clinical and molecular heterogeneity. The practical challenge presented by this diverse landscape is the difficulty in predicting leukemia progression. Despite extensive efforts employing various clinical and/or molecular prognostic tools, accurate prediction of disease progression remains elusive for a significant number of patients. Our group, along with others, described that activation-induced cytidine deaminase (AID) is abnormally expressed in the peripheral blood (PB) of patients with poor clinical outcomes, predominantly in unmutated cases (U-CLL). This enzyme is necessary to initiate somatic hypermutation (SHM) and class-switch recombination process in B- lymphocytes. Additionally, deamination of "off-target" genes by AID can result in oncogenic mutations or translocations. Nevertheless, the role of this enzyme in the origins and evolution of CLL as well as why AID is predominantly expressed in U-CLL, remains a topic of ongoing debate. Previous studies from our laboratory demonstrated the loss of superantigenic and polyreactive binding of B-cell receptor (BCR) following SHM, (Oppezzo et al., 2004 EJI). Furthermore, findings from (Hervé et al. 2005, JCI), provide evidence that both U-CLLs and M-CLLs may originate from self-reactive B cell precursors. Collectively, these studies highlight SHM as a pivotal process in the origins/development of B-cell lymphoid neoplasms by altering the original autoreactivity of the BCR. Based on this concept, we hypothesize that the absence of SHM in U-CLL may result in continuous BCR stimulation and constitutive AID expression, contributing to a poorer clinical outcome.In this work, we characterized CLL patients based on AID expression in PB, its association with immunoglobulin heavy chain gene (IgVH) status/use and disease progression, assessed as time to first treatment (TTFT). PB samples were collected from patients meeting the clinical/immunophenotypic criteria for CLL, following the iwCLL guidelines. The cohort included 279 patients from Uruguay and 33 from Argentina. Written informed consent was obtained, and the study was approved by the Institutional Ethics Committees of each institution. Peripheral blood mononuclear cells were isolated using Ficoll-Hypaque, and RNA extraction and cDNA synthesis performed. From 312 studied cases, 56% were Binet's stage A, 23% were stage B, and 21% were stage C. The IgHV gene status was mutated in 52% of cases, cytogenetic aberrations were observed in 70.5% of patients, including 13q14_deletion (36.5%), trisomy_12 (13%), 11q22_deletion (9.7%), and 17p_deletion (8.3%). The median age at diagnosis was 66 years old, and the median follow-up was 5 years. Our results identify a novel CLL subgroup characterized by clonal expression of an unmutated BCR with specific rearrangements (IgHV_1-02, 1-69, 3-30, 4-39), high expression of AID enzyme and earlier treatment. Moreover, our results suggest that AID expression in U-CLL could be linked with antigen restriction and clinical outcome. This subset constitutes approximately 38% of the U-CLL (15% of the total cohort) and can be identified in advance by integrating AID mRNA expression and IgVH profile assessment into routine testing. We propose a new and practical prognostic tool, assessed in PB, which enables the identification of a novel CLL entity requiring early treatment initiation, typically within the first year.

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CLL in young people, a subpopulation with particular characteristics. A study of GURU-LLC.

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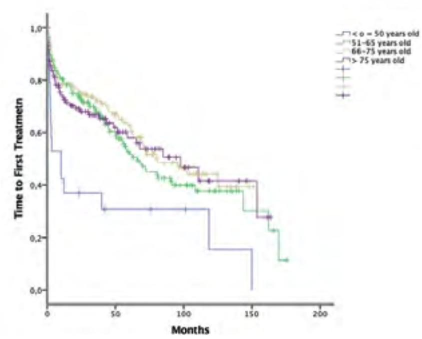
Introduction: Chronic lymphocytic leukemia(CLL) is the most prevalent leukemia in Western-world. In Uruguay approximately 180 cases/year are diagnosed according to SHU registry, median age at diagnosis: 73 years old(yo) and <65 represents 27%. 2021's SEER registry reports 9,2% CLL patients <55. We have no data of incidence and outcomes in younger population in our county. Parikh et.al in 2014 reported that CLL patients ≤55 yo have a higher prevalence of adverse clinical and biological characteristics and a shorter time to first treatment(TTFT): 4 years comparing to older ones. Therefore, this retrospective study seeks to understand how the young population with CLL in our country behaves. **Objective:** To study the biological characteristics and clinical outcomes of young CLL patients (≤50 yo) and compare them with those of the older population. **Results:** Retrospective study. Inclusion criteria: CLL diagnosed since 2000 and treated after 2010 or patients diagnosed after 2010 treated or not. Centers: CASMU,COSEM,Hospital Británico,Hospital de Clinicas,Hospital Maciel and Hospital Militar. 451 met inclusion criteria. Median age 68 yo(33-94), race: white: 445(98,7%). Public system: 228(50,6%). Median follow-up: 56 months(0,2-230). Table-1 shows characteristics according to age group. There aren't differences in sex, RAI, CD38, median lymphocytes, LDH or del17q. There was a trend of more unmutated-IGHV status in young CLL. There were differences in ECOG status, with worse ECOG in older patients. In those ≤50, 55% had Binet stage B/C at onset. As Parikh-2014 described, TTFT was shorter for younger patients. Interestingly, the media of our cohort was much lower than what was described by these authors. TTFT in ≤50 yo was 10,2 months (IC 95%_0-20-55), in 51-65 years: 65,85 (1,9-79,8), in 66-75 years: 80,9_(78-101) and > 75: 97_(55,3-139,9). p=0,004. 70% of ≤50-yo were treated. First line: FCR:8, FC:2, BR:1, Venetoclax-Obinutuzumab:1, Ibrutinib:1, CHOP-like:1. 40% of 51-65 needed treatment. FCR:29, FC:10, BR:4, Venetoclax-Obinutuzumab:6, Ibrutinib:2, CHOP-like/Clorambucilo:15.Patients treated with target agents(TA) had a trend of better OS than those treated with inmuno-chemotherapy: 100% versus 76% at 48-months,(p=0,3). Patients treated with TA had a trend of better TTNT than those treated with immuno+/-chemotherapy: 36 months in Ven-based 100%, ibrutinib 62% and FCR/BR: 82%,(p=0,7) Median OS: ≤50 and 51-65: not reached, 66-75: 121(101-142) months and >75: 51(37-66),p=0,0001. 3 years-OS was 80% in ≤50, 85% 51-65, 81% 66-76 and 54% >75 yo. **Conclusions:** Our data support previous results showing that young CLL patients need treatment early in their CLL journey compared with older ones. Surprisingly, in our cohort TTFT is shorter than other analyzed cohorts (Parikh Hemtologica-2014 and Cherng BJH-2021). At present we don't have a scientific explanation for this result. However, the fact that in Parikh's cohort, the subgroup of young CLL patients (<55 years old) includes only 3% with high Rai Stage and 54% with intermediate Rai (I-II) contrasts sharply with our cohort, where 55% of patients are in Binet stages B/C. This disparity could potentially be explained by differences in national healthcare systems, which may influence the stage at diagnosis and the overall clinical characteristics of patients in each country.

Table 1. Characteristics of CLL patients according to age

	≤ 50 years old N = 20 (%)	51-65 years old N = 165 (%)	66-75 years old N = 146 (%)	>75 years old N = 119	p
Median age (range)	46 (33-50)	60 (51-65)	70 (66-75)	81 (76-94)	
Sex					
- *Female*	5 (25)	56 (34)	57 (38)	44 (37)	0,576
- *Male*	15 (75)	109 (66)	89 (61)	75 (63)	
ECOG					
- *0-1*	17 (85)	135 (81,8)	115 (79)	53 (44,5)	0,0001
- *2-4*	0	2 (1,2)	5 (3,5)	27 (22,7)	
- *No data*	3 (15)	28 (17)	25 (17,1)	39 (32,8)	
Binet					
- *A*	9 (45)	104 (63)	98 (67,1)	85 (71,4)	0,038
- *B*	7 (35)	33 (20)	27 (18,5)	11 (9,2)	
- *C*	4 (20)	20 (12,2)	13 (8,9)	19 (16)	
- *No data*	0	8 (4,8)	(4,8)	4 (3,4)	
RAI					
- *0*	6 (30)	67 (40,6)	63 (43,2)	53 (44,5)	0,091
- *1*	2 (10)	34 (20,6)	36 (24,7)	25 (21)	
- *2*	6 (30)	24 (14,5)	17 (11,6)	18 (15,0)	
- *3*	4 (20)	11 (6,7)	4 (2,7)	12 (10,1)	
- *4*	1 (5)	5 (3,5)	4 (2,7)	6 (5)	
- *No data*	1 (5)	26 (17,1)	22 (15,1)	10 (8,4)	
CD38					
- *Positive*	4 (20)	15 (9,1)	16 (11)	15 (12,6)	0,458
- *No data*	6 (30)	58 (35)	51 (35)	41 (34)	
Median lymphocytosis (range)	25305 (3300-395.000)	16000 (1400-923850)	10100 (500-198000)	14200 (600-188850)	0,097
LDH					
- *High*	5 (20)	24 (14,5)	22 (15,1)	18 (15,1)	0,388
B2 microglobulin					
- *High*	4 (20)	56 (33,9)	54 (37)	42 (35,3)	0,015
Genetics					
- *Del 13q*	3 (15)	10 (6)	7 (4,8)	2 (1,7)	
- *Del 11q*	1 (5)	5 (3,0)	4 (2,7)	4 (3,4)	
- *+12 alone*	0	7 (4,2)	4 (2,7)	2 (1,7)	
- *Del 17p/TP53*	1 (5)	6 (3,6)	7 (4,8)	1 (0,8)	NS
- *Normal*	3 (15)	17 (10,3)	9 (6,5)	10 (8,4)	
- *No data*	12 (60)	116 (70,3)	115 (78,8)	100 (84)	
IGHV					
- *Mutated*	5 (25)	40 (24,2)	29 (19,9)	12 (10,1)	0,678
- *Unmutated*	8 (40)	38 (23,6)	31 (21,1)	12 (10,1)	
- *No data*	7 (35)	86 (52,2)	86 (58)	95 (79,8)	
Management at diagnosis					
- *Watch and wait*	11 (55)	138 (83,6)	120 (82,2)	97 (81,5)	0,021
- *Treatment*	9 (45)	27 (16,4)	26 (17,8)	22 (18,5)	



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Autonomous BCR Signaling and Genetic Aberrations in MBL Siblings of CLL Patients

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Introduction: Clonal expansion of CD5-expressing B cells, termed monoclonal B lymphocytosis (MBL), is a precursor condition for chronic lymphocytic leukemia (CLL) with an increased incidence in CLL siblings. In approximately 1% of MBL carriers, MBL progresses eventually to clinically overt CLL. The mechanisms driving benign MBL expansion and subsequent malignant progression are unknown, but both genetic and functional/environmental factors seem to play a role. Of these factors, autonomous signaling, which consists of the clonotypic BCR of CLL cells that signals without stimulation by a cognate antigen, serves as an indispensable oncogenic signal in CLL.

Objectives: The objectives of this study were functional characterization of the BCR of MBL in siblings of CLL patients and a comparison of genetic variants in MBL-CLL sibling pairs.

Methods: Screening of peripheral blood by flow cytometry detected 0.2-480 clonal CLL-phenotype cells per microliter (median: 37/µL) in 34 of 191 (17.8%) siblings of CLL patients. Clonal BCR was identified from highly purified MBL cells of 17 CLL siblings by ARTISAN PCR. Whole exome sequencing (WES) was performed with the SureSelect Human All Exon V7 kit and sequenced on the HiSeq2000 platform to 50x coverage. Variants were annotated for predicted pathogenicity. The comparison of allele frequency distribution was performed using the Wilcoxon rank sum test and kernel density estimation.

Results: Eleven MBL BCR, including all 5 assigned to a CLL stereotyped subset, were transduced into murine TKO cells arrested at the pro-B-cell stage by genetic deficiency of rag2, lambda5, and slp65. The autonomous BCR signal was less intense than the signal originating from the CLL BCR of their CLL siblings (p=0.015, Wilcoxon's matched-pair signed rank test). BCR signaling strength was correlated to the degree of MBL clonal expansion (p=0.034).
A polygenic CLL risk score (PRS) was higher in both CLL and MBL siblings compared to the general population, but no significant difference was found between CLL and MBL siblings. SNP array data showed recurrent CLL-associated CNVs in all CLL cases but only in a small fraction of MBL cells (p=0.003; Fisher's exact test). Likewise, variant allele frequencies (VAF) of non-shared variants as detected by WES were significantly lower in MBL samples than in CLL cases (p<0.0001). In particular, subclonal variants defined by a VAF of 0.1-0.33 were more prevalent in MBL compared to CLL siblings (p=0.014), whereas clonal variants (VAF 0.43-0.55) were equally abundant (p=0.65) in both conditions.
In this comprehensive comparison of MBL and CLL in siblings, CLL risk alleles were found with high and similar prevalence in CLL patients and MBL siblings, suggesting that CLL risk loci predispose to clonal expansion of CLL phenotype cells in both low-count MBL and CLL.

Conclusions: These findings support a stepwise CLL pathogenetic model wherein autonomous BCR signaling causes a non-malignant expansion of clonal CD5+ B cells in genetically predisposed individuals, followed by malignant progression to CLL after gradual acquisition of pathogenic genetic variants.

32 Quality of Life in patients with Chronic Lymphocytic Leukemia in Watch and Wait management. Data from the institutional register of the Italian Hospital of Buenos Aires.

Nancy Cristaldo[1]; Maria Orlova[1]; Julieta De Boeck[1]; Natalia Schutz[1]

[1] *Hospital Italiano de Buenos Aires.*

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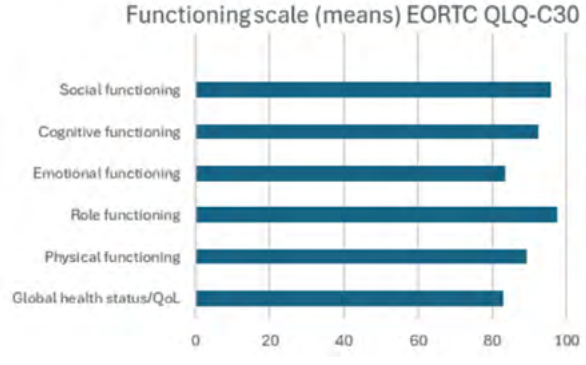
Introduction: Newly diagnosed chronic lymphocytic leukemia (CLL) patients are monitored regularly to detect treatment criteria for disease progression. However, studies have reported mixed results on the QoL between active surveillance patients and active treatment patients. In this study, we performed a cross-sectional study to examine the impact of active surveillance on health-related QoL in CLL patients on watch and wait management.

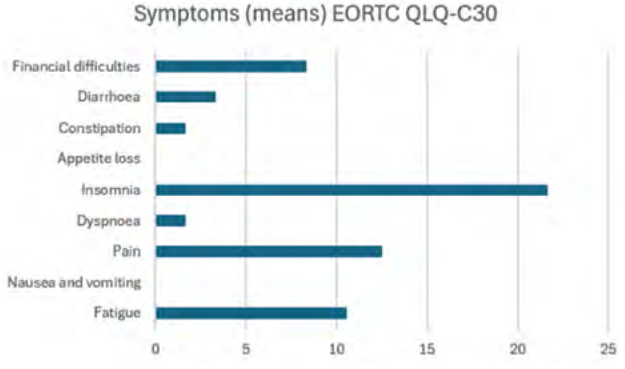
Objectives: Describe the quality of life in patients with CLL in watch and wait (WW).

Methods: We performed a cross-sectional of consecutive adults with CLL in watch and wait from the institutional registry of CLL of the Hospital Italiano de Buenos Aires, validated by the ethics committee. Specialist researchers conducted the telephone interview to assess the quality of life using the validated EORTC QLQ-C30 questionnaires in October 2024. Baseline characteristics, diagnosis, and follow-up were collected from the registry. Categorical variables are presented with percentage and absolute frequency. Quantitative variables with mean and standard deviation or median and interquartile range, according to distribution (Stata version 14)

Results: We included 20 patients with CLL on WW. The mean age was 74.1 years (SD=10.3), and 60% were male. The mean age to diagnosis was 64.7 years (SD=18.7), and the mean time in WW strategy was 9.6 years (SD=16.9). For EORTC QLQ-C30, all patients had functional scales above 80 over 100 (graph1), and the symptom with a higher mean was insomnia (graph2).

Conclusion: We concluded that for our small cohort in a LATAM single center with CLL in WW strategy, with a mean follow-up of 9.6 years, the impact on quality of life was mild, as shown in previous reports. This report is interesting because it shows data from Latin America. For the meeting, we intend to update the data with the score QLQ-CLL17 and gain more patients.





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Clinical features and evolution of treatment patterns in patients with Chronic Lymphocytic Leukemia in Uruguay. A multicenter observational study of the Uruguayan Group of CLL

Victoria Irigoin[1]; Carolina Oliver[2]; Ana Ines Landoni[3]; Virginia Lema[4]; Sabrina Ranero[5]; Victoria Remedi[3]; Rita Uria[6]; Raúl Gabús[3]; Gabriela De Galvez[1]; Pablo Muxi[7]

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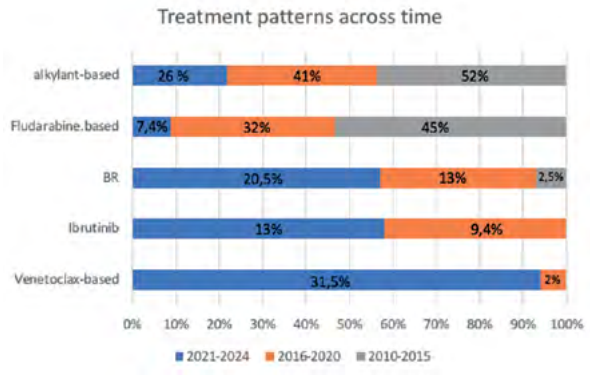
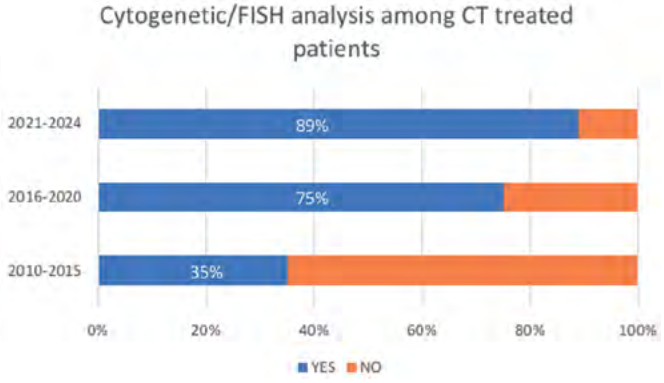
Introduction: Chronic Lymphocytic leukemia, the most frequent leukemia in adults in Western countries has an incidence in Uruguay of 4,17/100.000 per year. Data regarding incidence, age and gender is available but no information about clinical and biological characteristics or treatment selection has been published so far. The Uruguayan Group of CLL (GURU-LLC) gathers a group of clinicians and basic researchers in the field, aimed at developing investigation on CLL in our country. **Objectives:** Describe the clinical characteristics of patients and asses the evolution of treatment selection over time, following the advent of new drugs and recommendations. **Methodology:** Restrospective analysis of a cohort of patients assisted by physicians that participate in the GURU-LLC diagnosed or treated for the first time from 2010 onwards. **Results**: 447 patients from 6 institutions in Montevideo were included. Median age at diagnosis was 68 (33-94). 225 (50,3%) from public institutions, 222 (49,7%) private ones. 288 (64,4%) male. 295 (66%) Binet stage A, 78 (17%) B, 57 (13%) C, 17 (3,8%) no data at diagnosis. IgHV status was studied in 173 (38,7%), 19,5% had Mutated IgVH, 19,2% uIgHV 358 (80%) were observed at diagnosis. 196 patients received treatment during the study period. Median time to first treatment was 9,4 months (0-204). Conventional cytogenetic (CC) and FISH analysis performed in 17% and 37% before treatment. Analysis for p53 mutation done in 3% Altogether, 83 (42,3%) received alkylant-based therapy, 59 (30%) Fludarabine based treatment, 12 (6,1%) Ibrutinib monotherapy, 20 (10,2%) Bendamustine-Rtuximab, 18 (9,2%) Venetoclax and 2 (1,1%) other treatment. When we analyzed treatment patterns over time, out of 79 treated between 2010-2015, 53%, received alkylant-based therapy 44% Fludarabine, 2,5% BR. 54 were treated between 2016-2019, 40% received alkyants, 31,5% Fludarabine, 9,3% Ibrutinib, 13% BR, 1,9% Venetoclax. 53 treated between 2020-2024; 24,5% received alkylants, 7,5% Fludarabine, 13,2% Ibrutinib, 20% BR, 32% Venetoclax. Among chemotherapy (CT) treated patients between 2010-2015 60% didn´t have Cytogenetics (conventional or FISH) analysis before treatment, those treated with CT between 2016-2020 had no CG analysis in 24% and between 21021-2024 11 % of treated with CT had no CG performed before treatment Median Overall Survival among treated patients was 69,4 months (0,85-230) 104 months (65-143) among patients treated between 2010-2015, 211 months (72-350) between 2016-2019 and not reached among treated after 2020 (p=0,183) **Conclusions:** Epidemiology of this Uruguayan cohort resembles that in other western countries. Most patients were diagnosed in early stages and needed no treatment. Over time, the use of targeted therapies has increased following international guidelines and availability of new drugs in our country. Additionally we confirm a better cytogenetic and molecular characterization of the disease before treatment initiation in the last years. However, there is still room for improving the care of our patients, as we observe that even in the last years some patients treated with chemotherapy have no cytogenetic profile performed. We aim to gather information of treatment patterns in the resto of the country and to try to optimize the risk characterization and treatment of all our CLL patients.





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Real world experience of Venetoclax in patients with Chronic Lymphocytic Leukemia in Uruguay

Victoria Remedi[1]; Virginia Rodriguez[1]; Victoria Irigoin[2]; Carolina Oliver3; Mercedes Lassus[4]; Rafael Alonso[5]; Pablo Oppezzo[6]; Ana Inés Landoni[1]

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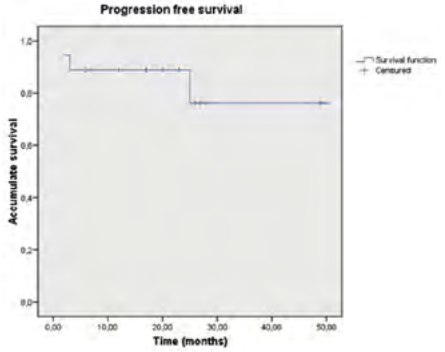
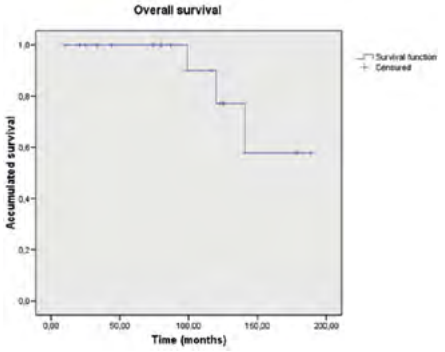
Introduction: Chronic lymphocytic Leukemia (CLL) is one of the most frequent types of leukemia among adult population of Caucasian origin, including Uruguay with an incidence of 4,17 / 100.000 per year. The gradual change in treatment from chemoimmune therapy to pathway inhibitors has been reflected in CLL patients all over the world, as well as in our country. Even though it has been shown the benefit of using Venetoclax in patients with Chronic Lymphocytic Leukemia, there is no published information of the real-world patients treated with Venetoclax in Uruguay. Objective: The main objective is to describe the experience of the administration of Venetoclax associated with anti CD-20 in real world CLL patients in Uruguay. We evaluated dose decrease, partial or total discontinuation of the treatment and clinical response. **Methods:** We analysed retrospectively 18 real world patients who had diagnosis of CLL according to the WHO classification and received Venetoclax associated with anti CD20 in the period from 2020 to 2024. We evaluated how many patients had a dose decrease, partial or total discontinuation of treatment and the impact it had on the clinical result of the treatment. The progression free survival and overall survival were analysed by Kaplan-Meier (IBM SPSS Statistics v15.0). **Results:** The analysed cohort is formed by 18 patients with the following characteristics: 24% are women, the median of age is 71 years old (35-81). Nine of the 21 patients had previous significant medical illness. Ninety percent were ECOG 0. The BINET classification shows 5 patients (24%) stage A, 8 patients (38%) stage B and 8 (38%) stage C at diagnosis. Fourteen percent of patients has del(17p) and 5% p53 mutation. Seven of the 18 patients (39%) received Venetoclax associated with Rituximab, 9 received Venetoclax associated with Obinutuzumab (50%). Two patients received Venetoclax monotherapy (11%). Of de 18 patients studied, 8 received Venetoclax associated with anti CD20 as first line of treatment (44%), being the rest their 2nd or 3rd line of treatment.Five of the 18 patients (28%) had to decrease the dose of Venetoclax, 3 of them in the first 3 months and the others after 3 months of treatment. From these, only 1 could return to full dose. The other 4 maintained the protocol treatment in a decreased dose. Of the 5 patients who required a decrease in dose, 3 of them were due to cytopenia and the other 2 due to infectious disease. None required a decrease in the dose of anti CD20 treatment. Four patients required partial discontinuation of treatment, in three of them the dose was reestablished in less than 1 month, the other required 2 months to return to the full dose. All achieved a complete remission after treatment. As far as total discontinuation of treatment is concerned, 3 patients from 18 (17%) had to discontinue treatment with Venetoclax and antiCD20 permanently. The reason to discontinue the treatment was in one case severe adverse skin reaction, one progressed under treatment and the third patient suffered sepsis and death. In terms of response, 15 achieved complete remission (CR) at the end of the treatment (83%), 2 partial response (11%) and 1 progressed (6%). After a median follow up of 17 months, the progression free survival (PFS) was not reached in the patients given Venetoclax plus anti-CD20. The median overall survival (OS) was not reached in this group of patients. **Conclusion:** twenty four percent of the patients required a decrease in treatment dose and 19% a partial discontinuation of Venetoclax. This did not affect the response rate, achieving complete remission in all cases. Further patients are required to increase the number of the sample.





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GURU-LLC-03. A multicentric, retrospective, observational study of the use of ibrutinib in the real world in Uruguay in patients with CLL and MCL. An investigator initiated study of the GURU-LLC (Uruguayan Group for the study of LLC, GURU_LLC)

Sabrina Ranero Ferrari[1]; Carolina Oliver[2]; Victoria Irigoin[3]; Ana Ines Landoni[4]; Virginia Lema[5]; Gimena Dos Santos[6]; Jorge Sclavi[7]; Andres Desiervo[8]; Marcelo Noble[9]; Patricia Segura[10]; Carolina Cordoba[11]; Mariana Stevenazzi[12]; Lucia Blanco[13]; Gabriela De Galvez[14]; Cristina Palermo[15]; Natalia Tejeira[16]; Victoria Toledo[17]; Pablo Muxi[18]; Raul Gabus[4]; Pablo Oppezzo[19]; Mercedes Lassus[20]; Rafael Alonso2[1]; Lilian Díaz Filgueira[22]; Cecilia Guillermo[6]

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We conducted a multicentric, retrospective, observational study of ibrutinib therapy in the real world (RW) in patients with CLL or MCL treated in Uruguay. All patients (pts) initiated therapy from 2017 until 31/5/2023 and were followed until 30/6/2024.

Primary objectives were the assessment of clinical/molecular patient profiles, efficacy and safety. Eighteen investigators participated and obtained approval from all corresponding Ethics Committees. All patients signed informed consent.

We enrolled 58 patients, 33 CLL/SLL, 25 MCL. Fourty were male. Median age was 68 years (52-87). Eighteen patients with CLL and all patients with MCL were pre-treated (13 transplants).

Most patients had ECOG scores 1-2. Twenty CLL patients had stage C and 19 MCL patients had stage IV or III. The most frequently associated diseases were hypogamaglobulinemia (8 CLL, 1 LM), infections (4 CLL, 2 LM) and hemolytic anemia (3CLL, 1 LM). The most frequent concomitant pathology was hypertension (twenty patients). Three patients had chronic atrial fibrillation and one also medicated for heart failure; they were initiated at a lower dose and they could be escalated during the first month.

Twenty-one CLL pts had p17del (20) or p53 mutations (1). In 10 patients, 1 had mutated and the rest unmutated IGvH.

Efficacy: 1) one CLL patient interrupted in month 1 due to Covid infection and is inevaluable, 1 progressed, 2 had SD and 29 had CR or PR (90.62%). By June 30/2024, 14 patients remain in treatment. Median overall survival has not been reached. Sixteen discontinued therapy: progression (8) adverse events (6), covid infections (1) and lung cancer (1).

2) MCL: One pt with renal failure died in week 1 and is unevauable, 4 progressed, 2 had SD and 18 had CR or PR (75%). Eleven are alive in response. Median overall survival is 31.4 months. Fourteen have disconued therapy: progression (10) and adverse events (4).

Six patients (3 MCL) initiated therapy at a reduced dose for medical reasons and could be escalated to full dose in the first month. Nine patients required dose reductions during therapy due mainly to cytopenia, diarrea or neumonía but 7 could return to full dose.

Six patients required transient discontinuation, one of them for atrial fibrillation. Six patients permanently discontinued therapy without progression: one who had covid during treatment developed taquicardia and reduced LVEF, 1 edema of upper limbs, 1 persistent anemia, 1 skin rash with pneumonia. Two patients with history of atrial fibrillation died; one developed acute myocardial infarction with shock and the another aggravation of cardiovascular symptoms.

Conclusions: Our results appear similar to real world literature albeit in a small sample. We plan to continue the RW evaluation of this and other targeted agents in the treatment of CLL

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Real World evidence Refractory/Relapsed Chronic Lymphocytic Leukemia in Uruguay (2000-2024)

Virginia Lema[1]; Victoria Irigoin[2]; Ana Ines Landoni[3]; Raul Gabus[3]; Sabrina Ranero[4]; Carolina Oliver[5]

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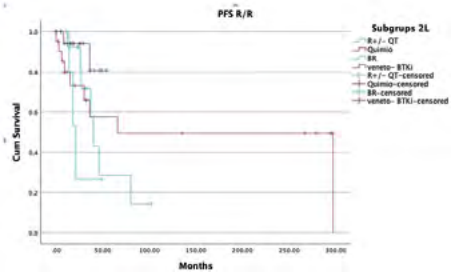
Introduction: In the last 10 years we have witnessed a great change in the paradigm treatment landscape for refractory or relapsed CLL worldwide. At 1L therapeutics, treatment options for relapsed/refractory (RR) patients and sequencing therapies. These advances include novel therapies such as Bruton's tyrosine kinase (BTK) inhibitors (e.g., Ibrutinib), BCL-2 inhibitors (e.g., Venetoclax). Being the current standard of care (SOC) for these patients. Improvements in the accessibility and optimization of cytogenetic and molecular study have allowed better characterization of patients and treatment sequencing with an impact on overall responses (ORR), progression-free survival (PFS) and overall survival (OS). In Uruguay, like in many countries, the approach to these cases typically involves various factors, including the availability of specific therapies, patient demographics, and healthcare infrastructure. Collecting real-world data on treatment outcomes, side effects, and overall patient experience is vital to apply treatment guidelines and improve care strategies. **Objectives:** Analyzing the treatment results in R/R) Chronic CLL patients, comparing immunochemotherapy/chemotherapy/BR with targeted therapies. Assess PFS between these 4 subgroups in 3 periods of time. Determine which treatment modality provides better outcomes for R/R CLL and establish evidence-based guidelines for managing R/R CLL in Uruguay **Methodology:** This multicenter retrospective analysis includes data from public and private healthcare centers in Uruguay, encompassing a follow-up period of 24 years (2000-2024) and involving 522 patients diagnosed with chronic lymphocytic leukemia (CLL). Focus on patients Relapsed/refractory status confirmed after first-line treatment. Analyze second-line treatment approaches over three defined time periods (2000-2014; 2015-2019;2020-2024) The documented treatment history was divided into first- and second-line treatments according to 4 subgroups, conventional chemotherapy, Immunochemotherapy, Bendamustine + Rituximab protocol and target therapies. Response assessments segmented by the treatment categories and time periods follow-up data for survival analysis (PFS) by log-rank tests to compare survival distributions and Kaplan-Meier survival curves for each treatment group. **Results:** The general characteristics of the R/R population are described in Table 1. Of the total of 522 patients, first-line treatment (1L) was given to n=251 patients (48%) an n=83 patients were R/R to 1L. With a median follow up of 22 months (0-297) from Relapse/Refractory the PFS by Subgroups was: 70% Rituximab+/- QT, 33% Bendamustina-Rituximab, 65% Conventional Chemo and 94% for de Venetoclax/BTKi group. While the differences in PFS aren't statistically significant, there appears to be a notable trend where the Venetoclax/BTK inhibitor group shows superior outcomes compared to the other treatments, especially the BR group, which shows a significant drop in PFS. (Figure1.) This reaffirms Venetoclax/BTKi protocols might be a more effective option for improving PFS in patients with relapsed/refractory CLL, despite the lack of statistical significance in the results Regarding the use of therapies per period we observed a shift in treatment preferences over time, with a notable decline in the use of conventional chemotherapy, immunochemotherapy, and BR with an increasing adoption of Venetoclax/BTKi. **Conclusions:** Collaboration among healthcare professionals, patients, and research institutions is essential to improve outcomes and advance treatment strategies in the region. The analysis reveals important trends in the management of R/R CLL in Uruguay suggesting a growing confidence in the efficacy of Venetoclax/BTKi for managing R/R CLL and demonstrating the need for ongoing evaluation of treatment efficacy through Local metrics like PFS and OS. The data will be valuable for guiding future clinical practices and improving patient outcomes in CLL management as well as increased awareness of the benefits of these newer therapies.

Table 1

Relapse/Refractory Chronic Lymphocytic Leukemia Population (n=83)
Median age 64 years (40-87)

Characteristics	2000-2014 (n=)	2015-2019 (n=)	2020-2024 (n=)
Feminine	3	5	7
Masculine	8	15	19
R/R	22	26	24
2L R+/- QT	5	8	3
2L QT	14	7	2
2L BR	1	4	2
2L Venetoclax/BTKi	0	4	16
No Data	2	3	1



44 Chronic Lymphatic Leukemia with Hyperleukocytosis

Ricardo Cañellas[1]; Carina Boveda[1]; Rodrigo Santacruz[1]

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Introduction
Chronic Lymphatic Leukemia (CLL) is a neoplasm with proliferation and accumulation of immunoincompetent clonal lymphocytes and B phenotype. It is the most common leukemia in adults in Western countries. The typical presentation is that of older adults with lymphocytosis between 15.000 and 150.000/mm3, many of whom do not require treatment at diagnosis.

Clinical case 1
43-year-old male, diagnosed by chance finding of Rai 1 Binet A CLL (adenopathy and mild lymphocytosis), without treatment criteria, Blood phenotype CD45+, CD19+, CD20+, CD5+, CD23+, CD43+, CD200++, KAPPA+, ZAP70-, CD38+. Normal karyotype, FISH (17p13.1) (tp53) not mutated, IGFV gene not mutated. LDH and B2M normal.

12 months after diagnosis, B symptoms and lymphadenopathy growth, lymphocytosis 200.000/mm3, without anemia or thrombopenia, polyadenopathy in all territories and splenomegaly by CT. Biopsy of cervical lymphadenopathy confirms CLL (not transformation to Richter syndrome) as well as flow cytometry in peripheral blood. Bendamustine type R immunochemotherapy is performed (initially without R) currently in progress with normalization of the blood count after cycle 2.

Clinical case 2
39-year-old woman, casually diagnosed with BINET B RAI II CLL (adenopathy and mild lymphocytosis < 20.000/mm3), without treatment criteria. Normal peripheral blood phenotype CD20+, CD23+, CD5+/CD10-/CYCLINE D1-), ZAPA70- LDH and B2M.
Nine months after diagnosis, cervical and axillary lymphadenopathy of up to 5 cm was found, lymphocytosis 327.00/mm3 without anemia or thrombopenia. Cytometry rules out transformation and FISH for CLL and molecular biology rules out TP53 (17p) and ATM (11q)
Immunochemotherapy type R FC was performed (without R in the first cycle) for 6 cycles, confirming a complete response by CT, normal blood count, with negative MRD in the marrow.

Discussion
These are 2 cases of young patients (< 45 years) initially without treatment criteria or poor prognostic factors who progress rapidly within 1 year. In both cases, immunochemotherapy was initially chosen without anti-CD20 (Rituximab) until lymphocytosis was <100.000/mm3 according to the availability of treatment in our public system. The response was excellent, reaching normalization of the blood count from cycle 2 onwards, in the second case even with negative MRD, the first case still undergoing treatment.

Biological and translational studies

POSTER SESSION

13 NF-KB Activation as a key driver in Chronic Lymphocytic Leukemia evolution to Richter's Syndrome: unraveling the influence of immune microenvironment dynamics

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Introduction: Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world and can evolve into a more aggressive condition known as Richter's syndrome (RS). The NF-κB pathway is central to CLL pathogenesis, primarily driven by B-cell receptor (BCR) signaling. Recent evidence suggests that BCR signaling is downregulated in RS, raising questions about how NF-κB activity is maintained and regulated during this transition.

Objectives: This study aims to elucidate the triggers and dynamics of NF-κB activation during the progression from CLL to RS.

Methodology: Single-cell RNA sequencing datasets were obtained from the Gene Expression Omnibus (accession numbers: GSE165087 and GSE183432) and Zenodo (accession code: 6631966). We selected paired peripheral blood samples from 4 patients with CLL and RS: 1 patient from GSE165087, 2 patients from GSE183432, and 1 patient from Zenodo. After quality control and preprocessing, we integrated the data using the Fast Integration with Reciprocal PCA (RPCA) method, resulting in a combined dataset of 49,879 cells. Cell types were annotated using the Azimuth reference database. We calculated canonical and non-canonical NF-kB activity scores using the AUCell algorithm with gene sets derived from the Gene Ontology Biological Process database. To infer and analyze cell-cell communication patterns, we employed the CellChat algorithm, comparing interaction networks across disease stages.

Results: A distinct expression pattern of NF-κB members was observed in RS compared to CLL, with a higher proportion of Malignant Cells expressing *NFKB1* (27.5% vs. 6.7%), *NFKB2* (21.5% vs. 8.3%), *RELA* (26.7% vs. 6.6%), *RELB* (40.7% vs. 25.9%), *NEMO* (19.1% vs. 4.8%), *NIK* (17.2% vs. 7.2%), *IKKα* (5.8% vs. 1.7%) and *IKKβ* (16.1% vs. 5.4%). Additionally, a significantly higher score for the Canonical NF-κB pathway was observed in RS ($p < 0.001$), along with an equivalent score for the Non-canonical NF-κB pathway. Analysis of the tumor microenvironment revealed a shift in cell type composition, with increased proportions of CD4+ T cells (11% vs. 4.8%), Double Negative T cells (dnT; 0.4% vs. 0.1%), and Gamma Delta T cells (gdT; 0.4% vs. 0.05%), accompanied by a slight decreased proportions of Dendritic Cells (DC; 0.05% vs. 0.14%) and Mucosal-associated invariant T cells (MAIT; 0.11% vs. 0.17%). Concurrently, intercelular communication patterns changed, showing increased activation of key NF-κB-related signaling complexes. Notably, BAFF and APRIL signaling (with monocytes and dendritic cells as senders and malignant cells as receivers) and LAIR1 signaling (with NK and MAIT cells as senders and malignant cells as receivers) were upregulated. These interactions were driven by specific ligand-receptor pairs, with these receptors being found to be expressed in a higher percentage of malignant RS cells, like BAFF-R (51.3% vs. 29.2%), TACI (30.6% vs. 20.2%), and LILRB4 (25.3% vs. 2.6%).

Conclusions: This study reveals increased NF-κB expression and activity in RS compared to CLL, partially attributable to changes in the tumor microenvironment composition and enrichment of NF-κB-associated signaling complexes.

These results suggest that NF-κB plays a critical role in the CLL to RS transition, modulated by alternative signaling pathways beyond BCR signaling, highlighting the importance of the tumor microenvironment in disease progression.

16 Double IGHV rearrangements in chronic lymphocytic leukemia patients. Their frequency and characteristics

Carmen Stanganelli[1]; Juana Cabrera[1]; Cecilia Rodriguez[2]; Silene Silvera[2]; Andrea Bender[3]; Evangelina Agrielo[3]; Raimundo Bezares[4]; Irma Slavutsky[5]

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Introduction: The IGHV (immunoglobulin heavy variable) mutational status, a robust prognostic marker in chronic lymphocytic leukemia (CLL) allows patients to be stratified into two groups: mutated (M) (<98% homology to the germline) and unmutated (U) (≥98%), with different outcome. Most cases usually develop a monoclonal disease but, occasionally, double productive IGHV rearrangements (DPR) have been detected.

Objectives: The aim of this study was to evaluate the frequency and characteristics of DPR in CLL patients from an Argentine cohort.

Methodology: A total of 1138 CLL patients (704 men; mean age: 65.8 years, range: 23-100 years; 58% M and 42% U) were analyzed. The study was approved by the Institutional Ethics Committees. All individuals provided their informed consent. RT-PCR and bidirectional sequencing were performed. Sequences were analyzed using ImMunoGeneTics (IMGT; http://imgt.cines.org). Stereotyped receptors were assessed using the database http://bat.infspire.org/arrest/assignsubsets/. For all test, $p<0.05$ was regarded as statistically significant.

Results: A total of 21/1138 (1.8%) cases showed DPR, 13 with concordant mutational status (7 double M and 6 double U) and 8 discordant, were observed (Table 1). In addition, 24/1138 (2.1%) cases with double productive (P) and non-productive (NP) rearrangements [22 with concordant mutational status (14 U and 8 M) and 2 discordant cases], were detected. The most represented VH families were VH3>VH4>VH1, with over-representation of the VH2, VH5 and VH7 families in the DPR group compared to cases with one rearrangement. The most frequent family combinations in the DPR group were VH1+VH3 and VH3+VH4 (6 each). VH2-5 gene was over-represented in DPR cases (p=0.04) (Figure 1). Three patients with DPR presented stereotyped receptors (14.3%), one of them showed stereotyped receptors in each rearrangement corresponding to different subsets (Case 10: subsets #31 and #3, both of adverse prognosis). Two cases were studied twice, one of them presented in the second sample (9 months later) the original P (VH3-23) and a new P rearrangement (VH4-4), while the other had a single NP rearrangement in the first study and a new P rearrangement in the second (6 months later), suggesting clonal evolution. Flow cytometry (FCM) detected one clone in patients with concordant mutational status and 2 clones (different light chains) in 5/8 (62.5%) discordant cases.

Conclusions: To our knowledge, this is the first analysis of DPR in CLL patients from Latin America. A similar frequency of IGHV DPR carriers to those described in the literature (~2%), as well as family distribution were observed. Cases with P and NP rearrangements were under-represented in our series compared to previous reports (8.4-15%). The expression of a single light chain and two IGHV rearrangements could correspond to the existence of two clones or a biallelic condition. Interestingly, the presence of two clones by FCM was only found in discordant cases. The co-occurrence of concordant mutational status could indicate the presence of common factors acting during maturation and selection, such as the presence of a common progenitor or reactivity to shared epitopes.

Table 1: Double productive rearrangements in Argentine CLL patients

Case	IGHV	Mutational Status	Subset
1	VH2-5/VH3-23	M/M	-
2	VH3-48/VH5-51	M/M	-
3	VH3-23/VH4-4	M/M	-/#71
4	VH3-72/VH4-34	M/M	-
5	VH5-51/VH3-30	M/M	-
6	VH3-15/VH4-39	M/M	-
7	VH3-33/VH4-34	M/M	-
8	VH3-33/VH1-46	U/U	-
9	VH1-69/VH3-30	U/U	-
10	VH3-48/VH1-69	U/U	#31/#3
11	VH1-69/VH3-30	U/U	-
12	VH2-5/VH3-23	U/U	-
13	VH4-59/VH2-5	U/U	-
14	VH7-4/VH4-38	M/U	#99/-
15	VH3-9/VH4-34	M/U	-
16	VH3-48/VH1-4	M/U	-
17	VH1-3/VH4-61	M/U	-
18	VH1-69/VH3-15	M/U	-
19	VH1-18/VH3-7	M/U	-
20	VH2-5/VH3-48	M/U	-
21	VH1-69/VH5-51	M/U	-

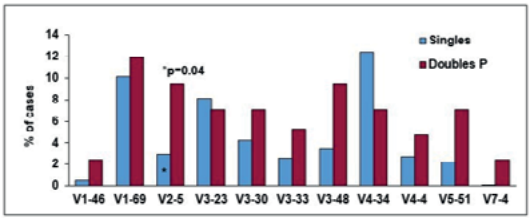

Figure 1: Distribution of IGHV genes in CLL patients with single and double productive rearrangements.

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Single nucleotide variants in genes involved in different signaling pathways in chronic lymphocytic leukemia patients

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Introduction: The analysis of the genomic architecture of chronic lymphocytic leukemia (CLL) is key to identifying novel drivers involved in mechanisms related to disease evolution. Microarray technology offers the possibility to evaluate a large number of single nucleotide variants (SNVs) that could reveal new genetic markers associated to the pathology.

Objective: To analyze by a microarray platform, SNVs of genes involved in different signaling pathways in order to identify biomarkers related to the clinical variability of CLL patients.

Methodology: Our retrospective study included 39 CLL patients (26 men; mean age: 67.9 years, range: 53-86 years; Rai stages: 0: 9.2%, I-II: 63.4%, III- IV: 27.4 %) and 8 controls (4 men; mean age: 64 years, range: 56-88 years). The study was approved by the Institutional Ethics Committees. All patients provided their written informed consent. Cytogenetics and FISH (fluorescence in situ hybridization) analysis were performed. For IGHV (immunoglobulin heavy variable) analysis, PCR and bidirectional sequencing were done. Genomic DNA was purified from peripheral blood cells using the Wizard" Genomic DNA Purification kit (Promega, USA). SNVs were analyzed using the Illumina Infinium Global Screening Array 24 v2.0 platform (774,295 variants). SNVs with a t.score >2.5 or <-2.5 were considered significant. Binary Discriminant Analysis (BDA), Principal Components Analysis and k-means clustering with RStudio software were used. The statistical evaluation was carried out considering the genetic models of penetrance (additive, recessive and dominant) using the SNPStat software. Time to first treatment, was estimated by the Kaplan-Meier method and compared with the Log-rank test. A $p<0.05$ was regarded as statistically significant.

Results: Patients were divided into two groups: favorable prognosis (FP) and adverse prognosis (AP), taking into account their genetic characteristics. The evaluation of karyotype ($p=0.025$), FISH ($p<0.001$), IGHV mutational status ($p<0.001$) as well as time to first treatment ($p=0.026$) showed significant differences between both groups. SNVs from 119 genes involved in different signaling pathways were analyzed. Bioinformatic analysis showed 25 SNVs significantly represented with respect to controls: 6 in the AP group and 19 in the FP group. The SNPStat analysis showed CARD11 rs1843939 and PAX5 rs702041 variants associated with an increased risk of adverse clinical outcome according to the dominant model (OR=5.2; $p=0.022$ and OR:5.14; $p=0.015$, respectively). For CARD11 rs1843939 and PAX5 rs702041 variants, the alternative allele was present in 39.4% and 50% cases, respectively. In both variants, the alternative allele was found in a greater proportion of patients with AP (55.6% and 66.7%, respectively), supporting the importance of the genetic profile in CLL characterization.

Conclusions: Even though the limited number of our series, these findings suggest that CARD11 rs1843939 and PAX5 rs702041 variants could be useful prognostic markers to identify CLL patients with increased risk of adverse prognosis. Interestingly, PAX5 gene is involved in the B-cell differentiation and maturation while CARD11 in NF-kB activation. Our results contribute to the genetic characterization of CLL and support the importance of deepening molecular studies in this pathology. More studies are necessary to confirm our results.

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Role of g protein-coupled receptor kinase 2 (GRK2) in the migration and activation of t cells from Chronic Lymphocytic Leukemia (CLL) patients.

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Background: The proliferation and resistance of leukemic cells to therapeutic agents primarily occur within lymphoid tissues, driven by interactions with the tumor microenvironment. GRK2 plays a central role in B and T cell homing to lymphoid organs by inducing the downregulation of the sphingosine-1 phosphate receptor-1 (S1PR1), which allows lymphocytes to overcome the S1P-mediated retention in the blood and to enter into lymphoid tissues. We have recently described that GRK2 inhibition increases leukemic cell migration towards S1P, inducing leukemic cell retention into the blood and reducing its presence in lymphoid tissues in the Eμ-TCL1 mouse model of CLL (XX iwCLL 2023). The impact of GRK2 inhibition on cells from tumour microenvironment was not studied yet. T-cells from CLL patients are key components of the tumor microenvironment providing survival and proliferative signals to the leukemic clone. Besides its role on T cell homing, GRK2 has also been shown to enhance TCR signalling through complex interactions with CD3 epsilon chain and other membrane receptors. Here we aim to evaluate the role of GRK2 in the migration, activation and survival of T cells from CLL patients.

Methods: Peripheral blood samples were collected from untreated CLL patients. All samples used in this study were obtained after informed consent in accordance with the Declaration of Helsinki and with Institutional Review Board approval. Cell viability was evaluated by flow cytometry (FC). T cell activation was induced by immobilized anti-CD3 mAb. T and leukemic B cell activation was evaluated by FC. Chemotaxis assay toward S1P, CXCL12 or CCL21 was carried out using Transwell migration assay. Venetoclax, a Bcl-2 inhibitor, was used to evaluate drug-induced apoptosis. For GRK2 inhibition, the commercially available compound CMPD101 was used. Statistical significance was determined using non-parametric tests with the GraphPad Prism software v7.

Results: We found that the GRK2 inhibitor (inhGRK2) (0.3-30 μM) did not affect the viability of T cells from CLL patients (not shown). As observed for leukemic cells, the migration towards S1P of T cells from CLL patients was significantly increased in the presence of the inhGRK2 (Fig. 1A). Interestingly, the migratory response to CXCL12 and CCL21 of T cells from CLL patients was decreased in the presence of the inhGRK2 (Fig. 1B-C). GRK2 inhibition also reduced the activation of T cells induced by the stimulation trough the TCR evaluated as the up-regulation of CD40L (Fig. 1D). Moreover, we found that activation and venetoclax-resistance of leukemic cells, induced by the co-culture with autologous activated T cells, was impaired when the GRK2 inhibitor was present (Fig. 1E-F).

Conclusion: Our previous research on GRK2 in CLL indicated that GRK2 inhibition could be a strategy to promote leukemic cell retention in the bloodstream, increasing their exposure to therapeutic agents and/or to overcome treatment resistance induced by the protective microenvironment. The findings presented here further underscore the therapeutic potential of GRK2 inhibition, revealing not only its direct effects on leukemic cells but also its impact on the tumor microenvironment, specifically through the reduction of T-cell-mediated activation and venetoclax resistance in leukemic cells.

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Expression and knock out generation of Histone 1.3 in Chronic Lymphocytic Leukemia

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Introduction: Chromatin activation has been associated to disease progression in chronic lymphocytic leukemia (CLL). Histones 1 (H1) modulate the conformation of the chromatin and limit its accessibility, playing a key role in the epigenetic regulation of transcription. We previously showed that overexpression of the mutagenic enzyme AID enhances CLL disease aggressiveness and introduces loss-of-function mutations in H1.3, a nuclear protein whose gene was previously described as a disease driver and was also found mutated in different B cell lymphomas. Interestingly, H1.3 levels are upregulated in ovarian and in pancreatic cancer. We hypothesize that H1.3 expression levels or mutant variants impact the phenotype and function of CLL cells.

Objective: To describe H1.3 expression profile and evaluate its role in CLL.

Methods: We studied H1.3 expression at the gene level (HIST1H1D) in CLL by analyzing available databases and initiated the evaluation of H1.3 at the protein level in highly purified CLL cells from primary patient samples. We generated H1.3 stable knock out (KO) clones by CRIPSPR/Cas9 technology and the limiting dilution technique on MEC-1 and OSU cell lines to study the impact of H1.3 absence in the levels of total RNA synthesis, cell activation and division.

Results: Gene databases of CLL samples showed no differences in HIST1H1D RNA expression between normal B cells and leukemic cells (n=8, ns) or when comparing IGVH mutated versus unmutated patients (n=58, ns). By contrast, databases show a downregulation of HIST1H1D in CLL cells activated with CD40L, by co-culture with T cells or through crosslinking of the BCR with anti-IgM (n=16, $p<0.05$). In addition, we obtained six H1.3 stable KO MEC-1 clones and two OSU H1.3 KO clones. Excision of the targeted gDNA was verified by end point PCR and lack of H1.3 expression was confirmed by WB. OSU clones KO for H1.3 did not sustain their proliferation in vitro, as opposite to MEC-1 H1.3 KO cells. Compared to WT MEC-1 cells, H1.3 KO MEC-1 cells showed increased activity of total RNA synthesis, upregulated expression of CD86, and decreased their levels of CD25. While proliferation was enhanced in one of the MEC-1 H1.3 KO clones, this did not occur in the other clones evaluated.

Conclusion: We conclude that H1.3 expression is downregulated in stimulated CLL cells and that its absence leads to activation and increased total RNA synthesis. We have generated new H1.3 KO tools which will allow us to further evaluate the role H1.3 in CLL. Experiments ongoing now in our lab include the H1.3 KO lines and also detection of CLL cases carrying the mutated form of HIST1H1D, for future studies such as RNA seq, ATAC-seq and Hi-C, as well as evaluations on the effect of Ibrutinib, which was shown to affect the chromatin conformation.

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Defects in the frequency and effector function of KIR+ and NKG2A+ virtual memory CD8+ T cells in patients with Chronic Lymphocytic Leukemia

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Introduction: The survival and proliferation of malignant Chronic Lymphocytic Leukemia (CLL) cells are heavily influenced by their interactions with both immune and non-immune cell populations. Recently, a subset of human CD8+ T cells expressing KIR or NKG2A receptors, known as Virtual Memory T (TVM) cells, has been identified. These cells exhibit a memory-like phenotype and possess robust cytotoxic activity and cytokine production capabilities. Given these properties, TVM cells may play a significant role in cancer immune surveillance, potentially impacting the control of CLL.

Objectives: The objective of this study was to assess the quantity, phenotypic profile, and functional characteristics of TVM cells in patients with CLL and healthy donors (HD).

Methodology: CLL patients and HD were recruited from the Hospital Nacional de Clínicas either at the time of diagnosis or during follow-up visits. None of the patients were undergoing treatment at the time of blood sample collection. Peripheral blood mononuclear cells (PBMCs) were isolated, and a 17-parameters flow cytometry analysis was performed to evaluate phenotypic markers, effector molecules, and transcription factor expression as part of the TVM cell signature.

Results: Our data reveal a significant decrease in the frequency of KIR+ TVM cells in CLL patients (p=0.009), while NKG2A+ TVM cells remain at similar levels compared to HD. Notably, two key transcription factors, Eomesodermin (Eomes) and Helios, which are master regulators of TVM cell function, are significantly reduced in KIR+ TVM cells (p=0.01 for Eomes and p=0.03 for Helios), but not in NKG2A+ TVM cells when compared to HD. Furthermore, KIR+ TVM cells in CLL patients show a marked reduction in Perforinhi (p=0.02) and Granzymehi (p=0.05) expression, while only Granzymehi expression is significantly reduced in NKG2A+ TVM cells (p=0.02). Interestingly, upon pre-stimulation with interleukin (IL)-15 for 48 hours followed by PMA/Ionomycin and plate-coated anti-CD16 stimulation for 5 hours, both KIR+ and NKG2A+ TVM cells from CLL patients and HD were capable of producing high levels of the inflammatory cytokine IFNg, as well as expressing the proliferation marker Ki67 and the degranulation marker CD107a at comparable levels. Importantly, the frequency of KIR+ TVM cells significantly increased following IL-15 stimulation in both CLL patients and HD.

Conclusions: In this study, we demonstrate a significant decline in both the frequency and function of a subset of CD8+ T cells, known as virtual memory T cells, which exhibit high cytotoxic potential against malignant B cells in CLL patients. Notably, we found that following IL-15 stimulation, KIR+ TVM cells from CLL patients not only increased in number but also exhibited functional characteristics comparable to those of TVM cells from healthy donors. These findings suggest the potential role of TVM cells in controlling B cell malignancies and highlight the need to explore whether different treatments could enhance or restore their effector functions in CLL patients.

30 The RNA-binding protein Musashi2 regulated by the NOTCH1/KLF4 pathway, modulates CLL cell migration and contributes to disease progression

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Post-transcriptional regulation is a crucial mechanism for cells to control gene expression, with RNA-binding proteins (RBPs) playing a key role in determining the fate of RNA molecules. Due to its significant function, any alteration in this process has been linked to various cancers. In chronic lymphocytic leukemia (CLL), we found that the RBP Musashi2(MSI2) promotes tumor cell survival and proliferation. CLL patients with elevated MSI2 levels experience shorter time to first treatment and reduced overall survival. This leads us to propose that MSI2, its regulators, or downstream targets could influence the progression of CLL in patients. Therefore, we studied the molecular mechanisms that induce MSI2 overexpression in CLL-B cells. In CLL-cells NOTCH1 suppresses the expression of the transcription factor Kruppel-likefactor 4(KLF4), a negative regulator of MSI2 in adenocarcinoma. Because low levels of KLF4were reported for CLL-B cells, we wondered whether MSI2 overexpression in CLL was due toalterations in NOTCH1/KLF4 pathway. To answer this, we determined mRNA expression levelsof KLF4/MSI2 in CLL-cells(n=16) and HD(n=3). Results showed a negative correlation betweenKLF4/MSI2($p=0.0028$). To further study the role of NOTCH1/KLF4/MSI2, poor outcome CLLpatients' cells were treated with NOTCH1 inhibitor(secretase) in-vitro andNOTCH1/KLF4/MSI2 levels were determined. NOTCH1 inhibition increased KLF4levels($p=0.01$) and downregulates MSI2 expression($p<0.05$). To confirm that KLF4 negativelyregulates MSI2 by binding to MSI2-promoter, chromatin immunoprecipitation using anti-KLF4, and PCR MSI2-promoter amplification was performed in CLL-cells(n=6). Blocking NOTCH1immunoprecipitated a fragment of the MSI2-promoter, showing that KLF4 binds and negativelyregulates MSI2 expression in CLL-cells. Supporting this data, we observed higher levels ofMSI2 in CLL-cells with NOTCH1-mutated (constitutive active NOTCH1) than NOTCH1-non-mutated, suggesting that NOTCH1 regulates MSI2 in CLL patients. Moreover, NOTCH1 activates survival genes, including oncoprotein c-MYC. Thus, weinvestigated the effects on c-MYC expression in CLL samples(n=13) in-vitro treated withNOTCH1 and MSI2 (Ro082750) inhibitors. The results show that combined inhibitorssignificantly reduce c-MYC expression($P\leq0.001$), suggesting a strategy to reduce tumorviability.Because MSI2 is highly expressed in dividing cells and regulates different targets in a cell type-specific manner, we studied MSI2´s role in activated/dividing CLL-cells. We analyzed theproteome in activated (CpG-ODN+IL15) CLL-B cells(n=12) treated with siRNA-MSI2(MSI2 low) and compare them to the control siRNA-CTR (MSI2 high). Results showed that the reduction ofMSI2 protein levels significantly increased the expression of 12 proteins($p\leq0.01$) involved incell-migration and cytoskeleton rearrangement. Cytoskeleton rearrangements of cells bound tofibronectin-coated slides with high and low MSI2 were analyzed by microscopy. Resultsshowed that high MSI2 levels inhibit cell-migration. Given that: 1-high MSI2 levels areassociated with poor outcomes, 2-MSI2 is higher in lymph nodes than in peripheral blood, particularly in dividing cells, and 3-MSI2 inhibits cell-migration in activated cells, wehypothesize that MSI2 may help retain cells in solid tissues where they receive survival signals,promoting disease progression. Further studies will be done to confirm the hypothesis. These results reveal novel insights into the molecular mechanisms regulating MSI2 expression,emphasizing the role of NOTCH1/KLF4 pathway. Additionally, we highlight the potentialfunction of MSI2 inhibiting CLL-cells migration within an active microenvironment supportingCLL progression.

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Unraveling the role of Musashi2 in c-MYC regulation and its implications for Chronic Lymphocytic Leukemia therapy.

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The regulation of gene expression sustains the cellular homeostasis, and its dysregulation is associated with various types of cancers. Therefore, understanding its role in cancer could give us tools for identifying new therapeutic targets. At the post-transcriptional level, RNA-binding proteins are crucial in controlling gene expression by regulating the temporal, spatial, and functional dynamics of messengers RNAs (mRNA). Among these proteins, the oncoprotein Musashi2 (MSI2) plays a significant role in these regulatory processes.

MSI2 binds to consensus sequences on target mRNAs, blocking protein translation, and in some cases, contributes to mRNA stability, positively regulating translation. Interestingly, elevated MSI2 levels have been reported in various cancers, including chronic lymphocytic leukemia (CLL). Our group has identified high MSI2 levels in CLL patients with poor prognosis. Notably, reducing MSI2 levels or inhibiting its function with Ro082750 eliminates both human and murine CLL cells. Given that MSI2 regulates cancer-associated biological processes, MSI2 and its target mRNAs represent promising therapeutic targets. Although MSI2 binds to consensus sequences in mRNAs, its binding and regulation of different mRNA molecules vary across tissues, affecting distinct cellular functions. Currently, the specific mRNAs that MSI2 binds in B-lymphocytes from CLL patients remains unknown. Interestingly, the MSI2 regulatory pathway is linked to the oncogene c-MYC in acute myeloid leukemia, an oncoprotein with higher mRNA expression in B-cells from CLL patients with poor prognosis. Additionally, it has been shown that MSI2 can bind to c-MYC mRNA in hepatocellular carcinoma. Based on this, we aimed to determine whether MSI2 regulates c-MYC expression in the tumor clones of CLL patients.

To investigate this, B-cells from 13 CLL patients were treated in-vitro with either the MSI2 inhibitor Ro082750 (5 μM) or a vehicle for 24 hours, and c-MYC expression levels were assessed by flow cytometry. Results showed that inhibiting MSI2 reduced c-MYC expression in 11 out of 13 samples ($p=0.0042$). Furthermore, we examined the effect of the MSI2 inhibitor on c-MYC levels in TCL1 mice. Animals treated with the MSI2 inhibitor showed reduced c-MYC expression in B220+CD5+ cells, reinforcing the role of MSI2 in positively regulating c-MYC. These data suggest that MSI2 directly or indirectly regulates c-MYC expression. To determine if MSI2 indeed binds to c-MYC mRNA, we immunoprecipitated MSI2 from CLL patient cells and amplified c-MYC by PCR, confirming that c-MYC mRNA directly binds to MSI2.
Our results provide insights into post-transcriptional regulation in CLL. Since it has been proposed that inhibiting translation could be an effective strategy for controlling CLL development by blocking the translation of several oncogenic pathways, including MYC, we are curious whether disrupting the MSI2–c-MYC mRNA axis might offer a more targeted therapeutic strategy for certain patients. Further studies will be done to confirm the idea.

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Sphingosine kinases as therapeutic targets for venetoclax resistance induced by activated T cells in Chronic Lymphocytic Leukemia

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Introduction: Venetoclax resistance is currently a major clinical problem. We previously showed that autologous activated T cells (aaT) from CLL patients favor the activation of CLL cells and the generation of venetoclax resistance (Elias et al. 2022;Elías et al. 2018). We also reported that the sphingosine kinase 1 and 2 (SPHK1/2) inhibitor, SKI-II enhanced the in vitro cell death triggered by fludarabine, bendamustine or ibrutinib and reduced the activation and proliferation of CLL cells (Almejún et al. 2017).

Objectives: to determine whether SKI-II (SPHK1/2 inhibitor) and opaganib (SPHK2 inhibitor) affect the activation, proliferation and venetoclax resistance on CLL cells induced by aaT cells. We also evaluated the effect of SPHK inhibitors on T cells.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from twenty-eight unrelated CLL patients that were free from clinically relevant infectious complications and were treatment naïve or without treatment for ≥3 months before the investigation began. All samples used in this study were obtained after informed consent in accordance with the Declaration of Helsinki and with Institutional Review Board approval from the Academia Nacional de Medicina, Buenos Aires, Argentina.

We evaluated the effect of SPHK inhibitors on the activation and generation of venetoclax resistance on CLL cells induced by aaT as shown on Fig.A.

Results: SKI-II and opaganib reduced the upregulation of CD86, PD-1 and PDL-1 as well as the upregulation of Ki-67 on CLL cells induced by aaT cells (Fig.B). When CD4+T cells were evaluated, we found that both inhibitors reduced the expression of CD40L induced upon CD3 crosslinking (Fig.C) without affecting their survival (not shown). In the case of CD8+T cells, only SKI-II reduced the upregulation of CD69 on this T cell subpopulation (Fig.D). SKI-II and opaganib did not affect the survival (not shown) or the production of TNFα, IFNγ and the degranulation of CD107a on CD8+T lymphocytes (Fig.E). More importantly, SKI-II and opaganib prevented the generation of venetoclax-resistance induced by aaT cells (Fig.F) by reducing the upregulation of BCL-XL and/or MCL-1 on malignant cells (Fig.G).

The presence of aaT cells enhanced SPHK2 expression on CLL cells, which was higher in those that survive to venetoclax (Fig.H.1), while SPHK1 expression was not consistently modified (Fig.H.2).

Finally, we studied the effect of SPHK inhibitors on already venetoclax-resistant cells as shown in Fig.I.1. Of note, venetoclax-resistant CLL cells from aCD3+venetoclax cultures died in response to opaganib alone (Fig.I.2). Moreover, even though 64% of already venetoclax-resistant cells survive to a second drug exposure, 50% of these cells died due to the presence of venetoclax in combination with SKI-II and 75% in combination with opaganib.

Conclusion: Our findings indicate that SHPK2 may be involved in CLL cells activation, proliferation and resistance to venetoclax, highlighting the therapeutic potential of SPHK inhibitors in combination with venetoclax. Our ongoing experiments are using CRISPR-Cas9 to edit SPHK2 in the MEC-1 cell line to evaluate its phenotype and sensitivity to venetoclax.

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Venetoclax resistant Chronic Lymphocytic Leukemia cells are sensitive to anti-CD20 monoclonal antibodies.

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Introduction: The treatment of chronic lymphocytic leukemia (CLL) patients with venetoclax (ven) based regimens has demonstrated efficacy and a safety profile, but the emergence of resistant cells and disease progression is a current complication.

Although the combination of ven with anti-CD20 monoclonal antibodies (mAbs) rituximab (Rtx) or obinutuzumab (Obz) represent a compelling therapeutic option due to their finite treatment duration and remarkable achievement of undetectable minimal residual disease, the evidence of the effect of these mAbs on Ven-resistant cells is still scarce (doi:10.1182/bloodadvances.2019000180, doi:1080/14737140.2023.2288899).

Objective: To compare the effect of anti-CD20 mAbs on ven resistant and control CLL cells.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from eleven unrelated CLL patients that were free from clinically relevant infectious complications and were treatment naïve or without treatment for ≥3 months before the investigation began. All samples used in this study were obtained after informed consent in accordance with the Declaration of Helsinki and with Institutional Review Board approval from the Academia Nacional de Medicina, Buenos Aires, Argentina.

Control and Ven-resistant CLL cells were employed as targets to evaluate the mechanisms of action of CD20 mAbs Rituxumab (Rx) or Obinutuzumab (Obz): direct cell death (DCD), complement dependent cytotoxicity (CDC), antibody dependent cell-mediated cytotoxicity (ADCC) and phagocytosis (ADCP), as shown in Figure A. We generated ven-resistant cells by culturing peripheral blood mononuclear cells from eleven unrelated CLL patients, with anti-CD3 antibody for 72h, with DMSO/Ven the last 24h (doi:10.3324/haematol.2018.188680). Then, viable CLL cells were purified and employed as targets.

Results: Coculture of CLL cells with aaT lymphocytes generate ven-resistant CLL cells (Fig B) which express lower CD20 levels compared to control cells (Fig C).

Despite the decrease in CD20 expression, CD20 mAbs induced DCD in control and Ven-resistant cells, being Ven-resistant cells more susceptible to Rx than control ones. Within ven-resistant cells, Obz was superior to Rx to induce DCD (Fig D.1), and Obz+Ven was superior to Rx+Ven or Ven alone (Fig D.2).

Regarding the CDC, unlike the positive control assessed with alemtuzumab, none of the anti-CD20 mAb were able to induce cell death by complement with any of the target cells employed (Fig E).

For ADCC, only Obz was able to induce CLL cytotoxicity (Fig F.1) and NK cell degranulation (Fig F.2), but these effects were reduced when ven-resistant cells were employed as target cells.

Finally, we noticed that both Rx and Obz were able to favor CLL phagocytoses by macrophages with no significant differences between control, activated or ven resistant CLL cells (Fig G).

Conclusions: Our in vitro results indicate that, despite the lower CD20 levels on ven-resistant cells, they are sensitive to Rx and Obz, being Obz superior to eliminate them by inducing higher DCD and ADCC.

39 Venetoclax resistance induced by autologous activated T cells on Chronic Lymphocytic Leukemia cells: CD4+ T cells and their extracellular vesicles as central players

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Introduction: CLL cells survive, activate and proliferate within lymphoid organs, interacting with their surrounding tumor microenvironment. This microenvironment includes a heterogeneous array of cell types, soluble signals, and extracellular vesicles (EVs) (doi: 10.1053/j.seminhematol.2023.12.004). Among the cells present in the microenvironment, CD4+ T cells are major players, providing activation, survival and proliferation signals to leukemic cells (doi: 10.1038/s41375-020-0873-2).

We previously reported that autologous activated T cells (aaT) from CLL patients induce the activation, proliferation and generation of venetoclax resistance on CLL cells through cell-cell contact and the production of secreted factors (doi: 10.3389/fonc.2023.1143881; doi: 10.1007/s00262-021-03043-x).

Objectives: We here aim to determine the contribution of CD4+ T cells and CD8+ T cells, as well as the role of EVs produced by T lymphocytes, in the activation of CLL cells and in the generation of venetoclax resistance.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from eight unrelated CLL patients that were free from clinically relevant infectious complications and were treatment naïve or without treatment for ≥3 months before the investigation began. All samples used in this study were obtained after informed consent in accordance with the Declaration of Helsinki and with Institutional Review Board approval from the Academia Nacional de Medicina, Buenos Aires, Argentina.

Purified CLL cells (pCLL) were cultured with autologous purified CD4+ (pCD4) or CD8+ T cells (pCD8) with anti-CD3 or isotype control for 72h, in the presence of venetoclax or DMSO for the last 24h (Figure A)

EVs from CD4+ T cells from healthy donors were obtained by a differential centrifugation process. pCLL were then cultured in the presence or absence of these EVs for 72h with venetoclax or DMSO for the last 24h (Figure B). Viability and expression of activation markers (CD86 and PD-1) on CD19+ cells were assessed by flow cytometry.

Results: As expected, we found that CD4+ T cells activated by CD3 crosslinking, were able to induce CD19+ cells activation, shown by an upregulation on CD86 and PD-1 expression. Of note, CD8+ T cells were also capable of inducing CLL cells activation, evidenced by an increase on CD86 expression, without a significant modulation of PD-1 expression on the leukemic clone (Figure C).

When analyzing the ability of these T cell subpopulations to generate venetoclax resistance, we observed that CD4+ but not CD8+ T cells, were able to induce venetoclax resistance con CLL cells (Figure D).

We then evaluated the effect of EVs produced by CD4+ T cells on leukemic cells, we found that they were able to activate the leukemic clone, leading to the upregulation of CD86 and PD-1 on CD19+ cells (Figure E). Finally, we found that these EVs were also able to generate venetoclax resistance on CLL cells (Figure F).

Conclusions: Both, activated CD4+ and CD8+ T cells induce the activation of CLL cells while only CD4+ T cells generate venetoclax resistance. EVs produced by activated CD4+ T cells induce leukemic cell activation and the generation of venetoclax resistance.

Development of new methods

POSTER SESSION

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Development and Validation of an 8-Color Flow Cytometry Assay for MRD Detection in CLL

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In chronic lymphocytic leukemia (CLL), measurable residual disease (MRD) status, assessed during and after treatment, is associated with improved progression-free and overall survival. Recently, the treatment landscape for CLL has shifted from chemoimmunotherapy to novel targeted therapies, including Bruton's tyrosine kinase inhibitors, BCL2 inhibitors like venetoclax, and anti-CD20 monoclonal antibodies. These modern therapies result in prolonged remissions, prompting the need for new therapeutic endpoints beyond traditional measures such as progression-free and overall survival. MRD detection is a sensitive method to evaluate disease burden and has shown significant prognostic value, although its role in routine clinical practice remains debated. The use of MRD as a prognostic tool, therapeutic target, and early intervention marker is increasingly integrated into clinical trials. However, comparative data on the optimal assays to detect undetectable MRD (U-MRD) and its impact on treatment decisions are limited. Monitoring MRD in CLL patients who have achieved complete remission poses challenges using conventional flow cytometry (FC) methods, especially in peripheral blood. Historically, FC assays relying on CD20 expression have been hindered during rituximab therapy due to antigen modulation.This study aims to optimize and validate an 8-color CLL-MRD flow cytometry assay for implementation in the Laboratorio de Citometría y Biología Molecular, Hospital de Clínicas.Method: A single-8 color tube assay using CD5, CD3, CD19, CD20, CD22, CD43, CD45, and CD38 antibodies was employed. To validate the assay's sensitivity, serial dilutions from 1:1 to 1:100.000 were performed using a CLL sample diluted with normal leukocytes. The limit of detection (LOD) and the limit of quantification (LOQ) was set at 20 and 50 CLL events respectively. A total of 12 peripheral blood samples from treated CLL patients in complete remission were analyzed using this single-tube approach, and a sequential gating strategy was developed to distinguish residual B-CLL populations from normal leukocytes. MRD positivity was defined when more than 0.01% of leukocytes were CLL cells. Results: The sensitivity of the assay was confirmed through matrix-spiking dilutions, showing a detection capability down to 0.001% of the total white blood cell (WBC) count, meeting the criterion for a MRD detection limit of 10-4 (MRD4). In the 12 samples analyzed, the median acquired leukocyte count was of 989.659 cells. (IQR 383.000- 1,584,594). The median MRD level was 0.011% (IQR 0.00–0.011%), with a median LOD of 0.006% (IQR 0.0033–0.012%) and a median LOQ of 0.022% (IQR 0.014–0.029%). Nine out of twelve patients (9/12) were MRD negative (<0.01%). Conclusion: The 8-color CLL-MRD assay has demonstrated reliable sensitivity for detecting MRD down to MRD4 levels. A bulk lysis protocol will be necessary to reach MRD5 levels. With the recent acquisition of a spectral flow cytometer, we are further refining the assay to reach MRD5 sensitivity, using a 12 color tube as the one proposed by Euroflow in order to significantly improve our ability to detect minimal residual disease. This enhanced precision will enable more accurate monitoring of disease burden, ultimately guiding therapeutic decisions and offering a valuable tool for both clinical use and ongoing research.

27 ImmuneREAD: a Third- Generation Method for IGHV Somatic Hypermutation, clonal architecture and intraclonal diversity in CLL.

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Introduction: Immunoglobulin somatic hypermutation is crucial for guiding therapeutic decisions in chronic lymphocytic leukemia (CLL). The current gold standard, BIOMED-2, which uses multiplex PCR and first-generation sequencing, has limitations in detecting multiple VDJ clones within the same sample and quantifying the size of the tumor compartment. We introduce a new technology that combines the Template-switching technique with third-generation sequencing, enabling stoichiometric amplification of the BCR repertoire and full coverage of VDJ rearrangements coverage. This provides deeper insights into clonal architecture and intraclonal diversity through an automated bioinformatics pipeline. ImmuneREAD integrates long-read, single-molecule sequencing with low upfront investment making for expanding testing accessibility.

Aims: Our aim was to develop a method that enables (i) IGHV somatic hypermutation analysis, (ii) estimation of the clonal space occupied by the malignant clone, and (iii) evaluation of the remaining non-clonal immune repertoire in CLL samples.

Methods: The study included 51 CLL patients and 12 healthy donors for control B-cell repertoires. BIOMED-2 was performed according to ERIC group recommendations. ImmuneREAD PCR amplicons were sequenced by ONT. BIOMED-2 amplicons were sequenced by Sanger and Nanopore technologies. Long-read sequencing was validated by the Sequel I PacBio platform. A Bioinformatic pipeline was developed for mutational status, clonal space and non-clonal B-cell repertoire characterization.

Results: In a pre-validation step in 10 samples, we compared BIOMED-2 vs ImmuneREAD, achieving 100% agreement in VDJ identification and mutational status classification. ImmuneREAD provided significantly higher VH gene coverage compared to BIOMED-2 (mean coverage 99.3% vs. 88.4%) with a 2.5 variants/kb error rate. In an expanded cohort, ImmuneREAD vs BIOMED-2 sanger showed equivalent mutational status classification in 96% of cases (49/51). The correlation of IGHV homology was r = 0.85, p <0.0001. ImmuneREAD's sensitivity was 91% and specificity 100%. In discordant cases, both methods identified distinct clonal IGHV rearrangements, potentially due to low read count in ONT sequencing. ImmuneREAD enabled full B-cell repertoire characterization, revealing overexpressed clonal reads in all CLL cases, while healthy donors showed no clonal expansion. The clonal Space (CS, i.e., proportion of clonal reads/non-clonal reads) was 0.66 for M-CLL and 0.71 for UM-CLL. One sample showed bi-clonality with two overexpressed clonal rearrangements. Healthy donors showed an average repertoire richness of 1178 vs 2806 in M-CLL and 2817 in UM-CLL as assessed by 0D Hill index. CLL showed a significantly lower number of common rearrangements (63 in UM-CLL,31 in M-CLL) compared with 1076 in healthy donors. CLL cases showed a higher mean amino acid charge and larger CDR3 mean length on non-clonal B-cells (charge 0.75 in M-CLL, 0.58 in UM-CLL vs 0.15 in healthy donors; CDR3 length 18.2 aa in M-CLL, 19.55 in UM-CLL and 15.8 in healthy donors). These data indicate a disturbance in the diversity and composition of the remaining non-clonal B-cell repertoire.

Conclusion: We present an amplicon NGS-based strategy to determine the IGHV mutational status and clonal space of the size tumor clone, which also characterizes the non-clonal B-cell repertoire in CLL patients. This method is fast, reliable, and shows high specificity and sensitivity relies on ONT sequencing which relatively low investment, making it suitable for small laboratories worldwide. ImmuneREAD offers a cost-effective approach to molecular testing for CLL.

29 Enhancing Prognostic Accuracy in Chronic Lymphocytic Leukemia through AI-Driven Analysis of Clonotype VDJ Sequences

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Introduction: Chronic lymphocytic leukemia (CLL) prognosis remains a significant challenge, as current clinical staging methods lack the precision needed to accurately predict disease progression and survival on an individual basis. Among prognostic markers, clonotype VDJ sequences are particularly informative, with the IGHV mutation status and V gene family usage serving as some of the strongest predictors (Cramer & Hallek, 2011; Pérez-Carretero et al., 2021).

Advances in artificial intelligence (AI), including efficient protein structure predictions like AlphaFold and RoseTTAfold, protein language models, and multi-modal learning process, have opened new opportunities to study proteins, their structures and functions. Taking advantage of these technologies, we explored prognosis classification in CLL using masked language approaches and transfer learning techniques applied to the VDJ sequence data. This AI-driven method aims to enhance the precision of prognosis, potentially offering a more individualized and accurate assessment of disease progression.

Methods: We analyzed 148 heavy chain clonotype sequences from patients with varying disease progression, focusing on the CDR3 region. Physicochemical encoders (Medina-Ortiz et al., 2022) and Fourier Transforms were applied as encoding strategies. Besides, LLM pre-trained models like ESM (Rives et al., 2021) and ProT5 were used as transfer learning approaches to facilitate embedding representation of CDR3 region by exploring their feature-extraction capabilities. Then, machine learning strategies were applied, including unsupervised learning algorithms for pattern recognition and supervised learning algorithms for generating classification systems.

Results: We employed 31 numerical representation strategies to represent the CDR3 sequences, including eight physicochemical-based encoders, Fourier Transforms, and pre-trained LLMs. Additionally, we trained over 100 clustering models for each numerical representation approach. Physicochemical and FFT-based encoders displayed random distributions, suggesting a limited ability to effectively separate subsets, even when applying non-linear dimensionality reduction methods such as kernel PCA. In contrast, pre-trained LLM-based approaches, such as ESM, ProT5, and ANKH (Elnaggar et al., 2023), facilitated the identification of well-defined clusters of CDR3 sequences correlated with CLL prognosis. Physicochemical characterization and statistical analyses revealed hydrophobicity and volume properties across distinct CLL prognosis categories. Moreover, pre-trained models used as feature extraction approaches enabled the implementation of classification models by employing traditional supervised learning algorithms in a low-sample context. The best-performing models were obtained by emulating multi-modal learning via ensemble strategies, underscoring the importance of integrating both sequence and structural contexts for effective IGHV analysis.

Conclusions: Our results indicate that integrating deep learning-based analysis of VDJ sequences can enhance the accuracy of CLL prognosis by complementing existing clinical methods. LLMs show potential for improved patient stratification and more precise tailoring of treatment strategies, supporting the advancement of personalized medicine in CLL management. However, explainability and interpretability strategies need to be incorporated to facilitate the understanding of the knowledge acquired by the machine learning strategies and to increase the trustworthiness of ML strategies for clinical integrations.

CLL registries

POSTER SESSION

19

Risk of second primary neoplasia in Chronic Lymphocytic Leukemia patients: population-based analysis from the National Cancer Registry of Uruguay

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Introduction: Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) is the most frequent leukemia among western adults. It affects mostly elderly people (median age of onset is about 70 years) and the course of the disease is frequently indolent, with high proportion of long-term survivors. Some recent research-draws attention to an increased risk of developinga second primary neoplasia (SPN) in general and skin cancer in particular, probably enhancedby a diminished immune condition, due to the disease, aging and treatments.

Objective: The aim of this study is to assess whether CLL/SLLpatients in Uruguaypresent increased risk of developing SPN, compared to the general population. We specifically ought to quantify their risk for all cancers excluded non- melanoma skin cancer (NMSC), melanoma and NMSC,

Methodology: The National Cancer Registry from Uruguay (NCRU) collects information on every new malignant tumor diagnosed in the country from 1991 on. All cases registered as CLL/SLL from 2001 to 2020 were extracted from the database. Person-years (PY) at risk for each age group and calendar year, were calculated from the date of CLL/SLL diagnosis until the occurrence of a SPN, death or end of follow up (December 31st,2022), whichever occurred first. In the event of multiple SPN in the same person, only the first was considered for calculation. In situ carcinomas and transformation to high grade lymphomas were excluded. Standardized incidence ratios (SIRs) and their confidence intervals (95%) were computed as the ratio of observed SPNs to expected tumors. The expected number of malignancies was based on age specific incidence rates for all cancers (NMSC excluded) for each age, sex and calendar year for the Uruguayan population. Specific SIRs for melanoma and NMSC were also calculated.

Results: From 2001 to 2020; 2100 new CLL cases were registered in the NCRU. Male: female ratio was 1.4, and median age at diagnosis was 72 and 75 respectively. Among this cohort, 198 people developed at least one SPN (excluding NMSC) and 104 developed NMSC along the follow up period. For those who develop a SPN, the median time from the CLL diagnosis was 2.14 years for males (range 0-15.5) and 2.38 years for females (range 0.9 to 16.7). The most frequent SPN were prostate, colorectal, lung, breast cancers, melanoma and other hematological malignancies (transformation excluded). Those entities together comprise more than half of all the SPNs. Calculated SIRs for all cancers were 1.49 for men (CI 95%; 1.24,1.77) and 1.67 for female (CI95%; 1.28,2.15). Among the STPs, 14 cases corresponded to melanoma. SIRs for melanoma were 4.65 for males (CI95%; 1.86,9.58) and 6.85 for females (CI95%; 1.84,17.56). Regarding NMSC, SIRs are 3.78 for males (CI95%; 2.95;4.77) and 3.38 for females (CI95%; 2.21;4.96)

Conclusions: The present study confirms that CLL/SLL patients are exposed to a significant increase ofrisk to develop SPNs, melanoma and NMSC, compared to their age-sex matched counterparts from the general population. Those findings are consistent with previous reports from other populations. We ought to raise awareness on specific health risks that CLL/SLL patients face and the need oftailoredmeasures address to early detection of SPNs in this population.

43 Chronic Lymphocytic Leukemia: A Decade of Real-World Data from the Peruvian National Cancer Center

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Purpose: Chronic lymphocytic leukemia (CLL) is relatively uncommon in Latin America, and there is a scarcity of studies focusing on Peruvian patients.

Methods: This retrospective study evaluated diagnosed chronic lymphocytic leukemia (CLL) patients at a single center from 2010 to 2021. Unfortunately, it was not possible to assess genetic risk due to the lack of molecular studies. During this period, BTK inhibitors, Venetoclax, and Obinutuzumab were unavailable. The treatment regimens included chemotherapy with Rituximab (FCR, RCHOP, RCVP) and therapies without Rituximab. Time to next treatment (TTNT) was defined as the duration from the start of frontline therapy to the initiation of the next treatment or death from any cause. Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier method, with survival curves compared using the Log-rank or Breslow test.

Results: A total of 142 patients with CLL were enrolled during the study period. The median age was 66.5 years (range: 29-90), with 31.7% of patients aged ≤ 60 years and 62.7% being male. Clinical presentations included B symptoms in 43%, bulky mass in 18.3%, and hepatosplenomegaly in 43.7%. According to the BINET criteria, 38.7% were classified as group C, 16.9% as RAI group IV (thrombocytopenia), and only 7% had Richter syndrome. Of the all patients, 35% (n=50) were observed as first management, 53.5% (n = 76) received first-line therapy, with the most common regimens being rituximab combined with fludarabine and cyclophosphamide (30.3%) and rituximab with chemotherapies (28.9%). Additionally, 46.1% of patients received a second line therapy. The median TTNT was 14 months (95% CI: 6.4, 21.6).

The median follow-up duration was 3.3 years (95% CI: 2.5-4.1 years). Recurrence or progression occurred in 23.2% of patients (2/66 in observe and 31/76 in first-line treatment), and by the study cut-off date, 54.2% of patients had died.

The median OS was 4.0 years (95% CI: 2.8-5.2), with a 3-year survival rate of 51.4%. The median PFS was 2.6 years (95% CI: 1.9-3.3), and 3-year rate was 44.0%. The OS did not present a significant difference between patients in observe and first-line therapy ($p = 0.827$); however, in relation to PFS they presented a significant difference ($p = 0.019$), reaching a 3-year PFS rate of 55.2% in observe and 38.2% in those in first-line treatment. The OS presented a significant difference in relation to the age group ($p = 0.001$), the BINET criteria group C ($p <0.001$) and RAI group III-IV ($p <0.001$). In relation to PFS, there was a significant difference in relation to BINET group C ($p < 0.001$), RAI group IV, and symptoms B ($p < 0.001$). The other variables did not present a significant difference ($p > 0.05$).

Conclusion: CLL is infrequent in Peruvian patients and the overall survival was inferior to the reported in large series treated with chemotherapy and Rituximab; however, we have considered the high percentages of patients treated not standardized therapies for CLL. In this cohort, patients with high tumor burden had poor prognosis.

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  - En renglón aparte se detallará la nómina de personas autoras, separados por comas, comenzando por el apellido completo e inicial del nombre.
  - Filiación institucional: para cada autor/a se detallará el nombre de la institución (sin abreviaturas) donde se realizó el trabajo,

Ejemplo:
Pérez V1; González C2
1 Servicio Hematología, Hospital Milstein. Buenos Aires, Argentina
2 Servicio de Hematología, Hospital Fernández. Buenos Aires, Argentina
Ciudad, país de origen y correo electrónico del autor/a responsable.

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- Haber realizado contribuciones sustanciales a la concepción y el diseño del estudio, a la recolección de los datos o al análisis y la interpretación de los mismos.
- Haber participado en la redacción del artículo o en la revisión crítica sustancial de su contenido intelectual.
- Haber aprobado la versión final del manuscrito.
- Ser responsable de todos los aspectos del manuscrito asegurando que las cuestiones relacionadas con la veracidad o integridad de todas las partes del manuscrito fueron adecuadamente investigadas y resueltas.

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"Todas las personas autoras han efectuado una contribución sustancial a la concepción o el diseño del estudio o a la recolección, análisis o interpretación de los datos; han participado en la redacción del artículo o en la revisión crítica de su contenido intelectual; han aprobado la versión final del manuscrito; y son capaces de responder respecto de todos los aspectos del manuscrito de cara a asegurar que las cuestiones relacionadas con la veracidad o integridad de todos sus contenidos han sido adecuadamente investigadas y resueltas."
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b) **Resumen y palabras clave**

- Resumen:
  - En español e inglés.
  - Estructura: Introducción, Material y Métodos, Resultados y Discusión.
  - Extensión: hasta 400 palabras.

- Palabras clave:
  - En español e inglés.
  - Cantidad: entre 3 y 5.
  - Utilizar términos del Medical Subjects Headings del Index Medicus.

c) **Introducción:** Breve resumen del estado del arte del tema a tratar y los objetivos del trabajo.

d) **Materiales y Métodos:** Debe detallar claramente la población utilizada en el trabajo (grupos controles y pacientes), las metodologías empleadas y los métodos estadísticos utilizados en la evaluación de los resultados. En esta sección se debe incluir una declaración que indique la aprobación del comité de ética Institucional o autoridad competente además se debe dejar constancia que se obtuvo de cada paciente el consentimiento informado por escrito y que el protocolo de estudio se realizó conforme a las normas éticas de la declaración de Helsinki 1975.

e) **Resultados:** Deberán estar expresados con claridad en forma cuantitativa, utilizando valores numéricos (expresados en las unidades internacionales habituales), tablas y/o gráficos. No se aceptarán tablas que ocupen un espacio mayor que el de una página de la Revista.

Las abreviaturas y símbolos deberán estar especificados en el texto o al pie de las tablas.

f) **Discusión:** Analiza los resultados y los hechos que tengan relación directa con los mismos, las relaciones entre éstos y el objetivo inicialmente propuesto y su confrontación con los conocimientos establecidos previamente.

g) **Referencias bibliográficas:**

Las personas autoras son responsables de verificar la exactitud e integridad de las referencias. Sólo se incluirán las referencias que hayan sido consignadas en el artículo, ordenadas numéricamente en forma correlativa. Se hará figurar inicialmente la nómina de autores separados por comas, comenzando por el apellido, seguido por las iniciales de los nombres. Cuando el número de autores sea mayor de 6, se hará mención sólo a los primeros 3 seguidos de la sigla «y col.»; a continuación se consignará el título del trabajo seguido del nombre de la revista en forma abreviada, según lo establezca por el «Index Medicus»; año de publicación, punto y coma, número de Volumen dos puntos, página inicial, guión, página final. **Incluya el DOI si corresponde.**

Ejemplo: Kaldor JM, Day EN, Clarke EA y col. Leukemia following Hodgkin's disease. N Engl. J Med 1990; 322:7-13. https://doi.org/15.1555/S0363-5023(10)8111-0

Cuando se trate de libros se harán figurar el nombre del autor/es, título del capítulo, título del libro, editor/es, año de aparición, páginas separadas por guión, agregando el número de edición si no fuera la primera edición, editorial, y ciudad. Ejemplo: Hughes TP and Goidman JM. Chronic myeloid leukemia.

Hematology: Basic Principles and Practice. R. Hoffman, El Benz, Sj Shatill, B Ftirie y EJCoben 1991, p 854-869. Churchill Livingstone, Edinburgh.

Datos respaldatorios

Para citar este tipo de datos, referidos en Depósito de datos, se deberá realizar con el siguiente formato:

López Cosar, H., Bentmiglia, C., Alfonsín, M., (2020). Estudio comparativo entre el método coagulométrico tradicional y un dispositivo portátil en la medición de la razón internacional normatizada y la toma de decisión médica.[Dataset] Versión de 22 de junio de 2021. SciELo Data. (enlace facilitado por el repositorio que contará con un identificador permanente de objeto digital, sea handle, DOI u otro)

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2) La sección **Yo opino** está destinada a expresar la opinión de un experto sobre un tema controvertido solicitado por el comité editor.
La disidencia respecto a esta opinión se podrá dar a través de la sección correo de lectores. La longitud no deberá superar las 3.000 palabras. Deberán ser escritas con el formato grafico de los artículos originales.

3) Los **Ateneos anatomo-clínicos** deberán ser escritos con el mismo formato gráfico y se procederá de la misma forma que los artículos originales.

4) Las **Editoriales** serán solicitadas por el Comité Editor. Tendrán título y texto con características de monografía, en lo posible con una extensión que no supere las 2.000 palabras, con un máximo de 5 citas bibliográficas, el nombre del autor, su dirección con código postal y dirección de mail.

5) Las **Actualizaciones y/o revisiones** deberán ser escritas con el formato gráfico de los artículos originales. La longitud no deberá superar las 5.000 palabras.

6) La sección **Hematología Pediátrica**: Estará destinada a revisiones de tópicos hematológicos y casos clínicos en niños. Deberán ser escritas con el formato grafico de los artículos originales.

7) La sección **Drogas nuevas en Hematología** será una actualización acerca de las nuevas drogas utilizadas por la especialidad. Serán solicitadas por el comité editor. La longitud no deberá superar las 3.000 palabras. Deberán ser escritas con el formato grafico de los artículos originales.

8) La sección **Comunicaciones breves** deberán ser escritas con el formato grafico de los artículos originales. La longitud no deberá superar las 2.000 palabras y su resumen no debe ser más extenso de las 200 palabras.

9) El **Laboratorio en Hematología** estará dedicada a realizar una ficha técnica de un ensayo utilizado en los laboratorios de Hematología. Será solicitado por el comité editor. Deberá expresar introducción fundamento del ensayo, Características pre analíticas y analíticas del mismo, valores de referencia y su utilidad clínica y hasta 4 citas bibliográficas. La longitud no deberá superar las 3.000 palabras. Deberán ser escritas con el formato grafico de los artículos originales.

10) La sección **Historia de la Hematología** deberán ser escritas con el formato grafico de los artículos originales. Está destinada a divulgar la evolución de la Hematología en Argentina. La longitud no deberá superar las 4.000 palabras. Deberán ser escritas con el formato grafico de los artículos originales

11) **Caso clínico.** En esta sección se admite un máximo de 8 referencias bibliográficas. Deberán ser escritas con el formato grafico de los artículos originales.

12) **Las Imágenes en Hematología:** estará constituido por material fotográfico en colores de excelente calidad destinado a exponer temas de diversa índole.
La longitud no deberá superar las 1000 palabras y se desarrollarán según el orden siguiente: Título, texto conciso, imagen, nombre del autor/es. Podrá agregarse hasta 4 citas bibliográficas. Deberán ser escritas con el formato grafico de los artículos originales.

13) En la sección **Correo de lectores** se publicarán opiniones sobre situaciones clínicas y experiencias que puedan relacionarse o no con los artículos publicados en la Revista, con sentido crítico, objetivo y/o educativo, aceptándose derecho a réplica en caso de opinar sobre algún trabajo publicado. La longitud no deberá superar las 1.000 palabras (hasta 4 citas bibliográficas).

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Se reconocen diferentes categorías de conflicto que detallamos:
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2) Consultor
3) Propiedad accionaria
4) Fondos de Investigación por estudios propios (La norma NO incluye a los protocolos de investigación de fase II a IV multicéntricos, nacionales o Internacionales)
5) Honorarios por conferencias (Speaker)
6) Miembro de Comité Asesor (Advisory Board)
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Hematología rige su política editorial sobre aspectos éticos de la publicación científica observando las directrices del Comité de Ética de las Publicaciones (Committee on Publication Ethics - COPE).
Cuando se realizan estudios clínicos en seres humanos, los procedimientos llevados a cabo deben estar explícitamente de acuerdo con el estándar de ética del comité responsable en experimentación humana, institucional o regional y con la Declaración de Helsinki de 1975, corregida en 1983 y revisada en 1989, los cuales deberán figurar explícitamente en la metodología del trabajo.
No utilizar los nombres de los pacientes, ni sus iniciales ni el número que les corresponde en el hospital, especialmente en el material ilustrativo.
Todos los trabajos de investigación que incluyan animales de experimentación deben haber sido realizados siguiendo las indicaciones de la "Guía para el cuidado y uso de animales de laboratorio" (http://www.nap.edu/readingroom/books/labrats/) perteneciente a la Academia Nacional de Ciencias de los Estados Unidos de Norteamérica y actualizada por la American Physiological Society (APS) (http://www.the-aps.org/committees/animal/index.htm).
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Si se aceptaran trabajos en modelos animales, los autores deberán enviar el certificado correspondiente de aprobación del proyecto emitido por la CICUAL (Comisión Institucional para el Cuidado y Uso de Animales de Laboratorio).

Sociedad Argentina de Hematología, Comité Editor de HEMATOLOGÍA
Julián Álvarez 146 - 1414 - C. A. de Bs. As. - Argentina
E-mail: sah@sah.org.ar /// revista@sah.org.ar

HEMATOLOGY JOURNAL REGULATIONS

HEMATOLOGÍA

The reception of articles will take place through the OJS system on the official website of Revista Hematología: www.revistahematologia.com.ar. You can access the instructions and ask for assistance with the indicated mail. Articles sent outside the system will not be accepted. There are no fees for submitting or processing articles (APC). **Every author must generate a persistent digital identifier (ORCID).**
We will accept the publication of articles from non-Spanish-speaking authors written in English.
The current sections of Revista Hematología are:

1. Original articles
2. My opinion
3. Anatomo-clinic discussion of the hematology fellowships
4. Editorial
5. Updates and/or reviews
6. Pediatric hematology
7. New drugs in hematology
8. Brief communications
9. Laboratory
10. History of hematology
11. Case reports
12. Images in hematology
13. Letters to the Editor

1) **Original articles** must be unpublished. They should not have been submitted simultaneously to another journal without knowing the decision of acceptance or denial from Revista Hematología.
The articles should be in Word format, double-spaced, in Times New Roman font 12, with wide margins of 3cm with a maximum of 4,000 words, including tables and references. All illustrations, figures and tables and their respective legend, should be placed in the appropriate places in the text, instead of at the end.
The articles arrangement should be as follows:
1. a) Cover: It will include the following items:
- Title (both in English and Spanish): with no abbreviations; it will be concise and precise.
- Authors:
  - The list of authors should be included in a separate line, separated by commas, beginning with the complete last name and the initials of the name.
  - Institutional affiliation: it will include the institution name (without abbreviations) where the work has been carried out for each author.

Example:
Pérez V1; González C2
1 Servicio Hematología, Hospital Milstein. Buenos Aires, Argentina
2 Servicio de Hematología, Hospital Fernández. Buenos Aires, Argentina
City, country of origin, and e-mail of the responsible author.

Authorship: Revista Hematología adheres to the International Committee of Medical Journal Editors (ICMJE) guidelines, which in the *Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals* delimits that to get the authorship of the studies, each of the participants must meet the following criteria:

- They must have made substantial contributions to the conception and design of the study or the acquisition, analysis, or interpretation of its data.
- They must have participated in drafting the work or revising it critically for important intellectual content.
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- They must have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

To the effects of complying with this requirement, the cover must include the following statement under the list of authors:
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Note: The statement of authorship should only be included in **research articles** with multiple authors, but not in those with only one author.

- If necessary, above the authorship declaration you can include the persons or institutions that have participated in the study who do not comply with the four mentioned criteria but that contributed to its development. They should be identified by name and last name/s or name of the institution, specifying the specific contribution to the research work.

1. b) **Summary and keywords**
- Summary:
  - Both in Spanish and English.
  - Structure: Introduction, Material and Methodology, Results and Discussion.
  - Length: up to 400 words.
- Keywords:
  - Both in Spanish and English.
  - Quantity: between 3 and 5.
  - Use terms from the Index Medicus Medical Subjects Headings.

c) **Introduction:** Summary of the state of the art of the topic and the goals of the work.

d) **Materials and Methodology:**It must detail the population used (control groups and patients), the methodology employed, and the statistical methods used to evaluate the results. This section should include a statement indicating the approval of the Institutional Ethics Committee or competent authority, as well as the written informed consent obtained from each patient, and that the study protocol was carried out following the ethical standards of the 1975 Declaration of Helsinki.

e) **Results:**They should be clearly expressed in quantitative form, using numeric values (in the usual international units), tables, and/or graphs. Tables that occupy more than one page will not be accepted.

Abbreviations and symbols must be specified in the text or under the tables.

f) **Discussion:** It analyses the results and the facts directly related to them, the relationship between them and the initially proposed goal, and their comparison with the previously established knowledge.

g) **Bibliographic references:**

The authors are responsible for checking the accuracy and integrity of the references. Only references mentioned in the article will be included, in sequential numerical order. The names of the authors must be listed at the beginning separated by commas, first the last name, then the initials of the names. If there are more than six authors, only the first three will be mentioned, followed by the acronym et al. Then, write the article title and the abbreviated name of the journal, according to the Medicus Index; year of publication, semicolon, volume number colon, first page, hyphen, last page. **Include the DOI, if applicable.**

Example: Kaldor JM, Day EN, Clarke EA, et al. Leukemia following Hodgkin's disease. N Engl. J Med 1990; 322:7-13. https://doi.org/15.1555/S0363-5023(10)8111-0

In the case of books, the name of the author/s, title of the book, publisher/s, year of publication, pages separated by a hyphen, adding the edition number if it is not the first edition, publishing house, and city. Example: Hughes TP and Goidman JM. Chronic myeloid leukemia.

Hematology: Basic Principles and Practice. R. Hoffman, El Benz, Sj Shatill, B Ftirie y EJCoben 1991, p 854-869. Churchill Livingstone, Edinburgh.

Supporting data

To quote this type of data, located in the Data depository, the following format must be used:

López Cosar, H., Bentmiglia, C., Alfonsín, M., (2020). Controlled study between the traditional coagulometric method and a portable device in the measurement of the normalized international ratio and medical decision-making. [Dataset] Version from June 22, 2021. SciELo Data. (link provided for the repository that will include a persistent digital object identifier, such as handle, DOI, or other)

References must be visible in the text in parentheses, and subscript. The journal adopts the criteria established by the APA Standards (www.normasapa.com)

2) **My opinion** section is destined to express an expert opinion about a controversial topic commissioned by the Editorial Committee.
Disagreement with this opinion can be expressed through the Letters to the Editor section. The length should not exceed 3,000 words. They should follow the graphic format of original articles.

3) **Anatomo-clinic studies** should be written with the same graphic format and follow the same guidelines as the original articles.

4) **Editorials** will be commissioned by the Editorial Committee. They will have a title and text with monograph characteristics, if possible, with a maximum length of 2,000 words, up to 5 bibliographic references, name of the author, address, zip code, and e-mail address.

5) **Updates and/or revisions** should follow the graphic format of the original articles. The length should not exceed 5,000 words.

6) **Pediatric Hematology** section: It will be intended for reviews of hematological topics and clinical cases in children. They should follow the graphic format of original articles.

7) **New drugs in Hematology** section will be an update on new drugs used by this specialty. They will be commissioned by the Editorial Committee. The length should not exceed 3,000 words. They should follow the graphic format of original articles.

8) **Brief communications** section should follow the graphic format of the original articles. The length should not exceed 2,000 words, and the abstract should not exceed 200 words.

9) **Laboratory in Hematology** is intended to perform a datasheet of a trial used in Hematology laboratories. It will be commissioned by the Editorial Committee. It should include an introduction, rationale for the trial, pre-analytical and analytical characteristics, reference values and their clinical benefit, and up to 4 bibliographic references. The length should not exceed 3,000 words. They should follow the graphic format of original articles.

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It should not exceed 1,000 words and should be developed in the following order: Title, concise text, image, and name of the authors. Up to four bibliographic references can be added. They should follow the graphic format of the original articles.

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Conflicts of interest:

Authors are solely responsible for the content, statements, and authorship of the published articles, and they must clarify in writing if there is any conflict of interest. All participants must include their disclosure in a footnote. From the first edition in 2013, all presentations in Revista Hematología must include a final paragraph in the manuscript that specifies the conflict of interest statement following the attached model.

It is NOT allowed to send to another journal the work submitted to Hematologia. The adapted model of conflict of interest proposed by the SAH Board of Directors is based on that of the American Society of Hematology and bears the same format as many prestigious journals of our specialty. We refer to all current activities and those carried out in the last year.

Different categories of conflicts of interest are recognized and detailed below:

1) Employee

2) Consultant

3) Share Ownership
4) Research funds for own studies (The standard does NOT include multicenter, national, or international Phase II to IV research protocols)
5) Conference fees (Speaker)
6) Advisory Board Member

Images:
Images must be submitted in jpg format, 300 dpi resolution, they can be sent in color.

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In the event of clinical studies performed on human subjects, the procedures carried out must follow the Ethics standard explicitly from the responsible committee in human experimentation, institutional or regional, and with the 1975 Declaration of Helsinki, amended in 1983 and revised in 1989, which should be explicitly stated in the methodology of the work.
Do not use the names of patients, their initials, or hospital number, especially in the illustrative material.
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Articles that do not comply with the Code of Ethics will not be considered for publication.

Animal models
If works in animal models are accepted, the authors should send the appropriate certificate of approval from the project issued by CICUAL (Institutional Committee for the Care and Use of Laboratory Animals).

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