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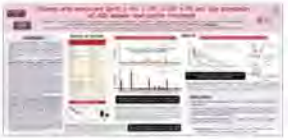
Category: General Abstract



1144 - Patients with unmutated IgHV_1-69; 1-02; 3-30; 4-39 and high expression of AID enzyme need earlier treatment.

Clinical and molecular heterogeneity is a hallmark of chronic lymphocytic leukemia (CLL). This point is even more complex considering that leukemic cells continuously interact with cells and/or soluble factors from their microenvironment constantly modifying the evolution of the disease. The practical problem of this heterogeneous landscape is that leukemia progression cannot be predicted and it still remains an important unmet clinical need. Among different prognostic tools validated in CLL the mutational IgHV status is recognized as the most reliable molecular prognostic factor. Patients expressing mutated IgHV (M-CLL) develop a more indolent disease, whereas unmutated IgHV (U-CLL) patients display a more aggressive one, often unresponsive to treatment. Despite this categorization and a myriad of other molecules proposed as prognostic markers (CD38, Zap-70, CD49d, CXCL3/4, LPL and others) disease progression and/or treatment requirement still cannot be accurately predicted in many patients.

We and others have described that activation-induced cytidine deaminase (AID) is over-expressed in peripheral blood (PB) of patients with poor clinical outcome and that it is predominantly expressed in U-CLL. Despite AID expression in CLL cells has been linked with disease progression, the role of AID in this leukemia and how its expression is involved during disease evolution remains controversial. To deepen into this question we studied AID expression in relationship with specific IgVH rearrangements that may contribute as prognostic markers. In a cohort of 270 CLL patients diagnosed according to IWCLL diagnostic criteria we analyzed the mutational IgHV status, the fluorescence in-situ hybridization (FISH) abnormalities and AID's mRNA expression by TaqMan Quantitative-PCR in the PB at diagnosis. Finally, we assessed the prognostic impact of IgHV rearrangements and AID expression levels in time to first treatment (TTFT). Of 270 patients, 172 were men and 98 women, (male-female ratio = 1.75). From these, 153 (57%) were Binet's stage A, 60 (22%) stage B and 57 (21%) were stage C. IgHV gene status was mutated in 53%, whereas typical cytogenetic aberrations were found in 68%: del(13q14) in 36%, trisomy 12 in 15%, del(11q22) in 10% and del(17p13) in 7%. Median age at diagnosis was 67 years old and median of follow up was 5 years. Our first observation was that positive AID patients in the unmutated group mostly express the rearrangements IgHV_1-02; 1-69; 3-30 and 4-39. However, negative AID U-CLL as well as the M-CLL counterpart (expressing AID or not), remain distributed among the different IgHV rearrangements without any significant selection in the use of a specific IgHV/D/J genes. Prompted by these findings, and also considering previous reports describing the poorest clinical outcome for U-CLL expressing IgHV_1-69, we focused on these 4 unmutated IgHV rearrangements (1-02; 1-69; 3-30 and 4-39, hereafter, Sub-group_1) and compared the prognostic impact on TTFT with those U-CLL, expressing AID or not, but carrying different IgHV rearrangements of the Sub-group_1, (hereafter, mentioned as Sub-group 2). Forest plot of the hazard ratio assessed for association with TTFT by univariate and multivariate analysis were performed. Our results showed that U-CLL group expressing AID with BCR rearrangements corresponding to Sub-group_1 maintained the highest hazard ratio in the final model even after being included in the multivariable analyses del(17p) or Trisomy12. Next, we performed Kaplan-Meier curves analyzing TTFT among the different groups (U-CLL/AIDpos/Sub-group_1; U-CLL/AIDneg/Sub-group_1; U-CLL/AIDpos/Sub-group_2; U-CLL/AIDneg/Sub-group_2). Our results showed statistically significant differences comparing TFTT between the different sub-groups of patients indicating that: U-CLL/AIDpos/Sub-group_1 needed earlier treatment (□ TFTT= 7 months, n=44), compared with: U-CLL/AIDpos/Sub-group_2, (□ TFTT= 26 months, n=27, p=*); U-CLL/AIDneg/Sub-group_2, (□ TFTT= 37 months, n=39, p=***), and U-CLL/AIDneg/Sub-group_1, (□ TFTT= 48 months, n=26, p=****), Log-rank (Mantel-Cox) test.

In conclusion, our results highlight the relevance of assessing AID expression in PB of CLL patients in relationship with the mutational IgHV profile with the final goal of identifying the U-CLL/AIDpos/Sub-group_1. Considering our previous study analyzing the South American cohort, n=900 (Stanganelli et al., Hematol. Oncol, 2019), the percentage of patients corresponding to the sub-group_1 is 27%. Hence, we can assume that the new entity described here (U-CLL/AIDpos/Sub-group_1) represents ~15% of the total CLL population, thus, accurate prediction of this sub-group could result in a useful tool to be incorporated in clinical practice after validation.

Primary Author, Presenting Author(s)

PO

Pablo Oppezzo, PhD

Institut Pasteur de Montevideo
Montevideo, Uruguay

Patients with unmutated IgHV_1-69; 1-02; 3-30; 4-39 and high expression of AID enzyme need earlier treatment

Ana Inés Landoni[1]; Jorge Souto[2]; Victoria Remedi[1]; Gimena dos Santos[2]; Sabrina Ranero[3]; Cecilia Guillermo[3]; Carolina Oliver[4]; Victoria Irigoin[5]; Lilian Diaz[6]; Rita Uria[2]; Eugenia Payque[2]; Juliana Querol[2]; Maria Elena Marquez[2]; Jorge Gonzales Puelma[7]; Irma Slavutsky[8]; Carmen Stanganelli[8]; Guillermo Dighiero[2]; Hugo Naya[9]; Marcelo Navarrete[7]; Raúl Gabus[1]; Florencia Palacios[2]; Pablo Oppezzo[2].

[1] Hospital Maciel, ASSE, Uruguay; [2] Research Laboratory on Chronic Lymphocytic Leukemia, Institut Pasteur, Uruguay; [3] Hematology Department, Hospital de Clínicas, Uruguay; [4] Hematology Department, CASMU, Uruguay; [5] Hematology Department, COSEM, Uruguay; [6] Department of Hematology and Transplant, SMI; [7] Universidad de Magallanes, Chile; [8] Academia Nacional de Medicina., Buenos Aires, Argentina; [9] Bioinformatics Unit, Institut Pasteur de Montevideo, Montevideo, Uruguay.

BACKGROUND

Clinical and molecular heterogeneity is a defining characteristic of chronic lymphocytic leukemia (CLL). 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.

We and others described that activation-induced cytidine deaminase (AID) is abnormally expressed in peripheral blood (PB) of patients with poor clinical outcome, predominantly in unmutated cases (U-CLL). While AID expression is primarily detected in the proliferative fraction of the tumor clone and has been associated with disease progression, the precise role of this enzyme during the CLL's evolution continues to be a subject of debate.

Building upon our prior research focused on AID's role in progressive and U-CLL, along with studies conducted by others laboratories, it is reasonable to assume that within this subgroup of patients exhibiting AID expression in the PB, an activated tumor microenvironment likely exists, driving a sustained activation of CLL cells. We think that this continuous stimulation leads to a range of biological alterations within the tumor clone, including an uncontrolled increase of AID expression within the proliferative fraction of these patients.

Our hypothesis propose that in these cases, specific antigens or auto-antigens, via T-dependent activation (CD40L and IL-4) could act as a primary driving forces behind three key processes: *1)* activation of CLL cells, *2)* sustained and uncontrolled AID expression and as a consequence, *3)* the emergence of a complex pattern of AID-induced "off-target" mutations within the tumor cell genome. The dynamic interplay of combinatorial and random mutational changes within a subset of CLL cells holds the potential to enhance the fitness of this "revitalized" tumor clone, ultimately contributing to a poor clinical outcome. Given this hypothesis, our primary aim in the work was to uncover the origins of this persistent tumor activation and specifically investigate if there exist any correlation between AID expression, specific IgVH rearrangements and need of early treatment.

Material and Methods

Characteristics of CLL cohort	N = 310			
Variable	**N**	**%**	**Missing**	$\bar{X}$
Age > 65 years	167	53.8	—	—
Male sex	188	60.6	—	—
Binet Stage A	175	56	—	—
Binet Stage B	71	23	—	—
Binet Stage C	64	21	—	—
Unmutated IgHV	149	48.6	—	—
FISH	—	—	39	—
FISH normal	80	29	—	—
Del(13q)	103	38	—	—
Trisomy 12	38	14	—	—
Del(11q)	26	10	—	—
Del(17 p)	24	9	—	—
AID expression (RQ-PCR)	250	—	60	—
Positive AID expression (Um)	88	62	*(6)*	—
Positive AID expression (Mut)	40	37	*(54)*	—
AID expression (nested-PCR)	310	—	—	—
Positive AID expression (Um)	90	60	—	—
Positive AID expression (Mut)	54	33	—	—
Follow-up cohort (years)	—	—	—	5
Age cohort (years)	—	—	—	67
OS _U-CLL(years)	—	—	—	7.6
OS_ Mut (years)	—	—	—	25

AID expression in CLL cohort

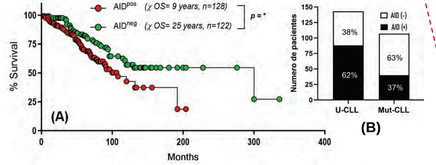
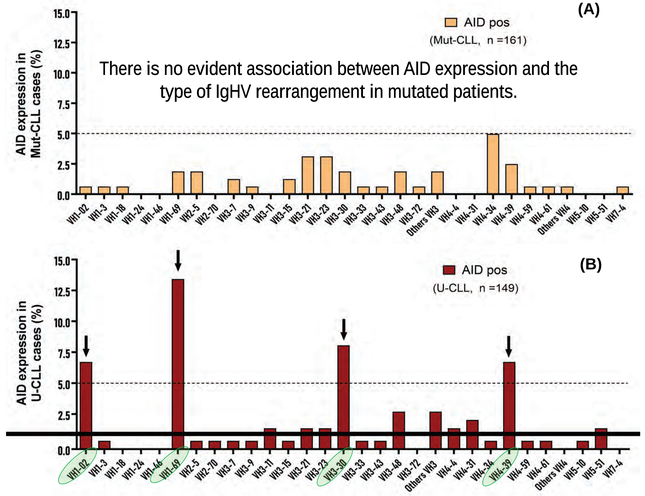


Table and Fig.1: Clinical and molecular features of CLL cohort. We assessed AID expression by q-PCR as described in Fig2 in 250 CLLs. Overall, AID positive cases showed poorer clinical outcome with positivity distribution. A) Survival curves showing differences between positive and negative AID patients. B) Percentages of AID expression in Mut and U-CLL. Survival curves were compared using Log-rank (Mantel-Cox) test, P values <0,05 = *, n=250.

AID expression evaluated by q-PCR in this cohort show similar data to those previously reported by Patten et al., Blood, 2012

AID expression is associated with specific IgHV rearrangements in U-CLL

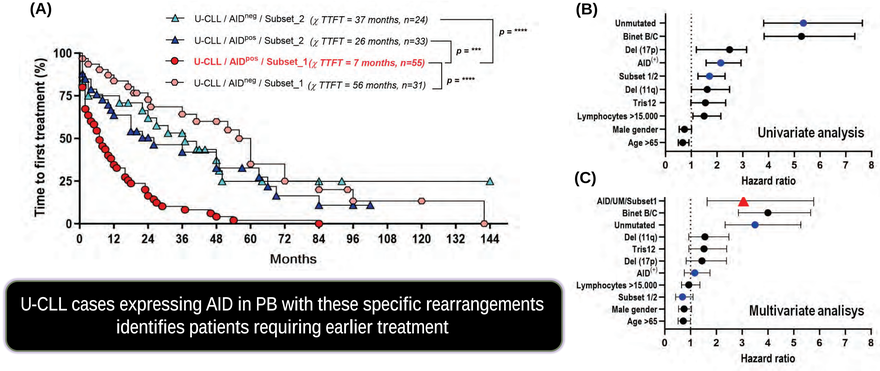


Patients with positive AID expression in the U-CLL subgroup predominantly exhibit the specific gene rearrangements IgHV *_1-02, 1-69, 3-30 and 4-39*.

Figure 2: Distribution of AID positive cases in M-CLL or U-CLL. AID expression was evaluated by nested PCR (n=310) and quantified using a TaqMan probe based qRT-PCR (n=250). IgHV genes are performed as previously described in *Pritsch O. et al., Br J Haematol., 1999*. For quantification the qRT-PCR assay was calibrated using cloned AID full length mRNA and the data was normalized against AID expression in peripheral blood of healthy donors (n=10). AID expression was heterogeneous, however Mut-CLL, (A) showed no cases of CLL patients exceeding 5% of AID expression, whereas U-CLL, (B) showed cases with higher expression albeit restricted to specific IgHV rearrangements.

RESULTS

AID expression and specific IgHV rearrangements impact in the clinical prognosis



U-CLL cases expressing AID in PB with these specific rearrangements identifies patients requiring earlier treatment

Figure 3: Prognostic value associating the variables AID, IgHV, and Subset_1 in U-CLL. A) Time to First treatment (TTFT) curves were compared using Log-rank (Mantel-Cox) test, P values <0,0001 = **** and 0,0001 = ***, n=143 U-CLL. B) Forest plot of the hazard ratio (HR) for the 10 covariates assessed for association with TTFT by univariable analysis. C) Forest plot of the HR for the 10 covariates assessed for association with TTFT by multivariable analysis compared with AID/U-CLL/Subset_1 score. Solid circle and triangle indicate the HR, horizontal lines indicate the 95% CIs. Subset_1 comprises patients with IgHV gene rearrangements *1-02, 1-69, 3-30, and 4-39*, while Subset_2 encompasses all other rearrangements.

CONCLUSIONS

- ✓ High expression of the mutagenic enzyme AID in U-CLL is associated with specific IgHV gene rearrangements.
- ✓ Patients in this subgroup represent approximately 38% of U-CLL cases (15% of the total) and can be identified in advance by integrating AID enzyme expression analysis into routine with IgHV assessments, all using peripheral blood.
- ✓ This approach enables the identification of a subgroup of patients with an unfavorable prognosis, requiring treatment within one year.

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