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

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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

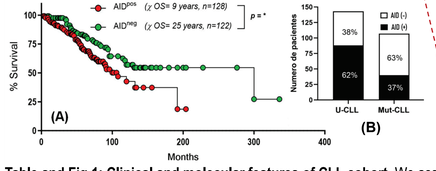
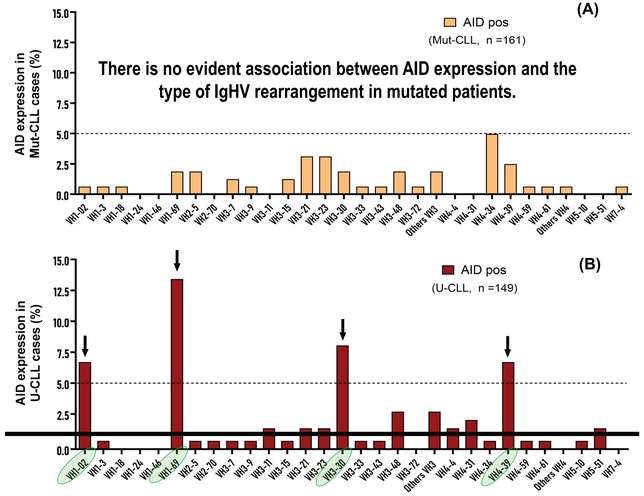


Table and Fig.1: Clinical and molecular features of CLL cohort. We assessed AID expression by q-PCR as described in Fig2 in 250 CLLs. Overall, AID positive cases showed poorer clinical outcome with positivity distribution. **A)** Survival curves showing differences between positive and negative AID patients. **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 is associated with specific IgHV rearrangements in U-CLL



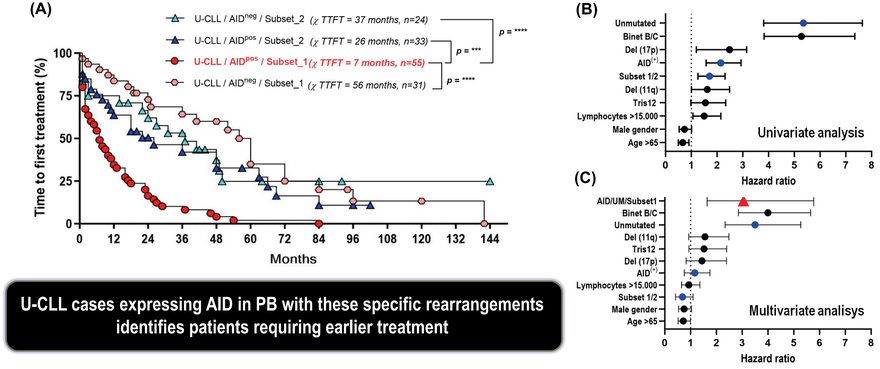
**Patients with positive AID expression in the U-CLL subgroup predominantly exhibit the specific gene rearrangements IgHV _*1-02, 1-69, 3-30 and 4-39.*

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.

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

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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