Characterizing Specificities of Chronic Lymphoid Leukemia Harboring a BCL2 Rearrangement, an update from the FILO group

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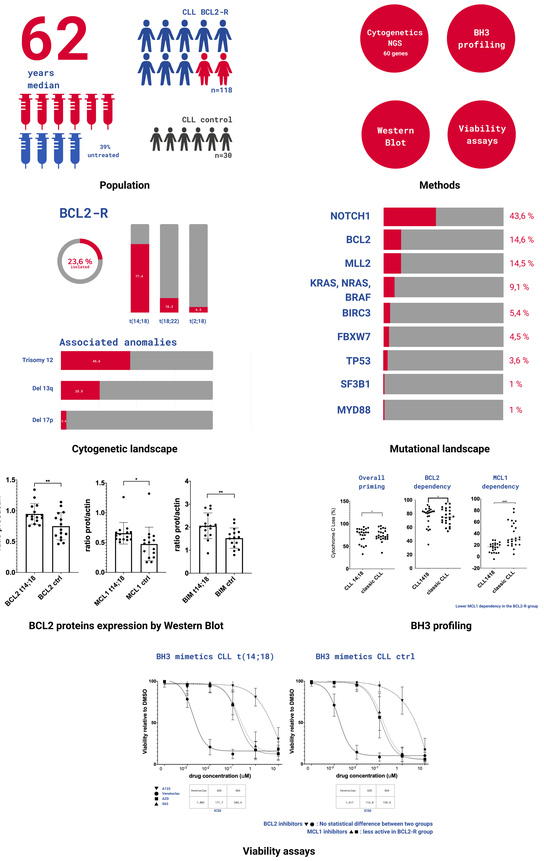
Introduction

Although survival dependence on BCL2 is a well-known aspect of the pathophysiology of chronic lymphocytic leukemia (CLL), the mechanisms of BCL-2 dysregulation are incompletely understood. Recurrent translocations involving BCL2 and immunoglobulin genes, including t(14;18)(q32;q21) and variants such as t(2;18) or t(18;22), are classically observed in follicular lymphoma or germinal center diffuse large B-cell lymphoma (GC DLBCL), but are uncommon (< 5%) in CLL and usually associated with an indolent clinical course. Here, **we characterize the mutational landscape and the functional BCL-2 family dependencies of BH3 proteins in BCL-2-rearranged (BCL2-R) CLL.**

Methods

Clinically annotated primary samples from BCL2-R CLL patients identified by karyotype were obtained from the French Innovative Leukemia Organization network and Dana-Farber Cancer Institute. Primary samples from CLL without BCL2 rearrangement were used as a control (ctrl CLL). Next generation sequencing (NGS) was performed using a custom-designed panel of 60 genes, including among others: BCL2, BIRC3, NOTCH1, FBXW7, MLL2, RAS pathway, SF3B1 and TP53. The mean coverage obtained was 2000X (limit of detection (LOD): 1%). Digital droplet PCR (ddPCR) was used to quantify NOTCH1 c.7544_7545delCT (LOD: 0.025%). Protein expression (Bcl2, Mcl1, Bim) was assessed by Western blot. Baseline BH3 profiling was performed as per Ryan et al., Bio Chem 2016. To mimic the lymph node microenvironment, viability assays were performed in co-culture with the stromal cell line NK.tert. Viability was assessed by AnnexinV/ Hoechst staining. Ex vivo drug treatments included: BCL2i (inhibitor): venetoclax; MCL-1i: AZD5991, S63845 and BCLXLi: A133. Statistical analyses were by unpaired and paired t-test with a two-tailed nominal p ≤ 0.05 considered as significant.

Graphics



Results

Genetics : karyotype and NGS assessment

In our cohort of 118 patients, the median age at diagnosis was **62 years**, and **79.5%** were male. Forty-six of them never received any treatment for CLL. BCL2-R were t(14;18) in 77.2%, t(18;22) in 16.3% and t(2;18) in 6.3% of patients. The translocation involving BCL2 gene was isolated in 23.6% of cases, and was associated with trisomy 12 in 45.4% of patients. **IgVH was hypermuted in 69% of cases.** The most frequently mutated genes in this cohort were in the **NOTCH pathway** (NOTCH1 mutation: **43.6 %**, mostly subclonal (mean of variant allelic frequency: 6.1%) and FBXW7: 4.5%)) and **RAS pathway** (KRAS, NRAS, BRAF: 9.1%). **BCL2 mutations** were observed in 14.6% of cases. No mutation previously described in venetoclax resistant CLL, such as F104L or G101V variant, were observed. Furthermore, **MLL2 mutations** were observed in 14.5% cases and were significantly associated with complex karyotype (p=0.01) and trisomy 12 (p=0.04). Others mutated genes were: BIRC3 (5.4%), TP53 (3.6%), SF3B1 (1%) and MYD88 L265P (1%). No mutations in EZH2, CREBBP or EP300 were found.

BCL-2 protein expression

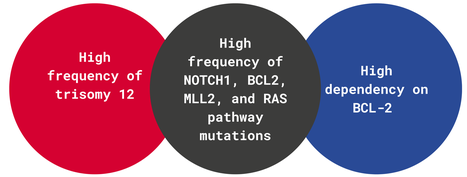
In 15 CLL representative samples from each group (BCL2-R and ctrl), Bcl2 protein expression was significantly higher in BCL2-R CLL (ratio Bcl2/actin 0.94 vs 0.74, p=0.009) as was expression of the pro-apoptotic protein Bim (ratio Bim/actin: 2.059 vs 1.524, p=0.007).

BH3 profiling and viability assays

BH3 profiling demonstrated that BCL2-R CLL and ctrl CLL samples (n=23 in each group) had comparable overall priming (cyto-C release 66.1% vs 63.3%, ns) and Bcl-2 dependence (cyto-C release 75.4% vs 76.3%, ns). Both also had low dependence on Bcl-xL (cyto-C release 8.2% vs 8.8%, ns). In contrast, Mcl-1 dependence was found to be significantly lower in BCL2-R CLL (cyto-C release 15.6% vs 37.4%, p < 0.0001). Consistent with our BH3 profiling results, the activity of venetoclax and the Bcl-xLi A133 did not differ significantly between the 2 groups (n=15). In contrast, **both Mcl-1i were less active in the BCL2-R group**: average viabilities after 24h treatment with AZD5991 were 76.4% vs 56.3% (p=0.006) and with S63845 77.3% vs 62.9% (p=0.02) in the BCL2-R vs ctrl group, respectively.

Conclusion

The genomic landscape of BCL2-R CLL is characterized by a high frequency of trisomy 12, subclonal NOTCH and RAS pathway mutations, as well as BCL2 and MLL2 mutations. Protein expression, BH3 profiling and viability assays data are consistent with nearly exclusive dependence on Bcl-2.



First study of a rare subpopulation of CLL with BCL2 rearrangements

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