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

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INTRODUCTION

Clonal Hematopoiesis (CH) is characterized by the accumulation of somatic mutations in hematopoietic stem cells (HSCs). The most frequently mutated genes are *DNMT3A*, *TET2*, and *ASXL1*, which provide a fitness advantage to HSCs. CH has been mainly studied in the context of myeloid neoplasms, whereas its clinical impact in chronic lymphocytic leukemia (CLL) needs further investigation.

AIMS

The aims of this study are:

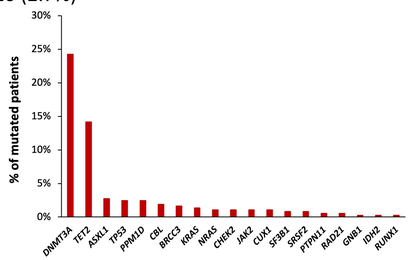
- to investigate the presence and the frequency of CH in a real-life cohort of CLL patients referring at our institution;
- to evaluate the clinical impact of CH at diagnosis of CLL;
- to examine the evolution of CH comparing baseline and post-therapy paired samples;
- To dissect the transcriptomic profile of T-cells of CLL patients according to CH status.

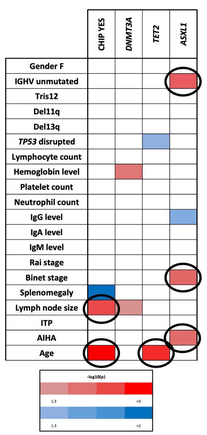
METHODS

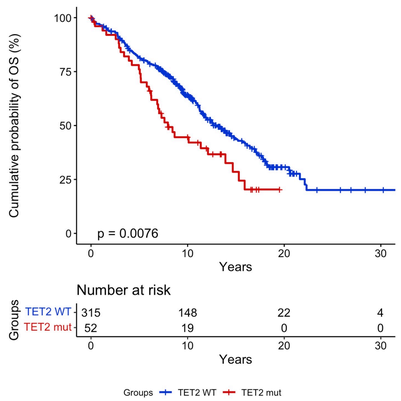
Tumor genomic DNA was extracted from granulocytes from CLL patients (N=367). Samples were analyzed by targeted next-generation sequencing (NGS), employing a custom panel of recurrently mutated genes in CH (N=28) and sequenced by MiSeq and NextSeq550 platforms (Illumina). scRNA sequencing was performed with the Singleron GEXSCOPE kit

RESULTS

A total of 167 (45.5%) patients showed at least 1 CH mutation, which were associated with several clinical and biological features. The most frequently mutated genes were ***DNMT3A*** in 89 (24.3%) patients, ***TET2*** in 52 (14.2%) and ***ASXL1*** in 10 (2.7%)



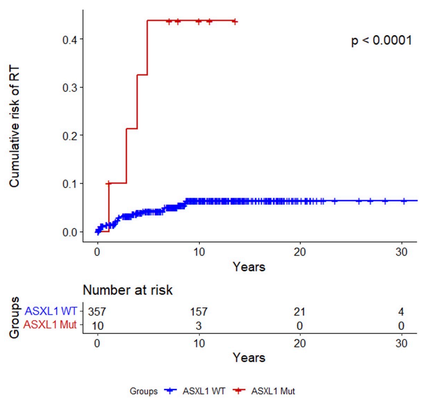




Variable	N	Hazard ratio	p
TP53 mut/del	357	3.06 (2.02, 4.62)	<0.001
IGHV unmutated	357	1.77 (1.33, 2.36)	<0.001
Age>65y	357	4.72 (3.20, 6.94)	<0.001
TET2 mut	357	1.64 (1.13, 2.39)	0.01

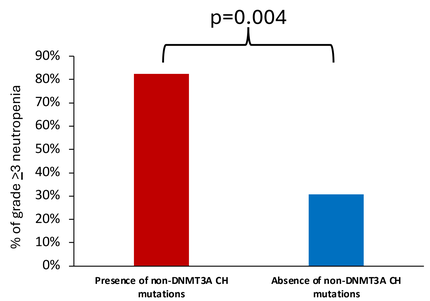
TET2 mutated patients displayed **shorter overall survival** (OS) compared to *TET2* wild-type patients. *TET2* mutations represent an independent predictor of shorter OS

ASXL1 mutated patients showed **increased risk of Richter transformation** (RT) compared to *ASXL1* wild-type patients. *ASXL1* mutations represent an independent predictor of shorter time to RT

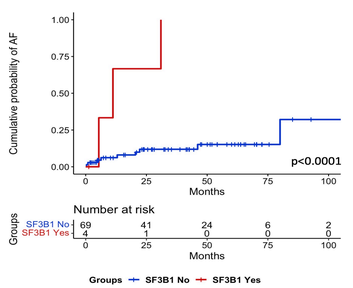


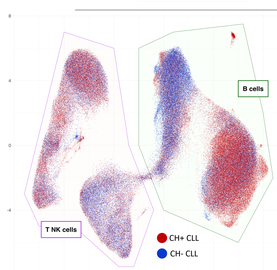
Variable	N	Hazard ratio	p
NOTCH1 Mut	302	1.96 (0.56, 6.91)	0.29
TP53 Mut/Del	302	3.97 (1.27, 12.43)	0.02
ASXL1 Mut	302	6.80 (1.54, 30.14)	0.01

At baseline, **non *DNMT3A* CH** mutations **associated with grade ≥3 neutropenia** in patients treated with **BCL2 inhibitors.** 82.4% of patients with non-DNMT3A CH mutations presented grade >3 neutropenia compared to 30.8% of patients without non-DNMT3A CH mutations (p=0.004)

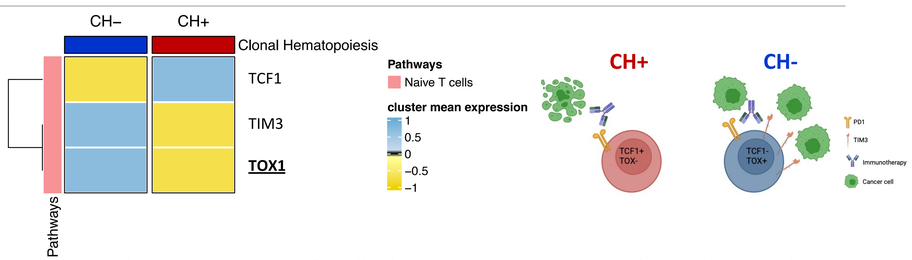


SF3B1 mutations identified only in the myeloid compartment associate with **higher risk of atrial fibrillation** in patients treated with **Bruton tyrosine kinase inhibitors**





Single-cell RNA sequencing of 7 CH+ and 6 CH- CLL revealed CH status-driven differences in the transcriptional landscape of both B and T cells



TOX, the master regulator of T-cell exhaustion induction, was significantly downregulated in T-cells of CH+ patients. Concordantly, TCF7, a critical transcription factor for maintaining multipotent progenitor T cells, was upregulated in CH+ CLL, suggesting that T-cells of CH+ patients may exhibit a less exhausted phenotype.

CONCLUSIONS

This study is the first to selectively analyze the myeloid compartment of CLL patients, aiming at carefully unraveling the interplay between CH and CLL. The findings demonstrate that CH holds potential clinical relevance for both CLL and RT, and impacts on pathway inhibitor toxicity, while also modulating the T-cell immune compartment of the patients with this leukemia.