Unveiling the Spectrum of Genetic Alterations in Relapsed/Refractory CLL Patients on Targeted Inhibitors: A Prospective Unicentric Study

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1. Introduction

- Next-Generation Sequencing (NGS) is pivotal in understanding CLL genomics.
- Presence of Variants of Uncertain Significance (VUS) and minor clones complicates interpretation.
- **Study aims:** Map mutations, track clonal evolution, specify parameters and identify associations.

2. Methods

Design: Prospective unicentric study.

Population: Relapsed or refractory CLL patients.

Sampling: 2019-2022, initiating treatment with oral tageted inhibitors.

Methodology:

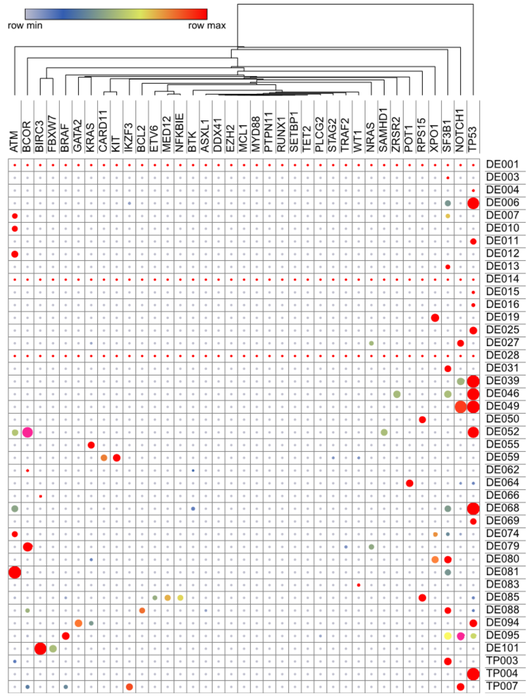
- CLL cell isolation: RosetteSep.
- NGS: Custom capture-based panel.
- Platforms: Illumina MiSeq.

Baseline Patient Characteristics	N (%)
Total number of patients	45
Median age	72y (range 45-86)
Male patients	32 (71%)
Median follow-up	22 months
Median no. of previous treatment lines	3 (range 2-5)
Median ECOG score	1 (range 0-2)
Treatment	
Bcl 2 inhibitor	14 (31%)
BTK inhibitor	25 (56%)
PI3K inhibitor	6 (13%)
Prognostic marker	
Unmutated IGHV	34 (76%)
Del17p and/or TP53 mutation	18 (40%)
Del11q	15 (33%)
Del13q	26 (58%)
Trisomy 12	5 (11%)
Complex karyotype	5 (11%)
Data at the start of treatment	
Rai modified risk: low / intermediate / high	1 (2%)/16 (35%)/ 29 (63%)
Lymphadenopathy (>5cm)	16 (36%)
Splenomegaly	18 (40%)
Thrombocytopenia (<100)	14 (31%)
Anemia (<100)	11 (24%)
B2M over ULN	28 (62%)
LDH over ULN	32 (71%)

Table 1. Baseline Patient Characteristics

3. Patients and Results

Figure 1. Heatmap of Genetic Mutation Distribution in the Studied Cohort (pre-treatment samples)



Patient	Baseline Mutation(s)	Change after 12 months
1	SF3B1	↓SF3B1, +XPO1, +KRAS
2	No mutations	no change
3	TP53, SF3B1, BCOR, ASXL1	↑TP53, ↑SF3B1, +BCL2, ↓BCOR, ↓ASXL1
4	XPO1	+BIRC3, ↑XPO1
5	TP53, NOTCH1 (VUS)	+TRAF2, -NOTCH1 (VUS)
6	CARD11, KIT, STAG2 (VUS), WT1	-STAG2, -WT1, -KIT, ↑CARD11
7	NRAS, NOTCH1, KRAS	-KRAS, ↓NRAS, ↑NOTCH1
8	ATM, SF3B1	↑SF3B1
9	IKZF3, BRAF, BCOR	+NOTCH1, ↓rest
10	TP53	+ATM, +MED12, +SF3B1, +XPO1, -TP53
11	No mutations	SF3B1, NRAS, BRAF, BIRC3
12	ATM, SF3B1	+TP53, ↔ATM (oncogenic → VUS), ↑SF3B1
13	TP53	+RPS15, ↑TP53
14	TP53	+NOTCH1
15	SF3B1	+NOTCH1, +KIT (VUS), ↓SF3B1
16	IKZF3, SF3B1, TP53	-IKZF3, +NOTCH1, +ATM, ↑SF3B1, ↑TP53

Table 2. Clonal Evolution and Genetic Mutations in Selected CLL Patients

Abbreviations: ECOG, performance status scale, IGHV immunoglobulin heavy chain variable region, TP53, tumour protein p53, BCL-2, B-cell lymphoma 2, BTK, Bruton tyrosine kinase, PI3K, phosphatidyl inositol 3-kinase, LDH, lactate dehydrogenase, ClCr G-C, creatinine clearance the Cockcroft and Gault formula, N, number, B2M, β2 microglobulin, ULN, upper limit of normal, "+", Mutation emerged; "-", Mutation disappeared or removed; "↑", Variant allele frequency (VAF) increased; "↓", Variant allele frequency (VAF) decreased; "↔", Mutation changed(additional details may be provided in parentheses)-

4. Conclusions

- Our results reveal a constantly evolving genomic landscape in CLL, affecting both disease progression and response to treatment.
- The data emphasize the critical role of ongoing genomic monitoring for understanding changing mutational profiles and their clinical impact.

Most Frequent Mutations:

- TP53: 40%
- SF3B1: 37%
- NOTCH1 & ATM: 19% each.

Clonal Evolution:

- Assessed in 16 patients.
- Genomic changes: 94%.
- New mutations: 69%.

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