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Tracking CLL cells with aberrations in the *TP53* gene using scRNA-seq in relapsed/refractory patients

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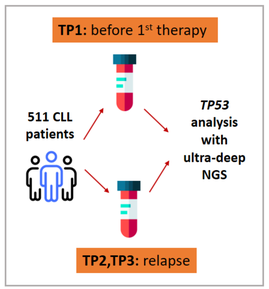
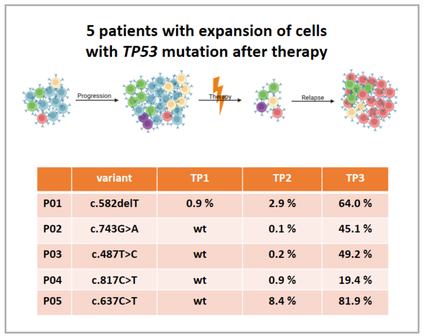
INTRODUCTION

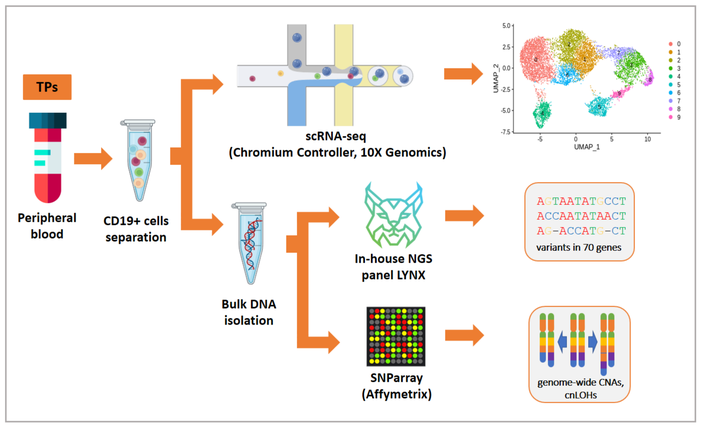
- *TP53* gene aberrations (mutation and/or deletion 17p) are the most important adverse prognostic and predictive markers in CLL
- Low-burden *TP53* mutations are often detectable in CLL cells prior to the therapy and expand upon the selective pressure of chemoimmunotherapy
- Other genomic alterations accompany aberrations in the *TP53* gene
- Bulk analysis (such as whole genome/exome sequencing or genomic array) cannot precisely determine the co-occurrence of abnormalities in individual cells
- Expression profiles of cells bearing *TP53* mutation are altered and may be distinguished from expression profiles of unaffected cells using single-cell RNA sequencing (scRNA-seq)

METHODS





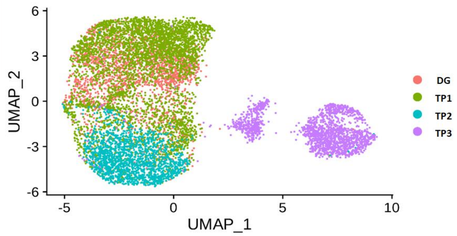
	variant	TP1	TP2	TP3
P01	c.582delT	0.9 %	2.9 %	64.0 %
P02	c.743G>A	wt	0.1 %	45.1 %
P03	c.487T>C	wt	0.2 %	49.2 %
P04	c.817C>T	wt	0.9 %	19.4 %
P05	c.637C>T	wt	8.4 %	81.9 %



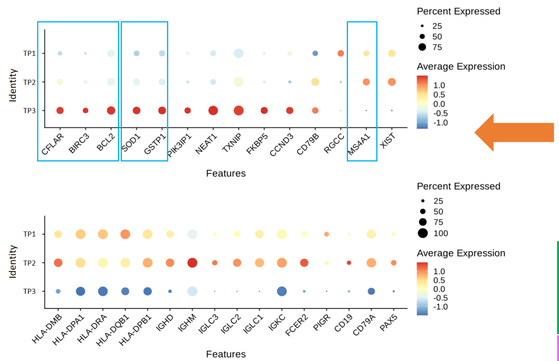
OBJECTIVES

We aimed to explore the possibility to identify and characterize populations of CLL cells resistant to treatment and causing refractoriness in samples from patients with disease relapse.
We tried to trace back the refractory cells in samples prior to the therapy using scRNA-seq in patients with *TP53* mutations.

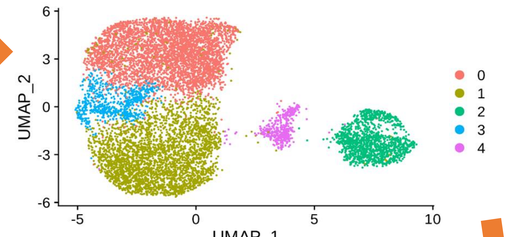
RESULTS



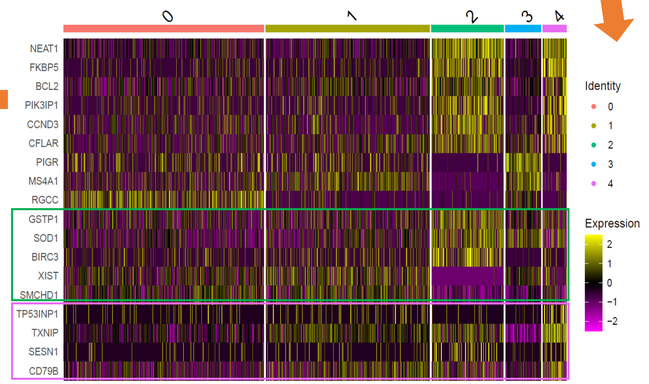
Clustered expression profiles of CLL cells (patient P01) from different time-points (TPs) Data analysis was carried out with the Seurat R package. Expression profiles of CLL cells are colored according to the TP; four TPs: DG – collected at diagnosis, TP1 – prior the first therapy, TP2 – the first relapse, TP3 – the second relapse.



Cells from advanced stages showed upregulation of negative apoptosis regulators (CFLAR, BIRC3, BCL2, YBX3) and downregulation of MS4A1 (encoding therapy target CD20), immunoglobulin genes, CD19, CD79A, and transcription factors PAX5 and TCF3, indicating a loss of original B-cell phenotype.



Differently expressed genes in CLL cells (patient P01) from different time-points (TPs) Expression profiles of CLL cells are colored according to similarly expressed genes (five groups).



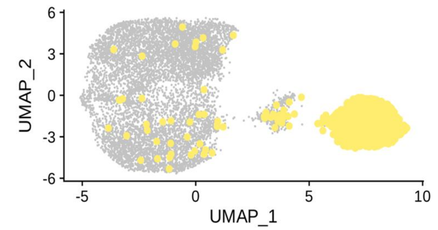
Differently expressed genes in diffetent TPs
(yellow – up-regulated; purple – down-regulated)

CONCLUSIONS

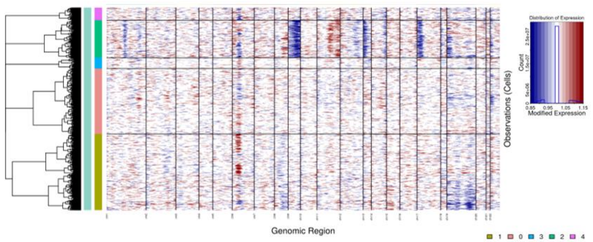
Our study highlights scRNA-seq as an efficient method for tracking rare cells potent for causing CLL refractoriness, confirming their presence as a subclone in pre-treatment stages of CLL.

The therapy selection pressure favors the preferential proliferation of rare resistant cells, leading to recurrent relapses.

scRNA-seq enables to identify expression profiles of malignant cells from patients with relapsed/refractory chronic lymphocytic leukemia (CLL) with high-burden *TP53* mutations in relapse and track them back in the cell populations sampled before therapy intervention with confirmed low-burden *TP53* mutation.



Expression profiles of CLL cells with complex chromosomal changes. Copy number aberrations (CNAs) were estimated via the InferCNV tool. Cells with complex chromosomal changes are marked in yellow. CNA results in relapse samples were verified using SNParray (Termo Fisher Sci).



The expansion of cells with *TP53* mutation was connected with a complex karyotype, as revealed by InferCNV and confirmed by SNParray and NGS. A complex karyotype comprised of structural as well as numerous chromosomal aberrations, including deletion 17p-. The InferCNV tool enabled the identification of subclonal cell populations bearing the same chromosomal changes

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