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Longitudinal CITE-Seq analyses of the peripheral blood identify transcriptomic signatures associated with progression and transformation under targeted therapies

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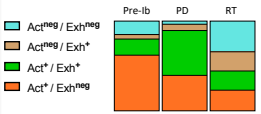
BACKGROUND and RATIONALE: *BTK/PLCG2* mutations account for only 60% of ibrutinib resistance. To understand co-evolution of normal immune bystander-tumor cells in CLL patients under covalent BTKi therapy, and hopefully help predict clinical relapses/transformations through transcriptomic signatures in discrete subsets of circulating cells, we have applied Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-Seq), a new powerful method to deeper analyze cellular protein and transcriptome in a single-cell sequencing approach, in 6 patients under therapy at various timepoints (before treatment, developing progressive disease (PD), and Richter's transformation (RT)).

Methods: Cells samples from patients with PD or RT under targeted therapies were obtained from the Hematology Department (IUCT-O) with informed consent and referenced in INSERM cell bank. Blood cells were cryopreserved in liquid nitrogen. For the study, CITESeq analyses have been done on samples from patients naïve of treatment (n=2), or resistant to ibrutinib (RT, n=3; PD n=3).

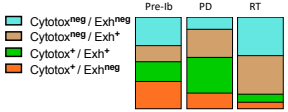
Cells from the different samples were thawed with a method preserving cell surface epitopes. Flow cytometry analyses (using the same antibodies as in CITESeq experiment), were done to evaluate the expression of cell surface proteins. For each sample, cells were processed according to the CITESeq manufacturer instructions. Raw data sets were computed with Cell Ranger 3.0 and then loaded in a R session with the Seurat 3.0 toolkit package to produce UMAP maps. Data were then analyzed using Single-Cell Signature Explorer. To assess differentiation trajectories, cells from each sample were injected into a corresponding reference and then classified by their mapping on the reference trajectory.

Gene score of T lymphocytes differentiates PD and RT in blood

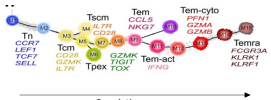
% of CD4 T cells according to activation and exhaustion signatures



% of CD8 T cells according to cytotoxic and exhaustion signatures



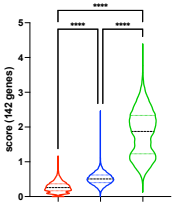
Differentiation status trajectory of CD8 T cells in healthy donors (Cerapio et al 2021)



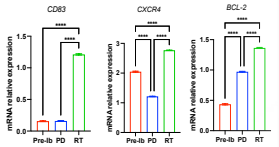
Differentiation status of CD8 T cells

B leukemic cells from RT exhibit a specific gene signature

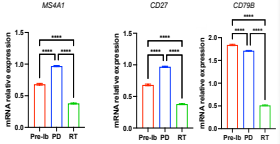
Specific "RT" gene signature in B leukemic cells



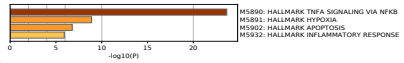
Up-regulated genes in RT



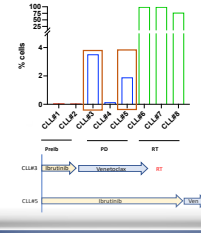
Down-regulated genes in RT



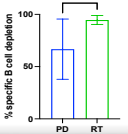
METSCAPE analysis of specific "RT" gene signature



Specific "RT" signature predicts RT evolution



B leukemic cells from PD and RT are equally sensitive to Glofitamab *in vitro*



Conclusion: Results showed an increase of genes involved in TNF signaling pathway in all cellular populations in RT patients. In these patients, we identified new phenotypic markers and differences in T lymphocytes sub-populations.

Despite this CD8 differential gene expression profile, B leukemic cells from PD and RT samples were equally and highly sensitive to Glofitamab *in vitro*.

Inside "RT" gene signature, we defined some genes that could be related to new therapeutic targets and/or phenotypic characteristics. This will be analyzed in a new cohort of patients after targeted therapies (PD, RT) or before treatment to validate a predictive "RT" blood signature.