Longitudinal Single-Cell Profiling of CLL Patients Exposed to BTK and BCL2 Inhibitors

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Introduction

Double Refractory CLL is an Emerging Group with High Risk

- Increasing use of BTKi and BCL2i leads to growing number of double exposed (2E) and double refractory (2R) patients
- High proportion of TP53 aberrations, RT and adverse risk features in 2E/2R pts.
- mOS is short with 8-22 months[1] in 2R pts.

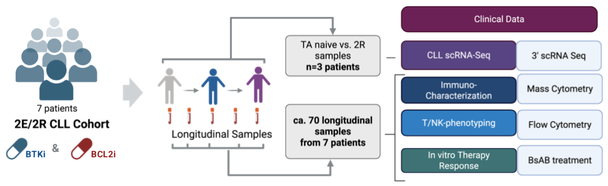
Treatment of Double Refractory CLL is an Unmet Need

- In 2E pts. re-treatment is possible
- Available treatment options are limited: e.g. CAR-T or Allo-SCT[2]
- Novel treatments in clinical trials i.a.
  - Bispecific antibodies
  - BTK-Degrader

Biology and Microenvironment of 2E/2R pts. is Largely Unknown

Methods

Longitudinal Multi-Omic Characterization of 2E/2R pts.

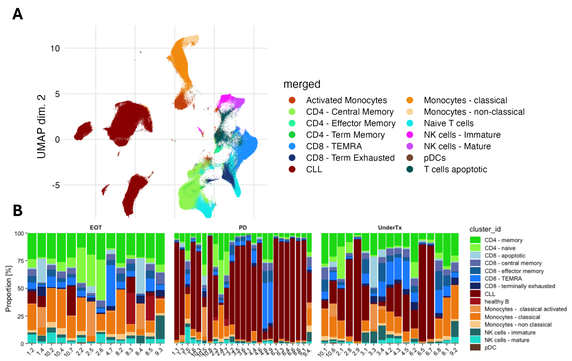


Graphical Overview of Multi-Omic Analyis: 70 longitudinal samples from 7 2E/2R patients were analyzed by Mass/Flow Cytometry and functional assays. Sample before targeted agents and 2R samples were subjected to scRNA-seq.

- Cohort of 2 patients with 2E or 2R CLL
- Longitudinal samples
- Multi-omic analysis by scRNA-Seq, Mass and Flow Cytometry Immunophenotyping and in vitro BsAB testing

Results

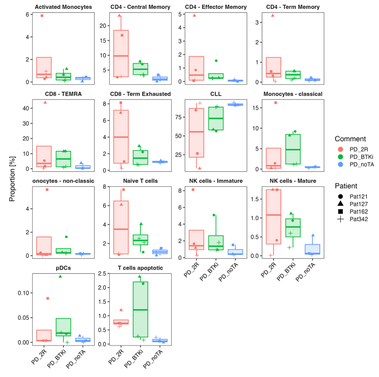
Longitudinal characterization of 2E/2R patient courses



Mass Cytometry: (A) UMAP representation of cellular subpopulations in longitudinal CLL samples (B) Abundance of subcluster in individual samples, grouped by treatment status.

- 50 marker assessment by Mass Cytometry characterizes longitudinal samples from 2E and 2R pts
- Identification of CLL cells and 13 microenvironmental subpopulations

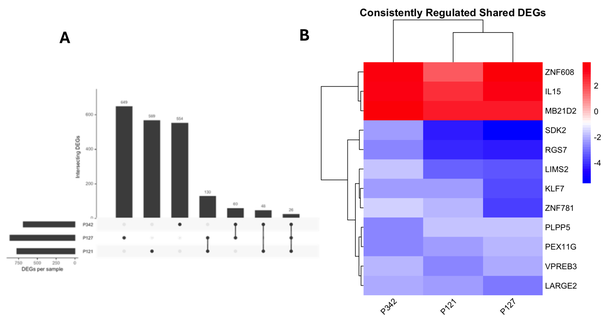
2R samples have higher TME proportion and lower CLL infiltration



Mass Cytometry II: Abundance of CLL and TME subpopulations separated by previous therapy status

- 2R samples have lower CLL infiltration compared to PD upon BTKi as well as PD before targeted agents
- 2R samples have higher T cellular, yet lower monocyte abundance

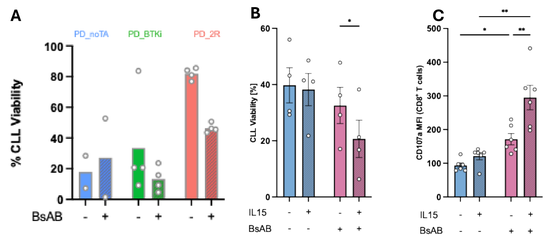
Differentially expressed genes in 2R samples



Differentially expressed DEG in scRNA-Seq of 2R CLL : (A): UpSet-Plot of DEG within CLL subpopulation from CLL PBMCs in 2R vs. non-targeted agent samples (B) Heatmap of shared DEGs from the 3 patients, which are consistently regulated

- 2R samples show large transcriptional difference with >500 DEG per sample
- Shared DEGs associate with neurodevelopment/synaptic function and cell-cell communication

Increased bispecific antibody function in 2R and IL15 treated PBMCs



In vitro Bispecific Antibody Assessment: (A) 2R PBMC samples show tendency for higher in vitro BsAB efficacy (B) IL15 addition increases cytotoxicity of BsAB in CLL PBMCs (C) CD8 cells from IL15 treated CLL PBMCs show increased CD107 expression

- 2R samples show tendency to increased BsAB efficacy
- Addition of IL15 increases efficacy of BsAB treatment and cytotoxicity markers on $CD8^+$ T cells

Conclusion

Unprecedented multi-omic analysis of 2E and 2R samples

2R samples show increased TME infiltration

Deregulated IL15 in 2R samples promotes BsAB efficacy *in vitro*

Outlook

- Comparison between 2E and 2R samples
- Advanced analysis of scRNA-Sequencing, incl. cell-cell interactions and TME adaptions
- In vitro validation experiments
- Clinical trials, targeting the tumor microenvironment in 2R patients (TaME-series of GCLLSG)

References

[1] Aronson et al. Am J Hematol. 2022
[2] Lewis et al. Blood 2023

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