Single-cell RNA-seq analysis reveals distinct tumor and immunosuppressive T cell phenotypes in CLL patients treated with ibrutinib.

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Introduction

The development of Bruton tyrosine kinase inhibitors (BTKIs) and their introduction into clinical practice represents a major advance in the treatment of chronic lymphocytic leukemia (CLL)[1]. Monotherapy with ibrutinib or other BTKIs generally do not induce complete remissions or undetectable minimal residual disease even with extended therapy. The reason why some patients relapse, and some have minimal residual disease is unknown[2-3]. Therefore, there is a need to understand the differences between ibrutinib sensitive and resistant CLL cells along with the immune microenvironment to identify novel therapeutic targets.

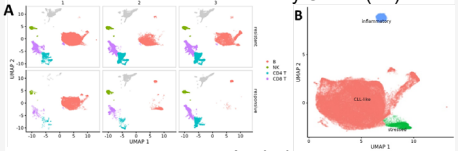
Methods

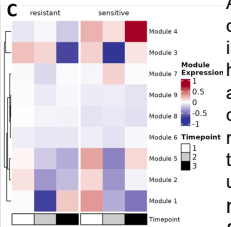
- Samples were collected from 8 patients at baseline (before treatment) (1), where BTK C481S is present with low variant allele frequency (VAF) (2) and at time of relapse (3). Data from these were compared to ibrutinib sensitive patient samples at baseline (1) (before treatment), 3yr (2) and 5yr (3) on treatment timepoints.
- Single cells were isolated using the 10X Genomics 5' immune profiling kit. Data were processed with CellRanger v3.1.0.
- Data were processed with CellRanger v3.1.0. Sample variation was reduced using Batchelor to aid clustering.
- Clustering was performed using the partitioning and Leiden methods.

Results

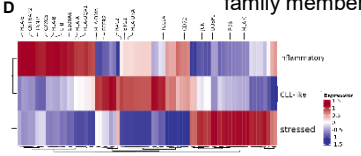
Single cell RNA-seq identifies changes in transcriptional heterogeneity within the B cell cluster

We identified distinct clusters of cells comprising disparate populations of T cells, B cells and NK cells in an integrated dataset of 10,000 cells from all samples (1A). Total B cells were grouped into three distinct subsets of clusters by UMAP (1B).



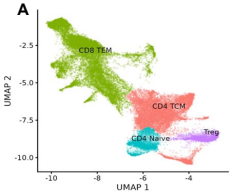


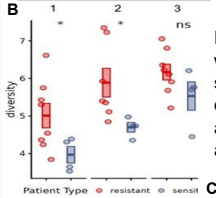
Analysis on these B cell clusters showed that ibrutinib sensitive samples had an increase in T cell activation, RNA splicing, cell adhesion and migration programs during therapy along with upregulation of MHC I molecules and TNF family members



Changes in T cell Phenotype in ibrutinib sensitive and resistant CLL samples

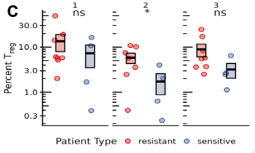
To identify immune cell landscape dynamics during CLL progression, we evaluated T cell distribution and heterogeneity across timepoints.

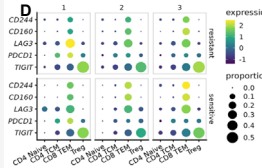




Four subsets of T cells were identified and found significant difference in the diversity between resistant and sensitive groups (2A and 2B).

We also observed significantly higher proportion of Tregs in the ibrutinib-resistant samples (2C).





These T cells have higher expression of checkpoint molecules and suppressive genes (2D).

Conclusion

- Based on pre- and post-treatment profiling, we identified unique transcriptional profile in CLL patients.
- Ibrutinib sensitive samples does have altered gene expression profile in which TNF family members and MHC class I molecules are upregulated overtime to treatment.
- Although overall the number of immune cells increase in long time ibrutinib therapy, they exhibit exhausted or non-functional phenotypes.
- At the single-cell level, these findings provide an insight into the molecular and cellular complexity of CLL during treatment.

References

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2. Woyach JA, Furman RR, Liu T, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med. 2014;370(24):2286-2294.
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