1102. Integrating Multi-Omics to Reveal the Heterogeneous Clonal Evolutionary Characteristics in CLL Patients with Zanubrutinib Resistance

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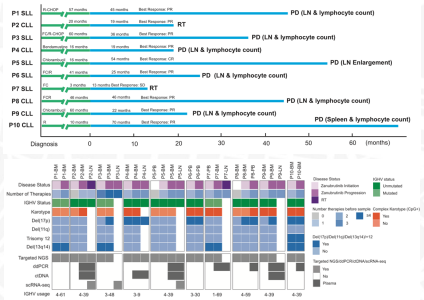
Introduction

- The drug-resistant mechanisms of the first-generation Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, has been extensively explored in Chronic Lymphocytic Leukemia (CLL) patients. However, the resistant mechanisms of the second-generation BTK inhibitor such as zanubrutinib remained largely unexplored.
- We integrated multi-omics to assess the heterogeneous clonal evolutionary characteristics in CLL patients with zanubrutinib resistance.

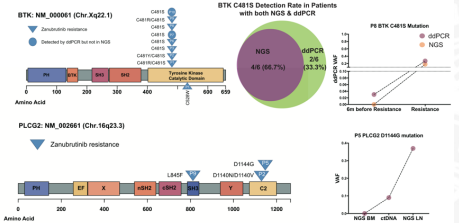
Methods

- We retrospectively identified 10 CLL patients with zanubrutinib resistance. Deep targeted-gene next generation sequencing (NGS) covering BTK (exon 1-19), PLCG2 (exon 1-33) and high sensitivity droplet digital PCR (ddPCR) detecting BTK mutation were assessed in available serial samples. Single-cell RNA sequencing (scRNA-seq) of matching peripheral blood (PB) and lymph node (LN) were performed in 3 zanubrutinib-resistant patients showing progressive lymphadenopathy.



Results

- Totally 10 patients were included in our study, the median time from zanubrutinib initiation to progression was 30.5 months, including 2 patients were both histologically confirmed as Richter Transformation (RT). 5 patients presented as LN enlargement at progression received PET-CT imaging scan and further underwent LN biopsy or puncture at the site of maximum Standard Uptake Value (SUVmax), 2 patients were diagnosed as RT, 2 as accelerated CLL (aCLL) and 1 as CLL progression.
- Deep targeted-gene NGS showing that BTK Cys481 mutation was detected in 8 of 10 patients (1 patients also harbored BTK Leu528Trp mutation with low frequency) and PLCG2 mutation was detected in one patient without BTK Cys481 mutation. For 4 patients with undetectable BTK mutation by NGS, ddPCR were performed and two patient was identified harboring BTK Cys481Ser mutation (progressed due to lymphadenopathy but NGS performed in bone marrow), suggesting spatial clonal heterogeneity in zanubrutinib-resistant patients. For one patient harboring both BTK Cys481 mutation and BTK Leu528Trp mutation, Cys481Ser (VAF 20.44%) and Cys481Arg (VAF 13.87%), BTK Leu528Trp (5.97%) mutations are in different cells. Crystal structure of $BTK^{Cys481Ser}$-Zanubrutinib suggested the disruption of hydrogen bond compared with BTK^{WT}-Zanubrutinib while $BTK^{Leu528Trp}$-Zanubrutinib suggested potential steric clashes compared with BTK^{WT}-Zanubrutinib, leading to the decrease of binding free energy between BTK and zanubrutinib.



Results

- For 3 of 5 patients who showed progressive lymphadenopathy during zanubrutinib resistance, 3 patients displayed different clinical manifestation with one RT, one aCLL and one disease progression. scRNA-seq of matched PB and LN were performed. Highest proportion of proliferating tumor cells was found in RT while lowest proportion of proliferating tumor cells was found in disease progression. Notably, RT patients displayed the most actively upregulated MYC, OXPHOS and G2M pathways and the downregulation of BCR signaling. aCLL showed the median activation of MYC, OXPHOS and G2M pathways and median downregulation level of BCR signaling, indicating its intermediate biological status between RT and disease progression. Moreover, analysis of BCL-2 anti-apoptotic family genes expression showed that tumor cells in RT showed significantly higher expression of MCL-1 while tumor cells in progressive CLL showed relatively higher expression of BCL-2, indicating the shift of BCL-2 family dependence among different disease characteristics

Conclusion

- Integrated multi-omics were performed in our zanubrutinib-resistant CLL patients' cohort. BTK Cys481 and Leu528 were two main BTK resistant mutations in zanubrutinib resistant CLL patients. Due to spatial heterogeneity and clonal evolution among patients, deep targeted-gene NGS and ddPCR should be used comprehensively to evaluate the emergence of resistant clones. Zanubrutinib-resistant RT showed highest upregulation of MYC, OXPHOS and G2M pathways and downregulation of BCR signaling. RT also showed upregulation of MCL-1, indicating underlying mechanism leading to insensitivity to venetoclax treatment following zanubrutinib resistance.