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Dynamic Single-Cell Profiling Reveals Novel Immune Regulatory Mechanism of ITKi Soquelitinib in Refractory T Cell Lymphoma

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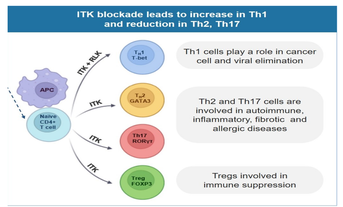
1. INTRODUCTION

Interleukin 2 inducible T cell kinase (ITK) plays an important role in the T cell receptor (TCR) signaling pathway.

The highly selective covalent ITK inhibitor, soquelitinib (SQL, formerly known as CPI-818), is being evaluated as a monotherapy in an ongoing Phase 1/1b trial in patients (pts) with refractory T-cell lymphoma (TCL) (NCT03952078).

Single cell RNA-sequencing and flow cytometry were performed on patient samples to identify potential prognostic biomarkers and to study immune regulatory mechanisms.



ITK blockade leads to increase in Th1 and reduction in Th2, Th17

2. METHODS

Paired peripheral blood and tumor tissue samples were collected from 8 TCL patients enrolled in the trial. .

The single cell RNA-seq and flow cytometry were used to dynamically analyze the changes of gene expression profiling, followed by signature genes enrichment to identify ITK related molecular and immune regulatory mechanisms in TCL patients treated with soquelitinib.

3. PTS，SAFETY & EFFICACY

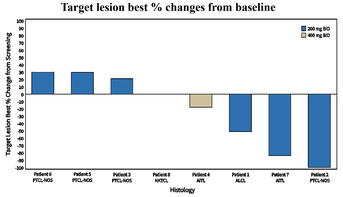
Patients for the assessment

Patient Characteristics	N=8
Age (yrs.), median (range)	57 (29, 76)
Gender, male N (%)	5 (62.5)
No. of prior therapies, median (range)	3 (1, 4)
HISTOLOGIES	
PTCL-NOS	4
AITL	2
ALCL	1
NKTCL	1

Summary of safety

Most common AEs (>1 patient) all causality were: anemia, bilirubin increased, neutrophil count decreased, WBC decreased, and pruritus. Grade 3+ AEs were reported in 1 patient: neutrophil count decreased and WBC decreased.

Target lesion best % changes from baseline



4. RESULTS

2 responding pts: The percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells post-SQL treatment

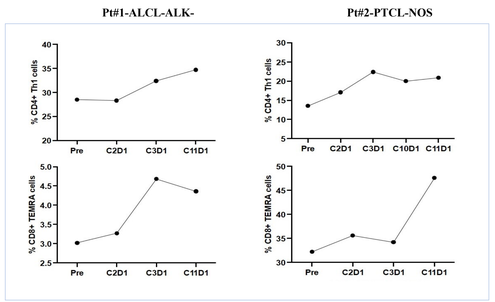


Figure 1. In the 2 patients with objective response (2 partial responses), the percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells was increased and sustained after soquelitinib treatment. Increased levels of normal CD4+ Th1 cells and CD8+ TEMRA cells correlated with the clinical responses.

3 PD pts: The percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells post-SQL treatment

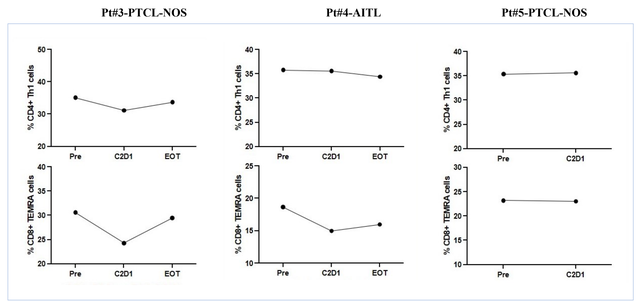


Figure 2. In the 3 patients with progressive disease (PD), the percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells was not increased after soquelitinib treatment.
Peripheral blood from PD patients were collected at various timepoints and T cell subsets evaluated by flow cytometry. Plots show percent change from baseline for CD4+ Th1 and for CD8+ TEMRA cells. EOT = end of treatment.

4 SD pts: The percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells post-SQL treatment

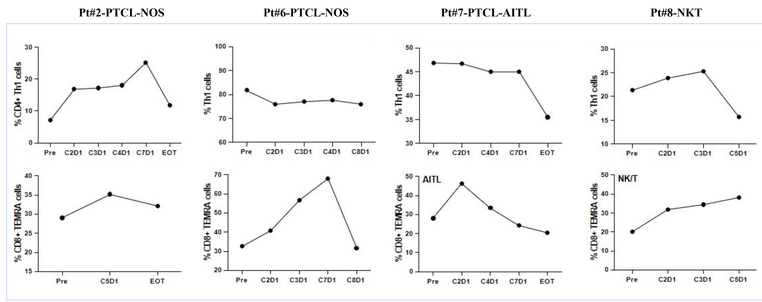


Figure 3. In the 4 patients who achieved stable disease (SD), the percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells was increased at the very beginning and decreased in the late phase of soquelitinib treatment.
Peripheral blood from SD patients was collected at various timepoints and T cell subsets evaluated by flow cytometry. Plots show percent change from baseline for CD4+ Th1 and for CD8+ TEMRA cells. Patient #2 sample obtained during period of stable disease.

SQL induced cytotoxic gene expression - the dynamic single-cell profiling

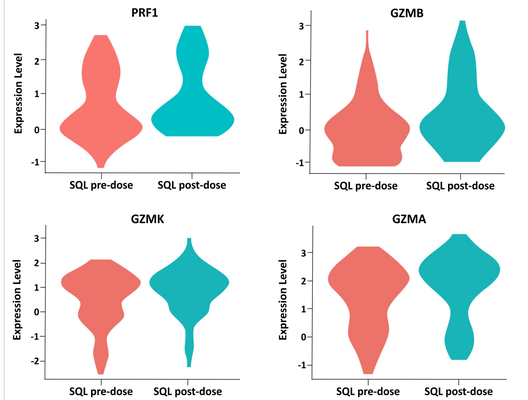


Figure 4. Tumor tissue from patients was collected at different time points and assayed by single-cell RNA-seq. Single-cell sequencing studies have shown that ITK inhibitor SQL can increase the expression PRF1 (perforin 1), GZMB (granzyme B), GZMK (granzyme K) and GZMA (granzyme A) mRNA in infiltrating CD8+ cells in tumor tissue. Violin plots showing the distribution of gene expression levels for selected differentially expressed genes over the time course.

SQL decreased exhaust gene expression - the dynamic single-cell profiling

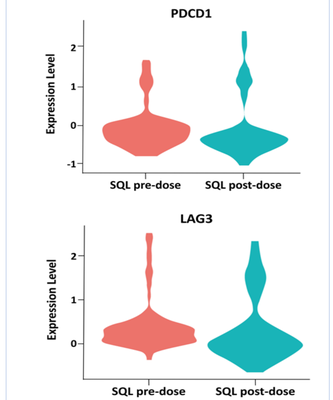


Figure 5. Tumor tissue from patients was collected at different time points and assayed by single-cell RNA-seq. Single-cell sequencing studies have shown that ITK inhibitors can downregulate the expression of various exhaustion molecules such as PDCD1 and LAG3. Violin plots showing the distribution of gene expression levels for selected differentially expressed genes over the time course.

The protein-protein interaction network analysis reveals that the hub gene TBX21 might play a central regulatory role

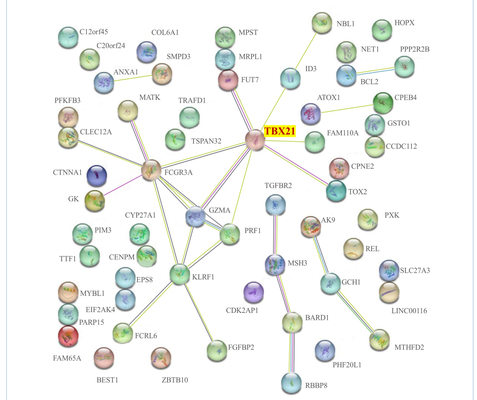


Figure 6. Single-cell sequencing studies further confirm that soquelitinib may primarily regulate TBX21 expression in tumor-infiltrating T cells, CD8+ TEMRA cell differentiation, and activation through the modulation of the tumor microenvironment.
Tumor tissues before and after treatment with soquelitinib were collected at different time points and assayed by single-cell RNA-seq. The upregulated and downregulated gene sets were enriched and the selected genes were constructed for protein-protein interaction networks.

SQL induced tumor infiltrated immune cells contribute to the anti-tumor effects of ITK inhibitor

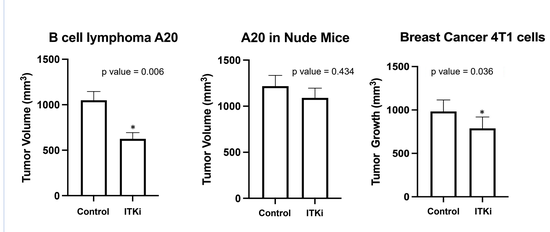


Figure 7. Human T cell in vitro analysis and TCL tumor bearing mouse model studies confirmed the immunomodulatory effect of soquelitinib.
Similar results were also observed in the murine syngeneic B cell lymphoma A20, breast cancer 4T1 models. Mice inoculated A20 or 4T1 tumors were treated with ITK inhibitor or vehicle daily for 10 days. Tumor measurements were performed after last dosing. Soquelitinib was not effective in nude mouse model indicating that competent host immune system is required for response.

5. CONCLUSIONS

Our findings reveal a novel immune regulatory mechanism by which the ITK inhibitor soquelitinib induced normal CD4+ Th1 cells and CD8+ TEMRA cells and reduced CD4+ Treg cells in the tumor micro-environment in responding patients. These findings demonstrate the potential of soquelitinib as a novel immunotherapy for the treatment of T-cell lymphomas and solid tumors.

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