

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

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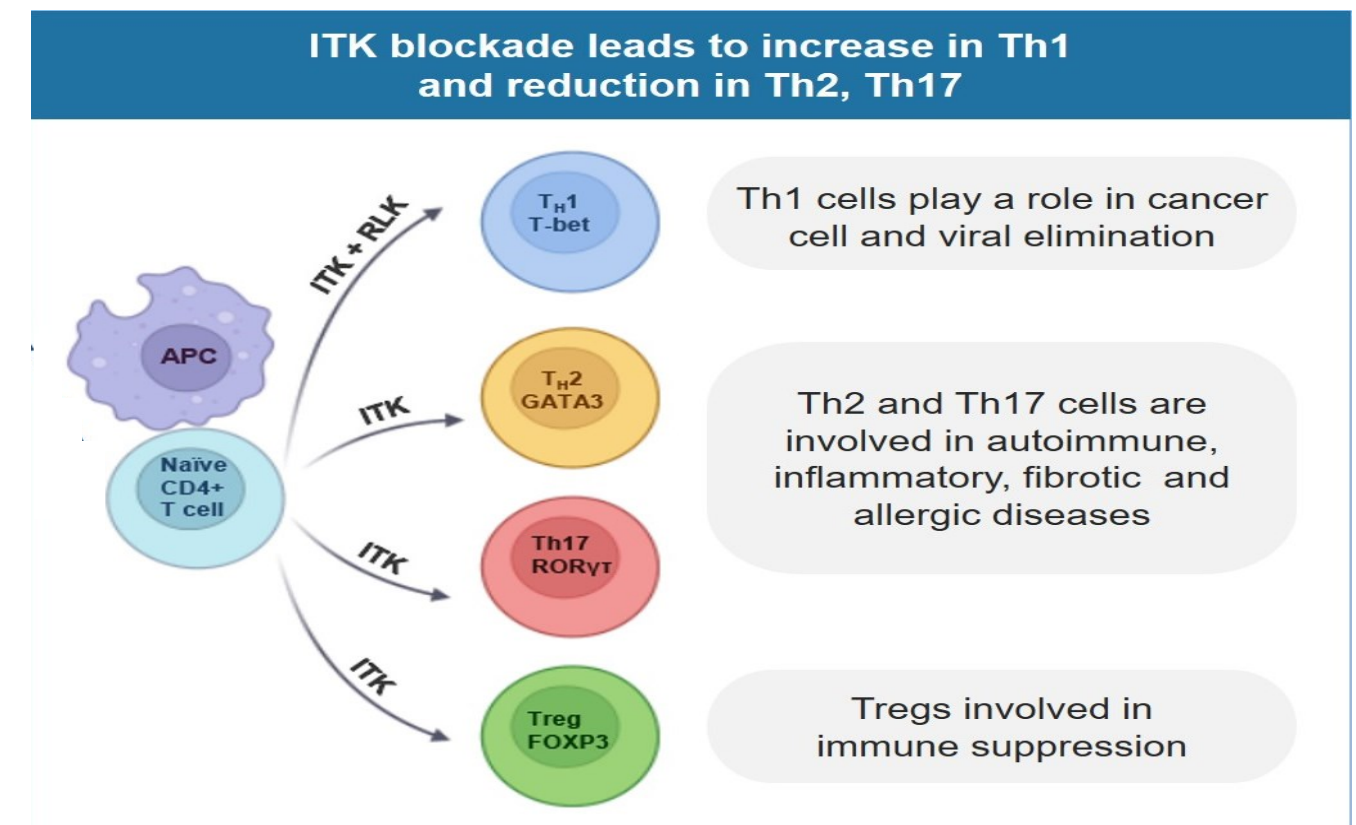
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## 1. INTRODUCTION

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 I/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

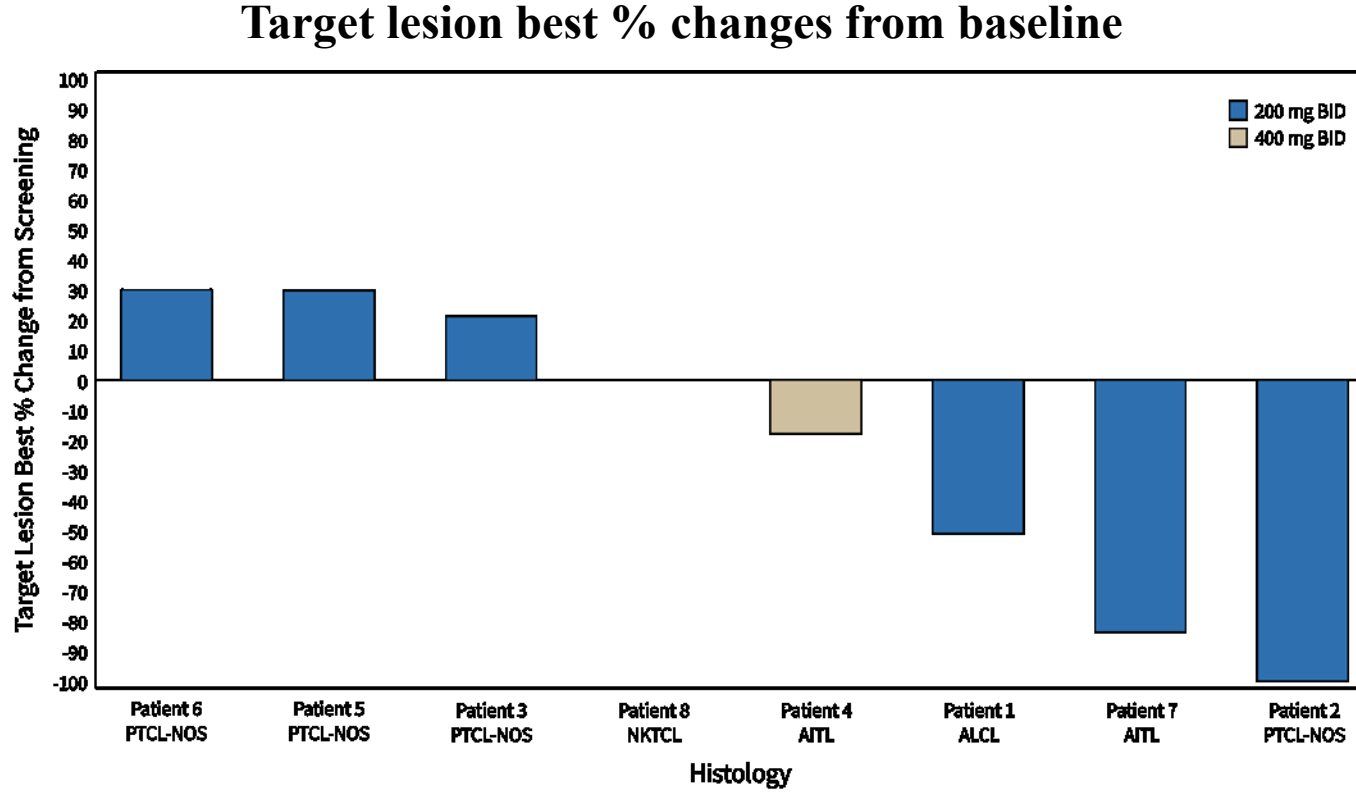
Paired peripheral blood and tumor tissue samples were collected from 8 TCL patients enrolled in the trial.

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

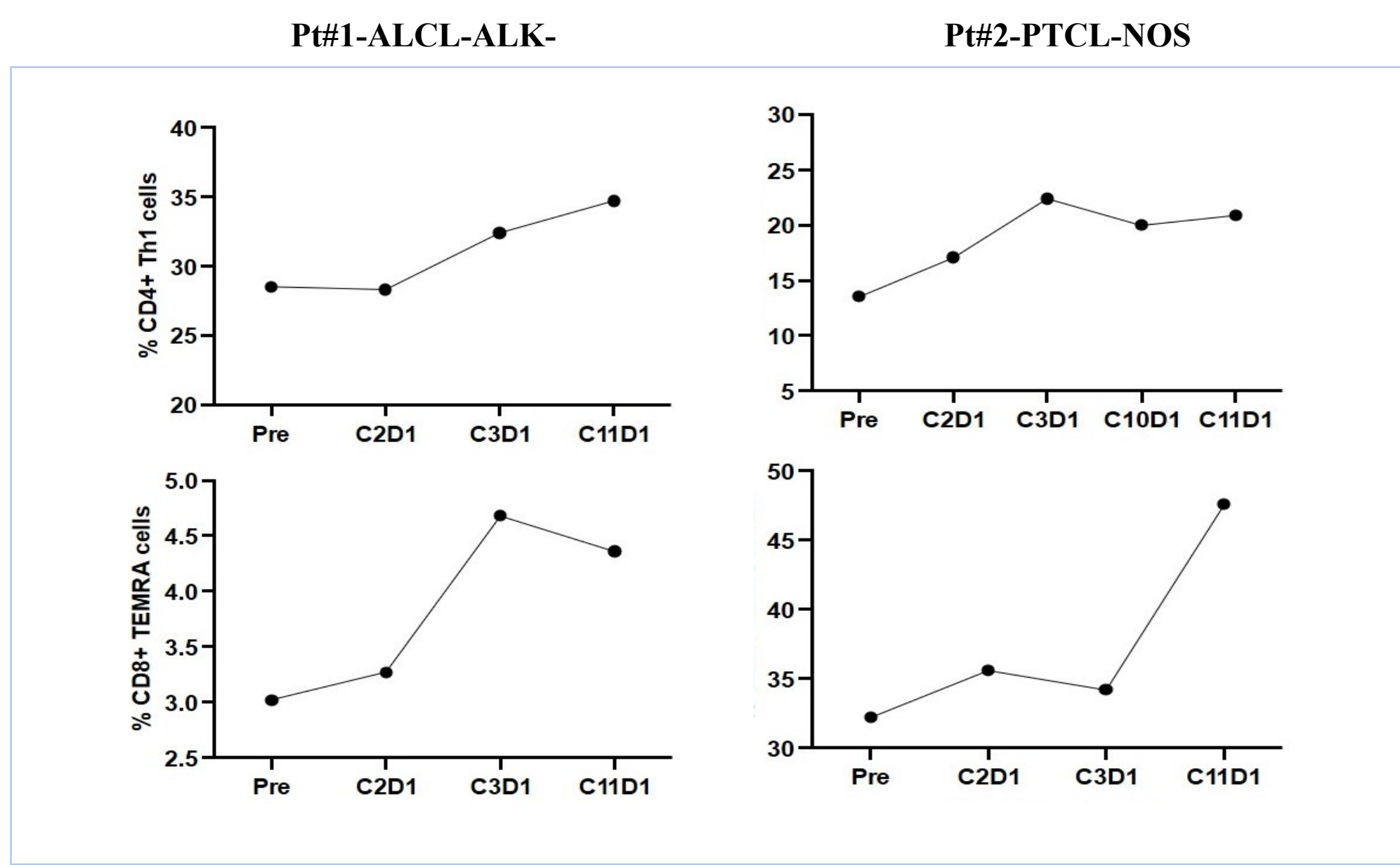
| Patients for the assessment            |             |
|----------------------------------------|-------------|
| Patient Characteristics                |             |
| Age (yrs.), median (range)             | 57 (29, 76) |
| Gender, male N (%)                     | 5 (62.5)    |
| No. of prior therapies, 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.



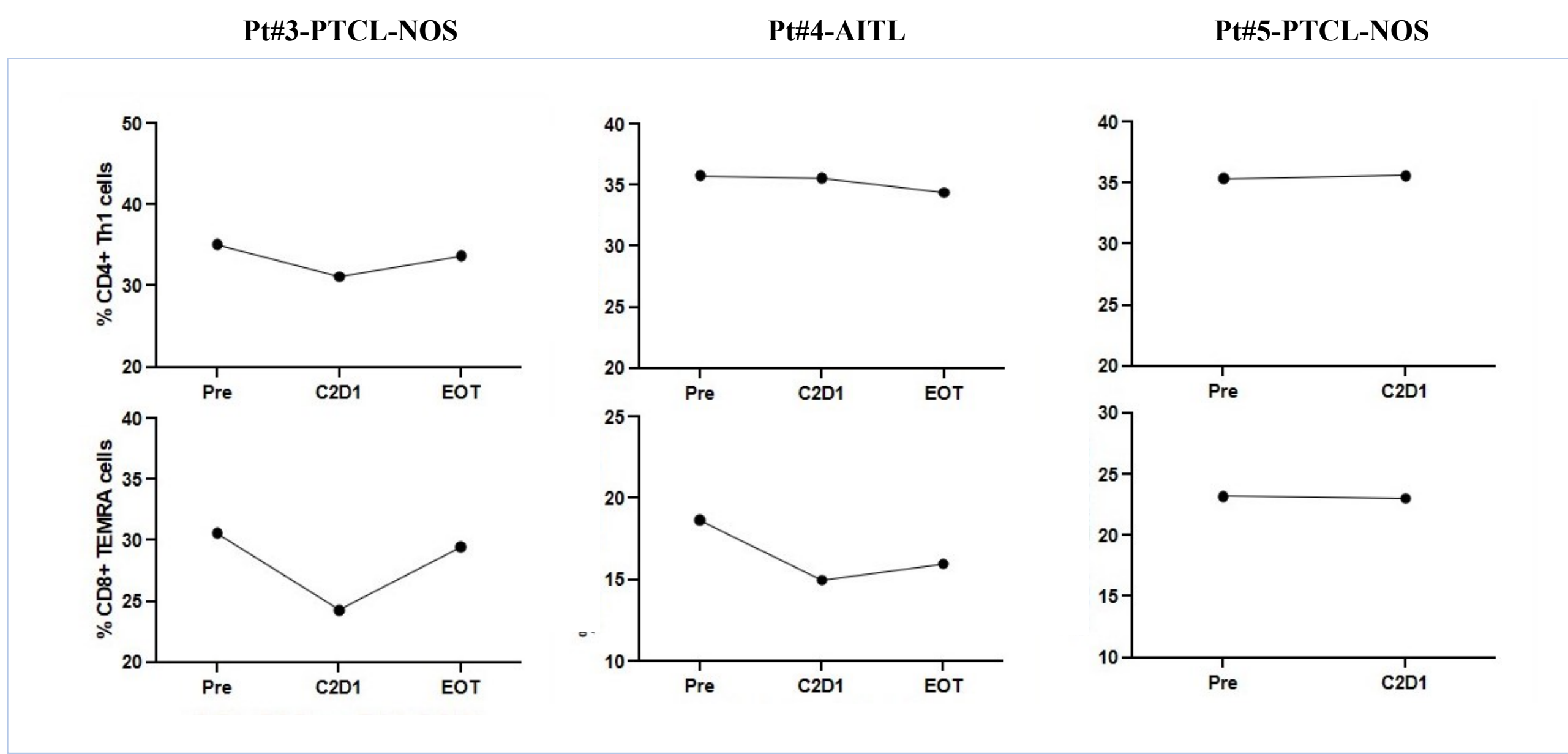
## 4. RESULTS

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



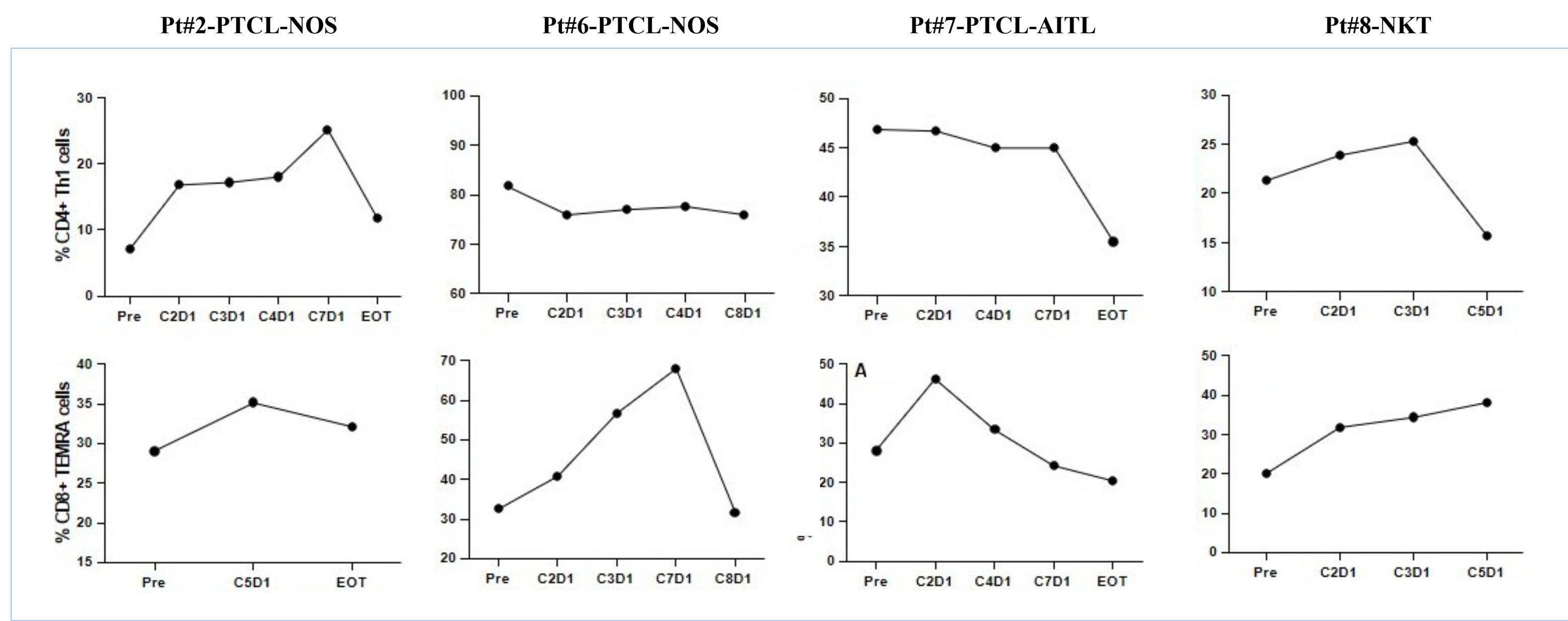
**Figure 1.** In the 2 patients with objective response (2 partial responses), the percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells was increased and sustained after soquelitinib treatment. Increased levels of normal CD4+ Th1 cells and CD8+ TEMRA cells correlated with the clinical responses.

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



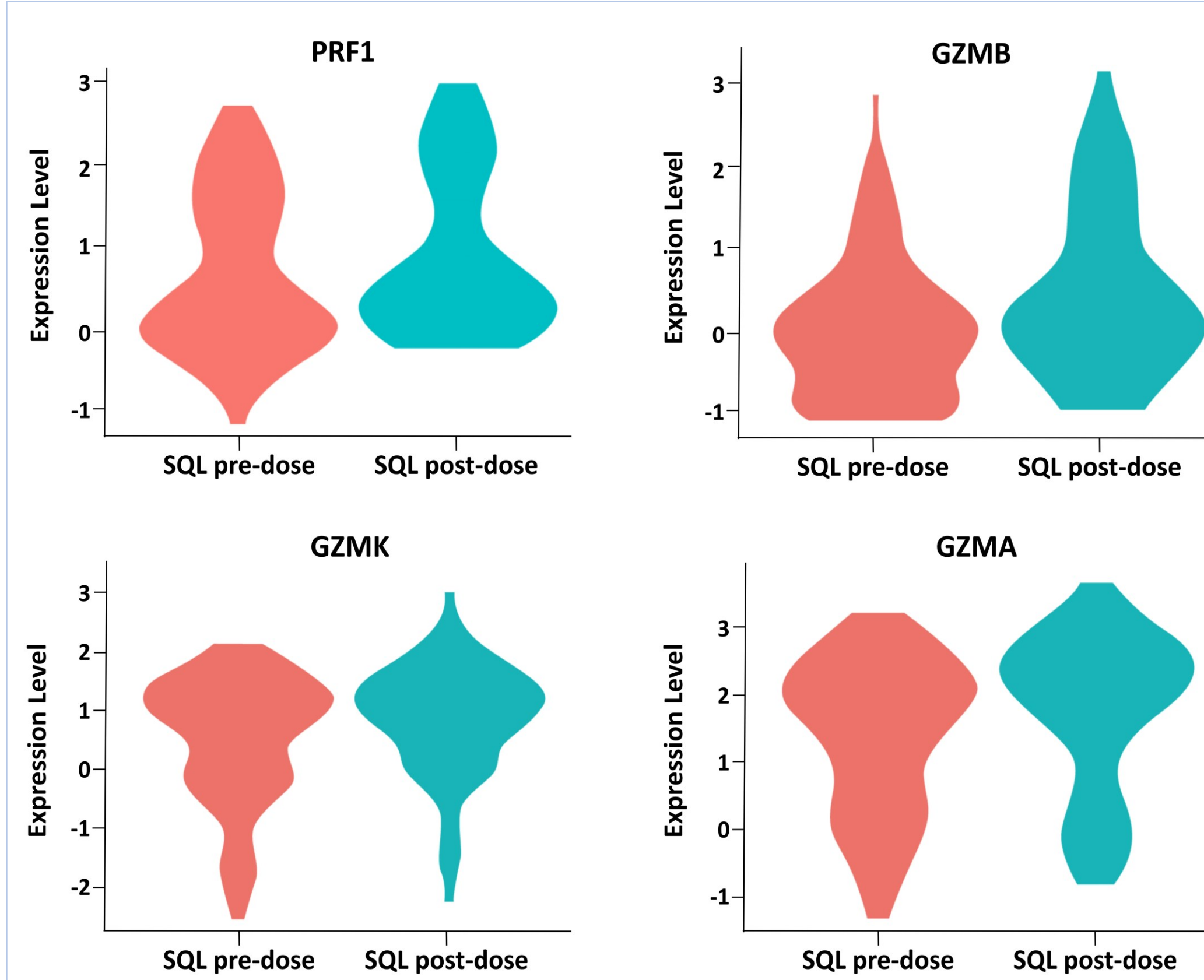
**Figure 2.** In the 3 patients with progressive disease (PD), the percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells was not increased after soquelitinib treatment. Peripheral blood from PD patients were collected at various timepoints and T cell subsets evaluated by flow cytometry. Plots show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



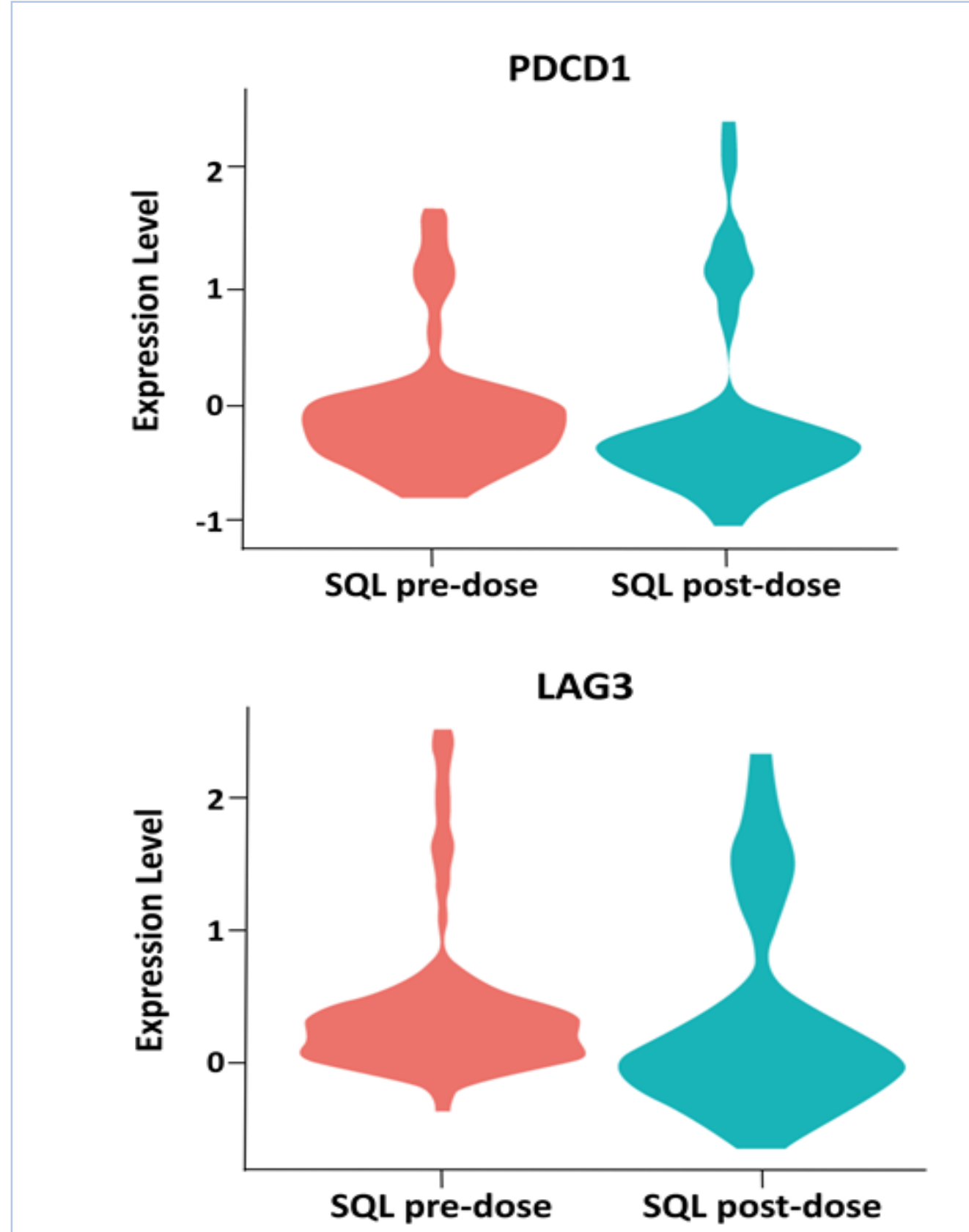
**Figure 3.** In the 4 patients who achieved stable disease (SD), the percentage of normal CD4+ Th1 cells and CD8+ TEMRA cells was increased at the very beginning and decreased in the late phase of soquelitinib treatment. Peripheral blood from SD patients was collected at various timepoints and T cell 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

