Single-Cell RNA Profiling of Myelofibrosis Patients Reveals Pelabresib-Induced Decrease in Megakaryocytic Progenitors and Normalization of CD4+ T cells in Peripheral Blood

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Background

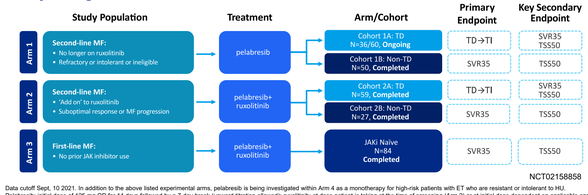
- Myelofibrosis (MF) is a disease characterized by abnormal differentiation of cells from megakaryocytic- and erythroid lineage, high expression of proinflammatory cytokines, bone marrow fibrosis and splenomegaly[1]
- Inflammatory signaling pathways implicated in MF pathogenesis include the constitutive activation of the JAK–STAT pathway.[2] Proteins that contain the bromodomain and extraterminal domain (BET proteins) are also heavily involved in the regulation of processes leading to neoplastic myeloproliferation in MF[3,4]
- Preclinical data indicate that **simultaneous** inhibition of the oncogenic JAK–STAT pathway and BET proteins has synergistic effects in MF[5]
- **Pelabresib (CPI-0610)** is an investigational, oral, small-molecule BET inhibitor with a potential wide therapeutic index
- **Ruxolitinib** is a JAK1/2 inhibitor currently approved for the treatment of intermediate- or high-risk MF[6]
- In the ongoing, open-label, Phase 2 MANIFEST study, the investigational BET inhibitor pelabresib in combination with the JAK inhibitor ruxolitinib is being evaluated in the treatment of MF
  - Arm 1: pelabresib as monotherapy in ruxolitinib-intolerant, ineligible or refractory MF patients
  - Arm 2: pelabresib in combination with ruxolitinib as an 'add-on' in MF pts with suboptimal response to ruxolitinib
  - Arm 3: pelabresib combination therapy with ruxolitinib in JAKi-naïve MF pts

Objective

- Pelabresib as monotherapy and in combination with ruxolitinib has demonstrated improved spleen volume reduction, symptoms and hemoglobin levels[7,8]
- Here, we present data on the effects of pelabresib on hematopoietic stem/progenitor cells and immune cell populations in patients with MF
- The current analysis aimed to characterize treatment-related effects of pelabresib monotherapy and in combination with ruxolitinib on CD34+ and CD34- blood cell populations, on the immune cell populations and on the inflammatory environment with the intent to correlate these changes to the observed clinical outcome

Materials and Methods

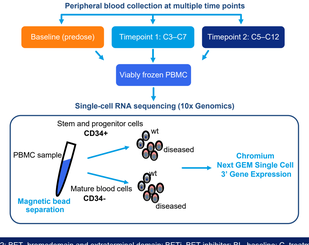
Study Design



Data cutoff Sept. 10 2021. In addition to the above listed experimental arms, pelabresib is being investigated within Arm 4 as a monotherapy for high-risk patients with ET who are resistant or intolerant to HU. Pelabresib: initial dose of 125 mg QD for 14 days followed by a 7-day break (upward titration allowed); ruxolitinib: at dose patient is taking at the time of screening (Arm 2) or at initial dose dependent on applicable approved package insert with upward titration allowed (Arm 3).

Single-Cell RNA Sequencing of Blood Samples From Patients With Myelofibrosis Enrolled in MANIFEST Trial

- Mononuclear cells from peripheral blood were collected at baseline, (ie before dosing) and at two consequent treatment time points, spanning from treatment Cycle 3 to Cycle 12
- After separating CD34+ stem and progenitor cells, and CD34- mature blood cells, cell populations were characterized by single-cell RNA sequencing
- Samples from a random pool of 20 patients were used:
  - Arm 1 (n=5)
  - Arm 2 (n=8)
  - Arm 3 (n=7)
- Patients had different baseline characteristics and clinical responses
- Control: 11 healthy donors (mobilized peripheral blood samples)



Results

Resolution of Individual Cell Populations in CD34+ and CD34- Fractions

- More than 370,000 cells were profiled, representing all major subpopulations of blood cells, as depicted in UMAP projections (Figure 1 and 2)

Figure 1: CD34+ HSPCs (UMAP) 234,904 cells

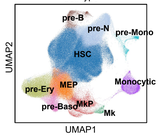
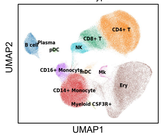


Figure 2: CD34- mature blood cells (UMAP) 135,970 cells

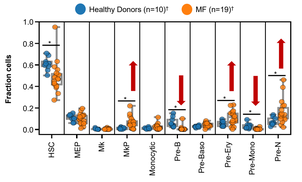


Patient samples from MANIFEST study and HD combined. **Cell numbers represent 77% of sequenced cells that passed the QC**. Not all cell fractions passed the QC for each patient/time point.

CD34+ Stem and Progenitor Cells

- When comparing HSPCs from patients with MF to cells obtained from healthy donors, significantly elevated numbers of megakaryocytic, neutrophilic and erythroid progenitors at baseline were observed (Figure 3)
- Baseline MF patient samples also presented decreased numbers of B-cell and monocytic progenitors compared with healthy donors (Figure 3)
- Treatment with pelabresib +/- ruxolitinib resulted in a decreased proportion of megakaryocytic, neutrophilic and erythroid progenitors and in the enrichment of immature myeloid cells (Figure 4)
- Treatment also resulted in increased numbers of cells from monocytic lineage
- Notably, the treatment-induced reductions of megakaryocytic-erythroid, erythroid and megakaryocytic progenitors were associated with spleen volume reduction at Week 24 (Figure 5)
- Enrichment of myeloid and maturing megakaryocytic cells were also associated with spleen volume reduction at Week 24 (Figure 5)

Figure 3: CD34+ Cells at Baseline in Myelofibrosis vs Healthy Donors



*T-test with Benjamini–Hochberg FDR correlation was used. FDR <10% was considered as significant.
†Patients from MANIFEST study/HD with cell fractions that passed the QC for each patient/time point.

Figure 4: Changes in CD34+ Cells in Myelofibrosis During Treatment (BL vs T2 [C5–C12])

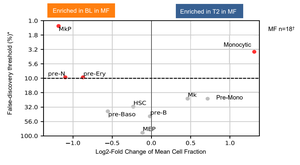
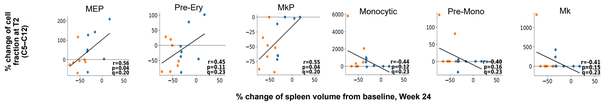


Figure 5: Changes in CD34+ Cell Populations During Treatment Associated With Spleen Volume Reduction at Week 24

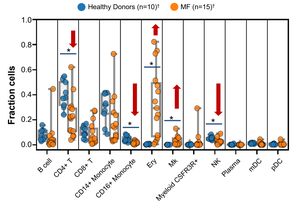


Samples from patients enrolled into MANIFEST study. *p*-Spearman correlation coefficient; p value: probability of zero correlation under normality assumptions; q value: Benjamini–Hochberg FDR-corrected p value.

CD34- Mature Blood Cells

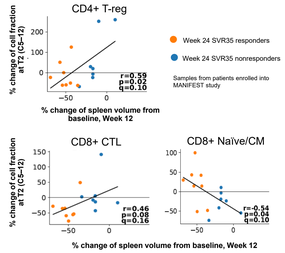
- At baseline, enriched fractions of nucleated erythroid cells and megakaryocytes were observed in patients with MF (Figure 6)
- Baseline samples also presented reduced numbers of NK, CD16+ monocytes and CD4+ T cells (Figure 6)
- To further evaluate the treatment-related effects on T cells, we subclustered sequenced T cells and defined all major subpopulations in the T-cell cluster
- Treatment-induced effects on T-cell subpopulations were observed (Figure 7):
  - Reduction of CD8+ CTLs was associated with spleen volume reduction at Week 12
  - Enrichment of immature CD8+ naïve/CM and loss of T-regulatory cells was associated with spleen volume reduction at Week 12 and also at Week 24

Figure 6: CD34- Mature Cells Comparison at Baseline vs Healthy Donors



*T-test with Benjamini–Hochberg FDR correlation was used. FDR <10% was considered as significant.
†Patients from MANIFEST study with paired BL-T2 (C5–12) samples that passed the QC for each patient/time point.

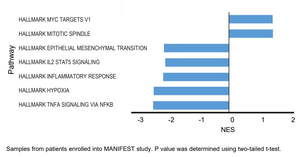
Figure 7: Treatment-Related Effects on T Cells and Spleen Volume



Pelabresib +/- Ruxolitinib May Downregulate the NF-κB-activated Proinflammatory Signaling in Monocytes

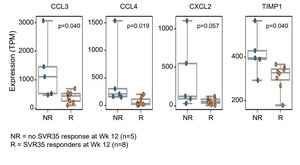
- Monocytic cells are known to be key drivers in the overproduction of inflammatory cytokines in MF via the NF-κB pathway which is also regulated by BET proteins
- In monocytes from patients with SVR35 response, the TNF-alpha signaling via NF-κB was significantly decreased (Figure 8) along with the downregulated expression of inflammatory and profibrotic genes (Figure 9) as compared with nonresponders, indicating potential targeting of the inflammatory pathophysiology typical of MF by pelabresib monotherapy and in combination with ruxolitinib
- Further pathway enrichment analyses in other key cell types are ongoing

Figure 8: GSEA Pathway Enrichment Analysis in Monocytes From Patients With SVR35R vs NR



Samples from patients enrolled into MANIFEST study. P value was determined using two-tailed t-test.

Figure 9: Decreased Levels of Proinflammatory Cytokines and Profibrotic Genes in CD14+ Monocytes of SVR35 Responders



NR = no SVR35 response at Wk 12 (n=5)
R = SVR35 responders at Wk 12 (n=8)

Conclusions

Preliminary results from single-cell profiling of peripheral blood from a subset of patients with MF from MANIFEST study supports immuno-modulatory activity of pelabresib +/- ruxolitinib treatment by:

- Improving the myeloid–lymphoid imbalance in both JAKi-naïve and r/r patients
- Decreasing NF-κB-induced proinflammatory and profibrotic signaling in monocytic cells

The observed changes:

- Were associated with an improved clinical outcome (SVR35) as observed in the MANIFEST study
- Support similar improvements shown in bone marrow and cytokine expression pattern
- Show in the MANIFEST study a potential disease-modifying effect of pelabresib, warranting further investigation

MANIFEST-2, a Phase 3, randomized, double-blind trial of pelabresib + ruxolitinib vs placebo + ruxolitinib in a JAKi-naïve MF patient population, has been initiated and is open for enrollment (NCT04603495)

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Data cut-off 10 Sep 21

Baso, basophil; BCL-2, B-cell lymphoma 2; BET, bromodomain and extraterminal domain; BETi, BET inhibitor; BL, baseline; C, treatment cycle; CCL, chemokine ligand; CD, cluster of differentiation; CM, central memory; c-Myc, cellular Myc; CSF3R, colony-stimulating factor 3 receptor; CTL, cytotoxic T lymphocytes; CXCL, chemokine (C-X-C motif) ligand; ery, erythroid; ET, essential thrombocythemia; FDR, false discovery rate; GEM, gel beads in emulsion; GSEA, Gene Set Enrichment Analysis; HD, healthy donors; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplant; HSPC, hematopoietic stem and progenitor cell; HU, hydroxyurea; JAK, Janus kinase; JAKi, JAK inhibitor; mDC, myeloid dendritic cell; MEP, megakaryocyte–erythroid progenitor; MF, myelofibrosis; Mk, megakaryocyte; MkP, megakaryocyte progenitor; mPB, mobilized peripheral blood; NES, normalized enrichment score, corrected for multiple testing; NF-κB, nuclear factor kappa B; NK, natural killer; NR, not reached; PBMC, peripheral blood mononuclear cells; pDC, plasmacytoid dendritic cell; QC, quality control; QD, once daily; R, reached; RNA, ribonucleic acid; r/r, relapsed/refractory; STAT, signal transducer and activator of transcription; SVR35, ≥35% spleen volume reduction from baseline; TD, transfusion dependent; TI, transfusion independent; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; TPM, transcript per million; T-reg, regulatory T cell; TSS50, ≥50% total symptom score reduction from baseline; UMAP, Uniform Manifold Approximation and Projection for Dimension Reduction; wk, week; wt, wild type.

Pelabresib (CPI-0610) is an investigational new drug and has not been approved by any regulatory authority