EHA EUROPEAN HEMATOLOGY ASSOCIATION
EHA2022 HYBRID VIRTUAL

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

Oksana Zavidij,[1] Nicholas J Haradhvala,[2] Rosana Meyer,[1] Jike Cui,[1] Srdan Verstovsek,[3] Stephen Oh,[4] Adam Mead,[5] Pietro Taverna[1]

[1]Constellation Pharmaceuticals, Inc. a MorphoSys Company, Boston, MA, USA; [2]Harvard Graduate Program in Biophysics, Harvard University, Boston, MA, USA; [3]Leukemia Department, University of Texas MD Anderson Cancer Center, Houston, TX, USA; [4]Hematology Division, Washington University, St Louis, MO, USA; [5]NIHR Biomedical Research Centre, University of Oxford, Oxford, UK.

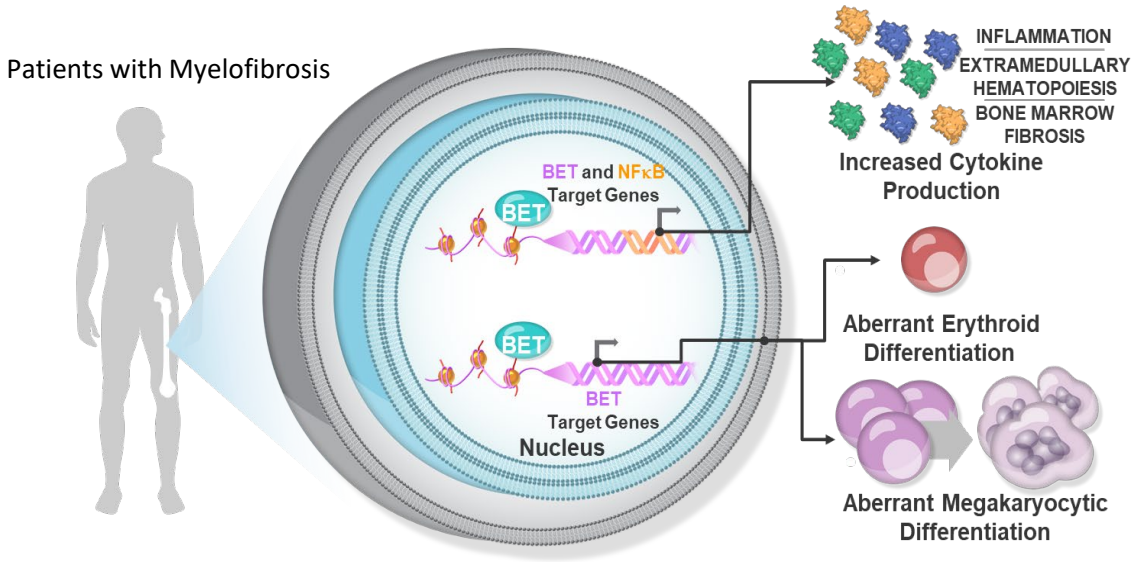
Disclaimer/Disclosure

- Pelabresib (CPI-0610) is an investigational compound that has not yet been approved by any regulatory authorities. The safety and efficacy of pelabresib is currently being evaluated in clinical trials for the treatment of myeloproliferative neoplasms

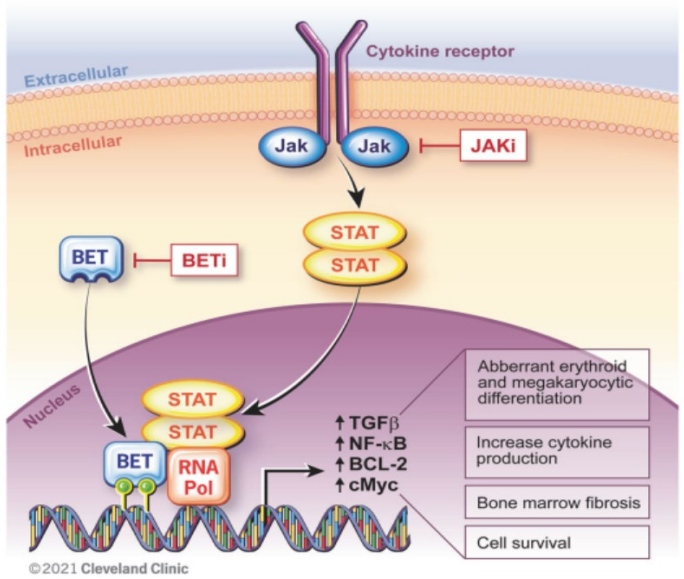
- I am an employee of Constellation Pharmaceuticals, Inc. a MorphoSys company, and I own equity in the company

A potential novel therapeutic approach for myelofibrosis:

Simultaneous inhibition of BET and JAK to address heterogenous disease pathology



Albrecht, et al. *J Med Chem* 2016

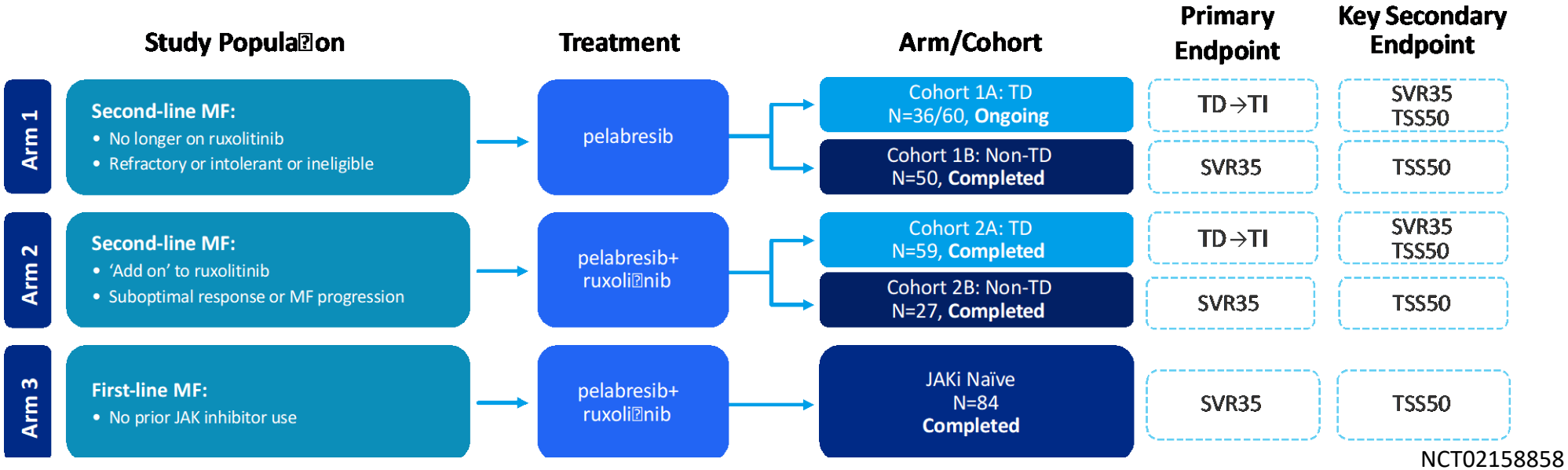


Reprinted with permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Leukemia, Paradigm shift: combination BET and JAK inhibition in myelofibrosis, John Mascarenhas, et al. Copyright ©2021.

- **Pelabresib (CPI-0610)**: an investigational, oral, small-molecule BET inhibitor with a potential wide therapeutic index
- **Ruxolitinib**: a JAK1/2 inhibitor currently approved for the treatment of intermediate or high-risk myelofibrosis
- Myelofibrosis is a heterogenous pathology, where the addition of **pelabresib** may improve the efficacy currently achieved by **ruxolitinib**

BCL-2, B-cell lymphoma 2; BET, bromodomain and extraterminal domain; BETi, BET inhibitor; c-Myc, cellular Myc; HSCT, hematopoietic stem cell transplant; JAK, Janus kinase; JAKi, JAK inhibitor; NF-κB, nuclear factor kappa B; RNA pol, ribonucleic acid polymerase; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor β.

MANIFEST is an ongoing, global, open-label Phase 2 study investigating pelabresib in myelofibrosis

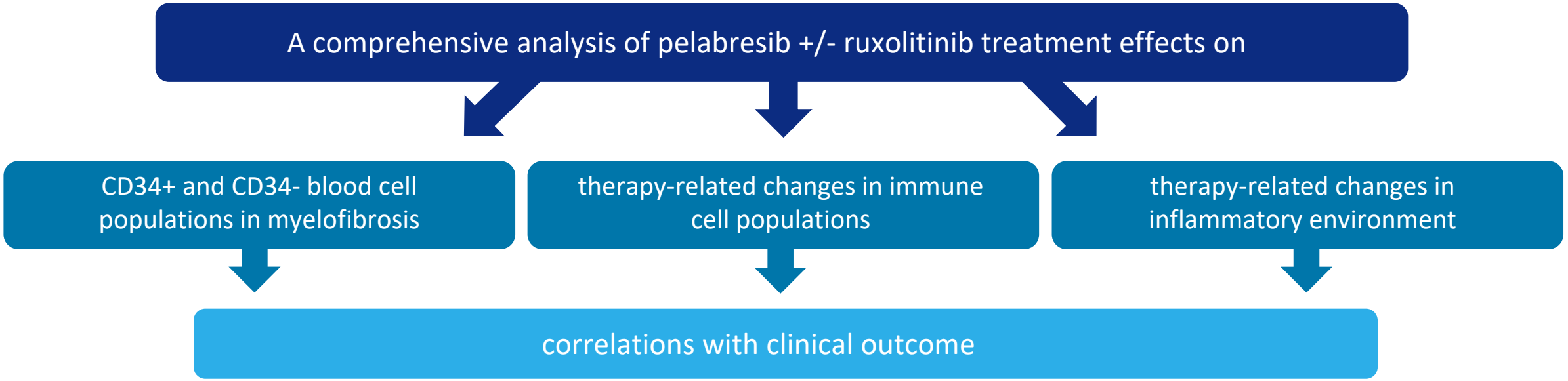


NCT02158858

- **Pelabresib monotherapy** demonstrated clinical activity through the achievement of splenic responses and symptoms improvement (Kremyanskaya, et al. ASH 2021)
- **Combination of pelabresib and ruxolitinib** in JAKi-naïve patients was generally well tolerated, and preliminary results showed improvements in splenomegaly and symptom burden, with associated biomarker results suggesting potential disease-modifying activity (Mascarenhas, et al. EHA 2022, S198)

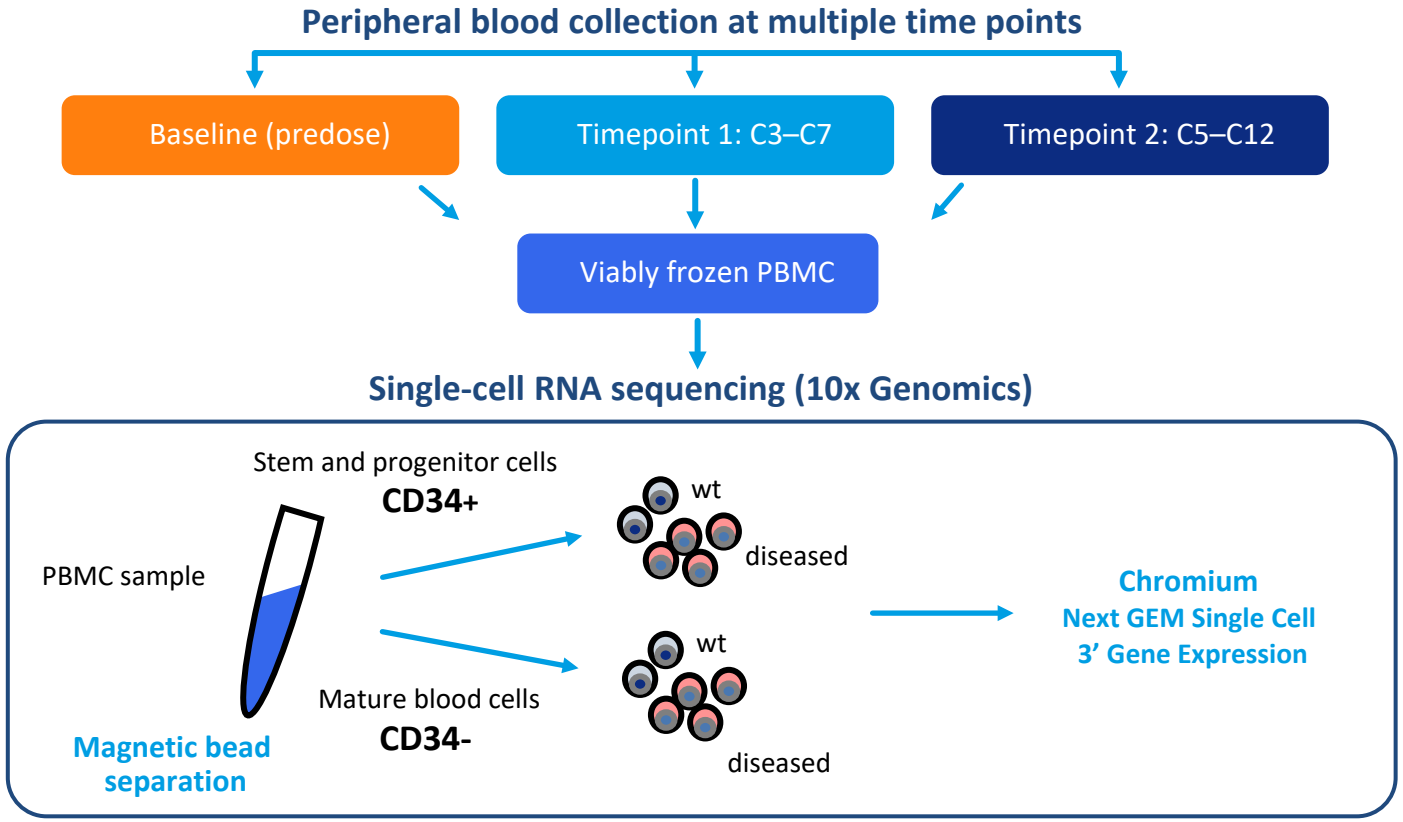
Data cut-off 10 Sep 21. In addition to the above listed experimental arms, pelabresib is being investigated within Arm 4 as a monotherapy for high-risk patients with ET who are resistant or intolerant to HU.
Pelabresib: initial dose of 125 mg QD for 14 days followed by a 7-day break (upward titration 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).
ET, essential thrombocythemia; HU, hydroxyurea; JAK, Janus kinase; JAKi, JAK inhibitor; MF, myelofibrosis; QD, once daily; SVR35, spleen volume reduction by 35%; TD, transfusion dependent; TI, transfusion independent; TSS50, total symptom score reduction by 50%.

Single-cell transcriptome sequencing in patients with myelofibrosis



CD, cluster of differentiation.

Single-cell RNA sequencing of blood samples from patients with myelofibrosis enrolled in MANIFEST trial



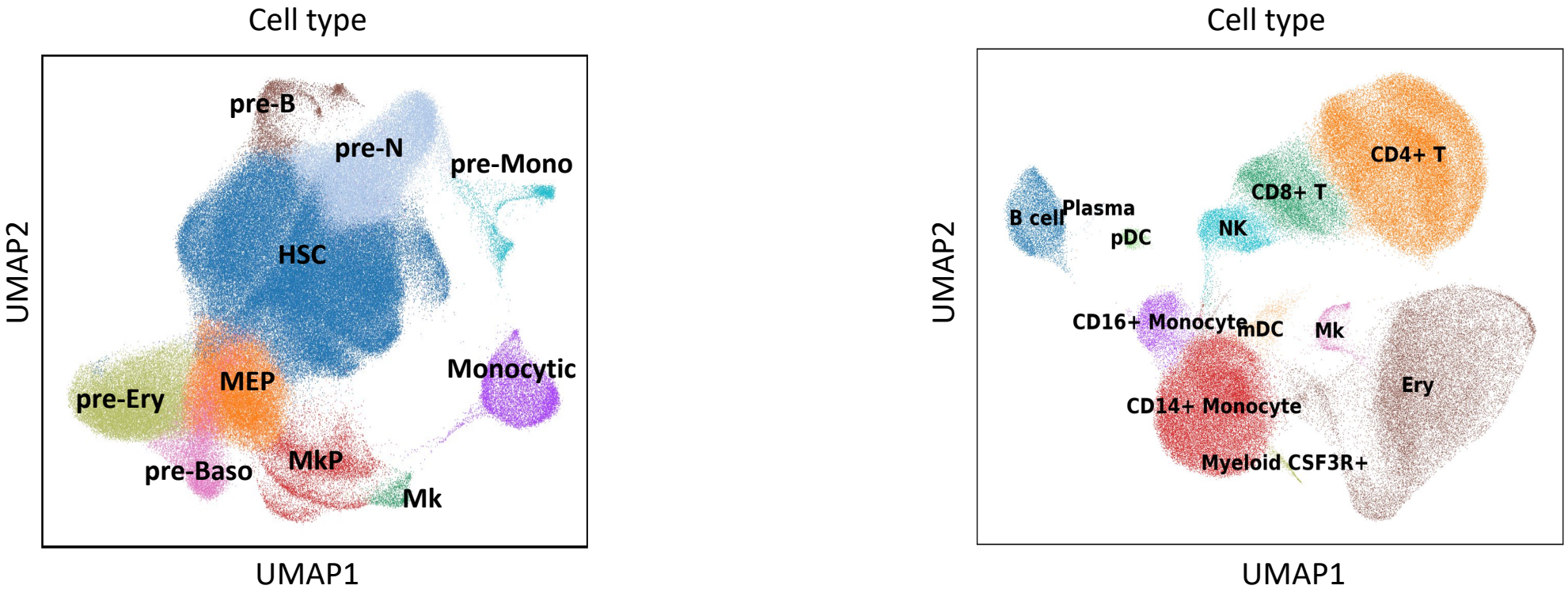
- Random pool of 20 patients:
  - Arm 1 (n=5)
  - Arm 2 (n=8)
  - Arm 3 (n=7)
- Control: 11 healthy donors (mPB)

C, treatment cycle; CD, cluster of differentiation; GEM, gel beads in emulsion; mPB, mobilized peripheral blood; PBMC, peripheral blood mononuclear cells; RNA, ribonucleic acid; wt, wild type.

Resolution of individual cell populations in CD34+ and CD34- fractions

CD34+ HSCP cells **234,904 cells**

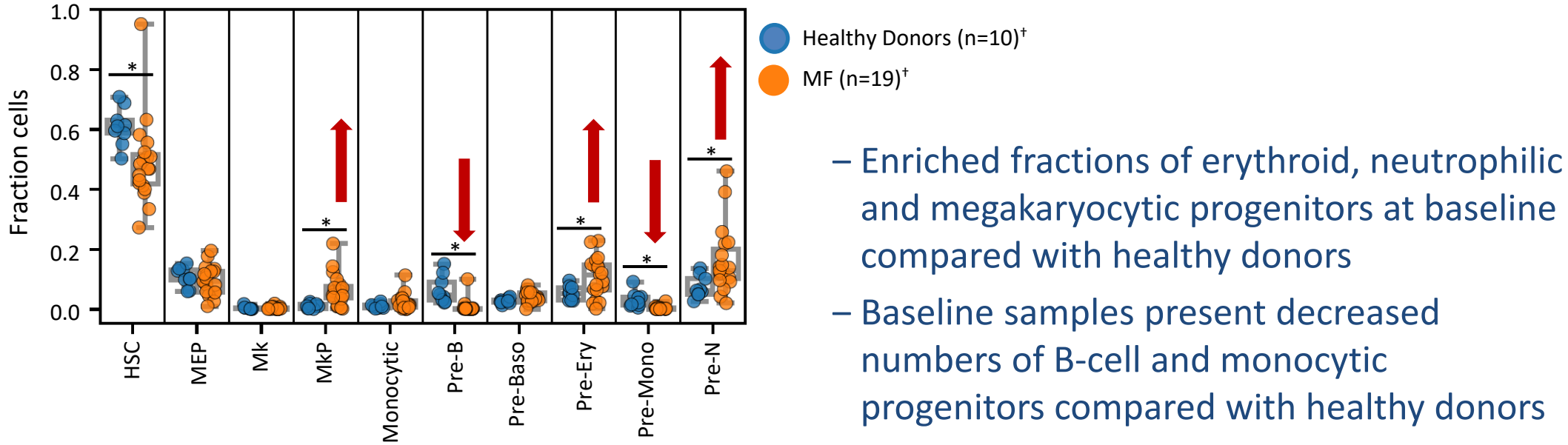
CD34- mature blood cells **135,970 cells**



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.
Baso, basophil; CD, cluster of differentiation; CSF3R, colony-stimulating factor 3 receptor; Ery, erythroid; HD, healthy donors; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cells; mDC, myeloid dendritic cell; MEP, megakaryocyte–erythroid progenitor; Mk, megakaryocyte; MkP, megakaryocyte progenitor; NK, natural killer; pDC, plasmacytoid dendritic cell; QC, quality control; UMAP, Uniform Manifold Approximation and Projection for Dimension Reduction.

Significantly elevated numbers of megakaryocytic, neutrophilic and erythroid progenitors at baseline in patients with myelofibrosis

CD34+ Cells at Baseline in Myelofibrosis vs Healthy Donors

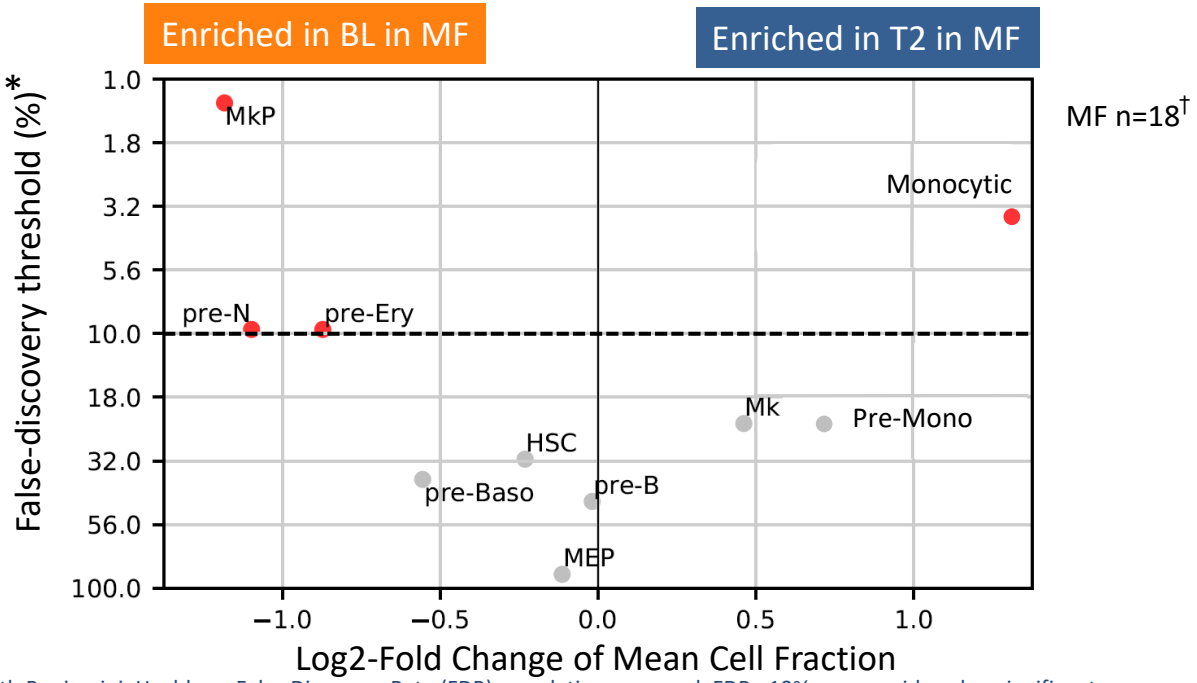


- Enriched fractions of erythroid, neutrophilic and megakaryocytic progenitors at baseline compared with healthy donors
- Baseline samples present decreased numbers of B-cell and monocytic progenitors compared with healthy donors

*T-test with Benjamini–Hochberg False Discovery Rate (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.
Baso, basophil; CD, cluster of differentiation; Ery, erythroid; HD, healthy donors; HSC, hematopoietic stem cell; MEP, megakaryocyte–erythroid progenitor; MF, myelofibrosis; Mk, megakaryocyte; MkP, megakaryocyte progenitor; QC, quality control.

Decreased numbers of megakaryocytic, neutrophilic and erythroid progenitors after pelabresib +/-ruxolitinib

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

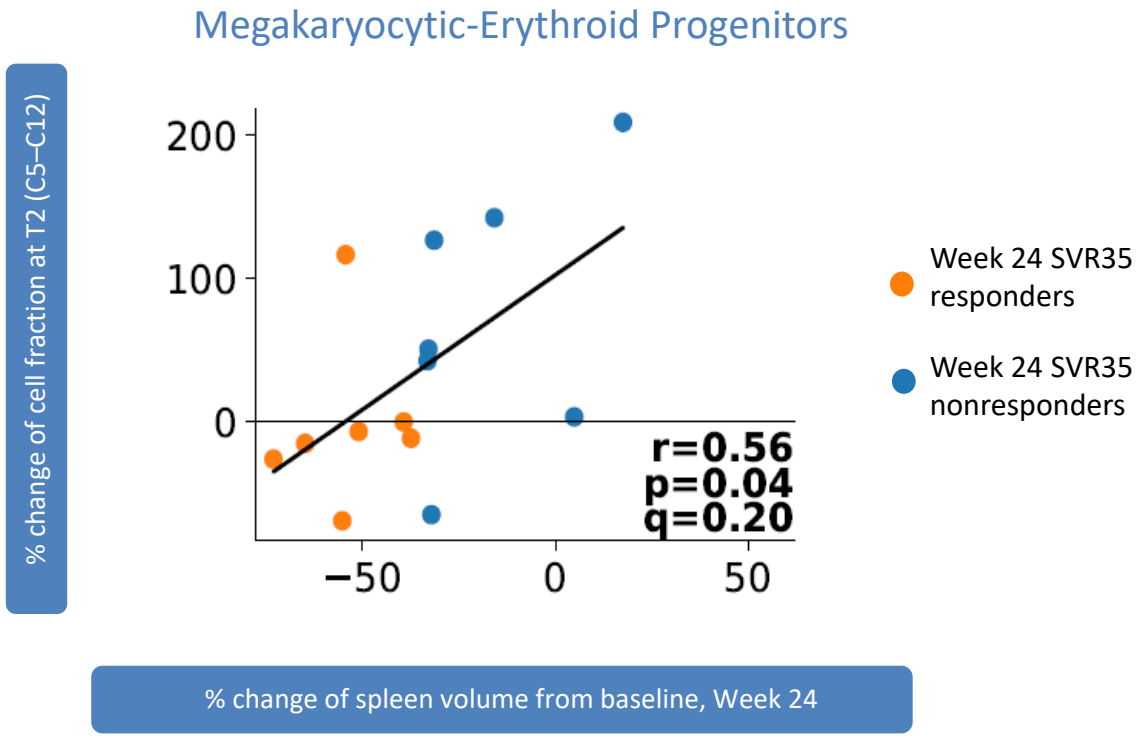


MF n=18†

– Treatment with pelabresib +/- ruxolitinib showed decreased proportion of megakaryocytic, neutrophilic and erythroid progenitors and enrichment of immature myeloid cells

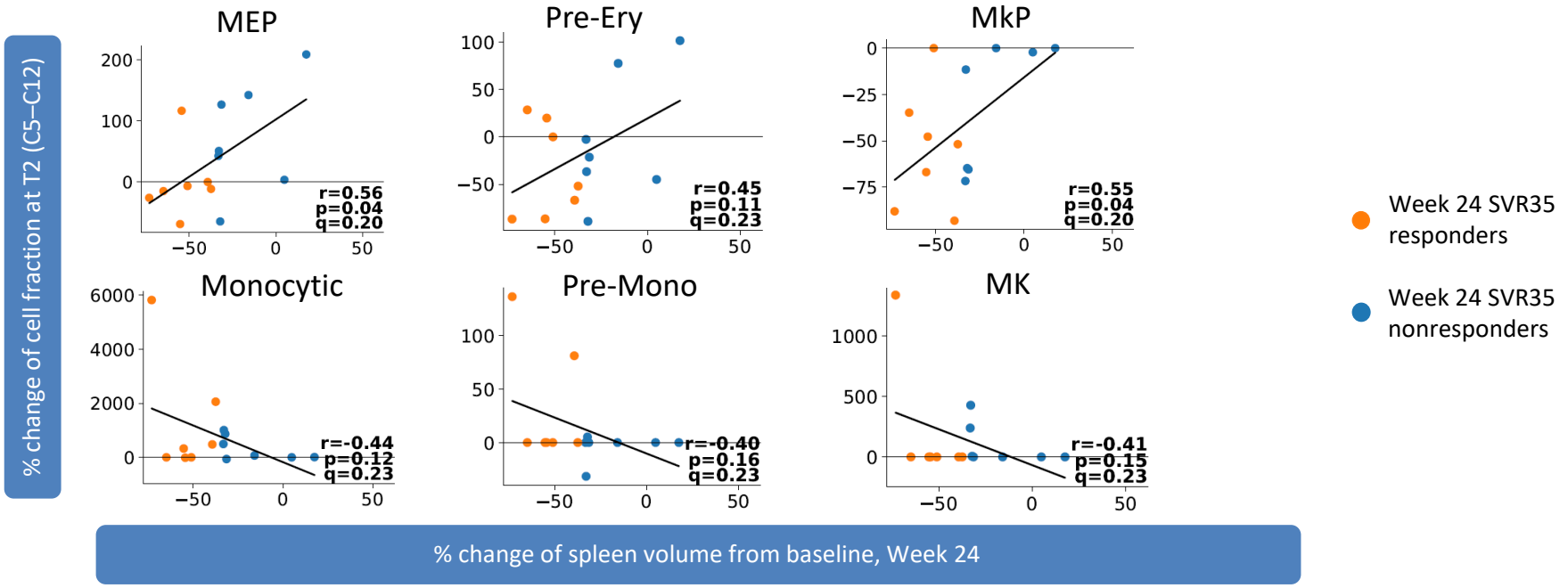
*T-test with Benjamini–Hochberg False Discovery Rate (FDR) correlation was used. FDR <10% was considered as significant.
†Patients from MANIFEST study/HD with cell fractions that passed the QC for each patient/time point.
Baso, basophil; BL, baseline; C, treatment cycle; CD, cluster of differentiation; Ery, erythroid; HD, healthy donors; HSC, hematopoietic stem cell; MEP, megakaryocyte–erythroid progenitor; MF, myelofibrosis; Mk, megakaryocyte; MkP, megakaryocyte progenitor; QC, quality control.

Treatment-induced changes in megakaryocytic-erythroid progenitors associated with spleen volume reduction at Week 24



Samples from patients enrolled into MANIFEST study. ρ-Spearman correlation coefficient; p value: probability of zero correlation under normality assumptions; q value: Benjamini–Hochberg FDR-corrected p value. C, treatment cycle; FDR, false discovery rate; SVR35, spleen volume reduction of 35%.

Changes in CD34+ cell populations during treatment associated with spleen volume reduction at Week 24

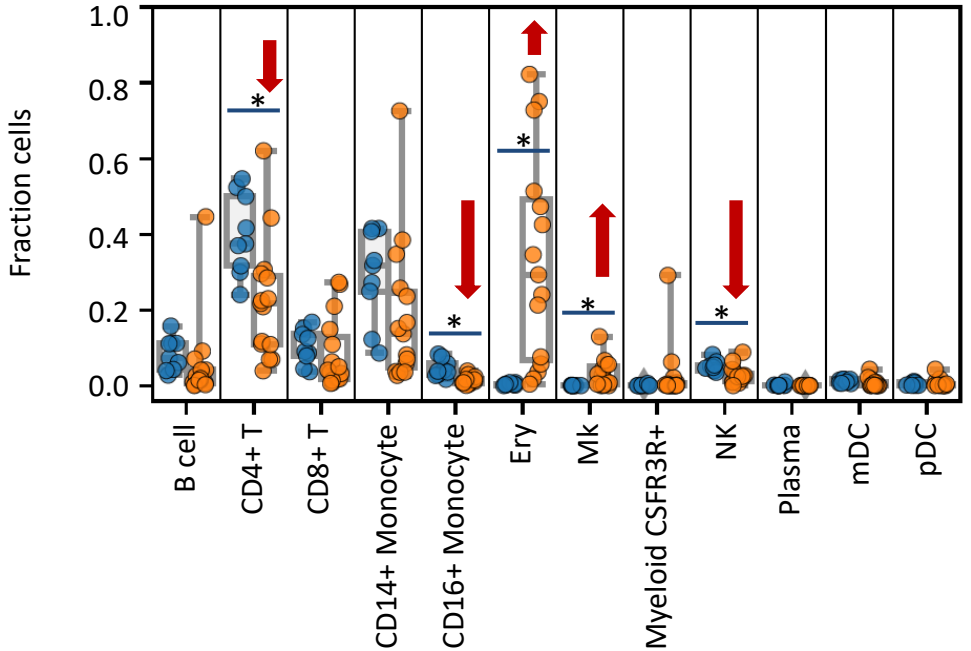


- Reduction of megakaryocytic-erythroid, erythroid and megakaryocytic progenitors associated with spleen volume reduction at Week 24
- Enrichment of myeloid and maturing megakaryocytic cells associated with spleen volume reduction at Week 24

Samples from patients enrolled into MANIFEST study. ρ-Spearman correlation coefficient; p value: probability of zero correlation under normality assumptions; q value: Benjamini–Hochberg FDR-corrected p value.
Baso, basophil; C, treatment cycle; CD, cluster of differentiation; Ery, erythroid; FDR, false discovery rate; HSC, hematopoietic stem cell; MEP, megakaryocyte–erythroid progenitor; Mk, megakaryocyte; MkP, megakaryocyte progenitor; SVR35, spleen volume reduction of 35%.

Significantly reduced numbers of CD4+ T cells and elevated number of megakaryocytic and erythroid cells at baseline

CD34- Mature Cells Comparison at Baseline vs Healthy Donors



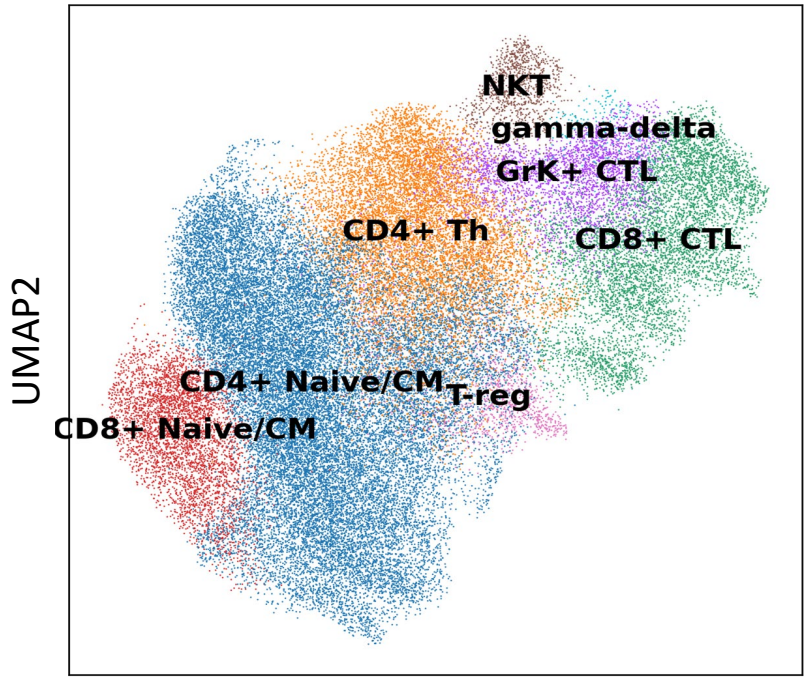
Healthy Donors (n=10)†
MF (n=15)†

- Enriched fractions of nucleated erythroid cells and megakaryocytes
- Baseline samples present reduced numbers of NK, CD16+ monocytes and CD4+ T cells

*T-test with Benjamini–Hochberg False Discovery Rate (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.
BL, baseline; C, treatment cycle; CD, cluster of differentiation; CSF3R, colony-stimulating factor 3 receptor; Ery, erythroid; FDR, false discovery rate; mDC, myeloid dendritic cell; MEP, megakaryocyte–erythroid progenitor; MF, myelofibrosis; Mk, megakaryocyte; NK, natural killer; pDC, plasmacytoid dendritic cell; QC, quality control; SVR35, spleen volume reduction of 35%.

Definition of major T-cell subpopulations

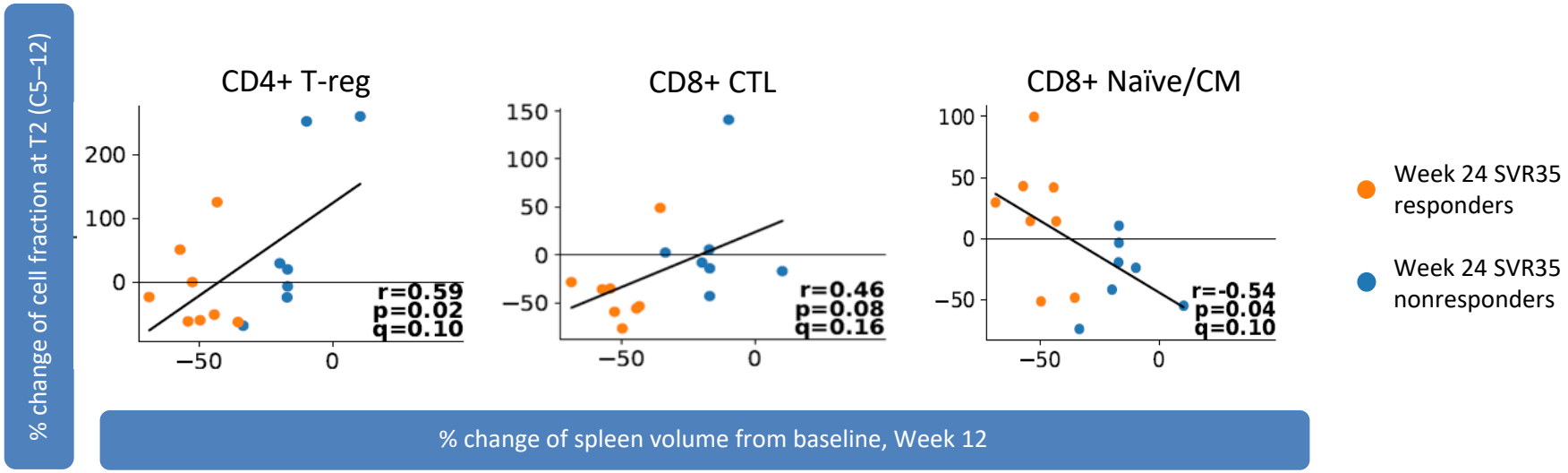
T-cell distribution **54,245 cells**



Cells from patients from MANIFEST study and HD combined.

CD, cluster of differentiation; CM, central memory; CTL, cytotoxic T lymphocytes; Ery, erythroid; GrK, granzyme K; HD, healthy donors; NKT, natural killer T cell; Th, T helper; T-reg, regulatory T cell; UMAP, Uniform Manifold Approximation and Projection for Dimension Reduction.

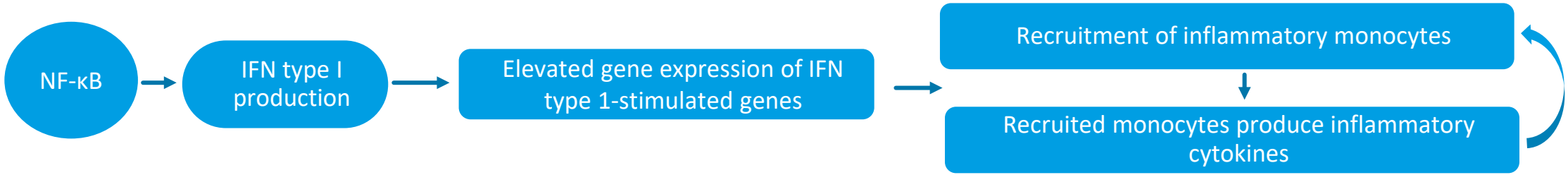
Changes in T-cell subpopulations associated with spleen volume reduction at Week 12



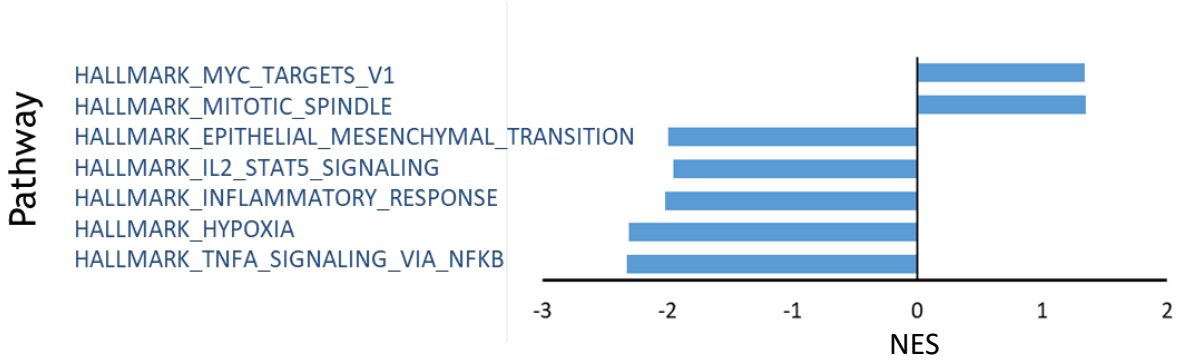
- Reduction of CD8+ CTLs associated with spleen volume reduction at Week 12
- Enrichment of immature CD8+ naïve/CM and loss of T regulatory cells are associated with spleen volume reduction at Week 12 and also at Week 24

Samples from patients enrolled into MANIFEST study.
C, treatment cycle; CD, cluster of differentiation; CM, central memory; CTL, cytotoxic T lymphocytes; SVR35, spleen volume reduction of 35%; T-reg, regulatory T cell.

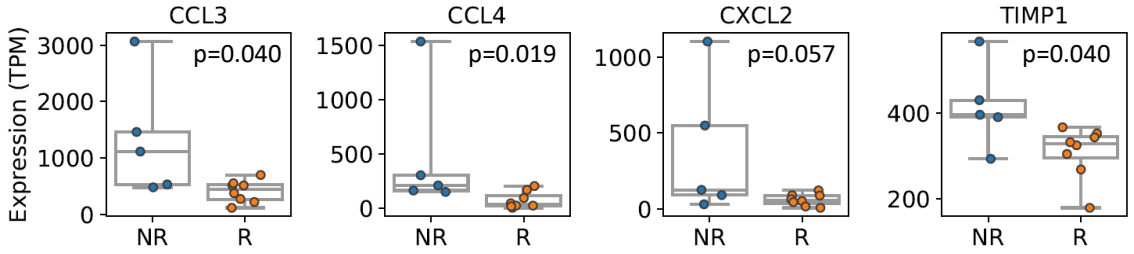
Pelabresib +/- ruxolitinib may downregulate the NF-κB-activated proinflammatory signaling in monocytes



GSEA pathway enrichment analysis in monocytes from patients with SVR35R vs NR



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)

Samples from patients enrolled into MANIFEST study. Subramanian A, et al. *PNAS* 2005; Mootha VK, et al. *Nature Genetics* 2003.

P value was determined using two-tailed t-test.

CCL, chemokine ligand; CD, cluster of differentiation; CXCL, chemokine (C-X-C motif) ligand; GSEA, Gene Set Enrichment Analysis, IFI, interferon-induced protein; IFN, interferon; IRF, interferon regulatory factor; NES, normalized enrichment score, corrected for multiple testing; NF-κB, nuclear factor kappa B; NR, not reached; R, reached; SVR35, spleen volume reduction of 35%; TIMP, tissue inhibitor of metalloproteinases; TPM, transcript per million; wk, week.

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 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

JAKi, Janus kinase inhibitor; MF, myelofibrosis; NF-κB, nuclear factor kappa B; r/r, relapsed/refractory; SVR35, spleen volume reduction of 35%; TSS50, total symptom score reduction of 50%.

Acknowledgements

– Patients, their families and clinical investigators from MANIFEST study
– Colleagues from Constellation and MorphoSys

MANIFEST 2

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