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

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Context: Abnormal differentiation of the megakaryocytic lineage and overproduction of proinflammatory cytokines, resulting in bone marrow fibrosis, anemia, symptoms, extramedullary hematopoiesis, and often hepatosplenomegaly, are key characteristics of myelofibrosis. Bromodomain and extraterminal domain (BET) proteins play a significant role in the regulation of neoplastic myeloproliferation and proinflammatory cytokine production. Pelabresib (CPI-0610) is an investigational, oral, small-molecule BET inhibitor.

Objective: Characterize cellular composition and transcriptional alterations in peripheral blood (PB) obtained from myelofibrosis patients enrolled in three arms (Arm 1: pelabresib monotherapy; Arm 2: pelabresib 'add-on' to ruxolitinib in patients with suboptimal ruxolitinib response; Arm 3: pelabresib with ruxolitinib in JAKi-naïve patients) of the ongoing MANIFEST Phase 2 study (NCT02158858).

Methods: We performed single-cell RNA sequencing on 234,904 CD34+ HSPCs and 135,970 CD34− mature PB cells. Baseline and on-treatment samples were obtained from a random pool of 20 patients (Arm 1: n=5; Arm 2: n=8; Arm 3: n=7). Mobilized PB cells from healthy donors (HD) were used as controls (n=11).

Results: Analysis of CD34+ HSPCs at baseline demonstrated increased numbers of megakaryocytic, neutrophilic, and erythroid progenitors in myelofibrosis patients compared with HDs and a significant reduction of myeloid and B-cell lineage progenitors. Pelabresib as monotherapy and combined with ruxolitinib led to significant reduction of megakaryocytic, neutrophilic, and erythroid progenitors compared with baseline. Analysis of CD34− cells from myelofibrosis patients identified a significantly lower proportion of CD4+ T cells and increased numbers of erythroid cells at baseline versus HD. Individual patients exhibited reduction in natural killer cells and CD16+ monocytes as well as elevated megakaryocytic lineage cells. Pelabresib as monotherapy and combined with ruxolitinib increased the proportion of CD4+ T cells, and, importantly, reduced megakaryocytic lineage cells in both treatment-naïve and ruxolitinib-relapsed/refractory patients. In myelofibrosis patients at baseline, larger spleen volume was observed in patients with lower numbers of CD4+ T cells and increased numbers of megakaryocytic and myeloid CSF3R+ cells.

Conclusions: Single-cell profiling of a subset of myelofibrosis patients in the MANIFEST study suggests that pelabresib alone and in combination with ruxolitinib induces improvement of the myeloid–lymphoid imbalance. This potential disease-modifying effect warrants further investigation.

Keywords: MPN, MANIFEST, pelabresib, myelofibrosis, ruxolitinib, single-cell RNA sequencing, Phase II