Sequential treatment of CD34+ cells from patients with primary myelofibrosis with chromatin modifying agents eliminate JAK2V617F positive NOD/SCID marrow repopulating cells

Running title: Treatment of PMF with chromatin modifying agents

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Abstract

Since primary myelofibrosis (PMF) originates at the level of the pluripotent hematopoietic stem cell (HSC), we examined the effects of various therapeutic agents on the in vitro and in vivo behavior of PMF CD34+ cells. Treatment of PMF CD34+ cells with chromatin modifying agents (CMA) but not hydroxyurea, JAK2 inhibitors or low doses of IFN-α led to the generation of greater numbers of CD34+CXCR4+ cells, which were capable of migrating in response to CXCL12 and resulted in a reduction in the proportion of HPCs that were JAK2V617F+. Furthermore, sequential treatment of PMF CD34+ cells but not normal CD34+ cells with decitabine [5-aza-2'-deoxycytidine (5azaD)], followed by suberoylanilide hydroxamic acid (SAHA) (5azaD/SAHA), or trichostatin A (5azaD/TSA) resulted in a higher degree of apoptosis. Two to six months after the transplantation of CMA treated JAK2V617F+ PMF CD34+ cells into NOD/SCID/IL2Rγnull mice, the percentage of JAK2V617F/JAK2total in hCD45+ marrow cells was dramatically reduced. These findings suggest that both PMF HPCs, short-term and long-term SCID repopulating cells (SRC) are JAK2V617F+ and that JAK2V617F+ HPC and SRC can be eliminated by sequential treatment with CMAs. Sequential treatment with CMAs, therefore, represents a possible effective means of treating PMF at the level of the malignant SRC.
Key words: CD34+ cells, PMF, HSC, SRC, chromatin modifying agents.
Introduction

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN), which is thought to originate at the level of a pluripotent hematopoietic stem cell (HSC).\textsuperscript{1-5} A gain of function mutation in the JH2 domain of the Janus kinase 2 (JAK2V617F) has been identified in the MPNs, which is present in the granulocytes of approximately 95\% of patients with polycythemia vera and 50\% of patients with either PMF or essential thrombocythemia. In about 10\% of patients with JAK2V617F negative PMF, an additional somatic mutation of the thrombopoietin receptor gene MPL has also been identified.\textsuperscript{6} Furthermore, malignant clones harboring additional genetic abnormalities including the TET oncogene family member 2 (TET2), the additional sex combs like gene and the gene for isocitrate dehydrogenase 1 as well as characteristic cytogenetic abnormalities have been observed in PMF indicating that multiple genetic events are likely responsible for the origins of this MPN.\textsuperscript{7}

Epigenetic modifications leading to the dysregulation of critical genes that contribute to cell proliferation, differentiation, cell death and trafficking have also been thought to play a role in the origins of PMF.\textsuperscript{8-9} Mutations in TET2, for instance, might contribute to the origins of PMF by altering chromatin structure. TET1 affects the conversion of 5-methylcytosine to 5-hydroxymethylcytosine and therefore influences epigenetic
regulation of transcription.\textsuperscript{10} Previously we had reported that the constitutive mobilization of CD34\textsuperscript{+} cells in PMF could be accounted by in part by the reduced expression of the chemokine receptor CXCR4 by PMF CD34\textsuperscript{+} cells which has been attributed to hypermethylation of its promoter.\textsuperscript{11} In addition, we have reported that the sequential treatment of PMF CD34\textsuperscript{+} cells with a DNA methyltransferase (DNMT) inhibitor (DNMTI), 5-aza-2'-deoxycytidine (5azaD), followed by an histone deacetylase (HDAC) inhibitor (HDACI), trichostatin A (TSA), resulted in an upregulation of CXCR4 expression by PMF CD34\textsuperscript{+} cells leading to correction of the abnormal cellular trafficking characteristic of PMF as well as a reduction of the burden of malignant HPC.\textsuperscript{12, 13} These preclinical studies illustrate the profound effects of chromatin modifying agents (CMA) on PMF HPC and led us to further evaluate these strategies this time using drugs that are currently approved for the treatment of other hematological malignancies and to determine if they affect malignant stem cells.

We hypothesize that curative drug therapies for PMF would ideally eliminate or at least reduce the burden of PMF HSCs/HPCs, allowing their normal counterparts to predominant. Currently, the standard surrogate assay for human HSCs assesses the ability of a putative HSC population to establish hematopoiesis in immunodeficient mice. In this report, we provide evidence that sequential treatment with 5azaD followed
by the HDACI, suberoylanilide hydroxamic acid (SAHA), not only affects PMF HPC but also stem cells. Sequential treatment with CMA, therefore, represents a promising therapeutic strategy for the treatment of PMF patients.

Materials and Methods

Description of patients and cell isolation procedures

Peripheral blood (PB) was collected from 32 patients who fulfilled the WHO diagnostic criteria for PMF. All patients signed an informed consent approved by the Institutional Review Board of the Mount Sinai School of Medicine (MSSM) in accordance with the Declaration of Helsinki.

Granulocytes were isolated by previously described techniques. CD34+ cells were selected from PB low-density mononuclear cells from PMF patients and cord blood (CB) collections provided by the New York Blood Center using a CD34+ cell selection kit (Stem Cell Technologies, Vancouver, Canada). The purity of the CD34+ cell population was analyzed using a FACS Canto Flow Cytometer (Becton Dickinson, San Jose, CA, USA). CD34+ cell populations with a purity ≥ 90% were used in all experiments. Due to limitation of the number of cells available from the individual patient, cells from subsets of patients were used for particular experiments as outlined in supplemental Table1.
JAK2V617F and MplW515L mutational analyses

JAK2V617F and MplW515L were detected by analyzing the PB granulocytes of patients with PMF by using a real-time quantitative PCR assay using an allelic discrimination method as described previously. The JAK2V617F status and allele burden of each patient studied is provided in Supplemental Table 1. Among the 32 PMF subjects, 18 patients were JAK2V617F positive while none possessed MplW515L. The JAK2V617F negative patients were further analyzed for marker chromosomal abnormalities using fluorescence in situ hybridization (FISH). Patient 32 was characterized by a marker chromosomal abnormality [Del (13)(q13)] that could be detected by FISH.

Treatment of CD34+ cells with various therapeutic agents

PMF CD34+ cells (1×10^5/ml) were cultured in Iscove's modified Dulbecco's medium (IMDM; Lonza, Walkersville, MD, USA) containing 30% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT) supplemented with 100 ng/ml stem cell factor (SCF), 100 ng/ml FLT-3 ligand (FL), 100 ng/ml thrombopoietin (TPO), and 50 ng/ml interleukin-3 (IL-3) (Amgen, Thousand Oaks, CA, USA) and incubated in a humidified incubator maintained at 37°C with 5% CO2. After an initial 16 hours of incubation, cells were either exposed to 5azaD (1uM, Pharmachemie B.V., GA Haarlem, Nederland) for
48 hours, or SAHA (1μM, gift of Merck) for 6 days, or one of two JAK2 inhibitors, JAK inhibitor 1 (100nM, Calbiochem, San Diego, CA, USA) or AZ1480 (100nM, gift of AstraZeneca), hydroxyurea (50μM, Sigma, St. Louis, MO, USA) or pegylated interferon-α 2a (Peg-IFN-α 2a, 125U, Roche, Nutley, NJ) alone for 3 days. In addition, CD34+ cells were exposed to 5azaD at a concentration of 1μM for 48 hours, and then washed and distributed to new culture plates containing SCF, FL, and TPO with either SAHA, for an additional 6-day culture period, or TSA (16.5nM, Sigma) for a 7-day culture period. 12, 13 In addition, cultures containing cytokines alone were performed in parallel. In some experiments, the cultured PMF CD34+ cells were re-isolated as described above after a period of culture and assayed for their ability to migrate in vitro in response to CXCL12 or to form hematopoietic colonies in semisolid media.

In order to determine if CMA were able to affect normal hematopoietic stem/progenitor cells, CB CD34+ cells (1×10^5/ml) were also cultured and treated with CMA in an identical fashion.

**Flow cytometric analysis of CD34+ cells**

Primary CD34+ cells and CD34+ cells isolated after incubation were labeled with anti-human CD34 monoclonal antibody (mAb) conjugated to allophycocyanin (APC) and CXCR4 mAb (clone# 12G5) conjugated to phycoerythrin (PE). All mAb were
purchased from Becton Dickinson Pharmingen. Each analysis was paired with a corresponding matched isotype control. Immediately before flow cytometric analysis, 1 µg/ml of propidium iodide (Sigma) was added in order to exclude nonviable cells. Cells were analyzed flow cytometrically and at least 10,000 viable cells were acquired from each sample (Cell-Quest software, Becton Dickinson).

The percentage of CD34+ cells undergoing apoptosis was determined using the Annexin V-FITC apoptosis detection kit (BD Pharmingen). CD34+ Annexin V+ PI- cells were regarded as cells undergoing apoptosis.

**Migration assay**

The migratory behavior of primary PMF CD34+ cells before and following culture was determined as previously described using 6.5-mm-diameter, 5-µm-pore transwell plates (Corning, NY). The percentage of cells migrating was calculated by determining the ratio of the number of cells recovered from the lower compartment to the total number of cells loaded in the upper compartment. Migrating cells were also assayed in semisolid media for their ability to form hematopoietic colonies and those colonies were plucked and their JAK2 status determined.

**Hematopoietic progenitor cell assays**
Primary CD34⁺ cells or CD34⁺ cells reisolated following culture with cytokines or cytokines plus various agents were assayed in semisolid media as described previously. Briefly, 5 ×10² cells were plated in duplicate culture dishes containing 1 ml IMDM with 1.1% methylcellulose, 30% FBS, 5 × 10⁻⁵ mol/L 2-mercaptoethanol (Stem Cell Technologies), to which SCF, thrombopoietin, IL-3, IL-6, granulocyte macrophage colony-stimulating factor (GM-CSF), each at 100 ng/ml, and 5 units/ml of erythropoietin (Amgen) were added. Colonies were enumerated after 12-14 days of incubation. Individual colonies were plucked and analyzed for the JAK2V617F using a nested allele-specific PCR as previously described and the percentage of JAK2V617F-positive colonies formed was determined.

**NOD/SCID Marrow Repopulating Cell Assay**

NOD/SCID/IL2Rnull mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All experiments were approved by the Animal Care Committee of the MSSM. PMF CD34⁺ cells from 7 patients with a granulocyte JAK2V617F allele burden ranging from 35% to 86% were treated with 5azaD/SAHA or 5azaD/TSA and their cellular phenotype analyzed, their ability to form hematopoietic colonies in vitro and the JAK2V617F status of individual colonies determined. Also, primary PMF CD34⁺ cells or the total number of cells generated after culture from the identical number of PMF
CD34⁺ cells in the presence of cytokines alone or cytokines plus 5azaD/SAHA or 5azaD/TSA (0.4-20.6 × 10⁵ CD34⁺ cells/mouse) were transplanted via the tail vein into eight- to nine-week-old sublethally irradiated (240 cGy) NOD/SCID/IL2Rnull mice. Two, four or six months after transplantation, mice were sacrificed and cells were recovered from the BM of femurs, tibias, humeri, spleens and the PB of the recipient mice. The presence of human CD45⁺, CD33⁺, Glycophorin A⁺, CD41a⁺, CD19⁺, CD3⁺ and CD34⁺ cells was determined by mAb staining and flow cytometric analysis. Each analysis was paired with a corresponding matched isotype control. Cells obtained from mice not receiving human cell transplants were analyzed in a similar fashion in parallel in order to exclude the possibility of false positive immunostaining. The antibodies utilized did not cross-react with murine cells. Human CD45⁺ cells in the BM of the recipient mice were further selected using CD45 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and the purity of human CD45⁺ cells were ≥ 90%. The JAK2V617F/JAK2total percentage in the genomic DNA of selected human CD45⁺ cells was determined by real-time quantitative polymerase chain reaction using the allelic discrimination method which is capable of detecting as few as 0.25% mutant allele in 40 ng of DNA isolated from ~6000 human engrafted cells.¹⁶

**Statistical analysis**
The results are reported as the mean ± SD of data obtained from 4-6 individual experiments. Statistical significance was determined using Student's t tests or paired-sample t tests. All P values were two-sided.

Results

5azaD/SAHA treatment increases the numbers of PMF CD34⁺CXCR4⁺ cells

Bogani C et al have previously reported that a short-term in vitro treatment with 5-azaD reduced CXCR4 promoter methylation, increased membrane expression of CXCR4 and resulted in improved migration of CD34⁺ cells in response to CXCL12 in vitro. In addition we have shown that sequential treatment of PMF CD34⁺ cells with 5azaD/TSA, resulted in an upregulation of CXCR4 expression by PMF CD34⁺ cells and correction of the abnormal cellular trafficking characteristic of PMF as well as a reduction of the burden of malignant HPC. We, therefore, explored if similar activity could be achieved with an HDACI which is currently approved for clinical use in order to develop a strategy for treating patients with PMF. SAHA is an HDACI currently approved to treat patients with cutaneous T-cell lymphoma (CTCL). We evaluated the effects of 5azaD or SAHA alone or the sequential treatment with these two CMA on PMF CD34⁺ cells.
CD34+ cells were first treated with cytokines alone or cytokines plus 5azaD for 2 days and were phenotypically characterized. As shown in Fig. 1A and 1B, a similar reduction in the total number of cells and CD34+ cells were observed in the cultures of incubated with cytokines alone or cytokines plus 5azaD alone. The percentage of CD34+ cells which expressed CXCR4 was, however, significantly greater (8.2±1.5%) following 5azaD treatment than that achieved with cells exposed to cytokines alone (3.6±0.6%; P<.05) or primary PMF CD34+ cells (1.6±0.3%; P<.01) (Fig. 1C). The number of CD34+ cells which expressed CXCR4 in cultures containing cytokines plus 5azaD (4.3±1.8×10^3) was 2-fold greater than that detected in cultures exposed to cytokines alone (2.1±0.9×10^3; P=.08; Fig. 1D).

We next examined the effect of SAHA treatment alone on PMF CD34+ cells. Incubation of CD34+ cells with cytokines alone resulted in a 5.8- to 10.2-fold increase, while treatment with the same cytokines and SAHA resulted in a 2- to 7-fold increase in the total number of cells as compared to the number of input cells (Fig 1 A; P=.11). The number of CD34+ cells in cultures containing cytokines alone (2.8±1.2×10^5) was statistically similar to the number in cultures exposed to cytokines plus SAHA (1.4±0.9×10^5; P=.37; Fig. 1B), and the absolute number of CD34+ cells expressing CXCR4 in cultures containing cytokines plus SAHA (3.4±2.8×10^3) was only half that
observed in the cells exposed to cytokines alone (Fig. 1D). These findings suggest that treatment with SAHA alone does not enhance the expression of CXCR4 by PMF CD34+ cells.

We next investigated the effects of the sequential treatment with 5azaD and SAHA on PMF CD34+ cells. PMF CD34+ cells cultured (Fig. 1A) in the presence of cytokines plus 5azaD/SAHA generated more total cells (10.0±1.9×10^5) than the number of primary cells used to initiate the cultures (1.1±0.01×10^5) (P<.001) but fewer cells than that observed in cultures containing cytokines alone (18.5±2.4×10^5, P<.001). The absolute number of CD34+ cells in cultures exposed to cytokines alone (1.5±0.5×10^5) was similar to that documented in cultures exposed to cytokines plus 5azaD/SAHA (0.9±0.2×10^5) (Fig.1B). The percentage of CD34+ cells which expressed CXCR4 was, however, significantly greater (22.3±1.9%) following 5azaD/SAHA treatment than that achieved following exposure to cytokines alone (6.2±1.1%; P<.001), primary PMF CD34+ cells (1.6±0.3%; P<.001) or cells exposed to 5azaD alone (P<.05) or SAHA alone (P<.01) (Fig. 1C). The number of CD34+ cells which expressed CXCR4 in cultures containing cytokines plus 5azaD/SAHA was greater than that present in primary CD34+ cells (P<.001) or cells exposed to cytokines alone (P<.001), 5azaD alone (P<.05) or SAHA alone (P<.05) (Fig. 1D). This effect of 5azaD/SAHA on
CXCR4 expression was observed irrespective of the JAK2 mutational status of the patients studied (data not shown).

**5azaD/SAHA treatment increases the migratory capacity of PMF CD34⁺ cells in response to CXCL12**

The reisolated 5azaD/SAHA treated PMF CD34⁺ cells had a greater capacity to migrate toward CXCL12 (19.2±3.96%) as compared with CD34⁺ cells exposed to cytokines alone (6.2±1.3%, \( P < .05 \)) or primary CD34⁺ cells (4.4±1.6%, \( P < .05 \)) (Fig. 2A).

The JAK2 mutational status of HPCs (CFU-GM, BFU-E and CFU-Mix) from 4 patients (JAK2V617F granulocyte allele burden ranging from 37% to 77%) that migrated in response to CXCL12 was then analyzed. Both the percentage and total number of JAK2 wild type colonies assayed from CD34⁺ cells treated with cytokines plus 5azaD/SAHA that had migrated was significantly greater (40.9±5.8%; 347±74.0) than JAK2 wild type colonies assayed from CD34⁺ cells exposed to cytokines alone that had migrated (11.3±5.3%, \( P < .001 \); 45±11.4, \( P < .05 \)) or primary PMF CD34⁺ cells that had migrated (7.2±3.9%, \( P < .01 \); 9.8±3.8, \( P < .05 \)) (Fig. 2B and 2C).

**5azaD/SAHA treatment reduces the percentage of malignant PMF HPCs**

We further investigate the effect of SAHA alone or 5azaD/SAHA on PMF HPC. As shown in Fig. 3A, a similar number of HPC were assayed from PMF CD34⁺ cells
treated with SAHA as that assayed from CD34+ cells exposed to cytokines alone or primary PMF CD34+ cells. Individual colonies from two JAK2V617F positive PMF patients (JAK2V617F allele burden of granulocytes was 60% and 77%) were plucked and analyzed for the JAK2V617F using a nested allele-specific PCR. As shown in Table 1, the treatment of PMF CD34+ cells with SAHA alone did not substantially reduce the percentage of JAK2V617F positive colonies or JAK2V617F homozygous colonies.

By contrast, 5azaD/SAHA treatment resulted in a marked reduction of all classes of assayable progenitor cells (CFU-GM, BFU-E, CFU-Mix), as compared with PMF CD34+ cells exposed to cytokines alone (P<.05) or primary PMF CD34+ cells (P=.08) (Fig. 3B and 3C). The inhibitory effect of 5azaD/SAHA treatment on PMF HPCs was similar to that previously reported with 5azaD/TSA treatment.12

We further analyzed the JAK2V617F status of HPCs assayed from PMF CD34+ cells treated with 5azaD/SAHA from 6 different patients (JAK2V617F granulocyte allelic burden of ranging from 35% to 80%). CD34+ cells were reisolated following treatment of cells from these same patients with either cytokines alone or cytokines plus 5azaD/SAHA. As shown in Table 2, 5azaD/SAHA treatment resulted in not only a reduction in the proportion of JAK2V617F+ HPCs (53.7±10.1%) as compared to cells exposed to cytokines alone (81.8±7.5%, P<.01) or primary PMF CD34+ cells
(82.5±8.3%, \(P<.01\)) but also a reduction in the proportion of JAK2V617F homozygous HPCs (13.4±6.5%) as compared to cells exposed to cytokines alone (33.4±9.7%, \(P<.05\)) or primary PMF CD34+ cells (55.3±15.1%, \(P=.09\)).

In addition, in a single case with JAK2V617F negative PMF but with a marker chromosomal abnormality [Del (13)(q13)] (Patient 32), the effect of 5azaD/SAHA treatment on the malignant clone was examined by performing FISH analysis of the cells within colonies. 53.8% of the colonies cloned from primary CD34+ cells from this patient were composed of cells where >90% contained the marker chromosomal abnormality as compared to 46.2% in cultures exposed to cytokines alone while only colonies containing cells lacking the chromosomal abnormality were observed in the culture exposed to cytokines plus 5azaD/SAHA. These data suggest that 5azaD/SAHA treatment can preferentially eliminate the malignant HPC of PMF patients with genetic defects other than JAK2V617F.

**5azaD/SAHA treatment does not affect normal CD34+ cells**

We next examined the effects of 5azaD/SAHA treatment on CB CD34+ cells. As shown in Fig 4A and 4B, the absolute number of CB CD34+ cells and assayable HPC in cultures exposed to cytokines alone was similar to that detected in cultures exposed to cytokines plus 5azaD/SAHA. The percentage of cells within the 5azaD/SAHA-treated
CB CD34⁺ cells which expressed CXCR4 was, however, 1.9 times greater than the cells exposed to cytokines alone (Fig. 4C). In addition, treatment of CB CD34⁺ cells in vitro with cytokines plus 5azaD/SAHA resulted in a 1.8-fold increase in the absolute numbers of CD34⁺CXCR4⁺ cells as compared to CD34⁺ cells exposed to cytokines alone (Fig. 4D). These findings suggest that 5azaD/SAHA treatment at the doses utilized is not toxic to normal CD34⁺ cells but does result in up-regulated CXCR4.

5azaD/SAHA treatment induces the apoptosis of PMF CD34⁺ cells

In order to explore additional mechanisms by which 5azaD/SAHA affects PMF CD34⁺ cells, we determined if sequential treatment with CMAs was associated with an increased degree of apoptosis. As shown in Fig. 5B, the percentage of CD34⁺Annexin V⁺PI⁻ cells were significantly greater in PMF CD34⁺ cells treated with cytokines plus 5azaD/SAHA (13.2±3.5%) as compared to that observed with PMF CD34⁺ cells treated with cytokines alone (0.9±0.9%, P<.05). Remarkably treatment of CB CB34⁺ cells with the same doses of 5azaD/SAHA in the presence of cytokines was not associated with the same degree of apoptosis (0.4±0.4%), indicating that PMF CD34⁺ cells are more sensitive than CB CD34⁺ cells to the actions of these CMA.

Effects of JAK2 inhibitors, Peg-IFN-α 2a or hydroxyurea on PMF CD34⁺ cells

The current standard of care for PMF patients include hydroxyurea, and interferon-α
(IFN-α). Recently several small molecule inhibitors of JAK2 have been evaluated in this patient population.\textsuperscript{21} We compared the effects of two small JAK2 inhibitors (JAK inhibitor 1 and AZ1480), hydroxyurea and Peg-IFN-α 2a on PMF CD34\textsuperscript{+} cells in order to assess the uniqueness of the effects of 5azaD/SAHA on PMF CD34\textsuperscript{+} cells. As shown in Table 3, incubation of PMF CD34\textsuperscript{+} cells (1×10\textsuperscript{5}) with cytokines alone for 3 days generated greater numbers of total cells (2.8±0.7×10\textsuperscript{5}) than in cultures performed in the presence of AZ1480 (1.7±0.4×10\textsuperscript{5}, \(P\,<\,0.05\)), hydroxyurea (0.7±0.2×10\textsuperscript{5}, \(P\,<\,0.05\)) or Peg-IFN-α 2a (1.7±0.5×10\textsuperscript{5}, \(P\,<\,0.05\)), while cultures performed in the presence of JAK inhibitor 1 contained a similar number of total cells (2.2±0.7×10\textsuperscript{5}). However, similar numbers of CD34\textsuperscript{+} cells were observed in cultures containing cytokines alone (0.6±0.3×10\textsuperscript{5}) as cultures with cytokines plus each of the JAK2 inhibitors, hydroxyurea or Peg-IFN-α 2a (Table 3). Similarly treatment with none of these agents altered CXCR4 expression by CD34\textsuperscript{+} cells. A similar number of HPC were assayed from CD34\textsuperscript{+} cells treated with cytokines plus each of these agents individually as that assayed from primary CD34\textsuperscript{+} cells or CD34\textsuperscript{+} cells exposed to cytokines alone. None of these agents substantially reduce the percentage of JAK2V617F colonies or JAK2V617F homozygous colonies (Table 4). These findings suggest that unlike 5azaD/SAHA, none of these agents was capable of eliminating JAK2V617F HPC or
upregulating CXCR4 expression by PMF CD34+ cells.

**Effects of sequential treatment with chromatin modifying agents on PMF SCID repopulating cells (SRC)**

Following transplantation, short-term SCID repopulating cells (SRC) are thought to contribute to hematopoiesis initially but their contribution to blood cell production persists for only a limited period of time, while long-term SRCs are thought to contribute to hematopoiesis after a delay but to be responsible for sustained blood cell production. These classes of SRC can be distinguished by monitoring the kinetics of engraftment in immunodeficient mice. We therefore evaluated the effect of sequential treatment with CMAAs on short-term SRCs assayed 2 months following transplantation and long-term SRCs, assayed 4 or 6 months following transplantation. CD34+ cells from 7 patients (JAK2V617F allele burden of granulocytes ranged from 35% to 86%) were transplanted and the JAK2V617F allele burden of hCD45+ cells was determined in the marrow of NOD/SCID/IL2Rγnull mice. Two months following transplantation, 4.4% of the marrow cells were hCD45+ cells in mice receiving primary CD34+ cells from Patient 4 as compared to 0.2% in mice receiving cells treated with cytokines alone or cytokines and 5azaD/TSA. The donor cells receiving primary PMF CD34+ cells were composed of myeloid cells belonging to multiple hematopoietic lineages (CD33+ cells, 4.3%;
glycophorin A⁺ cells, 0.8%; CD41a⁺ cells, 0.1% and CD34⁺ cells, 0.2%) but not CD19⁺ or CD3⁺ cells (Patient 4, Fig. 6). The percentage of JAK2V617F/JAK2_total in hCD45⁺ cells from BM of the two mice transplanted with primary CD34⁺ cells from this patient was 71.5% and 34.7% in mice transplanted with cells treated with cytokines alone and 6.8% in mice transplanted with cells treated with 5azaD/TSA (Table 5, Patient 4).

Four months after transplantation similar numbers of human CD45⁺ cells (0.04-0.1%) were detected in the BM of mice receiving either PMF CD34⁺ cells treated with cytokines alone or cytokines and 5azaD/TSA. A persistently marked reduction in the percentage of JAK2V617F/JAK2_total in hCD45⁺ cells was, however, observed in the BM of mice transplanted with PMF CD34⁺ cells treated with 5azaD/TSA (22.5%, 8.8%, 28.8%,) as compared to that observed in mice transplanted with the corresponding CD34⁺ cells treated with cytokines alone (74.5%, 82.4%, 79.9%) (Table 5, Patient 6, 9, 10).

Similarly, six months following transplantation, human marrow cell chimerism was also documented (Table 5, Patient 1, 3, 12). The engrafted cells in the BM of these mice were composed of myeloid cells belonging to multiple hematopoietic lineages (CD33⁺, Glycophorin A⁺, CD41a⁺, CD19⁺, and CD34⁺ cells) but not CD3⁺ cells (Patient 12). The percentage of JAK2V617F/JAK2_total in the gDNA of BM hCD45⁺ cells was 82.5%,
97.6% and 92.8% in mice transplanted with primary CD34+ cells as compared to 27.1%, 41.5% and 94.9% in mice transplanted with CD34+ cells treated with cytokines alone and 3.6%, 7.4% and 17.8% in mice transplanted with CD34+ cells treated with 5azaD/SAHA. A similar reduction of the percentage of JAK2V617F/JAK2_total in hCD45+ BM cells of mice transplanted with CMA treated CD34+ cells from an additional patient was also observed (Table 5, Patient 6). These findings suggest that both short-term and long-term PMF SRC are affected by the malignant process and that various classes of JAK2V617F+ SRC can each be eliminated by sequential treatment with CMAs.

**Discussion**

In the present report, we showed that the in vitro treatment with sequential 5azaD/SAHA or 5azaD/TSA but not 5azaD or SAHA alone had a profound effect on PMF CD34+ cells, resulting in a dramatic reduction in the number of JAK2V617F+ HPCs. These effects were not observed following the treatment with other agents including JAK2 inhibitors, hydroxyurea or peg-IFN-α 2a. We have chosen to use SAHA for these studies since clinical trials and animal models have shown that SAHA can induce growth arrest, and death of cancer cells in vitro and in vivo, at concentrations that have little or no toxicity for normal cells.23-27 We have demonstrated that both
short-term and long-term SRC in PMF are JAK2V617F⁺ and that JAK2V617F⁺ SRC can be eliminated by sequential treatment with CMAs, leading to engraftment by SRC with wild type JAK2. The degree of engraftment by 5azaD/SAHA or 5azaD/TSA treated PMF CD34⁺ cells might be underestimated in the present report since fewer PMF CD34⁺ cells treated with 5azaD/SAHA or 5azaD/TSA were transplanted into each mice as compared with the number of PMF CD34⁺ cells treated with cytokines alone or primary PMF CD34⁺ cells due to the effect of CMA on inducing PMF CD34⁺ cells apoptosis. Surprisingly, the percentage of JAK2V617F/JAK2total in hCD45⁺ cells of mice transplanted with PMF CD34⁺ cells treated with cytokines alone also appeared to be decreased as compared to that observed in hCD45⁺ BM cells of mice transplanted with primary PMF CD34⁺ cells, albeit to a lesser extent than that following CMA treatment. This observation is likely a consequence of the loss of the proliferative advantage of JAK2V617F positive HSC/HPC due to the addition of cytokines to in vitro cultures which have been reported by several groups. ¹⁶, ²⁸ Since the ability of primitive human hematopoietic cells to engraft sublethally irradiated immunodeficient mice is the standard surrogate in vivo assay for human HSC, our findings suggest that in PMF both short-term HSCs and long-term HSCs are involved by the malignant process and can be eliminated by in vitro treatment with CMAs.
Sequential treatment with 5azaD/SAHA at concentrations that resulted in the elimination of JAK2V617F+ HPC and SRC was associated with increased apoptosis of PMF CD34+ cells but not normal CD34+ cells. Our laboratory has previously reported that the sequential treatment of normal HSCs with 5azaD and TSA results in their ability to undergo symmetrical cell division with retention of their marrow-repopulating potential.29-31 This behavior is in contrast to stem cells exposed in vitro to cytokines alone which undergo progressive loss of their proliferative and self-renewal capacity. Sequential treatment of normal stem cells with 5azaD/TSA results in the up-regulation of expression of number of genes previously implicated in HSCs self-renewal.31 The effects of CMAs on MPN CD34+ cells could, therefore, be due not only to their ability to inducing apoptosis and terminal differentiation of malignant stem cells, but also their ability to promote preferential expansion of residual pool of JAK2 wild type stem cells. Such wild type PMF stem cells are not necessary normal since they could contain additional genetic abnormalities (TET2, the additional sex combs like gene and the gene for isocitrate dehydrogenase 1, etc).

We also showed that the sequential treatment of PMF CD34+ cells with 5azaD/SAHA resulted in the restoration of their capacity to migrate toward CXCL12 which was associated with the upregulation of CXCR4 expression. Moreover,
migratory 5azaD/SAHA treated PMF CD34+ cell contain greater number of JAK2V617F wild type CD34+ cells. These findings suggest that the correction of PMF CD34+ cell migration following the treatment of CMAs could, therefore, be due either to the increased expression of CXCR4 by normal CD34+ cells or an increased number of normal HSC/HPC surviving CMA treatment relative to the number of malignant HSC/HPC which has previously been reported by our laboratory to improve SRC homing.

In conclusion, our data clearly show that JAK2V617F+ SRC and HPC can be preferentially eliminated by sequential treatment with CMAs. Both SAHA and two DNMTIs are already approved to treat other hematological malignancies providing a path for their evaluation in this patient population. CMA therapy thus represents a promising treatment option for PMF patients, which requires careful evaluation in well constructed clinical trials.

Acknowledgment

This study was support by grants from the Myeloproliferative Disorders Foundation (to R.H.), National Cancer Institute (1P01CA108671) to R.H.

Authorship
Contributions: X.W. designed the experiments, performed the experiments, analyzed the data, and wrote the paper. W.Z., M. L. and Y. L. performed some of the experiments; J.T. performed FISH; M.X. reviewed the paper. V.N. analyzed FISH data; R.H. designed the experiments, interpreted the data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


Table 1. JAK2V617F status of hematopoietic progenitor cells assayed from PMF CD34⁺ cells treated with SAHA alone

<table>
<thead>
<tr>
<th>JAK2V617F allele burden (%)</th>
<th>PB granulocytes</th>
<th>Source of hematopoietic colonies assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary PMF CD34⁺ cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PMF CD34⁺ cells treated with cytokines alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PMF CD34⁺ cells treated with cytokines + SAHA alone</td>
</tr>
<tr>
<td>Patient 2</td>
<td>60*</td>
<td>90 (27/30)</td>
</tr>
<tr>
<td></td>
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<td>80 (24/30)</td>
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<td></td>
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<td>100 (34/34)</td>
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<td></td>
<td></td>
<td>62 (21/34)</td>
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<td></td>
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<td>100 (35/35)</td>
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<td>54 (19/35)</td>
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<td>Patient 6</td>
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<td>97 (29/30)</td>
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<td></td>
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<td>93 (28/30)</td>
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<td>88 (7/17)</td>
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<td>41 (15/17)</td>
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<tr>
<td></td>
<td></td>
<td>96 (21/22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>82 (18/22)</td>
</tr>
</tbody>
</table>

*Indicates the percentage of JAK2V617F allele obtained from granulocytes by real-time quantitative kinetic PCR assay using allelic discrimination method.

‡Each value represents the percentage of colonies (CFU-GM+BFU-E+CFU-Mix) exhibiting the JAK2V617F mutation or homozygous JAK2V617F allele. The numbers in parentheses denote the actual number of JAK2V617F positive or homozygous colonies/the total numbers of colonies (CFU-GM+BFU-E+CFU-Mix) analyzed.
Table 2. JAK2V617F status of hematopoietic progenitor cells assayed from PMF CD34+ cells treated with 5azaD/SAHA

<table>
<thead>
<tr>
<th>Source of hematopoietic colonies assayed</th>
<th>PB granulocytes</th>
<th>Primary PMF CD34+ cells</th>
<th>PMF CD34+ cells treated with cytokines alone</th>
<th>PMF CD34+ cells treated with cytokines + 5azaD/SAHA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>JAK2V617F allele burden (%)</td>
<td>% JAK2V617F</td>
<td>% homozygous JAK2V617F</td>
<td>% JAK2V617F</td>
</tr>
<tr>
<td>Patient 1</td>
<td>80*</td>
<td>80 (49/61)†</td>
<td>70 (43/61)</td>
<td>91 (42/46)</td>
</tr>
<tr>
<td>Patient 3</td>
<td>35</td>
<td>45 (37/83)</td>
<td>23 (19/83)</td>
<td>47 (27/58)</td>
</tr>
<tr>
<td>Patient 6</td>
<td>77</td>
<td>100 (30/30)</td>
<td>100 (30/30)</td>
<td>96 (44/46)</td>
</tr>
<tr>
<td>Patient 9</td>
<td>75</td>
<td>88 (22/25)</td>
<td>0 (0/25)</td>
<td>77 (10/13)</td>
</tr>
<tr>
<td>Patient 12</td>
<td>37</td>
<td>82 (45/55)</td>
<td>76 (42/55)</td>
<td>89 (34/38)</td>
</tr>
<tr>
<td>Patient 13</td>
<td>65</td>
<td>100 (65/65)</td>
<td>62 (40/65)</td>
<td>91 (31/34)</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>83°F</td>
<td>55°F</td>
<td>82°F</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>8.3</td>
<td>15.1</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Indicates the percentage of JAK2V617F allele obtained from granulocytes by real-time quantitative kinetic PCR assay using allelic discrimination method.

†Each value represents the percentage of colonies (CFU-GM+BFU-E+CFU-Mix) exhibiting the JAK2V617F mutation or homozygous JAK2V617F allele. The numbers in parentheses denote the actual number of JAK2V617F positive or homozygous colonies/the total numbers of colonies (CFU-GM+BFU-E+CFU-Mix) analyzed.

‡P < .01 vs. the proportion of JAK2V617F+ HPCs assayed from PMF CD34+ cells treated with 5azaD/SAHA.

§P = .09, ||P < .05 vs. the proportion of JAK2V617F homozygous HPCs assayed from PMF CD34+ cells treated with 5azaD/SAHA.
Table 3. Effects of other therapeutic agents on PMF CD34⁺ cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of total cells (×10⁵)</th>
<th>No. of CD34⁺ cells (×10⁵)</th>
<th>No. of CD34⁺CXCR4⁺ cells (×10³)</th>
<th>No. of CFU-GM</th>
<th>No. of BFU-E</th>
<th>No. of CFU-Mix</th>
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</thead>
<tbody>
<tr>
<td>Primary</td>
<td>1.0±0.1</td>
<td>1.0±0.0</td>
<td>0.9±0.3</td>
<td>7392±2226</td>
<td>2100±1152</td>
<td>360±162</td>
</tr>
<tr>
<td>Cytokines alone</td>
<td>2.8±0.7</td>
<td>0.6±0.3</td>
<td>3.6±1.9</td>
<td>9032±2880</td>
<td>4000±1326</td>
<td>732±458</td>
</tr>
<tr>
<td>Cytokines + JAK inhibitor 1 (100nM)</td>
<td>2.2±0.7</td>
<td>0.6±0.3</td>
<td>3.7±2.5</td>
<td>7002±1477</td>
<td>3203±1212</td>
<td>360±360</td>
</tr>
<tr>
<td>Cytokines + AZ1480 (100nM)</td>
<td>1.7±0.4*</td>
<td>0.4±0.2</td>
<td>1.0±0.4</td>
<td>6712±990</td>
<td>3184±1250</td>
<td>720±120</td>
</tr>
<tr>
<td>Cytokines + Hydroxyurea (50μM)</td>
<td>0.7±0.2*</td>
<td>0.1±0.0</td>
<td>0.6±0.2</td>
<td>2617±1140</td>
<td>770±450</td>
<td>104±104</td>
</tr>
<tr>
<td>Cytokines + Peg-IFN-α 2a (125U)</td>
<td>1.7±0.5*</td>
<td>0.4±0.2</td>
<td>1.6±0.6</td>
<td>6885±2187</td>
<td>1963±882</td>
<td>896±571</td>
</tr>
</tbody>
</table>

PMF CD34⁺ cells (1×10⁵) were treated with cytokines alone or cytokines plus various agents. *P<.05 vs. PMF CD34⁺ cells treated with cytokines alone. n=5.
Table 4. JAK2V617F status of hematopoietic progenitor cells assayed from PMF CD34+ cells treated with JAK inhibitor 1, AZ1480, Hydroxyurea or Peg-IFN-α 2a

The treatment of PMF CD34+ cells with each of JAK inhibitor 1, AZ1480, hydroxyurea or Peg-IFN-α 2a did not substantially reduce the percentage of JAK2V617F colonies or JAK2V617F homozygous colonies as compared with PMF CD34+ cells exposed to cytokines alone or primary PMF CD34+ cells.

*Indicates the percentage of JAK2V617F allele obtained from granulocytes by real-time quantitative kinetic PCR assay using allelic discrimination method.

†Each value represents the percentage of colonies (CFU-GM+BFU-E+CFU-Mix) exhibiting the JAK2V617F mutation or homozygous JAK2V617F allele. The numbers in parentheses denote the actual number of JAK2V617F positive or homozygous colonies/the total numbers of colonies (CFU-GM+BFU-E+CFU-Mix) analyzed.
Table 5. JAK2V617F+ SCID repopulating cells can be preferentially eliminated by sequential treatment with chromatin modifying agents

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Treatment</th>
<th>% JAK2V617F in granulocytes</th>
<th>Number of CD34+ cells transplanted (×10³)</th>
<th>Duration of engraftment (months)</th>
<th>CD45+ cells in BM (%)</th>
<th>% JAK2V617F in BM hCD45+ cells</th>
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<tr>
<td>4</td>
<td>Primary CD34+ cells</td>
<td>86</td>
<td>6.4</td>
<td>4</td>
<td>44.4</td>
<td>71.5</td>
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<tr>
<td></td>
<td>Cytokines alone</td>
<td></td>
<td>10</td>
<td>0.2</td>
<td>34.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytokines + 5azaD/TSA</td>
<td></td>
<td>4.2</td>
<td>0.2</td>
<td>6.8</td>
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<tr>
<td>6</td>
<td>Cytokines alone</td>
<td>77</td>
<td>2.1</td>
<td>0.04</td>
<td>74.5</td>
<td></td>
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<tr>
<td></td>
<td>Cytokines + 5azaD/TSA</td>
<td></td>
<td>1.5</td>
<td>0.1</td>
<td>22.5</td>
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<tr>
<td>9</td>
<td>Cytokines alone</td>
<td>75</td>
<td>5.1</td>
<td>0.1</td>
<td>82.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cytokines + 5azaD/TSA</td>
<td></td>
<td>3.5</td>
<td>0.1</td>
<td>8.8</td>
<td></td>
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<tr>
<td>10</td>
<td>Cytokines alone</td>
<td>70</td>
<td>0.8</td>
<td>0.1</td>
<td>79.9</td>
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<tr>
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<td>Cytokines + 5azaD/TSA</td>
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<td>0.9</td>
<td>0.1</td>
<td>28.8</td>
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<tr>
<td>1</td>
<td>Primary CD34+ cells</td>
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<td>0.2</td>
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<tr>
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<td>Cytokines alone</td>
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<td>14</td>
<td>0.1</td>
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<tr>
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<td>Cytokines + 5azaD/SAHA</td>
<td></td>
<td>7</td>
<td>0.1</td>
<td>3.6</td>
<td></td>
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<tr>
<td>3</td>
<td>Primary CD34+ cells</td>
<td>35</td>
<td>1.3</td>
<td>0.1</td>
<td>97.6</td>
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<td></td>
<td>Cytokines alone</td>
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<td>0.05</td>
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<td>Cytokines + 5azaD/SAHA</td>
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<tr>
<td>12</td>
<td>Primary CD34+ cells</td>
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<td>92.8</td>
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<td>Cytokines alone</td>
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<td>94.9</td>
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<td>Cytokines + 5azaD/SAHA</td>
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<td>0.05</td>
<td>84.4</td>
<td></td>
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<tr>
<td></td>
<td>Cytokines + 5azaD/SAHA</td>
<td></td>
<td>0.4</td>
<td>0.05</td>
<td>40.4</td>
<td></td>
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</table>

The percentage of JAK2V617F allele from granulocytes of PMF patients or from human CD45+ cells isolated from the BM of NOD/SCID/IL2Rγnull mice was determined by real-time quantitative kinetic PCR assay using allelic discrimination method.
Figure legends

Figure 1. Effects of chromatin modifying agents on PMF CD34⁺ Cells. PMF CD34⁺ cells were treated with cytokines plus 5azaD for 2 days (n=6), cytokines plus SAHA for 6 days (n=6) or cytokines plus 5azaD/SAHA for 8 days (n=22). The cultures containing cytokines alone were performed in parallel. The cultured cells were then phenotypically characterized. (A) The total number of cells generated after the culture of PMF CD34⁺ cells (1×10⁵) in the presence of cytokines plus CMA. ***: P<.001. (B) The number of CD34⁺ cells generated after the culture of PMF CD34⁺ cells (1×10⁵) in the presence of cytokines plus CMA. Both the percentage (C) and the number (D) of CD34⁺ cells expressing CXCR4 was significantly increased in the cultures containing cytokines plus 5azaD/SAHA as compared with cultures containing cytokines plus 5azaD alone or plus SAHA alone. *: P<.05, **: P<.01, ***: P<.001, #: P=.08. (E) A representative flow cytometric pattern demonstrating the analysis of the percentage of PMF CD34⁺ cells which express CXCR4 following the culture PMF CD34⁺ cells in the presence of cytokines alone or cytokines plus 5azaD/SAHA.

Figure 2. 5azaD/SAHA treatment increases the percentage of JAK2V617F wild type PMF CD34⁺ cells that migrate toward CXCL12. (A) The migratory behavior of primary PMF CD34⁺ cells or PMF CD34⁺ cells re-isolated after ex vivo culture in the presence of cytokines alone or cytokines plus 5azaD/SAHA was determined by the ratio of the number of cells recovered from the lower compartment to the total number of cells loaded in the upper compartment of a 6.5-mm-diameter, 5-µm-pore transwell plate (n=7). *: P<.05. JAK2V617F positive migrating cells were further assayed in semisolid media and analyzed for the JAK2V617F mutation using nested allele-specific PCR (n=4). Both the percentage (B) and the number (C) of JAK2V617F wild type colonies
(CFU-GM+BFU-E+CFU-Mix) that migrate toward CXCL12 from cultures treated with cytokines plus 5azaD/SAHA was significantly greater than that observed from cultures exposed to cytokines alone or primary PMF CD34+ cells. *: $P<.05$, **: $P<.01$, ***: $P<.001$.

**Figure 3.** 5azaD/SAHA treatment reduces the number of assayable PMF HPCs. (A) Total number of colonies assayed from the culture of PMF CD34+ cells ($1 \times 10^5$) in the presence of cytokines alone or cytokines plus SAHA for 6 days (n=6). (B) Total number of colonies assayed from the culture of PMF CD34+ cells ($1 \times 10^5$) in the presence of cytokines alone or cytokines plus 5azaD/SAHA for 8 days (n=15). *: $P<.05$.

(C) The degree of the expansion of progenitor cells after ex vivo culture was determined by dividing the number of colonies assayed from cultures performed in the presence of cytokines alone or cytokines plus 5azaD/SAHA by that assayed from input primary CD34+ cells (n=15). *: $P<.05$.

**Figure 4.** 5azaD/SAHA treatment does not reduce the number of HPC assayed from CB. CB CD34+ cells ($1 \times 10^5$; n=6) were treated with 5azaD/SAHA for 8 days in the same way as PMF CD34+ cells. (A) The absolute number of CD34+ cells in the culture of CB CD34+ cells ($1 \times 10^5$) treated with cytokines alone or cytokines plus 5azaD/SAHA. (B) Total number of colonies assayed from the culture of CB CD34+ cells ($1 \times 10^5$) in the presence of cytokines alone or cytokines plus 5azaD/SAHA. (C) and (D) The percentage and total number of CD34+ cells which expressed CXCR4 in CB CD34+ cells treated with cytokines alone or cytokines plus 5azaD/SAHA.

**Figure 5.** 5azaD/SAHA treatment induces the apoptosis of PMF CD34+ cells. (A) A representative flow cytometric pattern demonstrating the analysis of the percentage of PMF CD34+ cells which are Annexin V+ PI following the culture of PMF CD34+ cells
in the presence of cytokines alone or cytokines plus 5azaD/SAHA. (B) The percentage of CD34\(^+\) Annexin V\(^+\) PI cells were greater in PMF CD34\(^+\) cells treated with cytokines plus 5azaD/SAHA as compared to that observed in PMF CD34\(^+\) cells treated with cytokines alone (n=6). \(*: P<.05.\)

**Figure 6. Differentiative pattern of PMF CD34\(^+\) cells in NOD/SCID/IL2R\(^{null}\) mice 2 month following the transplantation.** Two months after transplantation, the mouse transplanted with primary CD34\(^+\) cells of patient 4 was sacrificed and cells were recovered from the BM of femurs, tibias, humeri of the recipient mice. The presence of human primary cells and various lineage cells in the BM of the recipient mice was determined by mAb staining and flow cytometric analysis.
Fig. 1 Wang X, et al.

A

B

C

D

E

Primary PMF
CD34+ cells

PMF CD34+ cells
treated with
cytokines alone

PMF CD34+ cells treated
with cytokines+5azaD/SAHA

Primary PMF
CD34+ cells

0.6%

1.7%

13.2%

CXCR4

CD34
Fig. 2 Wang X, et al.

A

Migration (% of input CD34+ cells)

B

% of JAK2 WT colonies that migrate toward CXCL12

C

No. of JAK2 WT colonies that migrate toward CXCL12

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Fig. 4 Wang X, et al.

A

No. of CD34+ cells (×10^6)

Cytokines alone  Cytokines + 5azaD/SAHA

B

No. of CFU-C (×10^3)

Primary  Cytokines alone  Cytokines + 5azaD/SAHA

C

% CD34+ cells expressing CXCR4

Cytokines alone  Cytokines + 5azaD/SAHA

D

Absolute No. of CD34+ cells expressing CXCR4 (×10^6)

Cytokines alone  Cytokines + 5azaD/SAHA

CFU-GM  BFU-E  CFU-Mix

CFU-GM  BFU-E  CFU-Mix

CFU-GM  BFU-E  CFU-Mix

CFU-GM  BFU-E  CFU-Mix
Percentage of CD34+Annexin V- PI- cells

A PMF CD34+ cells treated with cytokines alone

B PMF CD34+ cells treated with cytokines+5azaD/SAHA

Fig. 5 Wang X, et al.
Fig. 6 Wang X, et al.

CD45

CD33

Glycophorin A

CD41a

CD34

CD19

CD3
Sequential treatment of CD34+ cells from patients with primary myelofibrosis with chromatin modifying agents eliminate JAK2V617F positive NOD/SCID marrow repopulating cells

Xiaoli Wang, Wei Zhang, Joseph Tripodi, Min Lu, Mingjiang Xu, Vesna Najfeld, Yan Li and Ronald Hoffman