MPD-RC Newsletter

Project 1 Update (PI: Josef T. Prchal, MD)

We have published in 2 papers in 2014 next NGS of PV and shown that almost always JAK2V617F is associated with other somatic and at times also germline mutations. Unlike in acute leukemia, majority of these mutations involve epigenetic modifiers. During female embryogenesis, the most of genes in one X-chromosome are randomly inactivated. The X-inactive specific transcript gene (XIST), which encodes long non-coding RNA, is expressed only from the inactive X-chromosome and plays a crucial role in this process. Conditional deletion in female mice of Xist leads to aggressive MPN and eventual death from leukemia.

Using a quantitative, transcriptional clonality assay based on polymorphisms on 5 X-chromosome genes (MPP1, FH1L, IDS, BTK, and G6PD), we analyzed over 150 informative PV and JAK2V617F-positive ET females and all were clonal. However, we recently encountered 4 exceptional cases, these females appeared polyclonal using an IDS marker, and one female using a G6PD marker, whereas all of these females appeared clonal using at least 1 other X-chromosome marker in these cells. We are pursuing hypothesis and collecting supportive data that that reactivation of inactive of some X-chromosome genes in these females contributes to the PV/ET phenotype.

Project 2 Update (PI: Heike L. Pahl, PhD)

NF-E2 target genes participate in a novel regulatory loop regulating proliferation and histone methylation

We have demonstrated that activity of the transcription factor NF-E2 is aberrantly elevated in MPN patients. This confers a proliferative advantage by increasing expression of the cell cycle regulators CDK4, CDK6 and CyclinD3. We have now shown that these three cell cycle regulators as well as the histone methyltransferases MLL2 and MLL4 constitute direct NF-E2 target genes. Moreover, mRNA expression of all five target genes is significantly increased in primary cells of patients with PV compared to healthy controls. Correspondingly, primary cells from PV patients showed a significant elevation in global H3K4m1 levels, a chromatin mark conferred by the MLL proteins.

NF-E2 serves as a scaffold and is required for recruitment of MLL2 to the beta-globin locus and for chromatin remodeling at these sites. Here we show that NF-E2 likewise acts to direct MLL2 to the CDK6 gene.

Our data establish a novel interaction network where NF-E2 both regulates the expression levels of MLL2 while at the same time modulating its epigenetic activity at NF-E2 target genes, which include critical cell cycle regulators.

These data provide a molecular basis for pre-clinical investigation into the effects of CDK4/6 inhibitors as well as of histone methyltransferase inhibitors on MPN cell biology.
Who’s Who in the MPD-RC

We have had several new sites join our consortium since the last newsletter:

- University of Kansas
- Oregon Health & Science University
- University of Chicago
- Rush University
- Roswell Park
- Washington University
- Roswell Park
- Cleveland Clinic
- Massachusetts General Hospital

Project 3 Update (PI: Jerry Spivak, MD)

Identifying the initiating hematopoietic stem cell (HSC) population in polycythemia vera (PV) is central to our understanding of its pathogenesis and vital for developing animal models to study the disease in vivo. To this end, we exploited the fact that the most primitive HSC (CD34+CD38-) are rich in aldehyde dehydrogenase (ALDH)high and can be identified by flow cytometry on the basis of expression of this enzyme. Using single cell sorting and allele-specific PCR for JAK2 V617F, we genotyped circulating CD34+ cells from PV, essential thrombocytosis (ET) and primary myelofibrosis (PMF) patients. We found that JAK2 V617F was present in the circulating ALDHhigh, CD34+CD38- cells in all three MPN, indicating unequivocally that this is the initiating cell population in these three disorders. As expected, the CD34+CD38-ALDHhigh allelic burden was highest in PMF and lowest in ET. The CD34+CD38-ALDHhigh fraction also harbored additional lesions such as trisomy 8, trisomy 9, deletion of 5q and 11q, and ASXL1 and TET2 mutations. Two patterns of leukemia were observed. The first pattern in 7 patients was identical to that seen in de novo AML, with emergence of a unique CD34+CD38- fraction with lower ALDH expression (ALDHint); in 3 of these, the clonal leukemic CD34+CD38-ALDHint fraction was JAK2 V617F-negative, while the CD34+CD38-ALDHhigh fraction from these same cases remained JAK2 V617F-positive.

![Normal donor](image1.png) ![P. Vera patient](image2.png)

**PROJECT 4: Immunostaining with an antibody that recognizes GR of macrophages differentiated in culture from the blood of a normal donor and from a P. Vera patient.**

Project 4 Update (PI: Anna Rita Migliaccio, PhD)

In 1958, Marcel Bessis first described the presence in the marrow of islands formed by macrophages surrounded by erythroid cells. Ramos et al and Chow et al provided evidence that macrophages may represent a niche promoting erythroid differentiation by demonstrating that macrophage depletion in mice greatly impairs the response to erythroid challenges (Nat Med 2013;19:437-445 and 429-436).

In a paper in press in Haematologica, we describe that macrophages play an important role also in the expansion of erythroid cells observed in cultures of human CD34pos cells stimulated with dexamethasone, the ligand for the glucocorticoid receptor (GR). Live phase-contrast videomicroscopy allowed us to identify that physical interaction with macrophages promotes the proliferation of erythroid cells observed in these cultures. These interactions occurred also in cultures stimulated with a macrophage-specific form of dexamethasone covalently linked to CD163. Therefore, GR activation promotes the erythroid activity of the macrophage niches. In Polycythemia Vera, GR activation is hampered by the fact that the JAK2V617F mutation favors expression of the dominant-negative GRβ isoform (Varriacchio et al. Blood. 2011;118:425-36). It is possible that in these patients, reduced levels of GR activity may lead to macrophage dysfunction contributing to erythrocytosis. This hypothesis is currently under investigation.
Project 5 Update (PI: Ronald Hoffman, MD)

Myelofibrosis (MF) is characterized by the constitutive mobilization of hematopoietic stem cells (HSC) and hematopoietic progenitor cells (HPC) and the establishment of extramedullary hematopoiesis (EMH). The mechanisms underlying this abnormal HSC/HPC trafficking pattern remain poorly understood. We demonstrated that both splenic and peripheral blood (PB) MF CD34+ cells equally share a defective ability to home to the marrow but not the spleens of NOD/SCID mice. This trafficking pattern could not be attributed to discordant expression of integrins or chemokine receptors other than CXCR4 by CD34+ cells. The number of both splenic MF CD34+ cells and HPCs that migrated towards splenic MF plasma was, however, significantly greater than the number that migrated towards PB MF plasma. The concentration of the intact HSC/HPC chemo-attractant, CXCL12, was greater in splenic MF plasma than PB MF plasma as quantified using mass spectrometry. Concentrations of truncated products of CXCL12 which are not effective HSC chemo-attractants and are the product of proteolytic degradation by serine proteases were detected at similar levels in splenic and PB MF plasma. Equal amount of anti-CXCL12 neutralizing antibodies resulted in a less blocking of the migration of splenic MF CD34+ cells towards splenic MF plasma as compared to PB MF plasma. Our data indicate that the MF splenic microenvironment is characterized by enhanced levels of intact functional CXCL12, which contributes to the localization of MF CD34+ cells to the spleen and the establishment of EMH.

Our data indicate that the MF splenic microenvironment is characterized by enhanced levels of intact functional CXCL12 (Project 5)

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Project 6 Update

(PIs: Ronald Hoffman, MD & John Mascarenhas, MD)

MPD-RC Project 6 has been steadily increasing enrollment to the open & accruing clinical trials (listed on page 4). We are excited to announce that MPD-RC #115, a Phase I clinical trial entitled - Open Label Phase I Study of Single Agent Oral RG7388 in Patients with Polycythemia Vera and Essential Thrombocythemia (With pilot feasibility study in combination with pegylated interferon alfa 2a for patients who do not respond to the single agent at each dose level), will in the very near future be opening for enrollment at Icahn School of Medicine at Mount Sinai and will be then distributed to participating sites. In the next upcoming year, we are focused on enrollment to these clinical trials—your help is greatly appreciated and highly valued!
LIST OF OPEN & ACCRUING CLINICAL TRIALS

#111: Phase II study of Pegasys in ET & PV patients

#112: Phase III randomized study of Pegasys vs. Hydroxyurea in PV & ET patients

#114: Allogeneic transplant trial with RUX conditioning for MF patients

#109: Combination RUX & Decitabine for accelerated & blast phase MPN patients

In 2014, we enrolled 32 subjects to MPD-111, 21 subjects to MPD-112, 10 subjects to MPD-114, and 9 subjects to MPD-109!

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