AMERICAN SOCIETY OF HEMATOLOGY (ASH) MEETING REPORT ON MPN

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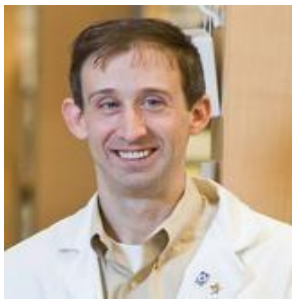


This report focuses on four areas from the ASH presentations:

- Clinical News for MF
- JAK inhibitors
- Combination Studies
- Novel agents
- Notable Basic Science Reports

Executive Summary

This year's ASH meeting was defined by many excellent basic science discoveries that shed further light into the biology of the MPNs, but few major clinical advances. Data from two important clinical trials of interferons revealed that pegasys and ropeginterferon, the latter a longer acting form of interferon, have anti-tumor activity similar to hydroxyurea (HU) at the one-year assessment. As for JAK inhibitors, a presentation on pacritinib revealed that this investigational agent has mixed results, but if approved would likely be helpful to patients with low platelet counts. Finally, results of several combination studies, both clinical and pre-clinical, were provided. These reports suggest that expanding on ruxolitinib may improve its activity in the MPNs. Below I've summarized several interesting abstracts, but this is by no means a complete listing of the important work that was presented this year.

Clinical News for MF

<u>Interferons</u>

1. Ropeginterferon Alpha 2b
   Dr. Gisslinger reported results of the PROUD-PV study comparing this new longer acting version of Interferon versus HU. Investigators found that ropeginterfron has a similar (i.e. non-inferior) activity to HU at 12 months. Although ropeginterferon didn't beat HU, it is well documented that interferons take time to show prominent activity, so the

study is encouraging. Also, ropeginterferon is administered every two weeks instead of every week as is Pegasys.

2. Pegasys
   Dr. Mascarenhas presented data from the Phase 3 MPNRC study of 168 patients treated with Pegasys or HU. The study did not show clear differences in the primary endpoint of complete hematologic remission nor in other outcomes. As with ropeginterferon, the data were analyzed at the one-year time point.

JAK inhibitors

1. Pacritinib
   In a late-breaking presentation, Dr. Mascarenhas presented an update on the Phase 3 trial of pacritinib in MF. This study of 311 patients compared pacritinib against best available therapy, which included ruxolitinib. Pacritinib was significantly more effective for spleen volume reduction and showed a trend toward superiority with respect to a reduction in total symptom score. The advantage to this agent is that it does not cause thrombocytopenia and thus may be beneficial to patients with low platelet counts. Time will tell whether the drug moves beyond the FDA clinical hold to approval.

2. Momelotinib
   Gilead reported mixed results from their Phase 3 study in mid-November, with non-inferiority to ruxolitinib for spleen volume reduction, but not for a response in total symptom score. At this ASH meeting, Dr. Tefferi presented a 6 year follow up study of patients treated with momelotinib at the Mayo Clinic. More than half of the patients had clinical improvement and nearly half showed an anemia response. However, 81% had discontinued therapy after a median of 2.3 years. Of note, the data demonstrated that patients with the *CALR* mutation had a significantly better spleen response and relapse-free survival as compared to patients with other mutations.

3. NS-018
   NS Pharma reported interim results of a Phase 1/2 study of the JAK inhibitor NS-018 in patients with MF, post-PV MF or post-ET MF. The phase 1 dose escalation aspect of the study has been completed. The data revealed that many patients achieved greater than 50% reduction in spleen volume, with a number of those individuals following discontinuation of a prior JAK inhibitor. There were also improvements in the total symptom score in many patients. At this point it is unclear when the drug will move forward to an advanced clinical trial.

Combination Studies

1. Ruxolitinib plus Azacytidine
   This study, presented by Dr. Daver, examined the effect of combining ruxolitinib with the DNA methyltransferase inhibitor 5-azacytidine (AZA). The combination led to reductions in spleen volume and

total symptom score, which were accompanied by a modest reduction in allele burden and degree of fibrosis. Further studies are warranted to determine if this is a viable and helpful combination.

2. Ruxolitinib plus a PI3K□ inhibitor
   Dr. Moyo presented data from a small phase 1 trial of the combination of the PI3-kinase □ inhibitor TGR-1202 with ruxolitinib in MF. In this small study, the investigators found the combination to be overall well tolerated with an 83% rate of clinical benefit and an improved hemoglobin in some patients. Further studies of this combination are being pursued.

3. Ruxolitinib plus PIM and CDK4/6 inhibitors
   Dr. Rampal presented pre-clinical data from a trial of a three drug combination in animal models of the MPNs. The combination of ruxolitinib, PIM447 (a drug that targets PIM kinases) and LEE011 (a drug that targets the cell cycle by inhibiting CDK4/6), was effective at suppressing the growth of *JAK2* mutant cells in vitro and in vivo. Treatment somewhat reduced the allele burden and prevented the development of fibrosis. The three-drug combination is currently being evaluated in a Phase 1 study.

4. Ruxolitinib plus Decitabine.
   Dr. Rampal presented data from the MPNRC phase 1 study of the combination of ruxolitinib and decitabine for patients with blast phase and post-MPN AML. The combination was found to be associated with cytopenias and infectious complications. Although the overall response rate was 57%, overall survival was poor. Nevertheless, there were several remissions, which support the opening of a Phase 2 study.

<u>Novel Agents</u>

1. Nuclear to cytoplasm transport inhibitor
   A presentation by Dr. Yan revealed that treatment of MPN cells lines with KPT-330 (selinexor), an inhibitor of nuclear to cytoplasmic transport (i.e. the shuttling of proteins such as tumor suppressors out of the cell nucleus), arrested the growth of the cells. The drug also inhibited the growth of CD34+ cells from MF patients. In a Jak2V617F animal model, treatment with KPT330 plus ruxolitinib led to decreased spleen size and improved splenic architecture, but was accompanied by toxicity. Although early, the pre-clinical data support further investigation of this novel strategy to target MPN cells.

2. Sotatercept (ACE-011) as a therapy for anemia in MF
   Dr. Bose presented data from a Phase 2 study of sotatercept, an activing receptor type IIA ligand trap, which allows for enhanced red cell production. In this study, 5 of 14 (36%) evaluable subjects responded to therapy. A combination study with ruxolitinib is in development.

Basic Science Reports

"Basic science" refers to research that is conducted pre-clinically, e.g. before it involved human or animal subjects. It is distinct from "translational science" in this way.

Genetics of Progression

1. CRISPR-Cas9 disruption of *DNMT3A* promotes progression of MF.
   DNMT3A is among the genes mutated in patients who progress from MPN to AML. In an elegant research study, Lane and colleagues used the CRISPR approach to mutate *DNMT3A* in a mouse model of Jak2V617F induced MPN. Although the combination of *DNMT3A* loss and Jak2V617F expression did not result in AML, the animals did show progression from a myeloproliferative stage to a more aggressive myelofibrotic and pancytopenic phase. These animals provide a novel and powerful model to study the progression of MPNs to MF.

2. Activation of *HMGA2* downstream of *EZH2* drives progression of MF.
   Previous studies have shown that combining Jak2V617F with an *EZH2* mutation in mice leads to a more aggressive and progressive disease akin to myelofibrotic progression. Two reports, by the Mohi and Takeishi laboratories, showed that increased expression of *HMGA2*, which is elevated in MF patients, with Jak2V617F leads to an accelerated disease similar to that seen with *EZH2* loss. The results point to *HMGA2* being a potential novel therapeutic target for MF.

3. Loss of *PTPN1* is associated with the MPNs
   Deletion of part of chromosome 20 is a common abnormality in the MPNs. The Mohi lab presented data which suggest that one of the critical genes in the region whose deficiency may contribute to the MPNs is *PTPN1*. In animal models, depletion of *PTPN1* led to an MPN-like disease, and combining the deficiency with Jak2V617F led to a more severe disease than Jak2V617F expression alone. *PTPN1* loss appears to promote MF by enhancing JAK/STAT signaling.

4. *JARID2* as a novel tumor suppressor in MF
   JARID2 is an epigenetic factor which controls the modifications of proteins that bind to DNA. Studies have shown that *JARID2* is mutated in a subset of MPN patients in blast phase of the disease. Using a novel animal model, Celik et al. discovered that *JARID2* participates in normal blood cell development and that its loss cooperated with Jak2V617F to promote a more severe disease with reduced survival.

Thrombosis

1. "Nuclear extracellular traps" (NETs) contribute to thrombosis in MPNs
   Neutrophils release DNA and proteins as a means to contain and kill pathogens, forming structures that are referred to as NETs. Dr. Ebert and colleagues reported that NETs are released from MPN neutrophils at a higher rate than healthy cells and that their formation is suppressed by treatment with ruxolitinib. Experiments in Jak2V617F mice suggest that the increase in NETs is associated with increased thrombus formation.

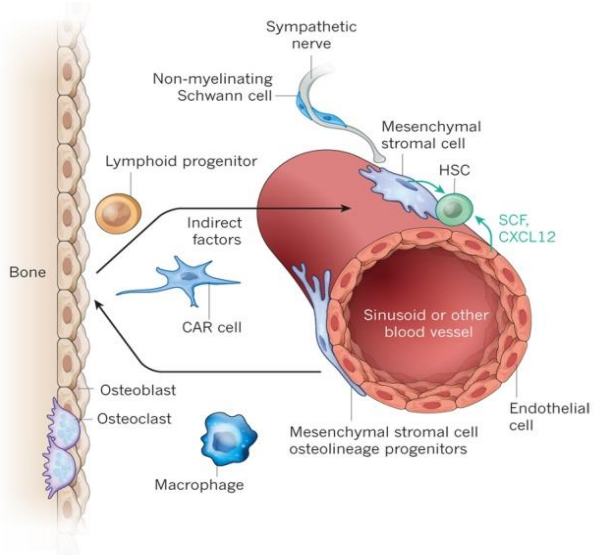
Further studies are needed to solidify this connection and determine whether targeting the NETs reduced thrombotic complications in vivo.

2. Plekstrin-2 is a novel player in thrombosis
   In seeking to better understand the contributions of Plekstrin-2, a gene that plays an important role in red blood cell development, in the MPNs, Ji and colleagues assayed the effect of removing it from Jak2V617F cells. They discovered that the loss of Plekstrin2 increased the survival of Jak2V617F mice, largely by reducing thrombotic complications. Although the mechanism by which Plekstrin-2 contributes to thrombosis is unclear, the study provides exciting new insights into the pathways that contribute to this aspect of the MPNs.

The Niche in MF

1. Characterization of the splenic niche
   Hoffman and colleagues studied the relationship between the splenic microenvironment and cytokines that affect the growth of blood cells. They found that a factor named lipocalin-2 promoted splenic cells to secrete additional factors, such as IL-8 and CXCL1, that in turn accelerate the growth of MPN cells while suppressing the growth of heathy cells. Furthermore, they discovered that Jak2V617F mutant endothelial cells within the spleen supported the expansion of MPN blood stem cells. These results demonstrate that the splenic endothelial cells provide a supportive microenvironment that encourages malignant hematopoiesis.

The Bone Marrow Niche as shown by Nature Magazine



2. Contributions of the JAK2 mutant vascular niche
   This study by Kaushansky and colleagues highlighted the impact of endothelial cells to MF. They reported that Jak2V617F mutant endothelial cells play an important role in the MPN niche and contribute to aberrant growth of MPN cells both in cell culture and animal models. These results provide new insights into the complex nature of the disease and suggests that targeting the supportive cells may lessen the disease burden.

We write these with the patient in mind, so that you can be an involved participant in your care. When you're finished reading this report, we would love to hear your feedback. Send us your comments at **info@mpnresearchfoundation.org**