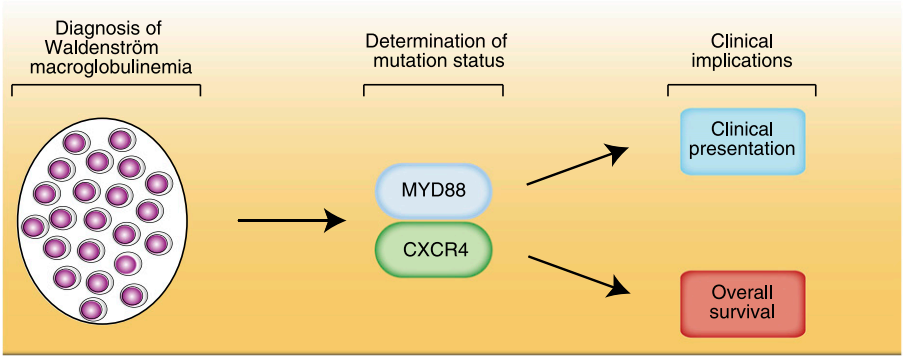

Determination of the *MYD88* and *CXCR4* mutation status in WM has clinical implications.

lymphoma subtypes such as the activated B cell–like subtype of diffuse large B-cell lymphoma (ABC DLBCL) or mucosa-associated lymphoid tissue lymphoma, leads to constitutive activation of the oncogenic nuclear factor-κB signaling pathway.[5-7] CXCR4 is a chemokine receptor that promotes survival of WM cells.[4] Different mutations affecting the C terminus of *CXCR4* can be identified in roughly 30% of WM patients.[4] Although these analyses provided important insights into the molecular pathogenesis of WM, it remained unclear if these genetic aberrations impact clinical presentation or survival of affected patients.

In this issue, Treon et al investigate the clinical implications of *MYD88* and *CXCR4* mutations in WM.[1] Virtually all patients harboring mutations in *CXCR4* also exhibited mutated *MYD88*. Patient samples harboring *CXCR4* mutations were further distinguished based on the mutation pattern (nonsense vs frameshift mutations). Interestingly, the mutation status of *CXCR4* and *MYD88* was associated with significant differences in clinical presentation and outcome (see figure).[1] Patients with mutated *MYD88* and *CXCR4* nonsense mutations exhibited bone marrow infiltration significantly more frequently, had higher serum immunoglobulin M levels, and presented with symptomatic disease, including hyperviscosity, syndrome more frequently. In contrast, patients with mutated *MYD88* and *CXCR4* frameshift or nonsense mutations presented less frequently with adenopathy. Finally, patients that were wild type for both *MYD88* and *CXCR4* were characterized by adverse survival compared with patients with mutated *MYD88* alone or patients that harbored both mutations. Taken together, these results suggest that the *MYD88* and *CXCR4* mutation status determines clinical presentation and outcome of patients diagnosed with WM (see figure).[1]

From a clinical standpoint, the results by Treon et al can potentially be highly relevant for WM treatment. A better understanding which oncogenic pathways are activated and used in WM is a prerequisite for the optimal utilization of novel therapeutic agents. To this end, the determination of the *MYD88* and *CXCR4* mutation status might help us identify novel subgroups of WM that differ with respect to clinical presentation and prognosis. Additionally, these subgroups might benefit differentially to novel therapeutic strategies. Bruton's tyrosine kinase, which interacts with *MYD88*, is a promising target for the treatment of WM, and encouraging results were obtained by using the Bruton's tyrosine kinase inhibitor ibrutinib in relapsed and refractory WM.[8] Similarly, interleukin-1 receptor–associated kinase inhibitors that are being developed for clinical use hold promise for the treatment of WM because *MYD88* coordinates the assembly of a signaling complex consisting of different members of the interleukin-1 receptor–associated kinase family.[5,6] Finally, the use of CXCR4 inhibitors such as plerixafor might be promising in WM patients with mutated *CXCR4*. Importantly, large and well-designed clinical trials with accompanying scientific programs are required to investigate if specific subtypes of WM respond preferentially to novel compounds. This approach will pave the way to more specific and less toxic treatment regimens for patients with WM.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● MYELOID NEOPLASIA

Comment on Goodwin et al, page 2838

Hallway gossip between Ras and PI3K pathways

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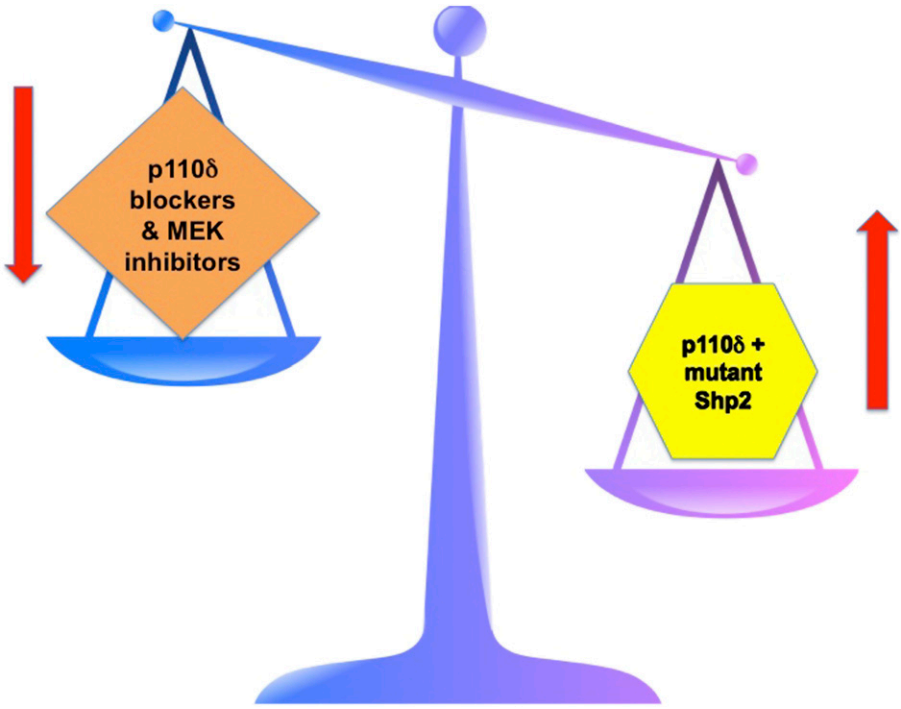
In this issue of *Blood*, Goodwin et al investigate the pathogenesis of juvenile myelomonocytic leukemia (JMML), demonstrating that mutant Shp2 induces granulocyte macrophage–colony-stimulating factor (GM-CSF) hypersensitivity and that the p110δ subunit of phosphatidylinositol 3-kinase (PI3K) further promotes this dysregulation.[1]

JMML has been classified by the World Health Organization as a mixed myeloproliferative neoplasm/myelodysplastic disorder. Many years ago, we demonstrated that a hallmark of JMML was that JMML progenitors displayed an in vitro cellular biology characteristic of a selective, hypersensitive growth pattern to GM-CSF while demonstrating a normal sensitivity to interleukin-3 (IL-3).[2] Subsequent investigations over the ensuing years by several groups have delineated mutations in *NF1*, *RAS*, *PTPN11*, and *CBL* in nearly 85% of JMML patients. These mutations are almost always exclusive of each other, and all of them hyperactivate and dysregulate the Ras signaling pathway, with an end result being the hallmark selective GM-CSF hypersensitivity. No one has yet been able to explain why there is selective hypersensitivity to GM-CSF with IL-3 sensitivity being spared, even though they share a common β subunit to their cell-surface receptor. More recently, additional but secondary mutations have been described in *SETBP1* and *JAK3*.[3] Given this knowledge of one specific pathway appearing to account for the disease phenotype, many have attempted to disrupt JMML signaling via Ras. But, just like other investigators in other cancers, efforts to normalize Ras signaling have fallen dismally short. In 2013, National Cancer Institute Director Harold Varmus initiated a new push to formulate agents capable of blocking Ras.

JMML is not the only proliferative disease of the marrow to show cytokine hypersensitivity. As far back as 1974, Prchal and Axelrad first described hypersensitivity of polycythemia vera (PV) progenitor cells to erythropoietin (Epo).[4] Abnormal erythroid colony growth in vitro was a hallmark of PV long before the *JAK2 V617F* mutation, observed in ~98% of PV patients, took center stage. Epo hypersensitivity has also been noted in primary and familial congenital polycythemia.[5] In at least some of these families, this Epo hypersensitivity is the result of Epo receptor mutations.[6] Additionally, a recent paper shows that the Cbl protein (encoded by the *CBL* gene) interacts with JAK2 and with the p85 regulatory subunit of PI3K.[7] An essential negative regulatory mechanism to turn off Epo signaling occurs when Epo induces Cbl to ubiquitinate the p85 subunit of PI3K, which ultimately leads to Epo receptor endocytosis, which terminates Epo signaling. Mutated Epo receptors, and/or Cbl deficiencies, result in Epo hypersensitivity.



The right side of the scale depicts JMML pathogenesis with Shp2 mutants favoring uncontrolled leukemic cell growth. This is further promoted by the p110δ subunit of PI3K. The left side of the scale depicts the goal of targeted therapy in JMML. By blocking p110δ PI3K and by inhibiting MEK, hopefully the "scales of cell growth" will once again balance out.

The contrary situation is found in JMML, where despite diligent searching, no mutations of either the α or the β subunit of the GM-CSF receptor have ever been found in any JMML patient. Rather, the mutations occur downstream in cytoplasmic signaling components. Several groups, including Goodwin, Chan, and colleagues, previously demonstrated that mutations in *PTPN11*, which encodes Shp2, induces GM-CSF hypersensitivity.[8] In this current paper, they convincingly demonstrate that the p110δ subunit of PI3K helps to promote this dysregulation.[1] Such crosstalk between the PI3K and Ras signaling pathways is not novel.[9] But importantly, demonstrating such crosstalk and further promotion of a dysregulated effect leading to more leukemogenic growth has significant implications in JMML, especially when we are thinking about targeted therapies. Goodwin et al hypothesized that blocking p110δ, along with blocking mitogen-activated protein kinase kinase (MEK) downstream in the Ras pathway, may prove effective in JMML. They present in vitro evidence in Figure 2 in their paper that this might work in a clinical setting. Further, the level of MEK blockade might be able to be reduced, thus dampening the untoward side effects of MEK inhibitors, which have been a recurring theme in clinical trials of various MEK inhibitors in various cancers. The concept for therapy in JMML is one of "balancing the scales" (see figure). *PTPN11* mutations in JMML lead to mutant Shp2 and subsequent hyperactivation of the Ras signaling pathway. In similar fashion, mutations in *NF1*, *RAS*, and *CBL* have similar effects in JMML. Thus, mutant Shp2 tips the scales in JMML in favor of uncontrolled growth that cannot be turned off. The PI3K pathway gets into the act by the p110δ subunit further promoting the effects of Shp2. So, the scales are even more tipped in favor of uncontrolled growth. The hope is that by specifically blocking the p110δ subunit of PI3K and by inhibiting MEK downstream of Ras, we may coordinately be able to in effect rebalance the scales and once again achieve a normal growth pattern.

Combining the specific p110δ inhibitor, idelalisib (which is not yet US Food and Drug Administration approved), with other agents has been recently reported wherein idelalisib was combined with rituximab in chronic lymphocytic leukemia.[10] Currently, the only known effective therapy for JMML is

allogeneic stem cell transplantation. Other forms of chemotherapy, even induction-type therapy, have not been consistently effective in JMML. Because we understand so much regarding JMML pathogenesis and dysregulated signaling pathways, certainly targeted therapy in this disease makes sense. Thus, the horizon appears brighter for effective therapeutics in JMML more so than it probably ever has been. But the cautionary note is that blocking 2 common signaling pathways will require careful study in clinical trials. In our efforts to balance the scales, we do not want to create new problems.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS

Comment on Jun et al, page 2843

Neutrophil energetics and oxygen sensing

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In this issue of *Blood*, Jun et al, through the study of neutrophils deficient in the glucose-6-phosphate transporter, describe a novel role for the peroxisome proliferator-activated receptor-γ (PPARG) pathway in the regulation of key neutrophil functions and link this to concomitant hypoxia-inducible factor (HIF) 1α stabilization.[1]

Glycogen storage disease type Ib (GSD-Ib) is a rare autosomal-recessive condition in which deficiency in the glucose-6-phosphate (G6P) transporter results in a defect in cycling of glucose and G6P between the endoplasmic reticulum and the cytoplasm. Patients with this condition characteristically display a phenotype of neutropenia and neutrophil dysfunction. In the current edition of *Blood*, Jun et al[1] report that viable peripheral blood neutrophils (ie, following exclusion of the significant subpopulation that displays biochemical markers of apoptosis) isolated from patients with GSD-Ib display defective superoxide generation, chemotaxis, and calcium mobilization. In keeping with other previously described disorders of G6P/glucose metabolism, they describe a reduction in basal levels of G6P, lactate, adenosine triphosphate (ATP), and reduced NAD phosphate in these cells. Interestingly, many (although not all) patient samples show increased expression of the oxygen-sensitive transcription factor HIF-1α and also of a nuclear receptor and transcriptional regulator, PPARG. The authors replicated the functional defects in neutrophils from healthy donors by treatment with a PPARG agonist, rosiglitazone, and partially rescued the functional defects observed in the GSD-Ib patient neutrophils with a PPARG antagonist, GW9662.

Together, these data raise interesting questions of broad interest with regard to the regulation of myeloid cell function and the interface with metabolic flux. GSD-Ib patient neutrophils show HIF-1α upregulation in the context of defective cellular energetics. Loss of HIF-1α has previously been linked to global deficiencies in myeloid cell function, where reduced ATP availability is associated with profound functional defects[2] and also with lack of survival under conditions of reduced oxygen availability.[3] It is, therefore, important to be mindful that although Jun et al describe increased expression of HIF-1α in GSD-Ib neutrophils, they do not demonstrate GSD-Ib neutrophil dysfunction to be a direct consequence of HIF-1α stabilization. Attempts to replicate the functional phenotypes in healthy neutrophils with the 2ME2 compound, whose actions include loss of HIF stabilization, were potentially confounded by other described actions of this compound.[4] What is clear is that key neutrophil functions are exquisitely sensitive to changes in intracellular ATP availability and that although the many hundreds of HIF target genes include those regulating cellular glycolysis, and thus ATP generation, the interactions are likely to be complex (see figure). For instance, metabolic intermediates have themselves been shown to regulate HIF stabilization, leading to the potential for disordered glucose cycling to result in disordered HIF activation[5] and inappropriate activation of proinflammatory signaling pathways.[6] Thus, the phenotype observed by Jun et al could equally represent a phenotype of disordered HIF stabilization as a consequence of defective glucose cycling, with the HIF-1α–mediated effects on glycolysis and glucose uptake observed in normal cells being ineffective because of the underlying cellular defect.

A further interesting finding in this paper is the upregulation of PPARG in neutrophils of some GSD-Ib patients. Divergent effects of HIF-1α on PPARG expression are described, with the ability of HIF-1α to either up- or downregulate PPARG in different cell types. Although the effects of PPARG activation have not, to our knowledge, been studied in neutrophils, in macrophages PPARG activation has been shown to be anti-inflammatory and to drive a phenotypic switch toward alternate activation.[7,8] PPARG also regulates cellular metabolism, increasing glucose uptake and lipid anabolism. In cardiomyocytes, an HIF-1α and PPARG axis