of these studies, although these generated some of the stated rationale for the reductions used. Long-term follow-up is vital to assess impact of the randomized dose reductions on fertility, the risk of second malignancies, and other late effects. In summary, "no pain, no gain" may still be true. Entirely novel approaches to therapy coupled with much more precise methods of subclassification are sorely needed.

The author declares no competing financial interests.

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NEOPLASIA

Comment on Aichberger et al, page 3031

Targeting the mast cell: beyond KIT

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In this issue of *Blood,* Aichberger and colleagues identify the antiapoptotic protein Mcl-1 as a novel therapeutic target in mastocytosis and show that inhibition of Mcl-1 potentiates the effects of KIT tyrosine inhibitors on survival of leukemic mast cell lines.

Most patients with systemic mastocytosis have an indolent variant of the disease that does not affect life expectancy, whereas patients in advanced disease categories such as aggressive systemic mastocytosis (ASM) and mast cell leukemia have poor prognosis and are candidates for mast cell cytoreductive therapies. Current options for mast cell cytoreductive therapy including IFN-α2b and cladribine, however, rarely result in complete or durable remissions.[1]

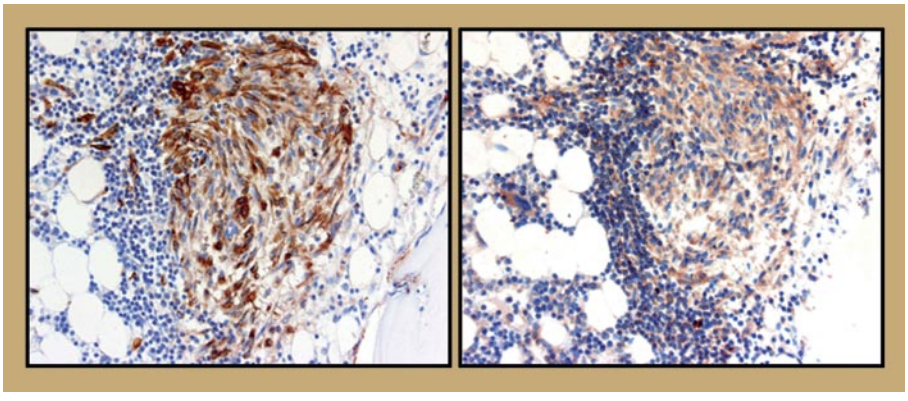
Systemic mastocytosis is associated with gain-of-function mutations of the tyrosine kinase domain of KIT, with one particular mutation, D816V, being detectable in more than 90% of cases.[2] Discovery of small-molecular-weight tyrosine kinase inhibitors capable of inhibiting KIT has consequently generated much interest in exploring their therapeutic potential in mastocytosis. Imatinib, the prototypical drug in this category, inhibits wild-type KIT but cannot bind to KIT bearing the D816V mutation, and is therefore not a good therapeutic option for the great majority of patients with mastocytosis.

Several "second-generation" tyrosine kinase inhibitors with the ability to overcome imatinib resistance conferred by D816V KIT have recently been described.[3] While these drugs effectively kill neoplastic mast cells in the test tube, emerging data from initial clinical trials have thus far shown low rates of complete remission, probably due to the inability of the drugs to reach or sustain sufficiently high tissue levels to result in complete inhibition of the mutated KIT without significant toxicity. Taken together with the possibility that neoplastic mast cells may have additional pathogenic survival mechanisms independent of KIT, these observations highlight the importance of continuing efforts to identify novel approaches to inhibit mast cell growth and survival.

Pathways involved in mast cell apoptosis constitute attractive targets for drug development as neoplastic mast cells are relatively resistant to apoptosis induced by SCF (KIT ligand) withdrawal compared with their normal counterparts.[4] Mcl-1, an antiapoptotic protein induced by SCF, belongs to Bcl-2 family and critically regulates hematopoietic cell survival.[5] The study by Aichberger and colleagues shows that Mcl-1 is expressed in mast cells and multiple other myeloid lineages in patients with mastocytosis (see figure), myelodysplastic syndromes (MDSs), and chronic myeloproliferative disorders as well as in normal bone marrow cells. Inhibition of Mcl-1 RNA expression in mast cell leukemia cell lines with or without D816V mutation resulted in induction of apoptosis. Moreover, Mcl-1 antisense oligonucleotides showed synergistic effects on neoplastic mast cell cytotoxicity when combined with PKC412, a tyrosine kinase inhibitor with activity against D816V KIT.

These results have potential clinical implications as pharmacologic inhibitors of Mcl-1/Bcl-2 are currently evaluated in early-phase clinical trials for hematologic neoplasias. However, it should be noted that complete deficiency of Mcl-1 expression in bone marrow results in abrogation of normal hematopoiesis in mice,[5] and in vivo safety and efficacy of regimens combining inhibitors of KIT and Mcl-1 remain to be determined. As the euphoria over the discovery of KIT tyrosine kinase inhibitors settles, our next challenge in the current state of clinical research is to find the optimal combination of drugs to inhibit mast cell survival in order to get another step closer to a cure for mastocytosis.

The author declares no competing financial interests.



Expression of Mcl-1 in neoplastic human mast cells. See the complete figure in the article beginning on page 3031.

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RED CELLS

Comment on Hsu et al, page 3088

The "NO" tipping point

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Pulmonary hypertension in sickle cell anemia, an important risk factor for early mortality, may be caused by intravascular hemolysis leading to decreased NO bioavailability.

Malcolm Gladwell in his bestseller *The Tipping Point: How Little Things Make a Big Difference* points out how little changes can have big effects.[1] For example, when a new hypothesis that proposes a different way of looking at things catches fire, that idea can ripple outward until a "tipping point" is reached, changing the world. Hsu and colleagues in this issue of *Blood* take the idea that intravascular hemolysis reaches a tipping point, leading to global impairment in nitric oxide (NO) bioavailability causing functional pulmonary hypertension in a murine model of sickle cell disease. These studies further amplify the critical physiological effects of plasma hemoglobin and oxidative stress on vascular responsiveness.

Pulmonary hypertension in patients with sickle cell disease is common and portends a poor survival.[2,3] One proposed mechanism for pulmonary hypertension suggests that intravascular hemolysis releases red blood cell (RBC) hemoglobin and arginase into the plasma, scavenging NO and decreasing arginine substrates, and ultimately leading to reduced NO bioavailability. This lack of NO can alter pulmonary vascular endothelial and vasomotor function. Of importance, the end stage of pulmonary hypertension in sickle cell disease is reflected by pathological intimal and smooth muscle changes in the pulmonary vasculature. Many other factors obviously play a critical role in the human disease including chronic hypoxemia, recurrent infections, fibrosis, recurrent thromboembolism, and heart failure.

To test this hypothesis in mice, Hsu et al use the Berkeley mouse expressing exclusively human α- and β^S-globins. These animals have anemia, sickle RBCs, intravascular and extravascular hemolysis, chronic inflammation, and evidence of excessive oxidative stress. These mice have elevated pulmonary arterial pressures and increased pulmonary vascular resistance. However, unlike the human sickle cell patient with pulmonary hypertension, there was no thickening of the pulmonary arteries, no plexogenic lesion, and no fibrosis noted, but increased intravascular leukocytes were present. These leukocytes may reflect the abnormal rheology in these animals and likely contribute reactive oxygen species promoting oxidative stress within the vessel wall.

Pulmonary hypertension in sickle mice was associated with a global and specific impairment in the pulmonary and vascular responsiveness to both exogenous and endogenous NO. Further proof that hemolysis and oxidative stress were responsible for pulmonary hypertension was shown in normal mice that received a transplant of sickle bone marrow or were induced to hemolyze with alloantibodies. Furthermore, in the sickle mouse, evidence for defects in NO synthase assembly and uncoupling is provided as well as evidence for increased NO consumption. Elevated plasma arginase activity, elevated reactive oxygen species production, and increased tyrosine nitrosylation were identified in the sickle mouse.

This tipping point study adds to the river of evidence that plasma-free hemoglobin, whether derived from hemolysis or hemolyzed RBC transfusion, or given as a therapeutic oxygen carrier, modulates vascular responsiveness.[4] However, care in extrapolating to human sickle cell disease is warranted as pulmonary hypertension takes years, not weeks, to develop in humans and is associated with pathological changes. Also, NO may not be the whole story in pulmonary hypertension in sickle cell patients. Altered vascular prostaglandins, cyclic AMP, and endothelin responses, as well as thromboembolism, probably all conspire. The critical roles of vascular inflammation and oxidative stress, which contribute to altered rheology in sickle cell disease, can also modulate oxidative sensitive NO synthase or tetrahydrobiopterin. Finally, the adaptation to an excess heme load in sickle cell disease and the induction of heme oxygenase-1 may have profound effects on these vascular responses through the release of its products that act as antioxidants (biliverdin/bilirubin, ferritin) or vasodilators (CO).[5]

The author declares no competing financial interests.

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IMMUNOBIOLOGY

Comment on Aksoy et al, page 2887

Brave new world: birth of immunity

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In this issue of *Blood,* Aksoy and colleagues contribute to the understanding of remarkable immunoregulatory controls at the time of birth by showing that impaired synthesis of interferon-β (IFNβ) and IFN-inducible factors elicited by lipopolysaccharide (LPS) depends on the transcriptional activity of interferon regulatory factor 3 (IRF-3) downstream of Toll-like receptor-4 (TLR4).

The human newborn is profoundly susceptible to intracellular bacterial pathogens that require Th1-dependent host defense pathways for eradication. The molecular basis for the newborn's attenuated response to such infections remains a mystery but is likely a consequence of the immunosuppression required for survival in utero.