study demonstrates that THPO/MPL/Bcl-xL signaling is required for RUNX1-RUNX1T1 cells to maintain a steady growth rate.

In a closely related paper, Pulikkan and colleagues identified THPO/MPL signaling as a key cooperating pathway in RUNX1-RUNX1T1–mediated leukemia.[2] Beginning with the observation that MPL is up-regulated in human AML cells that harbor the RUNX1-RUNX1T1 fusion, they assessed the effects of overexpression of MPL alone or MPL with RUNX1-RUNX1T1 in murine hematopoietic progenitor cells. While MPL alone caused a transient expansion in hematopoietic progenitors and expression of RUNX1-RUNX1T1 alone led to leukemia in recipient mice with a median latency of 140 days, coexpression of the 2 genes induced a fully penetrant myeloid leukemia with a mean latency of 50 days. Leukemia cells were found to be hypersensitive to THPO and were characterized by enhanced PI3K/AKT, ERK, and JAK/STAT signaling. Subsequent experiments revealed that JAK2 and PI3K activities are required for survival of the cells in vitro, and that the PI3K pathway in particular is crucial for leukemia progression in vivo. Thus, this study demonstrates that wild-type MPL cooperates with RUNX1-RUNX1T1 to increase survival and tumorigenesis of hematopoietic cells and that the JAK2/PI3K/AKT signaling axis is critical for this effect.

In comparing the 2 studies, there is an interesting difference. Chou and colleagues observed coordinate expression of RUNX1-RUNX1T1 and MPL in both human umbilical cord progenitors and murine fetal liver cells, whereas Pulikkan et al did not detect up-regulation of MPL gene expression on RUNX1-RUNX1T1 expression in hematopoietic progenitor cells. Nevertheless, human AML cells with the t(8;21) indeed appear to express higher levels of MPL compared with other subtypes. Precisely how RUNX1-RUNX1T1 leads to increased MPL is thus an open question. Does the fusion protein directly bind to the MPL gene and up-regulate expression or does it repress a critical negative regulator of MPL transcription (see figure)? Or are there both transcriptional and posttranscriptional mechanisms at work?

Together, these exciting papers demonstrate that increased signaling through wild-type MPL is a crucial cooperating event in RUNX1-RUNX1T1–induced leukemogenesis. Although the MPL/THPO axis is primarily associated with megakaryopoiesis, MPL is expressed on hematopoietic stem cells and these cells may thus depend on JAK/STAT signaling.[5,6] Activation of the JAK/STAT pathway through mutagenesis of *JAK2*, *MPL*, or *LNK* is associated with several hematologic malignancies, most notably the myeloproliferative neoplasms.[7] Although activation of STAT3 is common in de novo AML, mutations in *JAK2*, *MPL*, and other genes in the pathway are rare.[8] The observations provided in these reports by Chou et al and Pulikkan et al provide a mechanism by which increased expression of wild-type MPL can increase self-renewal and proliferation along with enhanced downstream signaling. These studies therefore provide incentives for rational testing of JAK, Bcl-xL, and PI3K/AKT inhibitors in AML cases with the t(8;21). These reports also beg the question of whether increased expression of wild-type MPL may contribute to myeloproliferative neoplasms that lack activating mutations in *JAK2* or *MPL*.

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

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● ● ● CLINICAL TRIALS

Comment on Josephson et al, page 748

PLADO and kids: earlier/increased bleeding after HSCT

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In this issue of *Blood*, Josephson et al report age group analyses of patients enrolled in the prophylactic platelet dose trial (PLADO) that evaluated the relationship between platelet dose per transfusion and bleeding in hospitalized patients with treatment-induced hypoproliferative thrombocytopenia.[1]

Patients, both pediatric and adult, who receive hematopoietic stem cell transplants (HSCTs) and/or chemotherapy for leukemia or other malignancies experience hypoproliferative thrombocytopenia rendering them at risk for bleeding. Although there is evidence that prophylactic platelet transfusions reduce the incidence of bleeding in these patients, there is a paucity of available information with regard to adequate platelet dosing per transfusion as well as a general lack of data that could be compared with current treatment and supportive care regimens. To this end, a secondary analysis of the PLADO clinical trial was conducted to determine whether bleeding outcomes differed among 4 distinct age groups: 3 pediatric (0-5 years, 6-12 years, and 13-18 years) and adults (> 18 years). Hospitalized patients were randomized to 1 of 3 platelet doses: 1.1×10^{11}, 2.2×10^{11}, or 4.4×10^{11} platelet/m^2/transfusion, administered for morning platelet counts of $< 10\,000/\mu L$, and daily hemostatic assessments were performed. The primary end point was the percentage of patients who developed grade 2 or higher bleeding based on the World Health Organization scale, and a total of 198 children (0-18 years) and

1044 adults were evaluated. Platelet dose was not predictive for bleeding in any age group; however, children in all age groups had a significantly higher risk of grade 2 or higher bleeding (grade 3 but not 4) versus adults, earlier bleeding after HSCT, and more days of grade 2 bleeding than adults. Moreover, the effect of age on grade 2 bleeding differed by disease treatment, with autologous transplants being the most pronounced. Josephson et al concluded that children were at higher risk of bleeding over a wide range of platelet counts, indicating that this increased bleeding risk was likely due to factors other than the platelet counts.

On the surface these data appear surprising because most hospitals that transfuse pediatric subjects employ specific dosing with a mL/kg or random donor equivalent/5-10 kg whereas adults often receive a single apheresis unit defined as $> 3 \times 10^{11}$ platelets/unit (*AABB Standards for Blood Banks and Transfusion Services*[2]). Thus, adults in a population with increasing body mass with successive generations could lead to "underdosing" in larger patients and increased bleeding due to relatively fewer platelets given per transfusion. In direct contrast, the PLADO analysis did not demonstrate that bleeding correlated with (1) the morning platelet count, (2) prophylactic platelet dose, or (3) that pediatric patients would benefit from a higher prophylactic transfusion trigger, suggesting that factors other than platelets appear to impart the bleeding risk in children. These factors likely include the treatment intensity as illustrated by the most pronounced bleeding differences in children receiving autologous HSCTs (vs adults) because the former received "conditioning" regimens for solid tumors, specifically neuroblastoma, brain tumors, and Wilms tumor, while the adults received pretransplant treatment for multiple myeloma or lymphoma. When analyzed against the other children in this study in the different disease categories, the children who underwent autologous/syngeneic HSCT had similar to increased bleeding risk whereas the adults who received autologous/syngeneic HSCT had markedly lower risk of bleeding than the other adult patients in the different treatment groups. Chemotherapy-induced mucositis may also have increased bleeding risk in children because they experienced more oral, nasal, and gastrointestinal bleeding versus adults who had more skin, soft tissue, and musculoskeletal bleeding.

Children are different from adults because they tolerate more intensive chemotherapy, may have discordant structure and function of their vascular endothelium, and may have different rates of vascular regeneration after treatment-based injury resulting in accelerated platelet consumption and increased bleeding events.[3-5] Josephson et al appropriately allude to these differences; however, other factors may impart bleeding risks including fevers, infection, and graft-versus-host disease (GVHD). The PLADO study design precluded GVHD because it was initiated immediately after HSCT, and patients were followed to day 36, which would be prior to when most patients develop GVHD, although 3 patients did manifest GVHD, which was likely acute GVHD. In addition, allergic rhinitis as a cause of epistaxis independent of platelet count was not studied and although it may represent the cause of epistaxis for many children, it would likely not impact the PLADO data because significant myelotoxicity is known to decrease allergic and atopic reactions. Future randomized controlled trials should account for the possible increased bleeding risks in febrile or infected patients as well as the effects of GVHD and aggressive treatment regimens for solid tumors, especially in pediatric patients, and must increase the sample size to ensure robust statistical analyses of the data.

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

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Shibeko et al, page 891

Factor VIIa: on its own and loving it

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In this issue of *Blood*, Shibeko et al have used a variety of experimental studies and mathematical models to investigate the mechanism of action of high-dose factor VIIa as a bypassing agent in hemophilia.[1]

These studies address a vexing intellectual problem regarding the use of factor VIIa as a bypassing agent in hemophilia. The activity of factor VIIa, like that of coagulation factors IXa and Xa, is significantly enhanced when bound to its coagulation cofactor. Thus, factor IXa and factor VIIIa form a functional complex in vivo and the absence of either protein results in hemophilia. Similarly, lipid surface factor Xa activity is enhanced several thousand-fold when bound to factor Va and the complex of factor Xa with factor Va is required for physiologic thrombin generation. Because factor VIIa activity is significantly enhanced in the presence of its cofactor, TF, one might suspect that relatively low doses of factor VIIa would saturate available TF and provide the needed hemostatic activity.

In treating hemophilia patients with inhibitors, some of the initial dosing strategies included administering as little as 35 μg/kg factor VIIa. This dose was predicted to lead to levels on the order of 0.5 mg/mL in plasma; a concentration roughly equal to the plasma concentration of factor VII (10nM). A dose of 35 μg/kg was effective at stopping bleeding in some patients but was suboptimal in many others.[2] Clinical studies comparing doses of 35 μg/kg against 70 μg/kg, 90 μg/kg, and 120 /kg suggested that increasing dose were, in general, associated with increasing efficacy.[2,3] Given that TF is almost certainly limiting in vivo, it was unclear why higher doses gave greater efficacy.

Biochemical studies approached this question and 2 theories emerged (see simplified