malignancies, and therefore ignores any possible genetic or familial predisposition to leukemia development. It is difficult to see, however, how this potential confounding factor can be bypassed in the real world.

Although this study is indeed reassuring, there is an ongoing need for surveillance and caution. A flurry of reports on possible effects of rhG-CSF on chromosomal integrity and gene expression have been published in recent years and have been recently reviewed. The effects described on gene expression were self-limiting and transient. In addition, these studies are small, have been subject to criticism, and have not been widely replicated so far, and should be viewed as inconclusive. Still, safeguarding donor safety is such a high priority that one can only welcome efforts to evaluate these leads. The United Kingdom Donor Registries will have a study to screen normal donors treated with recombinant human granulocyte colony-stimulating factor for long-term genetic damage using interphase fluorescence in situ hybridization and array comparative genomic hybridization analysis. Marrow donors will be used as negative controls and patients with hematologic malignancies as positive controls. The NMDP and University of Minnesota are working on a similar study. Conflict-of-interest disclosure: The author declares no competing financial interests.

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

In the first part of this study, the authors describe findings in rats treated with romiplostim at up to 10 times the recommended dose for treatment of patients with chronic ITP. All of the animals showed dose-dependent increases in platelet count with a relatively short treatment course (4 weeks); a subgroup of animals was evaluated 4 weeks after discontinuation of therapy. Within 4 weeks, many of the rats showed dose-dependent changes in bone marrow; both an increase in bone marrow fiber content and hyperostosis, suggestive of collagen fibrosis (although trichrome staining was not done). All of these changes resolved after a 4-week washout period with none of the animals showing residual changes. These findings suggest that, in animals at least, romiplostim actually does alter marrow fiber content, but if treatment is short, the changes induced are reversible. Certainly a longer course of therapy in this animal model is needed to determine whether eventually, these changes become progressive or irreversible.

Next, the authors present data from 2 clinical trials: a retrospective analysis of 271 patients with chronic ITP (treated June 2002 to January 2008) who received different doses of romiplostin and a smaller, prospective study in which bone marrow biopsies were obtained before initiation of treatment as well as at 3, 6, and 9 months after initiation of therapy. Photomicrographs were reviewed by an expert panel to determine level of bone marrow reticulin. In the retrospective study, bone marrow examination was left to the discretion of the investigators and only 11 patients had at least one sample. Of these, 10 patients showed reticulin fiber formation. Given the study design, one can say only that reticulin fiber formation in patients treated with this TPO-mimetic is somewhere between 4% (10 of 271 total patients enrolled) and 91% (10 of 11 who had bone marrow studies). The available data suggest that the real incidence may be closer to 5% to 10%. Of the 10 patients who were positive for reticulin staining, 6 received higher than the currently recommended 1 to 10 μg/kg dosing. Five of these patients had both a pretreatment and on-treatment marrow evaluation. Four of these showed an increase in reticulin formation on treatment, and 3 patients had subsequent bone marrow studies that

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TPO-mimetics and myelofibrosis? A reticulin question!

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In this issue of Blood, Kuter and colleagues present further information about the incidence and temporal course of bone marrow reticulin fiber formation after treatment with a TPO-mimetic.

Reticulin fiber formation is present to some degree in normal bone marrows of healthy adults, whereas collagen fibrosis (trichrome staining) is considered to be abnormal and associated with more severe disease. Both reticulin fibrosis and trichrome staining have been associated with myelofibrosis and other hematopoietic disorders. A study by Mufti et al showed that patients with immune thrombocytopenia (ITP) have normal reticulin fiber formation in the bone marrow so that excitement over potential benefits of thrombopoietin (TPO)–mimetic drugs in the treatment of ITP has been tempered by emerging reports of bone marrow fibrosis. These reports were especially concerning because previous animal data suggested that long-term stimulation of bone marrow with TPO (by virally induced overexpression) resulted in a phenotype that resembled primary myelofibrosis. In this issue of Blood, Kuter and colleagues present further information about the incidence and temporal course of bone marrow reticulin fiber formation after treatment with the TPO-mimetic romiplostim both in an animal model and in ITP patients. Romiplostim is a hybrid protein that contains a TPO receptor–binding region conjugated to the Fc portion of an antibody (peptibody).
showed a decrease in reticulin staining with discontinuation of the study drug.

The prospective study involved 10 patients who received doses of romiplostim that were between the recommended 2 to 10 μg/kg. Six patients had both pretreatment and posttreatment bone marrow studies that could be evaluated for reticulin fiber formation. Only one patient had clear increase in reticulin fiber formation upon treatment, which then remained stable for the remaining months. This patient remained on romiplostim and had no change in response to therapy or in other blood counts.

These preliminary studies suggest that bone marrow reticulin fiber formation after TPO-mimetics is real and can occur in some patients receiving the recommended dosage. This fibrosis is reversible after short-term treatment and may be dose related. This is consistent with a previous study in patients with acute myeloid leukemia treated with TPO. Preliminary evidence is also presented that perhaps long-term treatment may not cause continuously worsening fibrosis if the dose is within the presently recommended range. Certainly these studies are encouraging, but further studies will not only need to systematically and prospectively evaluate the consequences of short-term administration of these medications, but also address the long-term consequences of treatment with any TPO-mimetic drugs. For this reason, caution is still required when considering long-term management of patients with chronic ITP with TPO-mimetics, and patients should be made aware of this potential complication.

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**HIV infection: TRAILing the killers**

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Progressive loss of CD4+ T cells is a hallmark of HIV infection, but its mechanism remains poorly understood. In this issue of *Blood*, Stary and colleagues show that CD4+ T cells from viremic patients undergo apoptosis in response to death-inducing signals generated by TRAIL expressed on the surface of plasmacytoid dendritic cells.

HIV infection is characterized by progressive depletion of both infected and uninfected CD4+ T cells, which leads to the development of AIDS. Increased apoptosis is regarded as the primary cause of HIV-induced CD4+ T-cell loss, and multiple mechanisms have been brought forward to explain the immunopathogenesis of this apoptotic process.1 Whereas direct cytopathic effects affect the survival of infected CD4+ T cells, indirect mechanisms, such as activation-induced cell death, are likely to play a major role in the elimination of uninfected CD4+ T cells.1,2 Activation-induced cell death may involve an augmented responsiveness of CD4+ T cells to various inhibitory and death-inducing ligands such as PD-1L, CTLA-4, and FasL.3,4 Recent evidence points to an additional contribution of TNF-related apoptosis-inducing ligand (TRAIL).5,6

Soluble TRAIL is elevated in the plasma of HIV-infected patients and CD4+ T cells from these patients are more sensitive to TRAIL–induced death signals. A possible source of TRAIL is plasmacytoid dendritic cells (pDCs), a professional interferon (IFN)–producing dendritic cell subset that usually plays a key role in antiviral immunity.6 In infected patients, pDCs release massive amounts of IFN-α in response to HIV, but cannot achieve the control of the infection. Rather, in vitro studies indicate that pDCs up-regulate TRAIL expression in response to HIV–induced IFN-α and thereafter acquire cytotoxic activity on bystander CD4+ T cells.6 Of note, the up-regulation of TRAIL expression by HIV–exposed pDCs is highly dependent on sensing viral single-stranded RNA through the intracellular sensor Toll-like receptor 7 (TLR7).6

In this issue, Stary and colleagues sought to extend these in vitro findings to an in vivo setting and explored the presence of TRAIL–expressing killer pDCs in HIV-infected persons.7 They found that pDCs and CD4+ T cells from infected patients expressed TRAIL and its cognate receptor TRAIL–R1, respectively. TRAIL expression directly correlated with viremia, whereas there was an inverse correlation between TRAIL–expressing pDCs and CD4+ T cells. Remarkably, TRAIL–expressing pDCs were proximal to apoptotic CD4+ T cells in tissue sections from systemic lymph nodes. In vitro, pDCs from viremic patients, but not pDCs from aviremic or noninfected persons, triggered death of activated CD4+ T cells through a mechanism that required TRAIL, and to a lesser extent IFN-α. This study clearly shows that TRAIL–expressing killer pDCs are present in vivo and likely play an important role in the loss of CD4+ T cells.

Stary and colleagues’ findings also raise intriguing questions. For instance, it is remarkable that, although expressing comparable or higher surface levels of TRAIL, monocytes and myeloid dendritic cells (mDCs) from viremic patients do not have cytotoxic activity on activated CD4+ T cells. One possible interpretation is that monocytes...
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