

To the editor:

Priming reloaded?

The chemosensitizing effect of CXCR4 antagonists was recently demonstrated in 2 elegant studies using models of acute promyelocytic leukemia (APL)¹ and multiple myeloma.² Both studies show tumor reduction and prolonged survival in tumor-bearing/leukemic mice treated with CXCR4 antagonist AMD3100 in combination with chemotherapy compared with treatment with either drug alone. We studied the effects of 3 drugs antagonizing hematopoietic stem cell (HSC)–stroma interactions (granulocyte-colony stimulating factor [G-CSF], AMD3100,

and MOL27575, a small molecule VLA-4 antagonist³) in the aggressive MN1 leukemia model^{4,5} to investigate their role in leukemia stem cell (LSC) mobilization. Mice transplanted with *MN1-IRES-GFP*–transduced bone marrow cells were treated on 3 consecutive days with G-CSF (10 μ g/kg per day subcutaneously, last dose 24 hours before tissue harvest, $n = 4$), AMD3100, and MOL27575 (each 5 mg/kg per day subcutaneously, last dose 1 hour before tissue harvest, $n = 5$ and $n = 4$, respectively). AMD3100 treatment resulted in an increased proportion of leukemic cells (GFP⁺) in peripheral blood compared with control mice (Figure 1A). Next we determined the LSC frequency in peripheral blood and bone marrow in each treatment group by competitive repopulation unit (CRU) assays. 5, 50, 500, 5000, or 50 000 leukemic (GFP⁺) cells were transplanted to secondary mice along with a life-sparing dose of normal bone marrow cells (3 mice/cell dose). The LSC frequency was determined by Poisson statistics from the proportion of leukemic versus nonleukemic mice. The frequency of LSCs within the total leukemic cell population was not significantly different between bone marrow and peripheral blood in any of the treatment groups (Figure 1B). Thus, whereas AMD3100 increased the number of leukemic cells in peripheral blood, we did not observe a preferential mobilization of LSCs over their progeny. Treatment of MN1 mice starting 1 week after transplantation with cytarabine (5 or 50 mg/kg per day) with or without AMD3100 (5 mg/kg per day) for 5 consecutive days did not prolong survival of mice compared with solvent-treated mice ($n = 3$ evaluable mice per group), but was associated with fatal toxicity in the cytarabine 50 mg/kg group in 4 of 7 mice (data not shown).

Disruption of the CXCL12/CXCR4 axis releases HSCs from their niche, and is now being tested clinically to release LSCs from their niche environment to increase chemosensitivity.⁶ Twenty years ago the cell-cycle promoting effects of G-CSF in leukemic cells stimulated its use as a priming agent in AML patients undergoing induction chemotherapy. Only recently it has been shown that the stem cell–mobilizing effect of G-CSF is CXCR4-dependent.⁷ Priming with G- or GM-CSF in more than 4000 AML patients has not resulted in improved survival.⁸ However, it has been suggested that standard risk patients may benefit from G-CSF priming.⁹

Are 20 years of priming studies about to be reproduced under the concept of cell-to-microenvironment disruption? There may be additional functions of CXCR4 antagonists compared with G-CSF. However, G-CSF priming studies are instructive in 2 ways: first, a direct comparison of CXCR4 antagonists with G-CSF may give an early indication of superiority of CXCR4 antagonists, and, second, chemosensitizing effects of CXCR4 antagonists may be restricted to cytogenetic and molecular subgroups of AML. The latter is supported by the different treatment effects of AMD3100 in the MN1 model compared with the APL mouse model reported by Nervi et al.¹ We suggest

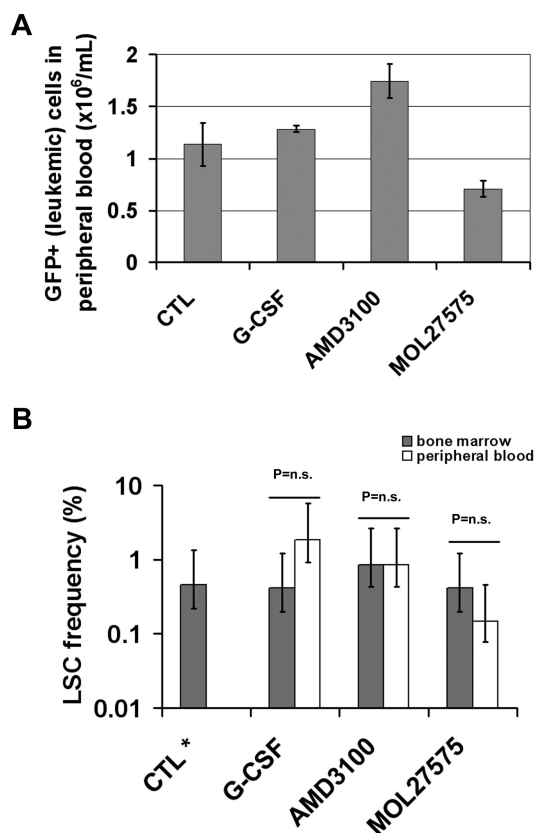


Figure 1. Leukemia stem cell mobilization in the MN1 leukemia model. (A) Number of transduced white blood cells in peripheral blood of control or drug treated mice. Eighteen or 19 days after transplantation of *MN1*-transduced bone marrow cells to lethally irradiated mice, solvent (CTL), G-CSF, AMD3100, or MOL27575 were injected subcutaneously for 3 consecutive days. Peripheral blood was harvested and pooled from 4 to 5 mice per group, and white blood cells were counted and immunophenotyped for the proportion of GFP⁺ (leukemic) cells (95% confidence intervals do not overlap between CTL and AMD3100 indicating a significant difference). (B) Results of CRU assays to determine the leukemia stem cell frequency in peripheral blood and bone marrow of G-CSF, AMD3100, or MOL27575 treated mice. Peripheral blood or bone marrow from 4 to 5 mice per treatment group were pooled and transplanted by limiting-dilution analysis (5 cell doses of GFP⁺ cells, 3 mice per cell dose). LSC frequency was calculated from the proportion of leukemic mice by Poisson statistics. Overlapping 95% confidence intervals indicate no statistically significant difference. *LSC frequency in bone marrow of a historical control group is shown for comparison.

that cytogenetic and molecular subgroups be carefully characterized in current and future trials using CXCR4 antagonists.

Michael Heuser

Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation,
Hannover Medical School,
Hannover, Germany
and Terry Fox Laboratory,
British Columbia Cancer Agency,
Vancouver, BC

Florian Kuchenbauer

Terry Fox Laboratory,
British Columbia Cancer Agency,
Vancouver, BC

Bob Argiropoulos

Terry Fox Laboratory,
British Columbia Cancer Agency,
Vancouver, BC

Sanja Sekulovic

Terry Fox Laboratory,
British Columbia Cancer Agency,
Vancouver, BC

Malina Leung

Terry Fox Laboratory,
British Columbia Cancer Agency,
Vancouver, BC

Marcin Stasiak

Gilead Sciences,
Seattle, WA

Arnold Ganser

Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation,
Hannover Medical School,
Hannover, Germany

R. Keith Humphries

Terry Fox Laboratory,
British Columbia Cancer Agency,
and Department of Medicine,
University of British Columbia,
Vancouver, BC

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Correspondence: R. Keith Humphries, MD, PhD, Terry Fox Laboratory, British Columbia Cancer Agency, 675 West 10th Ave, Vancouver, BC, V5Z 1L3 Canada; e-mail: khumphri@bccrc.ca.

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Response

Sensitization initiated

In their letter to the editor, Heuser et al showed that acute myelogenous leukemia (AML) cells are mobilized into the peripheral blood using the CXCR4 inhibitor AMD3100 (Plerixafor; Genzyme Inc).¹ Interestingly, they further studied the effect of this agent on the mobilization of leukemia stem cells (LSCs) in their model system. Their results showed no significant difference in the number of LSCs present in the bone marrow or peripheral blood using granulocyte colony-stimulating factor (G-CSF), AMD3100, or the VLA-4 inhibitor MOL27575. The authors also investigated the activity of AMD3100 in combination with low- or high-dose cytarabine and showed there was no additive effect of cytarabine. Although there are several possible explanations for the results, the investigators indicated that AMD3100 is nothing but a “reload” of G-CSF, and that the 20 years of experience using G-CSF to prime leukemia cells has failed to show improvement in the mortality of patients with AML. They also indicated that the activity of G-CSF and AMD3100 is similar through their effects on the CXCR4/SDF-1 axis.²

Several years ago, studies demonstrated that G-CSF induced a gradual decrease in SDF-1 in the bone marrow through degradation by neutrophil elastase,^{3,4} thereby leading to stem cell mobilization through the CXCR4/SDF-1 axis. More recent studies have shown that AMD3100 leads to mobilization of hematopoietic stem cells (HSC), even after failure of mobilization by G-CSF, indicating a

different level of activity of AMD3100 and G-CSF.^{2,5} In addition, AMD3100 enhanced the activity of G-CSF when used in combination, which led to the approval of this agent in the mobilization of HSCs in patients with multiple myeloma (MM) and lymphoma.⁶ Therefore, the notion that AMD3100 is just another G-CSF is not supported by many elegant scientific studies, which have shown that the inhibition of CXCR4 activity is biologically different from the neutralization of SDF-1 activity in the bone marrow. However, further studies are required to examine in depth the mechanistic differences in stem cell mobilization between G-CSF and AMD3100.

The interaction of cancer cells with their microenvironment in the bone marrow (BM) provides a protective environment and resistance to therapeutic agents.^{7,8} We have recently demonstrated that interrupting the CXCR4/SDF-1 axis through inhibition of CXCR4 by AMD3100 leads to mobilization of malignant cells from the BM and increases their sensitization to therapeutic agents.^{9,10} Contrasting with the nonadditive effect of AMD3100 and cytarabine in AML that was shown by the authors, we found in our study a significant enhancement of the effect of bortezomib, dexamethasone, doxorubicin and melphalan in vitro and the effect of bortezomib in vivo in MM.⁹ These differences may be explained by the timing and dosing of bortezomib and AMD3100, as well as the biologic differences between MM and AML cells. In our study,