Blood and Marrow TRANSPLANTATION

REVIEWS

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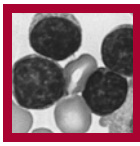

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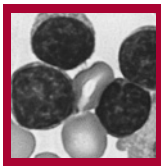
Perhaps You *Can* Get Blood Out of a Turnip After All

by John R. Wingard

The remarkable early success of G-CSF in effective stem cell mobilization was both surprising and fortuitous. Only years later do we have a good (yet still incomplete) idea of why and how it happens. For a number of years, many investigators (and companies) tested new agents to improve on G-CSF, but repeatedly these efforts have come up short in providing a clinically useful therapeutic. Indeed, it has seemed we are stuck. While G-CSF is effective in many normal donors and patients, there are some unmet needs: the mobilizing effect is hetereogenous in normal donors with an occasional healthy donor who cannot be mobilized; worse, heavily pretreated patients often cannot be mobilized successfully at all. As to the former, it has been suggested that polymorphisms of G-CSF receptors or secondary mediators is explanatory. As to the latter, the prevailing thought has been that there has been an "exhaustion" of the stem cell pool from prior therapy's stem cell toxicity, and if so, no therapeutic would likely be able to get blood out of a turnip.

Will a new contender, AMD-3100, prove these concepts wrong? In this transcript of a symposium held at the BMT Tandem Meetings in February 2005, studies of AMD-3100 are described. AMD-3100 is a selective antagonist of the CXCR4 chemokine receptor that blocks binding of stromal cell-derived factor 1α. This blocking results in interference of stem cell trafficking and retention in the marrow. Dr. Broxmeyer discusses the effects of AMD-3100 in preclinical animal models of short-term and long-term repopulation and describes early clinical studies of the effects of AMD-3100 in humans both alone and with G-CSF. Dr. DiPersio describes the effects of AMD-3100 in animals to explore effects on graft-versus-host disease, an important consideration for allogeneic HCT since AMD-3100 also alters T-cell trafficking. Dr. Flomenberg reports preliminary findings from a trial to evaluate the effects of the combination of AMD-3100 and G-CSF in comparison with G-CSF alone and notes an important observation, that successful mobilization was achieved with the combination in some patients who could not be mobilized with G-CSF alone.

So, will the old saying *"You can't get blood out of a turnip"* prove true, or will agents such as AMD-3100 change this? Only further clinical testing will tell. The greatest promise may be offering new hope for the hard-to-mobilize patient that every clinical transplanter sees all too often. At the very least, such agents affecting the CXCR4/SDF-1α axis have proven to be informative probes into stem cell homing and retention by the marrow, and animal and human testing with this class of agents are providing important new insights.



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American Society for Blood and Marrow Transplantation

Executive Office
American Society for Blood and Marrow Transplantation
85 West Algonquin Road, Suite 550
Arlington Heights, IL 60005-4425
(847) 427-0224; fax (847) 427-9656
e-mail: mail@asbmt.org

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PRELIMINARY APPLICATION

Be a part of a national organization established to promote education, research, and medical development in the field of blood and marrow transplantation.

Full Membership is open to individuals holding an MD or PhD degree with demonstrated expertise in blood and marrow transplantation as evidenced by either the publication of two papers on hematopoietic stem cell transplantation–related research as recorded by curriculum vitae, or documentation of two years of experience in clinical transplantation as recorded by curriculum vitae or letter from the director of a transplant center attesting to the experience of the candidate.

Associate Membership is open to individuals with an MD or PhD degree who otherwise do not meet the criteria for full membership.

Affiliate Membership is available to allied non-MD or non-PhD professionals who have an interest in blood and marrow transplantation. This category is especially appropriate for nursing and administrative staff of bone marrow transplant centers, collection centers, and processing laboratories, and for professional staff of corporations that provide products and services to the field of blood and marrow transplantation.

In-Training Membership is open to fellows-in-training in bone marrow transplantation programs. A letter from the transplant center director attesting to the applicant's training status is required.

Included in the membership fee is a one-year subscription to ***Biology of Blood and Marrow Transplantation.***

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Register Online for BMT Tandem Meetings in Hawaii

Online registration and housing reservations are open for the 2006 BMT Tandem Meetings that will be held February 16-20 in Honolulu.

On a single Web page, registrants can navigate to meeting pre-registration, housing reservations, preliminary program, abstract submission, travel discounts and local tours. Information also is provided that compares the cost of travel and lodging for Hawaii versus other U.S. convention cities.

The ASBMT Web site is at *www.asbmt.org*.

Updated Guidelines Released for Transplant Consultation Timing

The National Marrow Donor Program and ASBMT have updated and re-issued "Guidelines on Recommended Timing for Transplant Consultation."

The recommendations offer prognostic factors for patients at risk of disease progression using standard therapy, and provide criteria for identifying patients who should be evaluated for possible transplantation.

The guidelines, presented on page 14 of this issue of *Blood and Marrow Transplantation Reviews*, are based on current clinical practice, the medical literature and recent ASBMT evidence-based reviews. They are intended for use in patient counseling and initial discussion during development of a treatment plan that may include transplantation.

NMDP Launches Web Site To Guide Patients and Families

The National Marrow Donor Program (NMDP) Office of Patient Advocacy has launched a new Patient Resources Web site to provide information and resources to transplant patients and their families.

The patient resources site includes information that can help patients and their families:

- talk with their doctors
- choose a transplant center
- understand the role of the caregiver
- manage financial or insurance matters
- prepare for life after transplant
- connect with other organizations that can help

"Dealing with a major illness creates tremendous stress that can affect the whole family," Elizabeth Murphy, director of the Office of Patient Advocacy, said. "Patients and their families need information, resources and support to help them understand their treatment options and make informed decisions about their care. The office is available to provide that help and relieve some of that stress."

The Office of Patient Advocacy is staffed with trained case managers with a variety of backgrounds such as clinical social work, public health and patient education. They provide one-on-one guidance throughout the transplant process—from diagnosis through survivorship—and in some cases act as direct liaisons, connecting patients to other resources.

All NMDP patient advocacy services and resources are free and also available to patients and families outside of the United States. Call toll-free (888) 999-6743 or (612) 627-8140 outside of the United States or e-mail *patientinfo@nmdp.org*. Language interpreters are available.

ASBMT

Symposium Report

Blood and Marrow TRANSPLANTATION REVIEWS

CXCR4 Chemokine Receptor Blockade: A New Strategy for PBSC Mobilization

Adapted from a CME symposium presented at the American Society for Blood and Marrow Transplantation and the Center for International Blood and Marrow Transplant Research 2005 BMT Tandem Meetings, on February 13, 2005, in Keystone, Colorado. This program is supported by an unrestricted educational grant from AnorMED Inc.

MEDICAL COLLEGE OF WISCONSIN CME program

Faculty

Hal E. Broxmeyer, PhD
Chairman and Mary Margaret Walther Professor of Microbiology/Immunology
Indiana University School of Medicine, Indianapolis, Indiana

John DiPersio, MD, PhD
Chief, Division of Oncology
Washington University School of Medicine, St. Louis, Missouri

Neal Flomenberg, MD
Chief, Division of Medical Oncology
Thomas Jefferson University, Philadelphia, Pennsylvania

Faculty Disclosure

As an accredited CME provider, the Medical College of Wisconsin must ensure balance, independence, objectivity, and scientific rigor in all its individual or jointly sponsored educational activities. The authors who contributed to this publication have disclosed the following relationships:

Hal E. Broxmeyer, MD, has indicated that he is a consultant and a grant recipient from AnorMED Inc., is a member of the Scientific Advisory Board for ViaCell, Inc., and is on the Board of Directors for the National Disease Research Interchange (NDRI).

John DiPersio, MD, has indicated that he is a clinical investigator and is involved in preclinical laboratory studies for AnorMED Inc.

Neal Flomenberg, MD, has indicated that he is a clinical investigator for AnorMED Inc.

Continuing Medical Education Credit

The Medical College of Wisconsin is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The Medical College of Wisconsin designates this educational activity for a maximum of 1.0 category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in the educational activity.

Needs Assessment

The investigational drug AMD-3100 is a new stem cell mobilizing agent that has the potential to change current clinical practice in the field of stem cell mobilization. In the past year, clinicians have repeatedly demonstrated an interest in AMD-3100 and learning how the mechanism of action differs from currently available agents and approaches. This publication will provide physicians with an update of recent clinical trials of the investigational drug AMD-3100, as well as elucidate its proposed mechanism of action.

Target Audience

This program will be of value to physicians, data managers, nurses, and pharmacists who are involved in the care of recipients of blood and marrow transplants.

Learning Objectives

After completion of this activity, participants should be able to:

- Describe the role SDF-1α and the CXCR4 receptor play in the homing of stem cells to the bone marrow compartment.
- Assess the potential role a CXCR4 receptor antagonist could play in peripheral stem cell mobilization.
- Identify the mechanistic differences of mobilization via stimulation of the G-CSF receptor and blockade of the CXCR4 receptor.
- Discuss the current clinical data available for the CXCR4 receptor antagonist AMD-3100.

Rapid Mobilization of Murine & Human Hematopoietic Stem & Progenitor Cells with AMD-3100, a CXCR4 Antagonist

Hal E. Broxmeyer, PhD

Introduction

Although there have been recent advances in our understanding of homing and mobilization of hematopoietic stem (HSC) and progenitor (HPC) cells, there is still much that is unknown about this phenomenon [1]. It is believed that Stromal Derived Factor-1/CXCL12, a known chemotaxis protein produced in the bone marrow by stromal cells, is involved as an attractant for HSC and HPC to home into the marrow (Figure 1). A multi-institutional study was conducted hypothesizing that mobilization from the marrow would share some mechanistic functions with the homing process, but in a reverse order. Evidence from a number of studies indicated that SDF-1/CXCL12 can actually retain HPC and HSC in the bone marrow and nurture them. SDF-1/CXCL12 acts a survival factor. It was reasoned that cells could be mobilized by antagonizing the SDF-1/CXCL12-CXCR4 interaction, which would then result in the release of cells into the blood. CXCR4 is found on HSC and HPC and is the receptor for SDF-1/CXCL12.

The research team included Christie M. Orschell, D. Wade Clapp, Giao Hangoc, Scott Cooper, P. Artur Plett, Xiaxin Li, Barbara Graham-Evans, Timothy B. Campbell, Edward F. Srour, and me from the Indiana University School of Medicine; David C. Dale and W. Conrad Liles from the University of Washington; and Gary Bridger and Gary Calandra from AnorMED, Inc [2]. The CXCR4 antagonist, AMD-3100, is a bicyclam with 2 cyclotetradecane rings, 4 A's as substitutions and a phenylmethylene linker [3-5]. This small molecule has the ability to mobilize both mouse and human hematopoietic stem and progenitor cells and also to greatly enhance the mobilization induced by granulocyte colony stimulating factor (G-CSF) [2,6-8].

Mobilization Protocol Using AMD-3100 and/or G-CSF Induction

The first analysis was the use of AMD-3100 to mobilize HPC in mice. A starting dose of 5 mg/kg body weight of mouse was subcutaneously administered to observe the movement of cells into the blood of the granulocyte macrophage (CFU-GM), erythroid (BFU-E), and multipotential (CFU-GEMM) progenitors over time. It was clear that AMD-3100 was a very potent mobilizer of these progenitor cells, and mobilization peaked at about one hour in mice [2]. The next analysis conducted used a dose response to determine the maximum amount of AMD-3100 for mouse mobilization. Looking at different concentrations of AMD-3100, the optimal amount for mobilization of the granulocyte macrophage, erythroid and multipotential progenitors fell somewhere between 2.5-10 mg/kg [2].

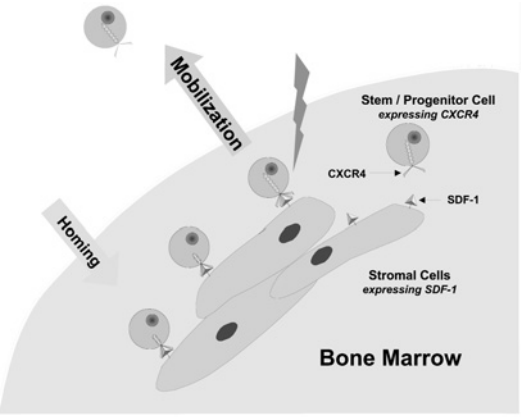

Figure 1. Bone marrow mobilization.

CXCR4 is a G protein coupled receptor [1]. Because G proteins can be easily desensitized so that they do not respond, it was necessary to determine an appropriate lag time for additional dosing with AMD-3100 to determine if AMD-3100 could include mobilization after multiple additions. Mice were administered AMD-3100 (5 mg/kg s.c.) either on day 1, or day 1 and 2, or day 1, 2, and 3. Mobilization was observed 1 hour after the last injection. Within the context of this 24-hour timing, it was found that mobilization occurred after the second or after the third injection of AMD-3100, as it did after the first injection and mobilization was the same on each day [2].

G-CSF is considered the gold standard for mobilization of HSC and HPC. However, there is great variation in humans in terms of their ability to respond to the G-CSF-induced mobilization. Interestingly, there are genetic strains of mice that also vary in response to this G-CSF-induced mobilization. For example, DBA/2 mice are excellent mobilizers, but C57Bl/6 mice are less responsive. The mechanisms for these differences in responsiveness are not known. We looked in animals to see if AMD-3100 could enhance the ability of G-CSF to mobilize in these different strains of mice.

Mice were injected with either control saline or G-CSF (2.5 μg/injection s.c.) 2 times a day for either 2 days or 4 days. Then each group was injected with either saline or AMD-3100 (5 mg/kg s.c.) 12 hours after the last injection of the saline or the G-CSF. Mice were bled one hour after the last injection and assessed for HPC per mL of blood.

Three different strains of mice were studied: C57/BL6, C3H/HEJ, and DBA/2. The C3H/HeJ mouse strain was chosen because they are relatively insensitive to endotoxin, so even if there was no endotoxin that showed up in the AMD-3100, it could be proven that the mobilization was due to the AMD-3100 and not perhaps from very small amounts of contaminating endotoxin. The granulocyte macrophage, erythroid, and multipotential progenitors were looked at. The C57Bl/6 mice had a lower ability to mobilize compared to DBA/2. This finding is essentially consistent with what has been published in the literature by a number of other groups who originally identified this different genetic sensitivity. The AMD-3100 had either a little better or a little worse mobilizing capacity compared to 2 days of G-CSF. When G-CSF was given first, followed by one injection of the AMD-3100, a tremendous synergy occurred in the release of these different types of progenitor cells into the blood [2].

We also looked at the potential for a more optimal dosage of G-CSF, administered for 4 days, followed by one dose of AMD-3100. Synergy or additive effects were still apparent [2].

Next, this information was translated into a genetic mouse model of disease, using Fanconi anemia complement C group gene knock-out (fancc -/-) mice as the model. Fanconi anemia is an autosomal recessive disorder. Many patients with Fanconi anemia who need to have their cells mobilized are not great mobilizers. The optimal dosage of 4 days of G-CSF was given. In each of the categories, the fancc -/- mice mobilized less of all 3 progenitor cells (CFU-GM, BFU-E, CFU-GEMM) compared to control littermate mice, which is consistent with results from many of the patients with Fanconi anemia who are mobilized with G-CSF. Interestingly, in the fancc -/- mice, there was incredible synergy when mice were administered 4 days of G-CSF, followed by one dose of AMD-3100. We don't know why this was happening, but the possibility exists that this

model can be used to determine the mechanisms that are involved and provide better insight into why these patients are poor mobilizers and the means to enhance mobilization.

Mobilizing Marrow Repopulating Stem Cells

In addition to mobilizing progenitors, it is important to mobilize long-term marrow repopulating stem cells. A competitive repopulating mouse stem cell assay was used to evaluate the mobilized cells for their content of stem cells. Figure 2 shows the assay using mice congenic for CD45 (CD45.2 or CD45.1). The competitive repopulating stem cell assay was set up specifically to measure competition. After one hour of mobilization either with saline or AMD-3100, donor blood cells from C57Bl/6 (CD45.2) mice were used at a ratio of 3:1, 2:1 or 1:1 of donor cells to competitor cells (B6.BoyJ, CD45.1) at a constant concentration of one half million low-density B6.BoyJ cells. These cells were then injected intravenously into lethally irradiated B6.BoyJ mice, and donor cell chimerism followed in recipients over time. A 3:1, 2:1; and 1:1 ratio of the AMD-3100 mobilized cells to competitor cells, all have significant stem cell activity above the control, indicating that AMD-3100 mobilizes a competitive repopulating mouse stem cell. Cells from the 3:1 ratio at 4 months were then transplanted into secondary lethally irradiated animals. The AMD-3100-mobilized cells that repopulated the primary mice in a competitive assay were also able to repopulate secondary mice in a noncompetitive assay. Because repopulation of secondary mice is a measure of the self-renewal capacity of the donor HSC, it was clear that AMD-3100 had mobilized long-term marrow competitive repopulating stem cells with self-renewal capacity.

Looking at the cells that were mobilized with the combination of G-CSF and AMD-3100, in terms of competitive repopulation and secondary repopulation, we used a 1.5:1 ratio of donor to competitor cells. Both the G-CSF and the AMD-3100 cells competitively engrafted. The combination produced greater than additive effects. Additionally, when a lower (1:1) donor to competitive cell ratio was used, the effect was clearly synergistic for mobilization of HSC. The mobilized cells were able to repopulate secondary recipients.

Clinical Correlations

David Dale and colleagues at the University of Washington, in collaboration with our group, studied healthy volunteers after giving AMD-3100 (80 mg/kg s.c.) and found that peak mobilization was at 6-9 hours for both the granulocyte-macrophage, erythroid, and multipotential progenitors [6]. They also found that this dose was the peak for CD34+ cell mobilization. We wanted to know if similar results regarding the desensitization issue in mice would occur in humans. It was found that mobilization did occur with AMD-3100 after each dose, indicating that similar results were found with humans and mice [2].

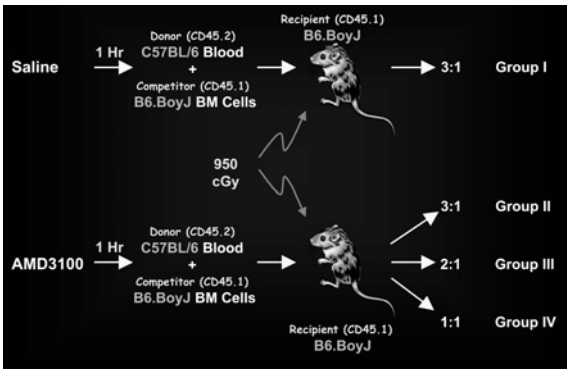

Figure 2. Competitive repopulating mouse stem cell assay.

The next step was to look at the influence of the combination of G-CSF and AMD-3100 on mobilization in humans. The protocol was set up to administer healthy human donors G-CSF 10 μg/kg per day, once a day for 4 days. On day 5, the subjects were split into 3 different groups and received G-CSF, AMD-3100, or the combination of G-CSF plus AMD-3100. AMD-3100 alone or with G-CSF gave a 3- or 4-fold increase in progenitor cells compared to that of G-CSF alone in these healthy human volunteers [2]. This was similar to the mobilization enhancement seen with CD34+ cells [8].

HSC mobilization is clinically important because increased numbers of these cells are needed for enhanced engraftment after transplantation. There are not many assays to measure human stem cells, but the one that most investigators believe to be the best assay, although not perfect, is putting human CD34+ cells into sublethally irradiated mice with a nonobese diabetic severe combined immunodeficiency (NOD/SCID) genotype. Mobilization with G-CSF plus AMD-3100 resulted in the greatest number of SCID repopulating cells (SRC) per kg body weight in an apheresis sample [2]. Looking at the expression of adhesion molecules CD49d, D49E, CD62L, as well as the CXCR4 receptor, we found that CD49 was increased in CD34+ cells mobilized by AMD-3100 plus G-CSF compared to G-CSF alone, and CD62L was decreased. The SRC assays were done by Christie Orschell, Edward Srour, and P. Artur Plett, and the adhesion molecule profile of the AMD-3100 plus G-CSF-mobilized CD34+ cells was similar to the phenotype of a very highly engrafting mouse stem cell [9].

Conclusions

These basic scientific and clinical laboratory studies support the hypothesis that the CXCL12-CXCR4 axis is involved in marrow retention of HSCs and HPCs and that antagonizing this axis results in rapid mobilization of HSC and HPC and suggests the clinical potential of AMD-3100 for HSC mobilization.

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Mobilization of Stem Cells Using AMD-3100: Early Experience in the Allogeneic Transplantation Setting

John DiPersio, MD, PhD

Introduction

The relative benefits of using peripheral blood as a source of stem cells for allogeneic transplantation is rapidly becoming the standard of care for both sibling matched and unrelated donor transplantation. Multiple studies over the past several years have demonstrated that it is a feasible and rational approach for patients with resistant and refractory hematologic malignancies.

A number of studies suggest that granulocyte colony stimulating factor (G-CSF) can be used to mobilize stem cells in both the autologous and allogeneic stem cell transplantation setting [1-3]. When administered to both autologous and allogeneic stem cell donors, G-CSF has been shown to promote the peripheral mobilization of a number of cell types including $CD34^+$ progenitors, granulocytes, monocytes, and other cells which do not express the G-CSF receptor such as B-cells, T-cells, and NK cells.

Data from Washington University on 21 consecutive allogeneic stem cell recipients who were infused with large numbers of G-CSF mobilized allogeneic peripheral blood stem cells revealed that CD34 cells from a donor circulate in the peripheral blood over several hours after infusion [unpublished data]. The peak number of $CD34^+$ stem cells occurred between 5 and 20 minutes after infusion and decreased to undetectable levels within 6 hours after infusion. Of interest was that CD34 cells appeared in large numbers in the peripheral blood during engraftment coincident with white cell recovery between 9 and 10 days after transplantation (Figure 1). Using molecular markers to distinguish between donor and recipient cells, these same investigators demonstrated that all the circulating CD34 cells in the peripheral blood of allogeneic stem cell recipients at the time of engraftment were of donor origin. These data suggest that donor CD34 stem cells rapidly disappear from circulation, expand in the bone marrow space, and then migrate into the peripheral blood at the time of neutrophil recovery. This is similar to what is seen in autologous stem cell transplantation patients after mobilization with chemotherapy plus G-CSF.

Multiple pieces of evidence suggest that a critical interaction between the chemokine receptor CXCR4 expressed on stem cells and its ligand, SDF1, expressed on the bone marrow microenvironment modulate critical stem cell homing and egress events [4,5]. Agents used to interrupt the interaction of CSCR4 and SDF1 could then be hypothesized to induce rapid mobilization of stem cells. The presence of this antagonist at the time of stem cell infusion might, however, alter stem cell homing, expansion, and repopulation in both autologous and allogeneic recipients. In addition to the obvious effects on stem cell homing and repopulation, CXCR4 antagonists might alter T-cell function because CXCR4 is expressed on all subsets of T-cells. In fact, CXCR4 is the co-receptor for HIV on $CD4^+$ T-cells. Therefore, the use of CXCR4 antagonists such as AMD-3100 might also alter the tracking and allo-reactivity of donor T-cells in the allogeneic setting. We performed a number of preclinical studies using mouse models to look at the role of CXCR4 blockade on stem cell homing and expansion using competitive repopulation studies as well as on T-cell allo-reactivity using a well defined H-2 disparate mismatched allogeneic transplant model.

Mouse Model

Recent studies by Broxmeyer and his colleagues at Indiana University demonstrated that AMD-3100 induced a 40-fold increase in the mobilization of hematopoietic progenitors within 1 hour after subcutaneous injection in the mouse [6]. We performed similar studies and found that the magnitude and temporal mobilization of hematopoietic progenitors is identical to the Broxmeyer study. Broxmeyer et al assayed the number and function of hematopoietic progenitors in the peripheral blood by removing a set volume of peripheral blood from AMD-3100 mobilized mice and demonstrated that the engraftment potential of peripheral blood increased commensurate with the increase in the number of measurable progenitor cells. Although this did demonstrate that AMD-3100 was a rapid and effective mobilization agent inducing the egress of functional progenitor cells in the mouse, it did not specifically compare the functional aspects of these stem cells in comparison to G-CSF mobilized stem cells or G-CSF + AMD-3100 mobilized stem cells in a competitive repopulation assay.

Figure 2 summarizes our murine competitive repopulation model. Recipient mice were B6, $H\text{-}2^d$, and Ly5.1/5.2 compound heterozygotes. These recipients were irradiated with an ablative bladed dose of radiation (900 cGy) and infused immediately after radiation with 1×10^6 unmanipulated bone marrow from these same compound heterozygotes. This allows for survival of recipient mice for analysis in the competitive repopulation assay. Comparisons were made on the ability of lim-

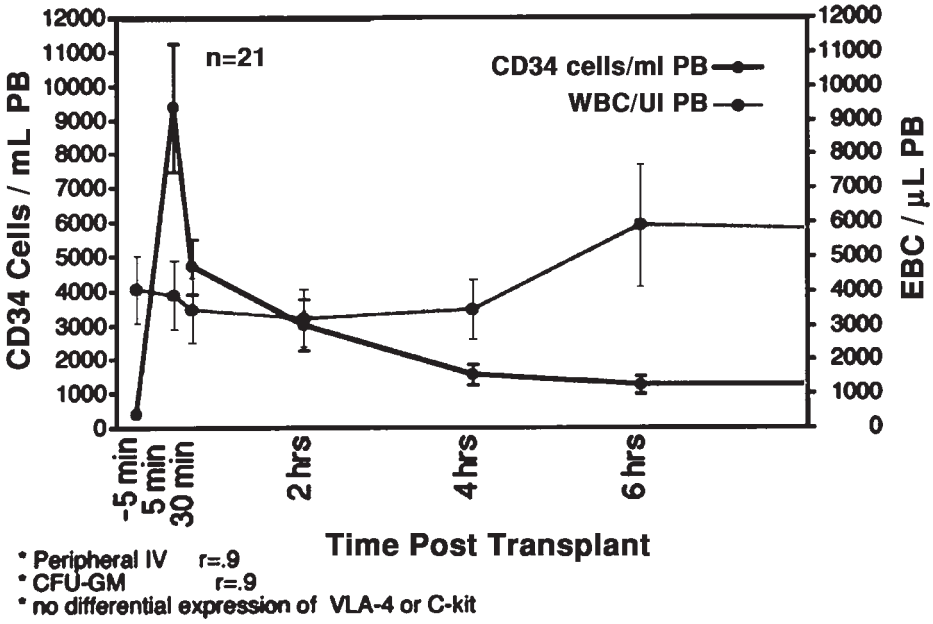

Figure 1. Circulating $CD34^+$ WBC: zero to 6 hours post allo PBSC infusion.

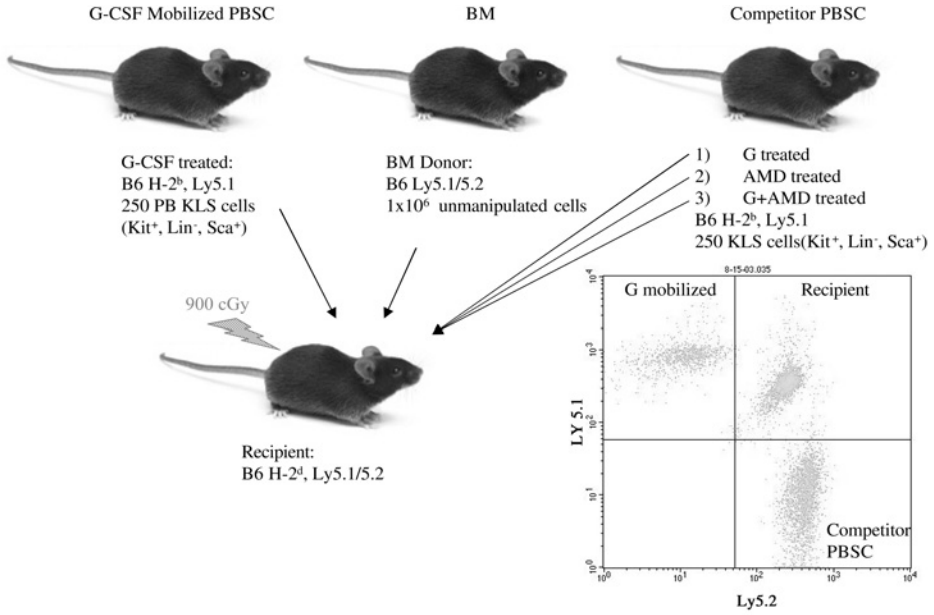

Figure 2. Murine competitive repopulation studies.

iting numbers (n = 250) of peripheral blood kit+, lin-, sca-1+ stem cells (KLS) from G-CSF mobilized mice (B6; H2b; Ly5.1) and the same number of limiting numbers of competitor KLS cells (B6; H2b; Ly5.2) mobilized by either G-CSF, AMD-3100, or G-CSF + AMD-3100 in a competitive repopulation experiment. Using FACS analysis (Ly5.1/Ly5.2), one can determine after transplantation the proportion of recipient cells which are contributed by the compound heterozygote bone marrow donor, G-CSF mobilized peripheral blood KLS cells and the competitor mobilized KLS cells. In this way one can directly compare the functional engraftment and repopulation potential of a G-CSF mobilized KLS cell to that of a competitor KLS cell mobilized by AMD-3100 or G-CSF + AMD-3100. Our studies demonstrated clearly that the repopulation potential of G-CSF mobilized KLS cells was identical for all lineages (B-cells, T-cells, and myeloid cells) to KLS cells mobilized with AMD-3100 and G-CSF + AMD-3100, both in short-term and long-term repopulation experiments. These studies provided reassuring evidence that AMD mobilized stem cells engrafted and expanded in recipients in an identical fashion to G-CSF mobilized stem cells.

To test the relative allo-reactivity and graft-versus-host disease (GVHD) potential of G-CSF mobilized T-cells, naïve unmanipulated T-cells, and T-cells mobilized with either G-CSF, AMD-3100, or the combination of G-CSF and AMD-3100, several murine allogeneic transplant models were utilized. Figure 3 shows that BALB/C (H-2^d) recipients were given 900 cGy of total body irradiation and infused with T-cell-depleted bone marrow from a fully disparate donor (B6, H-2^d) mice. These animals all engrafted and showed no signs of GVHD due to the T-cell depletion of the donor bone marrow. To test the function of allogeneic T-cells after various mobilizations we infused 2×10^6 splenic T-cells from H-2 disparate donors who had received either placebo control (naïve T-cells), G-CSF, AMD-3100, or G-CSF + AMD-3100 mobilized T-cells. Data presented in Figure 3 demonstrate that only those animals receiving T-cell-depleted bone marrow had evidence of mixed donor T-cell chimerism, with all of the animals having between 70% and 90% donor T-cells at day +27 posttransplantation. Naïve T-cells, G-CSF mobilized T-cells, AMD mobilized T-cells, and AMD + G-CSF mobilized T-cells all resulted in animals who developed progressive and lethal GVHD in full T-cell donor chimerism by day +27 posttransplantation (Figure 3). These data suggest that neither G-CSF, AMD-3100, nor the combination resulted in any significant dysfunction of T-cells resulting in decreased GVHD or donor T-cell engraftment.

Several less aggressive GVHD models were used to assess the ability of donor T-cells to promote donor T-cell engraftment. All of these models demonstrated clearly that T-cells mobilized with AMD-3100 and the combination of AMD-3100 + G-CSF were equal in their allo-reactivity and in their ability to induce donor T-cell engraftment to both G-CSF mobilized T-cells and naïve T-cells.

These data support the notion that T-cells mobilized with AMD-3100 were comparable to naïve T-cells, G-CSF mobilized T-cells, and the combination of AMD-3100 + G-CSF mobilized T-cells for their ability to induce lethal GVHD and donor T-cell engraftment in H-2 disparate recipients after both ablative and nonablative conditioning with TBI. These data, in conjunction with the competitive repopulation studies, suggest that AMD alone can induce functional hematopoietic stem cell and T-cell mobilization compared to animals mobilized with G-CSF, AMD-3100 + G-CSF and to animals who were treated with placebo control. These data provide the foundation and preliminary safety data for the use of AMD-3100 alone as an allogeneic stem cell mobilizing agent in humans undergoing allogeneic stem cell transplantation.

Pilot Human Trial Using AMD-3100 to Mobilize Allogeneic Stem Cells

Based on the preclinical murine studies described above, we hypothesized that the treatment of healthy HLA-matched sibling donors with AMD-3100 would result in the rapid mobilization of functional $CD34^+$ stem cells and allo-reactive lymphocytes which would allow for multi-lineage engraftment in allogeneic transplant recipients. A number of advantages exist for using AMD-3100, specifically that mobilization is extremely rapid (ie, peak $CD34^+$ cells in 6 to 9 hours compared to 5 days of G-CSF treatment). In addition, because AMD-3100 results in rapid mobilization of CD34 from the bone marrow to the

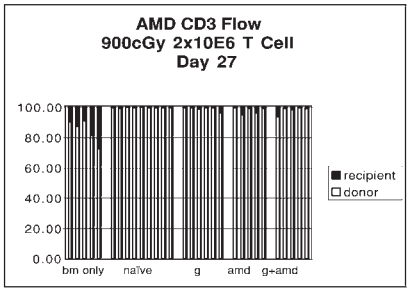

Figure 3. Day +30 T-cell chimerism.

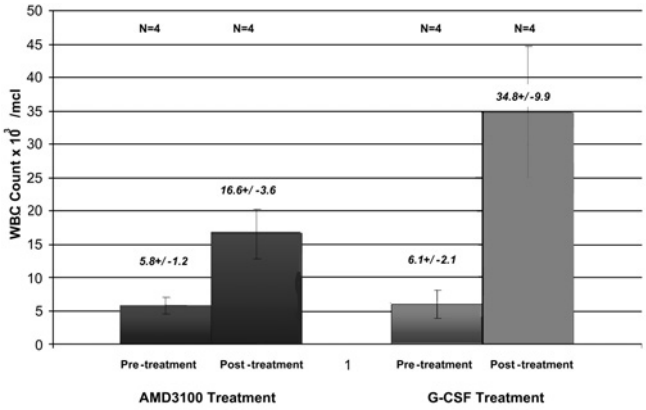

Figure 4. Summary of the mean +/- STEV values WBC count.

peripheral blood, reduced toxicities often seen after G-CSF mobilizations would be experienced by these normal donors. These symptoms primarily include bone pain.

Patients and Methods

Patients eligible for this study include those who are 18-65 years of age with a 6/6 HLA -A, B, and DR matched sibling donor. Patients also must have an advanced hematologic malignancy and healthy organ function.

The trial was designed using the optimal dose of AMD-3100 (240 μg/kg) given as a single SC injection 9 hours before the first apheresis procedure. Target CD34/kg was 2×10^6. If that target was not met after the first apheresis, patients could be given a second dose of AMD-3100 and apheresed 2 days later. The product was frozen and stored for infusion at the time of transplantation. Donors were then allowed a 1-week wash out period and were subsequently mobilized using standard dosages of G-CSF (10 μg/kg) daily for 5 days prior to the first apheresis. This apheresis product was frozen and stored as a backup.

Figure 4 summarizes the mean white cell counts generated before and after AMD-3100 and after G-CSF in treatment immediately prior to the first apheresis. G-CSF treatment of normal donors induced a significantly higher increase in the WBC compared to the AMD-3100 group ($P > .05$). Only 1 of 6 allogeneic donors did not reach a target number of 2×10^6 CD34/kg after AMD-3100. CD34 cells increased a mean of 6.4-fold in the AMD-3100-treated donors and 22.0-fold in the G-CSF-treated donors. Interestingly, there was an increase in CD3/kg and in CD4 and CD8 subsets after AMD-3100 mobilization compared to G-CSF mobilization. There was also a slight increase in B-cell mobilization after AMD-3100 when compared to G-CSF. Therefore, in spite of inferior CD34 mobilization, CD3 and CD3 subset

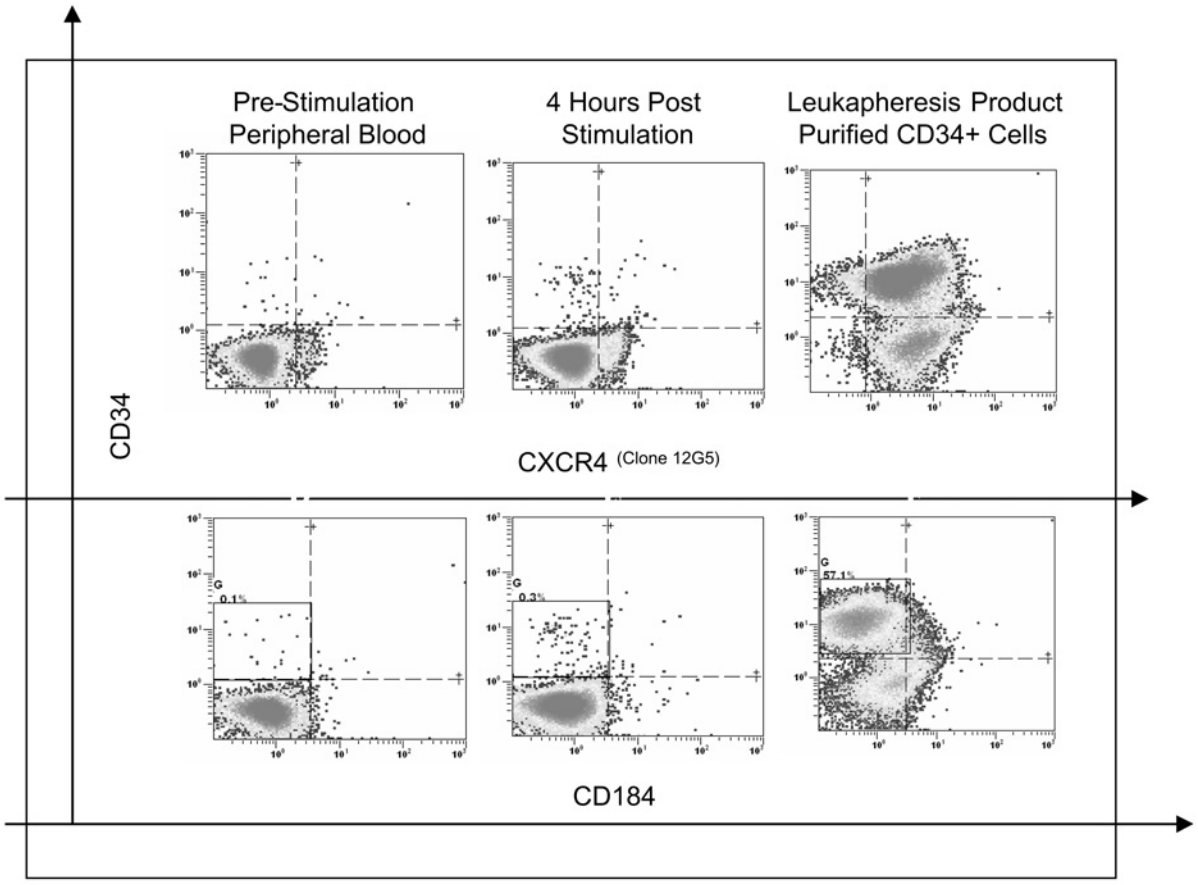

Figure 5. CXCR4 after AMD-3100 mobilization (patient #4).

mobilizations were superior after AMD-3100 mobilization.

One of the critical issues was to examine the expression of CXCR4 after AMD-3100 mobilization. If CXCR4 expression is essential for stem cell homing and engraftment then down regulation of this antigen might have a short-term and long-term impact on multi-lineage engraftment in these allogeneic recipients.

Figure 5 depicts CXCR4 expression in donor CD34 cells mobilized after AMD-3100 treatment. Two separate CXCR4 antibodies were used, one of which identifies CXCR4 expression in spite of AMD-3100 binding (clone 12G5). Using clone 12G5 it is clear that in the leukophereis products CXCR4 expression is maintained at high levels in mobilized CD34 cells consistent with persistent expression of CXCR4 in AMD-3100 mobilized CD34 cells.

Results and Conclusions

Although accrual to this trial is ongoing, preliminary results of the first 4 patients receiving transplants demonstrated that all 4 patients engrafted both neutrophils and platelets rapidly. All 4 patients had full donor engraftment with over 95% of T-cells of donor origin by day + 30. Only 1 of the 4 patients had grade 2 skin GVHD treated with topical steroids only. All 4 patients have remained alive and disease free 40-300 days posttransplantation. One patient was found to have residual lymphoma cells detected by bone marrow biopsy but remains clinically well, fully engrafted, and free of GVHD. There have been no significant complications from mobilization with AMD-3100 except for mild perioral paresthesias and some abdominal bloating (grade 1 toxicities).

In conclusion, the administration of AMD-3100 to mice results in rapid mobilization of hematopoietic stem cells, which are functionally equivalent to stem cells mobilized after G-CSF treatment. T-cells mobilized after AMD-3100 have equivalent GVHD potential, allo-reactivity and donor engraftment potential as naïve T-cells and T-cells mobilized with G-CSF. AMD-3100 successfully mobilizes adequate numbers of CD34 cells necessary for rapid engraftment of neutrophils and platelets insuring both short-term and long-term engraftment in human allogeneic transplant recipients. Five of 6 donors collected target numbers of CD34/kg after a maximum of 2 collections. No significant toxicities were associated with treatment of normal donors and all allogeneic transplant recipients remain alive, clinically well, fully engrafted 50-300 days posttransplantation. These preliminary studies suggest AMD alone may be an alternative mobilizing agent for allogeneic stem cell donors and infusion of these AMD-3100 mobilized stem cells results in multi-lineage long term engraftment with tolerable toxicities and rates of GVHD.

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Clinical Experience with AMD-3100 in Combination with G-CSF for Autologous Transplantation and Future Directions

Neal Flomenberg, MD

Introduction

AMD-3100 is a potent, selective antagonist of the CXCR4 chemokine receptor, blocking binding of its cognate ligand, stromal cell-derived factor 1α (SDF-1α). The rationale for use of AMD-3100 for the mobilization of hematopoietic progenitor cells (HPCs) in patients with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) was based on three known factors. The first was the recognition that the SDF-1α/CXCR4 interaction is an important mechanism for stem cell trafficking to and retention in the marrow. The next was the acknowledgment that G-CSF mobilization of HPCs may result, in part, from degradation of CXCR4 and SDF-1α as a consequence of enzymes released from PMNs and $CD34^+$ cells. Finally, based on the available murine and normal volunteer preclinical data, it became logical to test the hypothesis that the combination of AMD-3100 plus G-CSF would be superior to G-CSF alone as a clinical mobilizing regimen.

We at Thomas Jefferson University, Washington University, University of Rochester, Virginia Commonwealth University, Hackensack University Medical Center, Medical College of Wisconsin, and AnorMED, Inc. wanted to ask three questions in the initial trial. First, we wanted to know whether the combination of AMD-3100 and G-CSF actually mobilized more progenitor cells than G-CSF alone. Next, whether this would translate into fewer apheresis being required when the combination was used. And finally, we wanted to know about the pace of engraftment and the durability of engraftment when cells mobilized using AMD-3100 and G-CSF were used for transplantation.

AMD-3100–2101 Clinical Study Protocol

Figure 1 illustrates the design of the first clinical trial, known as AMD-3100–2101. Eligible patients were those with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) in their first or second remission, including those traditionally considered to be poor mobilizers. These represent the two most common clinical indications for auto-transplantion. The patients were to be randomized so that half would be mobilized with the combination of AMD-3100 plus G-CSF, of which G-CSF was taken 4 to 5 days followed by AMD-3100, administered subcutaneously. They would have a washout period of 13 to 16 days, depending on how many apheresis were required to collect a transplantable product, prior to undergoing a second mobilization with G-CSF alone. The other half of the

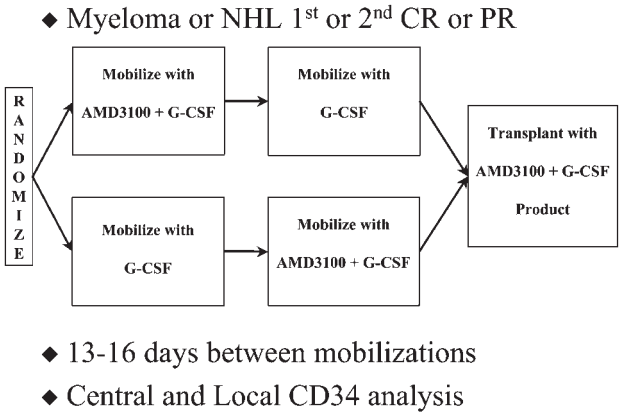

Figure 1. Initial study design.

patients would go through the regimens in the reverse sequence. The study was designed in this manner to ensure that there would be no sequence effect. Ideally, if enough AMD-3100 plus G-CSF (A plus G) mobilized cells could be collected, they would be utilized as the transplanted products with the G-CSF mobilized cells serving as a backup. A local CD34 analysis was used to guide the clinicians in terms of clinical care and a central analysis was used for the analysis of the study.

The inclusion criteria were initially limited to patients having 3 prior regimens and who were not to be too extensively pretreated. However, there were a number of patients in this study who had more than this chemotherapy exposure. Additionally, patients needed to demonstrate resolution of all acute toxicities from prior chemotherapy with reasonably normal hematopoietic function and decent renal, hepatic, pulmonary and cardiac function. They were required to be HIV-negative, capable of providing informed consent, and women agreeable to contraception. Patients could not have received Neulasta in the preceding 3 weeks and could not have had other cytokines or growth factors in the preceding week. A total of 25 patients enrolled, including 10 myeloma patients, most treated as part of initial therapy, with a median of 4 cycles as treatment. There were 15 NHL patients, typically treated as part of second line therapy and somewhat more heavily pretreated. There were 14 men and 11 women. The median age was 60 with a broad range of 18-70 years of age. A starting dose of 160 μg/kg of AMD-3100 was increased to 240 μg/kg in the final 17 patients once additional safety data became available.

Figure 2 shows the relative mobilization between the A plus G combination versus the G-CSF alone regimen. Illustrated is the ratio of CD34 cells that were collected per day of apheresis using the combination versus G-CSF alone. Fifteen patients, on the right in the figure, actually had between 3- and 50-fold more cells mobilized with the combination. In the dotted box are 6 patients who mobilized between 1½ to 2 times more CD34 cells per day. For the purposes of this study, we defined a significant increase as being 50% more cells with the combination, compared to G-CSF alone. Thus these patients also demonstrated a significant improvement in mobilization, although not as dramatic as the previous group. Four patients mobilized 10% to 40% more CD34 cells, and this group actually included those patients who were the very best mobilizers using G-CSF alone in the study. For the vast majority of patients, significantly more cells were collected with the combination.

Randomization was discontinued after 12 patients because of concerns about a sequence effect. In the study, 9 patients were successfully mobilized with one regimen and not with the other, and, of these, all were successfully mobilized with the combination but not with G-CSF alone. The first 4 of these patients were mobilized with AMD-3100 plus G-CSF first, and very respectable products were obtained with the combination. Two weeks later, when G-CSF alone was used, the products were dramatically inferior and clinically inadequate. In response to the possibility that a sequence effect was occurring, (ie, after reaching AMD-3100 plus G-CSF mobilization one might not be able to successfully mobilize with G-CSF alone), the randomization was discontinued. However, as subsequent patients were recruited to the trial, 5 additional patients were identified who were unable to mobilize an adequate product which was defined as 2×10^6 CD34 per kg using G-CSF alone as initial therapy, but who did mobilize an adequate product with A plus G subsequently. Thus, this result could not be similarly explained as the reverse sequence effect. In hindsight, all 9 patients had likely been poor mobilizers who did not mobilize an adequate product with G-CSF but succeeded with the combination.

Clinical Results

Of the 9 patients who failed to mobilize with G-CSF alone, 4 patients moved into an intermediate range, hitting the minimal target, but not did not reach the optimal target of 5×10^6 CD34 per kg. Five of these poor mobilizers actually did reach this optimal cell target. Of those who fell in an intermediate range with the G-CSF alone ($2\text{-}5 \times 10^6$ CD34 per kg), 1

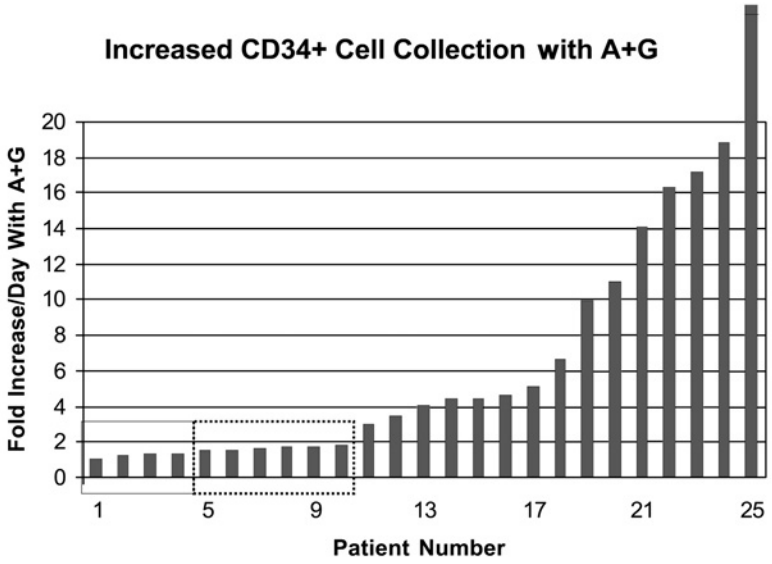

Figure 2. A+G mobilizes more CD34 Cells than G alone.

Regimen	Cells Collected		
	$<2 \times 10^6$	$2\text{-}5 \times 10^6$	$>5 \times 10^6$
G-CSF alone	20 (9)	3 (12)	2 (4)
AMD3100 + G-CSF	11 (0)	5 (10)	9 (15)

Figure 3. Patients reaching target in one (or two) aphereses.

Regimen	Successfully Mobilized When Other Arm Failed	Fewer Aphereses	Same Number of Aphereses But More Cells
AMD3100 + G-CSF	9	1 less = 6 (1*) 2 less = 4 (3*) 3 less = 2 (1*)	3
G-CSF	0	0	0
Total	9	12	3

* number of patients with ≥ 50 % more cells

Figure 4. Success of AMD-3100 + G-CSF versus G-CSF alone for mobilization.

remained in the intermediate range and 7 actually moved into the optimal range of above 5×10^6 CD34 per kg. All of the patients who produced an optimal product with G-CSF did so with a combination. Patients who produced the same range of mobilization with both regimens had superior mobilization with the combination (Figure 3). Figure 3 illustrates the ability to mobilize an adequate or optimal product in 1 or 2 days of apheresis, showing the 1-day mobilization and the 2-day mobilization (in parenthesis). After 1 day of collection using G-CSF alone, 20 patients failed to mobilize an acceptable product, while only 5 did so using the combination. Reciprocally, after 2 days of apheresis, the number of patients who produced an optimal product was skewed in favor of the combination.

Figure 4 highlights the clinical benefits in relation to the number of apheresis procedures required. Twelve patients in the combination arm required fewer aphereses to collect an adequate product, and 5 of these patients still mobilized more than 50% additional cells, despite undergoing fewer collections.

With regards to engraftment, 19 of the 24 patients in the study underwent transplantation with the AMD-3100 plus G-CSF cells only. Eighteen of these 19 demonstrated a relatively consistent pattern. Neutrophil recovery was relatively prompt in 10 to 11 days with a fairly tight range. Most patients demonstrated platelet recovery by day 16, and all except 1 had recovered by day 27. There have been no late graft failures. One patient was an outlier, developing early infections, delayed engraftment, and ultimately expiring due to complications of infection.

There were no serious or unusual events that occurred during AMD-3100 administration during mobilization. The most common adverse events that were seen included gastrointestinal upset and flatulence, some injection site irritation, and occasionally paresthesias. There were 7 severe adverse events that occurred during the trial. Four of them occurred during transplantation, including sepsis/renal failure resulting in the death of one patient, neutropenic colitis, catheter infection, and gastroenteritis. These were all thought to be transplantation-related complications not related to the mobilization, which occurred a number of weeks before. Three adverse events, abdominal pain, left internal jugular thrombosis and hematuria, did occur during G-CSF mobilization in patients where G-CSF mobilization occurred first; these events actually proceeded AMD-3100 by a couple of weeks and could not have been related to that agent.

Discussion

In this study, patients were given AMD-3100 early in the morning and waited 6 hours to begin the apheresis. We did this because of safety concerns in this first transplantation trial. Other studies have demonstrated that the CD34 cell increase induced by AMD-3100 after G-CSF pretreatment is relatively prompt with a fairly long plateau. Because of this long plateau, a number of subsequent studies, which now have amassed more than 75 patients, are looking at a simpler schedule where AMD-3100 is given at about 10 PM at night and then the patients can begin apheresis relatively promptly in the morning.

The most mature of these studies is AMD-3100–2105. Of the first 31 patients treated, 29 have mobilized over 4×10^6 CD34 cells/kg, which was defined as the optimal dose in this study. All 31 patients mobilized over 2×10^6 CD34 cells/kg. There is also an ongoing chemotherapy mobilization study. It has a complex design, but preliminary findings have concluded that they are mobilizing about twice the number of CD34 cells with AMD-3100 that would have been expected in its absence.

Phase III randomized studies of AMD-3100 plus G-CSF in comparison to G-CSF alone have been initiated both in non-Hodgkin's lymphoma and myeloma. They have begun to accrue the initial patients but are in the early stages. One clinical trial that is underway uses AMD-3100 alone for mobilization in multiple myeloma patients. Two additional ongoing studies that were reported at ASH and ASCO are looking for lymphoma cells and myeloma cells in products mobilized using AMD-3100. Thus far, neither of these has noted an increase in tumor cell mobilization. Although the studies are still ongoing, thus far they are encouraging.

Conclusion

In conclusion, AMD-3100 is a safe and well-tolerated drug in cancer patients. More cells are consistently collected with the combination of AMD-3100 plus G-CSF versus G-CSF alone. This has allowed some patients to undergo transplantation who otherwise would not have been able to, and for other patients it has resulted in fewer apheresis. These data have been corroborated in a number of trials using a somewhat logistically simpler dosing schedule. Engraftment in all of the studies has been prompt and durable, and thus far tumor mobilization does not appear to be an issue. For those more interested in chemo-mobilization or in using this agent alone, there are trials that are underway. Overall, the combination of AMD-3100 plus G-CSF is generally safe, effective, and superior to GCSF alone.

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Journal Watch

A scan of recent medical literature identified these articles of special importance in the science and clinical application of blood and marrow transplantation.

Lopez F, Parker P, Nademanee A, et al: Efficacy of mycophenolate mofetil in the treatment of chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2005;11: 307-313.

New approaches to primary and salvage therapy for chronic graft-vs-host disease (GVHD) are needed. Current treatment-consisting of prednisolone with or without cyclosporine or tacrolimus-carries high mortality and complication rates. The antimetabolite immunosuppressive drug mycophenolate mofetil (MMF) is currently used for prophylaxis of acute GVHD, and has shown promise in the treatment of both acute and chronic GVHD. Mycophenolate mofetil was evaluated as a first- or second-line therapy for chronic GVHD.

From 1999 to 2001, MMF was added to standard treatment with cyclosporine, tacrolimus, and/or prednisolone in 34 patients with chronic GVHD. Twenty-four patients received MMF as part of secondary or salvage therapy and 10 as first-line therapy. Outcome evaluation included clinical response rate, steroid tapering ability, and overall survival.

Response rates were 90% in patients receiving MMF as first-line therapy and 75% in those receiving second-line or salvage therapy. Complete remission was achieved in 35% of patients and partial remission in 44%. Another 15% of patients had stable disease, with just 6% experiencing disease progression after MMF therapy.

Of the 30 patients who had been receiving prednisolone, 73% had a reduction in prednisolone dose requirement, with a median decrease of 50%. Eighty-five percent of patients were still alive at a median follow-up of 24 months. Mycophenolate mofetil was generally well tolerated, although 3 patients experienced abdominal cramps requiring treatment discontinuation.

This retrospective study suggests that MMF may have a role in the treatment of chronic GVHD. Its shows evidence of therapeutic effect in both first-line and second-line salvage therapy, with good tolerability and a possible survival benefit. Added to standard treatment, this agent may help to reduce morbidity and improve survival even in high-risk patients with chronic GVHD.

Roy-Proulx G, Baron C, Perreault C: CD8 T-cell ability to exert immunodomination correlates with T-cell receptor:epitope association rate. Biol Blood Marrow Transplant. 2005;11:260-271.

CD8 T-cell responses to epitopes on antigen-presenting cells are subject to an immunodominance hierarchy, the mechanisms of which are not well understood. $H2D^b$ presents two antigens that lie on opposite ends of the immunodomination spectrum: $H7^a$ and HY. These two antigens induce similar CD8 cell responses when presented separately, but only $H7^a$ evokes a response when the two antigens appear on the same antigen-presenting cell. CD8 T cells specific for $H7^a$ and HY were used to study the mechanisms of immunodominance.

Nonimmune mice showed comparable rates of $H7^a$- and HY-specific T-cell precursors and similar levels of CD5. However, when harvested at the time of primary response, the $H7^a$-specific CD8 T-cell repertoire was very limited in terms of T-cell receptor (TCR) diversity. This was despite the fact that CD8 and TCR expression and tetramer decay rates were comparable between the $H7^a$- and HY-specific T cells. The TCR:epitope on-rate was dramatically faster for anti- $H7^a$ than for anti-HY T cells. In addition, primed CD8 T cells killed or inactivated the antigen-presenting cells, thus shortening the length of time antigens were presented.

These experiments using $H7^a$- and HY-specific CD8 T cells lend new insights into the underlying mechanisms of the immunodominance hierarchy. Immunodominant T cells exhibit functional priming after a relatively shorter period of antigen presentation, compared with T cells farther down the immunodominance scale. Further studies will be needed to test whether differences in the TCR:epitope on-rate also govern immunodominance in T cells specific for other antigens.

Peters BA, Diaz LA Jr, Polyak K, et al: Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nature Med. 2005;11:261-262.

Findings in murine tumor models suggest that endothelial cells derived from bone marrow contribute new blood vessel formation. However, few studies have assessed whether the same is true for tumor angiogenesis in humans. Tumors from bone marrow transplant recipients were analyzed to assess the role of bone marrow-derived endothelial cells in tumor neovascularization.

A search of transplant center databases identified 6 patients who developed primary cancers after receiving bone marrow from opposite-sex donors. The recipients were 4 females and 2 males; the cancers occurred 15 months to 15 years after transplantation. Multicolor fluorescence in situ hybridization with X or Y chromosome-specific probes was performed, along with fluorescent antibody staining, to identify donor cells in tumor specimens.

In all 6 patients, at least one tumor blood vessel was found to contain endothelial cells derived from donor bone marrow. Of 1,765 von Willebrand factor-positive, $CD45^-$ cells evaluated, just 27 were found to be of donor origin. Overall, the tumor vasculature included low percentages of bone marrow-derived endothelial cells: 1% to 12%, with an average of 4.9%. None of the vessels studied contained more than two bone marrow-derived cells.

This study of bone marrow transplant recipients who later developed cancer confirms that circulating bone marrow stem cells contribute to tumor angiogenesis in humans. However, the overall stem cell contribution to tumor endothelium is relatively low-only about 5%, on average. The findings point out some differences in the angiogenic process between mouse models and human tumors, with important implications for the clinical use of experimental treatments directed against angiogenesis.

Recommended Timing for Transplant Consultation

These guidelines have been developed and published jointly in 2005 by the National Marrow Donor Program (NMDP) and the American Society for Blood and Marrow Transplantation (ASBMT) and are based upon current clinical practice and the medical literature, as well as comprehensive evidence-based reviews.[1] Hematopoietic cell transplantation is a potential lifesaving treatment option for some patients. However, one of the critical factors in improved outcomes is the appropriate timing of the transplant. These guidelines indicate prognostic factors for patients at risk of disease progression using standard therapy and indicate which patients should be evaluated for transplantation. The guidelines provide a basis for initial discussions when developing a treatment plan that may include transplantation.

Adult Leukemias and Myelodysplasia

Acute Myelogenous Leukemia (AML)

High-risk AML including:
- Antecedent hematological disease [e.g. myelodysplasia]
- Treatment related leukemia
- Induction failure

CR1 with poor-risk cytogenetics

CR2 and beyond

Acute Lymphoblastic Leukemia (ALL)

High-risk ALL including:
- Poor-risk cytogenetics [e.g. Philadelphia chromosome positive, 11q23]
- High WBC (>30,000 - 50,000) at diagnosis
- CNS or testicular leukemia
- No CR within 4 weeks of initial treatment
- Induction failure

CR2 and beyond

Myelodysplastic Syndromes (MDS)

Intermediate-1 (INT-1), intermediate-2 (INT-2) or high IPSS score which includes either:
- >5% marrow blasts
- Other than good risk cytogenetics [good risk includes 5q- or normal]
- >1 lineage cytopenia

Chronic Myelogenous Leukemia (CML)

- No hematologic or minor cytogenetic response 3 months post-imatinib initiation
- No complete cytogenetic response 6 to 12 months post-imatinib initiation
- Disease progression
- Accelerated phase
- Blast crisis [myeloid or lymphoid]

Reference

1. Evidence-based Reviews, American Society of Blood and Marrow Transplantation. 2004. Published in *Biology of Blood and Marrow Transplantation* and available online at: http://www.asbmt.org/policystat/policy.html

Pediatric Acute Leukemias

Acute Myelogenous Leukemia (AML)

- Monosomy 5 or 7
- Age <2 years at diagnosis
- Induction failure

CR1 with HLA matched sibling donor

CR2 and beyond

High-Risk Acute Lymphoblastic Leukemia (ALL)

- Induction failure
- Philadelphia chromosome positive
- WBC > 100,000 at diagnosis
- 11q23 rearrangement
- Mature B cell phenotype (Burkitt's lymphoma)
- Infant at diagnosis

CR1 duration <18 months

CR3 and beyond

Lymphomas

Non-Hodgkin's Lymphoma

Follicular
- Poor response to initial treatment
- Initial remission duration <12 months
- Second relapse
- Transformation to diffuse large B-cell lymphoma

Diffuse Large B-Cell
- At first or subsequent relapse
- CR1 for patients with high or high-intermediate IPI risk
- No CR with initial treatment

Mantle Cell
- Following initial therapy

Hodgkin's Lymphoma

- No initial CR
- First or subsequent relapse

Multiple Myeloma

Multiple Myeloma

- After initiation of therapy
- At first progression

CXCR4 Chemokine Receptor Blockade: A New Strategy for PBSC Mobilization

CME Assessment Test

1. **AMD-3100 may mobilize CD34+ cells by:**
   a. Binding directly to the CD34 molecule.
   b. Altering the ratio of stromal cells to stem cells in the marrow.
   c. Blocking the interaction of SDF-1α with its receptor, CXCR4.
   d. Inducing a rapid proliferation of hematopoietic stem cells.
2. **Clinical observations using AMD-3100 together with G-CSF that have been noted to date include all of the following except:**
   a. The ability of some patients unable to successfully mobilize an autologous progenitor cell product with G-CSF alone to do so with the combination of AMD-3100 plus G-CSF.
   b. The ability of some patients to mobilize an adequate product with fewer apheresis procedures.
   c. Comparable time to ANC recovery using the combination compared to G-CSF alone.
   d. Better relapse rates after autotransplantation compared to patients mobilized with G-CSF alone.
3. **Toxicities observed in patients mobilized with AMD-3100 to date include:**
   a. GI upset and flatulence.
   b. Rebound neutropenia a week later.
   c. Injection site irritation.
   d. Paresthesias.
4. **Clinical laboratory studies support the hypothesis that:**
   a. The CXCL12-CXCR4 axis is involved in marrow retention of HSCs and HPCs.
   b. Antagonizing this axis results in rapid mobilization of HSC and HPC.
   c. There is the clinical potential of AMD-3100 for HSC mobilization.
   d. All of the above.
   e. None of the above.
5. **HSC mobilization is clinically important because:**
   a. It measures the self-renewal capacity.
   b. Increased numbers of these cells are needed for enhanced engraftment after transplantation.
   c. It is essential to multipotential progenitors.
   d. It influences long-term marrow competitive repopulating stem cells.
6. **What interactions are believed to be involved in the retention and homing of hematopoietic stem and progenitor cells in bone marrow?**
   a. Chemokine SDF-1/CXCL12 and its receptor: CXCR4.
   b. Adhesion molecules.
   c. Homing receptors.
   d. Mobilization enhancement.
7. **Which cytokines and compounds have not been successfully used to mobilize hematopoietic stem and progenitor cells to the blood?**
   a. Granulocyte-colony stimulating factor (G-CSF).
   b. Granulocyte macrophage colony stimulating factor (GM-CSF).
   c. Thrombopoeitin (TPO).
   d. The SDF-1/CXCL12-CXCR4 antagonist AMD-3100.
   e. All of the above.
8. **CXCR4 is the co-receptor for:**
   a. KLS cells.
   b. CD34+ stem cells.
   c. HIV on CD4+ T-cells.
   d. G-CSF.
9. **When administered to both autologous and allogeneic stem cell donors, G-CSF has been shown to promote the peripheral mobilization of:**
   a. CD34+ progenitors.
   b. Granulocytes.
   c. Monocytes.
   d. Cells which do not express the G-CSF receptor such as B-cells, T-cells, and NK cells.
   e. All of the above.
10. **In the pilot human trial using AMD-3100 to mobilize allogeneic stem cells, which of the following is not true of all 4 patients?**
    a. All had full donor engraftment with over 95% of T-cells of donor origin by day + 30.
    b. All had grade 2 skin GVHD.
    c. All engrafted both neutrophils and platelets rapidly.
    d. All have remained alive disease free between 40-300 days posttransplantation.

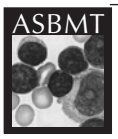
CME Assessment Test Answer Sheet

Release Date: September 30, 2005 Last Review Date: September 30, 2005 Expiration Date: September 30, 2006

Instructions

(1) Read the articles in the publication carefully. (2) Circle the correct response to each question on the Answer Sheet. (3) Complete the evaluation Form. (4) To receive CME credit, fax the completed Answer Sheet and Evaluation Form to the office of Continuing and Professional Education (414-456-6623) or mail to the Office of Continuing Education, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. No processing fee is required.

1.	A	B	C	D		**5.**	A	B	C	D		**8.**	A	B	C	D				
2.	A	B	C	D		**6.**	A	B	C	D		**9.**	A	B	C	D	E			
3.	A	B	C	D		**7.**	A	B	C	D	E	**10.**	A	B	C	D				
4.	A	B	C	D	E															



CME Evaluation Form

Please evaluate the effectiveness of this CME activity on a scale of 1 to 5, with 5 being the highest, by circling your choice. Fax *with the Answer Sheet* to the Office of Continuing and Professional Education, 414-456-6623, or mail to the Office of Continuing Education, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.

Overall Quality of the CME Activity	1	2	3	4	5
Articles in the publication were presented in a clear and effective manner.	1	2	3	4	5
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Educational objectives were achieved.	1	2	3	4	5
The CME activity provided a balanced, scientifically rigorous presentation of therapeutic options related to the topic, without commercial bias.	1	2	3	4	5

Please comment on the impact (if any) that this CME activity might have on your management of patients.

Would you benefit from additional CME programs on this topic? Yes No

I have read these articles on what is new in CMV and hematopoietic cell transplantation, published in *Blood and Marrow Transplantation Reviews*, and have answered the CME test questions and completed the Evaluation Form for this activity.

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