

INTERACTIVE UPDATES IN

The Use of Stem Cell Mobilization for the Treatment of Blood Related Cancers

Editor:

John F. DiPersio, MD, PhD, FACP

Contributors:

Luciano J. Costa, MD, PhD

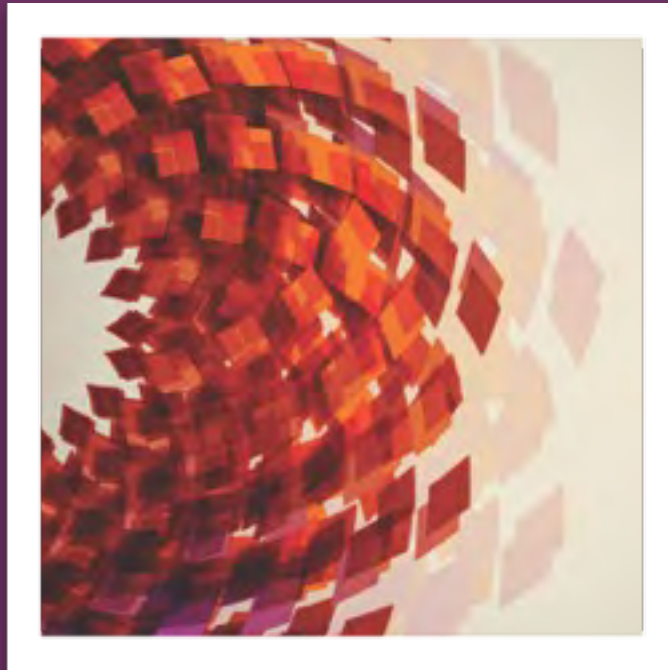
Sergio A. Giralt, MD, FACP

Edmund K. 'Ned' Waller, MD, PhD, FACP



Chapter 1

Program Overview & Introduction



Program Overview

- Needs Assessment
- Learning Objectives
- Target Audience
- Disclosures
- Commercial Support
- Accreditation Statement
- Credit Designation
- Off-Label/Investigational Use
- Evaluation & Post-Test
- Pre-Test

NEEDS ASSESSMENT

Autologous hematopoietic stem cell transplantation (aHSCT) is a well-established treatment for hematologic malignancies such as multiple myeloma (MM) and non-Hodgkin lymphoma (NHL). [1,2] Various changes in the field over the past decade, including the frequent use of tandem aHSCT in MM, the advent of novel therapies for the treatment of MM and NHL, plus the addition of new stem cell mobilization techniques, have led to the need to reassess current stem cell mobilization strategies.

Mobilization failures with traditional strategies are common and result in delays in treatment and increased cost and resource utilization. The mobilization of hematopoietic stem cells fails in approximately 10-20% of patients with MM and up to 20-30% of patients with NHL.[3,4] Poor mobilization can lead to poor engraftment, increased morbidity, greater resource utilization, and increased costs.[4,5] The cause of poor mobilization can be partially explained by clinical variables (i.e., age, underlying disease, prior therapies, underlying marrow function) and cannot be predicted.[6]

Methods to increase the circulating concentrations of hematopoietic stem cells (HSCs) have been found to be necessary to ensure adequate and successful collections. Novel mobilization regimens have changed the climate of stem cell transplantation such that aHSCT may now be performed in more than 90% of those patients in whom the procedure is indicated, with a minimal need for remobilization strategies.

The precise regimen that is most effective remains to be determined, however, and may vary depending on patient population and the specific goal of stem cell collection. [7,8]

1. The NCCN Clinical Practice Guidelines in Oncology for Non-Hodgkin's Lymphomas. V.1.2015 Accessed May 1, 2015.
2. The NCCN Clinical Practice Guidelines in Oncology for Multiple Myeloma. V.2.2015. Accessed May 1, 2015.
3. DiPersio JF, Micallef IN, Stiff PJ, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 2009;27(28):4767–4773.
4. Pusic I, Jiang SY, Landua S, et al. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. *Biol Blood Marrow Transplant*. 2008;14(9):1045–1056.
5. Gertz MA, Wolf RC, Micallef IN, Gastineau DA. Clinical impact and resource utilization after stem cell mobilization failure in patients with multiple myeloma and lymphoma. *Bone Marrow Transplant*. 2010;45(9):1396–1403.
6. Costa LJ, Nista EJ, Buadi FK, et al. Prediction of poor mobilization of autologous CD34+ cells with growth factor in multiple myeloma patients: implications for risk-stratification. *Biol Blood Marrow Transplant*. 2014;20(2):222- 228.
7. Duong HK, Savani BN, Copelan E, et al. Peripheral blood progenitor cell mobilization for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American

Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2014;20(9):1262–1273.

8. Giralt S, Costa L, Schriber J, et al. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. *Biol Blood Marrow Transplant*. 2014;20(3):295–308.

LEARNING OBJECTIVES

Upon completion of the program, participants should be able to:

1. Demonstrate improved knowledge of stem cell mobilization strategies in autologous hematopoietic stem cell transplant
2. Identify patients at risk of poor stem cell mobilization
3. Devise and evaluate strategies to increase mobilization success in autologous hematopoietic stem cell transplant
4. Assess the value of pharmaco-economics and resource utilization associated with stem cell mobilization strategies

TARGET AUDIENCE

This program has been designed for a targeted audience of hematologists, hematologists/oncologists, blood and marrow transplant specialists, and all healthcare professionals in the blood and marrow transplant community involved in the care and treatment of hematologic malignancies.

FACULTY DISCLOSURES

Luciano J. Costa, MD, PhD has received honorarium as a speaker for Sanofi.

John F. DiPersio, MD, PhD has no relevant financial relationships to disclose.

Sergio A. Giralt, MD has received honorarium as a speaker for Sanofi. Johnson & Johnson, and Celgene.

Edmund K. Waller, MD, PhD has received honorarium as a consultant from Novartis, Celldex and Seattle Genetics.

CJP DISCLOSURES

The employees of CJP Medical Communications have no financial relationships to disclose.

COMMERCIAL SUPPORT

This activity is supported by an educational grant from Sanofi.

ACCREDITATION STATEMENT

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education through the joint providership of the Medical College of Wisconsin and CJP Medical Communications. The Medical College of Wisconsin is accredited by the ACCME to provide continuing medical education for physicians.

CREDIT DESIGNATION

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 enduring material for a maximum of 3.0 *AMA PRA Category 1 Credits*™. Physicians should claim only credit commensurate with the extent of their participation in this program.

OFF-LABEL/INVESTIGATIONAL USE

This educational activity may contain discussion of published and/or investigational uses of agents that are not indicated by the FDA. The opinions expressed in the educational activity are those of the faculty and do not necessarily represent the views of the Medical College of Wisconsin, Carden Jennings Publishing or Sanofi.

Before prescribing any medication, physicians should consult primary references and full prescribing information. Please refer to the official prescribing information for each product for discussion of approved indications, contraindications, and warnings. Further, participants should appraise the information presented critically and are encouraged to consult appropriate resources for any product or device mentioned in this program.

EVALUATION & POST-TEST

Learners are to complete an evaluation and post-test in order to claim CME credit. Please click here to proceed to the online evaluation and post-test, and to submit your request for CME credit.

↓ **BEFORE MOVING FORWARD,
PLEASE TAKE THIS PRE-TEST**

PRE-TEST

The screenshot displays the Medical College of Wisconsin's Office of Continuing and Professional Education website. The page is titled "The Use of Stem Cell Mobilization for the Treatment of Blood Related Cancers". It includes a sidebar with navigation links such as Home, Calendar, Courses, News, and Discussions. The main content area features a large play button icon over the title, indicating a video or audio pre-test. To the right, there are course details including "Hours of Participation: 3.00", "Course opens: THU, Dec 31 2015", and "Course expires: SAT, Dec 31 2016". The page also has a top navigation bar with links for Home, Learning Groups, Course Catalog, About Us, and Contact Us.

Before moving forward, please take this pre-test.



Introduction

John F. DiPersio MD, PhD



John F. DiPersio MD, PhD

Chief, Division of Oncology
Deputy Director, Siteman Cancer Center
Washington University School of Medicine
St. Louis, Missouri

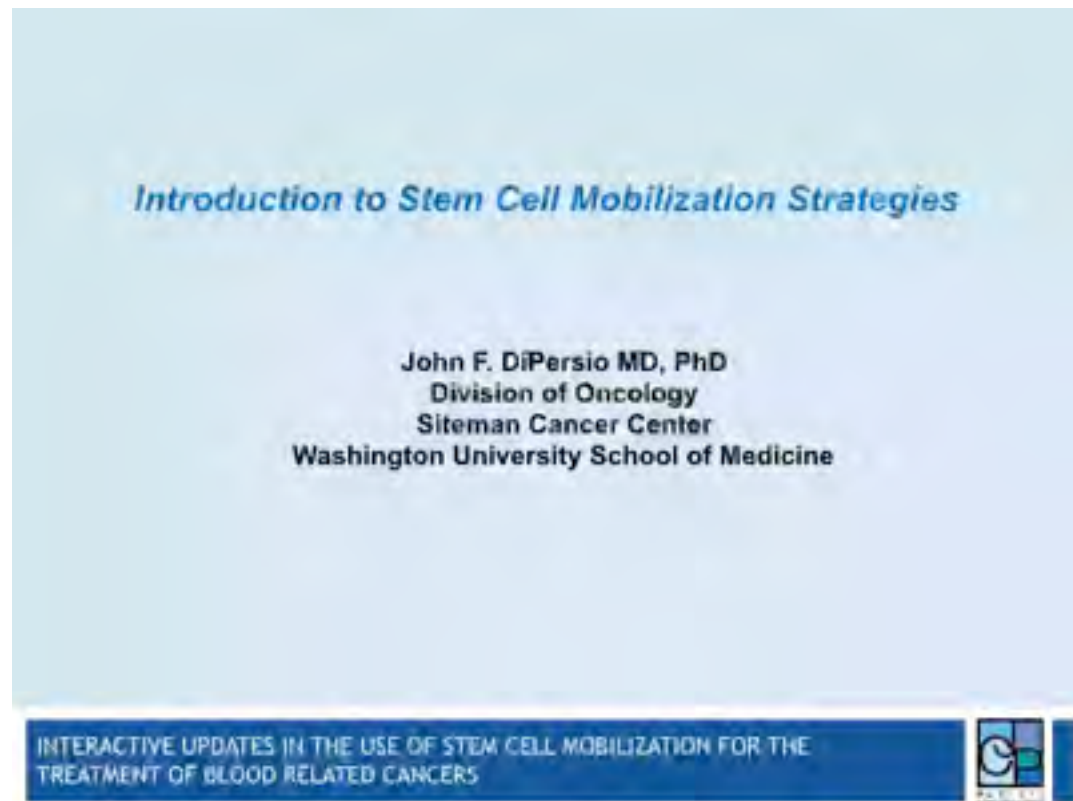
Dr. John F. DiPersio, Deputy Director, Alvin J. Siteman Cancer Center and Chief of the Division of Oncology at Washington University School of Medicine in St. Louis and the Virginia E. and Samuel J. Golman Professor of Medicine.

Dr. DiPersio's research focuses on fundamental and translational aspects of leukemia and stem cell biology. These studies include identification of genetic abnormalities in human leukemias, understanding processes involving stem cell and leukemia cell trafficking, and clinical and translational programs in both leukemia/myelodysplastic syndrome and stem cell transplantation.

Dr. DiPersio is Chair of ASH Scientific Committee on Hematopoiesis, a member of the Board of Scientific Counselors (Clinical Science and Epidemiology) of the National Cancer Institute, and the 2013 recipient of the Daniel P. Schuster Distinguished Translational Investigator Award from Washington University, the 19th Annual AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Cancer Research in 2014 and the 2014 recipient of the American Society of Hematology Mentor Award for Clinical Investigations. He has authored or co-authored more than 275 publications and over 60 invited reviews and book chapters.

Dr. DiPersio received his M.D. and Ph.D. from the University of Rochester and his B.A. in Biology from Williams College. He completed an internship and residency at Parkland Memorial Hospital and The University of Texas Southwestern Medical Center in Dallas. After serving as chief resident at Parkland Memorial Hospital, Dr. DiPersio completed a fellowship in the Division of Hematology/Oncology at the University of California, Los Angeles (UCLA).

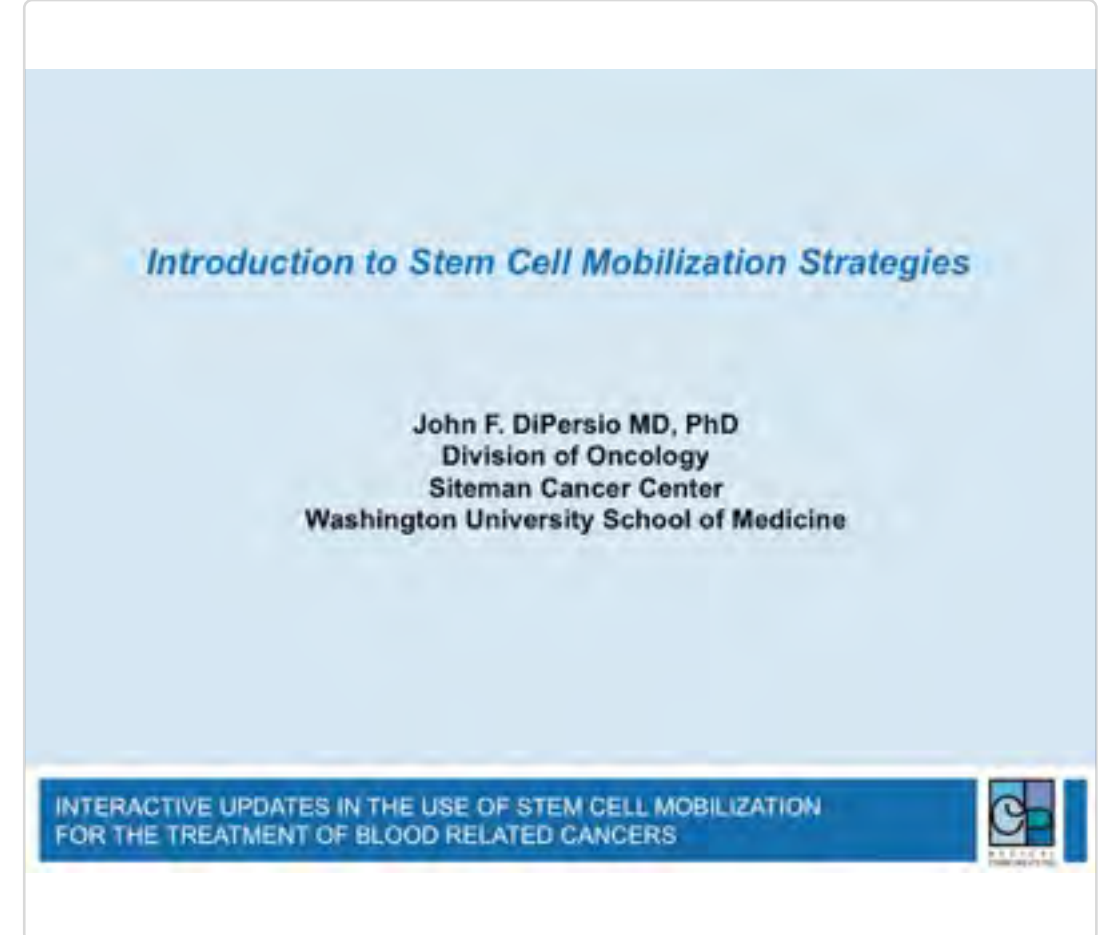
Movie 1.1



Introduction

John F. DiPersio MD, PhD

Interactive 1.1



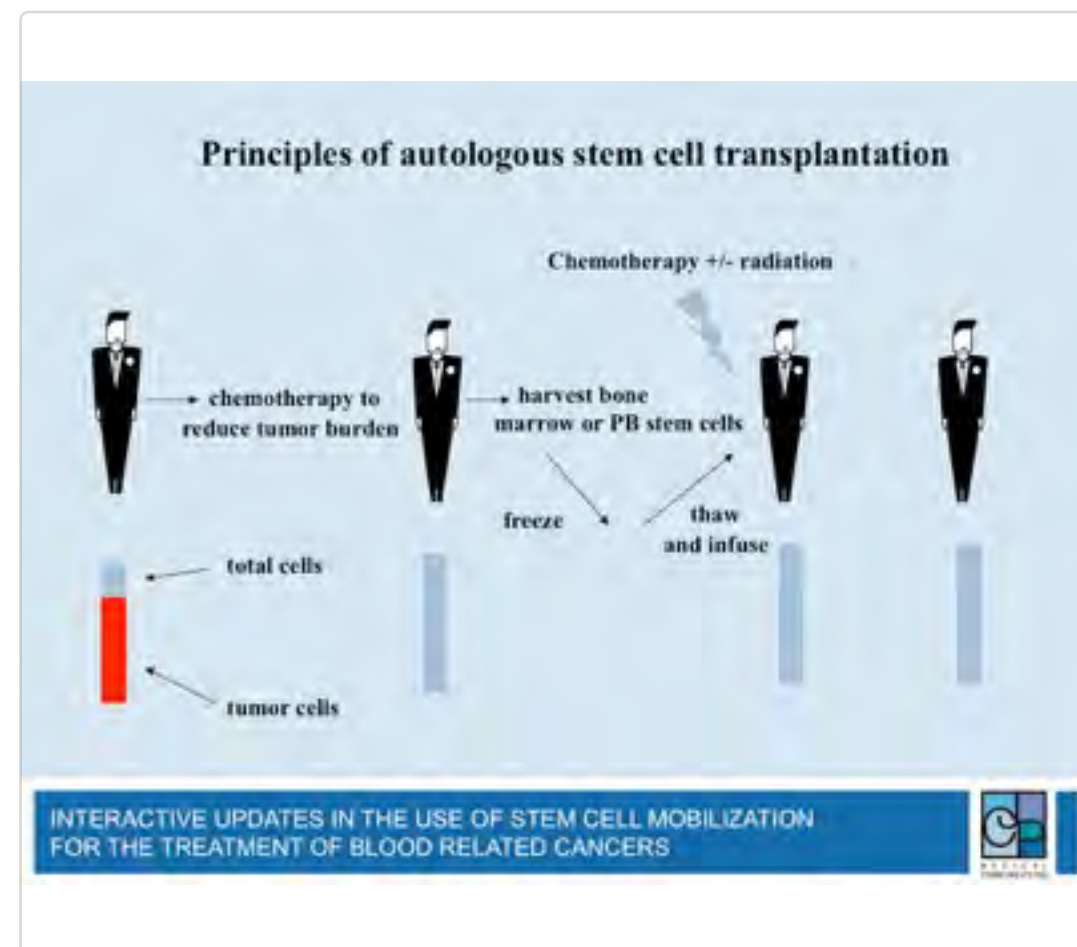
Good afternoon, my name is John DiPersio and I am Chief of the Division of Oncology and Deputy Director of the Siteman Cancer Center at Washington University School of Medicine. It is a pleasure to have this opportunity to introduce a topic of great importance to you, stem cell mobilization. Before

we get into the lectures I would like to give you a short introduction to some of the strategies and keys for stem cell mobilization as applied to both autologous and allogeneic stem cell transplantation. (**Interactive 1.1**)

The principles of autologous transplantation are illustrated here. A patient who has a malignant disease such as non-Hodgkin lymphoma or multiple myeloma comes into transplant with a fair amount of disease. The object of an autologous stem cell transplant is to give high doses of chemotherapy or chemotherapy and radiation therapy to dramatically reduce the burden of disease so that the patient's own immune system can maintain it or eliminate it after transplant. For some diseases like Hodgkin disease and non-Hodgkin lymphoma, this treatment can be curative in a fair percentage of patients. This treatment can put patients with multiple myeloma into remission for a long period of time but it is thought not to be curative in the vast majority of patients. In any event it can provide significant benefit for patients with hematologic malignancies such as non-Hodgkin lymphoma, Hodgkin disease, and multiple myeloma. (**Interactive 1.2**)

Before the high doses of chemotherapy and radiation therapy are given one must obtain stem cells, since performing this procedure in the absence of

Interactive 1.2



stem cell rescue of the patient would result in prolonged neutropenia and thrombocytopenia, and most adults would not survive this prolonged period of neutropenia.

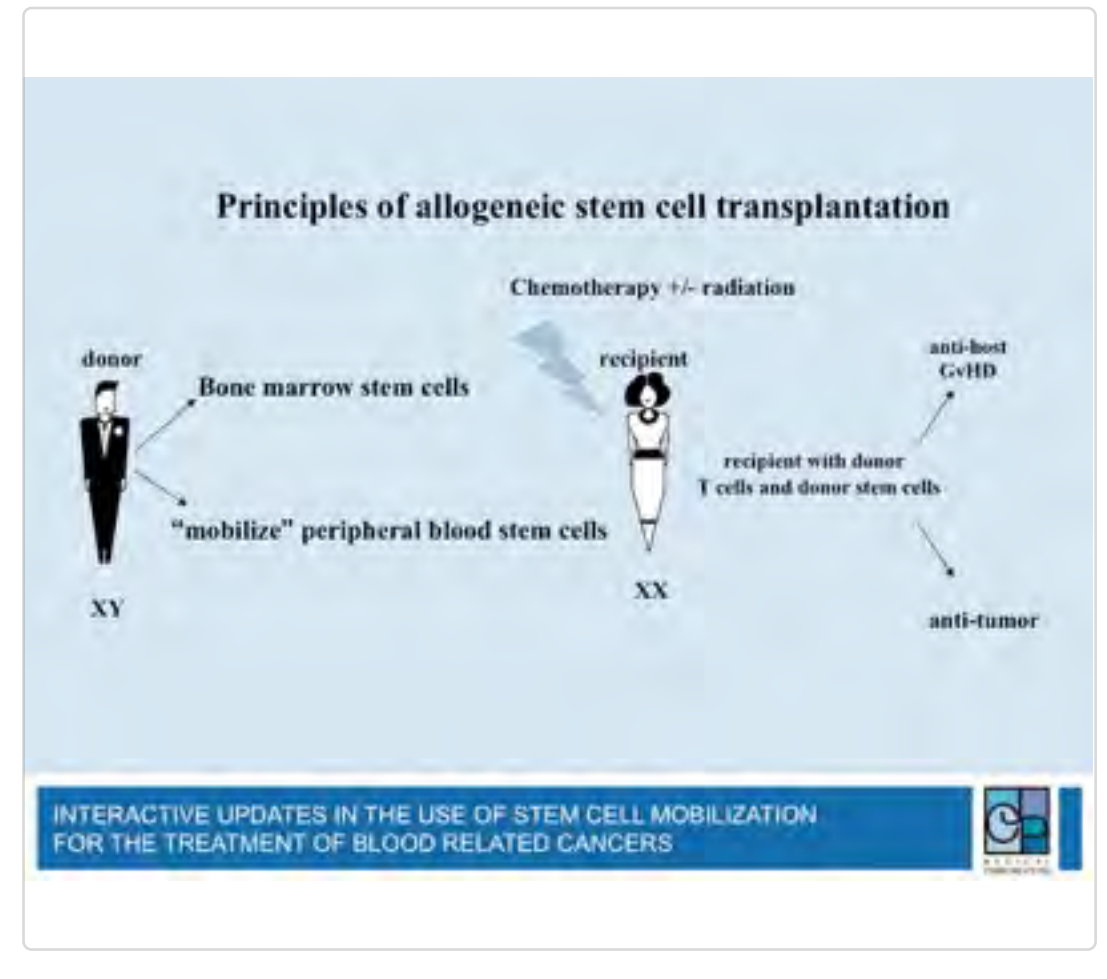
Stem cells are collected either from the bone marrow or more frequently from mobilized peripheral

blood. The stem cells are infused at the time of transplant immediately after chemotherapy where they home to the bone marrow, expand to the bone marrow, reproduce, give rise to differentiated progenitors such as myeloid, erythroid, lymphoid and megakaryocytic progenitors, and reconstitute the entire hematopoietic system of the recipient. Hopefully in that time the numbers of leukemic, lymphoma, and myeloma cells, etc. are so low that they can be maintained in that state for a long period of time, or they are completely gone and the patient's own immune system can fight them coming back.

The principles of allogeneic stem cell transplantation are slightly different. Chemotherapy is given to try and reduce the burden of disease in the recipient. However, the major reason to give chemotherapy or radiation therapy is to immune-suppress the recipient so that they can accept the peripheral stem cells. **(Interactive 1.3)**

The presence of T-cells in these products are important, not only to promote engraftment but also to eliminate the residual T-cells of the host so that

Interactive 1.3



the graft is not rejected. For the most part peripheral blood stem cells are used for this process, although in patients with non-malignant diseases, especially in children, the bone marrow is a frequent source of stem cells to reduce the risk of graft-versus-host disease (GvHD). These T-cells, which

mediate the graft versus tumor effect can cause GvHD, which can be mild, moderate, or life threatening. Patients usually require some type of GvHD prophylaxis or treatment to allow for the graft to be accepted by the recipient and also to reduce the risk of GvHD by these passively transferred, mature, native T-cells from the donor.

Here we review the basic steps. You have to have stem cells, the more stem cells the better. The large number of T-cells in peripheral blood cell products are problematic in that they may cause increased rates of acute, and more frequently chronic, GvHD. However, stem cells must be procured, either from the bone marrow or from the peripheral blood. If they come from the peripheral blood of allogeneic donors it is primarily done in the outpatient stem cell setting using G-CSF, G-CSF in combination with chemotherapy, a chemokine receptor antagonist alone, or G-CSF plus a chemokine receptor antagonist. Conditioning must be given to the recipient to eliminate the residual tumor and to promote engraftment. (**Interactive 1.4**)

Interactive 1.4

Basic Steps

1. Stem Cell Procurement
 - Post-chemotherapy and post-cytokine (auto)
 - Post-cytokine, chemokine and cytokine or chemokine alone
2. Conditioning
3. Engraftment
4. Reconstitution
5. Graft vs. Host Disease; Graft vs. Leukemia

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



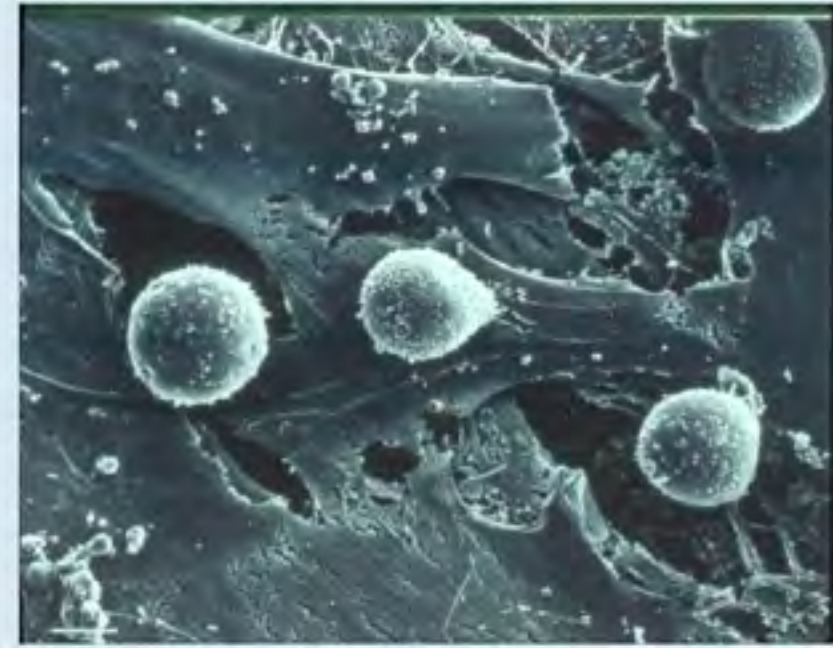
Engraftment occurs when the stem cells home to the bone marrow and expand and result in multilineage engraftment. There are many factors in the micro-environment that are key here, including soluble growth factors which promote reconstitution of all lineages. Again, there are issues relating to GvHD

and graft-versus-leukemia which must be considered with any transplant.

This is a scanning electron micrograph done in our lab looking at the interaction of highly purified human hematopoietic stem cells with a bone marrow stromal microenvironment which we constructed in vitro. You can see that the projections from the stem cells are the tethers that bind the stem cells to the microenvironment. To get these cells mobilized those tethers must be modified and the cells released into the microenvironment where they go into the vascular sinuses and out into the blood and then are collected by pheresis. (**Interactive 1.5**)

The biology of this has been elucidated over the past ten or twenty years by a number of investigators. This is a very simplistic representation of what might be happening. First of all, G-CSF is the most frequently used mobilizing agent. It directly downregulates the expression of SDF-1, which is one of those important tethers which binds to a receptor on the hematopoietic stem cell, CXCR4, a chemokine receptor. The downregulation of SDF-

Interactive 1.5



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



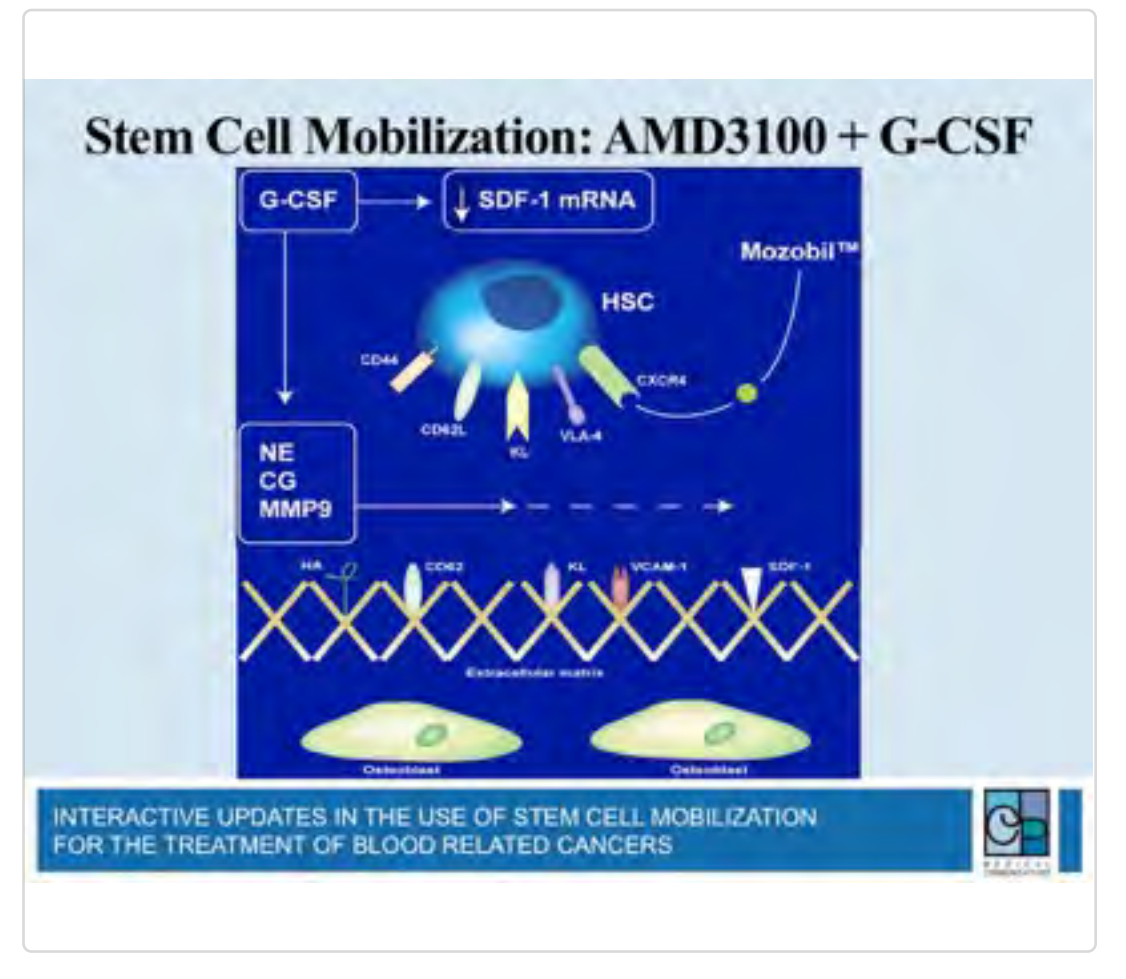
1, which occurs coincidentally with the elimination of osteoblasts, occurs over four to five days, which is exactly the temporal time frame that it takes for a stem cell to be released into the peripheral blood. So one can assume that the primary role of G-CSF is to down regulate SDF-1 over four to five

days. When it is low enough these cells will actually leave and go into the peripheral blood. (**Interactive 1.6**)

It is actually much more complicated than that and the exact mechanism of how this occurs is not clearly worked out. But a number of groups have shown that since the bone marrow microenvironment osteoblasts and stromal cells do not have receptors for G-CSF there must be some intermediate cell that induces these changes. Many groups have hypothesized that this intermediate cell is a monocyte and that G-CSF binds to a monocyte, which does several things - it releases factors which downregulate SDF-1 in the microenvironment but it also releases proteases. There I should note that none of these are known or unidentified yet. The proteases can clip the tethers and induce rapid release from the bone marrow. So that is our current understanding of mobilization. There are many other nuances that we don't have time to discuss today.

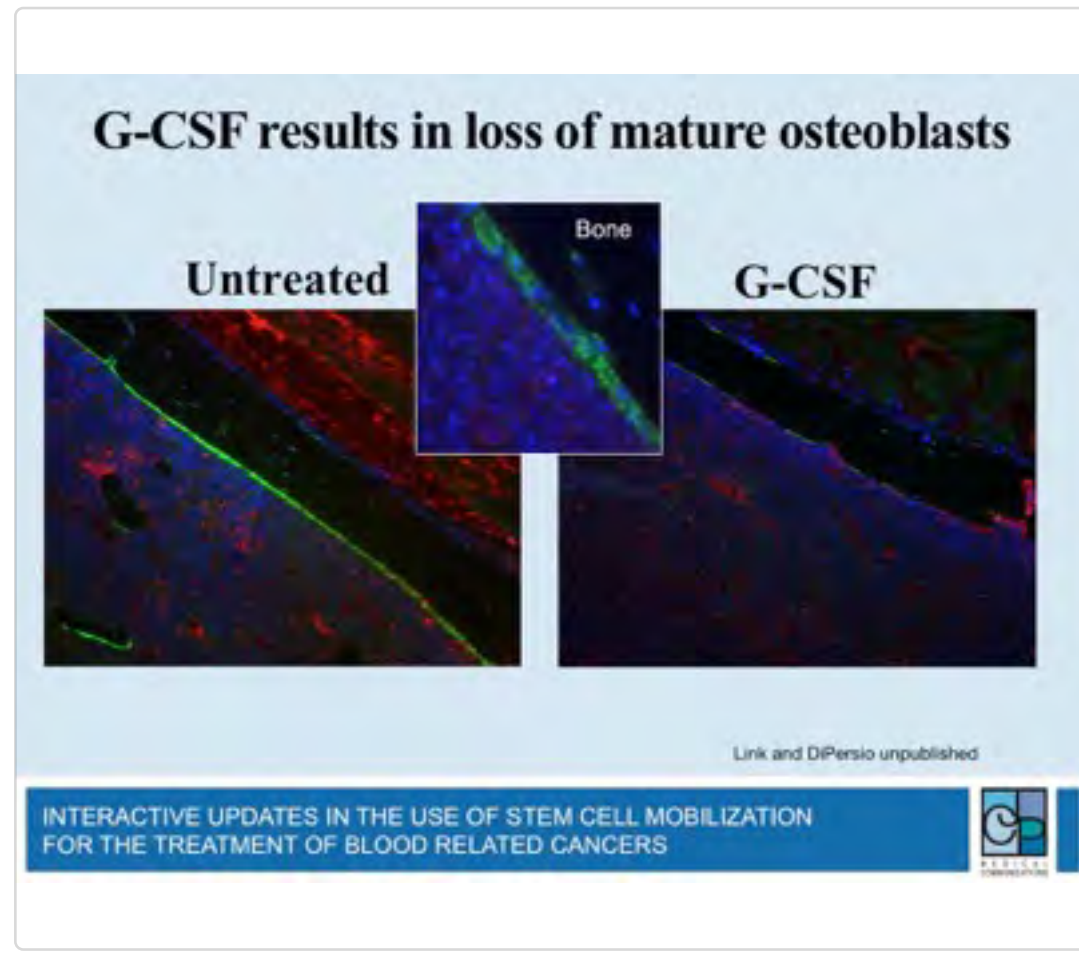
This is an example of bone marrow which shows the bone marrow osteoblast layer, the bone marrow

Interactive 1.6



itself, and the vascular sinusoids. The stem cells that are in this environment have to be released from the bone marrow osteoblasts and the mesenchymal cells and the nestin-positive cells into the vascular sinuses. You can see that G-CSF has a profound effect. After five days of G-CSF the osteoblastic layer is al-

Interactive 1.7



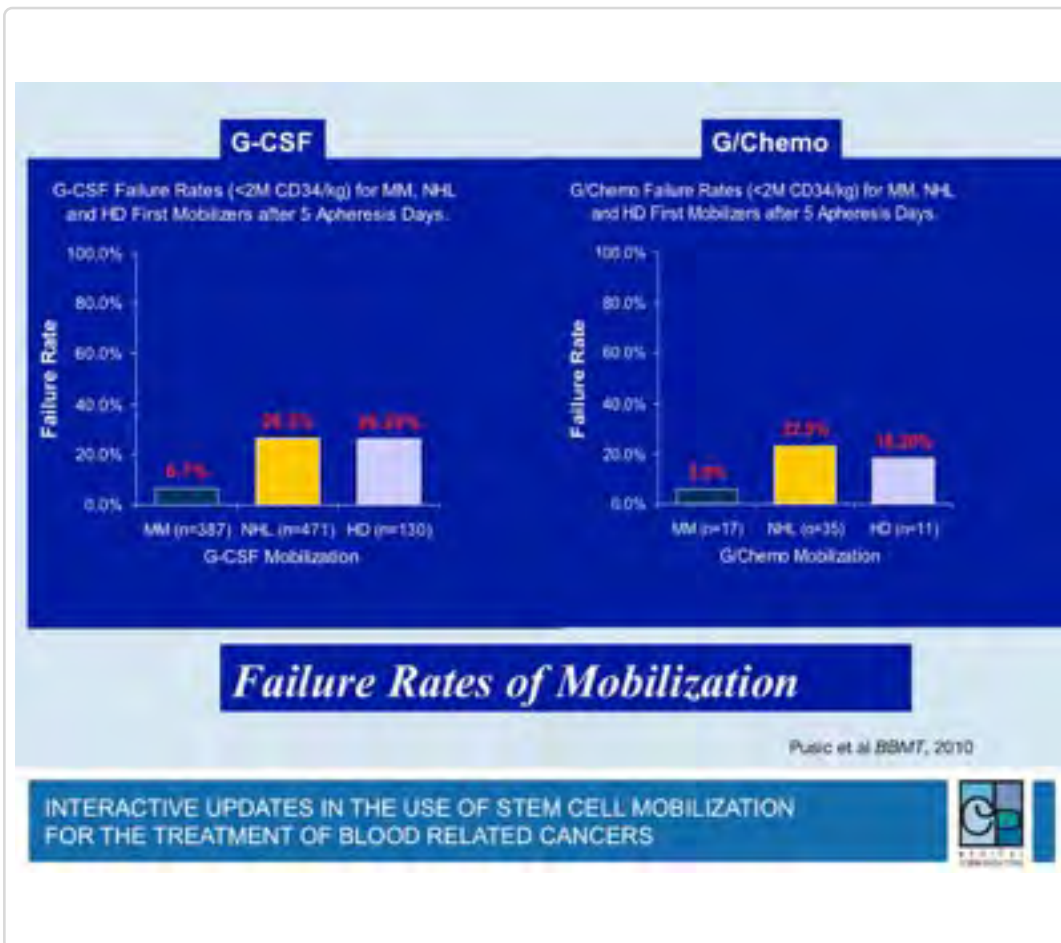
most gone. This is a time when SDF-1 levels are very low and the cells are then released into the peripheral blood. (**Interactive 1.7**)

Is there a problem with mobilization for patients undergoing autologous transplant? This is a study that we published in 2010 in BBMT and you can see

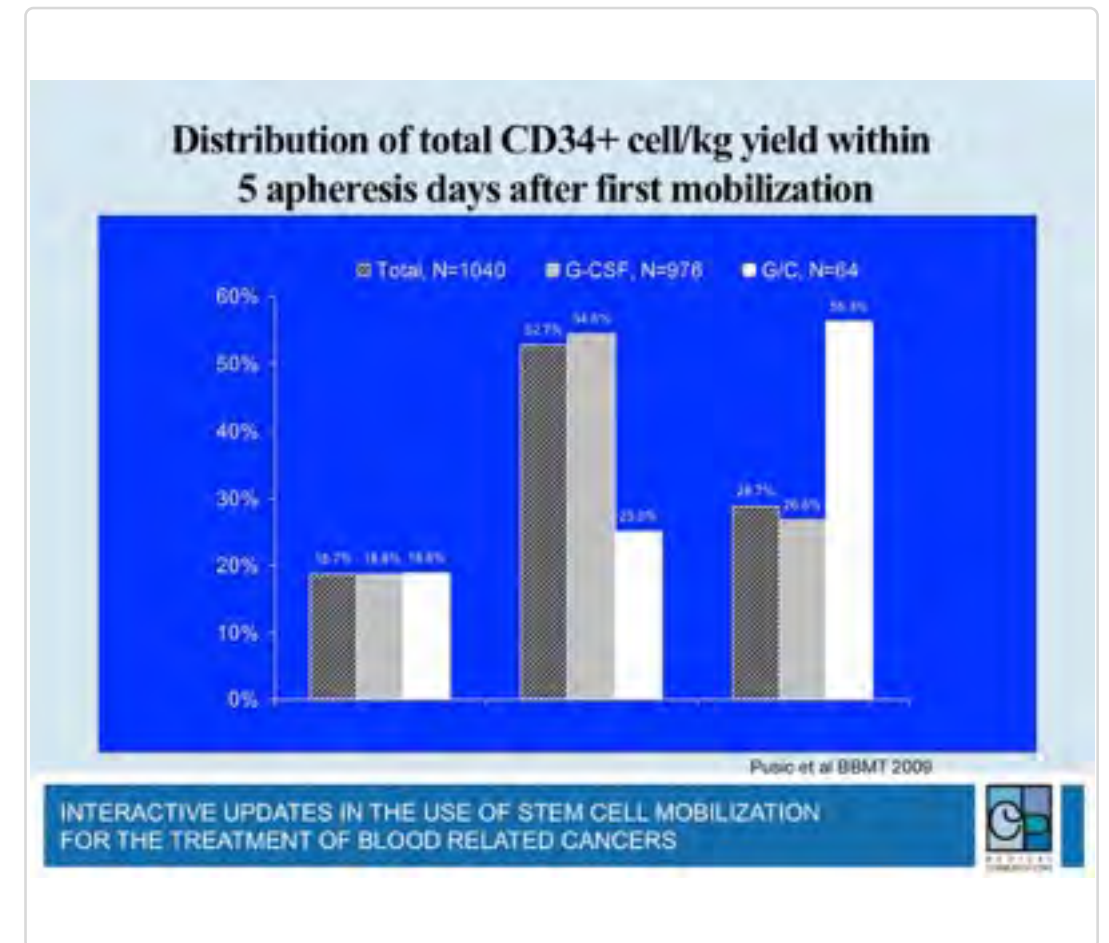
that patients with non-Hodgkin lymphoma and Hodgkin disease failed to mobilize adequate numbers of stem cells for transplant approximately 25% of the time, while this occurs in only 6% of multiple myeloma patients. Interestingly, G-CSF plus chemotherapy resulted in the same failure to reach this minimum number of stem cells for transplant. Patients that did not reach this minimum number had to be re-collected and some of those patients that needed to be re-collected actually relapsed and never got the transplant or had to undergo an allogeneic transplant with much higher morbidity and mortality. (**Interactive 1.8**) [1]

We were interested in what this looked like in our patients. You can see graphics representing the total number of patients, the G-CSF mobilized patients, and the G-CSF plus chemotherapy mobilized patients. The failure rate for all groups - myeloma, Hodgkin disease, and non-Hodgkin lymphoma - reaching the magic number of 2×10^6 CD34/kg was 18%. In patients that reached $2-5 \times 10^6$, you can see that there were higher failure rates in patients get-

Interactive 1.8



Interactive 1.9



ting G-CSF. The best mobilization, >5, occurred primarily in patients getting G-CSF plus chemotherapy. So our conclusion, from this retrospective study, was that failure rates are independent of the type of mobilization. However, with good mobilizers you want to get many cells and the best way to

do this is with G-CSF and chemotherapy, keeping in mind that chemotherapy has its own set of risks. **(Interactive 1.9)** [1]

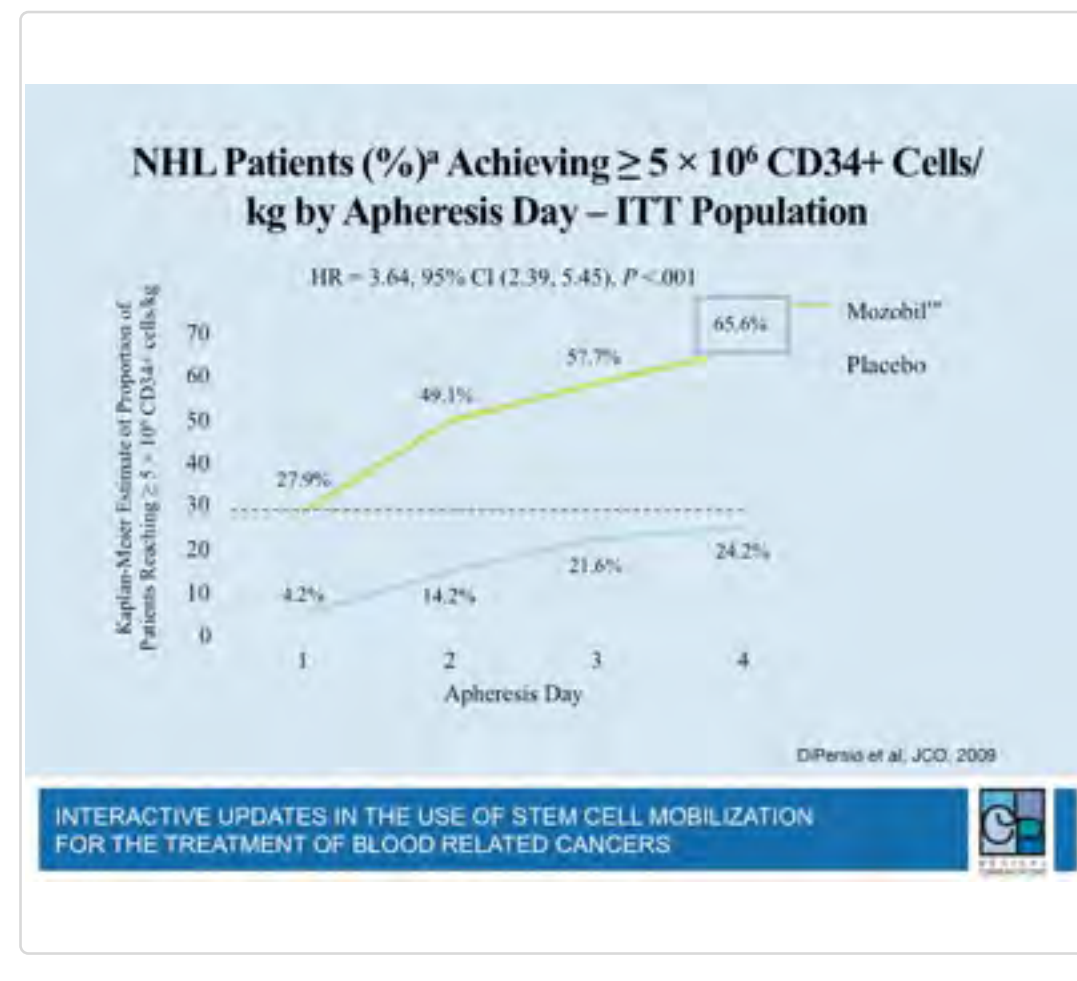
This was especially true when we did the first randomized study comparing plerixafor, a CXCR4 inhibitor, plus G-CSF versus G-CSF alone in pa-

tients with non-Hodgkin lymphoma. We looked at the number of patients who reached the optimal goal of $\geq 5 \times 10^6$ CD34+ cells/kg in four or fewer collections. You can see that the accumulated incidence of reaching that goal over 4 days in patients that received G-CSF alone was only 24%. The optimal number was reached in 65% of patients that received plerixafor and G-CSF. So this combination had a profound effect on increasing yields. **(Interactive 1.10) [2]**

This study was actually the study (in multiple myeloma patients along with a parallel study published in *Blood* in myeloma), which got plerixafor with G-CSF approved for the treatment of non-Hodgkin lymphoma and multiple myeloma for mobilization of stem cells in the oncology setting.

But what was more interesting in this study was not the number of patients who reached the optimal number, but the number of patients who improved from their minimum number, because patients that don't reach the minimum number are in trouble and need to be remobilized. So patients that received

Interactive 1.10



plerixafor plus G-CSF had an 86.7% chance of reaching the minimum number necessary for a transplant (2×10^6 CD34/kg) in four or fewer collections, while the placebo group had only a 47% chance of reaching this important milestone. Therefore, the addition of plerixafor to G-CSF for the first time showed im-

Interactive 1.11

Secondary Efficacy: Patients Achieving ≥ 2 million CD34+ cells/kg in 4 Days of Apheresis				
Intent-to-Treat Population				
Outcome	Plerixafor (n=150)	Placebo (n=148)	Estimate of Treatment Effect [95% CI]	p-value*
Success	130 (86.7%)	70 (47.3%)	39.4% ^A [29.7%, 49.1%]	<0.0001

^A Treatment effect estimated using difference in chance of success
* p-value of difference in proportions using Pearson's Chi-Squared test

DiPersio et al JCO, 2009

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS

how it should be given in patients is a subject of another talk in this series. (**Interactive 1.11**) [2]

In conclusion, I would like to emphasize that this entire session is focused on understanding the clinical and basic science aspects of stem cell mobilization and the role of different approaches to optimize stem cell collection. In the first talk, Dr. Waller from Emory University will be discussing how he predicts those patients who will be poor stem cell mobilizers and what he does with these patients. Dr. Giralt will discuss mobilization strategies for both myeloma and lymphoma. Dr. Costa will discuss a number of important pharmacoeconomic and predictive algorithms for stem cell mobilization that he has developed and strategies for optimizing stem cell mobilization using an approach which minimizes costs and toxicity to the patient. I will finish with two talks, one focused on the role of mobilized stem cells in allogeneic stem cell transplantation and the second on future and novel approaches for stem cell mobilization, which are both in preclinical development and are being

Interactive 1.12

OVERVIEW

- Identifying Patients at Risk: Predicting Poor Stem Cell Mobilization
(Dr. Waller)
- Current Stem Cell Mobilization Strategies in autologous Hematopoietic Stem Cell Transplants (aHSCT) for Multiple Myeloma (MM) and non-Hodgkin Lymphoma (NHL)
(Dr. Giralt)-
- Mobilization Algorithms to Optimize Patient Outcomes and the Pharmacoeconomics of Stem Cell Mobilization Strategies
(Dr. Costa) –
- Allo mobilization
(Dr. DiPersio)-
- Future novel approaches
(Dr. DiPersio)

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



tested in early phase clinical trials in the clinic. I want to thank you for your attention and I hope you enjoy the other lectures. (**Interactive 1.12**)

REFERENCES

1. Pusic I, Jiang SY, Landua S, et al. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. *Biol Blood Marrow Transplant*. 2008 Sep;14(9):1045-56. <http://www.ncbi.nlm.nih.gov/pubmed/18721768>
2. DiPersio JF, Micallef IN, Stiff PJ, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 2009 Oct 1;27(28):4767-73. <http://www.ncbi.nlm.nih.gov/pubmed/19720922>



Chapter 2

Identifying Patients at Risk: Predicting Poor Stem Cell Mobilization

Edmund K. 'Ned' Waller, MD, PhD, FACP



Edmund K. 'Ned' Waller, MD, PhD, FACP

Professor of Medicine, Pathology
and Hematology/Oncology
Winship Cancer Institute of Emory
School of Medicine
Director, Division of Stem Cell
Transplantation and Immunotherapy
Director, Bone Marrow and Stem Cell
Transplant Center
Atlanta, Georgia

Edmund K. Waller completed his undergraduate degree at Harvard University in 1978, his MD-PhD degree at Cornell-Rockefeller in 1985, and his clinical training in Oncology at Stanford University in 1991. As a post-doctoral Fellow at Stanford from 1991-1993, he studied the fate of human immune cells in immune-deficient mice with Dr. Irving Weissman, MD. He then characterized the stem cell activities of FACS-isolated progenitor cells as a research scientist at Becton Dickinson. Dr. Waller was recruited to Emory University in 1994. In 2005 he was promoted to a tenured Professor of Hematology and Medical Oncology, Medicine and Pathology at Emory University. He has served as the Director of the Bone Marrow and Stem Cell transplant Program since 1998. He also currently serves as the Director of the Division of Stem Cell Transplant and Immunotherapy. Dr. Waller is the author of over 191 peer reviewed articles with an h-index of 37. He has trained 30 post-doctoral fellows and directly supervised six graduate students.

Dr. Waller serves as Director of the Bone Marrow and Stem Cell Transplant Center of Emory University and as the Medical Director for the Stem Cell Processing Laboratory, Hemapheresis Department. Under his leadership, the annual number of stem cell transplants performed at Emory has risen from 93 in 2000 to over 350 in 2014. He was appointed as the Medical Director for Clinical Trials at the Winship Cancer Institute in 2005 and then served as the Associate Director of Clinical Research of the Winship Cancer Institute for an additional three years, through 2012, supporting the initial P30 grant submission and the successful renewal application. Under his management, the number of subjects accrued annually to therapeutic clinical trials at the Winship Cancer Institute rose from 218 in 2004 to over 500 in 2014.

Dr. Waller's research is funded by the National Cancer Institute and focuses on optimizing antitumor immunity in cancer patients. He has developed novel strategies of regulating immune responses by studying the interaction between T cells and dendritic cells in murine models and by using clinical samples from patients. Projects in his laboratory include graft engineering to select donor dendritic cell subsets that enhance graft versus leukemia activities and post-transplant immune reconstitution, novel drugs that target coinhibitory immune pathways and regulate graft-versus-host disease. The clinical translational focus of his research is in patients with hematological malignancies, especially patients undergoing autologous and allogeneic stem cell transplantation.

Movie 2.1

Identifying Patients at Risk: Predicting Poor Stem Cell Mobilization

Edmund K. Waller, MD, PhD, FACP
Emory University
Atlanta, GA 30036

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Identifying Patients at Risk: Predicting Poor Stem Cell Mobilization

Edmund K. 'Ned' Waller, MD, PhD, FACP

Interactive 2.1

Identifying Patients at Risk: Predicting Poor Stem Cell Mobilization

Edmund K. Waller, MD, PhD, FACP
Emory University
Atlanta, GA 30036

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



I'm Ned Waller and I direct the Bone Marrow and Stem Cell Transplant Program at Emory University in Atlanta. Today I'll speak about identifying patients at risk for poor stem cell mobilization. (**Interactive 2.1**)

To understand the problem of poor stem cell mobilization, it is useful to take a step back and look at the normal physiology of stem cells in the bone marrow. (**Interactive 2.2**)

What regulates the retention of hematopoietic stem cells in the bone marrow, and how are they re-

Interactive 2.2

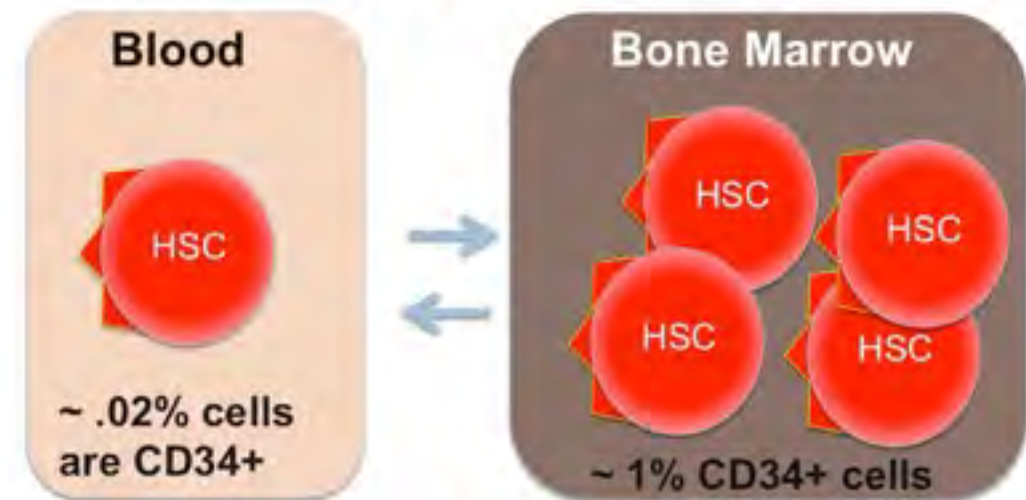
Brief primer on mobilization

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 2.3

Hematopoietic stem cells circulate between bone marrow and blood



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS

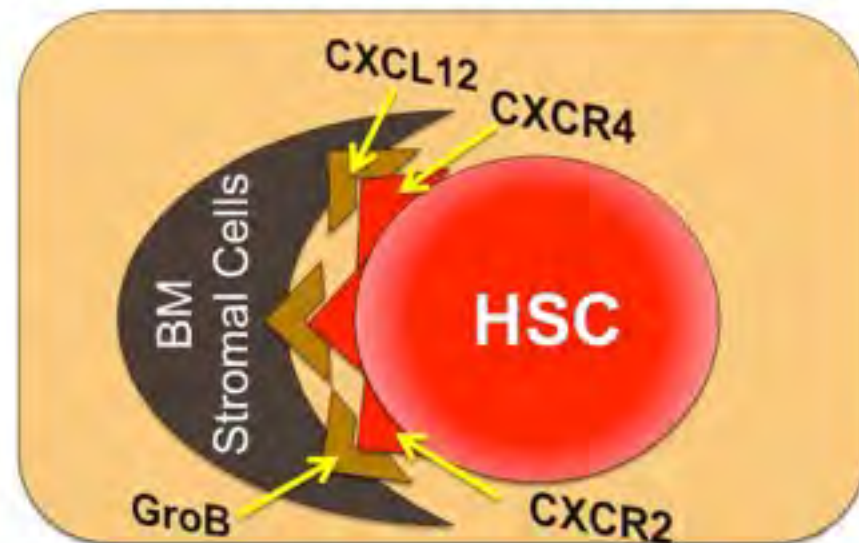


leased during mobilization procedures? The bone marrow contains hematopoietic stem cells which can differentiate to form all the formed cellular elements of the blood. (**Interactive 2.3**) Stem cells can be identified by the expression of the CD34 antigen. And CD34 positive cells constitute about 1% of

the nucleated cells in adult bone marrow. These CD34 positive stem cells normally circulate into the blood where they constitute a small fraction of leucocytes, generally about 0.02%, such that the absolute number of CD34 positive cells in the blood is usually one or two cells per microliter.

Interactive 2.4

Hematopoietic stem cells are tethered to stromal cells in the bone marrow



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Stem cells are retained within the bone marrow microenvironment because of adhesion molecules and chemokine receptors that tether them to stromal cells. In particular, the chemokine receptor CXCR4 binds to a ligand expressed on bone marrow stromal cells called CXCL12 or SDF1. In addition, the che-

mokine receptor CXCR2 binds to a ligand on stromal cells called GroB. These and other protein-protein interactions keep stem cells in close apposition to bone marrow stromal cells. (**Interactive 2.4**)

Decades ago it was noted that G-CSF mobilizes stem cells from the bone marrow into blood. The process usually takes four or five days of G-CSF mobilization and is accompanied by a significant rise of the white cell content of the blood. The mechanism for this process wasn't clear because stem cells do not express receptors for G-CSF. (**Interactive 2.5**) [1]

Work by Christopher and Link and others have shown that there is a complex interplay between bone marrow macrophages and osteoclasts that express the G-CSF receptor and osteoblasts that express the ligand for CXCL12; the tether that helps keep stem cells in the bone marrow microenvironment.

When G-CSF is administered to patients, it stimulates bone marrow macrophages through the G-CSF receptor such that they interact with the osteoblasts to downregulate levels of CXCL12 expression,

G-CSF induces monocytes to suppress CXCL12 synthesis by osteoblasts

BM macrophage/ Osteoclasts

G-CSFR

Osteoblast

CXCL12 mRNA

CXCR4

HSC

G-CSF

Christopher & Link (2011) *J Exp Med*.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

Administration of G-CSF also causes release of proteases into the bone marrow microenvironment, both from neutrophils which express the G-CSF receptor as well as osteoclasts themselves. The genera-

G-CSF induces proteases that cleave adhesion molecules in the BM microenvironment

Rank-L Activates Osteoclasts- Proteases released

Neutrophils

Fibronectin

VCAM1

VLA4

HSC

Proteases

G-CSF

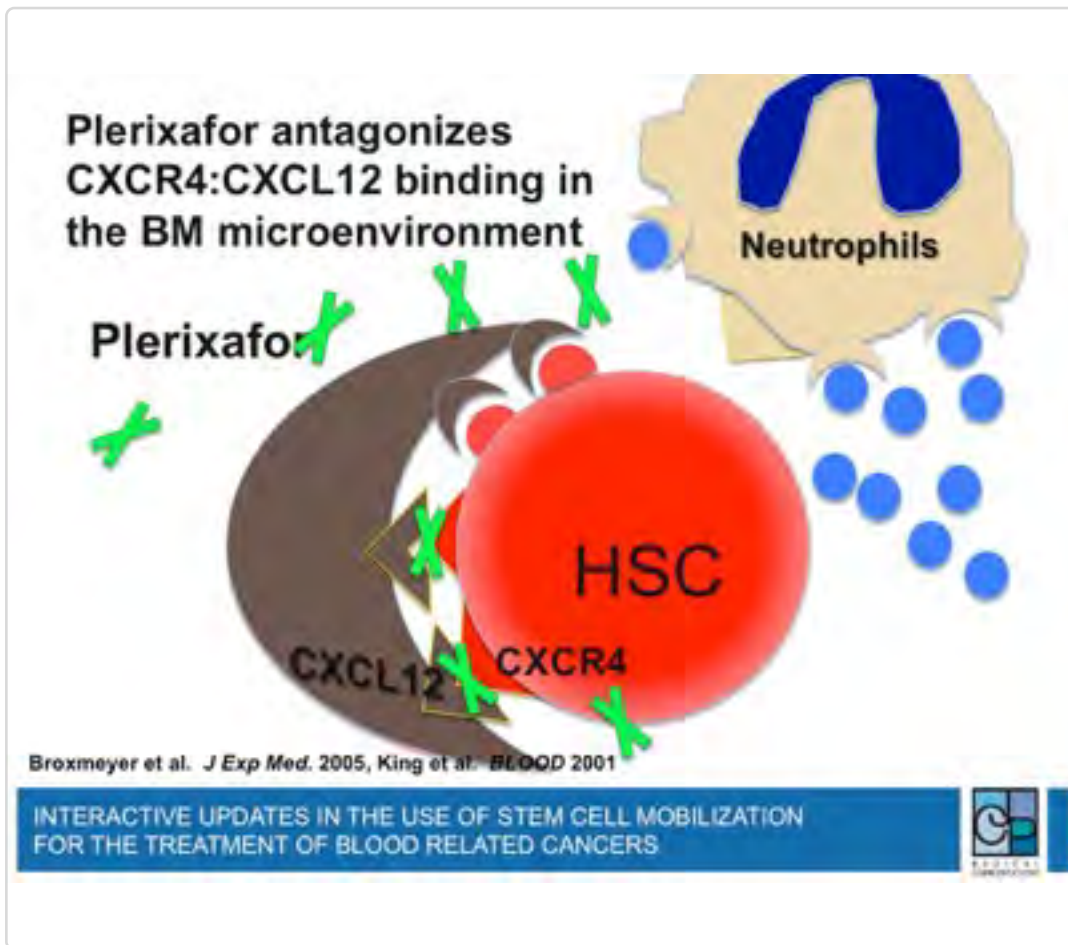
Shivtiel et al. *J Exp Med.* 2008; Nervi B, et al. *J Cell Biochem.* 2006; Kollet et al. *Nat Med* 2006

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

CEP

Fifteen years ago it was discovered that a small molecule antagonist to the binding of CXCL12 to the CXCR4

Interactive 2.7



chemokine receptor can mobilize stem cells from the bone marrow into the blood. (**Interactive 2.7**) [5,6]

This slide shows the activity of plerixafor and competitive binding to the CXCR4 receptor displacing CXCL12, thereby releasing stem cells from their attachment to bone marrow stromal cells and mobi-

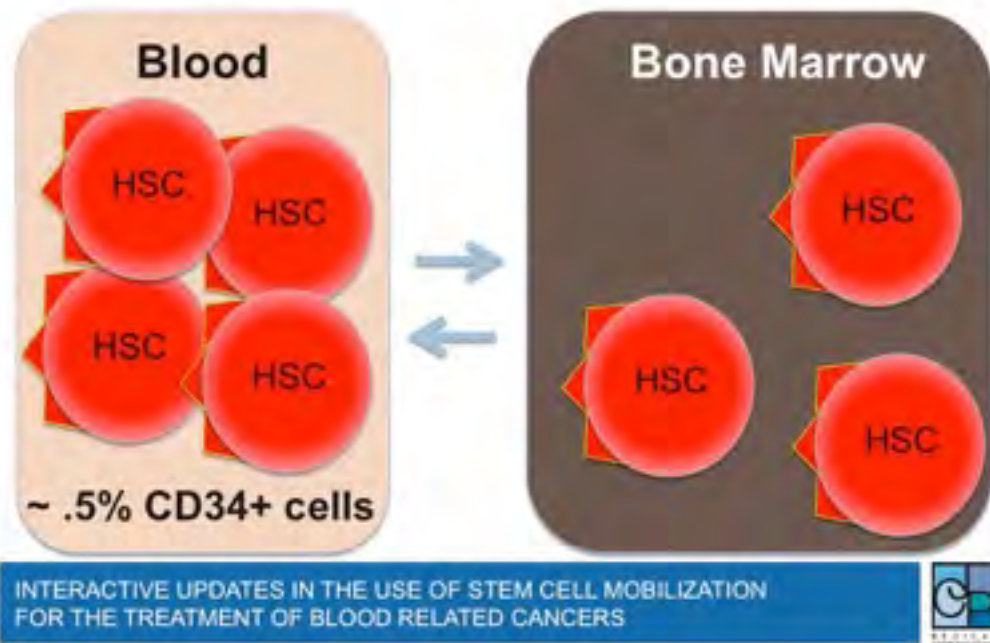
lizing them into the bloodstream. In contrast to the affected G-CSF, which usually takes four or five days to result in stem cell mobilization, plerixafor works faster, inducing a maximum number of bone marrow stem cells mobilized into the blood 8 to 10 hours after its administration.

The net result of stem cell mobilization is to increase the number of hematopoietic stem cells in the blood compartment, such that the absolute percentage of CD34 positive cells rises 20 to 100 times so that they constitute half or even one percent of the nucleated cells in the blood. With successful mobilization, the absolute number of CD34 positive cells in the blood rises above 20 cells per microliter. (**Interactive 2.8**)

So we have reviewed the physiologic process of stem cell mobilization, noting the activities of G-CSF in stem cell mobilization as well as competitive antagonists to the protein-protein interaction between CXCR4 and its ligand, CXCL12. With these drugs we can successfully mobilize stem cells. But how many stem cells do we need for a successful autologous stem cell transplant? A number of studies have ad-

Interactive 2.8

Net result of HSC mobilization



Interactive 2.9

What is the optimal CD34+ cell dose for patients undergoing hematopoietic stem cell transplantation?

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

dressed the question of the optimal CD34 cell dose for patients undergoing hematopoietic stem cell transplantation. (**Interactive 2.9**)

This slide shows a figure from a paper published 20 years ago by Bill Bensinger and his team at the Fred Hutchinson Cancer Research Center. They exam-

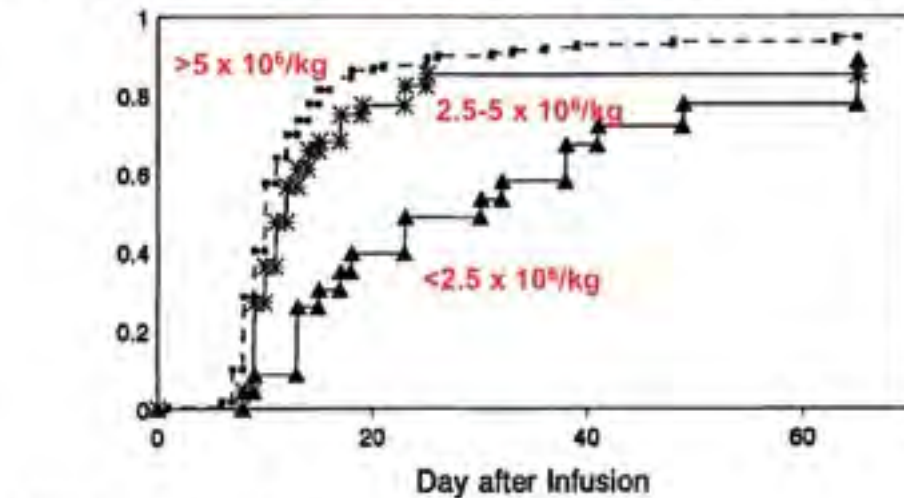
ined patients undergoing autologous stem cell transplantation with G-CSF mobilized stem cell products and correlated the content of CD34 positive cells in the stem cell graft with the kinetics of platelet recovery. Patients were stratified into three groups: those that had received a small number of stem cells (less

than 2.5 million CD34 positive cells/kg), those patients who received an intermediate number of stem cells (between 2.5 and 5 million CD34 positive cells/kg) and those patients who received a large number of stem cells (greater than 5 million CD34 positive cells/kg). (**Interactive 2.10**) [7]

This graph shows the probability that patients will have platelet engraftment, defined as a platelet count of greater than 20,000 cells per microliter without platelet transfusion over time after transplant. The top curve shows the fraction of patients who engrafted after receiving a large dose of CD34 positive cells, greater than 5 million/kg, and you can see that the median time to platelet engraftment was slightly less than 10 days and nearly all patients ultimately had successful platelet engraftment. In contrast, the lower curve shows those patients who received a low number of CD34 positive cells/kg. Here, the median time to platelet engraftment was nearly 30 days and even after two months only 80% of patients had had successful engraftment.

Interactive 2.10

Probability of platelet engraftment by CD34+ cell dose after auto-transplant



Bensinger (1985) JCO 13:2547-2555

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



These data suggest that a stem cell dose of at least 2.5 million cells/kg is necessary to achieve rapid and durable platelet engraftment, hallmarks of a successful autologous stem cell transplant.

In the same study Bensinger and his colleagues performed multivariate analysis to determine which

factors were associated with more rapid myeloid and platelet engraftment. (**Interactive 2.11**) [7]

In this table we see the covariates associated with the time to achieving absolute neutrophil cell count of greater than 1,000 per microliter, and a covariate associated with the time post-transplant to achieve a platelet count of greater than 20,000 per microliter. Neutrophil engraftment was associated with a CD34 positive stem cell dose of greater than 2.5 million cells/kg. It was also associated with the administration of growth factors post-transplant and the use of a TBI-based conditioning regimen. The time to platelet engraftment was associated with a stem cell dose of greater than 2.5 million cells/kg, a stem cell dose of greater than 5 million cells/kg as well as the use of post transplant cytokines and prior irradiation as part of the conditioning regimen.

Similar data have been presented by John Glaspy in this 1997 publication, *Blood*. Here again he looked at the kinetics of platelet engraftment in patients undergoing autologous stem cell transplant and correlated the probability of successful platelet engraft-

Interactive 2.11

Probability of platelet engraftment by CD34+ cell dose after auto-transplant

Table 6. Results of Multivariate Analysis on Tempo of Engraftment

Parameter Analyzed	Variable	P	Risk Ratio	95% Confidence Limits
Time to ANC > 1,000/ μ L	CD34 > 2.5×10^6 /kg	.0011	2.08	1.31-3.28
	Postinfusion CSF	.0001	2.25	1.66-3.06
	TBI regimen	.0337	1.35	1.02-1.79
Time to platelets > 20,000/ μ L	CD34 > 2.5×10^6 /kg	.0001	2.61	1.57-4.34
	Postinfusion CSF	.0251	0.59	0.43-0.82
	Prior radiation	.0238	0.70	0.53-0.93
	CD34 > 5.0×10^6 /kg	.0107	1.65	1.16-2.35

Berenson ET, Miller LD, et al. 1997;2547-2556

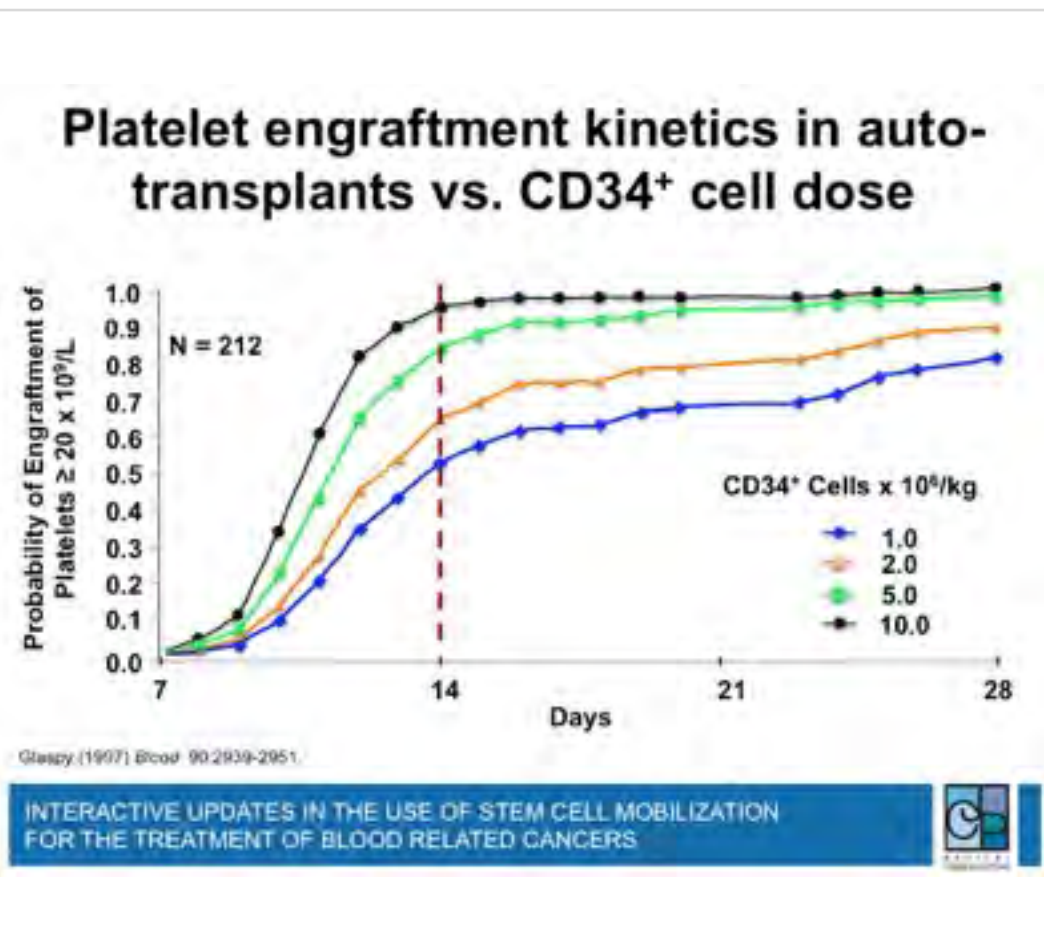
INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



ment with the dose of CD34 positive cells in the blood. (**Interactive 2.12**) [8]

In the blue curve we see those patients who received the lowest stem cell dose, containing only 1 million CD34 positive cells/kg. The median time to platelet engraftment was slightly greater than two

Interactive 2.12



weeks, and only 80% of those patients achieved a platelet count independent of transfusions of at least 20,000 by one month post-transplant.

With increasing doses of stem cells there was an increased rapidity of platelet engraftment and a higher proportion of patients who achieved a platelet count of

20,000 within the first month post-transplant. The optimal dose of stem cells from the study appeared to be 5 million CD34 positive cells or greater.

Finally, Pat Stiff analyzed the correlation between the stem cell dose in the autograft and durable hematopoietic engraftment measured 12 months post-transplant. In this study, he examined patients undergoing autologous stem cell transplant who had non Hodgkin lymphoma or multiple myeloma. He categorized patients according to whether the received stem cell dose was low (between 2 million and 4 million CD34 positive cells/kg), intermediate (between 4 and 6 million cells/kg), or high (greater than 6 million cells/kg). (**Interactive 2.13**)

Patients who received at least 4 million CD34 positive cells/kg had a greater than 80% probability of achieving durable platelet engraftment, with a platelet count in the normal range of $>150,000/\mu\text{L}$ at 12 months post transplant. In contrast, patients who had received a lower number of stem cells, between 2 million and 4 million cells/kg, had only a 56

Interactive 2.13

Higher CD34⁺ Cell Dose Leads to More Durable Hematopoietic Engraftment

Percent Patient Platelet Count > 150,000/ μ L

CD34 ⁺ Cell Dose Transplanted	2-4 $\times 10^6/\text{kg}$	$\geq 4-6$ $\times 10^6/\text{kg}$	> 6 $\times 10^6/\text{kg}$	P value
Non-Hodgkin Lymphoma	N = 76	N = 75	N = 66	
At 12 months	56%	81%	83%	0.020
Multiple Myeloma	N = 75	N = 82	N = 64	
At 12 months	74%	83%	81%	0.435

Stiff (2008) Blood 112:758-759. Abstract 2175

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



to 74% probability of achieving durable normal platelet counts 12 months post transplant.

From these data we would conclude that the optimal number of stem cells is at least 4 million CD34 positive cells/kg to achieve durable and normal hematopoiesis 12 months after transplant.

From Emory we have examined this question as well as in this publication with Mary Ninan, where we looked at thrombopoiesis as a covariate for long term survival after auto transplant. We examined a large number of patients undergoing autologous transplant mainly for lymphoma or multiple myeloma and asked which clinical factors are associated with long term survival. (**Interactive 2.14**) [9]

We found in a multivariable analysis that failure to achieve a normal platelet count after auto transplant, defined again as 150,000 cells per microliter, was associated with an increased risk of death. Another significant covariate associated with post-transplant death was a fluctuating platelet count that initially rose after engraftment and then fell, a phenomenon we called ISPT, or idiopathic secondary post-transplant thrombocytopenia. Patients who had evidence of initial platelet engraftment but then had declining platelet counts also had an increased risk of death post-transplant. Other significant clinical covariates associated with post-transplant death were the extent of prior chemotherapy as well as the disease status at transplant.

Interactive 2.14

Durable thrombopoiesis is highly significant in a multi-variate model for survival after auto-transplant

Table 4. Multivariable Model with Hazard Ratio for Death Post-Transplant, Stratified by Disease Diagnosis

	HR	95% CI		P-value
		Lower	Upper	
Maximal initial posttransplant platelet count <150,000/ μ L*	2.3	1.5	3.5	<.001
ISPT*	2.1	1.3	3.5	.003
Number of prior regimens	1.4	1.1	2.0	.022
Disease status at transplant*	2.0	1.4	2.9	<.001

Ninan & Waller (2007) BMMT 13:895-904

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



From these data we conclude that durable hematopoietic engraftment, particularly durable platelet engraftment, is a significant covariate for the long term success and disease control of the autologous transplant maneuver.

From the data we have looked at so far we can conclude that the optimal number of autologous CD34 positive cells in the graft are at least 4 million cells/kg. Patients that receive greater than 4 million CD34 positive cells/kg typically have rapid and durable platelet engraftment. We have also seen that failure to achieve normal platelet counts is associated with an increased risk of death post-transplant.

So we turn to the question of what clinical and laboratory factors can predict poor mobilization and identify the subset of patients who are unlikely to collect the optimal number of CD34 positive stem cells.

(Interactive 2.15)

One problem in collecting the optimal number of stem cells is timing, or when to start apheresis. For patients receiving G-CSF the answer is simple – start apheresis on the fifth day of G-CSF administration. For patients receiving the combination of G-CSF and plerixafor the answer again is simple – start apheresis the morning after plerixafor administration.

Among patients who receive chemotherapy based mobilization it is a bit trickier. Patients who receive

Interactive 2.15

What clinical and laboratory factors predict poor mobilization?

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



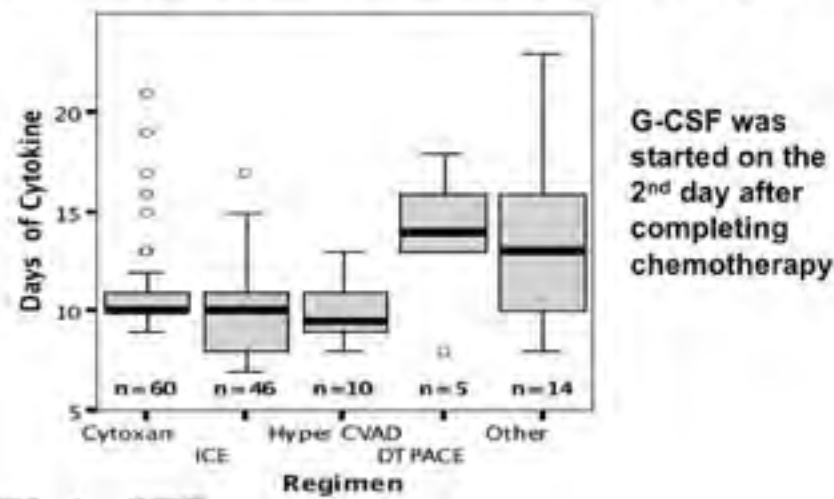
chemotherapy prior to stem cell mobilization have prolonged periods of low white blood cell counts. Mobilization is best initiated when the white blood cell count starts to recover after the effects of myelotoxic chemotherapy.

In order to collect the optimal number of stem cells, we need to be able to predict when that will occur. In this paper by Michelle Hicks using Emory data, we looked at the distribution of patients classified by the chemotherapy mobilization regimen on the x-axis according to the number of days of growth factor needed prior to the initiation of apheresis, as shown here on the y-axis. (**Interactive 2.16**) [10]

Growth factors began the second day after the last dose of chemotherapy. For patients receiving cytoxan mobilization, there was a tight clustering around 10 days of G-CSF administration, such that hematopoietic recovery after cytoxan was highly predictable and most patients required between 9 and 11 days of G-CSF before apheresis could be initiated. For patients receiving more complex regimens involving multiple drugs, the time to begin apheresis was still around 9 to 10 days of growth factor, the ICE and Hyper CVAD, but there was greater variation in the number of days of growth factor needed prior to hematopoietic recovery.

Interactive 2.16

Timing of the start of apheresis by days of G-CSF after chemotherapy



Hicks & Waller (2006) Transfusion 47:629-35

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Some patients mobilized more quickly after 8 days of G-CSF. Some patients required up to 14 days of G-CSF administration, making it difficult to schedule patients for apheresis. For patients receiving salvaged DT PACE or other salvage chemotherapy regimens, there was even a greater delay between the

last dose of chemotherapy and initiation of apheresis with a wide range of times, such that it became almost impossible to accurately predict when patients would be ready to start apheresis.

The same question of mobilization after chemotherapy has been examined in a recent paper by Xia. He looked at mobilization with G-CSF in non Hodgkin lymphoma patients after recovery from prior treatment with ICE or rituximab ICE chemotherapy. Eighty-eight patients received one-to-four cycles of chemotherapy, and G-CSF was started five days after chemotherapy at a standard dose of 5mcg/kg twice a day. (**Interactive 2.17**) [11]

Nearly three quarters of patients mobilized at least 15 CD34 positive cells/uL and collected greater than 2 million CD34 positive cells/kg in the apheresis product. Nearly a quarter of patients were poor mobilizers, with a stem cell content in the blood of less than 15 cells/uL, and 16% of patients were non-mobilizers with a stem cell dose so low they could not proceed to apheresis.

Interactive 2.17

Mobilization with G-CSF in NHL patients after recovery from prior ICE or R-ICE

88 NHL patients received 1-4 cycles of ICE or R-ICE, G-CSF started on day 5 @ 5 ug/kg BID

68 patients 77%% mobilized >15 CD34+ cells/uL and harvested > 2 x 10⁶ CD34+ cells/kg

30 patients (22.7%) were poor mobilizers with PB CD34 <15 CD34+ cells/uL.

14 patients (15.9%) were non-mobilizers with <7 CD34+ cells/uL and did not proceed to apheresis.

74 patients proceeded with apheresis: 52 (70.3%), 21 (28.4%), and 1 (1.4%) received 1, 2, and 3 days of apheresis, respectively.

Xia. (2014) Journal of Clinical Apheresis 29:322-330

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

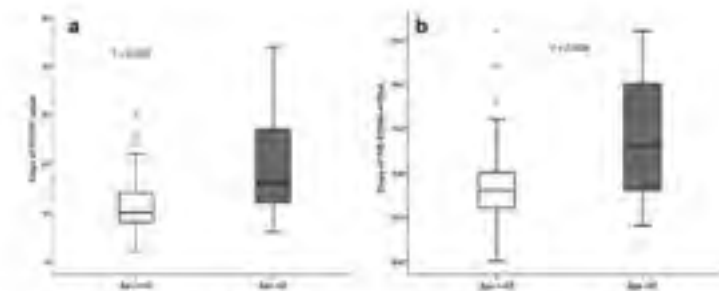


Interactive 2.18

Older age, BM NHL, and prior XRT were associated with mobilization failure

TABLE 3. Factors Associated with Non mobilization

Factor	% (Failed Mobilization)	Univariate	Odds ratio (95% CI)	Multivariate
Prior BM involvement				
Yes	46.7% (7/15)	0.002	8.42 (1.25-53.8)	0.021
No	9.7% (7/72)			
Prior radiotherapy				
Yes	44.3% (6/13)	0.007	5.61 (0.71-42.7)	0.115
No	9.2% (6/65)			
Number of induction chemotherapy regimens				
>1	36.1% (8/22)	0.004	5.12 (0.73-33.8)	0.124
1	9.1% (6/65)			
Hematological toxicity				
Platelets <10 ⁹ /L	22% (13/59)	0.011	4.31 (0.39-48.0)	0.177
Neutrophils <10 ⁹ /L	3% (1/33)			



Xia. (2014) Journal of Clinical Apheresis 29:322-330

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



These data suggest that even with chemotherapy mobilization a significant proportion of patients are non-mobilizers and are not able to undergo apheresis following G-CSF administration.

Xia looked at the covariates associated with mobilization failure and found in a multivariable analysis that

older age, bone marrow involvement by lymphoma, and prior radiation therapy were all associated with mobilization failure. (**Interactive 2.18**) [11]

We looked at our Emory data with this question and examined patients receiving rituximab ICE mobilization or cytoxan mobilization and found that 8%

Interactive 2.19

Mobilization with chemotherapy & G-CSF after recovery from R-ICE or Cy

TABLE 4. Emory University historical experience with chemomobilization with G-CSF and CY (all doses) or R-ICE

Mobilization regimen	Days of apheresis (mean)	Number collected in 1 day	Total collected	Never apheresed	Percentage apheresed collected in 1 day	Percentage of treated collected in 1 day	Median CD34/kg ($\times 10^6$)
R-ICE	1.7	25	46	8%	54%	50%	5.6
CY	1.7	31	58	6%	83%	50%	12.7
Total		56	104	7	54%	50%	

6% of cytoxan plus G-CSF and 8% R-ICE plus G-CSF chemo-mobilized patients never started apheresis

Joginder (2018) Transfusion

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



number between 6 and 12 million CD34 positive cell/kg. (**Interactive 2.19**) [12]

The fact that lymphoma patients are more difficult to mobilize has been confirmed in this recent publication by Russel. The study examined the fraction of patients who collected the optimal stem cell dose following mobilization with plerixafor and G-CSF and compared myeloma patients to lymphoma patients. (**Interactive 2.20**) [13]

For myeloma patients, 58% of patients collected the optimal stem cell dose after a single day of apheresis and 89% of patients collected the optimal stem cell dose after as many as four days of apheresis.

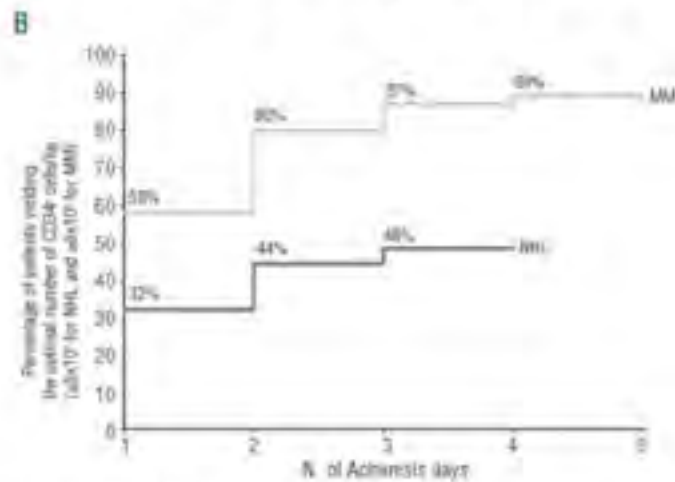
Lymphoma patients were more difficult to mobilize; only a third collected the optimal number of stem cells after a single day of apheresis and less than half were able to collect the optimal number of stem cells after four days of apheresis.

These data indicate that there is certainly a subset of patients who are difficult to mobilize either with G-CSF alone, G-CSF after chemotherapy, or after the combination of G-CSF and plerixafor. The target popu-

of lymphoma patients receiving rituximab and ICE were never able to undergo apheresis, and 6% of myeloma patients receiving cytoxan mobilization were never able to undergo apheresis. In both groups about half the patients could be successfully collected in a single day of apheresis, with a median

Interactive 2.20

Fewer NHL patients collect optimal dose of CD34+ cells with plerixafor + G-CSF



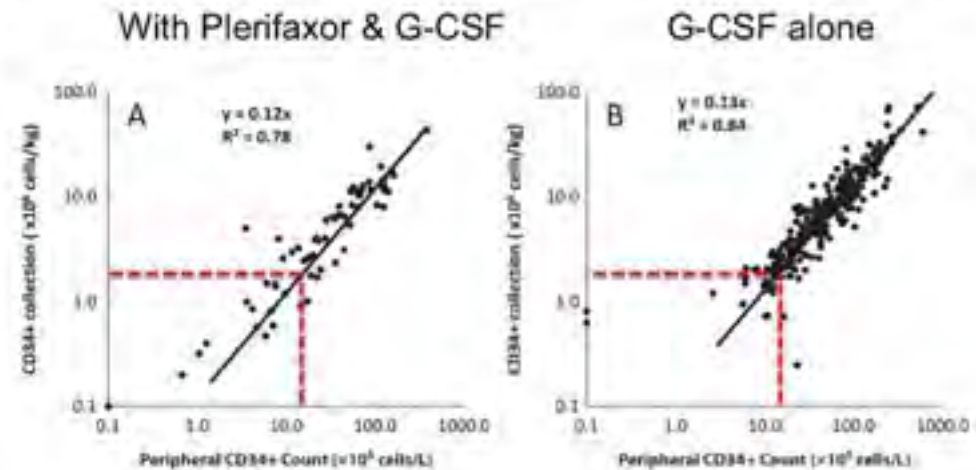
Russel (2013) Haematologica 98:172-178

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 2.21

Stem cell yield after 24 liter apheresis can be predicted by blood CD34+ cells



Li (2011) Transfusion

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



lation of difficult to mobilize patients includes older patients, those with lymphoma, those with prior marrow involvement by their cancer and those with prior radiation. However, it has been impossible to completely predict which patients will fail to mobilize and alternative clinical strategies are needed to indicate

those patients for whom apheresis will fail to yield an adequate number of stem cells.

The practical approach that we and others have taken is simply to measure the content of the CD34 positive cells in the blood and correlate that with the number of stem cells collected by apheresis. (**Interactive 2.21**) [14]

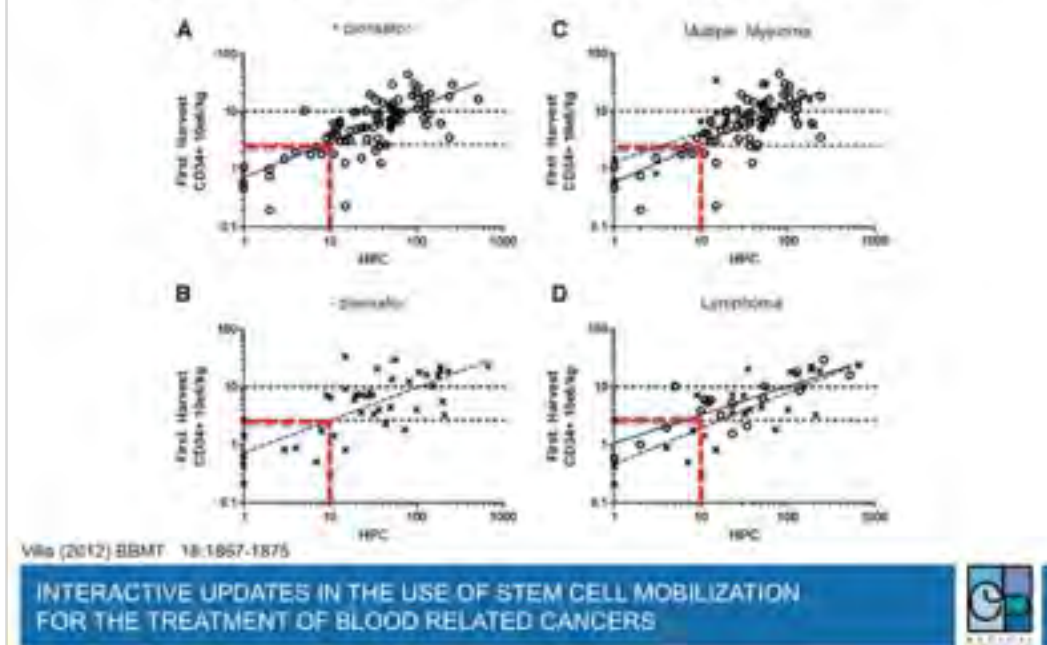
In this paper by Jie Li from our institution we measured the association between blood, stem cell content and the content of stem cells in the apheresis product in those patients undergoing large volume 24 liter apheresis procedures.

On the left we see the association between those patients who received plerixafor and G-CSF, and found that a stem cell content of 20 cells/uL was associated with a stem cell product containing at least 2 million CD34 positive cells/kg. Looking on the right at those patients who received G-CSF alone without plerixafor we found exactly the same association, such that a stem cell content of 20 cells/uL predicted mobilizing greater than 2 million CD34 positive cells/kg. Thus we can predict that those patients with a lower stem cell content in the blood are likely to fail to mobilize the minimum number of stem cells necessary for a successful autologous transplant.

The same question has been more recently addressed by Villa in a 2012 publication in BBMT. Here the authors looked at the association of blood CD34 cell counts with the content of stem cells in the aphere-

Interactive 2.22

Blood CD34+ cell counts predict yield after 18-24 liter apheresis



sis product after an 18 to 24 liter apheresis procedure. They looked at those patients with myeloma or lymphoma that received the combination of plerixafor with G-CSF or G-CSF alone. (**Interactive 2.22**) [15]

The data indicate that a stem cell content in the blood of greater than 10 cells/uL was typically associ-

ated with an apheresis product containing at least 2 million CD34 positive cells/kg. However there were a number of exceptions, with some patients having as many as 20 CD34 positive cells/uL in their blood but failed to collect the minimum number of stem cells following a single apheresis procedure.

To identify the threshold number of blood CD34 positive cells that could be used to predict successful apheresis, Villa and colleagues performed receiver operating curve analyses on the relationship between the content of stem cells in the blood and the content of stem cells in the apheresis product for patients undergoing stem cell mobilization. (**Interactive 2.23**) [15]

This slide shows the sensitivity curve in the solid line and the specificity curve in the dashed line, correlating the stem cell content in the blood on the abscissa or x-axis labeled here as HPC with the proportion of patients who achieved an apheresis product of at least 2.5 million CD34 positive cells/kg, shown on the y-axis.

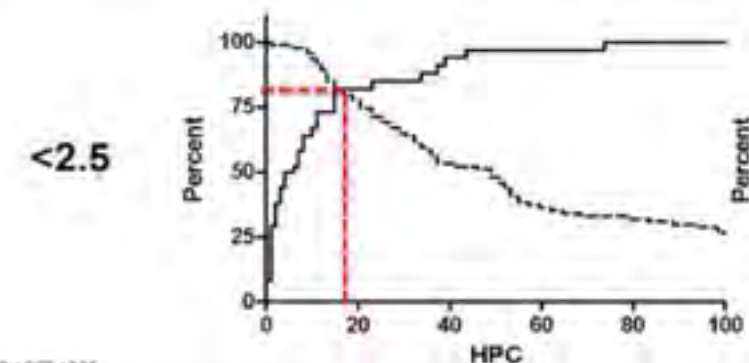
The sensitivity analysis, the solid curve, shows that a threshold number of 15 CD34 positive cells/uL in the blood identified 75% of patients who collected

Interactive 2.23

Blood CD34+ cell count <15/uL predicts low yield of stem cells with apheresis

Target CD34+
Yield ($\times 10^6$ cell/kg)

all patients



Villa (2012) BBMT 18:1867-1875

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



at least the minimum number of 2.5 million CD34 positive cells/kg. The specificity analysis, shown as the dashed curve, shows that a blood CD34 cell content of less than 15 cells/uL identified 75% of patients who failed to collect at least 2.5 million CD34 positive cells/kg.

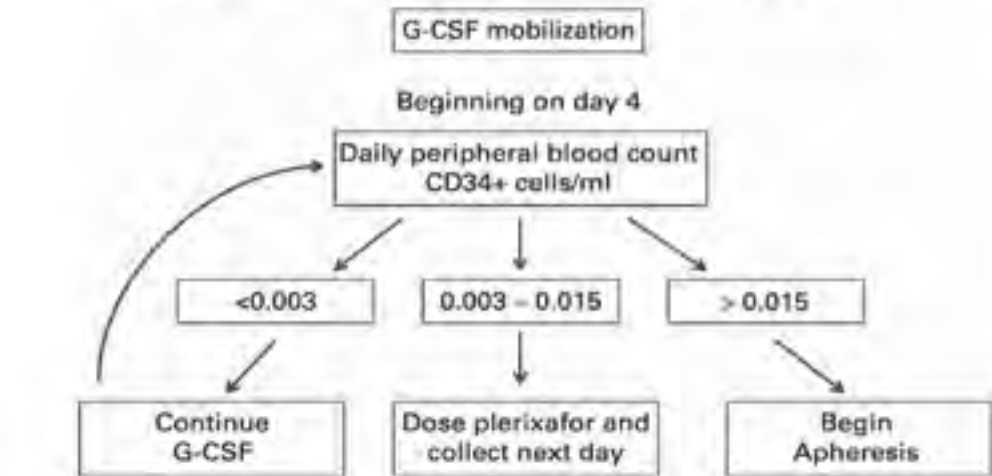
Thus, a target number of blood CD34 positive cells between 15 and 20 appears to be highly predictive in identifying those patients who will successfully collect at least the minimum number of CD34 positive cells.

Based upon this type of analysis, a number of investigators have put forth simple algorithms to predict successful mobilization and to identify those patients who might benefit from the addition of plerixafor to a G-CSF mobilization regimen. (**Interactive 2.24**) [16]

In this paper by Chen, patients receive G-CSF for four days. On the fourth day of G-CSF administration, a blood sample is obtained for enumeration of the CD34 cell content. Those patients who have very low numbers of CD34 positive cells, less than 3 cells/uL on the left, continue G-CSF for an additional day with a repeat evaluation on day 5 of growth factor administration. Patients with a high number of CD34 positive cells per microliter, greater than 15, begin apheresis immediately. The intermediate group in the middle column are those patients with a day 4 CD34 cell count between 3 and 15 cells/uL. These patients are

Interactive 2.24

Mobilization algorithm based upon day 4 blood CD34+ cell count.



Chen (2012) Bone Marrow Transplantation, 47:1526-1529

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



identified as benefitting from the addition of plerixafor. Plerixafor is administered and they begin apheresis on the following day.

The same question of predicting successful stem cell mobilization has been applied to allogeneic donors – those volunteer donors who are donating G-

Interactive 2.25

Predicting successful mobilization in allogeneic stem cell donors

N=360 sibling and unrelated donors
G-CSF administration for 5 days
Median peak CD34+ in PB: $54.4 \times 10^6 /L$ (range, 5-299 $\times 10^6$)

By multivariate analysis, poor mobilization associated with:

- 1) female sex ($p < 0.0005$)
- 2) older age ($p = 0.007$)
- 3) lower baseline WBC ($p < 0.0005$)
- 4) lower G-CSF dosage ($p < 0.0005$)

Bertani (2014) Transfusion

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



CSF mobilized stem cells for a brother or sister or an unrelated recipient. (**Interactive 2.25**) [17]

In this paper by Bertani, 360 sibling and unrelated donors were analyzed. All patients received G-CSF administration daily for five days. The median peak number of CD34 positive cells in the blood

was quite high – 54 cells/uL, with a range between 5 cells/uL and nearly 300 cells/uL. By multivariable analysis, poor mobilization was associated with female sex, older age, a lower baseline white blood cell count before beginning apheresis and lower G-CSF dosage.

We have taken these data into the clinical practice at Emory and developed the idea of “just-in-time” plerixafor administration. (**Interactive 2.26**)

This figure from a paper published four years ago by Jie Li and myself in *Transfusion* looked at two groups of patients, those patients who underwent apheresis before the FDA approval of plerixafor, and those patients who were collected after plerixafor was FDA-approved. (**Interactive 2.27**) [14]

The former group was divided between poor mobilizers who had a low CD34 cell content on the first day of apheresis, and good mobilizers, those patients who had a high CD34 cell content and didn't require plerixafor. Poor mobilizers typically had a CD34 cell count in their blood of less than 10 cells/uL with a white blood cell count between 10 and 30,000 cells/uL. Giving

Interactive 2.26

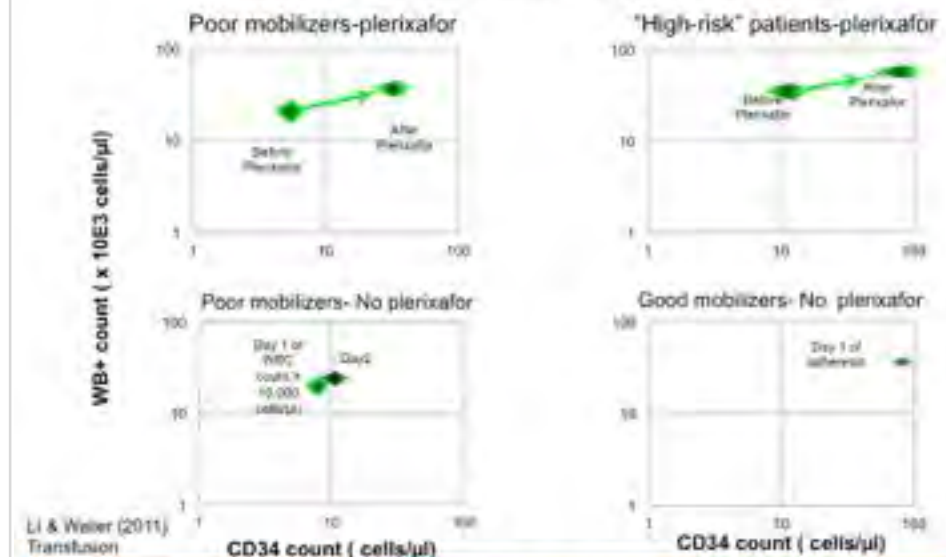
Pre-empting poor mobilization with “Just-in-time” Plerixafor administration

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 2.27

“Just-in-time” Plerixafor administration to poorly mobilizing patients



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



these patients an additional day of G-CSF raised their white cell count but did very little to mobilize stem cells in their blood and typically we had a very hard time collecting an adequate number of stem cells from this group of patients.

In contrast, the good mobilizers on the right had a high CD34 cell content in their blood, a high white count, and could be collected in a single day without the need for plerixafor.

In the top left corner, we see our experience with poor mobilizers who received just-in-time plerixafor.

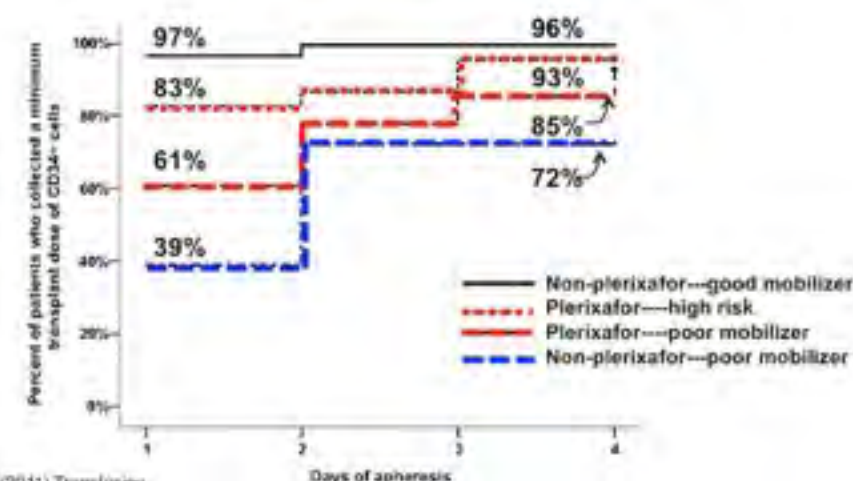
When we studied these patients who were potential candidates for apheresis, we noted that they had low CD34 cell counts, but a white count that was between 10,000 and 20,000 cells/uL. Waiting an additional day after treating them with plerixafor increased the CD34 cell count to greater than 30 cells/uL, with the white count rising to between 30,000 and 50,000 per microliter, allowing successful collection of stem cells by apheresis.

We also identified a group of high risk patients who were deemed likely to have problems with collection of adequate numbers of stem cells based upon prior radiation or treatment with lenalidomide. These patients had a CD34 cell count of an average of 10 cells/uL before plerixafor mobilization. The day after plerixafor, their CD34 cell count had risen to nearly 100 cells/uL, allowing them to be successfully collected with apheresis.

Here we see the cumulative proportion of patients who collected at least 2 million CD34 cells/kg based upon whether they were good mobilizers who did not need plerixafor treatment (shown in the black line on

Interactive 2.28

Cumulative proportion of patients who collected $\geq 2 \times 10^6$ CD34+ cells/kg



Li & Walter (2011) Transfusion

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



top), patients at high risk for mobilization failure (shown in the dashed red line) or patients who were poor mobilizers (shown in the heavy dashed red line in the middle). (**Interactive 2.28**) [14]

The group that were poor mobilizers prior to the availability of plerixafor had only a 39% probability

of collecting at least 2 million cells/kg after one day of apheresis, and only a 72% probability of collecting the minimal stem cell dose after four days of apheresis, as shown in the blue line on the bottom.

In contrast, the poor mobilizers treated with plerixafor had a 61% probability of collecting at least 2 million cells/kg on the first day of apheresis and an 85% probability of collecting at least 2 million cells/kg after four days of apheresis.

Does just-in-time plerixafor administration increase the probability of successfully collecting at least the minimum number of stem cells from patients who otherwise would be at very high risk for mobilization failure? (**Interactive 2.29**) [12]

The data I have reviewed so far looks at the question of stem cell collection after G-CSF alone or G-CSF with just-in-time administration of plerixafor. What about those patients who undergo chemotherapy mobilization? Can just-in-time plerixafor increase the efficiency of stem cell mobilization in this group as well?

This paper from our Emory experience, recently published in *Transfusion*, prospectively addresses

Interactive 2.29

Adding plerixafor to chemotherapy-mobilization increases efficiency of stem cell collection

TABLE 2. Mobilization and apheresis outcomes for subjects treated with plerixafor*

Characteristics	MM (n = 10)	Lymphoma (n = 23)
Total number of CD34+ cells collected ($\times 10^6$ /kg)	20.5 \pm 12.4	7.3 \pm 5.8
Patients with $\geq 3 \times 10^6$ CD34+ cells/kg collected	10 (100)	24 (100)
Number of apheresis days to reach $\geq 5 \times 10^6$ CD34+ cells/kg	1	2
Patients in which $\geq 5 \times 10^6$ CD34+ cells/kg were collected	9 (90)	17 (74)
Number of patients undergoing 1, 2, 3, or 4 days of apheresis		
1 day	0	13
2 days	0	6
3 days	1	2
4 days	0	2
Number of days for engraftment after transplant	12.4 \pm 1.62	12.105 \pm 1.63

100% patients collected at least 3×10^6 CD34+ cells/kg sufficient for transplant.

Jagtar (2015) Transfusion

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



that question in patients with multiple myeloma or lymphoma. All patients received mobilization chemotherapy, usually cytoxan for patients with myeloma or ICE for patients with lymphoma, and then began post-chemotherapy G-CSF mobilization. At the time the white blood cell count began to rise they were eli-

gible to receive plerixafor if the CD34 cell count was less than 30 cells/uL.

A quarter of patients mobilized with G-CSF alone and successfully collected stem cells without needing just-in-time plerixafor. The remaining 33 patients received plerixafor per protocol on the first day they had evidence for white cell recovery. One hundred percent of these patients collected at 3 million CD34 positive cells/kg, sufficient for one transplant.

The total number of stem cells collected was an average of 20 million cells/kg for myeloma patients and 7 million CD34 positive cells for lymphoma patients. Myeloma patients collected the target number of stem cells with the median of one day of apheresis. Lymphoma patients required a median number of two days of apheresis. And both groups of patients engrafted rapidly after transplantation.

So we have seen that the patients who are poor mobilizers can be predicted to some degree based upon their clinical history – older age, lymphomatous involvement of the marrow and extensive prior chemotherapy or radiation; and that the blood con-

tent of CD34 positive cells can more accurately predict those patients who will fail to mobilize the minimum number of CD34 positive cells with a large volume apheresis.

What to do then about those patients for whom mobilization has failed? Are there successful remobilization strategies that can still allow us to collect an adequate number of stem cells for their autologous transplant maneuver? (**Interactive 2.30**)

These data published by Gary Calandra were derived from the registration trials of plerixafor. In these studies, patients were randomized to receive G-CSF alone or G-CSF plus plerixafor. A substantial proportion of patients randomized to receive G-CSF alone failed to mobilize an adequate number of stem cells for transplantation. Those patients were eligible for a second trial with an attempt at remobilization using the combination of plerixafor and G-CSF. These data summarize the clinical experience of 115 patients distributed between myeloma, non Hodgkin lymphoma and Hodgkin lymphoma groups. (**Interactive 2.31**) [18]

Interactive 2.30

Re-mobilization strategies after failed mobilization

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 2.31

Salvage mobilization with plerixafox and G-CSF after failed G-CSF mobilization

Enrolled subjects	Myeloma N=35	NHL N=63	HL N=17	All N=115
Mean # CD34+ cells collected from all aphereses	4.44	2.97	4.54	3.51
% collecting > 2 10 ⁶ CD34+/kg	71.4%	60.3%	76.5%	66.1%
% Proceeding to transplant	77.1%	71.4%	100%	75.7%
Median day ANC engraftment	11 (9-17)	11 (8-36)	11 (9-25)	11 (8-36)
Median Day Platelet engraftment	21 (12-57)	18 (19-155)	15 (10-56)	18 (10-155)

Calandra (2008) Bone Marrow Transplantation 41:331-338

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



The mean number of CD34 positive stem cells collected from the second attempt at apheresis was between 3 and 5 million cells/kg, with 60 to 80% of patients collecting at least 2 million CD34 positive cells/kg and 70 to 100% of patients proceed to autologous transplant. Those patients who were

transplanted typically engrafted rapidly, with a median day of neutrophil engraftment of 11 and a median day of platelet engraftment between 15 and 21.

So salvage plerixafor administration with G-CSF after initial failure to mobilize with G-CSF alone can

be a successful strategy to collect sufficient stem cells for an autologous transplant.

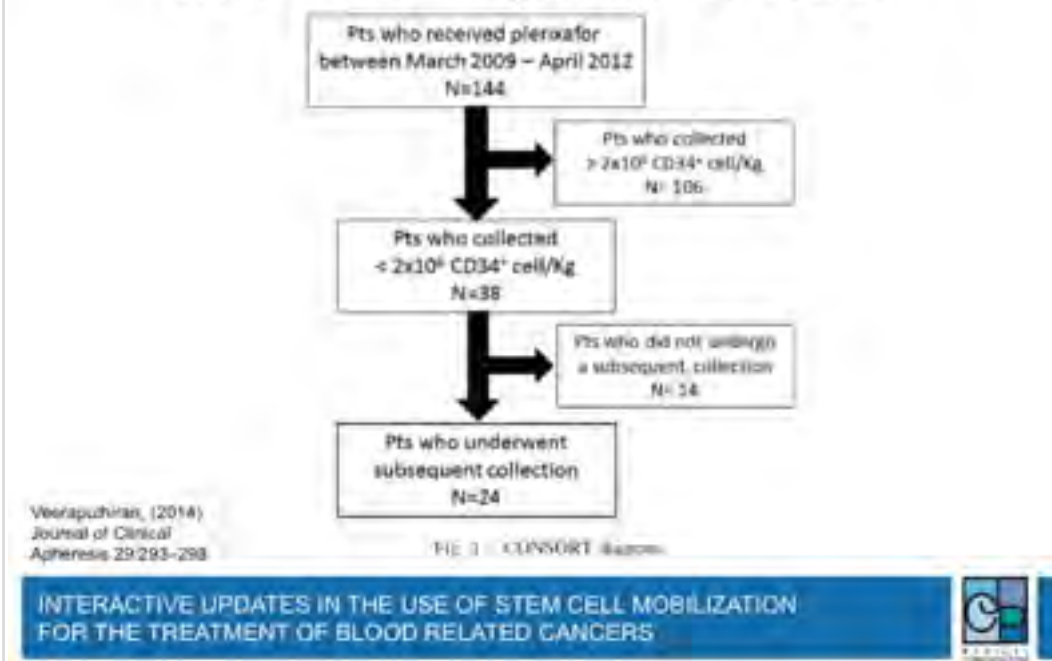
What to do then for those patients who failed to mobilize with a combination of G-CSF and plerixafor? This paper, recently published by Veeraputhiran in the *Journal of Clinical Apheresis*, looked at 144 patients who received plerixafor between 2009 and 2012. (**Interactive 2.32**) [19]

Most of those patients, 106, collected the minimum number of stem cells, greater than 2 million CD34 positive cells/kg and went on to an autologous transplant. There were 38 patients who failed to collect the minimum number of stem cells and 24 of those underwent a second attempt at stem cell collection.

Mobilizing these patients at a median time of three to four weeks after the first attempt at mobilization yielded a median number of 0.8 million CD34 positive cells/kg when the combination of GM-CSF and G-CSF was used, and yielded 1.8 million CD34 positive cells/kg when the combination of G-CSF plus plerixafor was used. (**Interactive 2.33**) [19]

Interactive 2.32

Re-mobilization attempts after mobilization with G-CSF & plerixafor failed



Some patients underwent a third attempt at mobilization with bone marrow harvest, yielding a very disappointing number of stem cells. These data indicate that a second or even third attempt at mobilization can be successful in a subset of patients who failed to mobilize with G-CSF and

Interactive 2.33

A second attempt at mobilization with the combination of G-CSF & GM-CSF

Median cell dose collected with first attempt $\leq 10^6$ CD34 ⁺ cells/kg (Range)	0.6 (0-1.2)	0.9 (0-1.5)	0.4 (0-1.4)
Median days between first mobilization and subsequent mobilization (Range)	15 (14-127)	23 (15-49)	21 (21-49)

TABLE II. Outcomes of Stem Cell Collection Strategies

Collection Strategies (No. of patients)	Highest Peripheral CD34 ⁺ count/gL Median (Range)	No. of Patients in Whom Apheresis Attempted	No. of Patients who collected $\geq 2 \times 10^6$ CD34 ⁺ cells/kg	Median CD34 ⁺ Cells Collected (Range)
Second attempt (N = 24)				
GM-CSF + G-CSF (19)	7.4 (0-33.3)	11	6	0.6 (0-7.2)
G-CSF + P (5)	20.6 (9.2-29.8)	3	1	1.8 (1.3-2.2)
CY + G-CSF (1)	0 (N/A)	0	0	0 (N/A)
Bone marrow harvest (1)	N/A	N/A	0	0.9 (N/A)
Third attempt (N = 6)				
Bone marrow harvest (3)	N/A	N/A	0	0.7 (0.6-1.3)
GM-CSF + G-CSF (1)	58 (N/A)	1	1	6.5 (N/A)
CY + G-CSF (1)	0 (N/A)	0	0	0.0 (N/A)
G-CSF + P (1)	27.5 (N/A)	1	1	4.2 (N/A)

GM-CSF, granulocyte macrophage colony stimulating factor; G-CSF, granulocyte colony stimulating factor; CY, cyclophosphamide.

Spengler et al. (2014) Journal of Clinical Apheresis 29:295-298

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



plerixafor. The addition of GM-CSF to G-CSF can be considered in this setting.

Thank you for your attention in this overview of predicting those patients who are at risk for poor mobilization. Please address any questions regarding the content of this material to the link in this iBook.

REFERENCES

1. Christopher MJ, Rao M, Liu F, et al. Expression of the G-CSF receptor in monocytic cells is sufficient to mediate hematopoietic progenitor mobilization by G-CSF in mice. *J Exp Med*. 2011 Feb 14;208(2):251-60. <http://www.ncbi.nlm.nih.gov/pubmed/21282380>
2. Shvitiel S, Kollet O, Lapid K, et al. CD45 regulates retention, motility, and numbers of hematopoietic progenitors, and affects osteoclast remodeling of metaphyseal trabecules. *J Exp Med*. 2008 Sep 29;205(10):2381-95. <http://www.ncbi.nlm.nih.gov/pubmed/18779349>
3. Nervi B, Link DC, DiPersio JF. Cytokines and hematopoietic stem cell mobilization. *J Cell Biochem*. 2006 Oct 15;99(3):690-705. <http://www.ncbi.nlm.nih.gov/pubmed/16888804>
4. Kollet O, Dar A, Shvitiel S, Kalinkovich A, et al. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nat Med*. 2006 Jun;12(6):657-64. <http://www.ncbi.nlm.nih.gov/pubmed/16715089>
5. Broxmeyer HE, Orschell CM, Clapp DW, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med*. 2005 Apr 18;201(8):1307-18. <http://www.ncbi.nlm.nih.gov/pubmed/15837815>
6. King AG, Horowitz D, Dillon SB, et al. Rapid mobilization of murine hematopoietic stem cells with enhanced engraftment properties and evaluation of hematopoietic progenitor cell mobilization in rhesus monkeys by a single injection of SB-251353, a specific truncated form of the human CXC chemokine GRObeta. *Blood*. 2001 Mar 15;97(6):1534-42. <http://www.ncbi.nlm.nih.gov/pubmed/11238087>
7. Bensinger W, Appelbaum F, Rowley S, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. *J Clin Oncol*. 1995 Oct;13(10):2547-55. <http://www.ncbi.nlm.nih.gov/pubmed/7595706>
8. Glaspy JA, Shpall EJ, LeMaistre CF, et al. Peripheral blood progenitor cell mobilization using stem cell factor in combination with filgrastim in breast cancer patients. *Blood*. 1997 Oct 15;90(8):2939-51. <http://www.ncbi.nlm.nih.gov/pubmed/9376574>
9. Ninan MJ, Flowers CR, Roback JD, et al. Posttransplant thrombopoiesis predicts survival in patients undergoing autologous hematopoietic progenitor cell transplantation. *Biol Blood Marrow Transplant*. 2007 Aug;13(8):895-904. <http://www.ncbi.nlm.nih.gov/pubmed/17640593>
10. Hicks ML, Lonial S, Langston A, et al. Optimizing the timing of chemotherapy for mobilizing autologous blood hematopoietic progenitor cells. *Transfusion*. 2007 Apr;47(4):629-35. <http://www.ncbi.nlm.nih.gov/pubmed/17381621>

11. Xia W, Ma CK, Reid C, et al. Factors determining pbsc mobilization efficiency and nonmobilization following ICE with or without rituximab (R-ICE) salvage therapy for refractory or relapsed lymphoma prior to autologous transplantation. *J Clin Apher*. 2014 Dec;29(6):322-30. <http://www.ncbi.nlm.nih.gov/pubmed/24944079>
12. Jagirdar N, Harvey RD, Nooka A, et al. Plerixafor in combination with granulocyte-colony-stimulating factor after chemotherapy increases mobilization efficiency in patients with lymphoma or myeloma: results of a Phase II clinical trial. *Transfusion*. 2015 Oct;55(10):2351-7. <http://www.ncbi.nlm.nih.gov/pubmed/26331348>
13. Russell N, Douglas K, Ho AD, et al. Plerixafor and granulocyte colony-stimulating factor for first-line steady-state autologous peripheral blood stem cell mobilization in lymphoma and multiple myeloma: results of the prospective PREDICT trial. *Haematologica*. 2013 Feb;98(2):172-8. <http://www.ncbi.nlm.nih.gov/pubmed/22983579>
14. Xu W, Zhu M, Li YP, et al. Peripheral blood stem cell collection from ABO incompatible allogeneic donors and its effect in hematopoietic stem cell transplantation. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2011 Oct;19(5):1237-40. <http://www.ncbi.nlm.nih.gov/pubmed/22040979>
15. Villa CH, Shore T, Van Besien K, et al. Addition of plerixafor to mobilization regimens in autologous peripheral blood stem cell transplants does not affect the correlation of preharvest hematopoietic precursor cell enumeration with first-harvest CD34+ stem cell yield. *Biol Blood Marrow Transplant*. 2012 Dec;18(12):1867-75. <http://www.ncbi.nlm.nih.gov/pubmed/22796644>
16. Chen AI, Bains T, Murray S, et al. Clinical experience with a simple algorithm for plerixafor utilization in autologous stem cell mobilization. *Bone Marrow Transplant*. 2012 Dec;47(12):1526-9. <http://www.ncbi.nlm.nih.gov/pubmed/22562080>
17. Bertani G, Santoleri L, Martino M, et al. Identification of hematopoietic progenitor cell donor characteristics predicting successful mobilization: results of an Italian multicenter study. *Transfusion*. 2014 Aug;54(8):2028-33. <http://www.ncbi.nlm.nih.gov/pubmed/24588265>
18. Calandra G, McCarty J, McGuirk J, et al. AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. *Bone Marrow Transplant*. 2008 Feb;41(4):331-8. <http://www.ncbi.nlm.nih.gov/pubmed/17994119>
19. Veeraputhiran M, Jain T, Cronin S, et al. Successful hematopoietic stem cell collection in patients who fail initial plerixafor mobilization for autologous stem cell transplant. *J Clin Apher*. 2014 Dec;29(6):293-8. <http://www.ncbi.nlm.nih.gov/pubmed/24700728>



Chapter 3

Stem Cell Mobilization Strategies for Myeloma and Non Hodgkin Lymphoma

Sergio A. Giralt, MD, FACP



Sergio A. Giralt, MD, FACP

Melvin Berlin Family Chair in
Myeloma Research
Chief, Adult Bone Marrow
Transplant Service
Division of Hematologic Oncology
Department of Medicine
Memorial Sloan-Kettering Cancer Center
New York, New York

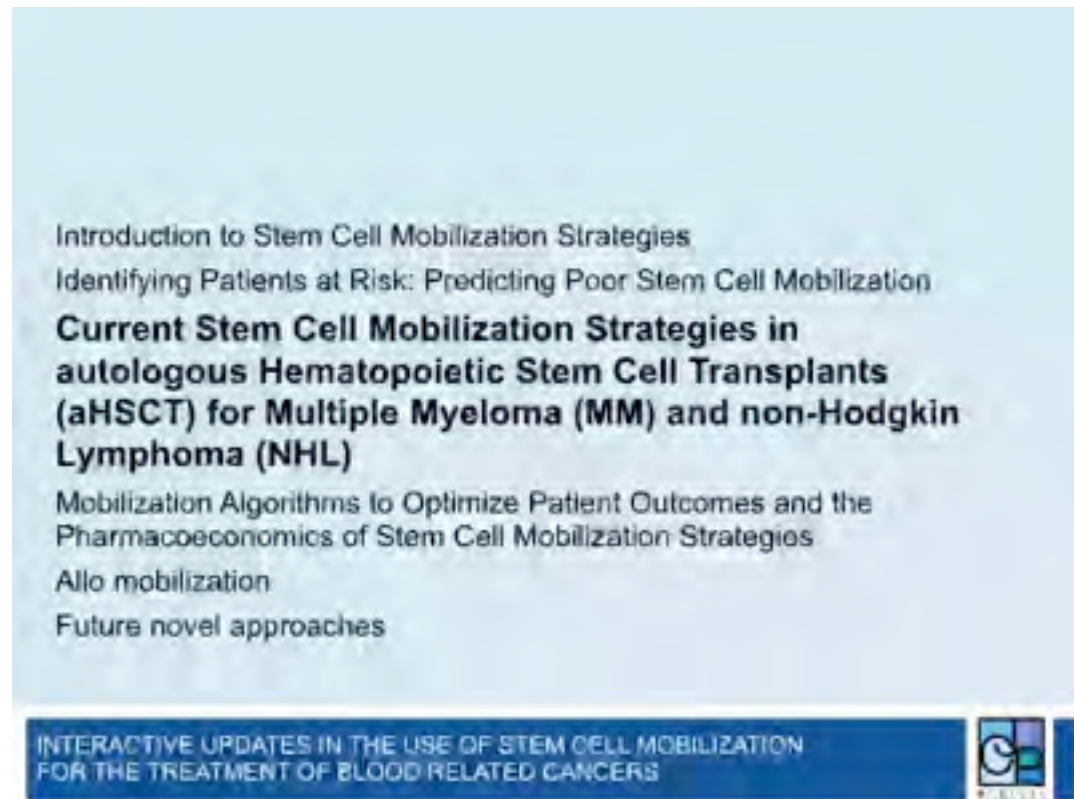
Sergio A. Giralt, MD, FACP, is Chief Attending Physician of the Adult Bone Marrow Transplant Service in the Department of Medicine at Memorial Sloan Kettering Cancer Center and Professor of Medicine at Weill Cornell Medical College. He previously was employed as a faculty member at the University of Texas MD Anderson Cancer Center.

Dr. Giralt received his medical degree from the Universidad Central de Venezuela in Caracas, Venezuela. He later completed a residency at Good Samaritan Hospital and a fellowship at The University of Texas MD Anderson Cancer Center. He is board certified in internal medicine with subspecialties in medical oncology and hematology.

Dr. Giralt's clinical and research activities include stem cell transplantation for patients with blood disorders and improving treatments for older patients who have acute and chronic leukemia. He was one of the pioneers of reduced intensity conditioning regimens, which have allowed access to hematopoietic cell transplantation to thousands of older patients with blood cancers. He has published and presented extensively on these topics in prestigious meetings and journals. Additionally, Dr. Giralt has served as the principle investigator for a number of clinical trials that examine new treatment approaches for multiple myeloma and other blood cancers that aim to reduce symptom burden and improve treatment tolerability.

Dr. Giralt is an active member of the American Medical Association, the American College of Physicians, the American Society of Hematology, the American Society of Clinical Oncology, the North American Society of Blood and Bone Marrow Transplantation, the International Society of Hematotherapy and Graft Engineering, the International Society of Haematology, and the Gerontological Society of America. He previously served as Chairperson on the Executive Board of the Center for International Blood and Marrow Transplant Research and on the Steering Committee of the Blood and Marrow Transplant Clinical Trials Network and is the past President of the American Society for Blood and Marrow Transplantation.

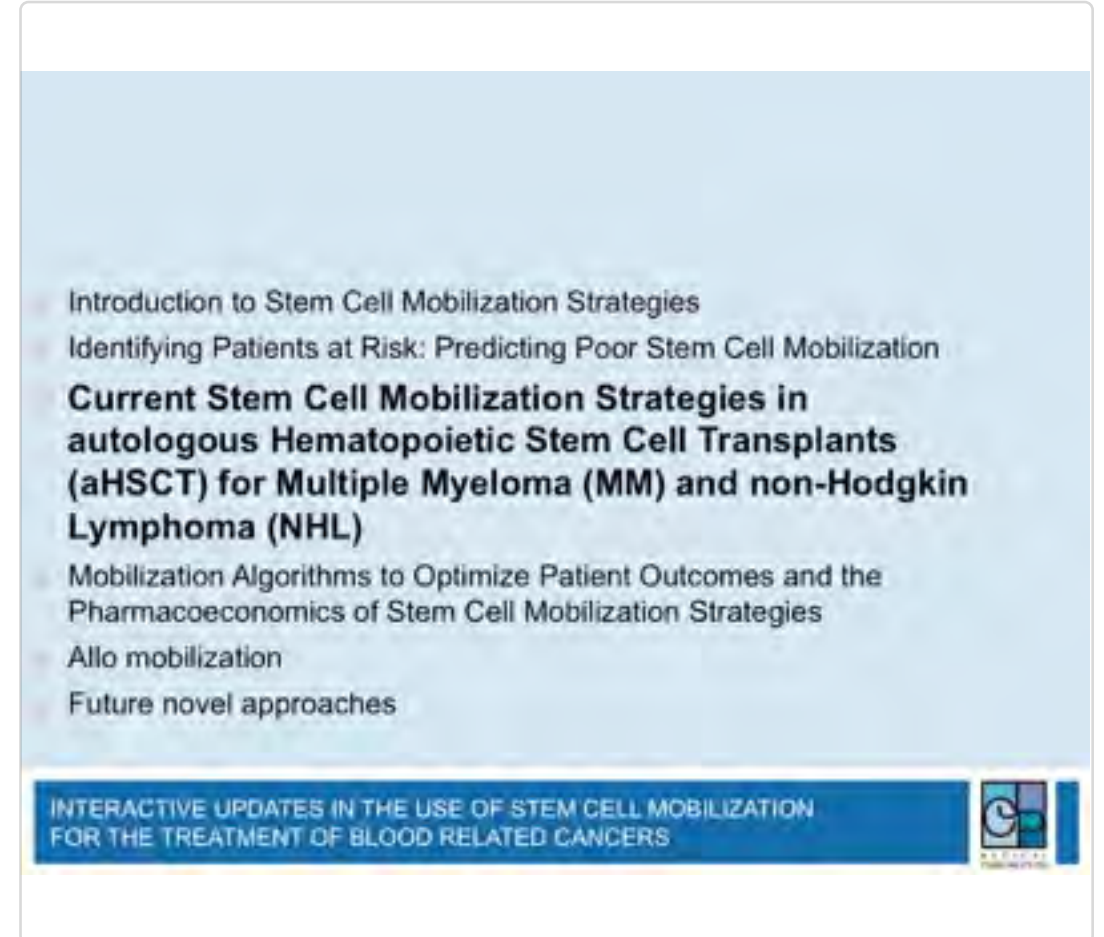
Movie 3.1



Stem Cell Mobilization Strategies for Myeloma and Non Hodgkin Lymphoma

Sergio A. Giralt, MD, FACP

Interactive 3.1



This is Sergio Giralt, Chief of the Adult BMT Service and Memorial Sloan-Kettering Cancer Center. I will be speaking about current stem cell mobilization strategies in autologous hematopoietic stem cell transplants for multiple myeloma (MM) and non-Hodgkin lymphoma (NHL). (**Interactive 3.1**)

Let's think about a case. This is a 64-year-old woman with stage 2A myeloma who received 6 cycles of lenalidomide, bortezomib and dexamethasone. She achieved a very good partial response (VGPR), with less than 5% clonal plasma cells. Prior to mobilization, her white count was 4,300 and her

Interactive 3.2

Stem cell mobilization strategies for
Myeloma and Non Hodgkin Lymphoma

- Sergio A. Giralt, MD
- Melvin Berlin Family Chair in Myeloma Research
- Chief, Adult Bone Marrow Transplant Service
- Division of Hematologic Oncology
- Department of Medicine
- Memorial Sloan-Kettering Cancer Center
- New York, New York

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS

platelet count 158,000. The question is: what would the ideal stem cell source be and what would the optimal collection strategy be? (**Interactive 3.2**)

If we look at the history of hematopoietic stem cell mobilization, the first observation – that small numbers of hematopoietic stem cells are found in

the peripheral blood during homeostasis – was made by Goodman and Hodgson in the early 1960s. This was also later confirmed by Dr. McCready and other investigators. In 1980, the finding of an increased number of stem cells in the peripheral blood of patients after chemotherapy led to the idea that these could be collected in sufficient enough amounts to be used for stem cell transplantation. Dr. Korbling and other collaborators showed that patients with chronic myelogenous leukemia could have sufficient autologous stem cells collected from the peripheral blood using apheresis techniques. (**Interactive 3.3**)

It wasn't until the early 1990s that chemomobilized peripheral blood stem cells (PBSCs) were first utilized in autologous hematopoietic stem cell transplantation (auto-HSCT), which was pioneered by Dr. Gianni, et al. The use of two colony stimulating factors, filgrastim, or granulocyte-colony stimulating factor (G-CSF), and sargramostim, or granulocyte-macrophage colony stimulating factor (GM-CSF), allowed mobilization of high numbers of PBSCs into the blood and, using apheresis, these could be used to support high-dose therapy after

Interactive 3.3

Case

- 64 year old woman stage 2A Myeloma
- Received 6 cycles of RVD (lenalidomide, bortezomib, dexamethasone)
- Achieves VGPR after 6 cycles. Marrow less than 5% clonal plasma cells.
- WBC prior to mobilization 4,300/uL; platelet count 158,000/uL
- What would the ideal stem cell source be?
- What would the optimal collection strategy be?

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



autologous stem cell transplants, and later after allogeneic stem cell transplants.

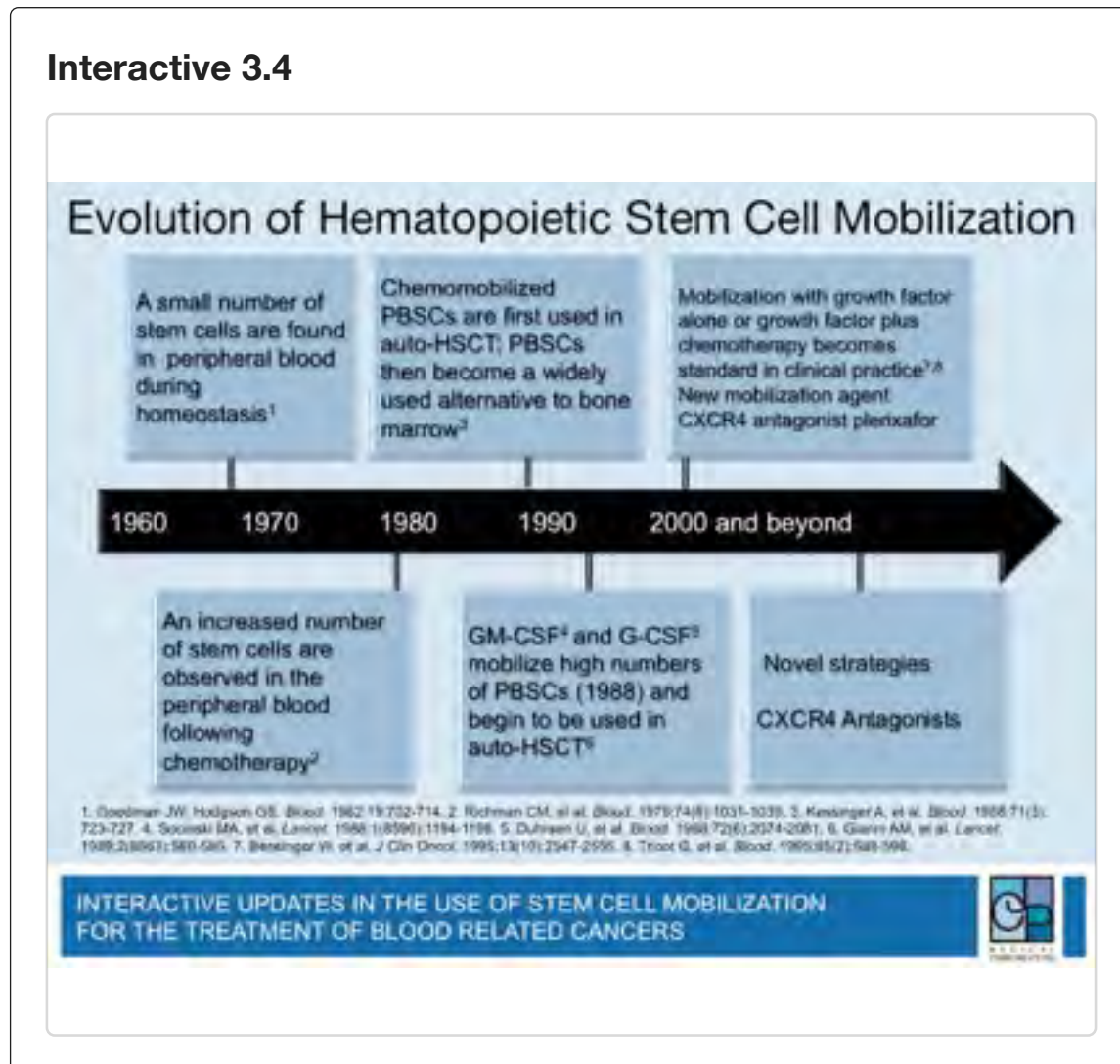
These observations, together with the fact that durable hematopoiesis was confirmed after autologous peripheral blood stem cell transplants in the early patients, led the field to replace bone marrow harvest

with autologous peripheral blood stem cell mobilization and collection, which is now the preferential source for more than 99% of all autologous transplants performed globally. After the year 2000, mobilization with growth factor alone or growth factor plus chemotherapy has become the standard of care.

Recently a new product, the CXCR4 antagonist plerixafor, has become commercially available. As we will see in this chapter, plerixafor has been shown to be more effective than filgrastim alone in collecting an adequate number of PBSCs.

In prior chapters we reviewed how different adhesion molecules maintain the hematopoietic stem cell (HSC) adhered to the extracellular matrix in the bone marrow. A variety of cytokines and chemokines have been shown to rupture these adhesion molecules from their ligands, allowing the stem cells to freeze themselves into the intramarrow space and eventually go out into the peripheral blood. This is actually a normal process that is regulated by the nervous system, by stress cytokines, and by other cellular-cellular interactions. The idea is to allow normal stem cells to be

Interactive 3.4



available in the peripheral blood that can be collected using apheresis.

What is the ideal mobilization regimen? It is one that is reliable (with a high likelihood of collecting a sufficient number of progenitors) and predictable (one we can use to predict the day of collection with precision). It is also one that is associated with a low failure rate, limited toxicity, and a limited number of days of apheresis required to get the appropriate cell count. In addition, it is preferably associated with low tumor contamination and low resource utilization. (**Interactive 3.5**) [9]

What defines successful stem cell collection? It allows for collection of a sufficient number of cells capable of prompt and durable hematopoietic reconstitution after a high-dose chemotherapy conditioning regimen. Such a mobilization regimen would be associated with minimal time of apheresis, optimal collection of CD34 positive cells, and minimal toxicity. Currently, we want enough stem cells to ensure a rapid neutrophil engraftment, which is an absolute neutrophil count of 500 for 3 consecutive days; and rapid platelet engraftment, in which the platelet count is

liberated and go to areas of tissue damage where they may be needed. (**Interactive 3.4**) [1,2,3,4,5,6,7,8]

Chemotherapy, and chemotherapy plus filgrastim or sargramostim, have been shown to increase the liberation of stem cells from their extracellular matrix and therefore enhance the number of cells

20,000 without transfusion support for the previous 7 days. (**Interactive 3.6**)

The generally defined target for a stem cell collection is a minimum of $\geq 2 \times 10^6$ CD34+ cells/kg to ensure a threshold effect for a rapid hematopoietic engraftment. Ninety-five percent of patients receiving $> 2.5 \times 10^6$ CD34+ cells/kg will have durable neu-

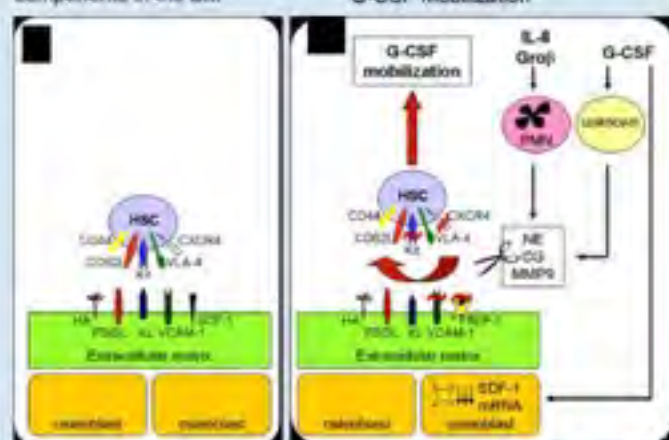
trophil engraftment by day 18. (**Interactive 3.7**) [10,11,12,13,14,15]

Data from breast cancer patients who underwent autologous transplants suggest that patients who receive more than five million CD34+ cells/kg have rapid and sustained platelet engraftment, more so than patients who receive less than five million

Interactive 3.5

Current Understanding of Mechanisms Governing Stem Cell Mobilization

Adhesive interactions between HSC and matrix components in the BM



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

Interactive 3.6

Ideal Mobilization Regimen

- **Reliable**
High likelihood of collecting sufficient number of progenitors
- **Predictable**
Able to predict day of collection with precision
- **Low failure rate**
- **Limited toxicity**
- **Limited number of days of apheresis required**
- **Low tumor contamination**
- **Low resource utilization**

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

Interactive 3.7

What is a “Successful” Stem Cell Collection?

- Mobilize sufficient number of cells capable of prompt and durable hematopoietic reconstitution
 - Minimize apheresis time¹
 - Optimize total CD34+ cell collection²
 - Minimize toxicity³
- Achieve adequate neutrophil and platelet engraftment
 - Neutrophil engraftment: first of 3 consecutive days where the absolute neutrophil count is $> 0.5 \text{ K}/\mu\text{L}$ ^{4,5}
 - Platelet engraftment: first day where the platelet count is $> 20 \text{ K}/\mu\text{L}$ without transfusion for previous 7 days^{4,5}

1. Gandy D, et al. Clin Lymphoma Myeloma. 2006;6:354-365. 2. Scott C, et al. Hematology. 1999;4:1185-1205. 3. Tassan H, et al. Clin Oncol. 1998;10:1547-1555. 4. Champlin R, et al. Blood. 2000;95:3702-3709. 5. Majumdar S, et al. Transfusion. 2008;48:827-832. 6. M. M. et al. Bone Marrow Transplant. 2002;30:748-752.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



CD34+ cells/kg. It is unclear if greater than five million CD34+ cells/kg will result in any better engraftment or will be associated with improved outcome. It is clear, however, in data we will show later on, that less than two million CD34+ cells is associated

with worse outcome, higher resource utilization, and poor platelet recovery post-transplant.

Current mobilization strategies include cytokine mobilization agents, such as G-CSF or GM-CSF. There are also chemotherapeutic and cytokine strategies, either after single agent chemotherapy with cyclophosphamide or etoposide (the most common), or disease-specific regimens, in which patients get high doses of filgrastim at the recovery period of standard chemotherapy for the disease, such as Rituxan ICE or Rituxan DHAP, among others. More recently, the combination of G-CSF plus CXCR4 antagonist plerixafor has also been utilized for stem cell collection, and in some centers patients have been getting chemotherapy plus G-CSF plus plerixafor, although this has not been studied in Phase III randomized trials. (**Interactive 3.8**) [16,17,18,19,20]

In chemo-based mobilization the advantage is that not only does the chemotherapy allow for stem cell collection, it also allows for further control of the underlying malignancy. This is particularly important for patients with NHL who may have kinetic fail-

Interactive 3.8

Defining a Target?

- Generally accepted that $\geq 2 \times 10^6$ CD34⁺ cells/kg is ensures a threshold effect for a rapid hematopoietic engraftment¹⁻²
- 95% of patients receiving $> 2.5 \times 10^6$ CD34⁺ cells/kg experience durable neutrophil engraftment by day 18
- 5×10^6 /kg may be threshold for rapid platelet engraftment³⁻⁴
- Unclear if $> 5 \times 10^6$ /kg will result in any better engraftment, may be associated with improved outcome⁵

1. Koller B, et al. Blood. 1997;89:2233-39. 2. Storer D, et al. Blood. 1993;81:310-17. 3. Hsu H, et al. Bone Marrow Transplant. 1997;18:383-10. 4. Neuwilch H, et al. Blood. 1995;86:3561-5. 5. Chittisen M, et al. J Clin Oncol. 1998;16:1522-32.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 3.9

Current Mobilization Strategies

- Using Cytokine Mobilizing Agents Alone
 - Most commonly used: G-CSF*,
 - GM-CSF*, pegylated filgrastim also used
- Chemotherapeutic agents + cytokines
 - After single agents
 - Cyclophosphamide and etoposide most common
 - Disease-specific regimens: (e.g. (R)ICE, (R)DHAP, many others)
- Cytokine plus CXCR4 antagonist (plerixafor*)
- Chemotherapy plus cytokine + CXCR4 antagonist

G-CSF*
or
GM-CSF*

*FDA Licensed

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



ure or who could progress if the chemotherapy was not being given on time. (**Interactive 3.9**)

The commonly used regimens are relatively predictable. They include cyclophosphamide at 2-4 grams/m², or standard lymphoma regimens such as RICE, ICE, or RDHAP, with or without Rituxan. Un-

fortunately, although relatively predictable, it is not an exact science, and the timing of leukapheresis has to be predetermined and prescheduled. Many centers are monitoring CD34 positive cell counts in the peripheral blood to predict the most likely time to put patients on apheresis and have an optimal collec-

tion. Various retrospective studies have shown that chemotherapy-based mobilization is more likely than G-CSF alone to result in high CD34 solid yields after each apheresis.

The disadvantages of chemotherapy-based mobilization include risk of infection. In addition, the time elapsed between chemo mobilization and the CD34 cell peak is not always predictable, and therefore spaces in apheresis units are sometimes left underutilized or patients are put on the machine who have a very poor apheresis yield. There is also a need for hospitalization, either to deliver the chemotherapy or due to neutropenic fevers that happen as a result of the chemotherapy. Many patients also require transfusion support, which is associated with increased costs. (**Interactive 3.10**)

What are the advantages of cytokine-only mobilization? There are generally very predictable kinetics of mobilization. It requires less resource utilization and is less toxic than chemotherapy mobilization. In addition, the need for hospitalization and transfusions are avoided, making tracking easier for the

Interactive 3.10

Advantages of Chemotherapy-Based Mobilization

- ▣ Can be used both to treat patient and collect CD34⁺ cells
- ▣ Commonly used regimens are relatively predictable
 - ▣ Timing of leukapheresis
 - ▣ CD34⁺ cell yields
- ▣ More likely than cytokine alone to result in very high CD34⁺ cell yields

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



pheresis and transplant units. (**Interactive 3.11**)
[**21,22,23,24,25,26**]

The limitation of cytokine-only mobilization is that it requires daily injections at least four days before apheresis and continued injections until the end of apheresis. That may present a problem for patients

Interactive 3.11

Disadvantages of Chemotherapy-Based Mobilization

- ❑ Risk of Infection
- ❑ Time elapsed between chemomobilization and CD34+ cell peak is unpredictable^{1,3}
- ❑ Criteria for initiating apheresis varies^{2,3,5,6}
- ❑ Need for hospitalization
- ❑ Need for transfusion support
- ❑ Increased costs

Wells ML, et al. *Transfusion*. 2007;47(4):628-635; Daston RR, et al. *J Clin Oncol*. 1998;16(4):1347-1353; Baigún MJ, et al. *Bone Marrow Transplant*. 2003;31(2):99-103; Nijmpe A, et al. *Transfusion*. 2000;40(11):1363-1370; Venzke A, et al. *Bone Marrow Transplant*. 1999;24(7):1019-1027; Venzke M, et al. *Bone Marrow Transplant*. 2004;19(5):395-404

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 3.12

Advantages of Cytokine-Only Mobilization

- ❑ Predictable kinetics of mobilization
- ❑ Lower toxicity and resource utilization compared to chemotherapy based
- ❑ Avoid need for hospitalization and transfusions
- ❑ Makes staffing easier

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



who do not want to self-inject, but instead require daily hospital visits or home healthcare agency involvement. Thirty-five percent of patients with NHL cannot mobilize enough stem cells with G-CSF alone, and even in myeloma patients, 20-23% may not collect sufficient cells to support tandem transplants if mobilized with

G-CSF alone. Very few patients are receiving GM-CSF as mobilization and this is generally viewed as being less effective than G-CSF. (**Interactive 3.12**)

The most common adverse event reported for cytokine-only mobilization is bone pain, which can be severe in 33% of the patients. On rare occasions splenic

rupture has been seen and it is important to remember that patients with underlying autoimmune disorders should not get filgrastim mobilization. Patients with sickle cell disease have to be carefully monitored, and the risks and benefits of cytokine mobilization versus bone marrow harvest needs to be carefully explained.

These are various studies comparing various cytokine mobilization strategies between G-CSF and a combination of chemotherapy and growth factor, either G-CSF or GM-CSF. All studies showed that chemo mobilization was associated with better stem cell collection than G-CSF alone, and that patients who received cytokine-only required more apheresis than those that received chemo mobilization. There was no difference in outcomes of the primary disease, whether the cells infused were obtained through chemotherapy or through cytokine-only mobilization. Please note that with exception of the study of Pusic et al., the number of patients analyzed was very small and none of these studies had a randomized design. (**Interactive 3.13**) [27,28,29,30,31,32]

Interactive 3.13

Limitations of Cytokine-Only Mobilization

- ❑ **Dosing**
 - ❑ Injected daily at least 4 days before apheresis and continued until end of apheresis¹
- ❑ **Insufficient stem cell mobilization and collection**
 - ❑ 35% of patients with NHL had unsuccessful PBSC mobilization with G-CSF alone (N = 52)²
 - ❑ Collections of PBSCs from 23% of MM patients who received G-CSF alone were insufficient to support tandem transplantations (N = 44)³
 - ❑ GM-CSF is less effective than G-CSF^{4,5}
- ❑ **Adverse events**
 - ❑ Bone pain reported in 33% of patients treated with G-CSF¹
 - ❑ Rare, but serious adverse events have occurred^{1,6}

1. Filgrastim prescribing information. Available at: http://www.neupogen.com/pdf/Neupogen_PI.pdf. Accessed 10/1/04. 2. Hestdal J, et al. *Hematol J*. 2000;1(6):357-373. 3. Cernian KL, et al. *J Clin Oncol*. 1998;16(4):1547-1553. 4. Meyer CH, et al. *J Clin Oncol*. 2000;18(1):43-53. 5. Kozak M, et al. *Blood*. 2000;95(10):3195-3204. 6. Filgrastim prescribing information. Available at: http://www.leukine.com/pdf/Leukine_PI.pdf.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



In general, cytokine mobilization is associated with less CD34 collection than chemo mobilization, but it is also associated with less toxicity. There are more mobilization failures in patients who receive cytokine mobilization alone versus those that receive

chemotherapy, and there are no differences in transplant outcomes. (**Interactive 3.14**) [33,34,35,36,37]

In 2007, Dr. John DiPersio reported, for the first time, the results of two randomized trials looking at the new CXCR4 antagonist, plerixafor, as a way of localizing stem cells for patients with multiple myeloma and NHL. The study design was the same for both diseases. Patients received 4 days of G-CSF at 10 mcg/kg/day and on the fifth day they received plerixafor 240 mcg/kg/day SQ, versus placebo. This was given in the evening of the day prior to each pheresis at 10 p.m. The primary endpoint for patients with multiple myeloma was collection of 6 million CD34+ cells/kg in 2 days. The primary endpoint for patients with NHL was collection of 5 million CD34 + cells/kg in 4 days. Patients were eligible to be rescued with plerixafor if they failed to collect 0.8×10^6 CD34+ cells/kg after 2 days or 2×10^6 CD34+ cells/kg after 4 days of apheresis. (**Interactive 3.15**)

This presentation resulted in two randomized trials that were published, one in the *Journal of Clinical Oncology* and the other one in *Blood*. The paper

Interactive 3.14

Study	Disease State(s)	N	Mobilization Regimen	Median Total Collected CD34+ Cells X 10 ⁶ /kg (Range)	Median Apheresis Sessions (Range)	Number of Mobilization Failures
Alegre A, et al. ¹	MM	22	G-CSF	4.85 (2.1–10.1)	3 (2–6)	NR
		18	Cy+GM-CSF	6.8 (1.8–34.8)	5 (4–12)	NR
Desikan KR, et al. ²	MM	22	G-CSF	5.8 (NR)	5	23%
		22	Cy+G-CSF	33.4 (NR)	7	18%
Dazzi C, et al. ³	NHL	12	G-CSF	2.89 (1.7–5.6)	16 total	NR
		12	Cy+G-CSF	6.41 (2.2–25.9)	15 total	NR
Narayanasami U, et al. ⁴	NHL, HD	22	G-CSF	2.5 (0.3–12.4)	NR (1–3)	1
		24	Cy+G-CSF	7.2 (0.3–44.8)	NR (1–3)	1
Pusic I, et al. ⁵	NHL, MM, HD	976	G-CSF	3.36 (NR)	2 (1–5)	182
		64	Chemo+G-CSF	5.43 (NR)	1.5 (1–5)	12

¹Alegre A, et al. *Blood Marrow Transplant* 1997;20:211–217; ²Desikan KR, et al. *J Clin Oncol* 1998;16:1547–1552; ³Dazzi C, et al. *Leuk Lymphoma* 2000;39:301–310; ⁴Narayanasami U, et al. *Blood* 2001;98:2059–2064; ⁵Pusic I, et al. *Biol Blood Marrow Transplant* 2008;14:1045–1056

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

in 2009 in the *Journal of Clinical Oncology* describes 298 patients with NHL that were randomly assigned to receive plerixafor G as the mobilization strategy, versus G or placebo. In the plerixafor group a total of 112 patients completed the study. In the G-CSF and placebo group only 68 patients completed

Interactive 3.15

G-CSF vs Chemotherapy + G-CSF

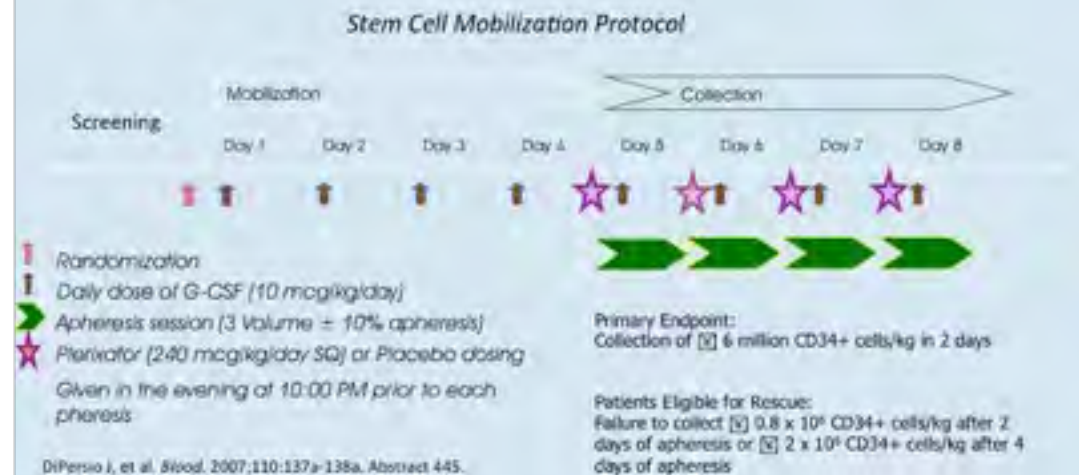
- ❑ Significantly more CD34+ cells/kg collected following chemotherapy
- ❑ Increased toxicity and time-to-transplant with chemotherapy mobilization
- ❑ Increased mobilization failures and/or less CD34+ cells with G-CSF alone vs. chemotherapy
- ❑ No difference in transplant outcomes

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 3.16

Details of Mobilization/Apheresis Period



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

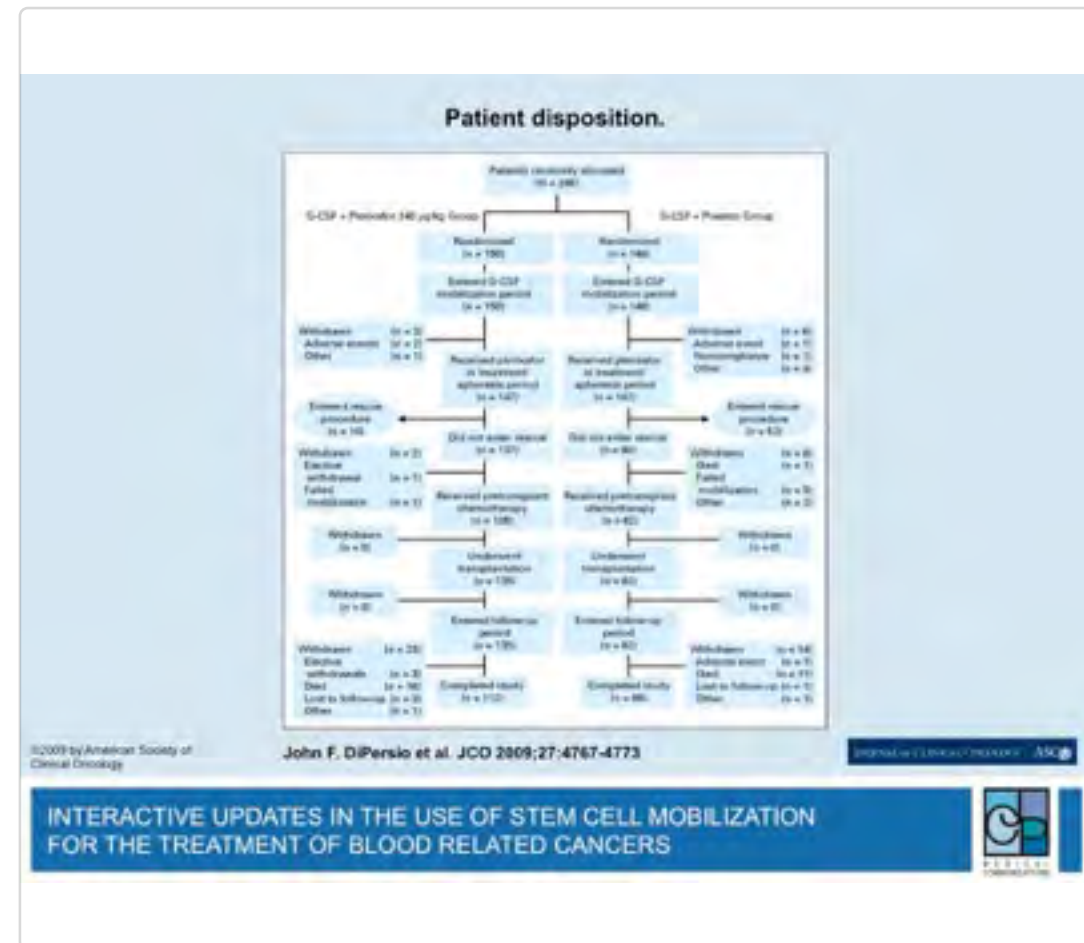


the study. This difference was due to a significantly increased risk of mobilization failure in patients who received the placebo, with 52 patients requiring a rescue procedure versus only 10 patients in the plerixafor group. In the plerixafor group 135 of the 150 patients actually went on to transplantation. In the placebo

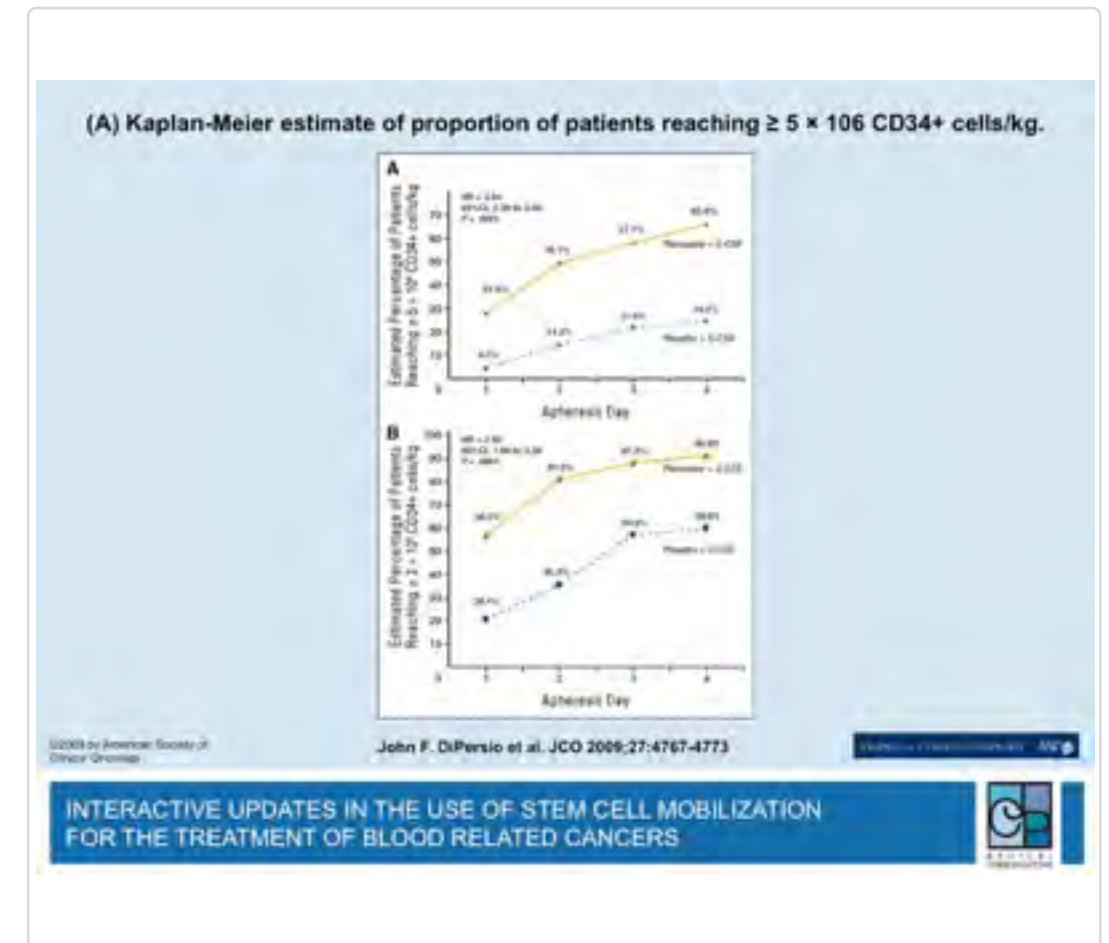
group only 82 of the 148 randomized patients underwent transplantation. (**Interactive 3.16**) [38]

As you can see, the probability of achieving the target goal of $\geq 5 \times 10^6$ CD34+ cells/kg within 4 days of apheresis was 65% for the plerixafor group versus only 24% for the placebo group. The possibility of

Interactive 3.17



Interactive 3.18



reaching the minimum dose required for at least one transplant was 90.9% in the plerixafor group versus 59.8% in the placebo group. (**Interactive 3.17**) [39]

Similar results were seen with the myeloma randomized study published in *Blood* in 2009. Three hundred and two patients were randomized to re-

ceive either G-CSF plus plerixafor or G-CSF plus placebo. As in the lymphoma trial, the yields were much better for patients that were randomized to the plerixafor. (**Interactive 3.18**) [39]

The probability of achieving the target goal of 6 million cells in 2 days or less of apheresis was 71% for the

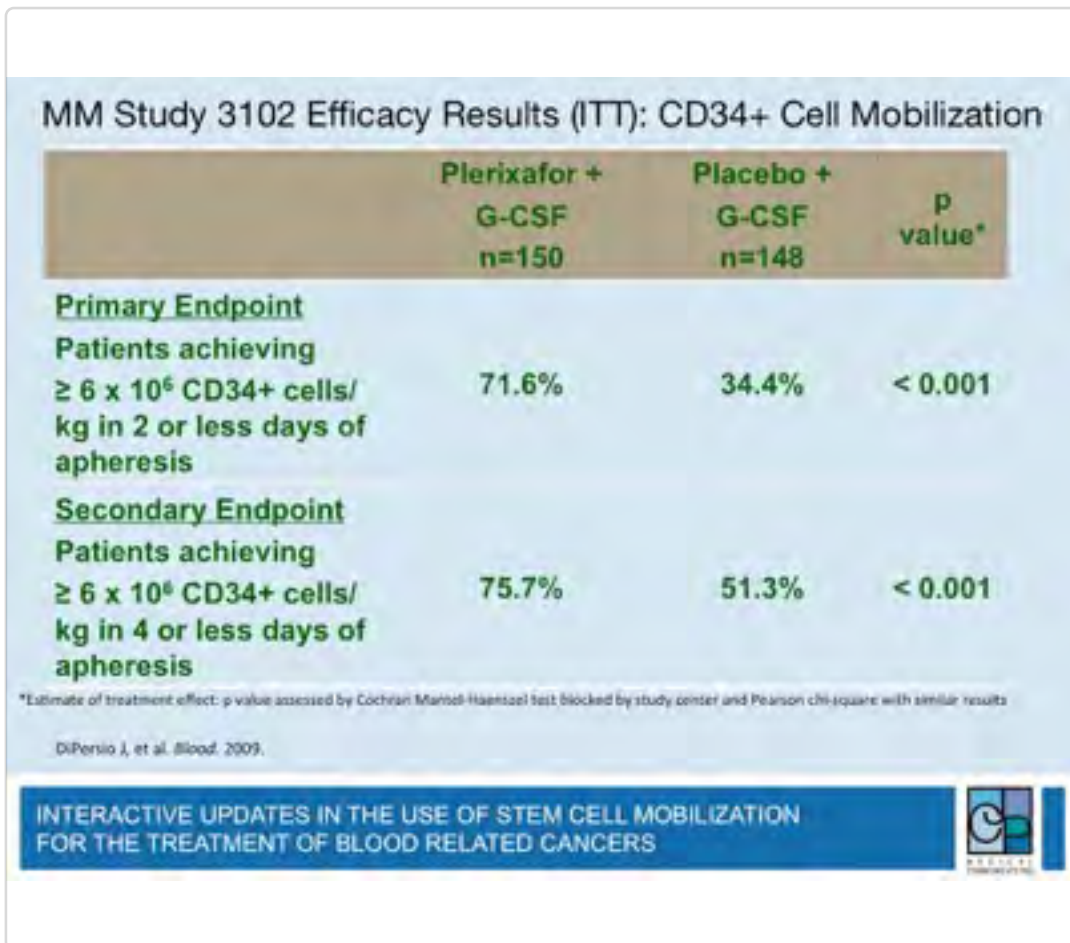
Patient disposition.

```
graph TD
    Root[20 patients randomized] --> G1[G-CSF + Filgrastim (n=10)]
    Root --> G2[G-CSF + Placebo (n=10)]
    
    G1 --> G1A[CR achieved]
    G1 --> G1B[CR not achieved]
    
    G2 --> G2A[CR achieved]
    G2 --> G2B[CR not achieved]
    
    G1A --> G1A1[CR achieved <br/> (n=8)]
    G1A --> G1A2[CR achieved <br/> (n=2)]
    
    G1B --> G1B1[CR not achieved <br/> (n=8)]
    G1B --> G1B2[CR not achieved <br/> (n=2)]
    
    G2A --> G2A1[CR achieved <br/> (n=8)]
    G2A --> G2A2[CR achieved <br/> (n=2)]
    
    G2B --> G2B1[CR not achieved <br/> (n=8)]
    G2B --> G2B2[CR not achieved <br/> (n=2)]
    
    G1A1 --> G1A1A[CR achieved <br/> (n=6)]
    G1A1 --> G1A1B[CR achieved <br/> (n=2)]
    
    G1A2 --> G1A2A[CR achieved <br/> (n=2)]
    
    G1B1 --> G1B1A[CR not achieved <br/> (n=6)]
    G1B1 --> G1B1B[CR not achieved <br/> (n=2)]
    
    G1B2 --> G1B2A[CR not achieved <br/> (n=2)]
    
    G2A1 --> G2A1A[CR achieved <br/> (n=6)]
    G2A1 --> G2A1B[CR achieved <br/> (n=2)]
    
    G2A2 --> G2A2A[CR achieved <br/> (n=2)]
    
    G2B1 --> G2B1A[CR not achieved <br/> (n=6)]
    G2B1 --> G2B1B[CR not achieved <br/> (n=2)]
    
    G2B2 --> G2B2A[CR not achieved <br/> (n=2)]
    
    G1A1A --> G1A1A1[CR achieved <br/> (n=4)]
    G1A1A --> G1A1A2[CR achieved <br/> (n=2)]
    
    G1A1B --> G1A1B1[CR achieved <br/> (n=2)]
    
    G1B1A --> G1B1A1[CR not achieved <br/> (n=4)]
    G1B1A --> G1B1A2[CR not achieved <br/> (n=2)]
    
    G1B1B --> G1B1B1[CR not achieved <br/> (n=2)]
    
    G2A1A --> G2A1A1[CR achieved <br/> (n=4)]
    G2A1A --> G2A1A2[CR achieved <br/> (n=2)]
    
    G2A1B --> G2A1B1[CR achieved <br/> (n=2)]
    
    G2B1A --> G2B1A1[CR not achieved <br/> (n=4)]
    G2B1A --> G2B1A2[CR not achieved <br/> (n=2)]
    
    G2B1B --> G2B1B1[CR not achieved <br/> (n=2)]
    
    G1A1A1 --> G1A1A1A[CR achieved <br/> (n=2)]
    G1A1A1 --> G1A1A1B[CR achieved <br/> (n=2)]
    
    G1A1A2 --> G1A1A2A[CR achieved <br/> (n=2)]
    
    G1B1A1 --> G1B1A1A[CR not achieved <br/> (n=2)]
    G1B1A1 --> G1B1A1B[CR not achieved <br/> (n=2)]
    
    G1B1A2 --> G1B1A2A[CR not achieved <br/> (n=2)]
    
    G2A1A1 --> G2A1A1A[CR achieved <br/> (n=2)]
    G2A1A1 --> G2A1A1B[CR achieved <br/> (n=2)]
    
    G2A1A2 --> G2A1A2A[CR achieved <br/> (n=2)]
    
    G2B1A1 --> G2B1A1A[CR not achieved <br/> (n=2)]
    G2B1A1 --> G2B1A1B[CR not achieved <br/> (n=2)]
    
    G2B1A2 --> G2B1A2A[CR not achieved <br/> (n=2)]
    
    G1A1A1A --> G1A1A1A1[CR achieved <br/> (n=1)]
    G1A1A1A --> G1A1A1A2[CR achieved <br/> (n=1)]
    
    G1A1A1B --> G1A1A1B1[CR achieved <br/> (n=1)]
    G1A1A1B --> G1A1A1B2[CR achieved <br/> (n=1)]
    
    G1A1A2A --> G1A1A2A1[CR achieved <br/> (n=1)]
    G1A1A2A --> G1A1A2A2[CR achieved <br/> (n=1)]
    
    G1B1A1A --> G1B1A1A1[CR not achieved <br/> (n=1)]
    G1B1A1A --> G1B1A1A2[CR not achieved <br/> (n=1)]
    
    G1B1A1B --> G1B1A1B1[CR not achieved <br/> (n=1)]
    G1B1A1B --> G1B1A1B2[CR not achieved <br/> (n=1)]
    
    G1B1A2A --> G1B1A2A1[CR not achieved <br/> (n=1)]
    G1B1A2A --> G1B1A2A2[CR not achieved <br/> (n=1)]
    
    G2A1A1A --> G2A1A1A1[CR achieved <br/> (n=1)]
    G2A1A1A --> G2A1A1A2[CR achieved <br/> (n=1)]
    
    G2A1A1B --> G2A1A1B1[CR achieved <br/> (n=1)]
    G2A1A1B --> G2A1A1B2[CR achieved <br/> (n=1)]
    
    G2A1A2A --> G2A1A2A1[CR achieved <br/> (n=1)]
    G2A1A2A --> G2A1A2A2[CR achieved <br/> (n=1)]
    
    G2B1A1A --> G2B1A1A1[CR not achieved <br/> (n=1)]
    G2B1A1A --> G2B1A1A2[CR not achieved <br/> (n=1)]
    
    G2B1A1B --> G2B1A1B1[CR not achieved <br/> (n=1)]
    G2B1A1B --> G2B1A1B2[CR not achieved <br/> (n=1)]
    
    G2B1A2A --> G2B1A2A1[CR not achieved <br/> (n=1)]
    G2B1A2A --> G2B1A2A2[CR not achieved <br/> (n=1)]
    
    G1A1A1A1 --> G1A1A1A1A[CR achieved <br/> (n=1)]
    G1A1A1A1 --> G1A1A1A1B[CR achieved <br/> (n=1)]
    
    G1A1A1A2 --> G1A1A1A2A[CR achieved <br/> (n=1)]
    G1A1A1A2 --> G1A1A1A2B[CR achieved <br/> (n=1)]
    
    G1A1A1B1 --> G1A1A1B1A[CR achieved <br/> (n=1)]
    G1A1A1B1 --> G1A1A1B1B[CR achieved <br/> (n=1)]
    
    G1A1A1B2 --> G1A1A1B2A[CR achieved <br/> (n=1)]
    G1A1A1B2 --> G1A1A1B2B[CR achieved <br/> (n=1)]
    
    G1A1A2A1 --> G1A1A2A1A[CR achieved <br/> (n=1)]
    G1A1A2A1 --> G1A1A2A1B[CR achieved <br/> (n=1)]
    
    G1A1A2A2 --> G1A1A2A2A[CR achieved <br/> (n=1)]
    G1A1A2A2 --> G1A1A2A2B[CR achieved <br/> (n=1)]
    
    G1B1A1A1 --> G1B1A1A1A[CR not achieved <br/> (n=1)]
    G1B1A1A1 --> G1B1A1A1B[CR not achieved <br/> (n=1)]
    
    G1B1A1A2 --> G1B1A1A2A[CR not achieved <br/> (n=1)]
    G1B1A1A2 --> G1B1A1A2B[CR not achieved <br/> (n=1)]
    
    G1B1A1B1 --> G1B1A1B1A[CR not achieved <br/> (n=1)]
    G1B1A1B1 --> G1B1A1B1B[CR not achieved <br/> (n=1)]
    
    G1B1A1B2 --> G1B1A1B2A[CR not achieved <br/> (n=1)]
    G1B1A1B2 --> G1B1A1B2B[CR not achieved <br/> (n=1)]
    
    G1B1A2A1 --> G1B1A2A1A[CR not achieved <br/> (n=1)]
    G1B1A2A1 --> G1B1A2A1B[CR not achieved <br/> (n=1)]
    
    G1B1A2A2 --> G1B1A2A2A[CR not achieved <br/> (n=1)]
    G1B1A2A2 --> G1B1A2A2B[CR not achieved <br/> (n=1)]
    
    G2A1A1A1 --> G2A1A1A1A[CR achieved <br/> (n=1)]
    G2A1A1A1 --> G2A1A1A1B[CR achieved <br/> (n=1)]
    
    G2A1A1A2 --> G2A1A1A2A[CR achieved <br/> (n=1)]
    G2A1A1A2 --> G2A1A1A2B[CR achieved <br/> (n=1)]
    
    G2A1A1B1 --> G2A1A1B1A[CR achieved <br/> (n=1)]
    G2A1A1B1 --> G2A1A1B1B[CR achieved <br/> (n=1)]
    
    G2A1A1B2 --> G2A1A1B2A[CR achieved <br/> (n=1)]
    G2A1A1B2 --> G2A1A1B2B[CR achieved <br/> (n=1)]
    
    G2A1A2A1 --> G2A1A2A1A[CR achieved <br/> (n=1)]
    G2A1A2A1 --> G2A1A2A1B[CR achieved <br/> (n=1)]
    
    G2A1A2A2 --> G2A1A2A2A[CR achieved <br/> (n=1)]
    G2A1A2A2 --> G2A1A2A2B[CR achieved <br/> (n=1)]
    
    G2B1A1A1 --> G2B1A1A1A[CR not achieved <br/> (n=1)]
    G2B1A1A1 --> G2B1A1A1B[CR not achieved <br/> (n=1)]
    
    G2B1A1A2 --> G2B1A1A2A[CR not achieved <br/> (n=1)]
    G2B1A1A2 --> G2B1A1A2B[CR not achieved <br/> (n=1)]
    
    G2B1A1B1 --> G2B1A1B1A[CR not achieved <br/> (n=1)]
    G2B1A1B1 --> G2B1A1B1B[CR not achieved <br/> (n=1)]
    
    G2B1A1B2 --> G2B1A1B2A[CR not achieved <br/> (n=1)]
    G2B1A1B2 --> G2B1A1B2B[CR not achieved <br/> (n=1)]
    
    G2B1A
```

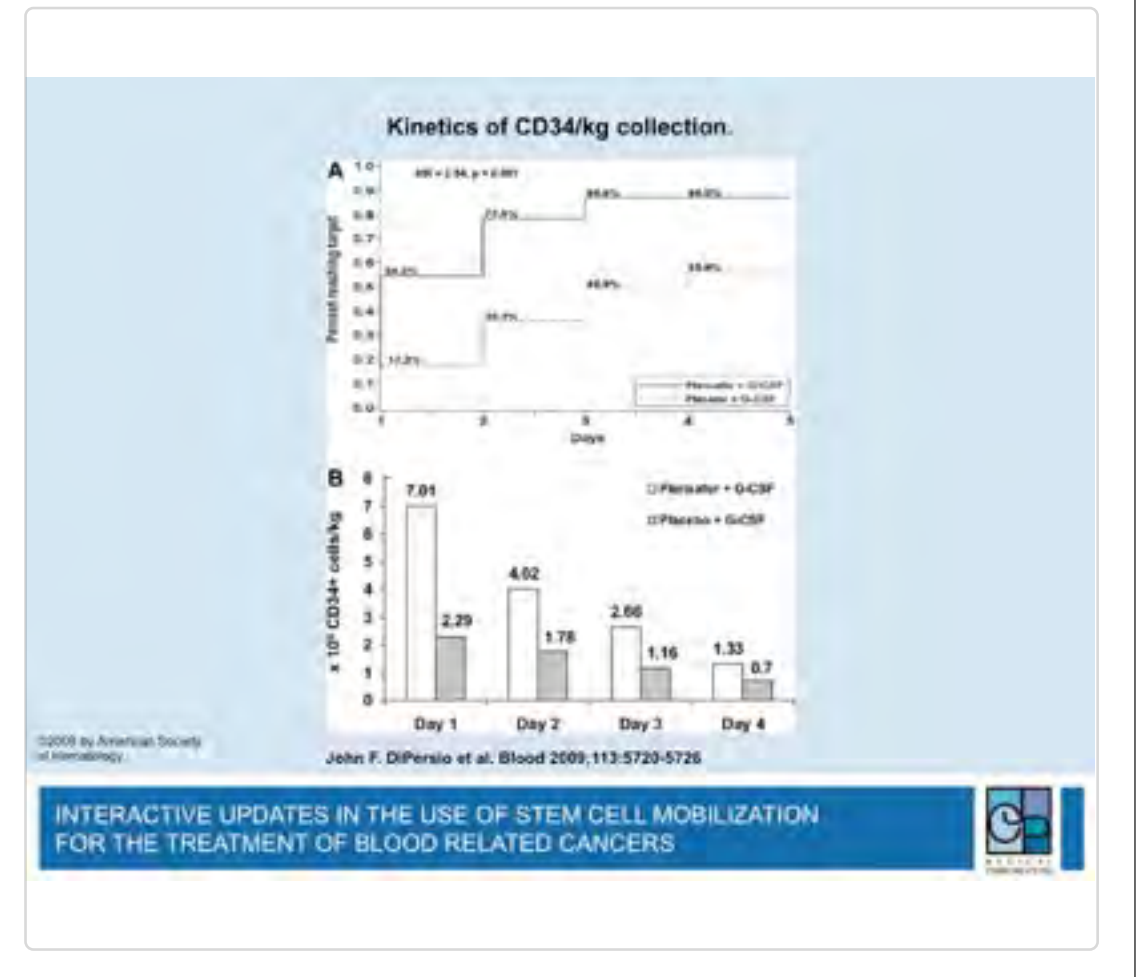
The kinetics of CD34 collection were also much quicker, with better yields on the first, second and third day for the plerixafor arm versus the placebo arm. In conclusion, plerixafor plus G-CSF is associated with a higher probability of achieving a target dose with a lower utilization of resources. In another chapter in this iBook Dr. Luciano Costa will talk about the pharmacoeconomic indications of adequate mobilization. (**Interactive 3.20**) [40]

What do we do with a poor mobilizer? Various strategies have been used for poor mobilization: one is to remobilize and the other is to harvest bone marrow. What if we were to proceed to a transplant with a less than optimal stem cell dose? First, there would be delayed, partial or failed stem cell engraftment,

Interactive 3.20



Interactive 3.21



an increased need for transfusions, and increased mortality rates. (**Interactive 3.22**) [41,42]

What do we do with a patient who did not collect enough cells? We can do more apheresis, we can re-collect, we can do a bone marrow harvest, or we can decide not to transplant these patients. All of these add

a significant burden on the patient and significantly increase use of resources. (**Interactive 3.23**) [42]

Of the remobilization strategies, plerixafor and G-CSF has emerged as the most effective strategy for patients who fail to mobilize with either chemo mobilization or G-CSF mobilization alone. As you can see

Interactive 3.22

Poor Mobilization

- Approximately 10-20% of patients do not collect adequate numbers of CD34+ cells to proceed to high-dose chemotherapy and ASCT using filgrastim alone.
- Pusic et al: 19% of patients collected $< 2 \times 10^6$ CD34+ cells/kg
- Gertz et al: 22% failure rate
- Failure rate is higher in NHL vs MM

Pusic J, et al. Bone Marrow Transplant. 2008;14:1085-1096
Gertz M, et al. Bone Marrow Transplant. 2010 Jan 11. epub.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 3.23

Outcome of Mobilization by Disease

	Disease		
	Hodgkin N = 93	NHL N = 686	MM N = 997
Optimal $\geq 5 \times 10^6$ CD34+ cells/kg	40	199	699
Low $\geq 2 < 5 \times 10^6$ CD34+ cells/kg	28	262	162
Poor $< 2 \times 10^6$ CD34+ cells/kg	8	119	48
Failed PB CD34 $< 10/\mu\text{L}$	17	105	86

27% 33% 14%

Gertz M, et al. Bone Marrow Transplant. 2010 Jan 11. epub.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



in this slide, G-CSF plus plerixafor was associated with an almost 75% success rate for remobilization and the ability to collect at least 2 million cells and take a patient to transplant. This compared favorably in this retrospective analysis to second mobilization with more cytokines or chemotherapy plus cyto-

kines – the failure was almost 70% for patients getting chemotherapy plus cytokines versus 80 plus percent for patients getting cytokines alone. Thus, plerixafor is emerging as the best salvage strategy for patients who fail to mobilize to an initial strategy

Interactive 3.24

Consequences of Suboptimal Mobilization

- ❑ **Failure to mobilize a sufficient number of CD34+ cells may result in:**
 - ❑ Increased number of days of apheresis
 - ❑ Need for bone marrow harvest
 - ❑ Ineligibility for transplantation
 - ❑ Additional burden on patients
- ❑ **Use of sub-optimal apheresis product may lead to:**
 - ❑ Delayed, partial, or failed stem cell engraftment¹
 - ❑ Increased need for transfusions²

1. Nandoriya et al. Blood 1994;83(12):3797-84. 2. Schiffer et al. Blood 1995;86(11):310-7

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 3.25

Management of Poorly Mobilizing Patients

Re-Mobilization Strategies

- ❑ Chemotherapy + G-CSF if G-CSF is used alone initially
- ❑ High-dose G-CSF
- ❑ Combine G-CSF with GM-CSF
- ❑ Combine G-CSF with SCF outside the United States
- ❑ Plerixafor + G-CSF

Novel Strategies

- ❑ Addition of growth hormone to G-CSF
- ❑ Defibrotide and G-CSF
- ❑ Parathyroid hormone
- ❑ CTCE-0214
- ❑ Enroll on clinical trial

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



that includes growth factor alone or growth factor with chemotherapy. (**Interactive 3.24**) [43,44]

In summary, there are few randomized trials exploring optimal mobilization strategies for myeloma and non-Hodgkin's lymphoma. Of the randomized trials that have been published, plerixafor plus G has

emerged as a superior strategy to G alone. There seems to be no clinical benefit with regard to transplant outcomes for patients who received cells that were mobilized with plerixafor versus those that were mobilized with G-CSF alone. Current mobilization strategies are all acceptable but result in different

CD34 yields and different cost benefit ratios. The advent of plerixafor, as well as other novel mobilization strategies that will be discussed later, opens the door to a series of interesting questions that should be explored in prospective trials. (**Interactive 3.25**)

REFERENCES

1. Goodman JW, Hodgson GS. Evidence for stem cells in the peripheral blood of mice. *Blood*. 1962 Jun;19:702-14. <http://www.ncbi.nlm.nih.gov/pubmed/13900318>
2. Richman CM, Weiner RS, Yankee RA. Increase in circulating stem cells following chemotherapy in man. *Blood*. 1976 Jun; 47(6):1031-9. <http://www.ncbi.nlm.nih.gov/pubmed/1276467>
3. Kessinger A, Armitage JO, Landmark JD, et al. Autologous peripheral hematopoietic stem cell transplantation restores hematopoietic function following marrow ablative therapy. *Blood*. 1988 Mar; 71(3):723-7. <http://www.ncbi.nlm.nih.gov/pubmed/2894230>
4. Socinski MA, Cannistra SA, Elias A, et al. Granulocyte-macrophage colony stimulating factor expands the circulating haemopoietic progenitor cell compartment in man. *Lancet*. 1988 May 28;1(8596): 1194-8. <http://www.ncbi.nlm.nih.gov/pubmed/2897009>
5. Dührsen U, Villeval JL, Boyd J, et al. Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood*. 1988 Dec;72(6):2074-81. <http://www.ncbi.nlm.nih.gov/pubmed/3264199>
6. Gianni AM, Siena S, Bregni M, et al. Granulocyte-macrophage colony-stimulating factor to harvest circulating haemopoietic stem cells for autotransplantation. *Lancet*. 1989 Sep 9;2(8663):580-5. <http://www.ncbi.nlm.nih.gov/pubmed/2570283>
7. Bensinger W, Appelbaum F, Rowley S, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. *J Clin Oncol*. 1995 Oct;13(10): 2547-55. <http://www.ncbi.nlm.nih.gov/pubmed/7595706>
8. Tricot G, Jagannath S, Vesole D, et al. Peripheral blood stem cell transplants for multiple myeloma: identification of favorable variables for rapid engraftment in 225 patients. *Blood*. 1995 Jan 15;85(2):588-96. <http://www.ncbi.nlm.nih.gov/pubmed/7529066>
9. Nervi B, Link DC, DiPersio JF. Cytokines and hematopoietic stem cell mobilization. *J Cell Biochem*. 2006 Oct 15;99(3):690-705. <http://www.ncbi.nlm.nih.gov/pubmed/16888804>
10. Dingli D, Nowakowski GS, Dispenzieri A, et al. Cyclophosphamide mobilization does not improve outcome in patients receiving stem cell transplantation for multiple myeloma. *Clin Lymphoma Myeloma*. 2006 Mar;6(5):384-8. <http://www.ncbi.nlm.nih.gov/pubmed/16640814>
11. Solá C, Maroto P, Salazar R, et al. Bone Marrow Transplantation: Prognostic Factors of Peripheral Blood Stem Cell Mobilization with Cyclophosphamide and Filgrastim (r-metHuG-CSF): The CD34+ Cell Dose Positively Affects the Time to Hematopoietic Recovery and Supportive Requirements after High-Dose Chemotherapy. *Hematology*. 1999;4(3): 195-209. <http://www.ncbi.nlm.nih.gov/pubmed/11399564>

12. Desikan KR, Barlogie B, Jagannath S, et al. Comparable engraftment kinetics following peripheral-blood stem-cell infusion mobilized with granulocyte colony-stimulating factor with or without cyclophosphamide in multiple myeloma. *J Clin Oncol*. 1998 Apr;16(4):1547-53. <http://www.ncbi.nlm.nih.gov/pubmed/10845900>
13. Champlin RE, Schmitz N, Horowitz MM, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). *Blood*. 2000 Jun 15;95(12):3702-9. <http://www.ncbi.nlm.nih.gov/pubmed/9552064>
14. Akk k CA, Liseth K, Nesthus I, et al. Autologous peripheral blood progenitor cells cryopreserved with 5 and 10 percent dimethyl sulfoxide alone give comparable hematopoietic reconstitution after transplantation. *Transfusion*. 2008 May;48(5):877-83. <http://www.ncbi.nlm.nih.gov/pubmed/18298599>
15. Ali MY, Oyama Y, Monreal J, et al. Reassessing the definition of myeloid engraftment after autotransplantation: it is not necessary to see 0.5 x 10(9)/l neutrophils on 3 consecutive days to define myeloid recovery. *Bone Marrow Transplant*. 2002 Dec;30(11):749-52. <http://www.ncbi.nlm.nih.gov/pubmed/12439697>
16. To LB, Haylock DN, Simmons PJ, et al. The biology and clinical uses of blood stem cells. *Blood*. 1997 Apr 1;89(7):2233-58. <http://www.ncbi.nlm.nih.gov/pubmed/9116266>
17. Schiller G, Vescio R, Freytes C, et al. Transplantation of CD34+ peripheral blood progenitor cells after high-dose chemotherapy for patients with advanced multiple myeloma. *Blood*. 1995 Jul 1;86(1):390-7. <http://www.ncbi.nlm.nih.gov/pubmed/7540888>
18. Kiss JE, Rybka WB, Winkelstein A, et al. Relationship of CD34+ cell dose to early and late hematopoiesis following autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant*. 1997 Feb;19(4):303-10. <http://www.ncbi.nlm.nih.gov/pubmed/9051238>
19. Weaver CH, Hazelton B, Birch R, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood*. 1995 Nov 15;86(10):3961-9.
20. Dercksen MW, Rodenhuis S, Dirkson MK, et al. Subsets of CD34+ cells and rapid hematopoietic recovery after peripheral-blood stem-cell transplantation. *J Clin Oncol*. 1995 Aug;13(8):1922-32. <http://www.ncbi.nlm.nih.gov/pubmed/7543561>
21. Hicks ML, Lonial S, Langston A, et al. Optimizing the timing of chemotherapy for mobilizing autologous blood hematopoietic progenitor cells. *Transfusion*. 2007 Apr;47(4):629-35. <http://www.ncbi.nlm.nih.gov/pubmed/17381621>
22. Desikan KR, Barlogie B, Jagannath S, et al. Comparable engraftment kinetics following peripheral-blood stem-cell infusion mobilized with granulocyte colony-stimulating factor with or without cyclophosphamide in multiple myeloma. *J Clin Oncol*. 1998 Apr;16(4):1547-53. <http://www.ncbi.nlm.nih.gov/pubmed/9552064>
23. Bargetzi MJ, Passweg J, Baertschi E, et al. Mobilization of peripheral blood progenitor cells with vinorelbine and granulocyte colony-stimulating factor in multiple myeloma patients is reliable and cost effective. *Bone Marrow Transplant*. 2003 Jan;

31(2):99-103. <http://www.ncbi.nlm.nih.gov/pubmed/12621490>

24. Humpe A, Riggert J, Meineke I, et al. A cell-kinetic model of CD34+ cell mobilization and harvest: development of a predictive algorithm for CD34+ cell yield in PBPC collections. *Transfusion*. 2000 Nov;40(11):1363-70. <http://www.ncbi.nlm.nih.gov/pubmed/11099666>

25. Venditti A, Battaglia A, Del Poeta G, et al. Enumeration of CD34+ hematopoietic progenitor cells for clinical transplantation: comparison of three different methods. *Bone Marrow Transplant*. 1999 Nov;24(9):1019-27. <http://www.ncbi.nlm.nih.gov/pubmed/10556963>

26. Arora M, Burns LJ, Barker JN, et al. Randomized comparison of granulocyte colony-stimulating factor versus granulocyte-macrophage colony-stimulating factor plus intensive chemotherapy for peripheral blood stem cell mobilization and autologous transplantation in multiple myeloma. *Biol Blood Marrow Transplant*. 2004 Jun;10(6):395-404. <http://www.ncbi.nlm.nih.gov/pubmed/15148493>

27. Filgrastim prescribing information. Available at http://www.neupogen.com/pdf/Neupogen_PI.pdf

28. Micallef IN, Apostolidis J, Rohatiner AZ, et al. Factors which predict unsuccessful mobilisation of peripheral blood progenitor cells following G-CSF alone in patients with non-Hodgkin's lymphoma. *Hematol J*. 2000;1(6):367-73. <http://www.ncbi.nlm.nih.gov/pubmed/11920216>

29. Desikan KR, Barlogie B, Jagannath S, et al. Comparable engraftment kinetics following peripheral-blood stem-cell infusion mobilized with granulocyte colony-stimulating factor with or without cyclophosphamide in multiple myeloma. *J Clin Oncol*. 1998 Apr;16(4):1547-53. <http://www.ncbi.nlm.nih.gov/pubmed/9552064>

30. Weaver CH, Schulman KA, Wilson-Relyea B, et al. Randomized trial of filgrastim, sargramostim, or sequential sargramostim and filgrastim after myelosuppressive chemotherapy for the harvesting of peripheral-blood stem cells. *J Clin Oncol*. 2000 Jan;18(1):43-53. <http://www.ncbi.nlm.nih.gov/pubmed/10623692>

31. Arora M, Burns LJ, Barker JN, et al. Randomized comparison of granulocyte colony-stimulating factor versus granulocyte-macrophage colony-stimulating factor plus intensive

chemotherapy for peripheral blood stem cell mobilization and autologous transplantation in multiple myeloma. *Biol Blood Marrow Transplant*. 2004 Jun;10(6):395-404. <http://www.ncbi.nlm.nih.gov/pubmed/15148493>

32. Sargramostim prescribing information. Available at http://www.leukine.com/hcp/Leukine_PI.pdf

33. Alegre A, Tomás JF, Martínez-Chamorro C, et al. Comparison of peripheral blood progenitor cell mobilization in patients with multiple myeloma: high-dose cyclophosphamide plus GM-CSF vs G-CSF alone. *Bone Marrow Transplant*. 1997 Aug;20(3):211-7. <http://www.ncbi.nlm.nih.gov/pubmed/9257889>

34. Desikan KR, Barlogie B, Jagannath S, et al. Comparable engraftment kinetics following peripheral-blood stem-cell infusion mobilized with granulocyte colony-stimulating factor with or without cyclophosphamide in multiple myeloma. *J Clin Oncol*. 1998 Apr;16(4):1547-53. <http://www.ncbi.nlm.nih.gov/pubmed/9552064>

35. Dazzi C, Cariello A, Rosti G, et al. Is there any difference in PBPC mobilization between cyclophosphamide plus G-CSF and G-CSF alone in patients with non-Hodgkin's Lymphoma? *Leuk Lymphoma*. 2000 Oct;

- 39(3-4):301-10. <http://www.ncbi.nlm.nih.gov/pubmed/11342310>
36. Narayanasami U, Kanteti R, Morelli J, et al. Randomized trial of filgrastim versus chemotherapy and filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation. *Blood*. 2001 Oct 1;98(7):2059-64. <http://www.ncbi.nlm.nih.gov/pubmed/11567990>
37. Pusic I, Jiang SY, Landua S, et al. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. *Biol Blood Marrow Transplant*. 2008 Sep;14(9):1045-56. <http://www.ncbi.nlm.nih.gov/pubmed/18721768>
38. DiPersio J, et al. *Blood*. 2007;110:137a-138a. Abstract 445
39. DiPersio JF, Micallef IN, Stiff PJ, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 2009 Oct 1;27(28):4767-73. <http://www.ncbi.nlm.nih.gov/pubmed/19720922>
40. DiPersio JF, Stadtmauer EA, Nademanee A, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. *Blood*. 2009 Jun 4;113(23):5720-6. <http://www.ncbi.nlm.nih.gov/pubmed/19363221>
41. Pusic I, Jiang SY, Landua S, et al. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. *Biol Blood Marrow Transplant*. 2008 Sep;14(9):1045-56. <http://www.ncbi.nlm.nih.gov/pubmed/18721768>
42. Gertz MA, Lacy MQ, Dispenzieri A, et al. Trends in day 100 and 2-year survival after auto-SCT for AL amyloidosis: outcomes before and after 2006. *Bone Marrow Transplant*. 2011 Jul;46(7):970-5. <http://www.ncbi.nlm.nih.gov/pubmed/20935685>
43. Haas R, Möhle R, Frühauf S, et al. Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. *Blood*. 1994 Jun 15;83(12):3787-94. <http://www.ncbi.nlm.nih.gov/pubmed/7515721>
44. Schiller G, Vescio R, Freytes C, et al. Transplantation of CD34+ peripheral blood progenitor cells after high-dose chemotherapy for patients with advanced multiple myeloma. *Blood*. 1995 Jul 1;86(1):390-7. <http://www.ncbi.nlm.nih.gov/pubmed/7540888>



Chapter 4

Allogeneic Stem Cell Mobilization

John F. DiPersio MD, PhD



John F. DiPersio MD, PhD

Chief, Division of Oncology
Deputy Director, Siteman Cancer Center
Washington University School of Medicine
St. Louis, Missouri

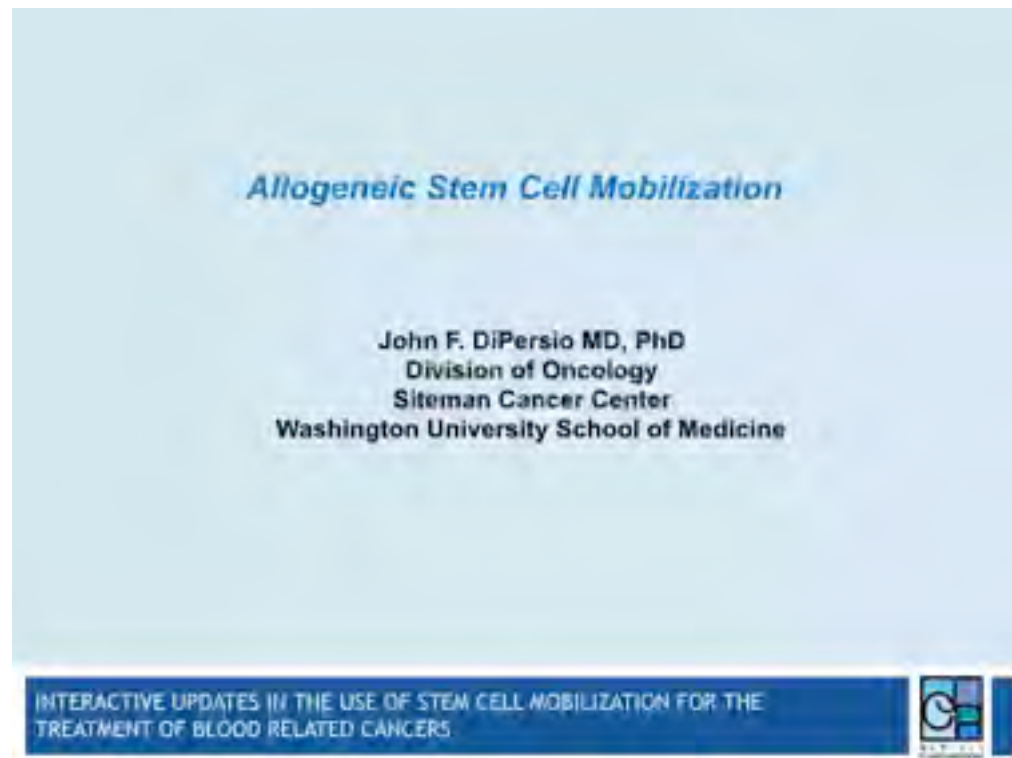
Dr. John F. DiPersio, Deputy Director, Alvin J. Siteman Cancer Center and Chief of the Division of Oncology at Washington University School of Medicine in St. Louis and the Virginia E. and Samuel J. Golman Professor of Medicine.

Dr. DiPersio's research focuses on fundamental and translational aspects of leukemia and stem cell biology. These studies include identification of genetic abnormalities in human leukemias, understanding processes involving stem cell and leukemia cell trafficking, and clinical and translational programs in both leukemia/myelodysplastic syndrome and stem cell transplantation.

Dr. DiPersio is Chair of ASH Scientific Committee on Hematopoiesis, a member of the Board of Scientific Counselors (Clinical Science and Epidemiology) of the National Cancer Institute, and the 2013 recipient of the Daniel P. Schuster Distinguished Translational Investigator Award from Washington University, the 19th Annual AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Cancer Research in 2014 and the 2014 recipient of the American Society of Hematology Mentor Award for Clinical Investigations. He has authored or co-authored more than 275 publications and over 60 invited reviews and book chapters.

Dr. DiPersio received his M.D. and Ph.D. from the University of Rochester and his B.A. in Biology from Williams College. He completed an internship and residency at Parkland Memorial Hospital and The University of Texas Southwestern Medical Center in Dallas. After serving as chief resident at Parkland Memorial Hospital, Dr. DiPersio completed a fellowship in the Division of Hematology/Oncology at the University of California, Los Angeles (UCLA).

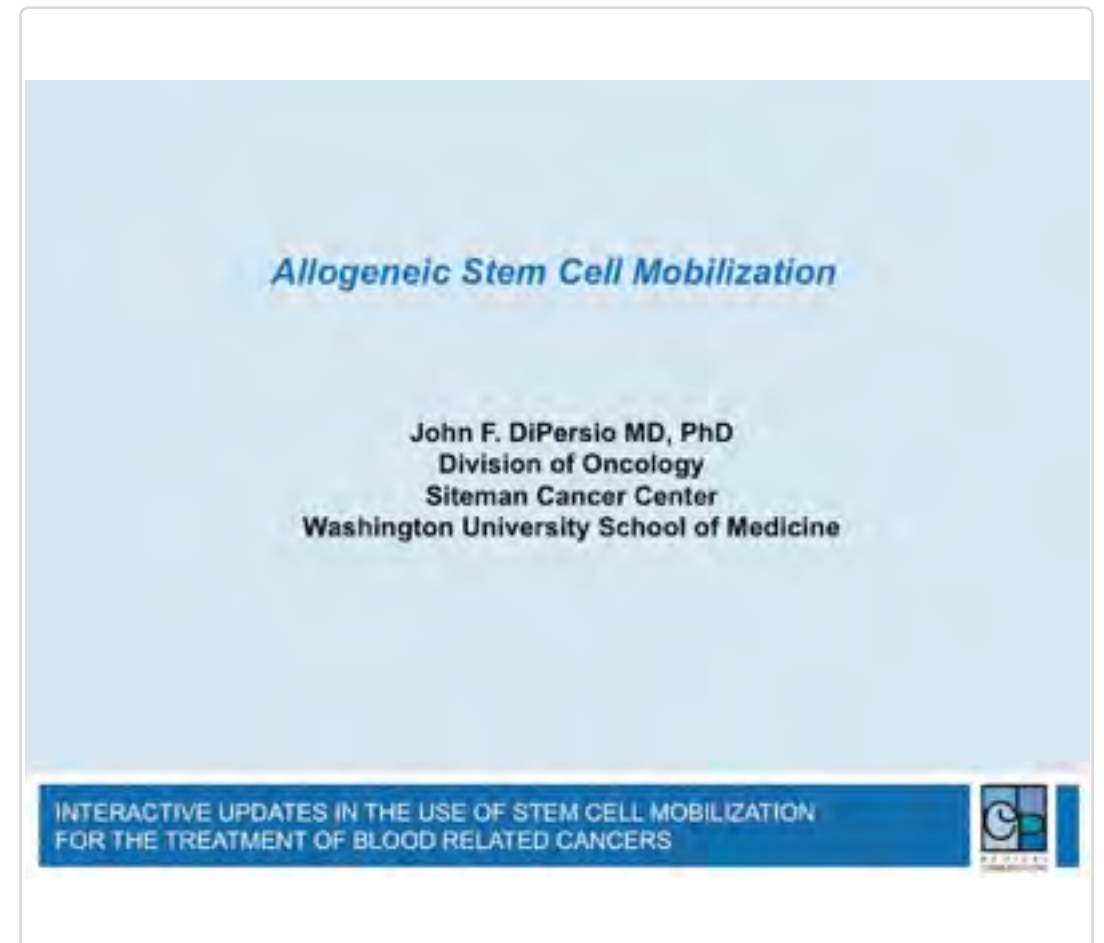
Movie 4.1



Allogeneic Stem Cell Mobilization

John F. DiPersio MD, PhD

Interactive 4.1



Hello, my name is John DiPersio and I am Chief of the Division of Oncology and Deputy Director of the Siteman Cancer Center at Washington University School of Medicine. I'd like to spend a few minutes this afternoon discussing allogeneic stem cell mobilization and its implications for patients undergoing complicated allogeneic stem cell transplants. (**Interactive 4.1**)

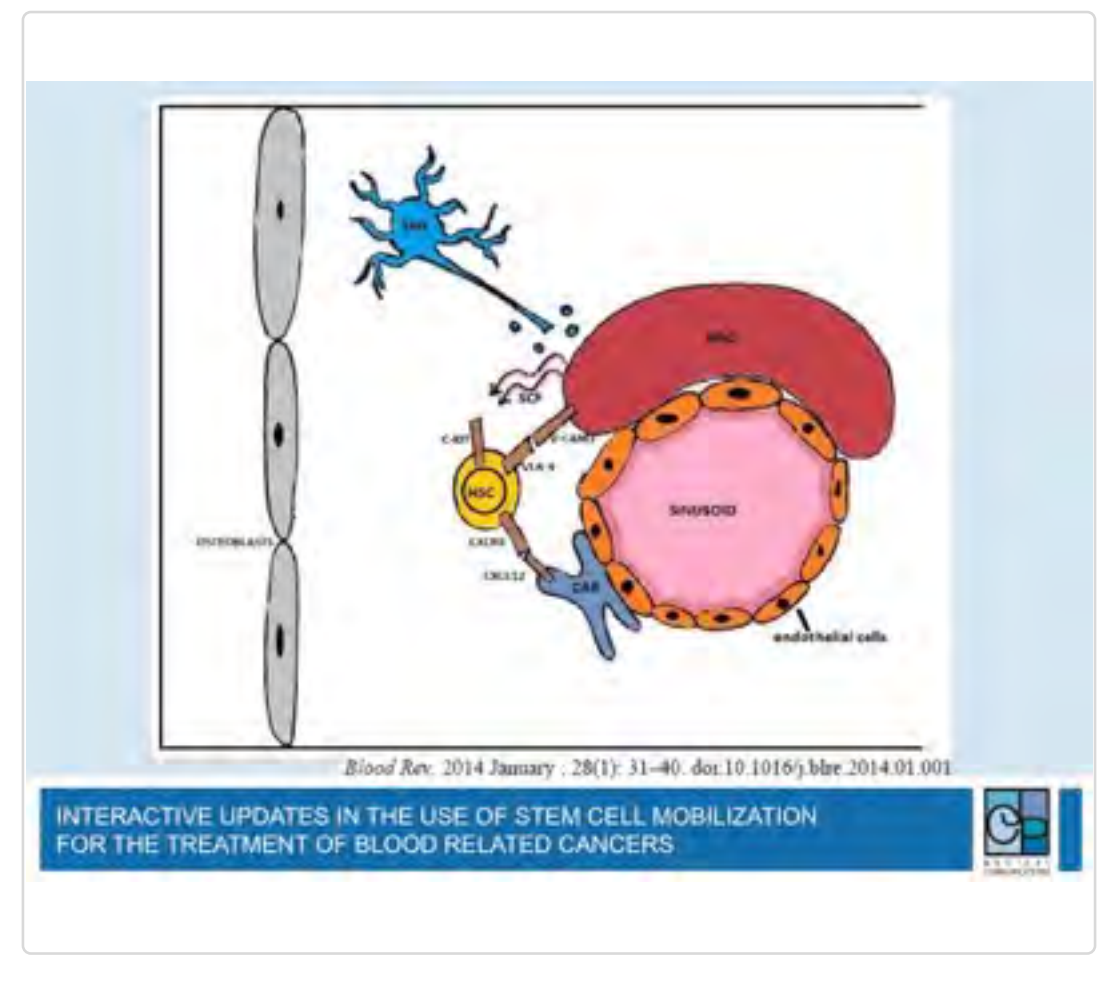
The basic biology of stem cell mobilization is complex and still under a great deal of examination and scrutiny by many scientists in the field. The hematopoietic stem cells live in both a vascular and osteoblastic niche, and these stem cells have tethers. These are primary chemokine receptors, integrins

and growth factor receptors, such as C-KIT, VLA-4 and CXCR4, which are the primary interactive tethers that bind these stem cells to supporting cells within the bone marrow microenvironment to the osteoblasts and promote stem cell homing and retention within the marrow space. They are also critical in the mobility of these stem cells from the bone marrow environment into the blood, where they can be collected by apheresis. (**Interactive 4.2**) [1]

As was previously discussed, G-CSF represents the primary way for which allogeneic donors are mobilized. Two cytokines are approved for the mobilization of allogeneic donors, G-CSF and GM-CSF. GM-CSF, as I will show you later, is a relatively inferior mobilizing agent, while G-CSF is a very efficient mobilizing agent. I'll provide you with some additional data where we have tested CXCR4 inhibitors by themselves for the first time for the mobilization of stem cells from allogeneic donors. (**Interactive 4.3**) [2]

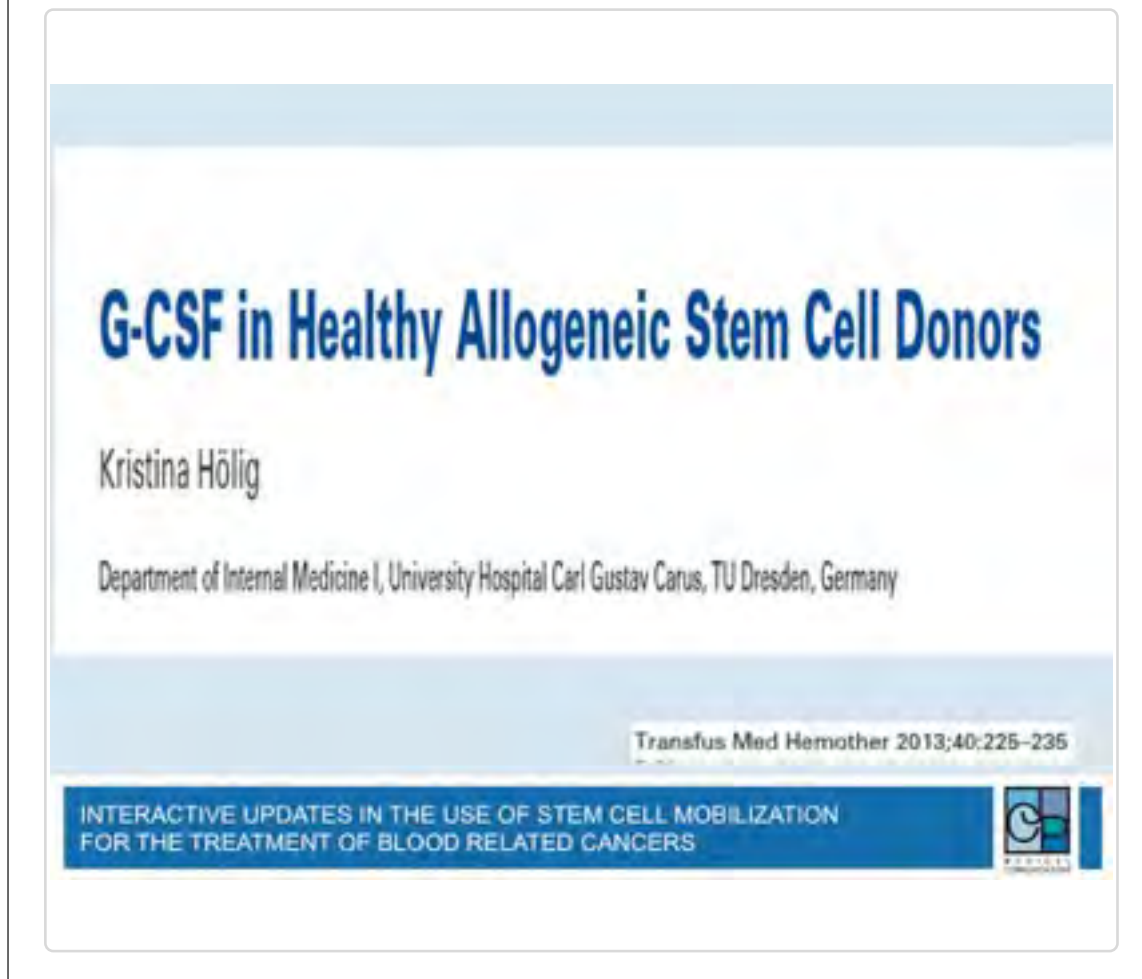
One of the largest reports using G-CSF and healthy allogeneic stem cell donors is outlined in this report by Kristina Hölig, who oversees the col-

Interactive 4.2



lections for the DKMS, the Deutschland Marrow Donor Registry. Her report, published in 2013, gives a good thumbnail overview of the impact of G-CSF on mobilization from normal allogeneic donors. But before I talk to you about G-CSF in general, I want to remind you that there are a number of biosimilars

Interactive 4.3



that have been tested both in the autologous and allogeneic setting.

When I refer to G-CSF, I'd like to have you consider G-CSF as not only Neupogen, for which most of these studies have been done, but also extend it to all the biosimilars, since there is no report of bio-

similars being significantly different than G-CSF in their ability to mobilize stem cells, either in the autologous or allogeneic setting. This is one overview from 2014 by Schmitt et al. which will confirm what I've just said. I suggest that you look over this paper and determine for yourself if you agree. (**Interactive 4.4**) [3]

Dr. Hölig's data, which looked at DKMS and the European Bone Marrow Transplant Registry, showed that mobilization of hematopoietic stem cells from normal donors conforms to this distribution, which is quite commonly seen in normal allogeneic donors. There is a wide range of productive mobilization, from a few donors in the 1-3% range who failed to mobilize sufficient numbers of stem cells, to 5% of donors who mobilized enormous numbers of stem cells after a single week of G-CSF mobilization. The median number stem cells mobilized from all of these unrelated donors (n=3,994) was 5.88×10^8 , approximately $5-7 \times 10^6$ CD34/kg after a single apheresis from an average 70-80 kg normal allogeneic donor. (**Interactive 4.5**) [2]

Interactive 4.4

Biosimilar G-CSF Based Mobilization of Peripheral Blood Hematopoietic Stem Cells for Autologous and Allogeneic Stem Cell Transplantation

Michael Schmitt¹, Amy Publicover², Kim H. Orchard², Matthias Götsche³, Lei Wang⁴, Aneta Schmitt⁵, Tije Maria¹, Panagiotis Tzifigiotis⁴, Reeba Kurukosel⁴, Arnon Nagler⁵

1. University Clinic Heidelberg, Internal Medicine V (Hematology, Oncology And Rheumatology) University of Heidelberg, 69120 Heidelberg, Germany.
2. University Hospital Southampton, Southampton, United Kingdom.
3. Research Group Biomolecular NMR Spectroscopy, Leibniz Institute for Age Research - Fritz Lipmann Institute o.V. (FLI), Jena, Germany
4. 2nd Department of Internal Medicine, Propædætic, Attikon General University Hospital, University of Athens, Greece.
5. Hematology Division, BMT and Cord Blood Bank, Tel-Aviv University, Chaim Sheba Medical Center, Tel-Hadassah, Israel.

Theranostics

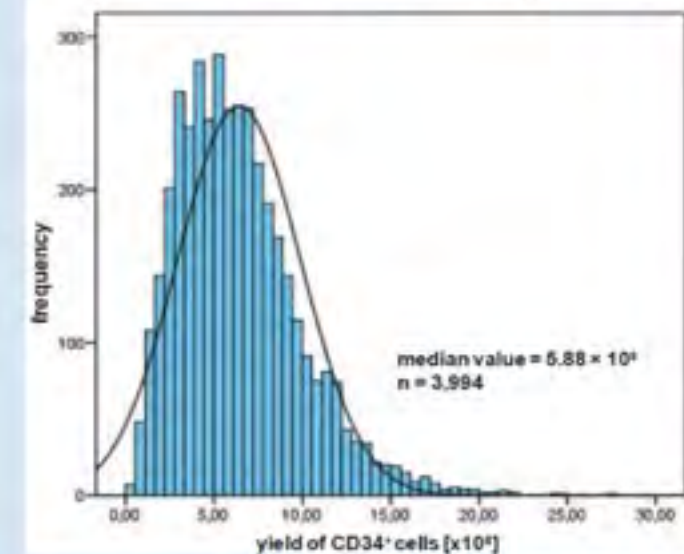
BMJ. 2016;29:doi:10.1136/bmj.2016.011111

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 4.5

Yield of CD34+ cells in the first leukapheresis ($\times 10^8$)



Transfus Med Hemother 2013;40:225-235

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



When Dr. Hölig looked at the side effects of G-CSF mobilization, she found that the most frequent side effects were headache and bone pain, with flu-like symptoms extremely uncommon. More severe complications were also relatively uncommon. As you know, some patients have been reported to have had splenic

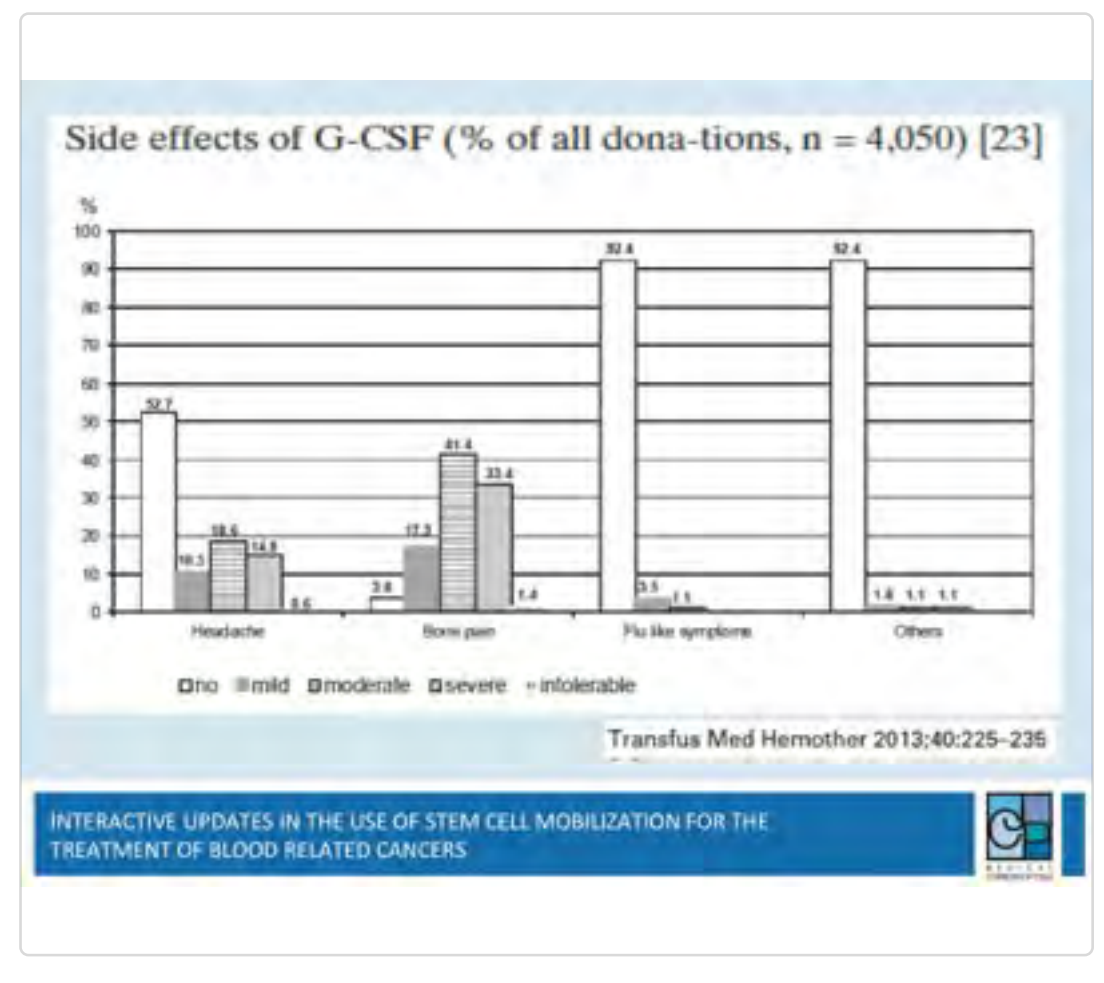
ruptures but this is exceedingly rare. And for the most part, with the exception of patients with multiple sclerosis and sickle cell anemia patients, high-dose G-CSF for mobilization is extremely safe, associated with limited or negligible long-term or short-term severe reactions. For the most part G-CSF is associated with headache

and bone pain, where 20-30% of patients may require transient use of narcotics. (**Interactive 4.6**) [2]

What predicts outcome for patients or for normal allogeneic donors collected with G-CSF? This is data from our center, looking at almost 1,000 normal allogeneic donors who were both female and male. Overall, there was no obvious difference in the collection of male and females and also no dramatic difference in the collection of stem cells with age, although statistically there appeared to be a slight decrease in the collection of hematopoietic stem cells over the age of 60 that obviously does not pass the eyeball test. For the most part, expectations for collection from male and females is relatively similar, although some have shown slightly inferior results from females. Our data suggest that females and males are quite similar and that age does not have a general impact on mobilization. (**Interactive 4.7**)

The only other comorbidity that we and others have determined to be predictive of outcome for normal allogeneic donors is diabetes. There are four separate reports, one of which was published this year by Fandini and our group, which suggest that

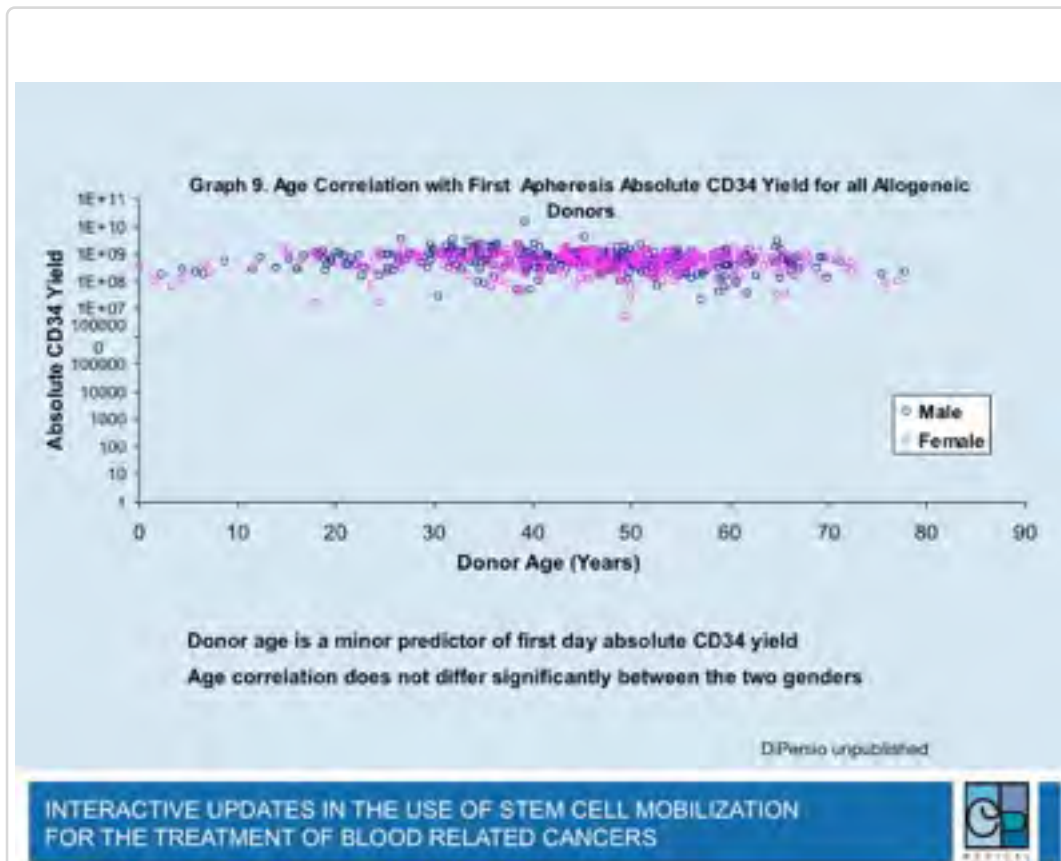
Interactive 4.6



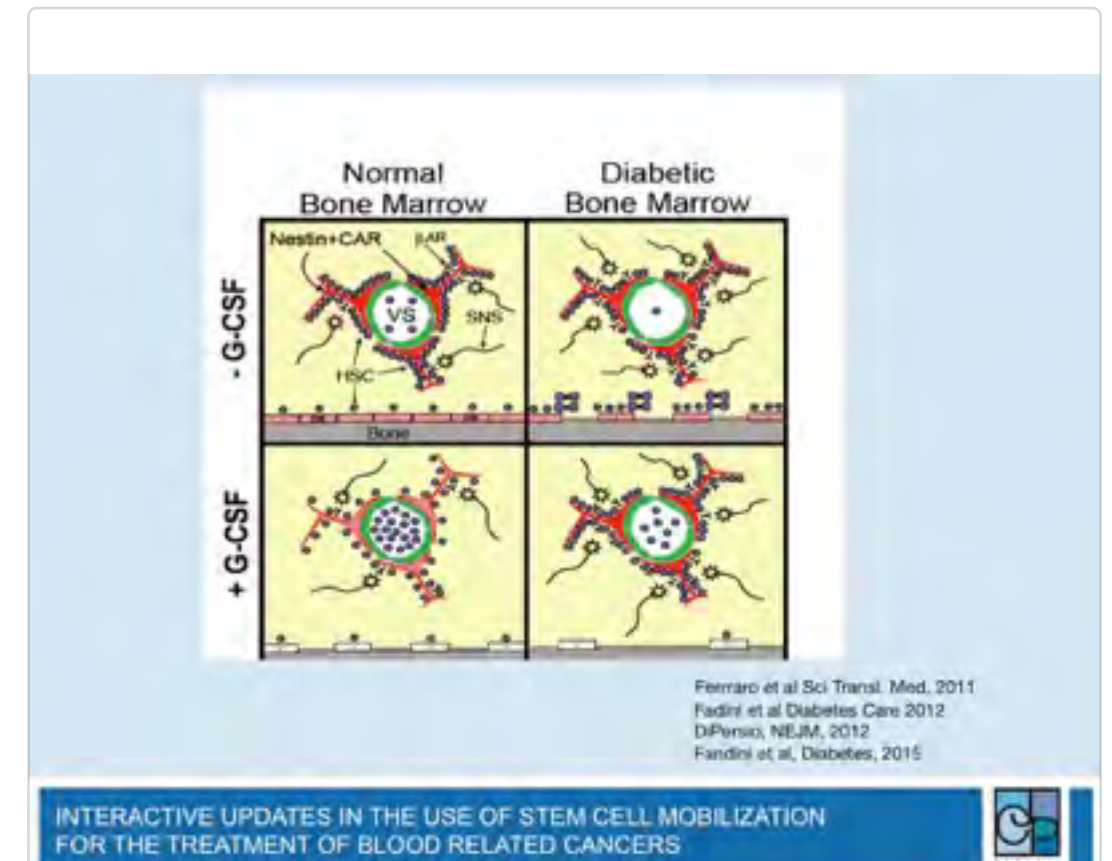
diabetes, due to progressive autonomic sympathetic denervation, results in decreased mobilization of stem cells into the vascular sinuses after G-CSF mobilization. (**Interactive 4.8**) [4,5,6]

The exact mechanisms of this are unclear. However, the beautiful work by Ferraro et al. in *Science*

Interactive 4.7



Interactive 4.8

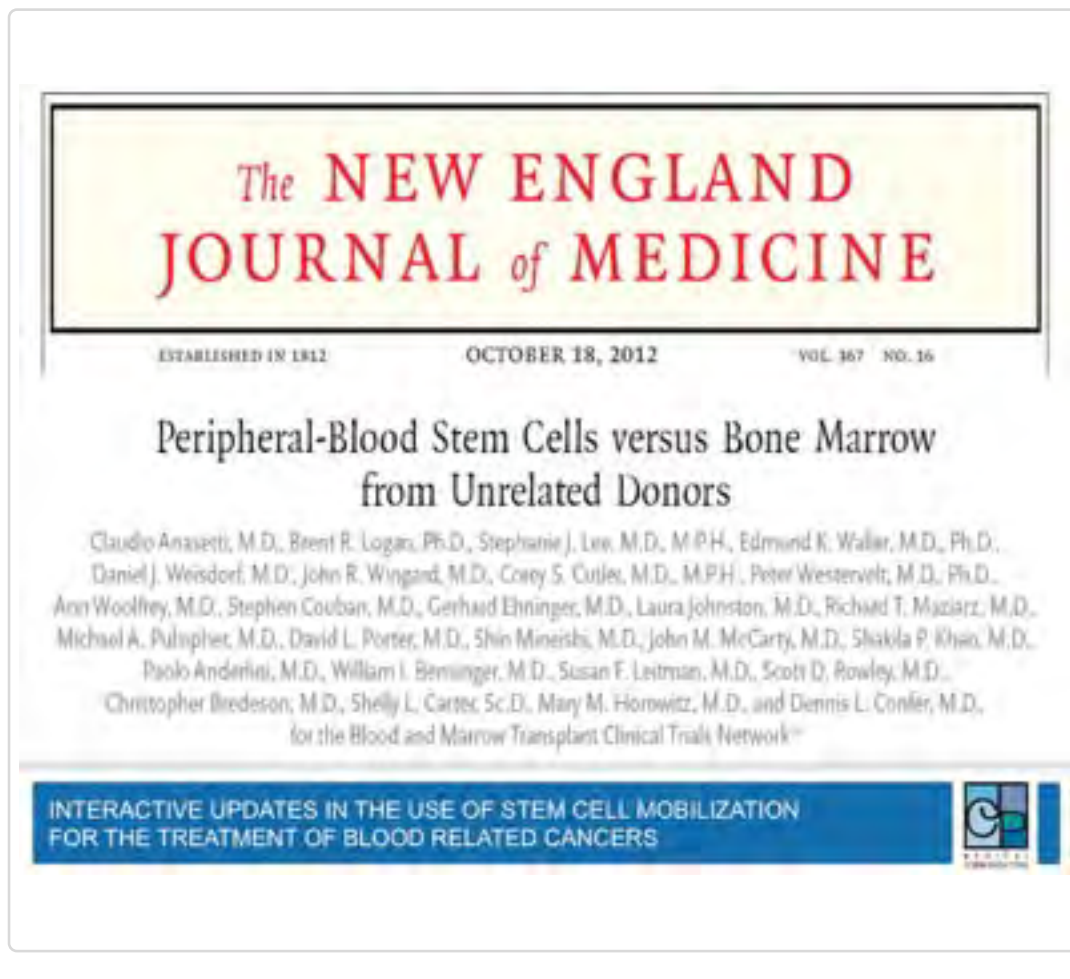


and *Translational Medicine* in 2011 and our review of this paper in *The New England Journal of Medicine* in 2012 suggest that the progressive autonomic sympathetic denervation seen in diabetes contributes to this decrease in mobilization. Several groups have validated these results in their own data sets.

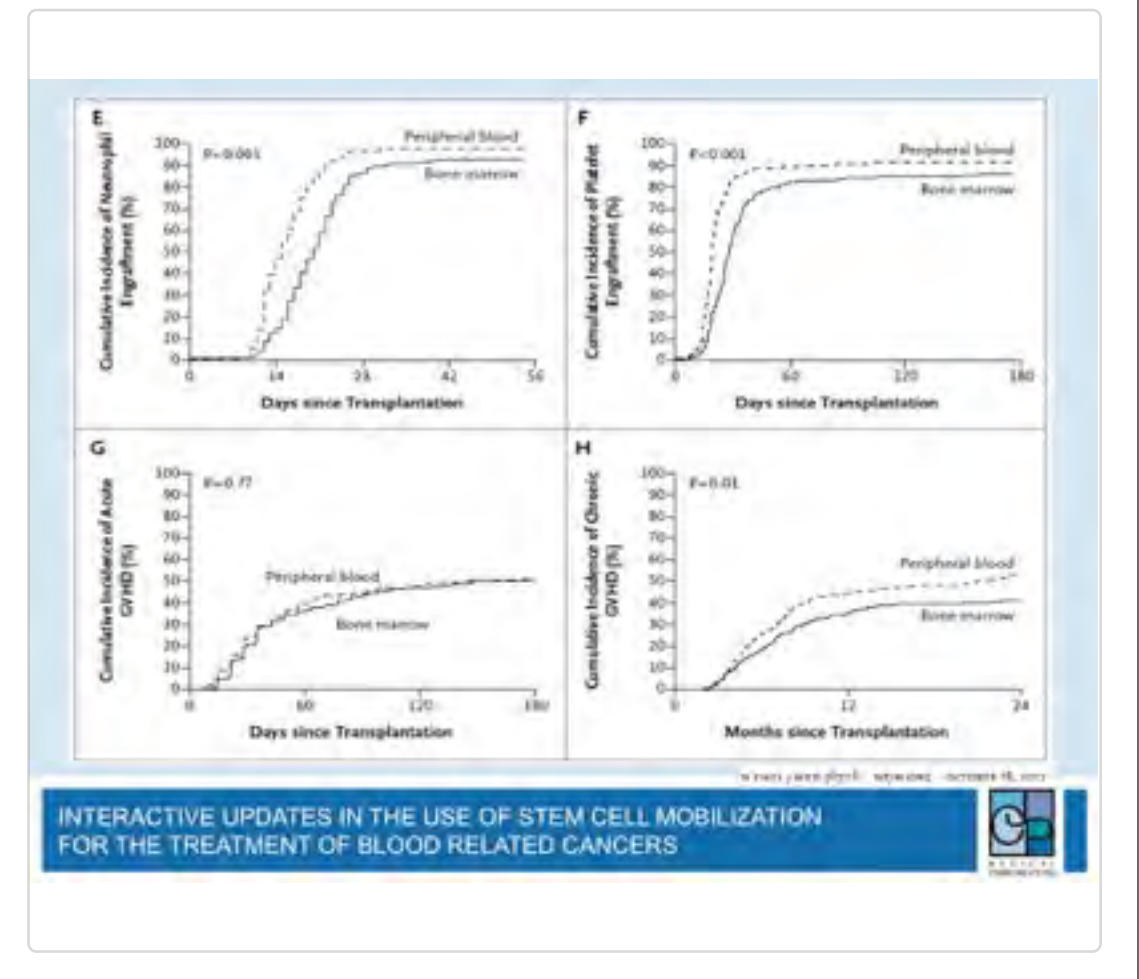
Now the question also emerges whether peripheral blood versus bone marrow is better for the outcome of patients transplanted with malignant diseases. (**Interactive 4.9**) [7]

Allogeneic stem cell transplants can be lumped into sib transplants or unrelated donor transplants.

Interactive 4.9



Interactive 4.10



In a large prospective randomized study published in *The New England Journal of Medicine* in 2012, the authors demonstrated clearly and unequivocally that there was no significant survival difference between transplant recipients who received peripheral blood stem cells and those who received bone

marrow from unrelated donors. In this study unrelated donors were collected in the operating room and bone marrow was collected or mobilized with G-CSF. Recipients of those transplants had similar long-term overall survival and disease-free survival. (**Interactive 4.10**) [7]

The data shown here is a summary of some of the major endpoints that were looked at in the study. First is neutrophil recovery. As expected, due to the large number of stem cells and the strong correlation between stem cell numbers and neutrophil and placebo recovery, there was a more rapid and more reproducible early recovery of both neutrophils and platelets in patients receiving peripheral blood as a source of stem cells for unrelated donor transplant.

However, the cumulative incidence of acute GVHD was identical in both groups and the cumulative incidence of chronic GVHD was statistically increased in the group of patients receiving peripheral blood stem cells mobilized with G-CSF. So although the overall and disease-free survival of these groups were the same, as well as acute GVHD, neutrophil and platelet recovery, the cumulative incidence of chronic GVHD was different in that peripheral blood allogeneic stem cell transplant recipients recovered their counts quicker and had an increased incidence of chronic GVHD.

This has led some centers to rethink their choice of peripheral blood versus bone marrow for recipients of unrelated transplants. I should also say that in the context of marrow failure, especially in patients with aplastic anemia and in younger patients and children, the preferred source of unrelated donor stem cells is bone marrow and not peripheral blood for the patients that have non-malignant diseases due to the excessive rates of chronic GVHD.

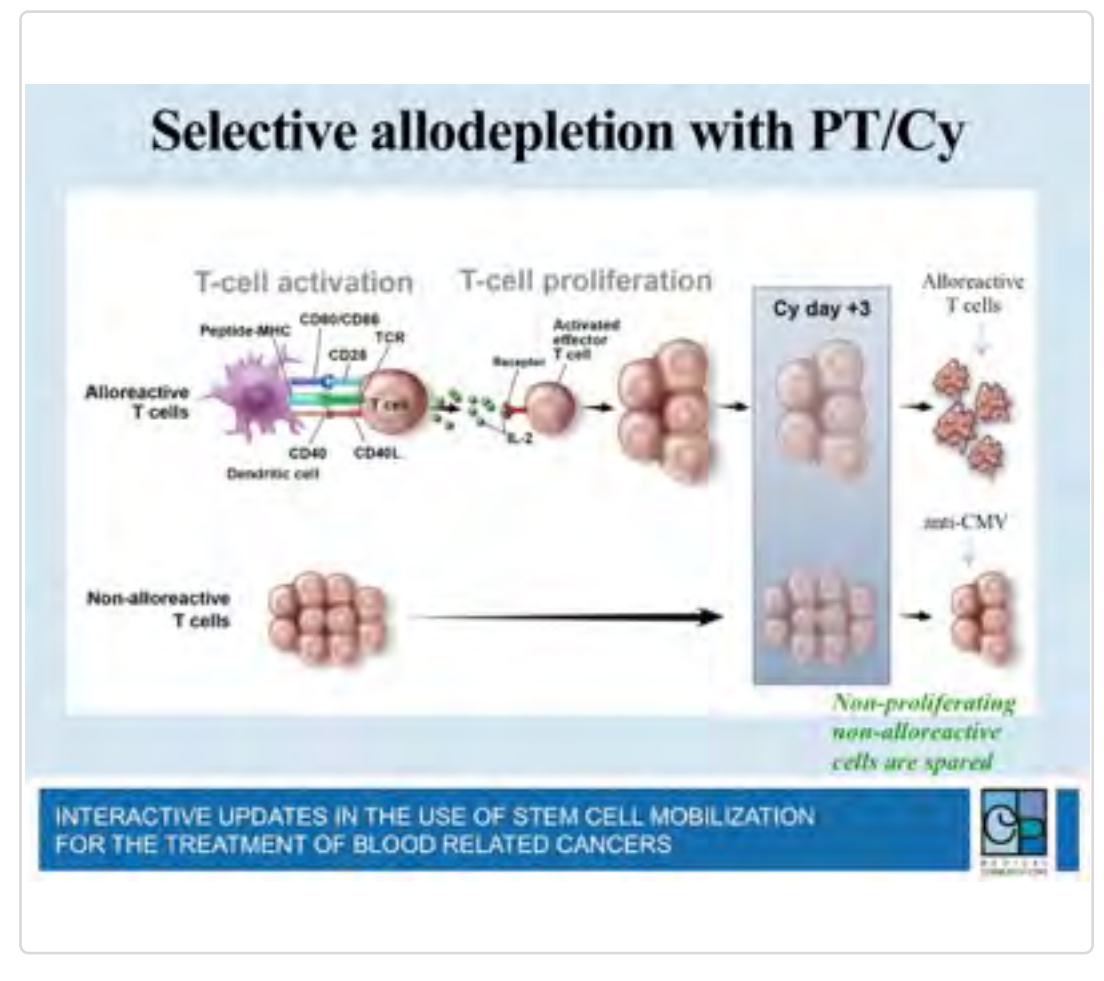
In addition to the use of allogeneic stem cell transplants from either matched sibs or unrelated donors, there has been an explosion of centers using haploidentical stem cell transplants for malignant hematologic diseases. This is a diagram from the Hopkins group showing some of the biology behind using post-transplant high-dose cytoxan to eliminate alloreactivity. In a typical stem cell graft or bone marrow graft, there are a number of non-alloreactive T cells, so-called memory T cells, which have already rearranged their TCRs for specific antigens that they have seen. The cells waiting to see those antigens are largely quiescent, and exposure of those cells to

high-dose cytoxan after transplant results in no major diminution of these cells, since they are quiescent and relatively resistant to cytoxan, unlike alloreactive cells. When they see allo APC they explode and proliferate rapidly from the moment those cells are infused into recipients, and those rapidly expanding T cells, which originated from the naïve population of T cells, are now very sensitive to high-dose cytoxan and are eliminated. In this way, the alloreactive T cells are preferentially eliminated by high-dose cytoxan. (**Interactive 4.11**)

Recent studies by the Hopkins group have clearly shown that the other explanation of this beneficial effect is that cytoxan has a preferential impact on expanding alloreactive T cells while it has no effect on expanding regulatory T cells, and thus, results in an increased ratio of T regulatory cells-to-T effector cells after transplant, mitigating GVHD, both acute and chronic. (**Interactive 4.12**) [8]

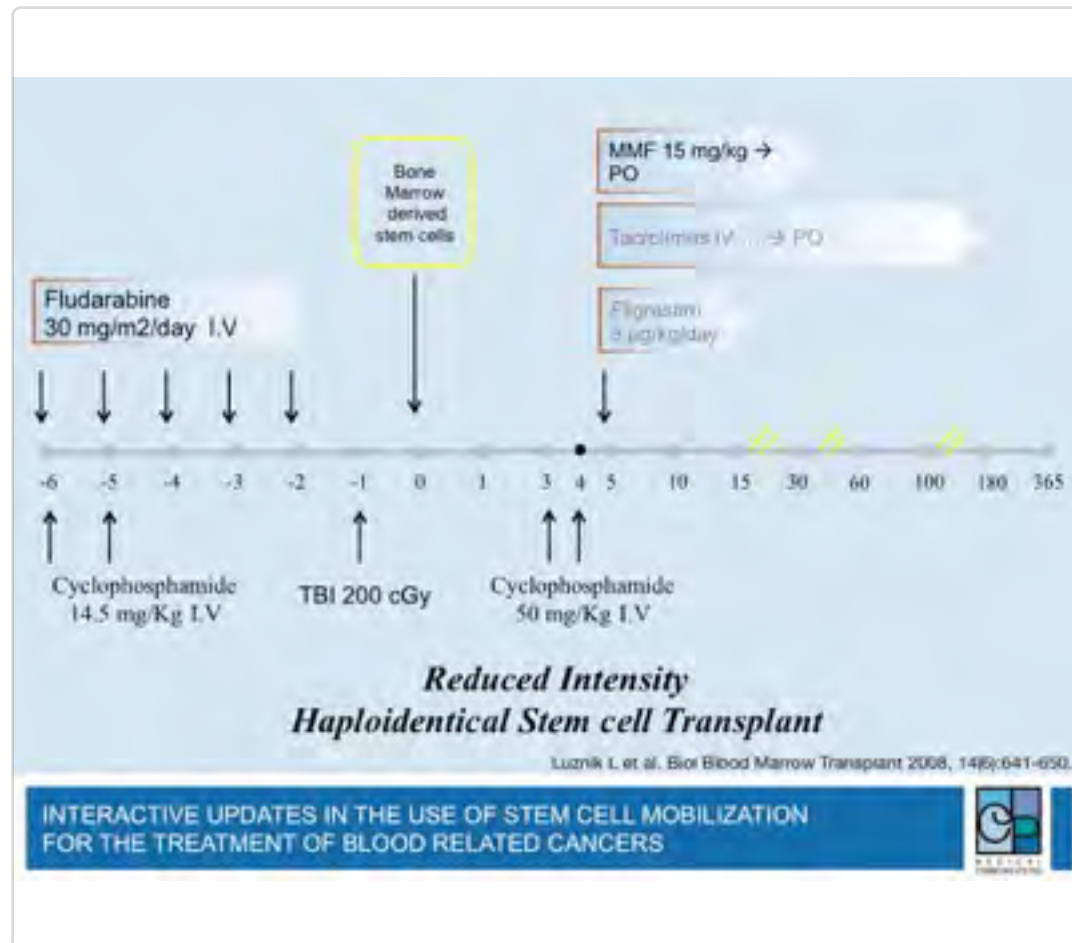
In addition to the non-myeloablative regimens pioneered by the Hopkins group using a combination of fludarabine and low-dose TBI followed by in-

Interactive 4.11

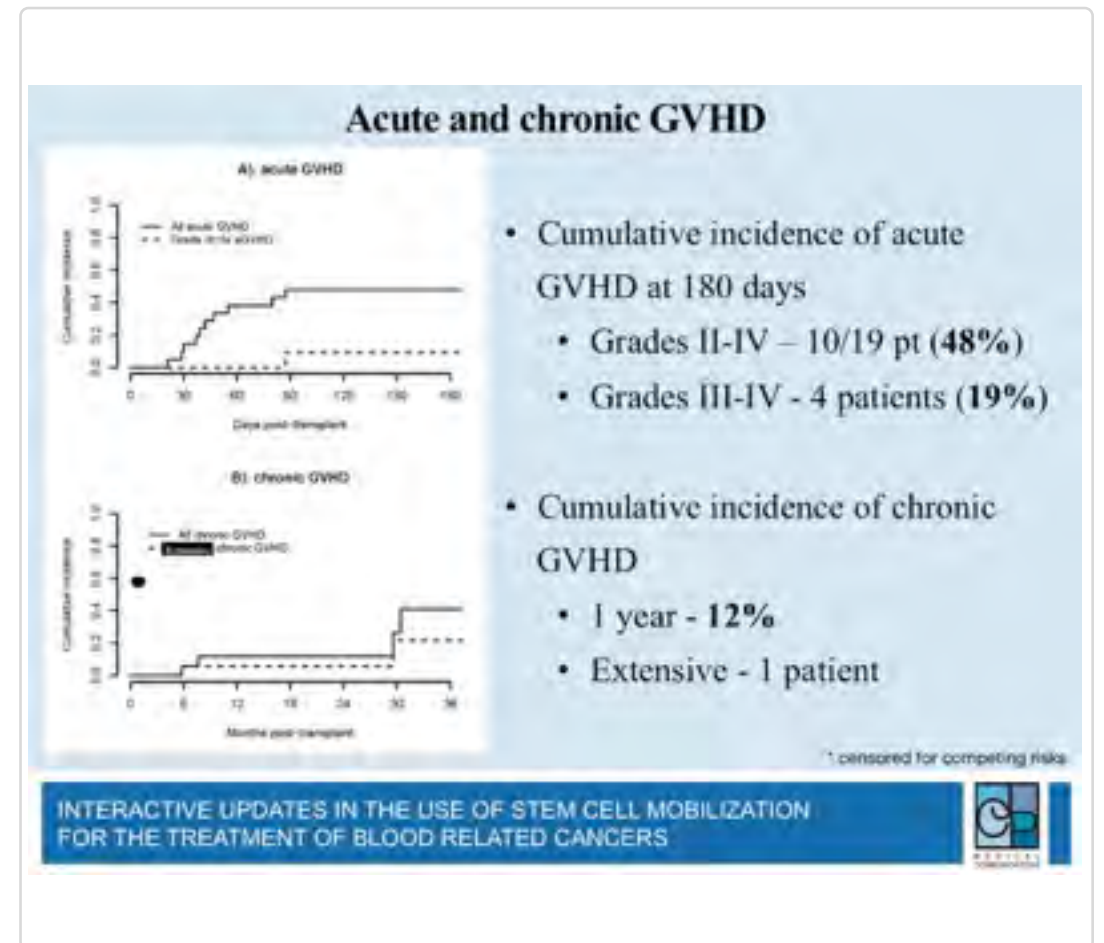


fusion of bone marrow stem cells, with high-dose cyclophosphamide being given on days three and four after transplant, other groups including our own and others around the world have used this same approach in fully ablative transplants.

Interactive 4.12



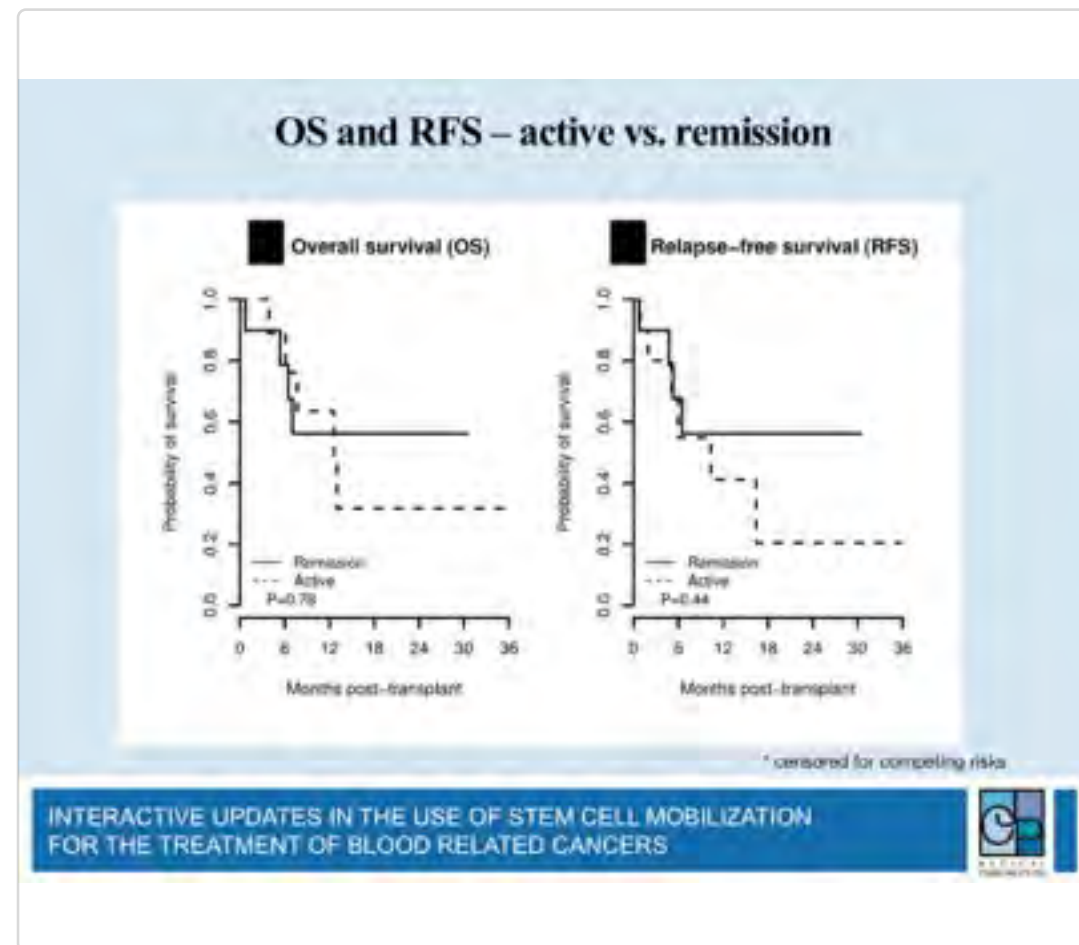
Interactive 4.13



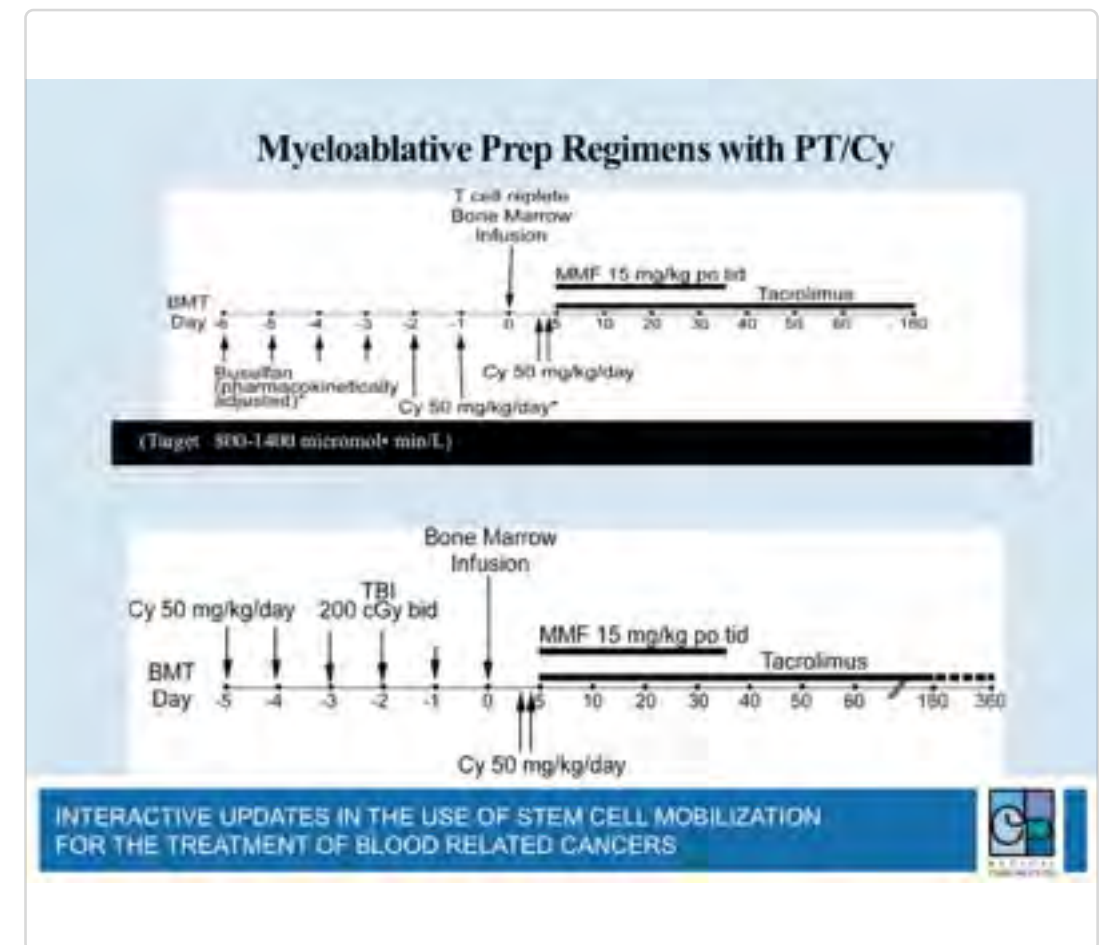
Of note is that acute and chronic GVHD using these reduced intensity regimens pioneered by Hopkins shows that the rates of acute and chronic GVHD are extremely low in patients receiving post-transplant Cytoxan. (**Interactive 4.13**)

The overall survival of patients is reasonable, with low-risk disease patients doing relatively well and patients with high risk disease doing, as expected, relatively poorly. The explanation for this poor outcome has to do with relapse-free survival. Relapse in those patients who come in with high

Interactive 4.14



Interactive 4.15



risk disease and get reduced intensity haploidentical transplant with post transplant Cytoxan have a significantly reduced relapse-free survival. Therefore the antileukemic effect of this approach in the context of bone marrow stem cells, a reduced intensity conditioning regimen by giving high-dose Cy-

toxan after transplant, may be markedly diminished. (**Interactive 4.14**)

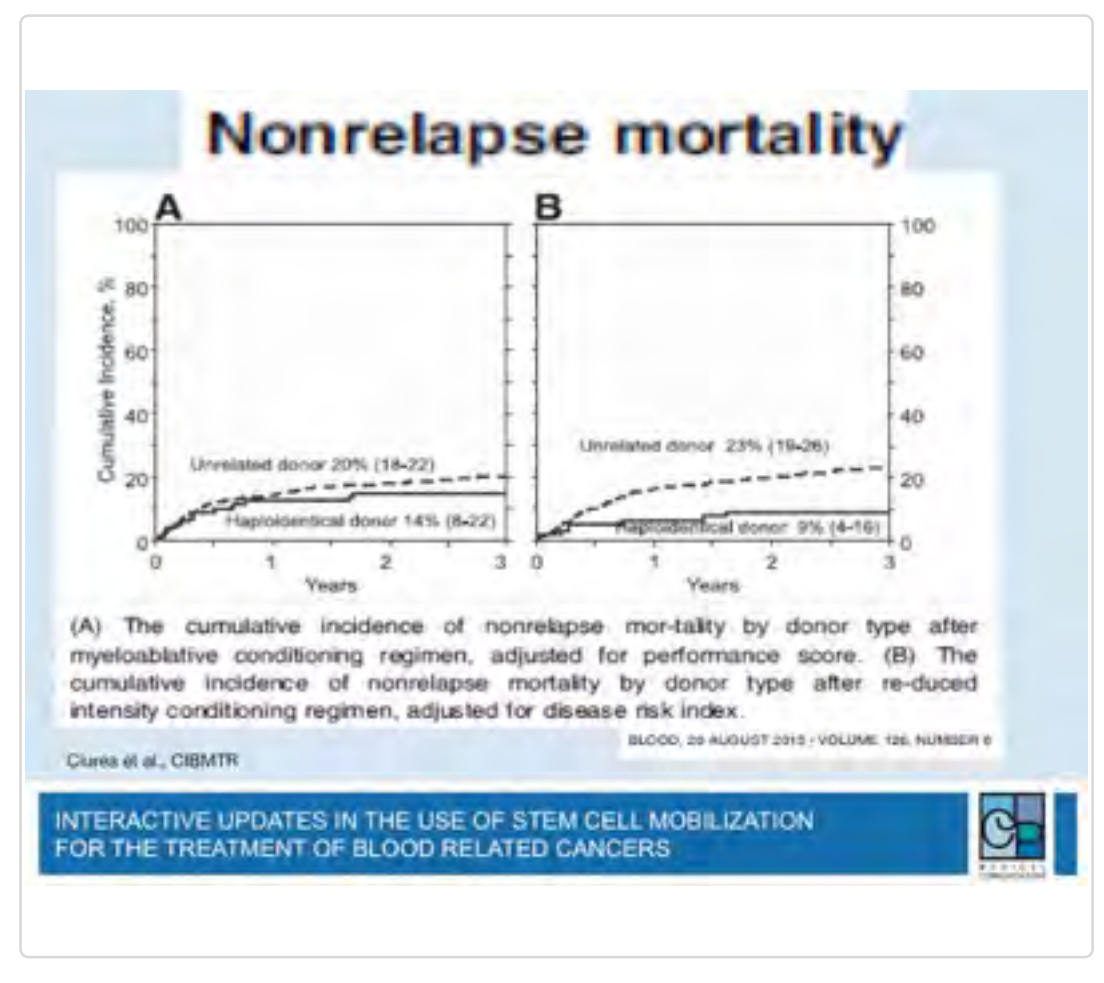
As I mentioned, other groups have used more aggressive ablative transplant regimens with post-transplant Cy and used mobilized peripheral blood or bone marrow infusions with similar outcomes. (**Interactive 4.15**)

Here are some preliminary data using a retrospective analysis of the CIBMTR database. Approximately 300 patients underwent haploidentical transplants for malignant hematologic disease with bone marrow as a source of stem cells. You can see that for haploidentical donors the cumulative index of non-relapse mortality is similar to unrelated donor transplants from matched donors. The cumulative incidence of non-relapse mortality by donor type after reduced intensity conditioning regimens is even lower in the haploidentical setting compared to unrelated donors. (**Interactive 4.16**) [9]

What is also interesting is that when using bone marrow as a source of stem cells there was a slightly increased risk of relapse (as was noted in the Hopkins series) which is inconsistent with this large registry retrospective analysis by the CIBMTR showing that haploidentical transplants have an increased relapse risk. (**Interactive 4.17**) [9]

However, the overall survivals were essentially the same when comparing unrelated donor transplants versus haploidentical transplants. So these data, al-

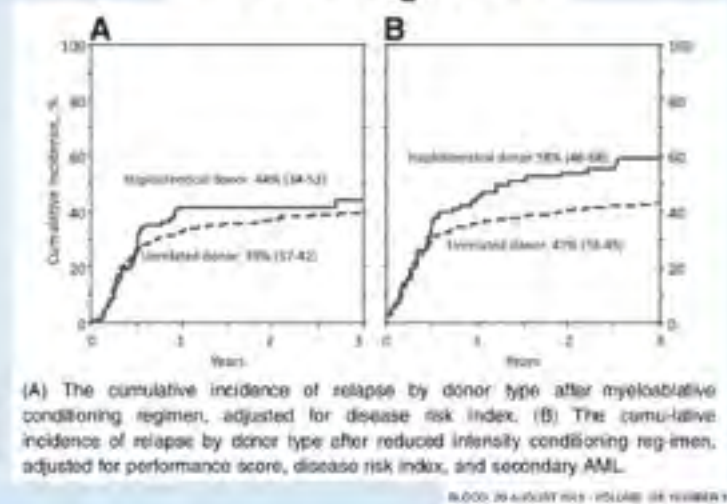
Interactive 4.16



though they are from retrospective analyses, suggest that haploidentical stem cell transplants using bone marrow as a source of stem cells result in similar outcomes to unrelated donor transplants. However, recent data needs to be generated using G-CSF mobilized peripheral blood to ensure that we can also say

Interactive 4.17

Relapse

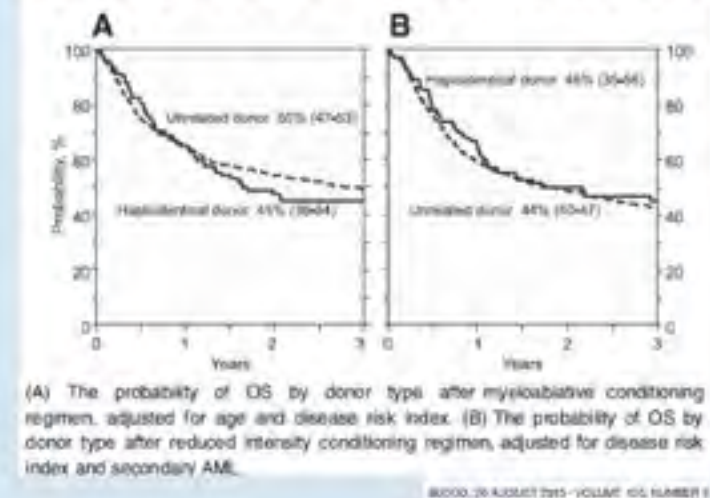


INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 4.18

Overall survival



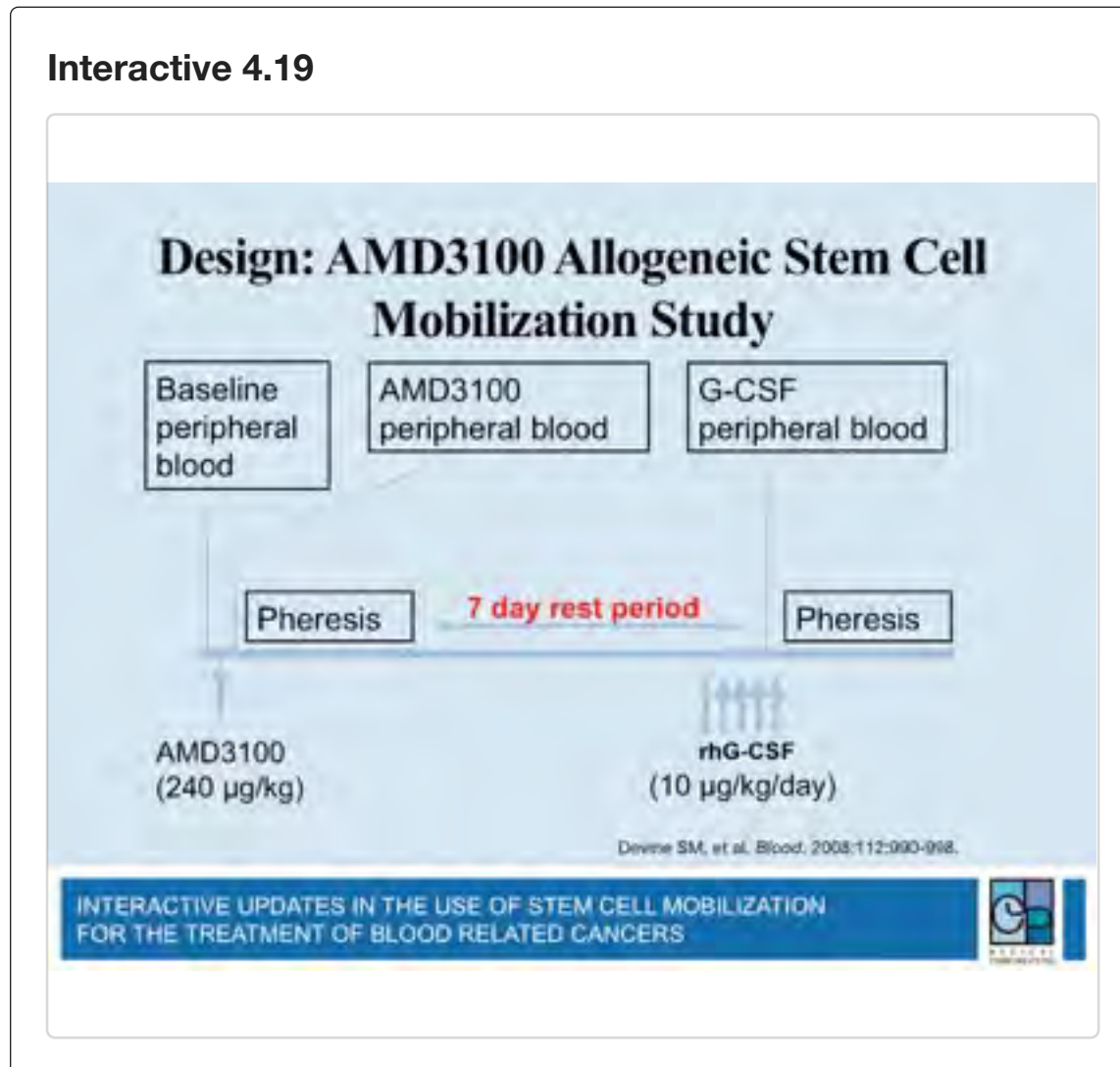
INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



the same about mobilized peripheral blood in the haplo setting; that that source of stem cells results in the same overall and disease-free survival as unrelated donor peripheral blood stem cell transplants. That has not yet been examined in a large retrospective analysis. (**Interactive 4.18**) [9]

What we were interested in doing some time ago was substituting G-CSF, (which as you know takes 5-6 days to mobilize enough stem cells in both normal sib donors and unrelated donors) and try to apply some of the basic science and preclinical studies we had done in the mouse with CXCR4 inhibitors and exploit

Interactive 4.19



some of the preliminary normal volunteer data done by David Dale at Seattle, using plerixafor as a mobilizing agent in normal donors. (**Interactive 4.19**) [10]

What we did was to give AMD3100 (as it was called in the old days and is now called plerixafor) to normal allo donors which were matched sibs, and collect their

stem cells 4 hours after a single injection, and use this pheresis product for stem cell transplantation. The FDA made us collect a G-CSF backup for the first eight patients, but since there were no problems with engraftment from these cells the FDA dropped the requirement for backup and let us proceed and finish our small phase 2 study.

We gave 240 mcg/kg of plerixafor to normal donors and the stem cell products that were collected were interestingly quite different than a G-CSF mobilized product. First of all, there were less CD34 cells per kilogram compared to a G-mobilized product. And there were far more T cells in this product than in a G-CSF mobilized product. This was published in 2008 by Steve Devine. The other interesting aspect is that the T cell numbers were increased for both CD4 and CD8 T cells, while NK cells were not different between a G mobilized product and the plerixafor mobilized product. (**Interactive 4.20**) [10]

We then did several other studies using subcutaneous and intravenous plerixafor in normal sibling donors. This is unpublished data and you can see that SC plerixa-

Interactive 4.20

Allograft Composition (n = 22)			
	AMD3100	G-CSF*	P Value
CD34 (x10 ⁶ /kg)	3.1 (1.2-6.3)	4.2 (2.5-18.7)	0.006
CD3 (x10 ⁸ /kg)	4.7 (1.5-7.8)	1.5 (1.2-6.8)	0.006
CD4 (x10 ⁸ /kg)	3.1 (1.0-5.7)	1.1 (0.7-3.2)	0.002
CD8 (x10 ⁸ /kg)	1.3 (0.4-3.4)	0.4 (0.3-3.4)	0.08
CD56 (x10 ⁷ /kg)	2.8 (0.2-4.6)	0.2 (0.1-0.5)	0.2

*includes 8 donors mobilized by both AMD3100 and G-CSF

Devine et al Blood, 2008

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

Interactive 4.21

Mobilization Failure Rates: SC versus IV Plerixafor			
	SC Plerixafor 0.24 mg/kg (n = 46)	IV Plerixafor 0.32 mg/kg (n = 29)	P Value
One apheresis Proportion of patients who fail to achieve $\geq 2 \times 10^6$ CD34+ cells/kg in a single apheresis	28%	34%	NS
Two aphereses Proportion of patients who fail to achieve $\geq 2 \times 10^6$ CD34+ cells/kg after two aphereses	7%	10%	NS

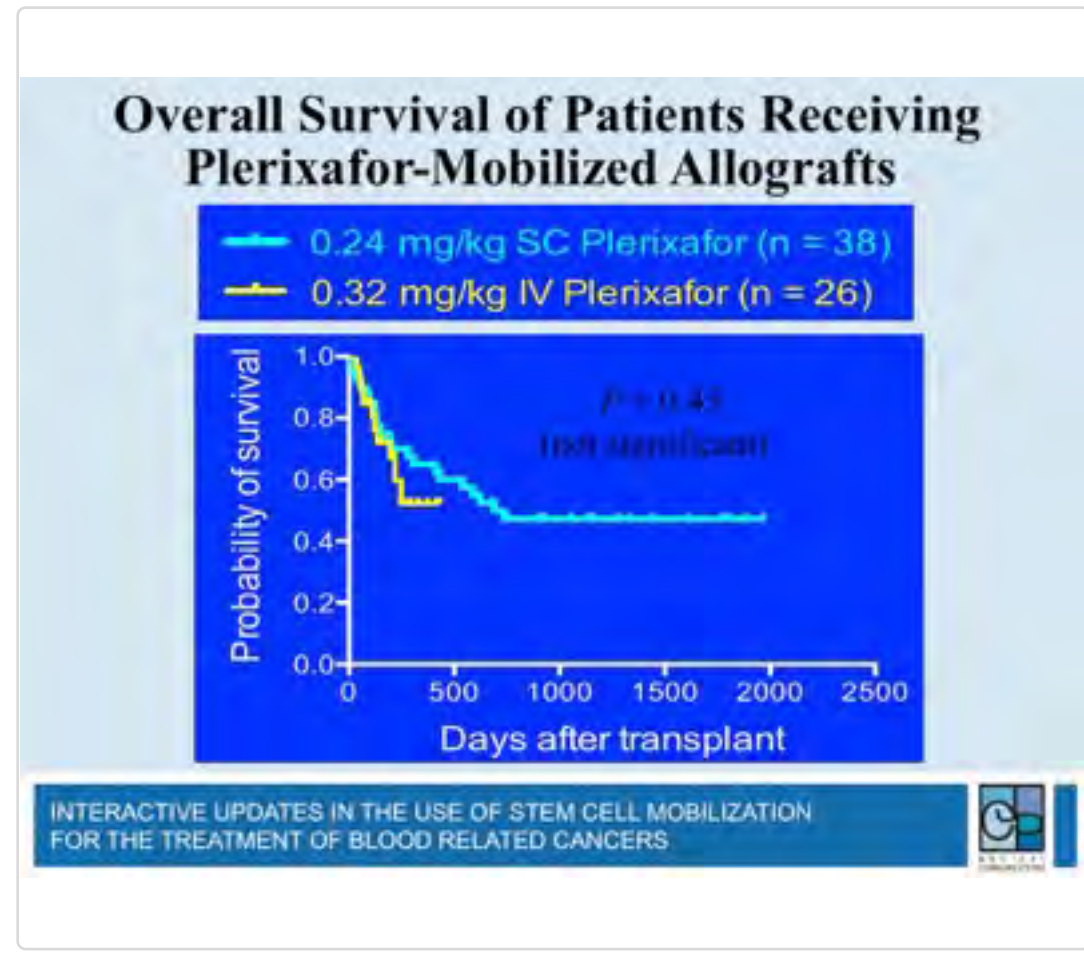
NS = Not significant by Chi-Square

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

for at 240 mcg/kg versus IV plerixafor at 320 mcg/kg results in significant failure to collect a minimum number of stem cells of 2×10^6 CD34 cells per kilogram during the first apheresis. Even after two collections, 7% of patients receiving SC plerixafor and 10% of patients receiving IV plerixafor did not collect the minimum number of

stem cells. One would have to assume that this is problematic as an efficient, robust, rapid mobilizing agent for normal donors, and that there will be significant donors that will have to undergo two collections, unlike with G-CSF, and some donors that don't collect sufficient numbers even after two collections. (**Interactive 4.21**)

Interactive 4.22

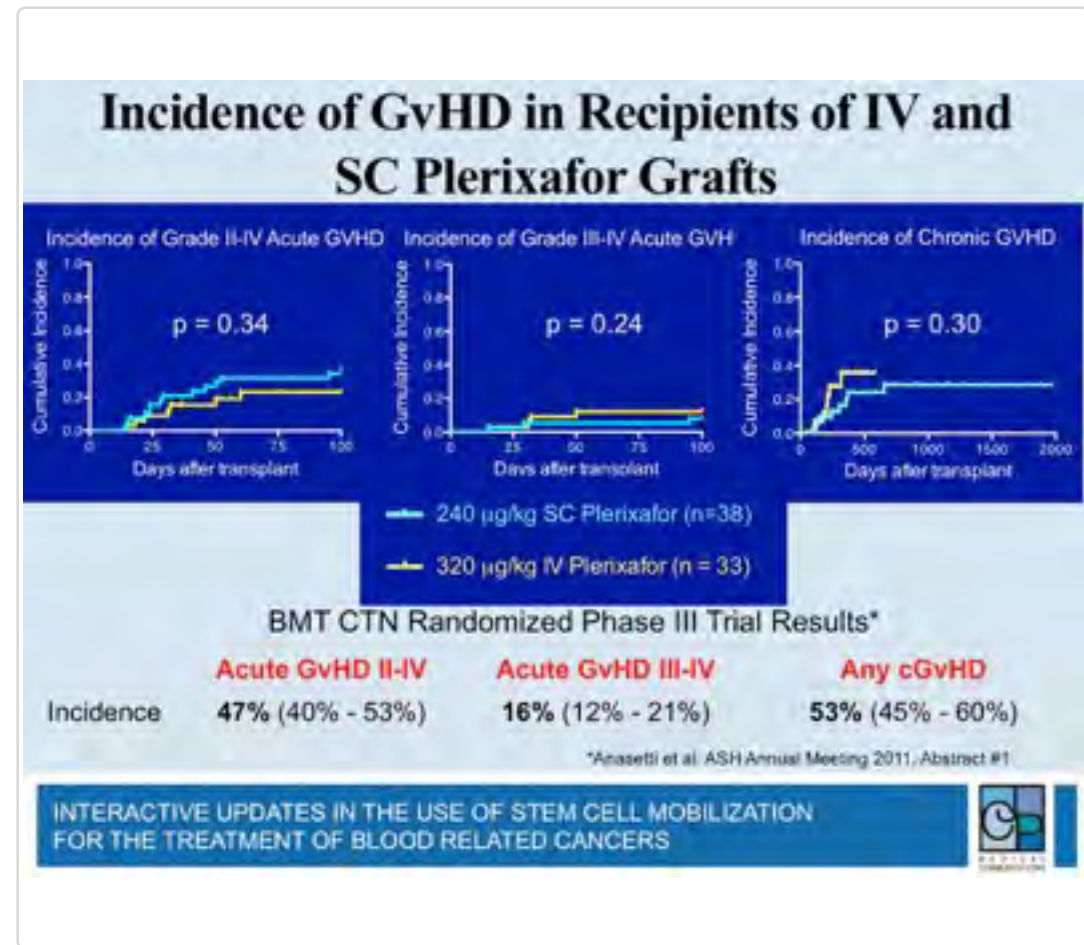


These data look quite a bit different than with normal donors, as is always the case, since normal donors are selected to be usually young males that are non-obese, do not have comorbid conditions like diabetes, etc., and normal allogeneic sibling donors are often the same age as their transplanted siblings.

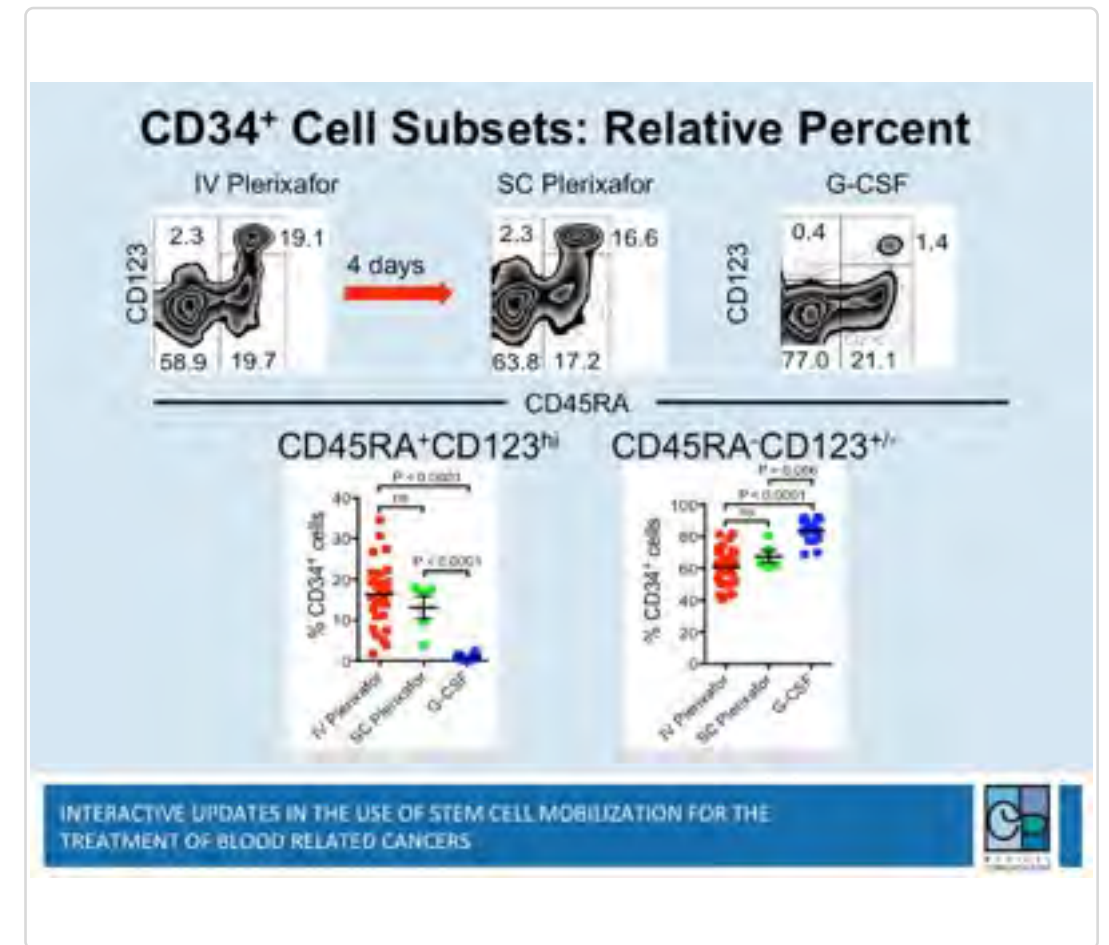
These ages range up in the 70s and they are more evenly distributed between male and female. Some are obese and some have diabetes. The overall survival of these groups, which were high-risk patients, looks relatively comparable with very long follow up. This is encouraging in that, even though we are mobilizing limited numbers of stem cells and most patients received approximately $2\text{-}2.5 \times 10^6$ CD34/kg, long term outcomes look very respectable for both groups. (**Interactive 4.22**)

What was of interest (and this is unpublished but presented at several ASH meetings in the past), is that the incidence of acute II-IV and III-IV GVHD appeared to be extremely low in both of these studies. Now obviously this has to be validated in larger trials, and perhaps ultimately in randomized trials if this were ever to be approved as a single agent to mobilize stem cells from normal donors. The incidence of chronic GVHD for both of these studies was also relatively low. And so perplexingly, even though there was significantly more T cells, there appeared

Interactive 4.23



Interactive 4.24



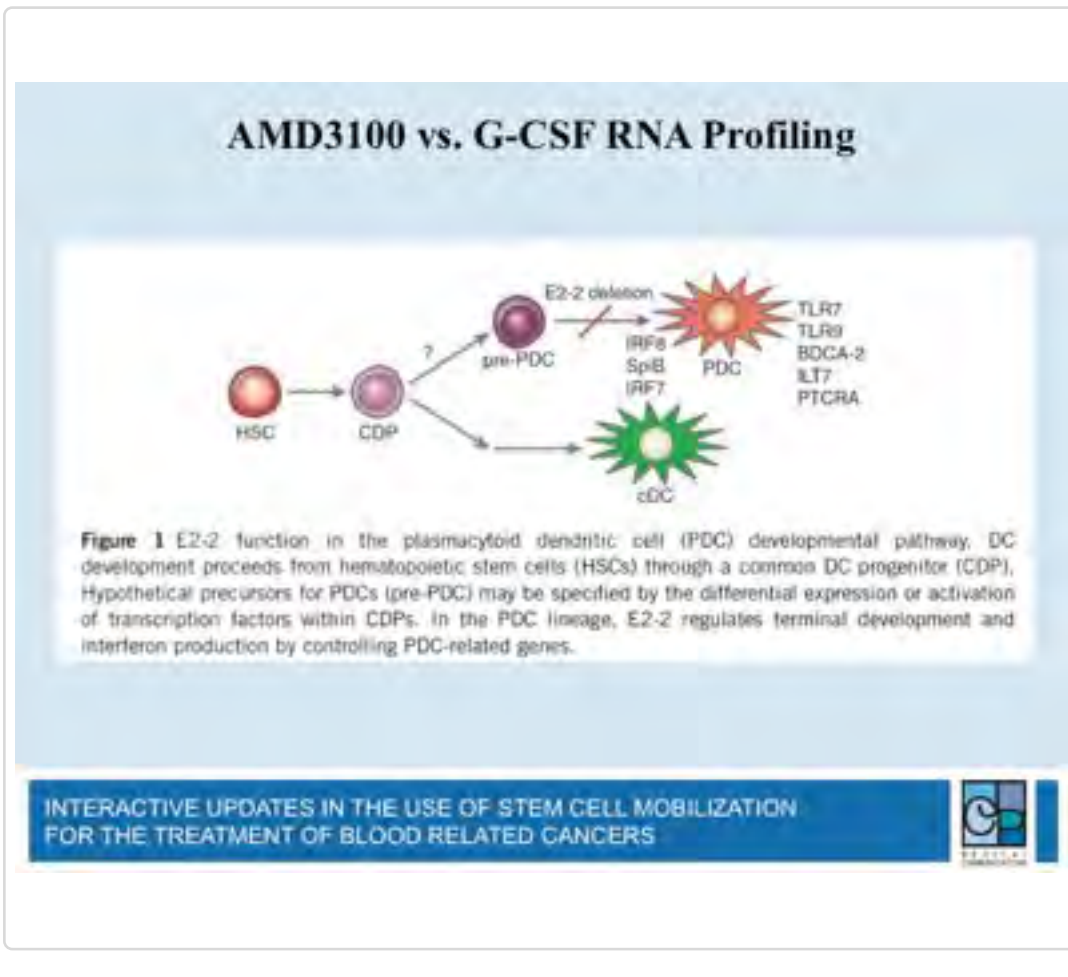
to be a relatively low incidence of both acute and chronic GVHD. (**Interactive 4.23**)

What is even more interesting is that when one actually looks at the phenotype of the kind of stem cells mobilized by IV plerixafor, SC plerixafor, or G-CSF from normal allogeneic donors, one can see, using

flow cytometry on CD34 selected cells for CD45RA on the X axis and CD123 on the Y axis that they are very different from G-CSF. (**Interactive 4.24**)

In the G-CSF population there is only a tiny population of these cells that are CD123 positive and bright and CD45RA positive and bright, and these

Interactive 4.25



at these cells we found that they were actually plasmacytoid dendritic precursor cells. (**Interactive 4.25**)

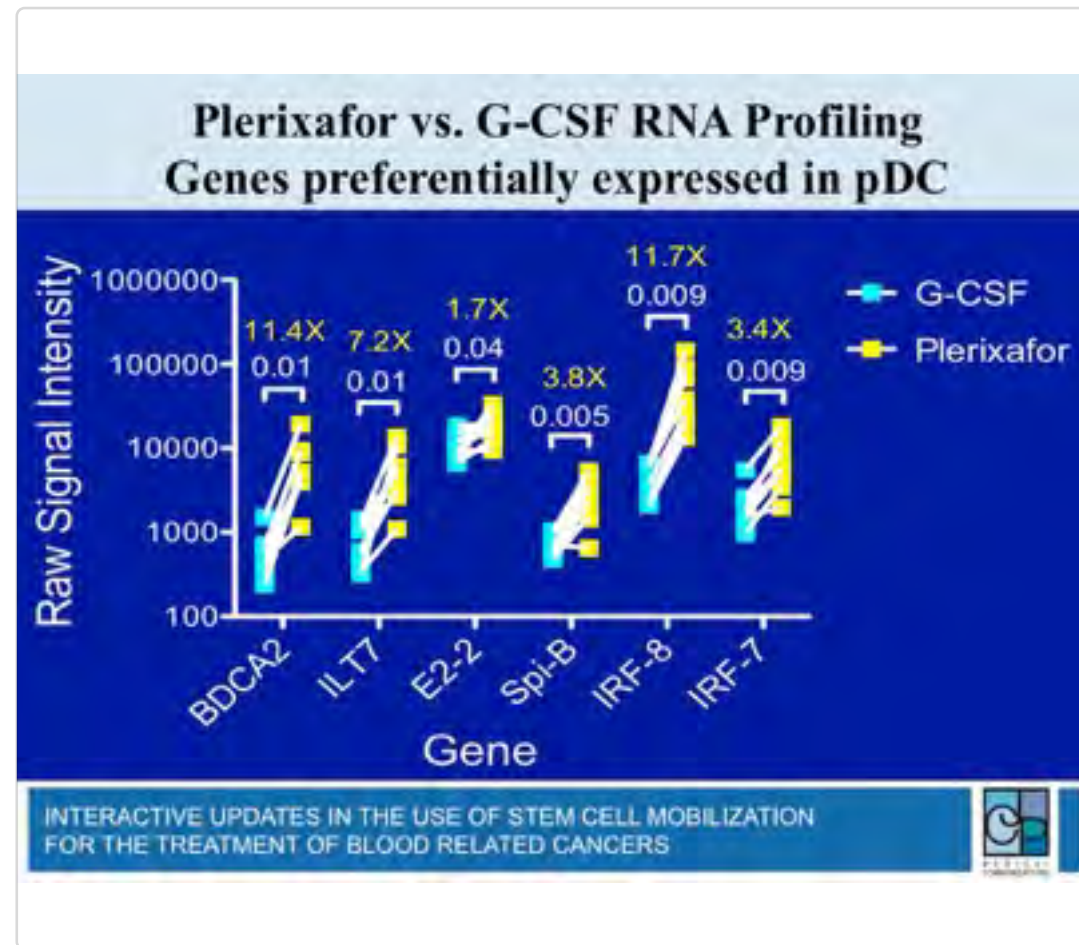
We figured this out by looking at all transcription factors involved in the development of these cells which were known to have that immunologic phenotype, and looked for the expression of those key transcription factors in the stem cells purified from G-CSF mobilized CD34-positive cells and plerixafor mobilized CD34 cells from normal allo donors. You can see that in every case, the expression of these plasmacytoid dendritic transcription factors was markedly elevated in patients that received plerixafor for mobilization in their hematopoietic stem cells. (**Interactive 4.26**)

When we examined these cells for biologic activity, we looked at the production of IFN-alpha, which is known to be expressed by these plasmacytoid dendritic cells and is very important for antiviral immunity. We found that there was a huge increase in the production of IFN-alpha in a plerixafor-mobilized stem cell compared to a G-CSF-mobilized stem cell when incubated in vitro for 24 hours.

cells are abundant in plerixafor mobilized allogeneic donors. The pictures on the bottom recapitulate what is shown on the top.

So the question is, what are these cells and what are they doing? Interestingly enough, when we looked

Interactive 4.26



When we looked at the incidence of CMV viremia in patients mobilized with G-CSF versus plerixafor we found that, for patients at risk of developing CMV viremia, about 60% receiving G-mobilized product developed viremia at some point after transplant while only 15% of our patients receiving plerixafor-mobilized prod-

ucts developed CMV viremia. There was an approximately equal incidence of CMV disease in the two groups. So there is reason to believe that in this stem cell subset it is not only progenitors that give rise to hematopoietic reconstitution but also plasmacytoid dendritic precursors that when infused into recipients, may have a protective effect against CMV viremia through the production of IFN-alpha. The most common growth factor that has been used for mobilization has been G-CSF, and there are very few papers looking at the role of GM-CSF by itself for mobilization. (**Interactive 4.27**) [11]

In this report by Devine et al., we looked at retrospective data from Washington University and showed that patients who received GM-CSF alone, again had a relatively low risk of grades 2-4 GVHD and no patients developed grades 3-4 GVHD compared to G-mobilized plus GM-mobilized products. This was suggestive that GM-CSF might be an effective mobilizing agent if you were primarily interested in reducing GVHD. (**Interactive 4.28**) [12]

Interactive 4.27

Incidence of CMV Viremia and Disease

	G-CSF*	plerixafor	Risk Difference (95% CI)
CMV viremia $\geq 10,000$ copies/mL	62% (43/69)	15% (12/80)	47% (23 - 69)
CMV disease with pre-emptive treatment	6% (4/69)	5% (4/80)	0.8% (-24 - 25)

*Ventricular et al. BMT 2006; 37:51-56

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 4.28

GM-CSF mobilized stem cell product was associated with less aGvHD in humans

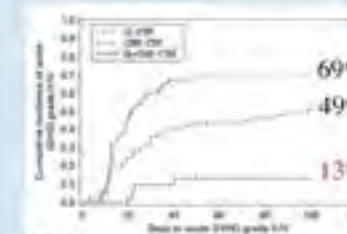


Figure 1 Incidence of grade 3-4 acute GVHD by cytotoxic group

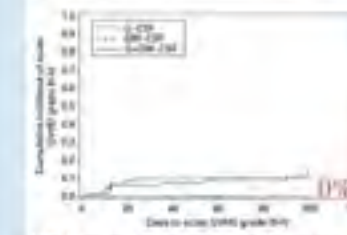


Figure 2 Incidence of grade 3-4 acute GVHD by cytotoxic group

Table 3 Univariate and multivariate analysis

Univariate	P-value	Multivariate	P-value
Donor age	0.48	Matched donor	0.002
Donor age	0.04	GM-CSF vs G-CSF	0.002
Sex mismatch	0.03	GM-CSF vs G-CSF	0.002
		Donor risk (high vs low)	0.002
		CD34 dose	0.002
		CD3 dose	0.12
		CD34 dose	0.002
		CD3 dose	0.002
		CD34 dose	0.002
		CD3 dose	0.002

GM-CSF = granulocyte colony-stimulating factor; G-CSF = granulocyte colony-stimulating factor

T cell dose and CD34 dose were not significant in multivariate analysis

Devine et al. BMT. 2005;36:531

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

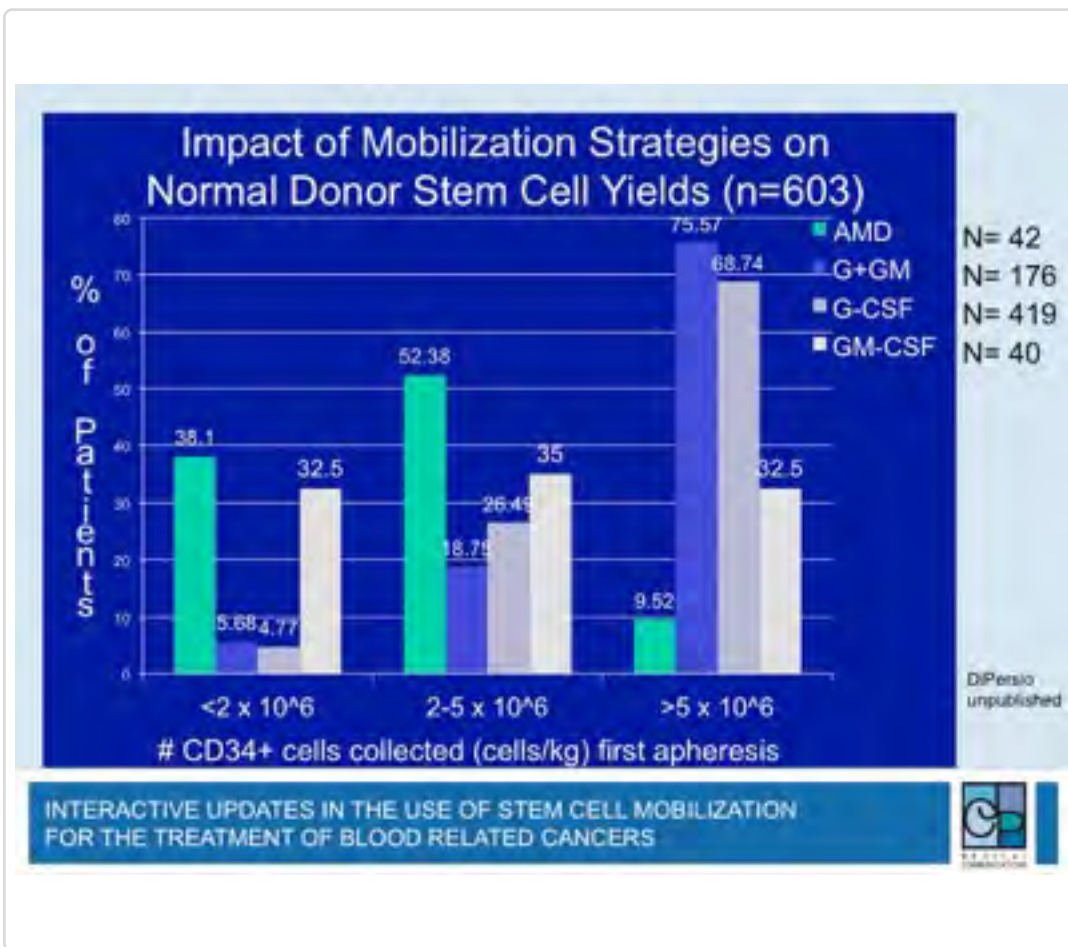


But when we compared the ability to mobilize stem cells with AMD3100 (plerixafor) versus G-CSF plus GM-CSF in 176 normal donors, versus G-CSF in 419 normal donors, versus GM-CSF alone in 40 donors, you can see that the failure rate to achieve the minimum number of stem cells needed for transplan-

tation after a single apheresis was 38% in the plerixafor-mobilized group and 32% in the GM-mobilized group and the other two groups had very low failure rates. (**Interactive 4.29**)

So even though GM-CSF results in the low incidence of acute and chronic GVHD, like plerixafor-

Interactive 4.29



failure rate gets higher and higher for the AMD groups and the GM groups.

In conclusion, G-CSF-mobilized peripheral blood stem cells are effective at reconstituting multi-lineage engraftment in autologous and allogeneic stem cell recipients. Most products today are mobilized with G-CSF; G-CSF plus chemotherapy for auto transplants and G-CSF by itself for allogeneic stem cell transplant patients. Successful mobilization of normal donors in a single collection occurs between 75 and 94% of the time with G-CSF after a single apheresis. (**Interactive 4.30**)

Biosimilars are as effective as Neupogen for both auto and especially for allogeneic blood stem cell mobilization. I did not show the data but I can summarize the studies that have been published. Recommended CD34/kg for MUD and Haplo transplant is greater than 5 X 10⁶ CD34/kg, and in many centers the number of stem cells infused is limited to 7-8 as a maximum. Plerixafor, the CXCR4 antagonist, when given either subcutaneously or intravenously alone, is similar to GM-CSF alone and results in failure to

Interactive 4.30

Conclusions

1. G-CSF mobilized peripheral blood stem cell is the dominant. Successful mobilization of normal donors occurs ~75-94% of the time in a single apheresis.
2. Biosimilars are as effective as neupogen as allo PBSC mobilizing agent.
3. Recommended CD34/kg for MUD and Haplo transplant is 5×10^6 CD34/kg
4. Plerixafor (SC and IV) alone (similar to GM-CSF) alone result in failure to mobilize 2×10^6 CD34/kg ~30-40% of the time after a single apheresis.
5. Plerixafor mobilization of allogeneic donors is associated with rapid and stable engraftment and limited acute and chronic GvHD.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



could add to plerixafor that would enhance its ability to quantitatively mobilize more stem cells in a rapid period of time would fulfill our goal of achieving the holy grail of robust and rapid mobilization within hours after administration of stem cell mobilizing agents. In the next talk we will discuss some of these potential approaches experimentally using preclinical models. Thank you very much.

mobilize an adequate number of stem cells in 30-40% of normal donors after a single apheresis, and so is probably not suitable for ongoing clinical use.

Plerixafor mobilization of allogeneic donors is associated with rapid and stable engraftment and limited acute and chronic GVHD. Any agents that we

1. Hopman RK, DiPersio JF. Advances in stem cell mobilization. *Blood Rev.* 2014 Jan;28(1):31-40. <http://www.ncbi.nlm.nih.gov/pubmed/24476957>

REFERENCES

2. Mozaheb Z, Khayami M, Sayadpoor D. Iron Balance in Regular Blood Donors. *Transfus Med Hemother*. 2011;38(3):190-194. <http://www.ncbi.nlm.nih.gov/pubmed/21760763>
3. Ferraro F, Lymperi S, Méndez-Ferrer S, et al. Diabetes impairs hematopoietic stem cell mobilization by altering niche function. *Sci Transl Med*. 2011 Oct 12;3(104):104ra101. <http://www.ncbi.nlm.nih.gov/pubmed/21998408>
4. Fadini GP, Albiero M, Vigili de Kreutzenberg S, et al. Diabetes impairs stem cell and proangiogenic cell mobilization in humans. *Diabetes Care*. 2013 Apr;36(4):943-9. <http://www.ncbi.nlm.nih.gov/pubmed/23111057>
5. Fadini GP, Fiala M, Cappellari R, et al. Diabetes Limits Stem Cell Mobilization Following G-CSF but Not Plerixafor. *Diabetes*. 2015 Aug;64(8):2969-77. <http://www.ncbi.nlm.nih.gov/pubmed/25804941>
6. Anasetti C, Logan BR, Lee SJ, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med*. 2012 Oct 18;367(16):1487-96. <http://www.ncbi.nlm.nih.gov/pubmed/23075175>
7. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008 Jun;14(6):641-50. <http://www.ncbi.nlm.nih.gov/pubmed/18489989>
8. Ciurea SO, Zhang MJ, Bacigalupo AA, et al. Haploidentical transplant with posttransplant cyclophosphamide vs matched unrelated donor transplant for acute myeloid leukemia. *Blood*. 2015 Aug 20;126(8):1033-40. <http://www.ncbi.nlm.nih.gov/pubmed/26130705>
9. Devine SM, Vij R, Rettig M, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood*. 2008 Aug 15;112(4):990-8. <http://www.ncbi.nlm.nih.gov/pubmed/18426988>
10. Verkruijse LA, Storch GA, Devine SM, et al. Once daily ganciclovir as initial pre-emptive therapy delayed until threshold CMV load > or =10000 copies/ml: a safe and effective strategy for allogeneic stem cell transplant patients. *Bone Marrow Transplant*. 2006 Jan;37(1):51-6. <http://www.ncbi.nlm.nih.gov/pubmed/18426988>
11. Devine SM, Brown RA, Mathews V, et al. Reduced risk of acute GVHD following mobilization of HLA-identical sibling donors with GM-CSF alone. *Bone Marrow Transplant*. 2005 Sep;36(6):531-8. <http://www.ncbi.nlm.nih.gov/pubmed/16025152>



Chapter 5

Mobilization Algorithms to Optimize Patient Outcomes

Luciano J. Costa, MD, PhD



Luciano J. Costa, MD, PhD

Associate Professor of Medicine
University of Alabama at Birmingham
Birmingham, Alabama

Luciano J. Costa obtained an MD and PhD at Universidade de Sao Paulo in Brazil where he also trained in Internal Medicine and Hematology. He subsequently completed fellowship in Hematology and Oncology (University of Colorado) and Blood and Marrow Transplantation (Mayo Clinic, Rochester).

Dr. Costa is currently an Associate Professor of Medicine and medical director of ambulatory care and the cell therapy collection facility for the UAB Blood and Marrow Transplant Program. His clinical research includes transplant and non-transplant management of lymphoproliferative and plasma cell disorders. He has also been deeply engaged in strategies for optimization of hematopoietic progenitor cell mobilization and in population outcomes of blood cancers.

Movie 5.1

MOBILIZATION ALGORITHMS TO OPTIMIZE PATIENT OUTCOMES

Luciano J. Costa, MD, PhD
Associate Professor of Medicine
University of Alabama at Birmingham

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Mobilization Algorithms to Optimize Patient Outcomes

Luciano J. Costa, MD, PhD

Interactive 5.1

MOBILIZATION ALGORITHMS TO OPTIMIZE PATIENT OUTCOMES

Luciano J. Costa, MD, PhD
Associate Professor of Medicine
University of Alabama at Birmingham

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



My name is Luciano Costa, and I'm an Associate Professor of Medicine at the University of Alabama at Birmingham, where I also serve as Medical Director for the apheresis collection facility. Today I will discuss mobilization algorithms to optimize patient outcomes. (**Interactive 5.1**)

Mobilization algorithms can be defined as a bundle of practices and procedures governing the uniform use of mobilization agents and the collection of hematopoietic progenitor cells in a transplant center. (**Interactive 5.2**)

Interactive 5.2

Mobilization algorithms – Bundle of practices and procedures governing the uniform use of mobilization agents and the collection of hematopoietic progenitor cell in a transplant center

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 5.3

Mobilization with algorithm



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



I like to make the analogy between mobilization and cutting grass. At a transplant center, you can have a uniform, coded practice that you call an algorithm, so you have uniform results. (**Interactive 5.3**)

If you don't do that, you'll end up with a case-by-case approach that might work for patient care,

but which will result in a nonuniform scenarios where the efficacy and outcomes are difficult to evaluate. (**Interactive 5.4**)

Why use mobilization algorithms? They increase the quality of the process. They improve communication in a setting where there is often involve-

Interactive 5.4

Mobilization without algorithm



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 5.5

Why to use a mobilization algorithm?

- ✓ Increase quality
- ✓ Improve communication
- ✓ Reduce resource utilization
- ✓ Reduce need for remobilization
- ✓ Improve patient outcomes

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



ment of the clinician, the advanced practice provider, the apheresis staff, and the cell processing staff. It's very helpful when you have a process that is simple and well known by all of those involved, so communication can occur effectively, which reduces the likelihood of processes or individual pa-

tient care practices that diverge from the intended plan of care. (**Interactive 5.5**)

As in many other fields, standardization often results in the reduction of resource utilization, so there is a good financial argument for standardization of mobilization. More importantly, it has been

shown that the adoption of algorithms in a practice reduces the need for remobilization, which is a big problem for both the transplant center as well as for the patient, as it increases costs and delays transplant, the ultimate goal of therapeutic intervention. So, when taken together, we can say that the adoption of mobilization algorithms are likely to improve patient outcomes.

When setting up an algorithm for a particular center there are concepts or priorities that are often conflicting. So the transplant center has to decide on its priorities and how to balance conflicting concepts. (**Interactive 5.6**)

One good example of this is the conflict between cost and resources vs. high target. There is variation among transplant centers in the target number of CD34 cells that are intended for collection and eventually, for transplantation. We all agree on the minimal number, or there's at least more agreement on the minimal number, which is 2×10^6 CD34 cells per kg of recipient. However, how that information is used in terms of stopping apheresis varies from

Interactive 5.6

Conflicting concepts in mobilization algorithms

Cost/Resources	vs.	High target
Uniformity	vs.	Adaptability
Complexity	vs.	Consistency
Control	vs.	Time Commitment
Belief (chemomobilization)	vs.	Practicality

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



center-to-center. Some centers aim at collecting 3×10^6 , 4×10^6 , or even 5×10^6 CD34 cells per transplant. Evidently, as you aim for a higher target, you are more likely to use multiple doses of expensive mobilizing agents, put the patient through more sessions of apheresis, and increase the cost.

Other conflicting concepts are uniformity versus adaptability. If you have a process, an algorithm that is extremely simple, it is likely to be well received by the staff. You are likely to have greater consistency; however, it also makes it difficult to adapt to different patient situations, such as age, type of disease, or the individual capacity to mobilize cells.

Along the same lines, you have the concept of complexity versus consistency. The more complex the algorithm, the more likely it is that there will be deviations from that algorithm within your transplant center, because it increases the odds that individual members of the team might overlook or might choose not to adopt a particular aspect of the algorithm.

Other conflicting concepts are those of control versus time commitment. The physician is one of many individuals involved in the mobilization process. If the physician chooses, for instance, to personally monitor and make adaptations during the mobilization process for each individual patient, that takes a lot of time. If the physician chooses to surrender that control, and instead benefit from having uniformity and a common

process, then less time would be committed to the day-to-day management of their patients.

Ultimately, there is a conflict between belief and practicality. For instance, if a transplant center has a firm belief that chemomobilization is the better method of mobilization, either because of greater yields or better disease control, that is the belief that will prevail when it comes to designing an algorithm, which can result in an algorithm that is not always practical.

I would like to review a few of the contemporary approaches to algorithms that have been published. Some institutions have an algorithm that is simple. It is one-size-fits-all, so essentially that institution chooses to adopt one method of mobilization for essentially all patients. (**Interactive 5.7**)

The most commonly seen of those approaches are with the use of filgrastim and plerixafor, essentially replicating the process described in the two Phase III clinical trials that led to the approval of plerixafor in the United States. That consists of four days of daily administration of filgrastim with the administration of plerixafor start-

Interactive 5.7

Contemporary approaches

- ✓ "One size fits all"
 - Filgrastim + Plerixafor
 - Chemotherapy + Filgrastim
- ✓ "Just in time" use of plerixafor
- ✓ Risk-based approaches

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



ing on the evening of the fourth day, and apheresis starting on the fifth day. Continued daily is the plerixafor administration and apheresis until the target is met, or until there are four consecutive days of apheresis. Another approach, also used by many sites, is the chemotherapy plus filgrastim as a universal method of mobilization.

Other algorithms, some of which we will review, use the concept of a "just in time" use of plerixafor. That consists of the use of either growth factor or, as published more recently, chemotherapy plus growth factor with the addition of plerixafor, based on the patient's actual capacity of mobilized cells, which is assessed by measuring the CD34 count in the peripheral blood at a given time point. If that count is lower than a certain threshold, then plerixafor is added. If it is above a certain threshold and the patient is a good mobilizer, collection is started without the use of plerixafor.

There are also risk-based approaches, which are intended to stratify patients based on the perceived risk of mobilization failure and assign them to different mobilization strategies. This is sometimes summarized in a document that we can call an algorithm, but many transplant centers and physicians do that in a less formal way by assigning patients with certain characteristics to specific mobilization practices. A common one is to use growth factor-based mobilization with most patients, but use chemomobilization in patients who have a high burden of multiple myeloma cells in the bone marrow or

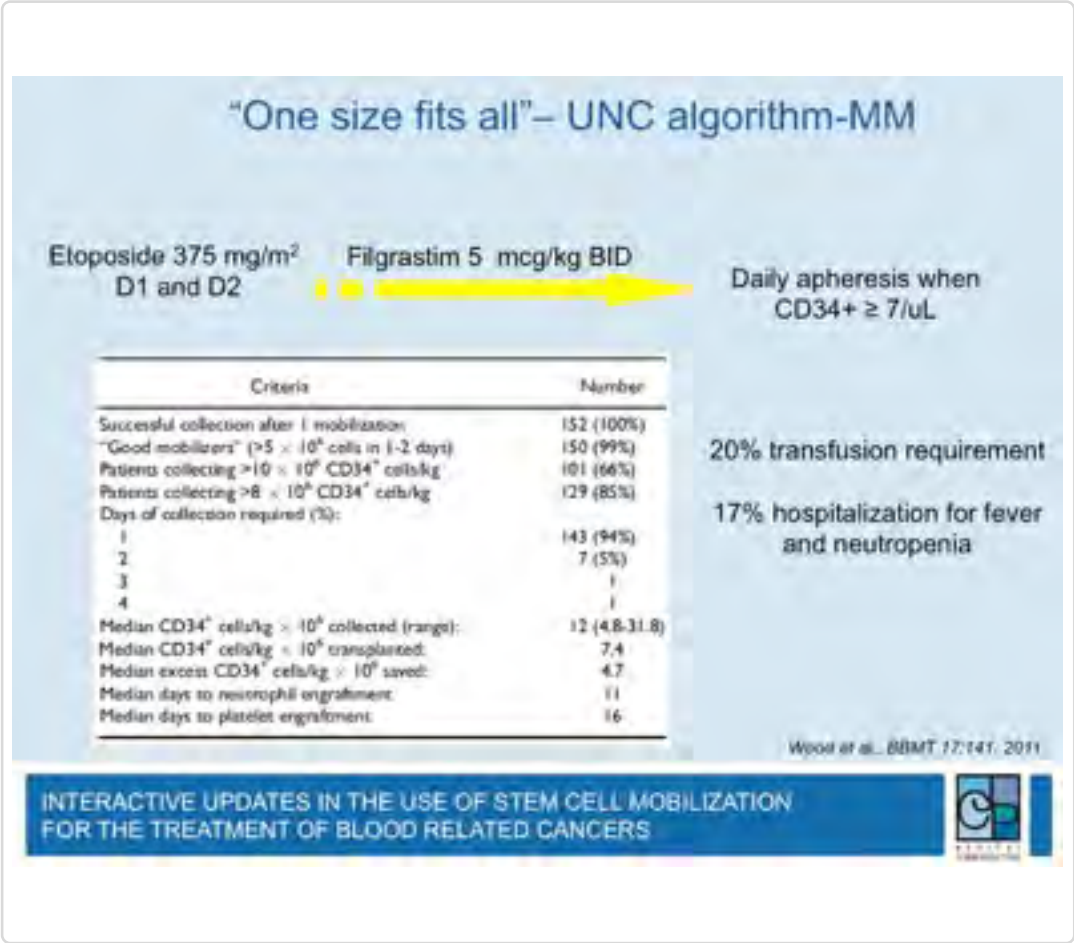
in patients who have received a certain number of cycles of drugs that impair mobilization, such as lenalidomide.

The first algorithm that I want to show as an example of “one size fits all” is the University of North Carolina algorithm in multiple myeloma that is chemotherapy-based, consistent with the administration of etoposide on days one and two, followed by filgrastim as growth factor, and starting apheresis when the CD34 count in peripheral blood is higher than 7. (**Interactive 5.8**) [1]

In this publication from 2011, 152 patients had 100% mobilization success, with 94% of the patients collecting all the necessary cells in one session. The median CD34 cells per kilo collected was 12, which is excellent; 20% of the patients, however, required transfusion, and 17% were hospitalized for fever and neutropenia, a number that is not very high compared to the literature in chemomobilization, but that is certainly much higher than what you would expect with growth factor alone. Nevertheless, this is an example of a “one size fits all” algorithm that is very successful, as all patients were able to collect.

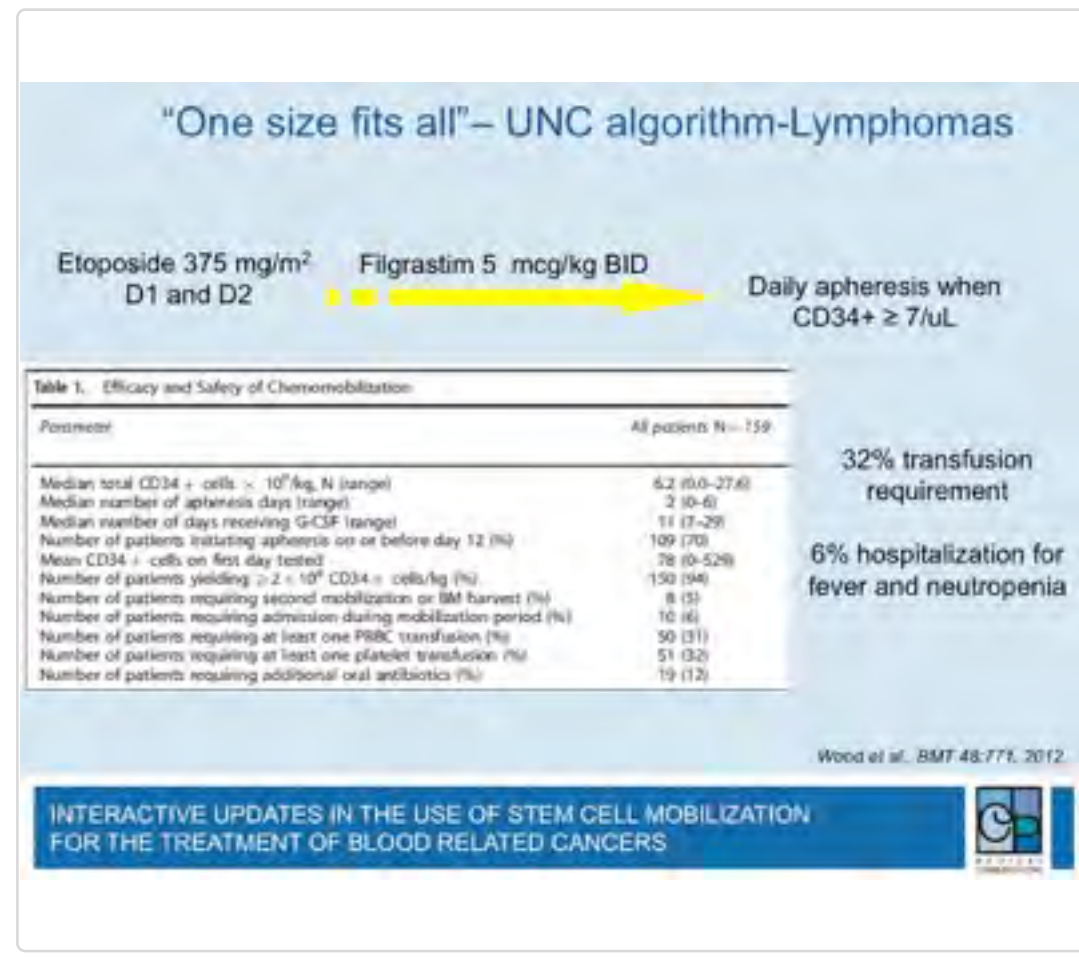
The same authors published their experience with exactly the same algorithm, but this time in pa-

Interactive 5.8



tients with lymphoma who are notoriously harder to mobilize than patients with multiple myeloma for various reasons. In this study with 159 patients, the median total CD34 cells per kilogram was 6.2. The median number of apheresis days was 2, as opposed to 1 in multiple myeloma. There were very few mobi-

Interactive 5.9

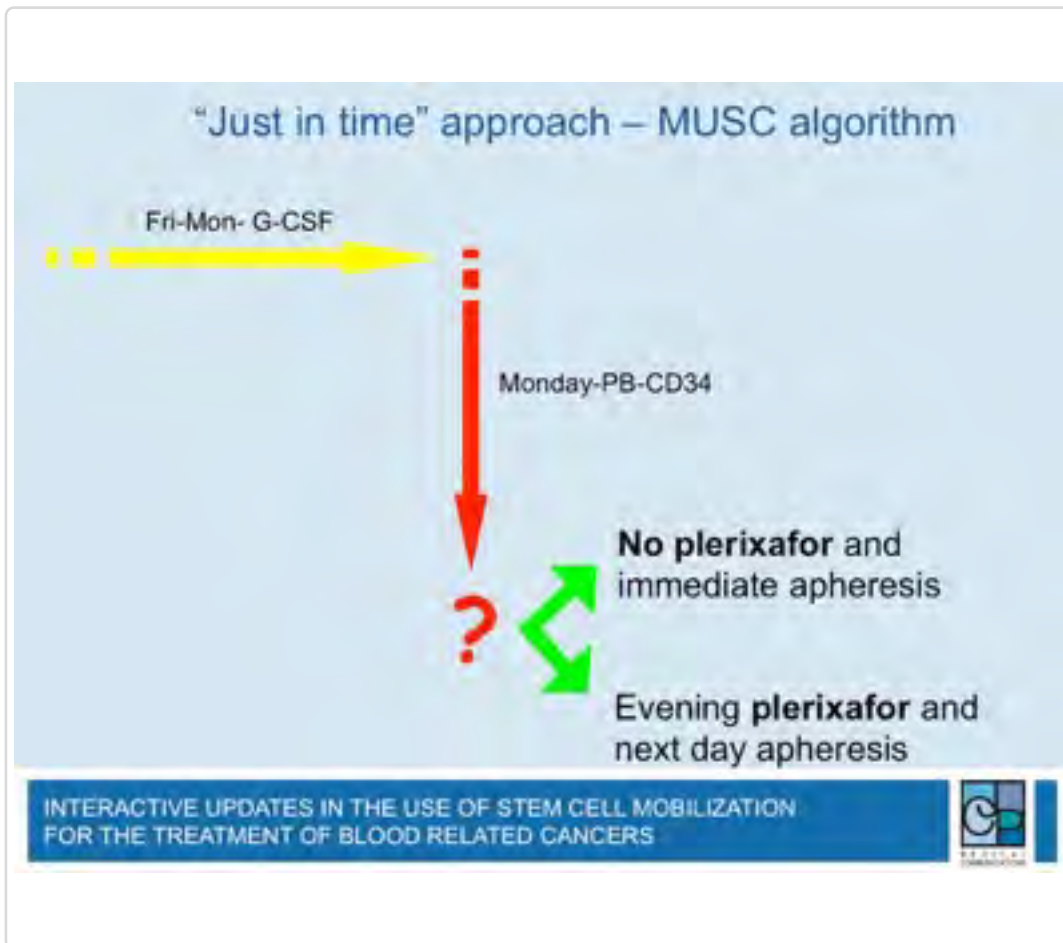


We will now discuss an algorithm with a “just in time” approach, which is an algorithm we developed while at the Medical University of South Carolina. Simply put, it consists of four days of filgrastim administration, very similar to how it was done in the Phase III trials for plerixafor, but instead of administering plerixafor on day four, what is done is the enumeration of peripheral blood CD34. If the patient is below a certain threshold then no apheresis is started, the patient receives plerixafor and starts collection on the subsequent day. However, if the CD34 in the peripheral blood is above a certain threshold, then apheresis is started on the same day. (**Interactive 5.10**)

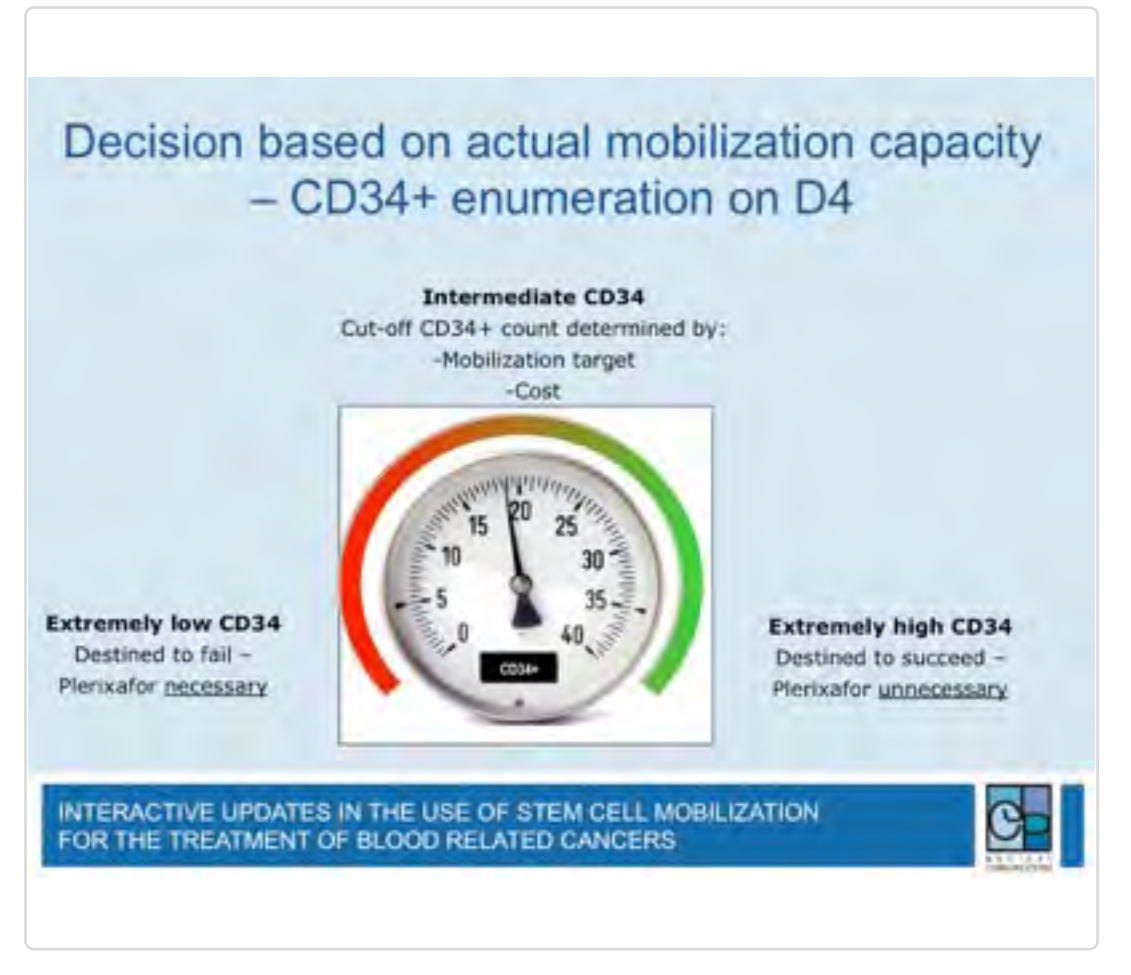
The biggest challenge with that approach was where to set the threshold. Many centers opted to arbitrarily set the threshold at 10 or 20 CD34/uL in similar outcomes. What we did was to integrate a few concepts into that decision, particularly the differences between mobilization targets for different patients, and importantly, the costs implicated in one approach versus the other. (**Interactive 5.11**)

lization failures, with 94% of the patients collecting more than 2×10^6 CD34 cells per kilo, which is considered the minimal necessary for a transplant. Thirty-two percent of the patients required transfusion and 6% required hospitalization due to fever and neutropenia. (**Interactive 5.9**) [2]

Interactive 5.10



Interactive 5.11



The concept here is simple. Patients who have extremely low CD34 counts are destined to fail with growth factor-only mobilization and plerixafor is then necessary. There is little reluctance about giving plerixafor in a patient that, for example, has a CD34 count of 6 or 5 on day four of growth factor.

On the other side, patients with an extremely high CD34 count, for example, a patient with a CD34 count of 50, are going to collect well without any addition of plerixafor, so plerixafor is unnecessary. The challenge is with patients with more intermediate CD34 counts, and essentially the question is where

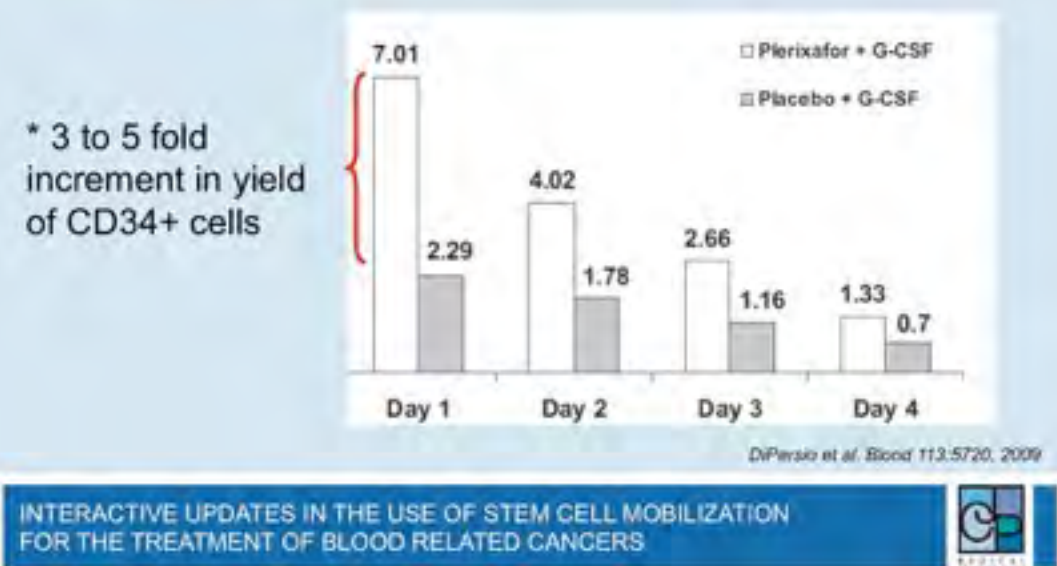
to set the bar. What is the number that will decide who gets plerixafor and who does not?

We acknowledge that that decision was to some extent one of economics, as different approaches might have different cost implications. So we constructed a decision model with certain elements. One of the elements is understanding how many cells we can collect on a patient given a certain CD34 count in the peripheral blood. We did that by retrospectively reviewing 50 cases on our site which showed a sharp correlation between peripheral blood CD34 and yield of collection on that given day. I call this the B-to-B relation – the relationship between what is in the blood and what is going to be in the bag. So by using this formula, the extraction from a linear regression, one can predict what the yield is going to be based on peripheral blood CD34 count. (**Interactive 5.12**) [3]

If that patient, instead of going through collection, receives plerixafor and goes to collection the next day, how many cells will we be able to collect? Here we are able to extrapolate from the literature, essentially going through the Phase III trials where a large pool of patients that had similar characteristics

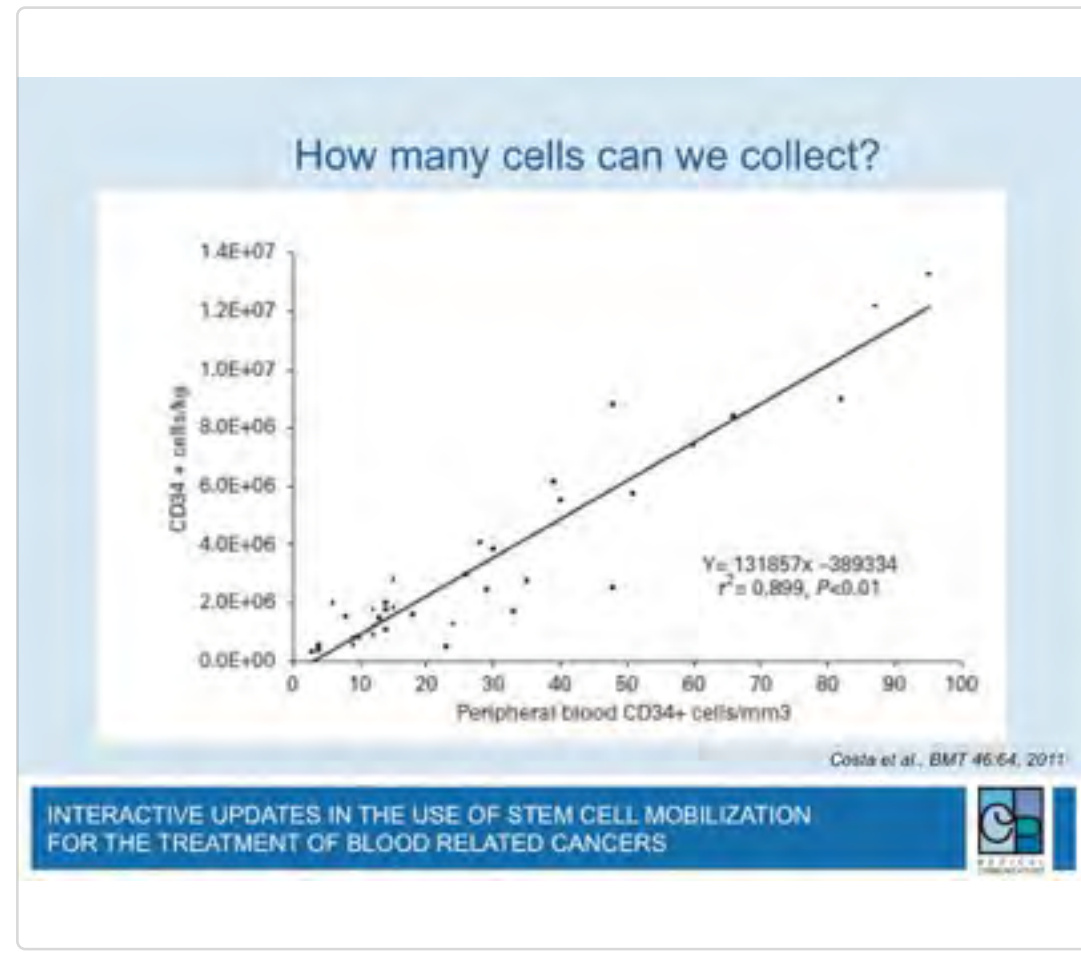
Interactive 5.12

What is the impact of plerixafor on ongoing G-CSF mobilization?



underwent collection on the first day. On the first day of apheresis patients who received plerixafor had a three-to-five fold increment in the yield of CD34 cells collected than patients who continued on growth factor only. (**Interactive 5.13**) [4]

Interactive 5.13

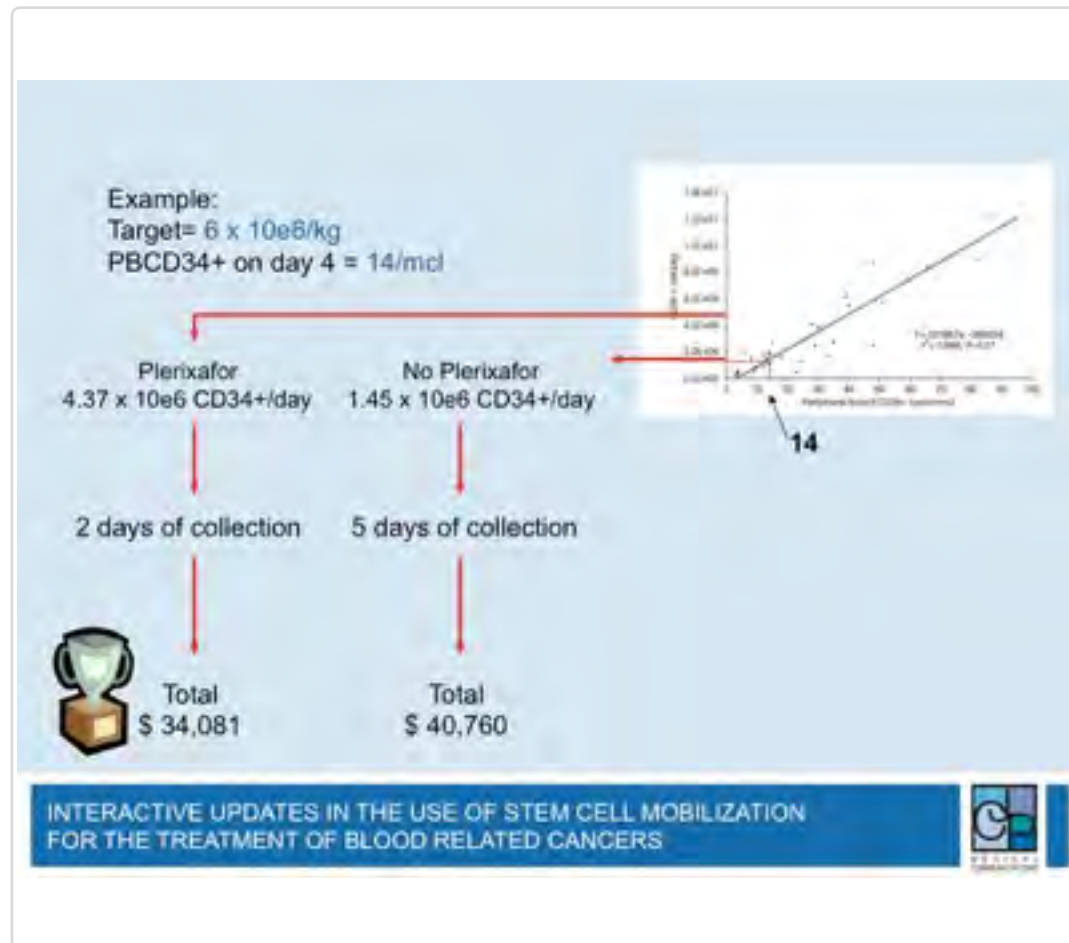


Based on those two pieces of information, we can project how much the patient would collect if he or she went straight to apheresis, versus how much he would collect if he received plerixafor and apheresis the next day. We can also project the cost of each approach. In this theoretical example, a multiple myeloma patient

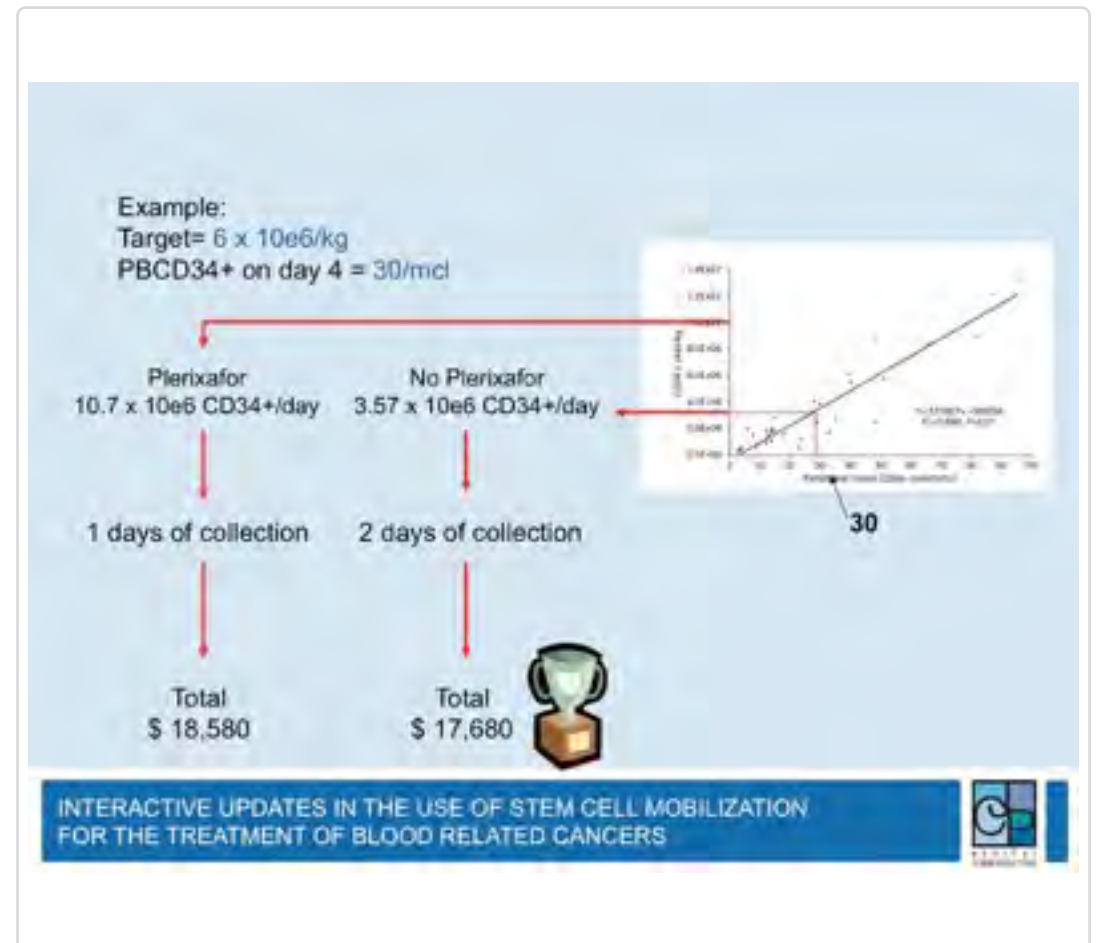
with a target of 6×10^6 per kilo has a CD34 count of 14 on day four. We know if that patient were to undergo collection, we would probably have a yield of 1.45, which means it would take five consecutive days of collection to meet our target. The total cost, when you add growth factor continuation, apheresis and cryopreservation, will be over \$40,000. However, if that patient receives plerixafor and collects the next day, it is projected that he will collect approximately 4.37, which means it will take two days of collection to reach the target of 6×10^6 . In that scenario, the extra cost of two days of plerixafor is more than overshadowed by the cost of three additional days of collection on the other approach, and the total projected cost is \$34,000, making the use of plerixafor in this case, cost effective and making this the winning approach. (**Interactive 5.14**)

This is another example: a patient with the same target, but with 30 cells in the peripheral blood on day four. If the patient does not receive plerixafor, it takes two days of collection with over \$17,000 in projected cost. If the patient does receive plerixafor, it takes one day of collection with a total cost of \$18,000, so in this

Interactive 5.14



Interactive 5.15

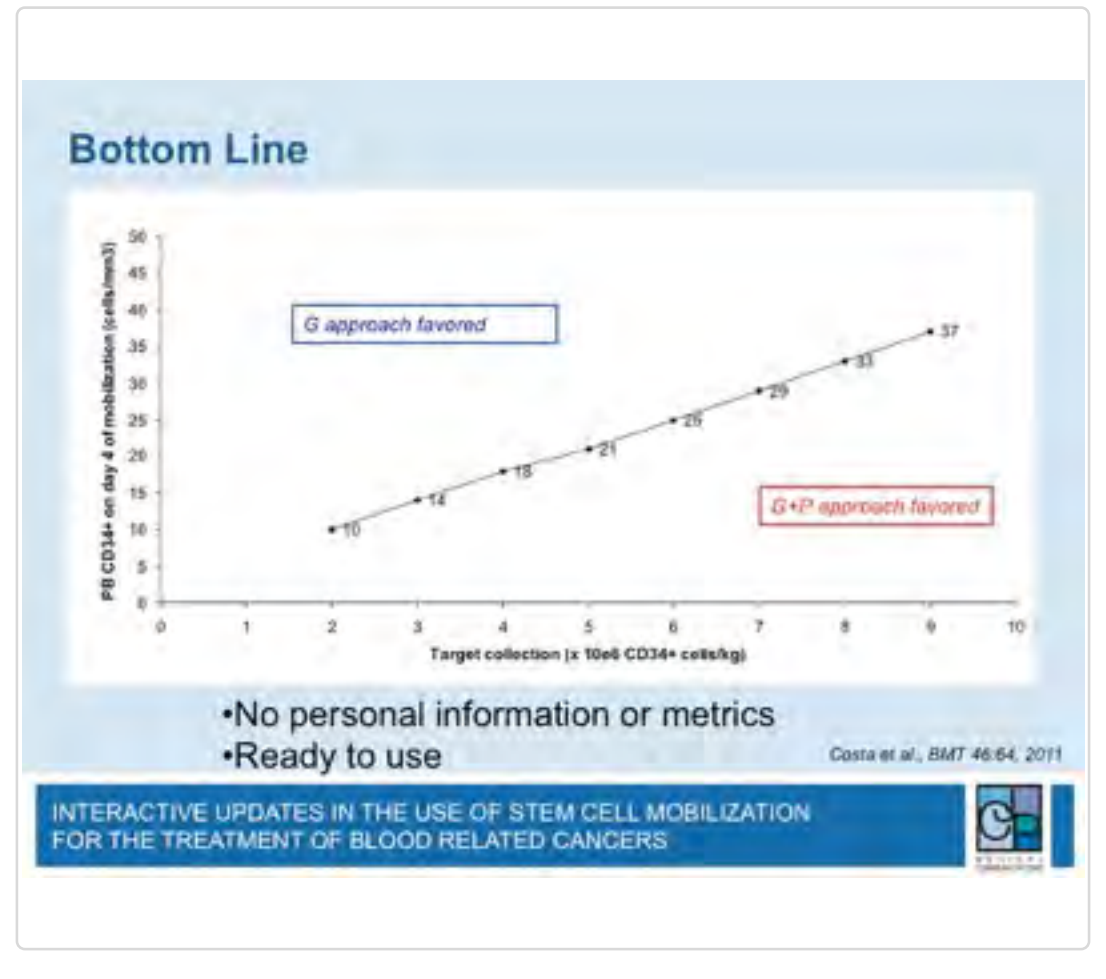


case, the winning and most cost-effective approach is to not use plerixafor. (**Interactive 5.15**)

Evidently there is a lot of calculation that goes into this and it would be totally impractical to do this on a patient-by-patient basis at the bedside. Fortunately, through a series of simulations, one can de-

termine the cutoff or threshold for each individual collection target, above which continuation of growth factor only and immediate apheresis is favored, and below which the addition of plerixafor and starting apheresis on the subsequent day is favored. (**Interactive 5.16**) [3]

Interactive 5.16



For instance, for 6×10^6 , a very common target for patients with multiple myeloma, the threshold is 25 CD34/ul. If you are collecting 3×10^6 CD34/kg for a lymphoma transplant, that threshold becomes 14 CD34/ul. So this information is ready to use. It does not require any personal information or metrics.

The background can be a bit complicated, but the day-by-day use is extremely simple.

When we started this algorithm with slightly over 30 patients we learned that we had a plerixafor use of 68%. Another way to put this is that we spared 32% of the patients from the unnecessary use of plerixafor. The correlation was proven to be very accurate, as well as the calculation of the projected number of apheresis – 94% of the patients met the target and only 1 out of those 37 patients required remobilization, so we were able to proceed to transplant in a short period of time, with a median interval for mobilization to transplant of 14 days. (**Interactive 5.17**) [3]

Other studies have used a similar approach. One well-known approach is that published by the Mayo group with Dr. Micalleff as the first author. Their initial approach was very similar to ours, but they used 10 as an arbitrary threshold. Patients who did not meet 10 after four or five days of growth factor were started on plerixafor and got apheresis the next day. Patients that exceeded 10 started immediate apheresis. In addition, patients who did not receive plerixa-

Interactive 5.17

Algorithm validation

Plerixafor use - 68%

Correlation PBCD34+ and aphaeresis' product- 103% of predicted

Actual number of apheresis sessions – 94% precision

Proportion meeting mobilization target – 94%

Proportion requiring remobilization - 3%

Interval mobilization-transplantation – 14 days

Costa et al., BMT 46:64, 2011

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 5.18

"Just in time" approach – MAYO algorithm



Micallef et al., BMT 19:87, 2013

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS

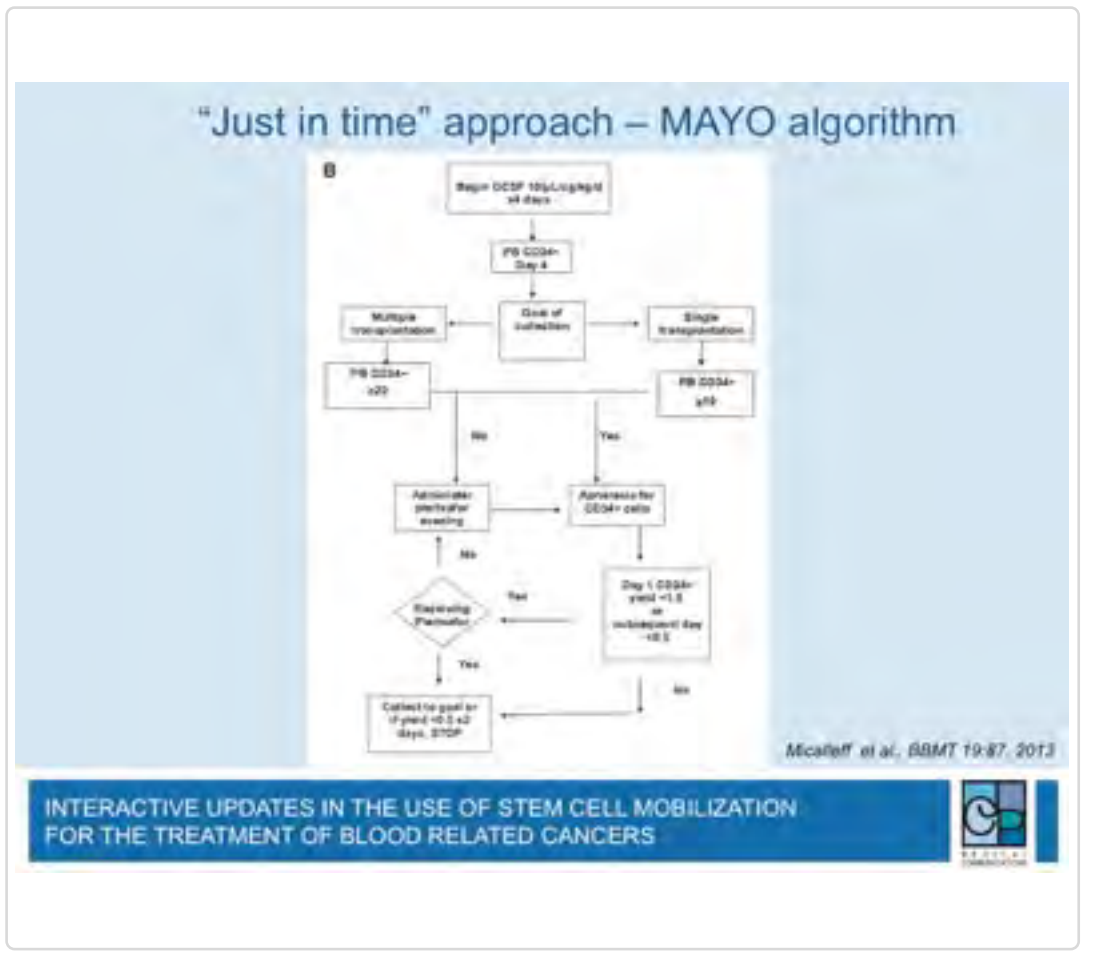


for initially but had a low apheresis yield on the first day had plerixafor added to their regimen and collected the subsequent day. (**Interactive 5.18**)

Later they realized that they would collect with many of those patients who had above 10, for example 12-15, but it would still take several days of collec-

tion, making collection more expensive, particularly with a higher target. So they adapted their protocol in a subsequent study and set the threshold to 20 for patients who are collecting for multiple transplantations, with multiple myeloma patients being the most typical scenario. (**Interactive 5.19**) [5]

Interactive 5.19



This is the comparison of the two approaches called plerixafor-1 and plerixafor-2. Essentially there is greater success, a 1% versus 5% mobilization failure, with more liberal use of plerixafor. The consequence of that is that plerixafor use does increase, and in this case, from 38% to 58%, and so does the

number of CD34 cells collected; here, from 6.1 to 7.8 x 10⁶ per kilogram. (**Interactive 5.20**) [5]

More recently, a few chemotherapy plus “just in time” plerixafor algorithms have been published. I chose to show one of them which was published by an Italian group in the *British Journal of Haematology*. That consisted of administration of cyclophosphamide, which is broadly the most common mobilizing chemotherapy agent used, followed by growth factor, in this case filgrastim. If the patient obtains 20 CD34 per microliter in peripheral blood at any point between days nine and twelve, the patient starts apheresis. If not, if the patient is greater than 10, but less than 20, the patient is rechecked daily until 20 is obtained and apheresis is started. If the patient does not obtain 20 by day sixteen, then the patient starts plerixafor for subsequent collection. If the patient is not on the plerixafor path, but the yield of collection is low, then plerixafor is added. (**Interactive 5.21**) [6]

In this same study they compared the outcomes of that approach to historical data on patients collecting with cyclophosphamide in growth factor mobili-

Interactive 5.20

"Just in time" approach – MAYO algorithm

Patient Cohorts	Plerixafor-1 (n = 215)	Plerixafor-2 (n = 98)	P Value
CD34 collected ($\times 10^6/\text{kg}$)			
Median	8.1	7.8	
Range	0.1-28	1.8-29.3	<.001
Mean	7	8.3	
$\geq 4 \times 10^6/\text{kg}$ (%)	181 (84%)	91 (93%)	<.001
$\geq 2 \times 10^6/\text{kg}$ (%)	205 (95%)	97 (99%)	<.001
Day-1 apheresis yield	2.4 (0.03-28)	3.7 (0.5-29)	<.001
Mobilization failures	10 (5%)	1 (1%)	<.001
Remobilizations	5	1	<.001
Plerixafor use	82 (38%)	57 (58%)	<.001
Indication for plerixafor use			
Day-4 CD34 <10	9	34	
Day-4 CD34 <20	-	10	
Day-5 CD34 <10	33	-	
Day-1 apheresis yield <1.5 $\times 10^6/\text{kg}$	-	10	
Any apheresis yield <0.5 $\times 10^6/\text{kg}$	40	3	
Days of plerixafor			
Median	3	2	.007

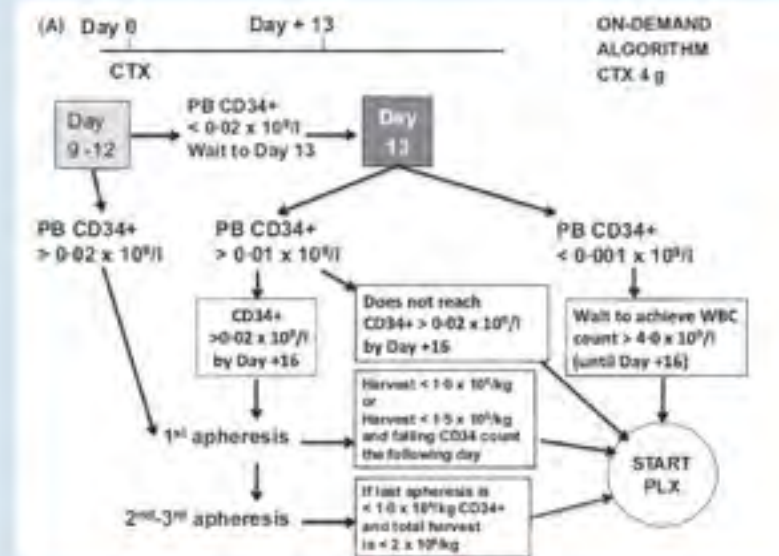
Micallef et al., *SBMT* 19:87, 2013

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 5.21

Chemotherapy + "Just in time" plerixafor



Miano et al., *BJH* 164:113, 2013

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



zation only. What was seen is that the on-demand approach was associated with a very low rate of mobilization failure, about 4%, being lower in myeloma and higher in lymphoma, compared to 15% and 26.5% in a historical group that was bias adjusted. Most importantly, 14.4% of patients required plerixa-

for (21.6% of lymphoma patients and 10.6% of multiple myeloma patients). (**Interactive 5.22**) [6]

Moving on from "just in time" plerixafor to risk-adapted plerixafor. One example of this is from the Moffitt group, where instead of going exclusively on the mobilization capacity, they chose an algorithm

Interactive 5.22

Chemotherapy + “Just in time” plerixafor

	Failure of successful apheresis harvest (CD34+ < 2 × 10 ⁶ /kg)		
	Overall	Myeloma	Lymphoma
On-demand prospective study	4.0% (4/102)	2.8% (2/72)	6.7% (2/30)
Historical controls group (unadjusted)	20.9% (50/240)	16.4% (30/184)	35.8% (20/56)
P	0.0001	0.003	0.003
Historical controls group (bias-adjusted)	17.4% (40/228)	13% (27/180)	26.5% (13/48)
PI	0.0008	0.006	0.02

14.4% of patients required plerixafor (21.6% of lymphomas and 10.6% of MM)

Mallon et al., BJH 164:113, 2013

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 5.23

Risk-adapted – Moffitt algorithm

High risk:

- 3 or more lines of therapy
- 2 lines of therapy + radioimmunconjugate or extensive XRT
- 4 or more cycles of HyperCVAD
- 4 or more cycles of lenalidomide
- Hypocellular bone marrow

Filgrastim 10 mcg/kg/day x 4 → Plerixafor from day 4, Apheresis from day 5

Standard risk:

- All others

Filgrastim 10 mcg/kg/day x 4 → Apheresis from day 5

CD34+ < 10⁶/ul or
Yield < 0.5 × 10⁶ CD34/kg

Add Plerixafor

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



that plans for plerixafor if the patient has a perceived high risk of mobilization failure. (**Interactive 5.23**)

The criteria used are: three or more lines of therapy; two lines of therapy with radioimmunoconjugate or extensive radiation; four or more cycles of hyperCVAD, four or more cycles of lenalidomide; or

hypocellular bone marrow. Other remaining patients receive straight mobilization with a growth factor, but if the patients have a lower yield, then plerixafor is added. The results of such an approach have not been compared with “just in time” use or with straight use of growth factor.

But the real question here is, can we clinically predict which patients will be poor mobilizers? There are multiple risk factors for poor mobilization, but the question remains, can clinical characteristics be effectively used for risk-stratification and predict who is going to be a poor mobilizer? (**Interactive 5.24**) [7]

A few years back we asked this question by combining 477 multiple myeloma patients undergoing growth factor mobilization from two institutions, the Mayo Clinic and the Medical University of South Carolina. We performed a multivariate linear regression analysis to indicate which factors are associated with poor mobilization using the CD34 on day four of growth factor as the readout for mobilization. Interestingly enough, the factors that were associated with mobilization were age; the duration of lenalidomide therapy; the platelet count prior to mobilization, likely a surrogate of bone marrow reserve; and the type of growth factor. Some of the patients received pegfilgrastim for mobilization, instead of filgrastim, and that affected the mobilization. Some

Interactive 5.24

But can clinical characteristics be effectively used for risk-stratification?

477 MM patients in 2 institution undergoing growth factor mobilization

Clinical Variables Associated with PB-CD34+ in the Training Cohort

	Univariate Linear Regression			Multiple Regression		
	Beta	95% CI	P	Beta	95% CI	P
Age (per year)	-.379	-.577 to -.182	<.001	-.332	-.529 to -.135	.011
Gender (male)	.990	-2.892 to 4.872	.62			
Duration of prior lenalidomide therapy	-1.396	-2.167 to -.625	<.001	-1.80	-2.96 to -.64	.003
% PC in the bone marrow	-.112	-.257 to .033	.33			
Platelet count (per 1,000/mm ³)	.066	.045 to .087	<.001	.066	.034 to .099	<.001
Interval from diagnosis to mobilization (per month)	-.059	-.158 to .039	.37			
Growth factor*	11.881	7.029 to 16.832	<.001	9.72	3.06 to 16.38	.004
Institution	-4.570	-10.665 to 1.525	.341			

Costa et al., *BBMT* 20:222, 2014

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



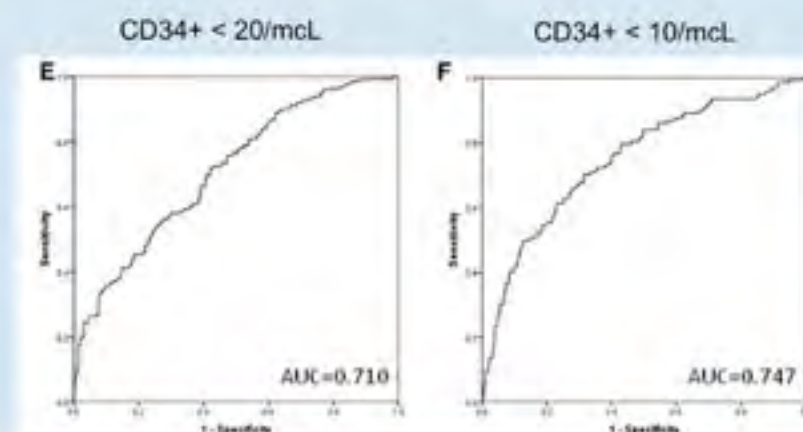
other factors that are often used by clinicians to stratify patients according to perceived risk of mobilization failure did not prevail in the multivariate analysis, particularly the percentage of plasma cells in the bone marrow.

Using the results of the multivariate analysis, we built a score to predict which patients would fail to reach 20 cells or which patients would fail to reach 10 CD34 cells after four days of growth factor. Essentially what you have is two receiver operating characteristic curves with a very low AUC of 0.71 and 0.74. The message here is that even if you use the best available model to factor in all the meaningful clinical characteristics and try to predict who is not going to collect properly, that prediction is still imperfect. (**Interactive 5.25**) [7]

So, any criteria you choose to say who gets one mobilization approach versus another would have either very low specificity or very low sensitivity, which translates into overtreatment of patients who are predicted to be poor mobilizers but would actually mobilize well, or mobilization failures on patients predicted to be good mobilizers, but who end up not mobilizing accurately. Essentially, we are making a point for the use of actual mobilization capacity with, for example, “just in time” use of

Interactive 5.25

But can clinical characteristics be effectively used for risk-stratification?



NO- and it will lead to mobilization failures and/or overtreatment

Costa et al., BMT 20:222, 2014

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



plerixafor, versus risk-stratification based on clinical characteristics.

In summary, some advice for developing and adopting mobilization algorithms. It is important to understand your site's peculiarities: what resources are available, what are the characteristics of your

Interactive 5.26

Developing and adopting a mobilization algorithm

- ✓ Understand your site's peculiarities
 - Resources available
 - Patient characteristics
 - Payment structure
- ✓ Define priorities
- ✓ Be fact-driven
- ✓ Educate
- ✓ Enforce
- ✓ Analyze
- ✓ Amend

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



portant that everyone involved in mobilization understands and embraces the process. Enforce by education and by performing audits. Understand how your process is actually performing. Lastly, amend if you notice that the performance is not what you expect. Amend your process and reanalyze, so you can get a mobilization algorithm that is more appropriate for your site, more appropriate for your patients, and finally one that is the most cost effective.

patients, and what is the payment structure, as these might influence what mobilization technique is used. (**Interactive 5.26**)

In addition, define priorities and be fact-driven, particularly the facts that can be learned from your own site's experience, and educate the staff. It is im-

REFERENCES

1. Wood WA, Whitley J, Moore D, et al. Chemomobilization with Etoposide is Highly Effective in Patients with Multiple Myeloma and Overcomes the Effects of Age and Prior Therapy. *Biol Blood Marrow Transplant*. 2011 Jan;17(1):141-6. <http://www.ncbi.nlm.nih.gov/pubmed/20637882>
2. Wood WA, Whitley J, Goyal R, et al. Effectiveness of etoposide chemomobilization in lymphoma patients undergoing auto-SCT. *Bone Marrow Transplant*. 2013 Jun;48(6):771-6. <http://www.ncbi.nlm.nih.gov/pubmed/23165501>
3. Costa LJ, Alexander ET, Hogan KR, et al. Development and validation of a decision-making algorithm to guide the use of plerixafor for autologous hematopoietic stem cell mobilization. *Bone Marrow Transplant*. 2011 Jan;46(1):64-9. <http://www.ncbi.nlm.nih.gov/pubmed/20383210>
4. DiPersio JF, Stadtmauer EA, Nademanee A, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. *Blood*. 2009 Jun 4;113(23):5720-6. <http://www.ncbi.nlm.nih.gov/pubmed/19363221>
5. Micallef IN, Sinha S, Gastineau DA, et al. Cost-effectiveness analysis of a risk-adapted algorithm of plerixafor use for autologous peripheral blood stem cell mobilization. *Biol Blood Marrow Transplant*. 2013 Jan;19(1):87-93. <http://www.ncbi.nlm.nih.gov/pubmed/22922211>
6. Milone G, Martino M, Spadaro A, et al. Plerixafor on-demand combined with chemotherapy and granulocyte colony-stimulating factor: significant improvement in peripheral blood stem cells mobilization and harvest with no increase in costs. *Br J Haematol*. 2014 Jan;164(1):113-23. <http://www.ncbi.nlm.nih.gov/pubmed/24138497>
7. Costa LJ, Nista EJ, Buadi FK, et al. Prediction of poor mobilization of autologous CD34+ cells with growth factor in multiple myeloma patients: implications for risk-stratification. *Biol Blood Marrow Transplant*. 2014 Feb;20(2):222-8. <http://www.ncbi.nlm.nih.gov/pubmed/24211319>



Chapter 6

Pharmacoeconomics of Stem Cell Mobilization Strategies

Luciano J. Costa, MD, PhD



Luciano J. Costa, MD, PhD

Associate Professor of Medicine
University of Alabama at Birmingham
Birmingham, Alabama

Luciano J. Costa obtained an MD and PhD at Universidade de Sao Paulo in Brazil where he also trained in Internal Medicine and Hematology. He subsequently completed fellowship in Hematology and Oncology (University of Colorado) and Blood and Marrow Transplantation (Mayo Clinic, Rochester).

Dr. Costa is currently an Associate Professor of Medicine and medical director of ambulatory care and the cell therapy collection facility for the UAB Blood and Marrow Transplant Program. His clinical research includes transplant and non-transplant management of lymphoproliferative and plasma cell disorders. He has also been deeply engaged in strategies for optimization of hematopoietic progenitor cell mobilization and in population outcomes of blood cancers.

Movie 6.1

PHARMACOECONOMICS OF STEM CELL MOBILIZATION STRATEGIES

Luciano J. Costa, MD, PhD
Associate Professor of Medicine
University of Alabama at Birmingham

Pharmacoeconomics of Stem Cell Mobilization Strategies

Luciano J. Costa, MD, PhD

Interactive 6.1

PHARMACOECONOMICS OF STEM CELL MOBILIZATION STRATEGIES

Luciano J. Costa, MD, PhD
Associate Professor of Medicine
University of Alabama at Birmingham

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS

My name is Luciano Costa, and I am an Associate Professor of Medicine at the University of Alabama at Birmingham, where I also serve as medical director for the apheresis collection facility. Today I will discuss the pharmacoeconomics of stem cell mobilization strategies. (**Interactive 6.1**)

The cost of mobilization is a substantial fraction of transplant costs, and in some series, it accounts for up to \$52,000 in costs. Multiple options exist for mobilization, particularly of autologous donors, making pharmacoeconomics even more relevant. Essentially, if you have multiple strategies that provide similar

clinical outcomes, the relative cost of each strategy might become the defining factor when choosing how to mobilize patients. (**Interactive 6.2**)

As we analyze pharmacoeconomics, it is important to keep in mind that remobilization sharply increases mobilization costs and it is often not accounted for in a pharmacoeconomic analysis comparing different mobilization strategies. We also need to keep in mind the burden of mobilization failure. And I like to say that the least cost-effective transplant is the one that never happens.

As we analyze cost effectiveness in healthcare we often use the concept of incremental cost-effectiveness ratio, which is the difference in cost between two strategies vs. the difference in quality adjusted life-years. The goal of mobilization is to obtain cells for transplant. The transplant itself is intended to be a curative strategy or to improve survival of the patient. If we have mobilization failure, there is no transplant, and therefore no gain in quality adjusted life-years. Therefore, the denominator of this equation is zero, making all the cost spent in mobilization

Interactive 6.2

Pharmacoeconomics of Mobilization

- ✓ Mobilization as substantial fraction of transplant cost
- ✓ Up to US\$52,000
- ✓ Multiple options makes pharmacoeconomics more relevant
- ✓ Remobilization sharply increases mobilization cost
- ✓ Burden of mobilization failure: "The least cost-effective transplant is the one that never happens"

$$ICER = \frac{\boxed{\text{cost}}}{\boxed{\text{QALY}}}$$

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



wasted cost from a societal standpoint. For transplant centers, most of the financial income comes from actually performing transplants. Therefore, it is not in the best interest of anyone to have mobilization failures.

There are several components of pharmacoeconomics in mobilization. The one that gets the

most attention is the one that is easiest to identify – the cost of mobilization agents, simply because there is a clear price tag attached. It is easy to find out how much a vial of a certain drug costs. (**Interactive 6.3**)

However, there are other less discussed costs that are also important, such as the costs of supportive care which include the cost of transfusions and antimicrobials. There is also the cost of monitoring, including associated multiple hospital visits and the necessity for multiple blood tests. There is the cost of hospitalizations for complications, which is particularly important in the case of chemomobilization, where patients often develop fever and neutropenia requiring IV antibiotics.

Then there is the cost of apheresis itself, which is often hard to identify in the hospital setting, but which is certainly not free. Included in that service is the cost of the personnel operating the machine, the cost of the machine and the kit that goes into the machine and the cost of maintaining the apheresis facility. There is also the cost of cryopreservation, so a strategy that takes multiple days of collection will have multiple days of apheresis and cryopreservation.

Interactive 6.3

Components of Pharmacoeconomics

- ✓ Mobilizing agents
- ✓ Supportive care (transfusion, antimicrobials)
- ✓ Monitoring
- ✓ Hospitalization for complications
- ✓ Apheresis
- ✓ Cryopreservation

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



We will now review the limitations of pharmacoeconomic studies. Often charge is used as a surrogate for cost as cost may be difficult to estimate. Cost and payment structure are highly variable among centers or patients in the same center, depending on what payer is associated with that pa-

Interactive 6.4

Limitations of Pharmacoeconomics Studies

- ✓ Charge often a surrogate of cost.
- ✓ Cost and payment structure highly variable between centers or patients in the same center.
- ✓ Payer vs. transplant center vs. patient's perspective.

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



tient. Also, cost can be seen from multiple points of view, that of society at large, the payer, the transplant center, and the patient. A strategy that might be more cost beneficial to the payer might not be the most cost effective for the transplant center, or vice versa. (**Interactive 6.4**)

Here we will review pharmacoeconomic comparisons of different mobilization strategies. These include chemomobilization with filgrastim plus plerixafor; chemomobilization vs. filgrastim plus “just in time” plerixafor; upfront filgrastim plus plerixafor vs. filgrastim plus “just in time” plerixafor; filgrastim vs. pegfilgrastim; and lastly TBO-filgrastim versus filgrastim. (**Interactive 6.5**)

An excellent and complete pharmacoeconomic analysis is one published by Paul Shaughnessy and colleagues. They performed a retrospective study of 33 patients who participated in the expanded access program (EAP) of plerixafor and G-CSF for initial mobilization of CD34⁺ cells, and compared outcomes to 33 matched controls mobilized with cyclophosphamide and G-CSF at 2 centers that participated in the EAP. The study analyzed outcomes of mobilization and costs. (**Interactive 6.6**) [1]

In this series, 100% of the patients were able to collect 2×10^6 CD34⁺ cells and proceed to transplant. Therefore there were no mobilization failures. The number of patients who collected 5 million cells,

Interactive 6.5

Pharmacoeconomic comparisons

- ✓ Chemomobilization vs. Filgrastim+P
- ✓ Chemomobilization vs Filgrastim + "just in time" P
- ✓ Upfront Filgrastim+P vs. Filgrastim + "just in time" P
- ✓ Filgrastim vs. Pegfilgrastin
- ✓ TBO filgrastim vs. Filgrastim

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 6.6

Filgrastim+P vs. Cy+ Filgrastim

	Plerixafor+G-CSF n = 33	Chemo+G-CSF n = 33	P Value
Median total CD34 ⁺ cells $\times 10^4/\text{kg}$ (range)	10.7 (3.5-37.9)	11.6 (2.1-49.3)	.5
Number of patients collecting $\geq 2 \times 10^6$ CD34 ⁺ cells/kg (%)	33 (100%)	33 (100%)	—
Number of patients collecting $\geq 5 \times 10^6$ CD34 ⁺ cells/kg (%)	31 (94%)	25 (76%)	.04
Number of HH patients collecting $\geq 3 \times 10^6$ CD34 ⁺ cells/kg (%)	13/13 (100%)	11/13 (85%)	.14
Number of HH patients collecting $\geq 5 \times 10^6$ CD34 ⁺ cells/kg (%)	20/20 (100%)	18/20 (90%)	.49
Median number of apheresis days (range)	1 (1-4)	1 (1-4)	.45
Number of patients initiating apheresis on scheduled day (%)	33 (100%)	29 (88%)	.04
Number of patients requiring weekend apheresis (%)	0	16 (48%)	$\leq .0001$
Total number of weekend apheresis procedures	0	19	$\leq .0001$

	Plerixafor+G-CSF n = 33	Chemo+G-CSF n = 33	P Value
Median G-CSF mcg dose/day (range)	700 (400-1440)	900 (400-1260)	.82
Median total number of G-CSF doses (range)	5 (4-8)	10 (6-17)	$\leq .0001$
Median plerixafor dose mg/day (range)	16.8 (3.3-24.4)	—	—
Median number of plerixafor doses (range)	1 (1-4)	—	—
Total number of mobilization-related hospitalizations (%)	0	19 (58)	$\leq .0001$
Median days of stay for mobilization-related hospitalization	0	1 (0-2)	$\leq .0001$
Total number of patients who received intrusions during mobilization (%)	0	4 (12)	.06

Shaughnessy et al. BBMT 17:729, 2011

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



however, was higher in the plerixafor group, and in that group there were no patients who required apheresis on the weekend. Sixteen (48%) control patients required weekend apheresis. There was also variation on what day apheresis was started, with

12% of the patients on chemomobilization not starting apheresis on the intended day.

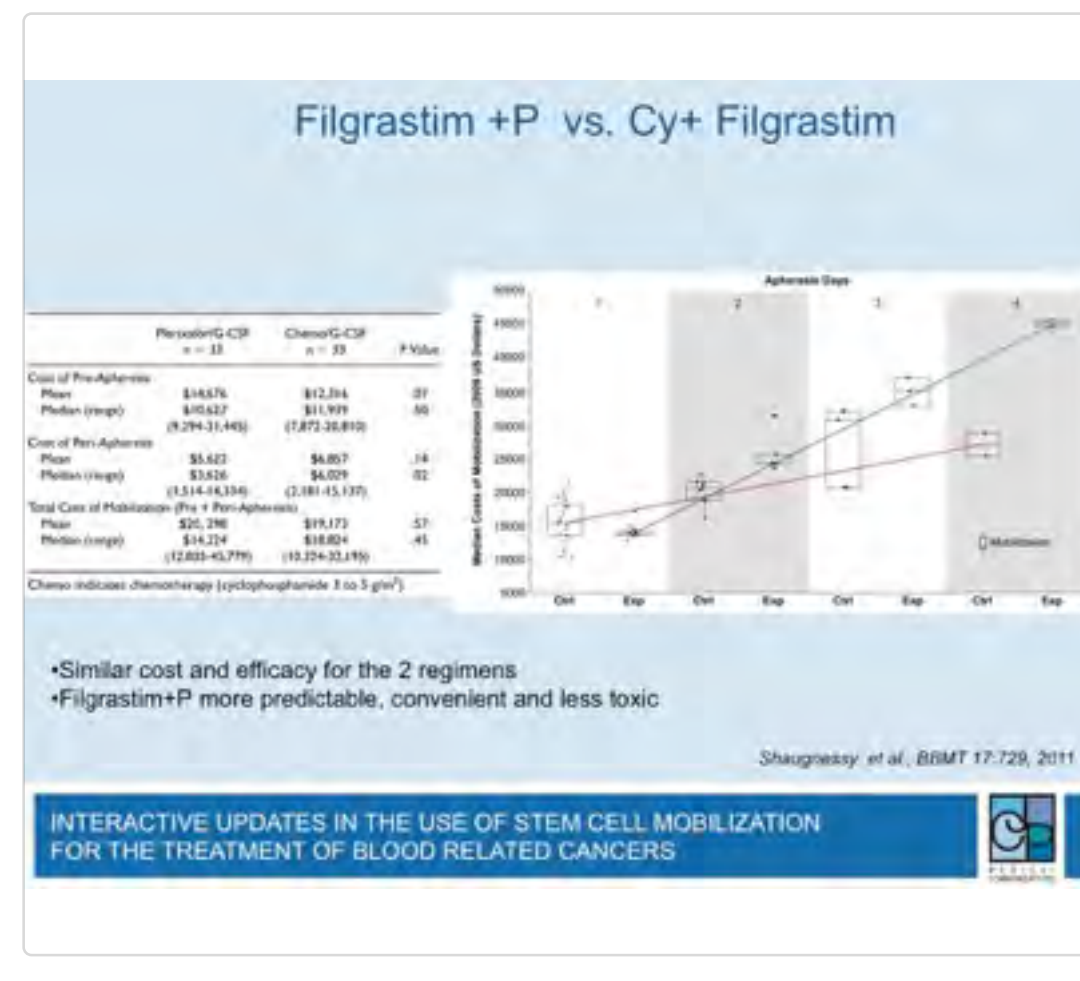
Not surprisingly, there was a higher use of growth factor in the chemotherapy plus growth factor group, ten vs. five doses. More importantly, over half of patients on chemomobilization required hos-

pitalization. Twelve percent of chemotherapy plus growth factor group required transfusions vs. 0% in the plerixafor plus growth factor group.

In this study, cost was divided into two categories, pre-apheresis, which is the cost of mobilization, and peri-apheresis, which is the cost of collection. The pre-apheresis cost was similar between the two groups. Some differences were seen in the peri-apheresis costs, with higher cost associated with the chemo/growth factor, probably reflecting the need for additional days of apheresis. Overall, the costs were very comparable – the mean cost of \$20,000 for plerixafor plus growth factor vs. \$19,000 for chemo plus growth factor. (**Interactive 6.7**) [1]

The pane on the right shows that the cost relationship is different depending on how many days of apheresis the patient requires. For patients who require only one day, the plerixafor approach seems more efficient, but as the patient requires additional days of apheresis, the relationships tend to invert as the cost of plerixafor escalates. In summary, there is similar cost and efficacy between the two regimens,

Interactive 6.7



but the filgrastim plus plerixafor regimen was more predictable, convenient, and less toxic.

While at University of South Carolina we performed a retrospective comparison between our mobilization algorithm with filgrastim and “just in time” plerixafor in 50 patients vs. a historical series

Interactive 6.8

Cy+ Filgrastim vs. Filgrastim + "JIT" P

Table 1 Characteristics of the patients

Characteristic	MA (N=36)	CY (N=45)	P
Disease			
Multiple myeloma	32 (64%)	33 (41%)	0.01
Lymphoma	18 (56%)	48 (59%)	
Age			
≤40	28 (56%)	49 (60%)	0.6
>40	22 (44%)	32 (40%)	
Gender			
Male	24 (48%)	42 (52%)	0.7
Female	26 (52%)	39 (48%)	
Prior lines of therapy			
Lymphomas			
≤2	16 (89%)	40 (83%)	0.3
>2	2 (11%)	8 (17%)	
Myeloma			
1	17 (53%)	13 (39%)	0.3
>1	15 (47%)	20 (61%)	
Prior lenalidomide	19 (59%)	7 (21%)	0.02

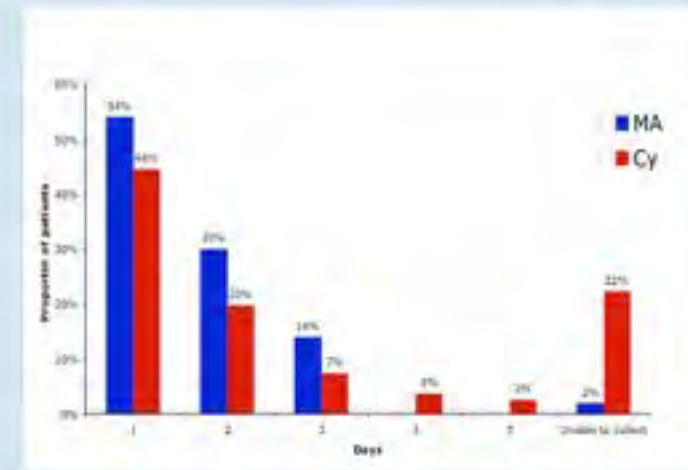
Costa et al., BMT 46:523, 2011

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 6.9

Cy+ Filgrastim vs. Filgrastim + "JIT" P



Number of apheresis sessions

Costa et al., BMT 46:523, 2011

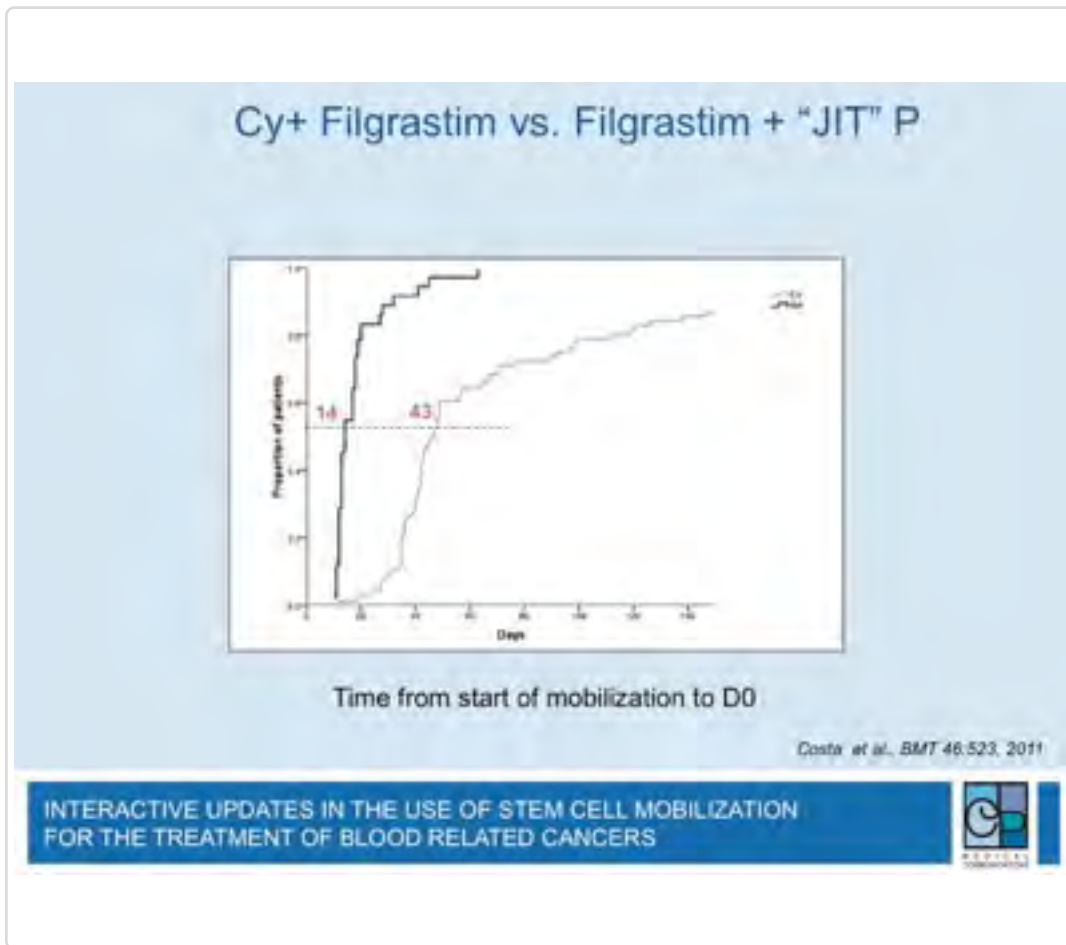
INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



in our center of 81 patients undergoing chemomobilization with 2 g/m² of cyclophosphamide. Populations were comparable, with the important difference being a higher proportion of multiple myeloma patients in the mobilization algorithm cohort. (**Interactive 6.8**) [2]

What was seen was that there was a higher proportion of patients completing collection in one and two days in filgrastim plus “just in time” plerixafor. But most importantly, 22% of the patients were unable to collect and did not undergo any apheresis in the cyclophosphamide group. (**Interactive 6.9**) [2]

Interactive 6.10



When you look at time, from the beginning of mobilization to day zero of transplant, you see a remarkable difference. It took a median of 14 days with filgrastim plus “just in time” plerixafor vs. 43 days when patients underwent chemomobilization with filgrastim. (**Interactive 6.10**) [2]

All but one patient with filgrastim and “just in time” plerixafor successfully completed mobilization in the sense of collection vs. only 77.8% with cyclophosphamide. Only one patient required hospitalization with filgrastim and “just in time” plerixafor vs. 30% of patients receiving chemomobilization. The estimated cost per patient that successfully completed mobilization was higher with chemomobilization, even without including the costs associated with subsequent mobilization attempts. (**Interactive 6.11**) [2]

To date, there is only one publication that I’m aware of that compares upfront filgrastim plus plerixafor, just as utilized on the Phase III trials that led to the approval of plerixafor vs. a “just in time” approach, where filgrastim is given for 4 days and plerixafor is added according to CD34⁺ mobilization into peripheral blood. (**Interactive 6.12**) [3]

In this series from University of West Virginia, upfront plerixafor was used for all patients up to a certain point. After which a transition was made to “just in time” plerixafor, with plerixafor being utilized only for patients who had a CD34⁺ count of less

Interactive 6.11

Cy+ Filgrastim vs. Filgrastim + "JIT" P

- ✓ All but one patient in MA (98%) successfully completed mobilization and stem cell collection vs. only 63 (77.8%) in the CY group ($P < .01$)
- ✓ Only one patient (2%) in MA and 24 (30%) patients in CY had complications requiring hospitalization during mobilization ($P < .01$)
- ✓ The estimated average cost per patient successfully completing mobilization:
 - MA - US\$ 23,893.28
 - CY - US\$ 29,423.31 (not including costs associated with subsequent mobilization attempts)

Corda et al., BMT 48:523, 2011

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 6.12

Filgrastim+P vs. Filgrastim + "JIT" P

Baseline characteristics	Routine G-CSF + plerixafor (n = 78)	JIT-P (n = 60)	P
Disease histology			0.23
Myeloma	45 (58%)	30 (50%)	
Lymphoma	31 (41%)	30 (50%)	
DLBCL	11 (35%)	9 (30%)	
tHL	9 (30%)	7 (23%)	
MCL	4 (13%)	9 (30%)	
FL	1 (3%)	1 (3%)	
Other*	6 (19%)	1 (3%)	
Median age, years (range)	61 (23–78)	59 (22–75)	0.48
Male sex	42 (55.3%)	34 (56.7%)	1.0
Race, Caucasian	74 (97.8%)	59 (98.3%)	1.0
Previous radiation	14 (18.4%)	12 (20%)	0.83
Lines of previous therapy, median (range)	1.6/1 (1–4)	1.8/2 (1–4)	0.25
Bone marrow cellularity, mean/median (range)	40/41 (10–90)	40/41 (5–95)	0.87
Pre-transplantation status			
Myeloma			0.83
CR/VGPR	28 (61%)	16 (53%)	
PR	18 (39%)	13 (43%)	
SD	0	1 (3%)	
Lymphoma			0.48
CR	20 (67%)	18 (60%)	
PR	9 (30%)	11 (37%)	
SD/BD	1 (3%)	1 (3%)	
KPS median (range)	80 (70–100)	80 (60–100)	0.64
HCT CI median (range)	2 (0–6)	2 (0–6)	0.39

Veltri L et al., Cytotherapy, 2015 (Epub)

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



than 10. This table shows that the two cohorts were comparable, without any major differences in disease characteristics or demographics.

Not surprisingly, the cohort that had plerixafor for all patients reached a higher median peak of CD34⁺. There were more cells collected on the first day of

apheresis, and there were more cells collected in how the apheresis performed. However, the portion of patients with mobilization failure was very similar, 5.3% with routine up-front G+B vs. 3.3% with “just in time” plerixafor. (**Interactive 6.13**) [3]

Interactive 6.13

Filgrastim+P vs. Filgrastim + "JIT" P			
Mobilization and apheresis results	Routine G+P (n = 70)	JIT-P (n = 90)	P
Peak PB CD34+ cell count (x10 ⁶ /L), mean/median (range)	77,541.5 (5-180)	13,129 (7-401)	<0.001
CD34+ cells > 10 ⁶ /kg collected on day 1, mean/median (range)	8,329 (0.2-14.4)	2,42.1 (0.4-6.7)	0.001
Total CD34+ cells > 10 ⁶ /kg collected, mean/median (range)	6,779.8 (0.2-22.2)	4,84.5 (1.7-8.5)	0.02
Total no. of apheresis sessions, mean/median (range)	2.22 (1-4)	2.42 (1-4)	0.06
Mobilization failure, n (%)	4 (5.7%)	2 (2.2%)	0.89
Patients requiring >1 apheresis sessions, n (%)	41 (60.3%)	54 (60%)	0.34
Patients requiring >2 apheresis sessions, n (%)	22 (38.9%)	27 (38.9%)	0.53

	Routine G+P (2014 USD)	JIT-P (2014 USD)	P
Average apheresis cost	9,292.09	10,336.82	0.68
Day 4 CD34+ flow cytometry, mean	Not performed	89.44	NA
Average G-CSF cost	4,460.34	4,686.11	0.01
Average plerixafor cost	13,766.77	8,449.26	<0.0001
Average total mobilization costs	27,513.29	23,380.44	0.01

Valeri L. et al. Cytotherapy, 2015 (Epub)

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

The cost analysis showed slightly higher costs for “just in time” plerixafor in terms of the cost of apheresis, which likely reflected a few patients who required additional apheresis they would not have otherwise required had they received plerixafor. However, that difference was not enough to over-

come the difference in cost of plerixafor, which was over \$13,000 for routine use vs. over \$8,000 for “just in time”. Overall, the average total mobilization cost was \$27,000 for routine use versus \$23,000 with “just in time”, illustrating that the “just in time” approach is likely more cost-effective.

Also while at the Medical University of South Carolina, we experienced a transition in growth factor, going from filgrastim with the dose of 10 mcg/kg/day, to an approach where we used a one-time flat dose of peg-filgrastim of 12 mg and utilized the same criteria of day four CD34⁺ count to add or not add plerixafor prior to collection on the subsequent day. (**Interactive 6.14**) [4]

We retrospectively compared our experience with both agents and found that the two cohorts were essentially very similar. A comparison of the two growth factors, the day four peripheral blood CD34⁺, which should be a direct readout of the growth factor mobilization capacity since this count happens before any plerixafor is given, was higher with pegfilgrastim at 28.7 than it was with filgrastim at 18.1. That resulted

Interactive 6.14

Characteristic	FIL N=74	PEG N=57	P
Diagnosis			
Multiple Myeloma	47 (64.5%)	38 (66.7%)	0.8
Lymphoma	27 (35.5%)	19 (33.3%)	
Age (year)	57.5 (+/- 11.9)	53.7 (+/- 12.1)	0.5
Weight (kg)	82.9 (+/- 18.9)	83.3 (+/- 16.4)	0.6
Gender			
Male	40 (54.1%)	30 (52.6%)	0.8
Female	34 (45.9%)	27 (47.4%)	
Prior lines of therapy- Lymphoma			
≤2	24 (88.9%)	15 (78.9%)	0.6
>2	3 (11.1%)	4 (21.1%)	
Prior lines of therapy- Myeloma			
≤1	29 (88.7%)	34 (89.5%)	0.005
>1	18 (38.3%)	4 (10.5%)	
Prior lenalidomide	30 (63.8%)	19 (50%)	0.3

Costa et al., Transfusion 52:2375, 2012

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

Interactive 6.15

Characteristic	FIL N=74	PEG N=57	P
Day 4 PB-CD34	18.1 (+/- 19.5)	28.7 (+/- 27)	0.01
Patients requiring plerixafor	50 (67.5%)	26 (45.6%)	0.01
Number of days of apheresis	1.62 (+/- 0.72)	1.68 (+/- 0.65)	0.6
Number of injections	13.12 (+/- 3.8)	2.68 (+/- 0.9)	<0.001
Number of CD34+ cells collected	7.26 (+/- 3.99)	7.54 (+/- 3.52)	0.6
Patients not meeting mobilization target	6 (8.1%)	5 (8.8%)	1
Mobilization failures (< 2 x 10 ⁶ CD34+/kg)	1 (1.3%)	1 (1.7)	1

Costa et al., Transfusion 52:2375, 2012

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

in lower use of plerixafor in the pegfilgrastim cohort than in the filgrastim cohort. (**Interactive 6.15**) [4]

All other variables were similar in terms of the number of days of apheresis, number of patients not meeting mobilization targets, and number of patients with mobilization failure, which was less than

2% in both groups. As one would expect, patients required fewer injections when they received pegfilgrastim than when they received filgrastim.

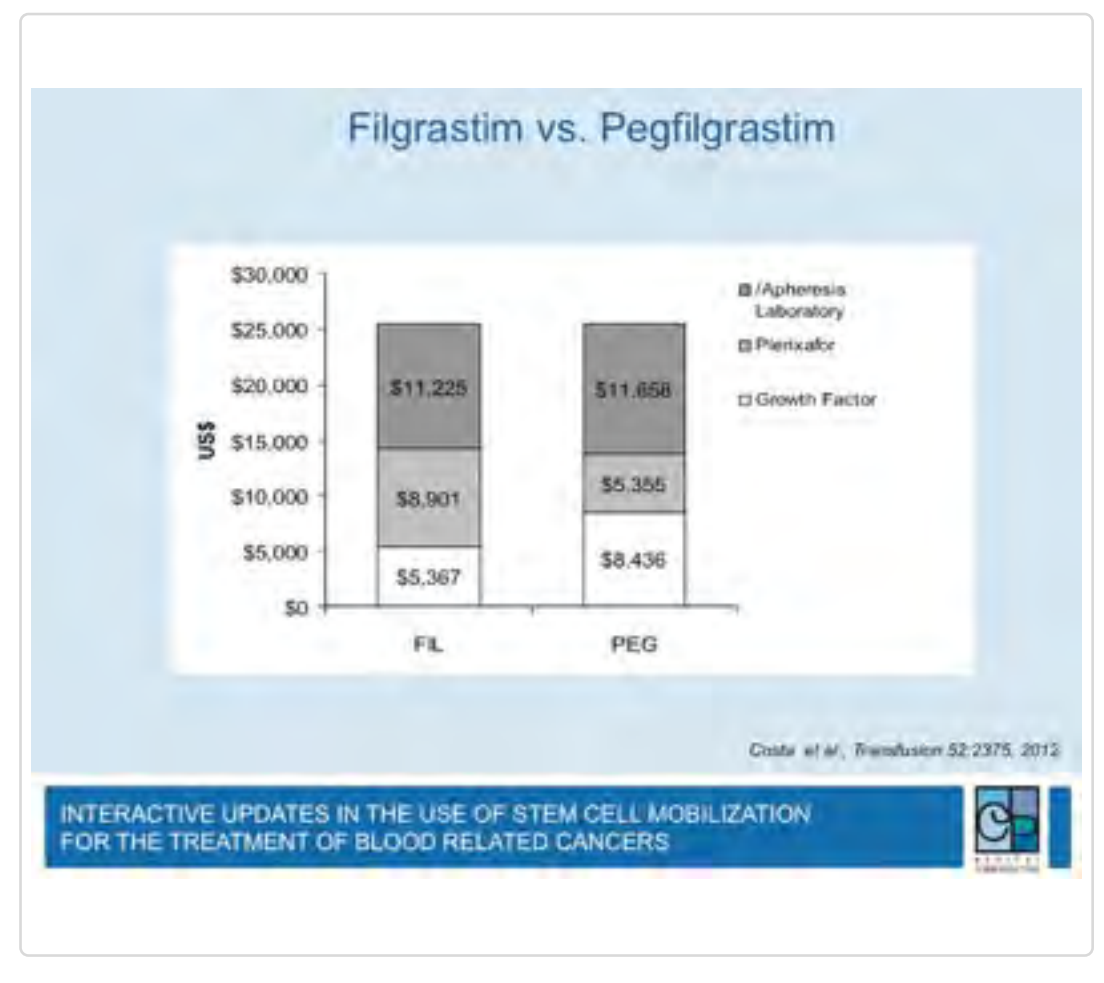
When we compared cost, the findings were quite interesting in that the cost of apheresis and laboratory utilization did not change, as these patients re-

quired a similar number of apheresis. However, the increase in cost of growth factor by going from filgrastim to pegfilgrastim is almost exactly matched by the decrease in cost with plerixafor, as fewer patients required plerixafor with the pegfilgrastim mobilization, leading to near identical costs per effective mobilization. It is important to keep in mind that in this comparison we used 12 mg of pegfilgrastim. We have unpublished data that suggest that similar results can be obtained with the use of 6 mg of pegfilgrastim in a way that would favor that approach over filgrastim. (**Interactive 6.16**) [4]

Also of great interest is the recent availability of TBO-filgrastim, which is another variation of G-CSF with a different manufacturing process that does not follow the path of bioequivalence and does not, therefore, share the same label indications with filgrastim. (**Interactive 6.17**) [5]

In this retrospective series, Dr. Elyan and colleagues followed the outcomes of their mobilizations wherein they transitioned from filgrastim to TBO-filgrastim at exactly the same dose, same schedule

Interactive 6.16



and same intended use of plerixafor on a “just in time” basis. The cohorts were very similar, with predominantly a mix of plasma cell disorders and lymphomas. (**Interactive 6.18**) [5]

The results of the mobilization were nearly identical, with both groups reaching 12.5 median CD34⁺

Interactive 6.17

TBO-Filgrastim vs. Filgrastim

Characteristic	Tbo-filgrastim n = 99 (51.5)	Filgrastim n = 86 (48.5)	P Value
Demographics			
Age, median (range) yr	57.2 (36-74.0)	61.5 (21-82.0)	<.01
Gender, male	58 (58.6)	48 (55.8)	.79
BMI, median (range)	29.3 (17.3-42.8)	28.9 (15.9-45.0)	.89
Diagnosis			
Plasma cell disorders	65 (65.7)	62 (72.1)	.13
Multiple myeloma	58 (58.6)	60 (70.0)	
Amyloidosis	5 (5.1)	1 (1.2)	
Waldenström's	1 (1.0)	0 (0.0)	
Lymphoma	34 (34.3)	24 (27.9)	.06
B cell NHL	21 (21.2)	14 (16.3)	
T cell NHL	4 (4.0)	4 (4.6)	
Hodgkin disease	9 (9.1)	6 (7.0)	
Prior therapy			
No. lines of prior therapy			<.01
1	59 (59.6)	36 (41.9)	
≥2	40 (40.4)	50 (58.1)	
Lenalidomide	38 (38.8)	26 (30.2)	.07
Reduction	20 (20.4)	18 (20.9)	.93
Baseline status at transplantation			
Plasma cell disorders			.03
CR	10 (10.4)	1 (1.2)	
VGPR	13 (13.1)	3 (3.5)	
IFR	29 (29.3)	30 (34.9)	
SD	13 (13.1)	19 (22.1)	
Lymphoma			.06
CR	15 (15.1)	10 (11.7)	
PR	14 (14.2)	9 (10.5)	
SD	5 (5.1)	5 (5.8)	

Elayan et al. BBMT
21:1921, 2015

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 6.18

TBO-Filgrastim vs. Filgrastim

Outcome	Tbo-filgrastim	Filgrastim	P Value
CD34 ⁺ day 4 cells/kg	12.5 (0-88.0)	12.5 (0-85.0)	.78
Plerixafor utilization, %	66 (66.7)	60 (69.8)	.84
Number of plerixafor doses	96 ± 87	108 ± 89	.11
Platelet count before mobilization	189.0 (61.0-380.0)	186.5 (67.0-324.0)	.91
CD34 ⁺ day 5 cells/kg	50 (0-292.0)	41 (4.0-374.0)	.15
CD34 ⁺ collected day 5, × 10 ⁶ cells/kg	3.95 (1.2-19.83)	3.78 (0.66-16.34)	.80
Apheresis equipment used			<.01
CORE	40 (43.9)	73 (84.9)	
Optia	53 (56.1)	7 (8.1)	
CORE + Optia	2 (2.0)	5 (5.8)	
Trasman	6 (6.4)	1 (1.2)	
Apheresis volumes, average	17.0 ± 3.3	16.7 ± 1.9	<.01
Total collection days	1.57 ± .70	1.60 ± .63	.24
CD34 ⁺ collected total, × 10 ⁶ cells/kg	5.85 (1.2-19.83)	5.56 (1.69-16.34)	.50
Collected 5 million CD34 ⁺ , %	96 (96.7)	52 (60.5)	.08
% Reached target collect in 1 collection day, %	40 (40.4)	31 (36.0)	.54

Average cost savings of \$964.25 per patient (AWP)

Elayan et al. BBMT 21:1921, 2015

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



cells/kg on day four. The plerixafor utilization was also nearly identical, with roughly two-thirds of the patients in each group requiring plerixafor. The same happened with the number of plerixafor doses. The apheresis resulted in near identical yields, and a similar proportion of patients in both groups

reached 5 X 10⁶ CD34⁺ cells/kg. About 40% of the patients in both groups met their target after only one day of collection.

This can be summarized by saying that TBO-filgrastim has very similar or near identical mobilization capacity to filgrastim when given at the same

Interactive 6.19

Pharmacoeconomic - Conclusions

- ✓ No prospective trial with PE endpoint
- ✓ G+P less toxic and not less CE than chemomobilization
- ✓ "Just in time" plerixafor likely more cost effective than "planned" plerixafor.
- ✓ Alternative GF (pegfilgrastim and tbo-filgrastim) may be more cost effective than filgrastim

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



to a retrospective analysis. Growth factor plus plerixafor is less toxic but not less cost-effective than chemomobilization. A “just in time” plerixafor approach is likely more cost-effective than planned plerixafor for all patients. (**Interactive 6.19**)

I’ll close by saying that alternative growth factors such as pegfilgrastim and TBO-filgrastim might be more cost-effective than filgrastim, and future research is necessary to bear this out.

doses. However, in this series, utilizing average wholesale prices, the use of TBO-filgrastim would have led to a savings of about \$960 per patient.

In conclusion, there are no prospective trials comparing two mobilization strategies with a pharmacoeconomic endpoint, so we are essentially limited

REFERENCES

1. Shaughnessy P, Islas-Ohlmayer M, Murphy J, et al. Cost and clinical analysis of autologous hematopoietic stem cell mobilization with G-CSF and plerixafor compared to G-CSF and cyclophosphamide. *Biol Blood Marrow Transplant*. 2011 May;17(5):729-36. <http://www.ncbi.nlm.nih.gov/pubmed/20813198>
2. Costa LJ, Miller AN, Alexander ET, et al. Growth factor and patient-adapted use of plerixafor is superior to CY and growth factor for autologous hematopoietic stem cells mobilization. *Bone Marrow Transplant*. 2011 Apr;46(4):523-8. <http://www.ncbi.nlm.nih.gov/pubmed/20622909>
3. Veltri L, Cumpston A, Shillingburg A, et al. Hematopoietic progenitor cell mobilization with "just-in-time" plerixafor approach is a cost-effective alternative to routine plerixafor use. *Cytotherapy*. 2015 Dec;17(12):1785-92. <http://www.ncbi.nlm.nih.gov/pubmed/20622909>
4. Costa LJ, Kramer C, Hogan KR, et al. Pegfilgrastim- versus filgrastim-based autologous hematopoietic stem cell mobilization in the setting of preemptive use of plerixafor: efficacy and cost analysis. *Transfusion*. 2012 Nov;52(11):2375-81. <http://www.ncbi.nlm.nih.gov/pubmed/22404694>
5. Elayan MM, Horowitz JG, Magraner JM, et al. Tbo-Filgrastim versus Filgrastim during Mobilization and Neutrophil Engraftment for Autologous Stem Cell Transplantation. *Biol Blood Marrow Transplant*. 2015 Nov;21(11):1921-5. <http://www.ncbi.nlm.nih.gov/pubmed/26033279>



Chapter 7

Future Novel Approaches for Stem Cell Mobilization

John F. DiPersio MD, PhD



John F. DiPersio MD, PhD

Chief, Division of Oncology
Deputy Director, Siteman Cancer Center
Washington University School of Medicine
St. Louis, Missouri

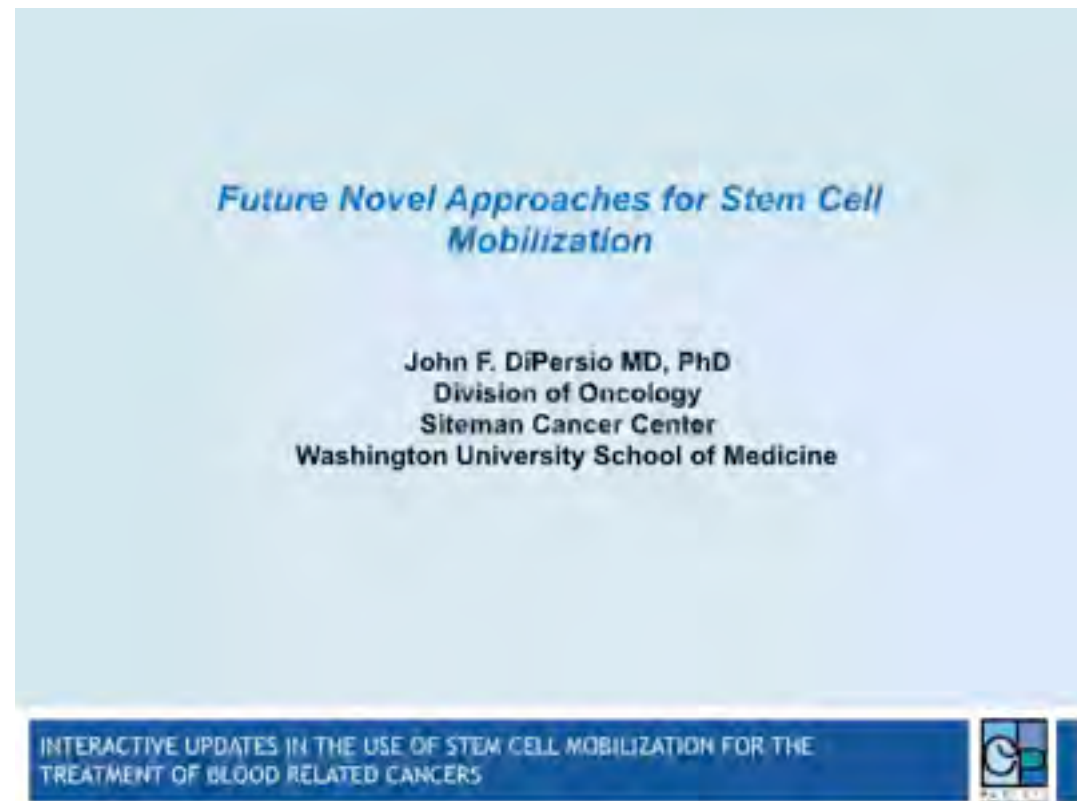
Dr. John F. DiPersio, Deputy Director, Alvin J. Siteman Cancer Center and Chief of the Division of Oncology at Washington University School of Medicine in St. Louis and the Virginia E. and Samuel J. Golman Professor of Medicine.

Dr. DiPersio's research focuses on fundamental and translational aspects of leukemia and stem cell biology. These studies include identification of genetic abnormalities in human leukemias, understanding processes involving stem cell and leukemia cell trafficking, and clinical and translational programs in both leukemia/myelodysplastic syndrome and stem cell transplantation.

Dr. DiPersio is Chair of ASH Scientific Committee on Hematopoiesis, a member of the Board of Scientific Counselors (Clinical Science and Epidemiology) of the National Cancer Institute, and the 2013 recipient of the Daniel P. Schuster Distinguished Translational Investigator Award from Washington University, the 19th Annual AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Cancer Research in 2014 and the 2014 recipient of the American Society of Hematology Mentor Award for Clinical Investigations. He has authored or co-authored more than 275 publications and over 60 invited reviews and book chapters.

Dr. DiPersio received his M.D. and Ph.D. from the University of Rochester and his B.A. in Biology from Williams College. He completed an internship and residency at Parkland Memorial Hospital and The University of Texas Southwestern Medical Center in Dallas. After serving as chief resident at Parkland Memorial Hospital, Dr. DiPersio completed a fellowship in the Division of Hematology/Oncology at the University of California, Los Angeles (UCLA).

Movie 7.1



Future Novel Approaches for Stem Cell Mobilization

John F. DiPersio MD, PhD

Interactive 7.1



Good afternoon, my name is John DiPersio and I am the Chief of the Division of Oncology and Deputy Director of the Siteman Cancer Center at Washington University School of Medicine. (**Interactive 7.1**)

I'd like to review some future novel approaches for stem cell mobilization. As a reminder, the bone

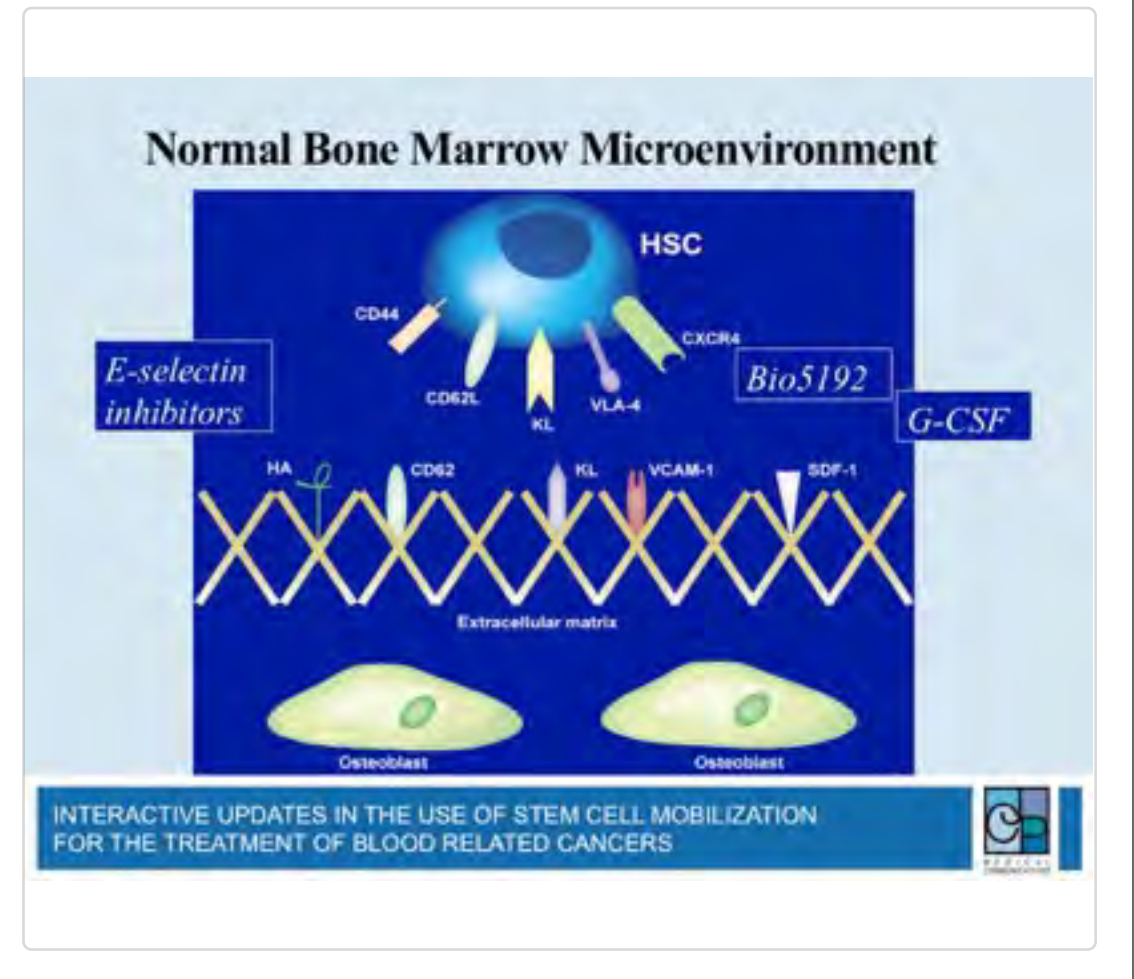
marrow microenvironment is a complicated structure with many types of cells. The major issue with stem cells is the tethering of these stem cells to the marrow microenvironment. We know that there are a number of important tethers that are key for both retention of stem cells in their marrow and also for

mobilization of those cells to the periphery. Those include primarily CXCR4 and VLA-4 but also other tethers such as CD62 and CD44. (**Interactive 7.2**)

There are a number of reagents that have been tested to interrupt these important niche tethers. They include G-CSF which downregulates SDF-1 in the marrow microenvironment and also directly injures and eliminates osteoblasts. There are also small molecular inhibitors of VLA-4. One, as noted on this slide, is Bio5192. There are a number of E-selectin inhibitors that target E-selectins expressed on the surface of stem cells. These may have an important role in the rolling and adhesion of stem cells into the marrow microenvironment.

I'd like to discuss some novel reagents that are under development in several laboratories, including some pharmaceutical companies. The first class of reagents is the VLA-4 antagonists. VLA-4 is expressed on hematopoietic stem cells and binds to its ligands in the marrow microenvironment. Its primary ligand is VCAM-1 and its secondary ligands include fibrinogen. These ligands bind to the activated

Interactive 7.2



state of VLA-4 which is activated through inside out signaling. That interaction creates an important bond by which the stem cells and other malignant cells use the marrow microenvironment as a shelter to protect them against the insults such as chemotherapy and other agents. This also is an important

retention pathway for stem cells. Interruption of this tether will release stem cells in the peripheral blood.

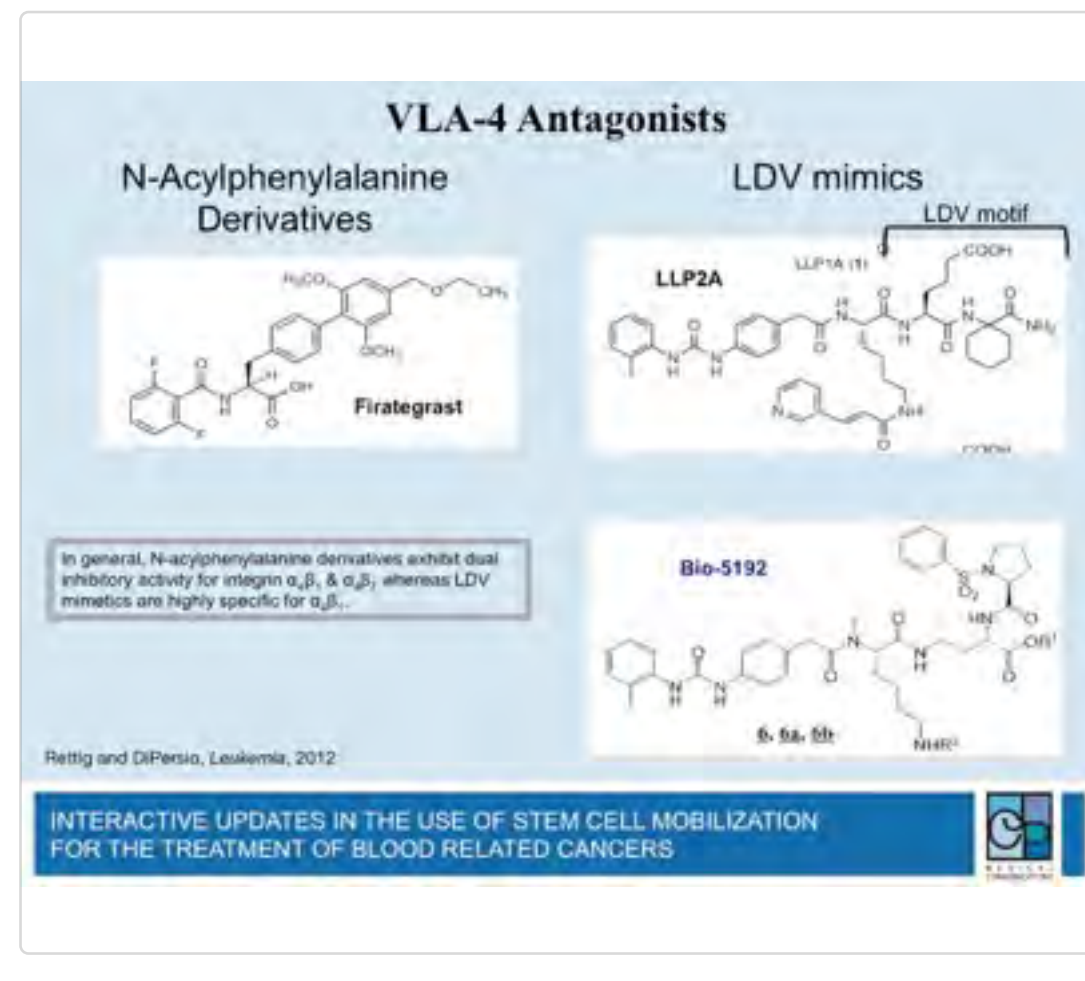
(Interactive 7.3) [1]

There are two primary chemical scaffolds which have been pursued. One is the N-acylphenylalanine derivative scaffold which has primarily been developed by GSK, and the drug is called Finategrast. This is a small molecular inhibitor of VLA-4. The second group of chemicals is based on the LDV mimetic pathway or scaffold. The prototypic drug is Bio5192, which was initially developed by Biogen.

Unfortunately neither of these drugs is being currently clinically developed. They were both tested in inflammatory disorders including rheumatoid arthritis and multiple sclerosis with negative clinical results. However, they both appeared to be safe. They have limited solubility and have been used primarily as oral agents.

Here's an example of what we had previously published looking at the role of one of these VLA-4 inhibitors, Bio5192, to rapidly and directly mobilize stem cells in the mouse. For comparison we used plerixa-

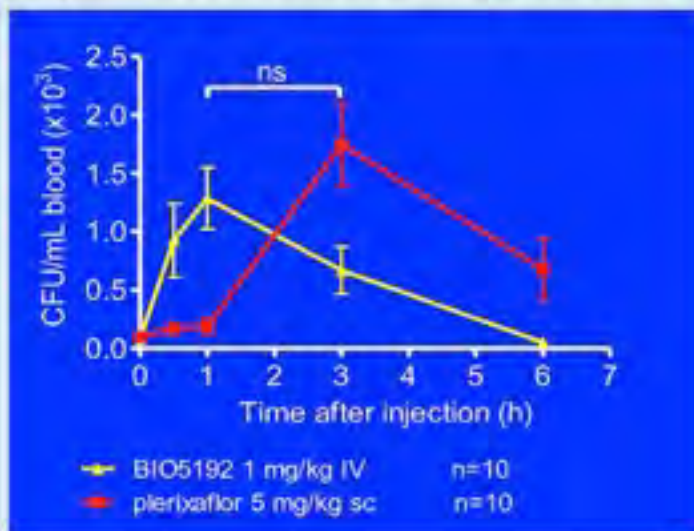
Interactive 7.3



for, which is the small molecule CXCR4 inhibitor currently approved for the treatment of patients being mobilized with myeloma and non Hodgkin lymphoma. In the mouse a subcutaneous injection of plerixafor induces peak mobilization in approximately two to three hours. Interestingly, this particular inhibi-

Interactive 7.4

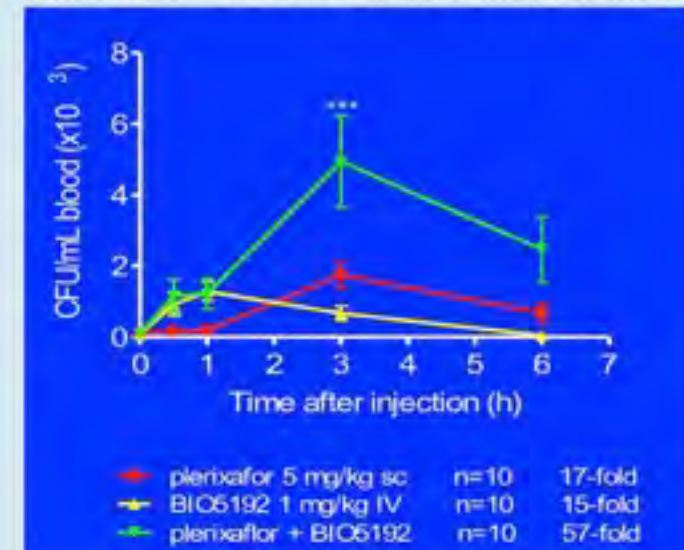
Kinetics of murine progenitors mobilization in response to BIO5192 and Plerixaflor



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

Interactive 7.5

Additive mobilization of murine progenitors after combination of Plerixaflor sc and BIO5192 iv



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

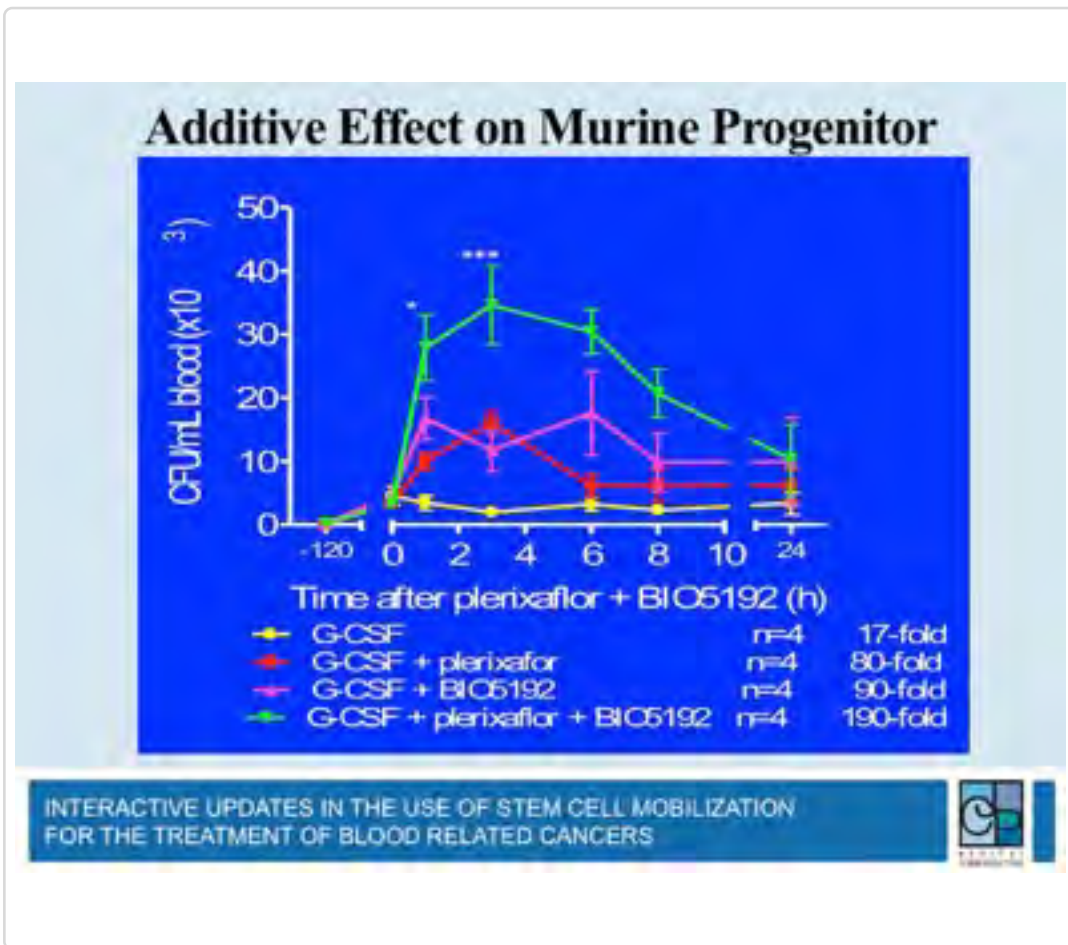
tor of VLA-4, Bio5192, does almost quantitatively the same, but in 15 to 30 minutes. (**Interactive 7.4**)

When we combined the VLA-4 inhibitor with the CXCR4 inhibitor we got additive or synergistic mobilization, as shown in the green line. The red line is CXCR4 inhibition in the mouse, the yellow line is the

VLA-4 mobilization and the green line is the two drugs given simultaneously, which results in a synergistic effect. (**Interactive 7.5**)

When you combine VLA-4 inhibitors with CXCR4 inhibitors with G-CSF you have, at least in the mouse, massive mobilization where the CFUs

Interactive 7.6



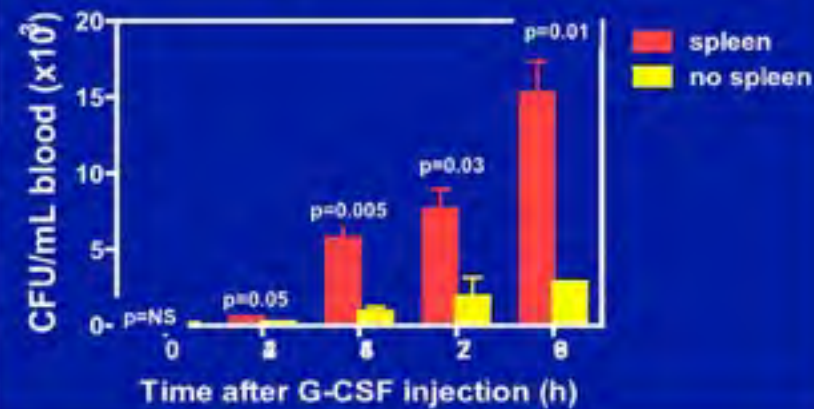
and the progenitor cells increase from 50 per ml, which is the normal circulating level in most strains of mice, to almost 40,000 per ml when you give all three drugs together, suggesting a dramatic synergistic mobilization and pathways for clinical development. (**Interactive 7.6**)

The interesting part of these experiments had to do with the removal of spleens to see if the spleen was contributing to the mobilization of stem cells, because the spleen is a hematopoietic organ in the mouse. When we removed the spleen, we found an interesting effect (the colors are reversed on this slide). The red bar is splenectomized mice and the yellow bar is wild type mice. You can see that mobilization with G-CSF is markedly enhanced when the spleen is removed because the spleen is a sieving organ. Although the colors are reversed, I want to remind you that the mobilization is dramatically increased when the spleen is removed when G-CSF is the mobilizing agent. Again, we think this is due to the inherent sieving activity, so any mobilized stem cells get trapped by the spleen. When you take the spleen out, it enhances mobilization. (**Interactive 7.7**) [2]

Looking at the same splenectomized and non-splenectomized mice mobilized with CXCR4 inhibitors or VLA-4 inhibitors we get a completely different effect. The first is that, in spite of the sieving action of the spleen, the mobilization of hematopoietic stem

Interactive 7.7

Increased mobilization of progenitors by G-CSF in splenectomized mice



Ramirez et al Blood, 2009

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

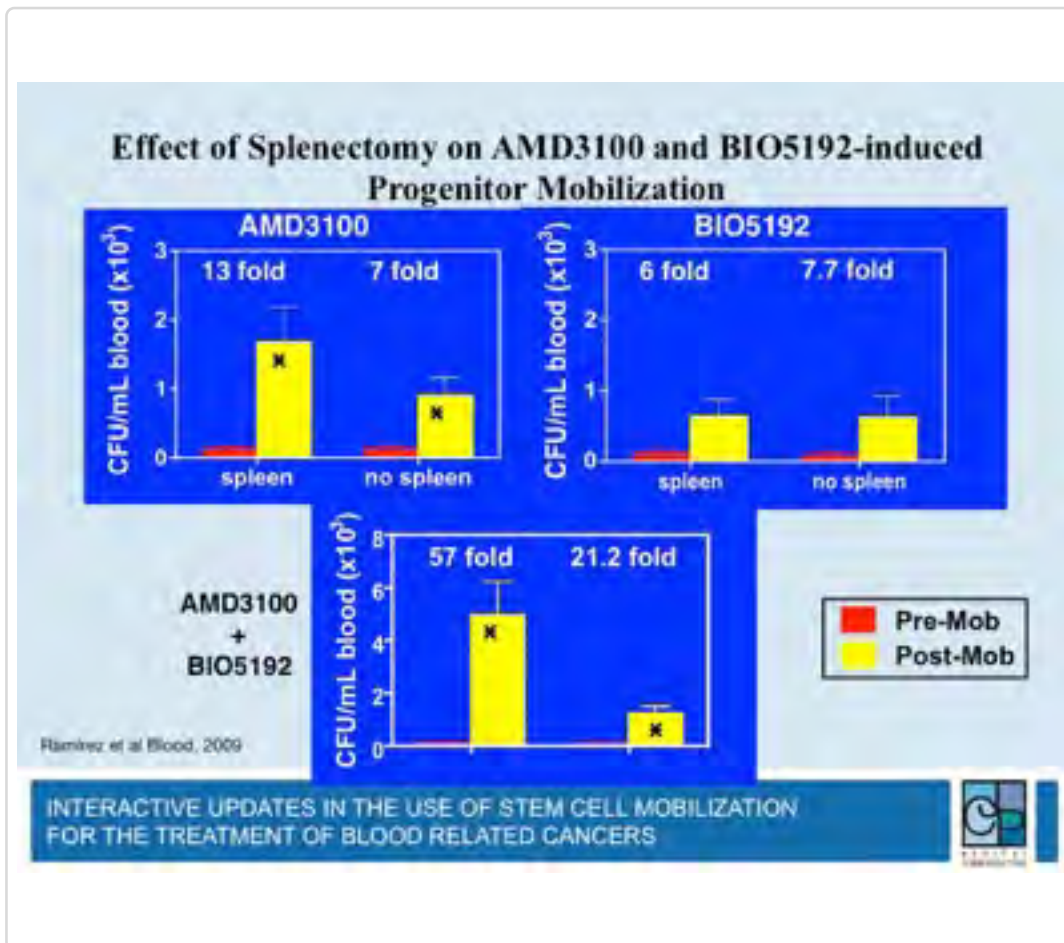


cells in the mouse drops when the spleen is removed, suggesting that most of the progenitors must come from peripheral or vascular stores. This is a so-called vascular niche. We assume that AMD3100 or plerixafor is mobilizing rapidly from these peripheral non-osteoblastic niches. One can see that when you do the

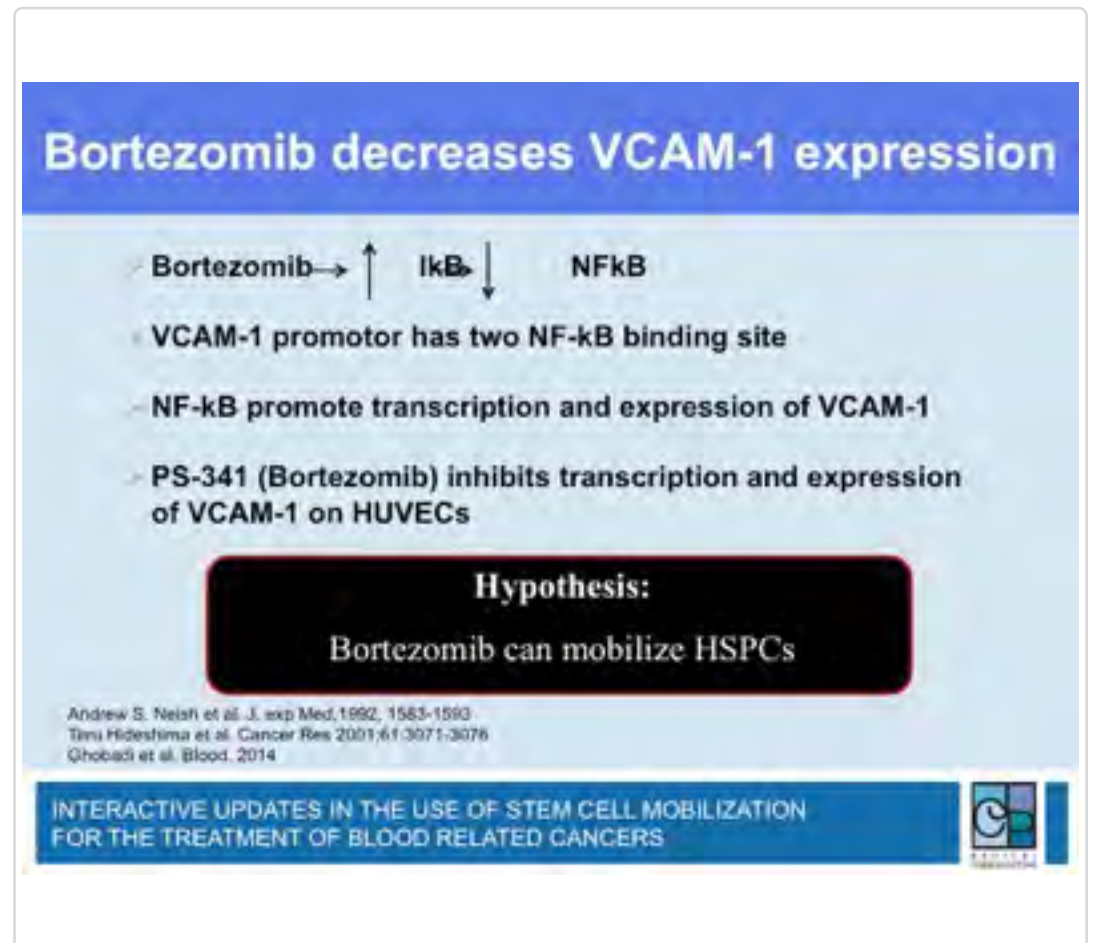
same experiment with the VLA-4 inhibitors you get the same mobilization whether you have the spleen or not, suggesting that most of these mobilized stem cells come from the osteoblastic niche in the bone marrow. When you combine the two, again, you see a significant drop off in mobilization, suggesting that a lot of the progenitors that are mobilized when CXCR4 is given subcutaneously come from these peripheral vascular stores. (**Interactive 7.8**) [2]

One of the interesting aspects of VLA-4 that has been known for some time is that the ligand for VLA-4 (VCAM-1), which is expressed in the microenvironment, has an important promoter binding site which binds NFkB which as you know is inhibited by proteasome inhibitors. The thought that we had was that if NFkB transcription factors bind to the VCAM-1 promoter and drive the expression of VCAM-1, then inhibition of that binding through the use of Bortezomid or other proteasome inhibitors would reduce the expression of VCAM-1 on human endothelial cells or bone marrow stromal cells. In fact that is the case, as we and others have shown that in previous studies that have been published.

Interactive 7.8



Interactive 7.9



Our hypothesis was that, like SDF-1 being downregulated by G-CSF, can we downregulate VCAM-1 with proteasome inhibitors, and if so would that result in mobilization of stem cells. (**Interactive 7.9**) [3,4,5]

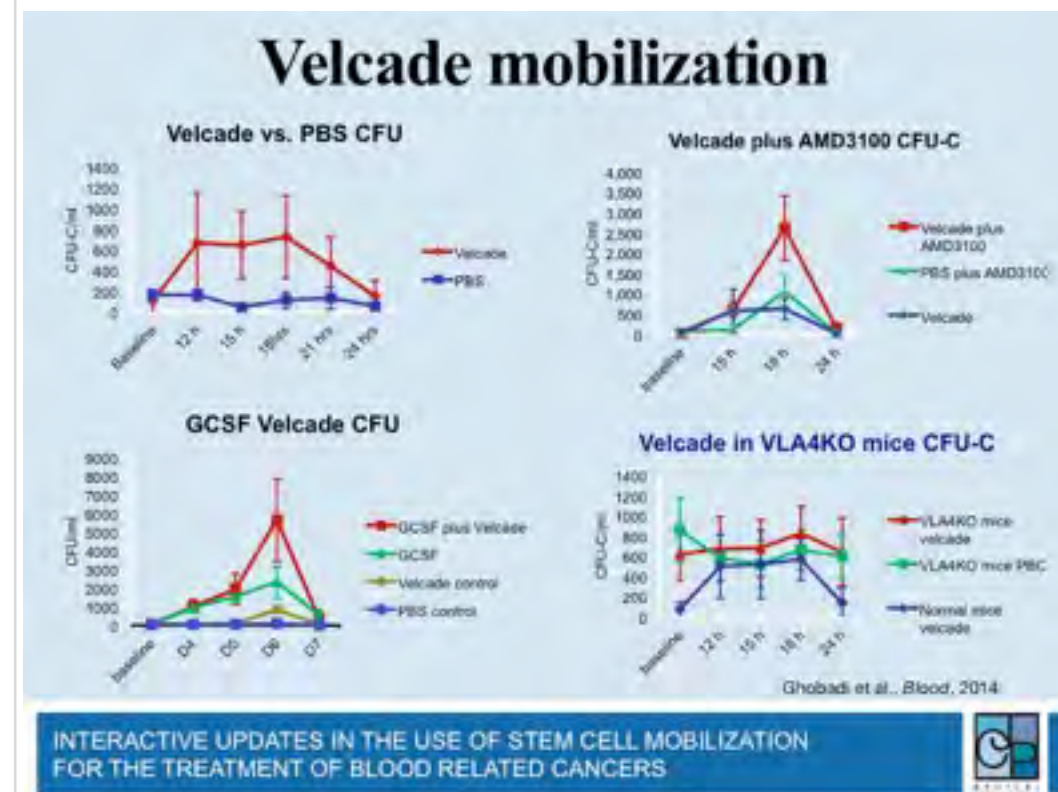
In fact, that is exactly what we saw. When we gave a single dose of Bortezomid to a mouse, we could

see that there was a robust and reproducible mobilization, but with odd and different kinetics than with CXCR4 inhibitors, which mobilized primarily within the first two to three hours in mice compared to G-CSF, which mobilized optimally between four and six days after treatment. You can

see in this setting that peak mobilization occurred between 12 and 18 hours after a single dose of Velcade. When we added AMD3100 or CXCR4 inhibitors to Velcade we saw that this effect was accentuated and that this was an additive effect. So there is reason to believe adding a CXCR4 inhibitor to Velcade would have an important, improved effect on mobilization. (**Interactive 7.10**) [5]

When we did the same experiment with G-CSF we again saw this additive effect of adding G-CSF with Velcade. The interesting part of these experiments was when we knocked out, or deleted, the VLA-4 locus in mouse bone marrow resulted in stem cells with no VLA-4 on their surface. These mice, compared to the wild type mice, have high resting levels of progenitors, as one might expect, because you're deleting that tether. You can see that there were few circulating cells at baseline in wild type mice, maybe 20 to 50 per milliliter. That increases to nearly 600 in mice that have the VLA-4 gene knocked out in their stem cells. When one gives Velcade to those mice (the red line) they do not mobi-

Interactive 7.10



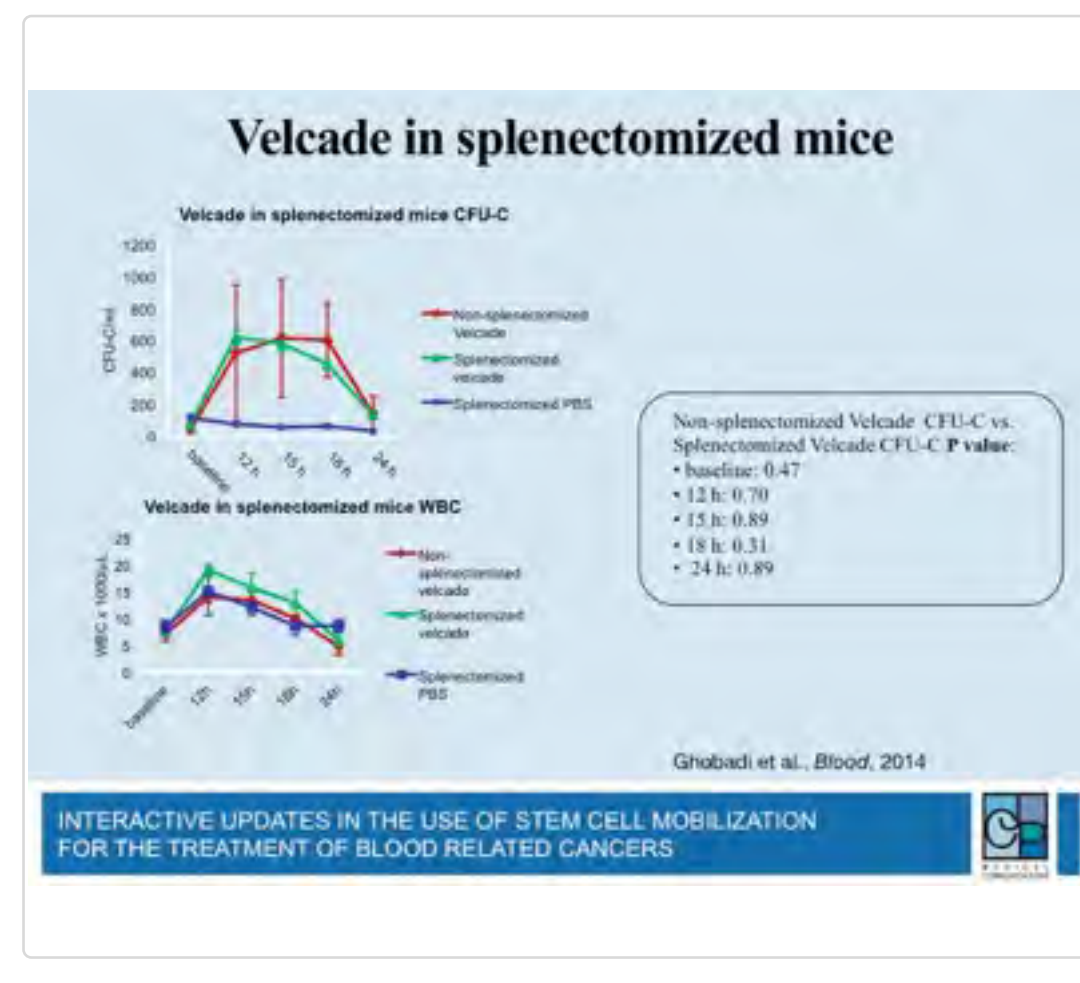
lize, suggesting to us that Velcade, as predicted, is primarily working through the VCAM-1, VLA-4 axis to mobilize stem cells.

We took the spleens out of these animals, and as expected, since we knew from previous experiments that VLA-4 inhibitors primarily mobilize from the

bone marrow, we saw identical mobilization with Velcade whether the animals had spleens or not. These data were suggestive that a blockade of VLA-4 and the pathway involving VLA-4 and VCAM-1 is important for stem cell mobilization and retention. Blockade with small molecule inhibitors rapidly induces mobilization of stem cells, primarily from bone marrow osteoblastic niches. With administration of Velcade, downregulation of VCAM-1 over 12-18 hours results in the intermediate kinetics for mobilization of stem cells from the bone marrow into the peripheral blood. Most importantly, either VLA-4 inhibitors or Velcade can be used additively or synergistically with CXCR4 inhibitors or G-CSF to enhance mobilization. (**Interactive 7.11**) [5]

One of the other interesting observations we and others have made, is if you give subcutaneous plerixafor or CXCR4 blocking agents to a mouse daily you get the same modest mobilization from about 50 to 600 progenitors per ml over two to three hours, which returns to baseline by seven to eight hours. Increasing the doses of the CXCR4 inhibitors does not

Interactive 7.11

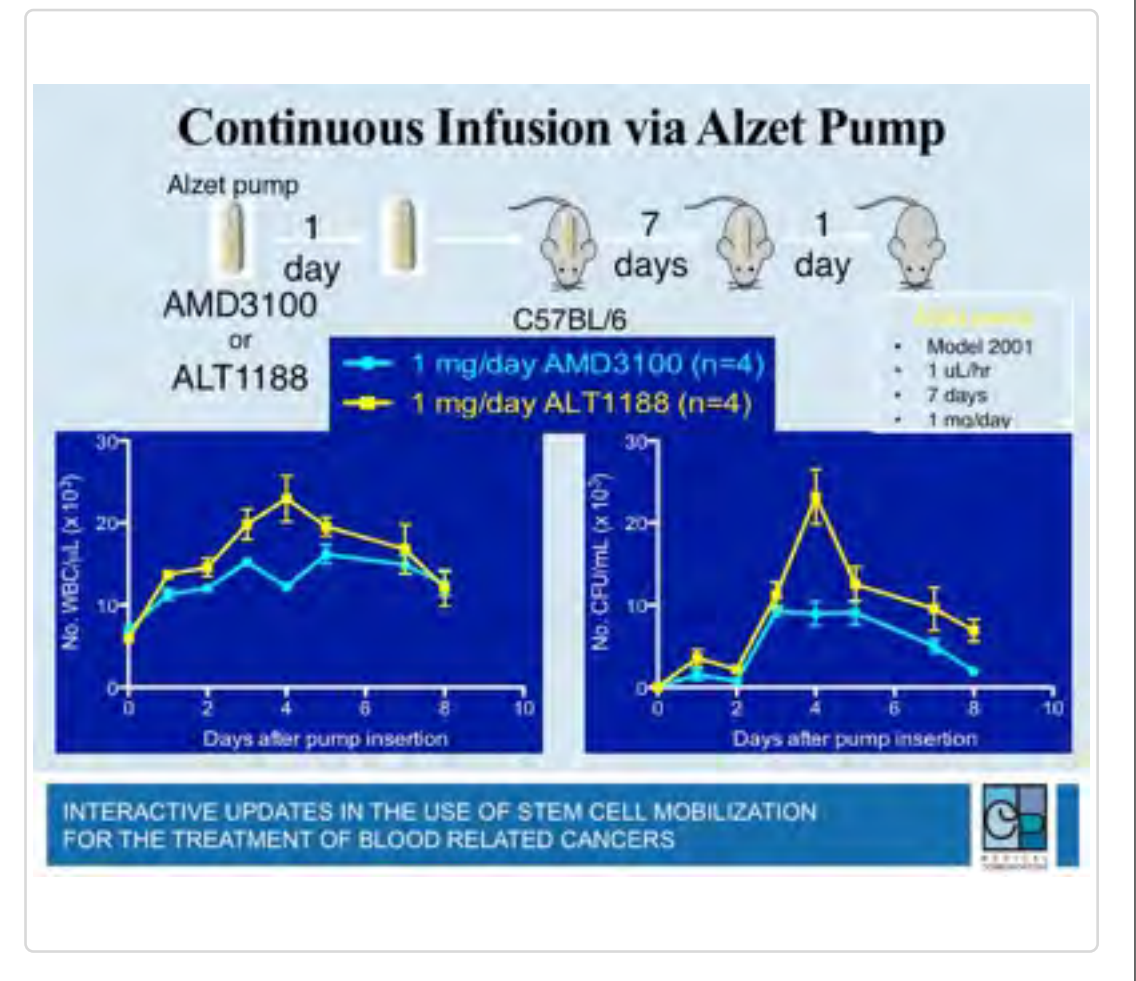


seem to further enhance mobilization and repeating the dose on a daily basis results in the same incremental increase in progenitors from 60 to 600 a day with no improvement in the peak of mobilization in the number of mobilized progenitors.

We gave the same dose of both AMD3100 (plerixafor) and an alternative active and small molecule inhibitor of CXCR4 which we were working with called ALT1188 to mice by continuous infusion via an Alzet pump. These pumps are inserted subcutaneously in mice and they deliver a daily dose of drug, the same dose that would be given by a single subcutaneous injection continuously over 24 hours. The pumps can work for 7 to 14 days depending on the type of pump used. (**Interactive 7.12**)

In this experiment we gave the drug for 7 days. You can see the usual small peak in mobilization, which is exactly what you see with the optimal dose of SC plerixafor or any other CXCR4 inhibitor. When this same daily dose is given over 7 days between days 2 and 3 there seems to be this monumental increase in progenitors which peaks at day 4 or 5. The pump is becoming less productive by day 5 and you can see the progenitors start to fall again. This represents at least a tenfold amount of mobilization compared to either plerixafor or ALT1188 alone or a five-to-ten fold better than optimal mobilization with G-CSF alone. So

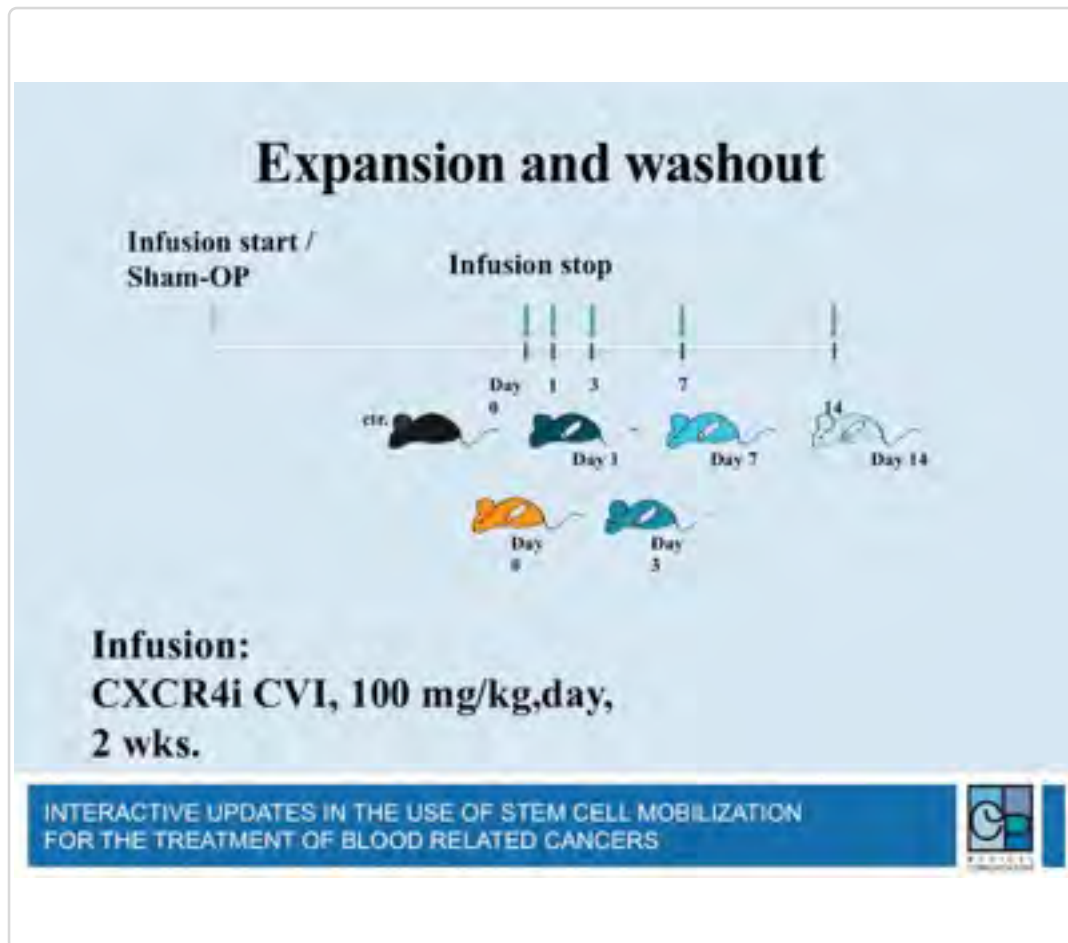
Interactive 7.12



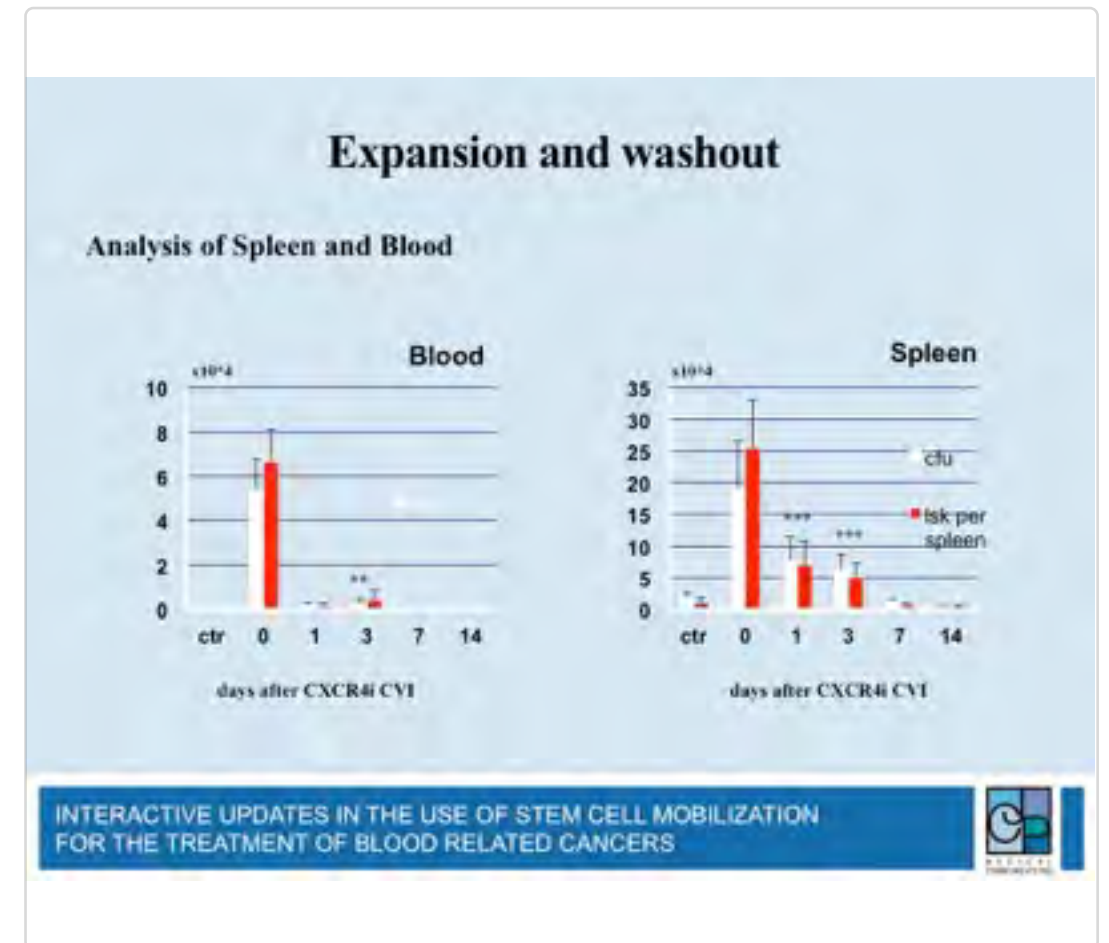
just giving these drugs by continuous infusion dramatically and profoundly increases mobilization.

We did some experiments to find out what was going on. We injected Alzet pumps into a series of mice, continued the infusions for 14 days instead of 7 days, and then looked at the mice at various times after the Alzet pumps

Interactive 7.13



Interactive 7.14



had been eliminated, for progenitors in the bone marrow and in the peripheral blood. (**Interactive 7.13**)

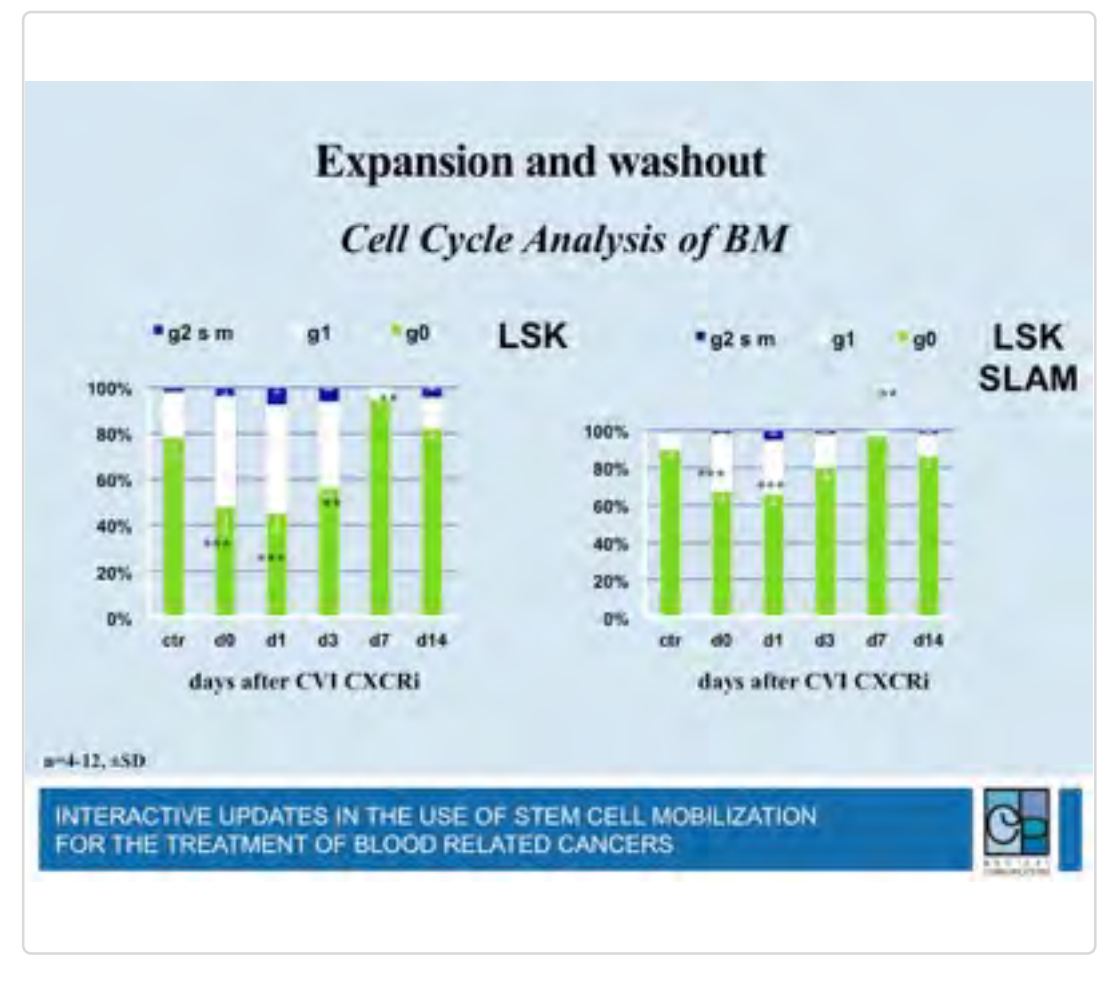
If you look at the progenitors, shown in the left panel, and “lsk”, which is the flow cytometry metric equivalent of progenitors on the right panel, you can see that after 14 days the number of progenitors in the

peripheral blood increases from approximately 60 to 80,000 and that when you take these pumps out they go back to the bone marrow relatively rapidly. The same thing is shown on the right except there is a different correlation between the flow cytometry and the colony forming units. (**Interactive 7.14**)

To find out what was going on we looked at the cycling kinetics in the bone marrow of these mice. We found that when we gave CXCR4 inhibitors continuously the percentage of cells that were in G1 and S phases dramatically increased after 14 days and these cells continued to cycle in the bone marrow for two or three days after the pump was removed until it returned to baseline. (**Interactive 7.15**)

Based on these results we think that continuous infusion of plerixafor, not higher doses, has a profound effect in the bone marrow, which is complete remodeling of the bone marrow resulting in rapid and massive proliferation and expansion of the bone marrow stem cells. These are then released into the peripheral blood and there is probably a component of rehoming to the bone marrow, which is blocked by continuous infusion of CXCR4 inhibitors. This is a potentially new way to give single agent CXCR4 inhibitors that could have profound effects on mobilization. These numbers, if they were repeated in humans, would represent a 10-100 fold increase in progenitors compared to G-CSF alone.

Interactive 7.15



The quest for rapid and robust mobilization designs is ongoing in many laboratories and companies. Several compounds in clinical development at this time are plerixafor, which has been approved; polyphor, a large peptide-based compound currently in development in Europe and the US for mobiliza-

tion and chemo sensitization; and the BiolineRX compound which is a high affinity CXCR4 inhibitor with a slow off-rate that is being developed for mobilization and chemo-sensitization. Cantex is a heparin derivative with no anti-coagulation properties that binds with high affinity to SDF-1. It induces dimerization of SDF-1 and prevents it from binding to CXCR4. It is used by continuous infusion to enhance mobilization and also as chemo-sensitization AML. Alteris is an effective small molecule inhibitor that I'll show you in the next slide; BMS makes an antibody to CXCR4; and Lilly also has an active CXCR4 small molecule inhibitor. (**Interactive 7.16**)

VLA-4 inhibitors are unfortunately not being clinically developed currently, but the previous ones were developed by Biogen and GSK for multiple sclerosis and other inflammatory diseases. Hopefully these can be taken to the clinic in the future in light of the data I've just shown you.

GroB and truncated GroB are made by GSK. They are the ligands for CXCR2, which is interestingly a receptor on monocytes and neutrophils and neutrophils,

Interactive 7.16

Quest for a Rapid and Robust Mobilization Design

- Other CXCR4 inhibitors
 - Plerixafor, Polyphor, BiolineRx, Cantex, Alteris, Cantex, BMS, Lilly
- VLA4 inhibitors (Biogen, GSK)
- Grob, truncated Grob (GSK)
- Velcade, proteasome inhibitors
- Flt3L, truncated Flt3L (CellDex)
- Continuous infusion CXCR4 inhibitors

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



not on stem cells. GroB itself is a mobilizing agent and GSK has decided not to develop it in the future.

Bortezomib, is a direct, rapid mobilizing agent in mice and is now being tested in humans. There have been several papers written about the role of Bortezomib to enhance the mobilization of G-CSF which

have been descriptive reports in small numbers of patients and the authors have not understood the biology behind this. The previous data I've shown you suggest some potential biology behind the role of Velcade as a mobilizing agent.

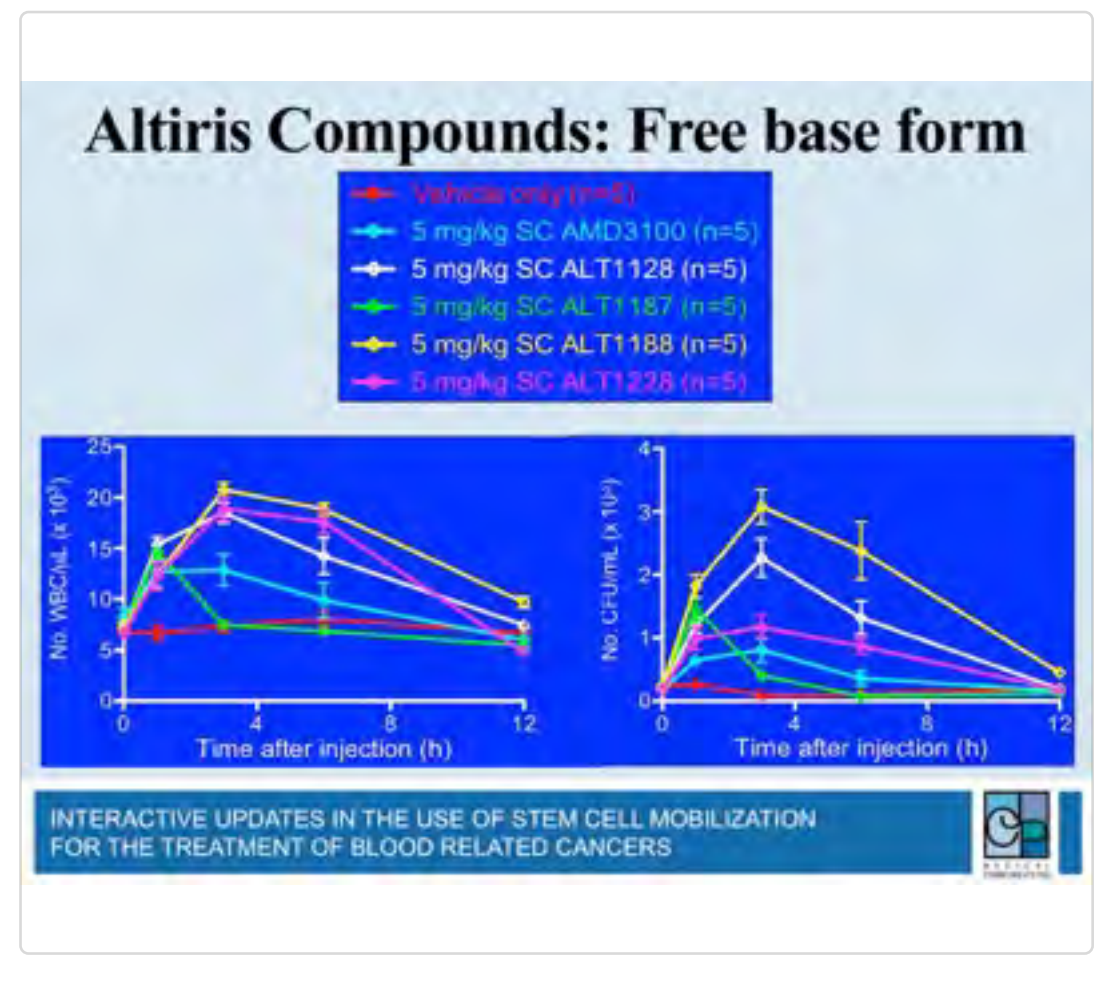
Flt3L is the ligand for a tyrosine kinase, expressed on stem cells. CellDex is the truncated Flt3 ligand and it mobilizes stem cells and also mobilizes and activates plasmacytoid dendritic cells.

Finally, I've suggested that giving CXCR4 inhibitors by a completely different mechanism and method robustly enhances mobilization.

This is an example of a small molecule inhibitor continuous IV infusion being developed that has similar properties to AMD3100 or plerixafor, except that it causes less toxicity in mice and can be given in higher doses. But even at equivalent doses you can see on the right panel that plerixafor mobilization with the same dose of ALT1188 is more potent than plerixafor and also works for longer periods of time. (**Interactive 7.17**)

The only other mobilizing agent that has not gained wide acceptance and not many people use it or

Interactive 7.17



are aware of it is granulocyte-macrophage colony-stimulating factor, GM-CSF. There is retrospective data from our center that was published some time ago. Looking at patients who had transplants with GM-CSF and only mobilized peripheral blood stem cells, when we compared GVHD grade II-IV and III-IV in patients

GM-CSF mobilized stem cell product was associated with less aGvHD in humans

Figure 1 Cumulative incidence of grade 3-4 acute GVHD by cytotoxic group

Figure 2 Incidence of grade 3-4 acute GVHD by cytotoxic group

Single-variable model	P-value	Multivariable model	P-value
Cytotoxic			
Recipient age	0.49	Mobilization regimen	
		GM-CSF vs G-CSF	0.012
		G-CSF vs G+GM-CSF	0.010
Donor age	0.44	Donor risk (high vs low)	0.039
Sex mismatch	0.51	CD34 dose	0.40
Mobilization regimen		CD3 dose	0.11
GM-CSF vs G-CSF	0.001		
G-CSF vs G+GM-CSF	0.001		
Donor risk (high vs low)	0.001		
CD34 dose	0.000		
CD3 dose	0.40		
CD4 dose	0.50		
CD8 dose	0.40		
CD19 dose	0.42		
CD11b dose	0.50		

G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor

T cell dose and CD34 dose were not significant in multivariate analysis

Devine et al. BMT. 2005;36:531

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

In a follow-up clinical trial which has not yet been published it does not show the same encouraging results. I think it is a weak mobilizing agent and

Donor Schema

Until $>2 \times 10^6$ CD34+ cells/kg^a
OR 2 days

The diagram illustrates a 6-day donor schema. A horizontal timeline is labeled 'Day' with markers for days 1 through 6. Above the timeline, a grey bar labeled 'Myelosuppression' spans from Day 1 to Day 4. A grey arrow labeled 'Apheresis (20L x 100L)' points from Day 4 to Day 5. Red downward arrows are positioned at Day 1 and Days 5, 5.5, 5.5, and 6. Black upward arrows are positioned at Days 1, 2, 3, 4, 5, and 6.

↑ GM-CSF (10 ug/kg SC) *Revised to 5mcg/kg SC after problems with catheter associated thrombosis*
Plerixafor (320 ug/kg IV) four hours before collection

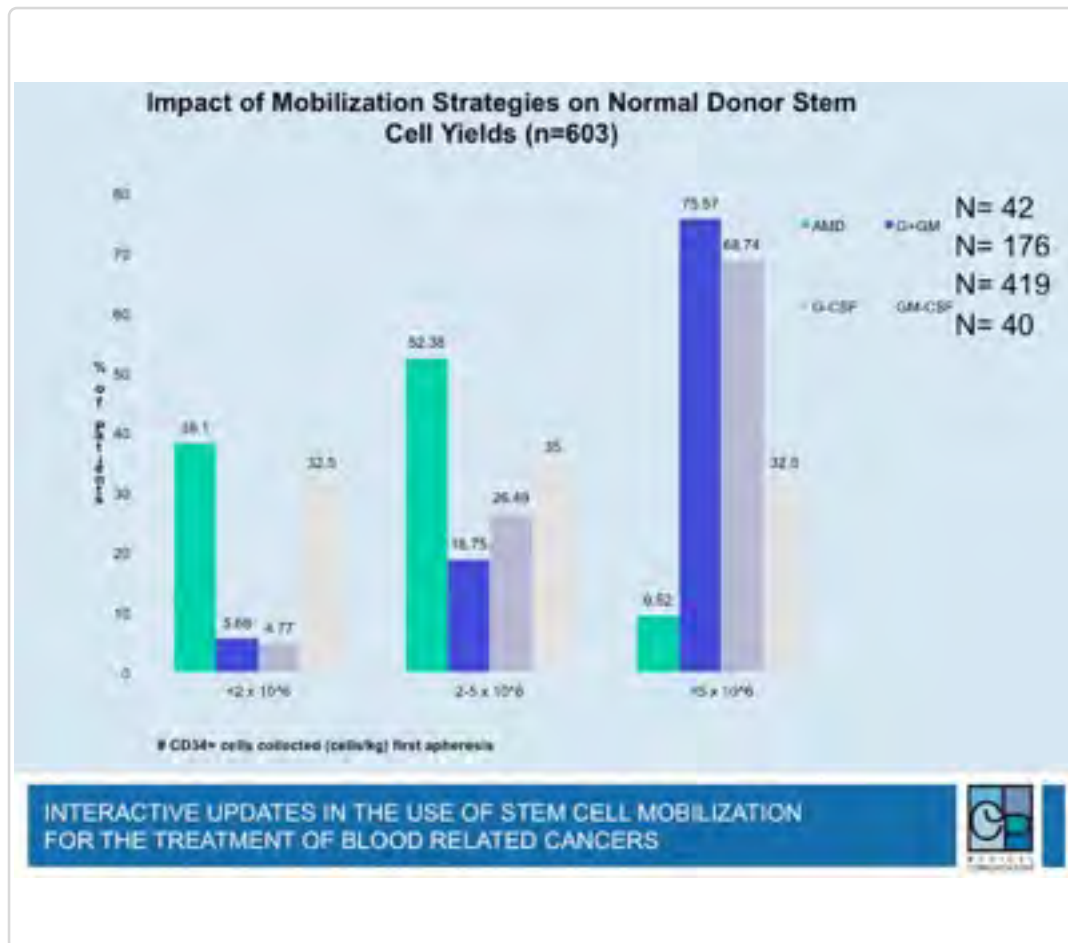
↓ Blood sample for correlative studies

^aminimum 2×10^6 /kg

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS

In the G-CSF group the failure rate to collect at least 2×10^6 CD34+ cells/kg after a single collection was only between 4 and 6% in these two groups. However the failure rate to collect at least 2×10^6

Interactive 7.20



Interactive 7.21

Best Blood Marrow Transplant 2017 (2017) 200-223

Biology

FLT3L and Plerixafor Combination Increases Hematopoietic Stem Cell Mobilization and Leads to Improved Transplantation Outcome

Shun He ^{1,3}, Jianhong Chu ², Sumithira Vasu ^{1,2,3}, Youcai Deng ², Shunzong Yuan ^{2,4}, Jianying Zhang ⁵, Zhijin Fan ², Craig C. Hofmeister ^{1,2}, Xiaoming He ^{2,6}, Henry C. Marsh ², Steven M. Devine ^{1,2,3}, Jianhua Yu ^{1,2,3,*}

¹Division of Hematology, Department of Internal Medicine, College of Medicine, Ohio State University, Columbus, Ohio
²The Ohio State University Comprehensive Cancer Center, Columbus, Ohio
³Blood and Marrow Transplantation Program, The James Cancer Hospital, Ohio State University, Columbus, Ohio
⁴Department of Lymphoma, PBA 307 Hospital, Beijing, China
⁵Center for Biostatistics, Department of Biomedical Informatics, Ohio State University, Columbus, Ohio
⁶Department of Biomedical Engineering, Ohio State University, Columbus, Ohio
^{*}Celltex Therapeutics, Inc., Norwell, Massachusetts

ASBMT
American Society for Blood and Marrow Transplantation

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS

CD34+ cells/kg is 38% in all plerixafor patients in all trials we've done and 32.5% in the GM-CSF mobilized group. Although we have shown in both these groups that there may be slightly less acute and chronic GVHD when patients are transplanted with these mobilizing agents, they are relatively ineffec-

tive and will probably not move forward in any setting as single agents to mobilize allogeneic stem cell donors. (**Interactive 7.20**)

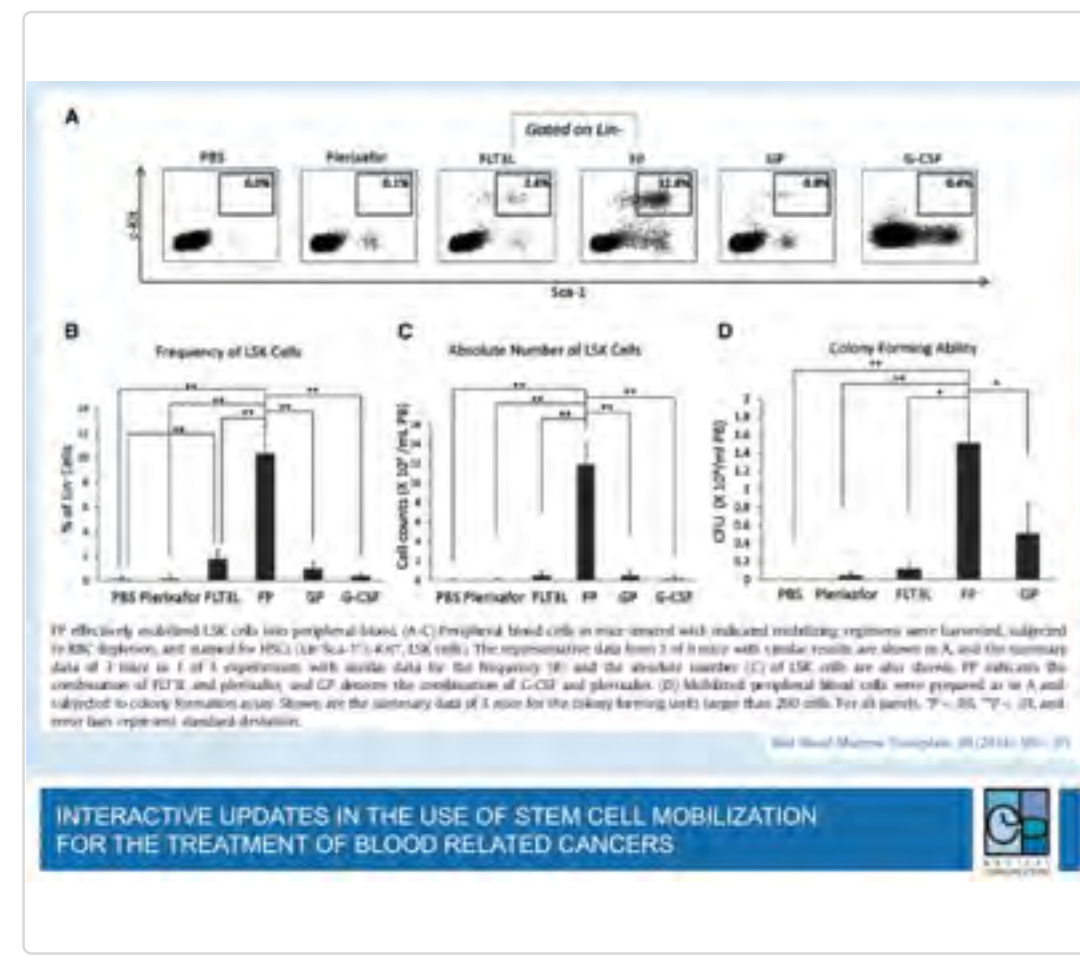
Flt3 ligand is also an effective agent. This is a paper from Ohio State University looking at a Flt3 ligand to mobilize stem cells in a mouse. (**Interactive 7.21**) [7]

They combined Flt3 ligand with G-CSF and plerixafor. On the top are the stem cells mobilized by each group: PBS, plerixafor alone, Flt3 ligand alone, Flt3 ligand plus plerixafor, G-CSF plus plerixafor, and G-CSF. The combination of Flt3 ligand and plerixafor was particularly effective in mobilizing hematopoietic stem cells and mobilizing plasmacytoid dendritic cells which have been shown by several groups to be associated with tolerance or inhibition of alloreactivity. (**Interactive 7.22**) [7]

These same authors did a transplant study in mice (shown in the right panel) which showed that mice that received Flt3 ligand plus plerixafor mobilized stem cells or Flt3 mobilized stem cells alone had much lower rates of GVHD and mortality than mice getting stem cells, T-cells, and splenocytes from mice receiving either PBS or G-CSF. (**Interactive 7.23**) [7]

The final way to use mobilizing agents is to think outside the box about the interaction between the microenvironment and the hematopoietic niche. There have been many papers published focused on diseases looking at the interaction of both the collagen matrix and the

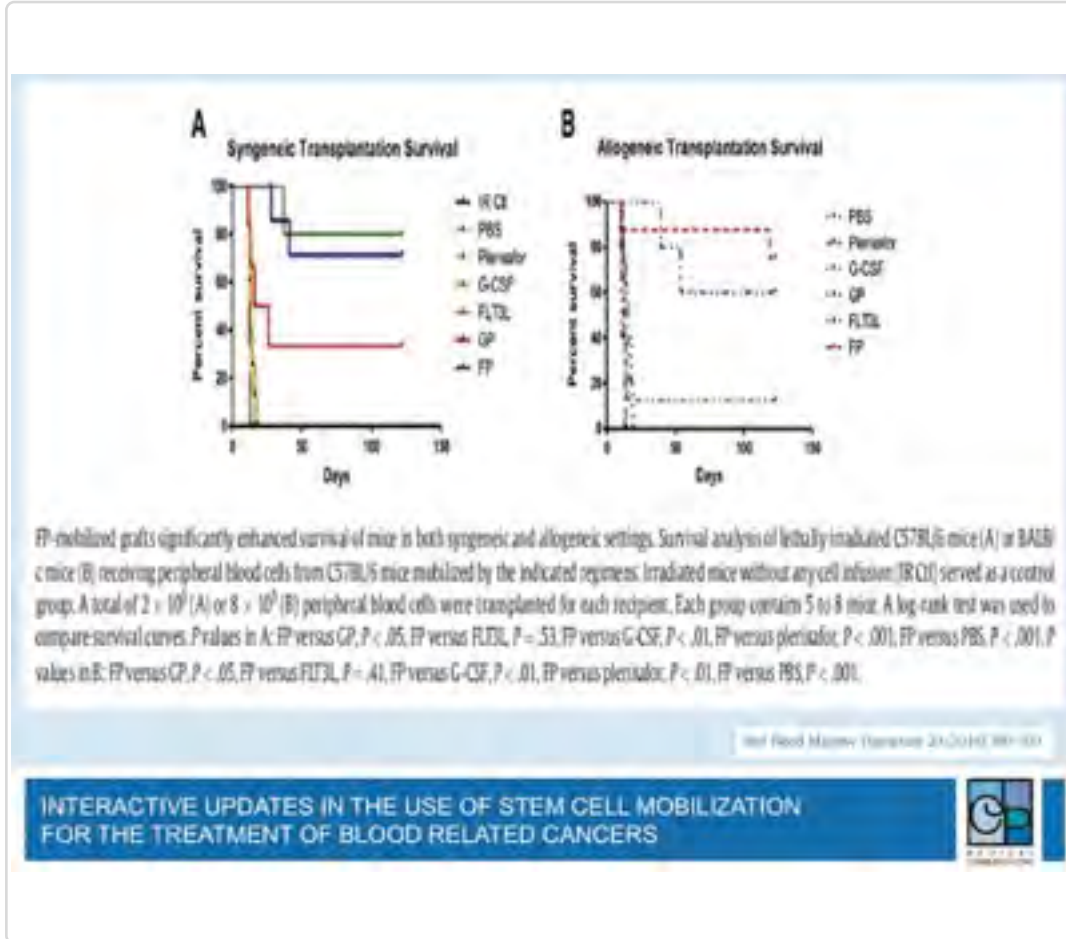
Interactive 7.22



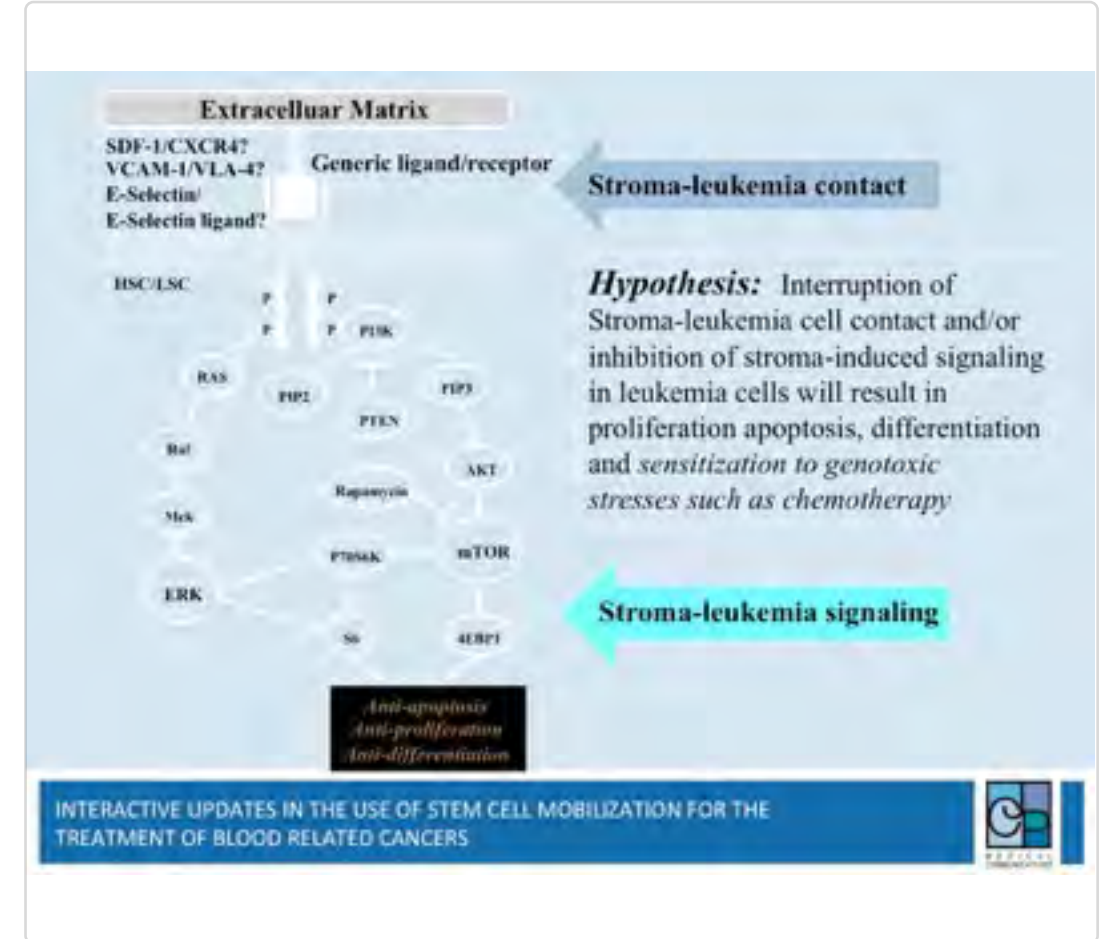
microenvironment, the microenvironment cells themselves, and the malignant cells. (**Interactive 7.24**)

This shows some of the pathways in a hematopoietic cell or a malignant leukemic cell that are activated when these cells interact with the microenvironment. We think that these interactions are mediated by the tethers

Interactive 7.23



Interactive 7.24



that I mentioned in the first slide. That includes SDF-1, CXCR4, VCAM-1, VLA-4, E-Selectin, and E-Selectin ligands, etc. Although one could inhibit one of these pathways that are inducing the following signals seen in all cells that bind to the microenvironment – anti-apoptosis, anti-proliferation and anti-differentiation –

one could also just interrupt this interaction so that these cells now become more sensitive to other genotoxic stresses like chemotherapy.

There are many studies looking at the role of CXCR4 inhibitors to sensitize leukemic and other cells to chemotherapy. This is one example in which

Interactive 7.25

Phase I/II Study of G-CSF + AMD3100 + MEC in Relapsed or Refractory AML

Eligibility Criteria

1. Dx of AML and either 1st refractory or relapsed disease
2. Age 18-65, ECOG PS \leq 2
3. Blast ct $<$ 30,000/mm³
4. No previous MEC salvage

AMD3100:	240-480mcg/kg IV qd/bid on d3-8
G-CSF	10 mcg/kg SQ on d1-8
Mitoxantrone	8 mg/m ² IV on d4-8
Etoposide	100 mg/m ² IV on d4-8
Cytarabine	1000 mg/m ² IV on d4-8



INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



Interactive 7.26

Response Evaluation (n=49)

Plerixafor Dose	Patients		Responses		Treatment Failures		Response Rate	
	Total	# Eval	CR	CRi	Death	PD	CR +CRi	CR
80 mcg/kg/day	3	3	1	0	0	2	33%	33%
160 mcg/kg/day	3	3	1	0	0	2	33%	33%
240 mcg/kg/day	43	40	17	3	2	19	50%	40%
Overall	49	46	19	3	2	23	48%	39%

Uy et al., Blood. 2012

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION FOR THE TREATMENT OF BLOOD RELATED CANCERS



we gave plerixafor immediately before MEC, which is salvage chemotherapy for AML, and looked at the rates of second remission in patients who had relapsed AML. (**Interactive 7.25**)

What we were able to show was that CR and CRi rates for patients that we would normally expect to

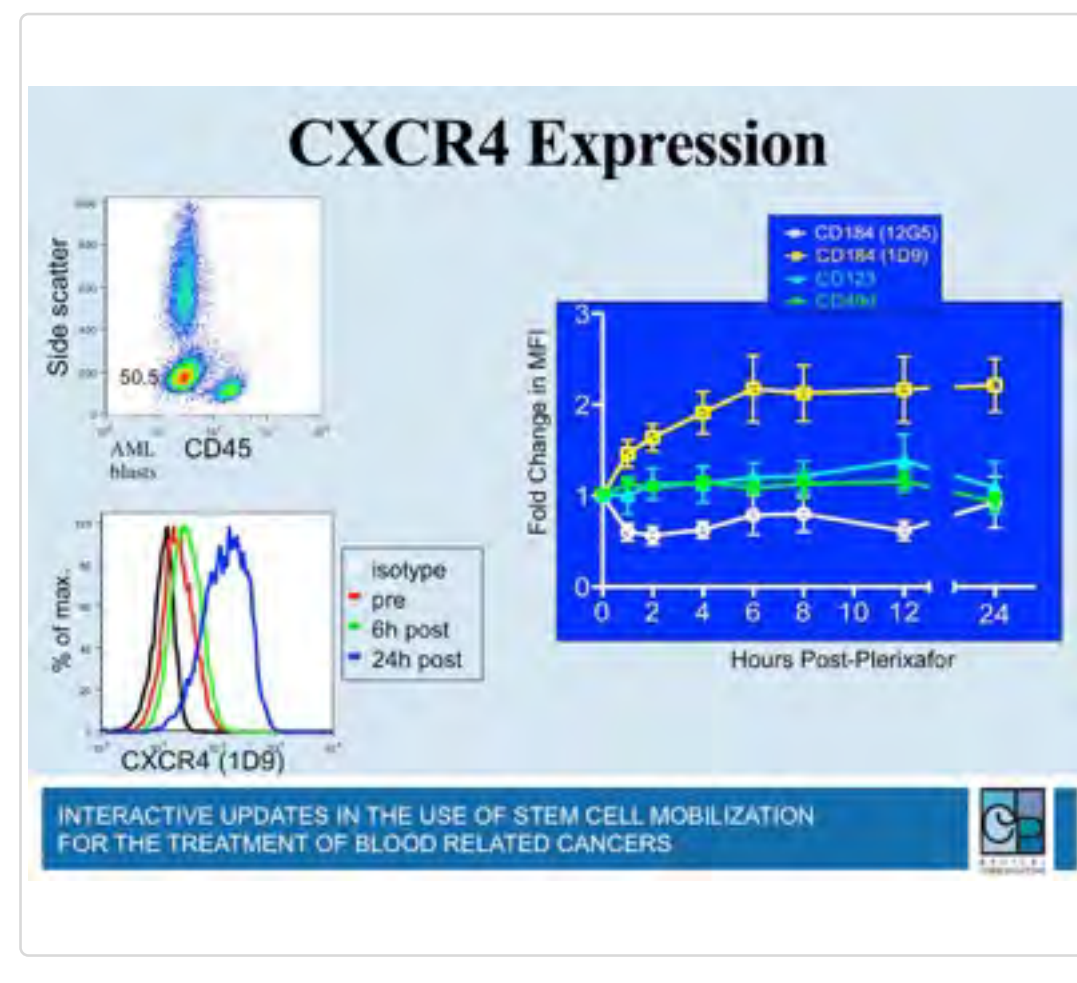
have CR rates in the 25-30% range had CR and CRi rates in the 50% range. These results are somewhat encouraging, but need to be taken with a grain of salt until proven in a prospective randomized trial. (**Interactive 7.26**) [8]

The other interesting observation we made was that when we gave a CXCR4 inhibitor to leukemic patients and looked at the CXCR4 expression on the leukemic cells we found something that we never expected. (**Interactive 7.27**)

First early on as expected there is a drop (the white line) of the binding of an antibody that binds to the plerixafor binding site on CXCR4. So giving plerixafor and the antibody which binds to that site on CXCR4 will not be able to bind; it stays low for a period of time. However, if you use a different antibody which identifies the CXCR4 but binds to a separate site on CXCR4 that the plerixafor does not bind to, the receptor goes up over time. This paradoxical increase in receptor expression, shown on the blue curve, is quite dramatic. These cells have between two and ten times more CXCR4 on the surface after administration of a CXCR4 inhibitor.

We are currently trying to understand the mechanisms of this, but this would work against any effective chemo-sensitization regimen and suggests that continuous blockade of CXCR4 is the preferred

Interactive 7.27



method to sensitize a cell to chemotherapy since subcutaneous dosing does block it temporarily but the receptor expression goes up rapidly. So these cells are more able to find their way back to the bone marrow and to be protected from chemotherapy.

Interactive 7.28

Conclusions

CXCR4 antagonists are rapid but weak stem cell mobilizers. They result in upregulation of the CXCR4.

VLA-4 antagonists are rapid and weak mobilizers but synergize with CXCR4 antagonists and G-CSF

Continuous infusion of CXCR4 inhibitors results in HSC expansion and robust mobilization

The use of niche disruption agents may provide a way to overcome chemoresistance in AML but must be proven in randomized studies

Bortezomib and other proteasome inhibitors mobilize stem cells in mice with unique kinetics and synergize with G-CSF and CXCR4 inhibitors and mediate their effects via the VCAM-1/VLA-4 axis

INTERACTIVE UPDATES IN THE USE OF STEM CELL MOBILIZATION
FOR THE TREATMENT OF BLOOD RELATED CANCERS



or stem cell expansion and robust mobilization. The use of niche disrupting agents like CXCR4 inhibitors and VLA-4 inhibitors may provide a way to overcome chemo-resistance in AML, but this must be proven in prospective randomized studies. Finally, Bortezomid and other proteasome inhibitors mobilize stem cells in mice with unique kinetics primarily from the bone marrow and synergize with G-CSF and CXCR4 inhibitors and mediate their effects by the VCAM-1/VLA-4 axis. (**Interactive 7.28**)

I want to thank you for attention and I hope you have enjoyed this session.

In conclusion, CXCR4 antagonists are rapid but weak stem cell mobilizers. They result in upregulation of the CXCR4 receptor after treatment. VLA-4 antagonists are rapid and weak mobilizers but synergize with CXCR4 antagonists and G-CSF. Continuous infusion of CXCR4 inhibitors results in monumental HSC

REFERENCES

1. Rettig MP, Ansstas G, DiPersio JF. Mobilization of hematopoietic stem and progenitor cells using inhibitors of CXCR4 and VLA-4. *Leukemia*. 2012 Jan;26(1):34-53. <http://www.ncbi.nlm.nih.gov/pubmed/21886173>
2. Ramirez P, Rettig MP, Uy GL, et al. BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. *Blood*. 2009 Aug 13;114(7):1340-3. <http://www.ncbi.nlm.nih.gov/pubmed/19571319>
3. Neish AS, Williams AJ, Palmer HJ, et al. Functional analysis of the human vascular cell adhesion molecule 1 promoter. *J Exp Med*. 1992 Dec 1;176(6):1583-93. <http://www.ncbi.nlm.nih.gov/pubmed/1281211>
4. Hideshima T, Richardson P, Chauhan D, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res*. 2001 Apr 1;61(7):3071-6. <http://www.ncbi.nlm.nih.gov/pubmed/11306489>
5. Ghobadi A, Rettig MP, Cooper ML, et al. Bortezomib is a rapid mobilizer of hematopoietic stem cells in mice via modulation of the VCAM-1/VLA-4 axis. *Blood*. 2014 Oct 23;124(17):2752-4. <http://www.ncbi.nlm.nih.gov/pubmed/25342668>
6. Devine SM, Brown RA, Mathews V, et al. Reduced risk of acute GVHD following mobilization of HLA-identical sibling donors with GM-CSF alone. *Bone Marrow Transplant*. 2005 Sep;36(6):531-8. <http://www.ncbi.nlm.nih.gov/pubmed/16025152>
7. He S, Chu J, Vasu S, et al. FLT3L and plerixafor combination increases hematopoietic stem cell mobilization and leads to improved transplantation outcome. *Biol Blood Marrow Transplant*. 2014 Mar; 20(3):309-13. <http://www.ncbi.nlm.nih.gov/pubmed/24365795>
8. Uy GL, Rettig MP, Motabi IH, et al. A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. *Blood*. 2012 Apr 26;119(17):3917-24. <http://www.ncbi.nlm.nih.gov/pubmed/22308295>



Chapter 8

Evaluation and Post-Test

Completing CME Evaluations and Claiming Credit

COMPLETING CME EVALUATIONS AND CLAIMING CREDIT

Learners are to complete an evaluation and post-test in order to claim CME credit.

BEFORE EXITING, PLEASE TAKE THIS POST-TEST



PRE-TEST

The screenshot displays the Medical College of Wisconsin's Office of Continuing and Professional Education website. The page is titled "The Use of Stem Cell Mobilization for the Treatment of Blood Related Cancers". It includes a sidebar with navigation links such as Home, Calendar, Courses, News, Discussions, Create content, and Admin. The main content area features a large play button icon over the text, which discusses the use of stem cell mobilization in the treatment of blood-related cancers. The course details section indicates that the course is 2.00 hours long, opens on Thursday, December 31, 2015, and expires on Saturday, December 31, 2016.

Before moving forward, please take this pre-test.

Publisher



Carden Jennings Publishing Co., Ltd.

375 Greenbrier Drive, Suite 100
Charlottesville, Virginia 22901
434-817-2000 • www.cjp.com

David Ern

Chief Executive Officer • dern@cjp.com

Marc Weathersby

Chief Marketing Officer • mweathersby@cjp.com

David Utz

Vice President, Production • dutz@cjp.com

Debbie Bretches

Designer • dbretches@cjp.com

Garland Branch

Product Manager • gtbranch@cjp.com

© 2015 Carden Jennings Publishing Co., Ltd.
All Rights Reserved

ISBN 978-1-891524-16-5

Carden Jennings Publishing Co., Ltd. presents this publication for informational and discussion purposes only. Readers are expected to use their own expertise and judgment while engaged in the practice of medicine. Please consult full prescribing information for any drugs or procedures discussed within this publication. The views and opinions expressed in these presentations are those of the authors and do not necessarily reflect the views of the commercial supporters or Carden Jennings Publishing Co., Ltd. Although great care has been taken in compiling and checking the information given in this publication to ensure accuracy, the authors and Carden Jennings Publishing Co., Ltd., and their agents shall not be responsible or in any way liable for the continued currency of the information or for any errors, omissions, or inaccuracies in this publication, whether arising from negligence or otherwise howsoever or for any consequences arising therefrom.

Except as provided herein, no part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or utilizing any storage or retrieval system without written permission from the Publisher.