Mobilization of PML/RARα negative peripheral blood stem cells with a combination of G-CSF and CXCR4 blockade in relapsed acute promyelocytic leukemia pretreated with arsenic trioxide

by Friedrich Stölzel, Martin Wermke, Christoph Röllig, Christian Thiede, Uwe Platzbecker, and Martin Bornhäuser

Haematologica 2009 [Epub ahead of print]

doi:10.3324/haematol.2009.016568

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. This paper will now undergo editing, proof correction and final approval by the authors. Please note that during this production process changes may be made, and errors may be identified and corrected. The final version of the manuscript will appear both in the print and the online journal. All legal disclaimers that apply to the journal also pertain to this production process.

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature. Haematologica is the official organ of the European Hematology Association (www.ehaweb.org).

Support Haematologica and Open Access Publishing by becoming a member of the European Hematology Association (EHA) and enjoying the benefits of this membership, which include free participation in the online CME program.
Mobilization of PML/RARα negative peripheral blood stem cells with a combination of G-CSF and CXCR4 blockade in relapsed acute promyelocytic leukemia pretreated with arsenic trioxide

Very recently Montesinos et al. reported on the incidence of central nervous system (CNS) involvement at first relapse in patients with acute promyelocytic leukemia (APL) who had been treated with all-trans retinoic acid (ATRA) and anthracycline monochemo-therapy without intrathecal prophylaxis.1 Although this study showed a relatively low incidence of CNS involvement at first relapse treatment options are generally discussed controversial. The introduction of ATRA and more recently arsenic trioxide (ATO) has changed treatment options and outcome for APL.2-3 In the setting of relapsed APL, ATO is presently regarded as the preferential remission induction therapy. However for patients achieving complete remission (CR) thereafter, appropriate consolidation strategies have not been defined so far.4 Autologous hematopoietic stem cell transplantation (HSCT) is one treatment option in relapsed APL. Here, we report a patient who had been diagnosed with relapsed APL involving the CNS and who achieved a second CR after ATO salvage therapy. Mobilization of peripheral blood stem cells (PBSC) was accomplished using a combination of granulocyte-colony stimulating factor (G-CSF) and CXCR4 blockade.

A 40-year old woman experienced extramedullary relapse of APL while on maintenance therapy after having achieved CR with ATRA containing induction chemotherapy. Due to multilocular CNS manifestation as well as molecular bone marrow involvement, ATO was started (5 cycles, 0.15 mg/kg, day 1-13) in parallel with local irradiation. Liposomal cytarabine (7 applications, 50 mg absolute/week) was applied intrathecally in order to treat meningeosis. After a molecular analysis of the bone marrow had shown negativity for PML-RARα transcripts after ATO and intrathecal therapy, G-CSF mobilization was started out of steady state in order to collect PBSC for autologous HSCT. While the WBC peaked at 31 Gpt/l, only 6/µL CD34+ cells could be measured in the peripheral blood. The corresponding apheresis yield was only 0.9×10^6/kg CD34+ PBSC. In order to achieve a target of >2×10^6/kg CD34+ PBSC the patient received the CXCR4 antagonist AMD3100 subcutaneously at a dose of 240 µg/kg 10 hours prior to the next apheresis additionally to G-CSF within a compassionate use program. CXCR4 blockade lead to an increase in WBC (44 Gpt/l) and CD34+ count (9/µL) with subsequent harvest of 1.2×10^6/kg CD34+ PBSC. Interestingly, both apheresis products were found to be PML-RARα-PCR negative (Figure 1). Sensitivity of nested PCR for PML-RARα was achieved according to the minimal target sensitivity of 10^-4.5 Three weeks later myeloablative conditioning containing 12 Gy total body irradiation (day -6 to -4) and 120 mg/kg of intravenous cyclophosphamide (day -3 to -2) was performed and followed by reinfusion of PBSC on day 0. Fast and stable trilineage engraftment was documented with neutrophils >0.5 Gpt/l and platelets > 50 Gpt/l on day +14 and +16, respectively. Three years later (day +1144 after autologous HSCT) the patient remains in complete hematologic remission without clinical signs of extramedullary disease.

Arsenic trioxide has recently been shown to play an emerging role in relapsed and refractory APL with the majority of patients achieving a complete molecular remission.6 Following molecular CR after ATO treatment, subsequent collection of PBSC and autologous HSCT after myeloablative chemotherapy is recommended but discussed controversially with regard to the best

Figure 1. PML-RARα specific PCR-Marker, lane 1) before ATO treatment, lane 2) G-CSF mobilized stem cells, lane 3) G-CSF + AMD3100 mobilized stem cells. Lanes were processed to secure cleanness since other patient samples were performed routinely in parallel. Graph: Treatment course after relapse. Local irradiation with 30 Gy starting December 2005 and 20 Gy starting in April 2006. Black arrows indicate intrathecal application of li-posomal cytarabine. White arrows pointing upward indicate apheresis #1 with G-CSF and apheresis #2 with G-CSF and AMD3100. White arrow pointing downward indicates autologous SCT after myeloablative conditioning with 12 Gy TBI and intravenous cyclophosphamide.
consolidation strategy.\textsuperscript{6,7} Harvesting a satisfactory amount of CD34\textsuperscript{+} PBSC after repetitive chemotherapy regimens might be challenging. Sequential therapy with ATO might even decrease the hematopoietic capacity. Application of AMD3100 in addition to G-CSF displays a possible option to compensate poor HSC mobilization. Albeit, leukemic blasts are known to express CXCR4 and could therefore become potential targets of AMD3100.\textsuperscript{8} Data in a murine model suggest that AMD3100 administration leads to an increased time-dependent mobilization of APL blasts by interrupting the CXCR4-SDF-1 axis.\textsuperscript{9} But for AML in general no clinical trials exist in order to confirm or disprove whether mobilizing leukemic (stem-) cells reflect a relevant problem in this setting. DiPersio et al. advised caution and stated that AMD3100 might not be intended for mobilization and harvest in patients with leukemia.\textsuperscript{10}

Our limited experience in this patient suggests at least that in case of molecular remission, no apparent mobilization of PML-RAR\textalpha positive cells occurred. Whether different subsets of leukemic stem- and progenitor cells might be differentially targeted by CXCR4 is unknown. Next leukemic blast mobilization in vitro was strictly time-dependent in the murine model with a peak of circulating APL blasts after 3 hours and return to baseline after 12 hours. Furthermore, APL blast mobilization in an in vivo model seems to be influenced by the respective microenvironment since extramedullary blasts with exclusive intraperitoneal expansion were not shown to be circulating after AMD3100 administration.\textsuperscript{9} Our case shows that even after intensified ATO treatment due to relapsed APL, PML-RAR\textalpha negative PBSC can be obtained by using the competitive CXCR4 antagonist AMD3100 to increase the number of harvested cells. These cells proved not to be contaminated by clonogenic APL cells allowing successful autologous HSCT which induced prolonged remission without further maintenance therapy.

Friedrich Stölzel, Martin Wermke, Christoph Röllig, Christian Thiede, Uwe Platzbecker, and Marine Bornhäuser
Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus Dresden, Germany

Key words: extramedullary blasts, PML-RAR\textalpha, all-trans retinoic acid.

Correspondence: Friedrich Stölzel, Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus Dresden Potschaperstr.74, 01307 Dresden, Germany. Phone: international +0049.351.4582321. Fax: international +0049.351.4585344. E-mail: friedrich.stoelzel@uniklinikum-dresden.de


References