

**Enhancement of G-CSF Mobilization of Human BM CD34+ Cells with AMD3100: Substantial Increase in the Frequency of Repopulating Cells through Tandem Administration of G-CSF and AMD3100.
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Mobilization of bone marrow (BM) hematopoietic stem cells (HSC) into the periphery can be accomplished by different modalities including the use of the CXCR-4 antagonist AMD3100. We previously demonstrated that in normal volunteers, AMD3100 induced efficient mobilization of CD34+ cells within six hours of administration and determined that the frequency of NOD/SCID repopulating cells (SRC) in the peripheral blood of these donors was 3.7-fold higher than that detected in comparable G-CSF mobilized peripheral blood. Given the short time required for mobilization of CD34+ cells with AMD3100, we investigated whether incorporation of AMD3100 into a regular 5-day schedule of G-CSF mobilization would augment the number of CD34+ in the peripheral blood and increase the frequency of SRC in these collections. Normal healthy volunteers received 4 daily injections of G-CSF (10^μg/kg). On the 5th day, subjects were divided into two groups. Those comprising the experimental arm of this study received an injection of AMD3100 (160^μg/kg) and a 5th injection of G-CSF (G-CSF/AMD3100 group) while subjects in the control group received only a 5th injection of G-CSF. Apheresis products from both groups were collected and CD34+ cells were selected using MACS columns and assessed for SRC content in limiting dilution analyses. The frequency of SRC in samples mobilized with G-CSF and one injection of AMD3100 (on day 5) was 1 in 72,118 ±10,793 cells (n=3) while that in G-CSF MPB was 1 in 144,277 ±64,459 cells (4 LDA values from 3 donors) (P=0.05). Chimerism in all mice receiving grafts of 75 x 10³ cells was 8-fold higher in recipients of G-CSF/AMD3100 cells (n=11) compared to control cells (n=16). In vitro migration assays demonstrated a 7-fold increase in the ability of G-CSF/AMD3100 CD34+ cells to respond to a chemotactic signal from SDF-1 than G-CSF-mobilized CD34+ cells. Comparison of adhesion molecule profiles revealed little differences in the expression of CD11a, CD43, CD44 and CXCR4 between G-CSF/AMD3100 and G-CSF mobilized cells. However, increased expression of CD49d and decreased expression of CD62L was observed on G-CSF/AMD3100 cells relative to G-CSF cells suggestive of the acquisition of an “engrafting” phenotype similar to that previously described by our group for murine HSC. Interestingly, in preliminary results, the frequency of SRC in a multiple myeloma patient mobilized with a single injection of AMD3100 (240^μg/kg) was 1 in 68,268 CD34+ cells, a value identical to that observed above for healthy volunteers. These results suggest that AMD3100 mobilizes a different class of primitive progenitor cells that can be assessed in NOD/SCID mice as SRC or that mobilization with G-CSF can be further augmented with AMD3100. The enhanced in vivo potential of G-CSF/AMD3100 cells may be attributed to a higher response to SDF-1, to differences in adhesion molecule expression that possibly play a role in the homing of these cells to the marrow, or both. Furthermore, AMD3100 is an efficient mobilizing agent in multiple myeloma patients suggesting the utility of this molecule in different clinical stem cell transplantation settings.