Modulating the Interaction of Stromal-cell-derived Factor-1/CXCL12 and its Receptor, CXCR4, for Enhanced Mobilisation, Homing and Engraftment of Haematopoietic Stem Cells

a report by

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Normal blood cell production – haematopoiesis – is crucial for the maintenance of health.[1] Haematopoiesis is initiated through rare populations of haematopoietic stem (HSC) and progenitor (HPC) cells that give rise to all blood-forming elements: HSCs/HPCs are found in the bone marrow of adults, where they are produced and nurtured. HSCs/HPCs are also found in high numbers in umbilical cord blood (CB) at birth, and circulate in the peripheral blood of adults – although in very low numbers. Numbers of HSCs/HPCs in adult blood can be enhanced by mobilising them out of the bone marrow into the circulation by agents such as granulocyte colony-stimulating factor (G-CSF). Because normal HSCs/HPCs can be used in a transplant setting to cure non-malignant and malignant blood-cell – as well as other non-blood-cell – disorders, knowledge of how HSC/HPC movement is regulated has clinical impact.

Stromal-cell-derived factor-1 (SDF-1)/CXCL12 is a member of the chemokine family of cytokines.[1–4] SDF-1/CXCL12 has a number of important functional effects on HSCs/HPCs,[1,5–7] including induction of chemotaxis (directed cell movement) *in vitro* and migration *in vivo* and enhancement of survival. The SDF-1/CXCL12 and its receptor, CXCR4, have been implicated in the retention of HSCs/HPCs within the bone marrow microenvironment.[7]

This article reviews recent studies that demonstrate the modulation of the SDF-1/CXCL12-CXCR4 axis for clinical advantage for HSC/HPC transplantation. It focuses first on the use of AMD3100, a low-molecular-weight antagonist of SDF-1/CXCL12 binding to CXCR4, for mobilisation of HSCs/HPCs into peripheral blood. It also focuses on ways to enhance the homing and engrafting capability of HSCs/HPCs that is limited by inhibition of a cell surface molecule, CD26/dipeptidylpeptidase IV (DPPIV).

Enhanced Mobilisation of Haematopoietic Stem and Progenitor Cells by AMD3100

G-CSF is the gold standard for mobilisation of HSCs/HPCs for use in autologous and allogeneic HSC transplantation.[9,10] However, not all patients respond well to the mobilising effects of G-CSF. Thus, additional agents are needed for mobilisation of HSCs/HPCs. In this context, AMD3100 has been of practical value for mobilising HSCs/HPCs in man,[11–17] mice,[11] dogs[18] and monkeys,[19] especially in synergy with G-CSF. Although the human studies the authors were involved in were published first,[12–14] the initial proof of principle that AMD3100 mobilises HSCs and HPCs was performed in mice, where we demonstrated that mobilisation by AMD3100 was rapid and maximal within one hour, and that a single dose of AMD3100 greatly enhanced mobilisation of HSCs/HPCs induced by G-CSF.[11] While it took one hour to maximally mobilise HSCs/HPCs to the blood of mice, it took six to nine hours to maximally mobilise human CD34+ cells (which contain HSCs/HPCs, but are not a pure population of these cells), HPC and CD34+ human cells with functional HSC capacity as assessed by their engraftment of mice with a non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) genotype.

It is postulated, but not yet definitively proved, that AMD3100 mobilises an earlier subset of HSCs than G-CSF,[11,19] and that cells mobilised with AMD3100, or the combination of AMD3100 plus G-CSF, are a higher-quality HSC than those mobilised by G-CSF.[11] This belief is supported by evaluation of the genomics of mobilised cells.[20] Structural analogues of AMD3100 are under investigation as next-generation mobilisers based on their ability to inhibit binding of SDF-1/CXCL12 to CXCR4.[21]

Chemokines macrophage inflammatory protein (MIP)-1α/CCL3[22] and GRO-β/CXCL2[23–25] have HSC/HPC-mobilising capability; both synergise with G-CSF in this capacity. Greatest mobilisation may entail combined effects of AMD3100 and G-CSF with either MIP-1α/CCL3 or GRO-β/CXCL2. Based on the minimal mobilising activity of MIP-1α/CCL3 alone in phase I clinical trials,[26] it is possible that GRO-β/CXCL2 may be a preferred reagent in double or triple combination treatment. Effects of GRO-β/CXCL2 are mediated by neutrophil-derived marrow matrix metalloproteinase 9, and GRO-β/CXCL2 mobilises early HSC characterised by enhanced homing and engrafting capabilities.[23–25]

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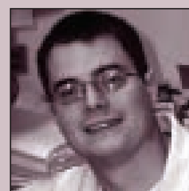

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Enhanced Homing/Engraftment of Haematopoietic Stem Cells by Inhibition of CD26/Dipeptidylpeptidase IV

Limited numbers of HSCs are a problem, especially when the source of HSC is from CB.[27] While CB is efficacious when used in transplants in children, its successes are less apparent in adults and higher-weight children, who may require more HSCs than present in a single collection of CB. Attempts to enhance the capacity of CB to treat adults and higher-weight children include *ex vivo* expansion of CB HSCs, use of multiple units of CB and intra-bone-marrow injection of cells. While mouse HSCs can be expanded *ex vivo* by combinations of growth factors/cytokines, there is little evidence at present that human HSCs have been successfully expanded *ex vivo*. Use of two CBs for transplantation has had some success, but only one of the CBs wins out; which one will win is not yet predictable, and there are indications that use of multiple CBs may enhance graft-versus-host disease (GVHD) in the recipients. This latter problem would distract from one of the advantages of single CB transplantation, where such efforts have shown less GVHD than that elicited by bone marrow transplantation. It is not clear whether intra-marrow injection will demonstrate clinical efficacy. Thus, we focused on enhancing the homing/engrafting capacity of limited numbers of HSCs.

> Granulocyte colony-stimulating factor is the gold standard for mobilisation of haematopoietic stem (HSC) and progenitor (HPC) cells for use in autologous and allogeneic HSC transplantation.

There is evidence that HSCs may not home with absolute efficiency and that this homing can be enhanced.[28] Inhibition of CD26/DPPIV has shown efficacy in enhancing homing and engraftment of mouse HSCs into lethally irradiated mice.[28] CD26 is a cell-surface dipeptidase expressed widely throughout the body.[29] It is a 110kDA glycoprotein with a small cytoplasmic region, a transmembrane section and an extracellular section containing the enzymatic activity.[29] The dipeptidylpeptidase region of CD26 cleaves the N-terminal dipeptide from various substrates, including chemokines, at the penultimate proline or alanine residue.[29–31] The action of CD26 on SDF-1/CXCL12 has biological consequences regarding HSC chemotaxis.[32]

> There is evidence that haematopoietic stem cells may not home with absolute efficiency and that this homing can be enhanced.

Immature HPCs/HSCs from human CB and mouse bone marrow express CD26 on their surface.[28,32,33] $CD34^+$ cells from human CB are positive for surface expression of CD26 (about 8% of the total population).[32] A higher percentage of $CD34^+CD38^-$ cells from human CB, a population enriched for HSCs compared with HPCs, expresses CD26 compared with the more mature $CD34^+CD38^+$ population.[34] CD26 is present on the surface of over 70% of mouse HSCs, phenotypically defined by their expression of c-kit and sca-1 antigens and lack of lineage antigens ($c\text{-}kit^+sca1^+lin^-$).[28,30] Both human and mouse haematopoietic CD26+ cells have functional CD26 peptidase activity measured by an *in vitro* enzymatic assay. That both mouse and human HSCs/HPCs express functional CD26 suggested that it may play a role in SDF-1/CXCL12-mediated functions such as chemotaxis and homing/engraftment.

HSCs/HPCs from mouse and human sources express CXCR4 and exhibit chemotaxis towards a positive gradient of SDF-1/CXCL12. Truncated SDF-1/CXCL12 blocked chemotaxis of mouse HSC and human $CD34^+$ cells to full-length SDF-1/CXCL12.[28,32] By using a selective inhibitor of CD26, such as Diprotin A or Val-Pyr,[33,35] the full-length form of SDF-1/CXCL12 was protected from truncation and significantly increased the percentage of cells able to migrate to SDF-1/CXCL12.[32] This led us to evaluate inhibition of CD26 for enhanced homing/engraftment of mouse BM HSCs.[28] Pre-treating donor HSCs/HPCs for transplant in a congenic mouse assay with Diprotin A or Val-Pyr for short periods before transplant significantly increased short-term homing, long-term engraftment, non-competitive and competitive repopulation of the donor cells and secondary repopulation of donor cells, the last being a measure of the self-renewal ability of donor HSCs.[28] Donor HSC from CD26-/- mice were also found to have increased homing and engraftment.[28] These effects have since been reproduced by at least two independent laboratories. The first showed that inhibition of CD26 enhanced engraftment of limited numbers of virally transduced HSC expressing a recombinant allogeneic MHC class I molecule.[36] The second group demonstrated that CD26 inhibition significantly increased homing and engraftment in the context of non-ablative, allogeneic *in utero* HSC transplantation.[37]

> While cord blood is efficacious when used in transplants in children, its successes are less apparent in adults and higher-weight children.

To assess clinical feasibility, we evaluated the effect of short pre-treatment of Diprotin A on engraftment of human CB $CD34^+$ cells in NOD/SCID mice.[34] CD26 inhibitor pre-treatment significantly enhanced $CD34^+$ cell engraftment, similar to that seen in mouse congenic transplant studies. Interestingly, pre-treatment of a less pure population of HSCs/HPCs (less than 40% $CD34^+$) led to greater enhancement of engraftment, suggesting effects of CD26 inhibition also on cells in this population that are not HSCs/HPCs. Differentiation of the human cells once engrafted in NOD/SCID animals was not significantly affected, suggesting that this treatment did not push cells towards one lineage as opposed to others.[34] Studies by two

independent laboratories published at the same time confirmed the enhancing effects of inhibition of CD26 on engraftment of human HSCs.[38,39] One study with CB cells suggested that CD34-CD26+ accessory cells negatively affect engraftment of the repopulating HSCs, and that inhibiting these cells as well as the CD34+CD26+ cells leads to dramatic increases in cell engraftment.[38] The other evaluated engraftment of G-CSF mobilised CD34+ cells.[39] However, these cells expressed little or no CD26, so the investigators found that engraftment was enhanced by pre-treating the recipient NOD/SCID mice rather than the donor cells.[39] We also found that *in vivo* treatment of recipient mice with Diprotin A enhances primary competitive and secondary non-competitive repopulating capacity of untreated congenic mouse bone marrow donor HSCs.[22] Testing inhibition of CD26/DPPIV in a clinical setting for enhanced engraftment of HSCs has not yet been initiated. This may entail treating either donor cells *ex vivo*, recipient *in vivo* or both *ex vivo* and *in vivo* efforts to inhibit CD26/DPPIV for enhanced engraftment of limited numbers of donor cells.

> While not yet in the clinic, inhibition of CD26/DPPIV shows promise for enhancing engraftment of limited numbers of haematopoietic stem and progenitor cells.

Conclusion

The SDF-1/CXCL12-CXCR4 axis is intimately involved in regulation of the movement and survival of HSCs/HPCs, and this is modulated by CD26/DPPIV. Manipulation of this axis by AMD3100 has already shown efficacy in enhancing mobilisation of human HSCs/HPCs induced by G-CSF, and is being increasingly used worldwide. While not yet in the clinic, inhibition of CD26/DPPIV shows promise for enhancing engraftment of limited numbers of HSC/HPC, especially for CB transplantation, where the limited numbers of CB collected are problematic for successful engraftment of adults and higher-weight children. ■

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