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The AMD3100 story: the path to the discovery of a stem cell mobilizer (Mozobil)

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ABSTRACT

AMD3100 was found to inhibit HIV-1 and HIV-2 within the 1-10 nM concentration range while not being toxic to the host cells at concentrations up to 500 μ M, thus achieving a selectivity index of approximately 100,000. The target of action was initially thought to be the viral envelope glycoprotein gp120. It appeared only to be the indirect target. The direct target of action turned out to be the co-receptor CXCR4 used by T-lymphotropic HIV strains (now referred to as X4 strains) to enter the cells. Initial (phase I) clinical trials undertaken with AMD3100, as a prelude to its development as a candidate anti-HIV drug for the treatment of AIDS, showed an unexpected side effect: an increase in the white blood cell counts. Apparently, AMD3100 specifically increased CD34+ hematopoietic stem cell counts in the peripheral blood. Stromal derived factor 1 (SDF-1), through its interaction with CXCR4, retains the stem cells in the bone marrow (a process referred to as “homing”), and AMD3100 specifically antagonizes this interaction. AMD3100 in combination with granulocyte colony-stimulating factor (G-CSF) resulted in the collection of more progenitor cells than G-CSF alone. At present, the major indication for clinical use of AMD3100 (MozobilTM) is the mobilization of hematopoietic stem cells from the bone marrow into the circulating blood for transplantation in patients with hematological malignancies such as non-Hodgkin’s lymphoma or multiple myeloma.

The start: an impurity showing anti-HIV activity

When evaluating a number of commercially available cyclam preparations for their potential inhibitory effects on the replication of human immunodeficiency virus (HIV), all these preparations (i.e. JM1498 (Fig. 1)), as expected, did not show marked anti-HIV activity [1]. However, one of these preparations proved quite effective, and, on further exploration, this activity appeared to reside in an impurity, which was characterized as a bicyclam in which the two cyclam rings were tethered by a direct carbon-carbon linkage (i.e. JM1657 (Fig. 1) [1]. As it did not prove feasible to re-synthesize JM1657, a program was launched to synthesize a bicyclam derivative with the cyclam rings tethered by an aliphatic bridge, the bicyclam derivative with the propyl bridge (i.e. JM2763) being about as active as JM1657 (Fig. 1).

A 100-fold increase in potency was noted upon replacing the aliphatic (i.e. propyl) bridge by an aromatic [i.e. 1,4-phenylene-bis(methylene)] bridge, as in JM2987 (Fig. 1) [2]. This compound proved inhibitory to the replication of HIV-1 and HIV-2 at an EC_{50} of 0.001-0.007 $\mu\text{g/ml}$, while not being toxic to the host cells at a concentration of $> 500 \mu\text{g/ml}$, thus achieving a selectivity index of $> 100,000$ (Fig. 1). Surprisingly, we did not detect any activity with JM2987 against the simian immunodeficiency virus (SIV) strains tested, which at that time (1994) was an enigmatic observation [this enigma was resolved, when several years later (1997) we demonstrated that the bicyclams specifically target the CXCR4 coreceptor of the T-lymphotropic X4 strains, while not being active against the M-macrophage tropic R5 strains (SIV strains apparently belong to this category)]. Being equipotent as the bromide salt (JM2987), the chloride salt (JM3100) (Fig. 1) was then used in all further experiments. The “JM” (for Johnson Matthey) designation was replaced by “AMD” (for AnorMED) when the further development of JM3100 was transferred from Johnson Matthey to AnorMED. From the beginning it was clear that the bicyclam derivatives (including AMD3100) interfered with an early process (viral entry) of the HIV replication cycle that had to be situated after the virus had been adsorbed to the cells but before the (reverse) transcription of the RNA genome [1,2]. It proved difficult to generate HIV strains resistant to AMD3100, and, on further analysis, these bicyclam-resistant HIV strains carried multiple mutations in the viral glycoprotein

(gp120), pointing to gp120 as the putative target for the antiviral activity of AMD3100 [3]. As it turned out, the viral glycoprotein gp120 was only an indirect target, the direct target being CXCR4, the receptor for the CXC chemokine SDF-1 (stromal derived factor), which also functions as the co-receptor (the principal receptor being CD4 (“CD” standing for “cluster of differentiation”) for the T-tropic X4 HIV strains.

CXCR4 as the direct target for the action of AMD3100

That the bicyclam AMD3100 interacted with CXCR4 as its direct target was unequivocally shown in a series of papers published in 1997-1998 [4-6]. More specifically, AMD3100 was demonstrated by Schols and colleagues [5] and Donzella and colleagues [6] to inhibit T-tropic HIV strains by selective antagonization of the SDF-1 chemokine receptor CXCR4. In fact, a nice correlation was found between the inhibitory effects of AMD3100 on (i) HIV-1 replication, (ii) binding of CXCR4 monoclonal antibody and (iii) SDF-1-mediated signal transduction (SDF-1-induced Ca^{2+} flux) (Fig. 2), as reviewed by De Clercq [7].

Inhibition of SDF-1-induced Ca^{2+} flux actually allows a very rapid (within 1-2 min) measurement of the blockade of CXCR4 by AMD3100 [8]. Thus, within the HIV-1 replicative cycle AMD3100 interferes with the binding of the HIV gp120 to the CXCR coreceptor after it has first been bound to the CD4 receptor, and before the HIV gp41 initiates the fusion of the viral envelope membrane with the cell membrane (Fig. 3) [9,10].

As reviewed previously [11], AMD3100, akin to SDF-1, specifically blocks those (T-tropic) virus strains using CXCR4 as the co-receptor, whereas neither AMD3100 nor SDF-1 exhibit any activity against the M-tropic virus strains using CCR5 as the co-receptor (Table 1) [5]. *Vice versa*, RANTES (the natural ligand of CCR5) effectively inhibited the M-tropic R5 virus strains, while not being active against the T-tropic R4 virus strains. AMD3100 is a highly specific CXCR4 antagonist inhibiting SDF-1-mediated Ca^{2+} flux in a number of cells expressing CXCR4, but has no inhibitory effect on chemokine-induced signalling from CXCR1, CXCR2, CXCR3, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8 or CCR9 [12]. The principal interaction points of AMD3100 with CXCR4 may be the aspartic

acid residues (particularly at positions 171 and 262) located at the extracellular side of CXCR4 (Fig. 4) [13]. Thus, AMD3100 is a specific antagonist of CXCR4, is not cross-reactive with other chemokine receptors, and is not an agonist of CXCR4 [14].

AMD3100 has shown antiviral activity (as measured by p24 production) in SCID-hu Thy/Liv mice infected with a CXCR4-using clinical HIV isolate (reduction of viral load by 0.9 log₁₀ HIV RNA copies/ml) [15], and proof of concept (POC) that AMD3100 is also effective in a patient infected with an X4 HIV-1 strain has been provided [16]. This was the only patient in this study that had a purely CXCR4-using virus. Overall, the average change in viral load across all patients was + 0.03 log₁₀ [17]. AMD3100 has the ability to suppress both X4-tropic and dual (X4 and R5) tropic variants that effectively use CXCR4 to enter the cells [18].

Stem cell mobilization by AMD3100

When AMD3100 was submitted to phase I clinical trials in normal volunteers (before embarking on the phase II clinical trials to evaluate its antiviral activity in HIV-infected patients), an unexpected side effect was noted: a rapid increase in white blood cell (WBC) counts peaking at 6 hours following the intravenous infusion of AMD3100 [19] (Fig. 5). The WBC response was clearly dose-dependent over the dosage range used (10, 20, 40 and 80 µg/kg) (Fig. 5B). These results were later confirmed by Lack et al. [20] over a dosage range of 40, 80, 160 and 240 µg/kg (Fig. 6B). On closer inspection, the white blood cells mobilized by AMD3100 appeared to carry the marker CD34, and thus could be characterized as hematopoietic stem cells [21]. The kinetics of mobilization of CD34⁺ cells into the peripheral blood (Fig. 7) [21] with a nadir at 6-9 hours following intravenous administration of AMD3100 was almost identical to that noted for the total white blood cell counts (Fig. 6B).

Circulating CD34⁺ cells increased 5-fold after administration of AMD3100 at 80 µg/kg and 15.5-fold after administration of AMD3100 at 240 µg/kg, both at 9 hours after injection. Myeloid progenitor cells-colony forming unit granulocyte-macrophage (CFU-GM); CFU-granulocyte, eosinophil, monocyte, megakaryocyte (CFU-GEMM); and burst forming

units-erythroid showed similar increases in mobilization to the blood with increasing doses of AMD3100 [22].

AMD3100 (administered at 160 µg/kg on day 5) was then found to act synergistically with granulocyte-colony stimulating factor (G-CSF administered at 10 µg/kg/day on days 1-4) [23]. Addition of AMD3100 (to G-CSF) resulted in a tripling of circulating CD34⁺ cells within 10 hours after administration (2.7-fold increase, range: 1.1-6.9-fold) while no mobilization occurred of B cells, tumor cells, or natural killer T-cell subsets such as CD2, CD3, CD4 or CD8 [24].

For the collection of CD34⁺ cells to be used for autologous transplantation, the target number of CD34⁺ cells is 4.0×10^6 /kg. With G-CSF (10 µg/kg/day for 5 days) the number of CD34⁺ cells collected was 3.73×10^6 /kg; with AMD3100 (240 µg/kg on day 5), the number of CD34⁺ cells collected was 3.02×10^6 /kg; with both G-CSF (10 µg/kg/day for 5 days) and AMD3100 (160 µg/kg on day 5) combined, the number of CD34⁺ cells collected was 9.88×10^6 /kg [23]. Broxmeyer and colleagues [25] confirmed that AMD3100 induced a rapid mobilization of hematopoietic progenitor cells (HPCs) and synergistically augmented G-CSF-induced mobilization of HPCs. Flomenberg et al. [26] further ascertained that the combination of AMD3100 plus G-CSF is generally safe, effective, and superior to G-CSF alone for autologous HPC mobilization.

Furthermore, the CD34⁺ peripheral blood progenitor cells (PBPCs) mobilized by AMD3100 in combination with G-CSF express significantly higher amounts of genes that potentially promote superior engraftment after myeloablative therapy than G-CSF-mobilized CD34⁺ PBPCs [27]. Indeed, AMD3100 mobilizes a population of hematopoietic stem cells with intrinsic characteristics different from those hematopoietic stem cells mobilized with G-CSF, suggesting that the mechanism of AMD3100-mediated hematopoietic stem cell mobilization is fundamentally different from G-CSF-mediated hematopoietic stem cell mobilization [28].

The ability of AMD3100 to mobilize hematopoietic progenitor stem cells from the bone marrow has been demonstrated in a number of experimental model systems: i.e. both

autologous and allogeneic peripheral blood mononuclear cells (PBMCs) in dogs [29]; hematopoietic progenitor stem cells (from the femoral bone marrow) in mice and humans [30]; and hematopoietic stem cells in Fanconi anemia knockout mice [31].

Holtan and colleagues reported that AMD3100 not only increases the autograft absolute lymphocyte counts (4.16×10^9 lymphocytes/kg *versus* 0.288×10^9 lymphocytes/kg), but also that with a median follow-up of 20 months, no relapses occurred in the AMD3100 group (compared with 15 of 29 in the control group) of non-Hodgkin lymphoma patients after autologous stem cell transplantation [32]. Calandra and colleagues [33] found that AMD3100 when given with G-CSF mobilized a sufficient number of CD34⁺ cells in non-Hodgkin's lymphoma, multiple myeloma, and Hodgkin's disease patients who could not deliver sufficient cells for autologous transplant following other mobilization regimens [33]. With one dose of AMD3100 (240 µg/kg) by subcutaneous injection, two-thirds of the donors collected an allograft with a number of CD34⁺ cells sufficient for transplantation [34], and, in this sense, AMD3100 may provide a more rapid and possibly less toxic and less cumbersome alternative to the traditional G-CSF-based CD34⁺ cell mobilization in normal donors.

Thus, in conclusion, AMD3100 is a rapid and efficient stem cell-mobilizing agent [35]; once in the circulating blood, stem cells can be collected for use in autologous stem cell transplantation in patients with haematological malignancies such as multiple myeloma or non-Hodgkin's lymphoma [36]. There is no increased risk that in the patients with multiple myeloma or non-Hodgkin's lymphoma AMD3100 may itself mobilize tumor cells [37-39].

Any potential of AMD3100 in the treatment of arthritis, ischemia, cancer or virus infections (other than HIV) ?

To the extent that in the pathogenesis of any given disease the CXCR4 interaction with SDF-1 plays a critical role, AMD3100, as a potent and selective CXCR4 antagonist, may be expected to interfere with the clinical course of this disease. Hence AMD3100 has been found to inhibit collagen-induced arthritis in mice, concomitantly with the inhibition of SDF-1-elicited intracellular Ca²⁺ flux in Mac-1⁺ cells harvested from the spleens of these mice [40].

Several human breast cancer cell lines have been found to express CXCR4 [41], and it is therefore surprising that the potential of AMD3100 in the treatment of breast cancer has not been further followed up in recent years.

Studies of Rubin et al. [42] have shown that systemic administration of AMD3100 inhibits the growth of intracranial glioblastoma and medulloblastoma xenographs. CXCR4 is apparently critical to the progression of diverse brain malignancies, thus providing a scientific rationale for clinical evaluation of AMD3100 in the treatment of malignant brain tumors [42].

CXCR4 has proven to be essential for the trans-endothelial migration of Waldenström macroglobulinemia (WM) cells, and this process is inhibited by AMD3100 [43]. AMD3100 may be useful in future clinical applications in the regulation of trafficking of WM cells and increasing sensitivity to therapeutic agents.

Furthermore, AMD3100 has been shown to effectively reduce tumor growth in nude mice inoculated with anaplastic thyroid carcinoma (ATC) cells and may well represent a novel potential strategy in the treatment of ATC, a rare thyroid cancer with extremely poor prognosis [44].

CXCR4 and SDF-1 are overexpressed in human pituitary adenomas, and as CXCR4 activation may contribute to pituitary cell proliferation, and, possibly, adenoma development as well, AMD3100 could play a role in suppressing the growth of pituitary adenomas in humans [45].

AMD3100 has also been reported to decrease invasion of human colorectal cancer cells *in vitro* [46]. Thus, blocking the interaction of CXCR4 with SDF-1 (CXCL12) may provide a novel means of preventing the invasion and metastasis of colorectal cancer.

AMD3100 is a potent and rapid mobilizer of angiogenic cells [47], which may stimulate angiogenesis at sites of ischemia through a paracrine mechanism [48], including ischemia in a diabetic environment [49].

Recently, McCandless et al. [50] reported that AMD3100 promoted the entry into the CNS parenchyma of CD8⁺ T-lymphocytes specific for WNV (West Nile virus), reduced viral load within the CNS, and significantly improved survival after WNV infection.

What about anti-HIV activity of CXCR4 antagonists ? From AMD3100 to AMD070.

Guided by AMD3100 as the prototype, several small-molecule CXCR4 antagonists (i.e. based on two aromatic amine moieties connected by a *para*-xylylene group) were synthesized, the most effective congener inhibiting the interaction of CXCR4 with SDF-1 at a concentration as low as 1.2 nM [51].

The identification of AMD070 as a potent, orally bioavailable CXCR4 antagonist which strongly inhibits HIV infectivity (at an EC₅₀ of 1-10 nM) dates already from more than 5 years ago, but has so far only been communicated in abstract form [52]. AMD070 was found to inhibit X4 HIV replication in 5 different CD4⁺ T-cell lines, CXCR4-transfected cell lines and peripheral blood mononuclear cells (PBMs). AMD070, akin to AMD3100, had no activity against R5 HIV-1 variants. Unlike AMD3100, AMD070 cannot be considered a bicyclam (Fig. 8). The structure of AMD070 was revealed by Kazmierski et al. in their report [53]. Proof of concept that AMD070 (originally referred to as AMD11070) can selectively inhibit X4-tropic virus in HIV-1-infected patients was provided by Saag and colleagues [54] and Moyle and colleagues [55]. Like AMD3100, AMD070 induced a dose-related elevation of the WBC counts, which can be attributed to CXCR4 blockade. Whether AMD070, which, unlike AMD3100 (which has to be given parenterally) can be administered orally, has any future potential as either an anti-HIV agent, or a stem cell-mobilizing agent, or, possibly, an agent inhibitory to any other CXCR4-mediated process, remains subject of further study.

What is for sure, is that if CRIs (coreceptor inhibitors), whether CXCR4 inhibitors or CCR5 inhibitors, are used as anti-HIV agents, they should be used in combination regimens, so as to prevent selection of CXCR4-using T-tropic variants if only CCR5 inhibitors are used, and to prevent selection of CCR5-using M-tropic variants if only CXCR4 inhibitors are used [56,57].

The immediate future use for AMD3100, now known as Mozobil™: hematopoietic stem cell mobilization

Genzyme Corporation announced on 17 June 2008 that it has submitted marketing applications in both the United States and the European Union for Mozobil™ (plerixafor injection) (previously known as AMD3100), a product candidate intended to enhance mobilization of hematopoietic stem cells for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma. On 15 December 2008 Genzyme Corporation announced that the US FDA had granted marketing approval for Mozobil™. European approval is expected in 2009. Additional global applications in up to 60 countries are expected to follow [58].

Genzyme plans to launch Mozobil in the U.S. and Europe in 2009. Upon commercial launch, Mozobil will be marketed and sold by Genzyme's existing Transplant sales force, which has a commercial presence in more than 55 countries worldwide. Approximately 55,000 stem cell transplants are performed each year for multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, and other conditions in markets where Genzyme has a commercial infrastructure, including the United States, Europe, Latin America and the Asian Pacific countries [59].

Numerous Genzyme and investigator-sponsored trials are planned or underway to study Mozobil's use in other settings such as allogeneic hematopoietic stem cell transplants. Genzyme is also studying the use of Mozobil to improve the efficacy of chemotherapy and/or immunotherapy in various types of hematologic malignancies such as chronic lymphocytic leukemia and acute myelogenous leukemia, and is pursuing preclinical work to explore the role that Mozobil may play in cord blood transplantation, solid organ transplantation, cardiovascular disease, renal ischemic disease, and a variety of additional types of solid tumor malignancies [59].

Conclusion

The bicyclam AMD3100 represents a typical example of the meandering path of drug discovery, hopping from one unexpected (“serendipitous”) observation to another, ultimately leading to the clinical use for which originally it was neither conceived nor developed. Bicyclam JM1657 (JM standing for Johnson Matthey) was originally identified as an impurity in a (mono)cyclam preparation evaluated for its potential inhibition of the replication of HIV (human immunodeficiency virus). While most of the commercially available cyclam preparations were virtually inactive against HIV, one particular preparation showed a marked anti-HIV activity, apparently due to an impurity. This impurity was purified and identified as the bicyclam JM1657 (with the 2 cyclam rings tethered through a direct carbon-carbon linkage). While re-synthesis of this compound failed, it was the onset of a large synthetic effort leading first to the synthesis of JM2763 (with the 2 cyclam rings tethered by an aliphatic bridge), and, then to the synthesis of JM3100 (later designated as AMD3100, AMD standing for AnorMED) (with the 2 cyclam rings tethered by an aromatic bridge). AMD3100 was found to inhibit HIV [both HIV-1 and HIV-2, but not SIV (simian immunodeficiency virus)], within the 1-10 nM concentration range while not being toxic to the host cells at concentrations up to 500 μ M, thus achieving a selectivity index of approximately 100,000. The target of action was initially thought to be the viral envelope glycoprotein gp120. It appeared only to be the indirect target. The direct target of action turned out to be the co-receptor CXCR4 used by T-lymphotropic HIV strains to enter the cells (the primary receptor being CD4). Consequently, the anti-HIV activity of AMD3100 was confined to those HIV strains (now referred to as X4 strains) using CXCR4 as co-receptor [the normal ligand for CXCR4 is the chemokine SDF-1 (stromal derived factor), now also referred to as CXCL12]. Initial (phase I) clinical trials undertaken with AMD3100, as a prelude to its development as a candidate anti-HIV drug for the treatment of AIDS, showed an unexpected side effect: an increase in the white blood cell (WBC) counts. On closer inspection it appeared that AMD3100 specifically increased CD34+ (hematopoietic stem) cell counts in the peripheral blood. SDF-1, through its interaction with CXCR4, retains the stem cells in the bone marrow

(a process referred to as “homing”). One single parenteral injection of AMD3100 (at a dose of 240 µg/kg) suffices to mobilize the stem cells from the bone marrow into the bloodstream, where they can be collected by leukapheresis. AMD3100 in combination with granulocyte colony-stimulating factor (G-CSF) results in the collection of more progenitor cells than G-CSF alone. AMD3100 is a more rapid mobilizer of hematopoietic stem cells than G-CSF. At present, the major indication for clinical use of AMD3100 (Mozobil™) is the mobilization of hematopoietic stem cells from the bone marrow into the circulating blood, from where the stem cells can be harvested for use in transplantation in patients with hematological malignancies such as non-Hodgkin’s lymphoma or multiple myeloma.

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References

- [1] De Clercq E, Yamamoto N, Pauwels R, Baba M, Schols D, Nakashima H, Balzarini J, Debyser Z, Murrer BA, Schwartz D, Thornton D, Bridger G, Fricker S, Henson G, Abrams M, Picker D. Potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclams interacting with a viral uncoating event. *Proc Natl Acad Sci USA* 1992; 89:5286-90.
- [2] De Clercq E, Yamamoto N, Pauwels R, Balzarini J, Witvrouw M, De Vreese K, Debyser Z, Rosenwirth B, Peichl P, Datema R, Thornton D, Skerlj R, Gaul F, Padmanabhan S, Bridger G, Henson G, Abrams M. Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. *Antimicrob Agents Chemother* 1994; 38:668-74.
- [3] De Vreese K, Van Nerum I, Vermeire K, Anné J, De Clercq E. Sensitivity of human immunodeficiency virus to bicyclam derivatives is influenced by the three-dimensional structure of gp120. *Antimicrob Agents Chemother* 1997; 41:2616-20.
- [4] Schols D, Esté JA, Henson G, De Clercq E. Bicyclams, a class of potent anti-HIV agents, are targeted at the HIV coreceptor fusin/CXCR-4. *Antiviral Res* 1997; 35:147-56.
- [5] Schols D, Struyf S, Van Damme J, Esté JA, Henson G, De Clercq E. Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J Exp Med* 1997; 186:1383-8.
- [6] Donzella GA, Schols D, Lin SW, Esté JA, Nagashima KA, Maddon PJ, Allaway GP, Sakmar TP, Henson G, De Clercq E, Moore JP. AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. *Nat Med* 1998; 4:72-7.
- [7] De Clercq E. Inhibition of HIV infection by bicyclams, highly potent and specific CXCR4 antagonists. *Mol Pharmacol* 2000; 57:833-9.
- [8] De Clercq E. Hamao Umezawa Memorial Award Lecture: "An Odyssey in the Viral Chemotherapy Field". *Int J Antimicrob Agents* 2001; 18:309-28.
- [9] De Clercq E. The bicyclam AMD3100 story. *Nature Rev Drug Discovery* 2003; 2:581-7.
- [10] Esté JA, Telenti A. HIV entry inhibitors. *Lancet* 2007; 370:81-8.
- [11] De Clercq E. Potential clinical application of the CXCR4 antagonist bicyclam AMD3100. *Mini Rev Med Chem* 2005; 5:805-24/
- [12] Hatse S, Princen K, Bridger G, De Clercq E, Schols D. Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. *FEBS Lett* 2002; 527:255-62.
- [13] Hatse S, Princen K, Gerlach L-O, Bridger G, Henson G, De Clercq E, Schwartz TW, Schols D. Mutation of Asp¹⁷¹ and Asp²⁶² of the chemokine receptor CXCR4 impairs its

- coreceptor function for human immunodeficiency virus-1 entry and abrogates the antagonistic activity of AMD3100. *Mol Pharmacol* 2001; 60:164-73.
- [14] Fricker SP, Anastassov V, Cox J, Darkes MC, Grujic O, Idzan SR, Labrecque J, Lau G, Mosi RM, Nelson KL, Qin L, Santucci Z, Wong RS. Characterization of the molecular pharmacology of AMD3100: a specific antagonist of the G-protein coupled chemokine receptor, CXCR4. *Biochem Pharmacol* 2006; 72:588-96.
- [15] Datema R, Rabin L, Hincenbergs M, Moreno MB, Warren S, Linquist V, Rosenwirth B, Seifert J, McCune JM. Antiviral efficacy in vivo of the anti-human immunodeficiency virus bicyclam SDZ SID 791 (JM 3100), an inhibitor of infectious cell entry *Antimicrob Agents Chemother* 1996; 40:750-4.
- [16] Schols D, Claes S, De Clercq E, Hendrix C, Bridger G, Calandra G, Henson GW, Fransen S, Huang W, Whitcomb JM, Petropoulos CJ, AMD-3100 HIV Study Group. AMD-3100, a CXCR4 antagonist, reduced HIV viral load and X4 virus levels in humans. Abstracts, 9th Conference on Retroviruses and Opportunistic Infections, Seattle, Washington, USA, 24-28 February 2002, p 53, no 2.
- [17] Hendrix CW, Collier AC, Lederman MM, Schols D, Pollard RB, Brown S, Jackson JB, Coombs RW, Giesby MJ, Flexner CW, Bridger GJ, Badel K, MacFarland RT, Henson GW, Calandra G. Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J Acquir Immune Defic Syndr* 2004; 37:1253-62.
- [18] Fransen S, Bridger G, Whitcomb JM, Toma J, Stawiski E, Parkin N, Petropoulos CJ, Huang W. Suppression of dualtropic human immunodeficiency virus type 1 by the CXCR4 antagonist AMD3100 is associated with efficiency of CXCR4 use and baseline virus composition. *Antimicrob Agents Chemother* 2008; 52:2608-15.
- [19] Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E, Bridger G, Henson GW. Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. *Antimicrob Agents Chemother* 2000; 44:1667-73.
- [20] Lack NA, Green B, Dale DC, Calandra GB, Lee H, MacFarland RT, Badel K, Liles WC, Bridger G. A pharmacokinetic-pharmacodynamic model for the mobilization of CD34+ hematopoietic progenitor cells by AMD3100. *Clin Pharmacol Ther* 2005; 77:427-36.
- [21] Liles WC, Broxmeyer HE, Rodger E, Wood B, Hübel K, Cooper S, Hangoc G, Bridger GJ, Henson GW, Calandra G, Dale DC. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood* 2003; 102:2728-2730.
- [22] Hübel K, Liles WC, Broxmeyer HE, Rodger E, Wood B, Cooper S, Hangoc G, Macfarland R, Bridger GJ, Henson GW, Calandra G, Dale DC. Leukocytosis and

- mobilization of CD34⁺ hematopoietic progenitor cells by AMD3100, a CXCR4 antagonist. *Support Cancer Ther* 2004; 1:165-72.
- [23] Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G, Christensen J, Wood B, Price TH, Dale DC. Augmented mobilization and collection of CD34⁺ hematopoietic cells from normal human volunteers stimulated with granulocyte-colony-stimulating factor by single-dose administration of AMD3100, a CXCR4 antagonist. *Transfusion* 2005; 45:295-300.
- [24] Reddy GK, Crawford J, Jain VK. The role of plerixafor (AMD3100) in mobilizing hematopoietic progenitor cells in patients with hematologic malignancies. *Support Cancer Ther* 2006; 3:73-6.
- [25] Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, Liles WC, Li X, Graham-Evans B, Campbell TB, Calandra G, Bridger G, Dale DC, Srouf EF. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 2005; 201:1307-18.
- [26] Flomenberg N, Devine SM, DiPersio JF, Liesveld JL, McCarty JM, Rowley SD, Vesole DH, Badel K, Calandra G. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood* 2005; 106: 1867-74.
- [27] Fruehauf S, Seeger T, Maier P, Li L, Weinhardt S, Laufs S, Wagner W, Eckstein V, Bridger G, Calandra G, Wenz F, Zeller WJ, Goldschmidt H, Ho AD. The CXCR4 antagonist AMD3100 releases a subset of G-CSF-primed peripheral blood progenitor cells with specific gene expression characteristics. *Exp Hematol* 2006; 34:1052-9.
- [28] Larochelle A, Krouse A, Metzger M, Orlic D, Donahue RE, Fricker S, Bridger G, Dunbar CE, Hematti P. AMD3100 mobilizes hematopoietic stem cells with long-term repopulating capacity in nonhuman primates. *Blood* 2006; 107:3772-8.
- [29] Burroughs L, Mielcarek M, Little MT, Bridger G, Macfarland R, Fricker S, Labrecque J, Sandmaier BM, Storb R. Durable engraftment of AMD3100-mobilized autologous and allogeneic peripheral-blood mononuclear cells in a canine transplantation model. *Blood* 2005; 106:4002-8.
- [30] Martin C, Bridger GJ, Rankin SM. Structural analogues of AMD3100 mobilise haematopoietic progenitor cells from bone marrow in vivo according to their ability to inhibit CXCL12 binding to CXCR4 in vitro. *Br J Haematol* 2006; 134:326-9.
- [31] Pulliam AC, Hobson MJ, Ciccone SL, Li Y, Chen S, Srouf EF, Yang FC, Broxmeyer HE, Clapp DW. AMD3100 synergizes with G-CSF to mobilize repopulating stem cells in Fanconi anemia knockout mice. *Exp Hematol* 2008; 36:1084-90.
- [32] Holtan SG, Porrata LF, Micallef IN, Padley DJ, Inwards DJ, Ansell SA, Johnston PB, Gastineau DA, Markovic SN. AMD3100 affects autograft lymphocyte collection and

- progression-free survival after autologous stem cell transplantation in non-Hodgkin lymphoma. *Clin Lymphoma Myeloma* 2007; 7:315-8.
- [33] Calandra G, McCarty J, McGuirk J, Tricot G, Crocker S-A, Badel K, Grove B, Dye A, Bridger G. 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; 41:331-8.
- [34] Devine SM, Vij R, Rettig M, Todt L, McGlauchlen K, Fisher N, Devine H, Link DC, Calandra G, Bridger G, Westervelt P, DiPersio JF. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood* 2008; 112:990-8.
- [35] Cashen AF, Nervi B, DiPersio J. AMD-3100: CXCR4 antagonist and rapid stem cell-mobilizing agent. *Future Oncol* 2007; 3:19-27.
- [36] Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K, Calandra G, DiPersio JF. Rapid mobilization of CD34⁺ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. *J Clin Oncol* 2004; 22:1095-102.
- [37] Devine S, Gazitt Y, Calandra G. Clinical use of AMD3100 to mobilize CD34⁺ cells in patients affected by non-Hodgkin's lymphoma or multiple myeloma (correspondence). *J Clin Oncol* 2005; 23:3872-3.
- [38] Flomenberg N, DiPersio J, Calandra G. Role of CXCR4 chemokine receptor blockade using AMD3100 for mobilization of autologous hematopoietic progenitor cells. *Acta Haematol* 2005; 114:198-205.
- [39] Gazitt Y, Freytes CO, Akay C, Badel K, Calandra G. Improved mobilization of peripheral blood CD34⁺ cells and dendritic cells by AMD3100 plus granulocyte-colony-stimulating factor in non-Hodgkin's lymphoma patients. *Stem Cells Dev* 2007; 16:657-66.
- [40] Matthys P, Hatse S, Vermeire K, Wuyts A, Bridger G, Henson GW, De Clercq E, Billiau A, Schols D. AMD3100, a potent and specific antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN-gamma receptor-deficient mice. *J Immunol* 2001; 167:4686-92.
- [41] Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; 410:50-6.
- [42] Rubin JB, Kung AL, Klein RS, Chan JA, Sun Y, Schmidt K, Kieran MW, Luster AD, Segal RA. A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc Natl Acad Sci USA* 2003; 100:13513-8.

- [43] Ngo HT, Leleu X, Lee J, Jia X, Melhem M, Runnels J, Moreau AS, Burwick N, Azab AK, Roccaro A, Azab F, Sacco A, Farag M, Sackstein R, Ghobrial IM. SDF-1/CXCR4 and VLA-4 interaction regulates homing in Waldenstrom macroglobulinemia. *Blood* 2008; 112:150-8.
- [44] De Falco V, Guarino V, Avilla E, Castellone MD, Salerno P, Salvatore G, Faviana P, Basolo F, Santoro M, Melillo RM. Biological role and potential therapeutic targeting of the chemokine receptor CXCR4 in undifferentiated thyroid cancer. *Cancer Res* 2007; 67:11821-9.
- [45] Barbieri F, Bajetto A, Stumm R, Pattarozzi A, Porcile C, Zona G, Dorcaratto A, Ravetti JL, Minuto F, Spaziante R, Schettini G, Ferone D, Florio T. Overexpression of stromal cell-derived factor 1 and its receptor CXCR4 induces autocrine/paracrine cell proliferation in human pituitary adenomas. *Clin Cancer Res* 2008; 14:5022-32.
- [46] Li JK, Yu L, Shen Y, Zhou LS, Wang YC, Zhang JH. Inhibition of CXCR4 activity with AMD3100 decreases invasion of human colorectal cancer cells in vitro. *World J Gastroenterol* 2008; 14:2308-13.
- [47] Shepherd RM, Capoccia BJ, Devine SM, DiPersio J, Trinkaus KM, Ingram D, Link DC. Angiogenic cells can be rapidly mobilized and efficiently harvested from the blood following treatment with AMD3100. *Blood* 2006; 108:3662-7.
- [48] Capoccia BJ, Shepherd RM, Link DC. G-CSF and AMD3100 mobilize monocytes into the blood that stimulate angiogenesis in vivo through a paracrine mechanism. *Blood* 2006; 108:2438-45.
- [49] Jiao C, Fricker S, Schattelman GC. The chemokine (C-X-C motif) receptor 4 inhibitor AMD3100 accelerates blood flow restoration in diabetic mice. *Diabetologia* 2006; 49:2786-9.
- [50] McCandless EE, Zhang B, Diamond MS, Klein RS. CXCR4 antagonism increases T cell trafficking in the central nervous system and improves survival from West Nile virus encephalitis. *Proc Natl Acad Sci USA* 2008; 105:11270-5.
- [51] Zhan W, Liang Z, Zhu A, Kurtkaya S, Shim H, Snyder JP, Liotta DC. Discovery of small molecule CXCR4 antagonists. *J Med Chem* 2007; 50:5655-64.
- [52] Schols D, Claes S, Hatse S, Princen K, Vermeire K, De Clercq E, Skerlj R, Bridger G, Calandra G. Anti-HIV activity profile of AMD070, an orally bioavailable CXCR4 antagonist H. 10th Conference on Retroviruses and Opportunistic Infections, Boston, MA, USA, 10-14 February 2003. Abstracts, p 257, no 563.
- [53] Kazmierski WM, Gudmundsson KS, Piscitelli SC. Small molecule CCR5 and CXCR4-based viral entry inhibitors for anti-HIV therapy currently in development. *Annu Reports Med. Chem* 2007; 42:301-20

- [54] Saag M, Rosenkranz S, Becker S, Klingman K, Kallungal B, Zadzilka A, Coakley E, Acosta E, Calandra G, Johnson V. Proof of concept of ARV activity of AMD11070 (an orally administered CXCR4 entry inhibitor); results of the first dosing cohort A studied in ACTG protocol A5210. 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, CA, USA, 25-28 February 2007. Abstracts, p 251, no 512.
- [55] Moyle G, DeJesus E, Boffito M, Wong R, Coakley E, Gibney C, Badel K, Calandra G, Bridger G, Becker S. CXCR4 antagonism: proof of activity with AMD11070. 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, CA, USA, 25-28 February 2007. Abstracts, p 250, no 511.
- [56] Moncunill G, Armand-Ugón M, Pauls E, Clotet B, Esté JA. HIV-1 escape to CCR5 coreceptor antagonism through selection of CXCR4-using variants in vitro. *AIDS* 2008; 22:23-31.
- [57] Nakata H, Steinberg SM, Koh Y, Maeda K, Takaoka Y, Tamamura H, Fujii N, Mitsuya H. Potent synergistic anti-human immunodeficiency virus (HIV) effects using combinations of the CCR5 inhibitor aplaviroc with other anti-HIV drugs. *Antimicrob Agents Chemother* 2008; 52:2111-9.
- [58] Genzyme press release, 17 June 2008.
- [59] <http://www.webwire.com/>, 18 September 2008.

Table 1. Anti-HIV activity profile of AMD3100 correlated with coreceptor use

Strain	Coreceptor used	EC ₅₀ (ng/ml)		
		AMD3100	SDF-1 α	RANTES
<u>T-tropic</u>				
HIV-1 IIIB	CXCR4	2	20	> 1,000
HIV-1 RF	CXCR4	5	50	> 1,000
HIV-1 NL4-3	CXCR4	3	100	> 1,000
HIV-2 ROD	CXCR4	7	55	> 1,000
<u>M-tropic</u>				
HIV-1 BaL	CCR5	> 25,000	> 1,000	25
HIV-1 SF-162	CCR5	> 25,000	> 1,000	5
HIV-1 ADA	CCR5 (CCR2b, CCR3)	> 25,000	> 1,000	10
HIV-1 JR-FL	CCR5 (CCR2b, CCR3)	> 25,000	> 1,000	4

According to Schols et al. [5].

Legends to the Figures

Fig. 1. Structures and HIV-inhibitory effects of JM1498, JM1657, JM2763, JM2987 and JM3100.

Fig. 2. Correlation between inhibitory effects of AMD3100 on HIV-1 replication, CXCR4 mAb binding and SDF-1-mediated signal transduction. According to De Clercq [7].

Fig. 3. Mechanism of action of bicyclams: inhibiting viral entry by blocking the CXCR4 receptor. During the viral adsorption process, a the viral envelope glycoprotein gp120 interacts with the CD4 receptor at the cell membrane. b Subsequently, gp120 interacts with the co-receptor CXCR4 for T-tropic (X4) HIV strains, whereupon c the viral glycoprotein gp41 anchors into the cell membrane. The bicyclams block the interaction between gp120 and CXCR4. According to De Clercq [9].

Fig. 4. Amino acid sequence and membrane organization of the chemokine receptor CXCR4. According to Hatse et al. [13].

Fig. 5A. Single-dose AMD3100 pharmacokinetics following 15-min. intravenous infusion (median and range). According to Hendrix et al. [19].

Fig. 5B. WBC ratio *versus* time compared to AMD3100 concentration *versus* time following single-dose intravenous AMD3100 administration. According to Hendrix et al. [19].

Fig. 6A. AMD3100 plasma concentrations *versus* time in healthy human volunteers. The points represent the experimental data (median). The error bars represent the interquartile range (shown for representative data sets) for 40 $\mu\text{g/kg}$ (●), 80 $\mu\text{g/kg}$ (○), 160 $\mu\text{g/kg}$ (■), 240

$\mu\text{g/kg}$ ($\blacklozenge$), and $320 \mu\text{g/kg}$ ($\diamond$). The lines represent the population-predicted concentrations from the pharmacokinetic model. According to Lack et al. [20].

Fig. 6B. Total white blood cell counts *versus* time. The points represent the experimental data (median). The error bars represent the interquartile range (shown for representative data sets) for $40 \mu\text{g/kg}$ ($\bullet$), $80 \mu\text{g/kg}$ (O), $160 \mu\text{g/kg}$ ($\blacksquare$), and $240 \mu\text{g/kg}$ ($\diamond$). According to Lack et al. [20].

Fig. 7. Dose-response analysis of AMD3100-induced mobilization of CD34^+ cells into peripheral blood. Healthy human volunteers received a single subcutaneous injection of AMD3100 at the following doses: $40 \mu\text{g/kg}$ ($n = 3$; solid line); $80 \mu\text{g/kg}$ ($n = 10$; O); $160 \mu\text{g/kg}$ ($n = 5$; $\blacktriangle$); and $240 \mu\text{g/kg}$ ($n = 5$; ∇). Peripheral venous blood was withdrawn at time intervals after drug administration, and FACS analysis was used to determine the concentration of CD34^+ cells. Each value represents the mean $\pm$ SEM. According to Liles et al. [21].

Fig. 8. Structures of AMD070 and AMD3100.

Anti-HIV activity of bicyclams in MT-4 cells

Compound	Structure	EC ₅₀ (μM)		CC ₅₀ (μM)
		HIV-1(III _B)	HIV-2(ROD)	
JM1498		399	150	1248
JM1657		0.144	1.01	319
JM2763		0.248	1.00	> 622
JM2987	8 HBr 2 H ₂ O	0.005	0.010	> 500
JM3100	8 HCl 2 H ₂ O	0.003	0.009	> 500

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

AMD3100 **AMD070**

Fig. 8

The AMD3100 story: the path to the discovery of a stem cell mobilizer (Mozobil)

Biochemical Pharmacology, ... 2009

Erik De Clercq

AMD3100, Mozobil, Plerixafor

1,1'-[1,4-phenylenebis(methylene)]-bis -1,4,8,11-tetraazacyclotetradecane

Originally discovered as an anti-HIV agent, then found to specifically act as an antagonist of the chemokine receptor CXCR4, and finally licensed for clinical use as a stem cell mobilizer.