Potential Clinical Applications of the CXCR4 Antagonist Bicyclam AMD3100

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Abstract: The bicyclam AMD3100 (originally called JM3100), in which the two cyclam rings are tethered by an aromatic bridge, emanated from JM2763, where the two cyclam moieties are tethered by an aliphatic linker – JM2763 in turn originated from JM1657, where the cyclam rings are directly linked to one another *via* a C-C bridge, and which was identified as an impurity, showing anti-HIV activity, in a commercial cyclam preparation. AMD3100 proved very effective against HIV-1 and HIV-2, inhibiting virus replication within the nM range, without toxicity for the host cells at concentrations that were > 100,000-fold higher than those required to inhibit HIV replication. The anti-HIV activity of AMD3100 appeared to be confined to the T-lymphotropic (X4) HIV strains, i.e. those strains that use the CXCR4 receptor to enter their target cells, and AMD3100 as of today still stands as one of the most potent and selective CXCR4 antagonists ever discovered. Hence, AMD3100 was found to interfere with a number of (patho)physiological processes which depend on the interaction of CXCR4 with its natural ligand, stromal derived factor (SDF-1) and which play an important role in rheumatoid, allergic and malignant diseases. AMD3100 has been shown to mobilize CD34+ stem cells from the bone marrow into the bloodstream and has also been shown to augment migration of bone marrow-derived endothelial progenitor cells into sites of neovascularization after myocardial infarction. Currently, AMD3100 is actively pursued as a stem cell mobilizer for transplantation in patients with multiple myeloma and non-Hodgkin's lymphoma.

1. INTRODUCTION

In 1985, shortly after the identification of human immunodeficiency virus (HIV), then termed LAV (for lymphadenopathy-associated virus) or HTLV-III (for human T-lymphotropic virus type III), as the causative agent of the acquired immune deficiency syndrome (AIDS), Rozenbaum *et al*. [1] reported evidence for the *in vivo* efficacy of HPA-23, a polyoxometalate, in reducing HIV levels, then measured by reverse transcriptase (RT) activity, in a single patient with AIDS. This observation triggered the search for other polyoxometalates that may be equally or even more effective than HPA-23 in suppressing HIV replication. Thus, hundreds of polyoxometalates, similar to HPA-23 [$(NH_4)_{18}(NaW_{21}Sb_9O_{86}17$], were examined for their anti-HIV activity, representative prototypes of this class of compounds being JM1493 [$H_4S_iW_{12}O_{40}$], JM1590 {$K_{13}[Ce(SiW_{11}O_{39})_2.26H_2O$} and JM2820 {$[Me_3NH]_8$ $[Si_2W_{18}Nb_6O_{77}]$} (Fig. **1**) [2,3]. Polyoxometa-lates can be viewed as globular or spherical polyanionic structures, their anionic charges being borne by the peripherally located oxygen atoms. Characteristically, polyoxometalates inhibit HIV replication in cell culture systems (i.e. MT-4 cells) at an EC_{50} (50% effective concentration) of approximately 1 μg/ml, without being cytotoxic to the host cells at concentrations up to a few hundred micrograms per ml [CC_{50} (50% cytotoxic concentration): ≥ 500 μg/ml], thus achieving a selectivity index (SI) of ≥ 500 [2-4].

Despite their favorable selectivity profile *in vitro* in cell culture, polyoxometalates have never been seriously pursued for their potential as systemic anti-HIV agents, because it was feared that if they had to be administered by a systemic (i.e. parenteral) route for prolonged periods of time, as would obviously be required in the treatment of HIV-infected patients, they may be deposited in the body, e.g. in the liver, thereby turning the latter in a kind of ceramic device. It was reasoned that, instead of incorporating the metal into a globular polyoxo panzer, it may be more appropriate to conceive metal complexes of organic molecules, akin to the natural hemoglobin component, the metaloporphyrin heme. In fact, metaloporphyrins have been studied, and found effective, as anti-HIV agents, however, with a potency and selectivity that was not higher than that of the polyoxometalates [5]. We then turned our attention to a simpler macrocyclic ring, namely cyclam (i.e. 1,4,8,11-tetra-azacyclotetradecane), that because of the presence of the four nitrogens in the center of the ring structure, should be able to coordinate metal ions. Before synthesizing any metal-cyclam complexes, we had to verify whether the starting material, the cyclam by itself, was devoid of anti-HIV activity, so that it could be readily ascertained whether complex formation with any of the envisaged metals would make the cyclam gaining anti-HIV activity.

2. DISCOVERY OF THE BICYCLAM AMD3100 AS A HIGHLY POTENT AND SELECTIVE HIV INHIBITOR

When several commercially available cyclam samples were analyzed for their anti-HIV activity, it turned out that, as expected, these samples were virtually devoid of an inhibitory effect against HIV replication in MT-4 cell cultures. One sample, though, showed distinct anti-HIV activity at an EC_{50} of about 10 μg/ml. As the other cyclam samples did not show anti-HIV activity at a concentration higher than 100 μg/ml, it was suspected that the active

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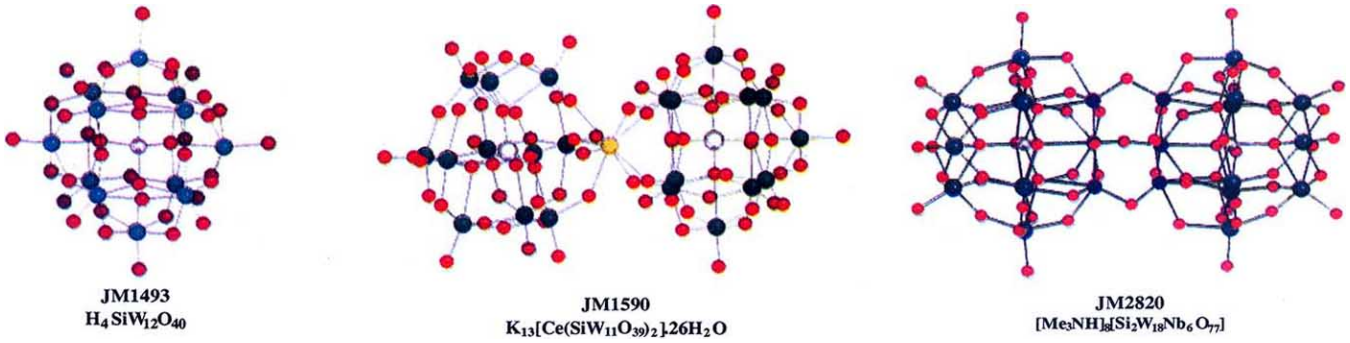
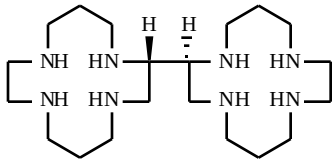
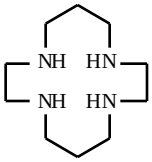


Fig. (1). Polyoxometalates JM1493, JM1590 and JM2820 [2,3].

sample may not have been completely pure, and this suspicion was corroborated by HPLC (high pressure liquid chromatography) analysis that revealed an impurity of about 1-2%. When the impurity was concentrated to about 10%, the EC_{50} was reduced by 10-fold, and, when the impurity was purified to homogeneity, its EC_{50} went further down by 10-fold, that is to 0.1-0.2 µg/ml. The "impurity" present in the active cyclam sample was finally characterized as the bicyclam JM1657, with the cyclam moieties tethered *via* a direct carbon-carbon bridge, thus creating two chiral centers (Table **1**) [6]. This molecule had activity against HIV-1 and HIV-2 in the 0.1-1 µM concentration range, while not being toxic to the host cells up to a thousand fold higher concentration. Pure (mono)cyclams such as JM1498 showed virtually no activity against HIV-1 or HIV-2 (Table **1**).

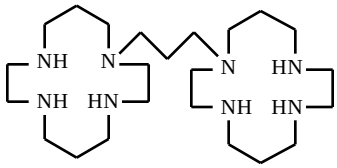
As it proved impossible to resynthesize JM1657, a synthetic program was started to make derivatives thereof, in which the two cyclam rings were tethered by an aliphatic bridge [$(CH_2)_n$ such as ethylene, propylene, …]. These efforts yielded JM2763 (Table **2**), which closely mimicked the anti-HIV potency and selectivity profile of the original

Table 1. Anti-HIV Activity of JM1657 in MT-4 Cells

Compound	Structure	EC_{50} (µM)		CC_{50} (µM)
		HIV-1(III_B)	HIV-2(ROD)	
JM1657		0.144	1.01	319
JM1498		399	150	1248

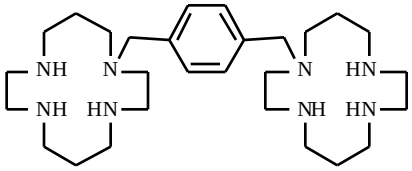
De Clercq *et al*. [6].

Table 2. Anti-HIV Activity of JM2763 in MT-4 Cells

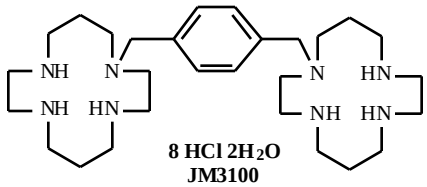
Structure	EC_{50} (µM)		CC_{50} (µM)
	HIV-1(III_B)	HIV-2(ROD)	
	0.248	1.00	> 622

De Clercq *et al*. [6].

Table 3. Anti-HIV Activity of JM2987 in MT-4 Cells

Structure	EC_{50} (μg/ml)	CC_{50} (μg/ml)	Selectivity index
	HIV-1(III_B)		
 8HBr $2H_2O$	0.005	> 500	> 100,000

De Clercq *et al*. [7].



8 HCl $2H_2O$
JM3100
AMD3100

1, 1'-[1,4-phenylene-bis(methylene)]-bis(1,4,8,11-tetraazacyclotetradecane) octahydrochloride dihydrate

Fig. (2). Structure of JM3100 (AMD3100).

"impurity" JM1657 [6]. Still the EC_{50} of this compound was in the 0.1-1 μM concentration range, and it was felt that, to be therapeutically useful, the antiviral potency should be further enhanced. A quantum jump in activity was achieved upon replacement of the aliphatic bridge by an aromatic bridge, as in JM2987 (the octahydrobromide dihydrate) (Table **3**) and JM3100 (the octahydrochloride dihydrate) (Fig. **2**), the aromatic bridge in JM2987 and JM3100 being 1,4-phenylene-bis(methylene). JM2987 and JM3100 proved active against HIV-1 and HIV-2 at a concentration of circa 0.005 μg/ml, and not being cytotoxic to the host cells at > 500 μg/ml, their selectivity index could be estimated at > 100,000, one of the highest selectivity indexes ever recorded for any anti-HIV agent. JM2987 and JM3100 showed similar activity against both HIV-1 (i.e. III_B) and HIV-2 (i.e. ROD) strains, but were notoriously inactive against several simian immunodeficiency virus (SIV) strains (i.e. MAC251, AGM-3, MND-GB1) (Table **4**) [7]. At the time this discrepancy was found, it did not make sense; later it should become quite clear why JM3100 discriminated between HIV-1 and HIV-2, on the one hand, and SIV, on the other hand.

3. MOLECULAR TARGET FOR ANTI-HIV ACTIVITY OF THE BICYCLAM AMD3100

3.1. Indirect Target: the Viral Envelope Glycoprotein gp120

From time-of-addition experiments, whereby the compounds are added at different time intervals after virus infection, it had become clear that the bicyclams must interact with an early (post-adsorption) event in the viral

Table 4. Anti-HIV Activity of Bicyclam JM2987

Virus	Strain	Cell line	EC_{50} (μg/ml)	CC_{50} (μg/ml)	Selectivity index
HIV-1	III_B	MT-4	0.005	> 500	> 100,000
	RF	MT-4	0.001	> 500	> 500,000
	HE	MT-4	0.003	> 500	> 167,000
HIV-2	ROD	MT-4	0.007	> 500	> 71,400
	EHO	MT-4	0.004	> 500	> 125,000
SIV	MAC-251	MT-4	> 100	> 100	
	AGM-3	MOLT-4	> 100	> 100	
	MND-GB1	MOLT-4	> 100	> 100	

De Clercq *et al*. [7].

Table 5. Mutations Detected in gp120 of Bicyclam-Resistant NL4-3 Viruses

$N_{269}Y\ R_{272}T\ S_{274}R\ Q_{278}H\ I_{288}V\ N_{293}H\ A_{297}T\ \Delta FNSTW$
$P_{385}L\ Q_{410}E\ S_{433}P\ V_{457}I$

	Passage no	Fold Resistance to		Mutations detected											
		JM2763	JM3100	$N_{269}Y$	$R_{272}T$	$S_{274}R$	$Q_{278}H$	$I_{288}V$	$N_{293}H$	$A_{297}T$	ΔFNSTW	$P_{385}L$	$Q_{410}E$	$S_{433}P$	$V_{457}I$
JM2763 resistant	16	2	3	0	0	+	+	0	0	0	0	0	0	0	0
	25	200	11	0	0	+	+	+	0	0	+	0	0	0	0
	28	200	6	0	0	+	+	+	0	+	+	+	0	0	0
JM3100 resistant	28	450	7	0	0	+	+	+	0	+	0	+	+	0	
				0	0	+	+	+	0	+	+	+	+	+	0
	42	>740	60	0	+	+	+	+	+	+	+	+	+	+	+
	60	>740	200	+	+	+	+	+	+	+	+	+	+	+	+

De Vreese *et al*. [11].

replicative cycle, that was tentatively identified as fusion/uncoating [6,7]. Following up on this lead, it was ascertained that JM3100 blocks entry of the virus into the cells, after the virus has been bound to the cell surface; using pseudotype virions containing the HIV-1 envelope, and monoclonal antibodies recognizing the viral envelope glycoprotein gp120, led us to conclude that gp120 was a most likely target for the anti-HIV activity of JM3100 [8]. If gp120 is indeed the molecular target of the bicyclams, it should be possible to generate viral resistance to the compound upon repeated passages of the virus in the presence of the compound, and this resistance should be associated with the emergence of mutations in the gp120 gene. It took more than 60 passages (300 days) in MT-4 cell cultures for the virus (i.e. the HIV-1 molecular clone NL4-3) to become resistant to JM3100 (i.e. 200-fold increase in EC_{50}) [9]. The bicyclam-resistant phenotype was rescued by transferring the envelope gp120 gene of the bicyclam-resistant virus into the NL4-3 parental genetic background [10]. Sequence analysis revealed the presence of a number of mutations (i.e. N269Y, R272T, S274R, Q278H, I288V, N293H, A297T, ΔFNSTW, P385L, Q410E, S433P and V457I) scattered over the whole gp120 glycoprotein, but primarily clustered in the V3 loop (Table **5**) [11]. It was postulated that, taken together, these mutations influenced the three-dimensional conformation of gp120 to such an extent that the virus lost at least part of its sensitivity to the bicyclams.

Table 6. Anti-HIV Activity Profile of AMD3100 Correlated with Coreceptor Use

Strain	Coreceptor used		EC_{50} (ng/ml)	
		AMD3100	SDF-1α	RANTES
T-tropic				
HIV-1 III_B	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
M-tropic				
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

Schols *et al*. [12].

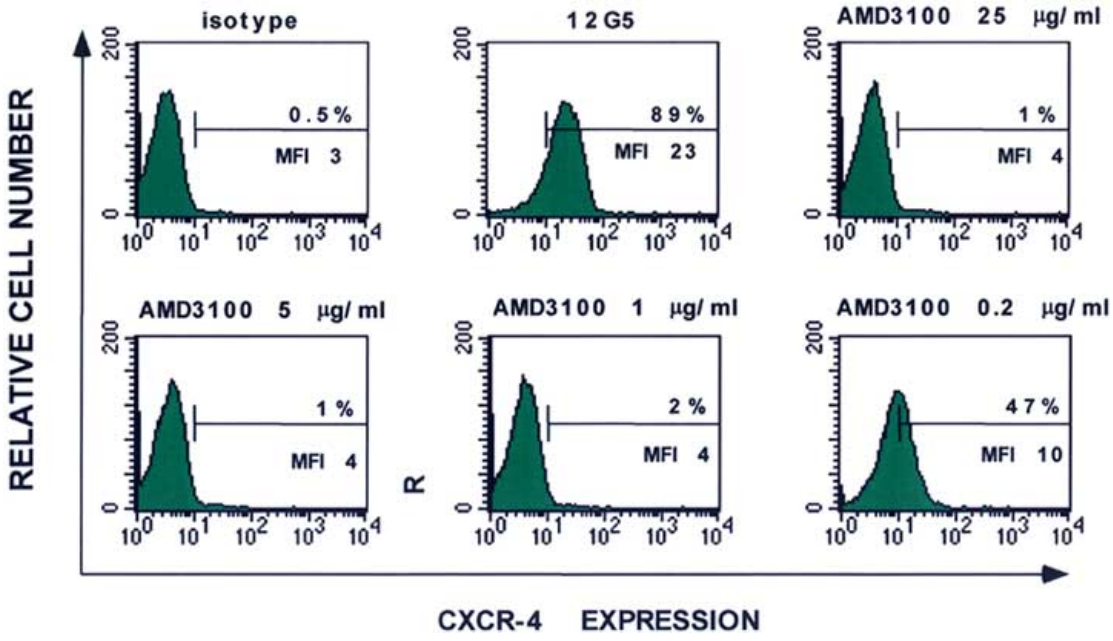


Fig. (3). Mechanism of action of bicyclams: interaction with monoclonal antibody binding to CXCR4 [13].

3.2. Direct Target: the Chemokine [Stromal Cell-Derived (SDF-1α)] Receptor CXCR4

Why would JM3100 (in the mean time called AMD3100), upon prolonged exposure, engender mutations in the viral gp120 ? The answer is that the viral gp120, after it has interacted with CD4, the primary receptor for HIV, interacts with CXCR4, the mandatory co-receptor for the so-called T-lymphotropic (or X4) HIV strains to enter the cells; and this is precisely where the bicyclams attack. That AMD3100 specifically interacts with CXCR4 could be readily deduced from its anti-HIV profile (Table **6**): AMD3100, like the natural ligand for CXCR4, SDF-1α, was found to inhibit the replication of T-lymphotropic (or X4) HIV strains, which are using the CXCR4 to enter the cells, but did not interfere with the replication of the macrophage (M)-tropic (or R5) HIV strains, which use the CCR5 receptor to enter the cells [12]. RANTES, a natural ligand for CCR5, on the other hand, blocked, as expected, the replication of the M-tropic HIV-1 strains, but not that of the T-tropic HIV strains. Then, it was shown that AMD3100 specifically prevents the binding of monoclonal antibody (i.e. 12G5) to CXCR4 (Fig. **3**) [13] and that AMD3100 also specifically inhibits signal transduction initiated by the interaction of SDF-1 with CXCR4, as monitored by Ca^{2+} flux [12-14], and illustrated in Fig. (**4**) [15]. In fact, a close correlation was found between the inhibitory effects of AMD3100 on (i) the replication of T-tropic HIV strains, (ii) the binding of monoclonal antibody

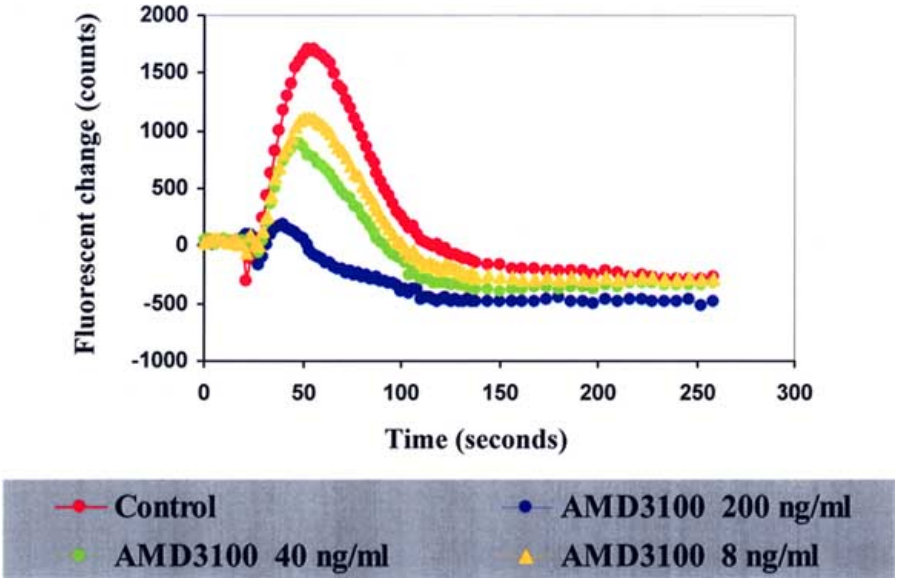


Fig. (4). Concentration-dependent inhibition of SDF-1-induced Ca^{2+} flux in Sup-T1 cells by AMD3100 [15].

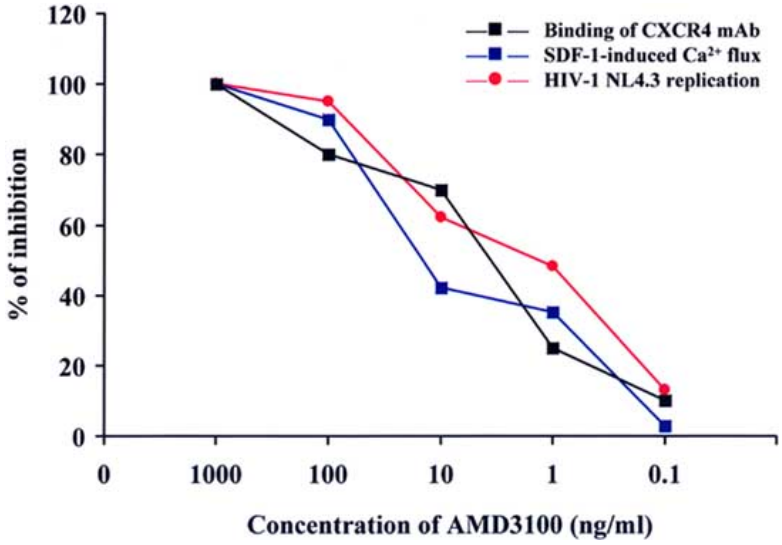


Fig. (5). Correlation between inhibitory effects of AMD3100 on HIV-1 replication , CXCR4 mAb binding and SDF-1-mediated signal transduction [16].

to CXCR4, and (iii) SDF-1-mediated signal transduction (as monitored by Ca^{2+} flux), all measured in the same (SUP-T1) cells (Fig. **5**) [16]. The interaction of AMD3100 with CXCR4 could obviously be withheld as the only common denominator of all three inhibitory effects. Also for various bicyclam analogs other than AMD3100, a close correlation was found between their anti-HIV-1 activity and their inhibitory effects on monoclonal antibody binding to CXCR4, on one hand (Fig. **6**), and SDF-1α-dependent intracellular Ca^{2+} flux, on the other hand (Fig. **7**) [17].

4. THE BICYCLAM AMD3100 AS COMPARED TO OTHER CXCR4 ANTAGONISTS

The bicyclam AMD3100 can be regarded as a bis-tetraazamacrocycle, and, in addition to AMD3100, numerous other phenylenebis(methylene)-linked bis-azamacrocycles have been described, among which the *p*-phenylenebis(methylene)-linked dimer of the py[*iso*-14]aneN_4 (AMD3329) (Fig. **8**) displayed the highest antiviral activity (EC_{50}: 0.8 and 1.6 nM against HIV-1 and

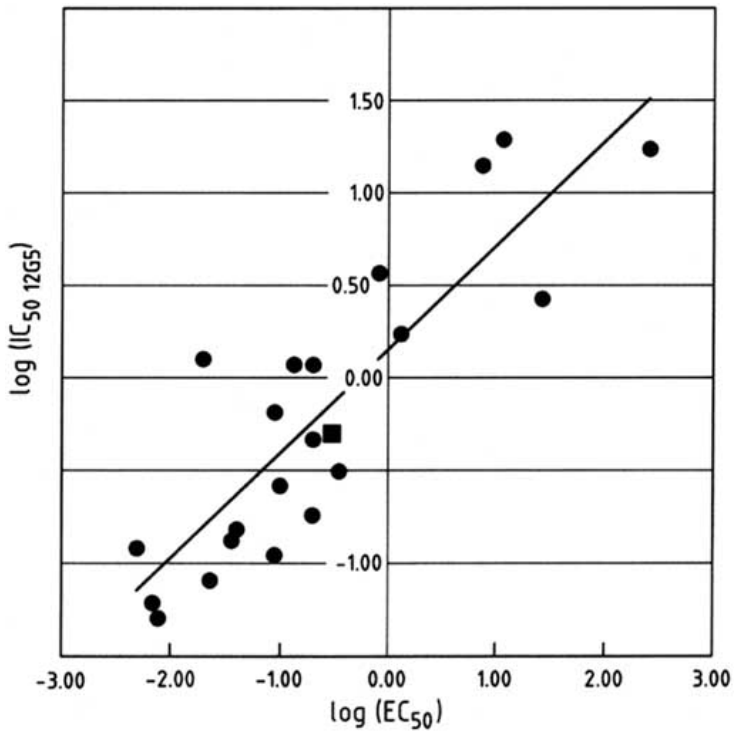


Fig. (6). Correlation between inhibitory effects of different AMD3100 analogues (●) and SDF-1α (■) on HIV-1 (NL4-3) replication and CXCR4 mAb (12G5) binding [17].

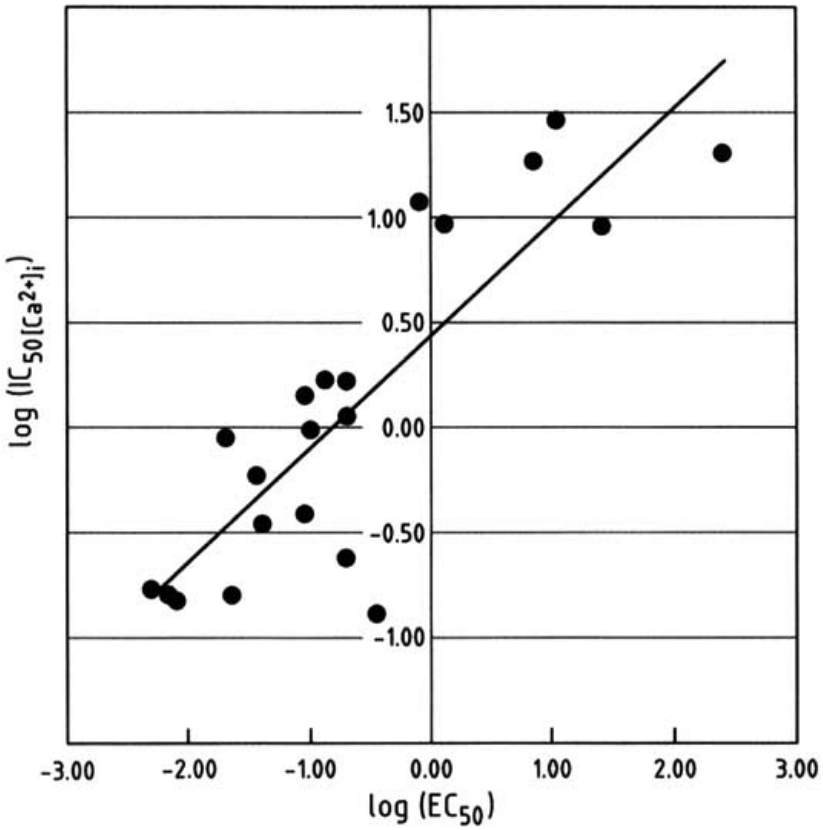
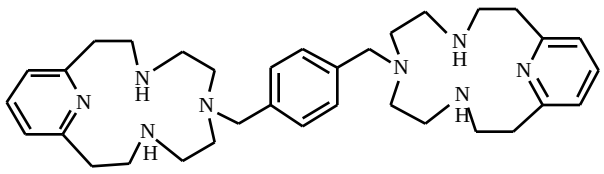


Fig. (7). Correlation between inhibitory effects of different AMD3100 analogues (●) on HIV-1 (NL4-3) replication and SDF-1α-induced intracellular Ca^{2+} flux [17].

HIV-2 replication, respectively; that is about 3- to 5-fold lower than the EC_{50} of AMD3100) [18]. AMD3329, also inhibited the binding of CXCR4 mAb and the SDF-1α-induced Ca^{2+} flux more potently than AMD3100.



AMD3329

Fig. (8). Structure of AMD3329.

The polyphemusins, e.g. [$Tyr^{5,12}$,Lys^{7}]polyphemusin II (designated T22), like the bicyclams, had been known for some time as inhibitors of HIV replication and HIV-cell fusion [19] before they were identified as CXCR4 antagonists [20]. T-22 contains eight basic amino acid residues (5 x Arg and 3 x Lys) for a total of 18 amino acid residues (Fig. **9**). Several analogs of T22 have been synthesized [21]; shortening the overall length from 18 to 14 amino acid residues yielded two derivatives, T134 and T140 (Fig. **9**), that retained high inhibitory activity against HIV-1 entry, as well as mAb(12G5) binding [22]. Starting from T140, which is not stable in (feline) serum due to the cleavage of the C-terminal Arg(COOH), new derivatives with a C-terminal Arg($CONH_2$) have been prepared, which possessed complete stability in feline serum [23].

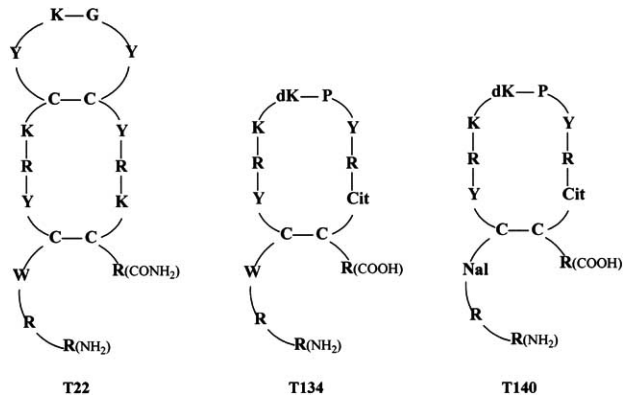
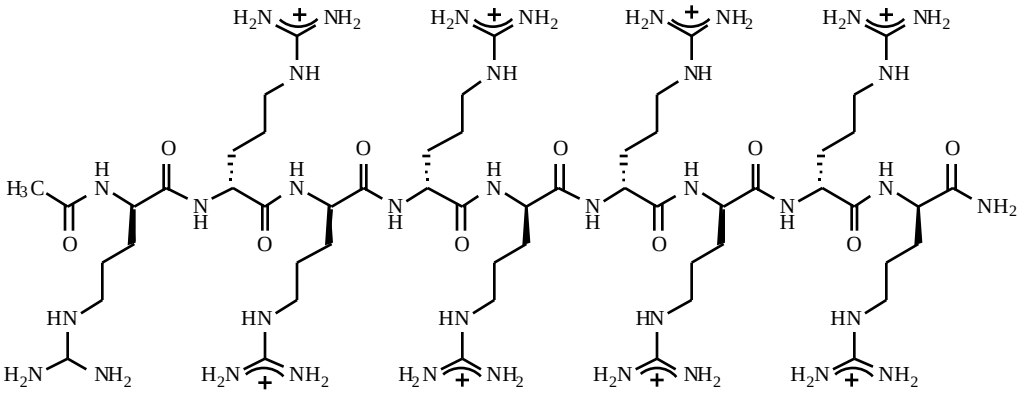


Fig. (9). Structure of T22, T134 and T140.

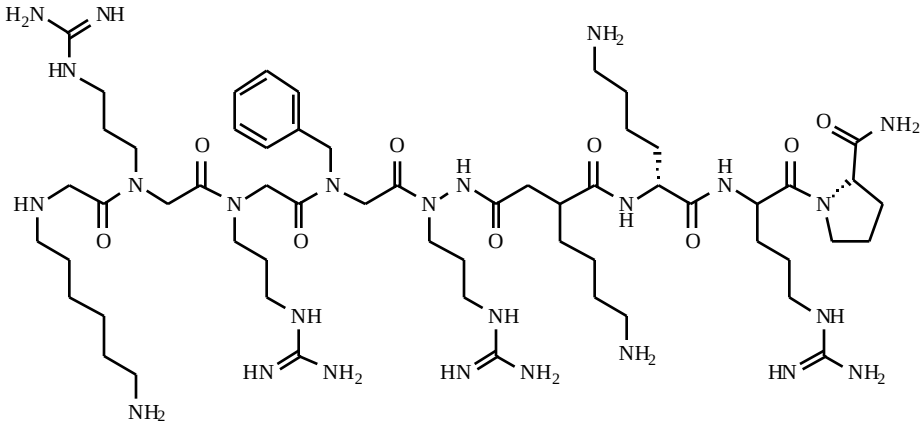
Cit = L-citrulline, Nal = L-3-(2-naphthyl)alanine.

The N-α-acetyl-nona-D-arginine amide acetate ALX-40-4C (Fig. **10**) was developed as a competitive inhibitor of the binding of the HIV Tat protein to its target, TAR, the Tat responsive region at the 5'-end of the viral RNA genome. Although designed as a Tat antagonist, ALX-40-4C was found to interfere with an initial virus-target cell interaction, involving HIV-1 gp120 V3 loop determinants of mainly T-cell line-adapted virus strains [24]. Consequently, ALX-40-4C was then shown to prevent cell entry of the T-tropic X4 virus strains through blockade of the CXCR4 receptor [25]. In the mean time, ALX-40-4C has been the subject of a phase I/II clinical trial in 40 asymptomatic HIV-infected



N-α-acetyl-nona-D-arginine amide acetate
ALX-40-4C

Fig. (10). Structure of ALX-40-4C.



CGP 64222

Fig. (11). Structure of CGP-64222.

patients [26]: the compound was well tolerated but, after a 1-month treatment period, did not achieve significant or consistent reductions in viral load.

Tat peptide analogs encompassing the Tat core domain (amino acid residues 36-50) or the basic domain (amino acids 48-56: RKKRRQRRR) have been reported to inhibit HIV replication, and, not unexpectedly, these Tat peptide analogs also block the Tat transactivation process [27,28]. The nonamer peptoid CGP-64222 (Fig. **11**) resembles the amino acid 48-56 sequence of Tat, and, consequently, was found to block the Tat/TAR interaction [29]. CGP-64222 was also found to suppress HIV-1 replication, but this results primarily from an action targeted at CXCR4 [30]. This is, after all, not surprising, given the structural similarity of CGP-64222 with the other peptidic CXCR4 antagonists T22, T134, T140 and ALX-40-4C (Figs. **9**, **10** and **11**).

Also, peptides derived from the stromal cell-derived factor 1 (SDF-1) were found to inhibit HIV entry into the cells through interaction with CXCR4 [31]: the highest affinity for CXCR4 was shown by a 10-residue substituted dimer, derived from the 5-14 sequence of SDF-1. This peptide dimer, dubbed S1D (Fig. **12**), is, like the N-terminal sequence of SDF-1 ((H$_2$N)KPVSLSYRCPCRF), polycationic, and, thus, again, basically similar in structure to the peptides T22, T134 and T140.

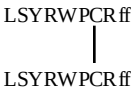
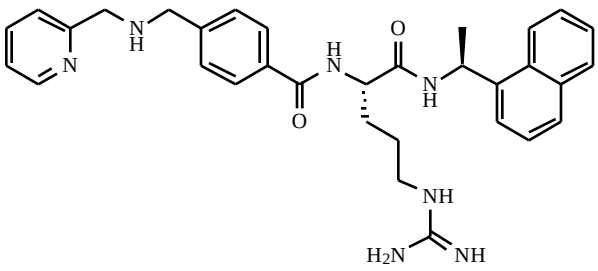


Fig. (12). Structure of S1D.

f = D-phenyladenine.

It thus appears that, while several substances have been demonstrated to exhibit a behavior similar to that of the bicyclams in that they inhibit the entry of T-tropic X4 HIV strains into the cells through a specific blockade of CXCR4, the bicyclams clearly differ from T22, T134, T140, ALX-40-4C, CGP-64222 and S1D in that the bicyclams are truly synthetic heterocyclic compounds, whereas all the others are essentially peptide analogs.

While the bicyclam AMD3100 and its congeners are not readily available by the oral route, attempts have been made to develop CXCR4 antagonists that are orally bioavailable. This has yielded, on the one hand, AMD070, an equally potent anti-HIV CXCR4 antagonist as AMD3100 [32], and, on other hand, KRH-1636, a duodenally absorbable CXCR4 antagonist with potent and selective anti-HIV-1 activity [33]. Interestingly, the latter compound (Fig. **13**) contains both cationic and peptidic features.



KRH-1636

Fig. (13). Structure of KRH-1636.

5. THE BICYCLAM AMD3100 AS A HIGHLY SELECTIVE INHIBITOR OF X4 HIV-1 STRAINS

Would the bicyclam AMD3100 gain (or loose) anti-HIV activity if complexed with metals, as originally envisaged when conceiving the cyclam metal complexes ? As it turned out, complex formation of AMD3100 with Zn or Ni did not markedly affect the anti-HIV activity of AMD3100, but complex formation with Cu, Co and Pd brought about an increasing loss in activity (in the order Cu < Co < Pd) [17]. Thus, "pure" AMD3100, without metals complexed to it, was further pursued for its potential to block infection by X4 HIV-1 strains. It was found that when exposed to a mixture of T-tropic (X4) and M-tropic (R5) HIV-1 strains, AMD3100 specifically impeded the replication of X4 strains, while allowing for the outgrowth of R5 virus [34] (Fig. **14**). These findings indicated that the selective blockade of the CXCR4 receptor by AMD3100, prevented the switch from the less pathogenic R5 HIV to the more pathogenic X4 HIV strains, a process that, when occurring *in vivo*, would herald the progression of AIDS.

It has, in fact, been demonstrated that antiretroviral therapy may shift HIV-1 populations from X4 to R5, and this shift in co-receptor usage may contribute to the clinical efficacy of the anti-HIV drugs [35]. Conversely, natural evolution would tend to favor a shift from M- to T-tropism, with higher cytopathic potential and progression to AIDS, as has been demonstrated for a patient infected with a *nef*-deleted HIV-1 strain [36].

While in the presence of AMD3100, a mixed population of R5 and X4 strains shifts towards predominantly R5, and thus CCR5-using, virus, the individual X4 strains made resistant to either SDF-1α or AMD3100, do not show such switch in co-receptor use and continued to use CXCR4 as co-receptor to enter their host cells [37], possibly by exploiting a different docking site on CXCR4. Vice versa, R5 HIV-1 strains made resistant to CCR5 antagonists (upon repeated passages in the presence of the compound, continue to use CCR5 for entering the host cells and do not switch to any other co-receptor [38].

Also, the apparent paradox that the bicyclams were inactive against SIV [7] was resolved when it was found that this happened only if SIV used CCR5 as co-receptor for entering human cells; if using CXCR4, as some SIV strains do, they are clearly sensitive to inhibition by AMD3100 [39].

6. THE BICYCLAM AMD3100 AS A TOOL TO PROBE CXCR4 USAGE BY HIV STRAINS

SIV isolates, like HIV-1, regularly use CCR5 as co-receptor for cell entry; but, unlike HIV-1, use of the CXCR4 co-receptor by SIV is rarely found and is essentially dependent on the virus strain [39]. SIV isolates, e.g. from sooty mangabey origin, demonstrate a complex co-receptor use pattern. When using CXCR4, they can be easily picked

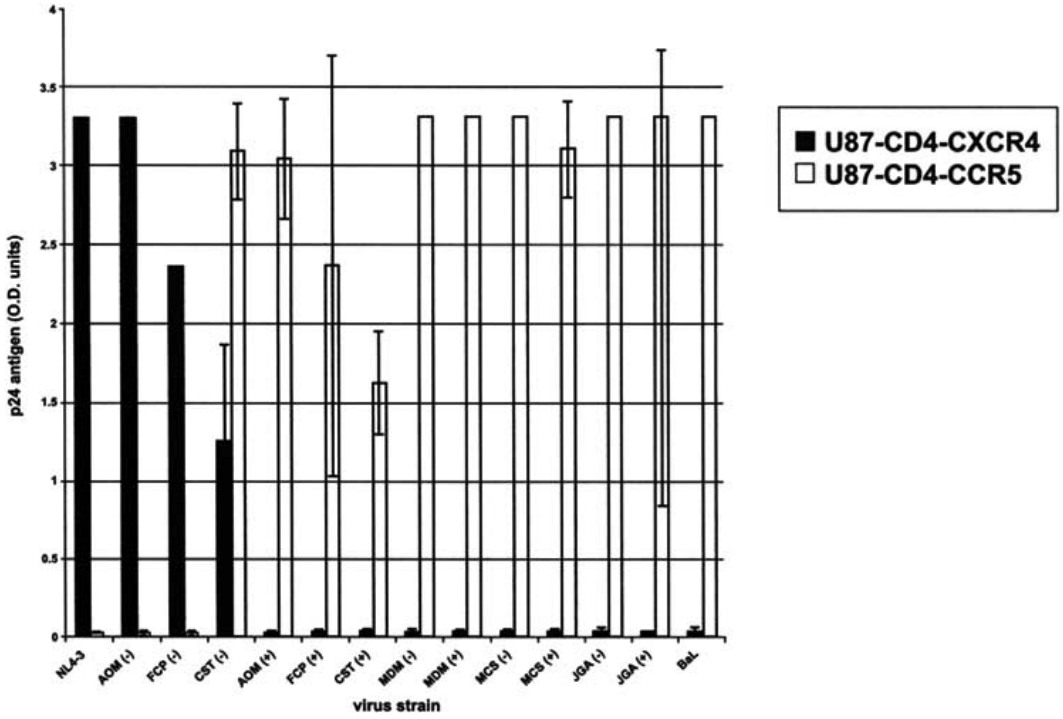


Fig. (14). Co-receptor use of clinical HIV strains in the absence (-) or presence (+) of AMD3100 [34].

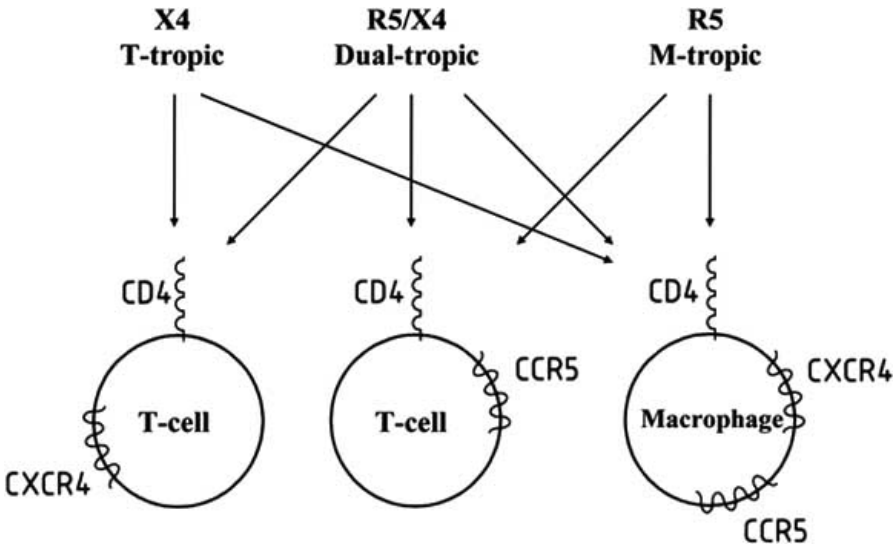


Fig. (15). Model for HIV cell tropism and co-receptor use.

up in a convenient [GHOST[3]] cell assay [40] with AMD3100 as probe [41].

Although the envelope glycoproteins of FIV (feline immunodeficiency virus) are substantially divergent from the HIV glycoproteins, FIV and HIV interact with CXCR4 in a highly similar manner; accordingly, AMD3100 was found to block primary FIV isolates and laboratory-adapted FIV strains with equal potency [42,43], in Crandell feline kidney (CRFK) cells, feline thymocytes [43] and feline peripheral blood mononuclear cells (PBMCs) [42]. Also CXCR4 is the primary receptor used by FIV to infect astrocytes; this process, that may play a role in the development of AIDS dementia complex, can be blocked by the bicyclam AMD3100 [44].

Whenever (and wherever) HIV strains are isolated that show X4 or dual (R5X4) tropism, they may be expected to be susceptible to AMD3100 [45], and, *vice versa*, when found sensitive to AMD3100, such HIV strains may be surmised of being CXCR4-tropic or dual tropic [46]. Only when they are purely CCR5-tropic, they cannot be inhibited

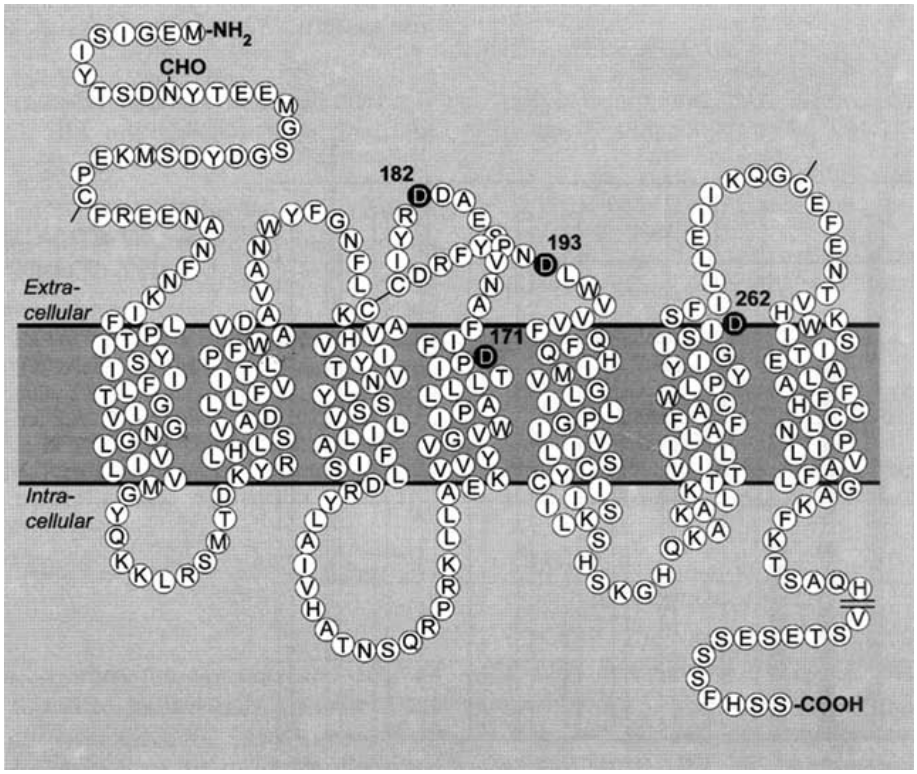


Fig. (16). The CXCR4 receptor with its crucial aspartic acid residues (at positions 171, 182, 193 and 262) involved in the interaction of CXCR4 with AMD3100 [55].

by AMD3100. It has been argued that CXCR4 and CCR5 are the primary targets for antiviral drug development (i.e. CXCR4 and CCR5 antagonists), the ability of HIV-1 strains to use co-receptors in addition to CCR5 and CXCR4 being irrelevant [47]. This notion was corroborated for a set of HIV-1 and HIV-2 isolates, except for one HIV-2 isolate that replicated in human PBMCs in the presence of AMD3100 and a CCR5 antagonist, and, therefore, may be using an alternative co-receptor to enter PBMCs [48].

Whereas R5 HIV strains infect (as expected) macrophages *via* CCR5, certain X4 HIV strains are also capable of infecting macrophages, which then occur *via* the use of CXCR4 [49,50]. In fact, some highly M-tropic isolates were found to enter microglia, primarily *via* CXCR4, which means that HIV-1 tropism for macrophages and microglia is not strictly linked to CCR5 usage [51]. To the extent that HIV infection of macrophages and microglia requires the usage of CXCR4, it can be readily blocked by CXCR4 antagonists such as AMD3100.

The bicyclam AMD3100 can thus be considered as a tool for identifying, and, consequently, inhibiting X4 T-tropic, whether single-tropic X4 or dual-tropic R5X4 HIV strains (Fig. **15**). AMD3100 has been found to efficiently suppress the replication of R5X4 dual-tropic HIV-1 primary isolates [52]. Dual-tropic viruses are not homogeneous in their co-receptor usage in lymphoid tissue, but probably comprise a continuum between the 2 polar variants that use CXCR4 or CCR5 exclusively; cytopathicity towards the general $CD4^+$ T-cell population in lymphoid tissue is associated with the usage of CXCR4 [53], and, therefore, susceptible to CXCR4 antagonists such as AMD3100. It should be emphasized that dual tropism may result from two distinct mechanisms: utilization of both CCR5 and CXCR4 on macrophages and T-cell lines, respectively (dual-tropic R5X4), or the ability to efficiently utilize CXCR4 on both macrophages and T-cell lines (dual-tropic X4) [54]. Both mechanisms are allowed by the model depicted in Fig. (**15**).

7. THE BICYCLAM AMD3100 AS A HIGHLY SELECTIVE CXCR4 ANTAGONIST: MECHANISM OF ACTION

The bicyclam AMD3100 is extremely specific in its affinity for the CXCR4 receptor: this depends at least in part on an electrostatic interaction between the basic (positively charged) nitrogens of the cyclam moieties, and the acid (negatively charged) carboxylates of the aspartic acid residues located at positions 171, 182, 193 and 262 of the CXCR4 receptor (Fig. **16**). In particular, the aspartate residues 171 and 262, situated at the junction of the transmembranous segments with the extracellular loops of CXCR4 have proven critical in the binding of AMD3100 with its receptor [55,56]. In addition, the Phe-X-Phe motifs in the second extracellular loop (ECL2) or the adjacent membrane-

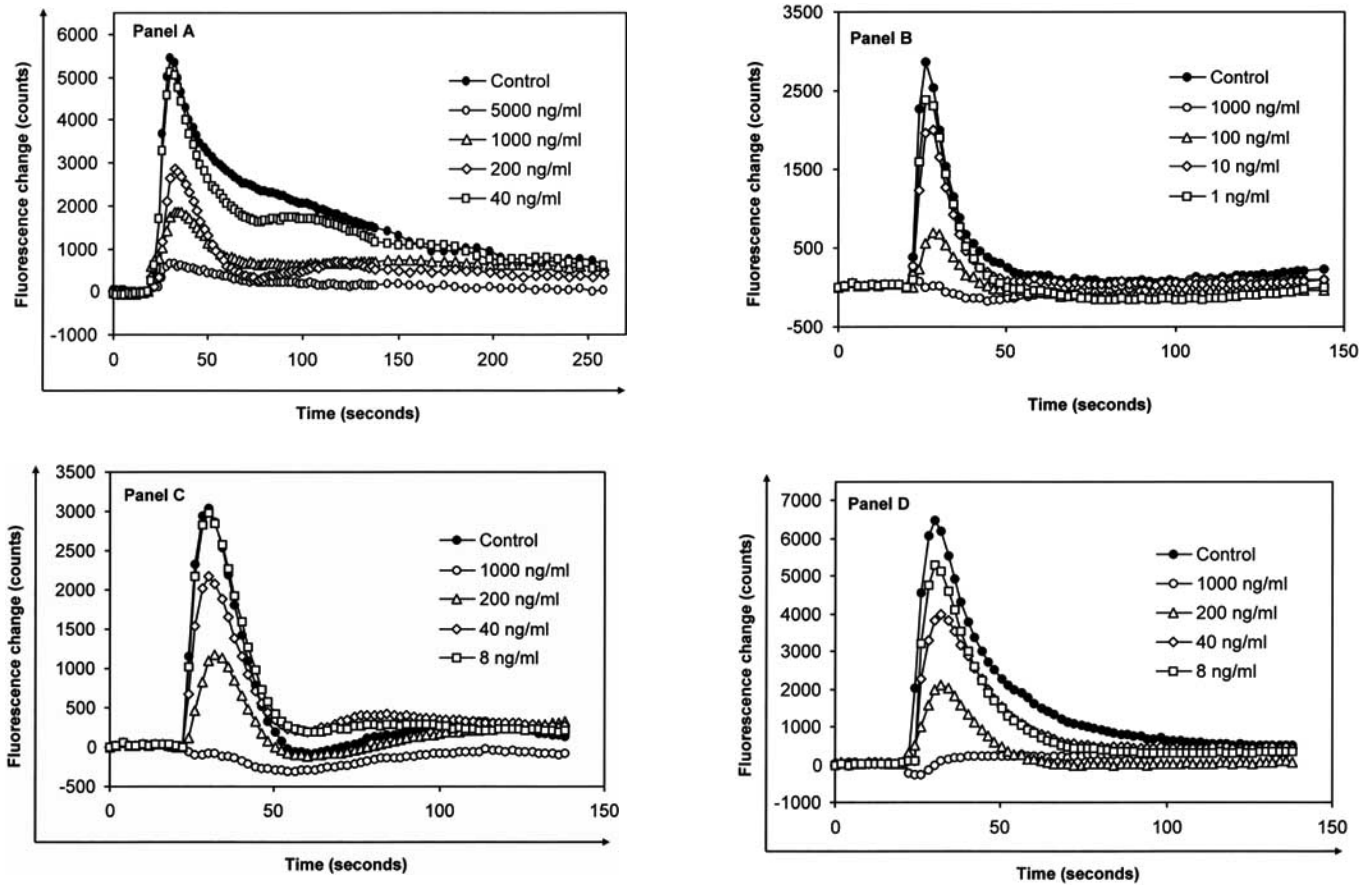


Fig. (17). Concentration-dependent inhibition of SDF-1/CXCR4-mediated intracellular calcium flux by AMD3100 in U87.CD4.CXCR4 cells (panel A), freshly isolated human PBMCs (panel B), human T-lymphoid HSB-2 cells (panel C) or murine B-lymphoblastic leukemia L1210 cells (panel D) [61].

spanning TM4 domain have been proposed to engage in hydrophobic interactions with the aromatic [phenylene-bis(methylene)] linker between the cyclam units of AMD3100 [57].

The affinity of AMD3100 for CXCR4 can be enhanced by any of the following metal ions: Cu^{2+}, Zn^{2+} or Ni^{2+}; and this enhanced affinity [58] is explained through an enhanced interaction with the aspartate in position 262. The first cyclam ring would bind to the carboxylate groups of Asp 262 (Zn(II) coordination) and Glu 288 (double H-bonding), whereas the second cyclam ring would bind, through Zn(II), with Asp 171 [59]. Remarkably, the introduction of CXCR4 domains stemming from the second extracellular loop (ECL2) into CCR5 was sufficient to allow utilization by X4 strains, and the viral entry by these CCR5/CXCR4 chimeric co-receptors was blocked by the bicyclam AMD3100 [60].

AMD3100 binds with extreme specificity to CXCR4, regardless and independent of the cell type that carries this receptor, i.e. U87.CD4.CXCR4 transfected cells, freshly isolated human peripheral blood mononuclear cells (PBMCs), human T-lymphoid HSB-2 cells or murine B-lymphoblastic leukemia L1210 cells (Fig. **17**) [61]. On the contrary, AMD3100 did not interact, as monitored by chemokine-induced signaling, with a variety of receptors other than CXCR4, *viz*. CXCR1, CXCR2, CXCR3, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8 and

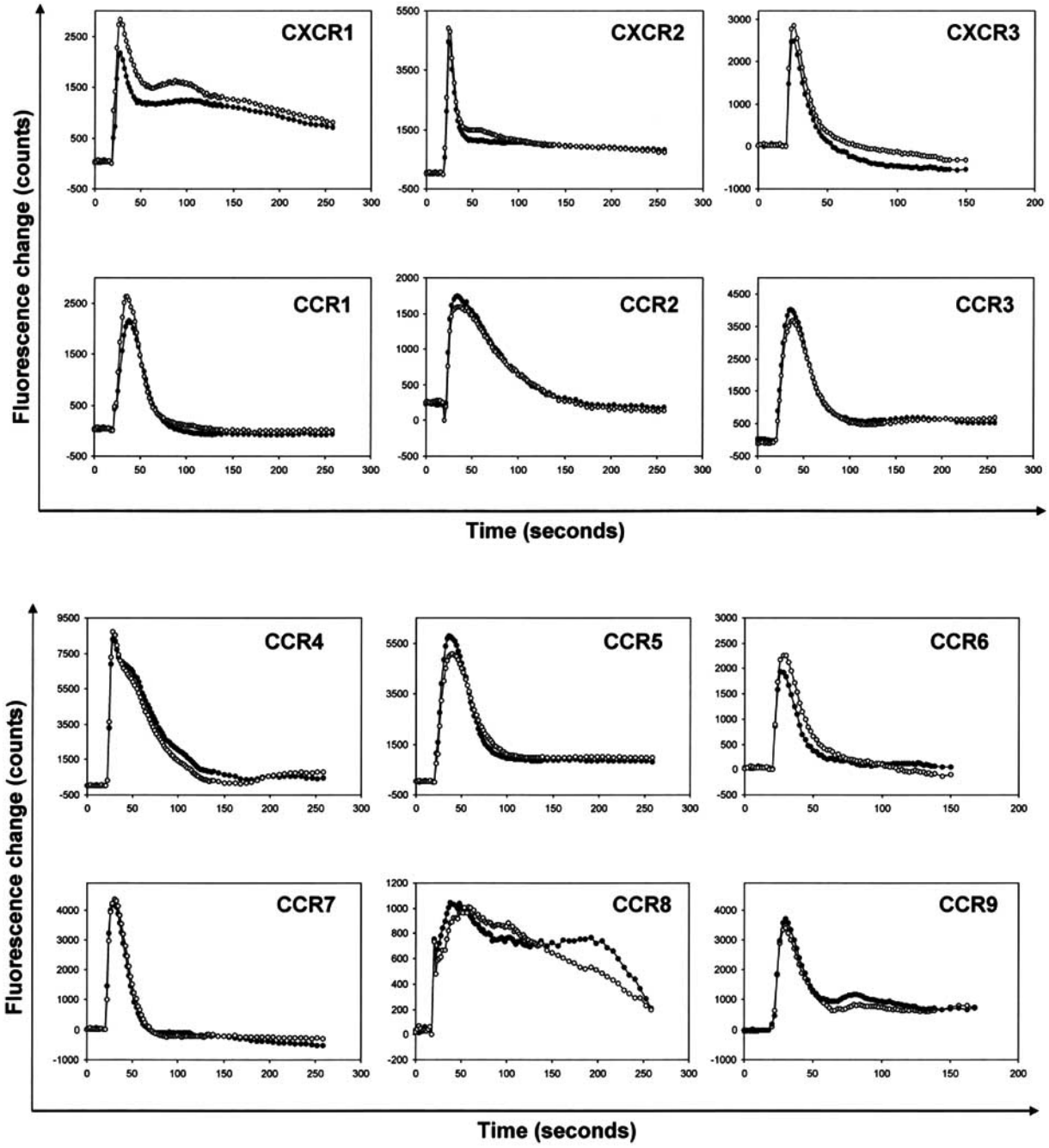
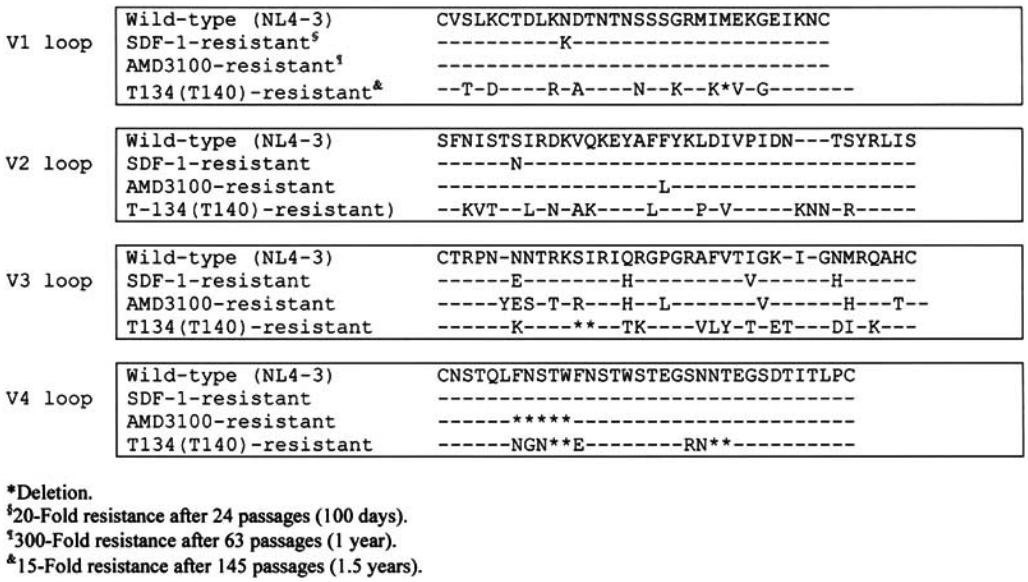


Fig. (18). Lack of inhibitory effect of AMD3100 (25 μg/ml) on chemokine-induced signaling mediated by CXCR1, CXCR2, CXCR3, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8 or CCR9 [61].



*Deletion.
§20-Fold resistance after 24 passages (100 days).
¶300-Fold resistance after 63 passages (1 year).
&15-Fold resistance after 145 passages (1.5 years).

Fig. (19). Amino acid substitutions in gp120 domains of HIV-1 strains resistant to CXCR4 antagonists [72]. Substituted amino acids are indicated by the single-letter code and deletions in resistant viruses are marked *. The top row shows the wild-type HIV NL4-3 amino acid sequences [73]. The gp120 amino acid substitution in the SDF-1α-resistant virus is derived from Schols *et al*. [37]. The gp120 amino acid substitution in the AMD3100-resistant virus is derived from De Vreese *et al*. [10].

CCR9 (Fig. **18**) [61]. This may provoke the prediction that AMD3100 would interfere with a number of pathophysiological processes mediated by CXCR4, but not any of the other CXCR or CCR receptors.

Through its antagonization of CXCR4, AMD3100 not only prevents HIV entry into the cells, but also prohibits apoptosis mediated by the interaction of the HIV-1 envelope with uninfected cells [62], thus protecting uninfected cells against cell death.

Recent studies have indicated that in human lymphoid tissues, *ex vivo* apoptosis of uninfected bystander cells may play a major role in lymphocyte depletion caused by X4 HIV-1 strains [63].

AMD3100 is not an all-inclusive antagonist of CXCR4: it still allows certain peptide agonists (i.e. RSVM and ASLW) to signal from agonist-binding sites that are not neutralized by AMD3100 (or T140, the other CXCR4 antagonist) [64]. Recent studies have suggested that, while T140 would act as an inverse agonist, AMD3100, as well as ALX-40-4C would behave as weak partial agonists [65]. The bio-pathological significance of these findings is unclear, however, as, in reality, any weak agonistic activity may be outweighed by the strong antagonistic character of AMD3100 and other CXCR4 antagonists.

8. FACTORS TO BE CONSIDERED TOWARDS CLINICAL USE: SYNERGY AND RESISTANCE DEVELOPMENT

In the successive steps leading to HIV-cell fusion, there is a high degree of cooperativity between the engagement of the envelope gp120 by CXCR4, and the subsequent formation of the gp41 six-helix bundle, the target for the fusion inhibitor enfuvirtide (T-20) [66]. In fact, strong synergy has been observed between T-20 and AMD3100 against an X4 HIV-1 isolate at least *in vitro*, in peripheral blood mononuclear cells [67]. Strong synergy has also been observed between the bicyclam AMD3100 and CCR5 antagonists, such as the spirodiketopiperazine E913, when evaluated against dual-tropic HIV-1 or 50:50 mixtures of R5 and X4 HIV-1 [68].

CXCR4 being a cellular target, it has proven very difficult for the virus to develop resistance to CXCR4 antagonists: *viz*. as mentioned above, it took more than 60

Table 7. Antiviral Activity of AMD3100 in SCID-hu Thy/Liv Mice [Infected with CXCR4-Using Virus (Clinical Isolate HIV-1 EW)]

Dose (mg/kg/day)	p24 (pg/million cells)	p value (treated *versus* untreated)
0	394 ± 107	---
0.3	280 ± 48	0.61
1	161 ± 46	0.028
3	122 ± 33	0.010
10	73 ± 15	0.003

Datema *et al*. [75].

passages (300 days) in cell culture for a molecular clone of HIV-1 to become resistant to AMD3100 [9], and the resulting virus still persisted in using CXCR4 as co-receptor to enter its target cells. Employing envelope chimeric virus technology (CVT), based on recombination of gp120 from resistant virus into a wild-type background, it was ascertained that the mutations in gp120 (Table **5)** [11]suffice to reproduce the phenotypic resistance profile towards AMD3100 [69].

After it was first reported that T134 would not have cross-drug resistance with AMD3100 [70], T134-resistant virus was generated starting with the HIV-1 molecular clone NL4-3: this virus was also cross-resistant to T140, ALX-40-4C and AMD3100 [71]. It contained the following mutations in the V3 loop of gp120: N269K, Q278T, R279K, A284V, F285L, V286Y, I288T, K290E, N293D, M294I and Q296K. Other mutations were located in the V1, V2 and V4 domains [71]. Fig. (**19**) presents an alignment of the different mutations arising in the V1, V2, V3 and V4 loop of gp120 upon repeated passage of HIV-1, in the presence of SDF-1, AMD3100 and T134, respectively [72,73]. As a rule, more mutations were required for acquiring 15-fold resistance to T134, than for the acquisition of 300-fold resistance to AMD3100.

In principle, HIV may develop resistance towards CXCR4 antagonists through a double mechanism: (i) a shift from X4 to R5 in a mixed X4/R5 virus population, involving a switch in co-receptor (CXCR4 → CCR5) use [34], and (ii) acquisition of multiple mutations in the envelope gp120 without a change in co-receptor use [37]. Regardless of the co-receptor used, AMD3100-resistant HIV strains were found to possess significantly reduced fitness as compared to wild-type virus [74].

9. CLINICAL EXPERIENCES WITH THE BICYCLAM AMD3100

9.1. *In Vivo* Efficacy Against T-Tropic X4 HIV-1 Infections

In vivo efficacy with AMD3100 was first demonstrated in SCID-hu Thy/Liv mice infected with a clinical isolate of X4 HIV-1 (using CXCR4 as the co-receptor) (Table **7**) [75]: in this animal model infection, the dosage required to achieve a statistically significant reduction (> 50%) in viral titer (as measured by p24 levels) was 1 mg/kg/day. This *in vivo* demonstration of anti-HIV efficacy in a small animal model, as closely related to the human situation as possible, prompted phase I/II clinical studies with AMD3100 to assess its antiviral efficacy in HIV-infected individuals. After phase I pharmacokinetic studies had assured the feasibility of intravenous dosing (in the range of 10-80 μg/kg) [76], AMD3100 was administered at a dosage of 2.5, 5, 10, 20, 40 or 80 μg/kg/hr for a period of 11 days to HIV-infected patients harboring a mixed population of CCR5- and CXCR4-using HIV variants [77]. All the patients, whether they had a proportion of less or more than 10% CXCR4-using (X4) HIV variants at entry, witnessed a reduction in the % X4 proportion at day 11 as compared to day 1. In most of the patients with less than 10% X4 virus on day 1, X4 virus strains were no longer detectable on day 11 [77]. In one patient (no 1-40), the only patient who had 100% X4 virus at entry, and where the AMD3100 dosage went up to 160 μg/kg/hr, a reduction in viral RNA of 0.89 $\log_{10}$ was noted on day 11 [77]. These phase I/II clinical studies clearly demonstrated that, as proof of principle, AMD3100 was effective in suppressing X4 HIV-1 replication *in vivo*, in HIV-1-infected individuals.

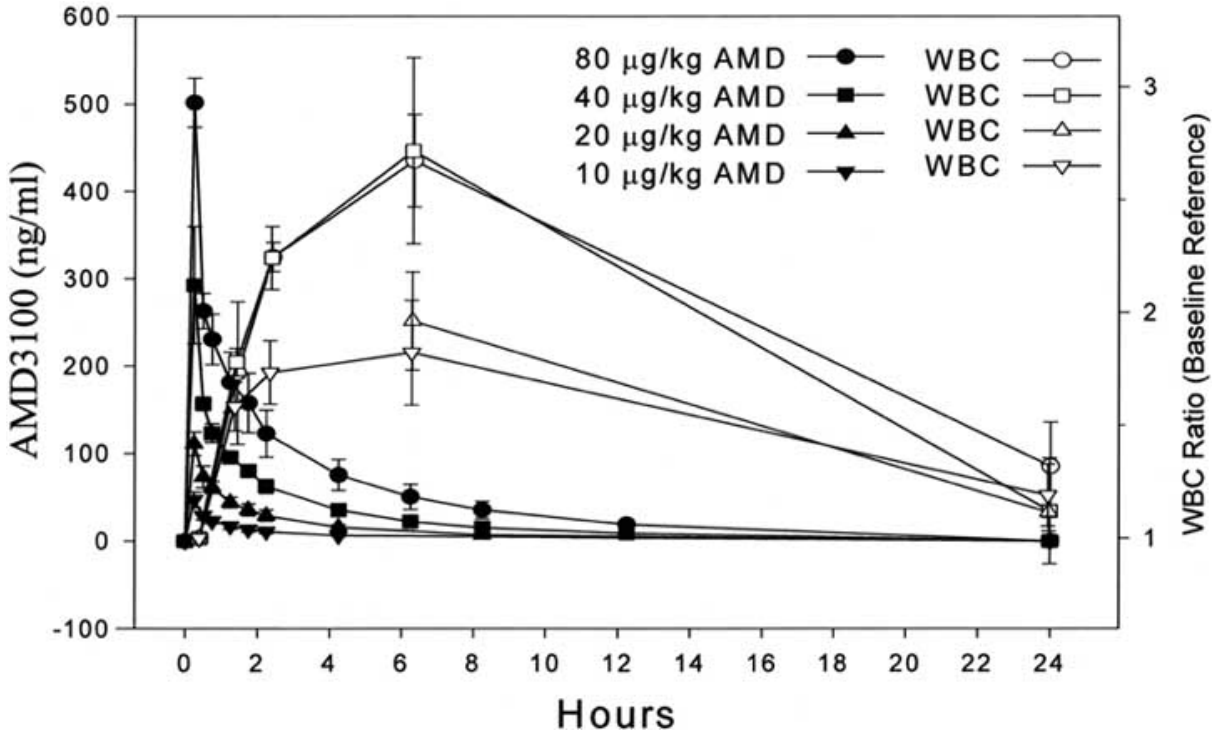


Fig. (20). White blood cell (WBC) counts *versus* time compared to AMD3100 plasma concentration *versus* time following single-dose intravenous AMD3100 administration [76].

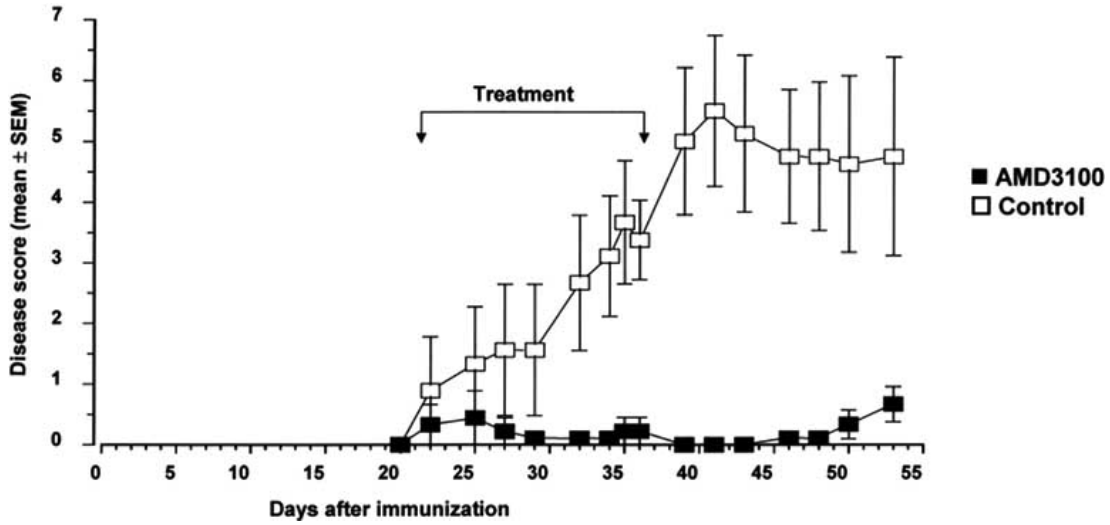


Fig. (21). Inhibition of collagen-induced arthritis by treatment with AMD3100. Mice were immunized with chicken collagen type II (CII) in CFA on day 0 and implanted on day 22 with osmotic minipumps delivering AMD3100 at a rate of 600 µg/day during a period of 14 days. Clinical symptoms of arthritis started to appear on day 22 [89].

9.2. *In Vivo* Mobilization of Hematopoietic ($CD34^+$) Stem Cells

An unexpected observation was made during the pharmacokinetic studies with single-dose intravenous AMD3100 administration [76]. While plasma drug concentrations showed the expected dose-dependent peak values and a gradual decline in function of time (Fig. **20**), an increase in white blood cell (WBC) counts was noted, reaching its peak value at approximately 6 hours after injection of AMD3100, and going up to 3 times the baseline value, even with doses as low as 40 or 80 µg/kg of AMD3100. This observation set the stage for a further exploration of what sort of white blood cells were exactly mobilized into the peripheral blood, and these appeared to be the hematopoietic ($CD34^+$) stem cells. After intravenous injection of single AMD3100 doses ranging from 40 to 240 µg/kg, $CD34^+$ cells in the peripheral blood went up from less than 5 cells per µl to up to 40 cells per µl, peaking at approximately 6 (to 9) hours after AMD3100 injection [78]. It was further found that AMD3100 acts synergistically with Neupogen [granulocyte-colony stimulating factor (G-CSF)] in mobilizing $CD34^+$ cells from the bone marrow into the peripheral blood [78,79]: with a combined treatment regimen of 5 injections of G-CSF at 10 µg/kg/day on days 1 to 5, and a single injection of AMD3100 at 160 µg/kg on day 5, $CD34^+$ cell could be further boosted up to 150 cells per µl. With this combined drug regimen, 9.88 x 10^6 $CD34^+$ cells per kg could be mobilized, as compared to 3.73 x 10^6/kg with G-CSF (10 µg/kg x 5 days) alone and 3.02 x 10^6/kg with AMD3100 (240 µg/kg on day 5) alone [78,79]. These observations point to the highly attractive usefulness of AMD3100, (whether or not) in combination with G-CSF, in collecting $CD34^+$ stem cells for transplantation purposes,

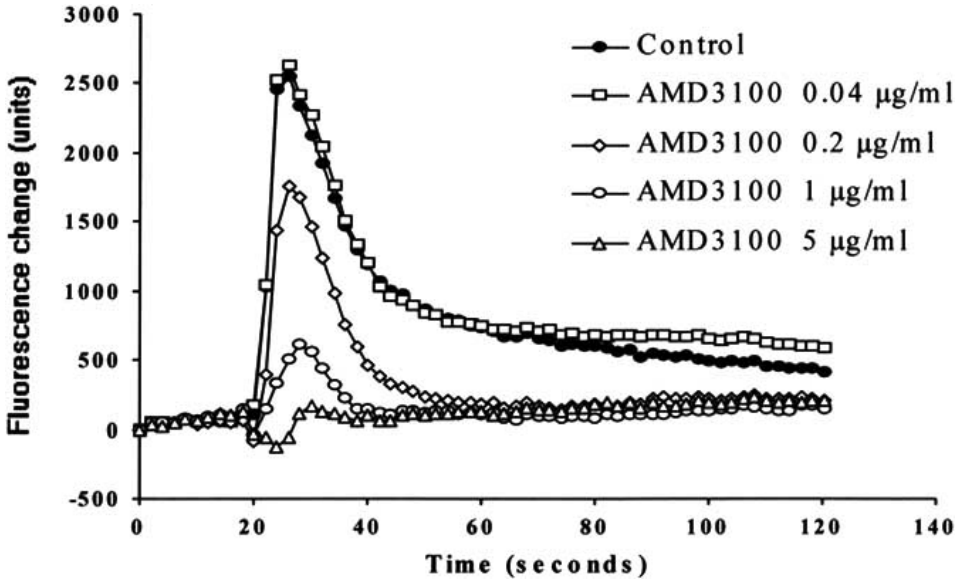


Fig. (22). Inhibition by AMD3100 of SDF-1-elicited intracellular Ca^{2+} flux in Mac-1^+ cells harvested from the spleens of mice on day 18 postimmunization with CII in CFA [89].

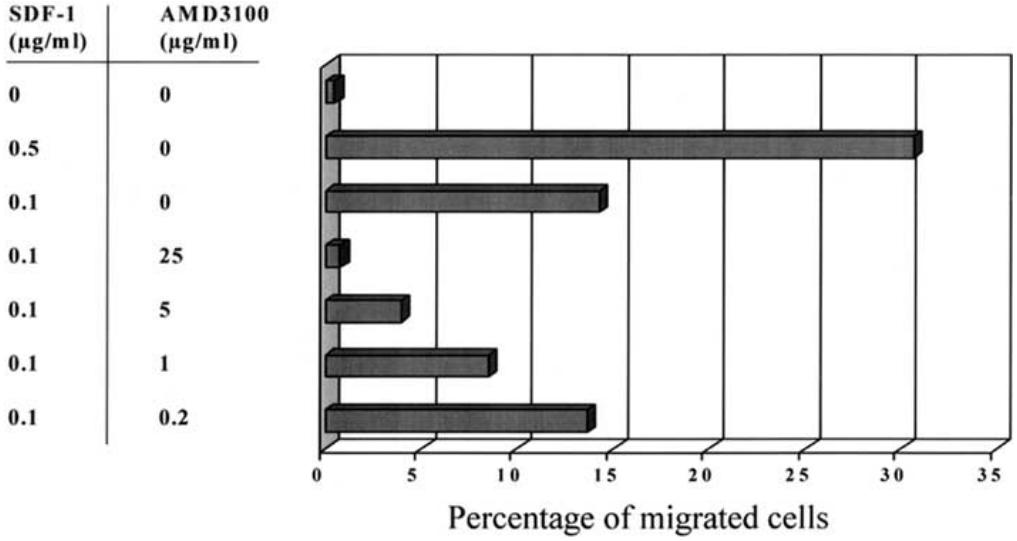


Fig. (23). Inhibition by AMD3100 of SDF-1-elicited chemotaxis of Mac-1^+ cells harvested from the spleens of mice on day 18 postimmunization with CII in CFA [89].

where the target $CD34^+$ cell transplantation dose is 2-5 x 10^6/kg [79,80].

10. AMD3100 AS A SPECIFIC INHIBITOR OF PATHOLOGICAL PROCESSES GENERATED THROUGH CXCR4

By blocking CXCR4, AMD3100 mobilizes $CD34^+$ stem cells from the bone marrow, where the stem cells are normally retained ("homed") in the stromal tissue through the agonistic action of the stromal cell-derived factor SDF-1. AMD3100 breaks up this agonistic effect, and thus releases the stem cells from the bone marrow into the bloodstream. This mobilization has been demonstrated in both men [80] and mice [81], is not only confined to the $CD34^+$ stem cells [78-80], but has also been noted (in mice) for the so-called competitive repopulating long-term marrow self-renewing stem cells [82]. Furthermore, AMD3100 has been shown to augment incorporation of bone marrow-derived endothelial progenitor cells into sites of neovascularization after myocardial infarction by mobilizing the endothelial progenitor cells from bone marrow into peripheral blood [83]. Thus, AMD3100 may well provide a novel strategy for preserving cardiac function in patients suffering from an acute myocardial infarction.

The chemokine receptor CXCR4 is expressed in developing vascular endothelial cells and considered to be essential for vascularization [84], as well as B-cell lymphopoiesis and bone-marrow myelopoiesis [85]. CXCR4 is also expressed on $CD34^+$ cells, including the more primitive, pluripotent progenitor cells, and may therefore, play a role in the homing of hematopoietic stem cells [86]. Leukemic blasts (mostly $CD34^+$) from patients with acute myeloblastic leukemia (AML) express variable amounts of CXCR4, and these cells may be involved in the trafficking of malignant hematopoietic cells [86]. Blockade of CXCR4 by antagonists such as AMD3100 may thus be expected to interrupt this trafficking.

The SDF-1/CXCR4 system is also operative in the migration of neuronal progenitors, may play a role in cortex development and influence neuronal functioning [87]; AMD3100 has been shown to block signaling from CXCR4 by SDF-1 and HIV gp120 in rat neurons and astrocytes.

SDF-1-CXCR4 interactions have also been shown to play a pivotal role in $CD4^+$ T-cell accumulation in rheumatoid arthritis synovium [88]. AMD3100 was found to suppress the clinical symptoms of collagen-induced arthritis in mice, even if treatment was delayed until the first symptoms appeared (Fig. **21**) [89]. Mac-1^+ cells are believed to play a crucial role in the pathogenesis of collagen-induced arthritis, and both the SDF-1-elicited intracellular Ca^{2+} flux (Fig. **22**) and SDF-1-elicited chemotaxis of the Mac-1^+ cells (harvested from the spleens), were markedly inhibited by AMD3100 (Fig. **23**) [89]. Along the same line, it has been shown that CXCR4 plays an important role in the development of cockroach allergen-induced inflammation and airway hyperreactivity in a mouse model of asthma [90], and that the treatment of the allergic mice with AMD3100 significantly reduces airway hyperreactivity, peribronchial eosinophilia and the overall pathological parameters related to asthmatic-type inflammation [90].

11. POTENTIAL ROLE OF THE BICYCLAM AMD3100 IN THE TREATMENT OF CANCER?

Human tumor cells can express a number of chemokine receptors, and of a series of seven human breast cancer cell lines, the most frequently and most strongly expressed chemokine receptor was CXCR4 (Fig. **24**) [91]. Lung colony formation after intravenous injection, as well as spontaneous lung metastasis after orthotopic injection, of the human breast cancer MDA-MB-231 cells in mice were significantly reduced by monoclonal antibody to CXCR4 (Fig. **25**) [91]. It is likely that AMD3100 may exhibit a similar inhibitory effect on tumor cell metastasis, at least to the extent that the latter is mediated by the agonistic interaction of SDF-1 with its receptor CXCR4.

There is a preferential expression of CXCR4 in both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (B-lineage) (ALL) [92], as well as other B-cell

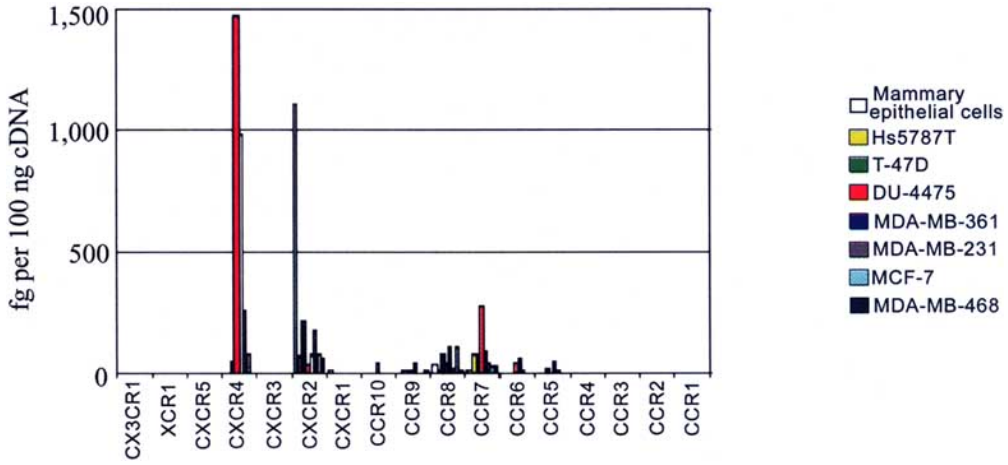


Fig. (24). Expression of chemokine receptors in human tumor cells. Quantitative RT-PCR analyses of all known chemokine receptors in seven human breast cancer cell lines [91].

malignancies [93], and, as this is accompanied by an increased functional response to SDF-1, the CXCR4-SDF-1 circuitry may contribute to the bone marrow infiltration of the neoplastic B cells [94,95], and, in childhood ALL, to extramedullary organ infiltration [96]. AMD3100 has been shown to inhibit SDF-1-dependent migration and proliferation of ALL cells in bone marrow, and should therefore be further entertained as a novel strategy for the treatment of ALL [97].

AMD3100 was found to inhibit the SDF-1-mediated migration of follicular non-Hodgkin's lymphoma (NHL) cells across endothelial and stromal cell layers; AMD3100 enhanced apoptosis and inhibited proliferation of NHL cells [98], and, hence, may be considered as a novel therapeutic modality for the treatment of non-Hodgkin's lymphoma. In fact, neutralization of CXCR4 by specific anti-CXCR4 antibodies have been shown to abrogate the growth of Burkitt's NHL tumors in mice [99], and taking together these studies [98,99], support further clinical trials of CXCR4 antibodies or CXCR4 antagonists in NHL patients.

The SDF-1/CXCR4 circuitry has also been shown to play a possible role in pancreatic cancer progression [100], and, likewise, kidney cancer progression may be mediated by CXCR4 expression and signaling [101]. In human melanoma cells, CXCR4 expression and signaling may contribute to cell motility during invasion, as well as regulation of cell proliferation and survival [102]. In neuroblastoma, the CXCR4-SDF-1 interactions may influence the ability of these tumors to preferentially form metastases in the bone marrow [103], and, likewise, prostate cancers, and perhaps other neoplasms as well, may use the SDF-1/CXCR4 pathway to spread to the bone [104].

In glioblastoma multiforme, SDF-1 and CXCR4 expression correlates with increasing tumor grade: CXCR4 is expressed particularly in regions of angiogenesis, on neovessel endothelial cells, and degenerative, necrotic, and microcystic areas [105]. CXCR4-mediated signaling in astroglioma cells allows these cells to express chemokines involved in angiogenesis and inflammation [106]. CXCR4 is overexpressed in, and required for proliferation of, glioblastoma cells, and specific anti-CXCR4 antibodies inhibit glioblastoma cell proliferation [107,108].

CXCR4 is also expressed on ovarian cancer cells: CXCR4 may influence cell migration in the peritoneum, a

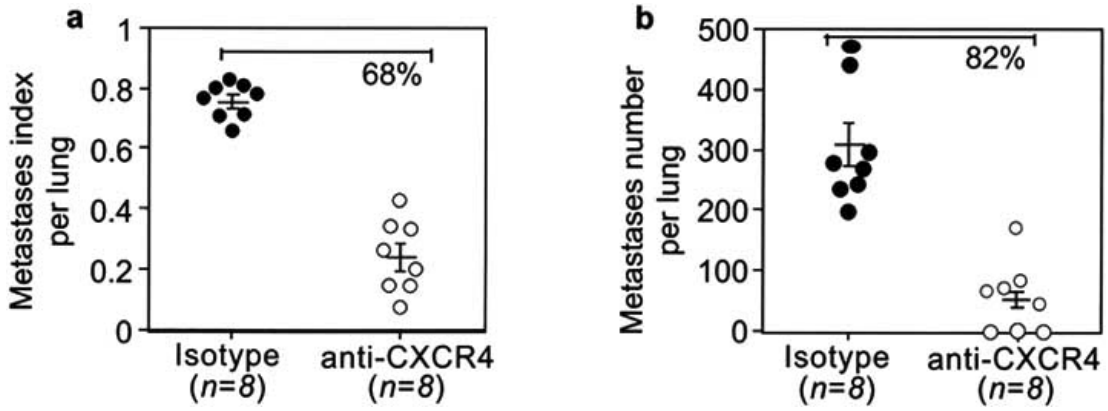


Fig. (25). Effect of CXCR4-neutralization on tumor metastasis in mice [91].

a. Lung colony formation after i.v. injection of MDA-MB-231 cells.

b. Spontaneous lung metastasis after orthotopic injection of MDA-MB-231 cells.

major route for ovarian cancer spread [109]. Ovarian cancer cells expressing CXCR4 form tumors in nude mice, and thus provide a useful clinical model to study the effect of CXCR4 antagonists on tumor growth and spread [109]. SDF-1 stimulates the *in vitro* growth of these cells, and both antibodies to CXCR4 and the bicyclam AMD3100 were found capable of abrogating the stimulatory effect of SDF-1 on the growth of ovarian cancer cells [110].

12. CONCLUSION

The development of the bicyclam AMD3100 has followed a meandrous course, starting with the serendipitous discovery of an impurity in a commercial cyclam preparation, finally leading to a product that may have multiple clinical applications in various fields such as AIDS, cancer, rheumatoid arthritis and stem cell transplantation. It all started with the identification of the bicyclam JM1657 as an impurity, showing anti-HIV activity, in one of several commercial samples of cyclams that were examined for their potential inhibitory effects on HIV replication. While the original compound JM1657 could not be re-synthesized, it served as a model for the synthesis of derivatives thereof, where the two cyclam rings were tethered by an aliphatic bridge (as in JM2763). A quantum increase in anti-HIV activity was noted when new derivatives were constructed with an aromatic bridge linking the two cyclam rings (as in JM3100, later dubbed AMD3100). AMD3100 was promptly recognized as a clinical candidate compound for the treatment of HIV infections. Mechanism of action studies pointed to the viral envelope glycoprotein gp120 as the most plausible target of action for AMD3100. It appeared to be an indirect target, after it was discovered that the direct target for AMD3100 was CXCR4, the receptor for the chemokine SDF-1α and co-receptor for the T-lymphotropic or X4 HIV strains. Phase I/II clinical trials provided the proof of principle: AMD3100 caused a reduction of X4 HIV-1 levels in HIV-infected individuals. During the phase I pharmacokinetic studies, a rather surprising observation was made: AMD3100 brought about a significant elevation in white blood cell counts, and follow-up studies revealed that AMD3100 effected a remarkable mobilization of hematopoietic progenitor cells, in particular $CD34^+$ stem cells, from the bone marrow into the bloodstream. Meanwhile, it was ascertained that AMD3100 is an extremely specific and effective CXCR4 antagonist, and, consequently, AMD3100 was found efficacious in different pathological disorders, depending on the interplay of CXCR4 with its monogamous agonist, SDF-1α, such as collagen-induced arthritis (in a mouse model), as well as tumor cell migration and proliferation of a number of tumor (i.e. leukemia, lymphoma, melanoma, neuroblastoma, glioblastoma, and prostate and ovarian cancer) cells. This now opens a variety of avenues for the potential clinical use of AMD3100 and its congeners: that is, in the treatment of HIV infections, rheumatoid diseases, allergic diseases, malignant diseases, and, in principle, many other diseases that may profit from stem cell mobilization.

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