



Multi-Drug Resistant HIV-1 is Sensitive to Inhibition by Chemokine Receptor Antagonists

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Background:

In a retrospective analysis of a Phase I/II study of the CXCR4 antagonist AMD3100, several subjects with X4 and dual-tropic virus at baseline developed R5 virus upon treatment. The total viral RNA in some patients remained high over the course of treatment despite receiving concomitant antiretroviral medications, suggesting that viruses in these patients were multi-drug resistant but sensitive to inhibition by chemokine receptor antagonists.

Methods:

Virus populations at baseline (day 0), on treatment (day 11) and off treatment (day 18, 39) were characterized by determining the co-receptor tropism of 30-40 envelope clones per time point using an envelope pseudo-virus infection assay (Fig. 1). Gp160 sequences were determined for at least 10 clones per time point. Sequence analysis of individual clones distinguished R5 viruses from X4 and dual-tropic viruses, largely based on differences in the V3 region. Viral stocks from patient samples at baseline were prepared by mixing patient lymphocytes with PHA-stimulated PBMC from uninfected donors. The viruses were tested for their sensitivity to inhibition by the concomitant antiviral medications they were receiving during AMD3100 treatment in PBMC, and the IC50 values were compared to those obtained with reference X4 and R5 strains. Viruses were also tested for sensitivity to inhibition by a combination of the novel oral available CXCR4 antagonist AMD070 and the CCR5 antagonist AMD887.

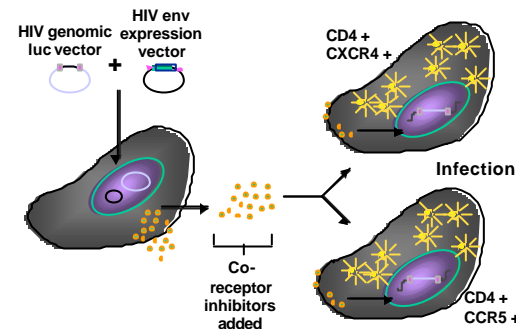


Fig. 1. Pseudo-virus infection assay

Results:

Clonal analysis was performed on the virus populations of three subjects exhibiting dual-tropism at baseline and predominantly R5 tropism with AMD3100 treatment.

In patient one, co-receptor utilization was 68%-R5 and 32%-X4 at baseline, 100%-R5 at day 11 (on treatment) and day 18 (off treatment), retaining to 65%-R5 and 35%-X4 on day 39 (off treatment). This patient received no other retroviral medications.

In patient two, co-receptor utilization was 62.5%-R5, 7.5%-X4 and 30%-dual at baseline, predominantly R5 at day 11 (85%) and day 18 (92%), retaining to 45%-R5 and 65%-dual by day 39. This patient received ddi, d4T, Kaletra (lopinavir/ritonavir) and amprenavir.

In patient three, the co-receptor utilization was 76%-R5, 14%-X4 and 10%-dual at baseline, 100%-R5 at day 11, and remaining R5 (100%) at day 18 and day 39. This patient received also combivir (AZT/3TC), abacavir and nelfinavir.

Sequence analysis of individual clones distinguished R5 viruses from X4 and dual-tropic viruses, largely based on differences in the V3 region, but outside of V3 also contributed to X4 usage.

During a short course of AMD3100 treatment X4 and dual-tropic variants were suppressed, accompanied by a concomitant increase in the proportion of R5 variants in the viral population.

Multi-RT- and PI-resistant virus remained equally sensitive to chemokine receptor inhibitors.

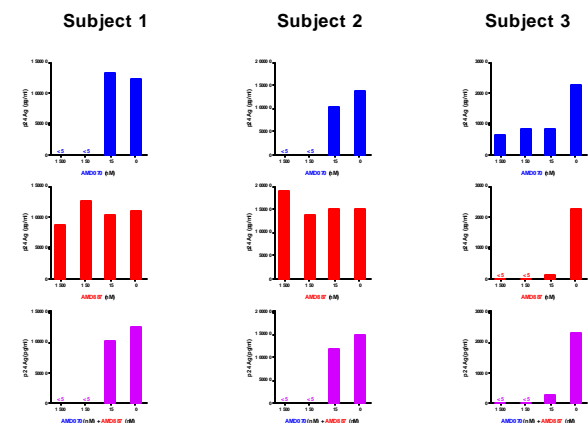
Tropism and V3 Sequence of Envelope Clones

Subject 1			Subject 2			Subject 3		
Sample	Tropism	V3 Loop Amino Acid Sequence	Sample	Tropism	V3 Loop Amino Acid Sequence	Sample	Tropism	V3 Loop Amino Acid Sequence
day 0_pool	R5/X4	CTRPSNTRKXXXGPRXXVYTTGEGDIRWAHC	day 0_pool	DM	CTRPNNTRKXXXGPRVWYTTGEGDIRWAHC	day 0_pool	DM	CTRPNNTRKXXXGPRVWYTTGEGDIRWAHC
day 0_c07	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c02	R5	CTRPNNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c02	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC
day 0_c08	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c05	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c04	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 0_c21	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c24	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c07	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 0_c26	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c26	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c24	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 0_c25	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c28	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c06	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 0_c26	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c12	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c17	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 0_c24	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c19	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 0_c27	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 0_c09	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 0_c31	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 0_c26	X4	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC
day 0_c13	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 0_c30	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 0_c30	X4	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC
day 0_c19	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 0_c38	X4	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC			
day 0_c30	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC						
day 0_c38	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC						
day 11_pool	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_pool	DM	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_pool	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c01	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c01	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c04	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c04	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c08	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c11	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c05	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c12	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c19	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c07	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c15	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c22	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c12	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c18	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c23	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c13	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c23	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c31	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c16	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c27	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c33	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c19	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c03	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c36	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 11_c24	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 11_c17	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC			
day 11_c25	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC						
day 18_pool	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_pool	DM	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_pool	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c01	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c03	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c02	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c03	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c04	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c03	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c06	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c09	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c05	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c09	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c12	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c06	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c04	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c42	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c07	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c16	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c43	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c08	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c17	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c46	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c12	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c20	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c52	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c13	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 18_c23	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c45	Dual	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 18_c14	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
						day 18_c15	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
						day 18_c36	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_pool	R5/X4	CTRPSNTRKXXXGPRXXVYTTGEGDIRWAHC	day 39_pool	DM	CTRPNTRKXXXGPRVWYTTGEGDIRWAHC	day 39_pool	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c12	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c02	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c01	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c14	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c07	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c02	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c18	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c10	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c04	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c32	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c37	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c06	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c37	R5	CTRPSNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c48	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC	day 39_c07	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c09	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c01	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c08	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c11	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c15	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c11	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c21	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c18	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c12	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c27	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c19	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c18	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
day 39_c41	X4	CTRPSNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c21	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC	day 39_c23	R5	CTRPNTRKSIKNGPRAFYTTGEGDIRWAHC
			day 39_c23	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC			
			day 39_c36	Dual	CTRPNTRKRVTLGPRVYVYTTGEGDIRWAHC			

compound	Subject 1	Subject 2	Subject 3	NL4.3 (X4)
AZT (nM)	0.87	9.62	2.99	3.1
d4T (nM)	50	95	64	156
3TC (nM)	1.4	78	3.3	1.3
ddC (nM)	38.7	21.1	50.1	71.6
ddI (nM)	0.317	1.56	1.37	0.87
abacavir (mM)	0.27	0.68	0.99	1.41
PMPA (mM)	0.17	0.34	0.48	0.36
nevirapine (ng/ml)	5.04	11.04	4.24	8.36
BHAP-E (ng/ml)	1.1	8.51	7.10	7.51
DMP-266 (ng/ml)	0.09	0.32	0.11	0.47
lopinavir (ng/ml)	3.80	468.5	17.9	9.3
saquinavir (ng/ml)	3.73	146.02	17.7	8.45
amprenavir (ng/ml)	5.34	138.77	17.8	3.2
indinavir (ng/ml)	4.88	345.41	17.1	15.13
nelfinavir (ng/ml)	4.35	482.36	17.8	10.78
ritonavir (ng/ml)	16.5	>2500	74.3	30.1
AMD3100 (nM)	12.5	15.6	4.2	16.4
AMD070 (nM)	5.1	9.2	34.4	10.2

Anti-HIV activity of antiviral drugs (RT inhibitors and PI inhibitors) against viruses isolated from the three subjects.

Anti-HIV activity of antiviral drugs and CXCR4 antagonists against the three patient isolates and the laboratory strain (NL4.3) in PBMC. Supernatant was collected after 810 days and viral replication was measured by p24 Ag ELISA (Perkin-Elmer) and IC50 values determined.



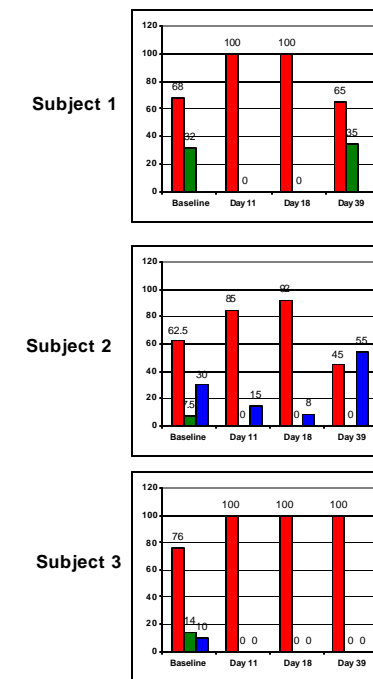
Anti-HIV activity of AMD887 (CCR5 antagonist) and AMD070 (CXCR4 antagonist) against three clinical isolates

Anti-HIV activity of AMD887, AMD070, and their combination against the three clinical isolates in PBMC. Supernatant was collected after 12 days and viral replication was measured by p24 Ag ELISA.

Shift in tropism during and after AMD3100 treatment

	RLUs X4 cells	RLUs R5 cells	Tropism	Viral Load
Subject 1				
BASELINE	2443	34494	R5/X4	53176
DAY 11	56	153409	R5	106411
DAY 18	66	94923	R5	547702
DAY 39	8232	104364	R5/X4	na
Subject 2				
BASELINE	48606	241910	D/M	326988
DAY 11	505	170994	D/M	338867
DAY 18	284	202086	D/M	96208
DAY 39	44667	144214	D/M	na
Subject 3				
BASELINE	2046	409212	D/M	380909
DAY 11	81	384574	R5	458127
DAY 18	83	469955	R5	482103
DAY 39	81	330739	R5	na

AMD3100 suppressed X4 and dual-tropic variants



Conclusion:

A 10-day treatment with the CXCR4 antagonist AMD3100 suppressed replication of X4 and dual-tropic variants, resulting in a predominance of R5 variants. The viruses in these patients were in some cases multi-drug resistant to their concomitant antiretroviral medications, but remained sensitive to inhibition with CXCR4 antagonists.