ACTIVITY OF ECD4-IG AGAINST CCR5 ANTAGONIST-RESISTANT HIV-1

Poster # 484

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ABSTRACT

Background: The anti-HIV molecule eCD4-Ig targets CD4 and chemokine coreceptor binding sites of HIV-1 gp120. eCD4-Ig neutralizes a broad range of HIV-1 isolates from various clades and protects humanized mice and macaques from infection with HIV and SHIV, respectively. To investigate whether eCD4-Ig can neutralize HIV-1 isolates resistant to the CCR5 antagonists maraviroc (MVC) and vicriviroc (VCV) we determined the eCD4-Ig susceptibility of infectious HIV-1 recombinants expressing env from MCV-susceptible and -resistant viruses.

Methods: HIV-1 resistant to VCV and MVC was identified from 3 participants in ACTG protocol A5211, a phase 2 trial of VCV. Infectious recombinant viruses carrying env genes of the paired baseline and MVC-resistant viruses cloned from plasma HIV-1 RNA were generated by transfection into 293T cells together with the recombination vector pNL4-3ΔEnv. Susceptibility to eCD4-Ig, MVC and the CXCR4 antagonist AMD3100 was determined by a standardized drug susceptibility assay on TZM-bl cells. HIV-1 recombinants expressing env from HIV-1 Bal, Hxb2 and 89.6, respectively, served as controls. Susceptibility was expressed as the 50% inhibitory concentrations (IC50) or percent maximum inhibition (PMI).

Results: As reported, VCV and MVC resistance emerged after 24, 103 and 138 weeks of VCV treatment (sub07, sub57 and sub85, respectively). At baseline, the IC50s for MVC ranged from 0.2 to 5.4 nM and IC50s to eCD4-Ig ranged from 0.07 to 4.3 μg/mL. The PMI for MVC of the paired resistant viruses ranged from 0% to approximately 45% (IC50s could not be calculated). By contrast, susceptibility to eCD4-Ig showed three distinct patterns: unchanged (sub57); increased susceptibility (-0.4-fold change in IC50; sub85); and decreased (+14.8-fold change in IC50; sub07). By comparison, the eCD4-Ig IC50 of recombinant viruses expressing env from Bal, Hxb2 or 89.6 ranged from 2-13 ng/mL.

Conclusions: Recombinant HIV-1 resistant to MVC showed disparate patterns of susceptibility to eCD4-Ig, suggesting that mutations conferring resistance to small-molecule CCR5 antagonists affect interactions with the CCR5mim1 sulfonated peptide moiety of eCD4-Ig in different ways. Analysis of additional HIV-1 isolates with varying susceptibility to CCR5 antagonists and eCD4-Ig may help refine our understanding of the interaction between this promising novel HIV-1 entry inhibitor and the CCR5mim1 binding domain on gp120.

RESULTS

HIV recombinants carrying Env gene from strains sensitivities to eCD4-Ig

Figure 1-1. Susceptibility to eCD4-Ig, MVC and AMD3100 of HIV-1 recombinants carrying Env gene from HIV-1 X4 strains Hxb2 (A,B and C) or 89.6 (D,E and F) assayed in TZM-bl cells.

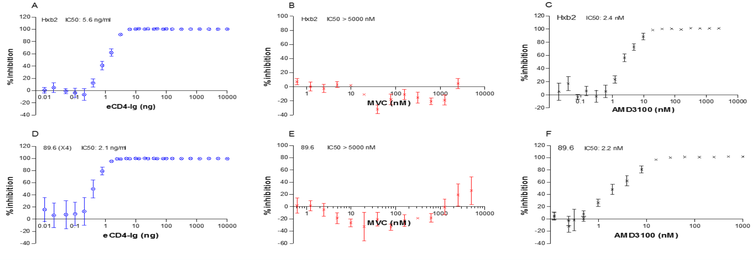
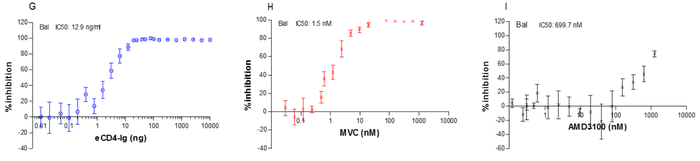


Figure 1-2. Susceptibility to eCD4-Ig, MVC and AMD3100 of HIV-1 recombinants carrying Env gene from HIV-1 R5 strain Bal (G,H and I) assayed in TZM-bl cells.



HIV recombinants carrying Env gene from patients sensitivities to eCD4-Ig

Figure 2. Susceptibility to eCD4-Ig and MVC of HIV-1 recombinants carrying Env gene from Subject 57 (A and D are baseline; B and E are MVC resistant; C and F are baseline and resistant comparison, FC: fold change in IC50) assayed in TZM-bl cells.

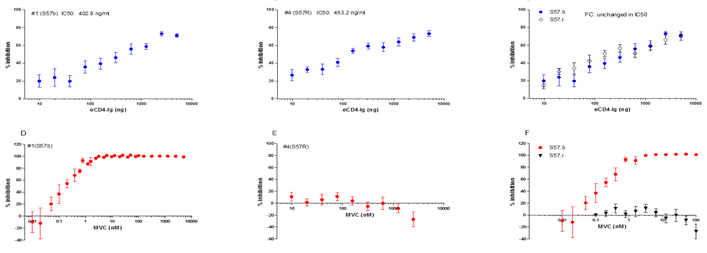


Figure 3. Susceptibility to eCD4-Ig and MVC of HIV-1 recombinants carrying Env gene from Subject 85 (A and D are baseline; B and E are MVC resistant; C and F are baseline and resistant comparison, FC: fold change in IC50) assayed in TZM-bl cells.

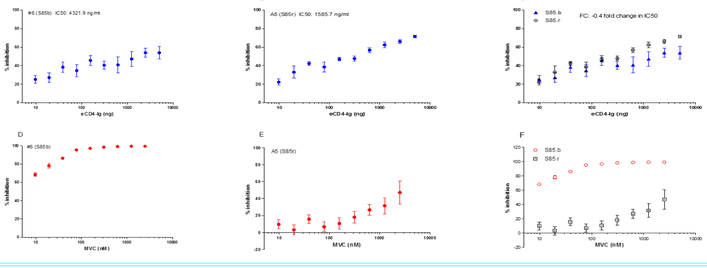
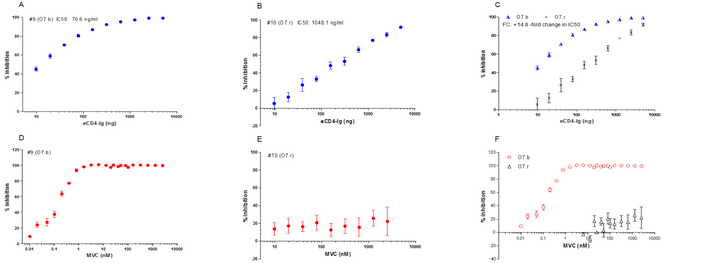


Figure 4. Susceptibility to eCD4-Ig and MVC of HIV-1 recombinants carrying Env gene from Subject O7 (A and D are baseline; B and E are MVC resistant; C and F are baseline and resistant comparison, FC: fold change in IC50) assayed in TZM-bl cells.



IC50 values of HIV-1 recombinants carrying HIV-1 Env gene to eCD4-Ig

Table 1. IC50 values of HIV-1 recombinants carrying HIV-1 interested Env gene to eCD4-Ig,MVC and AMD3100 by TZM-Black cell assays.

				Viral sensitivities				
					IC50 of eCD4-Ig		IC50 of MVC	IC50 of AMD3100
Subject	WT/Mutant	Env-gene	Recombinant virus	tropism	(ng/ml)	PMI*	(nM)	PMI*	(nM)
Subject 57	Baseline	572-31	S57.b**	**R5**	**402.8**	88.1	**0.17**	98.8	-
	Resistance	57-106-22	S57.r***	**R5**	**463.2**	85.9	**> 2500**	-52.3	-
Subject 85	Baseline	85-108	S85.b	**R5**	**4321.9**	56.1	**5.4**	99.4	-
	Resistance	85-138-81	S85.r	**R5**	**1585.7**	84.4	**> 2500**	47.2	-
Subject O7J	Baseline	W0	O7J.b	**R5**	**70.6**	99.1	**0.15**	99.6	-
	Resistance	W28	O7J.r	**R5**	**1048.1**	94.0	**> 2500**	40.9	-
HIV strain	Hxb2	Env	R-HIV-Hxb2	**X4**	**5.6**		**> 5000**		2.4
	89.6	Env	R-HIV-89.6	**X4**	**2.1**		**> 5000**		2.2
	Bal	Env	R-HIV-Bal	**R5**	**12.9**		**1.52**		699.7

Notes: PMI, percent maximum inhibition (PMI); MVC, Maraviroc, a CCR5 receptor antagonist; AMD3100, a CXCR4 receptor antagonist. percent maximum inhibition. b**, baseline; r***, resistance.*

CONCLUSIONS

- The eCD4-Ig IC50 of recombinant viruses expressing env from Bal, Hxb2 or 89.6 ranged from 2-13 ng/mL. As reported, VCV and MVC resistance emerged after 24, 103 and 138 weeks of VCV treatment (sub07, sub57 and sub85, respectively).
- At baseline, the IC50s to eCD4-Ig ranged from 0.07 to 4.3 μg/mL. The PMI for MVC of the paired resistant viruses ranged from 0% to approximately 45% (IC50s could not be calculated).
- Compared the resistant and baseline, susceptibility to eCD4-Ig showed three distinct patterns: unchanged (sub57); increased susceptibility (-0.4-fold change in IC50; sub85); and decreased (+14.8-fold change in IC50; sub07).
- Recombinant HIV-1 resistant to MVC showed disparate patterns of susceptibility to eCD4-Ig, suggesting that mutations conferring resistance to small-molecule CCR5 antagonists affect interactions with the CCR5mim1 sulfonated peptide moiety of eCD4-Ig in different ways.

ACKNOWLEDGMENTS

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