

POSTER 4**MULTIPLE MODES OF RESISTANCE TO HIV-1 ENTRY INHIBITOR C37**

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The HIV-1 entry inhibitor C37 targets the gp41 N-terminal heptad repeat region (N-HR), thereby blocking trimer-of-hairpins formation crucial to viral membrane fusion. Due to transient exposure of the C37 target, antiviral potency depends not only C37 binding affinity, but also on inhibitor association rate with gp41. As a consequence of this kinetic restriction, higher affinity C37 variants do not possess enhanced potency against nonresistant Env compared to the wild type C-peptide. Here, we describe the selection of HIV-1 resistant to both wild type C37 and a higher affinity variant C37_{KYI}. Through serial propagation of HIV-1_{NL4-3} in increasing inhibitor concentrations, two unique patterns of resistance involving both gp41 and gp120 emerged. For viruses propagated up to moderate levels of C37 (100 nM, $\sim 100 \times IC_{50}$), mutations were observed primarily in the gp41 N-HR. These mutations directly disrupt the binding affinities of both C37 and C37_{KYI}, but only conferred Env resistance to wild type peptide. Consistent with this observation, resistance to moderate levels of C37_{KYI} (100 nM, $\sim 200 \times IC_{50}$) appeared to be linked to additional mutations in the gp41 C-HR (C-terminal heptad repeat region) that temporally limit exposure of the C37 target, thereby imposing further kinetic restrictions on inhibitor binding. A similar mechanism was previously reported for resistance to the HIV-1 entry inhibitor 5-Helix. Increasing C37 and C37_{KYI} concentrations beyond 100 nM elicited nonconservative substitutions in gp120 constant regions. However, only at extremely high inhibitor levels ($\sim 5000 \times IC_{50}$) did the resistance profiles for the two inhibitors begin to converge, incorporating mutations from both affinity- and kinetically-driven mechanisms. The results demonstrate how two equipotent fusion inhibitors that have the same mechanism of action can elicit resistance through distinct pathways. The emergence of mutations in both gp41 and gp120 demonstrates the tremendous structural and functional plasticity of Env and might provide clues into the coupling between the two subunits.