

POSTER 41**ANTI-HIV-1 Gag APTAMERS SHOW POTENTIAL TO INHIBIT HIV-1 RELEASE**

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Gag is one of the key structural proteins of HIV-1 and plays essential roles in both early and late events. To perturb specific Gag-Gag, Gag-RNA and Gag-cellular protein interactions, we raised several anti-Gag RNA aptamers, characterized their in vitro interaction with Gag and tested their potential to inhibit HIV-1 replication. Aptamers were isolated using SELEX on a mutant Gag which lacked the p6 domain (and hence named DP6-Gag) and their dissociation constants ranged from ~80 to ~200nM. To identify the domains on Gag recognized by the aptamers, we performed binding assays with recombinant purified Gag components- MA, CA and NC. We found that several aptamers bound uniquely to either MA or NC. We also obtained aptamers that recognized the whole Gag protein. Binding assays performed with two other mutants of Gag, with mutations in their N-terminus, show that the N-terminal basic patch of Gag is important for the RNA-binding ability of Gag.

To test the potential of the aptamers to inhibit the assembly of HIV-1, 293T cells were co-transfected with the following: the envelope-deleted pNL4-3.Luc.R-E- molecular clone, VSV-G envelope expression constructs and plasmids that express the aptamers under the control of the CMV promoter. We observed a significant reduction, in the range of ~50 to ~90%, in the levels of viruses released 48h post-transfection with most of our aptamers. One aptamer, DP6-12, resulted in an inhibition of close to 90% comparable to the inhibition seen with most anti-HIV shRNAs. A dot-blot analysis of the RNA extracted from the released viruses revealed that the aptamers were encapsidated by the virions. However, when equal amount of viruses were used to infect 293T cells, we did not observe a significant change in their infectivity.

To obtain mechanistic insights into the basis for reduction in virus particles, we measured intracellular viral RNA levels and found that the reduction in p24 released correlates with reduction in viral mRNA. However, we did not observe a significant increase in the levels of interferon response genes that we measured: OAS2, PKR and MxA. Further studies to understand the nature of this late event block are currently in progress.