

POSTER 25**ENGINEERING APTAMERS TO HIV-1 RT FOR IMPROVED ANTIVIRAL BIOACTIVITY**

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RNA and ssDNA aptamers selected to bind HIV reverse transcriptase have been shown to inhibit RT competitively in enzymatic assays and to interfere with viral replication when expressed in mammalian cells. One of the challenges for developing these reagents for eventual use in gene therapy is to determine how antiviral bioactivity is governed by features of the aptamers, by the aptamer-expression system and by the virus itself. Our current work utilizes an aptamer-expression construct in which the expressed aptamer is expressed between two hammerhead ribozymes, which release the aptamer from the transcript upon self-cleavage. Although the RNA aptamers in our collection were originally selected as 118 or 134 nt transcripts ("full-length"), most are equally potent RT inhibitors when truncated to 35 to 50 nt transcripts ("cores") that contain a pseudoknot RNA secondary structural motif. Plasmids were assembled to direct the expression of single "full-length" aptamers, single "core" aptamers, or bicistronic transcripts in which two aptamers are connected by a short (6 to 15 nt) linker. Aptamer transcripts accumulate in the cytoplasm of 293T cells transfected with aptamer-expressing plasmids, as detected by fluorescence in situ hybridization (FISH). The amount of pseudotyped virus produced from 293T cells co-transfected with VSV-G-expressing, and env-deleted HIV-1 provirus plasmids was reduced when aptamer-expressing plasmids were also present. After normalizing to the amount of viral RNA released from these cells, subsequent infection of fresh cells varied greatly depending on the identity of the aptamers expressed in the virus-producing cells. Current work includes an evaluation of aptamer bioactivity against replication-competent HIV-1, and a comparison of aptamer expression from plasmids (delivery by transient transfection) and expression in stable cell lines. Finally, aptamer transcripts are detected in purified viral preps by RT-PCR, consistent with previous reports that aptamers to RT are packaged within the virions. We are exploring the determinants of this packaging and its potential impact on production of infectious virus. Our results thus far demonstrate that we have established a reliable experimental system in which to study inhibition of HIV-1 replication by expressed RNA aptamers.