Analysis of HIV-1 reverse transcriptase inhibition by multiple RNA aptamers
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Rapidly increasing resistance to currently available HIV-1 drugs has prompted the exploration of therapies less susceptible to resistance development. Anti-HIV-1 treatments often target reverse transcriptase (RT), the viral protein that polymerizes the integrated DNA copy of the viral RNA genome. For many such drugs, resistance requires only a few amino acid changes in the RT protein. Aptamers are small, single stranded nucleic acids that form unique three-dimensional structures allowing specific binding to molecular targets. When bound to proteins, aptamers often hinder their native function. RNA and DNA aptamers that bind to viral proteins and inhibit HIV-1 at several stages of infection have been isolated. Burke et al, (1996) have proposed that variability in anti-RT aptamer structures may overcome the development of resistance because of different contacts with the RT protein. Here, multiple anti-HIV-1 RT RNA aptamers (118 or 134 nucleotides in length) were screened for inhibition of RT-catalyzed DNA polymerization. Aptamer inhibition of RT activity was measured using an HIV-derived synthetic template and fluorescently-labeled primers, and by quantifying the amount of fully extended primer. Sixty-two aptamers were screened and grouped according to inhibition performance. In the “BEST” category, twenty-two aptamers displayed ~100 percent inhibition at the highest aptamer concentration (100 nM) and half-maximal inhibition (IC50) values of less than 3 nM. The “VERY GOOD” category contains twenty aptamers with greater than 90 percent inhibition at the highest concentration and IC50 values between 3 nM and 9.5 nM. Of the remaining samples, “MODEST” aptamers yielded 25 to 80 percent inhibition and “POOR” aptamers showed less than 20 percent inhibition at the highest aptamer concentration. Furthermore, sequence and structural analyses may reveal variations in the aptamer-protein interaction between potent aptamers. These data may define the critical interactions between the aptamer and RT, increasing inhibition potency while reducing susceptibility to resistance.