173g Computational Design of RNA Interference Gene Therapy Strategies to Treat HIV-1 Infections and Block Viral Escape

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Current treatments for HIV infections utilize combinations of chemotherapeutic drugs to block viral replication. However, HIV is a rapidly mutating virus, and as a consequence, viral strains that are insensitive to drugs eventually emerge, leading even treated HIV-positive patients to eventually progress to AIDS. An increasingly dire problem is that strains of HIV that resist most or all of the drugs currently applied have been detected, and the prevalence of these strains is constantly increasing. This ominous trend now appears to indicate that viral resistance is increasing at a rate that exceeds that at which new small molecule drugs are being developed. Consequently, much attention has been directed toward the use of genetic therapies to block HIV-1 infection, and a particularly promising class of potential approaches utilizes a powerful cellular gene regulatory pathway termed RNA interference.

RNA interference (RNAi) is a robust and highly evolutionarily conserved mechanism for down-regulating gene expression through targeted mRNA cleavage. Activation and targeting of this pathway is accomplished by treating a cell with short (~22 nucleotides) double stranded RNA molecules, called short interfering RNAs (siRNAs), that encode a sequence that matches a portion of the targeted gene. It has been shown that RNAi can be induced to effectively reduce the expression of human immunodeficiency virus 1 (HIV-1) genes and block viral replication *in vitro*. However, since even 1 or 2 nucleotides mismatched between the siRNA and the viral RNA are sufficient to significantly impair target degradation, the emergence of resistant strains is a major potential obstacle to long term suppression of this highly mutable virus. In fact, it was recently reported that HIV can evolve resistance to several different RNAi treatments in cell culture. In order to fully utilize this powerful technique therapeutically, treatments must be optimized to compensate for viral evolution. We seek to identify quantitative design strategies for using RNAi to suppress HIV-1 in a manner that minimizes the chances of viral escape. In the results presented here, we have focused on RNAi strategies that target the viral TAR RNA loop - a very highly evolutionarily conserved sequence of the viral RNA genome that participates in an essential viral gene regulatory function - a positive feedback loop with the viral transcription factor, Tat.

In order to quantitatively test a wide range of potential strategies and guide experimental design, we have developed an integrated computational and experimental approach. Our computational system uses a novel type of stochastic simulation that represents viruses as independent entities and incorporates molecular-level details describing the unique mechanisms of HIV-1 reproduction. This approach allows us to quantitatively evaluate clinically relevant questions that cannot be addressed using previously described theoretical methods. As we recently reported, these simulations have yielded counterintuitive predictions about the efficacies of antiviral RNAi strategies targeting the HIV-1 TAR loop. In particular, they predict that therapeutic efficacy is highly sensitive to heterogeneity in the delivery of RNAi-inducing species (such as siRNAs) to different cells within the target population. In addition, even though the entire TAR loop is relatively highly evolutionarily conserved, the therapeutic efficacy of a treatment is highly sensitive to the choice of subsequence within the TAR loop that is targeted by RNAi. These results also provide insights into the consequences of directing RNAi against a viral positive feedback loop – a regulatory motif that is utilized by many different types of viruses – such that these finding might be instructive in designing RNAi-based therapies to treat other important pathogens.

An important aspect of our modeling approach is that it enables us to directly validate our computational predictions in an *in vitro* experimental system. To this end, we have developed a lentivirus-based RNAi-inducing gene therapy vector for producing sustained and dose-dependent reductions (up to >95%) of target gene expression. Previous studies have reported that the HIV-1 TAR loop is refractory to RNAi-

mediated degradation, perhaps because it has a high degree of secondary structure. However, we optimized a hairpin RNA that induces both specific and effective RNAi against TAR. Using these tools, we created stable human T cell lines that serve as tunable RNAi challenges for a replicating HIV virus. In this manner, we can construct experimental scenarios that recreate the strategies tested in the computational model, and we are thereby quantitatively testing our model predictions. These studies should help to inform the design of effective RNAi-based therapies for suppressing HIV infections and improving the long-term outcomes of clinical antiviral applications.