Assisted suicide for retroviruses

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Antisense oligonucleotides activate the self-destruction of retroviruses *in vivo*.

Antisense technology has made little headway as a therapeutic approach to retroviral infection. But this may change if results reported in this issue by Moelling and colleagues[1] prove robust and generalizable. The authors demonstrate the therapeutic efficacy of antisense-mediated silencing[2] using a classic model of retroviral pathogenesis, infection with the murine spleen focus-forming virus. As the oligonucleotide targets a common feature of the Retroviridae, the strategy should in theory be applicable to any retrovirus.

The defining event of the retroviral replication cycle is conversion of the RNA genome into double-stranded DNA[3] (**Fig. 1**). This process, which requires a virally encoded reverse transcriptase with both DNA polymerase and RNase H activities, is followed by irreversible insertion of the newly synthesized DNA into the genome of the host cell to form a provirus that directs the production of progeny virions. During reverse transcription, the viral RNA serves as the template for polymerization of minus-strand DNA. RNase H–mediated cleavage of the viral RNA is necessary to free the minus strand for plus-strand DNA synthesis[3].

Only a short stretch of the viral RNA (typically 12–20 bases), known as the polypurine tract, is resistant to cleavage and remains firmly bound to its minus-strand DNA complement. The RNA-DNA duplex spanning the polypurine tract adopts an unusual local structure owing in part to atypical base pairing and the presence of unpaired bases. A specific interaction of the viral RNase H with this structure leads to precise cleavage at the 3′ end of the polypurine tract[4], and the free 3′ end of this short, remaining stretch of hybridized RNA then initiates plus-strand DNA synthesis. After plus-strand synthesis is underway, RNase H must cleave once again, at precisely the same site, so that the RNA primer can be displaced. If all goes well—for the virus—the result is a complete, double-stranded DNA copy of the viral genome.

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In previous studies using cultured cells, Moelling and her collaborators showed that antisense oligodeoxynucleotides against HIV-1 prevent viral replication, that the treatment causes aberrant cleavage of the viral RNA genome, and that the effect is mediated entirely by the viral reverse transcriptase[5–7]. The strategy works by inducing premature cleavage of the viral RNA by the viral RNase H, essentially programming the virus to destroy itself. But not all regions of the viral RNA make good targets. The oligonucleotide is most effective if it hybridizes to a sequence spanning the polypurine tract–U3 junction[7], thereby forming a structure that mimics the viral RNase H substrate and induces premature cleavage of the viral RNA before reverse transcription is complete (**Fig. 1**). No other antiretroviral strategy works in this way; all drugs approved for use against HIV-1, for example, simply block a step in the viral replication cycle[8,9]. The antisense treatment, in contrast, triggers the viral reverse transcriptase to destroy its own template, precluding reverse transcription and integration of the provirus.

In the new paper, the authors study the antisense treatment *in vivo*. Because there is no practical, small-animal model that supports the complete HIV-1 replication cycle, they used mice experimentally infected with the spleen focus forming virus, which in the presence of the helper virus Friend murine leukemia virus causes a severe erythroleukemia in mice[10]. The logic was sound, as the approach should work essentially the same way for murine leukemia virus as for HIV-1. All that was required was to tinker with the oligonucleotide sequence. The authors found that pretreatment of virions with oligonucleotides complementary to the murine leukemia virus polypurine tract prevents or reduces infection. Moreover, injecting mice with phosphorothioated oligonucleotides intravenously or intraperitoneally before, during or soon after infection reduces infection and prolongs survival. Although the results might be attributed exclusively to the viral RNase H activity, unrelated mechanisms cannot formally be excluded. For example, it is possible that hybridization of the oligonucleotide to viral mRNAs in infected cells could also recruit cellular RNases.

Of course, the approach of Moelling and colleagues will need to clear many hurdles on the way to becoming a viable therapeutic option. The polypurine tract sequences of simian immunodeficiency virus and HIV are very similar (for some strains they are identical), and studies in SIV-infected nonhuman primates would be a valuable next step. Among other considerations, it will be important to assay the ability of target viruses to generate resistance mutations. Although the polypurine tract is highly conserved, it might be wise to place your bets on the virus—currently, there isn't a single antiretroviral approved for use or in clinical trials that HIV-1 has not succeeded in evading.

Biodistribution is another potential concern. HIV-1 causes a systemic infection: the virus replicates in T-cells and tissue macrophages,

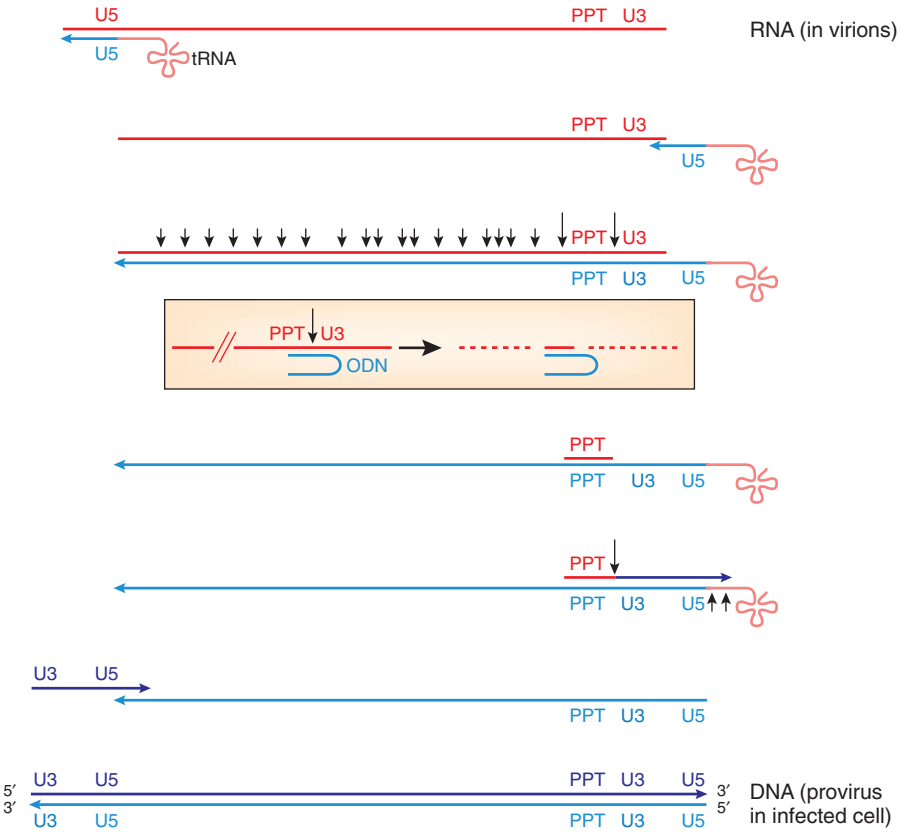

Figure 1 Steps in the reverse transcription of retroviral RNA (red) to produce double-stranded DNA (dark and light blue), and the site of interference by oligodeoxynucleotides targeted to the polypurine tract. A cellular tRNA primes synthesis of minus-strand DNA (light blue). The RNase H domain of the retroviral reverse transcriptase cleaves the RNA strand of the resulting duplex into short fragments without cleaving within the polypurine tract. Instead, cleavage occurs precisely at the polypurine tract–U3 junction. The 3′ end of the polypurine tract RNA then serves as a primer for plus-strand synthesis (dark blue). A second cut at the polypurine tract–U3 junction facilitates removal of the primer. In both cases, cleavage must occur precisely at this junction because the first few bases of U3 are subsequently required for integration. As indicated in the shaded box, an antisense oligodeoxynucleotide complementary to the polypurine tract creates an RNA-DNA duplex that mimics the structure recognized by the reverse transcriptase, leading to premature cleavage of viral RNA at the polypurine tract–U3 junction before reverse transcription. The most effective oligodeoxynucleotide design includes an antisense strand that is perfectly complementary to the polypurine tract and a passenger strand that is partially complementary to the antisense strand. PPT, polypurine tract. ODN, oligodeoxynucleotide. Vertical arrows denote cleavage of the viral RNA by RNase H. Short arrows represent non-specific digestion of viral RNA during minus-strand synthesis, whereas long arrows indicate sites of highly specific cleavage defined by the structure of RNA/DNA duplex at the site of polypurine tract.

with infected cells actively producing virions in lymphoid tissue and the circulatory and central nervous systems. In contrast, injected oligonucleotides accumulate mainly in the liver, and many of the treatments that have shown promise *in vivo* are those that target genes in situations amenable to localized administration[2]. Even if tissue distribution is a concern, anti-HIV oligonucleotide treatment could still move forward as a candidate for prophylaxis, for example, as a component of a vaginal microbicide. Oligonucleotide treatment might also be appropriate for emergency post-exposure prophylaxis, or in situations where viral replication has already been substantially reduced by treatment with other inhibitors. As with all pharmacologic agents, issues of toxicity, pharmacokinetics, large scale production and patient adherence will also need to be addressed.

Beyond these challenges, there is certainly cause for optimism. Research on using antisense oligonucleotides as therapeutic agents already has a rich history in both the academic and commercial arenas, and much is known about oligonucleotide design, synthesis and biological activity[2]. With the advent of RNAi-based therapies, research and development in this area is likely to expand further. Importantly, the pharmacokinetic behavior of oligonucleotides is probably not significantly affected by primary sequence but can still be fine tuned by a limitless array of chemical modifications and through incorporation of various nucleoside analogs. Thus, progress in one application of oligonucleotide therapy can lead readily to progress in others. For example, previous research on antisense therapeutics for genetic diseases and tumors, which require repeated dosing over long periods of time, may be relevant to HIV-1, which may also require long-term treatment.

The antisense approach may be applicable to a broad range of retroviral diseases. Polypurine tract identification is easy, and Moelling and colleagues have already demonstrated efficacy against two unrelated retroviruses—a primate lentivirus (HIV-1) and a gammaretrovirus of mice. Thus, the strategy should in principle be readily adapted to an array of retroviral pathogens of medical, environmental and agricultural importance, including bovine immunodeficiency virus, equine infectious anemia virus, feline immunodeficiency virus, feline leukemia virus, Koala retrovirus and the Jaagsiekte sheep retrovirus, to name just a few.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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