The discovery of the mechanism of RNA interference (RNAi) for knockdown of gene expression has spurred a variety of therapeutic applications. An asset of using small interfering RNA (siRNA) as therapeutics is their exquisite sequence-specific mechanism of action. It seems possible that the first RNAi therapy that could reach patients would aim at a debilitating eye disease called age-related macular degeneration, which afflicts 30–50 million people globally; the reason being that siRNAs can be delivered directly to the diseased tissue—that is, literally injected into the eye. To minimize systemic exposure and to make it less likely that the drug will have unanticipated, harmful effects elsewhere in the body, the therapeutic focus has been on local delivery. The disease is triggered by a protein called vascular endothelial growth factor (VEGF) that promotes blood vessel growth. Too much of this protein leads to the sprouting of excess blood vessels behind the retina. Macular degeneration is induced by leakage of the blood vessels, which causes clouding and frequently destroys vision. The new RNAi drugs were supposed to shut down VEGF expression or its receptor (VEGFR1). However, recent evidence indicates that the preclinical efficacy observed in mouse models of macular degeneration probably results from nonspecific side effects rather than RNAi-mediated sequence-specific gene knockdown. Such nonspecific effects may trouble other RNAi therapeutic applications.

The cellular mechanism of RNAi can be instructed to cleave specific mRNAs by addition of manmade siRNAs of which the antisense/guide strand has perfect sequence complementarity with the targeted mRNA. In our research to develop gene therapy against human immunodeficiency virus type 1 (HIV-1), we observed impressive and specific inhibition via the RNAi mechanism. In fact, we documented that mutant escape viruses are selected under RNAi pressure with a single point mutation in the 19-nucleotide target sequence, thus demonstrating the exquisite sequence-specificity of inhibition. Therapeutic application of “naked” siRNA molecules remains a challenge because mammalian cells do not spontaneously take up such molecules without cell-permeating entities. Nevertheless, siRNAs targeting the VEGF system that were injected directly into an affected eye did reduce angiogenesis in preclinical studies with mice. Based on these promising preclinical results, clinical trials have been approved that exploit RNAi to shut down the VEGF signaling pathway that promotes angiogenesis.

It is important to realize that although naked, unmodified siRNAs can be injected directly into the confined space of ocular tissue, there was little knowledge of how the siRNAs entered the target cells. The assumption has been that the siRNA enters an affected cell in some unexplained way, where it has a highly specific impact in
shutting down angiogenesis activity. Surprisingly, the recent study by Kleinman et al.\(^4\) indicates that this assumption is not correct. These authors describe that the anti-angiogenesis effect can be induced in the mouse model with unrelated siRNAs that differ significantly in nucleotide sequence. In their search for possible cell surface molecules that could mediate this general siRNA effect, Toll-like receptor 3 (TLR3) emerged as a possible candidate receptor. The siRNAs were indeed shown to act through TLR3, an innate immune system regulator present on the surface of many cell types. No effect was scored in TLR3-deficient mice and addition of soluble TLR3 protein reversed the inhibitory siRNA effects. Thus, the siRNAs operate without being taken up by the cell! Any double-stranded RNA of at least 21 nucleotides can bind directly to TLR3, inducing receptor dimerization and an intracellular signaling pathway that eventually leads to activation of the nuclear factor-κB transcription factor that, among other things, activates expression of interferon-γ and interleukin-12. These cytokines are the likely inducers of the anti-angiogenesis program. On a positive note, it was proposed that generic siRNAs could perhaps be harnessed to treat various diseases linked to angiogenic disorders that affect 8% of the world’s population.\(^4\)

This result indicates that the development of safe siRNA-based therapies might be more challenging than was initially anticipated. These surprising findings also reemphasize the importance of appropriate controls in RNAi experiments. It is not unusual that testing of a single siRNA candidate inhibitor should be accompanied by multiple siRNA controls to test for sequence-specificity, off-target effects on other mRNAs and TLR3-mediated cytokine responses. At least such nonspecific effects are becoming better defined in molecular terms, which should help in the design of more rigorous experimental standards. Clearly, this work does not relate to other therapeutic approaches that use delivery methods, e.g. siRNAs packaged into liposome or viral particles that shield the siRNAs from recognition by the innate immune system. Likewise, the recent findings do not relate to gene therapy approaches in which the siRNAs are expressed intracellularly, e.g. as short hairpin RNAs (shRNA) against HIV-1.

References