hairpin to form an intracellular siRNA. To date, small interfering RNAs have been used *in vitro* to target viral genes like *tat* and *rev* [31] and cellular genes like CCR5 [32] with great success.

Protein and nucleic acid-based strategies

Aptamers

In general, aptamers refer to short RNA, DNA, or protein sequences that bind a variety of specific target molecules, including nucleic acids and proteins. Small RNA aptamers have shown success *in vitro* against HIV Rev [33,34] and reverse transcriptase [35]. Aptamers can be used in conjunction with other therapies, such as ribozymes [35].

Application of bench-side treatment strategies to bedside

The early success of *in vitro* studies paved the way for clinical trials. All the above-mentioned strategies for gene therapy have shown good anti-HIV activity *in vitro*. However, not all of them have been tested in clinical trials. As reported in the literature, the trials conducted to date have used a suicide gene (HyTK), transdominant negative proteins (Rev M10, TdRev, or huM10), a chimeric receptor (CDζ), an RNA decoy (RRE), and ribozymes (anti-*tat*/*vpr* ribozyme and *tat* ribozyme, RRz2). A trial using siRNA was started recently and the results have not yet been published in literature. Following is an account of these clinical trials and their results (see Table 1 for summary).

Protein-based approaches used in the clinic

In 1996, Riddell *et al*. published one of the earliest trials, involving the use of a suicide gene [16]. This study enrolled six HIV-seropositive patients. Autologous $CD8^+$ T cells were genetically modified using a retrovirus-mediated gene transfer technique. The retrovirus, designated HyTK, comprised the hygromycin phosphotransferase gene (Hy) and the herpes virus thymidine kinase gene (TK) as a fusion gene under the control of the murine leukemia virus (MLV) long terminal repeat (LTR). Hygromycin was used to positively select for transduced autologous cells *in vitro* prior to infusion into the patient. Ganciclovir could have been used, if necessary, to negatively select (kill) the transduced cells in the patient. In four increasing doses, researchers transfused autologous HyTK-transduced $CD8^+$ cells at 14-day intervals. There were no significant side effects. However, five of six patients developed a CTL response to the foreign protein and thus rejected the modified $CD8^+$ cells, which cleared in response to each subsequent transfusion. The results of this trial suggested other strategies that would make modified cells less susceptible to the immune response and thus inspired further research [16].

In the same year, Woffendin *et al*. published the results of a pilot trial involving a transdominant negative protein approach [36]. For genetic modification of $CD4^+$ T cells, they used Rev M10 and a deletion mutant of Rev M10, which showed no antiviral activity. Woffendin and colleagues transduced the T cells using a non-viral vector and showed that following transfusion, there was a preferential survival of Rev M10 modified $CD4^+$ T-cells, as compared to cells that received the mutant. They also detected Rev M10 until 2 months post-infusion. Though there was increased survival of Rev M10-expressing cells, the overall numbers of transduced cells were low *in vivo* [36].

Trying to improve upon this trial, they conducted another pilot study in which they transduced $CD4^+$ cells with Rev M10, but this time they used retroviral vectors instead of a nonviral vector [37]. They detected Rev M10 for an average of 6 months post-infusion. In addition, cells transduced with Rev M10 survived longer than those transduced with the negative control vector. There were no detectable immune responses to the 'foreign proteins' (Rev M10 or MLV gp70 envelope protein). Though these studies with Rev M10 showed an improved efficacy of gene delivery with a retroviral vector, there was no effect on the patients' viral loads [37].

In 2002, Kang *et al*. published the results of another trial that used a transdominant negative mutant Rev protein (TdRev) [38]. This study had only two subjects. Both were HIV positive and had malignancies. One had leukemia and the other had refractory Hodgkin's lymphoma. Fourteen days prior to receiving gene therapy, the researchers stopped their cyclosporine and HAART medications. Six days prior to gene therapy, the patients received fludarabine-cyclophosphamide containing regimen (non-myeloablative) for five days and then cyclosporine and HAART was restarted. Each patient had an HIV negative sibling who was an HLA-compatible donor for bone marrow cells. The donor underwent blood apheresis followed by isolation of $CD34^+$ cells. The cells were genetically modified by using either TdRev or a control vector encoding human GP91phox. Following transplantation with these modified $CD34^+$ syngeneic cells, the patients developed CMV antigenemia, which was treated. By day 96 post-transplantation, both patients showed 100% transfer of either gene into lymphoid and myeloid lineages. Both patients developed acute graft versus host reaction beyond day 100, which was successfully treated. The patient with Hodgkin's disease died 12 months after transplantation due to relapse of the disease unrelated to any complication of the gene therapy [38]. In a follow-up paper published by the same group, the second patient showed persistence of TdRev at three years post-treatment and continued to be in remission. However, this might be due to the effect of HAART and not TdRev alone [39].

A 2005 study involving the transdominant negative protein approach was focused on the pediatric age group of