ticipants [41]. Of these, three did not receive any cell infusions. Patients received modified CD8+ cell infusions either alone or with IL-2. The group receiving IL-2 with the modified CD8+ cells showed higher levels of persistence of CD4ζ as compared to the group receiving no IL-2 supplementation. In order to test whether the IL2 was substituting for HIV-specific CD4+ T-cell help, 17 of the 30 participants received modified CD4+ cells along with modified CD8+ cells in a second series of infusions. Modified CD8+ and CD4+ cells were detected in the peripheral blood of these 17 patients for at least one year post-infusion, indicating that co-administration of the CD4+ cells may have increased survival of the modified CD8+ cells [41].

In the same year that Walker *et al*. reported their findings, Mitsuyasu and fellow researchers published the results of a similar chimeric receptor study [42]. Patients enrolled in this study were receiving anti-retroviral therapy (ART) and had viral loads between 1,000 and 100,000 copies/ml and CD4+ T cell counts greater than 50 per microliter. Following cell infusions, patients were followed for eight weeks. Eleven patients received CD4ζ-modified T cells along with IL-2, and thirteen patients received CD4ζ-modified T cells alone. In contrast to the previous study by Walker *et al*., administration of IL-2 did not increase the survival of the modified T cells. However, an increase in cell number was observed at eight weeks post-infusion; an average increase of 73 CD4+ cells per microliter was observed in the group receiving IL-2 as compared to the group that did not receive IL-2. They detected CD4ζ in 1 to 3% of the peripheral blood mononuclear cells (PBMCs) at eight weeks and 0.1% at one year post-infusion. CD4ζ-modified T-cells were also isolated from bulk rectal tissue and/or lamina propria lymphocytes in five of five patients at 14 days and two of three patients at one year, showing good tissue distribution. In addition, there was a transient decrease of greater than 0.5 log mean in rectal tissue-associated HIV RNA for at least 14 days [42].

Encouraged by data from this trial, the same group conducted a phase II randomized trial of CD4ζ gene-modified versus unmodified T cells in adult male HIV-positive patients in 2002 [43]. All participants were on combination ART. In 37 patients, there were no measurable viral loads (<50 copies per ml). In three patients, low levels were detected (53, 57 and 65 copies per ml). Only 40 of 42 enrolled patients proceeded to receive the study treatment. The researchers found good expression of CD4ζ for at least 24 weeks in all patients. However, no significant difference was found between patients receiving gene therapy versus the control group when the six following parameters were analyzed: plasma viral load, HIV co-culture on PBMCs, blood HIV DNA, rectal biopsy DNA, rectal biopsy RNA, and blood CD4+ cell count [43].

RNA-based approaches used in the clinic

A clinical trial involving RNA decoys was conducted in a pediatric population using an RRE decoy to modify CD34+ bone marrow cells. Kohn *et al*. (1999) showed that retroviral mediated CD34+ cell transduction had no significant adverse effects and that leukocytes containing an RRE decoy could be isolated from peripheral blood, even one year post-infusion; however, the numbers of transduced cells were extremely low [44]. Four HIV-positive patients, three teenagers, and one eight-year-old were enrolled in this pilot study. Bone marrow cells positive for CD34 were isolated from these patients and transduced with Moloney murine leukemia (MoMuLV) vector virus carrying the RRE decoy sequences. No change in the HIV viral load was observed among the participants [44].

In 2004, Amado *et al*. demonstrated long-term maintenance of a therapeutic transgene in a phase I clinical trial involving ribozymes [45]. They used MoMLV vector virus transduction of CD34+ HSCs for introduction of a ribozyme targeted to highly conserved regions in the HIV-1 *tat* and *vpr* genes. Ten patients participated in the study and researchers could detect the vector in naïve T cells for up to three years, the last time-point evaluated. There was an average increase of 10 CD4+ T cells per microliter from the beginning of the trial until the third year. In six patients, viral loads decreased by an average of 2.25 logs. Three patients had undetectable viral loads. One patient showed an increase of one log. The researchers found no correlation between the changes in viremia or CD4+ T cell counts with vector expression or detection in any cell type. However, during this trial, all patients were on ART, and the researchers attribute the changes in viral load as well as the cell numbers to individual viral susceptibility to ART [45].

More recently in 2005, Macpherson *et al*. published the results of a phase I clinical trial on ribozymes involving identical twins with discordant HIV status [46]. Again, one twin acted as the donor (HIV-negative) and the other was the recipient (HIV-positive) of genetically engineered CD4+ T cells expressing a ribozyme. Specifically, the cells were transduced with an anti-HIV-1 *tat* ribozyme (RRz2) and a control LNL6 retroviral vector (for cell marking). Patients were followed initially for 24 weeks and then at regular intervals over a 4-year period. PBMCs containing both RRz2 and LNL6 were detected consistently. However, the effect of this therapy on HIV-1 viral load or the CD4 count was not specifically addressed.

Lastly, Morgan *et al*. (2005) published data from a clinical trial involving anti-sense RNA in conjunction with a trans-dominant negative protein [47]. This study employed 10 pairs of twins. Like the earlier study involving twins with discordant HIV status, one twin served as the donor (HIV-