

1.0 Introduction

HIV-1 is the most important viral pathogen of humans. It is estimated that 32 million people will be living with HIV infection and that 3 million people will have died as a consequence of the infection during 1998 (1). Despite these sobering international statistics, morbidity and mortality related to HIV infection have been declining in the United States since 1996, largely because of improved antiretroviral therapy (2,3).

Combination antiretroviral therapy (CART) typically includes one protease inhibitor and two reverse transcriptase inhibitors, although other combinations are also used. The effectiveness of combination therapy has been demonstrated in clinical trials (4,5). The primary effect of CART is to suppress viral replication, as measured by plasma HIV-1 RNA, which decreases the rate of progression of immune deficiency (6,7,8,9).

In the future, there are several issues that will dampen the effect of CART on the epidemic. These include patients' ability to tolerate the agents, the long-term toxicity of the agents, the complicated and highly regimented treatment schedules, the development of resistance to the agents, and the effect of previous therapy on the response to a new regimen.

The antiretroviral agents currently approved for use in the United States are generally well-tolerated, but there are significant numbers of patients who are unable to tolerate them. In clinical trials, as many as 25% of participants failed to complete the study for this reason (4). These patients require novel therapeutic interventions.

The long-term toxicity related to the use of CART is not known at the present time. It has been suggested that CART, specifically protease inhibitors, may cause disturbances in lipid metabolism (10,11). Whether this effect is related to CART therapy, or is a consequence of HIV infection only being recognized now that patients are living longer, has not been determined. Patients who develop serious long-term toxicity may need to discontinue CART and will require new therapeutic options.

The schedules for CART therapy are complicated, and patient compliance is critical for treatment success. The compliance issues are related to the number of pills that must be taken daily, the timing of these medications around meals (since food affects absorption of the various agents), and the requirement for strict adherence to the regimen in order to maintain effective drug plasma concentrations. Problems with compliance have been shown to be associated with the rapid development of resistance that may or may not be controlled when compliance with CART is resumed, with obvious consequences for the patient (12,13).

Unfortunately, resistance to CART can develop even when the patient is compliant with therapy. The HIV-1 genome is single stranded, and the reverse transcriptase enzyme is error-prone (14,15). With an error rate of one base in every few thousand copied, multiple mutations develop with each replication. The more replicative events, the higher the probability of developing resistance. These scientific observations are supported by the clinical observation that the duration of response to CART is directly related to the nadir of the plasma HIV-1 RNA, which is an indirect measure of viral replication (16). Previous antiretroviral therapy also affects the response to CART. The results from many clinical trials suggest that the response to a new antiretroviral therapy, either monotherapy or CART, is related to previous therapy, particularly with similar agents (12). Previous therapy that does not control viral replication selects for viral resistance and results in a viral population at baseline that has already developed some or all of the mutations required for resistance to the new therapy. The treatment options available today have high degrees of cross-resistance within a class of agents, with the result that failure with one CART regimen diminishes the probability of having a durable therapeutic response to another regimen. Patients who develop resistance for any of these reasons require new treatment options.

New AIDS therapies can be divided into two categories: those that attempt to control viral replication and those that seek to reconstitute the immune system. Effective therapies have the potential to affect both variables. For example, there is an element of immune reconstitution that occurs with CART therapy (17). Nonetheless, each therapy primarily affects one or the other of these variables.

SyStemix has developed a novel therapeutic strategy that has immune reconstitution as its primary aim. This clinical program is based on the concept of protecting the cells that are the targets of HIV-1 infection with nucleic acid and protein sequences that inhibit viral replication, thereby allowing survival of the target cells. Prolonged survival of CD4⁺ T cells would be expected to lead to increased numbers of these cells, and the CD4⁺ count has been shown to be indicative of the magnitude of immunodeficiency in persons with HIV-1 infection (18).

The therapeutic sequences used by SyStemix include the gene for the transdominant negative Rev mutant, RevM10, and an antisense sequence pol-1 to the pol gene. Rev functions to facilitate the nuclear export of unspliced and singly-spliced RNA transcripts to the cytoplasm for translation to the viral structural proteins, Gag, Pol and Env. In order to complete this function, two domains of the protein are essential. The first is a domain that binds to the Rev responsive element (RRE) on unspliced and singly-spliced RNA transcripts. After binding, an activation domain assembles the required cofactors that make up the nuclear export complex (19). HIV-1 viruses with Rev deletions or mutations do not replicate. The RevM10 mutant has an intact binding domain that allows RevM10 to bind to RRE. It has two mutations in the activation domain that prevent assembly of the nuclear export complex, with the result that translation of structural proteins is blocked, thereby blocking viral replication (20,21).

The pol sequence encodes the reverse transcriptase, integrase, and RNase H proteins. The pol-1 antisense construct used by SyStemix is complementary to the region of pol that codes for reverse transcriptase (17). The antisense RNA binds to the complementary sequence, forming an RNA-RNA duplex that is then degraded and is not translated to protein. This block in viral protein synthesis has been shown to result in decreased viral replication. The combination of RevM10 and polAS has been shown to inhibit viral replication to a greater extent than either sequence alone (22).

The SyStemix treatment strategy is based on the transduction of hematopoietic stem cells (HSC) with vectors containing RevM10 and polAS. HSC are self-renewing so that a single treatment has the potential to result in the lifelong generation of gene-modified progeny. The cells that are the primary targets of HIV-1 infection, CD4⁺ T cells, monocytes and macrophages, and microglial cells, are all progeny of HSC. Thus, the use of gene-modified HSC has the potential to provide a lifelong population of target cells for HIV infection which are resistant to HIV replication and HIV-induced cell death.

There are three technical issues to resolve in order to implement this technology: obtaining sufficient numbers of HSC; successful transduction of the HSC; and engraftment of the gene-modified HSC. The mobilization of HSC from persons with HIV-1 infection has been shown to be both safe and feasible. In a previous clinical trial sponsored by SyStemix, 33 persons with HIV-1 infection received 5 days of G-CSF for HSC mobilization. There was no change in viral load during the course of the study (from day 0 to day 90), and the numbers of HSC mobilized in patients with ≥ 200 CD4⁺ T cells were comparable to those mobilized in patients without HIV-1 infection. These results are similar to data from trials in Denmark (23) and ACTG 285 (24).

As this therapy has developed, the transduction process used at SyStemix has been modified. The original process used vector packaged in PA317 cells with transduction efficiencies of <10% when measured by gene-marked colony-forming units (CFU). The use of a proprietary human packaging line, ProPak-A, and technical improvements in the transduction process, have resulted in transduction efficiencies >40%. The improved transduction process will be used in this clinical trial.

To date, the ability of RevM10polAS HSCIP to provide long-term engraftment and differentiation in patients with HIV-1 infection has not been fully evaluated. It has been proposed that since myelosuppression and hypocellular bone marrow often exist in HIV-1 infection, sufficient "space" may exist to allow for RevM10polAS HSCIP engraftment without myelosuppressive bone marrow conditioning. This phenomenon was not observed in an ongoing phase I/II clinical trial, SyStemix 104. However, the patients in the present study will receive higher doses of RevM10polAS HSCIP that may provide for engraftment without conditioning. The higher dose of RevM10polAS HSCIP to be used in this trial is expected to result in an increased number of gene marked PBMC (25).

The safety of hematopoietic stem cells transduced with RevM10 (RevM10-HSCIP) or RevM10polAS (RevM10polAS HSCIP) in HIV-1 infected persons is under investigation in an ongoing phase I/II clinical trial, SyStemix 104. In that study, the effect of bone marrow conditioning with cyclophosphamide prior to HSC infusion on engraftment is being evaluated in persons with CD4⁺ lymphocytes >100 and <500/mm³. Twenty-seven patients have been entered into this trial to date. Neither direct infusional toxicity nor replication-competent retrovirus has been seen. Results to date in that ongoing study indicate that cyclophosphamide given as a single dose, up to 2.4 g/m² is acceptably tolerated and that the 2.4 g/m² cohort had the highest rate of engraftment as determined by gene marking. The pilot phase of this trial will evaluate the effect of the increased RevM10polAS HSCIP dose obtained from 3 days of apheresis and the role of cyclophosphamide conditioning.

Differentiation of RevM10polAS HSCIP into CD4⁺ cell progeny may provide a selective advantage over native, untransduced CD4⁺ cells in the presence of HIV-1 infection. This may allow the RevM10polAS CD4⁺ cells to repopulate the immune system of patients with HIV-1 infection, resulting in immune restoration. The main study is designed to evaluate the safety of RevM10polAS HSCIP gene therapy and to determine if the CD4⁺ cell progeny of RevM10polAS HSCIP have a selective advantage over control cells transduced with an inactive control vector lacking the RevM10 and polAS sequences.

The use of RevM10polAS and the control vector will allow for comparison of the survival of CD4⁺ cells from known numbers of HSC. If RevM10polAS confers a selection advantage to CD4⁺ cells, the ratio of RevM10polAS to the control vector in CD4⁺ cells should increase with time. Demonstration of a selection advantage in RevM10polAS CD4⁺ cells is essential if this therapy is to provide immune reconstitution in patients with HIV-1 infection.