

2. INTRODUCTION

HIV-1 infection is characterized by progressive immunologic dysfunction that ultimately results in the development of opportunistic infections due to the profound defects in cellular immunity (1). The decline of immunologic function is attributed to viral replication in specific lymphocyte subsets of the CD4⁺ surface phenotype, as well as in cells of monocyte and glial origin (2-5). HIV-1 also has been detected in lymphoid precursor cells including cells of the thymus (6).

Antiretroviral therapy with nucleoside analogs results in a temporary suppression of viral replication and a transient increase in CD4⁺ cells in the peripheral blood (7). Antiviral chemotherapy is complicated by such shortcomings as incomplete control of viral replication (2,8), the emergence of viral strains of reduced susceptibility to antiretroviral drugs (8) the potential depletion of precursor cells of the hematopoietic system and thymus (7), as well as by the cost and cumulative toxicity of lifelong administration.

There has been increasing interest in the development of genetic therapies that have as a goal the replacement of the HIV-1 infected cellular reservoir with cells that are resistant to HIV-1 infection (9,10). One approach is based on the hypothesis that the transfer of HIV-resistance genes, RevM10 or **RevM10/polAS**, into hematopoietic stem cells (HSC) inhibits HIV-1 replication in progeny cells derived from the transduced stem cells. Expression of RevM10 in these progeny will result in competitive inhibition of the HIV-1 encoded wild-type protein and **expression of polAS (polymerase anti-sense sequence) will block the HIV-1 polymerase mRNA**. These effects serve to block infectious virion production, spread of virus infection and produce a decrease in the loss of the affected lymphocyte populations. The HIV-resistant progeny from RevM10-HSC or **RevM10/polAS-HSC** should have a selective survival advantage over the non-transduced HSC. Thus, even if only a fraction of the HSC carry the HIV-resistance gene, the gene-modified cells should undergo self-renewal, differentiate, and with time, potentially stabilize the patient's immune system.

The success of this approach depends on the feasibility of isolating stem cells and following transduction and infusion, their multilineage differentiation and self-renewing capacity. SyStemix defines the HSC as a self-renewing hematopoietic cell with the CD34⁺Thy-1⁺ phenotype and in on-going transplantation studies in multiple myeloma patients, has shown that these cells engraft within a clinically acceptable timeframe and display multilineage differentiation under myelosuppressive conditions.

Harvesting of hematopoietic stem cells has become more feasible with the development of strategies whereby such cells are mobilized into the peripheral blood with the use of colony stimulating factors such as G-CSF. A number of protocols have been successfully employed in which hematopoietic stem cells are mobilized into the peripheral blood by growth or colony-stimulating factors and harvested by apheresis. Under these conditions, a substantial number of stem cells have been harvested from most donors.

Little is known about the characteristics of the CD34⁺Thy1⁺ hematopoietic stem cell population in HIV-1 infected individuals. In particular, the yield, composition, and functional characteristics of such cells at various stages of HIV-1 disease have yet to be fully delineated, although preliminary data suggests that the characteristics of the mobilized populations resemble those of HIV-1 seronegative donors. The use of G-CSF for mobilization is supported by the drug's common use with little dose limiting toxicity in AIDS and severe AIDS-related complex. There has been no reported increase in p24 antigen levels or change in lymphocyte co-culture results. G-CSF did not enhance HIV-1 replication in monocyte/macrophages in *in vitro* assays (11, 12).

The physiology of autologous, transduced HSC to patients in the non-cancer setting has not been fully studied, and there is still a question regarding the need for additional "space" to allow the infused stem cells to home, engraft and, ultimately, differentiate. In the cancer setting, adequate "space" is generated by the cancer therapy itself, allowing for demonstrated gene marking post infusion (14). In developing strategies to achieve adequate engraftment resulting in 10-20% transduced cells in a non-ablative setting, research in the mouse model has focused on both the total number of engrafted cells infused, as well as increased number of doses and has demonstrated that both of these strategies result in maintenance of higher levels of infused cells (15, 16, 17). Using representative stem cell numbers in the mouse, researchers have found that lower doses of irradiation than that previously used in mice plus the use of G-CSF has resulted in increasing presence of transduced cells (18). In the large animal canine model, the ability to achieve the higher numbers of stem cells or the multiple dosing has not been found to be as feasible. In comparing both TBI and cyclophosphamide pre-conditioning, the cyclophosphamide pretreated animals were found to have evidence of up to 18% transduced cells in the circulation for over 1 year (19). In prior cord blood gene therapy trial for adenosine deaminase deficiency, no conditioning therapy was utilized and the expression of the gene in the target lymphocytes was delayed until a sufficient selective advantage was present (20). In the treatment of AIDS, adequate engraftment and subsequent selective differentiation of the transduced stem cells into the lymphocytic lineage would need to take place in a timely manner in order for a clinical benefit to be determined, and the time required in the ADA patients would represent too long of a wait (up to 3 years) in the presence of disease progression. The use of cyclophosphamide in HIV+ individuals has been demonstrated to be feasible and safe in the treatment of AIDS associated lymphoma (21). The need for "space" in the context of the current therapy will be addressed in an mild dose-escalating manner, with the initial Cohort receiving no conditioning and subsequent Cohorts receiving increasing doses of cyclophosphamide designed to maximally decrease circulating lymphocytes during the period of engraftment and potentially create additional "space" to allow selective differentiation of RevM10 or RevM10/polIAS containing resistant lymphocytes.