

# Feasibility of a Stem Cell Gene Therapy Approach with Non-Myeloablative Conditioning in Patients with HIV-1 Infection

Gian Paolo Rizzardi<sup>1\*</sup>, Silvia Nozza<sup>2</sup>, Lucia Turchetto<sup>1</sup>, Alexandre Harari<sup>3</sup>, Giuseppe Tambussi<sup>2</sup>, Fulvio Crippa<sup>2</sup>, Monica Salomoni<sup>1</sup>, Vega Rusconi<sup>2</sup>, Salvatore Toma<sup>1</sup>, Giuseppe Pantaleo<sup>3</sup>, Adriano Lazzarin<sup>2</sup>, and Claudio Bordignon<sup>1</sup>

<sup>1</sup>MolMed, Milan, Italy; <sup>2</sup>Div Inf Dis, San Raffaele Scientific Institute, Milan, Italy; <sup>3</sup>Div Immunology&Allergy, Ctr Hosp Univ Vaudois, Lausanne, Switzerland.

Gian Paolo Rizzardi, MD  
MolMed  
via Olgettina 58, 20132 Milan, Italy  
Tel +39-02-21277231 - Fax +39-02-21277239  
paolo.rizzardi@molmed.com

## INTRODUCTION

Several reasons warrant the development of innovative therapeutic strategies for HIV/AIDS. These include the inability of highly active antiretroviral therapy (HAART) to eradicate the virus, the drug-induced severe long-term toxicity occurring in patients who regularly take HAART, the development of HAART-resistant HIV-1 strains in the host, and the lack of an efficacious vaccine.

Therefore, there is the need not only to develop new potent antiretroviral drugs with a favourable toxicity profile, but also to design and implement alternative therapeutic approaches, including vaccines, immune and cell factor-based strategies, and gene therapy. Genetic engineering of hematopoietic stem cells (HSC) combined with nonmyeloablative conditioning proved safety and efficacy in the treatment of adenosine deaminase-deficient severe combined immunodeficiency. The feasibility of such an approach in HIV-1 infection remains, however, to be determined.

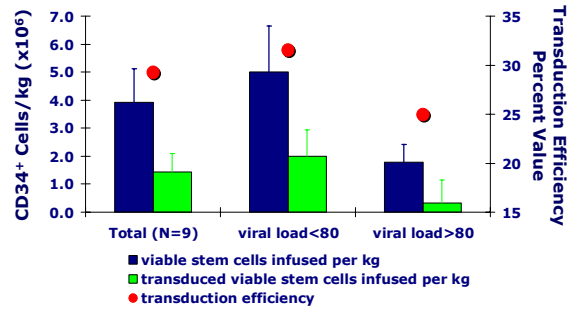
## STUDY DESIGN

In a 48-week open-label prospective trial, 18 patients with HIV-1 infection (mean±SEM age 36±1, range 19 to 40; HAART since ≥3 months; CD4<sup>+</sup>>200 cells/μL) have been enrolled in a HSC retroviral vector gene therapy trial using RevM10 and poIAS as anti-HIV genes. Patients have been stratified according to the extent of suppression of plasma HIV-1 RNA, either below (N=9) or above (N=9) 80 copies/ml (limit of the Naaba Organon assay). At baseline, mean±SEM baseline CD4<sup>+</sup> T cell counts were 577±42. After enrollment, patients underwent peripheral stem cell mobilisation with G-CSF (10 μg/kg/day for 5 days) followed by 2 sessions of aphaeresis. Then, stem cells have been purified with CliniMACS (Miltenyi Biotec) and frozen down. After about 4 weeks, stem cells have been thawed to undergo the 4-day transduction process on retroviral (TaKaRa Bio, Japan) plus cytokines. Cells were transduced using a retroviral vector, derived from the LXS1 vector backbone of the Fred Hutchinson Cancer Research Centre, containing the transdominant negative mutation RevM10 along with poIAS, an antisense to the reverse transcriptase sequence. Forty-eight hours before the infusion of fresh stem cells, patients received nonmyeloablative cyclophosphamide conditioning at 1.8 g/m<sup>2</sup> in a single dose. Of the 18 patients, 9 received fresh transduced CD34<sup>+</sup> cells and all study phases, while 9 did not undergo all study phases.

## RESULTS and DISCUSSION

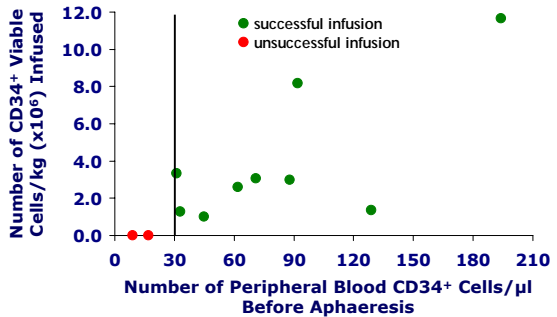
The first important result of the study is that G-CSF mobilisation, aphaeresis, and purification were efficient in patients with HIV-1 infection. Interestingly, stem cell figures were higher in patients with optimal suppression of HIV replication than in patients with detectable viral load at baseline. Also, the transduction process was efficient achieving 4.42±0.64x10<sup>6</sup> CD34<sup>+</sup> cells/kg after purification, and 3.93±1.2x10<sup>6</sup> viable CD34<sup>+</sup> cells/kg in the infusion product, 30% of which were transduced CD34<sup>+</sup> cells. Yet, the number of both total and engineered stem cells was higher in patients with optimally suppressed viral load than that measured in those with detectable levels of viremia (5 versus 1.8, and 2 versus 0.3 million stem cells/kg, respectively).

### Numbers of Total and Engineered CD34<sup>+</sup> Cells in the Infusion Product



In addition, it is worth noting that the number of stem cells/μL mobilised in the peripheral blood just before aphaeresis (mean, 72 cells/μL) significantly predicts the number of viable CD34<sup>+</sup> cells/kg infused (β 0.722, 95% CI 0.007-0.092, P=0.028, regression analysis). Since the transduction process failed in 2 patients because of a too low number of cells available, the ability of different simple variables to predict infusion success has been tested. Using the 25<sup>th</sup> percentile of the peripheral blood stem cell distribution as cut-off value, values >30 stem cells/μL predicted the success of gene transfer procedures (P=0.018, z<sup>2</sup> analysis, Fisher's exact test).

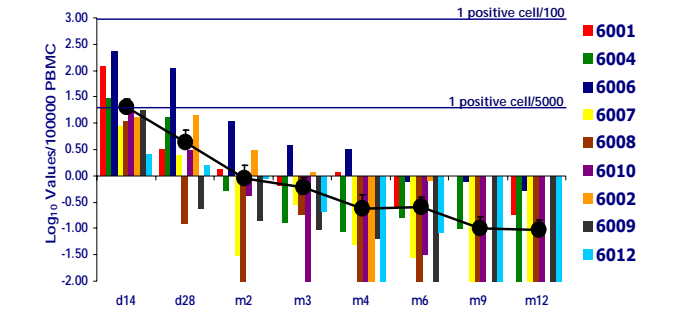
### Values >30 Stem Cells/μL Predicts Successful Infusion



Therefore, these procedures are overall well feasible in patients with HIV-1 infection, and the optimal suppression of virus replication increase their yield and efficiency.

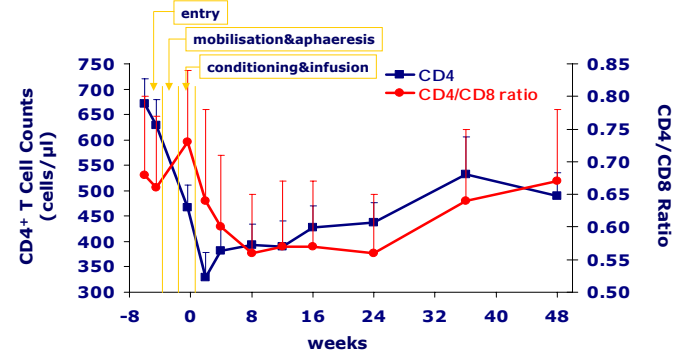
RevM10 gene marking *in vivo* at day 14 is predicted by the number of engineered stem cells infused (β 0.896, 95% CI 0.48-1.41, P=0.003, regression analysis). This is true throughout the follow-up, as by regression analyses at all time-points (not shown), suggesting that the stem cell dose contributes to the level of engraftment. RevM10 gene marking was detectable at around 1 positive cell over 5000 cells few weeks after infusion. However, it constantly decreased over time.

### Levels of PBMC Transgene Expression Constantly Decrease Over Time



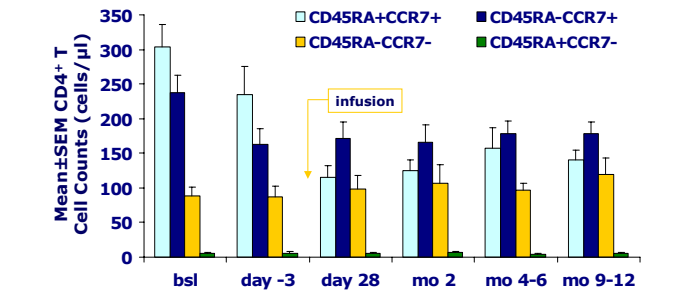
The most critical result of the study was the impact of cyclophosphamide conditioning on CD4<sup>+</sup> T cell counts. In fact, CD4<sup>+</sup> T cell counts (in blue) steeply declined following aphaeresis and conditioning, and they took about a year to recover. Likewise, the CD4/CD8 ratio (in red) completely restored over long-term follow-up. In order to dissect this phenomenon and to assess the feasibility of such an approach in patients with HIV-1 infection, several immunological parameters have been analysed.

### Changes in CD4<sup>+</sup> T Cell Counts and CD4/CD8 Ratio Over Time



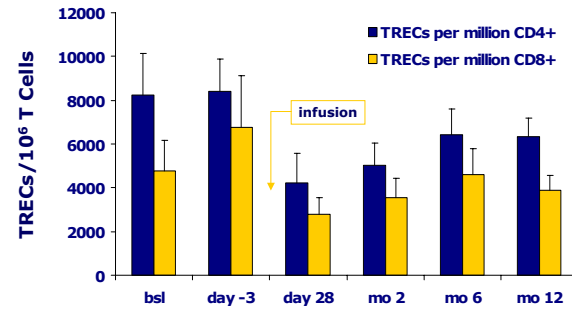
First, according to the varying expression of CD45RA and CCR7, it is possible to identify naive and central and effector memory CD4<sup>+</sup> T cells. Naive cells express both molecules, central memory cells no longer express CD45RA, and effector memory cells lack CCR7, while CD45RA is re-expressed in terminally differentiated effector memory cells. While both central memory CD4<sup>+</sup> T cells (in blue) and effector memory CD4<sup>+</sup> T cells (in yellow and green) did not significantly change over time, cyclophosphamide conditioning mostly affected the naive compartment (in light blue). It is important to note that naive CD4<sup>+</sup> T cells tended to restore during the follow-up. At variance, cyclophosphamide conditioning did not significantly alter phenotype patterns of naive and memory CD8<sup>+</sup> T cells over time.

### Naive and Memory CD4<sup>+</sup> T Cell Changes Following Treatment



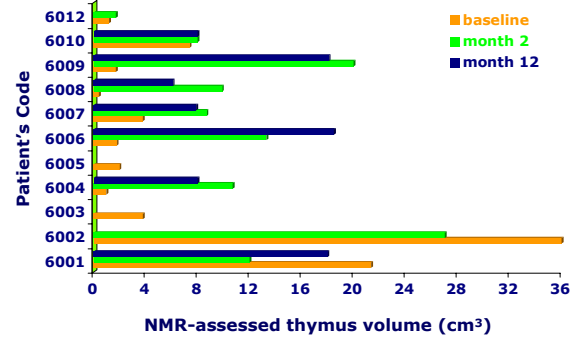
Secondly, the effect of cyclophosphamide conditioning was not only exerted in the periphery but also centrally, at the thymus level. In fact, both CD4 and CD8 TRECs significantly decreased after conditioning. It is worth noting that TREC levels increased thereafter, and interestingly, CD4 TREC levels significantly correlated with peripheral blood naive CD4<sup>+</sup> T cells, both at baseline and following infusion during the follow-up (not shown).

### Changes in TRECs/10<sup>6</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T Cells Over Time



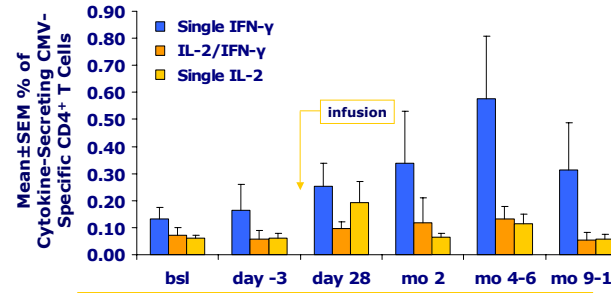
Furthermore, the thymic regeneration capacity measured by TREC is consistent with the significant increase in thymus volume measured in the majority of patients with nuclear magnetic resonance at months 2 and 12.

### Changes in Thymus Volume Over Time



Thirdly, on the basis of the ability to secrete IL-2 and IFN-γ, functionally distinct virus-specific cell populations within CD4<sup>+</sup> T cells can be identified: these are single IFN-γ, IL-2/IFN-γ, and single IL-2 secreting cells. Of note, in patients participating to this study, cytokine-secretion patterns in response to several common antigens, including tetanus toxoid, EBV, HSV, and CMV, were comparable at baseline to those observed in healthy donors (not shown). Interestingly, CMV-specific IFN-γ-secreting CD4<sup>+</sup> T cells were able to expand during the follow-up, in the absence of a clinically relevant CMV reactivation and with a persistently negative CMV plasma viremia at all time-points in all patients.

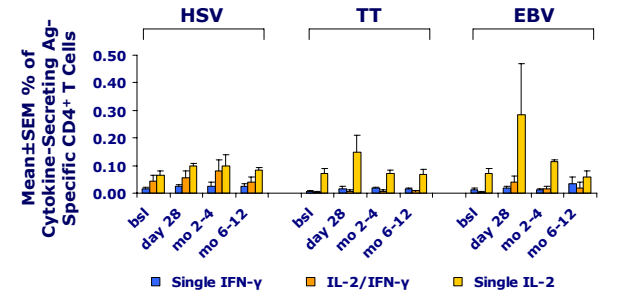
### Different Populations of CMV-Specific Cytokine-Secreting CD4<sup>+</sup> T Cells



CMV DNA levels were persistently below the limit of detection (500 copies/ml) in all patients at all time-points

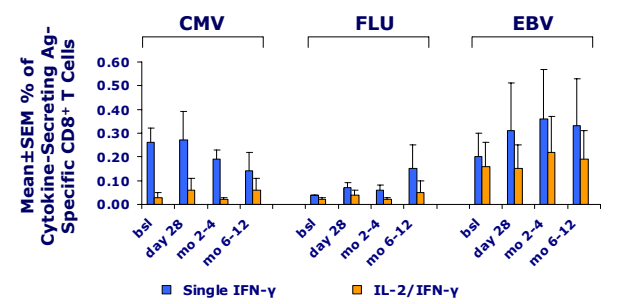
Furthermore, it is important to emphasise that no study procedure significantly affected the CD4<sup>+</sup> T cell specific immune responses against HSV, EBV and TT over time.

### Different Populations of Ag-Specific Cytokine-Secreting CD4<sup>+</sup> T Cells



Finally, no study procedure affected the CD8<sup>+</sup> T cell specific immune responses against CMV, FLU, and EBV.

### Different Populations of Ag-Specific Cytokine-Secreting CD8<sup>+</sup> T Cells



## CONCLUSIONS

- The procedures of *in vivo* stem cell mobilisation and aphaeresis, and *ex-vivo* stem cell purification and transduction are well feasible in patients with HIV infection, and the *in vivo* optimal suppression of virus replication increase their yield and efficiency
- Using simple variables, such as viral load at entry and the peripheral blood stem cell absolute and percent values before aphaeresis, it is possible to build-up a decision-making flowchart to increase the likelihood of success of gene transfer procedures
- Conditioning with cyclophosphamide aggresses mostly the CD4<sup>+</sup> T cell naïve compartment, while central memory and effector memory T cells are persistently maintained over time
- The thymus displays regeneration capacity in 35-year-old patients with HIV infection, regeneration capacity that correlates with the restoration of naïve CD4<sup>+</sup> T cells
- The magnitude and patterns of CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses against CMV, HSV, EBV, influenza and TT are maintained and effective throughout the follow-up
- Clinical gene therapy studies in ADA-SCID and in X-CGD showed clinical success using busulfan as drug for nonmyeloablative conditioning, and experimental data in macaques (C Dunbar, Blood 2004) suggest that busulfan is more toxic for stem cells than for lymphocytes, favouring engraftment and preventing T cell-specific toxicity

**Effective stem cell gene transfer is feasible in patients living with HIV/AIDS, and the use of non-lymphocyte-toxic conditioning regimen, such as busulfan, is advised**

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