Gene Therapy for Leukocyte Adhesion Deficiency Type I (LAD-I): Preclinical Studies and Clinical Trial Design

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Introduction

- **Leukocyte Adhesion Deficiency Type I** (LAD-I) is a primary immunodeficiency caused by mutations in the *ITGB2* gene, encoding the **CD18** subunit of β_2 integrins. The defective expression of β_2 on the leukocytes' surface limits their extravasation to inflamed sites.
- A **therapeutic Chim.hCD18-LV** was used carrying the human CD18 protein under the control of a CatG/cFes chimeric promoter.

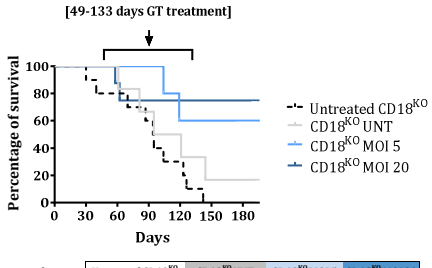
Objective

To improve the transduction of human CD34⁺ cells with the therapeutic lentiviral vector under conditions compatible with their clinical application.

Results

Ex vivo gene therapy in CD18^{KO} mice using the therapeutic Chim.hCD18-LV

Hematopoietic stem cells from CD18^{KO} mice were transduced with the therapeutic Chim.hCD18-LV at a multiplicity of infection (MOI) 5 and 20 and in the presence of Transduction Enhancers. Transduced cells were transplanted into irradiated CD18^{KO} recipients. Gene therapy (GT) treated mice showed an increased lifespan as compared to CD18^{KO} untreated mice or animals transplanted with untransduced (UNT) cells (**Figure 1**). Groups showed human CD18 (hCD18) expression in 1.9% (MOI 5) and 32.8% (MOI 20) peripheral blood cells from transplanted mice, and a mean vector copy number (VCN)/cell of 0.04 and 1.54 respectively (**Figure 2**). A functional assay based on lipopolysaccharide (LPS)-induced inflammation in the pads showed the preferential migration of corrected neutrophils (Gr1⁺CD11b⁺ cells), demonstrating the phenotypic correction of CD18^{KO} mice (**Figure 3**).



p value	Untreated CD18^{KO}	CD18^{KO} UNT	CD18^{KO} MOI 5	CD18^{KO} MOI 20
Untreated CD18^{KO}	-	ns	0.0171	0.0047
CD18^{KO} UNT		-	ns	0.0324
CD18^{KO} MOI 5			-	ns
CD18^{KO} MOI 20				-

Figure 1. Kaplan Meier survival analysis of gene therapy CD18^{KO} treated mice. Percentage of survival of untreated CD18^{KO} females or treated CD18^{KO} animals transplanted with untransduced cells (UNT) or cells transduced with Chim.hCD18-LV.

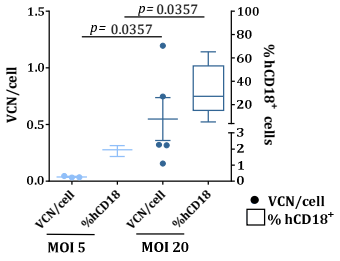


Figure 2. Analyses of vector copy number and hCD18 expression in the bone marrow of CD18^{KO} gene therapy-treated mice. Percentage of hCD18⁺ cells was determined by flow cytometry and it is represented in the right axis for MOI 5 (light blue) and 20 (dark blue). VCN/cell, in the left axis, was determined by QPCR and it is represented as dots.

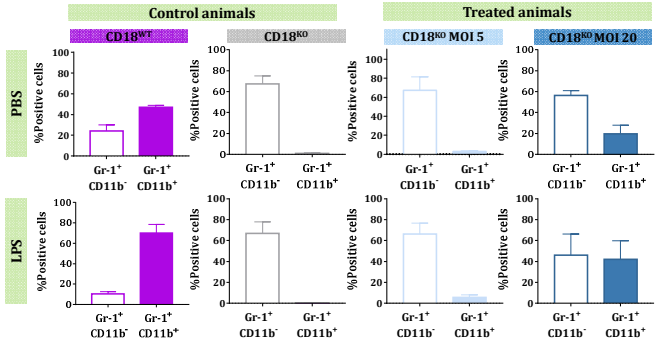


Figure 3. Neutrophil migration in a LPS-induced pad inflammation model. The figure represents the migration percentage of the Gr-1⁺CD11b⁻ and the Gr-1⁺CD11b⁺ populations (left and right bars of each graph respectively) in the phosphate-buffered saline (PBS) and in the LPS-treated pads. Control animals were transplanted with CD18^{WT} or CD18^{KO} untransduced cells.

Large-scale validation of genetically modified CD34⁺ cells with the Chim.hCD18-LV

To validate the transduction protocol, three independent validation runs were performed under large-scale Good Manufacturing Practices (GMP) conditions, as described in the protocol of **Figure 4**. VCN analyses in liquid cultures and in CFCs are summarized in **Table 1**. Cells were cryopreserved and thawed at different times post-transduction **(Figure 5).** Thawed cells were transplanted into immunodeficient NSG mice, showing stable engraftment over time, with an average of VCN per human engrafted cells (CD45⁺) of 1.08, 0.43 and 2.40 for runs I, II and III, respectively, at the end of the follow up **(Figure 7)**.

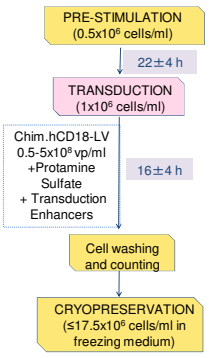


Figure 4. Transduction protocol. Vp, viral particles.

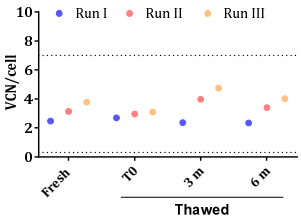
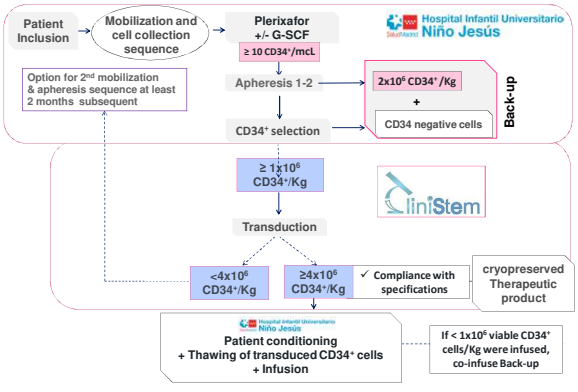


Figure 5. VCN/cell of cryopreserved CD34⁺ transduced cells under GMP conditions. Cells were cryopreserved and thawed the same day (T0) and at 3 and 6 months post-transduction (m). Each dot represent the average of VCN/cell.

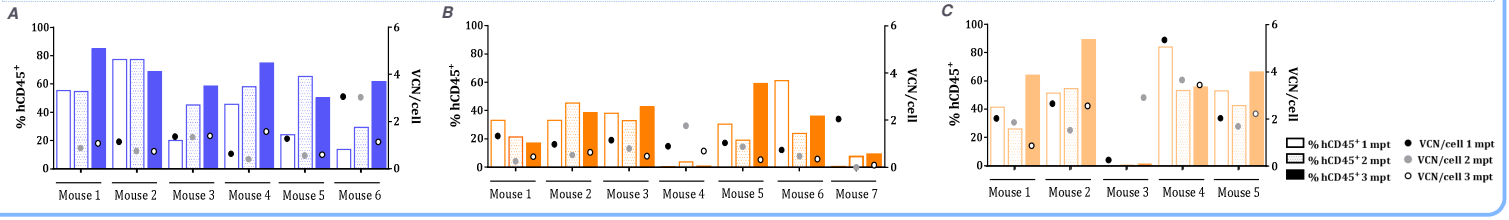
	RUN I	RUN II	RUN III
Source	CB	mPB	CB
Number of cells	14.6x10⁶	12x10⁶	12x10⁶
%CD34⁺	69.4%	92.3%	88.9%
VCN/cell	2.69	2.95	3.08
Viability (>50%)	75.03%	93.5%	95.8%
VCN/cell in colonies	2.28	2.49	4.92
% Transduction in colonies	71%	82.8%	86.1%
VCN/cell in positive colonies (>0.3 VCN/Cell)	3.21	2.99	5.71
Number of cryopreserved cells	6.8x10⁶	13.2x10⁶	8.05x10⁶

Table 1. Results from validation runs. Results of VCN and viability of the cryopreserved therapeutic product.



Clinical trial for LAD-I: Cell processing algorithm

Figure 6. Summary of NSG transplanted mice with LV:Chim.hCD18 transduced CD34⁺ cells under GMP conditions. Percentage of Human engraftment (hCD45⁺) was determined by flow cytometry and it is represented as bars in the right axis at different months post-transplant (mpt) per mouse in bone marrow samples. VCN/hCD45⁺, in the left axis, was determined by qPCR and it is represented as dots.
A) Validation run I. **B)** Validation run II. **C)** Validation run III.



Conclusions

CD18^{KO} mice treated with an optimized gene therapy protocol using transduction enhancers showed, for the first time, sustained engraftment and phenotypic correction of the extravasation defect.

The large-scale transduction of human CD34⁺ cells under optimized GMP conditions resulted in efficient transduction and preserved the repopulating properties of transduced hematopoietic stem cells in NSG mice.

Our preclinical studies have demonstrated the safety and efficacy of an optimized gene therapy protocol for LAD-I with the therapeutic LV:Chim.hCD18.

A Phase I Clinical Trial for severe LAD-I has been approved in Spain (NCT03825783) and in the US (NCT03812263) and is already opened and enrolling patients.