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Preclinical safety and efficacy of lentiviral-mediated gene therapy for leukocyte adhesion deficiency type I

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Leukocyte adhesion deficiency type I (LAD-I) is a primary immunodeficiency caused by mutations in the *ITGB2* gene, which encodes for the CD18 subunit of β_2-integrins. Deficient expression of β_2-integrins results in impaired neutrophil migration in response to bacterial and fungal infections. Using a lentiviral vector (LV) that mediates a preferential myeloid expression of human CD18 (Chim.hCD18-LV), we first demonstrated that gene therapy efficiently corrected the phenotype of mice with severe LAD-I. Next, we investigated if the ectopic hCD18 expression modified the phenotypic characteristics of human healthy donor hematopoietic stem cells and their progeny. Significantly, transduction of healthy CD34$^+$ cells with the Chim.hCD18-LV did not modify the membrane expression of CD18 nor the adhesion of physiological ligands to transduced cells. Additionally, we observed that the repopulating properties of healthy CD34$^+$ cells were preserved following transduction with the Chim.hCD18-LV, and that a safe polyclonal repopulation pattern was observed in transplanted immunodeficient NOD scid gamma (NSG) mice. In a final set of experiments, we demonstrated that transduction of CD34$^+$ cells from a severe LAD-I patient with the Chim.hCD18-LV restores the expression of β_2-integrins in these cells. These results offer additional preclinical safety and efficacy evidence supporting the gene therapy of patients with severe LAD-I.

INTRODUCTION

Leukocyte adhesion deficiency type I (LAD-I) is a primary immunodeficiency with an estimated prevalence of 1 in 1,000,000 births.[1] LAD-I is caused by mutations in the *ITGB2* gene, which encodes for CD18, the common subunit of the β_2-integrin receptor family. The consequence of these mutations is the reduced or absent expression of CD18, although the expression of a non-functional CD18 protein has also been reported in some LAD-I patients.[2–4] β_2-Integrins are required for normal leukocyte trafficking and extravasation to infected or inflammatory sites. Thus, defects in β_2-integrin expression limit or even prevent the ability of leukocytes to adhere to the endothelium and extravasate to infected areas, resulting in the absence of innate immune responses and impaired wound healing.

The severity of LAD-I is generally associated with the expression of CD18 in peripheral blood (PB) leukocytes. Patients with less than 2% of normal levels of CD18 suffer from a severe disease, while a moderate LAD-I disorder is associated with values ranging from 2% to 30% of normal CD18 levels.[1,3–7] The current curative treatment for LAD-I is based on the allogeneic transplantation of hematopoietic stem and progenitor cells (HSPCs) from HLA-compatible healthy donors (HDs). In the absence of this treatment, severe LAD-I patients have a fatal prognosis, with 60%–75% associated mortality before the age of 2 years.[4,8] Increases in the expression of CD18 correlate with an increased survival likelihood, with approximately 90% survival in early childhood in patients with at least 4% of normal levels of CD18.[4] Due to allogeneic HSPCs transplant-related complications and the frequent unavailability of an appropriate donor, *ex vivo* gene therapy (GT) based on the infusion of genetically modified autologous HSPCs has been proposed as a viable therapeutic option for patients with severe LAD-I.[9–13] In a previous study, we showed that a self-inactivating (SIN) lentiviral vector (LV), Chim.hCD18-LV, carrying the human *ITGB2* cDNA under the control of a chimeric promoter, drives a preferential transgene expression in myeloid cells, resembling the physiologic expression of CD18.[13] In that study, we demonstrated that the Chim.hCD18-LV restores the functionality of cells from mice with a hypomorphic mutation in CD18

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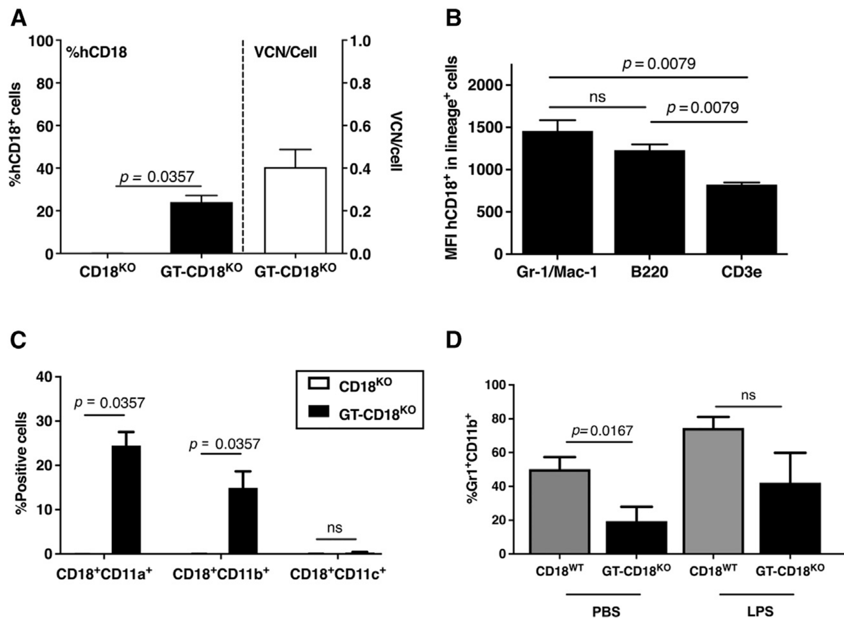


Figure 1. Phenotypic correction of mice with severe LAD-I by *ex vivo* GT with the therapeutic Chim.hCD18-LV

(A) Analysis of the percentage of $hCD18^{+}$ PB cells determined by flow cytometry (left axis) and VCN/cell (right axis) in $CD18^{KO}$ mice transplanted with either mock-transduced (n = 3; $CD18^{KO}$) or Chim.hCD18-LV-transduced $CD18^{KO}$ cells (n = 5; GT-$CD18^{KO}$). (B) Mean fluorescence intensity (MFI) of hCD18 measured in Gr-1^{+}/Mac-1^{+}, $B220^{+}$, and $CD3e^{+}$ PB cells from GT-treated $CD18^{KO}$ mice. (C) Percentage of PB cells positive for hCD18 co-expressed with the different murine α subunits (mCD11) in mock-transduced $CD18^{KO}$ and GT-treated $CD18^{KO}$ mice. (D) Evaluation of neutrophil migration in LPS-inflamed pads from control $CD18^{WT}$ (gray bars) and GT-$CD18^{KO}$ (black bars) mice. The panel represents the percentage of infiltrated neutrophils (Gr-$1^{+}$$CD11b^{+}$) in PBS- and LPS-treated pads in the mCD45.2^{+} population. The significance of differences resulting from the Mann-Whitney test is represented as a p value or ns (no statistical significance). Bars denote mean ± SEM.

($CD18^{HYP}$),[13] which supported the Orphan Drug designation from both the European Medicines Agency (EMA, EU/3/16/1753) and the Food and Drug Administration (FDA, DRU-2016-5430) for this therapeutic LV-based therapy.

To address an urgent medical need for patients affected with severe LAD-I, here we conducted preclinical efficacy and toxicity studies to support the GT of these patients by Chim.hCD18-LV-mediated GT.

RESULTS

Transplantation of syngeneic HSPCs transduced with the Chim.hCD18-LV corrects the phenotype of mice with severe LAD-I

In previous studies, we showed the efficacy of the Chim.hCD18-LV to correct the phenotype of mice with moderate LAD-I harboring a hypomorphic mutation in the *Itgb2* gene.[13] Here we first investigated the efficacy of this LV to correct the phenotype of a severe LAD-I mouse model, which is characterized by the null expression of CD18 and reduced lifespan (CD18 knockout mice [$CD18^{KO}$]).[14] With this purpose in mind, $Lin^{-}Sca1^{+}c\text{-}kit^{+}$ (LSK) cells from $CD18^{KO}$ mice were transduced with the Chim.hCD18-LV at a multiplicity of infection (MOI) of 20 transduction units (TU) per cell (see section "materials and methods"). Thereafter, a total of 15,000 mock-transduced cells (mock) or cells transduced with the Chim.hCD18-LV were transplanted into irradiated $CD18^{KO}$ mice.

In contrast to the early mortality characteristic of untreated $CD18^{KO}$ mice (most animals died before 5 months), all $CD18^{KO}$ mice transplanted with transduced $CD18^{KO}$ LSK cells were alive at the end of the follow-up, when they were euthanized to evaluate changes in the phenotype of their hematopoietic cells. While almost a null expression of the α and β subunits of β_2-integrins was observed in PB cells from untreated $CD18^{KO}$ mice, 24.1% ± 3.1% of PB cells from GT-treated mice became human CD18 (hCD18) positive (see Figure 1A and representative flow cytometry analyses in Figure S1). In these animals, a mean number of 0.4 ± 0.1 copies/cell was observed, indicating that a high proportion of transduced cells expressed the hCD18 (Figure 1A). A higher expression of hCD18 was observed in myeloid PB cells compared with T lymphocytes (Figure 1B), resembling the physiological expression of this integrin.[13] Importantly, the expression of hCD18 facilitated the membrane expression of CD11a and CD11b mouse α subunits in GT-treated $CD18^{KO}$ mice (see Figures 1C and S1). As expected, only a very low proportion of PB cells were positive for CD11c expression.

Given that neutrophils from $CD18^{KO}$ mice have an impaired ability to migrate and extravasate to inflamed tissues,[14] we also investigated whether GT restored the extravasation capacity of transduced $CD18^{KO}$ neutrophils. In these experiments, pads from wild-type (WT) mice (positive control) and also from GT-treated $CD18^{KO}$ mice were injected with lipopolysaccharide (LPS) to induce a local inflammation. While in no instance $Gr1^{+}CD11b^{+}$ neutrophils were found in pads from $CD18^{KO}$ mice (data not shown), in the case of GT-treated $CD18^{KO}$ mice, the proportion of leukocytes (CD45.2^{+} cells) consisting of infiltrating neutrophils reached 40% of values determined in PBS-treated pads from WT mice ($p < 0.05$). In LPS-treated pads, the proportion of infiltrating neutrophils increased in both groups, and no differences between the WT and the GT-treated $CD18^{KO}$ mice were observed (Figure 1D).

These results reveal that GT restored β_2-integrin expression and β_2-integrin-dependent migration of $CD18^{KO}$ neutrophils toward

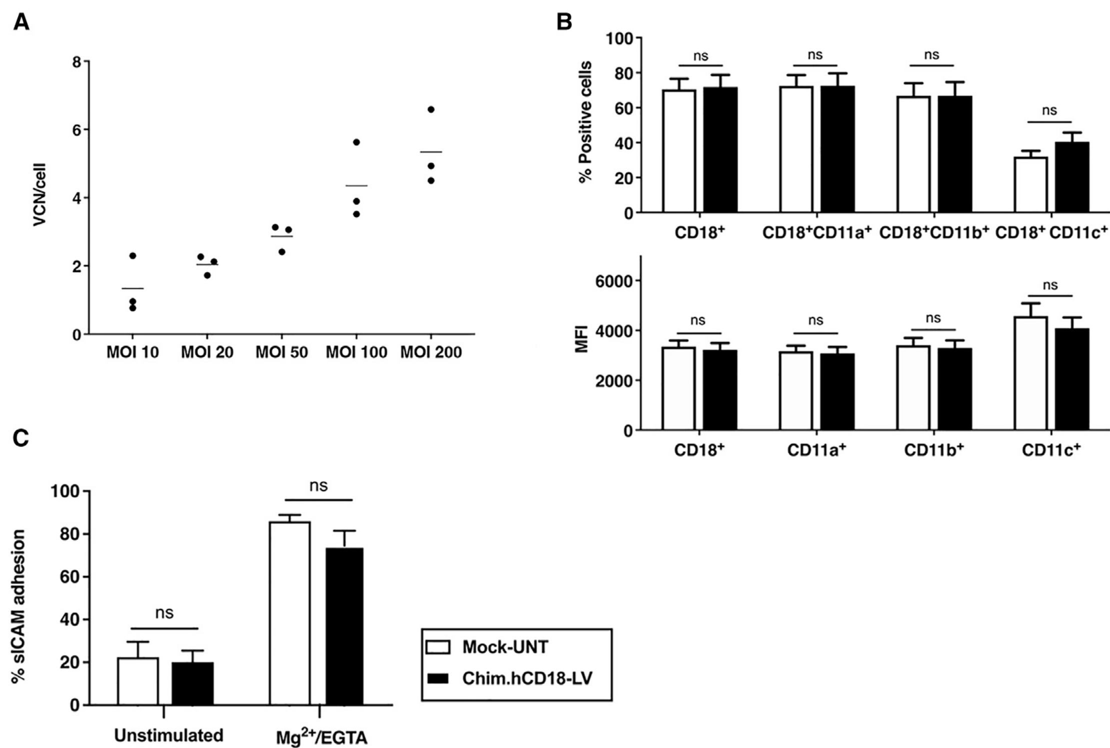


Figure 2. Preserved phenotype of healthy human CD34+ cells transduced with the Chim.hCD18-LV

(A) Analysis of the VCN/cell after transduction of HD mPB CD34$^+$ cells with the Chim.hCD18-LV at different MOIs (n = 3 for each MOI) after 14 days of liquid culture. (B) Percentage of cells positive for β2-integrins (upper), and MFI values on the double-positive CD18$^+$CD11$^+$ populations (bottom) after mock-transduction (mock-UNT, white bars) or transduction of HD CB CD34$^+$ cells with the Chim.hCD18-LV (Chim.hCD18-LV, black bars) (n = 6). (C) *In vitro* soluble ICAM adhesion assay performed in mock-UNT (white bars) or Chim.hCD18-LV cells (black bars) (n = 3 experiments). Cells were differentiated to the myeloid lineage and their adhesion capacity to sICAM-I was measured by the increase in the percentage of cells capable of binding sICAM-1 without activation or after Mg^{2+}/EGTA stimulation. Statistical analysis resulting from the Mann-Whitney test showed no significant differences between untransduced and LV-transduced groups. Bars denote mean ± SEM.

inflamed pads, providing evidence that GT results in the *in vivo* correction of the characteristic phenotype of mice with severe LAD-I.

Transduction of healthy human CD34$^+$ cells with the therapeutic Chim.hCD18-LV does not modify the phenotype of CD34$^+$-derived cells

Although we previously showed in lymphoblastic cells that the ectopic expression of CD18 does not result in supra-physiological membrane levels of this protein,[13] here we investigated whether the ectopic expression of hCD18 modified the phenotype of HD CD34$^+$ cells and their myeloid progeny.

In the first set of experiments, mobilized PB (mPB) CD34$^+$ cells from HDs were used to test the transduction efficacy of the therapeutic Chim.hCD18-LV in these clinically relevant cells. Data obtained from liquid cultures maintained for 14 days post transduction showed an MOI-dependent increase in the vector copy number (VCN)/cell (Figure 2A). As shown in this figure, transductions with an MOI of 50 TU/cell resulted in an average VCN/cell of 2.9 ± 0.2, which, as deduced from previous clinical experience, constitutes a good compromise of efficacy and safety for LV-mediated GT (see review by Tucci et al.[15]). This condition was thus selected to conduct subsequent experiments with cord blood (CB) and mPB CD34$^+$ cells.

In experiments shown in Figure 2B, we investigated whether the expression of β_2-integrins observed in the mock-transduced HD CB CD34$^+$ cells was modified by the transduction with the Chim.hCD18-LV. As shown in this figure, no changes in the proportion of β2-integrin-positive cells or in the expression levels of these integrins were noted in cells that had been transduced with the therapeutic LV with respect to mock-transduced cells (from now on referred to as mock).

Next, we studied whether the adhesion of HD hematopoietic cells to β_2-integrin ligands was modified by the ectopic expression of hCD18. Because Intercellular Adhesion Molecule I(ICAM-1) is the major ligand for CD11a:CD18 (LFA-1) and CD11b:CD18 (Mac-1) heterodimers, we evaluated the adhesion capacity of myeloid cells to this ligand by means of an ICAM-1 ligand-binding assay upon adequate stimulation. In brief, CB HD CD34$^+$ cells were transduced with the Chim.hCD18-LV (MOI 50 TU/cell) and then differentiated to

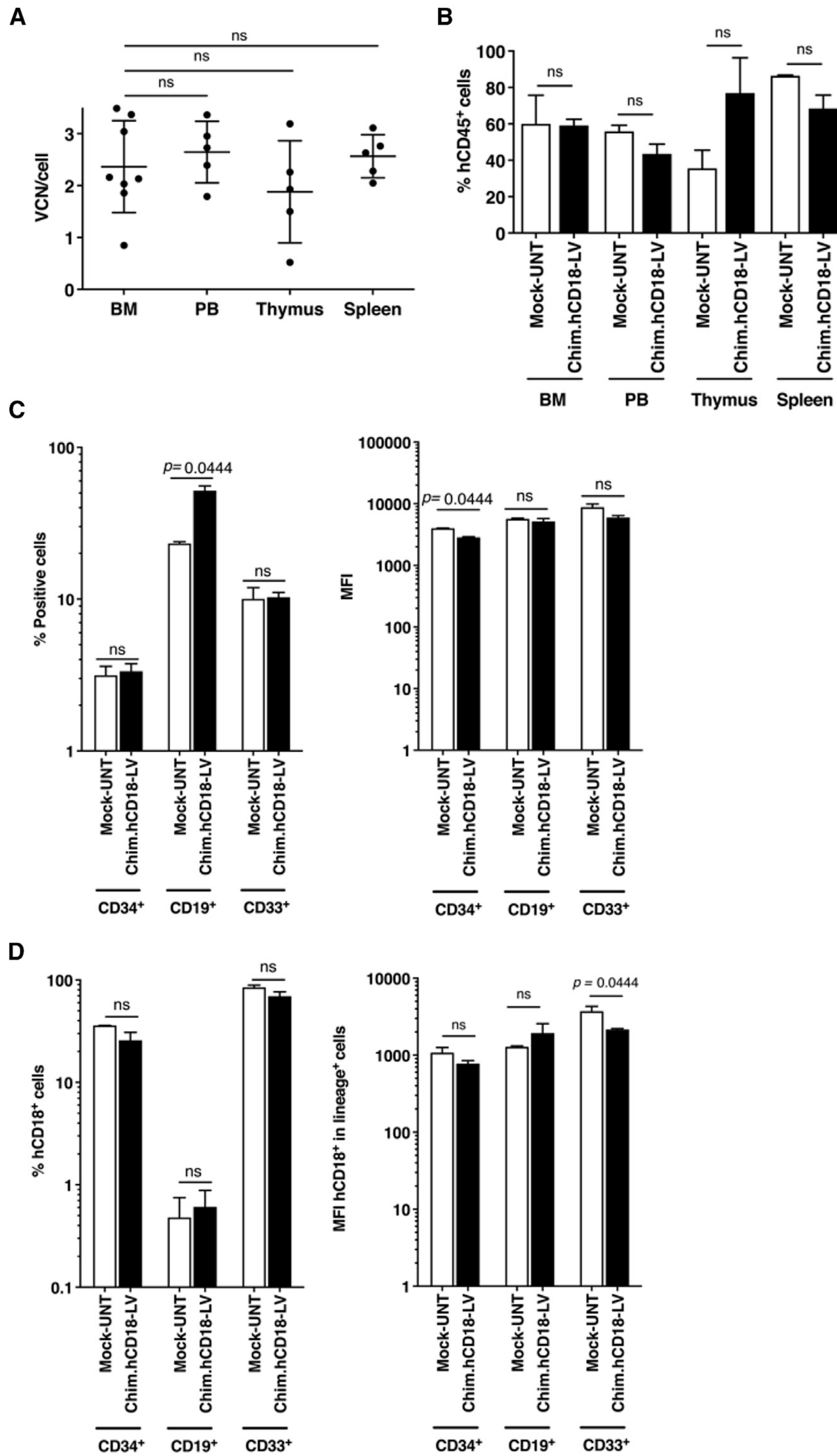


Figure 3. Analysis of the NSG repopulating properties and transduction efficacy of HD human CD34+ cells transduced with the Chim.hCD18-LV

Mock and Chim.hCD18-LV-transduced (MOI 50 TU/cell) HD CB $CD34^+$ cells were transplanted into NSG mice. Analyses were conducted 3 months post infusion. (A) VCN/human cell in hematopoietic tissues from transplanted mice. Each dot represents VCN/cell corresponding to one mouse. (B) Levels of engraftment of human cells (percentage of $hCD45^+$ cells) in hematopoietic organs from transplanted recipients. (C) Lineage distribution in the $hCD45^+$ population from the BM of transplanted mice. (Left) The percentage of human CD34, CD19, and CD33, and (right) the level of expression (MFI) corresponding to these markers. (D) Human CD18 expression in different human hematopoietic lineages engrafting the BM of recipient mice. (Left) The percentage of $hCD18^+$ cells, and (right) levels of CD18 expression (MFI) in the different human cell lineages. The significance of differences resulting from the Mann-Whitney test is represented as a p value or ns (n = 2 experiments). Bars denote mean ± SEM.

generate a homogeneous population of myeloid cells. These cells were then exposed to recombinant soluble ICAM-1 (sICAM-1) to study the adhesion of this ligand in the presence (stimulated) and absence (unstimulated) of activating agents (Mg^{2+}/EGTA). A PE-conjugated antibody was used to detect the ICAM protein bound to the activated cells. As shown in Figure 2C, no differences in the percentage of cells binding ICAM-1 were observed in mock and Chim.hCD18-LV-transduced cells, either with or without stimulation.

Altogether, these results indicate that transduction of HD $CD34^+$ cells with the therapeutic Chim.hCD18-LV (MOI of 50 TU/cell) results in clinically relevant numbers of proviral copies per cell, and also that the transduction with this LV does not alter the membrane expression of CD18, or the binding of these cells to the physiological ligand ICAM-1.

The efficient transduction of HDs $CD34^+$ cells with the Chim.hCD18-LV preserves their *in vivo* repopulating properties in NSG mice

To further investigate the safety associated with the ectopic expression of CD18 in human HSPCs, 1.3×10^5 to 1.6×10^5 HD CB $CD34^+$ cells that had been transduced with the Chim.hCD18-LV (MOI of 50 TU/cell) were transplanted into immunodeficient NOD scid gamma (NSG) mice to evaluate potential changes in the repopulating ability of transduced HSPCs. Consistent with the *in vitro* data shown in Figure 2, an average number of 2.4 ± 0.3 copies was observed in human cells engrafting the bone marrow (BM) of NSG recipients (Figure 3A). Comparable values were noted in PB, thymus, and spleen of transplanted mice (Figure 3A).

Table 1. GMP engineering runs corresponding to HD CD34$^+$ cells transduced with the Chim.hCD18-LV

		Liquid culture		Clonogenic assays				
					Average VCN/cell			
		Viability (%)	VCN/Cell	Transduction efficiency (%)	Total	BFU-E	CFU-GM	CFU-GEMM
Run I (CB)	fresh	93.7	2.5	85.0	2.6	2.7	1.4	3.8
	cryo (0 D)	75.0	2.7	71.0	2.1	3.1	0.9	2.3
	cryo (3 M)	80.4	2.4	ND	3.1	4.0	4.6	0.5
	cryo (6 M)	80.4	2.3	76.0	2.5	4.0	2.5	0.9
Run II (mPB)	fresh	95.1	3.1	88.0	1.8	2.0	2.3	1.1
	cryo (0 D)	93.5	3.0	83.0	2.5	1.8	2.4	3.3
	cryo (3 M)	86.4	4.0	89.0	2.0	2.5	0.5	3.0
	cryo (6 M)	92.3	3.4	92.0	1.9	2.8	0.9	2.0
Run III (CB)	fresh	98.0	3.8	93.0	4.8	6.1	0.7	7.6
	cryo (0 D)	95.8	3.1	86.0	4.7	6.7	2.7	4.6
	cryo (3 M)	93.8	4.8	90.0	3.6	5.5	1.8	3.5
	cryo (6 M)	96.4	4.0	93.0	2.1	2.2	1.3	2.8
Mean	fresh	95.6	3.1	88.7	3.1	3.6	1.5	4.2
	cryo (0 D)	88.1	2.9	80.0	3.1	3.9	2.0	3.4
	cryo (3 M)	86.9	3.7	89.5	2.9	4.0	2.3	2.3
	cryo (6 M)	89.7	3.3	87.0	2.2	3.0	1.6	1.9
SEM	fresh	1.3	0.4	2.3	0.9	1.3	0.5	1.9
	cryo (0 D)	6.6	0.1	4.6	0.8	1.5	0.5	0.7
	cryo (3 M)	3.9	0.7	0.5	0.5	0.9	1.2	0.9
	cryo (6 M)	4.8	0.5	5.5	0.2	0.6	0.5	0.6

Summary of results of three engineering runs corresponding to the transduction of HD CD34$^+$ cells with the Chim.hCD18-LV at the GMP-certified facility (CliniStem/CIEMAT). CB, cord blood; mPB, mobilized PB; cryo, cryopreserved and thawed cells at different time points; VCN, vector copy number; CFU-GEMM, granulocyte/erythroid/macrophage/megakaryocyte colony-forming units; CFU-GM, granulocyte/macrophage colony-forming units; BFU-E, burst-forming unit-erythroid. ND, no data (a very low efficiency on DNA recovery resulted in inadequate qPCR data).

When levels of engraftment were investigated, a similar proportion of hCD45$^+$ cells was observed in mice transplanted with mock and Chim.hCD18-LV-transduced samples (no significant differences between these groups were observed in any tissue) (Figure 3B). Additionally, no differences in the proportion of CD34$^+$ and CD33$^+$ cells were noted in the BM of NSG recipients transplanted with the mock and Chim.hCD18-LV samples (Figure 3C), while a higher proportion of CD19 cells was observed in mice transplanted with transduced cells. When levels of expression of the CD34, CD19, and CD33 lineage markers were investigated in these animals, only a slight but significant difference was observed in the CD34$^+$ population. Finally, a similar proportion of CD18$^+$ cells was found in the mock and Chim.hCD18-LV groups, and also similar levels of CD18 expression were noted in CD34 and CD19 cell populations, while a modestly weaker expression of CD18 was observed in myeloid CD33 cells (Figure 3D).

These studies together indicate that transduction of HDs human CD34$^+$ cells with the therapeutic Chim.hCD18-LV does not result in relevant changes in the repopulating properties of these cells.

Efficient and safe transduction of human CD34$^+$ cells with the Chim.hCD18-LV under GMP conditions

To evaluate the efficacy and safety of the transduction protocol in LAD-I clinical trials, three independent transduction runs were conducted under good manufacturing practice (GMP) using HD CB and mPB CD34$^+$ cells transduced with a GMP-produced lot of the Chim.hCD18-LV at an MOI of 50 TU/cell. Fourteen days after transduction, the VCN/cell was determined both in liquid cultures and in hematopoietic colonies generated in semisolid cultures. High cell viability was observed in the fresh transduced products in all instances (95.6% ± 1.3%), and a mean value of 3.13 ± 0.4 copies/cell was determined in fresh liquid cultures (Table 1), consistent with data obtained in the titering studies depicted in Figure 2A. VCN/cell determined in individual colonies showed a mean value of 3.08 ± 0.9 copies/cell and a mean transduction efficacy in colony-forming units (CFUs) of 88.7% ± 2.3% (Table 1). Analyses of VCN/cell in the different colony types showed higher VCN/cell values in erythroid (BFU-E) and mixed (CFU-GEMM) colonies compared with granulocyte-macrophage (CFU-GM) colonies (mean values in average VCN/cell column from Table 1), in line with previously published results.[16]

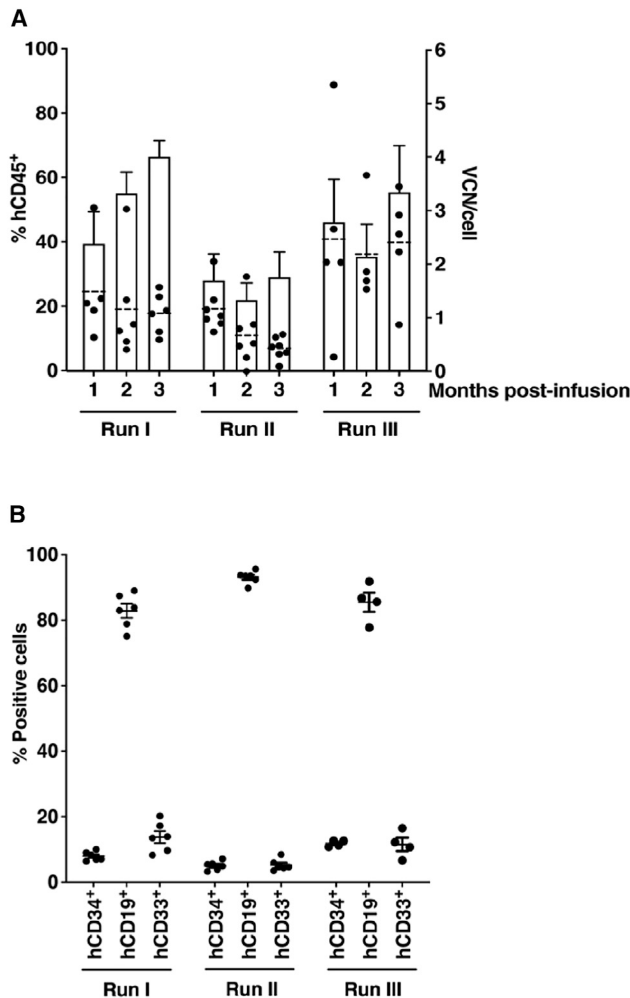


Figure 4. Analysis of the repopulating properties and gene marking of NSG repopulating cells after transduction under GMP conditions of HD human CD34+ cells with the therapeutic Chim.hCD18-LV

Healthy donor mPB and CB CD34+ cells were transduced with the Chim.hCD18-LV (MOI 50 TU/cell) using large-scale GMP conditions. Prior to transplantation into NSG mice, cells were cryopreserved and then thawed to resemble GT conditions to be used in LAD-I patients. Runs I (n = 6 mice) and III (n = 5 mice) were conducted with CB CD34+ cells, while run II (n = 7 mice) was carried out with mPB CD34+ cells. (A) Engraftment levels (%hCD45) in recipient BM samples are represented as bars on the left axis. VCN/human BM cells are shown as dots on the right axis (each dot represents one mouse). Analyses in BM samples from transplanted mice were conducted at different months post infusion. (B) Lineage distribution analyses in hCD45+ cells engrafting the BM of recipient NSG mice. Each dot represents one mouse. Bars denote mean ± SEM.

After the manufacturing of the medicinal product for clinical trials (CD34+ cells transduced with the Chim.hCD18-LV under GMP conditions), transduced cells will be cryopreserved until all quality control data are obtained prior to their infusion. To demonstrate the stability of transduced samples after the cryopreservation process, aliquots of transduced cells were cryopreserved and thawed at different times post cryopreservation (day 0, 3 months, and 6 months), to emulate conditions to be used in clinical trials. Thawed cells were then cultured both in liquid and semisolid cultures, as performed with fresh samples. As shown in Table 1, high cell viability was observed in all instances. Additionally, both the VCN/cell observed (range from 2.3 to 4.8 copies/cell) and the transduction efficacy (71.0%–93.0%) of cryopreserved cells were comparable with values determined prior to cryopreservation. The transduction efficacy and VCN/cell tested in hematopoietic colonies from cryopreserved samples thawed at day 0 were also similar to those detected in fresh cells (80.0% ± 2.3% and 3.08 ± 0.9 copies/cell, respectively. See Table 1; Figure S2), and was maintained up to 6 months of cryopreservation (87.0% ± 5.5% transduction efficiency and 2.2 ± 0.2 copies/cell). Finally, as observed in fresh samples, VCN values in BFU-E and CFU-GEMM colonies were, in most cases, higher than in CFU-GM colonies (Table 1).

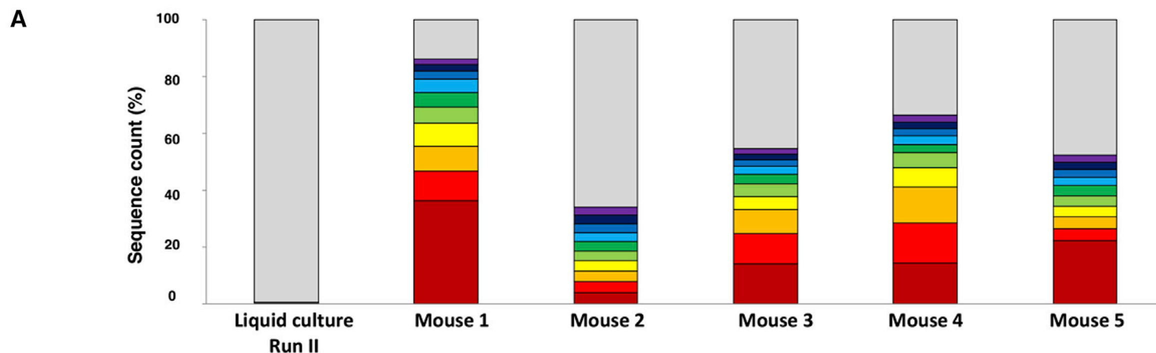
To confirm the functionality of HSPCs that had been transduced under GMP conditions, transduced CD34+ cells that remained cryopreserved for 3 months were thawed and transplanted into NSG mice. Mice were followed to monitor the engraftment of gene-corrected cells. Flow cytometry analyses conducted in BM from recipient mice at the end of the follow-up period (3 months post transplant) showed a mean value of 66.4% ± 5.0% and 55.3% ± 14.6% (runs I and III, respectively) and 29.0% ± 7.8% (run II) hCD45+ cells in mice transplanted with CB and mPB transduced cells, respectively (Figure 4A, see bars corresponding to the left y axis). Quantitative PCR analyses showed a mean value of 1.1 ± 0.2, 0.4 ± 0.1, and 2.4 ± 0.4 copies per hCD45+ cell in these run I, run II, and run III, respectively (Figure 4A, see dots corresponding to the right y axis). The presence of CD34+, CD19+, and CD33+ human cells showed that the differentiation capacity of transduced cells was also preserved after cryopreservation (Figure 4B).

To determine the clonal composition of human engrafted cells, aliquots of transduced CD34+ cells (prior to transplantation) and BM cells obtained from representative recipients at the end of the follow-up were used to perform integration site analyses (ISAs). Overall, ISAs shown in Figure 5 illustrate the polyclonal reconstitution pattern observed in transplanted NSG mice. Additionally, these studies also revealed the expected preferential integration of the provirus in coding sequences (not shown) and the near absence of preferred integrations in or near genes involved in serious adverse events noted in previous trials with gamma-retroviral vectors. In particular, no insertion sites (IS) in or near oncogenes involved in retroviral genotoxicity, such as *CCND2*, *LMO2*, *MECOM*, and *MN1*, were repeatedly observed or determined at significant frequencies in BM samples from recipient mice.

Restored membrane expression of β_2-integrins after Chim.hCD18-LV transduction of CD34+ cells from a patient with severe LAD-I

In the final set of experiments, we aimed at demonstrating the efficacy of the Chim.hCD18-LV to restore the expression of β_2-integrins in myeloid cells from a patient with severe LAD-I. Accordingly, mPB

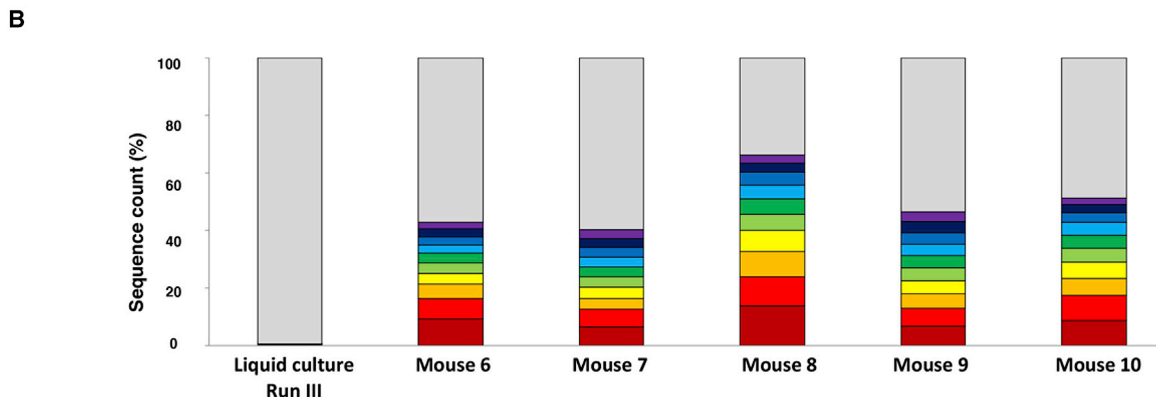
A



	Liquid culture Run II	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5
Seq Count 10 Strongest	1069	297323	147931	185562	267419	214054
Seq Count all other mapp. IS	285551	47812	285656	154345	135078	195172
Total Seq Counts Used	286620	345135	433587	339907	402497	409226

Rank	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]
Top1	NCOR1	0.088	YLPM1	36.359	EIF4G3	3.975	OSBPL8	13.974	XIAP	14.411	MS4A2	22.105
Top2	TET2	0.039	RPRD2	10.276	ARRDC2	3.721	TG	10.696	POLA2	14.089	CHD3	4.346
Top3	TNRC6C	0.037	NUP98	8.722	VWF	3.701	SAFB	8.379	BLM	12.643	RAB40C	4.075
Top4	FAM129B	0.033	BRD4	8.354	UBE2G1	3.679	RPS6KA5	4.669	FXR2	6.795	IL12RB1	3.918
Top5	AVL9	0.03	CKAP5	5.436	FNBP1L	3.394	ARHGAP25	4.417	DENND4A	5.23	SPATS2	3.575
Top6	ITGB2	0.03	CD5L	5.148	CAPZB	3.291	CDC20	3.44	C19orf57	2.929	GNG7	3.482
Top7	EXOSC9	0.029	SART3	4.777	ITGB2	3.272	IFT81	2.85	DAD1	2.926	PBRM1	2.929
Top8	STAT5B	0.029	S100A6	2.783	ZC3H6	3.108	PIAS1	2.178	CEP57L1	2.547	ITGAM	2.851
Top9	NT5C3A	0.029	FMO5	2.269	LINC01122	2.992	HN1L	2.021	FANCD2	2.454	TMEM87B	2.672
Top10	TSPAN16	0.029	THRB	2.023	EMD	2.985	ITGB2	1.968	DLG1	2.416	ANXA1	2.354
#All Other mapp. IS	43987	99.627	98	13.853	366	65.882	355	45.408	303	33.56	287	47.693

B



	Liquid culture Run III	Mouse 6	Mouse 7	Mouse 8	Mouse 9	Mouse 10
Seq Count 10 Strongest	1212	107741	94934	180624	128925	145577
Seq Count all other mapp. IS	242238	143387	140559	92218	147982	138188
Total Seq Counts Used	243450	251128	235493	272842	276907	283765

Rank	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]	Gene Name	Freq. [%]
Top1	LOC101928137	0.059	CHD3	9.32	CCDC23	6.362	NUDT3	13.592	COG5	6.738	SNX27	8.67
Top2	SIPA1L3	0.056	PACS1	6.931	NUDT3	6.149	MIER2	10.24	ATP5G2	6.034	ATF7	8.604
Top3	KIAA0922	0.054	NLRP1	5.176	WHSC1	3.88	ZNF609	8.805	EIF4G3	5.292	EML4	6.019
Top4	CAMSAP1	0.052	PTGIR	3.688	MIER2	3.808	VRK2	7.325	MYO1E	4.502	RNU6ATAC	5.761
Top5	CDRT7	0.049	FRMD8	3.56	ENTPD7	3.626	FXYD5	5.539	ZNF567	4.457	ST3GAL3	4.736
Top6	TUBA1A	0.049	ZIM2	3.37	ZNF609	3.525	TTBK2	5.301	SKAP2	4.3	SMG6	4.523
Top7	LOC100507557	0.047	MECOM	2.903	EHMT1	3.291	SNORD56B	4.88	PRKD2	3.964	AKAP8L	4.421
Top8	FANCL	0.044	LOC102724096	2.828	ETS1	3.248	ENTPD7	4.688	C1orf61	3.885	RPGRIP1L	3.434
Top9	JADE3	0.044	KIAA1468	2.652	ACTL7B	3.229	THUMPD2	2.963	EIF4G3	3.859	GSTP1	2.663
Top10	PLSCR4	0.044	STXBP2	2.475	TMCC1	3.195	TBC1D22B	2.868	OLA1	3.528	EHMT1	2.471
#All Other mapp. IS	26235	99.502	391	57.097	456	59.687	83	33.799	453	53.441	387	48.698

(legend on next page)

$CD34^+$ cells from a patient with a null expression of CD18 in PB cells were mock transduced or transduced with the Chim.hCD18-LV under conditions defined in the previous experiments. Clonogenic assays of mock and Chim.hCD18-LV-transduced $CD34^+$ cells showed similar numbers of total colonies and a similar distribution of the different colony types (Figure 6A). When transduced cells were maintained in liquid culture for 14 days, an average value of 2.5 ± 0.6 copies/cell was observed in five independent transductions, consistent with previous data obtained in HD $CD34^+$ cells (Figures 2A; Table 1). Quantitative PCR analyses performed in total hematopoietic colonies showed a transduction efficacy of 79.7%. Additionally, an average number of 2.5 ± 0.3 copies of the therapeutic provirus/cell was observed in these colonies (Figure 6B). Finally, flow cytometry analyses revealed that a mean of 70.6% ± 5.1% and 69.6% ± 5.2% of LAD-I cells became positive for CD18:CD11a and CD18:CD11b, respectively, in contrast with the null expression of β_2-integrins observed in mock-transduced cells (Figures 6C and 6D).

These results provide the first evidence that the therapeutic Chim.hCD18-LV facilitates the restoration of β_2-integrin expression in leukocytes in a patient with severe LAD-I.

DISCUSSION

GT is becoming an increasingly viable therapeutic option for patients with many different monogenic diseases. Currently, more than 300 patients have been infused with LV-modified HSPCs, and some of them have been followed for more than 10 years (see review by Tucci et al.[15]). In most of these patients, the clinical disorders were substantially reverted without gene therapy-associated severe adverse events, indicating that LV-mediated GT constitutes an efficient and safe therapeutic option for patients with monogenic hematopoietic diseases, including primary immunodeficiencies (PIDs).[15]

LAD-I is a PID characterized by defects in leukocyte migration associated with *ITGB2* gene mutations. LAD-I is frequently fatal for patients who suffer from the severe form of the disease, characterized by a very low expression of the CD18 β_2-integrin subunit in the membrane of PB leukocytes and with a concomitant reduction in the membrane expression of CD11 subunits. At present, the only curative option for patients with severe LAD-I is an allogeneic HSPC transplant.[4] However, as in other PIDs, identification, and access to compatible donors are serious limitations, as there are transplant-related toxicities.[17] GT is, thus, considered a relevant therapeutic alternative for patients with severe LAD-I.

In previous work, we described the characteristics of the Chim.hCD18-LV, which carries the human *ITGB2* cDNA driven by a chimeric myeloid promoter. This promoter results from the fusion of the c-Fes and Cathepsin G 5′-flanking regions, both expressed during the differentiation of granulocytes and monocytes, and thus facilitates a preferential expression of therapeutic genes in mature myeloid cells, rather than in cells of the HSPC compartment.[13,18] In our previous study, we demonstrated the efficacy of the Chim.hCD18-LV to correct the phenotype of mice with moderate LAD-I.[13] Now, we have confirmed the efficacy of *ex vivo* GT in a mouse model of severe LAD-I, characterized by the null expression of CD18 and a much more severe phenotype, including markedly reduced lifespan. Remarkably, while leukocytes from $CD18^{KO}$ mice do not show any expression of β_2-integrins in their membrane, the infusion of syngeneic HSPCs transduced with the Chim.hCD18-LV mediated the expression of hCD18 on $CD18^{KO}$ mice leukocytes, as well as the co-expression of several mouse CD11 subunits. This finding reveals that the ectopic expression of hCD18 results in the generation of functional heterodimers in the leukocyte membrane of mice with severe LAD-I. Our results also show that levels of β_2-integrins in the membrane of PB granulocytes were higher compared with those determined in PB T lymphocytes, resembling the physiologic expression of β_2-integrins in these cells.[13] These results are consistent with data obtained in $CD18^{HYP}$ LAD-I mice treated with the same LV[13] and also with data from GT-treated X-CGD mice, in which the therapeutic $gp91^{phox}$ gene was driven by the same promoter.[18] Whether these differences in lymphocyte versus granulocyte CD18 expression are reproduced in LAD-I patients will be elucidated via the ongoing GT clinical trial (NCT03812263).

In our experiments, the functional restoration of leukocytes from GT-treated $CD18^{KO}$ mice was demonstrated by the migration of $Gr\text{-}1^+CD11b^+$ neutrophils to inflamed LPS-treated pads and also the marked increase in the lifespan of these animals compared with untreated $CD18^{KO}$ mice.

Due to the fact that primitive HSPCs are characterized by a low/null expression of CD18,[19,20] we speculated that the ectopic expression of CD18 might induce detrimental effects in these cells. Nevertheless, the experiments conducted in this study demonstrate that transduction of HD human $CD34^+$ cells with the Chim.hCD18-LV does not mediate an overexpression in the membrane levels of β_2-integrins in human leukocytes, or in their binding to sICAM-1 upon Mg^{2+}/EGTA stimulation, most probably because of the tight transcriptional regulation of *ITGAL*, *ITGAM*, and *ITGAX* genes (encoding for CD11a, CD11b, and CD11c, respectively).[13] Although CD11d:CD18 is the least understood

Figure 5. ISAs of the Chim.CD18 lentiviral provirus in human transduced cells prior to and after transplantation into immunodeficient NSG mice
Cumulative retrieval frequencies of the 10 most prominent cell insertion sites (ISs) detected in samples from run II (A) and run III (B) from transduced CD34+ cells (14 days after *in vitro* culture) and from BM samples corresponding to NSG transplanted mice. For individual samples, sequence data from all S-EPTS/LM-PCR amplicons are combined. Sequence count (Seq Count) of the 10 most prominent IS and sequence count of all remaining ISs as well as total sequence count from all amplicons are shown at the bottom for each sample. RefSeq names of genes located closest to the respective IS are given in the table. Relative sequence count contributions of the 10 most prominent ISs and all remaining mappable ISs are shown (Freq).

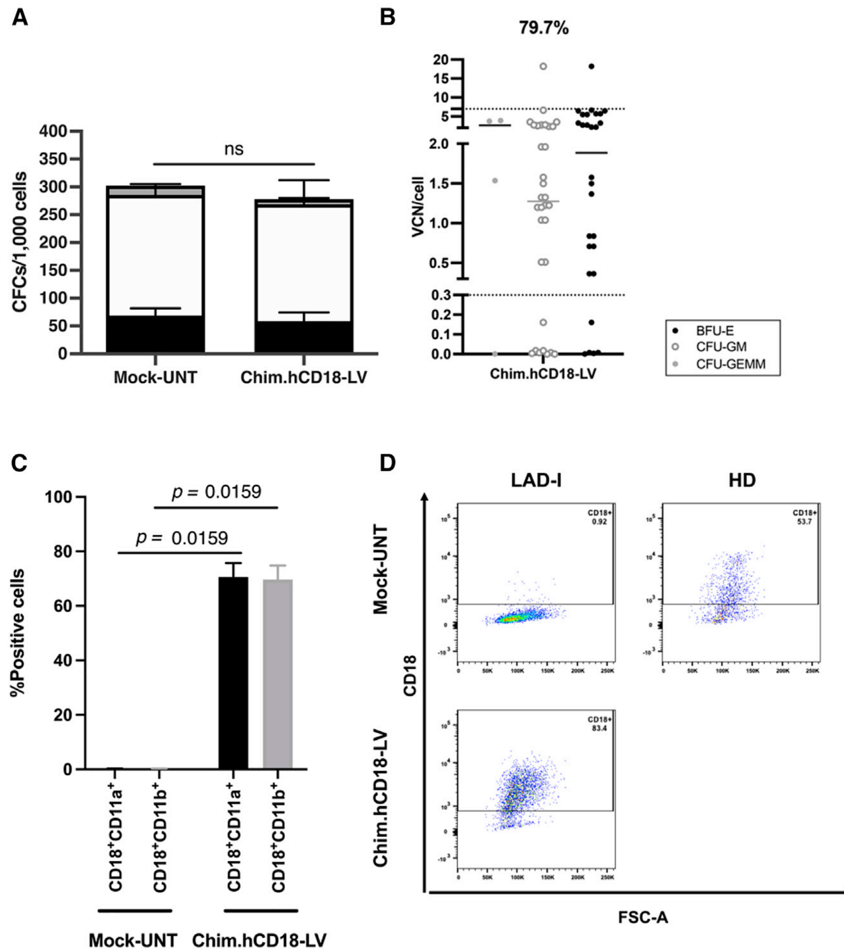


Figure 6. *In vitro* analysis of CD34+ cells from a patient with severe LAD-I after transduction with the GMP Chim.hCD18-LV

Mobilized PB CD34+ cells from a patient with severe LAD-I were transduced at an MOI 50 TU/cell with a GMP Chim.hCD18-LV. (A) Total number of the different colony types generated by mock- (mock-UNT) and Chim.hCD18-LV-transduced (Chim-hCD18-LV) CD34+ cells. No significant differences result from the analysis of the total colony number. (B) Analysis of the VCN/cell in individual colonies generated after transduction of CD34+ cells. At the top, the transduction efficiency of the respective CFUs is shown. The percentage of transduced CFUs is deduced from the ratio of positive colonies ($\geq$0.3 VCN per cell) versus the total number of colonies. Each dot represents a single colony. In (A) and (B), granulocyte/erythroid/macrophage/megakaryocyte colony-forming units (CFU-GEMMs), granulocyte/macrophage colony-forming units (CFU-GMs), and burst-forming unit-erythroid (BFU-E) are represented as gray, white, and black dots, respectively. (C) Percentage of transduced cells positive to the expression of β2-integrins (n = 5 independent transductions). (D) Representative flow cytometry analysis of CD18 expression in cells generated by mock-UNT and Chim-hCD18-LV LAD-I $CD34^+$ cells. As a control, HD CB $CD34^+$ cells are included. FSC, forward scatter. The significance of differences resulting from the Mann-Whitney test is represented as a p value or ns. Bars denote mean ± SEM.

β_2-integrin,[21] it is conceivable that the expression of this β_2-integrin will also be restored by means of the expression of CD18 in LAD-I patients.

The safety associated with the transduction of $CD34^+$ cells with the Chim.hCD18-LV was also shown by the observation that transduction of these cells with the therapeutic vector (either under laboratory or GMP conditions) does not modify their repopulating properties or their ability to generate myeloid and lymphoid cells in transplanted NSG recipients. As expected because of the higher frequency of SCID-repopulating cells, CB samples resulted in higher engraftment levels in NSG mice compared with mPB,[22] while, in all instances, multi-lineage hematopoietic reconstitution with cells harboring the therapeutic provirus was observed. Additionally, ISAs of human hematopoiesis engrafting NSG mice showed a polyclonal integration pattern, strongly suggesting the safety of the Chim.hCD18 LV-mediated GT.

Our final studies with primary $CD34^+$ cells from a patient with severe LAD-I confirmed the efficacy of the Chim.hCD18-LV to correct the phenotype of LAD-I cells, demonstrating for the first time the efficacy of this therapeutic LV to restore the expression of β_2-integrins in human LAD-I leukocytes.

Taken together, our data offer compelling preclinical evidence strongly suggesting that *ex vivo* GT with the Chim.hCD18-LV will constitute a safe and efficient therapy for patients with severe LAD-I.

MATERIALS AND METHODS

Animals

All experimental procedures were carried out according to Spanish and European regulations (Spanish RD 53/2013 and Law 6/2013, which translate and comply with the European Directive 2010/63/UE and with the European convention ETS-123).

NOD-scid *IL2Rg*^{null} (NSG) and C57BL/6J ($CD18^{WT}$) mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained at CIEMAT (registration number ES280790000183), while B6.129S7-*Itgb2*^{tm2Bay}/J ($CD18^{KO}$) were housed at Vivotecnia (Madrid, Spain) facilities (registration number ES289030000025) under quarantine. Mice were maintained under high standard conditions (high-efficiency particulate air [HEPA]-filtered air, a regulated temperature of 22°C, relative humidity of 55% ± 10%, light/dark cycle of 12 h, and food and ultraviolet-irradiated water *ad libitum*), and routinely screened for pathogens. The experimental procedures involving mice were carried out at the CIEMAT animal facility.

Ex vivo GT of $CD18^{KO}$ mice

BM cells were obtained from the femora and the tibiae of donor mice and stained for Lin^{-} phenotype using CD11b/Mac-1 (#11-0112-85, Thermo Fisher Scientific, Waltham, MA), CD45R/B220 (#1103206, BioLegend, San Diego, CA), CD3e (#5530262, BD/Becton, Dickinson and Company, NJ), Gr-1/Ly-6G (#108406, BioLegend, San Diego, CA), and Ter119 (#116206, BioLegend, San Diego, CA) monoclonal antibodies; as well as for C-KIT (#105813, BioLegend, San Diego, CA) and SCA-1 (#553335, BD/Becton, Dickinson and Company, Franklin Lakes, NJ). LSK cells (Lin^{-}, C-KIT^{+}, SCA-1^{+}) were isolated by fluorescence-activated cell sorting (FACS) using BD INFLUX (BD/Becton, Dickinson and Company, Franklin Lakes, NJ). These cells were suspended in StemSpan medium supplemented with 50 ng/mL recombinant mouse Stem Cell Factor (rmSCF), 25 ng/mL recombinant human FMS-like tyrosine kinase 3 (FLT3) ligand (rhFLT-3L), 10 ng/mL human interleukin (hIL)-3, 25 ng/mL hIL-6, and 20 ng/mL recombinant human Thrombopoietin (rhTPO) (EuroBioScience, Friesoythe, Germany). LSK from $CD18^{KO}$ mice were transduced overnight with the Chim.hCD18-LV at an MOI of 20 TU/cell in the presence of transduction enhancers and 4 μg/mL protamine sulfate (PS; Hospira Invicta, Madrid, Spain) in 2 μg/cm^2 retronectin (RN; Takara Bio, Otsu, Japan)-coated 96-well plate (Corning, New York, US). A total number of 15,000 LSK cells were transplanted into $CD18^{KO}$ recipients previously irradiated with 7 Gy (X-rays, 300 kV). Mice were followed up for 3 months and monthly bled for VCN analysis and evaluation of β_2-integrin levels using the following monoclonal antibodies: hCD18, #555923; mCD18, #553293; mCD11a, #558191; mCD11b, #553309; mCD11c, #550261; Streptavidin, #563262 (BD/Becton, Dickinson and Company, Franklin Lakes, NJ); and mCD18 #11-0112-85 (Thermo Fisher Scientific, Waltham, MA). Hematopoietic reconstitution was analyzed using the following monoclonal antibodies: mCD3e, #553060; CD11b/Mac-1, #553311; Gr-1/Ly-6G, #553128; CD45R/B220, #553091; and Streptavidin, #563262 (BD Pharmingen, San Diego, CA). Samples were analyzed in an LSRFortessa Cell Analyzer (BD/Becton, Dickinson and Company, Franklin Lakes, NJ).

Analysis of neutrophil migration in lipopolysaccharide-induced inflamed pads

Twenty micrograms of LPS derived from *Escherichia coli* (0111:B4, Merck KGaA, Darmstadt, Germany) were subcutaneously administered in the pad of one of the hind legs from WT, $CD18^{KO}$, and GT-$CD18^{KO}$ mice, while the other hind leg was used as control and injected with PBS. Twenty-four hours after PBS and LPS treatment, animals were culled, and each pad was surgically removed, mechanically disaggregated, and lysed. The cellular suspension was stained for flow cytometry analysis in an LSRFortessa Cell Analyzer using mCD45.2 (BioLegend, San Diego, CA, #109804) for gathering the hematopoietic cells; and mCD11b/Mac-1 (BioLegend San Diego, CA, #101218) and mGr-1/Ly-6G (BD/Becton, Dickinson and Company, Franklin Lakes, NJ, #553128) for the staining of neutrophils. Mouse $Gr1^{+}mCD11b^{+}$ cells presented in total $mCD45^{+}$ population from each of the treated pads were determined.

Transduction and culture of human HSPCs

CB samples from HDs were obtained from the Centro de Transfusión de la Comunidad de Madrid under Institutional Review Board approval and in compliance with the Helsinki Declaration. In all instances, samples from HDs and from LAD-I patients participating in the GT trial (ClinicalTrials.gov:NCT03812263) complied with all ethical regulations approved by the ethics committee at Hospital del Niño Jesús in Madrid and were obtained after informed consents were provided and following institutional regulations. Mononuclear cells were fractionated by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare, Chicago, IL), and $CD34^{+}$ cells were isolated with CD34 MicroBead Kit and QuadroMACS (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany). Mobilized HD PB $CD34^{+}$ cells were obtained from HemaCare, and mPB $CD34^{+}$ cells from a patient with severe LAD-I were provided by Hospital Universitario Infantil Niño Jesús. $CD34^{+}$ cells from either CB or mPB were pre-stimulated for 22 ± 4 h on 2 μg/cm^2 RN (Takara Bio, Kusatsu, Japan)-coated plates in X-VIVO 20 or Stem Cell Growth Medium (SCGM) containing 100 ng/mL Flt-3L, 100 ng/mL rhSCF, 100 ng/mL rhTPO, and 20 ng/mL hIL-3 (EuroBioScience, Friesoythe, Germany). Then, cells were transduced for 16 ± 4 h using a third-generation Chim.hCD18-LV produced under GMP-like or GMP conditions with the addition of 4 μg/mL PS (Hospira Invicta, Madrid, Spain) and transduction enhancers as previously described.[23,24] Cells were routinely analyzed for β_2-integrin expression in an LSRFortessa Cell Analyzer using the following monoclonal antibodies: hCD18, #555923; hCD11a, #551131; hCD11c, and #555392 (BD Pharmingen, San Diego, CA), and hCD11b and #CD11b29 (Thermo Fisher Scientific, Waltham, MA).

For human CFU assays, triplicates of 150 cells generated by transduced $CD34^{+}$ cells were seeded in 1 mL of methylcellulose-based medium (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany). Fourteen days after culture, colonies were scored and picked up for genomic DNA (gDNA) extraction to analyze the VCN in individual colonies.[16]

Soluble ICAM-1 binding assay

Soluble ICAM-1 binding assay was conducted after 10–12 days of myeloid differentiation in liquid cultures (Iscove′s Modified Dulbecco′s Medium (IMDM) supplemented with a 20% HyClone, 20 ng/mL rhSCF, 20 ng/mL rhIL-3, and 100 ng/mL PEG granulocyte colony-stimulating factor [G-CSF]). Two-hundred thousand cells were treated with 2% Fc receptor (FcR) blocking reagent (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) for 20 min at 4°C. An sICAM mixture containing 20 μg/mL of recombinant human ICAM-1/CD45 Fc Chimera (R&D System, Minneapolis, MN) and 40 μg/mL of PE-conjugated rabbit anti-human immunoglobulin (Ig) G Fc antibody (Thermo Fisher Scientific, Waltham, MA) in RPMI-1640 with GlutaMax medium (Thermo Fisher Scientific, Waltham, MA), 5% HyClone (GE Healthcare, Chicago, IL), and 50 mM HEPES (Thermo Fisher Scientific, Waltham, MA) was prepared and incubated for 30 min at room temperature. Cells were suspended in the sICAM mixture in the presence (stimulated) or absence (unstimulated) of

$MgCl_2$ 10 mM and EGTA 3 mM for 30′ at 37°C and finally analyzed by flow cytometry in an LSRFortessa Cell Analyzer.

Xenotransplants of human CD34+ cells

Immunodeficient NSG mice were irradiated with 1.5-Gy X-rays. Twenty-four hours later, irradiated mice were transplanted with mock-transduced (mock) or LV-transduced cells through the retro-orbital route.[25] Levels of engraftment and the lineage distribution in BM cells were analyzed on an LSRFortessa Cell Analyzer using the following monoclonal antibodies: hCD45, #304014 (BioLegend, San Diego, CA); hCD34, #555824 (BD/Becton, Dickinson and Company, Franklin Lakes, NJ); hCD33, #A07775 (Beckman Coulter, Brea, CA); hCD19, #363012 (BioLegend, San Diego, CA); and hCD18, #555923 (BD/Becton, Dickinson and Company, Franklin Lakes, NJ). Transplanted mice were culled at 3 months post transplant, and total BM cells and PB samples were analyzed. VCN analyses were performed after gDNA extraction on BM cell pellets. In some animals, the spleens and thymus were also excised for flow cytometry and VCN analyses.

ISAs

ISAs were performed using S-EPTS in Genewerk laboratories (Germany). Briefly, triplicates of 500 ng of gDNA were used as template DNAs for Shearing Extension Primer Tag Selection Ligation-Mediated PCR (S-EPTS/LM-PCR) and sheared to a median length of 400–500 bp using the Covaris M220 instrument. Primer extension was performed on the sheared DNA using a long terminal repeat (LTR)-specific biotinylated primer. The extension product was enriched by magnetic capture and then ligated to linker cassettes including a molecular barcode. The ligation product was amplified in two rounds of exponential PCR. PCR products were pooled and underwent deep sequencing by MiSeq technology (Illumina, San Diego, CA). S-EPTS/LM-PCR basics and preparation for deep sequencing were previously described.[26–28]

VCN analysis

Genomic DNA was amplified by qPCR using TaqMan master mix (Thermo Fisher Scientific, Waltham, MA) with specific primers (Grupo Tapper, Madrid, Spain) and probes (Merck KGaA, Darmstadt, Germany) in a 7500 Fast Real-Time PCR system or a QuantStudio 6 Flex Real-Time PCR (both from Thermo Fisher Scientific, Waltham, MA). Psi packaging sequence was used to quantify the viral genome, and either albumin gene (*Alb*) or titin (*Ttn*) genes were used to amplify the human or murine genome, respectively. The VCN/cell was calculated using a standard curve of plasmids containing both target sequences Psi/*ALB* (pRRL.PGK.eGFP/ALB) and Psi/*Ttn* (pRRL.PGK.eGFP/Ttn). Both constructs were provided by Dr. Sabine Charrier (Genethon, Evry, France).

Statistical analysis

Statistical analyses were conducted with GraphPad Prism software version 7.00 for Windows (GraphPad Software, http://www.graphpad.com). For comparison of qualitative and quantitative variables in which the sample size was below 30, or data did not follow a normal distribution deduced from Kolmogorov-Smirnov test, Kruskal-Wallis tests were performed to investigate the significance of differences. If samples showed significant differences ($p < 0.05$), a nonparametric two-tailed Mann-Whitney test was performed to obtain the p value. Only p values below 0.065 are represented in graphs.

Data availability statement

All data and supporting materials are available within the article and supplemental information.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtm.2022.07.016.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.A., J.A.B., and C.M.N.; investigation, C.M.N., E.A., C.D., B.D., M.G., P.R., and R.Y.; writing – original draft, C.M.N., E.A., and J.A.B.; writing – review and editing, C.M.N., E.A., J.A.B., G.R., J.D.S., and K.M.L.; visualization, C.M.N. and E.A.; collection of essential LAD-I patient samples, J.S.; supervision, E.A. and J.A.B.

DECLARATION OF INTERESTS

The Hematopoietic Innovative Therapies Division at CIEMAT receives funding from and has licensed the Chim.hCD18-LV to Rocket Pharmaceuticals. G.R., J.D.S., and K.M.L. are employees of Rocket Pharmaceuticals, Inc. E.A. has become a Rocket employee as of April

2020 (after this work was finished). J.A.B. and J.S. are consultants for Rocket Pharmaceuticals. E.A., J.A.B., P.R., and C.M.N. are inventors on patents on LVs filed by CIEMAT, CIBERER, and Fundación Jiménez Díaz, and may be entitled to receive financial benefits from the licensing of such patents. All the other authors work at the Hematopoietic Innovative Therapies Division at CIEMAT, which has a sponsored research agreement with Rocket Pharmaceuticals, Inc., for development of LAD-I GT.

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