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Long-Term and Real-World Safety and Efficacy of Retroviral Gene Therapy for Adenosine Deaminase Deficiency

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Abstract

Adenosine-deaminase (ADA) deficiency leads to severe combined immunodeficiency (SCID). Previous clinical trials showed that autologous CD34+ cell gene therapy (GT) following busulfan reduced-intensity conditioning is a promising therapeutic approach for ADA-SCID, but long-term data are warranted. Here, we report an unplanned analyses based on pre-specified and exploratory endpoints on long term safety and efficacy data of 43 patients with ADA-SCID who received retroviral e*x vivo* bone marrow-derived hematopoietic stem cell (HSC) GT. Twenty-two individuals (median follow-up 15.4 years) were treated in the context of clinical development or named patient program. Nineteen patients were treated post-marketing authorization (median follow up 3.2 years) and two additional patients received mobilized peripheral blood CD34+ cell GT. At data cut-off, all 43 patients were alive, with a median follow-up of 5.0 years

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Author Contributions statement:
M.M. contributed to the study design, patients' follow-up, data collection, interpretation and manuscript writing. F.B. contributed to the study design, patient follow-up, data collection and interpretation. C.F. contributed to data collection and analysis. P.M.V.R. and C.D.S. contributed to data analysis. M.G., A.C. and S. Z. contributed to data collection and regulatory applications. F.D., S.G., F.A.S. and I.M. , D.C. performed molecular and immunological analysis and interpretation. F.F., F.T., V.C., V.G., S.R., G.C., M.S., A.P., M.E.B. contributed to patient follow-up and data collection. C.F. and V.G. contributed to data collection and analysis. P.S. was responsible for the procedures under anesthesia of the patients. S.D. and M.C. assisted patients' and their families' as research nurse. M.L. coordinated the logistics, travels and cultural mediation of patients' and their families'. D.A.A., U.B., A.F., C.C., S.L., A.M., I.M., D.M., L.D.N., F.P., M.P., C.S., P.S., A.T., M.R., Z.K., M.G., L.L., M.D., A. P-N, and S.N.G. referred and followed patients for GT treatment and provided data. F.C. provided support to GT treatments within the Stem Cell Program of IRCCS Ospedale San Raffaele, Milan. M.P.C. and A.A. contributed to the study design, patients' follow-up, data collection and interpretation, manuscript writing and provided overall supervision.
Each author made substantial contributions to the present work, approved the submitted version and agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Inclusion & Ethics statement
Authors declare they did not discriminate against any individual on the basis of gender, race, age, religion, sexual orientation or disability status, and that they will continuously seek to include patients with rare diseases and in need for medical care in their research and clinical decisions.

Competing Interests statement:
The San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) is a joint venture between the Telethon Foundation and Ospedale San Raffaele (OSR). Gene therapy for ADA-SCID was developed at SR-Tiget and licensed to GlaxoSmithKline (GSK) in 2010. The treatments under NPP and Hospital Exemption were provided free of charge by GSK. Strimvelis Marketing Authorization in Europe occurred in 2016 (under GSK holding) and then transferred to Orchard Therapeutics (Netherlands) B.V. in 2018, which divested the program and transfer the authorization to Fondazione Telethon that became the holder in July 2023. The product, apart EU, is also currently approved in Iceland, Norway, Liechtenstein, and UK. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The authors received no specific funding for this work.
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(interquartile range 2.4-15.4) and 2 year intervention-free survival (no need for long-term enzyme replacement therapy or allogeneic HSC transplantation) of 88% (95% CI: 78.7%-98.4%). Most adverse events/reactions were related to disease background, busulfan conditioning or immune reconstitution; the safety profile of the real world experience was in line with pre-marketing cohort. One patient from the named patient program developed a T-cell leukemia related to treatment and is currently in remission. Long-term persistence of multilineage gene-corrected cells, metabolic detoxification, immune reconstitution and decreased infection rates were observed. Estimated mixed-effects models showed that higher dose of CD34+ cells infused and younger age at GT affected positively the plateau of CD3+ transduced cells, lymphocytes and CD4+CD45RA+ naïve T cells, whereas the cell dose positively influenced the final plateau of CD15+ transduced cells. These long-term data show that the risk-benefit of gene therapy in ADA remains favorable, and warrants for continuing long-term safety monitoring. Clinical trial registration: NCT00598481 [https://clinicaltrials.gov/study/NCT00598481];NCT03478670[https://clinicaltrials.gov/study/NCT03478670].

Keywords

ADA-SCID; immunodeficiency; ex vivo gene therapy; hematopoietic stem cell transplantation; retroviral vector

Introduction

Severe Combined Immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency is an ultra-rare genetic disease caused by accumulation of toxic purine degradation by-products. Allogeneic hematopoietic stem cell transplantation (HSCT) has been the standard curative option for ADA SCID patients, providing long-term benefit with a single intervention. Outcome depends on donor availability with an overall survival (OS) >90% after matched sibling (MSD)/matched family donor (MFD) HSCT and improved over the years to >85% after matched unrelated donor (MUD) HSCT[1–5]. TCRαβ/CD19-depleted haploidentical HSCT is a promising alternative option, although graft rejection remains a concern[6–8]. Enzyme replacement therapy (ERT) with pegylated-ADA (PEG-ADA) can be used to stabilize patients and initiate thymopoiesis with low immune reconstitution in the medium-long term, contributing to increased susceptibility to autoimmunity and lymphomas[9,10,14]. Gene therapy (GT) with autologous hematopoietic stem/progenitor cells (HSPC) engineered using a gammaretroviral (γ-RV) or lentiviral (LV) vector[11,12] has emerged as a treatment option for patients lacking a suitable MSD[13,14]. Results of the γ-RV GT trials[15] showed a favorable safety profile[16] with immune reconstitution, decreased infection rate and sustained metabolic correction[17].

Strimvelis is a medicinal product authorized in the European Union (EU) in 2016 which is based on an autologous CD34+ enriched cell fraction that contains bone marrow (BM) CD34+ cells transduced with γ-RV that encodes for ADA[18]. Strimvelis is indicated for the treatment of patients with ADA-SCID, for whom no suitable, matched related donor is available. This represents a unique experience of a medicinal product based on genetically modified HSPC that was available in the market, posing several challenges related to

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reimbursement, treatment access and logistics for referring and treating physicians, patients and caregivers.

In this paper, we report an unplanned analyses based on pre-specified and exploratory endpoints on the long-term safety and efficacy of the clinical development program and real-world experience following EU approval

Results

Patients' population and access to the approved treatment

The cohort of subjects comprises 43 patients treated with GT from 2000 to 2022 (Fig. 1), including 19 subjects who received the approved product Strimvelis (STRIM), 22 enrolled in the pre-marketing phase in clinical development program (CDP) or named patient program (NPP) and two additional subjects treated under hospital exemption (HE) with (mobilized peripheral blood) mPB-derived CD34+ cells (mPB-HE) (Table 1, Extended Data Tab. 2). Median age at diagnosis was comparable between STRIM and CDP+NPP (Extended Data Table. 1). Before GT, 4 CDP patients underwent an unconditioned unsuccessful haploidentical transplant and 40 subjects received ERT, without difference in duration between the two cohorts (Extended Data Table 1; Suppl. Fig 1).

In the STRIM cohort, 6 Italian patients from 4 different regions had treatment costs covered by the Italian National Health System; eight European patients were treated through the "S2 Form" route (Regulation 883/04 and Regulation 987/09) covered by respective national health care systems (Suppl. Table 1). Five non-EU patients had costs covered through government or special funding (Suppl. Table 1). Time from referral to treatment was shorter in STRIM vs CDP+NPP (median 9 vs 16 months), depending on the time for stabilization with ERT and/or management of underlying diseases (Extended Data Table 1). Nineteen additional patients who were initially referred for Strimvelis were not treated due to lack of eligibility, lack of funding or different treatment choices (Suppl. Table 2).

Characteristics of patients and drug product

The characteristics of the 19 STRIM patients and patients not previously described[14] are reported in Extended Data Table 1 and Extended Data Table 2. Median age at GT was 11 months (IQR 9-22.5) for STRIM and 20 months (IQR 13-29) for CDP+NPP (p=0.1133) cohorts, respectively (Fig. 2 A, Extended Data Table 1 and Suppl. Fig. 1). STRIM patients received a median dose of 11.6 x 10^6/kg (IQR 7.08-12.8) while CDP+NPP patients a median dose of 9.2 x 10^6/kg (IQR 6.44-11.3) (p=0.2048) (Fig. 2 B, Extended Data Table 1). We observed a higher vector copy number (VCN) (p=0.0135) in the drug product of the STRIM cohort compared to CDP+NPP patients (Fig. 2 C). Median estimated total area under the curve (AUC) of busulfan exposure was 21953 ng*h/ml for STRIM and 19590 ng*h/ml for CDP+NPP (p=0.1494) (Extended Data Table 1).

Survival

All patients (n=43) are alive with a median F-U of 5.0 years (Table 1). Intervention free survival (IFS) at 2 years is 88.0% (95% confidence interval (CI): 78.7%-98.4%), not

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significantly different between the two cohorts (p=0.1994). IFS at 2 years is 94.4% (95% CI: 84.4%-100%) for STRIM and 81.8% (95% CI: 67.2%-99.6%) for CDP+NPP (Fig. 2 D). CDP+NPP show also IFS at both 5 and 10 years of 77.3% (95% CI: 56.3%-93.9%). All 6 patients in whom GT failed were put on ERT and 5 of them were on ERT before GT. Subsequently, four patients underwent successfully a rescue HSCT: two from MSD (not available at the time of GT), one from a MUD, and one from a haploidentical donor[7] with TCRαβ/CD19 depletion (a STRIM patient); the other 2 patients are currently on long-term ERT. An additional patient who developed leukemia 4.7 years after GT required a secondary intervention (TCRαβ/CD19-depleted haploidentical HSCT) without restarting ERT.

Haematological reconstitution

All patients experienced transient neutropenia (median nadir 200/mm3 for both groups) from which they recovered (Suppl Table S1). There was a tendency for longer median duration of grade IV neutropenia (absolute neutrophil count <500/mm3) in the STRIM cohort (median 30 days) as compared to CDP+ NPP cohort (median 17.5 days) (p=0.0205, adj. p=0.0615) (Suppl Table S1), with a faster occurrence of neutrophil engraftment in the latter cohort (p=0.0082; adj p=0.0328) (Extended Data Fig. 2 A).

Considering the overall population, a significant correlation of total AUC was found with number of days of grade IV neutropenia (rho [95% CI] = 0.3701 [0.0618; 0.6139] and p= 0.0204) but not with nadir of neutropenia (rho [95% CI] =-0.2454 [-0.5207; 0.0759] and p=0.1320).

Median nadir of platelets was 92 x 10^9/L (range 31-210) for STRIM cohort and 90 x 10^9/L (range 14-314) for CDP+ NPP cohort. None of the STRIM patients and 5/22 CDP+NPP patients experienced grade IV thrombocytopenia (platelets <25 x 10^9/L).

Engraftment of gene corrected cells

Molecular analyses showed that in the STRIM group transduced T cells appeared 3-6 months post-treatment and stabilized at 1-2 years of F-U, similar to the CDP+NPP population. Median VCN at 1 year were 1.38 (IQR 0.77-1.61) for STRIM and 0.77 (IQR 0.62-1.0) for CDP+NPP, respectively (Extended Data Fig 1, A, B). CD19+ and CD56+ transduced cells were generally detectable within 2 months and showed a similar increase and stabilization in both cohorts (Extended Data Fig 1, C, D, E, F).

Transduced peripheral blood (PB) CD15+ myeloid cells, a surrogate parameter for the engraftment of gene corrected BM HSPC, appeared as early as 1 month and then stabilized with a wide heterogeneity among patients (Extended Data Fig 1, G, H). Median VCN at 1 year were 0.04 (IQR 0.01-0.06) and 0.01 (IQR 0.01-0.04) for STRIM and CDP+NPP cohorts, respectively. Longitudinal analyses of data up to 36 months after GT by means of nonlinear mixed effect (NLME) models showed a stabilization of CD15+ and CD3+ VCN levels during F-U with significant differences in the final estimated asymptote of VCN CD15+ (p=0.0394) for STRIM (VCN=0.03) vs CDP+NPP (VCN=0.01) and estimated VCN CD3+ (p=0.0001) for STRIM (VCN=1.18) vs CDP+NPP (VCN=0.61) (Extended Data Fig. 3 A, B, Suppl Table S4 a, b).

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In the CDP+NPP group we observed long-term persistence of gene corrected cells in multiple PB and BM cell lineages (Extended Data Fig 1 B, D, F, H) with the latest F-U ranging from 7 to 15.4 years.

Finally, by estimating a NLME model in the total population, we found that the neutrophil value was influenced by the level of transduced T cells (CD3+ VCN) and lymphocyte counts (Extended Data Fig 2 B, C) (Suppl Table S5 a, b) but not VCN in CD15+ cells (Suppl Table S5 c), suggesting that the expansion of corrected lymphocytes contributed to haematological recovery.

Immune reconstitution

Progressive immune reconstitution was observed in all patients treated with GT. Lymphocytes and T cells initially decreased in the initial 3 months after GT, mainly due to ERT discontinuation and busulfan administration, and then started to raise between 3 to 6 months after GT (Fig. 3 A-H).

The two cohorts showed no significant differences in the final plateau of the trend of lymphocyte, T cells and naïve T cells reconstitution, through an NLME model analysis of data up to 36 months after GT (Extended Data Fig 3 C, D, E; Suppl Table S4 c-e).

In line with previous observation[17], T-cell counts stabilized after 3 years in both groups and the kinetic of reconstitution was similar in both groups (Figure 3 C-H, Extended Data Fig 4). In the CDP+NPP cohort, T-cell counts progressively normalized over time in the majority of patients, while lymphocyte counts normalised in about half of the patients (Extended Data Fig 4). Median numbers of T-cell subpopulations in the CDP+NPP cohort, were in the normal range 8 years after GT and up to the latest available F-U (Fig. 3 D, F, H). Starting from 6 months post-GT, a steady increase of CD4+CD45RA+ naïve T-cells was observed in all patients (Fig. 3 I, J) without statistical difference between the 2 treatment groups by NLME model analysis (Extended Data Fig 3 E; Suppl Table S4 e). Naïve T-cells persisted long-term in the CDP+NPP population and active thymopoiesis was detected by T cell receptor excision circles (TRECs) analyses in the majority of patients (Suppl. Fig. 2).

Sustained and persistent in vitro T-cell function (including PHA and anti-CD3 stimulation), was demonstrated during the entire F-U (Extended Data Fig. 5). Both patients cohort showed significantly greater T-cell proliferative capacity (stimulation index) in response to anti-CD3 at 3 years of F-U compared to baseline (CDP+NPP adj $p<0.0001$; STRIM adj $p=0.0026$) (Suppl Table S6 b).

NK cells and B cells showed similar behavior in the early phase after treatment in the two cohorts (Fig. 3 K-N). In the long-term F-U, median B cells remained low, while NK cells reached levels at the lower limit of normal. Immunological improvement led to suspension of immunoglobulin (Ig) replacement therapy in 8/11 STRIM patients and 10/18 patients CDP+NPP population at 3 years of F-U (Fig. 4 A, B). At last F-U all STRIM patients (5/5) and nearly all CDP+NPP patients (16/17) were free from Ig supplementation (Fig. 4 B). Only one patient was still receiving Ig substitution due to anti-CD20 administration for the treatment of autoimmune haemolytic anemia. In patients who discontinued Ig, median IgG

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levels remained within normal range (Fig. 4 C, D). Median IgM and IgA levels increased over baseline at 1.5-2 years of F-U and were within normal range in long-term F-U cohort (Fig. 4 E, F, G, H). Median IgE levels increased over baseline after GT both in early and late F-U (Fig. 4 I, J). This was associated in some patients with a late onset phenotype and clinical manifestations of eczema and allergic asthma and could be ascribed to the known tendency to atopy in ADA-deficient patients[19] and/or to a cytokine imbalance towards Th2 phenotype.

Patients who were vaccinated displayed specific humoral response to the majority of vaccines, including live attenuated, as well as to native pathogens (Extended Data Table 3). Of note, similar rate of infections post-GT was observed between CDP+NPP and STRIM groups up to the last available observation, which remained very low long term (Fig. 4 K).

Deoxyadenosine nucleotide (dAXP) concentrations in PB red blood cells (RBC) progressively decreased both in STRIM and CDP+NPP patients to levels of <100 nmol/mL and remained low during the whole time of observation (Fig. 4 L, M). The estimated NLME model of data up to 36 months after GT showed a similar final plateau in both groups (Extended Data Fig.3 F; Suppl Table S4 f).

Engraftment and reconstitution according to treatment age

In order to evaluate if the age at treatment could have influenced the outcome of GT, we arbitrarily set an age threshold and divided the cohort in patients who underwent GT ≤2.5 yrs and >2.5 yrs. The dose of BM transduced CD34+ cells was significantly higher in the younger cohort considering all patients (p=0.0156) (Suppl. Fig 3 A, B). Patients treated ≤2.5 yrs of age showed a trend towards a higher engraftment of gene modified CD15+ and CD3+ cells (Suppl. Fig 4 A, B), had a more profound lymphopenia during the early phases post-GT but reached in the long-term higher lymphocyte, T-cell, naïve T cells, and B-cell counts than older patients (Suppl. Fig 4, C-I).

Univariate analyses showed a tendency for an association at 2 years post-GT for younger age at GT (treated as continuous variable) and higher CD34+ cell dose with lymphocyte and CD4+/CD45RA+ counts. Younger age and higher VCN in the product displayed a tendency for higher transduced CD15 + cells at 2 years of F-U (Suppl Table S3 a). Finally, a higher VCN in the DP showed a tendency for lower dAXP and higher ADA activity in MNC (Suppl Table S3 f, g).

Through NLME models we found that the final plateau of the kinetic of engraftment of transduced CD15+ and CD3+ cells was positively influenced by the dose of CD34+ cells/Kg infused whereas patients treated younger achieved a higher asymptote for transduced CD3+ cells (Fig 5 A, B; Suppl Table S7 a, b). On the other hand, the longitudinal trend of lymphocyte and CD4+CD45RA+ naïve cells count was significantly affected by the age at GT and dose of medicinal product, while CD3+ cell counts only by the age (Fig 5 C, D, E; Suppl Table S7, c-e).

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Outcome in patients treated with mPB-derived CD34+ cells GT

Two patients with a late onset phenotype referred for Strimvelis treatment displayed insufficient BM CD34+ cell content and were eventually treated at 4.6 and 10.8 years of age under HE (Fig 2 A), using CD34+ cells collected from mPB manufactured under the same transduction conditions of Strimvelis (mPB-HE). Patients mobilized an adequate number of CD34+ cells after G-CSF and Plerixafor and received a dose of 10 x 10^6/kg and 26 x 10^6/kg of drug product, respectively (Fig. 2 B). VCN was in the range of BM-derived product (Fig 2 C). The levels of gene corrected cells were comparable to patients treated with BM-derived CD34+ cells (Extended Data Fig. 6 A-D). Lymphocyte and subpopulations at 1 year were in the lower range of STRIM and CDP+NPP populations, and increased >3 years after GT, in line with other patients treated at older age. Thymic output, in vitro T-cell functions and dAXP in RBC were in line with the other patients' cohorts (Extended Data Fig. 6 E-N). One patient was able to discontinue Ig and received vaccination starting from 1 year after GT.

Safety

As of December 2022, a total of 17 patients out of 22 (77%) in CDP+NPP population reported 52 serious adverse events (SAEs) after treatment (of which 39 already reported[16]), of which 30/52 (58%) of infectious origin. All SAE eventually resolved and none was fatal.

In the STRIM population, 14 patients out of 19 (74%) reported 22 non-fatal SAEs post-treatment, maximum grade 3. Among these, 14 (64%) were of infectious origin, with no one being life-threatening and all having completely resolved (Extended Data Table 4 and Fig.4K). Of note, a SAE of macrophage activation syndrome following chickenpox vaccine administration occurred in a patient, with inadequate antibody response to vaccine, which resolved after treatment[20]. An additional SAE of lack of efficacy in another patient led to HSCT haplotransplant[7, 21] (Extended Data Table 4). One mPB-HE patient reported 3 SAEs, 2 of which infectious, all resolved (Extended Data Table 4).

One patient experienced a hypovolemic shock and refractory metabolic acidosis subsequent to BM harvest, complicated by multi organ failure, and did not receive Strimvelis. This was the only event of hypovolemic shock experienced in our centre in 20 years of BM harvests[22].

Signs of clonal expansion at the scheduled F-U were monitored through clinical visits and laboratory tests with no clinically relevant alterations reported. The analyses of TCR repertoire showed fluctuation over time; at latest follow up most TCR Vbeta families were represented at normal frequency, a minority was represented below normal, or rarely increased, without clinical impact (Suppl Fig. 5).

One event of lipofibroma was reported in a patient, not associated with GT.[16,23] None of the SAEs was related to treatment with the exception of one case of T-cell acute lymphoblastic leukemia (T-ALL) occurring 4.7 years after GT in a patient treated with autologous HSPC-GT under NPP (Cesana, Cicalese et al. manuscript submitted). Retroviral insertion site analysis on the leukemia sample identified a single dominant clone with an insertion near LIM-domain only 2 (LMO2) proto-oncogene. The patient underwent chemotherapy and,

after obtaining remission, human leukocyte antigen (HLA)-haploidentical transplantation, and is in clinical remission 2.5 years after transplantation.

Discussion

Here we report the extended follow up of ADA-SCID patients treated in the context of clinical development and NPP along with the post-marketing experience with Strimvelis, showing sustained efficacy for up to 22 years after GT.

Patients treated with commercial product showed excellent OS and IFS at 2 years (100% and 94.4 respectively) with a safety profile in line with the pre-marketing experience. No difference in the kinetics and levels of immune reconstitution, rate of severe infections and systemic detoxification were observed between the two groups.

To our knowledge, this is the first reported experience on patients treated with an approved medicinal product based on autologous genetically modified CD34+ cells. Despite the limitation of a fresh product available only in a single center due to its short shelf-life, patients from other EU countries had access to treatment covered by national health systems thanks to Social Security Regulation path (so called “S2 form route”), the only viable route for planned treatment abroad for this type of medicine in the EU. However, only about half of the patients eligible for GT was eventually treated with Strimvelis for different reasons. When funding was not granted, it is possible that the perception of high costs of the drug product, not subjected to local negotiation, and lack of clarity of the approval process represented a bottleneck towards reimbursement approval. This could be of concern for treatment access in the EU for other ATMPs for rare diseases for which qualified treatment centers are not available in the country of origin. The need for families to spend 4-6 months far from home with limited financial and logistical resources was dealt with a supporting program from Fondazione Telethon. The use of cryopreserved formulation could increase the network of treatment centers and mitigate partly these limitations for other ATMPs[11, 24].

The data in the overall population with a median follow up of 5.0 years confirm the durable efficacy of γ-retroviral vector GT. Five out of 6 failure cases occurred within two years post-treatment and one at 4.5 years post-GT, indicating a stability of treatment effect once engraftment of multilineage gene corrected HSPC and cellular and humoral immune reconstitution have occurred.

The patient population also included subjects treated at an older age, due to late diagnosis or late referral for a definitive treatment. The age at GT was an important variable influencing both the engraftment of transduced T cells and lymphocytes and naïve T cell counts, suggesting that younger patients have a more suitable thymic environment for seeding and maturation of HSPC. This is in agreement with a recent report on a cohort of 10 ADA-SCID patients treated with a different γ-retroviral vector and followed up to 11 years[25]. An older age might cause a progressive attrition on HSPC proliferation and differentiation[26], as well as on the supportive capacity thymic or BM microenvironment[27–30]. Ideally, GT should be performed as soon as possible after a newly diagnosed ADA-SCID patient is stabilized with ERT in order to prevent infections and organ damage[14]. The extension of the successful

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experience of SCID neonatal screening in the United States and a wider use of metabolic screening for ADA deficiency could facilitate the early diagnosis of ADA-SCID[31,32].

Most AEs in the STRIM population were related to disease background, busulfan conditioning or immune reconstitution, in line with data reported in the pre-approval experience[16]. Autoimmune manifestations post-GT were previously observed and may be related to an immune dysregulation during the early immune reconstitution post-GT.

Neutropenia is frequent in ADA-SCID[33] patients at diagnosis and the occurrence of prolonged neutropenia in some patients, in addition to busulfan effect, could be related to an intrinsic susceptibility of myeloid cells and/or an altered BM microenvironment[27,33] in the absence of ERT. The STRIM group displayed a more prolonged neutropenia, which did not result in an increased rate of bacterial infection; this could be linked to a policy change in the target AUC in order to favor the engraftment of corrected CD34+ cells. Interestingly, the neutrophil value was influenced by the level of transduced T cells and lymphocyte counts suggesting that the expansion of corrected lymphocytes contributed to haematological recovery.

Our analyses indicate that the dose of BM CD34+ cell dose influenced both the kinetics of transduced cells engraftment and immune reconstitution (lymphocytes and naïve T cell counts). The outcome of GT was favorable in the two patients who were treated with mPB-derived CD34+ cells, in line with the kinetic observed in patients of similar age treated with BM HSPC. The use of mPB allowed collection of higher amounts of HSPC and prevented general anesthesia and large blood volume depletion in these patients, allowing a safer procedure.[35]

In the future, our cohort of long-term surviving patients will also allow us to study the occurrence of multidisciplinary non-immunological disease-related features (i.e. neurological[36], psychiatric, metabolic, urological issues[37]) and compare it with other therapeutic approaches.

Following the single case of T-ALL associated with retroviral insertion in one of the ADA-SCID patients, EMA confirmed a favorable benefit-risk balance taking into account the balance between the intrinsic disease-related risk of hematopoietic malignancy and the challenges of allogeneic HSCT. All patients treated will be continued to be monitored long term up to 15-years post treatment. An insertion site analyses study is currently ongoing (#NCT04959890) as part of post-authorisation measures.

Lentiviral vectors have emerged in the past decade as a safe and effective platform for the treatment of genetic disorders including primary immunodeficiencies[11]. A recent study[12] reported results in 50 ADA-SCID subjects treated with LV-based GT with excellent event free survival and robust immune reconstitution at 24 to 36 months of follow up. In addition to the vector type, the main differences with our study relate to the age at treatment, which was lower than our CDP+NPP cohort, a shorter follow up, as well as for the use of mPB and cryopreserved product in a large fraction of the patients.

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Main limitations of our study include the use of non planned and exploratory analyses, a wide time range of observation (2000-2022) during which changes in other therapeutic approaches occurred, and a selection bias for patients in the STRIM cohort which include only those with access to Strimvelis with respect to those enrolled during clinical development which had no restrictions in country of origin.

In the absence of an HLA-identical related donor, which remains the standard of care, HSCT from matched unrelated donor or haploidentical donor are curative therapeutic options to be considered in case GT is not available or unsuccessful[1–8]. Indeed, worldwide experience with ADA-SCID gene therapy using γ-retroviral[25] or LV[5–12] demonstrate that this is an option with a high tolerability profile and excellent survival while allogeneic transplantation is still associated with 10-15% mortality in large multicenter studies[4,5].

In conclusion, our data show that γ-retroviral GT for ADA-SCID provides persistent benefit in a cohort of patients treated since year 2000 and that the use of an authorized product in the EU is feasible and displays a similar efficacy and safety profile. Long-term monitoring for this innovative technology is continuing and will be crucial to assess the maintenance of its safety profile.

Methods

Patients

Patients were referred for treatment by local physicians or by parents. Characteristics of patients treated in the CDP (n=18) including 2 pilot studies (Hadassah AD1117054; OSR AD1117056), a pivotal study (AD1115611) with a long-term follow-up (LTFU) component (STRIM-004), and a CUP (AD1117064) were reported in a previous interim analyses[17] (Fig.1). Patient 1 was treated at Hadassah Hebrew University Hospital in Jerusalem (Israel). All other patients were treated at San Raffaele Scientific Institute in Milan (Italy). Four additional patients were treated under NPP before marketing authorization (200893 NPP) and two patients with mPB-derived CD34+ cells, under HE.

The pivotal study (AD1115611) was approved by Ethics Committee of Ospedale San Raffaele on June 1st 2000, amended on July 19, 2011 with a long-term component follow up (subsequently named STRIM-004) and registered at www.clinicaltrials.gov as #NCT00598481. Protocol NCT03478670 (Strimvelis registry, STRIM-003) was approved by the Ethics Committee of Ospedale San Raffaele on 19/1/2017. The treatment of each patient in the Expanded Access Program was approved individually by the Ethics Committee of Ospedale San Raffaele, according to Italian Regulation and following two different frameworks: the Hospital Exemption framework (per the Italian Decree dated 16 Jan 2015) for mPB-HE patients and the Compassionate Use framework (per the Italian Ministerial Decree of 08 May 2003 now superseded by the Decree of 07 Sep 2017) for NPP patients. Written informed consent was signed by all patients' parents/guardians. The studies were carried out in accordance with the tenets of the Declaration of Helsinki. One of the patients who required a secondary intervention has been included only in aggregate analyses due to family's request for the definitive block of any treatment on the collected data (art 4, paragraph "o" of Italian Law Decree 196/2003).

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All studies were nonrandomized, single arm, and open label. Patients were screened to determine study eligibility.

The consent obtained by the patients or parents / tutor of the patients allow to include individual-level data in the publication, which are limited to strictly necessary information.

Strimvelis was approved by the European Medicine Agency (EMA) in 2016 and available to patients since the beginning of 2017. Thirty-eight ADA-SCID patients were referred to Ospedale San Raffaele for potential GT treatment including 8 subjects from Italy, 16 from other European countries and 14 from extra-European Countries. Overall, 19 subjects (STRIM cohort) were treated with Strimvelis (6 from Italy, 8 from Europe and 5 from extra-European countries). Two additional patients treated after data cut off were not included in this analyses (Extended Tab 2, Suppl. Table 1).

Treatment and follow up

Central venous catheter (CVC) placement, cryopreservation of BM back-up, and pre-conditioning with low dose Busulfan (0.5 mg/kg i.v. on 8 consecutive doses administered in 2 days, total dose 4 mg/kg) have been reported previously[15,17]. In CDP+NPP population, AUC was reduced if single AUC was above 4000 ng/ml*h and total AUC was targeted to 19200 ng/ml*h (range 19200-22400). In the STRIM population, in order to optimize the engraftment of the corrected CD34+ cells, total AUC was targeted to the upper range 22400 ng/ml*h. In STRIM patients ERT was discontinued at a median of 16 days (range 8-21) before GT, similarly to the historical population (median 18 days, range 5-18). CD34+ cell purification from BM and transduction protocol are reported elsewhere[15,17].

Adverse events (AEs) and SAEs were reported using Good Clinical Practice (GCP) guidelines. Adverse events in the initial follow up of the CDP population, including SAEs, were previously reported[16]. AEs that occurred after treatment failure (ERT > 3 months or HSCT after GT) were not considered in the analysis.

Clinical examinations and instrumental imaging were monitored annually or more frequently based on clinical needs. VCN in cell subpopulations was used to assess engraftment. From 2000 to 2012, the frequency of transduced cells and VCN were determined on genomic DNA by quantitative polymerase chain reaction (PCR) analysis for Neomycin resistance (NeoR) vector sequences, normalized for DNA content[15]. Subsequently, the evaluation of VCN/genome was performed by digital droplet PCR technology analysing the (long term repeat) LTR vector sequence (Primer Fw: 5’-GGCGCCAGTCTTCCGATA-3’; Primer Rv: 5’-TGCAAACAGCAAGAGGCTTTATT-3’), normalized to a region of the human Telomerase gene.[17]

Lymphocyte ADA activity, and RBC dAXP levels transgene function and metabolic detoxification[39], hemogram, cytofluorimetric analysis, TREC analysis[40], T cell proliferative capacity[17], Ig replacement administration, serum Ig levels and antibody response to vaccination[17], T-cell receptor V-beta repertoire[41], peripheral blood smears, cytogenetic karyotype analysis, BM morphology, and immunophenotype were assessed overtime.

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All patients are followed with at least annual visits for the initial 11 years and then at 13-and, and follow-up will include a complete blood count with differential, biochemistry and thyroid stimulating hormone (#NCT03478670).

Data collection

Data from NCT00598481 study were collected in paper Case Report Forms (CRF) and subsequently transferred to an electronic database by the Marketing Authorization Holder (MAH) until the study closure in 2019. NCT03478670 study was approved on January 19th 2017 opened and new data were collected in an electronic CRF.

Data listing used for tables and figures of the manuscript were provided from MAH.

No compensation was provided to the study participants, but only reimbursement of expenses, in line with guidelines of ethical committee.

No sex or gender analysis was performed because ADA SCID is an autosomal recessive ultra-rare genetic disease and no differences in sex was expected on the immunological and clinical outcome.

Statistical methods

Comparisons of numerical variables between groups identified by binary variables were performed with the Mann-Whitney test and, in case of categorical variables, with Fisher's exact test. Spearman's correlation coefficient was used to evaluate correlations between numerical variables at a fixed time-point and its confidence interval was computed by using the Fisher's transformation. Overall survival curves and IFS (no need to initiate PEG-ADA for ≥ 3 months or to perform an allogeneic HSCT) curves were estimated using the Kaplan-Meier estimator. The cumulative incidence curve was used to describe time to engraftment, to lymphocytes normalization and to CD3+ cell normalization, by considering the failure as a competing event. The comparison between the IFS curves of the two treatment groups was performed with the logrank test, while Gray's test for comparing cumulative incidence curves. The reverse Kaplan-Meier estimator was used for describing the follow-up.

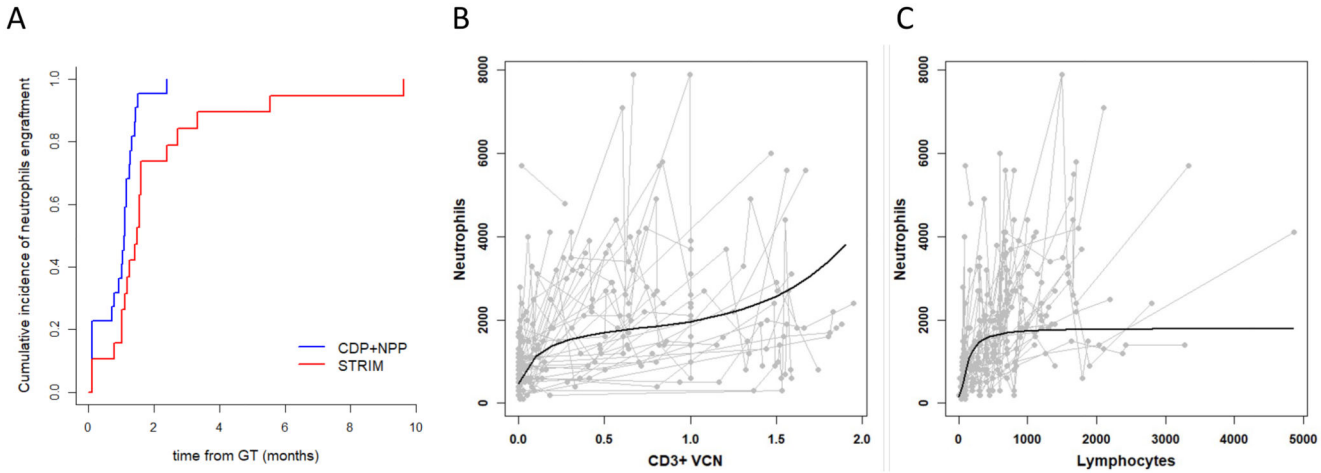
Univariate analyses were performed in order assess the influence of some baseline characteristics (ERT duration, age at GT, CD34+ cells/Kg, VCN in the product and total AUC) on clinical and biological outcomes. The method used depended on the type of outcome variable: median quantile regression for the duration of grade IV neutropenia, Fine-Gray proportional sub-distribution hazard regression model for cumulative incidence, linear regression for the values of biomarkers at 2-years of follow-up (after an appropriate transformation of the outcome in order to meet the assumptions of the model).

Mixed-effects models were used to model and compare the evolution over time of variables between different cohorts and/or with respect to other covariates. Details of the statistical analyses and final models are reported in Supplementary statistical methods.

Whenever present, missing data were not imputed. When required, the p-values were adjusted with Holm's correction to account for multiple testing and adjusted p-values were

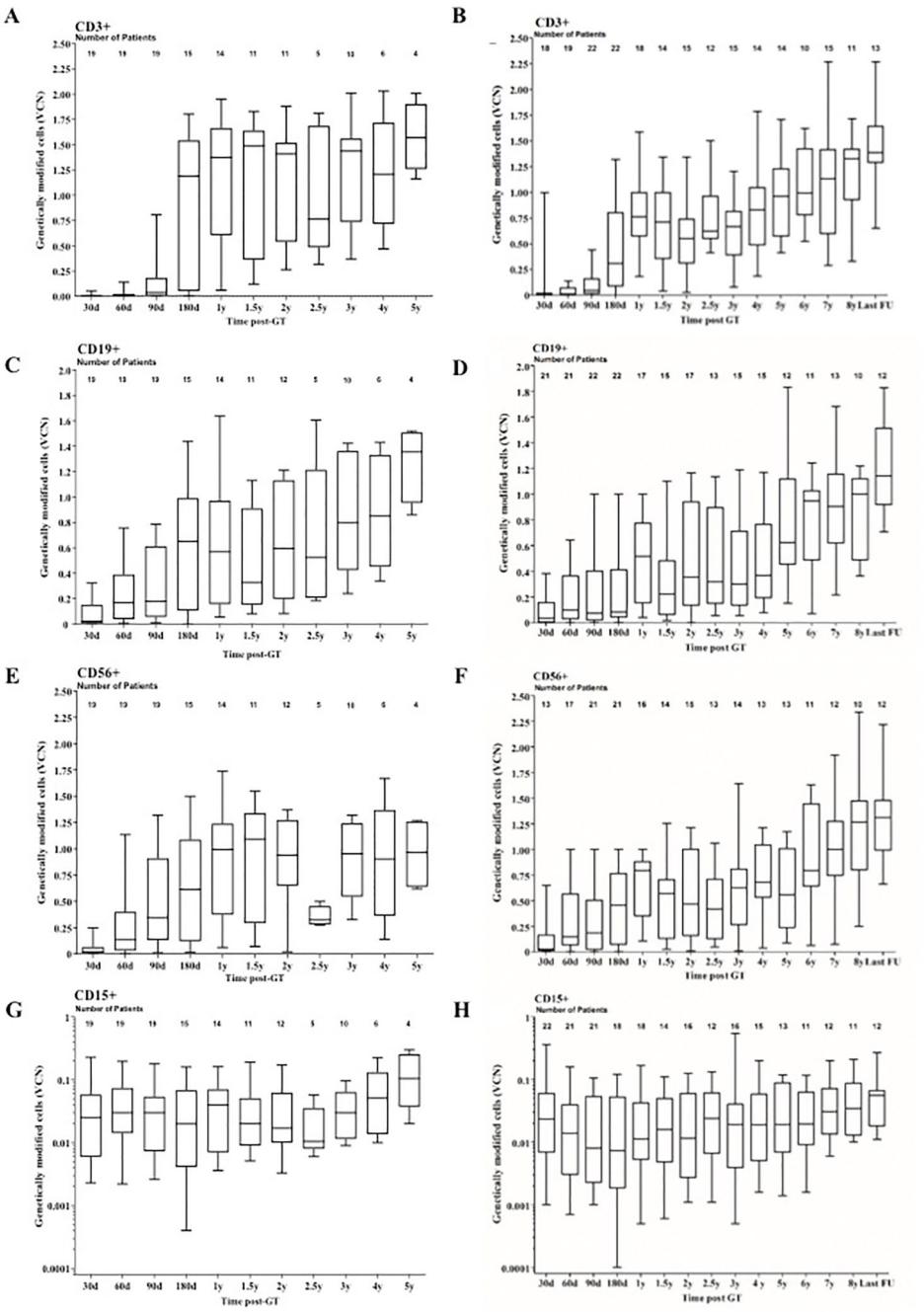
reported in the text as “adj. p”. For all tests, the significance level was set at 0.05 and the test was two-sided. CIs are computed at 95% confidence level. All statistical analyses were performed using R 3.6.2(https://www.R-project.org/). The R packages used were: stats 3.6.2; quantreg 5.85 for median quantile regression; survival 3.2-3, prodlim 2019.11.13 and cmprsk 2.2-10 for survival analysis; nlme 3.1-142 and phia 0.2-1 for mixed-effects model analysis.

Extended Data



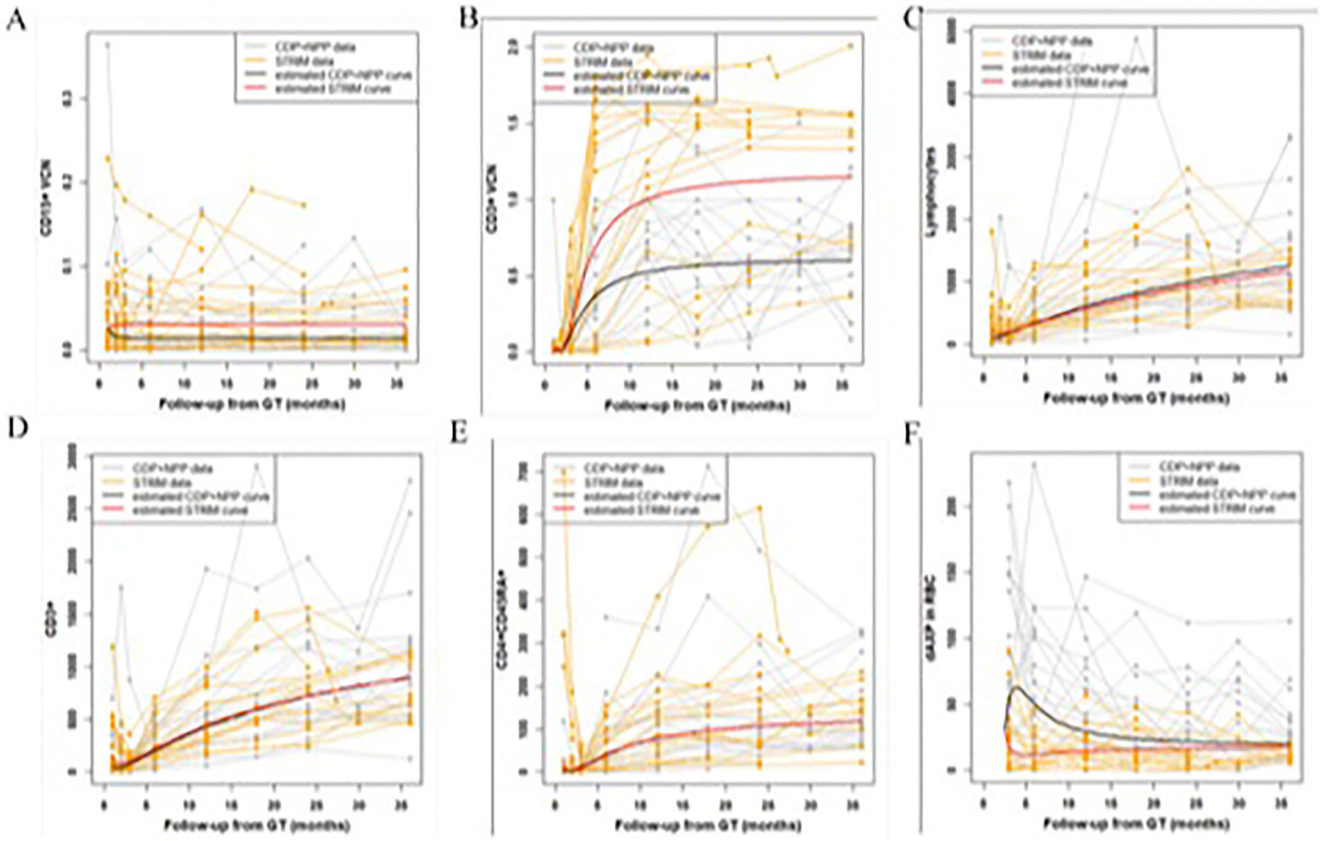
Extended Data Fig.1.

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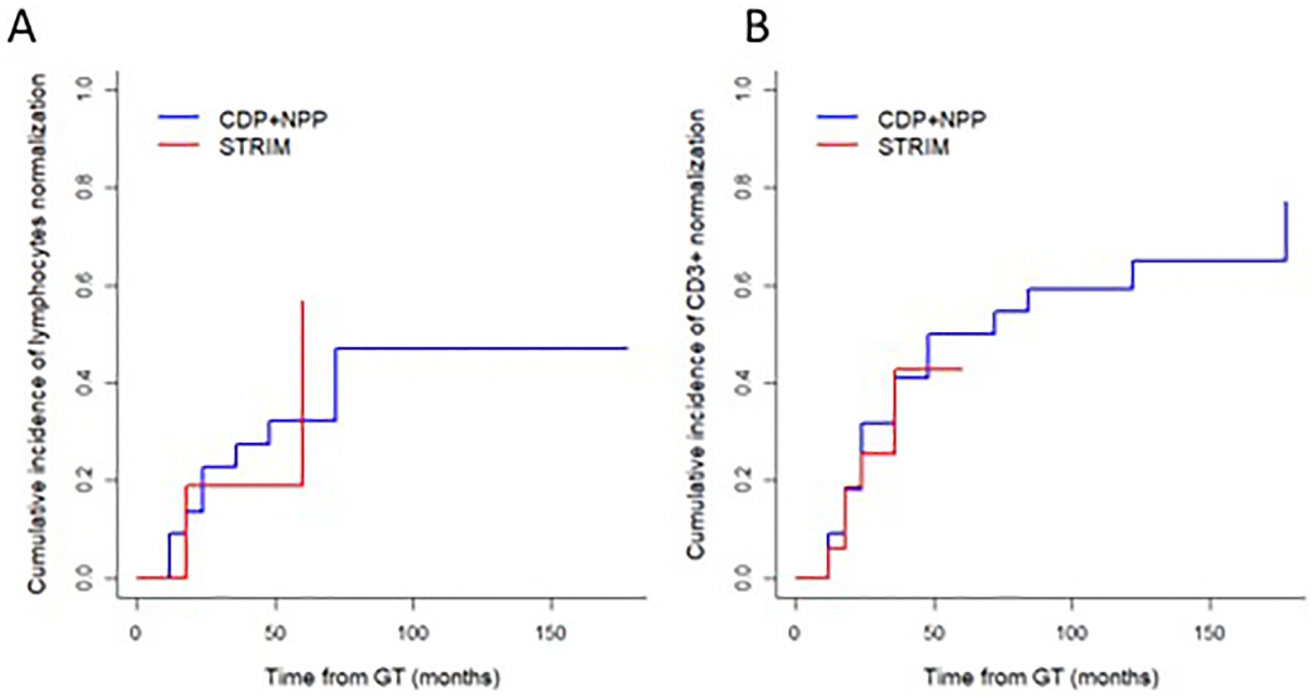
Extended Data Fig. 2.

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Extended Data Fig. 3.

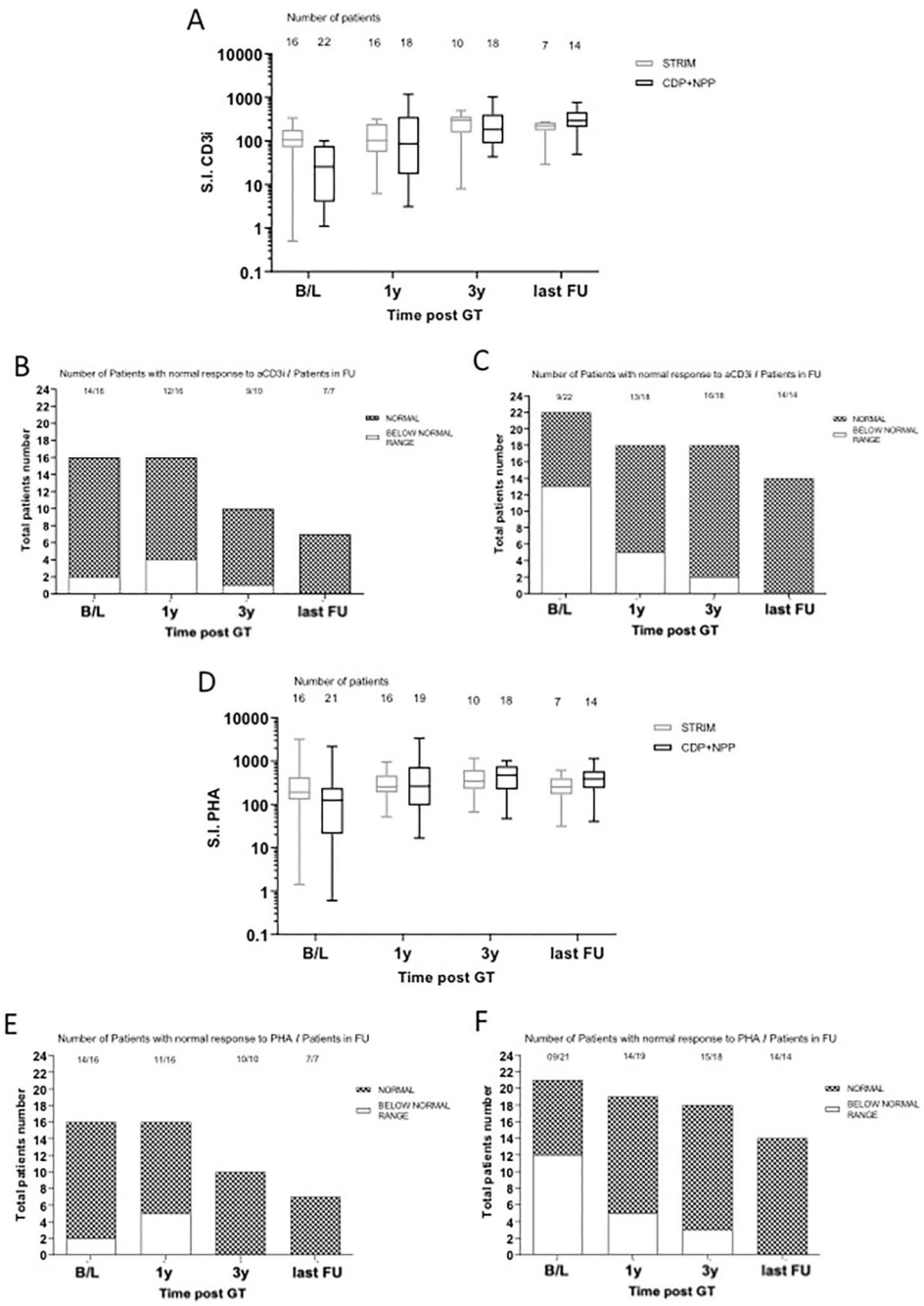
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Extended Data Fig. 4.

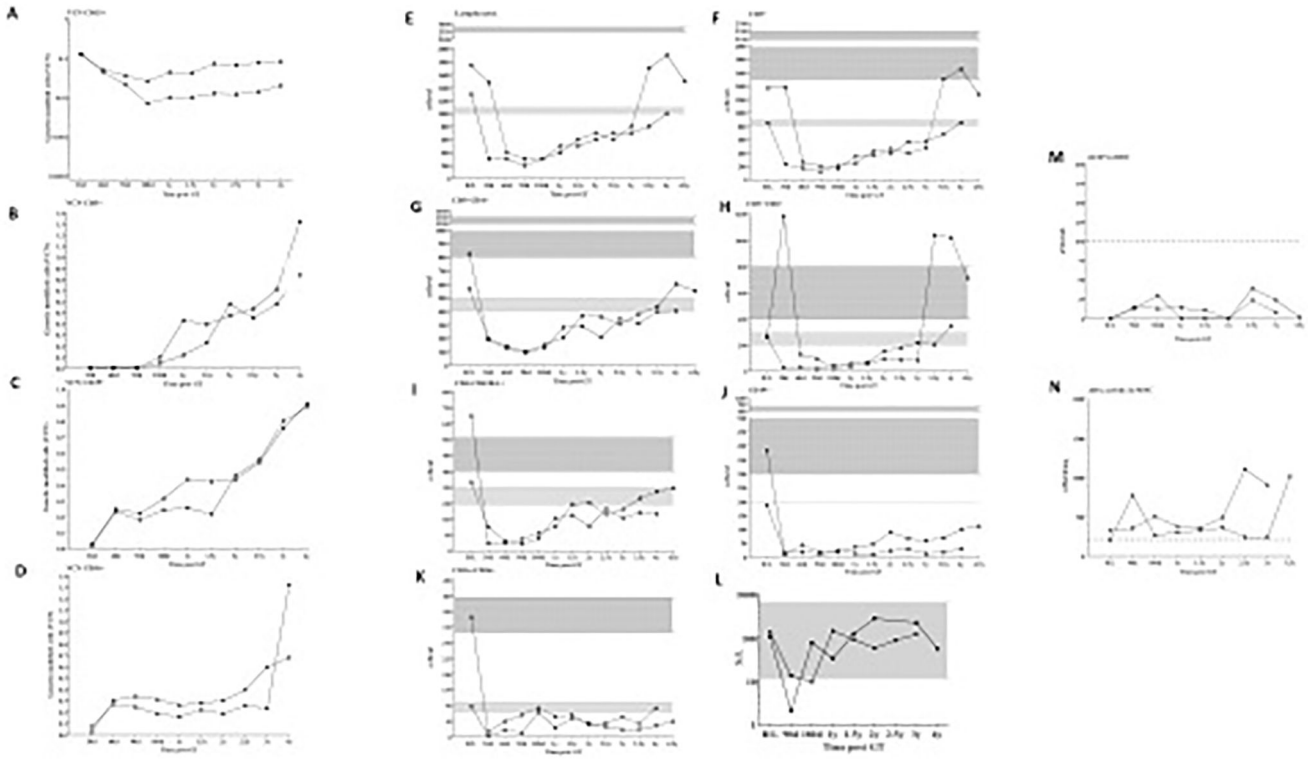
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Extended Data Fig.5.

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Extended Data Fig.6.

Extended Data Table 1

A	Patient no. §	Sex	Group of treatment	Age at onset *(month)*	Age at diagnosis *(month)*	ADA gene mutation	Previous treatment *(month duration)*	ERT dose *(UI/kg/ week)*	Relevant histo
	1	F	CDP	2	3	c.50A>C; H17P (homozygous)	None	-	-
	2	F	CDP	2	6	c.320T>C; c.632G>A	Haplo-SCT	-	-
	3	M	CDP	1	5	c.221G>T; c.845G>A	Haplo-SCT	-	-
	4	F	CDP	5	6	c.845G>A (homozygous)	Haplo-SCT; PEG-ADA*(for 2 mo)*	20	BCGitis Chiari t
	5	F	CDP	1	1	c.646G>A; c.956_960delAAGAG	PEG-ADA*(for 15 mo)*	40	Mild devel dela
	6	M	CDP	1	2	c.632G>A (homozygous)	PEG-ADA*(for 65 mo)*	30	Sensorin peripheral loss M developme Growth
	7	M	CDP	<1	2	c.646G>A; c.872C>T	PEG-ADA*(for 13 mo)*	22	Feedi disturb Developme
	8	F	CDP	<1	1	c.478+2T>C (homozygous)	PEG-ADA*(for 32 mo)*	60	Autoim haemolytic

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A	Patient no. §	Sex	Group of treatment	Age at onset *(month)*	Age at diagnosis *(month)*	ADA gene mutation	Previous treatment *(month duration)*	ERT dose *(UI/kg/ week)*	Relevant histo
									Psychon retarda Failur thriveMac activat syndro
	9	M	CDP	5	5	c.646G>A (homozygous)	PEG-ADA*(for 10 mo)*	41	Pneumo jiroveci pn requir intuba
	10	F	CDP	3	4	c.632G>A (homozygous)	Haplo-SCT; PEG-ADA*(for 11 mo)*	37	BCG Congenital hypopl Bilate peripheral loss Se mental reta Behavi proble
	11	M	CDP	4	7	c.646G > A; p.E319GfsX3	PEG-ADA*(for 8 mo)*	35	BCG Hyperte Mental reta
	12	M	CDP	<1	<1	c.606 + 5G>? (homozygous, no additional data available)	PEG-ADA*(for 12 mo)*	28	Alveo protein
	13	M	CDP	<1	<1*	c.646G>A; c.975+6Tdel	PEG-ADA*(for 1 mo)*	20	G6PDH de
	14	M	CDP	<1	1	c.466C>T (homozygous)	PEG-ADA*(for 71 mo)*	62	Bilateral loss Me retarda
	15	F	CDP	10	14	c.7C>T (homozygous)	PEG-ADA*(for 12 mo)*	18	-
	16	M	CDP	3	<1*	c.881C>A (homozygous)	PEG-ADA*(for 23 mo)*	19	Behavi proble
	17	M	CDP	<1	<1	c.956_960delAAGAG (homozygous)	PEG-ADA*(for 7 mo)*	30	Feedi disturb
	18	M	CDP	<1	<1*	c.466C>T; D123fsX132	PEG-ADA*(for 24 mo)*	20	-
	19	F	NPP	3	4	c.632G>A; c.646G>A	PEG-ADA*(for 6 mo)*	60	-
	20	M	NPP	4	22	c.467G>A; c.646G>A	PEG-ADA*(for 36 mo)*	30	Neuropsych abnorm
	21	M	NPP	2	4	c.455T>C; c.478+6T>C	PEG-ADA*(for 8 mo)*	21	-
B	Patient no.	Sex	Group of treatment	Age at onset*(month)*	Age at diagnosis*(month)*	ADA gene mutation	Previous treatment*(month duration)*	ERT dose*(UI/ kg/ week)*	Relevant histo
	23^	M	STRIM	<1	<1*	c.135 G>A (homozygous)	PEG-ADA*(for 6 mo)*	22	HCV infe
	24	F	STRIM	<1	<1	c.792 G>A (homozygous)	PEG-ADA*(for 6 mo)*	30	-

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A	Patient no. §	Sex	Group of treatment	Age at onset *(month)*	Age at diagnosis *(month)*	ADA gene mutation	Previous treatment *(month duration)*	ERT dose *(UI/kg/ week)*	Relevant histo
	25	F	STRIM	1	2	c.529G>A; c.1048delCTCTT	PEG-ADA*(for 69 mo)*	20	Charcot Toot type
	26	M	STRIM	1	2	c.43 C>G; c.320 T>C	PEG-ADA*(for 8 mo)*	45	-
	27	M	STRIM	2	5	c.302 G>A (homozygous)	PEG-ADA*(for 16 mo)*	30	-
	28°	F	STRIM	<1	12	c.302 G>A (homozygous)	PEG-ADA*(for 22 mo)*	25	BCGi
	29	M	STRIM	19	20	c.965T>C; c.22 G>A	PEG-ADA*(for 32 mo)*	30	Hypothyr
	30	F	STRIM	18	34	c.424 C>T; c.1078.20_1078-37delinsGATGTTG	PEG-ADA*(for 9 mo)*	44	-
	31	F	STRIM	<1	<1*	c.703C>T (homozygous)	PEG-ADA*(for 8 mo)*	45	-
	32	F	STRIM	2	3	c.7C>T (homozygous)	PEG-ADA*(for 4 mo)*	60	Anem
	33	F	STRIM	<1	7	c.730delG; c.254A>T	PEG-ADA*(for 5 mo)*	30	Psychon developme Growth
	34	F	STRIM	<1	7	c.730delG; c.254A>T	PEG-ADA*(for 3 mo)*	30	Congen complex malform Psychon developme Growth
	35	M	STRIM	<1	14	c.965T>C; p.F322S (homozygous)	PEG-ADA*(for 5 mo)*	60	Autoimm thrombocy
	36	M	STRIM	<1	2	c.956_960delAAGAG; p.E319Gfs*3 (homozygous)	PEG-ADA*(for 15 mo)*	60	Plasma stool aden infect
	37	M	STRIM	<1	<1•	c.7C>T; p.Q3Ter (homozygous)	PEG-ADA*(for 7 mo)*	34	-
	38	M	STRIM	<1	1•	c.955_959delGAAGA; p.E319Gfs*3 (homozygous)	PEG-ADA*(for 5 mo)*	60	Blood CM positiv
	39	M	STRIM	<1	2	c.58 G >A p.G20R; c.956_960delGAAGA	PEG-ADA*(for 7 mo)*	60	MRS coloniz
	40	F	STRIM	<1	4	c.646G>A; p.G216R c.778G>A; p.E260K	PEG-ADA*(for 11 mo)*	60	Klebsi bactere
	41	M	STRIM	<1	1•	c.678+1G>T; p.? (homozygous)	PEG-ADA*(for 9 mo)*	58	Alveo proteinos infection, hyperte
C	Patient no.	Sex	Group of treatment	Age at onset*(month)*	Age at diagnosis*(month)*	ADA gene mutation	Previous treatment*(month duration)*	ERT dose*(UI/ kg/ week)*	Relevant histo
	42	M	mPB-HE	25	47	c.529 G>A; c.1048delCTCTT	PEG-ADA*(for 78 mo)*	36	Charcot Tooth ty

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A	Patient no. §	Sex	Group of treatment	Age at onset *(month)*	Age at diagnosis *(month)*	ADA gene mutation	Previous treatment *(month duration)*	ERT dose *(UI/kg/ week)*	Relevant histo
	43	**F**	**mPB-HE**	**12**	**45**	**c.965T>C; c.22G>A**	**PEG-ADA***(for 10 mo)*	**18**	-

	Total patients	CDP+NPP	STRIM	P-value
Sex M, n (%)	41	13 (59.09%)	10 (52.63%)	0.7582
Age at diagnosis (months), median [IQR]	41	2.5 [1;5]	2 [1;7]	0.7919
ERT duration (months), median [IQR]	41	12.2 [6.53;23.33]	8.15 [5.75;13.42]	0.3810
Age at GT (months), median [IQR]	41	20 [13;29]	11 [9;22.5]	0.1133
Time between GT and diagnosis (months), median [IQR]	41	16 [8.75;26.25]	9 [7;14]	0.0629
CD34+ cells/Kg infused, median [IQR]	41	9.23 [6.44;11.3]	11.6 [7.08;12.8]	0.2048
VCN, median [IQR]	28	1.1 [0.5;1.6]	1.7 [1.5;2.05]	0.0135
Total AUC, median [IQR]	39	19589.5 [17704;26568.5]	21953 [20255;24303.5]	0.1494

Extended Data Table 3

Vaccinations response (Number of Patients responding / Patients vaccinated)		
	CDP+NPP(n)	STRIM (n)
Hepatitis B	9/13	8/10
Tetanus toxoid	13/13	8/11
Pertussis	12/13	6/10
Measles	9/9	2/3
Mumps	9/10	2/3
Rubella	10/10	2/3
Pneumococcus	12/12	7/11
Patients with native pathogens' infections (n)		
	CDP+NPP(n)	STRIM (n)
Chickenpox	7	1
Measles	1	0
VZV reactivation	0	1
Epstein-Barr-Virus primary infection	1*	1
Epstein-Barr-Virus reactivation	1	0
Haemophilus influenzae	3*	0

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Extended Data Tab 4

Event	F-U pest GT	Deration, days	Outcome	Maximun Toxicity Grade
CDP+NPP patients[A]				
T cell lymphoid leukemia	4 years 8 months	253	resolved	3
Measles	1 year 4 months	14	resolved	3
Abdominal pain	7 years 10 months	4	resolved	3
Piresia	7 years 10 months	4	resolved	3
Votting	7 years 10 months	4	resolved	3
Acute gastroenteritis	9 years	< 30	resolved	n.a.
Weight loss	9 years 1 month	12	resolved	3
Septic arthritis of left hip	10 years 7 months	21	resolved	3
Salmonellosis	13 years 9 months	< 30	resolved	3
Urinary tract infection	15 years	3	resolved	1
Urinary tract infection	15 years 1 month	3	resolved	2
STRIM patients				
Staphylococcus epidemida CVC infection	2 months	9	resolved	3
Sepocis	3.2 months	13	resolved	3
Gastroenteritis	3.5 months	5	resolved	3
Lack of efficacy	3.6 months	n.a.	not resolved	2
Dianboca	3.8 months	2	resolved	1
Gastroenteritis	5 months	8	resolved	2
Lactose inhance	22 months	11	resolved	2
Macrophage activation syndrome post chickenpox vaccination	26 months	541	resolved	3
Febrile gastroenters	27 months	2	resolved	2
Varicella-Zoster reactivation	3 years	14	resolved	2
Hemolytic anemia	2 years 5 months	37	resolved	3
Hemolytic anemia	2 years 8 months	42	resolved	3
Acute respiratory infection	2 years 8 months	13	resolved	3
Staphylococcus hominis MDR CVC infection	2 months	7	resolved	3
Upper respiratory tract infection	5 months	5	resolved	3
Multiscritive Staphylococcus epidermidis CVC infection	3 months	7	resolved	3
Upper respiratory tract infection	4 months	5	resolved	3
Fever	13 months	3	resolved	3
Hacrnalytic anemia	5 months		ongoing	3
Coral verster author recpies	2 months	13	resolved	3
Campylobacter and Clostridium stools infection	7 months	36	resolved	3

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Event	F-U pest GT	Deration, days	Outcome	Maximun Toxicity Grade
Porth a cath infection	1 months	15	resolved	4
mPH-HE patients				
Phlebias	3 years	10	resolved	3
CMV infection	3 years 4 months	15	resolved	3
Pneumonia	3 years 8 months	3	resolved	3

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Affiliations

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We thank the patients and families who have been treated both in the experimental and approved phases and those who are currently followed-up in the Strimvelis Registry, all the primary physicians from countries worldwide who referred patients and continued their follow-up with great commitment.

Availability of biological material

All patients' material is subjected to informed consent. All requests for biological material will be promptly reviewed by the corresponding author and forwarded to Fondazione Telethon (the sponsor and Strimvelis license holder) to verify if the request is subject to any intellectual property or confidentiality obligations.

Data Availability

The authors declare that data supporting the findings of this study are available within the paper and supplementary files. Because of the small number of participants in the studies and potential for identification, individual patient data beyond what is included in the manuscript will not be available. Requests of additional information will be forwarded by the corresponding author to Fondazione Telethon the holder of Strimvelis marketing license and current sponsor of the clinical trials.

Code Availability

All the statistical analyses were well detailed in the Statistical methods Section or in the Supplementary statistical methods and were performed with R 3.6.2 (https://www.R-project.org/). The R packages used were: stats 3.6.2; quantreg 5.85 for median quantile regression; survival 3.2-3, prodlim 2019.11.13 and cmprsk 2.2-10 for survival analysis; nlme 3.1-142 and phia 0.2-1 for mixed-effects model analysis.

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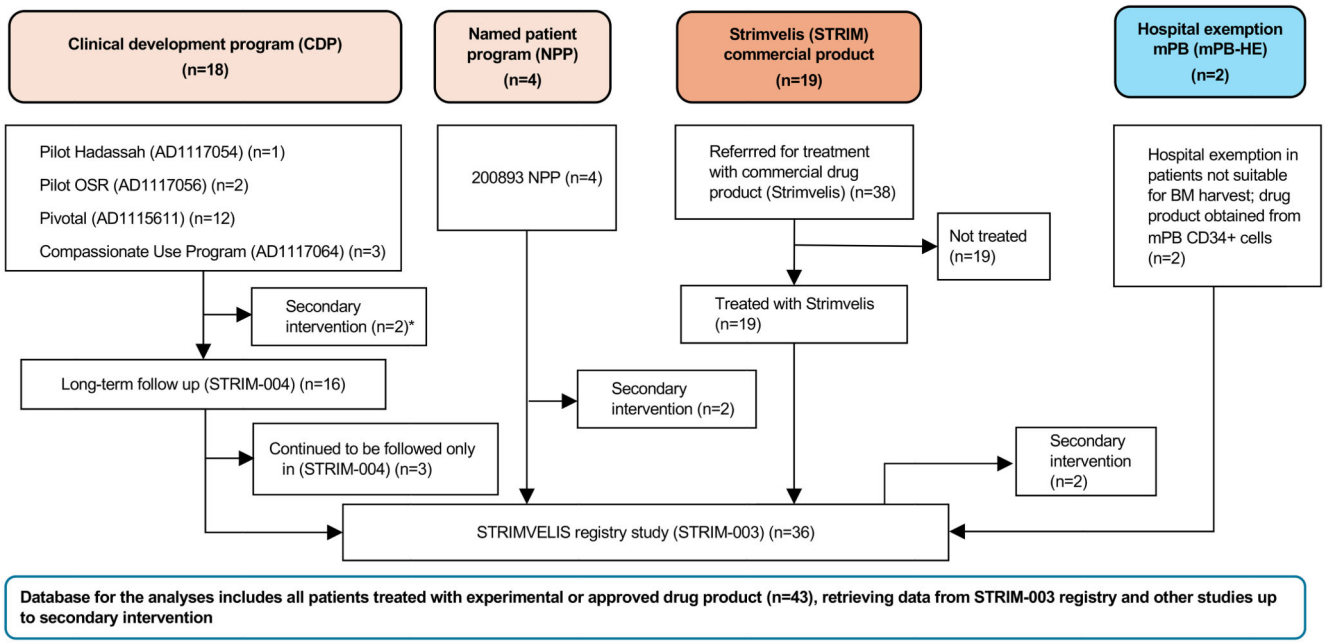


Figure 1. Flow diagram of patients treated γ-retroviral vector gene therapy included in the analyses

Data are from all patients treated with Strimvelis (experimental or approved) from 2000 to 09/2022, with a data cut off for analyses at 12/2022. Data were censored after secondary intervention (>3 months on PEG-ADA or allogeneic transplantation). Study AD1115611 is entitled "ADA gene transfer into hematopoietic stem/progenitor cells for the treatment of ADA SCID (NCT00598481)" and amended to include all subjects in long term follow up treated with the experimental drug product (STRIM-004). STRIM-003 is entitled "Adenosine Deaminase Severe Combined Immunodeficiency (ADA-SCID) Registry for Patients Treated With Strimvelis (Previously GSK2696273) Gene Therapy: Long-Term Prospective, Non-Interventional Follow-up of Safety and Effectiveness (NCT03478670). For patients not enrolled in the STRIM003 registry, data were retrieved from long-term follow up study or initial studies/program before intervention. One patient is described only in aggregated analyses due to data block requested by family.

*An additional patient had a secondary intervention (see Extended Data Table 2); PEG-ADA was started, but continued to be followed due to the persistence of gene corrected cells; data were censored for the analyses 3 months after PEG-ADA start.

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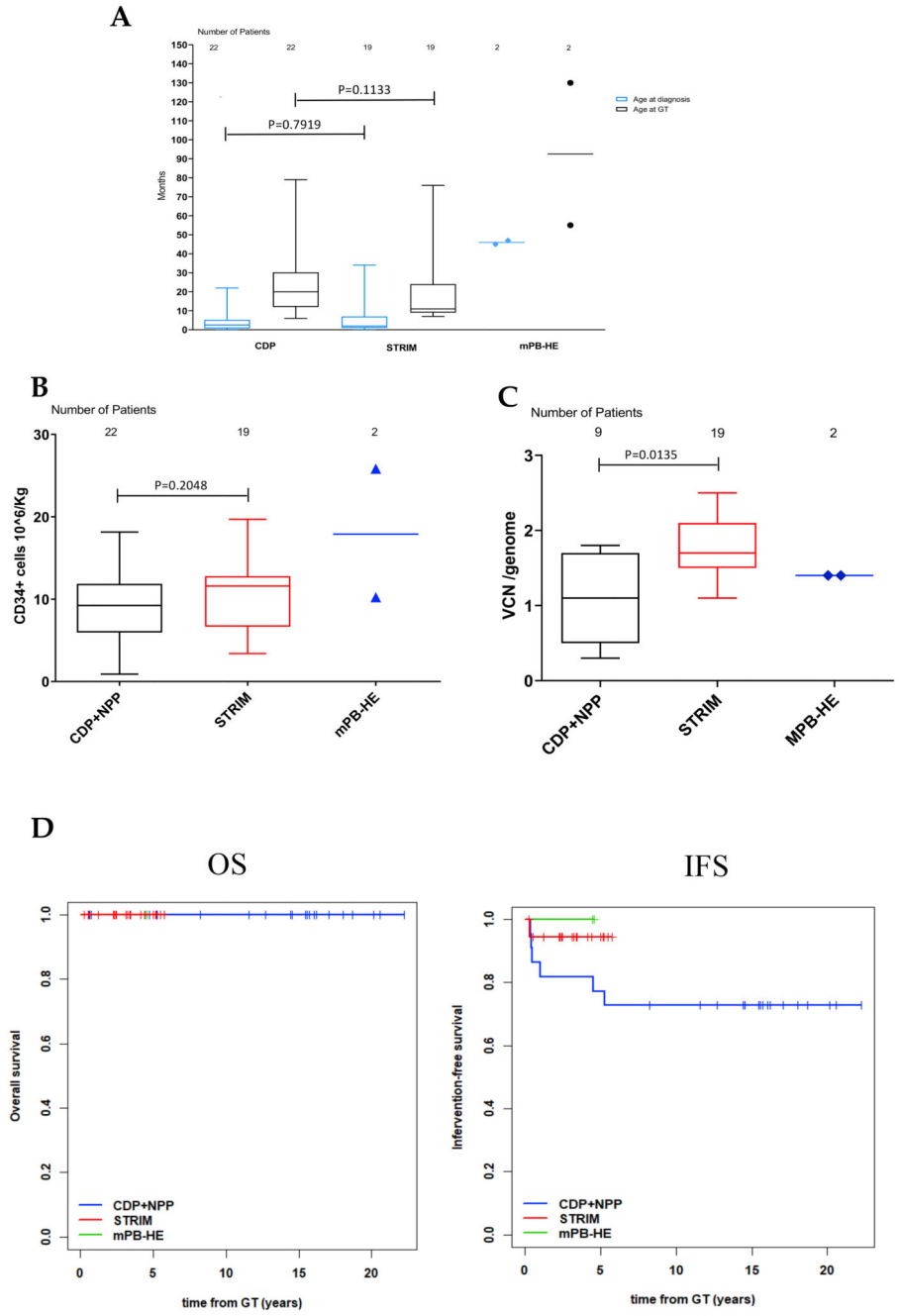


Figure 2. Patients' and γ-retroviral vector gene therapy drug product's characteristics, overall survival and intervention free survival after treatment.

(A) Age at diagnosis and GT (months). (B) Cell dose of CD34+ cells ($\times 10^6$ per kg). (C) Vector copy number (VCN) per genome in the drug product. CDP+NPP data are shown for patients (n=9) in whom the VCN analytical test was the same as the one currently employed for the STRIM cohort. In the plots, box and whiskers display the median, the first and the third quartile and the minimum and the maximum of the data. Comparison of numerical variables between groups in panels A, B and C was performed with Mann Whitney test (see Statistical Methods). (D) Kaplan–Meier curves showing OS for the entire cohort

(n=36) of ADA SCID patients treated pre- (CDP+NPP) and post-approval (Strimvelis), and IFS for CDP+NPP (in blue, n=22) and Strimvelis (in red, n=19). OS: Overall Survival; IFS: Intervention Free Survival, as no need of PEG-ADA >3 months or rescue allogeneic hematopoietic stem cell transplantation.

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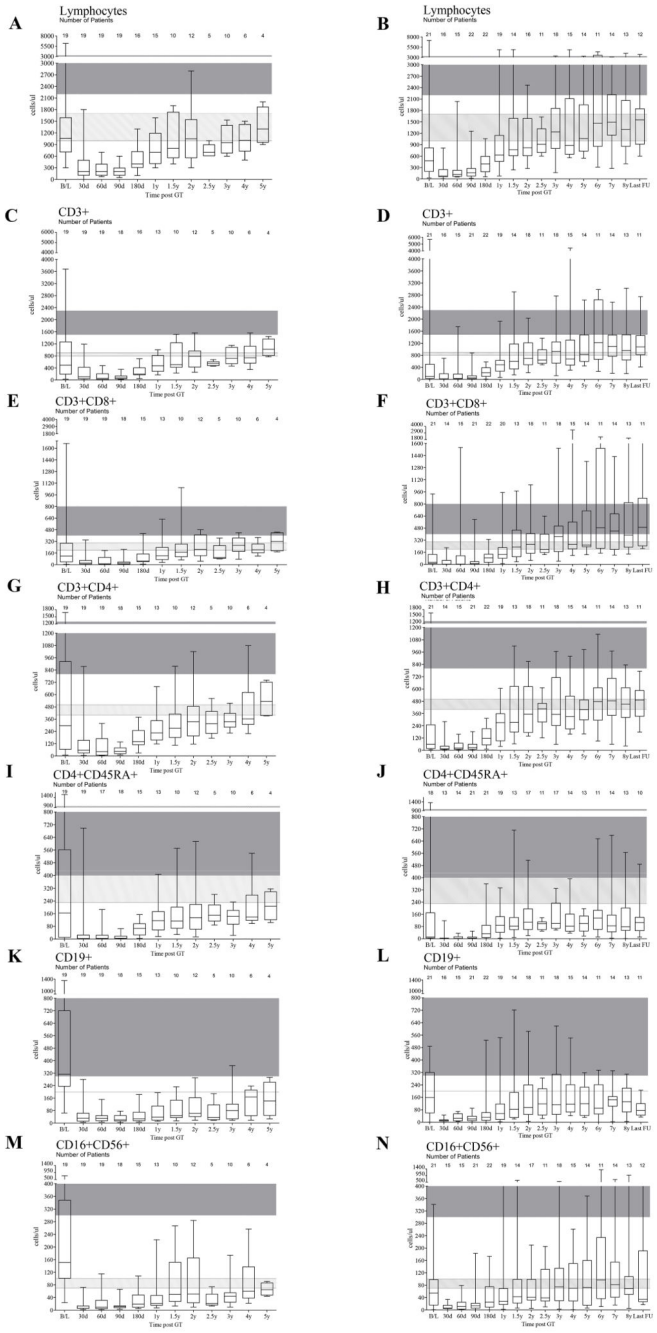


Figure 3. Immune reconstitution after gene therapy.

Absolute cell counts (cells/uL) of lymphocytes, CD3+, CD3+CD8+, CD3+CD4+, CD4+CD45RA+, CD19+, CD16+CD56+ in peripheral blood in STRIM (A, C, E, G, I, K, M) and CDP+NPP (B, D, F, H, J, L, N) patients are shown in the graphs. In the plots, box and whiskers display the median, the first and the third quartile and the minimum and the maximum of the data. For CDP+NPP population, the last available F-U after year 8 is reported The shaded dark and light grey regions represent median and fifth percentile values, respectively, in normal children. The top edges correspond to levels in children ages 2 to 5

years; bottom edges correspond to levels in children ages 10 to 16 years. Values for children ages 5 to 10 typically fall within the shaded areas.

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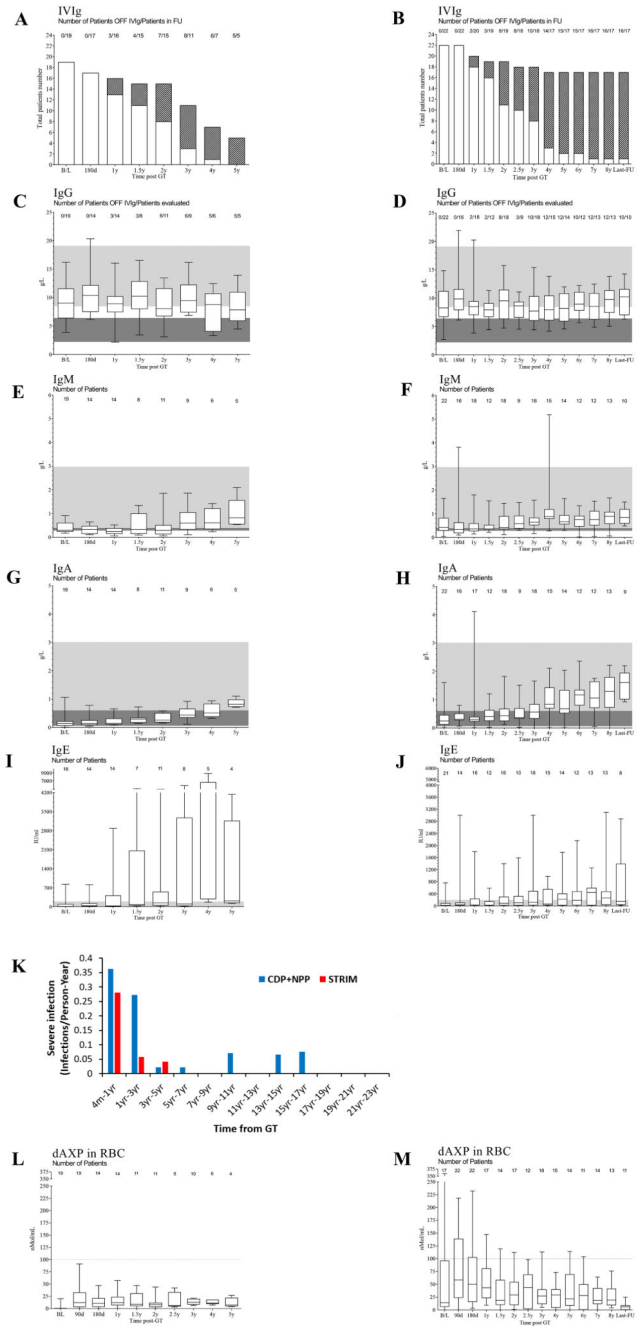


Figure 4. Humoral compartment restoration, incidence of infections and metabolic detoxification after gene therapy.

Patients off-Ig supplementation in the dark bars and still on-Ig in the light bars are reported at baseline and at subsequent timepoints after gene therapy in STRIM (A) and CDP+NPP (B) cohort, respectively. In (C) and (D), IgG levels were evaluated at baseline and at various timepoints after gene therapy in the STRIM and CDP+NPP groups. Patients off-Ig/patients evaluated are reported at each timepoint. Serum IgM (E, F) and IgA (G, H) levels. The shaded dark and light grey regions represent the fifth and ninety-fifth percentile values, respectively, in normal children aged 4 to 6 months and 12 to 16 years. Top edges

correspond to levels in children ages 12 to 16 years; bottom edges correspond to levels in children ages 4 to 6 months38. Top edges of the light grey region represent the ninety-fifth percentile in normal children aged 14 years and the bottom edges the ninety-fifth percentile in the ones aged 6 months38. Incidence of severe infections in the CDP+NPP (blue bars) and STRIM (red bars) cohorts (K). Severe infections in the 3 months immediately following GT were not included in the analysis. RBC dAXP levels measured in peripheral blood in the STRIM (L) and CDP+NPP (M) cohort. Dashed line indicates the lower reference value of dAXP for patients undergone successful hematopoietic stem cell transplantation (≤100 nmol/mL). In the plots, box and whiskers display the median, the first and the third quartile and the minimum and the maximum of the data. GT: gene therapy; Ig: intravenous immunoglobulins. dAXP: deoxyadenosine nucleotides; RBC: red blood cells.

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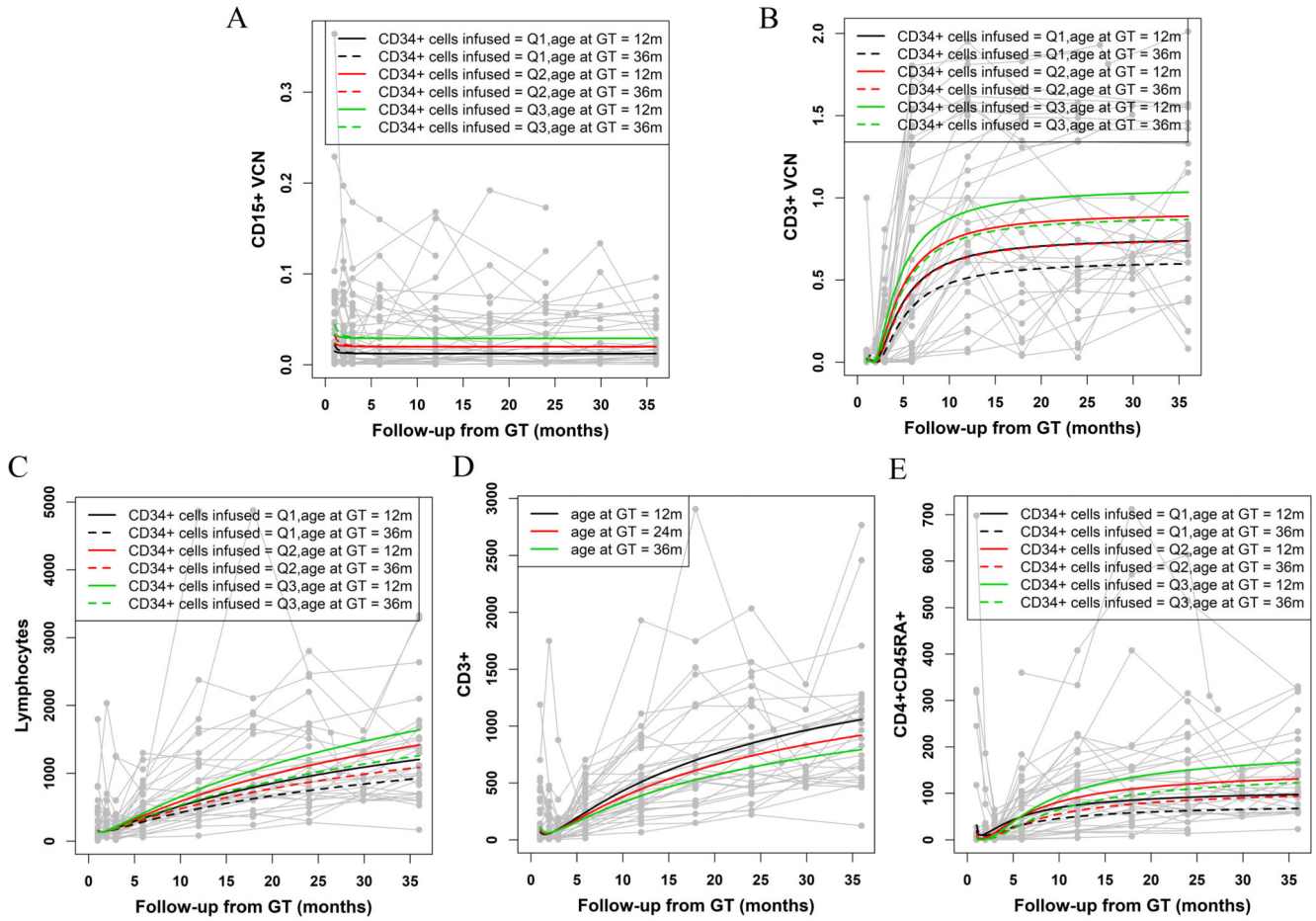


Figure 5. Longitudinal analysis of gene corrected cells, lymphocytes, CD3+ cells, CD4+CD45RA+ with respect to age at gene therapy and CD34+ cells infused.

The longitudinal trends were estimated by using mixed-effects models with fractional polynomials due to their nonlinear shape. The possible dependencies of the trend on age at gene therapy and CD34+ cells infused (as continuous variables) were tested within the model (see Supplementary Statistical Method and Suppl. Tables S7 A,B,C,D,E). Only data up to 36 months after gene therapy were used and data of both CDP+NPP and STRIM groups were considered together. The plots show the estimated curves for some specific values of the continuous covariates retained in the models (Q1, Q2 and Q3 denote the first, second and third observed quartiles of the variable, which are Q1=6.66, Q2=9.9, Q3=12.8 for CD34+ cells/Kg). Estimated longitudinal trend of CD15+ VCN (A), CD3+ VCN (B), lymphocytes (C) and CD3+ cells (D) and CD4+CD45RA+ naïve T-cells (E). GT: gene therapy; VCN: vector copy number.

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Table 1
Characteristics of ADA-SCID patients treated

	Total patients	Female	Male	Treatment period (years)	Alive at last FU	Median FU Years[§] (IQR)
CDP	18	6	12	2000-2011	18 (100%)	15.8 (12.7;18.1)
NPP	**4**	**2**	**2**	**2014-2016**	**4 (100%)**	**0.7; 8.3; 0.6; 5.3**
STRIM	19	9	10	2017-2022	19 (100%)	3.2 (2.3;5.0)
mPB-HE	**2**	**1**	**1**	**2018**	**2 (100%)**	**4.5; 4.5**
TOT	43	18	25	22	43 (100%)	5.0 (2.4;15.4)

Patients were censored for survival analyses at 3 month post-PEG-ADA initiation or allogeneic transplantation; they continued to be followed by local physician as per standard of care and are reported alive at current data cut off.

[§]Median FU on intervention-free pts; for groups with n<5, only individual data are provided (except for the Pt which withdrew consent to use of individual data).