Long-term and real-world safety and efficacy of retroviral gene therapy for adenosine deaminase deficiency

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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 analysis on long-term safety and efficacy data of 43 patients with ADA-SCID who received retroviral ex vivo bone marrow-derived hematopoietic stem cell 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 cutoff, all 43 patients were alive, with a median follow-up of 5.0 years (interquartile range 2.4–15.4) and 2 years intervention-free survival (no need for long-term enzyme replacement therapy or allogeneic hematopoietic stem cell transplantation) of 88% (95% confidence interval 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 premarketing cohort. One patient from the named patient program developed a T cell leukemia related to treatment 4.7 years after GT 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 $CD4^+$ $CD45RA^+$ naive T cells, whereas the cell dose positively influenced the final plateau of $CD15^+$ transduced cells. These long-term data suggest that the risk–benefit of GT in ADA remains favorable and warrant for continuing long-term safety monitoring. Clinical trial registration: NCT00598481, NCT03478670.

Severe combined immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency is an ultrarare genetic disease caused by accumulation of toxic purine degradation byproducts. Allogeneic hematopoietic stem cell transplantation (HSCT) has been the standard curative option for patients with ADA-SCID, providing long-term benefit with a single intervention. Outcome depends on donor availability with an overall survival (OS) >90% after matched sibling donor (MSD)/matched family donor (MFD) HSCT and improved over the years to >85% after matched unrelated donor

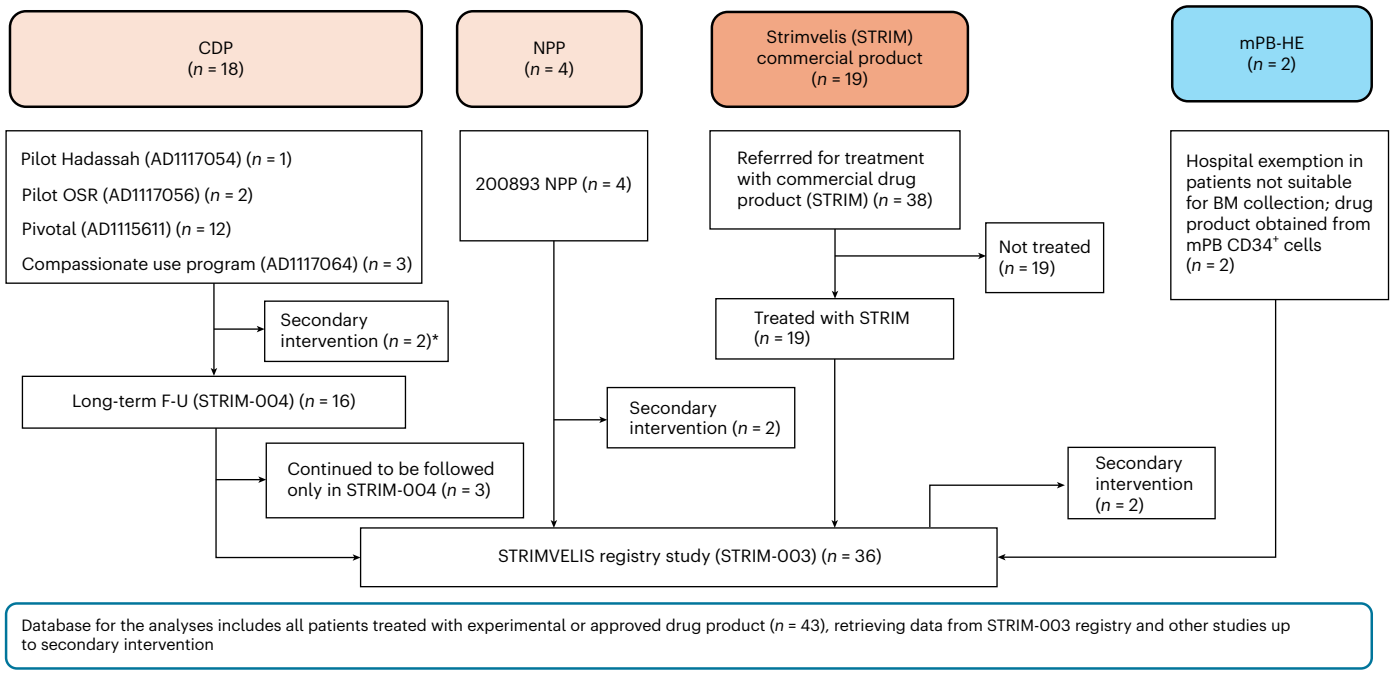

Fig. 1 | Flow diagram of patients treated γ-RV vector GT included in the analyses. Data are from all patients treated with STRIM (experimental or approved) from 2000 to September 2022, with a data cutoff for analyses at December 2022. Data were censored after secondary intervention (>3 months on PEG-ADA or allogeneic transplantation). Study AD1115611 is entitled '*ADA* gene transfer into HSPC for the treatment of ADA-SCID (NCT00598481)' and amended to include all patients in long-term F-U 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 F-U of safety and effectiveness (NCT03478670)'. For patients not enrolled in the STRIM-003 registry, data were retrieved from long-term F-U 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 (Extended Data Table 1); 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.

(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-to-long term, contributing to increased susceptibility to autoimmunity and lymphomas[9–11]. Gene therapy (GT) with autologous hematopoietic stem/progenitor cells (HSPC) engineered using a gammaretroviral (γ-RV) or lentiviral (LV) vector[12,13] has emerged as a treatment option for patients lacking a suitable MSD[11,14]. 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].

Ex vivo γ-RV HSPC-GT for the treatment of ADA-SCID (Strimvelis, STRIM) is a medicinal product authorized in the European Union (EU) in 2016 that 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]. STRIM 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 reimbursement, treatment access and logistics for referring and treating physicians, patients and caregivers.

In this Article, we report an unplanned analysis based on prespecified and exploratory endpoints on the long-term safety and efficacy of the clinical development program (CDP) and real-world experience following EU approval.

Results

Patients' population and access to the approved treatment

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

In the STRIM cohort, six Italian patients from four 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 (Supplementary Table 1). Five patients from outside the EU had costs covered through government or special funding (Supplementary Table 1). Time from referral to treatment was shorter in STRIM versus CDP + NPP (median 9 versus 16 months), depending on the time for stabilization with ERT and/or management of underlying diseases (Extended Data Table 2). Nineteen additional patients who were initially referred for STRIM were not treated due to lack of eligibility, lack of funding or different treatment choices (Supplementary Table 2).

Characteristics of patients and drug product

The characteristics of the 19 STRIM patients and patients not previously described[11] are reported in Extended Data Table 1. Median age at GT was 11 months (interquartile range (IQR) 9–22.5) for STRIM and 20 months (IQR 13–29) for CDP + NPP ($P = 0.1133$) cohorts, respectively (Fig. 2a, Extended Data Table 2 and Supplementary Fig. 1). STRIM patients received a median dose of 11.6×10^6 kg^{-1} (IQR 7.08–12.8) while CDP + NPP patients a median dose of 9.2×10^6 kg^{-1} (IQR 6.44–11.3) ($P = 0.2048$)

Table 1 | Characteristics of patients with ADA-SCID treated

	Total patients	Female	Male	Treatment period (years)	Alive at last FU	Median FU Years[a] (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)

CDP, clinical development program (n=18 patients). Pilot studies (SR-TIGET, Hadassah) (n=3 patients). Pivotal clinical study (n=12 patients). Compassionate use (n=3). NPP, named patient program (n=4 patients). One patient withdrew consent to use of individual data and is presented only in an aggregated manner in tables/graphs. NPP patients have been included in the long-term F-U analyses together with the CDP population. STRIM, Strimvelis commercial product (n=12). mPB-HE, patients who were not eligible for BM collection and received autologous CD34$^+$ cells from mPB under HE (n=2). Patients were censored for survival analyses at 3 months 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 cutoff. [a]Median FU on intervention-free patients; for groups with n<5, only individual data are provided (except for the patients who withdrew consent to use of individual data).

(Fig. 2b and Extended Data Table 2). 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. 2c). Median estimated total area under the curve (AUC) of busulfan exposure was 21,953 ng h^{-1} ml^{-1} for STRIM and 19,590 ng h^{-1} ml^{-1} for CDP + NPP (P = 0.1494) (Extended Data Table 2).

Survival and additional therapies

All patients (n = 43) are alive with a median follow-up (F-U) of 5.0 years (Table 1). Intervention-free survival (IFS) at 2 years is 88.0% (95% confidence interval (CI) 78.7% to 98.4%), not significantly different between the two cohorts (P = 0.1994). IFS at 2 years is 94.4% (95% CI 84.4% to 100%) for STRIM and 81.8% (95% CI 67.2% to 99.6%) for CDP + NPP (Fig. 2d). CDP + NPP show also IFS at both 5 and 10 years of 77.3% (95% CI 56.3% to 93.9%). All six patients in whom GT failed were put on ERT and five 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 an MUD and one from a haploidentical donor[7] with TCRαβ/CD19 depletion (a STRIM patient); the other two patients are currently on long-term ERT. An additional patient developed leukemia 4.7 years after GT and required a secondary intervention (TCRαβ/CD19-depleted haploidentical HSCT) without restarting ERT.

Hematological reconstitution

All patients experienced transient neutropenia (median nadir 200 mm^{-3} for both groups) from which they recovered (Supplementary Table S1). There was a tendency for longer median duration of grade IV neutropenia (absolute neutrophil count <500 mm^{-3}) in the STRIM cohort (median 30 days) as compared to CDP + NPP cohort (median 17.5 days) (P = 0.0205, adjusted P = 0.0615) (Supplementary Table S1), with a faster occurrence of neutrophil engraftment in the latter cohort (P = 0.0082, adjusted P = 0.0328; Extended Data Fig. 1a).

Considering the overall population, a significant correlation of total AUC was found with number of days of grade IV neutropenia (ρ (95% CI): 0.3701 (0.0618 to 0.6139) and P = 0.0204) but not with nadir of neutropenia (ρ (95% CI): −0.2454 (−0.5207 to 0.0759) and P = 0.1320).

Median nadir of platelets was 92 × 10^9 l^{-1} (range 31–210) for STRIM cohort and 90 × 10^9 l^{-1} (range 14–314) for CDP + NPP cohort. None of the STRIM patients and 5/22 CDP + NPP patients experienced grade IV thrombocytopenia (platelets <25 × 10^9 l^{-1}).

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 was 1.38 (IQR 0.77–1.61) for STRIM and 0.77 (IQR 0.62–1.0) for CDP + NPP, respectively (Extended Data Fig. 2a,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. 2c–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. 2g,h). Median VCN at 1 year was 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) versus CDP + NPP (VCN 0.01) and estimated VCN CD3$^+$ (P = 0.0001) for STRIM (VCN 1.18) versus CDP + NPP (VCN 0.61) (Extended Data Fig. 3a,b and Supplementary Table S4a,b).

In the CDP + NPP group we observed long-term persistence of gene-corrected cells in multiple PB and BM cell lineages (Extended Data Fig. 2b,d,f,h) with the latest F-U ranging from 7 to 15.4 years.

Finally, by estimating an 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. 1b,c and Supplementary Table S5a,b) but not VCN in CD15$^+$ cells (Supplementary Table S5c), suggesting that the expansion of corrected lymphocytes contributed to hematological 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 rise between 3 and 6 months after GT (Fig. 3a–h).

The two cohorts showed no significant differences in the final plateau of the trend of lymphocyte, T cell and naive T cell reconstitution, through an NLME model analysis of data up to 36 months after GT (Extended Data Fig. 3c,d,e and Supplementary Table S4c–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 (Fig. 3c–h and Extended Data Fig. 4). In the CDP + NPP cohort, T cell counts progressively normalized over time in the majority of patients, while lymphocyte counts normalized 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. 3d,f,h). Starting from 6 months post-GT, a steady increase of CD4$^+$ CD45RA$^+$ naive T cells was observed in all patients (Fig. 3i,j) without statistical difference between the two treatment groups by NLME model analysis (Extended Data Fig. 3e and Supplementary Table S4e). Naive T cells persisted long term in the CDP + NPP population, and active thymopoiesis was detected by T cell receptor excision circle (TRECs) analyses in the majority of patients (Supplementary Fig. 2).

Sustained and persistent in vitro T cell function (including phytohemagglutinin 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 adjusted P < 0.0001; STRIM adjusted P = 0.0026) (Supplementary Table S6b).

Natural killer cells and B cells showed similar behavior in the early phase after treatment in the two cohorts (Fig. 3k–n). In the long-term F-U, median B cells remained low, while natural killer 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. 4a,b). At last F-U, all STRIM patients (5/5) and nearly all CDP + NPP patients (16/17)

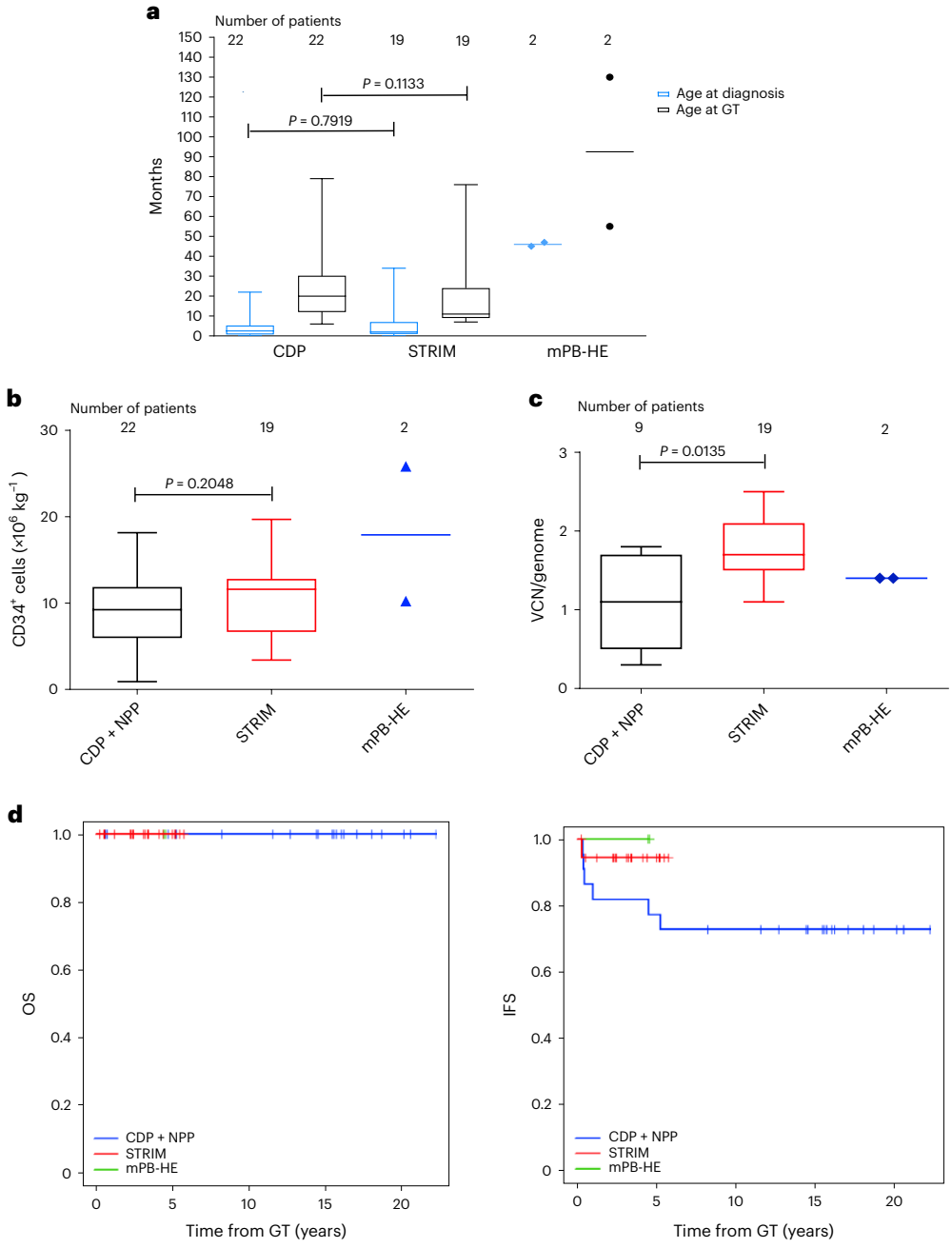

Fig. 2 | Patients' and γ-RV vector GT drug product's characteristics, OS and IFS after treatment. a, Age at diagnosis and GT (months). **b**, Cell dose of $CD34^+$ cells ($\times 10^6$ kg^{-1}). **c**, VCN/genome in the drug product. CDP + NPP data are shown for patients ($n = 9$) in whom the VCN analytical 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 **a**–**c** was performed with Mann–Whitney test (see 'Statistical methods'). **d**, Kaplan–Meier curves showing OS for the entire cohort ($n = 36$) of patients with ADA-SCID treated pre- (CDP + NPP) and post-approval (STRIM), and IFS for CDP + NPP (in blue, $n = 22$) and STRIM (in red, $n = 19$). IFS: intervention-free survival, as no need of PEG-ADA >3 months or rescue allogeneic HSCT.

were free from Ig supplementation (Fig. 4b). Only one patient was still receiving Ig substitution due to anti-CD20 administration for the treatment of autoimmune hemolytic anemia. In patients who discontinued Ig, median IgG levels remained within normal range (Fig. 4c,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. 4e–h). Median IgE levels increased over baseline after GT both in early and late F-U (Fig. 4i,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 toward 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. 4k).

Deoxyadenosine nucleotide (dAXP) concentrations in PB red blood cells (RBCs) progressively decreased both in STRIM and CDP + NPP patients to levels of <100 nmol ml^{-1} and remained low during the whole time of observation (Fig. 4l,m). The estimated NLME model of data up to 36 months after GT showed a similar final plateau in both groups (Extended Data Fig. 3f and Supplementary Table S4f).

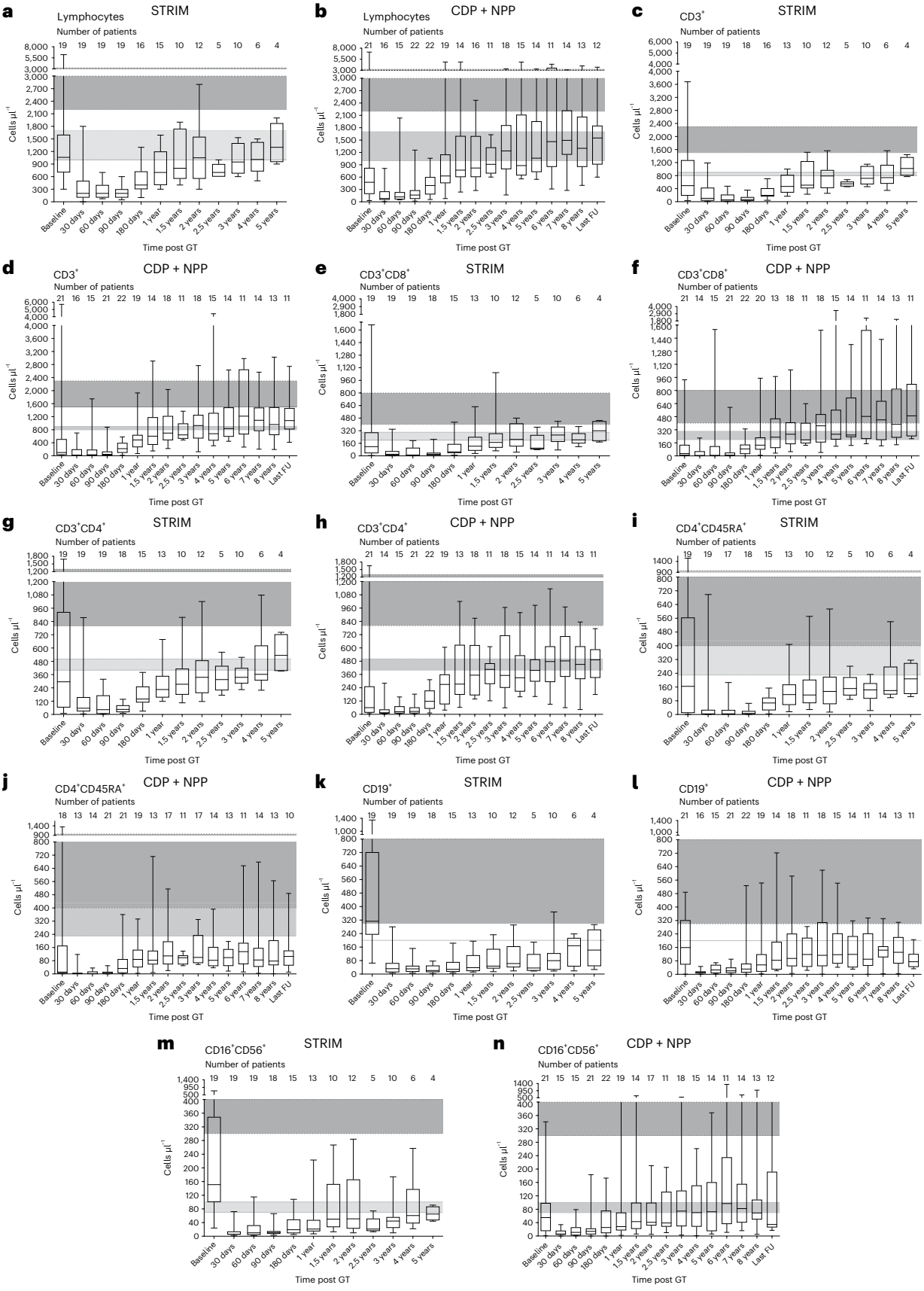

Fig. 3 | Immune reconstitution after GT. a–**n**, Absolute cell counts (cells µl^{-1}) of lymphocytes (**a**, **b**), CD3$^+$ (**c**, **d**), CD3$^+$CD8$^+$ (**e**, **f**), CD3$^+$CD4$^+$ (**g**, **h**), CD4$^+$CD45RA$^+$ (**i**, **j**), CD19$^+$ (**k**, **l**), CD16$^+$CD56$^+$ (**m**, **n**) in PB in STRIM (**a**, **c**, **e**, **g**, **i**, **k** and **m**) and CDP + NPP (**b**, **d**, **f**, **h**, **j**, **l** and **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-gray regions represent median and fifth percentile values, respectively, in normal children. The top edges correspond to levels in children ages 2–5 years; bottom edges correspond to levels in children ages 10–16 years. Values for children ages 5–10 typically fall within the shaded areas.

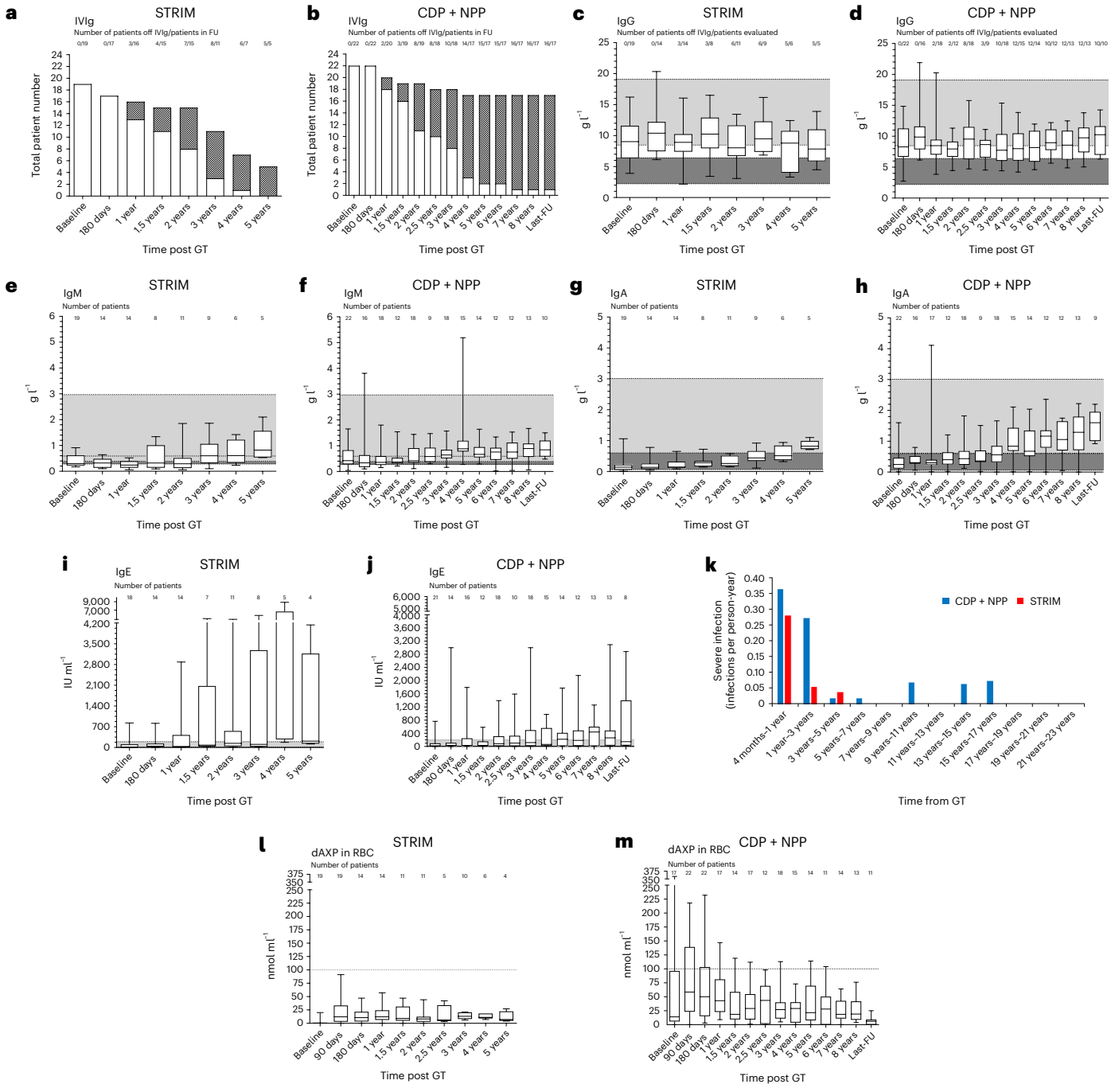

Fig. 4 | Humoral compartment restoration, incidence of infections and metabolic detoxification after GT. a,**b**, Patients off-Ig supplementation in the dark bars and still on-Ig in the light bars are reported at baseline and at subsequent time points after GT in STRIM (**a**) and CDP + NPP (**b**) cohort, respectively. **c**,**d**, IgG levels were evaluated at baseline and at various time points after GT in the STRIM (**c**) and CDP + NPP (**d**) groups. Patients off-Ig/patients evaluated are reported at each time point. **e**–**h**, Serum IgM (**e** and **f**) and IgA (**g** and **h**) and IgE (**i** and **j**) levels. The shaded dark- and light-gray regions represent the 5th and 95th percentile values, respectively, in normal children aged 4–6 months and 12–16 years. Top edges correspond to levels in children ages 12–16 years; bottom edges correspond to levels in children ages 4–6 months[38]. Top edges of the light-gray region represent the 95th percentile in normal children aged 14 years, and the bottom edges the 95th percentile in the ones aged 6 months[38]. **k**, Incidence of severe infections in the CDP + NPP (blue bars) and STRIM (red bars) cohorts. Severe infections in the 3 months immediately following GT were not included in the analysis. **l**,**m**, RBC dAXP levels measured in PB in the STRIM (**l**) and CDP + NPP (**m**) cohort. Dashed line indicates the lower reference value of dAXP for patients undergone successful HSCT (≤100 nmol ml^{-1}). In the plots, box and whiskers display the median, the first and the third quartile and the minimum and the maximum of the data. IVIg, intravenous immunoglobulins; Ig, immunoglobulins.

Engraftment and reconstitution according to treatment age

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 years and >2.5 years. The dose of BM transduced CD34$^+$ cells was significantly higher in the younger cohort considering all patients (P = 0.0156) (Supplementary Fig. 3a,b). Patients treated ≤2.5 years of age showed a trend toward a higher engraftment of gene-modified CD15$^+$ and CD3$^+$ cells (Supplementary Fig. 4a,b), had a more profound lymphopenia during the early phases post-GT but reached in the long term higher lymphocyte, T cell, naive T cell and B cell counts than older patients (Supplementary Fig. 4c–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 (Supplementary Table S3a). Finally, a higher VCN in the drug product showed a tendency for lower dAXP and higher ADA activity in mononuclear cells (Supplementary Table S3f,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^{-1} infused whereas patients treated younger achieved a higher asymptote for transduced $CD3^+$ cells (Fig. 5a,b and Supplementary Table S7a,b). On the other hand, the longitudinal trend of lymphocyte and $CD4^+$ $CD45RA^+$ naive cells count was significantly affected by the age at GT and dose of medicinal product, while $CD3^+$ cell counts only by the age (Fig. 5c–e and Supplementary Table S7c–e).

Outcome in patients treated with mPB-derived $CD34^+$ cell GT

Two patients with a late-onset phenotype referred for STRIM treatment displayed insufficient BM $CD34^+$ cell content and were eventually treated at 4.6 and 10.8 years of age under HE (Fig. 2a), using $CD34^+$ cells collected from mPB manufactured under the same transduction conditions of STRIM (mPB-HE). Patients mobilized an adequate number of $CD34^+$ cells after G-CSF and plerixafor and received a dose of 10×10^6 kg^{-1} and 26×10^6 kg^{-1} of drug product, respectively (Fig. 2b). VCN was in the range of BM-derived product (Fig. 2c). The levels of gene-corrected cells were comparable to patients treated with BM-derived $CD34^+$ cells (Extended Data Fig. 6a–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. 6e–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 are already reported[16]), of which 30/52 (58%) were of of infectious origin. All SAE eventually resolved, and none was fatal.

In the STRIM population, 14 patients out of 19 (74%) reported 22 nonfatal SAEs post-treatment, maximum grade 3. Among these, 14 (64%) were of infectious origin, with none being life-threatening and all having completely resolved (Fig. 4k and Extended Data Table 4). 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 three SAEs, two of which were infectious, all resolved (Extended Data Table 4).

One patient experienced a hypovolemic shock and refractory metabolic acidosis subsequent to BM collection, complicated by multi-organ failure, and did not receive STRIM. This was the only event of hypovolemic shock experienced in our center in 20 years of BM collections[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 F-U, most TCR Vβ families were represented at normal frequency, and a minority was represented below normal, or rarely increased, without clinical impact (Supplementary 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 occurring 4.7 years after GT in a patient treated with autologous HSPC-GT under NPP. Retroviral insertion site (RIS) analysis on the leukemia sample identified a single dominant clone with an insertion near LIM-domain only 2 (LMO2) proto-oncogene (full insertion site analysis in manuscript in preparation). 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 F-U of patients with ADA-SCID treated in the context of clinical development and NPP along with the post-marketing experience with STRIM, 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 premarketing experience. No difference in the kinetics and levels of immune reconstitution, rate of severe infections and systemic detoxification was 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 were eventually treated with STRIM 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 toward 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[12,24].

The data in the overall population with a median F-U of 5.0 years confirm the durable efficacy of γ-RV vector GT. Five out of six failure cases occurred within 2 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 individuals 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 naive 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 ten patients with ADA-SCID treated with a different γ-RV 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 patient with ADA-SCID is stabilized with ERT to prevent infections and organ damage[11]. The extension of the successful 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 adverse events (AEs) in the STRIM population were related to disease background, busulfan conditioning or immune reconstitution, in line with data reported in the preapproval experience[16]. Autoimmune manifestations post-GT were previously observed and may be related to an immune dysregulation during the early immune reconstitution post-GT.

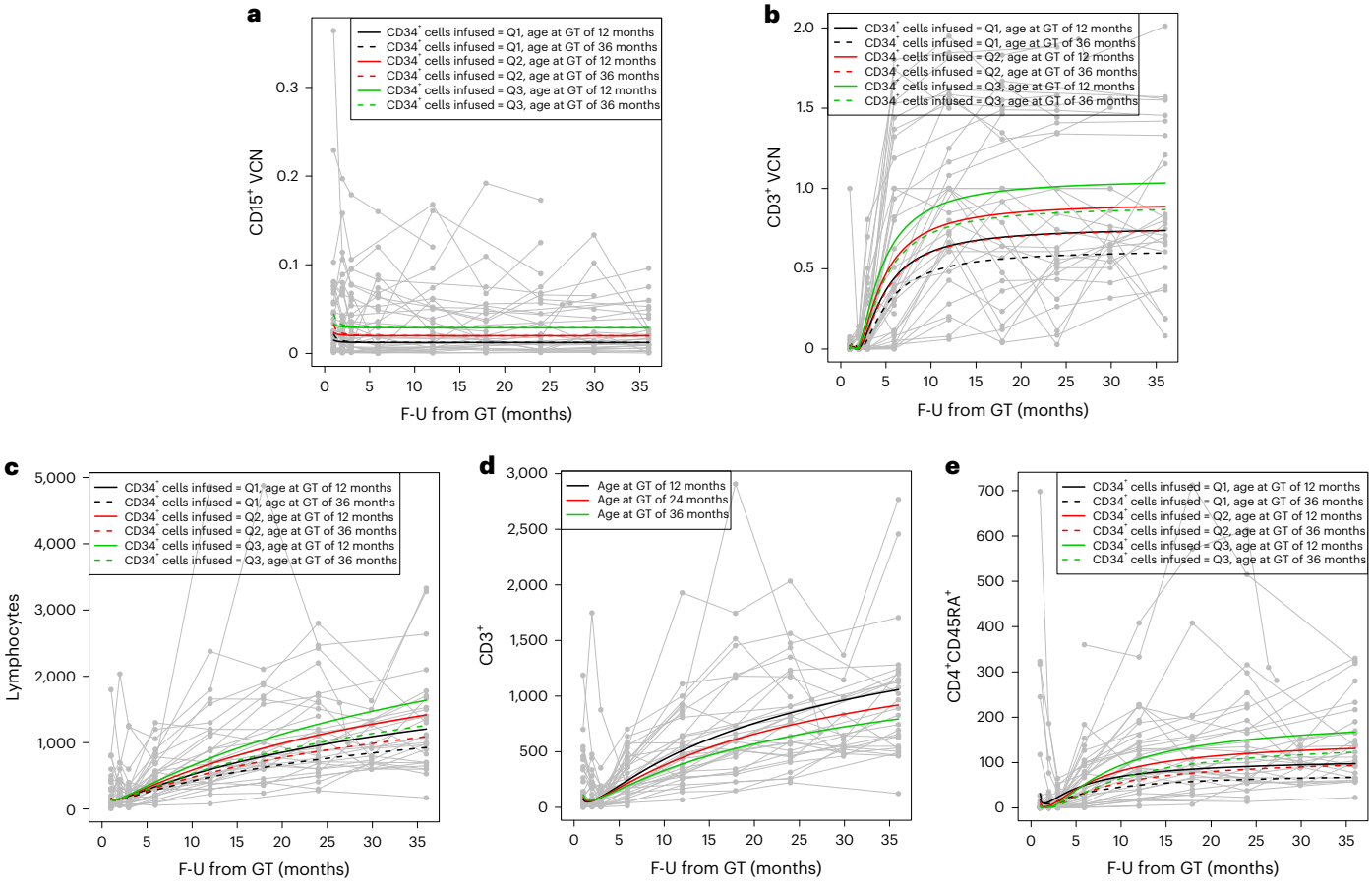

Fig. 5 | Longitudinal analysis of gene-corrected cells, lymphocytes, CD3$^+$ cells, CD4$^+$ CD45RA$^+$ with respect to age at GT and CD34$^+$ cells infused. **a**–**e**, The longitudinal trends were estimated by using mixed-effects models with fractional polynomials due to their nonlinear shape. The possible dependencies of the trend on age at GT and CD34$^+$ cells infused (as continuous variables) were tested within the model (Supplementary Statistical Methods and Supplementary Table S7a–e). Only data up to 36 months after GT 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 of 6.66, Q2 of 9.9 and Q3 of 12.8 for CD34$^+$ cells kg^{-1}). Estimated longitudinal trend of CD15$^+$ VCN (**a**), CD3$^+$ VCN (**b**), lymphocytes (**c**) and CD3$^+$ cells (**d**) and CD4$^+$ CD45RA$^+$ naive T cells (**e**).

Neutropenia is frequent in patients with ADA-SCID[33,34] 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 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 hematological 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 naive 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 kinetics 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 nonimmunological disease-related features (that is, neurological[36], psychiatric, metabolic and urological issues[37]) and compare it with other therapeutic approaches.

Following the single case of T cell acute lymphoblastic leukemia associated with retroviral insertion in one of the patients with ADA-SCID, 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-authorization measures.

LV vectors have emerged in the past decade as a safe and effective platform for the treatment of genetic disorders including primary immunodeficiencies[12]. A recent study[13] reported results in 50 patients with ADA-SCID treated with LV-based GT with excellent event-free survival and robust immune reconstitution at 24–36 months of F-U. 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 F-U, as well as for the use of mPB and cryopreserved product in a large fraction of the patients.

Main limitations of our study include the use of nonplanned 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 that includes only those with access to STRIM with respect to those enrolled during clinical development that had no restrictions in country of origin.

In the absence of an HLA-identical related donor, which remains the standard of care, HSCT from MUD or haploidentical donor is the curative therapeutic option to be considered in case GT is not available or unsuccessful[1–8]. Indeed, worldwide experience with ADA-SCID GT using γ-RV[17,25] or LV[12] demonstrates 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-RV 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. The case of a patient who developed leukemia calls for long-term monitoring, which is continuing and will be crucial to assess the therapy safety profile.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-023-02789-4.

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Methods

Study design

We performed for the purpose of this manuscript a preliminary analyses of some of the prespecified endpoints of the long-term study protocol (NCT03478670), as well as additional post-hoc measures as detailed below:

Prespecified measures

1. OS (number and causes of death).
2. IFS (intervention is defined as HSCT or >3 months of ERT).
3. Number of subjects with the use of medications/treatments of interest (subjects requiring ERT, HSCT, radiotherapy or cytotoxic agents).
4. Immune reconstitution (absolute peripheral lymphocyte).
5. Immune reconstitution (absolute $CD3^+$ T cells).
6. Immune reconstitution (absolute $CD19^+$ B cell counts).
7. Immune reconstitution (T cell functions by mitogen assessment (phytohemagglutinin and anti-CD3)).
8. dAXP levels in RBCs for the measurement of systemic metabolite detoxification.
9. VCN measured in PB mononuclear cells.
10. Number of subjects with severe infections. Severe infection is defined as an infection requiring hospitalization or prolonging hospitalization.
11. Percentage of subjects with severe infections. Severe infection is defined as an infection requiring hospitalization or prolonging hospitalization.
12. Number of subjects with nonimmunological manifestations of ADA-SCID (subjects will be examined for hepatic steatosis, cognitive deficits, behavioral abnormalities including suspected or diagnosed attention-deficit/hyperactivity disorder, autism or hearing impairment).
    Only relevant clinical history concomitant diseases of all patients before GT treatment are reported.
13. Pediatric development and quality of life data (determination of attendance at school, if appropriate for age; whether the child is in an age-appropriate grade/class at school; whether the child requires special educational support (for example, dedicated tutor); participation in sports as desired by child; requirement for hearing aid(s); adequate response to childhood vaccinations; severity of impact of a child's health on the guardian's intended employment and Karnofsky/Lansky performance status).
    Only available responses to vaccinations are reported in the current manuscript.
14. Number of subjects with AEs of interest (AEs and SAEs related to medical or surgical procedures, AEs and SAEs related to conditioning, hypersensitivity, autoimmunity and oncogenesis).
    We are reporting here the SAE of the STRIM (post-marketing) population and the SAE of the CDP + NPP population that are in addition to the 39 already reported in a previous manuscript[16].
15. Data from RIS analysis and replication competent retrovirus (RCR) (RIS and RCR will be performed when malignancy is suspected or after a diagnosis of malignancy).

Patient 21 (who developed leukemia) underwent RIS analyses and RCR. Results are described elsewhere (Cesana et al., manuscript in preparation).

The NCT03478670 study protocol outcome measures not included in the present manuscript are the following:

1. Growth percentile in body height.
2. Growth percentile in body weight.
3. Length of hospital stay.
4. Scores for Pediatric Quality of Life Questionnaire (Peds-QL).
    The Peds-QL is a generic Health-Related Quality of Life (HR QoL) instrument designed specifically for a pediatric population. It captures the following domains: general health/activities, feelings/emotional, social functioning and school functioning. Higher scores indicate better quality of life for all domains of the Peds-QL. This modular instrument uses a 5-point scale: from 0 (never) to 4 (almost always). Items are reversed scored and linearly transformed to a 0–100 scale as follows: 0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0. Four dimensions (physical, emotional, social and school functioning) are scored.
5. Scores for Ages and Stages Questionnaire-3 (ASQ-3). The ASQ-3 includes a series of questions designed to assess five areas of development: communication, gross motor, fine motor, problem solving and personal social. The questions target behaviors that are appropriate for particular developmental milestones.
6. Number of subjects with abnormal clinical laboratory blood test results as a safety measure (biochemistry, hematology and thyroid stimulating hormone parameters were assessed). Number of subjects with fertility- and pregnancy-related outcomes (labor and delivery information, full term pregnancy, cesarean section, abortion, miscarriage, ectopic and stillbirth rates will be assessed; both male and female fertility issues will be analyzed).

The following analyses were conducted post-hoc:

1. The analysis of neutropenia: comparison of the duration of grade IV neutropenia between STRIM versus CDP + NPP cohorts, univariate analysis for assessing the influence of some baseline characteristics (ERT duration, age at GT, $CD34^+$ cells kg^{-1}, VCN in the product and total AUC), and correlation analysis of AUC busulfan with duration of grade IV neutropenia or nadir of neutropenia).
2. Descriptive and inferential comparison between STRIM versus CDP + NPP cohorts of nadir of neutrophils and G-CSF doses.
3. Analysis of the cumulative incidence of neutrophil engraftment: descriptive and inferential comparison between STRIM versus CDP + NPP cohorts, univariate analysis for assessing the influence of some baseline characteristics (ERT duration, age at GT, $CD34^+$ cells kg^{-1}, VCN in the product and total AUC).
4. Analysis of the cumulative incidence of lymphocyte normalization and $CD3^+$ normalization: descriptive comparison between STRIM versus CDP + NPP cohorts and univariate analysis for assessing the influence of some baseline characteristics (ERT duration, age at GT, $CD34^+$ cells kg^{-1}, VCN in the product and total AUC).
5. Descriptive longitudinal analysis of $CD3^+$ $CD8^+$, $CD3^+$ $CD4^+$ and $CD16^+$ $CD56^+$ cells.
6. Analyses of $CD4^+$ $CD45^+$ RA^+ cells: longitudinal descriptive and mixed-effects model analyses, univariate analysis at 2 years post GT for assessing the influence of some baseline characteristics (ERT duration, age at GT, $CD34^+$ cells kg^{-1}, VCN in the product and total AUC).
7. Univariate analysis of mononuclear cell ADA activity on PB at 2 years post GT, for assessing the influence of some baseline characteristics (ERT duration, age at GT, $CD34^+$ cells kg^{-1}, VCN in the product and total AUC).
8. Mixed-effects model analysis of ANC with respect to VCN $CD3^+$, VCN $CD15^+$ and lymphocytes, by using longitudinal data.
9. Descriptive longitudinal analysis of TREC.
10. Descriptive longitudinal analysis of intravenous Ig, IgG, IgM and IgE.
11. Descriptive longitudinal analysis of $CD15^+$, $CD3^+$ $CD4^+$, $CD4^+$ $CD34^+$ RA^+, $CD3^+$ $CD8^+$, $CD16^+$ $CD56^+$ cells according to age at treatment.
12. Descriptive longitudinal analysis of $CD3^+$ $CD8^+$, $CD3^+$ $CD4^+$, $CD4^+$ $CD45RA^+$, $CD16^+$ $CD56^+$ cells on PB of mPB-HE patients.

Patients

Patients were referred for treatment by local physicians or by parents. Characteristics of patients treated in the CDP ($n = 18$) including two pilot studies (Hadassah AD1117054; OSR AD1117056), a pivotal study (AD1115611) with a long-term F-U component (STRIM-004) and a Compassionate Use Program (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 1 June 2000, amended on 19 July 2011 with a long-term component F-U (subsequently named STRIM-004) and registered at www.clinicaltrials.gov as NCT00598481. Protocol NCT03478670 (STRIM registry, STRIM-003) was approved by the Ethics Committee of Ospedale San Raffaele on 19 January 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 January 2015) for mPB-HE patients and the Compassionate Use framework (per the Italian Ministerial Decree of 8 May 2003 now superseded by the Decree of 7 September 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).

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 allows the inclusion of individual-level data in the publication, which are limited to strictly necessary information.

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

Treatment and F-U

Central venous catheter placement, cryopreservation of BM back-up and preconditioning with low-dose busulfan (0.5 mg kg^{-1} intravenously on eight consecutive doses administered in 2 days, total dose 4 mg kg^{-1}) have been reported previously[15,17]. In CDP + NPP population, AUC was reduced if single AUC was above 4,000 ng ml^{-1} h^{-1} and total AUC was targeted to 19,200 ng ml^{-1} h^{-1} (range 19,200–22,400 ng ml^{-1} h^{-1}). In the STRIM population, to optimize the engraftment of the corrected CD34$^+$ cells, total AUC was targeted to the upper range 22,400 ng ml^{-1} h^{-1}. In STRIM patients ERT was discontinued at a median of 16 days (range 8–21 days) before GT, similarly to the historical population (median 18 days, range 5–18 days). CD34$^+$ cell purification from BM and transduction protocol are reported elsewhere[15,17].

AEs and SAEs were reported using Good Clinical Practice guidelines. AEs in the initial F-U 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 on the basis of clinical needs. VCN in cell subpopulations was used to assess engraftment. From 2000 to 2012, the frequency of transduced cells and VCN was determined on genomic DNA by quantitative polymerase chain reaction analysis for neomycin resistance vector sequences, normalized for DNA content[15]. Subsequently, the evaluation of VCN/genome was performed by digital droplet polymerase chain reaction technology analyzing the (long-term repeat) vector sequence (primer forward: 5′-GGCGCCAGTCTTCCGATA-3′; primer reverse: 5′-TGCAAACAGCAAGAGGCTTTATT-3′), normalized to a region of the human telomerase gene[17].

Lymphocyte ADA activity, RBC dAXP level 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 Vβ repertoire[41], PB smears, cytogenetic karyotype analysis, BM morphology and immunophenotype were assessed over time.

All patients are followed with at least annual visits for the initial 11 years and then at years 13 and 15, and F-U 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 and subsequently transferred to an electronic database by the Marketing Authorization Holder until the study closure in 2019. NCT03478670 study was approved on 19 January 2017 opened and new data were collected in an electronic Case Report Form.

Data listing used for tables and figures of the manuscript were provided by the Marketing Authorization Holder.

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

No sex or gender analysis was performed because ADA-SCID is an autosomal recessive ultrarare genetic disease and no differences in sex were 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 95% CI was computed by using the Fisher's transformation. OS 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 lymphocyte 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 log-rank test, while Gray's test for comparing cumulative incidence curves. The reverse Kaplan–Meier estimator was used for describing the F-U.

Univariate analyses were performed to assess the influence of some baseline characteristics (ERT duration, age at GT, CD34$^+$ cells kg^{-1}, 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 subdistribution hazard regression model for cumulative incidence, and linear regression for the values of biomarkers at 2 years of F-U (after an appropriate transformation of the outcome 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. 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 the following: 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.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data supporting the findings of this study are available within the paper and its 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 should be addressed to aiuti.alessandro@hsr.it and will be shared with Fondazione Telethon (the sponsor and Strimvelis license holder) R&D Director, to verify if the request is subject to any intellectual property or confidentiality obligations. Criteria for request evaluations will be scientific merit of the request/intellectual property restrictions/data transfer agreement. The timeline of response will be from 2 to 4 weeks.

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Author contributions

M.M. contributed to the study design, patients' F-U, data collection, interpretation and manuscript writing. F.B. contributed to the study design, patient F-U, 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. Gabaldo, A.C. and S.Z. contributed to data collection and regulatory applications. F.D., S.G., F.A.S., I. Monti, D.C. and F. Carlucci performed molecular, biochemical and immunological analysis and interpretation. F.F., F.T., V.C., V. Gallo, S.R., G.C., M.S., A.P. and M.E.B. contributed to patient F-U and data collection. C.F. and V. Garella contributed to data collection and analysis. P. Silvani 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., U.B., A.F., C.C., S.L., A.M., I. Meyts, D.M., L.D.N., F.P., M.P., C.S., P. Stepensky, A.T., M.R., Z.K., M. Galicchio, L.L., M.D., A.P.-N. and S.N.G. referred and followed patients for GT treatment and provided data. F. Ciceri 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' F-U, data collection and interpretation, and 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 and resolved, and the resolution documented in the literature.

Competing interests

The San Raffaele Telethon Institute for Gene Therapy (SR-Tiget) is a joint venture between the Telethon Foundation and Ospedale San Raffaele (OSR). GT for ADA-SCID was developed at SR-Tiget and licensed to GlaxoSmithKline (GSK) in 2010. The treatments under NPP and HE 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 transferred the authorization to Fondazione Telethon that became the holder in July 2023. The product, apart from the EU, is also currently approved in Iceland, Norway, Liechtenstein and the United Kingdom. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. A.A. receives funding from Fondazione Telethon for other research projects. A.A. was the PI of pilot, pivotal and long-term F-U study of SR-Tiget clinical trials of gene therapy for ADA-SCID. M.P.C. and M.M. are PI and deputy PI, respectively, of the Strimvelis Registry, RIS and RMMs studies. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-023-02789-4.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-023-02789-4.

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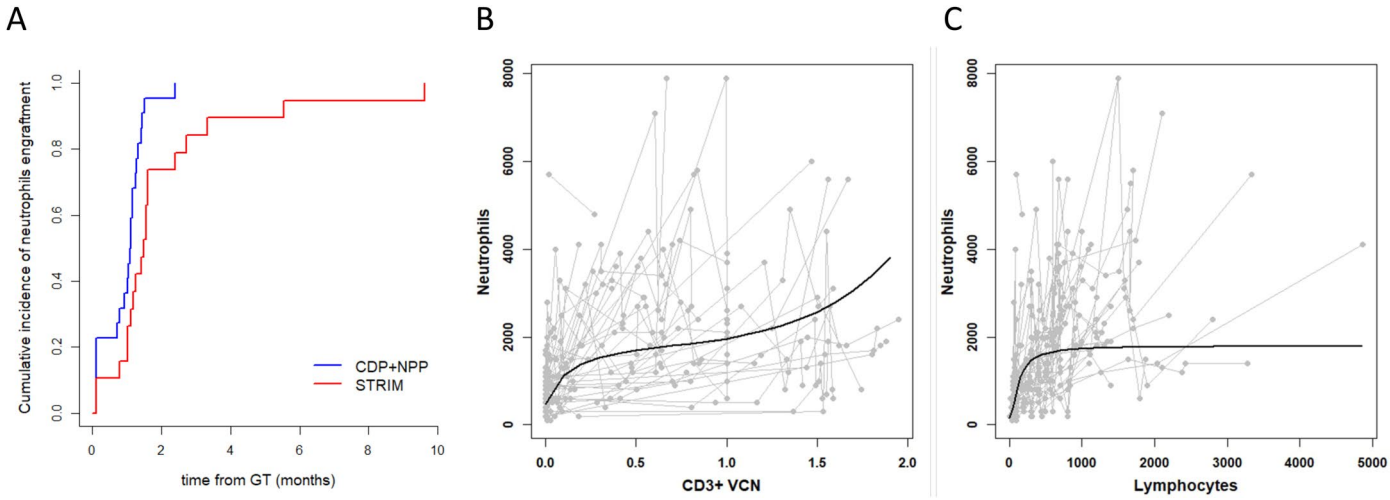
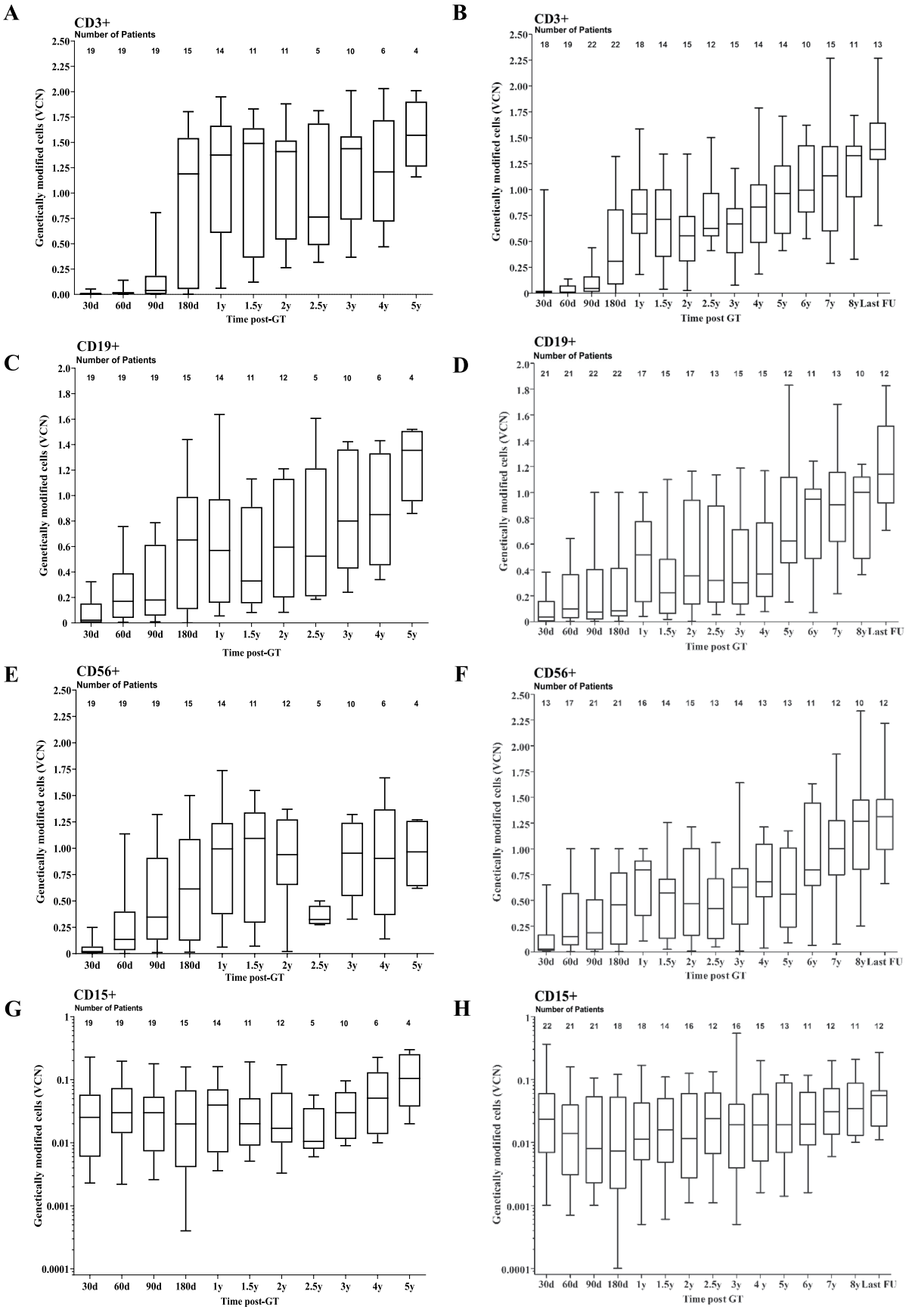
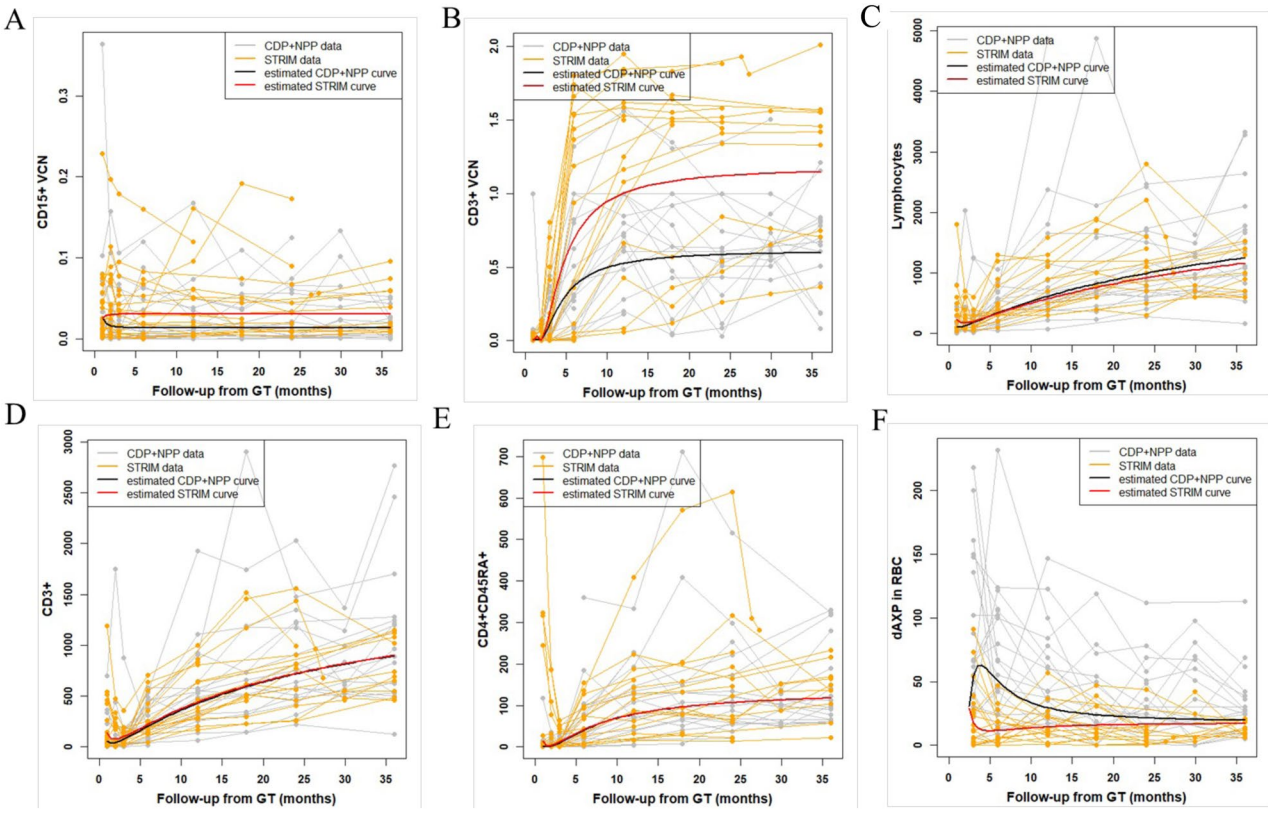


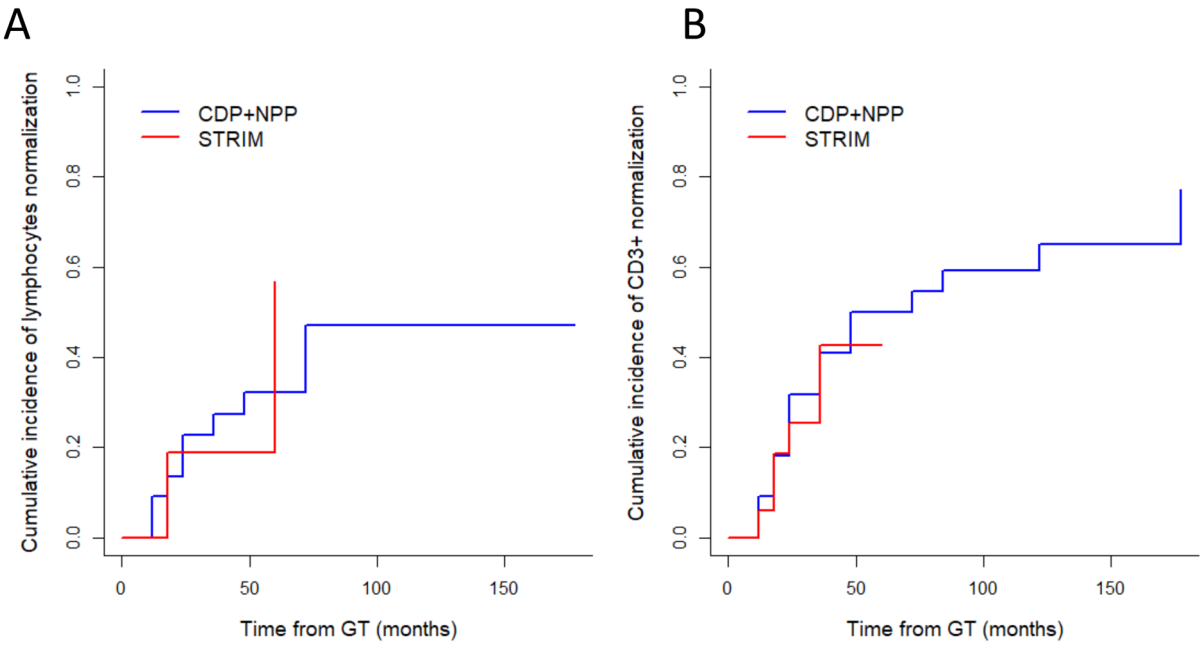
Extended Data Fig. 1 | In vivo engraftment of genetically corrected cells. (A) Cumulative incidence of neutrophil engraftment. Curves represent time to achieve neutrophil engraftment (3 consecutive days of ANC ≥ 500) after gene therapy in the CDP + NPP and STRIM cohort. (B; C) Longitudinal analysis of absolute neutrophil count with respect to CD3 + VCN (B) and lymphocytes (C) in CDP + NPP and STRIM patients. The neutrophil trend was estimated by using mixed-effects models with fractional polynomials, evaluating its dependency either on CD3 + VCN or lymphocytes. Only data up to 36 months after gene therapy were used and data of both CDP + NPP and Strimvelis groups were considered together. ANC: absolute neutrophil count; VCN: Vector Copy Number.



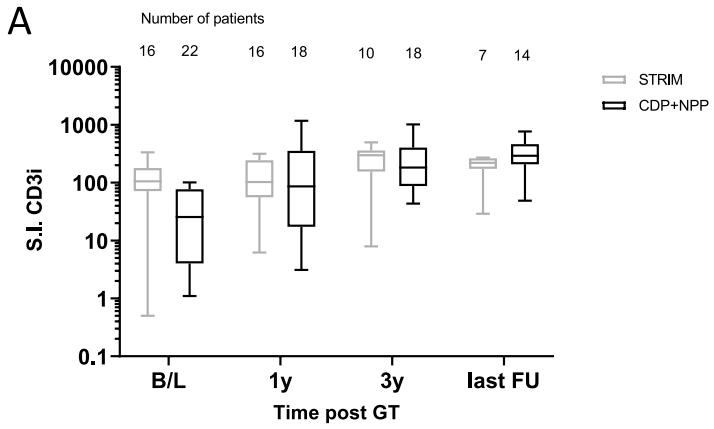
Extended Data Fig. 2 | Neutrophil engraftment and longitudinal analyses. Persistent multilineage engraftment of genetically corrected cells in peripheral blood in STRIM (A, C, E, G) and CDP + NPP (B, D, F, H) patients. In the plots, box and whiskers display the median, the first and the third quartile and the minimum and the maximum of the data. The number of patients contributing to each data point is indicated on the graph. CD3+ cells (A-B), CD19+ cells (C-D), CD56+ cells (E-F), CD15+ cells (G-H), were purified from peripheral blood. VCN=vector copy number; d=day; y=year.

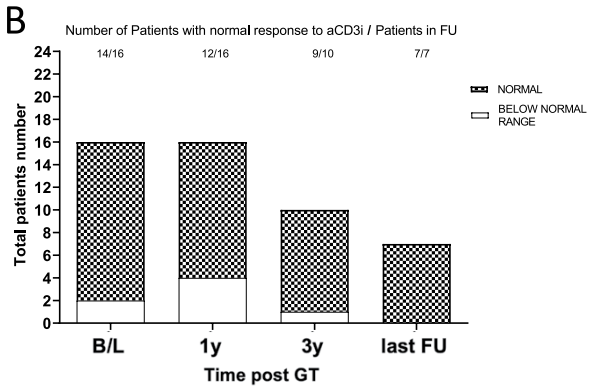


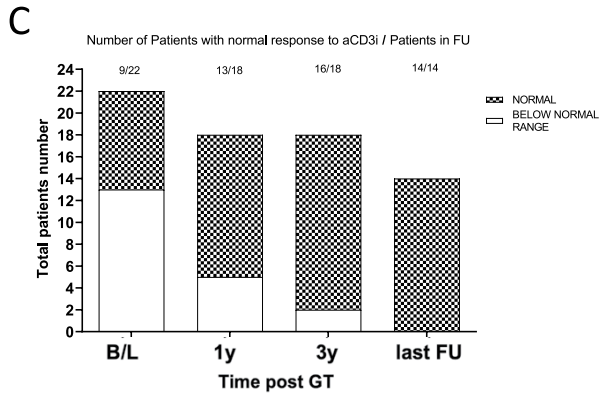
Extended Data Fig. 3 | Longitudinal analysis comparing engraftment, immune reconstitution and metabolic correction in CDP + NPP and Strimvelis patients. The longitudinal trends were estimated and compared between groups, by using mixed-effects models with fractional polynomials due to their nonlinear shape (see Supplementary Statistical Methods and Supplementary Tables S4a–f). Only data up to 36 months after gene therapy were used. Graphs represent estimated longitudinal trend of CD15+ cells VCN (A), CD3+ cells VCN (B), lymphocyte counts (C), CD3 + T cell counts (D) CD4 + CD45RA+ naïve T-cell counts (E) and dAXP in RBC (F). dAXP: deoxyadenosine nucleotides; RBC: red blood cells.

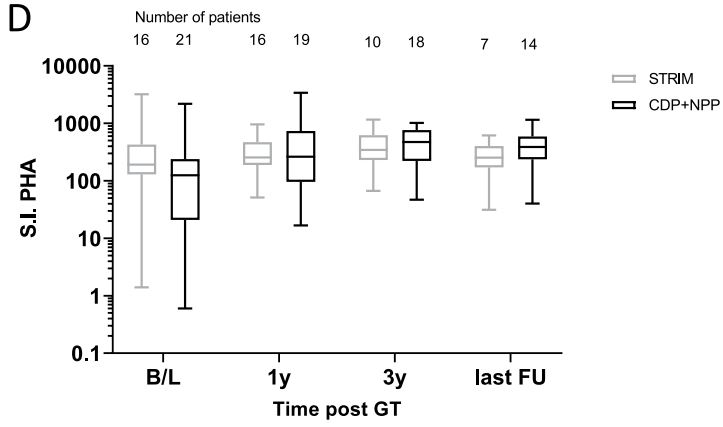


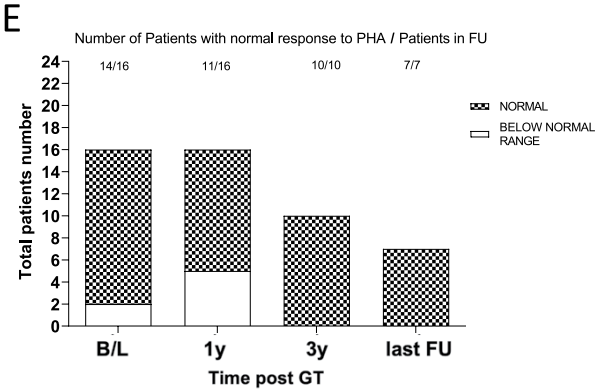
Extended Data Fig. 4 | Cumulative incidence of lymphocyte and T cells normalizations. Curves represent the cumulative incidence of achieving normal lymphocyte counts (A) or CD3 + T cells counts (B) after gene therapy in the CDP + NPP and STRIM cohort, according to normal age values.

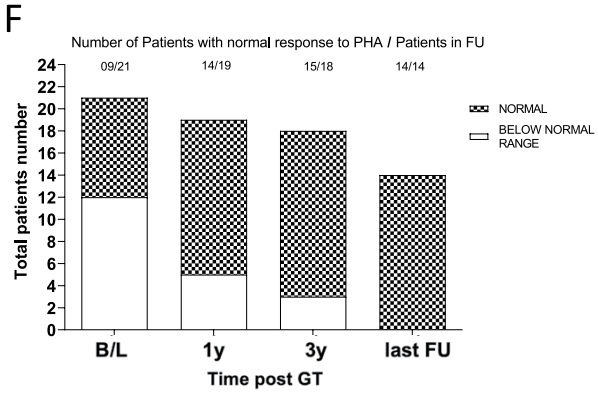




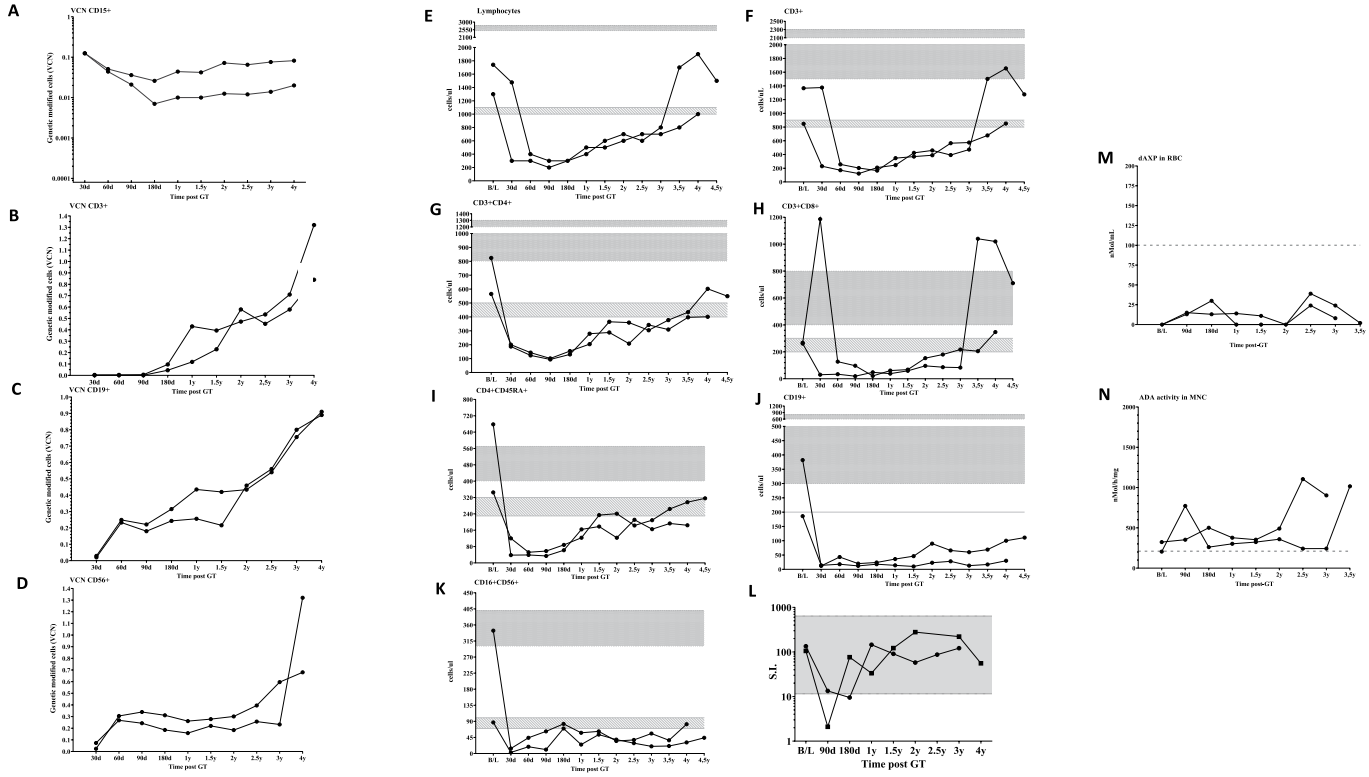








Extended Data Fig. 5 | In vitro T-cell functions in CDP + NPP and STRIM populations. Proliferative T-cell capacity assessed following challenge with anti-CD3 antibody (A, B, C) or PHA (D, E, F) in CDP + NPP and STRIM populations by observation period and expressed as Stimulation Index. In the plots, box and whiskers display the median, the first and the third quartile and the minimum and the maximum of the data. Analyses of data at the time-points baseline and 3 year are reported in the Supplementary Statistical Methods and Supplementary Tables S6a–d. SI: stimulation index; PHA: phytohemagglutinin.



Extended Data Fig. 6 | In vivo engraftment of genetically corrected cells, immune reconstitution and metabolic correction in patients receiving mobilized peripheral blood-derived CD34+ cells. Multilineage engraftment and immune reconstitution in 2 patients treated under hospital exemption (HE) with mobilized peripheral blood (MPB)-derived CD34+ cells up to 4 years of F-U. Graphs show: VCN in CD15+ (A), CD3+ (B), CD19+ (C) and CD56+ cells (D) purified from peripheral blood; absolute cell counts (cells/uL) of lymphocytes (E) CD3+ (F), CD3 + CD4+ (G), CD3 + CD8+ (H), CD4 + CD45RA+ (I), CD19+ (J), CD16 + CD56+ (K) in peripheral blood. 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 (Comans-Bitter, WM et al: Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulation. J Pediatr, 130: 3388-393 (1997)). in vitro T-cell proliferation response (expressed as SI) after anti-CD3i stimulation (L), by observation period. dAXP in red blood cells (RBC) (M) and ADA activity in mononuclear cells (MNC) (N). Dashed lines indicate the lower reference value of ADA activity/dAXP for patients undergone successful hematopoietic stem cell transplantation. VCN=vector copy number; d=day; y=year. SI: stimulation index.

Extended Data Table 1 | Baseline patient and drug product characteristics

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 clinical history	Age at GT *(month/year)*	Total AUC (ng/ml*h)	Infused CD34+ cells *(10^6/ kg)*	VCN transduced cells *(copies/genome)*	Follow-up *(year)*
1	F	CDP	2	3	c.50A>C; H17P (homozygous)	None	-	-	7 mo	nd	8.5	2.28	22.2
2	F	CDP	2	6	c.320T>C; c.632G>A	Haplo-SCT	-	-	2.4; 5.0 yr#	nd	0.9; 2.1	NR; 2.15	4.7¶
3	M	CDP	1	5	c.221G>T; c.845G>A	Haplo-SCT	-	-	1 yr	30664	6.7	0.85	20.6
4	F	CDP	5	6	c.845G>A (homozygous)	Haplo-SCT; PEG-ADA *(for 2 mo)*	20	BCGitis Arnold Chiari type I	1.9 yr	18640	3.8	NR	20.1
5	F	CDP	1	1	c.646G>A; c.956_960delAAGAG	PEG-ADA *(for 15 mo)*	40	Mild development delay	1.6 yr	17724	9.6	1.89	18.7
6	M	CDP	1	2	c.632G>A (homozygous)	PEG-ADA *(for 65 mo)*	30	Sensorineural peripheral hearing loss Mild development delay Growth delay	5.6 yr	18210	9.5	1.05	18.1
7	M	CDP	<1	2	c.646G>A; c.872C>T	PEG-ADA *(for 13 mo)*	22	Feeding disturbance Development delay	1.5 yr	11181	9.0	0.83	17.1
8	F	CDP	<1	1	c.478+2T>C (homozygous)	PEG-ADA *(for 32 mo)*	60	Autoimmune haemolytic anemia Psychomotor retardation Failure to thrive Macrophage activation syndrome	2.8 yr	23072	10.6	0.12	0.6¶
9	M	CDP	5	5	c.646G>A (homozygous)	PEG-ADA *(for 10 mo)*	41	Pneumocystis jiroveci pneumonia requiring intubation	1.4 yr	16427	13.6	0.57	16.2
10	F	CDP	3	4	c.632G>A (homozygous)	Haplo-SCT; PEG-ADA *(for 11 mo)*	37	BCGitis Congenital adrenal hypoplasia Bilateral peripheral hearing loss Severe mental retardation Behavioral problems	1.8 yr	19532	10.7	0.35	16.1
11	M	CDP	4	7	c.646G > A; p.E319GfsX3	PEG-ADA *(for 8 mo)*	35	BCGitis Hypertension Mental retardation	1.6 yr	20059	6.34	0.17	15.7
12	M	CDP	<1	<1	c.606 + 5G>? (homozygous, no additional data available)	PEG-ADA *(for 12 mo)*	28	Alveolar proteinosis	1.3 yr	19109	11.5	0.14	15.6
13	M	CDP	<1	<1*	c.646G>A; c.975+6Tdel	PEG-ADA *(for 1 mo)*	20	G6PDH deficiency	6 mo	33216	18.2	0.06	15.4
14	M	CDP	<1	1	c.466C>T (homozygous)	PEG-ADA *(for 71 mo)*	62	Bilateral hearing loss Mental retardation	6.1 yr	14772	6.0	0.54	14.5
15	F	CDP	10	14	c.7C>T (homozygous)	PEG-ADA *(for 12 mo)*	18	-	2.5 yr	17644	5.9	0.38	14.4
16	M	CDP	3	<1*	c.881C>A (homozygous)	PEG-ADA *(for 23 mo)*	19	Behavioral problems	2.3 yr	30368	6.9	0.17	12.7
17	M	CDP	<1	<1	c.956_960delAAGAG (homozygous)	PEG-ADA *(for 7 mo)*	30	Feeding disturbance	7 mo	16112	13.0	0.24	1.2¶
18	M	CDP	<1	<1*	c.466C>T; D123fsX132	PEG-ADA *(for 24 mo)*	20	-	2.1 yr	20448	9.9	0.11	11.6
19	F	NPP	3	4	c.632G>A; c.646G>A	PEG-ADA *(for 6 mo)*	60	-	11 mo	26882	16.9	1.1	8.3
20	M	NPP	4	22	c.467G>A; c.646G>A	PEG-ADA *(for 36 mo)*	30	Neuropsychological abnormality	5 yr	26464	4.6	1.8	0.6¶
21	M	NPP	2	4	c.455T>C; c.478+6T>C	PEG-ADA *(for 8 mo)*	21	-	1 yr	19647	14.4	1.8	5.3¶

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 clinical history	Age at GT *(month/year)*	Total AUC (ng/ml*h)	Infused CD34+ cells *(10^6/ kg)*	VCN transduced cells *(copies/genome)*	Follow-up *(year)*
23^	M	STRIM	<1	<1*	c.135 G>A (homozygous)	PEG-ADA *(for 6 mo)*	22	HCV infection^	9 mo	21764	12.6	1.6	5.5
24	F	STRIM	<1	<1	c.792 G>A (homozygous)	PEG-ADA *(for 6 mo)*	30	-	8 mo	25074	11.4	2.3	5.7
25	F	STRIM	1	2	c.529G>A; c.1048delCTCTT	PEG-ADA *(for 69 mo)*	20	Charcot Marie Tooth type 1	6 yr 4 mo	24924	11.7	1.9	5.1
26	M	STRIM	1	2	c.43 C>G; c.320 T>C	PEG-ADA *(for 8 mo)*	45	-	11 mo	20134	19.7	2.1	5.2
27	M	STRIM	2	5	c.302 G>A (homozygous)	PEG-ADA *(for 16 mo)*	30	-	2 yr	21003	11.7	2.5	5.0
28°	F	STRIM	<1	12	c.302 G>A (homozygous)	PEG-ADA *(for 22 mo)*	25	BCGitis°	3 yr	20021	4.8	1.6	0.5¶
29	M	STRIM	19	20	c.965T>C; c.22 G>A	PEG-ADA *(for 32 mo)*	30	Hypothyroidism	4 yr 6 mo	19602	6.3	1.6	4.4
30	F	STRIM	18	34	c.424 C>T; c.1078.20_1078-37delinsGATGTTG	PEG-ADA *(for 9 mo)*	44	-	3 yr 10 mo	21580	3.4	1.3	4.1
31	F	STRIM	<1	<1*	c.703C>T (homozygous)	PEG-ADA *(for 8 mo)*	45	-	8 mo	26150	12.8	1.2	3.4
32	F	STRIM	2	3	c.7C>T (homozygous)	PEG-ADA *(for 4 mo)*	60	Anemia	9 mo	18329	12.8	1.4	3.4
33	F	STRIM	<1	7	c.730delG; c.254A>T	PEG-ADA *(for 5 mo)*	30	Psychomotor development delay Growth delay	1 yr 1 mo	19858	7.5	1.5	3.1
34	F	STRIM	<1	7	c.730delG; c.254A>T	PEG-ADA *(for 3 mo)*	30	Congenital complex heart malformation Psychomotor development delay Growth delay	11 mo	20474	11.0	1.5	3.2
35	M	STRIM	<1	14	c.965T>C; p.F322S (homozygous)	PEG-ADA *(for 5 mo)*	60	Autoimmune thrombocytopenia	1 yr 9 mo	20988	6.7	1.1	2.5
36	M	STRIM	<1	2	c.956_960delAAGAG; p.E319Gfs*3 (homozygous)	PEG-ADA *(for 15 mo)*	60	Plasma and stool adenovirus infection	1 yr 5 mo	19328	6.6	2	2.3
37	M	STRIM	<1	<1•	c.7C>T; p.Q3Ter (homozygous)	PEG-ADA *(for 7 mo)*	34	-	7 mo	17171	9.7	2.3	2.3
38	M	STRIM	<1	1•	c.955_959delGAAGA; p.E319Gfs*3 (homozygous)	PEG-ADA *(for 5 mo)*	60	Blood CMV DNA positivity	7 mo	35304	18.6	1.7	2.4
39	M	STRIM	<1	2	c.58 G >A p.G20R; c.956_960delGAAGA	PEG-ADA *(for 7 mo)*	60	MRSA colonization	10 mo	23190.5	14.2	2.1	1.2
40	F	STRIM	<1	4	c.646G>A; p.G216R c.778G>A; p.E260K	PEG-ADA *(for 11 mo)*	60	Klebsiella bacteremia	1 yr 4 mo	20376	11.6	1.8	0.5
41	M	STRIM	<1	1•	c.678+1G>T; p.? (homozygous)	PEG-ADA *(for 9 mo)*	58	Alveolar proteinos, CMV infection, Arterial hypertension	10 mo	22840	13.4	1.8£	0.2

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 clinical history	Age at GT *(month/year)*	Total AUC (ng/ml*h)	Infused CD34+ cells *(10^6/ kg)*	VCN transduced cells *(copies/genome)*	Follow-up *(year)*
42	M	mPB-HE	25	47	c.529 G>A; c.1048delCTCTT	PEG-ADA *(for 78 mo)*	36	Charcot Marie Tooth type 1	10 yr 10 mo	18997	25.7	1.4	4.5
43	F	mPB-HE	12	45	c.965T>C; c.22G>A	PEG-ADA *(for 10 mo)*	18	-	4 yr 7 mo	22807	10.1	1.4	4.5

Patients were treated (A) during the clinical development/named patient program(CDP+NPP); (B) with approved drug product (STRIM), (C) from mobilized peripheral blood CD34+ cells under hospital exemption (mPB-HE). Data include patients previously reported in Cicalese et al.[17] and Pajno et al.[37]. *: suspected diagnosis due to familiar history, later molecularly confirmed; #: Second dose of GT did not include busulfan preconditioning; §: Patient 22 presented only in aggregate analyses due to requested block of data after treatment.: nd, not done; NR, not recorded. CDP: clinical development program; NPP: named patient program. ¶: Censored after ERT>3 months or allogeneic transplantation. STRIM, Strimvelis. *: suspected diagnosis due to familiar history, later molecularly confermed; •: suspected diagnosis due to neonatal screening, later confirmed by molecular analyses. £ this value correspond to infused product VCN (STEP 1 in manufacturing). ^Reported in Tucci et al.[42]. °Reported in Tucci et al.[7]. mPB-HE, mobilized peripheral blood, hospital exemption.

Extended Data Table 2 | Baseline characteristics and treatment information of patients receiving gene therapy

	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

Patients treated with BM-derived drug product are shown. Comparisons of numerical variables between CDP+NPP vs STRIM were performed with the Mann-Whitney test and, in case of categorical variables, with Fisher's exact test. All tests were two-sided. ERT, enzyme replacement therapy. VCN, vector copy number; IQR, Interquartile range.

Extended Data Table 3 | Vaccination response and native pathogens' infections

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

Vaccination responses in evaluable CDP+NPP and STRIM patients. *one patient experienced an additional infection from the same pathogen.

Extended Data Table 4 | Serious Adverse Events following gene therapy

Event	F-U post GT	Duration, days	Outcome	Maximun Toxicity Grade
CDP+NPP patients[&]				
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
Pirexia	7 years 10 months	4	resolved	3
Vomiting	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 epidermidis CVC infection	2 months	9	resolved	3
Sepsis	3.2 months	13	resolved	3
Gastroenteritis	3.5 months	5	resolved	3
Lack of efficacy	3.6 months	n.a.	not resolved	2
Diarrhoea	3.8 months	2	resolved	1
Gastroenteritis	5 months	8	resolved	2
Lactose intolerance	22 months	11	resolved	2
Macrophage activation syndrome post chickenpox vaccination	26 months	541	resolved	3
Febrile gastroenteritis	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
Staphyloccoccus hominis MDR CVC infection	2 months	7	resolved	3
Upper respiratory tract infection	5 months	5	resolved	3
Multisensitive Staphylococcus epidermidis CVC infection	3 months	7	resolved	3
Upper respiratory tract infection	4 months	5	resolved	3
Fever	13 months	3	resolved	3
Haemolytic anemia	5 months		ongoing	3
Central venous catheter sepsis	2 months	13	resolved	3
Campylobacter and Clostridium stools infection	7 months	36	resolved	3
Porth a cath infection	1 months	15	resolved	4
mPB-HE patients				
Phlebitis	3 years	10	resolved	3
CMV infection	3 years 4 months	15	resolved	3
Pneumonia	3 years 8 months	3	resolved	3

[&]Patient 22 presented 2 SAE but due to request of data block, they are presented only in aggregate analyses. F-U: follow-up; CVC: central venous catheter; MDR. Multidrug resistant, n.a.: not applicable.