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REVIEW

Gene therapy for severe combined immunodeficiencies and beyond

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Ex vivo retrovirally mediated gene therapy has been shown within the last 20 yr to correct the T cell immunodeficiency caused by γc-deficiency (SCID X1) and adenosine deaminase (ADA) deficiency. The rationale was brought up by the observation of the revertant of SCIDX1 and ADA deficiency as a kind of natural gene therapy. Nevertheless, the first attempts of gene therapy for SCID X1 were associated with insertional mutagenesis causing leukemia, because the viral enhancer induced transactivation of oncogenes. Removal of this element and use of a promoter instead led to safer but still efficacious gene therapy. It was observed that a fully diversified T cell repertoire could be generated by a limited set (<1,000) of progenitor cells. Further advances in gene transfer technology, including the use of lentiviral vectors, has led to success in the treatment of Wiskott–Aldrich syndrome, while further applications are pending. Genome editing of the mutated gene may be envisaged as an alternative strategy to treat SCID diseases.

Introduction

The concept of gene therapy emerged >50 yr ago (Friedmann and Roblin, 1972) at a time when (i) the basic principles of molecular biology had been determined and (ii) the first disease-causing genetic mutations were being discovered. Nevertheless, it took almost 30 yr and several key advances to become a reality. Once the biology of retroviruses was characterized (Varmus, 1988; Temin and Mizutani, 1970), it became clear that they could be used as vectors for integrating a transgene into targeted cells and enabling expression. Murine retroviruses were the first to be used to transduce hematopoietic stem cells (HSCs; Williams et al., 1984). The development of ad hoc vector packaging cell lines resulted in replication-incompetent vectors (Miller and Buttimore, 1986; Danos and Mulligan, 1988). The first attempts at correcting SCID caused by adenosine deaminase (ADA) deficiency, however, failed because the technology was not yet optimal (Blaese et al., 1995; Kohn et al., 1995; Hoogerbrugge et al., 1996).

Advances in HSC biology, the identification of genes associated with SCID, a better understanding of SCID pathophysiology, and empirical improvements in cell transduction protocols led to the first effective treatments. Gene therapy has thus become a reality, paving the way for the treatment of other diseases. (Cavazzana-Calvo et al., 2000; Aiuti et al., 2002, 2013). At the time of writing, regulatory authorities in Europe have approved one gene therapy product of primary immunodeficiency (PID), Strimvelis, to treat ADA SCID (Aiuti et al., 2017). Remarkably, SCID was the first condition to be corrected by gene therapy, just as it was the first ever indication for allogeneic HSC transplantation (HSCT; Gatti et al., 1968). It is instructive to look at why this was the case. SCIDs are inherited conditions characterized by a profound block in T cell development, variably associated with defects in other lymphoid (or more rarely myeloid) lineages (Fischer et al., 2015). Of the 16 genetic SCID diseases described to date, X-linked SCID (SCID X1) and ADA deficiency are the most frequent (Noguchi et al., 1993; Giblett et al., 1972; Valerio et al., 1984). Patients with untreated SCID develop a multitude of infectious complications and die within the first year of life. SCIDs can be successfully treated with allogeneic HSCT, which provides long-term correction of the T cell deficiency. In the early 1990s, however, HSCT with transplants from non-genoidentical donors was associated with relatively high mortality and morbidity rates, as a result of a graft-versus-host reaction or, when the donor's marrow graft was depleted of T cells, delayed T cell reconstitution (Antoine et al., 2003; Buckley et al., 1999).

"Natural" gene therapy in patients with SCID

Hirschhorn et al. (1996) first reported on the unexpected development of T lymphocytes in a patient with ADA deficiency; a

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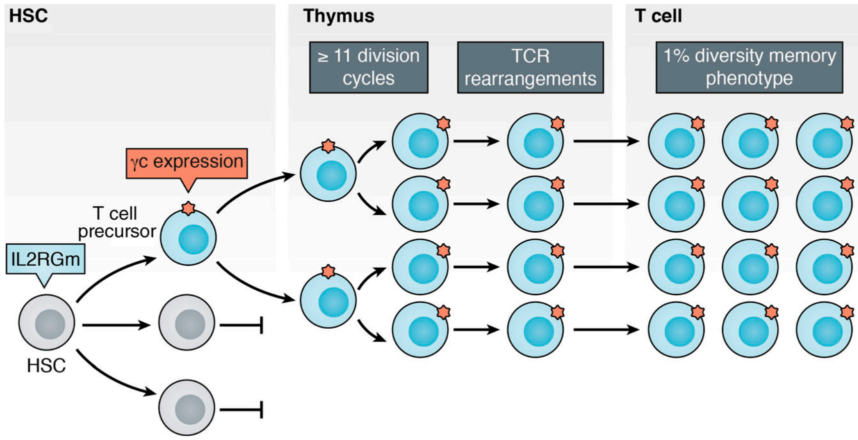

Figure 1. **Spontaneous partial correction of SCID-X1 by reversion of the *IL2RG* mutation in a T cell precursor.** The revertant cell (IL2RG wild type) was able to proliferate extensively before the TCRB variable diversity joining segments recombination stage. It generates a rather diverse repertoire, since 1,000 distinct TCRB sequences were expressed by mature CD4 and CD8 T cells. These cells expanded too, since the patient's T cell count was approximately half that of age-matched controls (Stephan et al., 1996; Revy et al., 2019). A similar schema can also account for partial correction of SCID-ADA (Hirschhorn et al., 1996).

revertant mutation in the *ADA* gene had initiated synthesis of ADA, leading to partial correction of the SCID phenotype. Soon after, it was found that an unusual patient with SCID X1 had only mild T cell lymphocytopenia at the age of 6 yr. The patient had a wild-type *IL2RG* gene sequence in his T cells but a mutated sequence in his neutrophils and epithelial cells (Stephan et al., 1996). This observation indicated that the *IL2RG* missense mutation had reverted during or before differentiation of the T cell lineage. At least one other similar case has since been reported (Speckmann et al., 2008). All the patients' T cells had a memory phenotype, indicating that the reversion had occurred well before the time of observation. Both CD4 and CD8 T cells were detectable. Interestingly, ∼1,000 unique TCR VB CDR3 sequences were found with the tools available 25 yr ago; this corresponded to an estimated 1% of the repertoire diversity of memory T cells (Bousso et al., 2000; Fig. 1). It can be inferred that the progeny cells divided ≥11 times. Thus, it was concluded that the expression of γc (the *IL2RG* gene product, a component of the IL2, IL4, IL7, IL9, IL15, and IL21 cytokine receptors) by a T cell precursor conferred the latter with the ability to survive by interaction with stroma cells producing IL7 and to proliferate extensively before the stage at which T cells undergo TCR rearrangements (Bousso et al., 2000). Since then, many other examples of T cell disease attenuation have been reported, highlighting the strong selective advantage of gene-corrected T cell precursors and the very long life span of mature memory T cells (Revy et al., 2019). Hence, SCID diseases were considered to be high-priority, highly appropriate targets for gene therapy. The small initial population of transduced precursor cells was expected to "auto-amplify" and give rise to a large pool of diversified T cells with a very long life span.

The first clinical trial of gene therapy for SCID X1

Based on the above findings, an MFG-derived murine retroviral vector (Rivière et al., 1995) containing the *IL2RG* cDNA was built (Hacein-Bey et al., 1996); in vitro, it restored γc expression in *IL2RG*-mutated B cell lines and T and natural killer (NK) cell development from *IL2RG*-mutated CD34$^+$ progenitor cells (Hacein-Bey et al., 1996, 1998; Cavazzana-Calvo et al., 1996). The potent enhancer activity of the vector's viral LTR was used to drive *IL2RG* expression, given that (i) γc is ubiquitously expressed by hematopoietic cells and (ii) γc membrane expression density is regulated by coexpression of the other distinct cytokine subunit coreceptors (Leonard et al., 2019). A clinical trial in 10 SCID X1 patients lacking an HLA-matched donor was initiated in Paris in March 1999. The transduction conditions included the introduction of a fibronectin fragment to favor cell–virus contact (Hanenberg et al., 1996). In eight of the patients, gene therapy led to the development of a normal T cell count within 3–6 mo (Cavazzana-Calvo et al., 2000; Hacein-Bey-Abina et al., 2002, 2010). The T cell subset distribution was also normal. In vitro functional assays showed that the T cells could be activated by antigens and could secrete cytokines. The vector copy number was ∼1 per cell. The T cells' surface levels of γc were normal or slightly below normal (Cavazzana-Calvo et al., 2000; Hacein-Bey-Abina et al., 2002, 2010). These results were accompanied by a lasting, clear-cut clinical benefit. After a median of 18 yr of follow up, all but one of the patients are doing well, with normal growth and development and no opportunistic infections. T cell receptor excision circles (generated by TCR B rearrangements during thymopoiesis) were persistently present 200 mo after gene therapy (Hacein-Bey-Abina et al., 2010). A similar study was performed in London (Gaspar et al., 2004, 2011), and the same outcomes were reported for all 10 patients enrolled. Taken as a whole, these proof-of-principle studies showed that gene therapy can provide sustained clinical benefit in a favorable setting characterized by the rapid expansion of transduced cells and long-lived progeny.

Adverse events and clinical trials based on second-generation vectors

The trials described above were also characterized by the occurrence of serious adverse events: six patients (five in Paris, one in London) developed T cell acute lymphoblastic leukemia 2–14 yr after treatment, being fatal in one (Hacein-Bey-Abina et al., 2003, 2008, 2010; Howe et al., 2008; Six, E., V. Gandermer, A. Magnani, C. Nobles, J. Everett, F. Male, C. Plantier, I. Hmitou, M. Semeraro, E. Magrin, et al. 2017. 20th Annual Meeting, American Society of Gene and Cell Therapy). Recovery in five patients was associated with the persistence of robust, transduced T cell precursors.

Analysis of γ retrovirus (γRV) integration sites (ISs) using linear amplification–mediated PCR (Schmidt et al., 2007) or nested ligation–mediated PCR (Wang et al., 2010) techniques revealed that in the leukemic cells, the γRV vector had inserted into the *LMO2* oncogene locus in five patients and into the *CCND2* locus in one patient (Hacein-Bey-Abina et al., 2003, 2008; Howe et al., 2008; Six, E., V. Gandermer, A. Magnani, C. Nobles, J. Everett, F. Male, C. Plantier, I. Hmitou, M. Semeraro, E. Magrin, et al. 2017. 20th Annual Meeting, American Society of Gene and Cell Therapy) resulting in an oncogenetic process by transactivation.

Safer vectors have been developed by removing the enhancer element from the vector's LTR and replacing it with a promoter. These vectors are referred to as self-inactivating (SIN). The first SIN vector was based on a lentivirus (LV, see below; Zufferey et al., 1998), but SIN γRV vectors have also been built (Thornhill et al., 2008). The elongation factor 1α S promoter has been incorporated into the SIN γRV vector (Thornhill et al., 2008). A clinical trial (referred to as SCID X1 trial 2) was initiated in Paris, London, and Boston. The level of safety was indeed greater. After 8 yr of follow-up (median 6.8 yr), none of the 13 treated SCID X1 patients had developed leukemia (Hacein-Bey-Abina et al., 2014). IS analysis in T cells with a vector copy number ranging from 1 to 3 per cell revealed that the frequency at which ISs were found in proto-oncogenes (and notably in *LMO2*) was much reduced (Hacein-Bey-Abina et al., 2014). Interestingly, no cases of leukemia have occurred in clinical trials based on the use of SIN vectors (whether γRV or LV constructs to transduce ex vivo CD34 hematopoietic progenitors in different diseases), since the 44 patients with ≥4 yr of follow-up are all leukemia free (Cartier et al., 2009; Cavazzana-Calvo et al., 2010; Aiuti et al., 2013; Biffi et al., 2013; Hacein-Bey-Abina et al., 2014; Ferrua et al., 2019; Sessa et al., 2016; Hacein-Bey Abina et al., 2015). T cell reconstitution was achieved in all but two patients treated with the SIN γRV-IL2RG vector associated with a major clinical benefit. One patient died from a disseminated adenovirus infection that was present before gene therapy, whereas the procedure failed in another patient (Hacein-Bey-Abina et al., 2014).

Additional and serendipitous findings

An IS analysis of the treated patients' T cells indicated that there were ~1,000 unique ISs, as estimated using Chao's methodology (Hacein-Bey-Abina et al., 2010; Clarke et al., 2018). After ≤20 yr of follow-up, the number of ISs was found to be quite stable. Furthermore, the number was in line with the Shannon diversity index, which also takes account of the abundance of clones (Clarke et al., 2018). Thus, the blood T cell population has derived from no more than 1,000 transduced progenitor cells. This finding confirms the rationale on which the gene therapy trials was based, i.e., the massive, IL-7–dependent amplification of transduced cells.

The next question relates to T cell diversity. High-throughput sequencing of T cell receptor beta chain (TCRB) variable diversity joining segments from blood T cells of patients treated in trials 1 or 2 showed that distribution of TCR VB (variable region of TCRB) and JB (joining region of TCRB) element usage and the number of unique TCRB CDR3 sequences were similar to control values in all but one of the seven patients tested (Clarke et al., 2018). These data made it possible to estimate the average number of cell divisions each transduced clone had undergone before rearrangement of the TCRB locus during thymus differentiation. The value is ~10; this fits with the estimate for the SCID X1 revertant discussed above (Bousso et al., 2000; Clarke et al., 2018; Fig. 2). These data (i) explain the efficacy of gene therapy in SCID X1 patients and (ii) demonstrate that human T precursor cells divide readily upon interaction with IL7 at the double-negative stage of T cell differentiation. It is hard to say whether this reflects the physiological situation or rather T cell development when only a few precursors are available. Another question relates to the nature of the CD34+ progenitors that were transduced ex vivo and then generated transduced mature cells in vivo. Given that no information can be retrieved directly by studying the initial population of progenitor cells, this matter can be indirectly addressed by determining whether different cell lineages share the same ISs. It is noteworthy that transduced CD34+ progenitor cells were not detected in bone marrow samples, suggesting that no or very few bona fide HSCs were transduced and differentiated. This is not surprising, given the absence of myeloablation and low availability of niches for transduced HSCs. More surprising, perhaps, was the persistent detection of naive/T cell receptor excision circles+ T cells. One can therefore hypothesize that transduced T precursor cells with self-renewal capacity persist in the body, likely in the thymus. This hypothesis is supported by similar findings for precursor cells transplanted into SCID mice in the absence of prior irradiation (Peaudecerf et al., 2012; Martins et al., 2012). It is remarkable that these SCID X1 gene therapy trials (Hacein-Bey-Abina et al., 2010, 2014; Gaspar et al., 2004, 2011) did not consistently lead to the differentiation of transduced B cells or NK cells. In contrast to the murine disease, γc deficiency in humans does not prevent B cell differentiation (DiSanto et al., 1995). Also, B cells that are deprived of competent IL4 and IL21 receptors do not make antibodies properly (Miggelbrink et al., 2018). In the gene therapy trials, the occupation of B cell differentiation niches may have hampered the differentiation of transduced B cell precursors. However, it is noteworthy that a minority of patients do not require IgG replacement therapy, suggesting that a few nondetectable transduced B cells are active (Hacein-Bey-Abina et al., 2010, 2014; Gaspar et al., 2011). The fact that IL15 is critical for NK cell differentiation explains why γc deficiency leads to a complete absence of this cell type (Leonard et al., 2019). NK cells reached control values in half of the patients within the first year after treatment; thereafter, however, very few NK cells persisted in the blood (Hacein-Bey-Abina et al., 2010, 2014). Thus, the NK cell deficiency is far less well corrected than the T cell deficiency. In the absence of HSCs, the low NK cell counts are probably due to the poor expansion capacity of NK cell precursors and the progeny's shorter life span. These data are reminiscent of those observed in SCID X1 patients having undergone nonmyeloablative HSCT, since NK cells and innate lymphoid cells are also barely detectable 10 to 40 yr after treatment (Vély et al., 2016; Vivier et al., 2018). Interestingly, the lack of NK does not appear to have clinical consequences in either setting.

SCID can also be treated with haploidentical HSCT. Until recently, successful transplantation required the thorough depletion

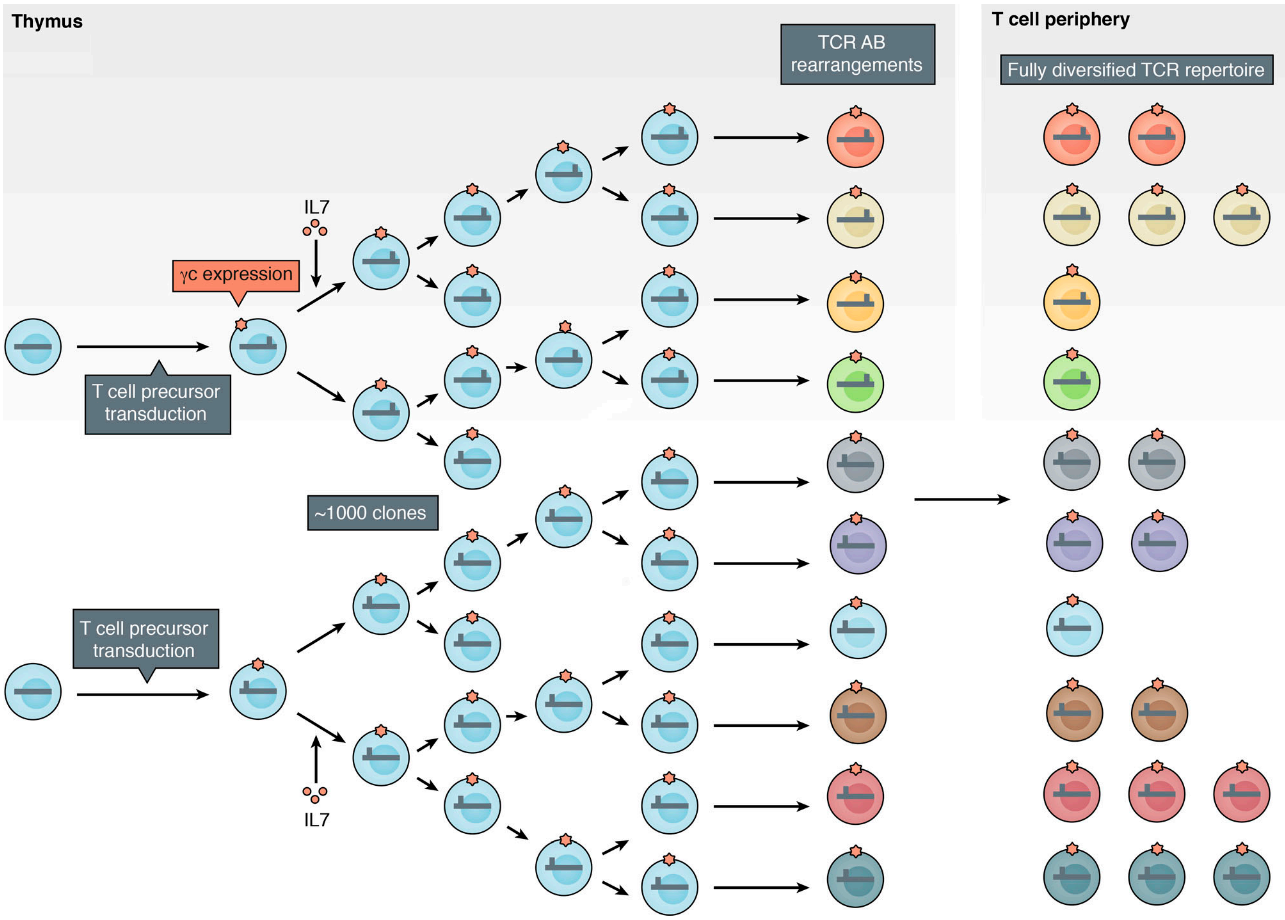

Figure 2. **A small number of transduced, γc-expressing T cell precursors can generate a fully diversified T cell repertoire.** Transduced T cell precursors can divide ≥10 times before undergoing TCR B then A rearrangements. This process eventually generates a fully diversified TCR repertoire (as indicated by different colors of the T cells in the figure). For the sake of clarity, T cell differentiation has been simplified, and additional expansion/selection steps have been ignored. Red star shape indicates γc expression. Number of cells in the periphery is variable, indicating diversity in TCR clonal abundance. Horizontal bars with short branches indicate arbitrary representation of two distinct ISs of the retroviral vector. All progeny cells carry the same insertion.

of donor marrow T cells in the graft, to prevent graft versus host disease (Haddad et al., 2018; Pai et al., 2014). Touzot et al. (2015) studied the characteristics of T cell reconstitution after transplantation compared with gene therapy. The results showed that naive T cell counts 2–5 yr after treatment were higher in patients having received autologous gene-modified CD34⁺ cells (Touzot et al., 2015). One possible explanation is that after haploidentical HSCT, subclinical graft versus host disease partially impairs thymus function. Nevertheless, recent advances in haploidentical HSCT (i.e., TCR αβ depletion, or even T cell–replete HSCT followed by the suppression of allogeneic T cells via in vivo cyclophosphamide treatment; Bertaina et al., 2014; Fernandes et al., 2018) may challenge this conclusion.

Gene therapy for SCID X1: Step 3

As indicated above, γRV vector–based gene therapy for SCID X1 led to sustained correction of the T cell deficiency only. The addition of mild myeloablation, as performed for gene therapy of ADA deficiency (Aiuti et al., 2002, 2009; Gaspar et al., 2011; see below), may promote the engraftment of transduced HSCs and thus the differentiation of B and NK cells. Furthermore, LV vectors derived from HIV (Naldini et al., 1996) are much more potent than γRV for transducing HSCs. Hence, the use of LV vectors was expected to significantly increase the number of transduced HSCs. This has now been confirmed (Fig. 3). In a recent publication on a multicenter study of SCID X1 (Mamcarz et al., 2019), the combination of mild chemotherapy with use of a SIN IL2RG LV vector resulted in the reconstitution of T cell, NK cell, and (to a lesser extent) B cell function in seven of the eight treated patients, with a follow-up period of ≤21 mo. These preliminary results are encouraging in terms of both safety and efficacy. It is likely that both modifications have contributed to improve immune reconstitution.

Gene therapy in older patients

In some SCID patients, the T cell count falls after HSCT, probably because of a lack of donor progenitor cells. Accordingly, attempts to restore T cell differentiation in these patients were made first by using γRV and then by combining mild myeloablation with SIN LV vectors. Despite a good CD34 cell transduction rate, the first strategy failed to modify the immunodeficiency phenotype: this was probably because the thymus no longer supported T cell

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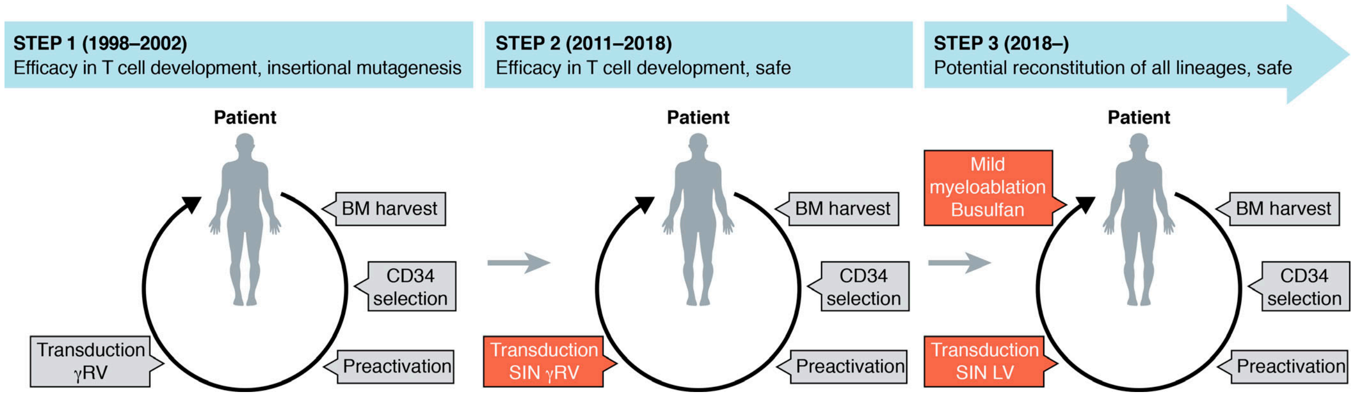

Figure 3. **The three steps in the progress of gene therapy for SCID X1 (1999–2019).** Step 1: First trial based on a γRV vector. Step 2: The LTR enhancer was removed from the vector while a promoter (elongation factor-1α) was inserted to drive IL2RG expression. Step 3: Usage of SIN LV vector instead of γRV should improve rate of transduction of HSC, thus correction of not only the T but also NK and B cell deficiency. Administration of low dose myeloablation (Busulfan) to the patient before autologous cell reinjection provides empty niches for relocalization of transduced HSC. Red text boxes indicate protocol modifications.

differentiation (Thrasher et al., 2005). The second strategy based on more efficient transduction of progenitor cells and greater accessibility to hematopoietic niches led to clinical improvement, the emergence of transduced B and NK cells, and a slight increase in T cell counts (De Ravin et al., 2016).

Gene therapy for ADA deficiency

The enzyme ADA is involved in purine metabolism. A lack of ADA activity leads to the accumulation of toxic deoxyadenosine and dATP (within the cell), and thus the premature death of lymphocyte progenitor cells (Kohn et al., 2019). It is noteworthy that ADA is expressed ubiquitously; hence, ADA deficiency is associated with a number of additional, nonimmune features, such as lung and brain disease (Kohn et al., 2019). ADA deficiency can be successfully treated with allogeneic HSCT, although the results to date are not as satisfactory as they have been for other forms of SCID (Kohn et al., 2019). Enzyme replacement therapy (ERT) has been developed with some success, although levels of long-term lymphocyte reconstitution are not optimal. In this setting, gene therapy has been considered a potential option. In the era of modern gene therapy, the combination of ex vivo γRV-mediated transduction of hematopoietic progenitor cells with mild chemotherapy has led to sustained improvements in immune functions (Aiuti et al., 2002; Gaspar et al., 2011; Candotti et al., 2012; Cooper et al., 2017; Shaw et al., 2017) for ≤18 yr. T cell reconstitution is less effective in ADA deficiency than in SCID X1. This difference is probably related to the effect of a lack of ADA on the thymic epithelial compartment. Furthermore, transduced B cells, NK lymphocytes, and myeloid cells are persistently detected. One striking feature of gene therapy for ADA deficiency (relative to SCID X1) is the absence of genotoxic effects among the 38 successfully treated patients, i.e., those able to discontinue ERT, even though the same type of vector was used. In fact, the ADA and SCID X1 trials featured the same pattern of vector integration and the same frequency of ISs within oncogenes such as *LMO2* (Selleri et al., 2011; Cicalese et al., 2016). However, the ADA trials are the exceptions to the rule, because the use of γRV vectors to transduce hematopoietic progenitor cells led to cases of leukemia for all the other diseases treated (Boztug et al., 2010; Stein et al., 2010). Thus, the explanation for this difference probably stems from the disease itself. As discussed above, a persistent ADA deficiency in nonhematopoietic thymic cells might create a deoxyadenosine-rich milieu that disfavors uncontrolled thymocyte proliferation. The safety and efficacy observed in the clinical trials led to the development of the very first gene therapy drug product to be approved and used (Strimvelis): a remarkable achievement that paves the way for further development.

Furthermore, HIV-derived SIN LV vectors for ADA were engineered and used in the clinic. SIN LV–mediated gene therapy for ADA deficiency has enabled at least 51 of the 53 treated patients to discontinue ERT (Kohn et al., 2019). Thus, the efficacy and safety criteria have been met. It is noteworthy that despite the higher frequency of HSC transduction, T cell reconstitution with SIN LV vectors does not appear to be better than that achieved with γRV vectors. This observation fits with the above-mentioned hypothesis, in which ADA-deficient thymic epithelial cells are the limiting factor in T cell reconstitution in this setting.

Extension of indications

Several other SCID gene therapy projects are now being developed. The target diseases include RAG-1 deficiency and Artemis deficiency (Pike-Overzet et al., 2011; Benjelloun et al., 2008). A clinical trial for Artemis deficiency has indeed been initiated (Punwani et al., 2017). Of note, RAG-1 SCID may not be easily corrected by gene therapy because (i) ectopic expression of RAG-1 may be harmful and (ii) if the number of corrected cells is too low, incomplete recovery as well as dysimmunity may be observed, as reported by one group but not another in a murine model (Pike-Overzet et al., 2014; van Til et al., 2014). Gene therapy of Omenn syndrome caused by hypomorphic mutation of RAG genes can also be envisaged (Capo et al., 2018).

Based on the success in the SCID trials and the improved safety and potency of RV vectors, it was thought that other PIDs could be targeted for gene therapy. The first one was Wiskott–Aldrich syndrome (WAS), an X-linked condition in which a PID affects all cell lineages but also modifies the platelet cytoskeleton, causing thrombocytopenia (Rivers and Thrasher, 2017). Depending on the exact mutation, WAS can be fatal, with death caused by bleeding, infections, severe autoimmune vasculitis, or lymphoma. WAS is caused by mutations in the *WASP* gene, which encodes a protein (WASp) expressed in the hematopoietic system and required for proper formation of the actin cytoskeleton (Rivers and Thrasher, 2017). A SIN LV vector containing the WASP cDNA under the control of the WASP promoter has been built by researchers in Milan (Aiuti et al., 2013). It has been used with success in two clinical trials; 23 patients have now been included in Europe and the United States. Myeloablation was applied so that the transduced HSCs could access hematopoietic niches more easily (Aiuti et al., 2013; Hacein-Bey Abina et al., 2015). The gene therapy's safety profile is excellent, since none of the treated patients has developed leukemia or experienced a serious adverse effect, after ≤8 yr of follow-up (median duration: 4 yr). The treatment appears to have effectively induced the differentiation of WASp-expressing leukocytes and platelets. This expression has translated into the high-quality reconstitution of immune functions, including T and B cell functions, and the control of infections, allergy, and autoimmunity (Aiuti et al., 2013; Hacein-Bey Abina et al., 2015; Ferrua et al., 2019). The level of WASp expression, however, did not usually reach that of wild-type cells. Correction of thrombocytopenia was, however, not as effective. This was enough to prevent severe bleeding but not to stop bleeding events completely. These results indicate that even after the transduction of multipotent HSCs and their differentiation into hematopoietic lineages (Scala et al., 2018), platelet production does not fully compensate for the destruction of WASp-deficient platelets (Sereni et al., 2019). It may well be that thrombocytopoiesis requires more progenitor cells than lymphopoiesis because of the high platelet turnover. More efficacious HSC transduction is therefore needed to ensure full recovery from WAS. Interestingly, longitudinal IS tracking has shown that following an early stage of uni(oligo) hematopoietic lineage differentiation, a steady state is reached with the multilineage differentiation ability of bona fide HSCs in a range of 1,000 per patient (Scala et al., 2018). Of note, an adult with WAS was successfully treated by the same procedure, stressing that not only young children and teenagers could benefit from such treatment (Morris et al., 2017).

Gene therapy for XL chronic granulomatous disease (CGD) is also being tested. This disease is caused by defective expression of the heavy glycoprotein (gp91 phox, encoded by *CYBB*) in the NADPH oxidase complex. The latter is notably required to generate the oxygen radicals that kill bacteria and fungi engulfed in phagolysosomes (Dinauer, 2019). A lack of NADPH oxidase exposes the individual to a lifelong threat of recurrent severe infections and inflammation (Dinauer, 2019). Although allogeneic HSCT is effective (Güngör et al., 2014), morbidity and mortality rates were up to recently still high in cases with an HLA mismatch. Gene therapy for CGD is a challenge because (i) the expression of NADPH oxidase does not provide the transduced cells with a selective advantage, and (ii) neutrophils, the most relevant transduced cells, have an extremely short life span: a few days, at most. Thus, to achieve the clinical objectives, a considerable number of HSCs must be transduced. Furthermore, the selective myeloid expression of gp91 phox is required to avoid the toxicity associated with ectopic expression. A SIN LV vector containing *CYBB* cDNA under the control of a chimeric promoter (restricting expression to myeloid cells) has been developed, and is being tested in the clinic (Brendel et al., 2018; Kohn, D.B., C. Booth, E.M. Kang, S.-Y. Pai, K.L. Shaw, G. Santilli, M. Armant, K. Buckland, U. Choi, S.S. De Ravin, et al.. 2018. American Society of Hematology 60th Annual Meeting).

Hemophagocytic lymphohistiocytosis (HLH; Ghosh et al., 2018; Panchal et al., 2018; Soheili et al., 2016), immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX, due to FOXP3 deficiency; Masiuk et al., 2019) and leukocyte adhesion deficiency are other potential targets. A clinical study for the latter disease has been initiated.

Conclusions and the future

After 20 yr, efficient (and now safe) gene therapy approaches have been developed for two SCIDs and WAS. Technological progress has generated tools that are likely to extend the indication for gene therapy to many other PIDs, as briefly discussed above. There are still limitations, however. The type of vector and envelope, the transduction protocol, the cell dose, the administration mode, the conditioning regimen, and the characteristics of the disease itself are all-important parameters that require further exploration. Reagents that displace HSCs from their niches in the absence of chemotherapy would be extremely valuable tools, for instance based on the usage of an anti-KIT antibody (Kwon et al., 2019). In the long term, reconstitution of an adequate stroma niche for HSCs at the time of transduction (Nakahara et al., 2019) might make the transduced cells more robust. The in vitro expansion of HSCs (Wilkinson et al., 2019) and their induction from pluripotent stem cells or another cell source could be alternative options, provided that (i) the techniques can be used safely, and (ii) adult HSCs (and not only embryonic-type HSCs) are readily generated.

The application of genome editing to SCID and other PIDs is obviously an attractive option, because the end product would (in most cases) result from expression of the right gene in the right place, i.e., in its physiological environment (Porteus, 2019; Naldini, 2019). This is important for both safety and efficacy. Ultimately, the most interesting application of genome editing would be the treatment of diseases caused by the mutation of a gene that is under strict transcriptional regulation (such as CD40L, or perhaps the RAG-1 and RAG-2 genes; Table 1), to preserve physiological expression patterns. The latter may also be critical for genes encoding kinases (such as JAK-3, which is deficient in a form of SCID) to prevent overexpression. When combined with a template for recombination, the available editing tools, i.e., zinc finger nucleases, transcription activator-like effector nucleases, and the bacterial nucleases such as Cas 9 and related proteins, have potential in these applications (Porteus, 2019; Naldini, 2019). In short, SCIDs might again be the first target disease because the genome editing of a small number of HSCs can be clinically sufficient, as discussed above and suggested by preclinical work on SCID X1 (Schiroli et al., 2017;

Table 1. **Gene therapy for PID, from SCID to other diseases**

	Selective advantage	Lifespan of transduced cells	Gene expression profile	Gene expression regulation
SCID	+++	++ (T)	Ubiquitous[a]	Loose regulation[b]
WAS	+	++ (T), − (platelets)	Ubiquitous	Loose regulation
HLH	−	+	Restricted	Loose regulation
IPEX	−	+	Restricted	Loose regulation
CGD and other myeloid defects	−	−	Restricted	Loose regulation
CD40L deficiency	−	+	Restricted	Tight regulation
XLA (BTK deficiency)	+	+/− (B)	Restricted	Tight regulation[c]

CD40L, CD40 ligand; XLA, X-linked agammaglobulinemia.

[a]Some genes associated with SCID have a restricted expression pattern, i.e., RAG-1, RAG-2, CD3 subunit.

[b]RAG-1, RAG-1 expression is limited in time. Permanent expression might be a problem in gene therapy of SCID caused by RAG-1 or RAG-2 deficiency.

[c]Overexpression of BTK might be harmful.

Pavel-Dinu et al., 2019). Of course, potential issues like off-target activity will have to be monitored carefully. It remains to be seen whether genome editing in SCID will be more beneficial than "conventional" additive gene therapy. Extension to other PIDs in which more HSCs (as in WAS) or many more HSCs (as in CGD) have to be corrected remains a daunting task. Indeed, correcting a mutation requires the introduction of a template for a homologous recombination (HR) event, while HR does not occur in noncycling cells, including most HSCs. Meanwhile, genome editing could perhaps be used to fix mutations in T cells in the context of HLH or IPEX. By virtue of their proliferation capacity, T cells will be much more prone to efficient recombination events. Lastly, gene (exome) inactivation of a gain-of-function mutation might be easier to achieve. In the future, targeting with an inactive Cas 9 base-editing enzyme to DNA cytosine or adenosine to be modified does not require HR. However, this approach may lead to a variety of off-targets on DNA and RNA (Rees and Liu, 2018; Grünewald et al., 2019).

Therefore, the development of gene therapy of PID is still dependent on the resolution of several significant bottlenecks. Looking back over the last 20 yr, however, we can be relatively certain that significant progress will be made over the next 20 yr, perhaps as a result of unexpected advances in basic science. We may thus expect to see the outcome of numerous gene therapy products available to treat PID in the path of Strimvelis. Affordability by societies of these therapies will also have to be secured (Fischer et al., 2019).

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