Gene Therapy for X-Linked Severe Combined Immunodeficiency: Where Do We Stand?

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More than 20 years ago, X-linked severe combined immunodeficiency (SCID-X1) appeared to be the best condition to test the feasibility of hematopoietic stem cell gene therapy. The seminal SCID-X1 clinical studies, based on first-generation gammaretroviral vectors, demonstrated good long-term immune reconstitution in most treated patients despite the occurrence of vector-related leukemia in a few of them. This gene therapy has successfully enabled correction of the T cell defect. Natural killer and B cell defects were only partially restored, most likely due to the absence of a conditioning regimen. The success of these pioneering trials paved the way for the extension of gene-based treatment to many other diseases of the hematopoietic system, but the unfortunate serious adverse events led to extensive investigations to define the retrovirus integration profiles. This review puts into perspective the clinical experience of gene therapy for SCID-X1, with the development and implementation of new generations of safer vectors such as self-inactivating gammaretroviral or lentiviral vectors as well as major advances in integrase knowledge.

INTRODUCTION

GENE THERAPY has proven to be a powerful strategy for the complementation of several monogenic, inherited diseases of the hematopoietic system. The several “back and forth” steps between the bench and the bedside over the last 20 years—emphasizing the complexity of this molecular medicine—have shown how important it is to integrate knowledge from a variety of complex fields when seeking to enhance therapeutic efficacy and reduce potential side effects. Here, we review the biological and medical history of this field, describe the main achievements to date, and summarize the remaining challenges in terms of safety, long-term reconstitution, and applications outside the field of severe combined immunodeficiency (SCID).

THE PATHOPHYSIOLOGY OF X-LINKED SCID

X-linked severe combined immunodeficiency (SCID-X1) is caused by mutations of the γ_c -encoding

gene and accounts for 30–40% of patients with SCID.¹ The γ_c chain is shared by several hematopoietic cytokine receptors, including the interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors.² Whereas defective IL-7 and IL-15 pathways are responsible for the early block in T and natural killer (NK) cell differentiation, respectively, abrogation of IL-21 receptor functions is the substrate for humoral dysfunctions. The IL-21 receptor is a major factor in the survival and proliferation of memory/switched B cells and (in contrast to the IL-4R) cannot be functionally replaced by other receptors.^{3,4} In view of the many physiological roles of γ_c , SCID-X1 is characterized by (1) the absence of circulating T and NK cells and (2) normal to elevated numbers of poorly functional mature B cells.⁵ Moreover, patients with SCID-X1 treated by allogeneic hematopoietic stem cell transplantation (HSCT) show a high incidence of human papillomavirus (HPV) disease, with a median onset of 8 years

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posttransplantation. The fact that patients with other forms of SCID do not have any signs of HPV disease implies that the lack of a common cytokine receptor γ chain or the lack of Janus kinase-3 (JAK-3) are the only genetic factors that predispose to this infection. It also suggests that γ_c /JAK-3-dependent signaling in keratinocytes has a role in anti-HPV immunity.⁶

Ever since gene therapy was first envisaged as a treatment for hematopoietic diseases, SCID-X1 has been considered a good model in view of (1) the severity of the disease, (2) the expectation that the restoration of γ_c expression will confer a selective advantage on the transduced lymphoid progenitors, (3) the long life span of T cells (with the potential for prolonged benefit in treated patients), and (4) the constitutive expression of the γ_c chain in all hematopoietic lineages (prompting researchers to hope that no adverse effects would be observed, even in the absence of tight regulation of the transgene by its own promoter). In fact, the γ_c chain forms a part of complex dimeric or trimeric receptors, and its function is controlled by the other subunits.

Moreover, the strong selective advantage expected in the context of this primary immunodeficiency is also based on the availability of T cell niches within the thymus (due to the absence of pro-T cell precursors). All these expectations have been confirmed by the results of gene therapy trials in SCID-X1.

PRECLINICAL EXPERIMENTS

Gammaretroviral vectors with long terminal repeat (LTR)-driven transgene expression were initially chosen for the stable integration of a functional copy of γ_c into the genome of CD34⁺ hematopoietic stem/progenitor cells (HSPCs). There were several reasons behind this choice:

1. Gammaretroviruses were the first retroviruses to be fully sequenced (in the second half of the 1990s).⁷
2. Their sequencing facilitated the establishment of stable packaging cell lines for the production of defective retroviral vectors (i.e., free of replication-competent retroviruses, which were considered at that time to represent the main risk of insertional mutagenesis).⁸
3. The integration pattern was expected to be random, and thus gammaretroviruses were expected to target primarily noncoding regions of the genome.

A second step toward the first clinical trial of SCID-X1 gene therapy resulted from better fundamental knowledge of the factors that drive the

survival and proliferation of HSPCs. The cloning of FLT-3 ligand (FLT-3L) and thrombopoietin significantly facilitated the use of the cytokine cocktails (which also included IL-3 and stem cell factor [SCF]) that improve the induction of CD34⁺ cell division and boost HSPC survival.^{9,10} When combined with the fibronectin fragment RetroNectin, the use of these cytokines raised the transduction efficiency with human CD34⁺ cells from 1–3% to as much as 30%—prompting us to thinking that it was time to move into the clinic.^{11,12}

Extensive *in vitro* experiments and *in vivo* complementation experiments in γ_c knockout mice enabled us to assemble the preclinical data required for submission of the first clinical trial protocol (which was approved in January 1999).^{13–16}

CLINICAL RESULTS

Table 1 summarizes the results of the various clinical trials. The first clinical trial of gene therapy for SCID-X1 was performed at Necker Children's Hospital (Paris, France) between 1999 and 2002. It was based on a conventional, amphotropic, murine leukemia virus (MLV)-based vector in which γ_c gene expression was driven by the LTR.^{17–19} Ten children under the age of 1 year were enrolled; all lacked an HLA-identical sibling. This trial was followed by a similar one with a gibbon-ape leukemia virus (GALV)-pseudotyped retrovirus (also performed on 10 patients) at Great Ormond Street Hospital (London, UK).^{20,21} Both trials omitted preconditioning of the patients before gene therapy.

When considering the total of 20 patients enrolled in the two trials, 17 had a stably corrected immunological phenotype.^{17–21} The time course and robustness of T lymphocyte reconstitution were reproducible (regardless of a patient's age and clinical status) but were directly related to the number of infused CD34⁺ γ_c ⁺ cells. In some patients, circulating CD3⁺TCR $\alpha\beta$ ⁺ could be detected (between 10 and 100/ μ l) as early as 1 month after the injection of transduced autologous CD34⁺ cells. Normal counts were achieved between 3 and 6 months posttherapy and remained stable over time for all patients (except those who developed monoclonal lymphoproliferative disease; see below). Circulating T cells expressed surface γ_c to the same extent as (or a slightly lower extent) than control T cells. Functional characteristics of the T cell population were also satisfactory. The cells proliferated *in vitro* in response to mitogens and vaccine antigens, and provided effective protection against infection *in vivo* (as evidenced by recovery from infections during the first few months after gene therapy).^{19,20}

Table 1. Summary of X-Linked severe combined immunodeficiency gene therapy trials

Vector	Centers	Pseudotyping	Conditioning	Inclusion criteria	Patients (n)	Results	SAEs	Ref(s)
Gammaretro	France	Ampho	No		10	Stably corrected immune function (T cells)	4 T-ALL	17, 19, 22
Gammaretro	UK	GALV	No		10	Stably corrected immune function (T cells)	1 T-ALL	20, 21, 24
Gammaretro	USA	GALV	No	Patients >10 yr old, post-HSCT	3	Partial clinical benefit	None	28
SIN-gammaretro	France	GALV	No	No HLA-genotypical donors Severe, ongoing, treatment-refractory infections	9	Stably corrected immune function (T cells)	None	27
SIN-lenti	UK, USA USA	VSVg	Nonmyeloablative busulfan	No HLA-genotypical donors Patients >2 yr old, No HLA-genotypical donors, mostly post-HSCT	5	Early immune recovery (T, B, and NK cell correction)	None	31

ampho, amphotropic; GALV, gibbon-ape leukemia virus; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; lenti, lentivirus; retro, retrovirus; NK, natural killer; SAEs, serious adverse events; SIN, self-inactivated; T-ALL, T-cell acute lymphocytic leukemia; VSVg, vesicular stomatitis virus G glycoprotein.

A spectratype analysis revealed a fully diversified TCR- V_{β} and TCR- V_{α} repertoire. Remarkably, 13 years after treatment, thymopoiesis is still ongoing in most patients (including those who developed leukemia and subsequently received chemotherapy), as evidenced by the stable detection of TCR excision circles in naive T cells.

In contrast to the reconstitution of the T cell lineage, reconstitution of NK cells was much weaker; 1 year after gene therapy, the NK cell counts had barely reached 10% of the control value (as is also observed in an allogeneic HSCT setting). This might have been due to weaker proliferative and/or survival capacities of the NK cell progenitors, relative to those of T cell progenitors. The patients' humoral functions have been partially restored, despite the continuing absence or undetectable level of transduced B cells.¹⁹ Accordingly, some of the patients no longer require immunoglobulin replacement therapy (five of eight patients in the Paris trial).¹⁹ However, virtually all of the patients show a defect in the memory B cell subpopulation and thus in the production of high-affinity antibodies and the response against recall antigens. These results (which are similar to those observed after allogeneic HSCT in the absence of myeloablation) indicate that the development of full humoral responses and the long-term maintenance of normal levels of memory B cells can be achieved only by stable engraftment of genetically modified HSPCs. This requires access to the hematopoietic niches by conditioning the patient before transplantation.¹⁹

Taken as a whole, these data demonstrate that genetic correction of T cell immunity restored the patients' general health status and enabled them to lead a normal life with long-lasting beneficial effects (median follow-up, 13 years).

Unfortunately, four patients in the French study and one patient in the U.K. trial developed T cell

leukemia 2 to 5.5 years after gene therapy. Four of them remain in long-term remission after chemotherapy^{22–24} and have fully recovered after treatment—thanks to good immunological reconstitution by the residual γ_c -corrected bone marrow CD34⁺ cells or thymic progenitors with a high self-renewal ability.^{19,20} The remaining patient died of chemotherapy-refractory leukemia, despite the deployment of a range of therapeutic approaches (conventional chemotherapy; the generation and administration of a specific monoclonal antibody directed against the γ_c -expressing leukemic clone, complemented with allogeneic transplantation from an unrelated, matched donor).^{19,22,23} In all cases, the adverse event was the result of insertional oncogenesis. Genetic analysis of the malignant cells showed that the retroviral vector had integrated within or near tumor-promoting genes (mainly the LIM domain only-2 gene, *LMO2*) and had caused transcriptional activation.

To improve the safety profile of gene therapy for SCID-X1 while maintaining the excellent immunologic outcome seen in previous trials, the original vector (MFG- γ_c) was modified to create a self-inactivating (SIN) gammaretrovirus in which (1) the *IL2RG* cDNA is under the control of the weak, human elongation factor-1 α short promoter, and (2) the strong viral enhancers in the LTRs have been deleted.^{25,26} This vector was used in an international study with participating centers in Paris, London, Boston, Cincinnati, and Los Angeles. In contrast to the first SCID-X1 trials, this trial was restricted to patients with severe, ongoing, treatment-refractory infections (for whom the prognosis after allogeneic HSCT would have been poor). In London and Boston all patients without an allogeneic HLA-identical donor have been treated without further restriction. A preliminary report on the first nine patients enrolled

since December 2010 was published.²⁷ All nine nonconditioned patients (aged between 3.9 and 10.5 months) received bone marrow-derived CD34⁺ cells transduced with the same GMP-grade SIN gammaretrovirus supernatant. After up to 48 months of follow-up, the overall survival was excellent: eight of the nine patients were alive and well but one had died of an overwhelming adenoviral infection (present on study entry) 4 months after gene therapy (i.e., before full reconstitution by genetically modified T cells). Of the remaining eight patients, seven displayed full T cell reconstitution and normalized T cell proliferation. Early immune recovery was as rapid as in the previous trials, but humoral function was not restored in these patients. An insertion site analysis of the patients' peripheral blood mononuclear cells revealed significantly less clustering within proto-oncogenes and genes involved in the serious adverse events (SAEs) in previous gene therapy trials²⁷—indicating that this modified SIN gammaretroviral vector is efficacious in the treatment of SCID-X1 and may have a better safety profile.

In addition to these 29 typical patients with SCID-X1 treated a few months after birth, gene therapy was attempted in five older patients with SCID-X1 (aged 10–20 years) as a rescue treatment option after HSCT failure. Three of them were included in the trial conducted at the National Institutes of Health (NIH, Bethesda, MD) in 2003.²⁸ No significant clinical benefit was achieved in these subjects; this was likely due to the age-dependent loss of thymic activity and/or a history of graft-versus-host disease (GvHD) and chronic infection.^{28,29}

Another clinical trial in SCID-X1 (based on an SIN lentiviral vector expressing the γ_c gene) is ongoing at St. Jude Children's Research Hospital (Memphis, TN) and at the NIH; the preliminary results appear to be promising.^{30,31}

After more than 16 years of follow-up for the first treated patients with SCID-X1 and more than 5 years for the patients in the second trial, some important conclusions can now be drawn:

1. The robustness of T cell reconstitution (i.e., required for a protective, stable T cell count) is directly linked to the number of transduced CD34⁺ γ_c ⁺ cells infused into the patient.
2. Patients with SCID-X1 massively infected with opportunistic pathogens run a high risk of dying soon after transplantation—even in the context of a successful gene-modified autologous transplantation. In this setting, methods for further accelerating T cell generation are needed (see below).

3. In the absence of myeloablation, ongoing normal B cell differentiation constitutes an obstacle to efficient recovery of humoral immunity.
4. A gene therapy approach is more likely to succeed in patients less than 1 year of age, because older patients present with thymus damage as a result of a long history of infections (and/or GvHD in those having first undergone HSCT).

With a view to gathering further evidence on the efficacy of immune reconstitution, we compared the results of gene therapy with those of conventional treatment (i.e., haploidentical allogeneic HSCT). Besides the higher rate of severe complications observed in patients who underwent haploidentical HSCT, the results for naive T cell and total CD3⁺ cell counts up to 5 years posttherapy³² argue in favor of gene therapy. The results emphasized the importance of continuous, clinical evaluation of the different approaches in terms of mortality and long-term morbidity when studying monogenic diseases of the lymphohematopoietic system.

TOWARD A COMPREHENSIVE INTEGROME ANALYSIS AND GREATER SAFETY

The development of leukemic SAEs after gene therapy has deeply challenged our understanding of gammaretroviral vector integration and has revealed the acute need for animal models that can predict the genotoxicity of integrating viral vectors. We and others have striven to use various strains of transgenic or knockout mice as animal models that reproduce the clinical observations. Montini and colleagues described a CDKN2^{-/-} model to test for toxicity specifically linked to various vectors.³³ In 2006, Shou and colleagues bred a γ_c ^{-/-} mouse into a p19Arf^{-/-} background and performed gammaretroviral gene therapy experiments for the correction of γ_c ^{-/-} deficiency.³⁴ The additional knockout of the tumor suppressor p19Arf revealed minor oncogenic effects in the course of gammaretroviral gene therapy. It could be shown that in the Arf^{-/-} γ_c ^{-/-} model, the γ_c ^{-/-} background of the donor cells and the transgene γ_c (but not the vector integration itself) have roles in leukemogenesis after retroviral gene therapy. However, these observations have yet to be confirmed. Our laboratory has developed two different models (an LMO2 transgenic mouse and an Arf^{-/-} Rag1^{-/-} tumor-prone gene therapy model) with a view to mimicking clinical observations (J. Hauer, unpublished data).

Neither of the two models could reproduce the SAEs observed in patients. In parallel, a number of other studies of animal models have yielded contradictory results^{35–37}—emphasizing that these models are unable to predict toxic events in clinical trials.

Another approach for tracking insertional mutagenesis is the *in vitro* immortalization assay developed by Baum's group. For example, the assay can reveal clonal imbalance driven by *MDS1* and *EVI1* complex locus protein (MECOM).^{38,39} However, this cell culture assay might not be suitable for detecting all mutagenesis events—especially in the lymphoid lineage. Other assays have also been developed for analyzing more specifically the transcriptional activation of *LMO2*.^{40,41}

Despite major progress in vector safety, the absence of a perfectly reliable *in vivo* or *in vitro* model for predicting SAEs means that we shall continue to adopt a cautious approach in all future clinical trials. It will certainly be essential to combine several different preclinical strategies (such as xenotransplantation and high-throughput integration site analysis) in order to ensure safety.⁴² In this context, the in-depth monitoring of retroviral integration site (RIS) patterns will help us to develop safer clinical trials.

Significant efforts have gone into the optimization of RIS analysis. The goal has been to enable better coverage and to increase sensitivity by using techniques such as ligation-mediated PCR (LM-PCR)⁴³ and linear amplification-mediated PCR (LAM-PCR).⁴⁴ Several drawbacks (such as the requirement for restriction enzymes) have been successfully circumvented by using more random DNA fragmentation with a combination of enzymes, acoustic shearing,⁴⁵ or nonrestrictive LAM-PCR (which works on circular DNA).⁴⁶ Much effort has also gone into the precise analysis of these databases, in order to accurately map RIS positions in the genome.^{47,48} Last, in the case of random shearing, integration sites can be quantified precisely through the enumeration of the various shear fragments containing a given integration site (in contrast to read counts, which can be biased by PCR amplification steps).⁴⁹

Studies of gammaretroviral vector integration in the human genome indicate that this process is not random; each type of retrovirus follows a unique set of precise rules, which explains the biased pattern of integration sites in transduced human cells. The Moloney MLV-derived vectors preferentially integrate into transcriptionally active promoters and regulatory regions, with a symmetrical accumulation around the transcription start site. Moreover, the integrations characteristically ac-

cumulate around enhancer and promoter regions; this is associated with modifications of histones H3K4me3 and H3K9me1, which are involved in chromatin configuration and binding to RNA polymerase II.⁵⁰ Transcriptional enhancers present in the MLV LTR were responsible for the activation of the proto-oncogene; they caused not only T cell acute lymphoblastic leukemia in the first two clinical trials for SCID-X1^{22–24} and in another gene therapy trial for Wiskott-Aldrich syndrome (WAS)⁵¹ but also myelodysplastic syndrome in patients treated for chronic granulomatous disease.^{52,53} This drawback led to the development of two distinct strategies.

The first was based on modified SIN retroviral vectors in which the enhancer regulatory sequences had been replaced by internal promoters capable of inducing limited (and, in some cases, tissue-specific) transcription of the therapeutic gene.^{25,26} In a trial using the SIN gammaretroviral vector, pyrosequencing enabled us to increase the retrieval of RISs by a factor of more than 50 (30,000 sites for the 9 patients treated in this trial,²⁷ relative to the 572 identified in the early MLV γ_c trials⁵⁴). Although the RIS distribution and diversity were similar in the two settings, we found that 19 RIS clusters detected in the first full MLV SCID-X1 trial were significantly less common in the second trial. Interestingly, the greatest reduction in the number of integrations was seen for the *MECOM*, *CCND2*, and *LMO2* clusters, suggesting that integration near these genes and gene activation mediated by the native LTR enhancer had favored clonal expansion. The significant reduction in these clusters suggests that SIN vectors are safer, as also confirmed by the absence of SAEs in the latest results from the corresponding clinical trials.

The second way to increase the safety of gene transfer vectors is based on HIV-derived vectors. This option constitutes a further step toward the prevention of insertional mutagenesis, because the integration profile of the HIV-derived vectors shows that they (1) do not target regulatory elements of the genome and (2) are randomly distributed across actively transcribed genes, with no preference for “dangerous” oncogenes.^{55,56}

Nevertheless, two papers show that lentiviral vector integration is responsible for an aberrantly spliced, chimeric transcript⁵⁷ and that lentiviral vectors integrate into a small chromosomal zone near the nuclear pores,⁵⁸ which further reduces the randomness of integration. It is impossible to say whether these discoveries may have clinical consequences. Integration profile studies in patients with HIV have also been highly informative,

showing the presence of clonally expanded T cells with integration in cancer genes such as *MLK2* or *BACH*.^{59,60} However, the positional effect of these integrations has been challenged by the demonstration that these expanded clones contain defective viruses and were more frequently associated with intergenic regions and lower gene expression levels.⁶¹

The tracking of integration site patterns in different settings will continue to be highly informative. The full integrome is becoming even more accessible by the new high-throughput sequencing approaches, and ongoing developments should enable rapid access to a comprehensive integration repertoire via specific capture approaches and direct genome sequencing.

We and others have shown that it is now possible to map approximately 10,000 RISs per patient in the new lentivirus-based gene therapy trials (for WAS and metachromatic leukodystrophy).^{62–64} The follow-up of these RISs will be critical in terms of both safety and basic science, because it constitutes a unique opportunity to better understand the dynamics of hematopoietic progenitors.

Many barcoding studies in the mouse and in the macaque have tracked various myeloid and lymphoid cell types over time and thus have generated a more precise map of the hematopoietic hierarchy.^{65–69} Similar work in humans can now be performed by efficient RIS analysis, as illustrated in a study highlighting the long-term survival of T memory stem cells.⁷⁰ The long-term follow-up of the dynamics of naive and memory T cell populations in the SCID-X1 trials will also enable us to better understand human thymopoiesis and immune responses. Overall, the clonal tracking of RISs in human gene therapy trials will pave the way to a deeper understanding of human hematopoiesis.

SOME BIOLOGICAL AND CLINICAL COMMENTS, AND EXPECTED IMPROVEMENTS

Self-inactivating vectors have formally demonstrated their improved safety profile (relative to LTR-driven retroviral vectors), as no SAEs have been observed since their first use in clinical trials in 2006.⁷¹ Another dogma has been also deeply challenged by the last 20 years of RIS analysis: changes due to the clonal dominance of a single integration site.⁷² Indeed, the thalassemia patient initially showing a dominant clone in the *HMG2* locus is still free of any complication 8 years after gene therapy⁷³ (M. Cavazzana and S. Hacein-Bey-Abina, unpublished observations). Interestingly, the abundance of this clone progressively decreased during

the follow-up period. A similar observation was made in one of our patients with SCID-X1 treated in the second trial based on the SIN gammaretroviral vector. The patient displayed a dominant clone that varied over time—albeit without any clinical impact.⁷² Furthermore, a growth advantage leading to clonal dominance has been observed in the absence of further transformation toward malignant clones.⁷⁴ Moreover, these observations are in line with arrayed lentiviral barcoding studies in the mouse, which showed that steady-state hematopoiesis can be maintained by a small number of relatively dominant clones.⁷⁵ Clonal hematopoiesis has also been documented in healthy individuals over the age of 65 years, due to somatic mutations, suggesting that clonal dominance is possible in the absence of integration site effects.⁷⁶

Given (1) the current inability to predict adverse events (despite intense work on RIS analysis) and (2) the absence of an accurate animal or *in vitro* model of toxicity, the continuous clinical follow-up of the patients is the only way to monitor the safety and efficacy of new vector designs and other technical improvements. Last, our ability to further increase the vector copy number per cell raises the question of how many vector copies can be “tolerated” without perturbing normal transcription. The development of “safe harbor” integration would obviate the risk of transactivation after integration.

Another major, unresolved concern is the time needed to generate functional T cells that can successfully fight the systemic, opportunistic viral infections responsible for poor outcomes in some patients treated by gene therapy.^{27–29}

One potential strategy (being developed by our laboratory) involves the infusion of T cell precursors able to seed the thymus and differentiate into mature T cells more rapidly than uncommitted CD34⁺ cells. To this end, we have developed an *in vitro* feeder cell-free culture system based on the use of a recombinant, modified DL-4 ligand that allows the generation of large numbers of CD34⁺CD7⁺ T cell precursors within 3–7 days. These precursors resemble their thymic *in vivo* counterparts in terms of the *in vitro* potential and expression profile. Once injected into NOD/SCID/ γ_c knockout mice, T cell precursors generated in this system were able to colonize the thymus and generate a diversified, functional T cell compartment.⁷⁷ In preliminary experiments, the combination of DL-4 culture and transduction of a SCID-X1 patient’s CD34⁺ cells enabled the production of gene-corrected T cell precursors. The latter rapidly generated a wave of mature T cells *in vitro* and

then *in vivo* in NOD/SCID/ γ_c knockout mice—suggesting that this strategy shortens T cell reconstitution, relative to uncultured CD34⁺ counterparts.

Because gene therapy has proved its effectiveness in several hereditary diseases of the hematopoietic system, the next major milestone will be the long-term safety of the new vectors under investigation. Another challenge is to develop a process that can be disseminated worldwide, that is, to regions of the world where some of the hereditary diseases that might be approached by gene therapy are more prevalent.

Since the first clinical trial in which we defined the whole gene therapy procedure,⁷⁸ a number of small and nonsignificant changes have been introduced into the transduction process. Hundreds of papers have been published on various cytokine cocktails, reagents, and drugs expected to make CD34⁺ cells cycle without losing their self-renewal capacity. A technical breakthrough has been described by Verhoeyen's group. They demonstrated that a baboon envelope pseudotyped lentiviral vector transduces more than 90% of CD34⁺ cells in 24 hours in the presence of low cytokine concentrations.⁷⁹ The impact of *ex vivo* cell culture and stimulation itself also appear to be difficult to measure. In light of the most recent publications, it would be useful to have a culture procedure that did not require active stimulation of human HSPCs.

Automation of the procedure would also constitute progress, so that any transplantation unit in

the world would be able to perform gene correction on autografts independently.

The development of an industrial process would be a further step toward the generalization of gene therapy for the many patients with hereditary diseases of the hematopoietic system. It would also pave the way to gene therapy for diseases outside the hematopoietic system.

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AUTHOR DISCLOSURE

There are no conflicts of interest.

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