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Review

Induced pluripotent stem cells and severe combined immunodeficiency: merely disease modeling or potentially a novel cure?

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For most, but not all, types of severe combined immunodeficiency (SCID) the underlying molecular defects are known, in principle allowing the cure of affected children via gene therapy. Typically such approaches have used autologous hematopoietic stem cells modified to express a therapeutic gene via γ-retroviral vectors. Insertional mutagenesis has emerged as a significant risk for successful application of this type of gene therapy. Therefore, lentiviral vectors with a self-inactivating design have been developed. Recent advances in stem cell technology using induced pluripotent stem cells (iPSCs) allow an entire different approach to gene therapy for SCID and other genetic disorders, namely by correction of the affected gene in patient-specific iPSCs followed by hematopoietic differentiation. Here, we review these recent advances in the field from an efficacy and safety point of view.

SEVERE COMBINED IMMUNODEFICIENCY

Immunodeficiencies invariably refer to defects in the immune system that lead to an increased risk of infections. Severe combined immunodeficiency (SCID) is a heterogeneous disease characterized by lack of T lymphocytes and sometimes also B and/or natural killer (NK) cells (1). Most infants develop opportunistic infections within the first 6 mo of life. The diagnosis is established by detecting lymphopenia, absence or very low numbers of T lymphocytes, and impaired T-cell proliferative responses to mitogens.

A number of genetic abnormalities can cause SCID. Worldwide, the most common form of SCID is X-linked SCID, caused by mutations in the gene coding for the Il2Rγ chain, resulting in SCID with a $T^-B^+NK^-$ phenotype, referring to the lack of T lymphocytes and NK cells, but the presence of B lymphocytes in these patients. The incidence is estimated to be roughly 1 in 65,000 live births (2) The lymphocytes of patients with X-linked SCID cannot respond to the several essential cytokines (interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21) needed for these cells to develop, survive, and fight infections. Although B cells are present, their function is severely impaired, not only because of a lack of T-cell help but also because of intrinsic B-cell defects.

Other forms of SCID are those with underlying deficiencies in the adenosine deaminase (*ADA*) gene, recombinase-activating genes (*RAG*), Artemis, or more rarely in the *CD3* genes, *ZAP70* and *IL7R* (**Figure 1**). For many types of SCID, the underlying molecular defect is unknown. ADA-SCID patients fail to make T cells, B cells, and NK cells, experience recurrent infections, and fail to thrive (3). The ADA enzyme is found throughout the body but is most active in lymphocytes. ADA converts deoxyadenosine into nontoxic deoxyinosine. Mutations in the *ADA* gene allow the buildup of deoxyadenosine to levels that are toxic to lymphocytes, in particular immature thymocytes.

The second or third most common form of SCID (depending on the genetic background of the population) is RAG-negative SCID. These patients have mutations in *RAG1* or *RAG2*, which are required for the assembly of the T-cell receptor and B-cell receptor (4–6). As a consequence, RAG-SCID patients lack B and T cells and develop many serious, life-threatening infections, especially pneumonia, meningitis, and sepsis, as neonates.

Replacing the affected bone marrow with allogeneic healthy (stem) cells is currently the only established therapy for SCID. However, this treatment is complicated by adverse immune reactions of the donor cells, a slow immune reconstitution, and a lack of suitable donors for most patients. An alternative to allogeneic stem cells is genetically modified autologous stem cells in which the genetic defect is functionally corrected (i.e., by gene therapy). It should be noted, however, that also for SCID, haploidentical stem cell transplantation has become a valuable option for patients lacking a human leukocyte antigen–identical donor (7). These issues have mainly been worked out in children with leukemia, for whom suitable donors can often be found, using one of the parents, if necessary. However, delayed immune recovery and the risk of graft-vs.-host disease remain substantial problems that warrant gene repair via gene therapy with autologous cells as an alternative.

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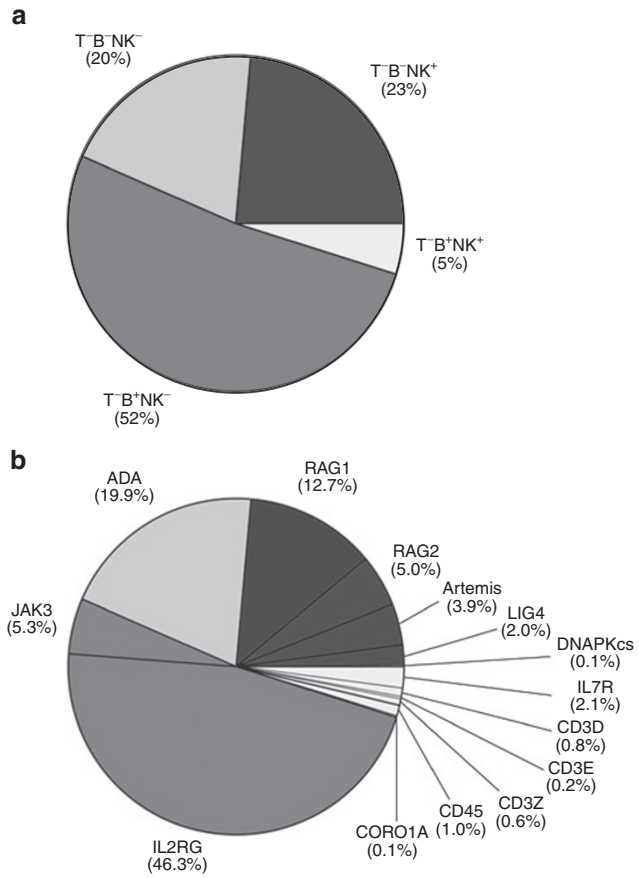

Figure 1. Mutation frequency in genes involved in severe combined immunodeficiency (SCID). Relative distribution of mutations in the genes known to be affected with SCID. (**a**) Genes are grouped according to the resulting absence and presence of B and natural killer (NK) cells. (**b**) The mutation frequencies of individual genes are plotted. Data were obtained from http://bioinf.uta.fi/IDbases/, http://www.esid.org/, and known cases in the literature.

GENE THERAPY FOR SCID

Seminal work by Fischer and Cavazzana-Calvo (8) in Paris as well as Thrasher and Gaspar (9) in London for X-linked SCID and Bordignon and Aiuti (10–12) in Milan for ADA-SCID, has shown the clinical efficacy of gene therapy for various types of SCID using gene-corrected autologous stem cells. This work is covered by a number of excellent reviews (13–18), but will be briefly discussed here because of its relevance for other approaches of gene therapy using novel types of stem cells.

The two gene therapy trials for X-linked SCID have shown the clinical feasibility of introducing a therapeutic gene into hematopoietic stem cells (HSCs) (8,9). Both X-linked SCID trials have been highly successful in many ways, showing long-lasting restoration of immunity. Immunodeficiency was restored and lymphocyte development was no longer blocked. However, the development of leukemia has appeared as a severe adverse effect in both trials (19). In all five reported cases (n = 4 of 10 children in the Paris trial and n = 1 of 10 in the London trial), T-cell acute lymphoblastic leukemia occurred as a direct consequence of insertional mutagenesis by the retroviral vector used to deliver the therapeutic gene (20–24). Such severe adverse effects have not been reported in any of the Italian ADA-SCID patients; however, in a German trial using similar technology for Wiskott–Aldrich syndrome, one similar serious adverse event resulting from insertional mutagenesis has been reported (25).

In all of these cases of leukemia development, the strong viral promoter/enhancer sequences in the long terminal repeat of the vector that was used to deliver the therapeutic gene activated a cellular oncogene upon insertion in the host genome (**Figure 2**). In most instances, the insertion affected the *LMO2* gene, a known T-cell acute lymphoblastic leukemia oncogene, which can block human thymopoiesis in an apparent preleukemic stage (26,27). Recent work by Thrasher and coworkers demonstrated that the insertion near *LMO2* is the first step in a multistep leukemogenesis program, involving somatic mutations in *NOTCH1* and other leukemic events such as loss of the *Arf* tumor suppressor gene, collectively leading to full-blown leukemia (19).

It is hoped that the development of novel vectors, especially those in which the viral promoter/enhancer sequences have been rendered inactive (self-inactivating vectors), will significantly reduce the incidence of insertional mutagenesis. This will likely promote the safety and thus further clinical development of cells, which are genetically modified. Most investigators in the field have moved to such vectors, either in a γ-retroviral or lentiviral (HIV) backbone. For instance, self-inactivating lentiviral vectors have been developed in preclinical models for ADA-SCID (28), RAG-SCID (29,30), Artemis-SCID (31,32), and agammaglobulinemias (33).

Another new development is the use of zinc-finger nucleases (34–36) or transcription activator–like effector nucleases (TALENS; ref. 37) to introduce specific genetic modifications in host genomes by homologous recombination (**Figure 3**). Gene correction of the endogenous genetic loci through homologous recombination overcomes the problems of insertional mutagenesis. However, in adult stem cells such as HSCs, this has proven to be technically challenging because the efficiency is low and HSCs cannot be efficiently expanded. In contrast, human and murine embryonic stem cells (ESCs) have *in vitro* unlimited self-renewal capacity, which would enable efficient genetic correction in a dish. Although adults do not possess pluripotent stem cells, recent exciting developments might have created novel opportunities for the treatment of SCID (38).

PLURIPOTENT STEM CELLS

Pluripotency is characterized by the ability to give rise to any cell type of our body. The best-characterized pluripotent stem cells are ESCs, which theoretically can self-renew indefinitely. The only way to isolate these cells is by culturing blastocysts and isolating the inner cell mass. The derivation of human ESC lines is, however, hindered by ethical concerns. Stem cell researchers have long been interested in generating pluripotent stem cells in an alternative way (39). In particular, a method to derive pluripotent cells from somatic cells would create tremendous opportunities. Such a

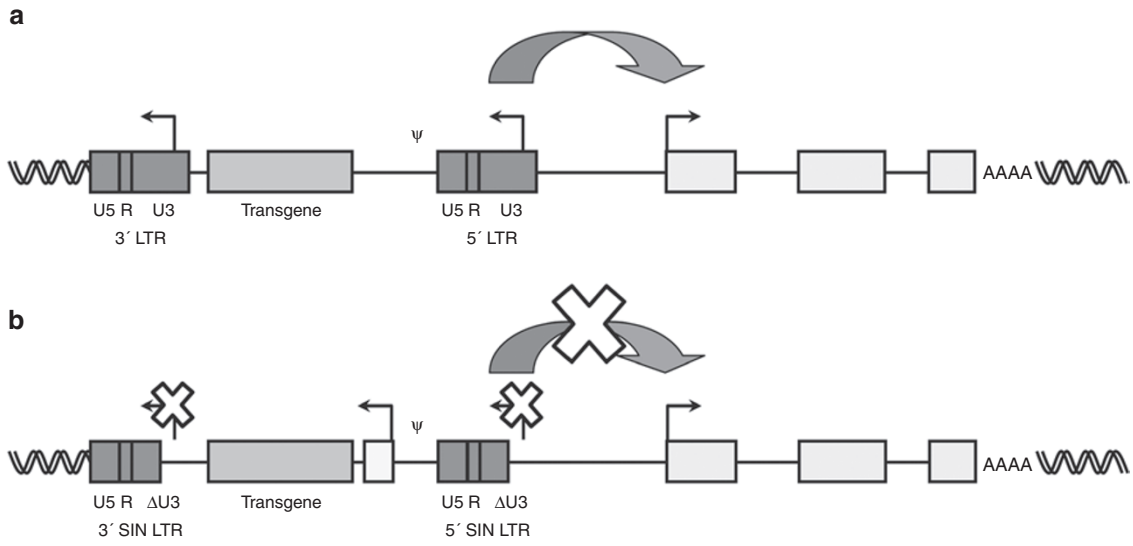

Figure 2. Insertional mutagenesis of integrating retroviral vectors. (**a**) An example of enhancer mutation as it may occur with a γ-retroviral or lentiviral vector integrated into the host genome. When integrated near a cellular gene, the enhancer element in the viral long terminal repeat (LTR) can upregulate this gene. U3, unique 3′ regulatory sequences; R, repeat sequence; U5, unique 5′ regulatory sequences; Ψ, packaging signal. (**b**) In the self-inactivation (SIN) versions of γ-retroviral or lentiviral vectors, viral enhancer and promoter sequences are deleted from the U3 region (ΔU3), eliminating the potential for activation of cellular genes by the viral LTRs. In this setting, an internal promoter is needed to drive the transgene expression.

method would not only bypass the ethical issues associated with ESCs but also allow the isolation of pluripotent stem cells from individuals with a specific genetic background. In addition, somatic cell–derived pluripotent cells create unique possibilities for cell replacement strategies because pluripotent stem cell banks that represent most haplotypes provide an inexhaustible source of any cell type. Provided that functional and safe HSCs can be generated from the banked pluripotent stem cells, congenital blood disorders that are currently only cured by bone marrow transplantations could be treated through this alternative source.

INDUCED PLURIPOTENT STEM CELLS

In 2006, Takahashi and Yamanaka used a simple but luminous approach to induce pluripotent stem cells from mouse tail-tip fibroblasts (40). Exploiting a candidate gene–based screen, they identified a tetrad of transcription factors—Oct4, Sox2, Klf4, and cMyc—which are sufficient to revert a fibroblast into a state mirroring pluripotency. Although these first so-called induced pluripotent stem cells (iPSCs) did not fully behave like mouse ESCs, it was a crucial step forward to the immediate generation of pluripotent stem cells from somatic tissues. Soon after, cells that resembled mouse ESCs in all their facets were generated from tail-tip fibroblasts (41). These cells are pluripotent *in vitro* and *in vivo*. They generate teratomas containing derivatives of all three germ layers and contribute to all tissues during mouse development in chimeric mice, and iPSC-derived germline cells give rise to new offspring. Even the most stringent pluripotency test available for mouse cells (i.e., the ability of one single mouse iPSC to create a complete, viable, and fertile mouse upon injection into a blastocyst of tetraploid cells (tetraploid complementation)) was successfully passed by a number of iPSC lines (42–44). iPSCs are currently routinely being produced from somatic cells from different species, including humans, and from several tissues (45–47). Although the gene cocktail that induces the pluripotent state may differ, the resulting iPSC lines are similar to the existing ESC lines with respect to behavior, epigenome, transcription profiles, and proteome.

SAFETY OF iPSCs

Many patient-specific iPSC lines have been created and their number is rapidly increasing (48). These lines can be used to model diseases in a dish as well as *in vivo*. However, the first generation of iPSCs was made using conventional retroviral and lentiviral vector technology. These vector systems insert the genome of the host cells, which may act as insertional mutagens by altering endogenous gene expression, similar to the adverse effects observed in gene therapy trials.

Reprogramming into a pluripotent state, however, is dependent on the complete silencing of the pluripotency genes. Because most of the pluripotency genes have been associated with tumorigenesis, incomplete silencing or reactivation of the inserted proviruses can cause tumors (49–52). As a consequence, iPSCs used for *in vivo* disease modeling should preferentially be generated by different methods, and when future therapeutic applications are considered, nonintegrating delivery systems are required. Safe vector systems alone are not sufficient. It is largely enigmatic whether the whole reprogramming and culturing procedure affects the (epi)genome of iPSCs. Genetic drift has been reported in iPSC lines as copy number variations altered during iPSC generation and culture (53,54). Whether the copy number variation changes constitute harmless natural selection occurring in any stressed and fast-growing cell population or represents a potentially dangerous selection process requires further investigation. Karyotyping has

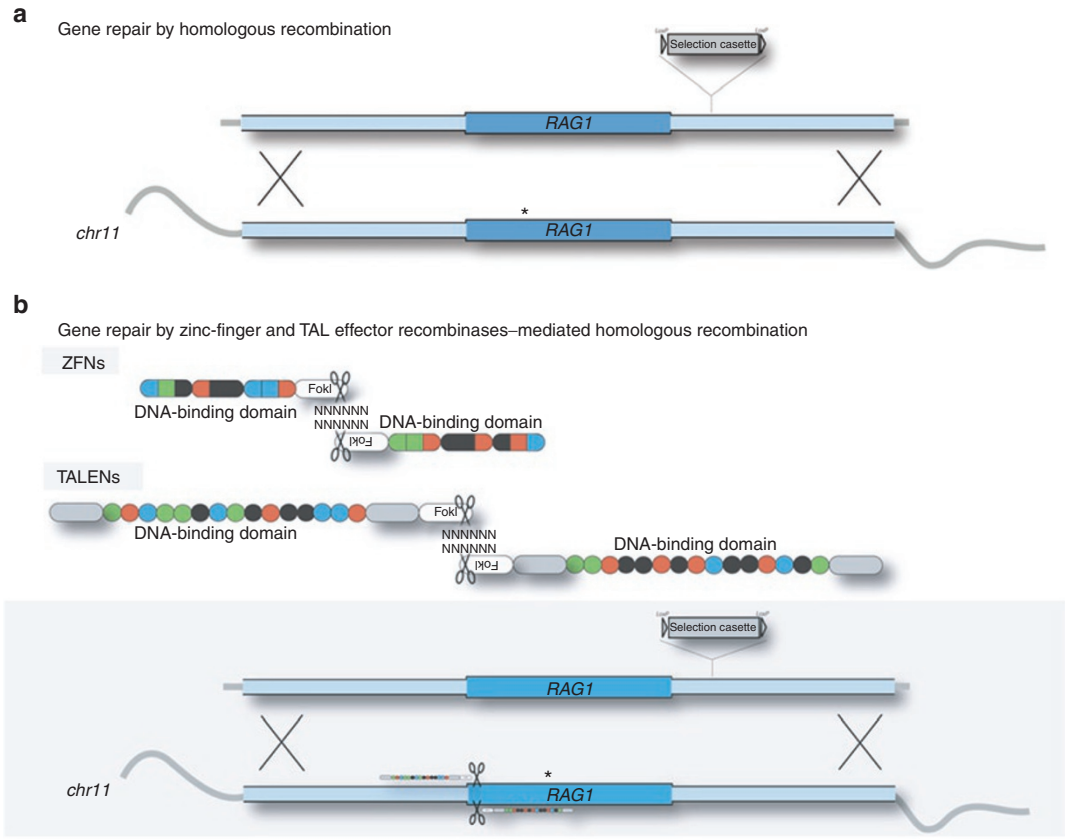

Figure 3. Repair of endogenous genes through homologous recombination. (**a**) Gene repair by homologous recombination. Conventional homologous recombination repair approaches are based on bringing a linearized targeting vector, in which a selection cassette is flanked by genomic sequences that are homologous to the locus/gene to be repaired, into the mutant cells. Proper homologous recombination events are rare in this setting, and positive selection of the cells using antibiotic selection markers is required to identify cells that have been correctly repaired. The strategy to repair one of the *RAG1* mutant alleles (chromosome 11) in the case of RAG1-SCID is depicted. (**b**) Gene repair by zinc-finger and transcription activator–like (TAL) effector recombinases–mediated homologous recombination. Engineered nucleases that bind and cleave the genomic DNA at specific places enhance the frequency of the homologous recombination. Two of these nucleases are zinc-finger nucleases (ZFNs) and TAL effector nucleases (TALENs). Both engineered proteins contain the *Fok*I nuclease domain, causing double-strand breaks, linked to a DNA-binding domain. DNA specificity is generated by either 3 zinc-fingers recognizing 9 nucleotides (ZFNs) or 15 TAL effector–derived repeat variable di-residues recognizing 15 nucleotides (A, green; T, red; G, black; C, blue). A TALEN strategy to repair one of the *RAG1* mutant alleles (chromosome 11) is depicted.

also revealed abnormalities in late-passage iPSC lines (55). In addition, single-point mutations potentially yielding a selective advantage have been observed in iPSC lines (56). These data may be caused by imperfect culture conditions or reprogramming stress but underscore the need for better characterization of iPSCs, their generation, and culture. Beside genomic issues, iPSCs may evoke an immune response upon transplantation of iPSCs as shown in a study using a syngeneic teratoma model (57). In conclusion, there are many safety issues concerning the generation and use of iPSCs that must be addressed before they can be utilized in transplantation settings.

THERAPEUTIC POTENTIAL OF iPSCs

Despite the uncertainties surrounding future iPSC use for cell replacement therapies, a proof-of-principle study using mouse iPSCs has clearly demonstrated the potential of iPSCs for therapeutic cell replacement purposes. In this study, iPSCs were derived from humanized sickle cell mice, in which the mouse α-globin genes were replaced with the human sickle

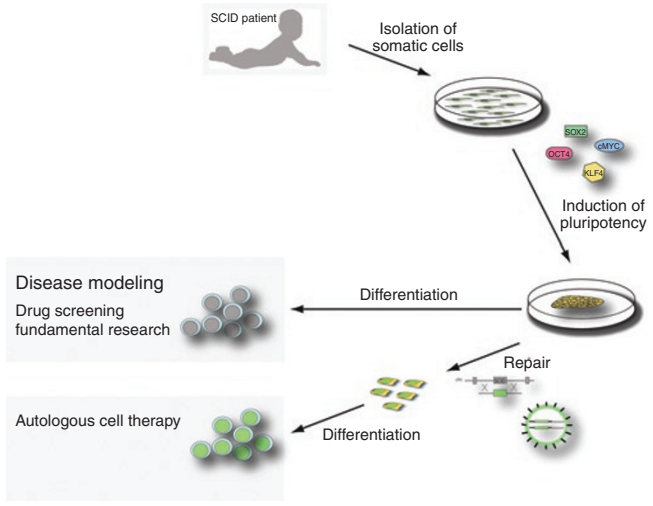

Figure 4. Use of SCID-specific induced pluripotent stem cells (iPSCs). Schematic representation of the use of iPSCs for severe combined immunodeficiency (SCID) diagnosis, drug screening, disease modeling, and therapy.

Table 1. Potential use of and obstacles for iPSCs in PIDs

Potential use of iPSCs for PIDs	Current obstacles for efficient usage
Disease modeling	Robust lymphocyte and HSC differentiation protocols do not yet exist
Drug screening	Human iPSC-derived HSC-like cells fail to repopulate in xenograft models
Therapy: iPSC-derived HSCs (iPSC banking)	Availability of clinical material
Therapy: transplantation of gene-repaired iPSCs	Uncertainty about safety and immunogenicity of iPSC-derived cells

HSC, hematopoietic stem cell; iPSC, induced pluripotent stem cell; PID, primary immune deficiency.

globin variants (58,59). The defective gene in the autologous iPSCs was repaired through homologous recombination, and HSCs were generated from the genetically corrected iPSCs. Transplantation of the *ex vivo*–generated corrected HSCs rescued the sickle cell phenotype. For a full *in vivo* functionality of the HSCs, the iPSCs required the expression of HoxB4. This brings forward the largest bottleneck concerning pluripotent stem cell–based hematopoietic studies because the evidence for the generation of pluripotent stem cell–derived HSCs that are functional in xenotransplantation protocols is scarce. Contribution to the mouse blood is in most cases low (<1%) and of relatively short duration (60–65). Furthermore, the functionality of the generated lymphoid populations has never been demonstrated.

Nevertheless, one could potentially envision a similar strategy for the treatment of SCID: iPSCs generated from fibroblasts would allow gene correction of the affected locus via homologous recombination (38). Subsequently, selected iPSC clones could be differentiated into HSC-like cells and, after rigorous safety and efficacy testing, used in transplantation (**Figure 4**). Although many ethical and technical hurdles must be overcome in this procedure, as have reviewed previously, the underlying principles are attractive.

iPSCs IN DISEASE MODELING FOR SCID

A more modest goal in using iPSCs is their use for disease modeling. Disease- and patient-specific pluripotent cells can be used to model disease (66). Good disease models have been rare but are essential for research on disease pathogenesis, drug testing, and drug discovery. For the diagnosis of increasingly more and more immunodeficiencies, bone marrow punctures are no longer required and, therefore, are not undertaken. This leads to a shortage of transplantable stem cells to be used in *in vitro* and *in vivo* disease models (e.g., in nonobese diabetic-SCID or other mouse models). Here, iPSC technology may come in handy because iPSCs can be generated from peripheral blood cells or fibroblasts and then used in model systems. For some types of SCID, a small-molecule activator of the defective signaling pathways (e.g., defective signaling in JAK3-SCID) could be of therapeutic benefit. In such cases, iPSC-based disease modeling could provide a useful tool for drug screening (**Table 1**).

CONCLUDING REMARKS

The high efficacy of gene therapy for SCID compared with transplantation of non–human leukocyte antigen–identical HSCs (in which graft-vs.-host disease remains a major problem) provides a clear rationale for gene therapy (20). This therapy is currently performed using novel self-inactivating vectors. In addition, new approaches with iPSCs are currently under investigation by various laboratories. As the technology in this rapidly expanding field becomes more advanced and safer, the use of autologous stem cells, via iPSCs containing genes repaired by homologous recombination (38), is already providing valuable insight into the pathogenesis of blood-borne diseases and may, when proven safe, provide a novel treatment modality.

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