

Preclinical modeling highlights the therapeutic potential of hematopoietic stem cell gene editing for correction of SCID-X1

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Abstract

Targeted genome editing in hematopoietic stem/progenitor cells (HSPCs) is an attractive strategy for treating immunohematological diseases. However, the limited efficiency of homology-directed editing in primitive HSPCs constrains the yield of corrected cells and might affect the feasibility and safety of clinical translation. These concerns need to be addressed in stringent preclinical models and overcome by developing more efficient editing methods. We generated a humanized X-linked severe combined immunodeficiency (SCID-X1) mouse model and evaluated the efficacy and safety of hematopoietic reconstitution from limited input of functional HSPCs, establishing thresholds for full correction upon different types of conditioning. Unexpectedly, conditioning before HSPC infusion was required to protect the mice from lymphoma developing when transplanting small numbers of progenitors. We then designed a one-size-fits-all IL2RG (interleukin-2 receptor common γ -chain) gene correction strategy and, using the same reagents suitable for correction of human HSPC, validated the edited human gene in the disease model in vivo, providing evidence of targeted gene editing in mouse HSPCs and demonstrating the functionality of the IL2RG-edited lymphoid progeny. Finally, we optimized editing reagents and protocol for human HSPCs and attained the threshold of IL2RG editing in long-term repopulating cells predicted to safely rescue the disease, using clinically relevant HSPC sources and highly specific zinc finger nucleases or CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9). Overall, our work establishes the rationale and guiding principles for clinical translation of SCID-X1 gene editing and provides a framework for developing gene correction for other diseases.

INTRODUCTION:

Targeted genome editing may enhance the precision of genetic engineering in cell and gene therapy. It exploits artificial nucleases, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and RNA-based CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) nucleases, to target a DNA double-strand break (DSB) or nick into a predetermined sequence of the genome (1, 2). Depending on the DSB repair pathway engaged, the outcome may be functional inactivation of the targeted locus by nonhomologous end joining (NHEJ), which often introduces small insertions or deletions (“indels”), or introduction of a new sequence by homology-directed repair (HDR) from an exogenous template DNA bearing homology to the sequences flanking the DSBs. The latter strategy has been used to knock in exogenous gene sequences, such as functional complementary DNA (cDNA), into inherited defective genes downstream of their own promoter, thus reconstituting the function and endogenous expression control of the mutant gene, without the risk of random insertional mutagenesis (3–6). Moreover, this approach has the advantage that most disease-causing mutations affecting the locus, including deletions, can be treated with the same engineered nucleases.

Notwithstanding the therapeutic potential of gene editing for the treatment of immune hematological diseases, a major challenge to its application comes from the limited efficiency of HDR in hematopoietic stem/progenitor cells (HSPCs). Despite recent reports of improvements in ex vivo gene editing protocols for human (7–11) and nonhuman primate HSPCs (12), HDR-mediated insertion of exogenous genes remains constrained in long-term se-

vere combined immunodeficiency (SCID)–repopulating HSPCs (7–11), likely because of low expression of the HDR machinery, cell quiescence, induced differentiation or apoptosis in response to DNA DSBs, and limited uptake of template DNA. A limited yield of edited hematopoietic stem cells (HSCs) may affect the feasibility and safety of clinical development of gene editing. Moreover, stem cell–based therapeutics will require highly specific targeted gene editing, which might be achieved with new or improved nuclease platforms.

We have been developing a gene editing strategy to correct mutations in the interleukin-2 receptor common γ -chain (IL2RG) gene, which are responsible for X-linked SCID (SCID-X1) (13), by knocking in a corrective cDNA into the affected locus (7). Although we have shown expression of a functional IL2RG from the edited allele in human HSPCs (7), it remains unknown whether the edited locus fully recapitulates the physiological gene expression pattern and its function during cell differentiation and in orchestrating an immune response in vivo. The knock-in strategy may fail to preserve or rescue some fine regulatory features associated with transcription and/or alternative splicing of intronic sequences not included within the corrective cDNA or induce an epigenetic memory of the DNA repair, which may affect gene regulation. Moreover, it remains unclear whether a low input of edited progenitors can safely and effectively rescue the disease, even in the presence of the growth advantage conferred by gene correction. Thus, there is a need to develop stringent preclinical models of disease correction by HSPC gene editing that suitably interrogate the features of the therapeutic cell product and its administration strategy, two parameters that may affect the timing and extent of immune reconstitution.

RESULTS

Establishing the threshold proportion of functional HSPCs required for immune reconstitution

To model SCID-X1 gene correction, we developed a mouse model carrying the human IL2RG gene with a disease-causing mutation (R226H), which abrogates β -chain expression (13), in place of the murine Il2rg gene (fig. S1A). By immunophenotypical and histological characterization, we found that these humanized SCID-X1 mice had impairments in lymphoid development that phenocopy those reported for Il2rg Δ/Δ mice (figs. S1 and S2 and table S1) (14–16). We then determined the lowest fraction of functional HSPCs required to rescue the immune function of SCID-X1 mice. We performed competitive transplantations with wild-type (WT) and IL2RG Δ/Δ HSPCs mixed at different ratios in SCID-X1 recipients conditioned by total body irradiation (TBI) and monitored lymphohematopoietic reconstitution over time (Fig. 1A). We found that B and T cells derived almost completely from WT cells, even in mice transplanted with the lowest fraction (1%) of functional HSPCs, demonstrating their strong selective advantage over defective cells. Selective advantage was also found within the Lin Δ Sca1 $^+$ bone marrow (BM) cells, which include progenitors endowed with lymphoid potential (fig. S3A) (17). Conversely, the WT chimerism observed within mature myeloid cells in the peripheral blood (PB), myeloid progenitors, and the more primitive KLS $^+$ (Kit $^+$ Lin Δ Sca1 $^+$) cells closely mirrored those of the input HSPC, as expected for cell types that are not dependent on IL2RG signaling for their survival and proliferation (Fig. 1B and fig. S3A). Consistent with the selective advantage of WT lymphoid cells, we found high amounts of B and T cell reconstitution, measured as percentage (Fig. 1C) and absolute counts (Fig. 1D) at long-term follow-up after transplantation. Administration of 10% WT cells almost completely rescued the B and T cell lineages, and even administration of as little as 1% WT cells was still sufficient to support a partial reconstitution of both compartments and normalization of the CD4/CD8 ratio in the PB (Fig. 1E). A similar dose-dependent correction of B and T cell compartments and phenotypes was found at end-point analysis in the BM and spleen

Immune reconstitution with clinically relevant and new conditioning strategies

Because TBI is rarely used for transplant conditioning for nonmalignant diseases, we tested whether transplantation of 10% WT HSPCs would achieve full immune reconstitution with more clinically relevant regimens. Treatment of the mice with a myeloablative dose of the alkylating agent treosulfan resulted in myeloid engraftment with WT cells approaching the input ratio, as observed after TBI, and full reconstitution of the lymphoid compartments (Fig. 3, A and B). We also explored a nongenotoxic strategy to selectively deplete hematopoietic cells with an immune toxin while sparing toxicity to the other organs, as recently reported (23). SCID-X1 mice were treated with a single dose of anti-CD45 antibodies conjugated to the protein synthesis inhibitor toxin saporin (CD45-SAP), which caused substantial depletion (\sim 70%) of the HSPC compartments and milder depletion of the more mature cell populations (fig. S6, A to C), and transplanted with 10% WT cells 3 days later. Myeloid engraftment with WT cells was lower for CD45-SAP than for TBI, used as a reference, but

stable in the follow-up, indicating successful HSC engraftment (Fig. 3C and fig. S6D). Notably, T cell reconstitution after CD45-SAP was faster and as robust as that achieved after TBI, despite the lower HSC engraftment, possibly because of the sparing of the thymus and BM environments, resulting in similar counts of circulating T cells at long-term follow-up (Fig. 3, D and E) and, upon LCMV challenge, similar expansion of total and virus-specific CD8 T cells and a considerable albeit less robust viral clearance (Fig. 3F). These findings confirm that an input of 10% functional HSPCs can support full lymphoid reconstitution across different conditioning strategies and highlight the potential advantages of a milder regimen that selectively targeted hematopoietic lineages and effectively rescued T lymphopoiesis even after engraftment of only a few percent of WT HSCs.

DISCUSSION

Here, we report preclinical studies geared toward clinical translation of targeted genome editing of HSCs and its application to the stable correction of SCID-X1 disease. The proportion of functional HSPCs in the cell product required to correct the disease in our mouse model (\sim 10%) was determined using cells expressing the WT murine Il2rg. This figure was true across different types of conditioning, including TBI, myeloablative chemotherapy, and a milder immunotoxin-based selective depletion of hematopoietic cells. Whereas the two myeloablative strategies (TBI and chemotherapy) established a WT HSC chimerism in the BM reflecting the input ratio, the biological conditioning was less efficient but still resulted in robust lymphoid reconstitution, likely because of better preservation of the tissue niches. This finding is encouraging from the perspective of clinical translation, because treatment of SCID-X1 would most likely rely on a mild conditioning regimen that may lower the proportion of engrafted HSCs from the administered cell product. Extrapolation of the mouse data to the human setting should also consider the following aspects. Whereas the thymus in SCID-X1 mice is hypomorphic but its overall structure is partially preserved, this organ is more severely affected by the disease in humans, possibly hindering the extent of reconstitution, even if the T cell compartment is successfully restored upon allogeneic transplantation or autologous gene therapy. On the other hand, the complete lack of T cells in SCID-X1 children might provide for lower competition in the thymus than observed in SCID-X1 mice, thus resulting in overestimation of the threshold in the mouse model. Overall, our prediction that engraftment of \geq 10% corrected HSPCs will provide benefits in human patients is in line with recent clinical data on LV-based gene therapy for SCID-X1, where patients showing a vector copy number \leq 0.1 in the HSC and myeloid compartments showed substantial clinical improvement (31).

TBI before transplant also protected the mice from an unexpectedly high incidence of thymic lymphoma during long-term follow-up. Our findings suggest that these tumors might arise as a consequence of sustained thymocyte proliferation from a limited input of administered progenitors that lack or have limited replenishment from the BM and a preserved thymic microenvironment allowing their rapid growth. The increasing rate of tumorigenesis in mice receiving a lower input of functional cells suggests that the fewer Il2rg $^{+/+}$ thymic progenitors contribute to thymopoiesis, the stronger the replicative stress they are sub-

jected to, raising concerns for the long-term safety of functional reconstitution by a limiting number of corrected progenitors. Our findings are consistent with a previous report of tumorigenesis from WT thymic progenitors growing in the absence of BM replacement in SCID mice (22) and further supported by a recent study in another model of SCID-X1, where thymic lymphomas arose upon limiting transplant doses after sublethal irradiation as conditioning (32). Although the lymphomagenesis observed in transplanted SCID-X1 mice might be a mouse-specific effect, it could help in explaining the high leukemia incidence observed in SCID-X1 gene therapy trials performed with early-generation vectors (33), at variance with gene therapy trials performed for adenosine deaminase-SCID with a similar type of vector but including administration of a mild conditioning regimen to allow some HSC engraftment. The inverse dependence of lymphomagenesis on input cell dose might help explain why no leukemic events have been reported in SCID patients after allogeneic BM transplantation, which is usually performed without conditioning but with the infusion of a full dose of functional donor cells (20).

MATERIALS AND METHODS

Study design

The objectives of this study were to establish the conditions for safe and effective correction of SCID-X1 in a humanized mouse model of the disease, used here as representative of functionally μ -chain-negative mice, and to validate a gene correction strategy for IL2RG in HSPCs. Kinetics and extent of hematopoietic reconstitution from limited input of WT or gene-edited HSPCs were analyzed in transplanted SCID-X1 mice upon different types of conditioning. Functionality of IL2RG-edited cells was verified using in vivo and in vitro functional studies. Optimization of treatment protocols and gene editing reagents was performed on mouse and human HSPCs to determine their effects on gene editing efficiency and in vivo repopulation capacity. Mice were randomized to treatment groups, without blinding. Criteria applied for mouse termination before the established end point were in accordance with the Institutional Animal Care and Use Committee protocol of the San Raffaele Scientific Institute. Cohort sizes were informed by previous experiments and by the total number of available treated cells; no outliers were excluded.

Conclusion:

Finally, we optimized editing reagents and protocol for human HSPCs and attained the threshold of IL2RG editing in long-term repopulating cells predicted to safely rescue the disease, using clinically relevant HSPC sources and highly specific zinc finger nucleases or CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9). Overall, our work establishes the rationale and guiding principles for clinical translation of SCID-X1 gene editing and provides a framework for developing gene correction for other diseases.