

## Preclinical Development of Gene Therapy for X-Linked Severe Combined Immunodeficiency (SCID-X1)

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### Abstract

X-linked severe combined immunodeficiency (SCID-X1) is caused by mutations in the gene encoding the interleukin-2 receptor  $\gamma$  chain (*IL2RG*), and is characterized by profound defects in T-, B- and NK-cell functions. Previous gene therapy clinical trials based on hematopoietic stem/progenitor cells (HSPCs) genetically corrected with MLV-derived retroviral vectors showed restoration of T-cell immunity but resulted in vector-induced leukemia through insertional mutagenesis. The use of an enhancer-less MLV vector to deliver the *IL2RG* gene caused no adverse events while retaining a significant clinical benefit. To increase the efficacy of gene therapy and further reduce potential genotoxicity, we developed a SIN lentiviral vector carrying a codon-optimized human *IL2RG* cDNA under the control of the human EF1 $\alpha$ -S promoter. Codon optimization resulted in a 3-fold increase in mRNA and a 1.5-fold increase in protein expression per integrated vector copy. The performance of the vector was demonstrated *in vitro* by the restoration of a normal level of *IL2RG* mRNA or protein in a *IL2RG*-deficient T-cell line, patient-derived EBV-immortalized B-cells and mobilized CD34<sup>+</sup> HSPCs, with no impact on viability or clonogenic capacity. An *in vitro* immortalization assay (IVIM) showed a safe genotoxic profile, while the *in vivo* safety and efficacy of the vector was tested in a preclinical model of SCID-X1 gene therapy based on transplantation of genetically corrected Lin<sup>-</sup> cells from *IL2rg*<sup>-/-</sup> donor mice into lethally-irradiated *IL2rg*<sup>-/-</sup>-*Rag2*<sup>-/-</sup> recipients. The study showed restoration of T, B and NK cell counts in bone marrow and peripheral blood, normalization of lymphoid organs (thymus and spleen) and a frequency of hematopoietic abnormalities comparable to that of control animals six months after transplantation. An extensive insertion site analysis carried out in bone marrow, thymus and peripheral blood of individual or groups of animals showed the expected genomic integration profile and no signs of clonal dominance in transduced cells. Interestingly, analysis of >100,000 integration sites in pre- and post-transplant murine cells showed that lentiviral vectors target at high frequency a substantially different set of genes compared to human CD34<sup>+</sup> cells, uncovering the limits in the predictive power of mouse-based genotoxicity studies. These studies will enable a multicenter phase-I/II clinical trial aimed at establishing the safety and clinical efficacy of lentiviral vector-mediated gene therapy for SCID-X1 in infants after non-myeloablative conditioning, and sustained restoration of both T- and B-cell immunity.