Defects in Recombination activating gene 1 and 2 (RAG) result in a broad spectrum of clinical manifestations (1). Hematopoietic stem cell transplantation (HSCT) is the treatment of choice when a suitable donor is available. Conventional gene therapy is an attractive therapeutic option; however, its applicability is constrained by the need of a physiological RAG1 expression. We set up a gene editing (GE) platform based on engineered nucleases to restore the expression of the corrective human RAG1 cDNA under the physiological control of its endogenous promoter. We identified the best performing CRISPR-Cas9 ribonuclease protein complex that allows a high level of cutting activity (by NHEJ-mutagenesis assay) and efficient editing (by homology directed repair assay) in K562 and NALM6 cell lines and developed an adeno-associated virus type 6 donor DNA carrying the human codon optimized RAG1 cDNA followed by BGH polyA sequence. We are validating this platform in human CD34+ cells obtained from cord blood (CB) and mobilized peripheral blood (mPB) from normal donors and RAG1 patients by in vitro and in vivo analyses. In parallel, we identified the minimal number of gene-targeted cells necessary to achieve therapeutic levels of immune reconstitution by competitive transplantation in Rag1-/- mice and tested non genotoxic conditioning mediated by antiCD45-Saporin (2). Overall, our work provides novel data supporting hematopoietic stem and progenitor cell (HSPC) gene editing and novel conditioning for treating Rag1 deficiency and pave the way towards the clinical application of GE in hematopoietic progenitors.

In parallel, we have focused our research on the correction of autosomal recessive osteopetrosis (ARO), a rare disease affecting osteoclast differentiation or function (3). Most patients present mutations in TCIRG1 gene, encoding the V-ATPase proton pump, necessary for bone resorption. Symptoms include dense and brittle bones, limited bone marrow (BM) cavity, anaemia and progressive nerve compression, leading to death in the first decade of life. Standard treatment is allogeneic HSCT, but availability of HLA-matched donor, toxicity of conditioning and significant transplant-related morbidity limit applicability. Gene therapy (GT) may represent an alternative therapy, alleviating the need for recipient conditioning and abrogating graft-versus-host disease. Since patients show increased number of circulating CD34+ cells in peripheral blood (PB), these cells could be exploited as a readily available cell source for autologous transplantation of gene-corrected HSPCs. We analyzed the peripheral blood of 5 TCIRG1-deficient patients finding a cellular composition resembling BM, enriched in progenitors such as erythroblasts and myeloblasts (4). Analysis of TCIRG1 CD34+ cell transcriptome indicated the expression of HSPC genes, similarly to conventional sources (CB and mPB CD34+ cells). To reduce the burden of CD34+ cell collection in low-weight, severely affected children, we applied an UM171-based ex vivo expansion protocol of HSPCs coupled with gene transfer. We generated a corrective lentiviral vector (LV), driving TCIRG1 expression under the control of the PGK promoter. Circulating CD34+ cells from TCIRG1-defective patients were transduced with LV and expanded up to 27-fold. Expanded cells maintained long-term engraftment and multi-lineage repopulating potential when transplanted in vivo both in primary and secondary NSG recipients. When CD34+ cells were differentiated in vitro, bone resorption capacity, measured as collagen type-I fragment release in culture supernatants, was restored only in GT cells, at levels comparable to healthy donor cells. These data provide evidence that expansion of circulating HSPCs coupled to GT could represent a feasible, readily available...
treatment for ARO patient, and on this basis we are moving to the clinical application of this innovative platform.

References:


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References:

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Contacts:

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Other: