Targeted gene correction for the treatment of Severe Combined Immunodeficiency caused by mutations in the IL7R gene

Marianna Romito[1], Rajeev Rai[1], Giorgia Santilli[1], Adrian Thrasher[1,2], Alessia Cavazza[1]

[1]Molecular and Cellular Immunology, Great Ormond Institute of Child Health, University College London, London, United Kingdom; [2]Department of Paediatric Immunology, Great Ormond Street Hospital, London, United Kingdom

Severe combined immunodeficiency (SCID) is a life-threatening syndrome characterized by a block in T and/or B cell development that has a fatal outcome in children by the second year of life. Mutations in the IL7R gene are responsible of the third most common form of SCID, and the majority of T-B+NK+ cases. Gene therapy has proved to be a powerful tool to treat rare genetic diseases affecting the hematopoietic system avoiding these complications. However, pre-clinical gene therapy studies using viral vectors to introduce a correct copy of *IL7R* in HSPCs showed that constitutive and unregulated expression of the gene predisposes to leukemia. CRISPR-mediated genome editing enables the correction of the mutated IL7R locus with a regulated and controlled transgene expression. To achieve this purpose, we have targeted several guide RNAs (gRNAs) in combination with Cas9 protein to the IL7R locus, detecting up to 70% of indels in hematopoietic stem and progenitor cells (HSPCs). CRISPR-system delivered with an Adeno-associated virus (AAV) donor template containing a GFP cDNA resulted in the knock-in of the cassette in up to 35% of HSPCs. As a proof of concept for our strategy, we showed that the delivery of an AAV encoding a codon optimized IL7R cDNA cassette in an IL7R-deficient model cell line restores IL7R expression. Our final goal is to correct IL7R-deficient HSPCs derived from SCID patients restoring T-cell production. For this purpose, successful lymphopoiesis will be assessed through ongoing optimizations of an *in vitro* T-cell differentiation protocols and *in vivo* xenotransplantation assays.