65

Clinical trial design and safety profile of HDR edited CD4 T-cells for the treatment of Hyper IgM1

Dr. Daniele Canarutto[1,2,10], Dr. Claudia Asperti[1], Dr. Stefano Beretta[1], Dr. Chiara Gaddoni[1], Dr. Simona Porcellini[1], Dr. Elisabetta Roverlli[1], Dr. Carolina Cuofano[1], Dr. Elisa Montaldo[1], Dr. Federica Fardella[1], Dr. Ilaria Mei[1], Dr. Ilaria Visigalli[1], Dr. Francesca Cecere[1], Dr. Michela Vezzoli[1], Dr. Francesca Ferrua[1,2], Dr. Valentina Vavassori[1], Dr. Samuele Ferrari[1], Dr. Sandra Ammann[17], Dr. Martina Ragazzi[14], Dr. Francesca Sanvito[1,15], Dr. Sean Russell[14], Dr. Stefano Zancan[14], Dr. Leonard Pattenden[14], Dr. Giuseppe Gaipa[12], Dr. Daniela Belotti[13], Dr. Benedetta Cabiati[12], Dr. Martina Galuzzi[12], Dr. Luis I. Gonzalez-Granado[3], Prof. Vassilios Lougaris[4], Prof. Raffaele Badolato[4], Prof. Andrea Finocchi[5], Prof. Ansgar Schulz[6], Dr. Wadih Abouchahla[7], Dr. Anna Schrimpton[8], Dr. Susana Lopes da Silva[16], Prof. Toni Cathomen[17], Dr. Paola Albertini[1], Dr. Anna Villa[1,9], Prof. Andrea Biondi[12], Prof. Alessandro Aiuti[1,2,10], Dr. Marina Radrizzani[1], Prof. Luigi Naldini[1,10]
[1]San Raffaele-Telethon Institute for Gene Therapy, IRCCS San Raffaele Scientific Institute, Milan, Italy, [2]Pediatric Immunohematology Unit and BMT Program, IRCCS San Raffaele Hospital, , Italy, [3]Primary Immunodeficiencies Unit, Department of Pediatrics, Research Institute, Madrid, Spain, [4]Pediatrics Clinic and Institute for Molecular Medicine A. Nocivelli, Department of Clinical and Experimental Sciences, University of Brescia and ASST-Spedali Civili of Brescia, Brescia, Italy, [5]Academic Department of Pediatrics (DPUO), Research Unit of Clinical Immunology and Vaccinology, Bambino Gesú Children's Hospital, Rome, Italy, [6]Department of Pediatrics, University Medical Center Ulm, Ulm, Germany, [7]CHU de Lille, Service d'hématologie pédiatrique, Université de Lille, , France, [8]Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom, [9]Milan Unit, Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, , Italy, [10]Vita-Salute San Raffaele University, , Italy, [11]Labratorio di Terapia Cellulare e Genica Stefano Verri, Fondazione IRCCS San Gerardo dei Tintori, , Italy, [12]Fondazione M. Tettamanti Onlus, , Italy, [13]School of Medicine and Surgery, University of Milano-Bicocca, , Italy, [14]Fondazione Telethon ETS, , Italy, [15]Pathology Unit, IRCCS San Raffaele Hospital, , Italy, [16]Hospitalar Graduada de Imunoalergologia ULSSM, , Portugal, [17]Institute for Transfusion Medicine and Gene Therapy, Medical Center – University of Freiburg, , Germany

Objective
We report progress of our academic clinical trial using homology-directed repair (HDR) gene editing for treating Hyper-IgM 1 (HIGM1).

Design and Methods
HIGM1, an X-linked immunodeficiency caused by CD40LG mutations, impairs CD4+ T cell function and has a median survival of 25 years. We developed FT018, an autologous CD4+ T-cell product that was HDR-edited at the CD40LG locus using Cas9 and an integrase-defective lentiviral vector (IDLV) encoding the corrective template and a LNGFR selector. Edited cells restore physiological CD40LG expression, CD40 binding, and signaling.

Results and conclusions

GLP biodistribution study showed normal behavior of edited cells. No off-target effects were detected by CAST-Seq, Optical Mapping, or target sequencing. Targeted nanopore sequencing revealed a majority of precise intended HDR events and the rest accounted by on-target concatemers not compromising functionality. Conventional single cell tracking tools, such as scRNA-seq, cannot capture genomic outcomes at single cell level, that are mostly transcriptional silent. Therefore, we developed a novel single cell DNA sequencing assay for characterization of editing outcomes at single cell level. We designed an amplicon panel covering the X chromosome, including the target Cas9 site, the donor IDLV template, and nominated off-targets. Three clusters were present in the cell product: 1. Unedited cells, with preservation of the amplicon covering the nuclease target site 2. Precisely edited cells, with no amplification of the nuclease target site and amplification of the repair template 3. Imprecisely edited cells, with no amplification of the nuclease target site and amplification of the repair template and other amplicons mapping onto the donor IDLV, compatible with previously characterized template concatemers. No off-target integrations or on-target large deletions were detected.

We designed a first-in-human, open label, phase I/II clinical trial to evaluate the tolerability and efficacy of FT018 for the treatment of Hyper IgM1, and is anticipated to start at the end of 2025/early 2026, following regulatory approval. We'll enroll patients with a mutation amenable to correction (95% of known mutations) for which HSCT is considered to be high risk. The first patients will be treated with a trial-boost regimen, without lymphodepletion. Dosing adjustments will be considered after treatment of the initial cohort. Endpoints include engraftment, CD40LG restoration, and clinical benefit.

In summary, we report on the advancement of our trial for the treatment of HIGM1 due to start enrolment soon, and present a novel technology for assessment of single cell HDR editing outcomes in clinical settings.