407. Somatic Mutation Tracking in Hematopoietic Stem Cell Gene Therapy Reveals Absence of Clonal Hematopoiesis

Francesco Gazzo[1,2], Andrea Calabria[1], Fabrizio Benedicenti[1], Maria Rosa Lidonnici[1], Samantha Scaramuzza[1], Francesca Tucci[1,3], Fabiola De Mattia[1], Sarah Marktel[1,3], Valeria Calbi[1,3], Francesca Fumagalli[1,3], Fabio Ciceri[3], Luigi M. Naldini[1,4], Alessandro Aiuti[1,3,4], Giuliana Ferrari[1,4], Eugenio Montini[1]

[1]San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy,[2]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milan, Italy,[3]Pediatric Immunohematology and BMT, San Raffaele Hospital, Milan, Italy,[4]Vita Salute San Raffaele University, Milan, Italy

In hematopoietic stem cell Gene Therapy (GT), patients' Hematopoietic Stem and Progenitor Cells (HSPCs) are genetically corrected *ex vivo* and reinfused to reconstitute the entire hematopoietic system and provide therapeutic benefit. During this process, HSPCs are subjected to tremendous pressures to sustain high levels of proliferation until the hematopoietic reconstitution is complete. So far it is unknown if this likely stressful condition may result in the accumulation of somatic mutations which may trigger decay of the hematopoietic functions and increased risk of oncogenesis. The impact of prolonged and heightened HSPCs proliferation rates on cellular fitness and safety remains an open question in GT. Indeed, HSPCs are not comprehensively geno-protected from DNA damage accumulation in HSPCs during aging and/or in specific disease conditions as it has been observed in sickle cell disease and Fanconi Anemia.Here we performed an analysis of somatic mutations in exons of 40 genes involved in clonal hematopoiesis and myeloid cancer in 23 GT patients treated for metachromatic leukodystrophy (MLD, 15 <3 years old, 8 3-12 years old) and 9 for β-thalassemia (β-Thal, 6 pediatric and 3 adults >30 years old). We used genomic DNA from HSPCs cells prior infusion and peripheral blood mononuclear cells harvested at 2 years after GT and at the last available time point (2.5-7.5 years after GT). Sequencing reads correctly aligned on the targeted exon panel resulted in an average coverage of 4,400 and 4,300 reads/base in β-Thal and MLD patients respectively.The average mutation rate in the adult β-Thal patients was >2 fold higher than the rate measured in the pediatric patients (11.3 ± 11 vs 5.6 ± 3.5). Moreover, the average somatic mutation rates in adult and pediatric β-Thal patients were both significantly higher than in MLD patients (1.6 ± 0.72, p-value=0.0136 vs adult β-Thal, p-value=0.012 vs pediatric β-Thal, by Kruskal-Wallis test). None of the mutations were pathological or likely pathological. Most somatic mutations (85 out of 96) exhibited a Variant Allele Frequency of less than 2%. The average number of mutations in both the clinical trials remained consistent across all time points, showing no statistically significant variations. Considering that the sequenced genomic interval corresponds to 76,715 bps and that we analyzed a total of 8,100 equivalent genomes per patient, the resulting mutation rate in β-Thal patients was $1.21x10^{-8}$ mutations/bp, while MLD patients resulted in a mutation rate of $2.6x10^{-9}$ mutations/bp. Five mutations (4 in β-Thal and 1 in MLD) were found at more than one time point, but none showed a progressive increase in abundance, suggesting that these mutations did not confer a selective advantage to the mutated cell clones.Our work revealed that no somatic mutations known to drive clonal hematopoiesis or myeloid cancer, nor accumulation of somatic mutations, were found in our GT patients, indicating that the GT treatment was neutral in these conditions. However, the underlying disease in β-Thal patients resulted in a significantly higher mutation burden than MLD patients, a finding worth of deeper analysis and that supports long-term follow-up assessments of the clonal composition of the hematopoietic system in GT patients.

Vector Product Engineering, Development, and Manufacturing (excluding AAV)

408. Single and Low Dose Self-Replicating RNA Vaccine Provides Effective Immune Protection Against Rabies in Healthy Volunteers

Christian Maine[1], Shigeki Miyake-Stoner[1], Brandon Essink[2], Guillermo Somodevilla[3], Yaquelin Rodriguez[3], Andrew Geall[1], Nathaniel Wang[1], Parinaz Aliahmad[1], Zelanna Goldberg[1]

[1]Replicate Bioscience Inc, San Diego, CA,[2]Velocity Clinical Research, Omaha, NE,[3]Cordova Research Institute, Miami, FL

Despite the unprecedented success of mRNA vaccines for COVID-19, their widespread expansion to other infectious diseases is limited by unacceptable side effects at doses required for protection. Self-replicating RNA (srRNA) technology is a fully synthetic RNA modality that is able to amplify in the host cell due to the presence of a virally derived replication machinery. Thus, lower amounts of srRNA lead to exponential protein expression, which enables lower clinical dosing and better tolerated vaccines. Replicate Bioscience has developed novel, proprietary srRNA vectors with improved performance for a variety of therapeutic applications. To demonstrate the superior performance of our technology, we designed and manufactured RBI-4000, a proprietary srRNA vector encoding the rabies glycoprotein encapsulated in a lipid nanoparticle. Rabies was selected as a target for this clinical validation as the general population is exposure-naïve, in contrast to influenza or SARS-CoV-2 where pre-existing exposure may confound a clean interpretation of the vaccine-generated immune responses. Additionally, an easy to interpret immune surrogate of protection has been defined by the WHO, where titers of rabies virus neutralizing antibodies (RVNA) $\geq$ 0.5 IU/mL are protective. RBI-4000 was evaluated in a Phase I clinical trial (NCT06048770). Healthy adults, age 18-45 and seronegative for rabies antibodies were recruited at two clinical sites in the U.S. Sequential cohorts (n=18 subjects) were dosed IM with 0.1, 1, or 10 micrograms (mcg) of RBI-4000 in a 2-dose prime-boost schedule or prime only for the highest dose. An active control cohort of 12 subjects received RabAvert, a commercial vaccine, per the recommended schedule. Enrollment is complete, and the study is ongoing. Interim analysis demonstrated that all doses were well tolerated, with no DLTs, SAEs, or safety signals. Acute reactogenicity was limited to grade 1 or 2 events, with a single exception of a time-limited grade 3 fever reported by 1 subject in the mid dose group, at