PROJECT 1

DoS: Alessio Cantore

Title: Evaluation of the efficacy and safety of gene therapy strategies for liver metabolic diseases

Curriculum: Gene and cell therapy

Link to OSR/UniSR personal page: https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/units/liver-gene-therapy.html

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

The liver is a relevant target organ for gene therapy, because of its high metabolic and secretory activity and involvement in many inherited and acquired diseases, including metabolic diseases and plasma protein deficiencies, such as hemophilia. Liver-directed gene therapy for the coagulation disorder hemophilia is among the most successful application of gene therapy. Indeed, it has been shown that a single intravenous administration of adeno-associated viral (AAV) vectors delivering a functional copy of a coagulation factor gene allowed multi-year clinical benefit in adult patients affected by hemophilia. However, because AAV vectors do not actively integrate into the host cell genome, they are lost upon cell division in liver growth, thus limiting their use in pediatric patients. On the contrary, lentiviral vectors (LV) integrate into the target cell chromatin and are maintained as cells divide, thus representing attractive vehicles for liver-directed gene therapy, given their potential for stable long-term expression after a single administration. The clinical success obtained by AAV-vector based liver gene therapy in hemophilia has indeed raised the expectation to broaden the indications to diseases that are more severe or lethal in childhood. In this context, LV represent an opportunity to address these challenges.
This project aims at evaluating the efficacy and safety of liver-directed gene therapy by hepatocyte-targeted LV for progressive familial intrahepatic cholestasis and maple syrup urine disease, two metabolic diseases, chosen as representative for the class with intrahepatic manifestations and liver damage and the class with extrahepatic manifestations, respectively. The former is due to accumulation of bile acids within hepatocytes and subsequent toxicity, ultimately leading to liver cirrhosis and failure, while the latter is caused by a defect in an enzyme involved in catabolism of branched chain amino acids, thus leading to their accumulation in the circulation and subsequent neurotoxicity. Both diseases severely affect children and contemplate liver transplant as the only curative solution, thus representing an unmet medical need and classifying as candidates for liver gene therapy. Specifically, the Ph.D. candidate will determine the threshold of genetically corrected hepatocytes required for disease correction and assess whether cross-correction or selective advantage occur in mouse models of these diseases. Moreover, since gene transfer with integrating vectors is associated with imperfect control of transgene expression and the risk of insertional mutagenesis, she/he will explore the possibility to perform in vivo gene

editing and compare advanced LV-mediated gene delivery and site-specific nuclease mediated gene editing strategies in terms of feasibility, efficiency, therapeutic efficacy, safety and durability.

Skills to be acquired by the student:

Wet skills

Generation and characterization of lentiviral vectors; design of gene editing strategy and reagents set up; cell cultures; molecular biology: molecular cloning, DNA and RNA extraction, quantitative PCR, sequencing; ELISA; western blot; flow cytometry; animal handling and post-mortem analysis.

Job-related skills

Experimental design and data analysis, interpretation and presentation; paper writing.

References (max. 3)

Milani M, Annoni A, Moalli F, Liu T, Cesana D, Calabria A, Bartolaccini S, Biffi M, Russo F, Visigalli I, Raimondi A, Patarroyo-White S, Drager D, Cristofori P, Ayuso E, Montini E, Peters R, Iannacone M, Cantore A*, Naldini L*. Phagocytosis-Shielded Lentiviral Vectors Improve Liver Gene Therapy in Non Human Primates. Sci Transl Med, In press. *Co-last, co-corresponding authors.

Milani M, Annoni A, Bartolaccini S, Biffi M, Russo F, Di Tomaso T, Raimondi A, Lengler J, Holmes MC, Scheiflinger F, Lombardo A, Cantore A*, Naldini L*. Genome editing for scalable production of alloantigen-free lentiviral vectors for in vivo gene therapy. EMBO Mol Med, 2017 Aug 23. *Co-last authors.

Cantore A, Ranzani M, Bartholomae CC, Volpin M, Valle PD, Sanvito F, Sergi LS, Gallina P, Benedicenti F, Bellinger D, Raymer R, Merricks E, Bellintani F, Martin S, Doglioni C, D'Angelo A, VandenDriessche T, Chuah MK, Schmidt M, Nichols T, Montini E, Naldini L. Liver-directed lentiviral gene therapy in a dog model of hemophilia B. Sci Transl Med. 2015 Mar 4;7(277):277ra28.