PROJECT 1

DoS: LUIGI NALDINI

Title: DEVELOPMENT OF GENE EDITING APPROACHES FOR CD40-LIGAND DEFICIENCY

Curriculum: CELL AND GENE THERAPY

Residency Program: PEDIATRICS

Link to OSR/UniSR personal page:

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

Hematopoietic Stem/Progenitor Cells (HSPC) gene therapy has provided clinical benefits in patients affected by a variety of genetic diseases. However, the use of semi-randomly integrating vectors poses the risk of insertional mutagenesis and ectopic/unregulated transgene expression. The latter issue is particularly relevant when dealing with tightly regulated genes active on cell proliferation, like the *CD40LG* gene whose expression on the surface of activated T cells leads to contact-dependent B cell activation, proliferation and immunoglobulin class-switching (1). Since *CD40LG* mutations cause the X-linked immunodeficiency with hyper-IgM (HIGM1), HSPC gene transfer was proposed as potential treatment. Although even small amounts of transduced cells restored immune function in a HIGM1 mouse model, constitutive expression of CD40LG led to lymphoproliferation and lymphomas (2). Gene repair strategies that preserve physiologic expression control of the corrected gene represent therefore a better suitable approach for the treatment of HIGM1. We recently developed a strategy based on CRISPR/Cas9 technology that enables efficient and specific gene correction of the *CD40LG* gene in human primary T cells and HSPC, thus allowing restoring both function and expression control of the edited gene. The resident/Ph.D. candidate will be engaged in fostering the transition of this cutting-edge strategy from the bench to the bed side. As a physician, he will be first involved in a T cell collection trial conducted on HIGM1 patients with genotyped *CD40LG* mutation, whose primary endpoint will be to obtain sufficient amounts of CD4 T cells from the peripheral blood to enable generating the predicted target cell dose of a gene therapy product and to assess functional recovery of the patient immune system after leukapheresis. The majority of the collected cells will be frozen and stored for future use by the participants, while a small fraction will be used to develop – in parallel with healthy donor cells - a clinical grade gene correction protocol that would allow treating large numbers of cells and achieving the required fraction of corrected cells to support clinical use. Ad hoc assays will be developed and validated by the candidate, to assess functional expression of the *CD40LG* gene as well as restoration of its tight regulation, and a GLP study will be conducted to evaluate the engraftment capacity and biodistribution of the gene edited T cells in a suitable xeno-transplantation mouse model. The candidate will then be involved in the design and future implementation of a first-in-human phase I/II trial in which the safety and efficacy of this advanced therapeutic medicinal product will be tested for the treatment of HIGM-1 patients. Overall, fulfilling the aims of this project will allow to position homology-based gene editing as new gold standard for precise T and HSC engineering, providing for safer and more efficacious therapeutic strategies.

Skills to be acquired by the student:

The student will gain a unique combination of expertise in: targeted genome editing, primary cell culture, flow cytometry and cell sorting, molecular biology, animal models, data analysis, clinical trial design, regulatory aspects of Advanced Therapeutic Medicinal Products (ATMPs), patient management, scientific communication.

References (max. 3)

1. Immunoglobulin class switch recombination deficiency type 1 or CD40 ligand deficiency: from bedside to bench and back again. Hirbod-Mobarakeh, A., Aghamohammadi, A., and Rezaei, N. (2014). **Expert Rev Clin Immunol** *10*, 91-105.
2. Thymic lymphoproliferative disease after successful correction of CD40 ligand deficiency by gene transfer in mice. Brown, M.P., Topham, D.J., Sangster, M.Y., Zhao, J., Flynn, K.J., Surman, S.L., Woodland, D.L., Doherty, P.C., Farr, A.G., Pattengale, P.K.*, et al.* (1998). **Nat Med** *4*, 1253-1260.
3. Targeted genome editing in human repopulating haematopoietic stem cells. Genovese P, Schiroli G, Escobar G, Di Tomaso T, Firrito C, Calabria A, Moi D, Mazzieri R, Bonini C, Holmes MC, Gregory PD, van der Burg M, Gentner B, Montini E, Lombardo A, Naldini L. **Nature**. 2014 Jun 12;510(7504):235-40.