**PROGETTO**

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| **Supervisor** | Alessio Cantore |
| Titolo/*Title*: | Dissecting hepatocyte heterogeneity in liver growth to improve in vivo gene therapy |
| *Curriculum*: | Terapia genica e cellulare |
| Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: | https://tinyurl.com/muj89n8d |

**Descrizione del progetto (max 3.000 caratteri spazi inclusi)**

**Background/gap of knowledge**

*In vivo* genetic engineering of hepatocytes may represent a definitive cure for monogenic metabolic diseases. Integration of the therapeutic transgene into the target cell genome is essential for long-term expression after a single dose early in life and can be achieved by semi-randomly integrating lentiviral vectors (LV) or site-specific genome editing. Maintenance of the genetic modification upon hepatocyte proliferation in liver growth and turnover requires targeting the cells underlying these processes. Little is known about post-natal liver growth and how different hepatocyte subsets contribute to it. Unexpectedly, we found that most hepatocytes are quiescent during liver growth, and a fraction of them generate most of the adult tissue.

**Rationale and hypothesis**

Our unpublished data show that only about 15% of hepatocytes proliferate in the newborn liver, giving rise to most of the adult liver, through growing clusters, proliferating at a progressively slower rate. Our longitudinal transcriptomics data also provide evidence of extensive and dynamic hepatocyte heterogeneity during post-natal liver growth. We hypothesize that the proliferating, i.e., clonogenic, hepatocyte subset is characterized by a distinct gene expression and epigenetic signature. We also hypothesize that different hepatocyte subsets will differently respond to different delivery vehicles and genetic modification, which may adversely affect the proliferation and maturation of hepatocyte subsets.

**Objectives and specific aims**

The overall goal of the project, thus, is to dissect hepatocyte heterogeneity in post-natal liver maturation and unravel its implications for *in vivo* gene engineering, to ultimately design and

develop safe, effective, and durable gene therapies to treat diseases of hepatic metabolism in pediatric patients.

**Expected outcomes**

The project will result in a comprehensive assessment of the molecular and epigenetic basis of proliferative heterogeneity in hepatocytes and the self-renewal potential of different hepatocyte subsets through multi-omic analysis and organoid assays. It will provide valuable insights into how different genetic engineering procedures impact hepatocyte biology, liver growth, and maturation. This knowledge will guide the future development of gene therapy technologies, offering critical considerations for the application of *in vivo* gene therapy to metabolic diseases in young children.

**Competenze che deve acquisire lo studente** (Max 600 caratteri spazi inclusi):

Wet skills: Generation and characterization of lentiviral vectors; design of gene editing and reagents set up; cell cultures; molecular biology: molecular cloning, DNA and RNA extraction, quantitative PCR, sequencing; single-cell and spatial transcriptomics; epigenomics; animal handling and post-mortem analysis;

Job-related skills: Experimental design and data analysis, interpretation, and presentation; paper writing.

**Bibliografia** (max. 15)

Zabaleta, N. et al. Gene therapy for liver diseases - progress and challenges. Nat Rev Gastroenterol Hepatol 20, 288-305 (2023).

Milani, M. et al. Phagocytosis-shielded lentiviral vectors improve liver gene therapy in nonhuman primates. Sci Transl Med 11 (2019).

Milani, M. et al. Liver-directed lentiviral gene therapy corrects hemophilia A mice and achieves normal-range factor VIII activity in non-human primates. Nat Commun 13, 2454 (2022).

Starinieri F, Milani M, et al. Spatio-temporal dynamics of the liver shapes hepatocytes heterogeneity and impacts in vivo gene transfer and editing. Manuscript submitted. https://doi.org/10.21203/rs.3.rs-3138832/v1 .