PROJECT 1

DoS: Gentner

Title: New gene therapy approaches for leukemia

Curriculum: Gene and Cell Therapy

Link to OSR/UniSR personal page: http://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/translational-stem-cell-and-leukemia.html

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

Genetically-engineered hematopoietic stem and progenitor cells (HSPC) represent a promising new treatment for genetic diseases. The possibility to equip them with novel functions and/or exploit their progeny as therapeutic vehicles that widely distribute throughout the body including sanctuary sites (e.g. the CNS) or tumors extends their potential use to nonhematopoietic genetic diseases and acquired disorders, such as cancer, as evidenced by 2 recent clinical studies (NCT03488394 and NCT03866109, respectively) to which the lab has contributed. Scope of this project is to study the interplay, dependencies and dissimilarities between normal HSPC and leukemia stem cells (LSC) in order to identify new cell and gene therapy approaches against leukemia. We will model the transplantation of human HSPC into primary leukemia xenografts, faithfully reproducing a clinical scenario where a full-blown leukemia is de-bulked by chemotherapy and molecularly-targeted agents in order to allow HSPC engraftment. We will then employ advanced genetic engineering and immunotherapeutic strategies to increase and prolong HSPC engraftment. To this end, the following approaches may be considered, alone or in combination: (I) Infusion of engineered T cells targeted against leukemia-specific antigens; (II) ex vivo expansion of normal HSPC, potentially paired with genetic modifications increasing their potency and competitiveness against leukemia; (III) engineering resistance of normal HSPC to immunotherapeutics directed against the leukemia, e.g. CAR-T cells against an LSC surface marker; (IV) genetic engineering of the myeloid cell compartment, e.g. to convert an immunosuppressive tumor microenvironment into an immune-stimulatory one. The project will leverage on the following assets available in the laboratory: (1) a huge and unique single cell RNA sequencing dataset on HSPC and leukemic stem cells (LSC), including matched HSC/LSC from the same marrow at the same timepoint. This dataset is a potent resource for LSC-specific surface markers and for defining functional HSPC states induced by the leukemia (; (2) unique expertise in HSPC ex vivo expansion, clonal marking and genetic engineering including lentiviral vectors and access to gene editing technology; (3) long-standing expertise in immunotherapy exploiting HSPC, and active collaborations with T cell experts giving access to state-of-the-art CAR and T cell editing technologies; (4) strong collaboration with the hematology and bone marrow transplantation unit enabling access to patient material.

Skills to be acquired by the student:

The student should already have some laboratory experience to successfully conduct the project in the planned timeframe. In particular, a solid background in cell biology, multicolor flow cytometry and basic molecular biology is of help. The student will learn how to prepare high quality single cell RNA sequencing libraries from primary patient samples using cell sorting and the 10x Genomics platform. She/he will tightly collaborate with a team of bioinformaticians to define the data analysis strategy. The student will apply state-of-the art assays to study hematopoietic stem and progenitor cells (HSPC), AML blasts and immune cells from tumor microenvironments, including in vitro and in vivo functional readouts. In vitro models of HSPC expansion and differentiation towards innate immune cell populations (e.g., monocytes/macrophages) will be applied. Mouse experiments will include xenotransplantation assays (human cells in NSG mice or derivatives thereof). Animal work will include IV injections, monitoring of animal well-being, sampling of blood and analysis, follow up (including in vivo imaging) and sampling of tumor lesions, necropsy with bone marrow and tumor harvest. For the genetic engineering part, the student is expected to master lentiviral vector cloning, production and cell transduction, as well as applying gene editing with the CrisprCas system. The student will participate to weekly lab meetings held in English, where she/he will learn to discuss science in an efficient way, a pre-requisite for participation to international congresses.

References (max. 3)

Efficient Ex Vivo Engineering and Expansion of Highly Purified Human Hematopoietic Stem and Progenitor Cell Populations for Gene Therapy.

Zonari E, Desantis G, Petrillo C, Boccalatte FE, Lidonnici MR, Kajaste-Rudnitski A, Aiuti A, Ferrari G, Naldini L, Gentner B.Stem Cell Reports. 2017 Apr 11;8(4):977-990. doi: 10.1016/j.stemcr.2017.02.010. Epub 2017 Mar 16.PMID: 28330619

Interferon gene therapy reprograms the leukemia microenvironment inducing protective immunity to multiple tumor antigens.

Escobar G, Barbarossa L, Barbiera G, Norelli M, Genua M, Ranghetti A, Plati T, Camisa B, Brombin C, Cittaro D, Annoni A, Bondanza A, Ostuni R, Gentner B*, Naldini L*. Nat Commun. 2018 Jul 24;9(1):2896. doi: 10.1038/s41467-018-05315-0.PMID: 30042420

miR-126 Regulates Distinct Self-Renewal Outcomes in Normal and Malignant Hematopoietic Stem Cells.

Lechman ER*, Gentner B*, Ng SW, Schoof EM, van Galen P, Kennedy JA, Nucera S, Ciceri F, Kaufmann KB, Takayama N, Dobson SM, Trotman-Grant A, Krivdova G, Elzinga J, Mitchell A, Nilsson B, Hermans KG, Eppert K, Marke R, Isserlin R, Voisin V, Bader GD, Zandstra PW, Golub TR, Ebert BL, Lu J, Minden M, Wang JC, Naldini L, Dick JE.Cancer Cell. 2016 Feb 8;29(2):214-28. doi: 10.1016/j.ccell.2015.12.011. Epub 2016 Jan 28.PMID: 26832662