PROGETTO

Supervisore:: _Daniela Cesana_____

Titolo/*Title*: cell-free DNA as an early molecular response biomarker of CAR-T cell activity and efficacy_____

Curriculum: _Medicina Molecolare___

Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento: ___https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/safety-of-gene-therapy-and-insertional-mutagenesis/daniela-cesana.html___

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Chimeric Antigen Receptor T-cell (CAR T) therapy has significantly succeeded in cancer treatment, particularly in hematological malignancies. However, a major hurdle is the heterogeneity of responses that can be achieved. The success of these applications depends on both intrinsic features of the cellular drug product, and inter-individual factors present at the time or after cell infusion.

Rationale and hypothesis

Liquid biopsies are emerging as a powerful approach to detect in a non-invasive fashion dynamic changes occurring in the host due to disease progression or specific treatment. Indeed, cell-free (cfDNA) fragments are released into the circulation from dying cells and can be traced back to the tissues and cell types they originated from using DNA methylation. Decoding changes in the cellular origins of cfDNA over time can reveal altered host tissue homeostasis and the site of damage. By applying optimized technologies and computational tools, the proposed investigation aims to characterize the epigenome and integrome of cfDNA to dissect the informative and predictive role of cfDNA in patients treated with engineered T-cells to fight hematological tumors.

Objectives and specific aims

The research proposal in subdivided in 3 specific aims:

Aim 1: Validation of technologies and computational tools for longitudinal cfDNA profiling studies. The candidate will test and validate omics technologies and computational tools required for longitudinal cfDNA profiling studies, such as methylation profile and

Aim 2. Profile cfDNA signatures in patients who received CAR-T for B-cell tumors. Here, the candidate will perform methylation profile studies on cfDNA longitudinally collected from patients who received CAR-T to reveal the extent of tissue damage induced by tumor presence and treatment. Furthermore, by using a dedicated patented technology, the candidate will retrieve vector integration sites (IS) from cfDNA to provide insights into the activity, clonality and persistence of engineered cells that are residing in peripheral organs.

Aim 3. Bioinformatics and statistical approaches for correlation studies. Correlation analyses between cfDNA-derived data and clinical, molecular and functional variables.

Expected outcomes

I expect to identify variations in the level and contribution of cfDNA production from different cell types depending on the engraftment and persistence of engineered cells in the periphery, disease condition (state, burden, and tumor type), and therapeutic outcome (Complete, Partial, and Non-Responder). Overall, our approach may highlight the role of cfDNA as a predictive biomarker of efficacy and toxicity of CAR-T cell immunotherapy approaches revealing mechanisms of therapeutic success and failure that have not yet been explored.

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

The student will learn cutting-edge molecular biology techniques allowing the retrieval of vector integration sites from different DNA sources (DNA and cfDNA extraction, PCR, real-time PCR, digital PCR, cloning, bacterial cultures, etc.). The student will learn the procedures for library preparation and sequencing and some information on cell culture, vector production, immune-staining, and fluorescence cell sorting analysis. The Student will strongly interact with a team of bioinformatics for integration analyses and learn state-of-the-art techniques for data analysis. Finally, the student will be required to communicate the progress of the project within the Institute and at international scientific meetings.

Bibliografia (max. 15)

1. Cesana D, Calabria A, Rudilosso L, et al. Retrieval of vector integration sites from cell-free DNA. Nat Med. 2021;27(8):1458-1470.

2. Loyfer N, Magenheim J, Peretz A, et al. A DNA methylation atlas of normal human cell types. Nature. 2023;613(7943):355-364.

3. Bachy E, Le Gouill S, Di Blasi R, et al. A real-world comparison of tisagenlecleucel and axicabtagene ciloleucel CAR T cells in relapsed or refractory diffuse large B cell lymphoma. Nat Med. 2022;28(10):2145-2154.

4. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med. 2018;24(5):563-571.

5. Sheih A, Voillet V, Hanafi LA, et al. Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy. Nat Commun. 2020;11(1):219.

6. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17(4):223-238.