PROGETTO

Supervisore:	**Chiara Bonini**
Titolo/*Title*:	**Uncover and overcome senescence and dysfunction of genetically engineered T lymphocytes for cancer immunotherapy**
Curriculum:	**Cell and Gene Therapy**
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/divisions/division-of-immunology-transplantation-and-infectious-diseases/experimental-hematology/chiara-bonini.html

Descrizione del progetto

Background/gap of knowledge

Advancements in gene transfer and genome editing technologies enable to control the expression of multiple genes in T cells, potentially creating precise innovative and long-lasting living drugs specific for tumor antigens and resistant to the immunosuppressive tumor microenvironment (1-3). Still, the success of these applications critically depends on the capacity to engineer T cells without compromising their functional properties.

Rationale and hypothesis

We hypothesize that concomitant exposure to nuclease-induced DNA Double strand breaks (DSB) and viral vectors, used for ex-vivo gene-editing, may lead to elevated DNA damage response (DDR) burden, to the establishment of a senescence program as reported for genetically-engineered hematopoietic stem cells (4) and to consequent impairment in T cell functionality.

We reason that temporary inhibition of DDR or senescence programs during ex-vivo genetic manipulation may improve T cell fitness, long-term persistence and anti-tumoral responses.

Objectives and specific aims

Here, we aim at developing innovative T cell based medicinal products to treat cancer and to investigate and potentially overcome adverse cellular signaling and senescence in the designed cellular products with the final goal of offering a cure to patients.

The proposed project will be implemented through the following steps:

AIM1 Development of first-generation advanced medicinal products for cancer treatment.
The candidate will use tools already available in the hosting lab designed to endow T cells to: 1. Specifically recognize tumor antigens selectively expressed by cancer cells and possibly relevant for oncogenesis and tumor progression. 2. Counteract the immunosuppressive tumor microenvironment. With the toolbox, we shall generate an array of engineered cellular products, that differ for antigen specificity and ability to counteract different and multiple immune suppressive signals. Tumor specific TCR will be transferred through lentiviral vectors or by recombinant adeno-associated virus serotype 6 (rAAV6)-mediated targeted integration of the exogenous TCR in the TRAC locus (1). The endogeneous TCR and genes encoding for inhibitory receptors will be disrupted by the use of CRISPR/Cas9 or base editing according to protocols already available. Cellular products and matched controls will be tested for viability, expansion, phenotype, effector functions, killing and exhaustion in vitro.

AIM2. Investigating senescence and T cell dysfunction to develop second generation T-cell based advanced medicinal products.
In collaboration with Di Micco's lab, we will evaluate several senescence markers in genetically engineered and unmanipulated T cells and we will assess the impact of distinct CRISPR-based platforms (CRISPR-Cas nucleases, base editors) and viral vector transduction on T cell senescence. We will then test selective T cell senescence inhibitors, to identify top candidates to be incorporated into in gene editing protocols to generate 2nd generation advanced cellular products to be tested in vitro (as in AIM1) and upon transfer into immunocompromised mice (AIM3).

AIM3: In vivo comparative analysis of advanced medicinal products.
Through AIM1 and AIM2 we will identify genome engineering and culturing tools that, assembled into workflows, can create advanced T cell products. In AIM3, we will assess their safety and efficacy in vivo. The workflows to be validated in AIM3 will be chosen according to the ability to:
- Redirect T cells towards relevant cancer antigens, granting the highest lysis in vitro.
- Preserve T cell vitality, expansion and function.
- Protect T cells from the tumor microenvironment suppressive signals.
- Avoid senescence and toxic activation of the DNA-damage response.

First and second-generation cellular products will be compared for safety and efficacy in xenograft models, already available in UO1 lab.

Expected outcomes

Our ultimate objective is the development and validation of biotechnological tools, to be combined by a modular approach, to generate innovative T-cell based advanced medicinal products for the treatment of hematological and solid tumors.

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

Hard skills: Genome editing and gene transfer with Crispr-Cas9 technology, base editing, lentiviral vectors, AAV6 vectors; functional testing *in vitro* and in complex xenograft mouse models. Advanced transcriptomics and proteomics analysis.

Soft skills: Critical analysis of scientific literature; Design and management of scientific projects; Data presentation and discussion at internal lab meetings; Scientific presentations at Institutional progress reports and at international conferences; Thesis and manuscript writing.

Bibliografia (max. 15)

1. Ruggiero, STM 2022;

2. Potenza, Balestrieri GUT 2023;

3. Cianciotti Frontiers in Immunology 2024

4. Schiroli et al., Cell Stem Cell, 2019