Damiano per l'Ematologia Associazione di volontariato
(Iscrizione Registro Regionale Umbria n. 837)

8 per mille
CHIESA VALDESE
UNIONE DELLE CHIESE METODISTE E VALDESI

Borsa di Studio "Damiano per l'Ematologia" 2023

Proclamazione del Vincitore

E' risultato vincitore il progetto " Impact of the treatment with targeted therapies on the generation of effective CAR T cells in patients with chronic lymphocytic leukemia". L'autrice è la Dr.ssa Giorgia Mancin, che svolgerà il suo lavoro presso la Divisione Universitaria di Ematologia, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, dell'Università degli Studi di Torino, diretta dal Prof. Benedetto Bruno, sotto la supervisione della Dr.ssa Marta Coscia.

Testo del progetto

a) Title: impact of the treatment with targeted therapies on the generation of effective CAR T cells in patients with chronic lymphocytic leukemia

b) Introduction: treatment approaches exploiting CAR T cells (T cells engineered to express a chimeric antigen receptor, CAR) are rapidly developing in the hematological field. However, in the setting of chronic lymphocytic leukemia (CLL), the efficacy of CAR T cell treatment appears suboptimal as compared to other diseases(1). This may be, at least in part, attributable to immune system dysfunctions - particularly those affecting the T-cell compartment - which characterize patients with CLL and limit the generation and expansion of effective CAR T cells. It has been recently shown that in the small subset of complete-responding patients with CLL, the therapeutic efficacy of CAR T cells depends on the composition of the cellular product and on the intrinsic T cell fitness(2, 3). Targeted drugs, such as BTK inhibitors (i.e. ibrutinib

and acalabrutinib) and the Bcl-2 inhibitor venetoclax, recently entered the therapeutic armamentarium of CLL, showing excellent results in terms of efficacy. Nevertheless, disease relapse still occurs, particularly in high-risk patients, who are therefore eligible for allogeneic transplant or treatment with CAR T cells. Interestingly, besides the direct anti-neoplastic effect, new targeted molecules may exert complex activities on the host immune system (4, 5). Of note, preliminary data obtained in small number of patients suggest that the combined administration of ibrutinib and CAR T cells results in high rates of durable responses, associated with a better tolerance(6, 7). However, little is known on the impact of treatment with targeted drugs on the efficiency of manufacturing and the final anti-tumor efficacy of CAR T cells. Based on these considerations, the objective of this project is to gain novel and original data on the effect of treatment with ibrutinib, acalabrutinib or venetoclax on the ability to generate CAR T cells from patients with CLL. To this aim, T lymphocytes will be isolated from patients before and during treatment and evaluate the efficiency of the process leading to CAR T cells production, and the immunophenotypic and functional features of the final cellular product. It can be anticipated that, during disease evolution, most patients with CLL eligible to CAR T cell therapy will be undergoing treatment with either targeted drug, thus rendering of utmost interest the definition of their immunomodulatory effects during long-term treatment. In addition, results from this project, providing specific information on the impact of these compounds on the generation and anti-tumor efficacy of CAR T cells, will help with the definition of a more rational design of combination regimens, particularly in terms of proper timing and sequencing, for the treatment of selected subsets of high-risk patients with CLL.

c) Aim(s) of the project:
To assess the impact of the treatment with targeted agents (ibrutinib, acalabrutinib or venetoclax) on the generation of CAR T cells in patients with CLL, in terms of manufacturing efficiency, immunophenotypic characteristics, and functional properties.
To correlate phenotypic and functional data on CAR T cells with main CLL prognostic factors (IGHV mutational status, FISH abnormalities, *TP53* mutation status) and outcome variables (response status, duration of response).
To this purpose, peripheral blood samples will be collected from patients with CLL before starting therapy with ibrutinib, acalabrutinib or venetoclax, and after treatment (i.e. 6-month timepoint). Anti-CD19 CAR T cells will be generated and tested for: (i) viability, expansion, and generation efficiency; (ii) phenotypic characteristics, in terms of CD4/CD8 composition, differentiation subset distribution, exhaustion markers and expression of immune check-point molecules; (iii) in vitro functional properties, in terms of proliferation ability, cytokines production, cytotoxic activity and killing of target cells. CAR T cells produced from the same patient at different timepoints will be compared. Phenotypic and functional data on CAR T cells will be also correlated with main CLL prognostic factors (e.g. IGHV mutational status, FISH abnormalities, *TP53* mutation status) and outcome variables (response status, duration of response).
d) Methods: all patients will provide written informed consent through an Institutional Review Board-approved protocol. IGHV mutational status, FISH and *TP53* mutational status will be assessed before

treatment start, according to clinical practice. PBMC will be isolated by density gradient centrifugation. The tumor cell frequency will be assessed by flow cytometry using population specific monoclonal antibodies (anti-CD19 and anti-CD5). If PBMC samples contain more than 90% of tumor cells, a portion of total cells will be cryopreserved without further manipulation to be used later as target cells. Otherwise, tumor cells will be isolated by immunomagnetic bead method prior to cryopreservation. T cells will be enriched by immune-magnetic beads method, and they will be activated with anti-CD3 and anti-CD28 agonists. For lentiviral particles production, 293T cell line (packaging cells) will be co-transduced with (i) a transfer plasmid containing the anti-CD19 CAR construct, (ii) an envelope plasmid, and (iii) a packaging plasmid. The assembled lentivirus will be then harvested from the culture supernatants and will be used to transduce activated T cells. Transduced cells will be cultured and expanded in specific culture medium containing cytokines (i.e. IL-7 and IL-15) for approximately 14 days. Manufacturing efficiency rate will be evaluated measuring cell viability (the percentage of AnnexinV and propidium propidium iodide negative cells measured by flow cytometry) and the percentage of transduction (evaluated by flow cytometry) of CAR T cells at the end of the expansion period (approximately Day 14 of culture). In addition, the expansion rate will be evaluated by the ratio between T-cell count at the end of the culture and T-cell count at the beginning of manufacturing process. Cell viability, transduction efficiency and rate of expansion will be compared between CAR T cells generated from CLL patients before and after during treatment with the targeted therapy. Quality controls will be performed to assess the validity of the process (e.g. T-cell enrichment evaluation and viability assessment). Cell viability will be evaluated by 7AAD staining. Phenotypic characteristics will be assessed by flow cytometry, evaluating: (i) CD4+ and CD8+ T cell composition and differentiation subsets using anti-CCR7 and anti-CD45RA or anti-CD27 and anti-CD45RO monoclonal antibodies, (ii) T helper cell subsets using anti-CD4, anti-CXCR3, anti-CCR6 and anti-CCR4 monoclonal antibodies, and (iii) the expression of immune checkpoint molecules (i.e. PD-1, Tim-3, CTLA-4, TIGIT, BTLA). To test in vitro functional properties, CAR T cells will be exposed to target cells (autologous CLL cells or CD19+ tumor cell lines) and evaluated for: (i) proliferation ability by CFSE assay, (ii) IFN-γ and TNF-α production by flow cytometry, (iii) cytotoxic activity by CD107a degranulation assay. CAR-T cell effector functions will be evaluated by a killing assay. Specific target cells (i.e. autologous CLL cells or CD19+ tumor cell line) will be labelled with a fluorescent dye and cultured with CAR T cells. At the end of the culture the viability of target cells will be assessed by flow cytometry (Propidium iodide staining) and the percentage of lysis will be calculated. The percentage of lysis obtained with CAR T cells generated from CLL patients before and during treatment with ibrutinib or acalabrutinib will be compared.

e) References

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Time to complete the project: 12 months. The project will be conducted in collaboration.

Giudizio del Comitato Scientifico:

Parametri	**Punti**
Rilevanza dell'argomento	3
Originalità del progetto	3
Innovazione	2
Metodi	3
Riproducibilità e applicabilità dei risultati al trattamento dei pazienti	2
Punteggio addizionale	0

CV della candidata

Il CV della Dr.ssa Giorgia Mancin è così riassumibile: Laurea magistrale in Cellular and Molecular Biology presso l'Università di Torino, laurea triennale in Scienze Biologiche presso la medesima università. Tirocinio curriculare presso il laboratorio di Ematologia Traslazionale, Divisione di Ematologia, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute dell'Università di Torino, con focus sugli effetti immunomodulanti del trattamento con ibrutinib in pazienti affetti da leucemia linfatica cronica..