DOCTORAT BIOLOGIE BRETAGNE SANTE LOIRE

Year 20 /20

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☒ **Requested** ☐ **Acquired**	**Funding origin:** Request of funding for a doctoral program and funding from the Ligue contre le Cancer.
Thesis title: Deciphering the interactions and functions of TIGIT to optimize tumor-specific T lymphocytes activation.		3 keywords: Immunotherapy Immune checkpoint Melanoma
Unit / team: **INCIT, Inserm UMR1302**		
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Socio-economic and scientific context (approximately 10 lines):
Therapeutic strategies blocking the interaction between coinhibitory receptors on tumor-infiltrating T cells and their ligands expressed on tumor cells and/or antigen-presenting cells have proven successful in multiple types of cancer. However, immune checkpoint inhibitor (ICi) therapy typically yields an overall response rate of 20% to 40% in patients with solid tumors, and although generally manageable, immune adverse events are frequent, due to the sustained activation of immune cells. In order to limit the occurrence of immune-related adverse events due to systemic injection of ICi, an alternative approach is to use genomic editing tools to invalidate IC expression in therapeutic T cells. This approach is currently evaluated in preclinical models and in clinical trials. Nonetheless, few studies documented the possible unexpected consequences of such gene edition on T cell fitness, that is a crucial limiting factor for adoptive cell transfer efficacy. Indeed, the question of the impact of genomic deletion of IC on essential properties of $CD8^+$ T lymphocytes, such as their persistence, is a crucial issue to address in order to consider the development of this therapeutic strategy.

Working hypothesis and aims (approximately 8 lines):

Among IC, TIGIT and PD-1, highly co-expressed on tumor-reactive T cells, appear to be interesting candidates. They exert a non-redundant inhibition of the activating receptor CD226, expressed on T cells. Beyond the competition of TIGIT with CD226, and unlike PD-1 for which the inhibitory signaling pathways are well known, the interactome of TIGIT has not yet been determined. The host team already documented that adoptive transfer of melanoma-specific $PD\text{-}1^{KO}$ T cells resulted in enhanced control of tumor growth of melanoma cells when transplanted into immunodeficient mice (*Marotte, 2020*). However, deletion of PD-1 in these cells also decreased their proliferative capabilities, preventing a complete tumor control, in addition to the maintained expression of TIGIT in these same cells.
Thus, the project aims to determine whether this characteristic is specific to PD-1 deletion or whether it can also be observed upon invalidation of TIGIT. Initial results obtained on T-cell clones confirmed that melanoma-specific $PD\text{-}1^{KO}$ T cells show a decreased proliferation while $TIGIT^{KO}$ cells retain this ability. Furthermore, another aspect of the project is to formally unveil the molecular basis of the T-cell intrinsic inhibitory function of TIGIT in melanoma-specific human $CD8^+$ CTL using quantitative interactomics. This part will be done in collaboration with the team of B. Malissen (CIML, Marseille, program funded by ARC) who recently characterized the interactome of PD-1 (*Celis-Gutierrez, 2019*).

Main milestones of the thesis (approximately 12 lines):

- **Impact of TIGIT and PD-1 deletion on polyclonal melanoma-specific T cell populations**

Although the differential impact of PD-1 and TIGIT deletion on the proliferative capacity of T cells has already been documented on T cell clones, we will confirm these results on polyclonal populations, specific to different tumor antigens, and on melanoma-derived TIL in order to increase their robustness. To do so, we set up a protocol to obtain very high editing efficiencies from effector-memory T cell populations.

These populations will be compared for 1) their anti-tumor reactivity using previously generated cell models (T2 lines and melanoma lines), and 2) for their proliferative capacities, via a CSFE proliferation assay and 3) the expression of cell-cycle related genes by RNAseq and qPCR.

- **Definition of TIGIT interactome (collaboration with B. Malissen, CIML, Marseille)**

The candidate will train and participate in the experiments necessary for the definition of the interactome of TIGIT, during regular stays in the team of B. Malissen, specialist in interactomics. The aim is to define the composition of the TIGIT signalosome assembling in therapeutic T cells, using high-dimensional phosphoproteomics.

- **Pre-clinical model**

The activity of IC-edited and unedited TILs on autologous tumor lines will be measured *in vivo* in immunodeficient mice. Obtained pre-clinical results could then allow the development of an adoptive therapy trial for patients with metastatic melanoma. Indeed, our team has a close collaboration with the clinical team of oncodermatology of the CHU of Nantes, with whom several adoptive transfer trials have been performed.

Scientific and technical skills required by the candidate (2 lines):
This project will combine cellular, molecular, and biochemical approaches to immuno-oncology. Particularly, the candidate should master cell culture with T cells and human tumor cell lines, as well as flow cytometry. They will also have to adapt to different work environments.

3 publications from the team related to the topic (last 5 years):

1. Marotte L, Capitao M, Deleine C, Beauvais T, Cadiou G, Perrin J, Chérel M, Scotet E, Guilloux Y, Bruchertseifer F, Morgenstern A, Jarry A, Gaschet J, Labarriere N. Anti-tumor efficacy of a combination therapy with PD-L1 targeted alpha therapy and adoptive cell transfer of PD-1 deficient melanoma-specific human T-lymphocytes. *Oncoimmunology*. 2021 Jun 27;10(1):1940676. doi:

10.1080/2162402X.2021.1940676.

2. Dréno B, Khammari A, Fortun A, Vignard V, Saiagh S, Beauvais T, Jouand N, Bercegay S, Simon S, Lang F, Labarrière N. Phase I/II clinical trial of adoptive cell transfer of sorted specific T cells for metastatic melanoma patients. *Cancer Immunol Immunother*. 2021 Oct;70(10):3015-3030. doi: 10.1007/s00262-021-02961-0.
3. Marotte L, Simon S, Vignard V, Dupre E, Gantier M, Cruard J, Alberge JB, Hussong M, Deleine C, Heslan JM, Shaffer JM, Beauvais T, Gaschet J, Scotet E, Fradin D, Jarry A, Nguyen TH, Labarriere N. Increased anti-tumor efficacy of PD-1 deficient melanoma-specific human lymphocytes. *J Immunother Cancer*, **2020**, Jan;8(1): e000311. doi: 10.1136/jitc-2019-000311.

National and international collaborations:
Part of the research program will be carried out in collaboration with the team of B. Malissen of CIML (Marseille). This part of the program is financed by a program labeled by the ARC and the stays and travels of the PhD student will be taken in charge in this context.