Triggering Haematological Adoptive T-cell Immunotherapy Strategies by HUnting Novel T-cell receptors

Reporting

Project Information

THAT IS HUNT

Grant agreement ID: 752717

Project website

DOI
10.3030/752717

Project closed

EC signature date
24 March 2017

Start date
1 January 2018

End date
31 December 2019

Funded under
EXCELLENT SCIENCE - Marie Skłodowska-Curie Actions

Total cost
€ 168 277,20

EU contribution
€ 168 277,20

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Periodic Reporting for period 1 - THAT IS HUNT (Triggering Haematological Adoptive T-cell Immunotherapy Strategies by HUnting Novel T-cellreceptors)

Reporting period: 2018-01-01 to 2019-12-31

Summary of the context and overall objectives of the project

Acute myeloid leukemia (AML) is an aggressive malignancy, still largely incurable. AML can be cured by allogeneic hematopoietic stem cell transplantation (HSCT), indicating that the disease is sensitive to immunotherapy. The efficacy of this therapeutic approach is partially related to the Graft versus Leukemia (GvL) effect, the ability of the allogeneic immune system to mount an immune response towards cancer cells. Unfortunately, the GvL effect is often counterbalanced by the onset of an inflammatory reaction against healthy tissues (Graft versus Host Disease, GvHD). Several strategies have been pursued with the aim of decreasing GvHD while benefiting of GvL. With these procedures, GvHD decreased but the immune reconstitution was slower with a consequent increase of the infection rate and of disease relapse. New treatments are needed, and adoptive T cell therapy may represent a promising approach. The infusion of T cells specifically able to recognize tumor antigens can mediate leukemia eradication while avoiding GvHD. Furthermore, it is nowadays possible to completely abrogate the endogenous T cell receptor (TCR) repertoire and to redirect T cell specificity towards tumor cells, further increasing the potential for human therapeutics of TCR-engineered T cells. What is then limiting the wide range exploitation of T cell-based immunotherapeutic approaches? To generate a potent and clinically relevant T cell engineered product, the identification of novel epitopes and the hunting of novel TCRs is fundamental. With the THAT IS HUNT proposal, we are addressing the paucity of tumor-specific TCRs currently available by exploiting a “from bed to benchside and back approach”. The plan was to identify in vitro a collection of novel TCRs and tumor epitopes by taking advantage of a) intrinsic biological features of the leukemic blasts, b) omics technologies and c) high dimensional flow cytometry.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far

The project has been developed along 4 different scientific objectives designed with 2 major aims: a) the longitudinal clonal tracking of tumor-specific T cells in AML patients and the b) the identification of an array of tumor-specific T cells, and TCRs, against a variety of tumor antigens, to be exploited for adoptive T cell therapy.

Concerning the first aim, we have taken advantage of multi-parametric flow cytometry to immunoprofile and longitudinally characterize the frequency and functional phenotype of tumor-specific T cells in patients after HSCT. T cells from peripheral blood (PB) and bone marrow (BM) were analyzed in terms of expression of activation and exhaustion markers, T cell memory phenotype and for reactivity against known tumor peptides. Results of our study highlighted the existence of a profound exhaustion signature associated with tumor-specific T cells both in BM as well as in PB samples, with the expression of multiple inhibitory receptors.

We identified 8 TCRs specific for 5 epitopes belonging to 4 tumor antigens and restricted to 2 HLA alleles highly frequent in the Caucasian population.

Coming to the second aim, we focused on the identification of tumor epitopes, and tumor-specific T cells, by employing different experimental strategies.

• Starting from peripheral blood mononuclear cells of healthy donors (HDs) we designed and implemented an innovative protocol for the rapid isolation of tumor-specific T cells and for the

characterization of a library of tumor-specific TCRs restricted to different HLA alleles. We selected as target antigens Cyclin A1 and Cathepsin G (CG), 2 proteins overexpressed in AML and relevant also for additional tumor entities. We achieved successful expansion of tumor-specific T cells from 15 consecutive HDs. TCR αβ sequencing led to the identification of 10 tumor TCRs specific for 10 epitopes presented by 6 HLA haplotypes.
• Starting from patient material, we enlarged our study to the identification of TCRs directed towards novel peptides of tumor antigens, independently of their HLA restriction. We exploited the exhaustion phenotype of tumor-specific T cells in AML patients and the possibility to instruct AML blasts to become potent leukemic antigen presenting cells. We identified 3 tumor-specific TCRs.
To identify novel leukemia epitopes, we employed a ligandome-based strategy.
Additionally, by interrogating the great amount of TCR sequences retrieved in our study, we sought for common molecular features. We have observed the existence of specific biases in the anti-tumor immune repertoire: the preferential usage of defined variable genes in the rearrangement process occurring during the generation of the TCRs and the presence of public clones generated mainly by convergent recombination events.

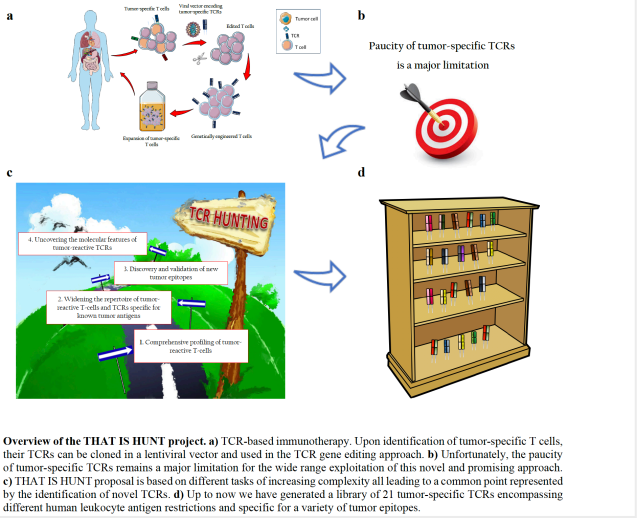
Overall, we have generated a library of 21 TCRs able to recognize 6 different tumor antigens (for a total of 15 peptides) and restricted to 7 HLA alleles, including the most frequent ones in the Caucasian population. These TCRs can be now exploited in the TCR gene editing strategy for the treatment of leukemia.

The results of this study have been presented at several national and international meetings.

Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

Results obtained in our project have shed lights on the molecular and cellular features of tumor-specific T cells in AML patients. Compared to previous studies, we here performed a deep immunoprofiling of this highly relevant tumor population starting from patient material stored at the biobank of San Raffaele Hospital. We characterized the phenotypic features of tumor infiltrating T cells and we molecularly studied their immune repertoire by TCR sequencing. With this analysis we were able to clearly identify the occurrence of molecular biases in the generation of tumor-specific T cells, represented by convergent recombination events, public clones and preferred selection of defined genes during the TCR rearrangement process. Previous studies have already reported the occurrence of such biases in the anti-viral immunity; a clear description of the events related to the anti-tumor immunity was still lacking.
Mostly relevant for the immunotherapy field, we have identified novel immunogenic tumor epitopes and consequently novel TCRs restricted to a variety of HLA alleles highly frequent in the Caucasian population. Furthermore, the comprehensive characterization of tumor-reactive T cell features and the establishment of a variety of approaches for the enrichment of tumor-specific T cells will streamline TCR hunting strategies in other tumor settings, including solid tumors. Altogether, these results will foster TCR-based strategies in the immunotherapy arena. Establishment of T cell-based approaches

as a standard-of-care treatment would also have an economic relevance. In fact, it would reduce the financial expenses and resources required for cancer therapy by moving from the one-pill a day for a life-time paradigm, to the concept of a living drug which provides long-term protection thanks to the ability of T cell to persist long term in the patients and patrol for tumor recurrence.



Overview of the THAT IS HUNT project. a) TCR-based immunotherapy. Upon identification of tumor-specific T cells, their TCRs can be cloned in a lentiviral vector and used in the TCR gene editing approach. **b)** Unfortunately, the paucity of tumor-specific TCRs remains a major limitation for the wide range exploitation of this novel and promising approach. **c)** THAT IS HUNT proposal is based on different tasks of increasing complexity all leading to a common point represented by the identification of novel TCRs. **d)** Up to now we have generated a library of 21 tumor-specific TCRs encompassing different human leukocyte antigen restrictions and specific for a variety of tumor epitopes.

THAT IS HUNT overview

Last update: 3 May 2024

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