ImmunoTools *special* Award 2015

Diana Campillo, PhD-student

Supervisor: Prof. Dr. Viggo Van Tendeloo

Institute: Antwerp University Hospital (UZA) and VAXINFECTIO institute (University of Antwerp), Laboratory of Experimental Hematology, Tumor Immunology Group (TIGR), Wilrijkstraat 10, B-2650 Edegem, Belgium

Wilm's tumor (WT1)-specific TCR-modified T cells for adoptive anti-cancer therapy

The selection of the best treatment for each type of cancer is essential for disease prognosis and progression. In this regard, treatment failure is one of the major problems due to the persistence of therapy-resistant tumor cells that can lead to a minimal residual disease (MRD) and, eventually, to cancer recurrence. In the case of acute myeloid leukemia (AML), it is characterized by the expansion of myeloid blasts in blood and bone marrow that can be spread out to other tissues. The major treatment of choice is chemotherapy, sometimes followed by stem cell transplant. The rapid progression and heterogeneity of this disease and the different responses to treatment make the eradication of all leukemic cells difficult. Therefore, there is an urgent need for novel treatment paradigms that could tackle the problem of MRD in the adjuvant setting or even provide a primary treatment option for the relapsed cancer patient.

Among all the different alternatives, adoptive immunotherapy by cytotoxic T lymphocyte (CTL) transfer uses CTL inherent capacity of recognizing peptides presented on MHC class I molecules and their effector antitumor activity to mediate tumor regression. Tumor cells are able to express self-antigens in an aberrant manner compared with normal cells. These tumor-associated antigens (TAAs) are highly expressed in tumor cells, but not in normal cells. It has been demonstrated that TAAs are recognized by specific T cell receptors (TCRs) leading to an immune response against the tumor cell. On this subject, Wilm's tumor gene 1 product (WT1) is highly expressed in AMLs, as well as in some solid tumor cells, whereas it is absent or only weakly expressed in the vast majority of normal cell types. This zinc finger transcription factor is used as a biomarker for cancer progression and prognosis. Due to its main restriction to tumor cells, it has been employed to develop different vaccines to fight against several cancers, including AML. An innovative strategy is based on the use of redirected T cells genetically engineered with a predefined WT1-specific TCR. This approach aims to select high-affinity WT1-specific T cell clones from a T cell population in order to obtain their TCRα and β chain sequences. Subsequently, the isolated TCRαβ chains can be introduced and

expressed into normal T cells from a different individual with the proper MHC haplotype. Hence, the TCR-modified T cells would acquire the capability of clearing WT1-bearing tumor cells after adoptively autologous transfer. However, these vaccines are not being as successful as initially described due to several reasons. The introduction of the exogenous TCRαβ chains could produce the formation of a neo-TCR due to mispairing of endogenous and inserted TCRαβ chains. This hybrid TCR could cause off-target side effects that might lead to severe tissue damage as a result of unwanted autoreactivity. Moreover, together with competition for CD3 molecules between the endogenous and the inserted TCR, mispaired TCRs could cause reduced expression of the transgene TCR and, hence, reduced function in WT1 antigen recognition. Our aim is to introduce a high-affinity WT1-specific TCR into normal T cells of patients using a clinically applicable transfection method and to incorporate a strategy to avoid TCR mispairing, thereby maximizing the killing of residual AML cells.

First, we will perform a screening of WT1-specific T cell clones from a patient that went into complete AML remission after WT1-specific dendritic cell vaccination developed by our group. After selecting the highest-affinity TCR, we will isolate and clone the TCR alpha and beta chains from that clone into an appropriate cloning vector. The specificity and the effectiveness of the introduced TCR will be tested (ImmunoTools). Secondly, the implementation and validation of a silencing strategy to block the expression of the endogenous TCR will be carried out in order to avoid TCR mispairing. After that, we will select the most effective T cell type for our vaccines by sorting relevant T cell subsets for adoptive immunotherapy (ImmunoTools). Our final goal is to design a clinically safe, adoptive TCR-redirected T cell immunotherapy for personalized AML treatment. In general, several anti-human antibodies, cytokines and ELISA kits will be crucial for the successful development of these experiments, including: CD3, CD8 and CD62L antibodies for T cell selection and sorting; IL-2, IL-15 and IL-21 for T cell *ex vivo* expansion; human IFN-gamma and TNF-alpha ELISA kits to assess for lymphocyte activation (ImmunoTools).