ImmunoTools *special* Award 2023

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Redirecting pre-existing antiviral T cells to eliminate cancer cells as a novel form of cancer immunotherapy

Immunotherapeutic approaches based on PD-1/PD-L1-inhibtiory molecules are unfortunately only effective in a minority of patients and only in selected cancer types. This may be caused by defects in (neo)antigen presentation and absence of appropriate T cell signaling. Consequently, anti-cancer cytotoxic T lymphocytes (CTLs) occur in very low frequencies, become increasingly impaired in the tumor microenvironment due to cancer immunoediting and decline with age.

In sharp contrast, CTLs directed against persistent viruses such as the cytomegalovirus (CMV), Human Papilloma Virus (HPV) and SARS-CoV2 are maintained at high frequencies in seropositive subjects. In case of CMV, up to 20% of the total CTL population can be comprised of anti-CMV T cells. Compared to endogenous anti-cancer CTLs, antiviral CTLs often display better breadth, polyclonality, frequency and functionality as they are generated against highly immunogenic ‘non-self’ epitopes from viral antigens. Moreover, antiviral CTLs are less prone to exhaustion and retain full capacity to migrate into virtually all tissues and even populate human tumors.

The aim of our research is to repurpose these endogenously present, constantly renewable antiviral CTLs to kill cancer cells in a target antigen-restricted manner.

To achieve this, we constructed a series of fusion proteins, coined ‘ReTARGs’, in which various cancer-directed antibody fragments (amongst others anti-CD19) are fused to a single-chained soluble HLA-I/β2m molecule genetically equipped with an HLA-matched virus peptide derived from a highly immunogenic virus protein. This ‘peptide makeover’ renders cancer cells targetable for cognate pre-existing antiviral T cells induced by natural infection (CMV, EBV) or (mass) vaccination (SARS-CoV2, HPV). In this way, we cannot

only redirect antiviral CTLs to attack cancer cells, but we also make the process of cancer cell recognition independent of (frequently impaired) endogenous HLA-I expression. My project aims to further improve this tumor-selective peptide exchange technology and broaden its clinical applicability, in particular for cancer patients who remain unresponsive to current forms of immunotherapy.

The efficacy of these 'ReTARG' fusion proteins will be examined on several human cancer cell lines (both solid tumors as well as leukemias/lymphomas) as well as on primary patient-derived cancer cells. We will investigate the cancer cell elimination capacity of antiviral CTLs by staining for Annexin V-positive (apoptotic) cells by flowcytometry.

The required antiviral CTLs will be obtained from peripheral blood mononuclear cells (PBMCs) from healthy HLA-matched seropositive volunteers by stimulation with a virus protein solution. Subsequently, cytokines IL-2, IL-7, IL-15 will be used to maintain and expand the population antiviral CTLs.

Moreover, we will assess how (multiple rounds of) ReTARG-mediated tumor cell elimination will affect the antiviral T cell population (in terms of activation and exhaustion). To distinguish different immune cell populations, we will use combinations of fluorescently labelled antibodies. We will detect CTLs by staining for CD3/CD8 and study T cell activation by examining CD25 surface expression and T cell exhaustion by CD279 surface expression.

Our research will contribute to the exploration of a potential complementary and/or alternative next-generation approach in cancer immunotherapy, which is of particular relevance for the vast majority of cancer patients who do not respond to currently available PD-1/PD-L1 immune-checkpoint inhibitors and/or to those who are not eligible for bispecific T cell engager (BiTE)-based approaches.

ImmunoTools *special* AWARD for **Anne van Wijngaarden** includes 9 reagents

FITC - conjugated anti-human CD25, Annexin V

PE - conjugated anti-human CD279

PerCP - conjugated anti-human CD8

APC - conjugated anti-human CD3, Annexin V

recombinant human cytokines: rh IL-2, rh IL-7, rh IL-15

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