# ImmunoTools *special* Award 2021

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## Repurposing inflationary anti-CMV CTLs to eliminate cancer cells - an alternate form of cancer immunotherapy

A major challenge in cancer immunotherapy remains the low response rate of patients to currently available treatment strategies. Cancer cells have intrinsic and/or acquired characteristics to escape immune surveillance by cytotoxic T lymphocytes (CTLs). This involves the limited reactivity of protective anti-cancer CTLs to self- and neoantigens. Moreover, cancer cells are capable of down-regulating their HLA-I complexes on the cell surface and thereby further reduce their immunogenicity. Consequently, anti-cancer CTLs occur in very low frequencies, become increasingly impaired in the tumor microenvironment due to cancer immunoediting and also decline with age.

In sharp contrast, CTLs directed against persistent viruses such as the cytomegalovirus (CMV) are abundantly present in CMV-seropositive subjects. CMV prevalence is particularly high, reaching up to 90% of the general population in many countries.

During viral infections T cells recognize viral peptides, proliferate and attack infected body cells. Once virus-infected cells are eliminated, the number of CTLs decreases and only a small memory T cell population remains. However, due to the lifelong persistence and occasional reactivation of CMV, the population of anti-CMV CTLs does not contract but accumulates instead and steadily increases with age; a phenomenon known as ‘memory T cell inflation’. Anti-CMV CTLs can comprise up to 20% of the total CTL population in elderly.

Unlike anti-cancer CTLs, anti-CMV CTLs are maintained at a *ready-to-go* state and do not become exhausted or senescent. Moreover, inflationary anti-CMV CTLs retain full capacity to migrate into virtually all tissues and also populate human tumors.

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

To achieve this, we constructed a series of fusion proteins, designated ‘Retargs’, in which various cancer-directed antibody fragments (amongst others anti-CD19) are fused to a single-chained soluble HLA-I/β2m molecule equipped with an HLA-matched CMV peptide derived from a highly immunogenic CMV protein. In this way, we cannot only redirect anti-CMV CTLs to attack cancer cells, but we also make the process of cancer cell recognition independent of (frequently impaired) endogenous HLA-I expression.

The efficacy of these ‘Retarg’ fusion proteins will be examined on several human cancer cell lines of different origin as well as on primary patient-derived cancer cells. I will investigate the capacity of anti-CMV CTLs to induce cancer cell elimination by staining for Annexin V-positive (apoptotic) cells by flowcytometry.

The required anti-CMV CTLs will be obtained from peripheral blood mononuclear cells (PBMCs) from healthy HLA-matched CMV-seropositive volunteers by stimulation with a CMV protein solution. Subsequently, growth factors (IL-2, IL-7, IL-15) will be used to expand/maintain anti-CMV CTLs.

Moreover, to be able to distinguish different immune cell populations, I will use combinations of fluorescently labelled antibodies. I will detect CTLs by staining for CD3/CD8 and study T cell activation by examining CD25/CD69 surface expression changes in the presence of our ‘Retarg’ fusion proteins and cancer cells.

This 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 **Isabel Britsch** includes 10 reagents

FITC - conjugated anti-human CD69, Annexin V

PE - conjugated anti-human CD25

PerCP - conjugated anti-human CD3

APC - conjugated anti-human CD8, CD19, Annexin-V APC

recombinant rh IL-2, rh IL-7, rh IL-15

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