ImmunoTools *special* Award 2015

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The use of chimeric antigen receptors (CARs) in adoptive T-cell transfer for cancer therapy

Harnessing the immune system to treat cancer by adoptive transfer of autologous T-cells bearing an engineered TCR has been widely used in the past decades. Chimeric antigen receptor (CAR) bearing T-cells are chimeric proteins combining the antigen recognition domain of an antibody with T-cell effector domains.

Several features underline the therapeutic use of CARs (1) They recognize their native targets without the need of prior antigen processing and HLA-dependent antigen presentation compared to normal T-cells (2). They can be used as a polyclonal T-cell population against sheer any antigen represented on a tumour cell consequently augmenting tumour-targeted T-cell numbers. (3) The use of autologous T-cells makes them compatible with any patient thus minimizing GvHD.

To date CAR T-cells have been tested successfully in a broad range of tumour settings. Targeting of erbB2 in colorectal and ovarian cancer *(Liu, 2015)*, GD2 for neuroblastoma *(Louis, 2011)*, carbonic anhydrase 9 (CAIX) *(Lamers, 2013)* in metastatic RCC, mesothelin for pancreatic, ovarian and some lung cancers *(Beatty, 2014)*, and CD20 *(Till, 2012)* and CD19, respectively, for B-cell malignancies like leukaemia and lymphoma *(Kochenderfer, 2015; Brentjens, 2013)*.

The vast majority of the clinical studies on CAR T-cells is being performed in the United States and several studies have been launched in china in the last two years. In Europe there are only few centres using CAR T-cells in adoptive immunotherapy (https://clinicaltrials.gov/ct2/results?term=car+t+cells). The objective of this study is to develop a protocol for optimal and maximal T-cell expansion together with transduction of T-cells with a lentiviral-based GPF expressing construct and furthermore, to expand the protocol to a GMP-compliant setting.

First, T-cells will be obtained from blood samples of healthy donors and lymphoma patients and activated by the agonistic antibody OKT-3 with or without the addition of

co-stimulatory CD28. In order to achieve appropriate T-cell expansion we will supplement cultures with T-cell growth supporting cytokines rh IL-2, rh IL-7, rh IL-15 and rh IL-21, respectively. This is especially important, as recent data suggest that combinations of these cytokines hold more promise than the sole use of IL-2 as they maintain the T-cells in a more undifferentiated state, in which they seem to perform better *in vivo (Pouw, 2010; Hinrichs, 2009)*. Extensive phenotypic characterization of the best expansion protocol can be performed thanks to ImmunoTools flow cytometry fluorophores. Expression of CD3, CD4, CD8 as well as the expression of CD25 and CD69, being T-cell activation markers, will be determined. Moreover a detailed phenotypic characterization of the differentiation state will be performed by CD27 and CD45Ra. Purity of the culture will be evaluated by staining for monocytes and dendritic cells. The protocol with the highest yield of T-cells and best phenotype will be chosen for transduction of T-cells.

Second, we want to generate CAR T-cells by means of lentiviral GFP expressing vector transduction. In order to determine the optimal transduction protocol, activated or resting T-cells will be used for transduction. Furthermore, the influence of stimulation directly after transduction will be tested. In order to enhance viral entry, cationic polymers, retronectin coated plates or spin infections can be applied. Important parameters for efficient transduction will be expression of GFP and furthermore evaluation of cell death. Therefore, virus titers will have to be optimized as well as several rounds of transduction will have to be considered. Adjusting viral concentrations, transduction time and transduction frequency, respectively, will give information about which protocol gives rise to stable transduced cells. Importantly, phenotypic characterization, as mentioned above, of stable transduced cells will be facilitated by ImmunoTools fluorophores.

Finally, the protocols giving the highest expansion and transduction efficiency will be used for further testing of functionality of T-cells and extended to GMP-compliant conditions.