Chimeric antigen receptors (CARs) for the treatment of CD19 positive B cell malignancies

Summary

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Status: open
Offered by: Medical University of Graz

Description

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Background

The most common types of hematological neoplasms in adults are aggressive B cell lymphomas, arising from a malignant transformed and clonally expanded mature B cell in the germinal center of secondary lymphoid follicles (1, 2). Despite the improvements in therapy, approximately one third of patients with advanced-stage DLBCL still are refractory to standard therapy or will relapse (3). Harnessing the immune system by e.g. cancer vaccination, administration of monoclonal antibodies and adoptive transfer of T-cells bearing an engineered TCR in the treatment of cancer 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. Fusion of the V¬L and VH chains of an antibody directed against a tumour-specific antigen with the signalling moiety of the TCR leads to specific antigen recognition and subsequent T-cell activation. To date CAR T-cells have been tested successfully in a broad range of tumour settings and the use of CD19 as antibody fragment is common in the treatment of various B-cell malignancies. CD19 is expressed on B-cells from the early to the mature state and its expression is maintained in transformed B-cells, being expressed in >95% of B-cell non-Hodgkin lymphomas (NHL), acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL). This broad expression pattern in B-cell malignancies taken together with the fact that its expression is restricted to B-cells excluding off-targets makes it a favourable antigen for adoptive CAR T-cell therapy (4, 5). Hence, the use of CD19-specific CAR T-cells for treatment of CLL, ALL and DLCBL is currently being investigated in several early phase clinical trials and shows promising results regarding engraftment, limited toxic-side effects and complete anti-tumour response, respectively (6-8).

Objectives

In this study, we will use a third generation adenovirus based construct bearing two co-stimulatory domains in order to improve T cell activation and cytotoxic activity together with a new gene delivery system for transient expression of the CAR construct. First, this study aims to develop a GMP-compliant protocol for the expansion, transduction and subsequent in-vitro validation of T-cells transduced with the CD19 CAR construct. Second, anti-CD19 expressing CAR T-cells will be tested ex-vivo in the PBMC of CD19+ lymphoma patients and healthy donors. Successfully generated autologous anti-CD19 CARs should specifically lyse more than 50% of CD19+ lymphoma cells in the fraction of lymphoma patients, whereas they should be ineffective in the PBMC of healthy individuals. Third, a GMP-compliant protocol, based on the proof-of-concept studies, will be established for the use in humans. Furthermore, we want to search for the identification and validation of novel markers on leukemic and lymphoma cells for targeted therapy with CAR T cells.

Methods

The PhD student will develop protocols for optimal T cell expansion of PBMC from healthy donors and lymphoma patients. This includes activation of T cells by agonistic antibodies and costimulatory molecules.
together with diverse cytokine supplementations. T-cell cultures will be phenotypically characterised regarding the expression of activation markers and different subpopulations using FACS analysis. Furthermore, a protocol for effective transduction of T cells with the vector system will be established. Therefore, the activation time, the use of transduction enhancers like cationic polymers and different cell culture media will be evaluated. Stable transduction will be determined by detection of GFP and the anti CD-19 construct by means of FACS analysis and Western Blotting, respectively. Transduced cells will be tested for their functionality using the Cr51 release assay, detection of inflammatory cytokines by the CBA human Th1/Th2 cytokine kit and cell lysis assays. Last, the student will validate and identify (novel) surface markers suitable for targeted gene therapy using CAR T cells.

Literature


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