Chimeric antigen receptors for the treatment of hematologic malignancies

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Introduction

- Adoptive T—cell therapy with chimeric antigen receptor (CAR) bearing T-cells combines the specificity of the antigen recognition (V_L and V_H) domains of an antibody with the signalling moiety of the TCR. Upon target recognition, directed against a tumour-specific antigen, this leads to activation of the cytotoxic effector functions of the T-cell and subsequent tumour cell lysis.

The use of CARs represents a powerful tool for cancer immunotherapy and CARs incorporating anti-CD19 are increasingly used for the therapy of CD19+ B-cell malignancies, especially in the setting of recurrent/ refractory leukemia and lymphomas.

Objectives

- In this study we will use a third generation construct bearing the CD28 and 4-1BB co-stimulatory domains in order to improve T cell activation and cytotoxic activity together with a SV40 based gene delivery system. This expression systems takes into advantage the transient, non-integrative, expression of the construct compared to the currently used lentiviral vectors. Importantly, this facilitates better *dosage* and *elimination of CARs* when desired. Moreover this systems *avoids development of autoimmune reactions*, an additional negative side effect of the currently used lentiviral-based methods.

Methods

- Development of a GMP-compliant protocol for the expansion and activation of T-cells including different activation protocols and determination of the subpopulations of T-cells, activation state and purity of the culture method
- Development of a GMP-compliant protocol for transduction with the SV40 based construct
- *In-vitro* validation of T-cells transduced with the CD19 CAR construct (Cr^{51} release and lysis of CD19+ cell lines)

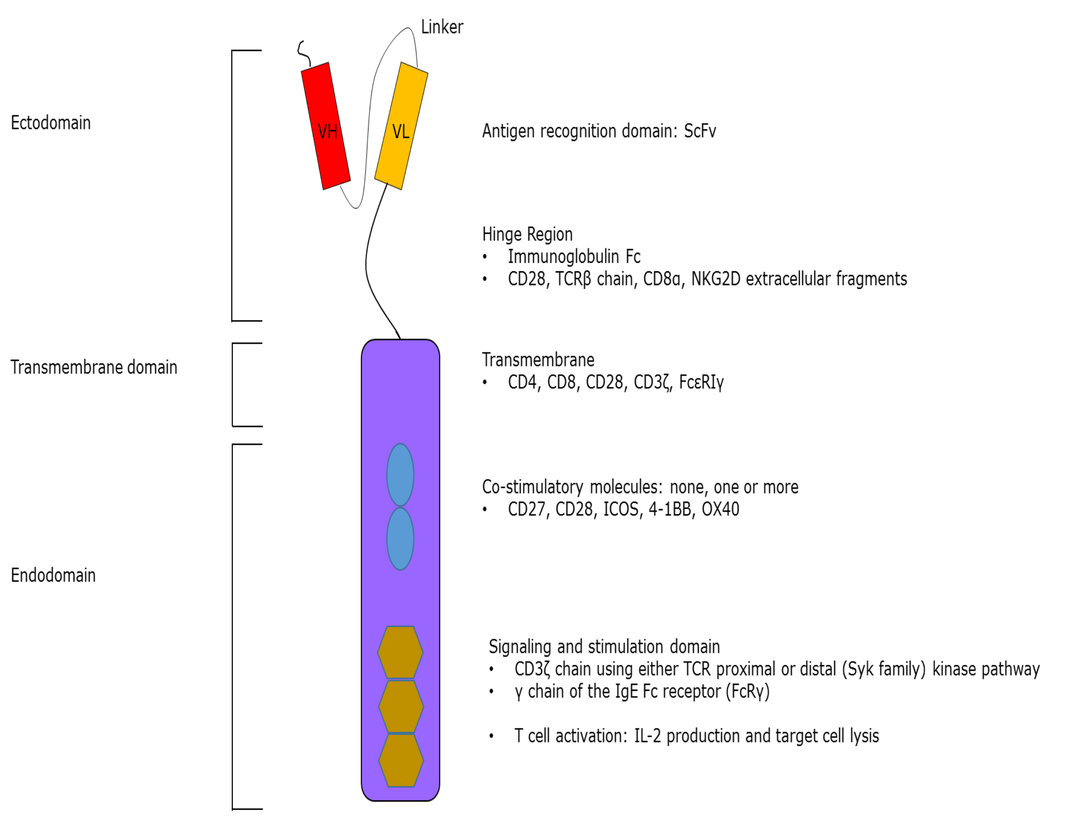

Figure 1. Structure of a typical CAR construct. Depicted are the different domains of the molecule together with the possible fragments used in CAR design

Number of Studies	Location	Sponsor	Phase
7	USA	Abramson Cancer Center of the University of Pennsylvania	0 to II
5		Baylor College of Medicine	I
1		Children's Hospital of Philadelphia	I
7		City of Hope Medical Cente	I
2		Fred Hutchinson Cancer Research Center	I/ II
1		Juno Therapeutics, Inc.	II
1		Kite Pharma, Inc.	n.k.
7		M.D. Anderson Cancer Center	I
1		Masonic Cancer Center, University of Minnesota	II
6		Memorial Sloan Kettering Cancer Center	I/ II
3		National Cancer Institute (NCI)	I
1		Novartis Pharmaceuticals	n.k.
1		Professor Robert Hawkins	I
2		Seattle Children's Hospital	I/ II
1	Asia	Beijing Doing Biomedical Co., Ltd.	I
2		Chinese PLA General Hospital	I/ II
1		Fuda Cancer Hospital, Guangzhou	I/ II
1		Jichi Medical University	I/ II
1		Peking University	I/ II
1		Shanghai Tongji Hospital, Tongji University School of Medicine	I/ II
1		Shenzhen Second People's Hospital	I
1		Southwest Hospital, China	I
2		University of Pennsylvania	I
3	Europe	University College, London	I/ II
1		Uppsala University	I / IIa

Table 1. Clinical trials using CD19 CAR T-cells. Data obtained from https://clinicaltrials.gov/

Outlook

In order to guarantee the maximal effectiveness and tolerability of the construct several mechanisms will be applied.
Those include

- Use of human scFv sequences
- Alternative spacer domain and hinge region sequences
- Alternative signalling peptide sequences

´Proof-of-concept`studies`
Successfully generated autologous anti-CD19 CARs should specifically lyse more than 50% of CD19+ lymphoma cell lines, whereas they should be ineffective CD19- lymphoma cell lines

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