SUMMARY

Background:

The CD19 chimeric antigen receptor (CAR) adoptive T-cell therapy for B-cell leukemia is a promising treatment for relapsed or refractory malignities. The overall response rate of CD19 CAR-T cells in clinical trials was greater than 80% for patients with B-cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkin´s lymphoma (NHL). However, CAR-T cell therapy of leukemias and solid tumors has been limited by a lot of factors such as antigen loss of tumor escape variants, reduced proliferation, persistence and tumor-infiltration of CAR-T cells *in vivo*, immunosuppressive tumor environment, absence of ideal antigens and on-target, off-tumor toxicities. Therefore, new strategies improving the safety and efficacy of CAR-T cells, including further T-cell modification to overcome the immune suppression, are tested.

Aims:

(i) Bispecific CARs designed to express two antigen-binding domains prevent of antigen escape. *(ii)* T-cells were genetically modified to express CAR along with an inducible IL-21 gene cassette driven by NFAT-responsive promoter. IL-21 directly enhances CAR-T cell activity and anti-tumor effects. *(iii)* Applying suicide epitope modification in CAR enables significantly increasing the therapeutic safety of CAR-T cells.

Methods:

CARs were constructed by using molecular biology methods. Stable CAR expression in primary T-cells was achieved by using lentiviral vectors or PiggyBac transposon system for efficient transgene transfer. Two CD19 and CD20 targeted bispecific CARs were designed and further the anti-tumor efficacy *in vitro* assessed. CD19 or PSMA CAR-T cells with inducible or constitutive IL-21 expression in all-in-one construct were generated. IL-21 secreted by CARs upon antigen stimulation was tested. Short peptide tags incorporated into extracellular part of CARs were compared and tested.

Results:

The *in vitro* experiments revealed specific activation of biCAR-T lymphocytes by cells overexpressing CD19 and/or CD20. Cytokine release and cytolytic activity of biCAR-T cells were comparable or improved to the responses of single CAR-T cells. T-cells modified to

produce IL-21 in NFAT promoter-controlled manner respond to CAR or TCR stimulation. Multi-epitope based CARs allowed positive activation of CAR-T cell expansion, easy cell detection and enrichment of CAR-T cells during manufacturing by magnetic bead-based selection. Furthermore, these constructs have the potential for antibody-based selection upon CAR-T cell infusion *in vivo*.

Conclusions:

Here, we report the successful design and construction of various vectors for CAR-T cell therapy. BiCAR-T cells, those promote *in vitro* anti-tumor efficacy against both CD19 and CD20 antigens, may reduce the rate of relapse in treatment of B-ALL and non-Hodgkin´s lymphoma. Induced IL-21 increased the number of infiltrating T-cells in tumor models *in vitro* and *in vivo*. CARs based on multi-epitope switching represent a promising strategy that could improve manufacturing procedure and clinical safety of CAR-T cells.

Keywords: chimeric antigen receptor, CAR-T cells, adoptive immunotherapy, PiggyBac transposon system, lentiviral transduction, bispecific CARs, IL-21, multi-epitope switching