ABSTRACT

Chimeric antigenic receptor (CAR) T cells are a paradigm-shifting cancer treatment option that is particularly effective against B-cell malignancies. Currently, several approved, commercially available CAR T-cell products exist. Nonetheless, this therapy is still prohibitively expensive. Thereby, there is an incentive to substitute the particularly costly retroviral/lentiviral vectors with cheaper non-viral alternatives. Moreover, ongoing research strives to further improve CAR T cells by enhancing their anti-tumor functions, expansion, and *in vivo* persistence. Additionally, broadening the spectrum of antigens and diseases of CAR T-cell applicability is highly sought after. This thesis addresses CD19-specific CAR T-cell manufacturing regarding cytokines used for the cultivation and vector for genetic alteration, new interleukin (IL)-21 secreting CAR construct testing, and analysis of CAR T cells from patients treated with tisagenlecleucel.

As part of this thesis, an alternative CAR T-cell cultivation protocol was developed. Replacing the traditionally used IL-2, which drives the effector differentiation of T cells, the protocol is based on the strongly pro-survival cytokines IL-4 and IL-7, with IL-21 supporting the stem cell memory-like T-cell phenotype retention and low inhibitory receptor expression. Moreover, the CAR T cells were successfully produced using a piggyBac transposon vector.

Furthermore, experiments with the addition of exogenous IL-21 confirmed its enhancing effect on CAR T-cell proliferation, reduction of apoptosis, and prevention of terminal differentiation. Therefore, CAR T cells inducibly secreting IL-21 were prepared. They exhibited increased tumor infiltration and inhibited tumor growth *in vivo* in a mouse model. Additionally, IL-21 decreased the immunosuppressive effects of chronic lymphocytic leukemia (CLL) cells during a co-culture. This suggests that IL-21-armed CAR T cells could improve therapy outcomes of CAR-resistant malignancies.

In this thesis, a novel production method using the piggyBac transposon vector was tested. Instead of the regularly used plasmids, enzymatically produced linear DNA encoding CAR and transposase coded by mRNA were used. This bacteria-free approach achieved efficient CAR T-cell production with controlled vector copy numbers, which makes it compatible with current good manufacturing practice (cGMP) standards and facilitates easier approval by a regulatory agency. The CAR T cells were functionally indistinguishable from those produced by standard and currently approved methods.

Lastly, a real-world study was conducted on patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) and B-cell acute lymphoblastic leukemia (B-ALL) treated with tisagenlecleucel. The immunophenotype of T cells in the apheretic material and CAR T cells in the product and peripheral blood samples was assessed by multiparametric flow cytometry. The treatment efficacy correlated with *in vivo* CAR T-cell expansion rather than the product immunophenotype. In addition, patients with

higher early-memory T-cell percentages in apheresis samples responded better to therapy. The worst outcomes were observed in patients with primary refractory disease and a large tumor load.

In conclusion, this thesis presents several methods for improving CAR T-cell manufacturing and enhancing CAR T-cell functions. These results were used to establish a manufacturing protocol for a phase I clinical trial (NCT05054257). It also provides insight into real-world CAR T cell usage and parameters affecting clinical outcomes.

Keywords: CAR T cells, memory phenotype, IL-21, piggyBac, tisagenlecleucel