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“Utilizing FRET biosensors to optimize the targeting efficacy of CAR-based T-cell immunotherapy for individual B-ALL patients”

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Human T cells can be genetically modified by retroviral or lentiviral vectors that contain recombinant DNA sequences encoding a chimeric antigen receptor (CAR). A CAR is typically composed of the following domains: an extracellular murine antibody-derived, single chain variable fragment antibody (scFv); a hinge region; the CD28 co-stimulatory receptor transmembrane and cytoplasmic signaling domains, and the cytoplasmic signaling domain of the CD3-zeta chain. T cells expressing a specific CAR construct subsequently recognize and bind the surface target molecule on tumor cells, resulting in tumor cell lysis. CAR constructs targeting the pan-B antigen CD19 have shown promising results in some B-cell acute lymphoblastic leukemia

(B-ALL) patients of on-going small-scaled clinical trials. However, many other patients show no or little responses. The underlying molecular mechanisms are unknown and novel approaches are needed to address the issue of heterogeneous responses. We hypothesize that the signals from key molecular pathways in the CAR-engineered T-cells provide critical information about T-cell activation and could be used to predict the efficacy of tumor lysis. Therefore, a reliable and convenient screening method is needed to monitor CAR-induced T-cell activation, which helps precisely gauge the activity of CAR construct. Fluorescence Resonance Energy Transfer (FRET) biosensors are powerful tools to monitor intracellular activities of single live cells. Our hypothesis is that conjugation of CAR and FRET biosensor allows us to monitor CAR-induced T cell activation. Aim 1 of this proposal will generate a CD19-CAR construct with Fyn FRET biosensor attached to the C-terminus, which is named as CD19(Fyn)-CAR. The CD19(Fyn)-CAR construct will be first characterized in human endothelial cells in the presence of stimulants. In Aim 2, the CD19(Fyn)-CAR will be introduced into human T cells and FRET signals will be detected in the presence of different types of CD19 antigen molecules. Aim 3 is to co-culture CD19(Fyn)-CAR engineered T cells with different CD19-expressing B-ALL neoplastic cells. FRET signals will be recorded and analyzed. Our hypothesis is that variations of FRET signals will be observed from different CD19+ cell lines or different patients. If true, it might provide an explanation of heterogeneous responses observed from clinical trials. This proposal aims at improving targeting efficacy of adoptive T-cell therapy specifically for B-ALL. Data generated from this study will ultimately help on individualized decisions for patients eligible for CAR-based T-cell immunotherapy, which will potentially improve the overall survival rate.