PRIMARY OR IPSC-DERIVED CELL-BASED CYTOTOXICITY ASSAYS TO ASSESS POTENTIAL SAFETY RISKS OF ENGINEERED T CELL THERAPIES *IN VITRO*

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Background Engineered T cell therapies, such as Chimeric Antigen Receptor (CAR) T cell therapies offer great promise in becoming a new wave of highly specific therapies against solid tumors. Solid tumors generally lack the expression of tumor specific target antigens. The alternatively selected target antigens are often expressed at low levels in healthy tissues throughout the human body as well, which poses the risk of activation of engineered T cell therapies against these tissues resulting in severe side effects. Therefore, assessing the safety of engineered T cell therapies is a critical step during early development and before filing for Investigational New Drug (IND) status.

Methods Using a set of assays, we have generated an *in vitro* safety profile for CAR-T cells targeting the 'Human Epidermal growth factor Receptor 2' (HER2) which is amplified and/or overexpressed in 20–30% of invasive breast carcinomas and ovarian cancers. To determine which tissues are most at risk for unwanted CAR-T cell reactivity, *in silico* analysis for expression of the HER2 gene was performed. Subsequently, HER2 protein expression in various tissues was validated by flow cytometry using a HER2 targeting antibody. Primary tissues and hiPSC-derived cells with high and low HER2 protein expression were selected, characterized, and utilized for *in vitro* co-culture assays to evaluate on-target off-tumor and/or off-target cytotoxicity of CAR-T cells. As readout we measured target cell viability by flow cytometry and/or high content analysis and T cell activation by cytokine release.

Results Our study generated high quality data that provided insight into the safety of the HER2 targeting CAR-T cells. Cytotoxicity of HER2 CAR-T cells against low HER2 expressing human healthy cardiomyocytes and renal cells was observed with a clear E:T ratio dependent effect which was confirmed by IFN γ secretion. HER2 negative neurons showed a clear absence of CAR-T response. This data suggests a safety risk of our HER2 targeting CAR-T cells against heart and renal tissue.

Conclusions Engineered T cell therapies have the capacity to fill tremendous unmet medical needs and are moving into the clinic at a high pace. Shortening the timeframe towards clinical application, requires assay panels which can be conducted quickly and in are in line with the rigorous safety tests required before FDA approval. Our strategy of using primary tissues and hiPSC derived cells to generate a safety profile for these therapies *in vitro* is robust and can be applied during both early-stage development and late-stage testing of the therapeutic product.

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