Targeting Solid Tumors with Prosthetic Antigen Receptor Modified T-cells

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Our laboratory has developed prosthetic antigen receptors (PARs) as a non-genetic system for enabling cells to bind multiple surface receptors simultaneously, and we have successfully employed them *in vitro* to redirect T cells to selectively kill leukemia cells. However, PAR efficacy has yet to be evaluated *In vivo* or against a solid tumor cancer model. To address these questions, we developed PARs selectively targeting epithelial cell adhesion molecule (EpCAM), which is overexpressed on multiple carcinoma and cancer stem cells.

PARs are formed by engineering fusion proteins that contain a single-chain variable fragment (scFv) fused to two *E. Coli* dihydrofolate reductase ($DHFR^2$) subunits. Upon the addition of a chemical dimerizer, bis-methotrexate, these fusion proteins spontaneously self-assemble into octomeric chemically self-assembled nanorings (CSANs). More specifically, the combination of an anti-CD3 fusion protein and anti-EpCAM fusion protein generates the formation of bispecific, multivalent, CSANs that stably bind to CD3+ T cell surfaces and selectively target the EpCAM receptor, thus behaving as PAR.

Our *in vitro* results show that PAR-functionalized CD3+ T-cells are able to selectively recognize EpCAM+ breast cancer cells and undergo subsequent activation and cytokine release. These directed cell-cell interactions led to the selective cell killing of the EpCAM+ cancer cells, with up to 90% cell death over 24 hours. Impressively, these selective cytotoxic effects are only observed in the presence of EpCAM+ target cells.

Furthermore, an orthotopic breast cancer model validated the ability of PAR therapy to redirect T-cell activity towards EpCAM+ breast cancer cells *in vivo.* More specifically, PAR-functionalized CD3+ T-cells were shown to eliminate tumor burden without the need of a daily dosing regimen. Following PAR treatment, the rapid deactivation of therapy was possible by an infusion of the FDA-approved antibiotic trimethoprim at clinically relevant concentrations. Additionally, there was no evidence of an immunogenic response to our protein based therapeutic. Taken together, our results demonstrate that PARs provide a promising platform for future use in cell-directed cancer immunotherapy.