Engineering CAR T Cells to Degrade Immunosuppressive Metabolites in the Tumor Microenvironment

Botchwey, Nicolas (School: Charles R. Drew Charter School)

Solid tumors contain a tumor microenvironment (TME) with high levels of immunosuppressive metabolites that interfere with T cell activation. While CAR T cells are effective in blood cancers, these metabolites limit their function in solid tumors. This project aims to engineer a CAR construct that includes a surface docking domain capable of binding an externally produced enzyme designed to degrade these metabolites. The hypothesis is that the docking domain would enable a stable tethering of the enzyme and that cells expressing this system would reduce metabolite levels more efficiently than controls. A CAR–docking domain plasmid was designed, assembled with Gibson Assembly, and validated by restriction enzyme digest and DNA sequencing. The confirmed construct will be packaged into lentiviral particles and delivered into mammalian cells to achieve surface expression. Flow cytometry will then be used to detect the docking domain on the cell surface. After adding the tethering protein and the metabolite-degrading enzyme, a second flow cytometry assay will measure the binding of the enzyme complex. A metabolite degradation assay was then performed by incubating engineered cells with the target metabolite and tracking absorbance changes over time. The project's expected outcome is successful expression of the docking domain and detectable tethering of the enzyme complex. A faster decrease in metabolite levels relative to controls would support the hypothesis. This engineering approach could contribute to strategies that improve CAR T cell performance in solid tumors by reducing local immunosuppression.

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