## 183 THERAPEUTIC T CELLS EXHIBIT DISTINCT VULNERABILITY TO GLUCOSE DEPRIVATION IN TUMORS WHICH CAN BE OVERCOME WITH AN ENGINEERED GLUCOSE TRANSPORTER

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**Background** While checkpoint blockade cancer immunotherapies reawaken dormant antitumor immunity, adoptive cell therapies (ACT) bolster an immune response through infusion of expanded tumor infiltrating lymphocytes (TIL), or healthy T cell redirected to tumors via chimeric antigen receptor (CAR), or T cell receptor (TCR) expression. However, the harsh, nutrient depleted tumor microenvironment (TME) creates metabolic barriers for T cell persistence and effector function. We and others have shown that glucose availability is key for T cell effector functions through multiple non-redundant mechanisms. We thus asked whether therapeutic T cells harbor increased sensitivity for glucose and whether this could be mitigated to increase efficacy.

**Methods** B16 and Pten deficient, *Braf* mutant melanomas were used as models in C57BL/6 mice. Tumor cell glucose uptake was inhibited using stable expression of shRNA to *Slc2a1*, encoding Glut1. Glut1 was retrovirally overexpressed in therapeutic, Pmel-1 (gp100-specific) T cells, and phosphomimetic mutations were engineered (S226D) into Glut1, stabilizing cell surface trafficking. Glucose uptake and glycolysis were measured using fluorescent glucose tracers and Seahorse analysis, respectively.

**Results** Here we sought to equip glucose sensitive therapeutic T cells with heightened ability to compete for glucose within the TME. Murine therapeutic T cells, expanded in hyperglycemic conditions *in vitro*, that infiltrate solid tumors compete poorly for glucose tracers compared to endogenous T cells. Knockdown or deletion of Glut1 in tumor cells sensitizes tumors to T cell therapies, but not checkpoint blockade, highlighting a role for glucose competition specifically in ACT. Overexpression of WT Glut1 in therapeutic T cells yields only modest increased glucose competition due to various modes of Glut1 regulation. We thus engineered a cell surface stabilized Glut1 construct. This construct's competitive advantage manifests robust increases in glucose uptake and glycolytic capacity, leading to superior effector functions even in extremely hypoglycemic conditions. This enhanced effector function manifests in therapeutic efficacy in highly glycolytic melanomas and with heightened competition for glucose tracers, tumor infiltration, and effector function *in vivo*.

**Conclusions** Our study suggests that, due to the hyperglycemic conditions of their *ex vivo* expansion, therapeutic T cells display a distinct metabolic disadvantage when they enter the nutrient poor TME in comparison to their endogenous counterparts. Overexpression of our surface engineered Glut1 in these therapeutic T cells rescues their ability to compete with highly glycolytic tumor cells for glucose, resulting in robust glycolytic metabolism and curative response to immunotherapy for cancer.

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