

## **Modeling the human prostate tumor microenvironment to develop a novel chimeric antigen receptor T cell therapy**

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The immune suppressive tumor microenvironment (TME) that inhibits T cell infiltration, survival, and anti-tumor activity has posed a major challenge for developing effective immunotherapies for solid tumors. Recently, immune checkpoint (IC) blockade (ICB) has been utilized in combination with chimeric antigen receptor (CAR) T cell therapy, with the notion that induction of immune responses with CAR T cells may instigate checkpoint pathways in immunologically cold tumors, like prostate cancer, that would otherwise not respond to ICB. While ICB is promising in multiple cancer types, toxicities and resistance are frequently observed, and novel strategies to engineer CAR T cells, with the ability to avoid exhaustion leading to improved safety and efficacy, are highly warranted. To recapitulate the TME in prostate cancer, we utilized a novel co-culture system with tumor cells, prostate stem cell antigen (PSCA)-directed CAR T cells, and polarized macrophages. We observed significant hampering of PSCA-CAR T cell activity with the presence of M2 macrophages coinciding with a robust induction of PDL1 in both tumor cells and macrophages. To further investigate the TME in vivo, we took advantage of "humanized" MISTRG mice, which are immunocompromised mice with knocked-in human genes that support human hematopoiesis and efficient tumor-infiltration of myeloid cell populations. Humanized MISTRG mice were intratibially engrafted with LAPC9 tumor cells to model bone metastatic disease. We observed PDL1 expression in tumor-associated macrophages infiltrating tumors following PSCA-CAR T cell therapy. To prevent suppression of CAR T cells from PDL1-mediated exhaustion, we developed a new engineering platform to simultaneously knockdown (KD) three IC receptors in T cells utilizing a lentiviral construct that has capacity to carry three shRNA sequences driven by three independent pol III promoters (termed HUSKY). We evaluated the strength of three pol III promoters in HUSKY and assigned the strongest promoter to drive PD-1-targeting shRNA (shPD-1). Our ongoing studies will continue to elucidate the TME-mediated suppression of PSCA-CAR T cell therapy for prostate cancer, and provide a novel, safe, and effective strategy to improve efficacy of our CAR T cells currently under clinical investigation to treat patients with prostate cancer.

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