Screening and Characterization of AlloCAR T Targeting DLL3 for the Treatment of Small Cell Lung Cancer

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Abstract
Small cell lung cancer (SCLC) is an aggressive disease with very limited treatment options. SCLC responsiveness to immuno-oncology agents suggests this indication may be amenable to a T cell-based therapy. Genetically modified T cells that express chimeric antigen receptors (CARs) have shown impressive efficacy in multiple hematological malignancies. To translate this approach for SCLC treatment, we are exploring Delta-like ligand 3 (DLL3) as a therapeutic target. A large panel of antibodies that bind to DLL3 were generated, formatted into CARs, and tested in vitro, in short-term and long-term cytotoxicity assays using target cells that express high, medium or low levels of DLL3. A subset of CAR T cells were highly active and displayed long-term killing potential. CAR T cells were engineered to contain an off-switch, by which CAR T cells are eliminated upon administration of rituximab. Multiple off-switch CAR formats were evaluated, and optimal formats determined independently. Lead DLL3 CARs in their optimal off-switch formats were tested in vivo and robust efficacy was seen in both subcutaneous and systemic models.

DLL3 is highly expressed in SCLC and several other types of neuroendocrine cancers, with limited normal tissue RNA expression in brain, pituitary and testis. To understand the potential for toxicity in pituitary and brain, subcutaneous or intracranial tumors expressing DLL3 were implanted in mice and human/mouse cross-reactive DLL3 CAR T cells were injected into tumor-bearing animals. T cell infiltration into intermediate and posterior pituitary was detected but no tissue damage in brain or pituitary was observed and hormone-secretion function of pituitary was not ablated. These studies decrease safety concerns associated with DLL3 CAR T.

Toxicity study with subcutaneous (SC) tumor showed T cell infiltration in pituitary but no tissue damage was observed

Toxicity study with intracranial (IC) tumor showed no brain damage in DLL3 CAR-treated animals

Conclusion
- DLL3 RNA is expressed in SCLC with normal tissue expression limited to brain, pituitary and testis
- CARs were screened, characterized, and ranked against targets using in vitro cytotoxicity assays
- Optimal RhoA/RhoK-based off-switch formats were determined independently for each CAR
- DLL3 CAR T cells are efficacious in multiple in vivo tumor models, including subcutaneous and systemic models
- Toxicity studies using subcutaneous and intracranial tumor models showed no tissue damage in brain or pituitary despite T cell infiltration in pituitary