tumors. Fate Therapeutics is currently using its proprietary induced pluripotent stem cell (iPSC) product platform to generate iPSC-derived CAR T cells and iPSC-derived CAR NK cells that secrete BiTEs for the treatment of solid tumors.

**Ethics Approval**

These studies were approved by Fate Therapeutics Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

**Abstracts**

**117 RAPID POINT-OF-CARE SUBCUTANEOUS CAR-T FROM BLOOD DRAW TO INJECTION IN 4 HOURS WITH MODIFIED LV ENCODING CARS AND SYNTHETIC DRIVER ELEMENTS ENABLES EFFICIENT CAR-T EXPANSION AND TUMOR REGRESSION**

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**Background**

Adoptive cellular therapy with chimeric antigen receptor (CAR)-T cells has demonstrated remarkable clinical activity in a number of hematologic malignancies, but product chain of custody, individualized manufacturing, preparative chemotherapy, and patient management present technical and logistical hurdles to broader implementation.

**Methods**

Lentiviral constructs for CARs (either CD19- or CD22-directed) co-expressed with a synthetic driver domain were identified from a >6 × 10 diversity combinatorial library of proliferative elements, transmembrane domains, leucine zippers, and an EGFR epitope screened for cellular expansion in a lymphoreplete model. Modified serum-free-lentiviral manufacturing process was developed to reduce complexity of CAR-T and to introduce CD3-activating elements into the viral envelope allowing activation and transduction of resting lymphocytes from peripheral blood.

**Results**

Four-hour exposure of as little as 1 ml of blood to the CD3-directed CD19-targeted CAR encoding lentivirus followed by subcutaneous injection in NSG mice bearing CD19+/CD22+ Raji cells resulted in tumor regression (figure 1) and robust CAR-T cell expansion as determined by flow cytometry (figure 2) and qPCR (table 1), with peak levels >10,000 CAR-T cells/ml and less than three CAR copies per genome. In contrast, administration of the same products intravenously failed to support significant CAR-T expansion or control tumor growth (figure 3). Regression of established Raji tumors was also observed in NSG-(KbDb) (IA) animals following SC administration of CD19 or CD22 CARs with driver domains. CAR-T cells contracted in peripheral blood following tumor regression.

Regression of Raji tumor from the initial median volume of 151 mm3 throughout 40 days post subcutaneous administration of the LV transduced (at MOI 1 or 5) CD19-directed CAR T product (1M or 5M cells) in the NSG mice

**Conclusions**

We conclude that through a synthetic subcutaneous lymph node approach with modified lentiviruses and driver domains, rPOC SC may enable CAR-T generation with reduced complexity, while maintaining the ability of CAR-T cells to expand, persist and exert anti-tumor activity.

**Ethics Approval**

All animal studies were IACUC approved.

**Abstracts**

**119 IL-6 IS CRITICAL FOR MEMORY RESPONSES ELICITED BY TH17 CELLS TO TUMORS**

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**Background**

Translation of novel T cell therapies is limited by cost and time-consuming protocols involving long-term T cell
expansion. We found that shortening ex vivo expansion of either TCR-specific murine Th17 cells or human CAR Th17 cells licenses the cell product to eradicate large tumors in low doses and generate long-lived memory against tumor. Th17 cells induce the systemic release of IL-6, IL-17, GM-CSF, and MCP-1 among other cytokines in tumor-bearing hosts, reminiscent of clinical cytokine release syndrome. As the toxicity of cytokine release is managed in patients through nonmyeloablative total body irradiation (5 Gy) preparative regimen. 

Methods Th17 cells were expanded ex vivo using the TRP-1 transgenic mouse model in which CD4+ T cells express a TCR that recognizes tyrosinase-related protein 1 on melanoma. Naïve CD4+ T cells were polarized to the Th17 phenotype and infused into mice with B16F10 melanoma after a nonmyeloablative total body irradiation (5 Gy) preparative regimen.

Results IL-6 blockade, targeting either IL-6R or neutralization of the cytokine, did not significantly impact the primary immune response of adoptively transferred Th17 cells against tumor. However, administering IL-6 blockade acutely after Th17 transfer resulted in a higher incidence of tumor relapse upon secondary tumor challenge, thereby compromising long-lived antitumor immunity. Mounting a secondary response to tumor was dependent on CD4+ T cells, but not CD8+ T cells, persisting in the host. Mechanistically, IL-6 blockade reduced pSTAT3 and Bcl2 in transferred T cells but did not greatly impact the concentration of other systemic cytokines. As a small fraction of Tregs remain in the Th17 cell product ex vivo, we examined the engraftment of those Tregs after transfer. IL-6 was critical to suppress engraftment of FoxP3+ donor T cells from the CD4+ T cell product. Thus, IL-6 promoted robust tumor infiltration by donor effector over regulatory cells for early Th17 cells relative to cell products expanded longer durations ex vivo.

Conclusions Overall, short-term expanded Th17 cells uniquely induced IL-6 unlike Th17 cells expanded longer ex vivo. IL-6 promoted Th17 survival, reduced engraftment of tumor-specific Tregs, and was critical to durable memory. This work may suggest that the universal strategy to inhibit IL-6 during cytokine release syndrome may come at the expense of long-term efficacy for specific cell therapy approaches.

REFERENCE

122 GUANYLYL CYCLASE C AS A TARGET FOR CAR-T CELL THERAPY IN A METASTATIC GASTRIC CANCER MODEL

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Background Gastric cancer is the sixth most common cancer and second-leading cause of cancer-related mortality worldwide. The heterogenous and genetically complex nature of this disease underlies the challenges in developing effective therapies for metastatic gastric cancer. In the majority of cases, stomach tumors evolve from intestinal metaplasia resulting in ectopic expression of the enterocyte differentiation antigen guanylyl cyclase C (GUCY2C) by ~50% of primary and metastatic gastric cancers.

Methods mRNA-generated MUC1C CAR-T cells were evaluated for specificity and function by degranulation assay against various solid tumor and normal cells and cell lines. Autologous and allogeneic MUC1C CAR-T cells were produced using the piggyBac® DNA Modification System, a non-viral CAR-T manufacturing method that produces CAR-T products with an exceptionally high percentage of T stem cell memory (TSCM) cells. To produce allogeneic cells, multiplex editing of both TRBC and B2M was performed with the Cas- CLOVER™ Site-Specific Gene Editing System to reduce or eliminate GvHD and host versus graftalloreactivity, respectively. To determine in vivo antitumor efficacy of MUC1C CART-T cells, we employed the MDA.MB.468 triple negative breast cancer model and the OVCA3 disseminated ovarian cancer model.

Results Specific degranulation of transiently-expressing CAR+ T cells was observed against multiple tumor cells, with no observable activity against normal human primary cells. In assay of stable P-MUC1C-101 CAR-T cells, more than 95% expressed CAR, and were comprised of an exceptionally high percentage of TSCM cells (CD45RA+CD62L+CD45RO-). In vitro, P-MUC1C-ALLO1 cells specifically proliferated, lysed, and secreted IFN-γ against MUC1C+ breast and ovarian tumor cell lines. In breast cancer in vivo xenograft model, both unedited (MUC1C CAR-T) and edited (P-MUC1C-ALLO1) MUC1C CAR-T eliminated established, triple negative MDA.MB.468 tumor cells to undetectable levels, demonstrating the efficacy of the MUC1C CAR-T and the robustness of the allogeneic platform. In the OVCA3 xenograft model, intraperitoneally administered MUC1C CAR-T eliminated established tumor cells to levels below the limit of detection.

Conclusions P-MUC1C-ALLO1 is Poseida’s allogeneic CAR TSCM product that has a potential to treat multiple MUC1C-expressing indications. P-MUC1C-ALLO1 displayed in vitro specificity for tumor vs normal cells, and in vivo efficacy against xenograft models of breast and ovarian cancer. We anticipate an IND filing and initiation of a Phase 1 clinical trial in 2021.