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.[1] Therapeutic 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 IL-6 blockade, we addressed the impact of IL-6 on efficacy and durability of Th17 cell therapy. We hypothesized that IL-6, induced by Th17 cells, was fueling the durable memory properties of this cell product.

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.[1] 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.[1]

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.

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120 P-MUC1C-ALLO1: AN ALLOGENEIC CAR-T FOR MULTIPLE SOLID TUMOR INDICATIONS

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Background MUC1 is a highly glycosylated protein that is expressed at the apical border of mucosal epithelium where it plays a protective role. MUC1 is comprised of an N-terminal subunit (MUC1N) tethered to a C-terminal subunit (MUC1C), forming a stable complex on the cell surface. A proteolytic 'stump' of MUC1C that may be aberrantly glycosylated is over-represented in cancer, making it an attractive therapeutic target. Here we report generation of allogeneic MUC1C-specific CAR T cells, P-MUC1C-ALLO1, that are designed to leverage the learnings of our P-BCMA-ALLO1 program. P-MUC1C-ALLO1 targets a MUC1C epitope and has the potential for efficacy against a wide range of solid tumors, without targeting normal epithelial cells.

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 (T_{SCM}) cells. To produce allogeneic cells, multiplex editing of both TRBC and B2M was performed with the CasCLOVER™ Site-Specific Gene Editing System to reduce or eliminate GvHD and host versus graft alloreactivity, respectively. To determine in vivo antitumor efficacy of MUC1C CAR-T cells, we employed the MDA.MB.468 triple negative breast cancer model and the OVCAR3 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 T_{SCM} 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 OVCAR3 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 T_{SCM} product that has a potential to treat multiple MUC1-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.

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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.[1] 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