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 Thera-
pic 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 PstAFA3 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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P-MUC1C-ALLO1: AN ALLOGENEIC CAR-T FOR MULTIPLE SOLID TUMOR INDICATIONS
Anna Kozlowska*, Yan Zhang, Jacqueline Fritz, Steven Wang, Rebecca Codde, Elvina Angus, Samad Ibitokou, Vanitra Richardson, Sumit Jain, Maximilian Richter, Deepak Patil, Yening Tan, Min Tong, Lu Yao, Majid Ghoddusi, Eric Ostertag, Julia Coronella, Devon Sherlock, Poseida, San Diego, CA, USA

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-spe-
cific CAR T cells, P-MUC1C-ALLO1, that are designed to lever-
eage the learnings of our PB-CMA-ALLO1 program. P-
MUC1C-ALLO1 targets a MUC1C epitope and has the poten-
tial 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 pro-
duced using the piggyBac® DNA Modification System, a non-
THERAPY IN A METASTATIC GASTRIC CANCER MODEL
Amanda Libby*, Trevor Baybutt, Megan Weidner, Robert Carlson, Alicja Zalewski, Scott Waldman, Adam Snook, Thomas Jefferson University, Philadelphia, PA, USA

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

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metastatic gastric cancers. In the context of the efficacy of GUCY2C-directed chimeric antigen receptor (CAR)-T cells against metastatic colorectal cancer in animal models, we hypothesized that this adoptive cell therapy may be effective against metastatic gastric cancer.

**Methods** Here, we explored the efficacy of GUCY2C-directed CAR-T cells for gastric cancer in a patient-derived xenograft (PDX) tumor model. Also, we interrogated translational GUCY2C biomarker assays using RT-qPCR, immunoblot analysis, and immunohistochemistry (IHC) for the intended purpose of identifying patients whose tumors express GUCY2C and could benefit from GUCY2C-directed CAR-T cell therapy.

**Results** GUCY2C-directed CAR-T cells significantly reduced subcutaneous T84 colorectal tumor growth, producing a 5-fold reduction in tumor volume, compared to control treated tumors. GUCY2C-directed CAR-T cells produced no response in tumors produced from the GUCY2C-deficient colorectal cancer cell line, SW480. Importantly, GUCY2C-directed CAR-T cells controlled gastric cancer PDX growth, maintaining a >12-fold reduction in tumor volume compared to control and in some cases produced complete tumor elimination. Furthermore, IHC based assays, indicate that antibodies developed in our laboratory may be suitable for development of a companion diagnostic for GUCY2C-directed CAR-T cells. Indeed, the commercial polyclonal antibody demonstrated robust, non-specific staining regardless of tissue type or GUCY2C mRNA profile, while novel monoclonal antibodies produced in our laboratory primarily detected protein localized to the membrane of glandular epithelial cells, demonstrating antigen specificity, and indicating their potential for further development in diagnostic companion assays to identify gastric cancer patients who may benefit from GUCY2C-directed CAR-T cell therapy.

**Conclusions** GUCY2C-directed CAR-T cells prevented the growth of, and at times eliminated, a subcutaneous gastric cancer PDX model. In the context of previously established safety in mouse models, additional studies defining the efficacy of GUCY2C-directed CAR-T cells in gastric cancer models may allow future translation of this therapy to patients with advanced gastric cancers. Concurrent development of a novel companion diagnostic IHC assay would permit identification of the ~50% of gastric cancer patients whose tumors express GUCY2C and could benefit from this therapy.

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**Ethics Approval** This study was approved by the Thomas Jefferson University Institutional Review Board (#14.0204) and the Institutional Animal Care and Use Committee (Protocol #01529).

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**Background** Solid tumors such as GBM are particularly difficult to treat, being largely resistant to traditional treatments, funding interest in alternative treatment approaches, including cell-based immunotherapy. Natural killer (NK) cells have emerged as promising effectors to target GBM through genetic modifications and ex vivo manipulation. However, immunosuppressive conditions within the tumor microenvironment (TME) further complicate NK cell-based treatments. Specifically, within the TME tumor cells release of high levels of ATP extracellularly. While intracellular ATP is necessary for cell metabolism, extracellular ATP is converted into adenosine (ADO) by ectonucleotidases CD39 and CD73, both overexpressed on GBM. Extracellular ADO induces immunometabolic suppression of NK cells through binding with A2A adenosine receptors (A2ARs) on NK cells, suppressing cytokine secretion, proliferation, and other functional activities.

Adding to the suppression of NK cells is the interaction between CD155, expressed highly on GBM and other solid tumors, and T cell immunoreceptor with Ig and ITIM domains (TIGIT) expressed on NK cells. This interaction signals inhibition of NK cell cytolytic function, allowing for cancer cell immune-evasion.

**Methods** To restore impaired NK cell anti-tumor activity, we have engineered NK cells to concomitantly target CD155 and CD73-induced immunosuppression on GBM using a tumor-responsive genetic construct. The construct is capable of blocking the immunosuppressive CD155/TIGIT interaction,