**INTERFERON GAMMA REDUCES CAR-T EXHAUSTION AND TOXICITY WITHOUT COMPROMISING THERAPEUTIC EFFICACY IN HEMATOLOGIC MALIGNANCIES**

Stefanie Bailey*, Sonika Vatsa, Amanda Bouffard, Rebecca Lanson, Irene Scarfo, Michael Karr, Andrea Schmitts, Marcola Maus. Massachusetts General Hospital, Charlestown, MA, USA

**Background** Chimeric antigen receptor (CAR) T cell therapy has shown remarkable efficacy in hematologic malignancies, ultimately leading to its FDA approval for relapsed/refractory acute lymphoblastic leukemia and large cell lymphomas in 2017. Despite the success of CAR T cells in the clinic, toxicities such as cytokine release syndrome (CRS) can be severe. Attempts to mitigate these effects have primarily focused on the blockade of macrophage-derived cytokines, such as IL-6 and IL-1β. Herein, we show that the pharmaceutical blockade or genetic deletion of interferon gamma (IFNg, a CAR-T-derived cytokine that strongly correlates with CRS in the clinic, appears to be a viable target for the reduction of CAR-T-associated toxicities.

**Methods** Pharmacologic (blocking antibody) and genetic (CRISPR/Cas9) approaches were used to block IFNg signaling and/or production by CAR T cells. In vitro CAR-T function and cytotoxicity was tested using ELISA, flow cytometry and short/long-term killing assays prior to their assessment in vivo. NSG mice were injected with Nalm6 or Jeko-1 cancer cells prior to treatment with IFNg-modified CART and tumor size and IFNg production were measured. To determine how the loss of IFNg might affect innate immune cells, CART, macrophages and tumor cells were co-cultured and assessed by flow cytometry, immunofluorescence, Luminex and RNA sequencing.

**Results** IFNg could be blocked using an anti-IFNg antibody or CRISPR/Cas9 editing of the CAR T cells without affecting T cell activation, proliferation or cytokine production (IL-2, TNFa, GM-CSF). Successful blockade of the IFNg signaling pathway was confirmed by reduced phosphorylation of JAK1, JAK2 and STAT1, even in the presence of exogenous IFNg. Loss of IFNg did not reduce the cytotoxic potential or persistence of CAR-T against hematologic malignancies in vitro or in vivo. When cultured with macrophages and cancer cells, IFNg knockout (IFNgKO) CAR-T yielded decreased levels of IL-1β, IL-6, IL-13, MCP1 and CXCL10, indicating a reduction in macrophage activation induced by CAR-T in the absence of IFNg. Serum from tumor-bearing mice treated with IFNgKO CAR-T elicited lower activation of macrophages in vitro compared to those treated with IFNg-producing CAR-T cells. Furthermore, IFNgKO CAR T cells co-cultured with tumor cells and macrophages demonstrated less exhaustion as shown by reduced expression of PD1, Tim3 and Lag3 and increased IFNgKO CAR-T expansion.

**Conclusions** Collectively, these data suggest that IFNg is not required for the efficacy of CAR-T in hematologic malignancies and can potentially be targeted to reduce toxicity and enhance CAR-T efficacy and persistence in the clinic.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0767

---

**NOVEL BIOLUMINESCENT BIOASSAYS FOR THE DISCOVERY AND DEVELOPMENT OF T CELL REDIRECTING CANCER THERAPIES**

Vanessa Ott*, Julia Gilden, Janisson Graier, Michael Slater, Pete Stecha, Jim Hartnett, Dan Lazar, Frank Fan, Mei Cong, Zhijie Jey Cheng. Promega, Madison, USA

**Background** Two main approaches for T cell-based therapies involve molecular T cell redirection by CD3 bispecific molecules such as bispecific T cell-engagers (BiTE) and cellular T cell redirection by genetic modification of T cells with chimeric antigen receptors (CAR) or transgenic T cell receptors (TCR). BiTEs redirect the cytotoxic activity of endogenous polyclonal T cells by simultaneously engaging CD3 on T cells and tumor antigens on target cells. BiTE potency studies have relied on primary cells, which measure target cell killing through redirected T cell cytotoxicity (RTTC) or cytokine release. However, these primary cell-based assays suffer from high donor-to-donor variability, as well as lengthy and hard to implement protocols.

**Methods** We have recently developed a new RTCC assay and cytokine immunoassays that are simple, sensitive and can quantitatively measure the potency of BiTEs and similar biologics. In this assay, preactivated cytotoxic T cells and target cells (both in cryopreserved thaw-and-use format) stably expressing a HaloTag-HiBiT fusion protein are co-incubated with a BiTE, which results in lysis of the target cells and subsequent release of the Halotag-HiBiT protein. These HiBiT proteins then bind to extracellular LgBiT provided in the detection reagent and form functional NanoLuc Luciferase to generate luminescence.

**Results** The assay is homogenous, highly sensitive, and has a robust assay window. Use of CAR-T has demonstrated promising results in treating leukemia, while the development of TCR-engineered T cells that can recognize intracellular tumor antigens, is still in early stages. To facilitate the screening and characterization of new transgenic TCRs, we used CRISPR/Cas9 to develop two TCRαβ-null reporter T cell lines, which are CD4+ or CD8+. Reintroduction of peptide-specific TCR α and β chains into TCRαβ-null reporter T cell lines results in peptide-dependent TCR activation and luciferase reporter expression. The select expression of CD4 or CD8 in the TCRαβ-null reporter T cell lines can enable the development of transgenic TCRs for both MHCI- and MHCIΙ-restricted tumor antigen targets.

**Conclusions** Together, these bioluminescent bioassays represent a new set of tools for the discovery and development of T cell-based immunotherapies.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0766

---

**RE-DIRECTED T CELL THERAPY TO CONTROL INVASIVE ASPERGILLOSIS**

1Karishma Bavisi*, 1Sebastian Wurster, 1Nathariel Albert, 1Sattva Neelapu, 1Dimitrios P Kontoyiannis, 2Pappanaicken Kumaresan. 1University of Texas MD Anderson Cancer Center, Houston, TX, USA; 2University of Texas MD Anderson Cancer C, Sugar Land, TX, USA

**Background** Opportunistic invasive fungal infections (IFI) are a major threat to immunocompromised populations such as patients with acute myeloid leukemia (AML) and allogenic hematopoietic stem cell transplant (HSCT) recipients(1,2). Specifically, Aspergillus fumigatus (AF) is responsible for high morbidity and mortality in cancer patients. As antifungal therapy has limited efficacy in immunocompromised patients, we...
sought to develop fungus-specific chimeric antigen receptor (CAR) T cells as a novel immune augmentation strategy to treat IFIs including invasive aspergillosis. To target fungal pathogens, we fused the pattern-recognition receptor Dectin-1 to activate T cells via chimeric CD28 and CD3ζ domains upon binding to β-1,3-glucan carbohydrates in the fungal cell wall (3). The generated Dectin-1 CAR+ T cells showed high specificity for β-1,3-glucan and inhibited the growth and branching of AF germlings in an in vitro co-culture assay. However, we found poor efficacy of Dectin-1 CAR+ T cells against mature AF hyphae, likely due to changes in the fungal cell wall that hamper T-cellular binding to β-1,3-glucan carbohydrates. To overcome this limitation, we have recently developed an AF-specific CAR (AF-CAR) based on a monoclonal antibody which recognizes a surface epitope of mature AF hyphae.

Methods Lentiviral vectors were used to generate AF-CAR expressing T cells from human peripheral blood mononuclear cells. Heat killed AF-293 hyphae was used for co-culture studies with No DNA T cells, and AF-CAR expressing T cells. Cell clusters, binding with AF hyphae were noticed in AF-CAR incubated wells whereas no such cell cluster were observed in NoDNA T cells incubated wells.

Results When co-incubated with AF hyphae, AF-CAR+ T cells efficiently targeted mature hyphae and formed lytic synapses with hyphal filaments. The released cytolytic granules damage hyphae and controls branch node formation. Furthermore, exposure to AF hyphae stimulated significant upregulation of activation markers CD69 and CD154 on AF-CAR+ T cells. The activated CAR T cell secretes proinflammatory cytokines which can boost innate immune system to fight against IFI.

Conclusions In summary, these results indicate that we have successfully generated a novel anti-Aspergillus CAR construct with good in-vitro targeting efficacy against mature AF hyphae. After thorough evaluation of fungicidal activity, cytokine response patterns, and release of cytotoxic mediators, we plan to embark on preclinical tolerability and efficacy studies in a murine model of invasive pulmonary aspergillosis. Thus, we report the production of Aspergillus specific CAR T cells to provide long term protection to immunocompromised patients, such as AML patients and HSCT recipients, from invasive Aspergillus infections.

Acknowledgements This study was supported by NIAID-R33 AI127381.

Ethics Approval This study was approved by IBC committee, University of Texas MD Anderson Cancer Center, Houston, Texas, 77030.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-STIC2020.0768

http://dx.doi.org/10.1136/jitc-2020-STIC2020.0768