842

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

Stefanie Bailey*, Sonika Vatsa, Amanda Boffard, Rebecca Larson, Irene Scarfo, Michael Karr, Andrea Schmidts, Marcela 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-1B. Herein, we show that the pharmaceutical blockade or genetic deletion of interferon gamma (IFNg, a CAR-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 CAR-T and tumor size and IFNg production were measured. To determine how the loss of IFNg might affect innate immune cells, CAR-T, 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 knock-out (IFNgKO) CAR-T yielded decreased levels of IL-1B, 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-modifying 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