The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

T-Bet Overexpression Increases 4-1BB-Costimulated CAR T Cell Potency

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Data from our lab and others has demonstrated that CAR T cells form a mixed Th1/Th2 population which, in contrast to canonical T cell differentiation, produce both interferon gamma (IFN-γ) and IL-4. Whereas a growing body of evidence has highlighted the importance of IFN-γ in the CAR T cell response, IL-4 can have immune-suppressive functions. IFN-γ from CD4+ CAR T cells (CAR4s), has been shown to promote cytotoxic effector functions of CD8+ CAR T cells (CAR8s), as well as bolster activation of endogenous immune cells including CD8 T cells and NK cells (Boulch et al *Sci Immunol.* 2021).

Given the importance of IFN-γ to effective anti-tumor immune responses, we generated murine CAR T cells that target CD19 with a CD28 costimulatory domain (1928z) and compared them to CAR T cells that overexpress T-bet (T-bet-1928z) in an effort to skew CAR T cells away from IL-4 production and towards IFN-γ production. Upon stimulation of CAR T cells with the murine B-cell acute lymphoblastic leukemia (B-ALL) cell line E2A-PBX, fewer T-bet-1928z CAR4s produced IL-4 compared to 1928z CAR4s. This suggests T-bet overexpression is a viable strategy to reduce Th2-like programming of effector functions in CAR4s. To determine the impact of T-bet overexpression on early CAR T cell efficacy *in vivo*, we adoptively transferred 1928z or T-bet-1928z CAR T cells into leukemia-bearing C57Bl6/J recipients and analyzed CAR T cells from the bone marrow at peak expansion of CAR T cells as well as post-contraction. Both CARs eliminated CD19+ cells from the bone marrow and mediated long-term survival. T-bet-1928z CAR4s demonstrated increased expansion at Day 4 compared to 1928z CAR4s (p=0.0412), although there was no significant difference in CAR8 numbers at this timepoint. At Day 11 post-CAR, we observed an increase in the number of T-bet-1928z CAR T cells present in the bone marrow (p<0.0001), observed in both CAR4s (p<0.0001) and CAR8s (p=0.0074). While levels of memory-associated transcription factors TCF1 and FOXO1 were the same between CARs, fewer T-bet-1928z CAR T cells expressed IL7Rα (p=0.0002) and eomesodermin (p<0.0001), which are associated with the ability of T cells to self-renew and persist in the bone marrow.

To determine the impact of T-bet on the persistence and renewal capacity of 1928z CAR T cells, we adoptively transferred 1928z or T-bet-1928z CAR T cells into leukemia-bearing RAG1-knockout recipients to eliminate endogenous T cell responses to E2A-PBX. After initial leukemia clearance, we rechallenged these mice with E2A-PBX and analyzed the bone marrow after 13 days. Both CARs were able to clear the leukemia rechallenge. While CAR4s were present in the bone marrow at similar numbers, the number of CAR8s was significantly higher among mice treated with T-bet-1928z CAR T cells (p=0.0317). Our results suggest that while T-bet may enhance *in vivo* expansion of CAR T cells, it provides no long-term benefit or deficit to the CAR T cell response.

4-1BB co-stimulated CAR T cells are less potent than CD28-costimulated CAR T cells and have reduced effector functions. We therefore hypothesized that T-bet overexpression would improve the efficacy of 4-1BB-costimulated CAR T cells by boosting effector functions and potency. To test this, we transduced T cells with a CAR targeting CD19 that contains a 4-1BB costimulatory domain (19BBz), or a 19BBz CAR and T-bet (19BBz-T-bet). We adoptively transferred 19BBz or 19BBz-T-bet CAR T cells into leukemia-bearing C57Bl6/J recipients. Despite no difference in CAR T cell numbers in the bone marrow, we observed fewer leukemia cells in mice treated with 19BBz-T-bet CAR T cells (p=0.0318) at Day 4 post-CAR. Treatment with 19BBz-T-bet CAR T cells also significantly improved survival of leukemia-bearing mice compared to 19BBz CAR T cells (p=0.0494).

Our data demonstrate T-bet overexpression improves CD28-costimulated CAR T cell expansion but does little to augment long-term responses and diminishes features associated with capacity for self-renewal. In contrast, T-bet overexpression

boosts the potency of 4-1BB-costimulated CAR T cells without impairing persistence, resulting in improved overall efficacy of 4-1BB-costimulated CAR T cell therapies.

Disclosures Fry: *Sana Biotechnology:* Current Employment, Current equity holder in publicly-traded company.
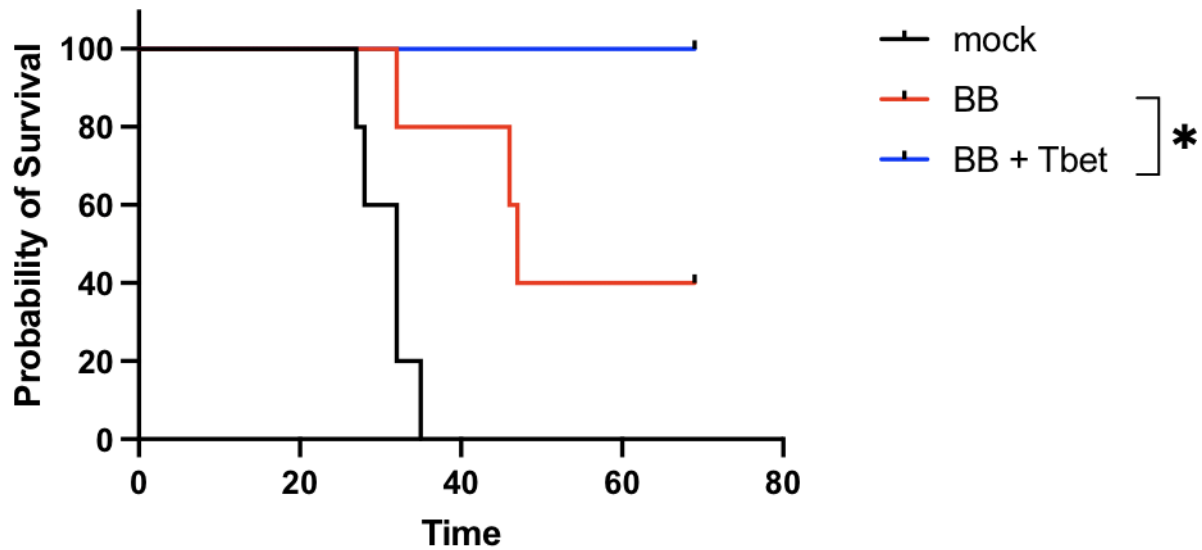

Figure 1. B6 mice were transplanted with 1×10^6 E2A-PBX leukemia cells (n=5 mice per group). Mice were then given 500cGy irradiation and transplanted with 1×10^6 19BBz or 19BBz-T-bet CAR T cells. Survival of the mice is plotted (*p=0.0494, Log-rank (Mantel-Cox) test).

Figure 1