The role of endogenous CD28 co-stimulation in tuning CAR-T cell performance

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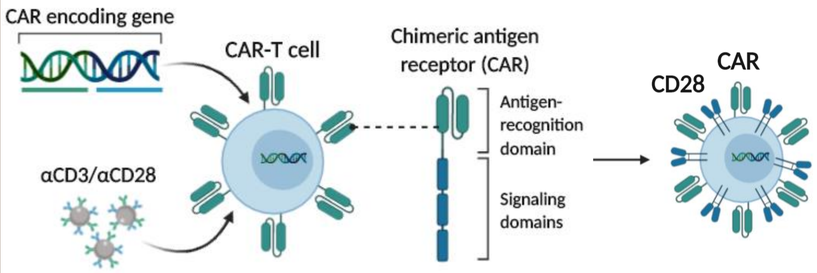
Abstract

Chimeric antigen receptor T (CAR-T) cells have proven extremely successful for the eradication of B cell leukemia and lymphoma, and more recently have entered clinical trials for multiple myeloma (MM) targeting B cell maturation antigen (BCMA). Although initial responses look promising, several institutes of relapse to αBCMA CAR-T therapy have been reported. These early clinical findings suggest that MM relapse following BCMA CAR-T cell therapy is due to defects in CAR-T cell function and/or acquired resistance of MM cells to CAR-T cell killing.

There is significant evidence in the CD19 CAR-T cell literature suggesting co-stimulation within the CAR greatly impacts CAR-T cell function, persistence and terminal differentiation. However, the consequences of endogenous CD28 signaling in CAR-T cells is largely unknown. Our preliminary data indicate that disruption of endogenous CD28 signaling does not impair CAR-T cell cytotoxic function. In fact, over-co-stimulation may be detrimental to subsequent CAR-T cell function and skew effector differentiation

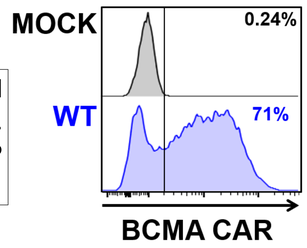
Importantly, we have previously shown that CD28 is expressed on and delivers pro-survival signals to MM, contributing to therapy resistance. Moreover, CD28 ligands CD80 and CD86 are also expressed on MM cells and bone marrow stromal cells. We hypothesize that CD80 and/or CD86 expression in the MM microenvironment reduces the efficacy of BCMA CAR-T therapy by providing a survival signal to MM cells while simultaneously limiting the persistence and function of CAR-T cells. We hypothesize that the combination of αBCMA CAR-T therapy and CTLA4-Ig will increase MM sensitivity to CAR-T therapy while also improving the durability of CAR-T responses through limitation of co-stimulatory signals.

Endogenous CD28 is required for CAR-T cell production

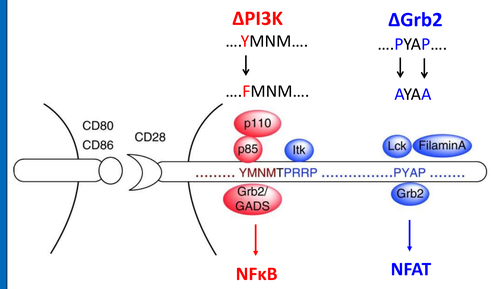


CD3⁺ T cells are activated with αCD3/αCD28/rIL-2/rIL-7 and transduced to express an αBCMA CAR at 24 and 48 hours post-activation. CD28 is necessary for initial expansion, but it's role in CAR-T cell function is largely unknown.

Figure 1: Efficiency of CAR-T cell transduction 7 days post activation. αBCMA CAR is co-expressed with GFP transduction marker.



CD28 signaling during T cell priming influences memory differentiation signatures



Boomer and Green *Cold Spring Harb Perspect Biol.* 2010

Amino acid mutations in the CD28 cytoplasmic signaling tail motifs, YMNM and PYAP, independently disrupt the downstream NFKB and NFAT signal transduction and have implications for T cell survival, proliferation and IL-2 secretion.

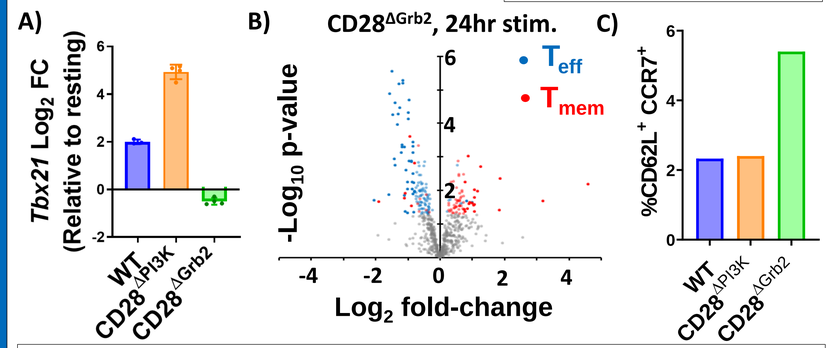
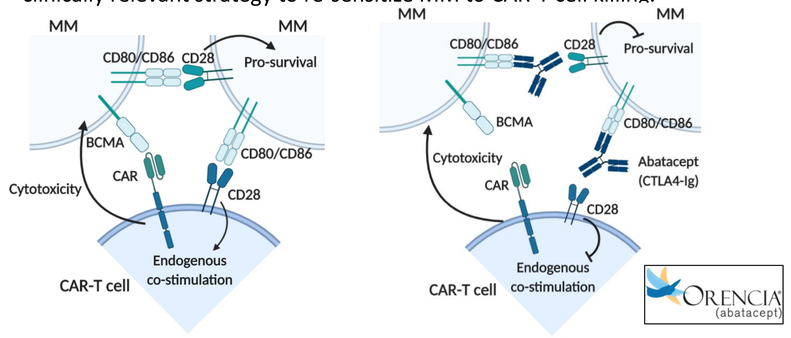


Figure 2: A) T-Bet expression in CD28 KI T cells at 24 hours post αCD3/αCD28/rIL-2 activation. **B)** Percentage of CD62L⁺, CCR7⁺ cells in the CAR T cell infusion product generated from CD28 KI T cells. **C)** Differentially expressed genes in $CD28^{\Delta Grb2}$ T cells 24 hours post αCD3/αCD28/rIL-2 activation. Effector and memory associated genes depicted in blue and red, respectively.

Blockade of CD28 on MM may improve sensitivity to αBCMA CAR-T cell killing

- CD28 on MM cells contributes to chemotherapeutic resistance. Blockade of CD28 ligands (CD80, CD86) interrupts critical pro-survival cues.
- Abatacept is FDA approved for the treatment of Rheumatoid Arthritis and is a clinically relevant strategy to re-sensitize MM to CAR-T cell killing.



Redundancies in CD28 signaling render endogenous CD28 is dispensable for CAR-T cell cytotoxic function

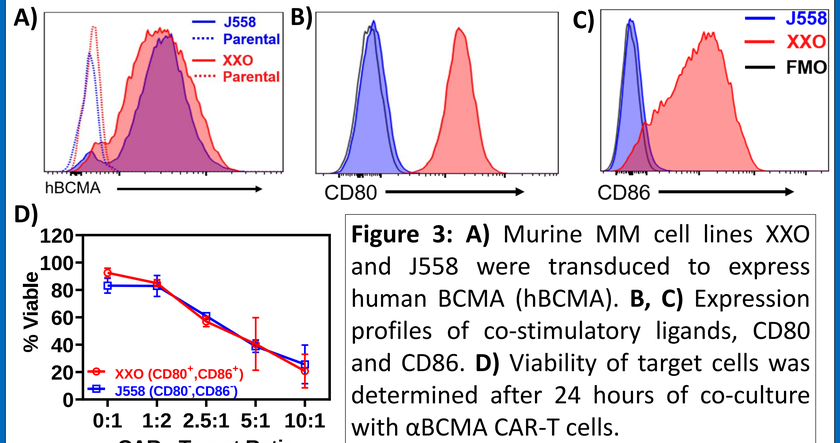
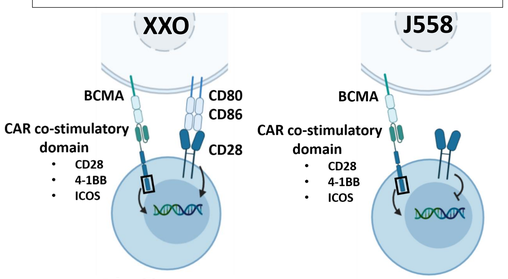


Figure 3: A) Murine MM cell lines XXO and J558 were transduced to express human BCMA (hBCMA). **B, C)** Expression profiles of co-stimulatory ligands, CD80 and CD86. **D)** Viability of target cells was determined after 24 hours of co-culture with αBCMA CAR-T cells.

Schematic illustrating co-stimulatory signals delivered to CAR-T cells through endogenous CD28 in XXO (L) and J558 (R) co-culture systems.



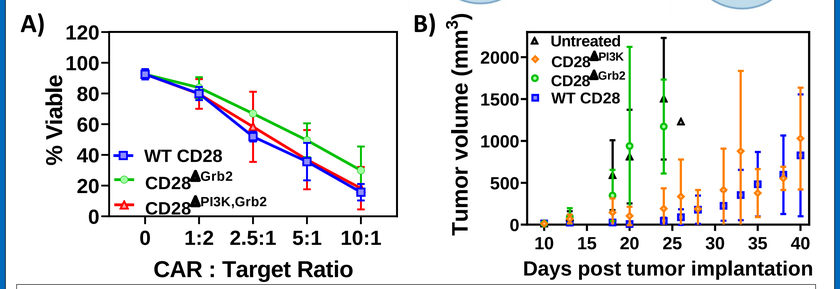


Figure 4: A) Viability of hBCMA⁺ target XXO cells following 24 hr. co-culture with CD28 KI CAR-T cells at varying effector : target ratios. **B)** Tumor burden in SCID mice following i.v. infusion of WT, $CD28^{\Delta PI3K}$, or $CD28^{\Delta Grb2}$ CAR T cells.

Conclusions and Future Directions

- The combination of CAR T cells with a CD28 antagonist represents a potentially clinically relevant treatment to reduce MM relapse.
- CD28 is critical to CAR-T cell production, however the impact of each motif on subsequent T cell differentiation has not been examined.
- Future work will investigate the impact of CD28 manipulation during the production process on the epigenetic landscape and phenotype of CAR-T cells.
- We also propose to investigate the effects of both endogenous and chimeric CD28 mutations to discern their relative contributions to CAR T function.