396 INVESTIGATING THE ROLE OF THE ENDOGENOUS CD28 CO-STIMULATORY RECEPTOR IN ACHIEVING OPTIMAL CAR-T CELL THERAPEUTIC EFFICACY

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Background Multiple myeloma (MM) remains an incurable hematological malignancy by conventional chemotherapeutic approaches. Chimeric antigen receptor T (CAR-T) cells targeting B cell maturation antigen (BCMA) on MM have shown remarkable efficacy in relapsed/refractory patients, leading to the recent FDA approval of two CAR-T cell products. Despite initially promising responses, many MM patients fail to achieve durable remissions leading to relapse in the months following treatment, highlighting the need to better understand mechanisms of CAR-T cell failure.

CD28, the prototypical T cell co-stimulatory molecule, is commonly expressed on MM and was previously shown to protect MM from chemotherapy-induced cell death, implicating its role in promoting therapy resistance. However, the role of the endogenous CD28 receptor on CAR-T cells is largely unexplored.

Methods To specifically target CD28 on the surface of adoptively transferred CAR-T cells we employed a novel, inducible $CD28^{-/-}$ genetically engineered mouse model as the T cell source.

Results We demonstrate that genetic deletion of the endogenous CD28 receptor does not impair CAR-T cell activation in response to stimulation by BCMA-expressing target cells, nor the subsequent cytotoxic response as assessed by intracellular cytokine production in two second-generation CAR-T cell models. However, the complete absence of a CD28 co-stimulatory signal has unveiled a previously uncharacterized role for the endogenous receptor in mediating an anti-tumor response *in vivo*. Specifically, $CD28^{-/-}$ BCMA-targeted 4-1BBζ CAR-T cells display a pronounced defect in their ability to control tumor growth in multiple disease models, while the function of $CD28^{-/-}$ 28ζ CAR-T cells is unaffected. Preliminary results suggest that CD28 deletion enhances persistence of the CAR-T cell population which may lend a more durable response to adoptive cellular therapies.

Conclusions These findings suggest that targeting the endogenous CD28 receptor with an FDA approved biologic CTLA4-Ig (Abatacept) may represent a viable clinical strategy to increase the efficacy of a potent 28ζ CAR-T cell design through two independent mechanisms: 1) Blockade of CD28 on MM abrogates a critical survival signal in the bone marrow microenvironment which may enhance their sensitivity to CAR-T cell mediated killing 2) Blockade of CD28 on CAR-T cells dampens the CAR-T cell activation signal which may promote differentiation into longer-lived memory T cell subsets for enhanced therapeutic durability as compared to effector T cells.

This work has contributed to the unveiling of a novel CD28 axis in CAR-T cell biology and provides insight into identifying optimal levels of co-stimulation while offering a therapeutic strategy to improve adoptive cellular immunotherapeuties.

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