391 ADVANCING ADOPTIVE T CELL THERAPY AGAINST AML THROUGH THE DEVELOPMENT OF CD40L-DUAL COSTIMULATORY RECEPTORS

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Background Acute myeloid leukemia (AML) is a malignant disease characterized by abnormal development of immature myeloid cells in the bone marrow. With standard chemotherapy and bone marrow transplantation, the overall 5-year survival rate for AML patients is 29.5%[1] – innovative, more targeted treatments are critically needed. Adoptive cell therapy (ACT) utilizing genetically modified T cells has revolutionized cancer treatment, enhancing patient immune responses against tumors and potentially providing long-term protection against tumor recurrence. Significant clinical benefits have been observed with T-cell receptor-engineered T cells (TCR-T cells) against some cancers, but enhanced persistence and antitumor TCR-T function are clearly needed. Receptors of the TNF receptor (TNFR) superfamily are emerging targets for cancer immunotherapies that license antigen-presenting cells, such as dendritic cells (DCs), to generate endogenous tumor-specific T-cell responses.[2] We developed Dual Costimulatory Receptors (DCR) that combine a costimulatory TNFR ligand ectodomain (CD40L) with a costimulatory endodomain (e.g., 4–1BB). We hypothesized that the co-expression of CD40L-based DCRs would improve the function of anti-tumoral T cells and promote the recruitment and activation of endogenous immune cells at the tumor site

Methods The anti-leukemic potential of CD40L-DCRs was assessed *in vitro* by co-culturing the engineered T cells with murine AML tumor cells (FBL) at a 1:1 ratio, using IncuCyte analysis to assess tumor lysis. To determine *in vivo* efficacy of CD40L-DCR ACT, FBL-bearing B6 mice were treated with 1e6-engineered T cells after pre-conditioning therapy. Mice were monitored for up to 100 days. To evaluate the ability of DCR-T cells to alter monocyte and/or macrophage programming, spleen and bone marrow were harvested 14 days post-ACT and myeloid cells were characterized using flow cytometry.

Results CD40L-DCRs cells displayed notable proliferation and maintained consistent CD40L expression after repeated stimulations, in contrast to mock-transduced cells. Our *in vivo* studies showed improved survival and therapeutic efficacy of several CD40L-DCR variants. Notably, we observed a high frequency of macrophages with a pro-tumoral phenotype in the tissues of mice that received a negative-control vector. In contrast, the groups that received CD40L-DCR TCR-T cells exhibited lower macrophage numbers but displayed higher levels of costimulatory molecules, consistent with antitumor macrophage subsets.

Conclusions Our findings strongly support the potential of CD40L-DCR in advancing the development of effective ACT strategies. By enhancing the function of endogenous immune cells, this innovative approach holds great promise for improving the efficacy of immunotherapy and achieving better outcomes for patients across a wide range of cancer types, including solid tumors.

REFERENCES

1. Tao J, Yang G, Zhou W, Qiu J, Chen G, Luo W, *et al*. Targeting hypoxic tumor microenvironment in pancreatic cancer. *J Hematol Oncol*. 2021;**14**(1):14. Epub 2021/01/14. doi: 10.1186/s13045–020-01030-w. PubMed PMID: 33436044; PubMed Central PMCID: PMCPMC7805044.
2. Moran AE, Kovacsovics-Bankowski M, Weinberg AD. The TNFRs OX40, 4–1BB, and CD40 as targets for cancer immunotherapy. *Curr Opin Immunol*. 2013;**25**(2):230–7. Epub 2013/02/19. doi: 10.1016/j.coi.2013.01.004. PubMed PMID: 23414607; PubMed Central PMCID: PMCPMC3815601.

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