394 USING CO-STIMULATORY CARS IN NATURAL KILLER CELLS TO SAFELY TARGET SOFT-TISSUE SARCOMAS AND THEIR INHIBITORY MICROENVIRONMENTS

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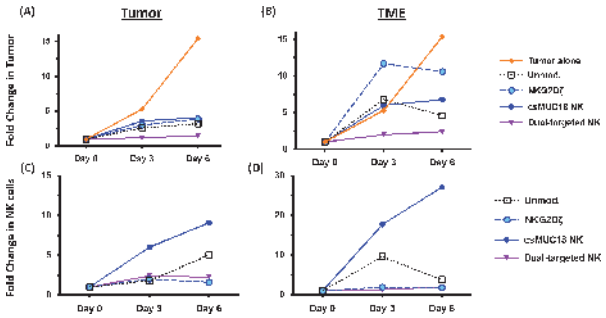
Background Outcomes for patients with refractory or relapsed soft-tissue (ST) sarcomas are poor. Immunotherapy with chimeric antigen receptor (CAR)-expressing lymphocytes has shown promise in pre-clinical models. However, efficacy has been limited by the suppressive tumor microenvironment (TME). Furthermore, because sarcoma associated antigens are also expressed on normal tissues, maximizing tumor killing while minimizing off-tumor toxicity has been challenging with traditional CAR approaches. The current study aimed to (1) design a natural killer (NK) cell immunotherapy that utilizes a "co-stimulation only" CAR to safely target the sarcoma antigen, MUC18; (2) define the optimal endodomain for the co-stimulatory (cs) MUC18 CAR that enhances NK cell proliferation without adding cytotoxicity; and (3) combine an optimal MUC18-csCAR with a cytotoxic CAR, NKG2D.ζ, that simultaneously eliminates sarcoma cells and inhibitory cells of the TME such as myeloid-derived suppressor cells (MDSCs) and M2 macrophages (M2s). By using this novel combinatorial antigen-recognition approach, we hypothesized that dual-targeted NK cells (co-expressing MUC18-csCAR and NKG2D.ζ) would be activated only within the TME co-expressing MUC18 and TME-associated ligands, but not in MUC18+ normal tissues, resulting in enhanced anti-tumor efficacy against MUC18+ ST sarcomas without toxicity.

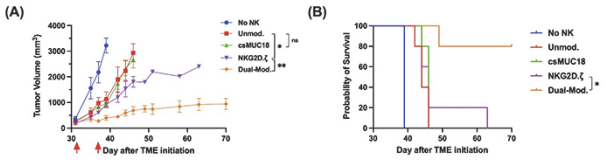
Methods We generated MUC18-cytotoxic and csCARs with 4-1BB, OX40, 2B4, and DNAM-1 endodomains and confirmed specificity and functionality using MUC18 overexpressing and knockout targets and a long-term TME co-culture comprised of alveolar rhabdomyosarcoma, Rh4, and inhibitory macrophages (M2s). Safety of MUC18-csCARs was tested against the MUC18+ liver sinusoidal endothelial cell (LSEC) line. Anti-tumor activity of dual-targeted NK cells compared to unmodified and singly-modified NK cells was assessed *in vivo* using a novel TME xenograft model with Rh4 and MDSCs.

Results MUC18 cytotoxic CAR-NK cells killed MUC+ high targets, while exhibiting low killing against an Rh4-MUC18 KO cell line, confirming CAR specificity and function. MUC18-OX40csCAR NK cells expanded without additional killing in the TME compared to NK cells with other co-stimulatory endodomains. MUC18-OX40csCAR NK cells did not exhibit killing of LSECs. Dual-targeted NK cells demonstrated enhanced tumor control in TME co-cultures (2.4-fold change in tumor vs. 4.6 by unmodified NK, 10.6 by NKG2D.ζ, and 6.8 by cs.MUC18) compared to either singly-modified NK population (figure 1). Dual-targeted NK cells demonstrated superior tumor control in the *in vivo* TME xenograft model compared to controls (p=0.007 versus NKG2D.ζ) and prolonged survival (p= <0.0001) (figure 2).

Conclusions Dual-targeted NK cells demonstrate enhanced anti-tumor activity without toxicity against normal tissue. Use of co-stimulation-only CARs in NK cells may allow exploitation of previously non-targetable sarcoma antigens.



Abstract 394 Figure 1 The dual-targeted CAR NK cells were compared to unmodified NK cells, NKG2D.? CAR NK cells, and csMUC18 CAR NK cells in a TME co-culture system. A) Rh4 fold expansion in the tumor alone conditions and B) in the TME conditions was determined for each time point. C) Fold change in NK cells in the tumor alone conditions. D) Fold change in NK cells in the TME conditions.



Abstract 394 Figure 2 (A) Mice (n=5 per treatment group) received two NK cells doses at 31 and 38 days post tumor inoculation and three times per week IL-2 and IL-15 to promote NK cell survival. (B) Survival probability of each treatment cohort.

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