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, 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) 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, 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) 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.