Targeting Ewing sarcoma, Osteosarcoma and Neuroblastoma with Anti-MCAM Chimeric Antigen Receptor Modified Natural Killer Cells

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Background

Pediatric patients with metastatic ES, OS and NB have a dismal average 5-year survival (<25%). Novel therapeutic approaches are desperately needed (Nayyar 2019). The melanoma cell adhesion molecule (MCAM) is highly expressed in pediatric solid tumors and constitutes a novel target for immunotherapy (Orentas, 2012). We previously demonstrated the anti-tumor efficacy of anti-CD20 CAR NK cells against Burkitt lymphoma in a humanized xenograft mouse model (Chu 2015). NKTR-255 is an investigational IL-15Rα-dependent, polymer-conjugated, recombinant human IL-15 agonist that retains the full spectrum of IL-15 biology, including expansion of NK cells (Miyazaki 2021, Robinson 2021).

Objective

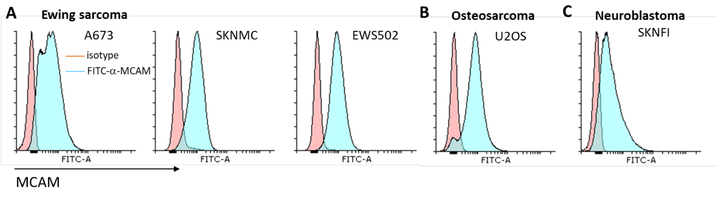
Here we developed an anti-MCAM CAR NK cell and investigated its in vitro and in vivo efficacy alone or in combination with NKTR-255 (provided by Nektar Therapeutics) in promoting NK cell cytotoxicity against ES, OS and NB.

Methods

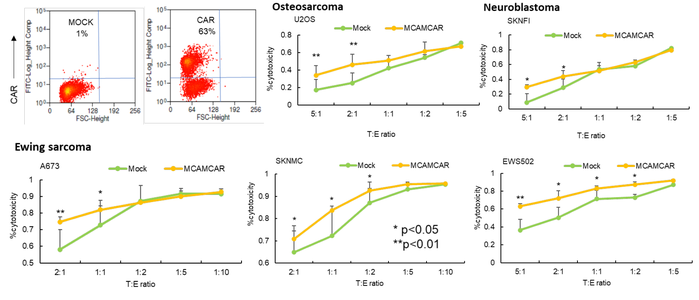
We engineered a CAR expressing NK cell targeting MCAM by electroporation of CAR mRNA into ex vivo expanded NK cells. In vitro cytotoxicity assays were performed to investigate the efficacy of anti-MCAM CAR NK cell against MCAM+ as well as CRISPR/Cas9 generated MCAM-knockout ES, OS and NB cells. Interferon (IFN)-γ and perforin levels were measured by ELISA assays. NB xenograft and ES patient derived xenograft (PDX) mouse models were used to evaluate the anti-tumor efficacy in vivo. Using an orthotopic mouse model of ES metastasis, we further investigated the anti-metastasis effect of CAR NK alone and/or in combination with NKTR-255 (0.3 mg/kg, iv).

Results

MCAM is highly expressed in ES, OS and NB cells

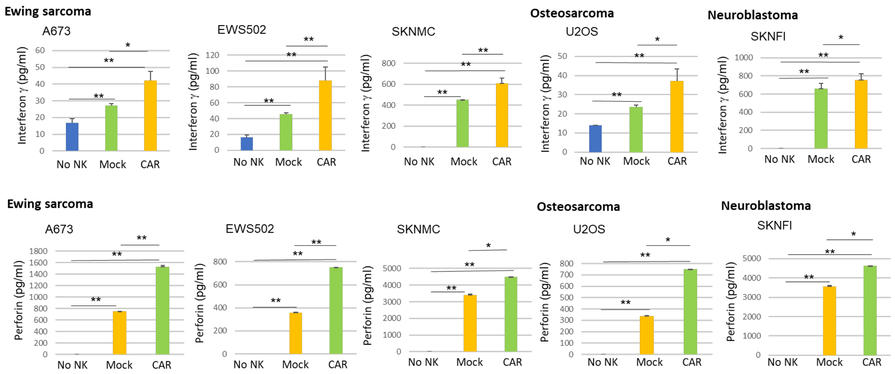


MCAM CAR significantly enhanced cytotoxic activity of NK cells against MCAM+ ES, OS and NB cells

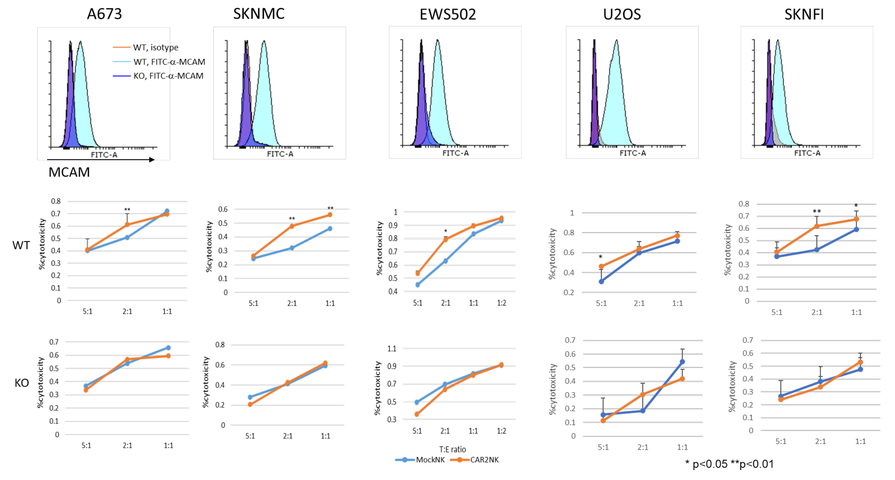


Results

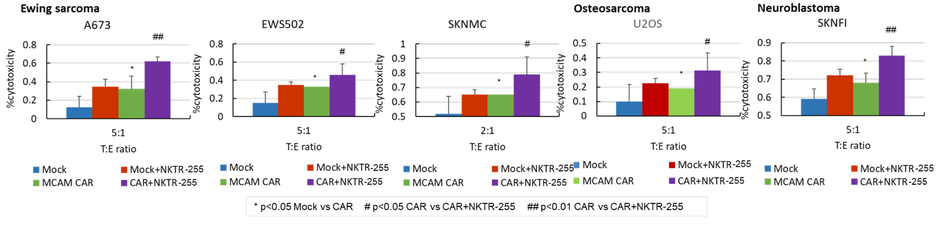
MCAM CAR significantly enhanced cytokine secretion of NK cells



MCAM CAR significantly enhanced cytotoxic activity of NK cells against MCAM+ but not MCAM KO ES, OS and NB cells

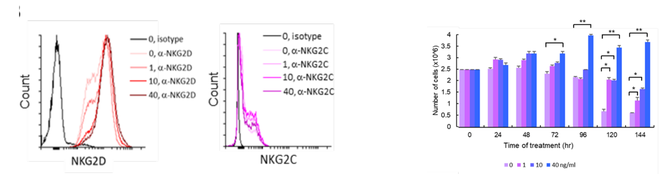


NKTR-255 further enhanced MCAM CAR NK cytotoxic activity against MCAM+ ES, OS and NB cells

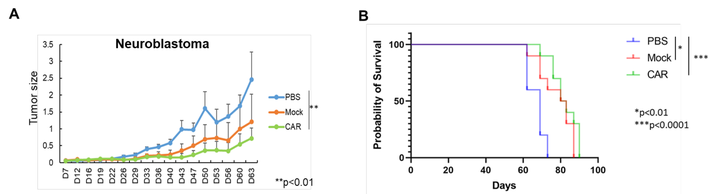


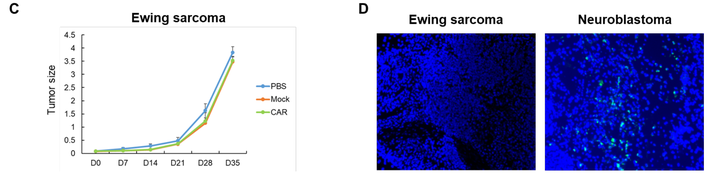
Results

NKTR-255 enhanced NK activating receptor expression and NK proliferation/expansion

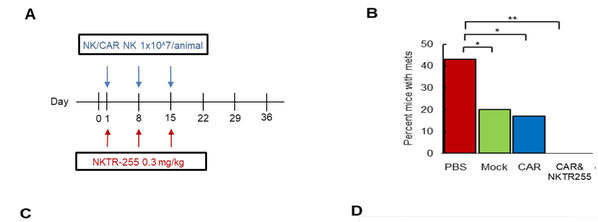


MCAM CAR NK cells significantly decreased tumor growth and prolonged animal survival in a NB xenograft but not ES PDX model





NKTR-255 further enhanced anti-metastasis effect of anti-MCAM CAR NK cells in an ES orthotopic mouse model



Conclusion

Our findings demonstrated efficacy of anti-MCAM CAR NK cells alone and/or in combination with NKTR-255 against malignant pediatric solid tumors in vitro and in vivo. Supported in part by U54 CA232561.