Peds-ONC Immunotherapy Center: Ex-vivo expansion of NK cells and electroporation with ROR1- and MCAM-specific chimeric antigen receptor (CAR) mRNA combined with IL-15 superagonist (N-803, ALT-803) to target and enhance function against neuroblastoma and Ewing sarcoma

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Background

Neuroblastoma (NB) and Ewing sarcoma (EWS) are malignant pediatric solid tumors. Despite significant survival improvements in multiple pediatric malignancies, children with relapsed/ metastatic NB and EWS continue to have dismal outcomes. The tyrosine kinase-like orphan receptor 1 (ROR1) and the melanoma cell adhesion molecule (MCAM) are novel cell surface proteins specifically overexpressed in NB and EWS. In collaboration with Dr. Dean Lee (Project 1), our group has developed a genetically engineered APC (K562) expressing mbIL-21 and 4-1BBL to expand peripheral blood mononuclear cells (PBMCs) into NK cells, leading to significant NK cell expansion and functional activation (Denman/Lee Plos One, 2012). ALT-803 is a novel IL-15 superagonist complex where mutant IL-15N72D is bound to an IL-15RαSu-Fc fusion protein. N-803 (ALT-803) has been shown to have superior biological activity, higher potency and longer half-life compared to IL-15, and is currently being tested in several clinical trials.

Objective

We hypothesized that ROR1- and/or MCAM-CAR expressed in ex-vivo expanded NK cells will enhance targeting against NB and/or EWS, and further enhance functional activity when combined with N-803/ALT803.

Methods

PBMC were expanded with lethally irradiated K562-4-1BBL-mbIL21 cells (exPBNK) (Denman/Lee Plos One, 2012)

ExPBNK cells were isolated using Miltenyi NK cell isolation kit (Chu/Cairo, Can Imm Res, 2015)

ExPBNK cells (aNK) were electroporated with anti-ROR1- or –MCAM-CAR mRNA with a 4-1BB signaling domain which is synthesized *in vitro*

Functional cytotoxic activity of anti-ROR1-and -MCAM CAR NK cells against NB and EWS cells were examined at different E:T ratios

IFN-γ levels were evaluated by ELISA assay.

Results

Figure 1. ROR-1 is highly expressed on different Neuroblastoma cell lines

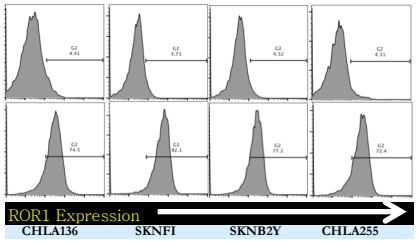


Figure 2. ROR-1 CAR is highly expressed on exPBNK cells following mRNA electroporation

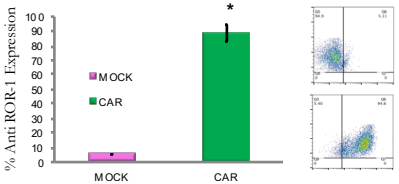


Figure 3. MCAM is highly expressed on Ewing sarcoma cells and primary tumors

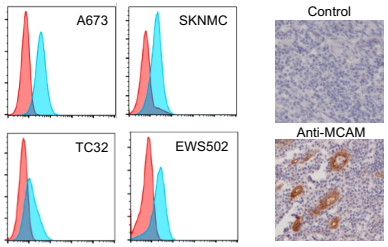


Figure 4. MCAM CAR expression on exPBNK cells following mRNA electroporation

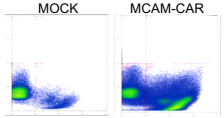


Figure 5. Significant Increase in In-vitro Cytotoxicity against NB and EWS cells by ROR1- and MCAM-CAR NK cells

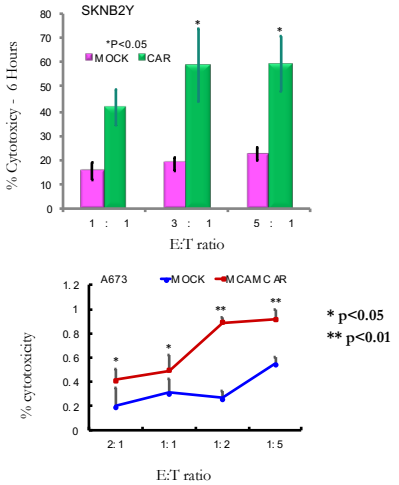


Figure 6. *In-vitro* Cytotoxicity against NB (SKNBE2) cells at 24 hours by ROR1-CAR NK cells in combination with ALT-803

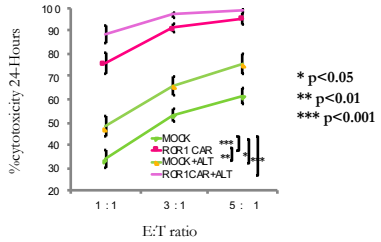


Figure 7. Addition of ALT-803 significantly improved the in vitro cytotoxicity against CHLA255 cells at low E:T ratio

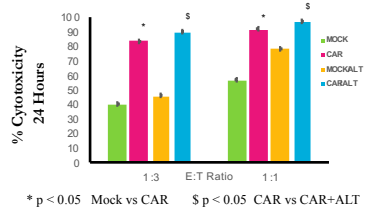
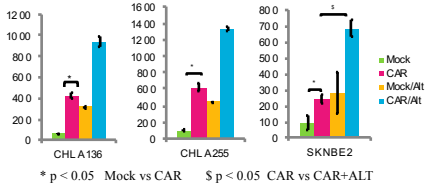


Figure 8. Enhancement of Interferon-gamma secretion against multiple neuroblastoma cell lines



Conclusions

These data demonstrated that anti-ROR1- and -MCAM-CAR expressed in ex-vivo expanded NK cells are highly active against NB and EWS cells, respectively. N-803 significantly increases in vitro cytotoxicity of anti-ROR1 CAR NK cells against ROR1 positive NB cells. In vivo studies using NB and EWS xenograft mouse models are currently in progress. Through interactions with other projects and shared resource cores (Dr. Elaine Mardis and Dr. Greg Behbehani), we will further investigate novel and innovative methodologies including SOCS3-deleted and/or TGFβ-imprinted NK cells (Project 1, Dr. Dean Lee) and myelolytic-virotherapy (Project 4, Dr. Tim Cripe) to circumvent chemotherapy resistance in pediatric solid tumors by CAR NK combinatorial immunotherapy.

Acknowledgements

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