TARGETING EWING SARCOMA WITH ANTI-IL1RAP CHIMERIC ANTIGEN RECEPTOR MODIFIED NATURAL KILLER CELLS

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Background Metastatic and recurrent/refractory Ewing sarcoma (ES) has a dismal prognosis, largely secondary to therapy resistance within the tumor microenvironment. ES has heightened natural killer (NK) cell sensitivity, and increased abundance of activated NK cells in ES patient tumors are correlated with extended survival. Solid tumors are resistant to NK in large part due to the small number of active NK cells and lack of specific tumor targeting of NK cells. Our group has developed a genetically engineered feeder cell to expand peripheral blood mononuclear cells (PBMCs) into NK cells, and demonstrated that expanded NK cells engineered to express chimeric antigen receptor (CAR) against various targets including CD20, GD2, ROR1 and MCAM had significantly enhanced cytotoxicity against lymphoma and sarcoma compared to mock NK. IL-1 receptor accessory protein (IL1RAP) is highly expressed on ES cells and minimally expressed in normal tissues as we previously reported.

Methods Here we developed an anti-IL1RAP CAR NK cell and investigated its efficacy in promoting NK cell cytotoxicity against ES. PBMCs were expanded into NK cells using K562-mbIL21-41BBL artificial antigen presenting cells. The IL1RAP antibody VH domain DNA was codon optimized and synthesized to create the anti-IL1RAP CAR. CAR NK cells were generated by non-viral electroporation of CAR mRNA into expanded NK cells. CAR expression was analyzed by flow cytometry using biotinylated IL1RAP protein. Anti-IL1RAP CAR NK cytotoxicity was evaluated in vitro by luciferase based cytotoxicity assay. CRISPR-Cas9 mediated IL1RAP knockout (KO) in ES cells was utilized to evaluate the specificity of CAR NK targeting.

Results Electroporation resulted in CAR expression in 70% of expanded NK cells and the CAR expression lasted for at least 6 days (figure 1). We found a significantly increased cytotoxicity of anti-IL1RAP CAR NK cells compared to mock NK cells against ES A673 and SKNMC cells at various effector to target ratios after co-culturing for 4 hours (\*p<0.01 and \*p<0.05) (figure 2). In the IL1RAP KO A673 cells, we did not observe the significant increase in cytotoxicity with IL1RAP CAR NK compared to mock NK as we did in the wildtype (WT) A673 cells (figure 3), demonstrating the enhanced cytotoxic activity of CAR NK cells is due to specific targeting of IL1RAP.

Conclusions These findings demonstrate enhanced efficacy of anti-IL1RAP CAR NK cells compared to mock NK cells against ES and support a preclinical evaluation of anti-IL1RAP CAR NK in limiting ES xenograft tumor growth and/or metastasis and prolonging animal survival in vivo.

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REFERENCES

Ethics Approval This study was approved by the New York Medical College Institutional Animal Care and Use Committee (protocol number 13911).

Abstract 303 Figure 1 Anti-IL1RAP CAR expression on ex vivo expanded NK cells on day 1 to 6 post electroporation

Abstract 303 Figure 2 In vitro cytotoxicity of anti-IL1RAP CAR NK and mock NK cells against ES A673 and SKNMC cells. *p<0.01, **p<0.05.

Abstract 303 Figure 3 In vitro cytotoxicity of anti-IL1RAP CAR NK and mock NK cells against A673 IL1RAP WT and KO cells. *p<0.05.