

POSTER PRESENTATION

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Chondroitin sulfate proteoglycan 4 specific chimeric antigen receptor therapy for pediatric solid tumors

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Chondroitin sulfate proteoglycan 4 (CSPG4) is a membrane bound proteoglycan, composed of an N-linked 280kDa glycoprotein, and a single 450kDa chondroitin sulfate linked proteoglycan [1]. CSPG4 expression has been documented on adult malignancies (melanoma, breast, mesothelioma) but has yet to be assessed on pediatric malignancies [2,3]. Furthermore, both monoclonal antibodies and chimeric antigen receptor (CAR) T cells against CSPG4 have shown modest anti-tumor efficacy in adult malignancies [2,3]. We screened a spectrum of pediatric solid tumor lines for CSPG4 expression to determine potential targets, then developed CSPG4-redirection CARs to target CSPG4+ pediatric solid tumors.

16 pediatric tumor lines were assessed for CSPG4 expression. CSPG4 was detected by flow cytometry in 69% of the cell lines assessed: osteosarcomas: 143b, HOS-MNNG, MG63; synovial sarcomas: SYO-I, HSSY-II, alveolar/embryonal rhabdomyosarcomas: Rh18c, Rh30/Rh36, medulloblastoma: DAOY, melanoma: Mel624, Mel1300. CSPG4 expression site density was estimated with BD PE Quantibrite beads by flow cytometry.

Using two different single chain variable fragments (scFv) known to target CSPG4 (225.28 and TP41.2), two anti-CSPG4 second generation CAR constructs were synthesized by transient transfection of retroviral packaging lines. Both constructs have identical intracellular signaling components (CD28, CD3z). Healthy donor T cells were transduced with viral supernatants, and assessed for CAR expression. Anti-tumor activity was measured by cytokine release and tumor specific lysis. Cell surface expression of the CSPG4-based CARs was detected by flow cytometry using conjugated Protein L to target the

expressed scFv light chain. 225.28 and TP41.2 based CAR T cells induced 25% and 31% lysis of target pediatric tumor lines, respectively (E:T ratio of 20:1). On further investigation, TP41.2 based CAR T cells were able to specifically lyse CSPG4+ cell lines 143b, Rh30, and Rh18c at 40%, 37%, and 16%, respectively (E:T ratio of 30:1). Additionally, the TP41.2 and 225.28 CAR T cells were assessed for Interferon gamma (IFN γ) production by flow cytometry. Compared to mock T cells coincubated with varying tumor lines (143b, Rh30, DND41), the TP41.2 and 225.28 CAR+ T cells had significantly positive populations for IFN γ production ($p < 0.0001$), predominantly in the CD8+ population.

High levels of CSPG4 are present on a variety of pediatric solid tumors. TP41.2 CSPG4-based CAR T cells target, generate significant levels of IFN γ , and lyse osteosarcoma *in vitro* at significant levels ($p = 0.01$). Targeting CSPG4 with CAR redirected T cells represents a new strategy for eliminating these pediatric malignancies in an antigen specific, cell-mediated manner.

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