ANTI-MYELOID POLY-PHARMACY COMBINED WITH FGFR4-TARGETED CHIMERIC ANTIGEN RECEPTORS (CARs) EFFECTIVELY TREATS ORTHOTOPIC RHABDOMYOSARCOMA, MODELING CAR EFFECTIVENESS FOR SOLID TUMORS

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Background Rhabdomyosarcoma (RMS) is the most common soft tissue cancer in children. Treatment outcomes have not improved in decades, especially for relapsed/refractory or metastatic disease. Previous work identified Fibroblast Growth Factor Receptor 4 (FGFR4, CD334) as being specifically upregulated in RMS, making it a candidate for targeting by CAR-T cells. A previous CAR designed to target FGFR4 showed in vitro efficacy, and some tumor control in a metastatic (intravenous) RMS model, but failed to control orthotopic (intramuscular, i.m.) disease in vivo. Impressively, even in the NSG mouse model system, i.m. disease is characterized by a collagen-rich stroma, replete with myeloid cells, that is induced by T cell therapy (figure 1A).

Methods A large-scale screening campaign for antibody-based binding domains, using the membrane proximal domain of FGFR4 as the bait molecule, yielded a number of candidates, that when expressed in the context of a CAR, exceed the activity of previous binders in vitro. CARs were ranked by direct detection on the T cell surface with recombinant target antigen, cytotoxicity, and cytokine production upon co-culture with target cell lines. Top candidate binders were then tested against orthotopic RMS tumors in NSG mice using the RMS cell line RH30, engineered to express truncated CD19. The presence of M2 macrophages (CD11b+, CD206+) and MDSC (F4/80-, CD11b+) was analyzed by immunohistochemical analysis of i.m. tumors from treated and untreated NSG mice. The presence of target antigen on the RH30_19 cell lines was quantified by flow cytometry using standardized bead assays.

Results The two highest ranking anti-FGFR4 CAR-T candidates failed to control i.m. RH30_19 on their own. Considering the low expression of target antigen present on tumors in vivo (700 FGFR4 per cell), we tested CD19 CAR-T (95,000 molecules per cell). Anti-CD19 CAR-T also failed. Gene expression analysis of RH30 identified several potential mechanisms of tumor resistance to CAR T therapy. Based on this data, the abundant presence of M2 macrophages, and the known presence of MDSC in NSG mice, we devised a pharmacologic strategy to augment CAR-T activity. The exposure of mice to anti-myeloid polypharmacy (targeting MDSC via ATRA, M2 macrophages via the CSF1R, IDO1, iNOS, MIF, TGFbeta, and PDL1) allowed both FGFR4 CAR-T and CD19 CAR-T to successfully clear orthotopic RMS tumors (figure 1B).

Conclusions RMS tumors can be cleared using an FGFR4-targeted CAR in combination with anti-myeloid poly-pharmacy, demonstrating that even extremely low copy number targets can be targeted by CAR-T by reversing an immunosuppressive microenvironment.

Abstract 161 Figure 1 T Cells induce myeloid-rich stroma in RMS which can be overcome with anti-myeloid poly-pharmacy. A. NSG mice with intramuscular RH30 tumors were left untreated (No T Cells) or received activated T Cells. Tumors were excised and stained as described in the figure. Upon T Cell treatment, a collagen-rich stroma forms, and is populated by M2-polarized macrophages (F4/80+CD206+), effectively excluding T Cells from the lesion. B. Anti-myeloid polypharmacy (PolyRx) treatment paired with CD19 or FGFR4 targeted CAR T therapy is able to control orthotopic RMS tumors.

Ethics Approval Animal experiments were conducted in an AAALAC accredited facility, following Seattle Children's Research Institute institutional animal care and use committee (IACUC) approval of procedures (IACUC00417).

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