ANTI-B7H3 CHIMERIC ANTIGEN RECEPTOR NK CELLS FOR HIGH-RISK PEDIATRIC BRAIN TUMORS

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Background Aggressive pediatric brain tumors such as atypical teratoid/rhabdoid tumor (AT/RT), MYC-amplified medulloblastoma, high grade glioma and diffuse midline glioma are major drivers of pediatric cancer mortality. These therapy-resistant tumors frequently overexpress the immune checkpoint cell surface molecule B7-H3 (CD276). B7-H3 is a pan-cancer antigen that is highly expressed in many tumor types. CAR-T cell studies have established that immunotherapeutic targeting of B7-H3 is safe in pediatric patients. We have developed B7-H3 targeted chimeric antigen receptor (CAR) natural killer (NK) cells as a tumor-specific cell therapy for these pediatric brain tumors. CAR-NK cells have several advantages over CAR-T cells. NK cells can be obtained from healthy donors and produced as an ‘off-the-shelf’ product. NK cells also have a lower risk of inflammatory toxicity and graft-versus-host disease compared to T-cells when transferred across the HLA barrier from healthy donors to patients.

Methods We have designed a library of variable affinity B7-H3-targeted CARs, produced replication incompetent g-retroviral vector, and used this for generation of B7-H3 CAR-NK cells. We verified B7-H3 expression in a panel of brain tumor cell lines, and further engineered firefly luciferase expressing AT/RT and medulloblastoma (CHLA06.ffLuc, D425.MED.ffLuc) as well as a CHLA06-derived B7-H3 knockout. We developed an orthotopic CHLA06.ffLuc xenograft model. We tested CAR-NK cell functionality using in vitro co-culture and cytotoxicity assays. In vivo study was performed in our xenograft with intratumoral deliver of CAR-NK cells. Parallel study using murine-specific B7-H3 CAR-NK cells and syngeneic immunocompetent murine CNS tumor models are planned.

Results B7-H3 targeted CAR-NK cells demonstrate target-specific cytotoxicity when compared to untransduced NK cells. Crispr/Cas9 mediated knockout of B7-H3 in target cells abolished the difference in CAR vs. no-CAR NK-mediated target killing. When delivered intracranially to CHLA-06 orthotopic xenograft bearing mice, B7-H3 CAR NK cells have evident antitumor activity and prolong survival.

Conclusions Clinical trials infusing NK cells directly into the brains of patients with refractory brain tumors are launching in 2023, and are aligned with our efforts to engineer B7-H3 CAR-NK cells, thereby accelerating translation of this approach to children with aggressive brain tumors. Targeting pediatric brain tumors with an anti-B7H3 CAR-NK cell therapy may provide a safe and effective treatment for patients who have extremely limited therapeutic options. As B7-H3 is a tumor associated antigen found to be overexpressed in a large variety of tumor types, this strategy may also be useful for treatment of other B7-H3 expressing cancers.

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REFERENCES

Ethics Approval Animal work and protocols were approved by Johns Hopkins University Animal Care and Use Committee (Protocol #MO22M138)

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