127 CAR T CELLS TARGETING THE INTEGRIN ALPHA V BETA 3 EXHIBIT ROBUST ANTI-TUMOR RESPONSES AGAINST DIFFUSE INTRINSIC PONTINE GLIOMA AND GLIOBLASTOMA

Dustin Cobb*, Jacopo de Rossi, Lixia Liu, Erin An, Daniel Lee. *University of Virginia, Charlottesville, VA, USA*

Background Diffuse intrinsic pontine glioma (DIPG) and glioblastoma (GBM) are two highly aggressive and mostly incurable gliomas with little therapeutic advancements made in the past several decades. Despite immense initial success of chimeric antigen receptor (CAR) T cells for the treatment of leukemia and lymphoma, significant headway into the application of CAR T cells against solid tumors, including gliomas, is still forthcoming. The integrin complex alpha v beta 3 (avb3) is present on multiple and diverse solid tumor types and tumor vasculature with limited expression throughout most normal tissues, qualifying it as an appealing target for CAR T cell-mediated immunotherapy.

Methods Patient-derived diffuse intrinsic pontine glioma (DIPG) cells and glioblastoma (GBM) cell lines were evaluated by flow cytometry for surface expression of avb3. Second-generation CAR T cells expressing an anti-avb3 scFv were generated by retroviral transduction containing either a CD28 or 4-1BB co-stimulatory domain and CD3zeta. CAR T cells were evaluated by flow cytometry for CAR expression, memory phenotype distribution, and inhibitory receptor profile. DIPG and GBM cell lines were orthotopically implanted into NSG mice via stereotactic injection and monitored with bioluminescent imaging to evaluate avb3 CAR T cell-mediated anti-tumor responses.

Results We found that patient-derived DIPG cells and GBM cell lines express high levels of surface avb3 by flow cytometry, while avb3 is minimally expressed on normal tissues by RNA sequencing and protein microarray. Second-generation CAR T cells expressing an anti-avb3 single-chain variable fragment (scFv) were designed and generated by retroviral transduction containing either a CD28 or 4-1BB co-stimulatory domain and CD3zeta. avb3 CAR T cells demonstrated efficient, antigen-specific tumor cell killing in both cytotoxicity assays and in in vivo models of orthotopically and stereotactically implanted DIPG and GBM tumors into relevant locations in the brain of NSG mice. Tumor responses were rapid and robust with systemic CAR T cell proliferation and long-lived persistence associated with long-term survival.

Conclusions These results highlight the potential of avb3 CAR T cells for immunotherapeutic treatment of deadly brain tumors with reduced risk of on-target, off-tumor mediated toxicity due to the restricted nature of avb3 expression in normal tissues.

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