244 TARGETING GANGLIOSIDES IN PEDIATRIC CANCER

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Background FDA-approved monoclonal antibodies (mAbs) that recognize the ganglioside GD2 (glycolipid) are used in the treatment of high-risk neuroblastoma (NBL). Despite successful integration of anti-GD2 mAbs into upfront treatment protocols, 40–50% of patients relapse, often with poor outcomes. We previously identified that downregulation of the sialyltransferase *ST8SIA1* reduces GD2 levels and renders resistance to anti-GD2 therapy. Interestingly, we discovered that the loss of GD2 synthesis as a result of *ST8SIA1* downregulation, is accompanied by a compensatory increase in GM2, another ganglioside. We have engineered anti-GM2 chimeric antigen receptors (CAR) and mAbs that recognize this antigen on NBL cells. Our overall hypothesis is that GD2 downregulation in NBL can be overcome by co-targeting GD2 and GM2.

Methods NSG mice were injected with GD2-high NBL cells and treated with anti-GD2 mAbs or CAR-T cells 7 days after tumor inoculation. Tumors were harvested at endpoint and analyzed by flow cytometry for ganglioside levels.

We developed GM2-specific CAR T-cells and mAbs and tested them *in vitro* and *in vivo*.

GM2-specific CAR T-cells were generated from primary human T cells by retroviral gene delivery. For *in vivo* experiments, NSG mice were injected with NBL cell lines and treated with CAR T-cells or mAbs 4–6 days after tumor inoculation. After meeting endpoint criteria, mice were euthanized and tumors were analyzed for ganglioside levels.

Results Tumors from mice treated with anti-GD2 therapy (mAb and CARs) showed reduced GD2 levels and increased GM2 at end point.

GM2-specific CAR T-cells showed robust *in vitro* activity (cytokine production and cytotoxicity) and a durable response *in vivo* with no signs of toxicity. Similar to our previous findings, regarding changes in ganglioside levels after treatment, tumors from mice treated with GM2-CAR T-cells, showed a reduction in GM2 and an increase in GD2 levels.

Anti-GM2 mAbs, alone or in combination with anti-GD2 mAbs, were well tolerated by mice, with no evidence of pain or weight loss. Dual administration of anti-GD2 and anti-GM2 mAbs, showed higher efficacy and better survival than targeting each ganglioside individually with mAbs.

Conclusions GM2 specific CAR T-cells and mAbs showed robust activity *in vitro* and *in vivo*, against multiple NBL lines, suggesting targeting GM2 could overcome anti-GD2 resistance in NBL.

Ethics Approval VERIFICATION OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) APPROVAL

Date: April 18, 2023

To: Robbie Majzner, Pediatrics - Hematology/Oncology Peng Xu, Cynthia A Klein, Alyssa Ray

Assurance Number: A3213–01

Approval Period: 04/18/2023 THROUGH 01/23/2026

Protocol ID: 33698

The IACUC approved this protocol transaction on 04/18/2023. Prior to initiation of animal studies, if this study involves biohazardous or radioactive agents, you must obtain Biosafety Panel or Radiological Safety Panel approval. The expiration date of this approval is 01/23/2026 at Midnight. If this project is to continue past that date, you must submit an updated protocol (renewal) in advance for IACUC re-approval. Proposed changes to approved research must be reviewed and approved prospectively by the IACUC. No changes may be initiated without prior approval by the IACUC, except where deemed necessary by veterinary staff. (Any such exceptions must be reported to the IACUC within 10 working days).

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0244