Letter to the Editor

Cancer Immunology Research

Neurotoxicity Associated with a High-Affinity GD2 CAR—Response

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We appreciate the concerns conveyed in the letter from Majzner and colleagues (1) related to our recently published article (2). We agree that caution must be used in extrapolating results from one CAR T-cell study to another due to the numerous differences in CAR T-cell design and methods of use that could affect function. Majzner and colleagues cite their own data on a E101K variant of the 14g2a-derived CAR (E101K-CAR) and a preclinical study by Hoseini and colleagues of a CAR that uses a single-chain variable fragment derived from GD2-specific clone, 3F8 (3). Neither study reported neurotoxicity similar to that observed in our studies.

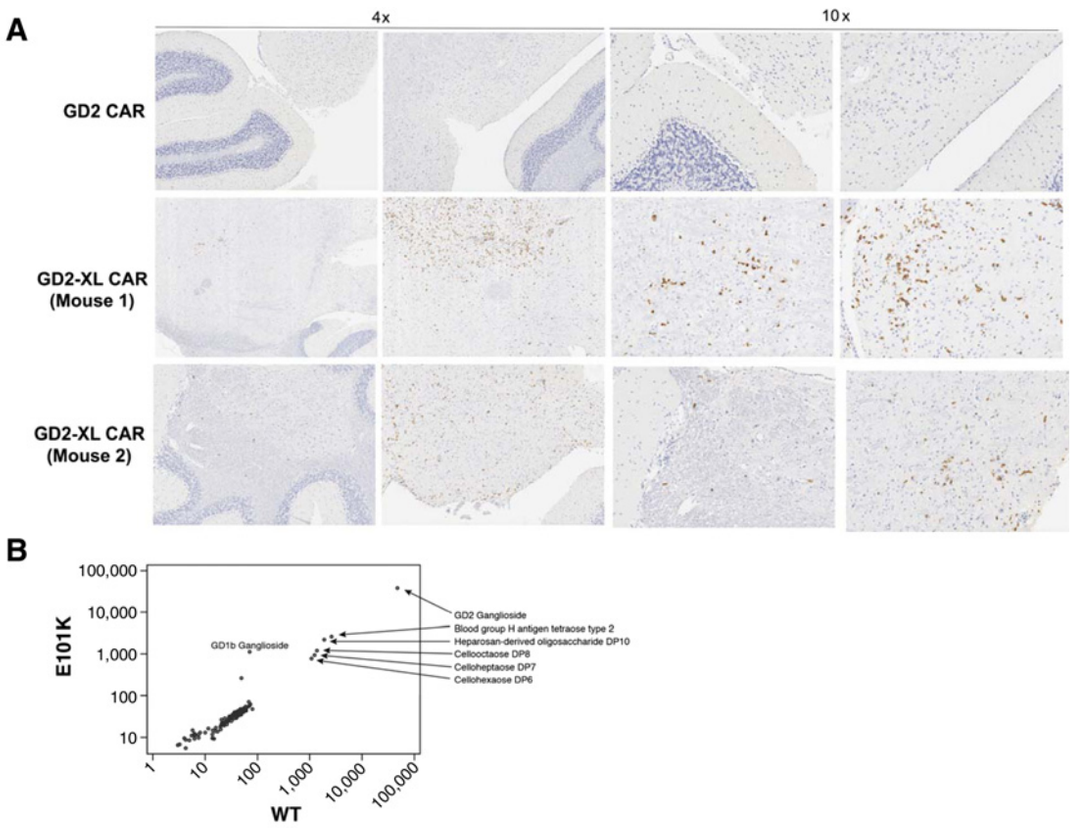

Figure 1.

A, Immunohistochemistry of mice treated with a 14g2a-derived CAR with a short linker between variable-light (VL) and variable-heavy (VH) chains (GD2 CAR) and two mice treated with 14g2a-derived CAR with a long linker between VL and VH (GD2-XL CAR) as described in Richman et al. (2). Both of GD-XL CAR-treated mice were non–tumor-bearing controls. **B,** Glycan microarray analysis was performed on 14g2a and 14g2a-E101K-mutant antibodies using the National Center for Functional Glycomics Defined Glycan Array v2. Normalized signal intensity for the original 14g2a clone (WT) or the E101K mutant (E101K) is plotted as indicated.

The E101K data provided used a CD28-costimulated second-generation CAR, whereas our studies used a 4-1BB–costimulated CAR. These investigators previously reported that the 4-1BB costimulatory domain enhanced the function of their original 14g2a-derived CAR (4). As we had noted (2), although neurologic dysfunction was not overt, a 14g2a-derived single-chain variable fragment (scFv) with a longer linker used between the light and heavy chains (GD2-XL) also exhibited T-cell infiltration within the central nervous system that was less intense, but similar in distribution to the E101K mutant, further illustrated in Fig. 1A.

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This scFv with a longer linker was the basis upon which our E101K-CAR was generated.

In addition to differences in scFv and CAR structure, the choice of gene-transfer vector (i.e., retroviral vs. lentiviral vector) or the T-cell culture conditions used to generate the CAR-T cells could contribute to the observed differences in toxicity, relative to our studies with the GD2-XL and E101K variant scFvs. Although the differences between our 3F8-based CAR T cells and those described by Hoseini and colleagues appear fewer (with similar overall CAR structure, vector, and T-cell culture system), the studies by Hoseini used the BALB/*c-Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ (DKO) strain for their *in vivo* xenograft studies. Our studies used the NODSCID-*Il2rg*$^{-/-}$ (NSG) strain of mice, which supports improved engraftment of human cells compared with the DKO BALB strain (5). Improved engraftment and T-cell persistence, important for CAR T-cell efficacy, may be contributing to differences in toxicity between these studies. The nature of the linker used in the 3F8 scFv is also not reported in Hoseini and colleagues, and differences in this structure or other features of their CAR (e.g., human vs. mouse framework) may further contribute to the activation-induced cell death noted with their CAR, which we have not observed with our constructs.

Majzner and colleagues also raise the possibility that the E101K mutation alters the 14g2a-derived scFv specificity for different gangliosides, explaining the increased toxicity. We explored this using the National Center for Functional Glycomics Defined Glycan Array v2 (ref. 6; Fig. 1B). The E101K variant shows selectivity toward GD2 similar to the original 14g2a clone; however, we agree that a selectively GD2-deficient model would be necessary to fully assess the functional specificity of these antibodies when incorporated into a CAR.

Finally, Majzner and colleagues suggest that our observed neurotoxicity might be a manifestation of severe cytokine release syndrome (CRS) similar to that described by Gust and colleagues (7), with CD19-specific CAR-T cells rather than on-target, off-tumor toxicity. We believe this mechanism of neurotoxicity is unlikely given the histopathologic analysis of E101K-CAR and 3F8-CAR mice. These mice exhibit a severe T-cell infiltration localized to the central nervous system, with evidence of T-cell proliferation and blast formation in the brain and spinal cord parenchyma consistent with local T-cell activation by antigen. The T-cell infiltration in the study by Gust and colleagues was confined to the perivascular spaces within the brain. Similar to the data presented by Majzner and colleagues, cytokine release by our GD2-specific CART cells is also highly antigen dependent. Combined with the observation of toxicity with focal CNS infiltration of CAR T cells even in non–tumor-bearing mice, these data in aggregate suggest that the CNS tissue is the source of antigen driving the activation of our GD2-targeted CAR T cells.

Disclosure of Potential Conflicts of Interest

M.C. Milone reports receiving commercial research support from Novartis. No potential conflicts of interest were disclosed by the other author.

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