Letter to the Editor

Cancer Immunology Research

Neurotoxicity Associated with a High-Affinity GD2 CAR—Letter

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We read with interest the article entitled, "High-affinity GD2-specific CAR T cells induce fatal encephalitis in a preclinical neuroblastoma model" by Richman and colleagues (1). In this report, the investigators generated a new chimeric antigen receptor (CAR) incorporating a mutated version (E101K) of the anti-GD2 14g2a scFv that enhances affinity for GD2 (2). This CAR demonstrated antitumor activity against neuroblastoma xenografts, but mice manifested high mortality, which the authors attributed to neurotoxicity. The authors present histologic evidence of T-cell infiltration in the cerebellum, basal ganglia, thalamus, midbrain, and spinal cord, in E101K-14g2a-BBz CAR (but not 14g2a-BBz CAR)–treated mice, which they state correlates with "brain regions known to contain GD2." They posit that the E101K-CAR induced neurotoxicity through on-target recognition of GD2. They conclude that "GD2 may be a difficult target antigen for CAR T-cell therapy without additional strategies that can control CAR T-cell function within the CNS." This article raises several concerns addressed below.

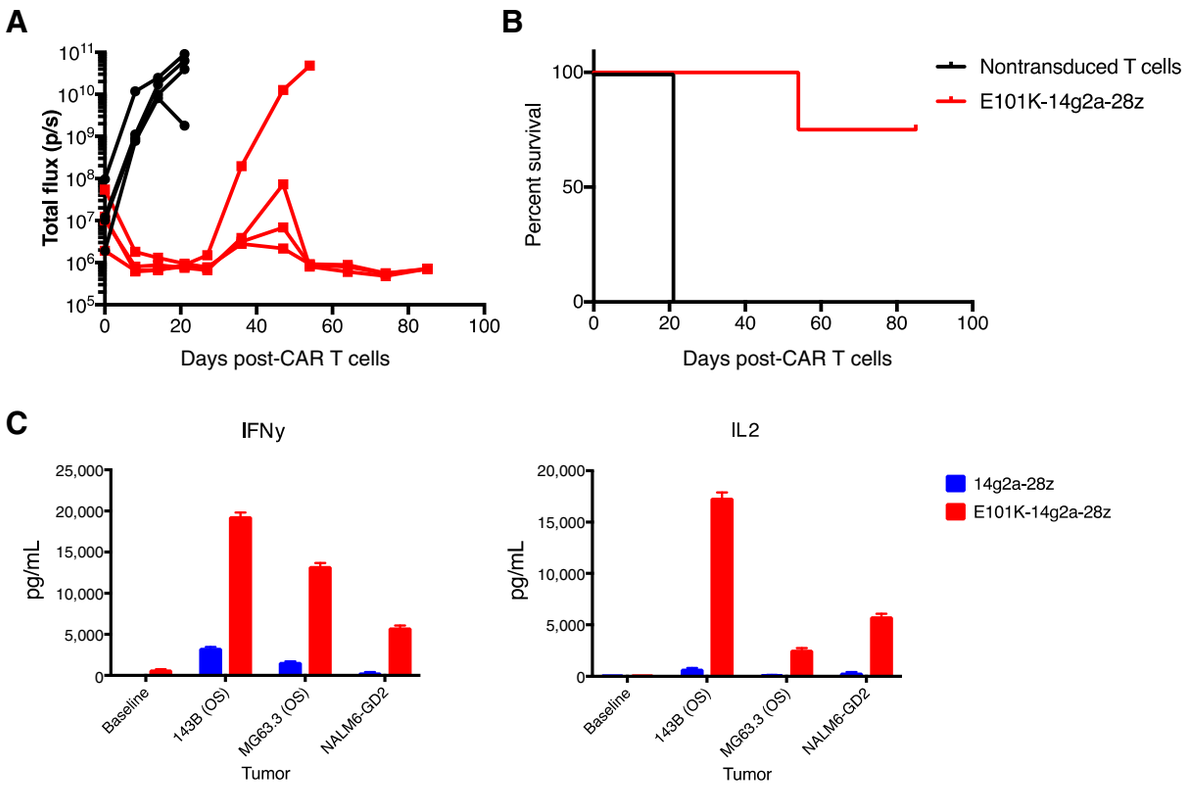

Figure 1.
5e6 E101K-14g2a-28z CAR T cells were infused into NSG mice 4 days after engraftment with 1×10^6 Nalm6 B-cell leukemia cells engineered to stably overexpress GD2 and GFP luciferase. These data show tumor regression (**A**) and survival of CAR-treated mice in comparison with those treated with nontransduced T cells (**B**). E101K-14g2a-28z CAR T cells had enhanced cytokine production compared with nonmutated 14g2a-28z CAR T cells after coculture of 1×10^5 CAR^+ T cells with 1×10^5 tumor cells for 24 hours (**C**). *In vitro* data are representative of three independent experiments with different T-cell donors, and *in vivo* data are representative of two independent experiments with different donors.

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We have also generated a CAR incorporating the E101K-14g2a scFv and have treated animals with E101K-14g2a-28z CARs without observing signs or symptoms of neurotoxicity (Fig. 1A and B). The authors also report neurotoxicity in mice treated with a CAR containing the anti-GD2 3F8 scFv. A previously published article on 3F8-based CARs reported no neurotoxicity, and treated mice survived more than 50 days (3). The E101K mutation alters a binding domain in 14g2a that could lead to recognition of other

gangliosides in the CNS. Many antibodies crossreact with multiple gangliosides (4–7). Furthermore, the authors saw no T-cell infiltration into the cerebral cortex, although the study cited by the authors that demonstrated GD2 expression in healthy brain tissue states that GD2 is expressed in the cortex (8). Proof of on-target toxicity could be obtained by studies in GD2-deficient mice, which have been generated previously (9).

An alternative explanation for the authors' findings is off-target, cytokine-mediated toxicity. Clinical data show that robust activation of CD19-CAR T cells can induce fatal neurotoxicity associated with CAR T-cell trafficking into the CNS, due to endothelial activation and breakdown of the blood:brain barrier (10). Our *in vitro* studies demonstrate that E101K-14g2a-28z CARs have higher tonic signaling than 14g2a-28z CARs and secrete more cytokines in response to $GD2^{+}$ cell lines (Fig. 1C). Therefore, it remains possible that the neurotoxicity could occur as a result of the potent activation of the E101K-14g2a CAR T cells rather than an on-target/off-tumor effect.

Generalizing toxicity concerns with the E101K-14g2a CAR to all CARs that target GD2 ignores an emerging body of literature that has demonstrated safety of GD2-CARs in clinical trials. The authors cite the work of Brenner and colleagues (11, 12), which demonstrated that a 14g2a-based GD2-CAR mediated antitumor responses and demonstrated long-term persistence in patients with neuroblastoma without neurotoxicity. The authors postulate that no neurotoxicity was seen because this construct was a first-generation CAR, containing only the intracellular TCRζ domain. However, the premise that neurotoxicity is dependent on a costimulatory domain remains unproven. A MAGE-A3 TCR that caused fatal neurotoxicity in early-phase clinical trials due to MAGE-A12 cross-reactivity did not contain an embedded costimulatory domain (13). Clinical trials of a third-generation GD2-CAR incorporating the 14g2a scFv, including one administered in combination with PD-1 inhibition, demonstrated T cell expansion after lymphodepletion, but no overt neurotoxicity in children or adults (14, 15). We have also found that a 14g2a-BBz CAR mediates strong activity in multiple xenograft models, including osteosarcoma (16), neuroblastoma (17), and diffuse intrinsic pontine glioma (18) without neurotoxicity. The pediatric oncology community is aware that any high potency CAR T-cell possess inherent risks and that the low-level expression of GD2 on vitals tissues poses a theoretical risk. We caution that these interesting findings with the E101K-14g2a CAR in a mouse model should not be overinterpreted as evidence that GD2-CARs pose additional risks beyond those already appreciated.

Disclosure of Potential Conflicts of Interest

R.G. Majzner and C.L. Mackall have a pending patent application for the use of GD2 CAR T cells to treat H3K27M mutant gliomas. No potential conflicts of interest were disclosed by the other authors.

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