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Dear CIRM Application Review Subcommittee,

Thank you for your service on the CIRM Governing Board.

I am the principal investigator of the TRAN-16912 proposal, which has been recommended for funding by the Grans Working Group (GWG). In this work, we aim to prepare an Investigational New Drug (IND) application for a phase-1 clinical trial that evaluates a novel chimeric antigen receptor (CAR)-T cell therapy that can combat glioblastoma via two distinct mechanisms:

(1) through the expression of a CAR that targets IL-13Rα2, which is found on the surface of some glioblastoma cells, and
(2) through the secretion of the cytokines IL-12 and IL-18, which can stimulate endogenous immune cells to attack tumor cells, including ones that may not express IL-13Rα2.

Glioblastoma accounts for close to half of all primary brain-tumor cases, and the annual incidence in the United States is estimated at 3.26 per 100,000 people. The median survival for newly diagnosed patients receiving standard of care is only 12–15 months. In fact, the median survival for all newly diagnosed patients is even shorter at 8 months, as a subset of patients have inoperable brain tumors or elect hospice over treatment because the burden associated with existing treatment options outweighs the very low probability of benefits that therapy could bring for some patients. Due to this dire lack of effective treatment options, the survival rate at 5 years post-diagnosis is less than 7%. For decades, glioblastoma survival rates and mortality statistics have remained unchanged. Therefore, new treatment options with greater long-term efficacy are urgently needed.

In the proposal, we presented compelling data showing that IL-13Rα2 CAR-T cells armored with IL-12 and IL-18 secretion can robustly eliminate IL-13Rα2–expressing tumor cells. We also presented the strategy of replacing IL-18 with DR-18, which is a decoy-resistant form of IL-18 that is even more potent. Finally, we showed that combining cytokine-secreting CAR-T cells ("CAR-12.DR18" T cells) with CAR-T cells that secrete a protein that blocks VEGF and angiogenesis ("CAR-αVEGF" T cells) results in strong anti-tumor activity without significant toxicity in multiple mouse studies.

We thank the GWG for recognizing the importance of this study and our team's experience and ability to shepherd this therapy to the clinic. I would also like to address some of their specific inquiries and concerns below. (Comments from the GWG are shown in ***bolded italics***.)

***Targeting IL-13RA2 is not a novel concept and has been used in the clinic.***

CAR-T cells targeting IL-13Rα2 have indeed been tested in over 70 human patients, with results showing a stellar safety record (Brown et al., Nature Medicine, 2024; Bagley et al., Nature Medicine, 2024). This is in fact the main reason that we chose IL-13Rα2 as the target antigen for the CAR. There are two main challenges that limit the efficacy of IL-13Rα2 CAR-T cell therapy for glioblastoma. First, most glioblastoma cells do not uniformly express IL-13Rα2, and

any tumor cell that does not express the target antigen can escape detection by the CAR-T cells, resulting in tumor progression or relapse. This is the “heterogeneity” problem noted by reviewers. Second, the CAR-T cells are insufficiently potent in some patients, such that even tumor cells that express IL-13Rα2 are not efficiently eliminated.

In contrast to conventional IL-13Rα2 CAR-T cell therapies that have been tested in the past, the therapy to be evaluated in this proposal utilize CAR-T cells that have been armored with the very potent cytokines IL-12 and IL-18 (or DR-18). We have shown that in mouse models where conventional IL-13Rα2 CAR-T cells fail the clear the tumor, our armored CAR-T cells reproducibly and efficiently achieve tumor control, indicating intrinsic superiority in the efficacy of armored CAR-T cells.

Furthermore, the cytokines induce a strong endogenous immune response, which we have characterized in detail at cellular and transcriptomic levels. This endogenous immune response can further amplify anti-tumor efficacy, including against tumor cells that do not express IL-13Rα2. We have now generated reproducible data sets demonstrating armored CAR-T cells’ ability to clear heterogeneous gliomas in multiple mouse studies. Therefore, we believe the proposed therapy presents a significant advancement over the CAR-T cell therapies that have been tested in previous clinical trials, and has the potential to significantly improve the care of patients with glioblastoma.

***It is impossible to know if this product would lead to long term cures.***

We fully agree that it is impossible to know any product would lead to long-term cures without extensive clinical testing. However, our preclinical data present a compelling case to evaluate this therapy clinically. Our animal studies are typically continued for 2+ months, which is sufficient time to observe any late relapses in mice. In these studies, we have observed long-term tumor control by our armored CAR-T cells.

***If approved, the product is likely to be expensive…must be given in specialized centers, possibly requiring patients to relocate for weeks if not months.***

Cost and accessibility of therapy are two key factors we have carefully evaluated in developing CAR-T cell therapy. To minimize cost and the time patients must wait for the therapy, our proposal outlines the ongoing development of an accelerated cell-manufacturing protocol that would cut down on reagent use as well as manufacturing time. It is undeniable that cellular therapies are complex and expensive. However, if we are able to generate a highly effective treatment—which we believe our armored CAR-T cells will be—then the cost of CAR-T cell therapy would be substantially lower than the combined costs of multiple cycles of therapy that are the current standard of care, not to mention the human cost in mental and physical health of both the patients and their caretakers.

***The team estimates that if the candidate needs to be replaced it would delay them by 15 months and $180k. They have not identified funding for this risk, outside of contingency funding for incurred costs associated with virus production.***

We respectfully disagree with this reviewer comment, as the contingency funding identified in the proposal is sufficient to cover this cost. We also note that since our proposal submission in July, we have performed follow-up studies that further minimized the likelihood that the candidate would need to be replaced.

There were comments on the topic of efficacy, including:

- ***It's not clear that armored CAR is overcoming heterogeneity.***
- ***Producing both cytokines under control of NFAT does not guarantee that you will get the correct levels of cytokines to maximize overall synergistic potential in GBM.***

As described above, we have now generated reproducible data sets indicating the armored CAR-T cells are indeed capable of overcoming heterogeneity. We already had one dataset at the time of proposal submission, but we did not include the data at that time because we felt it was necessary to reproduce the results before drawing conclusions. We have now confidently established that IL-13Rα2 CAR-T cells armored with IL-12 and DR-18 can indeed control heterogenous tumors, thus addressing a key challenge in the treatment of glioblastoma.

In our constructs, the stoichiometric ratio of the two cytokines is fixed because the two cytokines are co-expressed in a bicistronic cassette. However, there can indeed be donor-to-donor and batch-to-batch variability in CAR-T cell manufacturing, resulting in differences in cytokine production levels. In our animal studies, a new batch of CAR-T cells is produced for each study—similar to the fact that CAR-T cells are manufactured for each individual patient—and we have observed reproducible outcomes in our mouse studies. Therefore, we believe that our engineering approach is robust against the level of biological variability that is expected in cell manufacturing.

There were also comments on the topic of safety and toxicity, including:

- ***NFAT regulated cytokine expression has been tried in the clinic before. This is a leaky promoter and not specific to activated T cells. Potential systemic toxicity is a major concern.***
- ***More info and data on how cytokines might be tunable to prevent toxicity would be appreciated.***

The NFAT promoter is indeed leaky, meaning it allows low levels of gene expression even when the T cell is not activated. However, we have demonstrated through multiple *in vivo* studies using different tumor models that we are able to achieve acceptable safety profiles. Importantly, we have demonstrated that by combining CAR-12.DR18 and CAR-αVEGF T cells, we can significantly and reproducibly reduce the toxicity that is attributable to IL-12 and IL-18/DR-18 secretion. This is a novel finding not previously known through literature. For clinical translation, we have also incorporated additional measures to maximize safety, including using relatively small, split doses and not including lymphodepletion in our clinical protocol.

Finally, there were comments on the topic of product characterization, including:

- ***Product specific attributes, such as expression of the transgenes, expression per unit copy number, and percent cells expressed are limited in their description and/or are described as planned to be developed.***
- ***Product specific testing or the rLV to support controlled transduction with target expression profiles is limited in its descriptions.***

We acknowledge that due to space constraints we did not provide detailed characterization data on the preclinical products discussed in the proposal. However, our team has extensive experience with manufacturing and characterizing clinical-grade CAR-T cell products for an ongoing clinical trial (NCT04007029; Larson et al., Cancer Discovery, 2023). Furthermore, the application details new assays that will be developed and incorporated for the accelerated manufacturing process proposed for this armored CAR-T cell product. Finally, in-depth product-

release testing for the lentivirus will be performed following FDA-reviewed standard operating procedures by the Cincinnati Children's Hospital Medical Center, which will produce the clinical-grade lentivirus for this project.

Glioblastoma is a significant unmet medical need with no effective treatment options at this time. The armored CAR-T cell therapy described above addresses key challenges associated with glioblastoma, and our experienced team stands ready to bring a new, much-needed therapy to patients in California. We thank you again for your service, and please do not hesitate to reach out if you need any additional information.

Sincerely,

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