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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 TRAN1-11555 proposal, which has been recommended for funding by the Grants 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 bispecific chimeric antigen receptor (CAR)-T cell therapy that simultaneously targets BCMA and CS1, two antigens found on multiple myeloma cells. We thank the GWG for recognizing the importance of this study and for their many supportive comments, and I would also like to address some of their specific inquiries and concerns below. (Comments from the GWG are shown in italics.)

The major unmet need in the disease is the availability of therapies capable of producing prolonged remissions, especially in advanced patients. There is also a need for broadly applicable curative therapies. It is possible that the proposed approach can meet the first unmet need, but unlikely to address the second.

As the GWG reviewers noted, simultaneously targeting BCMA and CS1 has the potential to significantly increase durability of response by preventing tumor relapse due to antigen loss. Regarding broad applicability, as discussed in the Scientific Rationale section of our application, CS1 is present in 90%–97% of multiple myeloma (MM) cells, making CS1 a broadly applicable antigen for MM treatment. Single-input CAR-T cells that target BCMA alone have shown promising clinical efficacy, but BCMA expression on MM cells is far from uniform. By combining BCMA and CS1, we can broaden the eligible patient population to include those whose tumors have low or no BCMA expression, and increase the probability of both initial and durable response. Indeed, Figure 6 in our application shows *in vivo* data indicating that the BCMA/CS1 bispecific CAR-T cells significantly outperform single-input BCMA CAR-T cells in treating tumors with heterogeneous antigen expression.

- *There are some doubts about whether CS1 is a target that will likely improve efficacy.*
- *Antigen loss of CS1 may also occur.*
- *It is unclear whether CS1 is a useful target along with BCMA.*

Based on the widespread expression of CS1 on patient-derived MM samples and the *in vivo* data shown in Figure 6 of our application, we believe there is strong evidence that CS1 will indeed improve efficacy as a combination with BCMA, because the bispecific CAR can efficiently

target MM tumors even if some tumor cells have reduced or completely lost BCMA expression. It is true that loss of CS1 may also occur. However, the probability of losing both BCMA and CS1 to the extent that the tumor becomes undetectable by the bispecific CAR-T cells is substantially lower than the probability of losing BCMA alone. In fact, analysis of residual tumors recovered from animals shown in Figure 6 indicated that BCMA loss was far more common than CS1 loss under selective pressure exerted by CAR-T cell therapy (residual tumor analysis data not included in application due to space limitations; manuscript describing these results is currently under peer review). Therefore, the BCMA/CS1 bispecific CAR design allows us to retain the proven clinical efficacy of the BCMA CAR while broadening applicability and increasing therapeutic durability with the addition of CS1 targeting.

- *The available data does not support enrichment for T memory/stem cells as beneficial for CAR-T efficacy (bulk T-cells will contain requisite subsets).*
- *CD62L fractionation is not necessary and adds complexity.*

A bulk T-cell population will indeed contain some naïve/memory T cells, and enrichment for CD62L+ cells does increase manufacturing complexity. However, it is important to note that the relative proportion of different T-cell subtypes impacts the overall performance of a T cell product, as the different subtypes influence each other's relative growth and effector-function output. Therefore, even though bulk T cells would contain a fraction of naïve/memory T cells, a specifically enriched naïve/memory T cell population can significantly outperform bulk T cells in the therapeutic setting. Indeed, our *in vitro* and *in vivo* studies have provided compelling evidence that specific enrichment of naïve/memory T cells significantly increases the anti-tumor efficacy of the resulting cell product (please see Figures 4 and 5 of the application). Specifically, naïve/memory T cells showed substantially greater cytokine production, tumor-cell killing, and T-cell proliferation compared to bulk CD8+ T cells (Figure 4). Furthermore, naïve/memory T cells showed more rapid and durable tumor clearance than both bulk CD3+ and bulk CD8+ T cells *in vivo* (Figure 5). We would also like to note that we have established a robust GMP manufacturing process for naïve/memory T cells. This process, which includes CD62L enrichment, is now in use for a phase-I trial currently open for enrollment at UCLA, evaluating CD19/CD20 bispecific CAR-T cells as a treatment for relapsed/refractory B-cell lymphoma and leukemia. Therefore, we are confident that the incorporation of CD62L enrichment will not pose any significant barrier to cell manufacturing in the clinical setting.

The data regarding the fratricide experiment is confusing based on the proposed mechanism for how this product works. The results are unexpected and thus more investigation may be needed.

We apologize for not explaining the data regarding fratricide sufficiently clearly. CS1 is an antigen present not only on MM cells but also on a subset of T cells, particularly CD8+ T cells. However, CS1 expression level is substantially lower on T cells compared to MM cells. In developing the BCMA/CS1 bispecific CAR, we were cognizant that the CAR must

be effective against MM cells but not trigger toxicity toward T cells. This aim necessitated a calibration of CAR sensitivity toward different levels of CS1 expression. Results shown in Figure 7 of our application confirmed that our final CAR designs were indeed non-toxic to T cells, and human T cells expressing our bispecific CARs were fully capable of efficient *in vitro* expansion.

The rationale for the BCMA CAR-T cell and anti-PD1 antibody combination treatment in the proposed preclinical testing is unclear.

As part of the experiment shown in Figure 6, we harvested tumor cells from animals at the time of sacrifice and performed extensive analysis of their phenotypes. These results were not shown in the application due to space limitations. An important finding was that most animals still had residual CAR-T cells at the time of sacrifice, but those T cells had uniformly high expression of PD1, which is a marker of T-cell exhaustion. This observation, combined with the observation that the recovered tumor cells could still be killed by freshly cultured CAR-T cells, suggested that the adoptively transferred T cells had become functionally exhausted *in vivo*. To identify a solution to this obstacle, we performed a subsequent (and still ongoing) animal study to evaluate the treatment of MM tumor-bearing mice with BCMA/CS1 CAR-T cells alone or in combination with anti-PD1 antibody. Early results showed that co-administration of anti-PD1 accelerated the initial rate of tumor clearance compared to CAR-T cell alone. We are currently evaluating whether the beneficial effect of anti-PD1 is best achieved through short-term administration at early time points or sustained administration over long periods of time.

The CRS modeling study is not necessary.

Cytokine release syndrome (CRS) is a common and potentially fatal side effect associated with CAR-T cell therapy. It results from immune overstimulation triggered by T cells interacting with tumor cells, and can lead to systemic toxicity due to positive feedback loops involving inflammatory cytokines produced by both CAR-T cells and native immune cells. In theory, bispecific CAR-T cells—due to their ability to recognize more tumor cells than single-input CAR-T cells—could pose an increased risk of severe CRS. This is our rationale for proposing the CRS modeling study to compare potential toxicity of our bispecific CAR-T cells against clinically tested BCMA single-input CAR-T cells.

We acknowledge that CRS modeling has not been a required part of Investigational New Drug (IND) applications for CAR-T cell therapy to date. This, however, is largely due to the fact that CRS cannot be meaningfully evaluated *in vitro*, and no adequate animal model for CRS had been reported until 2018. To ensure the highest standard in our research, I personally visited the research group at the San Raffaele Hospital Scientific Institute in Milan, Italy to learn the appropriate animal model, and my laboratory has successfully established a humanized mouse model that recapitulates CRS-associated toxicities. We believe the proposed *in vivo* studies will provide valuable information that will either confirm the lack of toxicity of our constructs or forestall patient suffering and unnecessary clinical costs if any unexpected toxicities were to be detected.

Additional expertise in the biology of myeloma would be helpful.

Dr. Sarah M. Larson, the clinical PI for the proposed IND on our BCMA/CS1 bispecific CAR-T cell therapy trial, is a specialist in multiple myeloma and has extensive clinical as well as research experience on this disease. Furthermore, Dr. Larson has played leadership roles in multiple CAR-T cell therapy trials at UCLA. Dr. Larson and I have collaborated closely on a successful IND application focusing on CD19/CD20 CAR-T cell therapy for B-cell lymphoma and leukemia, and Dr. Larson will similarly serve as the clinical lead for our effort on the BCMA/CS1-targeted therapy.

In response to GWG reviewer comments, we have additionally engaged Dr. Alan K. Lichtenstein as a second MM expert for this important project. Dr. Lichtenstein has over 30 years of experience in studying the biology and molecular targeting of multiple myeloma, and has made pivotal discoveries on the impact of the PI3K/AKT/mTOR pathway and its therapeutic modulation on multiple myeloma (please see attached biosketch). Dr. Lichtenstein has also published extensively on apoptotic pathway molecules and their regulation in myeloma. Dr. Lichtenstein will serve as a valuable source of advice in our proposed project to treat multiple myeloma with BCMA/CS1 bispecific CAR-T cell therapy.

I will also be present at CIRM's Oakland office for the board meeting on October 31, 2019. Please do not hesitate to reach out if you need any additional information.

Sincerely,

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