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DEPT OF MICROBIOLOGY, IMMUNOLOGY & MOLECULAR GENETICS
DAVID GEFFEN SCHOOL OF MEDICINE AT UCLA
COLLEGE OF LETTERS & SCIENCE
BASIC BIOMEDICAL SCIENCES
LOS ANGELES, CA 90095-7363
PHONE: 310-825-2816

July 19, 2019

Dear CIRM Application Review Subcommittee,

Thank you for your service on the CIRM Governing Board.

I am the principal investigator of a TRAN1 application (TRAN1-11555) recommended for funding by the Grants Working Group (GWG) in the most recent round of reviews. Specifically, 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 an FDA-approved phase-I trial 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 clearly explaining the data regarding fratricide. 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 rate of tumor clearance compared to CAR-T cell alone. We are currently evaluating whether anti-PD1 administration would also extend overall survival and increases resistance to tumor re-challenge compared to single-agent CAR-T cell therapy.

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 July 24, 2019. Please do not hesitate to reach out if you need any additional information.

Sincerely,

Yvonne Y. Chen
Associate Professor
Department of Microbiology, Immunology & Molecular Genetics
University of California, Los Angeles
Email: yvonne.chen@ucla.edu
Phone: 310-825-2816

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Lichtenstein, Alan K.

eRA COMMONS USER NAME (credential, e.g., agency login): BBRIPI9

POSITION TITLE: Professor of Medicine in Residence, UCLA

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brooklyn College, Brooklyn, New York	BA	06/1968	Biology
George Washington University, Wash. D.C.	MD	06/1972	Medicine
U. of Pittsburgh, Pittsburgh, PA	Residency	06/1975	Internal Medicine
USC School of Medicine	Fellowship	06/1977	Hematology
USC School of Medicine	Fellowship	06/1978	Oncology
UCLA School of Medicine	Post-doc	06/1980	Tumor Immunology

A.Personal Statement

I have been studying the molecular biology and molecular targeting of multiple myeloma for over 30 years. My group has been at the forefront of many advances in myeloma during this time. We were one of the first to demonstrate an anti-apoptotic effect of IL-6 and IGF-1 on myeloma cells, the ameliorative effect of bis-phosphonates in patients, the importance of BCL-XL, BAX and BCL-2 expression in myeloma, the constitutive signaling in MM cells harboring RAS mutations, the potential for targeting aurora kinases and cox-2 in myeloma, effect of autophagy on myeloma cell viability, the key role of hepcidin in mediating the anemia of myeloma, the control of protein translation in myeloma, the important role of IRES-dependent myc translation in myeloma the importance of targeting the PI3K-AKT-mTOR pathway in this disease and the role of the mTOR inhibitor, DEPTOR, in myeloma. As DEPTOR is an mTOR binding protein, the current proposal is an extension of prior involvement with mTOR in myeloma. In addition, I am the lead clinician for all myeloma patients within a huge VA catchement area and, thus, encounter many new patients each year which will facilitate study of primary samples. I also have a significant experience with myeloma murine models which can be of assistance in this project.

B.Positions and Honors

Positions

1977-1978- Clinical Instructor in Medicine and Clinical Cancer Training Grant Fellow, USC Medical School
1978-1980- Post-doctoral Scholar, Department of Microbiology & Immunology, UCLA Medical School
1980-1987-Assistant Professor of Medicine in residence, UCLA Medical School
1987-1993-Associate Professor of Medicine in residence, UCLA Medical School
1984-2007-Chief of Hematology-Oncology, UCLA-VA West LA Medical Center
1993-present-Professor of Medicine in Residence, UCLA Medical School
1995-1996- Visiting Research Scientist, La Jolla Cancer Research Foundation (currently Burnham Institute),
2007-present-Assistant Chief of Hematology-Oncology, UCLA-VA West LA Medical Center

Honors and other experience-

1999-2001- member, NIH Special emphasis panel study section for PO1 submissions in “Monoclonal gammopathies”

2007-2014- Permanent Member, NIH study Section “Basic Mechanisms of Cancer Therapy”

Invited lecture and visiting professor:
Radiation Effects Research Foundation, Hiroshima, Japan, 6/1988
H Lee Moffitt Cancer Center, Tampa Florida, 11/1998
C.U.N.Y. Downstate Medical School, Brooklyn NY, 2/1999
University of Indiana Medical School and Cancer Center, 3/2006
Winship Cancer Institute, Emory University, Atlanta Georgia, 4/2009
Louisiana State University Medical School, Shreeveport, LA, 3/2010

Senior Faculty Research Award, Multiple Myeloma Research Foundation, 7/2000-6/2001, 7/2012-6/2013 and 9/2015-8/2017

Editorial Staff of BLOOD, 1/1999-2004

Journal Reviewer for Cancer Research, Immunology Letters, International Journal of Cancer, BLOOD, Journal of Immunology, Oncogene, American Journal of Pathology, Clinical Cancer Research, Journal of Biological Chemistry

Grant Reviewer for NIH, VA Merit Review Program, Israeli Cancer Research FDN, Canadian Cancer Society, and Cancer Federation of Flanders

C.Contribution to Science

1.My group was the first to identify the PI3K/AKT/mTOR pathway as being important in viability and proliferation of multiple myeloma cells. We showed that the pathway was stimulated by myeloma growth factors and it regulated responses to various new therapies. Subsequently, this early seminal work formed the basis for the development of new therapies for myeloma including AKT and mTOR inhibitors. Recently, we identified a myeloma-specific pathway which explained the ability of the mTOR-binding protein DEPTOR to control myeloma viability.

a.Tu, Y, Gardner, A, **Lichtenstein, A,** “The phosphatidylinositol 3-kinase/AKT kinase pathway in multiple myeloma plasma cells: Roles in cytokine-dependent survival and proliferative responses, Cancer Research 60:6763-6770, 2000

b. Hsu, J., Shi, Y., Krajewski, S., Renner, S., Fisher, M., Reed, JC., Franke, T., **Lichtenstein, A.** The AKT kinase is activated in multiple myeloma tumor cells. BLOOD 98:2853-2856, 2001

c. Shi, Y, Hsu, J-h, Hu, L, Gera, J, **Lichtenstein, A.** Signal pathways involved in activation of p70S6K and phosphorylation of 4E-BP1 following exposure of multiple myeloma tumor cells to IL-6. J of Biol Chem 277:15712-15720, 2002

d. Yang, Y, Bardeleben C, Frost P, Hoang B, Shi Y, Finn R, Gera J Lichtenstein, A. DEPTOR is linked to a TORC1-p21 survival/proliferation pathway in multiple myeloma cells. Genes & Cancer 5:407-419, 2014

e. Shi Y, Daniels-Wells TR, Frost P, Lee J, Finn R, Bardeleben C, Penichet M, Jung M, Gera J & **Lichtenstein**, A. Cytotoxic properties of a DEPTOR-mTOR inihibitor in multiple myeloma cells. Cancer Res 2016; 76:5822-5831

f. Lee J, Shi Y, Vega M, Yang Y, Gera J, Jung ME, **Lichtenstein A**. Structure-activity relationship study of small molecule inhibitors of the DEPTOR-mTOR interaction. Bioorg & Medicinal Chem Letters2017; 27:4714-4724

2. We have also investigated the important role of internal ribosome entry site (IRES) activity in cancer cells as a fail safe mechanism for protein translation. This work has demonstrated the role of the IRES in responses to mTOR inhibitors and depressed cap-dependent translation. It also provides an explanation for why AKT activity predicts for sensitivity to mTOR inhibitors. It has also opened the possibility of targeting IRES-trans-acting factors such as hnRNPA1.

a.Shi, Y, Frost PJ, Hoang, B, Sharma, S, Gera JF and **Lichtenstein, A.** IL-6-induced stimulation of c-myc translation in multiple myeloma cells is mediated by myc internal ribosome entry site function and the RNA-binding protein, hnRNP A1. Cancer Research 68:10215-10222, 2008

b.Shi, Y, Frost P, Hpoang, B, Benavides A, Gera J, & **Lichtenstein A.** IL-6-enhancement of c-myc translation in multiple myeloma cells: Critical role of cytoplasmic localization of the RNA-binding protein hnRNP A1. J Biol Chemistry, 286:67-78, 2011

c.Gera J and **Lichtenstein, A**.The mammalian target of rapamycin pathway as a therapeutic target in multiple myeloma. Leuk Lymphoma. 2011 52:1857-66

d. Shi, Y, Yang Y, Hoang B, Bardeleben C, Holmes B, Gera J and **Lichtenstein A.** Therapeutic potential of targeting IRES-dependent c-myc translation in multiple myeloma cells during ER stress. Oncogene, 2015; 35:1015-1024.

ENTIRE PUBLISHED CV: http://www.ncbi.nlm.nih.gov/sites/myncbi/1XWk6yWSae7k-/bibliography/47404224/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

RO1 CA 111448-06 Lichtenstein (PI) 06/01/05-5/31/19
Sensitivity of multiple myeloma cells to mTOR inhibitors
The goal of this study is to investigate how AKT regulates sensitivity to mTOR inhibitors and how therapeutic targeting of TORC2 can be effective in multiple myeloma
Role: PI

RO1 CA214246-01 Lichtenstein (PI) 03/01/17-2/28/22
Regulation of c-myc translation by hnRNP A1:role in multiple myeloma tumor responses
The goal of this study is to investigate the role of hnRNP A1 in regulating mRNA translational efficiency in multiple myeloma
Role: PI

RO1 CA211562 Lichtenstein (PI) 7/01/17-6/30/22
Targeting DEPTOR in multiple myeloma
The goal of this study is to evaluate inhibitors that prevent binding of DEPTOR to mTOR as novel therapeutics in multiple myeloma.
Role: PI

VA Merit Review Grant Lichtenstein (PI) 10/01/16-9/30/20
Targeting DEPTOR in multiple myeloma
The goal of this study is to evaluate inhibitors that prevent binding of DEPTOR to mTOR as novel therapeutics in multiple myeloma. The grant funding was terminated 7/1/17 because of overlap with NIH grant CA211562
Role: PI

Completed Research Support

Multiple Myeloma Research Foundation senior investigator award Lichtenstein (PI) 9/1/2015-8/31/2017
Regulation of c-myc translation by hnRNPA1
The goal of this study is to investigate the translation of c-myc in myeloma cells during ER stress
Role: PI

Multiple Myeloma Res Foundation senior investigator award Lichtenstein (PI) 7/1/2011-8/31/2013
Targeting protein:protein interactions in myeloma TORC complexes

The goal of this project was to investigate the role of different components of TORC1 and 2 complexes that promote myeloma cell survival
Role: PI

R21 CA168491 Lichtenstein (PI) 11/01/12-10/31/15 (1 yr no-cost extension)
Targeting DEPTOR in multiple myeloma
The goal of this study is to evaluate inhibitors that prevent binding of DEPTOR to mTOR as novel therapeutics in multiple myeloma. It will be terminated on 10/31/15

RO1 CA 96920 Lichtenstein (PI) 06/01/02-05/31/07
The AKT-mTOR pathway in multiple myeloma
The goal of this study was to evaluate AKT/mTOR signaling in multiple myeloma cells
Role: PI

Merit Review grant of the Veteran's Administration Lichtenstein (PI) 04/01/04-03/31/09
Targeting mTOR in mutant RAS-containing multiple myeloma cells
Role: PI

RO1 CA132778-01 Lichtenstein (PI) 06/01/09-5/31/15 (1 yr no-cost extension)
Regulation of c-myc translation by hnRNP A1:role in multiple myeloma tumor responses
The goal of this study is to investigate the role of hnRNP A1 in regulating mRNA translational efficiency in multiple myeloma
Role: Joint PI with Joe Gera

VA Merit Review Grant Lichtenstein (PI) 10/01/2012-09/30/2016
Targeting TORC2 in multiple myeloma
The goal of the study is to inhibit interactions between TORC2 components to inhibit AKT and SGK activation
Role: PI