Cell & Genome Engineering Shared Resource

Director: Olin Liang, Ph.D.

LEGORRETA CANCER CENTER
BROWN UNIVERSITY

Overview

Since its inception in October 2019, the Cell & Genome Engineering Shared Resource (CGE-SR) has served numerous investigators from the Warren Alpert Medical School of Brown University, Rhode Island Hospital, Women & Infants Hospital, the Providence VA Medical Center, 15 other U.S. and 7 international academic centers, as well as 6 U.S. biotech companies. Our mission is to share cell and genome engineering technologies for easy access and efficient use, with a focus on enhancing the competitiveness of Rhode Island investigators to secure federal research funding. The timely development of COVID-19 variant pseudoviruses by the CGE-SR was a prime example of our innovation. To genetically engineer oncolytic adenovirus encoding bi-specific T cell engagers (BiTEs) is cutting-edge, and the novel viro-immunotherapy combining CAR T cells has the potential to bring new hope in cancer treatments. The CGE-SR strives to make a positive impact as a catalyst on basic and translational cancer research to improve human health.

Key Services

- Lentivirus and retrovirus packaging
- Lentivirus-mediated stable gene over-expression in cells
- Lentivirus-mediated stable CRISPR/Cas9 gene knock-out in cells
- Lentivirus-mediated stable reporter cell lines with fluorescence protein, luciferase and/or antibiotic resistance
- Third generation human and mouse chimeric antigen receptor (CAR) T cells targeting solid tumor and senescent cells
- Oncolytic adenoviruses encoding bi-specific T cell engagers (Ad5-Δ24RGD-BiTEs and hybrid fiber Ad5/35-Δ24-BiTEs)
- Reprograming human CD34+ cells into induced pluripotent stem cells (iPSCs)
- SARS-CoV-2 variant pseudoviruses and other COVID-19 reagents

Value Added

Our proximity to and close interactions with the Legorreta Cancer Center investigators and four Ph.D. Graduate Programs at Brown Medical School (Pathobiology, Molecular Cell Biology, Neuroscience, and Therapeutic Sciences) have provided us with a sizable and steady user base in the long-term. Our Ph.D. level staff scientists directly interact in person with the investigators, and as such we have a distinct advantage over outside vendors in providing individualized customer experience during all stages of a project.

Major Technologies

- Third generation lentivirus with VSV-G pseudotyping, bi-cistronic expression of target and reporter from a single mRNA transcript
- Third generation human and mouse CAR T cells
- Oncolytic adenoviruses (Ad5 and Ad5/35) encoding BiTEs
- Lentivirus-based COVID-19 variant pseudoviruses

Major Personnel

- Eui-Young So, Ph.D., Technical Director/Manager
- Jamie Jeong, Ph.D., Research Scientist
- Moon Jung Choi, Ph.D., Research Scientist
- Bedia Akosman, M.D., Ph.D., Research Scientist

Recent Example of Scientific Impact

Glioblastoma Spheroid Killing by CAR T Cells

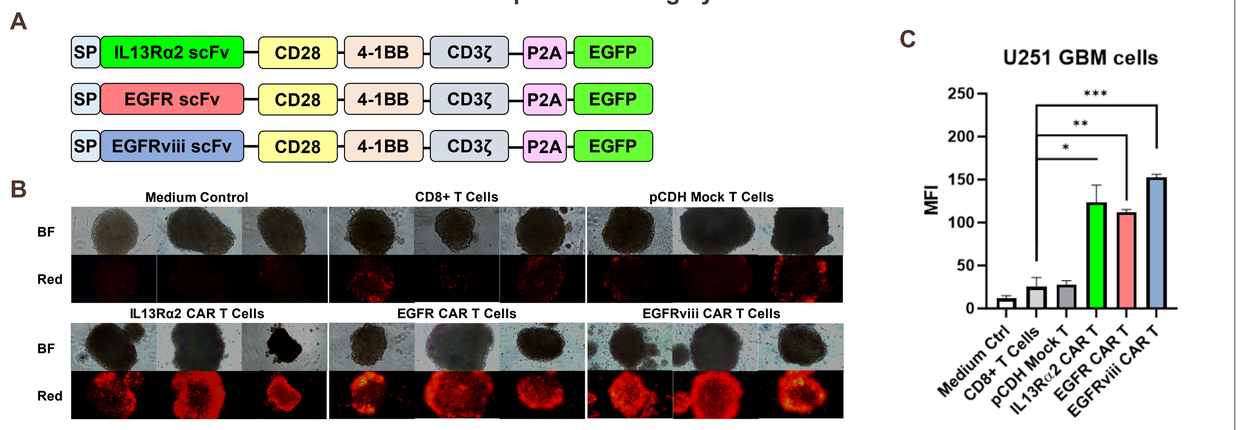

Figure 1. (A) Three 3rd generation lentiviral CAR T vectors targeting GBM tumor antigens IL13Rα2, EGFR and EGFRviii were made. (B) Spheroids were made from GBM cell line U251 using a 24 h hanging drop method in a 96-well-plate. Spheroids and T cells were co-cultured for 60 h followed by propidium iodine (PI) staining of dead cells. Briefly, spheroids were carefully washed 3 times with DPBS and incubated with 20 μg/ml PI at 37°C for 10 min. Spheroids were then carefully washed 3 times again with DPBS followed by visualization of dead GBM cells under red fluorescence microscope. (C) Mean Fluorescence Intensity (MFI) was calculated by using the NIH ImageJ software. MFI data are mean ± SEM. *** = P < 0.001, ** = P < 0.01, * = P < 0.05, Student's t-test, GraphPad Prism 10. BF: bright field..

Engineering Oncolytic Adenovirus Encoding BiTEs

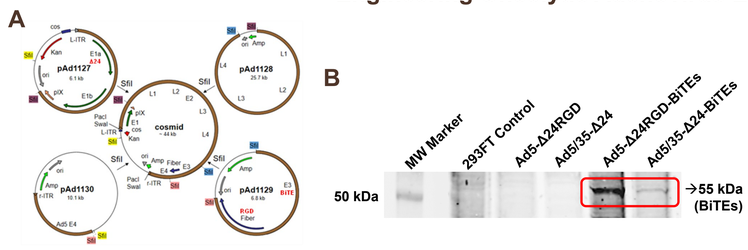

Figure 2. (A) An overview of oncolytic Ad5-Δ24RGD-BiTEs cloning. (B) Western Blot detection of BiTE expression by adenoviral vector Ad5-Δ24RGD-BiTEs but not Ad5-Δ24RGD control, and by Ad5/35-Δ24-BiTEs but not Ad5/35-Δ24 control. The cDNA sequence for anti-hCD3ε mAb OKT3 scFv was provided by Dr. Balyasnikova (Northwestern University). The cDNA for IL13Rα2 scFv was obtained from our CAR T vector IL13Rα2-scFv-h(CD28-41BB-CD3ζ)-2A-EGFP in the lab.

Reprogramming Human CD34+ Cells to iPSCs

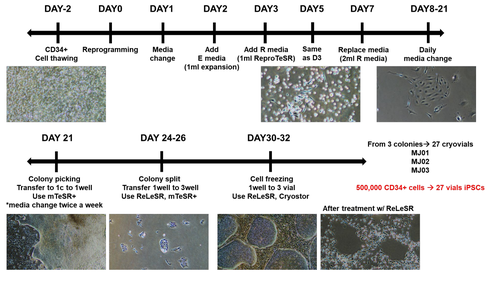

Figure 3. Our optimized workflow to reprogram human bone marrow-derived CD34+ cells into iPSCs. Expression of pluripotent stem cell markers Nanog, Oct4, SOX2, c-Myc, KLF4 and LIN2A by the iPSCs was confirmed by Western Blots (data not shown).

Development of COVID-19 Variant Pseudoviruses

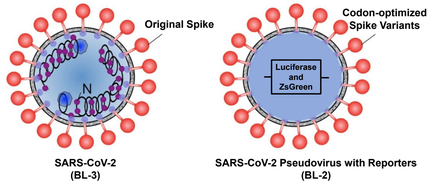

Figure 4. In response to the COVID-19 pandemic, we have rapidly developed a series of BSL-2 level SARS-CoV-2 pseudoviruses including the most recent highly infectious Delta and Omicron variants. Our SARS-CoV-2 pseudoviruses have drawn national attention and were requested by COVID-19 investigators from around the country. Thus, the CGE-SR has made a meaningful contribution to the global effort against the COVID-19 pandemic.

Current User Base

- Legorreta Cancer Center Cancer Biology Program
- Legorreta Cancer Center Cancer Therapeutic Program

- Molecular Microbiology and Immunology, Brown University
- Molecular Cell Biology and Biochemistry, Brown University
- Molecular Pharmacology and Physiology, Brown University
- Neuroscience, Brown University
- Pathology and Laboratory Medicine, Brown University
- Pathology and Laboratory Medicine, Rhode Island Hospital
- Orthopaedics, Rhode Island Hospital
- Infectious Diseases, Rhode Island Hospital
- Pathology and Laboratory Medicine, Providence VA Med. Center
- The Vascular Biology Laboratory, Providence VA Med. Center
- Perinatal Pathology, Women & Infants Hospital
- Pediatrics, Women & Infants Hospital

- Fred Hutchinson Cancer Research Center, Seattle, WA
- The Scripps Research Institute, La Jolla, CA
- Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Massachusetts General Hospital, Boston, MA
- Brigham & Women's Hospital, Boston, MA
- The National Institute of Allergy and Infectious Diseases, Bethesda, MD
- Emory University, Atlanta, GA
- New York University Langone Hospital, Mineola, NY
- Northwestern University, Chicago, IL
- University of Houston, Houston, TX

- McGill University, Montreal, Canada
- University of Calgary, Calgary, Canada
- University of Paris, Paris, France
- University of Tuebingen, Tuebingen, Germany
- Slovak Academy of Sciences, Bratislava, Slovakia
- University of South Australia, Adelaide, Australia
- Sungkyunkwan University, Suwon, South Korea.

Key Publications

- Walters, R. et al., *Circulation.* 2023,147(21):1606-1621.
- Petersen, M. et al., *Mol. Oncol.* 2023, doi: 10.1002/1878-0261.
- Jeong, E.-M. et al., *Cardiovasc. Res.* 2022, 118(16):3211-3224.
- Treaba, D. O. et al., *Br. J. Haematol.* 2022 doi: 10.1111/bjh.18513.
- Song, J. et al., *PLoS Biol.* 2022, 20(10): e3001805.
- Bigdelou, B. et al., *Front. Immunol.* 2022, 13:890517.
- Huntington, K. E. et al., *Pharmaceuticals* 2022, 15(5):618.
- Kamle, S. et al., *JCI Insight* 2021, 6(21):e148749.
- So, E.-Y. et al., *Am. J. Physiol. Cell Physiol.* 2021, 321(3):569.
- So, E.-Y. et al., *Aging* 2020, 12(24):25939-25955.
- Zhou, L. et al., *Oncotarget* 2020, 11(46):4201-4223.
- Klinger, J. R. et al., *Am. J. Respir. Cell Mol. Biol.* 2020, 62(5):577.
- So, E.-Y. et al., *J. Cell. Physiol.* 2020, 235(2):1425-1437.

Future Plans

- To further support basic cancer research with sophisticated genome editing and generation of iPSCs from patient samples.
- To align with translational cancer research with experimental CAR T cells targeting a variety of tumor-associated antigens.
- To interact with other LCC shared resources.
- To further increase user base.
- To work with LCC administration on fee structure.
- To become a self-sustainable research service facility.