Assessing large-scale CAR-T cell cytotoxicity ex vivo using 2-D and 3-D multimodal imaging techniques for industrial standards

Abstract #3187

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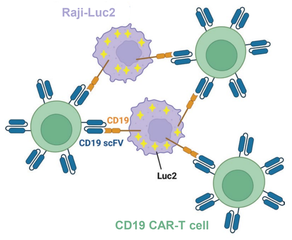
Abstract

CAR-T cell therapy is a revolutionary and highly effective treatment for hematologic malignancies, with rapid advancements particularly in applications to solid tumors. The development and refinement of this innovative therapy rely on efficient ex vivo assays to evaluate CAR-T cell cytotoxicity. Furthermore, advancement in the treatment of solid tumors using CAR-T cell therapy requires the development of ex vivo assays specifically using solid tumor models. Here, we report the development of luciferase reporter cancer cell lines that endogenously express high levels of key CAR-T target antigens such as CD19, CD20, BCMA, or HER2. Using targeted and mock-engineered CAR-T cells, we evaluated cytotoxicity against these reporter cells via a bioluminescence assay and phase contrast/fluorescence live imaging assays. Our multimodal imaging approach demonstrated significantly higher cancer cell killing by targeted CAR-T cells compared to mock CAR-T cells. To enhance our live imaging capabilities and enable assays for CAR-T cell infiltration into 3-D tumor models, we engineered GFP-Luc2 dual reporter cancer cell lines. Using 3-D confocal live imaging, we observed reduced spheroid size and decreased luciferase signal in Raji-GFP-Luc2 spheroids co-cultured with CD19 CAR-T cells versus mock CAR-T controls, indicating superior cancer cell killing when using targeted CAR-T cells. Upon embedding Raji-GFP-Luc2 spheroids in 3-D matrices to mimic a more physiologically relevant tumor microenvironment, we captured CAR-T cell infiltration into spheroids by time-lapse 3-D imaging. Our results highlight the utility of combining luciferase bioluminescence and live fluorescence imaging to assay CAR-T cell cytotoxicity and infiltration in 2-D and 3-D co-culture models. The luciferase assay provides sensitive, quantitative measurements, while live fluorescence imaging reveals the spatial and temporal dynamics of CAR-T cell–cancer cell interactions. These scalable assays hold significant potential for large-scale industrial applications in CAR-T cell therapy development.

Background

In CAR-T cell therapy, a cancer patient's T cells are isolated from their blood and transduced with a CAR (chimeric antigen receptor) construct that codes for a receptor that targets the patient's T cells to the cancer cells. For example, the lymphoblast line Raji (ATCC® CCL-86™) expresses endogenously high levels of CD19, an FDA-approved CAR-T target antigen. To develop ex vivo CAR-T cell cytotoxicity assays, we engineered Raji cells to express a transgene encoding the firefly luciferase transgene. CD19 CAR-T cells should target endogenously expressed CD19 on the surface of Raji-Luc2 (ATCC® CCL-86-Luc2™) cells, resulting in Raji-Luc2 cell death and loss of luciferase reporter expression. Similarly, Daudi-Luc2 (ATCC® CCL-213-Luc2™), BT-474-Luc2 (ATCC® HTB-20-Luc2™), and NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-Luc2™) are targets for CD20, HER2, or BCMA CAR-T cells, respectively. Figure created with Biorender.com.



Results

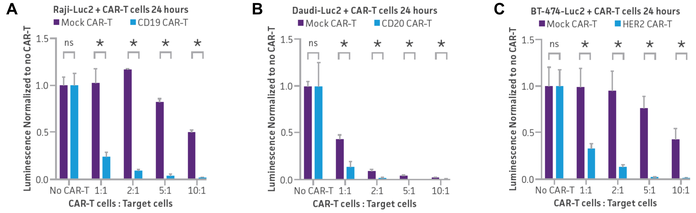


Figure 1: CAR-T cell in vitro killing assay of luciferase reporter lines in 2-D co-culture using bioluminescence. (A) Raji-Luc2 (ATCC® CCL-86-Luc2™), (B) Daudi-Luc2 (ATCC® CCL-213-Luc2™), or (C) BT-474-Luc2 (ATCC® HTB-20-Luc2™) cells were co-cultured with donor-matched mock (purple) or targeting (blue) CAR-T cells for 24 hours using varying ratios of CAR-T cells to target cells. Luciferase expression was detected using the Bright-Glo system (Promega Corporation). ns = not significant, * = p < 0.05, unpaired t-test. Error bars represent the standard deviation of three biological replicates.

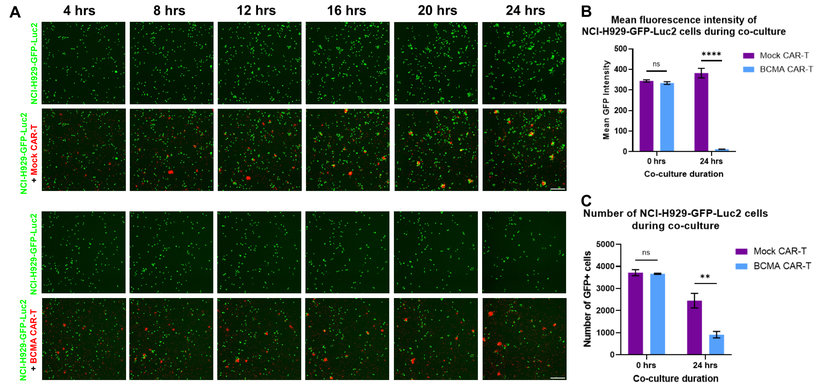


Figure 2: CAR-T cell in vitro killing assay of NCI-H929-GFP-Luc2 dual reporter cells in 2-D co-culture using live-cell imaging. (A) Mock (top) or BCMA (bottom) CAR-T cells (ProMab) were stained with Vybrant (Invitrogen) DiD dye and co-cultured with NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-Luc2™) cells at a 5:1 ratio of CAR-T cells to target cells. Images were acquired every 2 hours for 24 hours using the Leica Mica microscope. NCI-H929-GFP-Luc2 cells are in green, dyed CAR-T cells are in red. Scale bars, 200 µm. (B) Mean GFP fluorescence intensity or (C) number of GFP+ cells of entire NCI-H929-GFP-Luc2/CAR-T co-culture wells quantified at 0 hours and after 24 hours of co-culture with either mock (purple) or BCMA (blue) CAR-T cells at a 10:1 CAR-T cell to target cell ratio. Error bars indicate the standard deviation of three biological replicates. ns = not significant, ** = p < 0.005, **** = p < 0.0001, unpaired t-test.

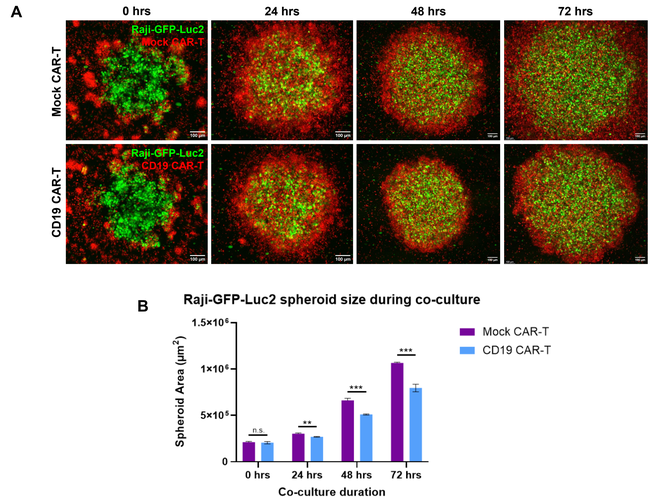


Figure 3: CD19 CAR-T cells infiltrate and kill Raji-GFP-Luc2 spheroids in 3-D co-culture. (A) Raji-GFP-Luc2 (ATCC® CCL-86-GFP-Luc2™) transduced pool cells (green) were formed into spheroids and Vybrant (Invitrogen) DiD-dyed mock or CD19 CAR-T cells (red) were added the next day. 5 µm Z stacks were acquired every 24 hours for 72 hours on the Leica Mica confocal microscope. Maximum projections are shown. Scale bars, 100 µm. (B) Raji-GFP-Luc2 spheroid area during co-culture with mock (purple) or CD19 (blue) CAR-T cells. Spheroid area was measured using the GFP channel only in ImageJ. n.s. = not significant, ** = p < 0.01, *** = p < 0.001. Error bars represent the standard deviation of three biological replicates.

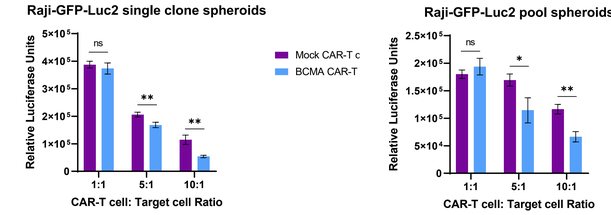


Figure 4: CD19 CAR-T cells kill Raji-GFP-Luc2 spheroids in a dose-dependent manner. Raji-GFP-Luc2 (ATCC® CCL-86-GFP-Luc2™) transduced pool (left) or single clone (right) cells were formed into spheroids and mock (purple) or CD19 (blue) CAR-T cells were added the next day. After 72 hours of co-culture, luciferase expression was detected for each well using the Bright-Glo system (Promega Corporation). * = p < 0.05, ** = p < 0.01, unpaired t-test. Error bars represent the standard deviation of three biological replicates.

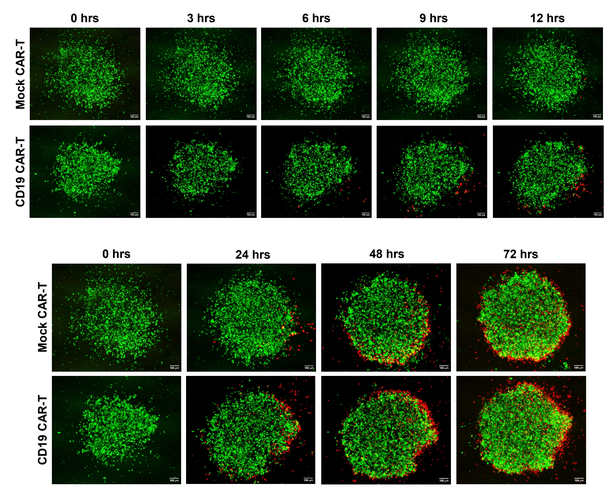


Figure 5: CAR-T cells infiltrate gel-embedded Raji-GFP-Luc2 spheroids within 12 hours. Raji-GFP-Luc2 (ATCC® CCL-86-GFP-Luc2™) transduced pool cells were formed into spheroids and embedded in a BME/collagen gel the next day. Vybrant (Invitrogen) DiD-dyed CAR-T cells were added the day after embedment at a 5:1 CAR-T cell: target cell ratio. 10 µm Z stacks were acquired (A) every 3 hours for 12 hours or (B) every 24 hours for 72 hours using the Leica Mica confocal microscope. Maximum projections are shown. Raji-GFP-Luc2 cells are labeled in green and CAR-T cells are labeled in red. Scale bars, 100 µm.

Conclusions

- CD19-, CD20-, and HER2-targeting CAR-T cells kill Raji-Luc2 (ATCC® CCL-86-Luc2™), Daudi-Luc2 (ATCC® CCL-213-Luc2™), and BT-474-Luc2 (ATCC® HTB-20-Luc2™) cells, respectively, in a dose-dependent manner
- NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-Luc2™) dual reporter cells show a decrease in GFP expression as assayed by live imaging
- CD19 CAR-T cells target and kill Raji-GFP-Luc2 (ATCC® CCL-86-GFP-Luc2™) cells in 3-D co-culture at higher levels compared to mock CAR-T cells
- Raji-GFP-Luc2 (ATCC® CCL-86-GFP-Luc2™) spheroids can be embedded to assay T cell infiltration in a solid tumor model

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