Evaluation of ex vivo CAR-T cell cytotoxicity and infiltration using multimodal 2-D and 3-D imaging approaches

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Poster 3723

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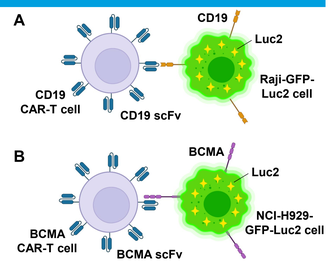
Abstract

Chimeric antigen receptor T (CAR-T) cell therapy has revolutionized cancer treatment, particularly for hematologic malignancies. Beyond CD19 and CD20, BCMA (B-cell maturation antigen)-targeted CAR-T therapies have become a major focus, especially for multiple myeloma. In fact, BCMA is considered one of the most validated targets in plasma cell malignancies. In pursuit of improving the efficacy and number of applications of this therapy, sensitive and robust cytotoxicity assays are required to efficiently evaluate different CAR-T constructs, effector-to-target cell ratios, and immune effector cells ex vivo. Furthermore, as CAR-T cell therapy is now under active investigation for the treatment of solid tumors, there is an urgent need for models that mimic the complexity of 3-D tumor environments. In this study, we engineered two luciferase-GFP dual reporter cancer cell lines, Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) and NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-LUC2™) and demonstrated their use in a streamlined combined bioluminescence and live-imaging assay. Notably, Raji-GFP-Luc2 and NCI-H929-GFP-Luc2 endogenously express high levels of the two FDA-approved CAR-T target antigens CD19 and BCMA, respectively. Using these lines in co-culture with mock and targeting CAR-T cells, we tracked reporter cancer and CAR-T cell interactions by fluorescence live imaging. Fluorescence quantification clearly demonstrated that CAR-T cells killed cancer cells at higher levels as compared to mock controls in both 2- and 3-D co-culture. We then evaluated dose-dependent cancer cell killing by targeting CAR-T cells in 2- and 3-D by luciferase assay. Importantly, we found that quantification of either transgene yields comparative results despite utilizing distinct assays and readouts. Finally, we embedded reporter cancer cell spheroids in 3-D matrices and performed time-lapse imaging of CAR-T cell infiltration. Bioluminescence assay of the embedded spheroids confirmed increased cancer cell killing by targeting CAR-T cells in a solid tumor model. Overall, our dual reporter cancer cell lines are powerful and effective tools for assaying CAR-T cell cytotoxicity. Furthermore, our multimodal imaging platform integrates bioluminescence and live-fluorescence imaging to quantitatively and visually assess CAR-T cell cytotoxicity. With scalability and sensitivity, this assay provides an avenue for standardizing CAR-T cytotoxicity testing and will aid in the expansion and improvement of this promising therapy.

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 encodes a receptor that targets the patient's T cells to the cancer cells. 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 GFP and luciferase transgenes. (A) CD19 CAR-T cells target endogenously expressed CD19 on the surface of Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) cells, resulting in Raji-GFP-Luc2 cell death and loss of expression of both reporters. (B) Similarly, the plasmacytoma line NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-LUC2™) is a target for BCMA CAR-T cells. Figure created with Biorender.com.



Results

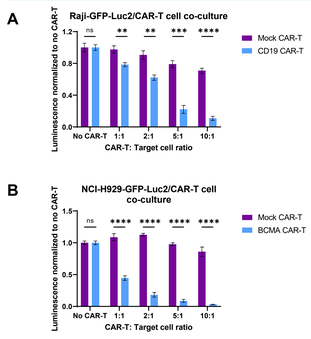


Figure 1: Targeting CAR-T cells kill dual reporter cancer cells in 2-D co-culture in a dose-dependent manner as measured by luminescence assay. (A) Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) cells were co-cultured with donor-matched mock (purple) or CD19 (blue) CAR-T cells (ProMab) for 24 hours using varying ratios of CAR-T cells to target cells. Luciferase expression was detected using the Bright-Glo system (Promega). Luminescence values were normalized to no CAR-T wells. ns = not significant, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, unpaired t-test. Error bars represent the standard deviation of three biological replicates. (B) NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-LUC2™) cells were co-cultured with donor-matched mock (purple) or BCMA (blue) CAR-T cells (ProMab) for 24 hours using varying ratios of CAR-T cells to target cells. Luciferase expression was detected using the Bright-Glo system (Promega). Luminescence values were normalized to no CAR-T wells. ns = not significant, **** = $p < 0.0001$, unpaired t-test. Error bars represent the standard deviation of three biological replicates.

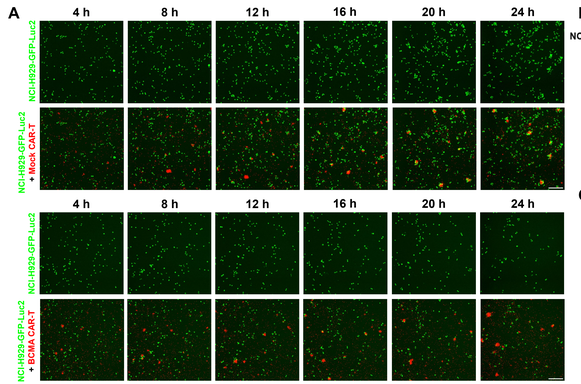


Figure 2: CAR-T cell in vitro killing assay of NCI-H929-GFP-Luc2 dual reporter cells in 2-D co-culture using fluorescence live imaging. (A) Mock (top) or BCMA (bottom) CAR-T cells were stained with Vybrant DiD dye (Invitrogen) and co-cultured with NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-LUC2™) cells at a 5:1 ratio of CAR-T cells to target cells. Images were acquired every 2 hours for 24 hours using a Leica Mica environmentally controlled 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.

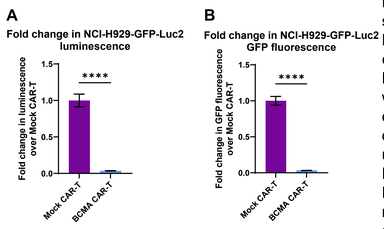


Figure 3: Luciferase and GFP transgenes show a similar fold decrease in expression when NCI-H929-GFP-Luc2 is co-cultured with BCMA CAR-T cells. (A) Luminescence values for NCI-H929-GFP-Luc2/BCMA CAR-T co-cultures at a 10:1 E:T ratio were normalized to the average values of mock CAR-T co-cultures. Error bars indicate the standard deviation of three biological replicates. **** = $p < 0.0001$, unpaired t-test. (B) Mean GFP fluorescence for NCI-H929-GFP-Luc2/BCMA CAR-T co-cultures at a 10:1 E:T ratio were normalized to the average values of mock CAR-T co-cultures. Error bars indicate the standard deviation of three biological replicates. **** = $p < 0.0001$, unpaired t-test.

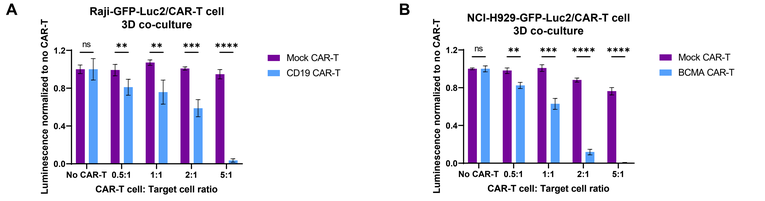


Figure 4: Targeting CAR-T cells kill dual reporter spheroids in a dose-dependent manner as measured by luminescence assay. (A) Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) or (B) NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-LUC2™) cells were formed into spheroids and the next day mock (purple) or targeting (blue) CAR-T cells were added at varying ratios of CAR-T cells to target cells. After 24 hours (Raji-GFP-Luc2) or 16 hours (NCI-H929-GFP-Luc2) of co-culture, luciferase expression was detected for each well using the Bright-Glo system. Luminescence values were normalized to no CAR-T wells. ns = not significant, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, unpaired t-test. Error bars represent the standard deviation of three biological replicates.

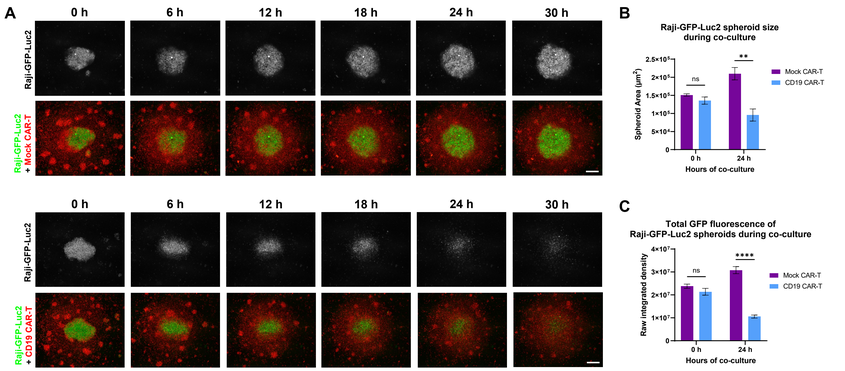


Figure 5: CAR-T cell in vitro killing assay of Raji-GFP-Luc2 dual reporter cells in 3-D co-culture using fluorescence live imaging. (A) Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) cells (green) were formed into spheroids and DiD-dyed mock (top) or CD19 (bottom) CAR-T cells (red) were added the next day. 5 µm Z stacks were acquired every 3 hours for 36 hours on a Leica Mica confocal microscope. Maximum projections are shown. Scale bars, 200 µm. (B) Raji-GFP-Luc2 spheroid area during co-culture with mock (purple) or CD19 (blue) CAR-T cells. Spheroid area was measured at 0 hours and after 24 hours of co-culture. n.s. = not significant, ** = $p < 0.005$. Error bars represent the standard deviation of three biological replicates. (C) Raw integrated density (sum of pixel values) of Raji-GFP-Luc2 spheroids during co-culture with mock (purple) or CD19 (blue) CAR-T cells. Raw integrated densities were measured using the GFP channel in ImageJ (NIH) at 0 hours and after 24 hours of co-culture. n.s. = not significant, **** = $p < 0.0001$. Error bars represent the standard deviation of three biological replicates.

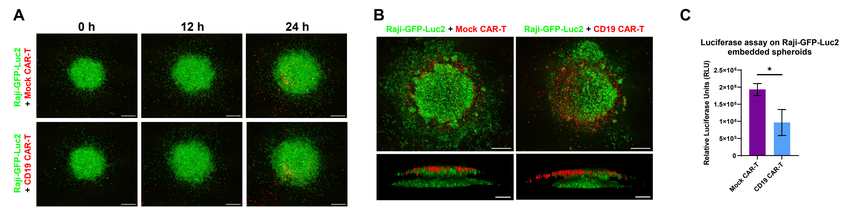


Figure 6: CAR-T cells infiltrate gel-embedded Raji-GFP-Luc2 spheroids. (A) Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) cells were formed into spheroids and embedded in a BME/collagen gel the next day. DiD-dyed CAR-T cells were added the day after embedment at a 10:1 CAR-T cell: target cell ratio. 7.5 µm Z stacks were acquired every 3 hours for 24 hours using a 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, 200 µm. (B) Maximum projections of Raji-GFP-Luc2 spheroids after co-culture with mock (left) or CD19 (right) CAR-T cells. Volume-filled side views are shown below. Raji-GFP-Luc2 cells are labeled in green and CAR-T cells are labeled in red. Scale bars, 200 µm. (C) Luciferase expression was detected for embedded spheroids after one week of co-culture with mock (purple) or CD19 (blue) CAR-T cells. * = $p < 0.05$, unpaired t-test. Error bars represent the standard deviation of three biological replicates.

Conclusions

- CD19- and BCMA-targeting CAR-T cells kill Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) and NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-LUC2™) cells, respectively, in a dose-dependent manner in both 2- and 3-D co-culture.
- NCI-H929-GFP-Luc2 (ATCC® CRL-3580-GFP-LUC2™) cells show a decrease in cell number and GFP fluorescence after 2-D co-culture with BCMA CAR-T cells as assayed by widefield live imaging.
- Raji-GFP-Luc2 (ATCC® CCL-86-GFP-LUC2™) spheroids show a decrease in GFP fluorescence and spheroid size after 3-D co-culture with CD19 CAR-T cells as assayed by confocal live imaging.
- 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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