High-throughput method to analyze the cytotoxicity of CAR-T cells in a 3D tumor spheroid model using Celigo® Image Cytometer

David Zurowski[1], Samir Patel[2], Daniel Hui[1], Mignane Ka[1], Charles Hernandez[2], Andrea Love[2], Bo Lin[2], Andrea Moore[1], and Leo Li-Ying Chan[2]
[1]Department of Analytical Development, Cell Therapy, Resilience, Inc. East Norriton, PA 19403, USA
[2]Department of Advanced Technology R&D, Revvity, Lawrence, MA 01843, USA

1 Introduction

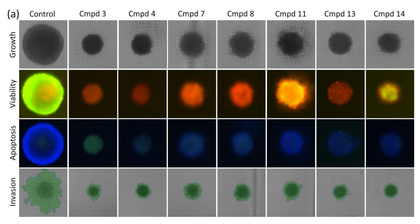
In the recent decade, chimeric antigen receptor (CAR)-T cell therapy has revolutionized strategies for cancer treatments due to its effective clinical efficacy. This has led to increased CAR construct development to target different tumor types. Robust and efficient *in vitro* potency assays can be beneficial to quickly identify potential CAR gene construct design candidates. *In vitro* cytotoxicity is commonly assessed using release assays (51Cr, calcein, and LDH). Now, image cytometry is being used due to ability to use different fluorescent labeling methods, ease-of-use, image acquisition for cell verification, and higher throughput performance.

2 Celigo® Image Cytometer for 3D tumor spheroid assays

Key Celigo Features

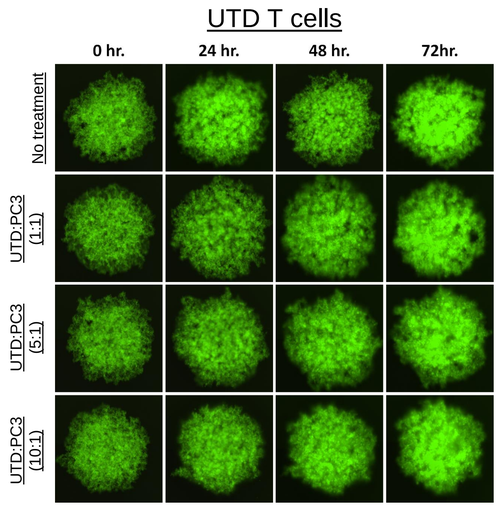
- Plate-based image cytometer that acquires brightfield and fluorescent whole well images of standard microplates
- Captured images are analyzed with the Celigo software to measure cell count, confluence, size, morphology, and fluorescence intensity

- Numerous applications: kinetic cell proliferation, GFP/RFP expression, tumor spheroid size change, DNA cell cycle, apoptosis, cytotoxicity, cell permeability, and transwell assays



3 PSMA CAR-T cells induce cytotoxicity in PC3 tumor spheroids

PC3-GFP+ cells were seeded into ultra-low attachment U-bottom plates to form loose spheroids. Either prostate-specific membrane antigen (PSMA) or un-transduced (UTD) T cells (effector cells) were added at different E:T (effector to target) ratios to verify killing capacity and were imaged on the Celigo to track GFP fluorescent signal over time.



Untreated spheroids (0 h) or un-transduced (UTD) T cells were seeded at different E:T (effector to target) ratios with the PC3-GFP+ spheroids and imaged over time (24, 48, and 72 h).

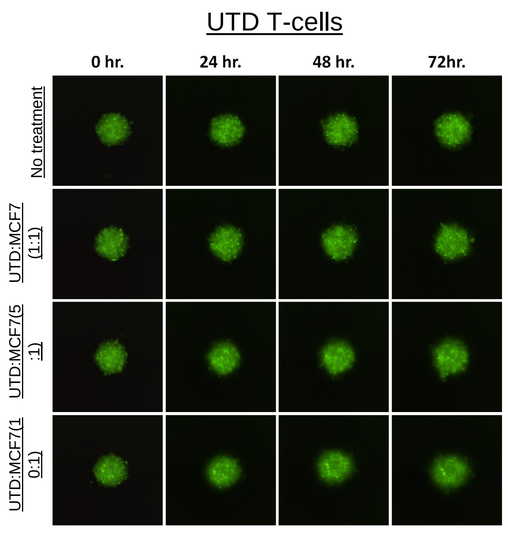
Untreated spheroids (0 h) or PSMA-specific CAR-T cells were seeded at different E:T (effector to target) ratios with the PC3-GFP+ spheroids and imaged over time (24, 48, and 72 h).

- Cytotoxicity was measured using the average GFP fluorescence
- A decrease in average GFP fluorescence indicates spheroid cell death
- PC3-GFP+ tumor spheroids exhibit a cytotoxic effect only when treated with PSMA CAR-T cells and not UTD T cells
- Reduction in GFP fluorescence at 72h is ~85-93%

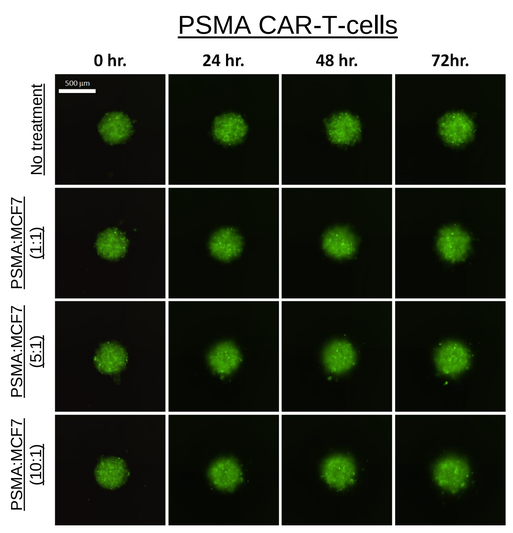
4 PSMA CAR-T cells are PC3 tumor spheroid-specific

MCF7-GFP+ cells were seeded into ultra-low attachment U-bottom plates to form loose spheroids. Either PSMA CAR or un-transduced T cells were added at different E:T (effector to target) ratios to verify killing capacity and were imaged on the Celigo to track GFP fluorescent signal over time.

- There was no decrease in GFP fluorescence over time
- MCF7-GFP+ tumor spheroids did not exhibit a cytotoxic effect when treated with either T cell population (un-transduced or PSMA) independent of E:T ratio
- Lack of PSMA CAR-T cells cytotoxicity indicate the specificity of the PSMA CAR to PC3 tumor spheroids

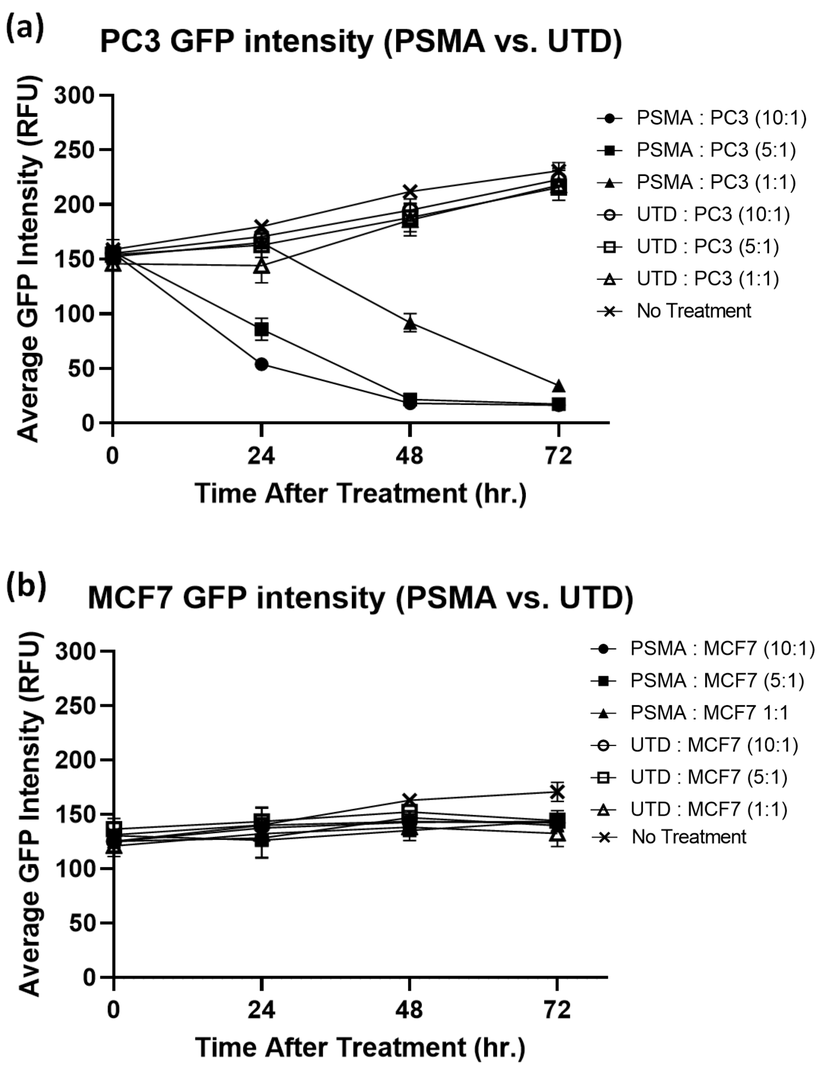


Untreated spheroids or un-transduced (UTD) T cells were seeded at different E:T (effector to target) ratios with the MCF7-GFP+ spheroids and imaged over time (24, 48, and 72 h).



Untreated spheroids (0 h) or PSMA-specific CAR-T cells were seeded at different E:T (effector to target) ratios with the MCF7-GFP+ spheroids and imaged over time (24, 48, and 72 h).

5 Time-dependent cytotoxicity of PSMA CAR-T cells



Time-dependent average GFP fluorescence intensity comparing PSMA CAR-T and un-transduced (UTD) T cell treatment at different E:T ratios of (a) PC3 or (b) MCF7 cells.

- Using the acquired images, the GFP fluorescence for both PC3-GFP+ and MCF7-GFP+ tumor spheroids was calculated in response to treatment with PSMA CAR-T cells and unstransduced (UTD) T cells for 72 h using the Celigo Image Cytometer Software
- PSMA CAR-T cells specifically target PC3-GFP+ tumor spheroids at all E:T ratios
- Higher E:T ratios result in earlier effective killing in PC3-GFP+ cells with PSMA CAR-T cells

6 Summary

In the last decade, in vitro assays for CAR-T cell therapy discovery have mainly been performed using 2D tumor cell model, however, it is critical to study the potency and specificity of CAR constructs against 3D tumor spheroid models that can better recapitulate the 3D spatial features and organizations of the solid tumors in cancer patients. The proposed plate-based image cytometry method can provide a robust and high-throughput method to characterize CAR-T cells treatment using a 3D spheroid model, which may improve the efficiency CAR-T cell discovery for treatment of solid tumors, and more rapidly identify suitable CAR construct candidates for downstream processes.

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