>> Visualizing the battle within: Exploring CAR-T cell killing dynamics through live fluorescence microscopy

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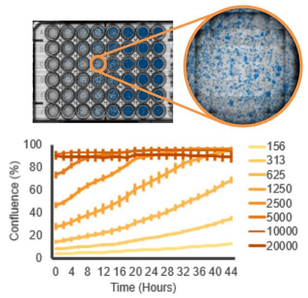
Omni: Dynamic Cell Tracking

Automated, Whole-Vessel Imaging and Analysis

Cell viability and cytotoxicity assays are often used to understand the mechanism and potency of novel drugs or cell therapies. Assessing the degree and dynamics of the target cell response in vitro is vital, but endpoint assays only provide a snapshot and can miss key indicators of cellular response. Therefore, live-cell imaging provides a non-invasive alternative as a measure of cytotoxicity.

Axion Biosystems' Omni platform offers live-cell imaging within an incubator for real-time tracking of cell proliferation, migration, colony formation, and organoid size. Here, we used the Omni assess the dynamics of HER2 CAR-T cell killing of two cancer cell lines that expresses high (SKOV3) and low (A549) levels of HER2.



The Omni Product Family

>> Assay your cells in brightfield and fluorescence – From label-free cell monitoring to fluorescence-based assays, the Omni adds dynamic visual results to any experiment.

>> Track every moment, straight from your incubator – The Omni operates within an incubator, automatically capturing images as your cells grow in their optimal environment.

>> See every cell – The Omni moves the camera, not the cells, capturing detailed brightfield images of the entire culture without disturbing the cells.

>> Monitor and analyze your cells remotely – The software allows you to monitor your cells and perform data analysis from your desktop.

>> Get started quickly – With an easy-to-install, maintenance-free device that does not require calibration, a short training is all it takes to start using the Omni.

Features	*Omni BR*	*Omni Pro 12*	*Omni FL*
Whole-well brightfield	✓	✓	✓
Automated acquisition	✓	✓	✓
Incubator compatible	✓	✓	✓
Fluorescence (Red)		✓	✓
Fluorescence (Green)		✓	✓
Number of plates	1	12	1
Plate handling	Manual	Automated	Manual

AI-Driven Imaging Software for Powerful, yet Simple, Analysis

Axion's software modules for the Omni platform enable simple assay setup, real-time cellular visualization, and fast analysis. Discover the module best suited for your research and transform your complex data into clear results.

Cell Confluence | Scratch Assay | Fluorescent Object Count | Clonogenic Assay | Organoid Analysis

Real-time Analysis of Cell Behavior

Assessing Cell Viability with the Confluency Module

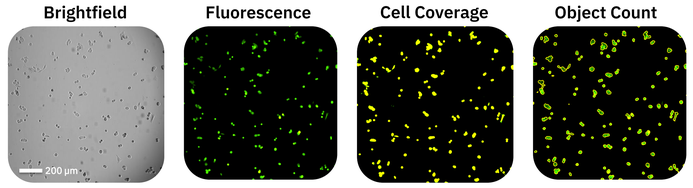
The Confluency Module measures the percentage of the surface area of a two-dimensional (2D) culture dish that is covered with cells. The module highlights the position of cells on the Omni platforms and automatically tracks and quantifies cell health and proliferation to improve experimental efficiency and reproducibility. Images are acquired and processed by the Confluency Module, which can automatically generate reports containing experimental results for cell coverage (brightfield or fluorescence) and fluorescent coverage ratios.

Metrics provided by the Confluency Module:

>> Cell coverage (%) - total cell coverage of an imaged area; when using fluorescence imaging, the percentage of red fluorescence and/or green fluorescence coverage is also assessed.

>> Confluence ratios (%) – the percentage of coverage ratios of green and red fluorescent objects.

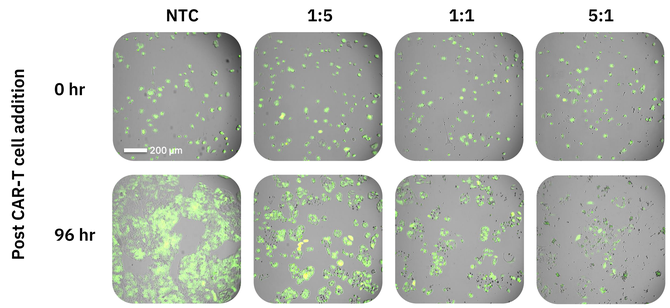
>> Object Count - Quantify the number of fluorescent objects in a culture and track them over time.

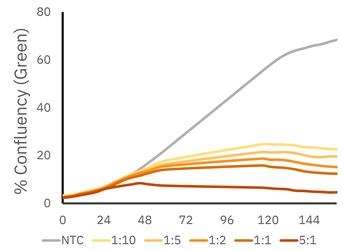


Tracking CAR-T cell killing of Fluorescent HER2+ Cancer Cells

Harnessing the potent cytotoxicity of chimeric antigen receptor (CAR) T cells against cancer has revolutionized the landscape of immunotherapy. One key aspect influencing CAR-T cell efficacy is the density of target antigens on cancer cells, often dictating the magnitude of cytotoxic response. Human epidermal growth factor receptor2 (HER2), a well-known biomarker overexpressed in various cancer and lung carcinomas, serves as a promising target for CAR-T cell therapy.

The Omni was used to monitor the killing of fluorescent HER2+ A549 cancer cells by HER2-CAR-T cells at various E:T ratios. CAR-T cells were added at 24 hours post initial cell seeding and images of the cells were captured every 4 hours by the Omni platform. The Confluency Module was used to assess changes in target cell confluency over time.





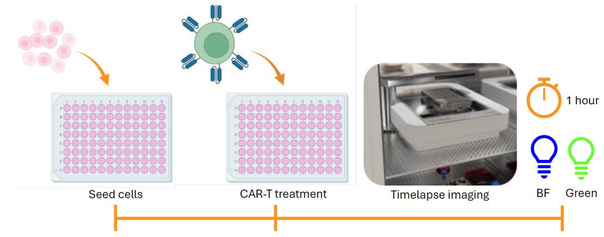
Fluorescent images captured by the Omni over the time course exhibit a decrease in fluorescence as compared to the no treatment control when co-cultured with CAR-T cells.

Over the entire time course, A549 cells demonstrated a dose-dependent response to increasing levels of E:T ratios of CAR-T cells, with A549 cells treated with the highest number of CAR-T cells (5:1) showing the greatest cytolysis.

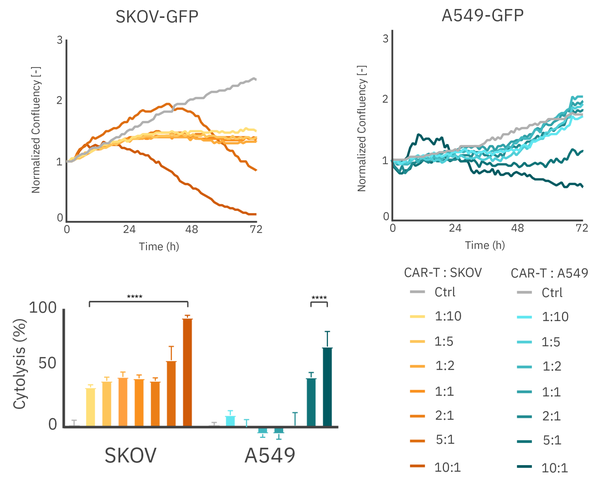
The Dynamics of CAR-T Cell Killing

HER2 Expression Levels Influence CAR-T Cytotoxicity

In this study, we delved into the intricate dynamics of CAR-T killing using fluorescence live-cell microscopy, focusing on SKOV3, ovarian carcinoma cells, and A549, lung adenocarcinoma cells. SKOV3 cells have a relative high expression of HER2 in comparison to A549 cells which exhibit lower HER2 expression. Our aim was to elucidate how variations in target antigen density influences the cytotoxicity of HER2 CAR-T cells, thereby shedding light on potential factors impacting treatment efficacy.



SKOV3-GFP and A549-GFP cells were seeded in a 96-well plate (50,000 cells/well) and were allowed to attach. After 24 hours, HER2 CAR-T cells were added at the following effector to target (E:T) ratios: 1:10, 1:5, 1:2, 1:1, 2:1, 5:1, and 10:1. Immediately afterwards, the well plate was placed on the Omni platform (37C and 5% CO_2, n = 4 per group) and a high-resolution brightfield and green fluorescent snapshot were made in each well for a duration of 72 hours with a time interval of 1 hour.



Addition of various E:T ratios of CAR-T cells to SKOV3 and A549 cells resulted in cell killing in a dose-dependent manner. Hourly monitoring reveled the lowest E:T ratios (1:10) already inhibited SKOV3 cell growth after 20 hours, while the first signs cell killing of A549 cells could be seen at an E:T ratio of 5:1 after 26 hours. Consistent with the images, the cytolysis bar plots demonstrate a significantly higher level of cell killing in SKOV3 cells compared to the A549 cells after 72 hours.

Conclusions

In this study, we investigated the heightened sensitivity of SKOV3 cells to HER2 CAR-T therapy, when compared to A549 cells. By utilizing the Omni FL, green fluorescence emitted by the cells was measured. Subsequently, growth curves and cytolysis percentage could easily be calculated. Growth curves depicting the temporal dynamics of cell death confirmed the heightened sensitivity of SKOV3-GFP cells to CAR-T therapy. A549-GFP cells exhibited comparatively lower levels of cytotoxicity, indicating reduced susceptibility to CAR-T cell-mediated killing. Overall, these results underscore the critical influence of target antigen density on the efficacy of CAR-T cell therapy, highlighting the differential sensitivity of cancer cells to immunotherapy based on HER2 expression levels.