>> Exploring CAR-T cell killing dynamics through live-cell fluorescence microscopy

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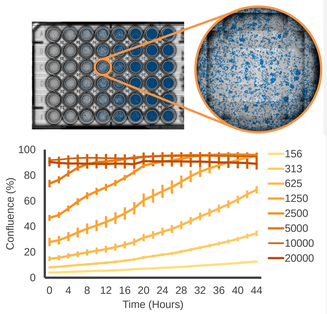
Omni: Kinetic cell tracking

Automated, whole-vessel imaging and analysis

Cell killing assays are often used to understand the mechanism and potency of novel cell therapies but are generally limited by endpoint measurements. An alternative, non-invasive method to analyze cell killing is live-cell imaging.

Here, we used the Omni to assess the kinetics of HER2 CAR-T cell killing in two cancer cell lines with different HER2 levels.

The Omni product family



>> From label-free cell monitoring to fluorescence-based assays, the Omni adds dynamic visual results to any experiment.

>> Automatically capturing images as your cells grow in their optimal environment.

>> Whole-well brightfield imaging capture high-resolution images of the entire culture

>> Monitor your cells and perform data analysis from your desktop.

>> Get started quickly! The platform is easy to use and requires no maintenance or calibration

AI-Driven imaging software for powerful, yet simple analysis

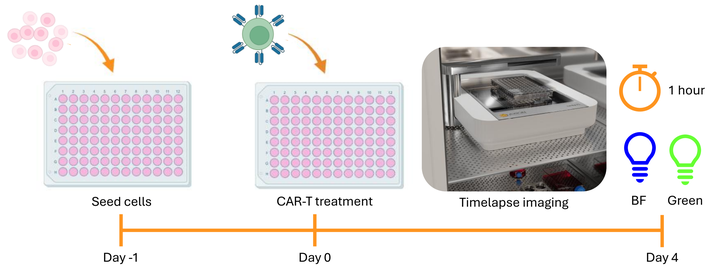
The Omni platform software modules simplify assay setup, offer real-time cellular visualization, and enable fast analysis.

Cell Confluence | **Scratch Assay** | **Fluorescence Analysis** | **iPSC Monitoring** | **Clonogenic Assay** | **Organoid Analysis**

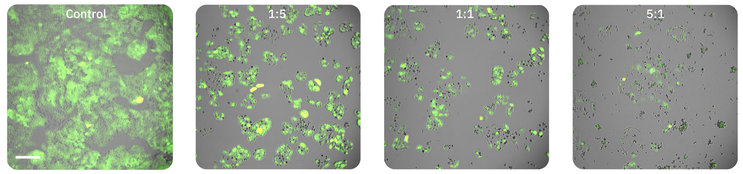
Real-time analysis of cell behavior

The dynamics of CAR-T cell killing

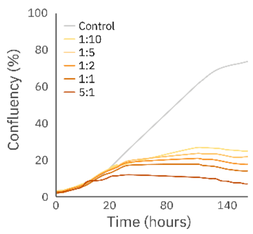
CAR-T cells have transformed immunotherapy by targeting antigens on cancer cells. The density of these antigens, such as human epidermal growth factor receptor 2 (HER2) which is overexpressed in various cancers, affects CAR-T cell efficacy and cytotoxic response. This makes HER2 a promising target for CAR-T cell therapy. Fluorescence live-cell imaging was used to analyze CAR-T cell killing of SKOV3 and A549 cancer cells, which have differing HER2 expression levels. Our aim was to understand how antigen density affects CAR-T-cell killing efficacy.



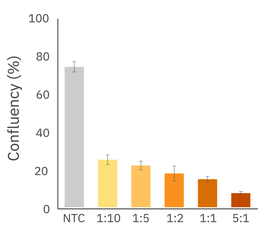
Experimental workflow: After 24 hours of culture, HER2 CAR-T cells were added to the cancer cells. High-resolution brightfield and green images were taken hourly for 96 hours.



A549-GFP



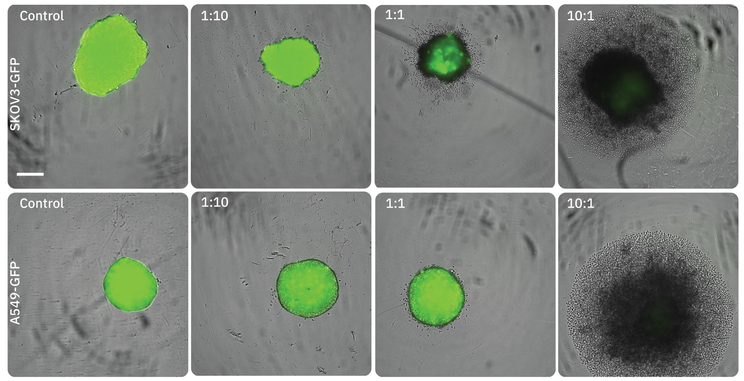
A549-GFP



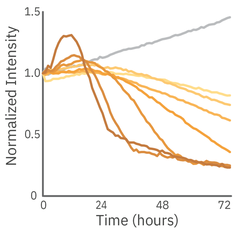
The cytotoxic potential of HER2 CAR T-cells was evaluated by comparing the fluorescence-based (green) confluency of A549-GFP cells at multiple time points and varying E:T ratios. As expected, A549 cells treated with CAR T-cells exhibited dose-dependent decrease in fluorescence confluency (%), with near-complete cell lysis observed in the 5:1 E:T ratio group at 160 hours.

Dynamic insight into cell viability

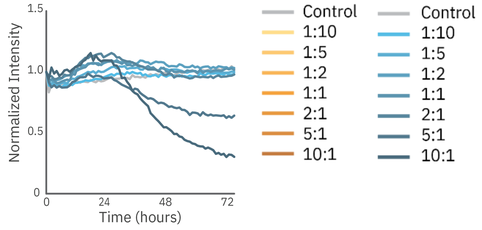
Differential sensitivity of cancer cells to HER2 CAR-T therapy



SKOV3-GFP



A549-GFP



The expression of the CAR within the immune cell population and its affinity for the target antigen are crucial factors in determining the potency of CAR T-cells. Immune cell-mediated killing was assessed by applying HER2-targeted CAR T-cells to both SKOV3-GFP and A549-GFP spheroids. SKOV3-GFP spheroids treated with CAR T-cells showed a dose-dependent decrease in fluorescence intensity. In contrast, A549 spheroids exhibited significant killing only at 5:1 and 10:1 E:T ratios.

Conclusion

The Omni platform enables real-time monitoring of CAR T-cell interactions with target cells, providing key insights into the cytotoxic potential of these engineered immune cells. Fluorescent metrics were used to track immune cell-mediated killing, with changes in fluorescence confluency and intensity reflecting the extent of target cell death. These findings suggest that the efficacy of CAR T-cell therapies may be influenced by the target antigen expression levels on cancer cells, emphasizing the need for optimized dosing and E:T ratios depending on tumor characteristics