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

Inge Thijssen[1], Skylar Dewees[2], Svenja Meiler[1], Danielle Califano[2], Stacie Chvatal[2]
[1]Axion BioSystems, Eindhoven, The Netherlands; [2]Axion BioSystems, Atlanta, GA, USA

Abstract
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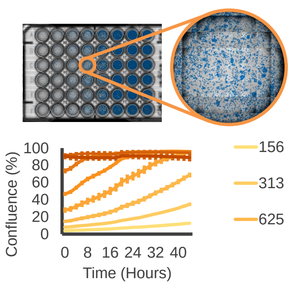
Omni: Kinetic cell tracking

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



>> Assay your cells in brightfield and fluorescence

>> Track every moment, straight from your incubator

>> See every cell by movement of the camera

>> Monitor and analyze your cells remotely

>> Get started quickly

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 | Clonogenic Assay | Organoid Analysis | iPSC Monitoring

Real-time analysis of cell behavior

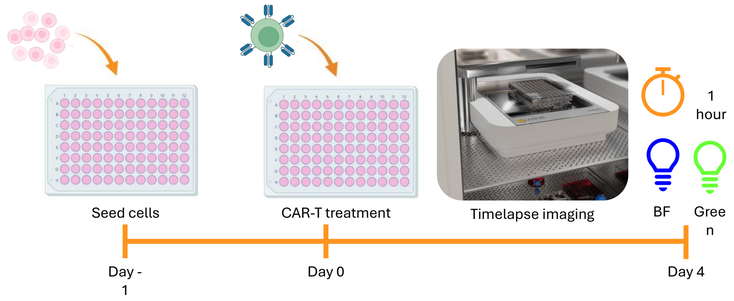
The dynamics of CAR-T cell killing

Introduction

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.

Methods

24 hours after seeding SKOV3 and A549 cells, HER2 CAR-T were added at various Effector:Target (E:T) ratios. The well plate was placed on the Omni and brightfield and green fluorescent snapshots were made hourly for 96 hours.
The green fluorescent intensity was determined in the Axion Portal and subsequently normalized to analyze cancer cell killing. Concurrently, cytolysis was quantified and analyzed.



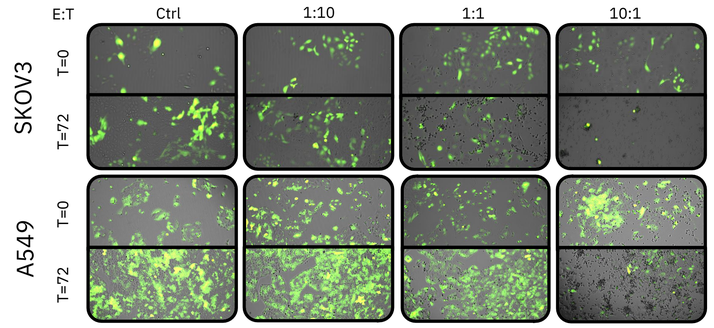
Experimental workflow: After 24 hours of culture, HER2 CAR-T cells were added to the cancer cells. High-resolution brightfield and green images were taken hourly for 72 hours.

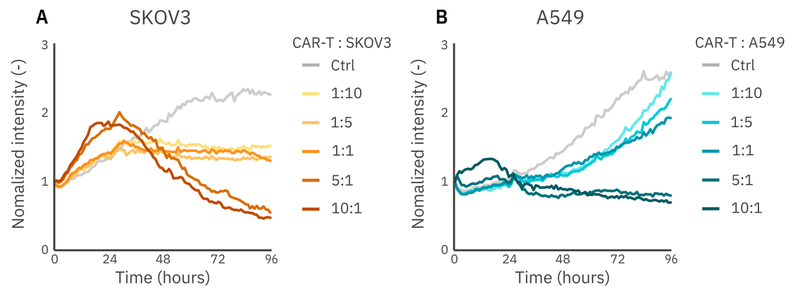
Results

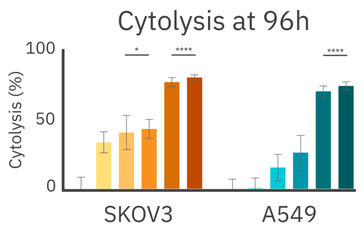
HER2 CAR-T cells killed the cancer cells in a dose-dependent manner, with SKOV3 cells showing higher sensitivity and earlier response at lower E:T ratios compared to A549 cells. Quantitative analysis indicated a significantly higher cytolysis for lower E:T ratios in SKOV3 cells, as confirmed by a decrease in green fluorescence in the images.

Dynamic insight into cell viability

Differential sensitivity of cancer cells to HER2 CAR-T therapy







Conclusion

This research highlights the importance of target antigen density in CAR-T cell efficacy. SKOV3 cells, with higher HER2 expression, were more susceptible to CAR-T cell killing compared to A549 cells. The Omni platform proved to be effective in providing real-time, kinetic insights into cell killing, emphasizing its potential in optimizing immunotherapy strategies.