In vitro real-time potency assay for immunotherapeutic development

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SITC 2025 Spring Scientific: Cellular Therapy for Solid Tumors
Abstract #: 91

Agilent Technologies

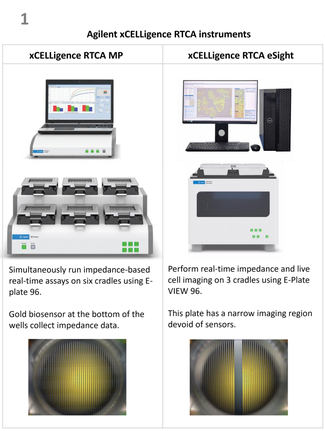
Introduction

Cancer immunotherapeutics have achieved remarkable efficacy and become promising treatments for hematologic malignancies. However, significant challenges remain in the clinical translation to many other types of cancers.

There is an urgent need for functional potency assays, that could model the interaction of immune cells with tumor cells and can be used to rapidly test the efficacy of different immunotherapy approaches like small molecules, biologics, cell therapies and/or combinations of these agents.

Here, we present an in vitro real-time and label-free cytotoxicity assay to assess the potency of cell therapy products.

Experimental materials and methods



Working principle:

Step 1: Add tumor cells. As the cells adhere to the floor of the wells, it impedes the flow of electric current between gold microelectrodes. Impedance is plotted as unitless "Cell Index".

Step 2: Add immune cells. Effector cells are added to the wells at around 24 hours.

Step 3: Monitor cytolysis in real-time. If the addition of effector cells result in killing of tumor cells, this cytolytic activity can be sensitively and precisely detected as a loss of impedance. In addition to impedance, the Agilent xCELLigence RTCA eSight uses E-Plate VIEW 96 for imaging cells in the brightfield, red, blue and green channels.

Results and Discussion

Cytotoxicity kinetics of NK92 cells targeting PC3 carcinoma cells

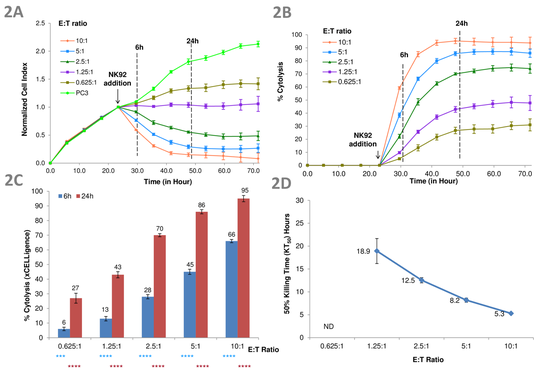


Figure 2. **Percent cytolysis and KT50 for cytolysis of PC3 cells by NK92 were determined through impedance measurements. (A)** Cell Index plot for nuclear-red labeled PC3 target cells treated with different E:T ratios of NK92 cytolytic cells. Samples have been internally normalized for the Cell Index value measured before NK92 addition (Normalized Cell Index plot). **(B)** The NCI data is converted to a percent cytolysis plot by the Agilent xCELLigence RTCA software. **(C)** Percent cytolysis measured at 6 and 24 hours after NK92 addition for the different E:T ratios. One way ANOVA result indicates significant difference between individual treatment and control at 6 hours (light blue) and at 24 hours (red); (*** p< 0.001) and (**** p<0.0001). (D) 50% Killing Time (KT50) for the same E:T ratios in (C). ND: Not Detected.

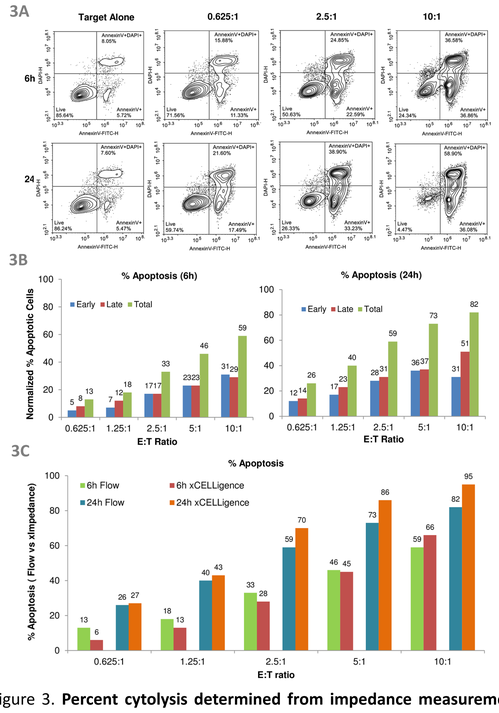


Figure 3. **Percent cytolysis determined from impedance measurements and flow cytometry correlate to confirm the cytolysis of PC3 cells by NK92. (A)** Flow cytometry was performed using cells from replica plates for the same experiment in Figure 2. There is an E:T ratio and time dependent increase of early apoptotic (annexin V+, DAPI-; bottom right of each plot), and late apoptotic (annexin V+, DAPI+; upper right of each plot) cells. **(B)** Charts show the percent apoptotic cells for the flow data. **(C)** Total apoptosis measured by flow cytometry is like the results of impedance analysis.

Results and Discussion

Using Bispecific T Cell Engager (BiTE) to target T47-D cells

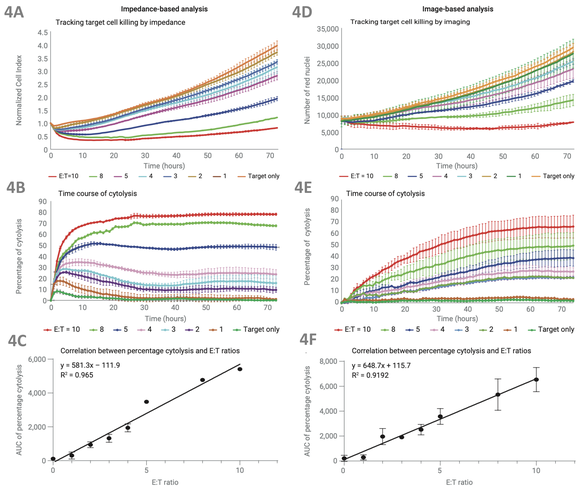


Figure 4. **Monitoring BiTE-mediated immune cell cytolysis of epithelial cell adhesion molecule-1 (EpCAM)-expressing T47-D tumor cells.** T47D red cells constitutively expressing mKate fluorescent were seeded at 8,000 cells per well. Impedance and live cell imaging data were collected every 15 minutes and two-hour intervals, respectively. Exposure time in red channel was 300 ms. Around 24 hours, PBMCs (40,000 cells/well) were premixed with EpCAM BiTE (80 ng/mL) and added to the wells containing T47D-Red cells. Increasing the E:T ratio increased time-dependent cytolysis of T47D-Red at different E:T ratios measured using impedance **(A–C)** and imaging **(D–F)** on the Agilent xCELLigence RTCA eSight. The images show **(A, D)** Normalized Cell Index **(B, E)** percentage cytolysis, and **(C, F)** the relationship between AUC of percentage cytolysis and E:T ratios derived from impedance and imaging, respectively. Cytolysis assays were run in triplicate; error bars represent standard deviation.

Cytotoxicity kinetics of EpCAM CAR T cells targeting A431 cells

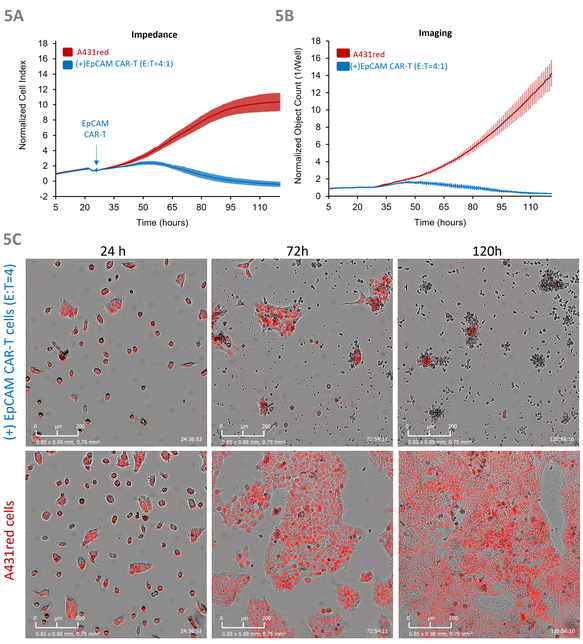


Figure 5. **EpCAM CAR T cells eliminate A431 cells at E:T of 4:1.** A431red expressing nuclear-localized red fluorescent protein (RFP) were generated by transducing parental cells with eLenti Red (Agilent Technologies). A431red (3000/well) were seeded on E-Plate VIEW 96 plate. Impedance was measured every 15 minutes. Images were acquired in bright-field at standard exposure and red channel at 150 ms. The addition of EpCAM CAR T cells result in killing of A431 cells detected by a loss of **(A)** impedance and **(B)** red fluorescence signal from the target cells. **(B)** Representative images from two wells at selected time points during the assay show the growth of A431 cells in the controls (lower panel) and the killing of target cells by EpCAM CAR T cells (upper panel).

Conclusions

- The Agilent xCELLigence RTCA instruments can be used to evaluate diverse cellular and antibody-based immunotherapies for solid tumors.
- These assays show a strong concordance between impedance, imaging, and flow cytometry data, confirming the reliability and robustness of impedance data collected by xCELLigence instruments.
- The real-time impedance assays are far simpler to carry out, provide analytical sensitivity and objectivity with less effort than end-point assays.
- The Agilent xCELLigence RTCA eSight combines microscopic live-cell imaging with real-time impedance detection to effectively evaluate CAR T cells for solid tumors.