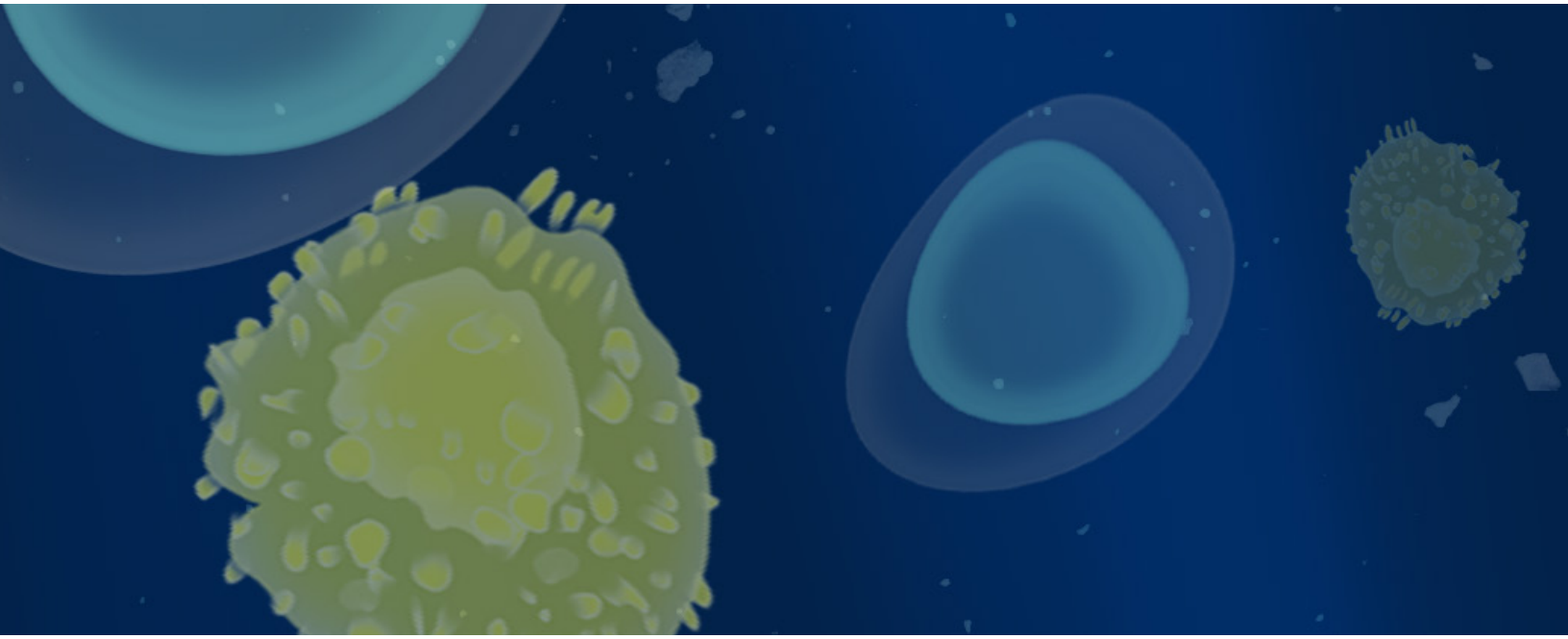
xCELLigence Immunotherapy Kits

Monitoring Liquid Cancer Killing in Real-Time



Introduction

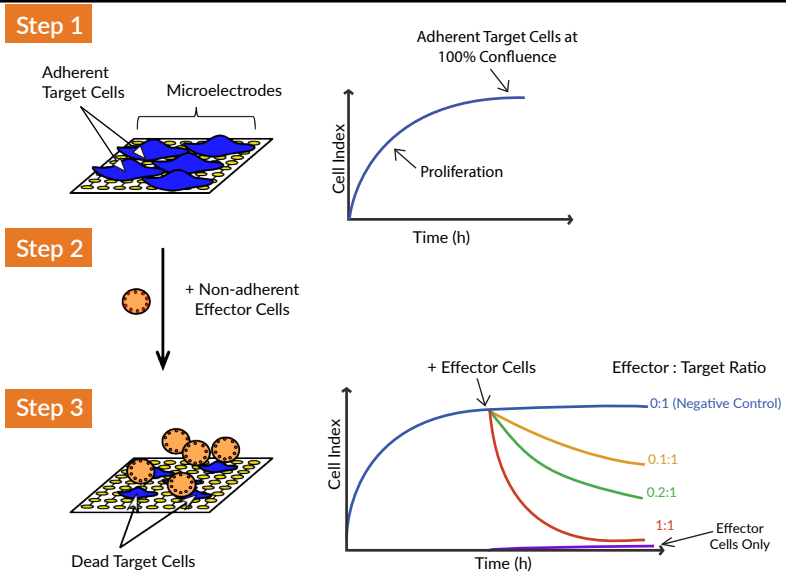
The Promise and Challenge of Cancer Immunotherapy

The high specificity and potent cytotoxicity of immune system effector cells make them promising agents for extirpating liquid cancers. However, realizing the full therapeutic potential of this approach will require the ability to quantitatively monitor the potency of immune cell-mediated killing of target liquid cancer cells under controlled conditions in vitro.

Using xCELLigence to Monitor the Efficacy of Immunotherapies That Target Liquid Cancers

Initially developed for analyzing adherent cells, ACEA's xCELLigence® Real-Time Cell Analysis (RTCA) instruments utilize gold microelectrodes embedded in the bottom of microtiter wells to non-invasively monitor cell number, cell size, and cell-substrate attachment quality using the principle of cellular impedance. In short, adherent cells act as insulators – impeding the flow of an alternating microampere electric current between electrodes. This impedance signal is measured automatically, at a frequency defined by the user (every 10 seconds, once per hour, etc.), and provides an extremely sensitive readout of cell health and behavior.

Over the past decade xCELLigence has been used extensively to study immune cell-mediated killing of adherent cancer cells. However, ~10% of all cancers are liquid in nature, are therefore non-adherent, and cannot be directly monitored by the standard impedance assay. Three different xCELLigence Immunotherapy Kits now enable the killing of liquid cancers (B cell cancers and Leukemia in particular) to be probed using xCELLigence. Each kit uses a cell type-specific antibody to immobilize target cells on the impedance electrodes, as outlined in **Figure 1** below:



Step 1: Adherent target cells (i.e. tumor cells) are first seeded in the wells of an electronic microtiter plate (E-Plate®). Adhesion of cells to the gold microelectrodes impedes the flow of electric current between electrodes. This impedance value, plotted as a unitless parameter called "Cell Index", increases as cells proliferate and then plateaus as cells approach 100% confluence.

Step 2: When added subsequently, non-adherent effector cells (i.e. immune cells) in suspension do not cause impedance changes in and of themselves (due to lack of adherence to the gold microelectrodes).

Step 3: If effector cells induce the destruction of the target adherent tumor cells, the corresponding cytolytic activity can be sensitively and precisely detected. The continuous acquisition of impedance data for each well of an E-Plate enables the generation of real-time killing curves for multiple conditions simultaneously.

Figure 1: Using an xCELLigence RTCA instrument and immunotherapy kit to monitor killing of liquid cancer target cells.

Immune Effector Cells and Target Cancer Cells That Have Successfully Been Utilized:

Kits	Effector Cells	Target Cells
B Cell Killing (anti-CD40) Assay	NK-92, TALL-104, CAR-T, primary CD8+ T cells	Daudi, Raji, Ramos, primary B cells
Leukemic Cell Killing (anti-CD29) Assay	NK-92	K562
B Cell Killing (anti-CD19) Assay	NK-92, primary CD8+ T cells	Raji, primary B Cells

Example Data

B Cell Killing (anti-CD40) Assay

Although three different xCELLigence Immunotherapy Kits are available, only data from the B Cell Killing (anti-CD40) Assay is presented here. The wells of an ACEA electronic microtiter plate were pre-coated with anti-CD40 antibody, enabling B cells to be immobilized on the plate bottom **(Figure 2A)**. Whereas antibody immobilized B cells generate a robust impedance signal and proliferate to the point of confluence (resulting in a plateaued impedance signal), the growth of untethered B cells is essentially undetectable **(Figure 2B)**. Importantly, with or without tethering antibody coating of the wells, effector cells such as the NK-92 cells used here produce minimal signal on their own **(Figure 2B)**. Addition of NK-92 cells on top of immobilized B cells results in target cell death in a dose dependent manner **(Figure 2C)**. The tethering and killing behaviors seen in **Figures 2B and C** have been observed in all three of the B cell lymphoma cell lines tested (Daudi, Raji, and Ramos), for multiple effector cell types (NK, T, CAR-T), and for combination therapies (CART + checkpoint inhibitors, etc.).

An important question is whether the physical immobilization of B cells via CD40 tethering affects the efficiency with which they are killed. To assess this, side-by-side four hour assays were performed for NK-92 cell-mediated killing of Raji B cells that were either immobilized (analyzed by xCELLigence) or in suspension (analyzed by flow cytometry). As seen in **Figure 2D**, the killing trends observed by these two methods correlate perfectly, with the magnitude of % cytolysis varying minimally. This is consistent with a large number of publications showing that xCELLigence data consistently recapitulates data obtained by traditional assays.

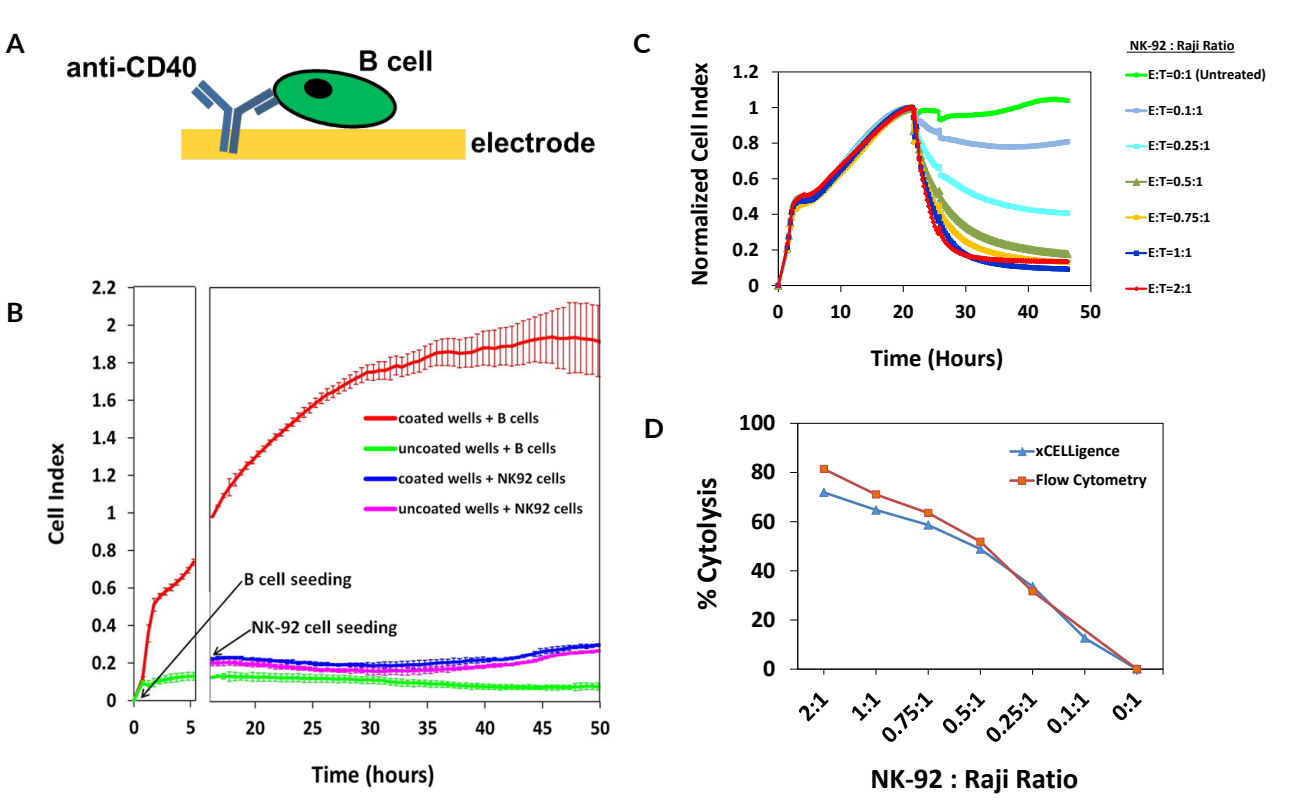


Figure 1. The xCELLigence® Immunotherapy Kit for monitoring B cell killing. **(A)** Precoating the wells of ACEA's electronic microtiter plates with B cell-specific antibody (anti-CD40) enables B cells to proliferate on, and be detected by, these sensors. **(B)** Controls showing the selective proliferation of Daudi B cells on electrodes coated with anti-CD40 antibody. As expected, with or without anti-CD40 coating non-adherent NK-92 effector cells produce minimal signal. Error bars are standard deviation. **(C)** The efficiency with which Raji B cells are killed is dependent on the number of NK-92 cells added per well. **(D)** The impact of B cell immobilization on killing efficiency. Raji B cells, either immobilized by antibody or in suspension, were treated with different numbers of NK-92 cells. % cytolysis was determined after 4 hours of treatment by xCELLigence® (tethered) or flow cytometry (in suspension). All panels are unpublished data from ACEA Biosciences.

Ordering Information

xCELLigence Immunotherapy B Cell Killing (anti-CD40) Assay

Complete Kit	Cat. No. 8100004
6 E-Plates View 96	
Tethering Reagent (anti-CD40) (250 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	
xIMT Software	

Tethering Kit	Cat. No. 8100005
Tethering Reagent (anti-CD40) (250 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	

Sample Kit	Cat. No. 8100006
2 E-Plates View 96	
Tethering Reagent (anti-CD40) (90 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	
Trial xIMT Software for 1-month usage	

xCELLigence Immunotherapy Leukemic Cell Killing (anti-CD29) Assay

Complete Kit	Cat. No. 8100007
6 E-Plates View 96	
Tethering Reagent (anti-CD29) (125 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	
xIMT Software	

Tethering Kit	Cat. No. 8100008
Tethering Reagent (anti-CD29) (125 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	

Sample Kit	Cat. No. 8100009
2 E-Plates View 96	
Tethering Reagent (anti-CD29) (45 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	
Trial xIMT Software for 1-month usage	

xCELLigence Immunotherapy B Cell Killing (anti-CD19) Assay

Complete Kit	Cat. No. 8100010
6 E-Plates View 96	
Tethering Reagent (anti-CD19) (250 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	
xIMT Software	

Tethering Kit	Cat. No. 8100011
Tethering Reagent (anti-CD19) (250 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	

Sample Kit	Cat. No. 8100012
2 E-Plates View 96	
Tethering Reagent (anti-CD19) (90 µL)	
10X Tethering Buffer (10 ml)	
Cytolysis Reagent (1.5 ml)	
Trial xIMT Software for 1-month usage	

xIMT Software Cat.No. 310100190

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