Utilization of a Label-Free Real-Time Cell Analysis Technology for Cancer Immunotherapy Applications

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VP, ACEA Biosciences
Outline of Presentation

- Introduction to ACEA Biosciences and its Line of Products
- xCELLigence® RTCA Systems and Principle of Detection
- General Introduction to Cancer Immunotherapy
- Applications of xCELLigence® RTCA System for Cancer Immunotherapy
  1. Natural Killer (NK) Cell Mediated Cytotoxicity
  2. T Cell Mediated Cytotoxicity
  3. Antibody-Dependent Cell Mediated Cytotoxicity (ADCC)
  4. Genetically engineered T-Cell cytolysis (including CAR-T)
  5. Macrophage mediated phagocytosis
  6. Complement mediated cytolysis (CMC)
- Summary & Conclusion
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ACEA Biosciences, Inc.

- Founded 2002
- Headquarters: San Diego, CA. USA
- Personnel 300+ FTEs, 30+ PhDs
- The technology inventor of the xCELLigence® Real-Time Cell Analysis Systems
- 29 Distributors Worldwide, Direct Sales/Support in the US
Innovation and Excellence

NovoCyte™
Flow Cytometer

RTCA-MP System
RTCA-HT System
RTCA-iCELLigence System
RTCA-CardioECR System

RTCA-SP System
RTCA-DP System
RTCA-Cardio System
NovoCyte

Solid Publication Track Record

Over 700 peer-reviewed publications citing ACEA’s Real-Time Cell Analysis Technology
Disease Related Application Areas

- Cancer
- Immunity
- Inflammation
- Infectious Diseases
- Safety Toxicity

Real-Time Cell Analysis
Simple Workflow

No cell labeling required, fully automated, physiological conditions

Seed Cells → Real-time monitoring at physiological conditions → Kinetic cell response curve
Real-Time Monitoring at Physiological Conditions

RTCA-DP (Dual Plate)
3 x E-Plate® 16
3 x CIM-Plate® 16

RTCA-SP (Single Plate)
1 x E-Plate® 96

RTCA-MP (Multiple Plates)
6x E-Plate® 96

Designed to be placed in regular tissue culture incubators.
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Impedance Biosensor Assay Principle

electrodes without cells

\[ Z = Z_0 \]

Add Cells

electrodes with cells attached

\[ Z = Z_{\text{cell}} \]

\[ \Delta T \]

Z = Z_{\text{cell2}}

cell number change
Immune Cell Killing – ASSAY PRINCIPLE

100% Confluence

Proliferation

No Adhesion Minimal Signal

Cell Index

Time (h)

Cell Index

Time (h)
Immune Cell Killing – ASSAY WORKFLOW

Adherent Tumor Cells

+ Non-adherent Effector Cells

Dead Target Cells

100% Confluence

Cell Index

Time (h)

Effector : Target Ratio

0:1 (negative control)

1:1

4:1

16:1

Cell Index

Time (h)
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Cancer Immunotherapy: Utilizing the Full Complement of Innate and Adaptive Immunity to Target Cancer
Cancer Immunotherapy and Different Ways of Targeting Tumors by the Immune System

1. NK-mediated cell cytotoxicity
2. T cell mediated cell cytotoxicity
3. Antibody-dependent cell cytotoxicity
4. Genetically engineered T-Cell cytolysis (e.g. CAR-T)
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1. NK-Mediated Cell Cytotoxicity

NK-cell

Target Cell

Grazyme and perforin

1. NK-mediated cell cytotoxicity
1.1 NK-92-Mediated Cytolysis of MCF7

- Higher sensitivity at lower E:T ratio
- Rapid detection of the NK lytic activity (2 hrs)
- Cell Index (CI) value correlated to a % of cytolysis

% Cytolysis = \[ \frac{CI \text{ (No Effector)} - CI \text{ (Effector)}}{CI \text{ (No Effector)}} \times 100 \]

Figure Adapted from ACEA’s xCELLigence Application Note #5
1.2 IL-2 Activated NK Cells from Patients Exhibit Differential Anti-tumor Activity

**PLoS ONE** 2013, 8(10): e76928
Phenotypic and Functional Characteristics of Blood Natural Killer Cells from Melanoma Patients at Different Clinical Stages.
2.2 NK cell-Mediated Cytolysis of Daudi B Cell Lymphoma
NK cell mediated cytolysis - References citing xCELLigence:

2. Cytotoxic T Lymphocyte-Mediated Cytotoxicity

1. NK-mediated cell cytotoxicity

2. T cell mediated cell cytotoxicity
2.1 CD8 T cell-Mediated Cytolysis of SKBR3 Tumor Cells

SKBR3 co-cultured with Her-2 neu p369 specific T Cell Clone
- Control
- T Cells:SKBR3 = 1.5 :1
- T Cells:SKBR3 = 40 :1

T Cells Non-Adherent Property is Useful in Cytolytic Assay!

Erskine CL, Henle AM, Knutson KL. Mayo Clinic, USA.

Determining optimal cytotoxic activity of human Her2neu specific CD8 T cells by comparing the Cr51 release assay to the xCELLigence system.
2.1 CD8 T cell-Mediated Cytolysis of SKBR3 Tumor Cells

Continuous Kinetic Readout!

*Killer T cell Activity is Dose Dependent*

\[5^{1}Cr\text{ measured at 5 hrs vs. xCELLigence}\]

2. Erskine CL, Henle AM, Knutson KL. Determining optimal cytotoxic activity of human Her2neu specific CD8 T cells by comparing the Cr51 release assay to the xCELLigence system. *J Vis Exp*. 2012 Aug 8;(66):e3683. (Mayo Clinic, USA)


3. Antibody-Dependent Cell Cytotoxicity (ADCC)

1. NK-mediated cell cytotoxicity
2. T cell mediated cell cytotoxicity
3. Antibody-dependent cell cytotoxicity
3.1 PBMC-Mediated Cytolysis of BT474 Cells in Presence and Absence of Trastuzumab

Understanding key assay parameters that affect measurements of trastuzumab-mediated ADCC against Her2 positive breast cancer cells
Kute T, Stehle Jr JR, Ornelles D, Walker N, Delbono O, Vaughn JP. Wake Forest University School of Medicine; USA.
3.3 Killing Tumors by Activating the Immune System via Bispecific T Cell Engager (BiTE)

Clinical validation of bispecific antibody approach:
Micromet: Bispecific T cell Engager (BiTE); scFv format

Cancer patients often mount weak tumor-specific T cell responses due to numerous immune escape mechanisms of tumor cells.
3.3 Anti-tumor Antigen / CD3 Antibodies Kill Tumor Antigen-Expressing Cells Dose-dependently

Slide Decks Courtesy of Dr. Judy Young (Genentech), USA
3.3 Relative Potencies of Bispecific Antibody Clones were Compared using % Cytolysis EC50 Values at 24 hr

\[
\text{% Cytolysis} = \frac{(\text{Cl}_{\text{target only}} - \text{Cl}_{\text{target, effector, bispecific Ab}}) \times 100}{\text{Cl}_{\text{target only}}}
\]

- Clone 1 EC50 = 5.8 ng/mL
- Clone 2 EC50 = 3.9 ng/mL
- Clone 3 EC50 = N/A
- Clone 4 EC50 = N/A

Effector:Target ratio = 4:1

Slide Decks Courtesy of Dr. Judy Young (Genentech), USA
3.3 xCELLigence vs. FACS: Comparison of EC50 calculated at 24 hr

EC50 values from % cytolysis calculations are similar between xCELLigence and FACS

<table>
<thead>
<tr>
<th>Effector: Target Ratio</th>
<th>xCELLigence EC50</th>
<th>FACS EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>0.99</td>
<td>0.79</td>
</tr>
<tr>
<td>2:1</td>
<td>0.83</td>
<td>0.68</td>
</tr>
<tr>
<td>4:1</td>
<td>0.75</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Slide Decks Courtesy of Dr. Judy Young (Genentech), USA
3.4 BiTE-Mediated Cytotoxicity in PC3 Prostate Cancer Cells

**A** EpCAM/CD3 BiTE

- BiTE concentration
  - 1 µg/ml
  - 0.5 µg/ml
  - 0.1 µg/ml
  - No BiTE

**C** PBMCs:PC3 Ratio

- BiTE Concentration
  - 1 µg/ml
  - 0.5 µg/ml
  - 0.1 µg/ml

**D** CD19/CD3 BiTE

- PBMCs:PC3 Ratio
  - 20:1
  - 10:1
  - 5:1
  - 2.5:1
  - 1:1

BiTE - Bi-specific T-cell Engager


5. Kute T, Stehle Jr JR, Ornelles D, Walker N, Delbono O, Vaughn JP. Understanding key assay parameters that affect measurements of trastuzumab-mediated ADCC against Her2 positive breast cancer cells. Oncoimmunology. 2012 Sep 1;1(6):810-821. (Wake Forest University School of Medicine, USA)


4. Genetically Engineered T Cell-Mediated Cytotoxicity

1. NK-mediated cell cytotoxicity
2. T cell mediated cell cytotoxicity
3. Antibody-dependent cell cytotoxicity
4. Genetically engineered T-Cell cytolysis
Developing the Right CAR for Targeting the Right Tumor
4.1 CAR.OT-I Cells are More Effective Long-term Killers When Activated via Their TCRs.

A

% Cytotoxicity

- MC57
- MC57 OVA<sub>257</sub>
- MC57 HER2

% Lysis

1:1 2:1 4:1 8:1 16:1 32:1

E:T ratio

B

1:1 E:T ratio (killing)

- MC57-OVA<sub>257</sub>
- MC57-HER2
- MC57

51Cr Release Assay Performed at 18 hrs

xCELLigence Reveals Kinetic Difference >20 hrs

MC57 = mouse fibrosarcoma;
HER2 is recognized by CAR expressed in CAR.OT-1 cells,
OVA257 (ovalbumin peptide) is recognized by TCR in CAR.OT-1 cells.


*CAR-T Cells Inflict Sequential Killing of Multiple Tumor Target Cells.* Darcy PK & Neeson PJ et al. University of Melbourne (Australia)
4.2 Glioblastoma cells treated with DAC are susceptible to lysis by T cells engineered to express the NY-ESO-1 TCR.
Genetically Modified T-cell (e.g., CAR-T cell) Mediated cell killing – References


Summary: Applications of xCELLigence System for Cancer Immunotherapy

Immune-Mediated Tumor Cell Killing

1. NK-mediated cell cytotoxicity (8 xCELL Pubs)
2. T cell mediated cell cytotoxicity (6 xCELL Pubs)
3. ADCC (9 xCELL Pubs)
4. Genetically engineered T-Cell cytolysis (3 xCELL Pubs)
5. Macrophage mediated phagocytosis (2 xCELL Pubs)
6. Complement mediated cytolysis (CMC) (2 xCELL Pubs)
Cytotoxicity Assay Methods that Distinguish between Adherent Targets and Non-adherent Effector Cells

### End Point Methods:

1) **FACS**
   - Laborious, Cell Removal Artifacts Possible
2) **Radioisotope Release**
   - Need to Label Target Cells
3) **Enzyme Release**
   - Dying Effector Cells Could Confound Reading
4) **ATP Production**
   - Need to Wash Effectors Out of Well First

### Impedance assay:

1) **Measurements in Real Time**
2) **No Labels or Secondary Readout Assays**
3) **Non-Labor Intensive Assay Development and Performance**
Thank you [word cloud with various translations]