Agilent case study: Tmunity Therapeutics

Building a CAR-T Toolbox for More Comprehensive Assessment of Cell Therapies

Scientists developing chimeric antigen receptor (CAR) T cell products strive to maximize specificity, potency, and persistence of these therapies, but the inability to predict treatment efficacy and response remains a significant challenge.

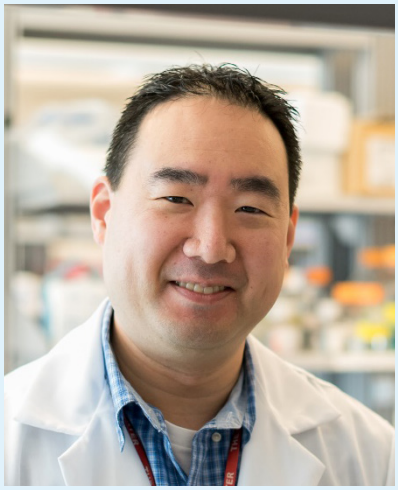
Daniel Hui, PhD, Associate Director of Analytical Development at Tmunity Therapeutics (Tmunity), addresses these challenges and highlights their multiplatform approach to build an innovative "toolbox" of orthogonal assays to provide a comprehensive set of data to characterize CAR-T cell therapeutic products.

World leaders in cell and gene therapy

Founded in 2015 by Drs. Carl June, Bruce Levine, and Anne Chew, members of the pioneering cell therapy group at the University of Pennsylvania (UPenn), Tmunity is a clinical-stage, vertically integrated biotech company focusing on developing the next generation of engineered T-cell therapies for solid tumors.

At the center of activity, the Analytical Development group is responsible for analytical testing to characterize and assess potency of the CAR-Ts for all phases of manufacturing and support the vector development group, process development, clinical operations, and translational medicine.

"Since autologous cell therapy is highly individualized medicine, I realized we would be better off developing an array of tools that capture as much information as possible," stated Hui. "We coined this approach our CAR-T toolbox."



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Designing a versatile CAR-T toolbox

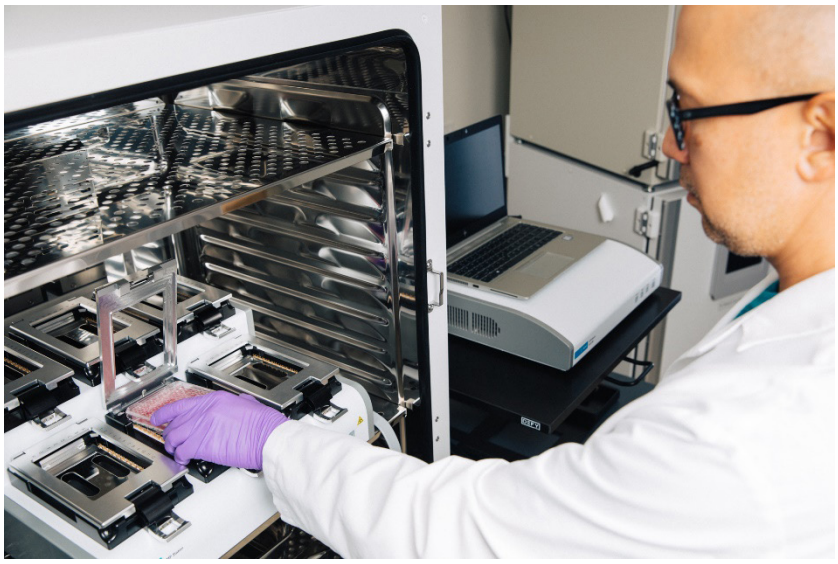
"Product characterization and potency determination is a very important piece of information for regulatory submission. I was new to CAR-T cells so I took a deep dive to see what we should look for in terms of potency, what the regulatory agencies require, and what the literature showed," said Hui.

The 2011 FDA guidance on cell and gene therapy products vaguely defines potency as the specific ability or capacity to effect a given result while the 2016 EMA guidance provides a little more detail asking for specific *in vitro* lysis of target cells, cytotoxicity, and *in vitro* cytokine production. In addition, the FDA says a single biological or analytical assay may not provide an adequate measure of potency and multiple complementary assays may be required.

Hui emphasized the importance of data collection and interpretation. "We want to use the data in a quantitative manner eventually moving towards a validated assay for GMP release of the CAR-T products," said Hui. "Cell-based assays are inherently challenging because of the variability with cell culture."

To address these challenges, "our partners at UPenn suggested the Agilent xCELLigence RTCA SP system to look at cell impedance as a measurement of cell killing," said Hui. "It was one of the first platforms we brought in-house."

"You can put together a comprehensive quantitative product report to compare to different production runs or products made at different facilities."



The Agilent xCELLigence RTCA MP instrument, placed in a standard CO_2 cell culture incubator, uses label-free cellular impedance to continuously monitor cell behavior.

Agilent xCELLigence RTCA – an essential power tool for cell analysis

Cell killing assays are simple to perform with the xCELLigence RTCA instrument, which uses specially designed 96-well plates that measure cellular impedance. As target cancer cells are grown on the plates, they impede the electron flow and generate a positive Cell Index.

When CAR-T effector cells are added, if they recognize the target cells, they can affect function and cause death. The readout is a decrease in cell index over time as cells are killed and electron flow is restored.

The xCELLigence RTCA platform can also normalize the data to accommodate different growth rates from various cell types. For example, different E:T ratios may have different values, but the software corrects for this and aligns all the different conditions into a single starting point to start comparing the data. "It makes the normal variability of a cell-based assay easier to deal with," added Hui.

"We normally test 4 to 5 samples/plate and use several E:T ratios. In a typical assay we see most of the activity within 72 hours. A nice feature is that the (xCELLigence) assay runs continually over time. You collect data continually and at any moment in time you can take a snapshot, calculate the data and compare to other end-point assays."

A test of target specificity of the Tmunity TnMUC-1 product using tissue-specific cancer cell lines for breast and pancreatic cancer demonstrated a nice killing curve as compared to control cell lines establishing proof-of-concept.

From data analysis to GMP release with RTCA Software Pro

The accompanying RTCA Software Pro Immunotherapy module provides automatic capture and intuitive analysis of immune cell killing with minimal effort. The latest version of the software now includes a compliance mode to support users in achieving requirements of FDA 21 CFR Part 11 to enable GMP release of cell therapy products.

With the immunotherapy module, easily calculate positive killing relative to control conditions and transform cell impedance data into % cytotoxicity to yield traditional killing curves. The measurement over time also adds another data dimension. "It is like doing an infinite number of cell-killing assays within one assay," said Hui. For instance, total cell killing over a set time period can be quantified by measuring total area under the curve. This might lend itself towards longer-term assays looking for persistent product effects.

"In addition, the KT_{50} value, the time it takes to reach 50% killing, provides a time element to determine how long it takes your product to reach a certain specified level of killing," said Hui. "The slope can be used as a reference to compare samples over time allowing evaluation of an inferior product on an individual basis." By performing multiple E:T ratios for pharmaceutical assessments, dose-response curves, EC_{50} or IC_{50}, can also be generated at a specific time point.

Summary

Measuring cell killing by impedance with xCELLigence RTCA is one of the many components in Tmunity's comprehensive analysis toolkit to measure the truest form of potency, cell killing. As Tmunity develops additional assays, data will be compared with orthogonal approaches.

Read the full article in GEN https://www.genengnews.com/sponsored/building-a-car-t-toolbox-for-more-comprehensive-assessment-of-cell-therapies/

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