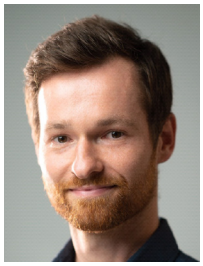
Case study

Immune cell - mediated killing of tumor cells with the AtlaZ platform

Tools:
AtlaZ

Dr. Michael Dudek, Ph.D.
featured by Nanion Technologies



Michael Dudek, Technical University of Munich, knows: attack wins games, defense wins championships. Equally, the immune system always wins under normal conditions, and the goal of his scientific career is to better understand auto-immune related diseases and to use the knowledge for discovering new treatment options.

Michael Dudek sees the immune system as a fascinating masterpiece of every living being. The tight regulated balance between resolving an infection or cancer but not damaging unaffected tissue, impressed him since he has started to study the immune system in more detail. CD8 T cells are equipped with a broad spectrum of weapons to fight against pathogens or cancer but they could also be responsible for deleterious tissue damage during chronic inflammation. In his recent studies he provided mechanistic insights into the tight balance of the functionality of CD8 T cells in organs like the liver and how this balance is out-of control during diseases like non-alcoholic steatohepatitis which could reach pandemic-like dimensions in the future (1). In-depth understanding of efficacies and responses against healthy or malfunctioning target cells like tumor cells to immune cells like CD8 T cells is highly relevant.

In the last decade immunotherapy, particularly T-cell therapy with chimeric antigen receptor (CAR) revolutionized the field of cancer treatments (2) because this "living drug" uses genetically modified T cells from the same patient to find and kill cancer cells, such as blood cancers. In general, identifying T cells that kill cancer cells *in vivo* or monitoring CAR T cell activity *in vitro* is critical to the development of successful cell therapies. *In vitro* assays are key for evaluating the potency of a treatment and also to understand the interactions between immune (effector) cells and cancer (target) cells.

Classical potency assays detect the ability of effector cells to kill target cells by flow cytometry or metabolic viability measurements (e.g. MTT or Alamar Blue (resazurin) dye) or membrane integrity (e.g. the chromium-release assay developed in 1968). However, these assays are limited to a single readout only, because the same sample cannot be measured at more than one time point.

Due to this limitation of endpoint assays, developing immune cell-based therapies has been notoriously difficult. With the AtlaZ platform, cell behavior and viability can be quantitatively measured rather than qualitatively. Readouts

Nanion's AtlaZ platform
AtlaZ allows for cellular research on cell adhesion and proliferation, cytotoxicity, GPCR, morphology and barrier function, label-free and in real-time. In up to six 96-well plates simultaneously or independently.

"The AtlaZ has a clean user interface with a cool design. The system allows for a robust and consistent data generation and analysis and there is no fear to miss out on any relevant data."

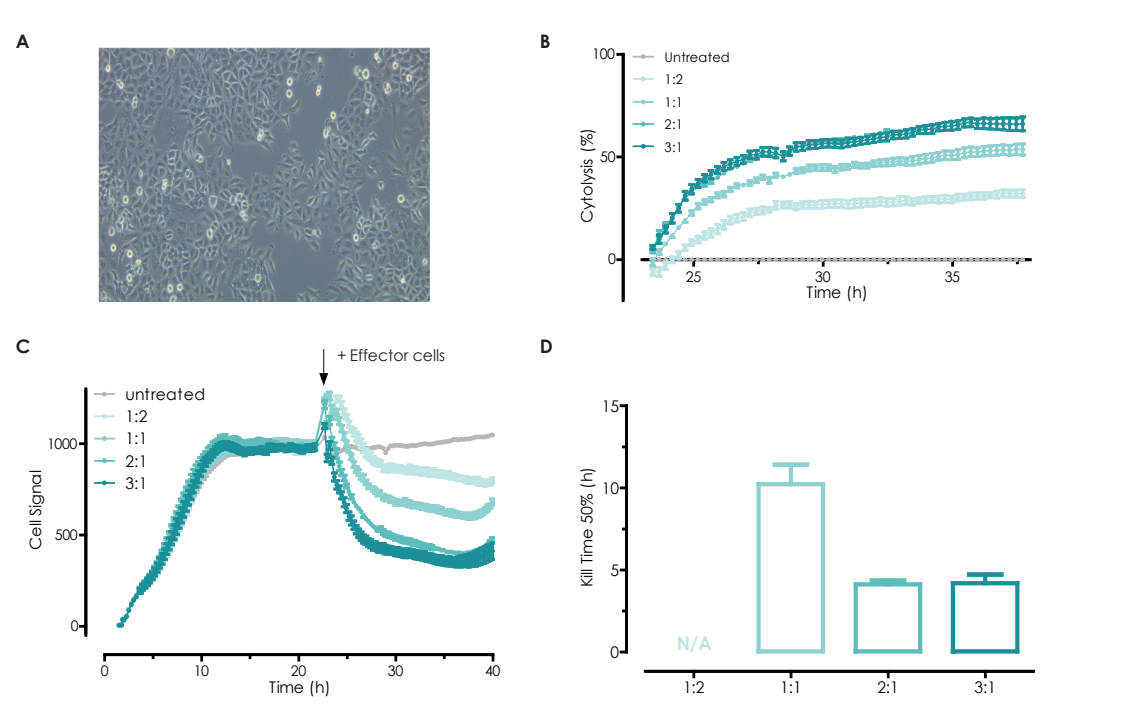
Michael Dudek, Ph.D., Technical University of Munich (TUM)

are in real-time, continuous and label free. Furthermore the system enables robust *in vitro* kinetic assays to accurately predict the *in vivo* behavior of therapies. Dr. Dudek recognized the great potential of the AtlaZ platform because the device does not only allow to precisely quantify effector responses against target cells, but also to detect novel killing mechanisms of immune cells that would not have been possible with other technologies.

Reading the impedance of planar gold-film electrodes that are used as growth substrate for adherent cells reveals changes in electrode coverage or cell behavior. Electrical impedance spectroscopy (3,4) as the methodology behind the AtlaZ system, in combination with the throughput of 6 x 96-wells allows for a so far unmet quantity and richness of information which can be gained from cells. AtlaZ allows to detect impedance signals at a huge spectrum of frequencies ranging from 0.1 kHz – 100 kHz. This allows a multitude of effects in cells to be detected, for example Transepithelial electrical resistance (TEER) signals representing barrier integrity can be recorded in parallel to cell adherance or cytolysis.

Here the human A549 cell line was used (Figure 1A), which is a widely utilized epithelian lund adenocarcinoma cell line derived from a primary lung tumor. The aim was to investigte immune cell - mediated cytolysis of A549 cells.

To investigate CD8 T cell mediated cytotoxicity, A549 cells were cultured for 4 days until they reached 80-90% confluency. With a seeding density of 40 k cells/well they were then directly plated on AtlaZ sensor plates without coating. The effector cells were obtained from the blood of healthy donors.



Immune cell - mediated killing of A549 cells. A *A549 cells at day 4 post plating.* ***B*** *Effector cells added at t = 24 h after plating of A549 cells. Increasing E:T cell ratios induced a ratio-dependent reduction of the viability of A549 cells, represented as a reducing Cell Signal, data are shown +/- SEM.* ***C*** *Cytolysis in percent (%) as calculated from the data seen in A.* ***D*** *Kill time 50 shows that 50% of A549 cells were killed after approx. 11 h (E:T = 1:1) or 4 h (E:T = 2:1 and 3:1). Data are shown +/- SEM.*

CD8 T cells were positively selected and purified using magnetic beads. After purification, the cells were stimulated with the cytokine IL-15 (10 ng/ml) for 2 days before being used for the co-culture experiments on AtlaZ. Effector cells were added to the AtlaZ sensor plate at effector to target (E:T) ratios of 1:1, 2:1, and 3:1 24 hours after target cell seeding at the desired final cell concentration.

The impedance signal of the cell monolayer was recorded every 15 minutes, and is plotted using the unitless parameter Cell Signal (Figure 1A). The data revealed that increasing E:T (Effector:Target) cell ratios induced a ratio-dependent reduction of the viability of A549 cells seen as a decrease of the Cell Signal. Uninterrupted attachment and growth of A549 cells was observed in wells with only A549 cells (Figure 1A and Figure 1B light grey line). The Kill Time 50 values were calculated to investigate at what timepoint and which ratios of effector cells killed the cancer cells to 50% (Figure 1C). These results demonstrate the capability of CD8 T cells to kill A549 cancer cells.

In summary, AtlaZ allows for label-free and real-time cellular research on cell adhesion, proliferation and cytolysis. Measurements can be performed in up to 6 x 96-well plates simultaneously or independently. Hence, multiple users can run their dedicated measurements independently, or larger screens in the same measruement mode can be executed. Michael Dudek along with Percy Knolle have diverse ongoing projects and collaborations with other departments, therefore the multi-user capability along with the outstanding capability to identify multiple diverse effects in cells over time in a 6 x 96 throughput format was key to decide on a longterm installation in the Institute of Molecular Immunology and Experimental Oncology of the Technical University of Munich (TUM). The AtlaZ system provides a versatile tool for *in vitro* cell monitoring addressing the demands for versatility, physiological relevance and throughput. Tackling the most significant challenge faced by not only TUM researchers when developing immune cell-based therapies, namely predicting treatment efficacy and quantify responses, the AtlaZ allows for reliable kinetic assays overcoming these hurdles.

Sebastian Lacher, graduate student at the Institute of Molecular Immunology (IMI) TUM is setting up an AtlaZ experiment. *Apart from Dr. Dudek, many researchers and students use AtlaZ technology in their experiments. Research at the Institute is diverse and agile - therefore in need of an easy, versatile and powerful technology in daily routines. AtlaZ is meeting that need. (Photo provided by: Michael Dudek).*

"My experience with Nanion´s technology was great, achieving highly reproducible data with the AtlaZ platform from day 1 was convincing for me."

Percy Knolle, Prof. Dr., Technical University of Munich (TUM)

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