AtlaZ as a High-Throughput System for Advanced Functional Cell Analysis to Develop Immunotherapies

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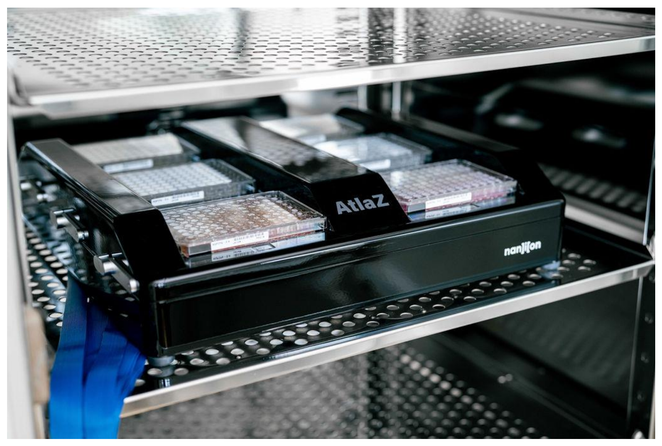
1 AtlaZ for sophisticated high-throughput quantitative live-cell analysis

Within classical treatment types for cancer, advancing therapies such as **immunotherapy** have emerged lately. Identifying T cells that kill cancer cells in vivo and monitoring **CAR-T** cell activity in vitro is critical to the development of successful cell therapies.
We here developed an in vitro system allowing for killing assays within immune-therapeutic efforts, and to search for pharmaceutical drugs or for cytotoxic effects of substances. The assay technology, Electric Cell-substrate Impedance Sensing (ECIS), offers possibilities to study the response of living cells to a stimulus in a label-free, time-resolved, and non-invasive manner. The impedance of planar gold-film electrodes that are used as growth substrate for cells reveals changes in e.g. electrode coverage or cell behavior. Real-time data provide insights regarding kinetics of cell responses. Advanced information content is obtained by using multi- frequency impedance readouts (0.1 kHz – 100 kHz): high frequency impedance is sensitive to differences in cell-confluency, making it useful for measuring proliferation or cytotoxicity, whereas low frequency impedance data reveal barrier integrity and allow to quantify cell adhesion.

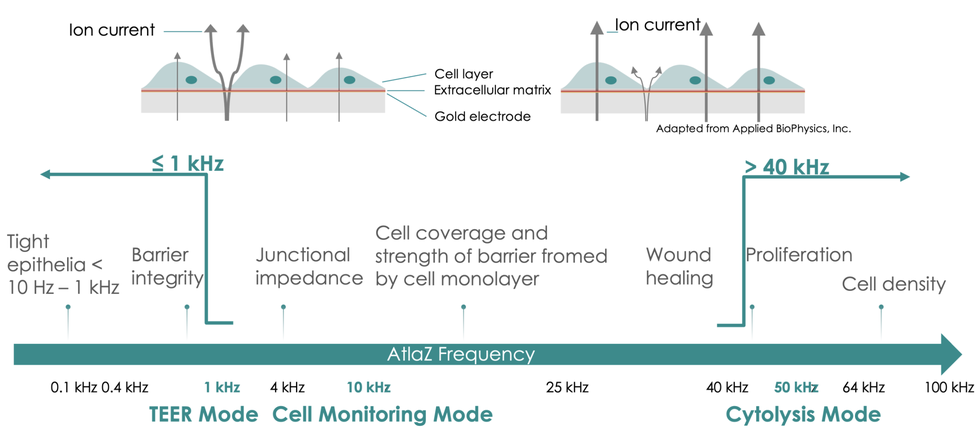


We used the **A549** epithelial lung adenocarcinoma cell line that was derived from a primary lung cancer. Effector cells co-cultured in the killing assay were purified human cytotoxic T- lymphocytes. We found that after 27 h the cytolysis of A549 cells gradually increases and reaches a maximum of 37%, 48%, 59% and 57% in the presence of the target to effector cell ratio 1:2, 1:1, 2:1 and 3:1, respectively. Respective Kill Time 50 values are shown. CAR T-cells directed to the receptor EGFR on the target cells A549 and **SKOV3** were evaluated as a next step. Furthermore, we investigated **H9C2** cardiac-like cells. We found that e.g. Erlotinib is, as expected, cardio-safe, whereas compounds with a different mechanism of action show toxic effects on the cells. For example, Vincristine which is interacting with tubulin proteins showed a concentration- and time-dependent effect on H9C2 cells.

Our aim with the newly developed 6 x 96-well platform, AtlaZ, is to elevate live cell analysis to a next level. To our knowledge, there is no platform available to quantify in vitro cell behavior like barrier function, proliferation, cytotoxicity, and more in such a time-resolved manner with this throughput.

2 Accelerating your research

..unique richness of information

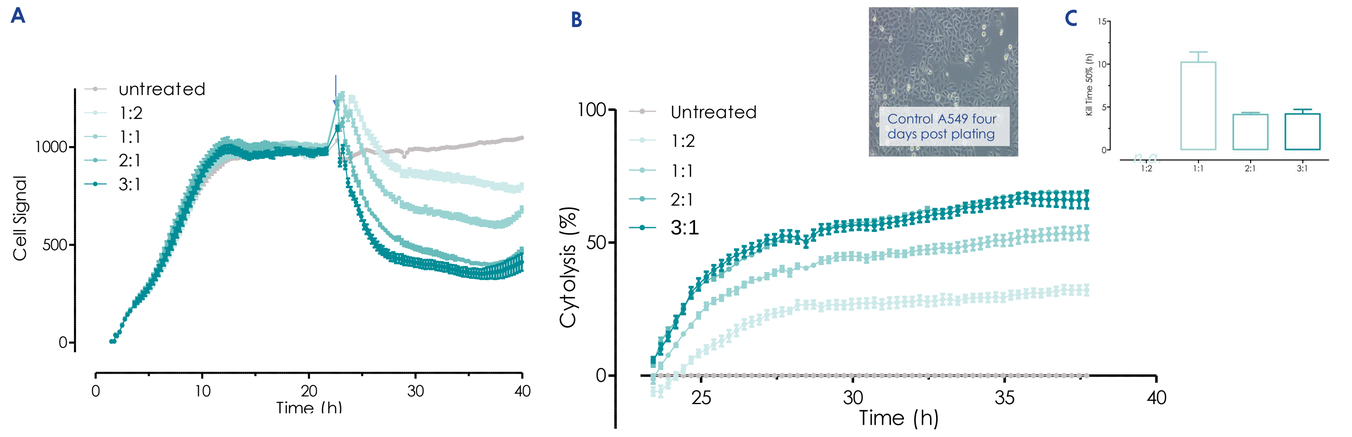


Many cell effects can be recorded in parallel in the same well. The Cell Signal is dominated by the paracellular cell layer resistance or capacitive currents across the cell membranes – depending on the readout frequency.

3 Immune-cell mediated killing assay

Real-time cell analysis

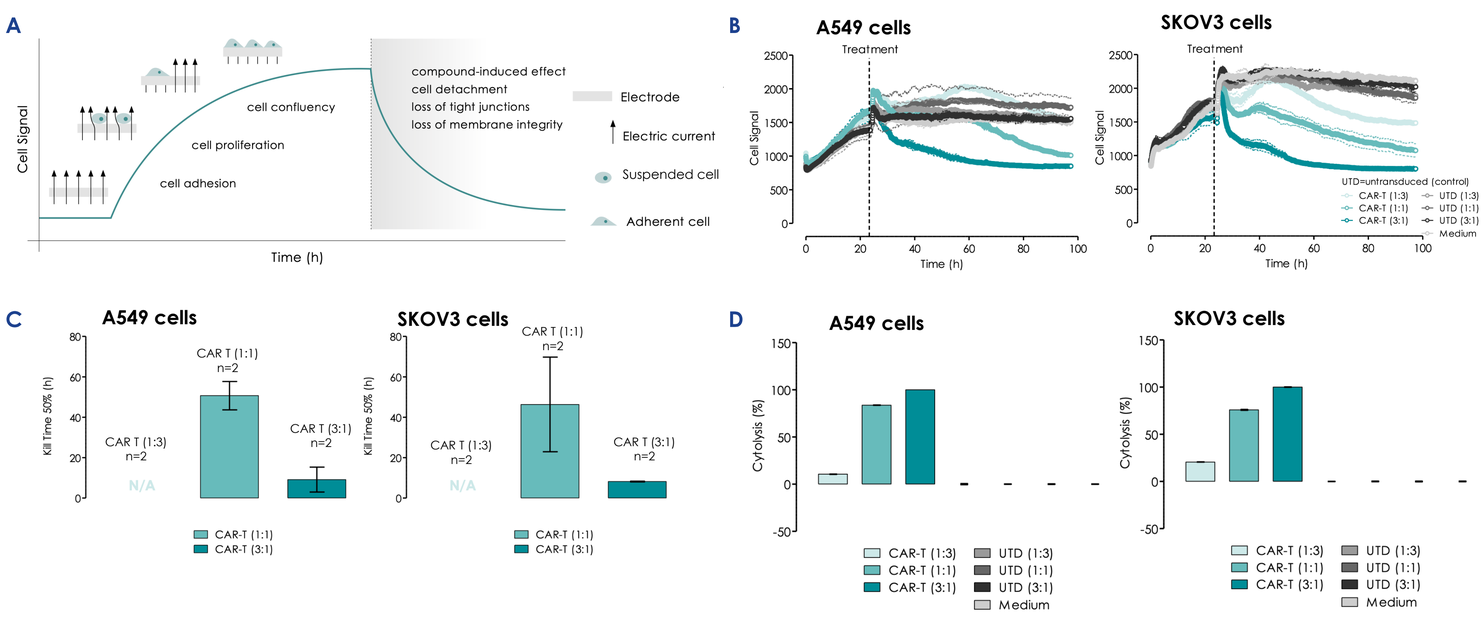
Automated graphing of results



A Effector cells were added at t = 24 h after plating of target A549 cancer cells. Increasing E:T cell ratios induced a ratio-dependent reduction of the viability of A549 cells, represented as a reducing Cell Signal. **B Cytolysis in percent** (%) as calculated from the data seen in Fig. 2. **C Kill time 50** shows that 50% of A549 cells were killed after approx. 13h (E:T = 1:1) or 6 hrs (E:T = 2:1 and 3:1).

Cancer cells *in vitro*: access kinetic and phenotypic information

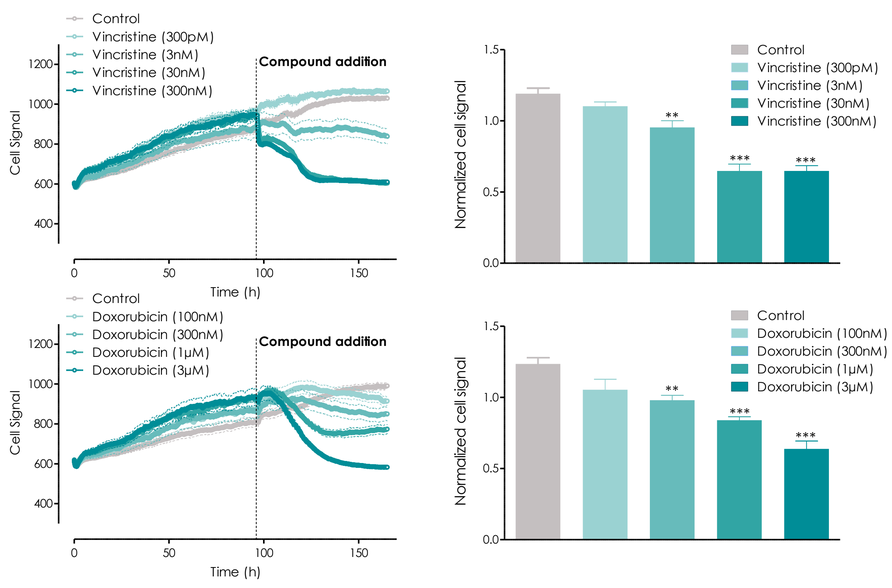
CAR T killing assay



A The Cell Signal value offers information on cell adherence, proliferation or cell death. **B** CAR T cells added in different ratios to A549 or SKOV3 cells at t = 24 h after plating. **C** Kill Time 50 values calculated from measurements as shown in B (+/- SD, n = 2 wells each. UTD = untransduced control cells). **D** The ratios 1:1 and 3:1 did induce a 84% and 100% cytolysis of A549 cells, and a 75% and 100% cytolysis in SKOV3 cells, respectively. The ratio 1:3 induced a 10% (A549) or 20% (SKOV3) cytolysis.

4 Cardiotoxicity

H9C2 differentiated towards cardiac phenotype



Vincristine, acting on myofilaments and Doxorubicin inducing apoptosis showed a time- and concentration dependent effect resulting in cell death/reduced adherence of H9C2 cells.

5 Conclusions

- AtlaZ is a quantitative live-cell analysis system and allows for cellular research on cell adhesion and proliferation, cytotoxicity, GPCR, morphology and barrier function, label-free and in real-time.
- Recordings can be performed in up to 6 x 96-well plates simultaneously or independently.
- Electrical impedance spectroscopy in combination with the throughput allows for a so far unmet quantity and richness of information which can be gained from cells, through the potential to access multiple kinetic and phenotypic information from in vitro cell cultures.