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 pharmacological drugs or other 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 27h the cytolyis 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. The respective KI 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 HPC2 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 is a cardiotoxic substance that interacts with tubulin proteins and shows a concentration- and time-dependent effect on HPC2 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

3 Immune-cell mediated killing assay

Real-time cell analysis

Automated graphing of results

4 Cardiotoxicity

HPC2 differentiated towards cardiac phenotype

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 fast and convenient quantification of rhodamine information which can be gained from cells, through the potential to access multiple kinetic and phenotypic information from in vitro cell cultures.