Integrated tumor models for immune oncology using live cell imaging for prediction of treatment efficacy *in vitro* and *in vivo*

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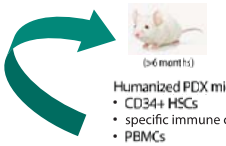
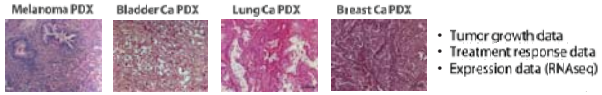
Background: The preclinical evaluation of novel cancer treatments demands comprehensive model systems in vitro that provide meaningful data before entering in vivo studies. Here we evaluate the capabilities of live cell imaging systems to evaluate novel immune therapies. Using integrated immune and tumor cell models in vitro we demonstrate, that these model systems can generate reliable data of pharmacodynamic activity of biologicals, small molecules or combinatorial approaches for further preclinical in vivo characterization.
Methods: Target tumor cell killing was assessed in vitro with immune cells (T- and NK-cells) and engagers. Tumor cells were transduced with a fluorescent marker to discriminate tumor cells from immune cells. The technology was used to determine inhibition of cell motility (re-invasion) after scratching of tumor cell monolayers. Cells were monitored using the IncuCyte. Dose-response-curves of single treatments and all combinations were generated in parallel. Active therapies were selected for further in vivo validation of immune cell killing. Humanized mice were generated by injection of CD34+ HSC or human immune cell subsets. Immune cell engraftment was monitored by FACS. To analyze the effect biologicals or small molecules, tumor cells were transplanted into these humanized mice. Tumor development and therapeutic effects were monitored by BLI measurements.
Results: Tumor cell killing by immune cells and monolayer scratch assay in 96 well format were successfully monitored in the IncuCyte. Here, data can be generated over time without the need of new samples at every time point compared with conventional end point measurements. Using antibodies directing immune cells to attack target cells extensive cell killing was observed over time. These data predicted in vivo treatment outcome in mice co-engrafted with human immune cells. After successful humanization of mice, immune cells can be directed to kill target tumor cells. Small molecule combinations were tested in vitro utilizing the metastasis/2D scratch assay. After setup of dose-response curves for two molecules combinatorial treatments were tested. Here we found a synergistic increase in efficacy. These combinations were tested in vivo to evaluate their abilities to inhibit cell motility and distant metastasis. Here we show that the in vitro assays predicted correctly the highest efficacy of combined treatments compared to mono-treatment.
Conclusion: The IncuCyte System provides data that translate our integrated model systems into in vivo studies. We have shown that activated immune cells can kill target tumor cells in vitro. These data have been validated in vivo using immune cell humanized mice. Further, immune cells, biologicals and small molecule based treatments can be tested either alone or in combination, allowing the preselection of active combinations for further development.

Available PDX models at EPO



Evaluation of treatments with:

- Cell-based therapies
- Antibody-based therapies
- Oncolytic microorganisms
- Immune modulators



- Tumor growth data
- Treatment response data
- Expression data (RNAseq)

PDX	n
Breast	39
Gastrointertinal	
Cholangio	2
Colon	183
Gastric	17
Oesophagus	4
Pancreatic	52
Glioma*	29

PDX	n
Gynaecologic	
Endometrial	9
Cervical	4
Ovarian	29
Haematological	
ALL*	10
AML	13
Lymphoma (B & T cell)	25

PDX	n
Head&Neck	85
Lung	58
NSCLC	47
SCLC	2
Melanoma	21
Mesothelioma	11
Neuroblartema*	22
Neuroendocrine	5

PDX	n
Sarcoma	31
Sarcomagaubartrix*	42
Urological	
Bladder	2
Prostate	1
Renal	41

* FCX available through the MITOCP4 platform

Experimental in vitro setup

Illustrative plate layouts

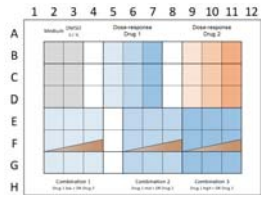
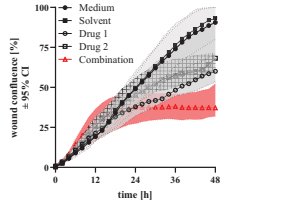


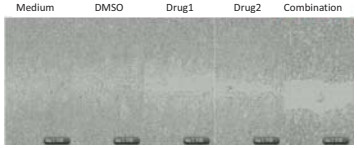
Figure 1: Drug synergy *in vitro* and *in vivo*. Two small molecule inhibitors were tested *in vitro* for their synergistic efficacy. Dose response curves of single molecules and all combinations of the tested concentrations were assessed. Next, these molecules were applied *in vivo*. Here, increased efficacy by combinaton of both molecules was sucesfully confirmed in a xenograft mouse model. Drug activity was shown by in vivo and ex vivo BLI as well as human satellite DNA qPCR.
A) Synergy analysis by combinatorial in vitro treatment using the scratch assay in the IncuCyte
B) In vivo validation of superior combination efficacy

Phenotypic inhibition by small molecule drugs

***In vitro* analysis**

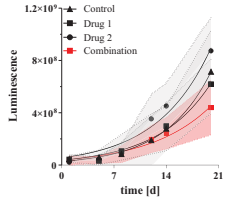
A

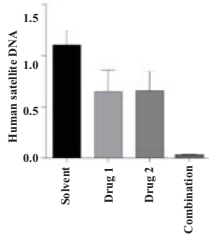




***In vivo* analysis**

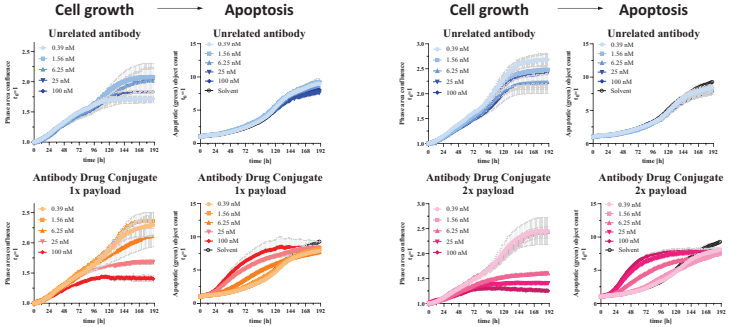
B



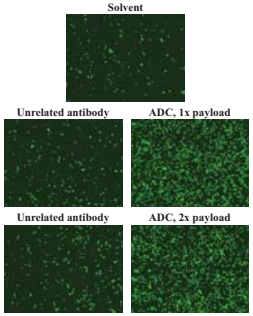


Cell killing by Antibody Drug Conjugates (ADCs)

A



B

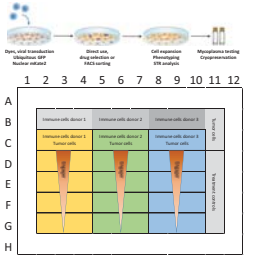


Alt wells including fluorescent caspase 3/7 staining

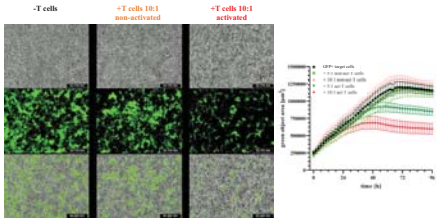
Figure 2: Cell killing efficacy by Antibody Drug Conjugates (ADCs). Antibody-drug conjugates were applied in vitro in increasing concentrations. Apoptosis induction was monitored a fluorescent caspase 3/7 dye. Similar effects were in vivo. There is a clear dose-response relationship and increased efficacy by a higher drug-antibody ratio.
A) Cell growth (phase contrast) and apoptosis induction (fluorescent marker) over time in the IncuCyte
B) Microphotographs at the experimental start (t=0 h) and 72 h

Immune cell engagement

A



Cell killing by T cells



B

Cell killing by NK cells

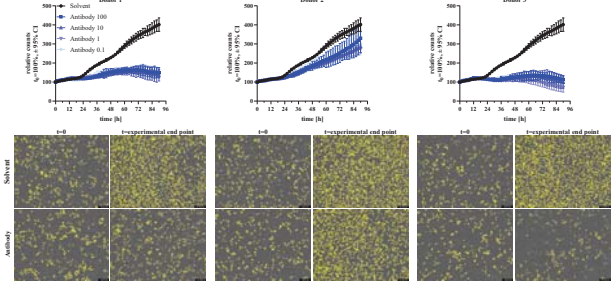


Figure 3: Immune cell efficacy *in vitro* and *in vivo*. Target cells were transduced with GFP or nuclear mKate2 to descriminate marker positive target cells from immune cells. During cocultivation of target and immune cells this ectopic fluorescence was monitored.
A) Cell killing by T cells monitored in the IncuCyte using fluorescent target cells
B) Cell killing by NK cells monitored in the IncuCyte using fluorescent target cells