Abstract Presentation
Number 4250

EXPERIMENTAL PHARMACOLOGY
& ONCOLOGY BERLIN-BUCH

Adherent and spheroid cell models of patient-derived xenograft (PDX) for drug development and translational research

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Introduction

The development of anti-cancer drugs usually begins *in vitro* and, in the best case, ends in clinical trials. Efficacy and toxicity tests are first performed in cell cultures of well-established cancer cell lines before the test substance is validated in animal studies as a prerequisite for clinical trials. Ethical considerations have led to increasing approaches for replacement methods for animal testing. A number of new approaches have been developed to simulate complex organ functions using cultured cells, including organ-on-a-chip systems and mini-organ-in 3D cultures. These models can already be used in cancer research to reduce the number of animal experiments. Pre-screens with patient-derived xenograft cell lines can help with the selection of *in vivo* models, for example. The toolbox of *in vitro* methods is filling up and the gap between *in vitro* and *in vivo* methods is closing.

Materials & Methods

We currently have a pool of more than 600 established PDX models from 21 tumor entities. From this pool, cancer tissue from glioblastoma, gastric, head and neck, lung and breast cancer were processed in single cell suspensions and cultured under defined conditions to obtain adherent cells or spheroids. The PDX *in vitro* cultures were analyzed for cellular impurities, cancer stem cell content and preservation of PDX characteristics *in vivo*. FACS analyses for tumor-specific markers, chemo-sensitivity tests and growth characteristics of PDX-derived cell lines (especially for glioblastoma) were analyzed.

Results

Of the PDX tissues of almost all entities in our library, ~80 % were obtained as adherent and/or spheroid PDX-derived *in vitro* cultures. Mouse cells were completely removed. A high percentage of these cultures showed enrichment of cancer stem cell characteristics and stem cell marker expression. How comparable are PDX and PDX derived cell lines? Tumor marker expression of selected markers correlate between the *in vivo* PDX and PDX derived *in vitro* cell culture models. RNAseq data (from *in vivo* PDX only so fare) were used to evaluate *in silico* the sensitivity of untested drugs and drug combinations to our PDX-derived glioblastoma cell lines. Initial screens with predicted candidates were performed. Promising conditions were successfully repeated in corresponding PDX animal models.

Conclusions

The newly developed technology for establishing cell cultures from PDX efficiently generates stably growing cell lines that have all the important characteristics of the original PDX. These cell lines can be used for initial pre-screens to optimize and improve the selection of pharmacologically active drugs or drug combinations before *in vivo* PDX studies are performed.

SCAN ME

Take a look at what else EPO can do!!

A) Established patient derived xenografts (PDX) in our library

❖ The 600 established PDX models are mostly molecularly characterized by transcriptome analyses (RNAseq & microarray: mutations and expression).

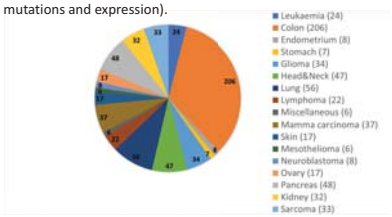

Figure 1: Diagram of the PDX models according to their entities

B) From PDX to PDX derived cell line

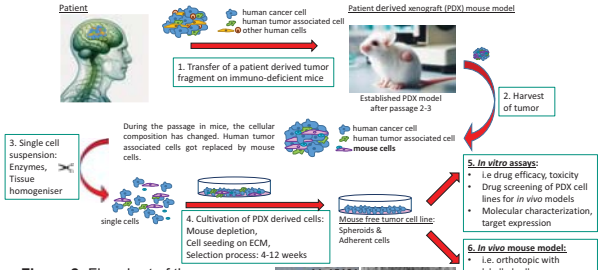

Figure 2: Flowchart of the necessary steps on the way from PDX to the in vitro assay.

C) Examples of PDX derived cell lines

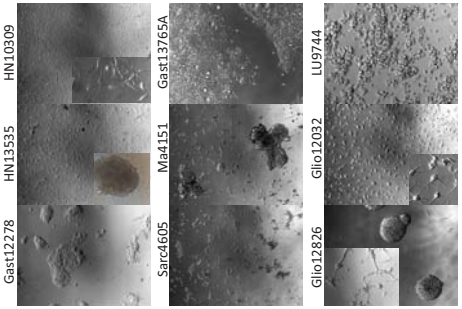

Figure 3: examples of PDX derived cells established from tumor tissue. The growth behavior is unique for almost every cell line. Currently, 26 cell lines were generated. The characterization is ongoing.

D) Pure human cell population in culture?

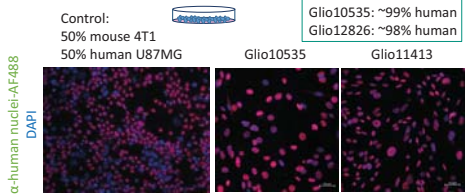

Figure 4: Immunofluorescence staining with a human specific nuclei IgG and DAPI. Double positive cells are human. DAPI only = mouse.

E) Tumor specific marker expression

PDX Glio10533 Glio11413

	relative expression level		x-fold
SOX10	500,08	5,05	56,91
DLK1	20,92	1974,57	54,21

In vivo RNAseq data: example of expression level for genes Sox10 and DLK 1

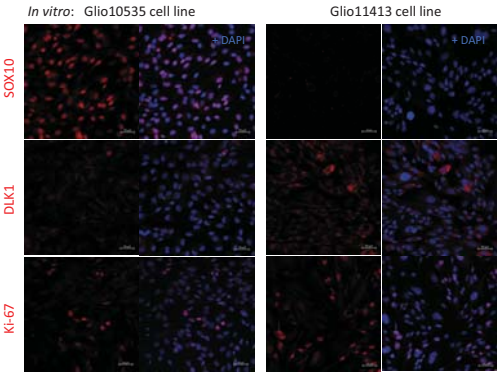

Figure 5: All PDX models in EPOs library are analyzed by RNAseq or micro-array. Can model specific enriched marker expressions be found in corresponding PDX derived cell lines? Example for Glio10535 and Glio11413 for SOX10 and DLK1. Ki-67: proliferation marker.

F) HLA, CD15 and CD133 expression

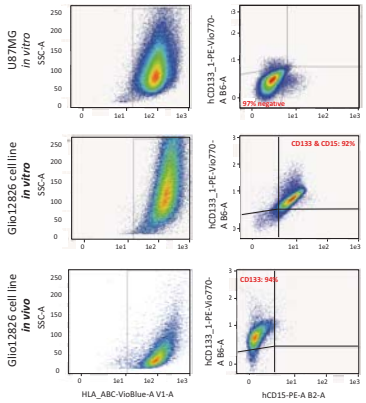

Figure 6: FACS analysis for human HLA, CD15 & CD133 in example for U87MG and Glio12826 derived cells *in vitro* and *in vivo*.

G) In vivo growth of PDX derived cells

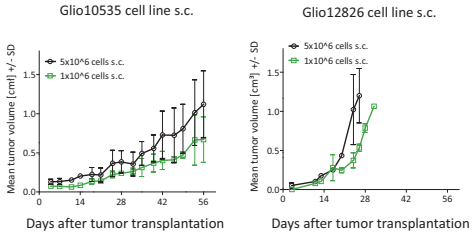

Figure 7: Growth behavior of two PDX-derived Glioblastoma cell lines *in vivo*.

H) Chemosensitivity of PDX-derived cells

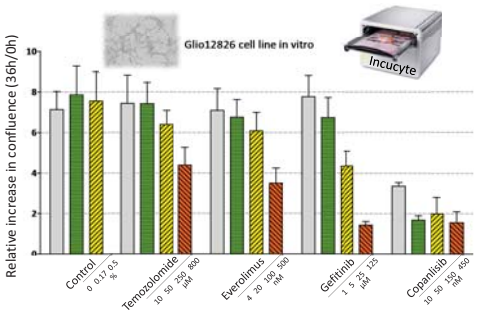

Figure 8: Real-time drug screening with PDX derived cell line Glio12826 in 96-well format. Temozolomide (SOC), Everolimus, Gefitinib and Copanlisib were tested in different concentrations. Glio12826 is a less sensitive PDX-model for Temozolomide *in vitro* and *in vivo*.

I) *in vivo* chemosensitivity of matching s.c. PDX

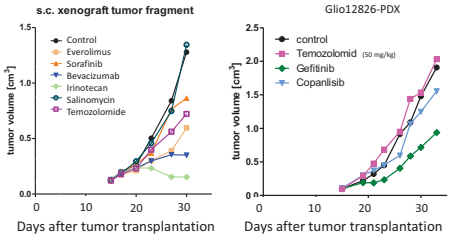

Figure 9: To fig. 8 corresponding *in vivo* PDX-model. Treatment of mice (n=3) with different drugs.

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