in vitro PDX models: 3D cultured patient-derived tumors for compound evaluation

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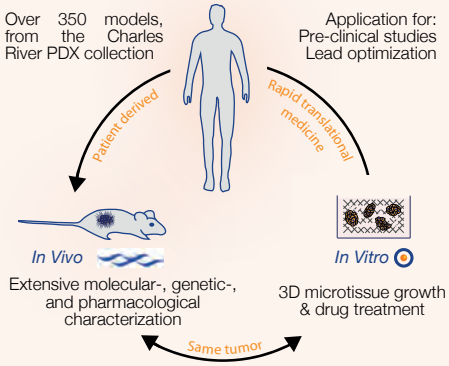
Background: Patient-derived xenograft (PDX) models in immune-compromised mice allow propagation of and compound testing in human-derived tumors ***in vivo***. To expand the potential of these human-relevant PDX models, we sought to develop 3D ***in vitro*** culture methods for PDX-derived tumor cells that show ***in vivo***-like growth characteristics, invasion and responses to therapeutics. In combination with advanced 3D image analysis methods, we created a unique high throughput in vitro PDX screening platform that not only allows efficient identification of active and selective molecules but also enables selection of the optimal PDX tumor models for subsequent validation of candidates ***in vivo***.

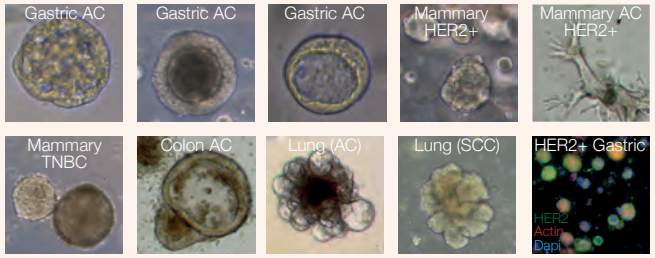
Results: Each PDX model has its own unique growth characteristics. Hydrogel and growth media composition were optimized to support growth of tumor tissues ***in vitro*** from cells derived from bladder, stomach, breast, pancreas, colon and lung cancer PDX tumors. Tumor tissues were cultured in a 384-well format and used to test chemotherapeutics (e.g. 5-FU, doxorubicin, paclitaxel, cisplatin), small molecules (e.g. erlotinib, lapatinib, trametinib, everolimus), antibodies (e.g. cetuximab, trastuzumab) and antibody-drug-conjugate (ADC, T-DM1) dose ranges. Using OcellO's 3D image analysis platform, Ominer, tumoroid growth, cell proliferation, apoptosis, invasion, cell polarity, differentiation and other aspects of cell and tissue architecture were analyzed and the effects of compound exposure on tumoroids was determined. By performing feature training based on reference compounds, we selected ±10 morphological features (out of more than 500) to generate a phenotypic signature that described the unique phenotypic change induced by each compound. Different compounds that target the same molecule were found to induce a similar morphological change whereas compounds with off-target effects could be discriminated. This approach enabled a high resolution evaluation and comparison of compound activity in an automated manner.

Conclusions: We established several PDX model-derived 3D tumor cultures in which standard-of-care and novel therapeutic agents (small molecules, antibodies and ADCs) can efficiently be screened, based on therapeutically relevant parameters and their changing morphological profile. This method enables both the ***in vitro*** selection of promising compounds in a pre-clinically relevant setting and the selection of optimum PDX tumor models for follow-up ***in vivo*** studies. This highly translational ***in vitro-in vivo*** PDX pipeline is expected to reduce attrition and increase efficiency in early drug-discovery.

Ex vivo propagation of human-relevant tumoroids



3D Tumoroid growth in extracellular matrix rich hydrogels

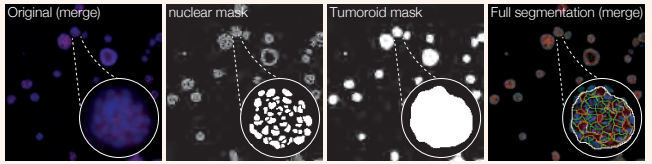


In vitro growth phenotypes of tumoroids cultured from murine PDX of various tissue origin and histotypes. Each model is grown in a optimized mix of extracellular matrix and growth factors.
Retention of a relevant tumor marker (HER2) in *in vitro* tumoroids is shown (IF).

Developed *in vitro* PDX

	Tumor Type	Model Number
1	Bladder (UC)	BXF 439
2	Bladder (UC)	BXF 1036
3	Stomach (Asian) (AC)	GXA 3038
4	Stomach (Asian) (AC)	GXA 3039
5	Stomach (Asian) (AC)	GXA 3054
6	Stomach (Asian) (AC)	GXA 3067
7	Stomach (AC)	GXF 281
8	Lung (NSCLC, AC)	LXFA 677
9	Lung (SCC)	LXFE 211
10	Lung (LCC)	LXFL 1072
11	Breast (HER2+)	MAXF 1162
12	Breast (HER2+)	MAXF 1322
13	Breast (TNBC)	MAXF 1384
14	Breast (HER2+)	MAXF 2499
15	Breast (TNBC)	MAXF MX1
16	Colon (AC)	CXF 1256
17	Colon (AC)	CXF 1753
18	Colon (AC)	CXF 1783
19	Colon (AC)	CXF 2061
20	Colon (AC)	CXF 2068
21	Colon (AC)	CXF 2049
22	Colon (AC)	CXF 2048
23	Colon (AC)	CXF 1103
24	Colon (AC)	CXF 609
25	Colon (AC)	CXF 2083
26	Pancreas (AC)	PAXF 1657
27	Pancreas (AC)	PAXF 1869
28	Pancreas (AC)	PAXF 1900
29	Pancreas (AC)	PAXF 2005
30	Pancreas (AC)	PAXF 2196

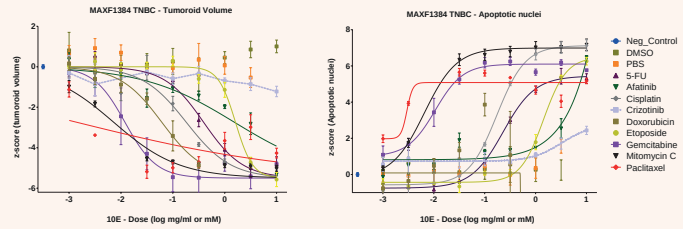
Image analysis and segmentation



Nuclei and actin signals are segmented using OcellO's analysis platform, Ominer. This allows for multiparametric measurement of morphological features to describe the change in the complete 3D structure, including:

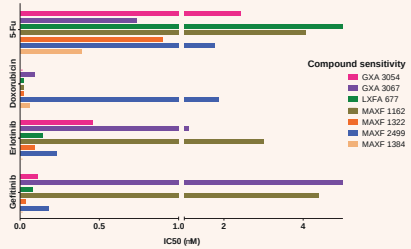
- Number of nuclei, shape, distribution
- Fraction apoptotic nuclei
- Tumoroid volume, roundness, lumen content, cell polarity
- Microtissue branching and invasion.

After segmentation: measurement of relevant features



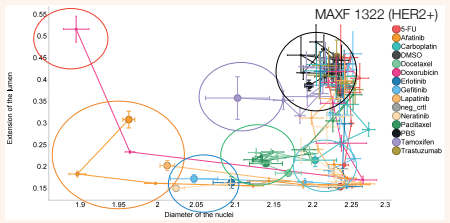
Dose dependent effects for two selected features (>500 are measured) in tumoroids derived from mammary model MAXF-1384 (TNBC). Depicted are end-points of a 7 day exposure to a panel of chemotherapeutic compounds and small molecules.

Differential drug responses



Model preselection; calculated IC50 values for reference compunds are compared amongst PDX models, identifying compound-sensitive models.

Morphologic clustering of compounds



Compound classes group in discriminatory clusters depending on the mechanism of action, allowing characterization of novel compounds by morphology.

Conclusions

Our platform allows drug sensitivity screening of Patient-derived tumor material for the purpose of pre-clinical drug development. In collaboration with Charles River, OcellO offers companion *in vitro - in vivo* PDX, combining human relevant tumor models with high-content compound evaluation.

- High-content pipeline
- Well-characterized PDX models
- Multiparametric analysis for selected features
- Amendable to small molecules, antibodies, ADC, etc
- Translational follow-up in **matching** *in vivo* models