High throughput screening of 30 PDX cell lines in a 3D ECM hydrogel platform, incorporating tumor, stroma and immune components to demonstrate simultaneous investigation of multiple anti-tumor modalities

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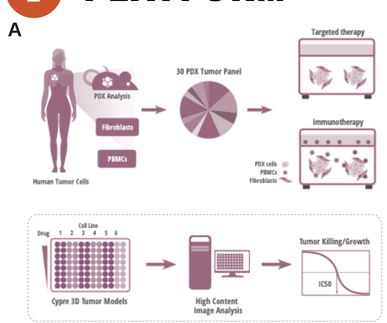
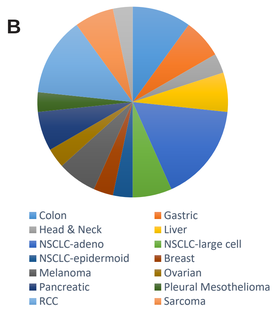
1 ABSTRACT

High throughput screening offers tangible benefits towards rapidly testing various permutations of novel or existing therapeutic agents. In particular, tumor panels that cover a range of histotypes and molecular subtypes have been previously developed, such as the NCI-60, however they utilize cell lines and, in some cases, a 2D cell culture format, which limit their translatability to preclinical and clinical trials. Moreover, the biological complexity of the tumor microenvironment (TME) has revealed a need for more translatable 3D *in vitro* tumor models that reflect the in vivo physiological outcome to therapies, particularly with the explosion of immunotherapy programs in drug discovery which target the immune compartment of the TME. Here, we describe for the first time a 3D *in vitro* PDX panel comprising 30 distinct PDX models in coculture with fibroblasts and PBMCs in engineered extracellular matrix hydrogels that display distinct similarities to the three compartments of the TME - tumor, stroma, and immune cells. The panel is constructed in a high throughput 96-well format and rapidly assays tumor growth delay and other endpoints such as tumor killing / apoptosis in a dose-dependent manner across various drug modalities such as small molecules, biologics and cell therapy. The panel has been tested against targeted therapy (Cisplatin, Cetuximab) and immunomodulatory agents (e.g. Pembrolizumab) and the results correlate to the corresponding *in vivo* data. Moreover, subsequent cytokine analysis and immunofluorescence staining of several models revealed protein signatures of cancer-associated fibroblasts and CD3+ sequestration in the tumor stroma in some 3D models, suggesting the fibroblasts' critical role in regulating the immune response. In short, the 30-PDX Panel described here represents a large step forward towards achieving translatable efficacy data at the earliest stages of drug discovery where little is known about the mechanism of action for a particular therapeutic agent or combination of agents.

2 PLATFORM





Cancer Type	Abbreviation	PDX Models
Colon	CXF (3)	94, 269, 1103
Gastric	GXF (2)	3023, 3067
Head & Neck	HNXF (1)	1853
Liver	LIXAH/ LIXFC (2)	575, 2050
Lung (NSCLC, adeno)	LXFA (5)	289, 526, 586, 629, 923
Lung (NSCLC, large cell)	LXFL (2)	430, 1674
Lung (NSCLC, epidermoid)	LXFE (1)	66
Breast	MAXFTN (1)	401
Melanoma	MEXF (2)	622, 1829
Ovarian	OVXF (1)	899
Pancreatic	PAXF (2)	1997, 2005
Pleural Mesothelioma	PXF (1)	698
RCC	RXF (4)	486, 1781, 2282, 2516
Sarcoma	SXFO, SXFS (2)	678, 1301
Uterus	UXF (1)	1138

A) A streamlined workflow for growing 3D patient-derived tumors models and assaying targeted and immunotherapy drugs using high content analysis and advanced analytics. 96-well format, 6-dose in duplicates, assayed for tumor size and/or apoptosis using high content imaging.
B) Fixed panel of 30-3D tumor models using low passage, PDX-derived cell lines across major solid tumor histotypes.

* Allogenic PBMCs and human dermal fibroblasts (HDFs) are used in the screening assay.
** Visit https://compendium.criver.com/ to learn more about the PDX lines in the panel.

4 CONCLUSION

- **The Cypre 30 PDX Panel is the 3D *in vitro* screening platform of the human tumor microenvironment (TME) comprising 30 PDX cell lines, fibroblasts and PBMCs in a patterned extracellular matrix hydrogel in 96-well plates.**
- **The 3D Panel rapidly assays both oncology and IO compounds in a 6-dose format, and moreover, demonstrates critical hallmarks of the TME such as immune infiltration through the tumor stroma.**
- **The Panel includes a range of histotypes such as colon, NSCLC, breast, pancreatic, gastric, melanoma, and renal cell carcinoma.**
- **As an example, Cisplatin, Cetuximab, and Pembrolizumab were screened in the Panel, revealing a subset of PDX responders.**
- **The Cypre 30 PDX Panel may be employed as a first step towards accelerating in vitro pharmacology of lead compounds and therapies, and for selecting PDX preclinical models in vivo.**

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3 RESULTS

Fig 1. Expemplary dose-response analysis of Cisplatin for 3D tumor growth/killing in the Cypre 30 PDX Panel.

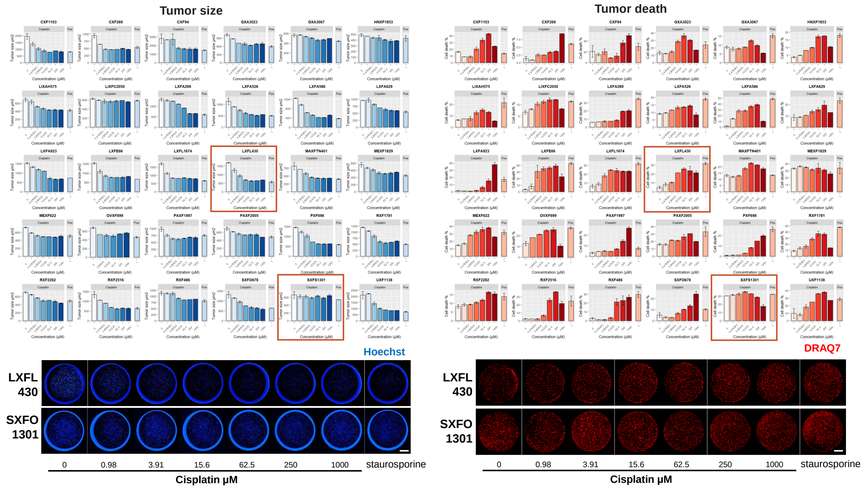

Fig 1. Dose-response profiles of Cisplatin with tumorsphere size and percentage of DRAQ7+ dead cells as the readouts. The Cypre 30 PDX panel was treated with 6 doses of cisplatin and staurosporine 1µM as positive control. The 3D assays were stained with Hoechst and DRAQ7 and analyzed with high content imaging. The lower panel shows the representative 2x images of responder LXFL430 and non-responder SXFO1301. scale bar = 1mm

Fig 2. The anti-tumor efficacy of Cisplatin, Cetuximab and Pembrolizumab in the Cypre 30-PDX Panel.

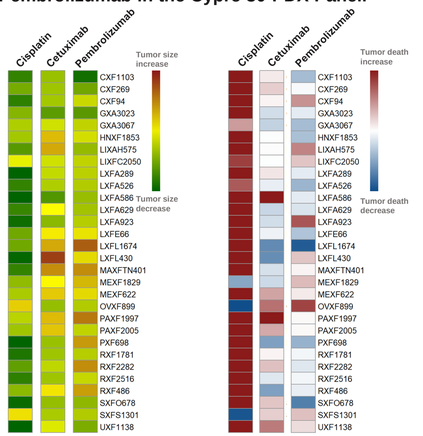

Fig 2. Screening the panel with three compounds – Oncology therapies, cisplatin and cetuximab; and IO therapy, Pembrolizumab – heatmaps were generated showing of percent change of tumor size and tumor death at E_{max}/E_0. Color scale is normalized within the panel for each drug.

Fig 3. Drug sensitivity analysis of Cisplatin, Cetuximab and Pembrolizumab in the Cypre 30-PDX Panel.

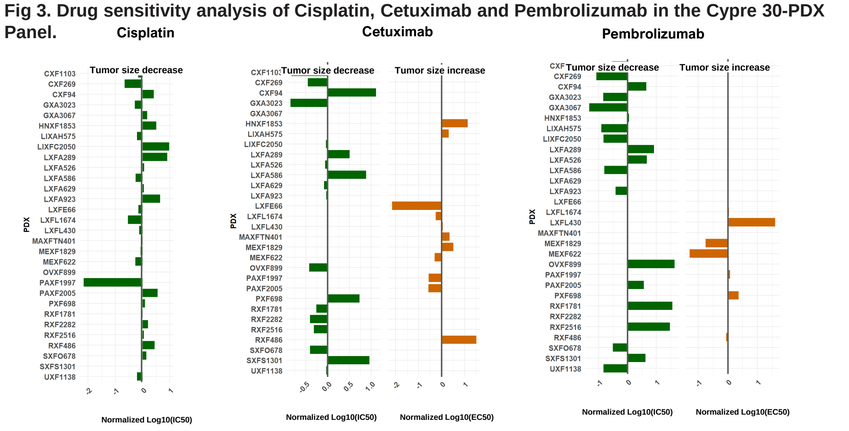

Fig 3. Panel screening reveals drug sensitivity of the 30 PDX lines to Cisplatin, Cetuximab and Pembrolizumab. EC50 were calculated from fitted dose response curves of each therapy across all the PDX lines and normalized. For cisplatin, the IC50 of tumor size reduction is shown in the bar graph. Bidirectional tumor size changes were uniquely observed in Cetuximab and Pembrolizumab screening. The PDXs are stratified into tumor size increase and tumor size decrease categories and the IC50/EC50 of each category are shown in the barplot. Individual Log10(EC50) value were normalized to mean_Log10(EC50) of the panel. Some PDXs were excluded due to a lack of IC50 response.

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