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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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 histologies and molecular subtypes have been previously developed, such as the NO-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 models that reflect the in vivo physiological outcome to therapies, particularly with the explosion of immunotherapies drug programs in drug discovery which target the immune components 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 assesses 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 preclinical monotypic cell line screening and immunofluorescence staining of several models revealed protein signatures of cancer-associated fibroblasts and CO2 +/− supplementation in the tumor growth 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.

PLATFORM
A) A streamlined workflow for growing 3D patient-derived tumors models and assaying targeted and immunotherapeutic 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) Panel of 30 PDX model systems using low passage, PDX-programmed cell lines across major solid tumor histotypes.

RESULTS

Fig 1. Exemplary dose-response analysis of Cisplatin for 3D tumor growth/killing in the Cypre 30 PDX Panel. Fig 2. The anti-tumor efficacy of Cisplatin, Cetuximab and Pembrolizumab in the Cypre 30-PDX Panel.

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

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 accelerated in vitro pharmacology of lead compounds and therapies, and for selecting PDX precritical models in vivo.