Cypre 3D *in vitro* tumor model platform for screening patient-derived NSCLC, CRC and RCC tumors with targeted therapy and immunotherapy

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1 INTRODUCTION

Significant challenges remain in developing *in vitro* oncology assays that correlate to *in vivo* outcomes, and in particular, including the stromal compartment to effectively assay immuno- therapy. Matrix–free systems, such as 3D tumor spheroid assays or 2D culture, fail to recreate critical features of the tumor microenvironment such as immune infiltration through extracellu- lar matrix-enriched stroma. Matrix-based systems such as basement membrane extract (BME) or Collagen I suffer from batch variability, as well as handling issue that impair their utilization in high-throughput assays.

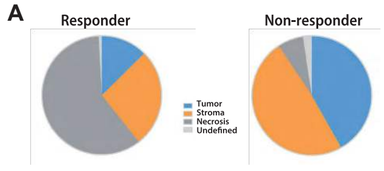
To address these challenges, we developed the novel Cypre 3D hydrogel platform to culture tumor cells and various stromal cells in a biocompatible and chemically-defined ECM hydrogel, layered in 96-well plates using a 3D patterning technology[1]. This system is highly reproducible, and the optical clarity of the system enables phenotypic screening such as spheroid size, tumor killing and T cell infiltration using high-content confocal imaging.

Here we performed both oncology and immuno-oncology screening on a selection of 15 PDX-derived cell lines from the Charles River Tumor Model Compendium, all of which have been previously characterized for their histopathological features and response to immune checkpoint inhibitors including Ipilimumab (a-CTLA4) and Nivolumab (a-PD-1)[2]. As over 30% of non-responder tumors display desmoplasia and the immune exclusion phenotype, fibroblasts were added to the culture to mimic stromal cues of the TME. IC50 of tumor killing/growth were determined in both tumor and tumor-fibroblasts conditions.

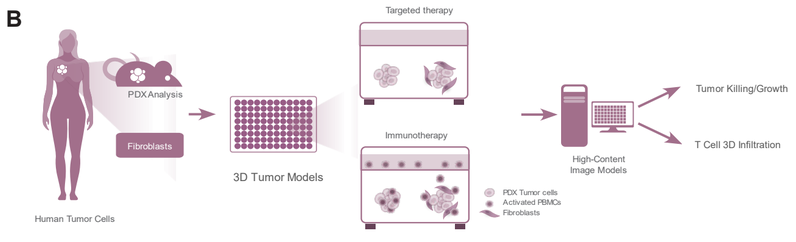
1.Hribar KC, Wheeler CJ, Bazarov A *et al.* A simple three-dimensional hydrogel platform enables ex vivo cell culture of patient and PDX tumors for assaying their response to clinically relavant therapies. *Mol. Cancer Ther.* 18(3), 718 (2019)
2.Roman S, Holt S, Schueler J, Immuno-oncology: developing integrated approaches toward clinical success of biologics and small-molecule modulators. F*uture Drug. Discov.* 2(2), FDD23 (2020)

2 RESULTS

Fig 1. a PDX-fibroblast *in vitro* platform for studying cancer stromal cells



(A) Classification of H&E histology staining between PDX tumors that respond to checkpoint inhibitors (responder) vs. those that did not respond to CPI (non-responder). The non-responder tumors have increased stroma.



(B) Using proprietary *in vitro* 3D assays, the Cypre immuno-oncology platform models key signatures of the tumor microenviroment. In this assay, PDXs of solid tumor origins are patterned into layers of Cypre's VersaGel®, a biocompatible and chemically defined extracellular matrix hydrogel, using the Symphony®3D hydrogel pattern- ing technology for standard 96-well plates. Fibroblasts are optionally co-cultured with the tumor cells to mimic the stromal region of the tumor microenvironment. The 3D tumor assays are then treated with targeted therapy or additionally with peripheral blood mononuclear cells (PBMCs) for immuno-oncology (IO) assays. The assays are subsequently analyzed via high-content confocal imaging analysis for tumor growth, immune cell infiltration and T cell-mediated tumor killing.

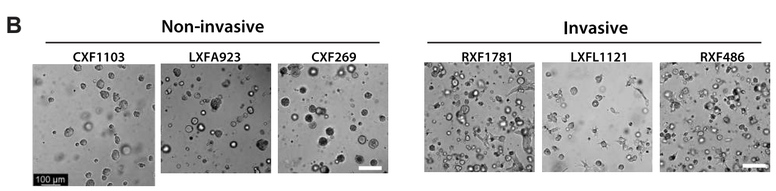
Fig 2. Pharmacological characterization of a panel of 15 PDX-derived cell lines in the 3D Tumor Model Platform

Table 1: The PDX lines and corresponding targeted therapy and immunotherapy

Cancer Type	Abbreviation	Models	Targeted Therapy	Immunotherapy
Colon	CXF (2)	1103, 269	Oxaliplatin, Cetuximab, 5-FU	Bevacizumab, Pembrolizumab
Lung (NSCLC, adeno)	LXFA (4)	289, 526, 923, 1647	Docetaxel, Cisplatin, Afatinib	Atezolizumab, Pembrolizumab
Lung (NSCLC, large cell)	LXFL(3)	529, 1121, 1674	Same as LXFA	Same as LXFA
Lung (NSCLC, epidermoid)	LXFE (2)	66, 2478	Same as LXFA	Same as LXFA
Renal cell	RXF (4)	2516, 486, 1781, 1183	Sunitinib,Temsirolimus, Pazopanib	Bevacizumab, Pembrolizumab

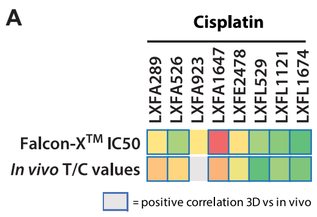
* Allogenic PBMCs and human dermal fibroblasts (HDFs) are used in the screening assay.

** Visit https://compendium.criver.com/ to learn more available CRL PDX lines.

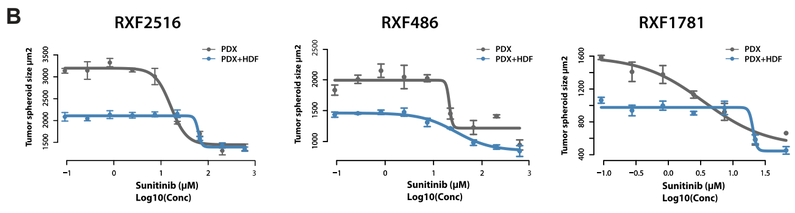


(B) The PDX lines cultured in the Cypre platform display a spectrum of the invasive phenotype. scale bar = 100µm

Fig 3. Preclinical profiling of targeted therapy using the Cypre platform



(A) *In vivo* drug response was well predicted by the standard Cypre oncology assay. Heat- map of *in vitro* IC50 values for cisplatin from the Cypre platform compared to *in vivo* IC50 values (mice). All seven NSCLC lines show a positive correlation between the Cypre plat- form and the corresponding tumor *in vivo*.

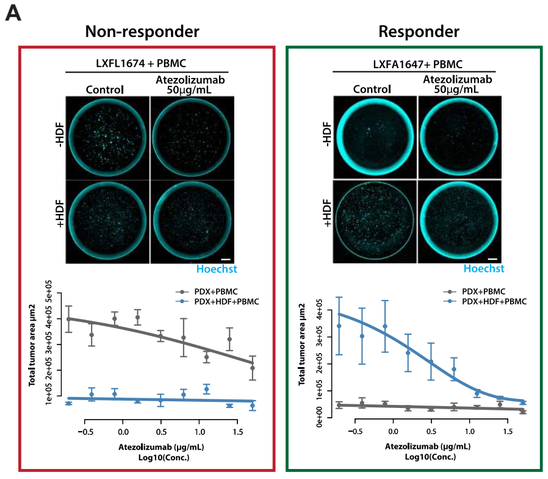


IC50 (µM)

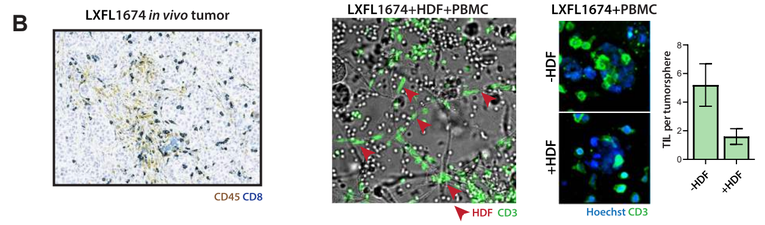
	RXF2516	RXF486	RXF1781
-HDF	10	20	3
+HDF	22	25	11

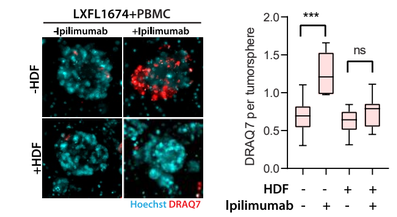
(B) Fibroblasts desensitize renal PDX lines responding to Sunitinib targeted therapy. Representative analysis of three Renal PDX lines in the standard oncology assay with Sunitinib, with tumorsphere sized as the output which generated IC50 value curves. RXF2516 and RXF1781 show a marked increased IC50 values when co-cultured with HDFs.

Fig 4. Fibroblasts modulate PDX lines responding to immune checkpoint inhibitors using the Cypre Immuno-Oncology platform



(A) Addition of fibroblasts modulates the T-cell killing in immunotherapy and predicts the *in vivo* response. Representative 2x images and analysis includes non-responder LXFL1674 (left) and responder LXFA1647 (right) cell lines with or without fibroblasts in Atezolmab IO assays, with tumorsphere size as the readout to generated IC50 value curve. Fibroblasts block the drug response in non-responder LXFL1674, but enable the drug response in responder LXFA1647. scale bar = 1mm





(B) Non-responder LXFL1674 displays immune exclusion phenotype in the Cypre IO assay. In LXFL1674 tumor *in vivo*, majority of immune cells adhere to the fibroblasts and preventing their infiltra- tion into the tumor. This immune exclusion phenotype was also observed in the ys

immune exclusion phenotype in the Cypre *in vitro* assay, whereby HDFs associated with CD3+ T cells and prevented their infiltration into LXFL1674 tumorspheres. As a result, HDFs blocked T-cell mediated tumor killing and contributed to immunotherapy resistance.

S CONCLUSIONS

- **Cypre is a novel 3D hydrogel patterning platform that generates 3D PDX tumor assays in a 96-well format.**
- **Immuno-Oncology assays with PDX, fibroblasts and PBMCs recapitulate the immune infiltration through the tumor stroma that is seen in the physiological TME.**
- **Fibroblasts modulate the PDX response to targeted therapy and immunotherapy.**
- **The optical clarity of Cypre hydrogels enable phenotypic high content screening of tumor growth, killing, and immune cell infiltration in situ, as well as downstream analyses such as cytokine, RNAseq, flow cytometry (in development).**
- **The Cypre *in vitro* platform supports the rapid screening of targeted therapy and immunotherapy against numerous PDX models from CRL Tumor Model Compendium and the pre-selection of PDX *in vivo* models for preclinical studies.**