

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

Bin Xue<sup>1</sup>, Julia Schöler<sup>2</sup>, Timothy Jensen<sup>3</sup>, Kolin C. Hribar<sup>1</sup>

<sup>1</sup> Cypr Inc. San Francisco, CA

<sup>2</sup> Charles River Discovery Research Services Germany, Freiburg, Germany

<sup>3</sup> Charles River Laboratories, Morrisville, NC

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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 extracellular 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 Cypr 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<sup>1</sup>. 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)<sup>2</sup>. 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 relevant 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. *Future Drug. Discov.* 2(2), FDD23 (2020)

## 2 RESULTS

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

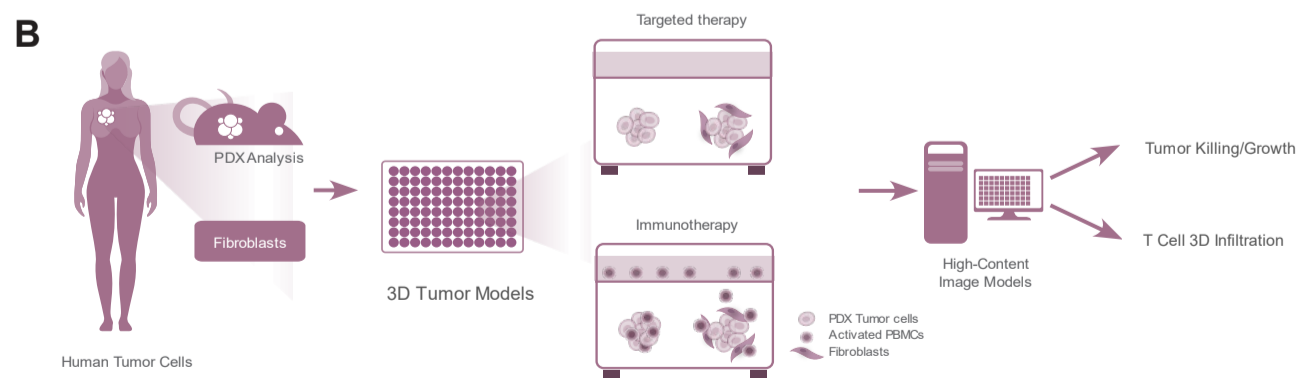
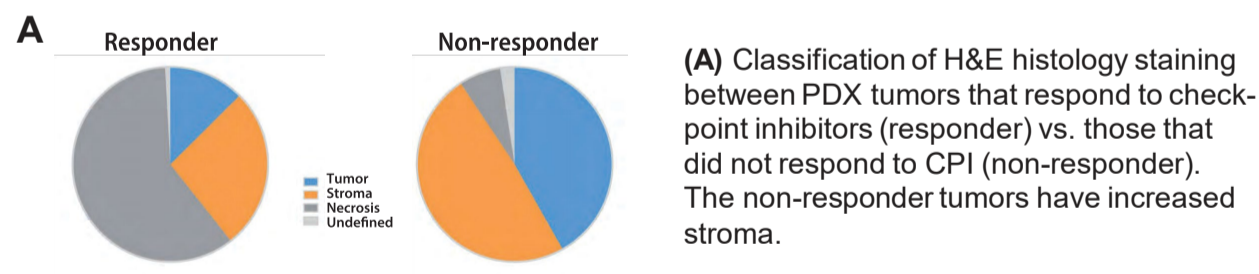


Fig 2. Pharmacological characterization of a panel of 15 PDX-derived cell lines in the 3D Tumor Model Platform

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, Temezirolimus, 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.

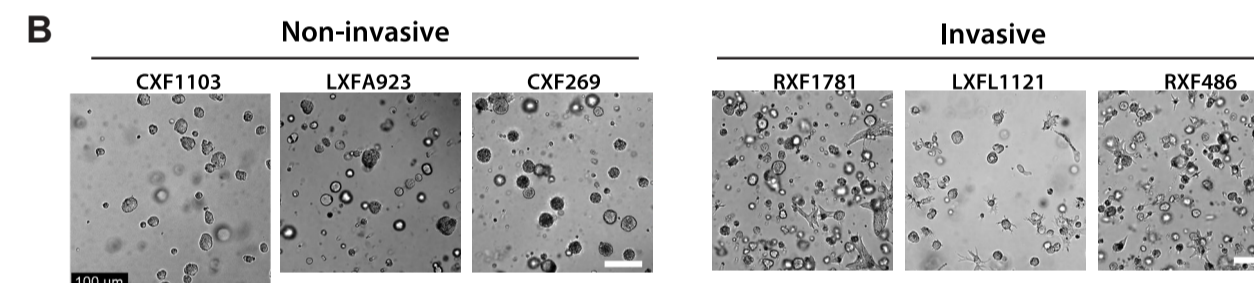
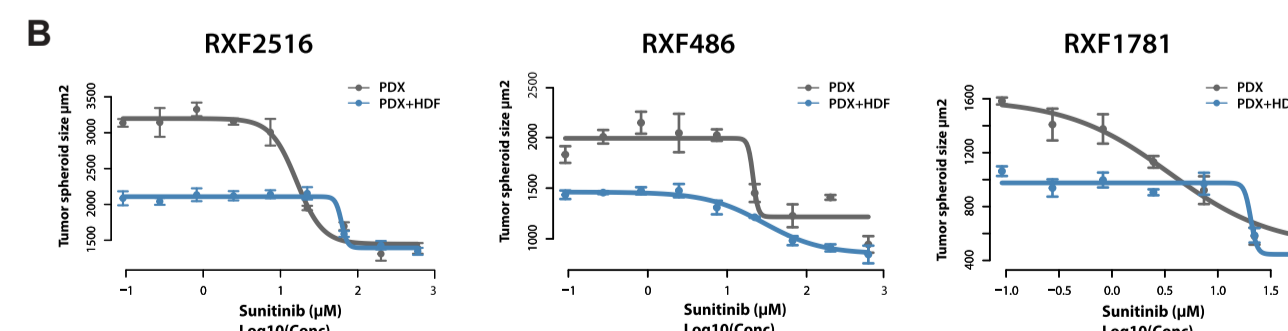
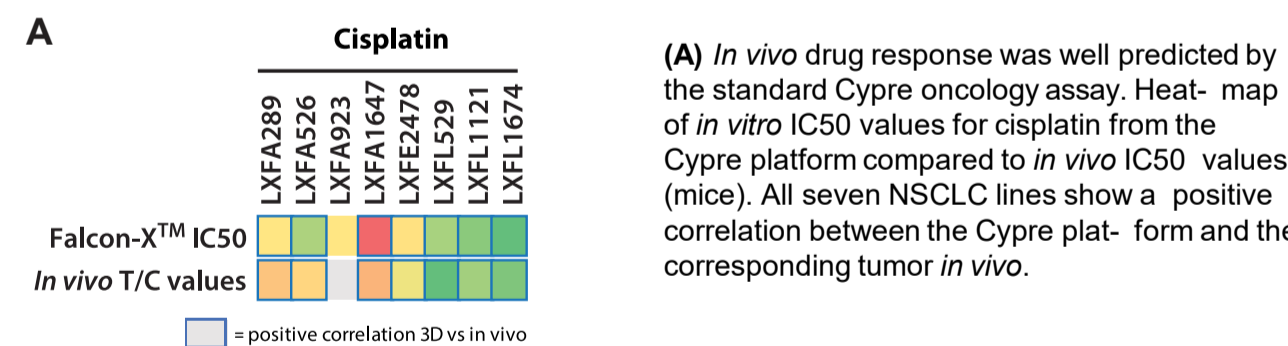
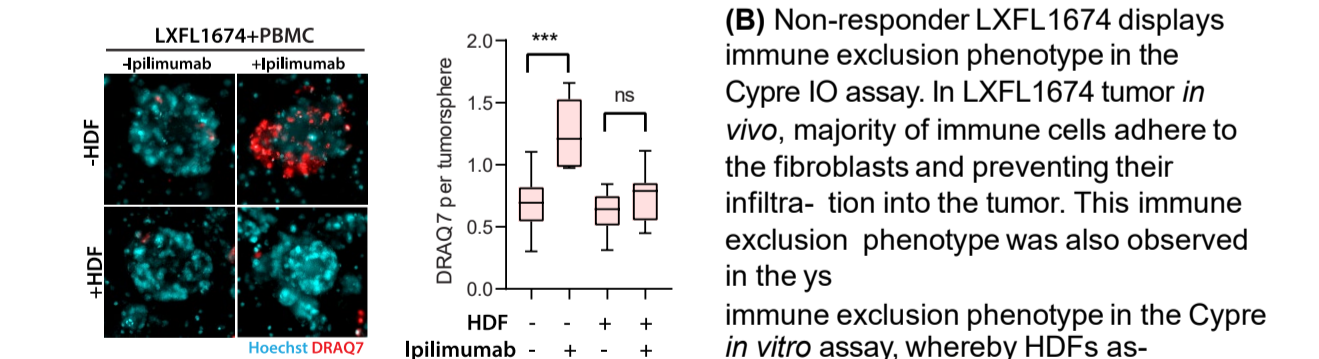
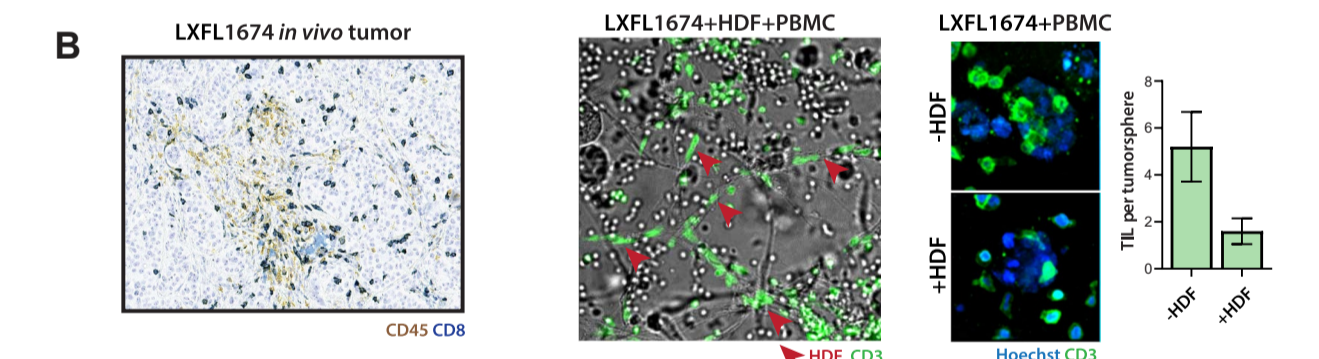
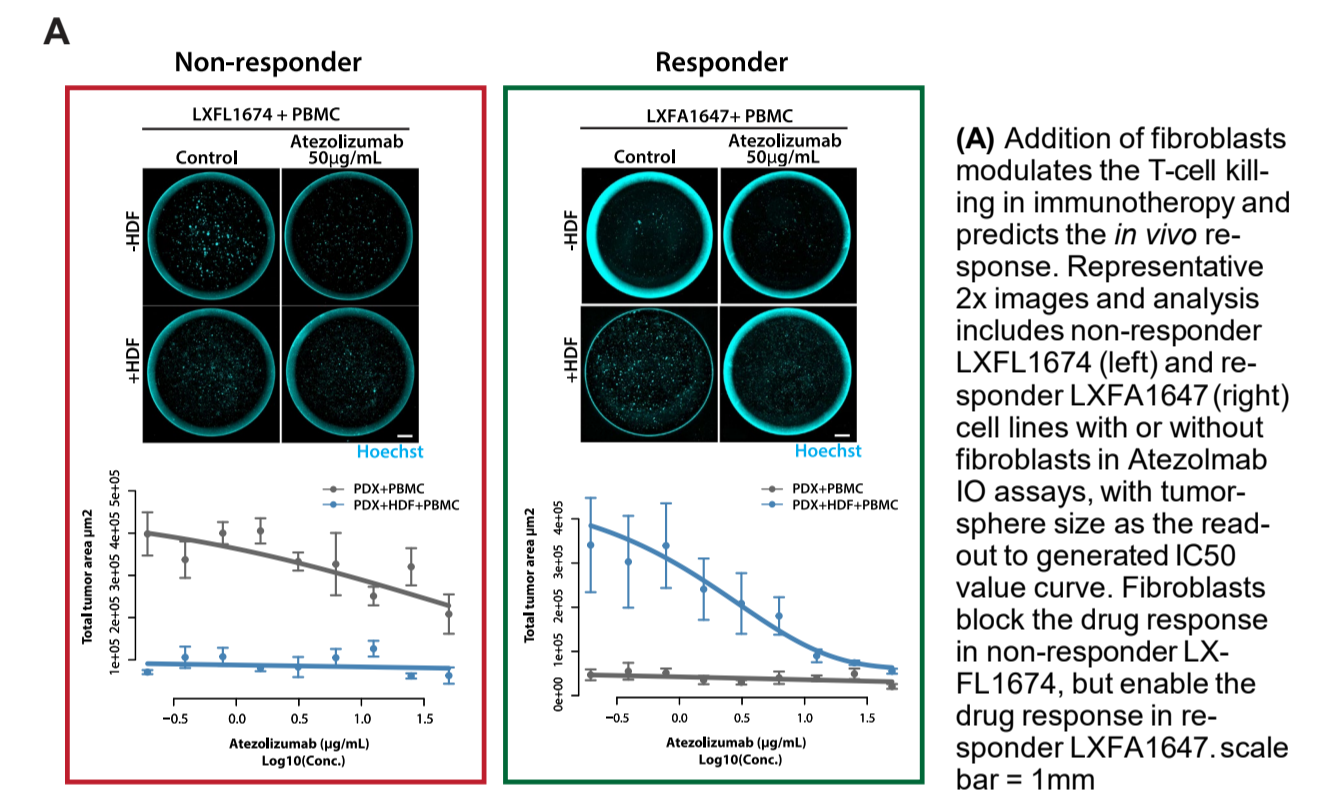


Fig 3. Preclinical profiling of targeted therapy using the Cypr platform



IC50 (µM)			
	RXF2516	RXF486	RXF1781
-HDF	10	20	3
+HDF	22	25	11

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



## CONCLUSIONS

- Cypr 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 Cypr 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 Cypr *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.