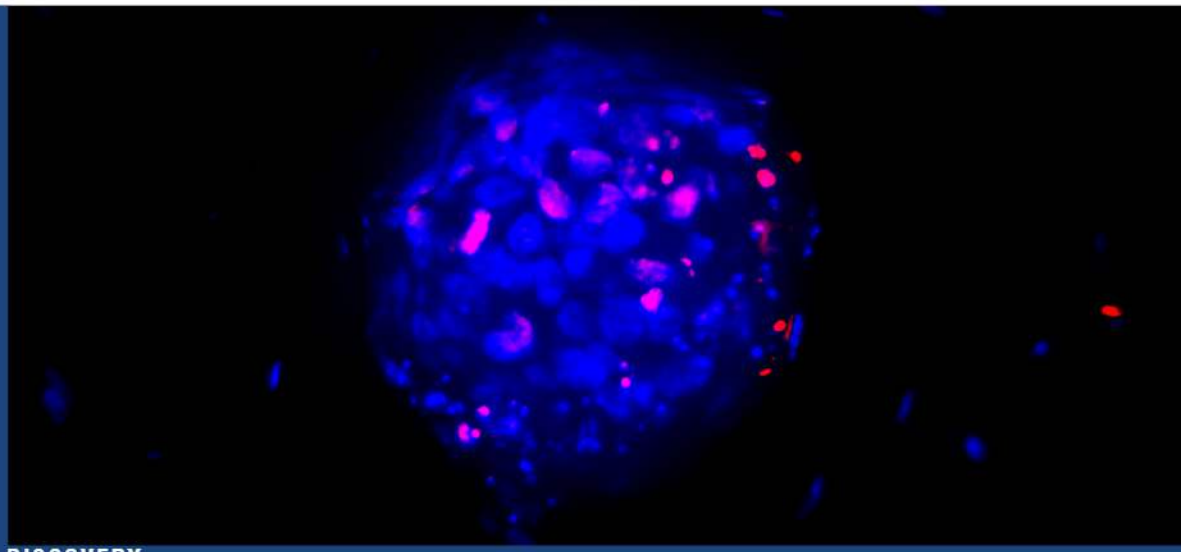
charles river

Summary

Cypre's 3D tumor assays rapidly screen cancer therapy and immunotherapy agents across a panel of genomically annotated PDX lines with endpoints of tumor growth, cytotoxicity, and T cell-mediated killing by infiltrating immune cells through the stromal matrix.

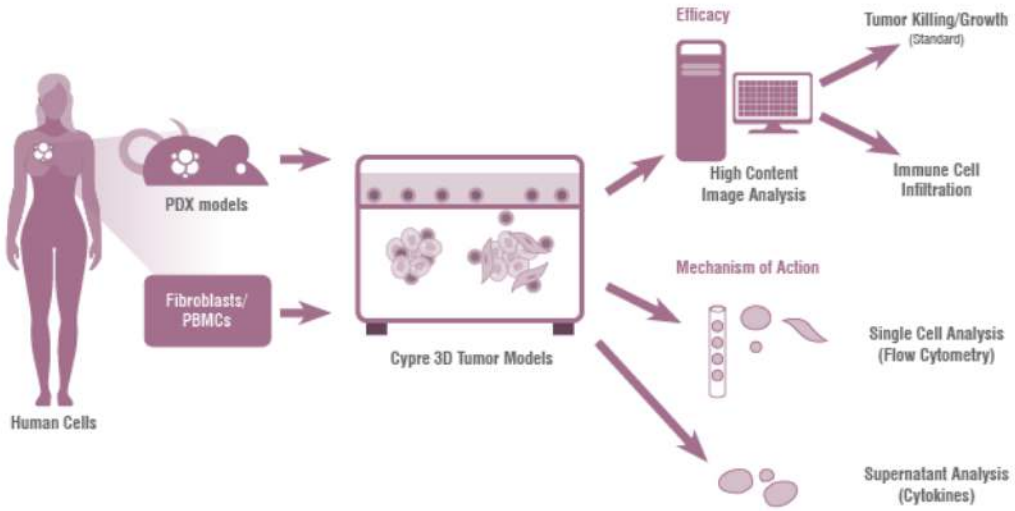


DISCOVERY

3D Engineered Hydrogel Oncology and Immuno-Oncology Assays Using the Cypre 3D Tumor Model Platform

Click to Learn More

Using proprietary *in vitro* 3D assays, Cypre's immuno-oncology platform models key signatures of the tumor microenvironment (TME), such as tumor growth and invasion, immune cell infiltration through the extracellular matrix stroma and stromal fibroblasts, and subsequent T cell-mediated tumor killing. Using Cypre's VersaGel® and Symphony® 3D hydrogel patterning technology, the platform reproducibly generates 3D patient-derived xenograft (PDX) tumor assays in 96-well plates that are subsequently analyzed via high content confocal image analysis. Cypre's platform ushers in new insights into the TME at unprecedented clarity, speed, and correlation to the PDX *in vivo* outcome, resulting in confidence to advance lead candidates to preclinical studies.



Contact an Oncology Expert

Visit https://www.criver.com/consult-pi-ds-hydrogel-3d-tumor-screens

Contact an Oncology Expert

Visit https://www.criver.com/consult-pi-ds-hydrogel-3d-tumor-screens

Assay Setup

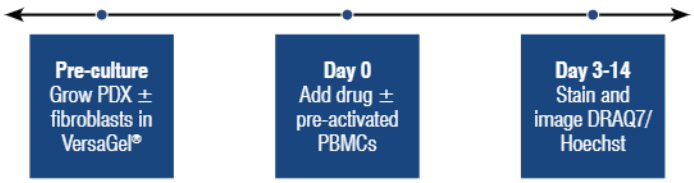
In this assay, PDX-derived tumor cell lines or *ex vivo* tissue of solid tumor origins (e.g., colon, NSCLC, renal, breast, pancreatic, gastric, melanoma) are engineered into layers of Cypre's VersaGel®, a biocompatible and chemically defined extracellular matrix hydrogel, using the Symphony® 3D gel patterning technology for standard 96-well plates. Fibroblasts are optionally co-cultured with the tumor cells to mimic the stromal region of the tumor microenvironment. Moreover, the platform has the capability of modulating the 3D stromal architecture as it relates to fibroblast and matrix density surrounding the tumor cells.

The 3D tumor assays are then treated with with test agents (oncology assays) or additionally with pre-activated PBMCs or cell therapies for immuno-oncology (IO) assays. PBMCs are initially added into the cell culture medium above the 3D gel. Over time, they gradually infiltrate and target tumorspheres inside the gel in response to immunomodulators.

The assay effectively recapitulates the immune infiltration through the stroma that is seen in the physiological TME prior to tumor killing, delivering translationally relevant results. Several assay versions are available:

- **Tumor killing:** High content confocal imaging is used to analyze drug-induced toxicity using the Hoechst nuclear and either Caspase 3/7 apoptosis or DRAQ7 dead cell stains.
- **Tumor growth delay:** High content confocal imaging is used to analyze tumor size reduction.
- **Immune cell infiltration:** 3D immunofluorescent images of infiltrating immune cells (e.g., CD3+) within the VersaGel® layer.
- **Additional Analytics:** Flow cytometry of digested 3D tumor models, or cytokine panels of the supernatants

PBMC (IO Assays only):	Allogeneic
Tumor:	PDX of solid tumor origin (e.g., colon, NSCLC, renal)
Fibroblast:	Human dermal fibroblast (HDF)
Negative control:	No drug
Analysis:	Endpoint
Readout:	Image analysis of tumor size and count using Hoechst stain (Oncology Growth Assay and IO Growth Assay); Dead cells using DRAQ7 or Caspase 3/7 stains (Oncology Cytotoxicity Assay); Custom IF analysis



Contact an Oncology Expert

Visit https://www.criver.com/consult-pi-ds-hydrogel-3d-tumor-screens

Cypre 3D Tumor Model Platform Assay Setup:

Assay Performance

Representative data shown in Figure 1 and Figure 2 below includes colon and NSCLC PDXs in VersaGel® with or without fibroblasts and with or without PBMCs, tested with Oxaliplatin or Afatinib (for the oncology assays) and Atezolizumab (for the IO assay).

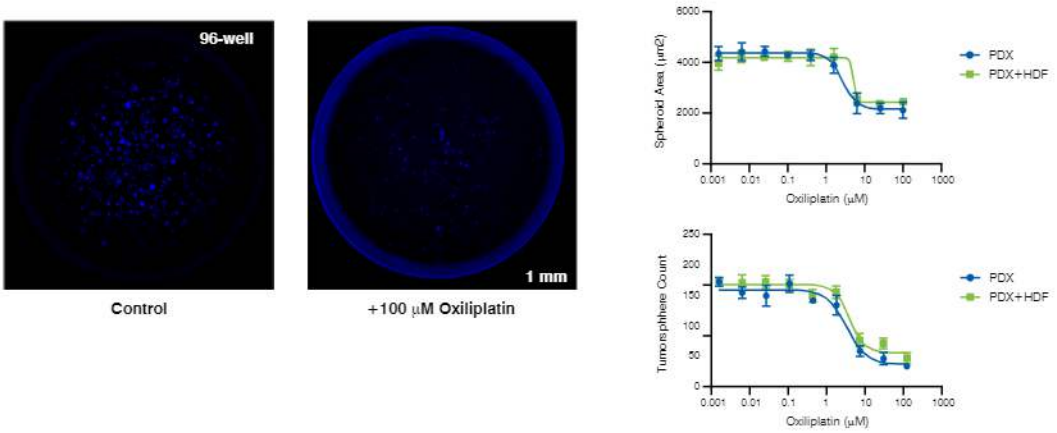


Figure 1: Oncology Growth Assay readout. Representative 2X confocal images (left) and analysis (right) in the Standard Oncology Assay, with tumorsphere size and count as the output which generate IC50 value curves.

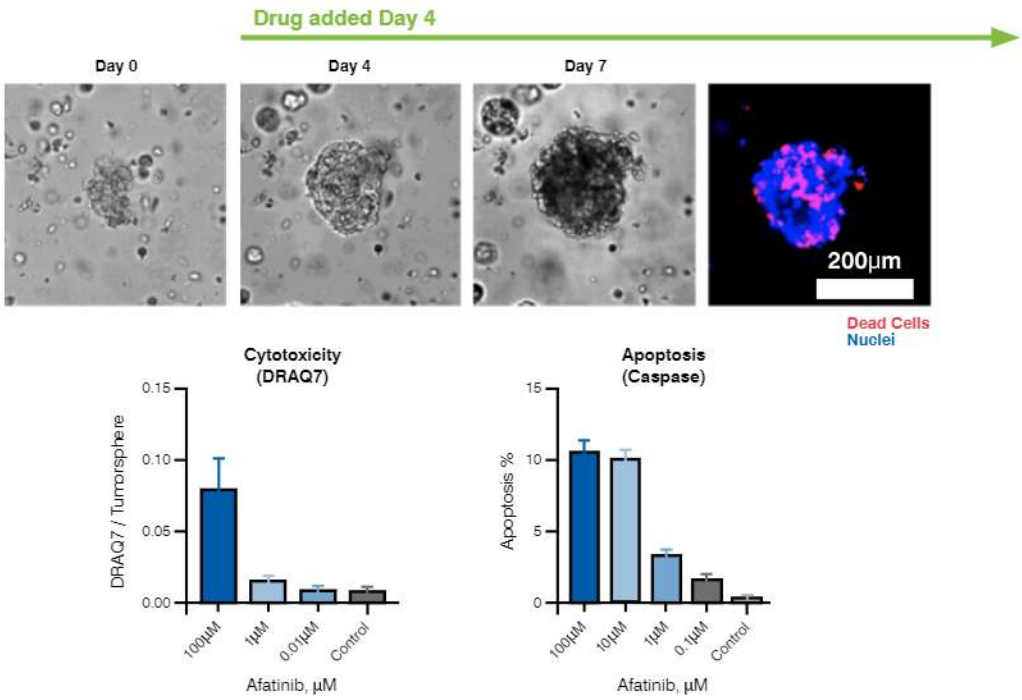


Figure 2: Oncology Growth Assay readout. Oncology Cytotoxicity Assay readout. Representative 20X confocal images (top) of an *ex vivo* NSCLC PDX grown over 7 days, and subsequent endpoint analysis (below) in the Standard Cytotoxicity Assay using the DRAQ7 dead cell or Caspase 3/7 apoptosis markers.

Contact an Oncology Expert
Visit https://www.criver.com/consult-pi-ds-hydrogel-3d-tumor-screens

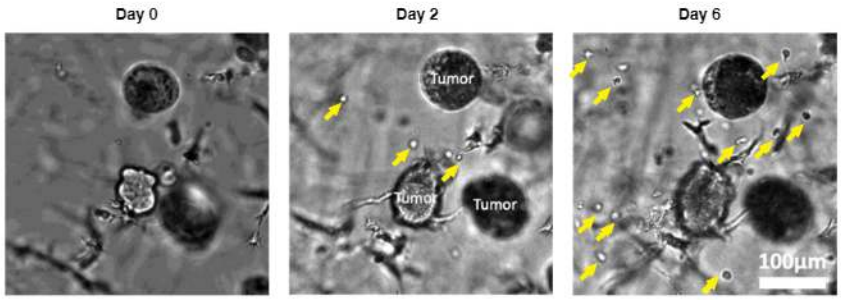


Figure 3: Immune infiltration: PBMCs migrating from the matrix surface into 3D tumor models consisting of PDX tumorspheres and fibroblasts, observed under transmitted light (TL) over several days.

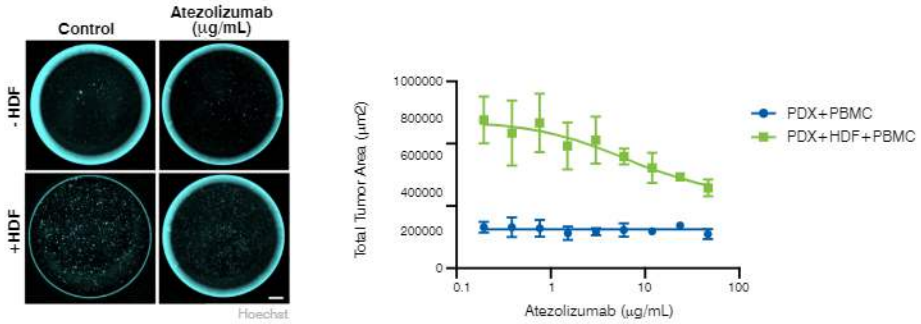


Figure 4: Immuno-Oncology (IO) Growth Assay readout. Representative 2X confocal images (scale bar = 1 mm) and quantitative data for total tumor area which generates an IC50 curve. In this instance, the co-embedding of fibroblasts with tumor cells allowed for stronger tumor growth and subsequent response to checkpoint inhibitors.

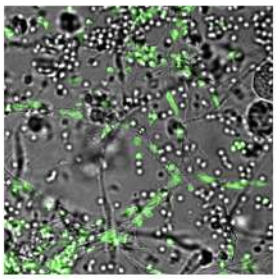


Figure 5: Custom IF staining. Representative image of immunofluorescent staining with CD3 marker of T cells trapped on fibroblasts (unlabeled) inside a 3D tumor model. Custom IF panels for more in depth phenotyping are available.

Contact an Oncology Expert

Visit https://www.criver.com/consult-pi-ds-hydrogel-3d-tumor-screens

3D PDX Tumor Panel

The 3D PDX Tumor Panel offers clients the ability to screen a range of histotypes in the Cypre 3D tumor model platform, including:

- NSCLC
- Colon
- Gastric
- Melanoma
- Breast
- Mesothelioma
- Pancreatic
- Renal
- Ovarian
- Uterus
- Liver
- Head and neck
- Sarcoma

Available for regular screening in a 6-dose format (or 3-dose format for cell therapy), clients receive tumor growth data for all 3D PDX against your small molecule, large molecule, or cell therapy. For immunotherapies, apoptosis markers (e.g. Caspase 3/7) will also be included in the endpoint readout. Screen 3D PDX tumors with confidence using the 3D PDX Tumor Panel prior to advancing to more in-depth *in vitro* pharmacology on the Cypre platform, such as 9-dose curves, combination screening, flow cytometry, RNAseq, and cytokine analysis, and later, *in vivo* pharmacology.

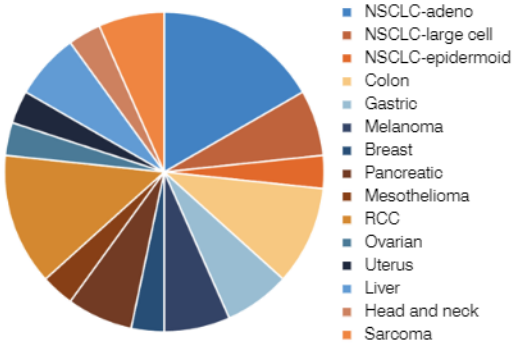


Figure 6: 3D PDX Tumor Panel. The range of histotypes in the 3D PDX Tumor Panel, available now for monthly screening with tumor growth and/or apoptosis provided.

Standard 3D PDX Panel Setup

Number 3D PDX Models:	30
Concentration/Cell Density:	6 (small molecule, antibody), 3 (cell therapy)
Repetition:	2
Model Type:	Oncology (PDX+HDF) or IO (PDX+HDF+PBMC or cell therapy)
Readout:	Image analysis of tumor growth (Oncology); growth + apoptosis (IO)

Summary

The Cypre 3D *in vitro* assay platform evaluates therapeutics against a panel of PDX-derived tumor cell lines or *ex vivo* tissue in a 3D matrix hydrogel microenvironment with accompanying stromal (fibroblasts) and immune cell compartments. The platform employs Cypre's 3D Symphony® and VersaGel® patterning technology to engineer 3D tumor assays in standard 96-well plates and uses high content imaging to quantitate endpoints such as tumor growth and killing. The Cypre 3D tumor model platform supports the selection of numerous PDX variants for rapid screening of therapeutic assets *in vitro* and pre-selecting their PDX *in vivo* models for preclinical studies. Even further, the 3D PDX Tumor Panel allows more streamlined access and throughput for efficacy screening where the mechanism may be unknown and prior to advancing to more complex *in vitro* pharmacology studies on the Cypre platform (e.g. 9-dose curves, immunofluorescence, flow cytometry, cytokines, RNAseq).

List of Assays Using Over 60 Validated PDX *In Vitro* Lines:

Standard 3D PDX Panel:

- Tumor Growth Assay covering all major tumor histotypes and screened monthly
- 2 Options:
  - Oncology Panel: PDX + Fibroblasts. Measured by tumor growth.
  - Immuno-Oncology Panel: PDX + Fibroblasts + PBMC (or your cell therapy). Measured by tumor growth and apoptosis

Custom Assays:

- Oncology Standard (PDX alone)
- Oncology Standard (PDX + HDF co-culture)
- Immuno-Oncology Standard (PDX + PBMC co-culture)
- Immuno-Oncology Standard (PDX + HDF + PBMC triple culture)
- Additional Analytics (e.g., IF, flow cytometry, cytokine panels, RNAseq)