CROWN BIOSCIENCE

3D Cell Culture Platform *In Vitro/Ex Vivo* Assays for Cancer Pharmacology

Capture the reality of tumor biology *in situ* by evaluating your compounds in Crown Biocience's extensive platform of 3D *ex vivo* and *in vitro* assays

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FACTSHEET

V1.0

Around 95% of new anticancer drugs eventually fail in clinical trials[1], despite robust indications of activity in existing *in vitro* preclinical models, suggesting that innovative models are required that better mimic tumor biology *in vivo*. To increase the speed and efficiency of the preclinical oncology research process a high proportion of drug discovery is currently performed using cell cultures. However, culturing cells on flat plasticware results in artificial 2D monolayering, with cells suffering additional stress as they spread and flatten on the plastic surface of the flask and fail to engage in efficient cell-cell and cell-matrix contacts. All of these factors are known to influence gene expression and protein function therefore raising the question as to whether a 2D cell culture model represents, with sufficient fidelity, the corresponding tumor *in situ*.

Cells that make up tissues follow complex and dynamic 3D arrangements, which are important for their physiology. The 3D architecture of a cell influences its ability to respond to external stimuli and activate the appropriate response. Cancer cells (much like their normal counterparts) benefit from such 3D interactions with each other and with the surrounding extracellular matrix by establishing a unique growing niche, the tumor microenvironment (TME). In order to investigate the efficacy of a new compound as an anticancer agent it is necessary to take into consideration the 3D nature of a tumor and its communications with the TME.

3D cell cultures represent a significant step forward towards capturing the realities of tumor biology in situ. In a 3D cell culture, tumor cells are grown to avoid contact with plastic allowing them to keep their native architecture and to establish interactions with each other, which minimizes stress and an artificial response. A comparison between 2D culturing and the improvement presented by 3D cell culture is shown in Table 1.

Crown Bioscience 3D Cell Culture Resources

At Crown Bioscience, we offer a variety of *ex vivo* and *in vitro* 3D assay systems for a range of *ex vivo* and *in vitro* models for preclinical oncology research.

Table 1: Comparison of 2D and 3D Cell Culture

	2D	3D
Cell Shape	Flat. Thickness: 3µm	Ellipsoid. Thickness: 10-30µm
Environment	About 50% of cell surface in contact with culture flask and very small proportion of cell in contact with neighboring cells	Nearly 100% of surface exposed to other cells or to the matrix
Cell Behavior	Differences in differentiation[2], drug metabolism[3], gene expression[4], proliferation rates[5], response to stimuli[6], and viability[7]	

Our *ex vivo* models are derived from Hu**Prime** and Hu**Kemia** - Crown Bioscience's collection of highly characterized patient-derived xenograft (PDX) models, the largest commercial PDX collection available.

Our models provide a rich resource for the derivation of primary cells and cell lines across a range of cancer types with many molecular targets.

Our *ex vivo* models include freshly isolated cells from PDX tumors (utilized in assays immediately on the day of isolation). We have currently validated almost 70 PDX-derived primary cells for 3D cell culture assays, with cells derived from both solid tumors and blood cancer models (detailed in Table 2).

We also provide fresh frozen PDX cells for 3D assay. Cell suspensions are isolated, frozen, and thawed as needed for assays. This can shorten assay timelines to two weeks, with no lag time while PDX tumor donors grow out. Crown Biocience provides fresh frozen PDX cells from a range of cancer types (shown in Table 3), with further models under development.

Hu**Prime** models have also been used to establish our proprietary **Prime**Panel cell line collection[8], consisting of mouse stromal-cell-depleted cancer cell cultures. Our cell lines are all early passage (<10) and maintain essential

Table 2: PDX-Derived Primary Cells Validated in 3D Cell Culture Assays

Tissue of Origin	HuPrime Identifier
ALL	AL5511, AL7443, AL7473
AML	AM5512, AM7440, AM7577
Brain	BN2276, BN2287, BN2331, BN2338
Breast	BR1282, BR1283, BR1458, BR1474
Colorectum	CR0029, CR1520, CR1560, CR1574, CR2161, CR2506, CR2518, CR2520, CR2524, CR2545, CR3085
Esophageal	ES0195, ES0201
Gallbladder	GL0440
Liver	LI0050, LI0574, LI0612, LI0752, LI0941, LI1037, LI1098, LI1646
Lung	LU0038, LU0299, LU0330, LU0367, LU0377, LU0387, LU0395, LU0743, LU0755, LU0884, LU1143, LU1144, LU1147, LU1155, LU1215, LU1271, LU1380, LU1423, LU1868, LU1901, LU2503, LU2505, LU2512, LU3075, LU6409
Ovarian	OV0205, OV0273, OV1658
Pancreas	PA1168
Stomach	GA0037, GA0087, GA0103, GA0114

Models in orange are newly validated

histopathological features and genetic profiles of the original patient tumors including genomic mutational status, biochemical signaling, and response to tumor cell autonomously targeted therapeutics. **PrimePanel** provides a versatile, cost-effective, high-throughput screening platform to assess drug efficacy prior to *in vivo* study. At Crown Bioscience we have established five **PrimePanel** cell lines for 3D use, detailed in Table 4.

To complement our *ex vivo* models, Crown Bioscience has established 3D cell cultures for >100 commercial cell lines that we have validated for *in vitro* drug efficacy studies, including breast, lung, ovary, and pancreatic cancer cell lines. A summary of our validated *in vitro* 3D cell lines is shown in Table 5.

Our 3D cell culture platforms utilizing the models detailed above for compound screening include the methylcellulose assay, including commercially available systems such as the 3D Alvetex® matrix (which can be used for cells which do not grow well in methylcellulose), soft agar colony formation, and the 3D tumor growth assay (3D TGA).

Table 3: PDX-Derived Fresh Frozen Cells Validated in 3D Cell Culture Assays

PDX Model Cancer Type	Model No.
Bladder	2
Brain	2
Breast	5
Cervical	1
Cholangiocarcinoma	3
Colorectal	18
Esophageal	7
Gallbladder	2
Gastric	10
Head and Neck	5

PDX Model Cancer Type	Model No.
Kidney	1
Liver	11
Lung	20
Lymphoma	3
Metastatic Carcinoma	1
Ovarian	6
Pancreatic	3
Sarcoma	3
Total	103

Table 4: Prime**Panel Cell Lines Validated in 3D Cell Culture Assays**

PrimePanel ID	Cancer Type	Patient and Disease Background
CR5063CL	Colon carcinoma	Female aged 27. Prior treatment with FOLFOX, Camptosar®, Erbitux®, FUDR®, Leucovorin
LU5143CL	NSCLC	Male, aged 78
LU5224CL	SCLC	Female, aged 48. Invasive high grade carcinoma
LU5381CL	NSCLC	Female, aged 57. Prior treatment with IFEX®, Taxol®, Gemzar®
ME5338CL	Melanoma	Metastatic melanoma (brain)

Table 5: In Vitro Cell Lines Validated in 3D Cell Culture Assays

	Methylcellulose	Alvetex	Soft Agar
Cancer Type	Cell Line		
Blood	Daudi, DoHH-2, EHEB, Jurkat (clone E6-1), JVM-13, JVM-2, JVM-3, K-562, Karpas299, Kasumi, MEC-1, MEC-2, MEG-01, Molm-16, MOLT-4, Namalwa, OCI-LY19, REH, SU-DHL-10, SU-DHL-5, SUP-B15, TF-1, THP-1, Toledo, U-937, WSU-DLCL-2		
Bone	CADO-ES1, RD-ES		
Brain & Nerves	A172, H4, IMR-32, LN18, LN229, SF126, SH-SY5Y, SK-N-SH, U-118 MG, U251, U-87 MG		
Breast	T-549, DU4475, MDA-MB-231, MDA-MB-436		MDA-MB-231
Colorectum	HT-29, HCT 116		HT-29, HCT-116
Esophageal	KYSE70, KYSE270, KYSE410, TE-1		
Head & Neck	FaDu, SW579		
Liver	HCCLM3, Hep G2, HLF, HUH-7, JHH-5, JHH-7, MHCC97H, PLC/PRF/5, SK-HEP-1, SNU-398, SNU-878, SNU-886	Hep 3B	Hep 3B, Hep G2, JHH-7, PLC/PRF/5, SK-HEP-1
Lung	A549, Calu-6, DMS53, DMS79, DMS114, EBC-1, H69AR, HCC4006, NCI-H1155, NCI-H1299, NCI-H1373, NCI-H1395, NCI-H1417, NCI-H1435, NCI-H1437, NCI-H157, NCI-H1581, NCI-H1650, NCI-H1688, NCI-H1703, NCI-H1792, NCI-H1836, NCI-H1975, NCI-H2052, NCI-H209, NCI-2227, NCI-H226, NCI-H23, NCI-H358, NCI-H446, NCI-H460, NCI-H520, NCI-H522, NCI-H526, NCI-H69, NCI-H82, SK-MES-1		NCI-H1299
Ovary	OVCAR-8, SK-OV-3, SW626, SW756		
Pancreas	AsPc-1, Capan-1, MIAPaCa-2, PANC-1, PL45		CFPAC-1, MIAPaCa-2
Prostate	PC-3		
Stomach	A3/KAW, AGS, AZ521, GTL-16, HGC-27, Hs 746T, IM95, IM95m, KATO III, MKN1, MKN45, MKN74, NCI-N87, NUGC-3, NUGC-4, OCUM-1, SCH, SNU-1, SNU-16, SNU-5, SNU-484, SNU601, SNU-620, SNU-638, SNU-668, SNU-719, YCC-2, YCC-7, YCC-10, YCC-11		AGS, AZ-521, GTL-16, HGC-27, MKN1

Ex Vivo Models in Methylcellulose Assays using HuPrime PDX Models

Our 3D clonogenic *in vitro*/*ex vivo* methylcellulose assay provides an intimate connection between low cost, high throughput *in vitro* drug screening, and clinically relevant in vivo drug efficacy studies with our unique **HuPrime** models (assay concept shown in Figure 1). All of the PDX-derived *ex vivo* models discussed above can be utilized in this assay.

For example, for freshly isolated primary cells inoculated into immunodeficient mice, xenografts are harvested once the tumor has reached the appropriate size (typically between 400mm3 and 800mm3). The harvested tumor is lysed and cells are seeded on methylcellulose or 3D Alvetex plates at the appropriate dilution to allow growth in 3D, with cell density optimized to provide highly reproducible results.

The anticancer agent to be tested can then be administered to cells starting from Day 2 up to Day 10 of culture. Endpoints include phase contrast imaging of cells and CellTiter-Glo® (CTG) assay to verify anticancer compond efficacy.

An example ex vivo efficacy study for cisplatin on freshly isolated primary cells from the gallbladder tumor model GL0440 is shown in Table 6. Cisplatin was administered to tumor cells at 0.1µM, 1µM, and 100µM and phase contrast images (10X) were taken for each dose to evaluate drug efficacy. Dose response curves can then be generated; Figure 2 shows the dose response to cisplatin for cells derived from the OV5397 ovarian cancer PDX model in the methylcellulose assay.

Figure 1: Ex Vivo Clonogenic Assay Using Freshly Isolated Cells From HuPrime PDX Models

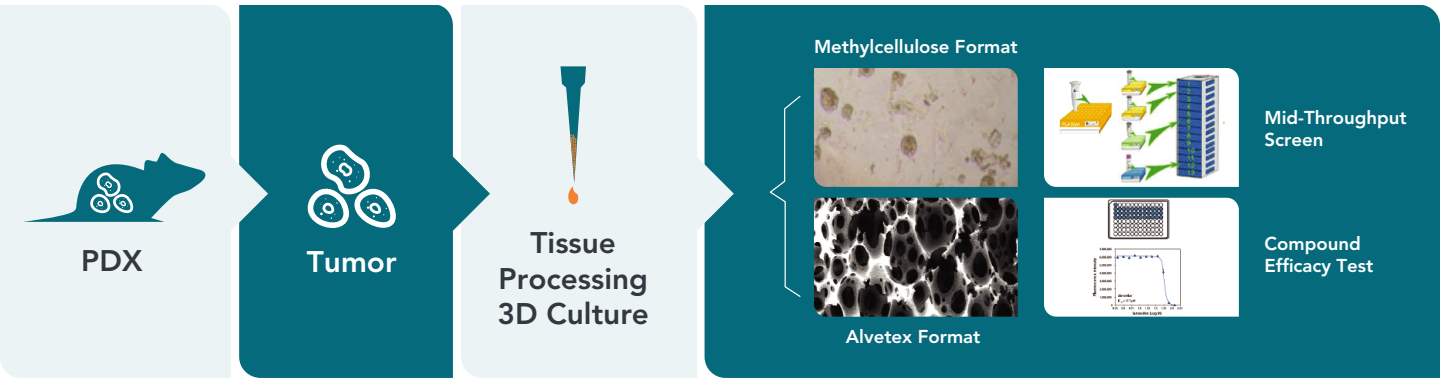
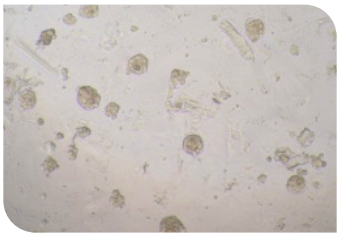


Table 6: Ex Vivo Efficacy Study of Cisplatin on Primary Cells Derived from GL0440 Grown in 3D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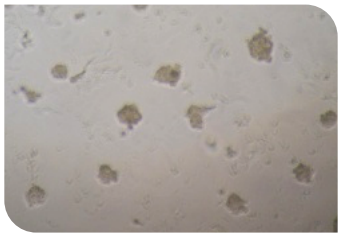
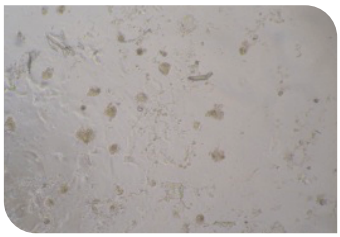

Cisplatin 0.1µM


Cisplatin 1µM

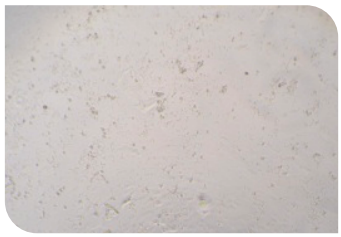
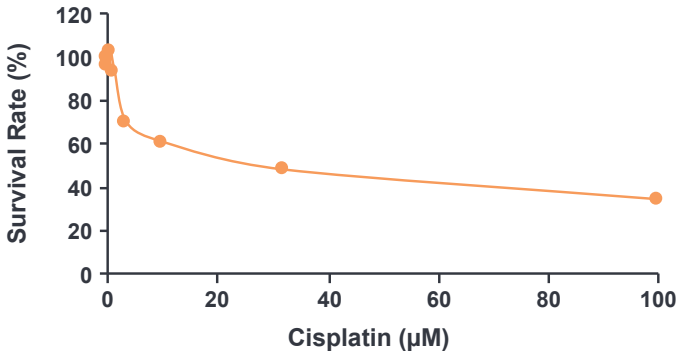
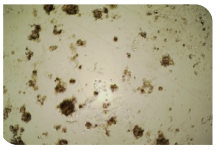
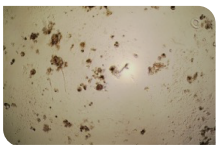
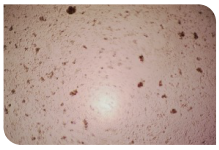

Cisplatin 100µM

Figure 2: Dose Response Curve for Cisplatin Effect on Primary Cells Derived from OV5397 Grown in 3D




10µM


1µM


100µM

Ex Vivo Models in 3D TGA Assays using HuPrime PDX models and PrimePanel Cells

3D TGA assays have become a proven platform for oncology drug development, with numerous publications covering research in a range of cancer types[9,10,11,12]. The 3D TGA assay utilizes a low stiffness laminin rich extracellular matrix (IrBME, Cultrex®) to embed tumor cells, and admixing with hMSCs (e.g. IL-6, HGF) and CAFs provides the paracrine signaling present in the TME of solid tumors. The addition of hormones (e.g. DHT/E2), restriction of glucose (≤7mM), and maintenance of an acidic pH (6.8) provide a 3D assay that is both "humanized" and TME-aligned for profiling of PDX-derived cells and drug panels[9].

Table 7 shows a comparison between tumor conditions in humans, 2D culture, and the humanized 3D cell culture environment, with the 3D culture more closely representing the human condition.

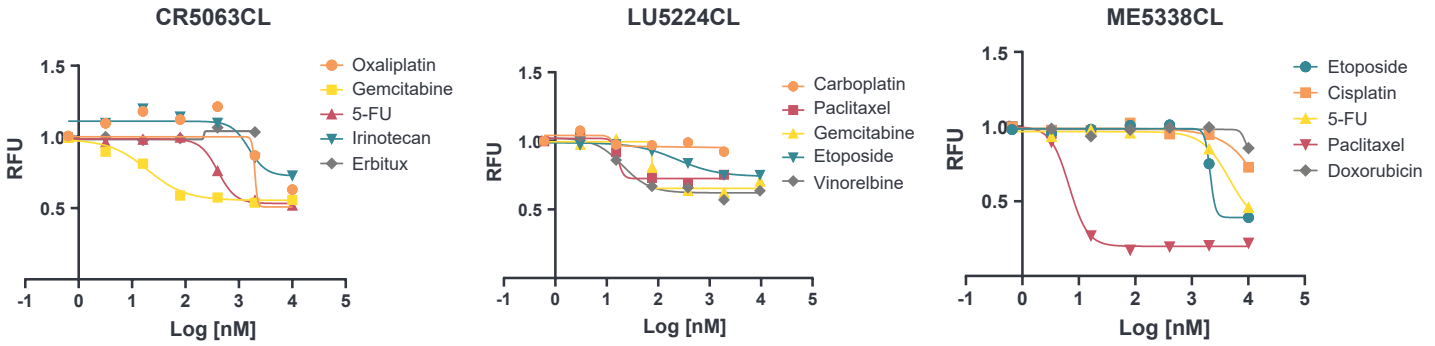
Our 3D TGA assay has been optimized to define a linear range for cell growth and for total cell population numbers using alamarBlue® colorimetric assay (further endpoints such as 3D Glo are also available). Individual cell types can also be monitored via fluorescent/bioluminescent read-outs if cells are transduced. The 3D TGA can be utilized with all of the *ex vivo* models described above for drug evaluation and further research as needed. Figure 3 shows example IC50 evaluations using our 3D TGA with selected colorectal, lung, and melanoma **PrimePanel** cell lines.

PDX tumor models are ideal for the preclinical assessment of anticancer agent efficacy because they closely mimic the pathological phenotypes of the original tumors. However, the labor-intensive, time-consuming, low throughput, and costly nature of in vivo animal studies can be limiting for their use in early stage drug development. PrimePanel cells are not generated from all PDX; however, the 3D TGA system enables the use of fresh PDX material in a 96-well format.

Table 7: Comparison of Tumor Conditions Across Humans, 2D, and 3D Culture[11]

Tumor Collection	Human	2D Culture	3D-TME Culture
Oxygen (Hypoxia)	0-5%	21%	≤5%
Stiffness	200-4,000 Pa	3,000,000,000 Pa	200-4,000 Pa
pH	acidic (≤7.0)	buffered (≥7.2)	acidic (≤7.0)
Dimensionality	3D	2D	3D
Glucose availability	<7mM	10-25mM	<7mM

Figure 3: Drug Panel IC_{50} Evaluation using PrimePanel Cell Lines in a 3D TGA



PrimePanel Cell Line	IC_{50} (μM)										
	5-FU	Carboplatin	Cisplatin	Doxorubicin	Erbitux	Etoposide	Gemcitabine	Irinotecan	Oxalipatin	Paclitaxel	Vinorelbine
CR5063CL	0.405	–	–	–	~0.157	–	0.020	1.54	~1.99	–	–
LU5224CL	–	~0.014	–	–	–	0.248	0.080	–	–	~0.017	0.021
ME5338CL	4.59	–	>100	8.67	–	~2.09	–	–	–	0.007	–

Correlation has been shown between response to anticancer agents in 3D TGA and in *in vivo* models and expected clinical outcome. LU6422 is a NSCLC PDX model of the adenocarcinoma subtype. The PDX model harbors a heterozygous c.2573T>G (L858R) in the intracellular kinase domain, which is associated with hypersensitivity to EGFR targeted therapies such as erlotinib in patients. *In vivo*, following subcutaneous implantation with MSCs and treatment with 25 and 50mg/kg erlotinib, complete regression of all tumor growth is observed for this PDX model (Figure 4A). Cells derived from LU6422 are also hypersensitive to erlotinib in 3D TGA, correlating with *in vivo* response (Figure 4B). These data were recently published with our collaborators in *Molecular Cancer Therapeutics* [9].

3D TGA Large Scale Screens

PDX models can be used in mouse clinical trials (**HuTrials**) to identify responder and non-responder populations, and to fully elucidate signatures and predictive biomarkers for patient stratification in the clinic. Cells derived from these PDX can also be used in 3D TGA assays, to perform large scale efficacy screens to potentially identify drugs and combinations most likely to benefit individual patients[9].

In Vitro Models in Methylcellulose and Soft Agar Assays using Cell Lines

To complement our *ex vivo* assays, CrownBio has validated a range of commercially available *in vitro* cell lines for 3D assay. Growing cells are harvested and seeded on methylcellulose, soft agar, or other commercially available matrices at the appropriate dilution to achieve a final cell density which is optimized for each cell line (e.g. 5,000 cells per well). Starting from Day 2, anticancer compounds are administered to each well according to desired concentration design. Cell growth is monitored daily until endpoint, when cell number is counted using CTG assay. Figure 5 shows a dose response curve for the head and neck cancer line FaDu, grown in 3D and treated with increasing doses of an experimental anticancer compound to test its efficacy.

We also provide soft agar assays in low-attachment 96 well plates. Cell lines are plated at 3×10^3 cells/well in 0.4% agar, over a base layer of 0.6% agar. The test agent is added with the growth media, and endpoints are CTG or imaging assays. Figure 6 shows comparison images for both endpoints from a soft agar assay, for cisplatin dose response. The calculated EC_{50} values are comparable, validating imaging as an endpoint for this assay.

Figure 4: Correlation of LU6422 PDX Response *In Vivo* and in 3D TGA

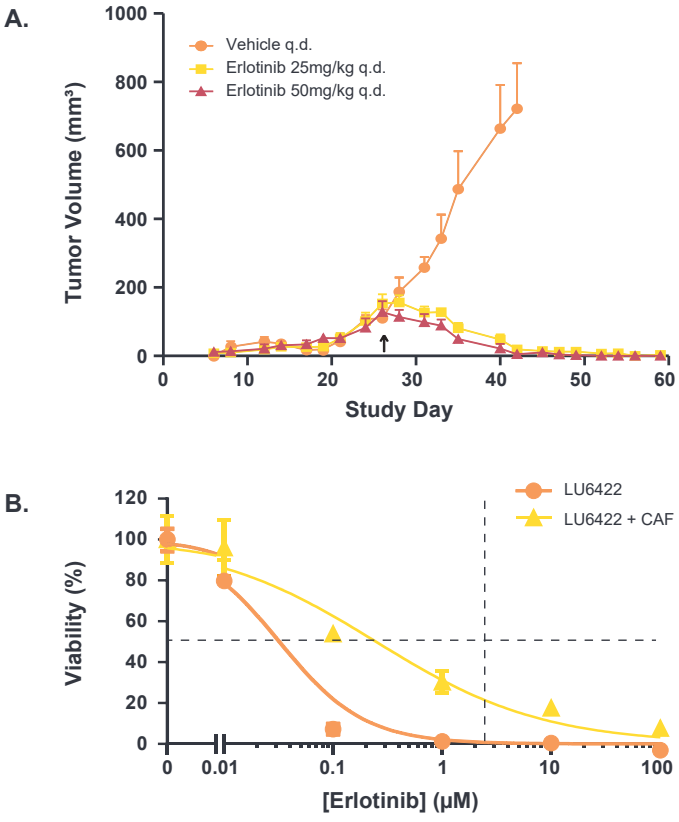


Figure 5: Dose Response Curve for the FaDu Head and Neck Cancer Cell Line

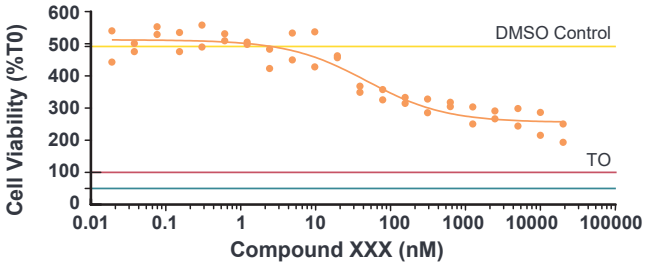
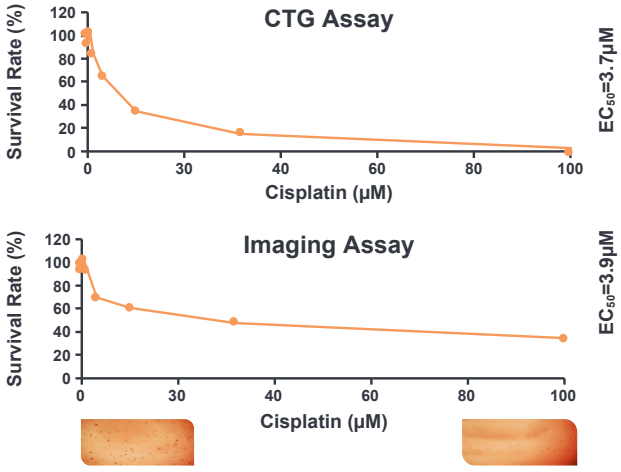
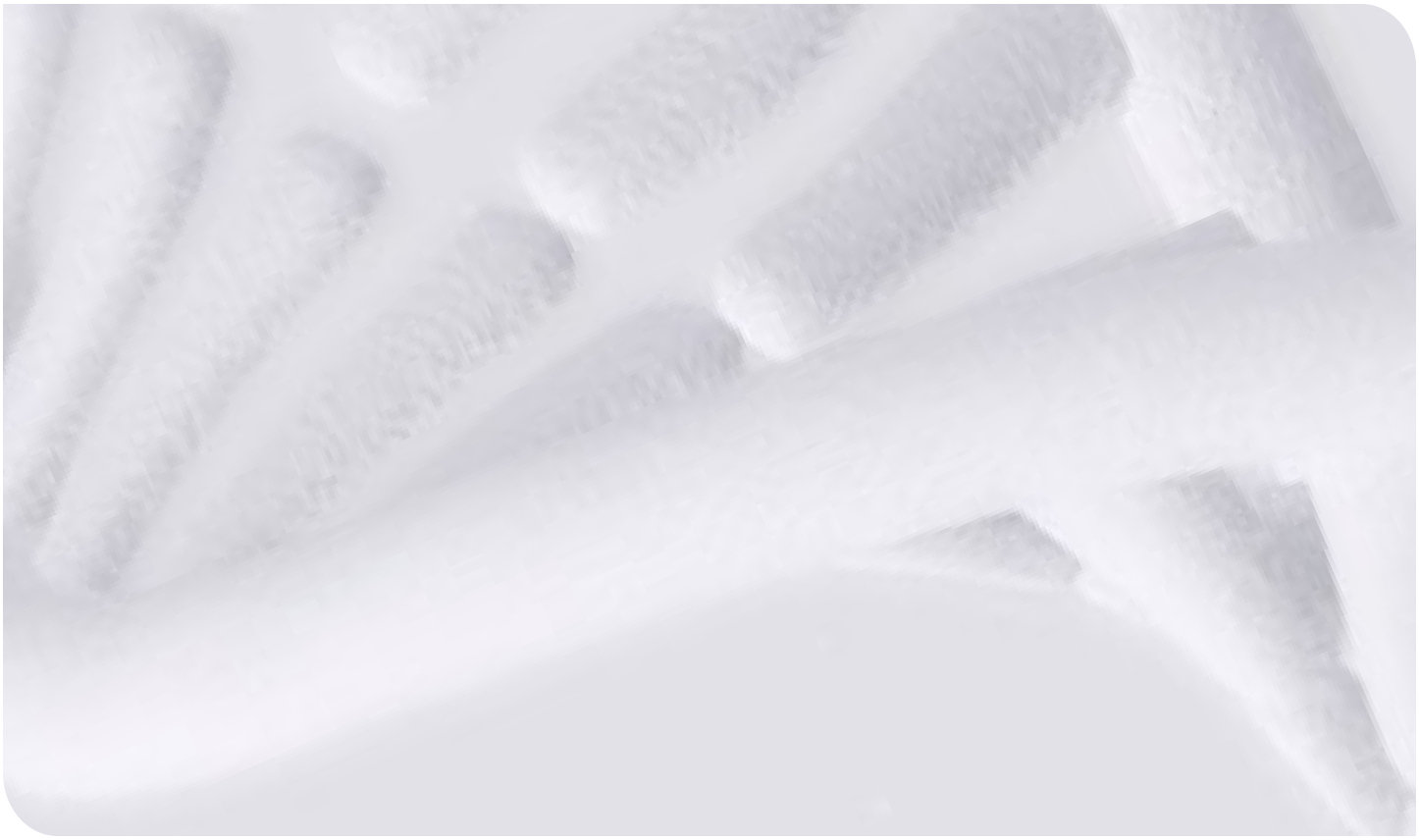


Figure 6: Dose Response Curve for CFPAC-1 Cancer Cell Line Treated with Cisplatin





Summary

Tumors are 3D pathological entities. Their 3D nature should be taken into consideration when assessing the efficacy of new anticancer agents as it is able to influence their response to external stimuli such as chemotherapy. Attrition rates in oncology drug development are significantly higher than in other disease areas, with only 5% of anti-cancer agents which show activity in existing preclinical models going on to be licensed after successful Phase III trials[1]. The majority of these failures are related to efficacy rather than the toxicity issues, which confirms that current preclinical oncology 2D cell culture systems do not represent, with sufficient fidelity, the corresponding 3D tumor *in situ*.

CrownBio has developed a variety of *ex vivo* and *in vitro* models for 3D culture and preclinical oncology research. Our *ex vivo* models are derived from our well-characterized and validated Hu**Prime** and Hu**Kemia** PDX models, and we provide freshly isolated cell lines, fresh frozen cells, as well as our proprietary **PrimePanel** cell lines which are early passage (<10) and maintain essential histopathological features and genetic profiles of the original patient tumors. To complement these resources, we also provide >100 commercial cell lines which we have validated for 3D culture.

Our *ex vivo* and *in vitro* models can be utilized across a range of 3D assays. CrownBio provides a 3D clonogenic *in vitro*/*ex vivo* methylcellulose assay which allows an intimate connection between low cost, high throughput in vitro drug screening and clinically relevant *in vivo* drug efficacy studies. Soft agar assays provide a similar function, with CellTiter-Glo or imaging endpoints as required.

We also offer the 3D TGA, which has become a proven platform for preclinical oncology drug development. 3D TGA is closely aligned to the TME with epithelial cells being cultured in combination with stromal cells and extra-cellular matrix in serum-free conditions, providing a more clinically relevant culture method. In combination with PDX models, the 3D TGA has the potential to accelerate research by providing a rapid and scalable *ex vivo* drug-sensitivity screen of multiple single and combinatorial agents[9].

CrownBio can be contacted at **busdev@crownbio.com** for any further questions or information on our 3D cell culture platforms or for any other information on other CrownBio products and services.

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