Establishing a Large Tumour Organoid Biobank using a Well-Characterised/Annotated Patient-Derived Xenograft (PDX) Library to Enable Drug Discovery and Translational Research

Xiaoxi Xu[1], Yuting Qiu[1], Lili Wang[1], Chunmei Li[1], Yan Liu[1], Peng Han[1], Zhongman Sun[1], Yaping Qu[1], Likun Zhang[1], Bonnie Chen[1], Davy Ouyang[1], Yujun Huang[2], Henry Li[2†]

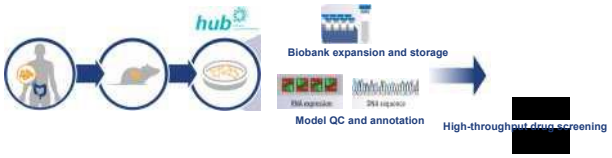
Crown Bioscience Inc., 21 Huoju Street, Changping District, Beijing, 102200, China; [2]Crown Bioscience Inc., 16550 West Bernardo Drive, Building 5, Suite 525, San Diego, CA 92127

INTRODUCTION & METHODS

Patient-derived xenografts (PDXs), a cancer stem cell (CSC) derived *in vivo* model, are an accepted model of choice for preclinical and translational research, due to their proven predictive power. Patient-derived cancer organoids (PDOs), are also CSC-derived 3D cultures of carcinoma, with defined structures, harbouring multicellular components of carcinoma, and mimicking cancer lesion structures/heterogeneity, both genomically and histopathologically. PDOs were first described by the Clevers Lab, and have proven to be a predictive model for preclinical research, similar to PDX.

We have used the Hubrecht Organoid Technology (HUB) approach to systematically create the worlds first biobank of organoids derived from a well-annotated PDX library (the world's largest PDX library with >2,500 models, covering a variety of carcinomas, and with extensive pathology, genomic, and treatment information), referred to as PDX-derived organoids (PDXOs). We have systematically profiled these PDXOs by WES (whole exome sequencing)/RNAseq (transcriptome sequencing), histopathology, and standard of care (SoC) treatment.

Fig 1. PDXO establishment, characterisation, and biobanking scheme



PDX tumour bearing animals were maintained at a CrownBio animal facility. To collect PDX cells, xenograft tumours were harvested when tumour volume reached 500-800mm³ and minced, followed by dissociation in 2.5mg/ml collagenase buffer at 37°C for 1-2h. After removal of red blood cells using red blood cell lysis buffer, tumour cells were washed with PBS and cultured in Matrigel® containing media. PDXOs were passaged either via trituration with a glass Pasteur pipet or dissociation with TrypLE for 5min at 37°C. Passage was performed weekly with a 1:2 to 1:3 ratio.

RESULTS

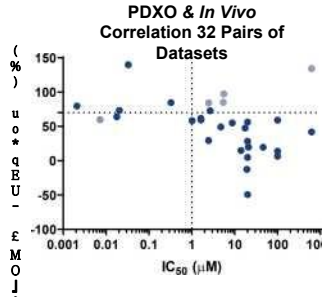
Table 1. Established PDXO models covering different cancer types

Cancer Type	Number of Established Models
Bladder cancer	4
Breast cancer	2
Cholangiocarcinoma	4
Colorectal cancer	19
Esophageal cancer	1
Gallbladder cancer	1
Gastric cancer	12
Kidney cancer	1
Liver cancer	7
Lung cancer	41
Melanoma	4
Ovarian cancer	6
Pancreatic cancer	12
Total	**114**

Table 2. Summary of PDXO models that currently have treatment response data

Cancer Type	Number of Models with Treatment Response Data
Breast cancer	1
Cholangiocarcinoma	1
Colorectal cancer	11
Gastric cancer	5
Kidney cancer	1
Liver cancer	1
Lung cancer	12
Melanoma	1
Ovarian cancer	1
Pancreatic cancer	4
Total	**38**

Fig 3. Predictive power of PDXO concordance for PDX *in vivo*. Z' value showed good production level of intra-plate variation. Pearson analysis reveals 81% data consistency between PDXO and PDX with a statistically significant Fisher's Exact test correlation (p=0.023). CC: cholangiocarcinoma; CR: colorectal; GA: gastric, LU: lung; OV: ovarian cancer



Fisher's Exact test, p value = 0.023

PDXO sensitivity	*In vivo* sensitivity response: Sensitive	Insensitive
Sensitive	4	2
Insensitive	4	22

	Percentage (%)
Positive prediction	67
Negative prediction	85
Overall prediction	81

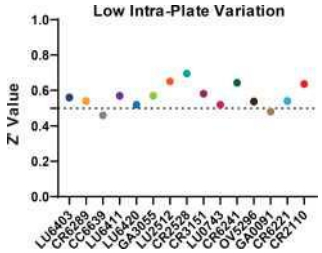
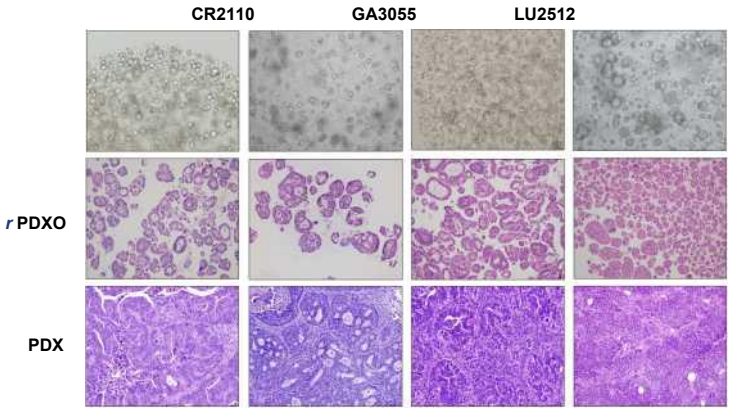


Fig 2. Brightfield morphology showed organoid structure (compact, cystic/luminal). H&E staining of PDXOs showed similar histopathology structures (such as carcinoid compact structures in LU6471, acinus/cavity structures in LU2512) as in the corresponding PDX models. CR: colorectal; GA: gastric, LU: lung cancer



SUMMARY

At present, we have established >100 PDXOs covering >10 cancer types, including bladder, breast, colorectal, gastric, liver, lung, ovarian, and pancreatic cancer, cholangiocarcinoma, etc

Histopathological analysis showed cellular/structural similarities (ductal, mucous, or carcinoid) between PDXO and the original PDX, suggesting that tissue specific structural features were maintained in the 3D organoids

A high throughput screening (HTS in 384 well) format was established using the PDXOs and SoC sensitivity testing was conducted. The preliminary results largely correlate to the SoC response seen *in vivo* for the corresponding PDXs In summary, we have successfully established a large biobank of PDXOs that mirror the original PDXs, creating a unique library of matched *in vitro/in vivo* models with high translational power and enabling HTS, therefore likely to become an important tool for future oncology drug discovery and development

REFERENCES

1. Boj *et al.* Organoid Models of Human and Mouse Ductal Pancreatic Cancer. *Cell* 2015;160(1):324-38.
2. Tiriac *et al.* Organoid Profiling Identifies Common Responders to Chemotherapy in Pancreatic Cancer. *Cancer Discov* 2018;8(9):1112-29.
3. Vlachogiannis *et al.* Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 2018; 359(6378): 920-6.