CrownBio
CONNECTING SCIENCE TO PATIENTS

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Modeling Human Cancers *In vitro & In vivo*

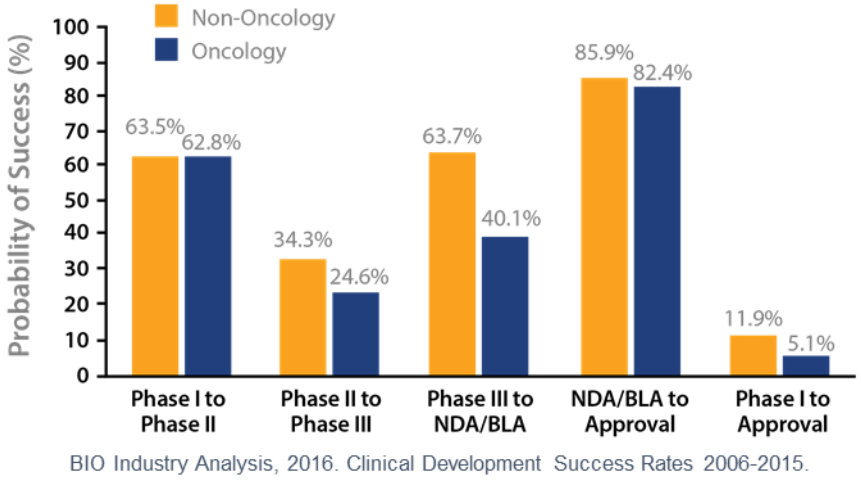
Matched patient-derived living biobanks

Henry Li, Ph.D.

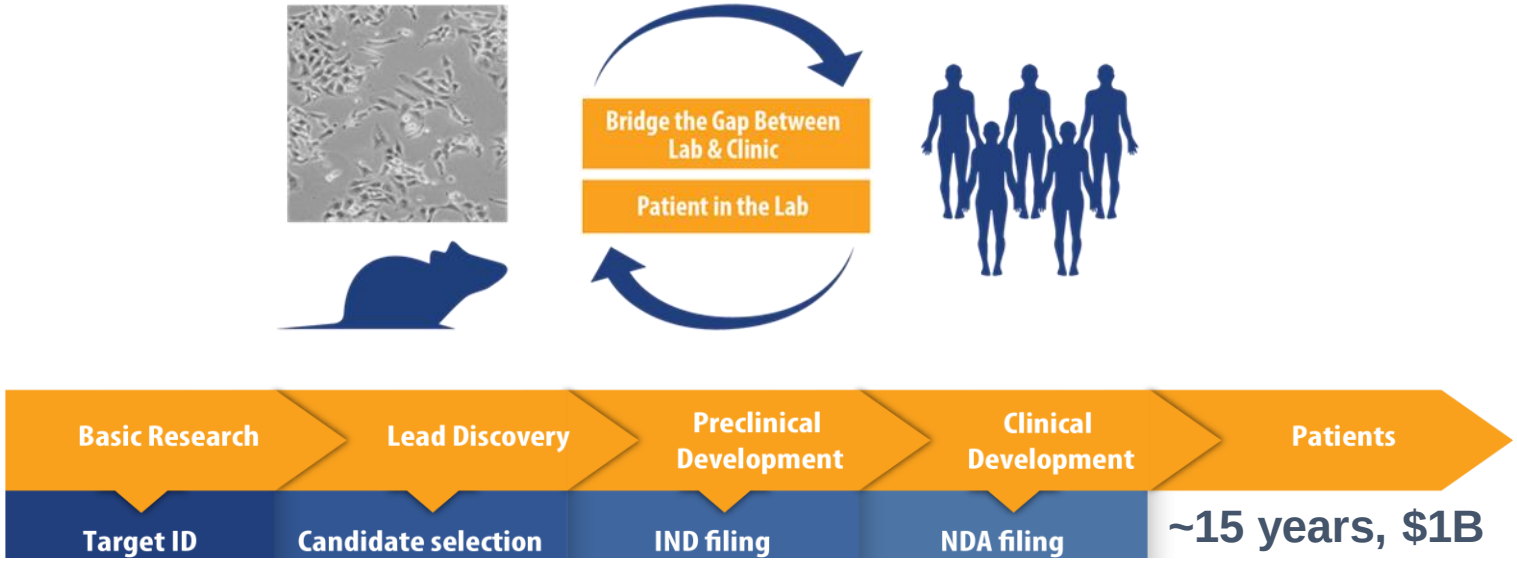
Chief Scientific Officer

Superior Preclinical Oncology Models Needed

- High attrition highlights the **gap** in translation from preclinical to clinical - there is a critical need for better modeling of human cancers
- High cost and long duration highlight the need for improved **workflows**

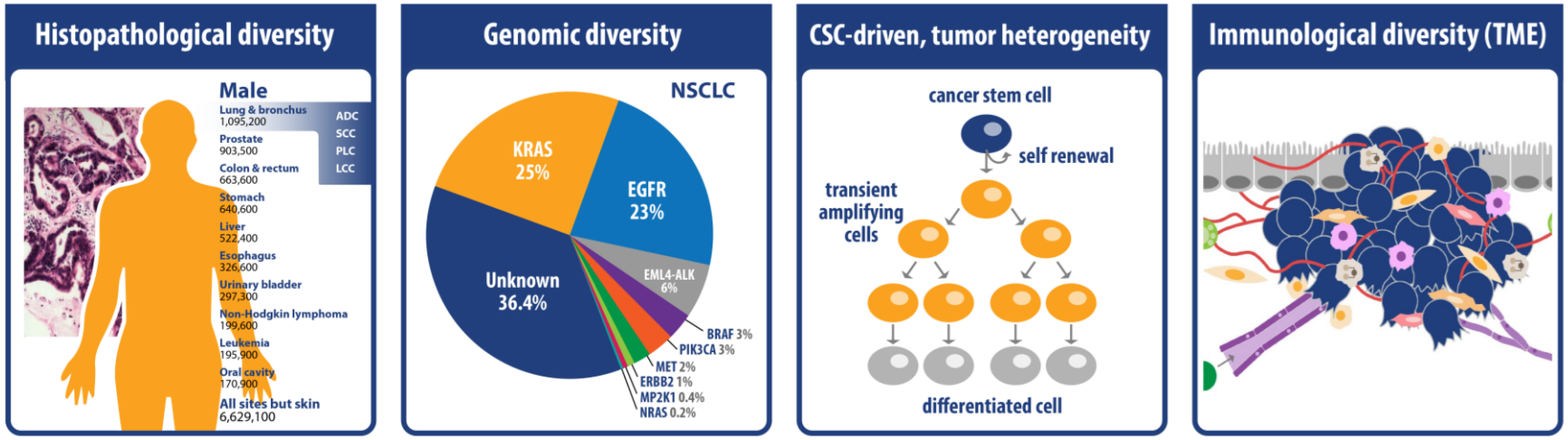


BIO Industry Analysis, 2016. Clinical Development Success Rates 2006-2015.



Complexity of Predictive Modeling of Human Cancers

Heterogeneity



Five Key Parameters:

1. Histopathology
2. Molecular pathology
3. CSC-theory
4. TME
5. Pharmacology

Key Considerations:

- Predictive power
- Diverse, extensive library
- Enabling efficient pharmacology to mimic population-based clinical trials
- Shorter timelines, reproducibility, cost

PDX: Today's Workhorse for Predictive Modeling

Contributions to novel drug approvals in 2020 from CrownBio's PDX models

Mirrors patient population:

- CSC-diseases (genetically stable)
- Inter- and intra- tumor heterogeneity
- Histology, molecular pathology
- TME
- Pharmacology (predictive for chemo/target therapy)
- "Arguably the gold-standard"


New cancer models could help scientists to devise better treatments.

BIOMEDICAL SCIENCE

US cancer institute overhauls cell lines

Veteran cells to be replaced by human tumours grown in mice.

Documented predictive power of PDX models:

- *Yen et al., 2017. AG-221, a First-in-Class Therapy Targeting Acute Myeloid Leukemia Harboring Oncogenic IDH2 Mutations. Cancer Discov.*
- *Walker et al, 2013. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. Cancer Discov.*
- *Corcoran et al, 2015. Combined BRAF and MEK Inhibition With Dabrafenib and Trametinib in BRAF V600-Mutant Colorectal Cancer. J Clin Oncol.*
- *Gao et al, (2015). High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med, 21, 1318.*

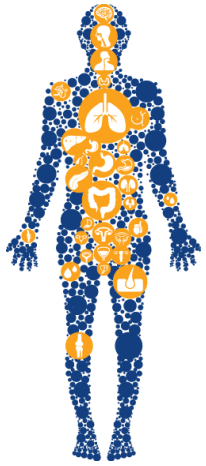
FDA approvals:

- 43 novel drug approvals by FDA since Nov 2019; 17 were novel oncology drug approvals
- CrownBio directly contributed to 7/17 of them; **6 were assessed using PDX models**

Approval Date	Drug Name	Active Ingredient	FDA-approved use (on approval date)
9/4/2020	Gavreto	pralsetinib (BLU-667)	Non-small lung cancer
5/15/2020	Qinlock	ripretinib (DCC-2618)	Advanced gastrointestinal-stromal tumors
5/8/2020	Retevmo	selpercatinib (LOXO-292)	Lung and thyroid cancers
5/6/2020	Tabrecta	capmatinib	Non-small cell lung cancer
4/17/2020	Tukysa	tucatinib	Advanced unresectable/metastatic HER2+ breast cancer
1/23/2020	Tazverik	tazemetostat	Epithelioid sarcoma
11/14/2019	Brukinsa	zanubrutinib	Mantle cell lymphoma

PDX Biobank

1. Hypothesis testing
2. Hypothesis generation



From **US**, **European**, and **Asian** populations

- 39.7% ASIAN
- 60% USA/EU
- 0.3% OTHERS

Acute lymphoblastic leukemia (ALL)	42	Fallopian	2	Mixed mullerian	20
Acute myeloid leukemia (AML)	6	Gallbladder	13	Ovarian	100
Adrenal	3	Gastric	163	Pancreatic	177
Bladder	38	Gastrointestinal stromal (GIST)	13	Peritoneal	2
Brain	32	Head and neck	126	Prostate	4
Breast	67	Kidney	35	Sarcoma	139
Cervical	25	Liver	144	Testis	1
Cholangiocarcinoma	24	Lung	428	Thyroid	6
Chondromyxoid fibroma	1	Lymphoma	44	Unclear primary site	18
Colorectal	378	Melanoma	262	Undergoing clinical confirmation	14
Esophageal	113	Mesothelioma	1	Uterine	18

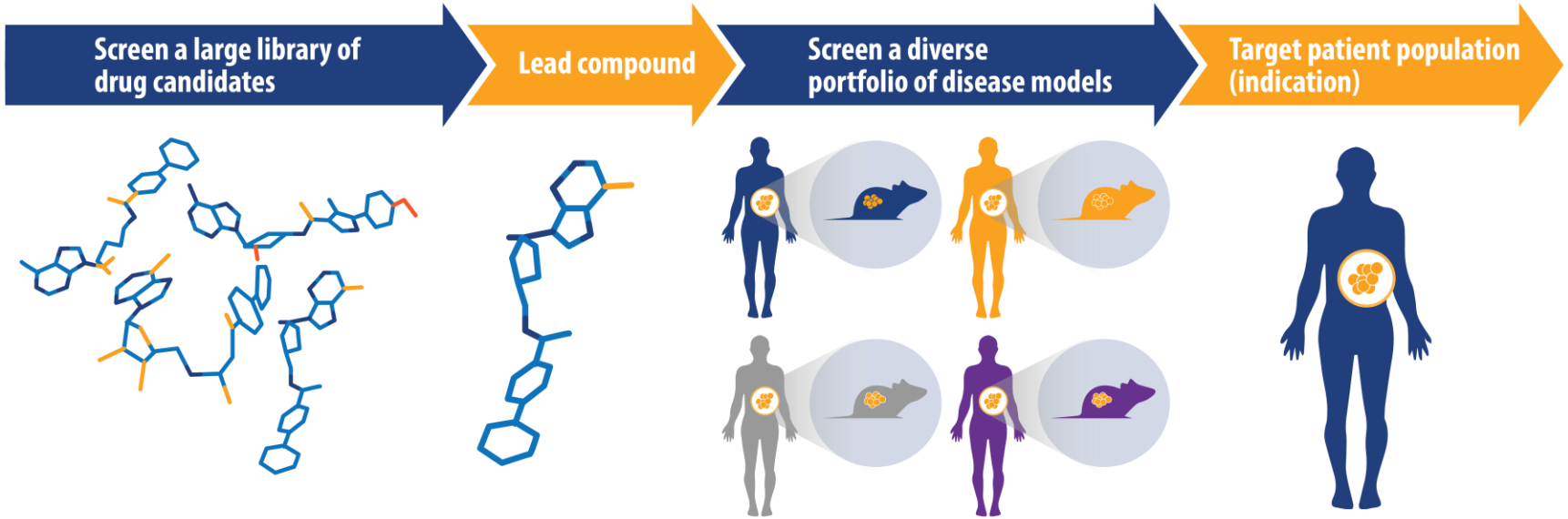
Largest comprehensively annotated PDX library:

HuBase™

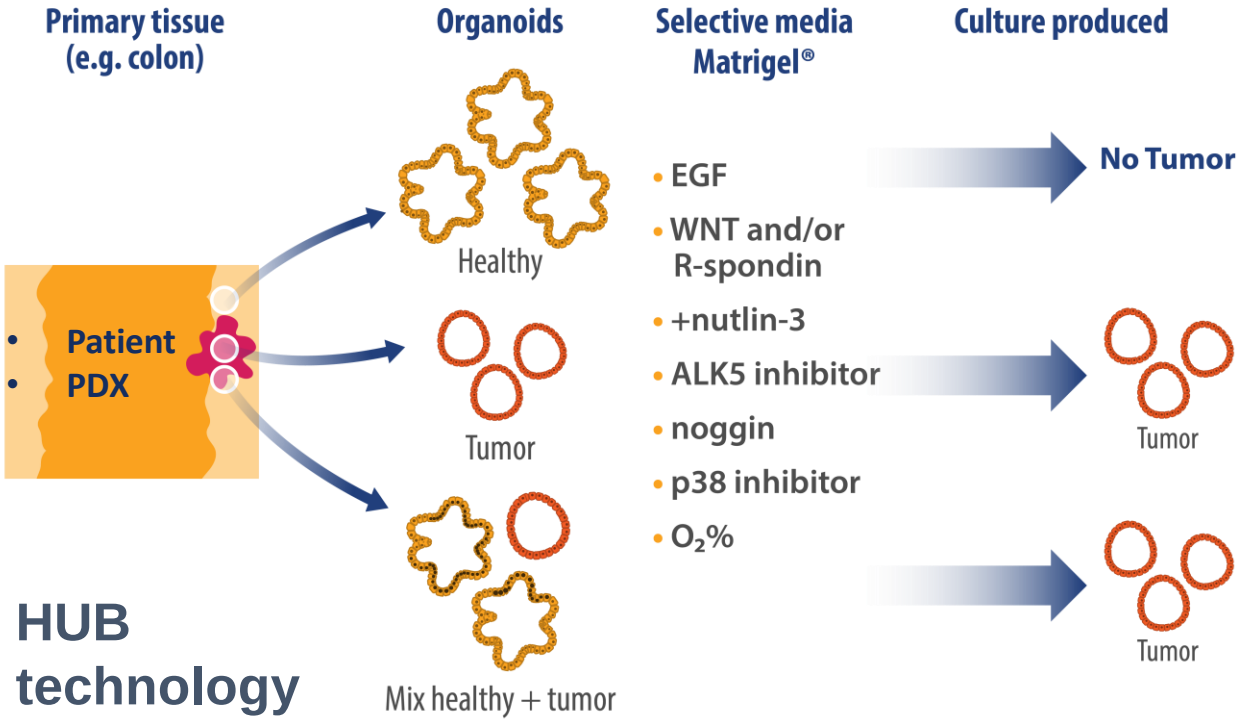
- >3,400 PDX
- ~10k users/40k individuals access/year
- >15,000 treatment datasets
- >200 mouse clinical trials/year
- RNA-Seq (>2363); WES (>1920); miRNA (193); proteomics data (> 50); SOC (430)
- Widely published, including >20 papers co-authored by CrownBio

Improved Oncology Drug Discovery Workflows Needed

- Oncology drug discovery requires selection on **two variables**
  - Lead compound identification
  - Disease indication
- Linear workflow is fundamentally time-consuming, risky and costly
- Matrix high-throughput screening (HTS) can improve workflow efficiencies
- PDX models, although widely adopted, have some limitations



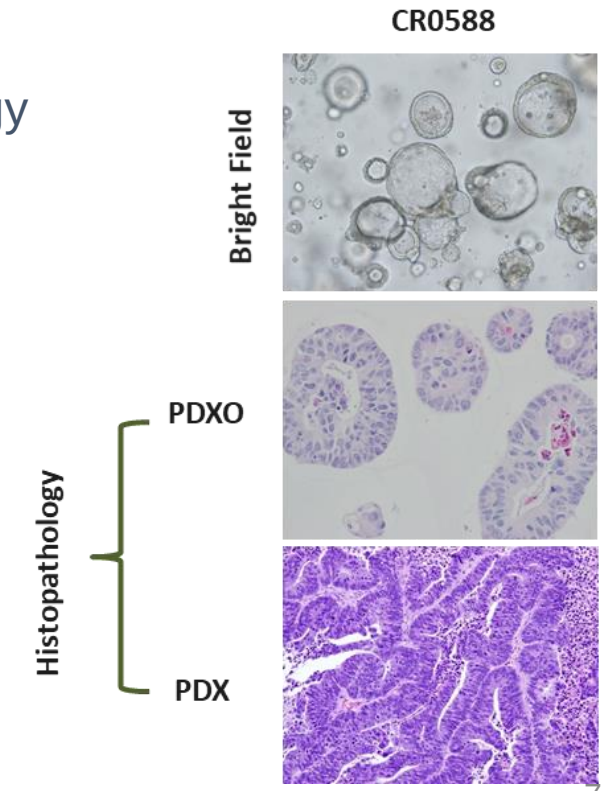
Tumor Organoids of Epithelial Origin: Patient-Derived Model *In Vitro*



HUB technology

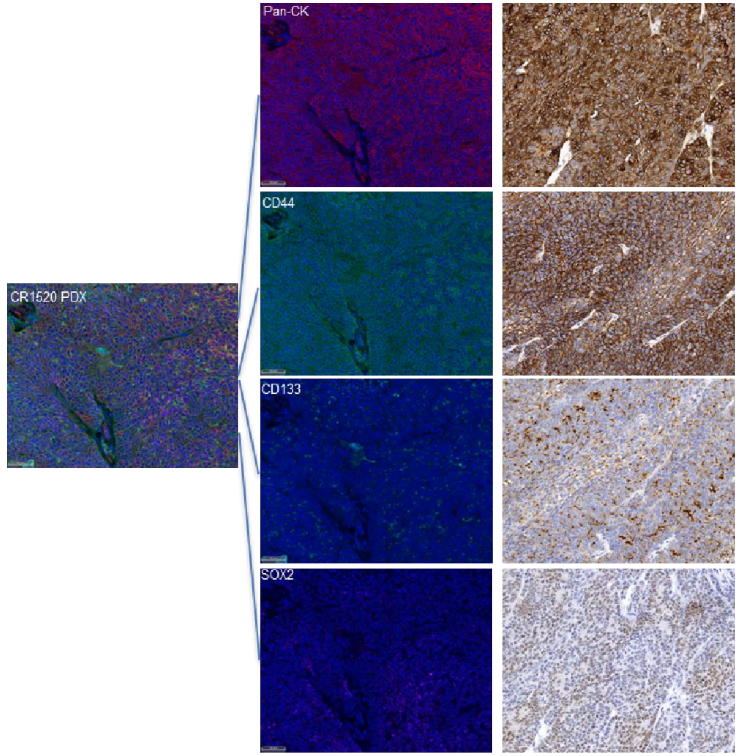
Five Key Parameters:

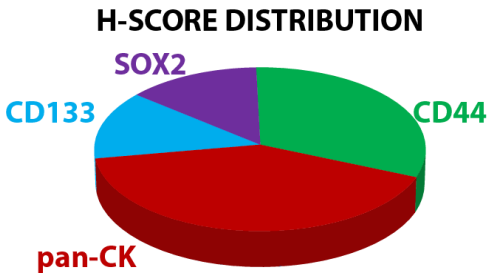
1. Histopathology
2. Molecular pathology
3. CSC-theory
4. TME
5. Pharmacology

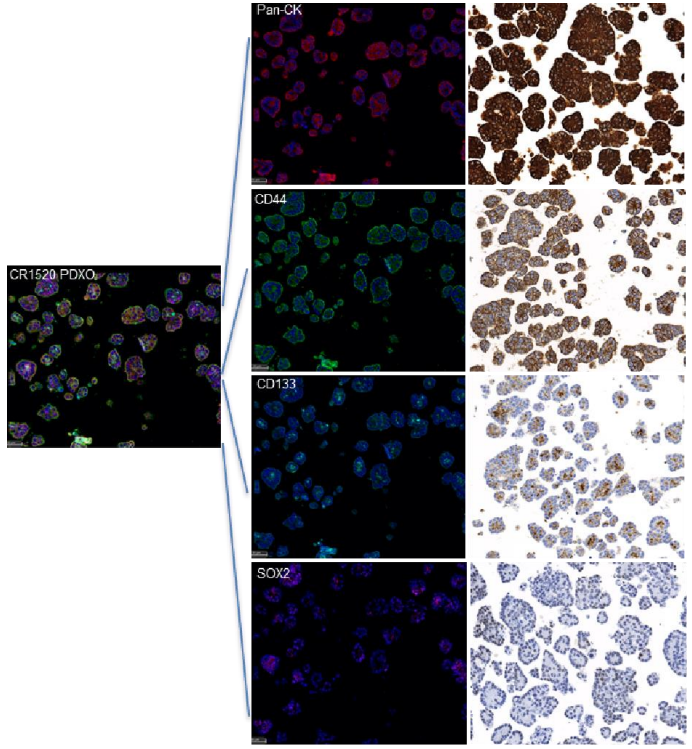


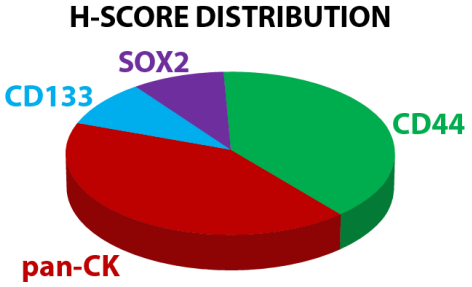
Similar CSC Components

Matched PDX/PDXO pair (CR1520)









Documented Predictive Power of PDO Models

RESEARCH | REPORTS

ORGANOIDS

Vlachogiannis *et al.*, *Science* **359**, 920–926 (2018)

Patient-derived organoids model treatment response of metastatic gastrointestinal cancers

- 90% positive prediction
- 100% negative prediction

nature medicine | RESOURCE

https://doi.org/10.1038/s41591-019-0584-2

A rectal cancer organoid platform to study individual responses to chemoradiation

Karuna Ganesh[1,2,21], Chao Wu[3,4,21], Kevin P. O'Rourke[5,6], Bryan C. Szeglin[4,7], Youyun Zheng[8,9], Charles-Etienne Gabriel Sauvé[4], Mohammad Adileh[4], Isaac Wasserman[4], Michael R. Marco[4], Amanda S. Kim[10], Maha Shady[8,9], Francisco Sanchez-Vega[4,11], Wouter R. Karthaus[3], Helen H. Won[8,9], Seo-Hyun Choi[4], Raphael Pelossof[4], Afsar Barlas[12], Peter Ntiamoah[8], Emmanouil Pappou[4], Arthur Elghouayel[4], James S. Strong[4], Chin-Tung Chen[4], Jennifer W. Harris[4], Martin R. Weiser[4], Garrett M. Nash[4], Jose G. Guillem[4], Iris H. Wei[4], Richard N. Kolesnick[1], Harini Veeraraghavan[13], Eduardo J. Ortiz[14], Iva Petkovska[14], Andrea Cercek[2], Katia O. Manova-Todorova[12], Leonard B. Saltz[2], Jessica A. Lavery[15], Ronald P. DeMatteo[16], Joan Massagué[5], Philip B. Paty[4], Rona Yaeger[2], Xi Chen[17], Sujata Patil[15], Hans Clevers[18], Michael F. Berger[8,9], Scott W. Lowe[5], Jinru Shia[8,19], Paul B. Romesser[10], Lukas E. Dow[20], Julio Garcia-Aguilar[4], Charles L. Sawyers[3,21]* and J. Joshua Smith[3,4,21]*

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients

Ooft *et al.*, *Sci. Transl. Med.* **11**, eaay2574 (2019)

"...PDO biobanks greatly expand the types of patient samples that can be propagated and studied..."

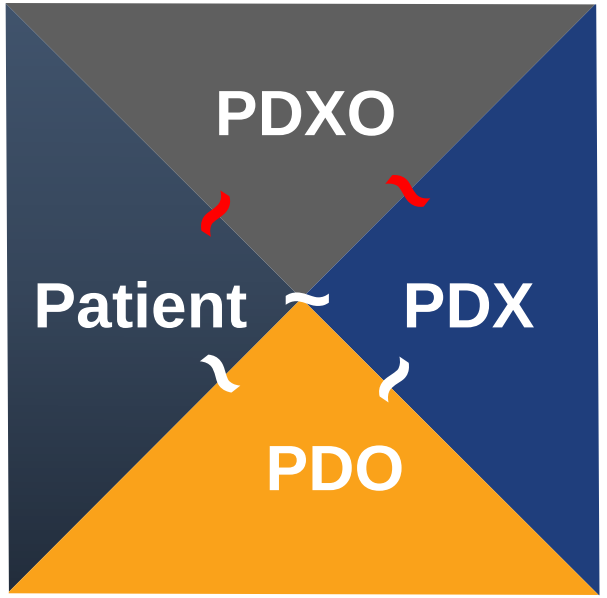
Clinical and Translational Report

Cell Stem Cell

Patient-Derived Organoids Predict Chemoradiation Responses of Locally Advanced Rectal Cancer

Yao et al., 2020, Cell Stem Cell *26*, 1–10

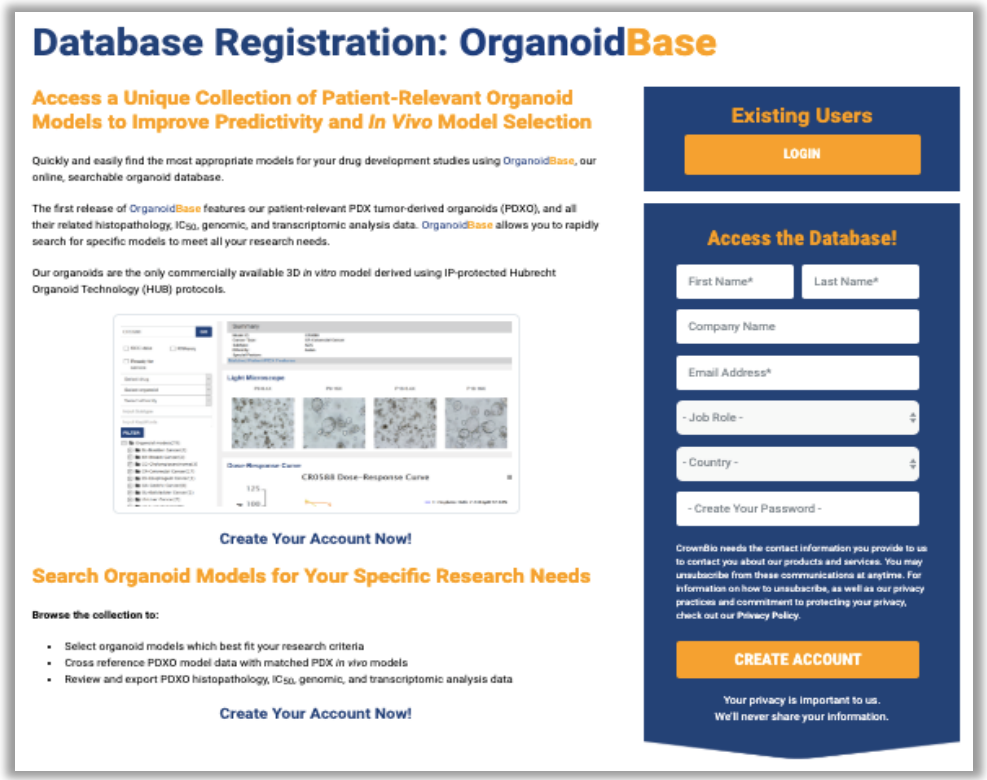
Bioequivalence among patient-derived models (?)



Organoid Biobank (PDO/PDXO)

Cancer type	PDO	PDXO	Sum
BL-Bladder Cancer		4	4
BR-Breast Cancer	13	5	18
BR-Breast Normal	6		6
CC-Cholangiocarcinoma		3	3
CR-Colorectal Cancer	87	59	146
CR-Colorectal Normal	16		16
CV-Cervical Cancer		3	3
ES-Esophageal Cancer		9	9
FT-Fallopian tube	4		4
GA-Gastric Cancer		26	26
GL-Gallbladder Cancer		2	2
HN-Head and Neck Cancer		2	2
LI-Liver Cancer		10	10
LU-Lung Cancer	22	49	71
LU-Lung Normal	12		12
ME-Melanoma		6	6
OV-Ovarian Cancer	8	10	18
PA-Pancreatic Cancer	25	40	65
PA-Pancreatic Normal	7		7
UT-Uterine Cancer		1	1
Sum	200	229	429

Growth kinetics	PDO	PDXO	Sum
Fast	42	33	75
Medium	76	143	219
Slow	59	53	112
Sum	**177**	**229**	**406**

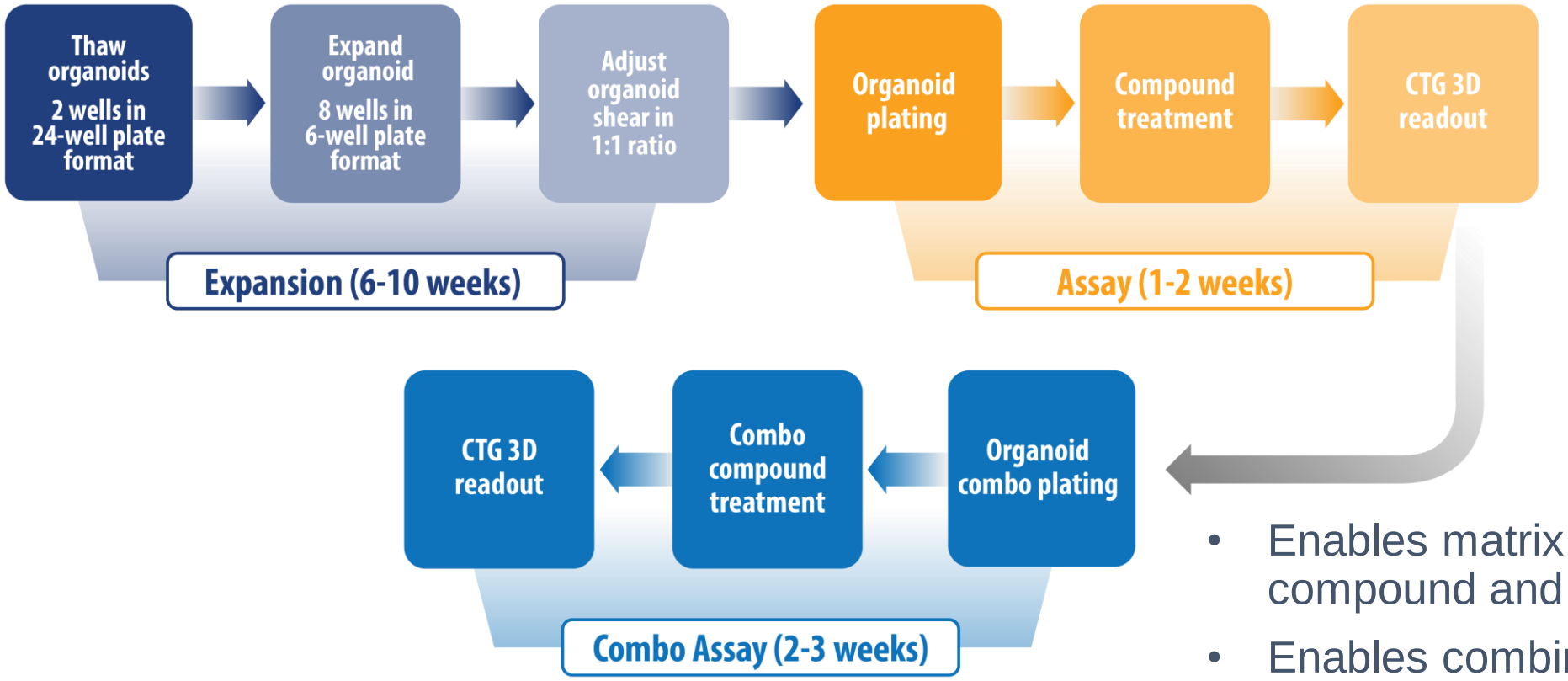


Data Featured:

- **Morphology:** light microscopy; histopathology (H&E, some IHC)
- **Genomic:** RNA-Seq and WES
- **Other info:** SOC & patient information
- **Login:** https://organoid.crownbio.com

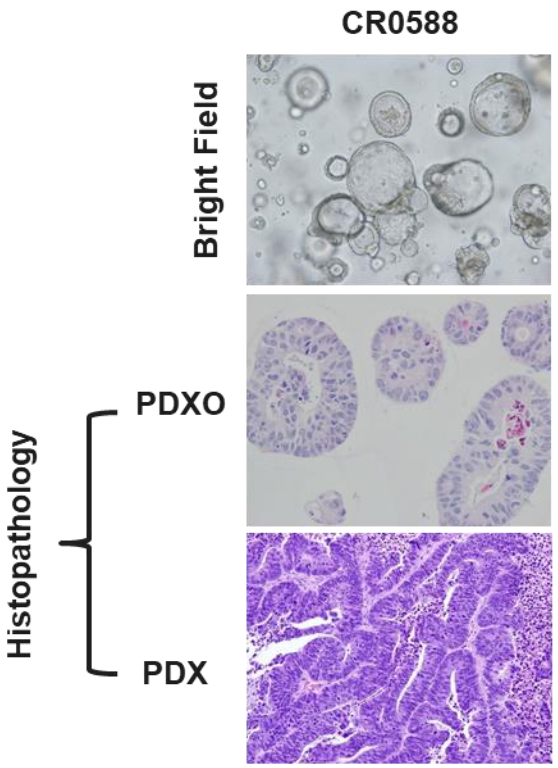
Updated Feb 2021

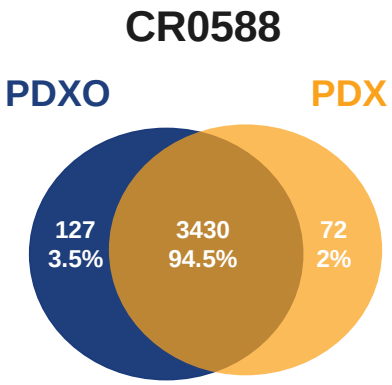
Organoid Screen Workflow



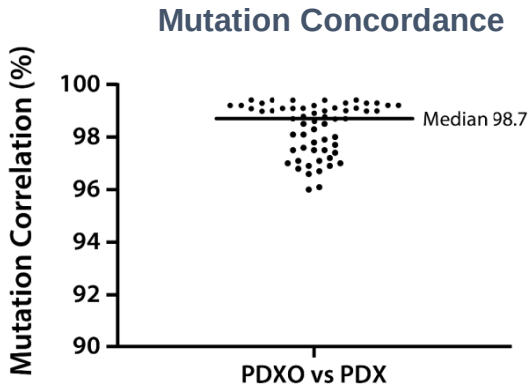
- Enables matrix screen for both compound and model library
- Enables combination therapy screening

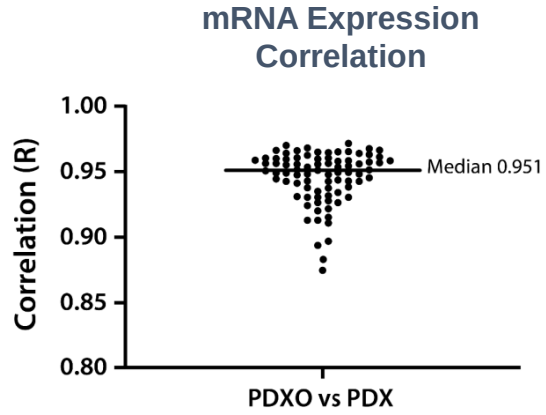
Biological Equivalence: High Correlation Between PDX/PDXO

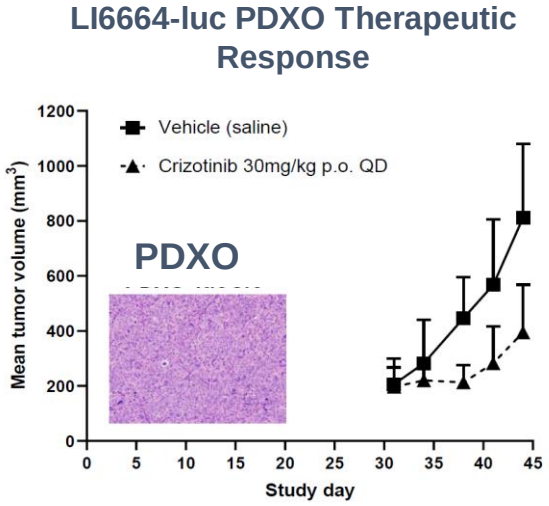


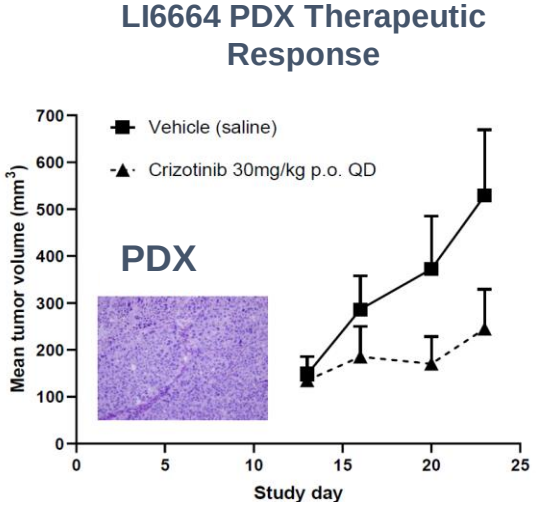


mRNA Expression Correlation	Mutation Concordance
R=0.97	94.52%









In Vitro PDXO Efficacy

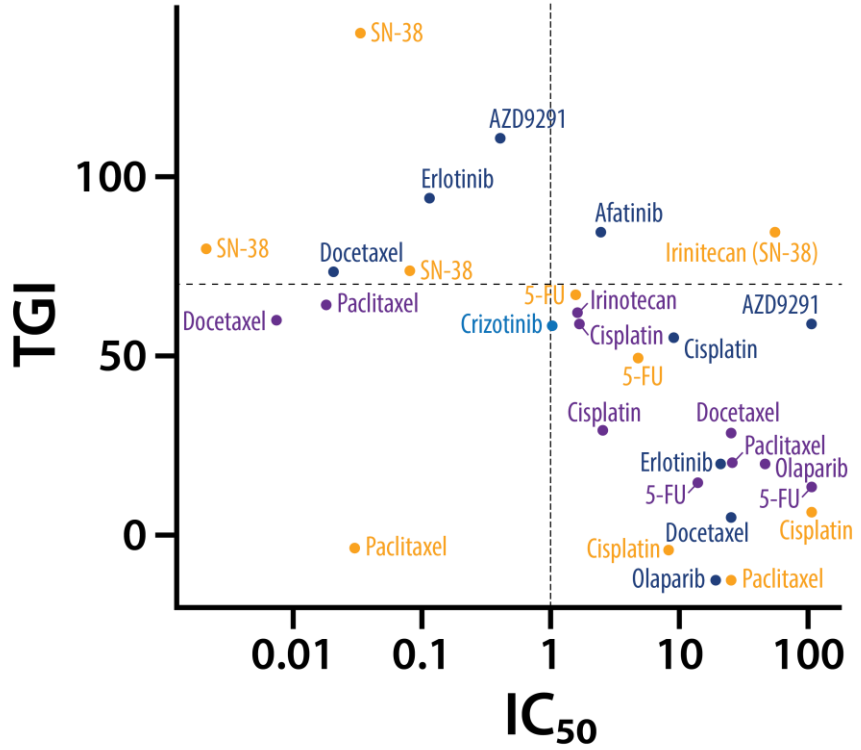
Enhanced predictivity of *in vivo* PDX efficacy

Overall prediction 86%
Fisher's exact test p value=0.001135

TGI	Sensitive (IC_{50}<1μM)	Resistant (IC_{50}≥1μM)
Sensitive (TGI≥70%)	6 (Positive prediction 75%)	2
Resistant (TGI<70%)	2	20 (Negative prediction 91%)

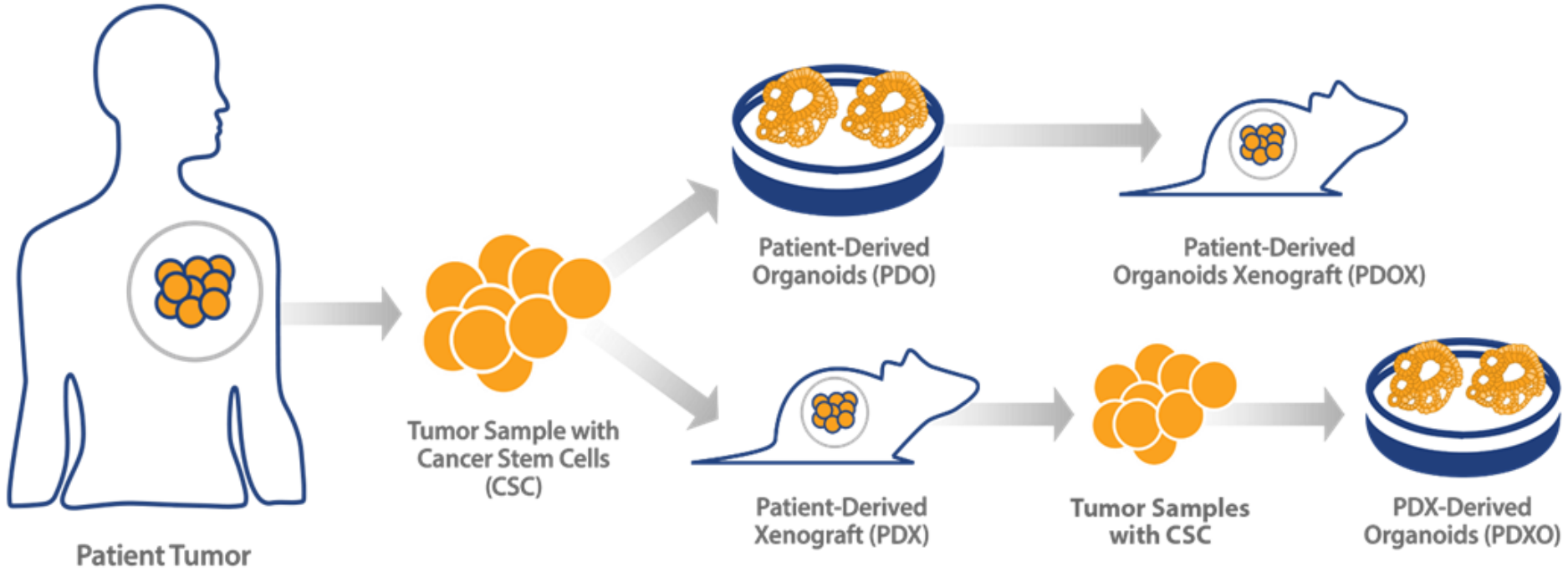
IC_{50}

Pharmacology Correlation: Paired *in vitro* PDXO and *in vivo* PDX

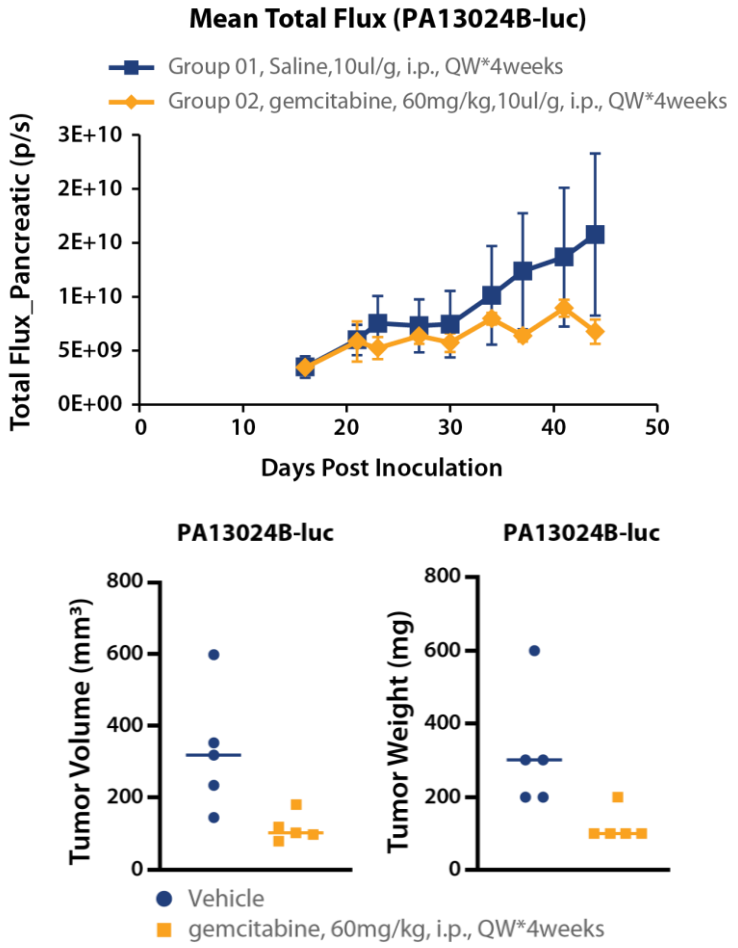


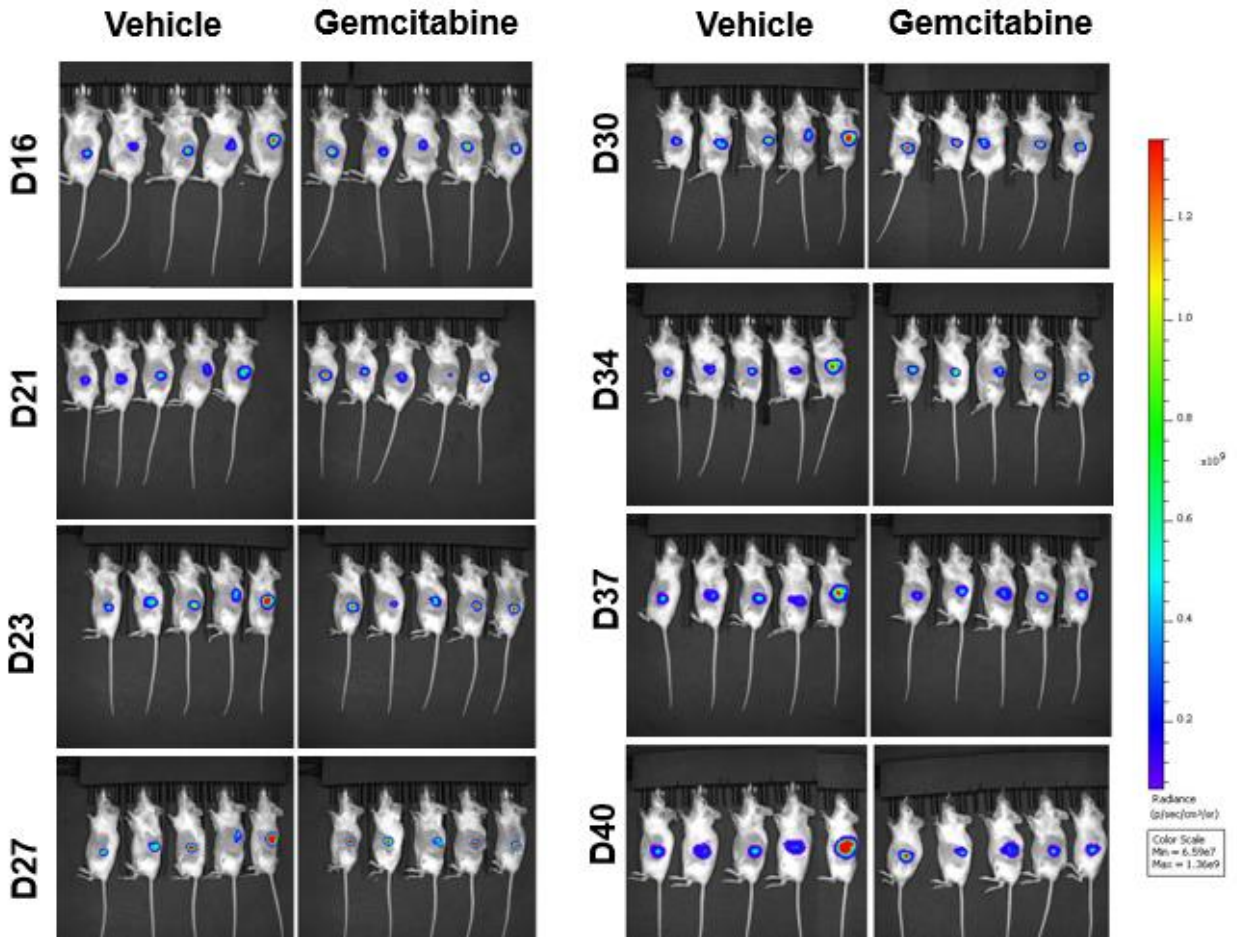
Matched Living Biobanks

Not only provides biologically equivalent model, but also enables ability to test more conditions in diverse applications



Engineered PDXO Models for Orthotopic Imaging





Big Data Analysis

Method 1

Big data view: different platforms and cancer types

Analysis: PC, DEG, PCC

	Cancer Type			
	CRC	LU	PA	Combined
TCGA	456	513	177	**1146**
PDO	45	13	25	**83**
PDXO	23	22	8	**53**
PDX	27	32	16	**75**
PDC	5	11	11	**27**
CCLE	54	171	41	**266**

Method 2

Individual paired datasets

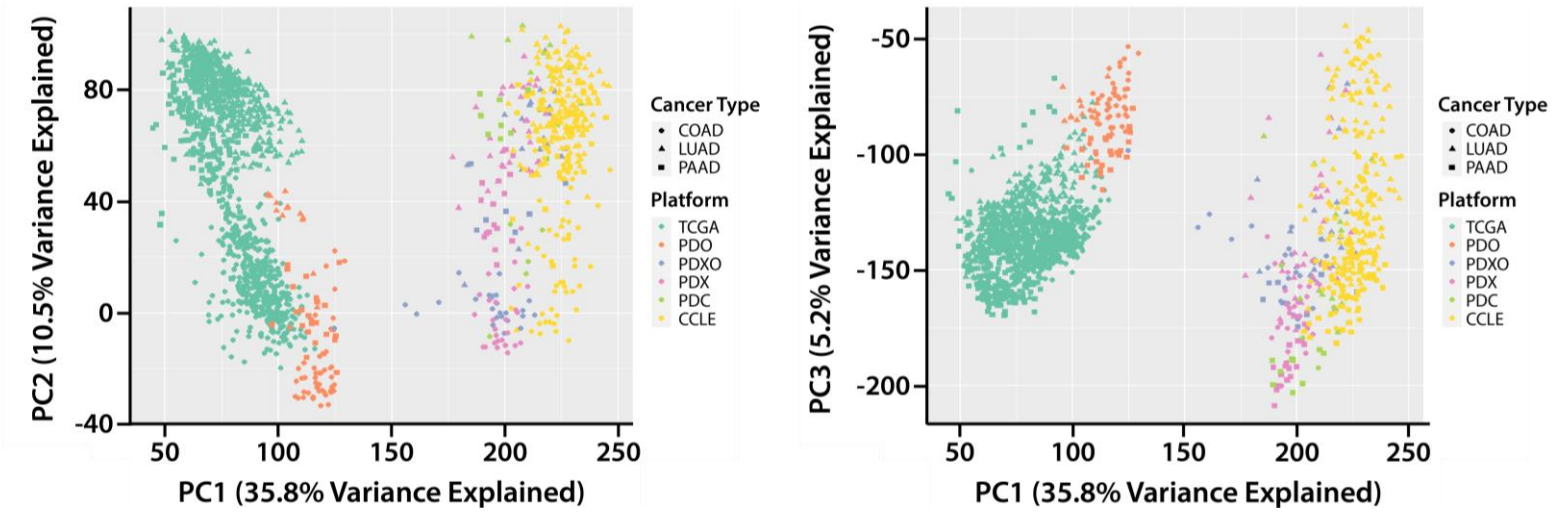
- 37 PDX-derived cell line/PDX pairs
- 83 PDXO/PDX pairs

Analysis

- DESeq2 to find DEGs
  - Whole dataset
  - Cancer type (at least two models)
- ORA and GSEA analysis
  - 50 MSigDB Hallmark genesets
  - 1329 canonical pathways in MSigDB
  - 203 KEGG pathways

mRNA Principle Component (PC) Analysis

Principle Component Analysis plots PCA plots

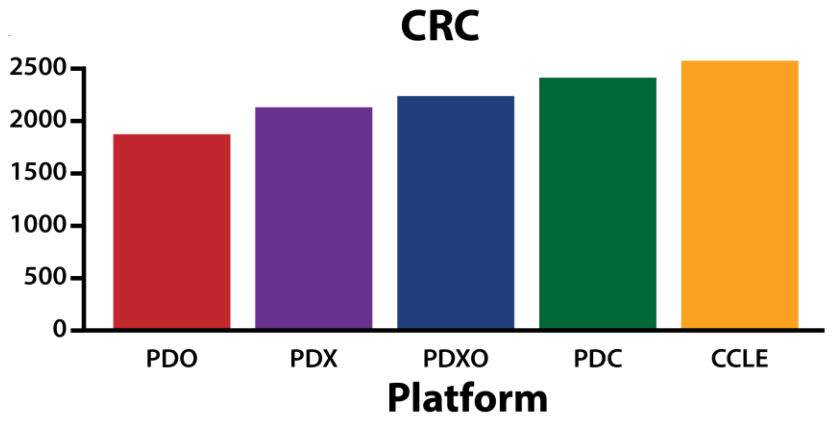


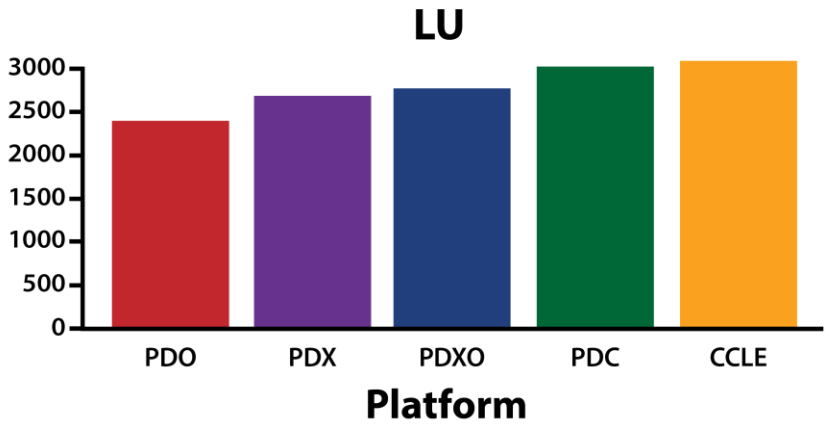
Top 5000 most varied genes of TCGA samples for PCA - PC1 contributes a large portion of variance (35.8%)

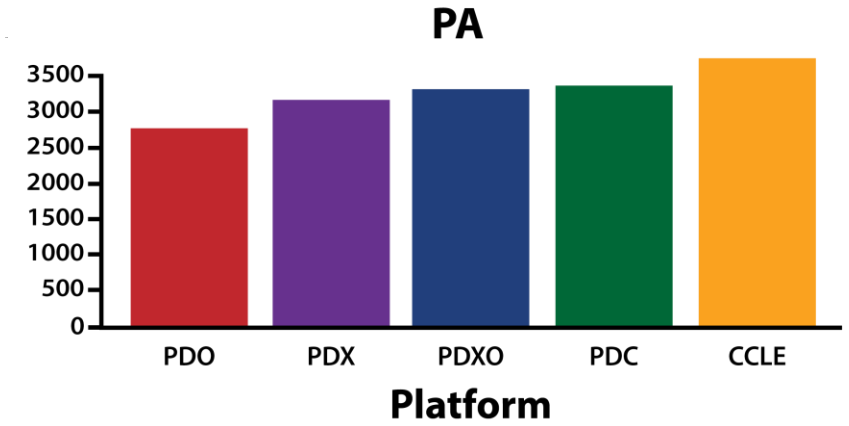
- PC1: TCGA>PDO>PDX/PDX-derived > CCLE (Platform)
- PC2: PDO > TCGA ~ PDX-derived/CCLE (Platform) (different among cancer types)
- PC3: PDX-derived > TCGA > CCLE > PDO

DEG# (Differentially Expressed Genes) Compared to TCGA

PDO > PDXO > PDC > CCLE

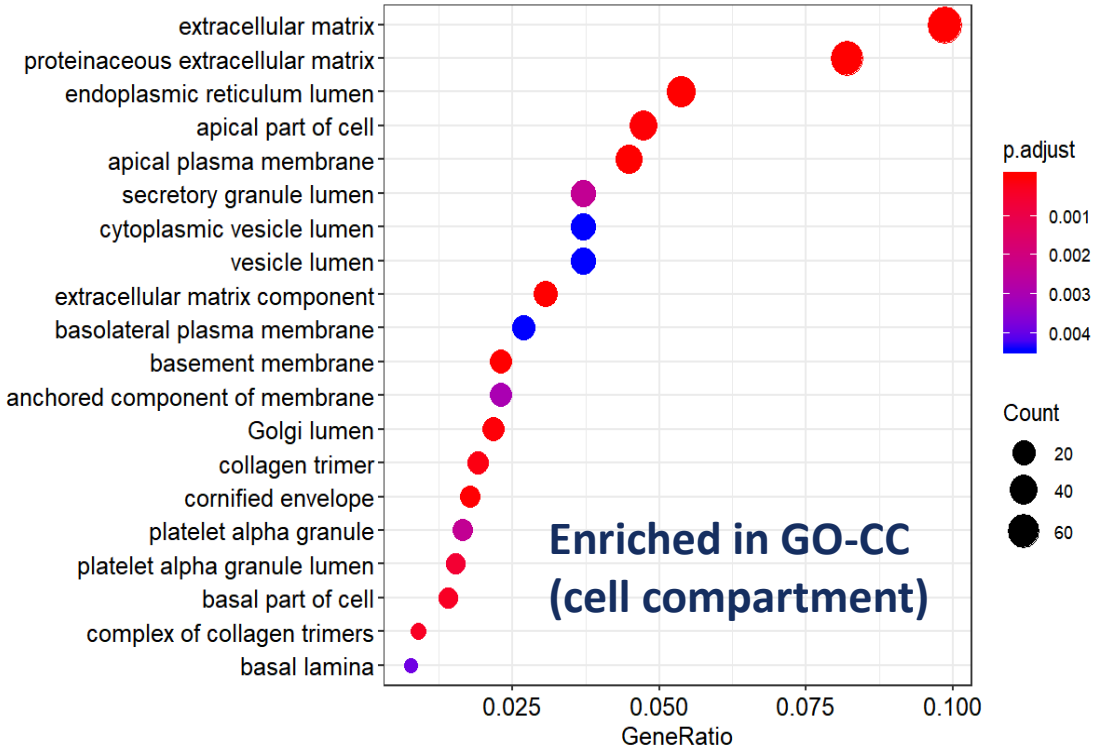


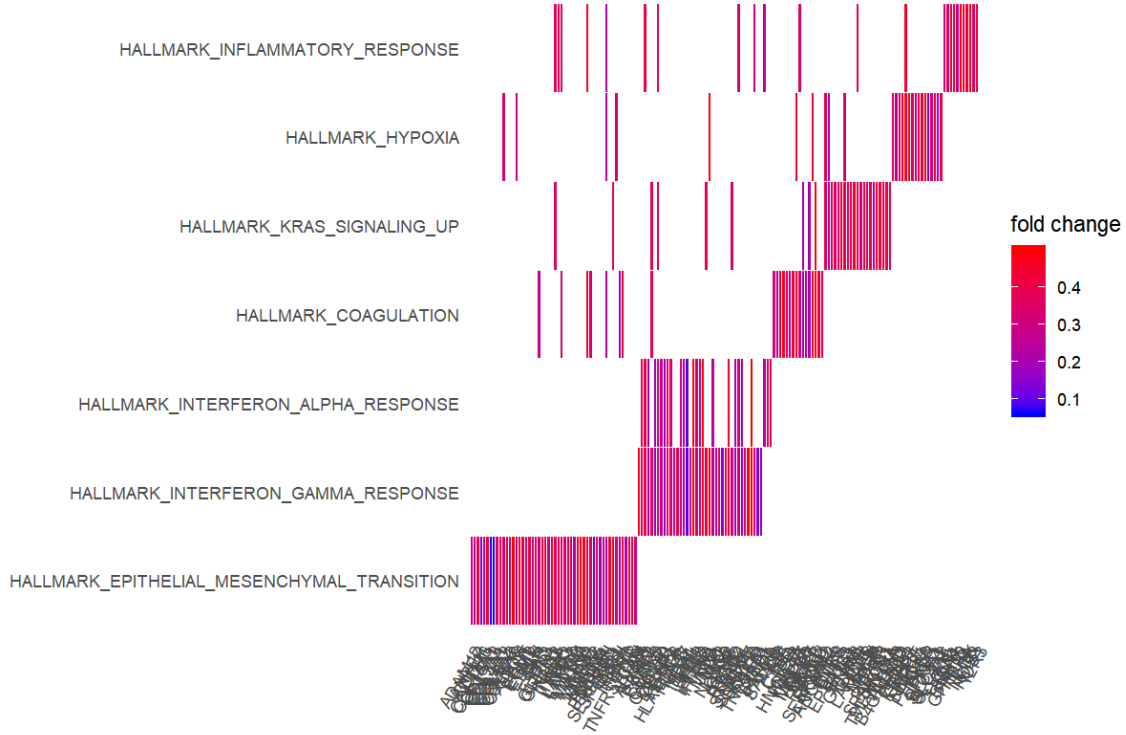




PDXO vs PDX – ORA, PDXO Down

Lowered EMT activity, reduced extracellular matrix-related expression





Summary

- Patient-derived models/biobanks - PDX/PDO are both clinically predictive and can be used in hypothesis testing & in drug development
- PDO enables matrix screening, significantly improving the drug discovery workflows
- Matched biobanks of both are largely bio-equivalent, complementing each other in applications
- Minor differences across the platforms will be investigated further

Clarity With CrownBio

Recognize your next clinical candidate when you see it.

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