

Comparison of PDX, PDC, and PDOrg models from the National Cancer Institute's Patient-Derived Models Repository (PDMR)

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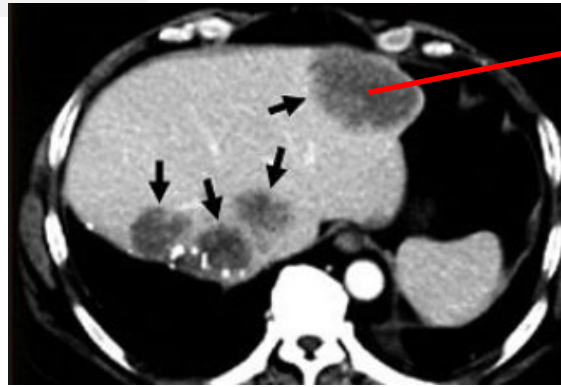
<https://pdmr.cancer.gov>

Overview

- Status of PDXs, organoids (PDOrgs), tumor cell lines (PDCs), and cancer associated fibroblasts (CAF) available today and in the upcoming 6-months
- Comparative analysis of WES and RNASeq data from matched PDXs, organoids (PDOrgs), and tumor cell lines (PDCs)
- Preliminary comparison of a set of PDX, PDOrg, and PDC models derived from a rapid autopsy case from one patient with Pancreatic Adenocarcinoma

NCI's Patient-Derived Models Repository

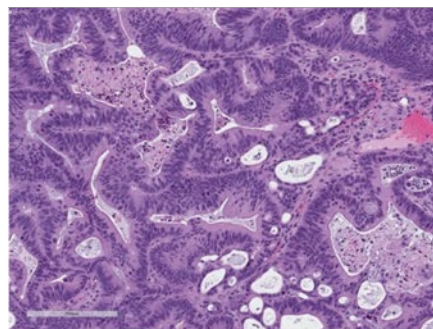
- A national repository of Patient-Derived Models (PDMs) to serve as a resource for academic discovery efforts and public-private partnerships for drug discovery
- Clinically-annotated & early-passage models with comprehensive molecular-characterization and quality control metrics
- Complement existing PDM collections and focus on under-represented model types such as rare cancers and models representing racial and ethnic minorities
- Provide models to the research community at a modest cost compared to other distributors
- Provide all related metadata including: deidentified patient clinical history and outcomes, model histology, WES and RNASeq of models, and SOPs through a public website: <https://pdmr.cancer.gov>



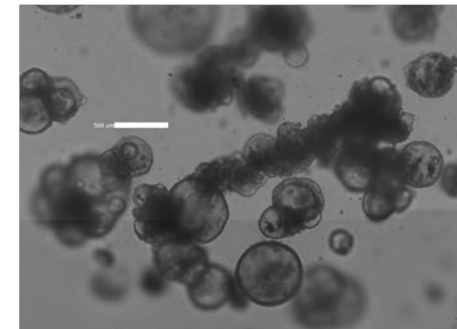
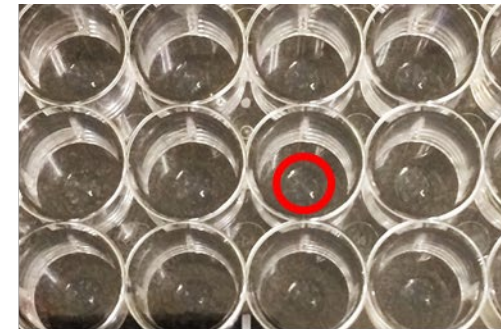
Ship overnight from Clinic to FNLCR

- Attempt to generate multiple patient-derived model types

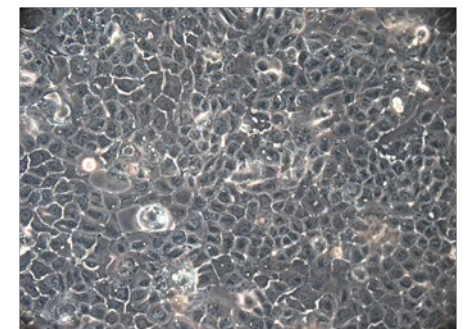
PDX



PDOrg Culture



PDC/CAF Culture





Model Development and Characterization

PDX Take-Rate from Tumor Tissue Implantations

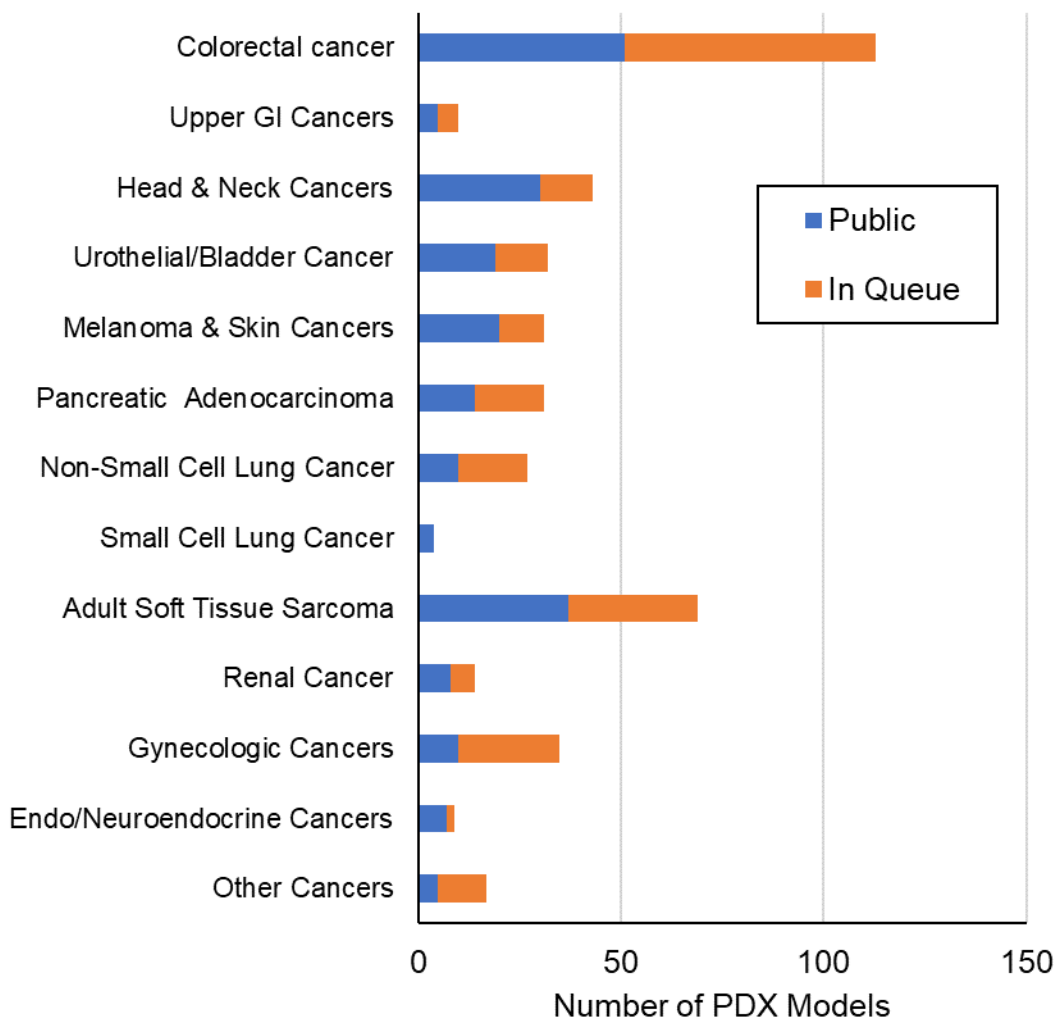
Body Location	Total Specimens Received	Total Assessable Specimens	%Take-Rate of Assessable Specimens	Histology-Confirmed Tumor	Discontinued	Not Yet Assessable: P0 tumors
Breast	307	238	38%	91	147	69
Digestive/ Gastrointestinal	692	614	52%	321	293	78
Endocrine/ Neuroendocrine	194	166	24%	40	126	28
Genitourinary	528	453	37%	168	285	75
Germ Cell	4	4	0%	0	4	0
Gynecologic	327	265	52%	137	128	62
Head and Neck	168	162	54%	88	74	6
Hematologic/Blood	20	13	54%	7	6	7
Musculoskeletal	385	351	34%	118	233	34
Neurologic	30	27	63%	17	10	3
Respiratory/Thoracic	225	198	48%	95	103	27
Skin	80	75	64%	48	27	5
Unknown Primary	20	19	26%	5	14	1
Totals	2980	2585	44%	1135	1450	395

All tumor material collected and shipped priority overnight in CO2-independent media for next-day implantation into NSG host mice

Rare Cancer Histology PDX Models Available

- Merkel Cell Carcinoma
- Mesothelioma
- Hurthle Cell Neoplasm of the Thyroid
- Malig. Periph. Nerve Sheath Tumor
- Salivary Gland SCC
- Pharyngeal SCC
- Nasopharyngeal SCC
- Laryngeal SCC
- Vaginal Cancer
- Cervical SCC
- Carcinosarcoma of the Uterus
- Synovial Sarcoma
- Liposarcoma
- Leiomyosarcoma – uterine and non-uterine
- Rhabdomyosarcoma
- Osteosarcoma
- Chondrosarcoma
- Malignant fibrous histiocytoma
- Fibrosarcoma – not infantile
- Ewing sarcoma/Peripheral PNET

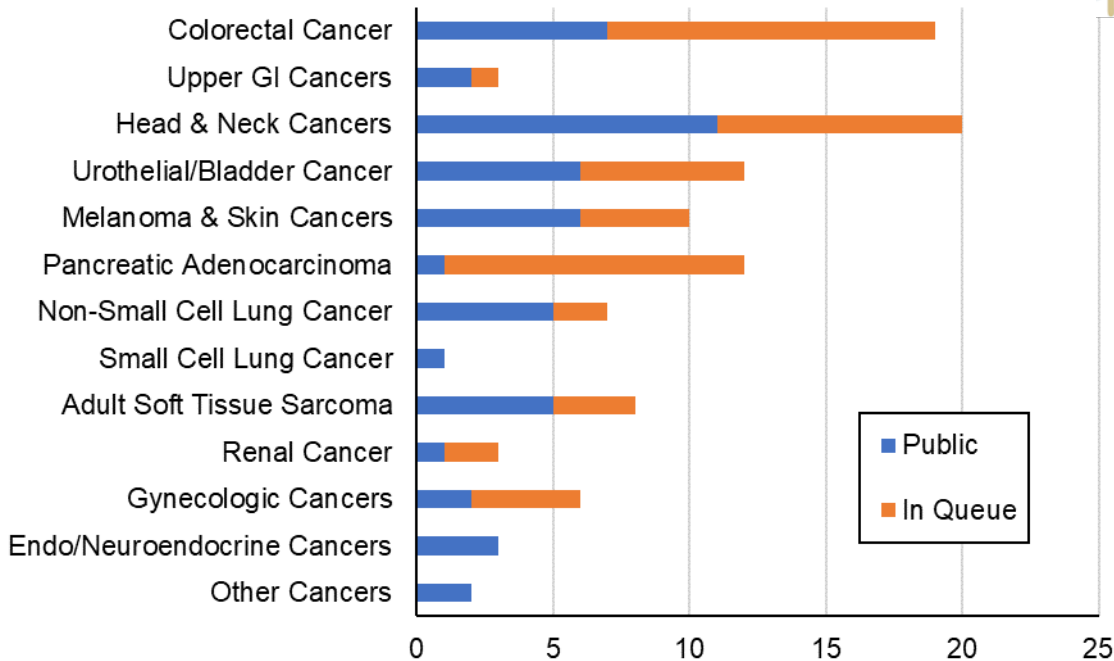
Patient-Derived Xenografts (PDXs)



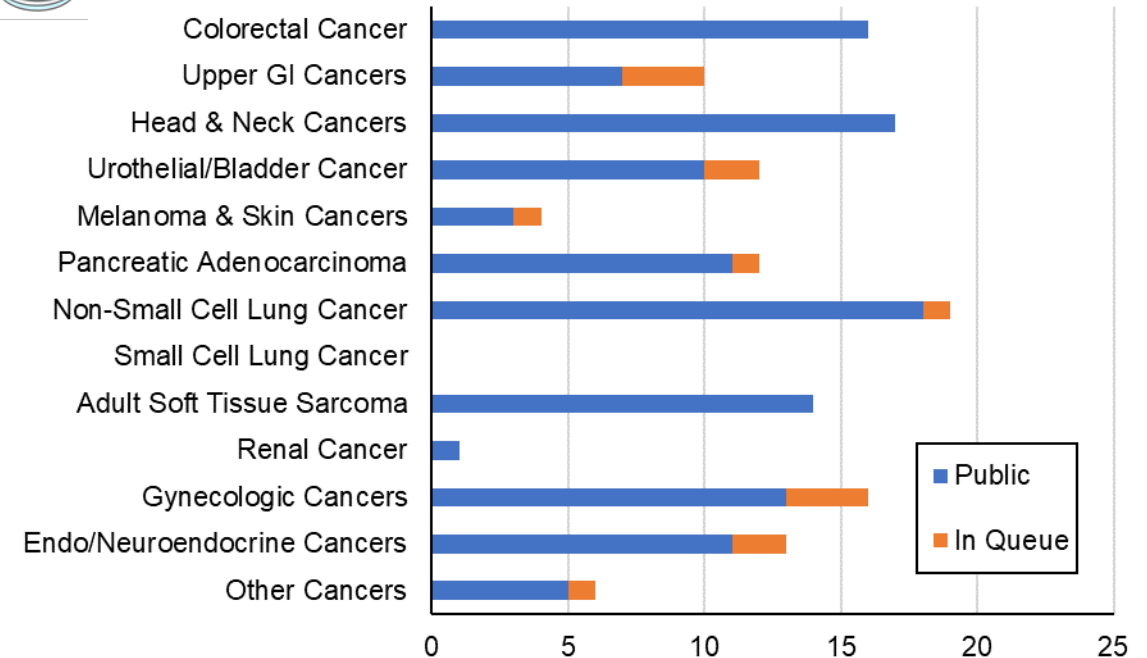
- **222 PDX models publicly available** (pdmr.cancer.gov).
 - ✓ 210 models going through final QC (final pathology, NGS, STR, regrowth from freeze,...)
 - ✓ ~200-300 models in Passage 1-4 expansion
 - ✓ ~600 models in Passage 0
- Clinically-annotated, early-passage, molecularly-characterized patient-derived models
- **Distribution Material**
 - ✓ Median Passage = 2
 - Range for NCI-generated models: 1-6
 - Range for Contributor models: 1-24
- Current distribution within the US (pdmr.cancer.gov).
 - ✓ Model information also available through PDX Finder at www.pdxfinder.org
- Specimens are from patients with both primary and metastatic disease from treatment naïve to heavily pre-treated.

Patient/PDX-Derived Cancer Cell Lines (PDCs) and Cancer Associated Fibroblast Cultures (CAFs)

52 PDCs



125 CAFs



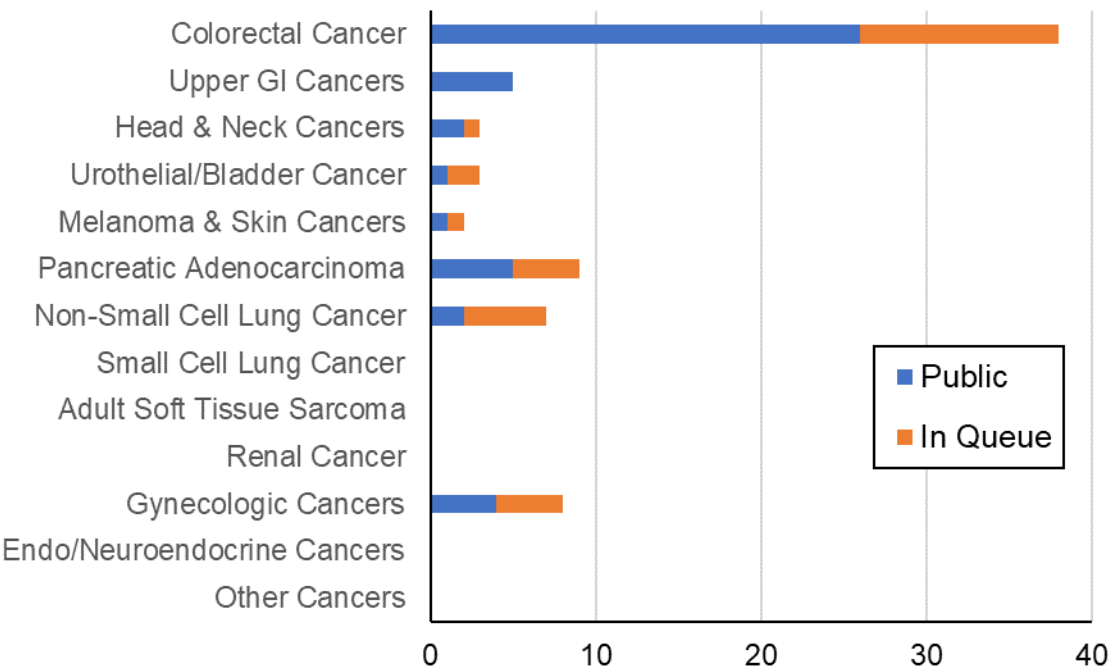
- **Adherent & Suspension Cultures**
- **Requires use of defined media**
- **Distribution Material**

✓ Median Passage = 20
• Range : 12-51

- **Not Transformed - Limited Lifespan**
- **Requires use of defined media**
- **Distribution Material**

✓ Median Passage = 14
• Range: 9-26

Patient/PDX-Derived Organoids (PDOrg)



- **Requires use of defined media**
- **Distribution Material**
 - ✓ Median Passage = 10
 - Range : 6-30

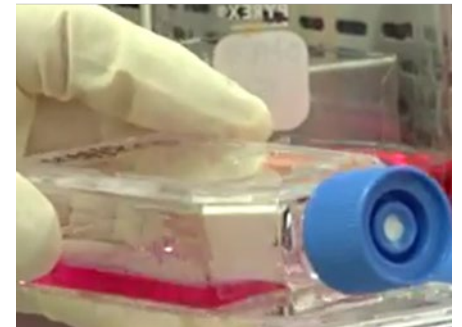
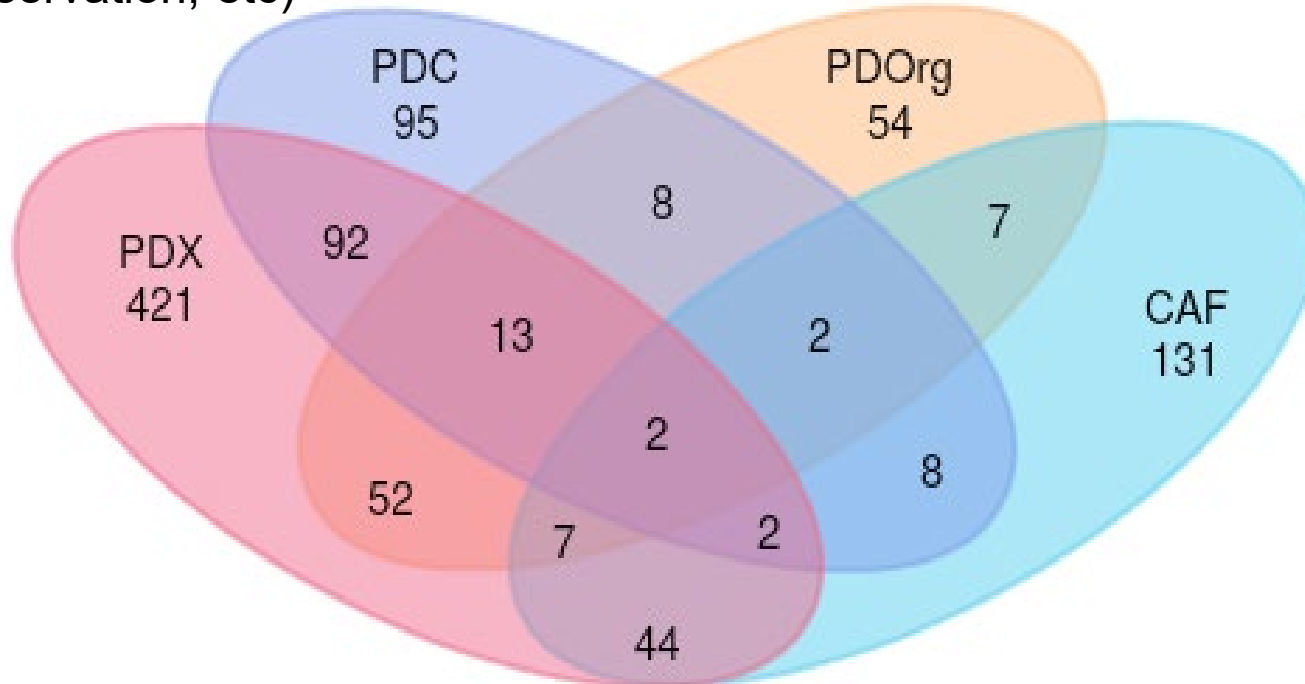
- First 46 models are publicly available with another 30 going through QC (NGS, tumorigenicity verification, STR, etc)
- **Goal:** Wherever possible develop a PDX, 2D *in vitro* PDC, and PDOrg culture for comparative preclinical studies
- Provide all related metadata and SOPs through the PDMR website and public database: pdmr.cancer.gov


Matched PDX, PDOrg, PDC, and CAF Models

Includes models that are either

- (1) Publicly Available or
- (2) Going through final QC for Public release (pathology confirmation of all contributing material, NGS, STR, regrowth from cryopreservation, etc)

PDX	421
PDC	95
PDOrg	54
CAF	131





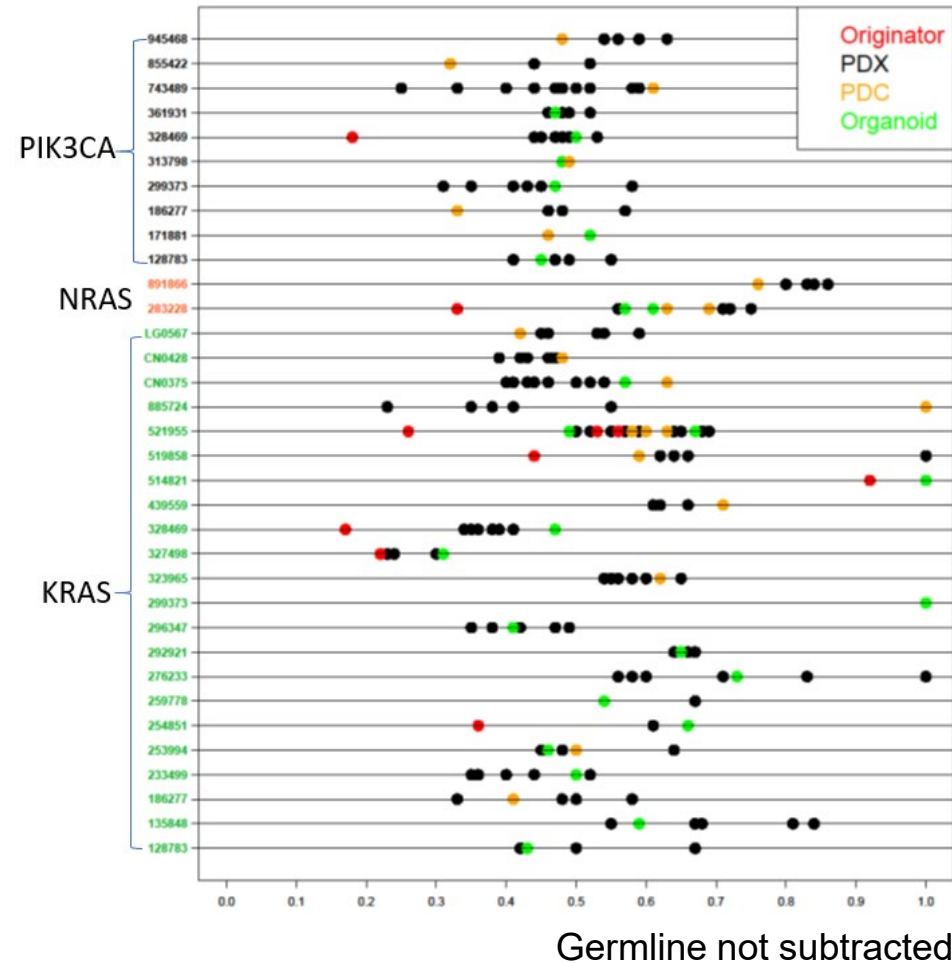
Molecular Characterization Comparison of PDX, PDC, and PDOrg Model Types

Comparing PDX, PDC, and PDOrg Model Types

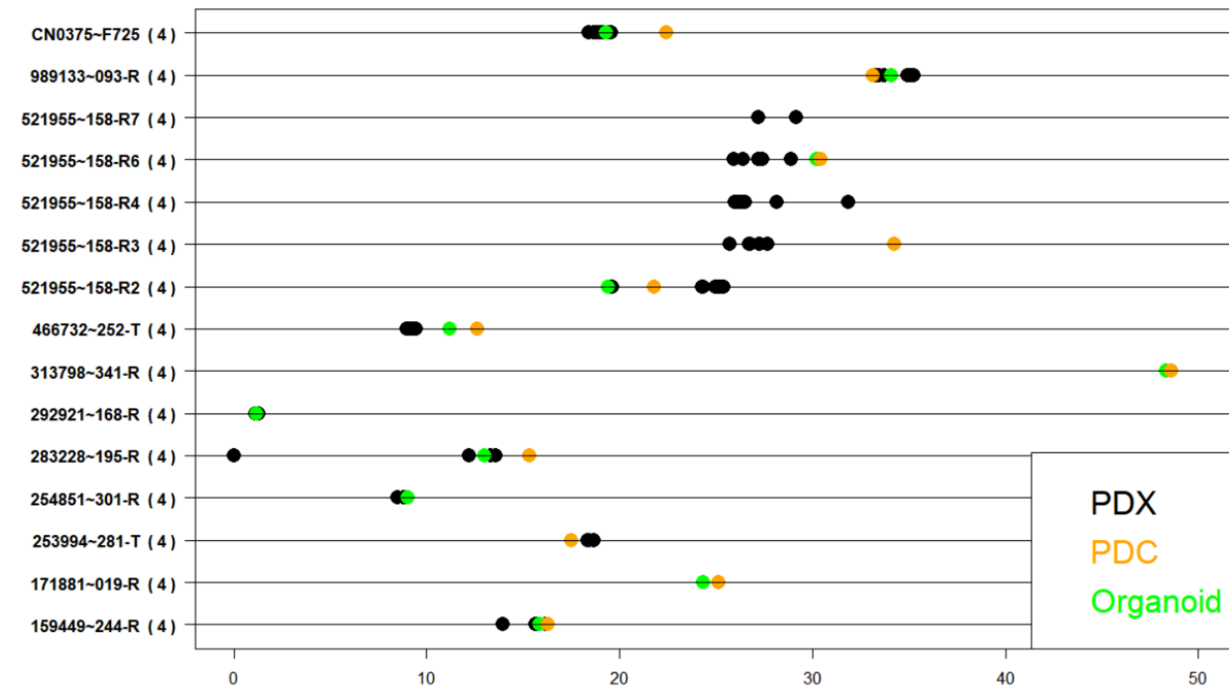
- **Data for Comparison**
 - 413 PDX samples from 75 PDX models
 - 43 PDOrg models
 - 73 PDC models
 - **Whole Exome Sequence Comparisons**
 - Driver Allele Frequency (VAF)
 - Loss of Heterozygosity (LOH)
 - Microsatellite Instability
 - **RNASeq**
 - RNASeq Correlation Matrix
 - tSNE Plots
-
- ✓ **Driver mutations are present in all model types (PDX, PDOrg, PDC)**
 - ✓ **Irrespective of model type (PDX, PDOrg, PDC), gene expression profiles cluster by histology and by patient**
 - ✓ **The majority of models are internally consistent for LOH; however, models can be found with differences at the LOH level between model types (PDX, PDOrg, PDC)**

Variant Allele Frequency and Loss of Heterozygosity is Consistent Across Model Types

Driver Allele Frequency Consistent Across Majority of Models

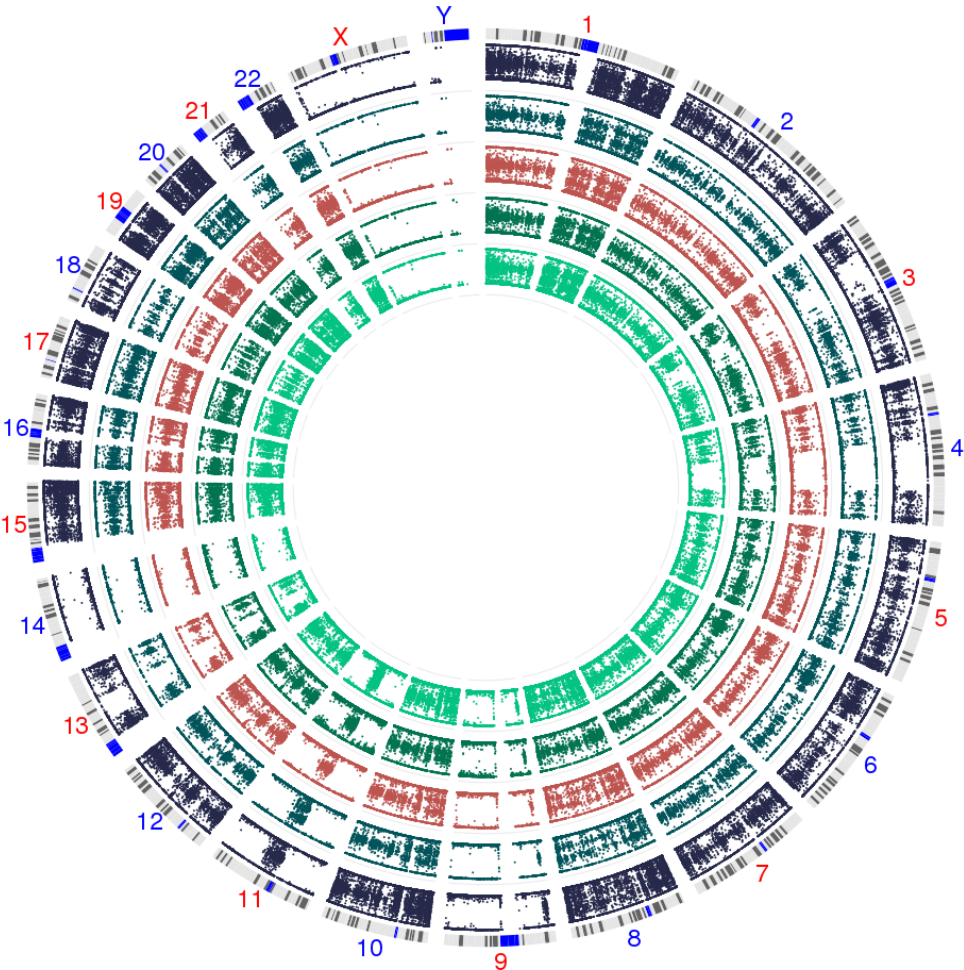


Percent LOH Consistent



Majority of Models have Consistent LOH Across Model Types

Originating Patient: 84yo, Treatment Naïve, Urothelial Transitional Cell Carcinoma



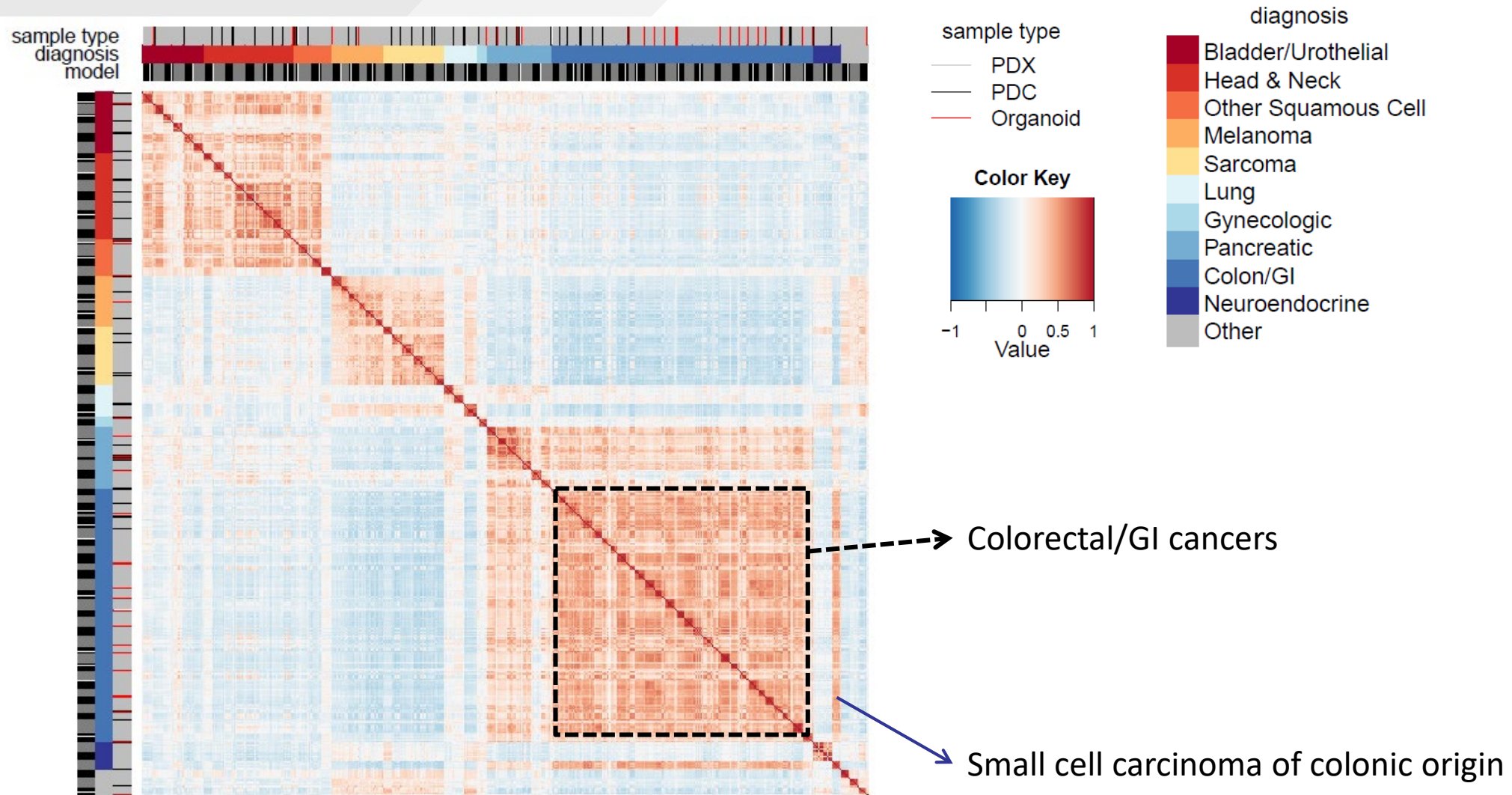
Model	Sample	Passage
159449-244-R	J1-PDC	Passage 19
159449-244-R	PDX	Passage 2
159449-244-R	PDX	Passage 1
159449-244-R	PDX	Passage 0
159449-244-R	V1-organoid	Passage 14

- ✓ LOH on 3p, 4q, 9,11,13, and 14 are preserved
- ✓ Imbalance on 1q, 8, and 10p are preserved

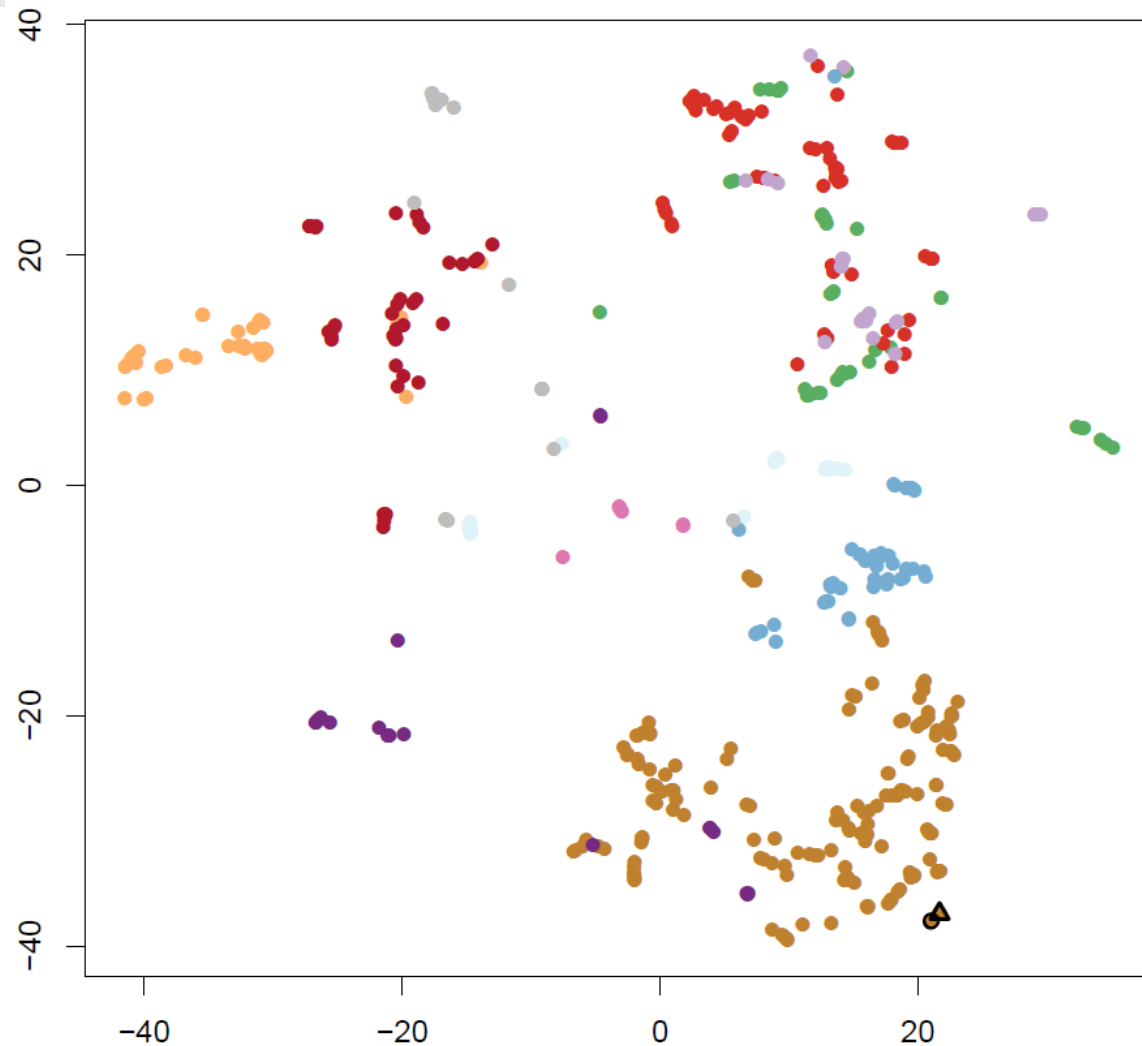
MSI-High Status is Consistent Across Model Types

Diagnosis	Number of Samples Sequenced				Number of MSI-H Samples			
	Originator	PDX	PDOrg	PDC	Originator	PDX	PDOrg	PDC
Cervical Adeno		6				6		
Colon Adeno	1	6			1	6		
Colon Adeno		4		1		4		1
Colon Adeno	1	6	1		1	6	1	
Colon Adeno		6	1			6	1	
Colon Adeno		4	1			4	1	
Colon Adeno		6	1			6	1	
Colon Adeno		6				6		
Colon Adeno	1	4		1	1	4		1
Colon Adeno	1	5			1	5		
Colon Adeno	1	6			1	6		
Colon Adeno	1	1			1	1		
Colon Adeno		4		1		4		1
Pancreatic Adeno		6	1			6	1	
Sm. Intest. Adeno		1				1		
Carcinosarcoma	1	4	1		1	4	1	
Endometriod Adeno		1				1		
Endometriod Adeno		4				4		
Endometriod Adeno	1	6			1	6		
Endometriod Adeno	1	3			1	3		
Salivary Gland Ca	1	5			1	3		
SCLC		5		1		3*		1
Bladder Ca	1	6			1	5*		
Uterine Ca		1				1		

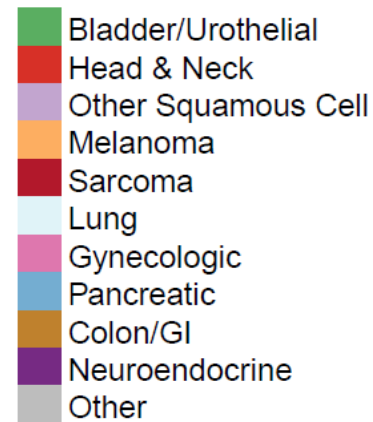
RNASeq Correlation Matrix



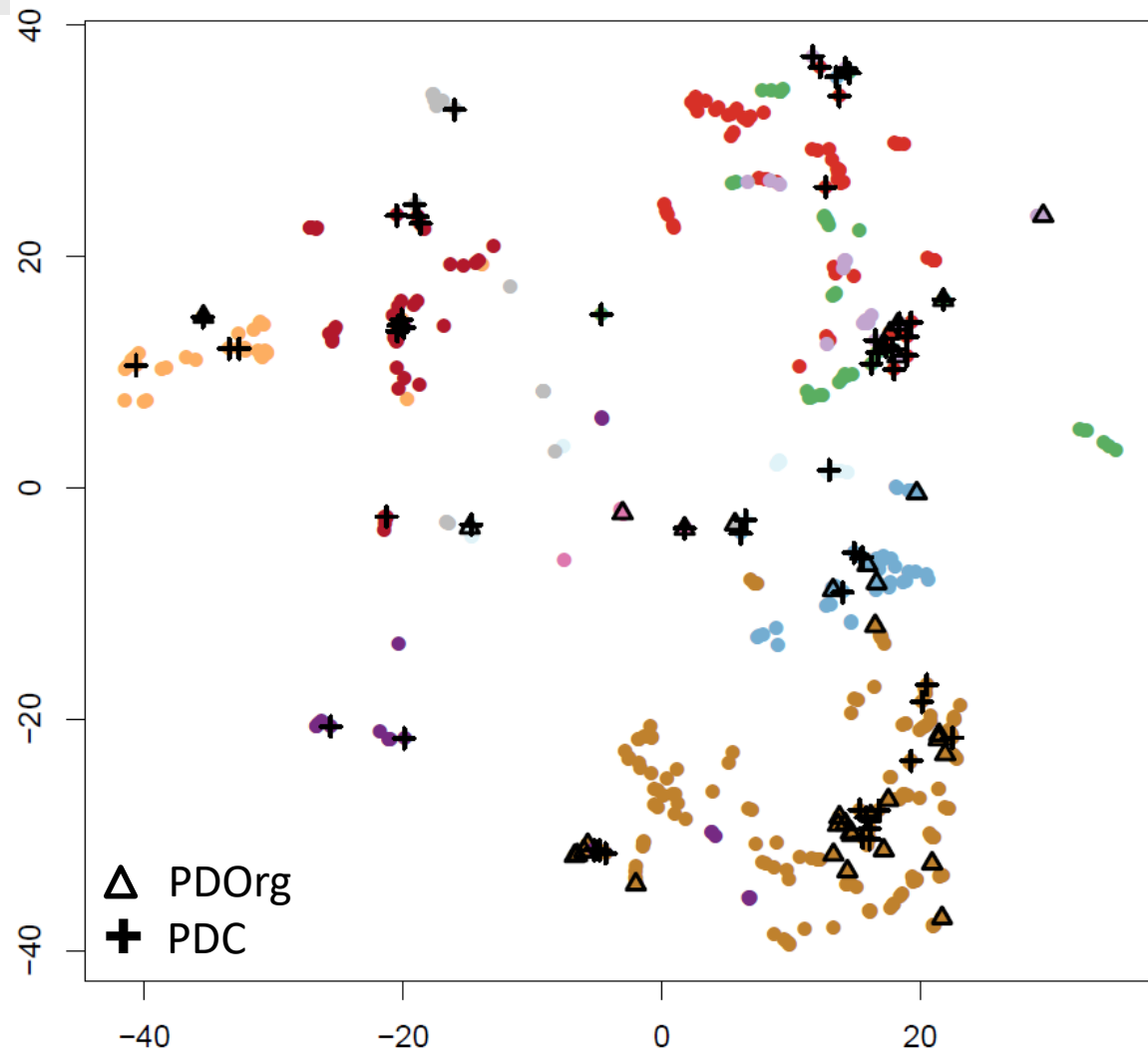
tSNE for all models w PDXs, PDCs, and/or PDOrgs



- Every point represents an individual PDX, PDC, or PDOrg. Within in one model 4-6 PDXs can be sequenced.
- Data represent
 - 413 PDX samples for 75 PDX models
 - 73 PDC models
 - 43 PDOrg models



tSNE for all models w PDXs, PDCs, and/or PDOrgs

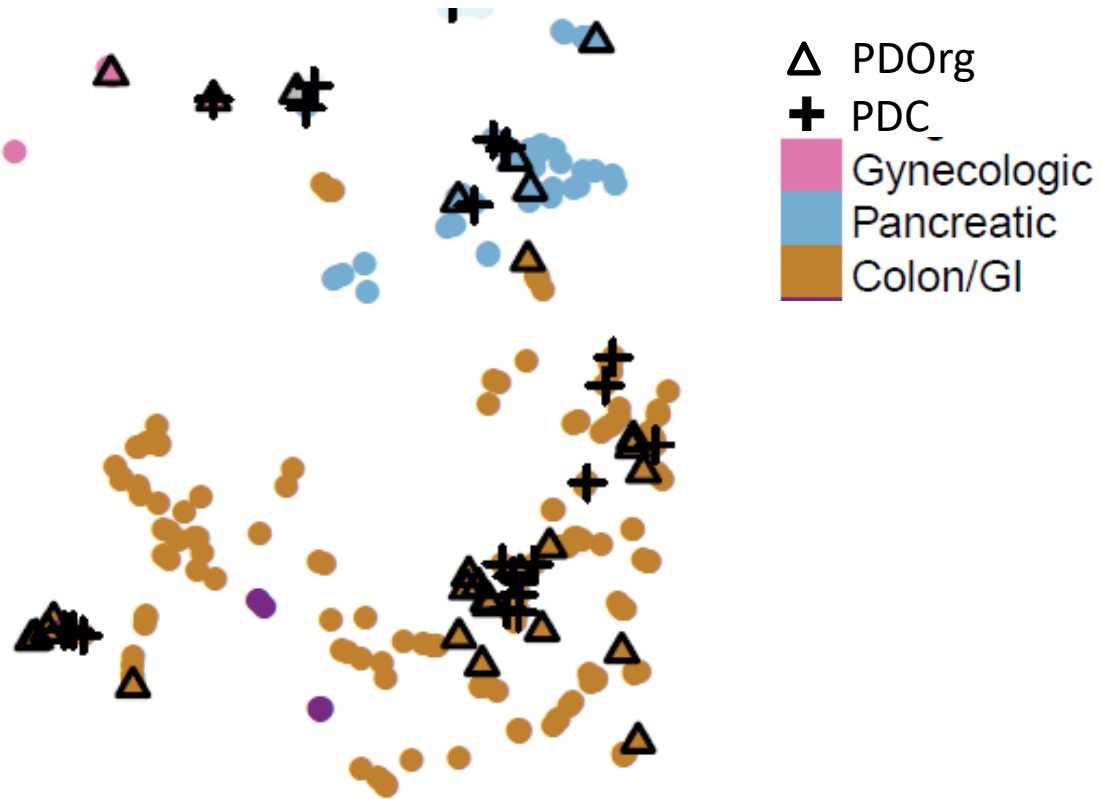


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 - 413 PDX samples for 75 PDX models
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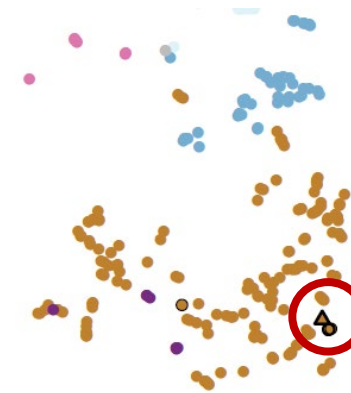


Expression Profiles Correlate Across the Majority of Models for Different Model Types: PDX, PDC, PDOrg

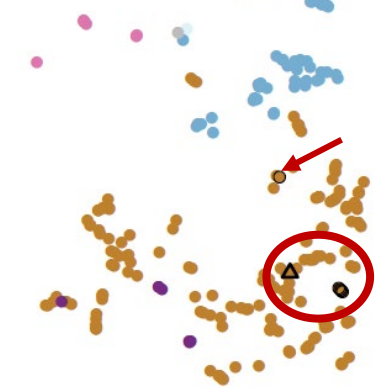
Colon/Digestive and Pancreatic Cancer Expression Clusters



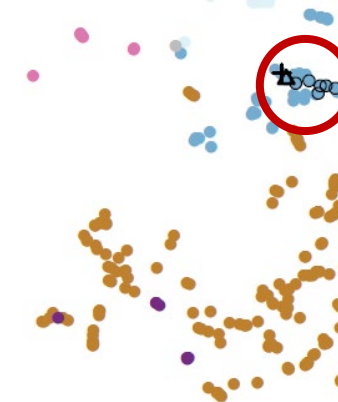
176247-063-R, Colon Adeno



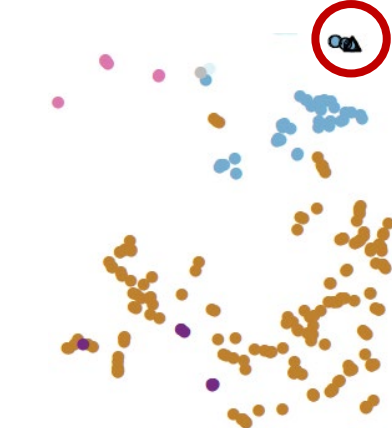
514821-333-R, Colon Adeno



521955-158-R6, Pancreatic Adeno



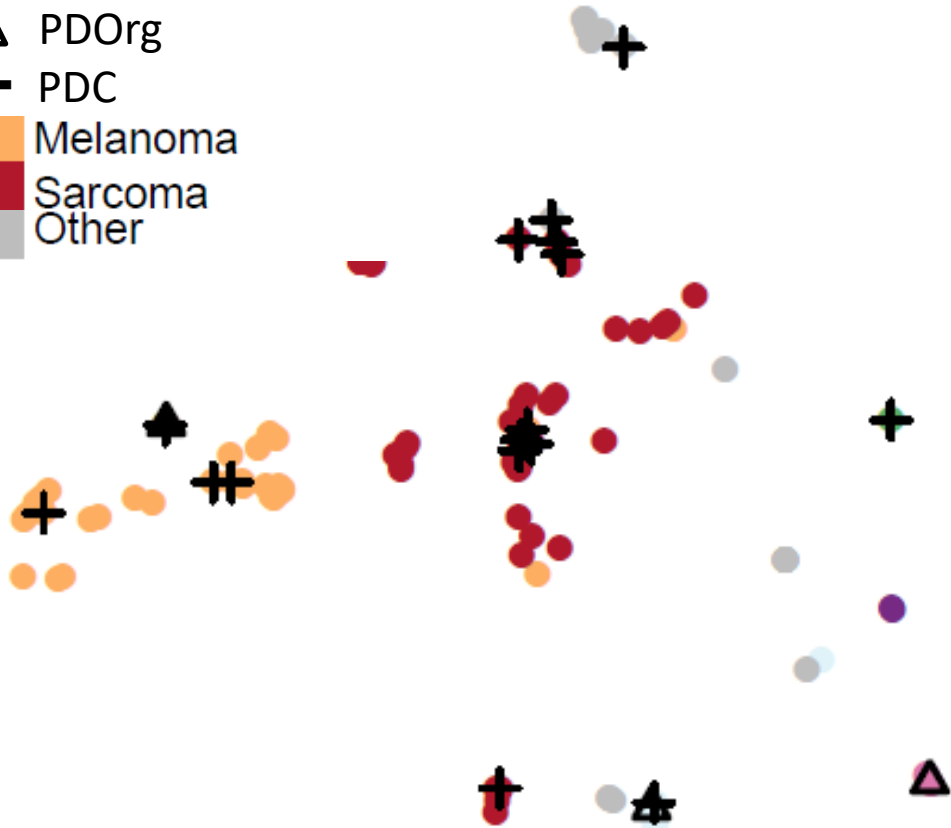
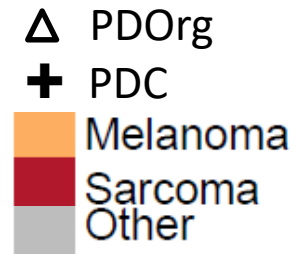
292921-168-R, Pancreatic Adeno



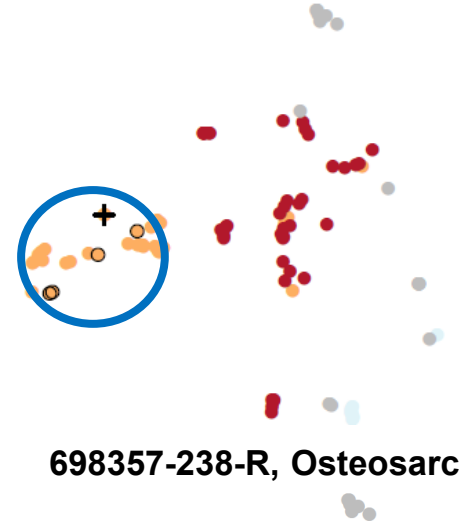
Expression Profiles Correlate Across the Majority of Models for Different Model Types: PDX, PDC, PDOrg

Melanoma and Sarcoma

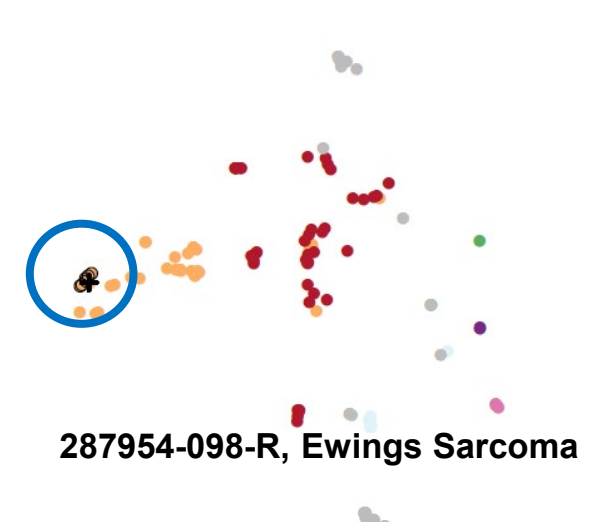
Expression Clusters



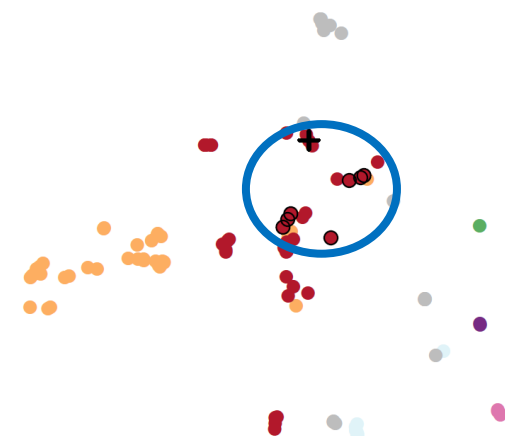
425362-245-T, Melanoma



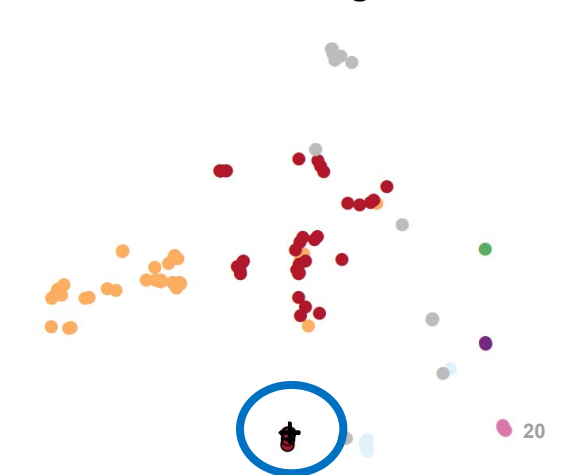
128128-338-R, Melanoma




698357-238-R, Osteosarcoma



287954-098-R, Ewings Sarcoma





Preliminary data from a set of Pancreatic Adenocarcinoma Models Derived from a Rapid Autopsy Case

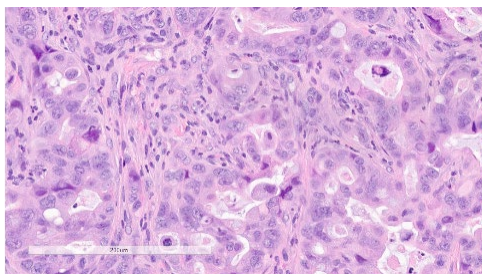
Rapid Autopsy Pancreatic Adenocarcinoma Models

521995-158-Rn

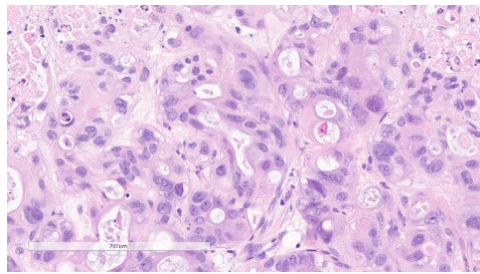
			Sequencing Status			
Model ID	Resection Site	Tissue Origin	PDX	PDOrg	PDC	Pt Tumor
521955-158-R2	Liver (A)	Metastatic Site	✓	✓	✓	⚡
521955-158-R3	Liver (B)	Metastatic Site	✓	🕒	✓	⚡
521955-158-R4	Colonic Fat	Metastatic Site	✓	🕒	🕒	⚡
521955-158-R5	Pancreas	Primary	⚡	🕒	🕒	⚡
521955-158-R6	Myometrium	Metastatic Site	✓	✓	✓	⚡
521955-158-R7	Colon	Metastatic Site	✓	🕒	⚡	⚡

A CAF has been generated and will be sequenced as a surrogate germline reference

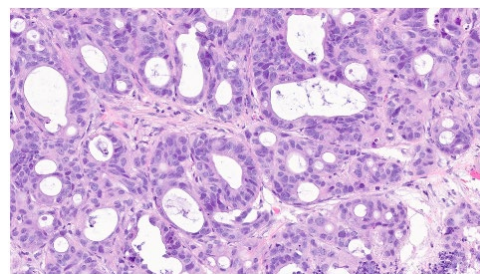
- ✓ NGS Complete
- ⚡ NGS In Progress
- 🕒 Model Dev't In Progress



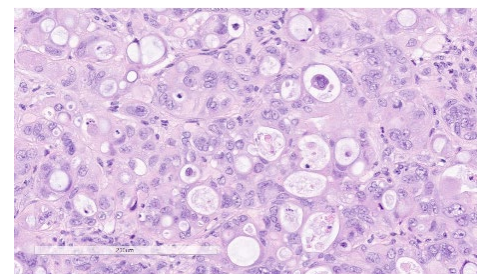
R2 – Liver met



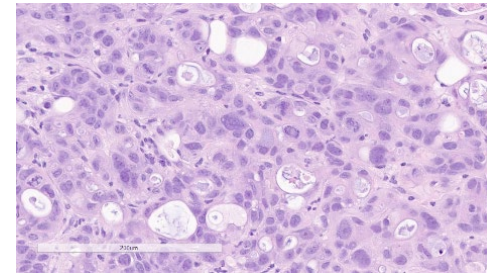
R5 – Pancreas, primary



R4 – Colonic Fat met



R6 – Myometrium met



R7 – Colon met

Rapid Autopsy Pancreatic Adenocarcinoma Models

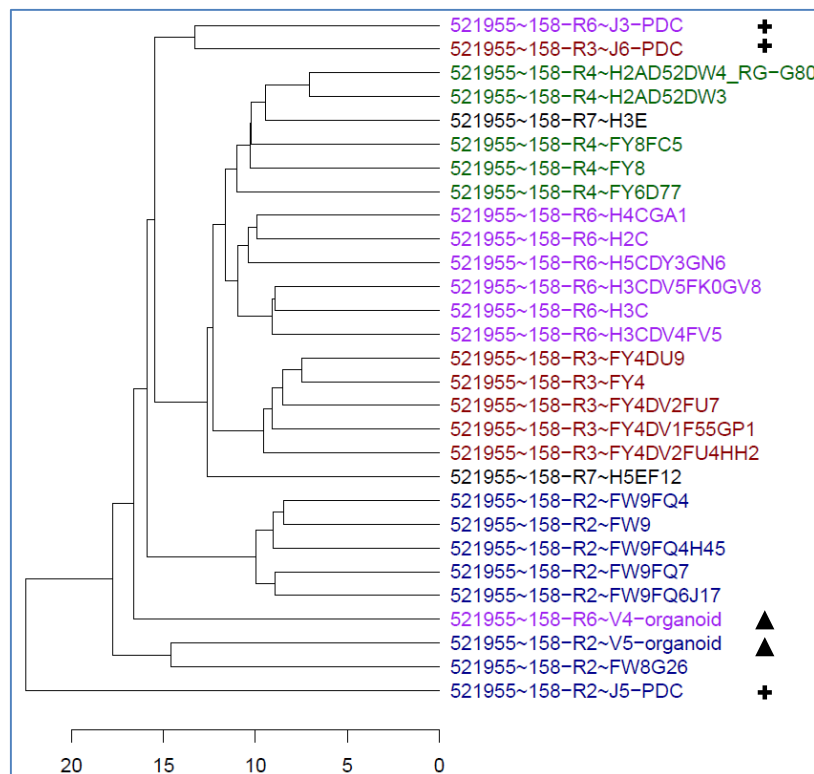
521995-158-Rn

Model ID	Resection Site	Tissue Origin	PDX	PDOrg	PDC	Pt Tumor
521955-158-R2	Liver (A)	Metastatic Site	✓	✓	✓	≈
521955-158-R3	Liver (B)	Metastatic Site	✓	⌚	✓	≈
521955-158-R4	Colonic Fat	Metastatic Site	✓	⌚	⌚	≈
521955-158-R5	Pancreas	Primary	≈	⌚	⌚	≈
521955-158-R6	Myometrium	Metastatic Site	✓	✓	✓	≈
521955-158-R7	Colon	Metastatic Site	✓	⌚	≈	≈

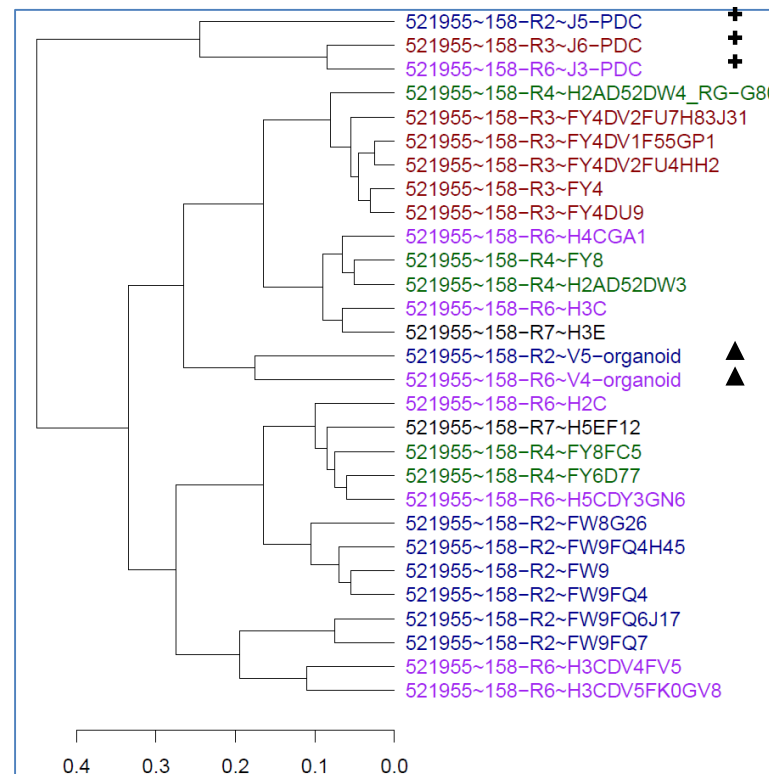
All driver mutations present

- KRAS-G12D (~60% AF)
- TP53-R158Sfs*8 (~100% AF)
- Homozygous Deletion of CDKN2A, CDKN2B, MAP2K4

WES



RNASeq



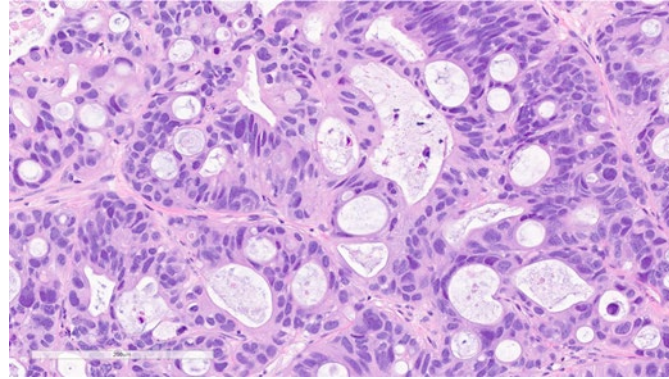
2D/3D Growth Conditions likely drive difference at expression level

- PDC grown on Matrigel-coated flasks in DMEM/F12 Complete Media + Y compound
- PDOrg grown in Panc media in BME2 domes

Δ PDOrg
+ PDC

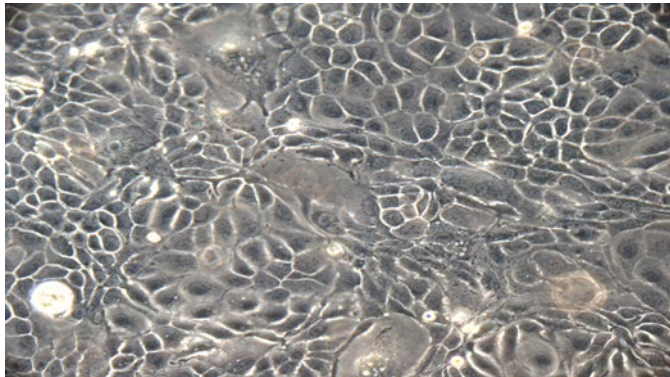
521955-158-R2, Adenocarcinoma - pancreas

Patient-Derived Xenograft
(PDX)

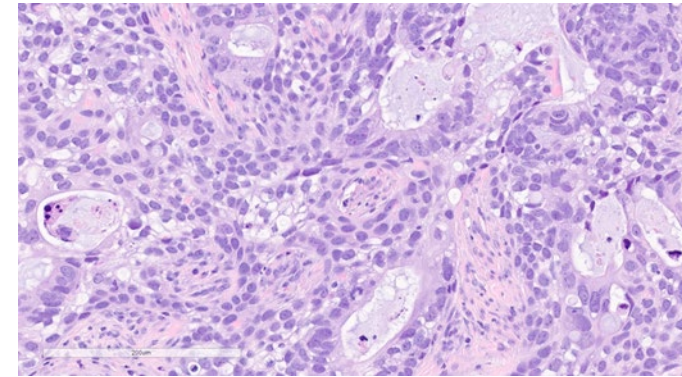


Glandular architecture is present: nests of glands with areas of back-to-back gland formation

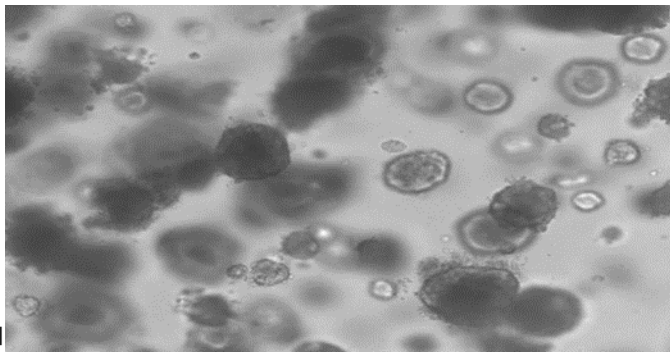
PDC Culture



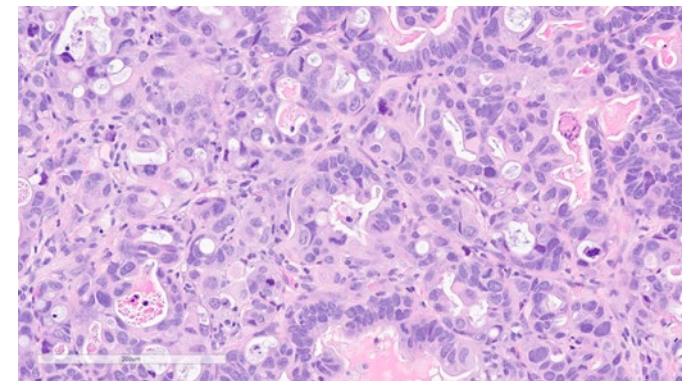
PDC-Derived
Xenograft



PDOrg Culture



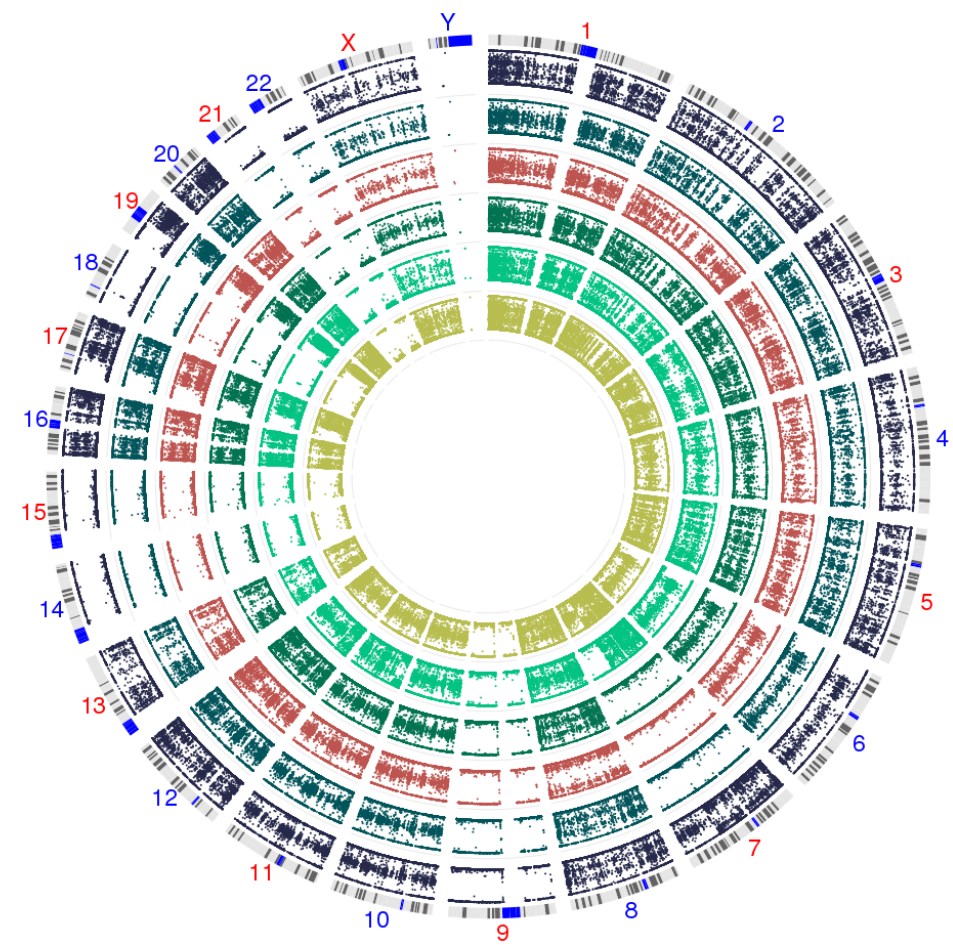
PDORG-Derived
Xenograft



Rapid Autopsy Pancreatic Adenocarcinoma Models

521995-158-R2

Model ID	Resection Site	Tissue Origin	PDX	PDOrg	PDC	Pt Tumor
521955-158-R2	Liver (A)	Metastatic Site	✓	✓	✓	⚡



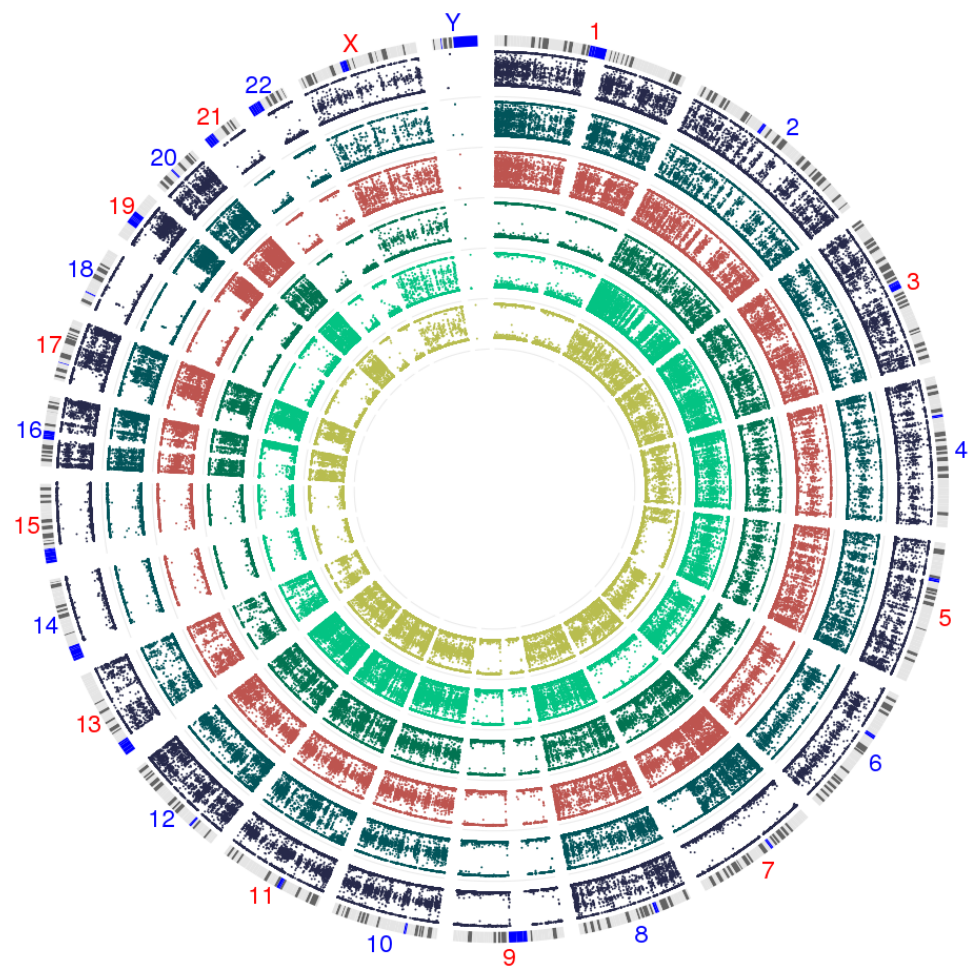
Model	Sample	Passage
521955-158-R2	PDX	Passage 1
521955-158-R2	PDX	Passage 1
521955-158-R2	PDX	Passage 1
521955-158-R2	PDX	Passage 0
521955-158-R2	J5-PDC	Passage 18
521955-158-R2	V5-organoid	Passage 14

✓ Differences in LOH observed on Chromosomes 7, 8, and 11

Rapid Autopsy Pancreatic Adenocarcinoma Models

521995-158-Rn

Model ID	Resection Site	Tissue Origin	PDX	PDOrg	PDC	Pt Tumor
521955-158-R2	Liver (A)	Metastatic Site	✓	✓	✓	≈
521955-158-R3	Liver (B)	Metastatic Site	✓	🕒	✓	≈
521955-158-R4	Colonic Fat	Metastatic Site	✓	🕒	🕒	≈



Model	Sample	Passage
521955-158-R2	PDX	Passage 0
521955-158-R2	PDC	Passage 18
521955-158-R2	PDOrg	Passage 14
521955-158-R3	PDX	Passage 0
521955-158-R3	PDC	Passage 25
521955-158-R4	PDX	Passage 0

✓ Differences in LOH observed on Multiple Chromosomes

Conclusions

- Driver mutations are maintained within a model across all model types: PDX, PDOrg, PDC
- Irrespective of model type (PDX, PDOrg, PDC), gene expression profiles cluster by histology and with other models from the same patient
- The majority of models are internally consistent for LOH; however, models can be found with differences at the LOH level between model types (PDX, PDOrg, PDC)

Acknowledgements

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Malorie Morris
Melanie Simpson
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In vivo & In vitro Teams

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Carrie Bonomi
Suzanne Borgel
Devynn Breen
John Carter
Kristen Cooley
Emily Delaney
Raymond Divelbiss
Kelly Dougherty
Kyle Georgius
Joe Geraghty
Marion Gibson
Tara Grinnage-Polley
Kelly Hedger
Sierra Hoffman
Candace Mallow
Chelsea McGlynn

Justine Mills
Tiffanie Miner
Jenna E. Moyer
Michael Mullendore
Matthew Murphy
Colleen Olkowski
Kevin Plater
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Nicki Scott
Luke H. Stockwin
Howard Stotler
Jesse Stottlemeyer
Savanna Styers
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Molecular Characterization

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Chris Karlovich
Kelly Benauer
Corrinne Camalier
Erin Cantu

Lily Chen

Biswajit Das

Vivekanand Datta
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Palmer Fliss
Brandie Fullmer
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Robin Harrington
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Wiem Lassoud
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