The National Cancer Institute Patient Derived Models Repository (PDMR)

An NCI Precision Oncology Initiative℠ Resource

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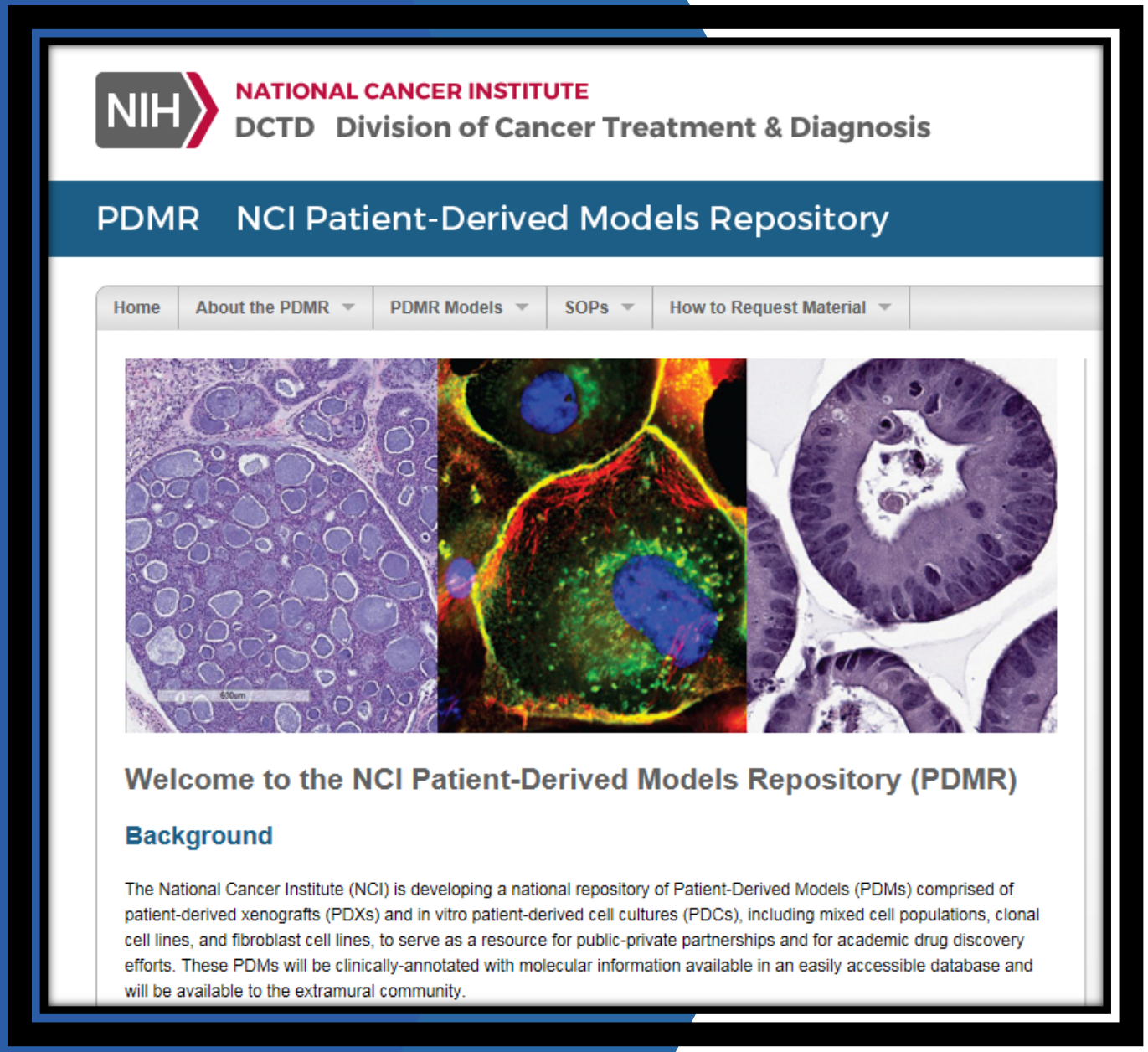
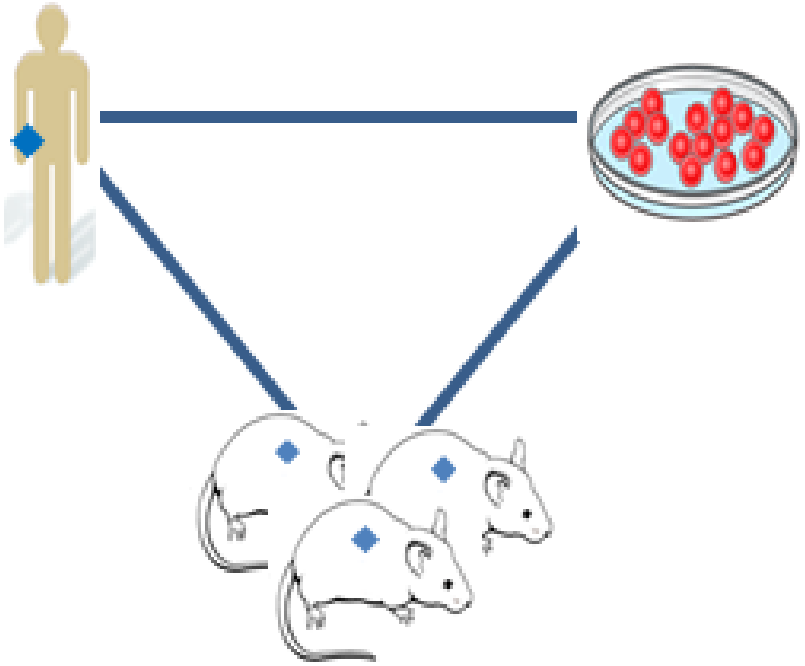
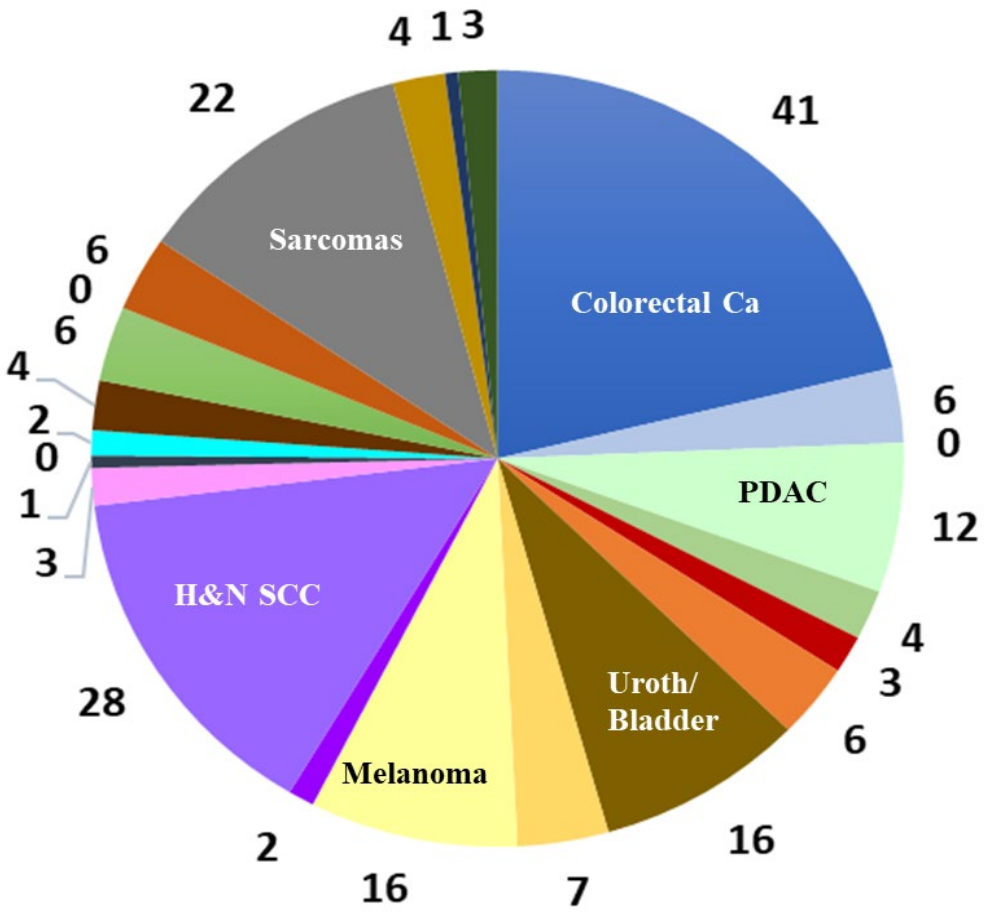
NCI Patient-Derived Models Repository





https://pdmr.cancer.gov

193 Patient-Derived Xenograft (PDX) Models Available for Distribution Across Solid Tumor Histologies: 10-22-2018



Require initial implantation in NSG Host strain

- Clinically-annotated, early-passage, molecularly-characterized patient-derived models
- Complement existing PDX collections and focus on under-represented model types such as rare cancers and models representing racial and ethnic minorities
- Provide all related metadata and SOPs through a public website
- Current distribution within the US (pdmr.cancer.gov).
  - ✓ Model information also available through PDX Finder at www.pdxfinder.org

PDX Take-Rate Across Tumor Histologies

Body Location	Total Specimens Received	Total Assessable Specimens	%Take-Rate of Assessable Specimens	Histology-Confirmed Tumor	Discontinued	*Not Yet Assessable: P0 tumors*
Breast	211	148	13%	19	129	63
Digestive/ Gastrointestinal	567	467	45%	212	255	100
Endocrine/Neuroendocrine	159	129	10%	13	116	30
Genitourinary	408	337	22%	74	263	71
Germ Cell	4	4	0%	0	4	0
Gynecologic	243	194	37%	72	122	49
Head and Neck	161	152	53%	81	71	9
Hematologic/Blood	13	7	29%	2	5	6
Musculoskeletal	338	306	28%	85	221	32
Neurologic	9	6	17%	1	5	3
Respiratory/Thoracic	164	133	35%	46	87	31
Skin	75	69	61%	42	27	6
Unknown Primary	20	16	19%	3	13	4
Totals	2372	1968	33%	650	1318	404

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

PDX Take-Rates for Specific Disease Types

	Total Specimens Received	Total Assessable Specimens	%Take-Rate of Assessable Specimens	Histology-Confirmed Tumor	Discontinued	*Not Yet Assessable: P0 tumors*
Small Cell Lung Cancer	12	9	44%	4	5	3
NSCLC – Adenocarcinoma	104	88	25%	22	66	16
NSCLC – Squamous Cell Ca	48	36	56%	20	16	12
Pancreatic Adenocarcinoma	243	196	30%	59	137	47
Colon adenocarcinoma	148	129	74%	95	34	19
Melanoma	52	49	69%	34	15	3
Head and Neck – Squamous	138	131	54%	71	60	7
Salivary Gland Cancer	21	20	50%	10	10	1
Urothelial/Bladder Ca	110	101	35%	35	66	9
Epithelial Ovarian Ca	89	78	29%	23	55	11
Gastroesophageal Cancer	28	21	29%	6	15	7
Gastrointestinal Stromal	32	26	23%	6	20	6
Malignant Peripheral Nerve Sheath	9	8	63%	5	3	1
Breast Ca, NOS	201	143	10%	15	128	58
Breast Cancer - Triple Negative	10	5	80%	4	1	5
Renal Ca	197	164	18%	29	135	33
Prostate Cancer	71	42	7%	3	39	29

Rare Histology PDX Models Available for Distribution and Under Development

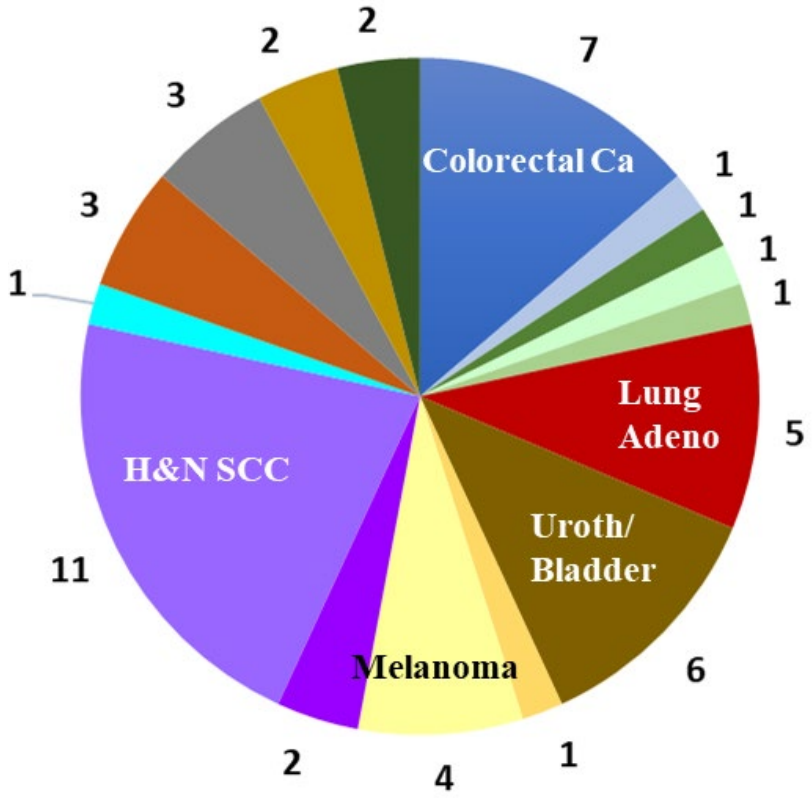
Rare Tumor PDX Models	#Models
Carcinosarcoma of the uterus	6
Gastric cancer, NOS	1
Gastrointestinal stromal tumor	3
Hurthle cell neoplasm (thyroid)	1
Leiomyosarcoma - uterus	4
Lip/oral cavity squam. cell car.	1
Lung cancer, NOS	1
Malig. periph. nerve sheath tum.	4
Merkel cell tumor	3
Mesothelioma	2
Miscellaneous neoplasm, NOS	1
Neuroendocrine cancer, NOS	3
Penile squamous car.(epidermoid)	1
Pharyngeal squam. cell carcinoma	17
Primary peritoneal carcinoma	1
Salivary gland cancer	7
Small cell car. (extrapulmonary)	1
Small cell lung cancer	4
Soft tissue neoplasm, NOS	3
Squamous cell car. - esophagus	1
Squamous cell carcinoma - anus	3
Urothelial/bladder cancer - sarcomatoid features	7
Vaginal cancer, NOS	2
Vulvar cancer, NOS	1

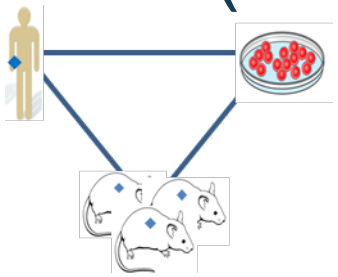
Other Sarcoma PDX Models	#Models
Alveolar soft part sarcoma	2
Chondrosarcoma	2
Ewing sarcoma/Peripheral PNET	3
Fibrosarcoma - not infantile	9
Leiomyosarcoma - not uterine	6
Liposarcoma	8
Malignant fibrous histiocytoma	9
Non-Rhabdo. soft tissue sarcoma	17
Osteosarcoma	3
Rhabdomyosarcoma, NOS	2
Synovial sarcoma	4

Used SWOG DART Study Tumor for model identification: S1609, "DART: Dual Anti-CTLA-4 and Anti-PD-1 blockade in rare tumors" Study Chairs: Drs. S.P. Patel, Y.K. Chae, and R. Kurzrock

Patient-Derived Cancer Cell Lines (PDCs) and Cancer-Associated Fibroblast Cultures (CAFs) Available 10-22-2018

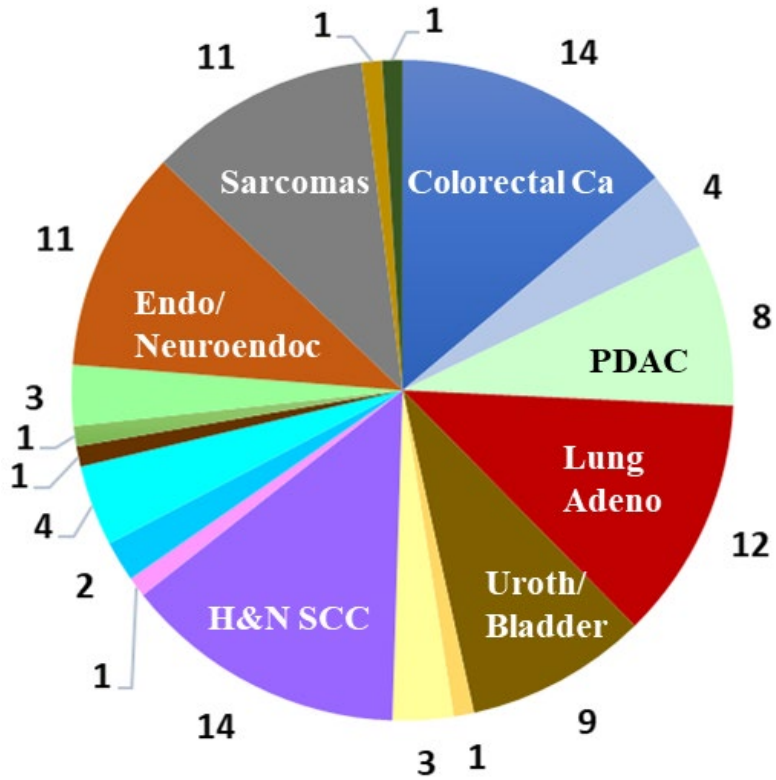
52 Conditionally-Reprogrammed PDCs





- Colorectal Adenocarcinoma
- Upper GI
- GI SCC
- Pancreatic Adenocarcinoma
- SCLC
- Lung Adeno
- Urothelial/Bladder
- Renal
- Skin - Melanoma
- Skin - Other
- H&N SCC
- Salivary Gland
- Breast
- Ovarian Origin
- Cervix/Vagina Ca
- Endomet/Carcinosarcoma - U
- Prostate
- Endo/Neuroendocrine
- Adult Soft Tissue Sarcoma
- Chond/Ost Sarcoma
- Misc

108 CAFs

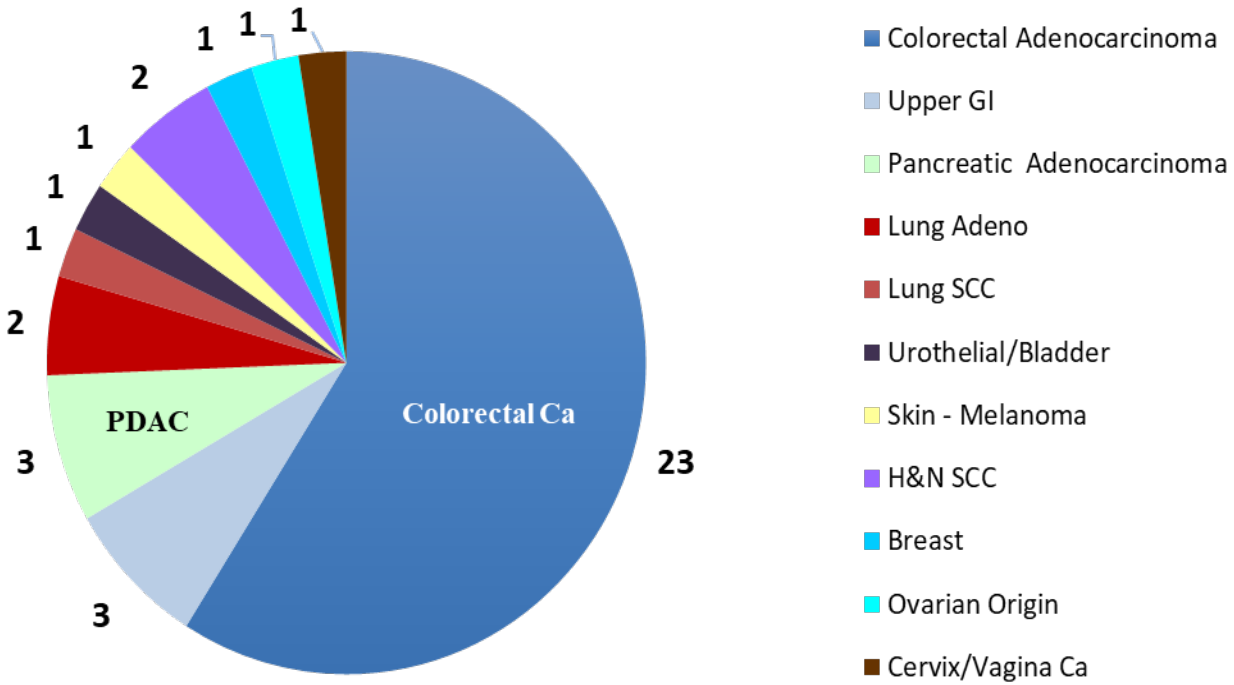


- Adherent & Suspension Cultures
- Requires use of defined media

- Not Transformed
- Limited Lifespan
- Requires use of defined media

In Development: Patient-Derived Organoids (PDOrg)

PDM Type	Patient ID	Specimen ID	Sample ID	Distribution Lot Name	CTEP SDC Code	CTEP SDC Description	Disease Body Location	Growth Properties	Max. Passage	Required Media	IDEXX Human Pathogen Testing Summary (h-IMPACT)
Organoid Culture	135848	042-T	V1-organoid	135848-042-T-V1-organoid	10009951	Adenocarcinoma - colon	Digestive/Gastrointestinal	Not Applicable	8	Media 6C/Colon 1B	Negative (IDEXX)
Organoid Culture	189374	263-R	V1-organoid	189374-263-R-V1-organoid	10038045	Adenocarcinoma - rectum	Digestive/Gastrointestinal	Not Applicable	11	Media 6B/Colon 1A	Negative (IDEXX)
Organoid Culture	683768	134-T	V1-organoid	683768-134-T-V1-organoid	10033159	Ovarian epithelial cancer	Gynecologic	Not Applicable	7	Breast	Negative (IDEXX)



Requires use of defined media

- Target release date of first ~50 models: January 2019
- Requires use of defined media
- 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

Distribution of Models

- Distribution of PDX models and derivatives, PDCs and CAF models
- Academic, Commercial, and Intramural

Material	Number of Vials Distributed
PDX Fragments – Viably Cryopreserved	218
DNA (Solution)	3
RNA (Solution)	17
Fresh-Frozen Fragment for Extraction	197
In Vitro PDCs – Viably Cryopreserved	98
In Vitro CAFs – Viably Cryopreserved	9
PDOrgs – Viably Cryopreserved	2

- Types of scientific inquiries proposed:
  - Methylome assessment
  - Target-specific inhibitors matched to molecular phenotypes
  - Small molecule agents
  - Angiogenesis
  - Proteogenomics
  - Radio-therapy
  - Small animal imaging studies
  - Biomarker assessment matched to molecular phenotypes
  - Academic preclinical core services
  - Commercial investigational agent validation

Stability of PDX Models: Genomics & Histomorphology

Biological Testing Branch, NCI

Molecular Characterization Laboratory, NCI-FNLCR

PDMR Assessment of Genetic Stability

Assessment of Genetic Stability

- PDMR has assessed Whole Exome Sequence (WES) stability between patient and PDX material up to P3-5 (depending on model) and observed no significant alterations in the majority of models. (P0 = first implanted host mouse)
  - ✓ Copy Number Alteration (CNA)
  - ✓ Variant Allele Frequency (VAF) of clinically actionable variations
  - ✓ Microsatellite Instability (MSI)
  - ✓ Tumor Mutation Burden (TMB)

Current PDMR Best Practices for Genetic Stability Assessment

- Use WES to assess stability/variation. All CNA assessment use WES not RNASeq inference
- When germline sequence is available, correct for tumor cellularity in the patient material WES

Additional comparisons

- Transcriptome profiles (RNASeq) of PDX and patient material cluster by model and by disease
- Histomorphology within a model is maintained through passages

Current PDMR Best Practices for Assessing Copy Number Alterations through Passages: Pipeline

OPTIMAL: Compare WES of PDX to Patient material when germline is available

a) Use germline sequence to correct for human stromal content (cellularity) in patient material

b) Remove mouse reads from PDX sequence

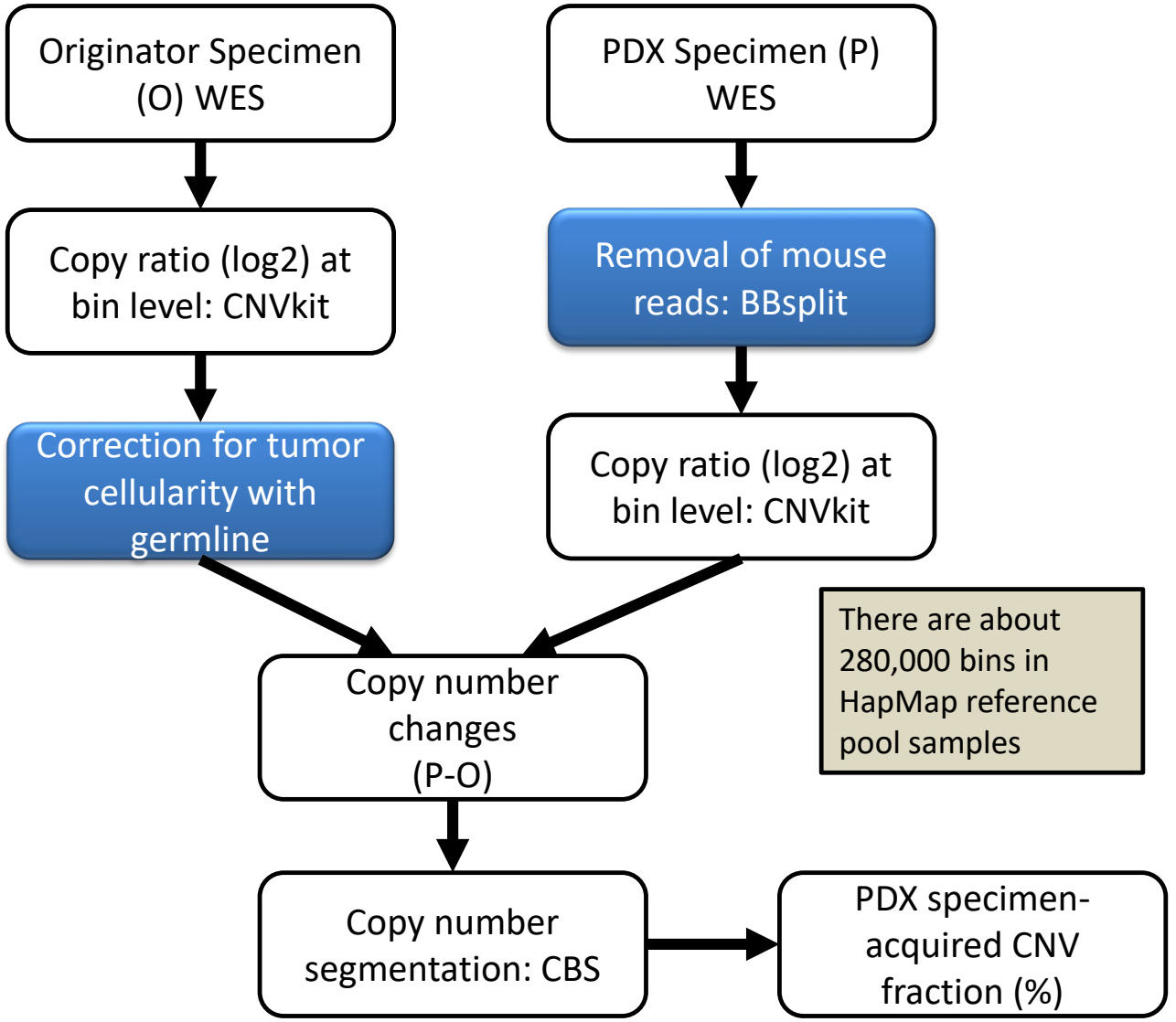
- Adjusting for tumor cellularity is key for assessment of CNA and VAF changes with passaging

ALTERNATE: Compare WES of PDX to Patient material

a) If no germline sequence is available, correction for cellularity to patient sequence cannot be performed

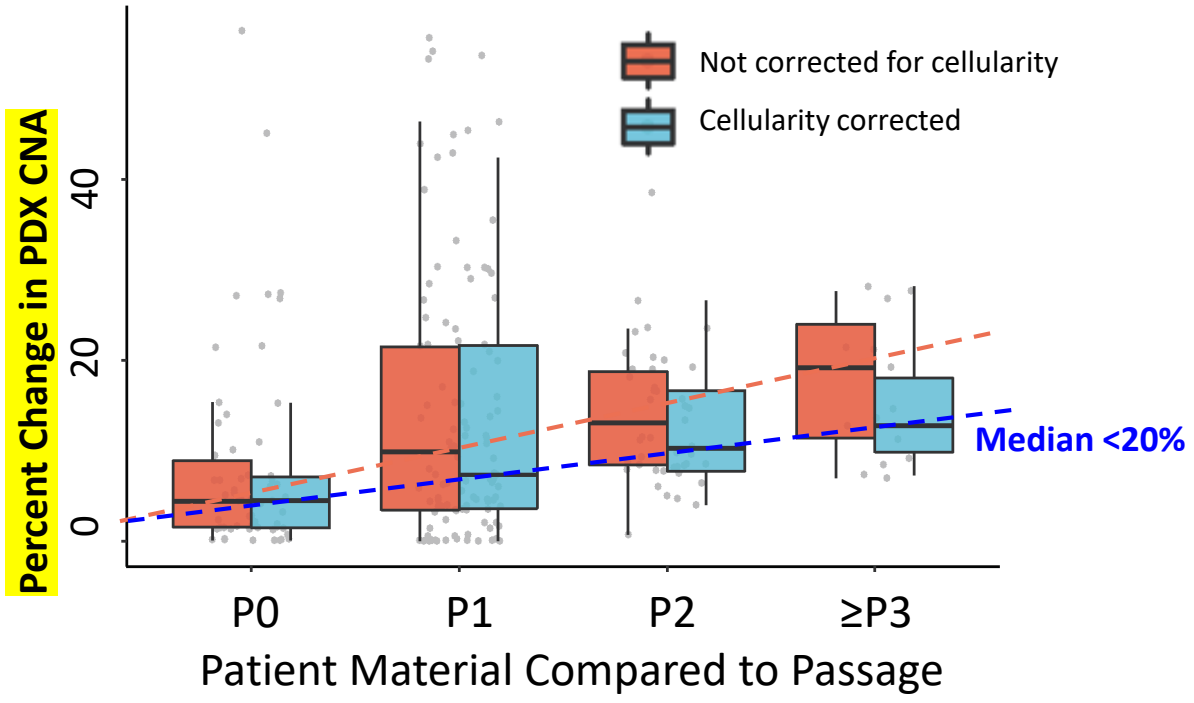
b) Mouse reads are removed from PDX sequence

- **Limitation**: Contaminating human stroma will dilute CNA, VAF, etc calls in comparison to the PDXs



Large Fraction of Models Have Stable CNA and VAF Across Passages

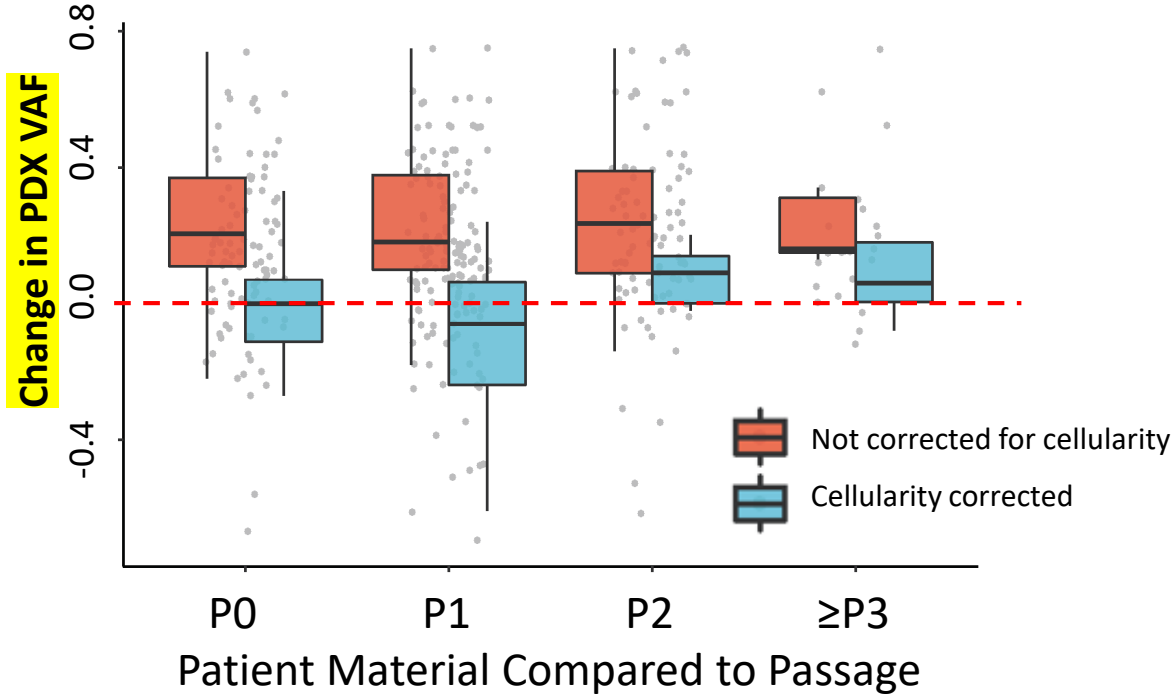
Copy Number Alteration (CNA)



Same 22 models used for Assessment with and without cellularity correction

Ploidy change cut-off set at ≥1.32

Variant Allele Frequency (VAF) of clinically actionable variations (oncoKB)



Change in VAF is inflated in PDX-passaged specimens in absence of cellularity correction in the sequenced patient material

Microsatellite instability (MSI) and high tumor mutational burden (TMB-high) status are consistent within PDX models

MSI status determined by MSINGS algorithm using WES data (57 models with Originator specimens used here).

	Total number of models	Concordant - Originator and PDX specimens	Disconcordant - Originator and PDX specimens	% of Discordant specimens (#of specimens discordant)
MSI-H	**8**	**6**	**2**	**25%-40% (n=1,2)***
MSI-S	**49**	**49**	**0**	

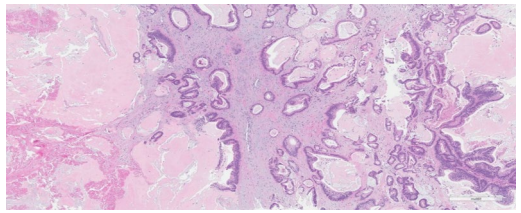
*These specimens are borderline MSI-S (very close to the cut-off value of MSI-H

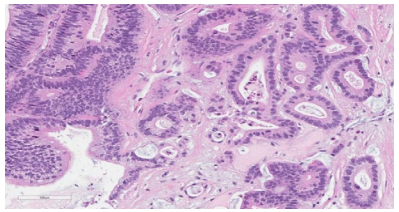
TMB-high status is determined as **>20 somatic mutations/MB** [22 models (with Originator and germline specimens available) used in this analysis]

	Total number of models	Concordant - Originator and PDX specimens	Disconcordant - Originator and PDX specimens	# of specimens discordant
TMB-high	**4**	**4**	**0**	**0**
TMB-low	**18**	**17**	**1***	**1***

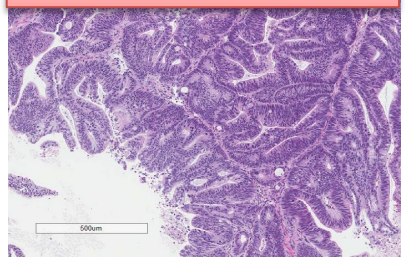
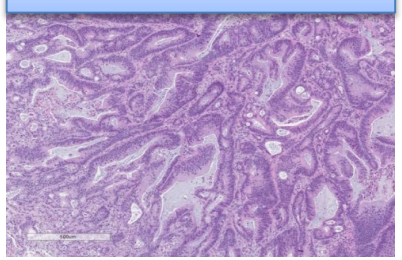
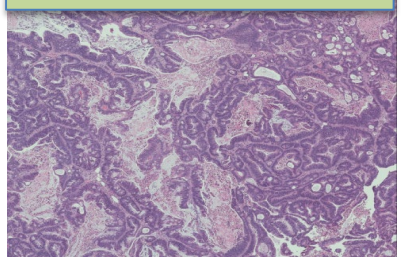
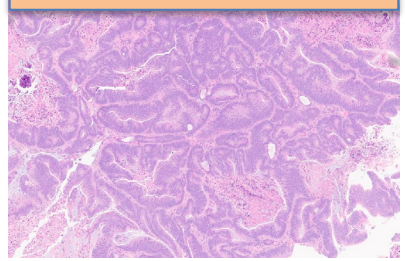
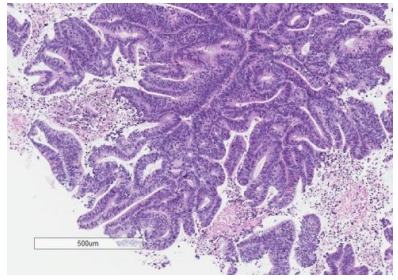
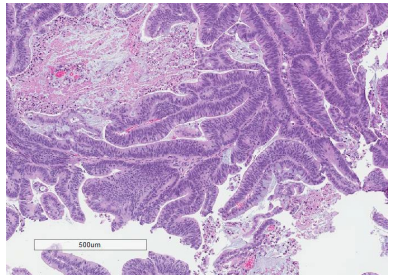
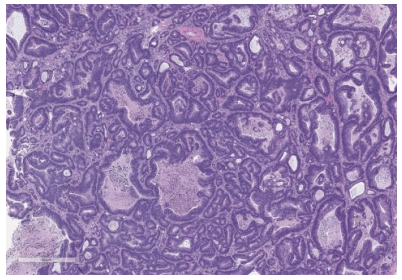
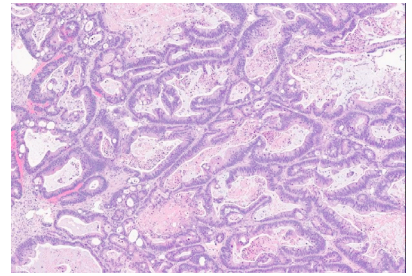
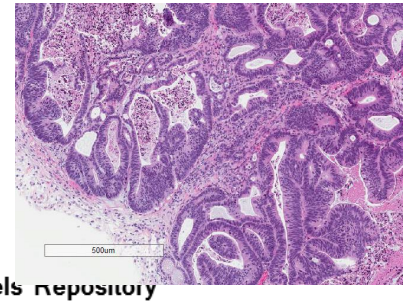
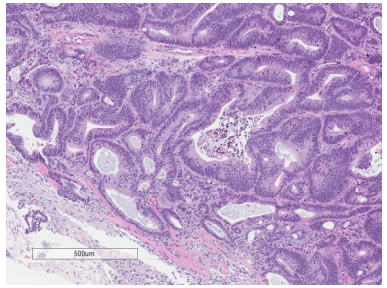
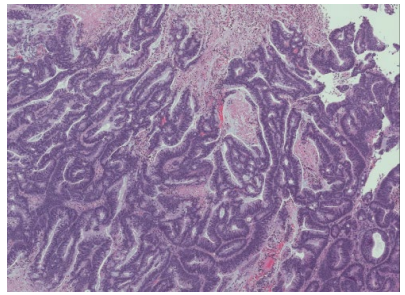
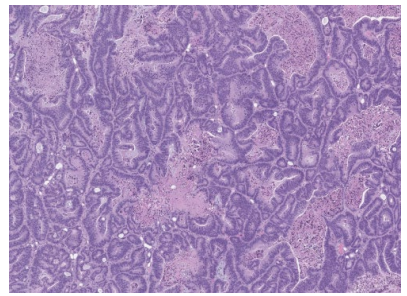
All specimens in this model have TMB very close to the cut-off (Originator has TMB = 19.6 mut/MB)

Histomorphology Maintained Across Passages





Patient: 172845
Diagnosis: Colon adenocarcinoma

	P0	P1	P2	P3
172845-121-T (Origin: Liver met #1)				
172845-142-T (Origin: Liver met #2)				
172845-121-B (Origin: CTCs)				

Assessing Metastasis in PDX Models

Biological Testing Branch, NCI
Small Animal Imaging Program, NCI-FNLCR
Cancer Imaging Program, NCI
Molecular Characterization Laboratory, NCI-FNLCR

Metastasis in PDX-bearing NSG Host Mice

- **Modalities**
  - ✓ MRI (non-contrast to evaluate tumor morphology and search for metastasis)
  - ✓ PET ([18F]FLT for cell proliferation and [18F]FDG for metabolism)
  - ✓ Ultrasound (3D volumes and microbubbles for tumor perfusion)

49 Models Assessed
3 Metastatic Pre-excision
12 Metastatic Post-excision

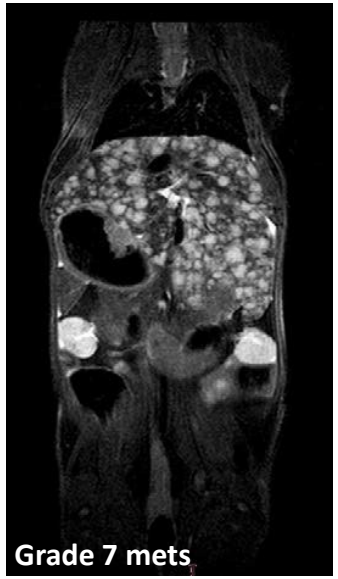
- **Timing**
  - ✓ Assess for *de novo*, pre-excision metastasis: while subcutaneous tumor in place
  - ✓ Assess post-excision for metastases

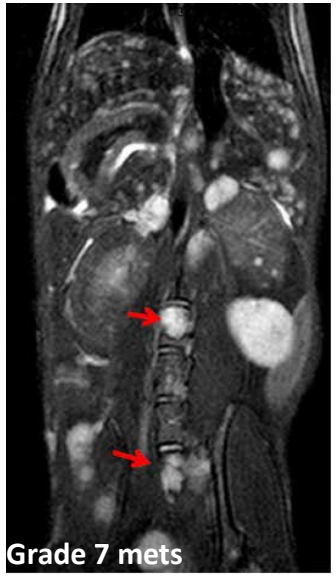
- **Characterization**
  - ✓ Pre-excision penetrance: X of Y implanted hosts with metastases identified at N days post-implant
  - ✓ Post-excision penetrance: X of Y implanted hosts with metastases identified at N days post-debulking
  - ✓ Location(s) of metastases

- **Developing a landing page** in The Cancer Imaging Archive (TCIA) for access to the imaging data. Shared hyperlinks will be included with the PDMR database

Bladder Model Responsive to Treatment at Metastatic Sites by MRI

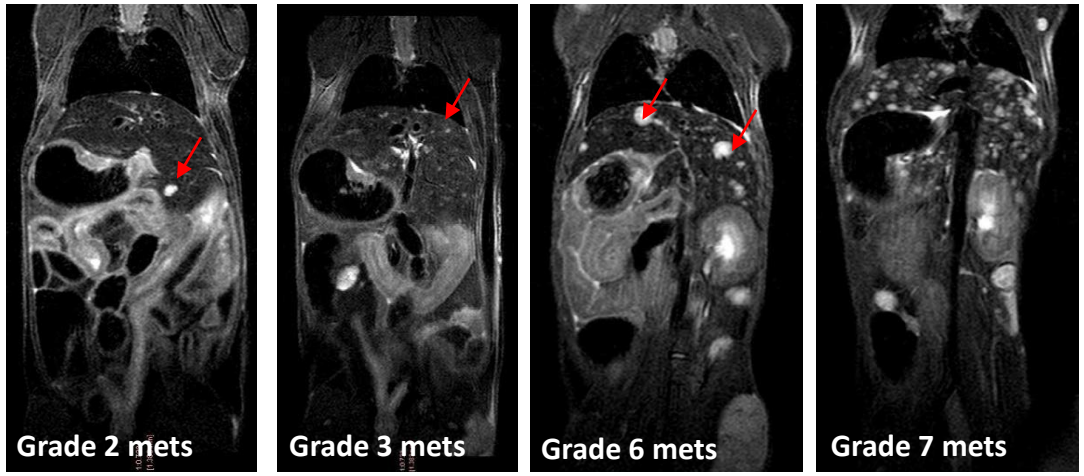
Pre- and Post-Excision Metastases	
Site(s)	Liver, Lymph Nodes, Spine



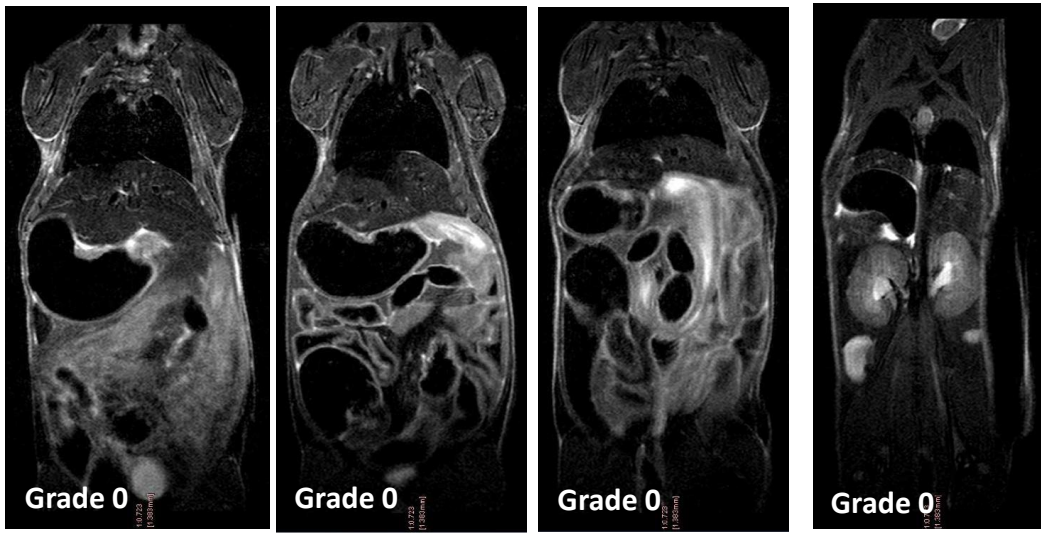


Drug Challenge with Imaging: Efficacy studies previously demonstrated subcutaneous PDX tumor has CR to Temozolomide

Control
Post-excision,
liver metastases



Treated
Post-excision



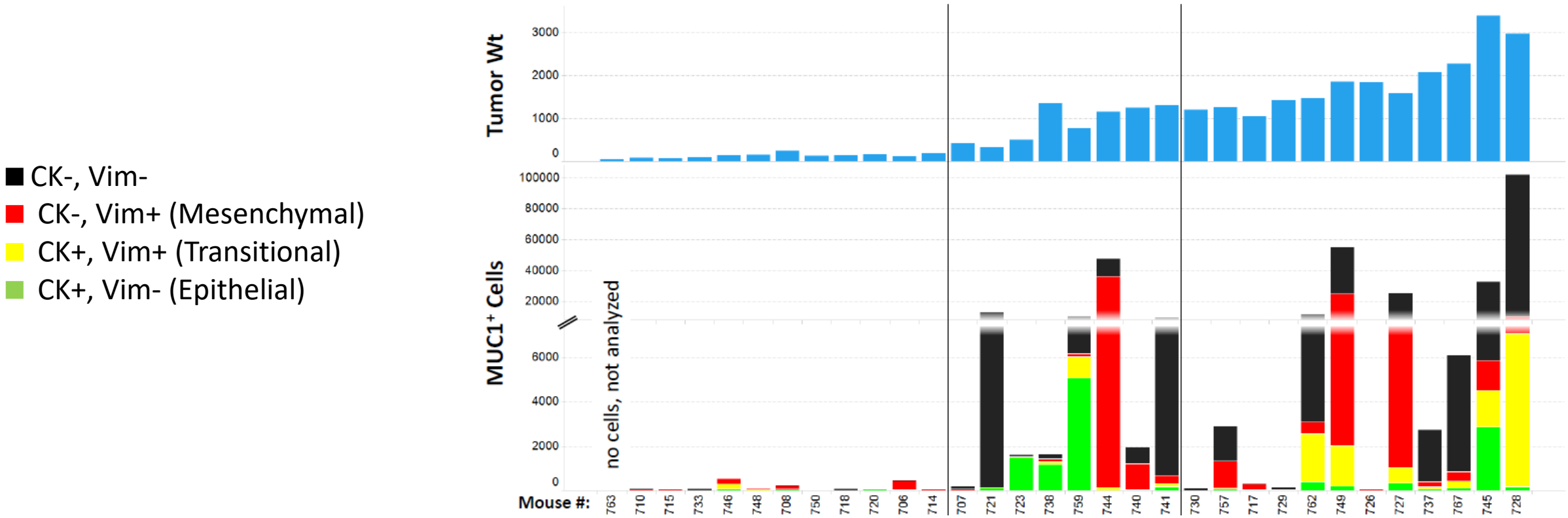
Circulating Tumor Cells in PDX-Bearing NGS Mice

Biological Testing Branch, NCI

Pharmacodynamic Assay Development and Implementation Section (PADIS), NCI-FNLCR

Circulating Tumor Cells in a Bladder Ca PDX Model

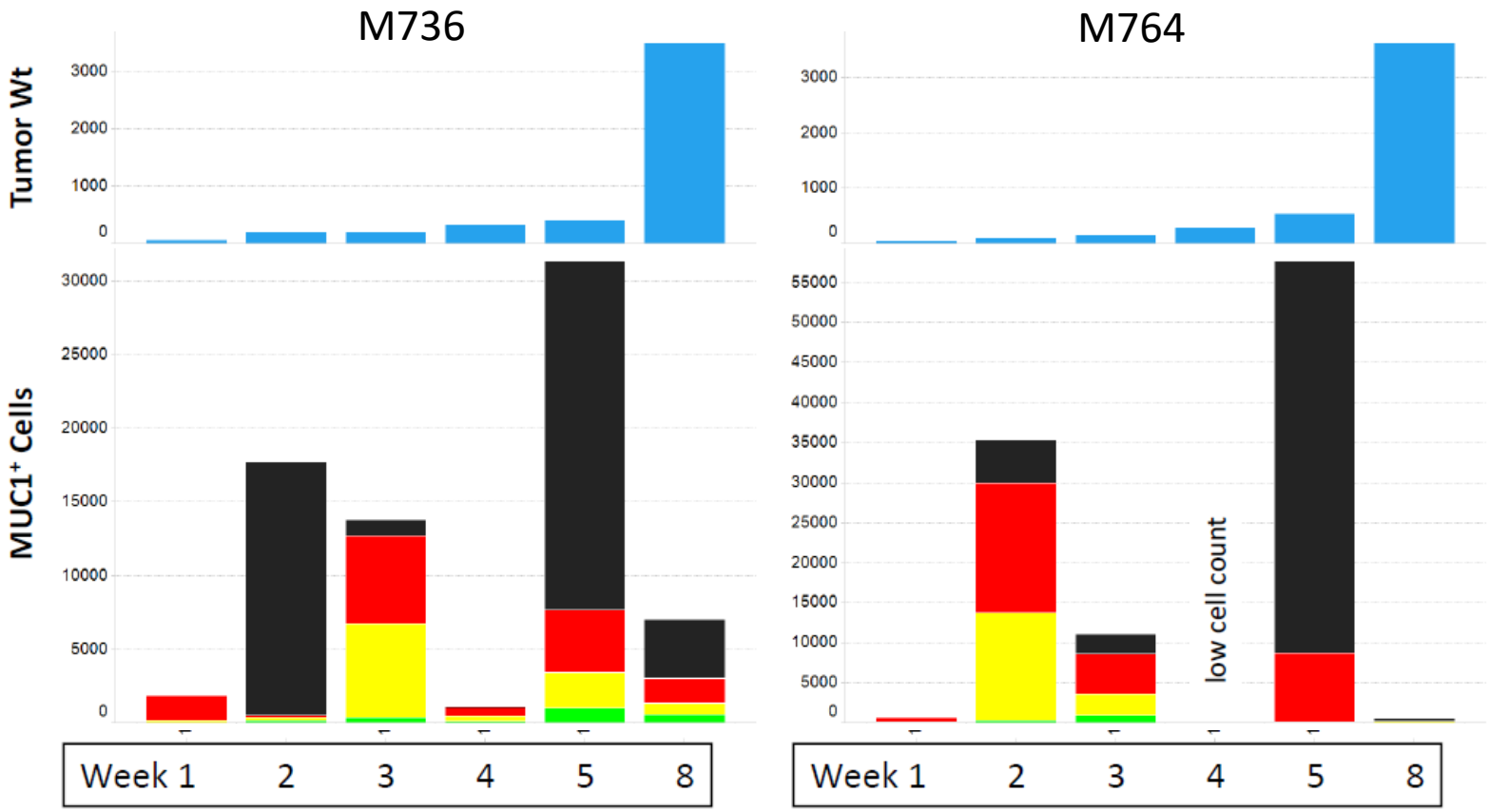
- **Known metastatic human Bladder Cancer Model (Metastatic to liver, lymph nodes, and spine. Pre-excision)**
- **Blood collected from 32 PDX-bearing mice with tumor size ranging from 50 mm^3 – 3300 mm^3**
- **CTCs enriched using Aviva BioSciences RedSift technology**
- **$MUC1^+$ Cell phenotype assessment using Amnis Platform (Multiparameter Analytical Cytometry)**



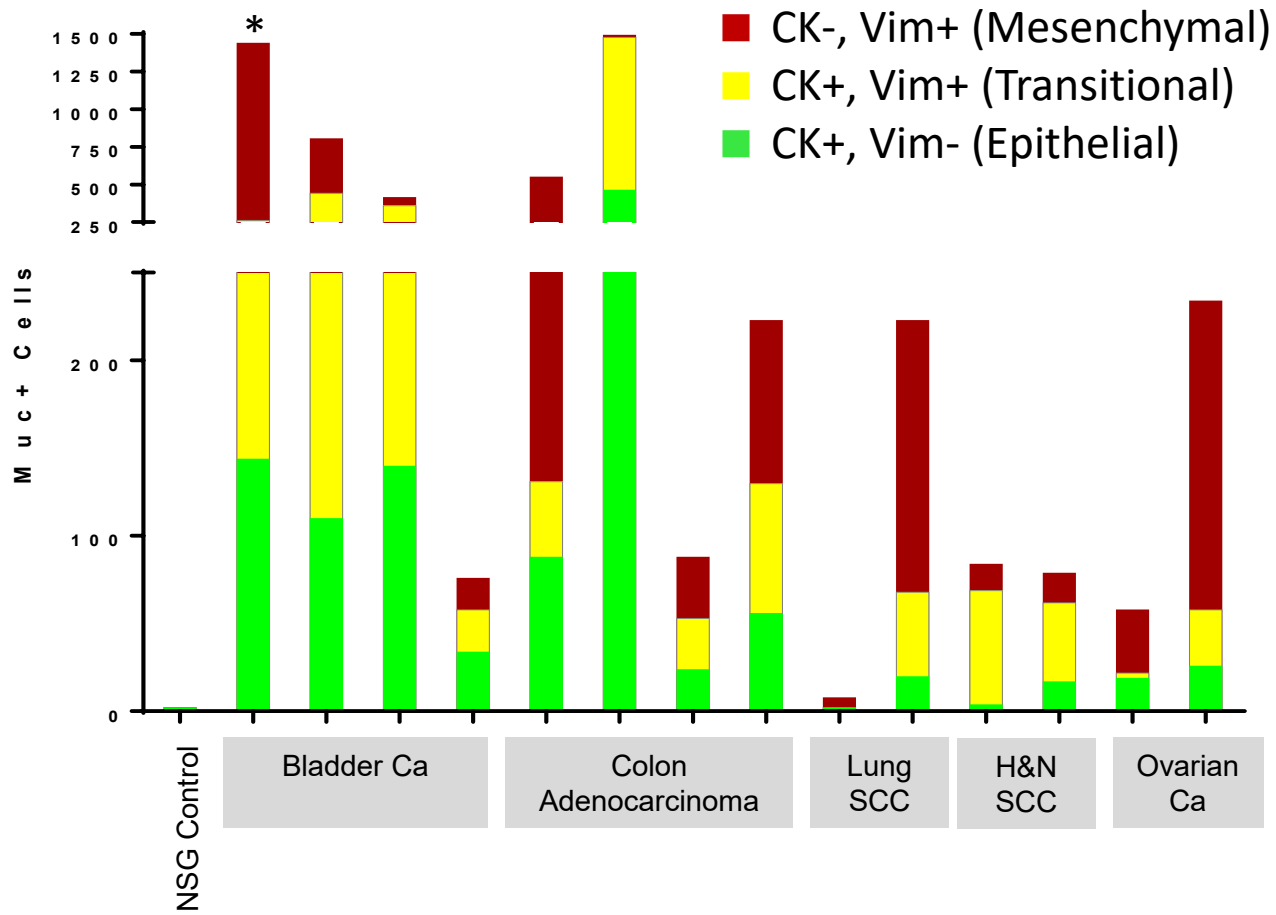
Circulating Tumor Cells in Two Bladder Ca PDX-Bearing Mice

Known metastatic Bladder Cancer Model (Metastatic to liver, lymph nodes, and spine. Pre-excision)
Blood collected once a week from 2 PDX-bearing mice over 8 weeks (timing)

- CK-, Vim-
- CK-, Vim+ (Mesenchymal)
- CK+, Vim+ (Transitional)
- CK+, Vim- (Epithelial)



PDX Circulating Tumor Cells Across Histologies



14 Models Assessed
12 Models In Queue
- 4 mice/model
- ~1500 mm^3

- **CTCs enriched using Aviva BioSciences RedSift technology**
- **Cell phenotype assessment using Amnis Platform**

Next Steps for CTCs in PDXs

- Expand assessment into other models that are known to be metastatic
  - ✓ Can CTC presence/quantity be used to identify pre-excision metastases or micro-metastases
  - ✓ Timing of different EMT populations in CTC population

- Tumorgenicity Assessment of CTCs from PDX-bearing Mice

- WES Heterogeneity Assessment
  - ✓ Compare genetics of CTCs to engrafted tumors and metastatic lesions

- Drug Study and Biomarker Assessment: PDX CTCs for Pharmacodynamics

PDX Preclinical Study with Standard of Care Agents

Biological Testing Branch, NCI

Molecular Characterization Laboratory, NCI-FNLCR

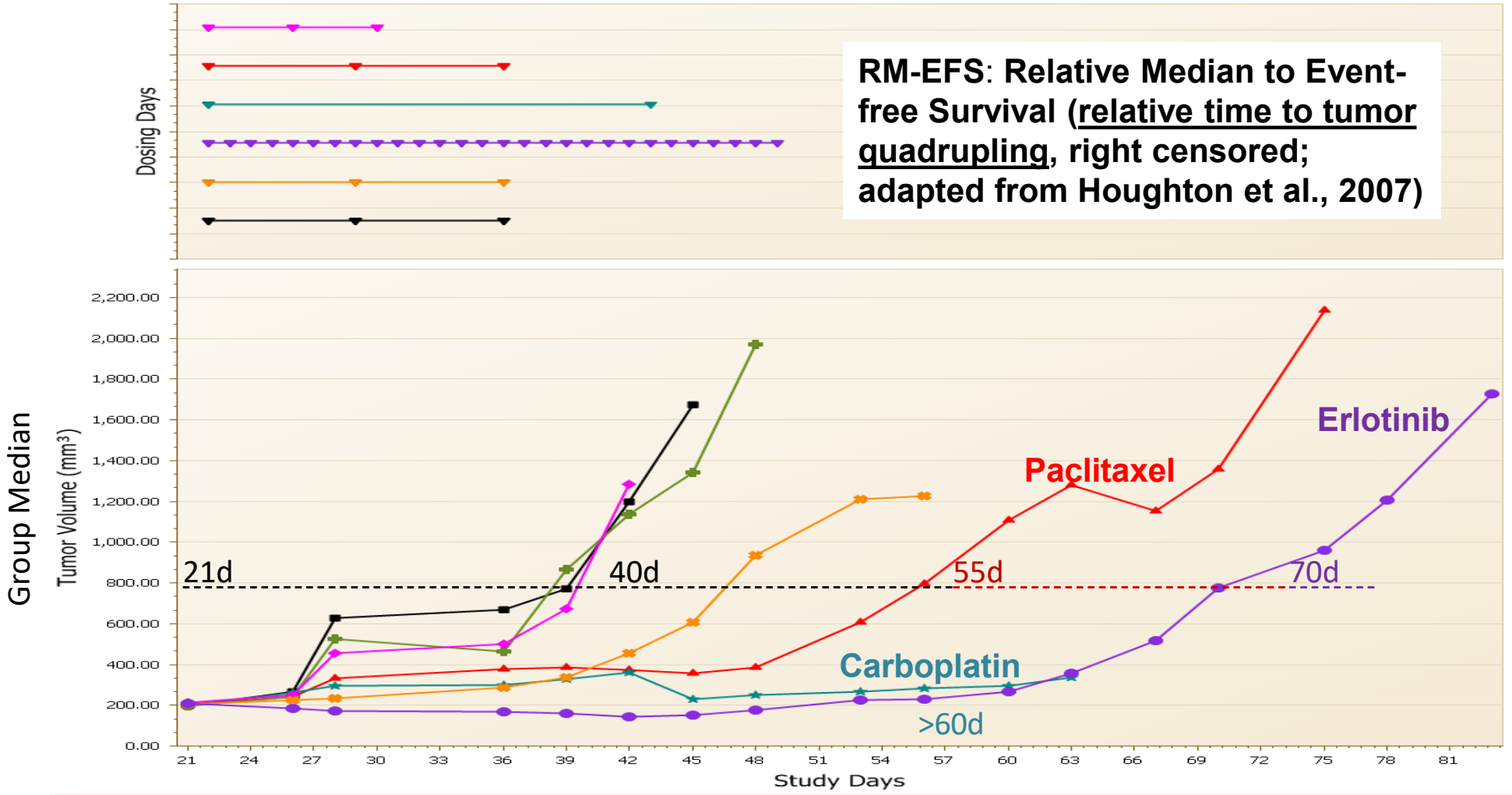
“Rolling” Study with Standard of Care Anticancer Agents

- Initial enrollment plan included: Colorectal Ca, Pancreatic Adenocarcinoma, H&N SCC, Urothelial/Bladder Ca, Lung Ca, and Melanoma Models (n = 36)
- “Rolled” in any available model that was actively growing in NSG mice and had pathology confirmed
- Screening study set-up: n-of-3 arms
- Total of 72 models enrolled in entire study

Arm	Agent	Dose	Route	Schedule
1	**Paclitaxel**	15 mg/kg	IV	Q7Dx3
2	**Carboplatin**	80 mg/kg	IV	Q21D
3	**Irinotecan**	100 mg/kg	IV	Q21D
4	**5-FU**	50 mg/kg	IP	Q4Dx3, Rest 2 Weeks, Repeat
5	**Gemcitabine**	150 mg/kg	IP	Q7Dx3
6	**Erlotinib**	50 mg/kg	PO	QDx28 [Feed Administration]
7	**Vemurafenib**	75 mg/kg	PO	BIDx56 [Feed Administration]

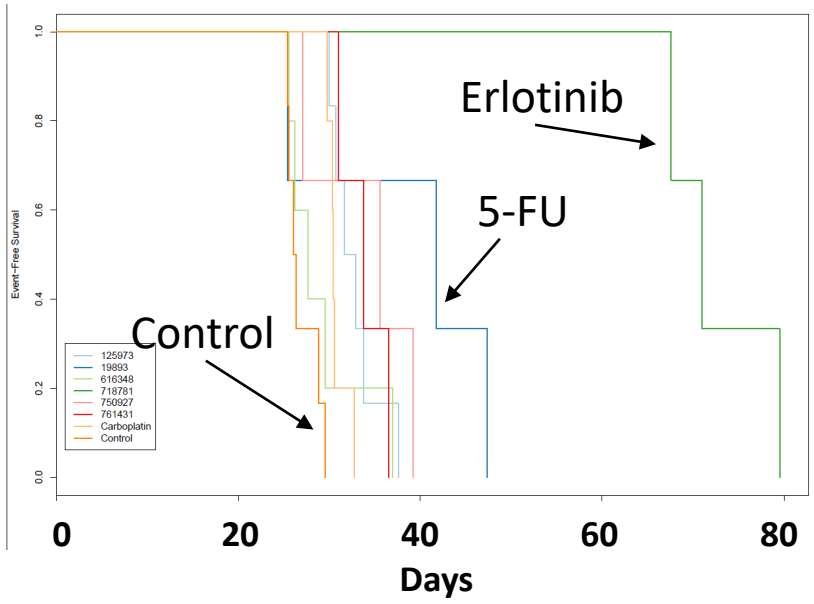
N-of-3 PDX Study: Response Across Drug Cohorts

Oral Squamous Cell Ca

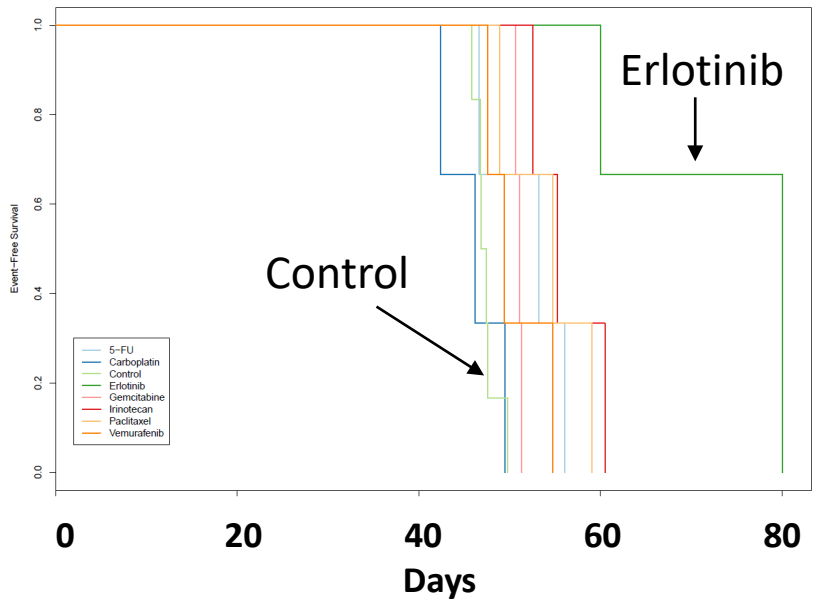


Agent
Paclitaxel
Carboplatin
Irinotecan
5-FU
Gemcitabine
Erlotinib*
Vemurafenib*

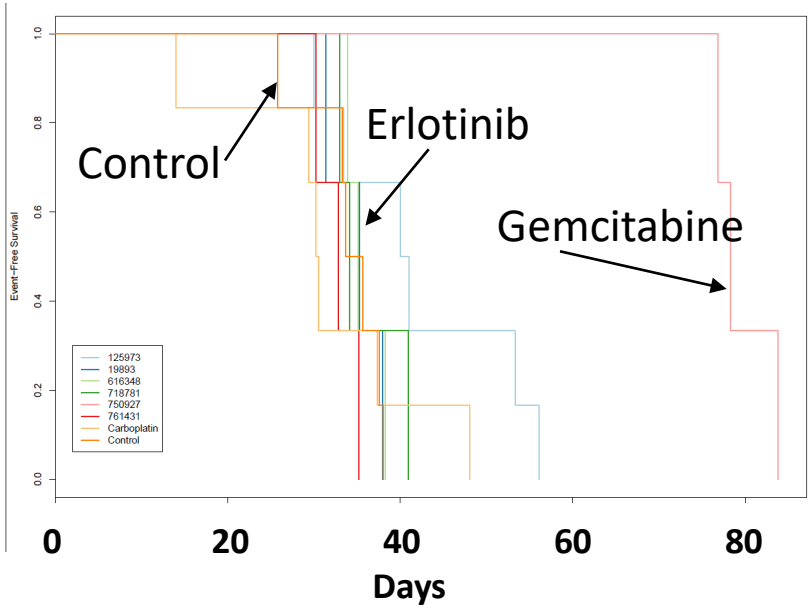
Response Assessment: Kaplan Meier Curves vs RMEFS



929823-356-R
Lip/Oral SCC
Erlotinib: RM-EFS = 2.71
5-FU: RM-EFS = 1.60
Study length: 79 days

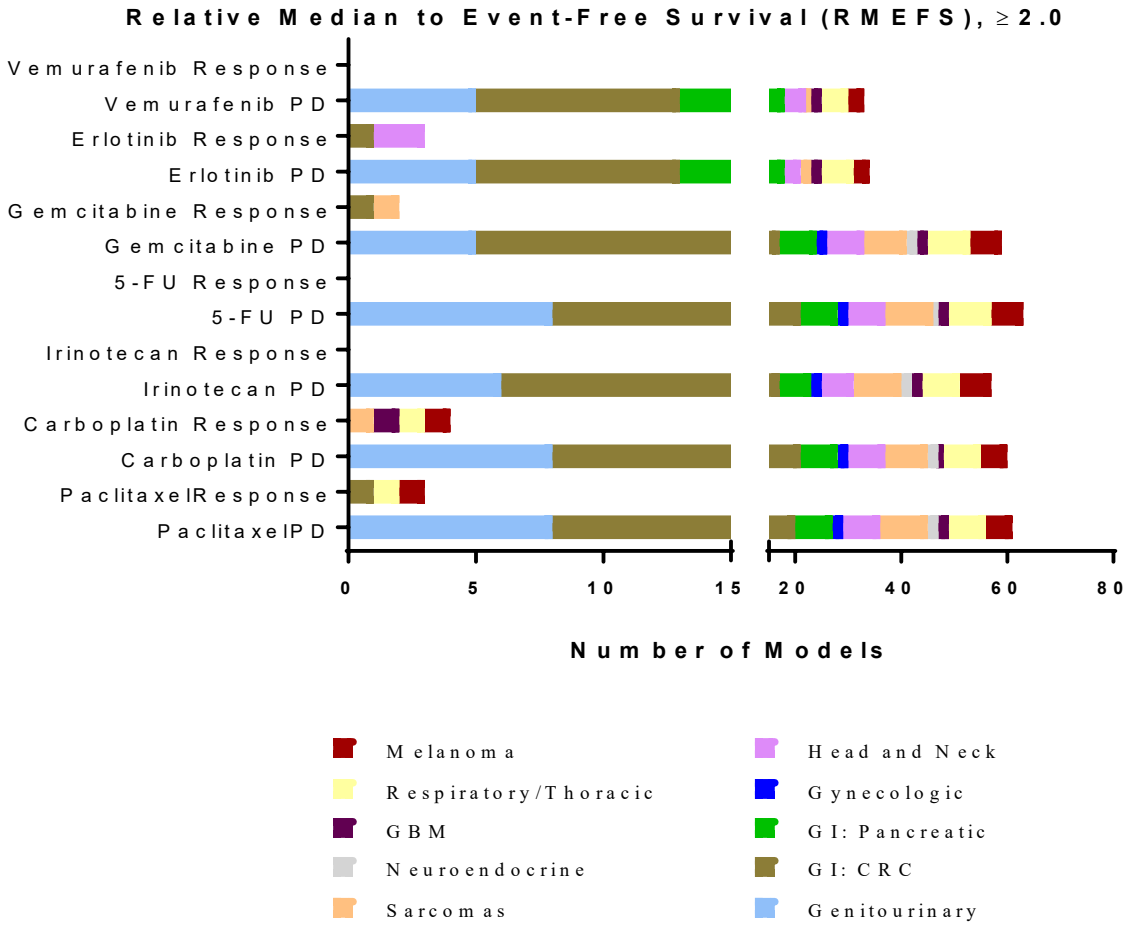


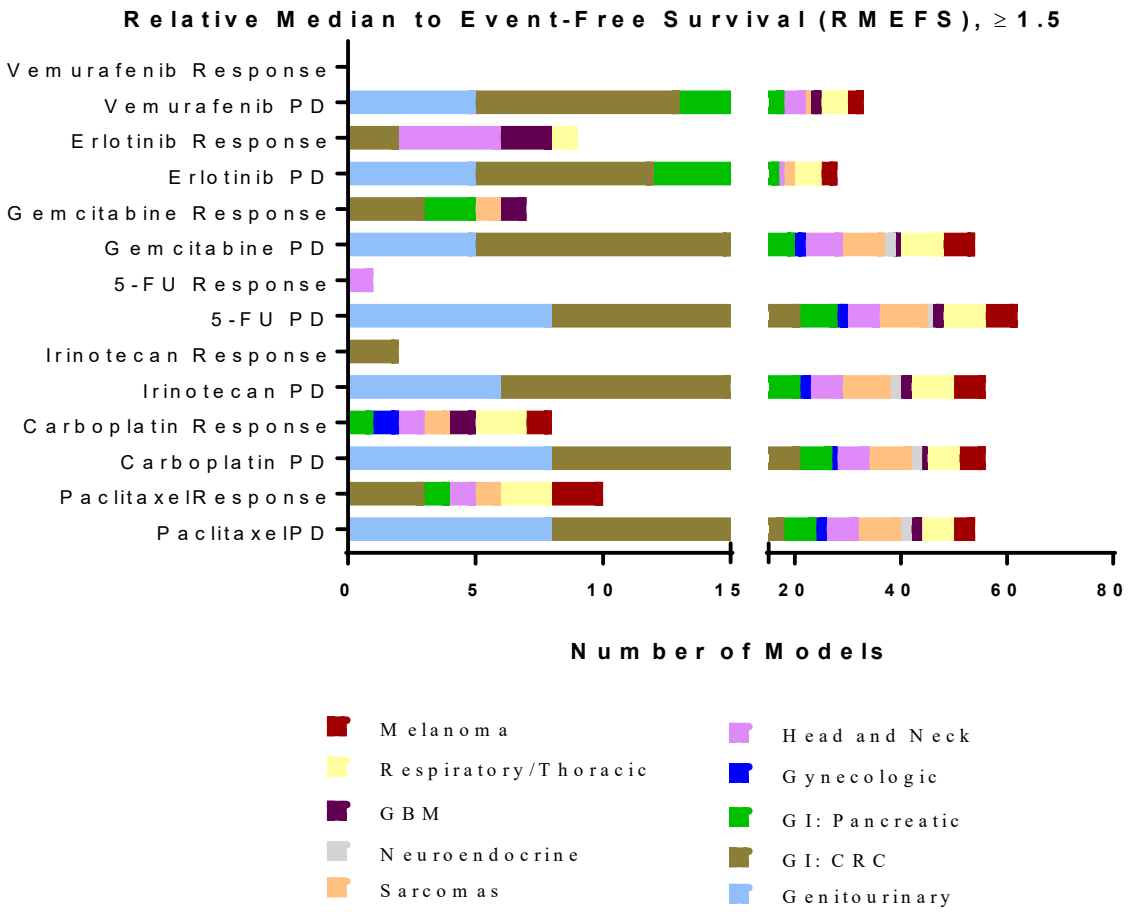
K62003-231-R
GBM
Erlotinib: RM-EFS = 1.70
Study length: 40 days



625472-104-R
Colon Adenocarcinoma
Erlotinib: RM-EFS = 0.94
Gemcitabine: RM-EFS = 2.3
Study length: 43 days

“Rolling” SoC Preclinical Results





RM-EFS: Relative Median to Event-free Survival (relative time to tumor quadrupling, right censored; adapted from Houghton et al., 2007)

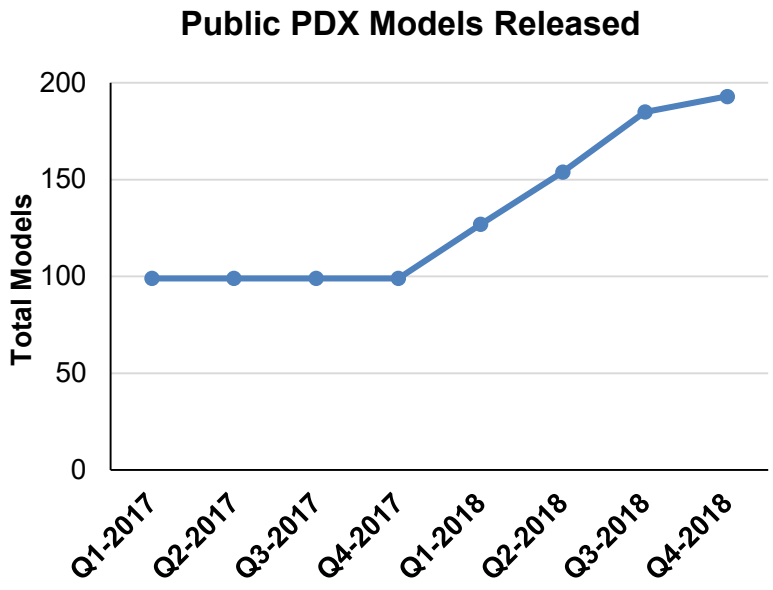
Comparison to Clinical Response Rate

RM-EFS	Paclitaxel	Carboplatin	Irinotecan	5-FU	Gemcitabine	Erlotinib	Vemurafenib
Cut-off ≥2	Lung SCC	Lung SCC	-None-	-None-	Colon Adeno	Gastric Ca	-None-
	Melanoma	Melanoma			Fibrosarcoma	H&N SCC	
	Rectal Adeno	GBM				H&N SCC	
		Sarcoma, NOS					
Added if Cut-off ≥1.5	H&N SCC	H&N SCC	Colon Adeno	Lip/oral SCC	Colon Adeno	H&N SCC	-None-
	Pancreatic Adeno	Pancreatic Adeno	Colon Adeno		Pancreatic Adeno	Lip/oral SCC	
	Lung Adeno	Lung SCC			Pancreatic Adeno	GBM	
	Colon Adeno				Colon Adeno	Lung SCC	
	Melanoma				GBM		
	Sarcoma NOS						
Phase II RR	**Paclitaxel**	**Carboplatin**	**Irinotecan**	**5-FU**	**Gemcitabine**	**Erlotinib**	**Vemurafenib**
	H&N 10%	H&N, Lung 10%	Colon 8-10%	Colon 10%	Colon <10%	SCC 10-15%	<5% V600neg
	Lung 10%	GBM 8%		Oral 10%	Sarcoma 10%		
	Colon 5%	Sarcoma 8%			Pancreas 8%		

Ongoing and Future Initiatives

1000 Patient-Derived Models

- 1000 PDXs across solid tumor histologies
  - ✓ Model generation to gap-fill as well as create models for rare tumor histologies, pediatric cancers, and models from patients of racial and ethnic minorities will be targeted over the next several years
- Plus 1000 models each: PDC, CAF, and PDOrg
  - ✓ Wherever possible matched to an existing PDX Model
- Clinically annotated, molecularly characterized, early passage
- Distributed to academic and commercial researchers.
  - ✓ Currently distribution is in the US, but working to establish a workflow for international distribution



Model Type	Current Public Models	Undergoing Final QC	Estimated Release Per Quarter
PDX	193	160	30-50
PDC	52	28	30-40
CAF	108	13	20-40
PDOrg	0	45	20-30

Preclinical Screening Efforts

- PDXNet Consortium
  - ✓ Network of 6 Preclinical Developmental Therapeutic Centers (PDTCs), 2 with a focus on racial and ethnic minority health disparities
  - ✓ Preclinical Developmental Coordinating Center to provide central data repository
  - ✓ NCI PDMR serves as a Hub for model retention and distribution to sites, and for SOP development

- PDMR
  - ✓ Systematic In Vivo Screening Study for Rare PDX Tumor Models
    - Novel Drug Combinations
    - Tumor types with limited in therapeutic options

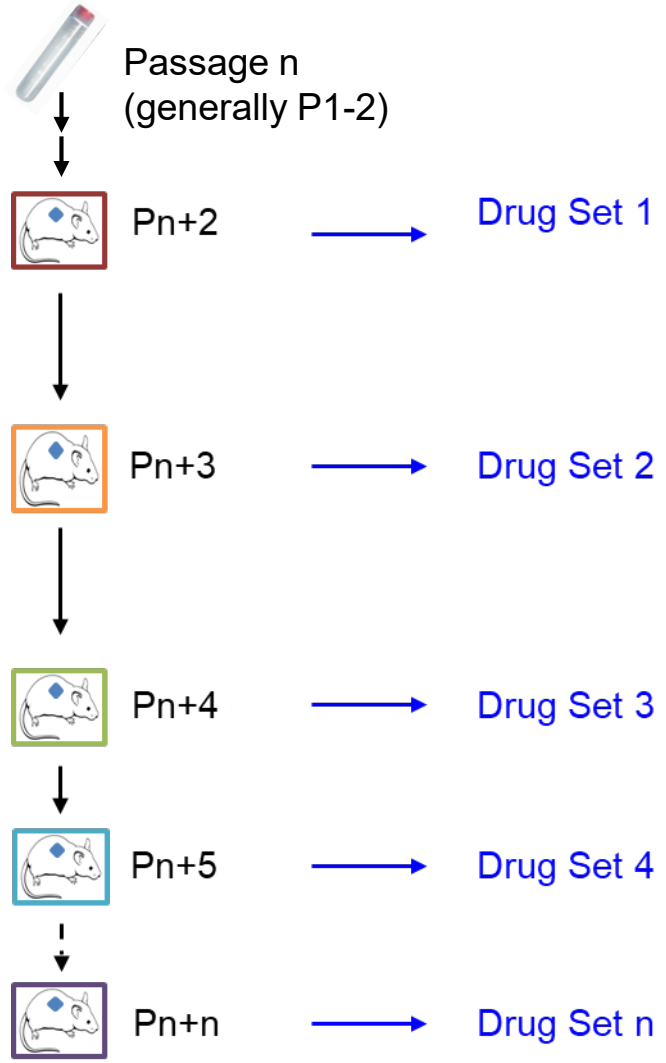
  - ✓ Goal is to have sufficient matched PDX, PDC, and PDOrg models to perform
    - Systematic screening efforts initially in PDCs and/or PDOrgs across tumor histologies
    - Followed by in vivo efficacy studies with selection narrowed by 2D/3D screening studies

Matched Models from Same Patient: Public or in Final QC

PDX	PDX-PDC	PDX-PDOrg	PDX-PDC-PDOrg	PDX-CAF	PDC-CAF	PDX-PDC-CAF
348	58	30	6	36	6	6

Screening Study for Rare Tumor PDX Models

- Screening Study
  - ✓ ~40 PDX Models of Rare Tumor histologies (SWOG DART Study)
  - ✓ 40-50 Drug combinations
    - Human-relevant dosing
  - ✓ N-of-4 study for efficacy
    - 5-7 drug combinations/passage
    - Repeat combination and single arms if efficacy observed in screening for validation of single versus combination effect
  - ✓ Iterative Passaging
    - QC: Shallow Seq for CNA at each passage, human tumor content assessment, pathology



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Question for FNLAC

Should an FNLCR Working Group to provide input regarding the area of cancer models for therapeutics development be initiated?

PDMR NCI Patient-Derived Models Repository
An NCI Precision Oncology Initiative℠ Resource

https://pdmr.cancer.gov