NCI Patient-Derived Models Repository Supporting Cancer Discovery & Therapeutics Development

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NCI Patient-Derived Models (PDM) Repository

- A national repository of PDMs to serve as a resource for academic discovery efforts and public-private partnerships for drug discovery comprised of:
  - clinically-annotated patient-derived xenografts (PDXs),
  - patient-derived tumor cell cultures (PDCs, including conditionally-reprogrammed tumor cell cultures) developed from 1º or metastatic tumors and/or PDXs,
- NCI to provide long-term home for >1000 PDX and PDC models each produced from tissues and blood supplied by NCI-designated Cancer Centers, NCTN & ETCTN
  - Target collections of tumors less prevalent in current resources (eg., Small Cell Lung, Pancreatic, Head/Neck, Ovarian & Bladder cancers; Prostate, Kidney, Sarcomas, Melanomas)
- Goals:
  - ✓ ~50 unique patient models (solid & derived tumor line) per disease (min) with sufficient size of each molecularly-characterized subgroup to power validation and/or efficacy studies
  - ✓ Comprehensive pre-competitive molecular characterization of samples and earliest passage PDXs: MPACT mutation panel, WES, RNAseq, copy number, histology, growth curves, and proteomics/phospho-proteomics (pilot study)
  - ✓ All models and associated data made available through a publicly available website

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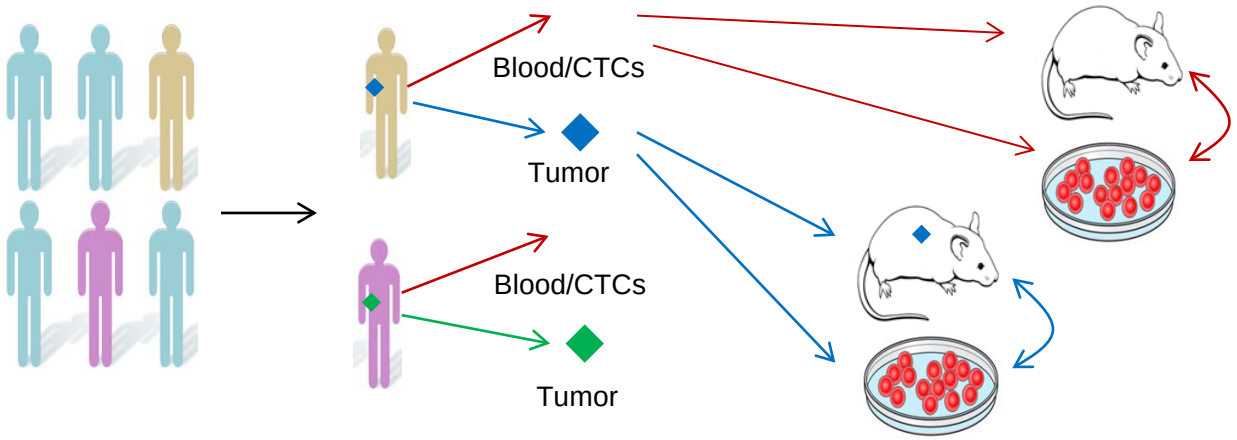
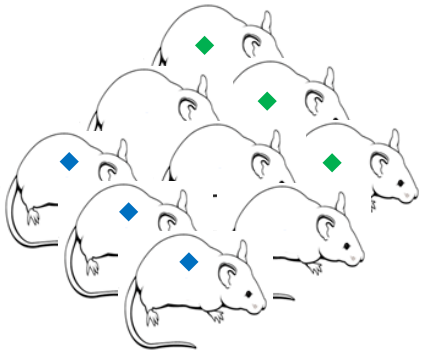
NCI Patient-Derived Models Repository: Multiple Avenues for Discovery

Develop PDX Models and PDC (Tumor & Fibroblast) Lines
DNA, RNA, Protein, WES, RNASeq, Targeted Sequencing

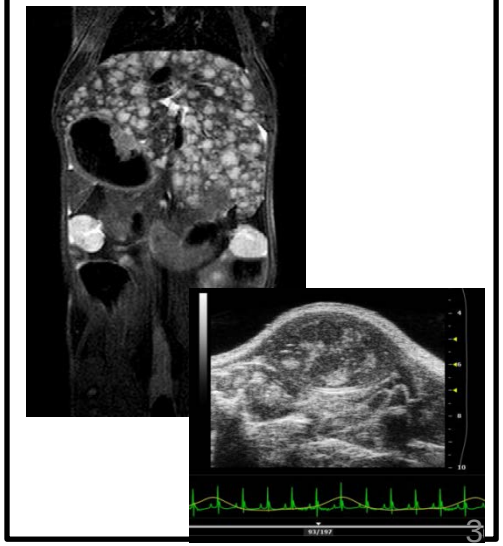


Tumor/Patient Heterogeneity

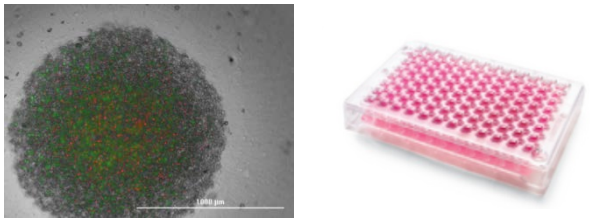


Preclinical Trial Modeling

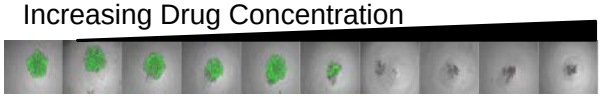
Live Tumor Imaging



3D Culture, 3D Pharmacodynamics



Increasing Drug Concentration



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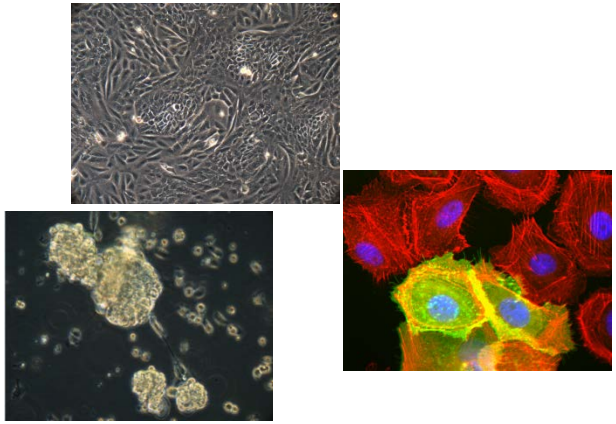
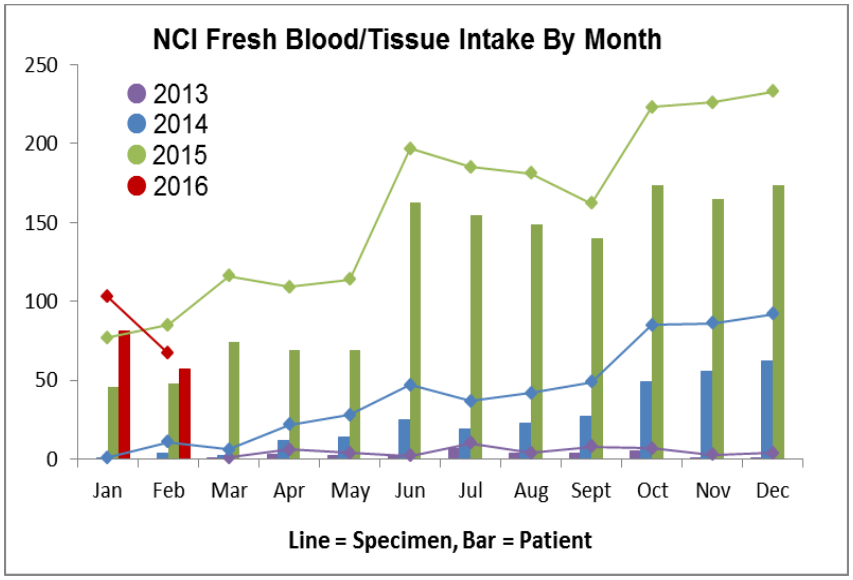
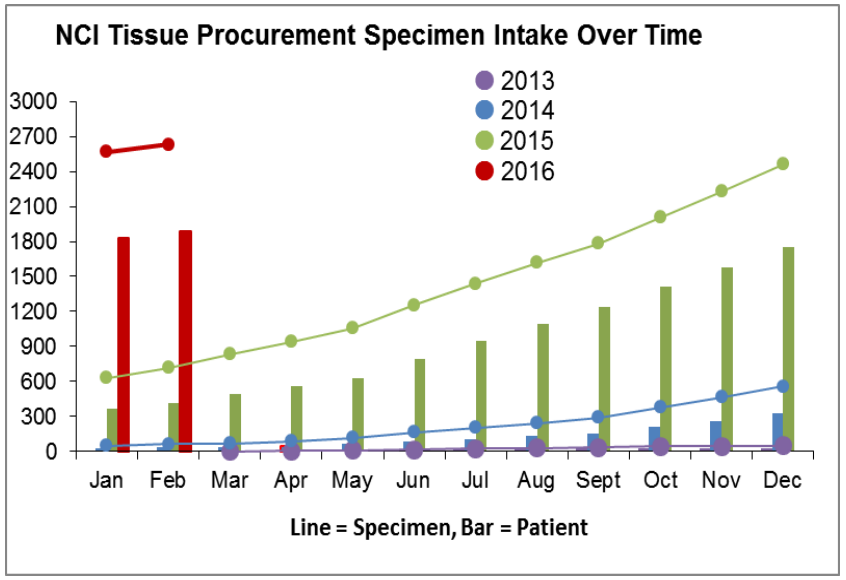
2D Culture, Spheroid growth



Specimen Acquisition for Model Development

- Currently receiving tissue (resections, biopsies) and blood samples for CTC enrichment from two separate tissue procurement protocols (06-C-0213 [NCI] and 9846 [CIRB])
- Clinical centers include 2 NCI clinics, 16 comprehensive cancer centers, and 23 ETCTN/NCORP centers.





Updated 2/3/2016

Lines Graph: Total Specimens
Bars Graph: Total Patients

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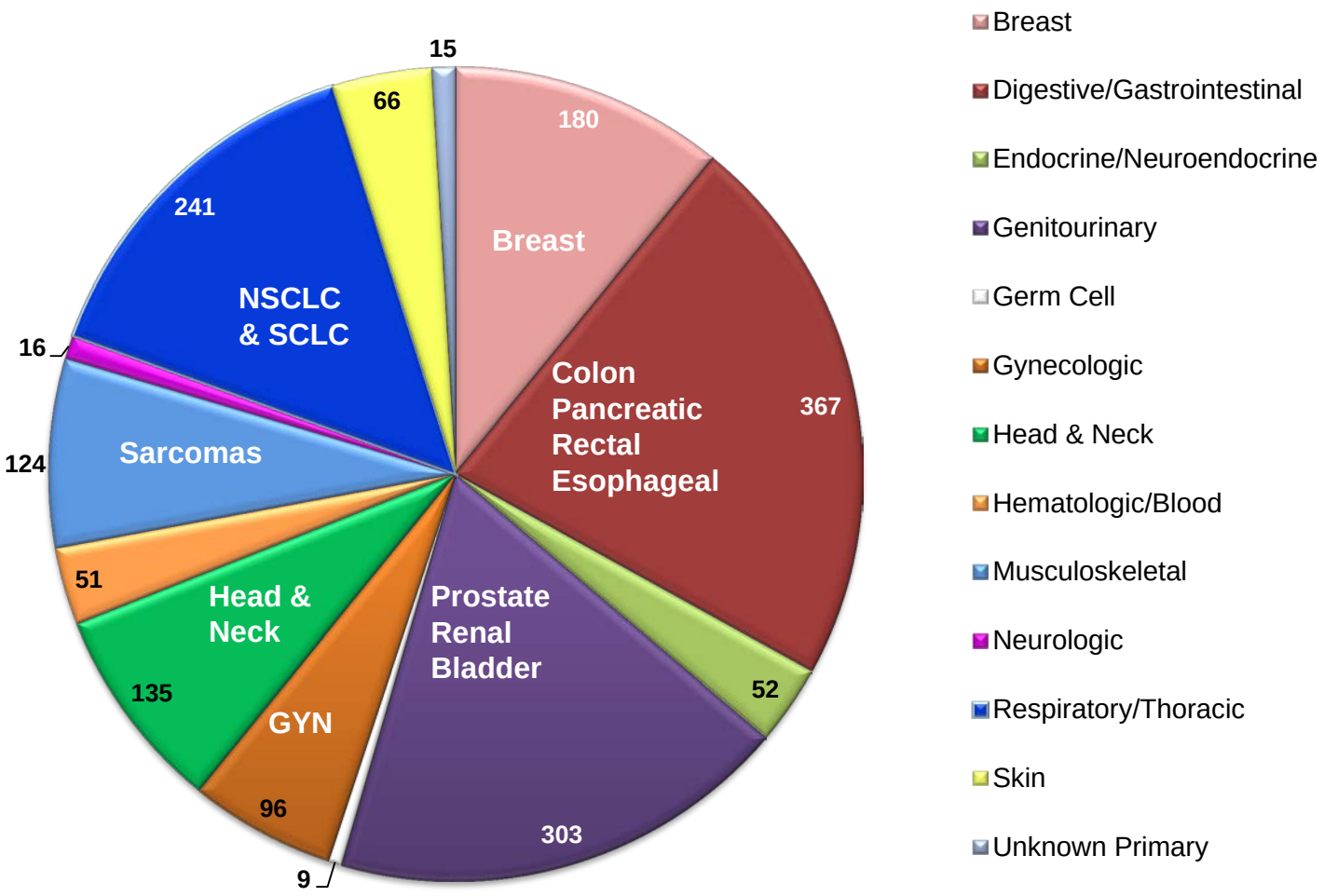
NCI Patient-Derived Models Repository Patients by Disease Location



As of Feb 3, 2016

Total Number of Specimens Received (tissue and blood): 2032

Total Number of Patients: 1655

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PDM *In Vivo* Quality Control Steps

Patient-Derived Xenografts (PDX)

- **Initial QC**
  - Verify P0 pathology matches patient diagnosis
  - Screen for xenograft-associated lymphoproliferative disease (XALD): includes both host-versus-graft response resulting in death or a passageable human lymphoid tumor (generally EBV+) which does not match the patient histology
  - Human:Mouse DNA ratio

- **Distribution Lot (DL) QC**
  - Verify pathology of all PDXs contributing to DL pool
  - Identifiler comparison to Passage 0
  - Whole Exome Sequencing , MPACT assay, and RNASeq of 6 PDXs performed; 1 deep sequence and 5 shallow sequence. Reviewed for concordance.
  - Verify regrowth of cryopreserved fragment

PDX Take-Rate from Tumor Tissue Implantations

Body Location	Total Received	Total Assessable Specimens	%Take-Rate of Assessable Specimens	Passageable Tumor*	Discontinued†	*Not Yet Assessable: P0 tumors*
Breast	32	4	25%	1	3	28
Digestive/ Gastrointestinal	129	80	70%	56	24	49
Endocrine/ Neuroendocrine	34	16	31%	5	11	18
Genitourinary	149	66	52%	34	32	83
Germ Cell	3	1	0%	0	1	2
Gynecologic	34	20	55%	11	9	14
Head and Neck	103	82	71%	58	24	21
Hematologic/Blood	1	0	--	0	0	1
Musculoskeletal	118	45	53%	24	21	73
Neurologic	4	2	50%	1	1	2
Respiratory/Thoracic	49	36	64%	23	13	13
Skin	38	18	83%	15	3	20
Unknown Primary	9	4	50%	2	2	5
Totals	**703**	**374**	**61%**	**230**	**144**	**329**

*Passageable Tumor — Includes any PDX where a palpable tumor has been passaged to at least P1 as well as Distributable PDXs. One or more of QC steps for PDX confirmation are pending for earlier passages.

†Discontinued — (1) Did not successfully grow palpable tumor in P0 (monitored 300 days), (2) Passaged tumor failed to grow in subsequent passages, (3) Mouse found dead/tumor not passageable, (4) Palpable tumors were 100% murine content, (5) xenograft-associated lymphoproliferative disease (XALD: host-versus-graft disease or human lymphoma out-growth)

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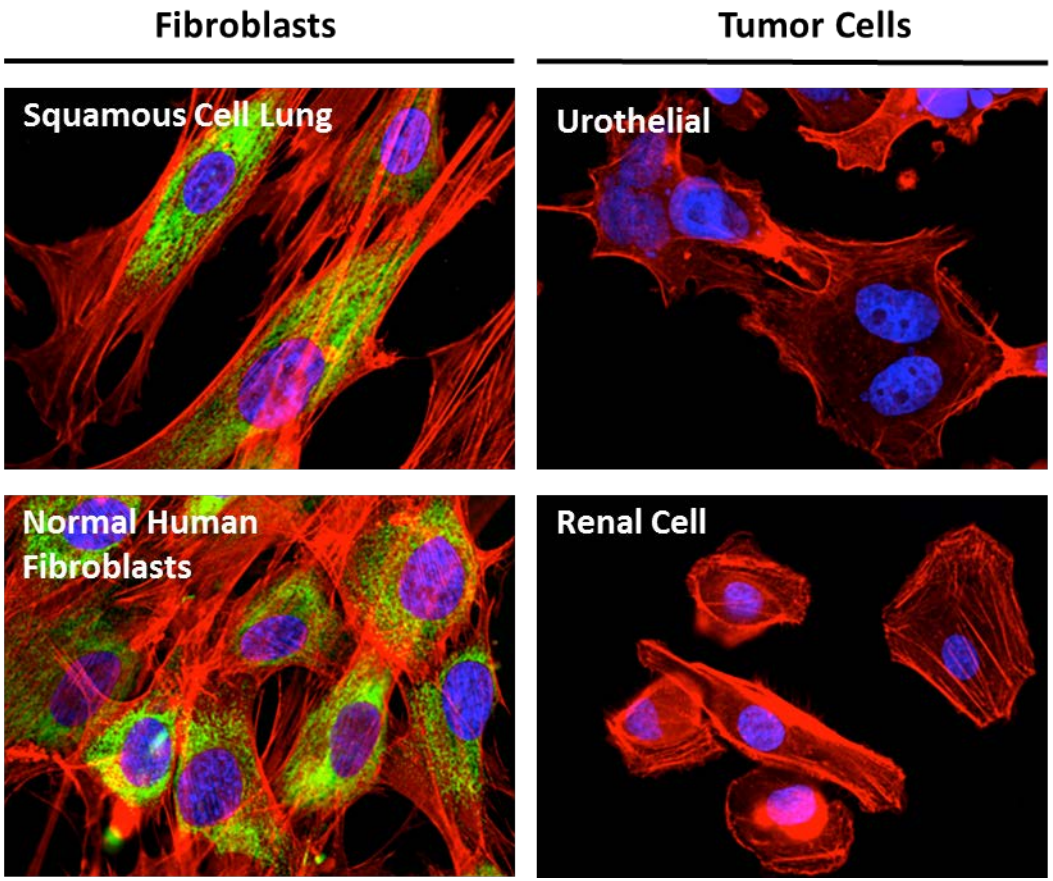
1/6/2016

In Vitro Models

Goal: Develop conditionally-reprogrammed patient-derived tumor cell cultures (PDCs), clonal cell lines, and cancer-associated fibroblasts (CAFs) from patient materials. Cultures maintained at an early passage and available for distribution.

Examples of patient-derived tumor cell cultures and a cancer associated fibroblast line and normal fibroblasts currently undergoing analysis.

Green: TE7 (fibroblasts),
Red: Phalloidin (actin filaments),
Blue: DAPI



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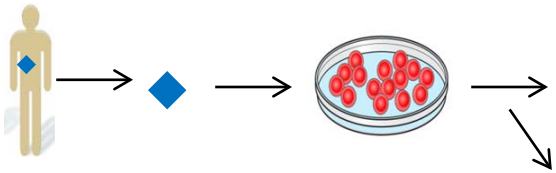
PDM *In Vitro* Quality Control Steps

Patient-Derived Cell (PDC) Cultures

- **Initial QC**
  - Use FACs sorting to isolate tumor cultures and cancer-associated fibroblast cultures
  - Determine doubling time, and optimal growth conditions
  - Perform qRT-PCR for tumor versus fibroblast cell phenotype
  - Human:mouse DNA ratio if tumor cells originated from a PDX rather than directly from human donor

- **Distribution Lot (DL) QC**
  - FACs and qRT-PCR analysis to verify purity
  - Identifiler comparison to early passage in vitro culture, and when possible to PDX
  - Whole Exome Sequencing , MPACT assay, and RNASeq of 6 PDXs performed. When possible, reviewed for concordance with PDX.
  - Karyotyping performed
  - Verify growth of cryopreserved vial for tumor lines and lack of growth for CAF lines as PDXs

In vitro Culture of Patient-Derived Tissue



Primary culture expanded in F12+Y+P/S (7-8 passages)

3-10 vials cryopreserved (~P4)

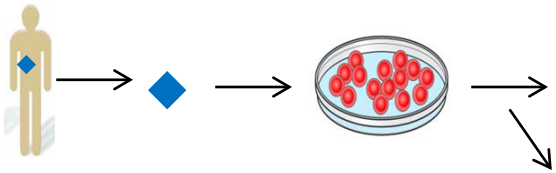
FACs separation of patient tumor and CAFs for in vitro culture

Final QC and stock vial preparation

	Grown to P4	Tumor Material Present After FACs	CAFs Present After FACs
Total Attempted Cultures	775	460	460
#In Evaluation	114	149	83
#Successful	460	--	--
#Discontinued (no growth)	201	102	45
#Discontinued (cell type not present)	--	107	80
Sorted, >**99%**Tumor Culture	--	**102**	N/A
Sorted, >**99%** CAF Culture	--	N/A	**252**

Cultures get re-screened after 3-4 passages to confirm purity of >**99%** and then go through final QC including: Genomic studies, IdentiFiler, karyotyping, tumorigenicity testing, growth rate assessment, and verification that distribution stocks will grow for up to 20 passages from freeze. CAF cultures have a shorter life-span and are assessed for growth up to 10 passages from freeze.

Patient-Derived *In Vitro* Cultures by Disease Location



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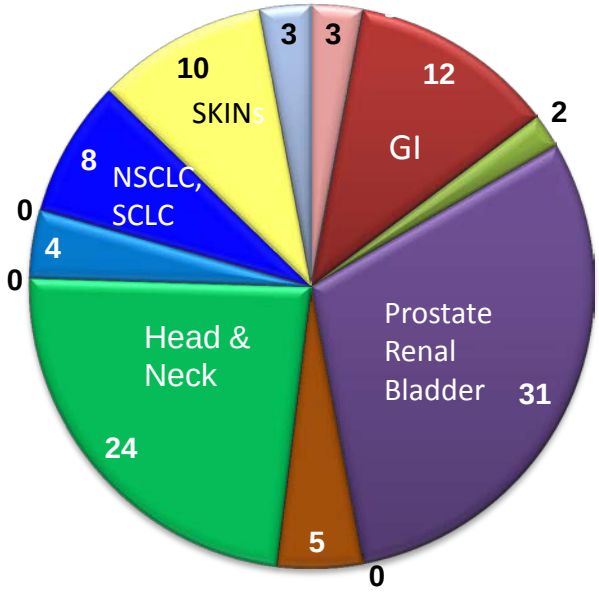
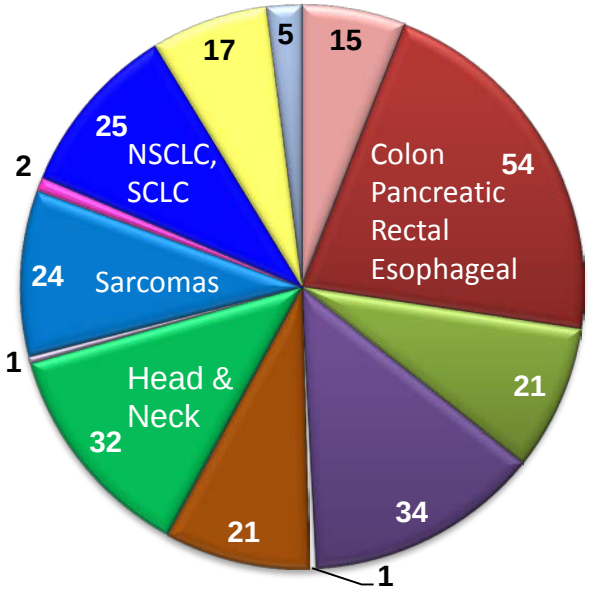
Final QC and stock vial preparation

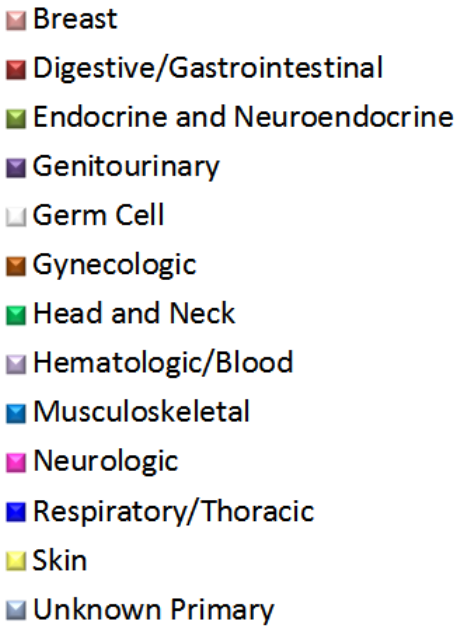
3-10 vials cryopreserved (~P4)

Sorted, >99% Tumor



Sorted, >99% CAF





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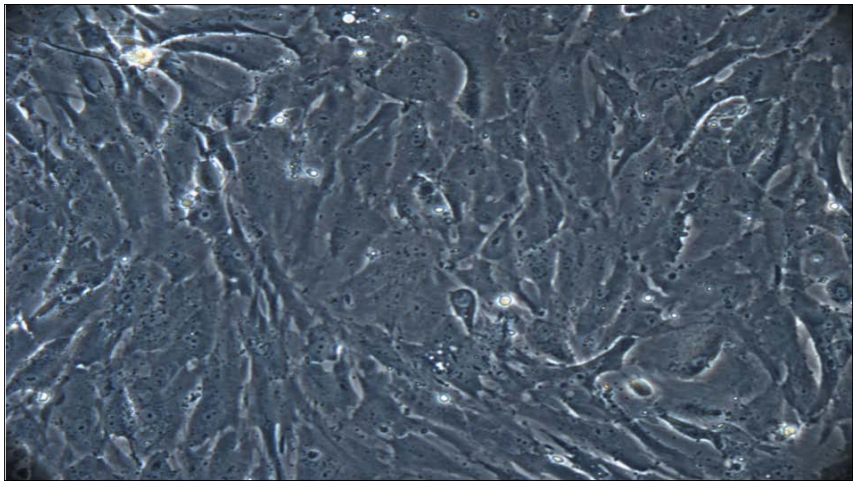
In Vitro Cultures from a Patient with NSCLC

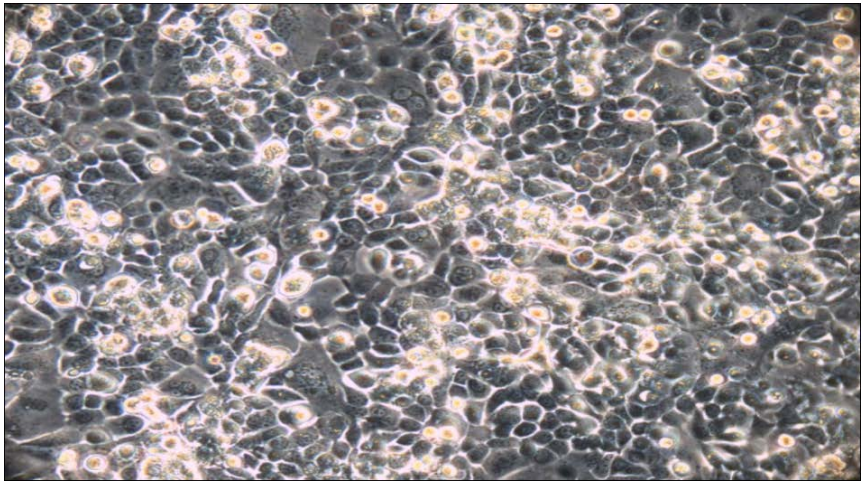
Fibroblast culture
CD90+, EPCAM-



Growth: Adherent Monolayer
Proliferation Rate: 57.3 h
Spheroid Formation: None
Soft Agar Growth: None

qRT-PCR Correlation with Fibroblast C1 & C2 Controls: 71%, 81%

Tumor culture
CD90- EPCAM+



Growth: Adherent Monolayer
Proliferation Rate: 25.5 h
Spheroid Formation: None
Soft Agar Growth: Yes

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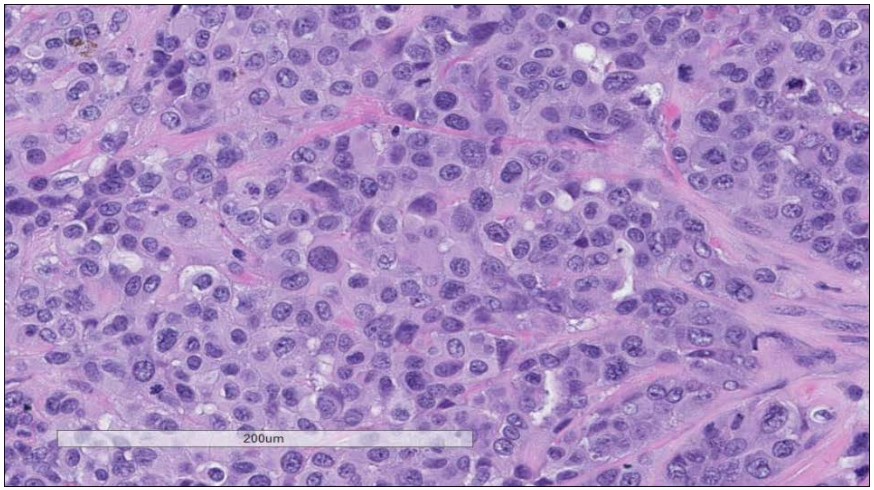
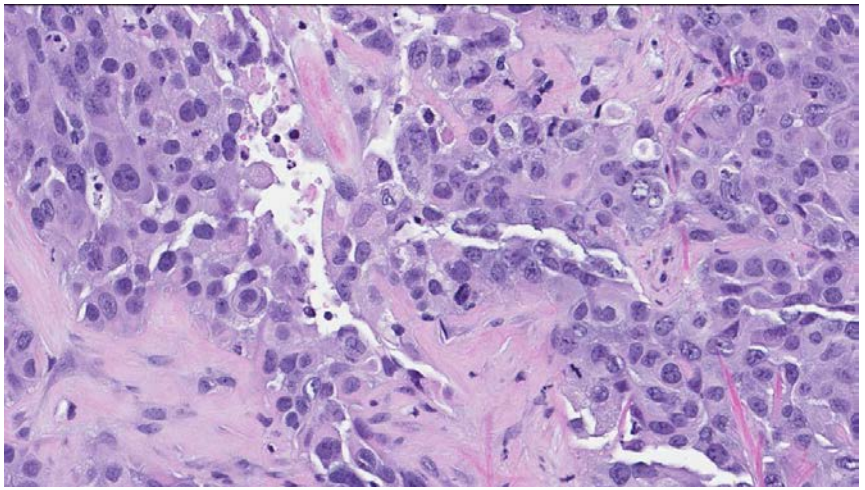
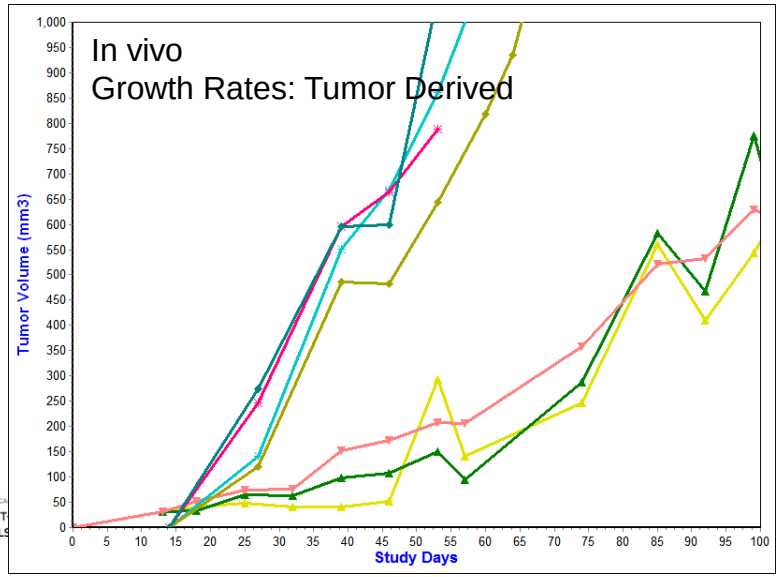
Comparison of NSCLC PDXs Developed Directly from Patient Tumor vs Tumor Cell Culture

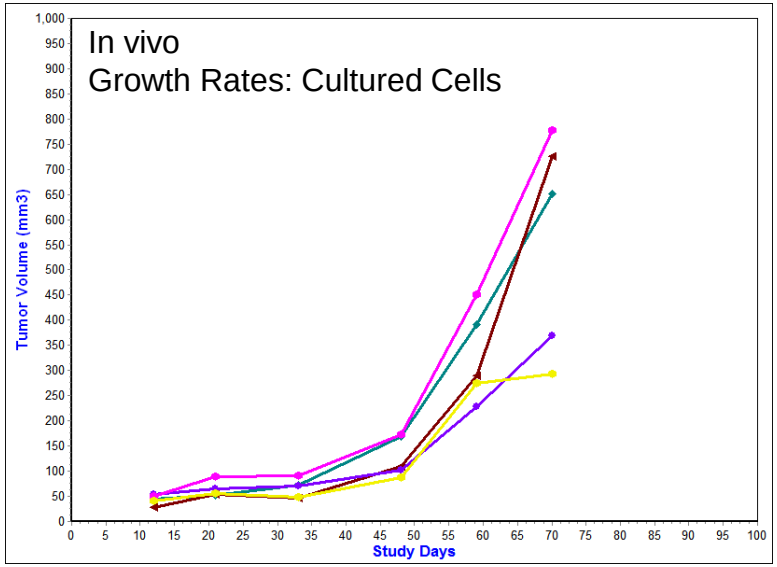
Tumor-Derived PDX (P0)



Tumor Culture-Derived PDX







CTC Patient-Derived Xenografts (CDXs)

Na-Heparin

Blood

Overnight Shipment

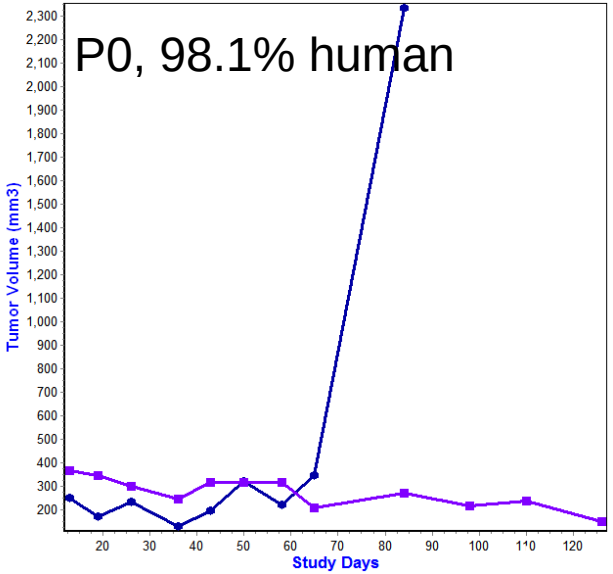
Multiple methodologies for isolation being tested

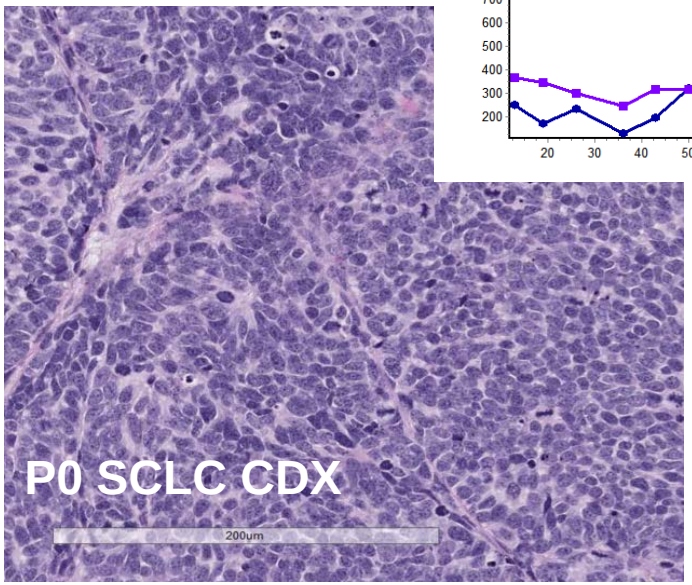
- Oncoquick isolation
- Large micron enrichment of CTC clusters + Oncoquick
- ApoStream enrichment

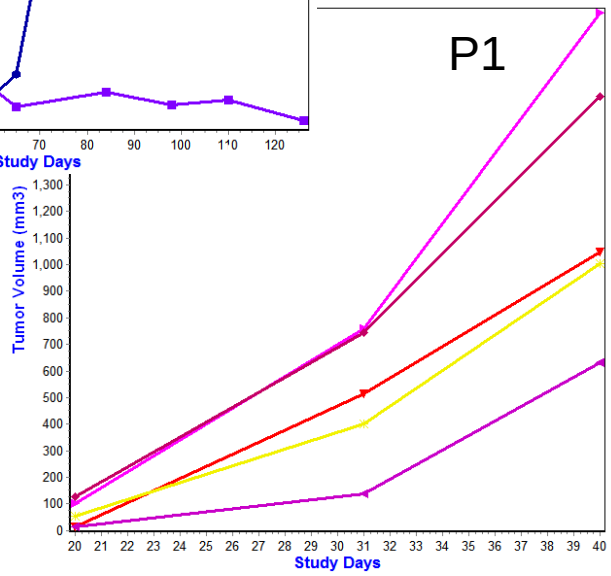
Rituxan

- Direct implantation (SC)
- Tail vein injection (IV)
- Short-term in vitro culture and then implantation

- Confirmed human, pathology confirmed
  - SCLC (CD56 (NCAM)+, NSE+, CK equivocal)
  - Colon Adenocarcinoma
- Confirmed human, pathology pending
  - Merkle Cell ca
  - Squamous Cell Lung ca
- In P0
  - B-cell Lymphoma
  - Prostate ca







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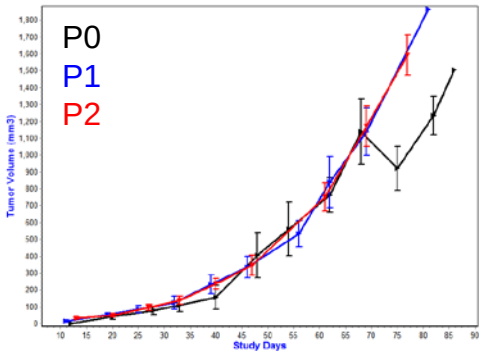
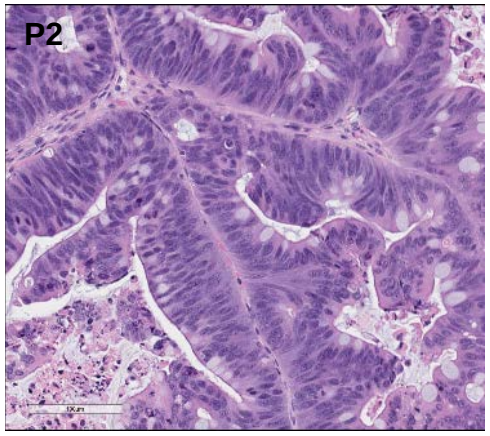
PDX Compared to CDX from Patient with Colon Adenocarcinoma

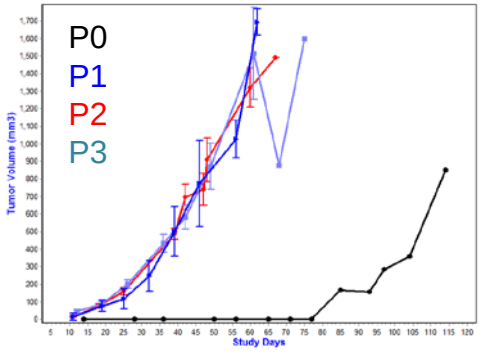
Source: Liver metastasis biopsy (121-T)

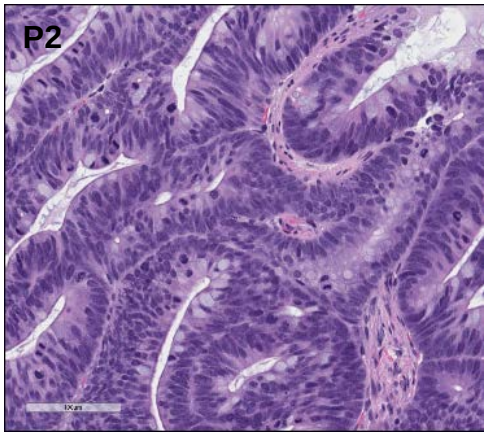




Gene Variant	Allele freq
KRAS-G12D	0.65
PIK3C-E545K	0.46

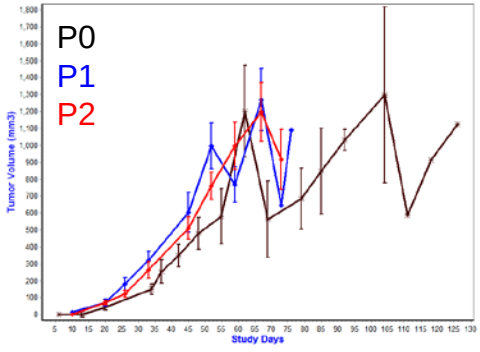
Source: Enriched CTCs (121-B)

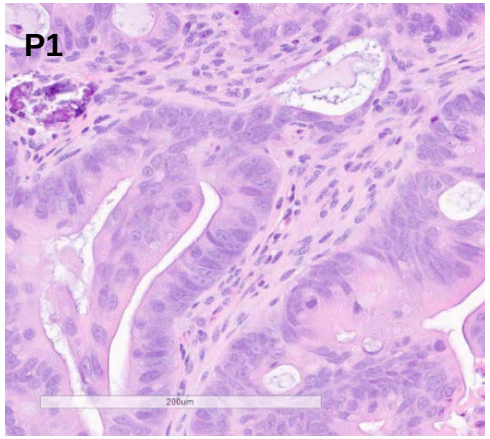




Gene Variant	Allele freq
KRAS-G12D	0.66
PIK3C-E545K	0.5

Source: Adrenal gland metastasis resection (288-R)





Gene Variant	Allele freq
KRAS-G12D	0.64
PIK3C-E545K	0.75

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Nude Rat PDXs: Implanted from Human PDXs Grown in NSG Mice

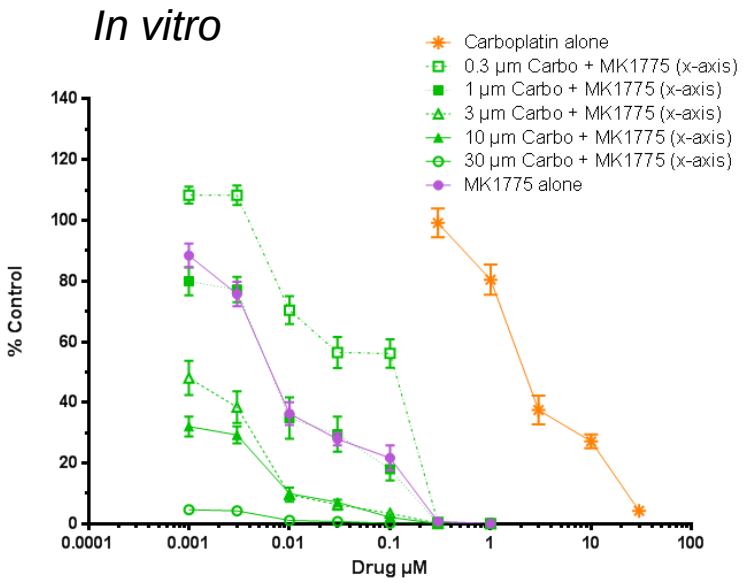
PDX ID	CTEP SDC Diagnosis	Growth in Rat (Passageable tumor)
172845-121-B	Adenocarcinoma - colon	No Growth
CN0330F216	Adenocarcinoma - colon	No Growth
CN0375F725	Adenocarcinoma - colon	Yes
CN0428F1126	Adenocarcinoma - colon	No Growth
CN0446F447	Adenocarcinoma - colon	Yes
466732-252-T	Adenocarcinoma - small intest.	Yes
ST0110F1568	GIST, poorly differentiated	No Growth
295223-140-R	H & N squamous cell car.	Yes
SA0426F1136	Leiomyosarcoma - not uterine	Yes
692163-330-T	Leiomyosarcoma - uterus	Yes
941425-263-T	Mesothelioma	Yes
LG0904F1496	Neuroendocrine cancer	Yes
LG0703F948	NSCLC, Adenocarcinoma	No Growth
LG0807F1297	NSCLC, Adenocarcinoma	Yes
LG1189F1952	NSCLC, Adenocarcinoma	Yes
114551-080-T	Salivary gland cancer, acinic	No Growth
275155-148-R	Salivary gland cancer, adenocarcinoma	Yes
LG0520F434	Squamous cell lung carcinoma	No Growth
LG0830F1385	Squamous cell lung carcinoma	Yes
416634-122-T	Transitional cell car. - uroth.	Yes
BL0269F402	Urothelial/bladder cancer	Yes
BL0293F563	Urothelial/bladder cancer	Yes
BL0382F1232	Urothelial/bladder cancer	Yes
BL0470F1820	Urothelial/bladder cancer	Yes
SA0350F605	Uterine cancer, undifferentiated sarcoma	Yes

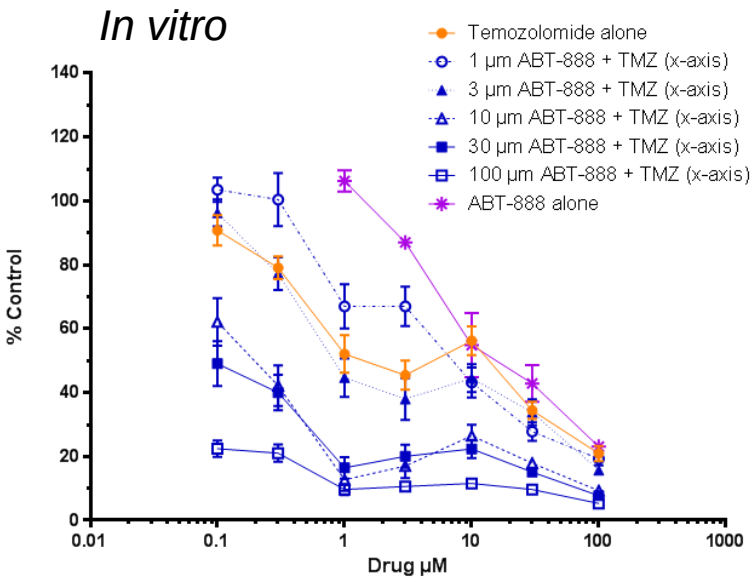
A total of 54 models have been implanted into nude rats

- Of the 25 assessable models (table) there is a 72% success rate growing PDXs.
- 29 additional models are still in P0 growth
- Possible now to assess CTCs

Of interest: Previous less successful attempts to grow traditional xenografts in nude rats have been started from in vitro culture and required a larger cell number implanted than normal to grow a xenograft.

Bladder Model BL3: *In Vitro* and *In Vivo* Response



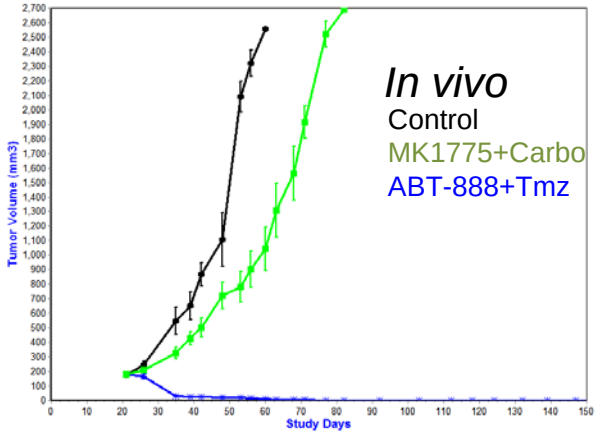


CI values: MK1775 + Carboplatin

ED50 3.52 = antagonistic

ED75 2.19 = antagonistic

ED90 1.4 = antagonistic



CI values: ABT-888 + Temozolomide

ED50 5.04 = antagonistic

ED75 0.65 = synergistic

ED90 0.47 = synergistic

In vitro data confirmed that BL3 was more sensitive to ABT-888 + Temozolomide than MK1775 + Carboplatin

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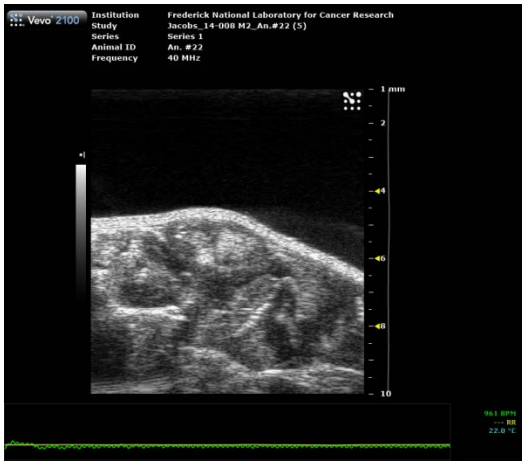
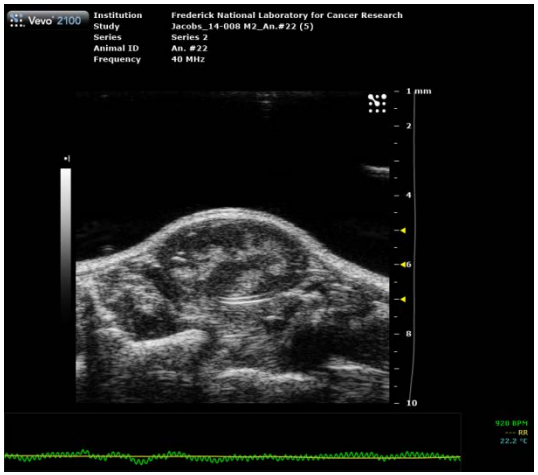
BL3 Pilot Imaging Study
Heterogeneity on B-mode Ultrasound



4/20/2015



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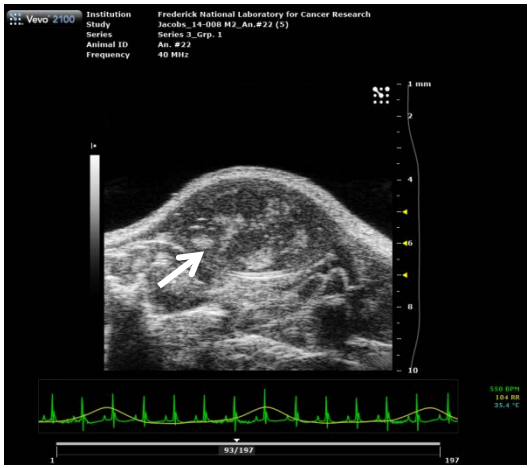
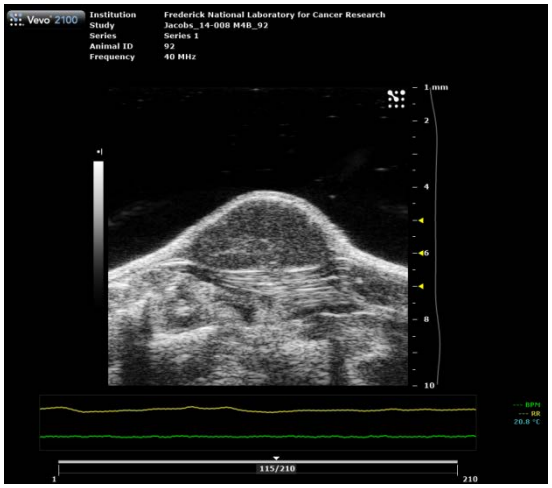


4/27/2015

Fragment Implants



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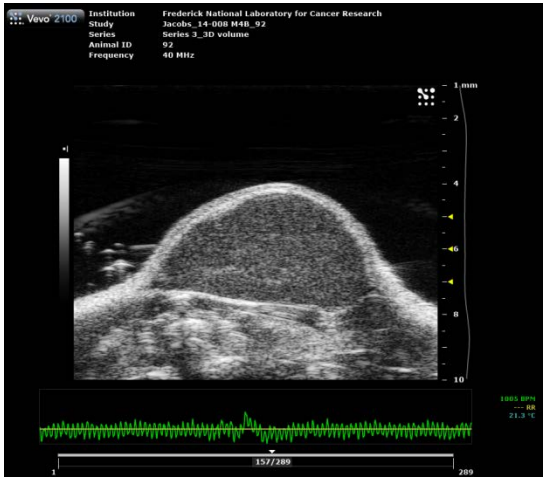

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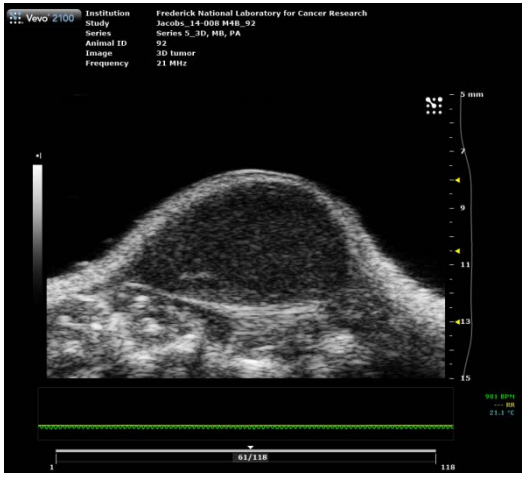

8/4/2015

Cells

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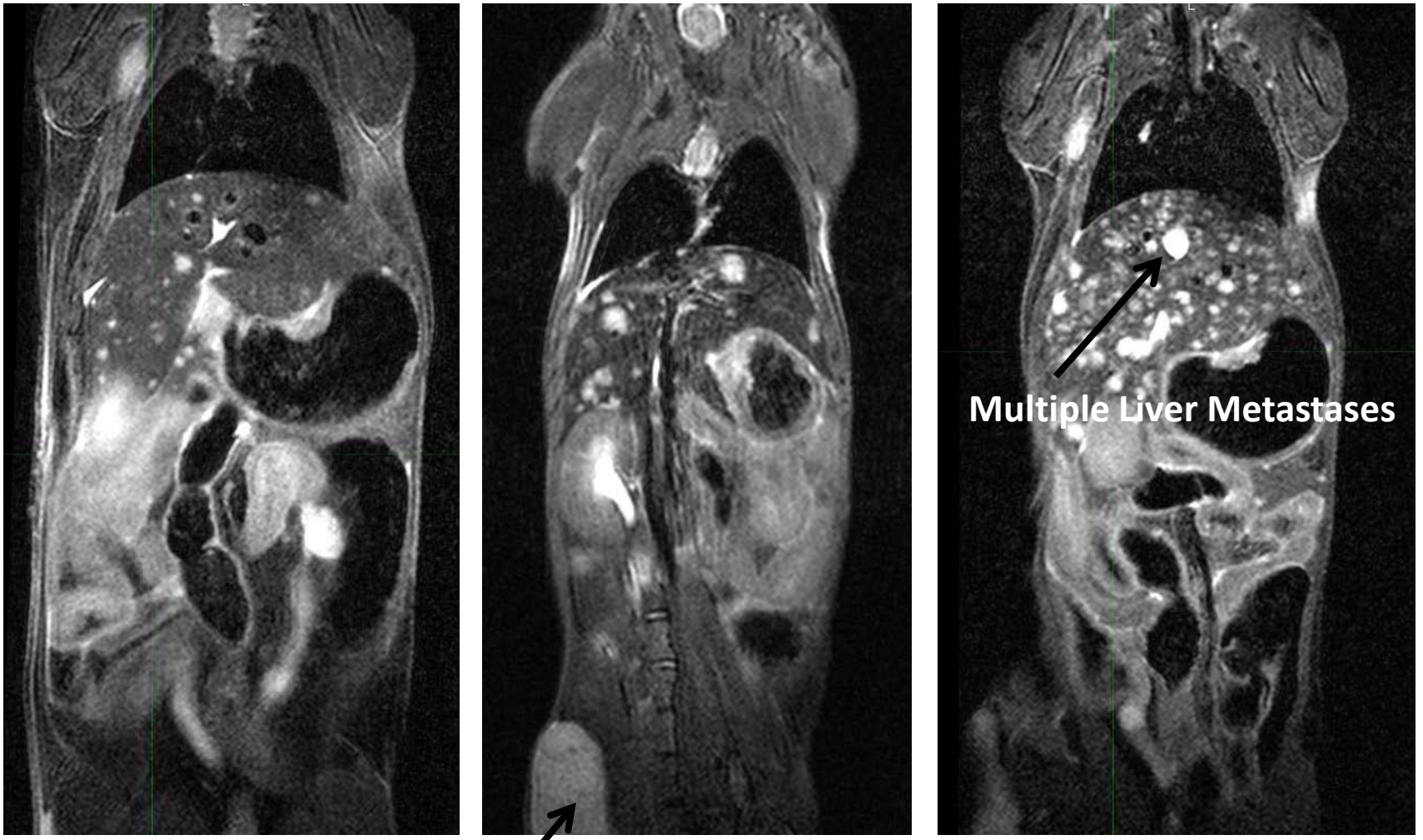
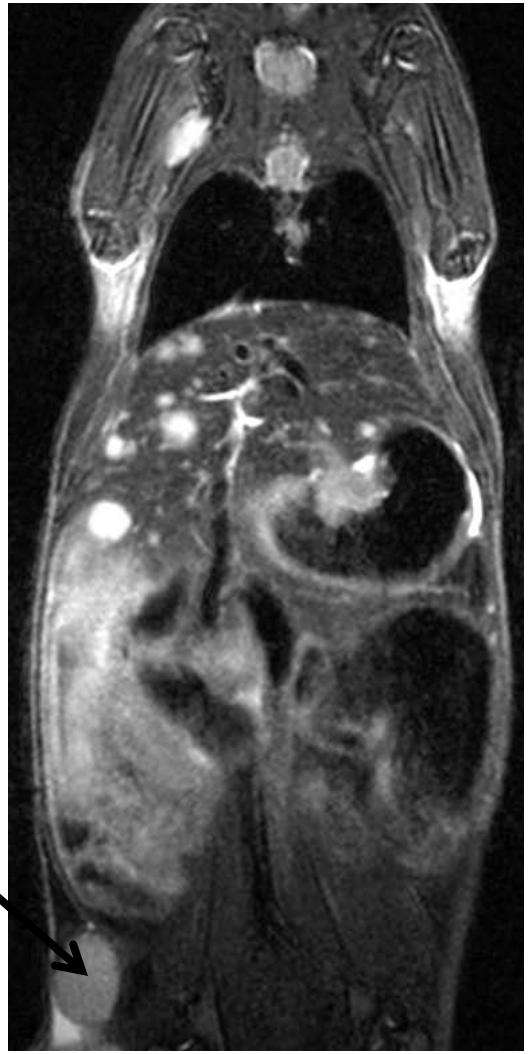
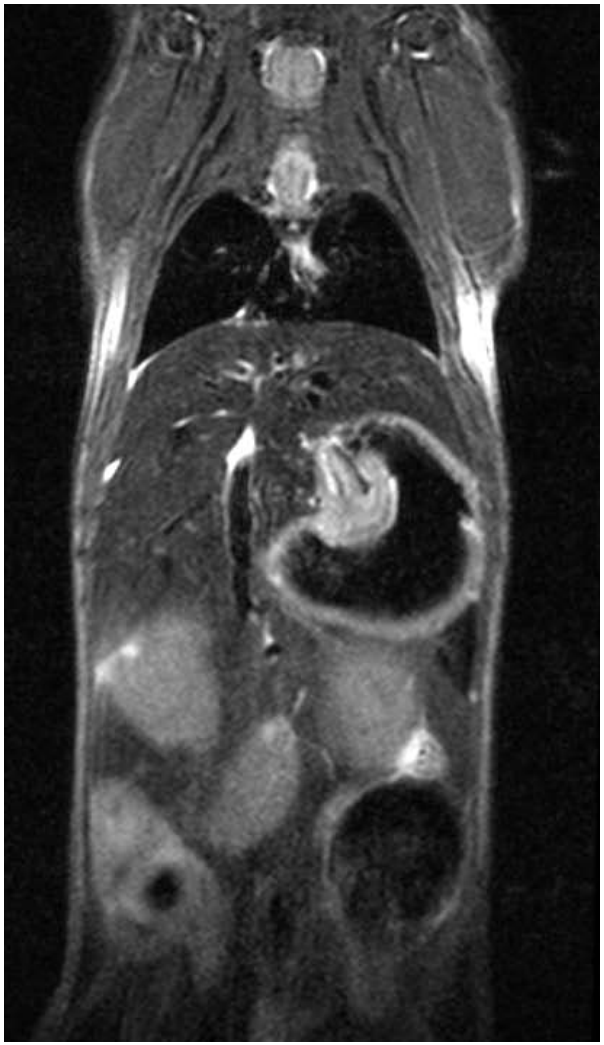
BL3 Bladder PDX Implanted in 3 NSG mice on 6/8/2015
MRI 7/30/2015



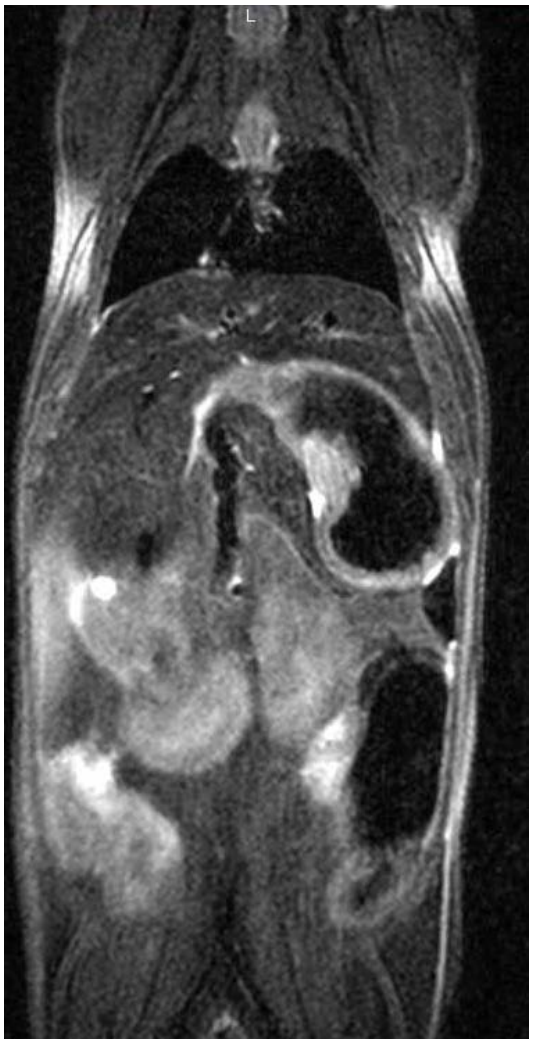
BL3 Bladder Tumor:
Single Cycle of ABT-888 + TMZ Begun 8/12/2015 (Daily X 5d)



8/12/2015: Pre-Dose

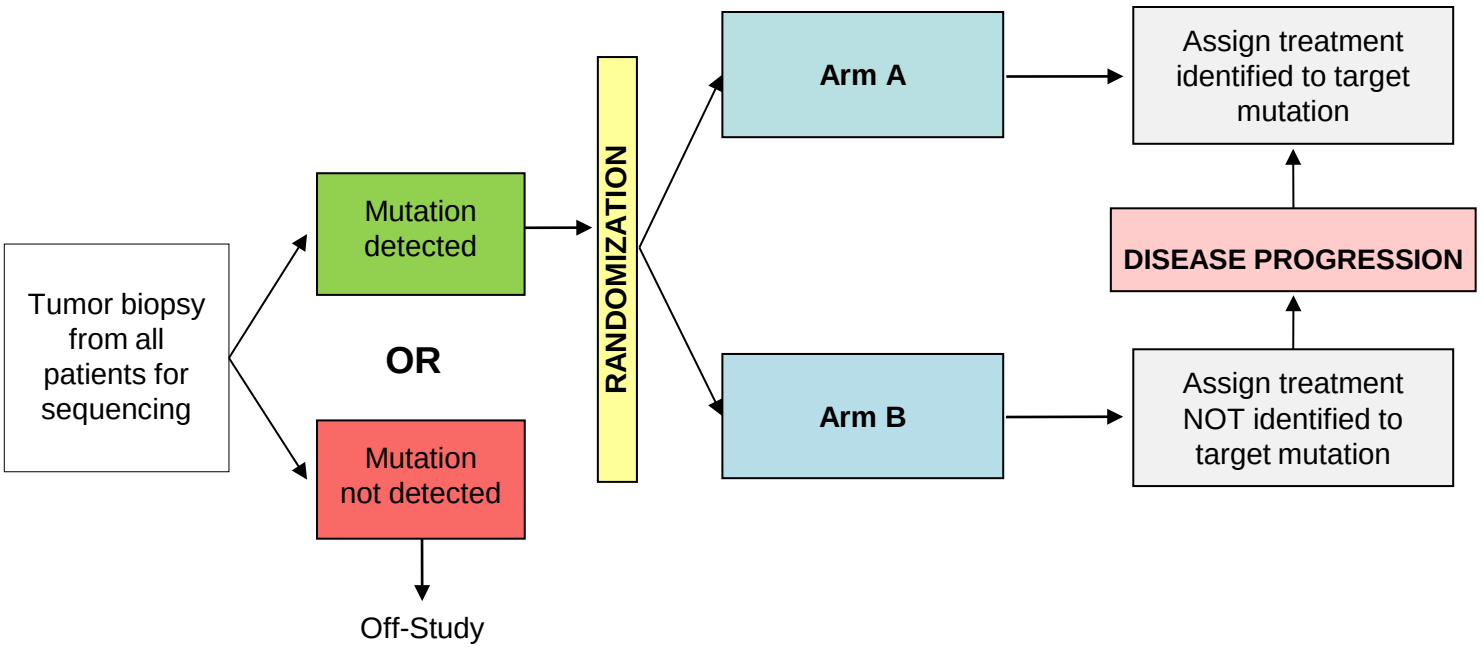


8/27/2015: CR



9/04/2015: CR

NCI's MPACT Clinical Trial: Study Design



- Fresh tumor biopsy on-study and at progression
- Primary endpoint response (CR + PR) and 4-month PFS improved for agents chosen on the basis of specific mutations
- Crossover from Arm B (non-mutation–directed) to Arm A (mutation-directed) treatment at progression
- Trial to be opened across NCI's Phase I/II network (>30 NCI-designated Cancer Centers)
- Accrual began Q1-2014: 60 patients accrued

Pre-Clinical MPACT

Can we predict the results of the MPACT trial*?*

- Perform proof-of-mechanism, pre-clinical trial using molecularly characterized PDX models carrying one (or more) of the MPACT actionable mutations

- Treat each 'patient-model' with all matched and unmatched agents to enhance statistical power, employing sample sizes that permit PD sampling, and that will allow estimation of variation across mice carrying identical PDXs

- Examine PD effects at treatment initiation, and molecular changes at the time of disease progression

- If pilot phase encouraging, continue pre-clinical MPACT study with PDX's generated from patients enrolled on the trial: retrospective correlation of preclinical result with therapeutic outcome on study

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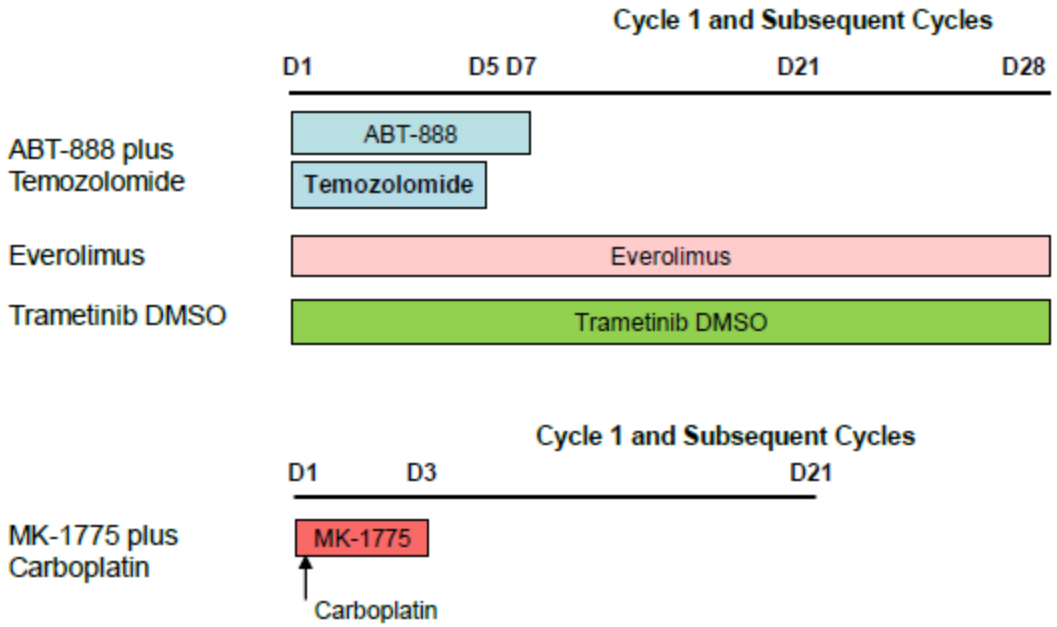
Preclinical MPACT: Modeling NCI-MPACT Clinical Trial 13-C-0105



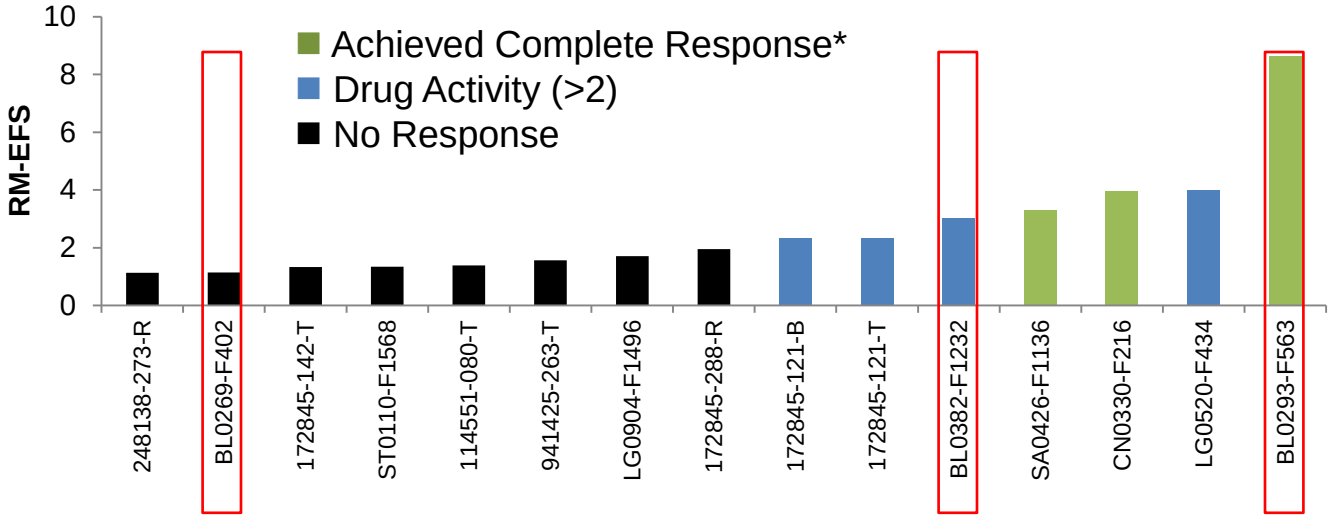
Preclinical trial dosing modeled after the <u>CLINICAL TRIAL</u>:

- Patients with specified mutations of interest will be assigned to receive **one** of the following study drugs or drug combinations at the assigned dose.
- **ABT-888** 40 mg orally BID qd days 1-7 plus **temozolomide** 150 mg/m2 orally qd days 1-5 (no food restrictions) in 28-day cycles
- **Everolimus** 10 mg orally each day (no food restrictions) in 28-day cycles
- **Trametinib DMSO:** 2 mg orally each day either one hour before or two hours after a meal in 28-day cycles
- **MK-1775** 225 mg orally BID for 5 doses either at least two hours before or two hours after a meal plus **carboplatin** (AUC 5) IV on day 1 every 3 weeks (21-day cycle)

Analysis of Preclinical MPACT

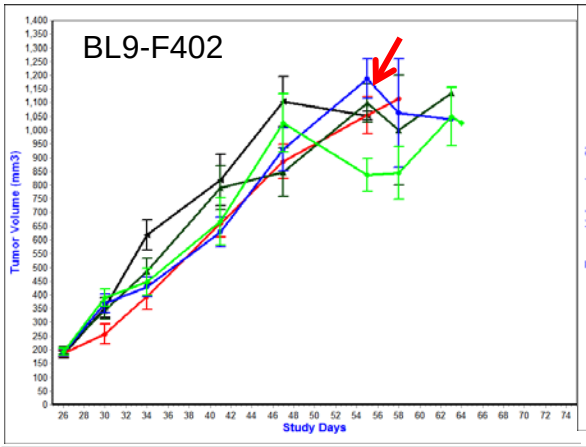
- To date 15 models have completed the Preclinical NCI-MPACT study, 3 are on-going and an additional 8 models are in the queue for tumor growth and treatment.
- Whole exome sequencing and RNASeq are being performed at baseline and at pre-defined times during the study.
- Preclinical Response Assessment:
  - While complete regressions and no response can be categorized fairly easily; what is/can be called a drug response in between those two extremes can be difficult to define. One complicating factor is the rate of growth between different models.
  - We are using a Relative Median to Event-Free Survival (RM-EFS) from staging to assign a numerical ranking to survival in the drug studies (based on Houghten et al. 2007). An event is defined quadrupling of the tumor volume from staging.
  - In addition to numerical assignment for the RM-EFS, we can bin the responses categorically. Bins include: (1) those models that achieve a complete response (CR) for >5 consecutive tumor measurements, (2) those that have a >2-fold change in RM-EFS, and (3) a 'no response' group for those with ≤2-fold change in RM-EFS.
- These criteria are being used with RNASeq data to evaluate mechanisms of response in the pre-clinical models.

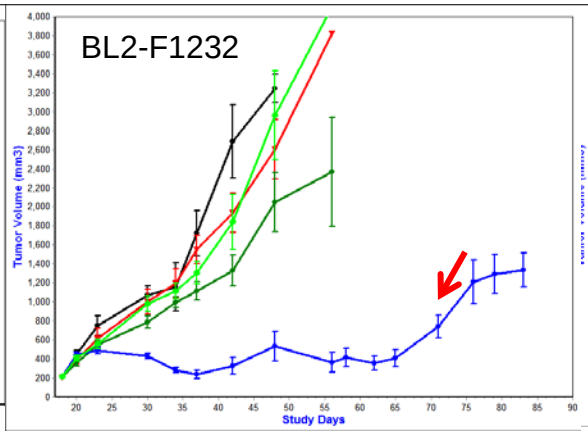
Preclinical Response in ABT-888 + Temozolomide Cohorts

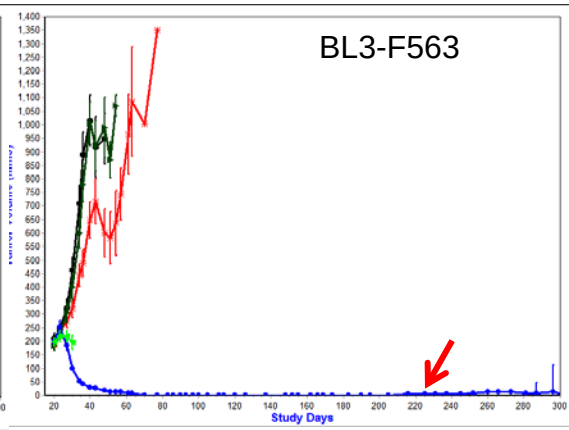


*CR. Tumor volume <60 mm3 for 5 consecutive time points

Drug
Vehicle
Everolimus
Trametinib
ABT-888 + Temozolmide
MK-1775 + Carboplatin







Bladder Cancer, sarcomatoid differentiation	Bladder Cancer, invasive	Bladder Cancer, transitional cell ca
TP53-R248Q	PIK3C-H1074R	TP53-E336 (stop codon)
MK1775+Carboplatin	Everolimus	MK1775+Carboplatin RM-EFS: >8.6

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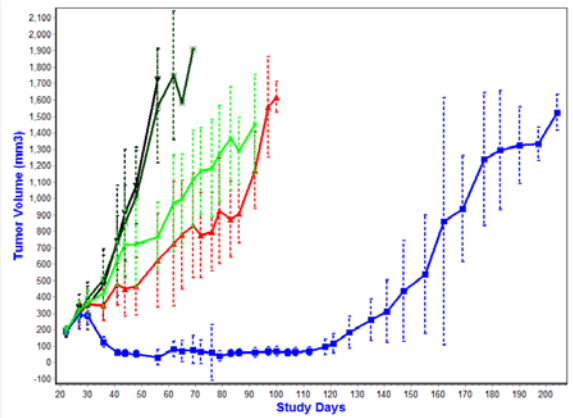
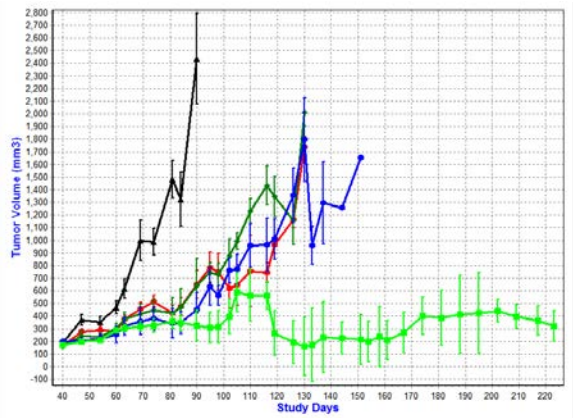
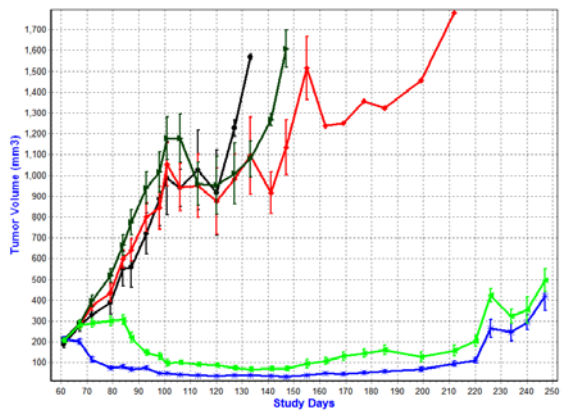
Preclinical MPACT Models

(Group) NSC	Drug
(G1)	vehicle
(G2) 733504	Everolimus
(G4) 758246	Trametinib
(G6) 752840	ABT-888
362856	Temozolmide
(G8) 754352	MK-1775
241240	Carboplatin







	LG0520	114551-080-T	SA0426
	Lung SCC	Acinic Salivary Gland Ca	Leiomyosarcoma, non-uterine
MPACT aMOI	ERCC1-Q67 (stop codon)	TP53-R175H	None
Assign:	ABT-888+Temozolomide	MK1775+Carboplatin	N/A

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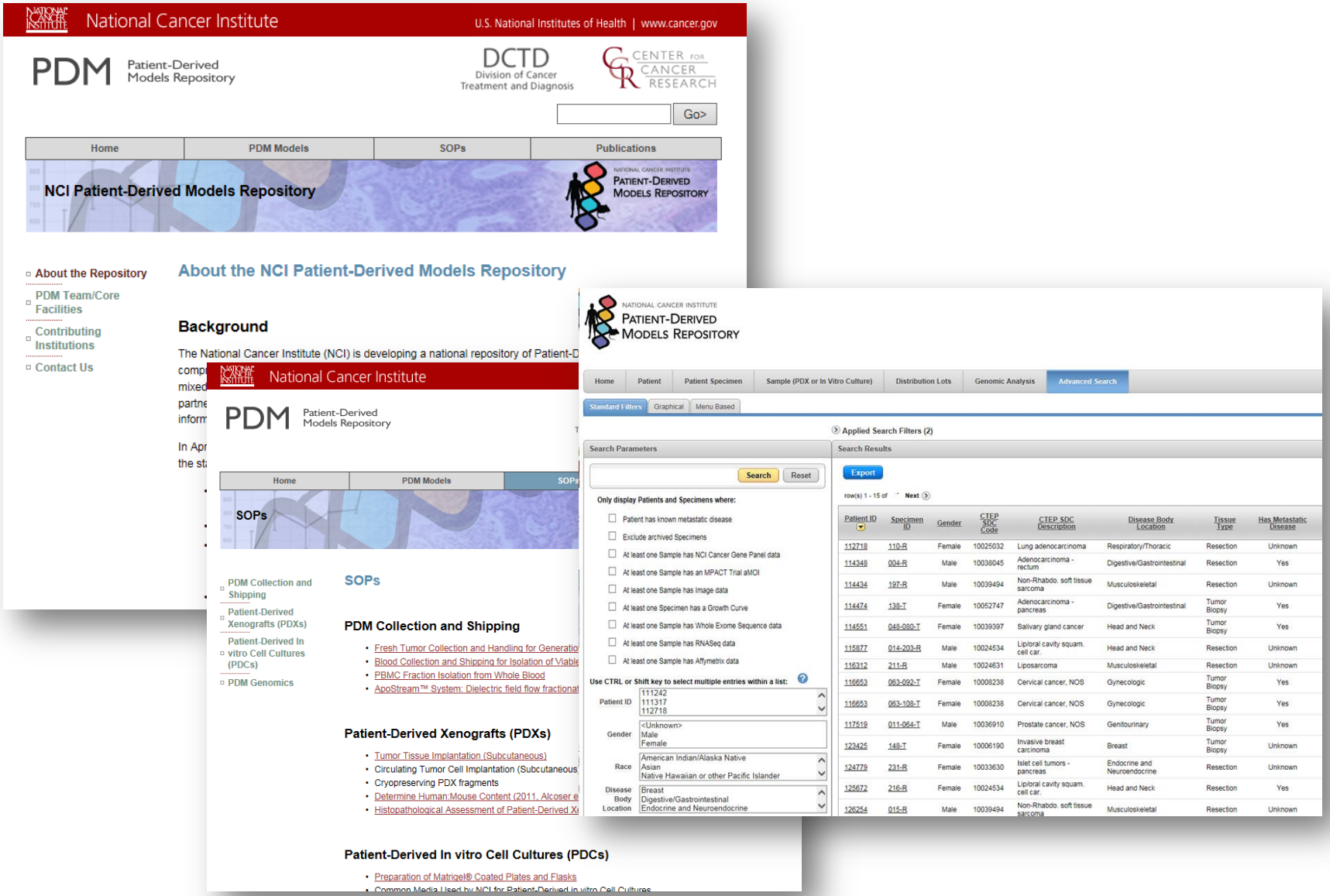
PATIENT-DERIVED MODELS REPOSITORY

NCI Patient-Derived Models Repository



NCI Patient-Derived Models Repository

- Public website with access to: PDM database, patient/model information, list of distributable models, SOPs
- Distribution of models will include: PDXs, conditionally-reprogrammed cell lines, DNA, RNA, whole cell lysates
- Use as core resource in support of extramural SCLC consortium
- Support development of extramural early phase pre-clinical clinical trials consortium
- Novel models to develop immunotherapy combinations and PD, for example in comparative oncology trials
- Support extramural studies that require in vivo use of investigational agents—performed at FNLCR with PI
- Provide all Standard Operating Procedures developed

Collection of Patient Medical Information

Patient Demographics

Gender	Female	**Metastatic Disease**	Yes
CTEP SDC Code	10009951 – Adenocarcinoma – colon	**Date of Diagnosis**	3/16/2010
Sub-type	--	**Age at Diagnosis**	43
Known Mutations	None Reported	**Race/Ethnicity**	Unknown

Current Therapy (at time of tissue/blood collection for PDX)

Date Started	Regimen	Best Response	Duration (Cycles)	Date of Progression/ Off Therapy	Comments	Reason for Off Therapy
5/1/2013	MK-2206, AZD6244	Stable Disease	6	10/29/2013	Adrenal mass unresponsive to study agents all other sites of disease (lung and liver) initially responded.	Disease Progression

Prior Therapies

Date Started	Regimen	Best Response	Duration (Months)	Comments
May-2010	FOLFOX, Bevacizumab	PR	3	
Oct-2010	Bevacizumab, Fluorouracil, Leucovorin	NA	15	
Oct-2010	Oxaliplatin	NA	5	
Mar-2012	FOLFIRI	NA	4	
Aug-2012	AT13387	Non-evaluable	1	
Nov-2012	LMP776	Disease Progression	1	

Specimens Collected

Specimen ID	Biopsy Site	Tissue Type	Growth Curve Available	Archived	Age at Sampling	Collection Date
121-B	Blood	Blood	Yes	No	46	5/2013
121-T	Liver	Tumor Biopsy	Yes	No	46	5/2013
142-T	Liver	Tumor Biopsy	Yes	No	46	5/2013
183-B	Blood	Blood	No	Yes	46	7/2013
288-R	Left adrenal	Resection	Yes	No	46	10/2013

PDX Information

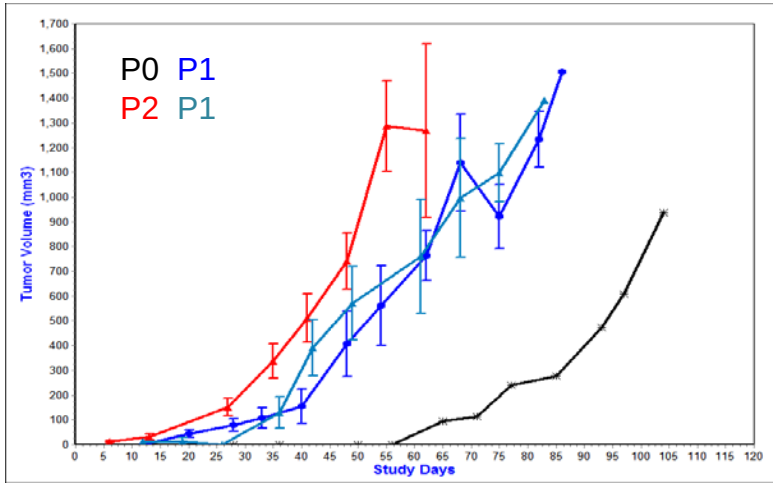
- PDX Growth Curves
- PDX/Patient H&E: incl. %tumor, %stroma, %necrosis, path notes
- NCI Cancer Gene Panel Results
- NGS Files
- Future: Preclinical Trial Results, Whole-Animal Imaging

NCI Cancer Gene Panel Results

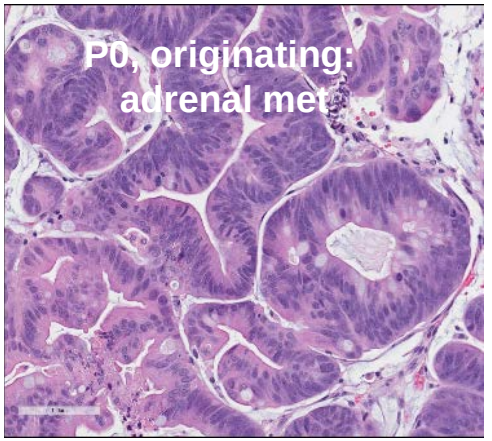
Gene	Pathway	AA Change (canonical transcript)	Cosmic ID	Allele Frequency	Read Depth	Impact	MPACT Trial aMOI
APC		-1535	-	0.79	1998	FRAME_SHIFT	No
KRAS	RAS	G12D	521	0.68	1601	nonsynonymous	Yes
NBN	DNA repair	E185Q	-	0.48	654	nonsynonymous	No
PIK3CA	PI3K	E545K	763	0.50	1102	nonsynonymous	Yes

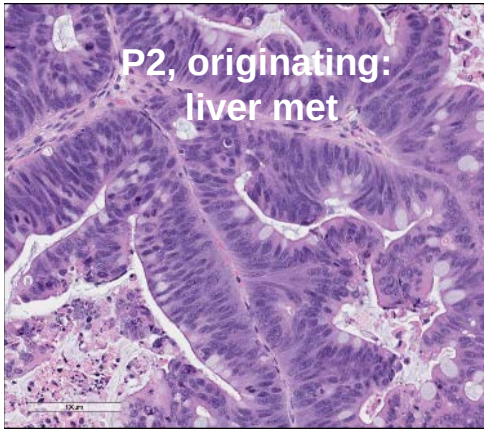
NGS Files

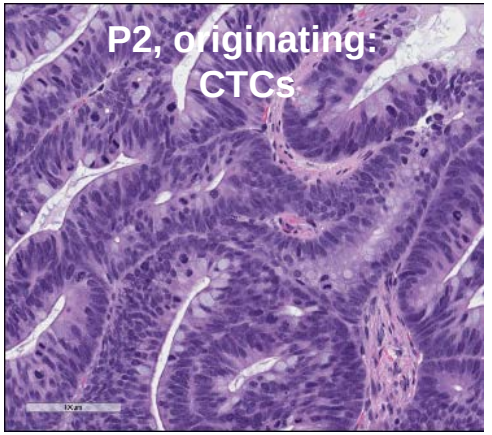
- WES VCF and FASTQ gz files
- RNASeq FASTQ gz files
- RNASeq TPM genes and isoforms tab-delimited files
- Affymetrix Cel files (if available)



Colon Adenocarcinoma







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Lessons Learned

Patient-Derived Xenografts (PDXs)

- Have determined K3-EDTA overnight shipping of blood samples results in loss of long-term viability of CTCs; currently testing Na-heparin collection tubes.
- Enriched CTCs resuspended in rituxan-containing media prior to implantation to minimize white blood cell driven lymphoproliferative disorders (independent of EBV status).
- A media from the shipped tumor tissue is cultured to inform any needed antibiotic/antimycotic treatment of mice. Fluconazole used to treat mice when yeast contamination is found in the tissue culture samples; piperacillin/tazobactam used when bacterial infections are suspected
- Matrigel plug used for all tissue and CTC implantations. Testing pre-culture of CTCs prior to implantation.
- Sarcoma tumor tissue implanted intramuscularly or near MFP to increase take-rate. Estrogen/Testosterone pellets used for all potentially hormone-dependent tumors: breast, ovarian, testicular, prostate
- There have now been several instances where a small sub-population of tumor cells become the primary PDX tumor.
    - For example: Patient enrolled with SCLC, histopathological analysis of patient tissue received indicates SCLC with nests of neuroendocrine cancer, and PDX histopath analysis confirms only neuroendocrine ca outgrowth.

Patient-Derived Cell Cultures (PDCs)

- While it has been reported that Y compound is needed to generate conditionally reprogrammed cancer lines, we have found that it strongly promotes human (and mouse) fibroblast growth resulting in the loss of tumor cells within 2-4 passages.
    - Y compound is now used for initial establishment of cultures from patient material;
    - It is removed in sorted tumor cells to ensure fibroblast contaminants die off; and
    - Y compound is left in fibroblast cultures continuously.
    - Y compound is not essential for establishment of patient-derived tumor cultures
- All Head & Neck, Colon, and Bladder tumor resections are initially cultured in presence of Fungizone until the shipping media is proven to be contaminant free. There is a high incidence of contamination resulting from primary-site tissue resections for these tumor types.
- Fibroblast cultures have a finite life-span and this varies from patient to patient. This results in a limited supply of these companion cells.
- In limited cases, human fibroblasts can be recovered from P0 PDXs.
- Following sorting to >99% purity, tumor cultures can grow out of “pure” fibroblast cultures and vice versa. Monitoring and diligent QC throughout the process are essential.

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Bishu Das
Han Si
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Beverley Teicher
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Larry Rubinstein

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