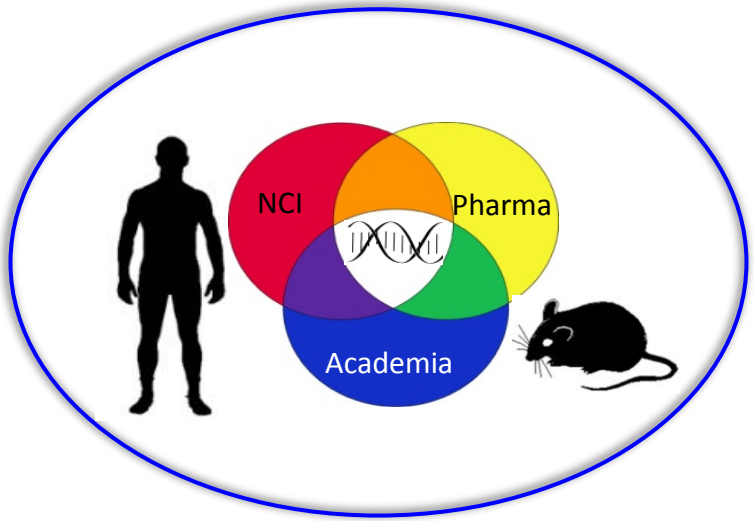
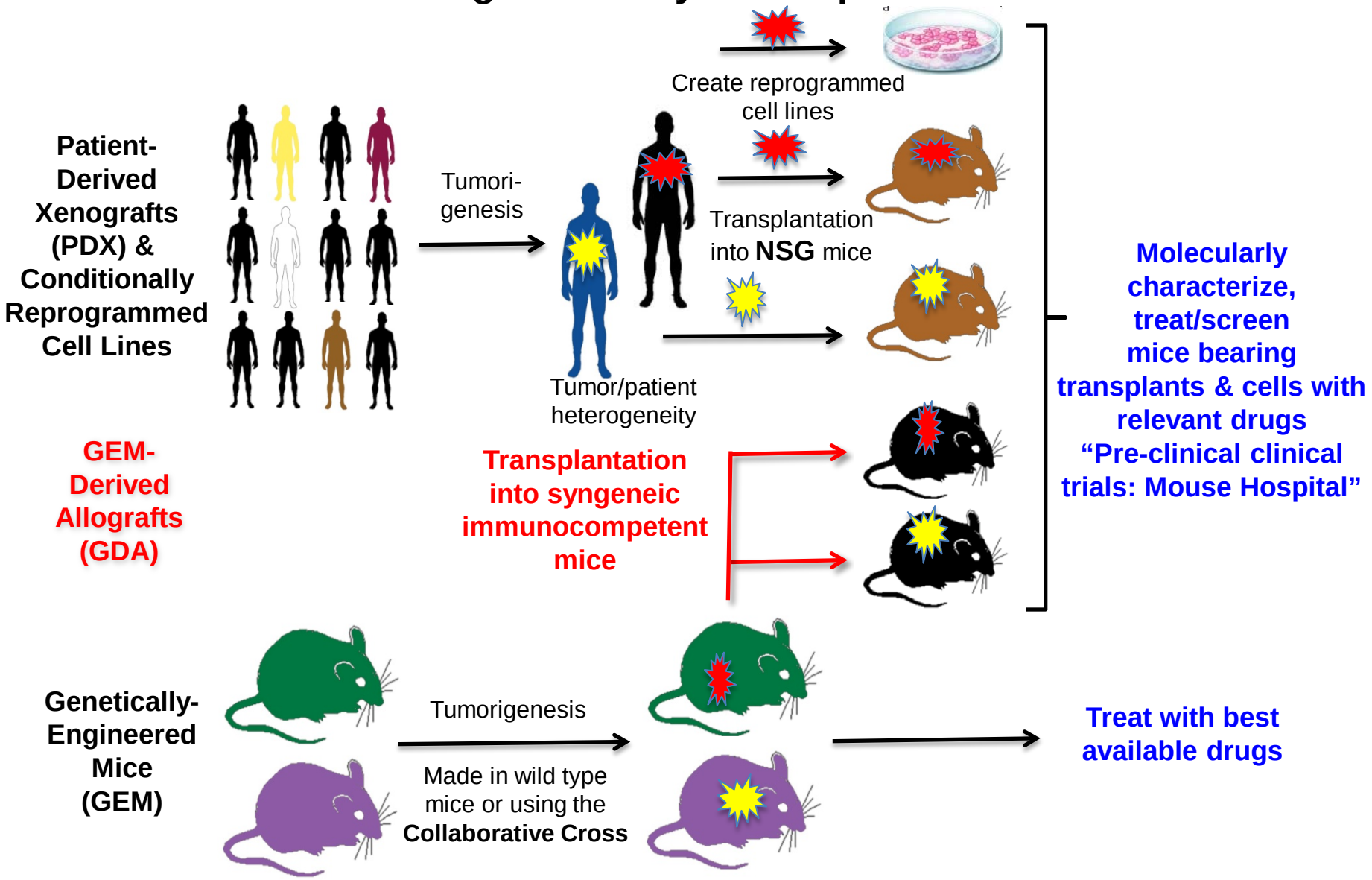
Preclinical Models Repository to Support Cancer Discovery & Therapeutics Development



James H. Doroshow, M.D.
FNL Advisory Board
September 24, 2013

Goal: **Develop a program attractive to academia and industry to create and produce clinically-annotated, patient-derived mouse and cell-based model systems for cancer discovery and therapeutics development in support of extramural investigators**

Emerging Human-Centered Preclinical Strategies in Drug Discovery/Development



Preclinical Models Repository: Why?

- Large expense
  - Drug development failures require improved preclinical models
  - Developing/maintaining patient derived xenograft (PDX) library with clinical and ‘omic annotation
- Need to interrogate models from both primary tumors and metastases, particularly from patients on clinical trials
- Preclinical models for development of therapeutics
  - Critical need to enhance reproducibility and transparency of preclinical data, and to justify (or not) investment in animal models used for preclinical therapeutics
  - Clarify the role of PDXs in target qualification
  - Need for development of models allowing comparative assessment of molecular predictors of drug efficacy: PDXs, conventional xenografts, conditionally-reprogrammed lines, and organoids
  - Establishment of predictive genomic signatures and/or proof of mechanism PD

Goal: Enhance ability of preclinical systems to predict success in the clinic in a timely fashion; facilitate extramural access to annotated models

Preclinical Models Repository: Why NCI?

- Large network of early phase academic trial sites currently funded to supply tumor samples. Over 5000 accruals/year and >100 active INDs
- Clinical annotation of specimens for patients on therapeutic trials are integral to NCI studies
- Facilities at FNL
  - Major facilities exist for animal production,
  - tumor model development,
  - tumor cell repository,
  - genomic characterization of human tumor biopsy specimens,
  - cell line screening

Preclinical Models Repository: What?

- Establish a quality-controlled repository of PDX models at NCI that have undergone detailed molecular characterization (>1000 models—initial goal)
  - Develop models from patients with recurrent disease; include complete clinical annotation; include pre-treatment and at-progression biopsies from patients at NCI-supported clinical trial sites
  - Obtain primary models, usually surgical samples (particularly of rare diseases) from NCI-designated Cancer Centers
  - Obtain established PDX models from Pharma partners, as available
  - Share SOPs for development, monitoring, and maintenance of reproducible models

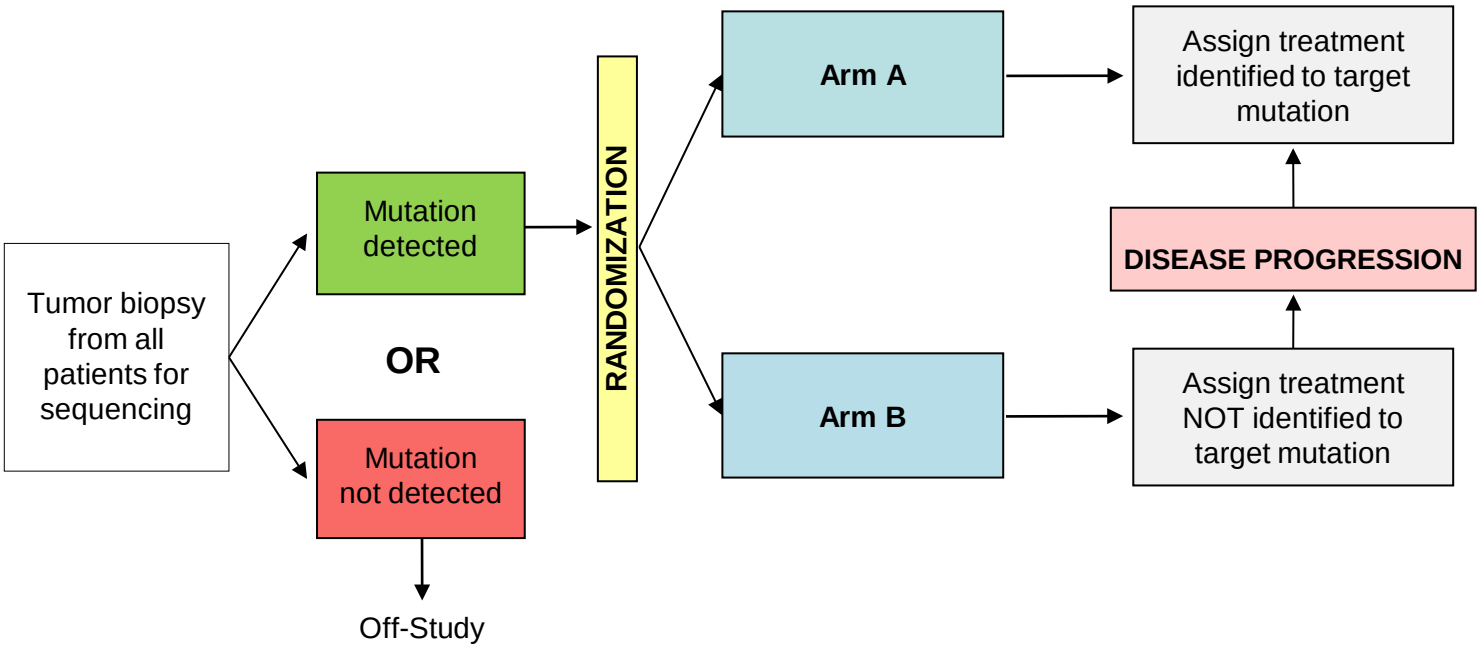
- Co-develop conditionally-reprogrammed lines (tumor and adjacent 'normal' tissue) from same patient samples used for PDX models

- Share molecular characterization data and models with extramural community
  - Molecular characterization data to be made publicly available according to NIH policy
  - Available to all academic and Pharma partners

Patient-Derived Xenograft (PDX) Repository

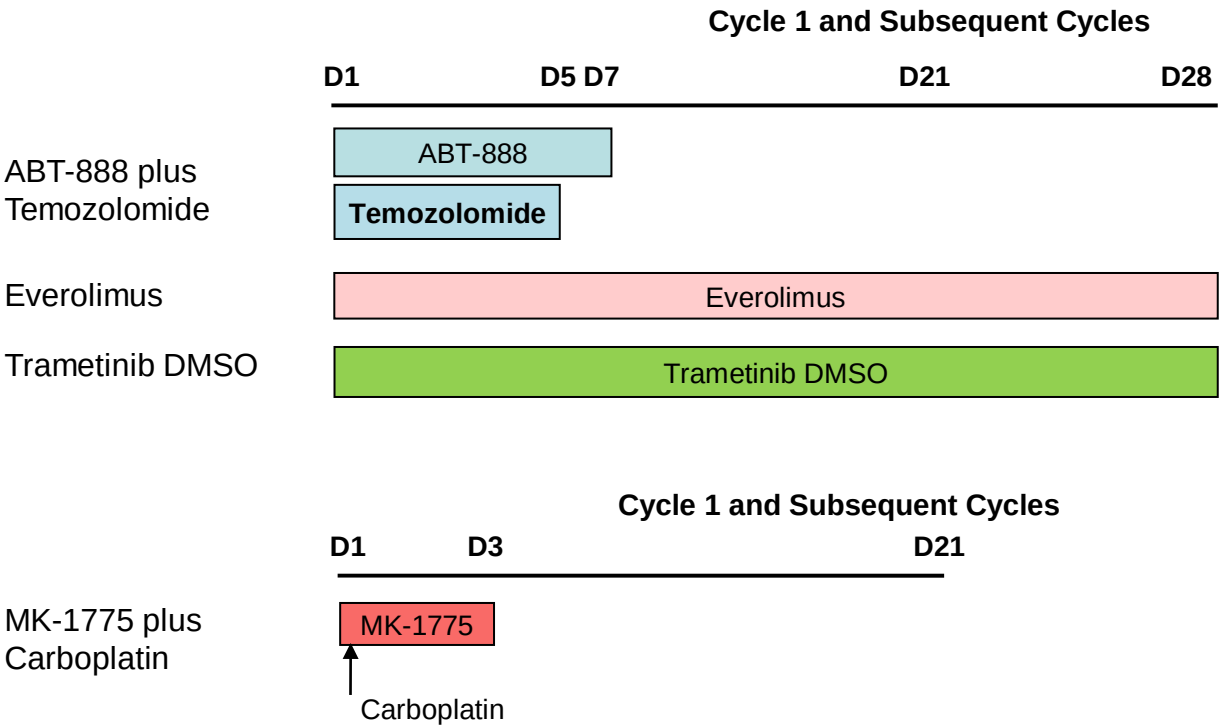
- NCI to provide long-term home for *>400-500 models* produced from primary tissues and blood supplied by *NCI designated Cancer Centers* or already developed at those sites; in negotiation with several *companies* that have indicated their willingness to share models with the NCI; additional *400-500 models* to be developed from *NCI-supported clinical trials*
- Repository size - should include sufficient number of biologically- and clinically-annotated models to reflect genetic diversity and effects of therapy for application in:
  - Target qualification
  - PD assay and predictive marker development
  - 'Preclinical' clinical trials
- Goals
  - >1000 clinically-annotated PDXs with 25% from pre- and post-treatment biopsies from the same patient
  - ~75-100 unique patient samples (solid tumor and tumor lines) per common disease such that the size of each molecularly-characterized subgroup is sufficient to power subsequent validation and/or efficacy studies
  - Comprehensive pre-competitive molecular characterization of samples and earliest passage PDXs where data not available
- Publicly-available repository
  - Molecular information in an easily accessible database
  - PDX models supplied to the extramural community at modest cost
  - Serve as a resource for public-private partnerships and for academic drug discovery efforts
  - Establish extramural group to provide input for optimal use of repository

NCI's M-PACT 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 open across NCI's Phase I/II network (>30 NCI-designated Cancer Centers)
- Accrual expected to begin Q1-2014

13-C-0105 MPACT Clinical Trial



Patients with specified mutations of interest will be assigned to receive **one** of the following study drugs or drug combinations at the assigned dose. Cycle length is +/- 1 day for scheduling:

- **ABT-888** 40 mg orally BID qd days 1-7 plus **temozolomide** 150 mg/m^2 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)

MPACT Assay

4 Drug Protocols, 3 Pathways, 22 Targeted Genes (aMOI)

Pathway	Treatment Protocol	Gain of Function Mutations	Loss of Function Mutations
RAS/RAF/MEK	Trametinib DMSO; MEK Inhibitor	BRAF KRAS NRAS HRAS	NF1
AKT/PI3K	Everolimus; mTor Inhibitor	AKT1 AKT2 AKT3 PIK3CA mTOR	PTEN FBXW7
DNA Repair	ABT-888 + Temozolomide; PARP Inhibitor		ATM ATR ERCC1 MLH1 MSH2 NBN RAD51
	MK-1775 + Carboplatin; Wee1 Inhibitor		PARP1 PARP2 TP53

Tissue Outputs from M-PACT Trial

PDX Models

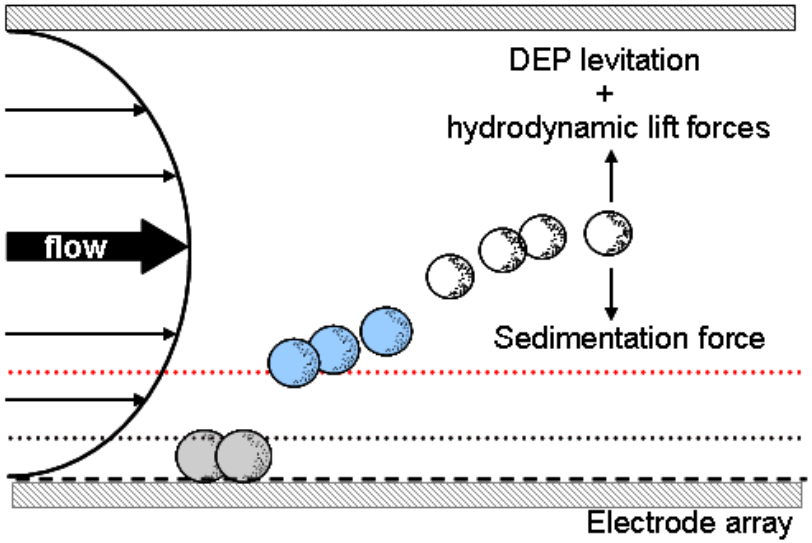
- Prospective collection of ~1000 clinically-annotated *on-study tissue biopsies* from sites of recurrence (not primary tumors), and *blood samples for CTCs* in context of clinical trial
  - Characterize mutational status with CLIA-approved panel
  - Available for whole exome sequencing
- Biopsies at disease progression for patients treated on study (~200 pts) with whole exome analysis
- Both on-study and at progression samples used for establishment of PDX models

Conditionally-Reprogrammed Lines

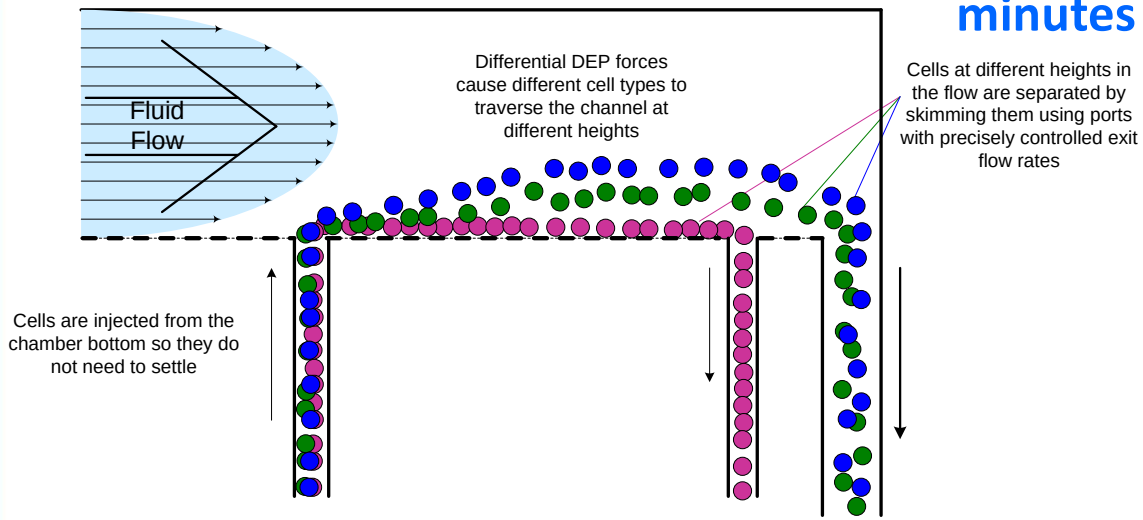
- Biopsies (3 passes) split by pathologist at time of acquisition for:
  - Genomics
  - PDX models
  - Initiation of conditionally-reprogrammed lines (J2 murine fibroblast co-culture with Rho-kinase inhibitor; Am. J. Pathol. 180: 599-607, 2012), and for frozen reserve specimen

National Cancer Institute

Dielectrophoretic Field-Flow Fractionation (DEP-FFF): Viable Circulating Tumor Cell Capture



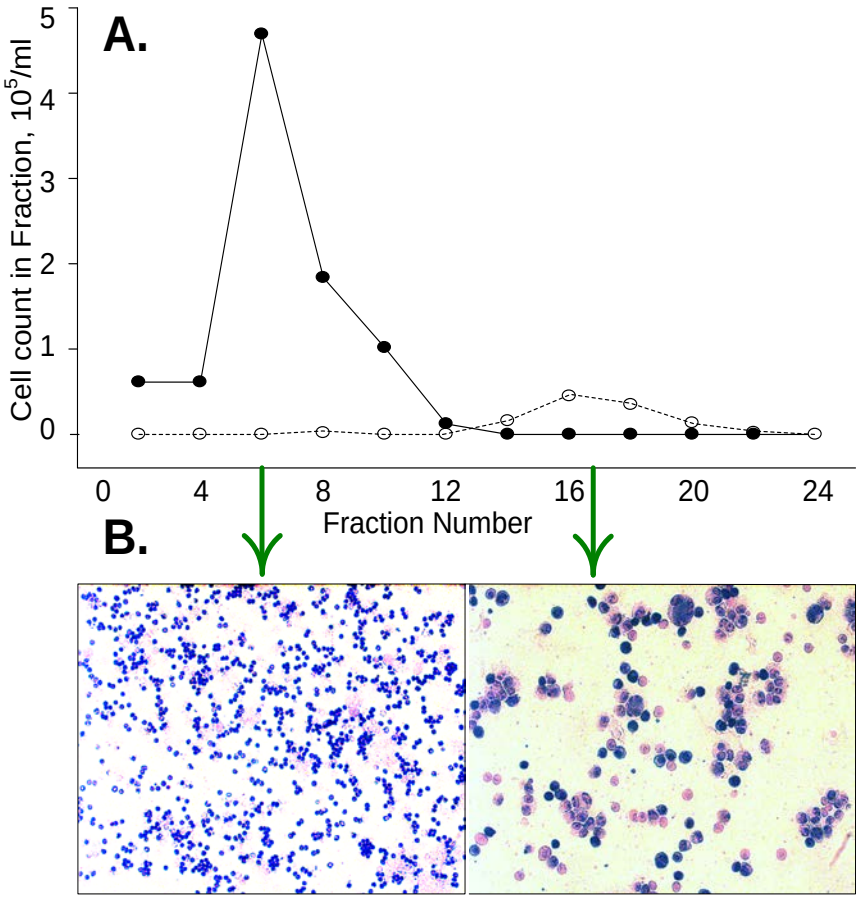
The force balance and levitation of various cells at different heights



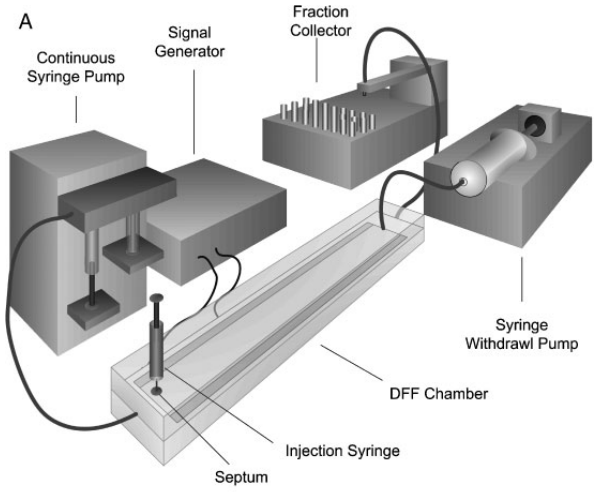
- **DEP-FFF utilizes balance of physical forces in a laminar flow chamber to isolate CTCs from blood cells**
- **Throughput is high compared to other systems; 1 ml of blood can be processed in <30 minutes**

National Cancer Institute

Cancer Cell Separation from Blood Cells using DEP-FFF



- **MDA-435 tumor cells isolated from blood cells with high purity and 2000-fold enrichment as observed by Wright staining**



Gascoyne, P.R., Noshari, J., Anderson, T.J., and Becker, F.F., *Isolation of rare cells from cell mixtures by dielectrophoresis*. Electrophoresis, 2009. 30(8): p. 1388-98.

National Cancer Institute

Specificity of Non-Small Cell Lung Cancer CTC Isolation by DEP-FFF

NSCLC	*CellSearch® Count per 7.5 ml blood*	*DEP-FFF Count (CK+/CD45–/DAPI+) per 7.5 ml blood*
Adenocarcinoma NSCLC 1	0	296
Adenocarcinoma NSCLC 2	5	81
Adenocarcinoma NSCLC 3	0	100
Adenocarcinoma NSCLC 4	0	47
Adenocarcinoma NSCLC 5	not available	461
Adenocarcinoma NSCLC 6	not available	26
Adenocarcinoma NSCLC 7	not available	487
Adenocarcinoma NSCLC 8	not available	10
Adenocarcinoma NSCLC 9	not available	541
median		*100*
range		*10 to 541*
mean		*228*

Normal Donor Blood

	DEP-FFF Count ($CK^+/CD45^-/DAPI^+$) per 7.5 ml blood
Normal Donor Blood 1	2
Normal Donor Blood 2	3

Low number of false-positive $CK^+/CD45^-/DAPI^+$ CTCs are recovered from normal donor blood by DEP-FFF isolation method

- **PDX initiation**
- **Single cell sequencing**
- **Molecular characterization**

PDX Research Challenges

Pros

- patient-derived tumors
- pre & post therapy samples possible
- clinical annotation
- experimental replicates
- some human stroma

Cons

- lacks immunobiology
- mouse/human stroma
- bypasses initiation/progression
- change possible with passage

Conditionally-Reprogrammed Cell Lines ('Georgetown Technique')

Pros

- potential to grow tumor & associated 'normal' tissue from same biopsy samples
- pre & post therapy samples
- clinical annotation
- potential to expand limited tumor resources to allow broader molecular characterization
- amenable to drug sensitivity/resistance testing

Cons

- technology just getting started
- long-term stability of lines unknown
- only preliminary evidence of clinical correlation
- changes in culture possible

PDX Models: Initial Progress

- Tissue/Model Acquisition:
  - 16 NCI-Designated Cancer Centers just reviewed and funded to *each* supply 20 tumor and 20 matched blood samples with clinical annotation in FY14; focus on less common malignancies: small cell lung cancer; head and neck cancer; sarcoma; thymic carcinoma; bladder cancer; melanoma; prostate cancer:

    Total ≈ 300 tumors & 300 blood samples for CTCs
  - Offered existing Cancer Center collections (>25 unique PDX models *each* ovary and breast)

    Total 50 (so far)
  - >300 unique PDXs from Pharma/Biotech: in negotiation
  - ≈ 1000 tumor biopsies from NCI MPACT Trial: starts Q1 2014
- PDX tumors in hand:
  - 27 GBMs
  - 31 Lung (adeno>squamous; 3 sclc's)
  - 7 Bladder
  - 5 Colon
  - 5 Sarcoma
  - 3 Head & Neck
  - 1 NHL
  - Take rate:
    - 70% for 18 gauge needle biopsies
    - 7/21 implants directly from CTCs growing as PDXs

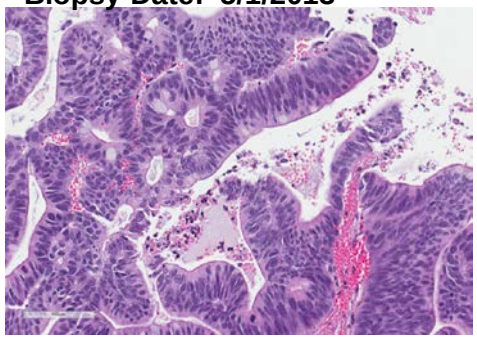
Histology Over Multiple Generations: 172845, Colorectal Cancer

172845-Patient
No H&E
Patient not delinked

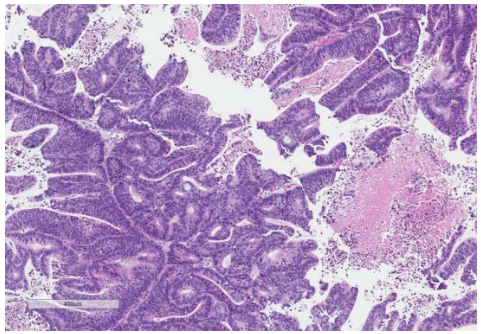
- PDX Sample obtained from DTC clinic (06-C-0213)
- Considered a “PDX tumor model” as a sample has not yet been frozen and re-established in mice
- In vitro cell culture with Rho Kinase inhibitor and fibroblast-conditioned F-media, not on mouse feeder layer.

P0, Biopsy Material

Biopsy Date: 5/1/2013

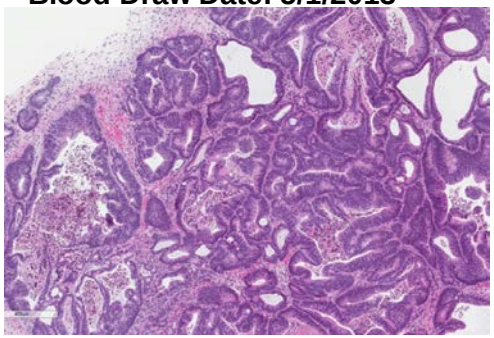


Biopsy Date: 5/22/2013



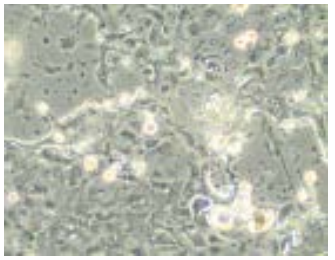
P0, CTC Material

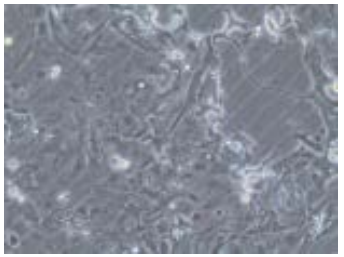
Blood Draw Date: 5/1/2013



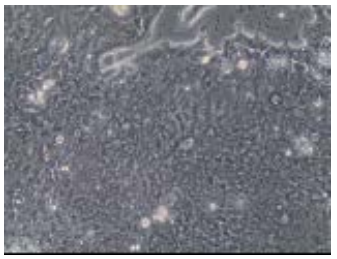
In Vitro Growth: Primary Cell, Mixed Population Cultures

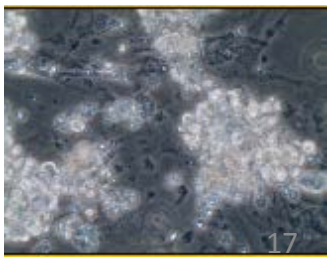
Biopsy Date: 5/1/2013





Blood Draw Date: 5/1/2013

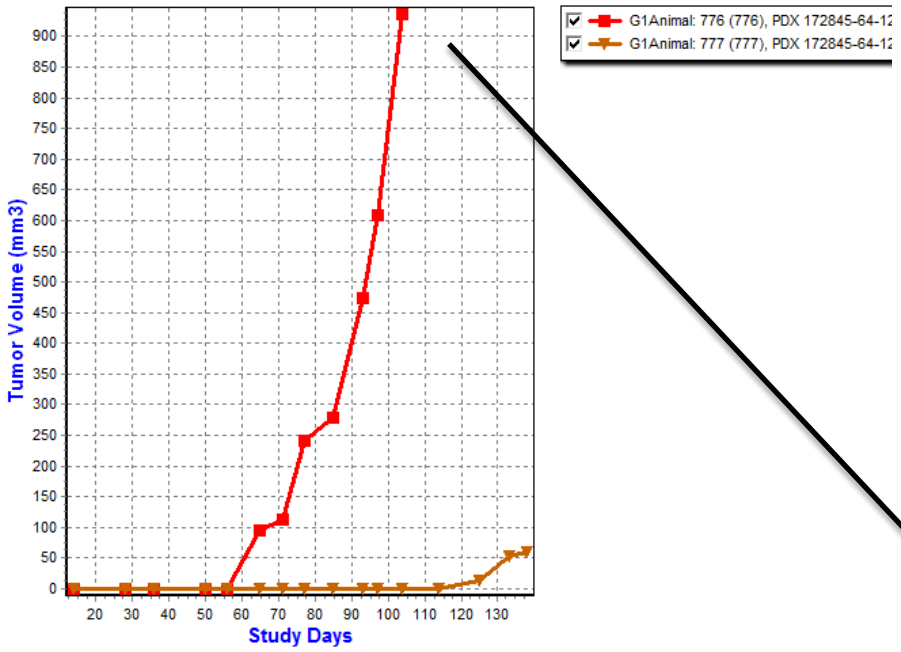




Growth Over Multiple Generations: 172845, Colorectal Cancer

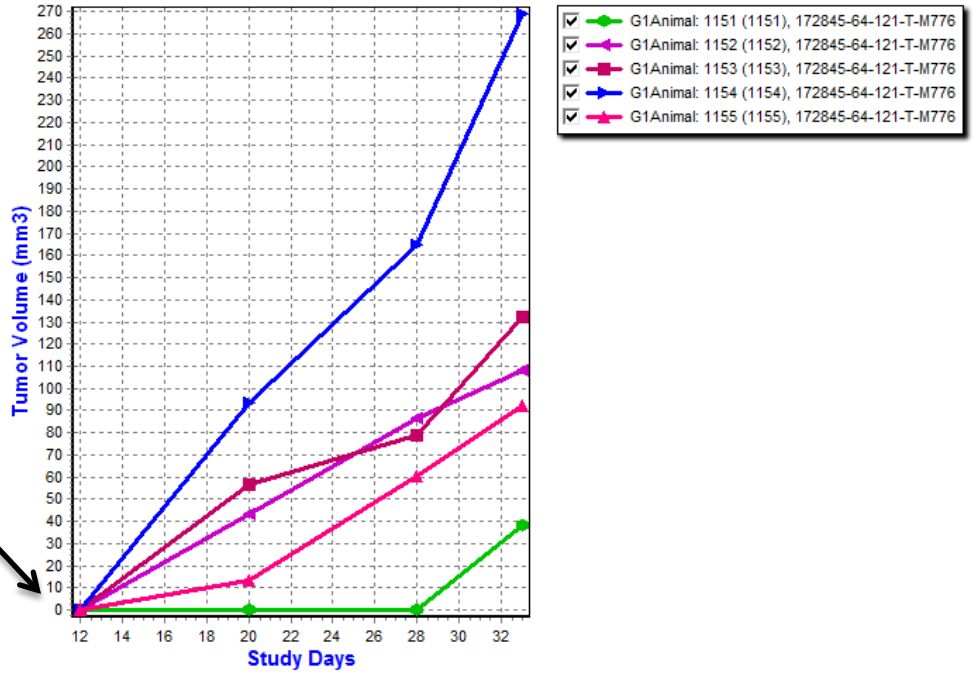
P0

Study Number: PDX 172845-64-121-T



P1

Study Number: PDX 172845-64-121-T-M776



MPACT Assay v1.0 Gene Panel

63 Gene Panel

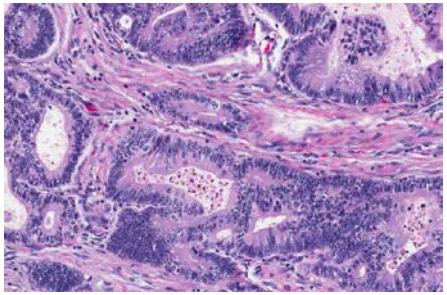
ABL1	ERBB2	IL3RA	NPM1	STK11
AKT1	ERBB4	JAK2	NRAS	TP53
AKT2	ERCC1	JAK3	PARP1	VHL
AKT3	FBXW7	KDR	PARP2	
ALK	FGFR1	KIT	PDGFRA	
APC	FGFR2	KRAS	PIK3CA	
ARHGAP5	FGFR3	MET	PTEN	
ATM	FLT3	MGMT	PTPN11	
ATR	GABRA6	MLH1	RAD51	
BRAF	GABRG2	MPL	RB1	
CDH1	GNAQ	MSH2	RET	
CDKN2A	GNAS	mTOR	SMAD4	
CSF1R	HNF1A	NBN	SMARCB1	
CTNNB1	HRAS	NF1	SMO	
EGFR	IDH1	NOTCH1	SRC	

Actionable Mutations for the MPACT Trial

Pathways: RAS/RAF/MEK AKT/PI3K DNA Repair

Histology Over Multiple Generations: CN0330F, Colon Cancer

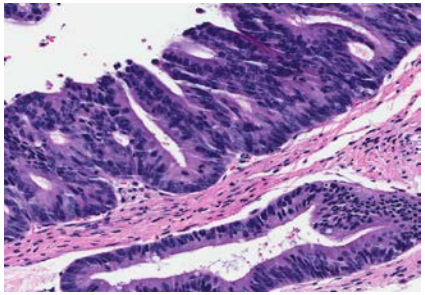
CN0330F-Patient



- PDX Sample obtained from Jackson Laboratories (P1). NCI generations start with P2
- Considered a "PDX xenoline" as tumor material was frozen and the ability to establish a new tumor was seen with P2
- MPACT aMOI include AKT-1 (E17K) and KRAS (G13D) gain of function mutations. Mutations also called through Jackson Lab ONCARTA screen

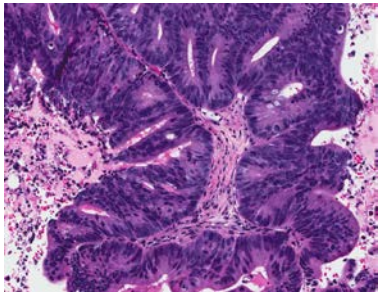
P0

CN0330F-216P0



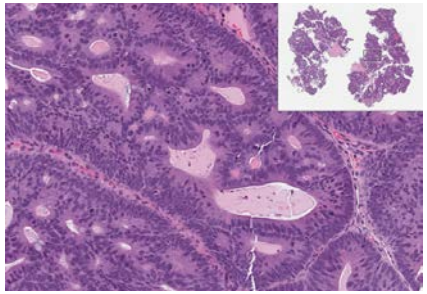
P1

CN0330F-216P1



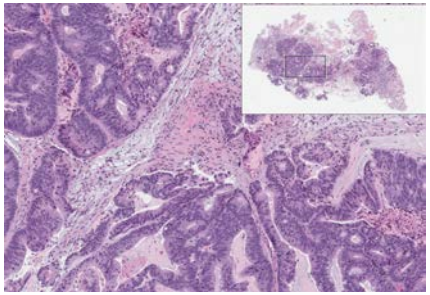
P2

CN0330F-216M517



P3 (4th mouse generation)

CN0330F-216M519M102



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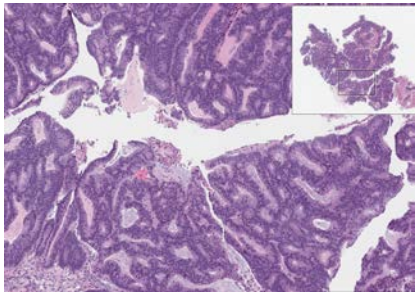

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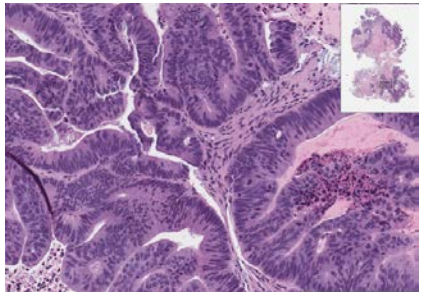


Variability Across 3 Generations : BL0269 (Bladder Cancer)

- High degree of overall gene expression similarity between BL0269F-402M601 initial in vivo passage (P1) at NCI, P2, and P3
  - One P3 tumor (of 6) began to over-express members of the GAGE/MAGE and SSX gene families located on the X-chromosome. Both of these gene families have been previously linked to increased aggressiveness or drug resistance
  - Other X-linked genes do not show similar increased expression levels in M251's gene expression profile, suggesting its unique gene expression is not due to grand chromosome level abnormalities (ie duplications)

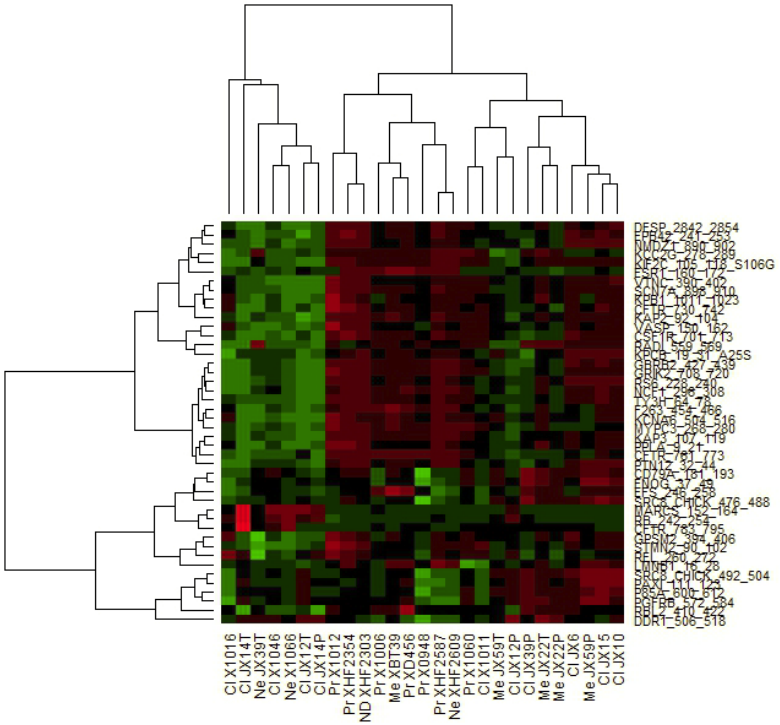
- If the tumors from the BL0269F-402M601-M633-M251 lineage are to be used in further experiments, the altered gene expression of GAGE/MAGE and SSX fusion genes verified by protein blots and drug resistance tendencies should be monitored

Glioblastoma PDX Project (UAB)

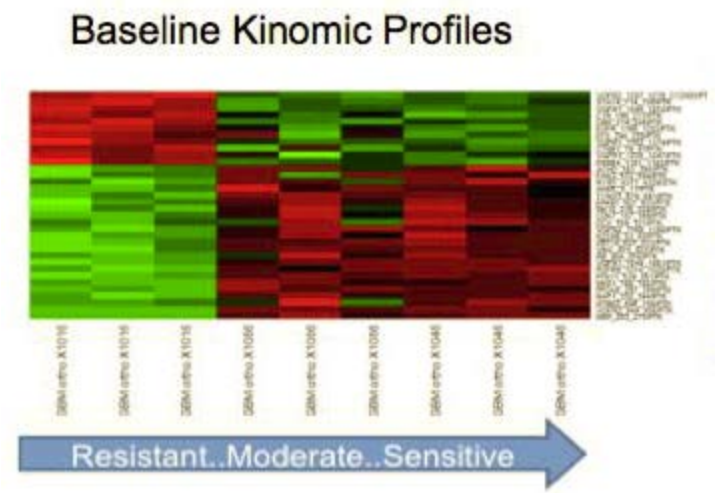
PDX ID	Diagnosis	Passages Received	Original Date Implanted	TMZ sensitivity, flank xenografts
GBM-X12P	GBM, Classical	p4,5, 6, 8, 9, 10, 15	6/24/2010	Sensitive
GBM-X12T	GBM, Classical	p10, 13, 15, 16, 17,18	6/23/2010	Resistant
GBM-X39P	GBM, Classical	p22	8/10/2010	Sensitive
GBMX39T	GBM, Neural	p20, 22	7/20/2010	Resistant
GBM-X14P	GBM, Classical	p10, 15	7/22/2010	Resistant
GBM-X14T	GBM, Classical	p15	9/1/2010	Sensitive
GBM-X22P	GBM, Mesenchymal	p18	8/22/2010	Sensitive
GBM-X59T	GBM, Mesenchymal	p17, 19, 20	8/19/2010	Resistant
GBM-X1066; 50601066	GBM, Neural	p1,2,3,4,5,15,17, 19, 20	8/31/2010	Sensitive
GBM-X1016; 60201016	GBM, Classical	p1,2,4,5,6,20	9/8/2010	ND
GBM-X1012	GBM, Proneural	p1,2,5,6	9/2/2010	ND
GBM-X6	GBM, Classical	p4,5,6,7,8,17	12/13/2010	Resistant
GBM-X10	GBM, Classical	p7,8,10,11,13,15	11/9/2010	Resistant
GBM-X15	GBM, Classical	p14,15,18	12/10/2010	Sensitive
GBM-X1011; 90301011	GBM, Classical	p2	1/20/2011	ND
GBM-X0948	GBM, Proneural	p1	1/31/2011	ND
GBM-X1006; 100301006	GBM, Proneural	p1,3,4,6	10/22/2010	ND
GBM-XBT39	GBM, Mesenchymal	p7,8	3/15/2011	ND
GBM-X1060; 101201060	GBM, Proneural	p2	3/31/2011	ND
GBM-X59P	GBM, Mesenchymal	p4,7	11/4/2010	Sensitive
GBM-X1046; 50501046	GBM, Classical	p2,4,3,5,6,18	8/18/2010	ND

Aliquots over several passages currently undergoing testing for transmissible spongiform encephalopathies (TSEs, aka prion disease) before growth in mice at FNLCR is initiated. Analysis being performed through collaboration with Andy Hughson (NIH/NIAID Rocky Mountain Laboratories).

Glioblastoma PDX Project (UAB)



Kinomic profiling of xenolines: 27 GBM tumors PTK and STK PamChip kinomic profiles were performed and the most highly variable substrates (var>0.3) were selected for hierarchical clustering displayed as a heatmap with phosphosubstrates indicated on the Y-axis and GBM xenoline name on the X-axis.



Heatmap of significantly different phospho-substrates from three different GBM PDXs with resistance, intermediate response or sensitivity to a specific JAK2 small molecule inhibitor.

Project Deliverables

Technology/Tools

- Together, develop a repository of reliable and relevant graft (PDX) models, methods, and SOPs optimized to inform future clinical trials. Bank tissues, fluids, and nucleic acids for future-use
- Tissue biomarker validation
- Clinical/preclinical database with positive ***and*** negative data

Preclinical Science

- Develop consensus with Cancer Centers and Pharma on the value of new preclinical models in directing successful clinical trial designs
- Genomic and network analysis of PDXs at baseline and following therapy to identify new targets
- Development of conditionally-reprogrammed lines from biopsies and PDXs

Patient-Derived Xenograft (PDX) Repository

Estimate for Development of 500 models; Tumor Fragment Origin

(Direct Costs Initial18-24 Months)

	Minimal Work-up	Full Work-up
Tissue Acquisition (per biopsy)	$3000	$3000
3 Mice/tumor fragment: passage 1	$120	$120
Identifiler (genetic confirmation of origin)	$135	$135
Master freeze (10 vials)	$15	$15
Labor	$180	$180
Whole exome sequencing; MPACT		$1,350
Histopathology	$75	$75
Human pathogen testing		$500
5 Mice/PDX: passage 2	$200	$200
Identifiler	$45	$ 45
Vial freeze (80 vials)	$120	$120
Labor	$360	$360
MPACT Panel & Whole exome sequencing; microarray?		$1,350
Histopathology	$75	$75
Mouse pathogen testing		$500
Estimate for 1 PDX Model	$4,325	$8,025
Approximate NCI Initial Investment (500 Models)	$2,162,500	$4,012,500

Summary

- Develop a PDX repository to support a national program to provide clinically-annotated, molecularly-characterized PDX models in multiple tumor types to extramural investigators
- Intellectual input from academia and Pharma colleagues
- Obtain both solid and liquid tissues primarily from NCI-designated Cancer Centers and NCI intramural clinics for new models; and currently-available PDX models from: Pharma/Biotech and Cancer Centers
- Ensure access to models and baseline molecular and clinical data for extramural community
- Longer-term goals:
  - Compare PDX, conditionally-reprogrammed lines, and other new culture systems to current xenograft models to optimize drug development across several histologies/genotypes
  - Understand mechanisms of drug resistance in patients using multiple model systems that allow comparison with actual clinical trial outcomes

Acknowledgements

Harold Varmus

Edward Harlow

Terry van Dyke

Robert Wiltrout

Joseph E. Tomaszewski

Sheila Prindiville

Raymond Petryshyn

Melinda Hollingshead

Glenn Merlino

Zoe Weaver-Ohler

Serguei Kozlov

Linda Weiss