

Biologically-Coordinated Clinical Trials

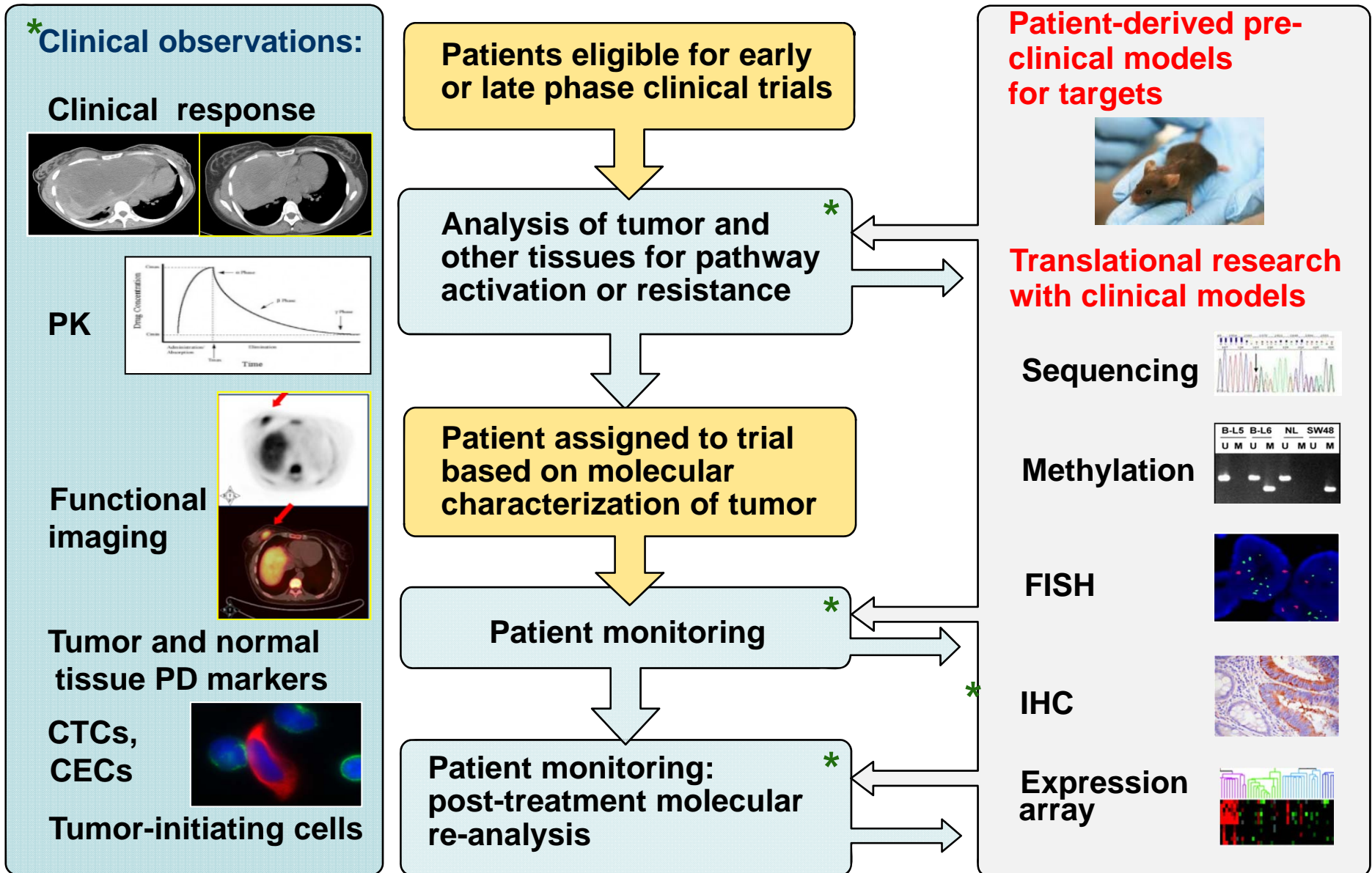
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Human Tumor Tissue Based Experimental Therapeutics



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; 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

M-PACT: Molecular Profilng based Assignment of Cancer Therapeutics

Pilot Trial to Assess the Utility of Genetic
Sequencing to Determine Therapy and
Improve Patient Outcome in Early Phase
Trials

NCI-Sponsored Clinical Trial

Objective

- Assess whether the response rate (CR+PR) and/or 4-month PFS is improved following treatment with agents chosen based on the presence of specific mutations in patient tumors.
 - Only patients with pre-defined mutations of interest will be eligible
 - Study treatments, regardless of cohort, will be chosen from the list of regimens defined in the protocol
 - Arm A: Receive treatment based on an study agent prospectively identified to work on that mutation/pathway
 - Arm B: Receive treatment with one of the study agents in the complementary set (identified to not work on one of the detected mutations/pathways)

Patient Population

- Patients with refractory solid tumors that have progressed on at least one line of standard therapy or for which no standard treatment is available that has been shown to improve survival.
- Adequate organ function (AST/ALT<3xULN, Bil < 1.5 xULN, S. Cr < 1.5 x ULN, platelets > 100K, ANC> 1500)
- Study regimens: As long as the same set of protocols are offered to a given set of patients, the number and actual treatments regimens can vary over time

Mutations in DNA repair pathways	Veliparib+ Temozolomide MK1775 + carboplatin
Mutations in the PI3K pathway; loss of PTEN, Akt amplification	mTOR inhibitor -Everolimus
Mutations in the RAS pathway	GSK 1120212 (MEK inhibitor)

Tumor Biopsy Assay Pre-Analytcs

- Fresh tumor biopsies (3 passes) will be obtained: (2) fixed in formalin; (1) not fixed (for PDXs). Routine diagnostic formalin-fixed paraffin-embedded (FFPE) tissue will be used for sequencing
- Adjacent section will be H&E stained, examined by pathologist for tumor content, % necrosis, inflammation, and scanned into high resolution image database
- RNA and DNA will be extracted from the same tissue section
- ~50ng of DNA will be used for targeted sequencing
 - If sufficient DNA is available, whole-exome sequencing will be performed for research purposes once patient is off-study and identifiable data has been unlinked.
- RNA will be available for gene expression profiling by either whole-transcriptome or miRNA microarray analysis

MPACT Assay

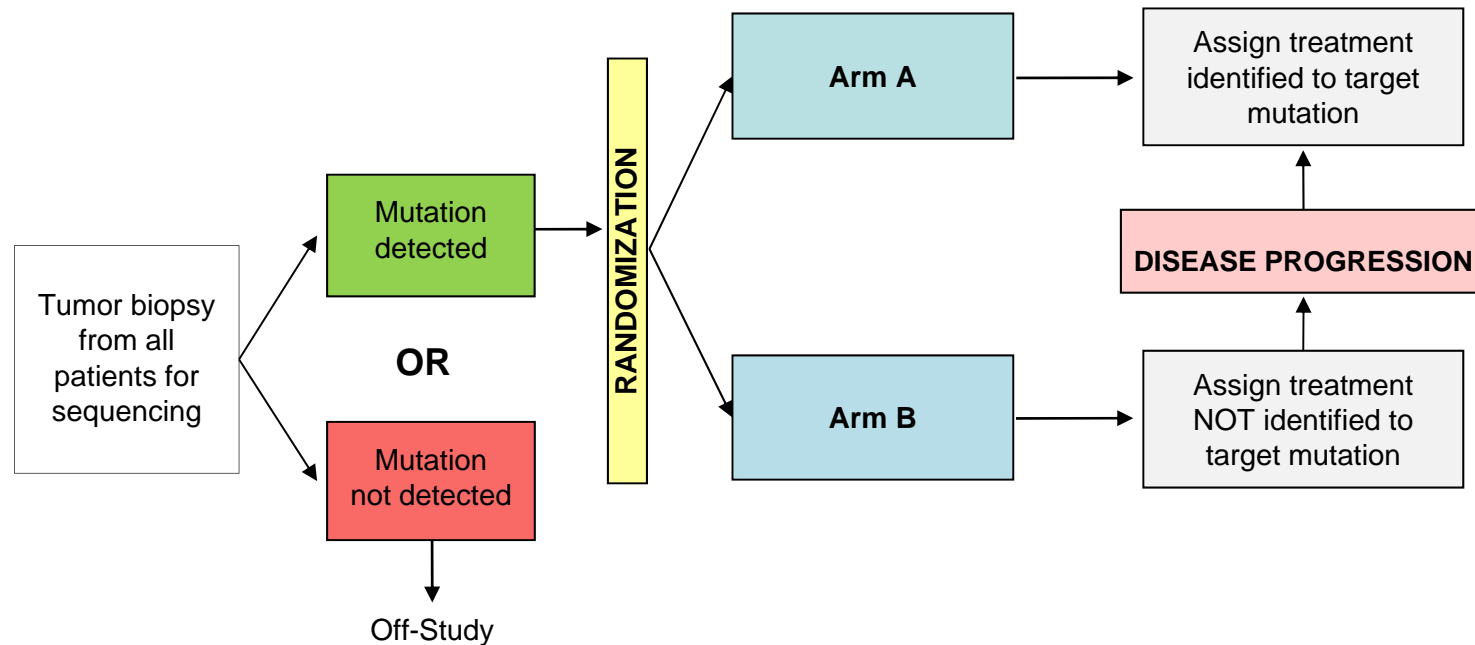


4 Drug Protocols, 3 Pathways, 22 Targeted Genes (aMOI)

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



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 to open across NCI's Phase I/II network (>30 NCI-designated Cancer Centers)
- IND approved by FDA 12/2013; accrual began January 2014 (3 pts accrued first week)



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

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

Preclinical MPACT Trial Design: For Each Pt.-Derived Model

Group	# Mice	NSC	Agent Name	Dose	Schedule
1	15	Klucel	Vehicle Control	0.1 ml/10 gm	QDx28
2	15	733504	Everolimus	1.94 mg/kg	QDx28
3	15	10% Cremophor/10% PEG 400	Vehicle Control	0.1 ml/10 gm	QDx28
4	15	758246	Trametinib	0.39 mg/kg	QDx28
5	15	12.6 gm Sorbitol and 0.62 gm Citric Acid Monohydrate per 120 ml Distilled Water	Vehicle Control	0.1 ml/10 gm	BIDx14, repeat every 4 weeks
		Klucel	Vehicle Control	0.1 ml/10 gm	QDx5 repeat every 4 weeks
6	15	752840	ABT-888	7.75 mg/kg	BIDx14, repeat every 4 weeks
		362856	Temozolomide	50 mg/kg	QDx5 repeat every 4 weeks (dose 2 hr after AM dose of NSC 752840)
7	15	Klucel	Vehicle Control	0.1 ml/10 gm	BIDx5, repeat every 3 weeks
		Water	Vehicle Control	0.1 ml/10 gm	Q21D
8	15	754352	MK-1775	20 mg/kg	BIDx5, repeat every 3 weeks
		241240	Carboplatin	80 mg/kg	Q21D (give 2 hr after first dose of MK-1775 in each cycle)

8 Study Groups: 4 therapeutic arms and 4 matched control groups

Preclinical MPACT Study Tumor Characteristics and Patient-Model Enrollment

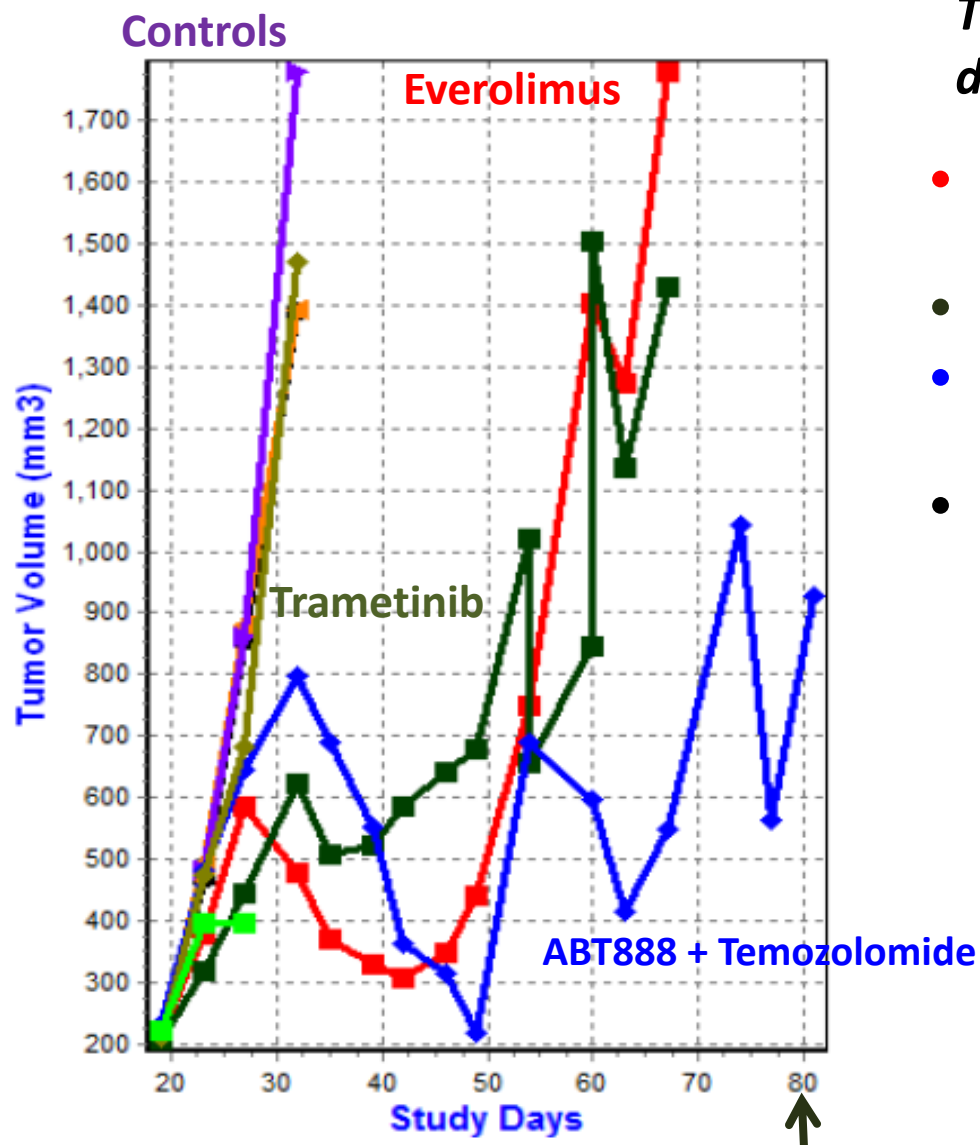
Model	Clinical Dx	MPACT aMOI	MPACT Treatment
BL0382F-1232	Bladder	TP53 Loss	MK-1775 + Carbo
BL0293F-563	Bladder	TP53 Loss	MK-1775 + Carbo
BL0269F-402	Bladder	PIK3CA Gain	Everolimus
BL0381F-1219	Lymphoma (EBV+)	No aMOI	N/A
CN0330F-1216	Colon adenocarcinoma	AKT1 Gain	Everolimus
"		KRAS Gain	Trametinib DMSO
172845-64-121-T	Colon adenocarcinoma	PIK3C Gain	Everolimus
"		KRAS Gain	Trametinib DMSO
172845-64-121-B	Colon adenocarcinoma	PIK3C Gain	Everolimus
"		KRAS Gain	Trametinib DMSO
114551-48-80-T	Acinic Salivary Gland tumor	TBD	TBD

Planned Analysis

- Growth Curves for all 8 Study Groups for all models enrolled
- MPACT Mutation Assay: Day 0 tumor and when >300mg tumor
- Gene Expression (Affymetrix): Day 0 tumor and on-drug biopsies
- H&E of Day 0 and >300mg tumors and End of Study
 - Will be used by Molecular Characterization Lab to select which specimens to process for enrichment, histology, sequencing, RNA analysis, etc
 - Samples for PD before and during treatment
- DNA and RNA will be harvested from all tumors and biopsies
- Whenever whole tumor is collected, extra tissue pieces will be cryopreserved in case additional analysis is warranted

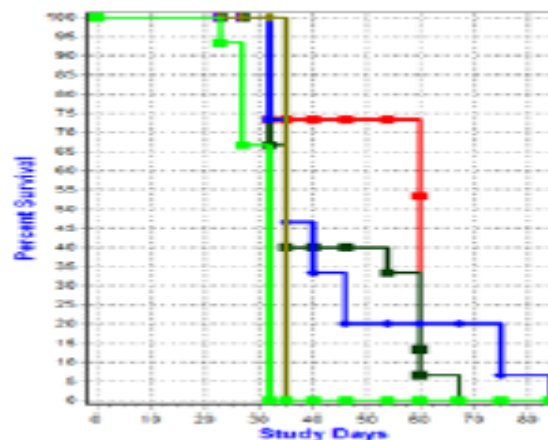
Mouse Pt.-Model No. 1- BL0382F1232, Bladder

Study Number: ZABR2-1



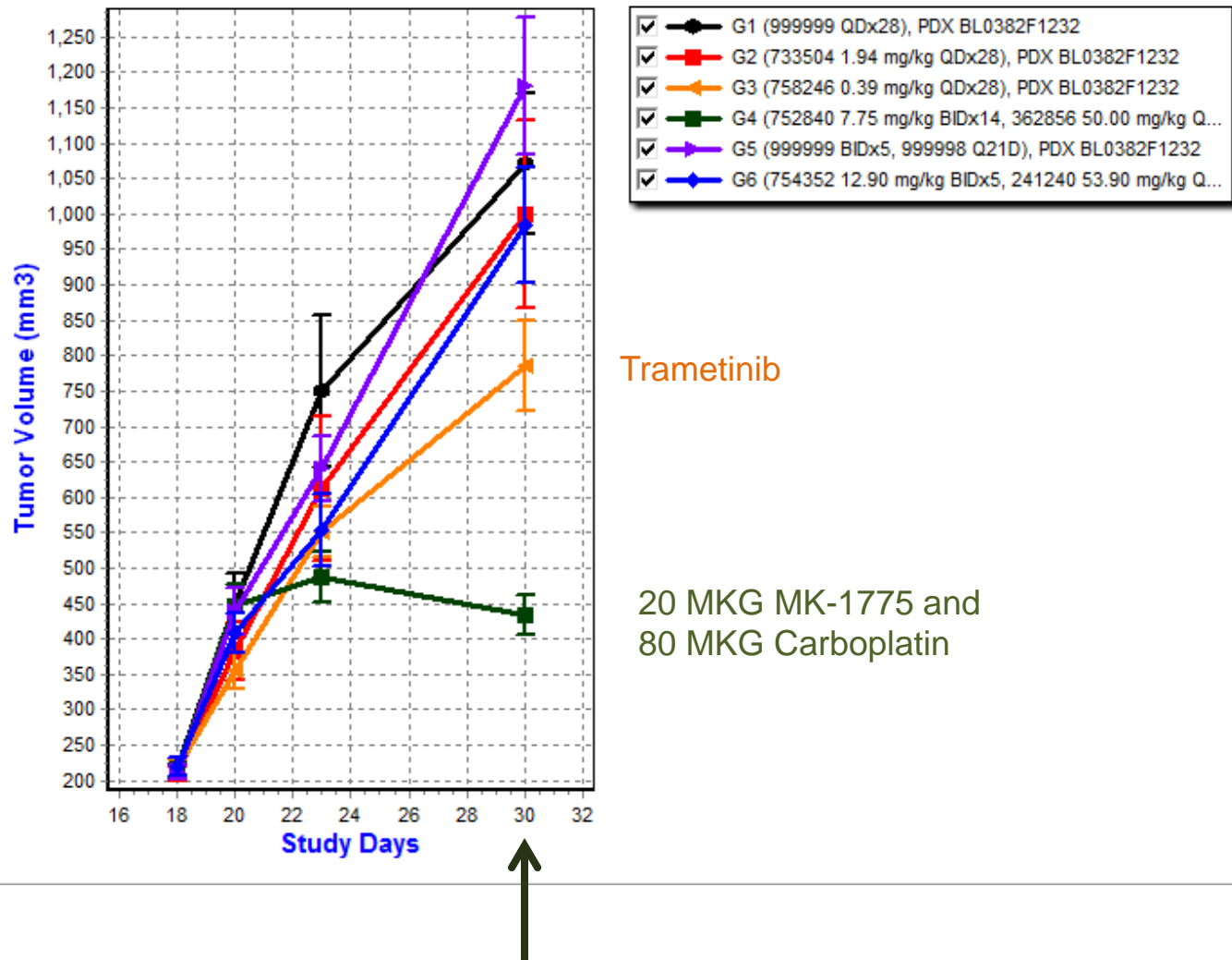
*TP53_E336**, 100%, Tetramer domain, MK17775, Wee1 inhibitor

- **Everolimus (daily) – delayed response then progression**
- Trametinib (daily) – < PR
- **ABT888 + Temozolomide – delayed objective response then progression**
- MK1775 + Carboplatin – treatment with reduced dose in progress

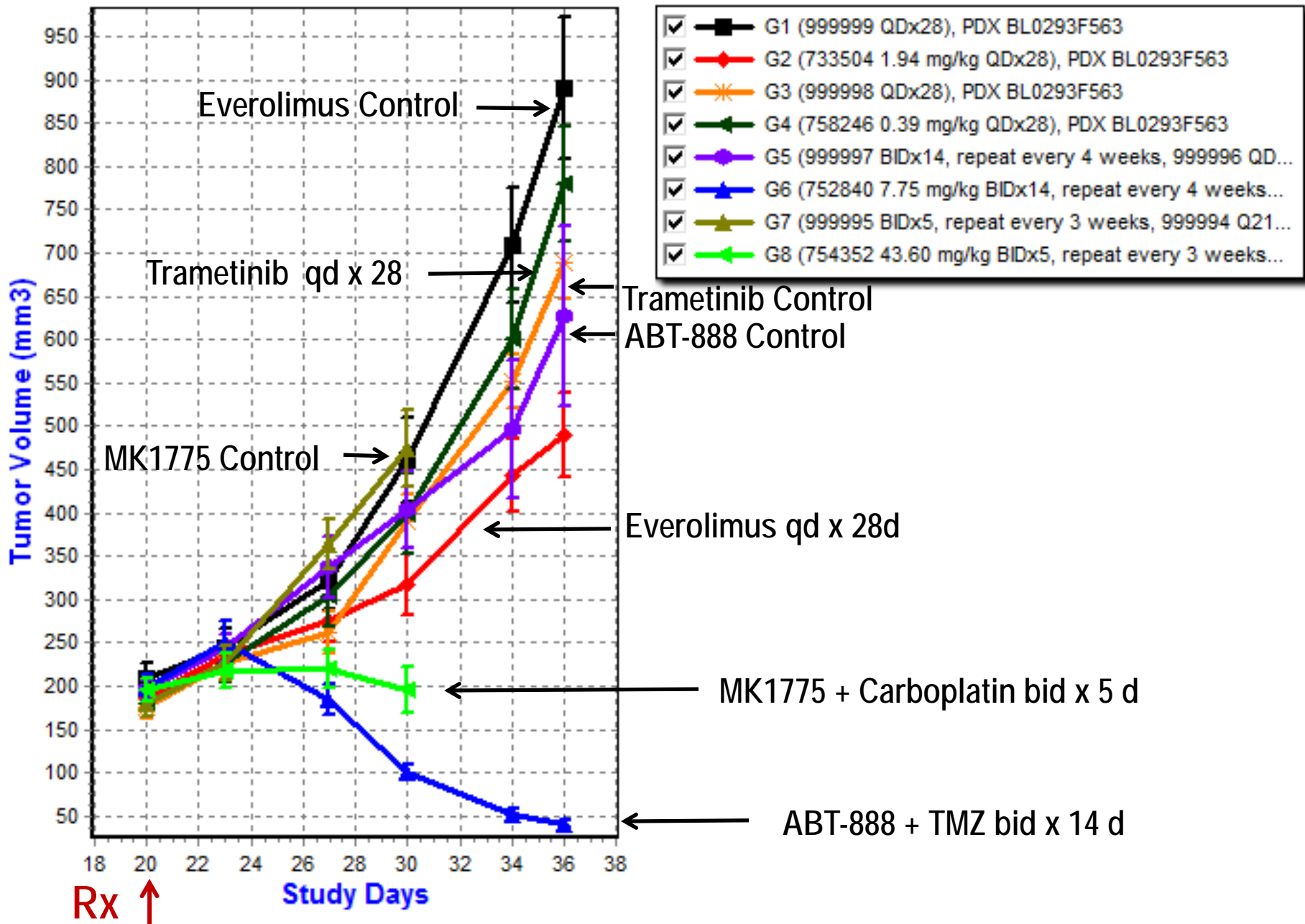


BL0382, Bladder, TP53 Loss: Repeat at Lower Carbo/MK-1775

Study Number: ZABR2-2

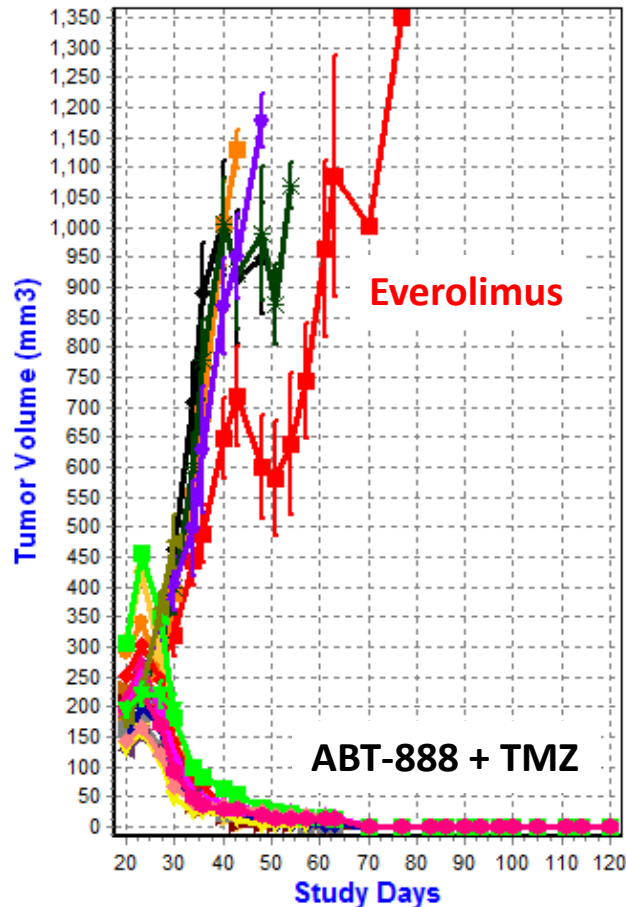


Mouse Pt.-Model No. 2: BL0293, Bladder, TP53 Loss



Mouse Pt.-Model No. 2: **BL0293**, Bladder, TP53 Loss, Longer Follow-Up

Study Number: ZADR2-1

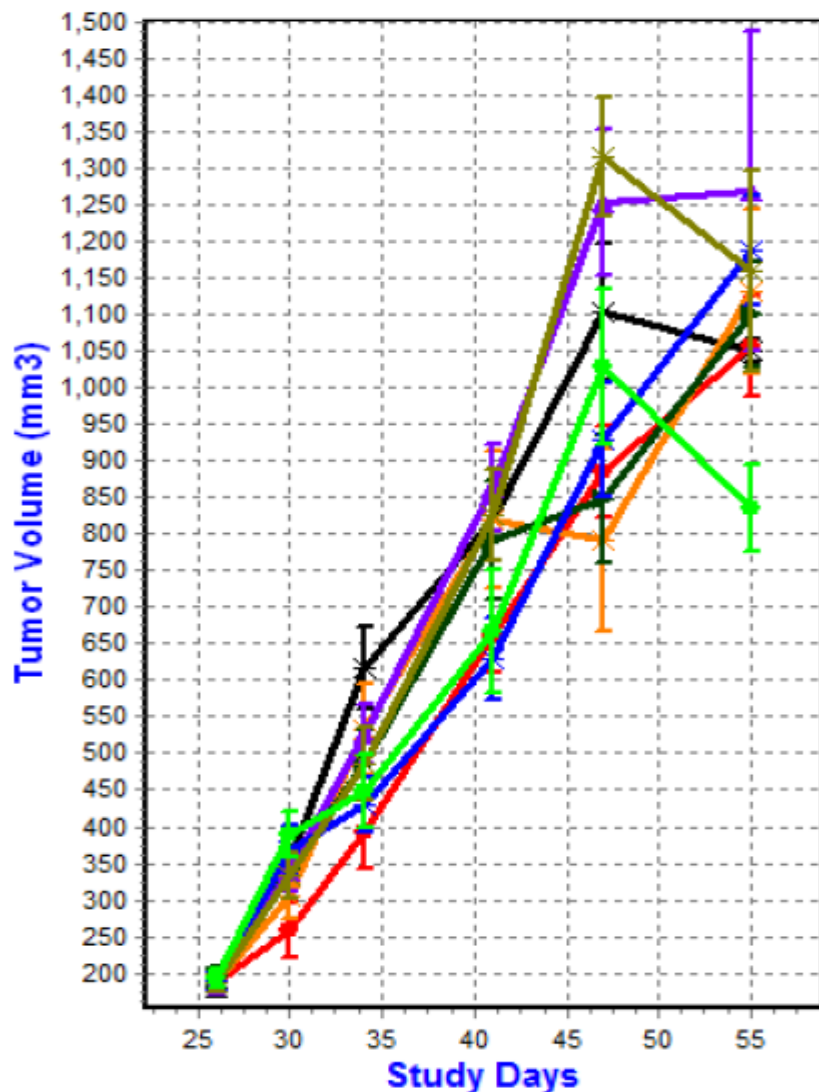


- G1 (999999 QDx28), PDX BL0293F563
- G2 (733504 1.94 mg/kg QDx28), PDX BL0293F563
- G3 (999998 QDx28), PDX BL0293F563
- G4 (758246 3.90 mg/kg QDx28), PDX BL0293F563
- G5 (999997 BIDx14, repeat every 4 weeks, 999996 QD...
- G6 (752840 7.75 mg/kg BIDx14, repeat every 4 weeks...
- G6Animal: 705 (705), PDX BL0293F563
- G6Animal: 716 (716), PDX BL0293F563
- G6Animal: 721 (721), PDX BL0293F563
- G6Animal: 725 (725), PDX BL0293F563
- G6Animal: 726 (726), PDX BL0293F563
- G6Animal: 729 (729), PDX BL0293F563
- G6Animal: 732 (732), PDX BL0293F563
- G6Animal: 737 (737), PDX BL0293F563
- G6Animal: 741 (741), PDX BL0293F563
- G6Animal: 756 (756), PDX BL0293F563
- G6Animal: 762 (762), PDX BL0293F563
- G6Animal: 791 (791), PDX BL0293F563
- G6Animal: 794 (794), PDX BL0293F563
- G6Animal: 797 (797), PDX BL0293F563
- G6Animal: 798 (798), PDX BL0293F563
- G7 (999995 BIDx5, repeat every 3 weeks, 999994 Q21...
- G8 (754352 43.60 mg/kg BIDx5, repeat every 3 weeks...

ABT+TMZ Group: ALL tumors complete regressions; carry mice forward until tumors regrow or mice die from advanced age. ABT+TMZ stopped after 2 of 3 planned cycles of treatment.

Mouse Pt.-Model No. 3: BL0269F402

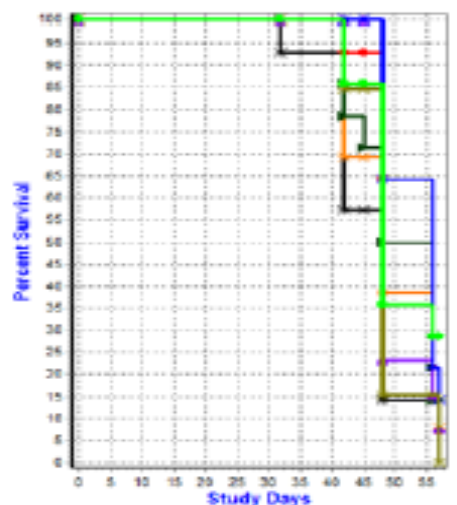
Study Number: ZACR2-1



PIK3CA_H1047R, 50%.

Everolimus, mTOR inhibitor

- Everolimus – No response
- Trametinib – No response
- ABT888 + Temozolomide – No response
- MK1775 + Carboplatin – No Response (at reduced dose)



WES Completed on 8 Pt.-Models, Data Analysis Underway

BLADDER

BL0293F563 (4)

*TP53_R248Q, 100%,
DNA binding domain
MK1775*

ZADR

BL0382F1232 (4)

TP53_E336, 100%,
Tetramer domain
MK1775*

ZABR

BL0269F402 (9)

*PIK3CA_H1047R, 50%
Everolimus*

ZACR

COLON

CN0330F1216 (2)

*AKT_E17K, 50%
KRAS_G12A, 65%
Trametinib*

ZAER

CN0428F1126 (7)

*KRAS_G12A, 50%
Trametinib*

PDX 172845 (7)

Biopsy & CTC
*PIK3CA_E545K, 50%
KRAS_G12D, 65%
Trametinib*
ZAFR – CTC; ZAGR - tumor

SARCOMA

SA0350F605 (3)

No aMOIs detected

STOMACH

ST-0110F1568 (2)

*TP53_R282W, 100%,
DNA domain
MK1775*

- 4 tumor types
- 8 patients
- 38 samples sequenced
- by WES
 - 17 variant called
 - 21 in pipeline

Questions

- Are differences in drug response between Pt.-Models **BL0382** and **BL0293** correlated with mutations in different TP53 domains or other genes of biological relevance?
- Are there any additional mutations in pathways of biological interest for these 2 PDX's?
- Are any relevant mutations associated with the lack of response to everolimus in Pt.-Model **BL0269** (PIK3CA H1047R)?
- Are there relevant expression profiles in untreated tumor that may be predictive of drug efficacy?
- Data for Mining: Variants in coding regions and gene expression profiles

Biologically-Coordinated Clinical Trials: Conclusions

- Feasible to generate PDXs from 18-gauge needle biopsy samples of metastatic sites from patients enrolled in NCI-supported clinical trials
 - ✓ Amount of tissue sufficient for genomics and PDX development
 - ✓ Can expand second passage PDX tissues for distribution that are genetically stable and will consistently re-grow following prolonged storage at -80° C (not shown)
- Treatment of individual patient models with genomically matched and unmatched agents **can** produce clinical results consistent with type of targeted therapy used (both response and resistance)
- Patient-Model tumor samples will be available from pre-clinical and clinical trials for study of mechanisms of targeted agent sensitivity and resistance, and heterogeneity of response
- PD effects of drugs in PDX models demonstrable (not shown)

Biologically-Coordinated Clinical Trials: Next Steps

How to Optimize Use of PDX Repository for Extramural Investigators?

- Develop method for prioritized distribution of clinically-annotated PDX tumors to extramural investigators
- Invite community to add value to PDX repository (open access to data)
 - ✓ analogous to molecular targets data available for NCI-60: WGS, epigenetics, protein phosphorylation, RNAseq, etc.
 - ✓ provide protein lysates, DNA, RNA, etc. as well as PDX tumor tissues for studies proposed by extramural PI's; only caveat that experimental data returned and made available for NCI's open access PDX database
- Perform preclinical in vivo studies that require access to NCI's investigational agent library and that could make optimal use of the PDX repository for investigators at FNL following peer review
- Consider other potential Phase II trials (clear therapeutic intent and expectation) for similar integrated, annotated model development if feasibility of pre-clinical MPACT study confirmed



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Questions?