National Cancer Institute

DCTD Division of Cancer Treatment and Diagnosis

Accelerating Cancer Diagnosis and Drug Development

National Cancer Advisory Board Meeting

Pharmacodynamics and Therapeutics Functional Work Group Update

Joseph E. Tomaszewski, PhD
Deputy Director, DCTD, NCI
June 27, 2011

Mission of Work Group

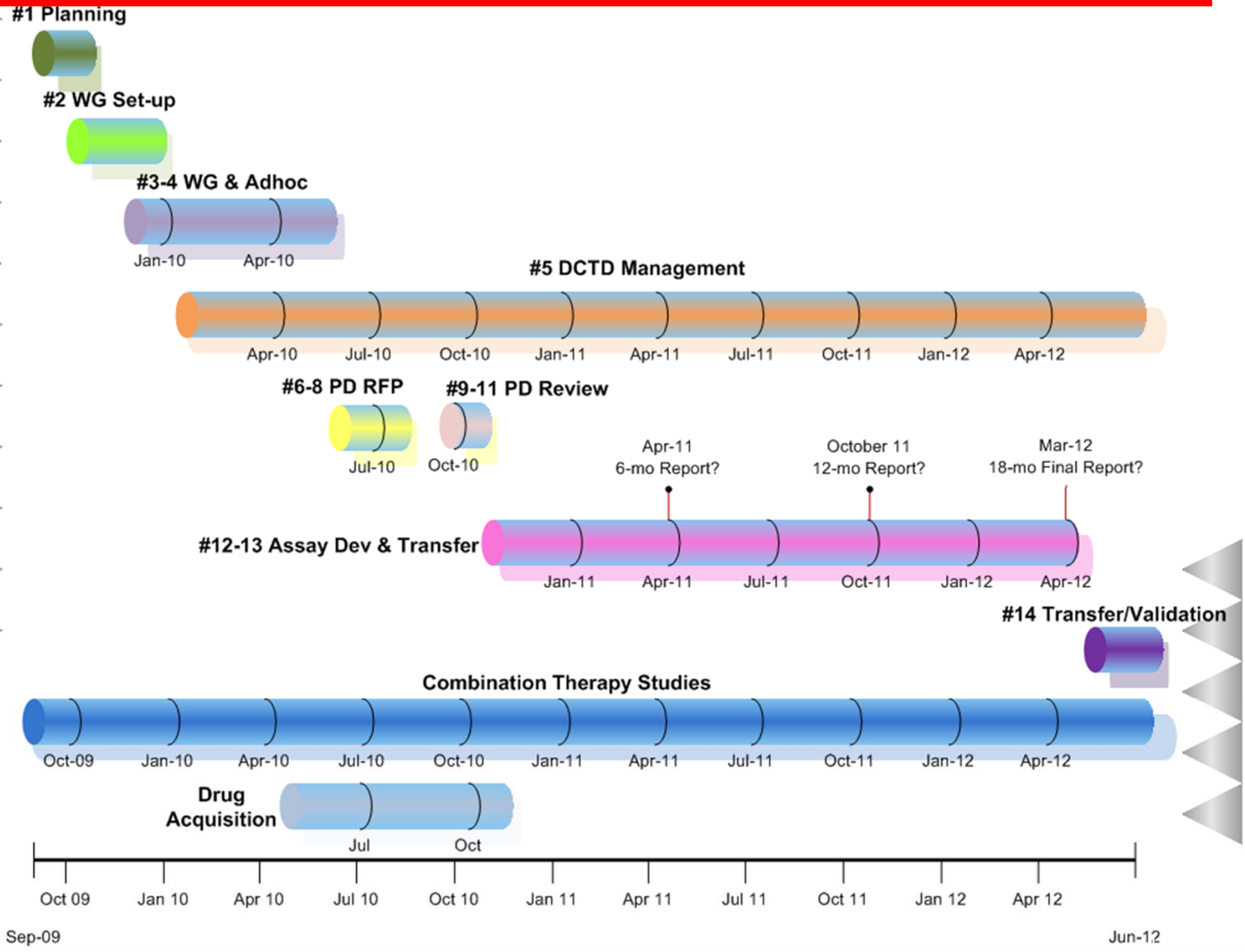
- The Pharmacodynamics and Therapeutics Functional Workgroup (PTF-WG) is to advise the National Cancer Advisory Board (NCAB), Director, NCI and the Director, Division of Cancer Treatment and Diagnosis (DCTD) on which molecular pathways and targets DCTD should concentrate on for the novel drug combinations and the development of pharmacodynamic assays to support early clinical trials. There are two primary goals:
  - *Building pharmacodynamic assay portfolio to support early clinical trials*
  - *Assessing promising drugs in novel combinations*

PTF-WG Membership

Steven Grant, MD, PhD – WG Chair	Medical College VA
Jon Askaa, DVM, PhD	Consultant
Kapil Bhalla, MD	Medical College GA
Daniel W. Chan, PhD, DABCC, FACB	Johns Hopkins University
Richard J. Cote, MD, FRCPath	University of Miami
Peter J. Houghton, PhD	Res Inst Nationwide Children's Hosp
Shirin Khambata-Ford, PhD	Bristol-Myers Squibb Co.
H. Kim Lyerly, MD	Duke Comp CA Center
Gary P. Nolan, PhD	Stanford School of Medicine
David L. Rimm, MD, PhD	Yale University School of Medicine
Neal Rosen, MD, PhD	MSKCC
Jeff Ross, MD	Albany Medical Center
Edward A. Sausville, MD, PhD	University MD School of Medicine
Gary K. Schwartz, MD	MSKCC
Craig B.Thompson, MD	University PA School of Medicine

ARRA Funded Pharmacodynamics and Therapeutic Initiatives

DCTD Activities Estimated Timeline: September 2009 – June 2012 (v2; 8/4/10)



Pharmacodynamic Assay Recommendations

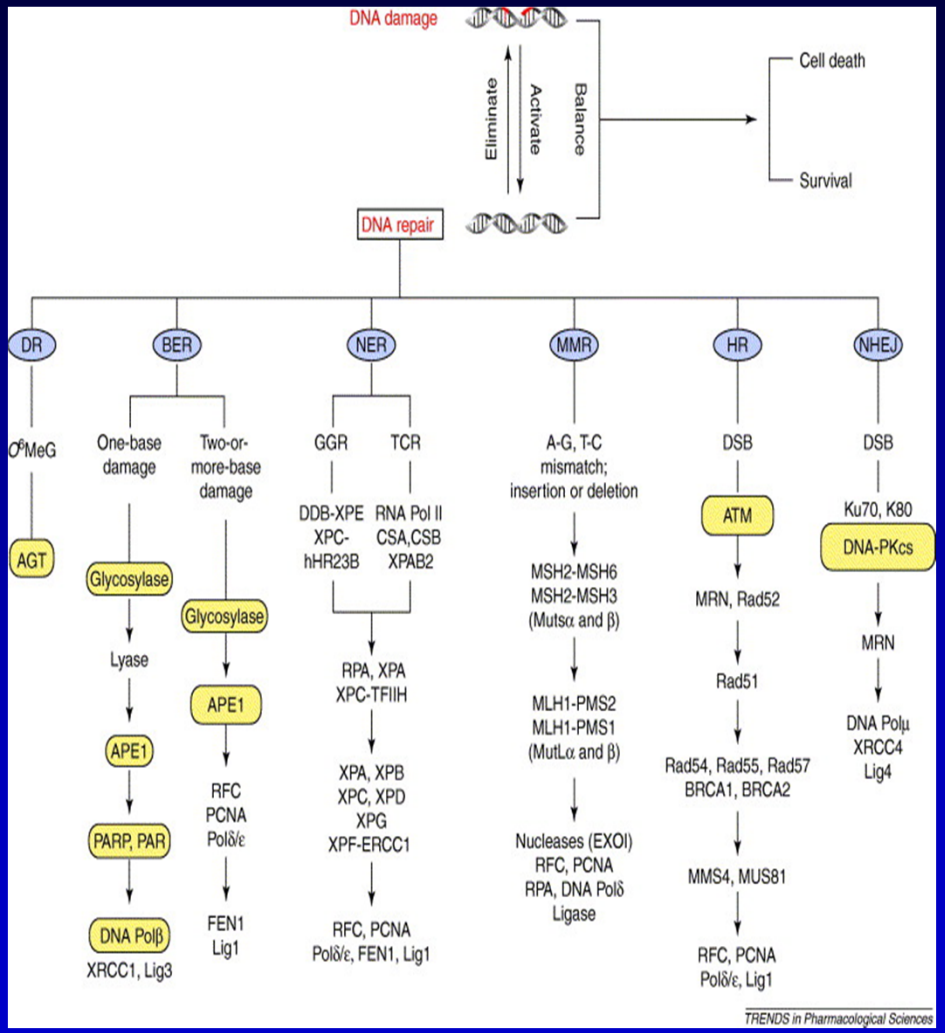
Single Assays *versus* Multiplexed

ARRA Pharmacodynamics Assay Program Expansion

- **Strategy**:
  - Develop methods to allow assessment of targeted agent activity downstream of the putative point of action and on related pathways
- **Goals**:
  - Understand why some targeted agents are effective and some are not
  - Understand off target toxicity
  - Understand basis for patient non-responsiveness to new agents

Critical Pathways for Development of Multiplexed Pharmacodynamic Assays

1. *Apoptosis
2. *Circulating tumor cells
3. RAS/RAF/MEK/ERK
4. PI3Kinase/AKT/mTOR
5. Glycolytic and mitochondrial energy metabolism
6. *DNA repair
7. *NOTCH
8. Autophagy
9. *EMT/SC
10. *Cleaved Casp3/Ki67/γH2AX



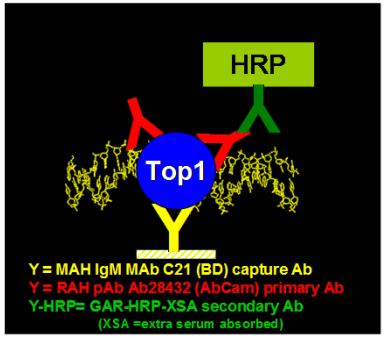
PANEL 1: Global Assessment of DNA Repair

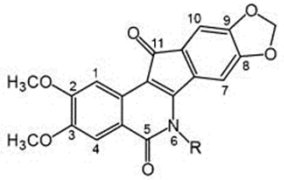
Assay Intent:	Quantitative immunoassay to measure analyte changes in cell/tissue lysates in response to DNA damage		
Pathway	**Analyte**	**Mandatory in Assay**	**Assay Readout**
Base Excision Repair (BER)	PARP 1	Yes	Increase in ratio of active PARP (ribosylated intact and cleaved) to total PARP (intact and cleaved)
	XRCC1	Yes	Change in ratio of pS371 to total XRCC1
Homologous Repair (HR)	BRCA1	Yes	Increase in ratio of pS308 to total BRCA1
	BRCA2	Yes	Increase in ratio of pS442 to total Brca2
	RAD51	Yes	Increase in ratio of pY315 to total RAD51
	Nbs1	Yes	Increase in ratio of pS1778 to total Nbs-1
	Mre11	-----	Proposed by Offeror
	53BP1	-----	Increase in ratio of pS343 to total 53BP1
	Chk2	Not pathway specific	Increase in ratio of pY68 and pS516 ratios vs. total Chk2
	ATM	Not pathway specific	Increase in ratio of pS1981 to total ATM
Fanconi A, C, & D	FANC	Yes	FANCD2 (pT691 and pS717)
Nucleotide Excision Repair (NER)	ERCC4/XPF	Yes	Change in ratio of pS283 to total ERCC4/XPF
	ERCC1	-----	Increase in total protein level.
Mismatch Excision Repair (MMR)	MSH2	Yes	Increase in total protein level
	MLH1	Yes	Increase in total protein level
Non-homologous End-joining (NHEJ)	ATM	Yes	Increase in ratio of pS367 and/or pS1981 to total ATM
	ATR	Yes	Increase in ratio of pS428 to total ATR
	DNA-PK	Yes	Increase in ratio of pT2609 to total DNA-PK
	Ku (Subunit of DNA-PK)	-----	Proposed by Offeror.

Panel 2: Global Assessment of PI3K-mTOR-PTEN Axis

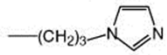
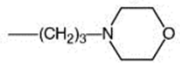
Assay Intent:	Quantitative immunoassay to measure analyte changes in cell/tissue lysates in response to targeted therapy		
Analyte	Qualified in Clinical Trials	Mandatory in Assay	Assay Readout
PI3K	-----	Yes	pY688 of p85 subunit and total pI3K
(pT308) Akt isoform 1	Yes	Yes	pT308 ratio to total AKT1 and total AKT1
(pS437) Akt isoform 1	Yes	Yes	pS473 ration vs. total AKT1
PRAS40	Yes	Yes	pT246vs. total PRAS40
PDK1	-----	-----	Proposed by Offeror
(pT320) SGK3	-----	Yes	pT320vs. total SKG3
PTEN	Yes	Yes	Total
mTORC1 (mTORC1 is a complex containing raptor and mLST8)	-----	Yes	Proposed by Offeror and total
Raptor	-----	Yes	Proposed by Offeror to include phosphorylated-forms (e.g., AMPK phosphorylation site, p90 ribosomal S6 kinases [RSKs 1 and 2 sites])
mTORC 2 (mTORC2 is a complex containing rictor, mLST8 and sin1	-----	Yes	Proposed by Offeror
Rictor	-----	-----	Proposed by Offeror
4EBP1	-----	-----	Proposed by Offeror
S6 Kinase 1: Ribosomal S6-H1 Kinase 1 = p70S6K1	Yes	-----	pS394, pT412, pT252, pT389, and total
MAPKAP K1 S6 Kinase 2 = p90S6K2	-----	-----	pY573, pS221 and total
EIF4G1	-----	-----	Total
mTOR	Yes	-----	pS2 448 and pS2 481 and total
LC3	Yes	-----	Total and type II
PIK3CA	-----	-----	Total
HER3	Yes	-----	pY1289 and total
HER2	Yes	-----	Phosphorylated (pY1112, pY1221, pY1222, pY1248, or pY877) and total
Mre11	-----	-----	Total
Myc	-----	-----	Total
Cdk	-----	-----	Total

Indenoisoquinoline Proof of Mechanism Randomized Phase I Trial

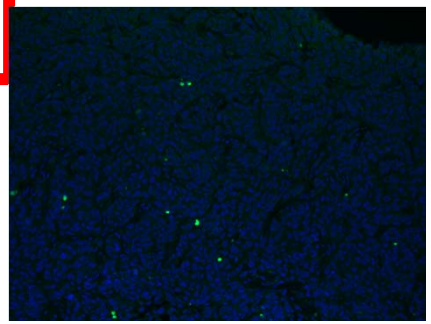




R =

$-CH_3$	NSC 314622
$-(CH_2)_3-NH-(CH_2)_2OH$	NSC 706744 (MJ-III-65)
$-(CH_2)_3-$ 	NSC 725776
$-(CH_2)_3-$ 	NSC 724998

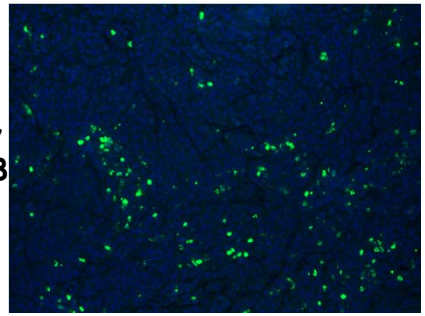
Vehicle



PBMCs
Hair Fol
Skin
CTCs
Tumor

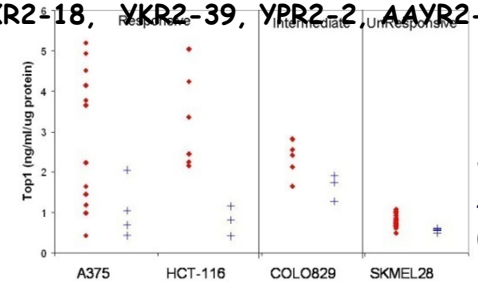
γ-H2AX

25 mg/kg iv
NSC 724998



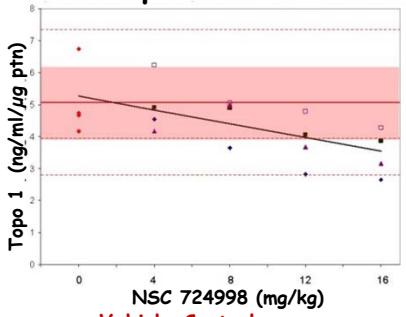
Topoisomerase I Levels in Xenograft Extracts

AAXR2-18, YKR2-39, YPR2-2, AAYR2-17



Vehicle Control ♦
4h Topotecan
(15 MG/KG) treated - +

Dose Response: Indenoisoquinoline Treated A375 Xenografts

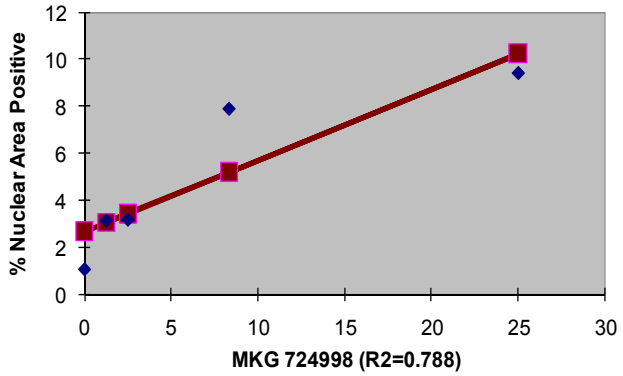


Vehicle Controls

Solid red line = Avg vehicle control Dashed red line = Avg ± 1 and 2 SD

Black line = Dose Response

Dose Response of gH2Ax to NSC 724998 at +2 Hours A375 Xenograft



New Therapeutic Molecules

Therapeutics, Investigational Agents and Positive Controls for PD, Combination and Comparative Studies

New Therapeutic Molecules

- Biologics
  - Ch14.18 anti-GD2 antibody – Neuroblastoma
  - IL-15
  - IL-7
  - Drug for Cancer Immunotherapy Network
- Synthetic Compounds/Drugs
  - Approved oncology drugs (89)
  - Other Synthetic Compounds (209/291)
    - Positive controls for specific pathway/kinase inhibition (NExT Portfolio)
    - Combination studies
    - Comparative studies
- Natural Products

Challenges to Development of Combination Targeted Therapeutics

- Incomplete understanding of mechanisms of action for a growing number of targeted agents available for trial
- Inability to assess target effect
  - Lack of assays, imaging tools
  - Lack of assay standardization
  - Lack of commercially-available agents formulated for in vitro use
  - Lack of available investigational agents for in vitro use
- Lack of preclinical models for combinations
  - To evaluate efficacy, schedule effects, biomarker utility, toxicity
- Clinical trials methodology
  - Need to screen large numbers of patients?
  - Need for tumor biopsies?
  - Is histologic homogeneity relevant?
  - Pharmacokinetic interactions? SD vs RR?
- Intellectual property & regulatory challenges to novel combinations

NCI "COMBO" Plates

- COMBO set 1
  - 87 compounds of diverse mechanism
  - Includes many older FDA-approved anticancer agents

- FDA-approved COMBO set:

A set of FDA-approved anticancer drugs to enable cancer research. This plated set (2 microtiter plates/set) contains most current FDA-approved anticancer drugs. The set consists of 89 agents and is intended to enable cancer research, drug discovery and combination drug studies. Details on the drugs included in this plated set can be found by clicking on Approved Oncology Drugs Plated Set (Plate 1, Plate 2.) Clickable links within the excel files will dynamically query the DTP databases to retrieve up to date DTP information, including NCI60 data, for each drug. Compounds in this set are provided as 20 microliters at 10mM in 100% DMSO; plates are shipped frozen, with dry ice. All proprietary agents in this set were obtained through commercial sources. All compounds were found to have satisfactory purity and identity. In general, substances were checked by both NMR and LC-MS. For recently-acquired compounds, the supplier's Certificate of Analysis was accepted.

Molec. Cancer Ther. 9:1451-1460, 2010

Statistics of Pilot Phase

Initial Studies

- 31 pairwise drug combinations x 60 cell lines
  = 1,759 evaluable experiments to date

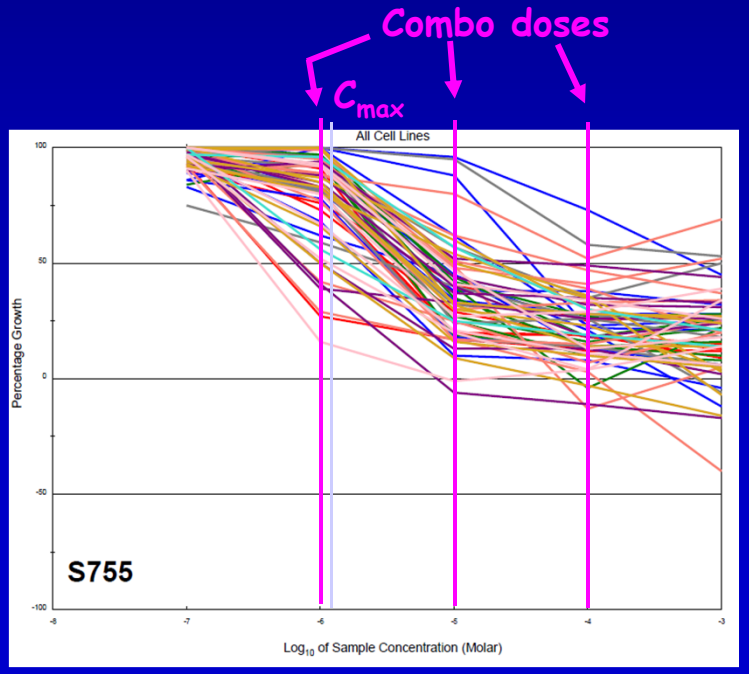
- 25,045 total dose combinations (5 concentrations of one drug; 3 of the second)
  - 11,287 (45%) better than or equal to expected additive value
  - 3,042 (12%) are better than both single agents at the same concentration
  - 1,129 (4.5%) are antagonistic

Goal: 100 commercially available drugs with 5000 unique combinations

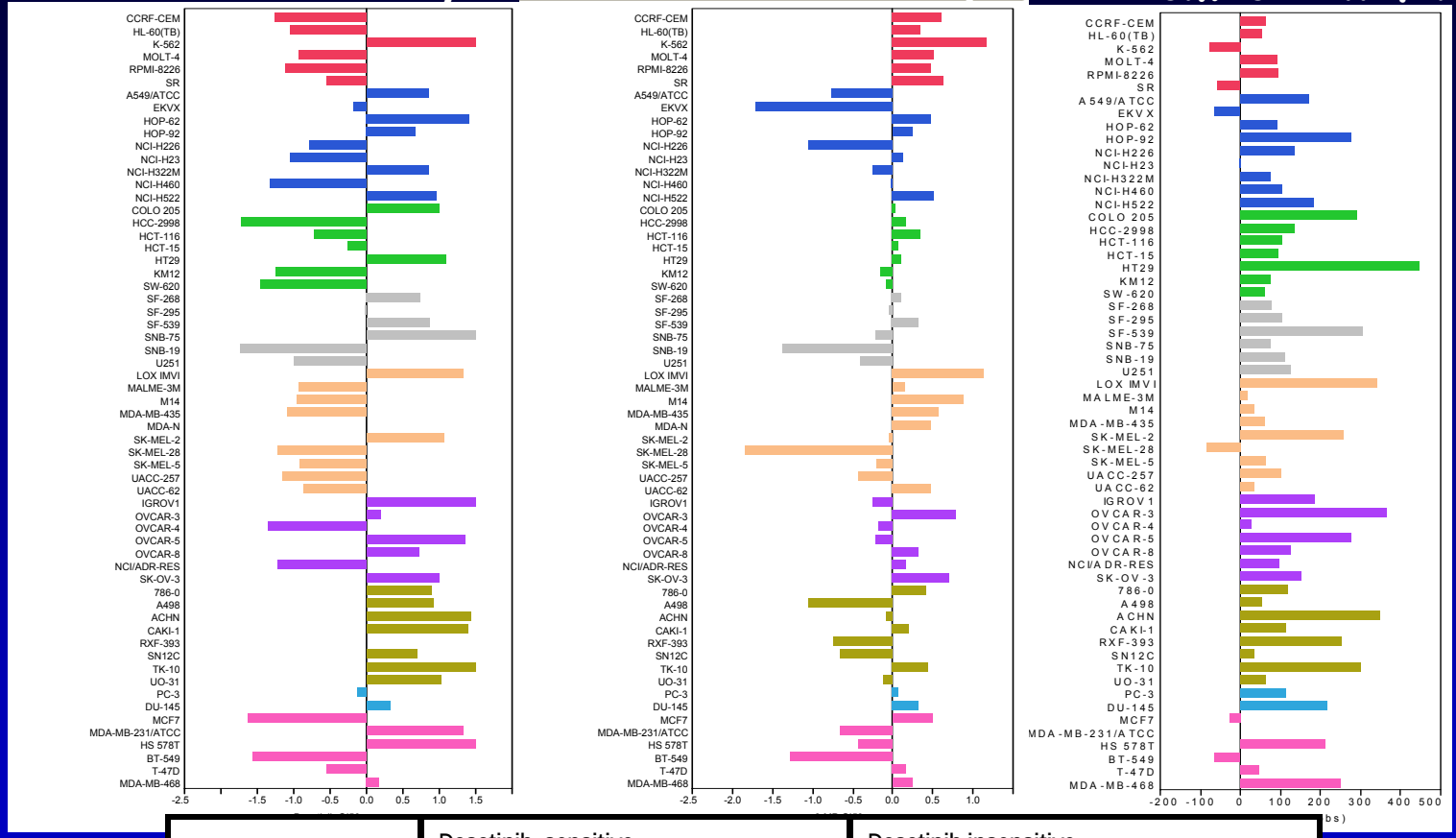
Using the NCI 60 Panel to Develop Combinations of Cancer Therapeutic Agents: Drug Concentrations Chosen Based on Cell Line Activity

Dasatinib: 4/5 concentrations are < Human C_{max}

6-MP: 1/3 concentrations are < Human C_{max}

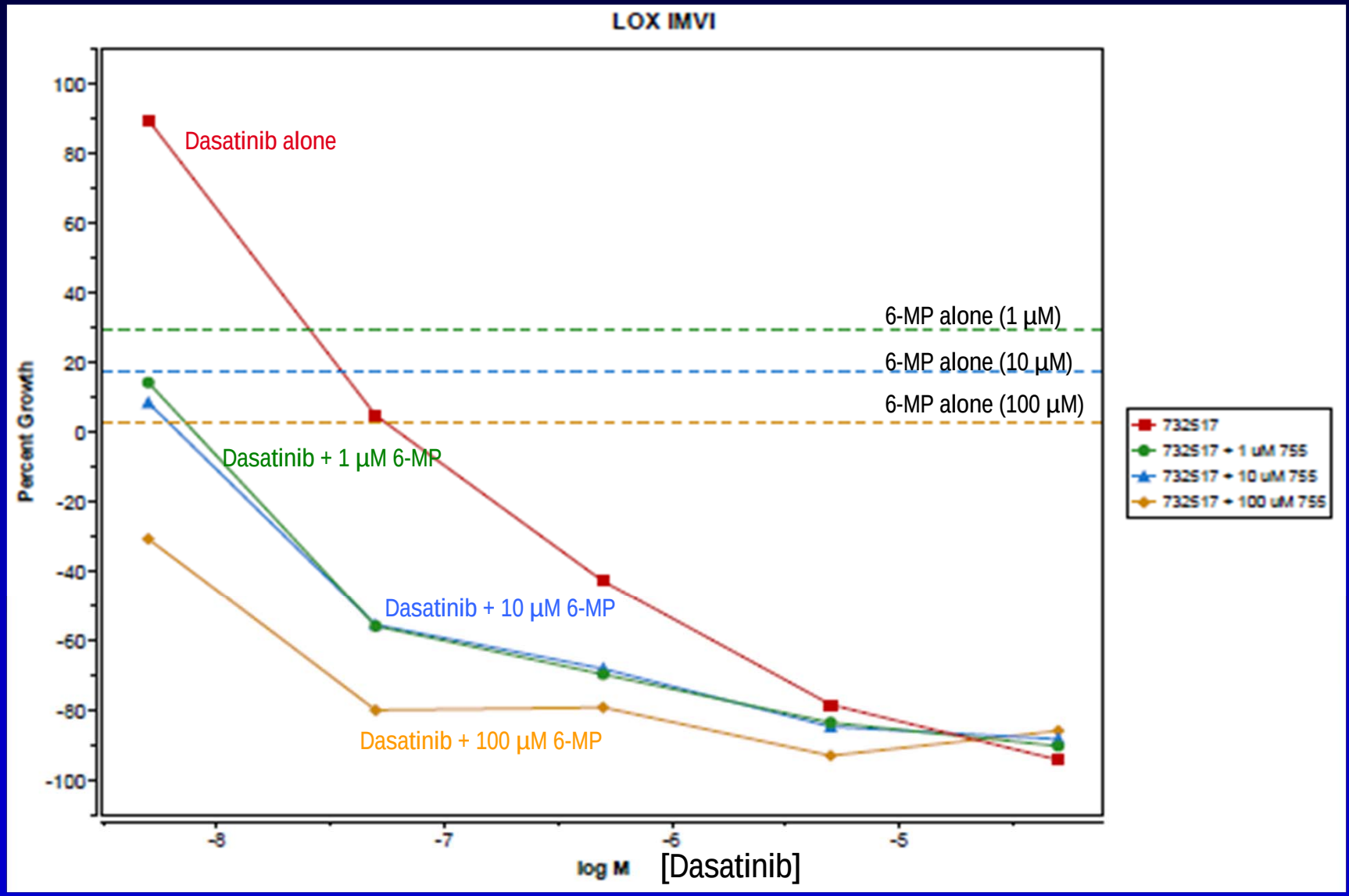


Growth Inhibition by Combination Is Not Predictable from Single Agent Activity

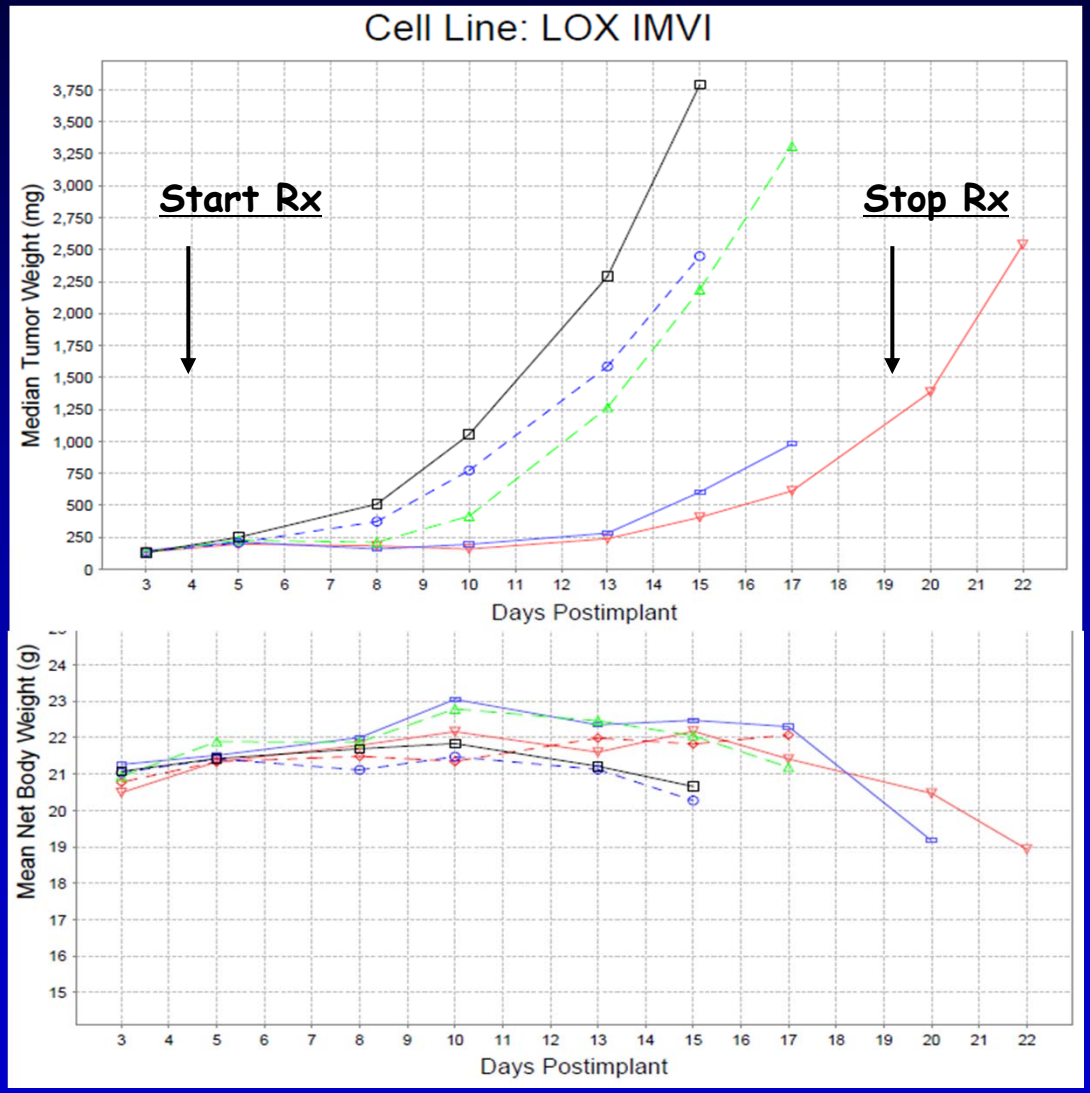


	Dasatinib sensitive	Dasatinib insensitive
6-MP sensitive	Combo benefit: LOX-IMVI No combo benefit: K-562	Combo benefit: OVCAR-3 No combo benefit: M14
6-MP insensitive	Combo benefit: HT29 No combo benefit: BT-549	Combo benefit: MDA-MB-468 No combo benefit: SK-MEL-28

Combination of Dasatinib (NSC 732517) and 6-MP (NSC 755) More Than Additive Across a Wide Range of Dasatinib and 6-MP Concentrations in LOX IMVI Melanoma Cells In Vitro



Dasatinib/6-MP Combination: LOX IMVI Melanoma Xenografts



- Untreated
- 6-Mercaptopurine 25 mg/kg PO QDx15, d3
- Dasatinib 50 mg/kg PO QDx15, d3
- Combo, 6-MP then Dasatinib
- Combo, Dasatinib then 6-MP 4 h later

10 mice per group

Why Is the Dasatinib/6-MP Combination of Interest?

- Unexpected result based on "standard" understanding of the mechanism of action of either agent:
  - Thiopurines (6-MP, 6-TG):
    - Inhibition of de novo purine synthesis
    - Incorporation into DNA
    - *ALSO*: Trigger mismatch repair-induced apoptosis that is dependent on homologous recombination apparatus and, thus, selectively kill BRCA2 defective cells (Cancer Res. 70: 6268, 2010; Molec. Cancer Res. 9: 206, 2011)
  - Dasatinib:
    - Inhibits BCR-ABL tyrosine kinase as will as c-KIT, EPHA, SRC, and PDGFR-β RTKs
    - *ALSO*: Inhibition of physiological c-ABL, absent translocation, strongly impairs DNA DSB repair (Oncogene 27: 4380, 2008)
- Suggests that "systematic" screening will provide novel, hypothesis-generating data that can be used to develop potential therapeutic combinations broadly

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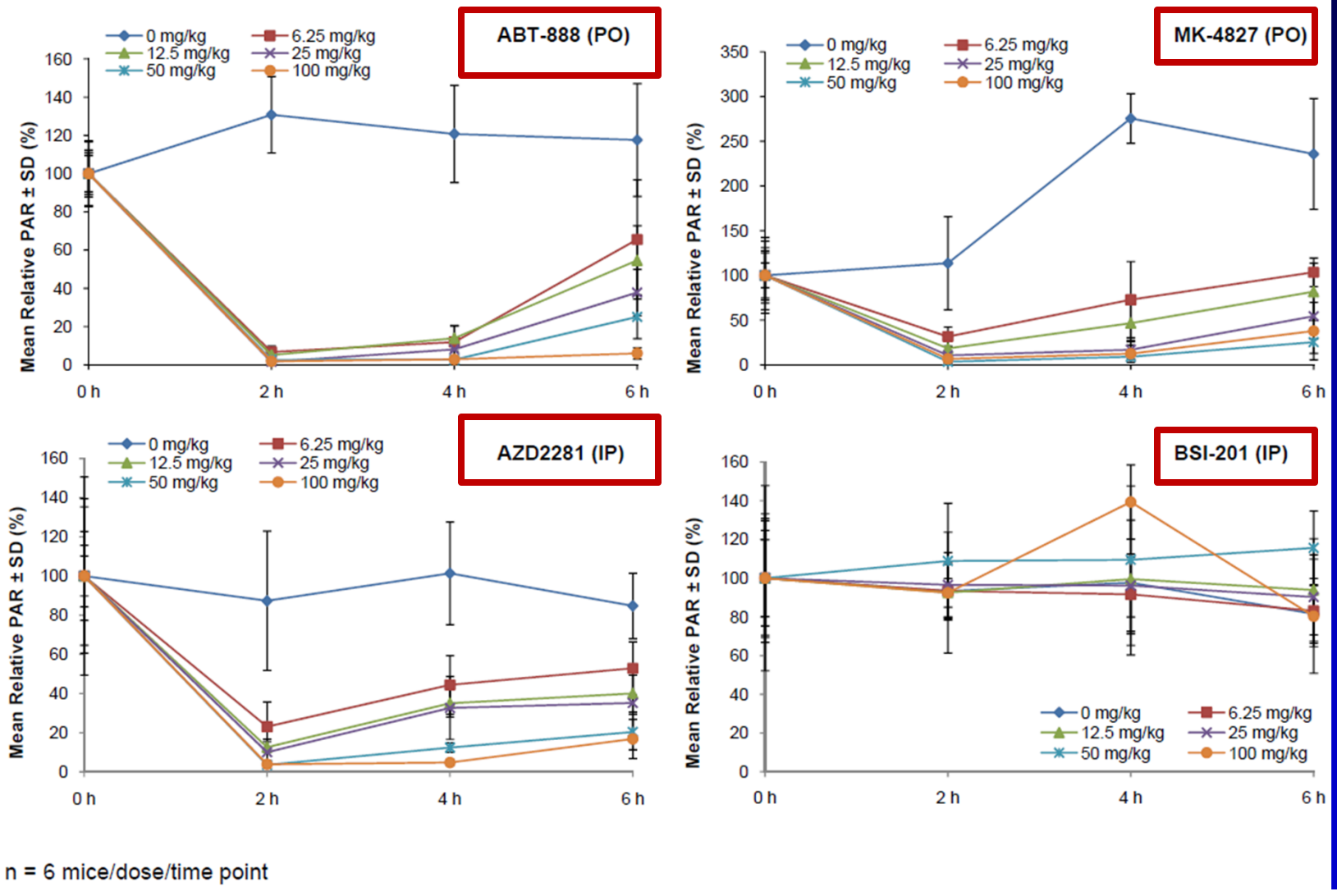
Comparative PD/Efficacy Studies

PARP Inhibitors

Comparative PARP Inhibitor Studies

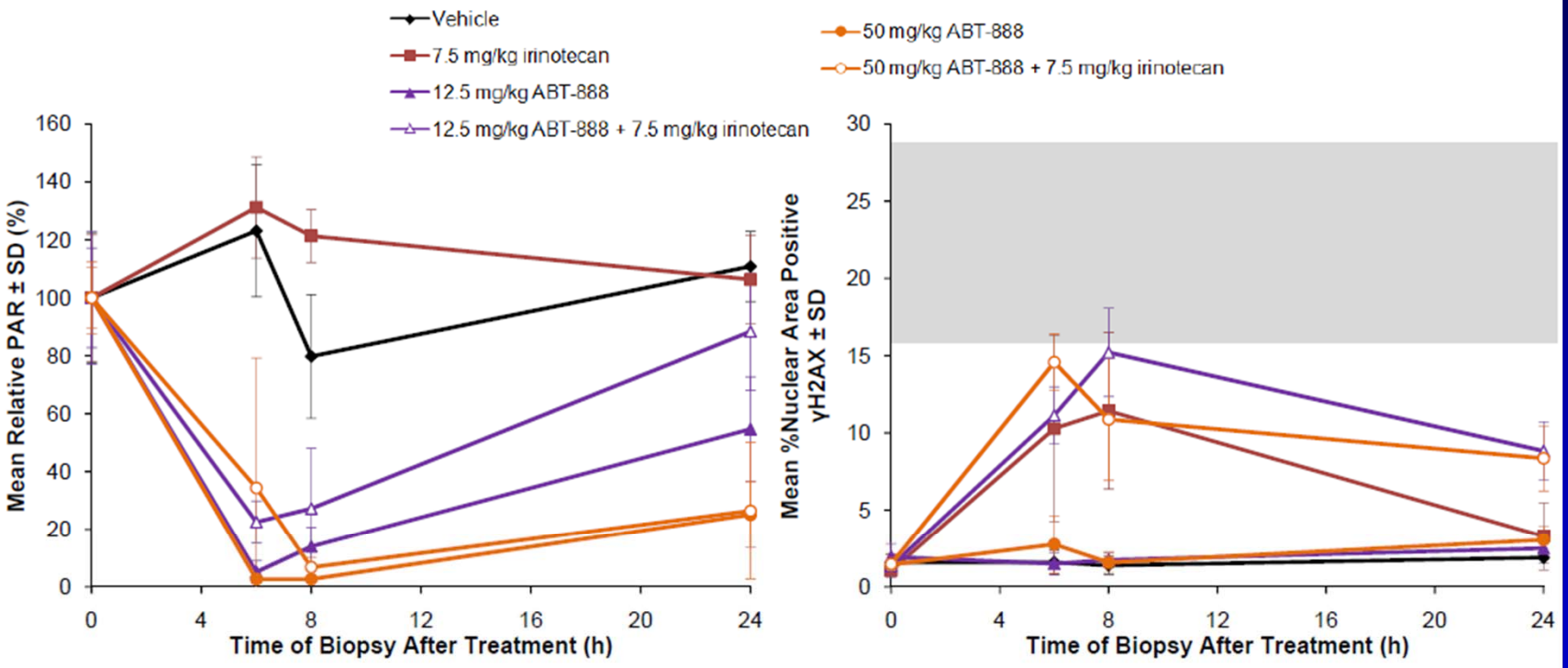
In Vivo Effects on PAR Levels in A375 Xenografts (BRCA1-intact)



n = 6 mice/dose/time point

Comparative PARP Inhibitor Studies

PAR and γH2AX Levels in ABT-888 Plus Irinotecan Combination In Vivo Studies



Addition of ABT-888 did not statistically increase γH2AX levels caused by irinotecan at 6 or 8 h; however, both doses of ABT-888 + 7.5 mg/kg irinotecan induced a significantly higher γH2AX %NAP than irinotecan alone ($p < 0.0002$, 2-sided Student's *t*-test).

Gray box indicates maximum γH2AX effect seen with irinotecan alone ± 1 standard deviation (75 mg/kg dose level).

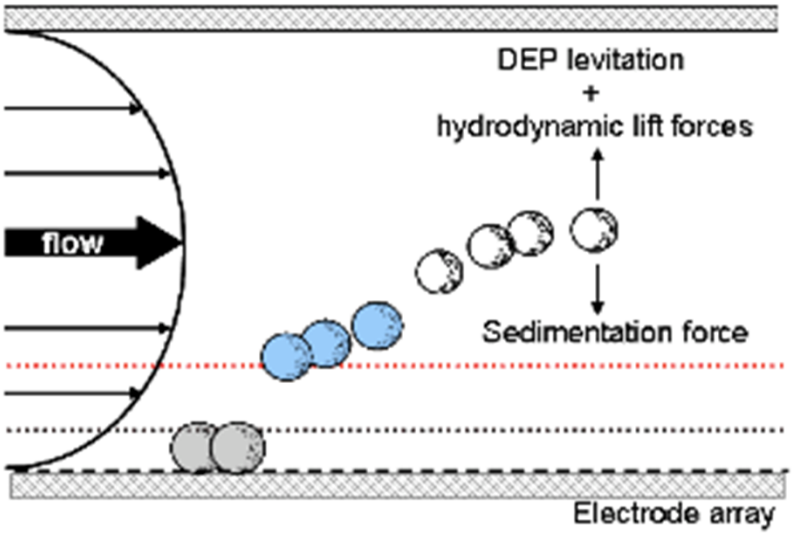
Biopsies from A375 xenografts collected at the indicated times after treatment; n = 6 mice/dose/time point.

Questions and Discussion?

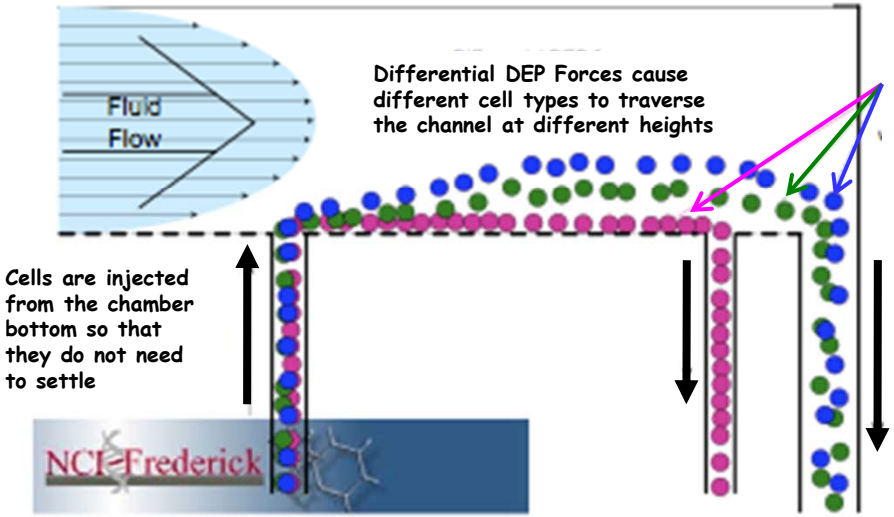
Thank you!

BACK UP SLIDES

Dielectrophoretic Field-Flow Fractionation (DEP-FFF)



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

APOCELL
molecular profiling & diagnostics

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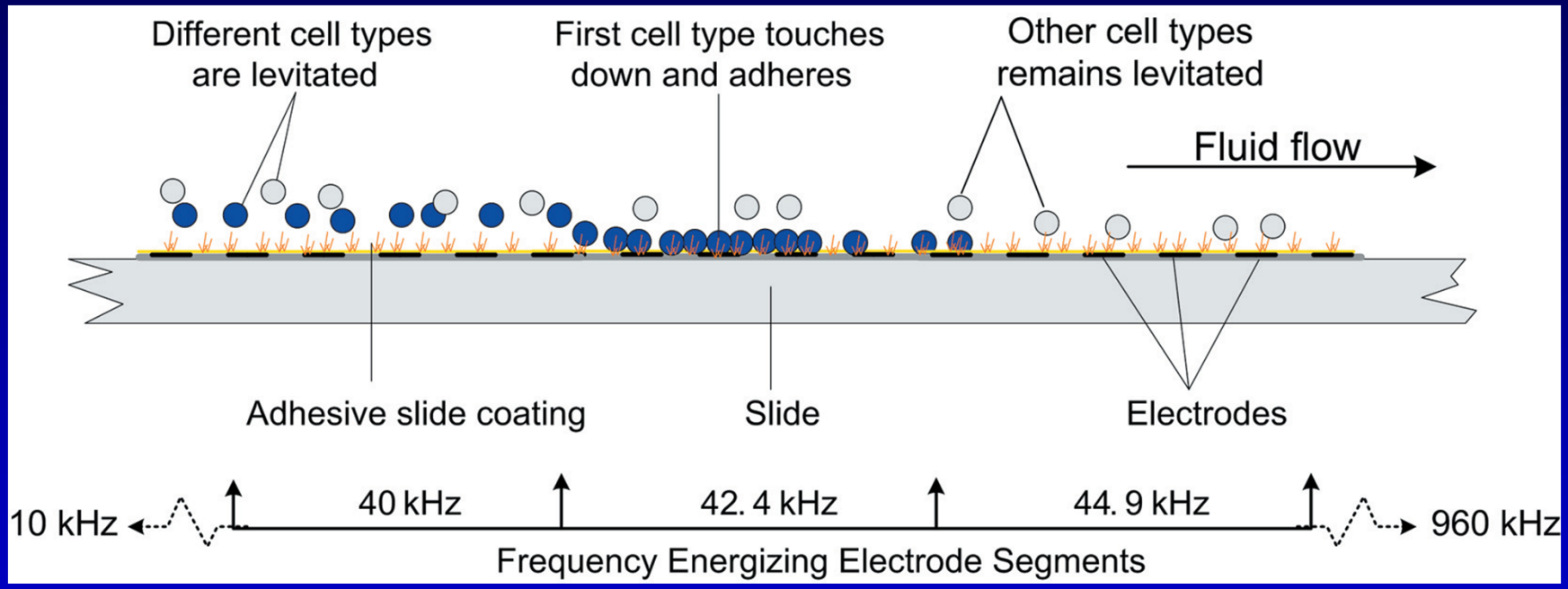
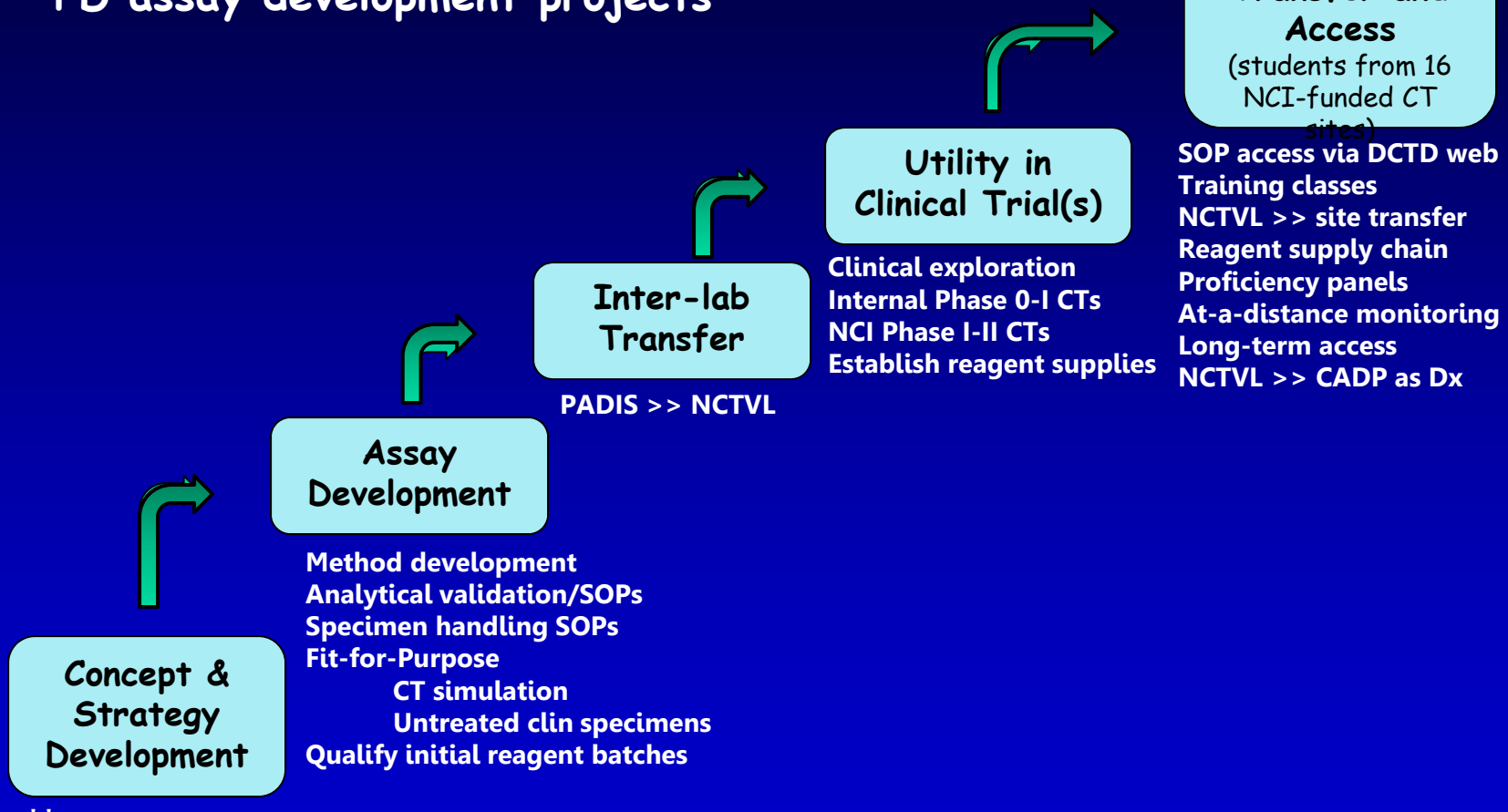
SAIC From Science to Solutions
SAIC-Frederick, Inc.
A subsidiary of Science Applications International Corporation

J. Sep. Sci. 2008, 31, 3732 – 3739

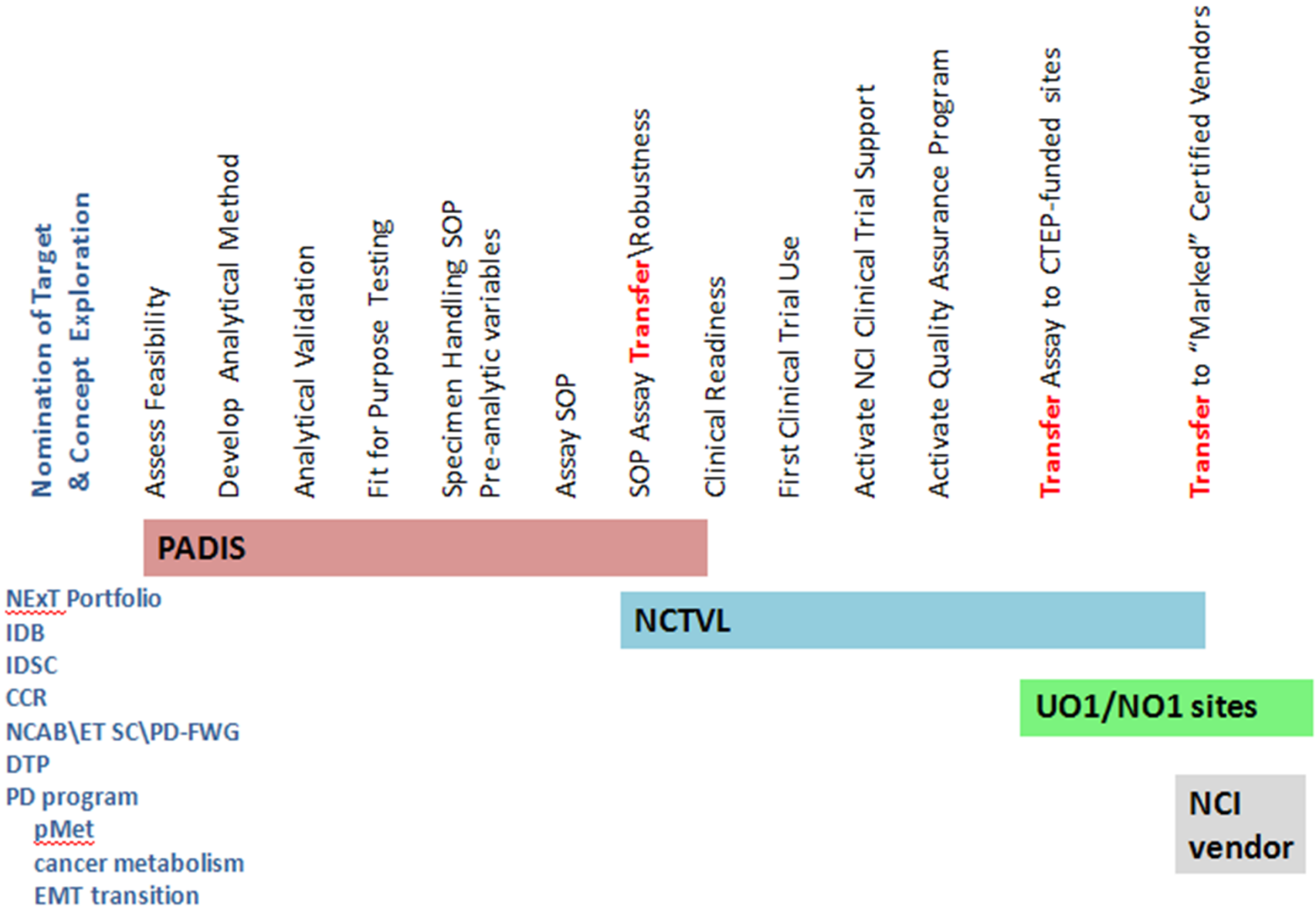


Proof of Mechanism/Pharmacodynamics

Systematic approach to
PD assay development projects



Strategy – Robust, Tissue-Based Assays of Drug MOA



PD Biomarker SOPs Available on DCTD Web Site

http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

DCTD Biomarker SOPs Available

BIOMARKERS

Validated Assays, Specimen Handling Procedures and Reagent Sources for Clinical Pharmacodynamic Studies and Training Certification.

Method	Target(s), Platform	Description
Circulating Tumor Cells	**γH2AX, Cell Search**	Detection of histone H2AX phosphorylated at Ser139 in CTCs using the Cell Search System
Immunoassays	**Poly(ADP-Ribose), ELISA**	Detection of PAR in needle tumor biopsies and PBMCs
Immunofluorescence Assay	**γH2AX, Bond-Max**	Detection of histone H2AX phosphorylated at Ser139 in paraffin-embedded tumor biopsies using the Bond-Max System

Biomarker SOP Details

BIOMARKERS

Validated Assays, Specimen Handling Procedures, and Reagent Sources for Immunofluorescence Analysis of γH2AX in Tumor Biopsies

- Solid Tumor Assessment
    1. **Specimen Collection**
    2. **Sample Preparation**
    3. **Assay SOP**
    4. **Image Capture**
    5. **Image and Data Analysis**
- **Request Key Reagents**
- **Quality Control & Troubleshooting Guide**
- **NCI Publications**
- **Training and Certification**
- **Biomarkers FAQ**

Last Updated: 01/03/11

RESEARCH-USE ONLY (RUO) PROTOCOLS

The following assays have been analytically validated on preclinical samples and/or cell lines. They are not intended for use with clinical samples.

DCTD Programs

Cancer Diagnosis Program

Cancer Imaging Program

Cancer Therapy Evaluation Program

Developmental Therapeutics Program

Radiation Research Program

Translational Research Program

Biometric Research Branch

Method	Assay
Immunofluorescence Assay	MET; TK domain (Custom Reagents)
	Cadherin (E - epithelial) Protein
Immunohistochemical Assay	ER Protein
RT-qPCR	Cadherin (E - epithelial) mRNA
	Catenin (alpha) mRNA
	Catenin (beta) mRNA

Other DCTD Resources

DTP Reference Guide for New Users

DTP Resources for NCI Intramural Researchers

Drug Development Interactive Timeline

Research-Use Only Biomarker Protocols

NCI/NIH Resources

NCI-Wide Research Resources

Mouse Models of Human Cancers Consortium

Type 1 Diabetes Rapid Access to Intervention Development Program

NIH RAID Pilot Program

PD Biomarker Portfolio and Other Available Assays

●	In Progress	✓	Completed	X	Dropped
●	Delayed	CA	Commercially Available	NA/UIN	Not Applicable or Uninformative
●	Technical Difficulty	H	On Hold	R	Ready

PD Assay Portfolio: DCTD/SAIC-F

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
Akt1	ERK/AKT inhibitors	IA	✓	●							
E-Cadherin mRNA	Multiple	RT-qPCR	CA	✓	✓	✓	✓	R			
E-Cadherin Protein	Multiple	IFA	✓	✓	✓	UIN	✓	H			
α-Catenin mRNA	Multiple	RT-qPCR	CA	✓	✓	✓	✓	R			
α-Catenin Protein	Multiple	IFA	✓	✓	H	UIN	✓	R			
β-Catenin mRNA	Multiple	RT-qPCR	CA	✓	✓	✓	✓	R			
β-Catenin Protein	Multiple	IFA	✓	✓	H	UIN	✓	R			
DNA Methylation DNMT1 Activity	Methylation Inhibitors	IA	✓	✓	●	●					
DNA Methylation Global	Methylation Inhibitors	Microarray CCR	●								
DNA Methylation Me-CpG LINE1	Methylation Inhibitors	Pyro-sequence	✓	✓	✓	✓	✓	✓	H		

KEY:

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PD Assay Portfolio: DCTD/SAIC-F

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
ER Protein	**Methylation Inhibitors**	**IHC Ventana**	✓	✓	✓	**UIN**	✓	**R**			
ERK 1/2 & AKT	**Kinase Inhibitors**	**IA**	✓	✓	✓	●					
γ-H2AX Protein (tumor)	DNA Damaging Agents	IFA	✓	✓	✓	✓	✓	✓	✓	●	●
γ-H2AX Protein (tumor)	DNA Damaging Agents	IA	✓	✓	H (calibrator)	✓	✓	H			
HIF-1 alpha	**Multiple**	**IA**	✓	✓	✓	●	●				
Ki-67	**Multiple**	**IFA multiplex**	✓	✓	✓	●	✓	**H**			
MET TK Domain & DockingSite	Kinase inhibitors	IFA Commercial	✓	✓	UIN	X					
MET TK Domain & DockingSite	Kinase Inhibitors	IFA Custom Reagents	✓	✓	✓	✓	✓	●	●		
MET Intact	Kinase Inhibitors	IFA Custom Reagents	✓	✓	✓	✓	✓	✓	●		

PD Assay Portfolio: DCTD/SAIC-F

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
PARG mRNA	PARP Inhibitors	RT-qPCR	✓	✓	✓	✓	✓	✓	✓	R	
PARP 1 mRNA	PARP Inhibitors	RT-qPCR	✓	✓	✓	✓	✓	✓	✓	R	
PARP 1,2 Activity (PAR levels)	PARP Inhibitors	IA	✓	✓	✓	✓	✓	✓	✓	●	●
PARP 2 mRNA	PARP Inhibitors	RT-qPCR	✓	✓	✓	✓	✓	✓	✓	R	
PR Protein	Methylation Inhibitors	IHC Ventana	✓	✓	✓	UIN	✓	R			
Top 1 Cleavable Complex	TOPO Inhibitors	IA	✓	✓	●						
Top 1 Protein	TOPO Inhibitors	IA	✓	✓	✓	✓	✓	●			
Tyrosinase mRNA Melanoma Marker	Melanoma Drugs	RT-qPCR	✓	✓	✓	✓	✓	R			

KEY:

- ● In Progress
- ● Delayed
- ● Technical Difficulty
- ✓ Completed
- CA Commercially Available
- H On Hold
- X Dropped
- NA/UIN Not Applicable or Uninformative
- R Ready

PD Assay Portfolio: DCTD/SAIC-F

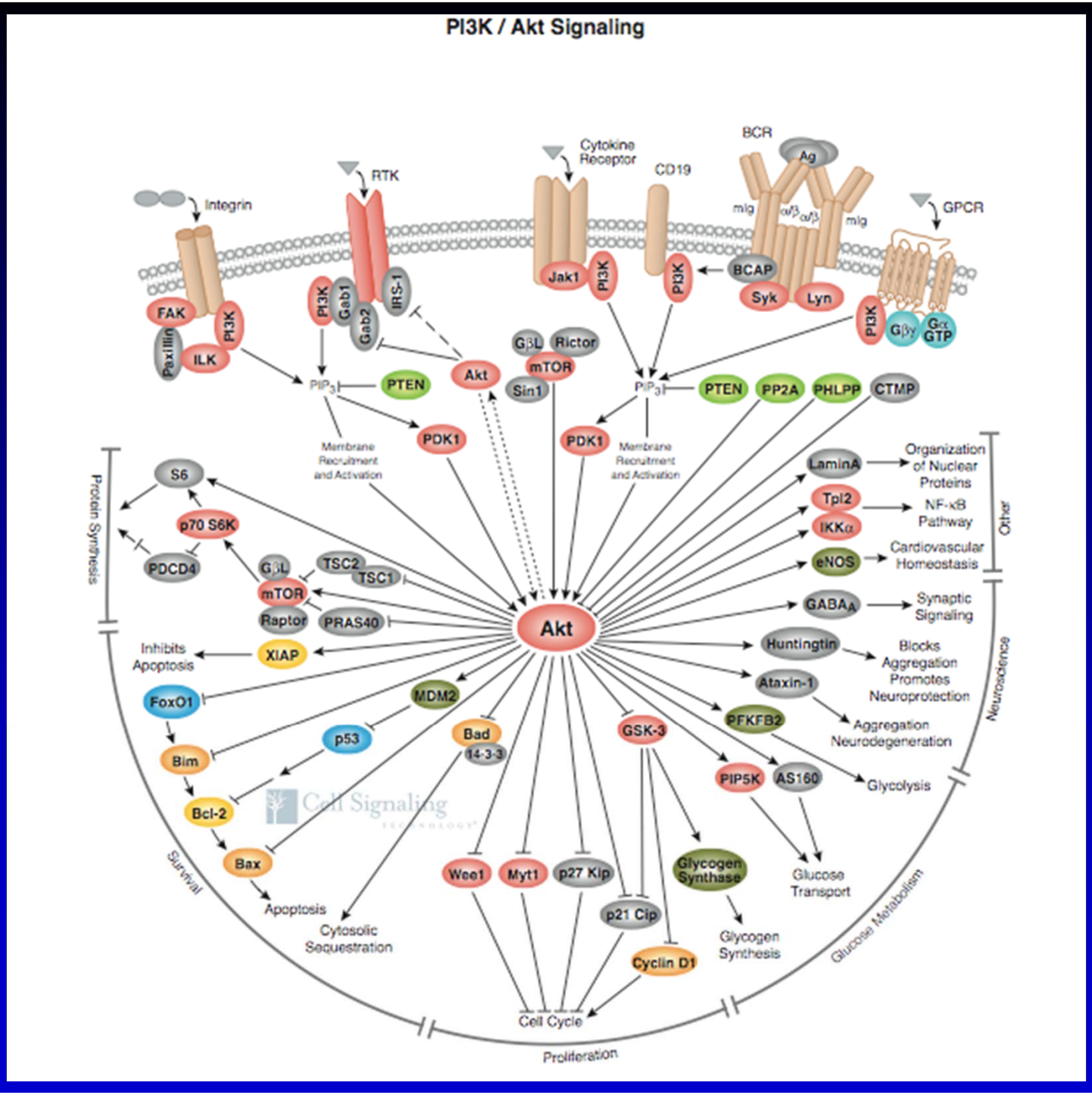
Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Surrogate Assays											
γ-H2AX (skin)	DNA Damaging Agents	IFA	✓	✓	✓	✓	✓	NA	✓	●	
γ-H2AX (MNC)	DNA Damaging Agents	qIFA (cytospin)	✓	✓	✓	✓	✓	✓	✓	●	●
γ-H2AX (CTC)	DNA Damaging Agents	CellSearch IFA	✓	✓	✓	NA	✓	NA	NA	●	●
p16INK4a & GSTP1 (CTC)	Methylation Inhibitors	CellSearch IFA	✓	✓	✓	✓	✓	✓	●		
RASSF1A (CTC)	Methylation Inhibitors	CellSearch IFA	✓	✓	✓	●					
Normalization (Denominator) Assays											
CBC Differential (% of total)	Denominator (Normalization)	Beckman Coulter ACT	CA	✓	CA	✓	NA	NA	NA	R	
Number of PBMCs (% of total)	Denominator (Normalization)	Beckman Coulter Vicell	✓	✓	✓	NA	✓	✓	✓	R	
Number of PBMCs (cell #)	Denominator (Normalization)	Cellometer, Nexcelom	✓	✓	X						
Tissue Cellularity Actin mRNA	Denominator (Normalization)	RT-qPCR	✓	✓	✓	✓	✓	✓	✓	R	
Tissue Cellularity Actin Protein	Denominator (Normalization)	IA	✓	✓	✓	NA	✓	✓	✓	R	

PD Assay Portfolio: New Single-Plex Assays

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
Cadherin (N-nerve) Protein	Multiple	IFA	✓	●							
CQAP1/2	Multiple QAP inhibitors	IFA	✓	●							
GSK3 (alpha/beta)	GSK & AZD inhibitors	IA	✓	●							
γ-Secretase (Notch Pathway)	Gamma-Secretase Inhibitors	Activity	✓	✓	●	●					
HIF-1 alpha	Multiple	IFA	Planned								
STAT3	JAK/STAT inhibitors	IFA	Planned								
XIAP	Multiple QAP inhibitors	IFA	✓	H							

KEY:

●	In Progress	✓	Completed	X	Dropped
●	Delayed	CA	Commercially Available	NA/UIN	Not Applicable or Uninformative
●	Technical Difficulty	H	On Hold	R	Ready


PI3K / Akt Signaling
Integrin
RTK
Cytokine Receptor
CD19
BCR
Ag
mIg
GPCR
FAK
Paxillin
ILK
PI3K
Gab1
Gab2
IRS-1
Jak1
BCAP
Syk
Lyn
Gβγ
Gα GTP
GβL
Rictor
mTOR
Sin1
Akt
PIP3
PTEN
PDK1
Membrane Recruitment and Activation
PP2A
PHLPP
CTMP
Protein Synthesis
S6
p70 S6K
PDCD4
TSC2
TSC1
Raptor
PRAS40
Inhibits Apoptosis
XIAP
FoxO1
MDM2
p53
Bim
Bcl-2
Bax
Apoptosis
Cytosolic Sequestration
Survival
Bad
14-3-3
Wee1
Myt1
p27 Kip
p21 Cip
Cyclin D1
Cell Cycle
Proliferation
GSK-3
Glycogen Synthase
Glycogen Synthesis
PIP5K
AS160
Glucose Transport
Glycolysis
Glucose Metabolism
Huntingtin
Blocks Aggregation Promotes Neuroprotection
Ataxin-1
Aggregation Neurodegeneration
Neurodegeneration
PFKFB2
LaminA
Organization of Nuclear Proteins
Tpl2
IKKα
NF-κB Pathway
eNOS
Cardiovascular Homeostasis
Other
GABAA
Synaptic Signaling
Cell Signaling TECHNOLOGY

PD Assay Portfolio: ARRA

Apoptosis	IA Multiplex	Development
PI3K/AKT/mTOR/ERK	IA Multiplex	Planned
RAS/Raf/ERK	IA Multiplex	Planned
CTC	DEF-FFF	Development

ARRA Funding Assay Delivery date: September 2012

PD Assay Portfolio: New Multiplex

Apoptosis	IFA	Planned
Autophagy	IFA	Planned
DNA Damage Repair	IA	Planned
EMT Stem Cells	IFA	Feasibility
Glycolysis	IA	Planned
Cleaved Casp3/Ki67/YH2AX	IFA	Anal Valid
NOTCH	IFA	Planned

PD Assay Portfolio: SBIR

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory// Feasibility (Ph1)	Development (PhII)	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
Akt1 (total/phospho) (Rockland)	**ERK/AKT inhibitors**	**IA**	✓								
AMPK - α1 (total, pT-174, pS-485) (MSD)	**Multiple (metabolic drugs)**	**IA multiplex**	✓								
AMPK Activity (Bioassay Systems)	**Multiple (metabolic drugs)**	**Activity**	✓	●							
ERK activity (Bioassay Systems)	**ERK/AKT \inhibitors**	**Activity**	✓	●							
Total and Phospho ERK2 & MEK (CTS)	**MAPK Pathway Inhibtors**	**IA**	✓ ERK2								
HIF-2 alpha (Bioo Sci)	**Multiple**	**IA**	✓								
MET - HGF (MSD)	**Kinase Inhibitors MET/HGF Competitors**	**IA multiplex**	●								
P21 (MSD)	**Multiple**	**IA**	✓								
PI3K & MAPK Pathways (MSD)	**PI3K & MAPK Inhibitors**	**IA multiplex**	✓	●							
Wnt - Frizzled (MSD)	**Wnt Pathway Inhibitors**	**IA multiplex**	●								
Aby Reagents VEGF, AKT1, GSK2-beta, JNK1, JNK2, ERK2 **(Singulex)**	**Multiple**	**IA Multiplex / Reagents**	✓								

Delivery date SBIR 2010 ~ September 2011

Strategy – Robust, Tissue-Based Assays of Drug MOA

- **Assembled key team of Dx assay experts to create assays** for quantifying drug effect that achieve the same level of performance required of Dx assays (note: Dx assay directs medical intervention, but PD assays requires drug treatment prior to assay)
  - diagnostic immunoassays
  - diagnostic immunohistochemistry/microscopy
  - clinical chemistry
- **Adopted principles and practices of assay development from Dx testing field,** but pushed these into dynamic post-translational/biochemical changes in tissue biomatrix – many of them rapid and unstable during tissue processing
- **Utilize common clinical instrument platforms**
  - Good market penetration, so ready export of assays to extramural clinical labs
  - Experienced instrument operators in the field, requiring only assay training
    - Liquid immunoassays in calibratable readers, e.g., sandwich-IA, ELISA, RIA
    - Quantitative microscopy assays (objective, whole-slide) on digital pathology systems, e.g., qIFA, qIHC
    - Specialized assays on "marked" Dx instruments, e.g., CTCs on Veridex CellSearch system
  - Removes instrument validation/performance as a source of assay problems

Strategy – Robust, Tissue-Based Assays of Drug MOA

- **(i) Fit-for-Purpose testing, (ii) Inter-lab, SOP-based transfer and (iii) Clinical readiness proven prior to any clinical trial use of a validated assay**
  - FFP testing is replicating the clinical trial protocol in an animal model (in-life tumor needle Bx)
    - Anticipated tissue specimen type and size yield high proportion of evaluable results
    - Assay readout moves in the correct direction in responsive and resistant preclinical models
    - Biological variability at baseline is sufficiently small to be able to demonstrate a 90% drug effect
    - Optimal time window after drug administration to sample tumor for PD response
    - Clinical trial protocol results in proof of significant drug action on intended target
  - Robustness of validated assay proven by SOP-based transfer from development to clinical lab (PADIS/Kinders→NCTVL/Ji) using common set of relevant specimen types (e.g., tumor Bx, PBMC, skin) prepared under a specimen handling SOP
  - Clinical readiness proven by
    - Successful analysis of relevant clinical specimen type(s)
    - Confirmation of sufficiently low biological variability in clinical disease
    - Stability of reference materials with assay values falling within the calibration curve
- **1st Clinical Use in a clinical trial with tightly controlled specimen handling**
- **Establish quality-controlled supply chains for key reagents** to support Phase I/II trials (up to 5 yrs, up to 20 clinical trials) with consistent data using multiple lots

Strategy – Robust, Tissue-Based Assays of Drug MOA

- **Demonstrate clinical utility in ECTs using NCTVL support**
  - demonstrable drug effect in tumor
  - high proportion of patients evaluable for PD effect
  - peer review acceptance of assay and its first preclinical and clinical application
- **Transfer SOP assay to NCI-funded extramural sites** for required use in NCI-sponsored Phase I/II trials (CTEP/IDB, CCC investigator trials), while maintianing Quality Assurance in the field
  - Public access to the assay and specimen handling SOPs at DCTD Biomarkers webspace
  - NCI-based training of certified assay operators
  - Certified Operators provided access to supply chain of qualified, key reagents for assay setup offsite
  - Web-based assay results calculator to monitor assay performance on reference materials and calibrators in the field
- **Permanent access to the assay via DCTD-certified vendor(s) that achieve NCI's assay quality and performance under an active Quality Assurance Program**
  - Key UO1/NO1 studies would be eligible for a voucher to redeem for assays
  - Other NCI/DCTD-funded ECTs would be eligible for "best pricing" for government studies
  - NCI-funded basic researchers would also be eligible for government pricing structure
  - Pharma and Diagnostic companies would have "central lab access" to Quality-Assured validated assay

Exporting Validated, Fit-for-Purpose Pharmacodynamic Assays to Extramural Clinical Trial Sites

Clinical Instrument Platform	Prototype Assay	Specimen Type(s)	Trained Assay Operators
Validatable plate reader (e.g., Tecan)	PAR-IA	Tumor Bx (≥ 18 Ga) PBMCs	6 training courses 10 universities 3 pharma 6 NIH internal
Quantitative Microscopy	γH2Ax	Tumor Bx (≥ 18 Ga)	1 training course 4 universities
Veridex CellSearch	γH2Ax	Circulating Tumor Cells	2 training courses 4 universities 3 pharma 1 small business

Exporting Validated, Fit-for-Purpose Pharmacodynamic Assays to Extramural Clinical Trial Sites

- SOP-based: rigorous procedures documented for specimen handling and assays
  - Analytical performance on par with diagnostic assays
  - Assays developed only for clinical instrumentation
  - Version control, change-control and uniformity at all sites via internet access to current SOPs
    - http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

- Lab-based training of assay operators from outside institutions creates certified field staff
  - Training sessions conducted at NCI-Frederick laboratory facilities
  - PARP pharmacodynamics (sandwich immunoassay)
    - 6 training classes since Oct 2008
    - 18 certified operators from 13 institutions
    - Several NCI clinical trial sites:

      Emory, Karmanos, NYU Med Center, Dana Farber, Ohio State, City of Hope, Univ Pittsburgh/Magee, Johns Hopkins, Univ Wisconsin
    - Several companies:

      Astra-Zeneca, Sanofi-Aventis

- Quality control of the supply of key reagents (batch consistency)
  - Specifications for acceptable range of performance in the assay
  - Specifications for acceptable range of physico/chemical properties

- Provide sites with QC'd key reagents for assay setup; use restricted to trained assay operators

- Monitor assay performance in the field by tracking reported assay results for analytical standards and reference materials

Validated Pharmacodynamic Assays: Fit-for-Purpose Studies

Kinders RJ, Hollingshead M, Khin S, Rubinstein L, Tomaszewski JE, Doroshow JH, Parchment RE; the National Cancer Institute Phase 0 Clinical Trials Team. Preclinical Modeling of a Phase 0 Clinical Trial: Qualification of a Pharmacodynamic Assay of Poly (ADP-Ribose) Polymerase in Tumor Biopsies of Mouse Xenografts. Clin Cancer Res 14:6877-6885, 2008.

Rapisarda A, Hollingshead M, Uranchimeg B, Bonomi CA, Borgel SD, Carter JP, Gehrs B, Raffeld M, Kinders RJ, Parchment R, Anver MR, Shoemaker RH, Melillo G. Increased antitumor activity of bevacizumab in combination with hypoxia inducible factor-1 inhibition. Mol Cancer Ther 8:1867-1877, 2009. [PMID: 19584228}

Pfister TD, Reinhold WC, Agama K, Gupta S, Khin SA, Kinders RJ, Parchment RE, Tomaszewski JE, Doroshow JH, Pommier Y. Topoisomerase I levels in the NCI-60 cancer cell line panel determined by validated ELISA and microarray analysis and correlation with indenoisoquinoline sensitivity. Mol Cancer Ther 8:1878-84, 2009. [PMID: 19584232]

Wang LH, Pfister TD, Parchment RE, Kummar S, Rubinstein L, Evrard YA, Gutierrez ME, Murgo AJ, Tomaszewski JE, Doroshow JH, Kinders RJ. Monitoring drug-induced gammaH2AX as a pharmacodynamic biomarker in individual circulating tumor cells. Clin Cancer Res 16:1073-1084, 2010. [PMID: 20103672]

Reinhold WC, Mergny JL, Liu H, Ryan M, Pfister TD, Kinders R, Parchment R, Doroshow J, Weinstein JN, Pommier Y. Exon array analyses across the NCI-60 reveal potential regulation of TOP1 by transcription pausing at guanosine quartets in the first intron. Cancer Res 70:2191-2203, 2010. [PMID: 20215517]

Validated Pharmacodynamic Assays: Clinical Trial Use

Kummar S, Kinders R, Rubinstein L, Parchment RE, Murgo AJ, Collins J, Pickeral O, Low J, Steinberg SM, Gutierrez M, Yang S, Helman L, Wiltrout R, Tomaszewski JE, Doroshow JH.. Compressing drug development timelines in oncology using phase '0' trials. Nature Reviews Cancer 7(2):131-139, 2007.

Kinders R, Parchment RE, Ji J, Kummar S, Murgo AJ, Gutierrez M, Collins J, Rubinstein L, Pickeral O, Steinberg SM, Yang S, Hollingshead M, Chen A, Helman L, Wiltrout R, Simpson M, Tomaszewski JE, and Doroshow JH. Phase 0 clinical trials in cancer drug development: from FDA guidance to clinical practice. Molec Interv 7(6):325-334, 2007.

Kummar S, Rubinstein L, Kinders R, Parchment RE, Gutierrez ME, Murgo AJ, Ji J, Mroczkowski B, Pickeral OK, Simpson M, Hollingshead M, Yang SX, Helman L, Wiltrout R, Collins J, Tomaszewski JE and Doroshow JH. Phase 0 Clinical Trials: Conceptions and Misconceptions. Cancer Journal 14(3): 133–137, 2008.

Doroshow JH, Parchment RE. Oncologic phase 0 trials incorporating clinical pharmacodynamics: from concept to patient. Clin Cancer Res 14:3658-3663, 2008.

Murgo AJ, Kummar S, Rubinstein L, Gutierrez M, Collins J, Kinders R, Parchment RE, Ji J, Steinberg SM, Yang SX, Hollingshead M, Chen A, Helman L, Wiltrout R, Tomaszewski JE, Doroshow JH. Designing phase 0 cancer clinical trials. Clin Cancer Res 14:3675-3682, 2008.

Kummar S, Kinders R, Gutierrez ME, Rubinstein L, Parchment RE, Phillips LR, Ji J, Monks A, Low JA, Chen A, Murgo AJ, Collins J, Steinberg SM, Eliopoulos H, Giranda VL, Gordon G, Helman L, Wiltrout R, Tomaszewski JE, Doroshow JH. Phase 0 clinical trial of the poly (ADP-ribose) polymerase inhibitor ABT-888 in patients with advanced malignancies. Journal of Clinical Oncology 27:2705-2711, 2009. [PMID: 19364967]

Dancey JE, Dobbin KK, Groshen S, Jessup JM, Hruszkewycz AH, Koehler M, Parchment R, Ratain MJ, Shankar LK, Stadler WM, True LD, Gravell A and Grever MR (on behalf of the Biomarkers Task Force of the NCI Investigational Drug Steering Committee). Guidelines for the Development and Incorporation of Biomarker Studies in Early Clinical Trials of Novel Agents. Clinical Cancer Research 16:1745-1755, 2010. [PMID: 20215558]

Rubinstein LV, Steinberg SM, Kummar S, Kinders R, Parchment RE, Murgo AJ, Tomaszewski JE, Doroshow JH. The statistics of phase 0 trials. Stat Med 29:1072-6, 2010 [PMID: 20419759]

Panel 3: Assessment of the MET Receptor Family (ALK and RON (total, phospho-signaling/regulatory sites, and splice variants**) and Signaling Molecules

Therapeutic Area	Anti-cancer agents targeting the MET receptor family.	
Assay Intent:	Quantitative immunoassay to measure total, phosphorylated, and splice variants of the MET receptor family in cell/tissue lysates	
Analyte	**Mandatory in Assay**	**Assay Readout**
RON	Yes	pY1238, pY1239, pY1353 and pY1360, and total
*Delta RON 165 kDa	Yes	phosphorylated (same as RON) and total
*RON 160 kDa	Yes	phosphorylated (same as RON) and total
*RON 155	Yes	phosphorylated (same as RON) and total
ALK	Yes	pY1278, pY1282, and pY1283 and total
c-CBL	No	pY700 and total
Gab1	No	pY627 and total

Panel 4: Immunoassay Assessment of Mitochondrial Respiration and Oxidative Stress

Therapeutic Area	**Global assessment of baseline and drug induced changes to mitochondrial respiration and oxidative stress in cell/tissue lysates**	
Assay Intent:	**Quantitative immunoassay to measure mitochondrial function and oxidative stress, normalized to mitochondrial content**	
Analyte	**Mandatory in Assay**	**Assay Readout**
Mitochondrial function and stress markers	**Yes**	**Offeror proposed**
Markers of mitochondrial content	**Yes**	**Offeror proposed (normalization factor)**

Offerors are asked to nominate markers of mitochondrial function and content. These markers should be measurable via immunoassays of cell/tissue lysates and use purified authentic calibrators. The proposed targets must be scientifically supported in the literature as reliable metrics of mitochondrial function, stress, and content. Nominated analytes are subject to approval by DCTD officials and outside experts.

PD Assay Portfolio: New Multiplex Panels

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
Apoptosis Panel (SMAC, Bcl-xl, Bcl-2, BAD, BAX, Caspase 3, FADD/pro-Caspase 8, DR4/DR5, cQAP1/2, XQAP, other QAPs, Mcl-1)	**Multiple**	**IFA multiplex**	**Planned**								
Autophagy (LC3-II, Annexin V, Nuclear apobodies, ATG4β, Mitochondria)	**Multiple & Autophagy Inhibitors**	**IFA multiplex**	**Planned**								
DNA Damage Repair (BER, HR, NER, MMR, and NHEJ) (1st Phase - chk1, BRACA, ERCC, ATR, ...)	**Multiple**	**IA multiplex**	**Planned**								
EMT/Stem Cell Proteins Weinberg/CPTC: FOXQ1, LBX1, SLUG, SNAIL, ZEB1, ZEB2, FOXC2, GSC, SOC9, CD133, FOX03, NANOG DCTD/SAIC: ALDH 1A1, OCT 3/4, NANOG, CD44v6	**Stem Cell Inhibitors**	**IFA multi-channel**	●								
Glycolysis Control (GSK3α/β, AXIN, βCatenin, PKA, LKB1, AMPK, PKCβ, AKT2, HK2, PFK1, PDK1, LDH-A, ULK1, GYS1, PDH-A1, PDP-1, BIM1)	**Multiple**	**IA multiplex**	**Planned**								

PD Assay Portfolio: New Multiplex Panels

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
MIXED Cleaved Caspase 3, Ki67 & γH2AX	**Multiple**	**IFA multiplex**	✓	✓	●						
Notch Panel (Notch 1-4, secretases, presenelin, NUMB, Delta 1-4, Jagged 1 & 2, Hes1, Hey1)	**Notch Inhibitors**	**IFA multiplex**	**Planned**								
PI3K Pathway (PTEN, PI3K, Akts, mTORs, PRAS40, PDK1, SGK3, Raptor, Rictor, 4EBP1, S6 K1, p90S6K2)	**PI3K Pathway inhibitors**	**IA multiplex**	**Planned**								
RAS Pathway (RAS, RAFs, MEK, ERK, STAT3, JUN, JAK2, ALK, PIM))	**Ras Pathway inhibitors**	**IA multiplex**	**Planned**								

PD Assay Portfolio: ARRA

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Exploratory/ Feasibility	Development	Analytical Validation	Fit for Purpose	Specimen SOPs	Assay Transfer	Clinical Readiness	Support NCI Trials/QAP	Transfer Community/ Licensing – Certif Marking
Biopsy Assays											
Apoptosis Award (9)	**Multiple**	**IA multiplex**	●								
Possible Contract PI3K/AKT/mTOR/PTEN Pathway	**PI3K/Akt/ mTor Inhibitors**	**IA multiplex**	**H**								
Possible Contract RAS Pathway and STATs	**RAS-RAF and STAT Inhibitors**	**IA multiplex**	**H**								

ARRA Funding Assay Delivery date: September 2012