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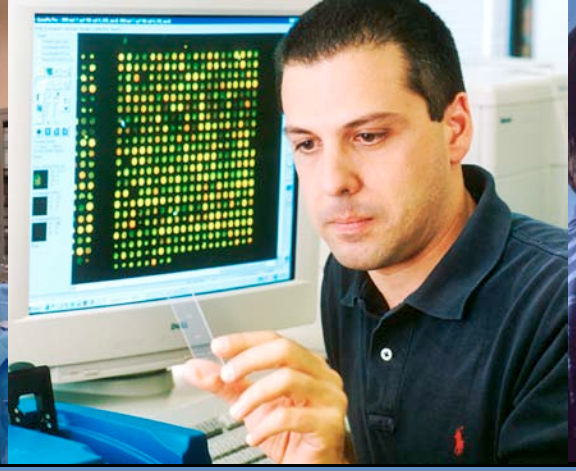

Facilitating Collaborations to Develop Combination Investigational Cancer Therapies: NCI Perspectives on Preclinical Issues in Co-Development

James H. Doroshow, M.D.
Deputy Director for Clinical & Translational Research
National Cancer Institute

Institute of Medicine, NAS
Washington, DC
June 13, 2011

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Challenges to Development of Combination Targeted Therapeutics

- Incomplete understanding of mechanisms of action for a growing number of targeted agents available for trial
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  - Pharmacokinetic interactions? SD vs RR?
- Intellectual property & regulatory challenges to novel combinations

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Lack of Molecular Markers with Proven Clinical Utility

Drug	Biomarker
Anti-estrogens	ER, PR, genomic signature
Trastuzumab	Her2 FISH, IHC
EGFR small molecule inhibitors	Mutation status
B-Raf, ALK inhibitors	Mutation status
Anti-VEGF/VEGFR agents	??
IGF-I receptor antagonists	??
Src inhibitors	??
Cdk/Cyclin D1 inhibitors	??
HDAC/DNMT inhibitors	??
Anti CTLA-4 Antibody	??

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Role of Proof of Mechanism Studies in Early Phase Trials of Molecularly Targeted Combinations

- Demonstration of mechanism(s) of action (or resistance) of the combination in tumor early in development provides:
  - Evaluation of the actual versus presumed sites of target engagement
  - Evidence to support further development of the combination
  - Demonstration of the relationship of drug schedule and systemic exposure to target effects
  - Data to determine the relevance of the biomarker chosen to represent modulation of the target—downstream effects can be studied as well as direct target inhibition
- Ability to investigate molecular effects of the combination of agents in surrogate (non-malignant) tissues
  - Evaluate the relevance of the non-malignant tissue as a marker of target engagement
  - Opportunity to study molecular toxicology and other safety signals in a range of normal organs
- Not necessarily predictive of clinical benefit--requires larger, later stage trials

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Pharmacodynamic Assay Development

Concept		Feasibility & Development						Validation		Launch	
Target	Application	Platform	Feasibility	Development	Analytical Validation	Preclinical Modeling	Specimen SOPs	Assay Transfer	Clinical Validation	Support NCI Clinical Trials	Transfer to Scientific Community
Biopsy Assays											
γ-H2AX Protein (tumor)	DNA Damaging Agents	ELISA	✓	✓	●	●					
γ-H2AX Protein (tumor)	DNA Damaging Agents	qIFA	✓	✓	✓	✓	✓	✓	✓	●	
Top 1 Protein	TOPO Inhibitors	ELISA	✓	✓	✓	✓	✓	✓	✓	●	
MET TK domain and Grb2 Docking Site	Kinase Inhibitors	IFA **Commercial Reagents**	✓	✓	●	●	●	●			
MET TK domain and Grb2 Docking Site	Kinase Inhibitors	IFA **Custom Reagents**	✓	✓	✓	●	●	●			
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	
Stem Cell Proteins -ALDH 1A1 -OCT 3/4 -NANOG -CD44v6	Tumor Stem Cell 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

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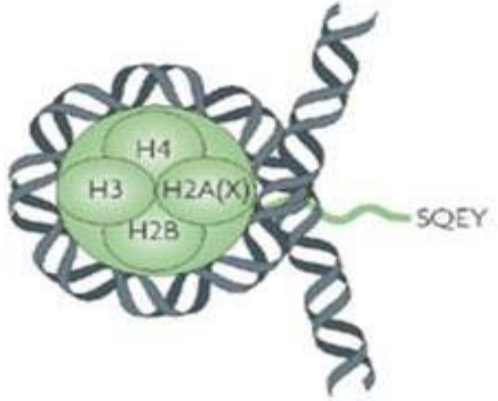
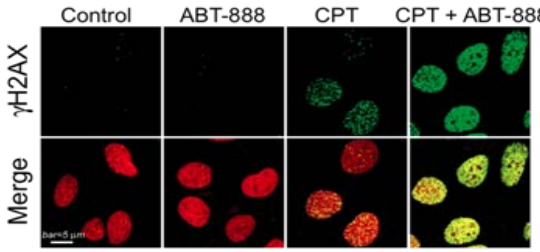
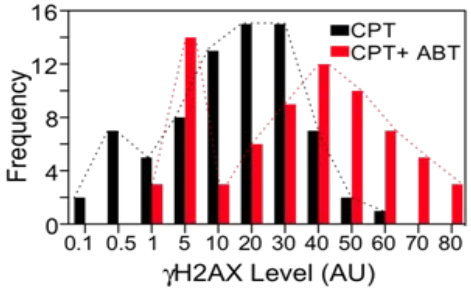
γH2AX: Pharmacodynamic Marker of DNA Double Strand Breaks

Nucleosome with H2AX Tail



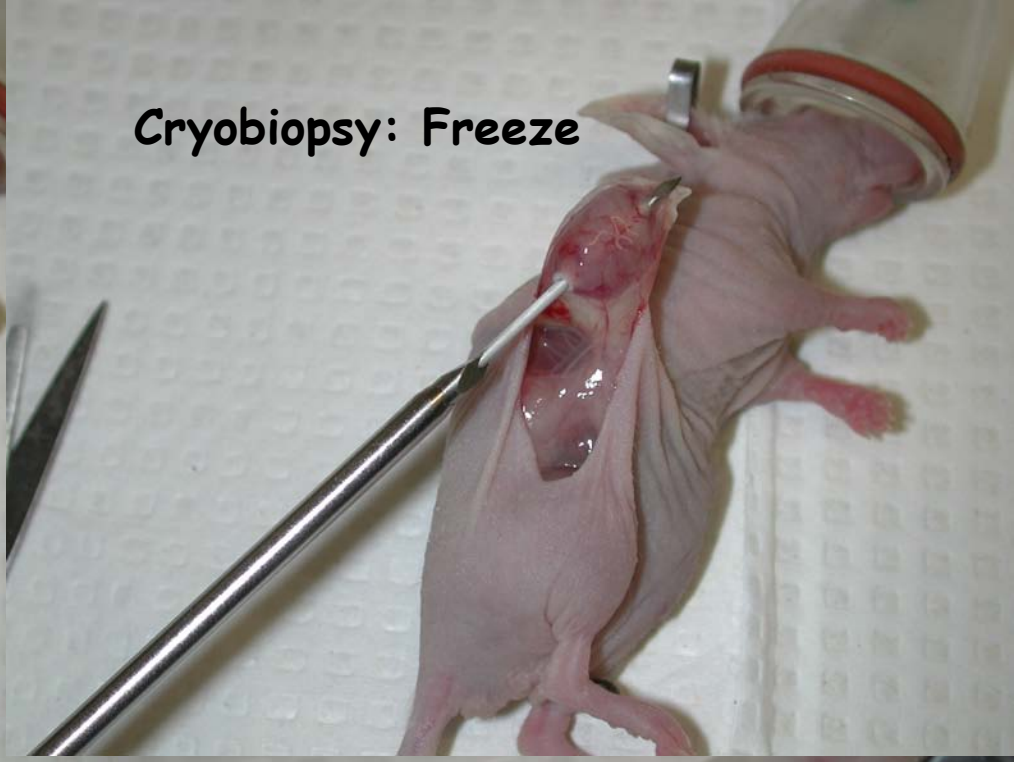




- H2AX: Histone protein phosphorylated by ATM, ATR, or DNA-PK on serine c-4 following DNA DSBs (many sources) in geographic proximity to the DSB, forming foci
- DNA repair proteins accumulate around the phosphorylated γH2AX focus—platform for DNA damage response
- Detect by counting immuno-fluorescent foci: each focus contains hundreds of γH2AX molecules
- Decay of foci reflects DNA rejoining
- Downstream indicator of the effects of the formation of DNA-topoisomerase I covalent complex

Nucl. Acids Res. 39: 3607-3620, 2011


Standard 18 gauge Bx


Cryobiopsy: Freeze


Cryobiopsy: Excise


Excisional Biopsy

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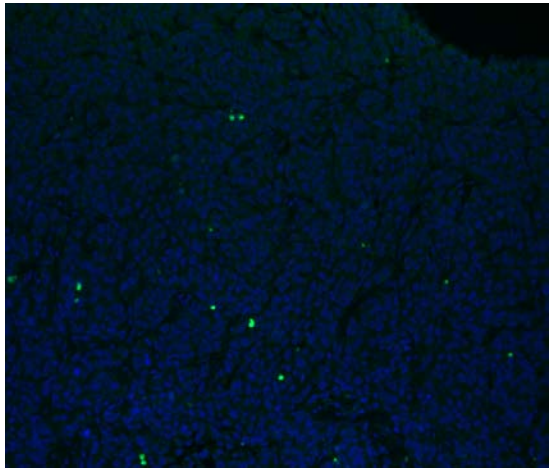
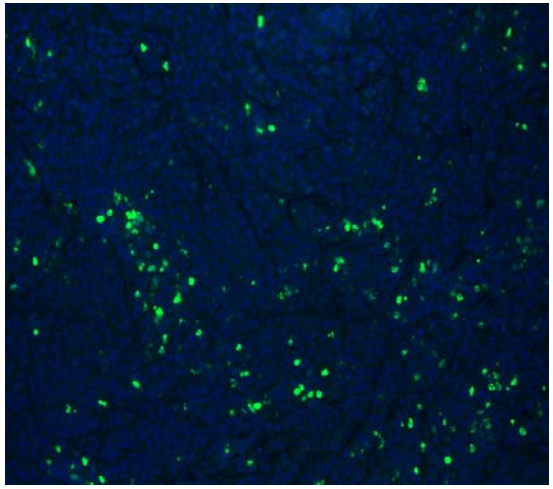
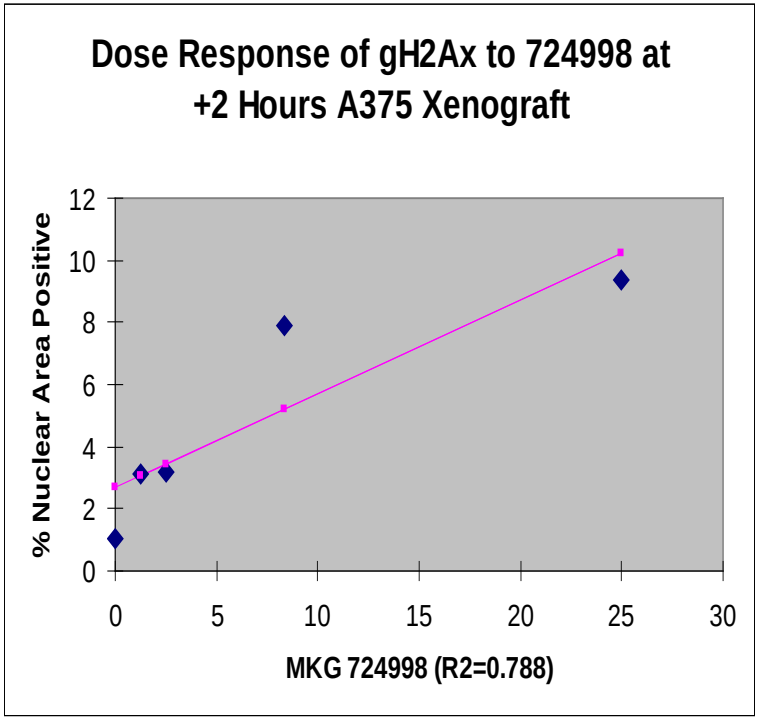
Quantitative Immunofluorescence Assay: γH2AX Measured in A375 Xenografts after Top1 Inhibitor Therapy (18G Biopsy)

Vehicle



25 mg/kg iv
NSC 724998





Clin. Cancer Res. 16: 5447-57, 2010

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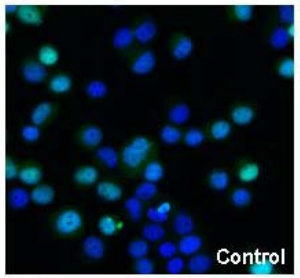
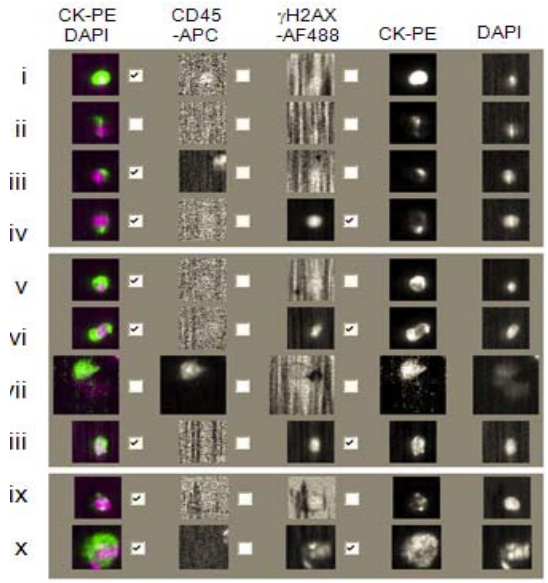
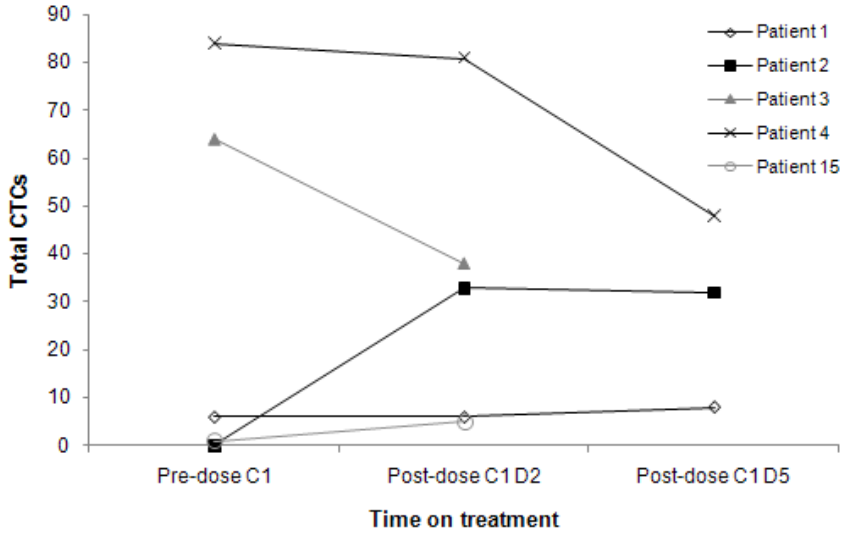
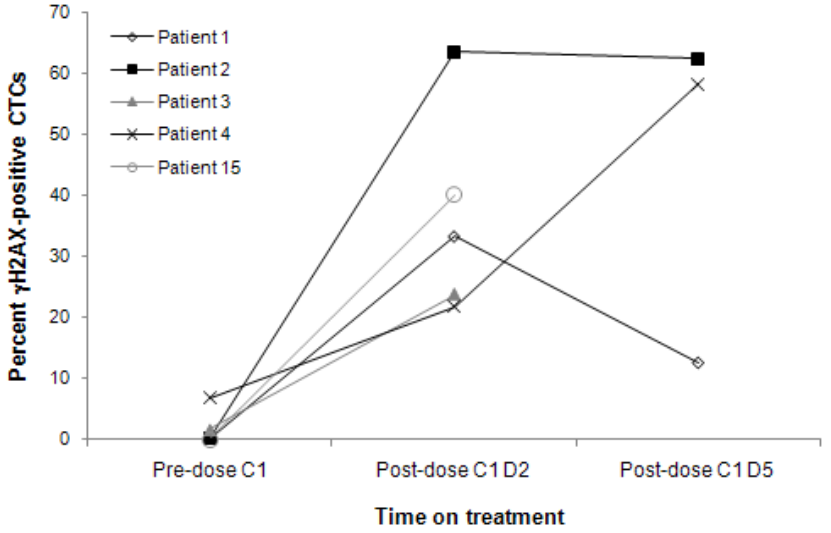
γ-H2AX: A Non-Invasive Marker of DNA Double Strand Breaks in CTCs









Clin. Cancer Res. 16: 1073-1084, 2010

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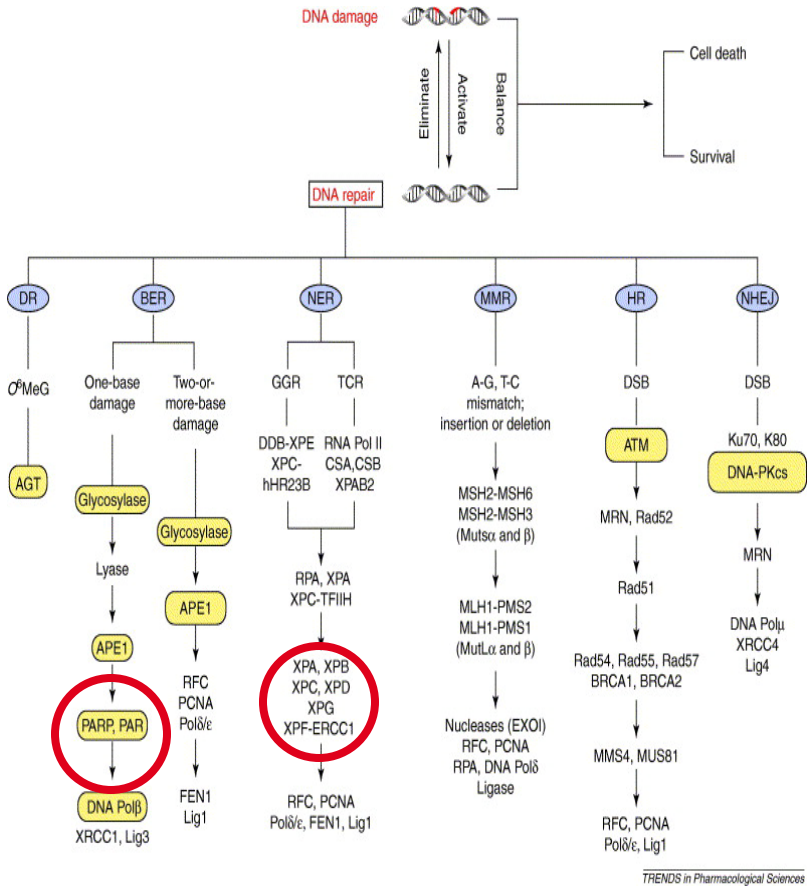
Critical Pathways for Development of Multiplexed Pharmacodynamic Assays

- RAS/RAF/MEK/ERK
- PI3Kinase/AKT/mTOR
- Glycolytic and mitochondrial energy metabolism
- DNA repair
- Circulating tumor cells
- NOTCH
- Apoptosis
- Autophagy
- EMT



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NCI "COMBO Plates"

Plated Compounds for Combination Studies

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

- FDA-approved COMBO set:

Approved Oncology Drugs Set Information: 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 88 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.

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

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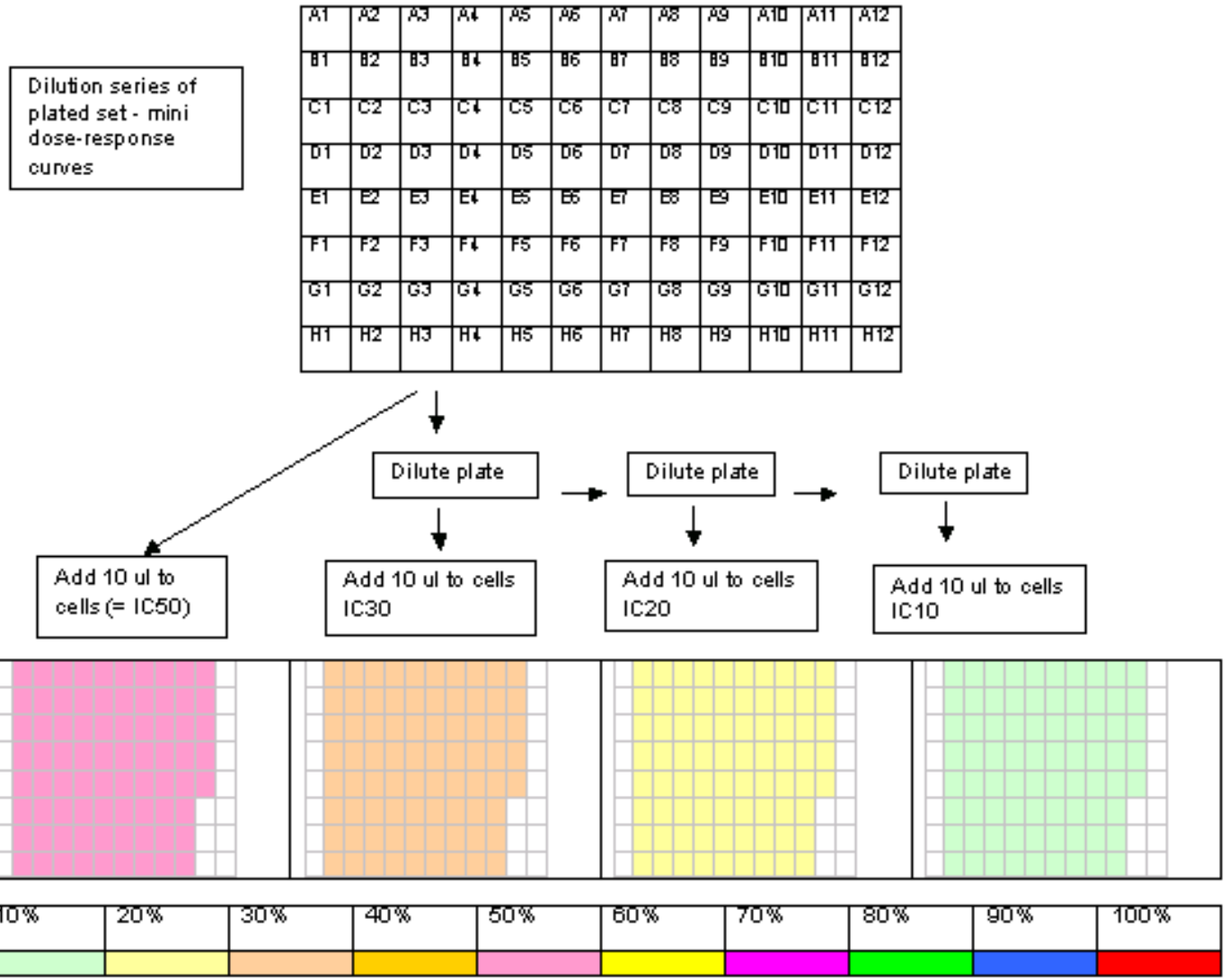
Combination Plate: Dilution Series



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  - To evaluate efficacy, schedule effects, biomarker utility, toxicity
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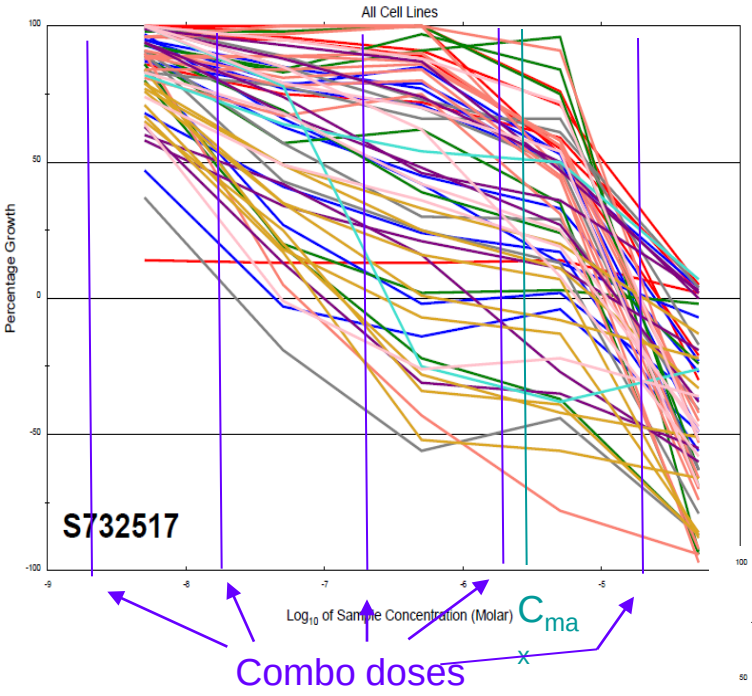
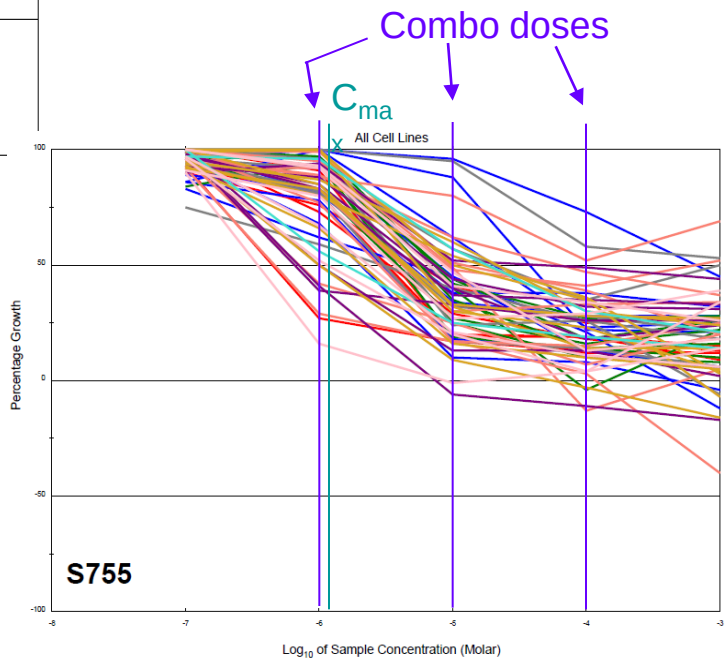
Using the NCI 60 Panel to Develop Combinations of Cancer Therapeutic Agents: Drug Concentrations Chosen Based on Cell Line Activity



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

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



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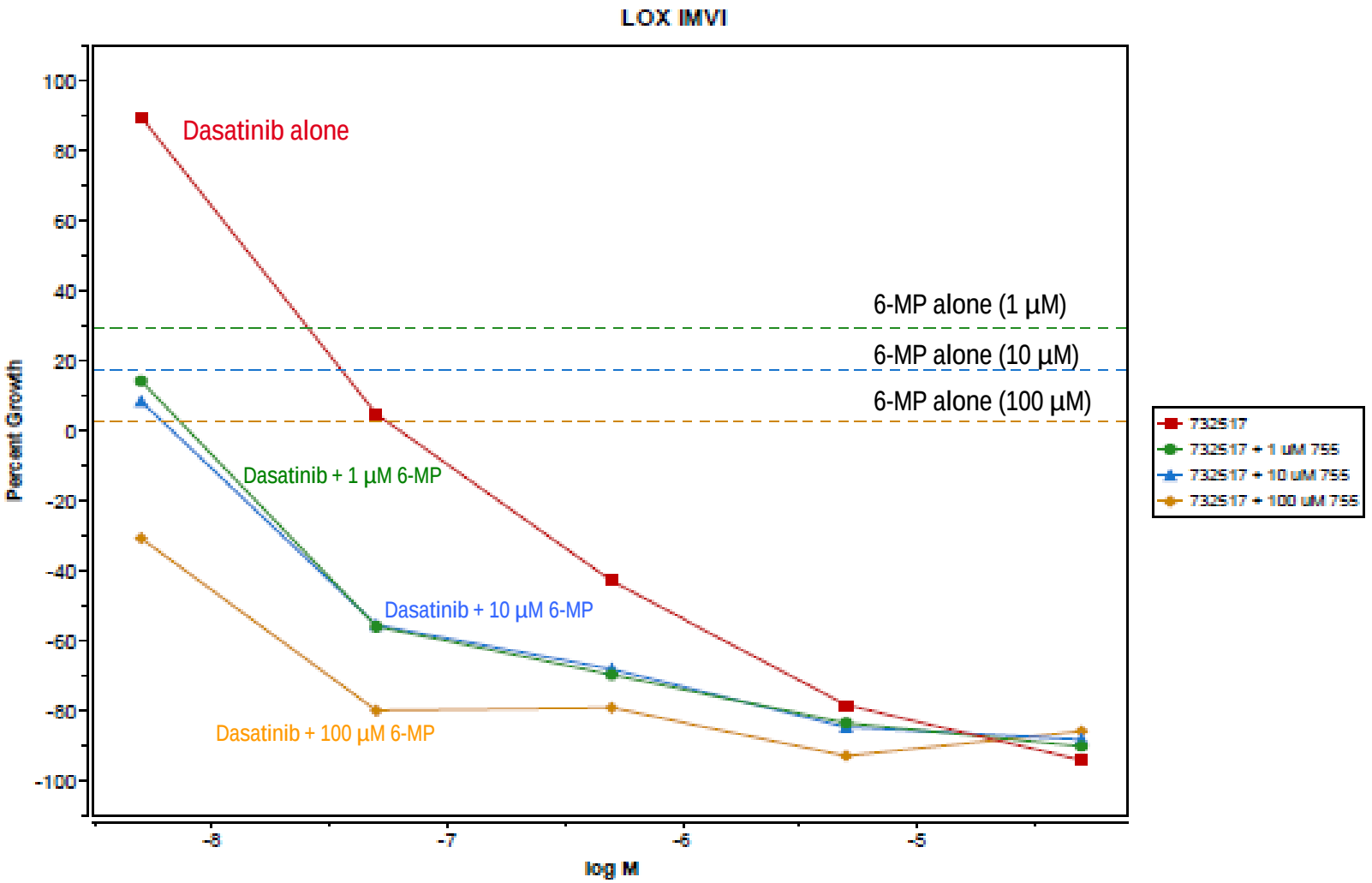
Combination of Dasatinib (NSC 732517) and 6-MP (NSC 755) More Than Additive Across a Wide Range of Dasatinib and 6-MP Concentrations in LOX IMVI Melanoma Cells In Vitro



[Dasatinib]

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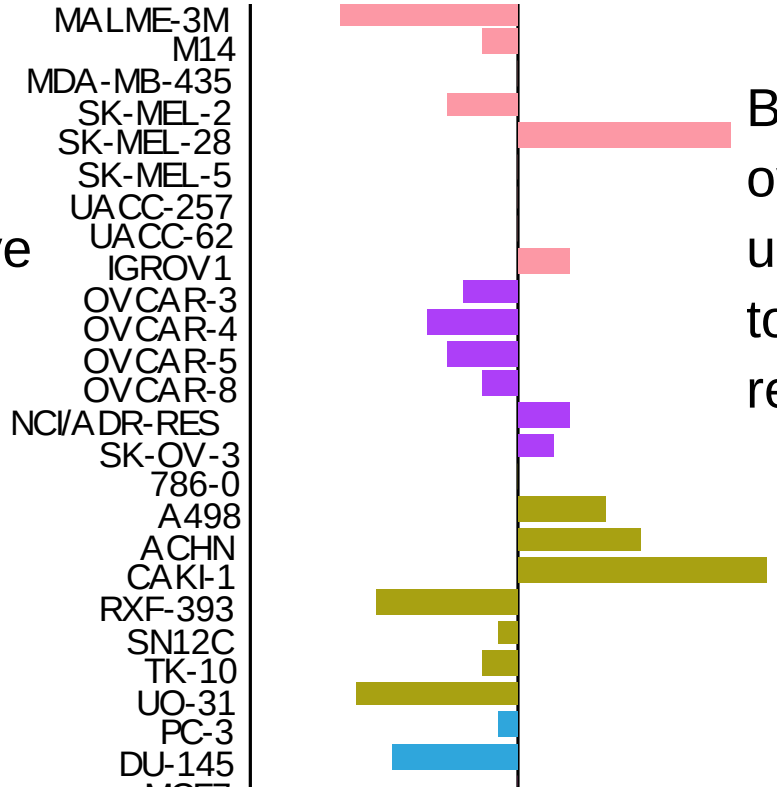
Modeling Therapeutic Combinations in NCI 60

For any 2-drug combination:

Bars to *left* indicate loss of benefit relative to best single-agent results



Bars to *right* indicate overall benefit to using combo relative to best single-agent results

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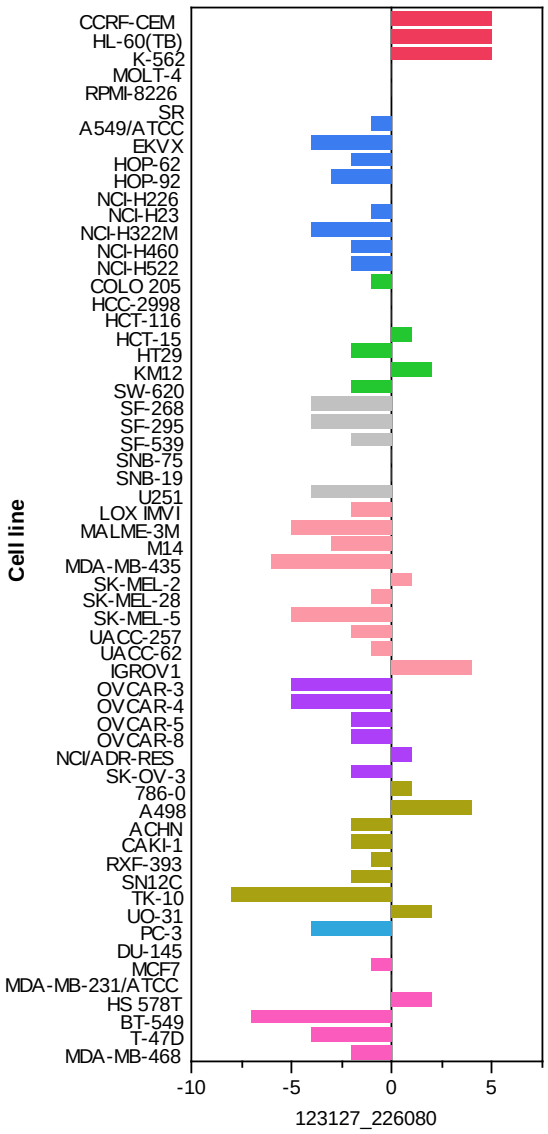
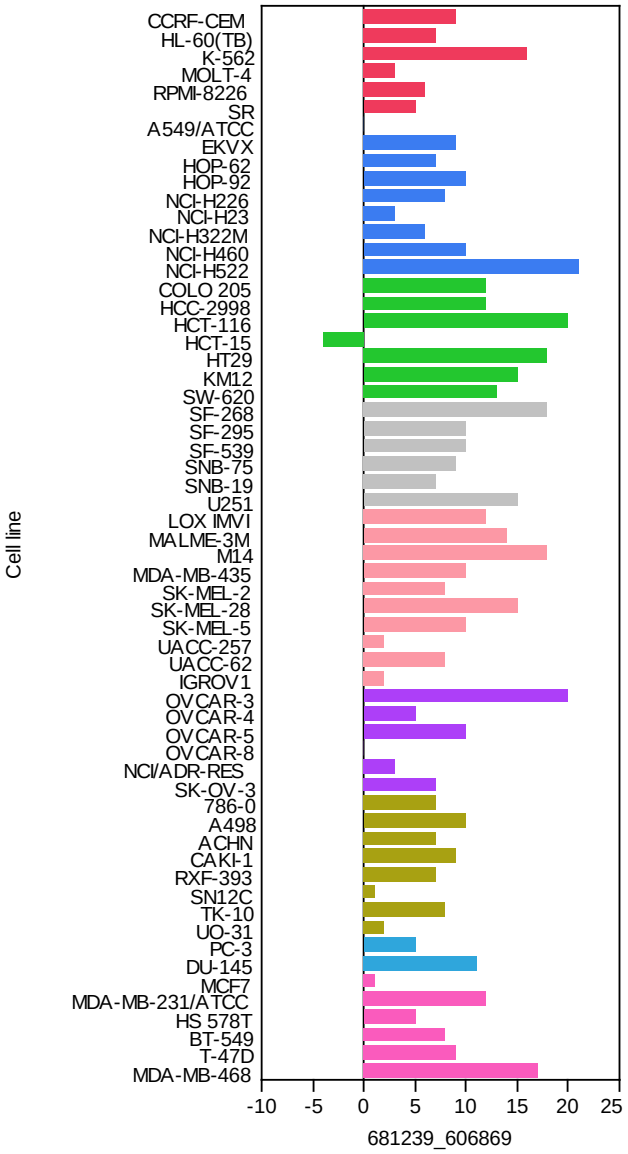
Doxorubicin + Rapamycin



Bortezomib + Cladribine (2CDA)



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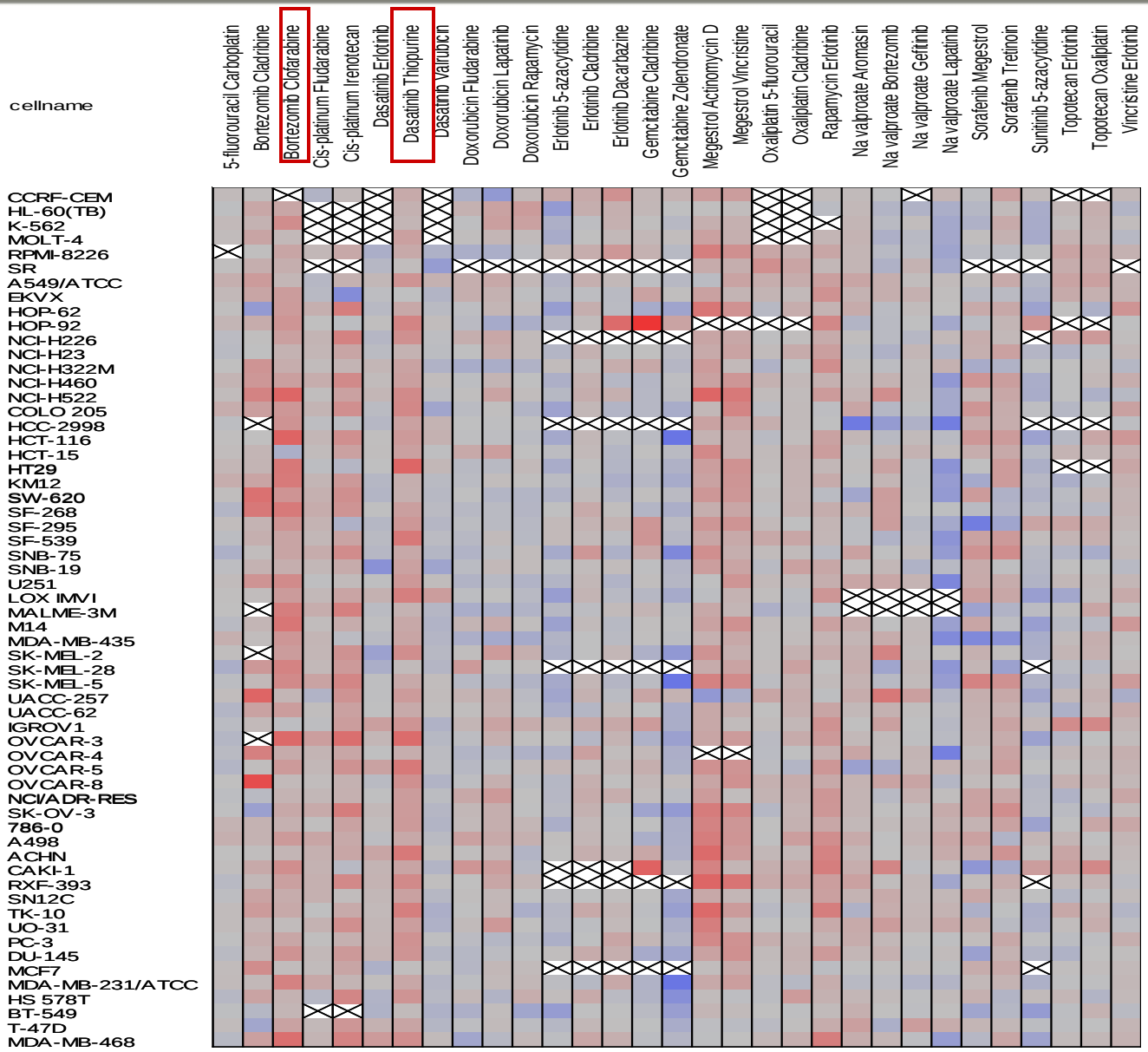
Growth Inhibition Across NCI 60 for Commercially Available Therapeutic Agents



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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

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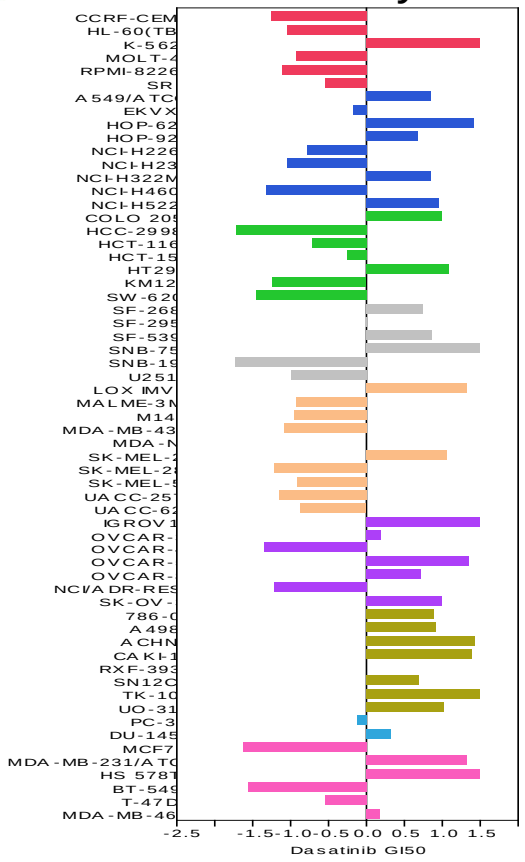
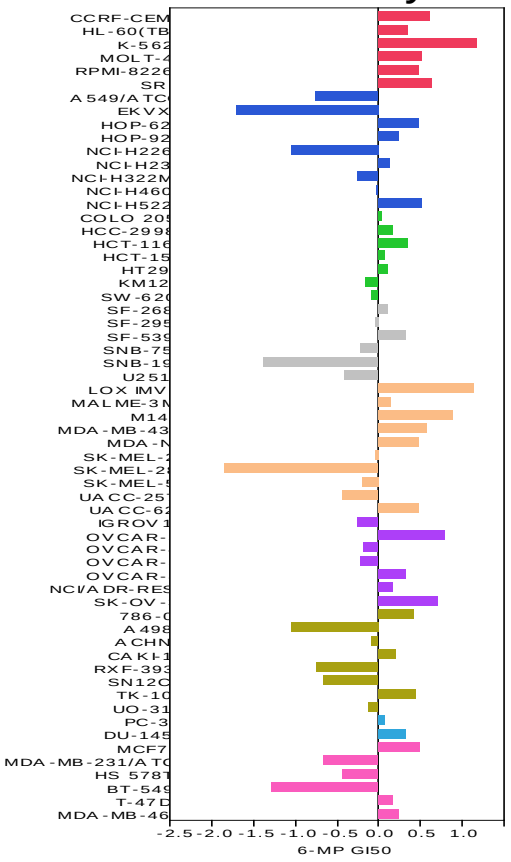
Growth Inhibition by Combination Is Not Predictable from Single Agent Activity

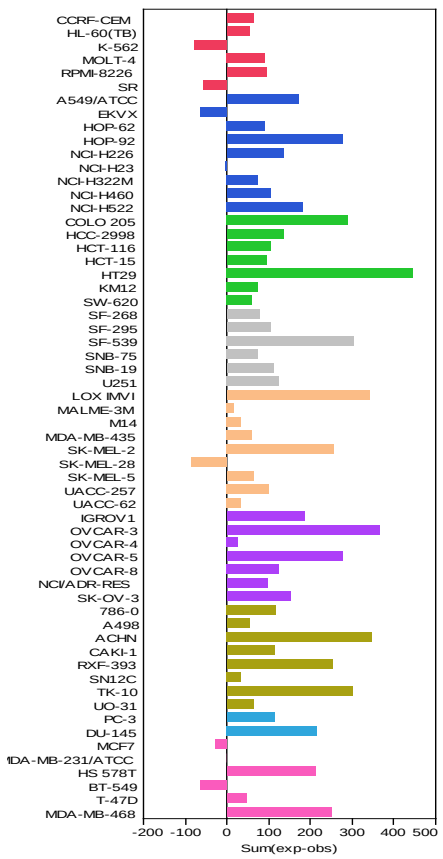
Dasatinib activity



6-MP activity



Combo benefit



	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

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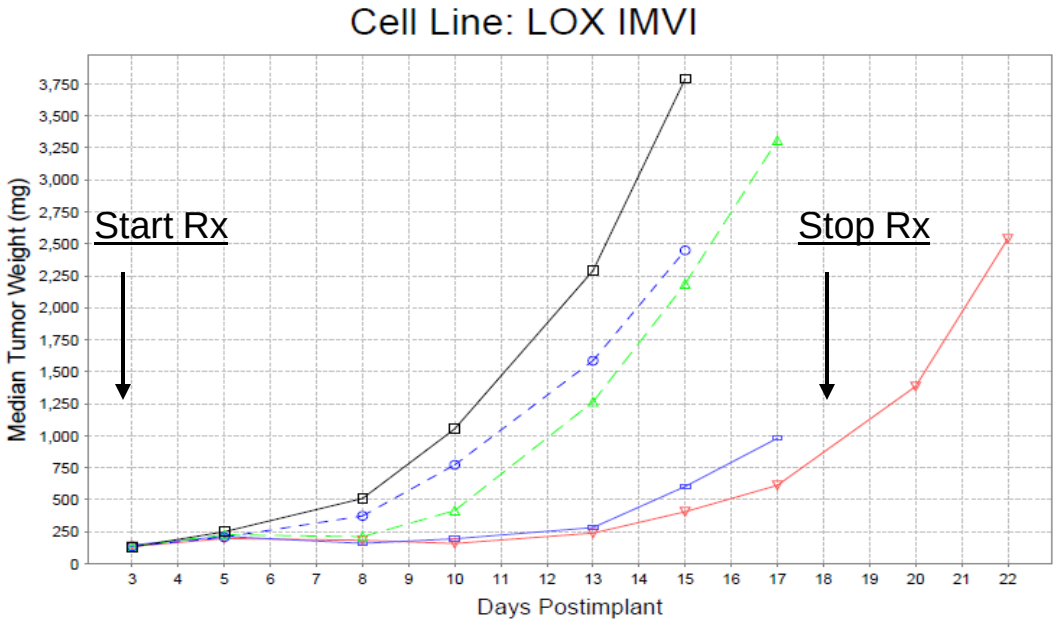
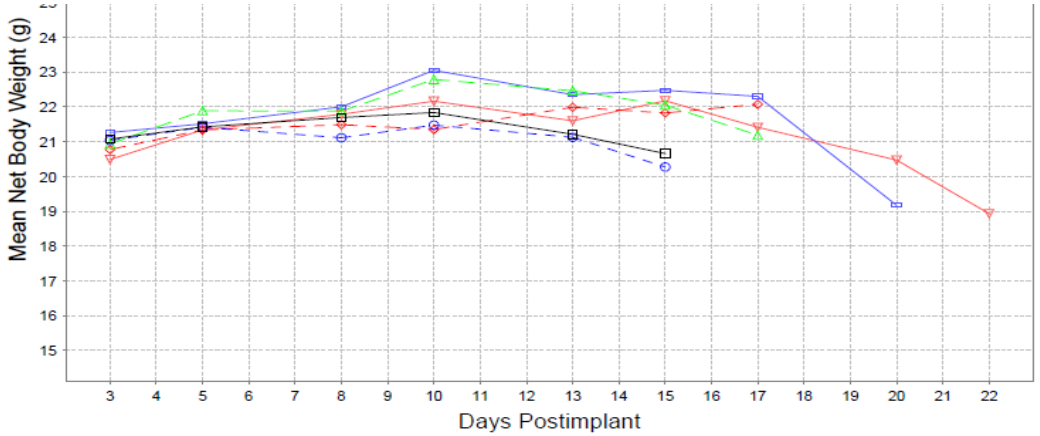
Dasatinib/6-MP Combination: LOX IMVI Melanoma Xenografts





- 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

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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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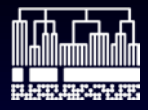
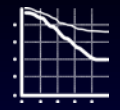
DCTD Division of Cancer Treatment and Diagnosis






CAM

Accelerating Cancer Diagnosis and Drug Development

- **Developmental Therapeutics**

Jerry Collins
Joe Tomaszewski
Melinda Hollingshead
Ralph Parchment
Robert Kinders
Tom Pfister
Jay Ji

- **Center for Cancer Research**

Yves Pommier
Lee Helman
Bob Wiltrout
Shivaani Kummar
William Bonner

- **DCTD**

Jason Cristofaro
Barbara Mrochowski
Michael Difilippantonio

- **CTEP**

Jamie Zweibel
Jeff Abrams

- **Cancer Imaging**

Paula Jacobs

- **Cancer Diagnosis**

Barbara Conley

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Toxicities of Molecular Targeted Combinations

Targets	Regimen	Full doses of individual agents	MTD of the combination (% of full dose)	Main DLTs within 1-2 cycles or after prolonged therapy
VEGF + VEGFR	Bevacizumab + Sorafenib	•BV: 10 mg/kg q2w •Sorafenib: 400mg BID	•BV 5 mg/kg q2w (50%) + Sorafenib 200 mg BID, 5 days on/2 days off (35%) •In patients with RCC: BV 5 mg/kg q2w + sorafenib 200 mg QD (25%)	Hand and foot syndrome; Hypertension; Proteinuria; Thrombocytopenia;
	Bevacizumab + Sunitinib	•BV: 10 mg/kg q2w •Sunitinib: 50 mg/D, 4wks on, 2 wks off	•Full doses based on cycle toxicities •Intolerable with prolonged therapy in RCC patients	HTN, headache. Thrombotic microangiopathy after prolonged therapy in RCC patients
VEGF + mTOR	Bevacizumab + Temsirolimus	•BV: 10 mg/kg q2w •Tem: 25 mg qw	Full doses	Mucositis, hyperlipidemia
	Bevacizumab + Everolimus	•BV: 10 mg/kg q2w •Eve: 10 mg/d	Full doses	
	Temsirolimus + Sorafenib	•Tem: 25 mg qw •Sorafenib: 400 mg BID	Tem: 25 mg qw (100%) + Sorafenib 400 mg BID	Hand-foot syndrome; thrombocytopenia
	Temsirolimus + Sunitinib	•Tem 25 mg qw •Sunitinib: 50 mg/D, 4wks on, 2 wks off	Intolerable despite 40-50% dose reduction of both agents (MTD exceeded)	Rash, thrombocytopenia, asthenia, diarrhea, stomatitis,
VEGF + EGFR	Erlotinib + Bevacizumab	•Erlotinib: 150 mg/d •BV: 10 mg/kg q2w	Full doses	No DLT
	Erlotinib + Sorafenib	•Erlotinib: 150 mg/d •Sorafenib 400 mg BID	Erlotinib: 100 mg/d (67%) + Sorafenib 400 mg BID (100%)	LFT abnl, HFS, diarrhea, lipase abnl
	Tipifarnib + Sorafenib	•Tipifarnib 300 mg BID •Sorafenib: 400 mg BID	Tipifarnib 100mg BID (33%) + Sorafenib 400mg am/200mg pm BID (75%)	Rash, Fever, Diarrhea

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Pre-Clinical Toxicology for Molecular Targeted Combinations