University of Pennsylvania High-Throughput Screening Core

A Discovery Platform for Translation of Innovative Basic Science in Academia

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Philadelphia LRIG Spring Meeting

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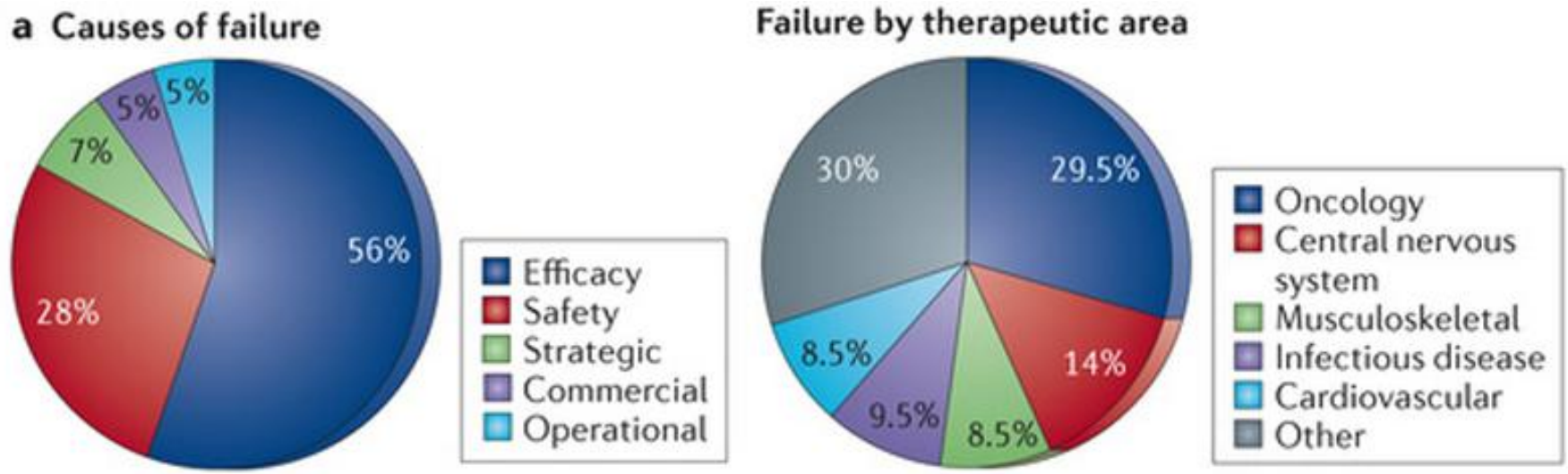
Perelman School of Medicine
University of Pennsylvania

Attrition in Drug Development

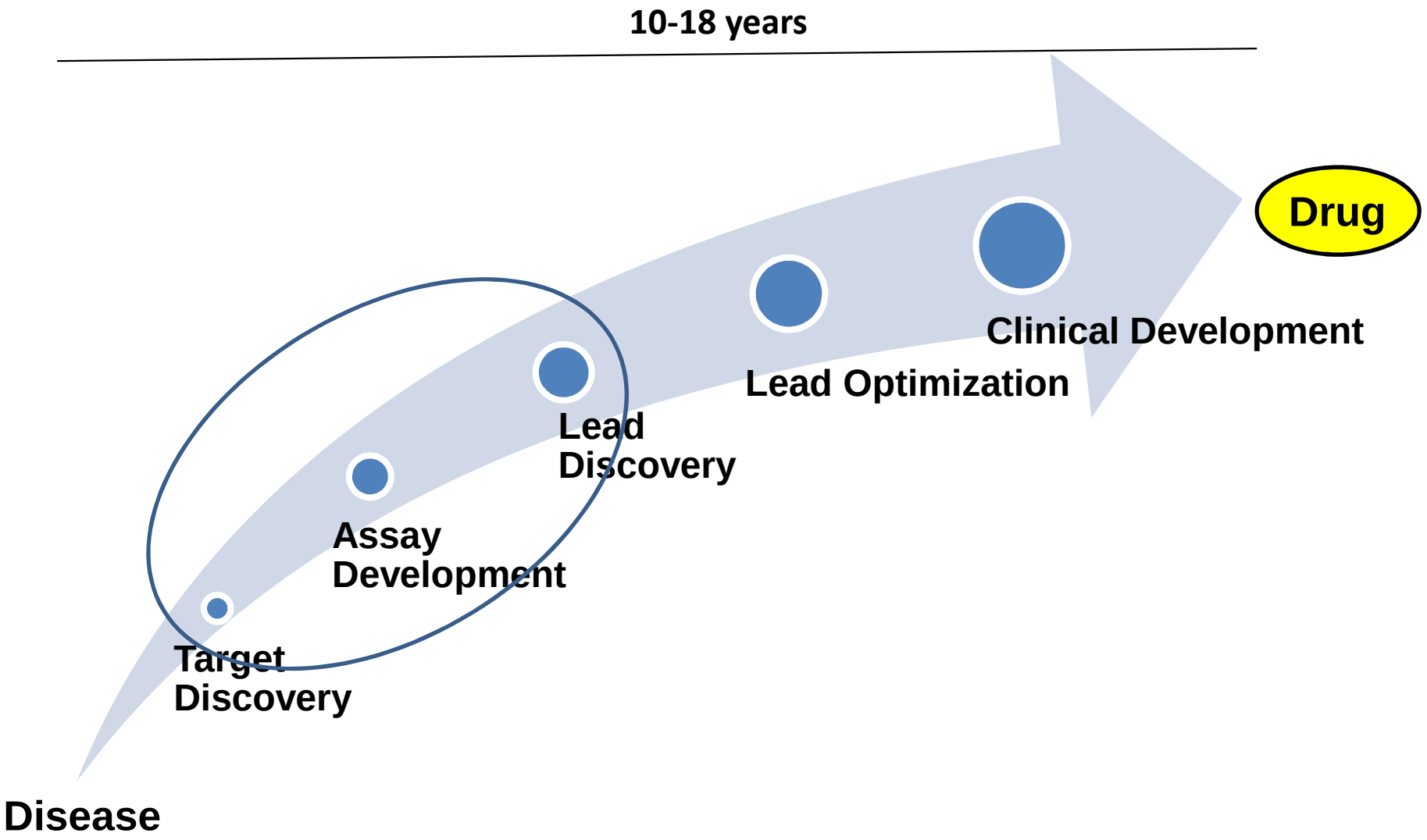
Phase II/III failures 2011-2012



N=145/105

Arrowsmith, Nat Rev Drug Disc. 12, 2013

Steps to Developing a New Drug



Mission

- **To provide SOM investigators the ability to apply genetic approaches to identify cellular components of signaling pathways and study mechanisms of protein function in cellular models of human disease. –Target Identification/Validation**
- **To provide high-throughput screening of chemical libraries in high-density (i.e. 384) microtiter well plates to identify and assess the activity of organic small molecule modulators of protein function or disease biology.- Discovery**
- **Develop novel technology to support HTS at Penn (e.g. new assays, unusual cell types, unique biology)**
- **Seed collaborative research programs in thematic areas of unmet medical challenge.**

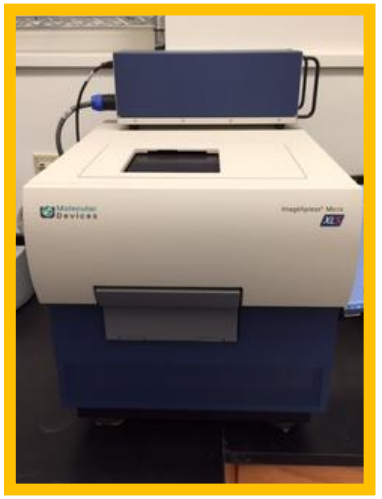
SOM Screening Core Infrastructure

- **Automated pipetting workstations**
  - Janus Verispan 8-tip and MDT
  - Bulk Reagent Dispensers
  - ELx405 microplate washer
- **Detection Systems**
  - EnVision multi-mode microplate reader
  - ImageXpress Micro XLS
  - FLIPR Tetra screening system
- **BSL2 Tissue Culture** capabilities (hood &incubators)
- **Accessories** (Plate sealers, Barcode readers & Stackers)
- **Informatics**

EnVision

Dispensor

Janus



ImageXpress

SOM Screening Core Library Resources

Chemical Libraries

Bioactives, FDA approved, and FDA-like compounds

- SelleckChem Bioactives (~2100)
  - Kinase Inhibitors, Epigenetic Inhibitors, Cancer compounds, GPCR/Ion Channel, Metabolism, Microbiology, FDA approved/FDA-like
- LOPAC (1280): Library of Pharmacologically Active Compounds

Natural Products

- Microsource Purified Natural Products (800)

Diversity sets

- *TBD (50,000-100,000) compounds*

SOM Screening Core Library Resources

Genetic Libraries

siRNA

- human genome-wide, human drugable genome, human GO categories
- user-defined human and mouse

Non-coding RNAs

- lncRNAs (human)
- miRNA mimics/antagonists (human)

Human TRC 2.0 and Mouse TRC1.0 Lentivirus shRNA library

- Screening pools: GO categories; user-defined sets
- Order groups/individuals

MGC cDNA collection (CMV-driven)

- 18,000 full length, sequenced, mouse and human (arrayed);
- user-defined sets
- Order groups/individuals

What services can we provide?

- **Assay Development** (biochemical, cell, & high-content)
  - Consultation (technology assessment, assay design);
  - Optimization & miniaturization;
  - validation
- **High-throughput screening**
  - Pharmacologically active cmpds,
  - diversity collections,
  - focused libraries (e.g. annotated inhibitors),
  - siRNA, cDNA, shRNA
- **Pharmacological profiling**
  - Pathway inhibitor screening,
  - Structure-activity relationship studies,
  - synergism studies,
  - molecular mechanism of action
- **Grant preparation**
  - Letters of support, experimental design section

Assays

- **Enzymatic**
  - Luminescent and Flouresence Intensity based
  - Radioactive
  - Flouresence polarization (FP)
- **Protein: Protein, Protein: Nucleic Acid interactions**
  - ELISA
  - Flouresence polarization (FP)
  - Alphascreen technology
- **Biophysical**
  - Thermal stability/Differential Scanning Flourimetry (DSF)
  - Label-free SPR
- **Luciferase Reporter Gene Assays**
- **Metabolic Viability** (Cell Titer Glo, MTS, Alomar Blue)
- **AlphaLISA (biomarker expression-e.g. P-antigens, secreted proteins)**
- **Phenotypic/High Content Assays**
  - Live/Dead
  - Autophagy
  - EdU incorporation
  - Viral Infection
  - Migration/invasion
  - Lipid accumulation

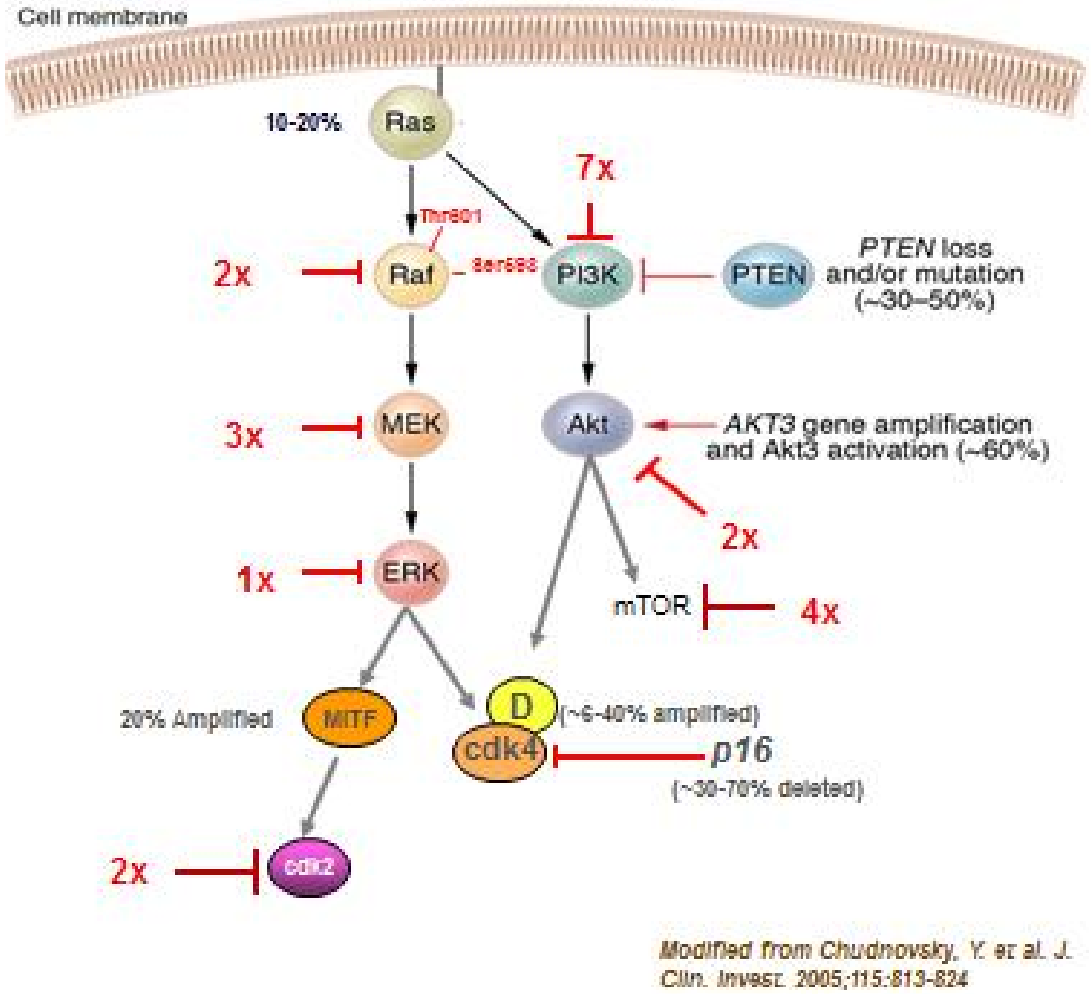
Funding Opportunities

- **NIH**
- **NCAT/TRND opportunities**
- **Foundations (**e.g. Welcome Trust, Melanoma Research Foundation, Leukemia/Lymphoma Society, Gates,Cystic Fibrosis, StandUp2Cancer, Children's Tumor Foundation**)**
- **Commercial** (e.g. Bayer Grants4targets, Astrazeneca Openinnovation)
- **Institute/Center/Program Pilot project funds**
  - Institute for Immunology
  - Center for Orphan Disease Research
  - Institute for Regenerative Medicine

Profiling and Sensitivities

- Phenotypic profiling of cell lines
  - FDA and FDA-likes
  - Annotated gene family (e.g. kinome)
  - Synthetic lethality screens
  - Synergy studies (combinations gene-gene; gene-drug; drug-drug)
- Across tumors (e.g. melanomas)
- Of a particular patient tumor line (to define responsiveness)

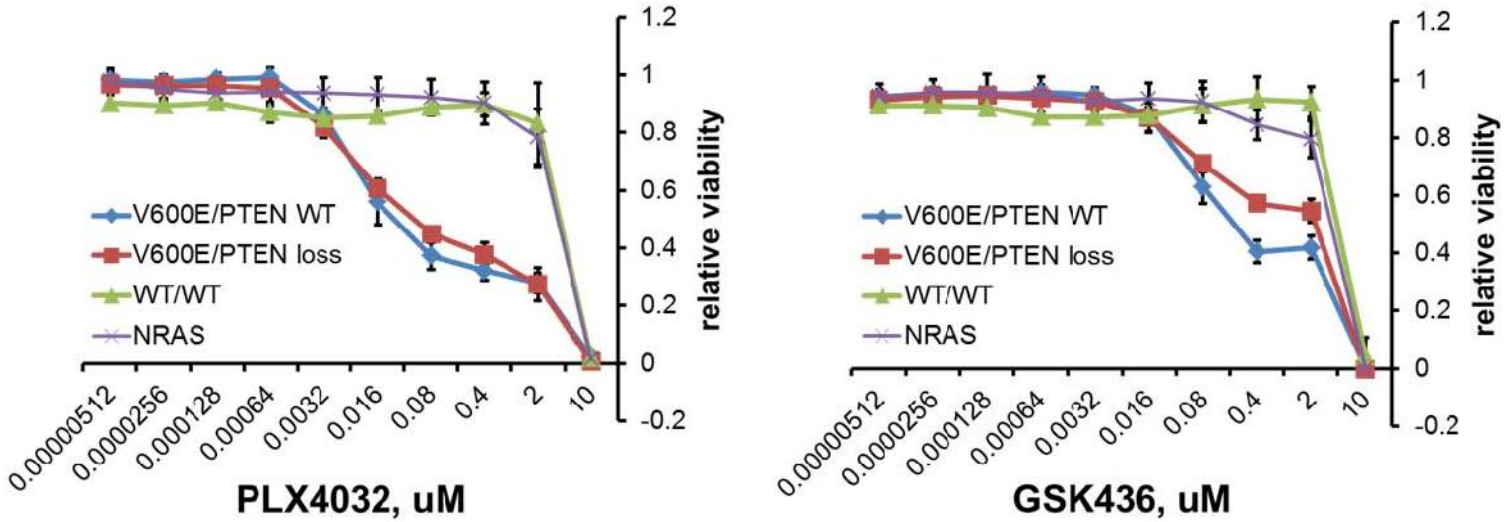
Targets for Melanoma Therapy



Modified from Chudnovsky, Y. et al. J. Clin. Invest. 2005;115:813-824

How can we better predict initial responses to molecular targeted therapies and/or design rational therapeutic combinations for durable responses?

Profiling Drug Activity



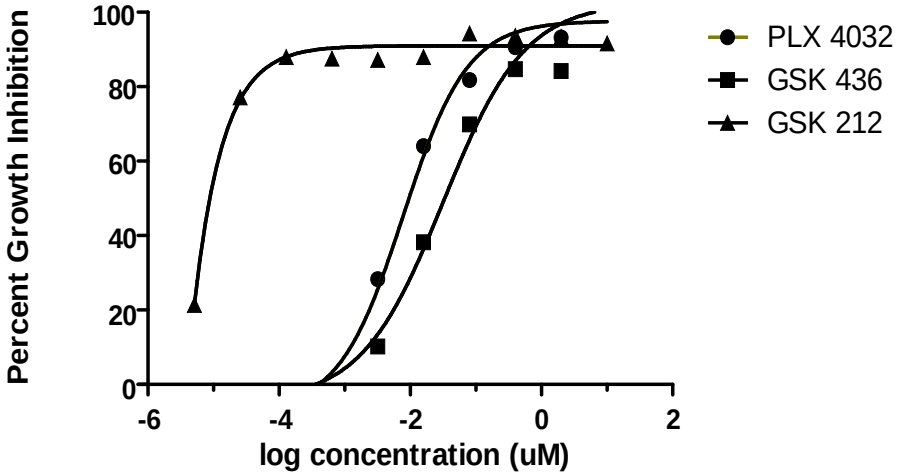
Meenhard Herlyn

THE WISTAR INSTITUTE
TODAY'S DISCOVERIES – TOMORROW'S CURES

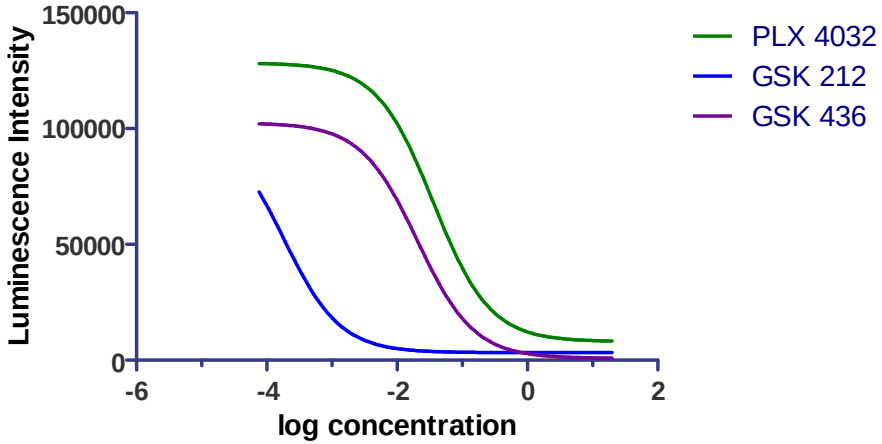
Multiplex analysis of Cell Viability and Biomarker levels

WM164 ($BRAF_{V600E}$, $PTEN_{WT}$)

Viability (resazurin)



AlphaLISA (pERK)



Grace Heck

Improving Clinical Efficacy with Combinations- Synergy screens

- Drug combinations present opportunities to develop therapeutic strategies with clinical efficacy.

  - A combination drug product (with low doses of each drug) may achieve a desired level of efficacy with a low side effects profile if each compound is associated with biologically different and independent side effects.

  - A disease may have two biological pathways which each can be blocked by a different drug

    - Improved efficacy for altered physiology (asthma: bronchoconstriction and inflammation)
    - Improved management of infectious agents such as bacteria and viruses (HIV: HAART).
    - Improved kill rates for cancer cells/ **reduce resistance**.

Searching for Drug Synergy

Drug B

Drug A

Fraction Affected

WM88	2	0.4	0.08	0.016	0.0032	0
2	100	100	100	99	99	81
0.4	100	100	99	99	99	69
0.08	100	100	99	99	97	56
0.016	100	99	98	97	95	44
0.0032	100	99	97	97	94	36
0	99	90	86	75	53	7

WM164	2	0.4	0.08	0.016	0.0032	0
2	97	93	89	87	84	67
0.4	98	92	88	86	82	62
0.08	98	91	87	83	76	51
0.016	97	88	83	80	65	25
0.0032	97	84	78	78	64	16
0	92	66	61	44	24	4

451Lu	2	0.4	0.08	0.016	0.0032	0
2	88	78	75	70	71	57
0.4	87	77	73	70	64	51
0.08	87	74	72	68	58	39
0.016	87	72	66	66	51	15
0.0032	85	69	63	62	53	8
0	77	53	48	32	19	0

Percent Inhibition

Drug B

Percent Excess over Bliss

WM88	2	0.4	0.08	0.016	0.0032	0
2	1	2	2	4	8	0
0.4	0	3	4	7	13	0
0.08	1	4	6	10	18	0
0.016	1	5	6	11	22	0
0.0032	1	5	6	13	24	0
0	0	0	0	0	0	0

WM164	2	0.4	0.08	0.016	0.0032	0
2	0	4	3	6	10	0
0.4	1	5	3	7	12	0
0.08	1	8	6	11	15	0
0.016	3	13	12	23	22	0
0.0032	3	13	11	25	28	0
0	0	0	0	0	0	0

451Lu	2	0.4	0.08	0.016	0.0032	0
2	-2	-1	-3	-1	6	0
0.4	-2	0	-2	3	3	0
0.08	1	3	4	9	7	0
0.016	6	12	11	24	20	0
0.0032	7	12	12	25	28	0
0	0	0	0	0	0	0

% excess over Bliss

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Acknowledgments

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