DRUG DISCOVERY

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National Cancer Institute

OUTLINE OF PRESENTATION

- ***General Introduction***
- Definition of Drug Targets
- Generating Diversity
- Definition of Lead Structures
- Qualifying Leads for Transition to Early Trials

DRUG DISCOVERY: WHERE HAS IT WORKED?

Majority of Drug Targets:	% Top Sales
- G-Protein Coupled Receptors	18
- Nuclear (Hormone) Receptors	10
- Ion Channels	16
- Enzymes	~50

Problem:

How to choose target likely to succeed especially if directed at new target (e.g. protein-protein interactions)?

Nature 384 suppl 11:5, 1996

DRUG DISCOVERY: A SUCCESSION OF STYLES

Antiquity to 1960s:

Mixtures of natural products vs. bioassays
(e.g., digitalis, rauwolfia, penicillins, anthracyclines, vinca, taxol, camptothecins)

1930s to present:

Pure compounds vs. bioassays
(e.g., sulfas, diuretics, hypoglycemics, antiHBP)

1960s to present:

Pure compounds vs. pure enzymes
(e.g., ACE inhibitors, cholesterol-lowering statins, RT and protease inhibitors)

1980s to present:

Combinatorial methods to bring mixtures of compounds vs. many targets

WHY COMPOUNDS FAIL AND SLOW DOWN IN DEVELOPMENT

Reasons for failure

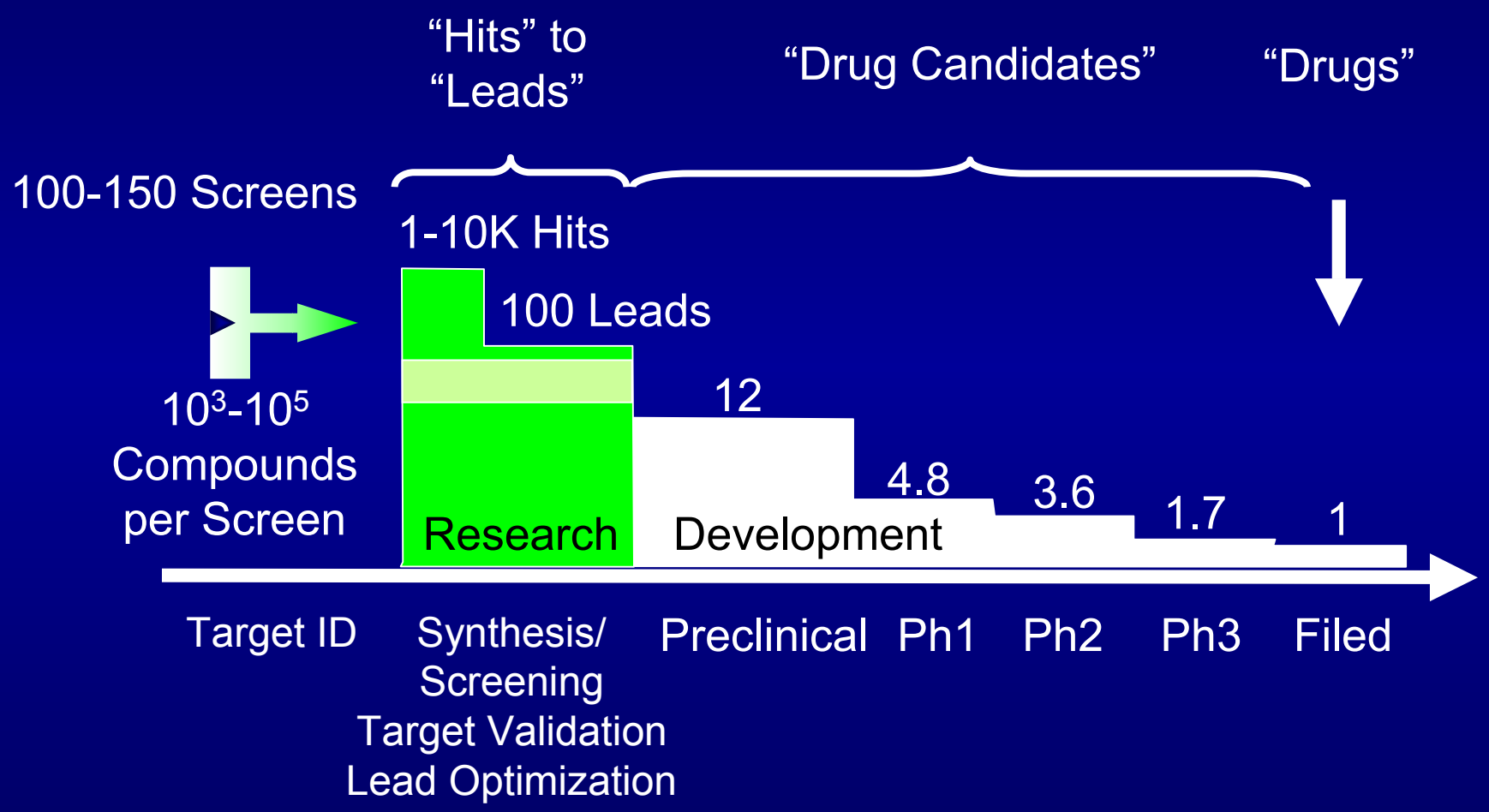
- Toxicity, 22%
- Lack of efficacy, 31%
- Market reasons, 6%
- Poor biopharmaceutical properties, 41%

Reasons for slowdown

- Synthetic complexity
- Low potency
- Ambiguous toxicity finding
- Inherently time-intensive target indication
- Poor biopharmaceutical properties

Modern Drug Discovery
January/February 1999
Modern Drug Discovery, **1999**, 2 *(1)*, 55-60.

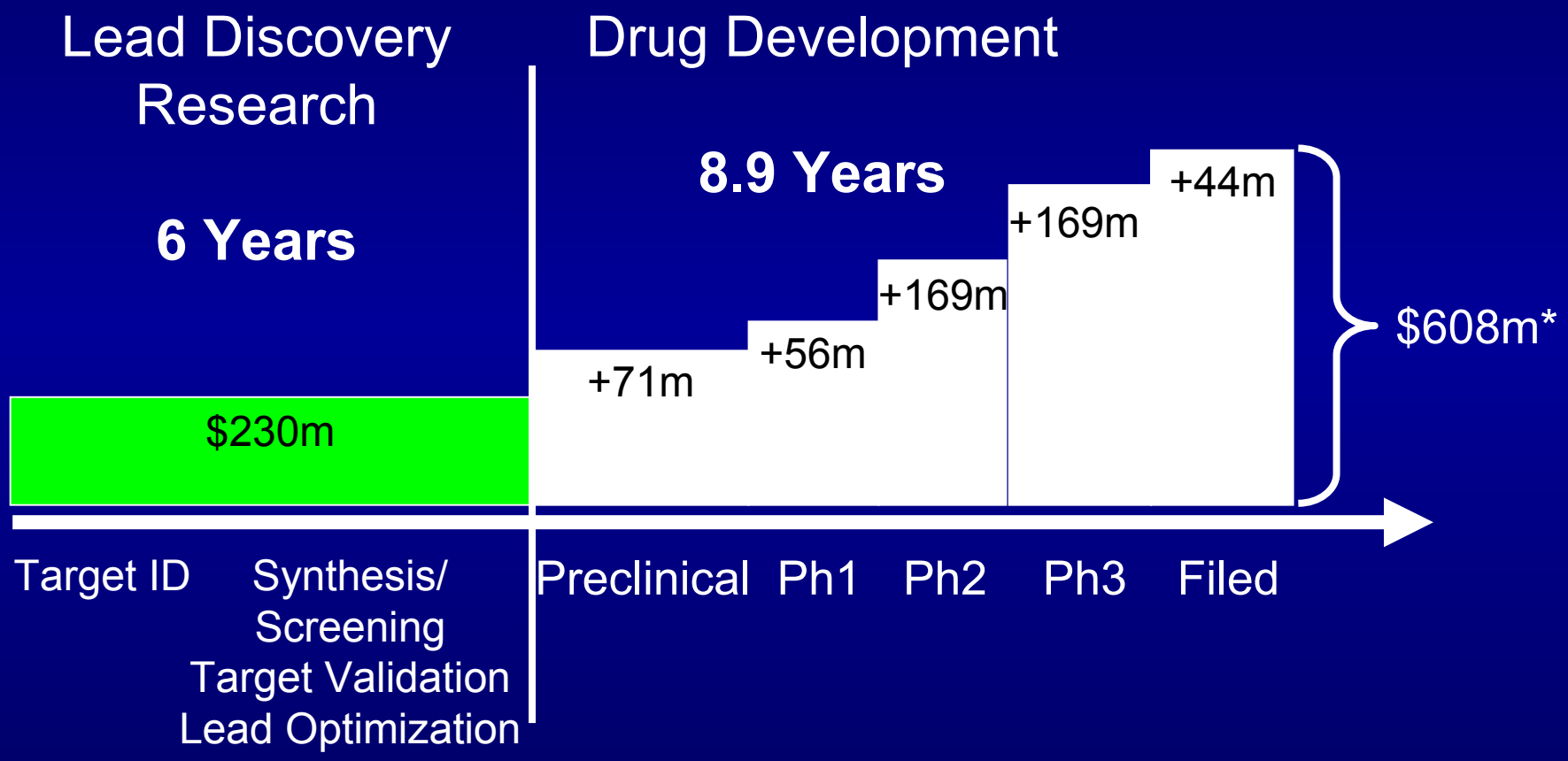
TRADITIONAL PHARMACEUTICAL R&D
Suffers High Attrition*



** Tufts CSDD, H&Q 1998; The Pfizer Journal, 1/2000*

TRADITIONAL PHARMACEUTICAL R&D

Costly* and Time Consuming**



** Lehman Brothers, 1997; ** Tufts CSDD*

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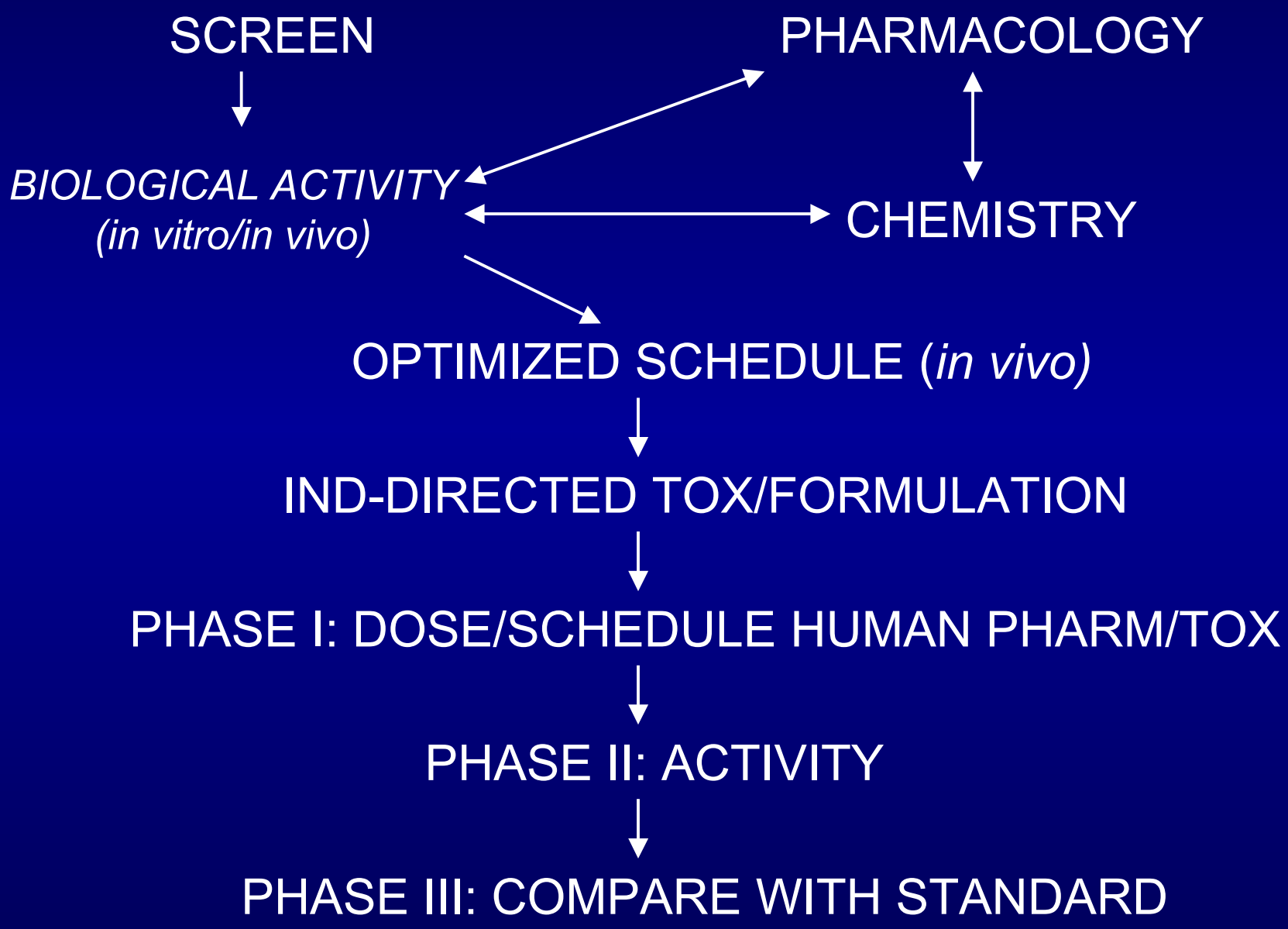
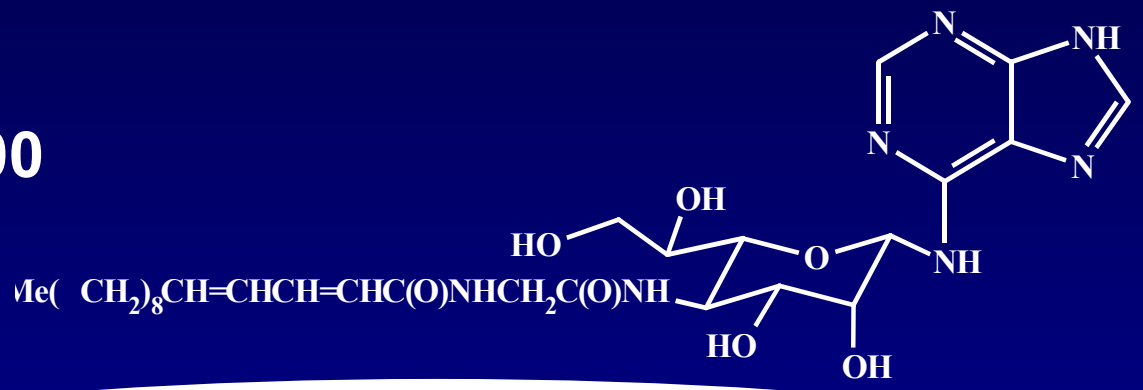
"EMPIRICAL" DRUG DISCOVERY



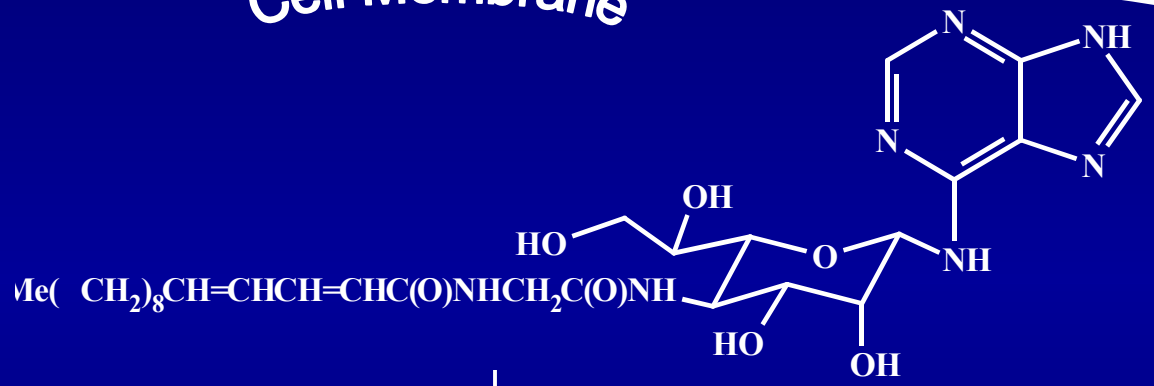
PROBLEMS WITH EMPIRICAL MODELS

- Lack of predictive power *in vivo*
- Poor correlation of non-human with human pharmacology
- Divorced from biology: How to optimize?

KRN5500

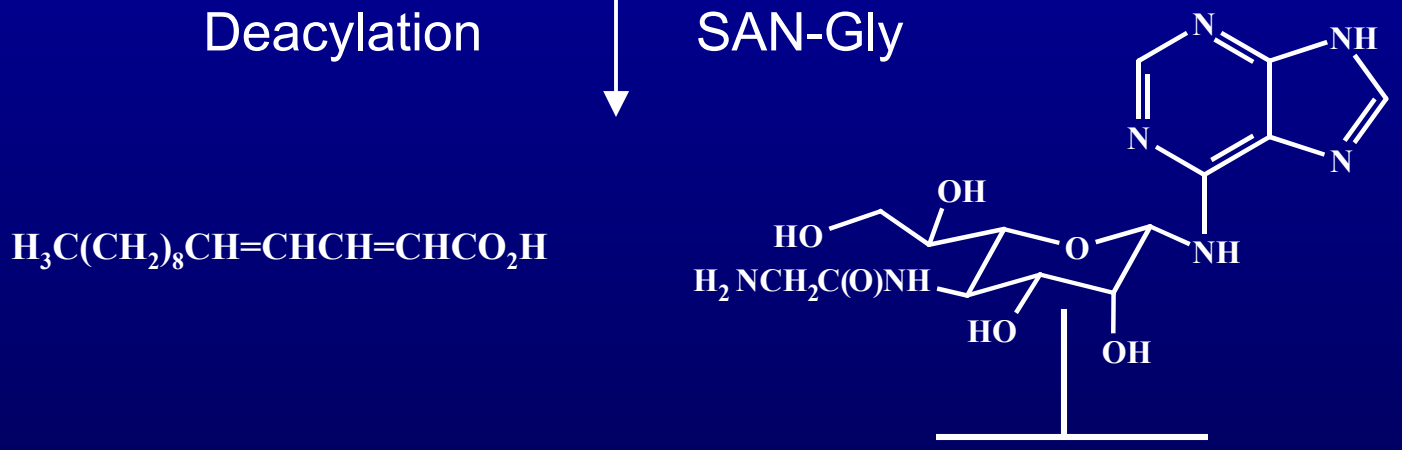


Cell Membrane



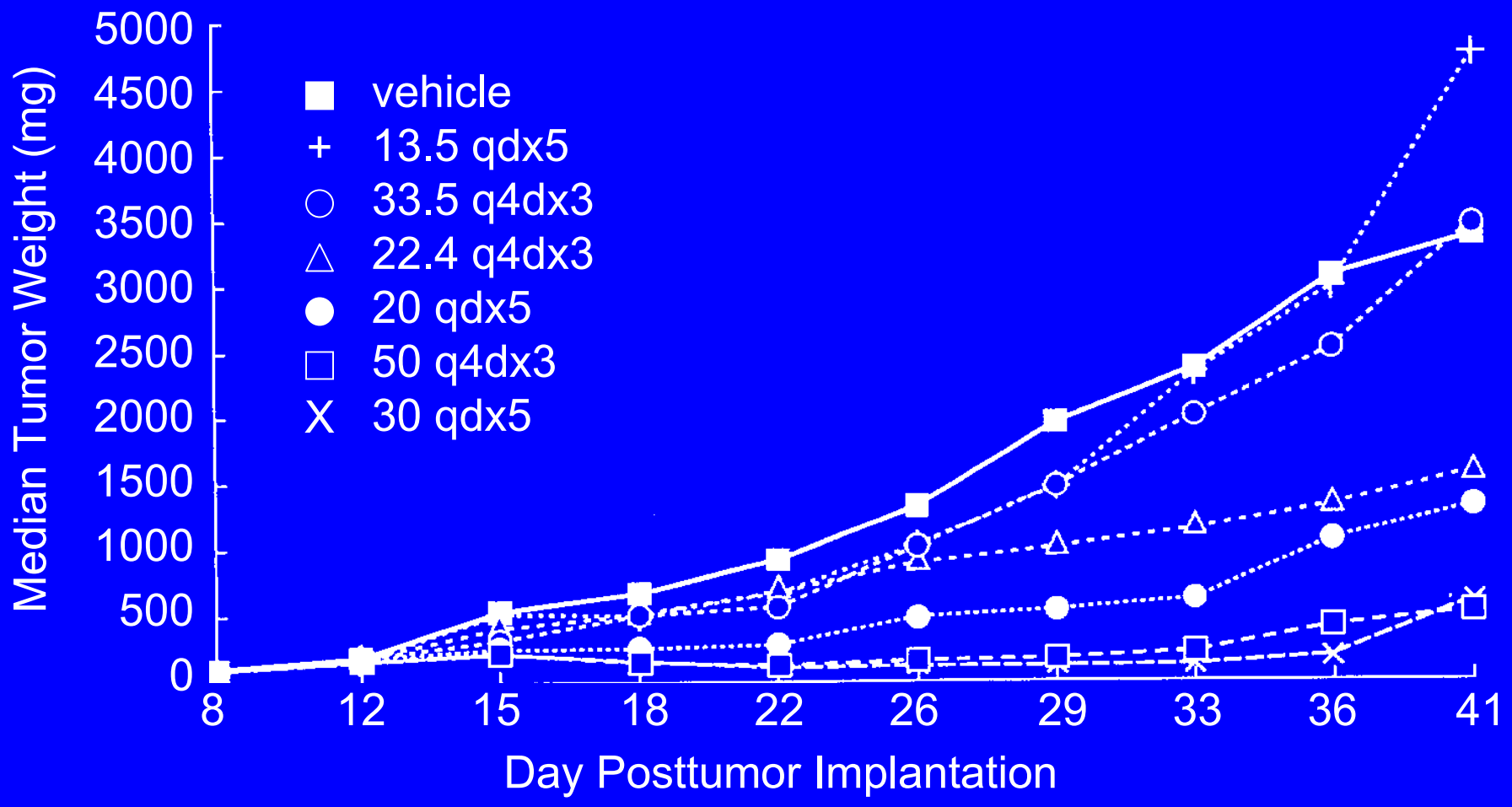
Deacylation

SAN-Gly

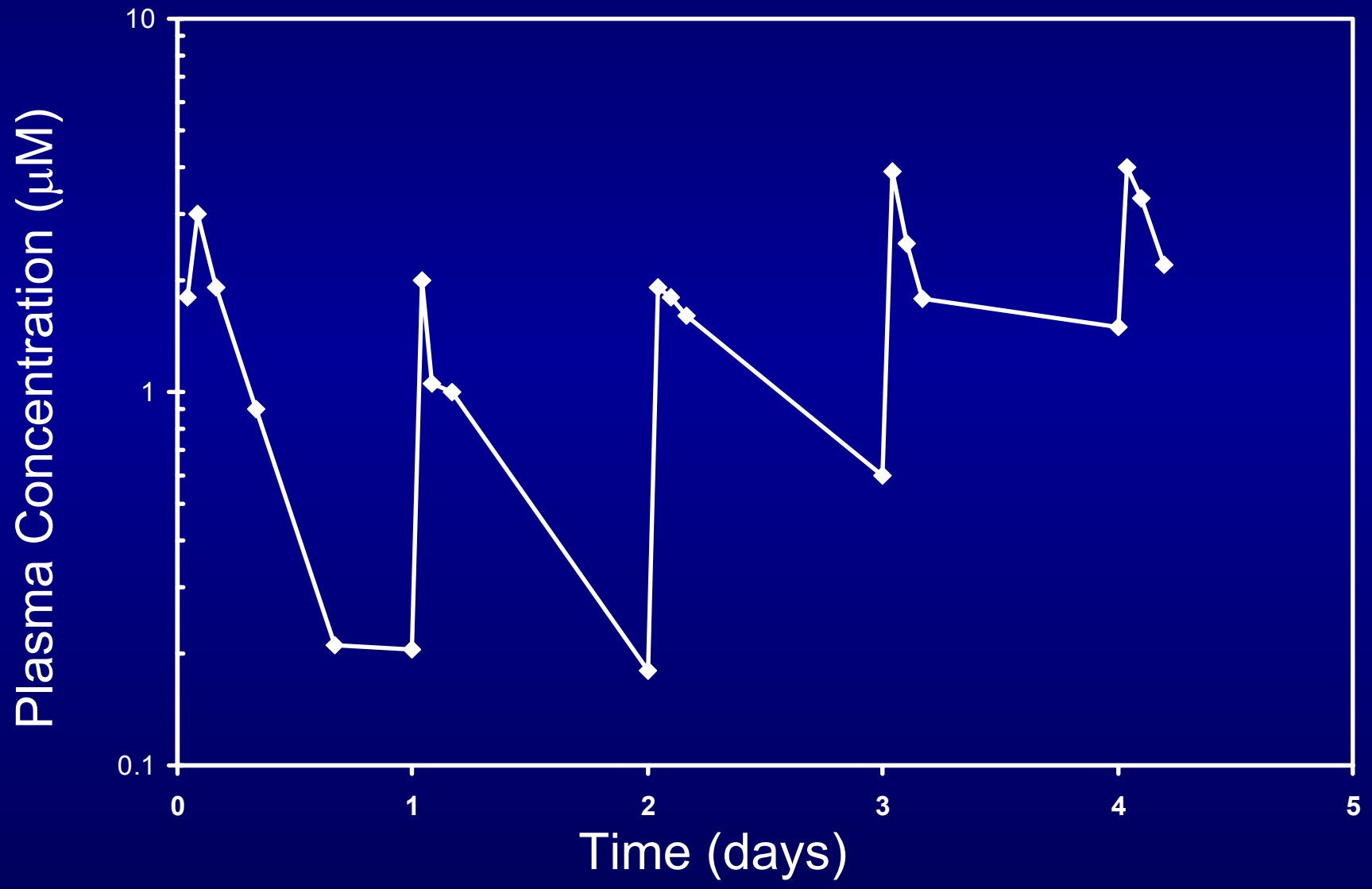


Protein Synthesis

EFFECT OF KRN5500 ON COLO-205 ATHYMIC MOUSE XENOGRAFTS



KRN5500 PLASMA CONCENTRATIONS ON EFFECTIVE SCHEDULE(20 MG/KG/D) IN MICE



SUMMARY OF KRN-5500 PHASE I

- 26 patients as IV once per day over 5 days
- Dose limiting toxicity = interstitial pneumonitis
- MTD = 2.9 mg/M^2/d x 5
- Achieve only 0.75 - 1 μM at 3.7 mg/M^2/d x 5
- 4/6 patients with >25% incr C_{max} have grade 4 toxicity

Data of J. P. Eder, DFCI

"RATIONAL" DRUG DISCOVERY

MOLECULAR TARGET SCREEN ⟷ PHARMACOLOGY

Biochemical
Engineered cell
Animal (yeast/worm/fish)

(to affect target)

↕

CHEMISTRY

↓

TARGET-DEPENDENT *IN VIVO* MODEL

↓

IND DIRECTED TOX/FORM

↓

PHASE I: DOSE/SCHEDULE: HUMAN PHARM/TOX;
? AFFECT TARGET

↓

PHASE II: ACTIVITY = ? AFFECT TARGET

↓

PHASE III: COMPARE WITH STANDARD; STRATIFY
BY TARGET?

MOLECULAR TARGET DEFINITION - HOW TO?

- **BIOLOGY:**
  - * Cytogenetics → Breakpoints → Molecules (bcr-abl)
  - * "Positive" selection from tumor DNA → Active oncogenes (signal transduction)
  - * Tumor gene expression profiling (CGAP)

- **" RETROFIT" ACTIVE MOLECULES:**
  - * Binding partners (geldanamycin, rapamycin, fumagillin)
  - * Computational algorithm (molecule ↔ target)
    - COMPARE
    - Cluster analysis

- **"CLASSICAL:"**
  - * Cell metabolism / Biochemistry
  - * Suggest single targets → Inefficient; Medicinal Chemistry possible

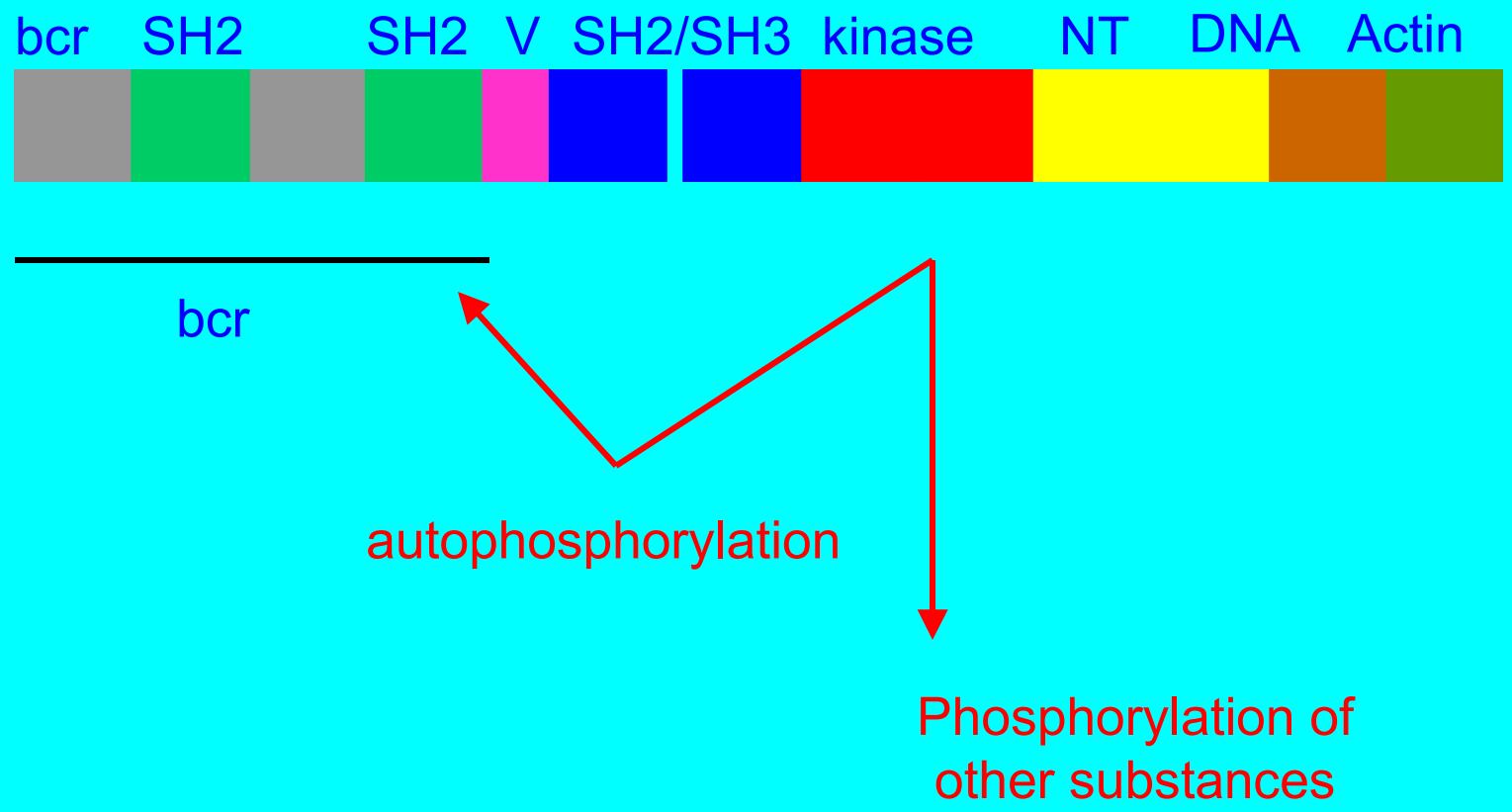
- **CHEMICAL GENETICS:**
  - * Libraries of molecules and precisely defined organisms

bcr-abl AS TARGET: RATIONALE

- Apparently pathogenetic in t9:Q22 (Ph+) CML/ALL
- Absence in normal tissues
- Modulate signal transduction events downstream

  Maintenance of chronic phase
  Adjunct to bone marrow transplantation

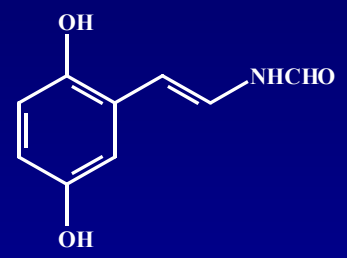
bcr-abl FUSION PROTEIN



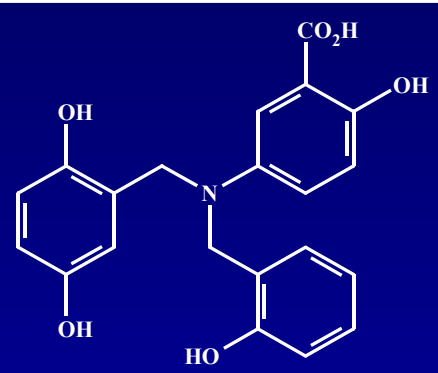
McWhirter JR, EMBO *12:1533, 1993*

EXAMPLE OF "RATIONAL" APPROACH: bcr-abl directed agents

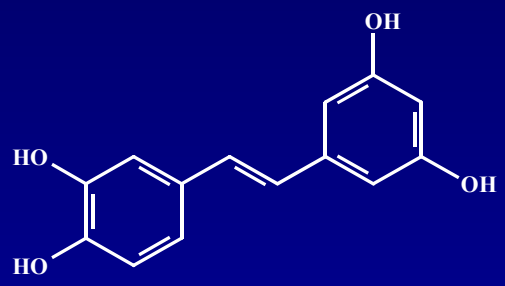
Natural product empiric lead



erbstatin



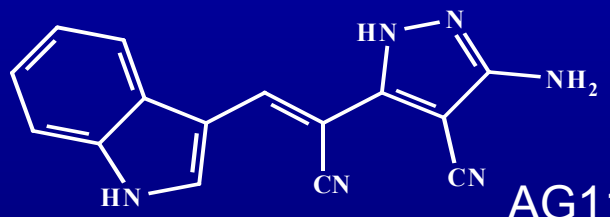
lavendustin



piceatannol

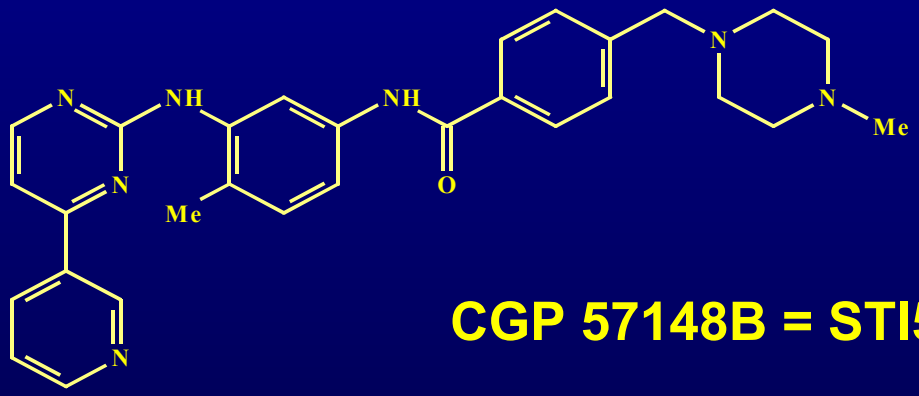
1st generation synthetic

AG957



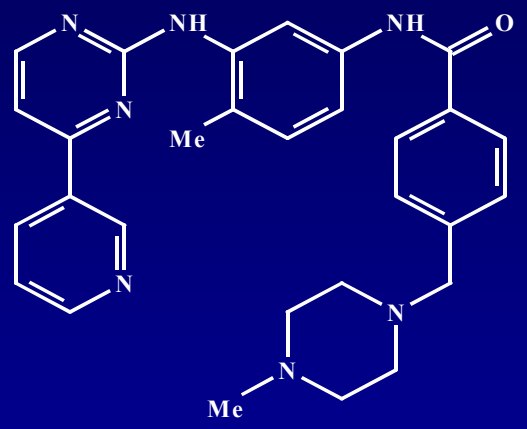
AG1112

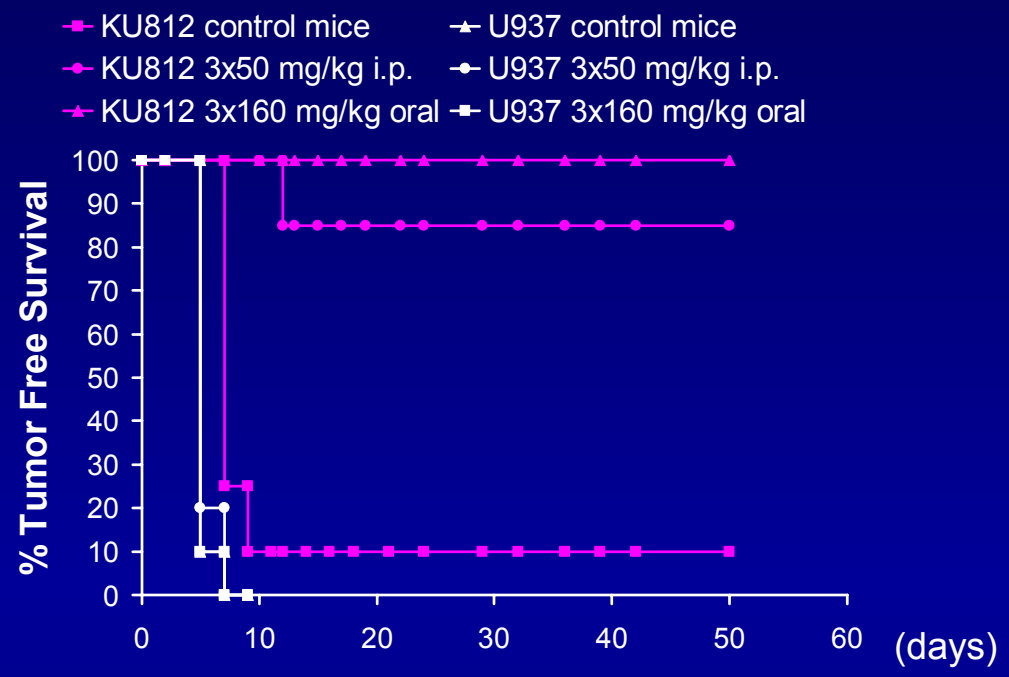
2nd generation synthetic; in clinic

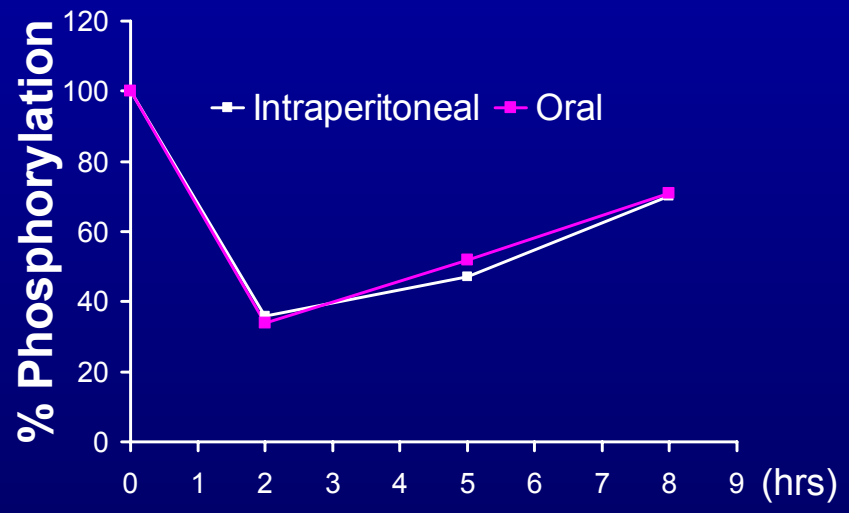


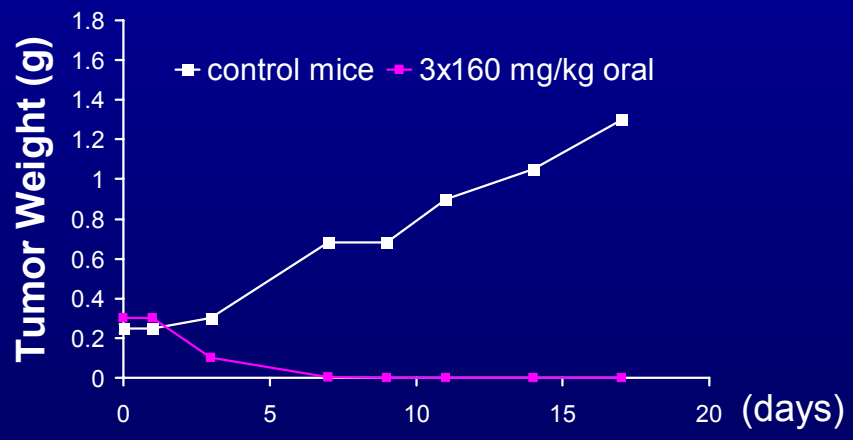
CGP 57148B = STI571

STI571: An oral in vivo bcr-abl kinase inhibitor









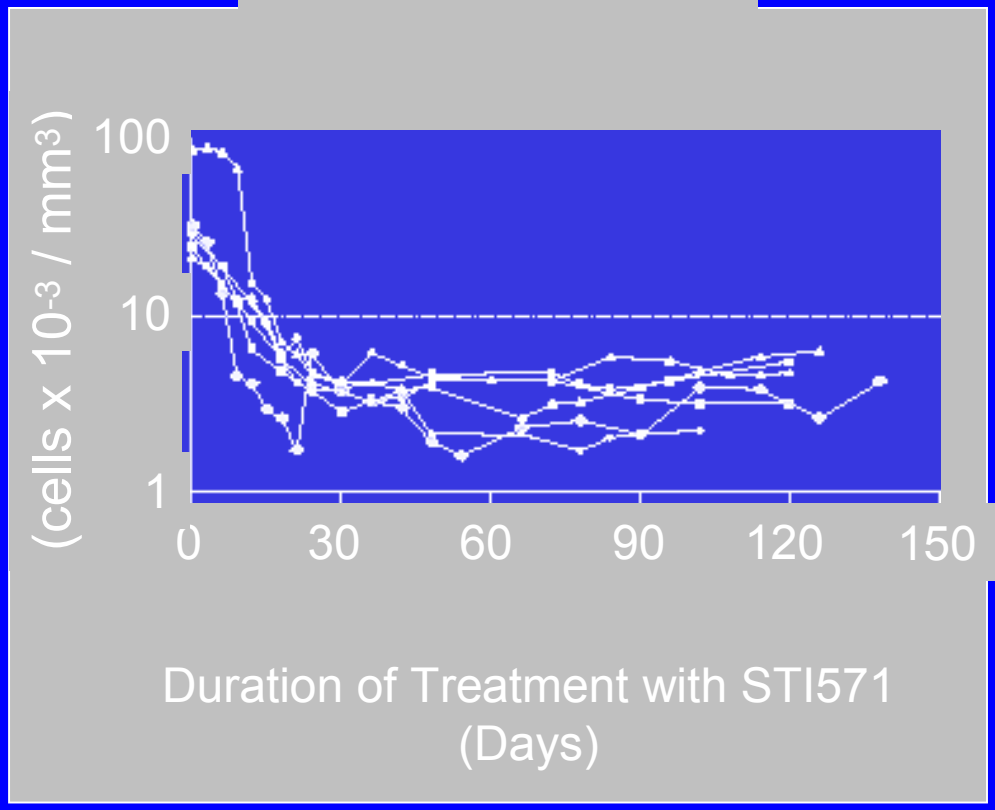
Tyr phosphorylation *in vivo*

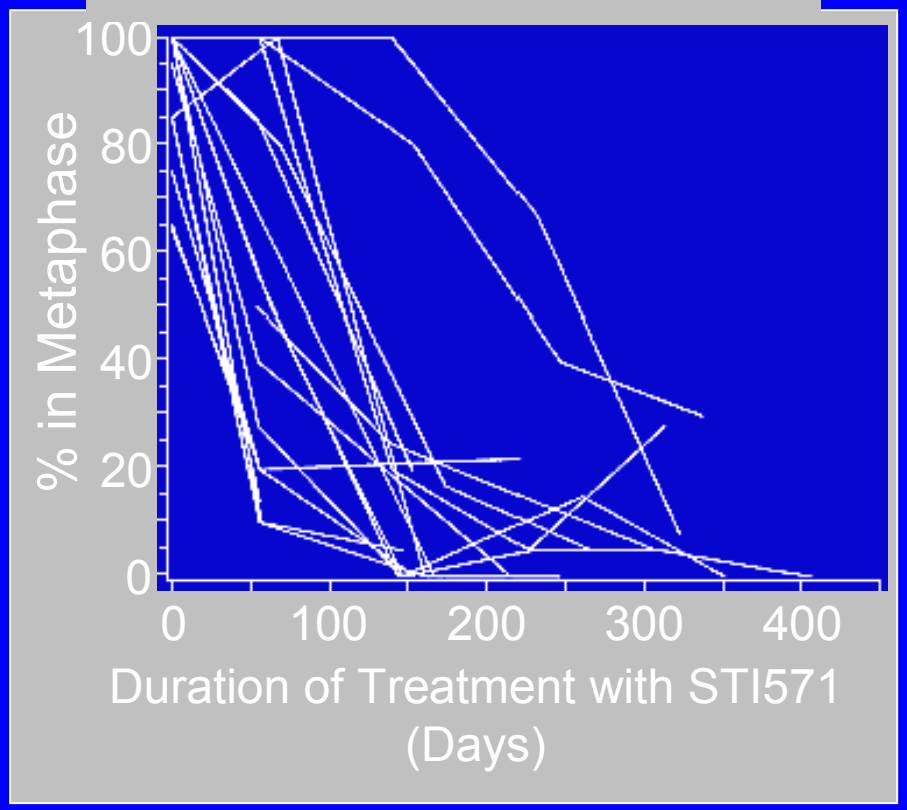
Antitumor activity *in vivo*

le Coutre et al, JNCI 91:163, 1999

EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

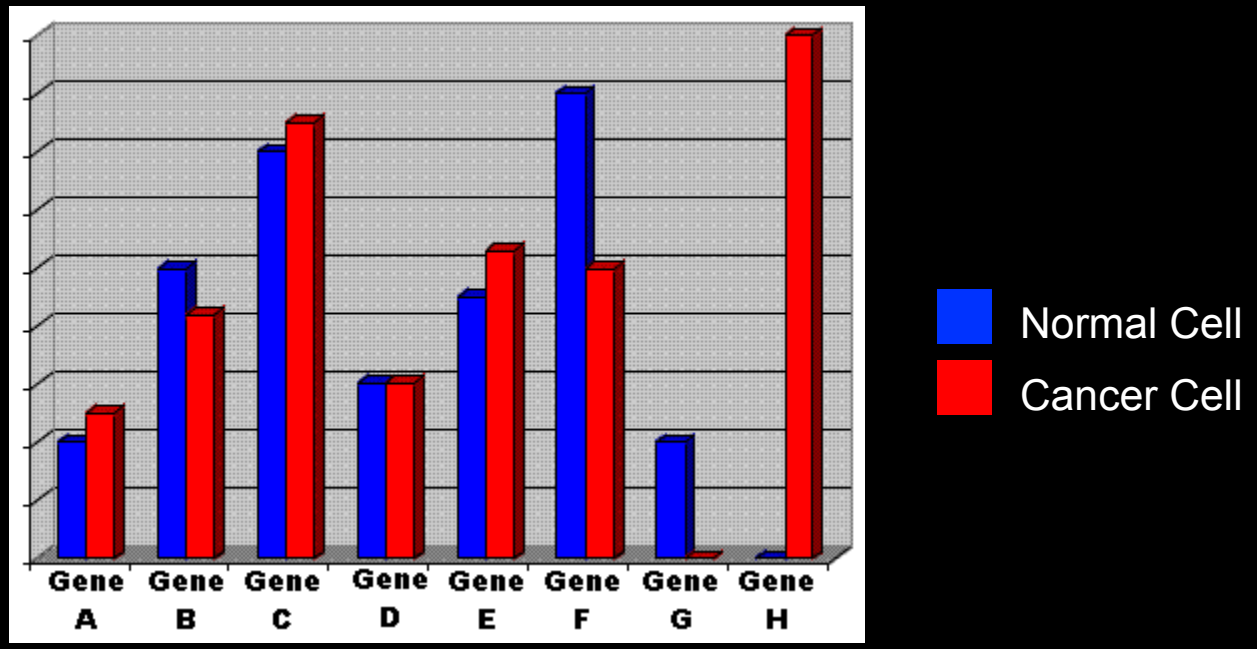
BRIAN J.DRUKER,M.D.,MOSHE TALPAZ,M.D.,DEBRA J.RESTA,R.N.,BIN PENG,PH.D.,
ELISABETH BUCHDUNGER,PH.D.,JOHN M.FORD,M.D.,NICHOLAS B.LYDON,PH.D.,HAGOP KANTARJIAN,M.D.,
RENAUD CAPDEVILLE,M.D.,SAYURI OHNO-JONES,B.S.,AND CHARLES L.SAWYERS,M.D.





NEJM 344: 1031, 2001

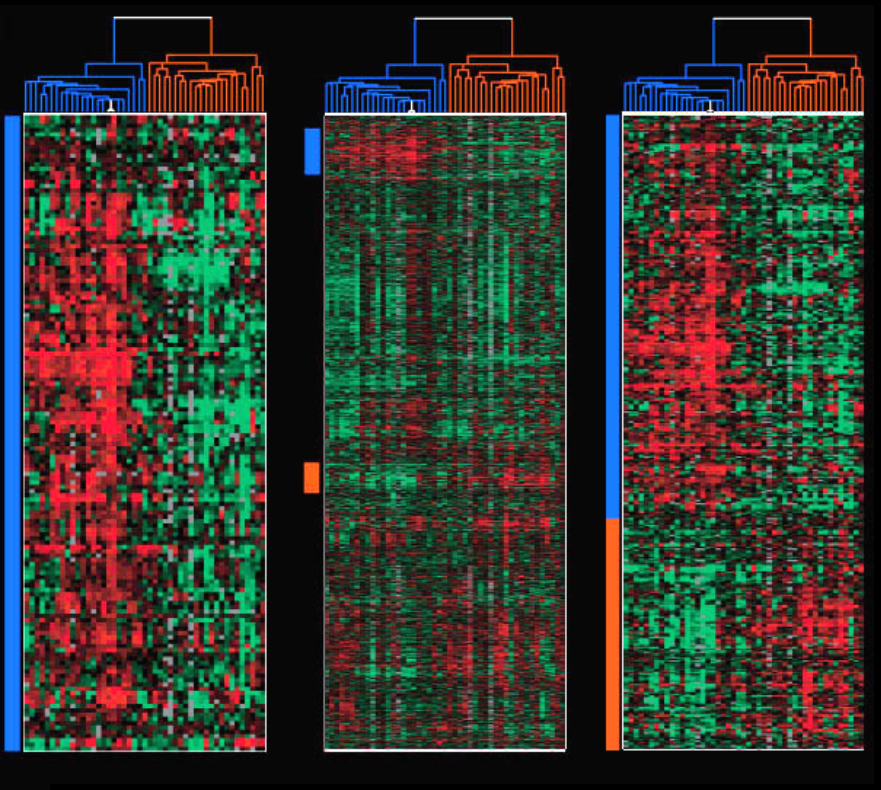
Gene Expression: The Cell's Fingerprint

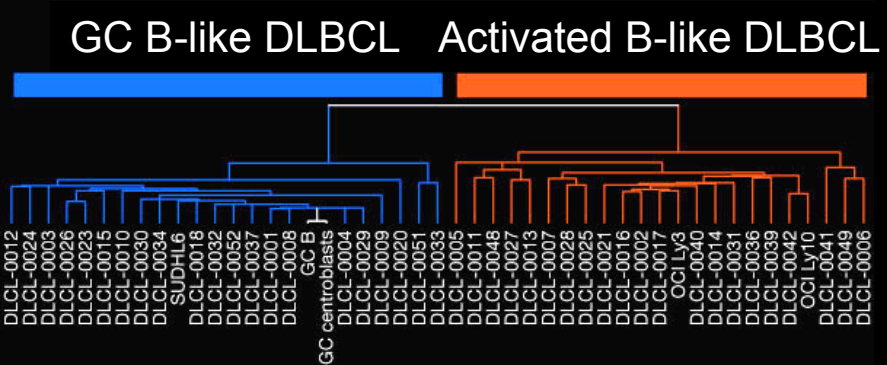


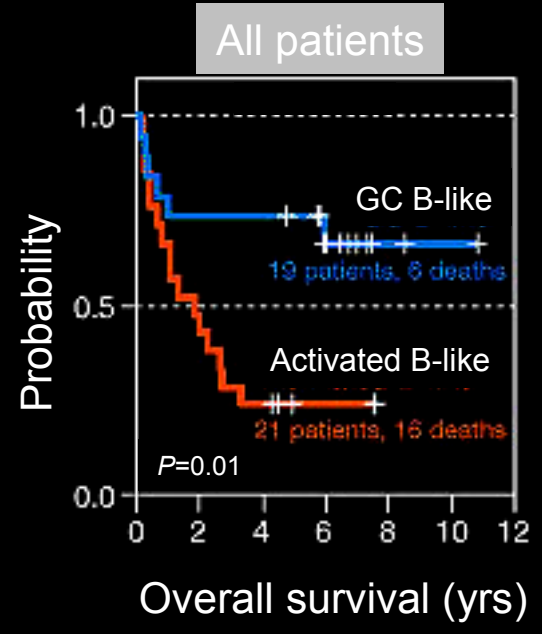
Establishing for a cell the repertoire of genes expressed, together with the amount of gene products produced for each, yields a powerful "fingerprint". Comparing the fingerprints of a normal versus a cancer cell will highlight genes that by their suspicious absence or presence (such as Gene H) deserve further scientific scrutiny to determine whether such suspects play a role in cancer, or can be exploited in a test for early detection.

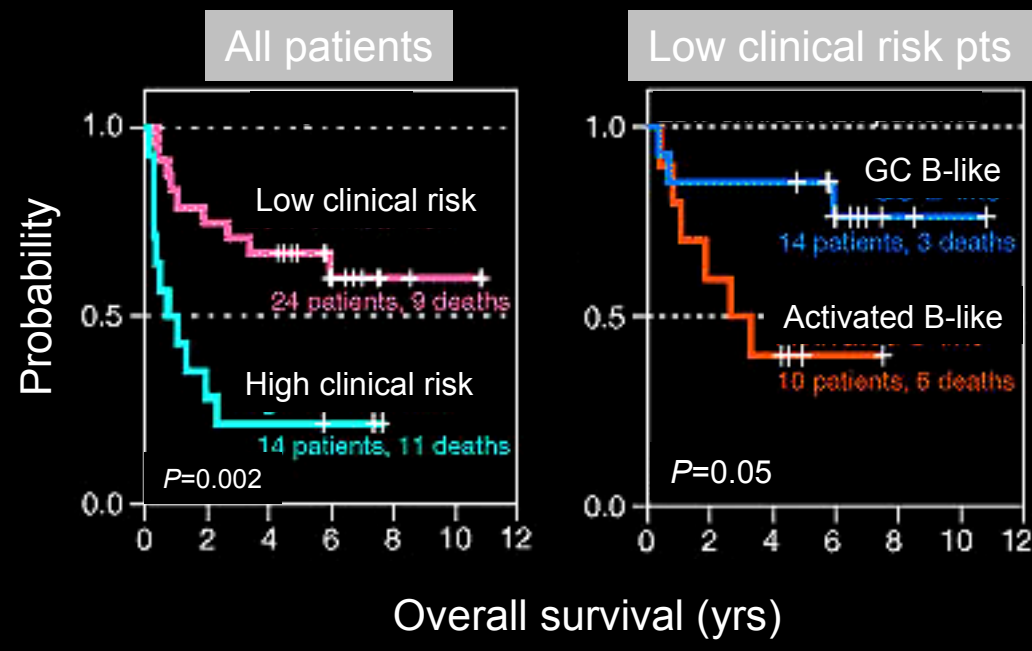
http://cgap.nci.nih.gov

Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling



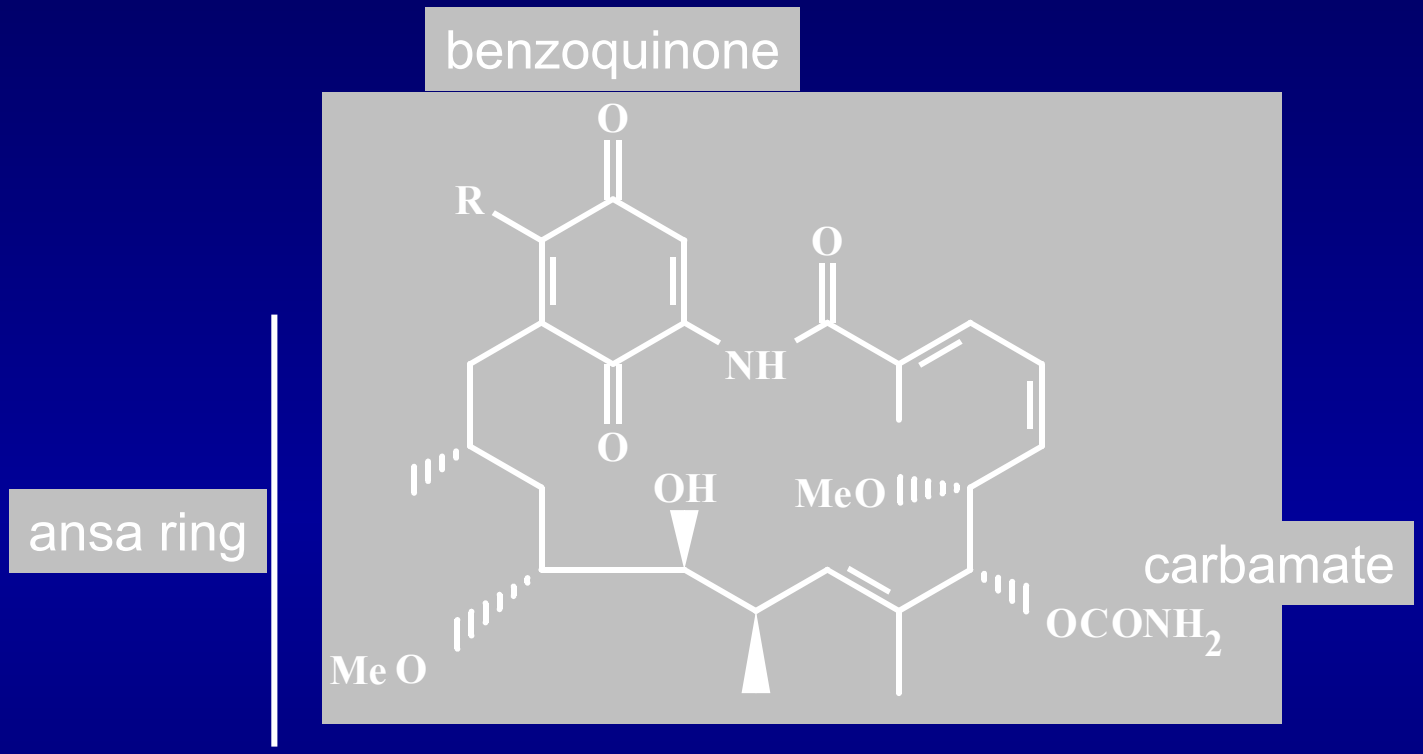






Alizadeh et al, Nature 403: 503, 2000

GELDANAMYCIN STRUCTURE



	NSC	R
Geldanamycin	122750	OMe
17-AAG	330507	$NHCH_2CH{=}CH_2$

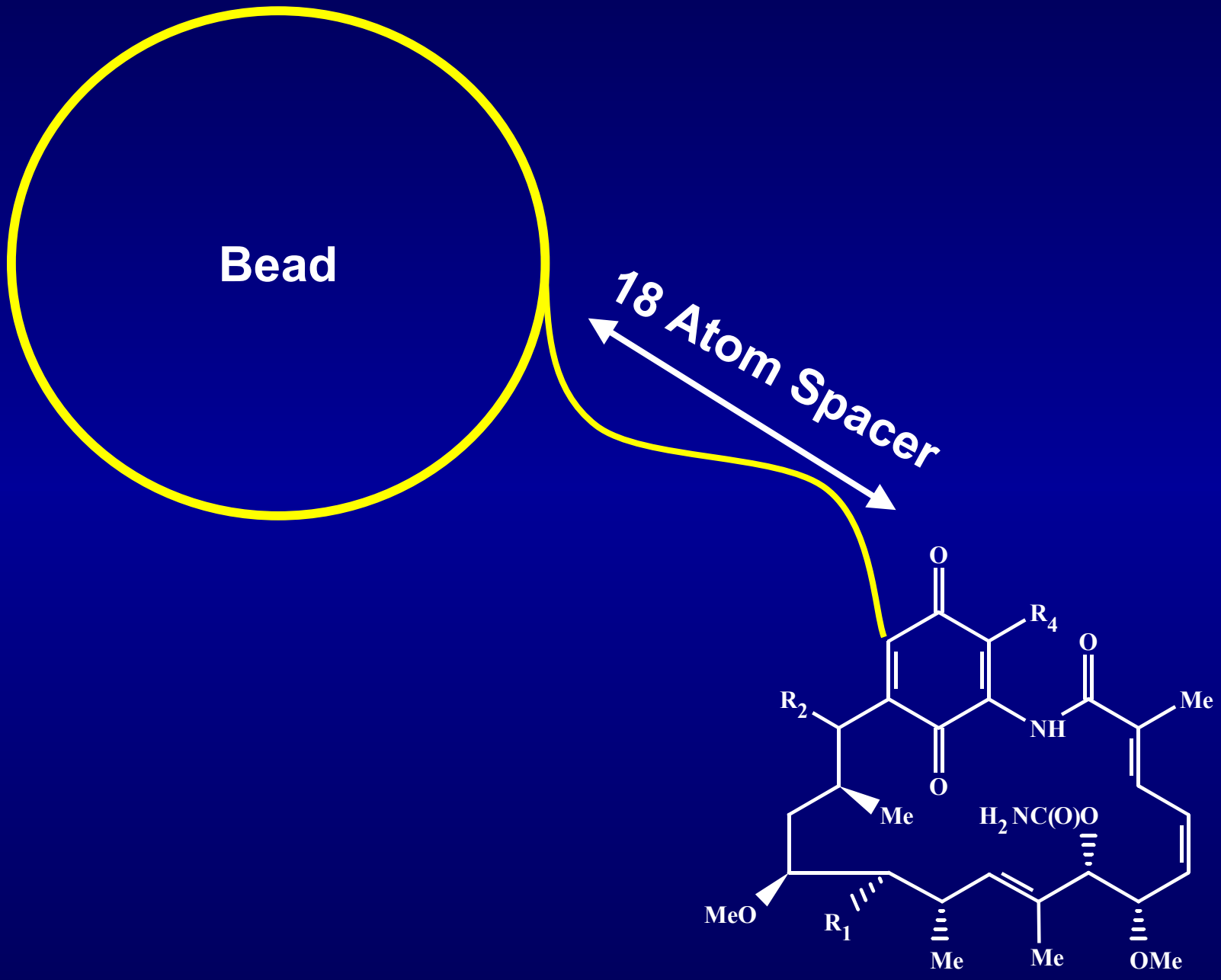
BENZOQUINOID ANSAMYCINS INITIAL CELL PHARMACOLOGY - I

- "Reverse" transformed phenotype of src-transformed rat kidney cell line
  - decrease tyrosine phosphorylation of pp60src
  - not inhibit pp60 immune complex kinase directly but these were inhibited from drug-treated cells
  - thus alter "intracellular environment" of src

(Uehara et al, MCB 6: 2198, 1986)

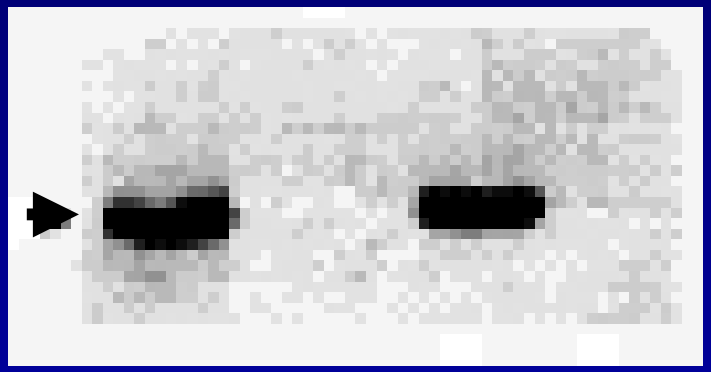
- Decrease steady state phosphorylation levels to 10% of control
  - decrease steady state level of pp60src by 30%
  - accelerate turnover of pp60src

(Uehara et al, Cancer Res 49: 780, 1989)


Bead
18 Atom Spacer
O
R4
O
R2
NH
Me
O
Me
H2NC(O)O
MeO
R1
Me
Me
OMe

GELDANAMYCIN BEADS IDENTIFY HSP90 AS BINDING PARTNER

R. Lysate



1 2 3 4

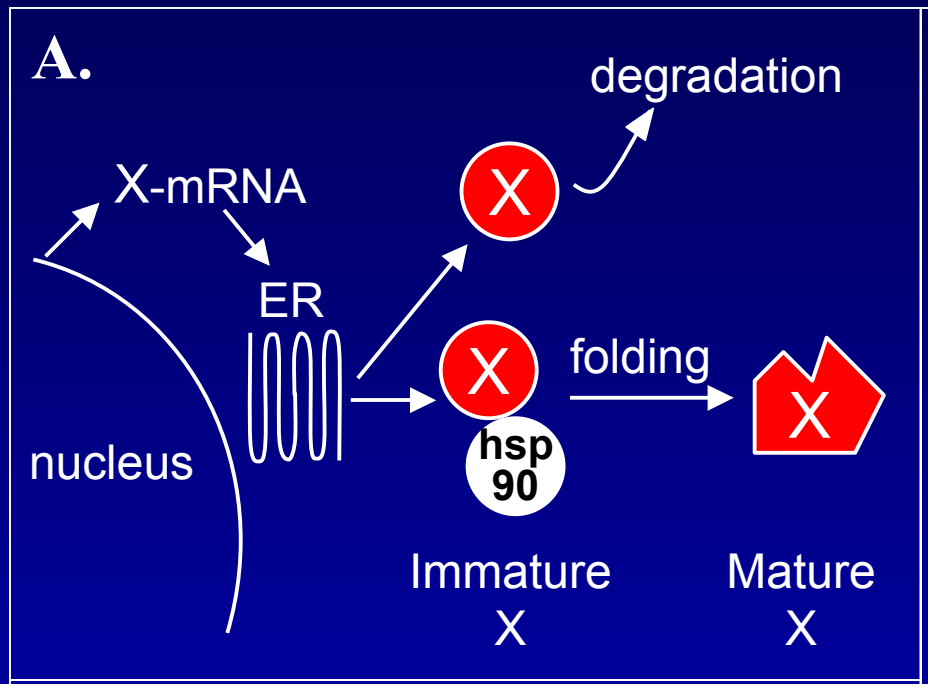
1) Bead-Geld

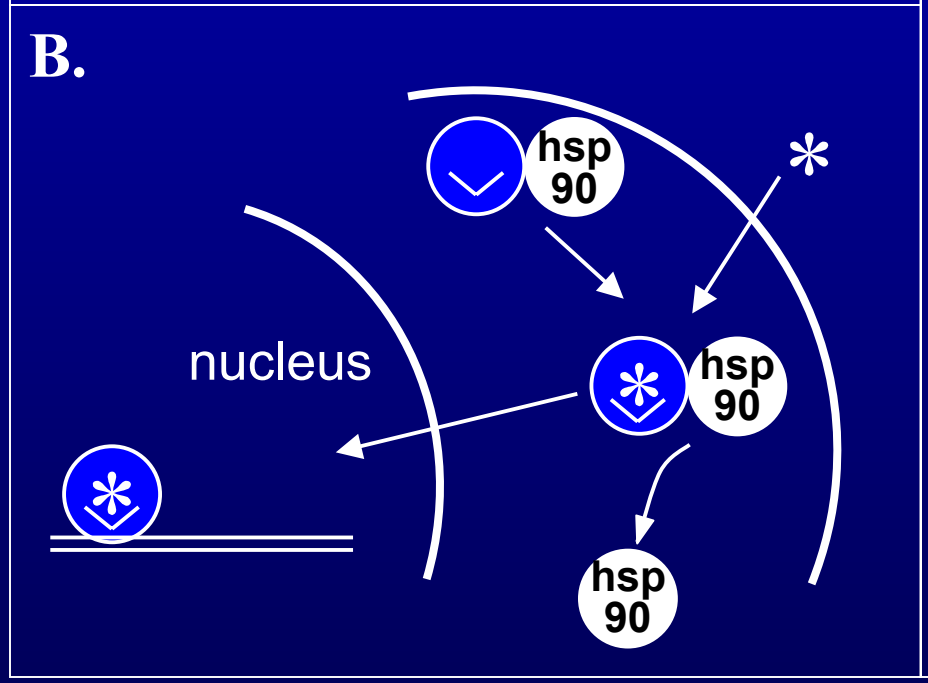
2) Bead-Geld + Geld

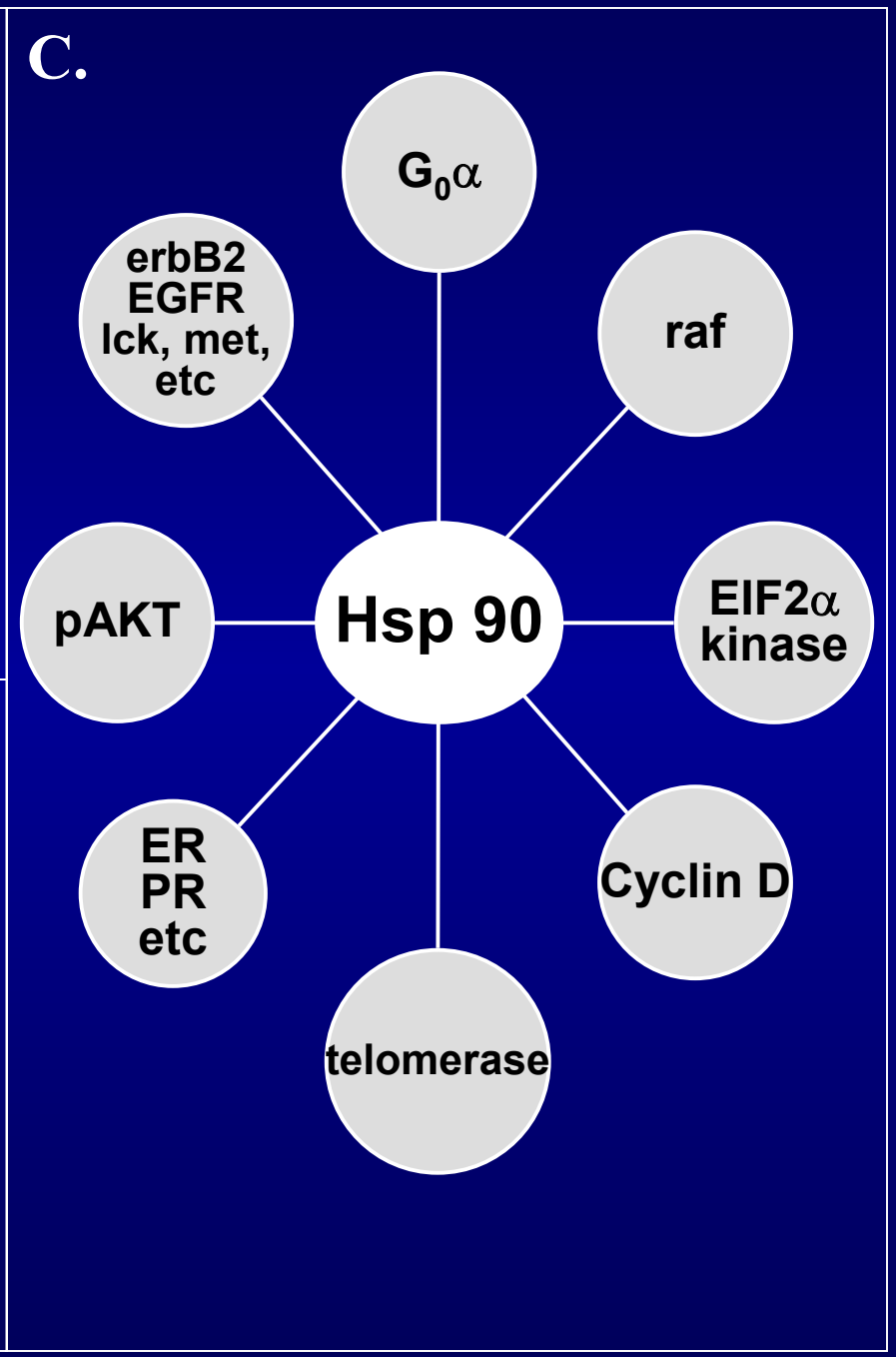
3) Bead-Geld + Geldampicin

4) Bead

Neckers et al, PNAS 91:8324, 1994


A.
degradation
X-mRNA
ER
X
X
folding
X
hsp
90
nucleus
Immature
X
Mature
X


B.
hsp
90
*
nucleus
*
hsp
90
hsp
90


C.
$G_0\alpha$
erbB2
EGFR
lck, met,
etc
raf
pAKT
Hsp 90
EIF2α
kinase
ER
PR
etc
Cyclin D
telomerase

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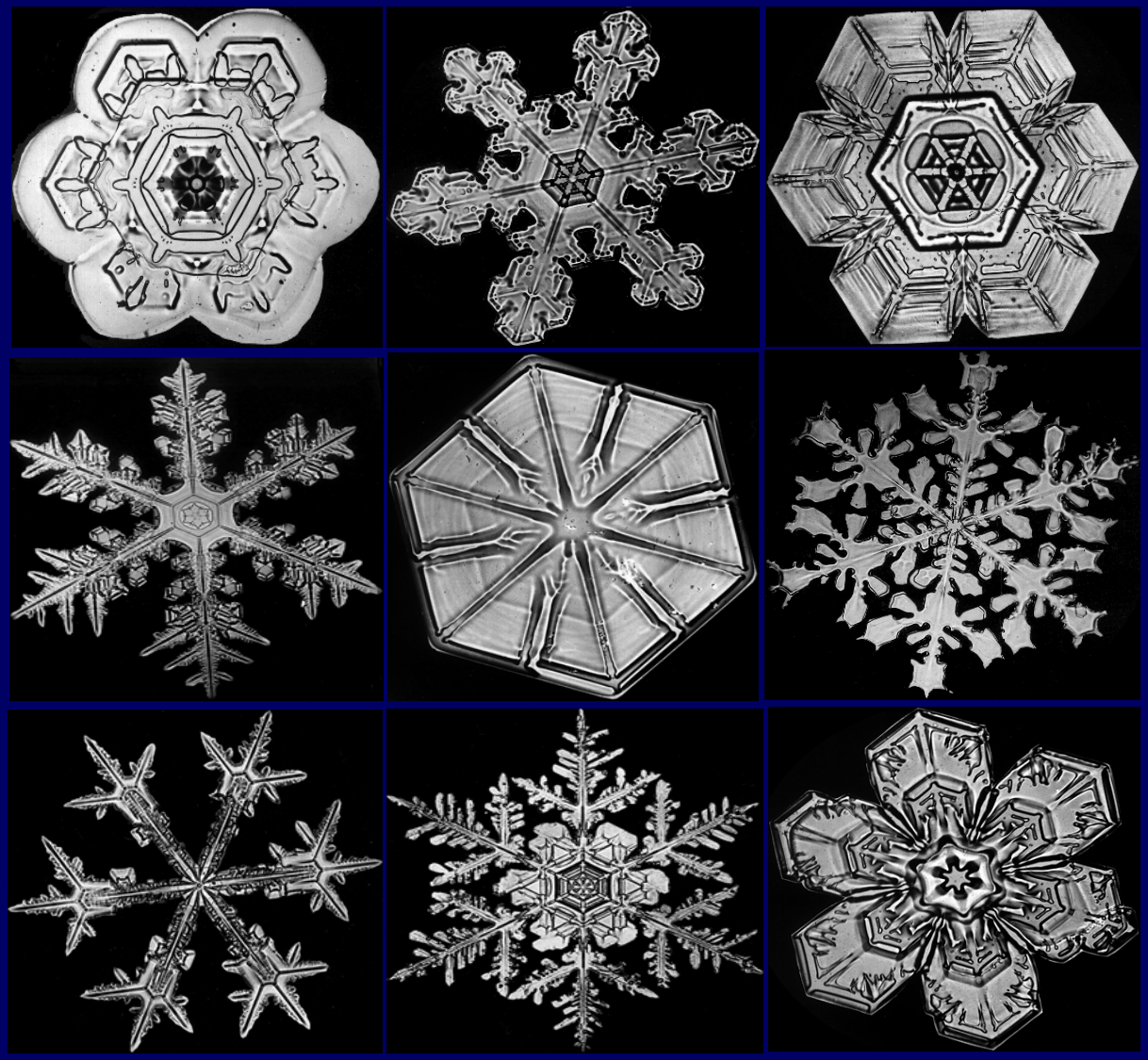
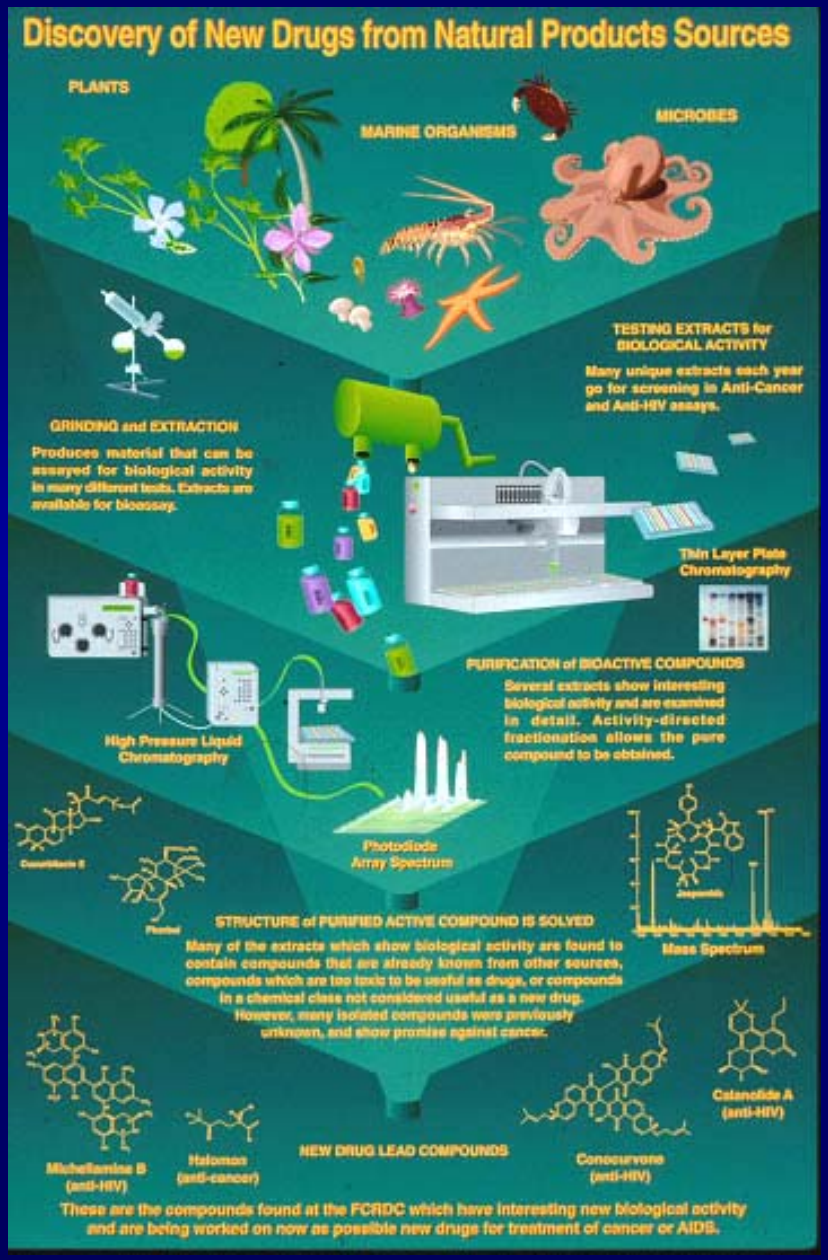
Diversity



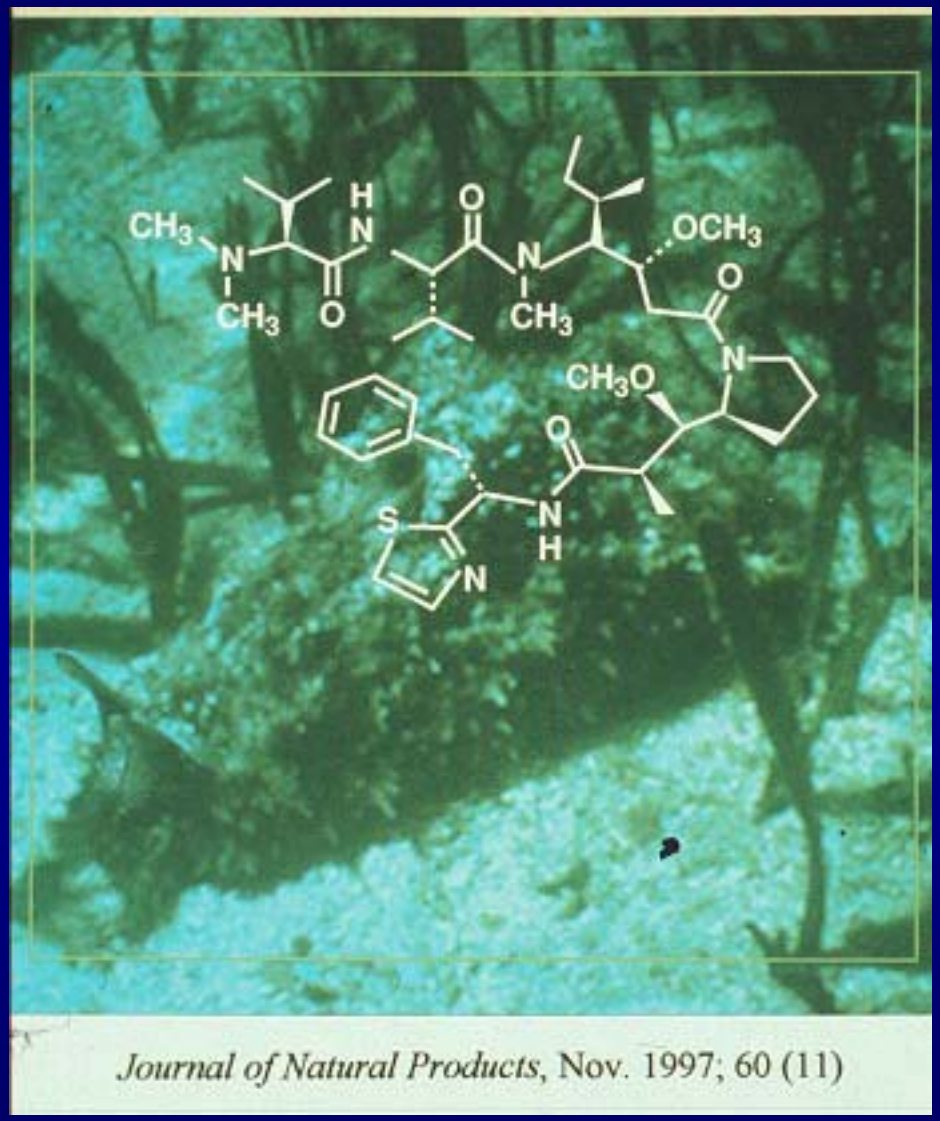
It is estimated that there are 10^{40} compounds in all of "chemical space". Since the Big Bang, there have only been 10^{17} seconds.

- Peter Wipf

Natural Product Isolation Tree



“You are what you eat”



Journal of Natural Products, Nov. 1997; 60 (11)

Dolabella auricularia

Dolastatins come from a *Symploca* species that they graze on

TRIPEPTIDE COMBINATORIAL LIBRARY

X X X

Four amino acids in each position

$4^3 = 64$

A = Alanine
R = Arginine
T = Threonine
W = Tryptophan

after R. Houghten, 1999

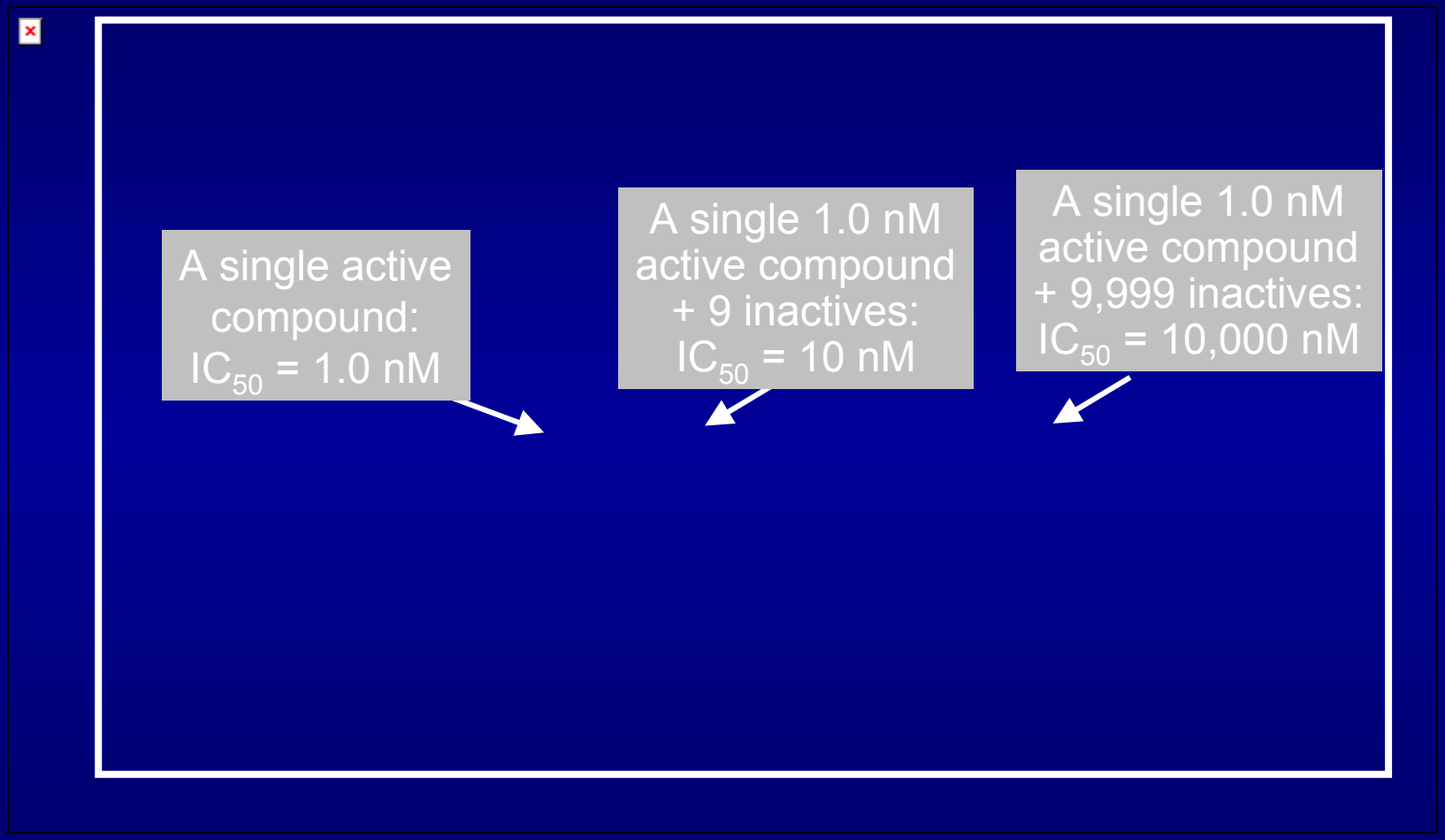
NUMBER OF PEPTIDES POSSIBLE WITH INCREASING LENGTH

Length	Peptide	Number
2	Ac – OO – NH_2	400
3	Ac – OOO – NH_2	8,000
4	Ac – OOOO – NH_2	160,000
5	Ac – OOOOO – NH_2	3,200,000
6	Ac – OOOOOO – NH_2	64,000,000
7	Ac – OOOOOOO – NH_2	1,280,000,000
8	Ac – OOOOOOOO – NH_2	25,600,000,000

O = Individual Defined Amino Acid

after R. Houghten, 1999

IC_{50} OF MIXTURES



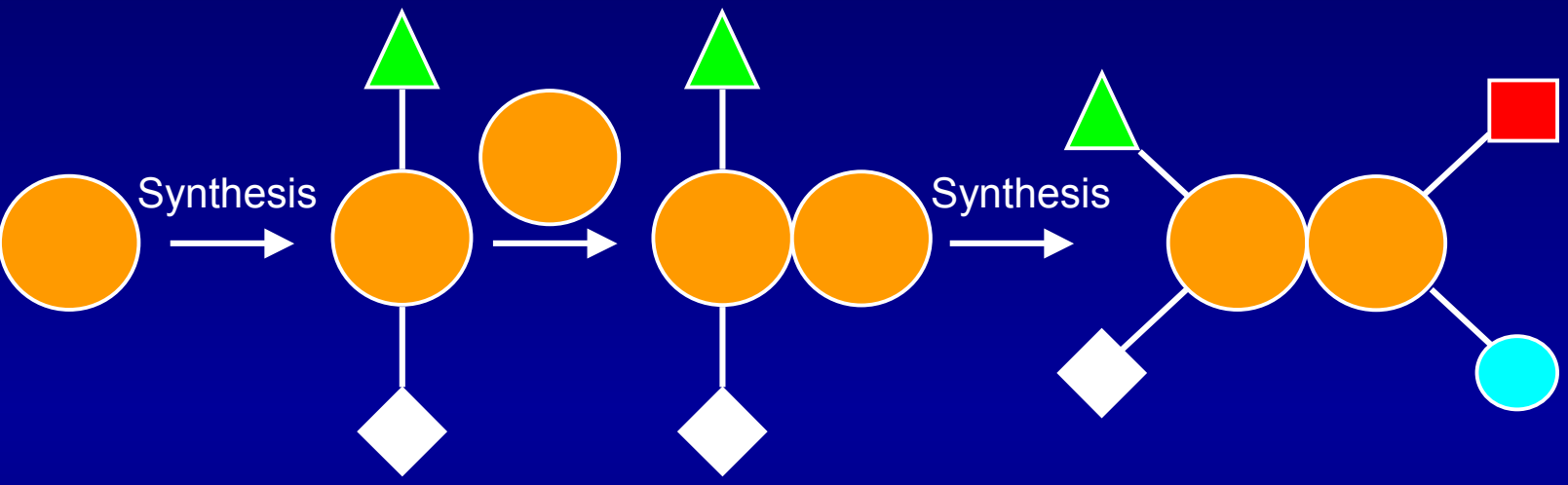
Log Concentration

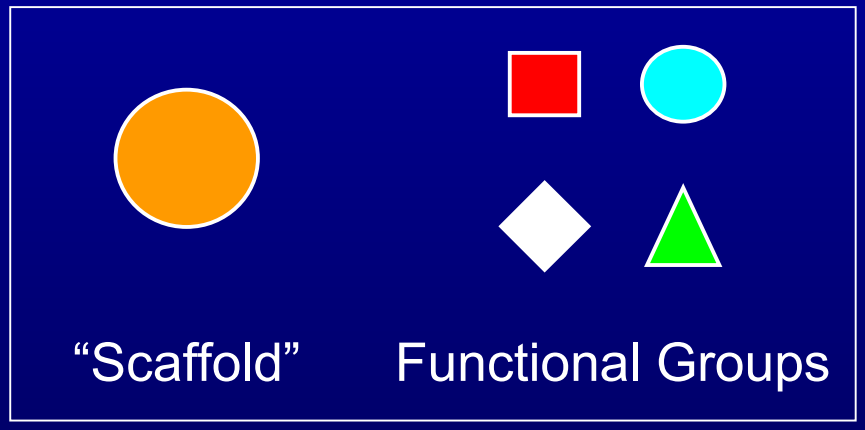
COMBINATORIAL LIBRARIES: THE MIXTURE QUESTION

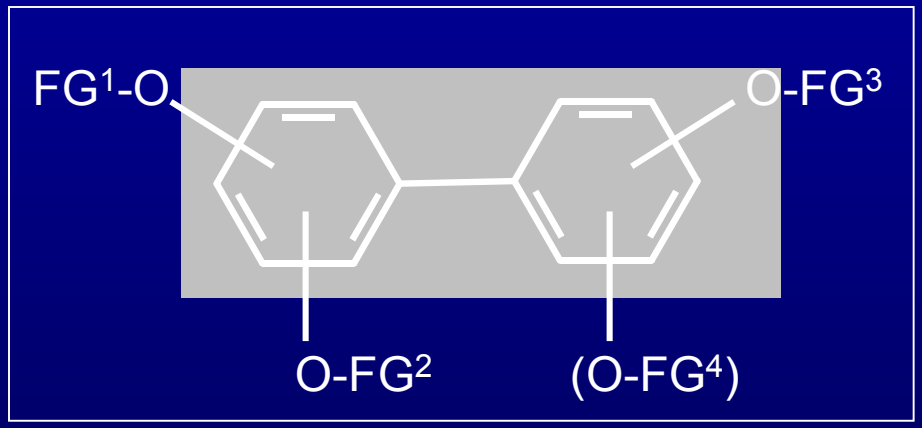
	Natural Product Extracts	Synthetic Combinatorial Mixtures
Direct screening of compound mixtures	Yes	Yes
Discovery of highly active compounds	Yes	Yes
Equal concentrations of compounds	No	Yes
Chemical structures known	No	Yes
Synthetic pathway known	No	Yes
Structure – activity relationship known	No	Yes

after R. Houghten, 1999

NON-PEPTIDE "COMBINATORIAL" STRATEGIES COMBINE "SCAFFOLDS" (OR "BACKBONES") WITH "FUNCTIONAL GROUPS"







The Chemical Generation of Molecular Diversity from
http://www.netsci.org/Science/Combichem/feature01.html

THE RULE OF FIVE

An awareness tool for discovery chemists:

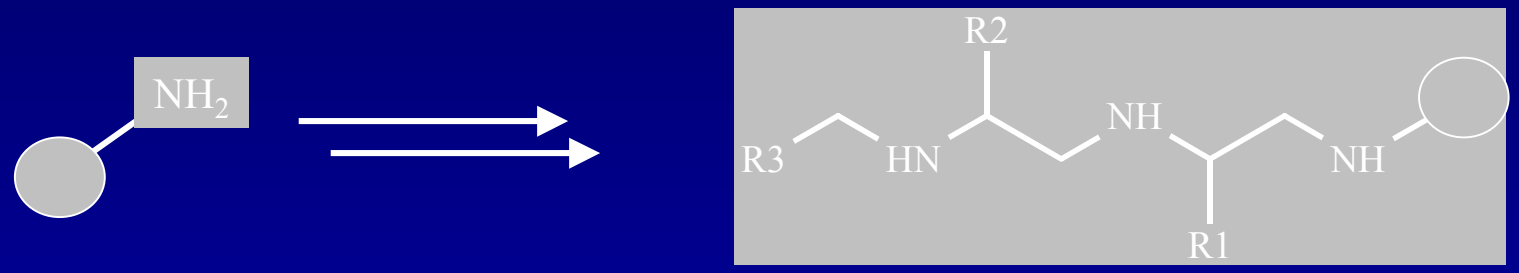
Compounds with two or more of the following characteristics are flagged as likely to have poor oral absorption

- More than 5 H-bond donors
- Molecular weight >500
- c log P > 5
- Sum of N's and O's (a rough measure of H-bond acceptors) > 10

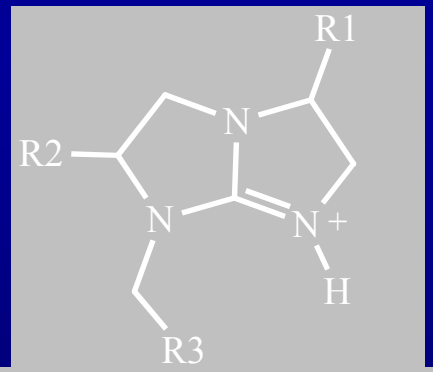
Modern Drug Discovery
January/February 1999

Modern Drug Discovery, **1999**, 2 *(1)*, 55-60.

COMBINATORIAL LIBRARIES OF BICYCLIC GUANIDINES FROM REDUCED ACYLATED DIPEPTIDES



1. $CSIm_2$
2. HF/anisole



R1 x R2 x R3 = 49 x 51 x 42 = 104,958 compounds

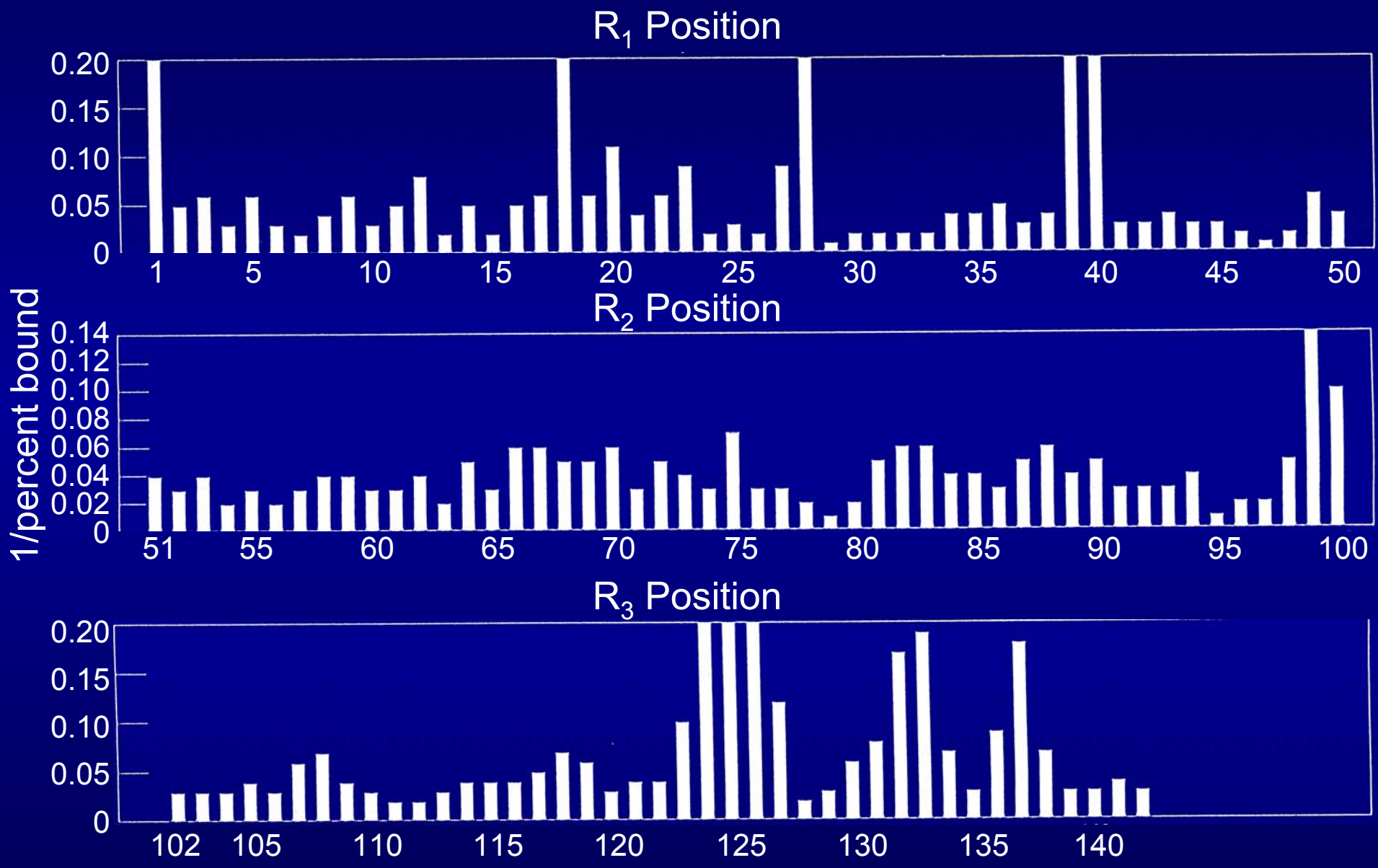
after R. Houghten, 1999

BIOASSAYS
(READY APPLICATION OF SOLUBLE LIBRARIES)

- Soluble Acceptors
  - antibodies
  - enzymes
- Membrane-bound Receptors
  - tissue homogenate
  - functional cell based
- Microorganisms: Disruption of Function
  - bacteria
  - fungi
  - virus
- Differentiation
  - stem cells
- *In Vivo*

after R. Houghten, 1999

POSITIONAL SCANNING BICYCLIC GUANIDINE LIBRARY (κ RECEPTOR)



after R. Houghten, 1999

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-***Structure based design***

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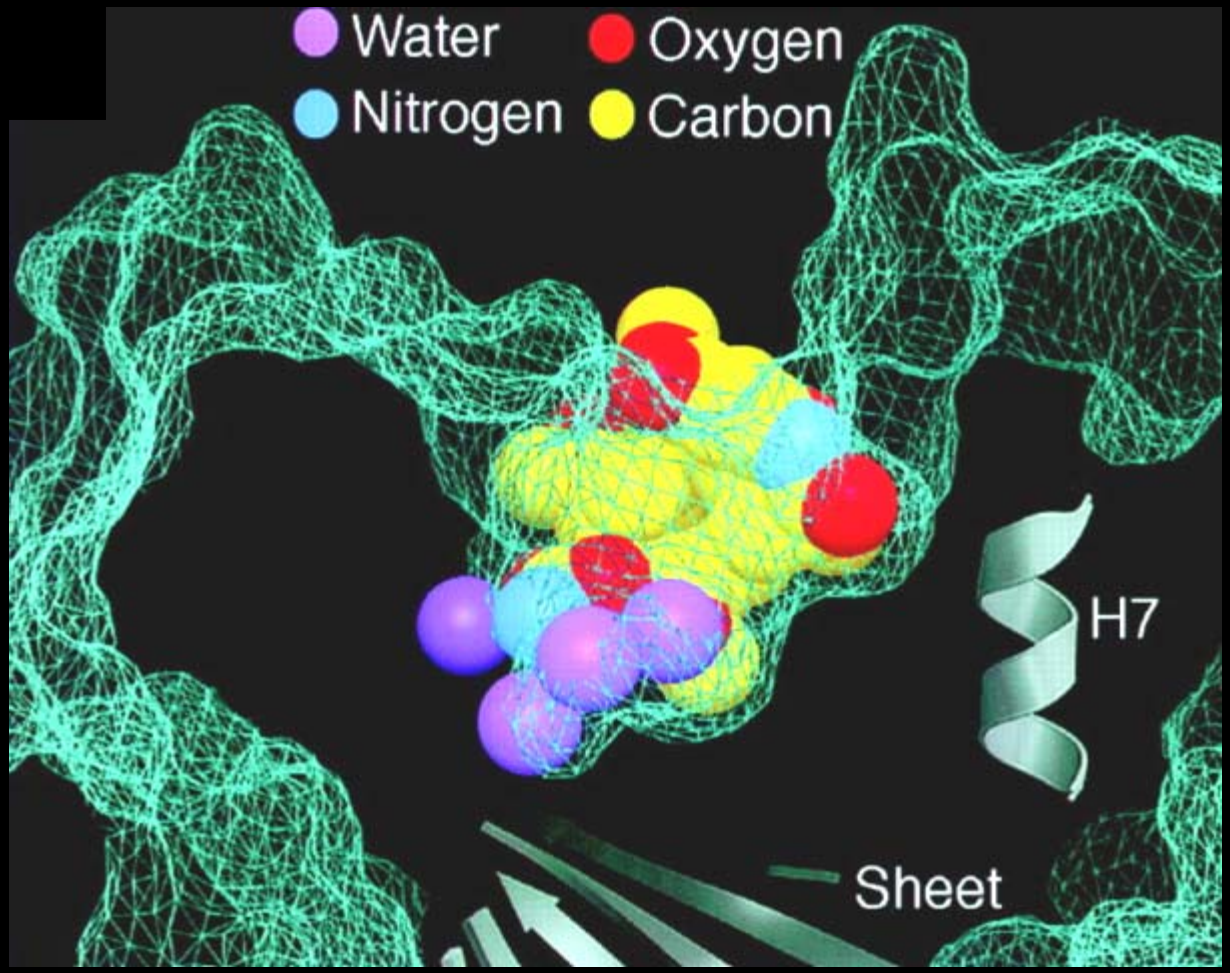
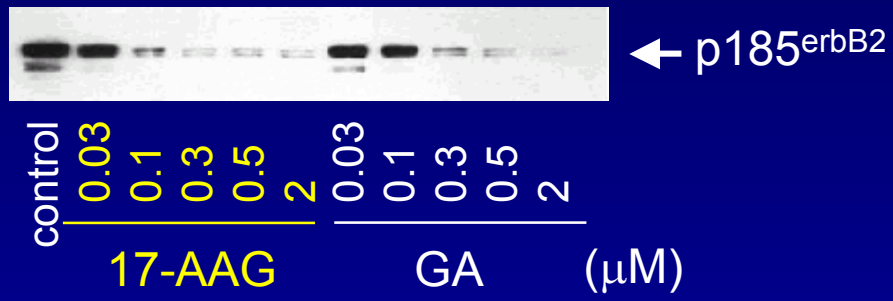
-Cell-based Screen

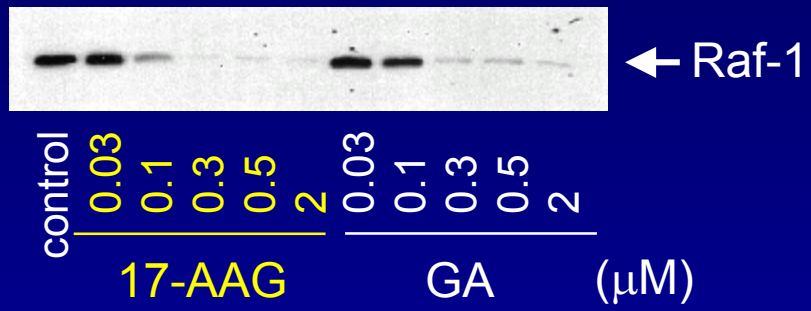
THREE DIMENSIONAL VIEW OF GELDANAMYCIN BINDING POCKET IN AMINO TERMINUS OF HSP90

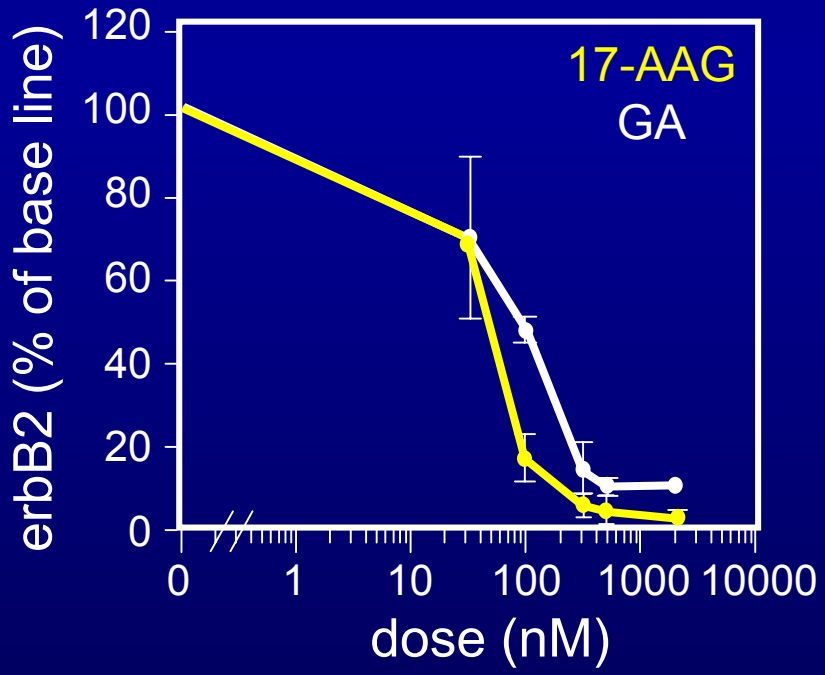


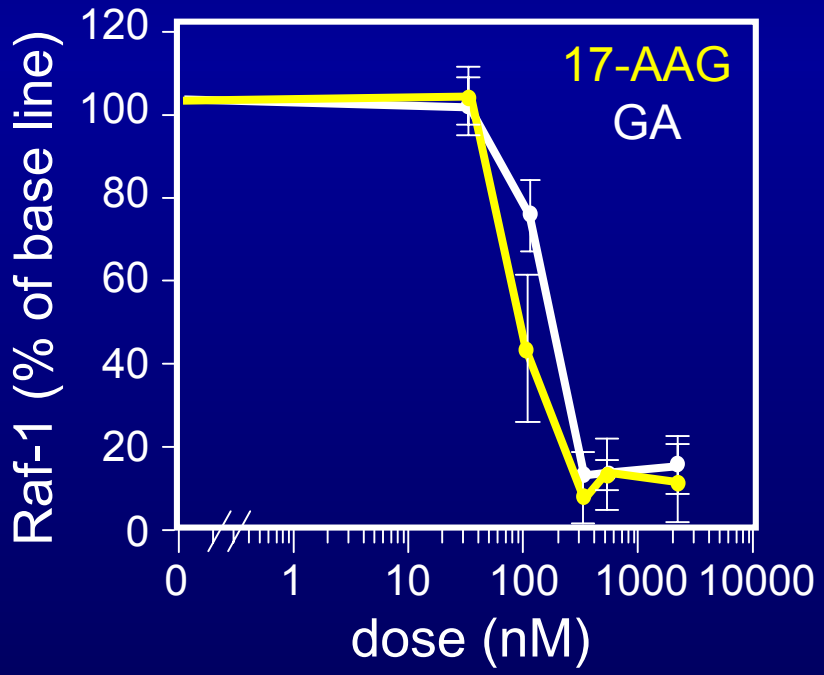
Stebbins et al, Cell 89:239, 1997

17-AAG BINDS TO HSP90 & SHARES IMPORTANT BIOLOGIC ACTIVITIES WITH GELDANAMYCIN





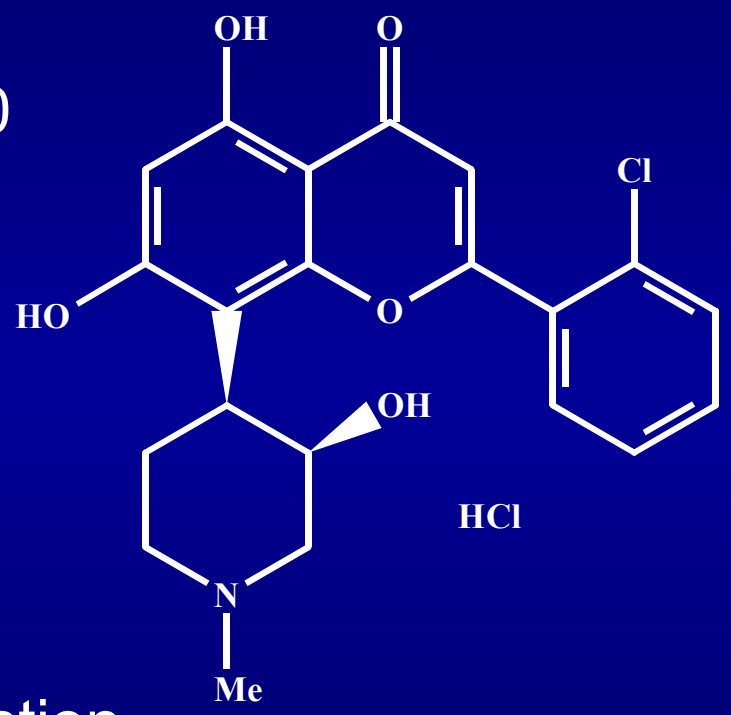


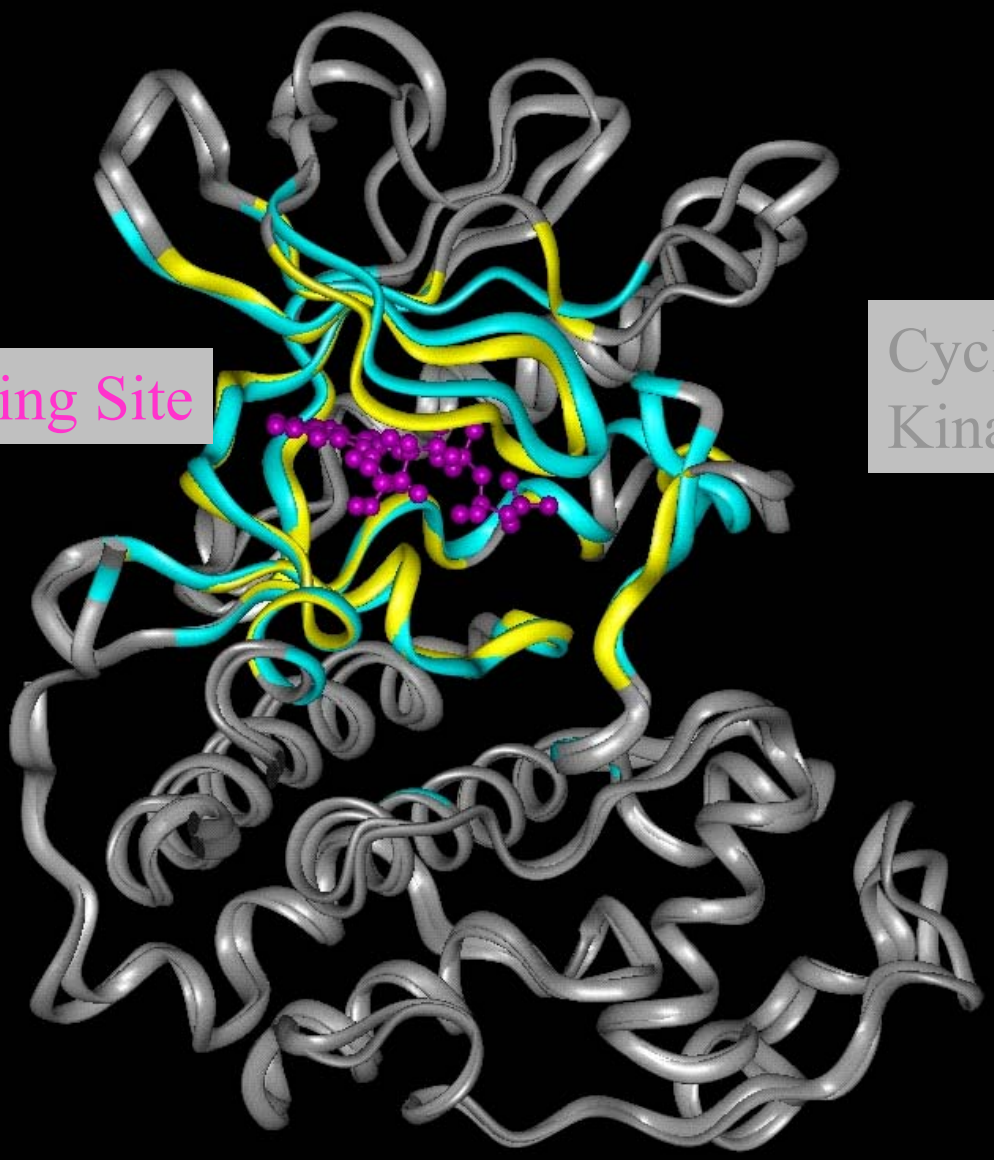


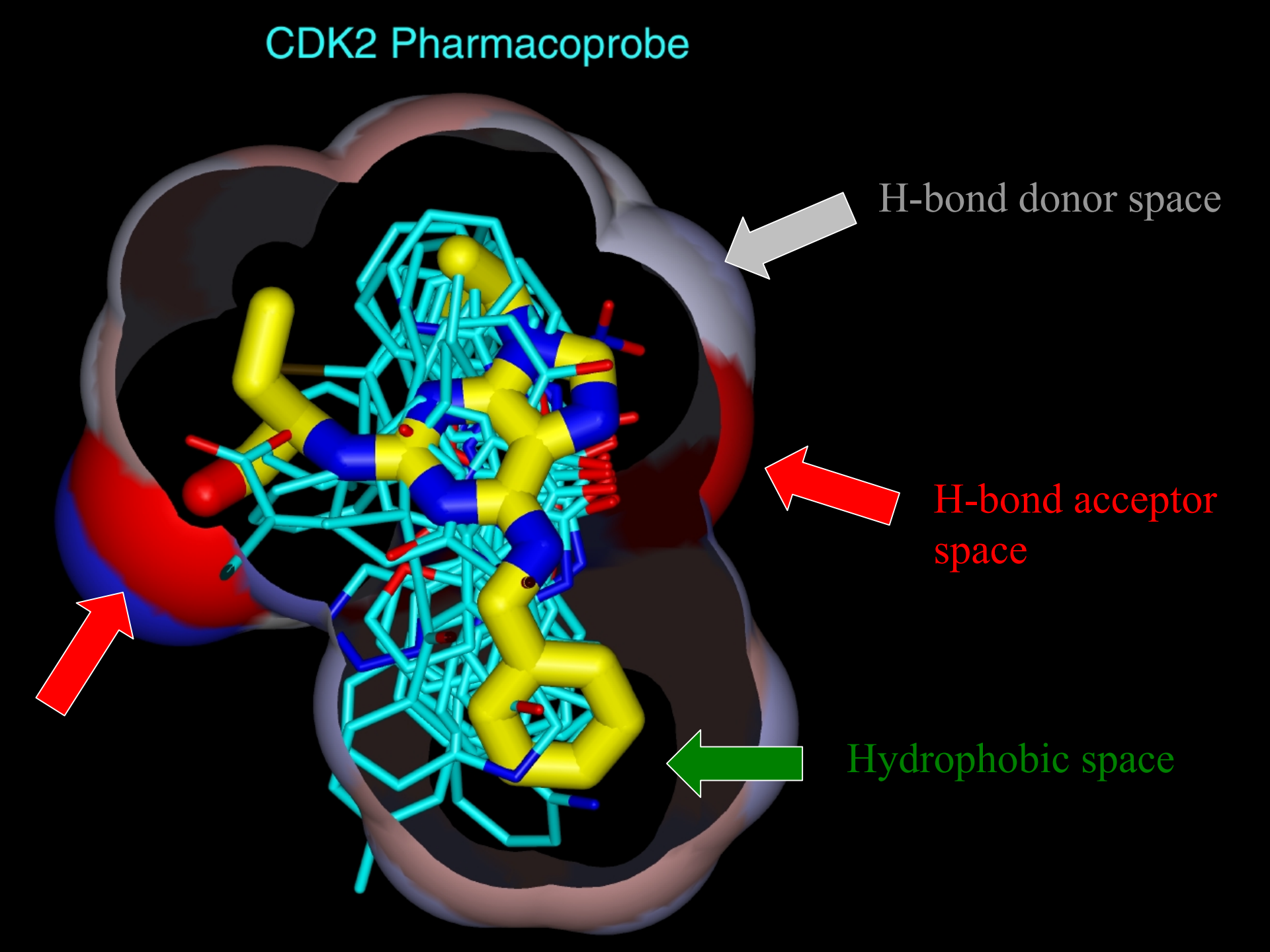
Schulte & Neckers, Cancer Chemother Pharmacol 42: 273, 1998

FLAVOPIRIDOL PRECLINICAL SUMMARY

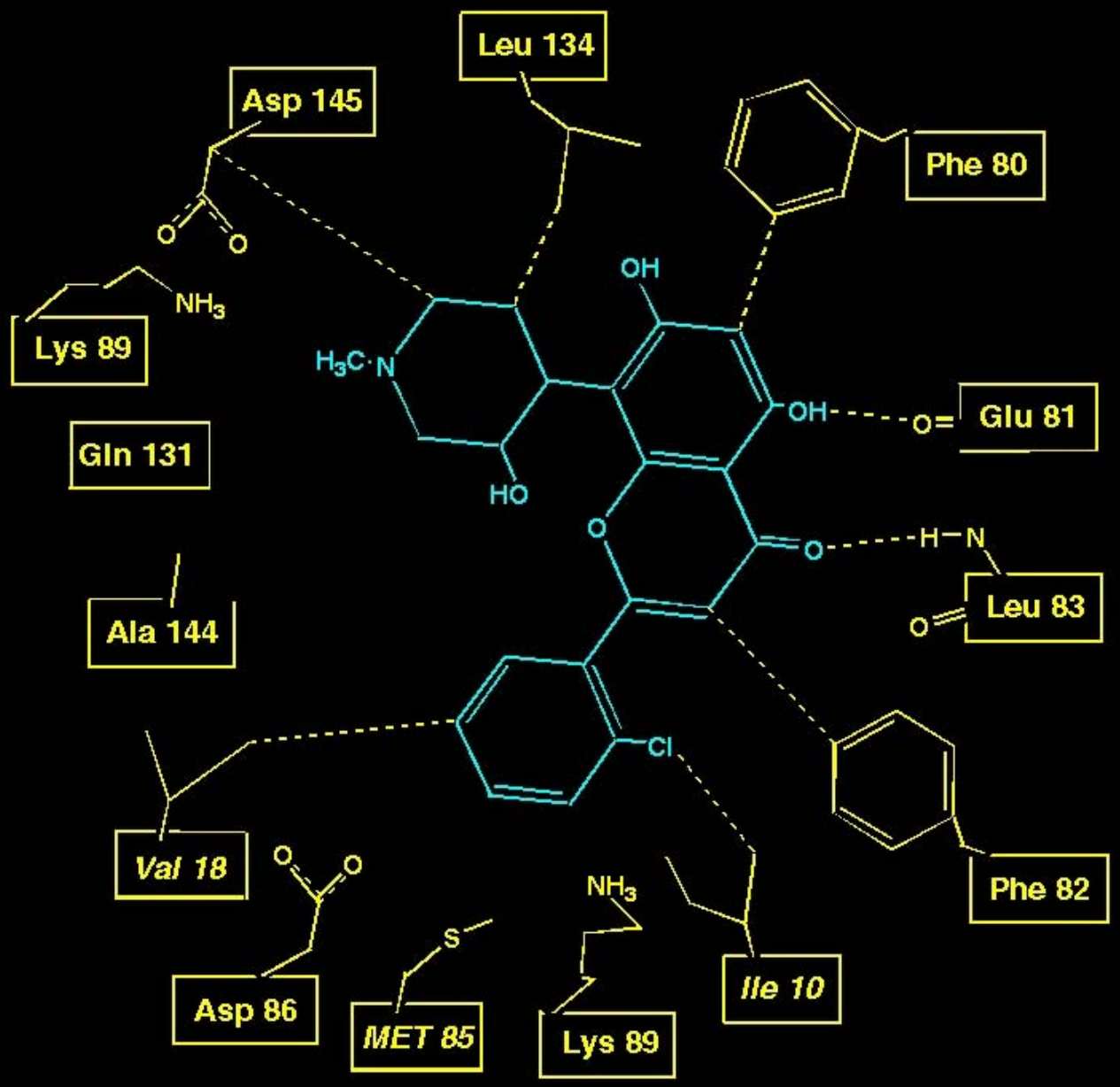
- Pan CDK (1,2,4,6,7,9) inhibitor
- Δ vs other kinases ~10 (PKC) ≥ 100 (EGFR, PKA, PKC, etc.)EXCEPT GSK-3
- Induces apoptosis efficiently in certain cell types (hematopoietic, squamous)
- Most active with frequent administration
- Binds to Ald dehydrogenase, glycogen, phosphorylase (et al)



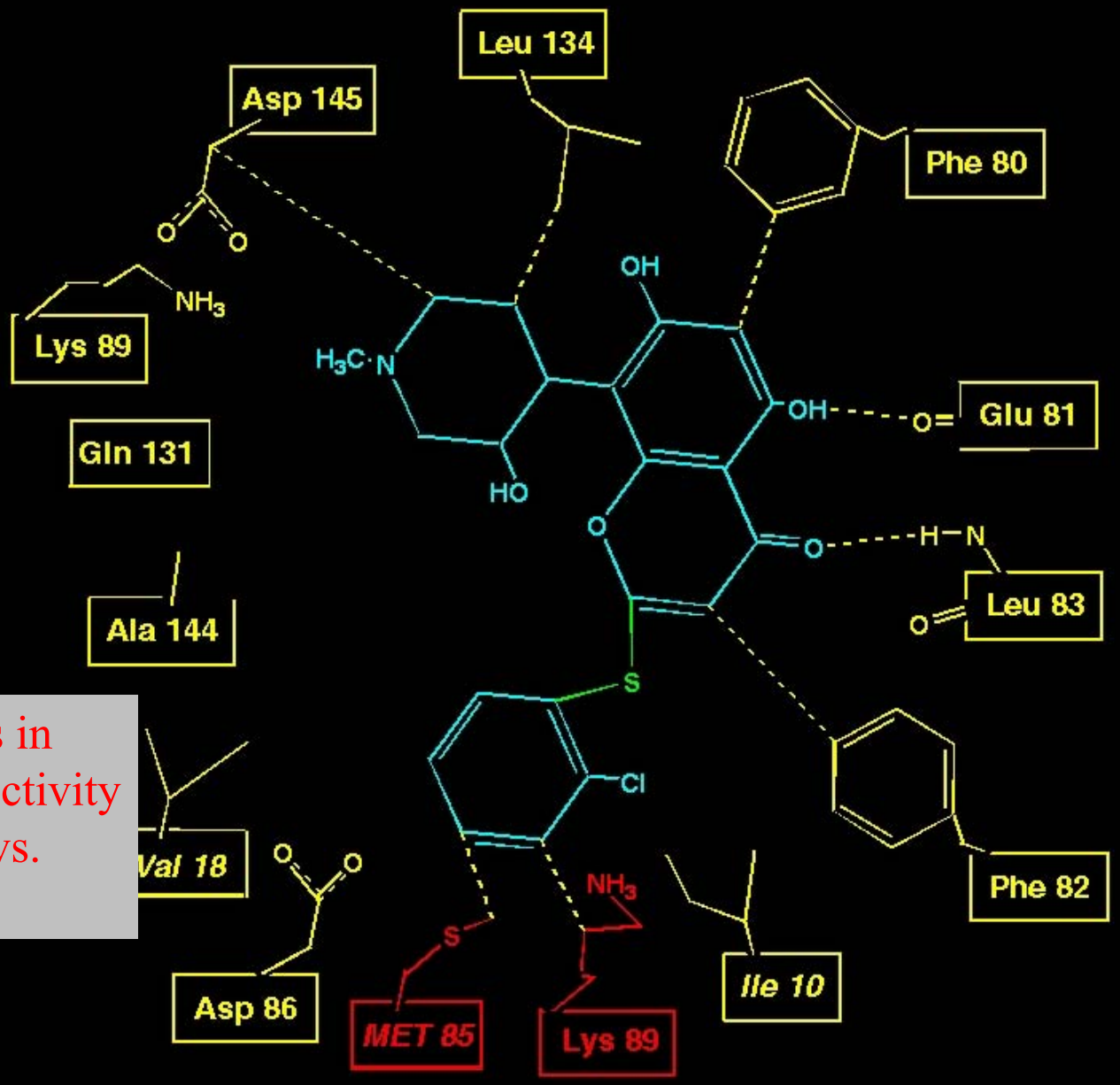

ATP Binding Site
Cyclin-Dependent
Kinase 2


CDK2 Pharmacoprobe
H-bond donor space
H-bond acceptor space
Hydrophobic space

Flavopiridol in the CDK1 ATP binding site



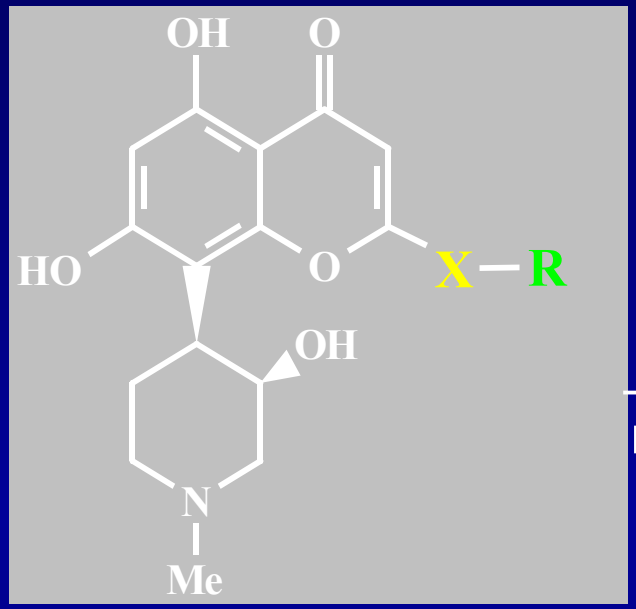
Thioflavopiridol in the CDK1 ATP binding site



This results in greater selectivity for CDK1 vs. CDK2

Thio-ether addition places the chloro-phenyl ring in closer proximity to M85 and K89

THIO- AND OXOFLAVOPIRIDOLS CYCLIN-DEPENDENT KINASE ACTIVITY DATA



X	R	IC_{50} (μM) CDK1/ Cyc B1	CDK2/ Cyc E	CDK4/ Cyc D1
none	2-Cl Ph	0.03	0.17	0.10
S	Et (±)	0.46	3.93	2.06
S	2-Cl Ph (-)	0.11	2.10	16.2
O	2-Cl Ph (-)	0.13	2.11	6.15
S	2-Cl Ph (+)	6.10	4.40	>25
S	Ph (±)	0.44	6.59	4.10
S	*tert*-Bu (±)	0.08	1.07	2.07
S	4,6-diMe pyr. (±)	6.40	40.4	82.5
NH	Ph (±)	16.3	>25	>25
none	N-piperidyl (±)	2.50	9.69	3.70

J Med Chem 43: 4126, 2000

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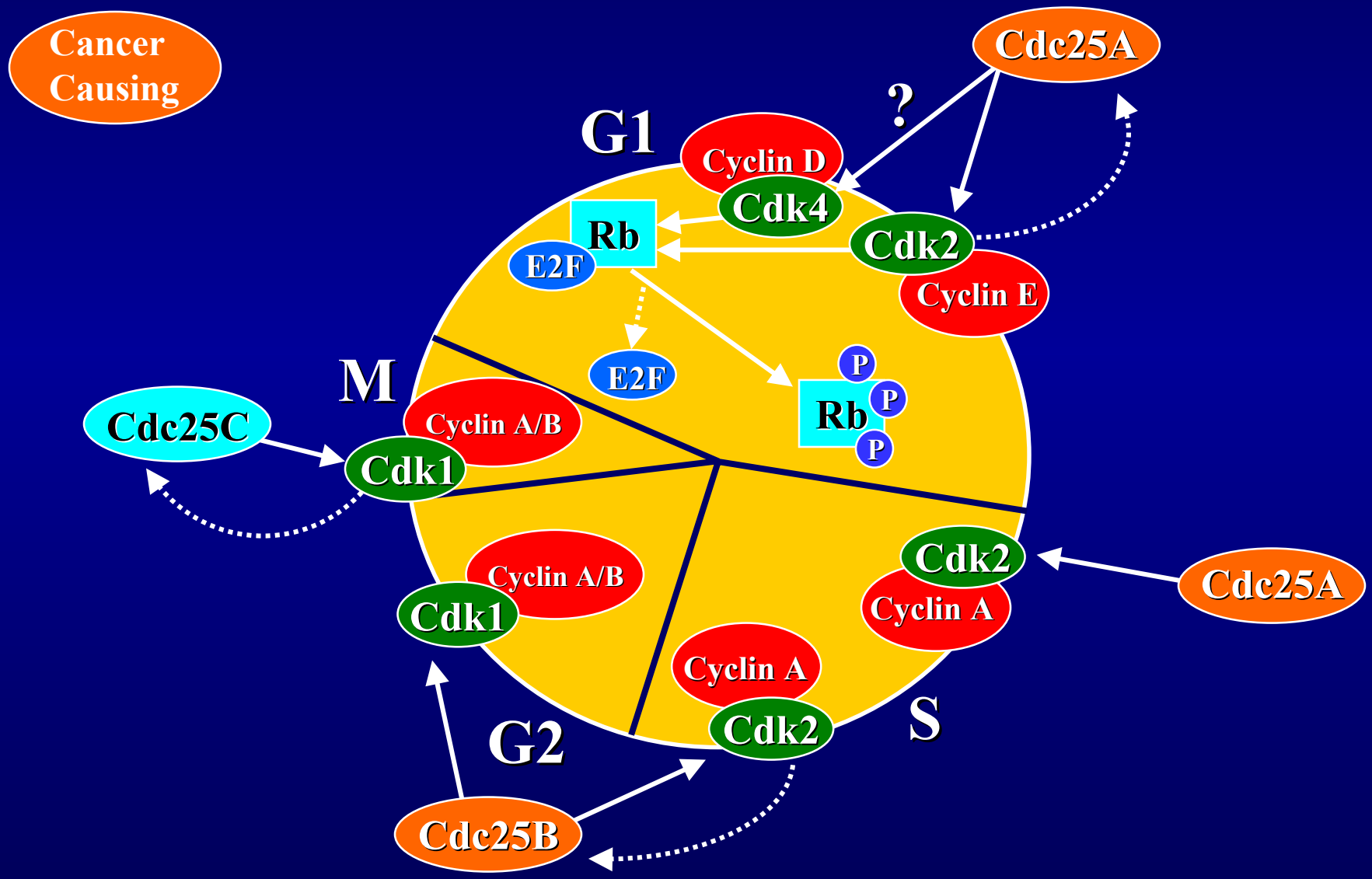
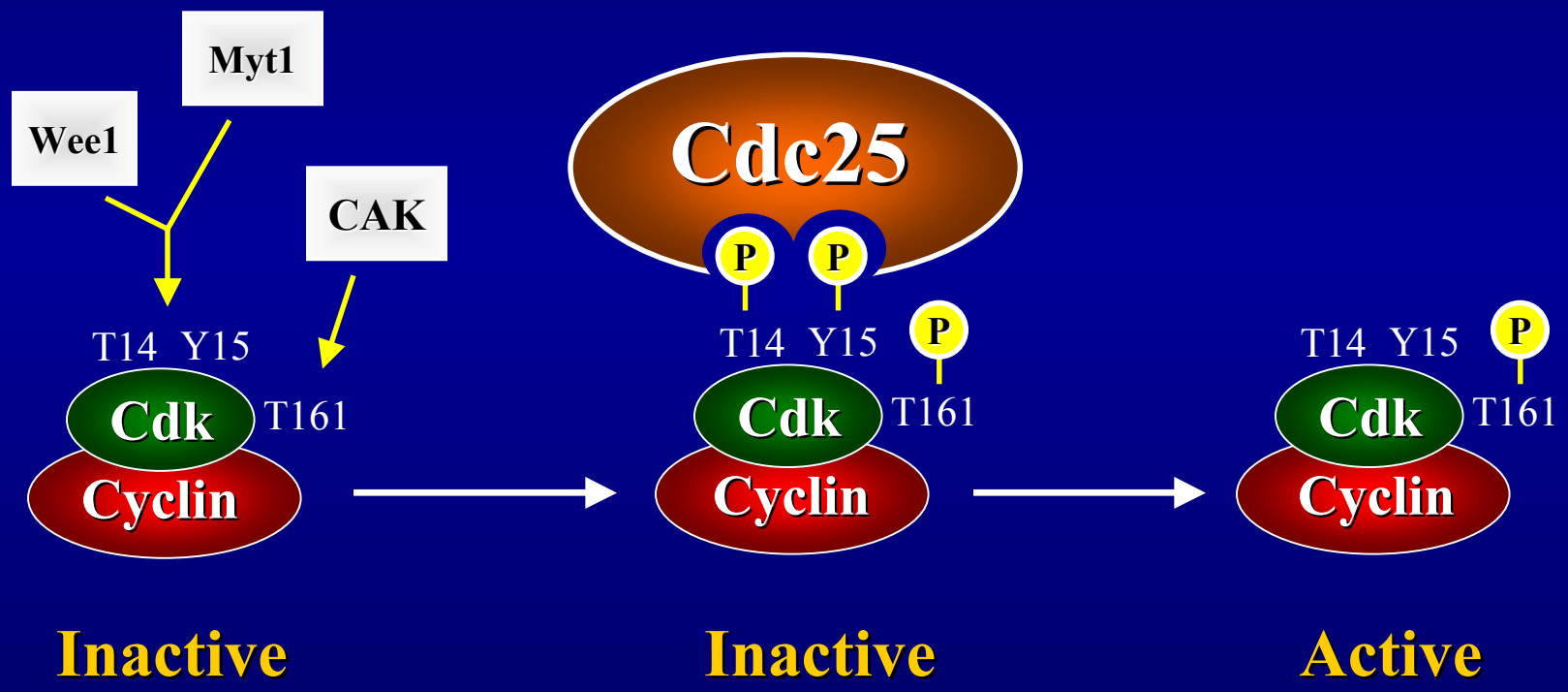
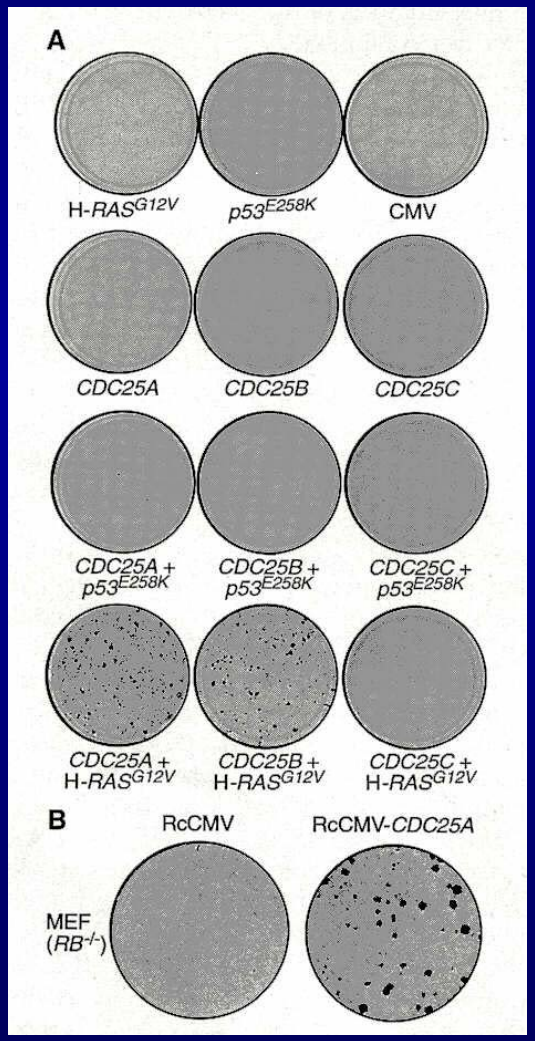
Cell cycle regulation by Cdc25 phosphatases

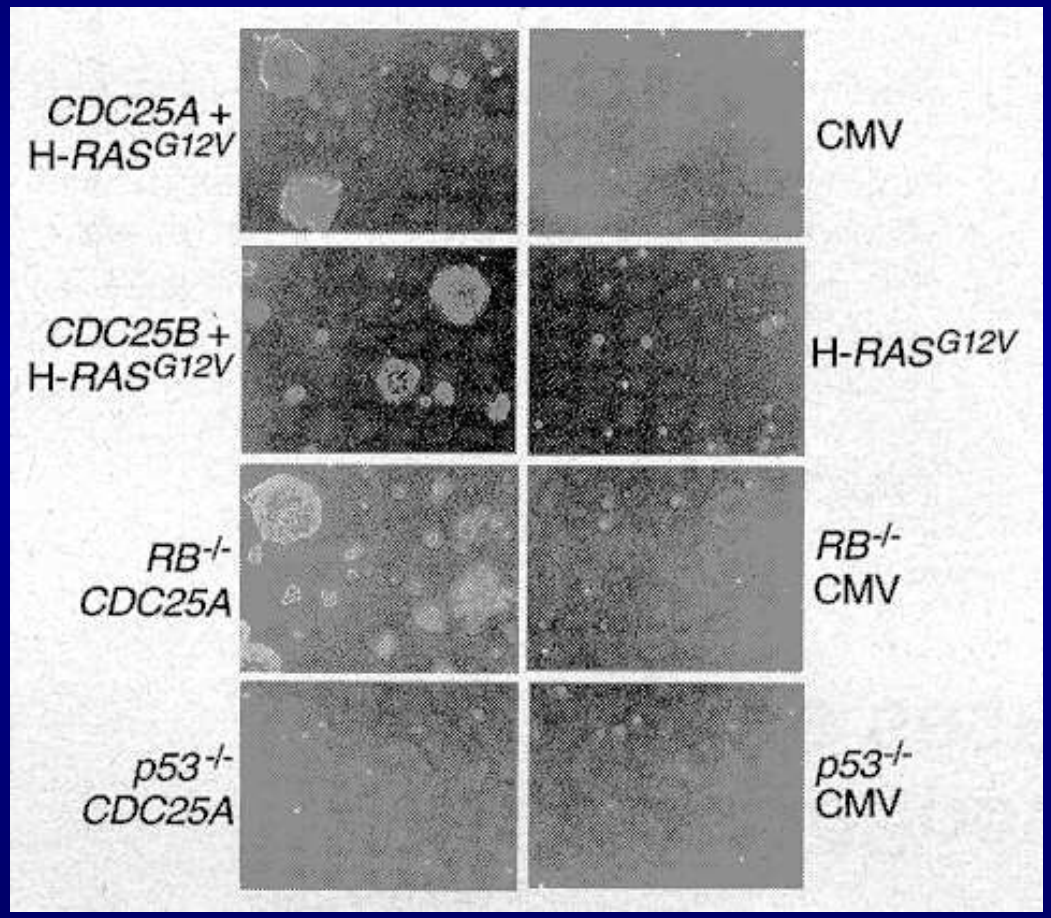


Regulation of Cell Cycle Progression by Cdc25: Cdk Activation



Oncogenic Cooperation between Cdc25A and H-Ras^{G12V} or Rb$^{-/-}$





Galaktionov et al., Science 269: 1575, 1995.

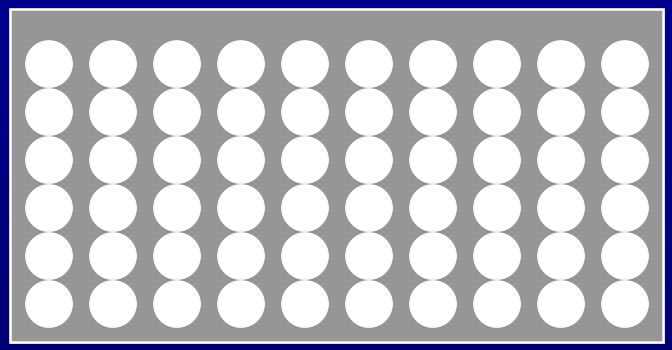
CDC25 Phosphatases and Cancer

- CDC25A and B overexpressed in many cultured cancer cell lines.
- Cdc25A suppresses apoptosis.
- Overexpression of CDC25A or B has been detected in human breast, head and neck, cervical, skin, lymph, lung and gastric cancers.
- Human CDC25A & B cooperated with Ha-Ras^{G12V} and CDC25A cooperated with Rb $^{-/-}$ in the oncogenic focus transformation of mouse embryonic fibroblasts and tumor formation in nude mice. Thus, Cdc25A & B may be human oncogenes.

Method for identifying Cdc25 phosphatase inhibitors

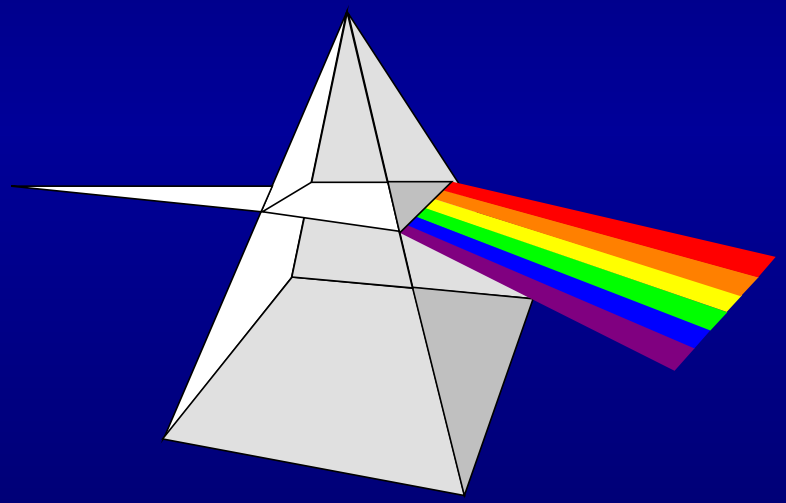
GST-Cdc25 in assay buffer

Fluorescein diphosphate



Incubate 1h

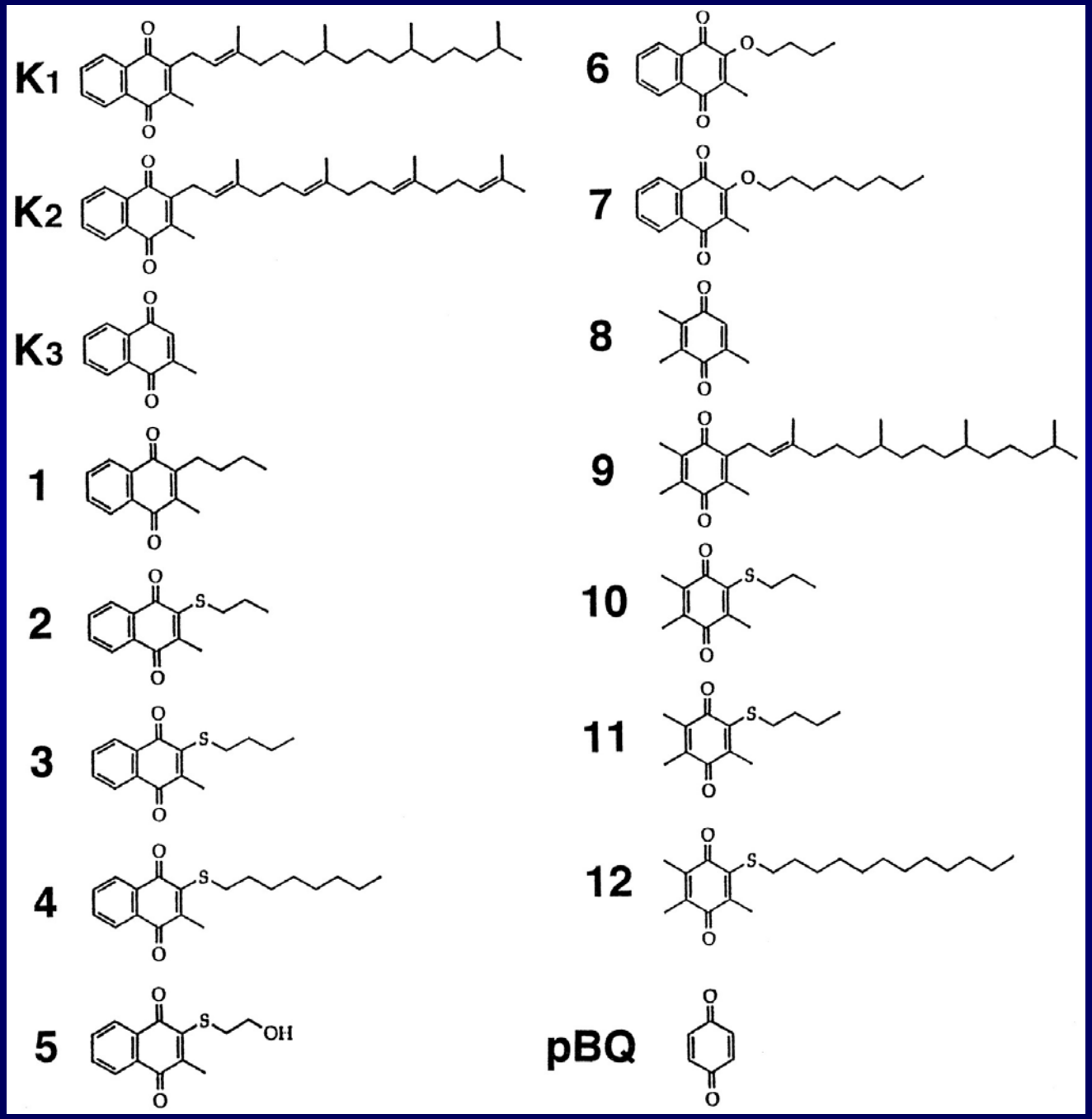
RT



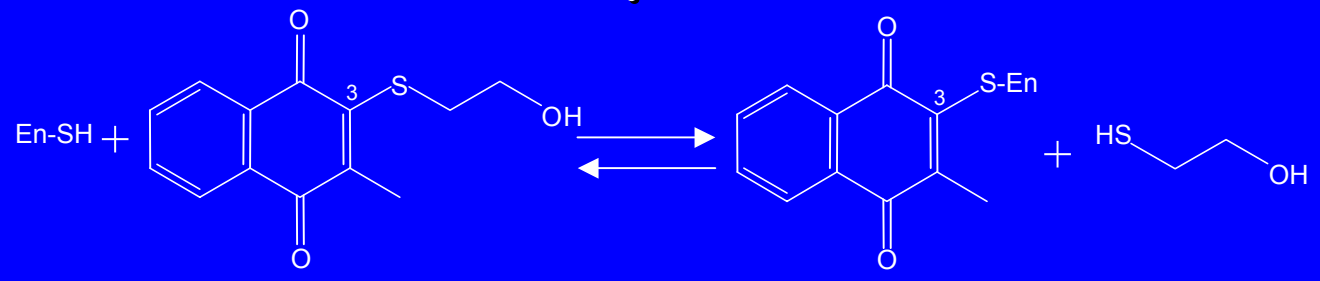
Read product
(fluorescein monophosphate)
on cytoflour II

Chemical Screening Approach

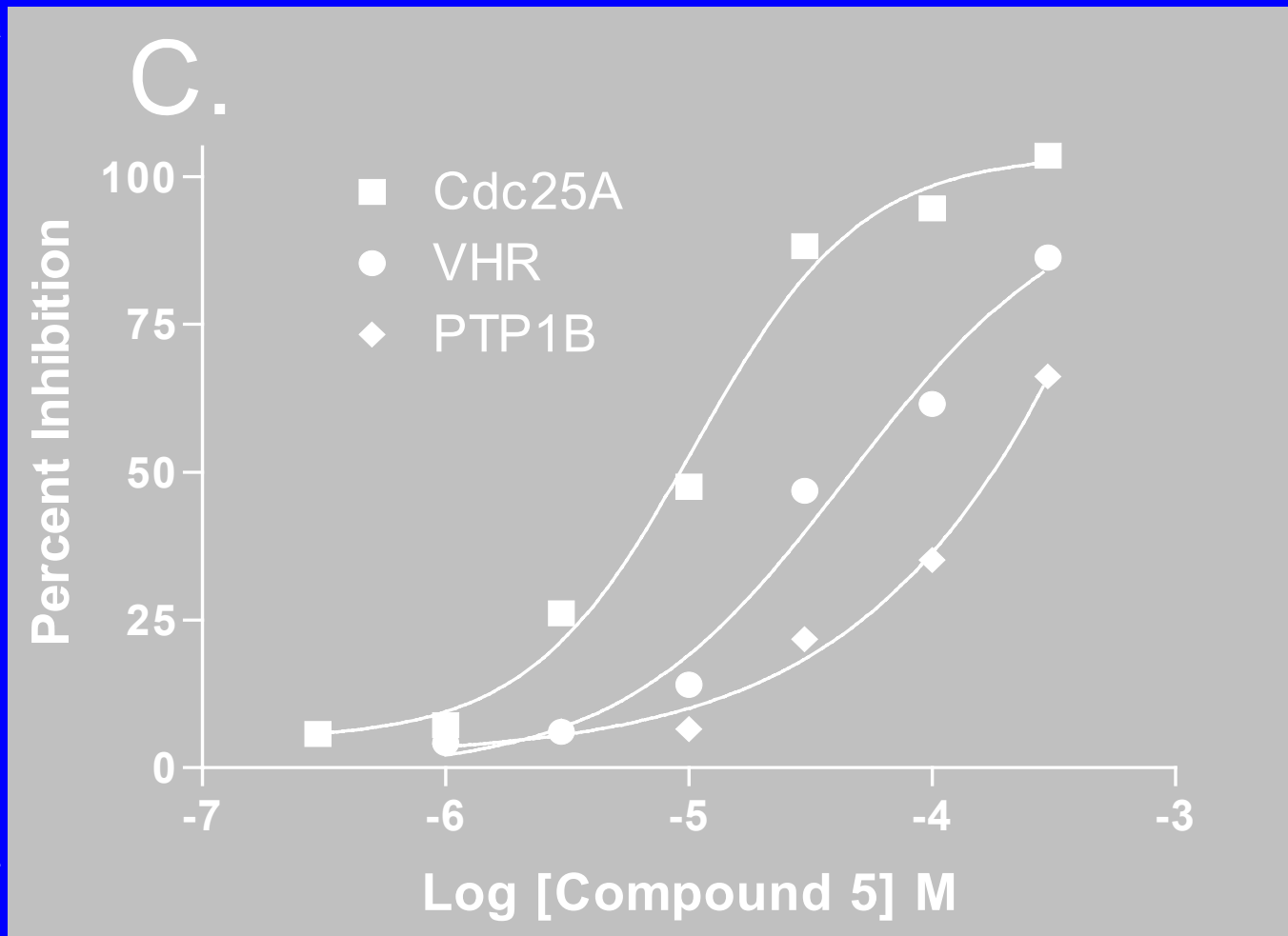
- Targeted Array Libraries
- Diverse Chemical Libraries


K1
K2
K3
1
2
3
4
5
6
7
8
9
10
11
12
pBQ
O
S
OH

Compound 5 inhibits Cdc25

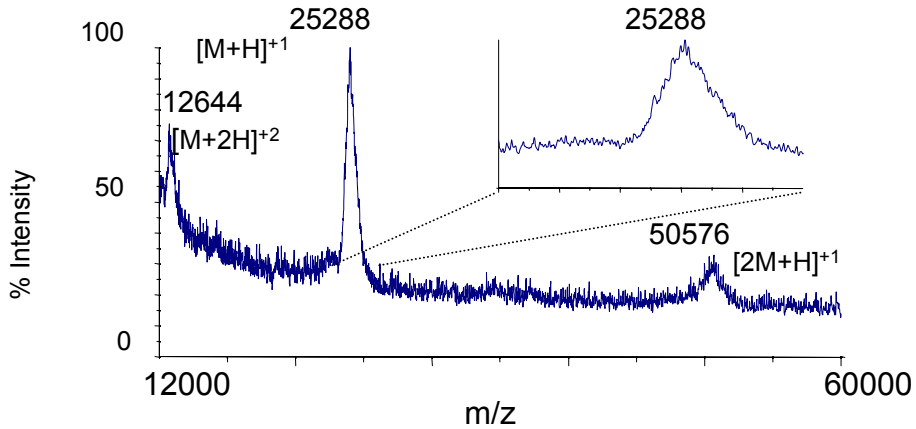


$MW_{monoisotopic}$ = 248.1 Da

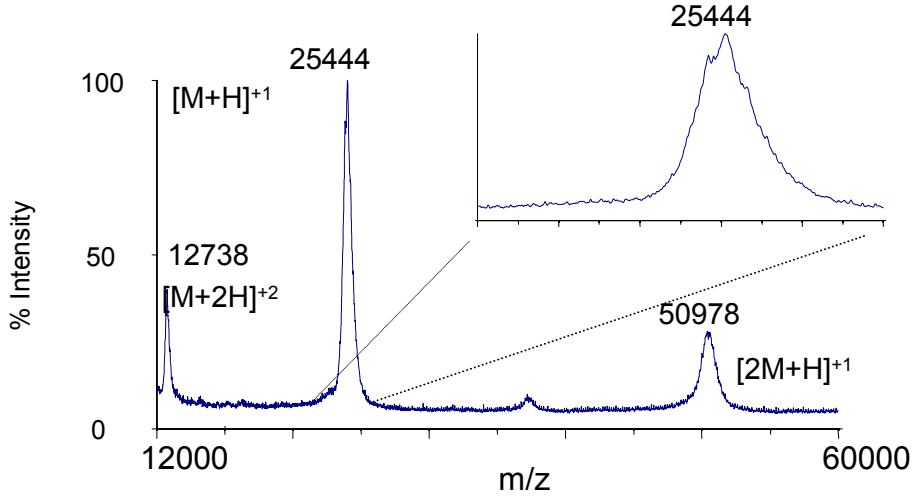


$Cdc25B_2$ K_i ~ 2 μM

MALDI-TOF ANALYSES
Compound 5 binds tightly to the catalytic domain of Cdc25A



DMSO



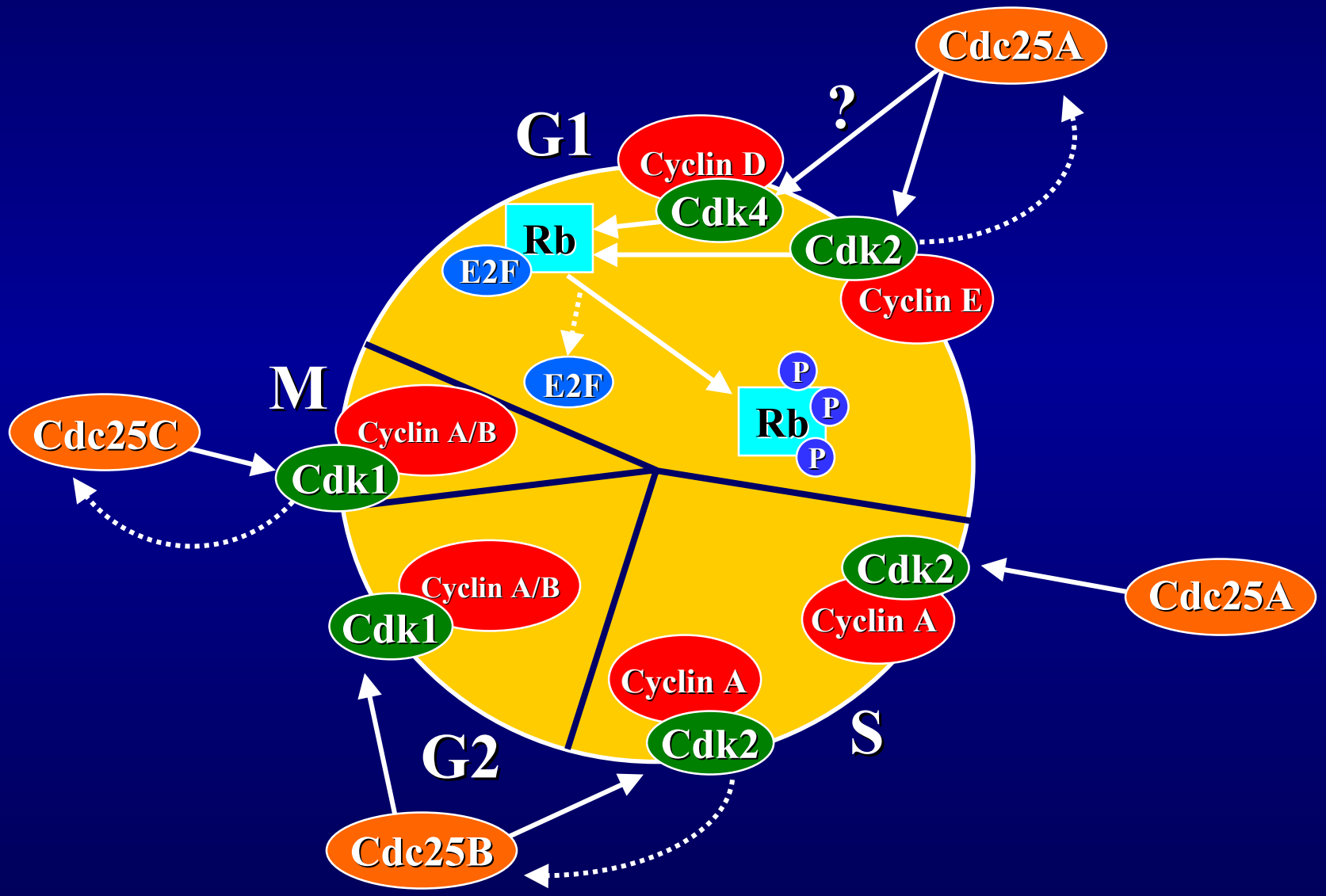
Compound 5

Lixia Pu

Compound Validation

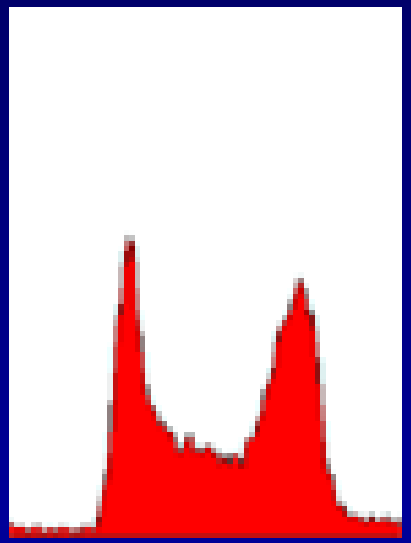
- Cellular: Cell Cycle
- Biochemical: Substrate phosphorylation
- Genetic: Chemical complementation

Cell cycle regulation by Cdc25 phosphatases



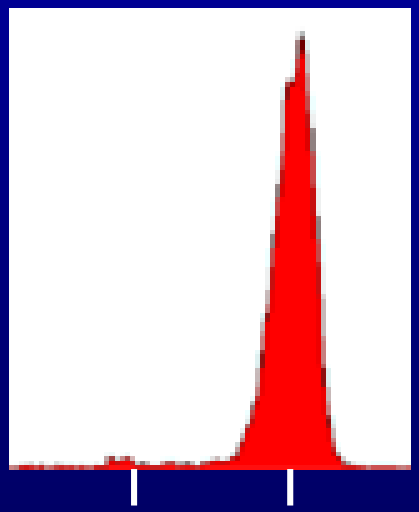
tsFT210 Cell System

32º 17 h



Functional Cdk1

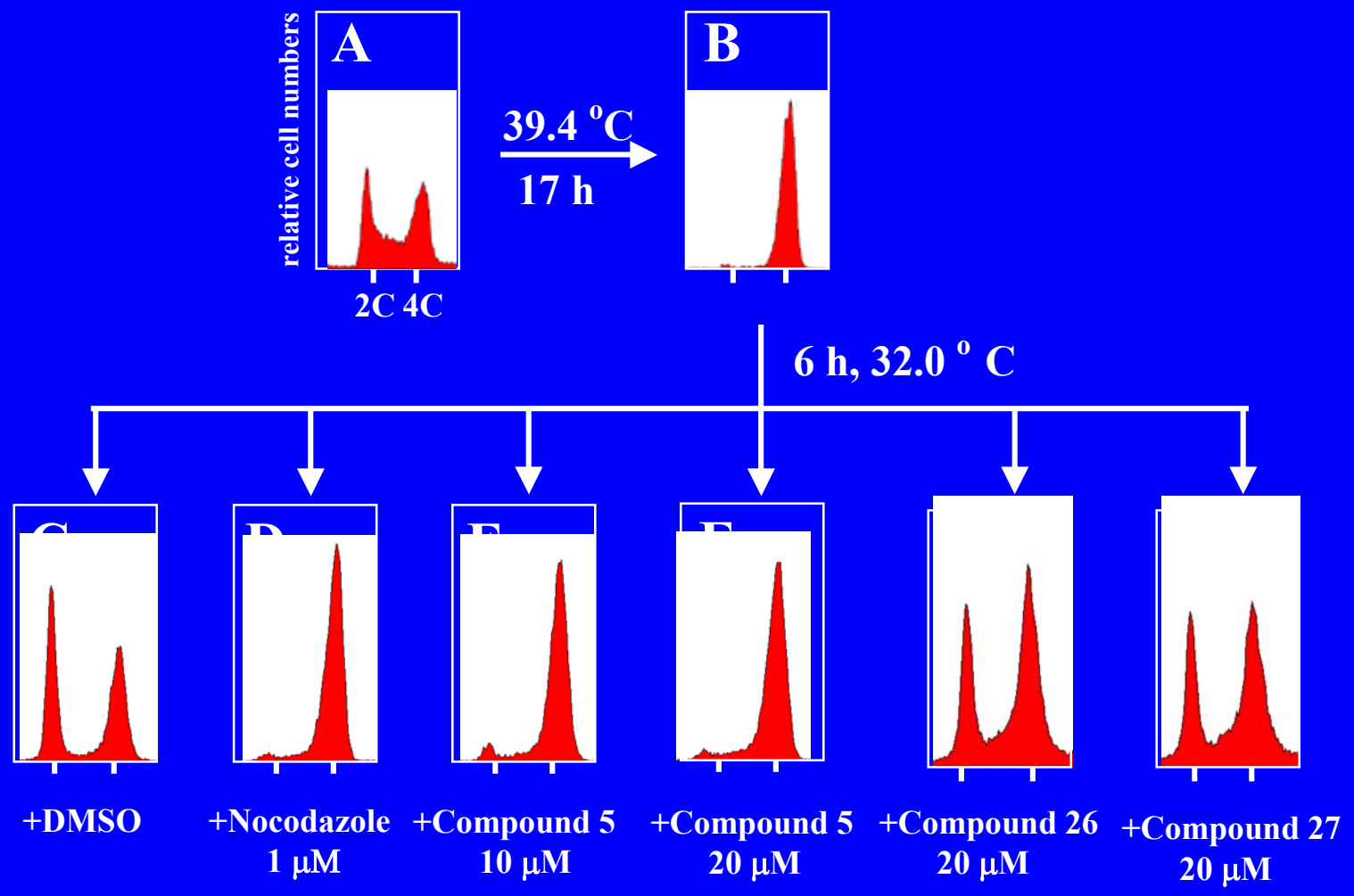
tsFT210 cells
Cdk1 mutants



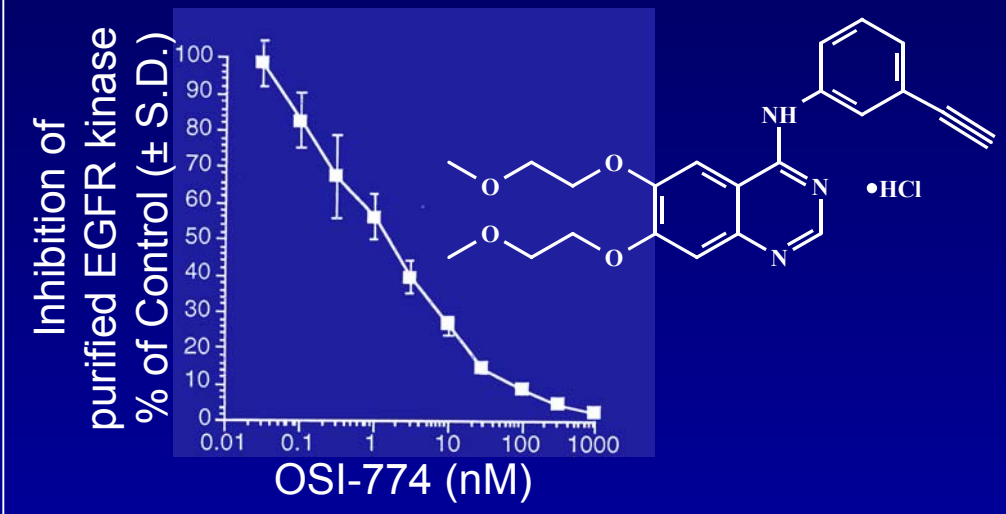
No functional Cdk1

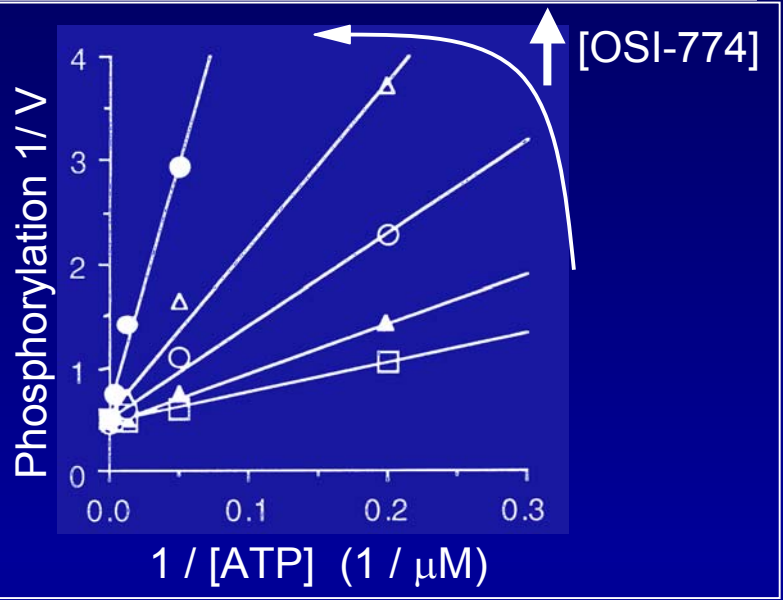
39.4º 17 h

Compound 5 causes G2/M arrest

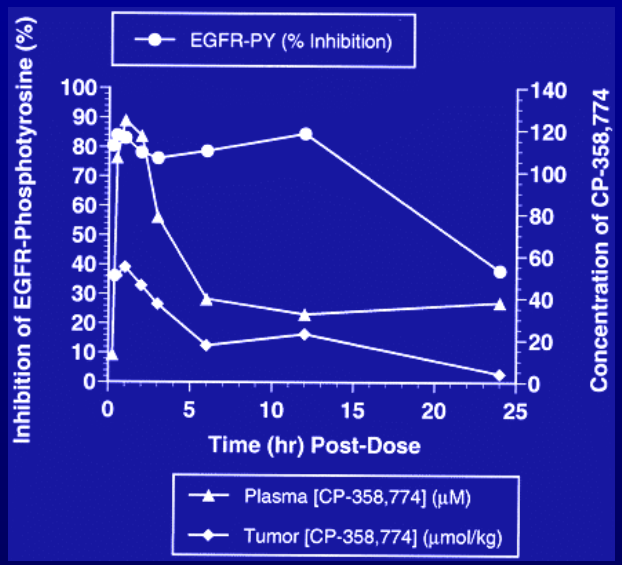


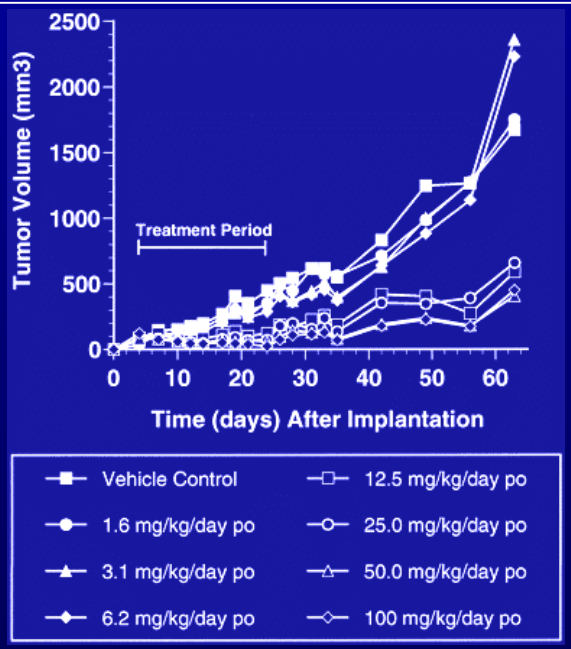
OSI-774:
AN EGF-R PROTEIN KINASE ANTAGONIST





Moyer et al, Cancer Res 57: 4838, 1997





Pollack et al, J Pharmacol Exp Ther 291: 739, 1999

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C/EBPα AS A TARGET FOR DEVELOPMENT OF NOVEL CANCER THERAPEUTICS

- The transcription factor C/EBPα plays key roles in regulation of differentation of various cell lineages (adipocytes, keratinocytes, etc.)

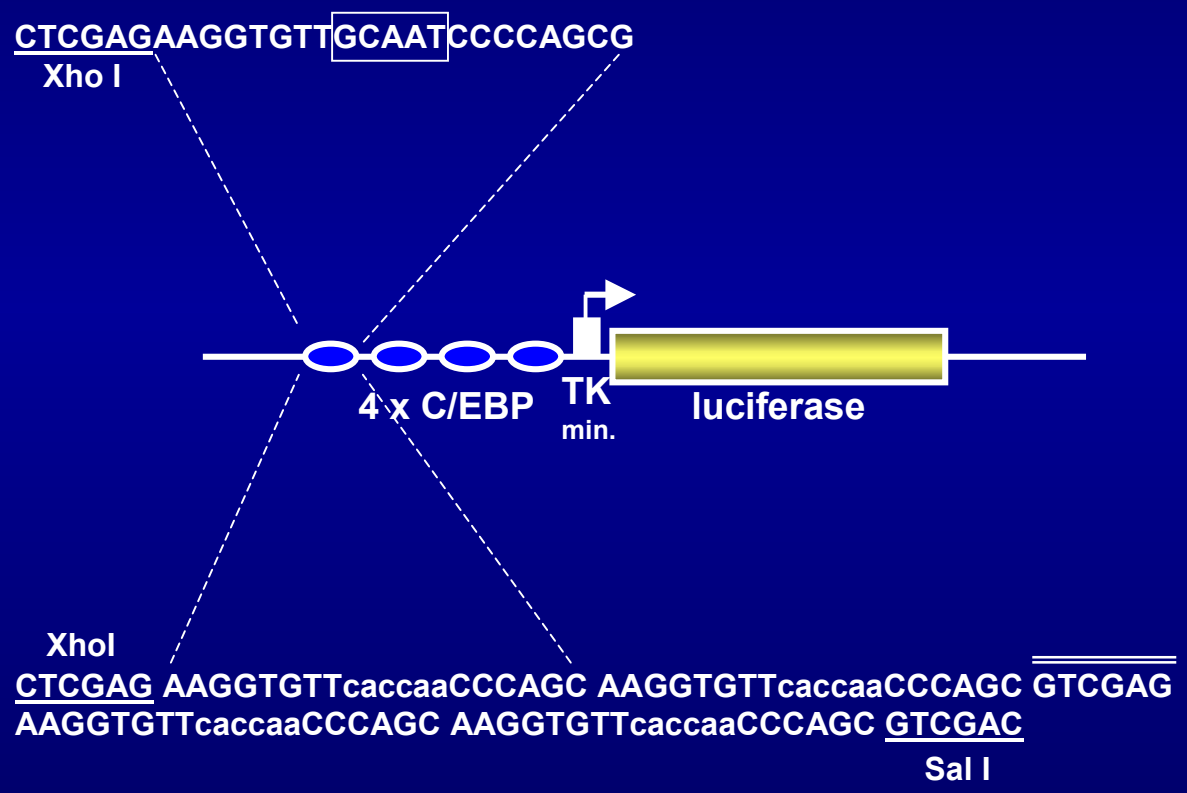
- Mutations in CEBPA (the gene coding for C/EBPα) are associated with development of AML [t(8;21) - subtypes M1 and M2]

- CEBPA knock-out mice show no mature neutrophils

- Conditional expression of CEBPA is sufficient to trigger neutrophilic differentiation

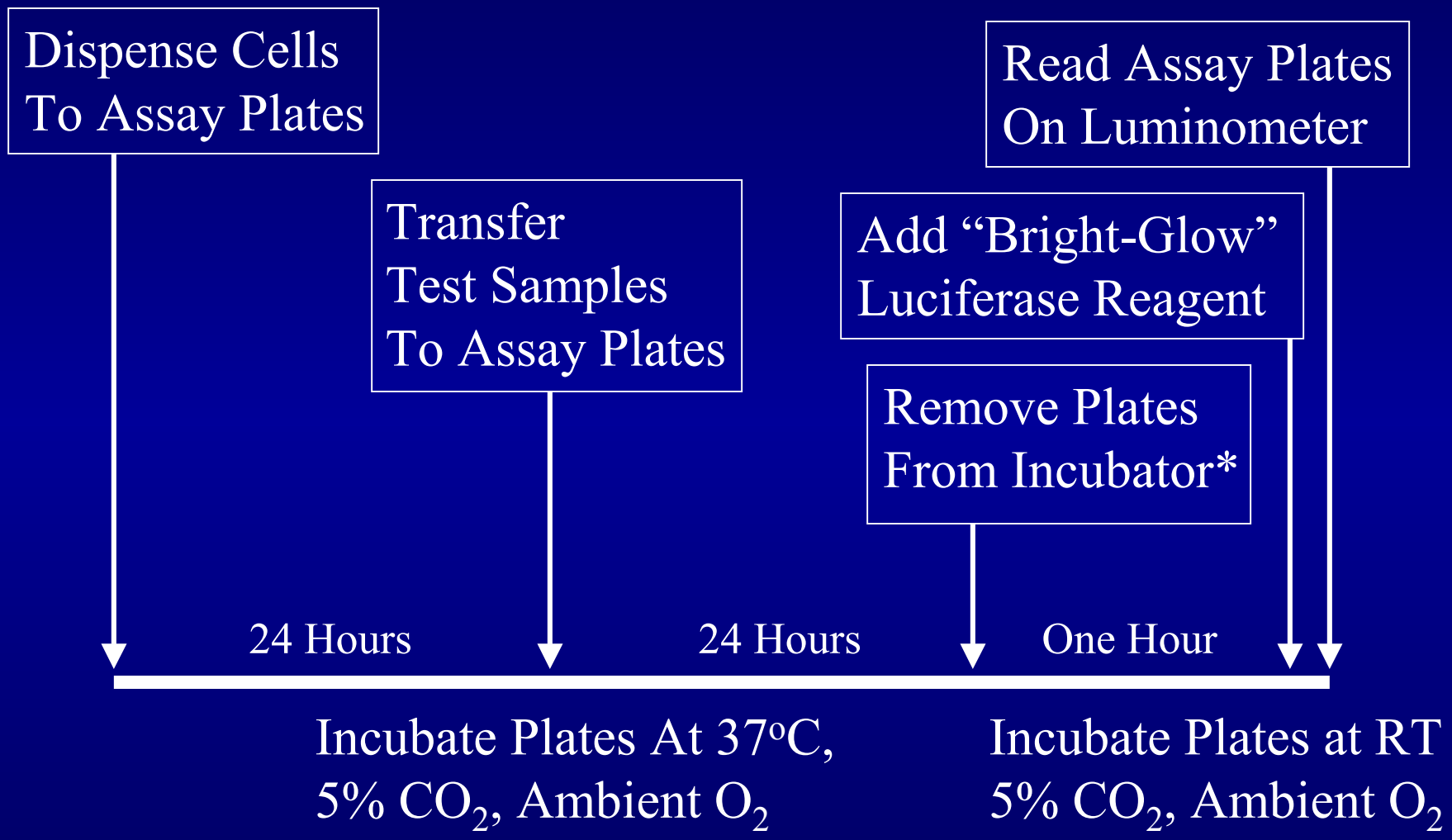
- Pharmacologic modulators of CEBPA could act as differentiation inducers and thus limit proliferation of AML cells

CEBP Reporter Construct*

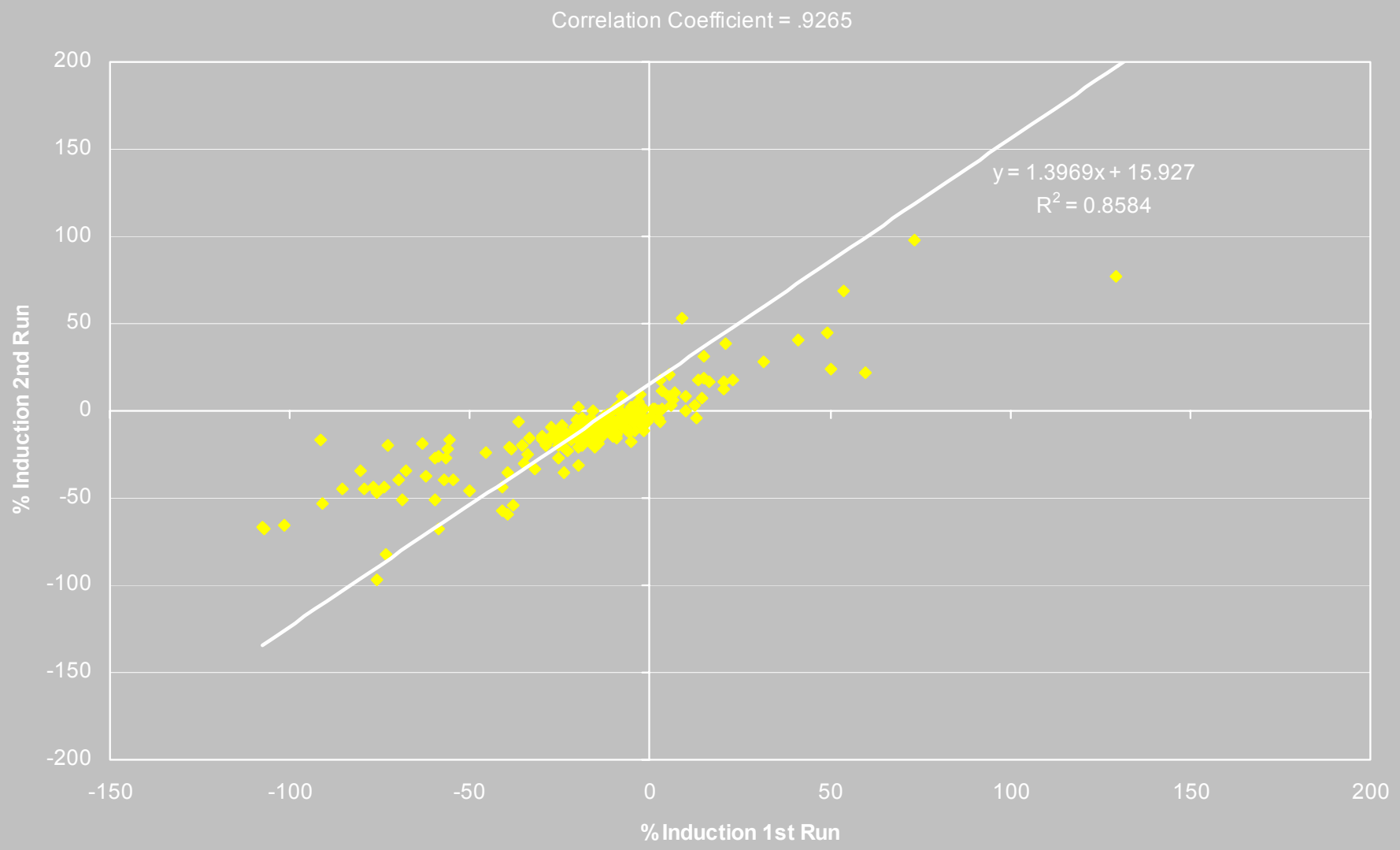


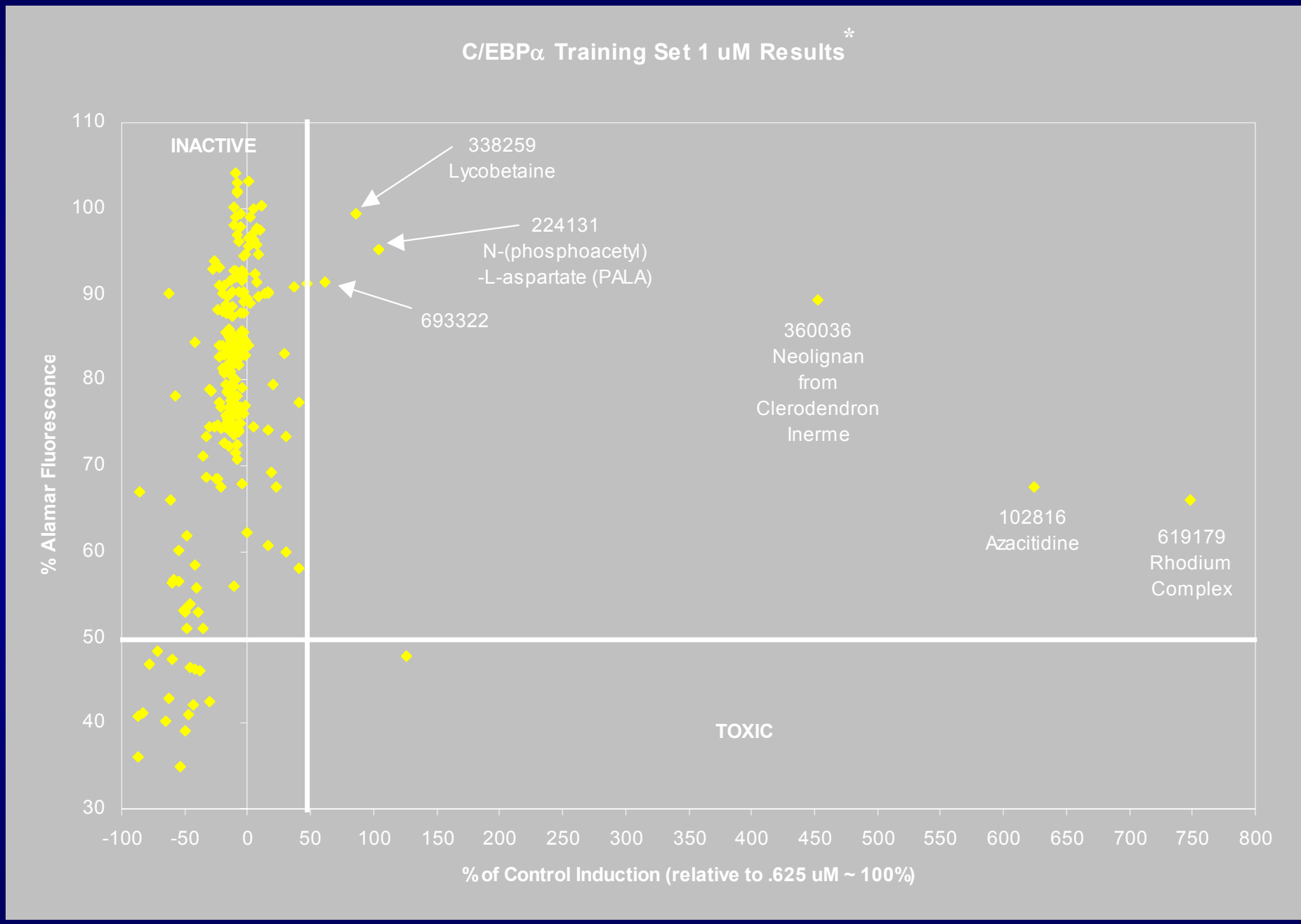
*Host cell for this construct is U-937

CEBPA Assay Timeline

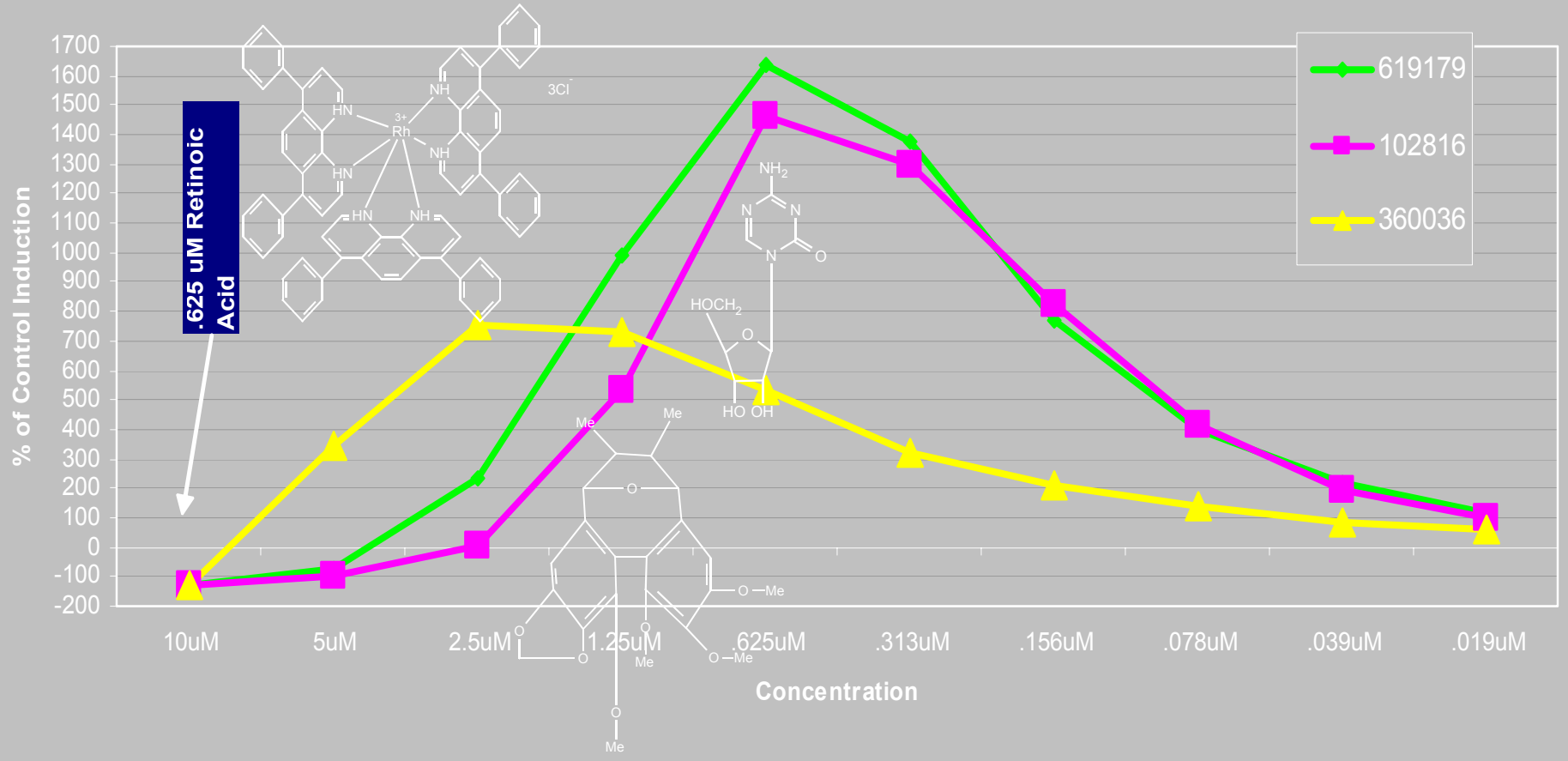


*Sister plates processed for Alamar blue toxicity assay

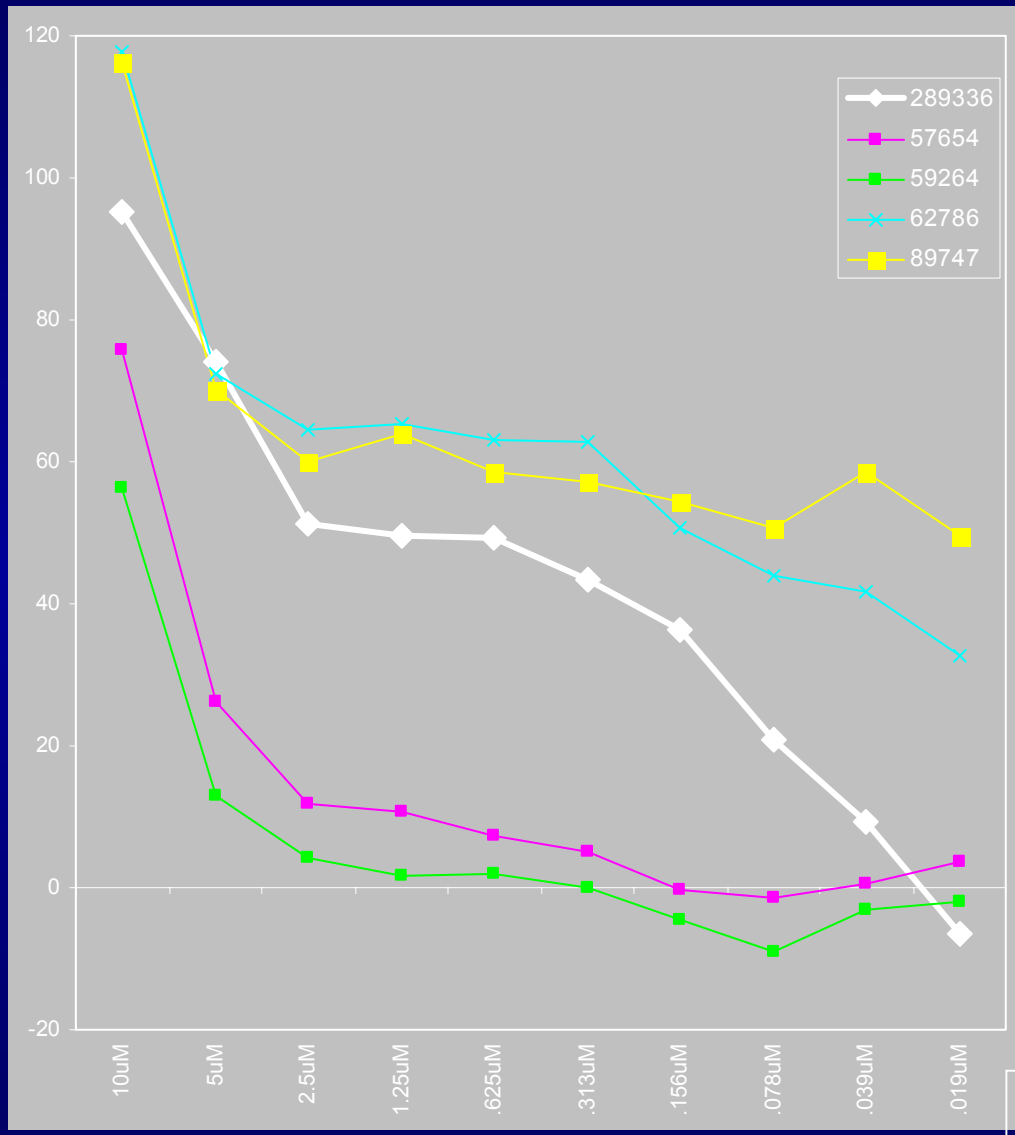

C/EBPa Training Set: 1st Run compared to 2nd Run % Induction
Correlation Coefficient = .9265
y = 1.3969x + 15.927
R2 = 0.8584
% Induction 2nd Run
% Induction 1st Run
200
150
100
50
0
-50
-100
-150
-200
-150
-100
-50
0
50
100
150
200

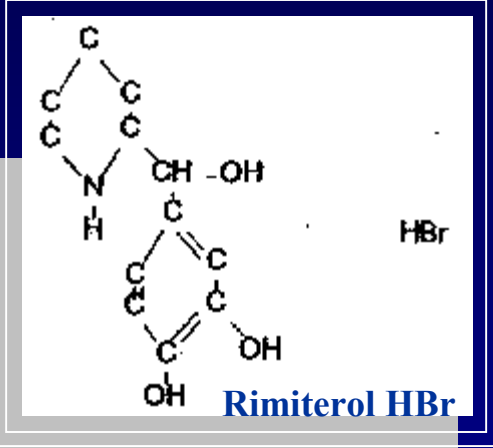


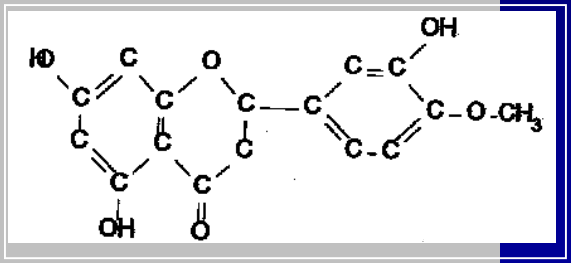
*Data averaged from two independent assays

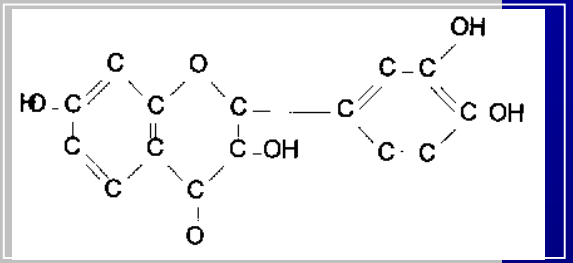

C/EBPa Screen: % Concentration Response Graphs
% Induction (relative to .625 uM retinoic acid induction) for seven select compounds
% of Control Induction
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
.625 uM Retinoic Acid
10uM
5uM
2.5uM
1.25uM
.625uM
.313uM
.156uM
.078uM
.039uM
.019uM
Concentration
619179
102816
360036
3Cl
Rh
3+
HN
NH
NH2
HOCH2
HO OH
Me
O
O—Me

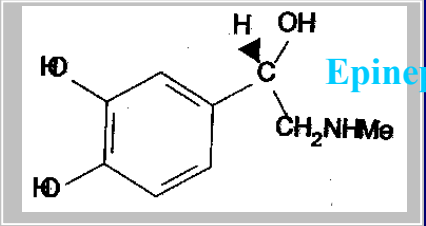
C/EBPa % Induction Dose Response Curves

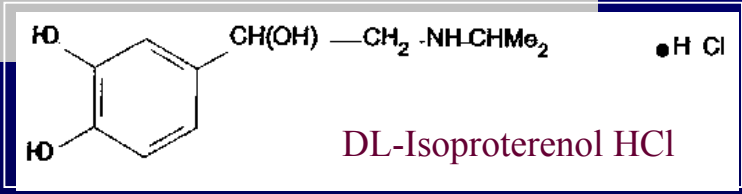




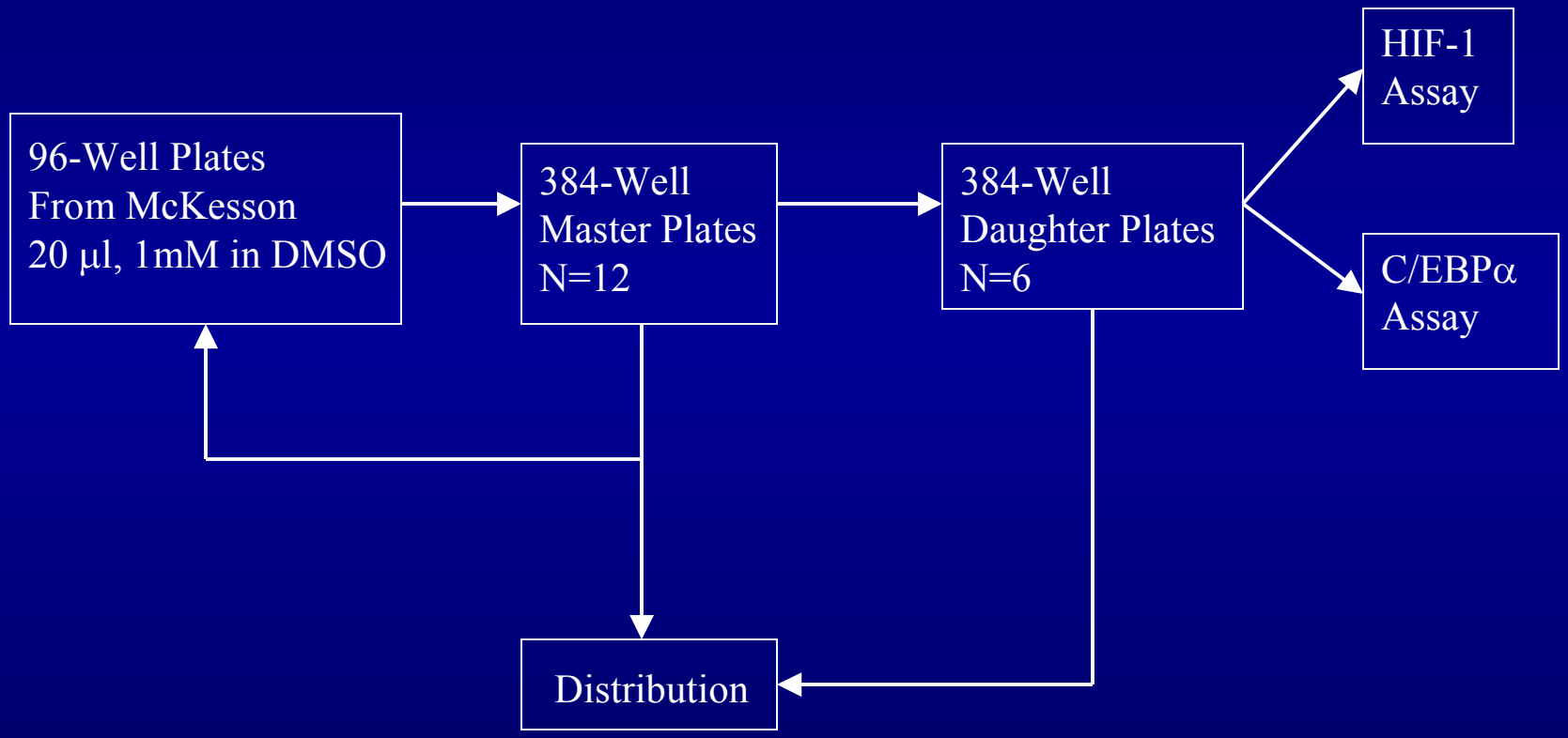








Sample Handling for the HIF-1 and C/EBPα Screen of the NCI Repository*



*Screening of the entire ~140,000 compound open repository at the rate of 10-20,000 compounds per month will take up to one year.

NCI IN VITRO DRUG SCREEN

1985 Hypothesis:

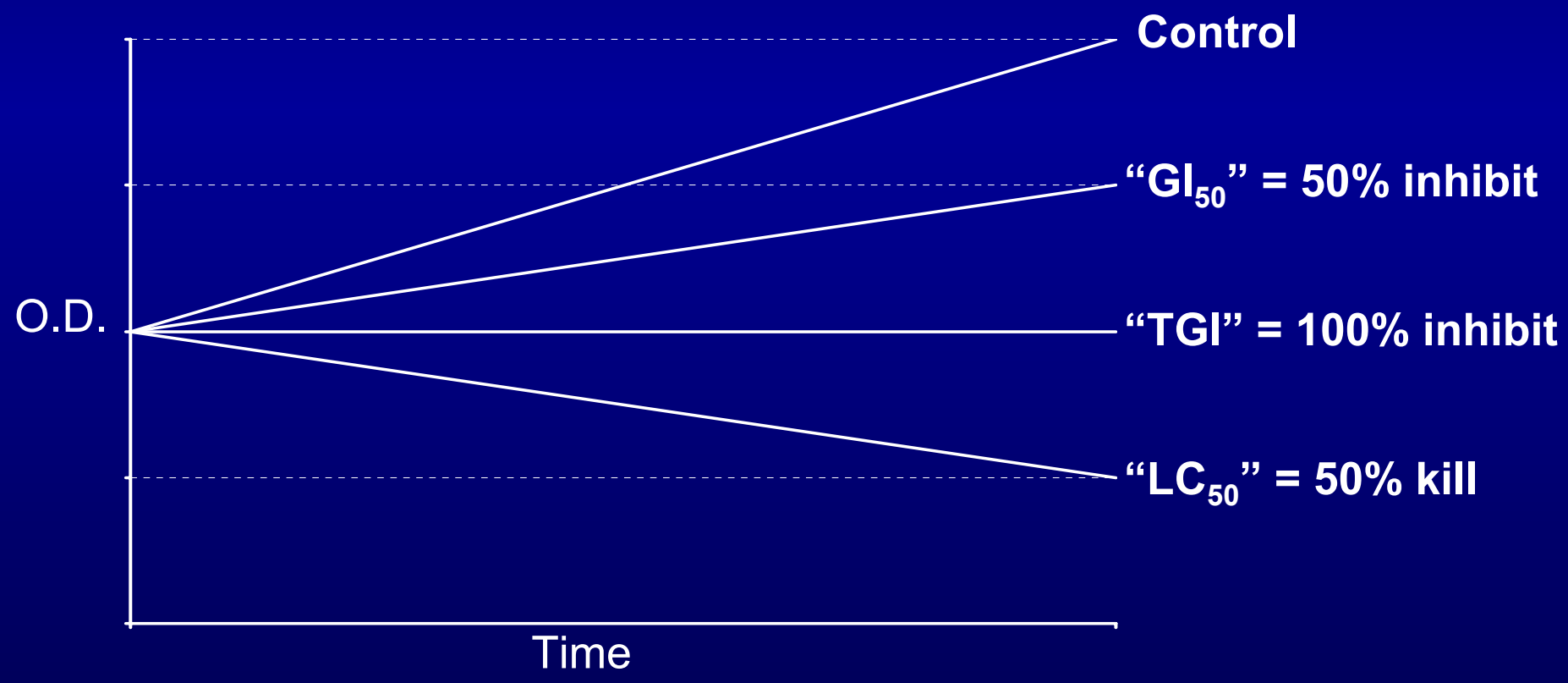
- Cell type specific agents
- Activity in solid tumors

Emerging Realities:

- Unique patterns of activity, cut across cell types
  AND
  Cell type selective patterns found
- Correlations of compound activity
  - relate to molecular “target” expression
  - generate hypothesis re: molecular target

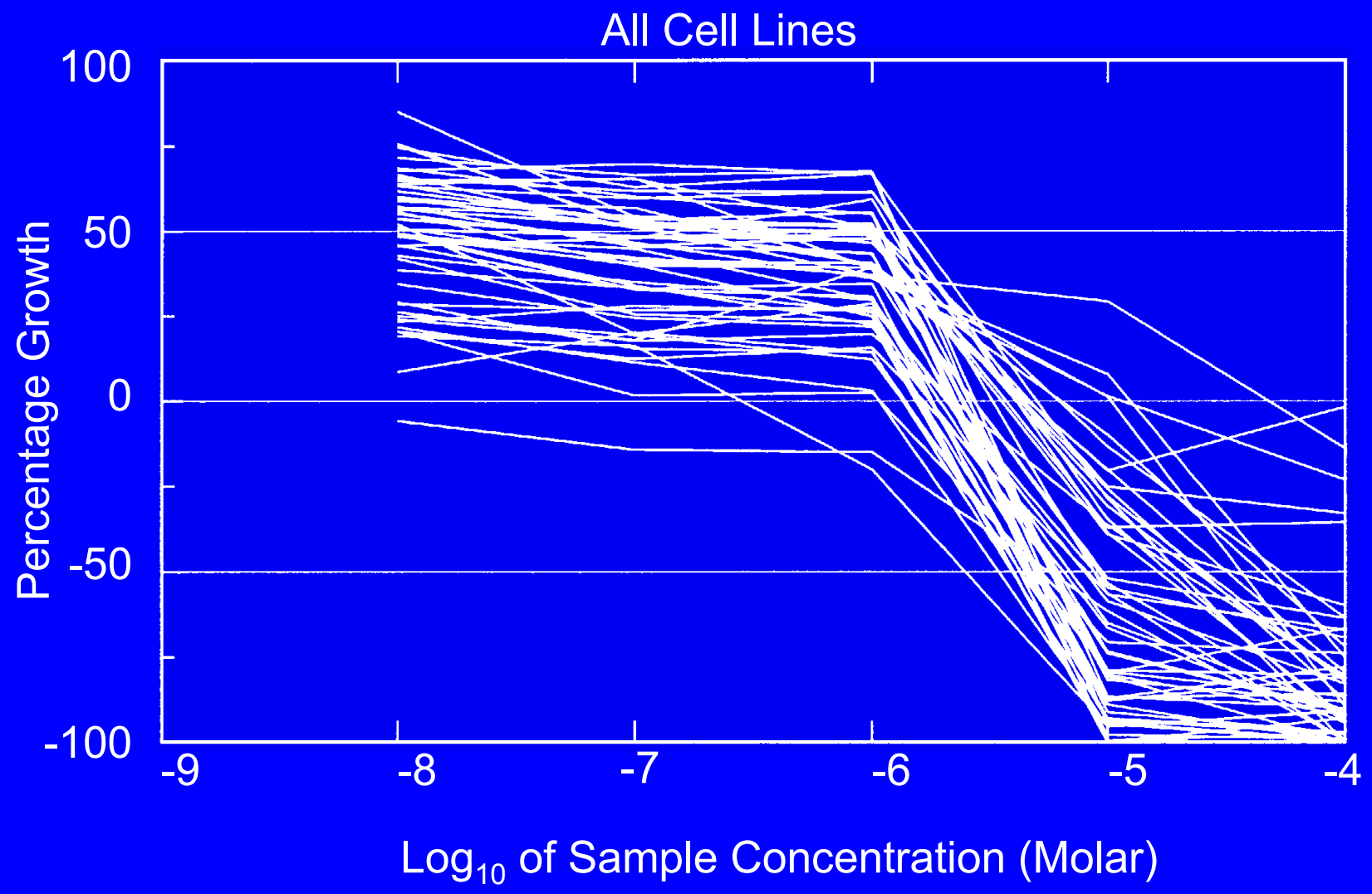
NCI IN VITRO CANCER CELL LINE SCREEN

- 60 cell lines
  (8 breast, 2 prostate, 8 renal, 6 ovary, 7 colon, 6 brain, 9 lung, 8 melanoma, 6 hematopoietic)
- 48 hr exposure; protein stain O.D.



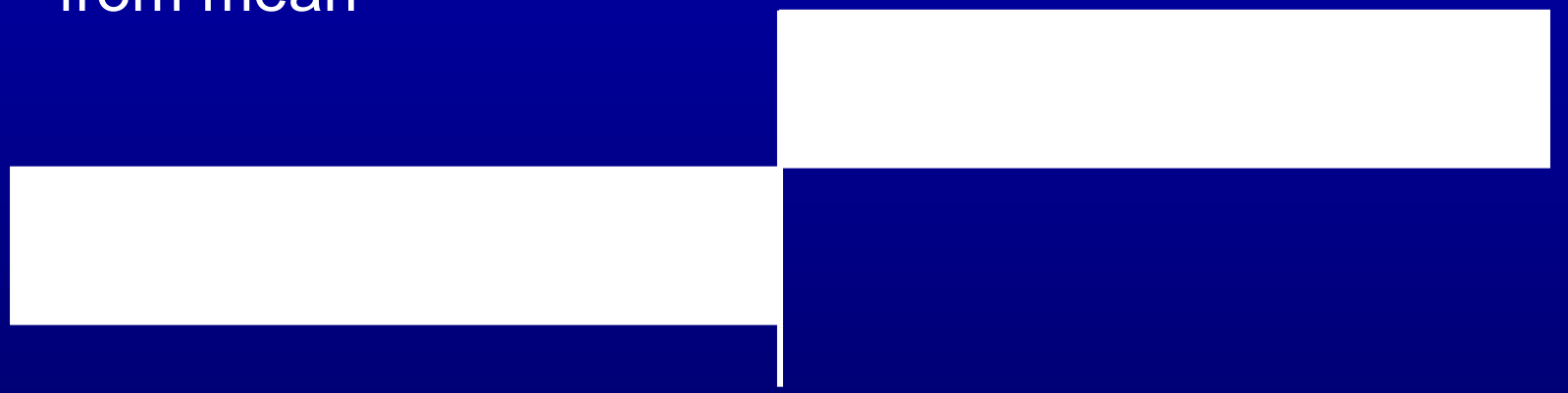
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC: 643248-Q/2 ***(a rapamycin)***	**Exp. ID:** 9503SC35-46



PATTERN RECOGNITION ALGORITHM: COMPARE

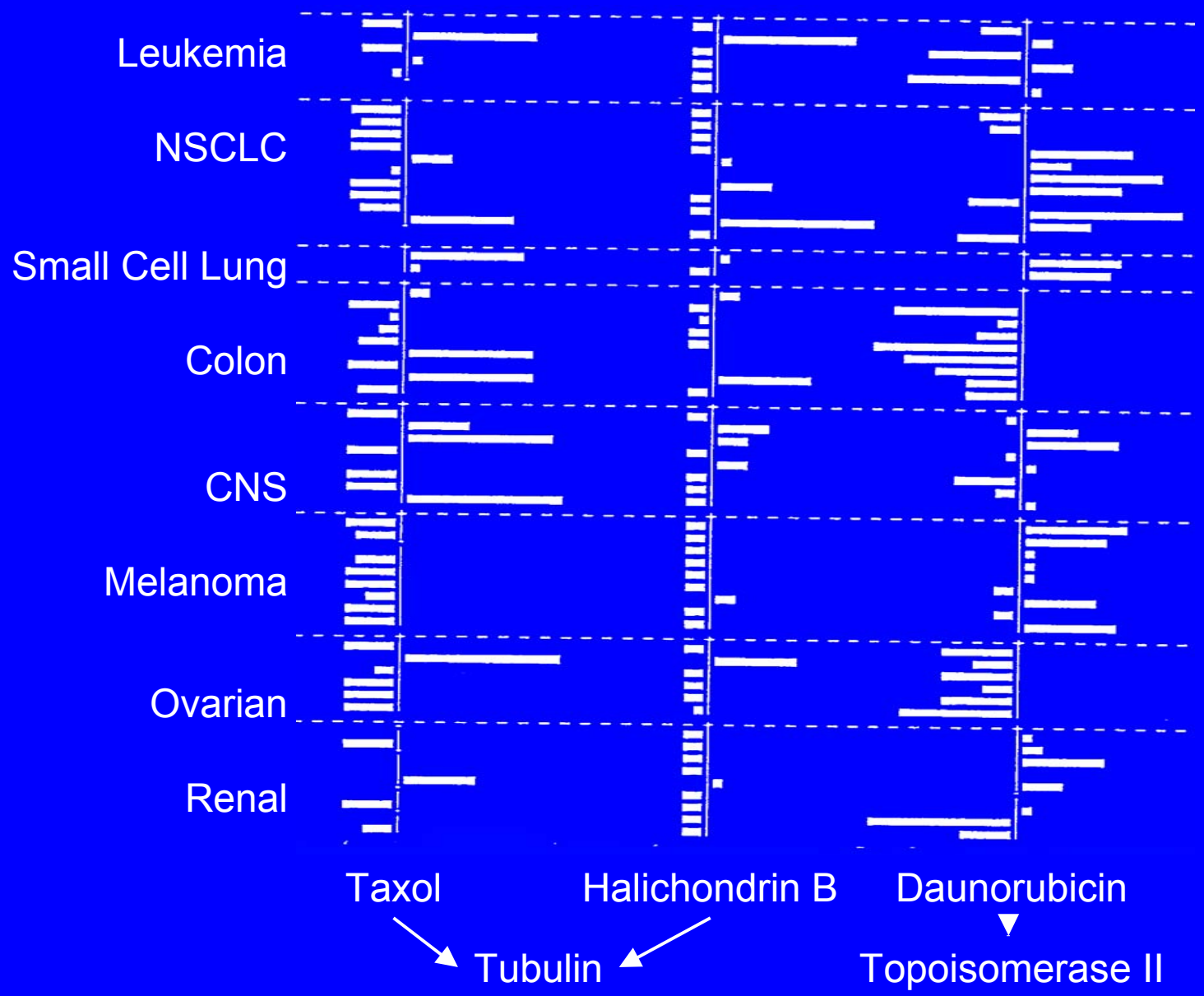
- Goal: COMPARE degree of similarity of a new compound to standard agents
- Calculate mean GI_{50}, TGI or LC_{50}
- Display behavior of particular cell line as deflection from mean



- Calculate Pearson correlation coefficient:

1 = identity ; 0 = no correlation

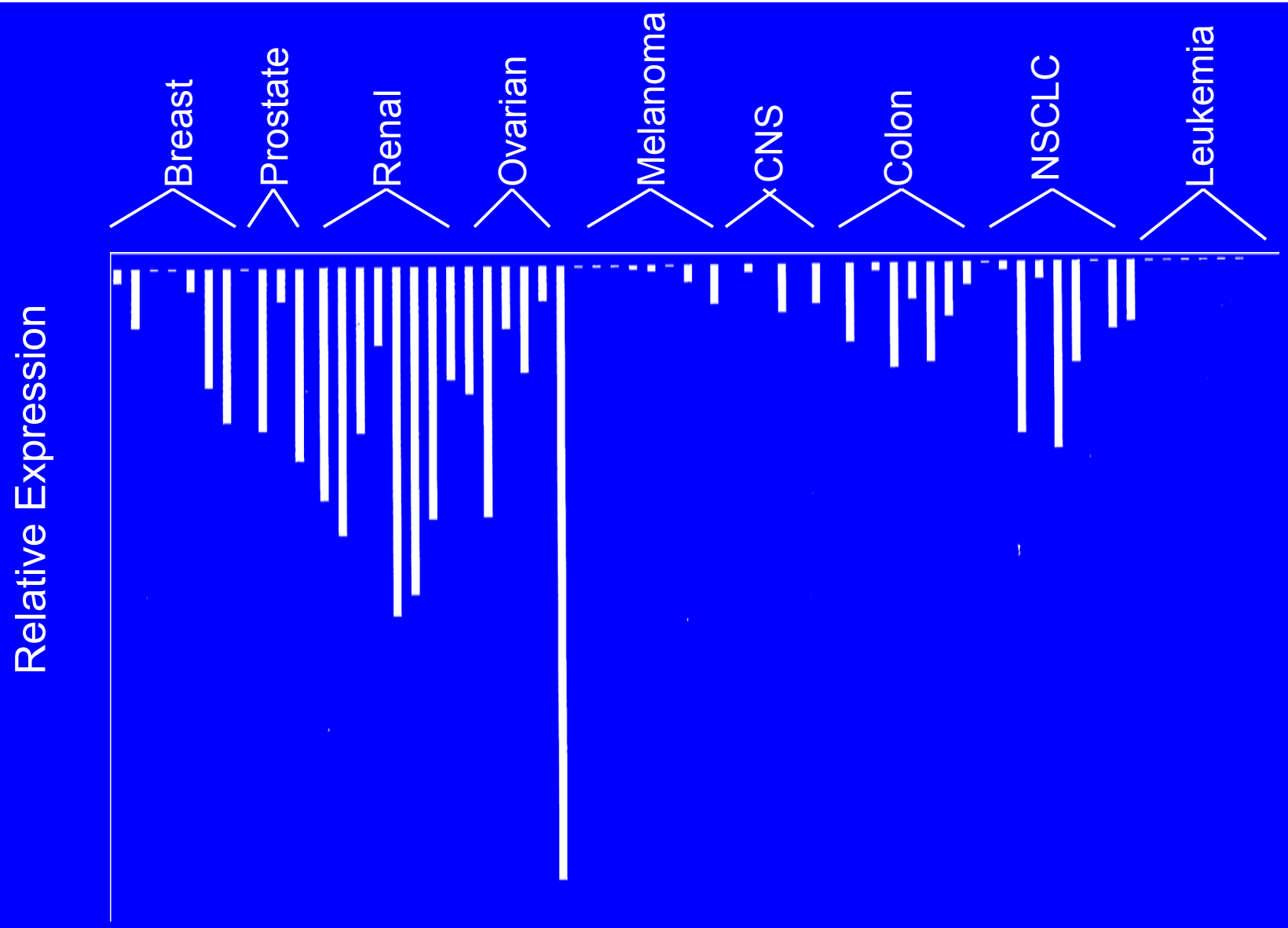
AGENTS WITH SIMILAR MECHANISMS HAVE SIMILAR MEAN GRAPHS



THE COMPARE ALGORITHM
Seed: Rubidazone

164011	1.000	Rubidazone
82151	0.921	Daunomycin
123127	0.915	Adriamycin
665934	0.891	Epipodophyllotoxin analogue
Discreet	0.880	Gyrase-To-TOPO analogue
Discreet	0.867	AMSA analogue
267469	0.865	Deoxydoxorubicin
305884	0.865	Acodazole HCL
665935	0.864	Epipodophyllotoxin analogue
668380	0.861	Azatoxin analogue
639659	0.854	Adriamycin analogue
644946	0.850	Epipodophyllotoxin analogue
254681	0.848	Daunomycin analogue
Discreet	0.847	Epipodophyllotoxin analogue
Discreet	0.843	Epipodophyllotoxin analogue
180510	0.842	Daunomycin analogue
Discreet	0.837	Epipodophyllotoxin analogue
Discreet	0.833	Gyrase-To-TOPO analogue

RELATIVE EGF RECEPTOR mRNA EXPRESSION

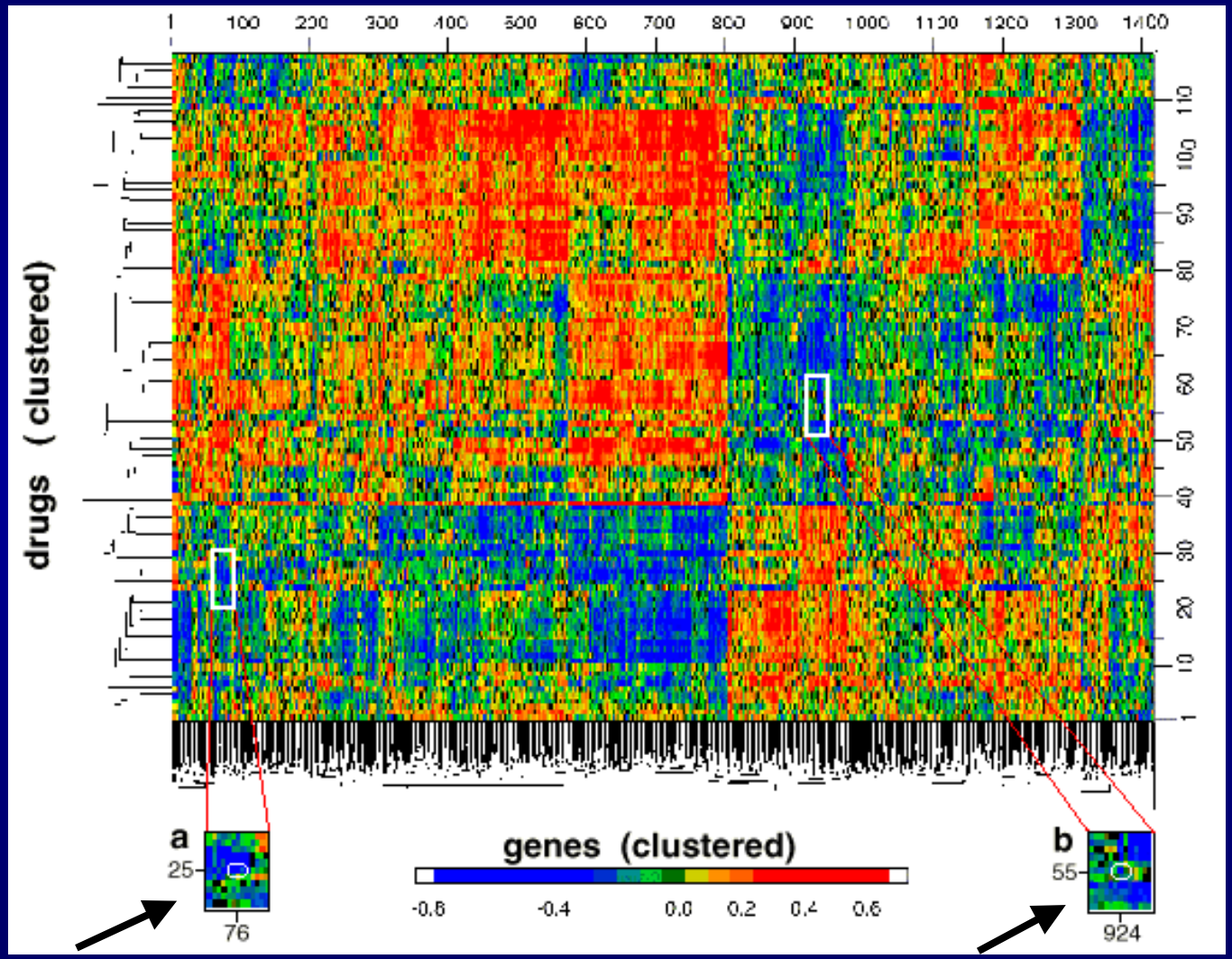


COMPARE ANALYSIS: EGF RECEPTOR

RANK	CORRELATION	CHEMICAL NAME
1	0.71	TGFα-PE40
2	0.66	Toxin-Δ53L, MW=43K
7	0.57	EGFR Tyrosine Kinase Inhibitor
88	0.43	EGFR Tyrosine Kinase Inhibitor

40,421 COMPOUNDS IN THE NCI DATABASE

DRUG TARGET CLUSTERINGS REVEAL CLUES TO MECHANISM



5FU/DPYD

L-Asparaginase/ASNS

Nature Genetics 24: 236, 2000; http://dtp.nci.nih.gov

OUTLINE OF PRESENTATION

- General Introduction
- Definition of Drug Targets
- Generating Diversity
- Definition of Lead Structures
- ***Qualifying Lead for Transition to Early Trials***

GOALS OF PRECLINICAL DRUG STUDIES

Regulatory framework

- IND = “Investigational New Drug” application = approval by FDA to conduct human studies; main criterion : SAFETY AND LIKELY REVERSIBLE TOXICITY = allows *start* of Phase I trials

- NDA = “New Drug Application” = basis for sale to public; main criteria: SAFETY AND SOME MEASURE OF EFFICACY = *result* of Phase II/III trials

COMPONENTS OF AN IND

The goal of the pre-clinical process

- "Form 1571"
- Table of Contents
- Intro Statement / Plan
- Investigator Brochure
- Clinical Protocol
- Chemistry, Manufacture, Control
- Pharmacology/ Toxicology
- Prior Human Experience
- Additional Info - Data monitoring, Quality Assurance

WHAT TO DO AFTER THE LEAD COMPOUND IS IDENTIFIED?

- If "*empirical*" lead, OPTIMIZE: schedule; formulation vs. biological effect after identifying a "predictive" model; ideally bring series of close analogs through pharmacology and perhaps early toxicology

- If "*rational*" lead, capitalize on target-directed effects in optimizing process

- IN EITHER CASE, KEY TASKS ARE: ACTIVITY; PHARMACOLOGY; FORMULATION; SAFETY TESTING

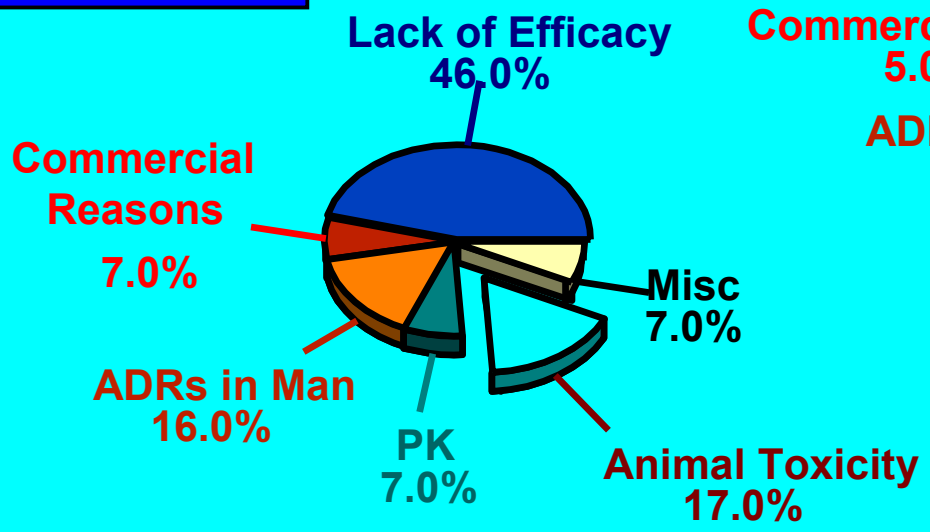
OBJECTIVES OF PRECLINICAL PHARMACOLOGY STUDIES FOR ANTI-NEOPLASTIC DRUGS

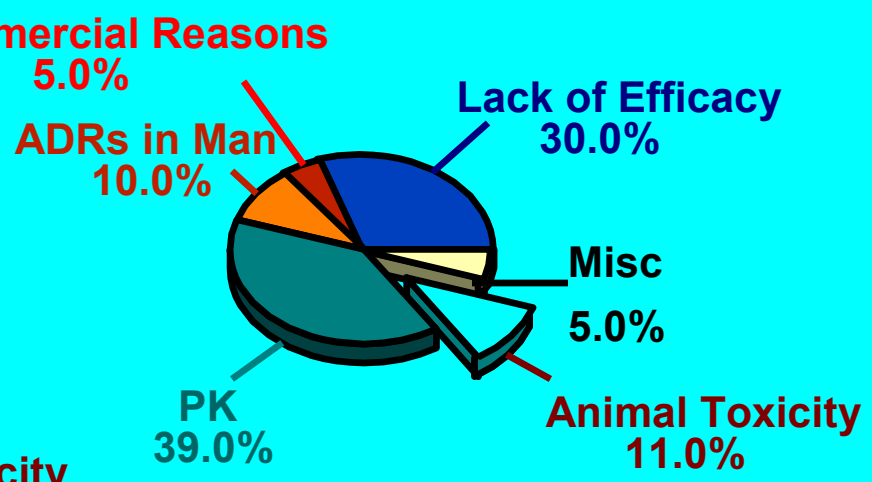
- Development of Sensitive Analytical Methods for Drugs in Biological Fluids & Tissues
- Determine *In Vitro* Stability and Protein Binding
- Determine Pharmacokinetics in Rodents (& Dogs)
- Identification and Analysis of Metabolites
- Define Optimal Dose Schedule and Blood Sampling Times
- Define C_P and/or AUC with Efficacy, Safety & Toxicity
- Analog Evaluation - Determine Optimal Development Candidate

REASONS FOR TERMINATION OF DEVELOPMENT OF NCEs

(Excluding Anti-Infectives)

J. Ormerod (1994)

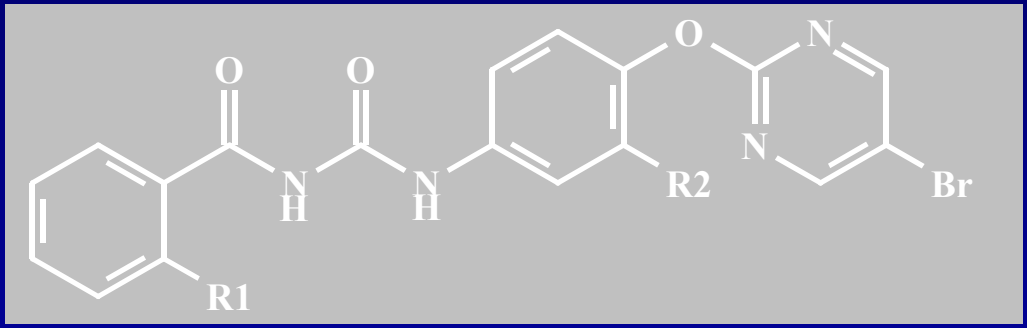




Data taken from 7 UK-owned Companies (1964 - 1985)

BENZOYLPHENYLUREAS

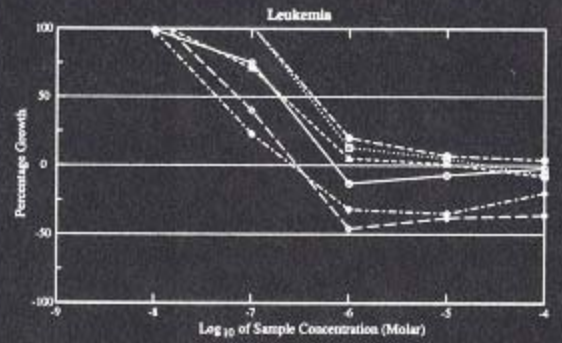
Ishihara Sangyo Kaisha, Ltd.

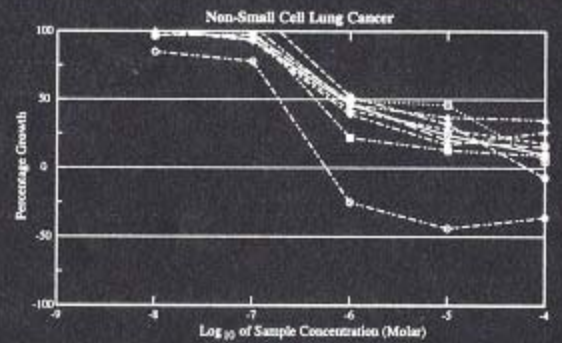


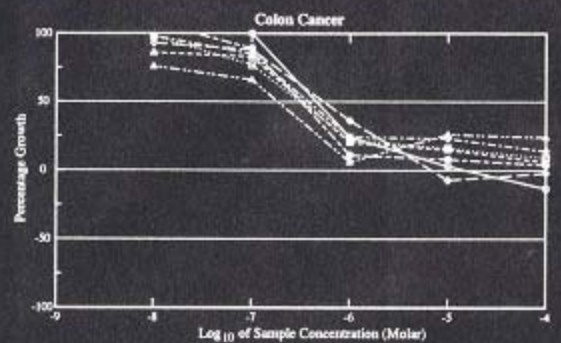
NSC	R1	R2
624548	NO_2	Cl
639828	NH_2	Cl
639829	$N(CH_3)_2$	CH_3
647884	NH_2	CH_3
654259	$NCOCH_2NH_2$. HCl	CH_3
654261	$NCOCH_2N(CH_3)_2$. HCl	CH_3

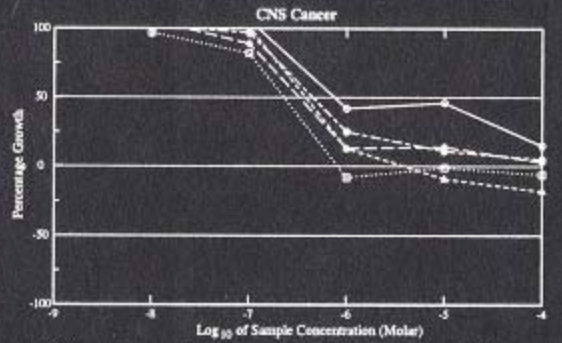
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

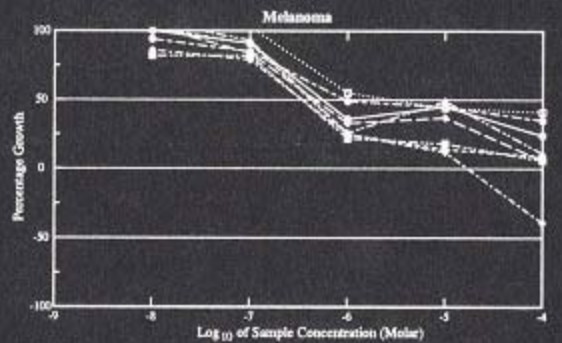
NSC: 639829 / 2		SSPL:	Exp. ID: 9412MD97-43
Report Date: May 30, 2001		Test Date: December 5, 1994	

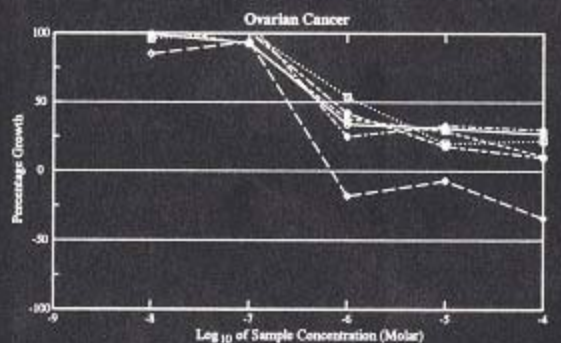


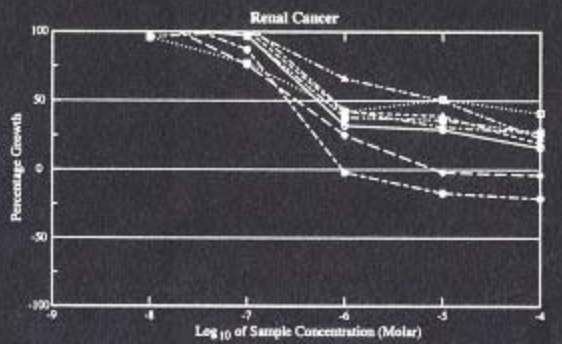


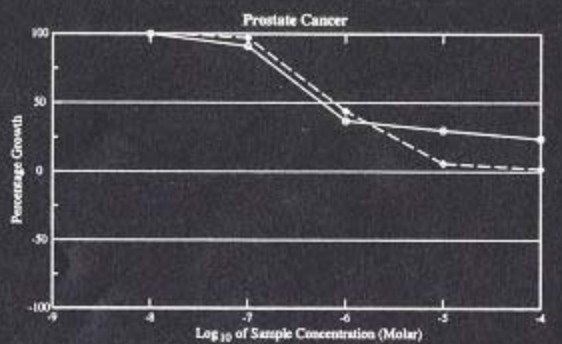


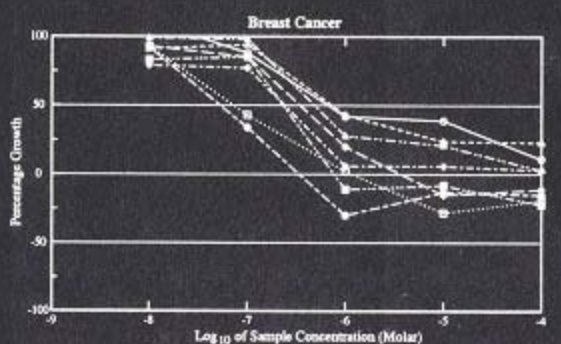












Parent Compound - NSC 639829

COMPARE - Correlation at GI50

	NSC	LCONC	(MAX X)	PEARSON CORR. COEFF.	(N)	CHEM_NAME
1)	332598	-9.00	9	0.664	66	RHIZOXIN
2)	49842	-5.60	129	0.613	72	VINBLASTINE SULFATE
3)	153858	-4.00	15	0.592	69	MAYTANSINE
4)	83265	-3.90	15	0.546	67	S-TRITYL-L-CYSTEINE
5)	157365	2.30	15	0.534	67	NEOCARZINOSTATIN
6)	303861	-2.60	15	0.517	67	L-CYSTEINE ANALOG
7)	67574	-3.00	63	0.517	69	VINCRISTINE SULFATE
8)	192965	-2.00	11	0.496	67	SPIROGERMANIUM
9)	79037	-3.30	59	0.478	69	CCNU
10)	125973	-4.60	21	0.459	70	PACLITAXEL
11)	352122	-3.60	14	0.457	67	TRIMETREXATE

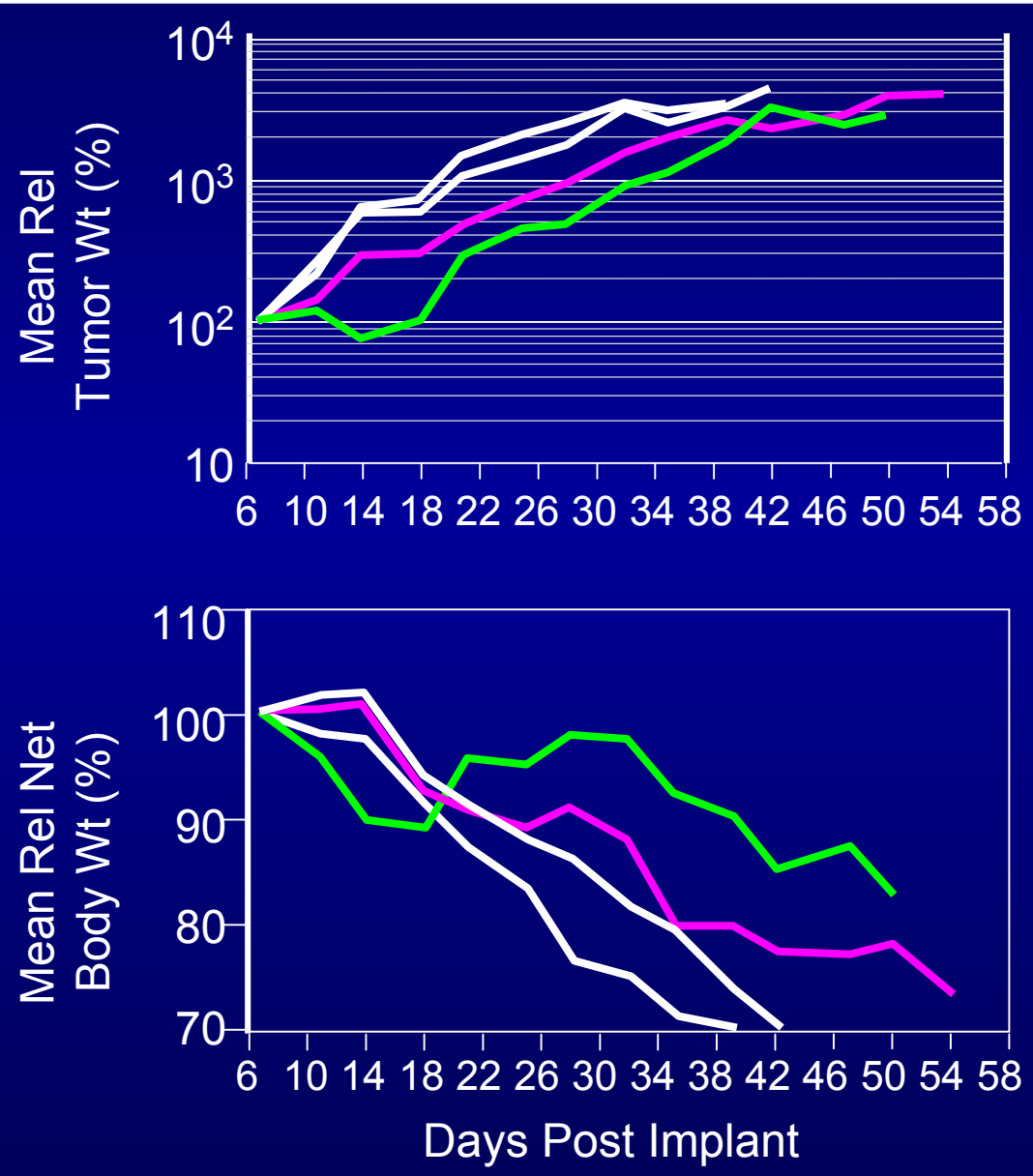
COMPARE - Correlation at TGI

	NSC	LCONC	(MAX X)	PEARSON CORR. COEFF.	(N)	CHEM_NAME
1)	332598	-9.00	9	0.644	66	RHIZOXIN
2)	49842	-5.60	130	0.625	70	VINBLASTINE SULFATE
3)	125973	-4.60	20	0.566	69	PACLITAXEL
4)	153858	-4.00	15	0.548	68	MAYTANSINE
5)	67574	-3.00	64	0.535	68	VINCRISTINE SULFATE
6)	83265	-3.90	15	0.495	66	S-TRITYL-L-CYSTEINE
7)	330500	-3.30	12	0.489	66	MACBECIN II
8)	649890	-6.00	2	0.473	59	FLAVOPIRIDOL
9)	127716	-3.43	11	0.418	66	5-AZADEOXYCYTIDINE
10)	79037	-3.30	58	0.402	68	CCNU
11)	32065	-2.60	58	0.392	68	HYDROXYUREA

COMPARE - Correlation at LC50

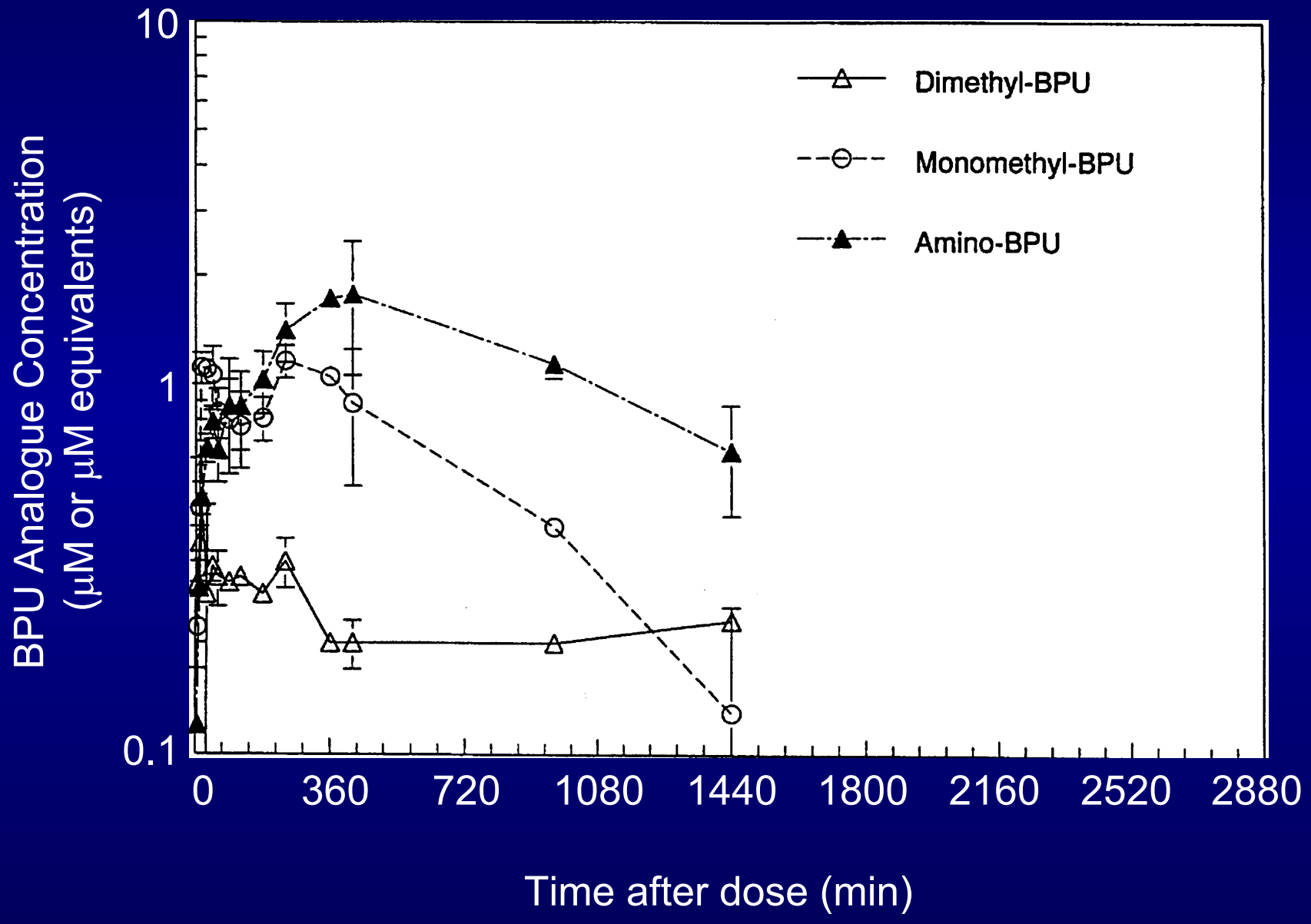
	NSC	LCONC	(MAX X)	PEARSON CORR. COEFF.	(N)	CHEM_NAME
1)	363812	0.60	13	0.674	67	TETRAPLATIN
2)	284356	0.60	13	0.642	67	MITINDOMIDE
3)	321803	0.60	15	0.570	67	NITROESTRONE
4)	314622	0.00	5	0.538	60	TOPO1A
5)	291643	0.30	15	0.538	67	PYRIMIDINE-5-GLYCODIALDEHYDE
6)	119875	0.30	126	0.505	70	CIS-PLATINIUM
7)	126771	0.60	15	0.494	67	DICHLOROALLY LAWSONE
8)	71261	0.90	15	0.472	67	B-TGDR
9)	118994	0.60	14	0.448	67	DIGLYCOALDEHYDE
10)	271674	0.00	16	0.448	69	CARBOXYPHTHALATO PLATINIUM
11)	336628	0.90	13	0.434	67	MERBARONE

RESPONSE OF ADV STAGE PC-3 XENOGRAFTS TO THE BENZOYLPHENYLUREA NSC 639829

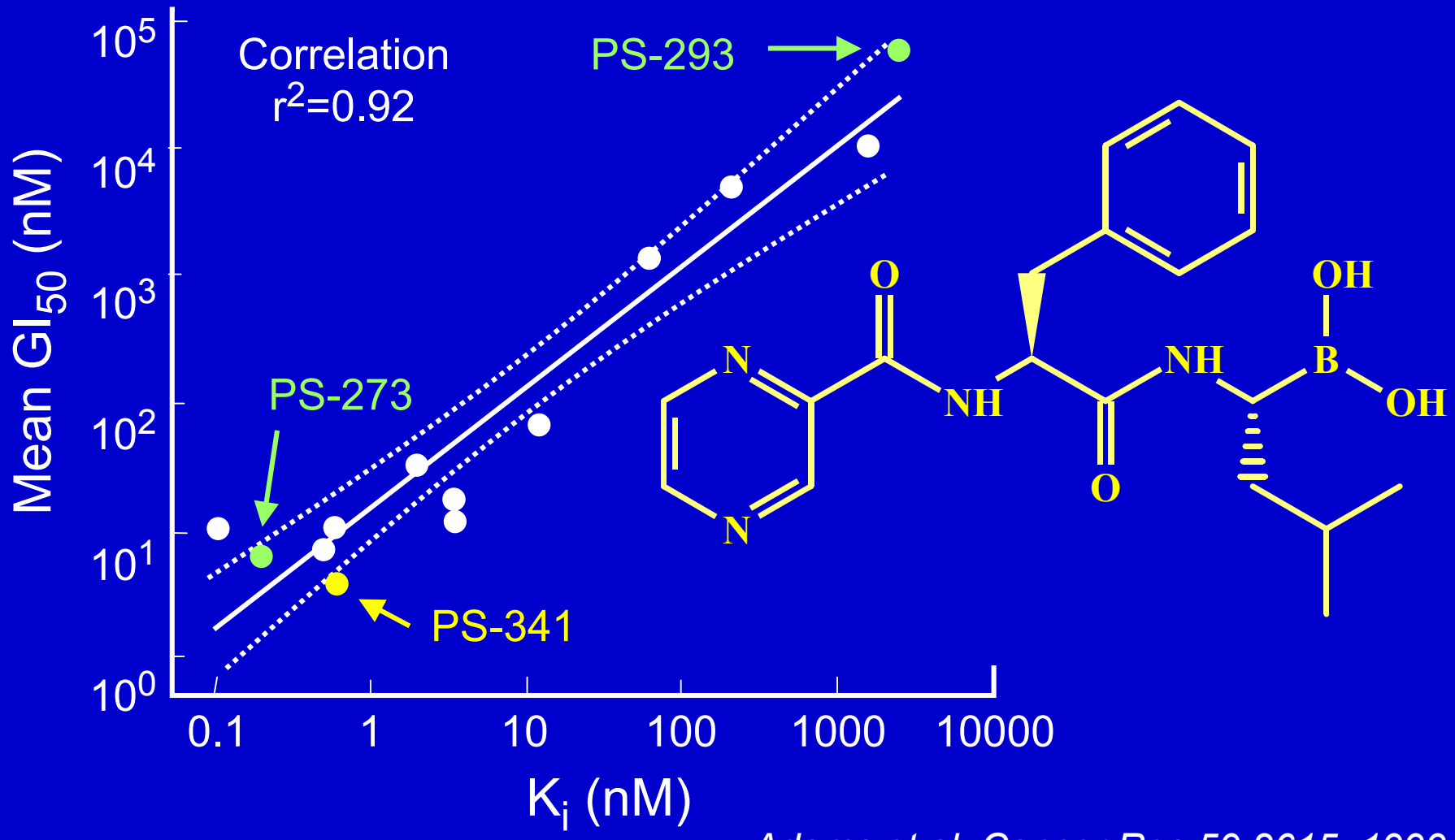


Schedule:
PO, Q4D x 3, Day 7

Control, Saline Tw 80
NSC 639829, 45 mg/kg/dose
NSC 639829, 30 mg/kg/dose
NSC 639829, 20 mg/kg/dose

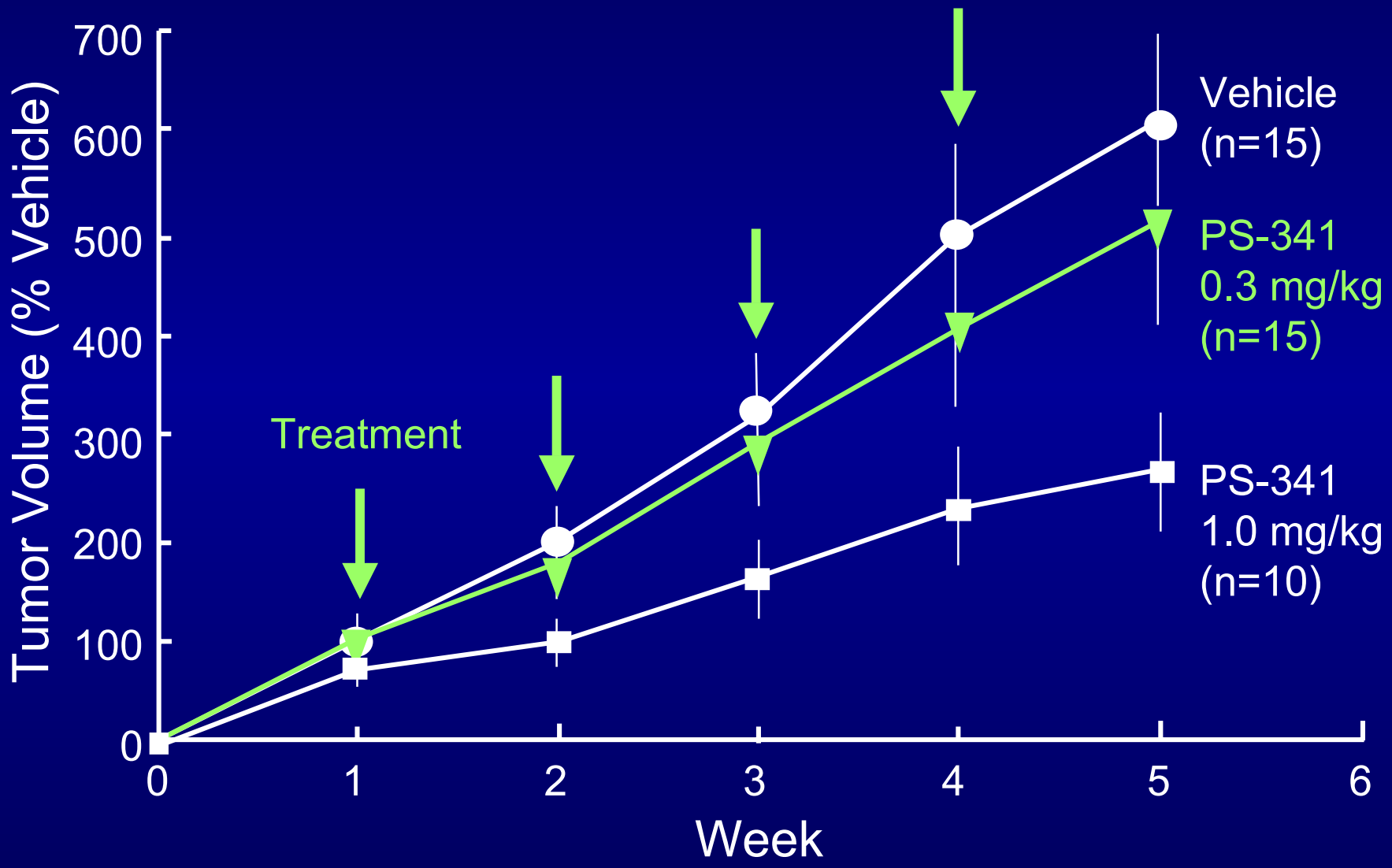

10
1
0.1
BPU Analogue Concentration
(μM or μM equivalents)
Dimethyl-BPU
Monomethyl-BPU
Amino-BPU
0
360
720
1080
1440
1800
2160
2520
2880
Time after dose (min)

CORRELATION BETWEEN 20S PROTEASOME INHIBITORY POTENCY & GROWTH INHIBITION FOR 13 DIPEPTIDE BORONIC ACIDS



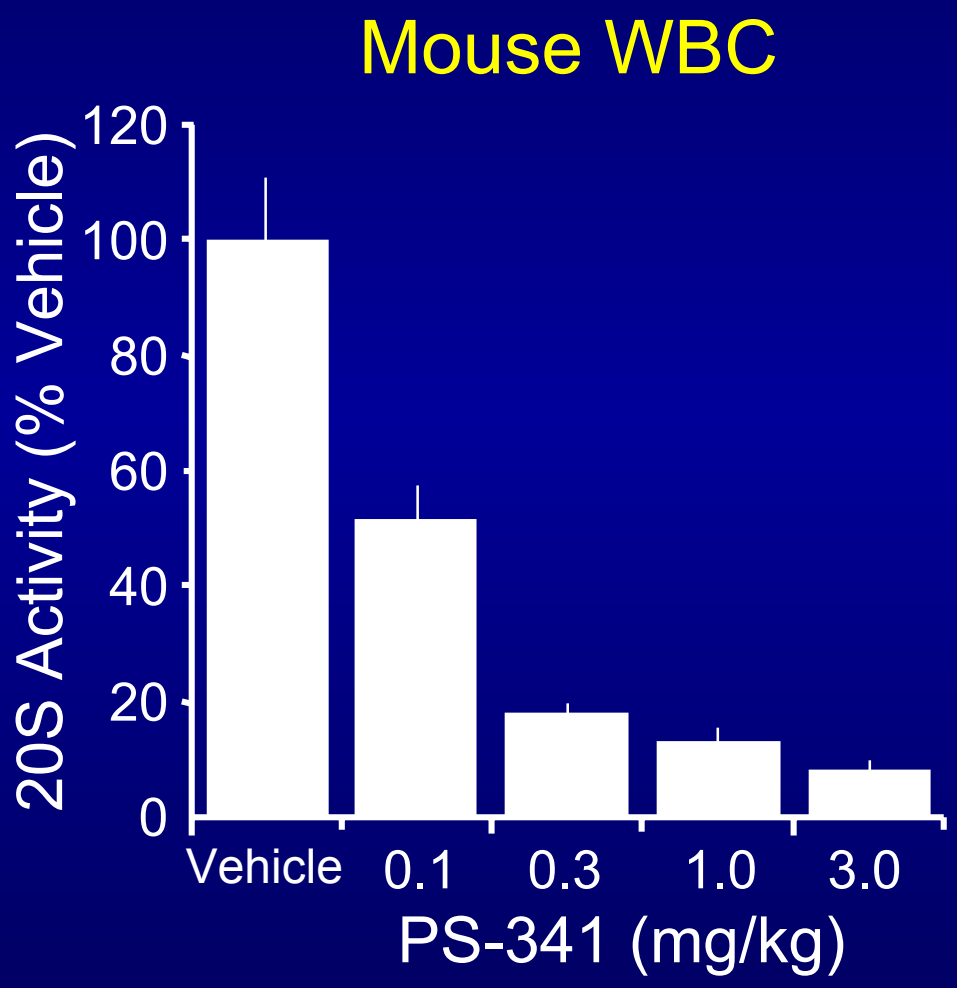
Adams et al, Cancer Res 59:2615, 1999

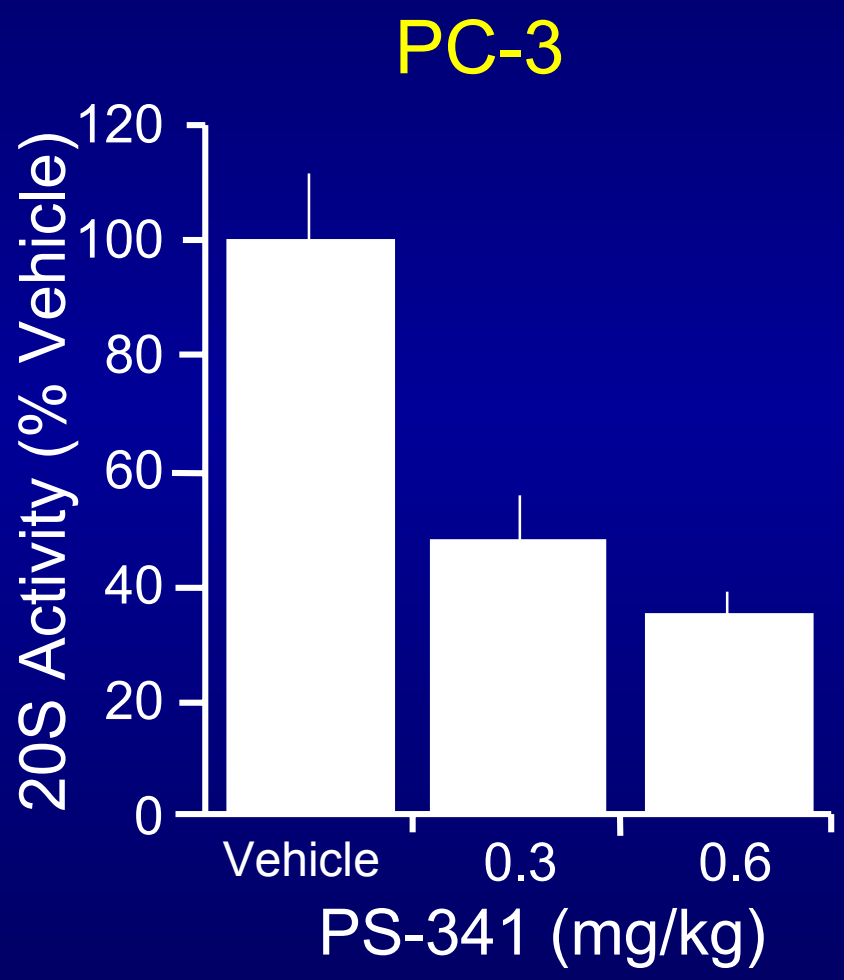
EFFECT OF PS-341 ON PC-3 TUMOR GROWTH IN MICE



Adams et al, Cancer Res 59:2615, 1999

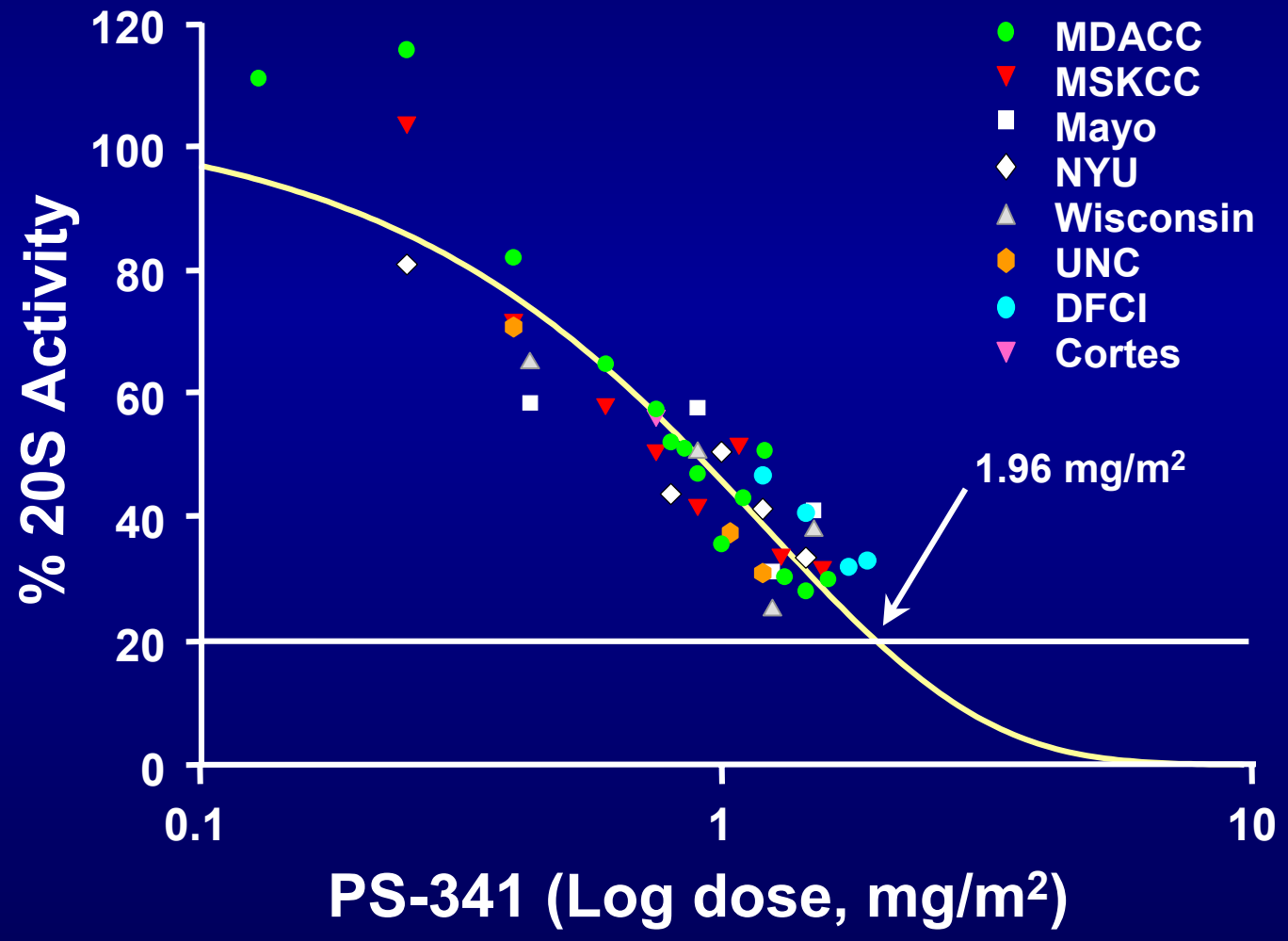
EFFECT OF PS-341 ON 20S PROTEASOME ACTIVITY





Adams et al, Cancer Res 59:2615, 1999

Ex Vivo Proteasome Activity: 1 Hour Post Treatment



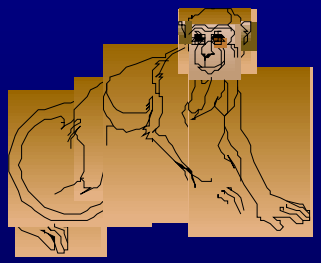
OBJECTIVES OF PRECLINICAL TOXICOLOGY STUDIES

- DETERMINE IN APPROPRIATE ANIMAL MODELS:
  - The Maximum Tolerated Dose (MTD)
  - Dose Limiting Toxicities (DLT)
  - Schedule-Dependent Toxicity
  - Reversibility of Adverse Effects
  - A Safe Clinical Starting Dose

FDA PRECLINICAL PHARMACOLOGY & TOXICOLOGY REQUIREMENTS: ONCOLOGY Rx

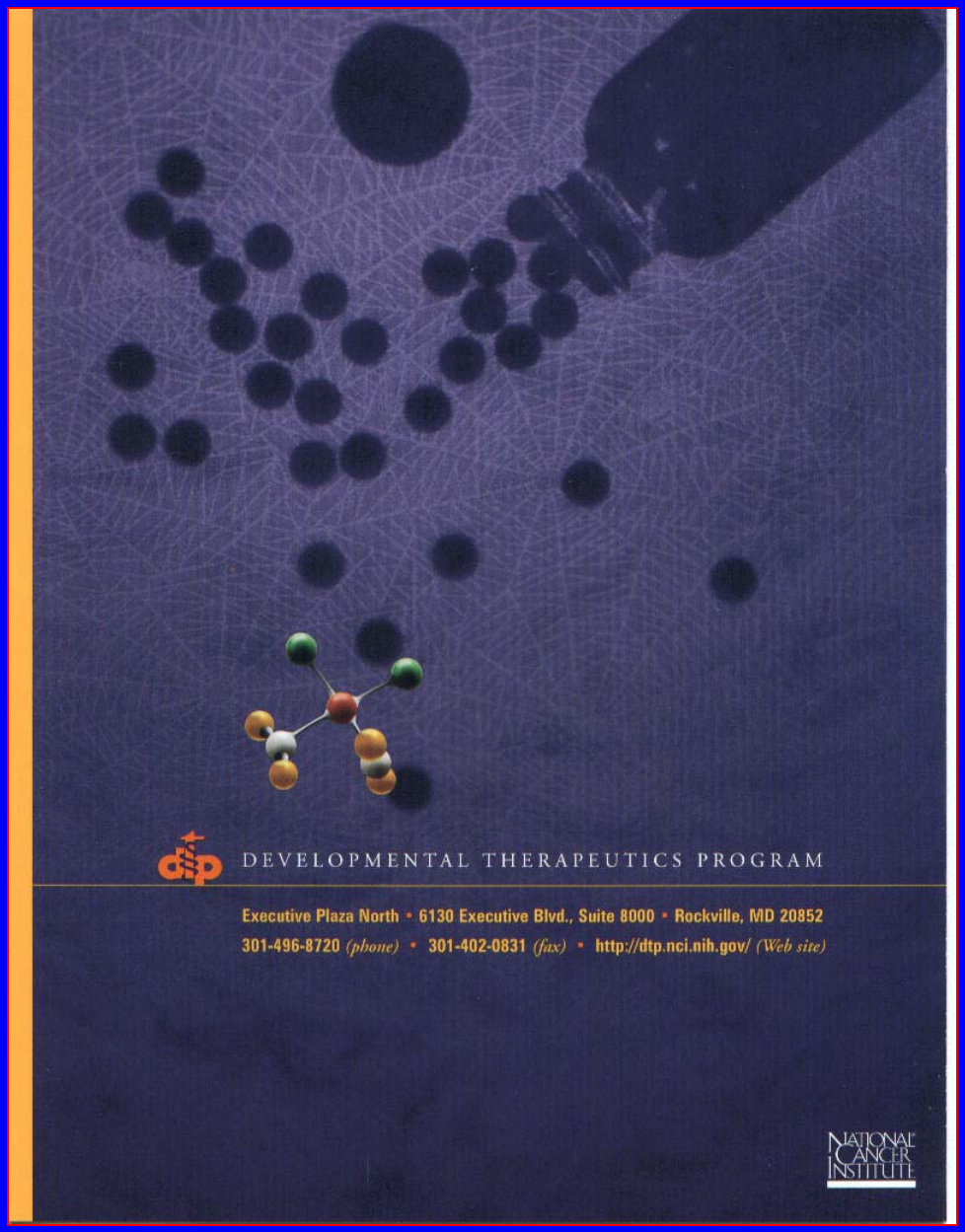
- DRUGS
  - Two Species - Rodent & Non-rodent
  - Clinical Route & Schedule
    - Follow NCI Guidelines
  - Pharmacokinetics - Optional

- BIOLOGICALS
  - Most Relevant Species
  - Clinical Route & Schedule



Questions and Discussion

Thank you!



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Millenium
J. Lazo
U. Pittsburgh