DRUG DISCOVERY

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OUTLINE OF PRESENTATION

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

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

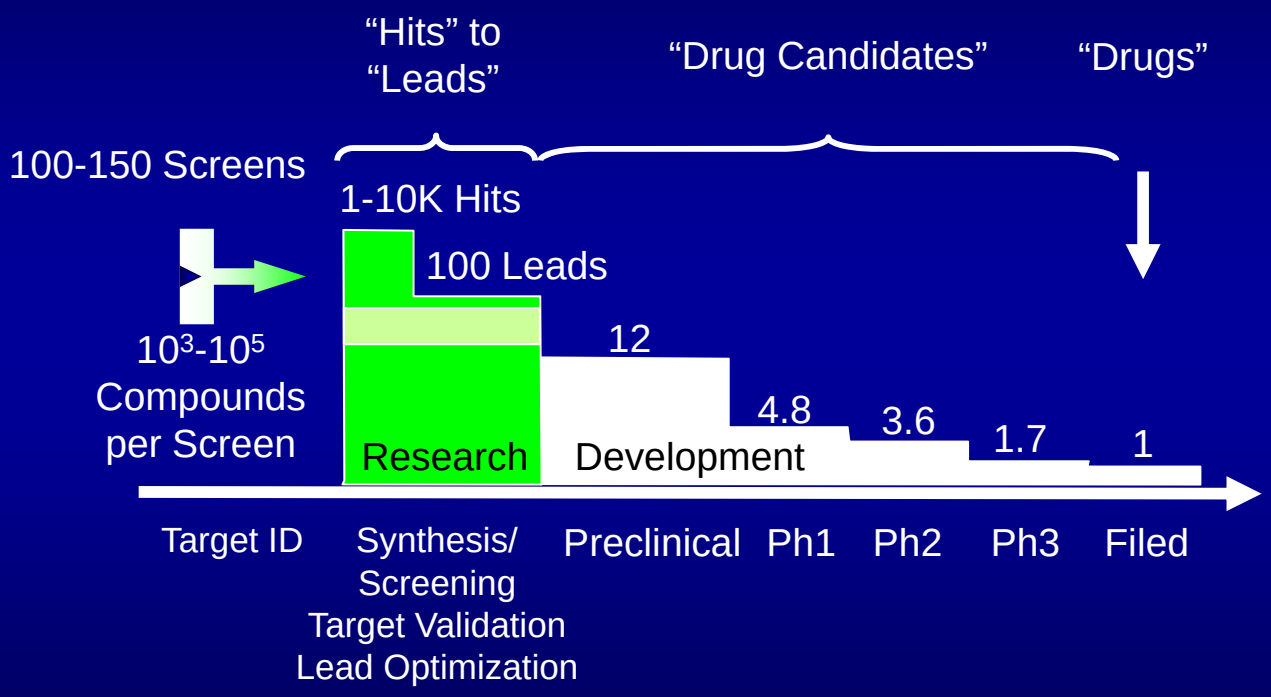
- Toxicity, 22%
- Lack of efficacy, 31%
- Market reasons, 6%
- Poor biopharmaceutical properties, 41%

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*

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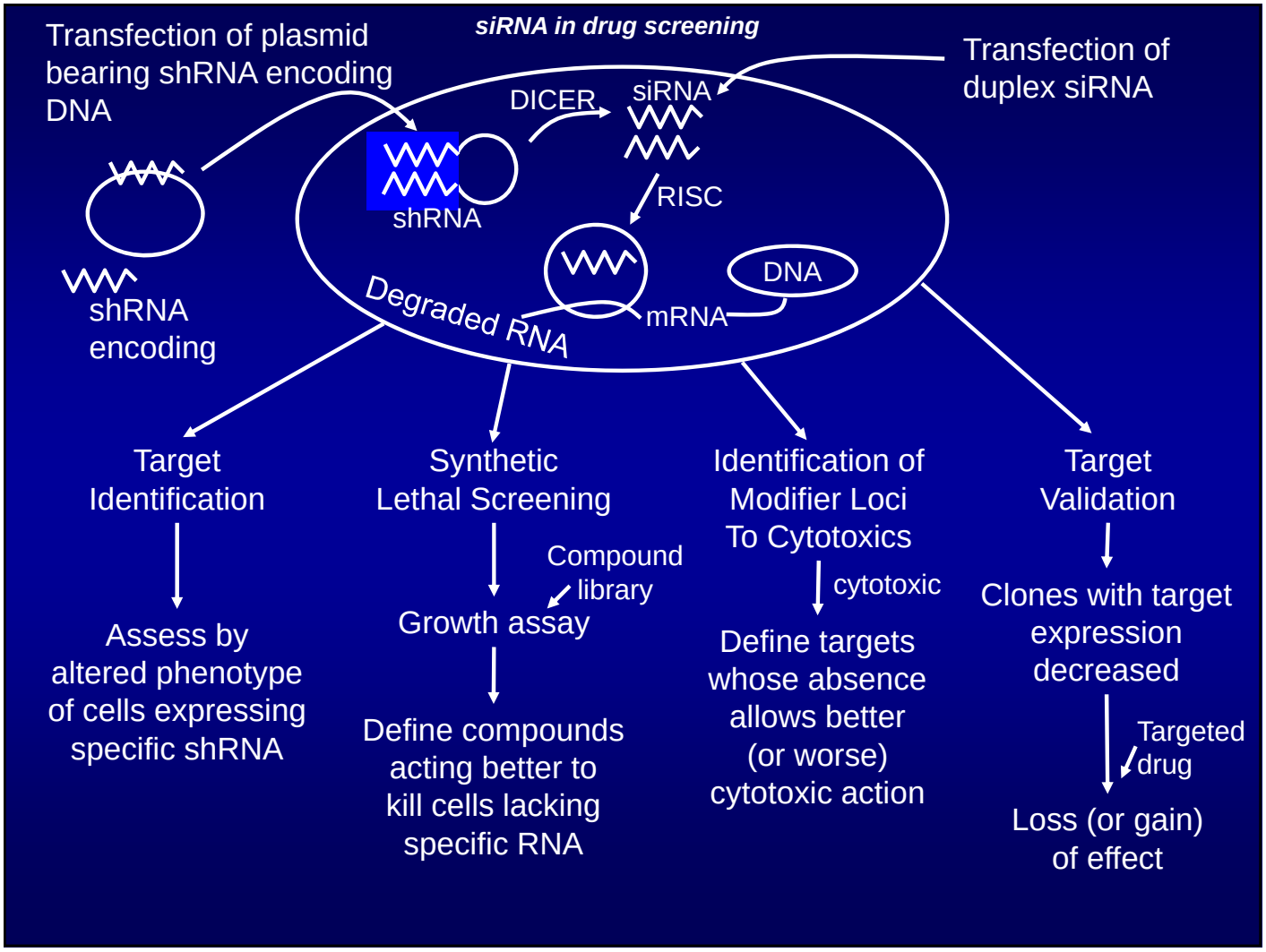
TWO CONTRASTING DRUG-DISCOVERY "PHILOSOPHIES"

- "EMPIRICAL": Recognize initial drug lead by functionally useful effect
  -E.g. : penicillin (anti-bacterial effect)
  rauwolfia (anti-hypertensive)
  taxol (anti-tumor)
  digoxin (cardiotonic / antiarrythmic)

- "RATIONAL": Recognize drug by design or screen against drug target's function
  -E.g.: HIV-protease inhibitor (anti-infection)
  metoprolol (anti-hypertensive)
  methotrexate (anti-tumor)

PROBLEM:
HOW TO RECOGNIZE DISEASE RELEVANT TARGETS?


siRNA in drug screening
Transfection of plasmid bearing shRNA encoding DNA
Transfection of duplex siRNA
DICER
siRNA
shRNA
RISC
shRNA encoding
DNA
mRNA
Degraded RNA
Target Identification
Assess by altered phenotype of cells expressing specific shRNA
Synthetic Lethal Screening
Compound library
Growth assay
Define compounds acting better to kill cells lacking specific RNA
Identification of Modifier Loci To Cytotoxics
cytotoxic
Define targets whose absence allows better (or worse) cytotoxic action
Target Validation
Clones with target expression decreased
Targeted drug
Loss (or gain) of effect

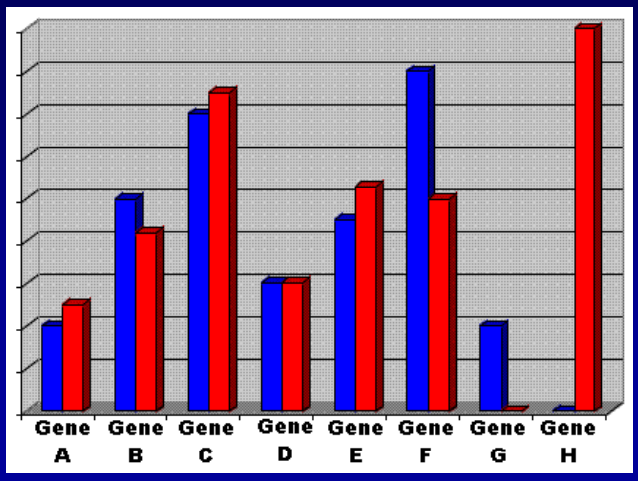
MOLECULAR TARGET DEFINITION - HOW TO?

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

Cancer Genome Anatomy Project
PROCESS

- Tumor material (archival)
- "Laser capture microdissection" of tumor cells from defined sections
- Creation of tumor-derived cDNA libraries
- Sequence to establish uniqueness
- Deposit in public domain

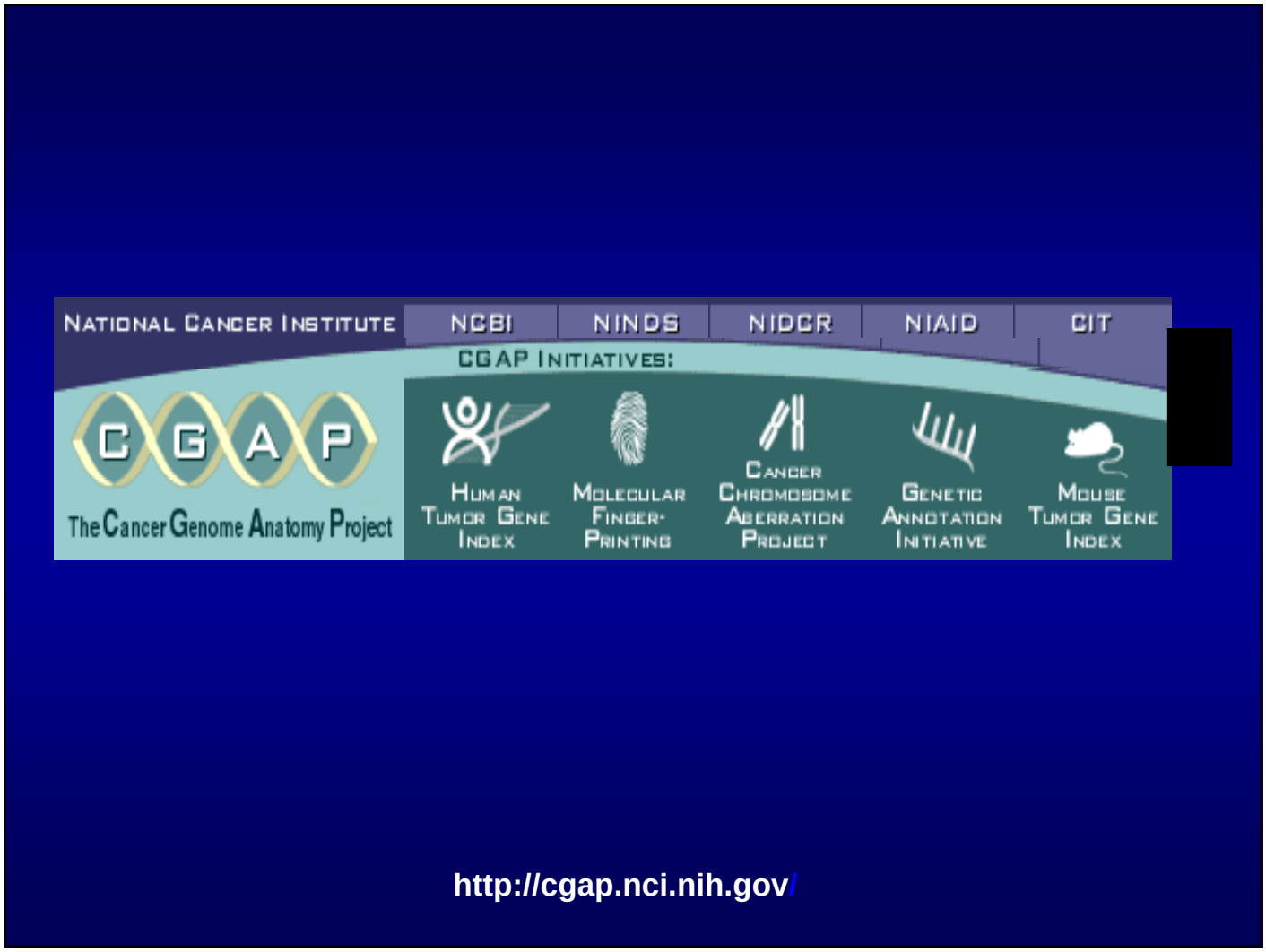
Gene Expression: The Cell's Fingerprint



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.

National Cancer Institute
CGAP
The Cancer Genome Anatomy Project

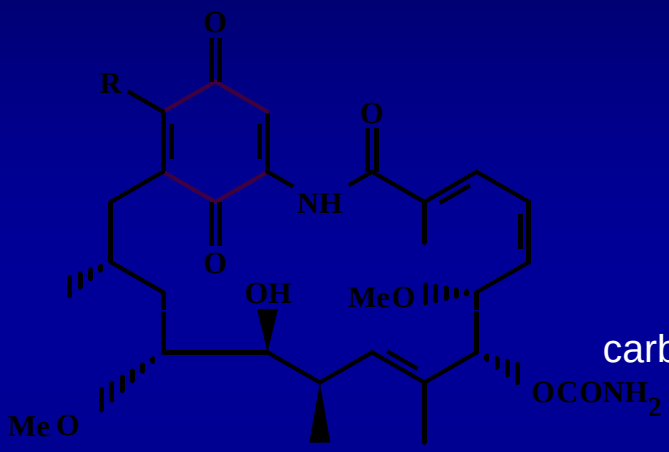
http://cgap.nci.nih.gov


National Cancer Institute
NCBI
NINDS
NIDCR
NIAID
CIT
CGAP Initiatives:
CGAP
The Cancer Genome Anatomy Project
Human Tumor Gene Index
Molecular Finger-Printing
Cancer Chromosome Aberration Project
Genetic Annotation Initiative
Mouse Tumor Gene Index
http://cgap.nci.nih.gov/

GELDANAMYCIN: EXAMPLE OF BINDING PARTNER DEFINING TARGET

benzoquinone

ansa ring



carbamate

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

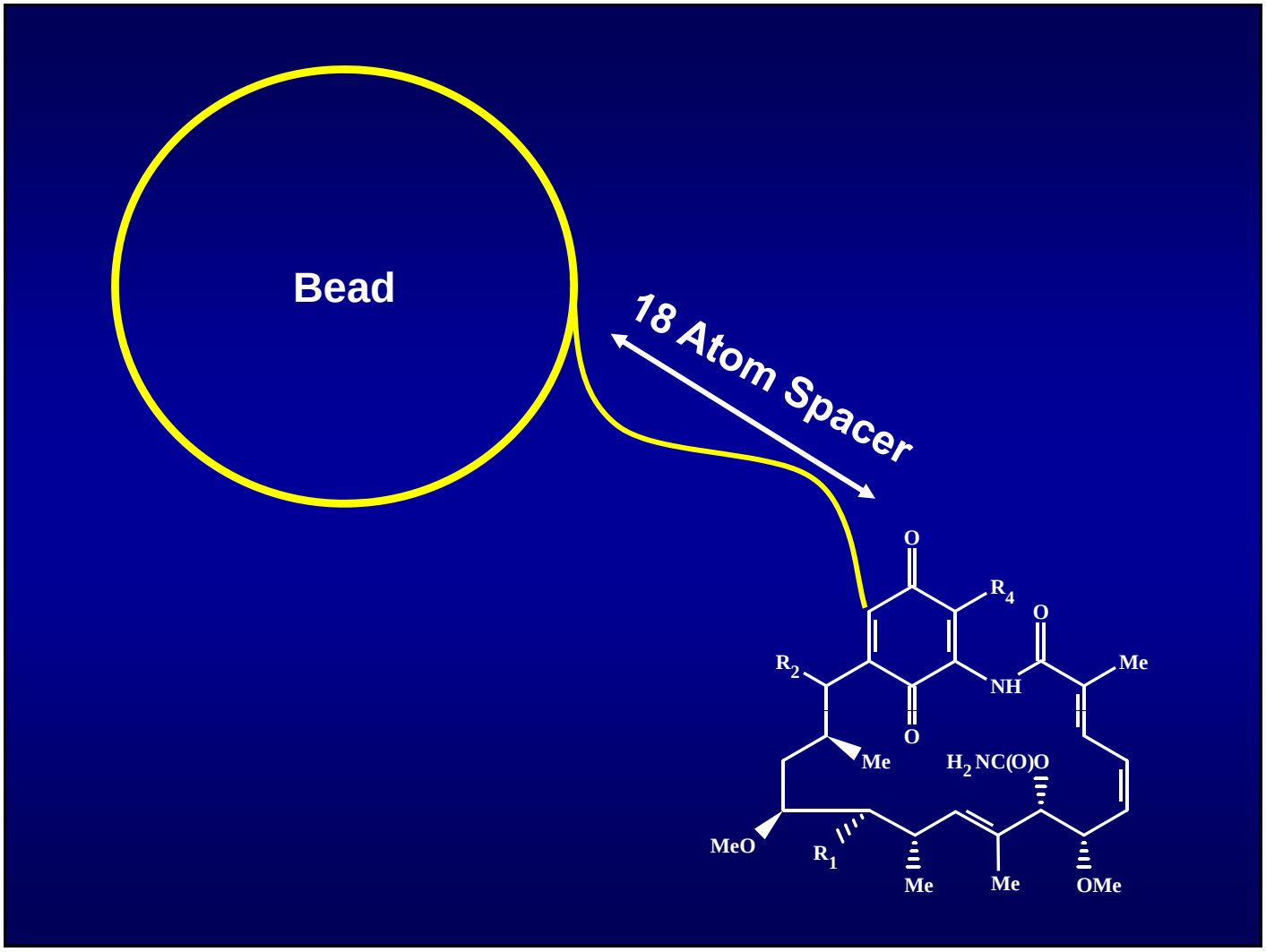
BENZOQUINOID ANSAMYCINS
INITIAL CELL PHARMACOLOGY - I

- "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)

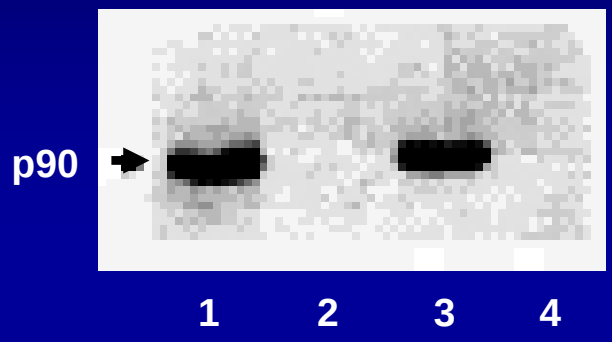
- Decrease steady state phosphorylation levels to 10% of control
  - decrease steady state level of pp60src by 30%
  - accelerate turnover of pp60src

  (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

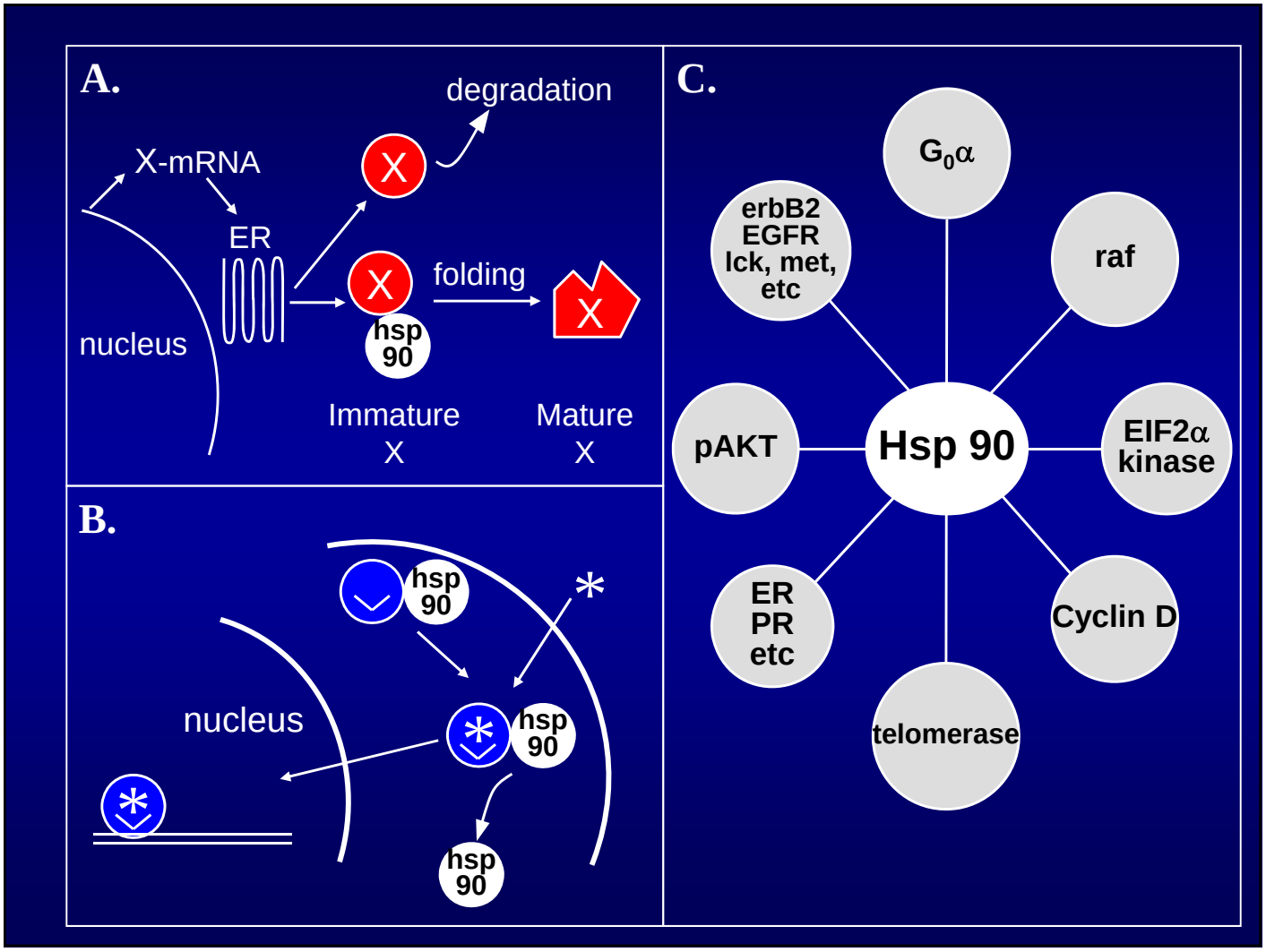
GELDANAMYCIN BEADS IDENTIFY HSP90 AS BINDING PARTNER

R. Lysate



1) Bead-Geld
2) Bead-Geld + Geld
3) Bead-Geld + Geldampicin
4) Bead

Neckers et al, PNAS 91:8324, 1994


A.
degradation
X-mRNA
X
ER
X
folding
X
nucleus
hsp
90
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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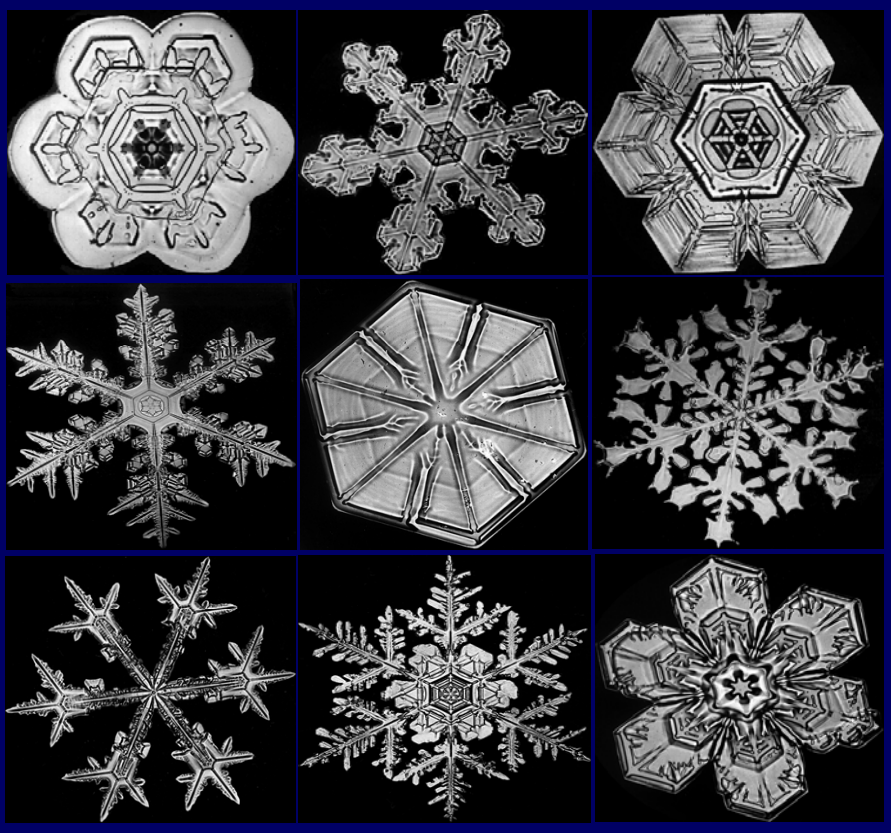
Diversity



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

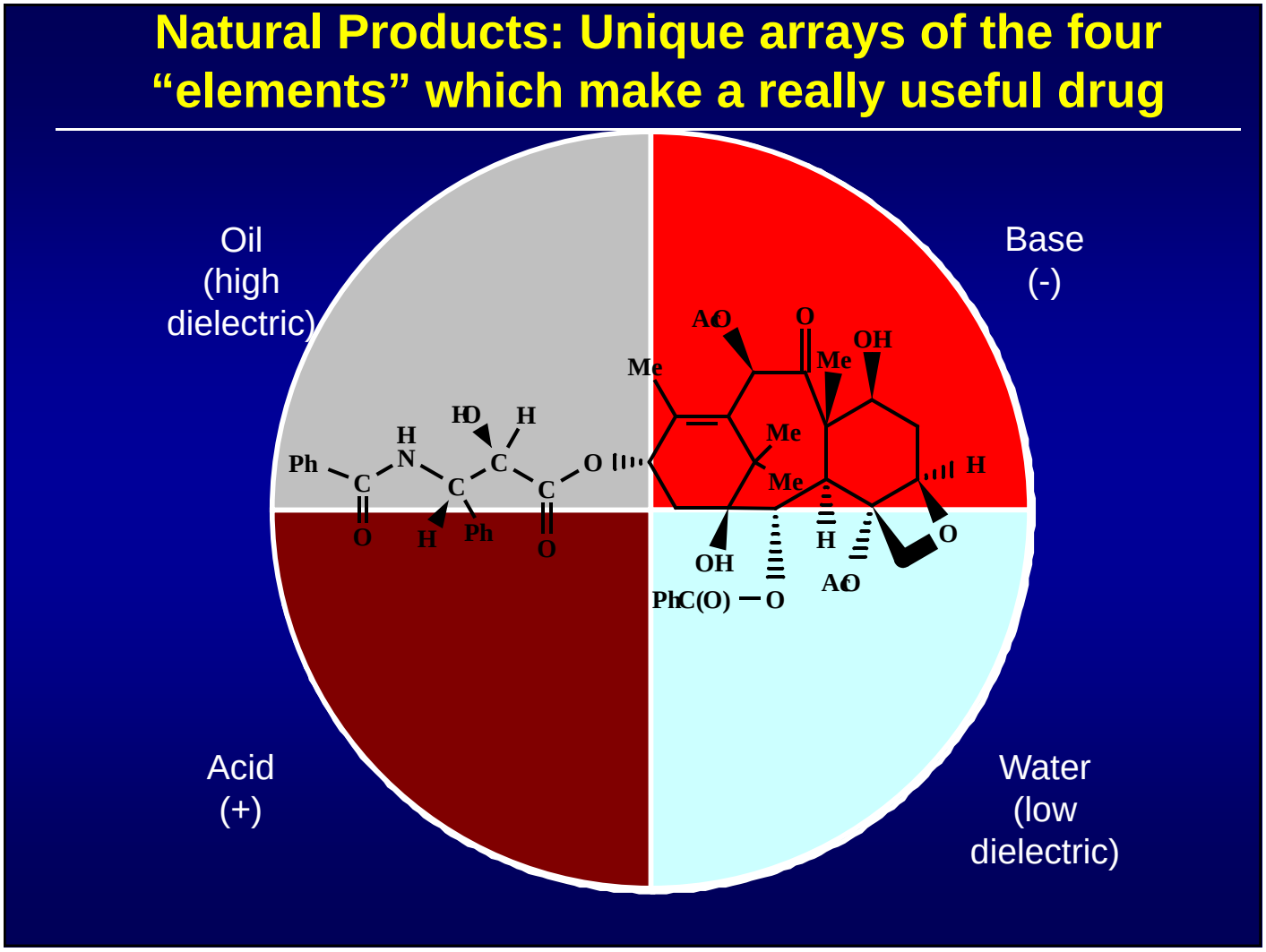
SOURCES OF DIVERSITY

- "Natural Products" = entities derived from plants, animals, bacteria, etc. May have "ethnopharmacognosy" to suggest use
  - "pure compound" collections
  - extracts: aqueous/organic
  - genetically altered producer organisms

- Target non-selected chemical compound libraries
  -peptide / protein
  -non-peptide

- Target-directed chemical compound libraries
  - "classical" medicinal chemistry / bona fide crystal structure - derived
  - "docked" lead structures into model

Natural Products: Unique arrays of the four "elements" which make a really useful drug



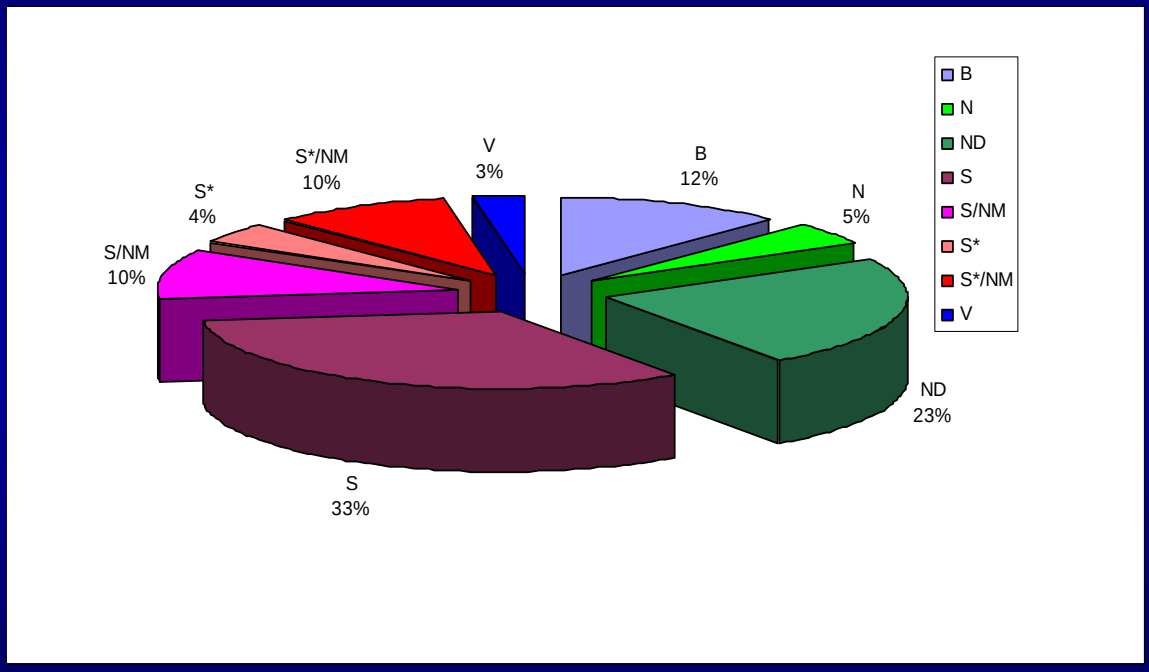
Sources of "Modern Drugs"

If one looks at the current drug scene from a chemical perspective (data from 1981 – 2002) then the following slides show reasonable approximations of the sources of drugs currently approved, World-wide, by the FDA or equivalent body.

Codes are:

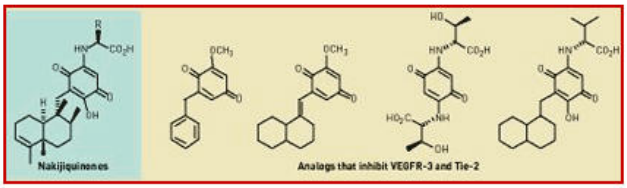
N	Natural Product
ND	Natural Product Derivative
S*	Natural Product Pharmacophore
S	Synthetic Compound
B/V	Biological / Vaccine
(NM)	Natural Product Mimic as a subdivision

Sources of Drugs (1981-2002); Extended Subdivisions n = 1031



Newman et al, J. Nat. Prod., 2003, 66, 1027-1037

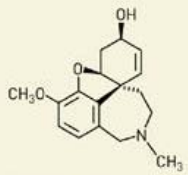
EXAMPLES OF NP LEAD GENERATION OF NOVEL SCAFFOLDS



GUIDED BY NATURE A compound library developed around nakijiquinones, which are natural inhibitors of the receptor tyrosine kinase called Her-2/Neu, produced analogs that inhibit two other receptor tyrosine kinases, VEGFR-3 and Tie-2.

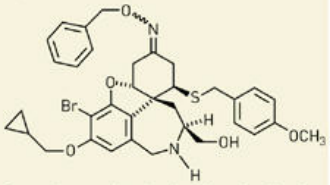
NATURE LEADS

A library based on a natural product ...



Galanthamine, an antidementia drug

... turns up a new compound with a different activity

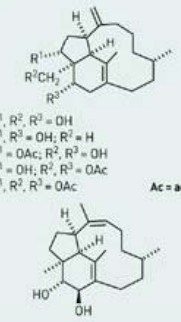


Secramine, a galanthamine-based molecule that blocks protein trafficking

INSECT CHYMISTRY

Nasute termites ...

... are rich in trinervitane compounds



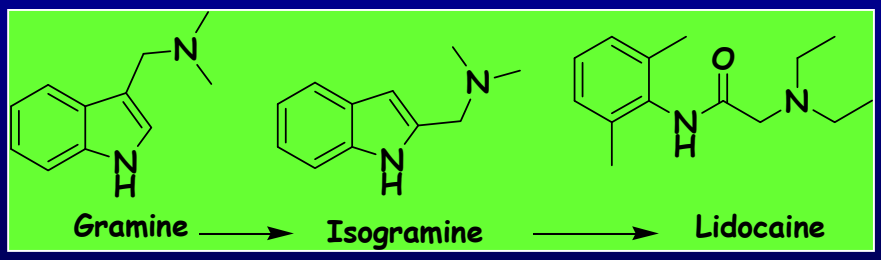
CSIRO PHOTO

Discovery of Lidocaine

*Central Asian camels refused to eat a certain type of reed

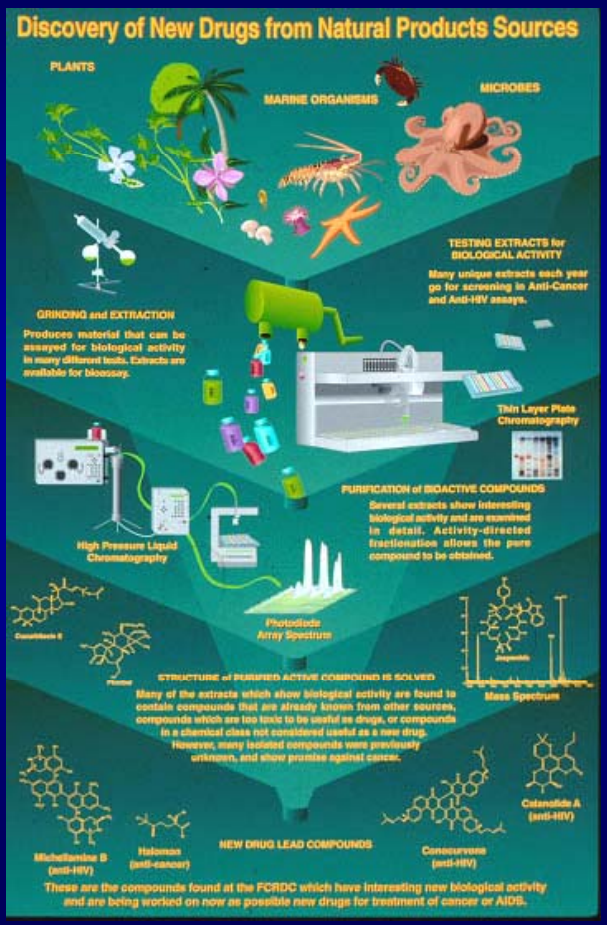
*Characterization of gramine as the antifeedant principle led to the synthesis of isogramine

*Taste-test: numbness; therefore, lead for anesthetic agent development

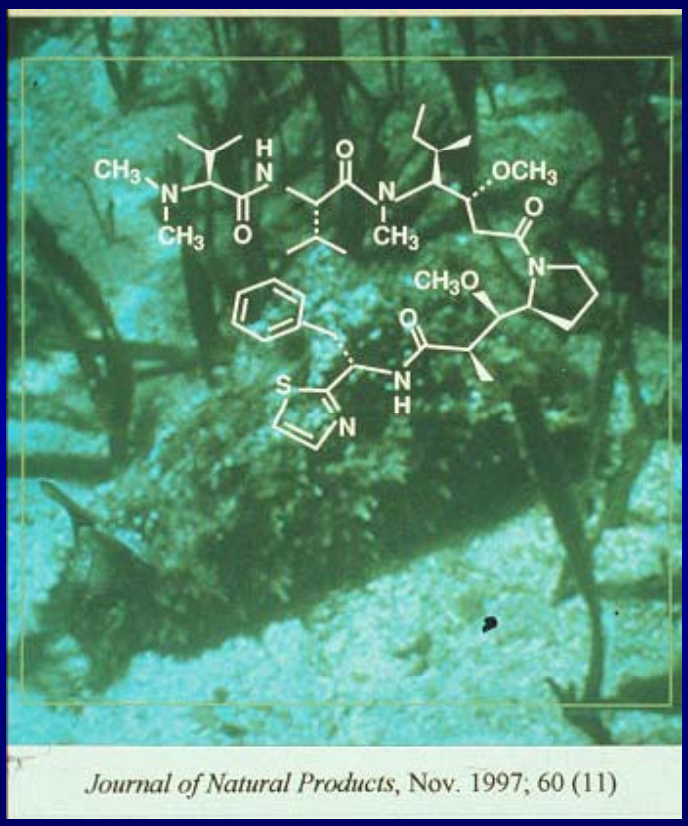


Courtesy of N. R. Farnsworth

Natural Product Isolation Tree



“You are what you eat”



Dolabella auricularia

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

"Non-culturable" versus "Cultured" microbes

- The microbial World has only just been scratched.
  - Much less than 1% of the available organisms have even been seen, let alone identified.

- In soil, there are estimates of > 1000 species per gram
  - very few can be cultured
  - these may not be representative of the "Soil meta-Genome"

- Over 1000 microbes per mL of seawater can be seen and only ~ 1% can be cultured using current methods.

SOURCES OF DIVERSITY

- "Natural Products" = entities derived from plants, animals, bacteria, etc. May have "ethnopharmacognosy" to suggest use
  - "pure compound" collections
  - extracts: aqueous/organic
  - genetically altered producer organisms

- Target non-selected chemical compound libraries
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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

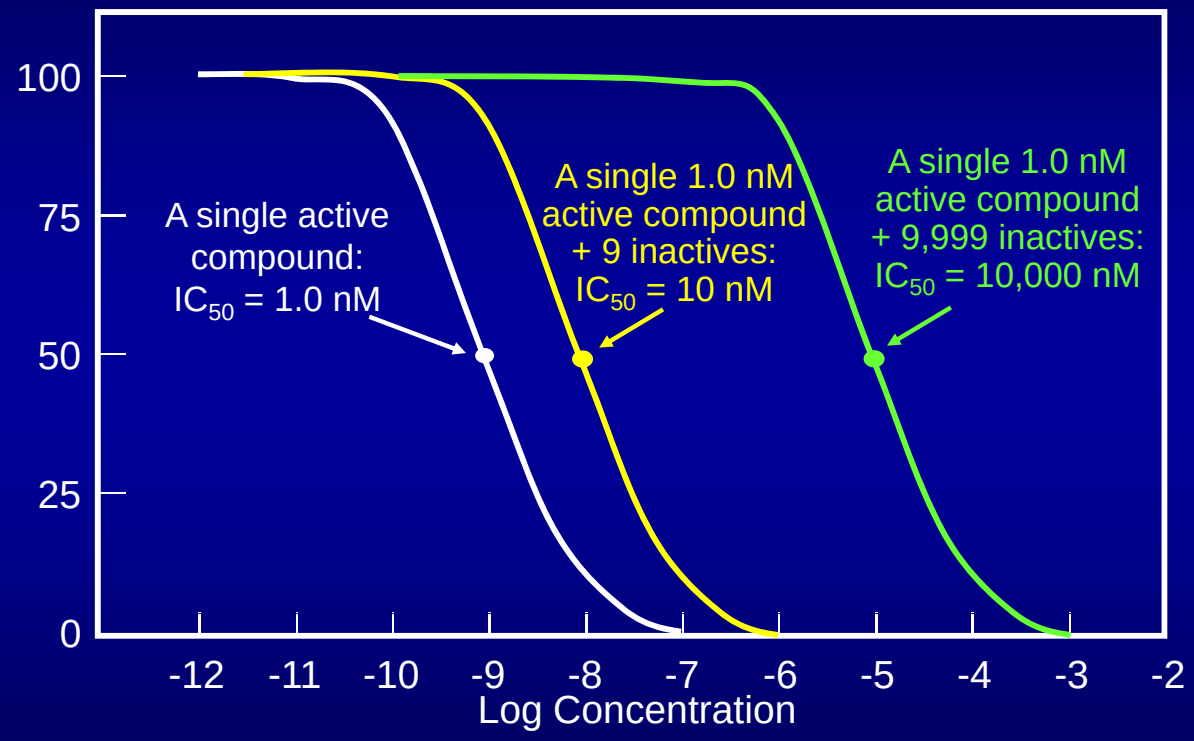
NUMBER OF PEPTIDES POSSIBLE WITH INCREASING LENGTH

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

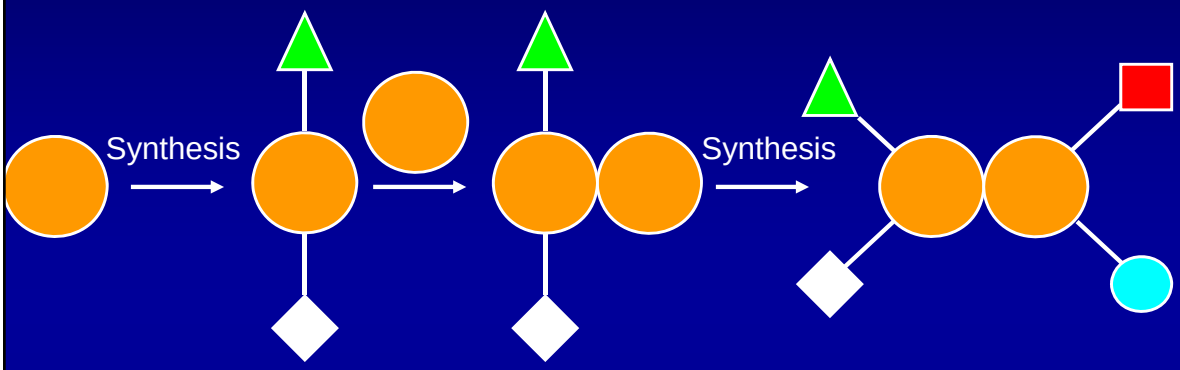


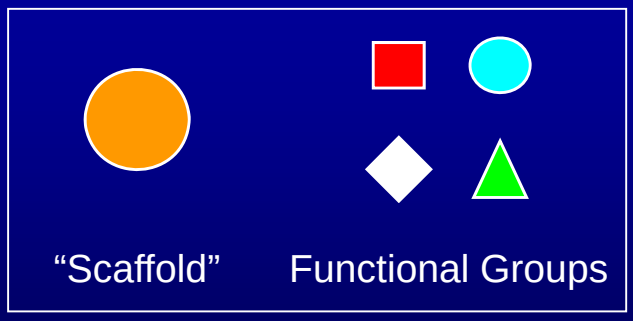
COMBINATORIAL LIBRARIES: THE MIXTURE QUESTION

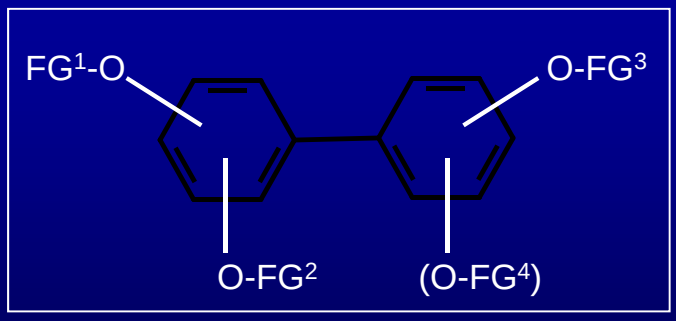
	Natural Product Extracts	Synthetic Combinatorial Mixtures
Direct screening of compound mixtures	Yes	Yes
Discovery of highly active compounds	Yes	Yes
Equal concentrations of compounds	No	Yes
Chemical structures known	No	Yes
Synthetic pathway known	No	Yes
Structure – activity relationship known	No	Yes

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

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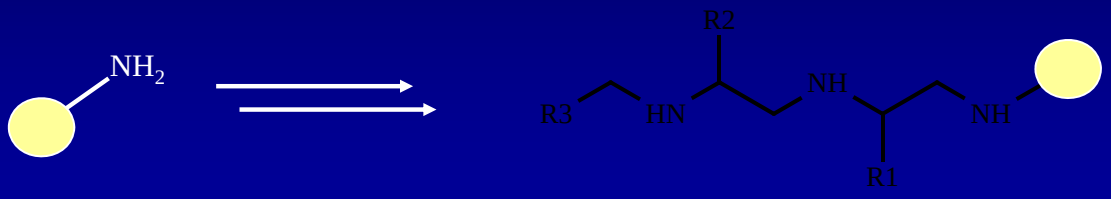
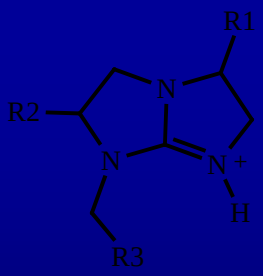
COMBINATORIAL LIBRARIES OF BICYCLIC GUANIDINES FROM REDUCED ACYLATED DIPEPTIDES



1. $CSIm_2$
2. HF/anisole



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

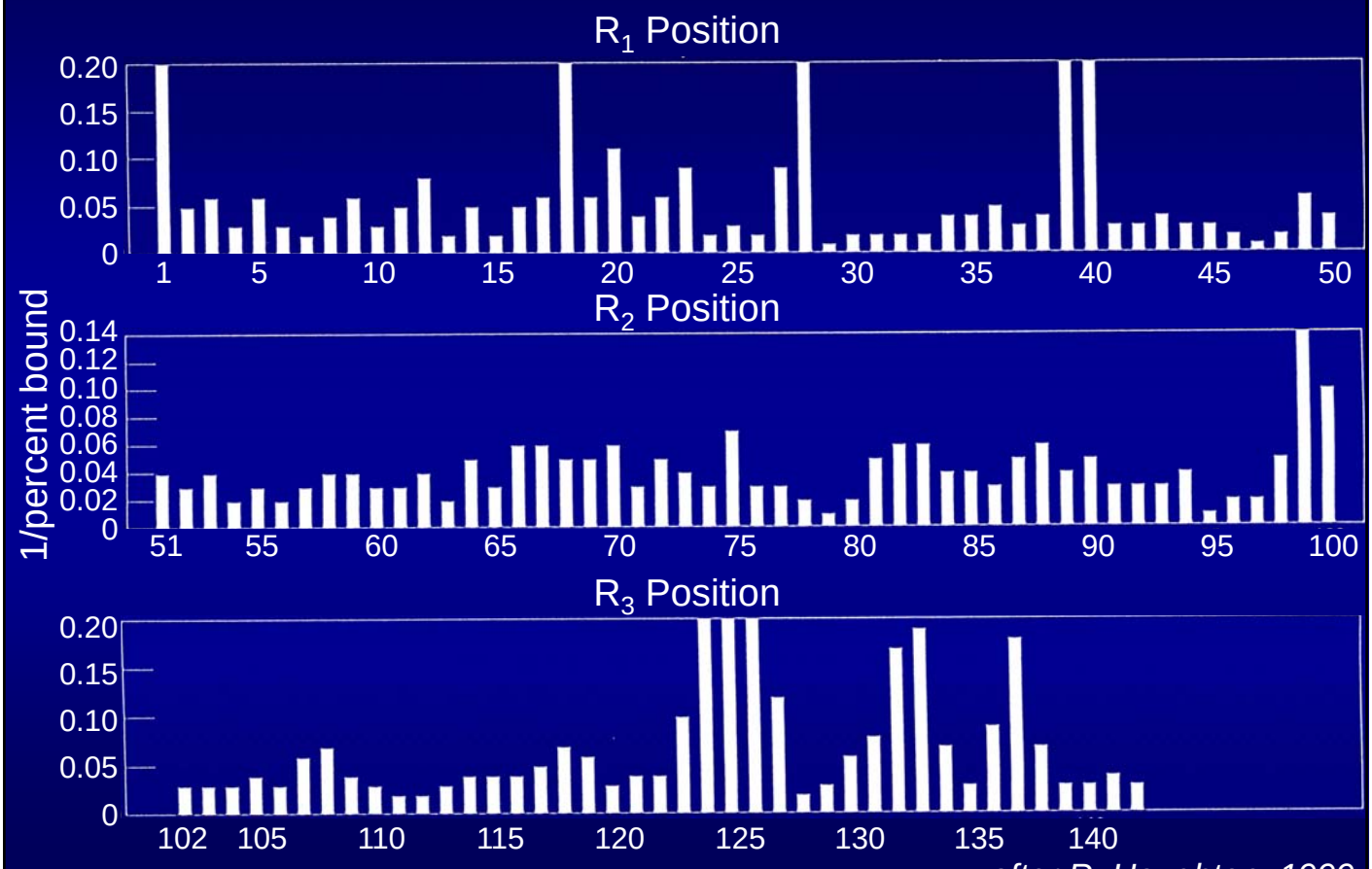
after R. Houghten, 1999

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)



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ONCE YOU HAVE A TARGET AND CADIDATE DRUG MOLECULES: HOW TO DESIGN A DRUG SCREEN?

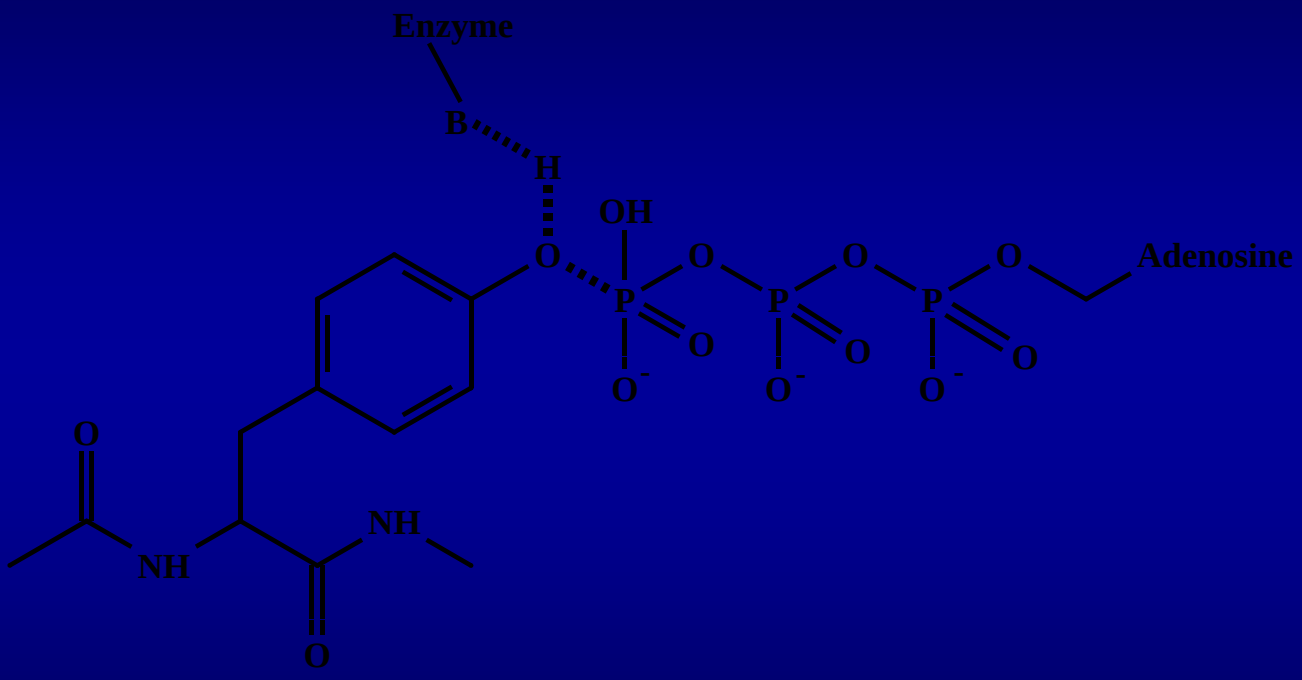
- Biochemical "Pure target" Screen (binding, functional):
  - •Advantage: "Pure" Structural / Functional Outcomes
  - •Disadvantage: Out of cellular / biochemical context
- Cell-Based
  - •Advantage: Readout in a "living" system;
  - •Disadvantage: Must deconvolute mechanism

CASE 1: TYROSINE KINASES AS BIOCHEMICAL SCREENING TARGET

COMMON ELEMENTS / REPEATED THEMES

- Overexpressed or activated in cancer (e.g, EGFR, Her2/neu, etc)
- Altered activity by mutation (e.g., c-*kit*)
- Altered activity by translocation(e.g., *bcr-abl*)
- Overexpression associated with
  - advanced stage
  - inferior prognosis

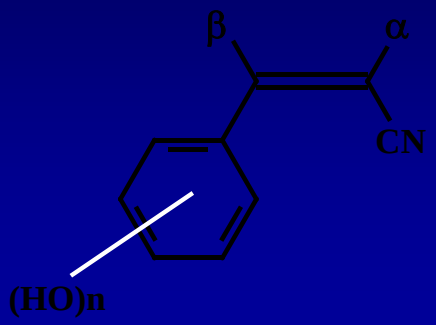
PROPOSED ENZYMATIC MECHANISM FOR TPKs



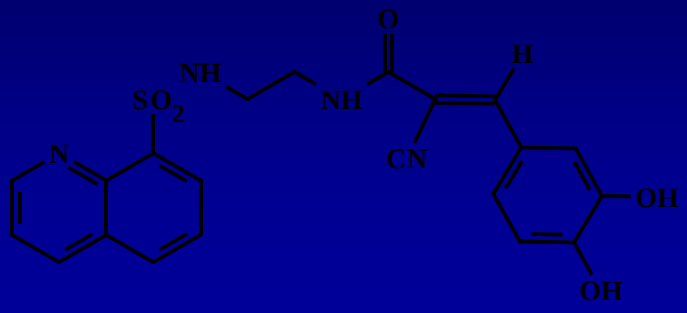
Levitsky, FASEB J 6: 3275, 1992

STRUCTURAL CLASSES OF TYRPHOSTIN:
mimic the kinase transition state

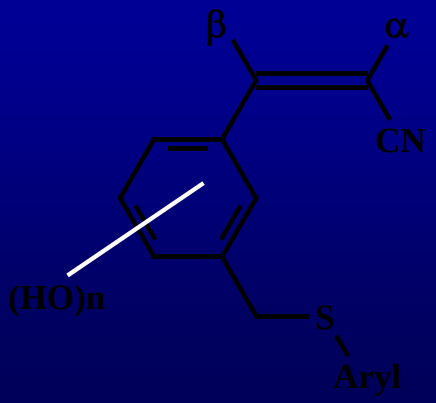
Benzene malononitrile



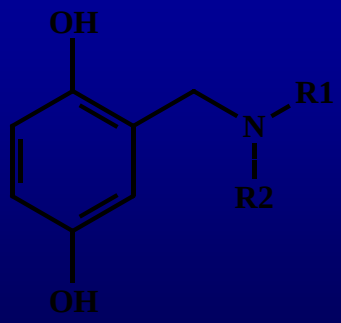
Bisubstrate quinoline



S-Arylbenzene malononitrile



Lavendustin-based



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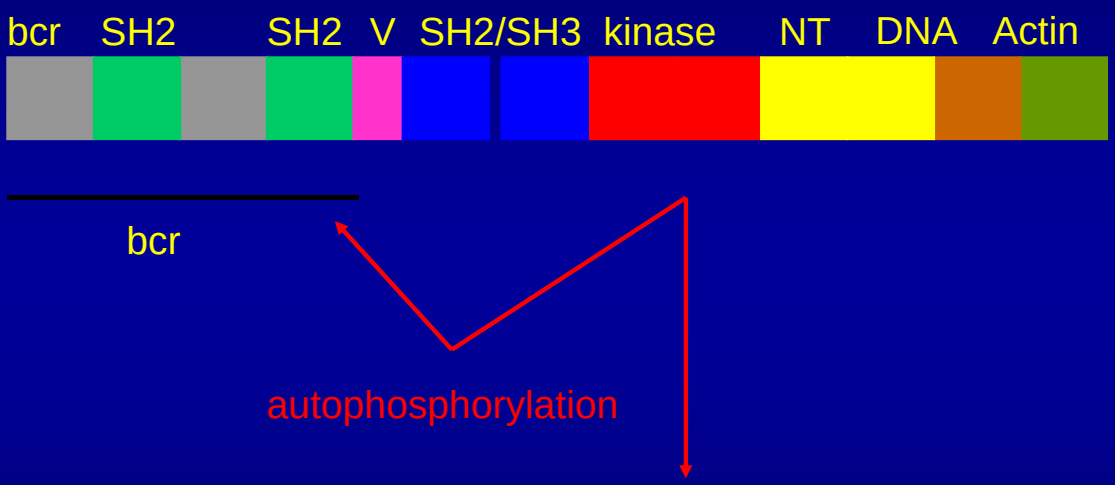
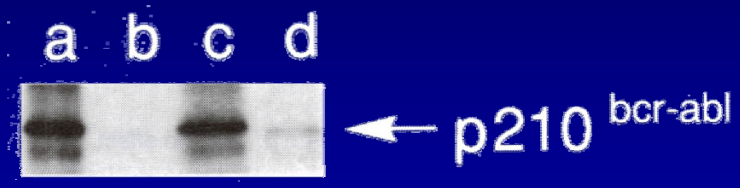
bcr-abl FUSION PROTEIN



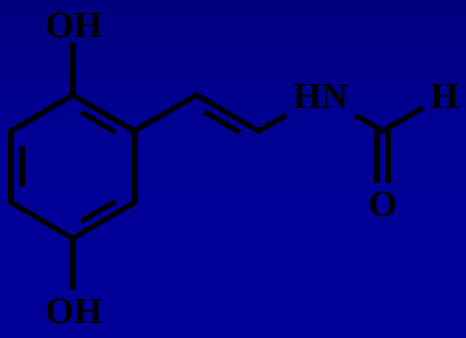
McWhirter JR, EMBO *12:1533, 1993*

INITIAL TYRPHOSTIN SCEEN: CORRELATE $p210^{bcr/abl}$ AUTOKINASE WITH K562 GROWTH INHIBITION

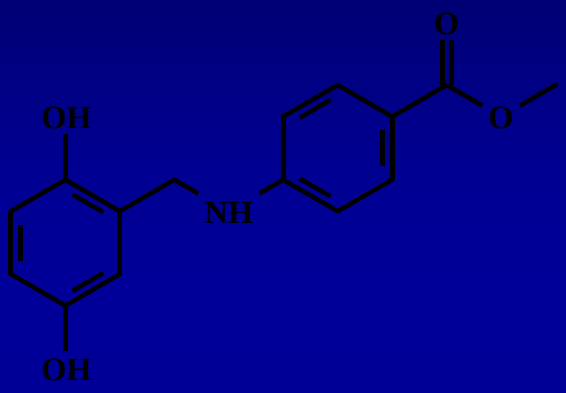


	IC50, K562
a – control	-
b – 50 μM AG957	15
c – 50 μM AG555	9.2
d – 50 μM AG1318	21

Kaur et al, Anti-Cancer Drugs, 5: 213, 1994



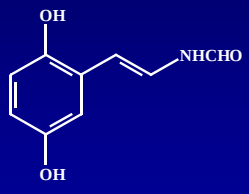
Erbstatin
NSC 606641



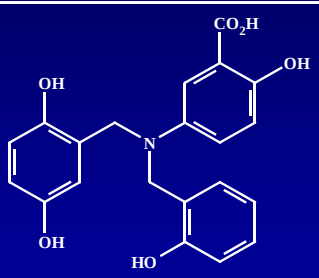
AG957
NSC 654705

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

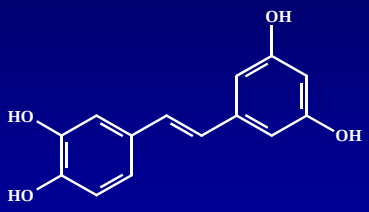
Natural product empiric lead



erbstatin



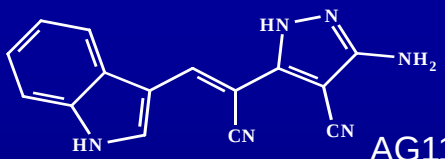
lavendustin



piceatannol

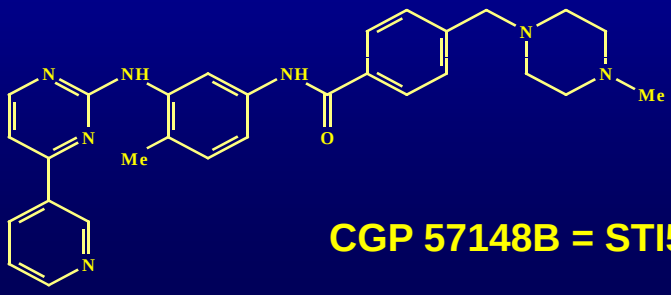
1st generation synthetic

AG957



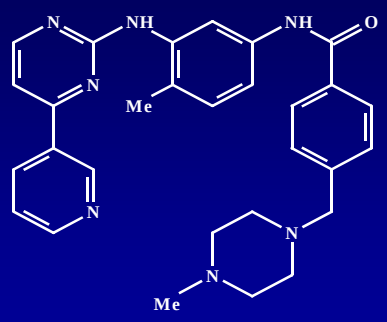
AG1112

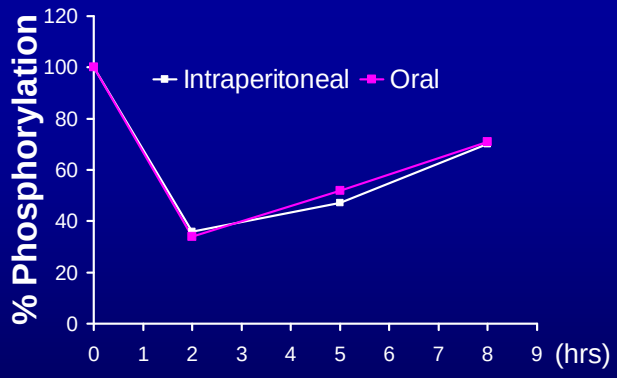
2nd generation synthetic; in clinic



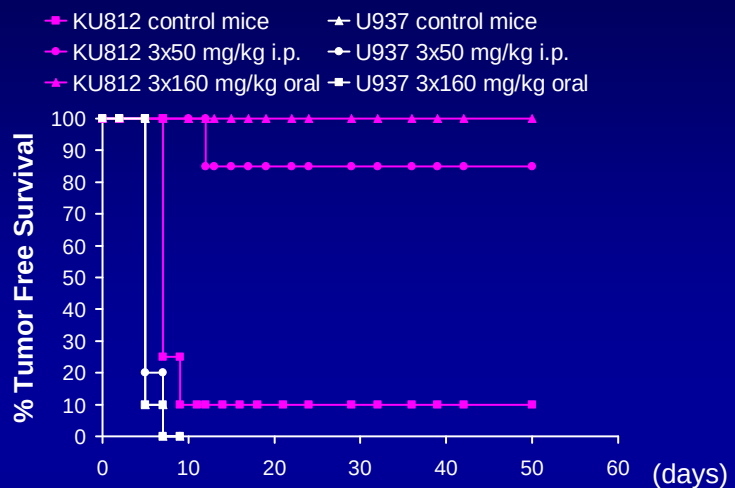
CGP 57148B = STI571

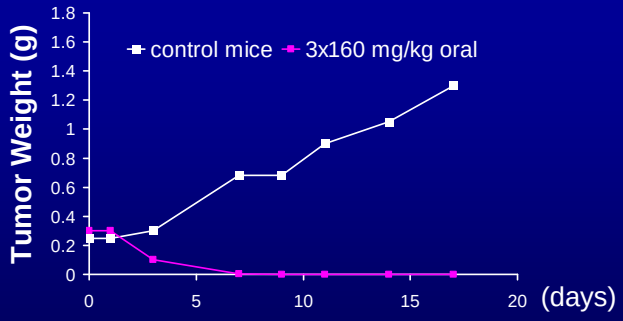
STI571: An oral in vivo bcr-abl kinase inhibitor





Tyr phosphorylation *in vivo*





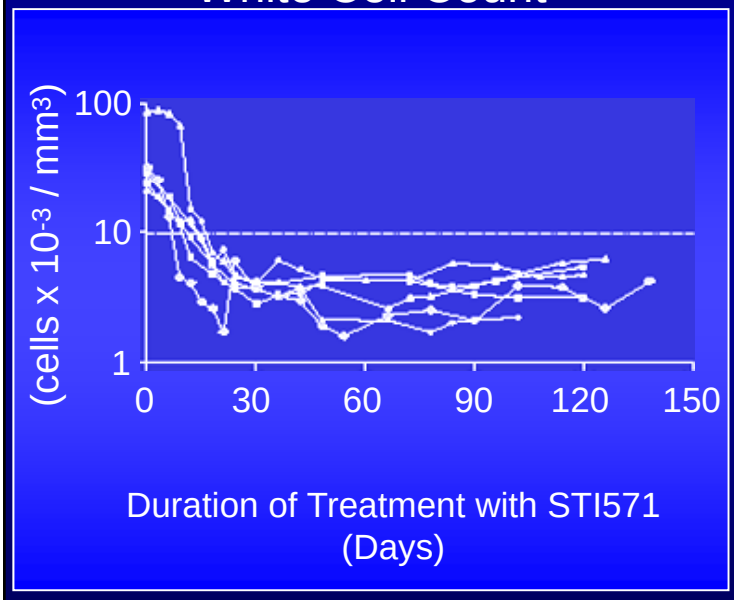
Antitumor activity *in vivo*

le Coutre et al, JNCI 91:163, 1999

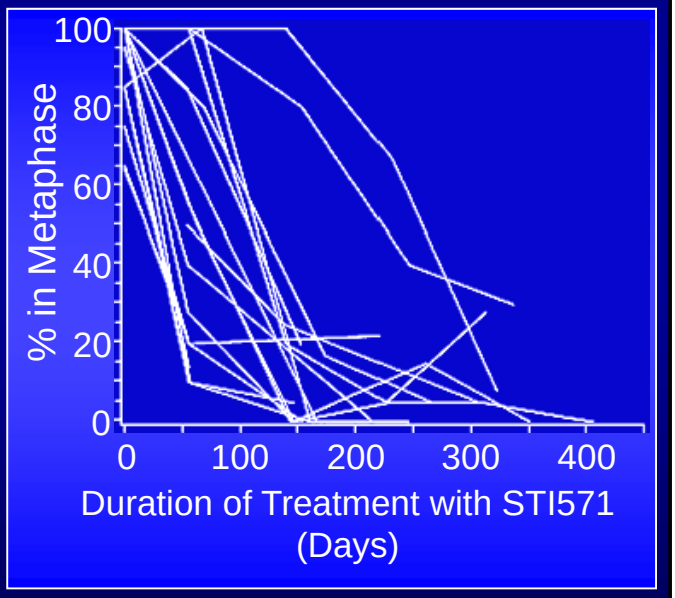
EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

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.

White Cell Count

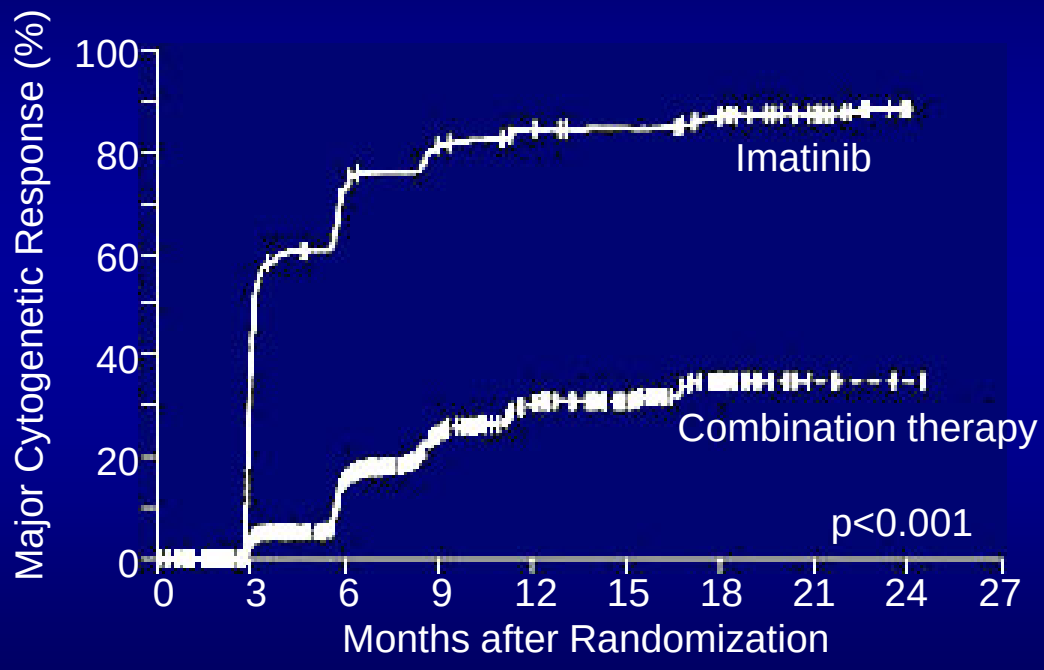


Ph Chromosome + Cells



NEJM 344: 1031, 2001

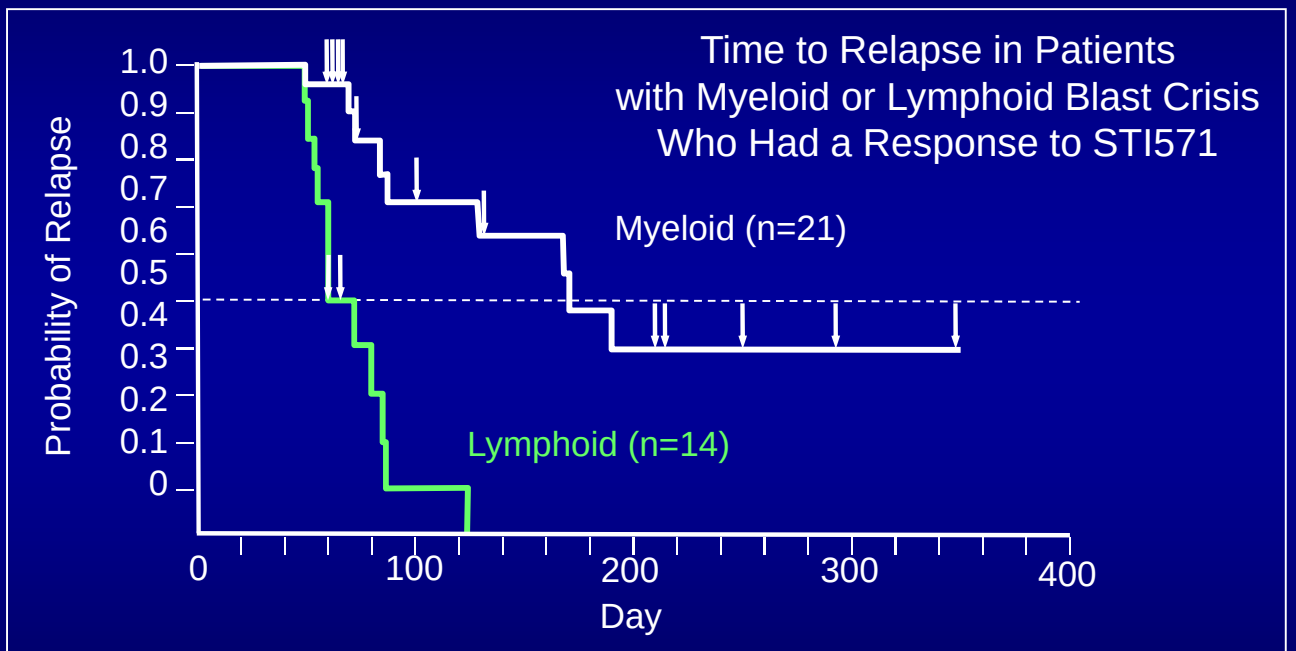
TIME TO A MAJOR CYTOGENETIC RESPONSE FOR IMATINIB VS. INTERFERON AND LOW-DOSE CYTARABINE IN CHRONIC-PHASE CML



Druker et al, NEJM 348: 994, 2003

IMATINIB IN BLAST CRISIS OF CML AND ALL WITH THE PHILADELPHIA CHROMOSOME

BRIAN J.DRUKER,M.D.,CHARLES L.SAWYERS,M.D.,HAGOP KANTARJIAN,M.D.,DEBRA J.RESTA,R.N.,
SOFIA FERNANDES REESE,M.D.,JOHN M.FORD,M.D.,RENAUD CAPDEVILLE,M.D.,AND MOSHE TALPAZ,M.D.

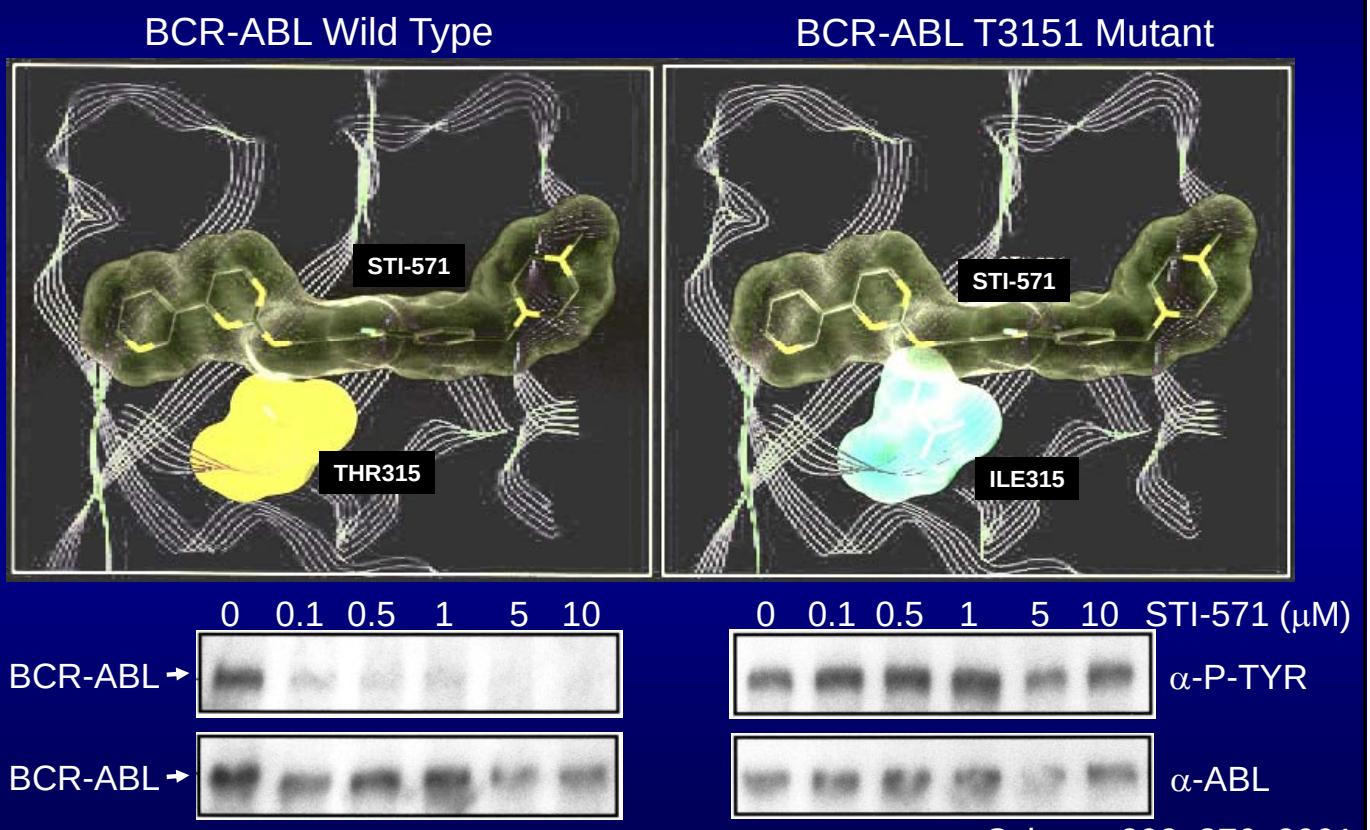


- **Yellow arrows indicate patients still enrolled in the study and in remission at the time of the last follow-up**
- **White arrows indicate the day on which patients were removed from the study**

NEJM 344: 1038, 2001

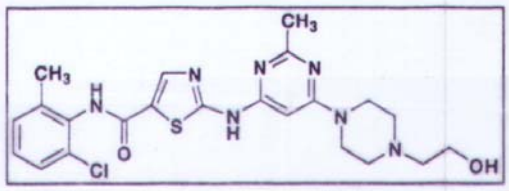
Clinical Resistance to STI-571 Cancer Therapy Caused by BCR-ABL Gene Mutation or Amplification

Mercedes E. Gorre,[1, 3] Mansoor Mohammed,[2] Katharine Ellwood,[1]
Nicholas Hsu,[1] Ron Paquette,[1] P. Nagesh Rao,[2] Charles L. Sawyers[1, 3*]



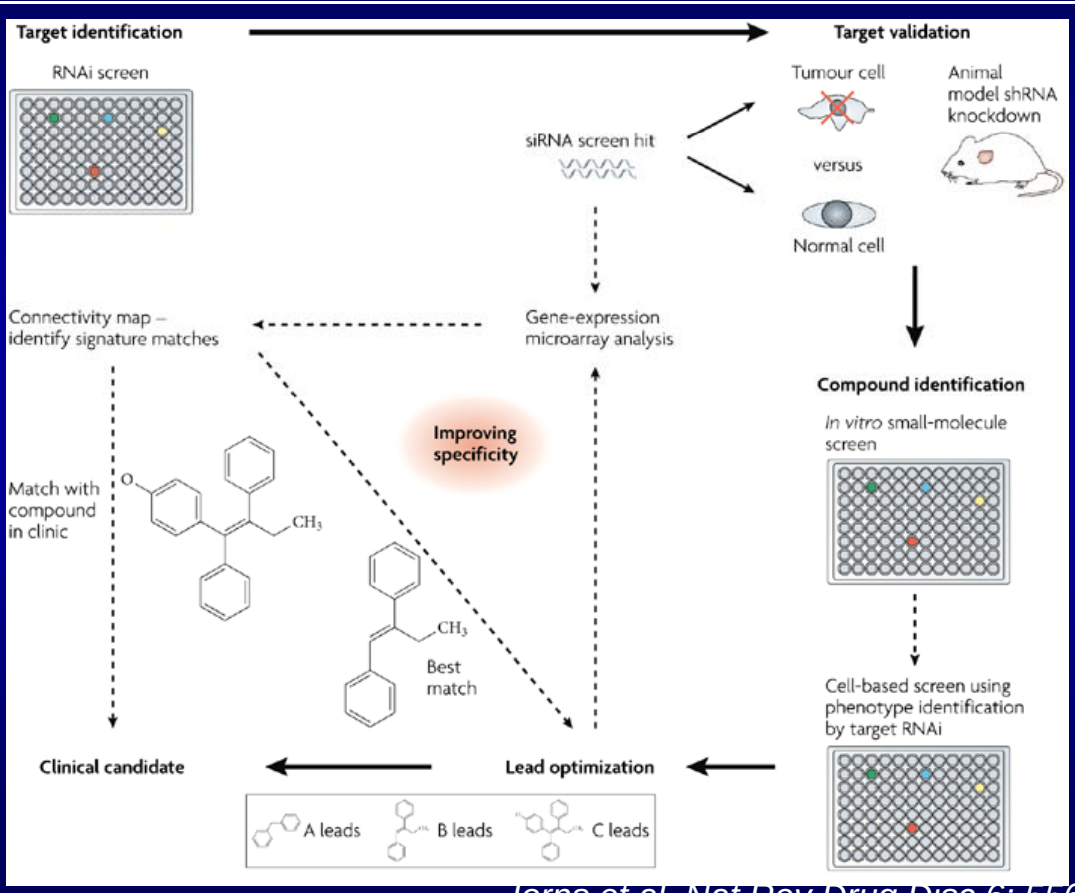
Science 293: 876, 2001

DASATINIB (BMS-354825) ACTIVE AGAINST MOST IMATINIB RESISTANT MUTANTS



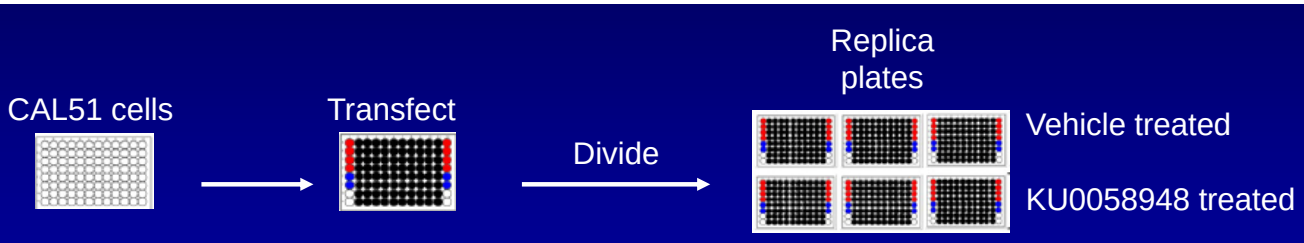
Ba/F3 Clone	BMS-354825 IC_{50}, nM (fold WT IC_{50})	Imatinib IC_{50}, nM (fold WT IC_{50})
p210 WT	1.34 (1)	323 (1)
L248R	16 (12)	>10,000 (>30)
Y253H	10 (7.5)	>10,000 (>30)
E255K	13 (9.7)	8,400 (26)
V299L	18 (13.4)	540 (1.7)
T315I	>1,000 (>750)	>10,000 (>30)
T315A	125 (93)	760 (2.4)
F317L	18 (13.4)	810 (2.5)
F317V	53 (40)	350 (1.1)

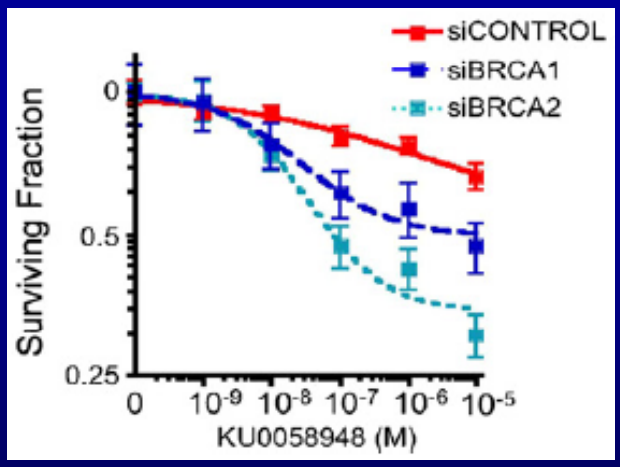
CASE 2: UTILIZING RNAi IN CELL BASED SCREENS TO ENHANCE DRUG DISCOVERY



Iorns et al, Nat Rev Drug Disc 6: 556 (2007)

DEVELOPMENT OF HTS
PARP INHIBITOR SENSITIVITY SCREEN



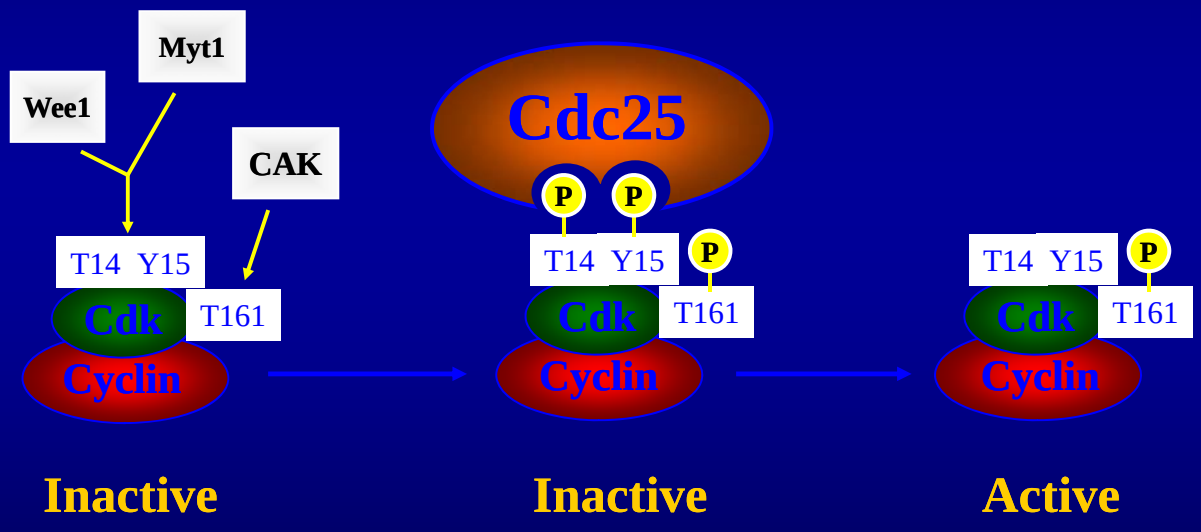


Lord et al, DNA Repair 7: 2010 (2008)

CASE 3: 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.

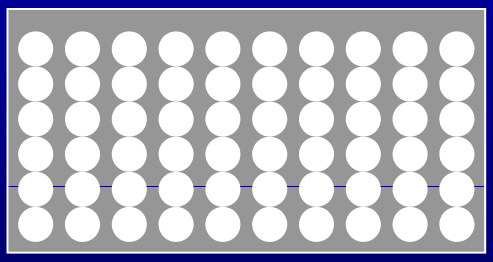
Regulation of Cell Cycle Progression by Cdc25: Cdk Activation



Method for identifying Cdc25 phosphatase inhibitors

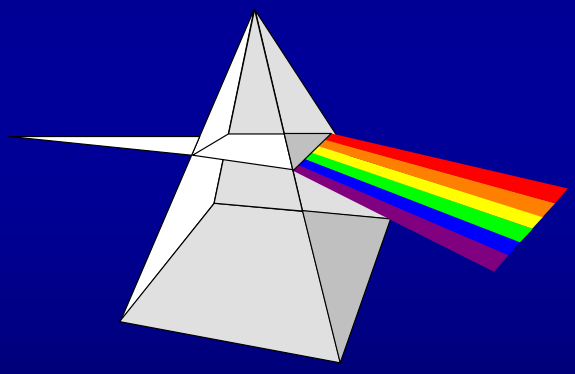
GST-Cdc25 in assay buffer

Fluorescein diphosphate



Incubate 1h

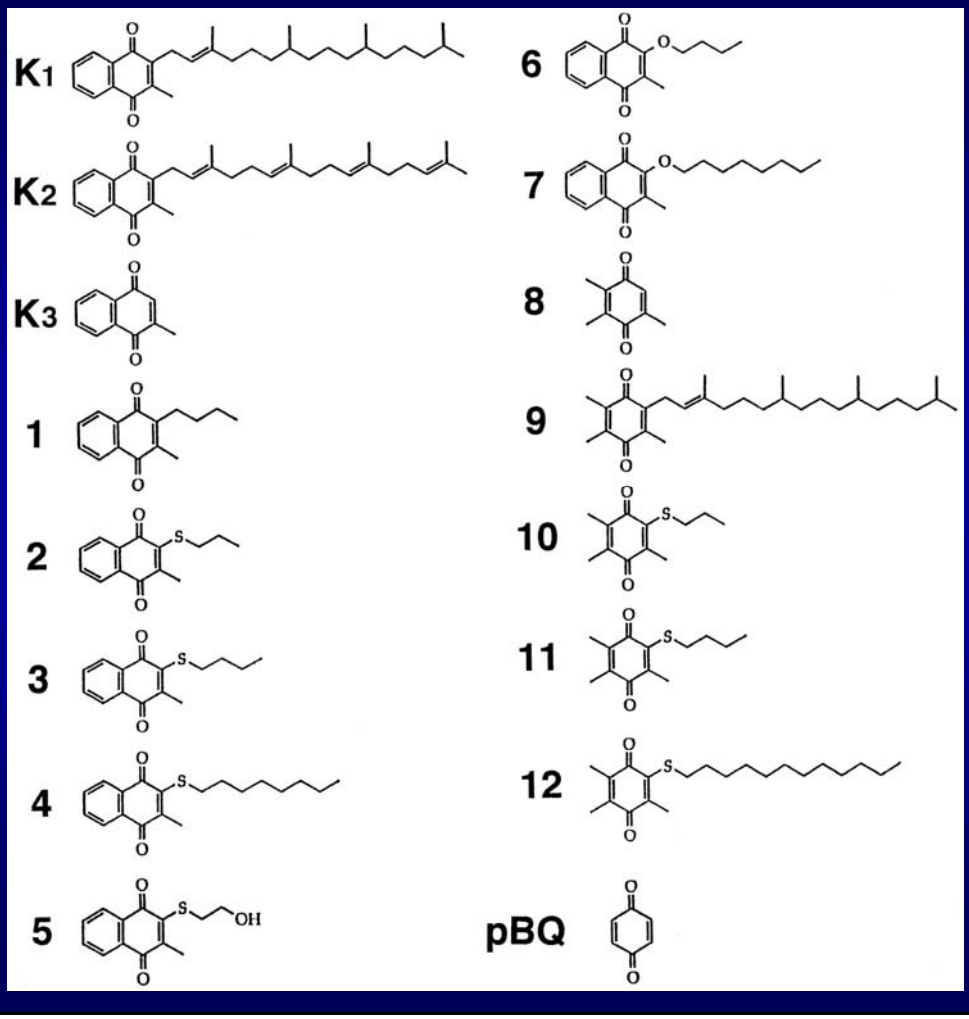
RT



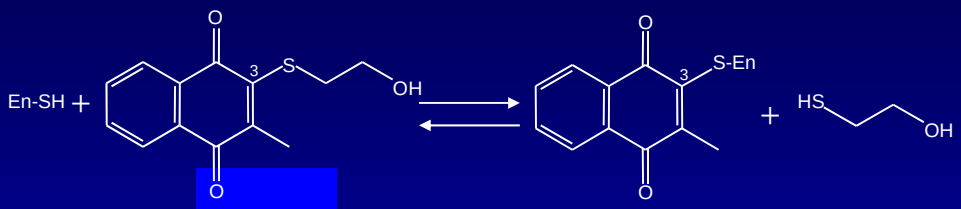
Read product
(fluorescein monophosphate)
on cytoflour II

Chemical Screening Approach

- Targeted Array Libraries
- Diverse Chemical Libraries

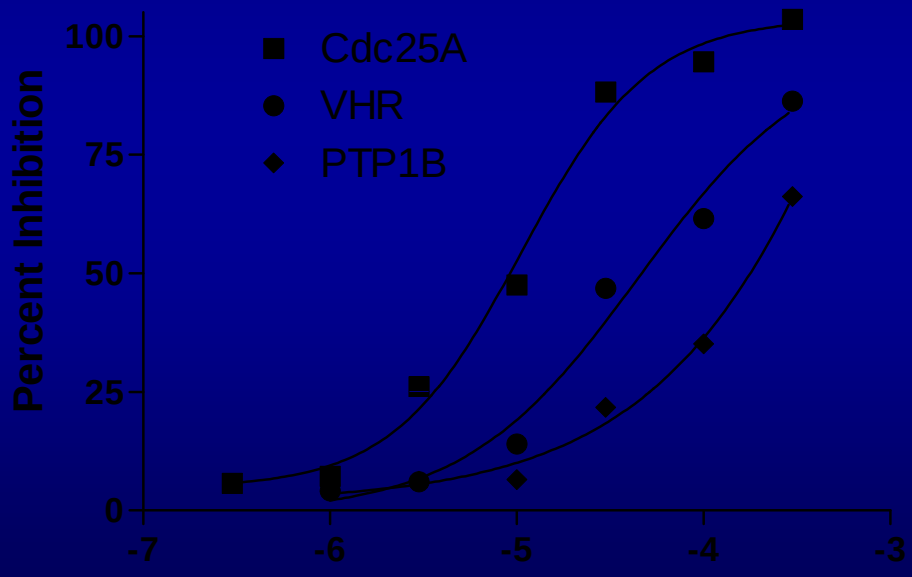

K1
K2
K3
1
2
3
4
5
OH
6
7
8
9
10
11
12
pBQ

Compound 5 inhibits Cdc25



Cdc25A **Cpd 5** **Adduct** **Mercaptoethanol**

$MW_{monoisotopic}$ = 248.1 Da



$Cdc25B_2$ K_i ~ 2 μM

Compound Validation

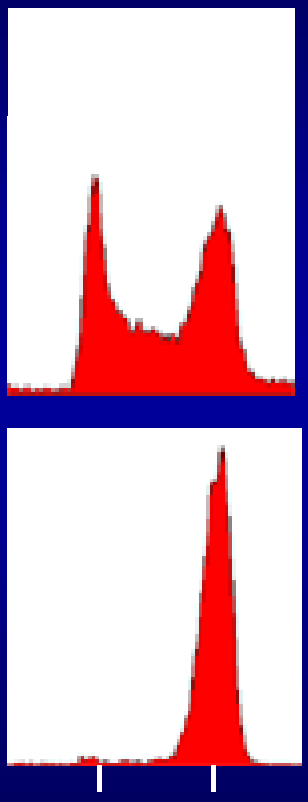
- Cellular: Cell Cycle
- Biochemical: Substrate phosphorylation
- Genetic: Chemical complementation

tsFT210 Cell System

32º 17 h

tsFT210 cells
Cdk1 mutants

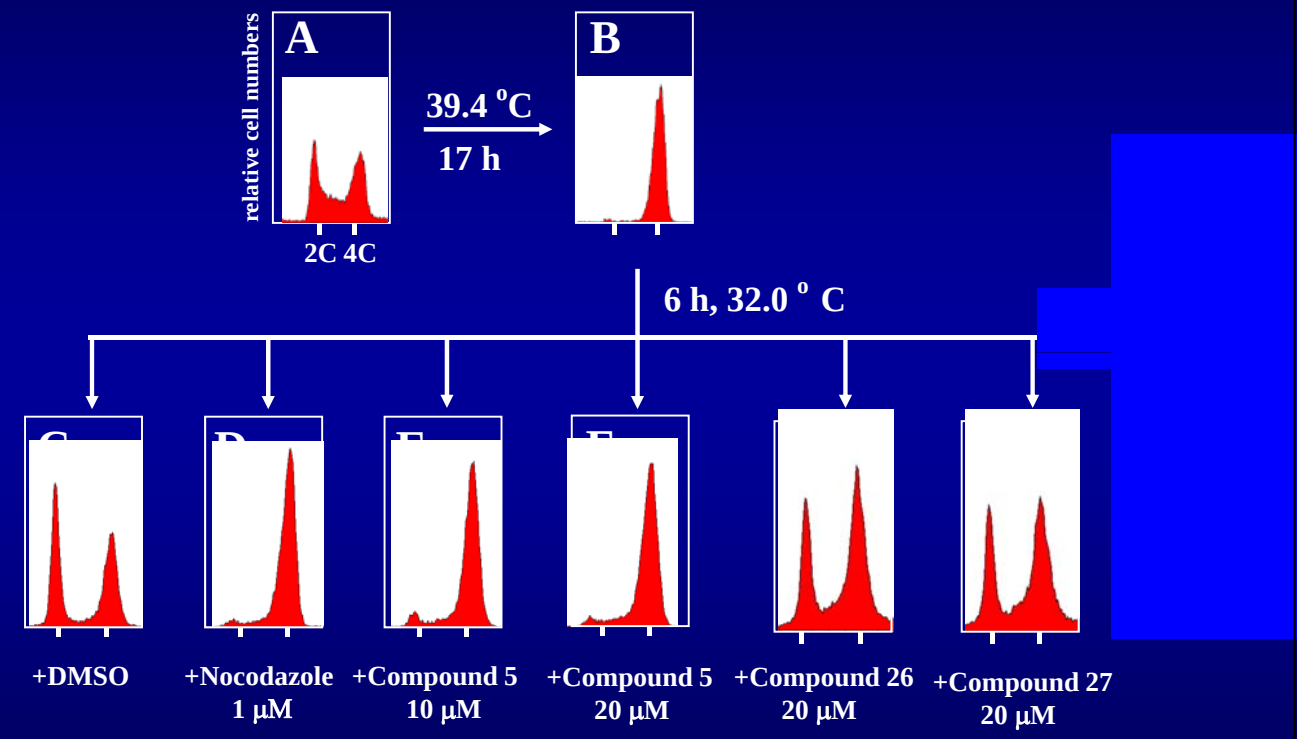
39.4º 17 h



Functional Cdk1

No functional Cdk1

Compound 5 causes G2/M arrest



CASE 4: NMR-BASED SCREENING

1. Screen “fragment” like molecules with “leadlike” properties (MW <300; ClogP ~1.5)
2. Characterize ***binding*** and portion of molecule to which they bind
3. Ligands with weak affinities can be defined (~K_D = 5mM)
4. Lead to high affinity binders through iterative screening
5. Can label protein of interest with isotopes “sensitive” to ligand effects (e.g. N15) and utilize proton resonances of drug to simultaneously allow definition of ligand and receptor binding sites

Hajduk et al. J Med Chem 48: 2518, 2005

NMR AS MEANS OF DEFINING BINDING SITES

E.G., BLEOMYCIN BIMDING TO DNA

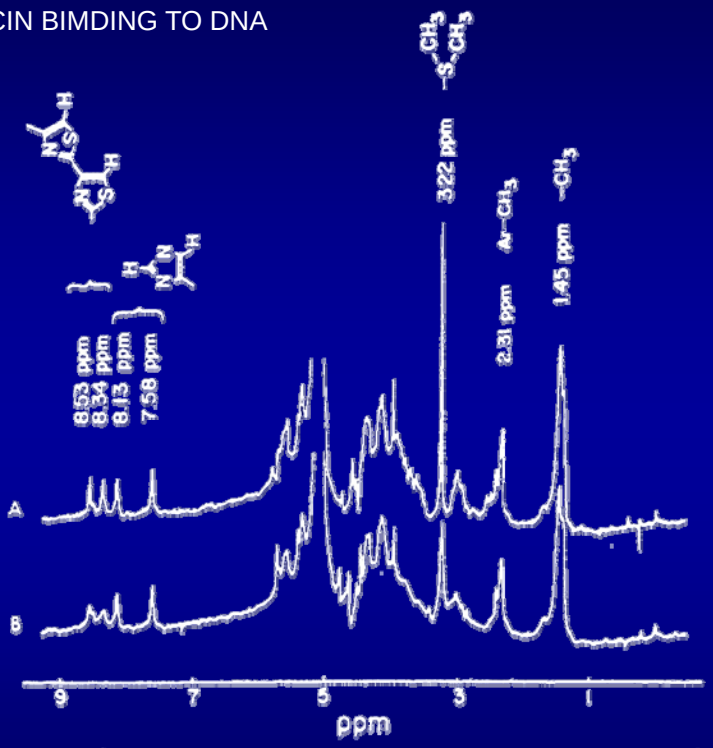
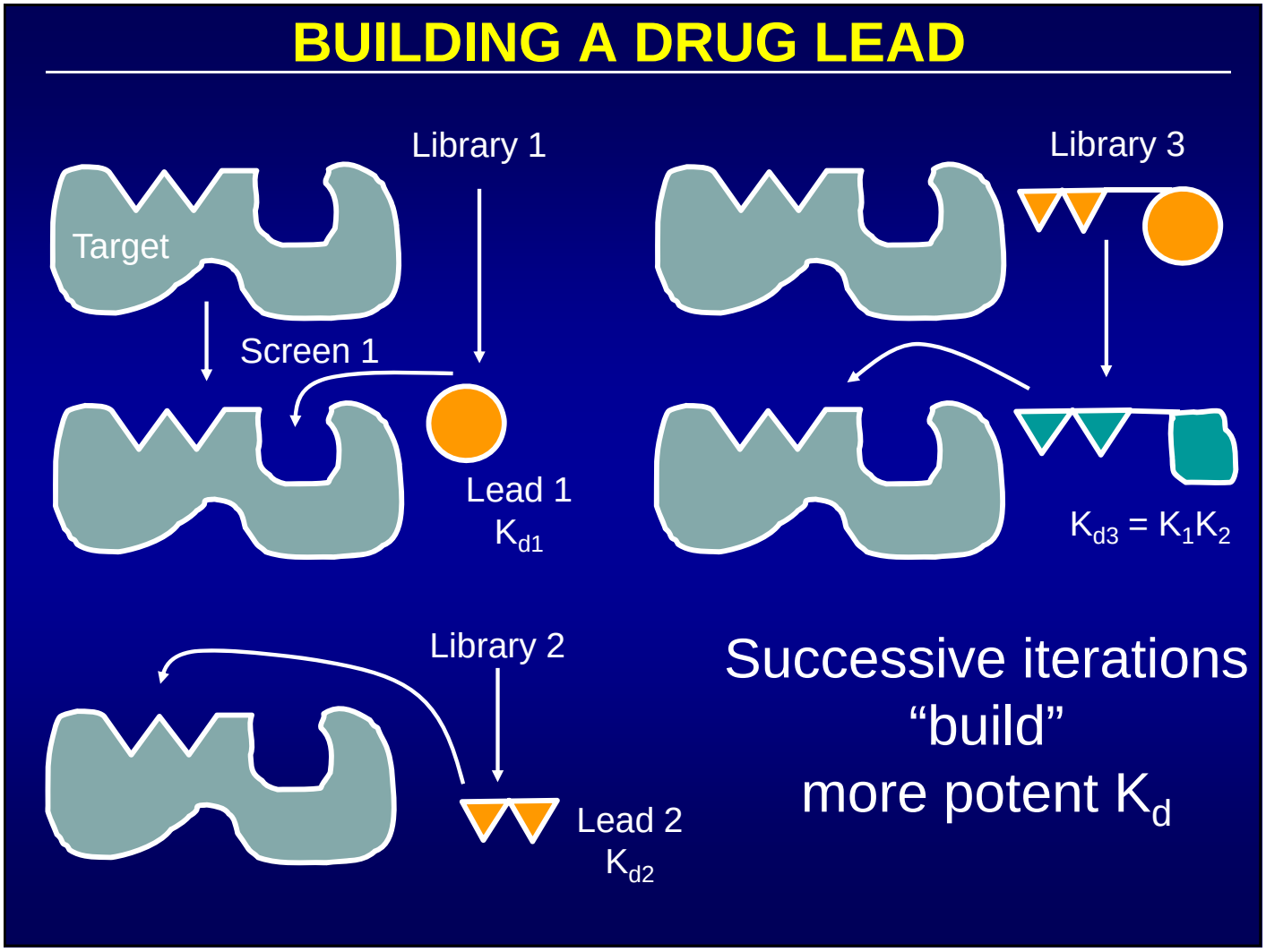


FIGURE 7: ^1H NMR spectra of bleomycin at 100-MHz resolution. Each spectrum is an average of 512 scans. (A) With 6 mM bleomycin in D_2O at pD 8.4; (B) 6 mM bleomycin and 3.5 mM calf thymus DNA in D_2O, pD 8.4.

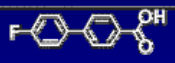
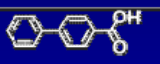
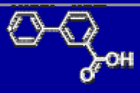
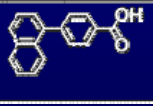
Horwitz et al. Biochemistry 16: 3641, 1977

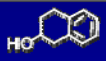
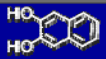
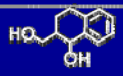
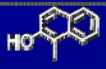
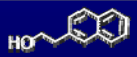
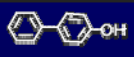
BUILDING A DRUG LEAD



AFFINITIES OF

SELECTED BIARYL COMPOUNDS FOR BCL-XL

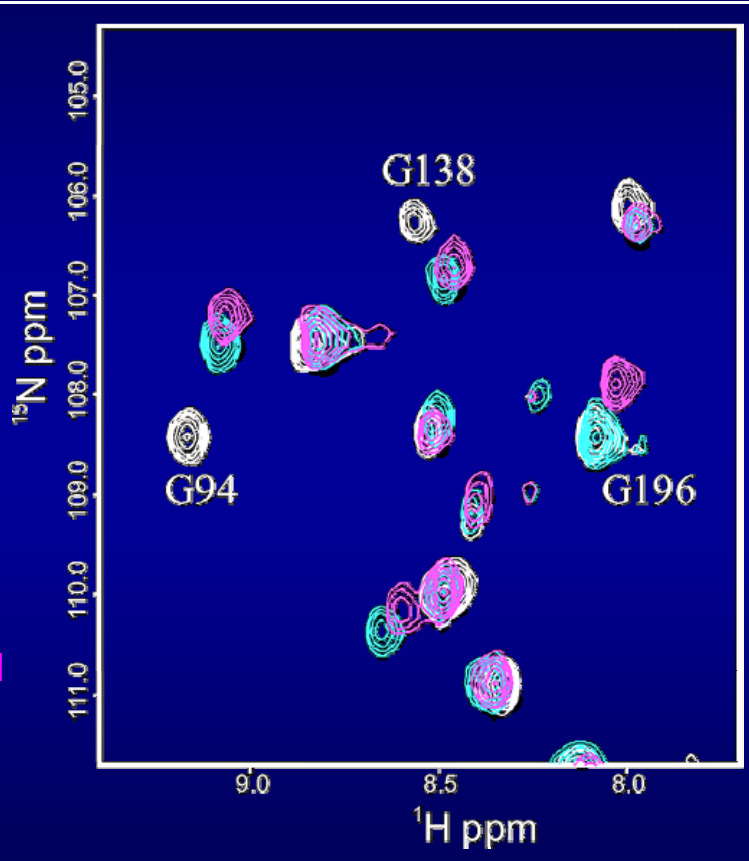
No.	Structure	NMR K_d (μM)
1		300 ± 30
2		1200 ± 530
3		> 5000
4		> 5000
5		> 5000
6		2000 ± 1600
7		1990 ± 990
8		383 ± 117
9		238 ± 110
10		250 ± 139

No.	Structure	NMR K_d (μM)
11		4300 ± 1600
12		13000 ± 7000
13		5000 ± 2000
14		2000 ± 440
15		11000 ± 4800
16		13000 ± 4500
17		9000 ± 2000
18		4000 ± 2050
19		6000 ± 1970
20		6000 ±2000

Petros et al. J Med Chem 49: 656, 2006

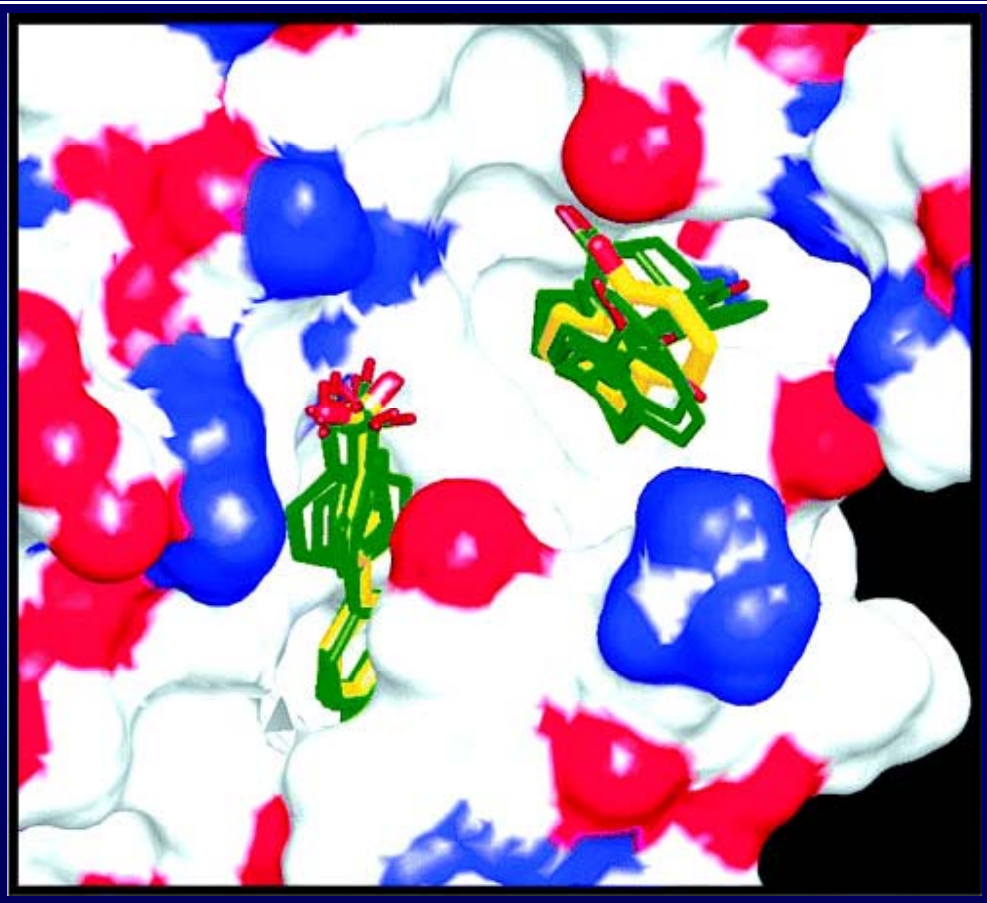
SECTION FROM A ^{15}N HSQC SPECTRUM OF BCL-XL IN THE PRESENCE AND ABSENCE OF COMPOUND

alone (white)
2 mM biaryl acid **1** (cyan)
2 mM biaryl acid **1** and 5 mM naphthol derivative **11** (pink)



Petros et al. J Med Chem 49: 656, 2006

SUPERPOSITION OF SEVEN LOW-ENERGY STRUCTURES CALCULATED FOR BCL-XL COMPLEXED TO 1 AND 11



Petros et al. J Med Chem 49: 656, 2006

OUTLINE OF PRESENTATION

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

STEPS IN CANCER DRUG DISCOVERY & DEVELOPMENT

- DEFINE DRUG TARGET OR DEFINE AN "ACTIVE" DRUG
- OPTIMIZE EVIDENCE OF ACTIVITY IN ANIMAL MODELS OF CANCER (DOSE / SCHEDULE)
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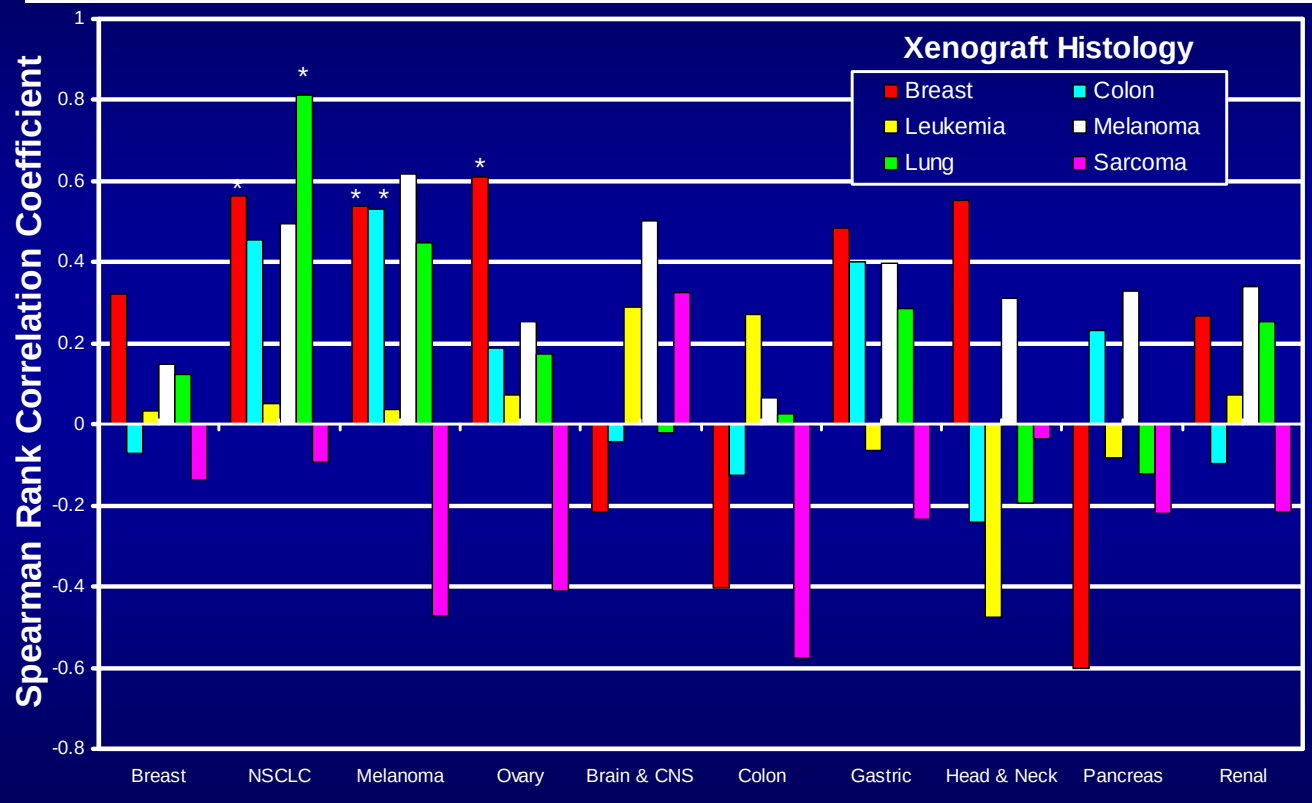
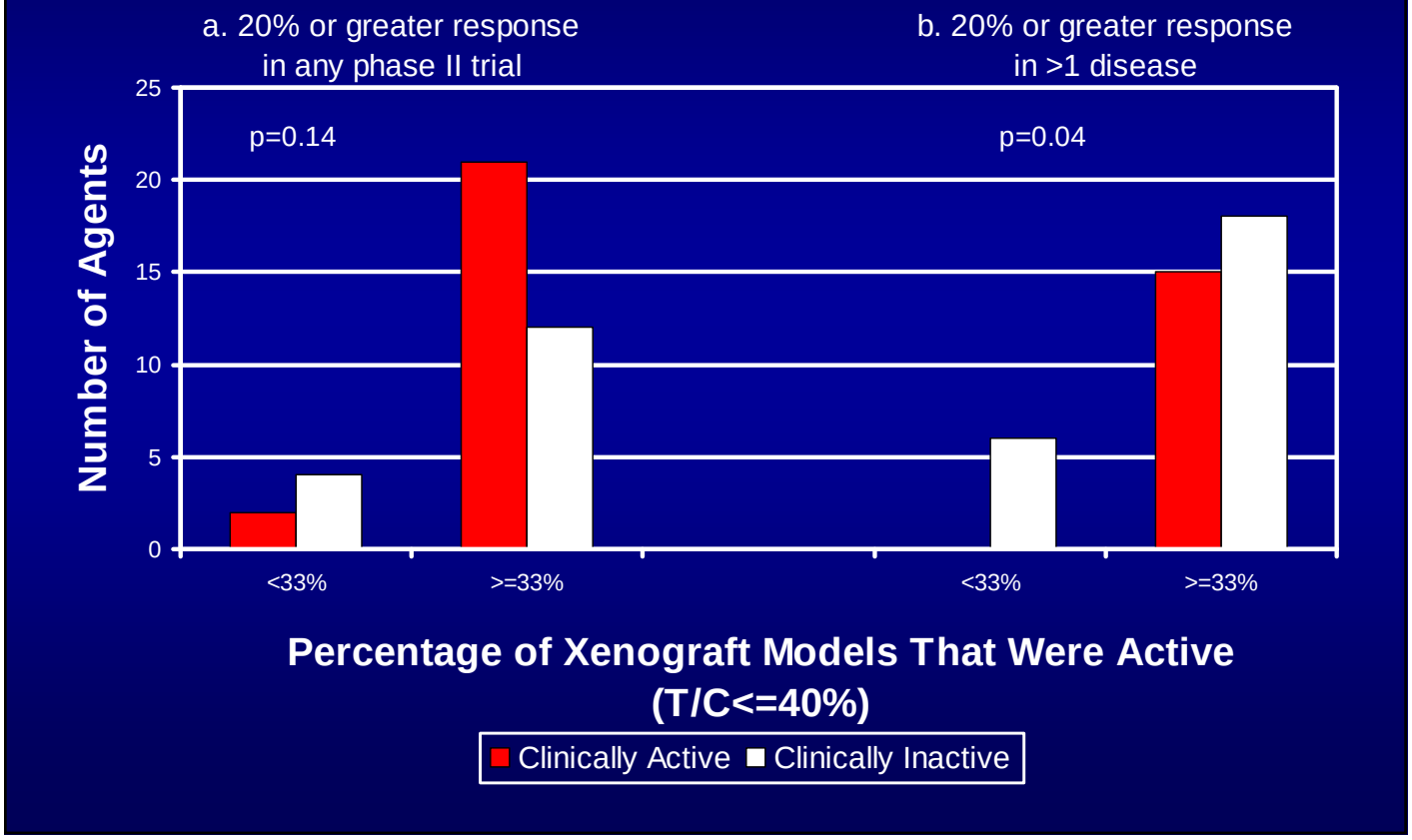
CORRELATION OF *IN VIVO* ACTIVITY WITH CLINICAL ACTIVITY BY DISEASE TYPE



% *IN VIVO* ACTIVITY vs CLINICAL ACTIVITY (39 AGENTS)



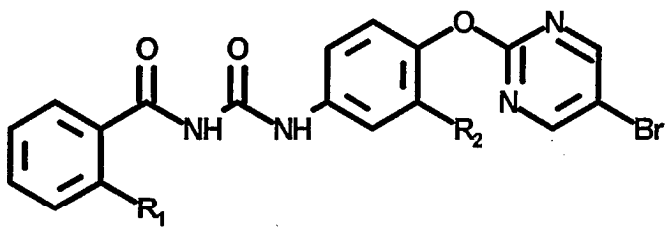
PROBLEMS WITH EMPIRICAL MODELS

- Lack of predictive power *in vivo*
- Poor correlation of non-human with human pharmacology
- Divorced from biology
- Inefficient: many compounds screened;
  developed, but have “late” = clinical trials outcome
  at Phase III to define “validation” of compound action

Figure 1

BENZOYLPHENYLUREAS

(Ishihara Sangyo Kaisha, Ltd.)



NSC	R_1	R_2
624548	NO_2	Cl
639828	NH_2	Cl
639829	$N(CH_3)_2$	CH_3
647884	NH_2	CH_3
654259	$NCOCH_2NH_2 \cdot HCl$	CH_3
654261	$NCOCH_2N(CH_3)_2 \cdot HCl$	CH_3

National Cancer Institute Developmental Therapeutics Program Dose Response Curves	NSC: 639829 -Y / 2	SSPL:	Exp. ID: 9412MD97-43
	Report Date: April 26, 2000	Test Date: December 5, 1994	

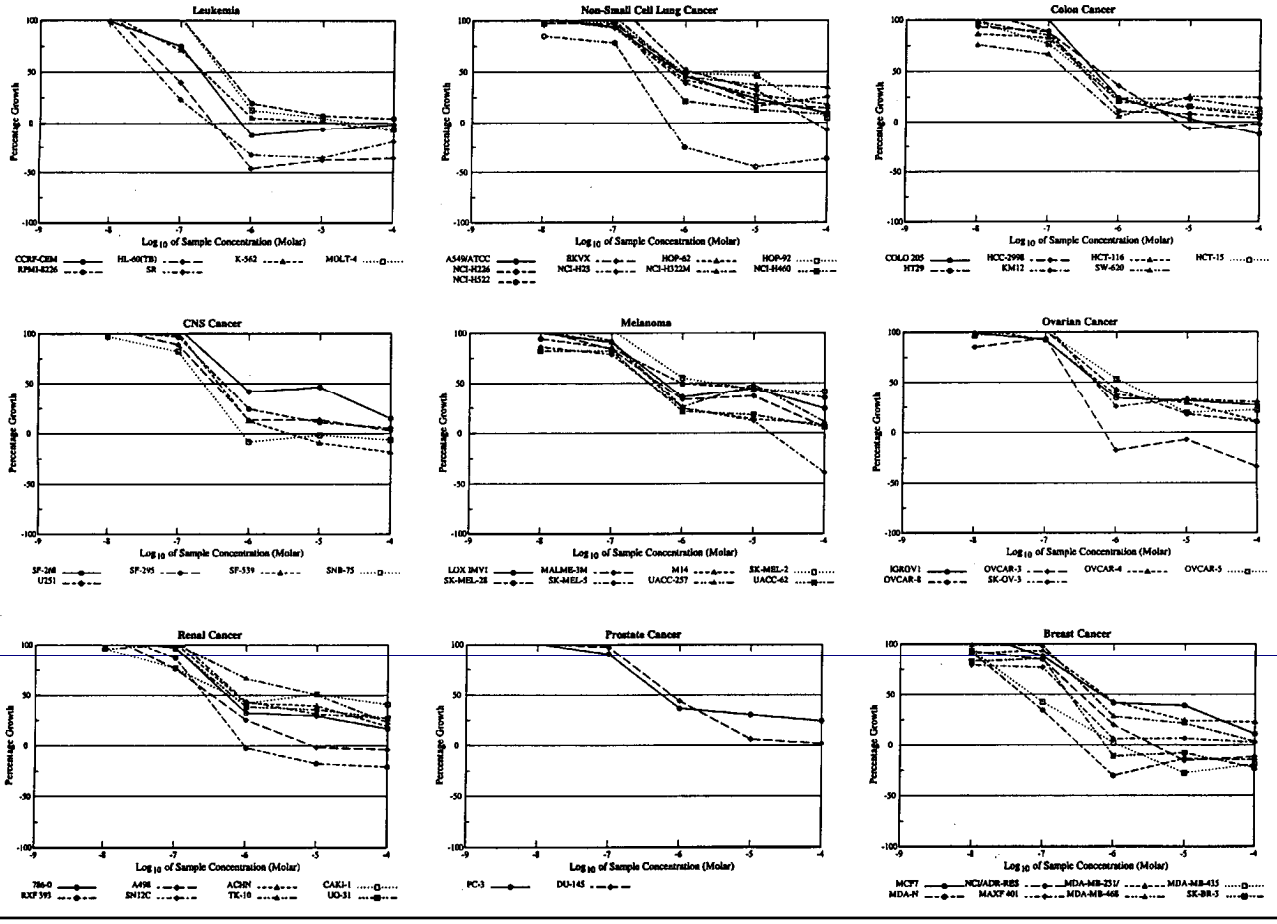


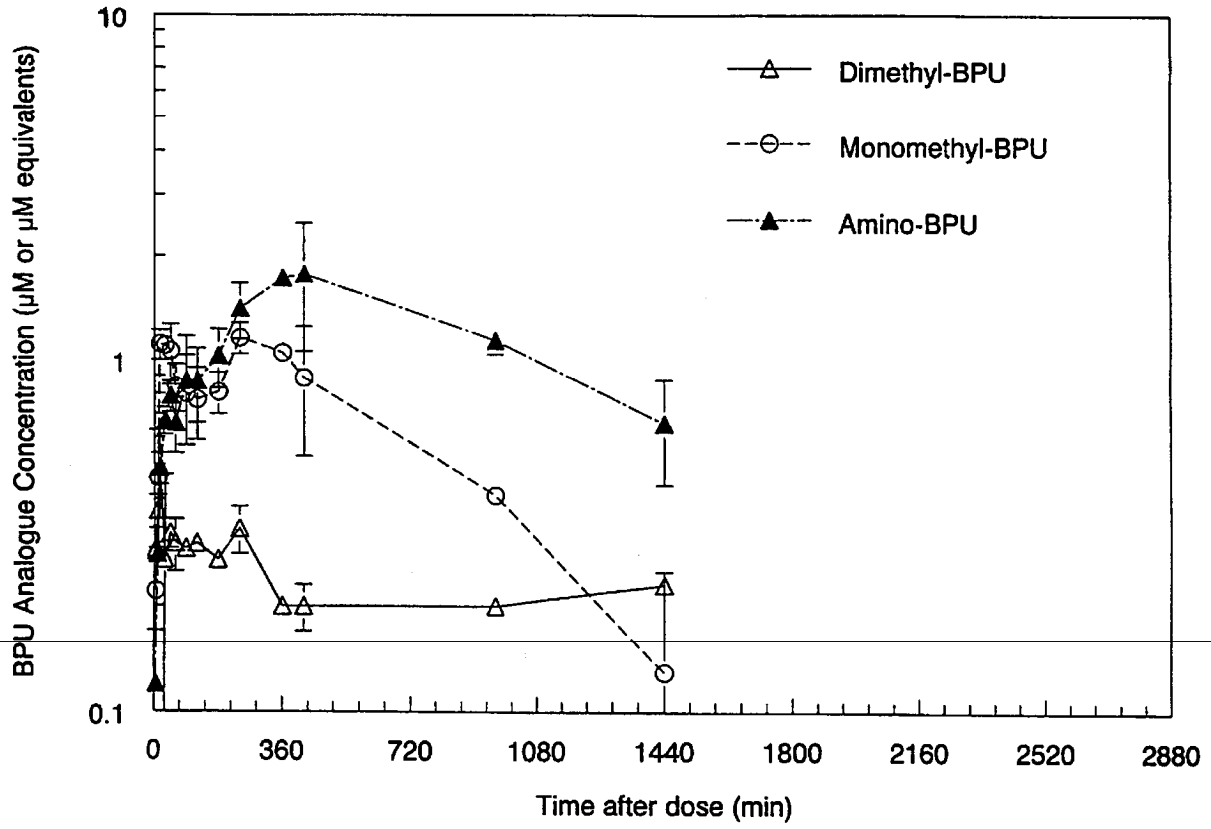
Figure 4

Efficacy Testing of NSC 639829 in Human Tumor Xenografts

Model	Stage/ Implant Site	Treatment Route	Treatment Schedule	MTD (mg/kg /dose)	BW Loss %	Activity Optimal %T/C	Growth Delay %[(T-C)/C]C
AS-283 (SCID mice)	Early-SC	IP	QD X5	15	3.7	**0**	**43**
	Adv-SC	PO	QD X5	8	10.1	**18**	**65**
	Adv-SC	PO	Q4D X3	18	16.2	**21**	**88**
NCI-H522	Adv-SC	IP	Q4D X3	20	0.0	**19**	**57**
		PO	Q4D X3	45	0.9	**19**	**83**
OVCAR-3	Adv-SC	IP	Q4D X3	20	1.5	**21**	**75**
		PO	Q4D X3	>45	2.3	**25**	**71**
MDA-MB-231	Adv-SC	IP	QD X5	>12	0.0	106	-23
	Early-SC	PO	Q4D X3	>30	0.9	**37**	**32**
		PO	Q7D X3	100	0.7	63	**37**
MDA-MB-435	Early-SC	IP	QD X5	12	0.0	**33**	**>29**
		IP	Q4D X3	30	0.1	**11**	**>29**
	Early-SC	IP	QD X5	12	12.2	**13**	**>43**
		IP	Q7D X3	>30	3.7	53	**>33**
		PO	Q7D X3	>67.5	8.6	**38**	**>58**
MDA-N	Early-SC	IP	Q7D X3	>25	4.6	65	16

(BPUTAB2.WPD)

Figure 13


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

STEPS IN CANCER DRUG DISCOVERY & DEVELOPMENT

- DEFINE DRUG TARGET OR DEFINE AN "ACTIVE" DRUG
- OPTIMIZE EVIDENCE OF ACTIVITY IN ANIMAL MODELS OF CANCER (DOSE / SCHEDULE)
- RELATE ACTIVITY (OR LACK THEREOF) IN ANIMAL MODELS TO CONCENTRATIONS AND DURATIONS OF DRUG EXPOSURE
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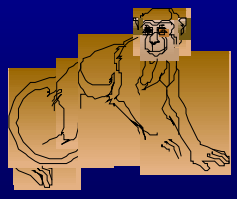
FDA PRECLINICAL PHARMACOLOGY & TOXICOLOGY REQUIREMENTS

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



- BIOLOGICALS
  - Most Relevant Species
  - Clinical Route & Schedule



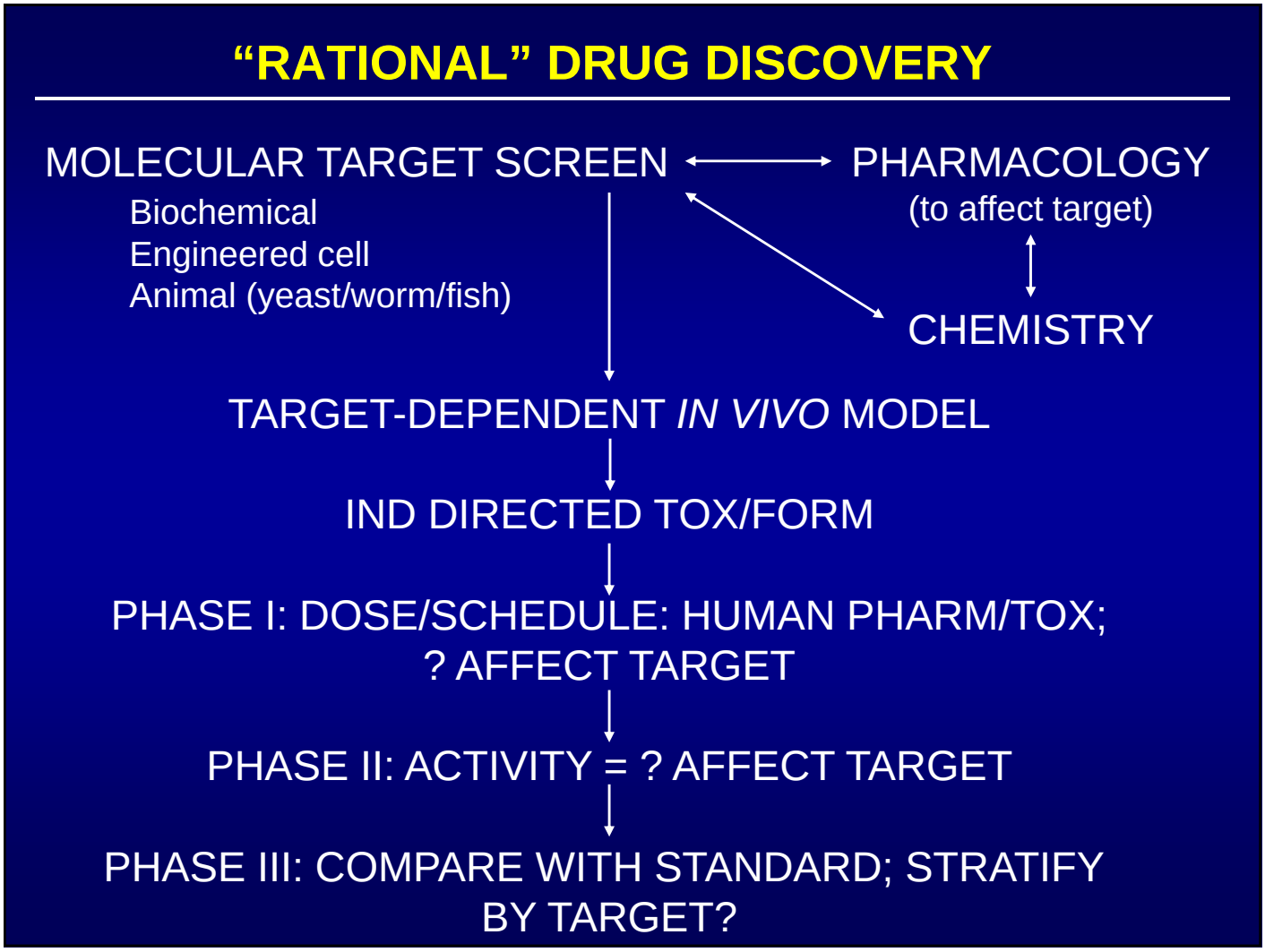
BENZOYLPHENYLUREA
PRECLINICAL MTD & DLTs

Schedule q4Dx3, *po*	RAT	DOG
MTD (Total Dose)	360 mg/m^2	> 150 < 240 mg/m^2
DLT	Bone Marrow GI Tract	Bone Marrow, GI Tract

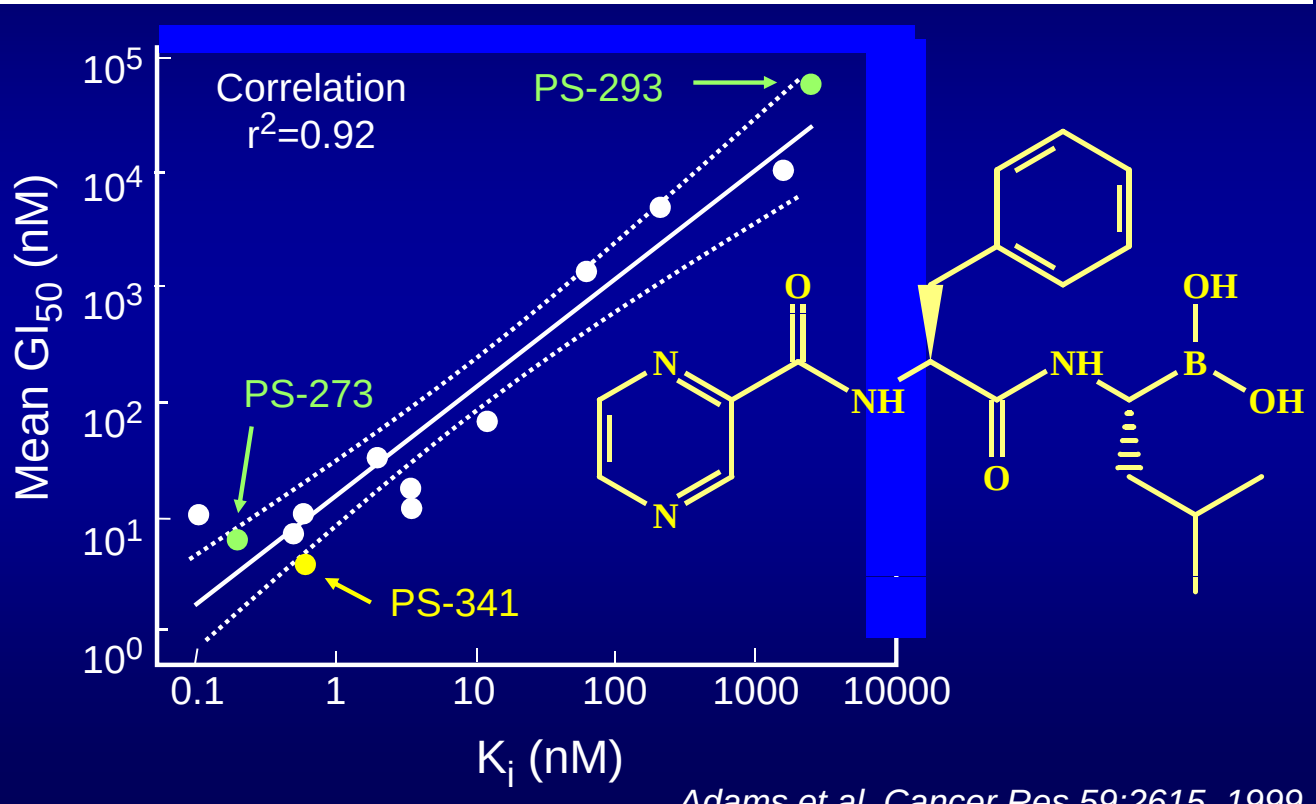
Starting Dose: 24 mg/m^2

PROBLEMS WITH "MTD" DRIVEN ENDPOINTS

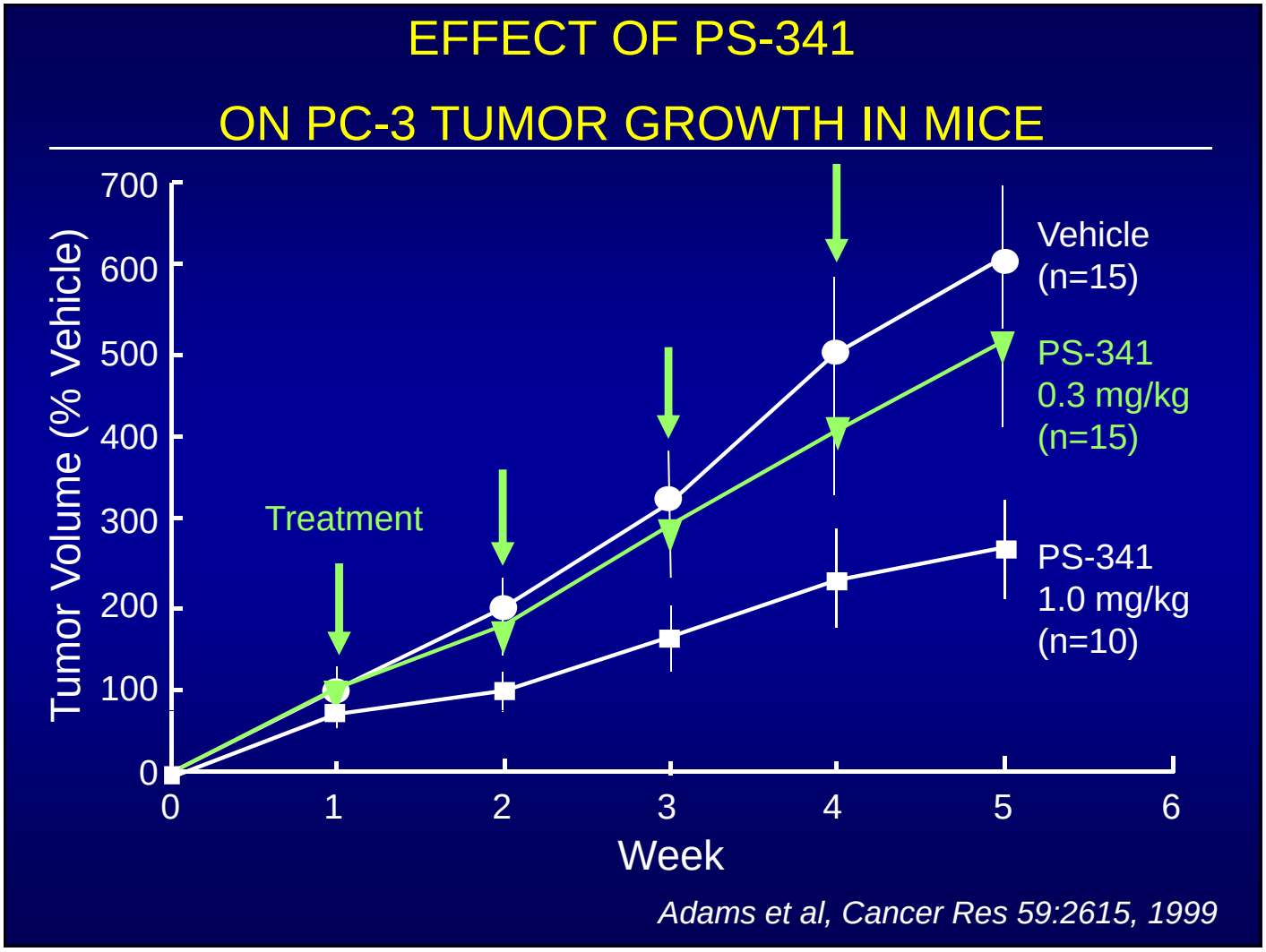
- Drugs regulating pathways important in oncogenesis are effective by combining with high affinity binding sites; therefore must distinguish "targeted" vs "non-targeted" toxicity related to these binding sites
- Whether dosing beyond effect on desired target "buys" therapeutic value not clear
- Therefore must define in pre-clinical studies "*BIOLOGICALLY EFFECTIVE DOSE"* and *"MAXIMUM TOLERATED DOSE"*
- *Use BIOLOGIC rather than TOXIC endpoints in PhaseI?*


"RATIONAL" DRUG DISCOVERY
MOLECULAR TARGET SCREEN
Biochemical
Engineered cell
Animal (yeast/worm/fish)
PHARMACOLOGY
(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?

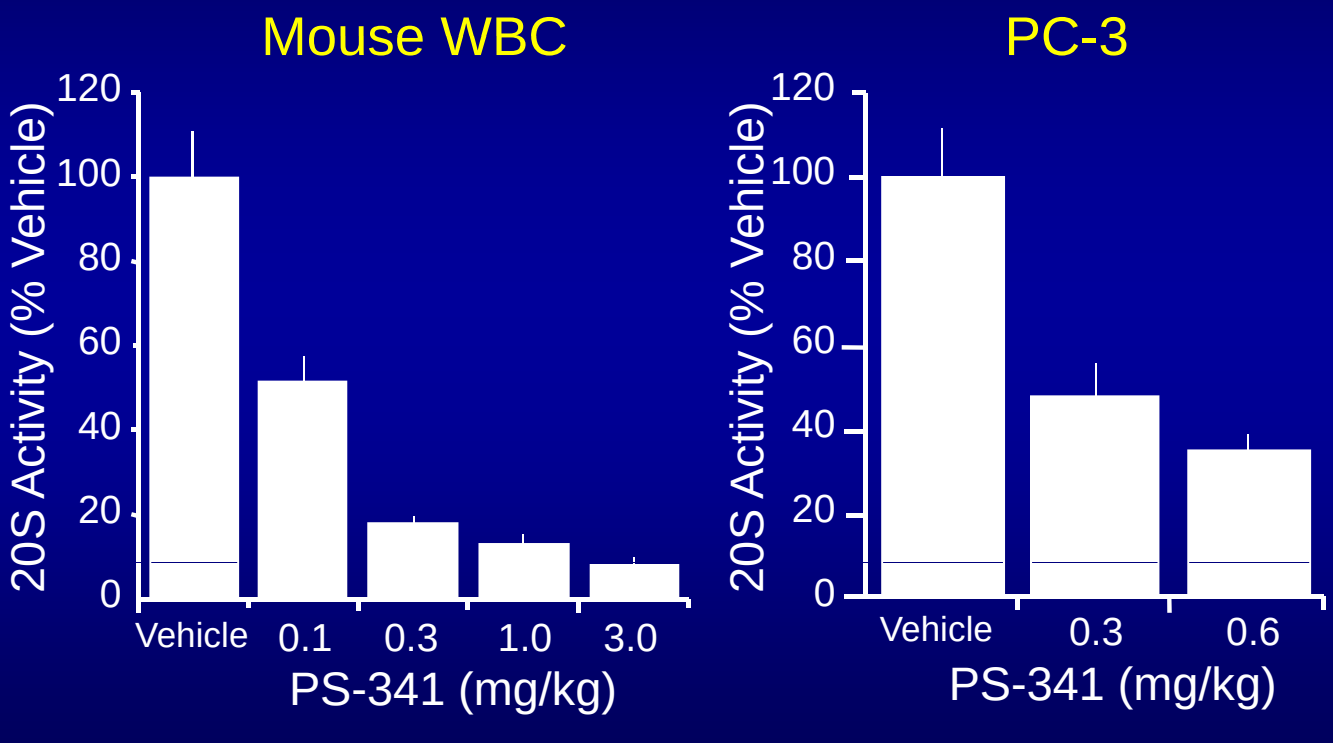
CORRELATION BETWEEN 20S PROTEASOME INHIBITORY POTENCY & GROWTH INHIBITION FOR 13 DIPEPTIDE BORONIC ACIDS



Adams et al, Cancer Res 59:2615, 1999


EFFECT OF PS-341
ON PC-3 TUMOR GROWTH IN MICE
Tumor Volume (% Vehicle)
700
600
500
400
300
200
100
0
0
1
2
3
4
5
6
Week
Treatment
Vehicle
(n=15)
PS-341
0.3 mg/kg
(n=15)
PS-341
1.0 mg/kg
(n=10)
Adams et al, Cancer Res 59:2615, 1999

EFFECT OF PS-341 ON 20S PROTEASOME ACTIVITY



Adams et al, Cancer Res 59:2615, 1999

PS-341: INTERSPECIES

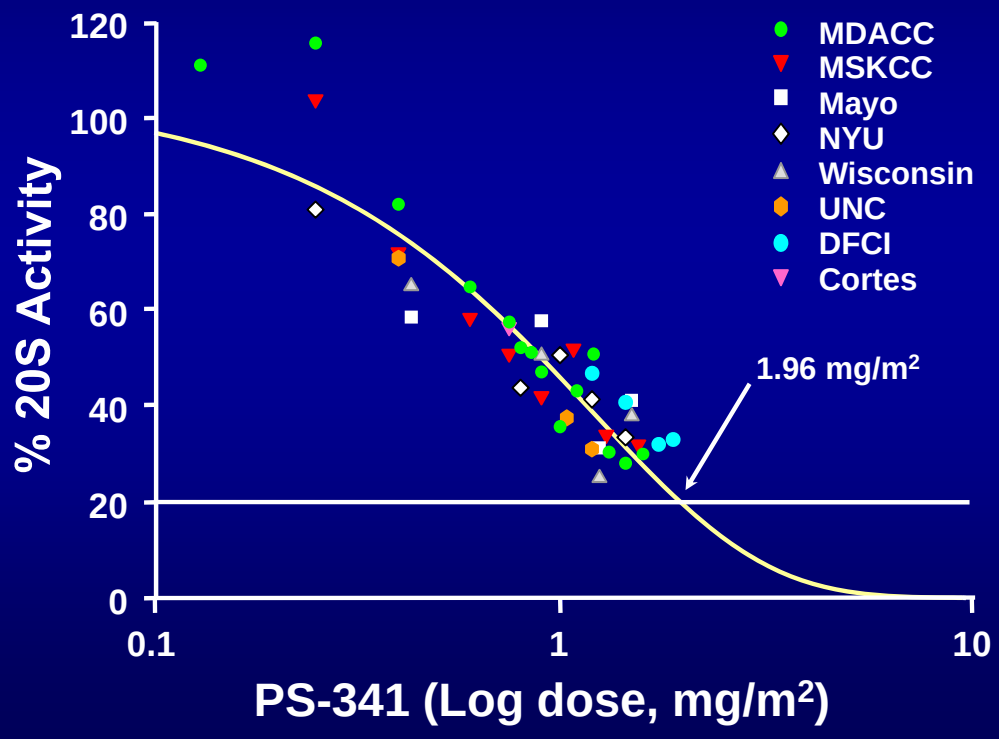
Q: Is the 'safe' dose in animals in the efficacy range for man?

Species	Dose (mg/kg)	Dose (mg/m^2)	% 20S Proteasome Inhibition*
Mouse	1.0	3.0	80
Rat	0.25	1.5	80
NHP	0.067	0.8	70

*In white blood cells at 1.0 h, post-dose

Ref: Adams, *et al, Cancer Res* 59:2615, 1999

Ex Vivo Proteasome Activity: 1 Hour Post Treatment



PRECLINICAL DRUG STUDIES: SUMMARY

- Aid and promote clinical trials design
- Assure likely safety of initially explored regimen
- Provide scientific basis for assessing clinical effects of agent
- Increasingly to focus on correlating molecular effects of agents on intended targets along with “usual” pharmacologic / toxicologic endpoints