DRUG DISCOVERY
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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?

<table>
<thead>
<tr>
<th>Majority of Drug Targets:</th>
<th>% Top Sales</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-Protein Coupled Receptors</td>
<td>18</td>
</tr>
<tr>
<td>Nuclear (Hormone) Receptors</td>
<td>10</td>
</tr>
<tr>
<td>Ion Channels</td>
<td>16</td>
</tr>
<tr>
<td>Enzymes</td>
<td>approximately 50</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reasons for failure</th>
<th>Reasons for slowdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity, 22%</td>
<td>Synthetic complexity</td>
</tr>
<tr>
<td>Lack of efficacy, 31%</td>
<td>Low potency</td>
</tr>
<tr>
<td>Market reasons, 6%</td>
<td>Ambiguous toxicity finding</td>
</tr>
<tr>
<td>Poor biopharmaceutical properties, 41%</td>
<td>Inherently time-intensive target indication</td>
</tr>
<tr>
<td></td>
<td>Poor biopharmaceutical properties</td>
</tr>
</tbody>
</table>

Modern Drug Discovery
January/February 1999

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Diagram illustrating the flow from initial candidate compound screening ($10^3 - 10^5$ compounds per screen) through “hits” and “leads” (100 leads), lead optimization, pre-clinical development (12 drug candidates) and clinical development (4-5 drug candidates) that results in only one NDA filing.

*Tufts CSDD, H&Q 1998; The Pfizer Journal, 1/2000*
TRADITIONAL PHARMACEUTICAL R&D
Costly* and Time Consuming**

<table>
<thead>
<tr>
<th>Lead Discovery Research</th>
<th>Drug Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 years</td>
<td>8.9 Years</td>
</tr>
<tr>
<td>$230m</td>
<td>+71m +56m +169m +169m +44m</td>
</tr>
</tbody>
</table>

Target ID | Synthesis/Screening | Preclinical Ph1 Ph2 Ph3 Filed
Target Validation | Lead Optimization

* Lehman Brothers, 1997; ** Tufts CSDD
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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 biochemical target’s function
E.g.: HIV-protease inhibitor (anti-infection)
      metoprolol (anti-hypertensive)
      methotrexate (anti-tumor)
“EMPIRICAL” DRUG DISCOVERY

SCREEN \(\rightarrow\) Pharmacology
\(\downarrow\)
\(\rightarrow\)
\(\downarrow\)
\(\rightarrow\)
\(\downarrow\)
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\(\downarrow\)
\(\rightarrow\)
\(\downarrow\)

BIOLOGICAL ACTIVITY (in vitro/in vivo)

OPTIMIZED SCHEDULE (in vivo)

IND-DIRECTED TOX/FORMULATION

PHASE I: DOSE/SCHEDULE HUMAN PHARM/TOX

PHASE II: ACTIVITY

PHASE III: COMPARE WITH STANDARD
PROBLEMS WITH EMPIRICAL MODELS

Lead optimization difficult without known biochemical target--How to optimize?

Value of screen depend on predictive value of screening model with biology of disease
E.g.: acid hypo-secretion or H2 receptor binding assay HIGHLY correlate with useful anti-ulcer Rx

Counter E.g.: anti-tumor activity in > 33% mouse models of cancer have at best 50% chance of >1 P2 trial for non=targeted cancer Rx’s

Divorced from mechanism: an intriguing lead must be “deconvoluted”
KRN5500

Chemical Structure (prodrug outside cell)

Cell Membrane

Chemical Structure (prodrug inside cell)

Deacylation ↓ SAN-Gly

Chemical Structure (active metabolite)

↓
Protein Synthesis (blocked)
EFFECT OF KRN5500 ON COLO-205 ATHYMIC MOUSE XENOGRAFTS

Line chart showing median tumor Weight (mg) in mice versus time (day posttumor Implantation). A dose-response relationship is shown for KRN5500 inhibition of tumor growth.
Line chart showing plasma concentration (µM) in mice by Time (days) from 0 to 5 days. The plasma concentration in the mice goes up and down (peaks and troughs) quite a bit over time but overall it increases somewhat from the level on day 1. In addition, on days 3 and 4 peaks and trough levels are higher.
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/M2/d x 5

Achieve only 0.75 - 1 μM at 3.7 mg/M2/d x 5

4/6 patients with >25% incr Cmax have

Data of J. P. Eder, DFCI
“RATIONAL” DRUG DISCOVERY

Flow chart showing steps from molecular target screen including Biochemical, Engineered cell, and Animal (yeast/worm/fish) to pharmacology (to affect target) to chemistry and then proceeds to the following steps in the order shown:

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?
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

Schematic representation of regions in fusion protein.

*McWhirter JR*, EMBO 12:1533, 1993
EXAMPLE OF “RATIONAL” APPROACH:
bcr-abl directed agents

Natural product
empiric lead       Chemical structure of erbstatin, lavendustin and piceatannol

1st generation
Synthetic          Chemical structure for AG957 and AG1112

2nd generation
synthetic;
in clinic         Chemical structure of CGP 57148B = ST1571
STI571: An oral in vivo bcr-abl kinase inhibitor

Graphic illustration of antitumor activity in vivo in KU812 and U937 mice.

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.

Line chart showing white blood cells (cells x 10^3 /mm^3) over duration of treatment (days) with STI571. There is a significant drop in white cell count from day 1 by or before 30 days of the study. That lower white cell count continues through the period followed of approximately 100 - 140 days.

The second graph shows a decline in percent in metaphase for Ph chromosome t cells.

NEJM 344: 1031, 2001
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
Gene Expression: The Cell’s Fingerprint

Bar chart comparing normal cells with cancer cells in Genes A through H expression. The bar chart shows that cancer cells out number normal cells in Genes A, C, E, and H. In H, few cells are normal and the vast majority are cancer cells. Normal cells out number cancer cells in Genes B, F, and G and with F there are significantly more normal cells. For Gene D the normal and cancer cells appear to be approximately equal.

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

At the bottom left of the slide is a logo from the National Cancer Institute - The Cancer Genome Anatomy Project.
This is information from a National Cancer Institute (NCI) document or website for the Cancer Genome Anatomy Project. It lists 5 different NIH ICs that are part of the CGAP initiatives

http://cgap.nci.nih.gov/
Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling

Graphic illustration of different survival probability based on gene expression in lymphoma cells.

GELDANAMYCIN: EXAMPLE OF BINDING PARTNER DEFINING TARGET

Chemical structure of benzoquinone (ansa ring and carbamate moieties)

<table>
<thead>
<tr>
<th>NSC</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geldanamycin</td>
<td>122750 OMe</td>
</tr>
<tr>
<td>17-AAG</td>
<td>330507 NHCH₂CH=CH₂</td>
</tr>
</tbody>
</table>
“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

Diagram of ansamycin molecule linked to a bead by 18 atom spacer.
GELDANAMYCIN BEADS
IDENTIFY HSP90 AS BINDING PARTNER

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

Neckers et al, PNAS 91:8324, 1994
Three graphic illustrations of the role of HSP 90 in cell function.
OUTLINE OF PRESENTATION

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Definition of Drug Targets

*Generating Diversity*

Definition of Lead Structures

Qualifying Lead for Transition to Early Trials
Diversity

Graphic illustration of 9 different snowflakes which vary widely one from another.

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

A circle is shown which is divided into four equal parts. Going clockwise from the top right segment they are labeled Base (-), Water (low dielectric), Acid (+), and Oil (high dielectric).
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

A pie chart is shown and broken down as follows:

B = 12%
N = 5%
ND = 23%
S = 33%
S/NM = 10%
S*/NM = 4%
S*/NM = 10%
V = 3%

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
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

Chemical structures of Gramine → Isogramine → Lidocaine

*Courtesy of N. R. Farnsworth*
Natural Product Isolation Tree

Flow chart illustration
“You are what you eat”

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

*Dolabella auricularia*
Dolastatins come from a *Symphocca* species that they graze on
“Non-culturale” 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 approximately 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
peptide / protein

non-peptide

Target-directed chemical compound libraries
“classical” medicinal chemistry / bona fide crystal structure – derived

“docked” lead structures into model
TRIPEPTIDE COMBINATORIAL LIBRARY

XXX

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

Chart showing the length, peptide and number possible with increasing length, going from 400 possible peptides with 2 amino acids to over 25 billion with 8 amino acids

after R. Houghten, 1999
IC$_{50}$ OF MIXTURES

A chart showing log concentration of a single active compound: IC$_{50} = 1.0$ nM, a single 1.0 nM active compound + 9 inactives: IC$_{50} = 10$ nM, and a single 1.0 nM active compound + 9,999 inactives: IC$_{50} = 10,000$ nM.
COMBINATORIAL LIBRARIES:  
THE MIXTURE QUESTION

<table>
<thead>
<tr>
<th></th>
<th>Natural Product Extracts</th>
<th>Synthetic Combinatorial Mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct screening of compound mixture</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Discovery of highly active compounds</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Equal concentrations of compounds</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Chemical structure known</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Synthetic pathway known</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Structure – activity relationship known</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*after R. Houghten, 1999*
NON-PEPTIDE “COMBINATORIAL” STRATEGIES
COMBINE “SCAFFOLDS” (OR “BACKBONES”) 
WITH “FUNCTIONAL GROUPS”

Graphic illustration and example of chemical structure

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
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COMBINATORIAL LIBRARIES OF BICYCLIC GUANIDINES FROM REDUCED ACYLATED DIPEPTIDES

Chemical structure and synthesis

1. CSI m₂
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)

1/percent bound for \( R_1 \) position, \( R_2 \) position, and \( R_3 \) position.

after R. Houghten, 1999
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Definition of Drug Targets

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Qualifying Lead for Transition to Early Trials

"RATIONAL":

-Structure based design
  Biochemical Screen
  Target-driven
  Cell-based Screen
"EMPIRICAL"
  Bioassay of effect
NMR-BASED SCREENING

Screen “fragment” like molecules with “leadlike” properties (MW <300; ClogP ~1.5)

Characterize binding and portion of molecule to which they bind

Ligands with weak affinities can be defined (~KD = 5mM)

Lead to high affinity binders through iterative screening

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 BINDING TO DNA

NMR recording

1H NMR spectra of bleomycin at 100-MHz resolution. Each spectrum is an average of 512 scans. With 6 mM bleomycin in D₂O at pD 8.4; 6 mM bleomycin and 3.5 mM calf thymus DNA in D₂O, pD 8.4.

Horwitz et al, Biochemistry 16: 3641, 1977
BUILDING A DRUG LEAD

Graphic illustration of target molecule, screening of compound libraries and selection of lead compounds.

Successive iterations “build” more potent $K_d$
Illustration of 20 chemical structures and their respective NMR $K_d$ (µM)

_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

Plot of $^{15}$N ppm over $^1$H ppm

- 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

Molecular model

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

Molecular model

Stebbins et al, Cell 89:239, 1997
17-AAG BINDS TO HSP90 & SHARES IMPORTANT BIOLOGIC ACTIVITIES WITH GELDANAMYCIN

Two graphs. One shows erbB2 (% of base line) for 17-AAG GA over dose (nM). The other chart shows Raf-1 (% of base line) for 17-AAG GA over dose (nM).

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Cell cycle regulation by Cdc25 phosphatases

Graphic illustration
Regulation of Cell Cycle Progression by Cdc25: Cdk Activation

Graphic illustration
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-RasG12V 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

Graphic illustration of GST-Cdc25 in assay buffer with fluorescein diphosphate. There is another graphic illustration showing what happens after incubating one hour/RT. It becomes Read product (fluorescein monophosphate) on cytoflour II.
Chemical Screening Approach

Targeted Array Libraries

Diverse Chemical Libraries
Chemical structures showing lead compounds $K_1$, $K_2$, and $K_3$ and 13 analog structures for screening (including pBQ)
**Compound 5 inhibits Cdc25**

Chemical structure

Percent inhibition over log [Compound 5] M for Cdc25A, VHR, and PTP1B

Cdc25B\textsubscript{2} $K_i$ approximately 2 µM
MALDI-TOF ANALYSES
Compound 5 binds tightly to the catalytic domain of Cdc25A

Two graphs, one shows the % intensity of m/z for DMSO.

The other shows % intensity for m/z for Compound 5.

Lixia PU
Compound Validation

Cellular: Cell Cycle

Biochemical: Substrate phosphorylation

Genetic: Chemical complementation
tsFT210 Cell System

Graphic illustration with functional and nonfunctional Cdk1.
Compound 5 causes G2/M arrest

Graphic illustrations
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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 differentiation 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*

Graphic illustration (CEBP, TK, luciferase)

*Host cell for this construct is U-937
CEBPA Assay Timeline

Graphic illustration of assay procedure

*Sister plates processed for Alamar blue toxicity assay
C/EBPa Training Set: 1\textsuperscript{st} Run compared to 2\textsuperscript{nd} Run \% Induction
Correlation Coefficient = .9265

Scatter plot shows \% induction 2\textsuperscript{nd} run compared with \% induction 1\textsuperscript{st} Run.
C/EBα Training Set 1 uM Results*

% Alamar Fluorescence over % of control induction (relative to .625 uM approximately 100%)

*Data averaged from two independent assays
C/EBPa Screen: % Concentration Response Graphs

% induction (relative to .625 uM retinoic acid induction) for seven select compounds.
Categories of Confirmed Actives in CEPBα HTS

β-adrenergic agonists
Toxic compounds (stress signaling)
Retinoids
HDAC Inhibitors
Novel Drug Lead - Sterol mesylate
C/EBPa Frequency of Fold Induction for OSR Compounds in HTS

(Total of 135640 compounds tested)

Histogram showing frequently over fold induction

NSC67657 when screened AT 10μM scored 1.4 fold induction relative to RA control.
C/EBPa % Induction Dose Response Curves

Line chart showing % induction (test-cells only)/(Ret – Cells Only)* 100 by concentration of test compounds

Chemical structures for rimiterol HBr, Epinephrine, and DL-Isoproterenol HC1
NSC 67657, a novel sterol mesylate inducer of CEBPα with potential anti-leukemic activity

Chemical structure of NSC67657

Basis for Interest
Identified in a DTP high-throughput screen of > 140,000 compounds

Induced CEBP-luciferase activity at low concentrations: 50% activation at 40 nM

Induced differentiation in U937 cells as measured by CD11b or CD11c antigens or NBT staining

Induced morphologic differentiation in HL60 cells

Induced cell surface markers of monocytic differentiation in AML patient blasts ex vivo
Secondary testing of NSC67657 in C/EBPa (U937) cells

Scatter charts indicating the following:

- Dose-dependent increase of luciferase reporter activity (max. 1.6 fold)
- Based on control induction of retinoic acid (1µM)
- Activity occurs at non-toxic concentrations
Evidence for Morphologic Differentiation in HL60 Cells

Untreated control - largely myeloblasts

1 mM ATRA – Reduced cell numbers, segmented and cells resembling neutrophils

20 mM NSC 67657 – Reduced cell numbers, segmented and cells resembling neutrophils
GENERATION OF SAR AROUND
STEROID MESYLATE LEAD

Related compounds available from the DTP Repository were tested in concentration-
response format

No compounds with comparable activity were found (most were completely inactive)

Three compounds which showed some activity provided an initial SAR model
SAR data and chemical structures for NSC 67657
138980
260627
622259
Hierarchical cluster of 51 genes dysregulated >3 fold over control by NSC 67657 in HL60 cells.

Heat map of gene activation.

When compared to ATRA treated cells, several genes of the monocyte/macrophage lineage were uniquely up regulated by NSC 67657.
NSC 67657 induces differentiation in different cell lines compared to ATRA

**HL60 cells:**
Can differentiate to either granulocytes or monocyte/macrophages

**NB4 cells:**
Can only differentiate into granulocytes

ATRA induces differentiation (measured by NBT reduction after 7 days) in both HL60 and NB4 cell lines, while NSC 67657 induced differentiation only in HL60 cells. This supports the monocyte/macrophage lineage specific differentiation proposed from the gene expression studies.
NSC 67657 induces a different pattern of cell surface markers compared to ATRA

Four graphs. Two show NB4 cells over CD14 and CD18. The other shows HL60 cells over CD14 and CD 18.

NSC 67657 induced CD14 expression only in HL60, not NB4 cells. ATRA does not induce CD14 expression in either cell line (5 day incubation).
INITIAL STRUCTURE-ACTIVITY MODEL

Chemical structure of steroid ring and substitutions at R_2, R_3, R_6, R_{12} and R_{23}. 
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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.

Graphic illustration

O.D. over time showing the following:
    Control
    “GI\textsubscript{50}” = 50\% inhibit
    “TGI’’ = 100\% inhibit
    “LC\textsubscript{50}” = 50\% kill
Line chart showing all cell lines by percentage growth over Log_{10} of sample concentration (Molar). There is a significant drop at -6 molar of all cell lines that continues until approximately -5 and then in most cases levels off by -4 molar.
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 (resistant or sensitive) as deflection from mean

Calculate Pearson correlation coefficient:
1 = identity; 0 = no correlation
AGENTS WITH SIMILAR MECHANISMS HAVE SIMILAR MEAN GRAPHS

Leukemia

NSCLC

Small Cell Lung

Colon

CNS

Melanoma

Ovarian

Renal

All of the above over Taxol, Halichondrin B, and Daunorubicin

↓  ↓  ↓

Tubulin  Topoisomerase II
# THE COMPARE ALGORITHM

**Seed: Rubidazone**

<table>
<thead>
<tr>
<th>ID</th>
<th>Score</th>
<th>Compound</th>
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</thead>
<tbody>
<tr>
<td>164011</td>
<td>1.000</td>
<td>Rubidazone</td>
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<tr>
<td>82151</td>
<td>0.921</td>
<td>Daunomycin</td>
</tr>
<tr>
<td>123127</td>
<td>0.915</td>
<td>Adriamycin</td>
</tr>
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<td>665934</td>
<td>0.891</td>
<td>Epipodophyllotoxin analogue</td>
</tr>
<tr>
<td>Discreet</td>
<td>0.880</td>
<td>Gyrase-To-TOPO analogue</td>
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<tr>
<td>Discreet</td>
<td>0.867</td>
<td>AMSA analogue</td>
</tr>
<tr>
<td>267469</td>
<td>0.865</td>
<td>Deoxydoxorubicin</td>
</tr>
<tr>
<td>305884</td>
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<td>Acodazole HCL</td>
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<tr>
<td>665935</td>
<td>0.864</td>
<td>Epipodophyllotoxin analogue</td>
</tr>
<tr>
<td>668380</td>
<td>0.861</td>
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<tr>
<td>Discreet</td>
<td>0.833</td>
<td>Gyrase-To-TOPO analogue</td>
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RELATIVE EGF RECEPTOR mRNA EXPRESSION

Relative expression for breast, prostate, renal, ovarian, melanoma, CNS, colon, NSCLC, Leukemia.
### COMPARE ANALYSIS: EGF RECEPTOR

<table>
<thead>
<tr>
<th>RANK</th>
<th>CORRELATION</th>
<th>CHEMICAL NAME</th>
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<tbody>
<tr>
<td>1</td>
<td>0.71</td>
<td>TGFα-PE40</td>
</tr>
<tr>
<td>2</td>
<td>0.66</td>
<td>Toxin-Δ53L, MW=43K</td>
</tr>
<tr>
<td>7</td>
<td>0.57</td>
<td>EGFR Tyrosine Kinase Inhibitor</td>
</tr>
<tr>
<td>88</td>
<td>0.43</td>
<td>EGFR Tyrosine Kinase Inhibitor</td>
</tr>
</tbody>
</table>

*40,421 COMPOUNDS IN THE NCI DATABASE*
Heat map.

Drugs (clustered) over genes (clustered)

5FU/DPYD    L-Asparaginase/ASNS

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*

<table>
<thead>
<tr>
<th>Component</th>
<th>Purpose/Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Form 1571”</td>
<td>Pharmacology/Toxicology</td>
</tr>
<tr>
<td>Table of Contents</td>
<td></td>
</tr>
<tr>
<td>Intro Statement / Plan</td>
<td>Prior human experience</td>
</tr>
<tr>
<td>Investigator Brochure</td>
<td>Additional info – data monitoring, Quality assurance</td>
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<tr>
<td>Clinical Protocol</td>
<td></td>
</tr>
<tr>
<td>Chemistry,</td>
<td></td>
</tr>
<tr>
<td>Manufacture, Control</td>
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</table>
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
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
CORRELATION BETWEEN 20S PROTEASOME INHIBITORY POTENCY & GROWTH INHIBITION FOR 13 DIPEPTIDE BORONIC ACIDS

Chemical structure and plot of Mean FI_{50} (nM) over K_j (nM)

Adams et al, Cancer Res 59:2615, 1999
EFFECT OF PS-341
ON PC-3 TUMOR GROWTH IN MICE

Plot of tumor volume (% vehicle) over Week (1 through 6) when treatment is administered at week 1.

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

Two bar charts. One shows 20S activity (% vehicle) for mouse WBC over PS-341 (mg/kg). The other one shows 20S activity (% vehicle) over PS-341 (mg/kg).

Adams et al, Cancer Res 59:2615, 1999
**PS-341: INTERSPECIES**

**Q: Is the ‘safe’ dose in animals in the efficacy range for man?**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>Dose (mg/m²)</th>
<th>% 20S Proteasome Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>1.0</td>
<td>3.0</td>
<td>80</td>
</tr>
<tr>
<td>Rat</td>
<td>0.25</td>
<td>1.5</td>
<td>80</td>
</tr>
<tr>
<td>NHP</td>
<td>0.067</td>
<td>0.8</td>
<td>70</td>
</tr>
</tbody>
</table>

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

Ex Vivo Proteasome Activity:
1 Hour Post Treatment

Scatter chart showing % 20S activity over PS-341 (Log dose, mg/m²)
ACKNOWLEDGEMENTS

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