DRUG DISCOVERY

Edward A. Sausville, M.D., Ph.D.
Associate Director for Clinical Research
Greenebaum Cancer Center
University of Maryland at Baltimore

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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:
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	**Reasons for slowdown**
Toxicity, 22%	Synthetic complexity
Lack of efficacy, 31%	Low potency
Market reasons, 6%	Ambiguous toxicity finding
Poor biopharmaceutical properties, 41%	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*

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*

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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

Diagram of this process

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)
* 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

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/

GELDANAMYCIN: EXAMPLE OF BINDING PARTNER DEFINING TARGET

Chemical structure of benzoquinone (ansa ring and carbamate moieties)

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

BENZOQUINOID ANSAMYCINS INITIAL CELL PHARMACOLOGY –

"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)

Graphic illustration of a bead and an 18 atom spacer

GELDANAMYCIN BEADS
IDENTIFY HSP90 AS BINDING PARTNER

1) Bead-Geld

2) Bead-Geld + Geld

3) Bead-Geld + Geldampicin

4) Bead

Neckers et al, PNAS 91:8324, 1994

Three graphic illustrations of the role of HSP 90 in cell function.

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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* = 4%
S*/NM = 10%
V = 3%

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 antidemintia 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 *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 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

	Natural Product Extracts	Synthetic Combinatorial Mixtures
Direct screening of compound mixture	Yes	Yes
Discovery of highly active compounds	Yes	Yes
Equal concentrations of compounds	**No**	Yes
Chemical structure 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”

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

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COMBINATORIAL LIBRARIES OF BICYCLIC GUANIDINES FROM REDUCED ACYLATED DIPEPTIDES

Chemical structure and synthesis

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)

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

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- **Definition of Drug Targets**
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- ***Definition of Lead Structures***
- **Qualifying Lead for Transition to Early Trials**

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

- 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

Chemical structures of Benzene malononitrile, Bisubstrate quinoline, S-Arylbenzene malononitrile and Lavendustin-based

Levitsky, FASEB J 6: 3275, 1992

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

Chemical structures of

Erbstatin

NSC 606641

and

AG957

NSC 654705

EXAMPLE OF “RATIONAL” APPROACH: bcr-abl directed agents

Natural product empiric lead

erbstatin lavendustin piceatannol

1st generation Synthetic

AG058 AG1112

2nd generation synthetic; in clinic

CGP 57148B = STI571

STI571: An oral in vivo bcr-abl kinase inhibitor

Plot showing Tyr phosphorylation *in vivo* .

Another plot showing 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.

Plot of white cell count and duration of Treatment with STI571

Plot of Ph Chromosome + Cells Duration of Treatment with STI571

NEJM 344: 1031, 2001

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

Plot showing major cytogenetic response (%) over months after randomization for Imatinib

and Combination therapy.

Druker et al, NEJM 348: 994, 2003

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

Chart showing Time to Relapse in Patients
with Myeloid or Lymphoid Blast Crisis
Who Had a Response to STI571

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

Chemical structure

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

Flow chart

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

Graphic illustration

Method for identifying Cdc25 phosphatase inhibitors

Graphic illustration

Chemical Screening Approach

- Targeted Array Libraries
- Diverse Chemical Libraries

Chemical structures

Compound 5 inhibits Cdc25

$Cdc25B_2$ K_i ~ 2 μM Log [Compound 5] M

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

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 BINDING TO DNA

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

AFFINITIES OF SELECTED BIARYL COMPOUNDS FOR BCL-XL

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

2 mM biaryl acid **1**

2 mM biaryl acid **1** and 5 mM naphthol derivative **11**

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

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

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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
- DEFINE IN ANIMALS A SAFE STARTING DOSE FOR HUMAN CLINICAL TRIALS
- THIS INFORMATION ASSEMBLED INTO AN "INVESTIGATIONAL NEW DRUG" ("IND") APPLICATION TO THE FDA

CORRELATION OF *IN VIVO* ACTIVITY WITH CLINICAL ACTIVITY BY DISEASE TYPE

Xenograft histology

% *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)

Chemical structure

NSC	R_1	R2
624548	NO_2	Cl
639828	NH_2	Cl
639829	$N(CH_3)_2$	CH_3
647884	NH_2	CH_3
654259	$NCOCH_2NH_2$ x HCl	CH_3
654261	$NCOCH_2N(CH_3)_2$ x HCl	CH_3

National Cancer Institute Developmental Therapeutics Program

Dose Response Curves

Figure 4

Efficacy Testing of NSC 639829 in Human Tumor Xenografts

Figure 13

BPU Analogue Concentration (μM or μM equivalents) over time after dose (min) for Dimethyl-BPU, Monomethyl-BPU, and Amino-BPU.

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FDA PRECLINICAL PHARMACOLOGY & TOXICOLOGY REQUIREMENTS

Graphic illustrations of a mouse a rat, a dog, and a monkey.

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

Flow chart

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

Chart

Adams et al, Cancer Res 59:2615, 1999

Effect of PS-341 on 20S Proteasome Activity

Two bar charts, one of Mouse WBC and the other of PC-3

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