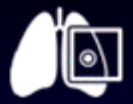
National Cancer Institute

DCTD Division of Cancer Treatment and Diagnosis


∫ CAM

Accelerating Cancer Diagnosis and Drug Development

NIEHS Scientific Advisory Committee for Alternative Methods Meeting

NCI Research, Development, Translation and Validation

Joseph E. Tomaszewski, PhD
Deputy Director, DCTD, NCI
Chief, TPB, DTP, DCTD, NCI
June 18, 2008

Outline of Presentation

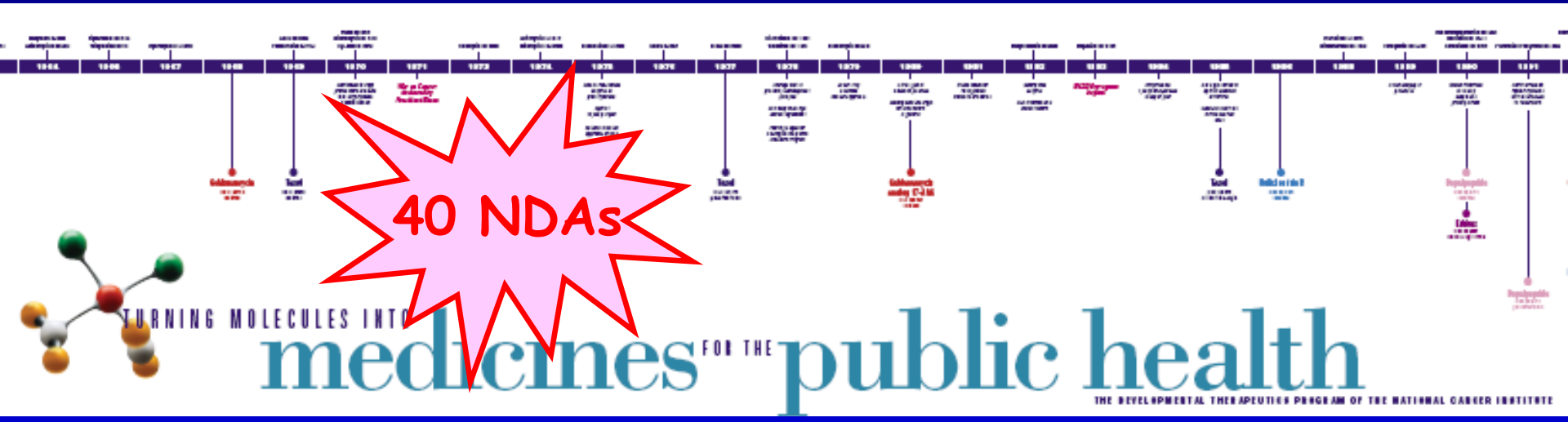
- Why is toxicity testing important?
- Why is the prediction of human toxicity and sensitivity important?
- Is animal data sufficient to enter the clinic safely?
- Can *in vitro* toxicity data increase the safety margin in the clinic?
- *In vitro* assays under development.
- Future in silico and HTS evaluations

Question:

Why is toxicity testing important to the NCI?

From Bench to Bedside: The Concept

1955 to 2005: 50TH Anniversary of the creation of the Cancer Chemotherapy National Service Center (CCNSC)… The predecessor of DTP



Taxol: 1969-1992 **Erbitux:** 1990-2004 **Velcade:** 1995-2003

http://dtp.nci.nih.gov/

DTP Organizational Structure

Discovery
Acquisition and
In vitro testing of
New compounds;
Natural Products,
Grants; Web Site,
Data Management
And Storage

To

Development
Biologics; *In vivo* models;
Formulation,
Bulk Synthesis, Stability
Testing, Pharmacology and
Range-Finding Tox, IND-
Directed Toxicology

Drug Synthesis & Chemistry
Ven Narayanan

Natural Products
(Dave Newman)

Screening Technologies
Bob Shoemaker

Information Technology
Dan Zaharevitz

Biological Testing
Melinda Hollingshead

Grants & Contracts Operations
Mary Wolpert

Biological Resources
Steve Creekmore

Pharmaceutical Resources
Rao Vishnuvajjala

Toxicology & Pharmacology
Joseph Tomaszewsk

DTP - Chronological Change In Responsibilities

Cancer (1°Sm Molec) Drugs	1955 - Present
AIDS Drugs	1986 - 1997
Cancer Biologicals	1992 - Present
Cancer Vaccines	1993 - Present
Cancer Gene Therapy	1995 - Present
Imaging Agents	1996 - Present
AIDS-Related Agents	2000 - 2004
Other Therapeutics	2002 - Present

Drug Development Programs Supported By DTP

- DCTD DDG (Former DNC) – NCI IND
- DTP NCDDGs
- NCI CCR (DCS / DBS)
- NIH Clinical Center PET Dept
- NCI RAID – Investigator IND
- NCI / NIAID AIDS IIP (Until 2004)
- DCTD DCIDE
- DCTD R*A*N*D
- Other NIH I/Cs (*e.g.*, NIMH, NINDS)
- 2004 – NIDDK T1D (Type 1 Diabetes) RAID
- 2005 – NIH RAID (All Therapeutics)
- 2005 – DCTD-CCR JDC Phase 0 (NIH CC)
- 2007 – (NCI Chemical Biology Consortium)

Drug Development Supported by the Division of Cancer Prevention

- Supports preclinical chemopreventive drug development for the NCI
  - ← Biomarkers
  - ← Pharmacokinetics
  - ← Pharmacodynamics
  - ← Toxicology (Rodent and Non-rodent)
- Rapid Access to Prevention Intervention Development (RAPID) Program

http://prevention.cancer.gov/programs-resources/groups/cad/programs

NCI Nanotechnology Characterization Laboratory (NCL)

- Physicochemical characterization
- *In Vitro*
  - Sterility
  - Targeting
  - Immunology
  - Toxicity
  - Metabolic Stability
- *In Vivo*
  - Disposition
  - Immunotoxicity
  - Dose-Range Finding Toxicity
  - Efficacy
  - GLP Studies

http://ncl.cancer.gov/index.asp

Question:

Why is the prediction of human toxicity and sensitivity in drug development important?

Cancer Drugs and Toxicity

- Cancer drugs are some of the most toxic compounds that we purposely administered to man, terminal cancer patients in Phase 1.
- Phase 1 conducted in terminal cancer patients

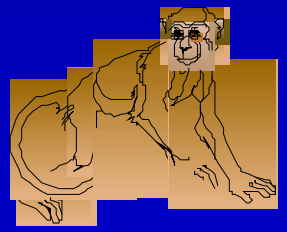
- <u>Needs for the clinic</u>:
  - ← Predict a safe Starting Dose (SD)
  - ← Predict Maximum Tolerated Doses (MTDs)
  - ← Predict Dose Limiting Toxicities (DLTs)

Preclinical Pharmacology and Toxicology

- **Small Molecules**
  - ← Two Species - Rodent & Non-rodent
  - ← Clinical Route & Schedule
    - ∪ Follow NCI Guidelines
  - ← Pharmacokinetics/PD – Optional
  - ← Identity, stability, >98% purity



- **Biologicals**
  - Most Relevant Species
  - Clinical Route & Schedule
  - Biodistribution




Study Designs are Agent-Directed, Not Simply Designed to Check a Regulatory Box.

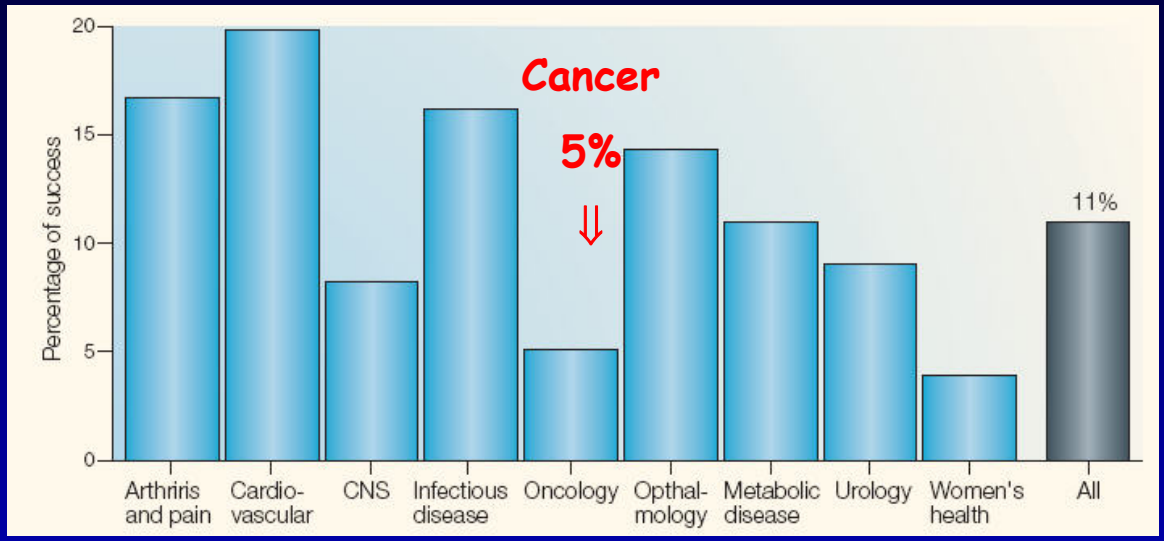
Cost of Drug Development Failures

- "The main causes of failure in the clinic include safety problems and lack of effectiveness: inability to predict these failures before human testing or early in clinical trials dramatically escalates costs. For example, for a pharmaceutical, a 10-percent improvement in predicting failures before clinical trials could save $100 million in development costs per drug."

- (Source: Boston Consulting Group as referenced in Challenge and Opportunity on the Critical Path to New Medical Products http://www.fda.gov).

Can The Pharmaceutical Industry Reduce Attrition Rates?

Kola and Landis, Nature Reviews Drug Discovery, 3: 711-715, 2004.



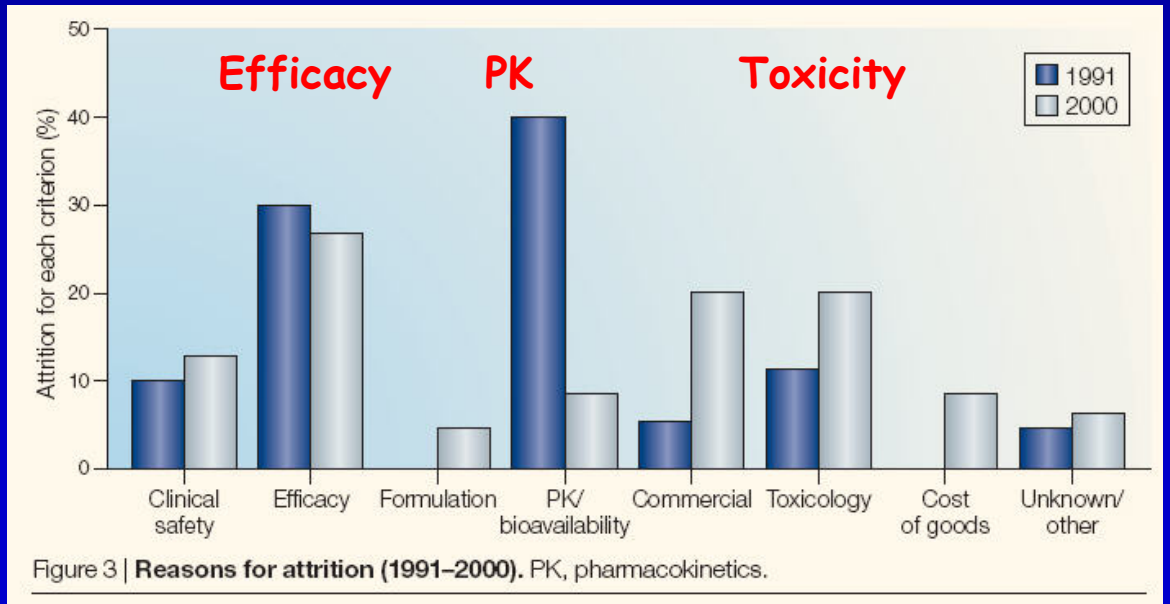
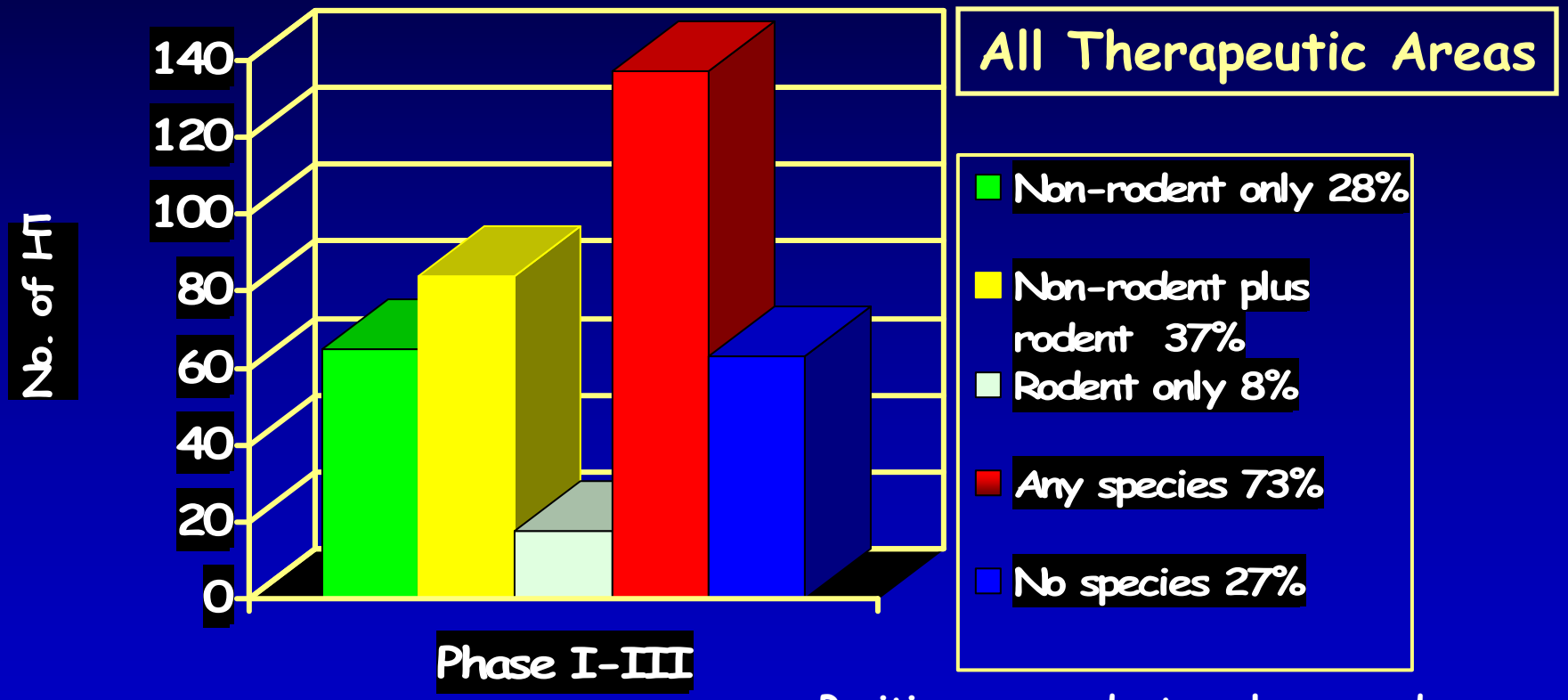


Figure 3 | **Reasons for attrition (1991–2000).** PK, pharmacokinetics.

Question:

First, is animal data sufficient to enter the clinic safely and will it predict human toxicity???

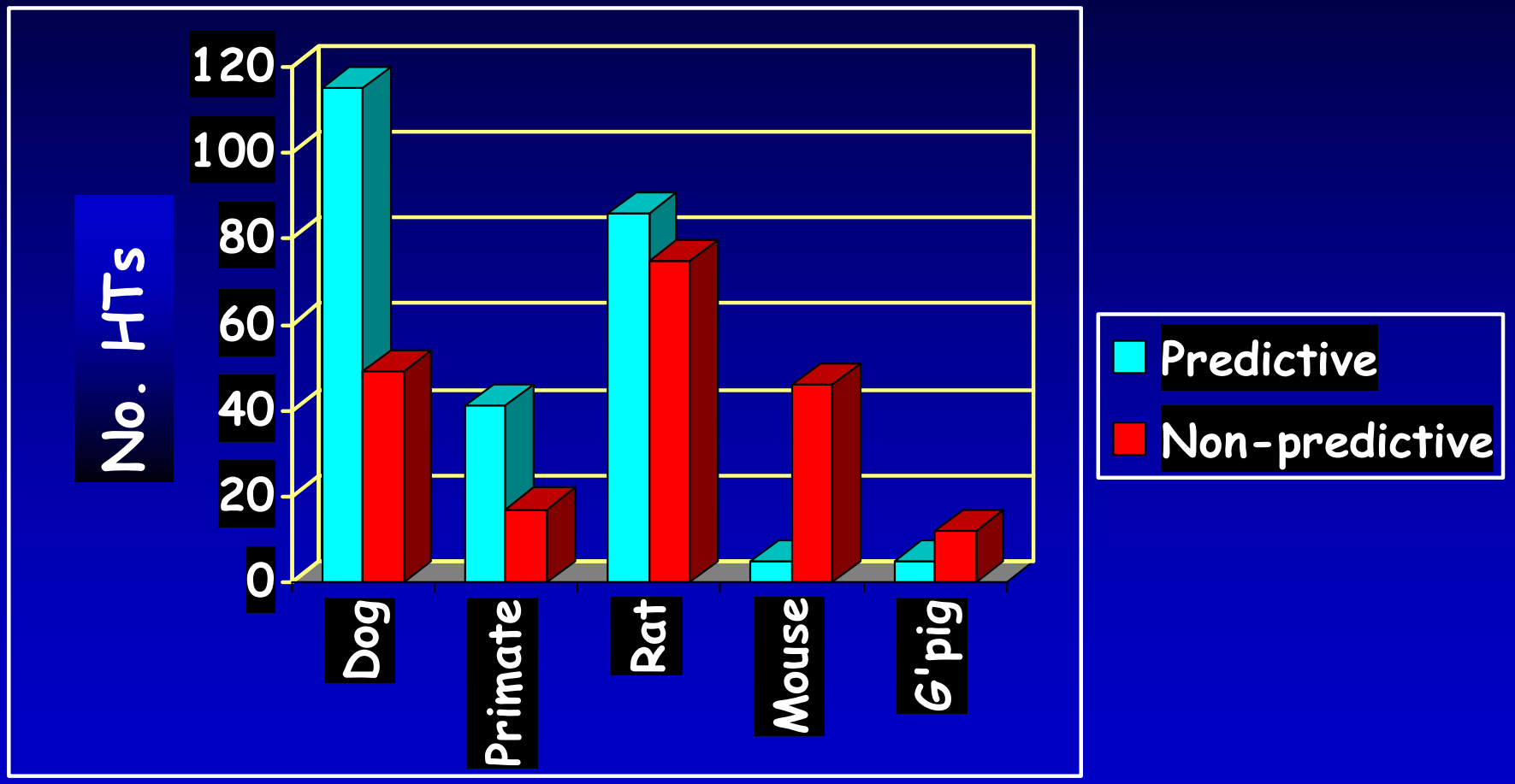
Preclinical Species In Which Human Toxicity Predicted (n = 230)



Positive non-rodents only were dog (53), primate (12) or both (2). Rodents only were rat (13), mouse (3), guinea pig (4) and rabbit (1).

Ref: Olson H, *et al*, *Reg. Tox. Pharm.* 32:56-67, 2000 (ILSI/HESI).

Human Toxicity Prediction / Non-prediction By Species



Ref: Olson H, *et al*, *Reg. Tox. Pharm.* 32:56-67, 2000 (ILSI/HESI).

Smith A and Tomaszewski JE, 2002. Preclinical and Clinical Toxicity Correlations for Cancer Drugs Developed by the NCI. [Abstract No. 19]. *Eur J Cancer*, 38: S12.

- New oncology compounds with preclinical toxicity data and a completed Phase I clinical trial (from 1983 – 2002).

- Route/Schedule matched.
- Basis of Starting Dose (SD) determined.
- MTD and DLTs from preclinical and clinical studies compiled & evaluated.

SD – Margin of Safety
[Basis: 1/10 Rodent; 1/6 NR]

Margin of Safety (Clinical MTD/SD)	37 Drugs / 47 Schedules					
	Ms #	Ms %	Rat #	Rat %	Dog #	Dog %
Unsafe (< 1)	4	13.8	2	6.5	4	8.7
Low (< 2)	1	3.4	6	19.4	4	8.7
Ideal (2-10)	13	44.8	9	29.0	20	43.5
<Ideal (10-20)	5	17.2	5	16.1	12	26.1
Too Large (>20)	6	20.1	9	29.0	6	13.0

SD – Margin of Safety [2]
[Basis: Most Sens Sp + Other Info]

Margin of Safety (Clinical MTD/SD)	Drugs - 37		Schedules - 47	
	#	%	#	%
Unsafe (< 1)	0.5	1	1	2
Low (< 2)	2	5	2	4
Ideal (2-10)	17	46	21	45
<Ideal (10-20)	7	19	9	19
Too Large (>20)	10.5	28	14	30

Conclusions - SD

- High degree of safety of starting dose (98-99%) for new oncology agents using toxicity data from rodents and non-rodents.
- Human safety is increased with non-rodent data.

MTD – Species Predictions

	Mouse	Rat	Dog
Total Possible †	32	32	45
Within 4X	23 (71.9%)	20 (62.5%)	37 (82.2%)
Best	10 (31.3%)	6 (18.8%)	24 (53.3%)

† Based on Number of Studies Conducted

Conclusions - MTD

- Dog > Mouse > Rat at estimating clinical MTD.
- Across all drugs/schedules the median of clinical MTD/preclinical MTD was 1.0 for either species.
- Wide variability.

DLT – Species Predictions

70 Human DLTs

	Mouse	Rat	Dog
Total Possible †	34	51	70
Total Predicted	9	28	40
% Correct Prediction	26.5	54.9	57.1

† Based on Number of Studies Conducted

Conclusions

- DLTs are well predicted in relation to bone marrow and GI toxicity, other toxicities aren't predicted as well.

…and that's a serious problem!!!

Question:

Can *in vitro* toxicity data increase the safety margin in the clinic?

Bone Marrow Assay Conditions

Parameter / Species	Murine	Canine	Rat	Human
Strain	CD2F1 (Male)	Beagle	F344 (Male)	Hip Patient
Source	Femur	Femur Aspirate	Femur	Iliac Crest Aspirate
Conditions	IMDM 20% FBS r-Mu-GM_{CSF}	IMDM 20% FBS r-Ca-GM_{CSF}	IMDM 20% FBS R-Ra-GM_{CSF}	IMDM 20% FBS r-Hu-GM_{CSF}
No. of Cells	$1.0x10^5$ /mL	$5.0x10^5$ /mL	$1.0x10^5$ /mL	$1.0x10^5$ /mL
Incubation Time	7 Days	12-14 Days	9-11 Days	14 Days

In Vitro Bone Marrow Assay Endpoint

- IC_{50} *versus* IC_{90}
- Traditional Cytotoxic Oncology Phase I Clinical Endpoint = MTD
  (If no PD Marker available)
- Allows for Determination of DLT
- Grade 1 Myelosuppression $\cong IC_{35}$
- MTD Correlates with IC_{90}

Quantitative Analysis of NCI + ECVAM Bone Marrow Assay Results – 51 Drugs *

- Mouse Data Alone Accurately Predicted Human MTD for 40/51 Drugs (78%)

  In Vivo, 33/48 or 69%

- Mouse + Dog (NCI Only) Data Accurately Predicted Human MTD for 45/51 Drugs (88%)

  In Vivo, 42/48 or 88%

* Also includes 4 Drugs from WSU

Current Use of Bone Marrow Assay at the NCI

- Prospective Studies During Discovery / Early Development
- Use Limited Murine *vs.* Human Protocol
- Used to Select Development Candidate
- Mouse and Human CFU-GM assay using Ms marrow and Hu cord blood has been validated by ECVAM (http://ecvam-dbalm.jrc.ec.europa.eu/public_view_doc.cfm?id=6E7E72104B2DEFD6BE979B3B139176C67180BB0BC12CB10496CDA74B54630A05A3291B895581F634).
- Rat marrow assay under evaluation.

Topotecan vs. Indenoisoquinolines Human vs. Mouse Bone Marrow

Drug	Mouse IC90 (nM) $\mu \pm SD$ (range)	Human IC90 (nM) $\mu \pm SD$ (range)	Ratio Mouse/Human
Topotecan HCl (Hycamtin)	120 ± 50 (64 - 160)	5.9 ± 5.1 (1.7 - 15)	20.3
Topotecan	Mouse MTD 70	Human MTD Pred=6.3 Act=7.5	Predicted=11.1 Actual=9.3
NSC 724998	29 ± 12 (18 - 41)	27 ± 14 (7.1 - 45)	1.07
NSC 706744	47 ± 6 (47 - 48)	8.1 ± 2.9 (4.4 - 11)	5.8
NSC 725776	26 ± 3 (23 - 30)	6.6 ± 2 (2 - 10)	3.9

ECVAM - Prediction of Human Maximum Tolerated Dose (MTD)

Drug	IC90 Ratio (Hu/Mu)	Actual Mu LD10 (1)	Predicted Hu MTD (1)	Actual Hu MTD (1)	Successful Prediction?
Adriamycin	0.926	11.1 13.5	10.2 12.5	22.5 5	Yes Yes
Bleomycin	0.428	27.9	11.9	15	Yes
Etoposide	0.912	23.1	21.1	54	Yes
Fludarabine	0.034	1008.9	34.3	25	Yes
5-Fluorouracil	5.98	66 96	394 574	740 1295	Yes Yes
Myleran	0.21	90	18.9	24.2	Yes
Taxol	1.19	69.6	82.8	40	Yes
Teniposide	1.6	15.9	25.4	80	Yes
Thioguanine	2.86	27.3 156	78 446	35 1000	Yes Yes
Thorazine	1.03	158	162	79.3	Yes

(1) Dose expressed as $mg/m^2/dose$.

(2) Ref: Pessina, *Tox Sci*, 75: 355-367 (2003).

Additional *In Vitro* Assays in Development/Validation

- Human and animal Liver slices
- Human and animal Lung slices
- Others (heart, kidney, GI, *etc*) will be developed as time and resources permit

Evaluation of Pulmonary Toxicity Using Rat and Human Lung Slices

1. Isolate human/dog/rat organ cores

For Rat: Aseptically remove lung, inflate airways with PBS containing 0.8% agarose

For Human: inflate airways with PBS containing 1.5% agarose through the primary bronchi.

Cool to ice-cold temperature for agarose gelling, dissociate lobes and core (8mm diameter).

2. Slice cores with Krumdieck slicer

In thermostatically controlled cold V-7 for lung, using or 500 (lung) micron depth

3. Mount slices in vials

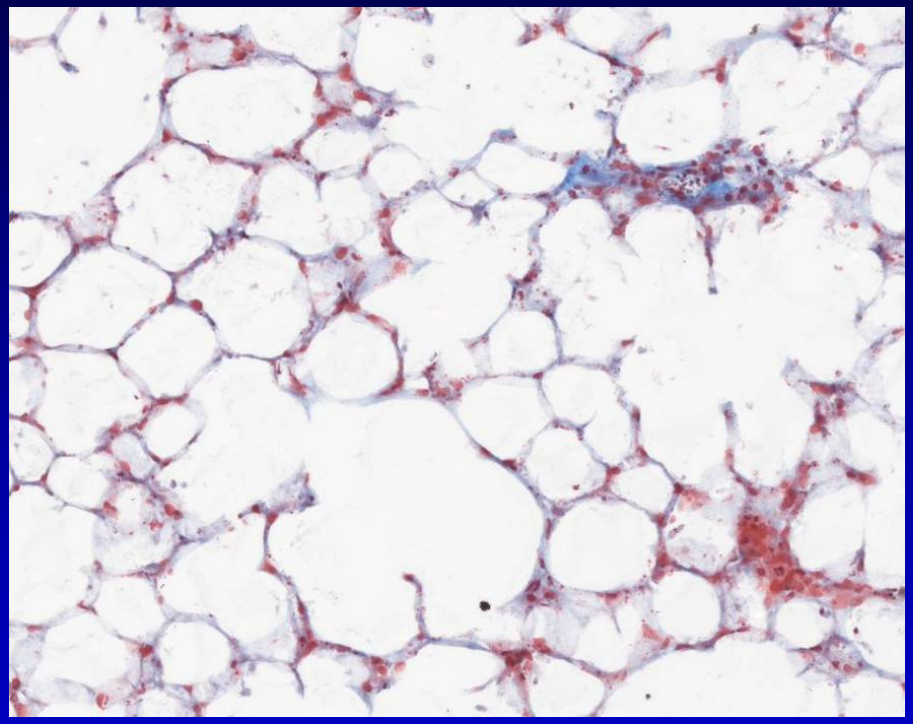
Slices mounted onto 0.45 µM HATF paper (surfactant-free) within titanium roller inserts and placed in vials

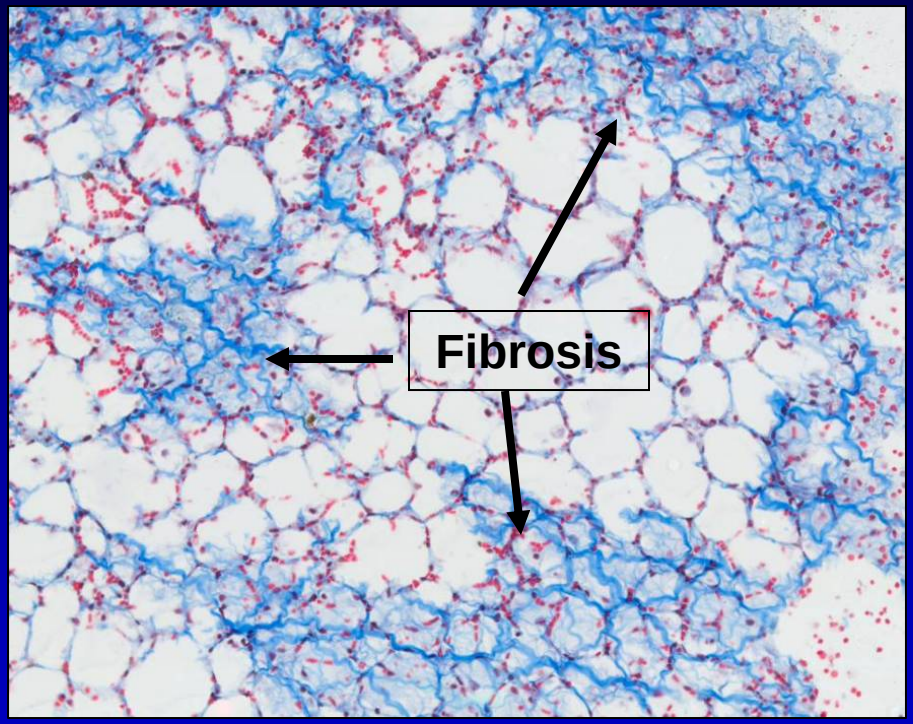
4. Rotation in roller drum

5% CO2/95% air (lung)

7 rpm rotation speed, 37° in humidified incubator

BCNU-Induced Changes In Peripheral Lung (Sprague Dawley Rat)





Control D28

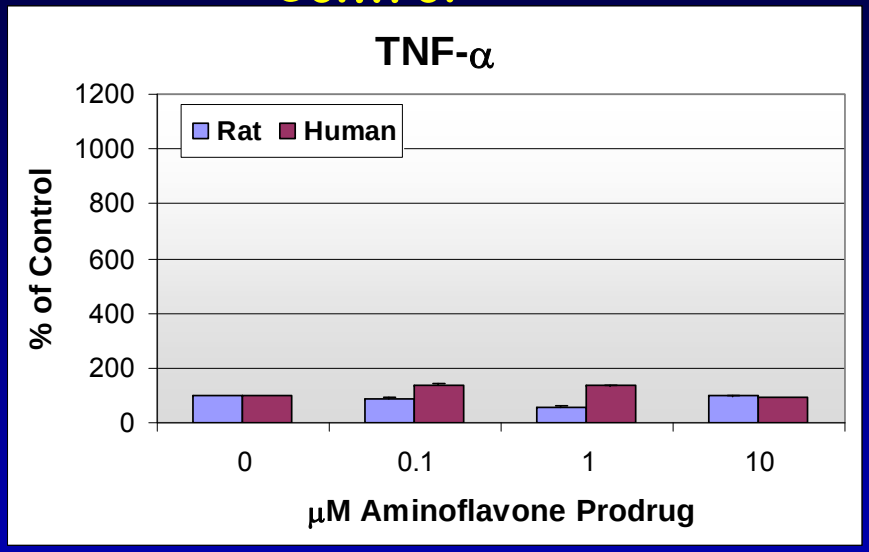
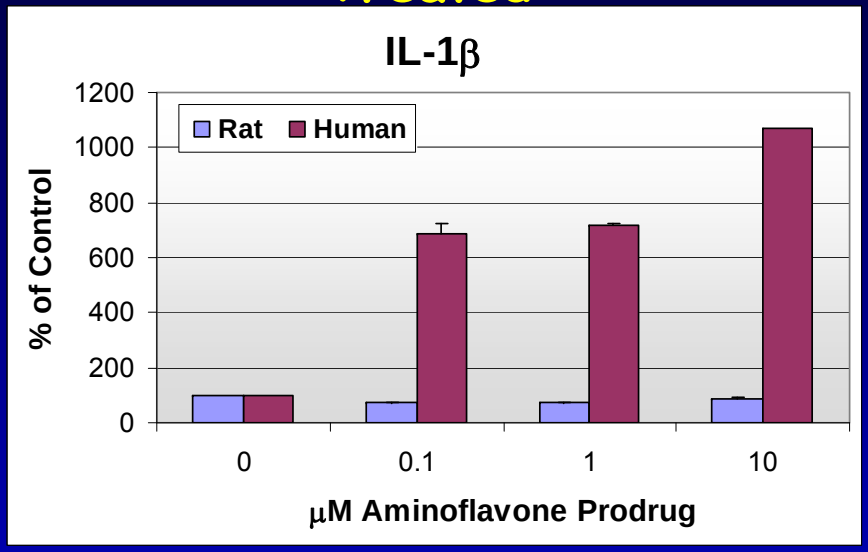
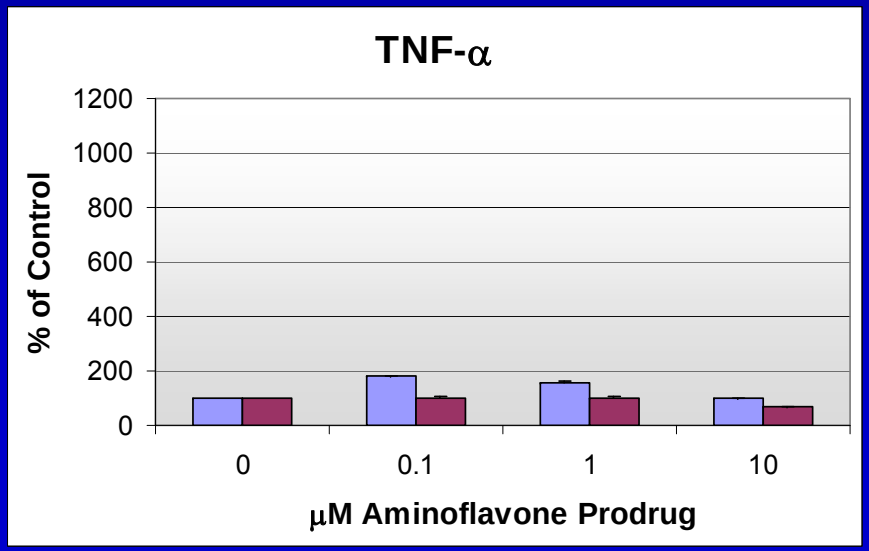
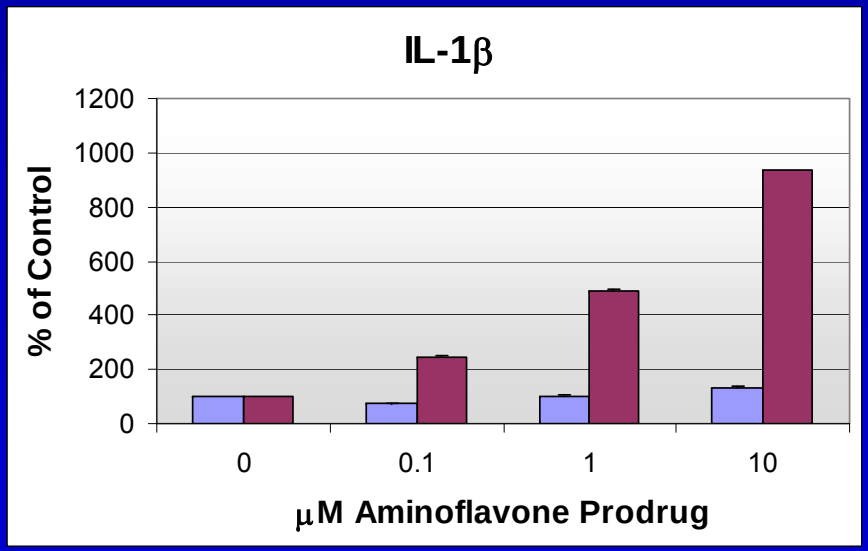
100 μM BCNU D28

Masson's Trichrome Stain

Increased collagen staining with BCNU

Aminoflavone Prodrug: Cytokine Response

7D Exposure, 10 μM Aminoflavone Prodrug

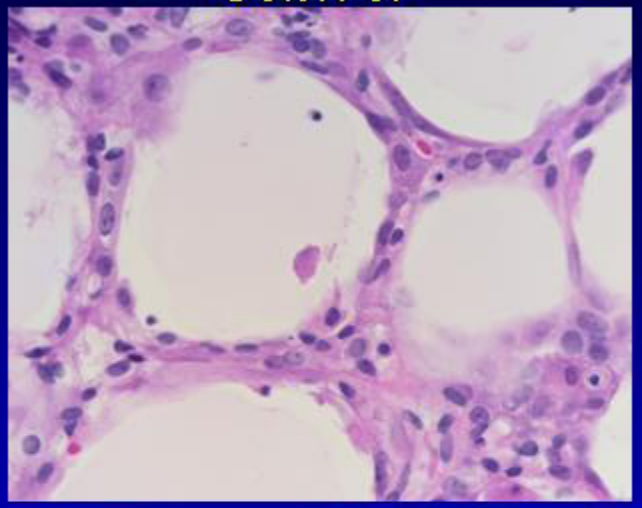
	Control	Treated
Day 1		
Day 3		

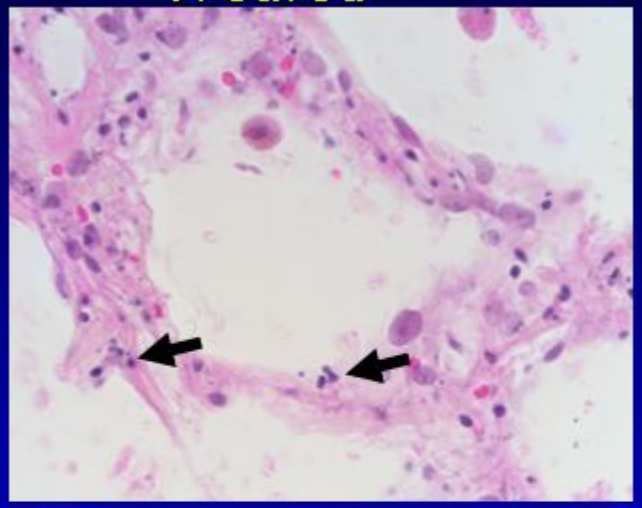
Aminoflavone Prodrug: Histology & Viability

7D Exposure, 10 μM Aminoflavone Prodrug

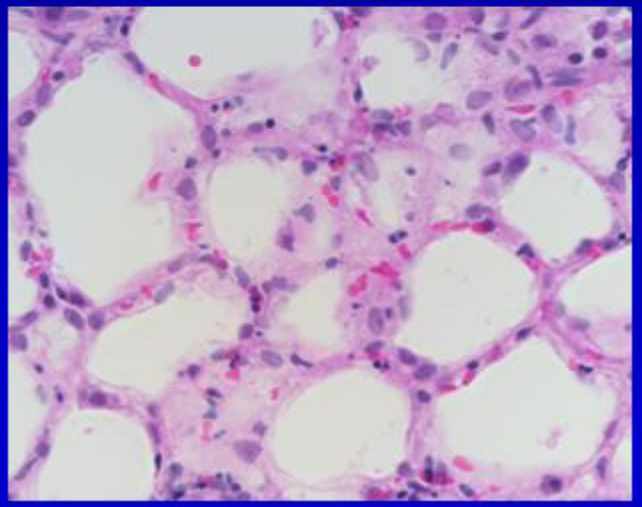
Control | Treated

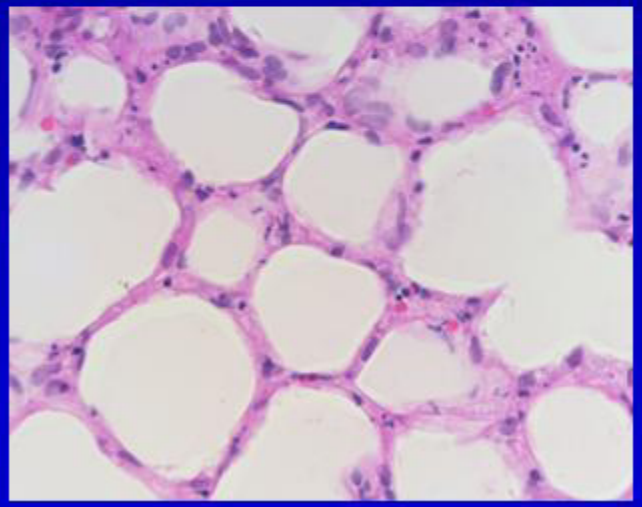
Human



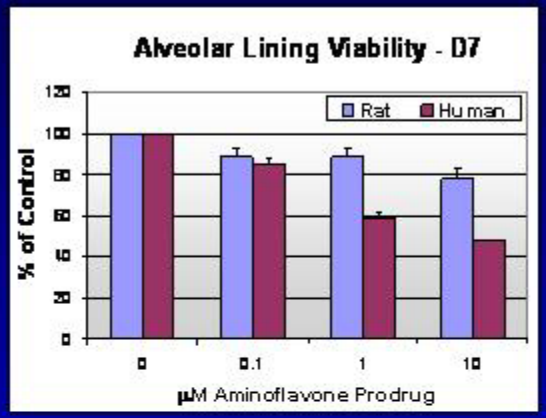


Rat





H&E, Magnification: 630x



- injury to the lining pneumocytes & possibly the endothelial cell

- necrotic cells (arrows) with nuclear fragments.

Control slice shows alveoli with lining cells that are mostly viable.

Gel & 17-AAG Toxicity (Beagle Dog) *Medium (Day 5)*

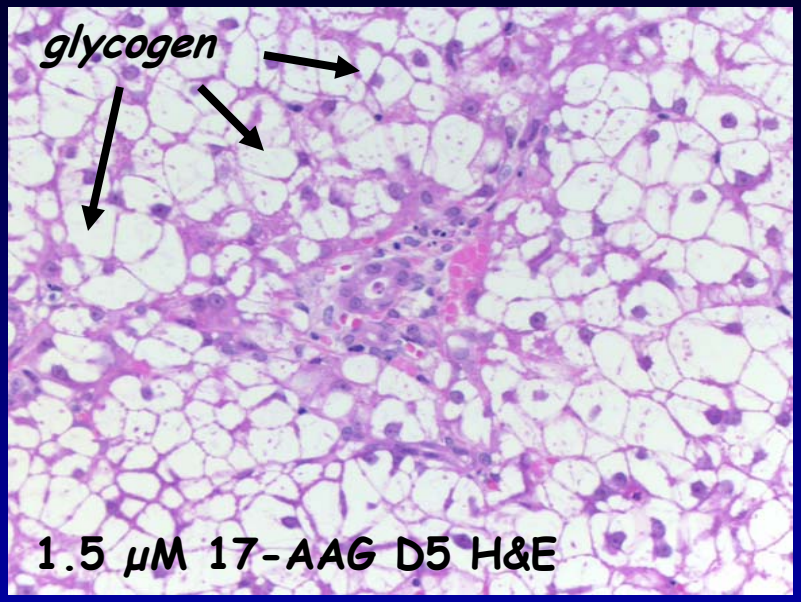
Concentration-dependent Changes in Medium: Comparison of GEL and 17-AAG (n=8)

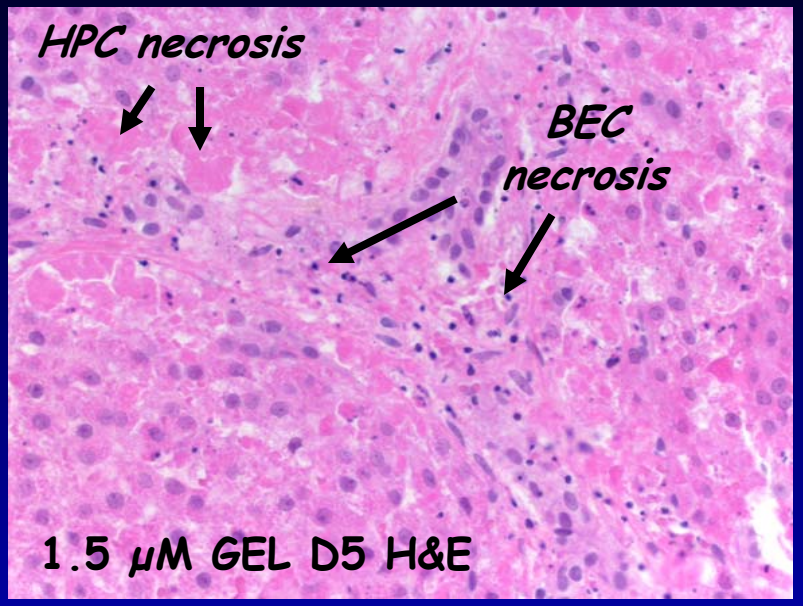
Treatment	Biochemistry (% Control±SD of ratio)				Histology (% Control±SD ratio)			
	Clinical Biomarkers				Viability		Score	Fold
	AST	ALT	ALP	LDH	HPC	BEC	Glyc.	BrdU
0.1 µM GEL	77 ± 15	85 ± 14	126 ± 19	89 ± 15	96 ±2	94 ± 2	88 ± 5	1.0 x
0.1 µM 17-AAG	88 ± 16	96 ± 15	104 ± 17	77 ± 15	101 ± 2	97 ± 1	88 ± 7	1.4 x
0.5 µM GEL	198 ± 48	176 ± 36	61 ± 16	185 ± 42	81 ± 4	83 ± 2	75 ± 7	0.7 x
0.5 µM 17-AAG	83 ± 15	86 ± 13	124 ± 14	88 ± 14	96 ± 3	95 ± 1	88 ± 5	1.3 x
1.5 µM GEL	368 ± 62	330 ± 51	47 ± 8	301 ± 42	45 ±11	55 ± 3	19 ± 8	0.1 x
1.5 µM 17-AAG	85 ± 15	90 ± 13	136 ± 26	101 ± 15	94 ± 3	91 ± 2	84 ± 7	0.7 x
5 µM GEL	365 ± 52	341 ± 40	40 ± 9	292 ± 39	34 ± 9	41 ± 3	16 ± 7	0.0 x
5 µM 17-AAG	234 ± 43	214 ± 34	66 ± 16	234 ± 43	76 ± 5	67 ± 6	66 ± 9	0.5 x

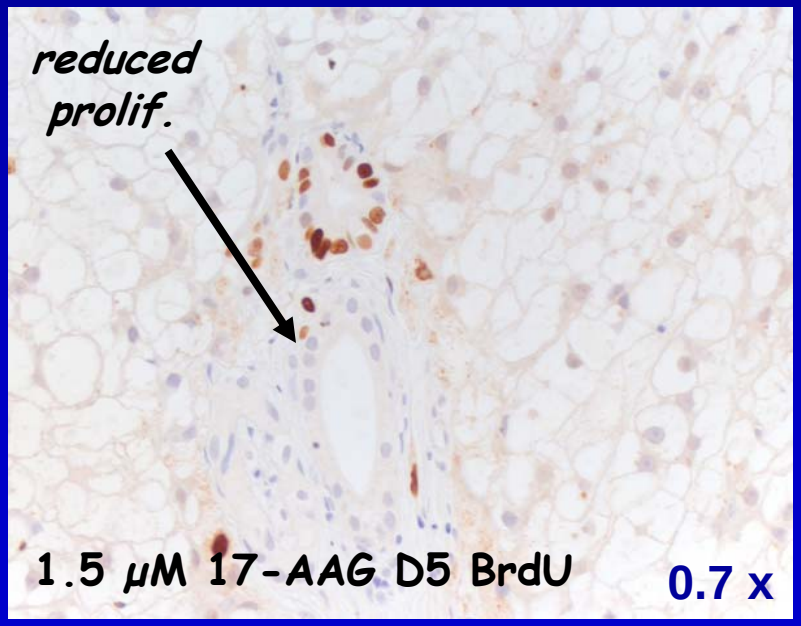
compound differences noted - all biomarkers

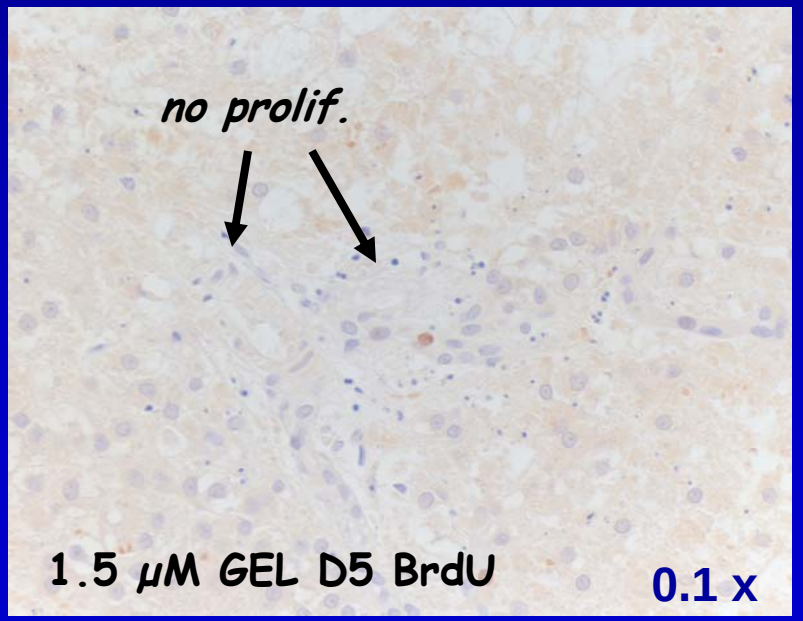
Increasing compound-dependent biomarkers in medium – parallels increasing toxicity

Gel versus 17-AAG Toxicity (Dog)









Summary: Gel and 17-AAG Toxicity

- Compound induced toxicities were time- and concentration-dependent
- GEL is *more toxic in both rat and dog* liver slices (biochem & histology)
- Concentrations of GEL that caused overt hepatobiliary necrosis caused BEC proliferation (hyperplasia) and minimal hepatocellular necrosis when 17-AAG was applied
- Differences in toxicity are similar to *in vivo* studies using dog and rat

(Page, J. et al. (1997) Comparison of geldanamycin (NSC-122750) and 17-allylaminogeldanamycin (NSC-330507D) toxicity in rats. Proc Am Assn Cancer Res 38:308.)

(Sausville et al. (2003) Clinical development of 17-allylamino, 17-demethoxygeldanamycin. Curr. Cancer Drug Targets. Oct 3(5):377-83. Review)

Future In Silico and HTS Evaluations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Optimization
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Version 2.3 ADMET Predictor™ is an advanced computer program that enables pharmaceutical researchers to estimate ADME properties (such as permeability, solubility, lipophilicity, diffusivity, etc.) of new chemical entities (NCE's) from their molecular structure.
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ADMET Modeler
ADMET Modeler™ has now been integrated into ADMET Predictor 2.0. This is a powerful software program that reduces the time needed to create high quality predictive artificial neural network and support vector machine models from several months to a few days by combining the most efficient machine-learning methods currently available into a single program. It eliminates the drudgery associated with the various steps in the model building process, and gives modelers the ability to investigate numerous architectures in much less time than would be required using existing tools.
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Version 2.2 DDDPlus™ (Dose Disintegration and Dissolution) is a new tool for formulation scientists that simulates the in vitro disintegration and dissolution of dosage forms in USP Paddle, Basket, and Flow-Through experiments.


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CeeTox, Inc. provides a unique and robust in vitro toxicity screening approach designed to improve the drug discovery process. We have developed a proprietary algorithm that incorporates multiple- endpoint analysis, dose-response profiles, Pgp interaction, solubility, metabolic stability, and in vivo validation to provide an estimate of the sustained blood concentration in a rat 14-day repeat dose study where toxicity would first be expected to occur. We can improve the efficiency of early discovery research by providing data that allows teams to optimize safety and drug attributes simultaneously. The result is a reduced development time, a higher probability of success in preclinical animal testing and safer drugs. We provide a high throughput screening that rank orders large numbers of "hits" based on toxicity. We combine the results of 9 different biochemical assays to predict the point at which toxicity will first occur in vivo and give detailed information on sub-cellular sites of toxicity, all designed to expedite the lead optimization process. We provide in vitro assays that evaluate CYP enzyme induction, metabolic activation and CYP inhibition to evaluate the drug-drug interaction potential of a compound. We also evaluate cardiotoxicity, lipidosis and endocrine interactions and we assess anti-tumor drug candidates.


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Quantum Pharmaceuticals is a drug design and preclinical contract research organization (CRO). We offer hit identification services, hit-to-lead programs, lead optimization services, as well as antibody optimization and humanization services. Our clients are biotech companies and scientific organizations around the world.
We focus on employing our breakthrough computational technologies and providing drug discovery services. We use our partner companies to produce chemical synthesis, in-vitro and vivo tests, and other non-computational activities.
Quantum Pharmaceuticals develops industry leading computational drug design technologies based on applying quantum, molecular and statistical physics in molecular modeling. Their applying in drug design is proved to be reliable, and provides substantial time and cost savings.
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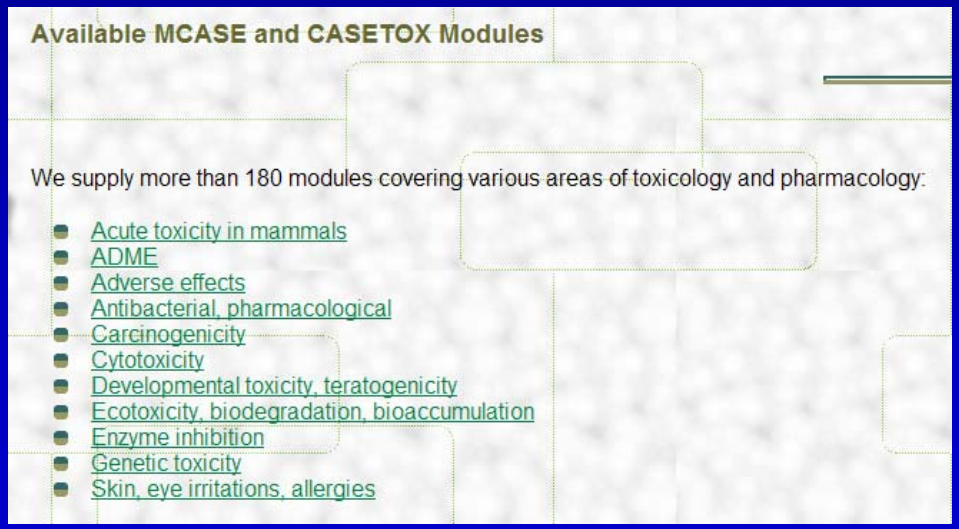
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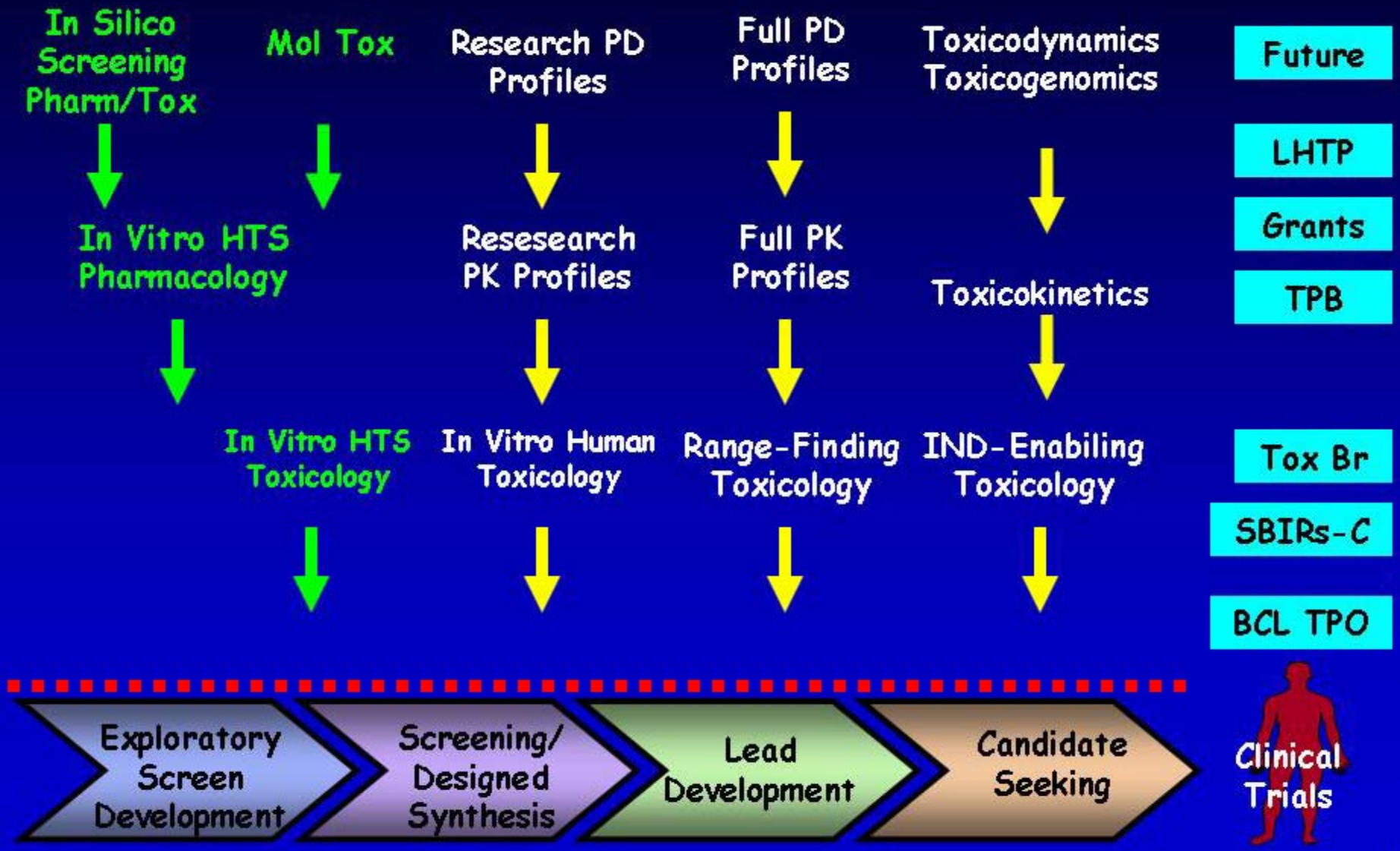
Evolution of TPB in Drug Discovery and Development



In Vitro and *In Silico* Assays *versus* Animal Studies

- To replace animal useage, assays must predict human:
  - ←Metabolism
  - ←Pharmacokinetics
  - ←Pharmacodynamics
  - ←Sensitivity
  - ←Safe starting dose for Phase 0/1
  - ←Maximum tolerated doses
  - ←Dose limiting toxicities

Concluding Thoughts

- In order to validate in vitro or in silico assays, both animal and human data is required.
- Cancer drugs are ideal for this purpose since there is a wealth of both animal and human data available.

Questions,

Comments,

Discussion!

Thank you!

Contact Information

- Phone No: (301) 496-6711 (OD)
  (301) 496-8777 (TPB)
- Fax No: (301) 496-0826 (OD)
  (301) 480-4836 (TPB)
- E-mail: tomaszej@mail.nih.gov
  tpb@dtpax2.ncifcrf.gov

- Web Address:
  http://dtp.nci.nih.gov/branches/tpb/index.html
  http://www.cancer.gov/dctd/