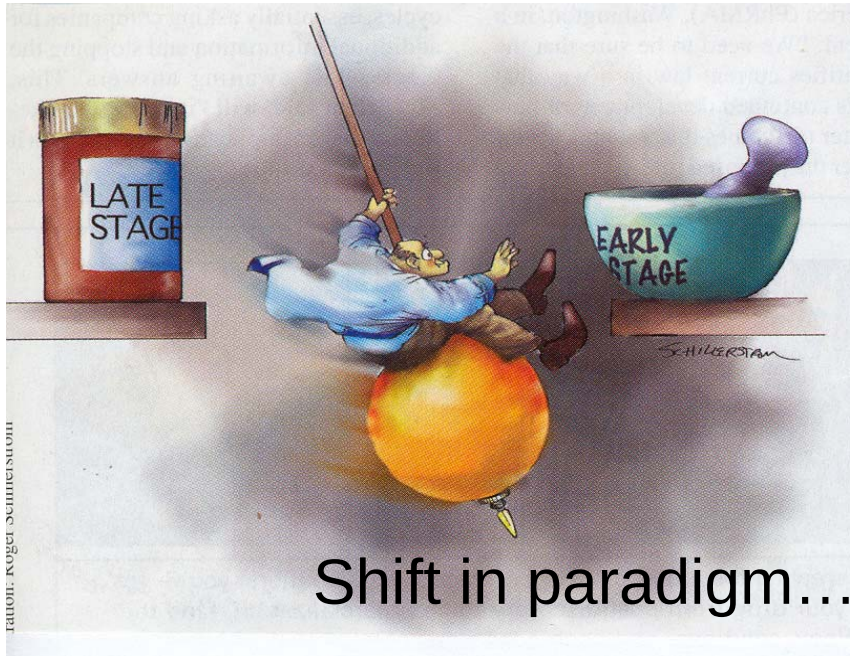
Integrating Molecular Toxicology Earlier in the Drug Development Process

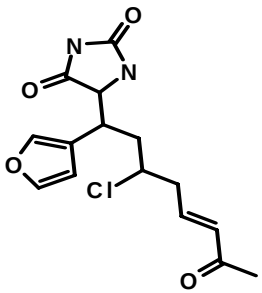
Jonathan Hitchcock

Molecular Toxicology, Drug Safety R&D
Pfizer Global Research & Development
Sandwich, Kent UK



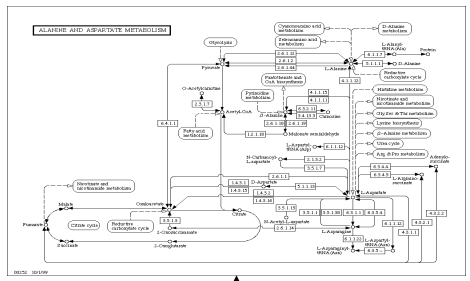
Shift in paradigm….

Structure



~omics

Pathways



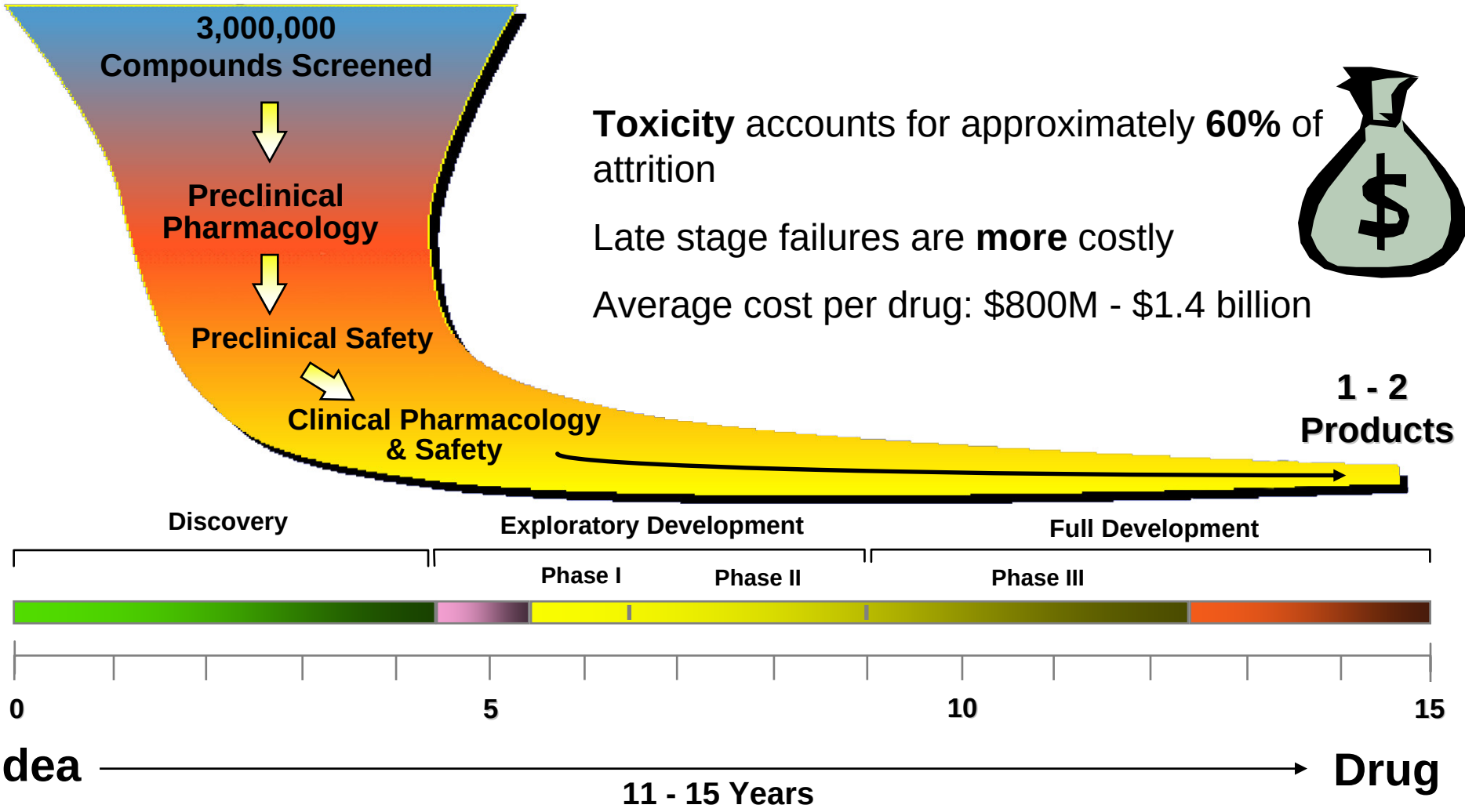
Toxicity

Integrated approach to Toxicology

Outline of Presentation

- Introduction
  - Safety Attrition
  - Value of earlier toxicology integration
- Molecular Toxicology:
  - Past Perspective
  - Examples
  - Future challenges and opportunities
- Concluding remarks/summary

Compound Attrition on the R&D Process



Toxicity accounts for approximately **60%** of attrition

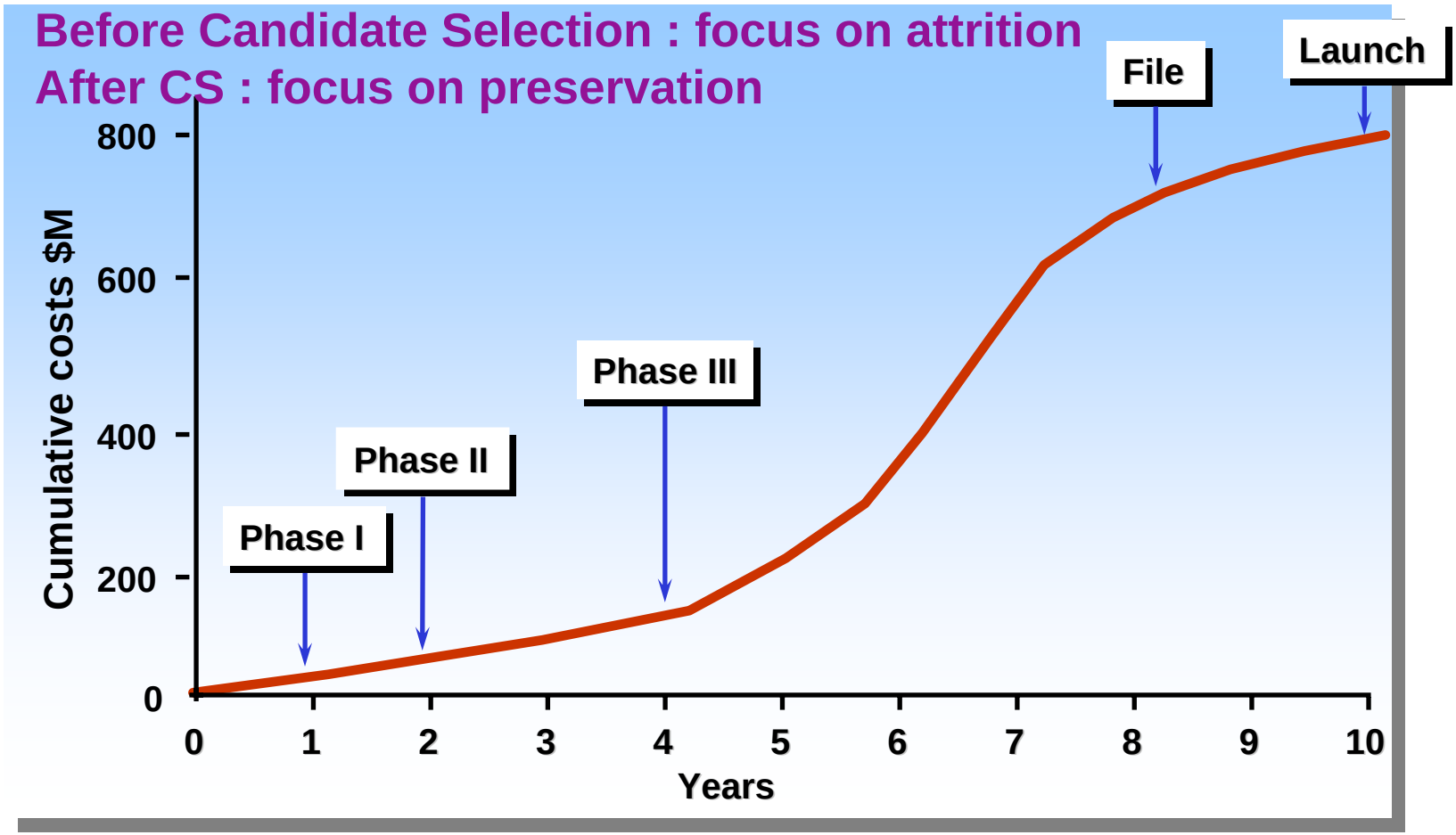
Late stage failures are **more** costly

Average cost per drug: $800M - $1.4 billion

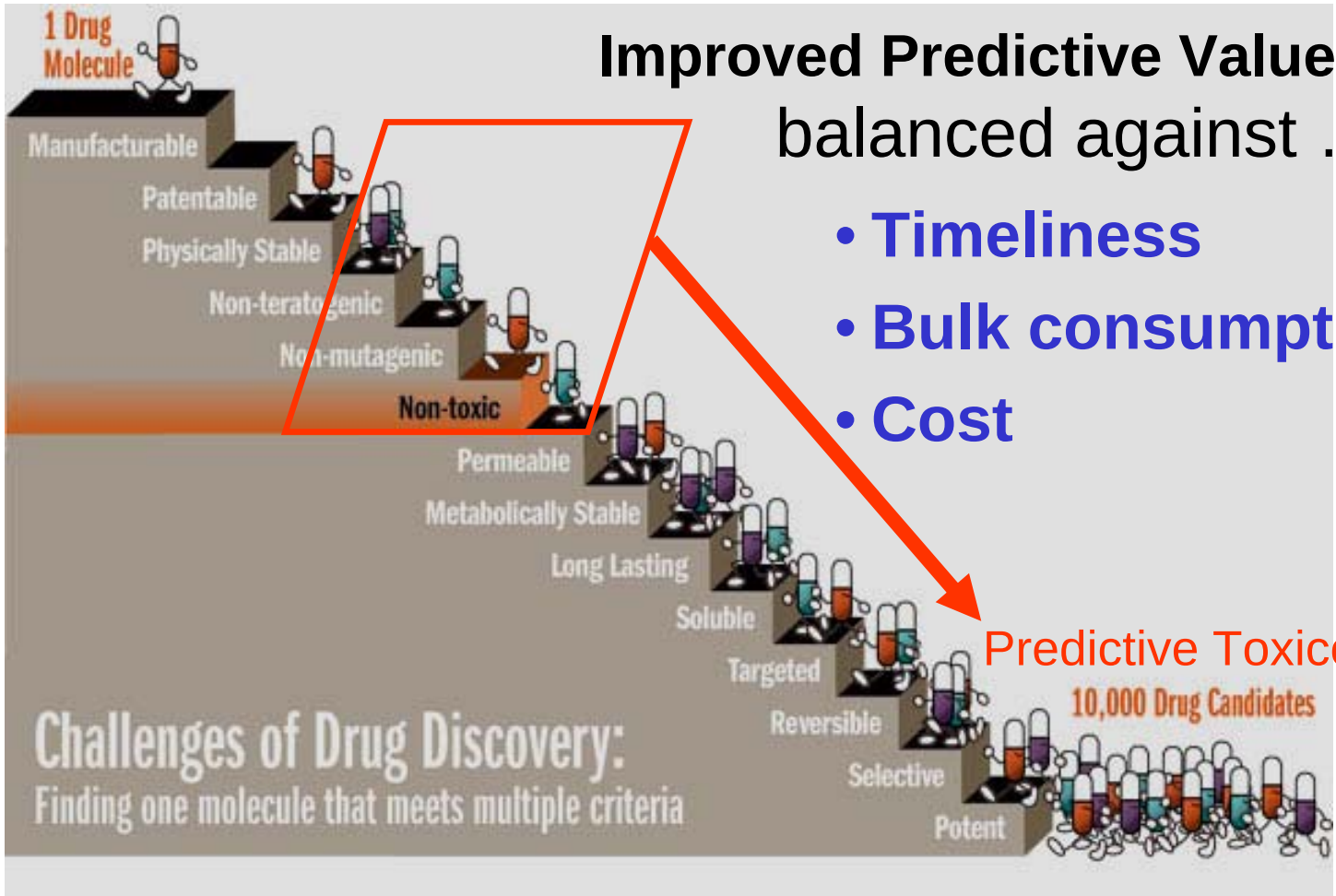
Late-Stage Attrition is Costly

Early stage attrition
Safety, Gentox, PK, Tox, QT
Screens to manage it

Late stage attrition
Idiosyncratic tox. Very
expensive. Difficult to screen for



Value of Earlier Integration…



(Source: Abbott Laboratories) From Drug Discovery & Development July 2003

Outline of Presentation

- Introduction
  - Safety Attrition
  - Value of earlier toxicology integration
- Molecular Toxicology integration:
  - Past Perspective
  - Examples
  - Future challenges and opportunities
- Concluding remarks/summary

Why Molecular Toxicology?

13 years,
$250 million
>1100 scientists

Molecular Technology

1985 **Discovery of PCR** (*Kary B Mullis*)

1995 **TaqMan™** (*Livak KJ*)
SAGE (*Velculescu VE*)
DNA microarray (*Schena M*)

1996 **Molecular Beacons** (*Tyagi S*)
SSH (*Diatchenko L*)

1998 **RNA interference (*Fire A & Mello C*)**

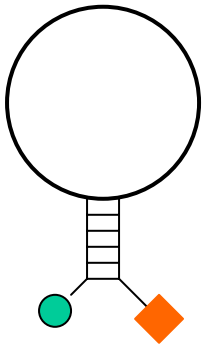
1999 **Affymetrix Genechip** (*Lipshutz RJ*)

Luminex Multi-Analyte System

2000 **Protein microarray** (*Snyder M*)

2004 **Inducible RNAi**

PCR amplification

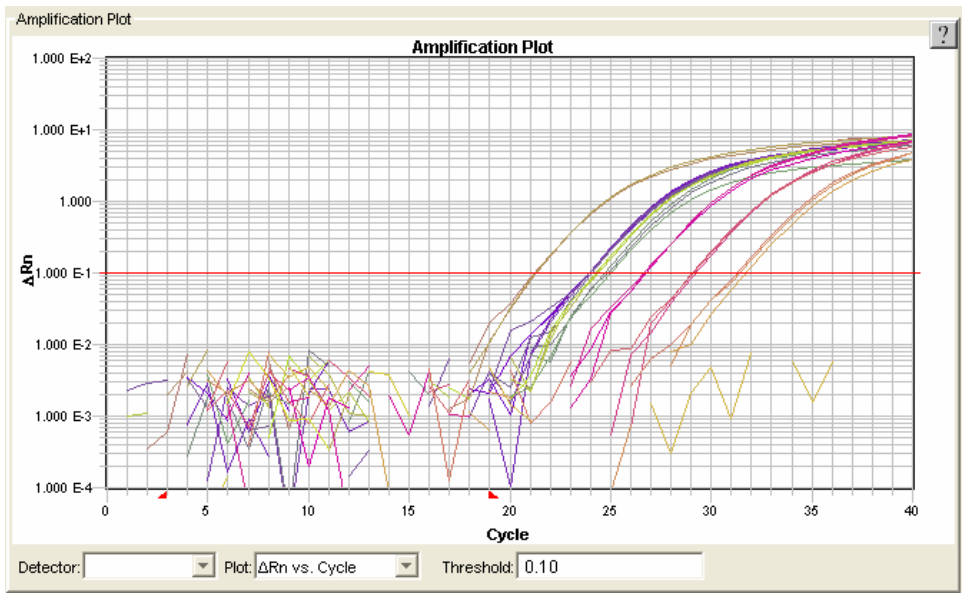


1985 **Discovery of PCR** (*Kary B Mullis*)

1995 **TaqMan™** (*Livak KJ*)

1996 **Molecular Beacons** (*Tyagi S*)

- Ability to amplify very small amounts of genetic material.
- Quantitative gene expression analysis of specific genes.
- Measure multiple genes in the same sample.



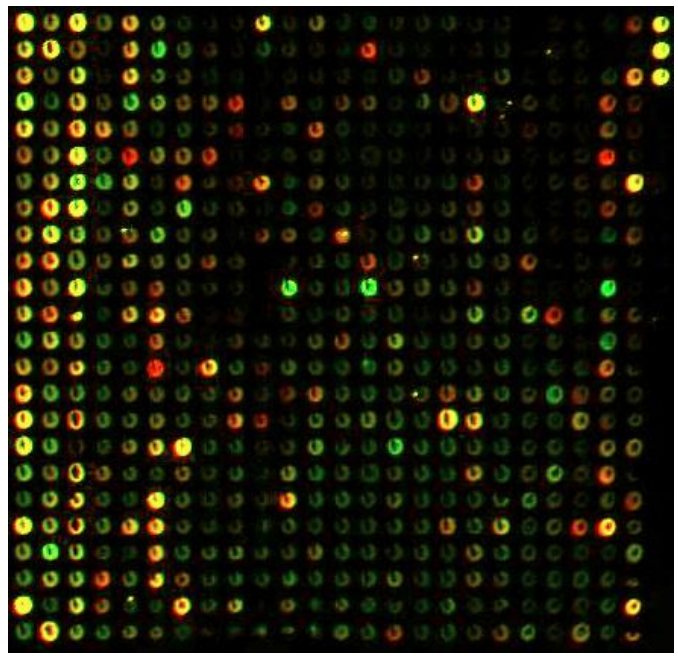
Gene Expression Microarrays

1995 **SAGE** (*Velculescu VE*)
DNA microarray (*Schena M*)

1996 **SSH** (*Diatchenko L*)

1999 **Affymetrix Genechip** (*Lipshutz RJ*)

- Ability to measure thousands of genes in one go!
- Improved analysis and pathway software to put results into context.
- RNA amplification techniques ensure even smallest of samples can be analysed.



RNA Interference (RNAi)

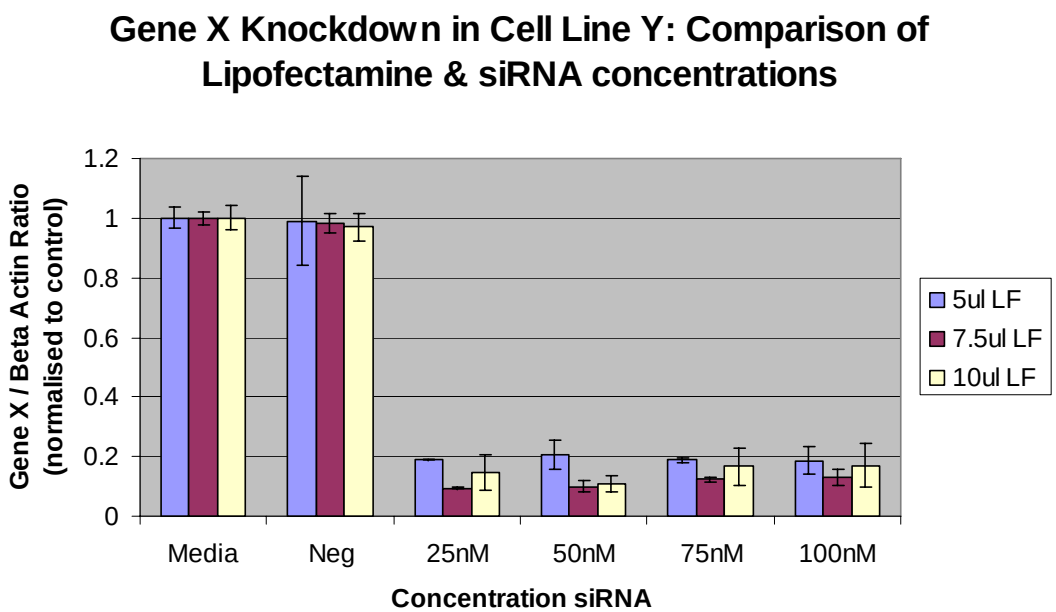
1998 **RNA interference (*Fire A & Mello C*)**

2004 **Inducible RNAi**

- Ability to knockdown expression of a particular gene of interest.

- Increased application in Drug Discovery for the evaluation of Target safety.

- Inducible RNAi can be used when evaluating key cell survival pathways.



Deeper Knowledge of Targets and Pathways

- Molecular understanding of biological processes is continually developing.

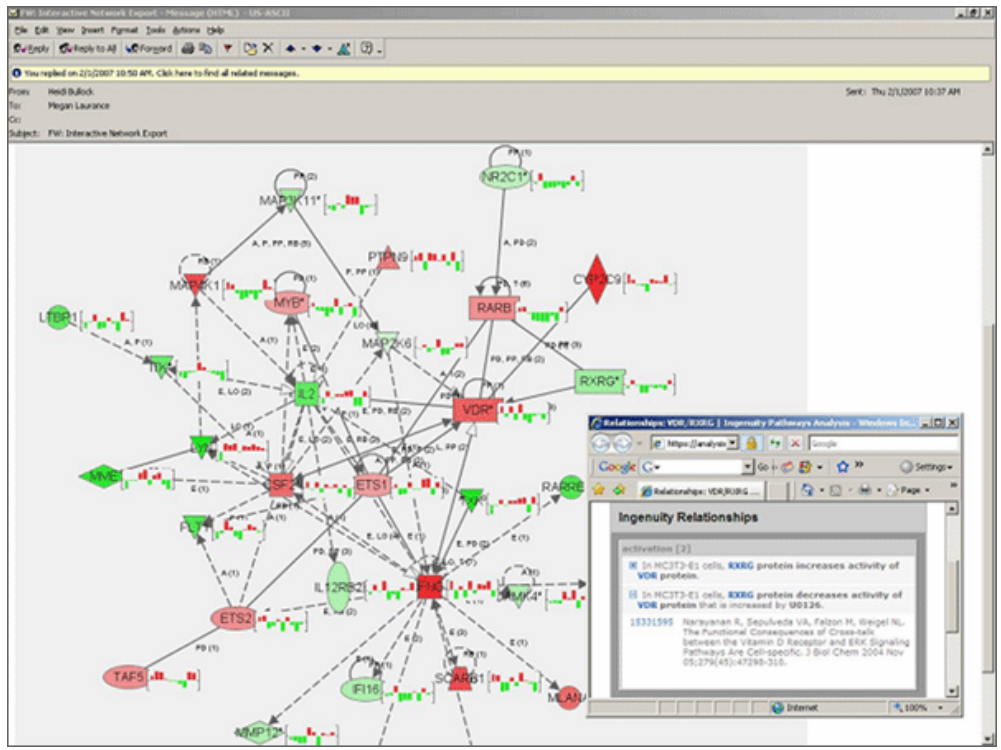
- Potential ‘target related’ effects can be evaluated before compound has been synthesised.

- Endpoints from multiple assays can be analysed in an integrated manner.

MetaCore

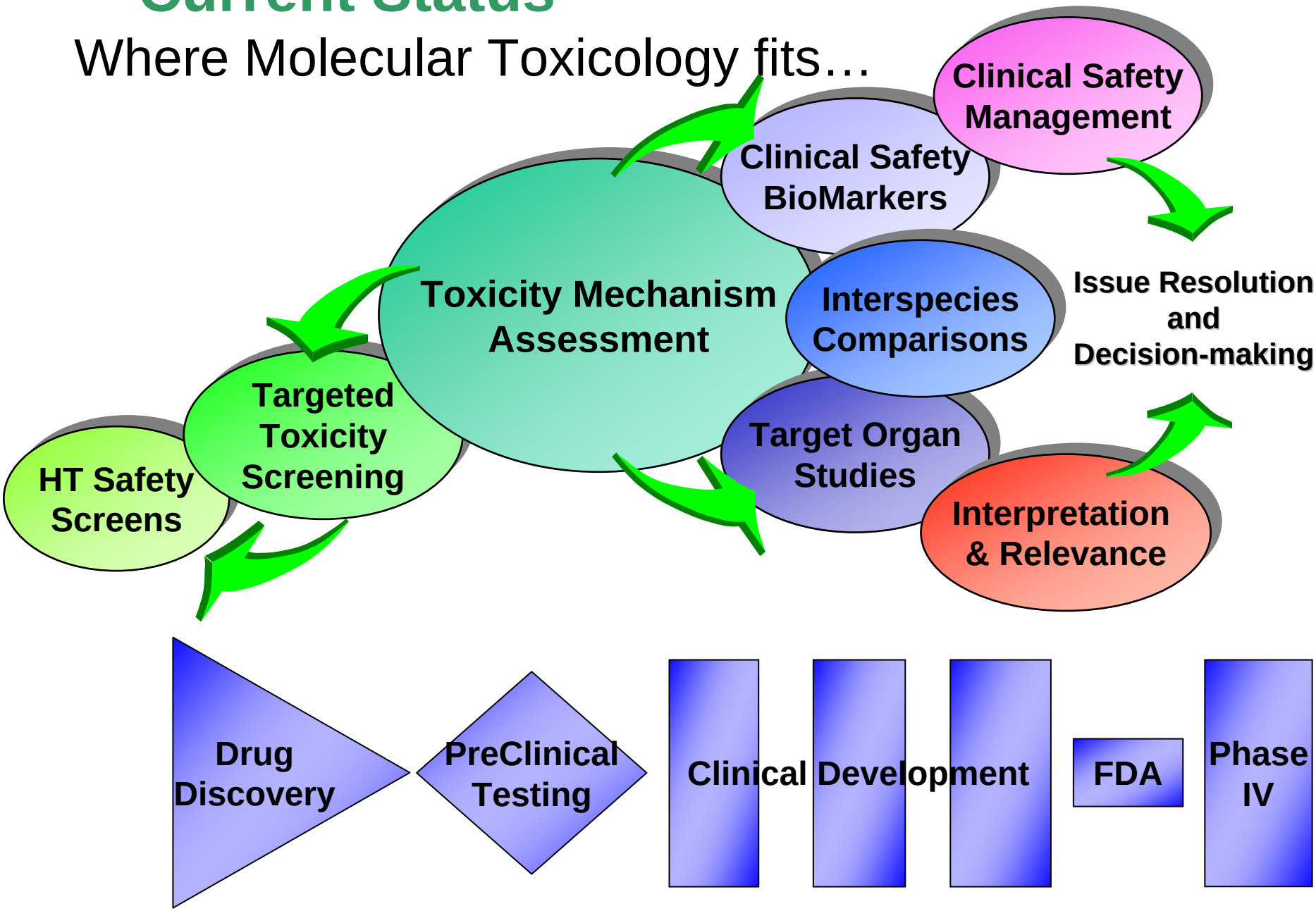
INGENUITY SYSTEMS

Cambridge Cell Networks



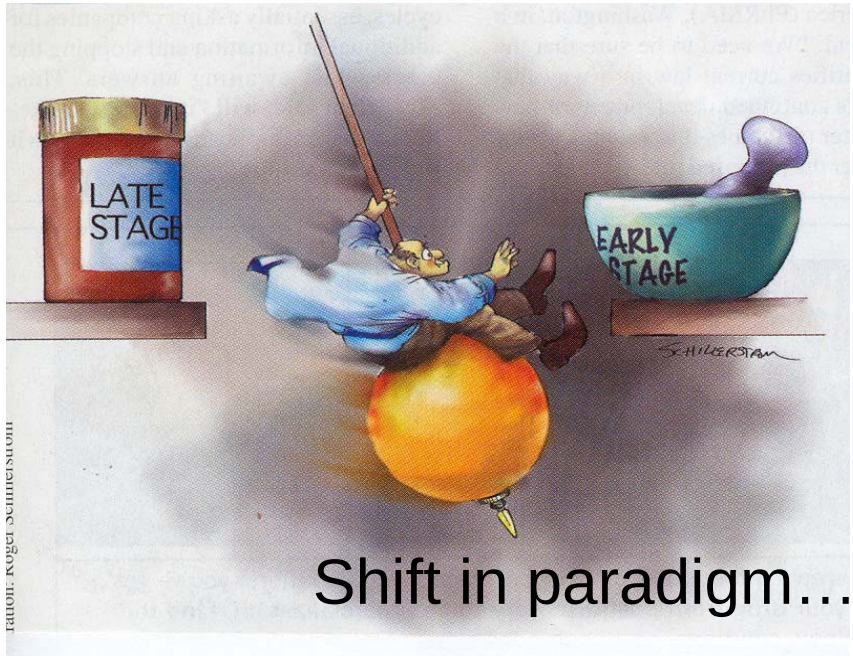
Current Status

Where Molecular Toxicology fits…



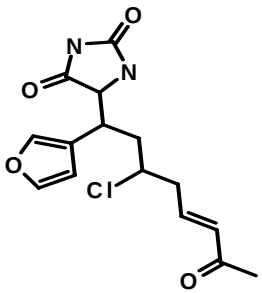
Outline of Presentation

- Introduction
  - Safety Attrition
  - Value of earlier toxicology integration
- Molecular Toxicology integration:
  - Past Perspective
  - Examples
  - Future challenges and opportunities
- Concluding remarks/summary



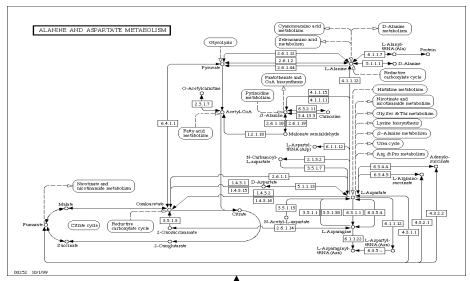
Shift in paradigm….

Structure



~omics

Pathways



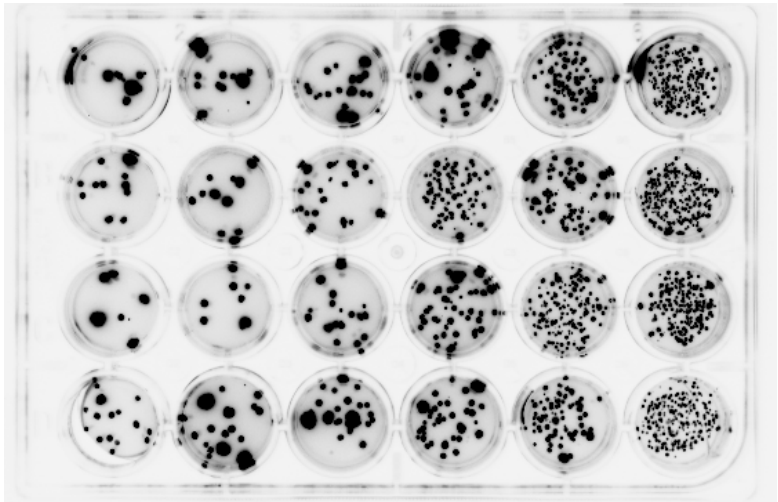
Toxicity

Integrated approach to Toxicology

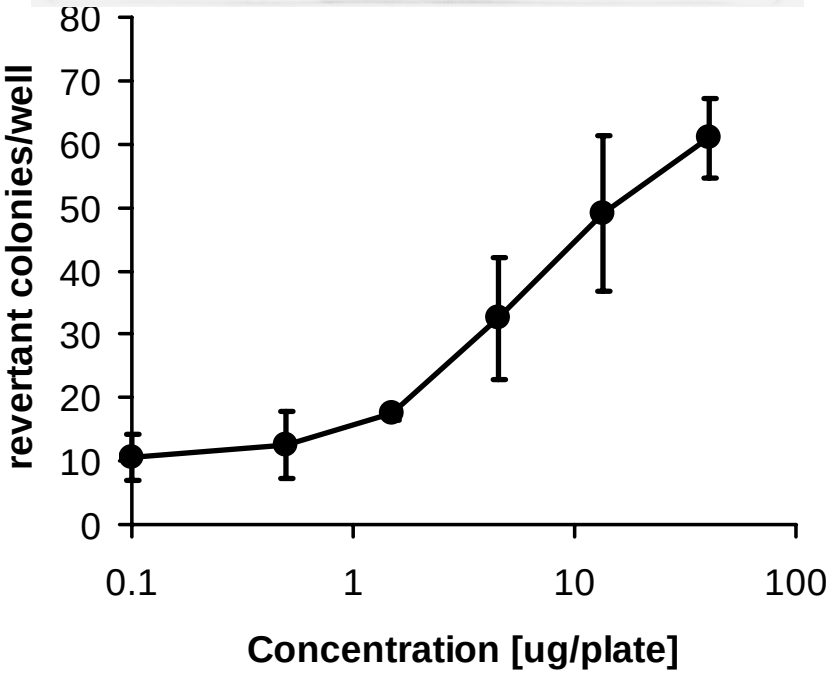
Predictive Screens – Biolum Ames

- **Re-engineered Salmonella reverse mutation assay**
  - Genetically modified AMES *Salmonella* strains
    - Bioluminescent detection of bacterial reversion
    - Simple, Robust & amenable to automation
  - Proprietary technology, Patent Application No. 60/258,073 filed on December 22, 2000.
- **Assay principle**
  - Bioluminescent sensor for revertant cells
    - Measurement of metabolic activities
    - Eliminates problems associated with high concentrations of cells, or contamination.

Bioluminescent detection of revertants



1 - **Image of bioluminescent colonies of revertants TA100lux treated with MMS**



2- **Number of revertant colonies detected (n=4wells, ±SD)**

Biolum Ames: Key attributes

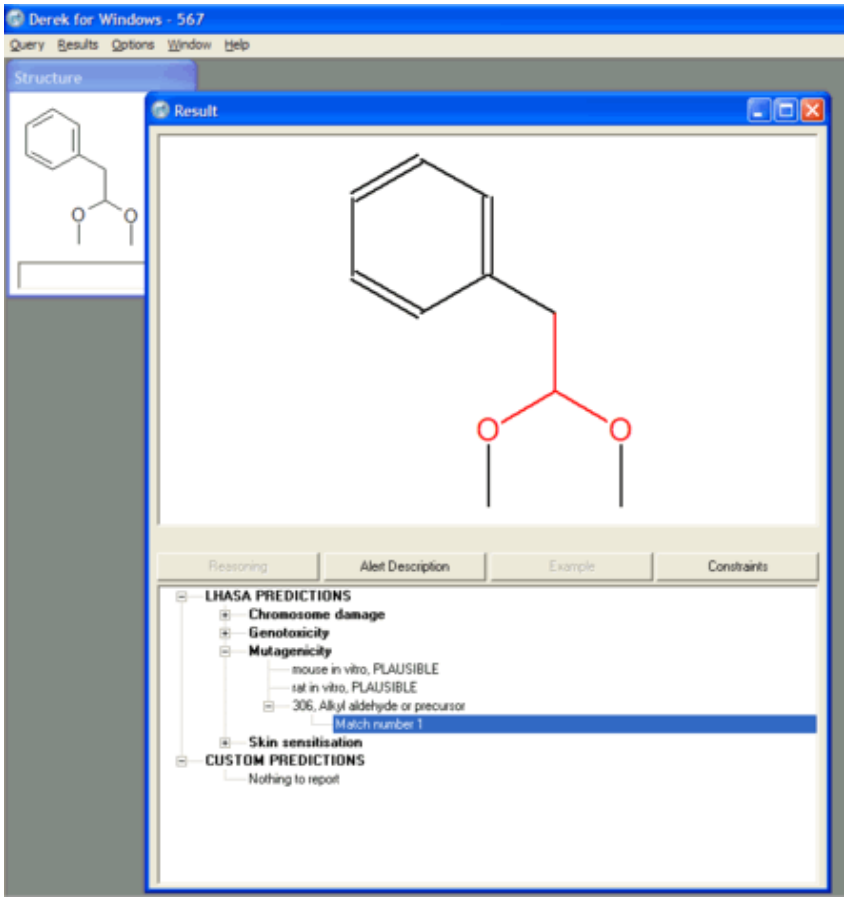
Sensitive & Predictive

- **High concordance with standard assay**
  - Sensitive, high concordance with NTP data (94-95%)
  - low false positive rate

Quick & Economical

- **Low bulk requirement (5mg instead of up to 1g)**
- **Increased throughput**
  - currently 24 well plate (potential for < 48 well)
  - 2 day assay instead of 3, 20 compounds/wk
- **Automation capability**
  - Fully automated scoring
  - Software produces report

Use of In Silico Tools
Predict before the Experiment!



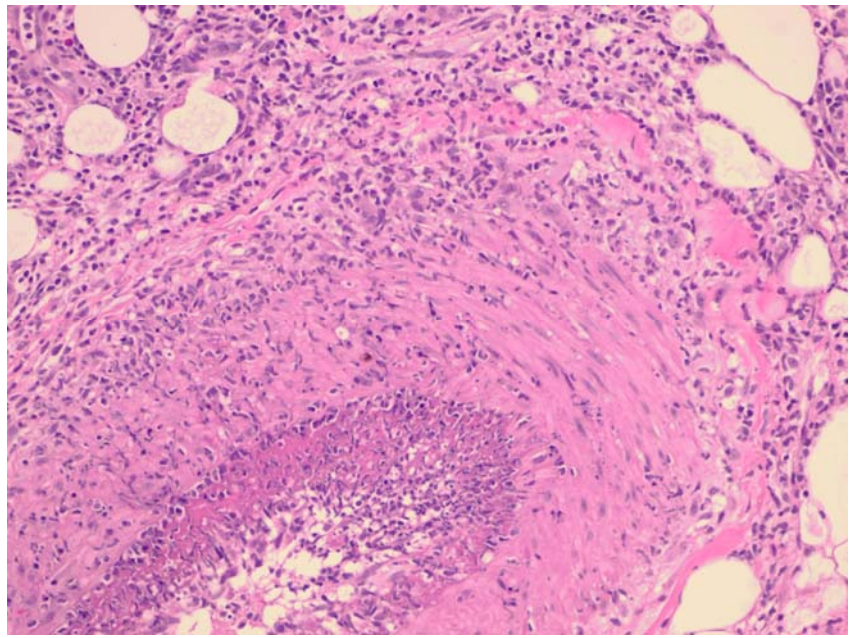
- DEREK stands for **D**eductive **E**stimation of **R**isk based on **E**xisting **K**nowledge
- DEREK (Lhasa Ltd) is an expert knowledge base system
- Several rule bases, consisting of descriptions of molecular substructures (structural alerts) have been associated with toxic end points.

Use of In Silico Tools
What can DEREK tell me?

- DEREK has only been “validated” for genotoxicity
- Rules do exist for other toxicology endpoints, currently under assessment:
  - Skin & respiratory sensitisation
  - Carcinogenicity
  - Reproductive & Developmental
  - Irritancy
  - Thyroid toxicity
- Alerts should always be put in context and DEREK should not be used as a sole source for go/no go decisions for compounds

Genomic Biomarker Development
Vasculitis

- Vasculitis is a major safety issue associated with a variety of drugs; basic mechanism of toxicity is unknown
- Vasculitis can only be confirmed histopathologically
- Mechanistic insights are of great value towards understanding relevance and clinical significance
- Specific biomarkers needed for clinical risk management



Genomic Biomarker Development
Example: **Vasculitis**

In vivo Study Design

- PDE 4 inhibitor 3 days, 2 doses
- Male Sprague-Dawley rats: 10 per treatment, 6 control animals
- Standard Endpoints: **Histopathology,** Clinical Pathology
- Additional Endpoints:
  - **Genomics: Expression profiling (Affymetrix) & Taqman**
  - Proteomics
  - Metabonomics

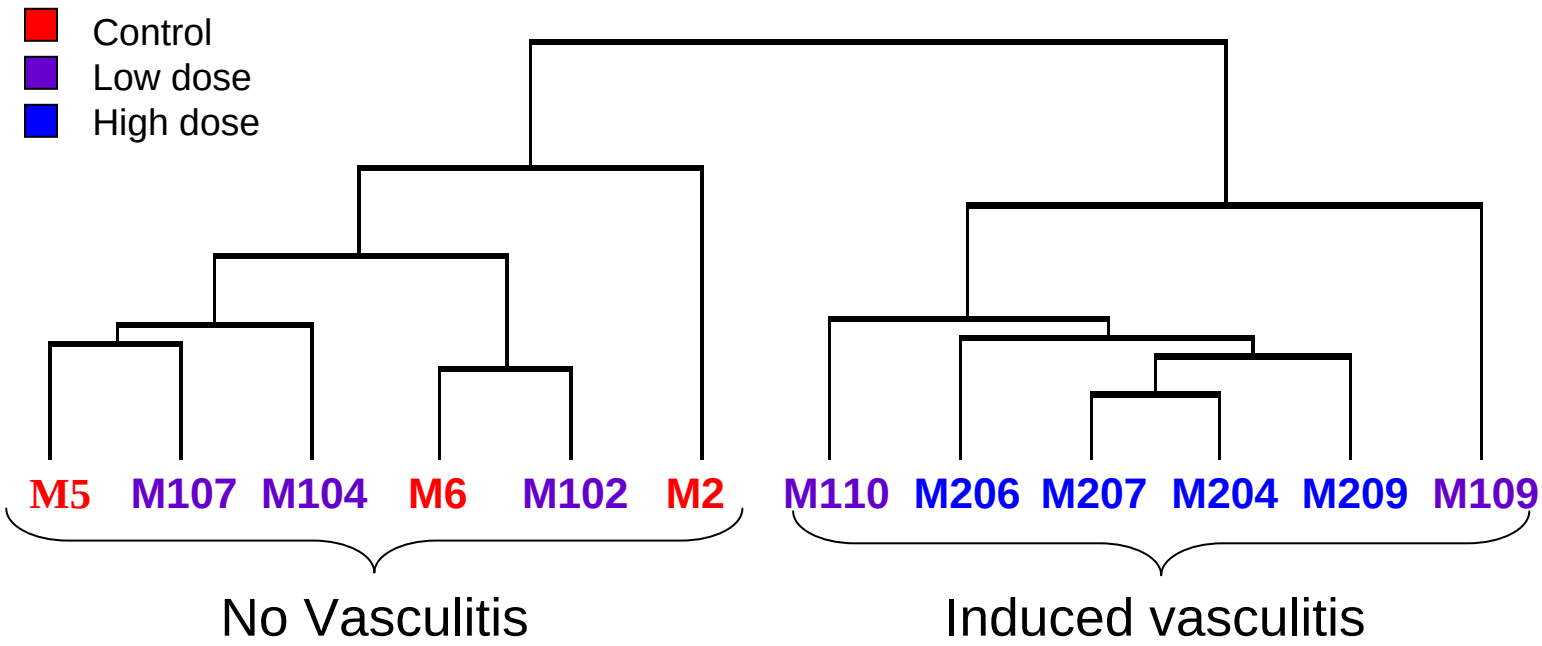
Histopathology: Degree of Vasculitis Observed

Doses	Rat number	Vasculitis *
Control	M2	-
	M5	-
	M6	-
40 mg/kg	M102	-
	M104	-
	M107	-
	M109	1
	M110	1
80 mg/kg	M204	3+
	M206	3+
	M207	3+
	M209	3+

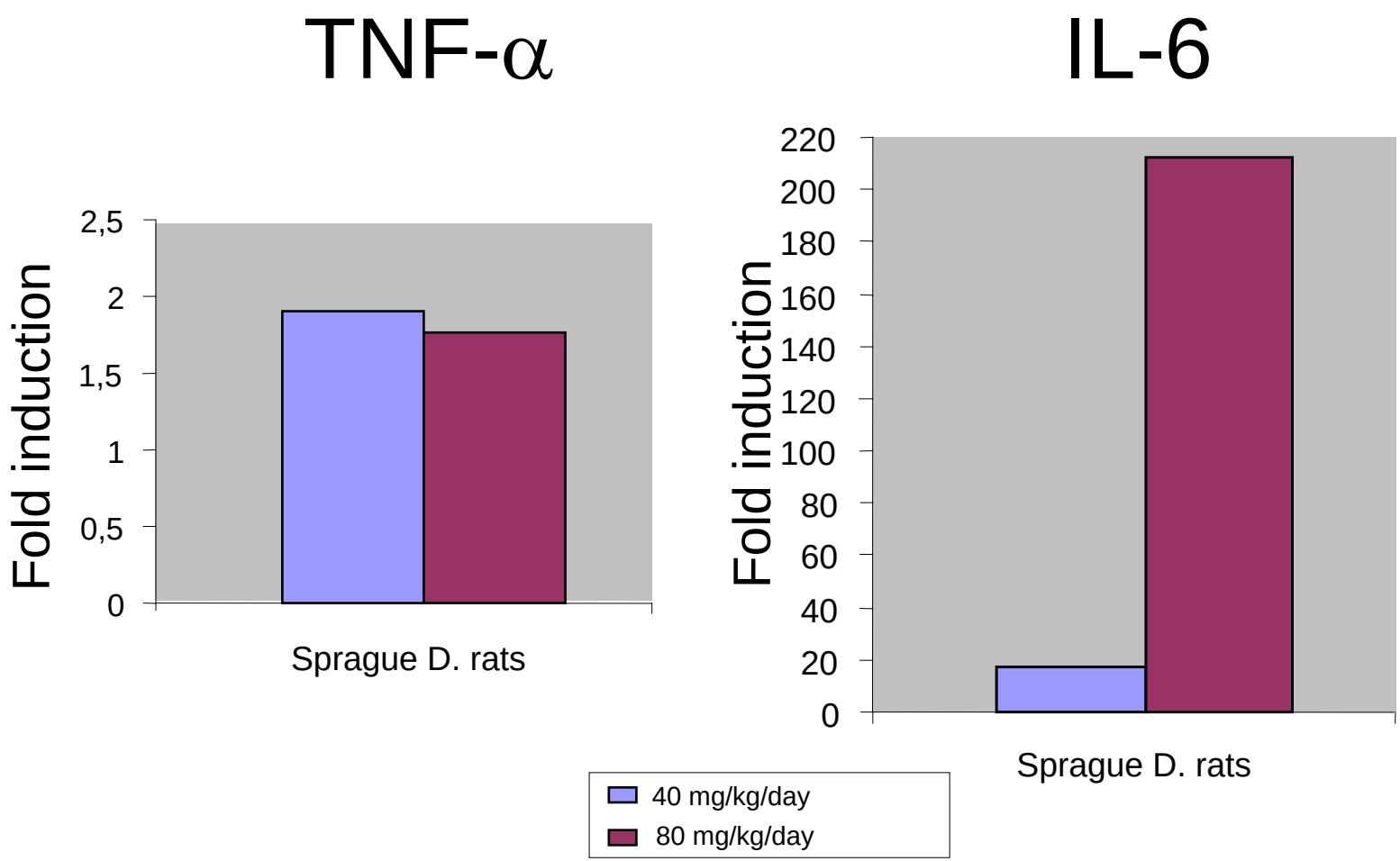
Incidence of vasculitis:
40mg/kg/day:6/10
80mg/kg/day:10/10

+ indicates fibrinoid necrosis

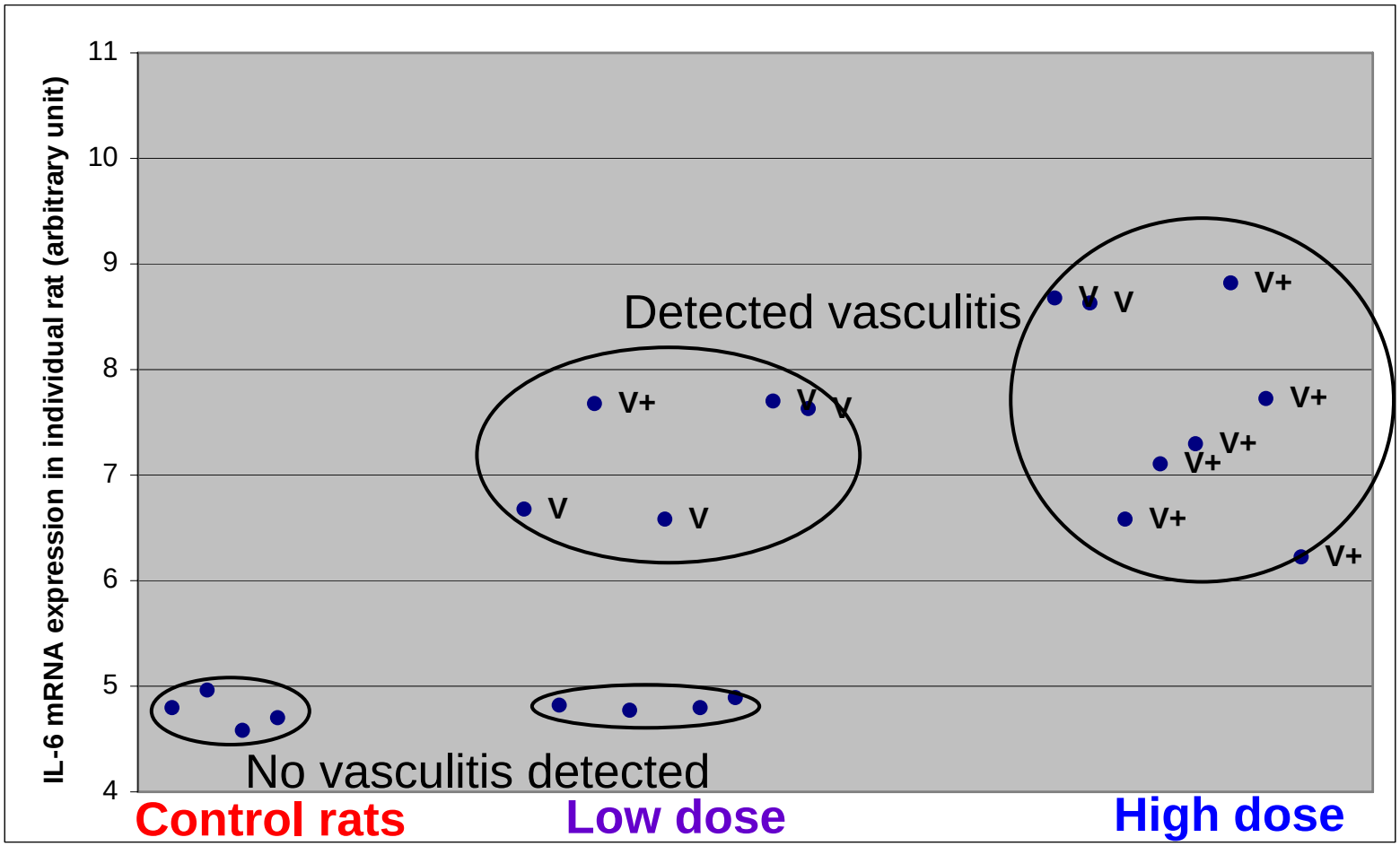
Hierarchical Clustering (Affymetrix Data)



Fold induction of mRNA expression in mesenteric tissue (Taqman)



Correlation between IL-6 mRNA Expression and Vasculitis for Male Sprague-Dawley Rats



What makes a Good Biomarker?

- Predictive and specific.
  - How? Well validated using a large data set and traditional endpoints
- Accessible and Translatable
  - Non-invasive (blood, urine sample), measurable in animal models and in the clinic.
- Highly sensitive.
  - can we pick up changes before traditional endpoints?

Profile of gene/protein changes often used as opposed to individual ‘biomarkers’.

Future Challenges and Opportunities

Freedom to operate (Regulatory Acceptance)

In November 2003 the FDA released ‘voluntary submission guidelines’ to cover regulatory use of genomic-based data generated and submitted under an IND

- **Genuinely encourages the application of molecular data.**
  - Presents illustrative examples that cover many real life questions.
  - Biomarker definitions need expansion.
- **Limited understanding within the agency of dealing with data sets of this kind.**
  - Opportunity to educate and influence the regulators
  - FDA now actually performs own analysis of molecular (specifically genomic) data.

Illustrative Example Relating to Safety

"Vasculitis is a major drug-related nonclinical safety signal and the basic mechanism of toxicity is unknown…normally confirmed by histopathology. A sponsor can use new rat gene chip microarray technologies for expression profiling of 8000 known sequence genes to ***investigate the mechanism of toxicity and possibly see a pattern of genetic biomarkers*** *in treated rats that is different from controls ."*

THESE ARE RESEARCH DATA:

VOLUNTARY SUBMISSION ENCOURAGED

Future Challenges and Opportunities

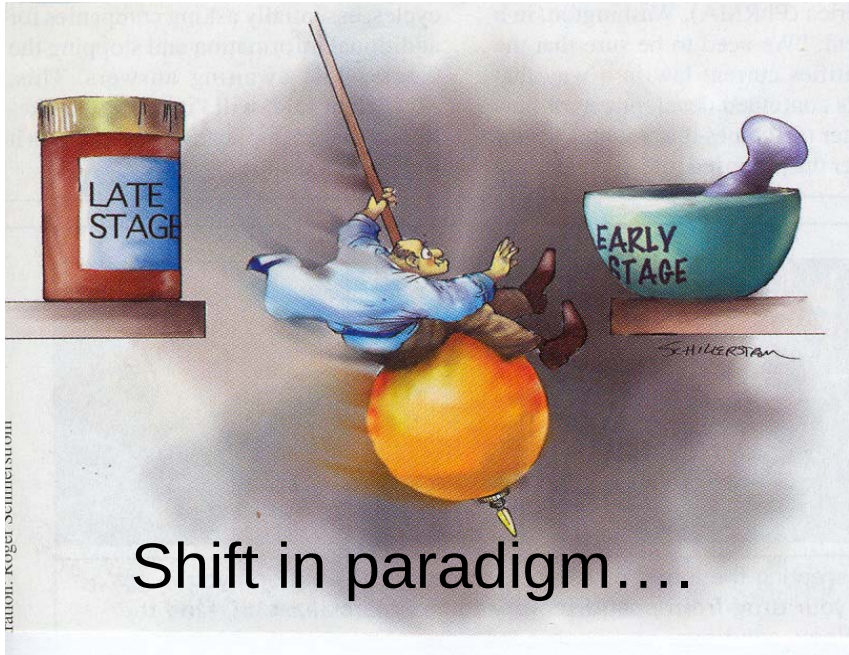
Challenges:

- Linking to other “omics” i.e. metabonomics, proteomics.
- Deeper knowledge of Targets and Pathways
  - improve understanding of large data sets
- Demonstration of ‘added value’ of Molecular Toxicology
  - Integration within development process

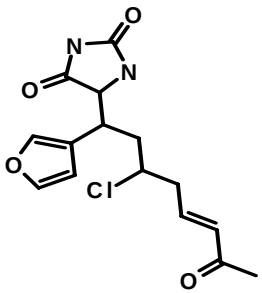
Opportunities:

- Pharmacogenomics
  - Drugs and dosage chosen based on genetic composition – ‘personalised medicine’
  - Potential Benefits: minimal / no side effects
- Discovery of novel targets
  - Improved understanding will lead to new targets
  - Improved predictivity of screens should lead to safer medicines.

In Summary …

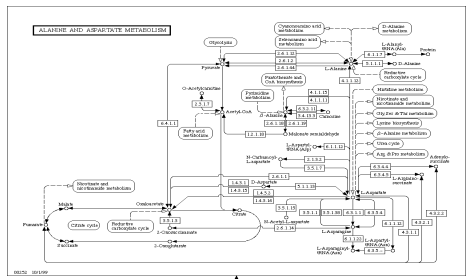


Structure



~omics

Pathways



Toxicity

Integrated approach to Toxicology

Acknowledgements

Fiona Spence

Lab Lead, Molecular Toxicology DSRD - Sandwich

Michael Lawton

Jiri Aubrecht