# Principles of PKPD
# - Preclinical Models in Toxicity Testing/Drug Development-

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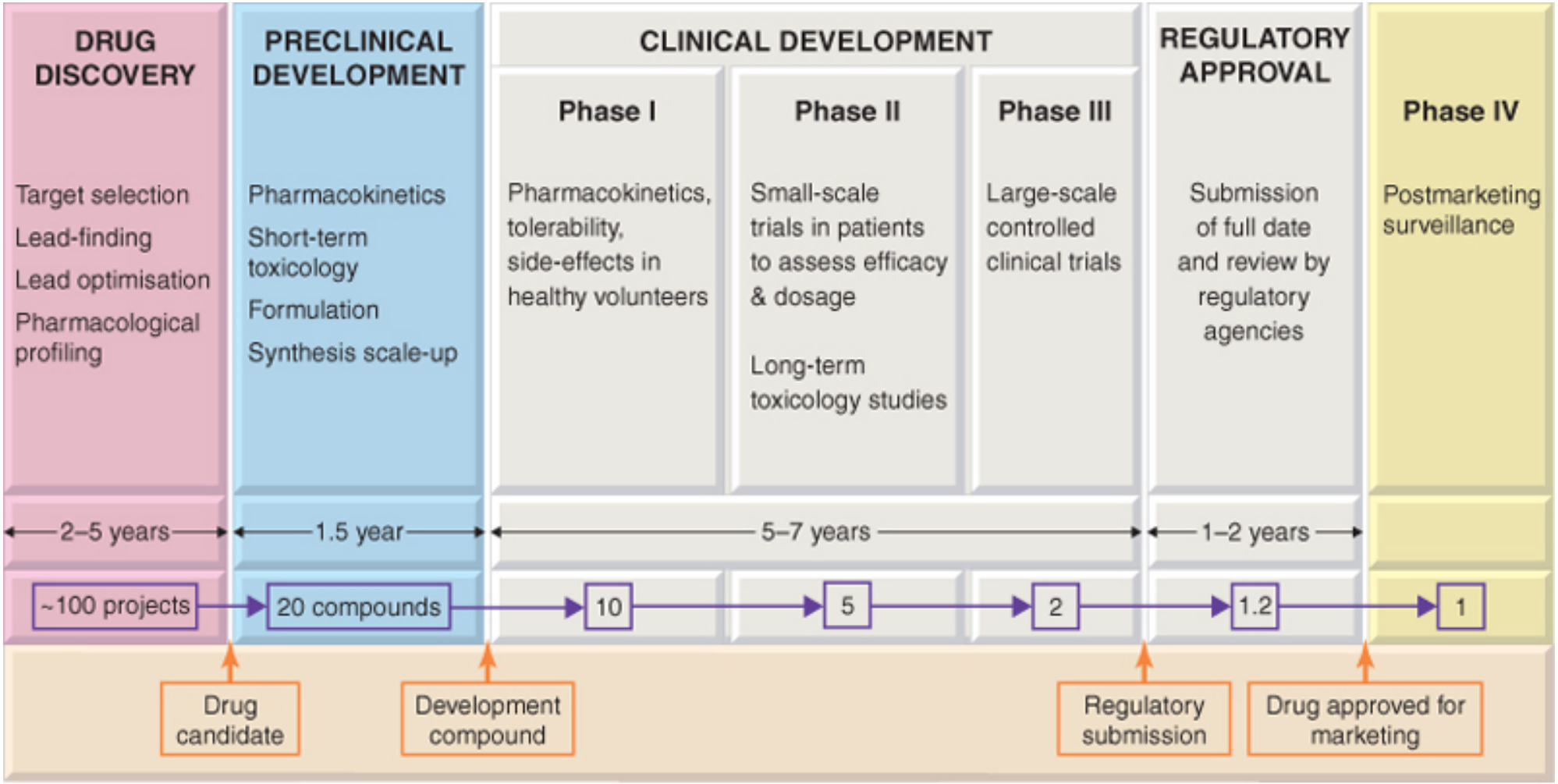
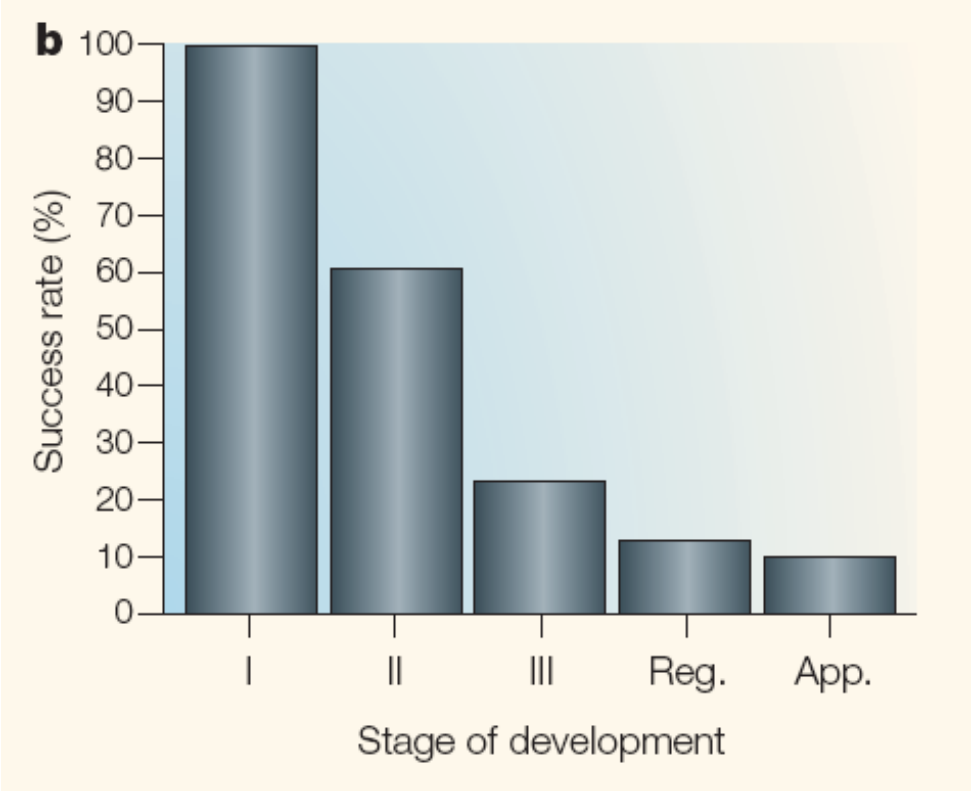
# Stages of Development of a New Drug



# The Drug Discovery Pipeline

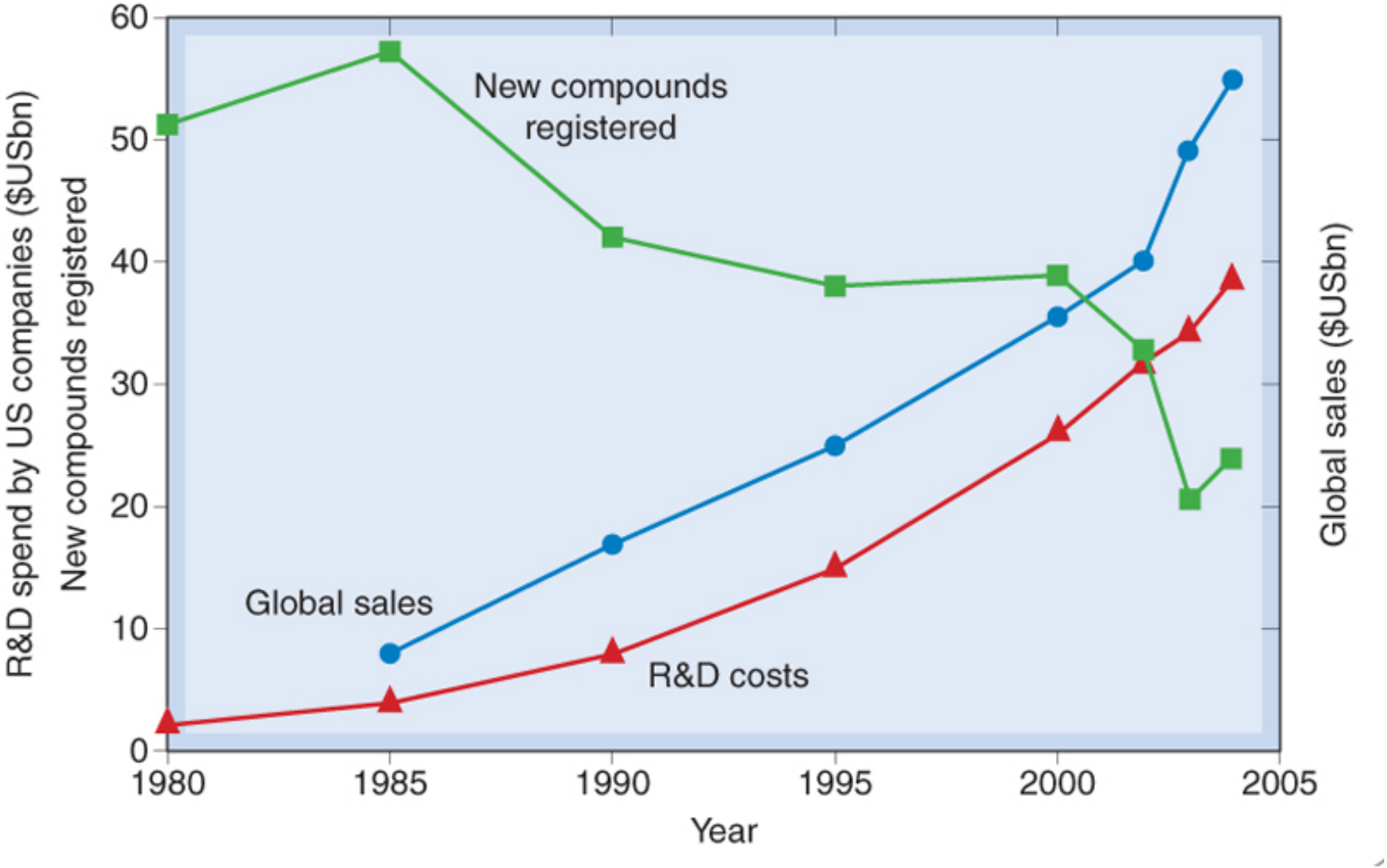
Discovery → Development → Pre-clinical → Clinical

## Increasingly High Drug Attrition Rates

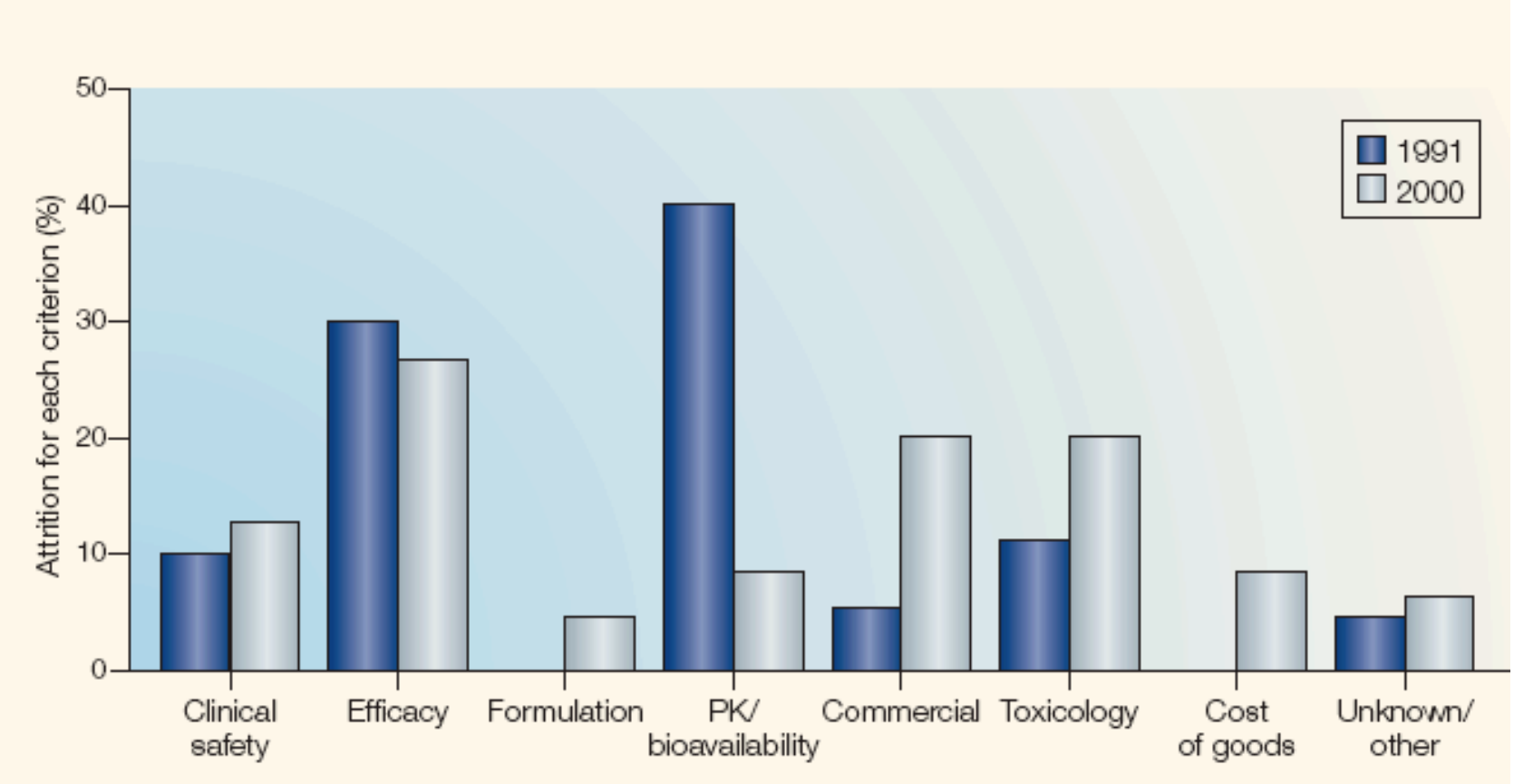



Kola and Landis, Nature Reviews Drug Discovery, 2004

# R&D Spend, Sales and New Drug Registration



# Reasons for Attrition




Efficacy and Toxicity/Clin Safety = Major Reasons
PK/bioavail change – can identify/remedy but also shift to later stages – fail for other reasons

# Recommended Reading

- Nature Reviews, Drug Discovery: A guide to drug discovery – article archive on http://www.nature.com/nrd/series/drugdiscovery/index.html

# The Pre-clinical Stage

Steps before a new compound can be tested in humans

Laboratory and animal studies to -

Scale up and optimise chemical synthesis

Choose formulation

Investigate formulation stability

Prove biological efficacy in animal models

Conduct safety studies (toxic threshold/genotoxicity?)

Evaluate drug metabolism (ADME)

# The Pre-clinical Stage - Chemistry

Chemical Development

- synthesis scale up
- synthesis modifications

Pharmaceutical Development

- formulation
- stability

# Chemical Synthesis

Research chemists use expensive and “dangerous” reagents, starting material and solvents in small quantities

Development chemists have to scale up the synthesis using reagents and solvents which are environmentally friendly, available in bulk and at low cost

Often an intermediate step is taken for intial pre-clinical studies in specialised research laboratories – “kilo lab”

# Pre-formulation Tasks

Determination of the physico-chemical properties of a drug

Solubility

pK values

Partition coefficient

Chemical stability profile

Crystal form and polymorphism

Particle size, shape and surface area

# Formulation

This is carried out after pre-formulation studies or sometimes concurrently

The route of administration has to be considered

The dose form has to be confirmed – tablet, intra-venous, skin patch, cream, buccal dose, suppository, etc

Formulations are tested for physical and chemical stability at higher temperatures

Often need to develop new analytical methodology

HPLC for quantifying degradation products is common

# The Pre-clinical Phase - Biology

Pharmacology

Molecular mechanisms of action and targets

Mechanisms of drug resistance

Determinants of response

Intracellular pharmacodynamics

Molecular pharmacology (primary/secondary)

# The Pre-clinical Phase - Biology

*In vitro* studies using isolated cell systems

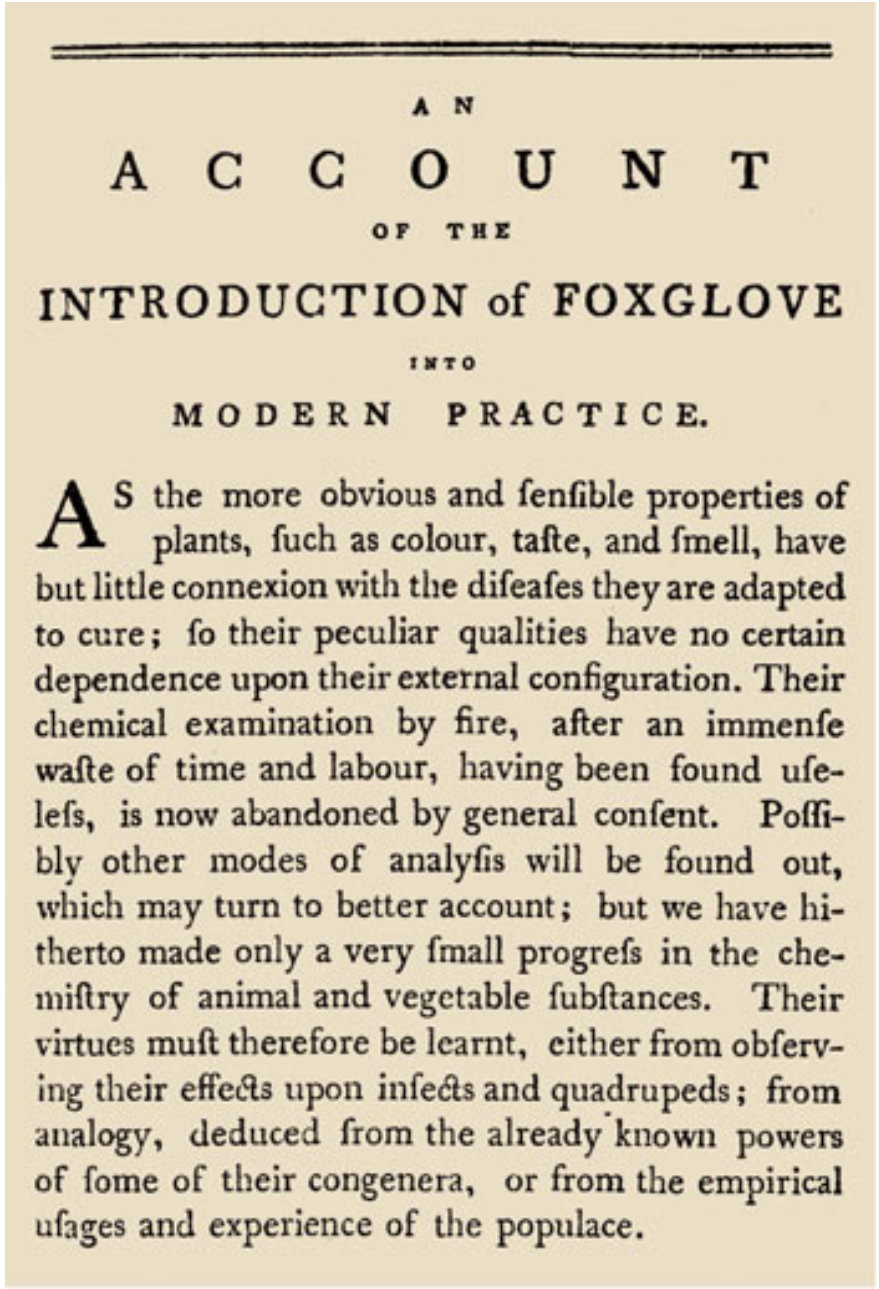
- Used for study of substrates/inhibitors and metabolism of drugs – cytotoxicity/efficacy/ safety/genetic toxicity
- Genetic toxicity eg Ames Test - A bacterial 'reverse mutation' mutagenicity assay that is designed to identify frame- shift and base-pair-substitution point mutations.
- Metabolite-mediated toxicity: Glutathione binding assays or covalent binding (radiolabel)
- Can guide *in vivo* study design

# *In vivo* signal generation

*in vitro assays often can not reliably predict safety margins, as there is no efficacy end point and pharmacokinetic parameters are not faithfully reproduced in vitro*

*In vivo* studies using animal models

- Proof of therapeutic principle
- Animal pharmacokinetics and pharmacodynamics
- Toxicology (single and repeat dose studies)
- Starting dose and schedule for clinical trials



AN

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INTRODUCTION of FOXGLOVE

INTO

MODERN PRACTICE.

AS the more obvious and ſenſible properties of plants, ſuch as colour, taſte, and ſmell, have but little connexion with the diſeaſes they are adapted to cure; ſo their peculiar qualities have no certain dependence upon their external configuration. Their chemical examination by fire, after an immenſe waſte of time and labour, having been found uſeleſs, is now abandoned by general conſent. Poſſibly other modes of analyſis will be found out, which may turn to better account; but we have hitherto made only a very ſmall progreſs in the chemiſtry of animal and vegetable ſubſtances. Their virtues muſt therefore be learnt, either from obſerving their effects upon inſects and quadrupeds; from analogy, deduced from the already known powers of ſome of their congenera, or from the empirical uſages and experience of the populace.

Nature Reviews | Drug Discovery

# Early Record of need for *In Vivo* Animal Models for Toxicity Testing

William Withering laments the lack of chemical methods available to study drugs and accepts that the second-best method is the study in whole animals and in humans. (Oxford Univ. Press, London, 1785).

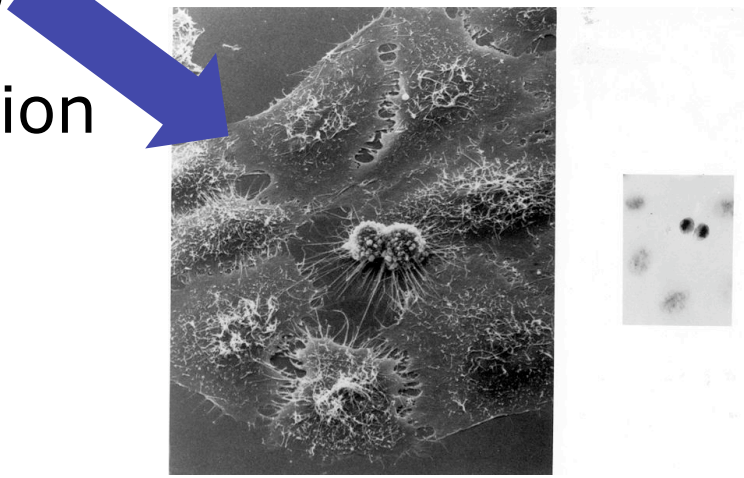
See: Cohen, Nature Reviews Drug Discovery, 2010, Epub

# *In Vitro* Preclinical Model Systems

- Sub-cellular fractions
  - Microsomal, S9
  - Tissue derived and recombinant
  - Liver, Kidney, intestine, lung etc
  - E Coli, yeast, insect cells etc
- Whole cell systems
  - Hepatocytes etc.
  - Mammalian Cell lines

# Cell Lines and Cell Culture

- Primary Cells – explanted directly donor, short survival times (Hayflick limit), senescence
- Secondary Cells – explanted, cultured and divide/ grow for time (eg MRC5 cells – human lung fibroblasts)
- Immortalized cell lines - can provide unlimited tissue amounts - transformed cells as growth properties altered (HeLa cells)
- Cancer Cell Lines – rapid division



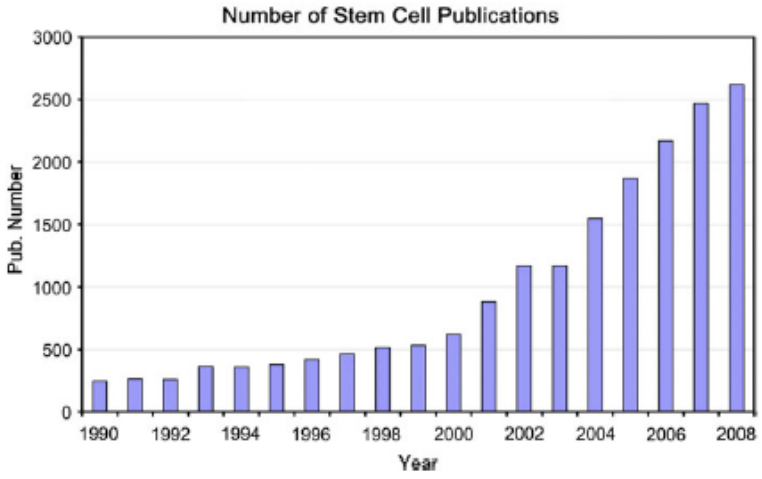
# Limitations of *In Vitro* Models

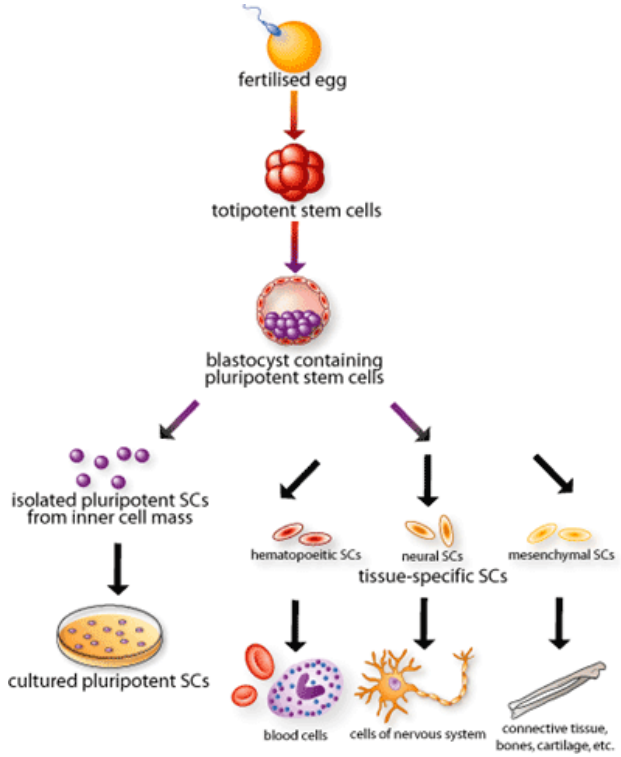
- Sub-cellular fractions need addn co-factors eg CYP/ FMO – NADPH; NAT – AcetylCoA
- In primary culture, use of special substrates (collagen, laminin, extracellular matrix preparations), growth factors and soluble media supplements, is complex
- Difficulties in obtaining adequate tissue amounts - immortalized cell lines
- lack of metabolism, differences in sensitivity of cell types, culture conditions and the context of the ultimate target tissue *in vivo*
- Stability and/or viability major issues

TABLE 1.—Advantages and limitations of different in vitro liver models.

| Model | Advantages | Limitations |
|---|---|---|
| Isolated cells | All species including humans<br>Whole livers or biopsies as source<br>Information on cellular toxicity<br>Cryopreservation<br>Several compounds at different concentrations | No bile measurement<br>No cell-to-cell interaction<br>No preserved anatomy |
| Liver slices | Lobular structure partly preserved<br>All species including humans<br>Whole livers or biopsies as source<br>Information on cellular toxicity<br>Several compounds at different concentrations | No bile measurement<br>No cell-to-cell interaction<br>No preserved anatomy |
| Isolated organs | Closest to in vivo conditions<br>Anatomy preserved<br>Bile flow preserved<br>Hematodynamic parameters can be assessed | Short-term viability (2–4 h)<br>Only a few compounds can be assessed with one organ<br>No studies on human liver<br>High number of animals used<br>Complexity of the setup |

# Stem Cells – Novel *In Vitro* Models




- Much research - regenerative medicine
- Delivered new assays to predict embryo-fetal developmental toxicity *in vitro*
- mixtures of cells much more representative of tissues *in vitro* – can monitor alterations in homeostasis

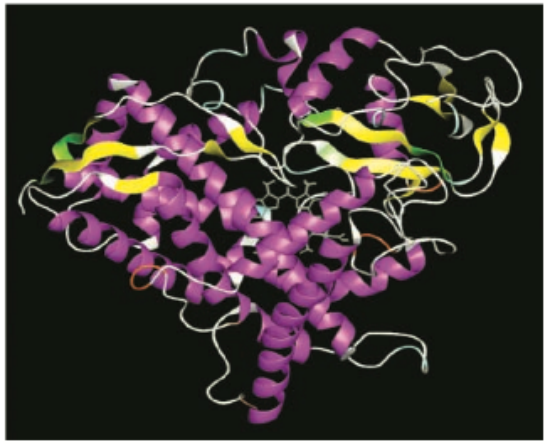
Stem Cell Res. 2010 Jul;5(1):4-22. Epub 2010 Mar 4.

**Generating hepatic cell lineages from pluripotent stem cells for drug toxicity screening.**

Baxter MA, Rowe C, Alder J, Harrison S, Hanley KP, Park BK, Kitteringham NR, Goldring CE, Hanley NA.

Endocrinology & Diabetes, School of Biomedicine, Manchester Academic Health Science Centre, University of Manchester, Oxford Road, Manchester, M13 9PT, UK.

# *In Silico* Models



- Computer Based Approach – Data Modelling (eg Protein binding) & Molecular Modelling
- Prediction – QSAR – multivariate modelling eg PLS

- Physicochemical properties (eg lipophilicity/H bonding)

- absorption, distribution, metabolism, excretion (ADME) and toxicity (T) ADMET

- Van de Waterbeemd and Gifford, Nature Reviews, ADMET IN SILICO MODELLING: TOWARDS PREDICTION PARADISE?, 2003, 2, p192

# In Vivo Animal Models

one rodent and one non-rodent, have to be included in preclinical safety studies before human testing

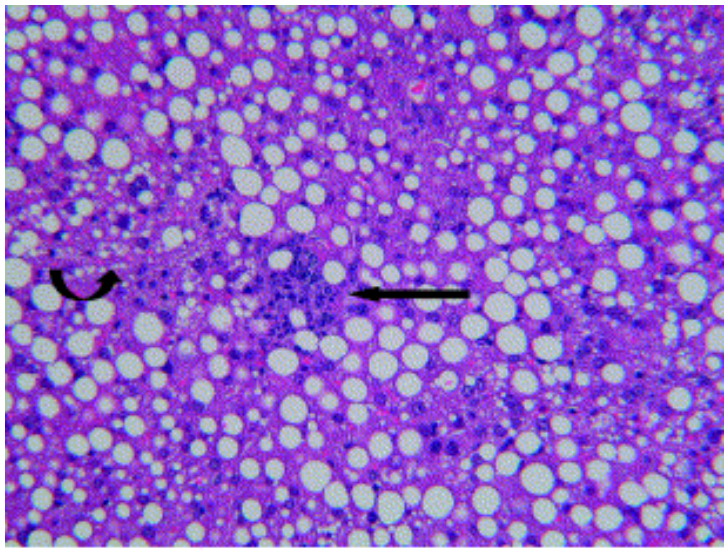
Considerations: Species, Strain, Sex, Age, Diet, Housing, Dosage, Sample Collection, Termination, Time-points etc…

C elegans, fruit-flies and zebra fish – new models: faster, cheaper, more ethical

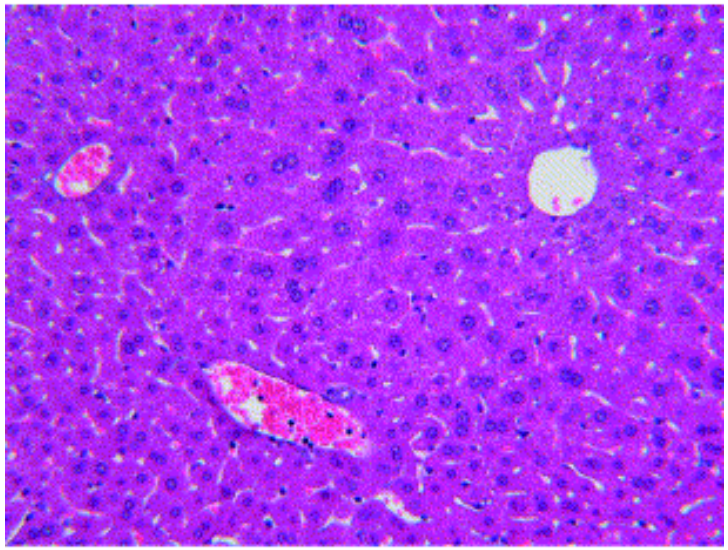
# Genetic/Transgenic Models

- Selective Breeding – animals with characteristics of a disease, eg genetically obese mice – responsible genes often not identified
- Transgenic models – deliberate genetic manipulation of germ-line
- Inactivate individual genes (knock-out)/introduce new genes (knock-in)/over-express
- Phenotypic changes that can mimic human disease
- E.g. Alzheimer's Disease mouse model – overexpresses mutated forms of amyloid precursor protein

# Metabolic/Diet-Induced Models of Disease




MCD




CONTROL

- Methionine/choline deficient (MCD) diet – histological steatohepatitis very similar to human non-alcoholic steatohepatitis (NASH)

# The 3Rs - Replacement, Refinement and Reduction

- a widely accepted ethical framework for conducting scientific experiments using animals humanely
- Replacement: Established animal cell lines, Animal cells, tissues and organs collected from animals killed by a humane, nematodes etc.
- Reduction: Improved experimental desdign and statistical analysis, modern imaging techniques, sharing data and resources
- Refinement: improvements to scientific procedures and husbandry which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable.

# Translatability from animal to man??

- Huge species differences eg morphine is a strong emetic in dogs, it does not have this effect in rats, and to a much lesser extent in humans
- Anatomical and physiological differences e.g. physical size
- Nonhuman primates – ethical issues and high costs
- APAP is a good example - an *in vivo* mouse model resembling human APAP toxicity – need more of these models

# Translatability from animal to man??

- Differences in age (young animals Vs variable aged human populations)
- Genetic background homogeneous (animal) Vs heterogeneous (man)
- Healthy Animal Vs Diseased Patient
- Therapeutic Vs Toxic Dose – how does high drug concentrations used in *in vitro/in vivo* models relate to therapeutic doses?
- Environmental Factors: Controlled Vs Variable – e.g. nutrition, concomitant therapies…

* Are these *in vitro/in vivo/in silico* models predictive of adverse drug reactions in human populations??

Regulatory Toxicology and Pharmacology **32**, 56–67 (2000)
doi:10.1006/rtph.2000.1399, available online at http://www.idealibrary.com on IDEAL®

# Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals

Harry Olson,[1] Graham Betton,[2] Denise Robinson,[3] Karluss Thomas,[3] Alastair Monro,[1] Gerald Kolaja,[4] Patrick Lilly,[5] James Sanders,[6] Glenn Sipes,[7] William Bracken,[8] Michael Dorato,[9] Koen Van Deun,[10] Peter Smith,[11] Bruce Berger,[12] and Allen Heller[13]

**TABLE 2**
**Distribution of Compounds by Therapeutic Class**

| Therapeutic class | No. of compounds |
|---|---|
| Anticancer | 14 |
| Anti-infection | 21 |
| Anti-inflammatory | 15 |
| Antiviral | 8 |
| Cardiovascular | 16 |
| Endocrine | 10 |
| Gastrointestinal | 9 |
| Hematology | 1 |
| Immunology | 2 |
| Impotence | 2 |
| Metabolism | 5 |
| Neurologic | 31 |
| Renal | 2 |
| Respiratory | 13 |
| Trauma | 1 |
| Total | 150 |

221 separate cases of compounds associated with significant human toxicity (HT) were recorded. A total of 150 compounds contributed to this series with multiple HTs being recorded in 47 cases.

70% concordance in one or more preclinical animal model – target organ toxicity in both animal and human

Remaining 30% - no relationship between human and animal

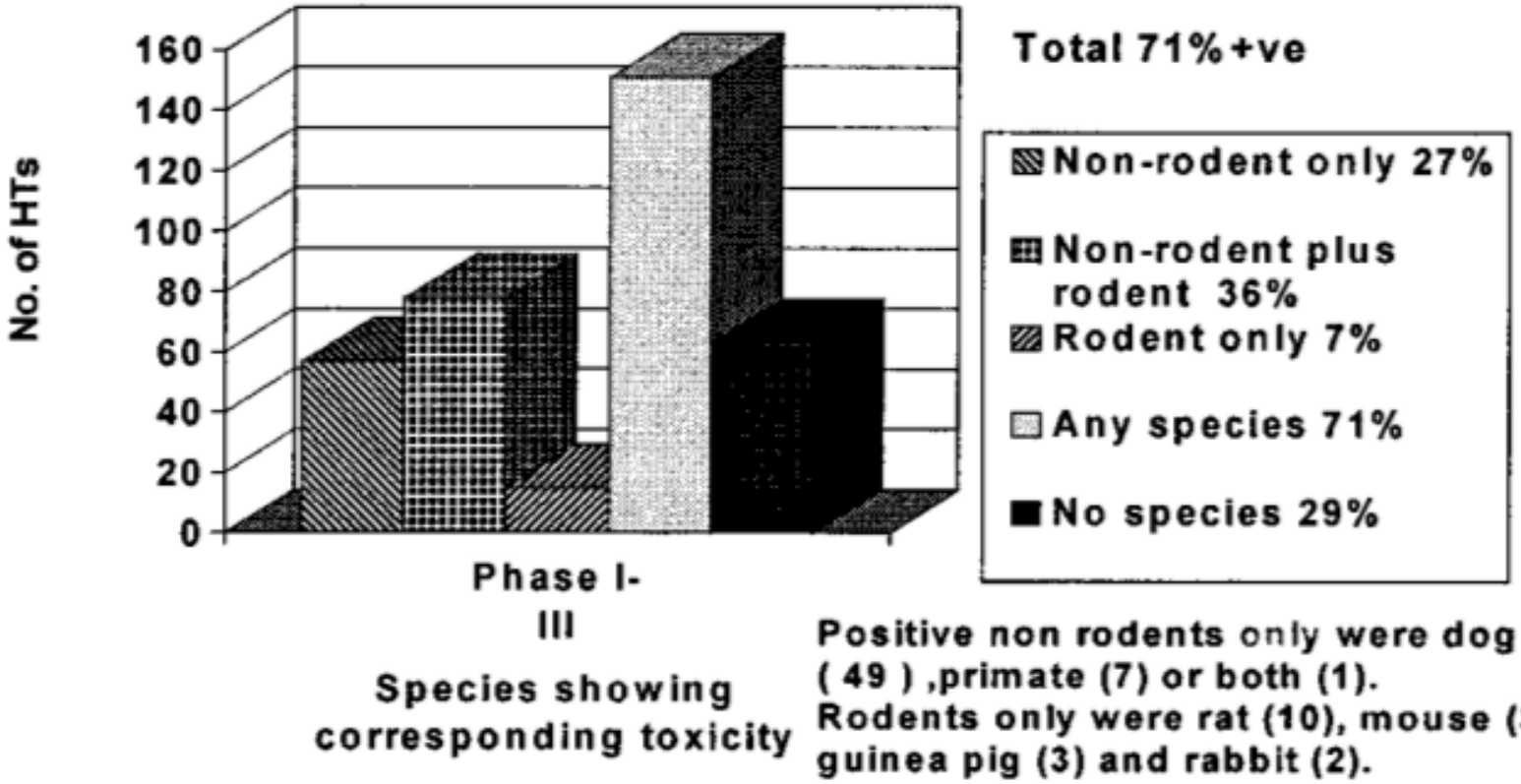



**FIG. 3.** Concordance of human toxicity from animals.

- Best concordance for hematological, gastro- intestinal, and cardiovascular toxicities and the least for cutaneous toxicity
- Dependence on therapeutic class

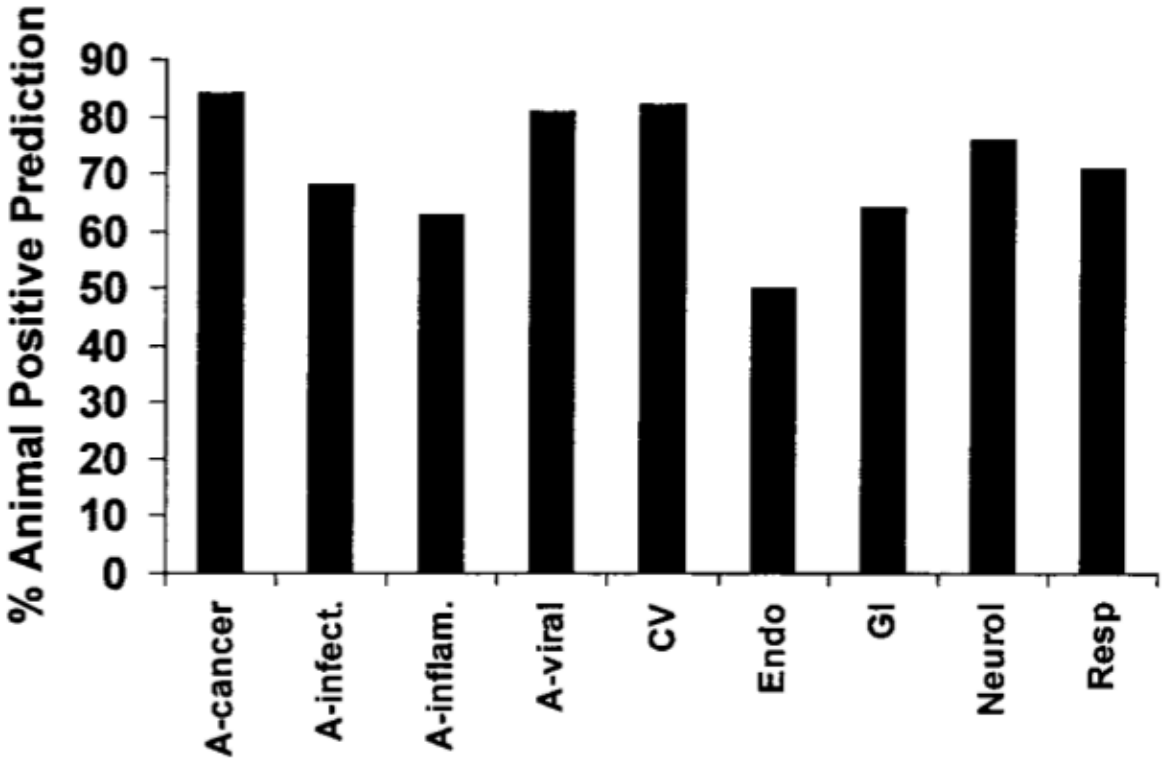



**FIG. 6.** Preclinical concordance for HTs by therapeutic class.

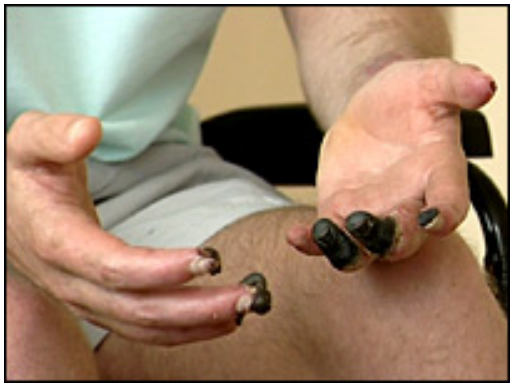
94% detected within one month in animals

Liver toxicity never reported after single dose in animals

# Lack of Pharmacology: CD28 agonist TGN1412

- A good example of a proto- typical new drug, resulting from increasing biological knowledge in immunology and the physiology of T cell activation.
- TGN1412 caused serious damage to six healthy volunteers in a first-in-human trial
- Molecular, cellular and whole organism studies were done in several species, but no integration of results

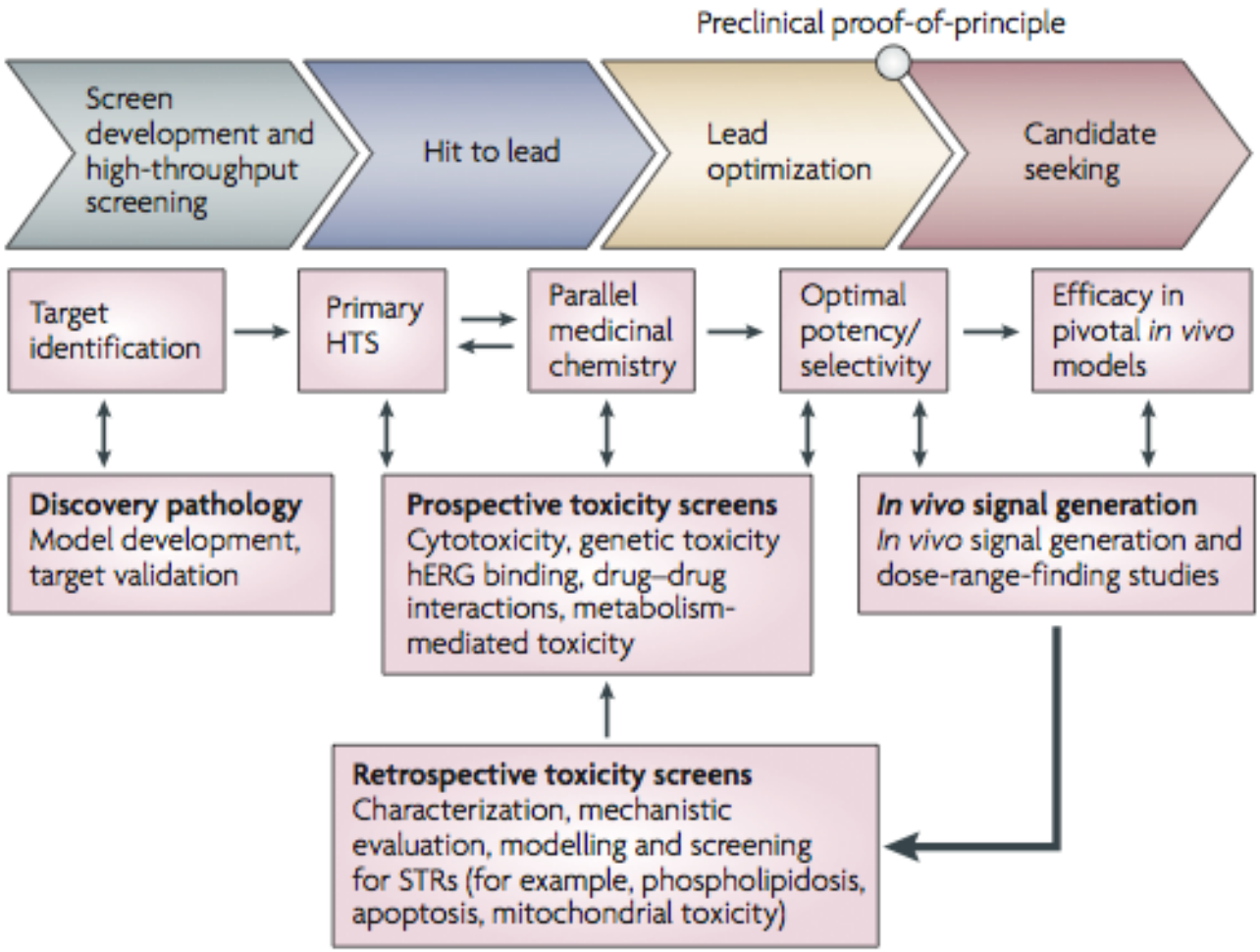
- cytokines interleukin-10 and interferon-$\gamma$ that are produced by regulatory T cells — the target cell population to be stimulated and increased — were not measured (but pro-inflammatory cytokines were)



# CD28 agonist TGN1412

- Toxicology tested in an animal species but pharmacological effects not included.
- Mice equipped with a human immune system, showed the severe depletion of T cells that was seen in the volunteers, not included in information submitted to the regulatory authorities
- calculated starting dose was too high as it was based on the NOAEL approach only, disregarding the pharmacological and immunological effects.

# Discovery Pathology




- Linking many end-points
- Mechanistic studies
- Detailed histopathology /clinical chemistry
- Newer 'omics

# Established and Novel Biomarkers for DILI

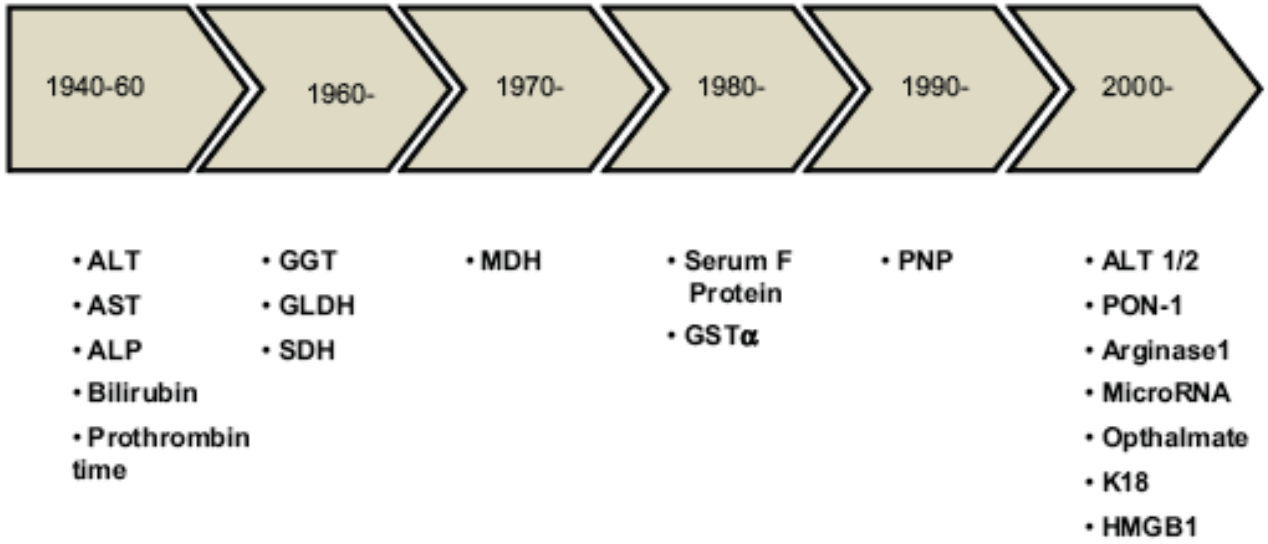



**Table 1.** Standard panel of currently used blood clinical chemistry parameters to assess hepatic function and injury.

| Biomarker | Injury type | Normal reference range |
|---|---|---|
| Alanine aminotransferase (ALT) | Hepatocellular | 5–40 IU $l^{-1}$ |
| Aspartate aminotransferase (AST) | Hepatocellular | 10–40 IU $l^{-1}$ |
| Alkaline phosphatase (AP) | Cholestatic | 30–120 IU $l^{-1}$ |
| Gamma glutamyl-transpeptidase (GGT) | Cholestatic | 0–51 IU $l^{-1}$ |
| Total bilirubin | Hepatic function | 2–14 μmol $l^{-1}$ |
| Direct (conjugated)bilirubin | Hepatic function | 0–4 μmol $l^{-1}$ |
| Pro-thrombin time (coagulation test) | Hepatic function | Local laboratory reference value |

Antoine et al., 2009, Xenobiotica, 39(8): 565–577

# Established and Novel Biomarkers for DILI

**Table 2.** Summary of established and novel biomarkers to drug-induced liver injury (DILI) with organ or mechanism specificity for non-invasive assessment not routinely used as part of a standard clinical liver function test.

| Biomarker | Established/novel | Organ specific | Mechanism specific | Compartment | Reference |
|---|---|---|---|---|---|
| Alanine amino-transferase 1 (ALT1) | Established | Yes — ALT1 | No — necrosis (leakage) | Blood | Lindblom et al. (2007) |
| Glutamate dehydrogenase (GLDH) | Established | No | Yes — mitochondrial damage | Blood | Carakostas et al. (1986) |
| Sorbital dehydrogenase (SDH) | Established | Relatively | No — necrosis (leakage) | Blood | Khayrollah et al. (1982) |
| β-Hydroxy-cortisol | Established | Relatively | Yes — CYP3A4 metabolism | Urine | Park & Kitteringham (1990) |
| Drug mercapturate | Established/novel | Relatively | Yes — potential reactive metabolite | Urine | Wagner et al. (2007) |
| Keratin-18 (K18) — fragmented | Novel | No (epithelial cells only) | Yes — apoptosis not necrosis | Blood | Cummings et al. (2006) |
| Keratin-18 (K18) — full length | Novel | No (epithelial cells only) | Yes — necrosis not apoptosis | Blood | Cummings et al. (2006) |
| Ophthalmic acid | Novel | No | Yes — oxidative stress | Blood/urine | Soga et al. (2006) |
| High mobility group box protein 1 (HMGB1) | Novel | No | Yes — necrosis (not apoptosis) and inflammation | Blood | Scaffidi et al. (2002) |
| Cytochrome c | Novel | No | Yes — mitochondrial damage | Blood/urine | Miller et al. (2008) |
| Serum F protein (HPD) | Novel | Relatively | No — necrosis (leakage) | Blood | Foster et al. (1989) |
| Arginase 1 | Novel | Relatively | No — necrosis (leakage) | Blood | Ashamiss et al. (2004) |
| Malate dehydrogenase | Novel | Relatively | Yes — mitochondrial damage | Blood | Zieve et al. (1985) |
| Purine nucleoside phosphorylase (PNP) | Novel | Relatively | No — necrosis (leakage) | Blood | Ohuchi et al. (1995) |
| Micro RNAs (miRNA) | Novel | Yes — depending on coded protein | No — potential | Blood | Wang et al. (2009) |
| Paraoxonase 1 (PON-1) | Novel | Relatively | Yes — liver function | Blood | Meneses-Lorente et al. (2004) |

# Personalised Medicine / Healthcare

- Tailor medicine to individual
- 'Omics technologies – integration of genetic, protein and metabolic status of individuals
- Prediction of efficacy/toxicity
- Examples:

- TPMT polymorphism and azathioprine/6-mercaptopurine treatment for IBD
- Herceptin screening: HER2 status in cancer patients
- Pharmacometabonomics – prediction of APAP metabolism in humans and rats