GENERAL TOXICOLOGY: NON-RODENT SPECIES IN SAFETY STUDIES AND PLANNING CARCINOGENICITY STUDIES

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EVERY STEP OF THE WAY

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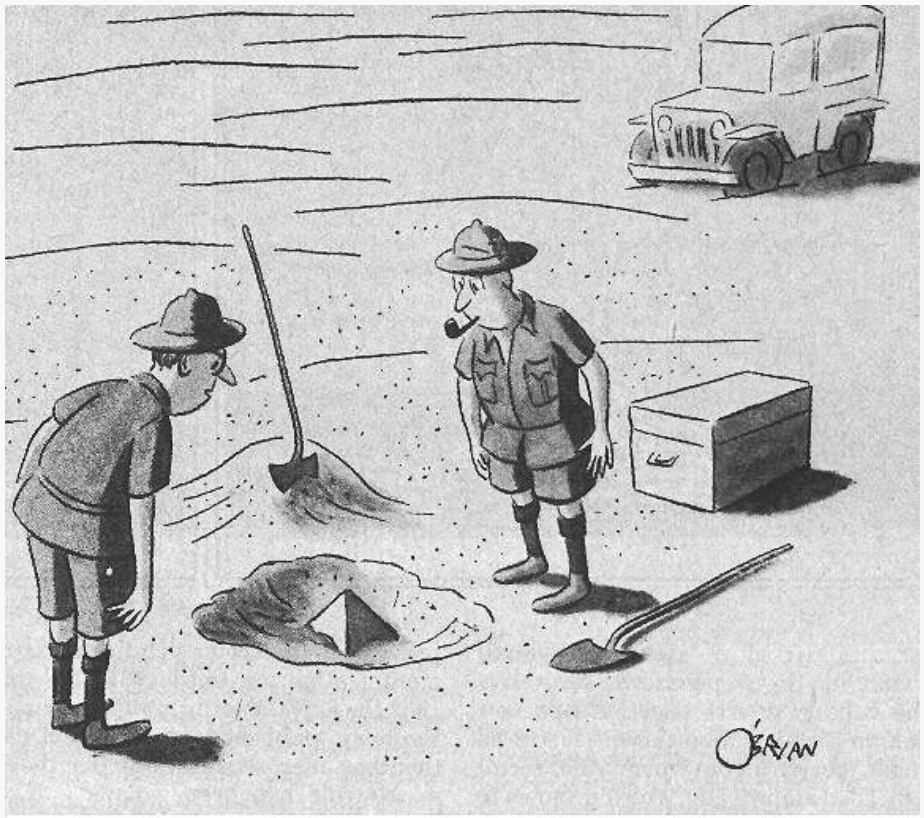
OUTLINE

- Brief overview of non-rodent species
  - Small molecules
  - Biologicals
- Brief overview of planning a carcinogenicity study
  - Planning and study design considerations
  - Transgenic mouse model

THE TOXICOLOGIST WILL SEARCH FOR THE UNEXPECTED



"This could be the discovery of the century. Depending, of course, on how far down it goes."

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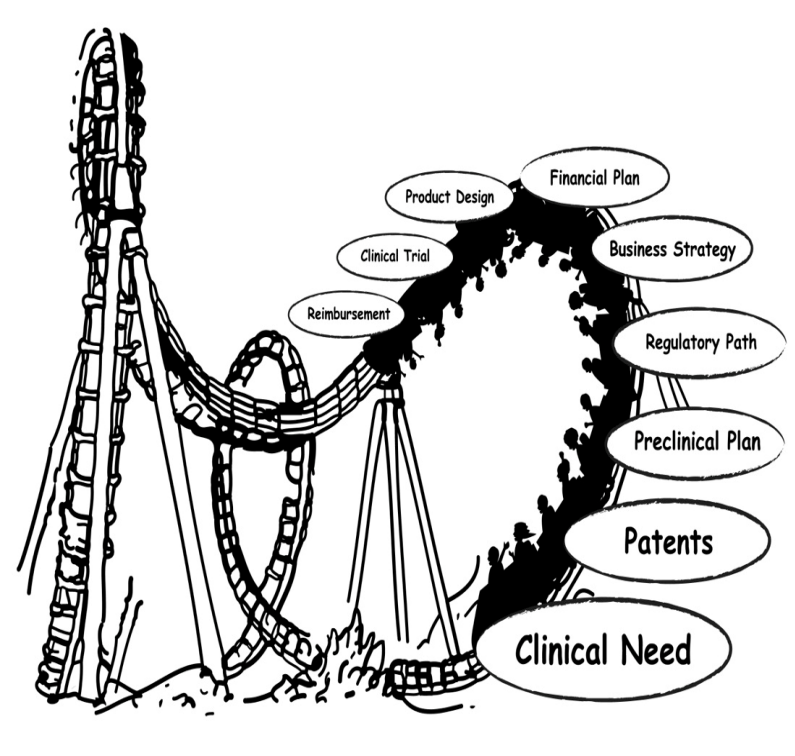
DRUG DISCOVERY AND DEVELOPMENT

How are drugs discovered and developed?

The Roller Coaster Ride to the Clinic!



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NON-CLINICAL DRUG SAFETY EVALUATION

What are we trying to achieve?

For small molecules and biological products the principles remain the same

Safety of the volunteer or patient

GOALS OF NON-CLINICAL SAFETY TESTING

Characterisation of toxicity

- Identify target organs
- Dose dependence and relationship to exposure (toxicokinetics)

Risk assessment

- Safety margins between toxicology and efficacy studies
- Monitorability and reversibility of the observed toxicity
- Mechanism of toxicity
  - Relevance to humans
  - Relationship to pharmacology

Guidance for clinical trials

- Studies designed to characterise potential adverse effects that might occur in the clinical trial to be supported
  - Duration
  - Same route of administration
- Estimation of a safe starting dose & guide dose escalation
- Parameters for clinical monitoring

RISK COMES FROM NOT KNOWING WHAT YOU'RE DOING!

Warren Buffett

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REGULATORY GUIDELINES

Regulatory guidelines are like the London Underground!

There is almost always more than one way to reach an objective and the recommended route might not be the one you should follow!

Guidelines are generally written in order to provide an element of flexibility and not to place undue legislative restraints on scientific progress

NEVER FOLLOW A REGULATORY GUIDELINE IF THERE IS A GOOD SCIENTIFIC RATIONALE NOT TO! GOOD SCIENCE IS FAR MORE IMPORTANT THAN A RIGOROUS ADHERENCE TO A GUIDELINE

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REGULATORY GUIDELINES

- ICH Harmonised Tripartite Guidelines (www.ich.org/products/guidelines.htmlguidelines)
  - M3 (R2) GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS
  - S1A GUIDELINE ON THE NEED FOR CARCINOGENICITY STUDIES OF PHARMACEUTICALS
  - S1B TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS
  - S1C DOSE SELECTION FOR CARCINOGENICITY STUDIES OF PHARMACEUTICALS
  - S2 (R1) GUIDANCE ON GENOTOXICITY TESTING AND DATA INTERPRETATION FOR PHARMACEUTICALS INTENDED FOR HUMAN USE
  - S3A NOTE FOR GUIDANCE ON TOXICOKINETICS: THE ASSESSMENT OF SYSTEMIC EXPOSURE IN TOXICITY STUDIES
  - S3B GUIDANCE FOR REPEATED DOSE TISSUE DISTRIBUTION STUDIES
  - S4 DURATION OF CHRONIC TOXICITY TESTING IN ANIMALS (RODENT AND ON RODENT TOXICITY TESTING)
  - S5 (R2) DETECTION OF TOXICITY TO REPRODUCTION FOR MEDICINAL PRODUCTS & TOXICITY TO MALE FERTILITY
  - S6 (R1) PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS
  - S7A SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICALS
  - S7B THE NON-CLINICAL EVALUATION OF THE POTENTIAL FOR DELAYED VENTRICULAR REPOLARIZATION (QT INTERVAL PROLONGATION) BY HUMAN PHARMACEUTICALS
  - S8 IMMUNOTOXICITY STUDIES FOR HUMAN PHARMACEUTICALS
  - S9 NONCLINICAL EVALUATION FOR ANTICANCER PHARMACEUTICALS
  - S10 PHOTOSAFETY EVALUATION OF PHARMACEUTICALS

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

FDA
U.S Food and Drug Administration

ICH
harmonisation for better health

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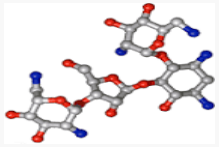
Non-Rodent Species in Safety Studies

SMALL MOLECULES AND BIOLOGICALS ARE DIFFERENT

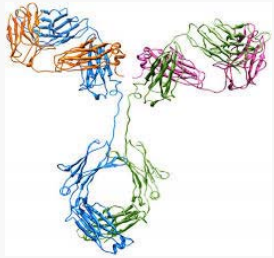
Small Molecules



- Chemically synthesized
- Low molecular weight <1kDa
- May be metabolised to toxic intermediate
- Usually not immunogenic
- Can interact with multiple cells or organs
- Generally active in many species

Toxicity often off-target

Biologicals



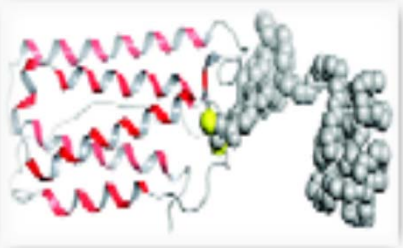
- Derived from living cells
- High molecular weight >5kDa
- Degraded to non-toxic amino acids or peptides
- May initiate immune response to “foreign” protein
- Usually highly targeted to specific cellular receptors
- Activity often limited to species possessing relevant receptors/mechanism of action

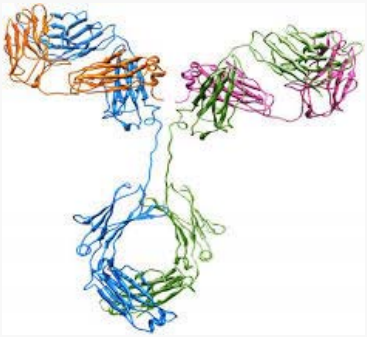
Toxicity often exaggerated pharmacology

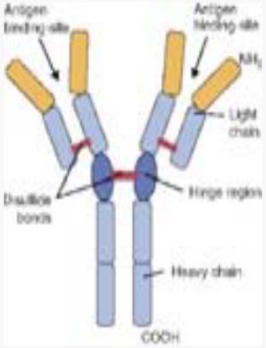
PROTEIN THERAPEUTICS “BIOLOGICS”

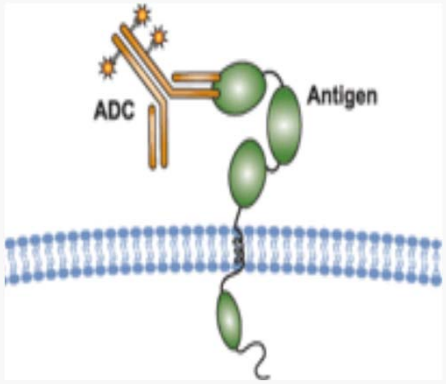
Current generation

Recombinant proteins (hormones, cytokines, growth factors, clotting factors…), pegylated proteins, antibodies (mAbs), antibody fragments (e.g. Fabs), antibody drug conjugates (ADCs)









Next generation

- Engineered antibodies, bispecifics, ADCs, antibody fragments and related products, above proteins with non-natural amino-acids / post-translational modifications (e.g. altered glycosylation pattern), new protein scaffolds (e.g. fibronectins, anticalins, fynomers, ...)

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SPECIES SELECTION

The challenge !!!

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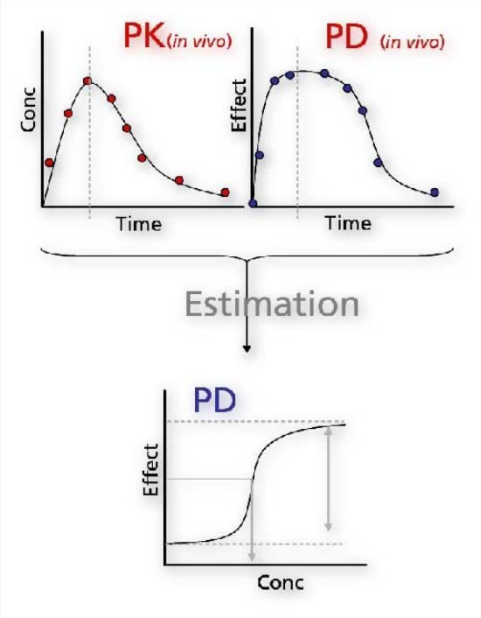
CONSIDERATIONS SELECTING ANIMAL SPECIES

Small molecules

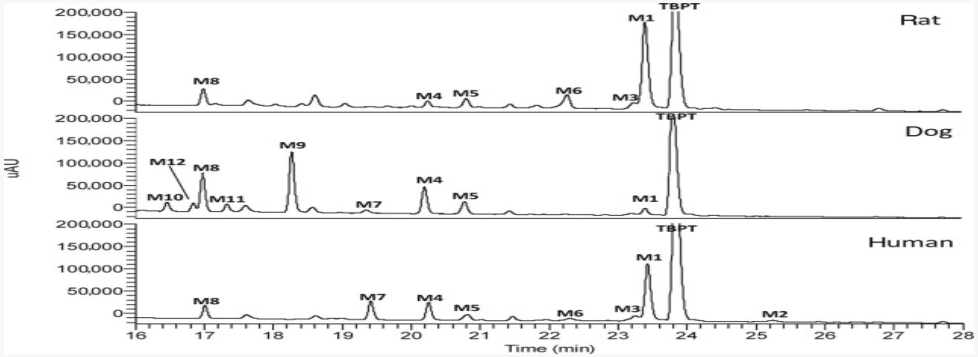


Pharmacologically responsive?

To address concerns around exaggerated pharmacology

Appropriate local and systemic exposure to the drug

- Ensure adequate exposure margins in the relevant tissues/compartments is achievable using the proposed route of administration in man
- Supplement by alternative route if needed and feasible
- Take into account local vs systemic exposure, half-life, exposure profiles, max/min levels, protein binding etc.
- Coverage of human relevant metabolites?
- Compare cross-species man
  - Microsomes
  - Hepatocytes
  - In vivo data



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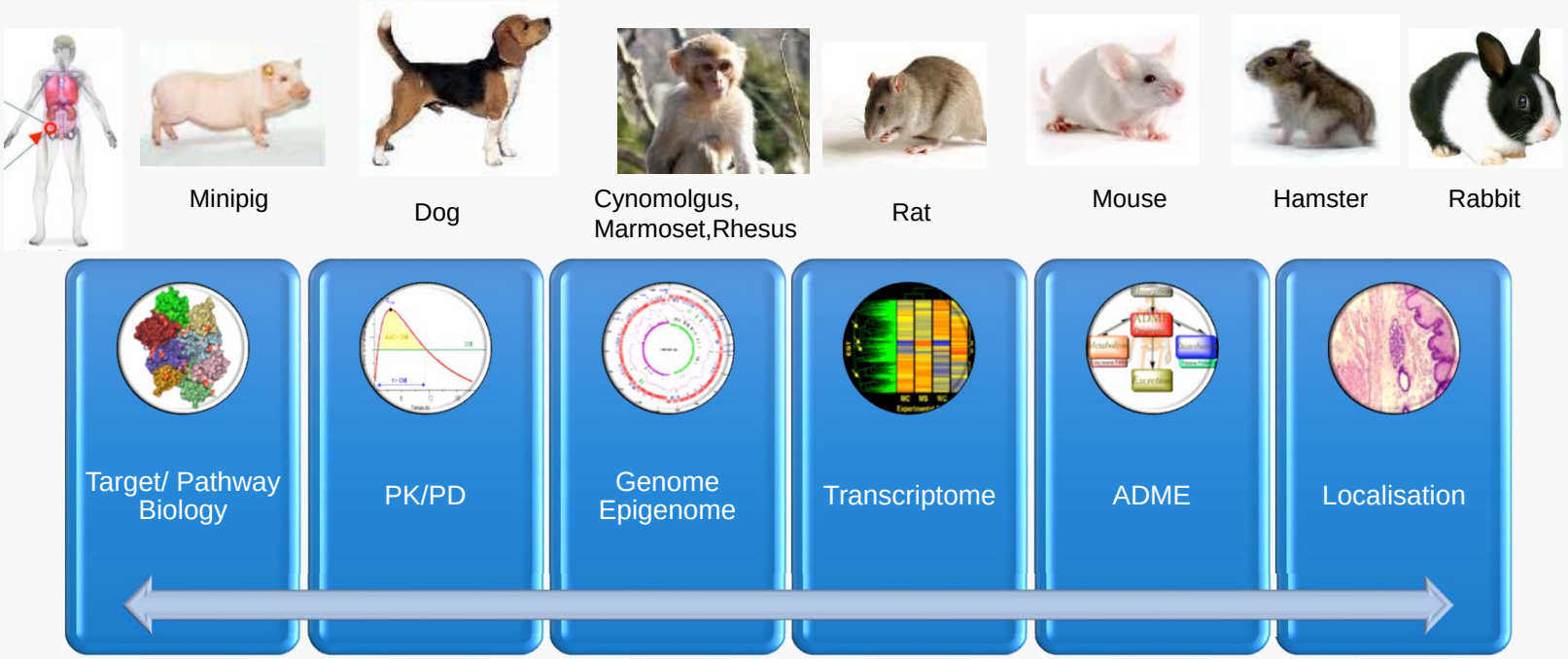
CONSIDERATIONS SELECTING ANIMAL SPECIES

Key drivers of comparative biology

Tissue and species specificity of target pathway biology

- Target specificity, potency and off-target potential
- Characterising PK/PD
- Predicting safety liabilities
- Opportunity for new indications

Comparative Biology at Molecular, Biochemical, Cellular and Tissue Levels



Integration of gene sequence, gene expression (e.g. splice variants, post translational modifications, sub-cellular localisation) **and endogenous protein interactions & functions (e.g. cofactors, enzyme activity) at molecular/ biochemical/cellular/tissue levels**

(Adapted from J Moggs, Novartis)

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HOW TO SELECT THE APPROPRIATE SPECIES FOR SAFETY ASSESSMENT?

Large molecules - Biologicals

ICH S6 (R1) Addendum

'A number of factors should be taken into account when determining species relevancy. Comparisons of **target sequence homology** between species can be an appropriate starting point, followed by in vitro assays to make qualitative and quantitative cross-species comparisons of **relative target binding affinities** and **receptor/ligand occupancy and kinetics.**

Assessments of **functional activity are also recommended. Functional activity can be demonstrated in species-specific** cell-based systems and/or in vivo pharmacology or toxicology studies. Modulation of a known biologic response or of a pharmacodynamic (PD) marker can provide evidence for functional activity to support species relevance.

Consideration of **species differences in target binding and functional activity** in the context of the intended dosing regime should provide confidence that a model is capable of demonstrating potentially adverse consequences of target modulation. When the target is expressed at very low levels in typical healthy preclinical species (e.g., inflammatory cytokines or tumor antigens), binding affinity and activity in cell-based systems can be sufficient to guide species selection.'

REGULATORY TOXICOLOGY TESTING FOR BIOLOGICALS

An overview

ICH S6 (R1) guideline recognises that conventional approaches to toxicity studies used for small molecule drugs are often **NOT** appropriate for biologicals

Does **NOT** provide a “one size fits all” or “cookbook” approach to toxicology study design

- Provides a framework for design of animal studies to address safety of biotechnology derived products, based on characteristics of the product and intended clinical use

Pilot Studies: Early De-risking Strategy Conduct “two-phase” pilots

- Single dose and repeat dose DRF
- Enriched design with clinical pathology, PK, biomarkers (limited or full pathology evaluation)
- Determine the (lower dose) MTD
- Learned toxicity may be cumulative

Allow time for pilot data analysis in program plans

Use data to refine and de-risk GLP plans

PRECLINICAL STUDIES FOR SMALL MOLECULES

Genotoxicity

- Ames test (mutagenicity only sufficient for single dose clinical trials)
- Chromosome aberration or mouse lymphoma
- Rodent Micronucleus Assay

Repeat dose toxicology (rodent and non-rodent)

- Rat single dose and 7-day dose range-finding toxicity study
- Non-rodent maximum tolerated repeat dose study (MTD)
- 14 or 28-day rat toxicity study including TK
- 14 or 28-day non-rodent toxicity study including TK

Safety pharmacology (ICH S7A and S7B)

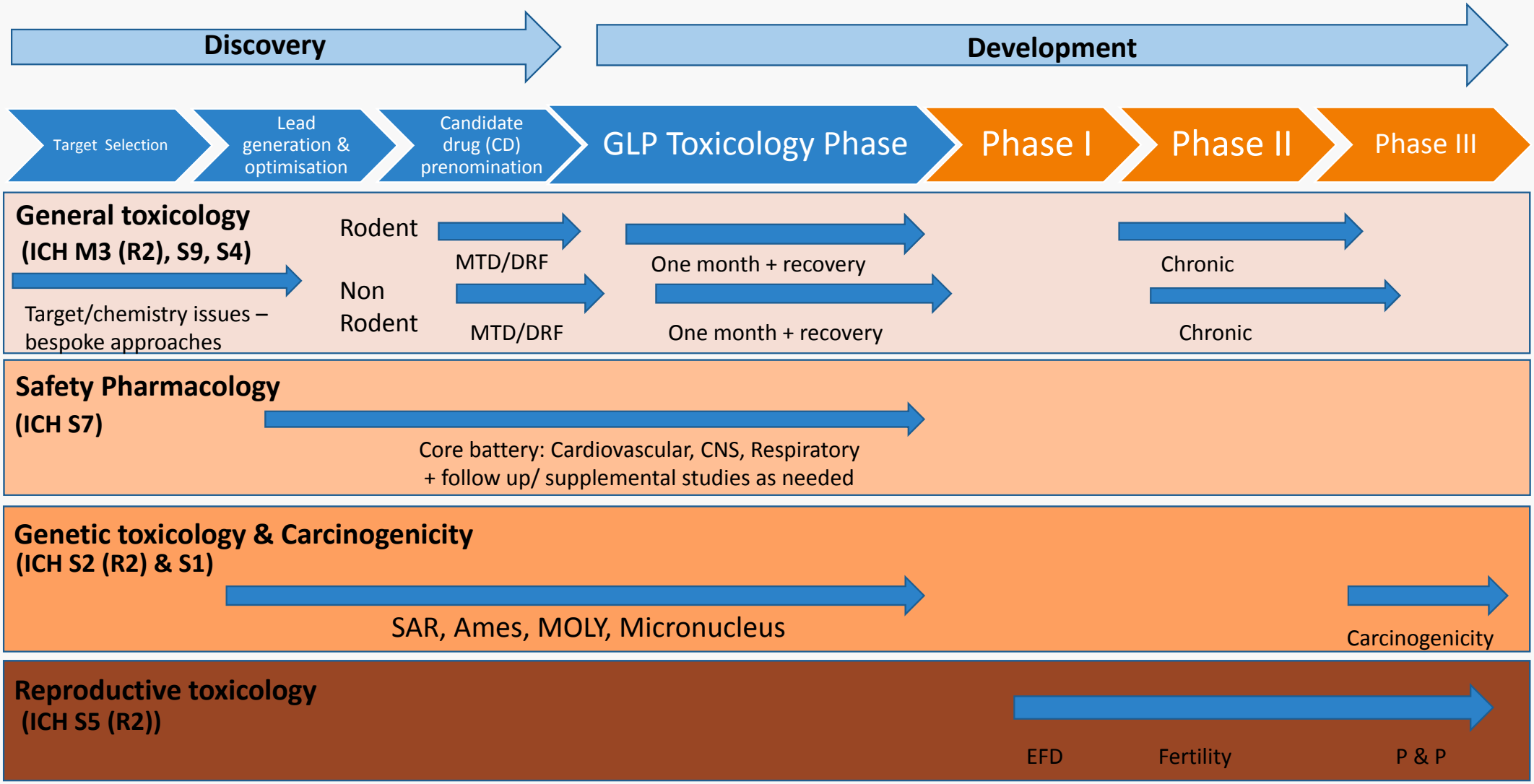
- hERG cardio electrophysiology study
- Cardiovascular telemetry study
- Irwin Screen or Functional Observational Battery (FOB)

ADME

- Confirmation of species suitability (e.g. in vitro metabolic profiling)
- Plasma/RBC binding study (to put hERG results into context)

REGULATORY TOXICOLOGY TESTING FOR SMALL MOLECULES

An overview



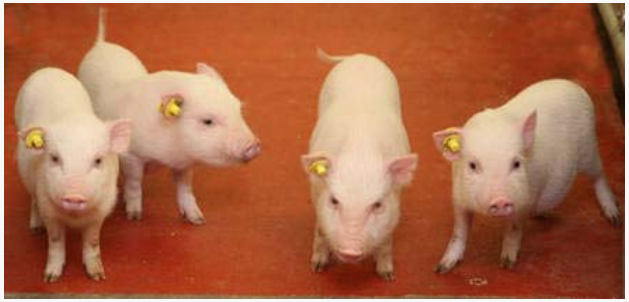
COMMONLY USED SPECIES IN TOXICOLOGY

Domestic Rabbit (Oryctolagus cuniculus)

- New Zealand White Rabbit most common
- Dutch Belted Rabbit – has pigmented skin and eye
- Non-rodent animal model
- Reproductive toxicology
- Ocular toxicology
- Vascular irritation

COMMONLY USED SPECIES IN TOXICOLOGY

Pig (Sus scrofa)



- Minipig breeds
  - Göttingen minipig
  - Yucatan minipig
  - Hanford minipig
  - Sinclair minipig
- Cardiology
- Dermal toxicology
- Organ transplantation and surgical research

COMMONLY USED SPECIES IN TOXICOLOGY

Domesticated Dog (Canis familiaris)

- Most widely used non-rodent research animal
- Cardiovascular and circulatory research
- Oncology research
- Extensive historical database for small molecule development
- Organ transplantation
- Pressures to limit use

SPECIES SELECTION

Non-human primate (NHP)

- Phylogenetic and physiologic homology with humans
- Marmoset and rhesus monkey occasionally used
- Pharmacological activity of biologicals in monkeys often resembles that in humans than dogs, rabbits and rodents
- Primate immune system is similar to humans
- Biologicals less immunogenic following chronic dosing in primates than lower species
  - Antigenicity of humanised protein therapeutics decreased
- Old and New World species
  - Differences in physiology need to be considered
- Costly and limited resource
- Scientific justification for use

NHP identified as most suitable and relevant toxicology non-rodent species for biologicals

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IMPORTANT “MONKEY BUSINESS”

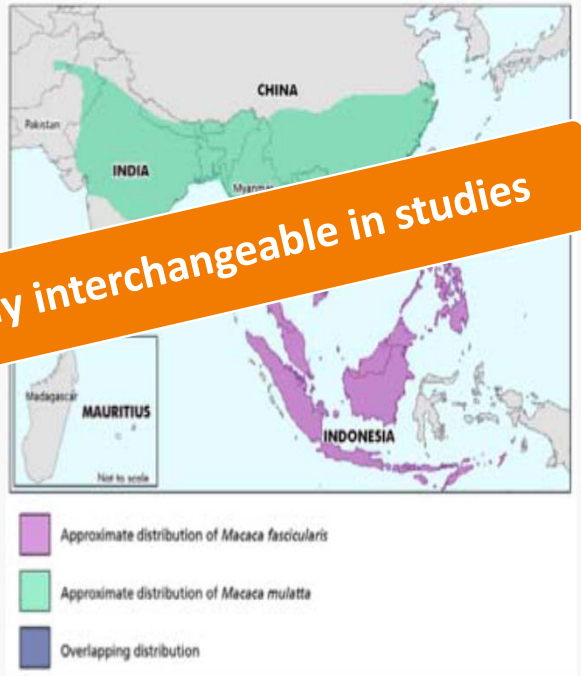
Protein Sequence Differences between Species / Subspecies

10 subspecies of cynomolgus:

Crab-eating Macaque, *Macaca fascicularis fascicularis*, synonym *Macaca irus*

- Burmese Long-tailed Macaque, *Macaca fascicularis aurea*
- Nicobar Long-tailed Macaque, *Macaca fascicularis umbrosa*
- Dark-crowned Long-tailed Macaque, *Macaca fascicularis atriceps*
- Con Song Long-tailed Macaque, *Macaca fascicularis condorensis*
- Simeulue Long-tailed Macaque, *Macaca fascicularis*
- Lasia Long-tailed Macaque, *Maca*
- Maratua Long *ularis tua*
- Ken *Macaca fascicularis karimondjawae*
- Philip Long-tailed Macaque, *Macaca fascicularis philippinensis*

Cynomolgus macaques from different origins are not necessarily interchangeable in studies



While the differences between species are intuitive, there are also variations between subspecies but even between races or breeds as a result of **geographical** distribution or isolation.

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Carcinogenicity Planning

CARCINOGENICITY TESTING

The objectives of carcinogenicity studies

- Identification of carcinogenic properties of a test item
- Identification of target organs
- Characterisation of dose response relationship
- Identification of NOEL or NOAEL
- Prediction of carcinogenic effects of test item at human dose levels

CARCINOGENICITY TESTING

Overview

- Studies in rodents with life time exposure ~ 2 years
- Expense (up to £1 million per bioassay) – depends on complexity of study
- Usually rats and mice (2 studies)
- Large number of animals required (≥ 480 animals/study)
- Tumour incidence – histopathological evaluation of most tissues
- Duration - between study initiation and final report (~3 years)
- General lack of useful mechanistic data
- Options for alternative tests, e.g. 6 month test in transgenic mice models sensitive to tumour development
- ICH S1 revision under discussion
    - Comprehensive and integrated approach to address risk of human carcinogenicity
    - Weight of evidence to justify a waiver request

CARCINOGENICITY TESTING

Things to think about!

- Advisable to conduct studies in the same strain throughout the development programme
- Will carcinogenicity studies be required?
- Survival (regulatory guidelines indicates survival statistics of the strain should be considered)
  - 25 animals/sex/group desired at scheduled necropsy
  - If survival does become an issue talk with FDA
- Availability of historical background data
- Sponsor preference
  - Considerations of partner influence or potential licensing
- Testing laboratory experience with particular rat and mouse strains
- Test item availability
  - Long term stability issues

CARCINOGENICITY TESTING

Study design considerations

Selection of model
- Rat, mouse

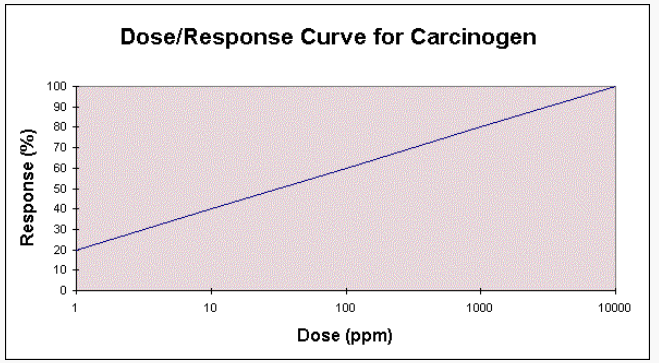
Route of administration
- Intended or expected human exposure

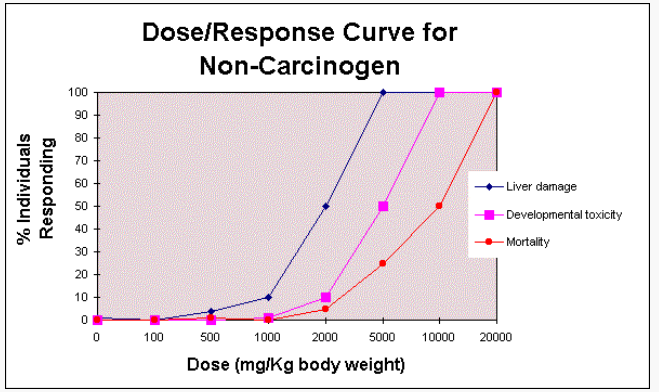
Number of animals
- At least 50/sex/group

Dose groups and dose level selection
- Three treated dose groups, untreated and/or vehicle control
- Dose levels – MTD, 25-fold AUC ratio, dose-limiting pharmacodynamics effects, saturation of absorption, MFD, limit dose

Housing
- Single or group housed

Endpoints and evaluations
- Interim kill, haematology parameters, toxicokinetics





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CHOICE OF RAT STRAIN

Outbred strains

Sprague-Dawley

- Relatively large in size, rapid growth, high fertility, long life spans and genetic diversity that is analogous to human population
- Experience with Sprague-Dawley studies indicates 50/sex/group would not achieve the survival to satisfy the definition of a lifetime study
- We would recommend 60+/sex/group

Han Wistar

- Shown a low rate of mortality and preferable alternative to Sprague-Dawley
- Experience with Han Wistar studies indicates 50/sex/group is sufficient

EXPERIENCE: NUMBER OF RAT STUDIES BY STRAIN (FROM 2010)

Edinburgh

Han Wistar

- Popular choice particularly if carcinogenicity studies part of development programme

Sprague-Dawley

- Still the common choice for reproductive toxicology studies
- Conduct reproductive endpoints (such as male fertility assessment) on Han Wistar toxicology studies

Duration (weeks)	Number of Studies	No. with Sprague Dawley	No. with Han Wistar
4	154	58	96
13	91	16	75
26	20	11	9
104	14	4	10

CHOICE OF MOUSE STRAIN

CD-1® IGS Mouse Crl:CD1(ICR)

Manufacturer: Charles River Laboratories

General multipurpose model

- safety and efficacy testing

Alternative – transgenic model

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TRANSGENIC MOUSE MODELS

Alternatives to the 2 year mouse bioassay

Acceptance by regulatory agencies of three primary transgenic mouse models

- $p53^{+/-}$ model
- Tg.AC model
- rasH2 model

A transgenic mouse with an activated/overexpressed oncogene or an inactivated tumor suppressor gene should be much more susceptible to carcinogens than a normal mouse, resulting in a more rapid induction of tumorigenesis.

Each model has a shorter testing duration (6 months), uses fewer animals (25/sex/group), and costs much less than a standard 2-year mouse carcinogenicity bioassay.

TRANSGENIC MOUSE STUDY

Talk with the regulatory agency

Secure regulatory agency approval before transgenic mouse

A 28-day range-finding study with full histopathology should be performed before actual carcinogenicity study.

Range-finding study in parental strain (e.g. CByB6F1) of the transgenic mouse rather than a more commonly used mouse strain (e.g. CD-1).

For studies intended for FDA submission, the results of the 28-day range-finding study and the proposed protocol for the 6-month transgenic mouse carcinogenicity study should be submitted to the FDA Carcinogenicity Assessment Committee for prior review.

SUMMARY

Guidelines are just that…..guidelines…..**avoid** 'box ticking tox'…..

Do employ innovative, science-based approaches to achieve into man quickly and efficiently while ensuring patient/volunteer safety

And **do** think beyond Phase I – ensure your non-clinical strategy is well set up for future clinical success in Phase II and beyond….

Pick the right animal model(s)

It is wasteful of resources (time, money), unethical from an animal welfare point of view, and potentially dangerous to humans to perform safety testing in an inappropriate animal model

ANY QUESTIONS?

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