Nonclinical safety assessment of potential new medicines:
Toxicology, safety pharmacology and pharmacokinetic considerations

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Candidate to Market

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Outline

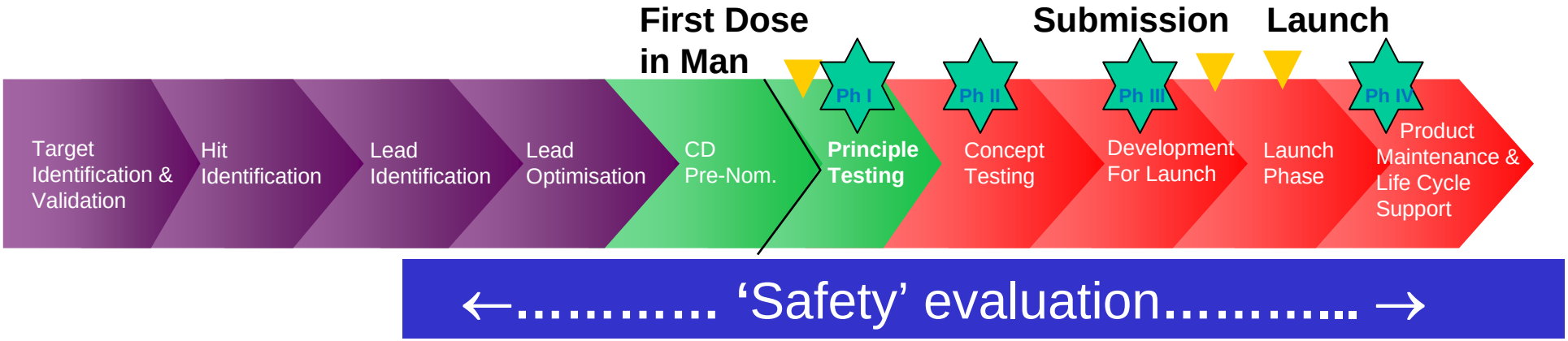
Safety evaluation

- Why is it important in the pharmaceutical industry?
- What studies are needed to support first dose to humans?
- Pharmacokinetic considerations
- Safety Assessment and Risk/Benefit

Safety evaluation in the Pharmaceutical Industry

- Discovery toxicology (CD selection)
- Testing for safety to administer to man (nonclinical, regulatory)
- Safety in man, monitoring adverse events (clinical)
- Safety of workers (occupational)
- Environmental impact
- Notification (transport of intermediates)

Safety evaluation in the Pharmaceutical Industry



Nonclinical

Toxicology : Target organ toxicity.

Safety Pharmacology :
Functional safety, off-target liability

TK: ADME

Clinical

Phase I Healthy volunteers or patients : tolerance, PK, pharmacology (Proof of Principle).

Phase II Early patient studies : tolerance, PK, pharmacology, "Efficacy" (Proof of Concept), dose range, drug interactions, special patient populations (WoCBP, paeds)

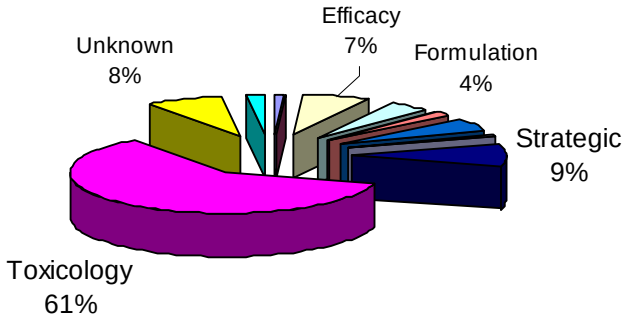
Phase III "Proving trials" double blind efficacy against disease target

Phase IV Post marketing surveillance, Market Positioning

What are the main objectives of nonclinical safety assessment?

- To understand toxicity associated with a compound
- To provide appropriate information for a compound to proceed safely through clinical trials to registration
- Indicate likely risk to man for the indication, patient population, dose and duration required by the clinicians
- A high proportion of CD's cease development before first dose to man because of unacceptable nonclinical toxicity

Toxicity (non-human), in the early development phase, is a major cause for attrition in development



Safety Testing
What needs to be done and when?

- **First Dose in Man is a key Milestone for a new Pharmaceutical**
  - provides human safety/tolerability and PK data
  - can provide efficacy, biomarker activity or proof of principle
  - can be in patients or volunteers
- **Nonclinical safety testing is highly regulated to assure human safety** (especially for healthy volunteers)
- **Regulatory approval needed Regulatory Approval required to dose man**
  - Investigational New Drug (IND) In USA; JIND in Japan;
  - Investigational Medicinal Product Dossier (IMPD) in EU
  - IND/IMPD includes all nonclinical pharmacology, toxicology, DMPK data

WW 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

- S 1A GUIDELINE ON THE NEED FOR CARCINOGENICITY STUDIES OF PHARMACEUTICALS
- S 1B TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS
- S 1C 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 NON RODENT TOXICITY ESTING)

- 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

FDA U.S. Food and Drug Administration

emea

pmda

Good Laboratory Practice (UK, EU, FDA)

- WW Regulatory Authorities will not accept data unless it complies with GLP and all test facilities must have a GLP certificate from a monitoring authority
- GLP ensures accurate recording and reporting of study data using validated methods

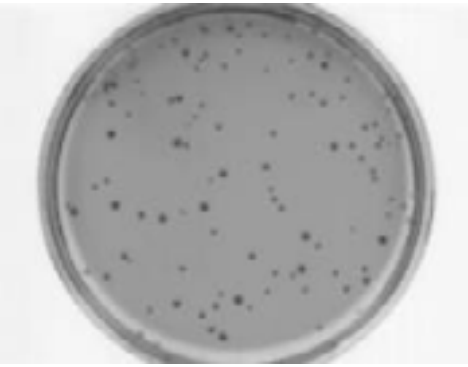
http://www.mhra.gov.uk/Howweregulate/Medicines/Inspectionandstandards/GoodLaboratoryPractice/index.htm

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58&showFR=1

Safety evaluation: requirements for first dose to humans in Phase I

- Genetic toxicology - damage to DNA and chromosomes
- Secondary/Safety Pharmacology - functional changes at multiples of therapeutic dose; potential off-target activity
- General toxicology - target organ toxicity
- Reproductive toxicology? - only if WOCB included in early trials; not normally needed for FTiM
- Others as required
  - e.g. in vitro phototoxicity, local tolerance (i.v.)
- Carcinogenicity studies
  - if compound to be administered continuously for >6 months)
  - not required for FTiM

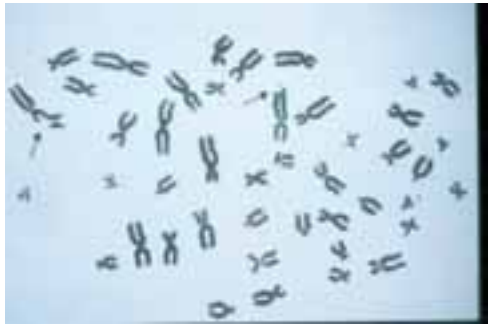
Genetic Toxicology (in vitro and in vivo)



Ames Test (bacterial)

S typhimurium or E Coli containing a defective (mutant) gene making it unable to synthesize the amino acid histidine from the ingredients in its culture medium

The mutation in the histidine (his) operon can be reversed and enables growth in absence of histidine

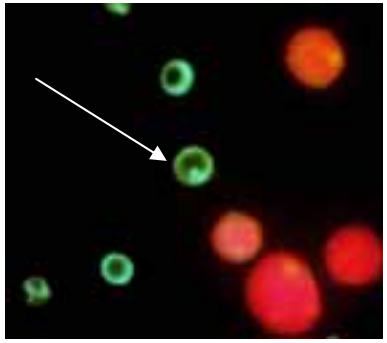


Mouse lymphoma Tk assay:

Mammalian cell equivalent of Ames; but also detects chromosomal damage

Human peripheral lymphocytes

Chromosome breakage



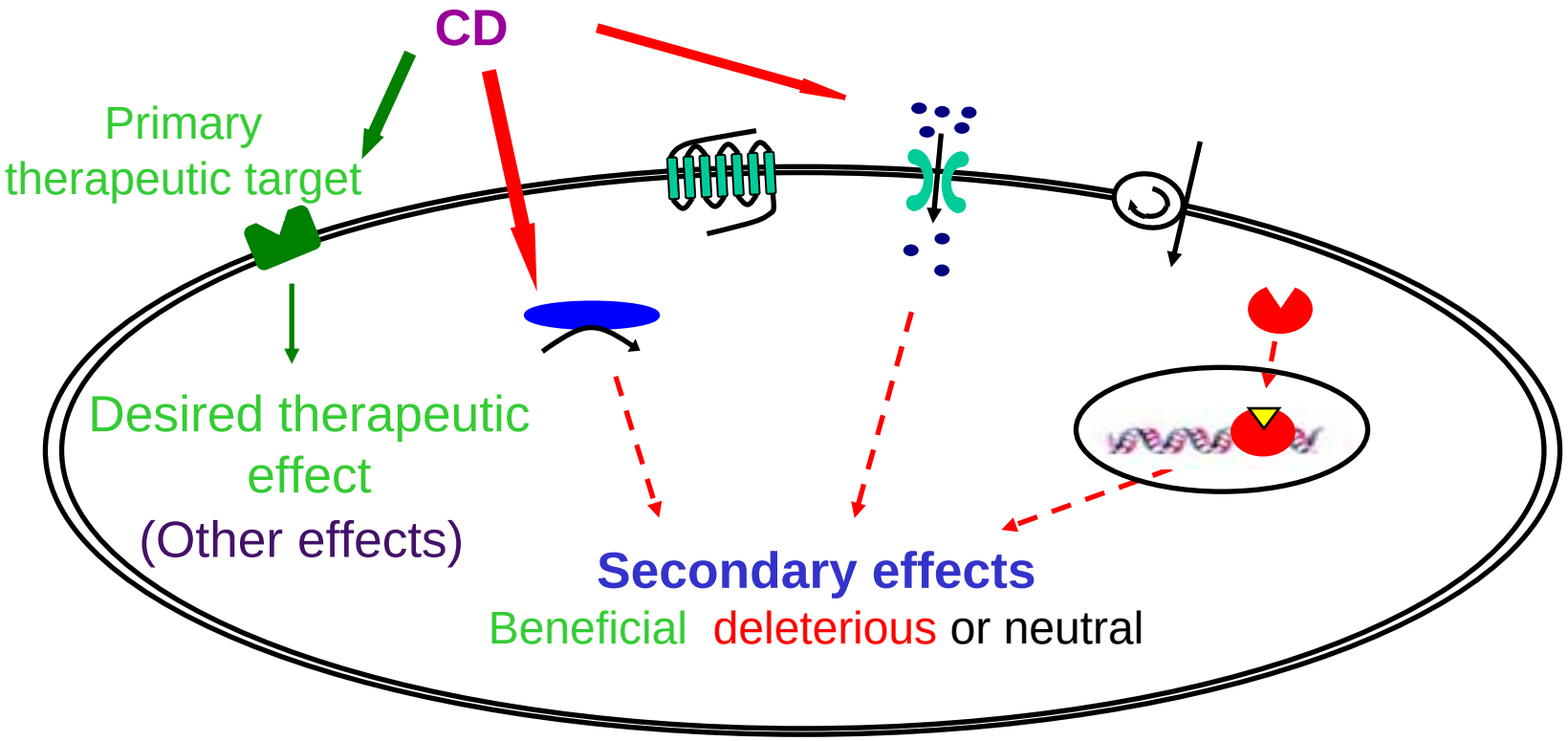
In vivo rodent bone marrow micronucleus test

Micronuclei may be formed by:

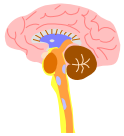
- loss of whole chromosomes during cell division (aneugens)
- chromosome breakage (clastogens)

Secondary Pharmacology studies

- To identify undesirable off-target and/or pharmacodynamic properties of a substance that may have relevance to its human safety
- Use radioligand binding and enzyme assays, covering a diverse range of receptors, ion channels, transporters and enzymes (>350 targets for FTiM), with functional assays to determine if agonist or antagonist (IC_{50})



ICH S7A : Core battery of safety pharmacology studies



Central nervous system

In vivo: motor activity, behavioural changes, coordination, sensory/motor reflex responses, body temperature (e.g. using FOB).

Cardiovascular system

In vivo : blood pressure, heart rate, ECG, repolarization and conductance abnormalities
In vitro (hERG, plus other cardiac ion channels)

Respiratory system

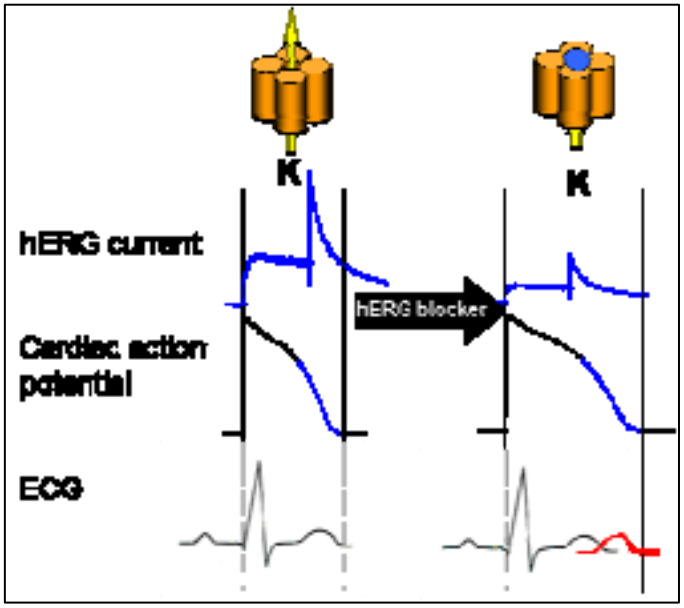
In vivo: Respiratory rate and tidal volume or haemoglobin oxygen saturation.

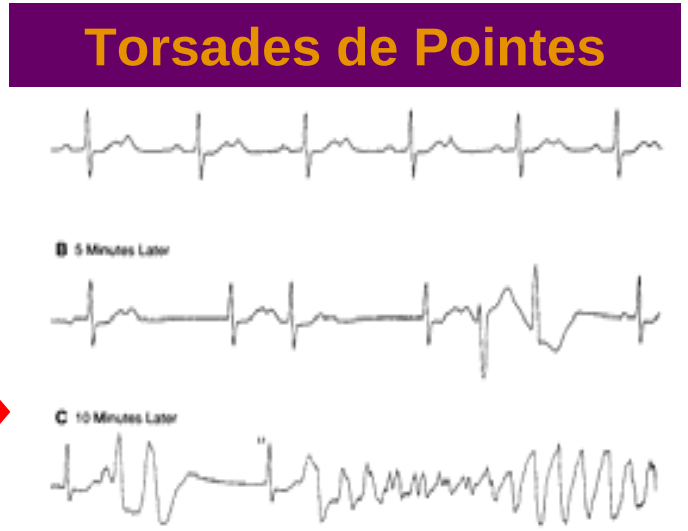
Others

If required (e.g. GI tract motility, Renal function)

Pivotal importance of hERG

- Block of hERG-encoded channel prolongs action potential
- Seen as QT interval prolongation on ECG – confirmed by dog telemetry
- <u>May</u> lead to potentially fatal arrhythmia (Torsades de Pointes (TdP))
- Regulatory and competitive pressure to minimise QT prolongation risk
- Design molecules with low hERG activity





In vivo 'General' Toxicology studies

- Pivotal Repeat dose toxicology studies - target organ toxicity
- Prior to FITM, Regulatory requirement to :
  - examine toxicity in 2 species (rodent and non-rodent)
  - must dose to MTD, Max feasible dose or Limit Dose (1g/kg); usually greatly in excess of proposed therapeutic dose
  - often dose for longer than intended human exposure
- Maximum tolerated dose (MTD):
  'High dose used in toxicity testing that is expected to produce limited toxicity when administered for the duration of the test period. It should not induce (a) overt toxicity, for example appreciable death of cells or organ dysfunction, or (b) reduce the life span of the animals except as the result of neoplastic development or (c) 10 % or greater retardation of body weight gain as compared with control animals'

- Should also define dose levels that cause no significant effects:
  no observed effect level **(NOEL)**
  no-observed-adverse-effect-level **(NOAEL)**

Species selection

- Two species required (default = rat and dog)
- Non-human primates or minipigs recognised alternative non-rodent species
- Biotechnology compounds tend to use primates
- Reproductive toxicology uses rodents and rabbits
- In the absence of human data, they remain the best we have:
  - ILSI consortium (2000) assessed predictivity of animal toxicity data for subsequent human toxicities (HT); rodents predicted ~50% HTs, dogs ~60%, combination both predicted >70%.

Regulatory Toxicology and Pharmacology 32, 56–67 (2000)
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Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals

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Duration of Repeat Dose Toxicology Studies Depends on Clinical Trial Duration

Table 1 **Recommended Duration of Repeated Dose Toxicity Studies to Support the Conduct of Clinical Trials**

Maximum Duration of Clinical Trial	Recommended Minimum Duration of Repeated-Dose Toxicity Studies to Support Clinical Trials	
	Rodents	Non-rodents
Up to 2 weeks	2 weeks[a]	2 weeks[a]
Between 2 weeks and 6 months	Same as clinical trial[b]	Same as clinical trial[b]
> 6 months	6 months[b, c]	9 months[b, c, d]

Table 2 **Recommended Duration of Repeated-Dose Toxicity Studies to Support Marketing**

Duration of Indicated Treatment	Rodent	Non-rodent
Up to 2 weeks	1 month	1 month
>2 weeks to 1 month	3 months	3 months
>1 month to 3 months	6 months	6 months
>3 months	6 months[c]	9 months[c,d]

ICH M3 (R2) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (2009)

Repeat Dose Toxicology (GLP) for FTIM

- Duration of dosing typically 1 month, by clinical route
- 1 Control and 3 Test Groups (low, mid and high dose)
- Dose / bodyweight (mg/kg/day), usually oral by gavage or intravenous
- High dose must be MTD
- Low dose usually a multiple (x 100) of proposed human therapeutic dose, based on free plasma levels, or multiple of ED50 in pharmacology model
- Mid dose selected to establish dose-response relationship
- Mid or Low dose usually = NOEL/NOAEL
- Additional groups to assess recovery over 1 month
- Need to demonstrate exposure (additional rodents for Toxicokinetics)

One Month Repeat Dose Study Designs

Group	Rats (No./Sex)	Dogs (No./Sex)	Dose levels
Main test – dose days 1-28, kill Day 29-30			
1	10 M + 10 F	3 M + 3 F	0 (Vehicle Control)
2	10 M + 10 F	3 M + 3 F	Low dose
3	10 M + 10 F	3 M + 3 F	Mid dose
4	10 M + 10 F	3 M + 3 F	High dose
Recovery – dose Days 1-28, retain undosed and kill Day 57			
5	5 M + 5 F	-	0 (Vehicle Control)
6	5 M + 5 F	3 M + 3 F	High dose
Satellite animals: Toxicokinetics Day 1 and 28			
7	4 M + 4 F	-	0 (Vehicle Control)
8	6 M + 6 F	-	Low dose
9	6 M + 6 F	-	Mid dose
10	6 M + 6 F	-	High dose

Repeat dose studies parameters assessed

	Rat	Dog
Clinical observations; food/water consumption	Daily; starting pre-study	Daily; starting pre-study
Body weights	Daily; starting pre-study	> Twice weekly from pre-study
Ophthalmoscopy	Pre-study, week 4 and end of recovery (week 8)	Pre-study, week 4 and end of recovery (week 8)
ECG/BP	N/A	Pre-study, week 4 and end of recovery (week 8)
Clinical pathology	Weeks 2 and/or 4 and end of recovery (week 8)	Pre-study, weeks 2 and/or 4 and end of recovery (week 8)
Toxicokinetics	Day 1 and 28 (steady state)	Day 1 and 28 (steady state)
Necropsy, OW, BM, Histopathology	Main test (week 5) and recovery kill (week 9)	Main test (week 5) and recovery kill (week 9)

Clinical pathology parameters are useful early indicators of toxicity

HAEMATOLOGY

- Erythrocytes
- Haemoglobin
- Haematocrit
- Mean corpuscular haemoglobin
- Mean corpuscular haemoglobin concentration
- Mean red cell volume
- Red cell distribution width
- Reticulocytes
- Platelets
- Leucocytes
- Neutrophils
- Lymphocytes
- Monocytes
- Basophils
- Eosinophils
- Large unstained cells

COAGULATION

- Prothrombin time
- Activated partial thromboplastin time

OTHERS as required (e.g. hormones)

PLASMA CHEMISTRY

- Albumin
- Albumin/globulin ratio
- Alanine aminotransferase
- Alkaline phosphatase
- Aspartate aminotransferase
- Bilirubin (total)
- Calcium
- Cholesterol
- Creatinine
- Troponin T or I
- Glucose
- Glutamate dehydrogenase
- Phosphate (inorganic)
- Potassium
- Sodium
- Total protein
- Triglycerides
- Urea
- Creatine kinase

URINE CHEMISTRY

- Appearance
- Volume
- Specific gravity
- PH
- Protein
- Glucose
- Total protein (quantitative)
- Ketones
- Bilirubin
- Blood
- Cytological examination, urinary sediment
- Urinary protein electrophoresis

Typical 1 month study – tissues examined

Adrenal glands
Aorta (thoracic)
Bile duct
Bladder (gall)
Bladder (urinary)
Brain
Cervix
Epididymides
Eyes
Femoral head (bone and marrow)
Harderian gland
Heart
Intestine - duodenum (pyloric sphincter)
Intestine - jejunum
Intestine - ileum
Intestine - caecum
Intestine - colon
Intestine - rectum
Kidneys
Lacrimal glands
Larynx
Liver
Lungs
Lymph node – axillary, mandibular,
Lymph node - mesenteric
Muscle - skeletal
Nerve – sciatic
Skin/site of mammary gland (abdominal)
Oesophagus
Optic nerves
Ovaries
Pancreas
Parathyroid glands
Pituitary
Prostate
Salivary gland – parotid
Salivary gland –sublingual
Salivary gland – submandibular
Salivary gland – submaxillary
Seminal vesicles
Spinal cord (lumbar and cervical)
Spleen
Sternum (bone and marrow)
Stomach
Testes
Thymus
Thyroid glands
Tongue
Trachea
Uterus
Vagina
Abnormal tissues

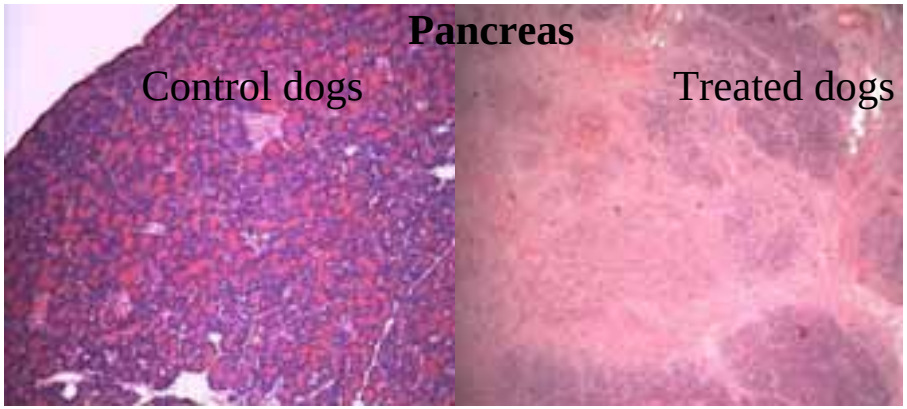
Rat only
Dog only
Both

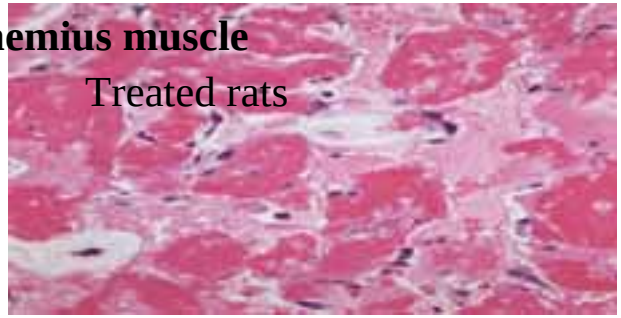
Tissues sectioned, slides stained (H & E, special stains) and examined by light microscopy and EM if required

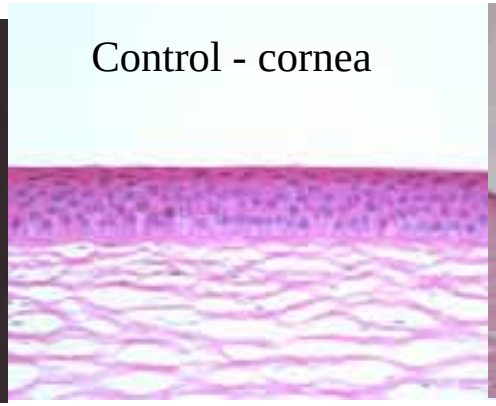
Pathology is key data

- In-life data can ‘suggest’ possible target organ toxicities, but some biochemical markers are less specific than others. For some changes, no in-life markers are available.
- Histopathology usually determines whether drug can be used in volunteers or patients, or whether compound can progress at all
- Can influence clinical study design (exclusions, monitoring)
- Showing reversibility of pathological lesions very important

Pathology = Key data on toxicology studies







IRESSA : approved in 36 countries for treatment of patients with advanced NSCLC.

Reproductive Toxicology

- Women of child bearing potential (WOCBP)
  - usually excluded from Phase I trials but included in Phase II/III
  - could include post-menopausal women and/or adequate contraception
- Aim: to define adverse effects on reproductive function at doses known to be toxic to the animal

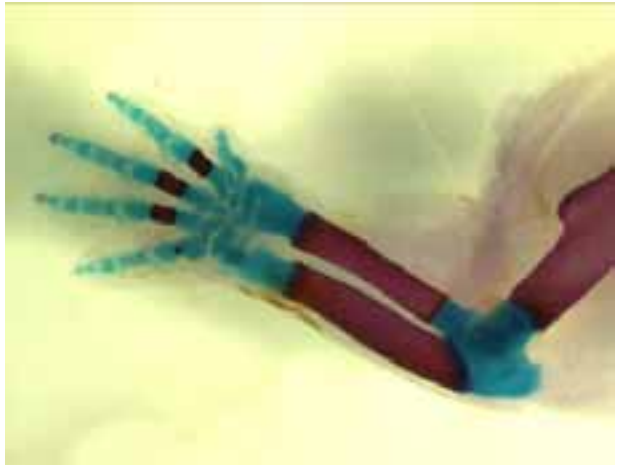
ICH 6 stages from development to sexual maturity :

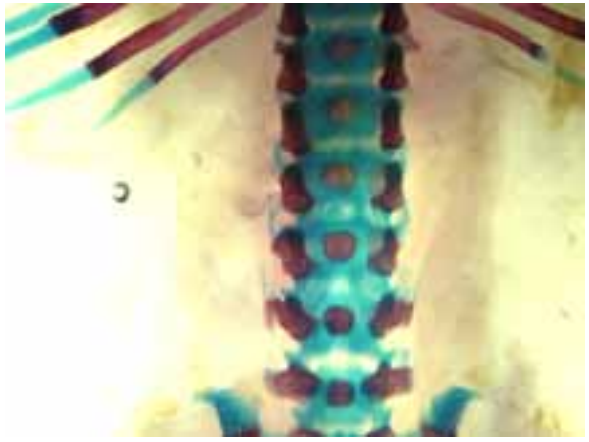
A - Premating to conception
B - Conception to implantation
C - Implantation to closure of the hard palate
D - Closure of the hard palate to the end of pregnancy
E - Birth to weaning
F - Weaning to sexual maturity

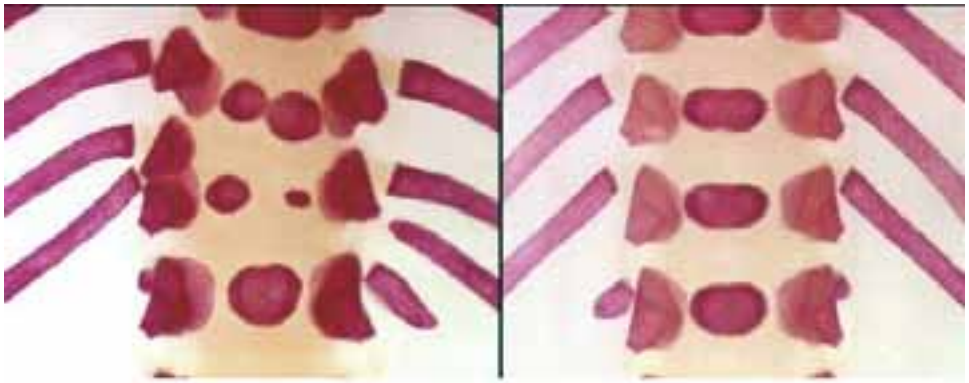
Typically covered by 3 types of study

- Fertility (rodent)
- Embryo-fetal development (rodent and rabbit)
- Pre-and post natal development (rodent)

Foetal Examinations

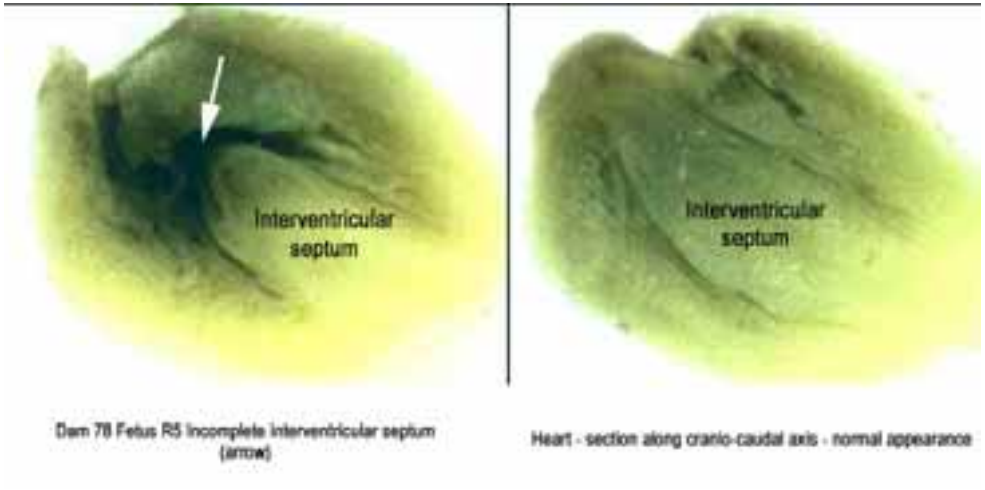






Dam 73 Fetus R6 Absent 13th thoracic centrum, absent 13th right neural arch, fused 12th and 13th left neural arches, 13th bilateral ribs arising from same neural arch (dorsal aspect)

Normal thoraco-lumbar vertebrae (dorsal aspect)



Dam 78 Fetus R5 Incomplete interventricular septum (arrow)

Heart - section along cranio-caudal axis - normal appearance

Pharmacokinetic considerations

ADME studies prior to FTiM conducted to:

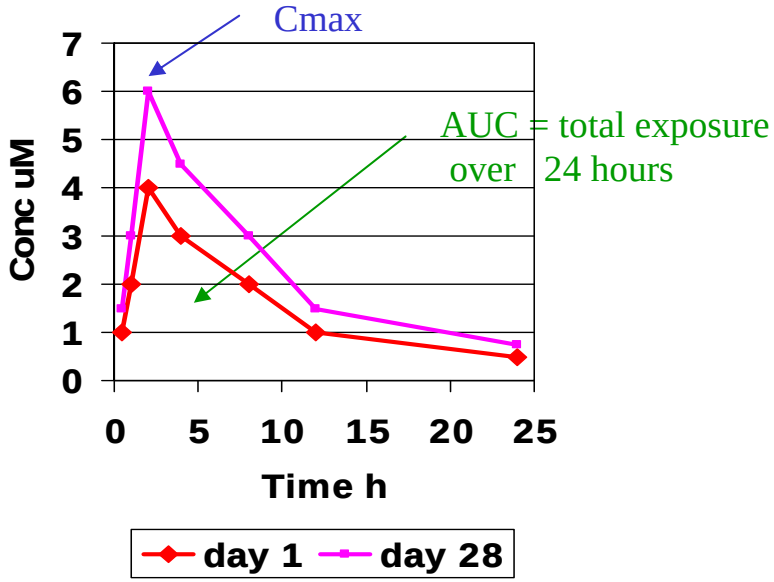
- Demonstrate exposure of animals to drug
- Confirm that toxicology species chosen are relevant to man (metabolic routes) and exposed to any metabolites expected to be seen in man
- Explore drug behaviour (metabolism and kinetics) in pre-clinical species and inform predictions to man
- Understand routes of elimination and tissue distribution
- Predict potential drug-drug interactions
- Understand effects on efflux/influx transporters
- Determine which CYPs are responsible for drug metabolism plus any potential for CYP induction/inhibition
- Determine cross-species plasma protein binding (only unbound drug can interact with target)

Toxicokinetic blood sampling

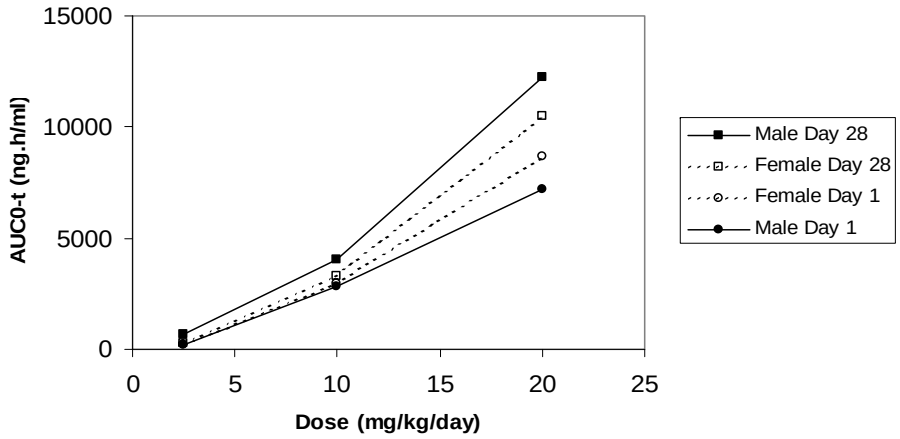
Included in pivotal studies :

- Evidence that dosed animals are exposed to drug, control animals were not
- Information on multiple dose kinetics (accumulation or effects on absorption or clearance) and dose proportionality/linearity
- Peak plasma levels or total exposure can produce different toxicities

e.g. Accumulation on repeat dosing



e.g. Lack of linearity – more than proportional increase in AUC with dose.

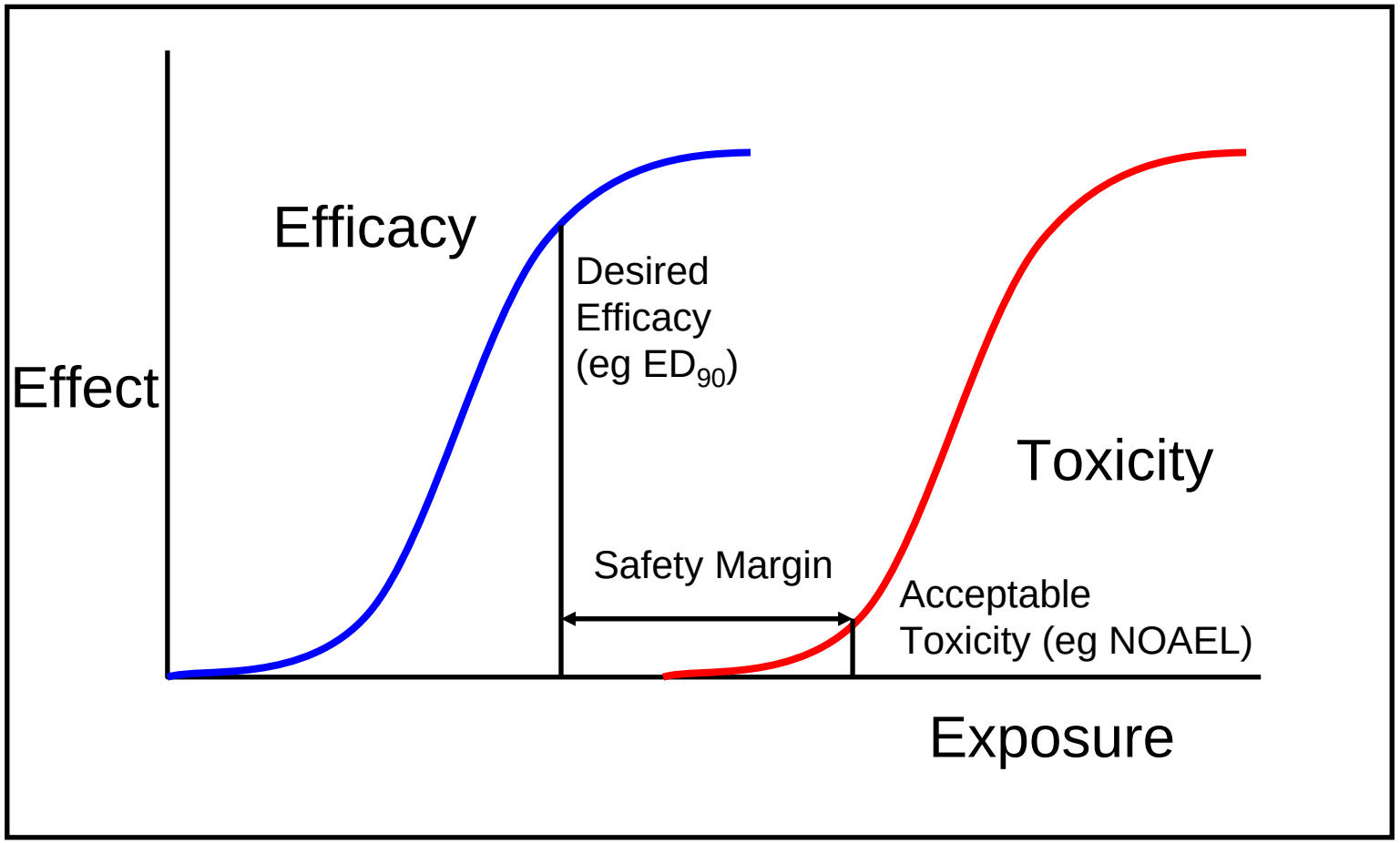


Safety/Risk Assessment

- Before first dose to man, need to consider:
  - What doses produce (a) toxicity and (b) no effects in animals ?
  - Were the animal models relevant for human toxicity ?
  - Did effects differ following single and multiple dosing ?
  - Did exposure change following single and multiple dosing ?
  - Were the toxic responses reversible ?
  - What were the target organs/systems ?
  - Are there biomarkers to enable clinical monitoring ?
  - Was the toxicity expected for this chemical class ?
  - Are toxic metabolites produced ?

- Risk assessment depends on therapeutic indication, patient population

Safety margins



For benign indications, 100-fold margin may be appropriate

For terminal conditions, much lower margin may be acceptable

Dose for first human volunteer trial based on no effects doses:

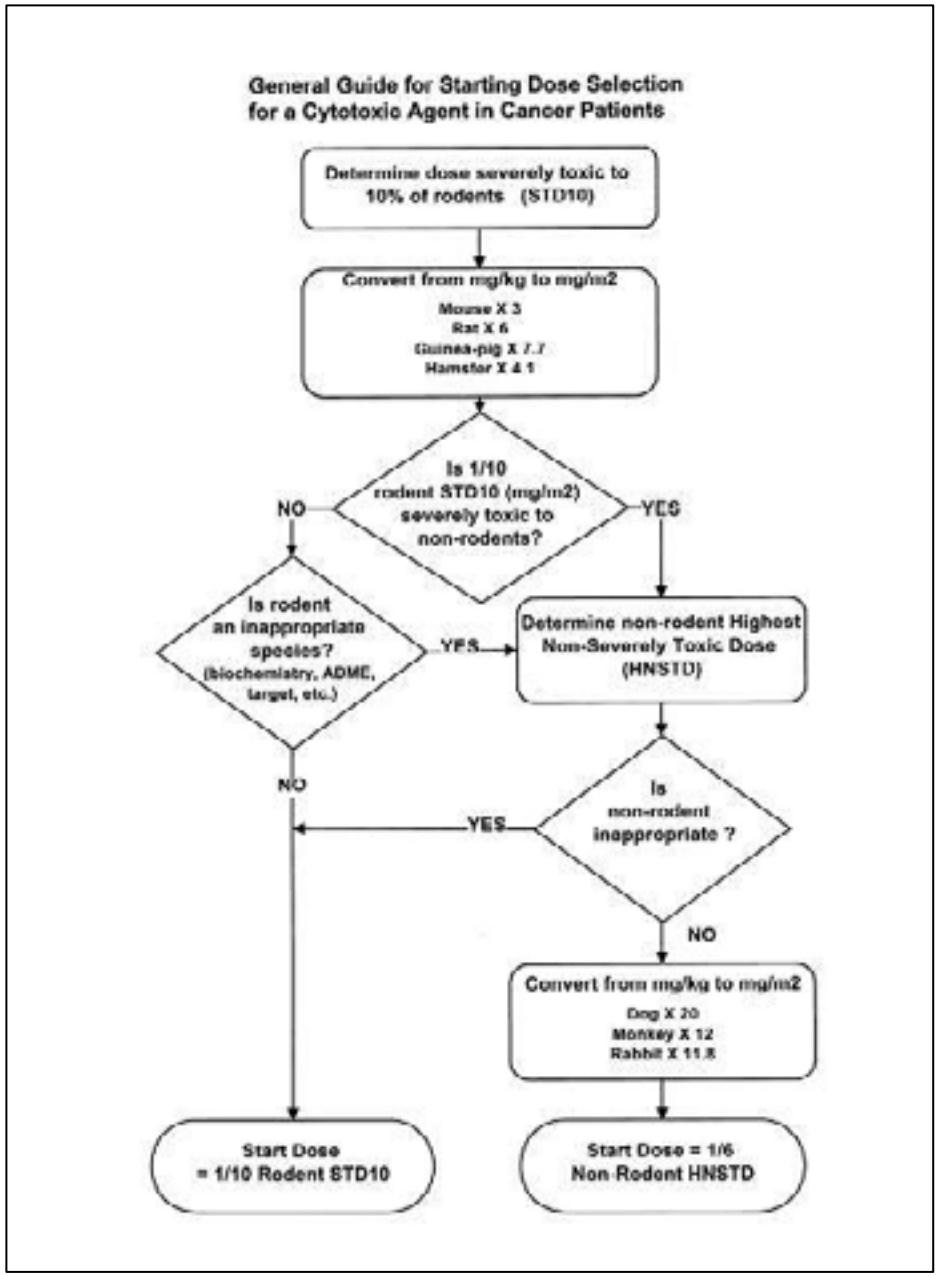
Apply safety margin of at least 10 to HED at NOEL in most sensitive species.

FDA may expect much >10 fold margin, if severe toxicity, steep dose response, irreversible or non-monitorable toxicities etc.

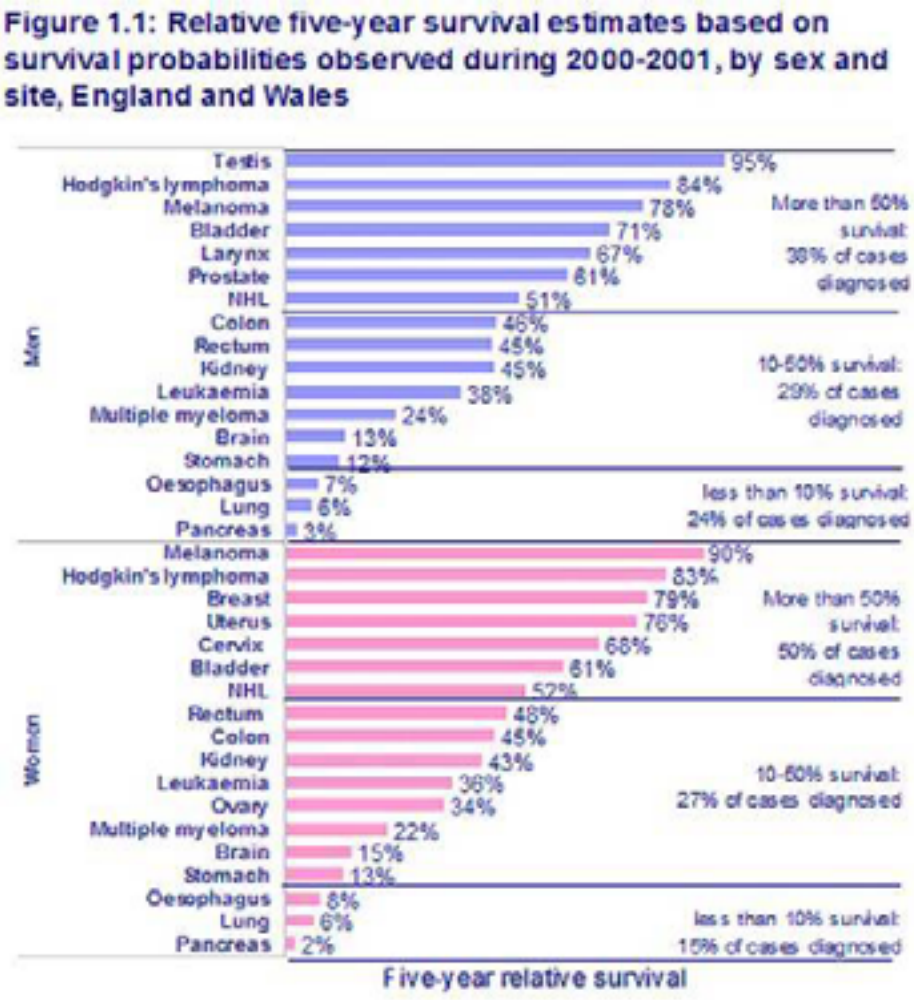
http://www.fda.gov/cber/gdlns/dose.pdf

Dose for first human cancer patient trial can be based on toxic findings :- FDA flowchart guide for cytotoxics start dose in cancer patients

www.fda.gov/cder/cancer/docs/doseflow.pdf



Risk/Benefit assessments



Risk : Benfit : greater risk of treatment-related toxicity is acceptable in oncology because of life-threatening disease

For some anti-cancer drugs, efficacy often occurs at close to toxic doses. Phase I can start with small safety margins and dose to MTD

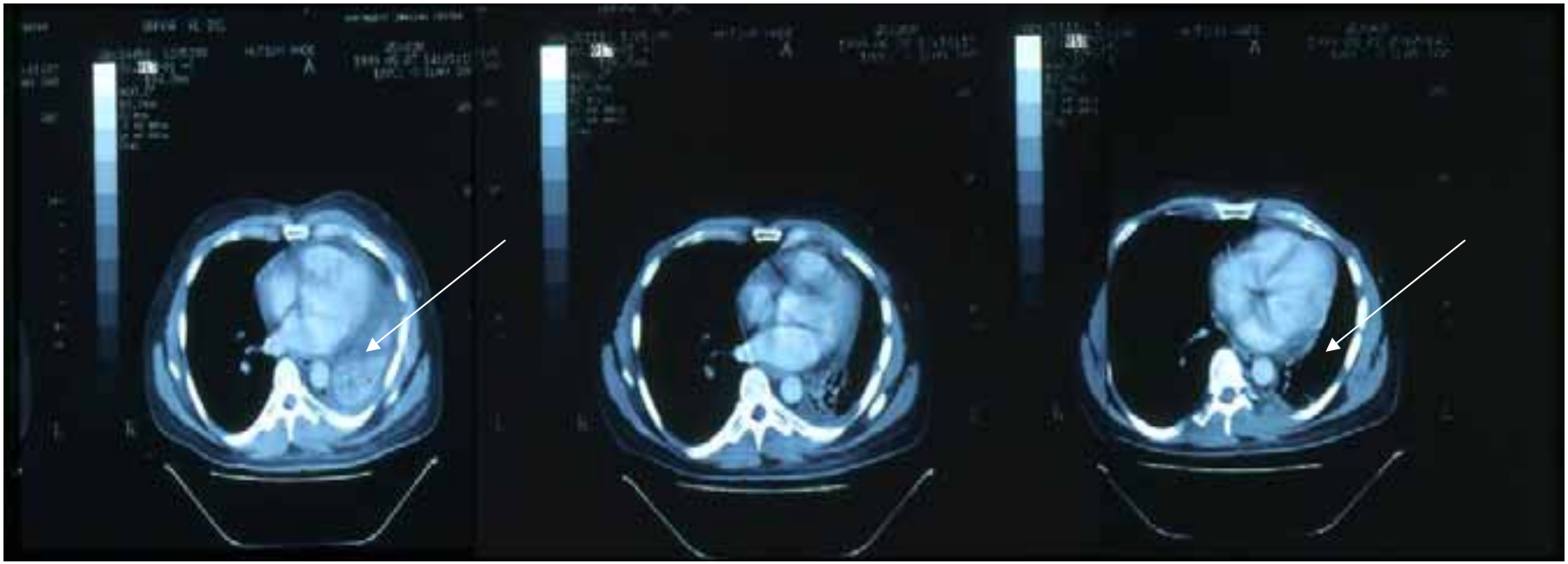
Risks - Clinical AEs with Platinum cytotoxics

Clinical toxicity	Cis	Carbo	Oxali
Nausea/vomiting	+++	++	++
Bone marrow suppression (neutopenia, thrombocytopenia, anaemia)	+	+++	+
Renal dysfunction/tubular damage – electrolyte disturbances	+++	+	- ?
Neurotoxicity (sensory peripheral neuropathy, loss of deep tendon reflexes, numbness)	+++	++	+++
Ototoxicity (tinnitus, bilateral high-frequency hearing loss)	+++	+ ?	-
Retinal toxicity (visual disturbances, blurred vision)	++	- ?	?
Hypersensitivity/anaphylaxis (rash, GI discomfort, bronchspasm, tachycardia)	++	++	++

But benefits can out-weigh the risks

45 y.o with Refractory NSCLC

Pretreatment **3 Months**



Concluding remarks

- It's a regulatory expectation that we characterise the toxicity profile for a compound before dosing to man
- One month studies in 2 species are key to really understanding the safety issues for a compound.
- Toxicology and PK data is used to help set a safe starting dose for volunteer or patient Phase 1 trials.
- Understanding target organ toxicity assists in Phase 1 study design
  - which patients to exclude
  - what monitoring is required in clinic (e.g. LFTs, ECGs etc)
  - useful in oncology trials as humans often dosed to MTD
  - important to define reversibility/recovery

- Nonclinical safety testing has a key role in drug discovery and development

Thank you all for your attention

…. any Questions ?