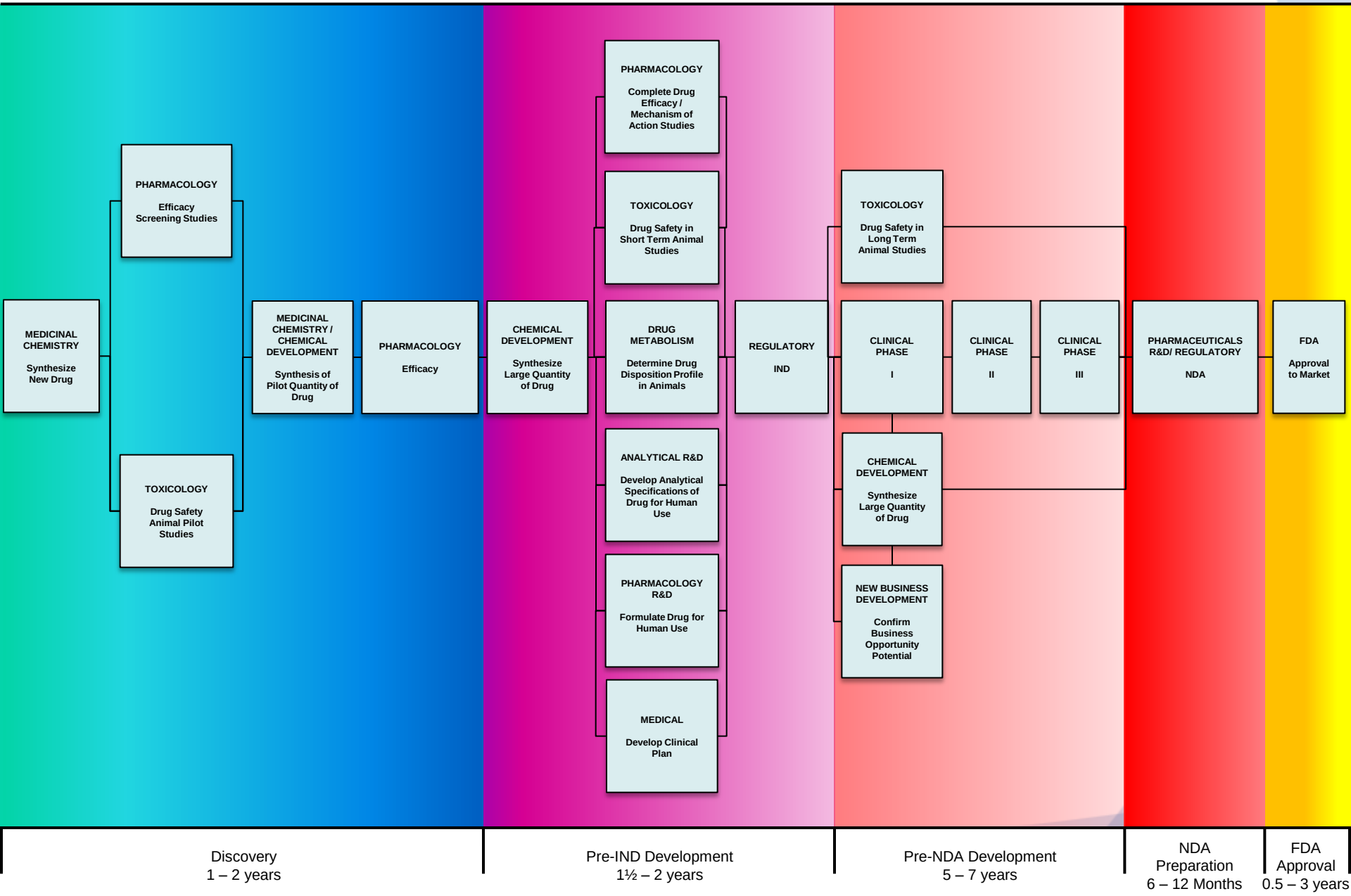
Animal Use for Testing Purposes

Drug Development Process



Test Guidelines

- The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Safety Topics Guidelines
- ICH Harmonised Tripartite Guideline S6. Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.
- Organisation for Economic Co-operation and Development, OECD Guidelines for the Testing of Chemicals / Section 4: Health Effects
- Japanese Ministry of Health, Labor, and Welfare, 1990 Guidelines for Toxicity Studies of Drugs Manual
- European Medicines Agency (EMEA) CPMP/CHMP guidance and points-to-consider documents

Test Guidelines (Continued)

- FDA Center for Food Safety and Applied Nutrition, FDA Redbook
- The FDA Center for Drug Evaluation and Research (CDER) provides no specific test guidelines
- US EPA Office of Prevention, Pesticides and Toxic Substances 870 Series (Health Effects Test Guidelines)
- ICH Harmonised Tripartite Guideline S8. Notes for Guidance on Immunotoxicity Testing of Human Pharmaceuticals.

Role of Regulatory Agencies

- Agencies' role is to protect health requiring proper evaluation to approve or restrict the use of compounds

- Testing standards and requirements are not static and do evolve

Duration of Preclinical Toxicology Studies

- The duration of preclinical toxicology studies is dependent on the intended duration of clinical trials.

- The duration of required preclinical repeated-dose toxicology studies is specified by ICH Guideline M3 (R2), *Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals.*

Drug Discovery and Development

- Efficacy
  - Does the compound impact the disease state?
    - Animal models of disease
    - In vitro animal and human preparations
    - Clinical assessment in patients (Phase II, III, IV)

- Safety
  - Does the compound have unwanted (side) effects
    - Normal animal models
    - In vitro animal and human preparations
    - Clinical assessment in healthy volunteers (Phase I) and patients (Phase II, III, IV)

Toxicology Study Durations

- Durations needed to support Phase I, II and III trials

Duration of Clinical Trial	Minimum Duration of Repeat Dose Toxicity Studies	
	Rodents	Non-Rodents
Single dose	2-4 Weeks	2 Weeks
Up to 2 Weeks	2-4 Weeks	2 Weeks
Up to 1 Month	1 Month	1 Month
Up to 3 months	3 Months	3 Months
Up to 6 months	6 Months	6 Months
> 6 Months	6 Months	9 Months

Requirements for Non-Clinical Safety Package in Support of First in Human Studies

	Europe: EMEA Position Paper on microdosing	Europe: ICH M3 Guidance	US: Single dose acute toxicity guidance (ICH M3)
Toxicology			
Study design and duration	Single dose with extended observation in 1 species (must be comparable to human) up to limit dose (1000x)	2 week repeat toxicology in 2 species (one non-rodent)	Single dose toxicology with extended observation in 2 species (one non-rodent)
Clinical route	[Generally] 2 routes including the clinical route (one IV)	2 routes including the clinical route (one IV)	2 routes including the clinical route (one IV)
Safety Pharmacology			
	Assessments from toxicology study plus “all available information from screening”	Standard battery (CNS; CV; respiratory)	Standard battery (CNS; CV; respiratory)
Genotoxicity			
	Mutation and chromosomal damage (abridged testing acceptable if NCE from a well known class)	Mutation and chromosomal damage	Mutation and chromosomal damage

charles river

Refinements in Animal Testing – Applying the 3Rs

- Study Design modifications
  - Reduction in number of animals required for TK sampling
  - Reduce the number of animals required for the recovery phase
  - Minimize the volume of blood required for assessment
  - Reduce fasting times or eliminate fasting times

Applying the 3Rs

How could we reduce blood volume requirements in order reduce population?

- Optimization of analytical procedures
    - combine with capillary microsampling (blood sample or dried blood spot)
- Reduce TK collection timepoints to 6 instead of 8
- Start with slightly older/larger animals 7 to 8 weeks at start of treatment instead of average of 6 weeks at dose initiation (permissible blood volumes ↑ by 33 to 50% for females and males, respectively)
- For mice, consider feasibility of collection of one TK timepoint collected in vivo and second one terminal along with ADA sample collected at termination
- Use additional matrices to assist exposure assessment eg. Tissue samples (biopsy or terminal).
- Performing preliminary repeat (2 dose) study to assess potential for immunogenicity?

Typical Design with inclusion of PK, PD and Immunogenicity Endpoints

Group	Dose Level	Animal Numbers					
Identification	(mg/kg/dose)	Main and Recovery		Toxicokinetic (and Immunogenicity)		Pharmacodynamic	
		Males	Females	Males	Females	Males	Females
Control	0	10 + 5	10 + 5	3	3	-	-
Low dose	1X	10	10	9 / 6 / 3	9 / 6 / 3	3 / 0	3 / 0
Mid dose	3X	10	10	9 / 6 / 3	9 / 6 / 3	3 / 0	3 / 0
High dose	10X	10 + 5	10 + 5	9 / 6 / 3	9 / 6 / 3	3 / 0	3 / 0

Total population = 80/sex = 160 rats if reduce TK timepoints from 8 to 6 = 10% reduction
Total population = 62/sex = 124 if start study with slightly older rats (7 to 8 weeks old) and 6 time points; assumes PD sampling done from main study/recovery rats = 30% reduction
Microsampling could mean elimination of TK population/IMG population = 44% reduction

charles river

Possible Changes in Design for the Large Animal Pivotal Repeat Dose Toxicology Study to Support Early Clinical Development

Dose group	Number of animals in main dose groups		Number of animals in recovery groups		Number of animals
Historically					
Control	4 M	4F	2M	2F	48
Low	4M	4F	2M	2F	
Mid	4M	4F	2M	2F	
High	4M	4F	2M	2F	
Current situation					
Control	3 M	3F	2M	2F	40
Low	3M	3F	2M	2F	
Mid	3M	3F	2M	2F	
High	3M	3F	2M	2F	
Proposal 1					
Control	3 M	3F	2M	2F	32
Low	3M	3F	0	0	
Mid	3M	3F	0	0	
High	3M	3F	2M	2F	
Proposal 2					
Control	3M	3F	2M	2F	26
Low	3M	3F	0	0	
High	3M	3F	2M	2F	

M= male F=female

Refinements in Animal Testing

- Housing for Rodents
  - Shift from single housed rodents to social housing
  - Change from stainless steel grid cages to poycarbonate bins with bedding
  - Addition of chewing blocks, nylabones
  - Addition of nesting material
  - Hiding devices
  - Housing for Large Animals

    -cage modifications to allow social housed dogs and primates

    -addition of enrichment materials such as chew toys, foraging materials, mirrors, perches

Refinements in Animal Testing

- Modifying techniques
- - in rats modified the blood collection technique from retro-orbital sinus to jugular vein
- Further refined technique from using a restraint board to a 1-hand technique (resulting in less stress on the animal)
- Implanted catheter when multiple blood collections are required
- Microsampling techniques
- -sampling done via tail snip

Study Plan/Protocol Review Process

- Standard study plan/protocol templates

  - reviewed by IACUC committee for all standard study types

  - templates reflect the regulatory requirements for basic parameters

  -additional parameters required must be reviewed and approved prior to ordering the animals/prior to animal arrival

IACUC Review Process

- IACUC approval form is submitted by the Study Director
- Study plan is attached to the form
- Additional information included on the form should include the following:
- Route of administration
- Duration of treatment
- Duration of recovery
- Clinical signs expected or seen in previous studies

IACUC Review Process

- Total number of animals (including spares)
- Blood sampling procedures including site of sampling, volumes required and number of samples per animal/occasion
- Use of restraint device
- Use of anesthesia
- Use of analgesics
- Housing – social or single, if single, justificaition for it

IACUC Review Process

- Food and/or water restriction – either as per standard procedures or restricted due to scientific reason
- Any special features of the study plan which are not routinely done

Post-Approval Monitoring Process

- Administrative policy outlining the PAM of IACUC approved study plans
- A selection of study plans are reviewed each month, approximately 10% per year
- PAM form is filled out by the IACUC representative indicating the area to be monitored as well as the timeline/frequency of the monitoring requested
- PAM specialist is responsible for ensuring that the monitoring process occurs

Post-Approval Monitoring Process

- PAM specialist and a member of the IACUC group will visit the study room and observed the selected procedures during the study
- Observations are recorded on a form and submitted to the IACUC and copied to Test Facility Management (TFM) and appropriate scientific staff
- Any corrective actions are appropriately documented and monitored by both the IACUC and TFM

Rodent Oral Toxicity Studies Based on Regulatory Guidelines

Study Parameter	EPA OPPTS Guidelines		FDA Redbook		OECD Guidelines	
	Subchronic	Chronic	Subchronic	Chronic	Subchronic	Chronic
Study Duration	≥ 90 days	≥ 12 mos	≥ 90 days	≥ 12 mos	≥ 90 days	≥ 12 mos
No. of Groups	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4
No. of Animals/Sex/Group	≥ 10	≥ 20	≥ 20	≥ 20	≥ 10	≥ 20
Age of Animals (at start of study)	≤ 8-9 wks	≤ 8 wks	≤ 6 wks	≤ 6 wks	≤ 9 wks	ASAP
Body Weight Measurement Frequency through 13 weeks Frequency after 13 weeks	 weekly NA	 weekly every 4th wk	 weekly NA	 weekly monthly	 weekly NA	 weekly every 4th wk
Feed Consumption measurement Frequency through 13 weeks Frequency after 13 weeks	 weekly NA	 weekly every 4th wk	 weekly NA	 weekly monthly	 weekly NA	 weekly every 3rd mo
Clinical Observations Mortality and morbidity (times/day) General condition (times/day) Detailed clinical findings (frequency)	 2 1 weekly	 2 1 weekly	 2 n daily	 2 n daily	 2 1 weekly	 2 1 daily
Neurotoxicity Evaluation (at term)	y	y	y	n	y	n

charles river

Design of Rodent Toxicology Studies (Continued)

Study Parameter	EPA OPPTS Guidelines		FDA Redbook		OECD Guidelines	
	Subchronic	Chronic	Subchronic	Chronic	Subchronic	Chronic
Ophthalmology N of animals pretest N/sex//high dose and control at term	 All Surviving	 All 10	 All Surviving	 All Surviving	 All Surviving	 n n
Hematology & Clinical Chemistry (N/sex/group) Intermediate time(s) Term	 Surviving n y	 10 6 mo y	 10 wk 1, mid y	 10 every 3rd mo y	 Surviving n y	 10 3 & 6 mo y
Urinalysis (No./sex/group) Intermediate time(s) Term	 Surviving n o	 10 n y	 NA n n	 NA n n	 Surviving n o	 10 3 & 6 mo y
Gross Necropsy and Tissue Collection	All	All	All	All	All	All
Organ Weights (No./sex/group at term) Adrenals, kidneys, liver Brain Testes/ovaries Epididymides, heart, uterus Spleen, thymus Thyroids, parathyroids	Surviving y y y/y y y y	Surviving y y y/y y y n	Surviving y y y/y y y y	Surviving y y y/y y y y	Surviving y y y/y y y y	10 y y y/y n n n

charles river

Reproductive Toxicology

- Embryofetal development (Seg II, ICH-3)
- Male and female fertility (Seg I, ICH-1)
- Pre and post natal development (Seg III, ICH-2)
- Usually done post IND
- FDA urges including women on clinical trials
- Timing is ultimately determined by the inclusion of Women of Child Bearing Potential (WOCBP) on a clinical trial

Carcinogenicity Studies

- Mainly required for small molecule programs
- Required for clinical administration of over 6+ months
- Purpose is to investigate the potential to cause tumors
- Study design: minimum of 50/sex/group (4 groups)
- 2 year study in rats and mice (life-time studies, bioassays)
- Usually start around time of Phase III
- Limited routes of exposure
- Limited study endpoints
  - Palpate for subcutaneous tumor formation
  - Evaluate tissues for microscopic evidence of tumor formation

Choice of Non-Rodent Species

- Pharmacologically relevant – share similar physiology to humans and have similar TA pharmacodynamic parameters to humans
- Species
  - Dogs
  - Nonhuman Primates
    - Used when dogs are inappropriate
    - Biologicals
  - Minipigs
    - in the USA, dermal products
    - popular in Europe in lieu of dogs and primates

Non-Rodent Oral Toxicity Studies Based on Regulatory Guidelines

Study Parameter	EPA OPPTS Guidelines		FDA Redbook		OECD Guidelines	
	Subchronic	Chronic	Subchronic	Chronic	Subchronic	Chronic
Study Duration	≥ 90 days	≥ 12 mos	≥ 90 days	≥ 12 mos	≥ 90 days	≥ 12 mos
No. of Groups	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4
No. of Animals/Sex/Group	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4	≥ 4
Age of Animals (at start of study) – not typically used for NHPs	4 - 6 mos	4 - 6 mos	4 - 6 mos	4 - 6 mos	4 - 6 mos	---
Body Weight Measurement Frequency through 13 weeks Frequency after 13 weeks	 weekly NA	 weekly every 4th wk	 weekly NA	 weekly weekly	 weekly NA	 weekly every 4th wk
Feed Consumption measurement Frequency through 13 weeks Frequency after 13 weeks	 weekly NA	 weekly every 4th wk	 weekly NA	 weekly weekly	 weekly NA	 weekly every 3rd mo
Clinical Observations Mortality and morbidity (times/day) General condition (times/day) Detailed clinical findings (frequency)	 2 1 weekly	 2 1 weekly	 2 n daily	 2 n daily	 2 1 weekly	 2 1 daily
Neurotoxicity Evaluation (at term)	y	y	y	y	n	n

charles river

Design of Non-Rodent Toxicology Studies (Continued)

Study Parameter	EPA OPPTS Guidelines		FDA Redbook		OECD Guidelines	
	Subchronic	Chronic	Subchronic	Chronic	Subchronic	Chronic
Ophthalmology N of animals pretest N/sex//high dose and control at term	All Surviving	All Surviving	All Surviving	All Surviving	All Surviving	n n
Hematology & Clinical Chemistry (N/sex/group) Pretest Intermediate time(s) Term	Surviving All monthly y	Surviving All 6 mo y	Surviving All monthly y	Surviving All every 3rd mo y	Surviving All monthly y	Surviving All 3 & 6 mo y
Urinalysis (No./sex/group) Pretest Intermediate time(s) Term	Surviving All monthly y	Surviving All 6 mo Y	Surviving All monthly Y	Surviving All every 3rd mo Y	Surviving All monthly y	Surviving All 3 & 6 mo y
Gross Necropsy and Tissue Collection	All	All	All	All	All	All
Organ Weights (N/sex/group at term) Adrenals, kidneys, liver Brain Testes/ovaries Epididymides, heart, uterus Spleen, thymus Thyroids, parathyroids	Surviving y y y/y y y y	Surviving y y y/y y y y	Surviving y y y/y y y y	Surviving y y y/y y y y	Surviving y y y/y y y y	Surviving y y y/y n n y

charles river

Design of Non-Rodent Toxicology Studies (Continued)

Study Parameter	EPA OPPTS Guidelines		FDA Redbook		OECD Guidelines	
	Subchronic	Chronic	Subchronic	Chronic	Subchronic	Chronic
Histopathology						
All tissues (all high-dose and control)	y	y	y	y	y	y
All tissues (all animals dead)	y	y	y	y	y	y
Target tissues and gross lesions (all)	y	y	y	y	y	y

Parameters adapted from Wilson *et al.,* Chapter 19, *Principles and Methods of Toxicology*, 4th Edition, Hayes.

charles river