In Vitro and In Vivo Toxicology Studies to Push Your Drug into Development

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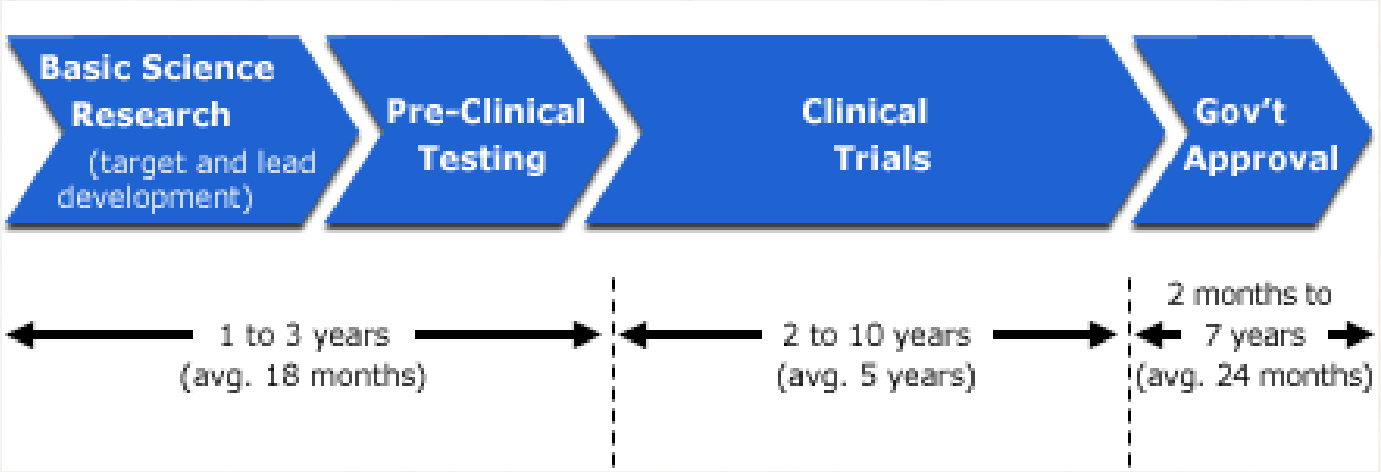
“Turning Your Research Into A Therapeutic

What You Should Think About and When"

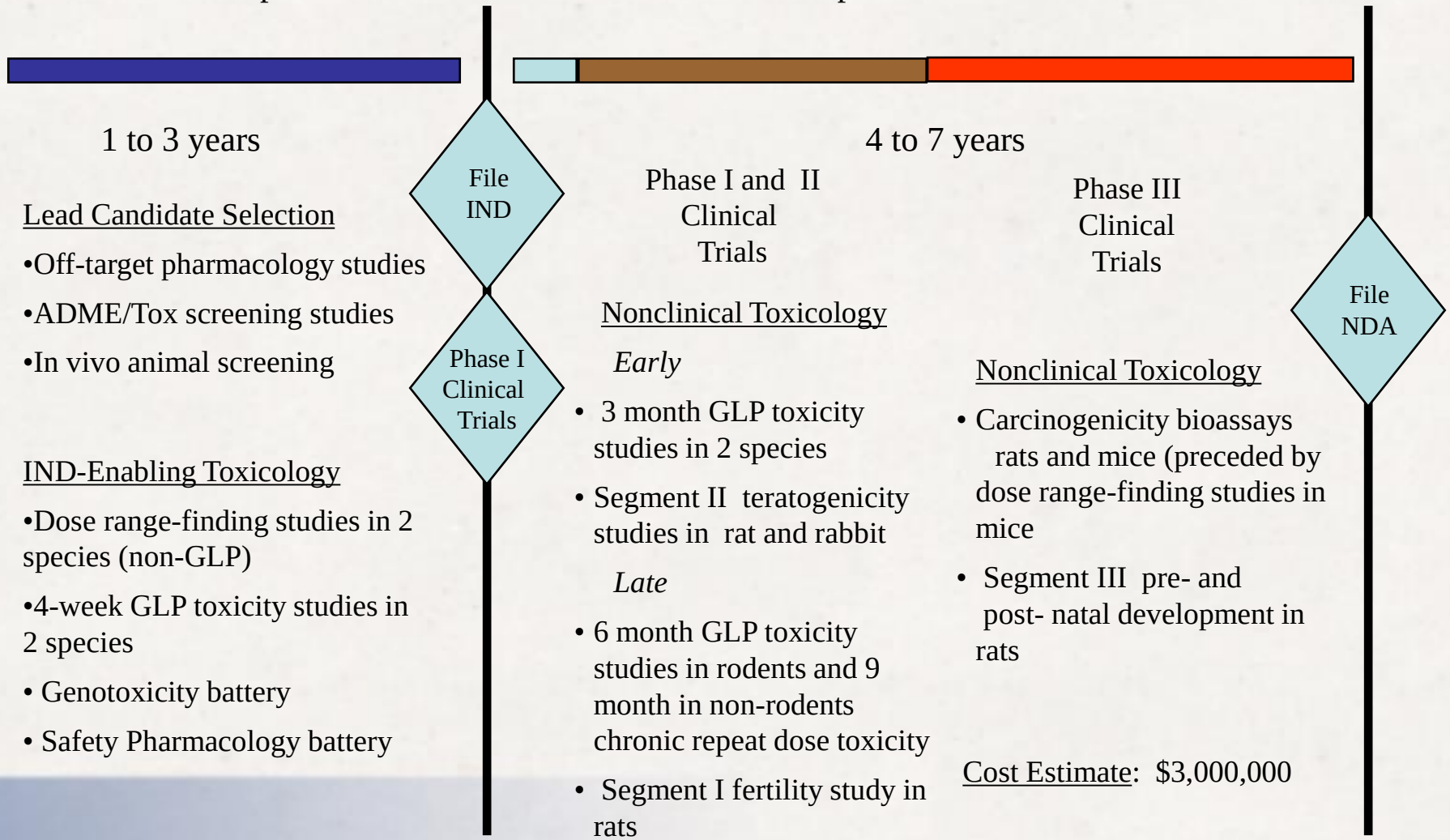
Path to Drug Development

- My therapeutic kills cancer cells
  - Throw my drug on cells and they stop dividing
  - IP dose my drug and tumor shrinks



- Do my preclinical tox/PK
- Make my drug GMP
- Get FDA blessing and enroll sites!
- Run clinical trials

Basic Outline of Studies and Costs



Screen to Kill Your Drug EARLY

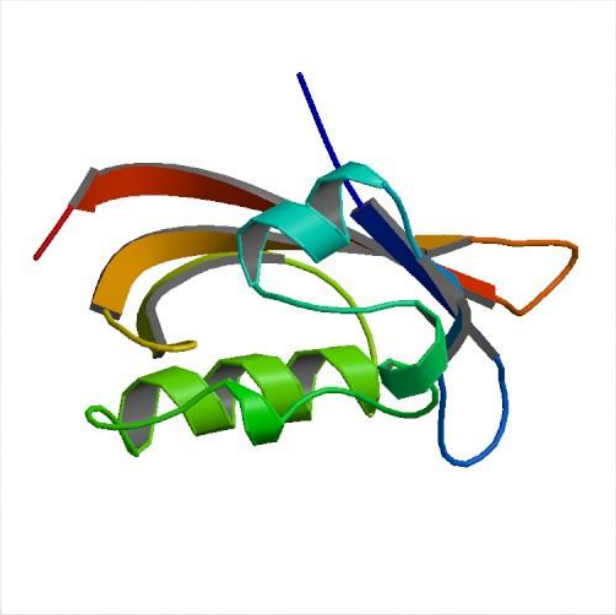
- What should be apparent from the previous slide is that you want to kill a drug early *before* you spend a lot of money in preclinical (and clinical) work

In Vitro and In Vivo Screening

1) Off-target pharmacology
   - Screening for inhibition of multiple targets; i.e. transporters, ion channels, neurotransmitters
2) ADME screening
   - CYP-P450 inhibition, protein binding, microsomal stability, reactive intermediates
3) In vitro toxicology
   - hERG inhibition and Ames mutagenicity screening
4) In vivo toxicology
   - Target organ screening, bioavailability, initial dose selection

In Vitro Toxicology Screening

- hERG assay
  - Inhibition of potassium channel
  - ICH S7 Guidance
- Ames mutagenicity
  - Limited panel of strains
  - ICH S2 Guidance
- Neither of these are conducted GLP at this point
- ~$1000-2000 each
- A positive could be enough to kill your drug



Getting Started on In Vivo Toxicology/PK

- Look at pharmacology and ADME
  - Which species have the pharmacological target present?
  - In which species is the metabolism/metabolite profile relevant to human
- In vivo dose setting
  - Doses in mice can be scaled to rats and non-rodent species
  - You may use estimates of volume of distribution, oral bioavailability and half-life to estimate minimum effective plasma concentrations in your toxicology species
  - You need to have an eye on your clinical dose as your in vivo toxicology doses need to support the starting clinical dose in the clinic
  - FDA Guidance for Industry "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Normal Healthy Volunteers", July 2005)

In Vivo Toxicology and PK

- Studies usually conducted in two species (rodent and non-rodent)
  - Rat is primary rodent species, mice used less frequently
  - Dogs are the primary choice for small drug molecules and seem to be the preferred species, at least by FDA
  - Monkeys are commonly used as the non-rodent species (and sometimes as the only species) for biological products
  - Additional non-rodent species used less frequently include rabbits and pigs

In Vivo Toxicology and PK

- We're not looking for a research paper and we're not looking to show our drug is safe in animals
- Goals:
  - Identify/characterize target organ toxicities
  - Maximum Tolerated Dose (MTD), No Observable Effect Level (NOEL) & Lowest Observable Effect Level (LOEL)
  - Assess time to toxicity and reversibility of effects
  - At what blood levels (tissue levels) are toxicities observed
  - Do systemic exposures vary with number of doses?

- Characterize toxicity profile in animals and not humans!

In Vivo – Rodent Dose Range Finding

- Rats preferred over mice
  - Single dose, 5 days, 7 days, 10 days, 14 days
  - There is no “standard” dose range-finding study design
- For Non-GLP dose range-finding study:
  - Group size of 3-5/sex
  - At least 3 dose levels and vehicle control
  - Clinical pathology at termination
  - Target organ histopathology (control and high dose, confirmed target tissues in low and mid dose)
  - Consider PK (but requires additional groups of rats)
  - In the background, you are doing bioanalytical method development

In Vivo – Dog Dose Range-Finding

- Beagle dogs are preferred non-rodent species
  - Non-rodent study may be first a dose escalation study to determine MTD and then followed by repeated doses for 5-14 days
  - Again, there is no “standard” dose range-finding study design
- For non-GLP dose range-finding studies:
  - Group size of 1-2/sex
  - MTD may have no control group to limit numbers
  - Dose escalation as in a clinical study (1-3 daily doses); wash-out period between dose levels
  - Clinical pathology at baseline and after each dose level
  - Generally, terminate for histopathology
  - Collect samples for PK

General IND-Enabling Toxicology Plan for Non-cancer Therapeutics

1) 4-week GLP toxicology studies
   - Two species, protocol elements to follow
2) ICH S2 Genotoxicity battery (not for cancer IND)
   - Requirement depends upon indication and molecule
3) ICH S7 Safety pharmacology battery
   - Requirement depends upon indication and molecule

What You Intend to do in the Clinic Drives IND-Enabling Preclinical Development

- Preclinical Toxicology and PK
  - Treatment duration and regimen determines what the toxicology program looks like
    - How often and how long?
  - Treatment indication determines what toxicology program looks like
    - Genotoxicity, reproductive toxicology, safety pharmacology
  - Route and excipients are considerations
  - How does the study support my clinical development?

While there are standard studies, the overall preclinical program is not conducted "cookbook" with no eye to clinical development

GLP Toxicology Studies

ICH M3(R2) Guidance on Nonclinical Safety Studies

Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies	
	Rodents	Non-rodents
Up to 2 weeks*	2 weeks	2 weeks
Between 2 weeks and 6 months	same as clinical	same as clinical
> 6 months	6 months	9 months

*In the US, as an alternative to 2 week studies, single dose toxicity studies with extended examinations can support single dose human trials. Studies of less than 14 days can be supported with toxicity studies of the same duration as proposed for clinical study

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4-Week GLP Toxicology in Rats – Protocol Elements

- Control and low, mid, and high dose drug treatment groups
- N=10/sex/group, with an additional 5/sex/group in control and high dose groups to evaluate reversibility over 2-4 weeks
- Parameters evaluated will be clinical observations, body weight, food consumption, ophthalmological exams, clinical pathology, organ weights and histopathology (all tissues, control and high dose groups and target organs in low and mid dose and recovery
- Studies will usually include satellite animals for systemic exposures/pharmacokinetics as recommended in ICH S3
- No Observable Adverse Effect Level (NOAEL) or STD10 for cancer drugs, converted to human equivalent dose (HED) and safety factor or 10 applied to give starting clinical dose

4-Week GLP Toxicology in Dogss – Protocol Elements

- Control and low, mid, and high dose drug treatment groups
- N=4/sex/group with additional 2/sex/group in control and high dose
- Parameters evaluated will be clinical observations, body weight, food consumption, ophthalmological and ECG exams, clinical pathology, organ weights and histopathology (all tissues, all animals)
- Studies will usually include systemic exposures/pharmacokinetics as recommended in ICH S3
- NOAEL converted to HED and safety factor or 10 applied to give starting clinical dose, or for cancer drugs, HNSTD converted to HED and safety factor of 6 applied to give starting clinical dose

Genetic Toxicity Battery ICH S2

- Bacterial Mutagenicity
  - Ames assay; point mutations
- In Vitro Mammalian Mutagenicity
  - Chromosome aberration, mouse lymphoma, in vitro micronucleus assay – any of these are acceptable; chromosome aberrations
- In Vivo Mammalian Mutagenicity
  - Mouse or rat in vivo micronuclei formation. Whole animal so ADME properties taken into consideration; chromosome aberrations
- GLP, so requires compound meeting GLP characterization

Safety Pharmacology Core Battery ICH S7

- Pulmonary function
  - Usually rats. Respiratory parameters, plethysmography
- Central nervous system
  - Functional observation battery, sometimes bolted on to GLP toxicology study
- Cardiovascular
  - In vitro hERG assay (ion channel inhibition
  - In vivo dog or monkey, telemetry in instrumented animals
- GLP strongly recommended, so requires compound meeting GLP characterization

Later Stage Toxicology Studies

1) Subchronic and Chronic GLP toxicology studies
   - 3 month studies (both species)
   - 6 month (rat) and 9 month (nonrodent) studies
2) Reproductive Toxicity
   - Rats and rabbits

– When to conduct the longer duration toxicology studies and your reproductive toxicology studies depends on your clinical development plan

Special Studies

- Topical drugs may require local tolerance studies including contact hypersensitivity, phototoxicity, and photoallergy studies in the guinea pig or rabbit.
- Drug classes with known cardiovascular effects may require early screening of cardiotoxicity beyond hERG.
- A drug or biologic that may cause an immune response in humans might be screened for such a response in non-human primates, or the rodent specific homologue might be tested in rodent studies. Failure to account for immunogenicity might lead to inaccurate predictions of safe clinical doses.

Summary

- The previous slides are a generalized overview, not an absolute requirement, of the package of non-clinical toxicity testing to be conducted in support of the IND and first in man clinical trials
- The process is very dynamic, there are no rules that apply to all drugs – the goal of the package of studies is to support the clinical development of the drug, not to do research
- Communicate with FDA before you conduct the definitive GLP studies with a pre-IND meeting

ICH Safety Guidances

International Conference on Harmonsation (ICH) www.ich.org

S1 Topic: Carcinogenicity

S2 Topic: Genotoxicity

S3 Topic: Toxicokinetics and tissue distribution

S4 Topic: Duration of chronic toxicity testing

S5 Topic: Reproductive toxicity testing

S6 Topic: Safety assessment of biotechnology derived drugs

S7 Topic: Safety pharmacology evaluation

S8 Topic: Immunotoxicity testing

S9 Topic: Nonclinical evaluation of anticancer drugs

S10 Topic: Photosafety

S11 Topic: Nonclinical Pediatric Safety

S12 Topic: Non-clinical Biodistribution Studies

Additional Guidances

FDA Estimating the Maximum Safe Starting Dose

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers

Note: this guidance is useful for Table 1, conversion of animal doses to human equivalent doses. HOWEVER, for the starting dose calculations, you DO NOT use the NOAEL as in this FDA guidance, you use the ICH S9 Guidance on setting initial clinical dose. The ICH guidance allows for a more aggressive initial dose in the clinic (because the indication is cancer)