Juvenile Animal Studies: A CDER Perspective

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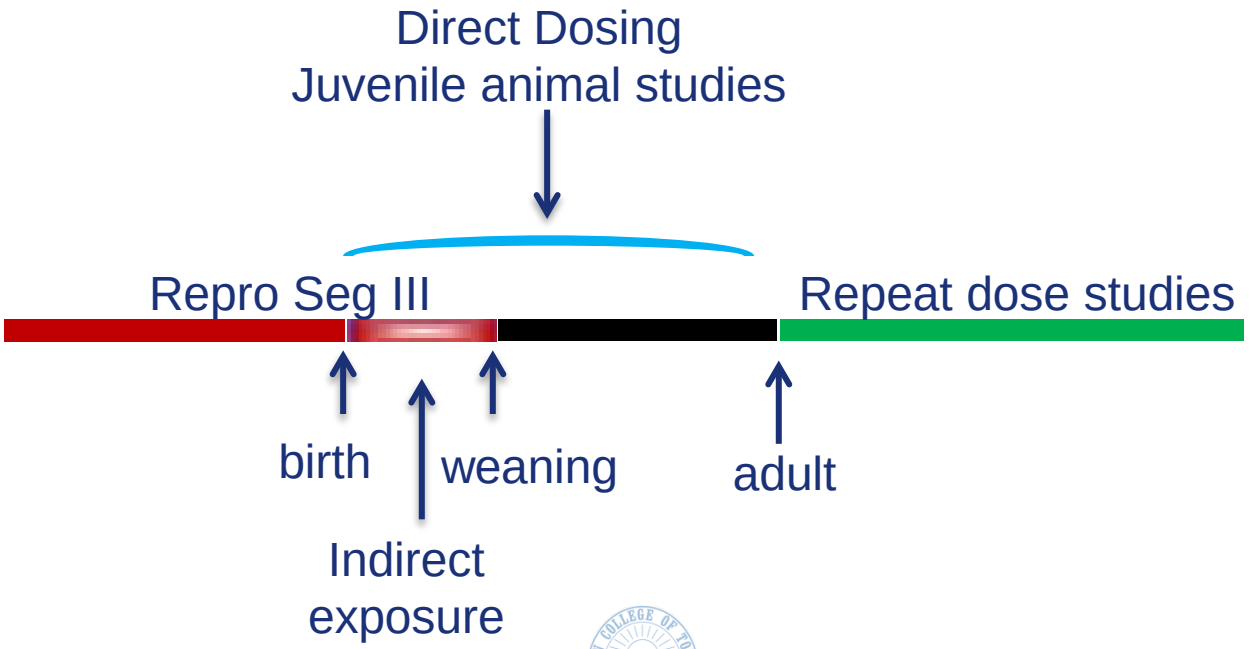
Non-clinical Studies Conducted for Safety Assessment of a Drug

- Safety pharmacology
- Pharmacokinetics/Toxicokinetics
  - ADME: (absorption, distribution, metabolism, elimination)
- General toxicology
- Genotoxicity
- Carcinogenicity
- Reproductive toxicology
- Local tolerance
- Special studies:
  - Juvenile Animal Study

GLP

Juvenile Animal Studies (JAS) in Pediatric Drug Development

- Juvenile Animal Studies are conducted when existing data from animals and humans are insufficient to support the proposed clinical trials in children
- Juvenile animal studies are conducted on a case-by-case basis



Juveniles are not Little Adults

- Continued development in juveniles compared to adults (CNS, reproductive, pulmonary, renal, skeletal, immune)

- Differences in PK (absorption, distribution, metabolism, excretion) and/or PD (receptor expression and function)

  Drug's effect on developmental stage and effect of developmental stage on drug

Regulatory Background

- Guidance for Industry- Nonclinical Safety Evaluation of Pediatric Drug Products, 2006
- ICH M3R2, 2009 and M3(R2) Q&A (R2), 2012
- Pediatric Study Plans (PSPs), Pediatric Research Equity Act (**PREA**)
  - Nonclinical data, complete or planned, to support studies in children are to be discussed in PSPs
- Written Requests (WR), Best Pharmaceuticals for Children Act (**BPCA**)
  - Juvenile animal studies can be included as part of a WR

Pediatric Research Equity Act (PREA) and Best Pharmaceuticals for Children Act (BPCA)

PREA *

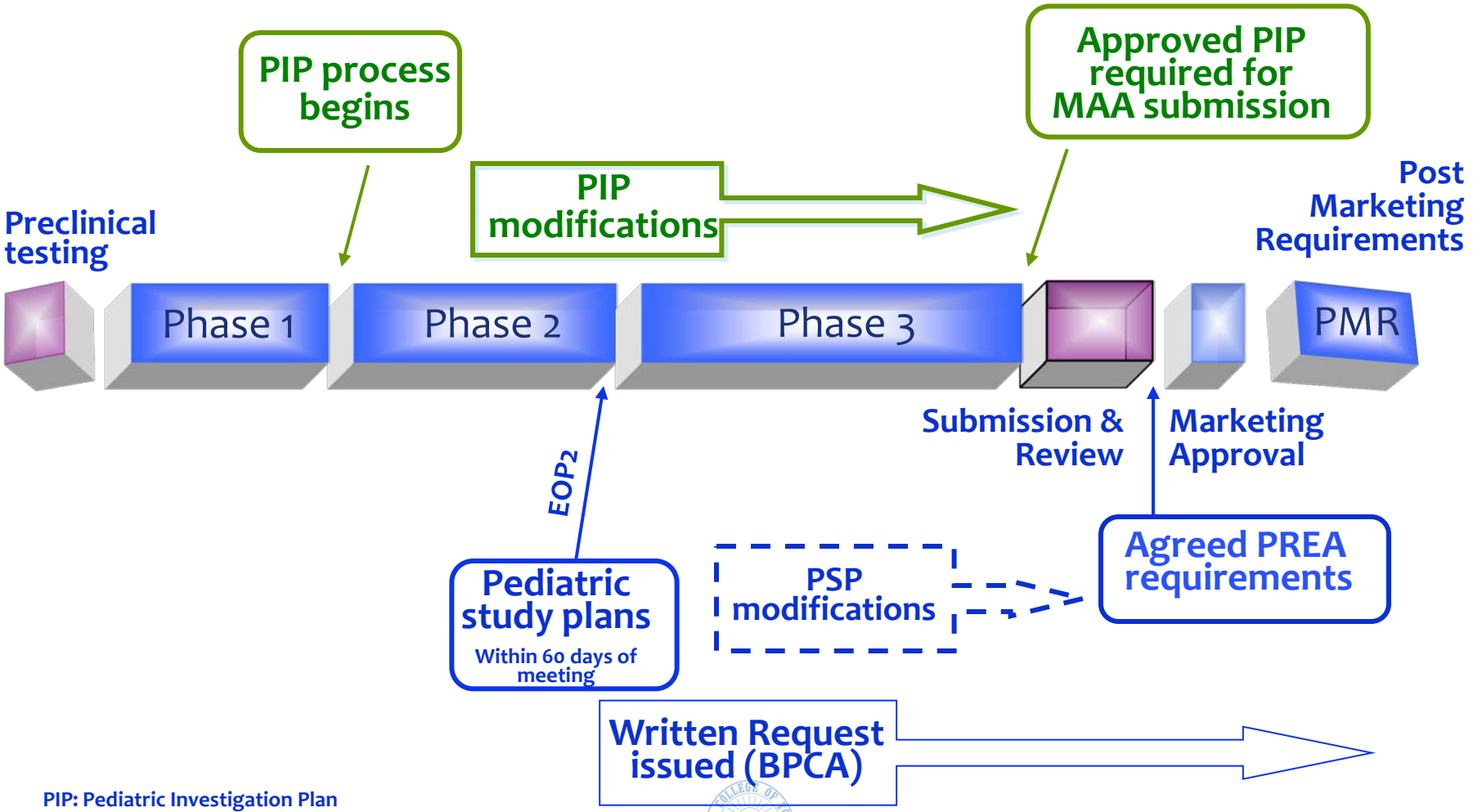
- Drug & Biologics
- Studies-mandatory & apply to indication
- Orphan indications exempt
- Trigger:
  - New Indication
  - New Active Ingredient
  - New Dosage Form
  - New Dosing Regimen
  - New Route

BPCA *

- Drugs & Biologics
- Studies-voluntary & apply to active moiety
- Written Request may be issued for drugs with orphan indications
- Trigger: public health need

*** Permanent extension by FDA Safety and Innovation Act (FDASIA), 2012**

Pediatric Planning in the Drug Development Process - Timing



PIP: Pediatric Investigation Plan
MAA: Marketing Authorization Application

Why Conduct Juvenile Animal Studies?

- Address pediatric safety concerns that cannot be assessed by clinical or standard toxicology studies due to developmental and/or drug sensitivity differences in juveniles compared to adults
- Assess safety concerns that cannot be adequately, ethically, or safely studied in pediatric trials
  - Serious adverse effects that are irreversible
- Provide needed information to allow for adequate clinical monitoring
- May inform about safe doses/exposures in particular developmental/pediatric age groups

A Need or No Need for JAS?

- Determining the need for JAS is part of the pediatric development plan
- A scientific justification supporting the need/no need for these studies is to be included in the PSP and PIP:
  - What to be considered:
    - Indication,
    - Age of pediatric population,
    - The extent and timing of exposure to the drug,
    - Pharmacology of drug (both primary and secondary),
    - Distribution of the drug in the body,
    - Receptor/binding site distribution,
    - Maturity/immaturity of system/s affected by drug distribution,
    - What toxicities are identified from adult animals,
    - PK/PD differences between adults and juveniles.

Considerations for the Design of Juvenile Animal Studies

- Animals should be treated throughout the stages of development that are comparable to the timing of exposure in the intended pediatric population
- Temporal developmental differences between animals and humans (use of the appropriate model)
- Potential differences in pharmacological and toxicological profiles between mature & immature systems (differences in ADME)
  - Dose range finding studies can be helpful in designing definitive JAS

Considerations for the Design of Juvenile Animal Studies, cont'd

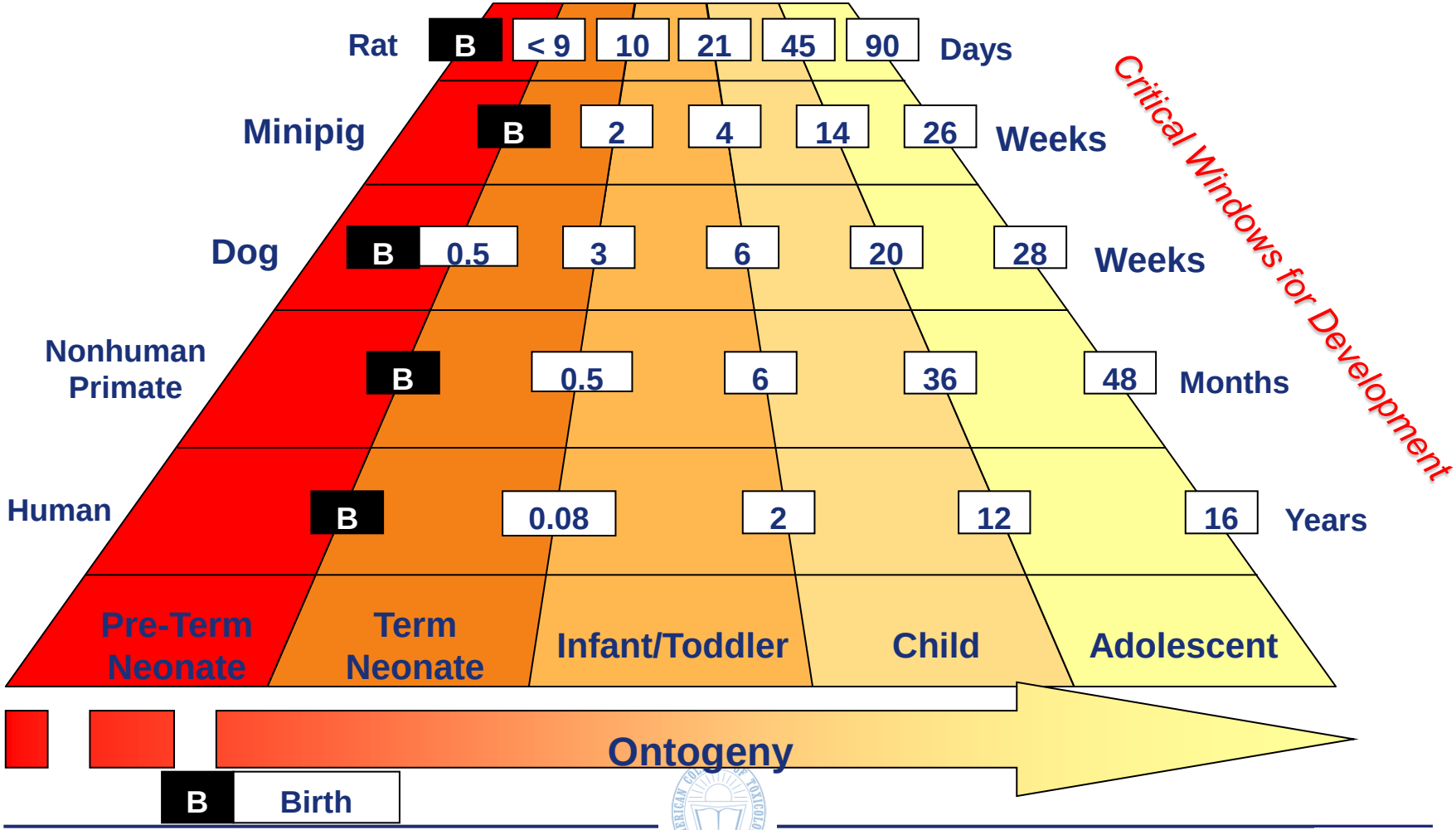
- Use of available data from adult animals and humans to identify potential targets
- Special attention to systems that undergo developmental changes during treatment period
- Attempt to distinguish between acute and permanent effects of the drug by including a recovery group at the end of treatment period
- Usually one relevant species, preferably rodent. A study in non-rodent species can be requested when scientifically justified

Age of Animals at Start of Dosing

- A very critical point to consider
  - What is the lower limit of the age range during which pediatric patients would be treated?
  - How does the maturity and development of the affected system/s in humans compare to that in animals?

Comparative Age Categories Based on Overall CNS & Reproductive Development

Buelke-Sam, 2001



General Toxicity Screening Study Design

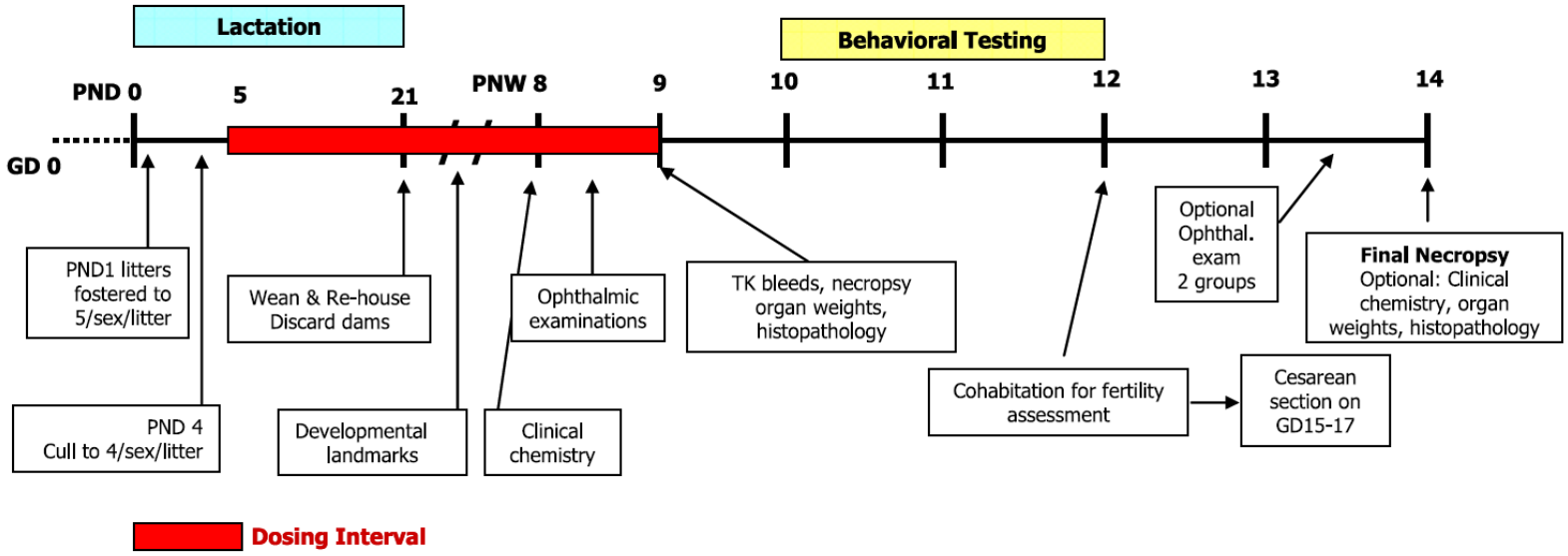


Fig. 3. Example of a general toxicity screening study in juvenile rats. GD, gestation day; PND, postnatal day; PNW, postnatal week; TK, toxicokinetics.

Birth Defects Research (Part B) 86:463–469, 2009

Histopathology

- Standard histopathology to be conducted at the end of treatment; more specific or expanded assessments of certain organs (e.g., brain) may be warranted
- If an effect is observed at the end of treatment, the recovery should be evaluated at the end of a drug-free period

Toxicokinetics

- Important in order to compare the human to animal plasma levels
- Parent compound and significant metabolites

Evaluation of this Study Design

- Findings from this study can be very valuable, especially if the target/s or the receptors/binding sites are not clearly identified (a reason for a screening)
- Histopathology findings can help identify target/s for more focused investigations
- Conducting general toxicity studies in juvenile animals without including endpoints valuable to investigate developing systems (CNS, reproductive, skeletal) might give the false impression of a lack of an effect

Targeted CNS and Reproduction study design

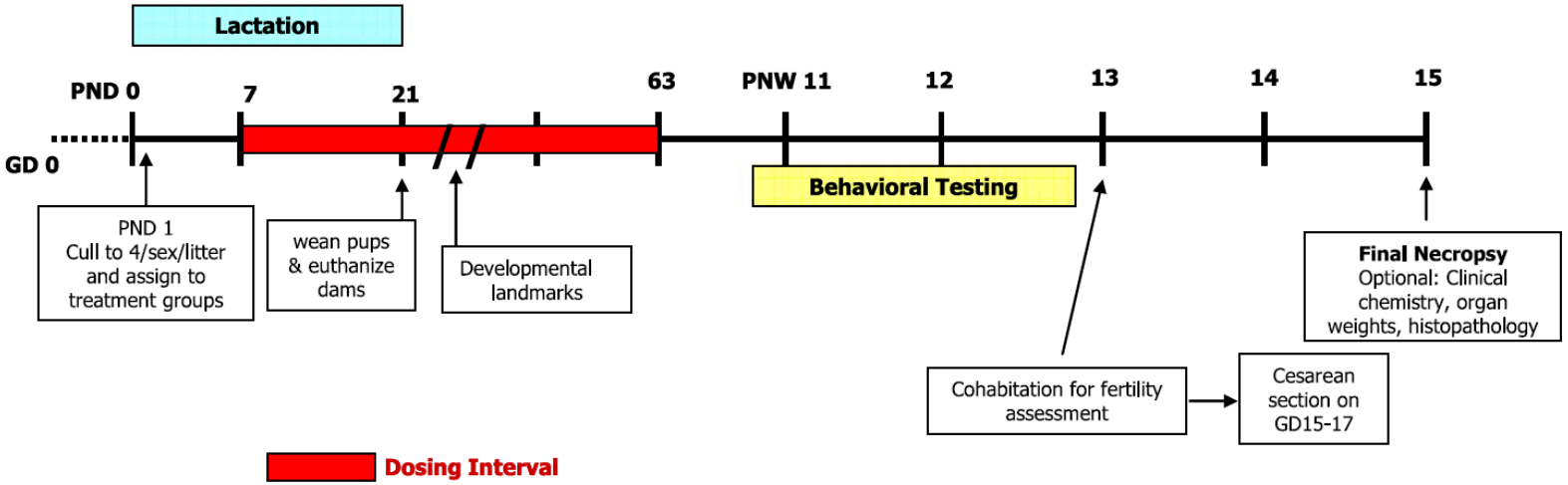
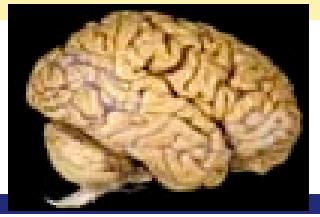


Fig. 1. Example of a targeted CNS and reproduction study design. GD, gestation day; PND, postnatal day; PNW, postnatal week.

Birth Defects Research (Part B) 86:463–469, 2009

Targeted CNS study Design



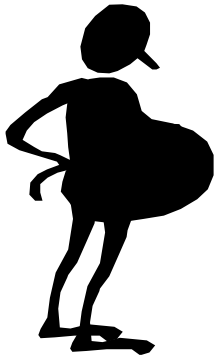
- **Neurobehavioral evaluation:**
  - Assessment of the effect of treatment on cognitive function (learning and memory, usually a complex maze test or other sensitive and reliable tests)
    - Cincinnati water Maze, Morris Water Maze, Biel Maze
  - Other CNS assessment tests (locomotor activity using an automated system, startle habituation, FOB test)
  - Test for direct effect of drug and long term effect (different animals are to be used for testing during treatment and after the recovery period)

Neurohistopathology

- Evaluation of at least seven brain slices as described by:
  - Bolon et al. Toxicol. Pathol 41 (2013), 1028-1048
  - Rao et al. Toxicol. Pathol 39 (2011). 463-470

- Use of Special staining might be warranted

Targeted Reproductive Study



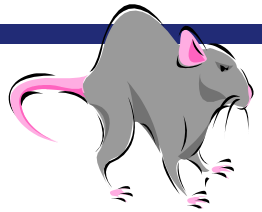
- **Reproductive evaluation**
  - Attainment of sexual maturation
    - Individual body weight recorded on day of acquisition
  - After an adequate recovery period animals are mated and effects on fertility in males & females are assessed
    - animals in this group may be used for neurobehavioral testing after the recovery period to reduce the use of animals

Other Systems

- **Immune system:**
  - T-cell dependent antibody response (TDAR)
  - Immunophenotyping of lymphocytes
  - Organ weights, histopathology
- **Skeletal system evaluations**:
  - Bone length
  - Bone Density

Other systems as warranted, using endpoints specified for the evaluation of that system

Summary of the General Design of Juvenile Study



- Studies conducted in young animals of an age range developmentally comparable to that during which exposure would occur in humans
- Design emphasizes assessment of effects on growth and development, with other standard toxicologic endpoints included as appropriate for risk characterization
- Choice of endpoints informed but not defined by adult animal data
- Purpose is to identify age-related toxicity (i.e., unique developmental effects as well as differences in sensitivity)
- Standard histopathology to be conducted at end of treatment; more specific or expanded assessments of certain organs (e.g., brain) may be warranted
- Neurobehavioral and reproduction function and other relevant end points are usually assessed
- When an effect is seen during treatment, recovery should be assessed

Pediatric Only Indications

- In some circumstances a long-term juvenile toxicity study could be adapted to replace the standard chronic study and a separate juvenile animal study (e.g., 12-month dog study or 6-month study in rodents). The study can be designed to initiate dosing in the appropriate age and address developmental concerns.
- A 12-month study in dogs is important to follow up on the effect on reproductive system in these animals as sexual maturation occurs starting ~10 months

Study Design for Biologics

- Generally similar to that for small molecules
- Immunogenicity and species specificity need to be considered, as for adult animals
- Species: rodents, if possible; however, NHP may be requested (might be the only relevant species)
- For NHP, study duration is usually 1 year and design is modified to accommodate characteristics unique to this species (e.g. reproduction)
- Non-standard dosing regimens and routes may be used

Study Design – Take Home Message

- ***It is important to conduct a well designed, informative study with appropriate endpoints and not just conduct a study!***
  - Consult with the Division regarding the study design before starting your study. Most Divisions are willing to review the protocol and provide feed back on the study design.
  - It is important to provide your rationale for the study in the context of the pediatric trials that you are planning to support and the use of the drug in the pediatric population.

Timing of Juvenile Studies Relative to Clinical Testing

- Based on what you know and what you need to know
  - Prior knowledge
    - Prior clinical data in adults or older pediatric age groups
    - What toxicology has been done
    - Known potential hazards

- The Guidance* provides different situations regarding the timing of these studies relative to clinical testing

- It is important to consult with the Division regarding the timing of these studies in the context of the clinical program that they will support because the timing of these studies is considered on a case-by-case basis.

* Guidance for Industry- Nonclinical Safety Evaluation of Pediatric Drug Products, 2006

Case Studies

Case Study #1- Vigabatrin

- MOA: a γ-aminobutyric acid transaminase (GABA-T) inhibitor, (↑ GABA)
- Adjunctive therapy for refractory complex partial seizures in adults and infantile spasms in pediatric patients
- Species - rat
- Multiple dose studies (0, 5, 15,or 50 mg/kg/day, starting on PND 4)
  - Standard toxicological endpoints with added assessments for neurotoxicity (neurobehavioral & histological) and retinal toxicity (ophthalmoscopy & electroretinograms) based on previous adult findings. Reproductive endpoints
  - Mortality and neurobehavioral deficits, convulsions, brain lesion that was unique, retinal and brain lesions at exposures lower than those used in adult rats and lower than projected clinical doses

Case Study #1- Vigabatrin

- Pediatric Use Section
  - Notes abnormal MRI signal changes in infants treated for infantile spasms
  - Description of juvenile rat studies

Value - increased sensitivity, unique toxicity, possible clinical correlate

Sabril Label

8.4 Pediatric Use

Abnormal MRI signal changes were observed in infants *[see Warnings and Precautions (5.3) and (5.4)]*.

Oral administration of vigabatrin (5, 15, or 50 mg/kg) to young rats during the neonatal and juvenile periods of development (postnatal days 4-65) produced neurobehavioral (convulsions, neuromotor impairment, learning deficits) and neurohistopathological (brain vacuolation, decreased myelination, and retinal dysplasia) abnormalities in treated animals. The no-effect dose for developmental neurotoxicity in juvenile rats (5 mg/kg) was associated with plasma vigabatrin exposures (AUC) less than 1/30 of those measured in pediatric patients receiving an oral dose of 50 mg/kg.

CASE Study #2- Linaclotide (Linzess)

- A 14-aa peptide guanylate cyclase-C (GC-C) agonist.
- First-in-class NME for the treatment of:
  - Irritable bowel syndrome with constipation (IBS-C)
  - Chronic idiopathic constipation (CIC)
    - IBS-C: 290 mcg orally once daily (5 mcg/kg/day)
    - CIC: 145 mcg orally once daily (2.4 mcg/kg/day)

CASE Study #2- Linaclotide (Linzess)

- MOA: acts locally on the luminal surface of the intestinal epithelium.
- Activation of GC-C results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP).
- Elevation in intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, mainly through activation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, resulting in increased intestinal fluid and accelerated transit.

CASE Study #2- Linaclotide

- Limited oral bioavailability (< 0.20% in all species tested)

Toxicity	Species	NOAEL (mg/kg) M/F	Multiples of maximum recommended clinical dose
13-week oral	Rat	M: 50; F: 100	10,400; 20,800
	Mouse	Not determined	-
	Monkey	Not determined	-
26-week oral	Mouse	M & F: 20	4,170
39-week oral	Monkey	M & F: 5	1,040
9-week oral juvenile	Mouse (7-day old)	M & F: 0.003 (< 9 days) M & F: 0.010 (≥ 9 days)	0.6 2
5-day Oral Juvenile	Rabbit (14-day old)	M & F: 40 (tolerated dose)	8,330

CASE Study #2- Linaclotide

Drug-Related Deaths in a Dose-Ranging Study in Neonatal/Juvenile Mice

	Age at Start of Dosing		
Dose (mcg/kg/day)	Age 7 Days	Age 14 Days	Age 21 Days
0.1	0/16	0/16	0/16
10	0/16	0/16	0/16
50	16/16	0/16	NT
100	16/16	11/16	0/16
600	NT	NT	16/16

NT: not tested
All deaths occurred within 24 hr after the first dose.
Surviving animals received 5 daily doses.

CASE Study #3- Linaclotide

Drug-Related Deaths in a 9-Week Study in Mice Commencing on Postpartum Day 7

Dose (mcg/kg/day)	Deaths	Time of Death
Control	0/40	
1	0/40	
3	0/40	
10	5/40	1 - 2 days
30	16/16*	8 - 24 hr

* Toxicokinetic group (no main study group)

CASE Study #2- Linaclotide

Value: Data from both range finding and definitive studies were utilized in the labeling to limit use in pediatric patients.

Updated Linzess Labeling

8.4 Pediatric Use

LINZESS is contraindicated in children under 6 years of age. The safety and effectiveness of LINZESS in pediatric patients under 18 years of age have not been established. In neonatal mice, increased fluid secretion as a consequence of GC-C agonism resulted in mortality due to dehydration. Due to increased intestinal expression of GC-C, children under 6 years of age may be more likely than older children and adults to develop diarrhea and its potentially serious consequences.

Avoid use of LINZESS in pediatric patients 6 through 17 years of age. Although there were no deaths in older juvenile mice, given the deaths in young juvenile mice and the lack of clinical safety and efficacy data in pediatric patients, avoid the use of LINZESS in pediatric patients 6 through 17 years of age *[see Contraindications (4), Warnings and Precautions (5.1, 5.2) and Nonclinical Toxicology (13.2)]*.

CASE Study #2- Linaclotide

- *A PMR was requested to evaluate the cause of death in neonatal and juvenile mice*
- *Label was currently updated based on the findings from the PMR*

Updated Linzess Labeling

13.2 Animal Toxicology and/or Pharmacology

In toxicology studies in neonatal mice, linaclotide caused deaths at 10 mcg/kg/day after oral administration of 1 or 2 daily doses on post-natal day 7. These deaths were due to rapid and severe dehydration produced by significant fluid shifts into the intestinal lumen resulting from GC-C agonism in neonatal mice. Supplemental subcutaneous fluid administration prevented death after linaclotide administration in neonatal mice *[see Contraindications (4) and Warnings and Precautions (5.1)]*.

In studies conducted without supplemental fluid administration, tolerability to linaclotide increases with age in juvenile mice. In 2-week-old mice, linaclotide was well tolerated at a dose of 50 mcg/kg/day, but deaths occurred after a single oral dose of 100 mcg/kg. In 3-week-old mice, linaclotide was well tolerated at 100 mcg/kg/day, but deaths occurred after a single oral dose of 600 mcg/kg. Linaclotide was well tolerated and did not cause death in 4-week-old juvenile mice at a dose of 1,000 mcg/kg/day for 7 days and in 6-week-old juvenile mice at a dose of 20,000 mcg/kg/day for 28 days

Linaclotide did not cause death in adult mice, rats, rabbits and monkeys at dose levels up to 5,000 mcg/kg/day. The maximum recommended dose in adults is approximately 5 mcg/kg/day, based on a 60kg body weight. Animal and human doses of linaclotide should not be compared directly for evaluating relative exposure *[see Nonclinical Toxicology (13.1)]*

Case Study #3-Kalydeco

- A potentiator of the cystic fibrosis transmembrane regulator (CFTR); a protein present at the surface of epithelial cells in multiple organs
- Facilitates increased Cl transport by potentiating the channel-open probability (gating) of the CFTR protein
- Pediatric population ≥ 6 years who have specific mutations in the CFTR gene

➢ Study design:

- Species: rats
- Daily oral gavage dosing from PND 7-35 followed by a 28-day recovery period
- Standard toxicological endpoints with ophthalmic exams conducted at the end of dosing and end of recovery phase. A full histopathology evaluation was requested by the Division

Case Study #3-Kalydeco

- Findings:
- Bilateral cataracts were observed at HD at the end of the treatment period
- Bilateral cataracts and corneal crystals were also observed at the end of the recovery period
- No NOAEL was identified for these findings
- These findings were not seen in adult animals

- *Value: Findings resulted in labeling changes and affected safety decision (PMR study to monitor for formation of cataracts in children 2-11 years)*

Labeling Kalydeco (Ivacaftor)

13.2 Animal Toxicology and/or Pharmacology

Cataracts were seen in juvenile rats dosed with ivacaftor from postnatal day 7-35 at dose levels of 10 mg/kg/day and higher (*approximately 0.12 times the MRHD* based on summed AUCs of ivacaftor and its metabolites). *This finding has not been observed in older animals*.

Current Labeling Based on PMR Findings

- *Changes to the labeling based on the PMR*

5 WARNINGS AND PRECAUTIONS

5.3 Cataracts

Cataracts were seen in juvenile rats dosed with ivacaftor at dose levels of 10 mg/kg/day [*see Animal Toxicology and/or Pharmacology (13.2)*]. Cases of non-congenital lens opacities/cataracts have also been reported in pediatric patients up to 12 years of age treated with KALYDECO. Although other risk factors were present in some cases (such as corticosteroid use and/or exposure to radiation), a possible risk attributable to KALYDECO cannot be excluded. Baseline and follow-up ophthalmological examinations are recommended in pediatric patients initiating KALYDECO treatment.

Continuing Questions

- When is it necessary to conduct studies in two animal species?
- Does any specific clinical age group trigger studies? (e.g., neonates)
- Which endpoints provide consistently meaningful data?
- Are there any new endpoints to be considered?
  - How to regularly address the developing immune system

Future Considerations

- An ongoing effort to evaluate all applications that have juvenile animal studies
- Evaluation of what endpoints were included and other concepts of the study
- Evaluation of how the data were used for regulatory decision making and for the labeling

Future Considerations

- Final Concept Paper

- S11: Nonclinical Safety Testing in Support of Development of Pediatric Medicines dated 3 September 2014
- *Endorsed by the ICH Steering Committee on 10 November 2014*

Conclusion

- What is the ‘value’ of the juvenile animal study?
  - Safety assessment
  - To aid in characterizing the risks
  - Detect unique toxicity, increased sensitivity
- Consideration of and inclusion of juvenile animal studies in pediatric development plans will increase with the incorporation of pediatric plans earlier in drug development.
  - Important for Division to review nonclinical as well as clinical pediatric plans
- Post-FDAAA and with FDASIA, if a study is done, relevant data will be placed in the label
  - WR template includes a statement on nonclinical toxicology

❖ Discussions on ICH harmonization is coming, stay tuned…

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References

Comparative Organ System Development Literature

Birth Defects Research (Part B) Volume 68:

- Bone development
- Renal development
- Lung development
- Male reproductive system
- Female reproductive system
- Heart development
- Immune system development
- CNS development

Birth Defects Research (Part B) Volume 74 (2), 132-156:

- Gastrointestinal development

Birth Defects Research (Part B) Volume 77(5), 471-484:

- Postnatal growth and morphological development of the brain

Birth Defects Research (Part B) Volume 86(6), 463-469:

- Juvenile Animal Toxicity Study Designs