Use of Minipigs in Juvenile Toxicity Studies

WIL RESEARCH

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Introduction

- The potential for juvenile toxicity of pharmaceuticals in humans is frequently evaluated in animal models, and is often assessed using the rat. The rationale for not using the rat will be discussed, and a case study will be presented, illustrating the basis for using the minipig as a juvenile toxicity model.

Default Species: The Rat

- ICH M3 (R2) – Nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals
  - "The conduct of any juvenile animal toxicity studies should be considered only when previous animal data and human safety data, including effects from other drugs of the pharmacological class, are judged to be insufficient to support pediatric studies. If a study is warranted, one relevant species, preferably rodent, is generally considered adequate. A study in a nonrodent species can be appropriate when scientifically justified."

Default Nonrodent Species

- FDA Guidance Document on Nonclinical Safety Evaluation of Pediatric Drug Products
  - “Traditionally, rats and dogs have been the rodent and nonrodent species of choice. In some circumstances, however, other species may be more appropriate for testing.”

Why Not the Rat?

- Pharmacokinetics
  - Exposure
  - Metabolic profile
- Pharmacodynamics
  - Target organ differences
  - Pharmacologic response
- Sensitivity
- Logistical/practical limitations in working with small species shortly after birth (i.e., intravenous or dermal exposures)

Alternative Species

- Dog
  - Well-characterized nonrodent species
  - Long gestation length (ca. 63 days)
  - Comparable adult data in the dog frequently available
- Mini-Pig
  - Use increasing in adult studies
  - Long gestation length (ca. 112 days)
  - More developed at birth than other often-used species
- NHP
  - Typical species of choice for biologics
  - Long gestation length (ca. 165 days)
  - Impractical to carry study to sexual maturity

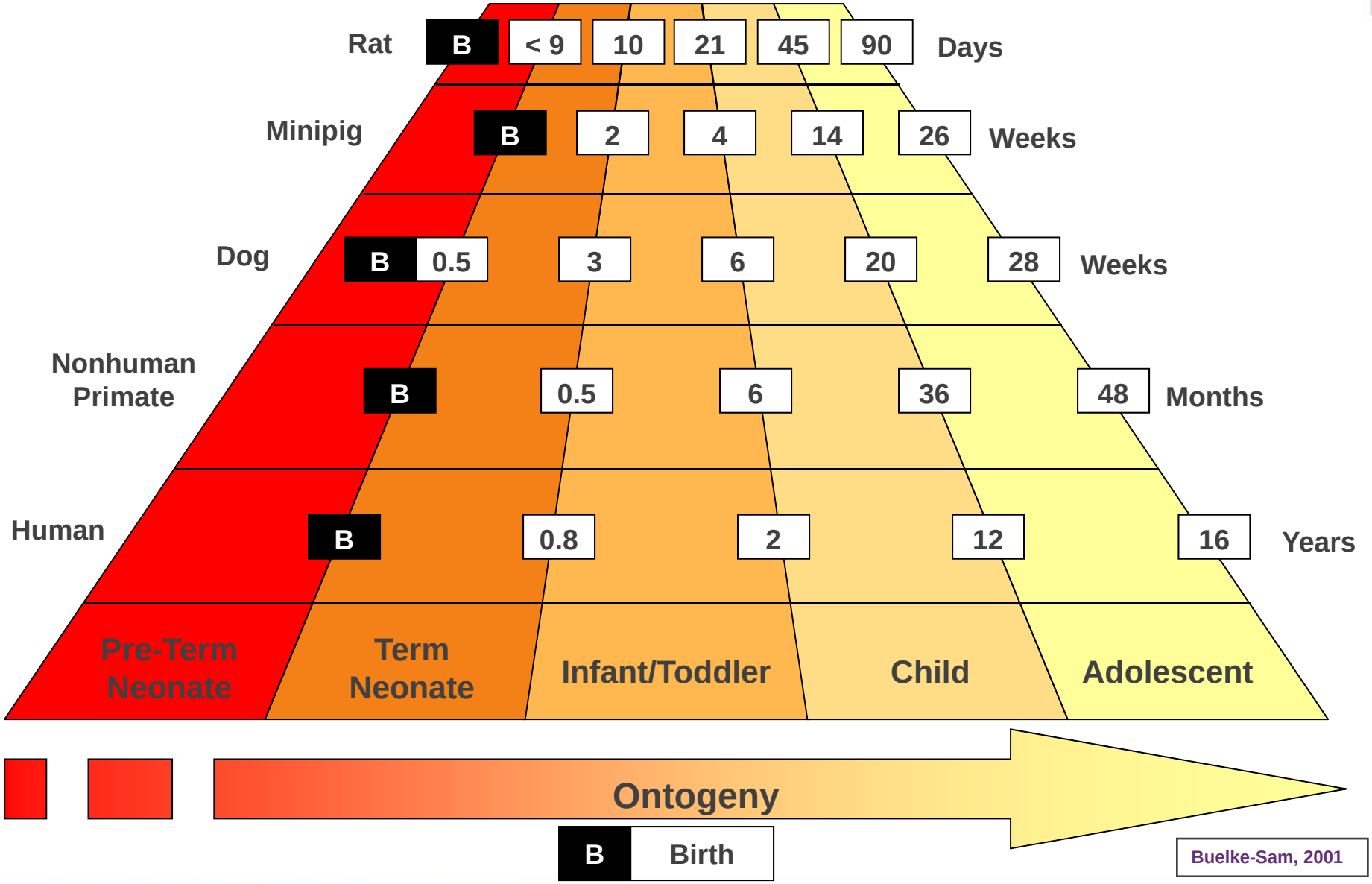
Why Minipigs?

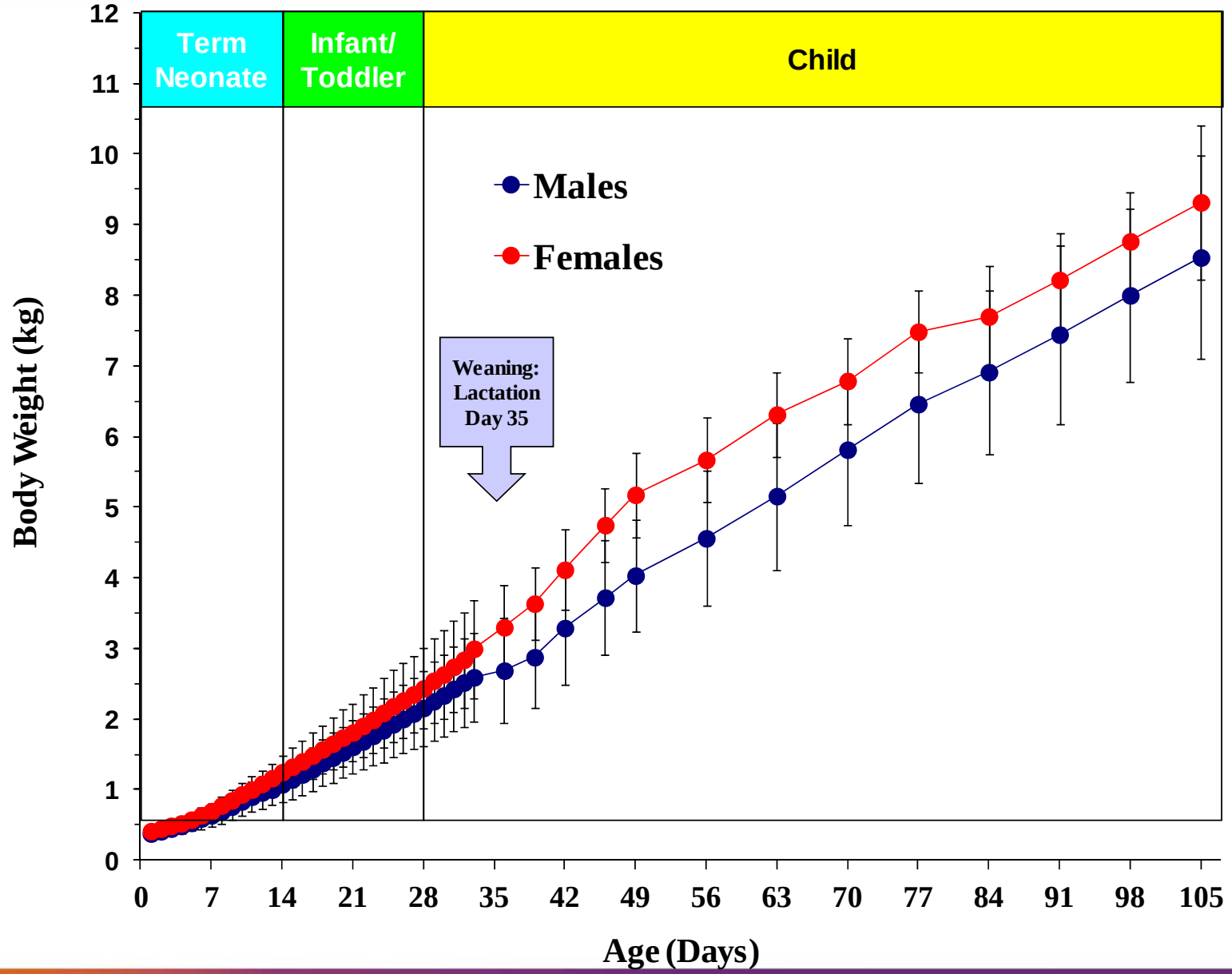
Similarity between human and minipig…

- skin
- cardiovascular system
- urogenital system
- gastrointestinal tract
- nasal cavity
- reproductive sensitivity
- metabolism

Configuration for Farrowing Gottingen Minipigs

Comparative Age Categories Based on Overall CNS and Reproductive Development




Term Neonate
Infant/ Toddler
Child
Males
Females
Weaning: Lactation Day 35
Body Weight (kg)
Age (Days)
0
1
2
3
4
5
6
7
8
9
10
11
12
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
105

Serum Chemistry Parameters

Parameter (Units)	Sex	Age in Weeks: 1	2	4-5	8-9	12-13	20-25
		Term Neonate		Infant/Toddler	Child		Adolescent
Albumin (g/dL)	M	2.6 ± 0.21 (9)	3.3 ± 0.41 (8)	4.1 ± 0.30 (10)	4.2 ± 0.30 (11)	4.7 ± 0.38 (11)	5.1 ± 0.28 (20)
	F	2.6 ± 0.61 (5)	3.3 ± 0.39 (6)	4.1 ± 0.14 (6)	4.4 ± 0.33 (7)	4.9 ± 0.11 (7)	5.0 ± 0.23 (20)
Albumin/ Globulin Ratio	M	1.00 ± 0.295 (9)	2.37 ± 0.767 (8)	3.83 ± 0.825 (10)	4.10 ± 0.769 (11)	4.91 ± 1.818 (11)	4.33 ± 0.721 (20)
	F	1.05 ± 0.105 (5)	2.33 ± 0.623 (6)	3.41 ± 0.777 (6)	3.54 ± 0.845 (7)	3.93 ± 0.678 (7)	3.30 ± 0.372 (20)
Total Bilirubin (mg/dL)	M	0.23 ± 0.106 (9)	0.29 ± 0.134 (8)	0.10 ± 0.037 (10)	0.05 ± 0.025 (11)	0.04 ± 0.046 (11)	0.09 ± 0.031 (20)
	F	0.26 ± 0.087 (5)	0.26 ± 0.043 (6)	0.08 ± 0.031 (6)	0.07 ± 0.036 (7)	0.06 ± 0.047 (7)	0.10 ± 0.039 (20)
Indirect Bilirubin (mg/dL)	M	0.20 ± 0.109 (9)	0.20 ± 0.104 (8)	0.07 ± 0.031 (10)	0.03 ± 0.022 (10)	0.03 ± 0.033 (7)	NA
	F	0.23 ± 0.064 (5)	0.21 ± 0.045 (6)	0.07 ± 0.024 (6)	0.05 ± 0.040 (7)	0.06 ± 0.040 (5)	NA
Alkaline Phosphatase (U/L)	M	1094 ± 485.3 (9)	1101 ± 403.4 (8)	388 ± 61.9 (10)	272 ± 57.4 (11)	205 ± 33.8 (11)	136 ± 31.9 (20)
	F	1342 ± 470.4 (5)	1041 ± 358.3 (6)	406 ± 97.6 (6)	260 ± 56.0 (7)	190 ± 35.4 (7)	150 ± 23.6 (20)
Cholesterol (mg/dL)	M	148 ± 91.7 (9)	116 ± 32.7 (8)	159 ± 47.7 (10)	88 ± 12.4 (11)	76 ± 19.3 (11)	70 ± 9.7 (20)
	F	165 ± 68.2 (5)	181 ± 21.7 (6)	220 ± 55.3 (6)	107 ± 11.9 (7)	93 ± 13.4 (7)	89 ± 10.3 (20)
Triglycerides (mg/dL)	M	261 ± 270.4 (9)	185 ± 39.2 (8)	128 ± 51.2 (10)	46 ± 13.0 (11)	38 ± 15.6 (11)	23 ± 7.7 (20)
	F	203 ± 44.5 (5)	198 ± 85.5 (6)	116 ± 49.6 (6)	47 ± 9.9 (7)	33 ± 4.4 (7)	39 ± 8.5 (20)

Hematology Parameters

Parameter (Units)	Sex	Age in Weeks: 1	2	4-5	8-9	12-13	20-25
		Term Neonate		Infant/ Toddler	Child		Adolescent
Red Cells (mil/uL)							
	M	4.85 ± 0.800 (8)	5.00 ± 0.354 (5)	6.13 ± 0.926 (10)	8.04 ± 0.600 (11)	9.18 ± 0.730 (11)	8.60 ± 0.634 (18)
	F	5.50 ± 0.976 (6)	5.35 ± 0.477 (6)	6.57 ± 0.736 (6)	8.09 ± 0.637 (7)	9.54 ± 0.553 (7)	9.17 ± 0.733 (18)
Hemoglobin (g/dL)							
	M	9.4 ± 1.47 (8)	9.9 ± 0.85 (5)	9.7 ± 2.13 (10)	12.8 ± 0.90 (11)	14.6 ± 1.09 (11)	14.3 ± 1.22 (18)
	F	10.4 ± 1.58 (6)	10.3 ± 0.48 (6)	10.8 ± 1.46 (6)	13.0 ± 1.00 (7)	14.9 ± 0.92 (7)	15.0 ± 1.00 (18)
Hematocrit (%)							
	M	30.7 ± 3.82 (8)	32.3 ± 2.82 (5)	30.3 ± 6.98 (10)	39.1 ± 2.42 (11)	42.8 ± 2.68 (11)	40.5 ± 4.24 (18)
	F	33.3 ± 4.97 (6)	33.7 ± 2.24 (6)	33.7 ± 5.17 (6)	39.2 ± 3.05 (7)	43.2 ± 2.81 (7)	43.3 ± 3.99 (18)
Platelets (thous/uL)							
	M	831 ± 193.2 (8)	804 ± 124.8 (5)	872 ± 271.1 (10)	672 ± 132.1 (11)	594 ± 125.6 (11)	546 ± 96.1 (18)
	F	856 ± 191.3 (6)	814 ± 310.0 (6)	931 ± 114.1 (6)	721 ± 99.8 (7)	616 ± 106.1 (7)	553 ± 116.3 (18)
Reticulocytes (%)							
	M	18.3 ± 8.95 (8)	18.6 ± 5.77 (5)	8.2 ± 2.24 (10)	3.0 ± 0.65 (11)	1.9 ± 0.52 (11)	1.3 ± 0.59 (18)
	F	14.5 ± 4.50 (6)	16.1 ± 2.64 (6)	7.5 ± 1.84 (6)	2.6 ± 0.97 (7)	1.2 ± 0.42 (7)	1.4 ± 0.87 (18)
Reticulocytes Absolute (thous/uL)							
	M	835.5 ± 319.16 (8)	917.8 ± 237.64 (5)	501.3 ± 144.53 (10)	239.1 ± 53.68 (11)	170.4 ± 43.76 (11)	101.4 ± 48.68 (16)
	F	765.5 ± 126.87 (6)	858.5 ± 133.18 (6)	492.1 ± 130.85 (6)	203.2 ± 74.48 (7)	119.7 ± 42.07 (7)	111.8 ± 53.18 (16)

ECG Parameters

		Age in Weeks					
		1-2	4	7	10-11	14-15	17-38
Parameter (Units)	Sex	Term Neonate	Infant/ Toddler	Child			Adolescent/Adult
Heart Rate (bpm)							
	M	229 ± 31.7 (9)	206 ± 43.6 (6)	160 ± 39.7 (7)	152 ± 49.6 (11)	136 ± 36.0 (12)	113 ± 21.9 (50)
	F	240 ± 21.5 (5)	232 ± 26.6 (3)	154 ± 20.9 (4)	146 ± 33.6 (7)	123 ± 24.0 (7)	112 ± 20.2 (49)
QRS Interval (ms)							
	M	24 ± 3.9 (9)	31 ± 10.4 (6)	26 ± 3.6 (7)	33 ± 7.6 (11)	36 ± 6.0 (12)	33 ± 4.7 (41)
	F	23 ± 5.8 (5)	30 ± 5.3 (3)	28 ± 4.0 (4)	32 ± 9.0 (7)	38 ± 10.0 (7)	31 ± 3.1 (40)
PR Interval (ms)							
	M	63 ± 10.4 (9)	61 ± 10.4 (6)	75 ± 6.8 (7)	73 ± 11.5 (11)	78 ± 12.9 (12)	96 ± 10.0 (50)
	F	64 ± 8.7 (5)	65 ± 6.7 (3)	78 ± 7.3 (4)	79 ± 10.2 (7)	80 ± 10.5 (7)	92 ± 10.4 (48)
QT Interval (ms)							
	M	139 ± 15.7 (9)	154 ± 16.9 (6)	195 ± 40.7 (7)	209 ± 38.3 (11)	230 ± 25.6 (12)	246 ± 23.0 (50)
	F	131 ± 9.8 (5)	144 ± 11.0 (3)	200 ± 10.8 (4)	218 ± 22.4 (7)	236 ± 14.5 (7)	253 ± 24.8 (49)
QT_F Interval (ms)							
	M	217 ± 16.4 (9)	230 ± 15.7 (6)	265 ± 40.7 (7)	278 ± 31.1 (11)	298 ± 20.3 (12)	301 ± 21.8 (41)
	F	208 ± 12.7 (5)	226 ± 8.7 (3)	273 ± 4.7 (4)	290 ± 17.7 (7)	297 ± 20.8 (7)	304 ± 25.9 (40)
QT_V Interval (ms)							
	M	203 ± 13.0 (9)	214 ± 12.3 (6)	247 ± 35.0 (7)	259 ± 30.2 (11)	276 ± 19.2 (12)	270 ± 10.7 (9)
	F	196 ± 9.4 (5)	209 ± 9.0 (3)	253 ± 6.7 (4)	268 ± 16.6 (7)	278 ± 14.4 (7)	301 ± 14.9 (9)

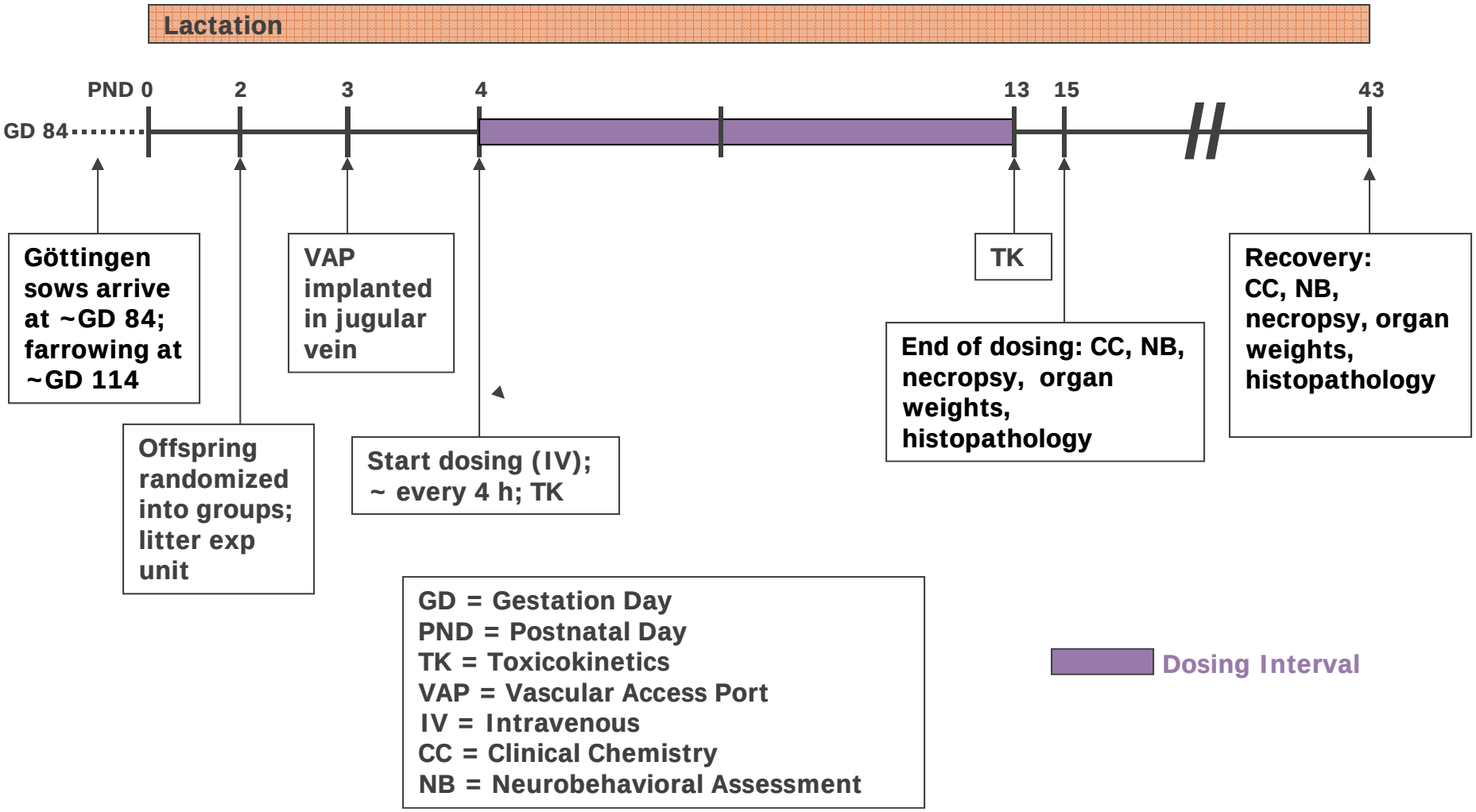
Case Study

- Intravenous drug for treatment of hypoxia in newborns
- Published literature on pig as a model for assessment of hypoxia in newborns
- Repeated intravenous injection not practical shortly after birth in the rat but is feasible in the minipig
- Availability of data from adult toxicity studies in the minipig
- Ability to perform experiments during critical developmental period in minipig comparable to human equivalent neonatal/infant age ranges
- Relative ease of blood sampling and ability to collect sufficient blood volume from serial sampling in individual animals
- The minipig is accepted and widely used as a surrogate to humans for nonclinical toxicity studies

Treatment Regimen/Study Design in Minipigs

- Need to cover period immediately after birth
  - Dose administration from PND 4-12 in the minipig offered coverage for term human neonate through 1 month of age
- Because drug is intended only for administration during the first 24 hours after birth, a protracted treatment period was not needed
  - 10-day dosing period was requested by FDA to cover variability in developmental stage at birth in humans
- Study design was to administer intravenously by slow bolus injection once every 4 hours over a 240-hour treatment period from PND 4-13, followed by a 4-week recovery period
  - Continuous infusion not feasible in neonatal piglets given the weight of the infusion pump (20-50% of PND 4 body weight)
  - Intermittent (every 4 hours) injections was considered an acceptable surrogate for continuous infusion

Juvenile Toxicity Study in Minipigs Outline



Study Execution

- Surgically implanted a vascular access port (VAP) on PND 3 and fitted each piglet with infusion jacket
- Jacket pockets contained the infusion catheters between individual doses; piglets remained in jackets until necropsy on PND 15
- For each injection, piglets were held in place by dosing technicians and injection was administered over approximately 45-60 seconds

Sow with piglets on PND 7; jacketed piglets surgically implanted with catheters on PND 3

Case Study Results

- There were no effects on survival or clinical condition
- No changes in mean body weights and body weight gains throughout the treatment and recovery periods
- No neurobehavioral findings
- No changes in clinical pathology parameters or absolute/relative organ weights
- No microscopic findings in any tissue that were related to test article administration
- The no-observed-adverse-effect level was considered to be the high dose

Conclusions

- Strong scientific rationale is paramount when using a nonrodent species for juvenile toxicology
- Juvenile studies can be successfully performed in nonrodent species when the rat's use is contraindicated
- Minipigs are appropriate alternative models to the rat and dog for juvenile studies

Acknowledgments

- Donald Stump, PhD, DABT, Vice President, Non-Clinical Safety Science, WIL Research
- Eric Padgett, PhD, Alcon Pharmaceuticals – Study Director for the case study presented
- Bennett Varsho, MPH, DABT, Director, Nonclinical Safety Operations, WIL Research