



# MINIPIGS IN SAFETY ASSESSMENT OF DRUGS

Roy Forster, CiToxLAB France

# Outline

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- Features of minipig models
- Study types
  - ▣ Local tolerance, repeated dose toxicology, PK/TK, reproductive toxicology, safety pharmacology
- Deployment of the minipig model
  - ▣ Small molecules (and others), Biologics
- Conclusions

# Minipigs?

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- Minipigs are strains of domestic pigs that are markedly smaller than farmyard varieties, and thus better adapted to laboratory housing.

# Why pigs?

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- The pig closely resembles man in many features of its anatomy, physiology, biochemistry and lifestyle. In particular, the cardio-vascular system, skin and digestive tract are considered to be very good models for man.
- Because of these similarities the toxic effects of chemicals and drugs in pigs may resemble the effects in man more closely than do some other commonly used laboratory animals.
- The pig also has some features that make it a very practical model in toxicology and safety assessment.
- Finally, since the pig is a food animal, testing in the minipig may be more acceptable to the public than the use of dogs or monkeys.

# 1. General features of minipigs

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# Breeds of Minipigs

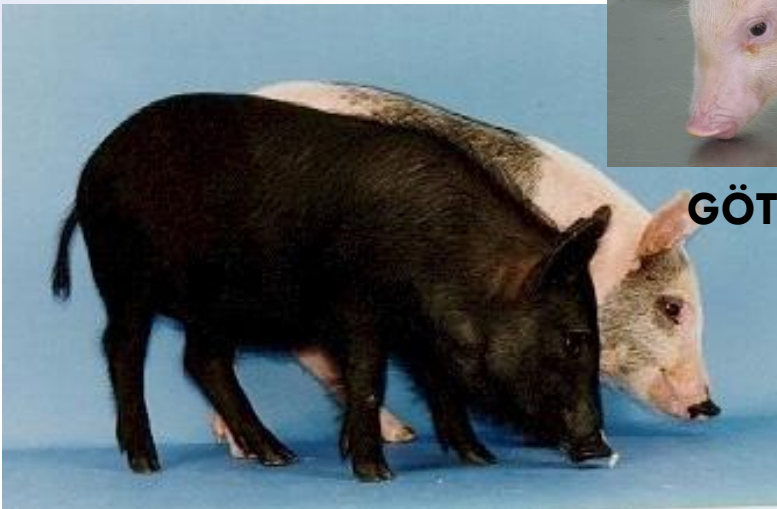
(Weight at 6 months)

**YUCATAN (32 Kg)**



**Micro-Yucatan (20 kg)**

**SINCLAIR (22 kg)**



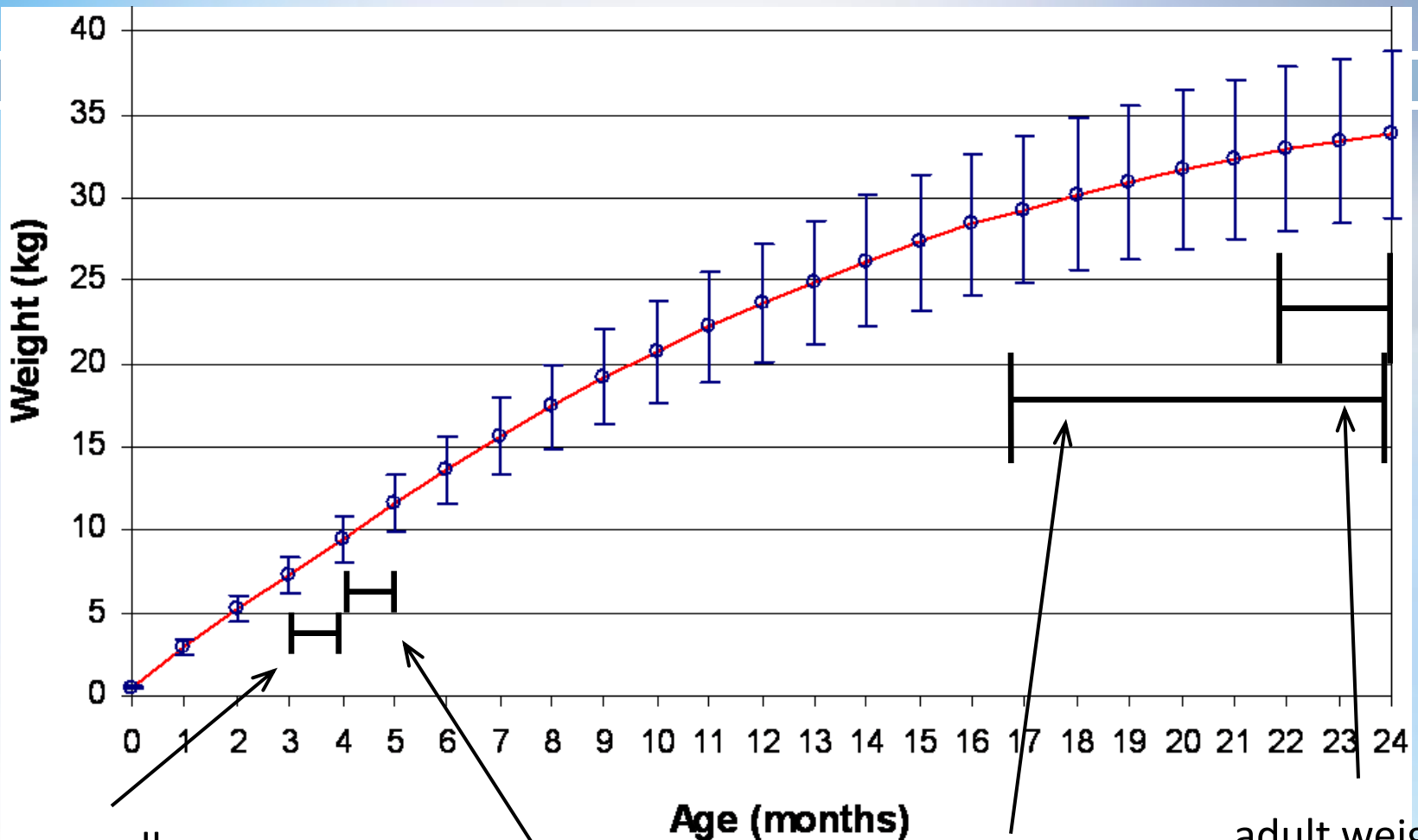
**GÖTTINGEN (13 kg)**

**HANFORD (37 kg)**



# Growth Curve - Göttingen minipig

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male sexually  
mature at 3-4 m

female sexually  
mature at 4-5 m

skeletal  
development  
completed

adult weight  
around 35 kg

8 What does it mean to be small and white?



# Dominant white skin colour

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Minipig eye fundus



# Origins & genetic management

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- The population history is documented back to the 1960's.
- The strain was caesarian-derived in 1992 and claims defined microbiological status. The Göttingen minipig is bred in a (sanitary) barriered unit.
- Genetic management is assured by the Institute of Animal Breeding of the University of Göttingen
- Two of the founder strains showed island dwarfism. Proportional dwarfism is probably mediated by modulation of actions of GH and IGF-1 and is not associated with any genetic defects
- The Göttingen minipig is not albino. The light skin colour is due to a “dominant white” status. The retina is pigmented and melanin is present in proportions similar to humans (Durairaj et al, 2012).

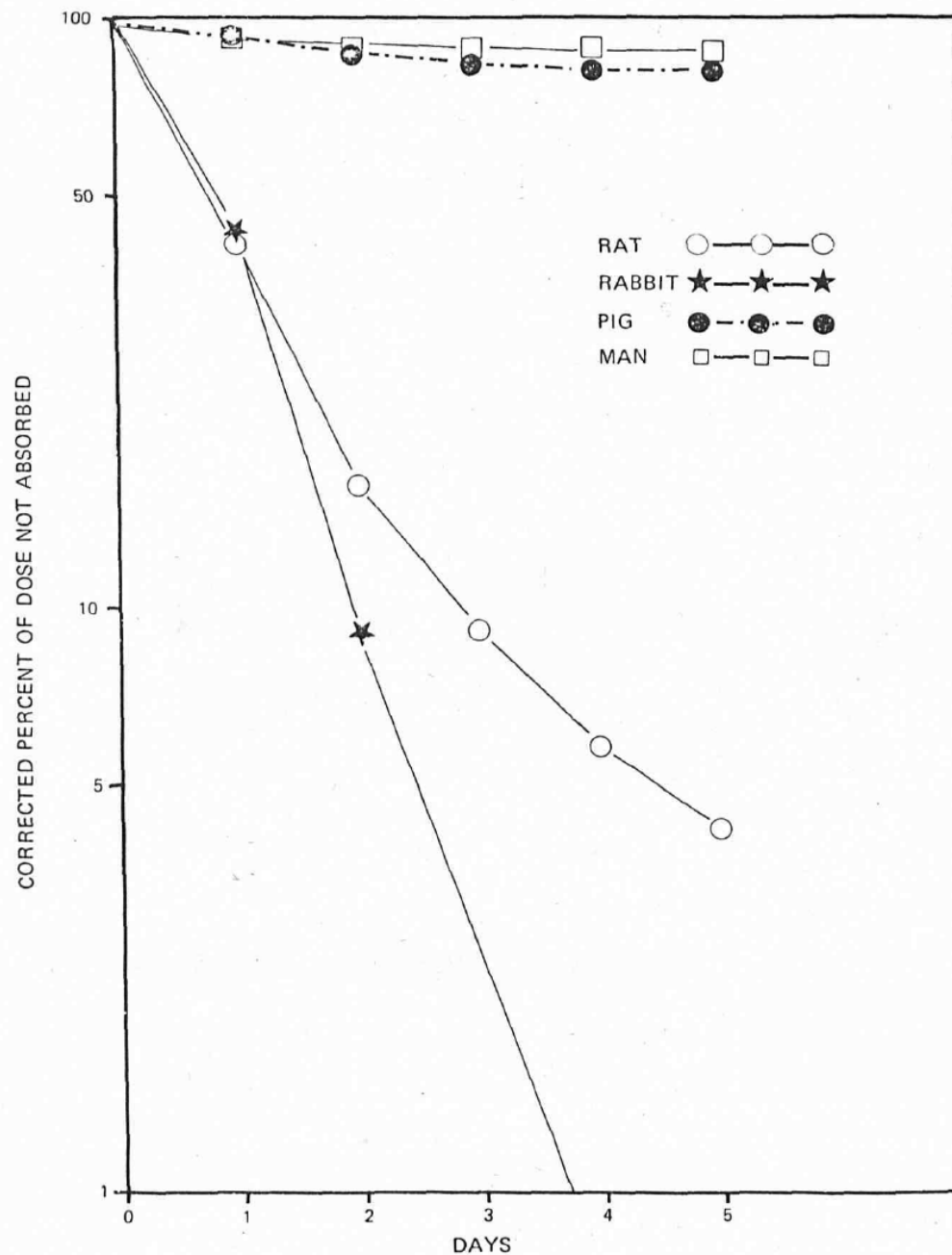
## 2. Minipig as a toxicology model

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# Similarities with human skin

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- Sparse hair covering
- Skin is closely attached to underlying structures
- Epidermis has an identical number of cell layers in stratum corneum and viable zones
- Presence of a rete ridge structure, like humans
- Skin texture and thickness varies over the surface of the body
  
- The epidermal thickness in the minipig is very close to that of humans
  - Minipig 70 - 140  $\mu\text{m}$
  - Human 50 - 120  $\mu\text{m}$
  - Rat 10 - 20  $\mu\text{m}$



In vivo skin permeability,  
radiolabeled Haloprogin

(Bartek, LaBudde, Maibach, 1972)

# Local tolerance studies

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- Minipig skin is considered a good predictor of cutaneous local tolerance in humans
  - ▣ The rabbit correlates poorly (Steinberg et al, 1975) and overpredicts tolerance issues (Jacova et al, 2010)
- Minipig models are available for a wide range of cutaneous tolerance endpoints, including
  - ▣ Tolerance on broken (scarified) skin or mucous membranes
  - ▣ Wound healing models
  - ▣ Phototoxicity (Swindle 2008, Sinclair Tech Bull)
  - ▣ Chemical vesication (Swindle 2008, Sinclair Tech Bull)
  - ▣ Contact allergic dermatitis (Swindle 2008, Sinclair Tech Bull)
  - ▣ Oral Photosensitisation (Yoshikawa et al 2013)

# Repeat dose (general) toxicology

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- All the usual dose routes can be used :
  - ▣ Dermal, oral, intravenous (bolus and infusion), im, sc, ocular/IVT and other
  - ▣ Although experience is limited for intranasal and inhalation routes.
- Blood sampling can also be conveniently achieved.



# Pharmacokinetics

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- Pharmacokinetics and ADME studies are therefore also feasible for the “usual” dose routes:
  - ▣ including mass balance and whole body autoradiography
  - ▣ punch biopsy, skin stripping and skin residence techniques
  - ▣ the minipig is also a convenient model for ocular pharmacokinetics

# Mass balance study

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**Table 10.1 Mass Balance of <sup>14</sup>C-Radiolabeled Drug after Dermal Administration to Two Female and Two Male Minipigs**

Animal No.	F1	F2	M1	M2	Mean	SD
Urine	0.027	0.008	0.055	0.156	0.062	0.066
Cage wash	0.039	0.069	0.066	0.178	0.088	0.061
Feces	0.057	0.042	0.101	0.191	0.098	0.067
Total excretion (% absorbed)	0.123	0.119	0.222	0.525	0.247	0.191
Strip 2–15	3.80	5.11	6.88	1.12	4.23	2.43
% in skin	3.80	5.11	6.88	1.12	4.23	2.43
Strip 1	2.82	2.28	4.81	0.710	2.66	1.69
Swab	2.07	2.77	7.21	4.99	4.26	2.33
Bandage	91.8	81.4	76.9	77.7	81.9	6.89
% not absorbed	96.7	86.4	88.9	83.4	88.8	5.71
Total recovery	101	91.7	96.0	85.0	93.3	6.65

*Note:* Cumulative excretion of drug and metabolites in % of dose until 7 days postdose.  
F = female; M = male, SD = standard deviation

(from: Preusser and Skaanild, 2012)

# General toxicology studies

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The minipig is a useful non-rodent model :

- ▣ Questions of practicality and feasibility are largely overcome
- ▣ Pharmacokinetic behaviour of drugs in minipigs is a good model for humans (Bronner et al, 2006, Zheng et al 2012)
- ▣ Metabolism is also generally similar to humans (Dalgaard , 2012, Bode et al 2010)
- ▣ Test item needs are similar to the dog
- ▣ The use of the minipig model is underpinned by the extensive use of pigs and minipigs in biomedical research
- ▣ Regulatory acceptance is not an issue

# General toxicology studies

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Study types where minipigs used.

Type of study	Number of companies
Safety Pharmacology—cardiovascular	5
<3m dermal repeat dose toxicity	7
>3m dermal repeat dose toxicity	4
<3m oral repeat dose toxicity	12
>3m oral repeat dose toxicity	5
<3m parenteral repeat dose toxicity	4
>3m parenteral repeat dose toxicity	0
Reproductive toxicity	1
Immunotoxicity	0
Other	1—model for adverse event, Bridging studies to compare formulations (requested from FDA if clinical dermal irritation study is positive) 1—GI tolerability

Studies performed by 13/22 industry respondents: (Bode et al., 2010)

# Reproductive toxicology

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# Reproductive toxicology

- Minipigs are suited to performance of Segment II and juvenile animal studies
  - ▣ Polyestrus
  - ▣ Short developmental cycle
  - ▣ Large litter size
- These features compare very favourably to the dog and NHP

Sexual maturity (females)	Circa 4 months
Estrus cycle	21-22 days
Duration of estrus	3 days
Gestational period	112-114 days
Average litter size	5-6
Bodywt of newborn piglets	350-400 grams

# Standard study design

- Group size: 14–18 Göttingen minipigs per group.
- Primiparous pregnant females: age 6 to 8 months.
- Exposure: Gestation Days (GD) 11–35 (organogenesis).
- Route of administration as appropriate: (oral, subcutaneous, or intravenous etc.).
- Fetuses: Collected by caesarean section on GD 109–111.
- Examination of fetuses: (follows that for rabbits)
  - o external and visceral examination of fresh tissue at necropsy
  - o skeletal examination of Alizarin stained bones
  - o Heads from half of the fetuses are fixed in Bouin's fixative, sectioned and examined for abnormalities and described according to the terminology published by [Wise et al. \(1997\)](#).

# Reproductive toxicology

- More data is needed on placental transfer and antibody transfer of antibodies in the pig
  - ▣ Fetal exposure of small molecules resembles humans (Wiest et al 1996; also human teratogens)
  - ▣ Maternal antibody transfer is entirely via colostrum and not placenta

Placental type	Species
Diffuse, epitheliochorial	Horses, pigs
Cotyledonary, epitheliochorial	Ruminants (cattle, sheep etc)
Zonary, endotheliochorial	Carnivores (dog, cat)
Discoid, hemochorial	Humans, primates, rodents

# Segment II embryofetal studies

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The minipig can be a useful alternative to rabbits or cynomolgus monkeys for segment II studies :

- ▣ Accepted study designs and reference (control) data are available
- ▣ The minipig has demonstrated sensitivity to human teratogens (thalidomide, hydroxyurea, pyrimethamine, ethanol and others; Bode et al 2010)
- ▣ The minipig has larger litter size and shorter gestational period than primates
- ▣ Placental passage: fetal exposure of small molecules resembles humans (Wiest et al 1996) but there is no placental transfer of maternal antibodies



# Juvenile toxicity studies

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- The minipig is a convenient model for juvenile toxicity studies because of:
  - ▣ Large litter size
  - ▣ Piglets are easy to cross foster
  - ▣ Size of piglets permits all dosing routes from an early age
  - ▣ Size of piglets permits other investigations and assays (blood sampling, ECG and ophthalmoscopy etc)
  - ▣ Cognitive models are under development (Grand 2010)
  - ▣ Rapid development of piglets to sexual maturity means that the entire juvenile period can be covered

(McAnulty, Barrow and Marsden, 2012)

# Safety pharmacology

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# Safety pharmacology

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All core battery studies can be performed in minipigs

- Cardiovascular function by telemetry

- ▣ There is a strong background of prior experience with pigs and minipigs in cardiovascular research and hemodynamics studies, occlusion, reperfusion, and atherosclerosis models.
- ▣ Expression profile of cardiac ion channels similar to humans (Laursen et al, 2010)

- Respiratory function

- ▣ using pneumotachometer or RIP approaches (Authier 2012, Milano 2012)

- CNS function

- ▣ using FOB (Authier 2012) or cognitive testing (Gieling et al 2013)

# Nonclinical drug safety

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The minipig can be a useful non-rodent alternative to the dog or monkey for:

- ❑ Efficacy studies
- ❑ Local tolerance
- ❑ Repeat dose (general) toxicology
- ❑ Pharmacokinetics/ADME
- ❑ Reproductive toxicology
- ❑ Juvenile toxicology
- ❑ Safety pharmacology

# 3. Deployment of the minipig

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# Porcine models in biomedical research

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- ❑ Transplantation
- ❑ Diabetes and obesity
- ❑ Blood substitutes
- ❑ Dental models, oral flora and disease
- ❑ Wound healing
- ❑ Traumatology
- ❑ Acute radiation syndrome
- ❑ Medical devices

# Deployment: small molecules

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- The minipig is the preferred non-rodent model for dermally administered drugs

# Deployment: small molecules

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- Because of anatomical or physiological similarities to man, the minipig is often selected for classes of drugs such as gastrointestinal and dietary therapeutics, insulin, ocular drugs, anemia therapies and medical devices
- The minipig may also be selected as non-rodent model on the basis of drug metabolism
  - eg by aldehyde oxidases, N-acetyl transferases, CYP2C9 (Dalgaard, 2012)

# Deployment: small molecules

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The minipig can be an advantageous non-rodent model for drugs **not well tolerated** by dogs:

- ▣ The minipig is less susceptible to vomiting
- ▣ Minipigs do not develop the characteristic canine cardiac lesions seen in dogs with vasodilators (eg Minoxidil) or sympathomimetics (Lehmann, 1998)

# Deployment: small molecules

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The minipig can be an advantageous non-rodent model for drugs **not well tolerated** by dogs:

- ▣ Dogs are more particularly sensitive to GI lesions induced by NSAIDs. The minipig was used for the development of the NSAID meloxicam (Lehmann, 1998)
- ▣ The low capacity of renal organic acid transporters in the dog makes it susceptible to toxicities of this class (Bode et al, 2010)

# Deployment: small molecules

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The minipig may be an inappropriate choice of non-rodent model:

- The low capacity of methemoglobin reductase in the minipig makes it susceptible to methemoglobin toxicity (Bode et al, 2010)

**Table 3.** Indications for Which Minipig Was Used to Varying Levels in Toxicological and/or Pharmacological Assessment<sup>a</sup>

Indications	Generic Name	Comments
Psoriasis	Calcipotriene; calcitriol	Vitamin D analogues
	Calcipotriene-betamethasone	Vitamin D analogue-corticosteroid
	Clobetasol, fluocinonide	Corticosteroids
	Tazarotene	Retinoid
Atopic dermatitis	Pimecrolimus, tacrolimus	Calcineurin inhibitors
Acne vulgaris	Adapalene-benzoyl peroxide	Retinoid-oxidizing agent
	Tazarotene	Retinoid
	Clindamycin-benzoyl peroxide	Antibiotic-oxidizing agent
	Clindamycin-tretinoin	Antibiotic-retinoid
Actinic keratosis and renal carcinoma	Didofenac	Cyclooxygenase inhibitor
	Fluorouracil	Thymidylate synthase inhibitor
	Methyl aminolevulinate	Photosensitizer
	Everolimus	Kinase inhibitor (mTOR)
Antiviral	Kunecatechins (HPV)	Immunomodulator
	Enfuvirtide (HIV)	Fusion inhibitor
Lentigenes and Melasma	Mequinol-tretinoin	Tyrosinase inhibitor-retinoid
	Fluocinolone acetonide-hydroquinone-tretinoin	Corticosteroid-tyrosinase inhibitor-retinoid
Hirsutism and androgenic alopecia	Eflornithine	Ornithine decarboxylase inhibitor
	Minoxidil	Vasodilator
Diabetes	Insulin detemir	Insulin analogue
Parkinson and Alzheimer disease	Rotigotine; pramipexole	Dopamine agonists
	Selegiline	Monoamine oxidase inhibitor
	Rivastigmine	Cholinesterase inhibitor
Osteoporosis	Calcitonin; alendronate; risedronate	Osteoclast-mediated bone resorption
Rheumatoid arthritis	Meloxicam	Nonsteroidal anti-inflammatory
Hypertension, heart failure and arrhythmia	Carvedilol	Vasodilator (antiadrenergic $\beta$ blocker)
	Dronedarone	Antiarrhythmic
Anesthesia and analgesia	Lidocaine	Local anesthetic (sodium channel blocker)
	Tapentadol	Centrally acting analgesic
Antibiotics	Retapamulin	Pleuromutilin antibiotic
	Quinupristin-dalfopristin	Streptogramin antibiotics
	Telavancin	Glycopeptide antibiotic
Imaging	Sulfur hexafluoride	Contrast agent for cardiac and vascular imaging
Prevention of sunburn	Anthelios	UV protection
Miscellaneous	Lanreotide	Somatostatin analogue
	Ecallatide	Kallikrein inhibitor
	Iron sucrose	Iron supplement

(Ganderup, 2012)

# 43 cases by route and indication

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**Table 39.1 Marketed Drug Products, Broken Down by Route of Intended Clinical Administration**

Route of Administration	Number of Products (%)
Dermal	25 (58)
Oral	8 (19)
Intravenous	5 (12)
Subcutaneous	4 (9)
Intramuscular	1 (2)

*Note:* A total of 43 cases are covered in detail. The number of marketed drug products identified is larger, as some products, for example, sunscreen, are covered as one case but in fact cover four separate marketed and approved products. Also, several of the products indicated for osteoporosis are approved in several formulations and/or dosage forms.

(Ganderup, 2012)

# Deployment of minipig: biologics

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□ Species selection will be influenced by:

- *Pharmacological activity*
- *Size and test item needs*
- *Availability of reagents*

And also

- *Pressure to justify NHP use*

Concerns differ for

- *Species restricted biologics*
- Non-restricted biologics

# Deployment of minipig: biologics

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## □ Monoclonal antibodies:

- ▣ Zheng et al (2012) found minipig to be a translational model for the sc pharmacokinetics of 9 mabs
- ▣ Immunogenicity of adalimumab and infliximab studies in minipigs (Penninks 2011)

## □ r-proteins

- ▣ Chen et al (2000) studied the plasma and lymph pharmacokinetics of recombinant hu-interleukin-2 and PEG-modified interleukin-2 in pigs.
- ▣ Immunogenicity and pharmacokinetics of r-hu-IL1r studied in minipigs (Penninks 2011)

# Deployment of minipig: biologics

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## □ Antisense:

- ▣ Two antisense projects (Bode, 2010; Forster, unpublished)

## □ Gene therapy

- ▣ Minipig projects with hu-Ad5 and lentivirus vectors (Bode 2010, Glud et al, 2010), DNA vaccine (Dincer et al, 2006)

## □ Vaccine, nanoparticle, cytokine studies

# Deployment: proceed with caution

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## General toxicology

- Evaluate carefully any therapeutic acting on IGF/IGFr
- Also any therapeutics acting through cKIT pathways

## Reproductive toxicology

- No placental transfer of antibodies

# 4. Conclusions

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# Conclusions

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- Minipigs are versatile animal models for preclinical toxicology of candidate drugs with numerous advantageous features
- There appears to be growing interest in the use of minipig models
- We may see increasing use of minipigs for the preclinical studies of biologics
- We need a systematic evaluation of the predictivity of models models for human toxicities
- *Acknowledgements: My thanks to many friends and colleagues who provided material and ideas that have gone into this presentation, with particular thanks to Andrew Makin, Simon Authier, Lars Ellegaard and Paul Barrow.*

# Further reading

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- Rethink project :
  - ▣ Special Issue of the Journal of Pharmacological and Toxicological Methods (Vol 62 (3), 2010)
  - ▣ The articles may be downloaded from the project website: [www.rethink-eu.dk](http://www.rethink-eu.dk)
  
- and
  - ▣ The Minipig in Biomedical Research, McAnulty, Dayan, Ganderup & Hastings (Eds) CRC Press 2012