
Minipigs in human relevant safety assessment - learnings from Roche (Safety Pharmacology & Toxicology/Pathology)

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Roche Innovation Center Basel

Webinar 13 June 2019

pRED Innovation Center Basel ...

Pharma Research and Early Development -pRED Innovation Centers



... today



... and tomorrow



Matching a patient with the best treatment based on their specific biological markers is the essence of **personalized healthcare**



EMA FIH Guideline 2017 – Non-clinical aspects

Relevance of the animal model has to be explored prior to FIH

- **Need to justify or dis-qualify animal species for toxicity testing**
 - Pharmacological activity
 - Metabolite pattern in animals compared to man
 - Limitations for PD readout of certain targets in healthy animals
- **Is a species relevant?**
 - 👍 Go with a rodent and a non-rodent, or go with single species
 - 👎 Go with a surrogate/homologue or in vitro data only
 - use of in vitro human cell systems or human-derived material could provide relevant information about translational differences



20 July 2017
EMA/CHMP/SWP/28367/07 Rev. 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products

Trigger: BIA 10-2474 FAAH irreversible antagonist

Company: Bial-Portela

Brain damage/hemorrhage with 5th dose in MAD on January 10, 2016

1 HV died, 3 with permanent neurological symptoms, one not affected

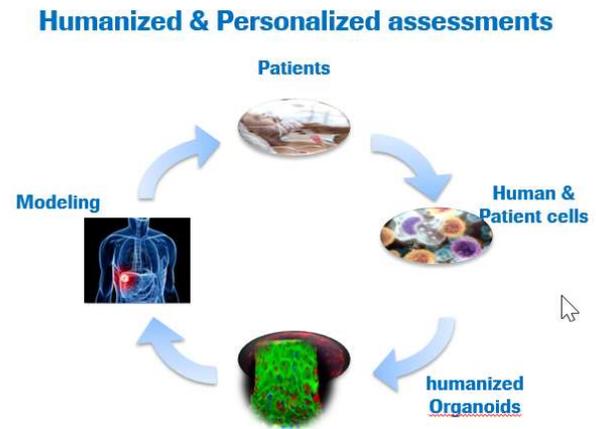
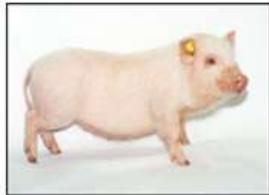
Consequence: multiple Phase I hurdles

It will be always a case-by-case approach

What defines a responder species?

Human Relevant Safety Assessment

- Is target expression / tissue distribution in animals comparable to human?
- Degree of target / pathway homology between species?
- Do we expect same / similar target mediated effects as in human (pathway/regulatory mechanisms)?



Maximize the likelihood of identifying responses that are similar to those expected in human

Species Selection for non-clinical safety studies

Some points to consider especially for small molecules

- Is metabolite pattern similar to man?
- Can animals be exposed high enough?
- At least one species used for toxicity testing should be a responder species
- Animal species considered responder as long as the same degree of agonism/antagonism (as in human) can be reached in preclinical safety studies

		
Availability/quality/cost	✓	☹
Space requirements	3.65 m² for 5 animals	15 m² for 5 animals
Age of sexual maturity	3-4 months (M) 4-5 months (F)	4-5 yrs
Weight at sexual maturity	~10 kg	4-6 kg
API requirements	☹ (⊗ chronic)	☺
Stock animals	☹	✓

The perfect animal model does not exist

Timing of assessment of responder status



- **At Target Identification /Assessment**

- Evidence from the literature and data bases as to expression, tissue distribution and degree of homology of a given target in human and animal species of interest

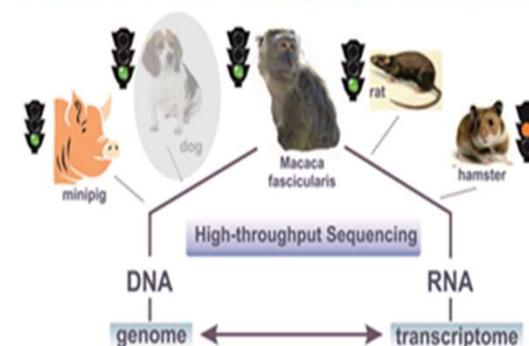
- **During LI or early LO-phase** (if above info missing/insufficient)

- Recommendation to clone the target for binding/functional assays in animal species *and/or*
- Establishment of functional assay to compare activity, if feasible

- **Selling point in the past:**

- Information needed for informed selection of the tox-species to assure human relevant safety assessment and also to avoid producing potentially irrelevant findings
- Judging potency for safety margin calculation for human starting dose

Genome based characterization of animal models



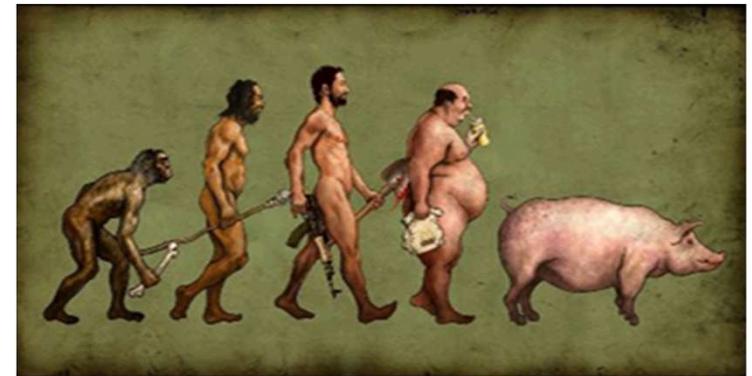
[green light indicates complete genome and protein annotation]

New guideline confirms importance of investigations on relevance of animal species

Non-rodent species selection at Roche

Some general aspects

- **Minipig and non-human primate are non-rodent species used at Roche**, unless scientific reasons speak against it
- **Dog** has not been used at Roche anymore for several years
- With increasing ethical concerns relating to the use of primates and increasing problems with regard to supply of non-human primates the use of Minipigs is rising in general
- Species selection should always be based on **scientific criteria**



Number of studies performed at Roche Innovation Center Basel
In-house and at CROs from 2010 to 2019

Study Type	Number of Studies
PK studies	29
Telemetry studies	25
Ascending dose studies	9
Dose range finding studies	21
4-week studies	10
13-week studies	1
39-week studies	1

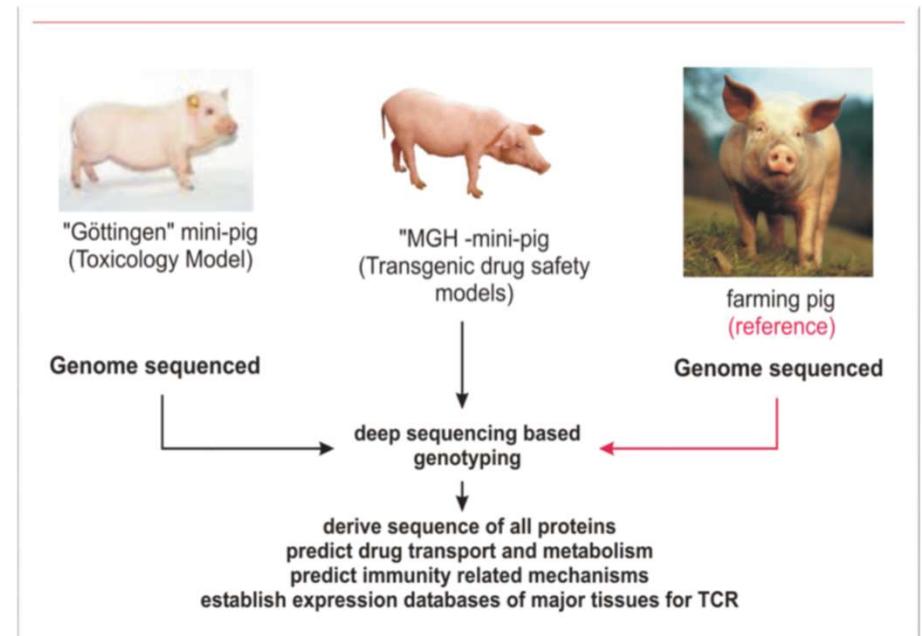
Data and facts are essential to convince scientists to use the Minipig

“Pig Genomics” in Pharmaceutical Sciences

Collaboration LMU and TU München, Ellegaard and PS Basel

Sequencing of Minipig genome

- Genome size and content comparable to primates and other animal models
- Tissue gene expression profiles are highly similar to humans
- Variability of gene expression levels is comparable to other animal models



Minipigs are as suitable for safety assessment as dogs and non-human primates

Moving beyond standard small molecule drugs

Characterization of Minipig for Single Stranded Oligonucleotide safety testing

In-house characterization of 4 naked SSOs in 2 or 4 week toxicity studies in Minipig:

- So far the Cynomolgus monkey has been broadly used as non-rodent model for assessing SSO safety
- Very sparse data published on Minipig in safety assessment of SSOs
- Duration and dose regimen comparable to Cyno
- Assessing SSO safety with focus on tissue exposure, target organs of toxicity, target engagement and pharmacodynamic effects

[Toxicol Sci.](#) 2017 May; 157(1): 112–128.

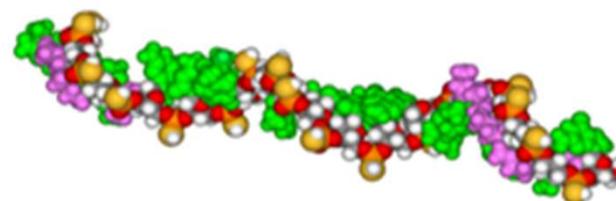
Published online 2017 Jan 25. doi: [10.1093/toxsci/kfx025](https://doi.org/10.1093/toxsci/kfx025)

PMCID: PMC5414856

PMID: [28123102](https://pubmed.ncbi.nlm.nih.gov/28123102/)

From the Cover: The Minipig is a Suitable Non-Rodent Model in the Safety Assessment of Single Stranded Oligonucleotides

[Annamaria Braendli-Baiocco](#)^{✉*}, [Matthias Festag](#)^{*}, [Kamille Dumong Erichsen](#)[†], [Robert Persson](#)[†], [Michael J. Mihatsch](#)[‡], [Niels Fisker](#)[†], [Juergen Funk](#)^{*}, [Susanne Mohr](#)^{*}, [Rainer Constien](#)[§], [Corinne Ploix](#)^{*}, [Kevin Brady](#)^{*}, [Marco Berrera](#)^{*}, [Bernd Altmann](#)^{*}, [Barbara Lenz](#)^{*}, [Mudher Albassam](#)[¶], [Georg Schmitt](#)^{*}, [Thomas Weiser](#)^{*}, [Franz Schuler](#)^{*}, [Thomas Singer](#)^{*}, and [Yann Tessier](#)[†]



Comparison of safety profiles in Minipig, Cyno & human

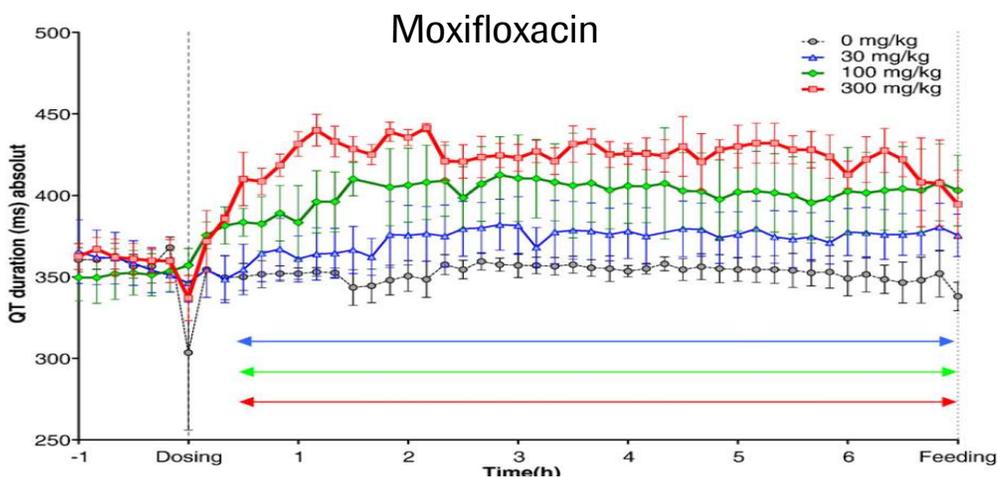
Safety Pharmacology

Minipig well established for cardiovascular assessment

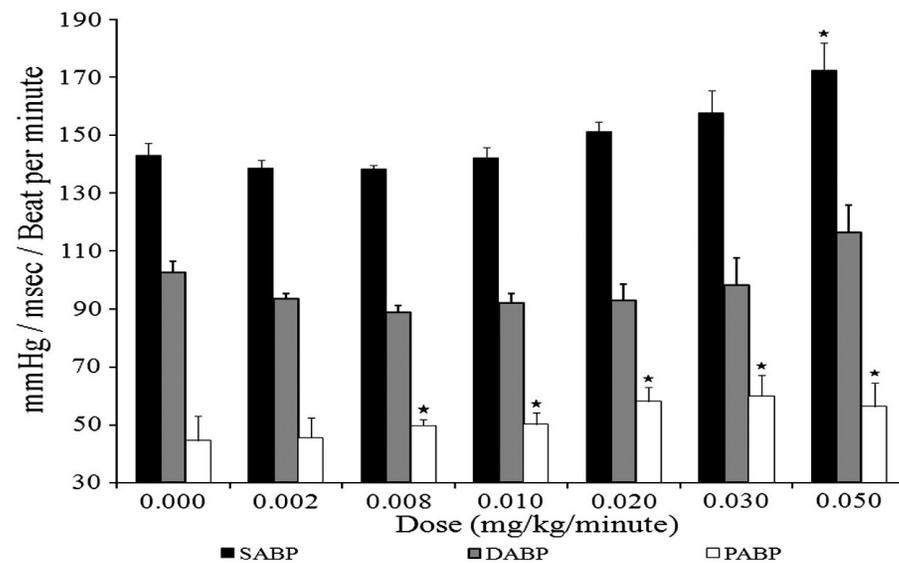
	Mean (day, 1-7 h)
SAP mm Hg	122
DAP mm Hg	86
LVP mm Hg	113
LV dP/dt mm Hg/s	2282
HR bpm	56
Temp. °C	37.0

	Mean
PR (ms)	125
QRS (ms)	56
QT (ms)	320
RR (ms)	861

Stubhan et al, JPTM 2008; Markert et al., JPTM 2009



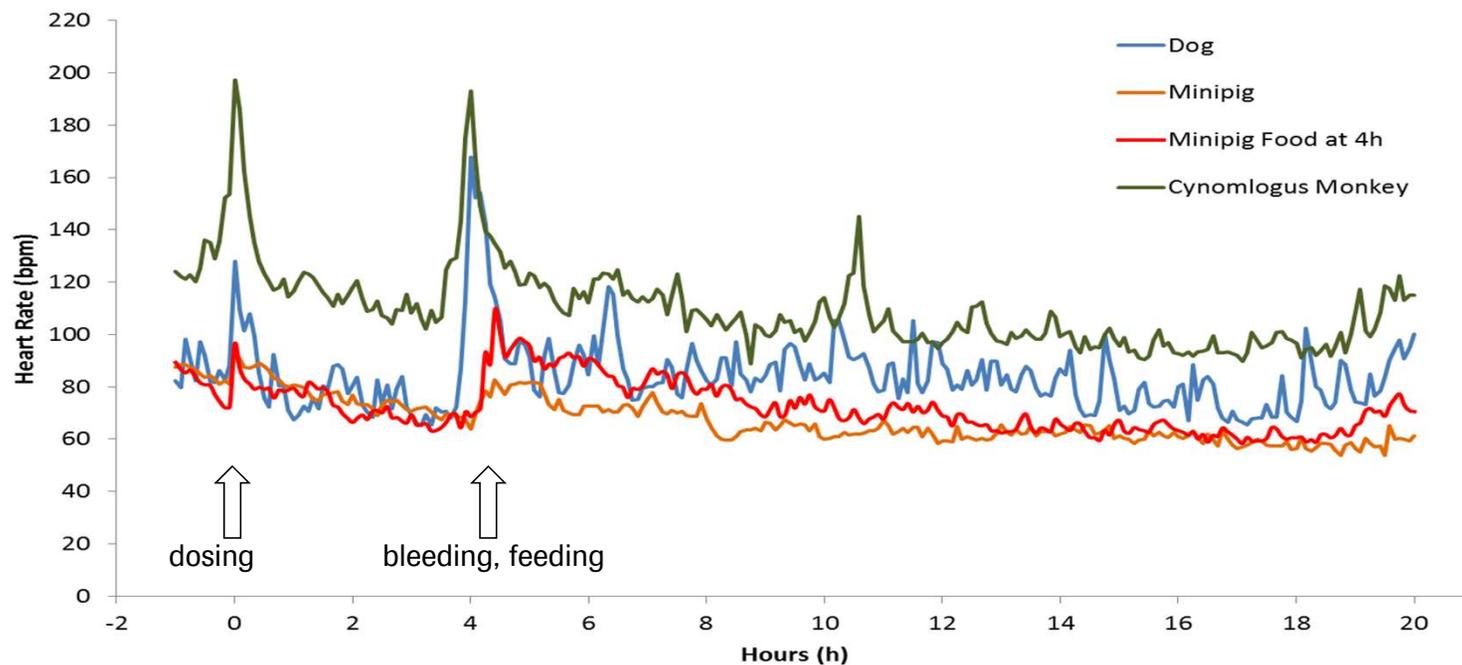
Dopamin 15 min after i.v. Infusion, effect on blood pressure



Authier et al, JPTM 2011

Species Differences in Heart Rates

Minipig, Cynomolgus Monkey, Dog



Minipigs less excited after handling than dogs and monkeys, but more influenced by food!

Feeding of Minipig should occur well before compound administration

Overnight fasting dependent on food

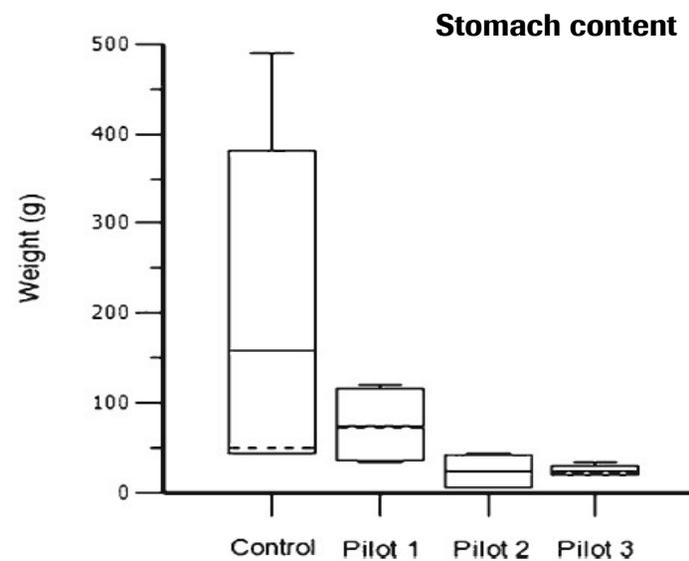
Pharm Res (2014) 31:2696–2707
DOI 10.1007/s11095-014-1367-6

RESEARCH PAPER

Pharmacokinetics of Paracetamol in Göttingen Minipigs: In Vivo Studies and Modeling to Elucidate Physiological Determinants of Absorption

Claudia Suenderhauf • Gerald Tuffin • Helle Lorentsen • Hans-Peter Grimm • Christophe Flament • Neil Parrott

- 4 groups of 4 Minipigs were given either:
 - Control: Straw and pellet diet
 - Pilot 1: Cotton towels and pellet diet
 - Pilot 2: Cotton towels and fluid diet
 - Pilot 3: Cotton towel and fluid diet + 0.2 mg/kg of metoclopramide
- Stomach and gastric content were weighed after an overnight fast



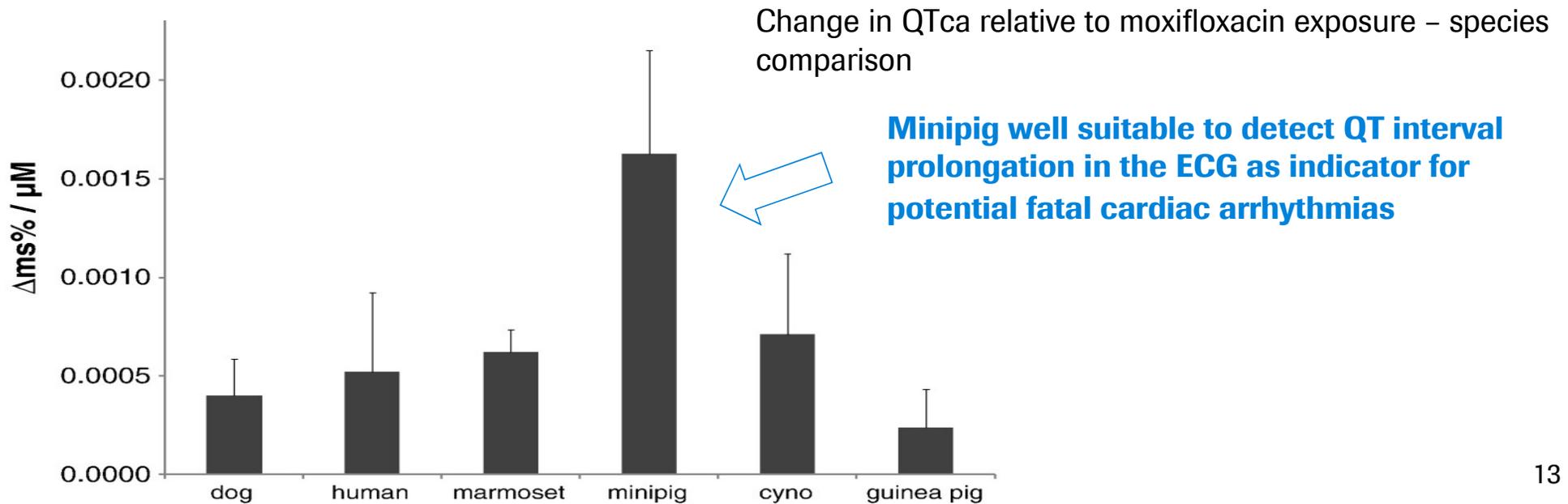
Prolonged gastric emptying with solid food, longest with straw



Appraisal of state-of-the-art

Preclinical QT safety assessment: Cross-species comparisons and human translation from an industry consortium

Henry Holzgrefe ^{c,*}, Georg Ferber ^h, Pascal Champeroux ^b, Michael Gill ^a, Masaki Honda ^d,
Andrea Greiter-Wilke ^e, Theodore Baird ^f, Olivier Meyer ^e, Muriel Saulnier ^g

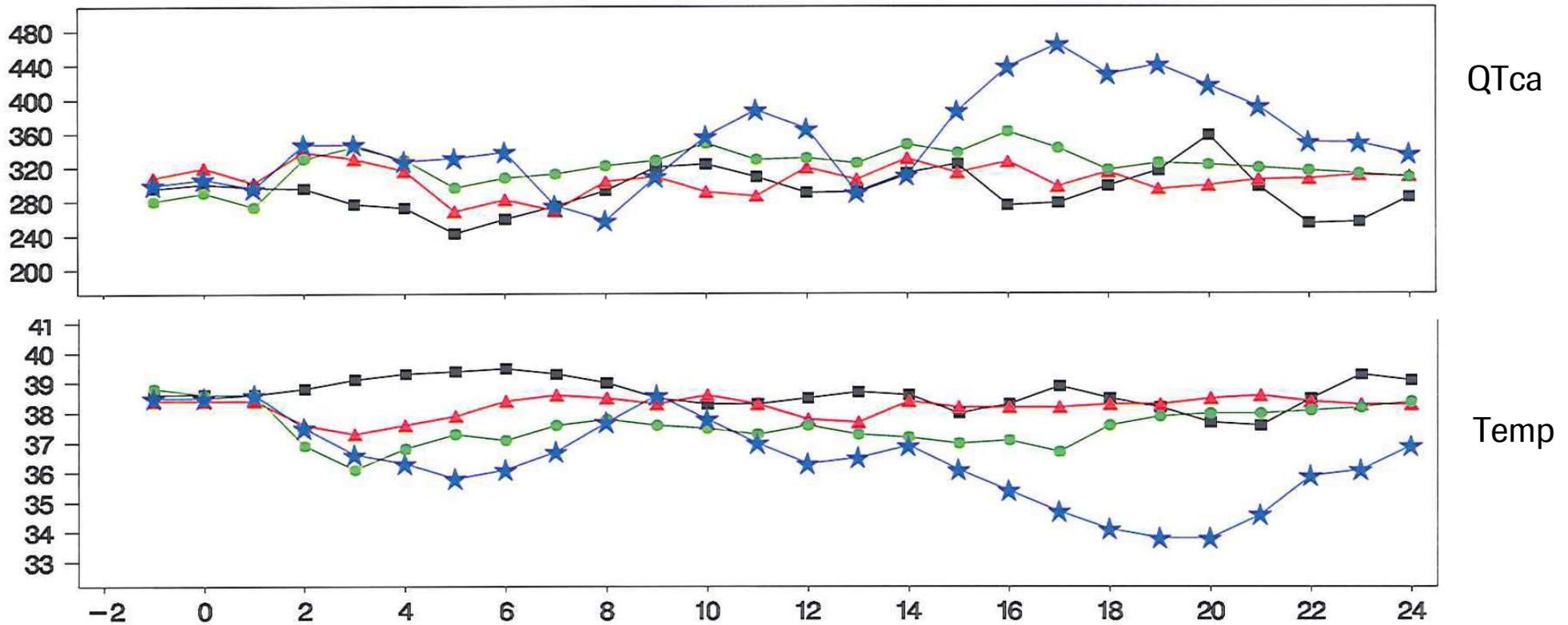


Individual Animal in case study

QT and Body Temperature



Roche



The Minipig in Safety Pharmacology

- The Minipig is suitable to detect QT prolongation as published and also proven in internal studies with reference compounds (invasive telemetry as well as jacketed ECGs)
- Sensitive to body temperature changes which influences the QT interval (decrease in body temperature leads to QT prolongation and vice versa)
- Less prone to CNS driven heart rate changes and vomiting compared to dog
 - Important aspects for safety assessment of CNS active drugs
- The minipig is suitable for Functional Observational Battery (FOB) and respiratory assessment if needed for toxicity studies (based on literature)

The Minipig is a well recognized and valuable species in safety pharmacology



The Minipig is a Suitable Non-Rodent Model in the Safety Assessment of Single Stranded Oligonucleotides

Annamaria Braendli-Baiocco,^{*,1,2} Matthias Festag,^{*,1} Kamille Dumong Erichsen,[†] Robert Persson,[†] Michael J. Mihatsch,[‡] Niels Fisker,[†] Juergen Funk,^{*} Susanne Mohr,^{*} Rainer Constien,[§] Corinne Ploix,^{*} Kevin Brady,^{*} Marco Berrera,^{*} Bernd Altmann,^{*} Barbara Lenz,^{*} Mudher Albassam,[¶] Georg Schmitt,^{*} Thomas Weiser,^{*} Franz Schuler,^{*} Thomas Singer,^{*} and Yann Tessier[†]

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Characterization of Minipig for SSOs safety testing

Comparison of safety profiles in Minipig, Cyno & human

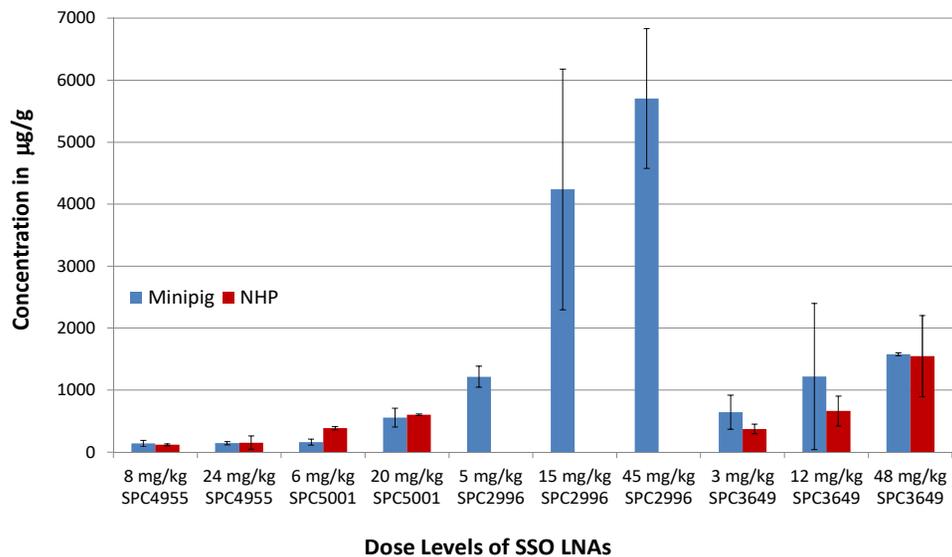
- Characterization of 4 Single stranded Oligonucleotides (SSO) in Minipig for assessing SSO safety relative to Cyno & human
 - Duration & dose regimen comparable to Cyno
- **2 or 4 week toxicity studies in female Minipigs to assess safety with focus on:**
 - Tissue exposure
 - Target organs of toxicity
 - Injection site reactions
 - Binding to plasma proteins
 - Target engagement & pharmacodynamic effects

No unexpected findings in Minipig

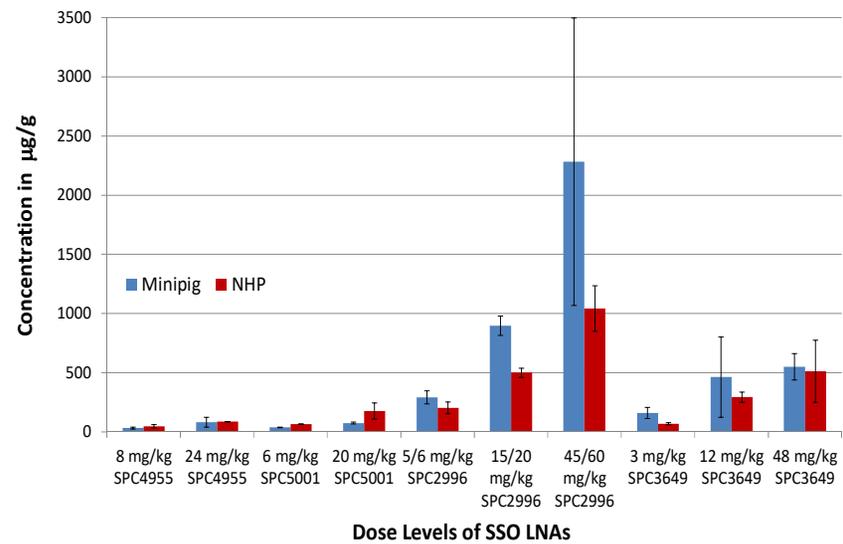
Tissue exposure

Comparison between Minipig & Cyno

Kidney Concentration



Liver Concentration



Braendli-Baiocco et al. Toxicol Sci. 2017;157:112-128

Tissue exposure in Minipig were within 2-fold of the Cyno values

Target organs in Minipig - Kidney

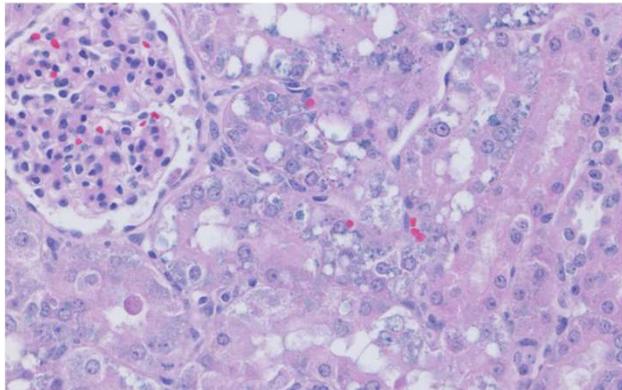
Similar findings in Minipig & Cyno



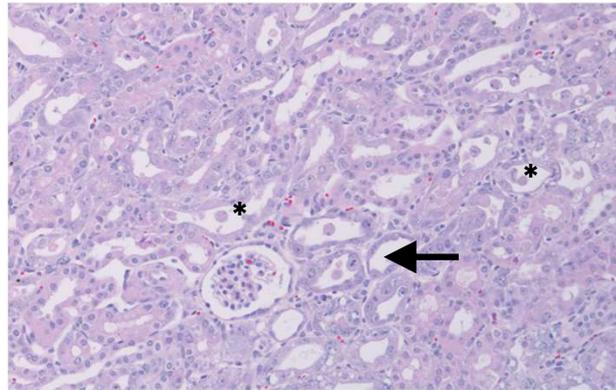
Control animal; kidney*



High dose: Pale kidneys due to kidney damage*



Basophilic granules*

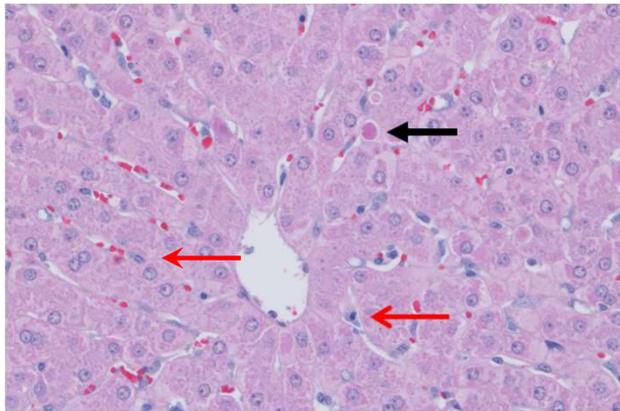


Tubular degeneration/regeneration: mitosis (arrow), single cell necrosis (asterisk)*

*Pictures from Minipig studies (Annamaria Braendli-Baiocco)

Target organs in Minipig – Liver & Lymph node

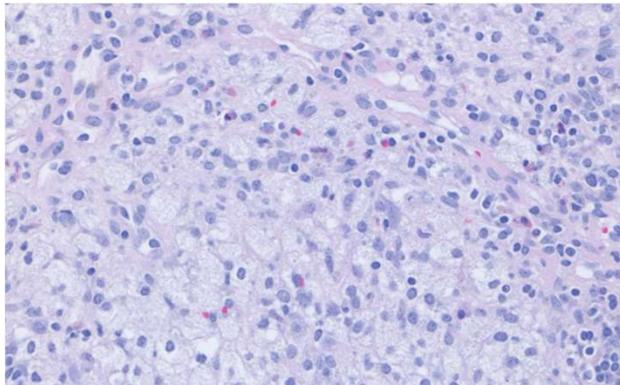
Similar findings in Minipig & Cyno



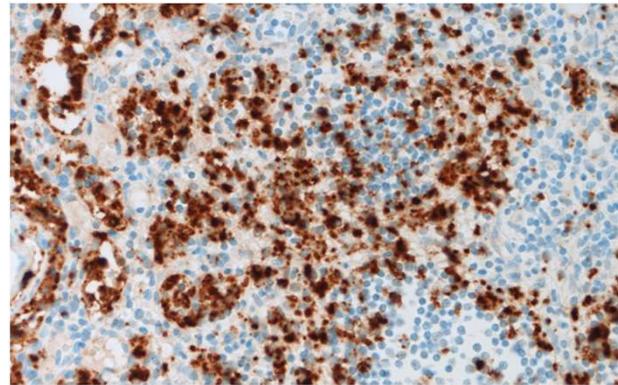
Apoptosis (black arrow), Kupffer cells (red arrow)*



SSO accumulation in Kupffer cells (IHC for SSO)*



Vacuolated macrophages in Lymph node*



SSO accumulation in macrophages (ISH for SSO)*

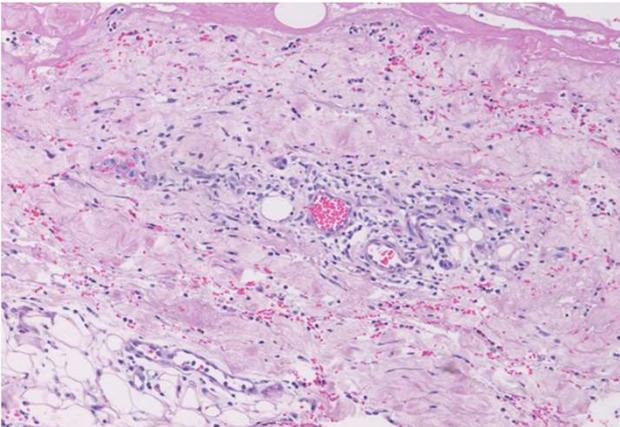
*Pictures from Minipig studies (Braendli-Baiocco et al. Toxicol Sci. 2017;157:112-128)

Target organs in Minipig – Injection Site

Histopathological findings



- All clinically tested SSO (sc) are associated with injection site reaction (ISR)
- Severity varies between SSO
- Severity increases at higher dose
- Some cause persistent lesions



- No clinical signs at injection site in Minipig
- Histopathology: Perivascular mononuclear inflammatory cell infiltration, hemorrhage & edema in Minipig
- Histological changes nearly completely reversible

Binding to plasma proteins: Effect on coagulation

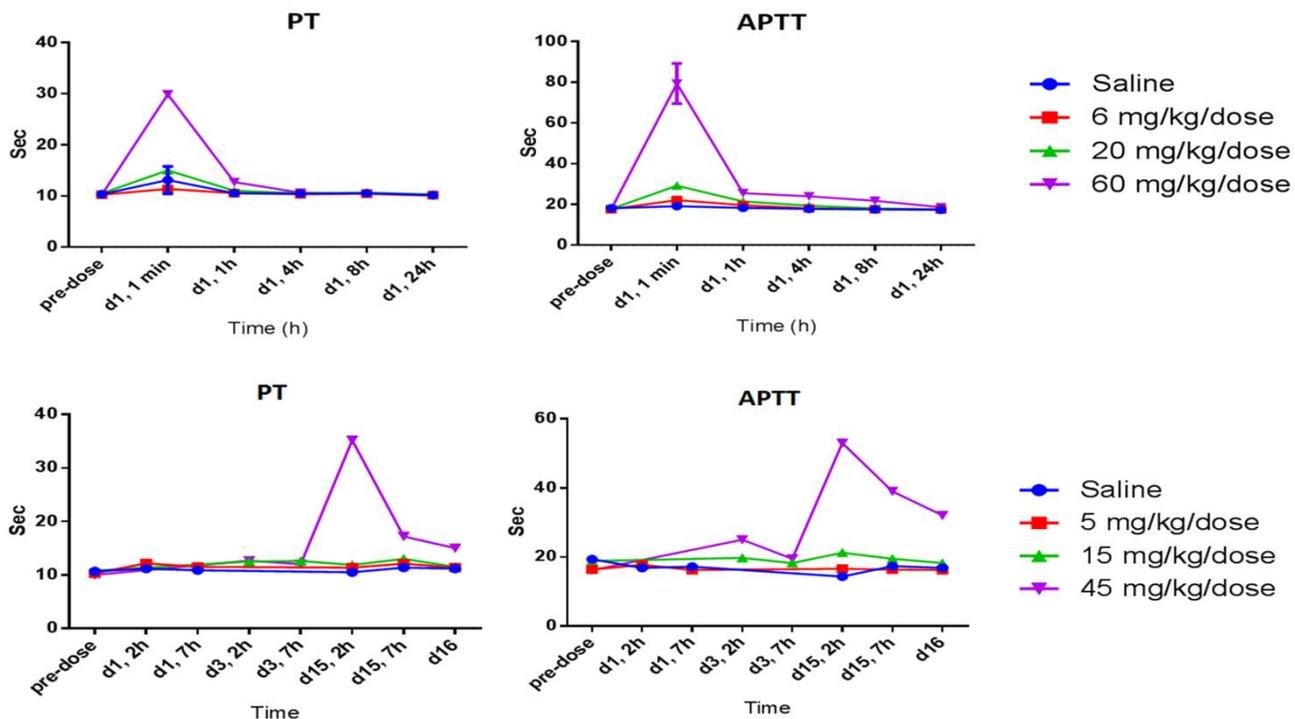
Similar findings in Minipig & Cyno

Human:
 C_{max} (4 mg/kg, d1,1-2 min): 28-84 µg/mL
 C_{max} (6 mg/kg, d1,1-2 min): 52 µg/mL

Cyno:
 C_{max} (60 mg/kg, d1,1-2 min):
 1886 µg/mL

Minipig:
 C_{max} (45 mg/kg, d1,15 min):
 541 µg/mL

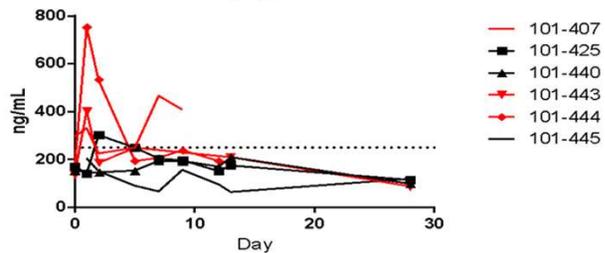
No coagulation alterations in humans



Binding to plasma proteins: Complement Activation

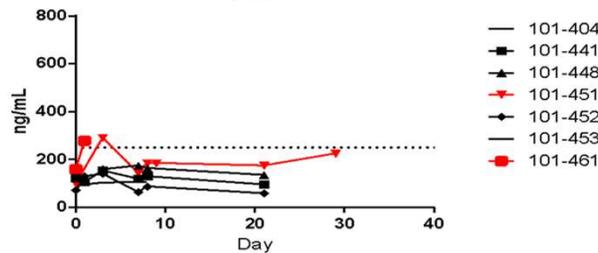
Similar findings in Minipig & Cyno

Human C5b-9 complement activation
4 mpk_6 inf



IV infusion on Days 0, 2, 5, 7, 9, 12

Human C5b-9 complement activation
6 mpk_3 inf



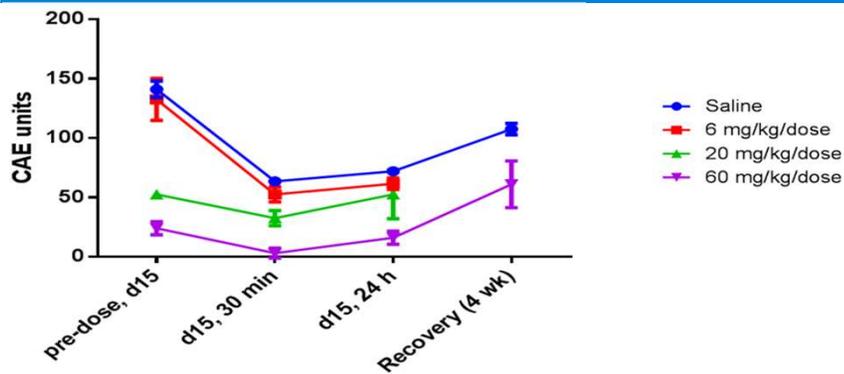
IV infusion on Days 0, 3, 7

Human

- Humans: 3/6 pts with increased C5b-9, no dose response

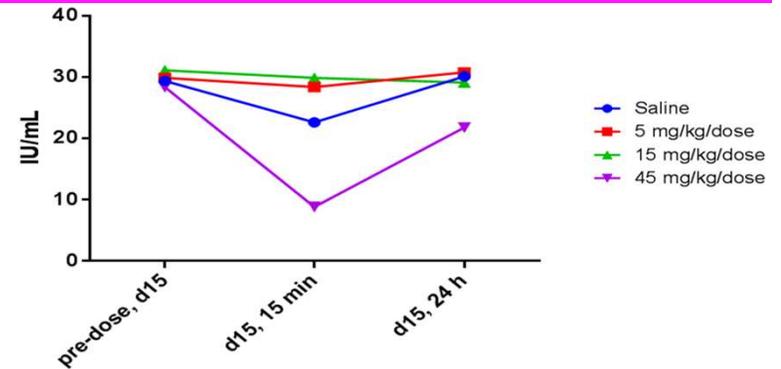
Total complement

Cyno



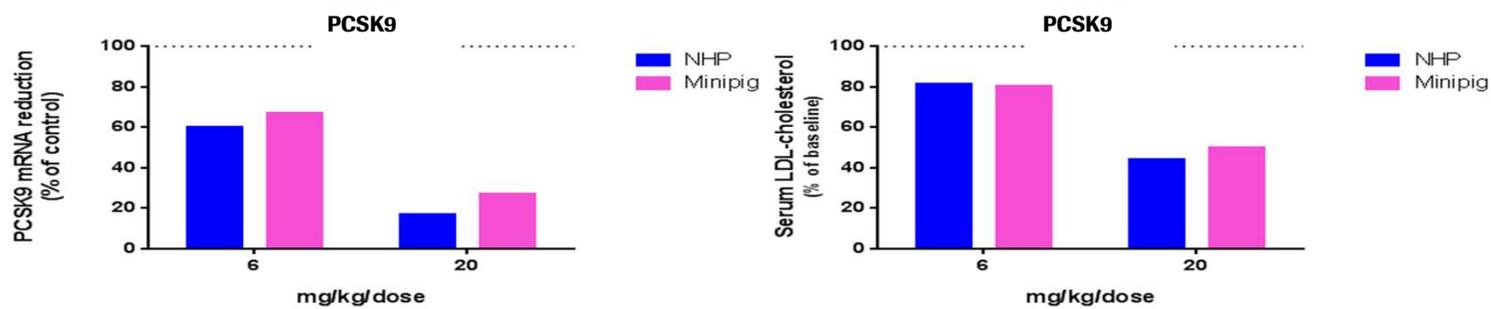
Braendli-Baiocco et al. Toxicol Sci. 2017;157:112-128

Minipig

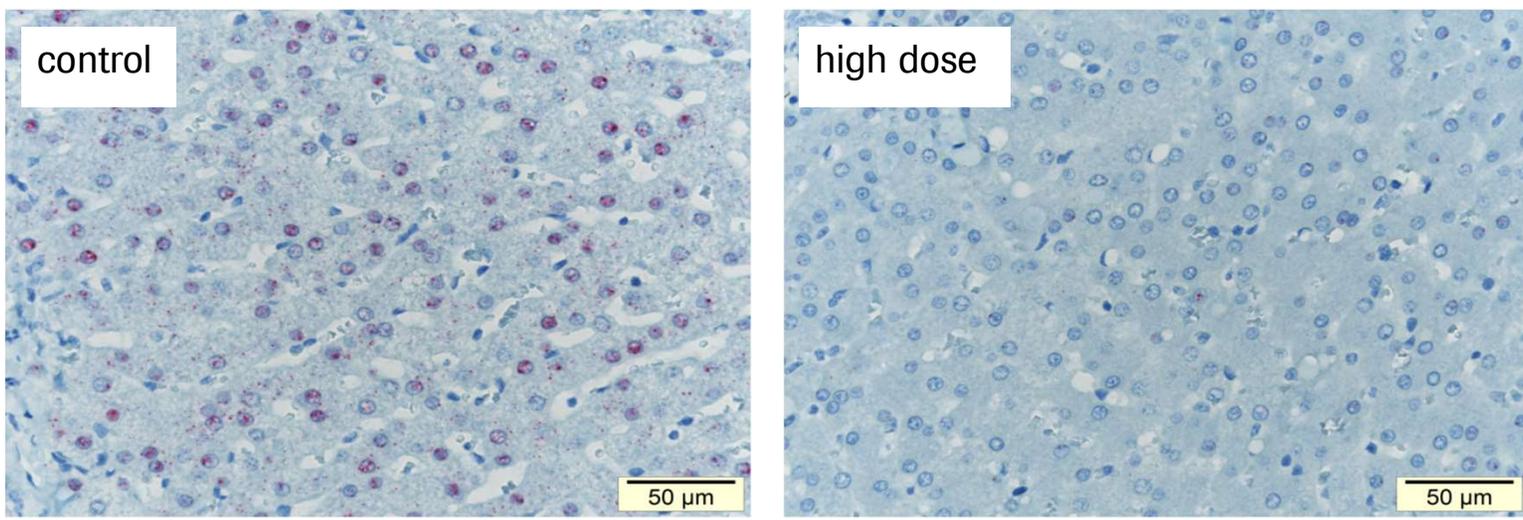


Target Engagement & Pharmacodynamics in Minipig

PD effects seen with SSO



ISH – PCSK9 mRNA expression in Minipig liver

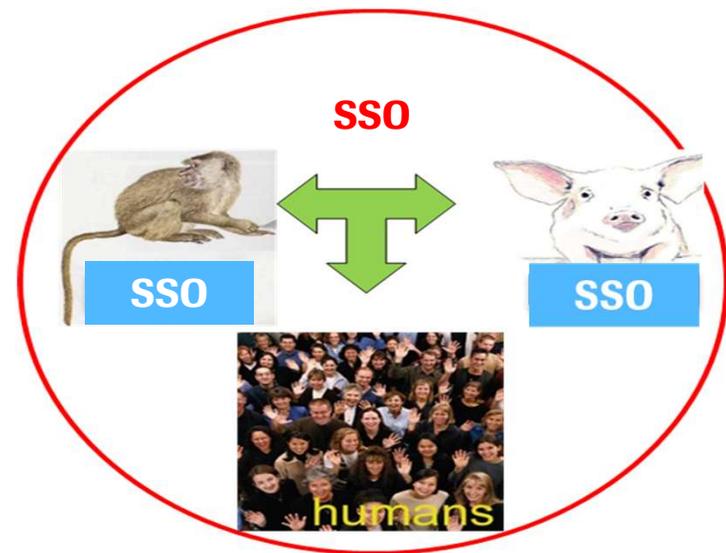


Braendli-Baiocco et al. Toxicol Sci. 2017;157:112-128

Characterization of Minipig for SSOs safety testing

Minipig considered as predictive as Cyno for assessing SSO safety

- Target organs of toxicity same as in Cyno & human (kidney & liver)
 - Minipig kidney appears to be more sensitive
- Effects on coagulation & complement activation similar to Cyno & human
- Based on clinical observations, Minipig and Cyno not predictive for Injection site reactions observed in human
- Pharmacokinetics, pharmacodynamics and target engagement generally similar to Cyno and human
- **No unexpected additional findings in Minipig**



Design oligos that are conserved in Minipig and/or Cyno whenever possible

Regulatory use of Minipig (pharmapendium[™] Nov 2018)



- Total **379 documents** listed
- **First:** Naproxen (analgetic), FDA approval package, **1976**
- **Latest non dermal:**
 - Lidocaine (analgesic): Postmarketing 9 mth study required by FDA **2018**
 - Levodopa (Parkinson's): intrainestinal infusion, FDA approval, **2014**
 - Afatinib dimaleate (Kinase inh), FDA approval, **2013**
- Used as non-rodent (dermal and oral route) in :
 - Dermatologics (e.g. Retinoids, Corticosteroids)
 - Immunosuppressives (e.g. Pimecrolimus)
 - NSAIDs (e.g. Diclofenac)
 - Cholinesterase inhibitors (e.g. Rivastigmine)
 - Analgetics (e.g. Meloxicam)
 - Antiparkinson (e.g. Pramipexol)
 - Vasodilators (e.g. Minoxidil)

Modified from G. Schmitt

Round table with Chinese KOLs on Minipig as non-rodent species

Minipig as non-clinical safety species accepted by CFDA



- Minipig used as non-rodent species in a HBV small molecule program at RICS (Roche Innovation Center Shanghai)
 - First non-dermatology compound using Minipig as non clinical safety species in China
- Two round table discussions were organized to get insight and guidance from two leading Chinese KOLs on the decision making process for CFDA acceptance of Minipig
- Successful pre-CTA meeting with Center for Drug Evaluation (CDE) in Feb 2017
- Roche submitted comprehensive data/literature package on Minipig to CFDA
 - Scientific rationale for species selection (specific for the current development compound)
 - Background / historical control data on Minipig
 - Successful examples in the clinics/market with tox data from Minipigs

CTA package with Minipig as non-rodent safety species approved by CFDA early 2018

Acknowledgements to numerous colleagues @ Roche

- Annamaria Braendli-Baiocco
- Georg Schmitt
- Jianxun Xie
- Martin Ebeling
- Matthias Festag
- Yann Tessier
- Thierry Lave
- Thomas Singer



...and many more colleagues

Doing now what patients need next