Therapeutic Antibody Testing in Minipigs

Considerations for selecting the minipig as a pharmacologically relevant species

Jerome Egli, Toxicology Project Leader
Roche Pharma Research & Early Development

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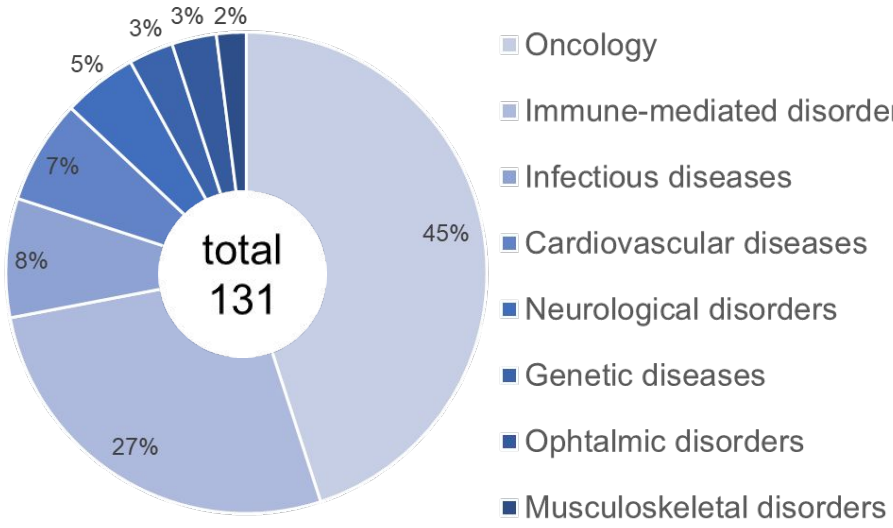
Therapeutic antibodies

Antibodies are an important class of therapeutics that come in many flavors

Antibody indications (2022)



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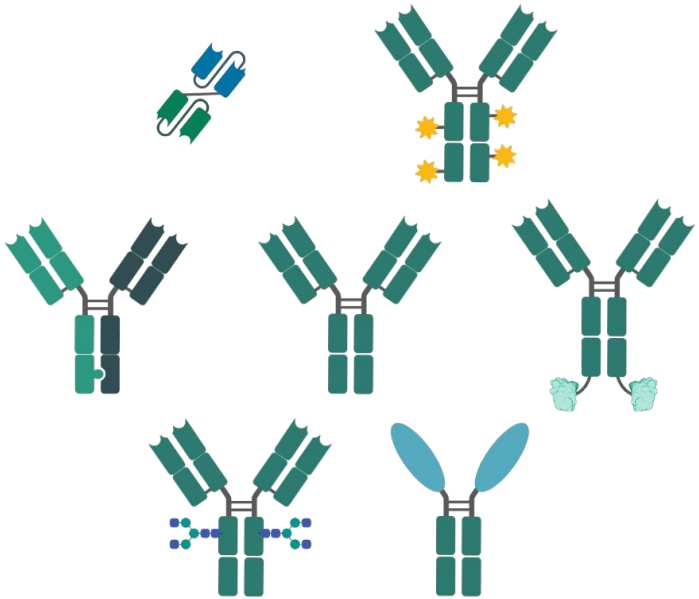
Kaplon H., et al. mAbs 2022 Antibodies to watch in 2022

High diversity of antibodies in development



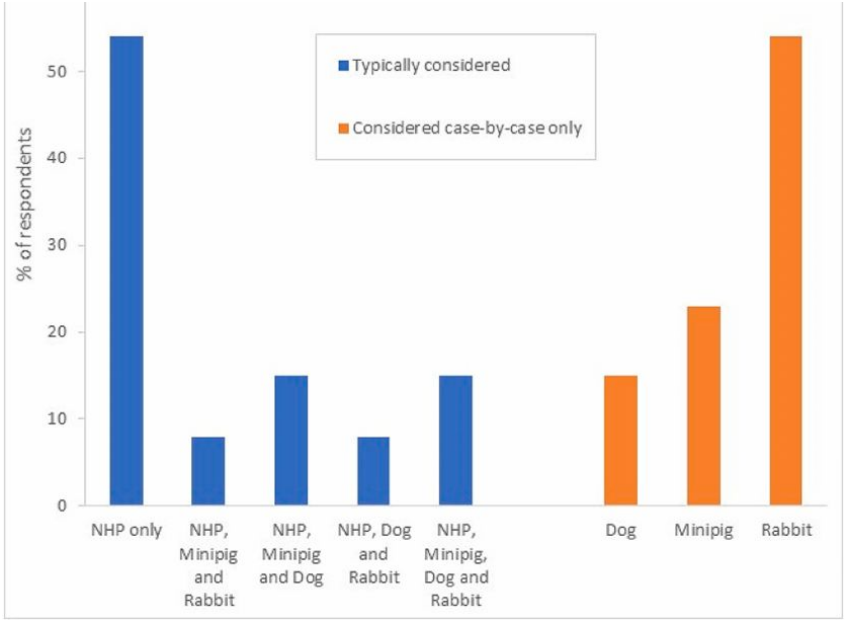
*"Safety evaluation programs should include the use of **relevant species**. A relevant species is one in which the test material is **pharmacologically active** due to the expression of the receptor or an epitope (in the case of monoclonal antibodies)."*

ICH guideline S6 (R1): preclinical safety evaluation of biotechnology-derived pharmaceuticals

The minipig as a tox species

Minipigs are a valuable animal model with potential to replace non-human primates

Non-rodent species considered for biologics



[Namdari R. et al. Species selection for nonclinical safety assessment of drug candidates: Examples of current industry practice. RTP 2021]

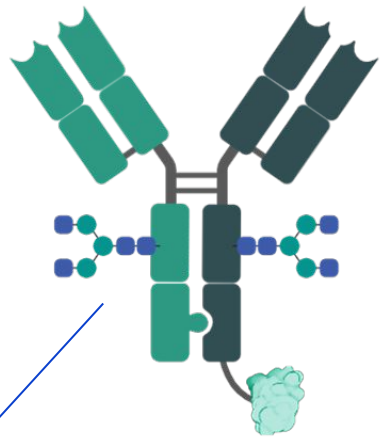
- Current shortage of non-human primate availability is a challenge for the industry
- Minipigs are highly similar to humans in anatomy, genetics, physiology, and immune system
  [Ganderup, N.C., et al., Int J Toxicol, 2012]
  [Pabst, R., Cell Tissue Res. 2020]
- Accepted animal model for regulatory tox studies, mainly used for small molecules
- However, the minipig is currently rarely used in regulatory tox studies with therapeutic antibodies
  [IQ DruSafe survey 2022; Presented at MRF 2023]
- Minipigs represent an important alternative to non-human primates

Pharmacologic activity of antibodies

Antibodies closely interact with the animal species to mediate effects

Target binding
Target biology
- ➢ Efficacy / potency
- ➢ On-target toxicity

Interaction with FcγR
Complement binding
- ➢ Impact on efficacy (e.g., ADCC)
- ➢ Impact on safety (e.g., immune cell activation)



Interaction with FcRn
- ➢ Impact on PK

Pharmacokinetics

Placental transfer

Immunogenicity

Practical considerations

FcRn: neonatal Fc receptor
FcγR: Fc gamma receptor
PK: Pharmacokinetic
ADCC: Antibody-dependent cellular cytotoxicity

Target

Target binding and biology must be investigated on a case-by-case basis

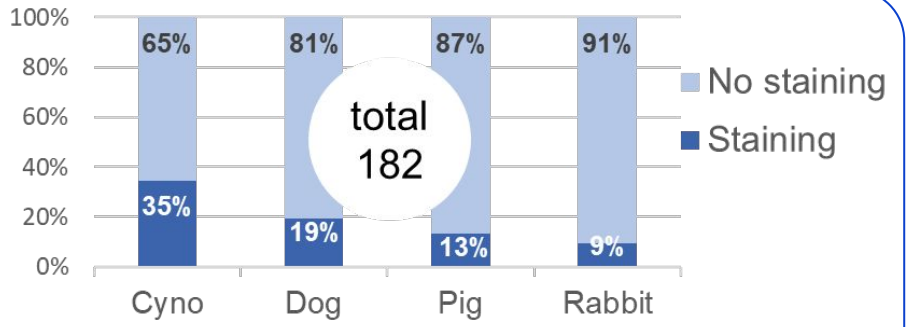
Considerations for selection of the minipig as a preclinical species

- Target homology
  - Sequence similarity to the human target (in silico)
  - Similar expression level and distribution to human target (RNA sequencing, IHC, microarray, flow)
- Target binding
  - Similar binding affinity to human target (SPR, ELISA, flow cytometry)
  - Target occupancy
- Target biology / potency
  - Similar downstream effects of target modulation
  - In vitro and in vivo kinetics, potency, biomarker

IHC: Immunohistochemistry
SPR: Surface plasmon resonance
ELISA: Enzyme-linked immunoassay

Likelihood of cross-reactivity of anti-human antibodies with pig targets

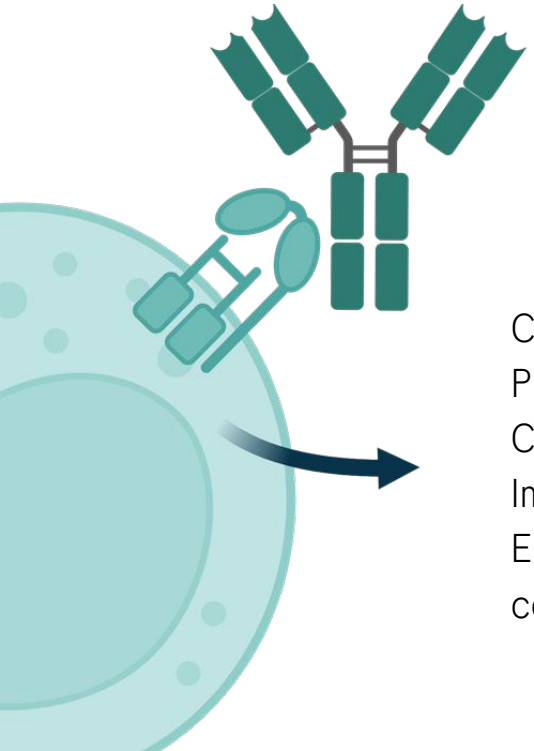
- "There is a clear orthology of sequences between most human and swine cytokines, chemokines, and growth factors" [Dawson et al., Res. Vet. Sci. 2020]



- 24/182 flow cytometry antibodies against human leukocyte differentiation antigens cross-react with pig [Saalmüller A., et al. Cellular Immunology 2005]

FcγRs and effector functions

Antibodies mediate effector functions by interaction with FcγRs



Human antibodies interact with porcine immune system via FcγRs

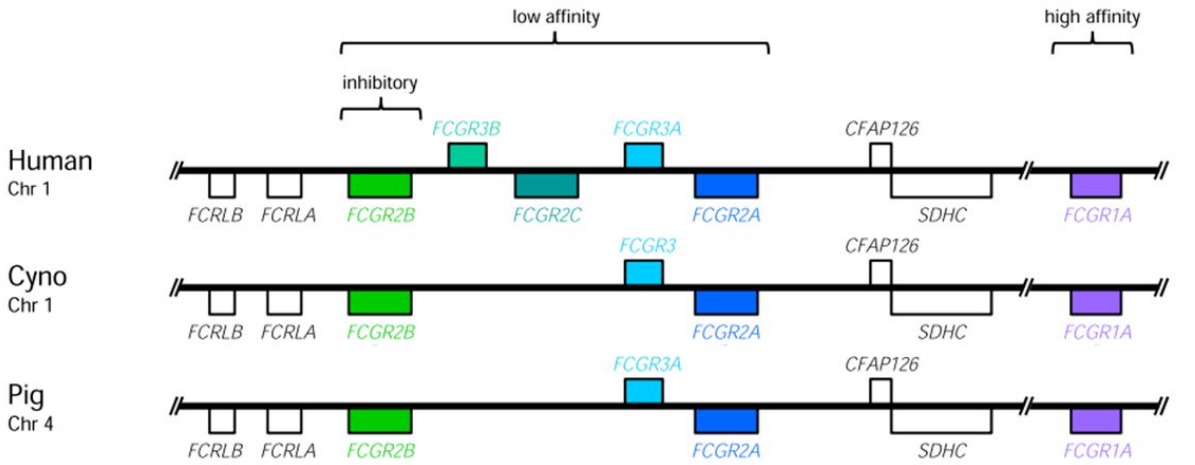
Cytotoxicity (ADCC)
Phagocytosis (ADCP)
Cytokine induction
Immunosuppression
Endocytosis (clearance of immune complexes, antigen uptake)

Impact on safety and efficacy of therapeutic antibody

FcγR: Fc gamma receptor
ADCC: Antibody-dependent cellular cytotoxicity
ADCP: Antibody-dependent cellular phagocytosis

FcγR expression

Similar set of FcγRs in pig and non-human primates



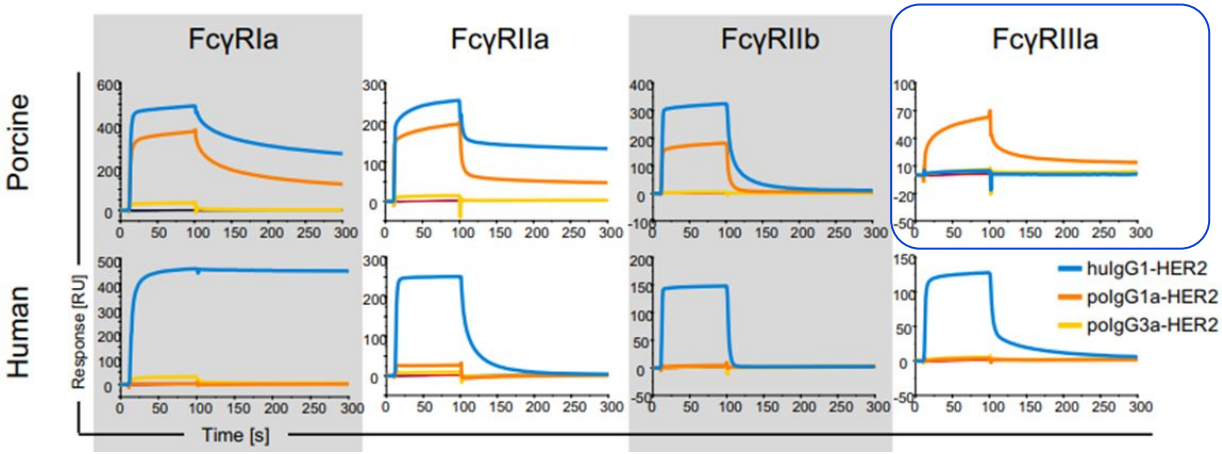
- FcγR genes are conserved between human, cynomolgus monkey and minipigs
  - FcγRIIa recently discovered in minipigs
  - FcγRIIc and FcγRIIIb are only expressed in humans
- Similar expression pattern in human and minipig
  - Differences in FcγRIIb expression in monocytes

Egli J., et al., Immunogenet 2019

FcγR: Fc gamma receptor

FcγR interaction

Therapeutic IgG1 antibodies interact with porcine FcγRs, except FcγRIII



Egli J., et al., Pharm Res 2019

- Human IgG1 binds to porcine FcγRIa, IIa, IIb
- Human IgG1 binding to porcine FcγRIIIa is absent [1] or 8x reduced compared to human FcγRIIIa binding [2]

[1] Egli J., et al., Pharm Res 2019
[2] Bhatti M.M., et al. PLoS One. 2019

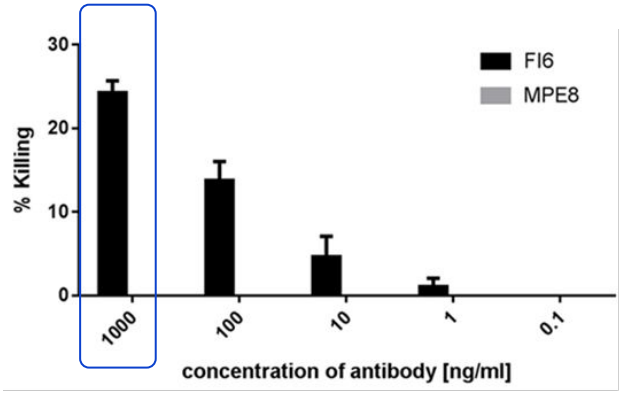
- No / reduced NK cell-mediated ADCC expected

FcγR: Fc gamma receptor
ADCC: Antibody-dependent cellular cytotoxicity

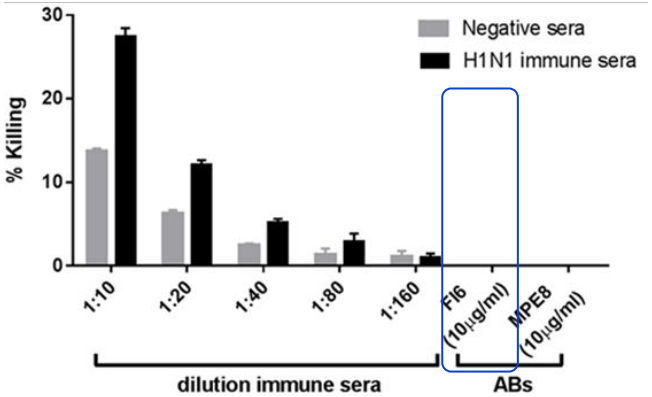
FcγR interaction and efficacy

Limited suitability of minipigs for antibodies dependent on FcγR interaction

Human PBMC



Pig PBMC



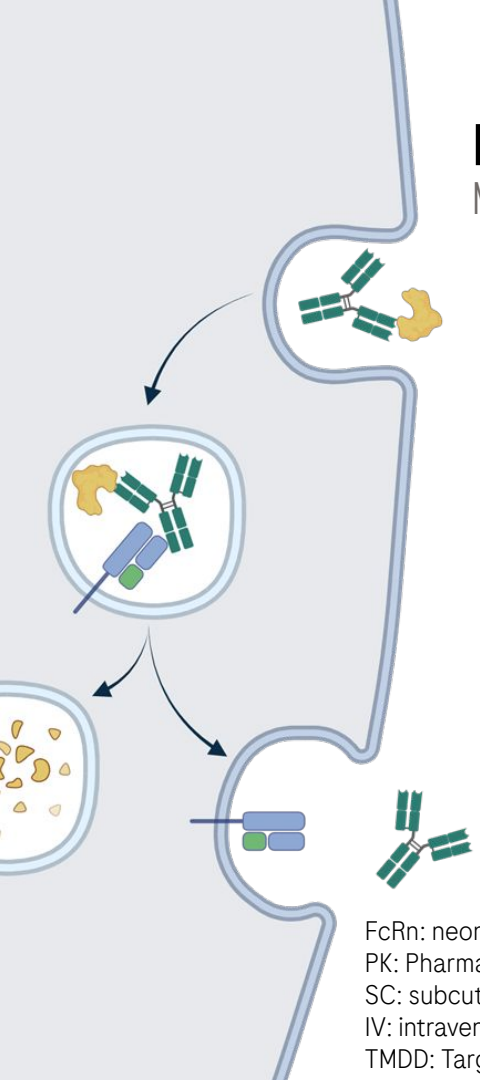
- Lack of efficacy of hemagglutinin-specific **human IgG1 antibody (F16)** administered IV or by aerosol in landrace pigs
  - Mode of action: reduction of viral load via FcγR-interaction and ADCC
- Killing of H1HA-expressing cells with F16 in the context of human PBMCs but not with porcine PBMCs
- Minipigs are not an adequate model to study ADCC of human antibodies

[Morgan, S.B., et al., Front. Immunol., 2018]

FcγR: Fc gamma receptor
ADCC: Antibody-dependent cellular cytotoxicity

FcRn and PK

Minipigs are a translatable model for disposition of human antibodies



- FcRn promotes antibody recycling and prolongs half-live
- **Minipig FcRn binds human antibodies** [Zheng Y., et al. Mabs. 2012]
- PK studies reported with therapeutic antibodies in minipigs
  - **Glycoform-dependent PK** of **human model antibody** upon SC and IV administration in line with humans [Falck D., et al., Mabs 2022]
  - Suitable model **PK, PD, and immunogenicity** of **natalizumab** upon IV dosing [Grabowski T., et al. Biomed. Pharmacother. 2022]
  - Predictive **linear clearance** and weak correlation to human SC bioavailability of **adalimumab** and **8 human antibodies** [Zheng Y., et al. Mabs. 2012]
  - Translatable **bioavailability** of **tocilizumab** after SC administration behind the ear [Richter W., et al. AAPS Journal 2020]
- Caveat: TMDD only reflected if antibody binds porcine target

FcRn: neonatal Fc receptor
PK: Pharmacokinetic
SC: subcutaneous
IV: intravenous
TMDD: Target-mediated drug disposition

Minipig considered to be more predictive for human SC bioavailability than the non-human primate

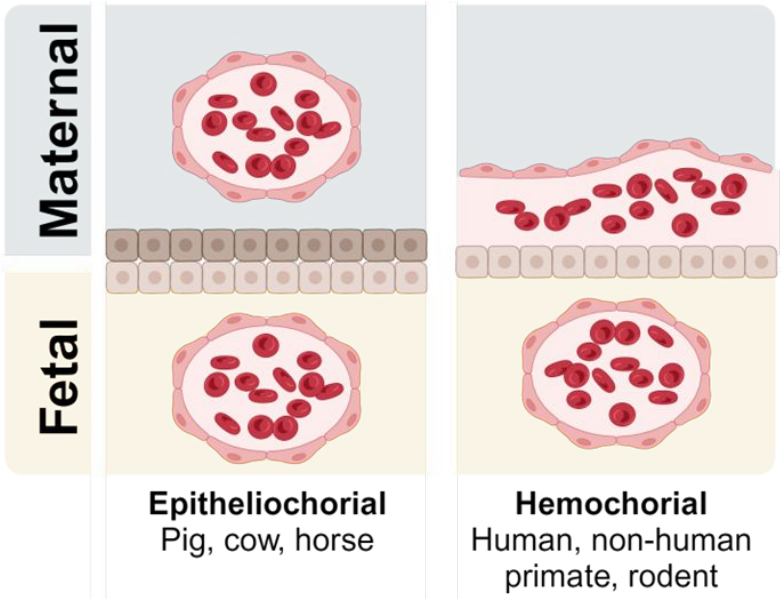
Placental transfer

Limited suitability of minipigs for DART studies with antibodies

- In humans, IgG is actively transported via FcRn from the mother to the fetus
- Minipig fetal (but not maternal) placenta and the fetal jejunum expresses FcRn
  [Jacobsen B., et al. Toxicol. Pathol. 2016]
- No placental transfer of chimeric IgG1 antibody **basiliximab** in minipigs
  [Hey A., et al. Reprod. Toxicol. 2020]
- Maternal IgG is transferred to the newborn via colostrum
  [Ke C., et al. Immunology 2021]

Minipigs are of limited use for developmental and reproductive tox studies with antibodies

Different morphology of placental barrier in pig and human



DART: Developmental and Reproductive Toxicology

Immunogenicity

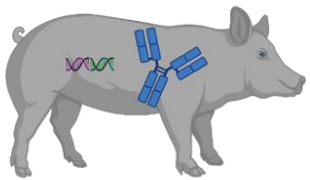
Anti-drug antibodies (ADA) in minipigs limit the translatable potential

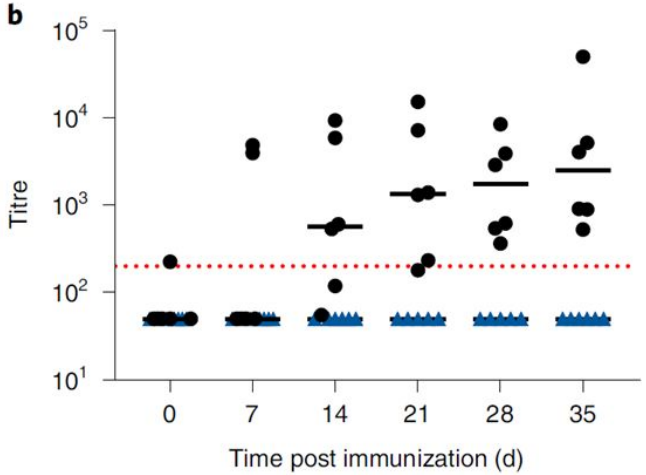
- Human antibodies are foreign proteins in animals
- Most human biotherapeutics induce anti-drug antibodies in animals
  - Impact on exposure, efficacy, safety in preclinical studies
  - Immunogenicity limits the use of minipigs in preclinical tox studies
- **Adalimumab** but likely not **infliximab** induced ADA in minipigs leading to decreased plasma levels of the drug
  [Mierlo G.J.D., et al., J Immunotoxicol. 2014]
- **Human IgG4 antibody** targeting follicle stimulating hormone receptor (FSHR) in **8-weeks regulatory toxicology study** with daily SC administration triggered ADA in 4 out of 10 (5m/5f) minipigs.
  [Presented by Cristina Longobardi & Pascal Clayette at Ellegaard and ERBC Symposium 2023]

ADA: Anti-drug antibody
SC: subcutaneous

Immunogenicity

Humanized IgG1/4 Göttingen Minipigs for immunogenicity and long-term studies





- Transgenic minipigs expressing human IgG1/4 antibodies as self-proteins
  - No ADA responses against **bevacizumab** and **daratumumab**
  - **Atezolizumab** and **cergutuzumab amunaleukin** with high clinical ADA rates broke tolerance in transgenic minipigs
- Allows immunogenicity risk assessment via various drug delivery routes
  - E.g., ADAs tested in ocular fluids after intravitreal injection of **human IgG1 Fab fragment** in wt minipigs
    [Wessels U., et al. Bioanalysis 2018]
  - Caveat: immune responses are dependent on endogenous pig MHCII-TCR interactions
- Endogenous IgG expression can allow long-term pharmacological studies
  - Caveat: ADA formation against immunogenic antibodies

Flisikowska T. and **Egli J.**, et al. Nat. Biomed. Eng. 2022

ADA: Anti-drug antibody
Wt: wild-type

Practical considerations

- Established methods for drug delivery
  - Many methods established for minipigs, including IV, SC, oral, dermal, ocular, intrathecal, pulmonary via inhalation, etc.
- Use of active pharmaceutical ingredient
  - Minipigs gain weight quickly and are rather large compared to NHP
  - Smaller Göttingen Micropig (Growth hormone receptor KO) recently introduced
    [Presented by Arne Hinrichs at Minipig Research Forum 2023]
- Availability of biomarkers, assays, and safety pharmacology readouts
  - Large range of flow cytometry markers, cytokine panels, and biomarker assays available
- Availability of background data and use cases
- The minipig is generally accepted by regulatory bodies, also for biopharmaceuticals
  - Full non-clinical development of recombinant protein in minipig to support non-oncology clinical trials. Accepted by health authorities for first in human and phase II studies
    [Presented by Thierry Flandre at BioSafe meeting 2023]

Summary

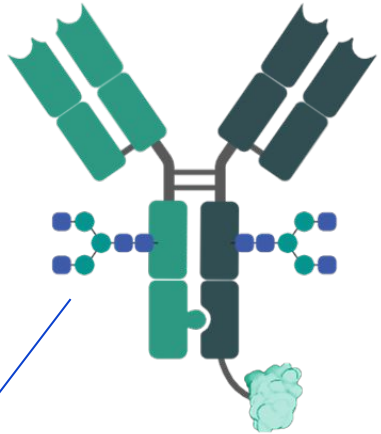
? Target binding
Target biology

Y ➢ Multiple conserved targets with high genetic similarity

Interaction with FcγR
Complement binding

➢ No / low interaction of

X human IgG with porcine FcγRIIIa limiting ADCC effector functions



Y Interaction with FcRn

➢ Human IgG interacts with porcine FcRn

Y Pharmacokinetics

X Placental transfer

Y/? Immunogenicity

Y/? Practical considerations

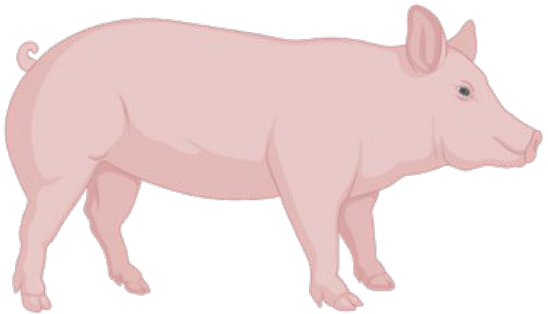
FcRn: neonatal Fc receptor
FcγR: Fc gamma receptor
PK: Pharmacokinetic
ADCC: Antibody-dependent cellular cytotoxicity

Conclusion

- Numerous studies with human antibodies in minipigs reported
- Minipigs are well suited for antibody testing in
  - PK studies (also without Target Mediated Drug Disposition)
  - IV vs SC bridging and local tolerability studies
  - Immunogenicity studies for special applications (e.g., intravitreal, intrathecal dosing)
  - Regulatory tox studies if pharmacologically relevant (e.g., oncology indications where DART studies are not required, and efficacy is not related to effector functions)
- Case-by-case evaluation of the suitability of minipigs is needed for every program

Acknowledgements

Thank you!



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Contribution to original work on minipig FcγR and IgG humanized minipigs

Alex Odermatt
Andreas Roller
Angelika Schnieke
Antonio Iglesias
Barbara Kessler
Benjamin Loos
Christian Spick
Eckhard Wolf
Erich Küng
Felix Weber
Guy Georges
Krzysztof Flisikowski
Marlene Stumbaum
Martin Ebeling
Mayuko Kurome
Nils Grabole
Roland Schmucki
Stefan Seeber
Stephan Reichl
Tatiana Flisikowska
Thomas Singer
Tilman Schlothauer
Valeri
Zakhartchenko

Contribution to aspects of the use of minipigs in preclinical studies

Susanne Mohr
Sven Kronenberg

Doing now what patients need next