Humanized Göttingen Minipigs

for the toxicological testing of therapeutic antibodies

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Overview

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A humanized minipig model for the toxicological testing of therapeutic recombinant antibodies

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Check for updates

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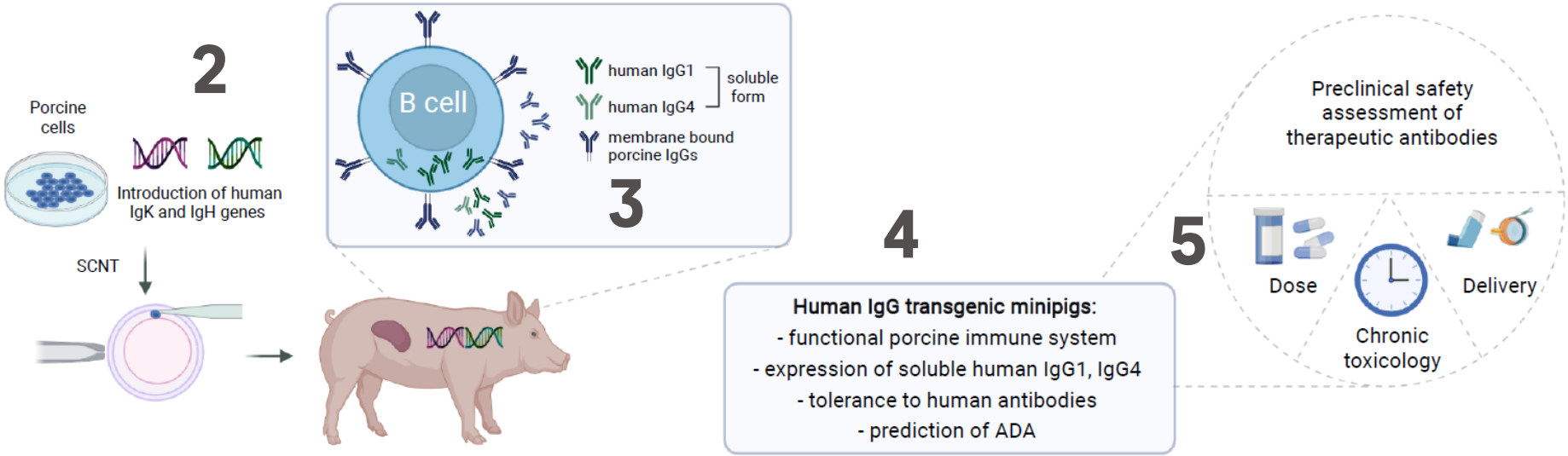
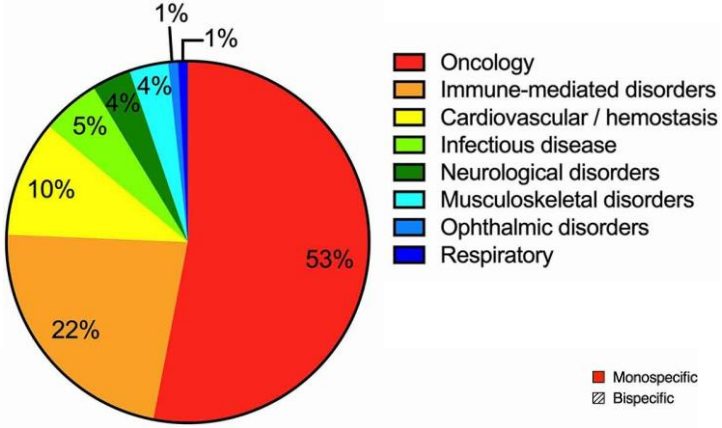
1 Why transgenic minipigs?

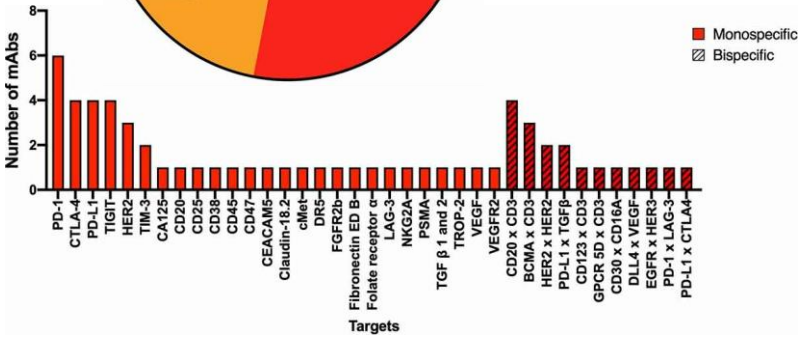


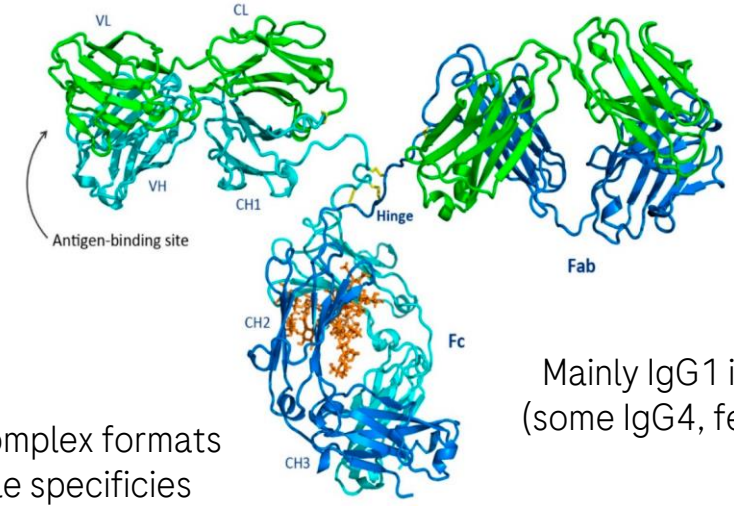
Why human IgG transgenic minipigs?

Therapeutic antibodies in development

Mainly human IgG backbones







Some complex formats
Multiple specificies
Fusion to cargo

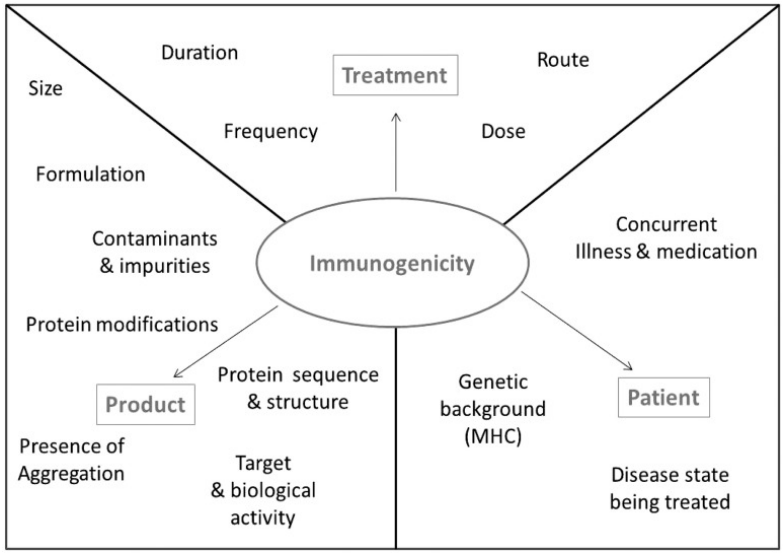
Mainly IgG1 isotype
(some IgG4, few IgG2)

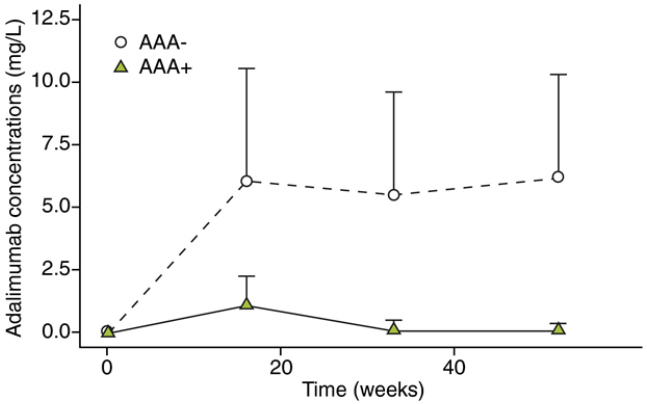
Human or humanized
backbone

Kaplon H., et al. mAbs 2022 Antibodies to watch in 2022.
Chiu M.L., et al. Antibodies 2019 Antibody Structure and Function: The Basis for Engineering Therapeutics.

Therapeutic antibodies in development

Immunogenicity poses a risk for the safety and efficacy of therapeutic antibodies





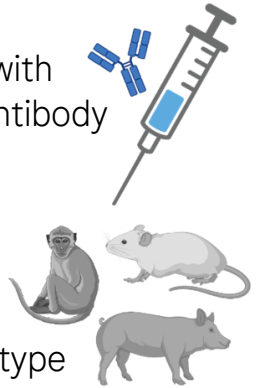
- Many factors can contribute to the immunogenicity of therapeutic antibodies despite human sequence
- Anti-drug antibodies (ADA) can lead to loss of exposure, reduced efficacy and in some cases even adverse events

Ratanji K.D., et al. J. Immunotoxicol. 2013
Danese S, et al., Nat Rev Gastroenterol Hepatol 2015

Animal models and immunogenicity

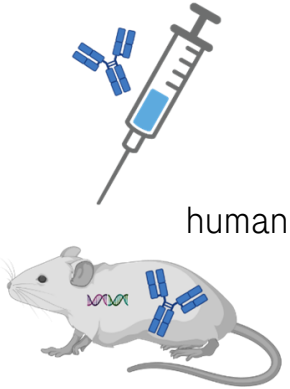
Expression of IgG as self-protein limits xenogeneic immune responses in animal models

Immunization with human IgG1 antibody



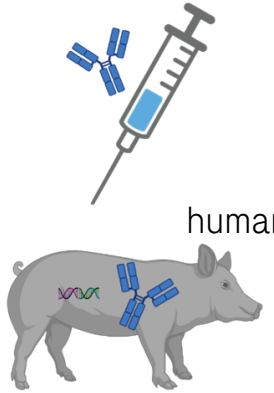
Wild type

Response to foreign antigen
Low predictivity for evaluation of immunogenicity[1]



human IgG1

Tolerant to human IgG1
Suitable to study immunogenicity[2]



human IgG1 / IgG4

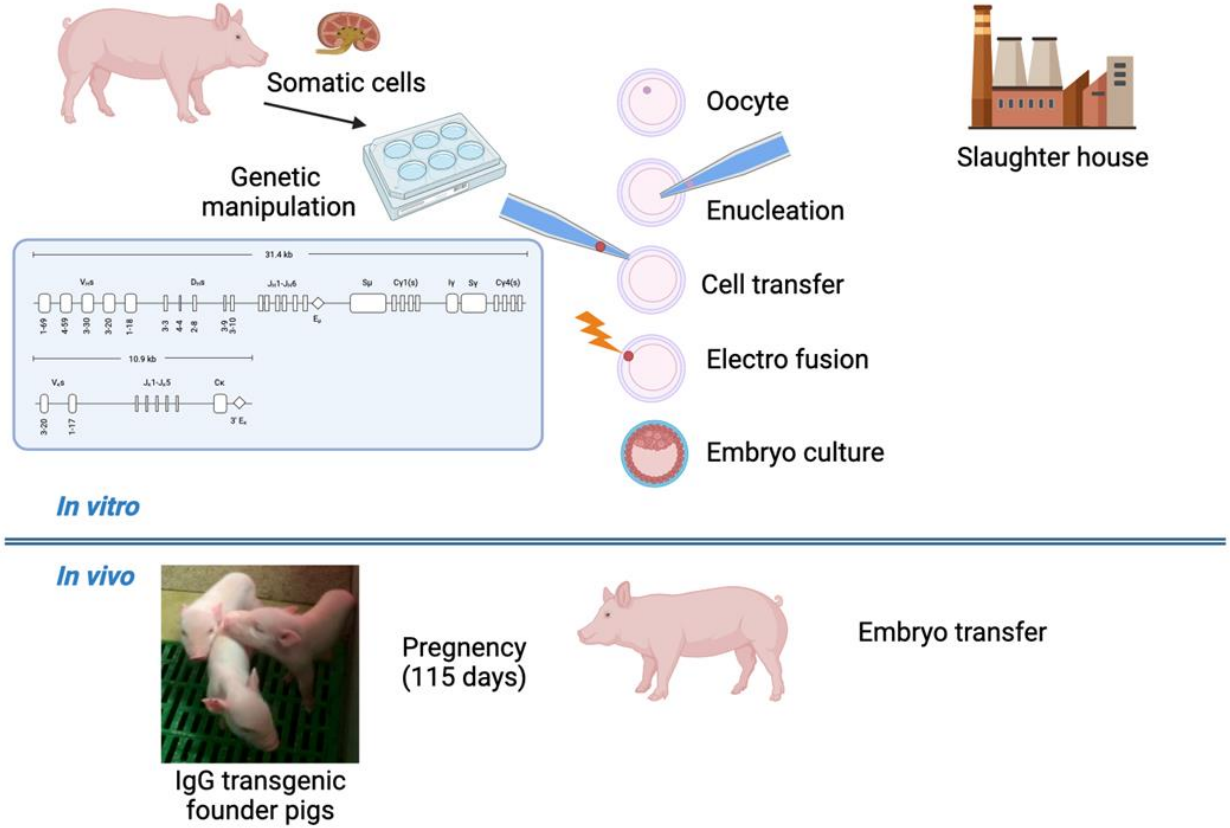
Tolerant to human IgG
High degree of similarity to humans
Outbread background
Suited to replace NHP

[1] van Meer PJK, et al. MAbs 2013 Immunogenicity of mAbs in non-human primates during nonclinical safety assessment
[2] Bessa et al. Pharm Res 2015 The immunogenicity of antibody aggregates in a novel transgenic mouse model

Generation and characterization of the minipig model

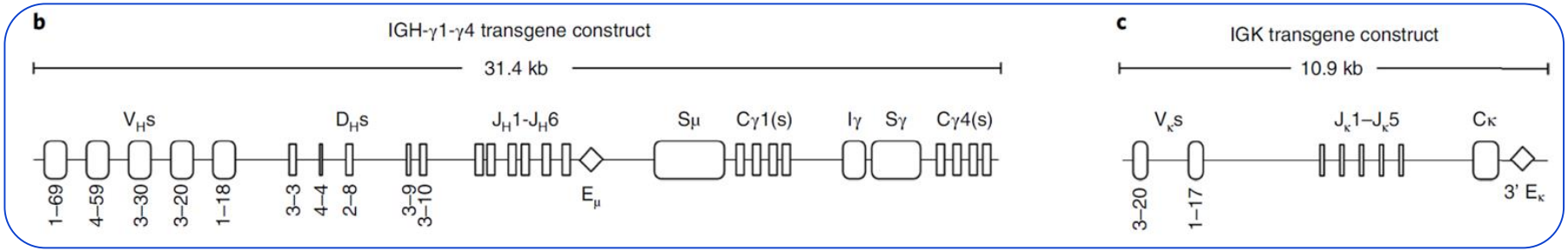
Generation of human IgG transgenic minipigs

Mini-repertoire of human soluble IgG1 and IgG4 heavy chains and human Ig *kappa* light chain

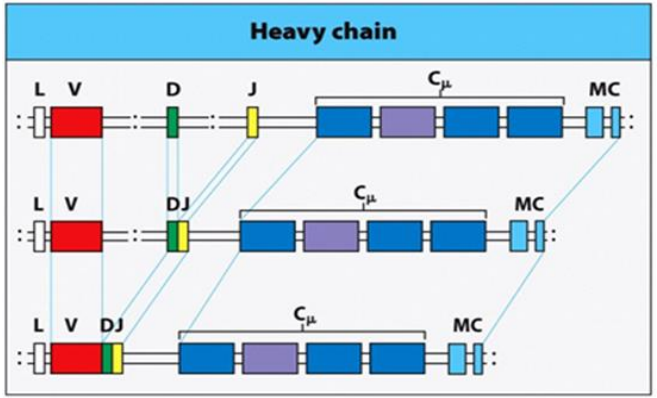


Transgene organization

Generation of polyclonal IgG1 and IgG4 antibodies



1. **V(D)J rearrangement** → multiple clones



2. **Isotype switching** → IgG1 and IgG4 backbone

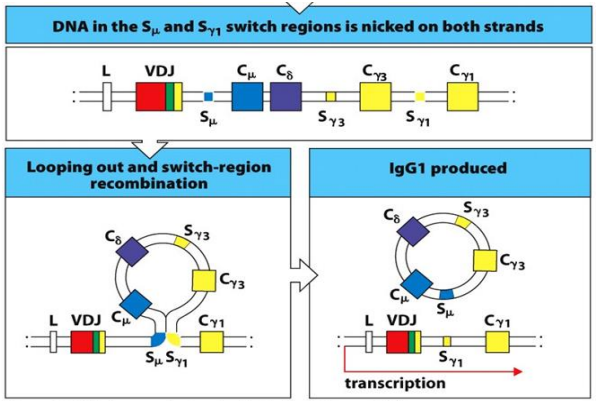


Figure 4.30 The Immune System, 3ed. (© Garland Science 2009)

Human IgG expression in transgenic minipigs

Recombination of V(D)J elements leads to the expression of human IgG1 and IgG4 RNA

- Targeted sequencing of human IgG RNA in the blood of human IgG transgenic minipigs reveals the expression of functional arrangements containing all transgenic VH and Vκ gene segments
- A minor amount of sequences contained the isotype switch variant Cγ4

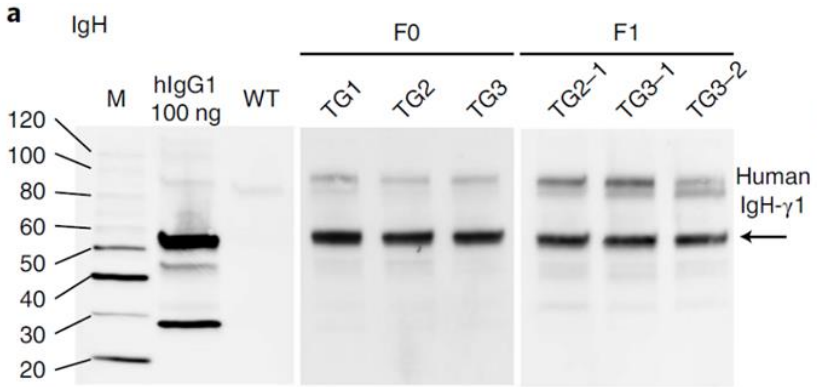
C

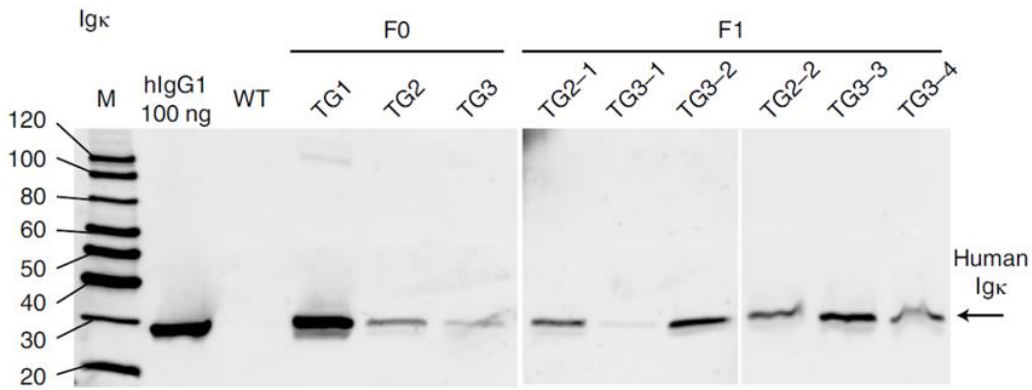
	VH	N(D)N	JH	Cγ	
VH1-69	TAVYYCA	KGPVCGWFDP	WGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH1-69	TAVYYCA	KDLGS	YWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH1-69	TAVYYCA	ANWGY	YFDYWGQGTLVTVSS(4)	ASTKGPSVFPLAPCSRSTSES	
VH1-69	TAVYYCA	KD	DYWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH4-59	TAVYYCAR	DLGI	DYWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH4-59	TAVYYCA	SN	WGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH4-59	TAVYYCAR	EG	YYYMDVWGKGTTVTVSS(6)	ASTKGPSVFPLAPSSKSTSGG	
VH3-30	TAVYYCAK	DPY	YFDYWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH3-30	TAVYYCA	NMVRE	FDYWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH3-30	TAVYYCAK	DQLANWGS	FDIWGQGTMVTVSS(3)	ASTKGPSVFPLAPSSKSTSGG	
VH3-23	TAVYYCAK	DRG	FDYWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH3-23	TAVYYCAK	LY	YFDYWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH3-23	TAVYYCA	ITGV	DYWGQGTLVTVSS(4)	ASTKGPSVFPLAPSSKSTSGG	
VH3-23	TAVYYCAK	DYYGSGSY	DAFDIWGQGTMVTVSS(3)	ASTKGPSVFPLAPSSKSTSGG	D3-9
VH1-18	TAVYYCAR	AGMGV	WGKGTTVTVSS(6)	ASTKGPSVFPLAPSSKSTSGG	
VH1-18	TAVYYCAR	DHWGL	YFDLWGRGTLVTVSS(2)	ASTKGPSVFPLAPSSKGTSGG	
VH1-18	TAVYYCAR	DHWGL	YFDLWGRGTLVTVSS(2)	ASTKSPSVFPLAPCSRSTSES	

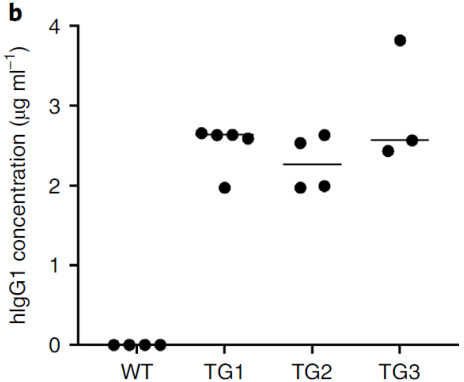
	Vk	N	Jk
Vk3-20	FAVYYCQQYGSSP	PNWVTNWG	TFGPGTKVDIK(3)
Vk3-20	FAVYYCQQYGSSP		YTFGQGTKLEIK(2)
Vk3-20	FAVYYCQQY	GRSRS	FGGGTKVEIK(4)
Vk3-20	FAVYYCQQYGSS	DYNHTPR	TFGQGTKVEIK(1)
Vk3-20	FAVYYCQQYGSS	RECE	WTFGQGTKVEIK(1)
Vk1-17	FATYYCLQHNSY	E	YTFGQGTKLEIK(2)
Vk1-17	FATYYCLQHNSYP	RAAGFP	FTFGPGTKVDIK(3)
Vk1-17	FATYYCLQHNSY	TW	TFGQGTKVEIK(1)
Vk1-17	FATYYCLQHNSYP	VIDGE	YTFGQGTKLEIK(2)
Vk1-17	FATYYCLQHNSYP	HKSQ	LTFGGGTKVEIK(4)

Human IgG expression in transgenic minipigs

Only human IgG1 detected on protein level







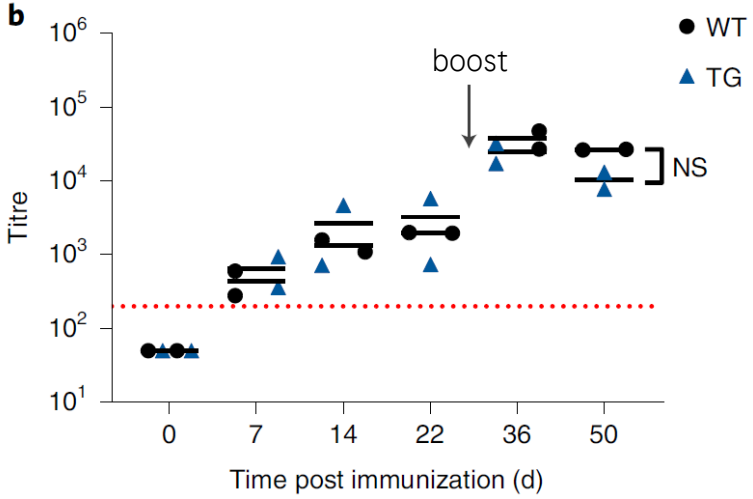
- Human IgG1 heavy chain and Ig kappa light chain detected in founder and filial generation
- Quantification reveals concentration of human IgG1 in serum in the range of 2-4 ug/ml
  (human endogenous IgG1 concentration approx. 10 mg/ml)

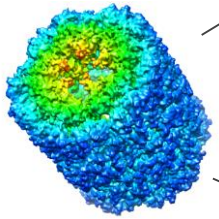
Functional validation of human IgG transgenic minipigs

Transgenic minipigs are capable to mount immune responses

Adequate T cell-dependent antigen response (TDAR) and memory response detected

Pig ADA against **KLH**





T cell dependent antigen

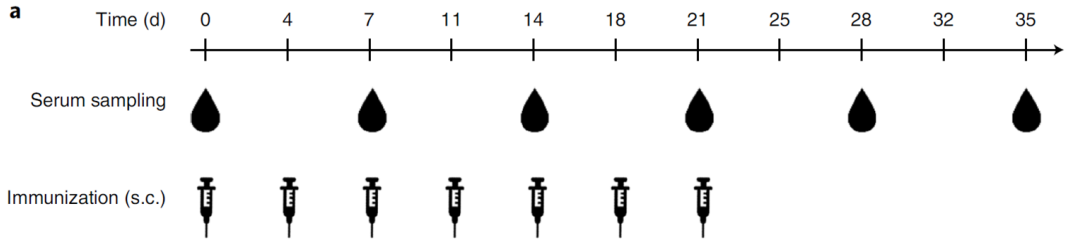
Xenobiotic from keyhole limpet

Clinical ADA incidence: 100%

Therapeutic antibodies and clinical ADA incidence

Testing of human IgG1 antibodies with low and high clinical immunogenicity

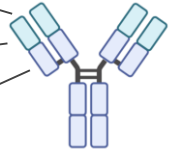
	Specificity	Cross-reactivity to pig	Clinical immunogenicity
Bevacizumab (Avastin)	VEGF	yes	0.63%
Daratumumab (Darzalex)	CD38	no	0%
Atezolizumab (Tecentriq)	PD-L1	yes	30%
Cergutuzumab amunaleukin	CEA / IL-2R	IL-2R: yes	79%

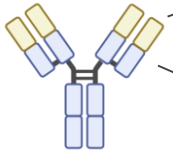


Expression of hIgG1 induces immune tolerance

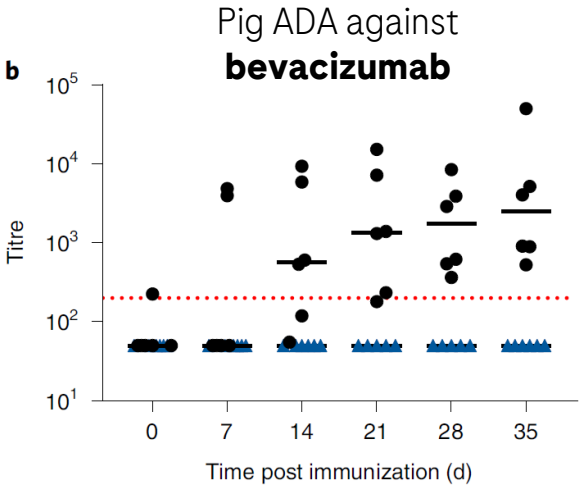
Human IgG1 antibodies with low clinical immunogenicity are well-tolerated in transgenic minipigs

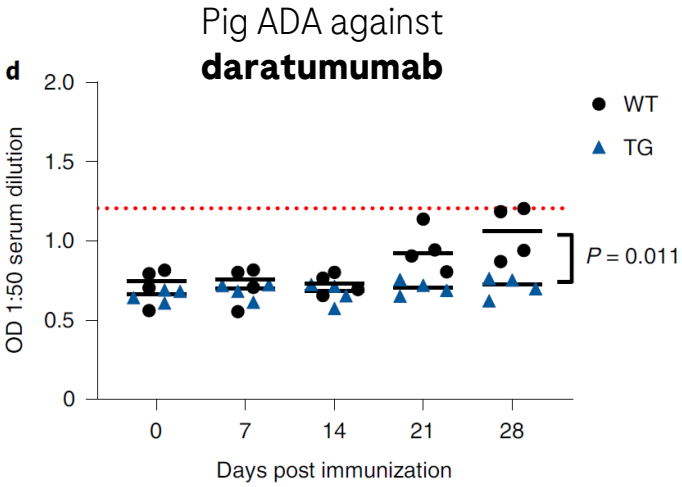
Anti-VEGF
Pig cross-reactivity: YES
Clinical ADA incidence: 0.63%





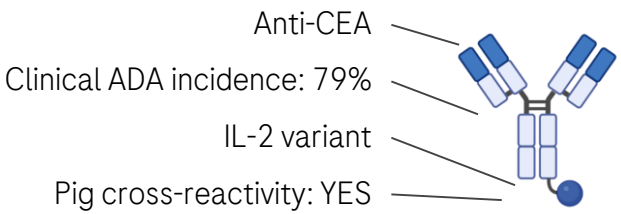
Anti-CD38
Pig cross-reactivity: NO
Clinical ADA incidence: 0%

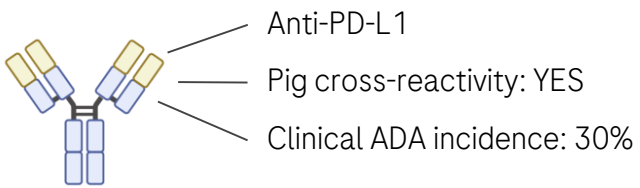


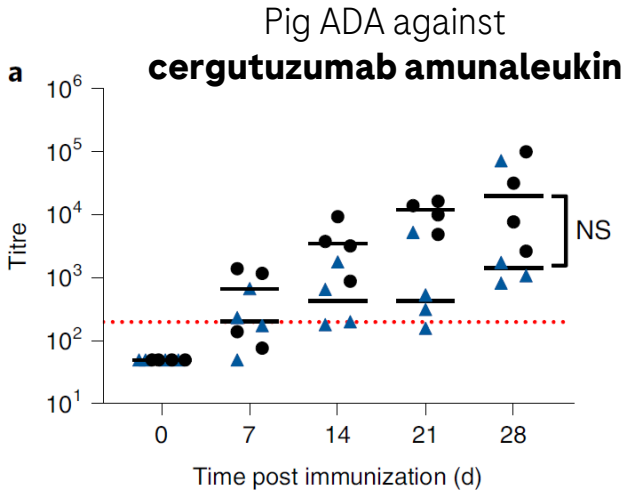


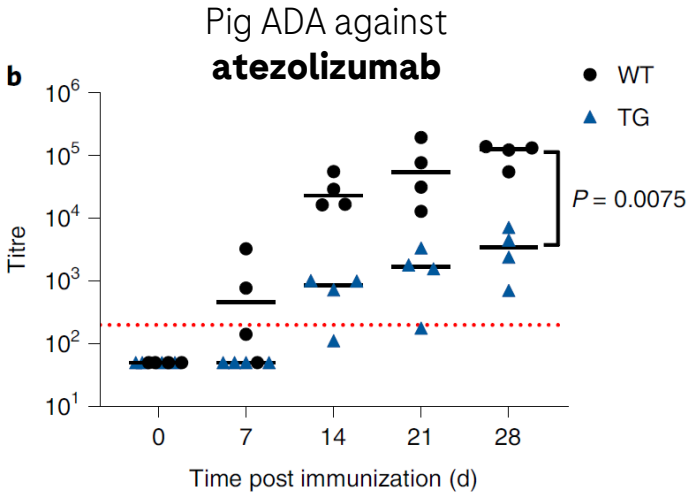
Tolerance to human IgG1 can be broken

Antibodies with high clinical immunogenicity can induce ADA in transgenic minipigs









Potential use of human IgG transgenic minipigs

Potential use of human IgG transgenic minipigs

Transgenic minipigs can help reduce or replace the use of NHP in pre-clinical sutdies

Chances

- Size and anatomy of minipigs allows immunogenicity risk assessment using various drug delivery routes
  - Inhalation studies
  - Intravitreal injection
  - CNS applications
- Endogenous IgG expression allows long term pharmacological studies
  - Chronic toxicity studies
  - Biodistribution

Risks

- Potentially high demand of API for systemic exposure
- Antibody cross-reactivity can be critical
- Limited availability of assays
- Immune responses are dependent on endogenous pig MHCII-TCR interactions
- Does not improve long term tox studies with immunogenic antibodies

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THANK YOU

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