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Membrane Protein Solutions

Harnessing Divergent Species

to Access Difficult and Conserved Antibody Targets

April 25, 2024
Antibody Society Webinar

Ross Chambers PhD
VP of Antibody Discovery
Integral Molecular, Philadelphia PA

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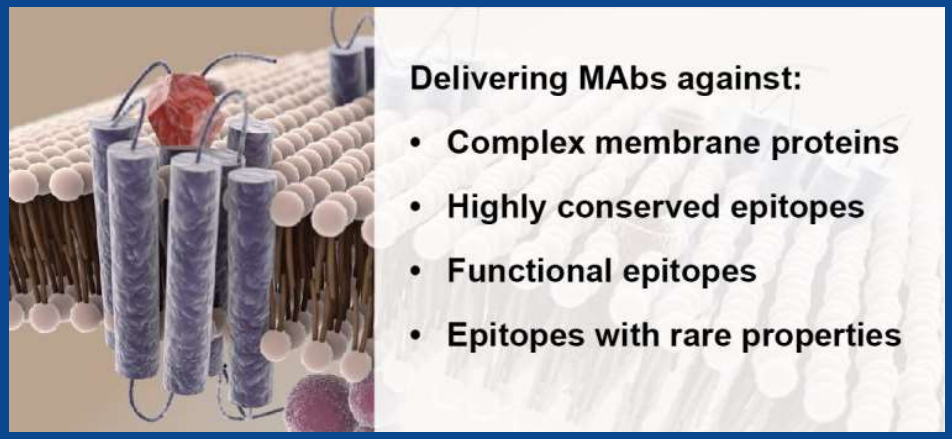
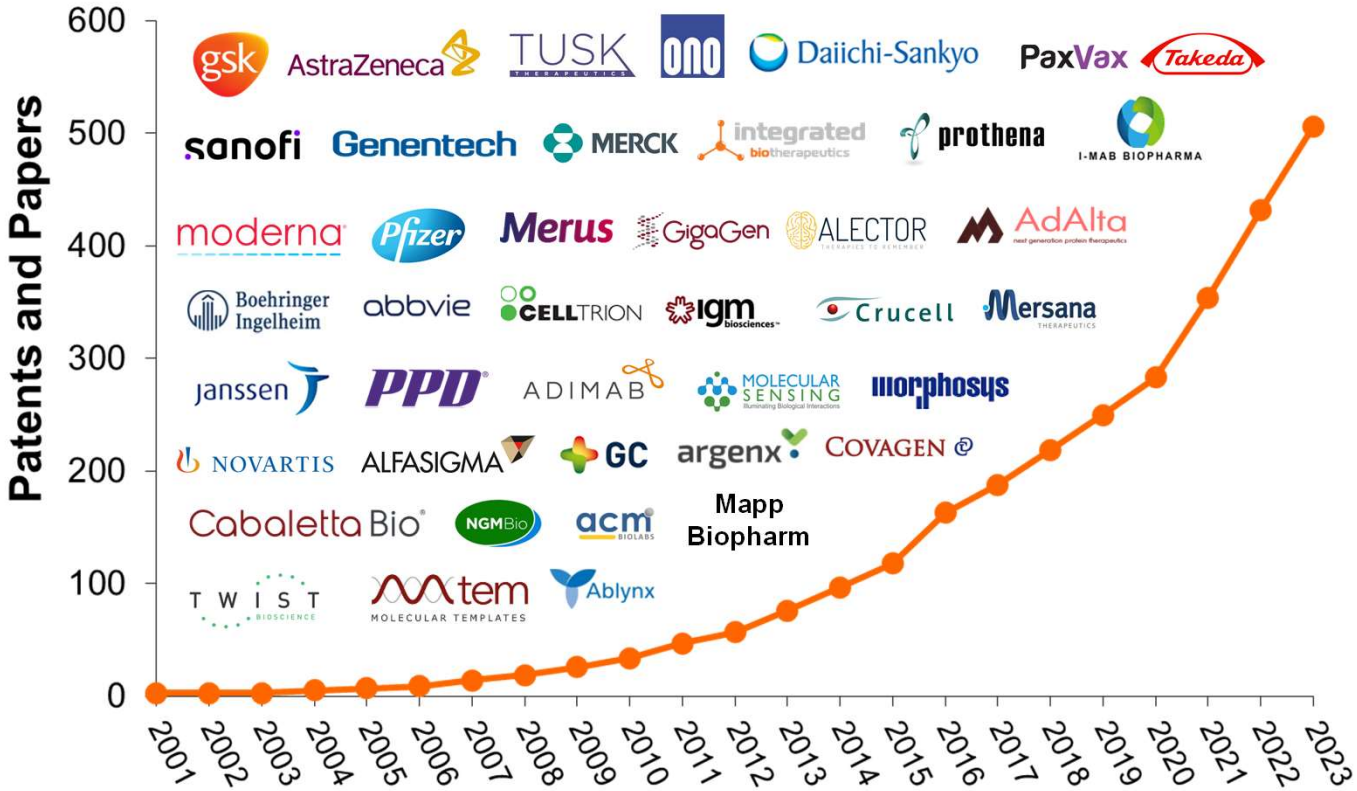
The Industry Leader in Delivering Lead Antibodies Against Undruggable Targets

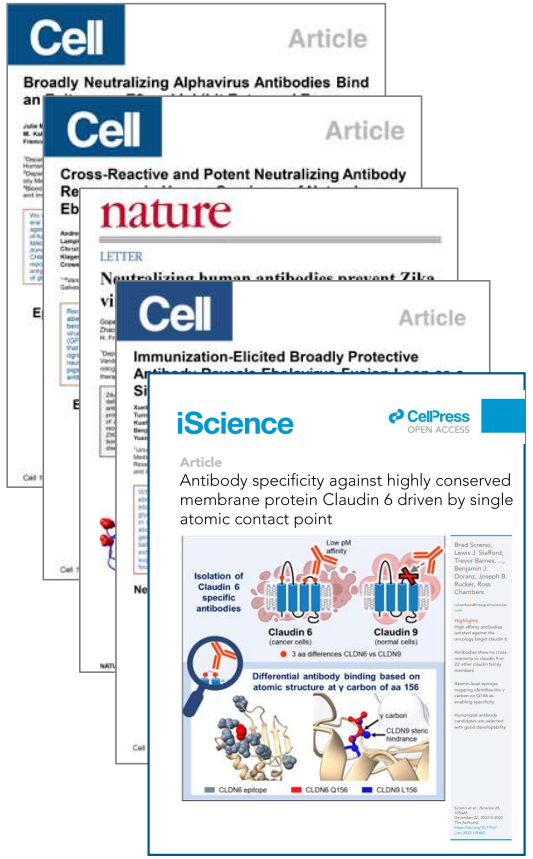


- 20+ years working with challenging protein targets
- Industry-leading epitope diversity
- Pipeline of therapeutic antibodies against complex targets
- Therapeutic MAbs licensed to AstraZeneca, Context Therapeutics, Cartexell, and others

Trusted by 600+ Companies

500+ publications and patents, including *Cell*, *Science*, *Nature*





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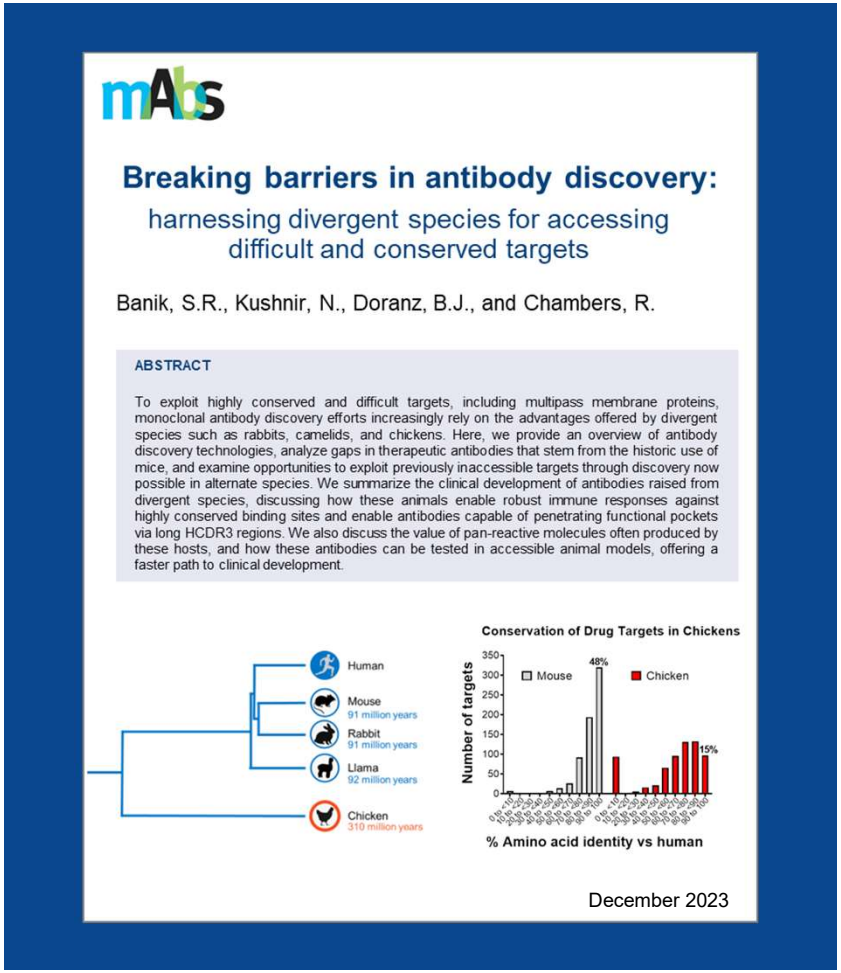
The Industry Leader in Delivering Lead Antibodies Against Undruggable Targets



mAbs

Breaking barriers in antibody discovery:

harnessing divergent species for accessing difficult and conserved targets

Banik, S.R., Kushnir, N., Doranz, B.J., and Chambers, R.

ABSTRACT

To exploit highly conserved and difficult targets, including multipass membrane proteins, monoclonal antibody discovery efforts increasingly rely on the advantages offered by divergent species such as rabbits, camelids, and chickens. Here, we provide an overview of antibody discovery technologies, analyze gaps in therapeutic antibodies that stem from the historic use of mice, and examine opportunities to exploit previously inaccessible targets through discovery now possible in alternate species. We summarize the clinical development of antibodies raised from divergent species, discussing how these animals enable robust immune responses against highly conserved binding sites and enable antibodies capable of penetrating functional pockets via long HCDR3 regions. We also discuss the value of pan-reactive molecules often produced by these hosts, and how these antibodies can be tested in accessible animal models, offering a faster path to clinical development.

December 2023

How Divergent Species Can Access Conserved Targets

Gaps in antibody space & role of divergent species

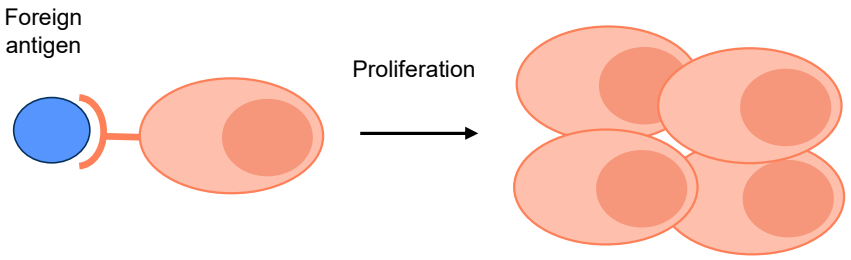
Rabbits, Camelids, Chickens

Chicken immunization has delivered:

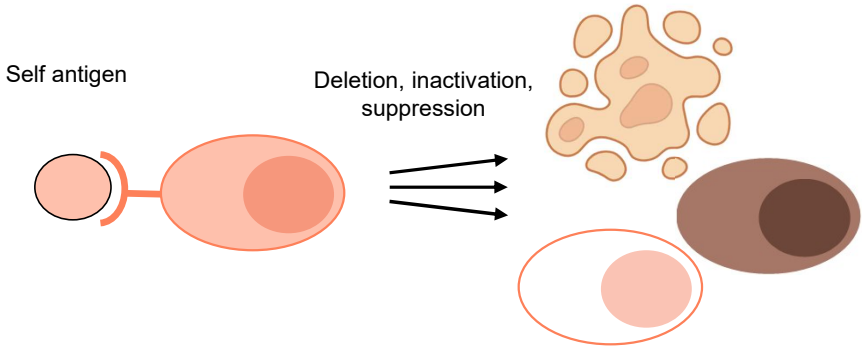
Antibodies against conserved targets
Agonist antibody
Exquisite specificity

Why Are Conserved Targets Difficult for Antibody Discovery?

Robust Immune Reaction



Immune Tolerance



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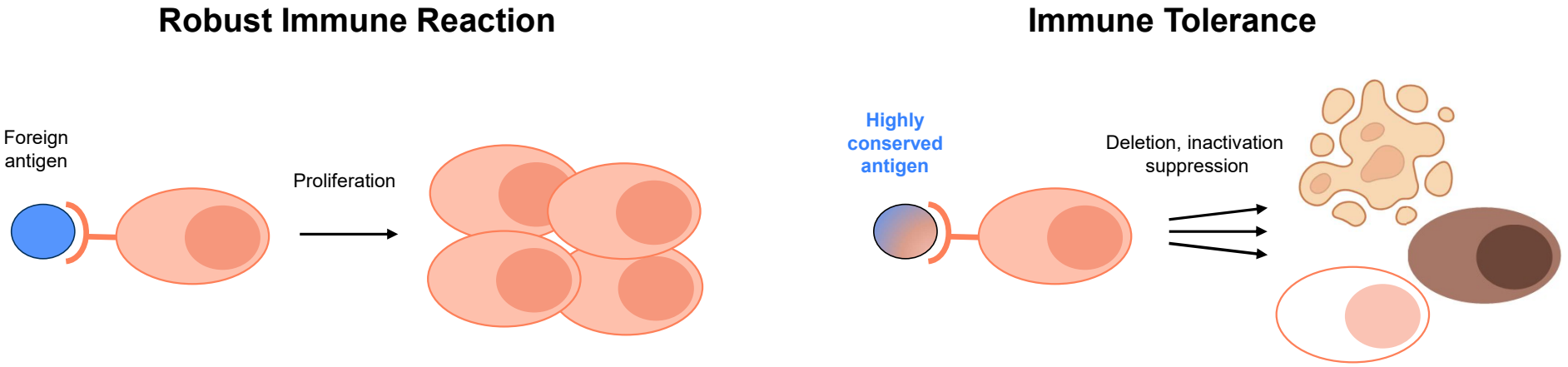
Why Are Conserved Targets Difficult for Antibody Discovery?



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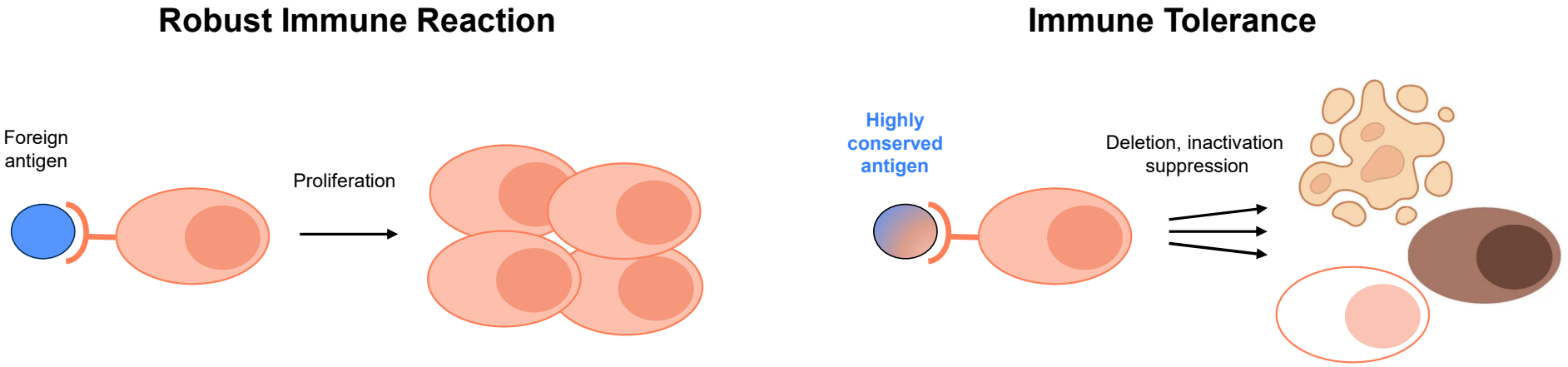

- Immune system recognizes self-antigen
  - Tolerance prevents immune system from attacking own body
- Conserved proteins resemble self-antigen

Conserved proteins make poor immunogens for therapeutic discovery

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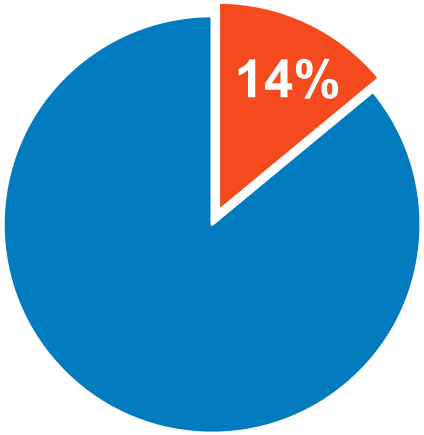
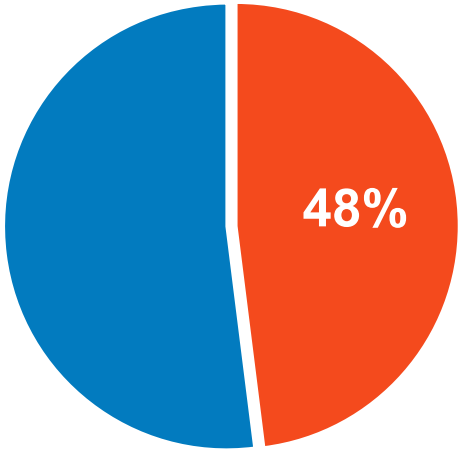
Many Highly Conserved Targets Currently Undruggable by MAbs

MAb Targets



All Drug Targets



Percent Identity
Human vs. Mouse Targets

- High conservation (≥ 90%)
- Low/med conservation

Banik et al., 2023, *mAbs*

Untapped opportunities for MAb discovery

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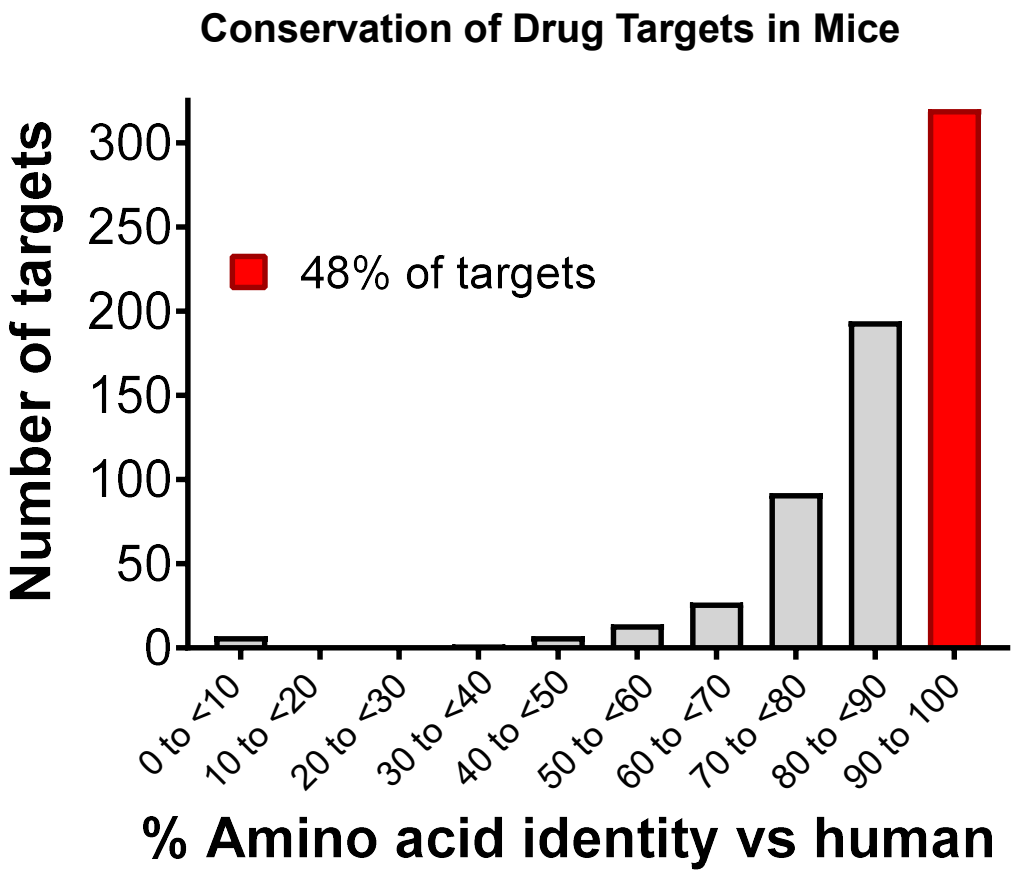
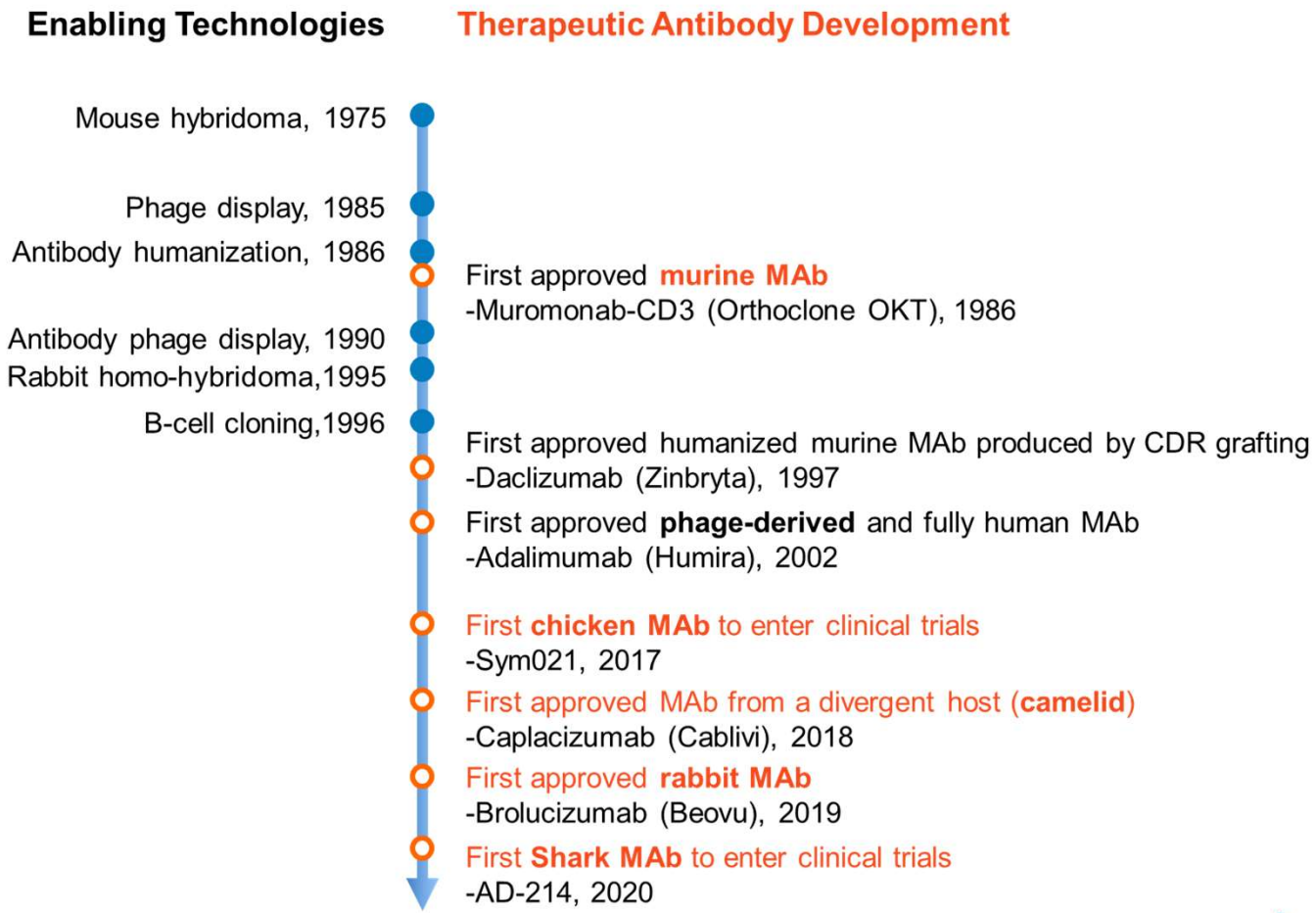
~Half of Drug Targets May Be Poor Immunogens in Mice

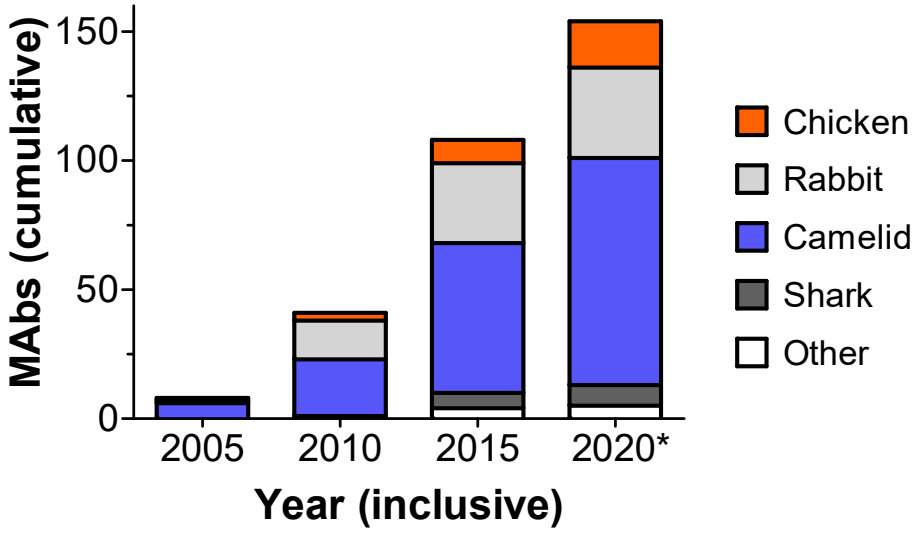


Antibody Enabling Technologies and Therapeutics



Divergent Species Increasingly Used for MAb Discovery

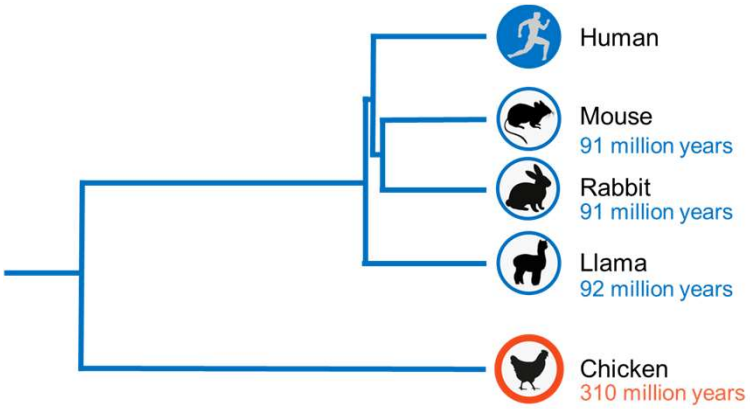
MAbs From Divergent Species in Clinical and Preclinical Development



Advantages of Divergent Species

1. Less immune tolerance
2. Long HCDR3 regions
3. Cross species-reactive antibodies

1. Divergent Species Avoid Immune Tolerance



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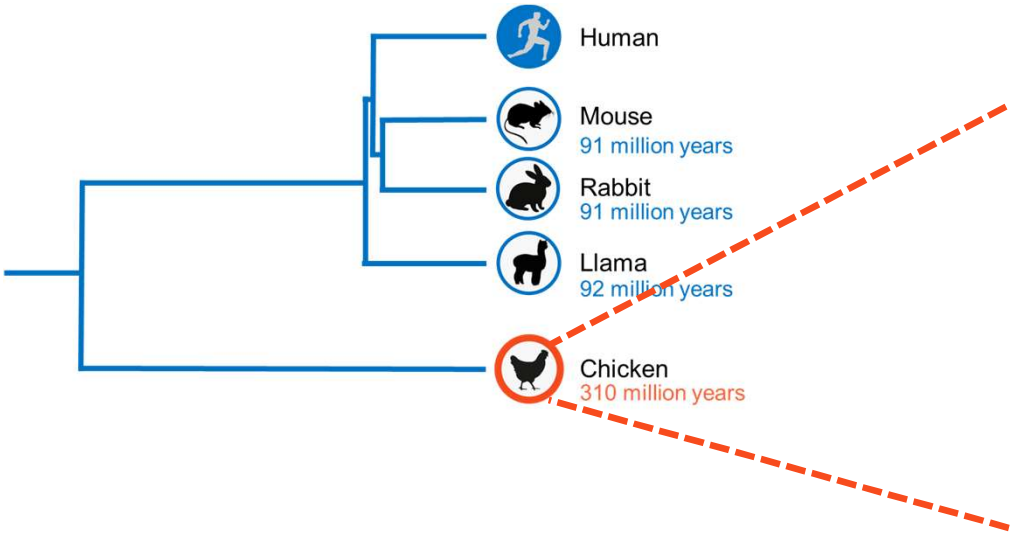

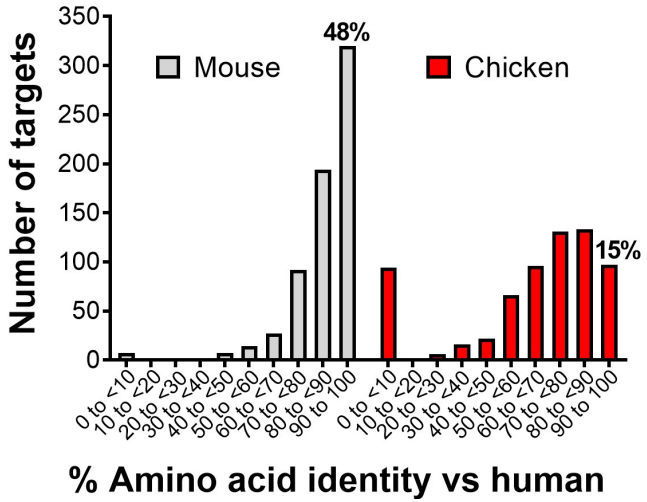

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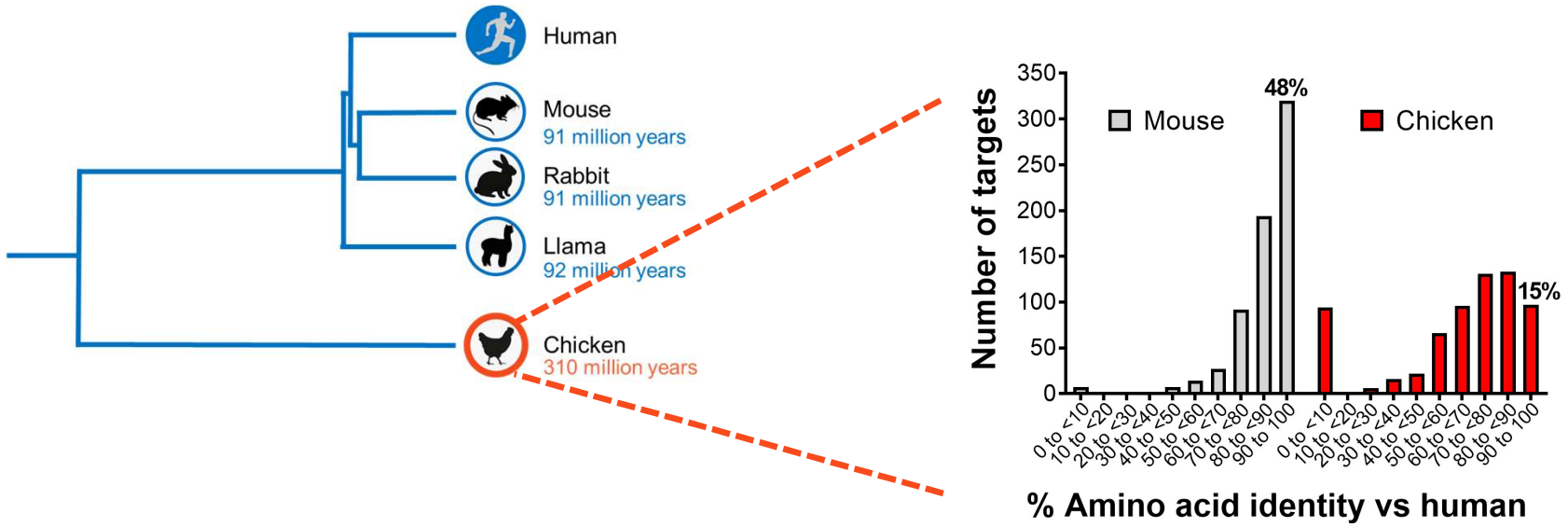

- Target antigen must be 'foreign' for host to mount effective immune response
- Sequence divergence important at epitopes of interest
- Chickens: long evolutionary distance + MAb structure similar to humans

Divergent species enable access to more targets and more epitopes

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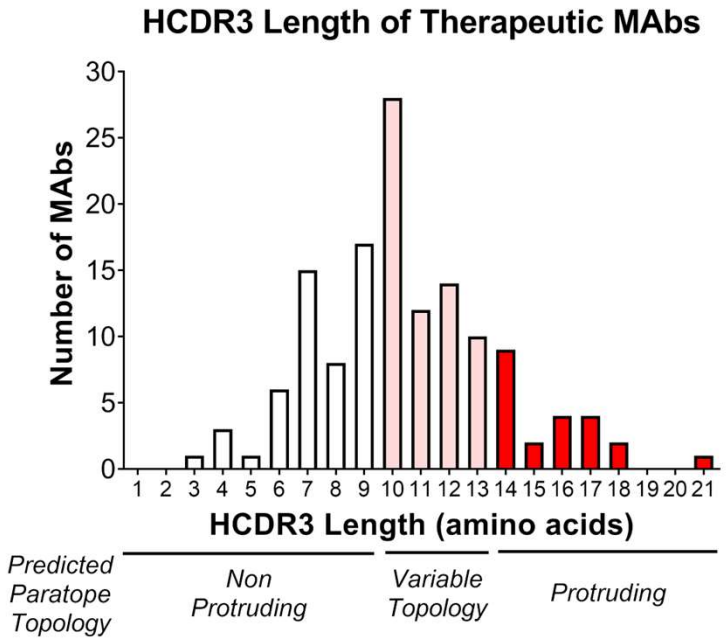
2. Longer HCDR3 Can Form Protruding Structures

- HCDR3 region correlates with paratope shape
- Ramsland et al., studied 50 antibody crystal structures

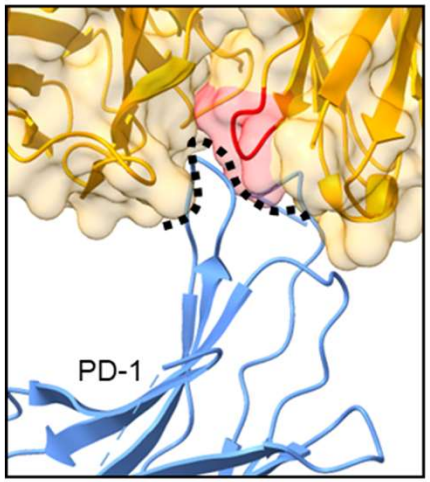
HCDR3:

- **1-9 aa:** predominantly non-protruding
- **10-13 aa:** variable
- **14+ aa:** predominantly protruding



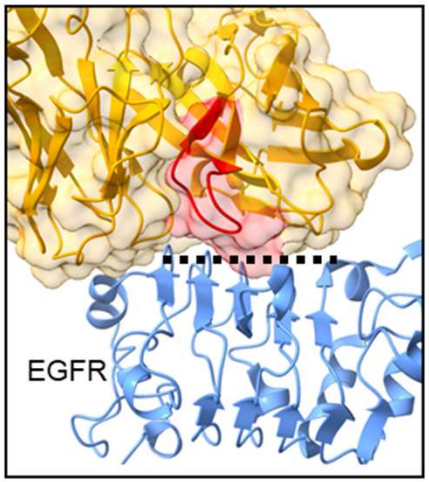
Longer HCDR3 Can Access Pockets

Concave paratope
4 aa HCDR3



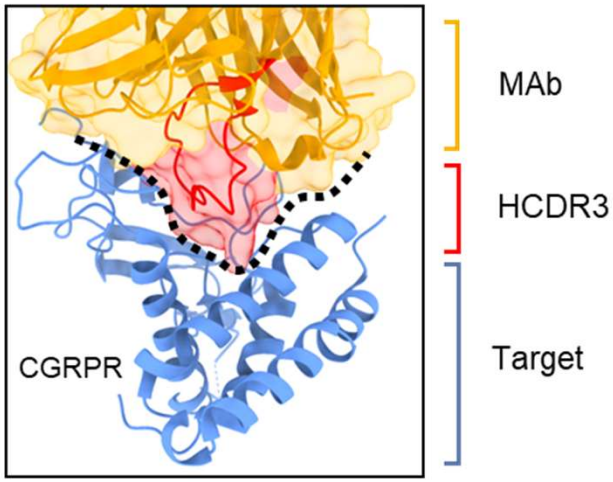
Nivolumab

Flat paratope
11 aa HCDR3



Cetuximab

Convex paratope
21 aa HCDR3



Erenumab

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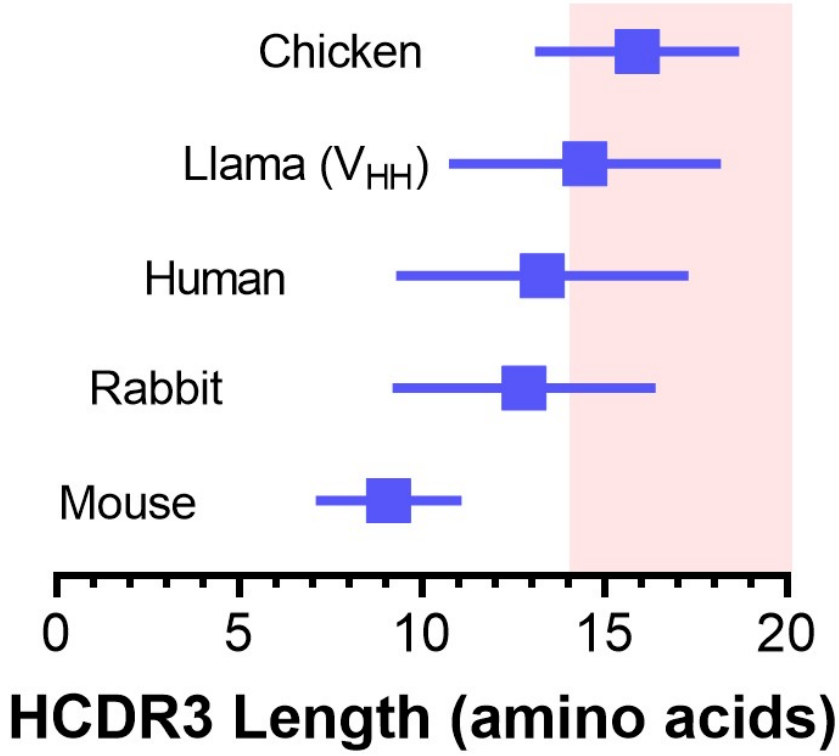
Divergent Species Can Access Recessed Epitopes

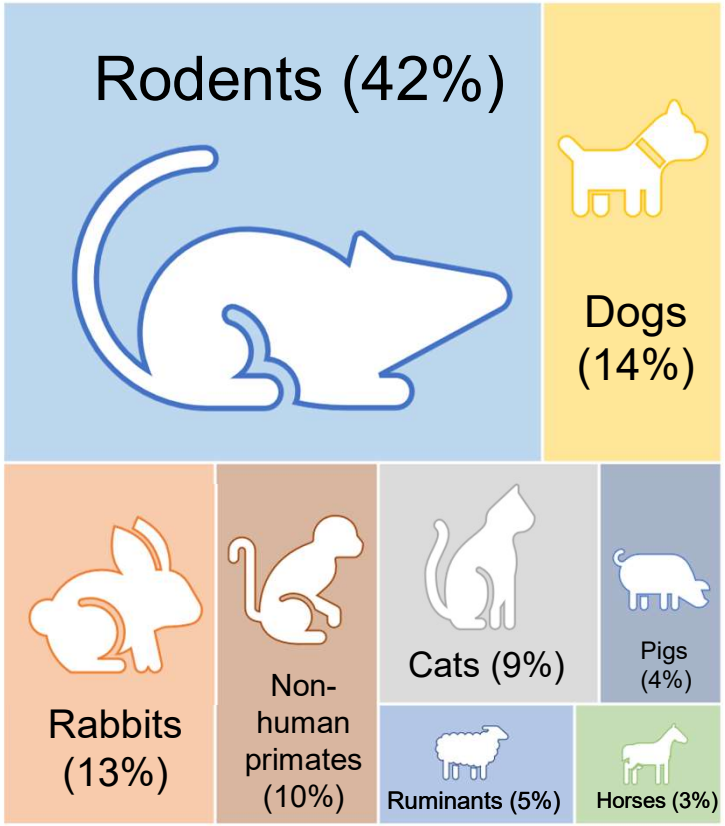
- HCDR3 of antibody is most important for contacting the epitope
- Mouse HCDR3 relatively short
  - Tend to bind flat epitopes
- Longer sequences can protrude into functionally important recessed epitopes



**Kabat numbering used throughout*

3. Cross Species-Reactivity Could Expedite MAb Development

- Many well-validated preclinical animal models
- Most mouse-derived MAbs reactive only in primates
  - Testing limited to non-human primates (NHP)
  - NHP experiments resource intensive
  - NHPs in short supply
  - NHP testing not actually required
- Ability to test MAbs in lower mammals could greatly streamline studies



Mammalian species used in animal models

*in Nobel prize work (physiology/medicine)
Adapted from (Jota Baptista et al., 2021, Pharmacology)

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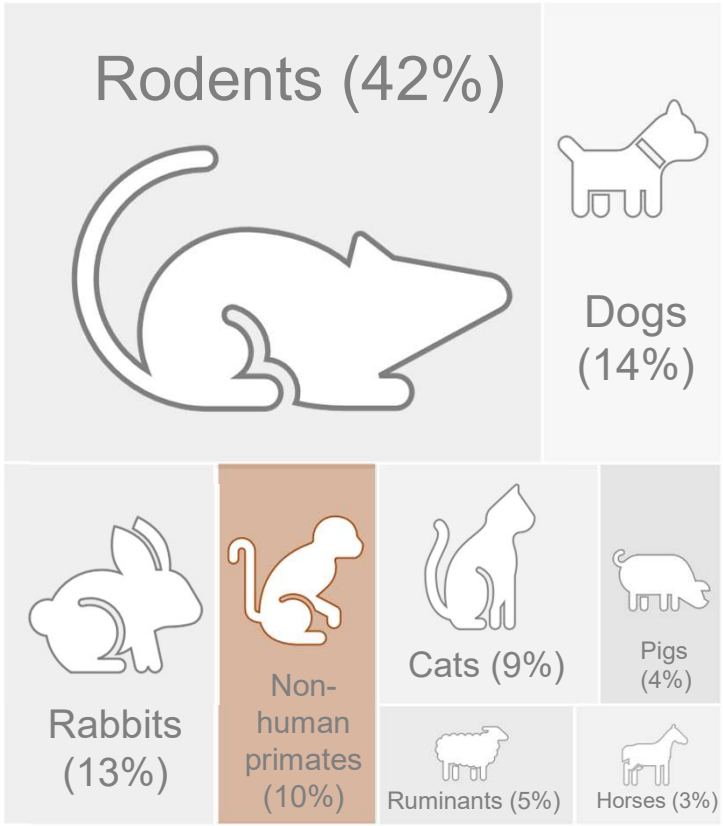

Mammalian species used in animal models

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Adapted from (Jota Baptista et al., 2021, Pharmacology)

Cross Species-Reactive MAbs From Divergent Species

	Clinical Stage	Immunization host	% Identity vs host	% Identity vs mouse	Reactivity in non-primates
Caplacizumab (von Willebrand factor)	Approved	Camelid	81	83	Guinea pig, mini-pig, pig,
Brolucizumab (VEGF-A)	Approved	Rabbit	88	84	Rat, mouse, dog, pig, cat
Eptinezumab (CGRP)	Approved	Rabbit	89	89	Rat, rabbit
Sym021 (PD1)	Clinical trials	Chicken	35	59	Mouse
CTIM-76 (Claudin 6)	Preclinical	Chicken	61	88	Mouse
IM-68-27G10 (GPRC5D)	Preclinical	Chicken	49	82	Mouse
B30 (BDNF)	Discovery	Chicken	94	100	Rat
pT231/pS235_1 (Tau)	Discovery	Chicken	84	90	Mouse
AC1 (CD20)	Discovery	Chicken	No ortholog	75	Mouse
YW33 (Integrin α11β1)	Discovery	Chicken	78/85	90/93	Mouse, rat
MAb panel (SIRPα)	Discovery	Chicken	42	66	Mouse
MAb panel (SLC2A4)	Discovery	Chicken	65 (Paralog)	95	Mouse
MAb panel (GIPR)	Discovery	Chicken	50	82	Mouse, rat
MAb panel (Kv1.3)	Discovery	Chicken	84	96	Mouse
MAb panel (Claudin18.2)	Discovery	Chicken	76	90	Mouse

Banik et al., 2023, *mAbs*

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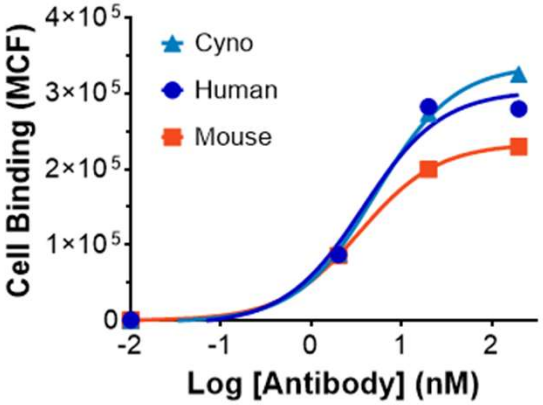
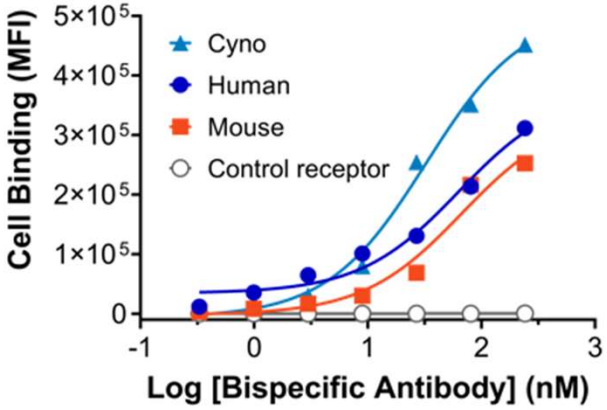
Our Experience Generating Species Cross-Reactive MAbs

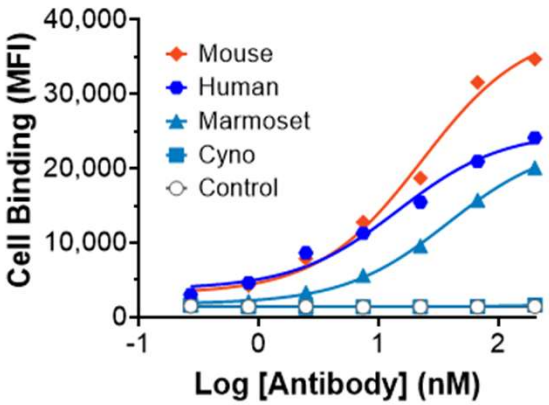
CLDN18.2
(90% identical to mouse)



GPRC5D
(82% identical to mouse)



CCR8
(71% identical to mouse)



(Antibodies raised in chickens)

How Divergent Species Can Access Conserved Targets

Gaps in antibody space &
role of divergent species

Rabbits, Camelids, Chickens

Chicken immunization has delivered:

Antibodies against conserved targets
Agonist antibody
Exquisite specificity

Rabbit MAb Discovery

- Well-established role as immunological hosts
- Single VH (VH1) and VL (VK1) framework
  - Cloning, humanization, and engineering relatively straightforward
- MAb discovery technologies
  - B cell cloning
    (e.g. eptinezumab, crovalimab, clazakizumab)
  - Rabbit hybridomas
    (e.g. APX005M/sotigalimab)

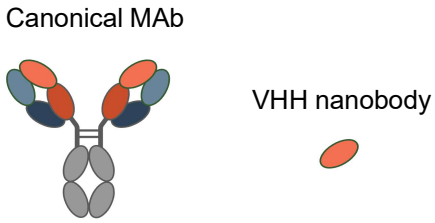
	Mouse	Rabbit
Evolutionary distance from humans, years	91 million	91 million
Animal host class	mammalia	mammalia
Robust immune response against conserved proteins	-	-
Cross-reactive MAbs for preclinical models	-	+/-
Canonical IgG	+	+
HCDR3 length (aa)	9.1±2.0	12.8±3.6
Long paratope with average HCDR3 >14 aa	-	-
Nanobodies from host	-	-
Immunization costs and animal logistics	$	$
Access to humanized animals	+	-
Diverse accessible B cell repertoire (spleen, marrow)	+	+

Rabbit MAbs

INN	Brand name	Other names	Most advanced phase	Target(s)	Indications of clinical trials
Eptinezumab	Vyepti	ALD403	Approved in US and EU	Calcitonin gene-related peptide (CGRP)	Migraine prevention
Brolucizumab	Beovu	RTH258, ESBA1008, DLX-1008	Approved in US and EU	VEGF-A	Diabetic macular edema, neovascular age-related macular degeneration
Crovalimab		SKY59, RG6107, RO7112689	Regulatory review in US, EU, China and Japan	Complement C5	Sickle cell disease, atypical hemolytic uremic syndrome, paroxysmal nocturnal hemoglobinuria
Suvemcitug		APX003, BD0801; TK001, Sevacizumab (not WHO assigned INN)	Phase 3	VEGF	Ovarian cancer, macular degeneration
Clazakizumab		CSL300, ALD518; BMS-945429	Phase 3	IL-6	Subjects with end stage kidney disease undergoing dialysis, COVID-19, acute GvHD, psoriatic arthritis; Crohn's disease, rheumatoid arthritis, oral mucositis, non-small cell lung cancer-related fatigue and cachexia
		9MW0211	Phase 2/3	VEGF	Macular degeneration
Sotigalimab		APX005M	Phase 2 (non-commercial sponsor)	CD40	Ovarian cancer, melanoma, gastro-esophageal cancer, pancreatic cancer, solid tumors, non-small cell lung cancer, renal cancer
		LU AG09222, ALD1910	Phase 2	PACAP-38	Allergic rhinitis, migraine
		TRK-950	Phase 2 pending	CAPRIN-1	Gastric cancer, solid tumors
		YYB101	Phase 1/2	HGF	Colorectal cancer
		ASKB589	Phase 1/2	Claudin 18.2	Solid tumors
		CLM-101, NOV-110501, YYB-101	Phase 1/2	HGF	Colorectal cancer, solid tumors
		QX005N, SNC005	Phase 1	IL-4R	Atopic dermatitis

(Antibody Society, December 2023)

Camelid MAb Discovery



- Small heavy-chain only MAbs
  - Can be engineered into new formats (bispecifics)
  - Long HCDR3
- MAb discovery techniques
  - Phage display (caplacizumab, envafolimab, and ozoralizumab)
  - Yeast display
- Complex logistics due to large host animals

	Mouse	Camelid
Evolutionary distance from humans, years	91 million	92 million
Animal host class	mammalia	mammalia
Robust immune response against conserved proteins	-	-
Cross-reactive MAbs for preclinical models	-	+/-
Canonical IgG	+	+
HCDR3 length (aa)	9.1±2.0	14.5±3.7
Long paratope with average HCDR3 >14 aa	-	+
Nanobodies from host	-	+
Immunization costs and animal logistics	$	$$$
Access to humanized animals	+	-
Diverse accessible B cell repertoire (spleen, marrow)	+	-

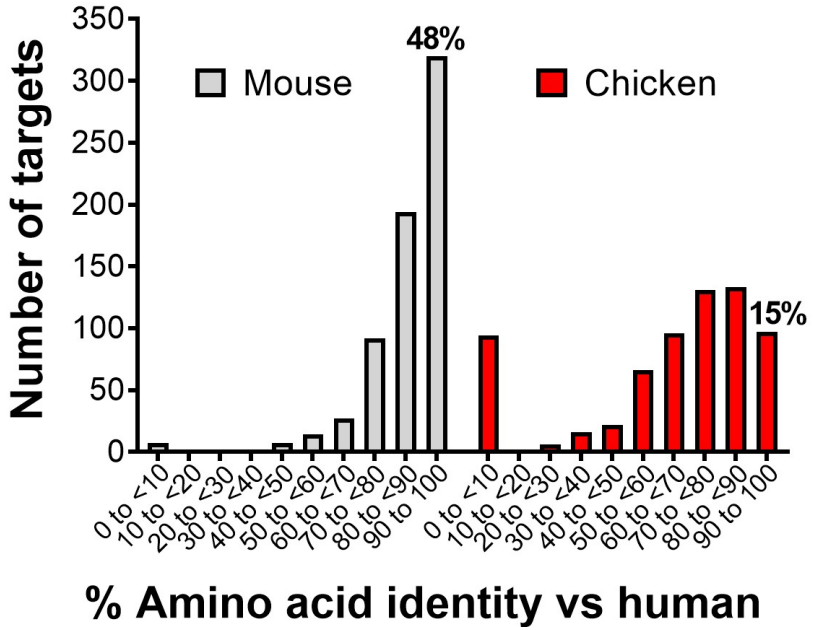
Camelid MAbs

INN	Brand name	Other names	Most advanced phase	Target(s)	Indications of clinical trials
Caplacizumab	Cablivi	ALX-0081 (IV), ALX-0681	Approved in EU and US	von Willebrand factor	Acquired thrombotic thrombocytopenic purpura, unstable angina; non ST segment elevation myocardial infarction (NSTEMI); stable angina (associated with high risk PCI), thrombotic thrombocytopenic purpura
Ozoralizumab		TS-152, ATN-103	Approved in Japan	TNF, albumin	Rheumatoid arthritis
Envafolimab	ENWEIDA	KN035, ASC22	Approved in China	PD-L1	HIV infection, metastatic or recurrent non-microsatellite highly unstable (non-MSI-H)/non-DNA mismatch repair defect (non-dMMR) endometrial cancer, undifferentiated pleomorphic sarcoma, hepatitis B, bile tract carcinoma, solid tumors
Netakimab	Efleira	BCD-085	Approved in Russia	IL-17	Ankylosing spondylitis, psoriasis, psoriatic arthritis
Levilimab	Ilsira	BCD-089	Approved in Russia	IL-6R	COVID-19, rheumatoid arthritis
Gefurulimab		ALXN1720	Phase 3	C5	Myasthenia gravis
Erfonrilimab		KN046	Phase 3	PD-L1/CTLA-4	Multiple tumor types
		PM8002	Phase 2/3	PD-L1, VEGF	Hepatocellular carcinoma, small cell lung cancer, non-small cell lung cancer, solid tumors
		LMN-201	Phase 2/3 pending	C. difficile exotoxin B	Clostridioides difficile infection
Ozekibart		JCT205, INBRX-109	Phase 2, pivotal	DR5	Chondrosarcoma, solid tumors including sarcomas
		BI 836880	Phase 2	VEGF, Ang2	Head and neck cancer, liver cancer, anal canal squamous cell carcinoma, macular degeneration, non-small cell lung cancer, solid tumors
		PM8001	Phase 2	PD-L1, TGFβ	Cancer
Sonelokimab		M1095, MSB0010841, ALX-0761	Phase 2	IL-17A, IL17F, HSA	Psoriatic arthritis, hidradenitis suppurativa, psoriasis
		LMN-101	Phase 2	FlaA	Campylobacter jejuni infection
Livmoniplimab		ABBV-151, ARGX-115	Phase 2	GARP-TGFβ1 complex	Solid tumors
Cusatuzumab		ARGX-110, JNJ-74494550	Phase 2	CD70	Cutaneous T-cell lymphoma, AML, hematological and solid cancers, Waldenström's macroglobulinemia
		LEO 138559, ARGX112, LP0145	Phase 2	IL-22R	Atopic dermatitis
		SAR442970	Phase 2	TNF, OX40L	Hidradenitis suppurativa
Tarperprumig		ALXN1820	Phase 2	Properdin	Sickle cell disease

+ many more early stage (Antibody Society, 2023)

Chicken MAb Discovery

- Many conserved targets immunologically accessible
  - Only 15% of drug targets appear highly conserved (>90% identity)
- Canonical antibodies despite phylogenetic distance
- Skewed distribution of HCDR3s with longer sequences
  - ~90% with HCDR3 ≥13+ amino acids
- Easy to humanize, only 1 germline gene for heavy and light chain
- MAb discovery techniques:
  - Phage display (numerous preclinical examples)
  - B cell cloning (Sym021)
- First chicken MAb in clinic
  - Sym021, targeting PD-1
    (Cyno and mouse cross reactive)



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	Mouse	Chicken
Evolutionary distance from humans, years	91 million	310 million
Animal host class	mammalia	aves
Robust immune response against conserved proteins	-	+
Cross-reactive MAbs for preclinical models	-	+
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Long paratope with average HCDR3 >14 aa	-	+
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Access to humanized animals	+	+
Diverse accessible B cell repertoire (spleen, marrow)	+	+

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Gaps in antibody space & role of divergent species

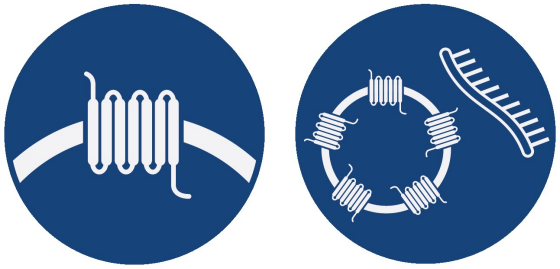
Rabbits, Camelids, Chickens

Chicken immunization has delivered:

Antibodies against conserved targets
Agonist antibody
Exquisite specificity

Chickens Are Integral to Our Platform

Native Antigen



- Pioneers in DNA & mRNA immunization for MAb discovery
- Inventors of Lipoparticle technology
- 20+ years membrane protein expertise

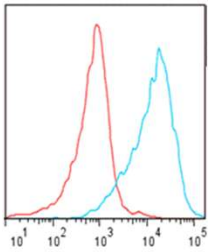
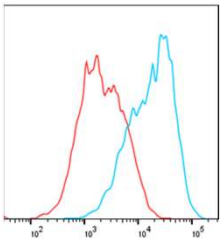
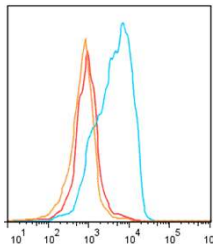
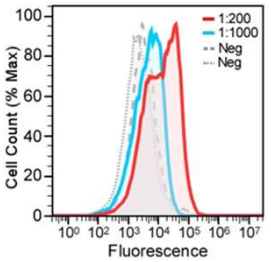
Chicken MAb Discovery

- Access conserved & difficult epitopes
- Diverse antibody candidate panels
- Humanized, pM affinity, developable

Preclinical Leads

- Bispecific/therapeutic format
- Functional POC *in vitro* & *in vivo*
- Deliverable: 12-18 months to IND

High-Titer Responses to Conserved Targets

	CLDN6	Kv1.3	CB1	SLC2A4
% Mouse identity	88	96	97	95
% Chicken identity	61	84	94	65 (paralog)
Serum titer				

Chickens deliver diverse MAbs against wide range of targets, with **95% success**

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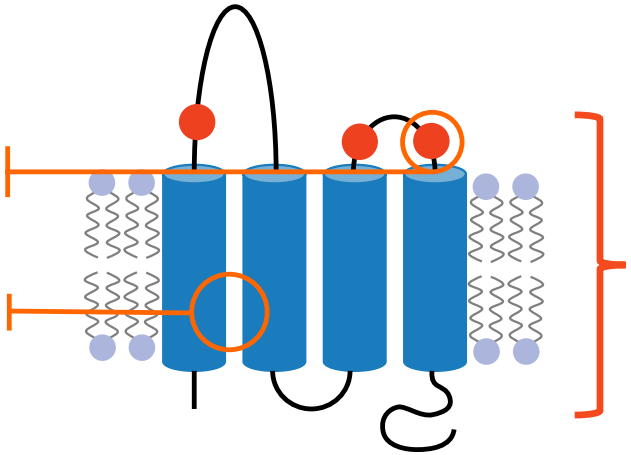
v0121

Claudin 6 Program for Oncology – Enabled by Chickens

Challenges

Only 3 extracellular residues different from CLDN9 (widely expressed)

Structurally complex antigen



ECL identity: 95%

Total aa identity: 88%

Therapeutic Potential

- Expressed in ovarian, NSC lung, teratomas, gastric tumors
- Not expressed in normal adult tissues

Current Status

- Configured as a bispecific
- Potent antitumor effects in animals, and good developability
- IND filed, entering clinical trials mid-2024

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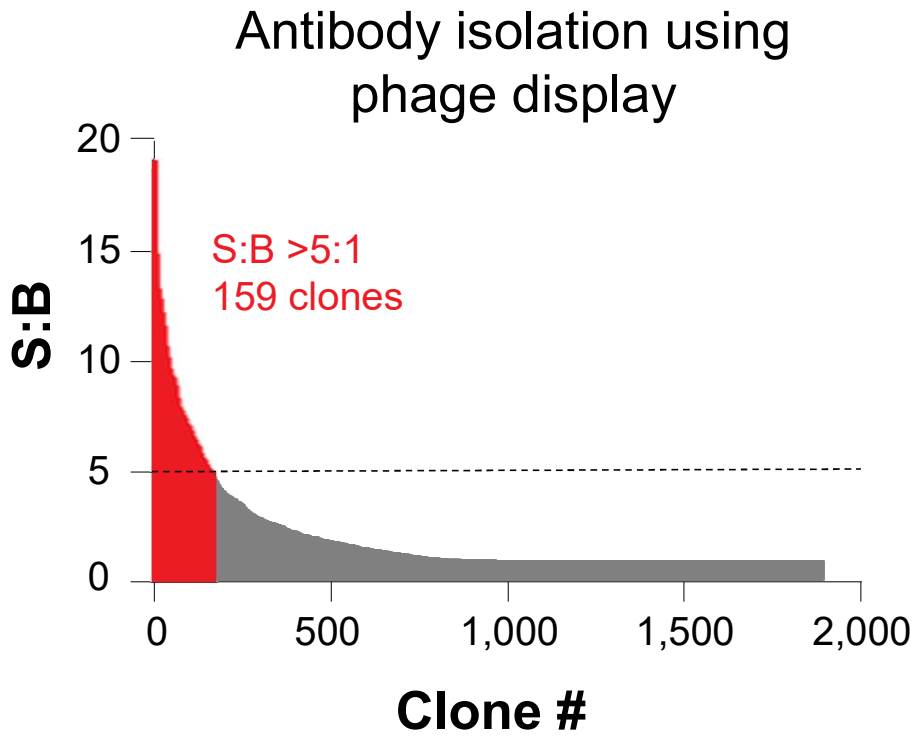
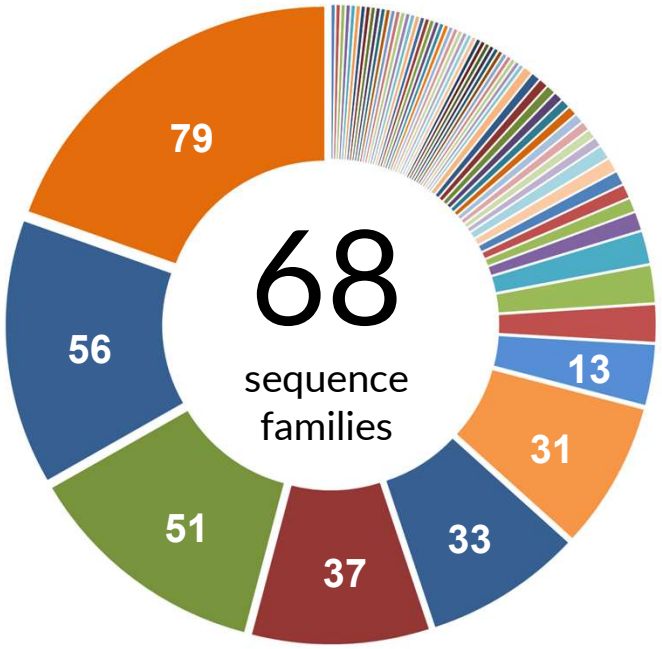
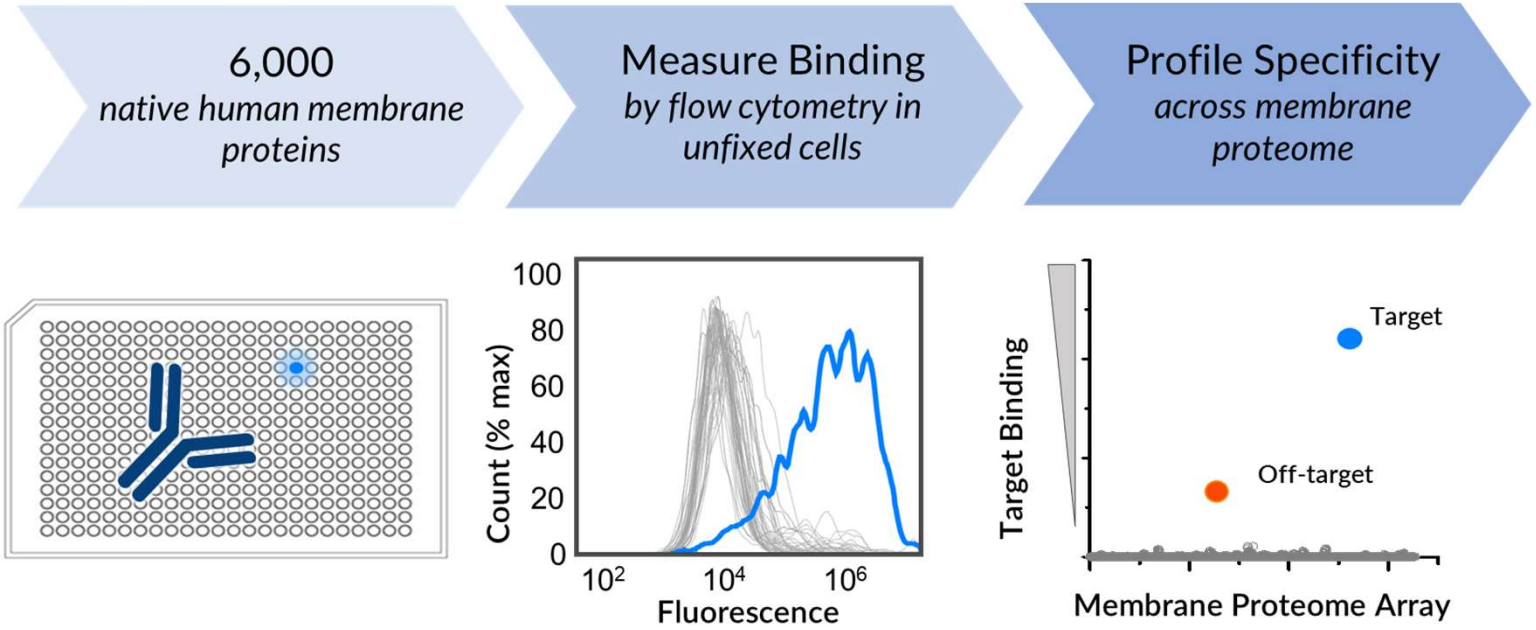
Antibody Diversity From Chicken Immunization





CLND6 MAb Discovery: A Search for Specificity



- MPA encompasses human membrane proteome including 24 claudin family members
- Specificity profiling tool under review by FDA, for qualification as a Drug Development Tool

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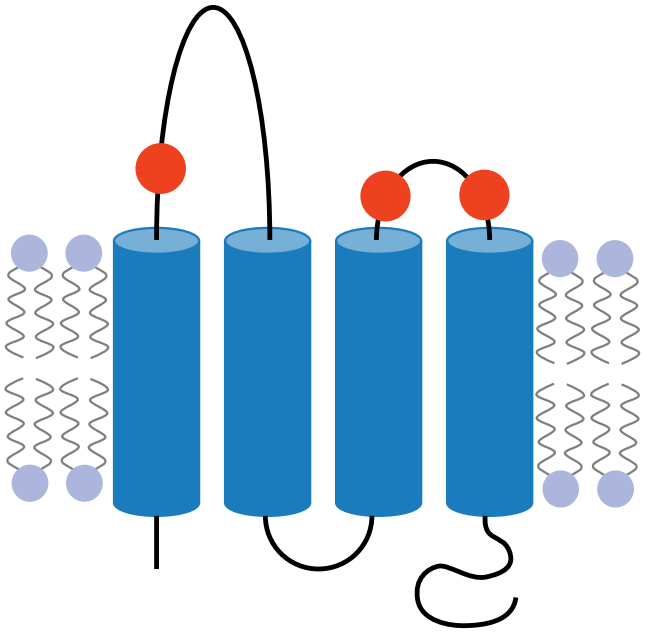
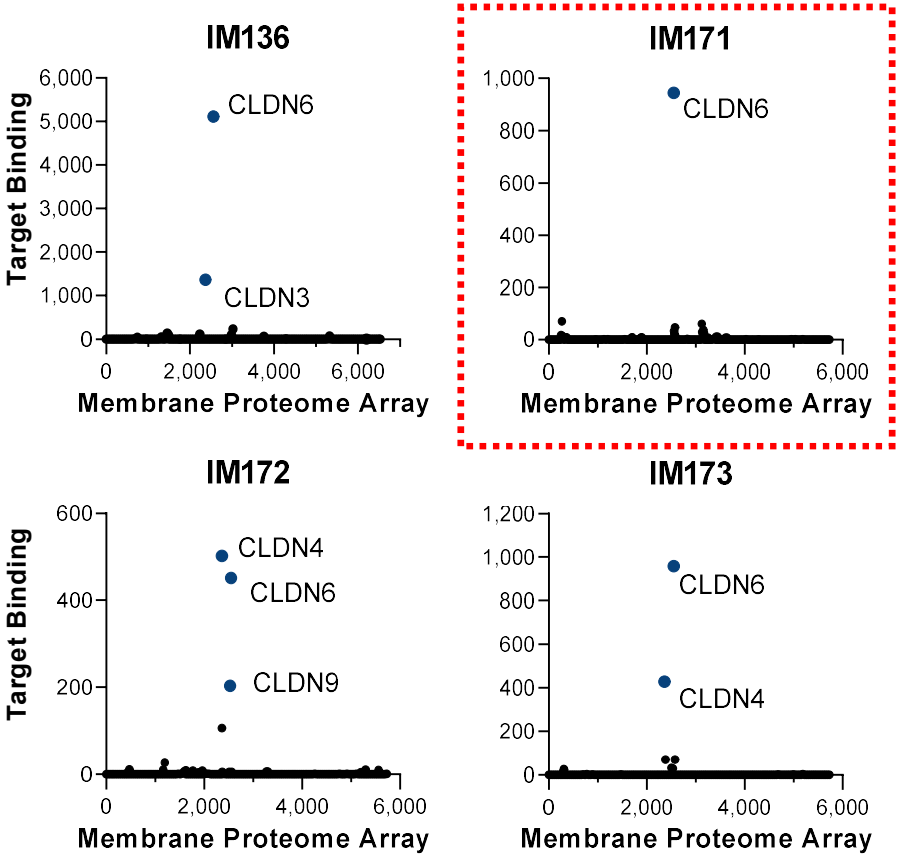
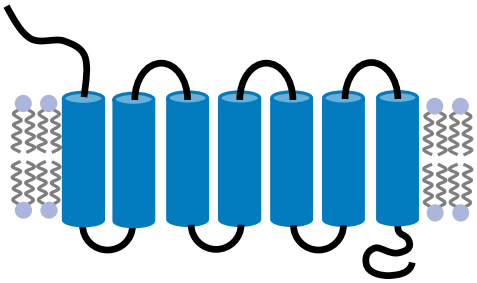
CLND6 MAb Discovery: A Search for Specificity



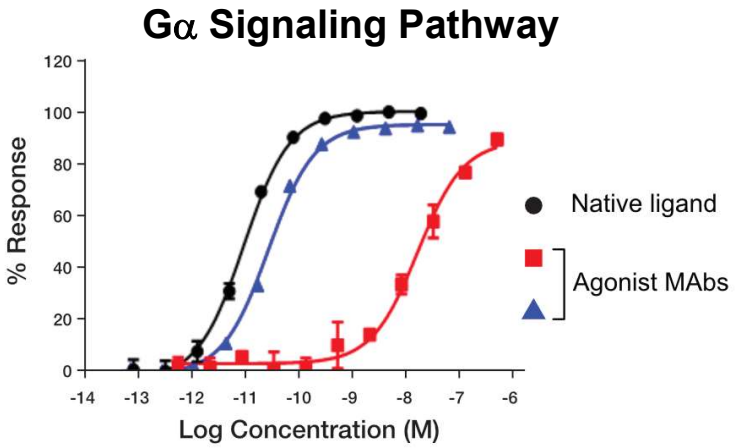


Screnci et al., 2022, *iScience*

GPCR Agonist MAb Enabled by Chickens



- Therapeutic antibody discovery project completed for Merck
- Merck sought an agonist antibody



- ✓ Human
- ✓ Non-human primate
- ✓ Mouse
- ✓ Rat
- ✓ Dog

“ The unique CDR diversity generated in chickens provided an interaction capable of activating a GPCR that no other rodent or human phage display derived antibodies could do even to the same epitope region. ”

-Director of Antibody Discovery, Merck

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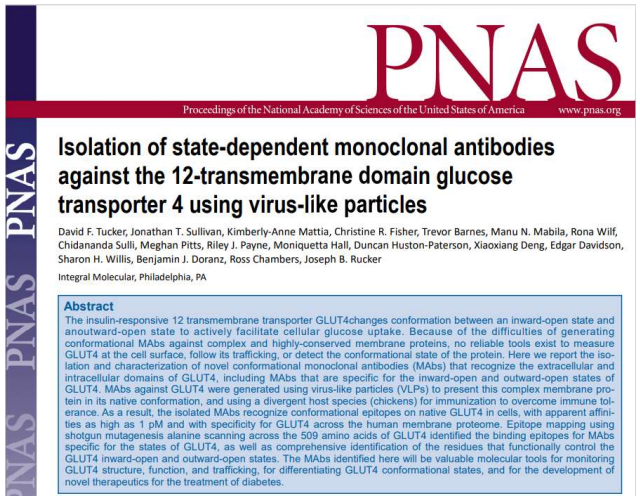
SLC2A4 MAb: Unique Activity and Long HCDR3



PNAS

Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

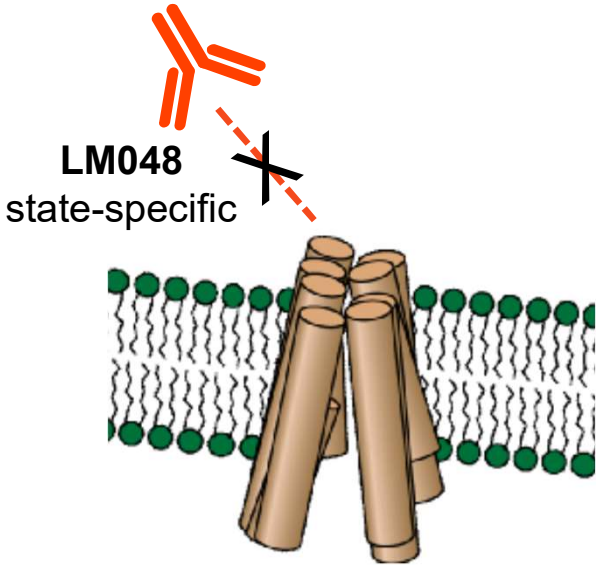
Isolation of state-dependent monoclonal antibodies against the 12-transmembrane domain glucose transporter 4 using virus-like particles

David F. Tucker, Jonathan T. Sullivan, Kimberly-Anne Mattia, Christine R. Fisher, Trevor Barnes, Manu N. Mabila, Rona Wilf, Chidananda Sulli, Meghan Pitts, Riley J. Payne, Moniquetta Hall, Duncan Huston-Paterson, Xiaoxiang Deng, Edgar Davidson, Sharon H. Willis, Benjamin J. Doranz, Ross Chambers, Joseph B. Rucker

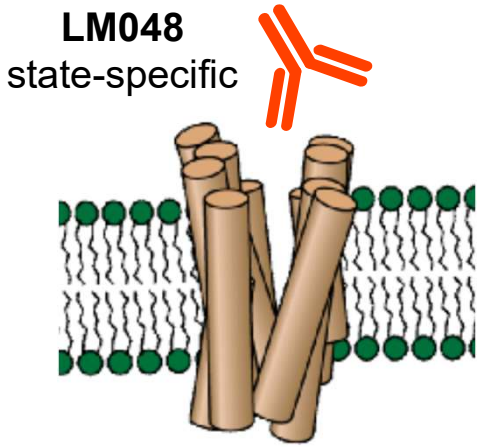
Integral Molecular, Philadelphia, PA

Abstract

The insulin-responsive 12 transmembrane transporter GLUT4changes conformation between an inward-open state and anoutward-open state to actively facilitate cellular glucose uptake. Because of the difficulties of generating conformational MAbs against complex and highly-conserved membrane proteins, no reliable tools exist to measure GLUT4 at the cell surface, follow its trafficking, or detect the conformational state of the protein. Here we report the isolation and characterization of novel conformational monoclonal antibodies (MAbs) that recognize the extracellular and intracellular domains of GLUT4, including MAbs that are specific for the inward-open and outward-open states of GLUT4. MAbs against GLUT4 were generated using virus-like particles (VLPs) to present this complex membrane protein in its native conformation, and using a divergent host species (chickens) for immunization to overcome immune tolerance. As a result, the isolated MAbs recognize conformational epitopes on native GLUT4 in cells, with apparent affinities as high as 1 pM and with specificity for GLUT4 across the human membrane proteome. Epitope mapping using shotgun mutagenesis alanine scanning across the 509 amino acids of GLUT4 identified the binding epitopes for MAbs specific for the states of GLUT4, as well as comprehensive identification of the residues that functionally control the GLUT4 inward-open and outward-open states. The MAbs identified here will be valuable molecular tools for monitoring GLUT4 structure, function, and trafficking, for differentiating GLUT4 conformational states, and for the development of novel therapeutics for the treatment of diabetes.



inward-open



outward-open

SLC2A4 State-Specific MAb (LM048)

- 26 aa HCDR3
- Binds in cavity formed by ECL4 and ECL6
- Rare property of binding state-specific conformation

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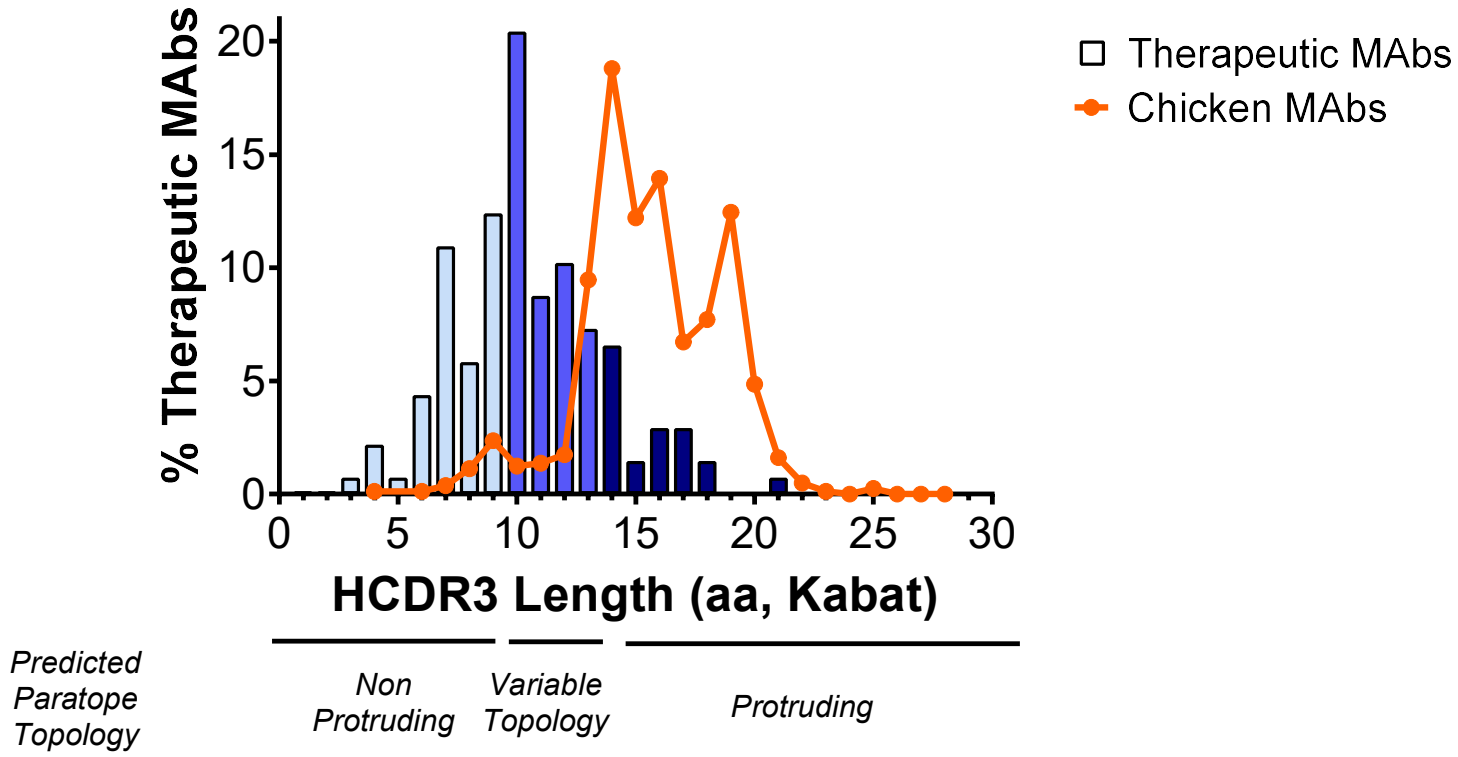
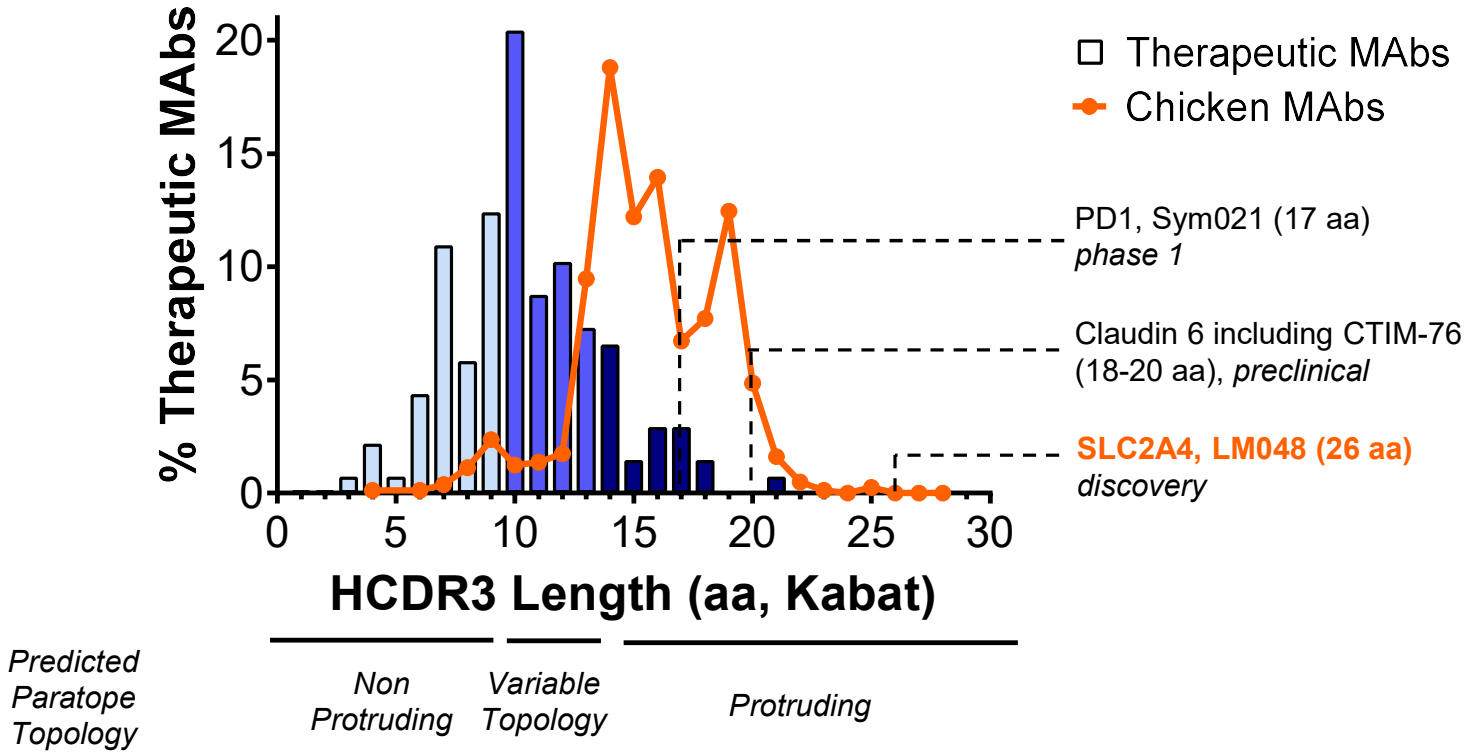
Chicken MAbs Have Long HCDR3



Chicken MAbs Have Long HCDR3



Integral Molecular: Preclinical Pipeline of Chicken-Derived MAbs

Target	Indication	Modality	Discovery	Preclinical	Phase 1	Partner
CLDN6	solid tumors	TCE				context therapeutics
Undisclosed	oncology	undisclosed				AstraZeneca
CLDN18.2	solid tumors	CAR-T				CARTEXELL
CLDN18.2	solid tumors	ADC				Undisclosed
CLDN18.2	solid tumors	mRNA TCE				
GPRC5D	multiple myeloma	TCE				
CCR8	solid tumors	ADC				
KV1.3	autoimmune	multiple				

TCE: T- cell engager (multi-specific antibody)
ADC: Antibody-drug conjugate
CAR-T: Chimeric antigen receptor T cell

Partnered
Internal (available)

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Divergent Species Enable MAb Discovery

- Many valuable drug targets unfeasible in mice due to conservation
- Divergent species are increasingly used

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Evolutionarily divergent species provide:

- Robust immune response with broad epitope coverage
- Long HCDR3 regions can access functional pockets
- Access to more animal models because of species cross reactivity
- See Banik et al., 2023, mAbs

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Chicken MAbs have demonstrated

- Exquisite epitope specificity
- Agonist & state specific activity

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Tell us what M Ab you wish you had!

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Membrane Protein Solutions

THANK YOU

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