

Copyright and Permission: Slides may be viewed and shared. Copying, modification, or reuse of slide content requires permission from the author.

The Current Landscape of Non-animal Preclinical Assessment for Monoclonal Antibodies and Biopharmaceuticals

Dr. Leander Grode



SLS Europe - We bring your product development to life



- Scientific Officer, Medical Expert, Project Managers, Clinical Project Managers, Quality Assurance Managers
- Interdisciplinary and multicultural team
- Academic qualifications: natural and life sciences & medicine (Ph.D/MD rate in project management and consulting: 100%)
- Fields of expertise: Vaccines, Antibodies, Biologicals, ATMPs, Small Molecules, Medical Devices and Diagnostics
- Medical expertise in clinical development of vaccines, monoclonal antibodies, and small molecules

SLS Europe Today - Core Competencies & Experience



Regulatory Consulting

- Clinical Trial Information System (**CTIS**) and submissions
- **Scientific advice** meetings in Europe, US, and globally
- Investigational new drug (**IND**) submissions
- Preparations of **clinical trial application** packages
- Pediatric investigational plans (**PIP**)
- ATMP classifications



Marketing Authorization

- **Marketing Authorization Application** globally
- **Marketing Authorization Holder** (Sponsor) in Europe
- **Import and Release**
- In house **Pharmacovigilance** System



Clinical Trial Management

- Full **sponsor** responsibility
- **Representative of the sponsor** in EU
- Clinical project management
- Quality oversight
- Site identification and feasibility
- Data management and biostatistics



Translational Project Management

- **Reverse-planning** approach for product development
- Preparation of Target Product Profile (**TPP**)
- Validation
- Budgeting
- **Risk analysis** (including gap analysis, red flag analysis)



Quality Assurance Implementation

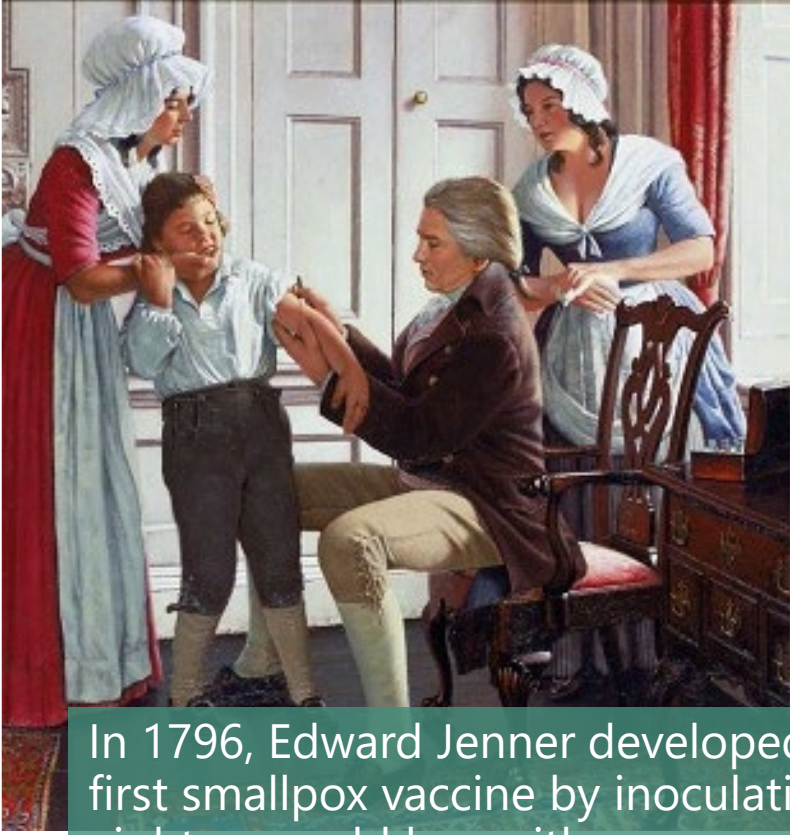
- Tailor-made clinical **QMS** consultancy following TransCelerate approach
- In house training courses
- Support in ensuring **compliance with GxP**
- Preparations for audits and inspections



Medical Expertise

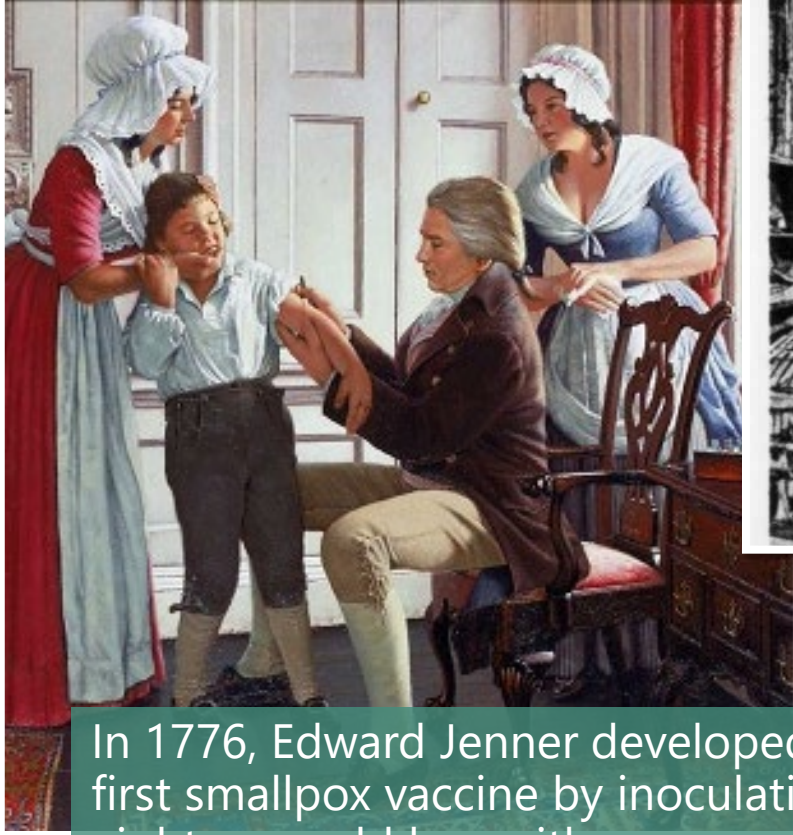
- Conduct of >70 clinical trials
- Thereof: 12 with monoclonal antibodies,
38 small molecules,
7 vaccines,
6 biologicals,
2 medical devices

Brief History on Biopharmaceutical Development

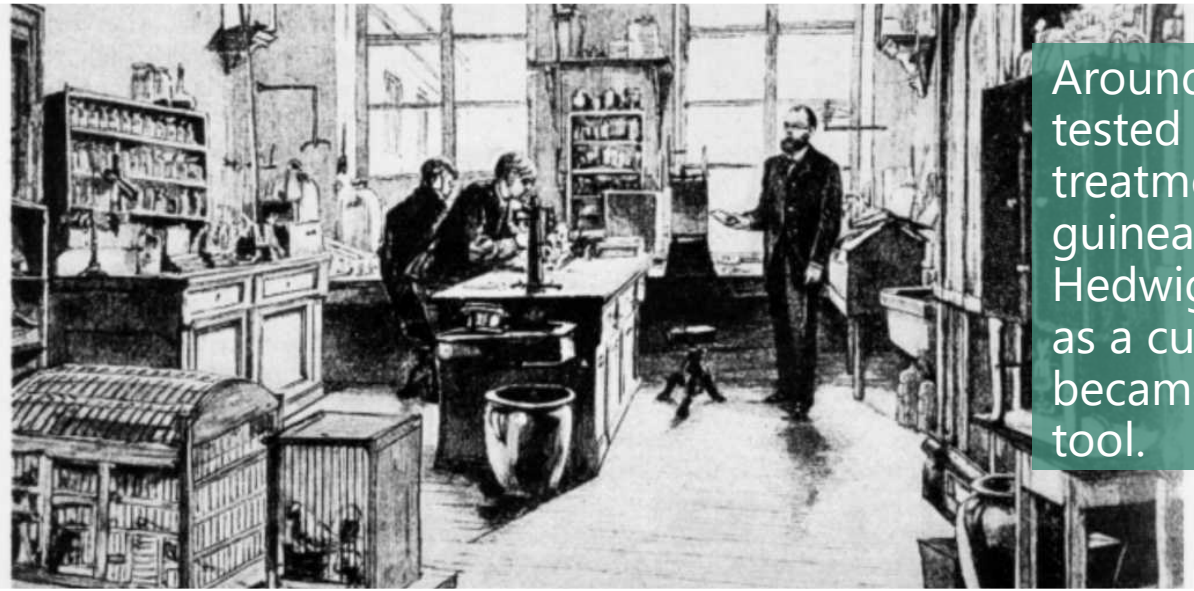


In 1796, Edward Jenner developed the first smallpox vaccine by inoculating an eight-year-old boy with cowpox, which successfully provided immunity to smallpox.

Brief History on Biopharmaceutical Development



In 1776, Edward Jenner developed the first smallpox vaccine by inoculating an eight-year-old boy with cowpox, which successfully provided immunity to smallpox.

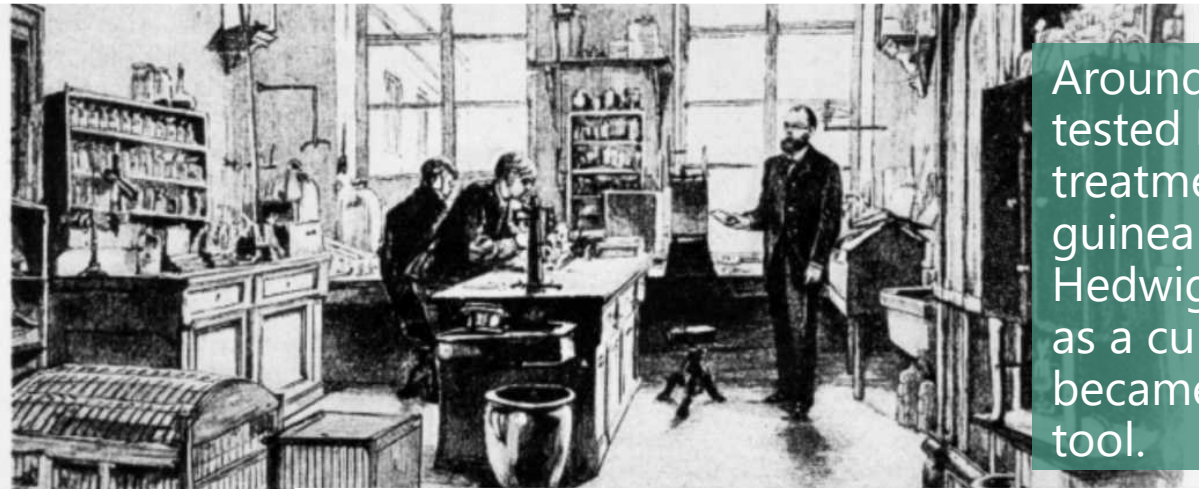


Around 1890, Robert Koch tested his tuberculosis treatment, tuberculin, on guinea pigs and his wife, Hedwig Freiberg, but it failed as a cure, although it later became useful as a diagnostic tool.

Brief History on Biopharmaceutical Development



In 1776, Edward Jenner developed the first smallpox vaccine by inoculating an eight-year-old boy with cowpox, which successfully provided immunity to smallpox.



Around 1890, Robert Koch tested his tuberculosis treatment, tuberculin, on guinea pigs and his wife, Hedwig Freiberg, but it failed as a cure, although it later became useful as a diagnostic tool.



In 2006, the TeGenero/TGN1412 disaster revealed critical flaws in drug testing procedures, prompting significant changes to enhance safety and risk assessment in nonclinical and clinical trials.

Traditional Biopharmaceutical Development Relies Heavily on Animal Models



- **Purpose:** Determine safety and efficacy of new compounds before clinical trials.
- **Focus:** Safety profile, including toxicity testing using animals.
- **Outcome:** Safe dose determination for human trials.

Image taken from: <https://www.scmp.com/magazines/style/news-trends/article/3004229/china-announces-end-post-market-animal-testing-cosmetic>

Challenges in Pharmaceutical Development

Repeated animal testing required to assess efficacy and side effects.

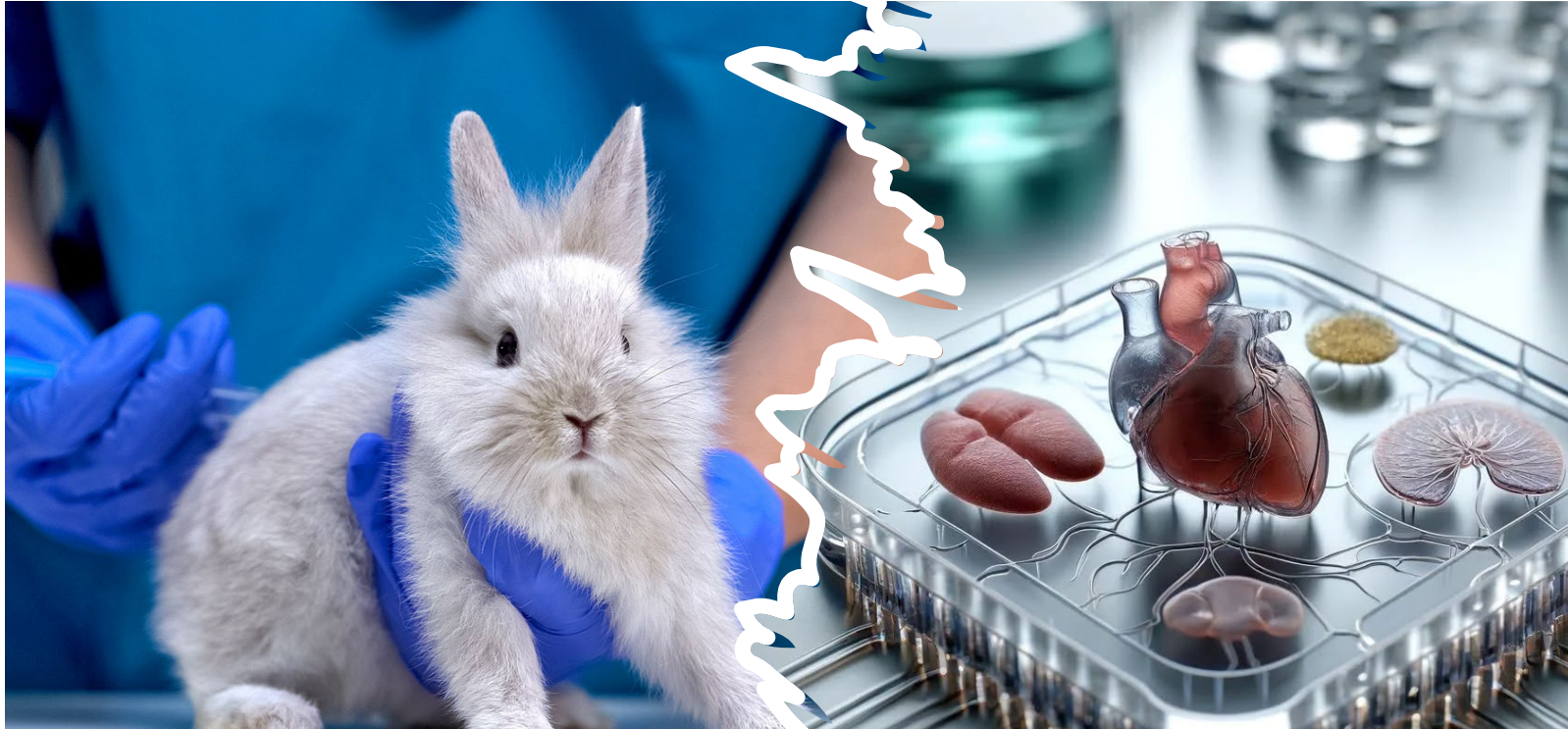
Animal experiments raise significant **ethical issues**.

Up to 85% of drug candidates **fail** in clinical trials despite preliminary animal testing.

High rate of failure increases development costs and raises questions about the **value** of animal experiments.

→ **Need for alternatives to minimize animal use, for ethical considerations and for more relevant data**

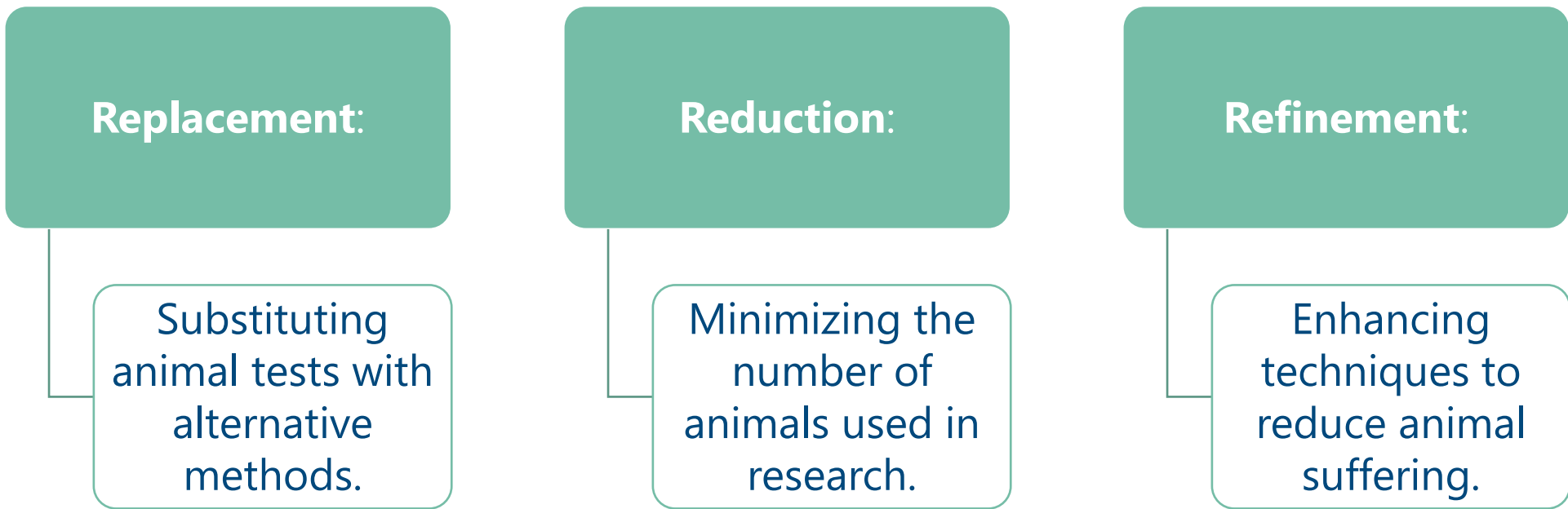
Shift in Paradigm: Growing Focus on Non-animal Preclinical Assessment Methods



→ Explore advancements, regulatory frameworks, and practical considerations for non-animal methods.

3R Principle

In 1959, the British scientists William Russel and Rex Burch formulated the 3R principle as the principle of experimental scientific work:



Non-Animal Assessment Methods

In Vitro Techniques

Cell-Based Assays:
Evaluate drug interactions, efficacy, and toxicity in human cells.

Organ-on-a-Chip Systems: Simulate human organ functions and complex biological interactions.

Computational Models

Predictive Modeling:
Forecast biological responses and potential toxicity.

In Silico Approaches:
Use data and simulations to model drug behavior

Regulatory Pathways & Guidelines

ICH S6(R1): biologics already allow flexible, scientifically justified nonclinical packages

Toxicology species should be **pharmacologically relevant**.

If no relevant species exists, conventional animal toxicology may provide limited value.

Alternative approaches can be justified using:

- target biology and tissue expression
- in vitro pharmacology and safety assays
- cross-reactivity / off-tissue binding assessment
- clinical risk mitigation, sentinel dosing, and cautious escalation

FDA 2025: explicit regulatory shift for mAbs and other drugs

FDA announced a plan to reduce, refine, or potentially replace animal testing using NAMs, including AI/computational toxicity models, cell-line systems, and organoid-based testing.

Implementation began immediately for IND applications, where inclusion of NAM data is encouraged.

FDA specifically identifies mAbs as an initial focus area.

FDA draft mAb guidance, December 2025

Draft guidance covers monospecific antibodies.

Describes when general toxicology studies are not warranted or may be limited to short-term studies.

Addresses chronic toxicology, reproductive/developmental/juvenile toxicity, and unnecessary NHP use.

Important caveat: Draft; not for implementation; non-binding recommendations.

EMA: NAM acceptance is possible but context-dependent

EMA supports NAMs for regulatory decision-making when scientifically sound.

Acceptance depends on a defined context of use, robust methodology, relevance, reliability, and early regulatory interaction.

Regulatory routes include Innovation Task Force briefing meetings, scientific advice, CHMP qualification, and voluntary data submission / safe harbour.

Regulatory Framework on Animal Research



European Union

- Directive **2010/63/EU** embeds the 3Rs and states the final objective of replacing animal procedures when scientifically possible.
- EMA now provides a structured pathway for NAM regulatory acceptance
- EMA explicitly states that NAMs may either **replace an existing regulatory requirement** or contribute to a **weight-of-evidence** package.



European Pharmacopoeia

- In June 2024, the Ph. Eur. Commission adopted deletion of the rabbit pyrogen test from **57 texts** and introduced general chapter **5.1.13 Pyrogenicity**.
- Implementation date: **1 July 2025**.
- Developers are responsible for selecting a suitable in vitro test, for example **monocyte activation test**, based on product-specific risk assessment.



United States

- FDA Modernization Act 2.0 removed the statutory expectation that animal data are always required before human trials.
- FDA's 2025 roadmap goes further by encouraging NAM data in INDs and prioritising mAbs as a starting point.

Replacement of Animal Tests – Rabbit Pyrogen Test Largely Eliminated



Paul-Ehrlich-Institut

17,980 followers

1mo •

Replacement of animal tests – The European Pharmacopoeia Commission, with the participation of the Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, and the Federal Institute for Drugs and Medical Devices ([Bundesinstitut für Arzneimittel und Medizinprodukte](#)), removed the rabbit pyrogen test from 57 pharmacopoeial monographs during its 179th session in June 2024. This change is a milestone in the development of the European Pharmacopoeia in terms of the replacement, reduction, and refinement (RRR) of previously legally prescribed animal experiments in medicinal product testing.

The rabbit pyrogen test was first developed in the 1940s. The test involves measuring the increase in rabbits' body temperature after they have been injected intravenously with the test substance. Fever-causing substances known as pyrogens and endotoxins can be detected with this test. Since its creation, the rabbit pyrogen test has been an important test in medicinal product development for the early detection of impurities that could cause life-threatening side effects in patients. It took about 20 years to develop alternative methods and to be able to provide evidence of their equivalence to the animal model with substantial data.



<https://www.pei.de/EN/newsroom/hp-news/2024/240718-rabbit-pyrogentest.html>

Regulatory and Community Sentiment: NAMs Are Moving from Advocacy to Regulatory Practice

01

Stronger regulatory appetite for NAMs, especially when animal models are mechanistically weak.

02

mAbs are a leading use case because:

- they are often highly target-specific
- animal relevance is frequently constrained by species cross-reactivity
- long-term NHP studies are ethically and scientifically scrutinised

03

Regulators still expect:

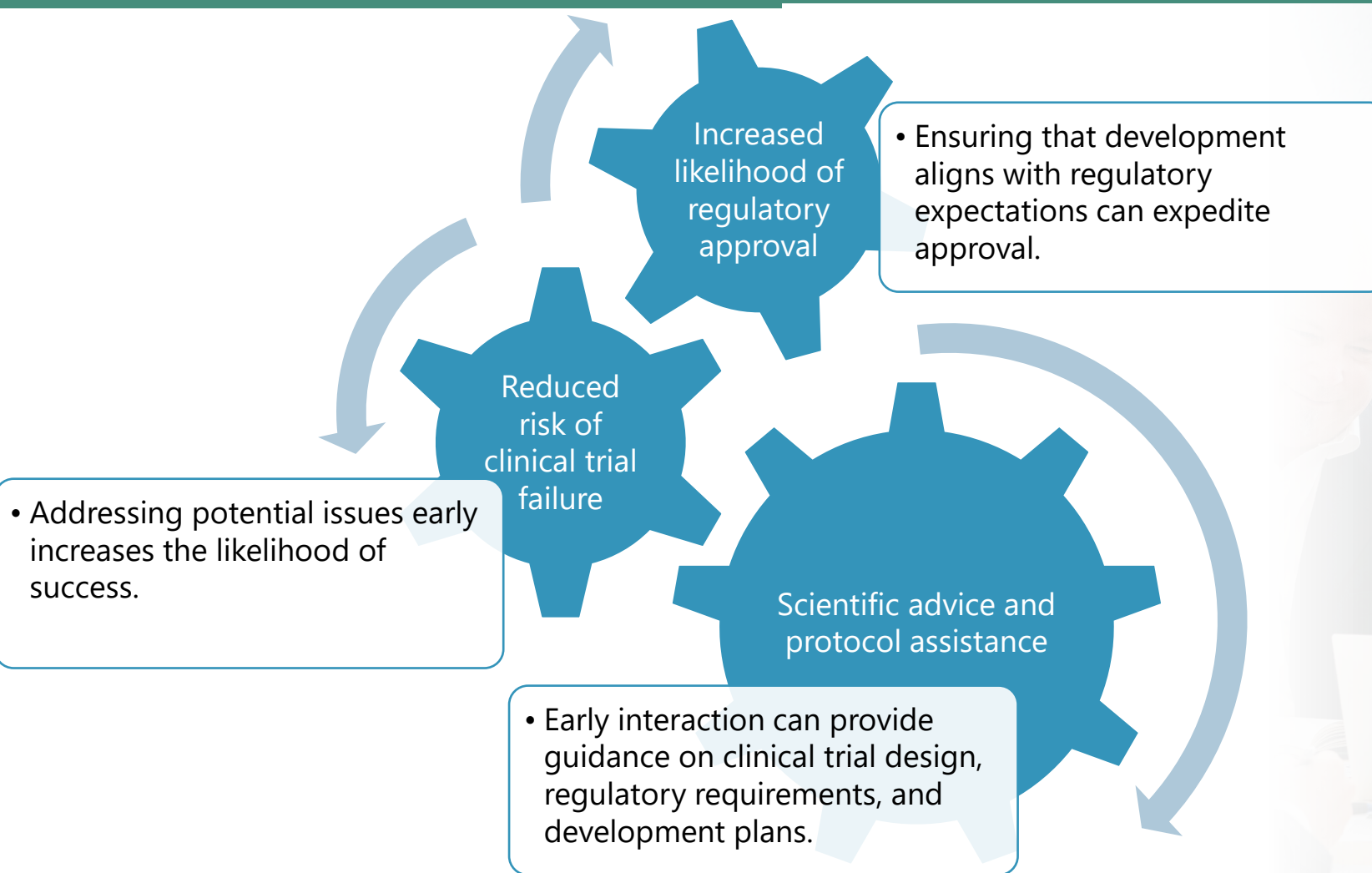
- clear context of use
- assay validation / robustness
- product-specific risk assessment
- conservative clinical risk mitigation
- early scientific advice

Human Recombinant mAbs Against Foreign Antigens — A Stronger Case for Non-Animal FIH Packages

Non-Animal WoE Package for Foreign-Antigen mAbs

Risk question	Non-animal evidence
Does the antibody bind human tissue unexpectedly?	Human tissue cross-reactivity / off-tissue binding
Is there unintended immune activation?	Cytokine release assays; Fc-function characterisation
Is the product sufficiently characterised?	Purity, aggregates, variants, Fc profile, endotoxin/pyrogenicity
Is pharmacology understood?	In vitro antigen binding and neutralisation potency
Is exposure predictable?	PK modelling, prior class knowledge, clinical PK monitoring
Is immunogenicity controlled?	ADA assays and clinical immunogenicity plan
Is FIH risk managed?	Sentinel dosing, low starting dose, slow escalation, stopping rules

Early Engagement with Regulators



Scientific Advice

- ✎ **At any stage of a medicine's development**, a developer can ask guidance and direction from regulatory agencies on the best methods and study designs to generate robust information on how well a medicine works and how safe it is.



Scientific Advice at National Level in Europe



Practical Conclusion: Where Non-Animal FIH Packages Are Most Plausible

Most plausible near-term use case

- Well-characterised human recombinant mAbs
- Directed against **foreign antigens or toxins**
- No intended binding to human tissue targets
- Low-risk Fc design and controlled product quality
- Human-relevant in vitro safety and cross-reactivity package
- Conservative clinical risk mitigation

Regulatory position

- FDA and EMA increasingly support NAMs and WoE approaches.
- Acceptance remains **case-by-case** and depends on context of use.
- Early scientific advice is essential.

17th | International SLS Days

Collaborate to **Innovate:**
AT THE INTERSECTIONS OF
SCIENCE, INNOVATION, AND DEVELOPMENT



September 24-25, 2026



TWINCORE, Hannover



Traditional Get-together
Barbeque at the
TWINCORE Terasse

CONFIRMED SPEAKERS

Ingrid de Visser-Kamerling
Infecta

Ellen Goel
Life Science Factory

Umesh Shaligram
Serum Institute of India

Sven Hammerschmidt
University of Greifswald

Zahra Ghodrati
Dense Immune

Tim Reinhard
Osborne & Clarke

Nina Hobi
Alveolix

Esther Lange
Revvity

Wolfgang Weiß
Weickmann & Weickmann

REGISTRATION IS NOW OPEN



SLS-Days@SLS-EU.com



SLS-EU.com/SLS-Days-2026

For program updates and registration information



Thank you for your attention



SLS Europe GmbH

Ahrensburger Str. 1

30659 Hannover Germany

Phone: +49 511 - 169908 0

Fax: +49 511 - 169908 29

E-Mail: info@sls-eu.com



PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.



Dr. Jessica Perrin



Advisor at the Science Consortium on animal-free approaches to assess the toxicity of pharmaceuticals and medical devices.



PhD in biotechnology from Heidelberg University, Germany

Master's degree in biotechnology from the Mannheim University of Applied Sciences, Germany.



18 years of experience working in the pharmaceutical and biotechnology industries, focusing on the discovery and development of innovative human medicines. (Cellzome and GlaxoSmithKline)

Copyright and Permission: Slides may be viewed and shared. Copying, modification, or reuse of slide content requires permission from the author.

Approaching the development and regulatory review of therapeutic monoclonal antibodies without using animal studies

ESTIV Congress, 1 July 2026

Jessica Perrin, PhD
Regulatory Scientist, Pharmaceuticals and Medical Devices

Outline



1. Introduction

Our work



2. Background

Regulatory landscape of NAMs for pharmaceuticals and antibodies



3. Non-animal preclinical safety strategy

Diphtheria mAb antitoxin



4. Endpoint-focused animal replacement opportunities

Pyrogenicity testing for mAb

Introduction

Our work





Funding



Training, workshops, webinars



Retrospective data reviews



Standards making organizations



Publications and presentations



Regulated products



Agrochemicals



Household Products



Industrial Chemicals



Medical Devices



Nanomaterials



Novel Foods



Pharmaceuticals



Tobacco



Avian Toxicity



Carcinogenicity



Endocrine Disruption



Developmental
Neurotoxicity



Eye Irritation



Fish Toxicity



Pyrogenicity



Inhalation Toxicity



Skin sensitization



Skin Irritation

Regulated endpoints

Background: Regulators encourage submission of non-animal strategies

FDA publishes a roadmap and draft guidance documents



Hoeg et al., Eliminating Unnecessary Animal Testing, 2026

EMA: Building Regulatory Pathways for NAM Acceptance

Broader approach to NAM qualification and regulatory acceptance

Dedicated mechanisms for scientific dialogue



Guideline under revision:



[Concept paper](#)

[Draft reflection paper](#)

Regulatory acceptance of new approach methodologies: how to interact with EMA

EMA works with new approach methodology (NAM) developers to facilitate the **regulatory acceptance** of these methodologies.

There are several types of regulatory interactions that EMA makes available to developers.

Select the expandable panels below for information on scope and outcome of regulatory interactions between EMA and NAM developers:

Expand section

Collapse section

Briefing meetings within EMA's Innovation Task Force (ITF)



Scientific advice



Qualification



Voluntary submission of data



MHRA: Openness to Scientifically Justified Deviations



Medicines & Healthcare products
Regulatory Agency

“[...] the MHRA is open to considering proposals that omit animal studies, if alternative methods address safety.”

Policy paper

Replacing animals in science: A strategy to support the development, validation and uptake of alternative methods

Published 11 November 2025

In Basket 1: Phasing out preclinical animal testing of biologicals where no pharmacologically relevant animal models exist

By the end of 2026 we will define and apply guidance that permits first in human clinical trial submission for biological therapies where no pharmacologically relevant preclinical animal model exists based only on non-animal data.

EURL ECVAM Recommendation on Antibodies

Animals should no longer be used for the development and production of antibodies



“EURL ECVAM recommends that animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and **therapeutic** applications.”

Directive 2010/63/EU

“If a scientifically satisfactory non-animal method exists, it should be used instead of an animal method.”

Non-animal preclinical safety strategy

Diphtheria mAb antitoxin case study

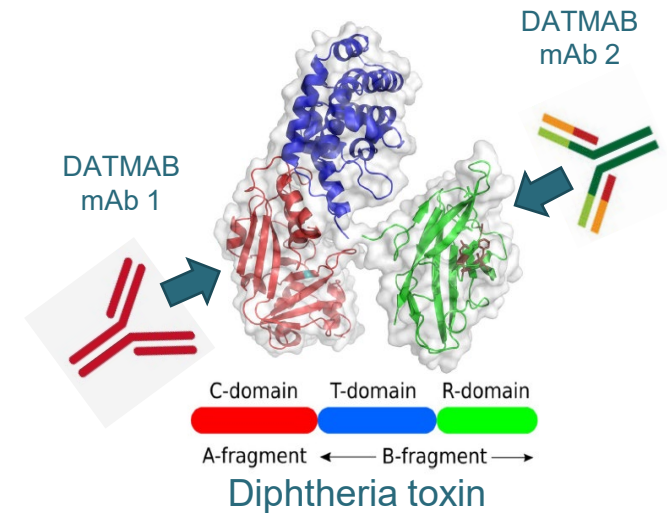
Diphtheria: serious, vaccine preventable disease, toxin mediated clinical illness

Treatment: Equine diphtheria antitoxin (DAT)



DATMAB

**Diphtheria
Antitoxin
Monoclonal
Antibodies**



Proof-of-concept data DATMAB works as well as equine DAT

The questions: How to get more human-relevant DATMAB data?
Is DATMAB safe enough for clinical trials?

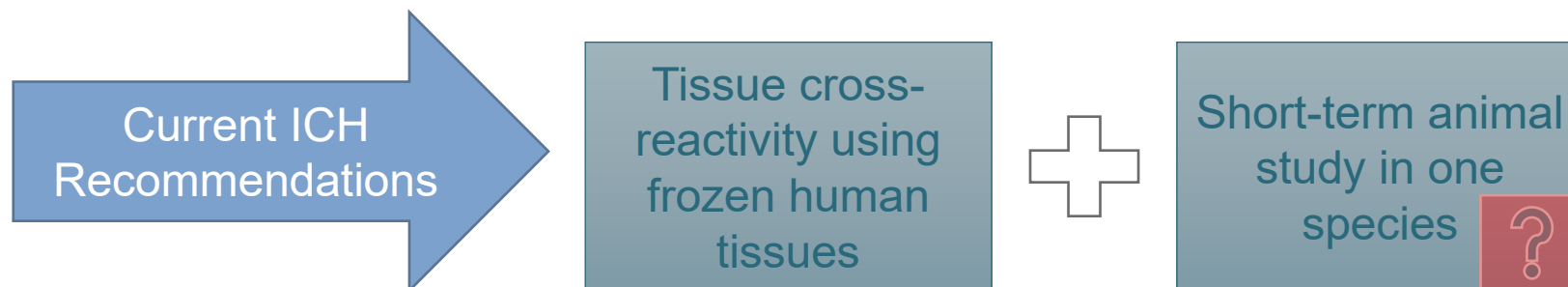
Non-animal preclinical safety strategy

DATMAB as a case study

The answers begin with ICH guidance

Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
S6(R1) 2011

- **DATMAB is in a clear drug class**
“Purified human recombinant mAbs directed against foreign antigens”
- ICH recommends a uniform set of preclinical assessments for compounds in this class



Non-animal preclinical safety strategy

DATMAB as a case study

The answers continue in ICH guidance... sort of...

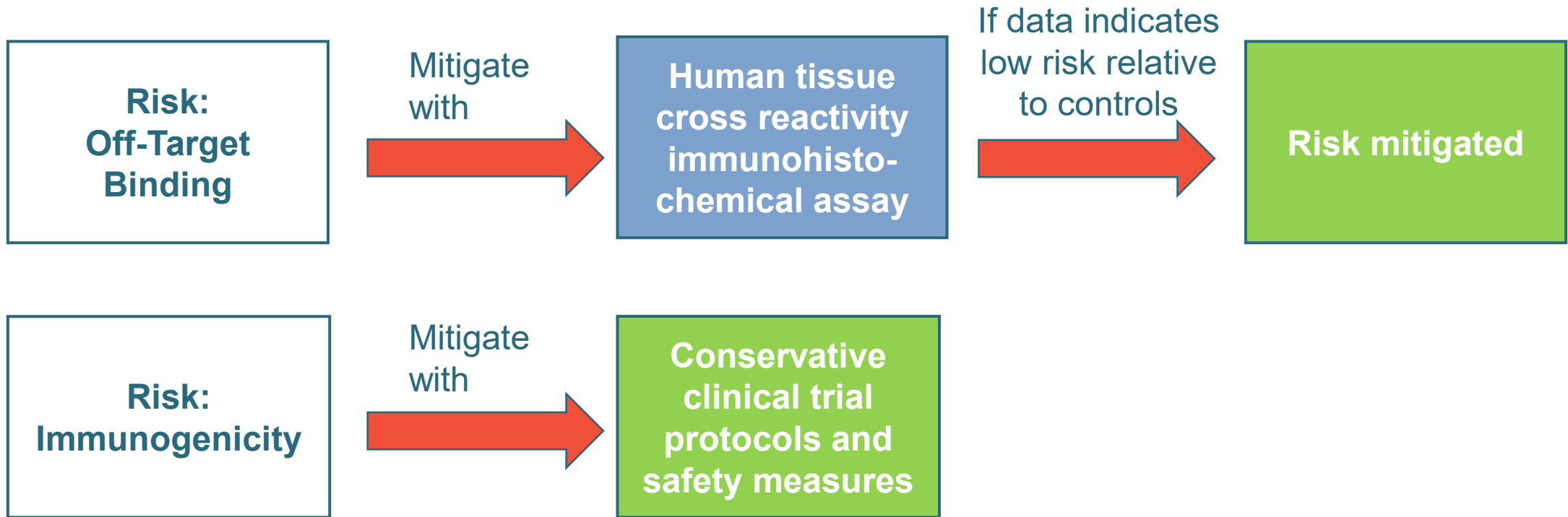
It's not very clear what safety discernment the short term animal study is adding.

Is this an animal study for the sake of an animal study? Let's consider the risks, mitigations:

Potential Risk	Degree of Risk for Target Drug Class	Rationale
Excessive on-target activity (exaggerated pharmacology)	Negligible	Drug within the target class has high specificity / affinity for an antigen foreign to humans
Off-target binding	Low (to be mitigated by preclinical non-animal assay)	Low risk for antibody with high specificity / affinity for non-human target
Genotoxicity, Carcinogenicity, Reproductive Toxicity	Negligible	Testing for these is not recommended by ICH S6(R1) for the target drug class
Toxic metabolite or reactive intermediate	Negligible	Per ICH S6(R1), metabolism of therapeutic proteins is well-understood and gives no safety risk
Immunogenicity	Low (to be fully mitigated by a conservative clinical trial protocol / human safety measures)	Unwanted human immune response is unlikely for a fully human antibody with a non-human target Immunogenicity will be fully investigated in humans during clinical trials

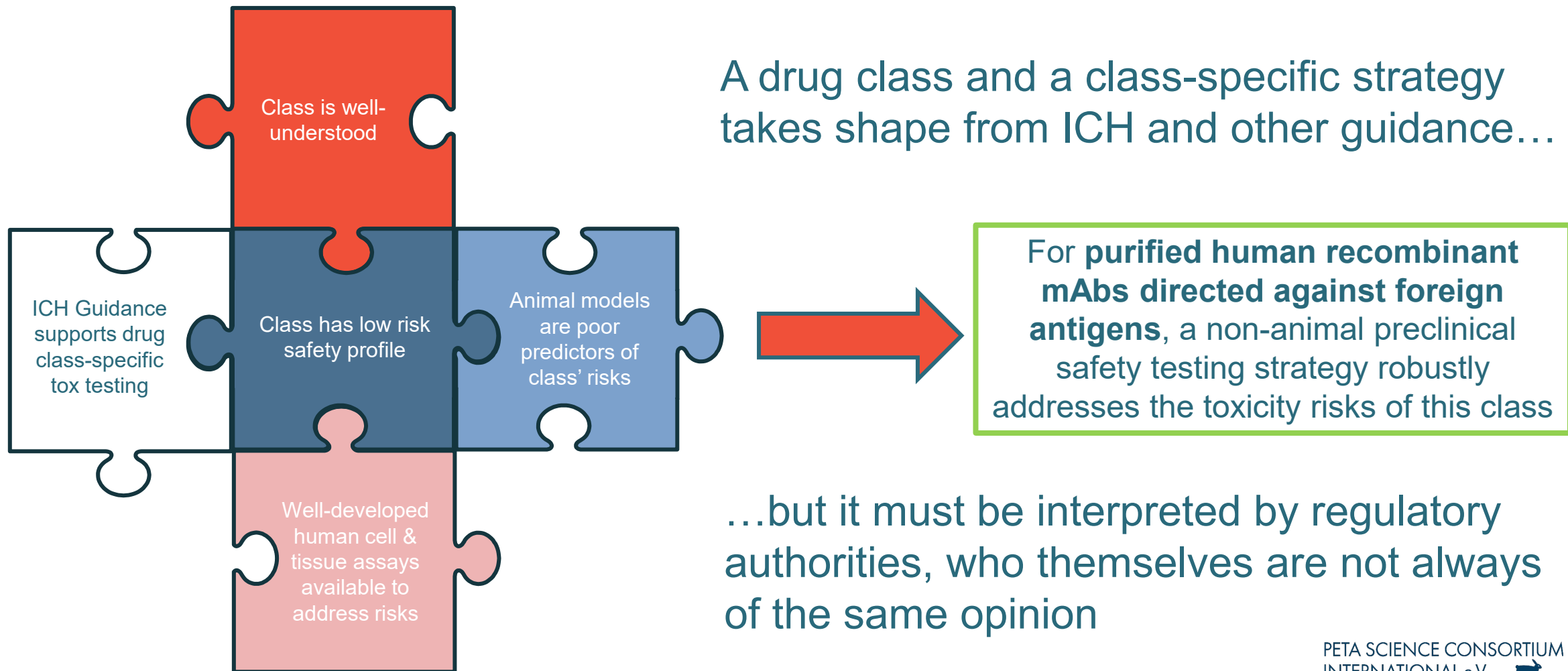
Weight-of-evidence strategy

DATMAB as a case study



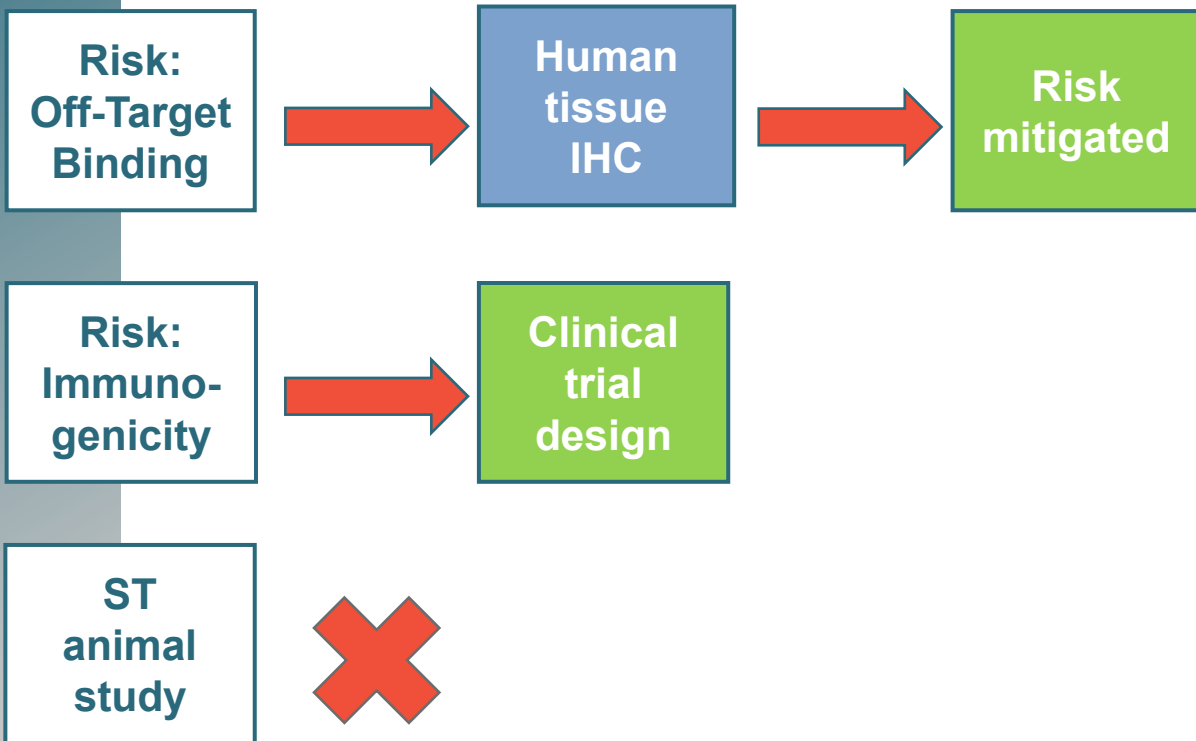
Non-animal preclinical safety strategy

DATMAB as a case study



Non-animal preclinical safety strategy

Build regulatory consensus



FDA Pre-IND Meeting (May 2022)

Approach appears reasonable, agency welcomes further communication prior to IND submission

EMA Scientific Advice (May 2023)

“...animal studies are of limited informative value” for DATMAB, “...could be acceptable to proceed to a FIH clinical trial in the absence of animal toxicity data.”

PEI Scientific Advice (April 2024)

Rationale is acceptable for FIH trials

Extending the strategy

Different drug classes, similar opportunities

Immuno-oncology therapeutic manufacturers and regulators are embracing non-animal preclinical evaluation approaches—**manufacturers bring these strategies to the regulators, and they react.**

FDA, May 29, 2026

An FDA oncology analysis of CD3 bispecific constructs and first-in-human dose selection

Article in *Regulatory Toxicology and Pharmacology* - September 2017

FIH dose selection using animal data is not acceptable,
FIH dose selection based on *in vitro* activity data is an acceptable approach

RESEARCH ARTICLE

An approved *in vitro* approach to preclinical safety and efficacy evaluation of engineered T cell receptor anti-CD3 bispecific (ImmTAC) molecules

“Animal models are not deemed suitable for ImmTAC testing for a number of reasons...”



Front. Immunol., 17 April 2026
Sec. Cancer Immunity and Immunotherapy
Volume 17 - 2026 | <https://doi.org/10.3389/fimmu.2026.1736584>

Translational strategy to support the first-in-human study of a TCR-like T cell bispecific with an *in vitro*-based safety approach

**Oncology Pharmaceuticals:
Streamlined Nonclinical Safety
Studies for Biologics and
Conjugated Products
Guidance for Industry**

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

“...we envision a safety assessment based on alternative or animal-free methods becoming a future standard for most large molecules.”

End-point focused replacement

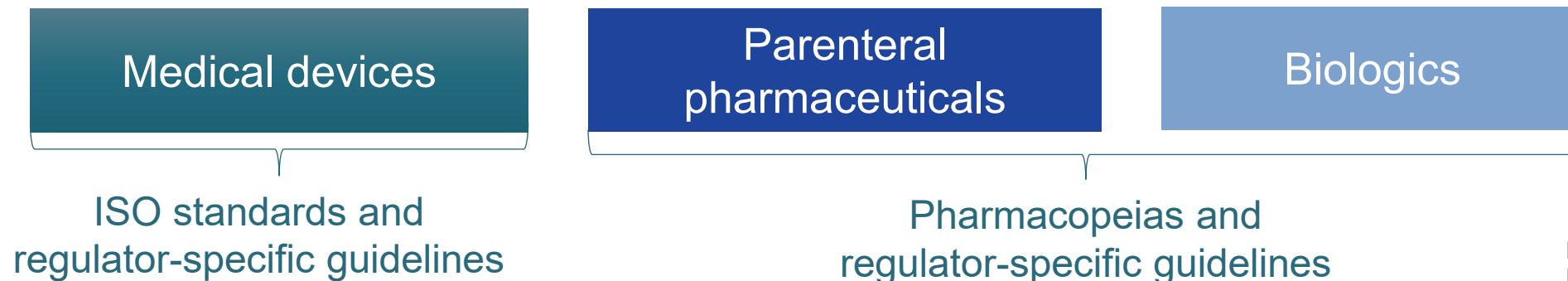
Pyrogenicity testing

Pyrogenicity: capacity of a substance to induce **fever** when introduced into the bloodstream, lymphatic system, or cerebrospinal fluid of an organism.

Pyrogens:

- Endotoxins: lipopolysaccharides derived from gram-negative bacteria
- Non-endotoxin: cytokines, gram-positive bacteria, fungi, yeast, viruses, parasites + non-biological sources

Testing required for many products:



Pyrogenicity tests

edqm

European Directorate for the Quality of Medicines & Health Authorities

COUNCIL OF EUROPE

CONSEIL DE L'EUROPE

2024

European Pharmacopoeia
bids adieu to rabbit pyrogen
test in its monographs

**Rabbit pyrogen test
(RPT)**

animal-based

USP <151>
Eur. Pharm. 2.6.8



**Monocyte activation
test (MAT)**

X
Eur. Pharm. 2.6.30



**Bacterial endotoxin
test (BET)**

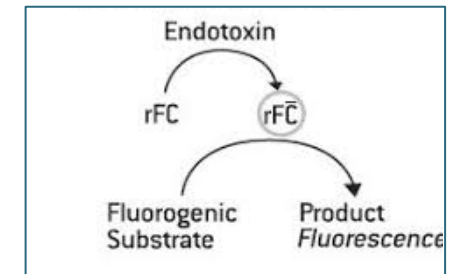
animal-based

USP <85>
Eur. Pharm. 2.6.14



**Recombinant
Factor C (rFC)**

USP <86>
Eur. Pharm. 2.6.32



Food for Thought ...

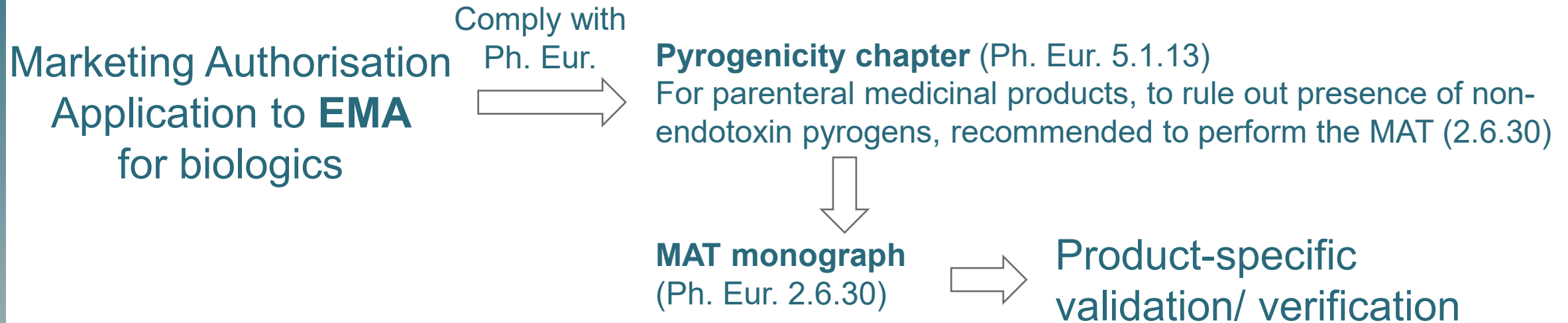
**Pyrogen Testing at a Turning Point –
On Occasion of the 30th Anniversary of the Whole
Blood Monocyte Activation Test**

Thomas Hartung

Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT), Doerenkamp-Zbinden Chair for Evidence-Based Toxicology, Baltimore, MD, USA; CAAT-Europe, University of Konstanz, Konstanz, Germany

= limulus amebocyte
lysate test (LAL)

MAT: Practical experience on product-specific verification



Practical Experience from Product Sponsors

Research Article

Validation of the Monocyte Activation Test with Three Therapeutic Monoclonal Antibodies

Ruth Daniels¹, Wim Van der Elst², Nele Dieltjens¹, Tinne Appels¹, Chi K. So⁴, Thomas Nys³, Liesbeth Voeten¹, Philip Breugelmans¹, Marijke W. A. Molenaar-de Backer⁵, Eelo Gitz⁶, Stephen Poole⁷ and Mehul Patel⁴

¹Microbiology CoE, Janssen R&D BE, Beerse, Belgium; ²Nonclinical Statistics, Janssen R&D BE, Beerse, Belgium; ³Discovery Biology, Janssen R&D BE, Beerse, Belgium; ⁴Large Molecule Analytical Development, Janssen R&D US, Malvern, PA, US; ⁵Sanquin Diagnostics, Dept Virology and MAT services, Amsterdam, The Netherlands; ⁶Sanquin Reagents, Amsterdam, The Netherlands; ⁷Independent consultant, London, United Kingdom

[Daniels et al., Altex, 2022](#)

~~Method validation~~

Product-specific validation of 3 monoclonal antibodies, including a successful product license application to the EMA

MAT: Validation expectations differs between regulators

Biologicals Licence
Application to
FDA CBER



Guidance for Industry
Pyrogen and Endotoxins Testing:
Questions and Answers
(Edition 2)

MAT is not a USP
compendial method



MAT is an alternative method



Method validation?

*shown to achieve
equivalent or better results*



Product-specific
validation/ verification

MAT: Method validation without additional RPT with FDA



Article

Validation of the Monocyte Activation Test Demonstrating Equivalence to the Rabbit Pyrogen Test

Luisa Burgmaier ^{1,2,†}, Jonas van den Berg ^{3,†}, Maria Gajewi ¹, Ruth Röder ¹, Johannes Reich ^{1,*}  and Sven M. Deutschmann ⁴

Method validation

- Product independent
- Comparison MAT and RPT, based on existing, published RPT data (requested by the FDA)
- *“The sensitivity of the HaemoMAT® kit for both endotoxins and NEPs is concluded to be at least non-inferior, if not superior, when compared to the RTP.”*

Product-specific verification

On 3 batches of pilot product

- Test for interfering factors
- Batch-to-batch comparability
- Interference in the detection system



Method validation and release test results approved by the FDA



rFC: From first acceptance by the FDA to USP chapter

Nov 2018 **News Release**

FDA Approves First Drug Using the Recombinant Factor C Assay for Endotoxin Testing

Emgality™ (Eli Lilly, Galcanezumab): mAb for the prevention of migraine in adults

Nov 2024 USP publishes Chapter <86> *Bacterial Endotoxins Test Using Recombinant Reagents*



“Please note that regulatory authorities may require supplemental data prior to acceptance.”

Mar 2026



Guidance for Industry **Pyrogen and Endotoxins Testing:** **Questions and Answers** **(Edition 2)**

“Alternate bacterial endotoxin test (BET)”

Alternative procedures should be validated as described in USP <1225>

Conclusions

- Regulators increasingly support NAM-based submissions.
 - DATMAB demonstrates a regulatory-acceptable animal free development strategy.
 - Pyrogen testing is an immediate opportunity for animal replacement for pharmaceuticals.
-
- The industry needs to pioneer the proposed interaction format and recent guidelines, even though in draft form: **Examples help build trust.**
 - Contact us if you want to explore non-animal approaches in a regulatory context.

Thank you for your attention

Jessica Perrin, PhD
JPerrin@thePSCI.eu

www.thePSCI.eu

Subscribe to our email distribution
list at www.thepsci.eu/email-list





Dr. Esther Wenzel

„The future of antibodies is animal-free“

- Co-Founder and CSO/COO at Abcalis GmbH
- PhD in biotechnology from Technische Universität Braunschweig
- 12 years of experience working with animal-free antibody development and translating recombinant antibody technologies into practical solutions for research & diagnostics
- Leads a laboratory that is free of animal-derived products across all departments
- Received multiple awards (selection):
 - 1st ECEAE prize for Animal-free Antibodies
 - Lower Saxony Innovation Award
 - The NIH Replication Prize 2026



Winner Team - NIH Replication Prize 2026

TEAM WINNER | Antibody Team
Team Members: Valérie Allamand · Jeffery Bajramovic · Hannah Cable · Pierre Cosson · Doris Lou Demy · Stefan Dübel · Mark Fowler · Katherine Groff · Michael Hust · Ravindran Kumaran · Jens Kurreck · Raphael Munoz Ruiz · Samira Rafiq · Ouarda Saib · Esther Wenzel · Kilian Zikens



23RD INTERNATIONAL CONGRESS

ESTIV

MAASTRICHT 2026

29 JUNE - 2 JULY



Copyright and Permission: Slides may be viewed and shared. Copying, modification, or reuse of slide content requires permission from the author.

**BEYOND THE SADDLE:
FULLY HUMAN ANTIBODIES AGAINST DIPHTHERIA TOXIN
FROM ANIMAL-FREE DISCOVERY PLATFORMS AND OTHER EXAMPLES**

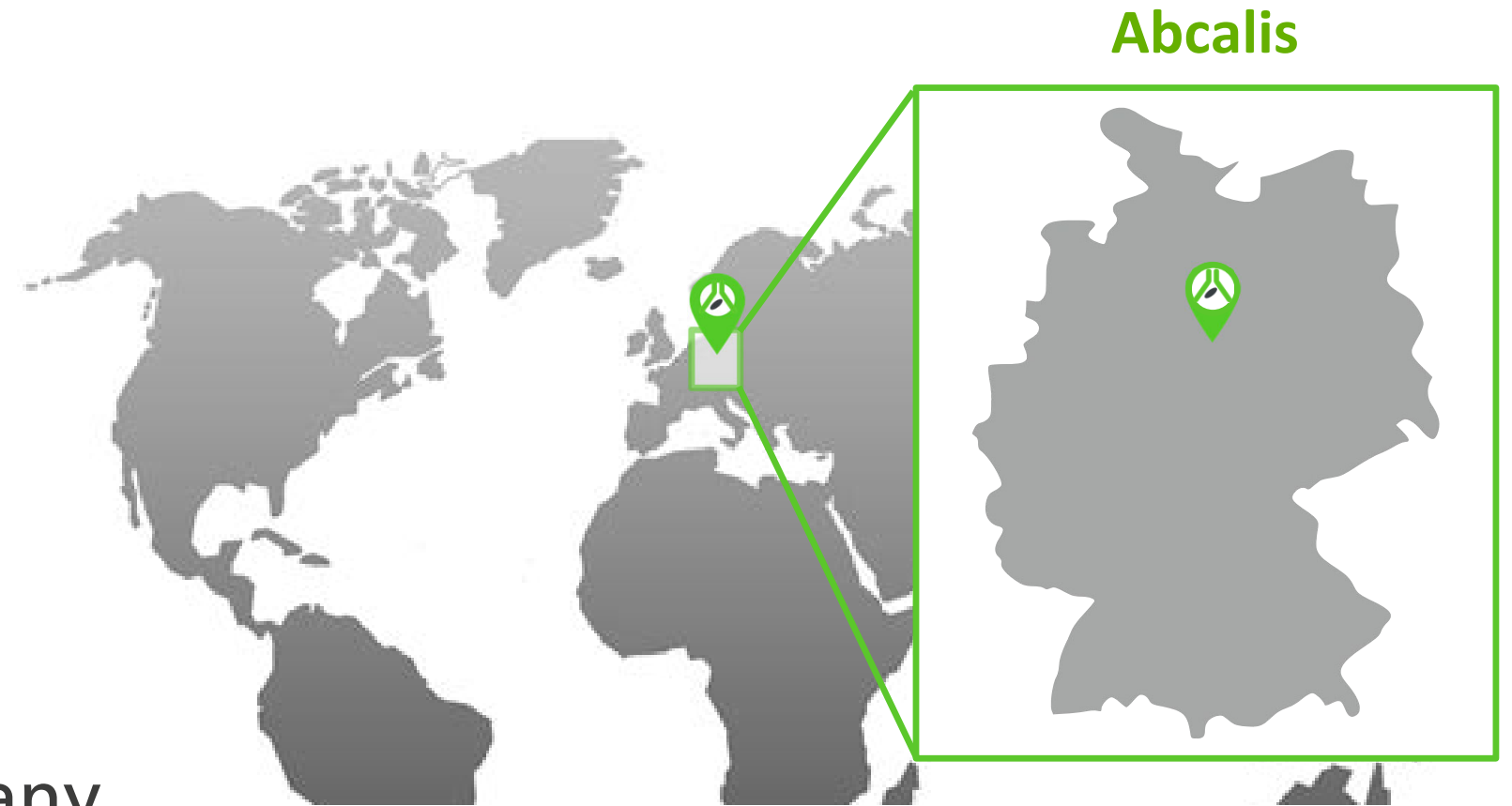
www.abcalis.com

Dr. Esther Wenzel



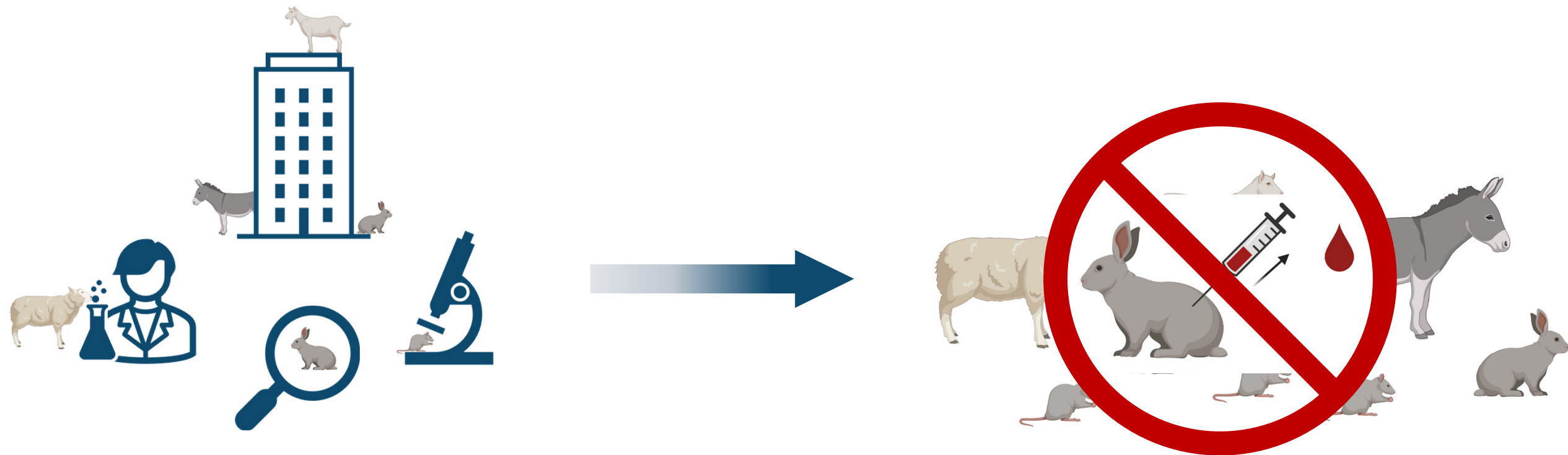
Abcalis - Animal-free antibodies for diagnostic and research

- **Abcalis** founded in 2019
- Based in **Braunschweig - Germany**
 - Between Hanover and Berlin
- **Animal-free antibody service** company
 - Animal-free catalogue antibodies
 - Animal-free antibody discovery service



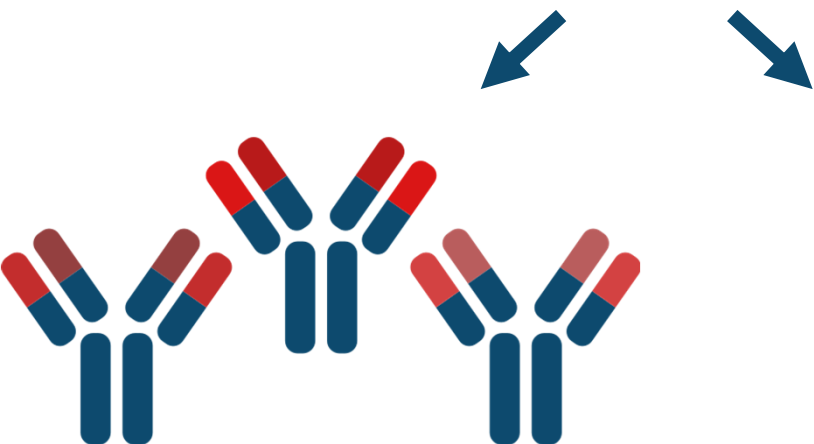
Vision

A world without the use of laboratory animals

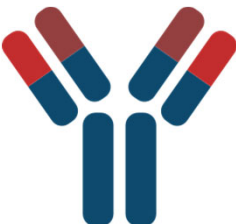


Production of antibodies

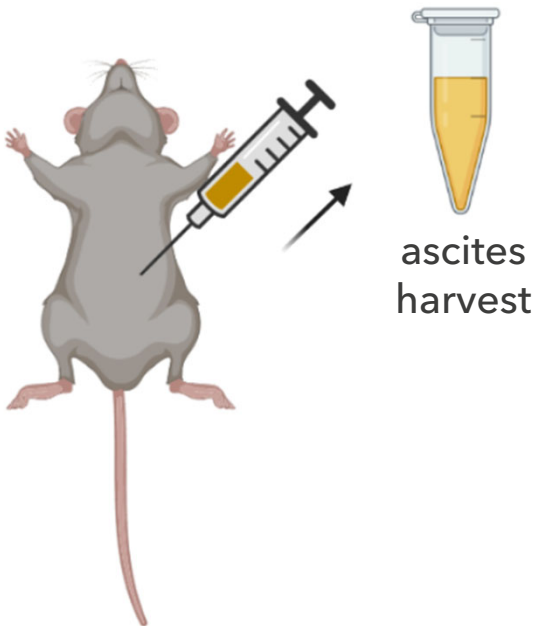
animal source



polyclonal

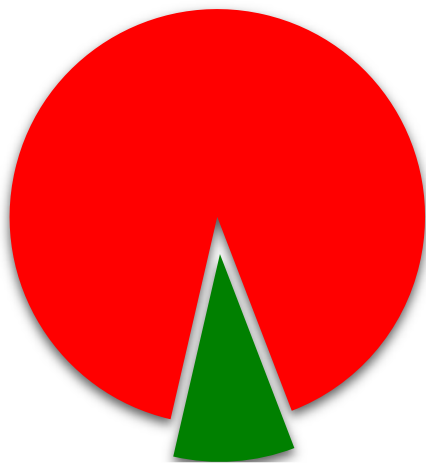


monoclonal



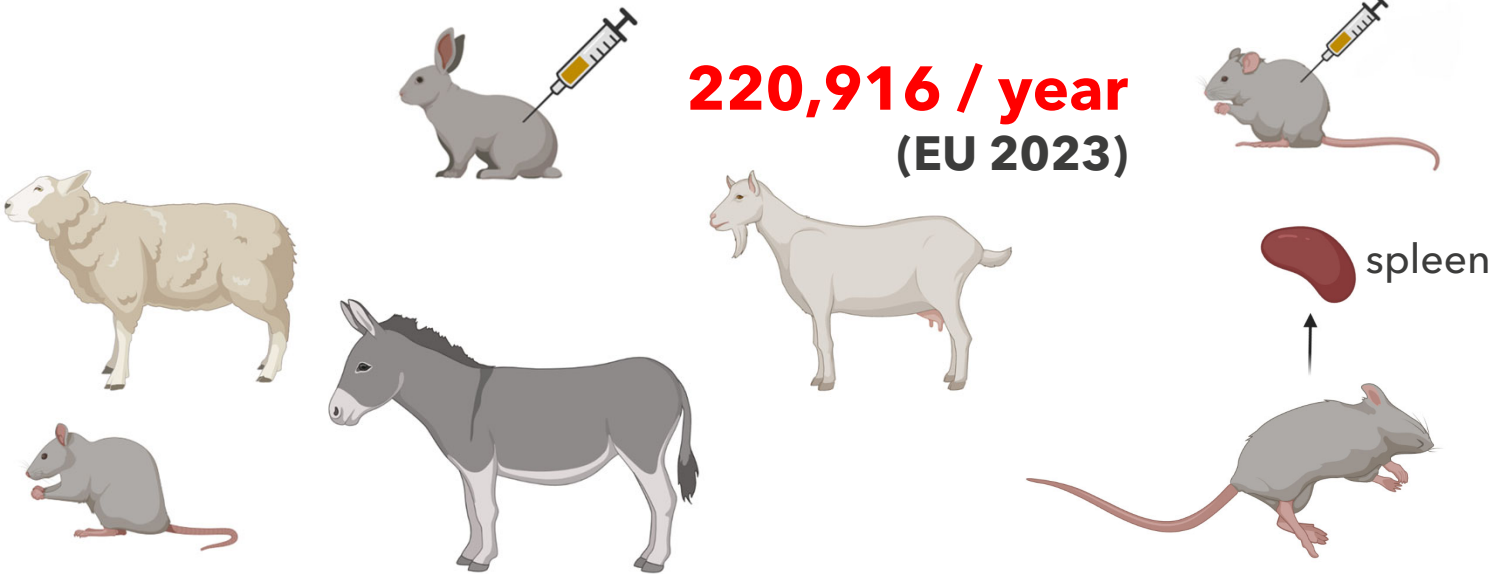
ascites
harvest

Total use of animals
EU (2023)
8.07 Million



Animals used
for antibody
production

**Replacement
is technically
possible**



220,916 / year
(EU 2023)

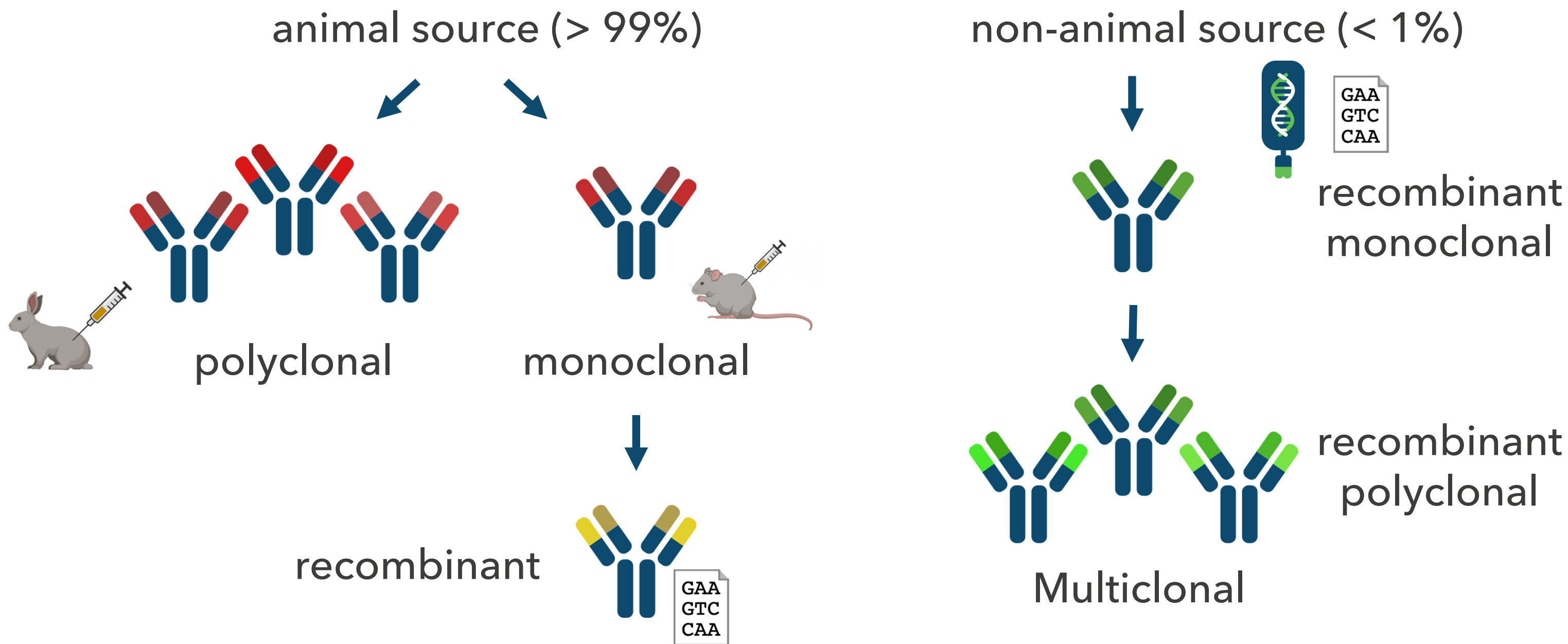
35,981 / year
(EU 2023)

**For regulatory use and
Routine Production**
**+ X for individual research
antibodies e.g. from academia**

EU Statistics Database on the Use of Animals for Scientific Purposes Under Directive 2010/63/EU. Section 2: Details of all uses of animals for research, testing, routine production and education and training purposes in the EU. https://webgate.ec.europa.eu/envdataportal/content/alures/section2_number-of-uses.html

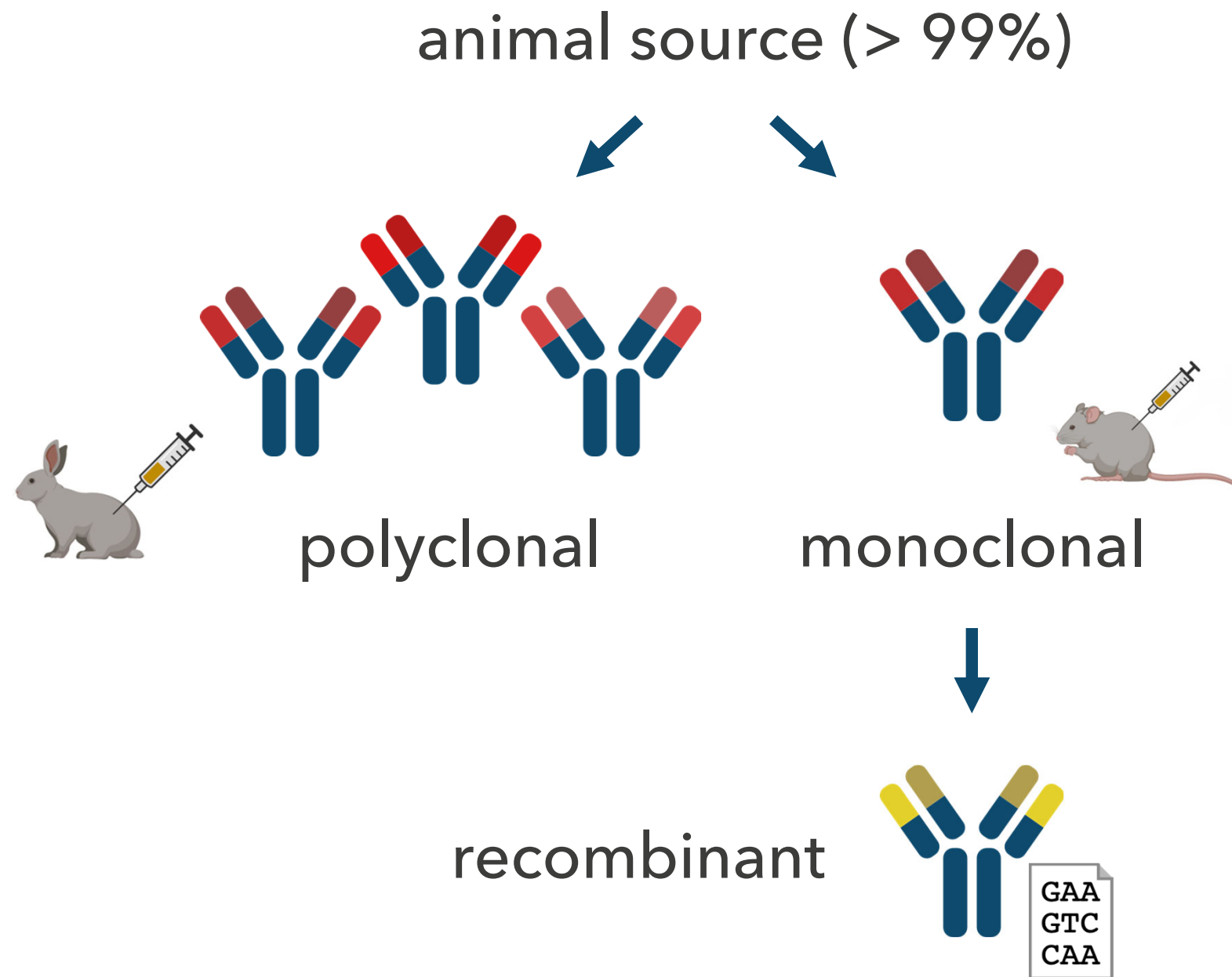
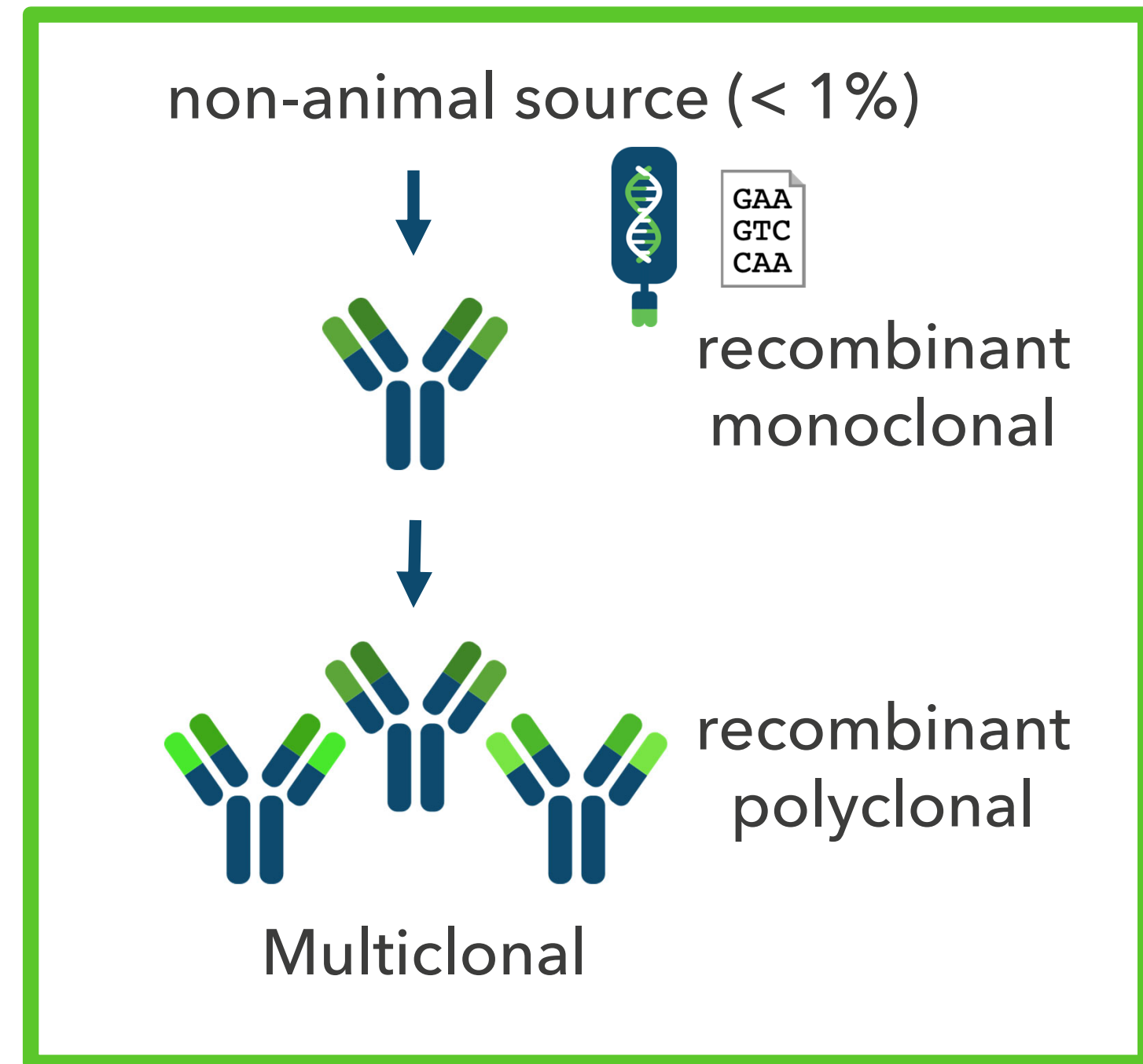
Barroso, J., M. Halder, and M. Whelan. EURL ECVAM recommendation on non-animal-derived antibodies (Publications Office of the European Union, 2020).

Off-the-shelf research/ diagnostics antibodies - Shares on the market



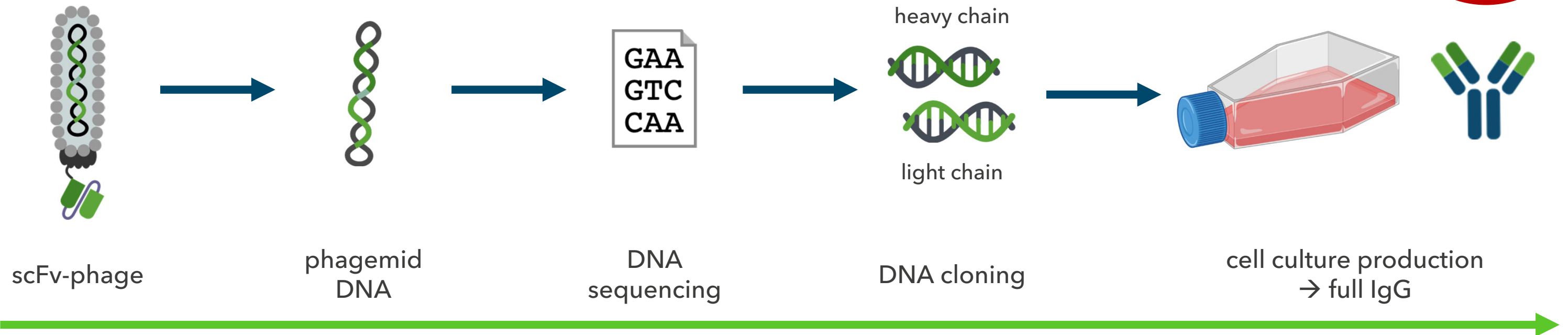
non-animal derived antibodies → **NADA**

Off-the-shelf research/ diagnostics antibodies - Shares on the market



non-animal derived antibodies → **NADA**

antibody phage display - **animal-free** antibody selection



animal-component free

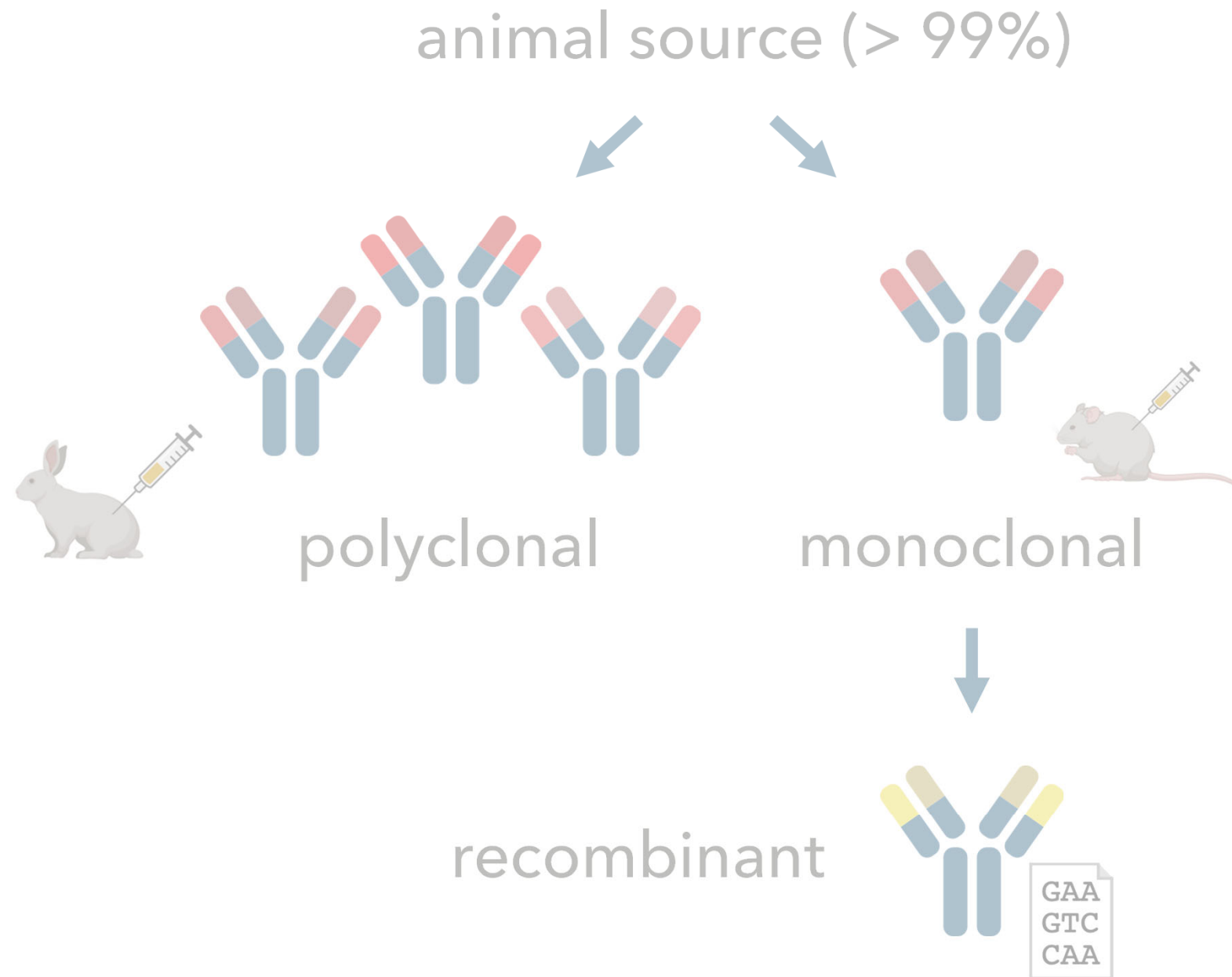
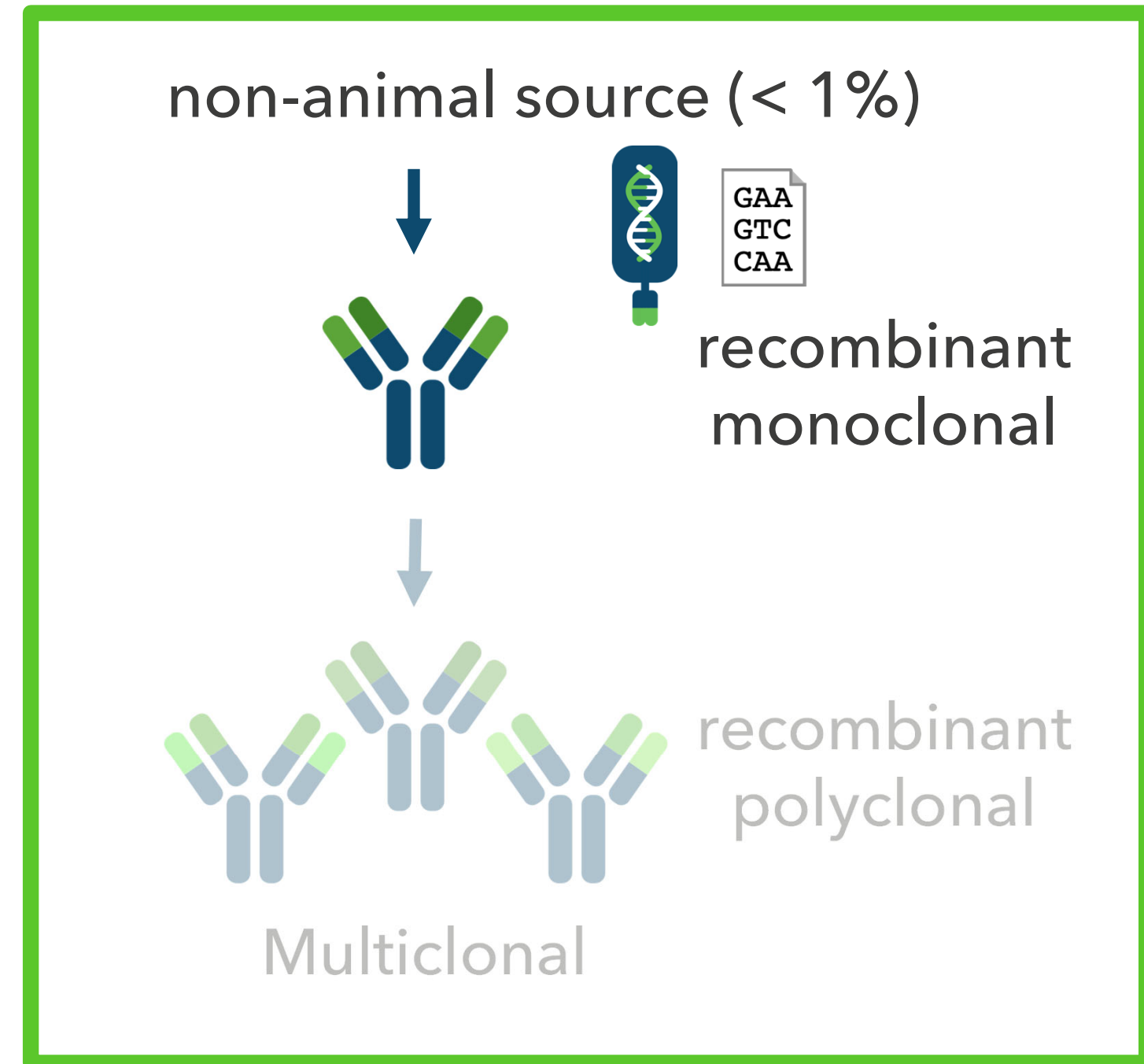
- ✓ Blocking reagents (no BSA or Skim Milk)
- ✓ Enzymes (no animal-extracted enzymes or BSA containing reagents)
- ✓ Cell culture medium (no FCS/ FBS)
- ✓ Stabilizers (no BSA or other animal-extracted proteins)

EXAMPLES

Animal-free antibodies from phage display



Animal-free monoclonal/ primary antibodies

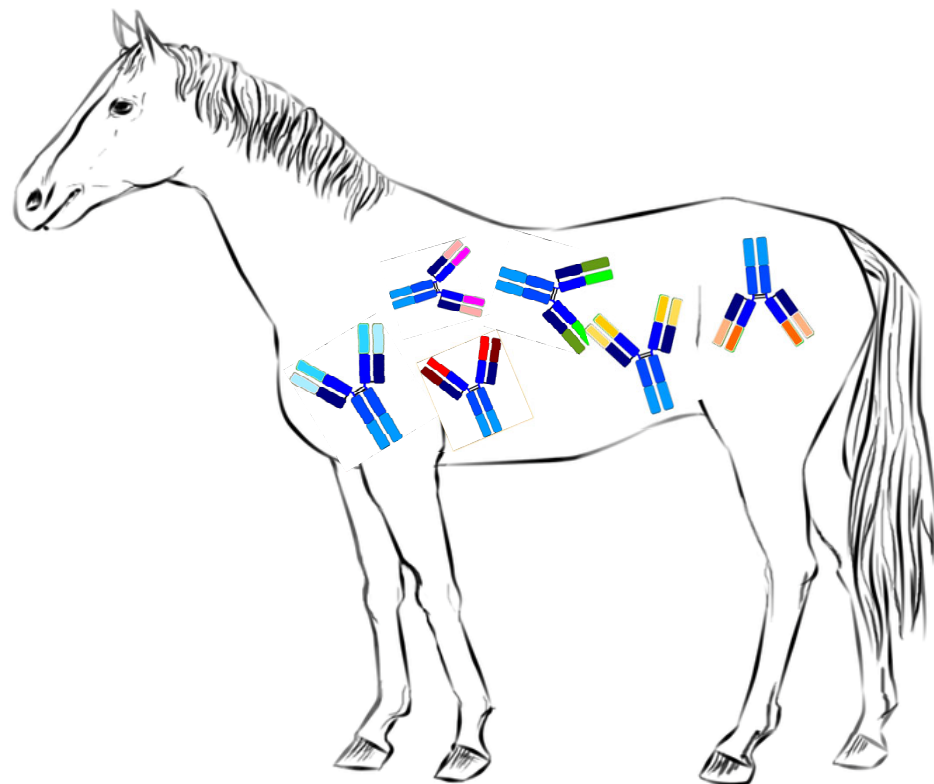


non-animal derived antibodies → **NADA**

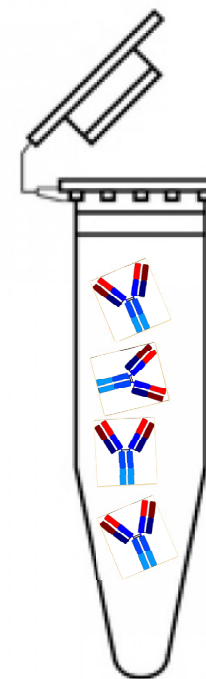
Anti-Diphtheria toxin



Development of a human diphtheria anti-toxin antibody product



**Horse Polyclonal Antibodies
(equine serum)**



**Human Monoclonal antibody
(recombinant)**



adapted from Wenzel, et al. (2020) Sci. Rep. 10, 571

Anti-Diphtheria toxin



INFECTIOUS DISEASES

Life-saving diphtheria drug is running out

Two children's deaths in Europe spur search for new sources of antitoxin

By Kai Kupferschmidt

When a little girl was transferred to the university hospital in Antwerp, Belgium, on 10 March 2016 with severe tonsillitis, doctors suspected a very unusual cause: diphtheria. Once a scourge known as “the strangling angel of children” which killed hundreds of thousands annually, diphtheria is almost unheard of today in Europe thanks to a safe and effective vaccine. But the 5-year-old, whose family was from Chechnya, was unvaccinated and had a hallmark symptom: a dense, gray layer of dead cells and bacteria in the throat known as a pseudomembrane.

In years past, she would have been given an antiserum that neutralizes the deadly toxin produced by *Corynebacterium diphtheriae*, in addition to antibiotics. But no antiserum was available in Belgium, and it was scarce across Europe. On 15 March, when a national reference lab confirmed the doctors’ presumptive diagnosis, they were still empty-handed. On 16 March, af-



A horse is bled to produce diphtheria antiserum in this 19th century illustration. The method is still in use today.

toxin in cell culture, which could help shore up production and improve quality. In November 2016, People for the Ethical Treatment of Animals (PETA) announced it will fund such work by Michael Hust, a researcher at the Braunschweig University of Technology in Germany, in hopes of ending the use of horses “as living factories.” In the United States, nonprofit vaccine manufacturer MassBiologics is working on a similar project.

Diphtheria, transmitted primarily through coughing, usually starts with a sore throat and fever, but often develops into a severe infection that can block the airways. “The children simply cannot breathe anymore,” says Anja Takla, a researcher at the Robert Koch Institute in

toxin has plummeted. Many production facilities have shuttered; just in the past few years, companies in Australia, Poland, and Switzerland have ceased production. Until recently, many European countries relied on the Institute of Immunology in Croatia for antitoxin, but production there has stopped as well, and vials have largely expired. Now, BulBio in Bulgaria is the only remaining European producer, but “they do not currently have stock to share,” an ECDC risk assessment last year noted.

The antitoxin is also still produced in Russia, India, and Thailand, but production

“It’s sad, something like this can happen today in the world.”
Michael Hust
University of Technology

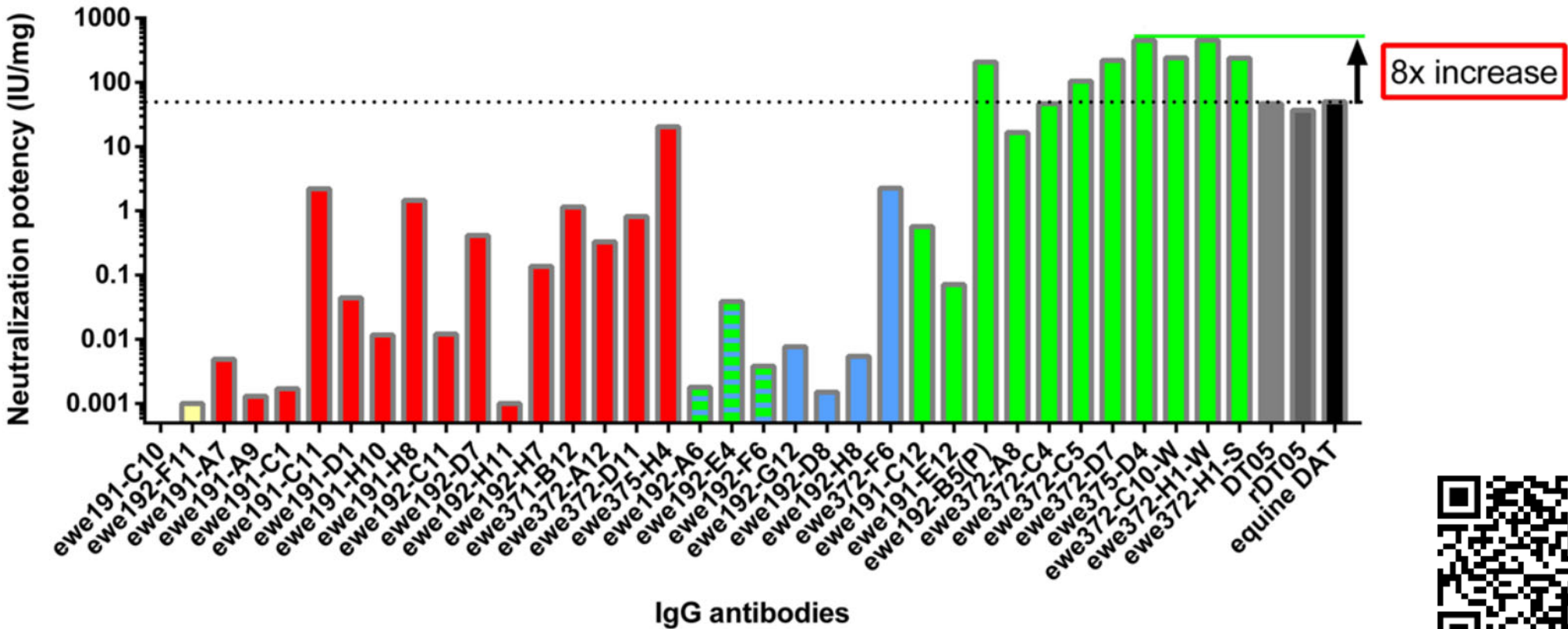


de.wikipedia.org

in vitro neutralization

SCIENCE,
13. Jan. 2017

Neutralization potency of IgG antibodies expressed as IU/mg determined by **Vero cell neutralization assay** with a toxin dose level of 4x MCD



adapted from Wenzel, et al. (2020) Sci. Rep. 10, 571

Anti-Diphtheria toxin



INFECTIOUS DISEASES
Life-saving diphtheria drug is running out
Two children's deaths in Europe spur search for new sources of antitoxin
By Kai Kupferschmidt

When a little girl was transferred to the university hospital in Antwerp, Belgium, on 10 March 2015 with severe tonsillitis, doctors suspected a very unusual cause: diphtheria. Once a scourge known as “the strangling angel of children” which killed hundreds of thousands annually, diphtheria is almost unheard of today in Europe thanks to a safe and effective vaccine. But the 5-year-old, whose family was from Chechnya, was unvaccinated and had a hallmark symptom: a dense, gray layer of dead cells and bacteria in the throat known as a pseudomembrane. In years past, she would have been given an antiserum that neutralizes the deadly toxin produced by *Corynebacterium diphtheriae*, in addition to antibiotics. But no antiserum was available in Belgium, and it was scarce across Europe. On 15 March, when a national reference lab confirmed the doctors’ presumptive diagnosis, they were still empty-handed. On 16 March, af-



A horse is bled to produce diphtheria antiserum in this 19th century illustration. The method is still in use today.

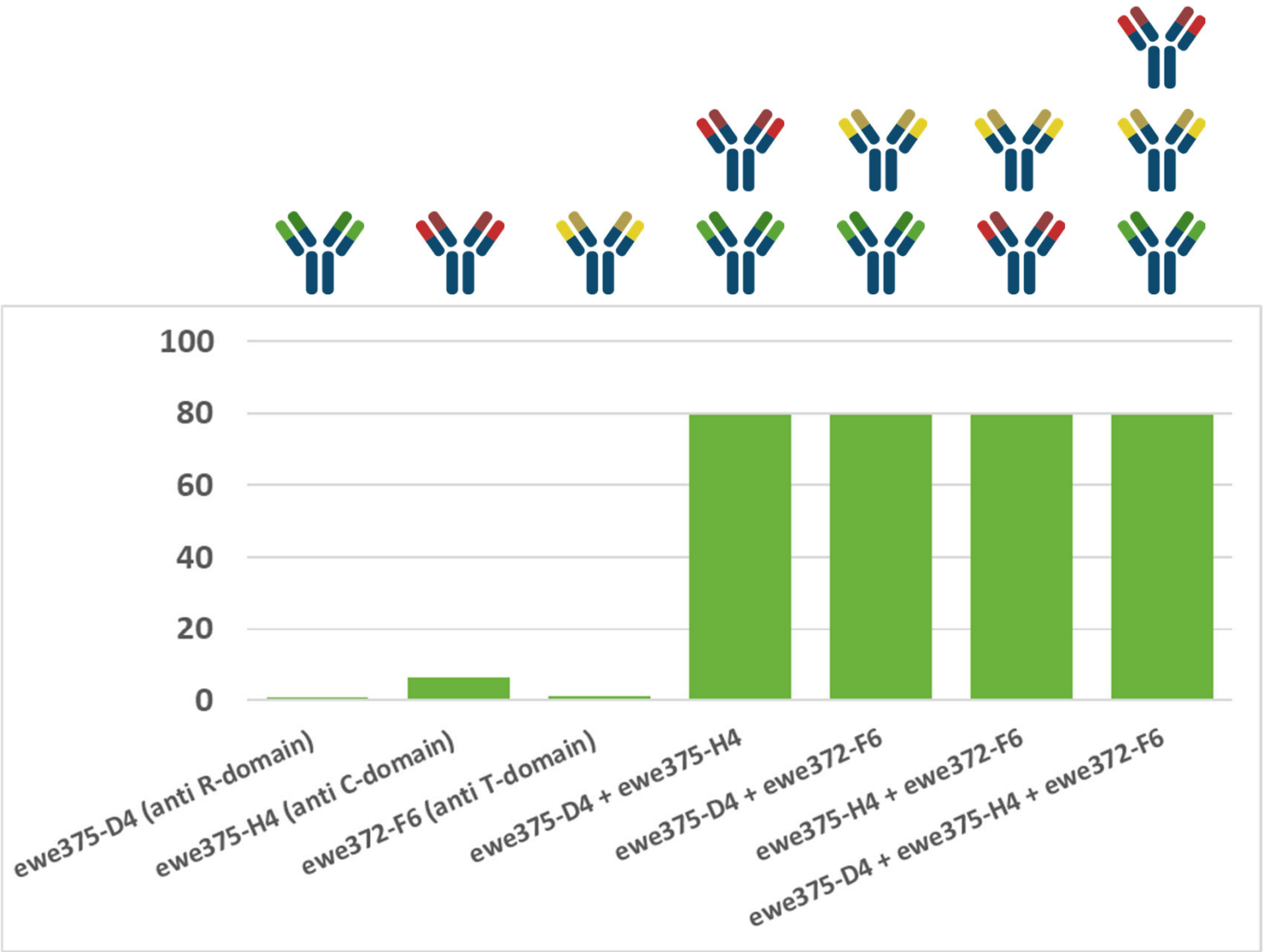
toxin in cell culture, which could help shore up production and improve quality. In November 2016, People for the Ethical Treatment of Animals (PETA) announced it will fund such work by Michael Hust, a researcher at the Braunschweig University of Technology in Germany, in hopes of ending the use of horses “as living factories.” In the United States, nonprofit vaccine manufacturer MassBiologics is working on a similar project. Diphtheria, transmitted primarily through coughing, usually starts with a sore throat and fever, but often develops into a severe infection that can block the airways. “The children simply cannot breathe anymore,” says Anja Takla, a researcher at the Robert Koch Institute in Berlin. “It’s sad, something this can happen today in Germany.” Michael Hust, University of Technology in Braunschweig



de.wikipedia.org

SCIENCE,
13. Jan. 2017

in vivo neutralisation [IU/mg]



Multiclonal concept

in vivo neutralization

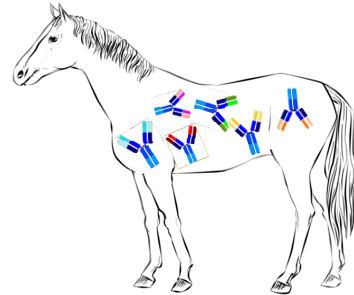


non-lethal guinea pig intradermal challenge assay (at Lr/100 toxin dose level)



adapted from Wenzel, et al. (2020) Sci. Rep. 10, 571

Anti-Diphtheria toxin



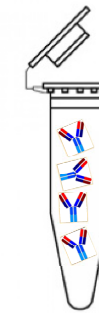
Horse Polyclonal Antibodies (equine serum)

- Disadvantage: 
 - Serum sickness
 - Stability of serum
 - Quality and variations of batches
 - Production in horses + animal welfare rules

Summary



NIBSC
Confidence in Biological Medicines



Human Monoclonal antibody (recombinant)

- Solution: 
 - Human product
 - Lyophilized, stable IgG
 - Recombinant, defined Product
 - Production in cell culture



adapted from Wenzel, et al. (2020) Sci. Rep. 10, 571

Blockbuster therapeutics

- **14** animal-free phage display-derived therapeutic antibodies are **approved** (Jul. 2022)
- **53** animal free phage display-derived therapeutic antibodies are **in clinical trials** (Aug. 2020)



(Atezolizumab, Avelumab, Belimumab, Necitumumab, Ramucirumab, Guselkumab, Lanadelumab, Emapalumab, Raxibacumab)

Shim H. Curr Pharm Des. 2016, 22:6538-59
Alfaleh et al., 2020, doi: 10.3389/fimmu.2020.01986

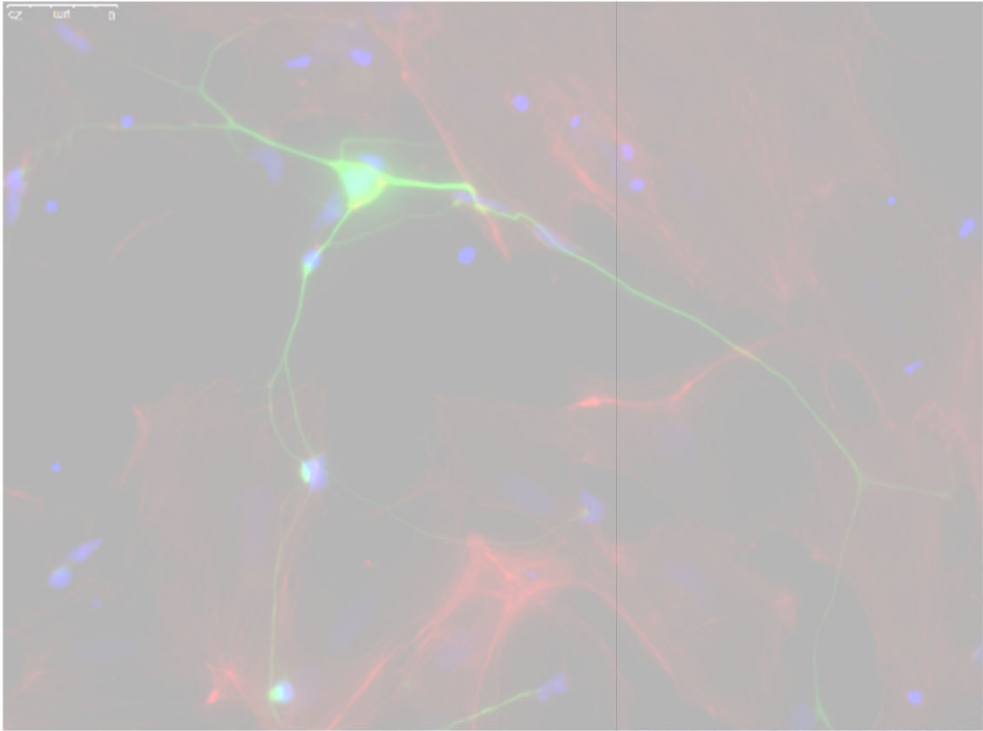
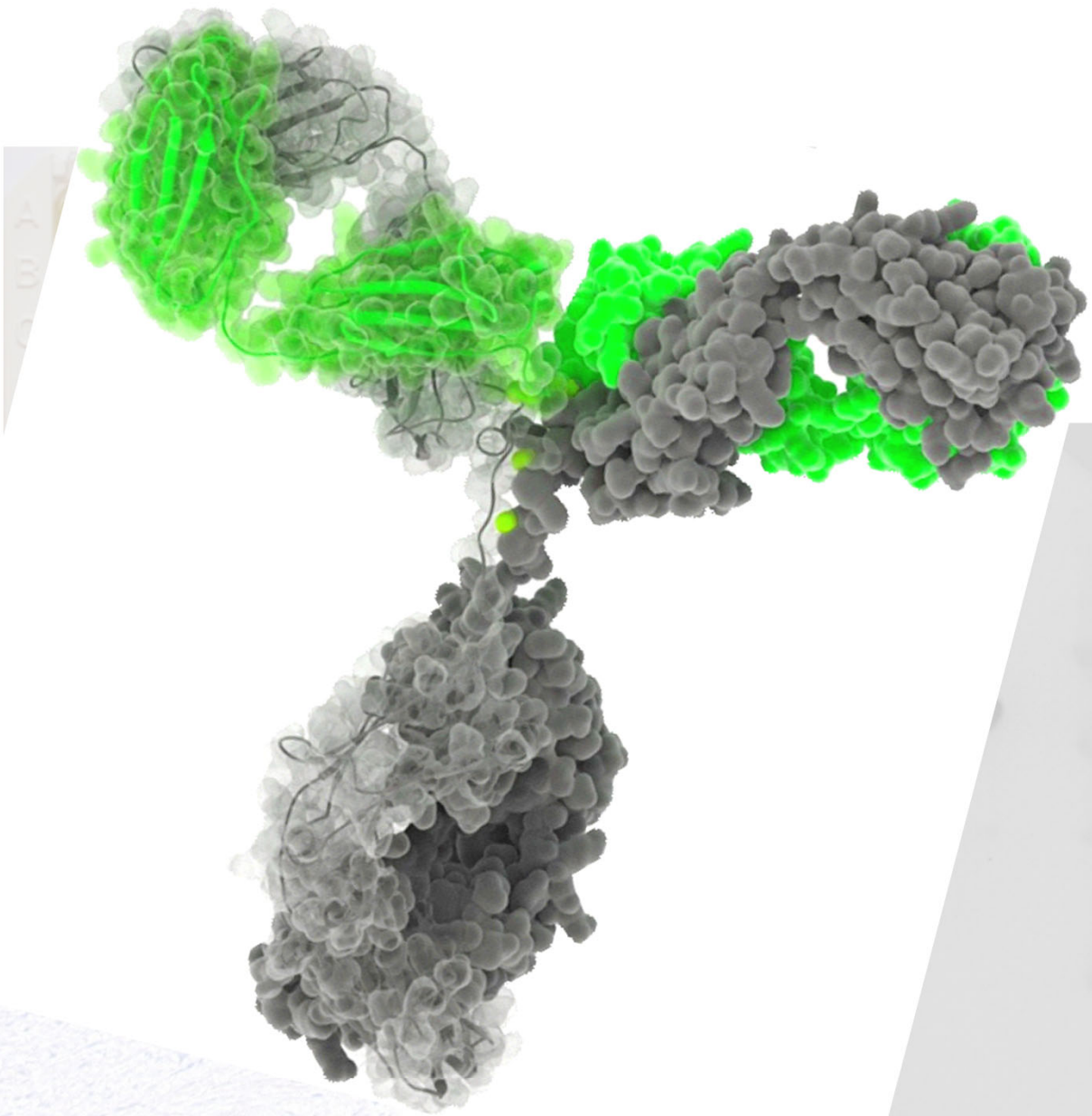
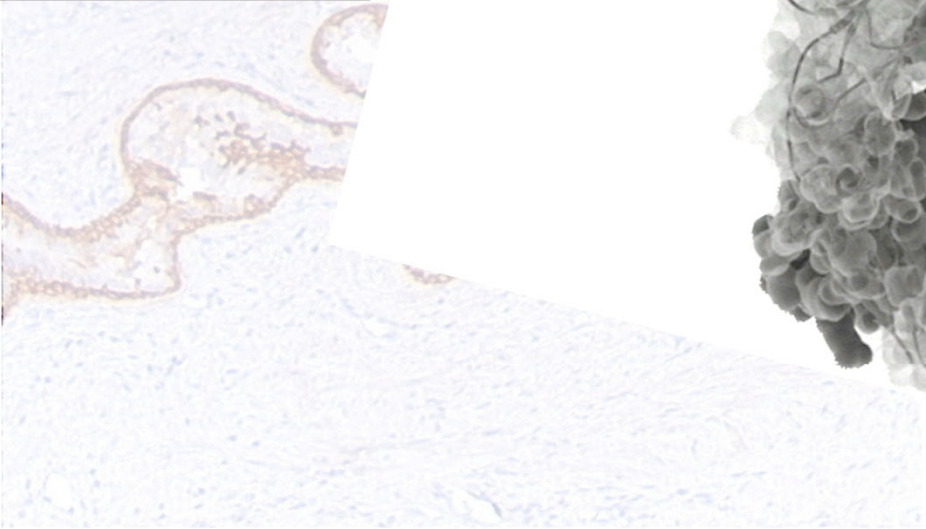
Fact:

- **Non-animal derived antibodies** have the **largest market share** in the segment that requires the **highest antibody quality** → Therapy

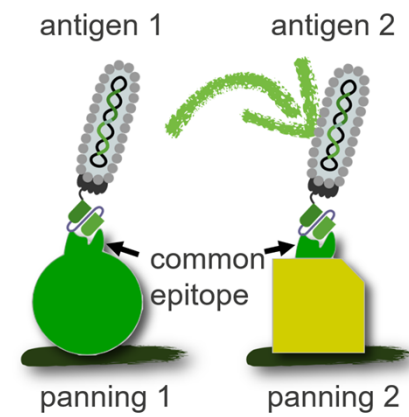
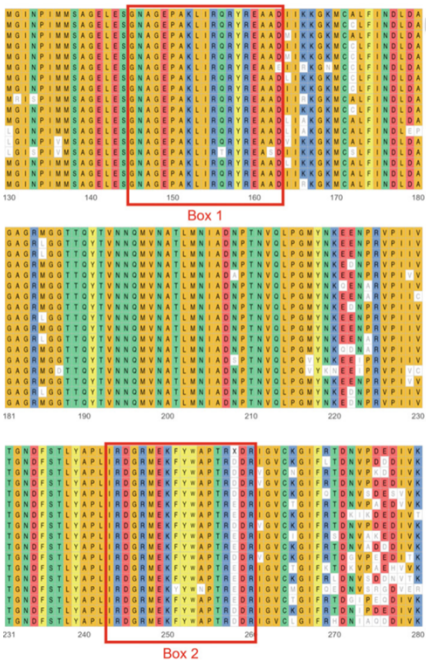
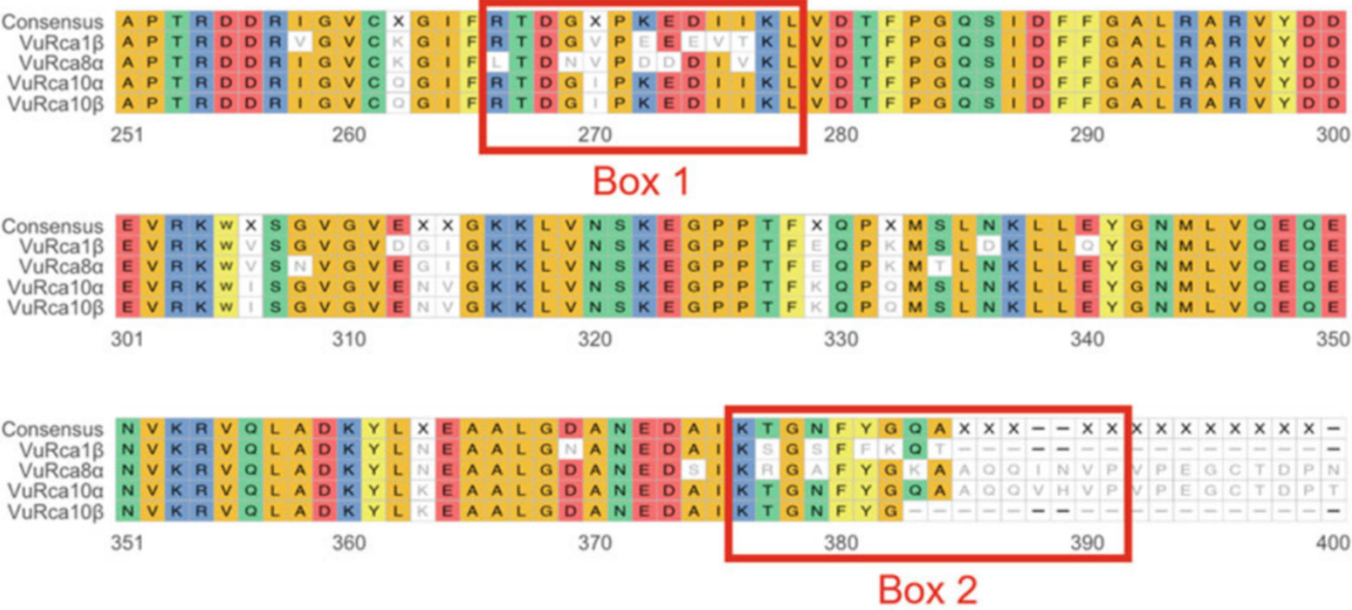
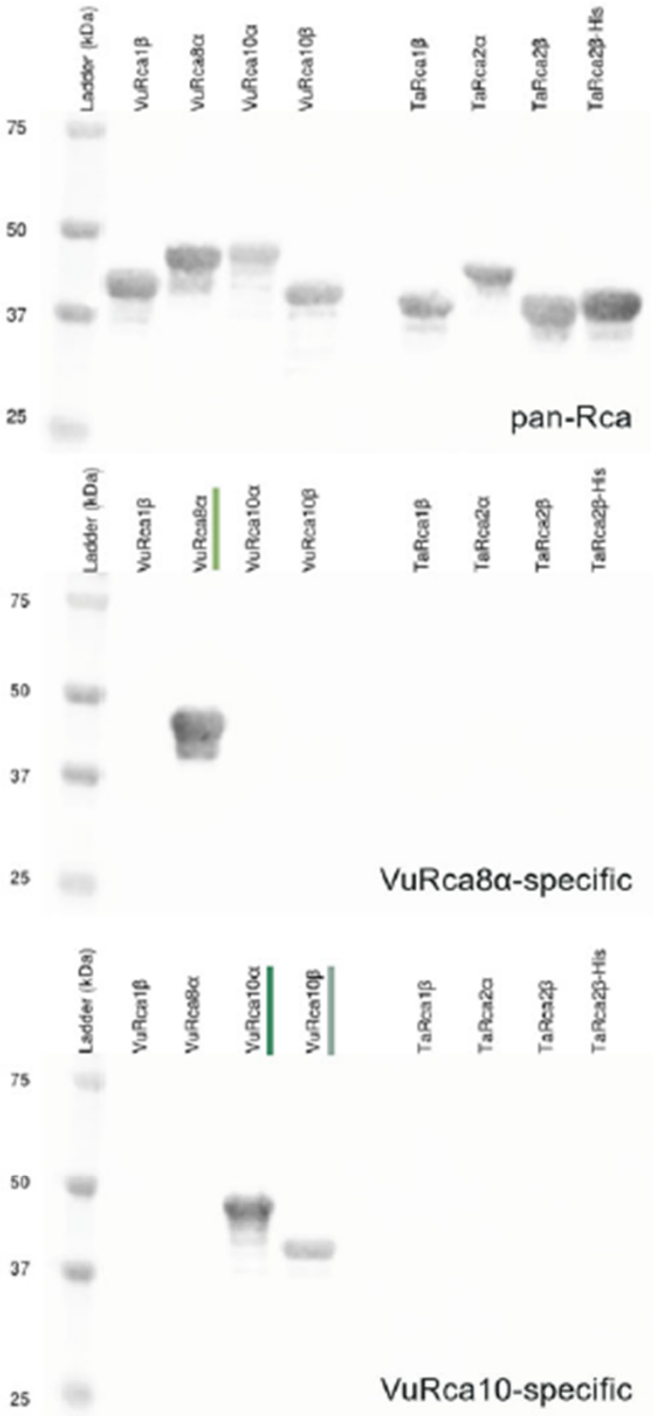
Last time ESTIV 2018 - Berlin



Daily life of a scientist – Research & Diagnostics

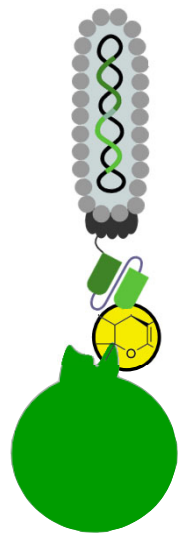


anti-photosynthetic enzyme Rubisco activase (anti-Rca)

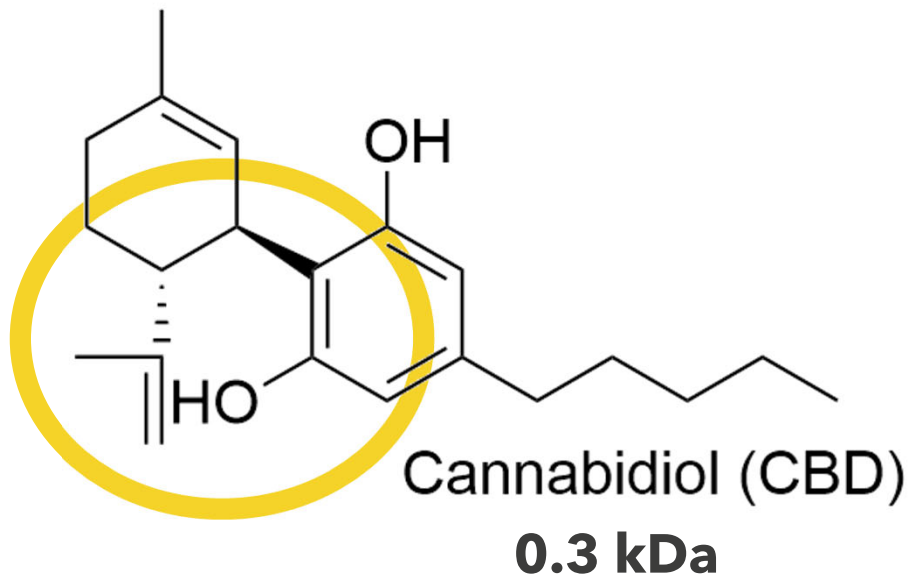
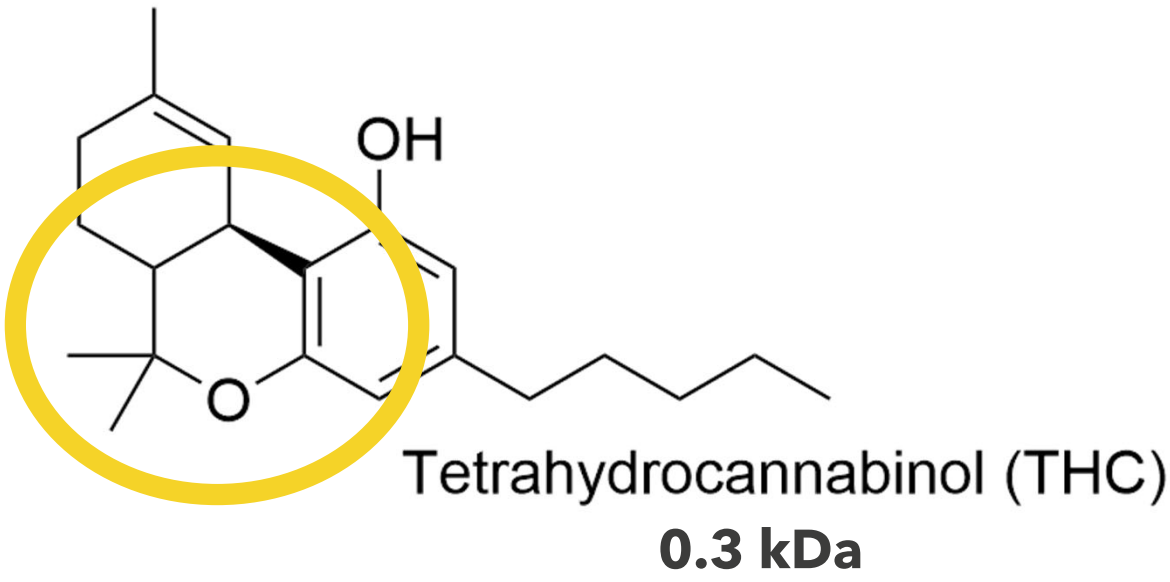
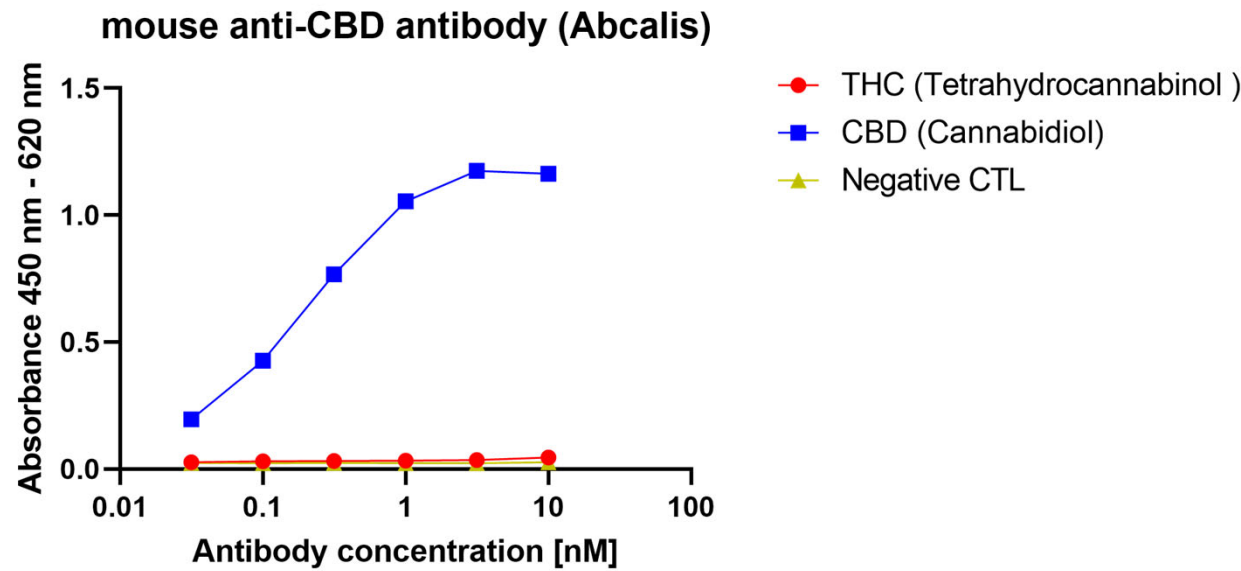
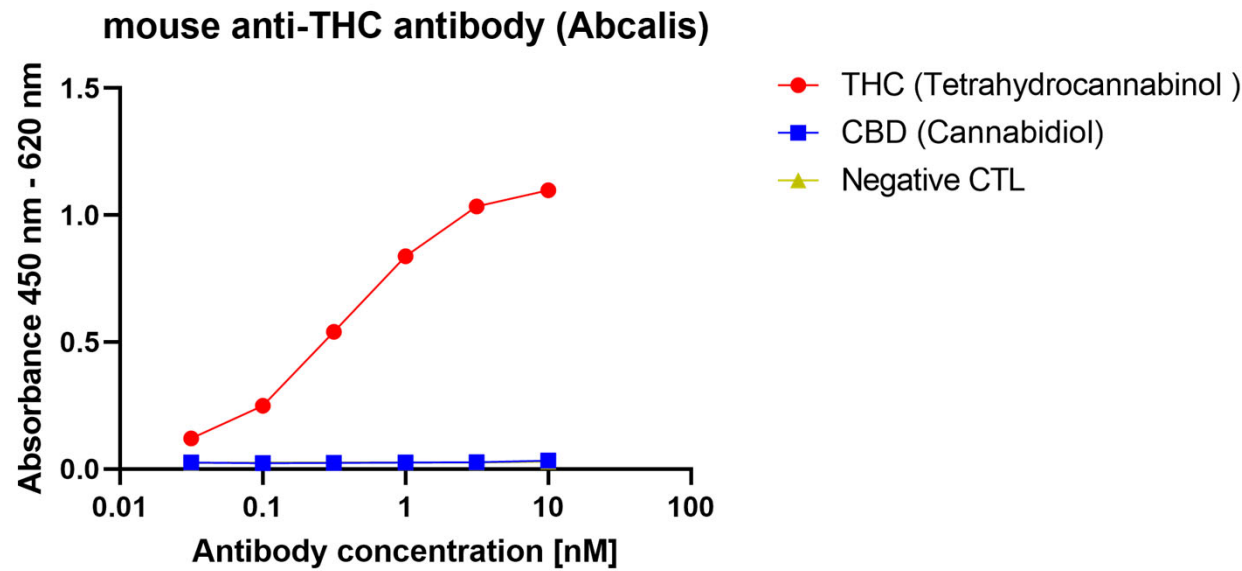


Bloemers, D., Carmo-Silva, E. (2024).
https://doi.org/10.1007/978-1-0716-3790-6_21

Extreme fine specificity by design



Antibody detects difference in small protein (THC/ CBD)

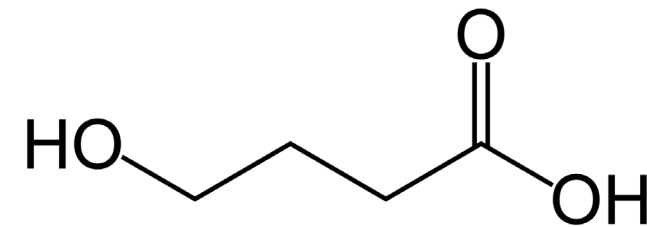


Specificity by design



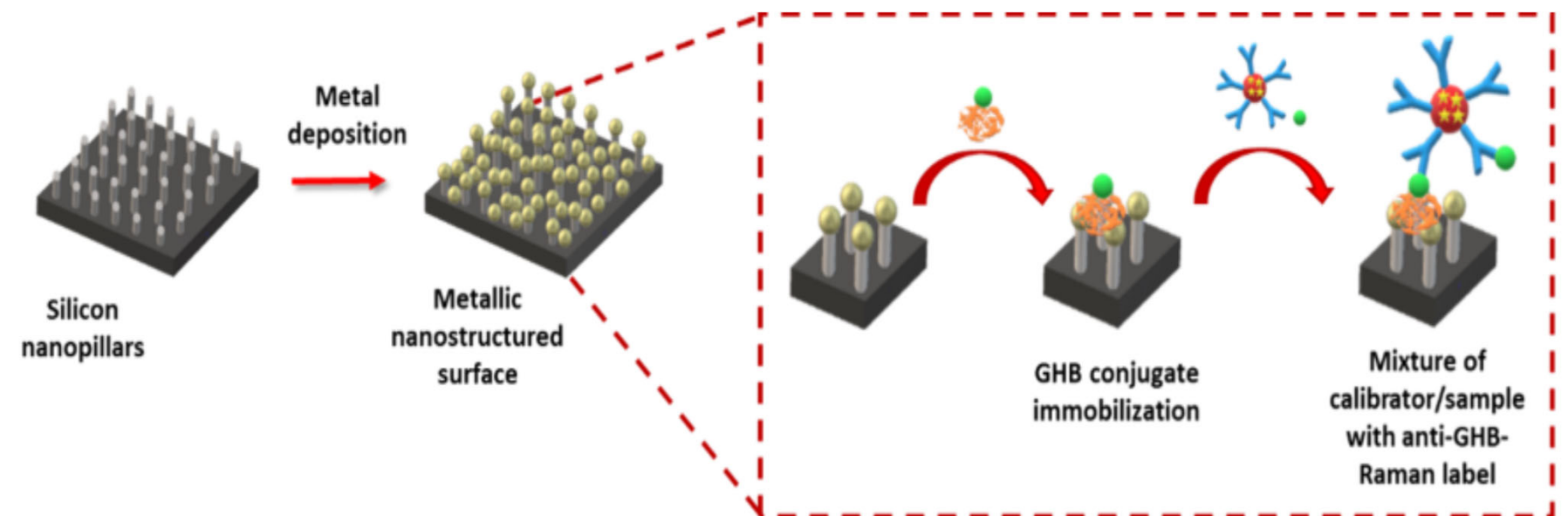
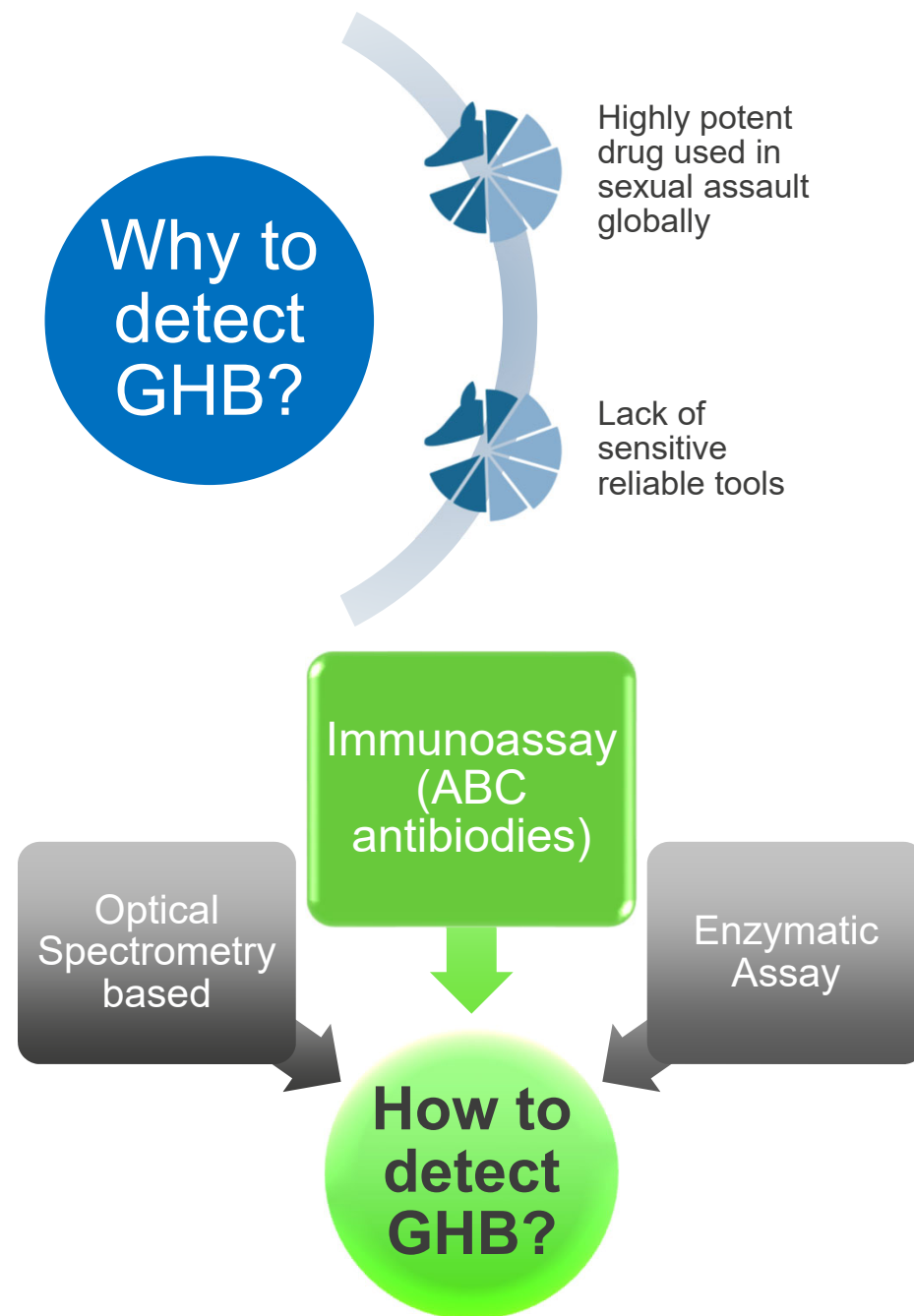
Funded by
the European Union

Grant Agreement number 101168416



Gamma-Hydroxybutyric acid (GHB)
the knockout drug

Proposed plan of Immunoassay to detect GHB in ARMADILLO platform



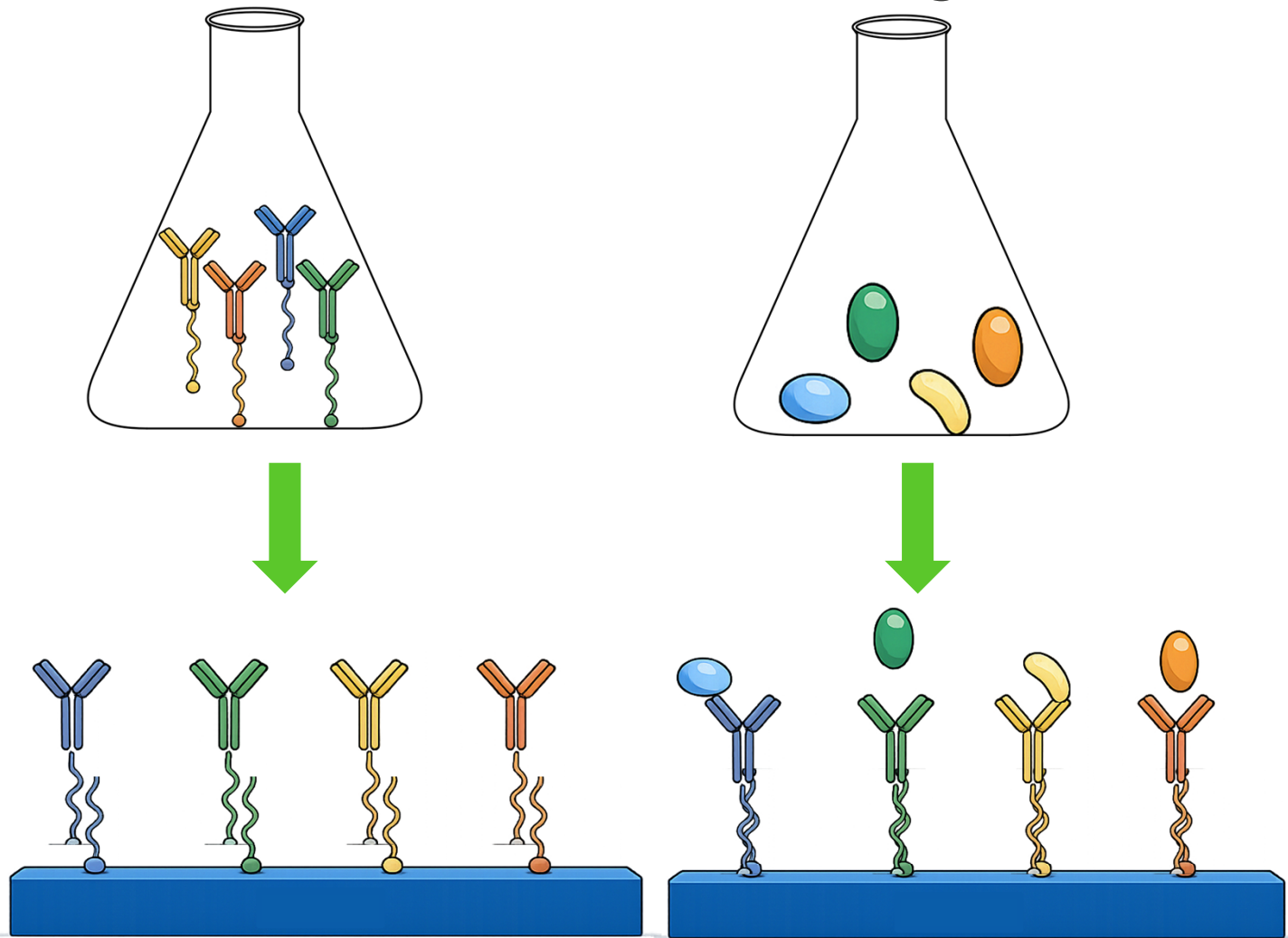
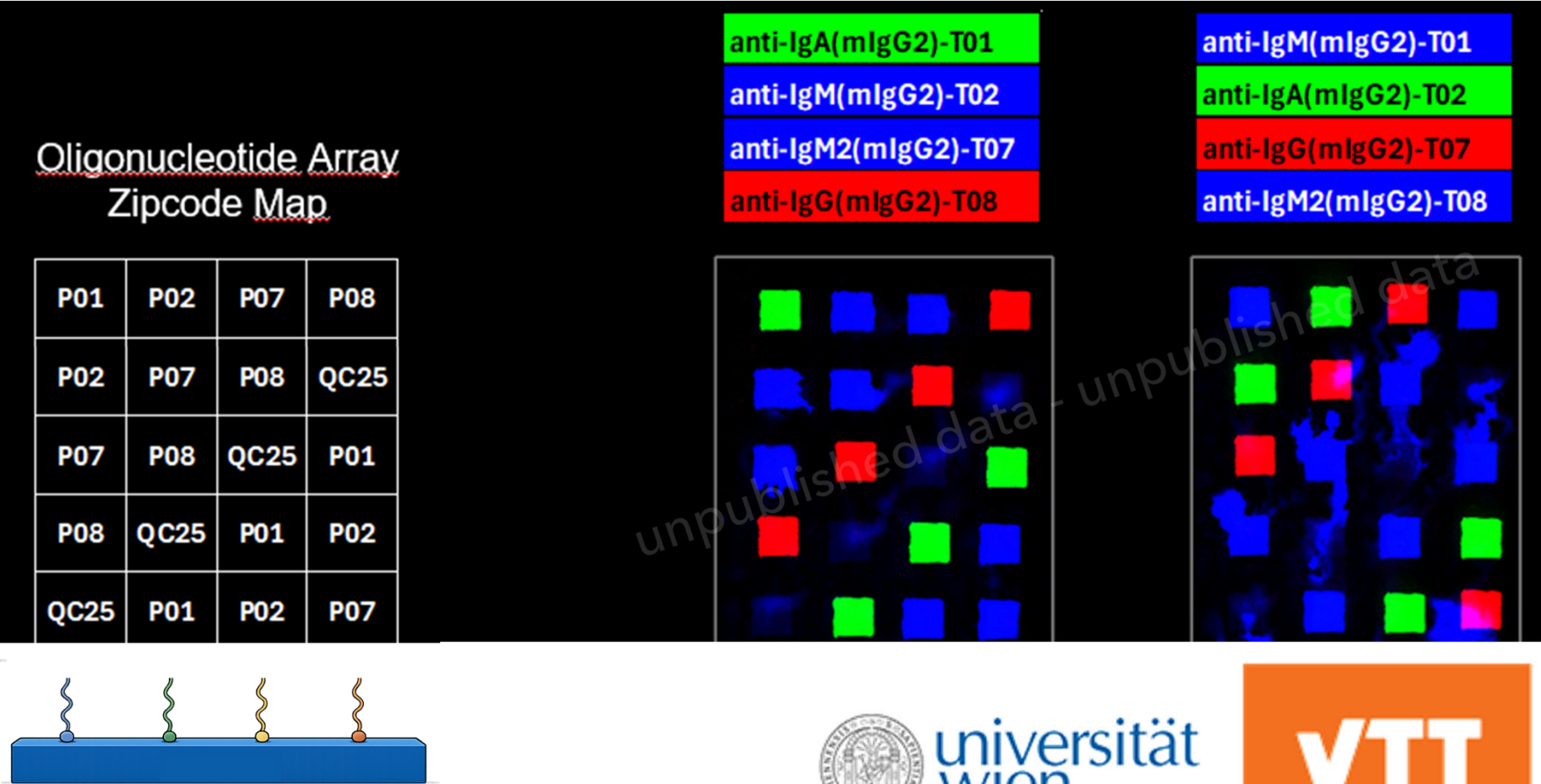
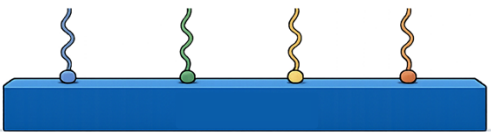
Specificity by design



Funded by
the European Union

Grant Agreement number 101070589

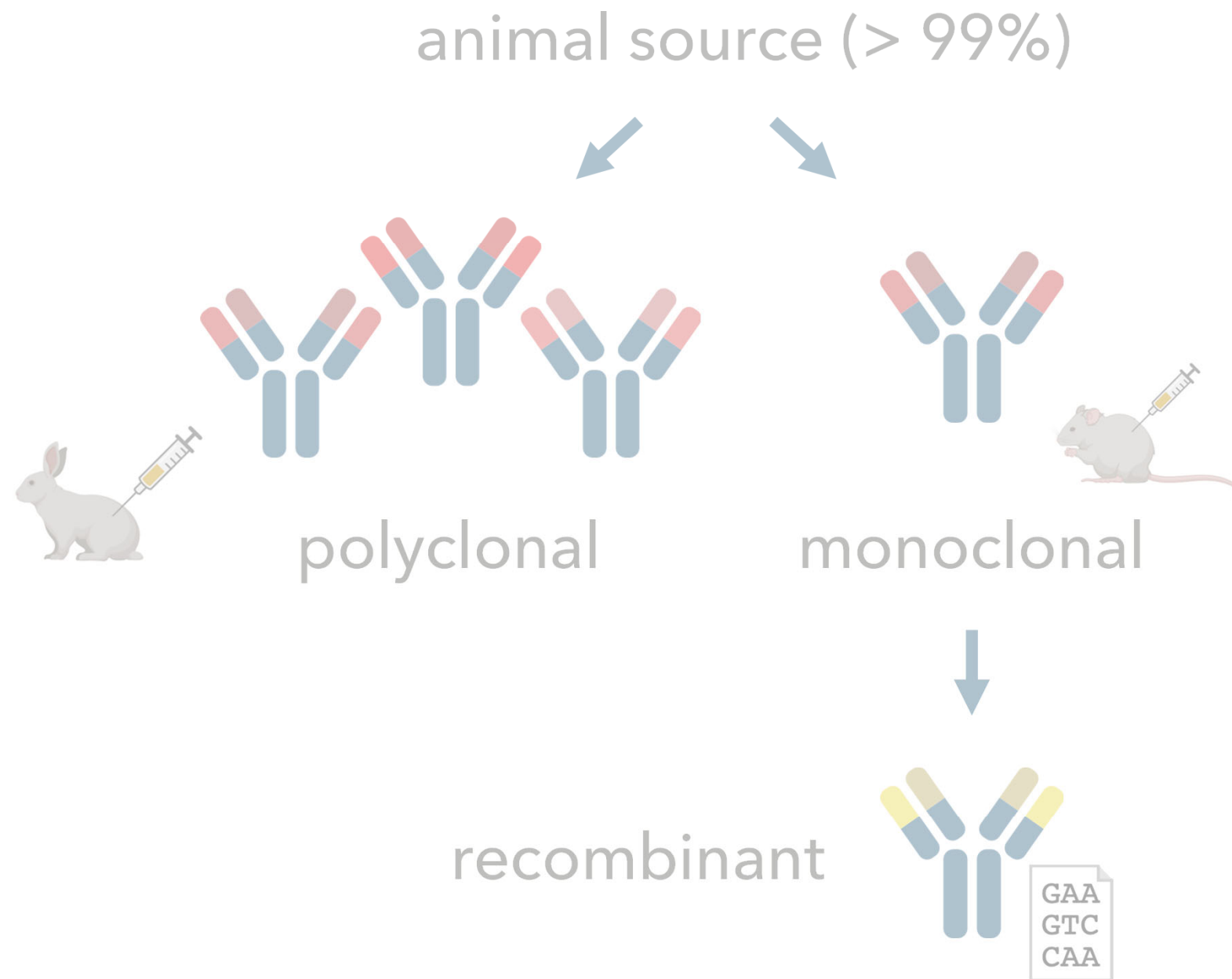
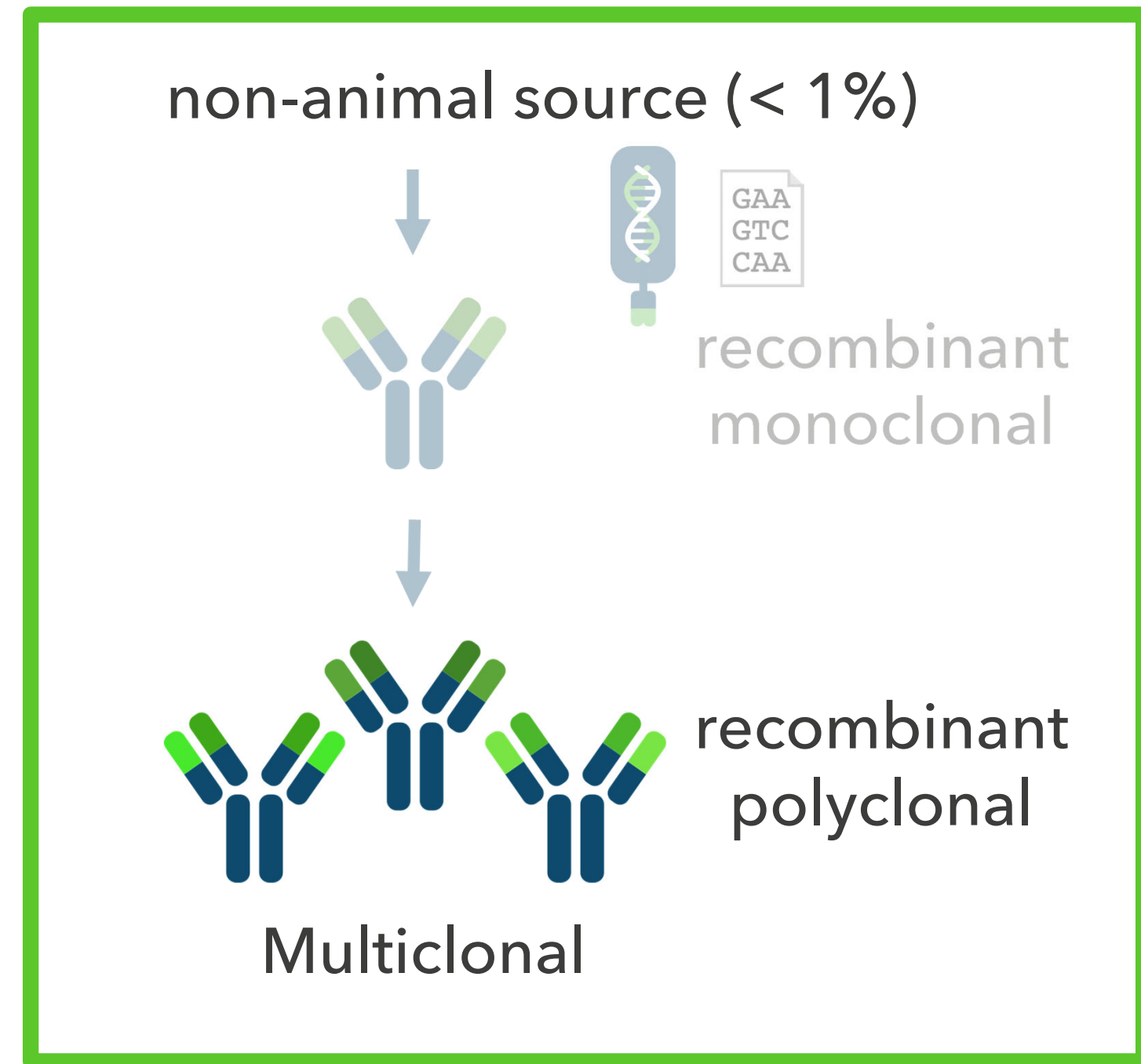
Self-assembling molecular binders



Unpublished data



Animal-free multiclonal/ secondary antibodies



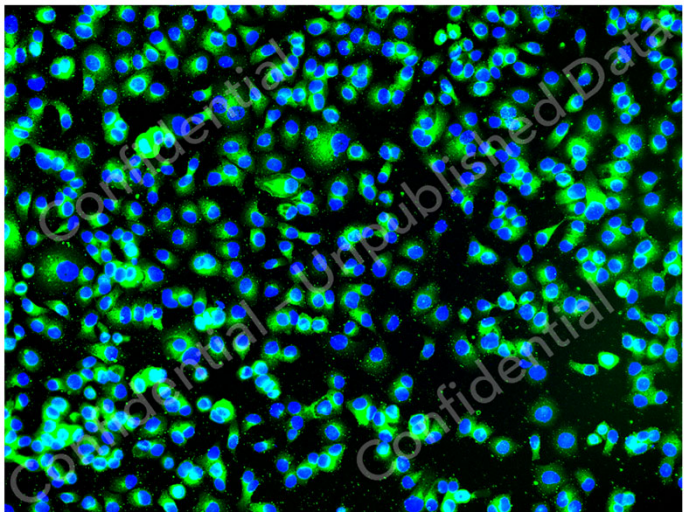
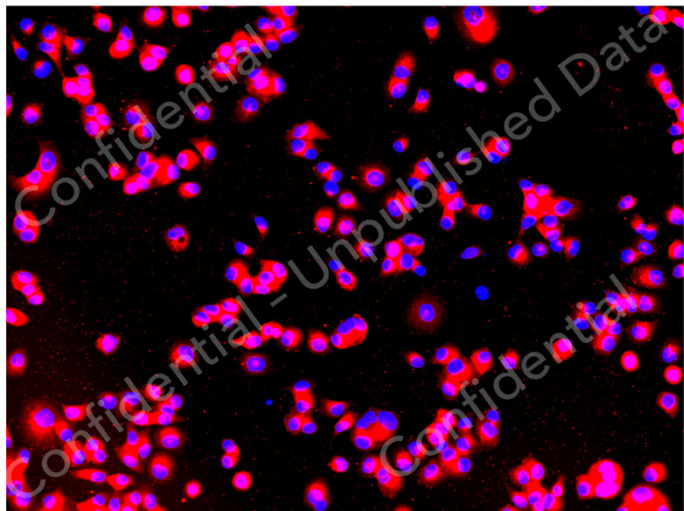
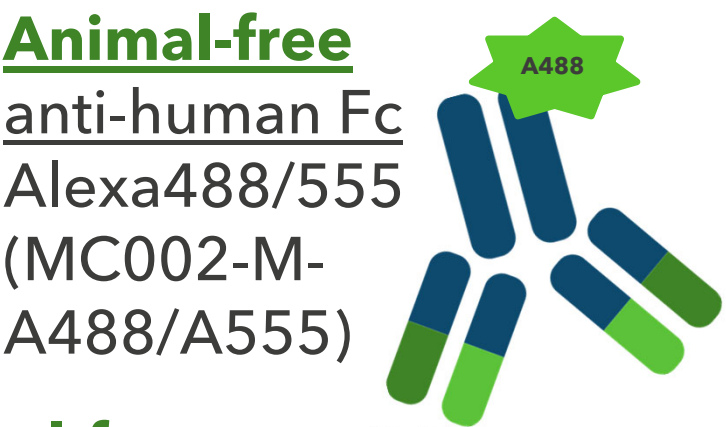
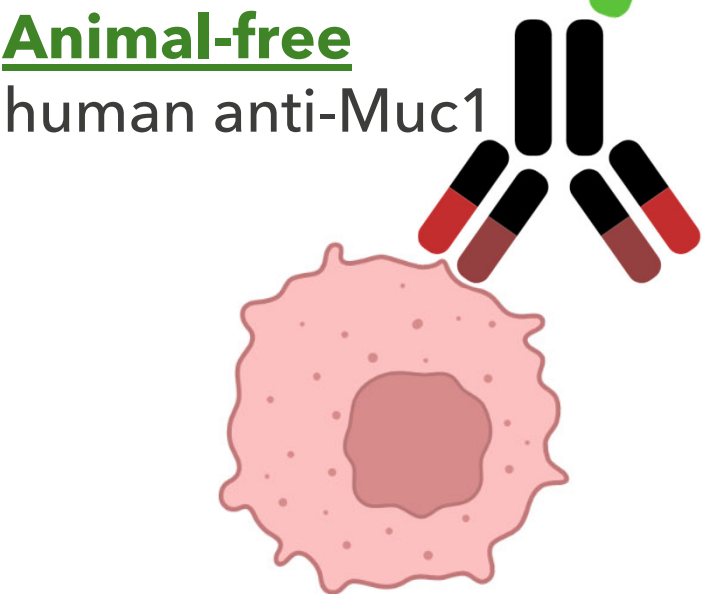
non-animal derived antibodies → **NADA**

Animal-free secondary antibodies - Specificity by design



Completely animal-component free assay

Animal-free
blocking reagents



Breast cancer (T-47D)

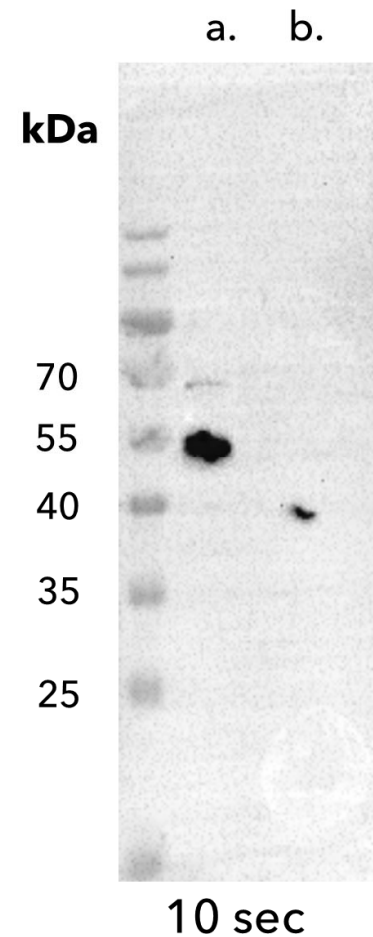
Unpublished data

Animal-free secondary antibodies - Specificity **by design**

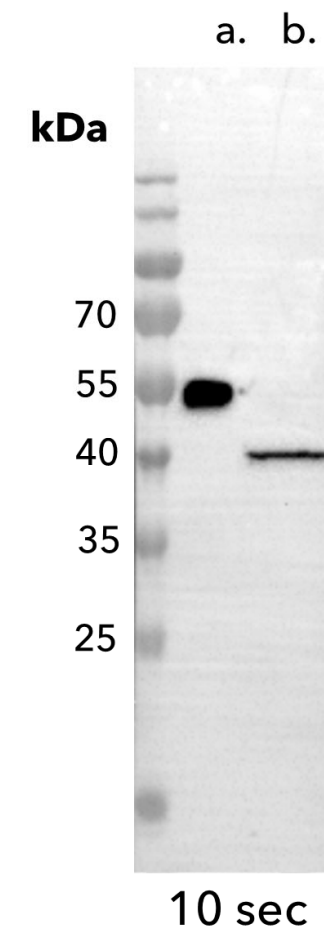


Completely animal-component free assay

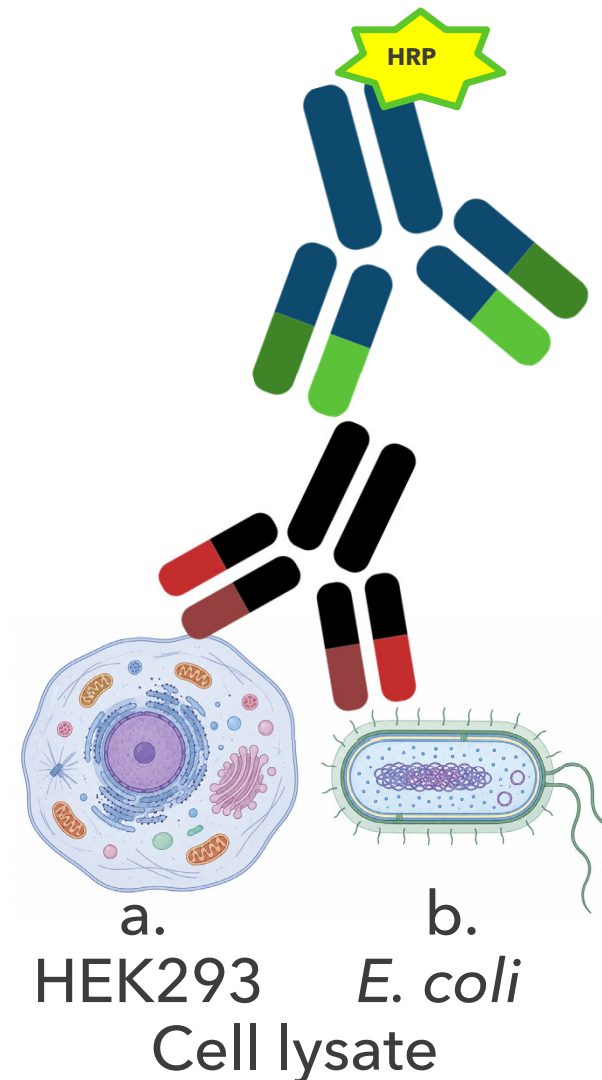
5 % Milk Powder



5 % Oat Powder

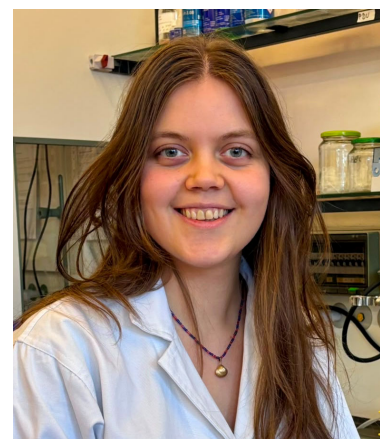


Animal-free
blocking reagents



Animal-free
anti-rabbit Fc HRP
(rbMC001-M-HRP)

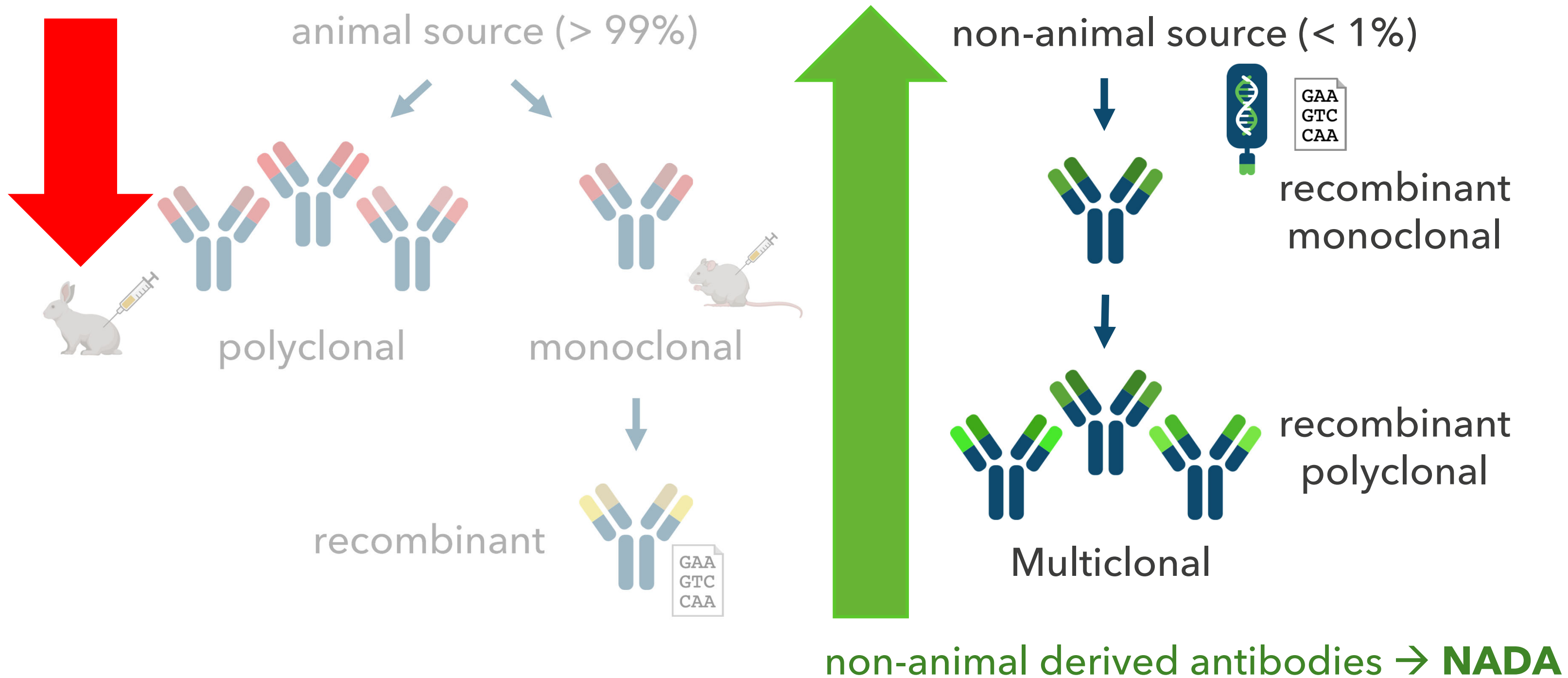
Animal-free
rabbit anti- β -tubulin/
FtsZ (*E. coli*)
(ABK681-1-B01-Rb)



Maja Kochzius

Unpublished data

Goal - Increase non-animal derived antibodies



What can we do?

- **improve information** about **animal-free antibody** availability and sources etc.



What can we do?

- improve information about animal-free antibody availability and sources etc.



PETA SCIENCE CONSORTIUM
INTERNATIONAL e.V. 
Advancing 21st Century Toxicology

<https://www.thepsci.eu/antibody-availability/>

Availability of Animal-Free Antibodies

Animal-free antibodies are available from the sources listed below. Most companies offer a selection of catalog antibodies and custom antibody development, and generate antibody fragments and full immunoglobulin format. Note that this list is not comprehensive and that a number of the suppliers listed sell animal-derived antibodies in addition to animal-free recombinant antibodies.

[Abcalis](#) offers animal-free recombinant monoclonal antibodies and multiclonal antibodies produced in animal-serum free media.

[Abcam](#) produces a selection of antibodies using phage display recombinant antibody technology.

[Absolute Antibody](#) sequences and produces antibodies developed via phage display.

[Adimab](#) offers therapeutic antibody discovery and an antibody discovery platform transfer service.

[AdipoGen Life Sciences](#) generates and characterises animal-free recombinant monoclonal antibodies (RecMAbs™).

[AvantGen](#) provides a complete antibody discovery service using human antibody libraries.

[Bio-Rad](#) uses its Human Combinatorial Antibody Libraries (HuCAL) library to offer animal-free recombinant monoclonal antibodies. Catalog HuCAL antibodies can be found [here](#) by searching the HuCAL isotype.

Animal Free Research UK Recombinant Antibodies & Mimetics Database



What can we do?

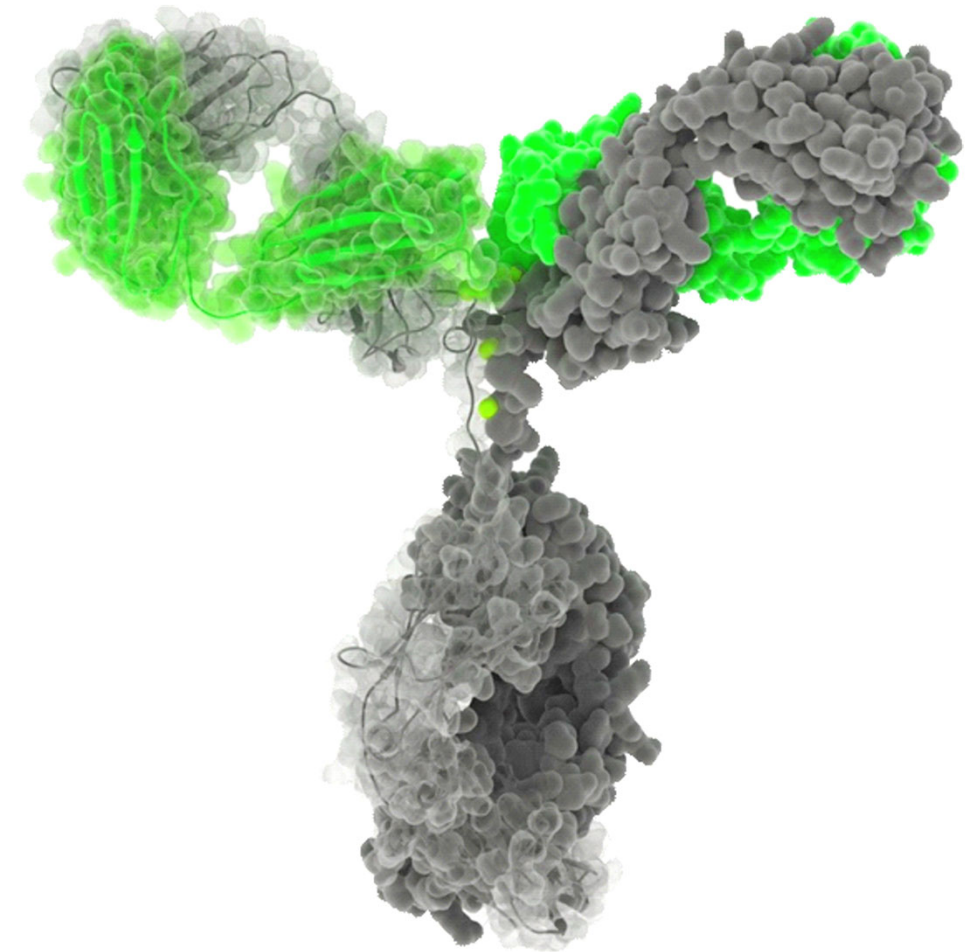
- **improve information** about **animal-free antibody** availability and sources etc.
- **improve information** about advantages of recombinant Abs:
 - fully defined
 - highly reproducible
 - can be engineered with great flexibility
- provide **equal chances** to **animal-free antibodies**
 - **Try it out!**



Thank you very much for your attention!

Contact me:

✉ esther@abcalis.com
 [/estherwenzel](#)



Questions ?