# Structure of antibodies

General structure of antibodies

Structural differences between isotypes

Half-life

# Monoclonal antibodies

Monoclonal vs polyclonal antibody

Development

Therapeutic applications

# All about monoclonal antibody drugs — the experimental treatment that may have turned Trump's COVID-19 around

*It's challenging and expensive to make. It's laborious, time-consuming, and can cost thousands of dollars per dose*

**Sharon Kirkey**
Oct 06, 2020 • Last Updated 22 hours ago • 4 minute read

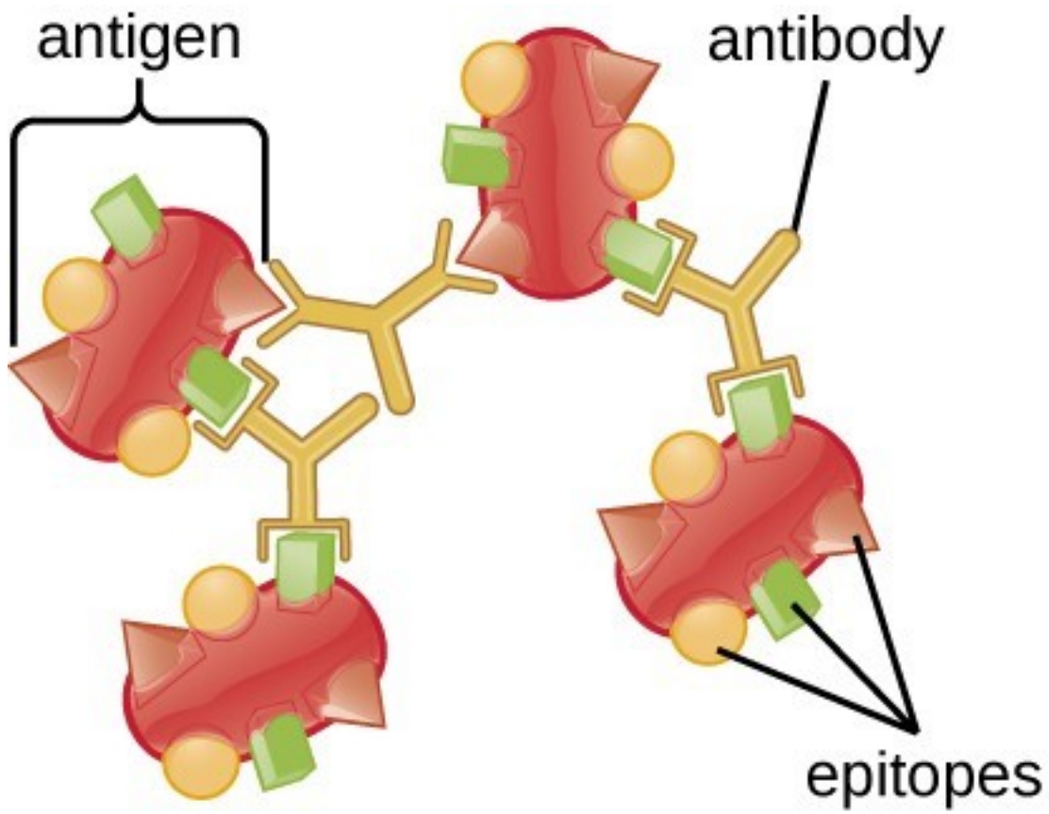
U.S. President Donald Trump leaves Walter Reed National Military Medical Center after a fourth day of treatment for COVID-19 to return to the White House, October 5, 2020. PHOTO BY JONATHAN ERNST/REUTERS

# What’s a monoclonal antibody (mAb)?

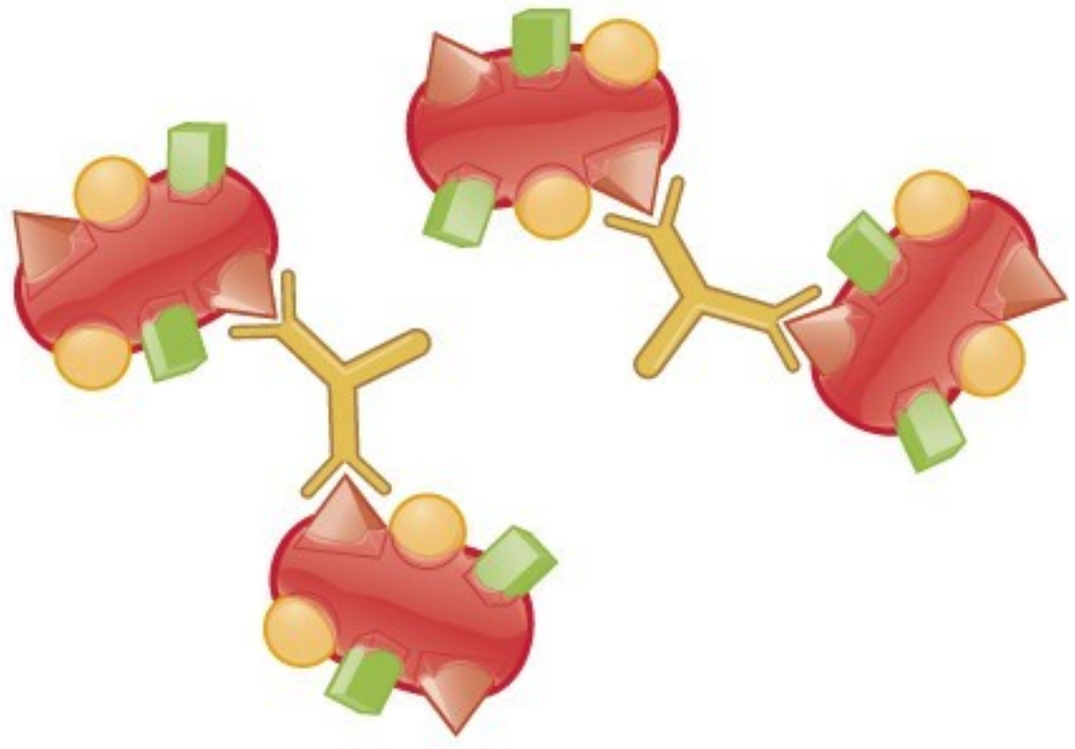
An antibody preparation derived by a single B cell clone

An antibody preparation containing identical antibody molecules

# Monoclonal vs polyclonal



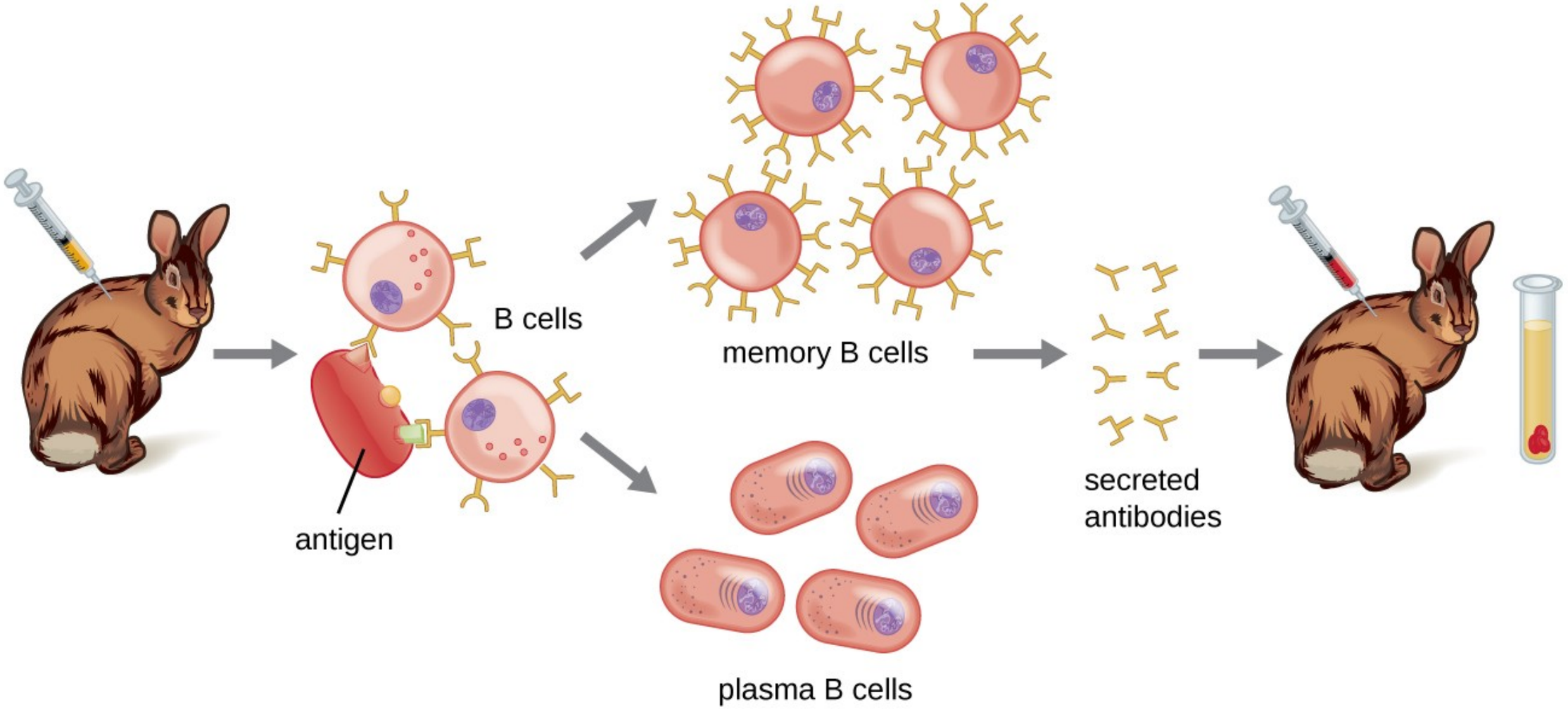

polyclonal antiserum



monoclonal antibodies

# Development of polyclonal antibodies

1. Inject antigen into rabbit.
2. Antigen activates B cells.
3. Plasma B cells produce polyclonal antibodies.
4. Obtain antiserum from rabbit containing polyclonal antibodies.



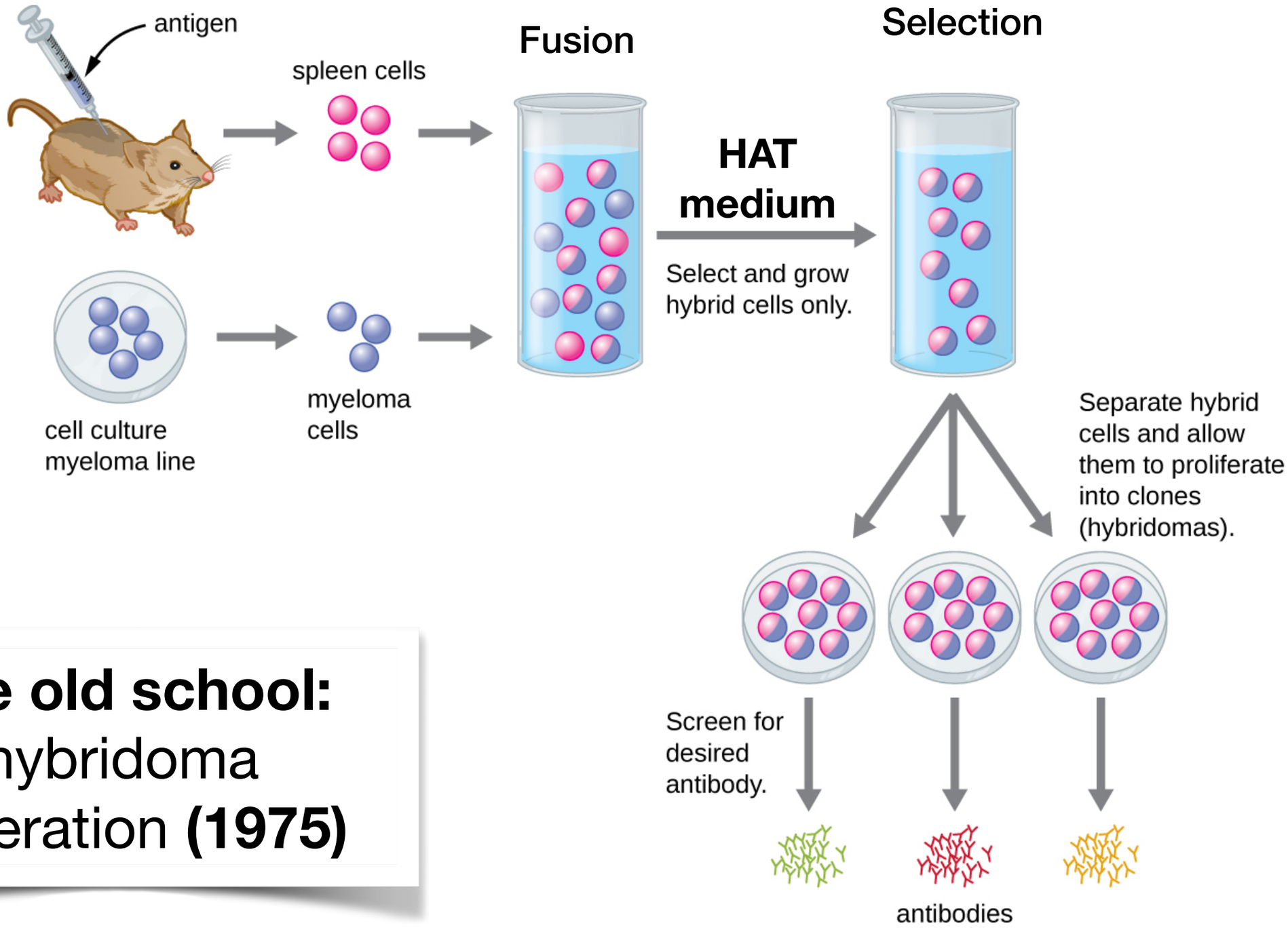
# Development of monoclonal antibodies

The old school: hybridoma generation (1975)

A more recent approach: single-cell BCR sequencing and recombinant Ig expression

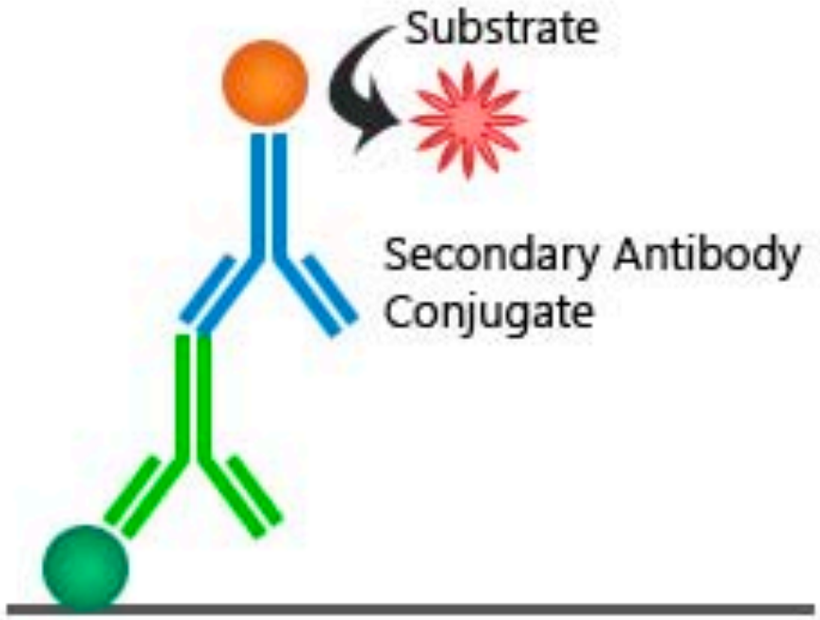
An evergreen: in vitro, by directed evolution methods

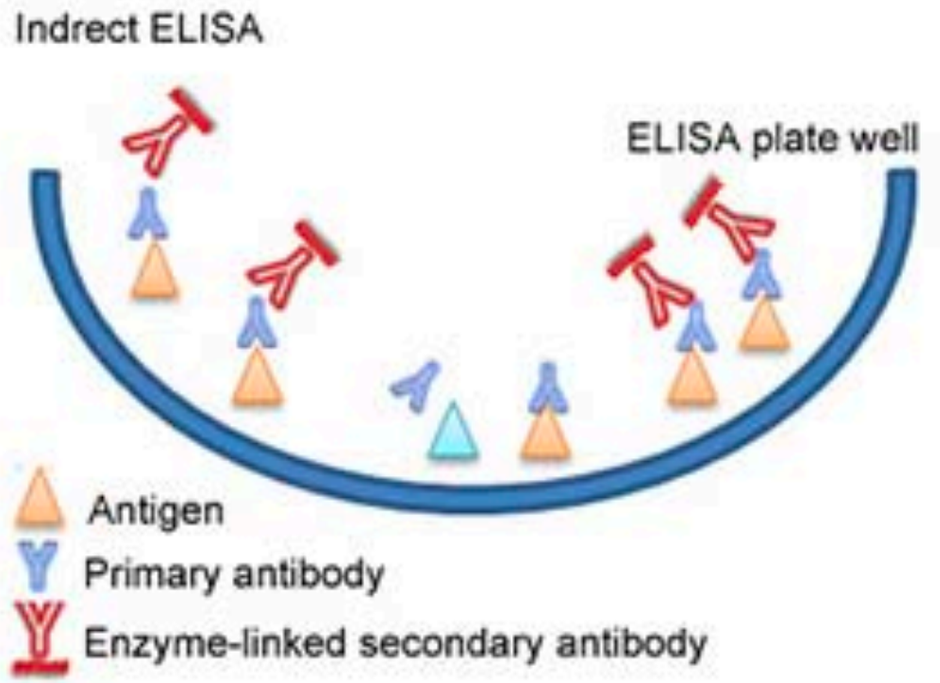
# Hybridoma generation



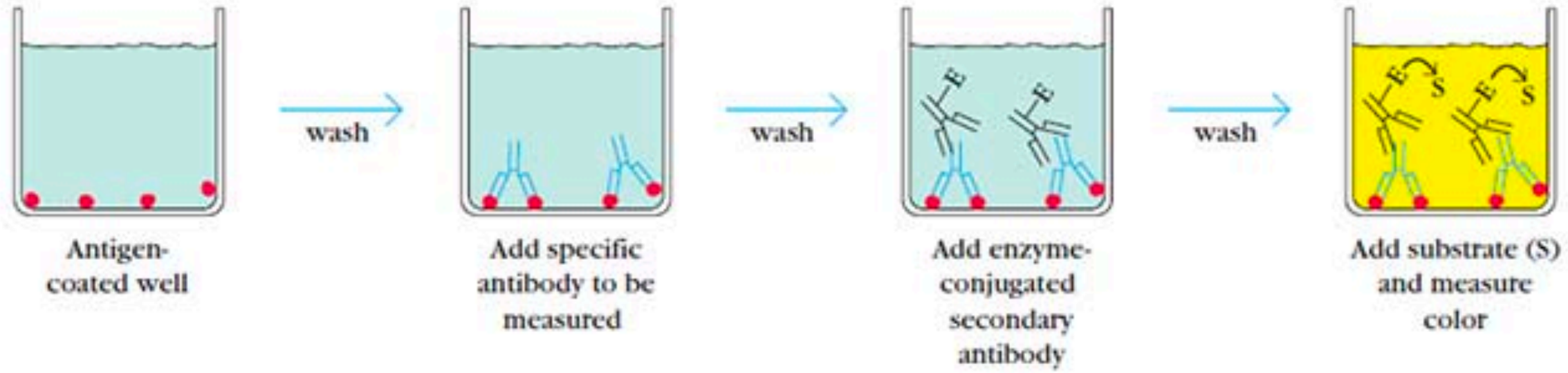

**The old school:** hybridoma generation **(1975)**

# Enzyme Linked Immunosorbent test (ELISA)

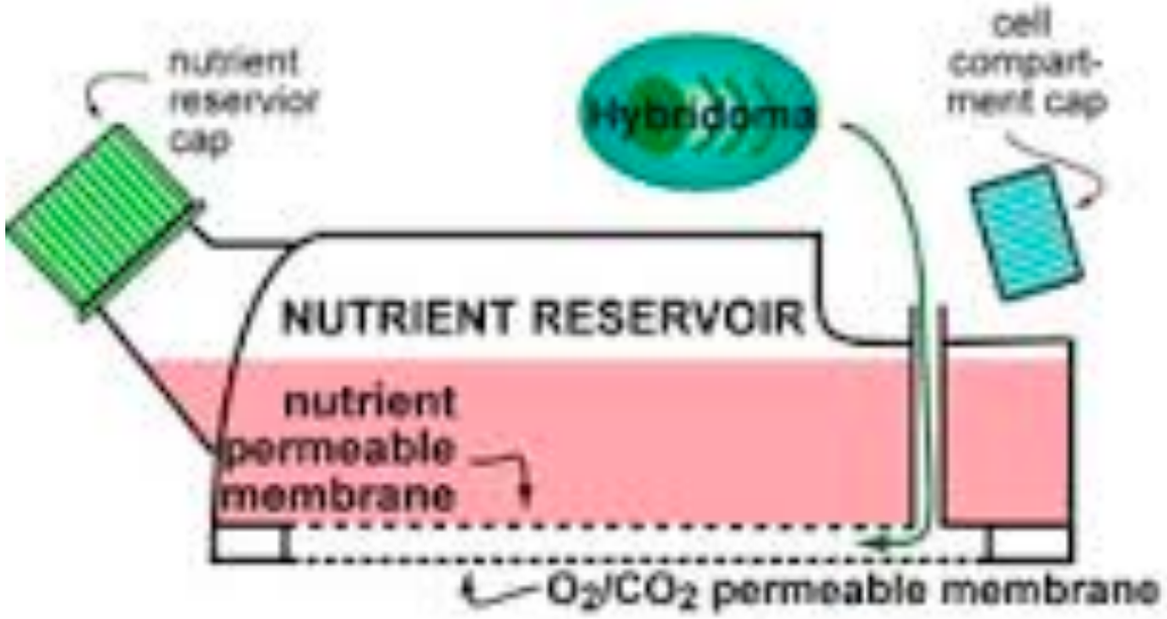


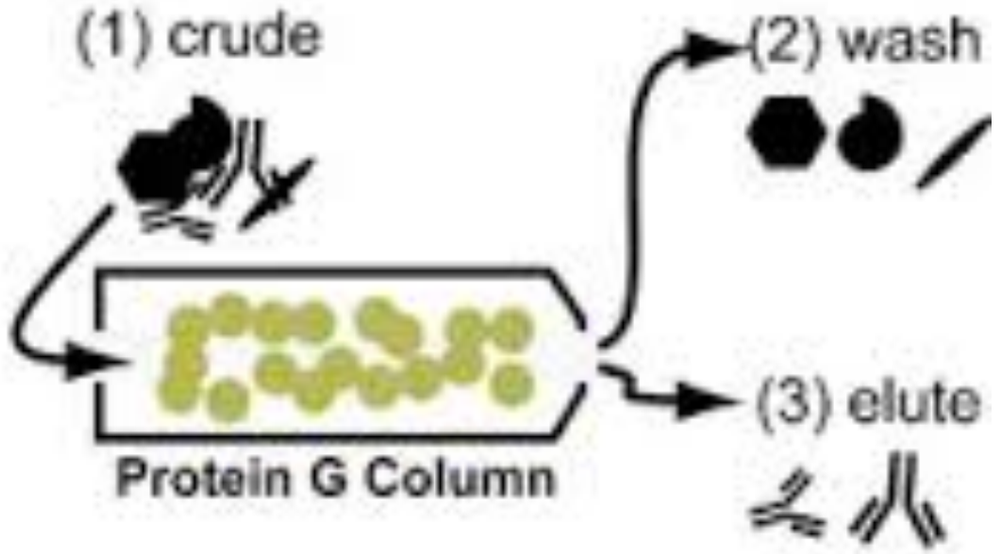

## INDIRECT ELISA



## 1. CELLine Bioreactor Culture




## 2. Antibody Purification



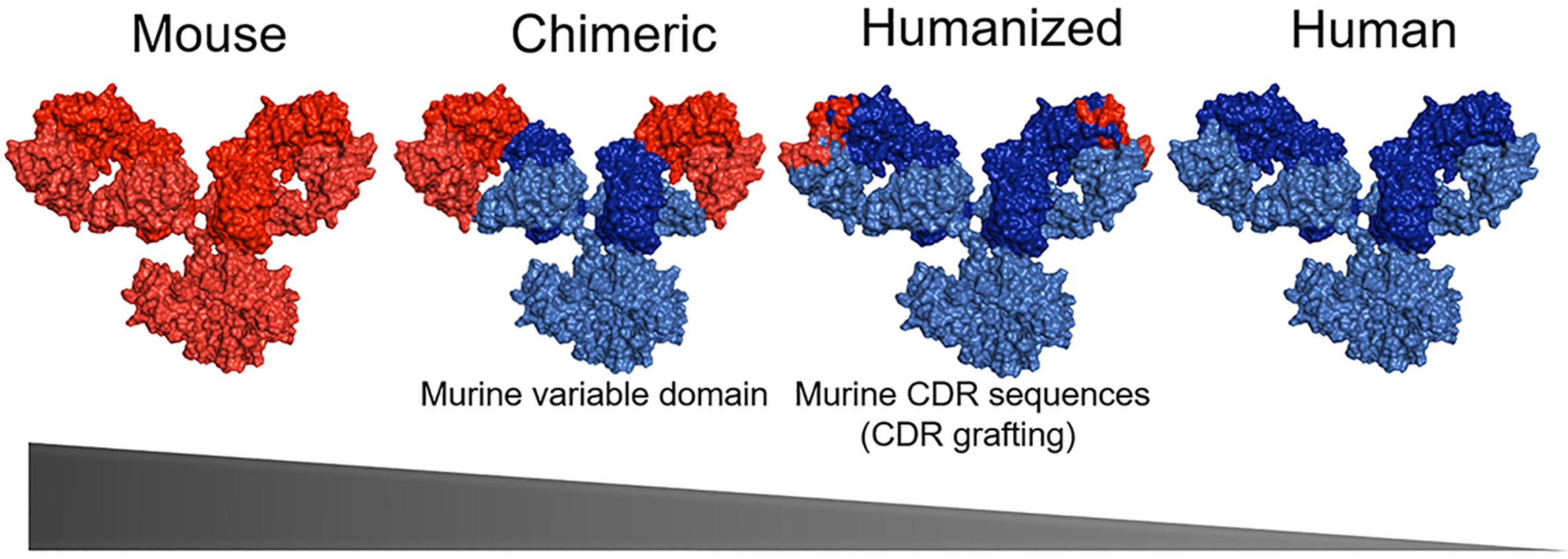
# Limitation in using mouse in Humans:

- Adverse reactions due to heterologous proteins
- High Immunogenicity
- Inneficiency of effector functions
- Half-life tipicaly<20hr

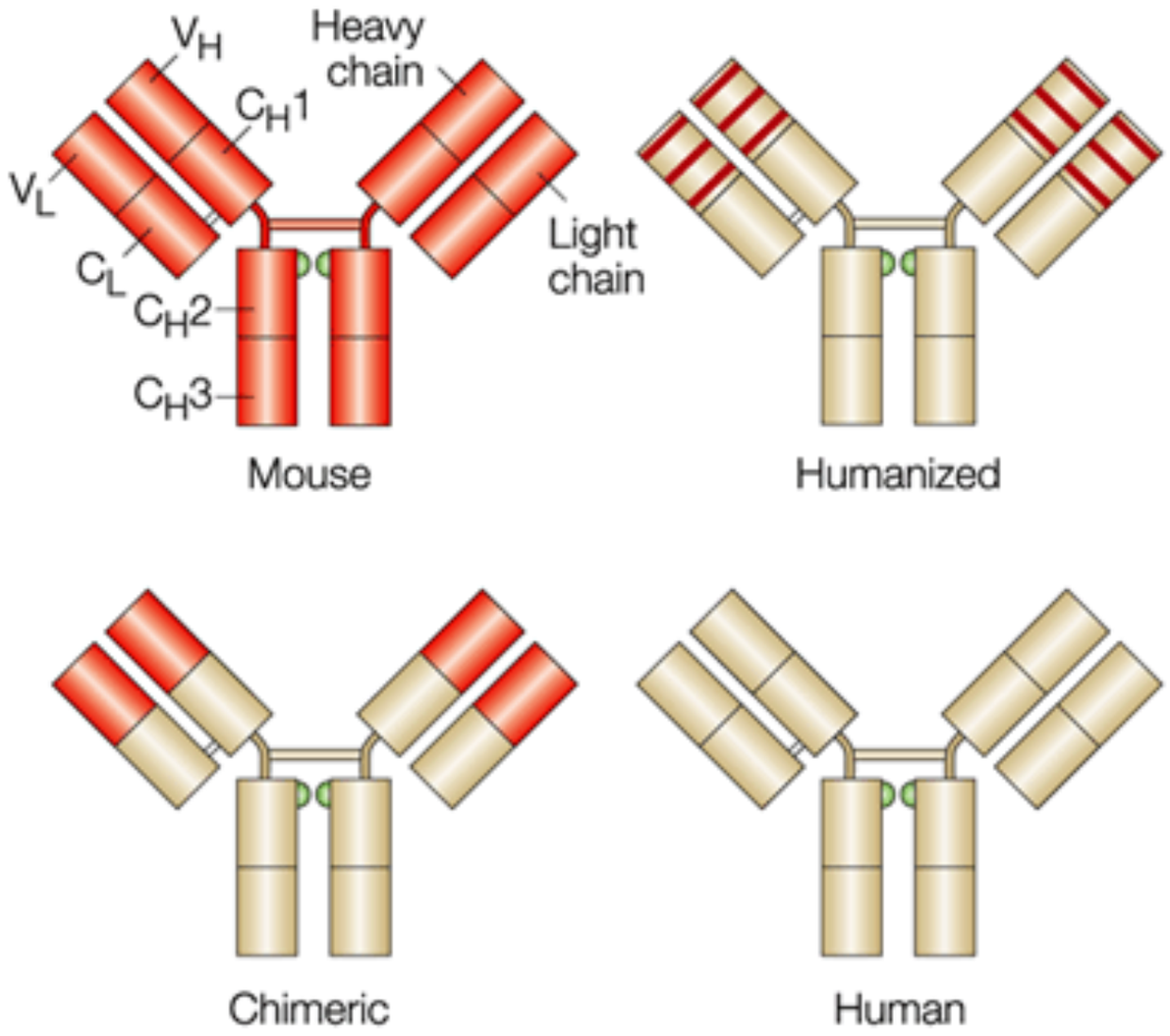
# Overcoming limitations of mouse antibodies

- Chimerization
- Humanization
- Development of human Ab through phage display libraries or transgenic mice
- Modificationof Ab properties (molecular size, affinity, specificity and valency

# Grafting of antigen binding site on human backbone

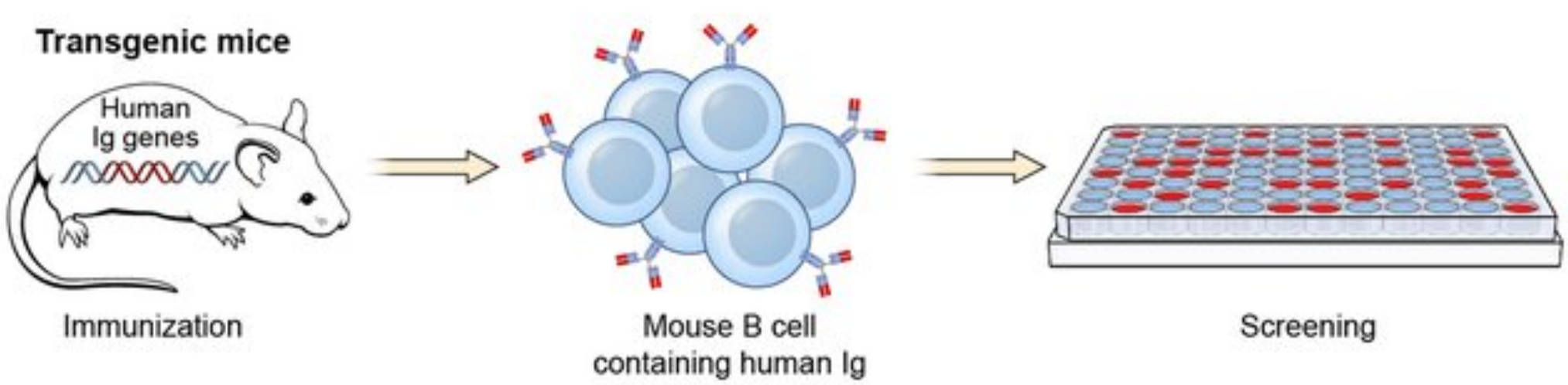


# Evolution of therapeutic mAb




**Nature Reviews** | **Cancer**

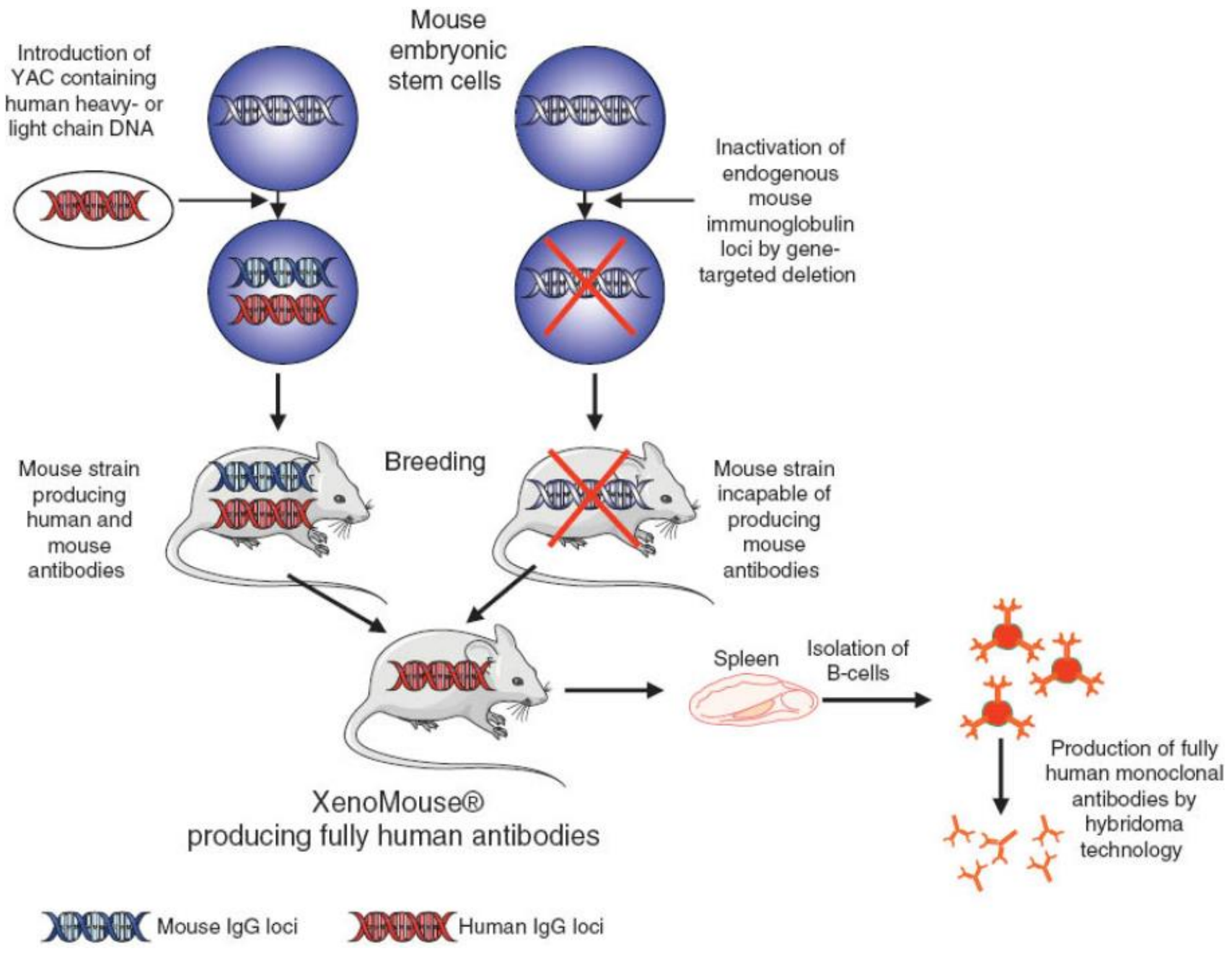
# Generation of HUMANized mAb



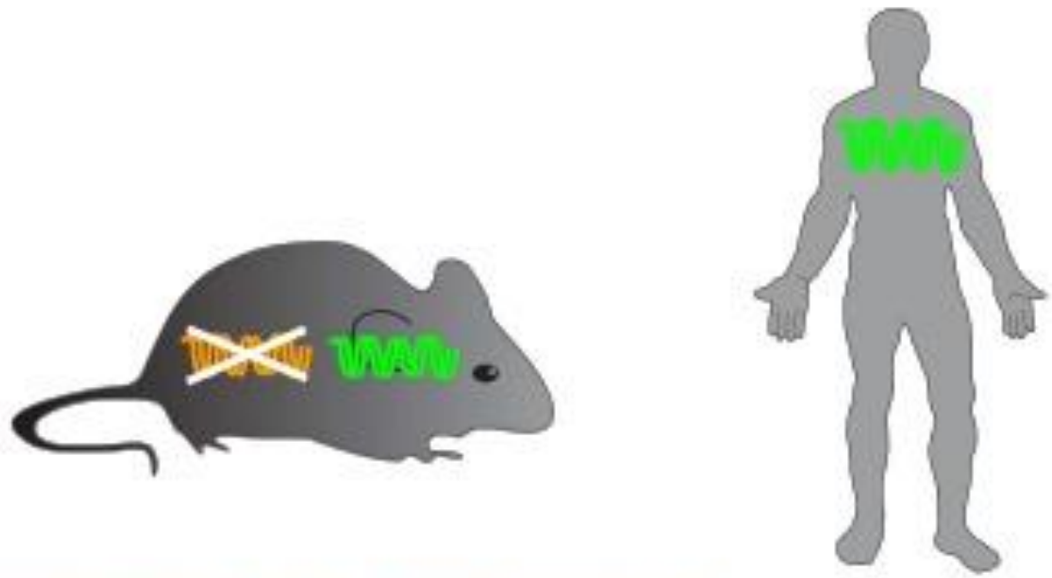
# Development of fully human mAb:

®

# XenoMouse



# PANITUMUMAB

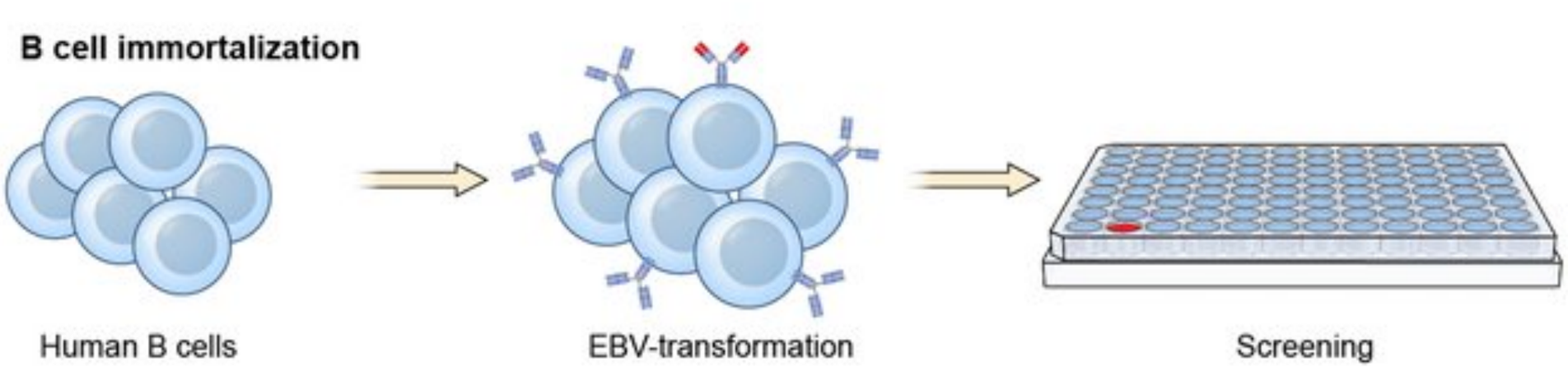


- XenoMouse generates **fully human** antibodies
- Panitumumab is a fully human IgG2 directed **against EGFr**
- High Affinity, $K_d = 5 \times 10^{-11}$ M

**Vectibix (anti-EGFr)**

# Generation of HUMAN mAb

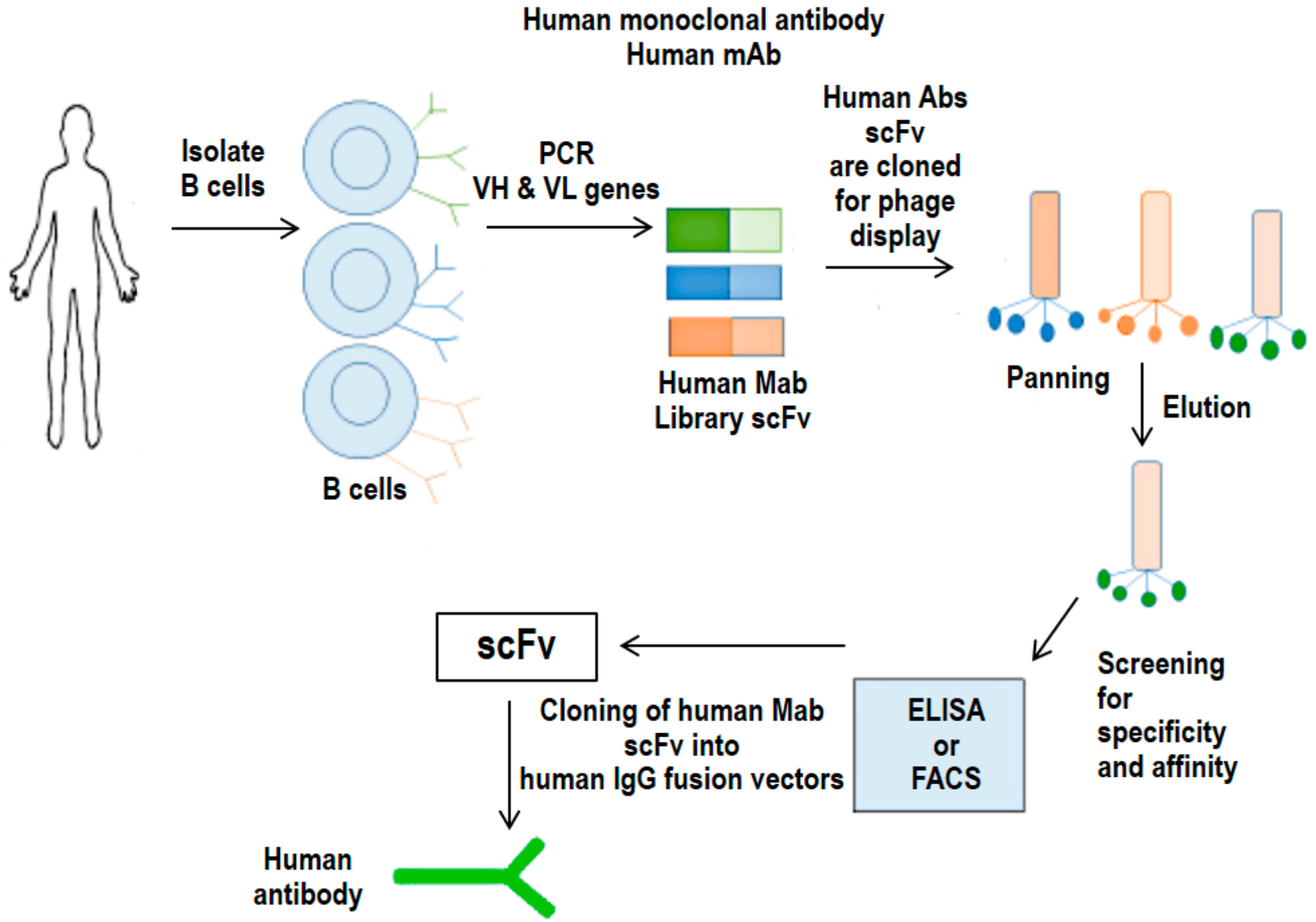
**B cell isolation from immune individuals**



# Generation of HUMAN mAb

In vitro, by directed evolution methods

Phage and yeast display



Science **Ebola** REPORTS

Cite as: D. Corti *et al.*, *Science* 10.1126/science.aad5224 (2016).

# Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody

**Davide Corti,[1,2*] John Misasi,[3*] Sabue Mulangu,[3] Daphne A. Stanley,[3] Masaru Kanekiyo,[3] Suzanne Wollen,[4] Aurélie Ploquin,[3] Nicole A. Doria-Rose,[3] Ryan P. Staupe,[3] Michael Bailey,[3] Wei Shi,[3] Misook Choe,[3] Hadar Marcus,[3] Emily A. Thompson,[3] Alberto Cagigi,[3] Chiara Silacci,[1] Blanca Fernandez-Rodriguez,[1] Laurent Perez,[1] Federica Sallusto,[1] Fabrizia Vanzetta,[2] Gloria Agatic,[2] Elisabetta Cameroni,[2] Neville Kisalu,[5†] Ingelise Gordon,[3] Julie E. Ledgerwood,[3] John R. Mascola,[3] Barney S. Graham,[3] Jean-Jacques Muyembe-Tamfun,[5] John C. Trefry,[4‡] Antonio Lanzavecchia,[1,6‡] Nancy J. Sullivan[3‡§]**

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Ebola virus disease in humans is highly lethal, with case fatality rates ranging from 25-90%. There is no licensed treatment or vaccine against the virus, underscoring the need for efficacious countermeasures. Here, we demonstrate that a human survivor of the 1995 Kikwit Ebola virus disease outbreak maintained circulating antibodies against the Ebola virus surface glycoprotein for more than a decade after infection. From this survivor we isolated monoclonal antibodies (mAb) that neutralize recent and previous outbreak variants of Ebola virus, and mediate antibody-dependent cell-mediated cytotoxicity in vitro. Strikingly, monotherapy with mAb114 protected macaques when given as late as five days after challenge. Treatment with a single human mAb suggests a simplified therapeutic strategy for human Ebola infection may be possible.



Ebola virus disease (EVD) causes severe illness characterized by rapid onset of fever, vomiting, diarrhea and bleeding diathesis (*1*, *2*). The challenges of a large outbreak and the failure of traditional quarantine and contact tracing measures (*3*, *4*) to control the 2014 West Africa outbreak highlights the urgency for therapies. The success in non-human primates (NHP) of ZMapp, a cocktail of three chimeric monoclonal antibodies (mAbs) derived from immunized mice (*5*–*7*), illustrated the potential of mAb therapies against EVD, and it is currently being evaluated in human trials. To date, efforts in NHP to simplify the ZMapp regimen to contain fewer mAbs have not been successful (*7*). We sought to isolate mAbs from human EVD survivors, with the goal of identifying antibodies that confer clinical protection either as single or dual-combination agents.

We obtained blood from two survivors of the 1995 Kikwit EVD outbreak (*8*) eleven years after infection. To determine if the subjects retained circulating antibodies against Ebola virus (EBOV) glycoprotein (GP), we assessed GP-specific antibodies by ELISA (Fig. 1A) (*9*). The reciprocal $EC_{90}$ titer for subject 1 (S1) was 2,326, greater than ten-fold higher than control sera. Moreover, serum from the more severely ill subject, S1, displayed potent virus neutralizing activity (Fig. 1B), indicating that S1 maintained serologic memory against EBOV GP more than a decade following infection and suggesting the potential to clone immunoglobulins with potent neutralizing activity from S1's memory B cells.

Therefore, we sorted memory B-cells from S1's peripheral blood mononuclear cells, and immortalized individual clones with Epstein-Barr virus (*10*). Forty clone supernatants displayed a range of GP-binding (Fig. 1C), and two, 100 and 114, expressed antibodies with markedly higher neutralizing activity than all others (Fig. 1D). A second immortalization yielded 21 clones, from which the GP-specific clones 165 and 166 were rescued (fig. S1).

mAb100, mAb114, mAb165 and mAb166 sequences were PCR-amplified and antibodies produced by transient transfection. We assessed ELISA binding to EBOV GP and observed that mAb114, mAb165 and mAb166 displayed nearly 50% higher maximal binding than KZ52, a prototypic EBOV



Science **Zika** REPORTS

Cite as: K. Stettler *et al.*, *Science* 10.1126/science.aaf8505 (2016).

# Specificity, cross-reactivity and function of antibodies elicited by Zika virus infection

**Karin Stettler,[1*] Martina Beltramello,[1*] Diego A. Espinosa,[2†] Victoria Graham,[3†] Antonino Cassotta,[4,5†] Siro Bianchi,[1†] Fabrizia Vanzetta,[1†] Andrea Minola,[1] Stefano Jaconi,[1] Federico Mele,[4] Mathilde Foglierini,[4] Mattia Pedotti,[4] Luca Simonelli,[4] Stuart Dowall,[3] Barry Atkinson,[3] Elena Percivalle,[6] Cameron P. Simmons,[7,8] Luca Varani,[4] Johannes Blum,[9,10] Fausto Baldanti,[6] Elisabetta Cameroni,[1] Roger Hewson,[3] Eva Harris,[2] Antonio Lanzavecchia,[4,5] Federica Sallusto,[4‡§] Davide Corti[1‡§]**

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Zika virus (ZIKV), a mosquito-borne flavivirus with homology to Dengue virus (DENV), has become a public health emergency. By characterizing memory cells from ZIKV-infected patients, we dissected ZIKV-specific and DENV-crossreactive immune responses. Antibodies to NS1 were largely ZIKV-specific and were used to develop a serological diagnostic tool. In contrast, antibodies against E protein domain I/II (EDI/II) were cross-reactive and, while poorly neutralizing, potently enhanced ZIKV and DENV infection in vitro and lethally enhanced DENV disease in mice. Memory T cells against NS1 or E proteins were poorly crossreactive, even in donors pre-exposed to DENV. The most potent neutralizing antibodies were ZIKV-specific and targeted EDIII or quaternary epitopes on infectious virus. An EDIII-specific antibody protected mice from lethal ZIKV infection, illustrating the potential for antibody-based therapy.



After its introduction into Brazil in 2015, ZIKV has spread rapidly and in February 2016 the World Health Organization (WHO) declared it a Public Health Emergency of International Concern (*1*–*3*). The main route of ZIKV infection is through bites by *Aedes* mosquitos, but the virus may also be sexually (*4*) and vertically transmitted (*5*). While most of the ZIKV infections are asymptomatic or cause only mild symptoms, there is evidence that ZIKV infection can lead to neurological complications, such as Guillain-Barré Syndrome in adults (*6*) and congenital birth defects including microcephaly in the developing fetus (*5*, *7*, *8*), likely through its ability to infect human neural progenitor cells (*9*).

While flavivirus envelope (E) proteins mediate fusion and are the main target of neutralizing antibodies, the non-structural protein 1 (NS1) is secreted by infected cells and is involved in immune evasion and pathogenesis (*10*). Two recent structural studies showed a high level of structural similarity between the E protein of ZIKV and that of other flaviviruses, such as dengue virus (DENV), yellow fever virus (YFV) and West Nile virus (WNV) but also revealed unique features that may be related to the ZIKV neurotropism (*11*, *12*). Similarly, the structural analysis of ZIKV NS1 revealed conserved features with NS1 of other flaviviruses although with different electrostatic characteristics (*13*).

A phenomenon that is characteristic of certain flaviviruses is the disease-enhancing activity of cross-reactive antibodies elicited by previous infection by heterologous viruses, termed antibody dependent enhancement (ADE). In the case of DENV, for which 4 serotypes are known, there is epidemiological evidence that a primary infection protects from reinfection with the same serotype, but represents a risk factor for the development of severe disease upon reinfection with a different serotype (*14*). The exacerbated disease is triggered by E and prM-specific antibodies that fail to neutralize the incoming virus but instead enhance its capture by Fc receptor-expressing ($FcR^+$) cells, leading to enhanced viral replication and activation of cross-reactive memory T cells. The resulting cytokine storm is thought to be the basis of the most severe form of disease known as dengue hemorragic fever/dengue shock syndrome (*15*, *16*).



..but also HIV and Influenza

New Results

3 comments

# A human monoclonal antibody blocking SARS-CoV-2 infection

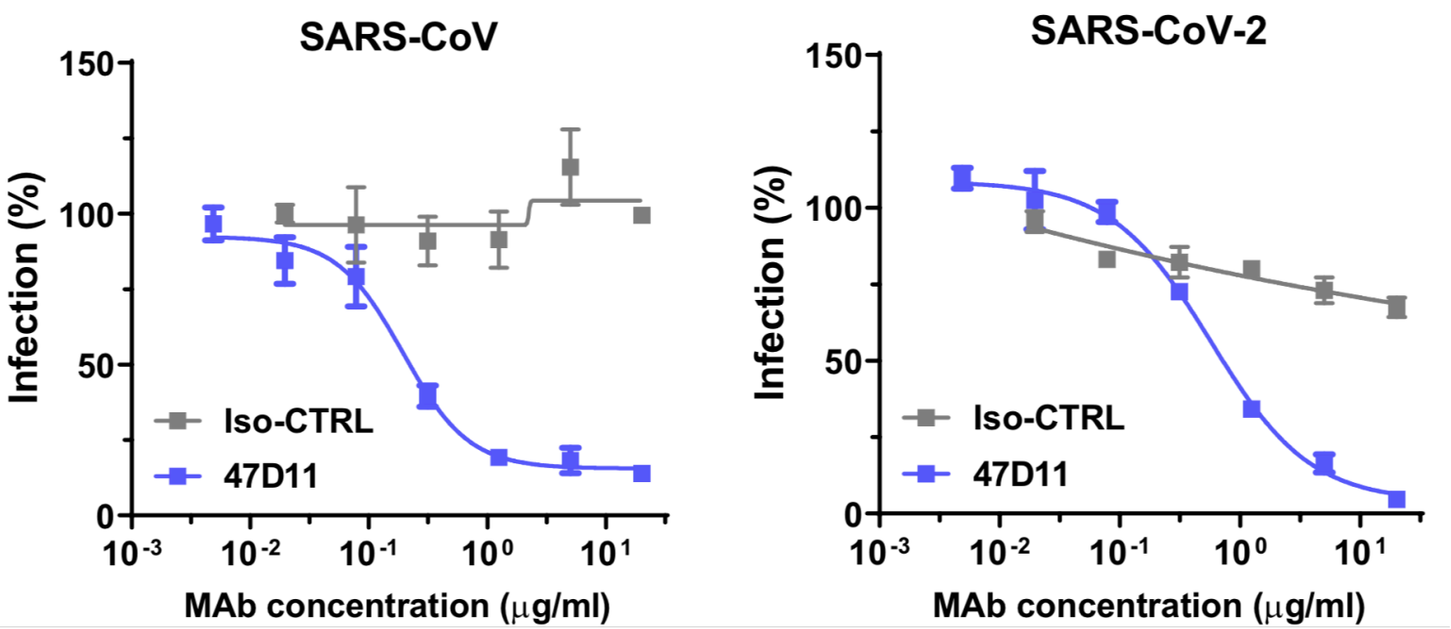
Chunyan Wang, Wentao Li, Dubravka Drabek, Nisreen M.A. Okba, Rien van Haperen,
Albert D.M.E. Osterhaus, Frank J.M. van Kuppeveld, Bart L. Haagmans, Frank Grosveld, Berend-Jan Bosch

**doi:** https://doi.org/10.1101/2020.03.11.987958



Generated using humanized mouse

Block SARS-CoV-2 infection in vitro



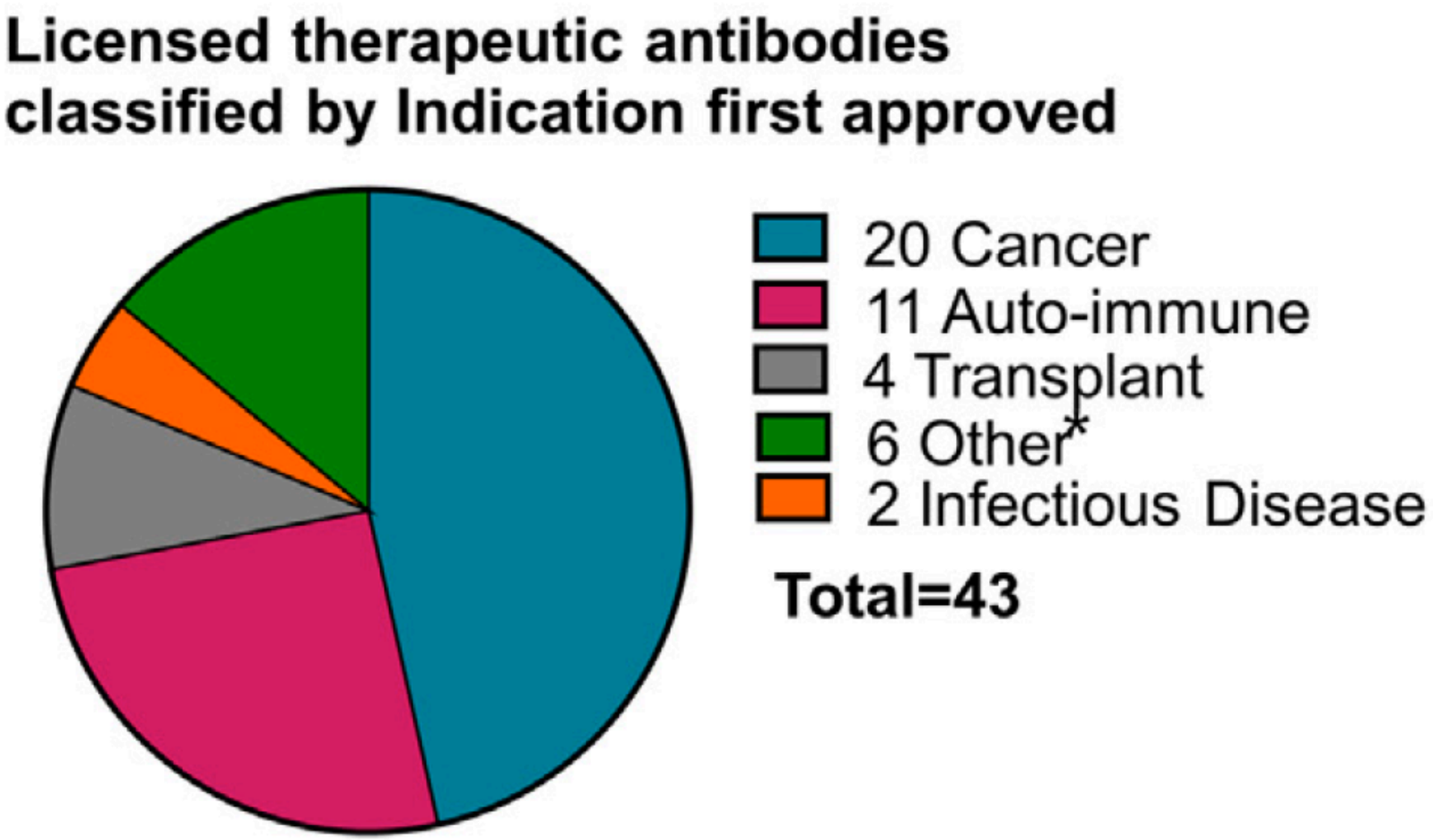
# Application of monoclonal antibodies

Therapy

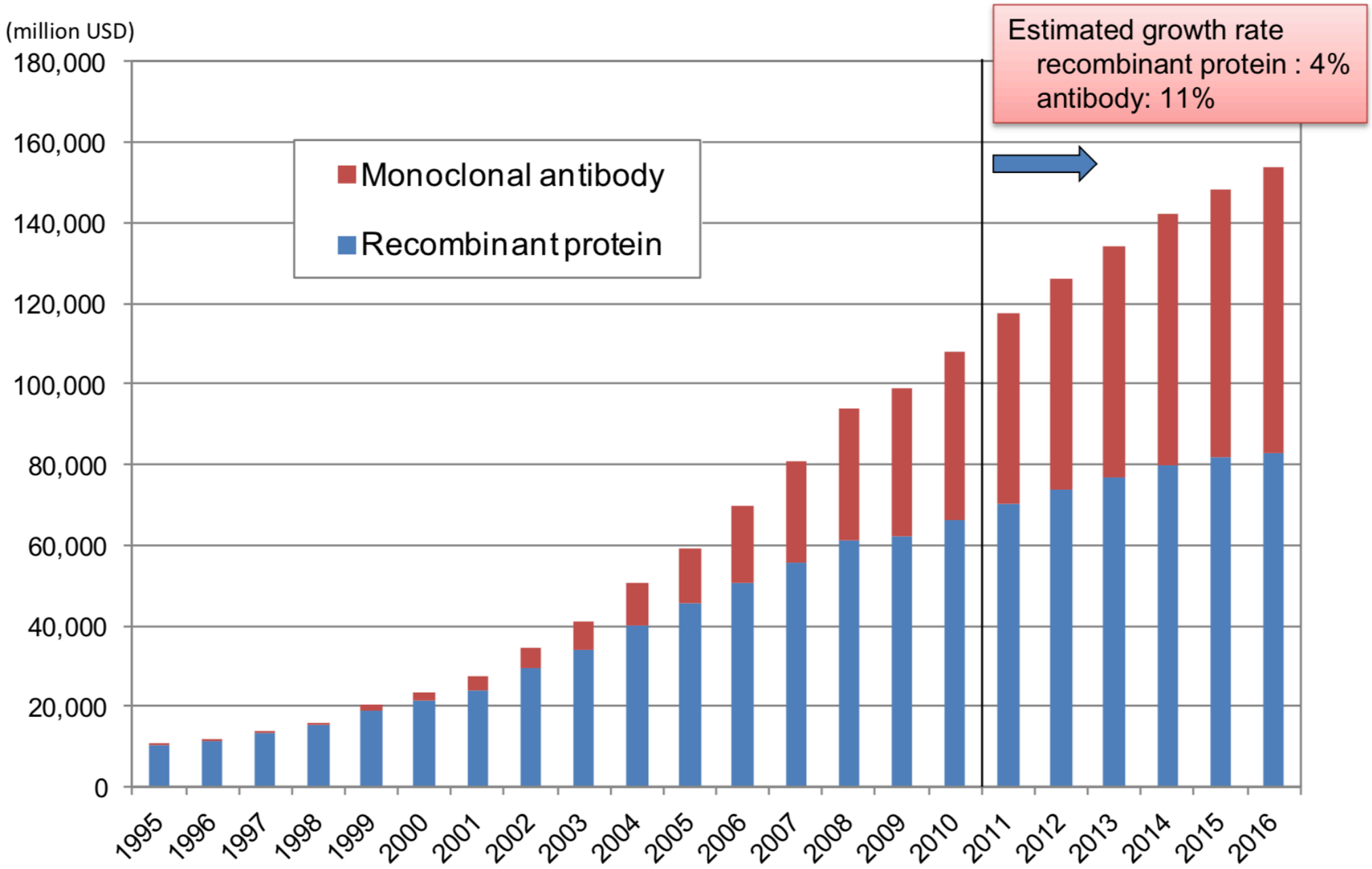
Diagnostics

Research

# Therapeutic antibodies



# Therapeutic antibodies drive growth of biopharmaceutical market



# Some mAbs used in clinics

**TABLE 5.3** Examples of Monoclonal Antibodies in Clinical Use

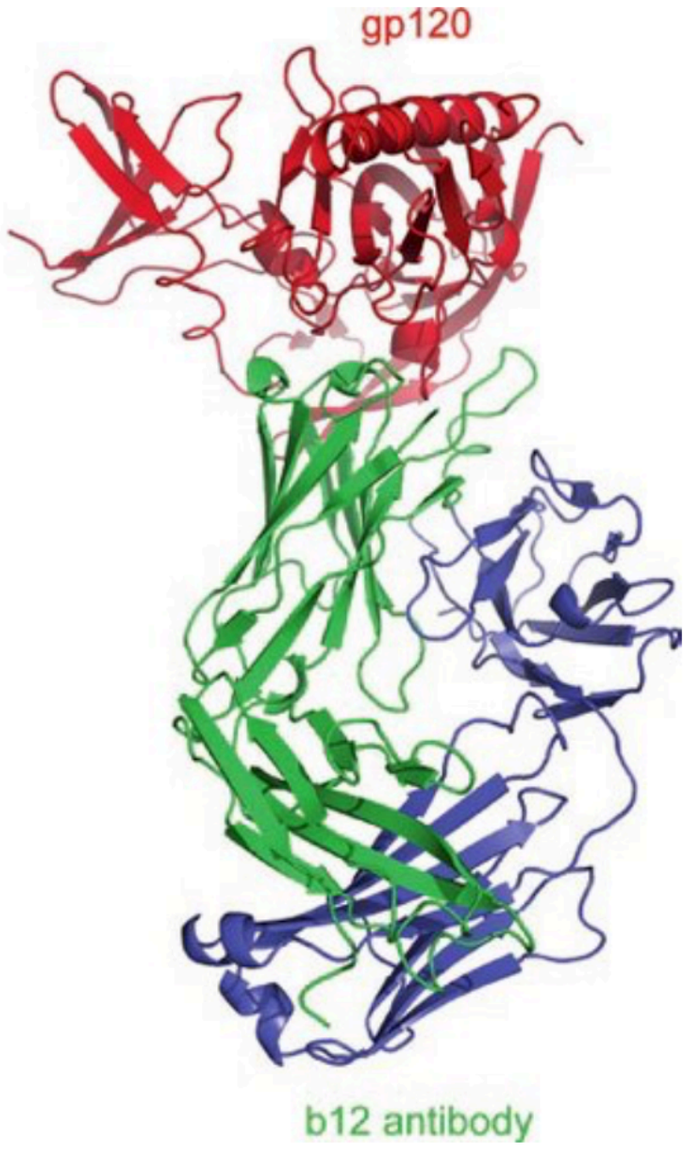
| Target | Effect | Diseases |
|---|---|---|
| **Inflammatory (Immunological) Diseases** | | |
| α4 integrins | Blocking of immune cell egress to intestine and CNS | Crohn's disease, multiple sclerosis |
| CD20 | Depletion of B cells | B cell lymphomas, rheumatoid arthritis, multiple sclerosis, other autoimmune diseases |
| IgE | Blocking of IgE function | Allergy related asthma |
| TNF | Blocking of inflammation | Rheumatoid arthritis, Crohn's disease, psoriasis |
| **Other Diseases** | | |
| C5 | Blocking of complement-mediated lysis | Paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome |
| Glycoprotein IIb/IIIa | Inhibition of platelet aggregation | Cardiovascular disease |
| RANK ligand | Blocking of RANK signaling | Postmenopausal osteoporosis, bone metastases of solid tumors |
| RSV F protein | Blocking of viral entry | Respiratory syncytial virus infection |
| **Cancer (see Table 18.1, Chapter 18)** | | |

*RANK*, receptor activator of nuclear factor-κB; *RSV*, respiratory syncytial virus; *TNF*, tumor necrosis factor. Additional anti-cytokine antibodies in clinical use are listed in Table 19.5, Chapter 19.

# Cross-reactive antibodies, sometimes to self

## Cardiolipin Polyspecific Autoreactivity in Two Broadly Neutralizing HIV-1 Antibodies

Barton F. Haynes,[1]* Judith Fleming,[1] E. William St. Clair,[1]
Herman Katinger,[2] Gabriela Stiegler,[2] Renate Kunert,[2]
James Robinson,[3] Richard M. Scearce,[1] Kelly Plonk,[1]
Herman F. Staats,[1] Thomas L. Ortel,[1]
Hua-Xin Liao,[1] S. Munir Alam[1]

# Effector mechanisms of humoral immunity

**Defense against  extracellular bacteria, fungi, and viruses when they are released from infected cells.**

**Mediated by secreted antibodies**

(How do antibodies do their job?)

# Antibodies production sites

Bone marrow (home of long-lived plasma cells)

Secondary lymphoid organs

Tissues

> sites of inflammation
> intestine lamina propria

# Antibodies act distant from their production sites

Antibodies are soluble molecules that are transported in the blood through the body

Antibodies can reach:

- Lumen of of the gastrointestinal and respiratory tracts (poly-Ig receptor)
- Placenta (FcRn)

Antibodies can NOT reach (in physiologic condition):

- Brain
- Eye
- Testis

# Main functions of antibodies

| | |
|---|---|
| Neutralization | The pathogen is not destroyed, but its capability to infect cells is impaired |
| Elimination | The pathogen itself, or cells infected with it, are destroyed |

# Main functions of antibodies

| | |
|---|---|
| Neutralization | The pathogen is not destroyed, but its capability to infect cells is impaired |
| | **Antibodies** — **Antigen binding site** |
| Elimination | The pathogen itself, or cells infected with it, are destroyed |
| | **Antibodies + other components (macrophages, NKs, complement)** — **Antigen binding site + Fc portion** |

# Different isotypes are recognized by different FcR

Different isotypes (IgM, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 and IgE) are characterized by **different Fc portion**

Different Fc portions are recognized by different Fc receptors (**FcR**)

**Specific FcRs are expressed by distinct immune cells, and that's how different isotypes can perform different effector functions**

# How many Fc Receptors?

| | FcR | Affinity for Immunoglobulin | Cell Distribution | Function |
|---|---|---|---|---|
| FcγR | FcγRI (CD64) | High ($K_d$ ~$10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG | Macrophages, neutrophils; also eosinophils | Phagocytosis; activation of phagocytes |
| | FcγRIIA (CD32) | Low ($K_d$ ~$10^{-7}$ M) | Macrophages, neutrophils, dendritic cells, eosinophils, platelets | Phagocytosis; cell activation |
| | FcγRIIB (CD32) | Low ($K_d$ ~$10^{-7}$ M) | B lymphocytes, macrophages, dendritic cells, other cells | Feedback inhibition of various cellular responses |
| | FcγRIIC (CD32) | Low ($K_d$ ~$10^{-7}$ M) | Macrophages, neutrophils, NK cells | Phagocytosis, cell activation |
| | FcγRIIIA (CD16) | Low ($K_d$ ~$10^{-6}$ M) | NK cells, macrophages, dendritic cells | Antibody-dependent cell-mediated cytotoxicity |
| | FcγRIIIB (CD16) | Low ($K_d$ ~$10^{-6}$ M); GPI-linked protein | Neutrophils | Phagocytosis (inefficient) |
| FcεR | FcεRI | High ($K_d$ ~$10^{-10}$ M); binds monomeric IgE | Mast cells, basophils, eosinophils | Cell activation (degranulation) |
| | FcεRII (CD23) | Low ($K_d$ ~$10^{-7}$ M) | B lymphocytes, eosinophils, Langerhans cells | Unknown |
| FcαR | FcαR (CD89) | Low ($K_d$ ~$10^{-6}$ M) | Neutrophils, eosinophils, monocytes | Cell activation? |