labmed. Ticino

Corso di formazione continua di labmed per tecnici in analisi biomediche

La scoperta e lo sviluppo di anticorpi monoclonali umani per la cura di malattie infettive

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Statements in this presentation that are not statements of historical fact are forward-looking statements. Such forward-looking statements include, without limitation, statements regarding our research and clinical development plans, expected manufacturing capabilities, strategy, regulatory matters, market size and opportunity and our ability to complete certain milestones. Words such as "believe," "anticipate," "plan," "expect," "intend," "will," "may," "goal," "potential" and similar expressions are intended to identify forward-looking statements, though not all forward-looking statements necessarily contain these identifying words. These forward-looking statements are based on the beliefs of the management of Vir Biotechnology, Inc. (the "Company") as well as assumptions made by and information currently available to the Company. Such statements reflect the current views of the Company with respect to future events and are subject to known and unknown risks, including business, regulatory, economic and competitive risks, uncertainties, contingencies and assumptions about the Company, including, without limitation, risks inherent in developing products and technologies, future results from the Company's ongoing and planned clinical trials, the Company's ability to obtain adequate financing to fund its planned clinical trials and other expenses, trends in the industry, the legal and regulatory framework for the industry and future expenditures. In light of these risks and uncertainties, the events or circumstances referred to in the forward-looking statements may not occur. The actual results may vary from the anticipated results and the variations may be material. Other factors that may cause the Company's actual results to differ from current expectations are discussed under the "Risk Factors" section of the Company's prospectus dated October 10, 2019 filed pursuant to Rule 424(b)(4) with the Securities and Exchange Commission. These forward-looking statements should not be taken as forecasts or promises nor should they be taken as implying any indication, assurance or guarantee that the assumptions on which such forward-looking statements have been made are correct or exhaustive or, in the case of the assumptions, fully stated in this presentation. You are cautioned not to place undue reliance on these forward-looking statements, which speak only as of the date this presentation is given. The Company claims the protection of the safe harbor for forward-looking statements contained in the Private Securities Litigation Reform Act of 1995 for all forward-looking statements.

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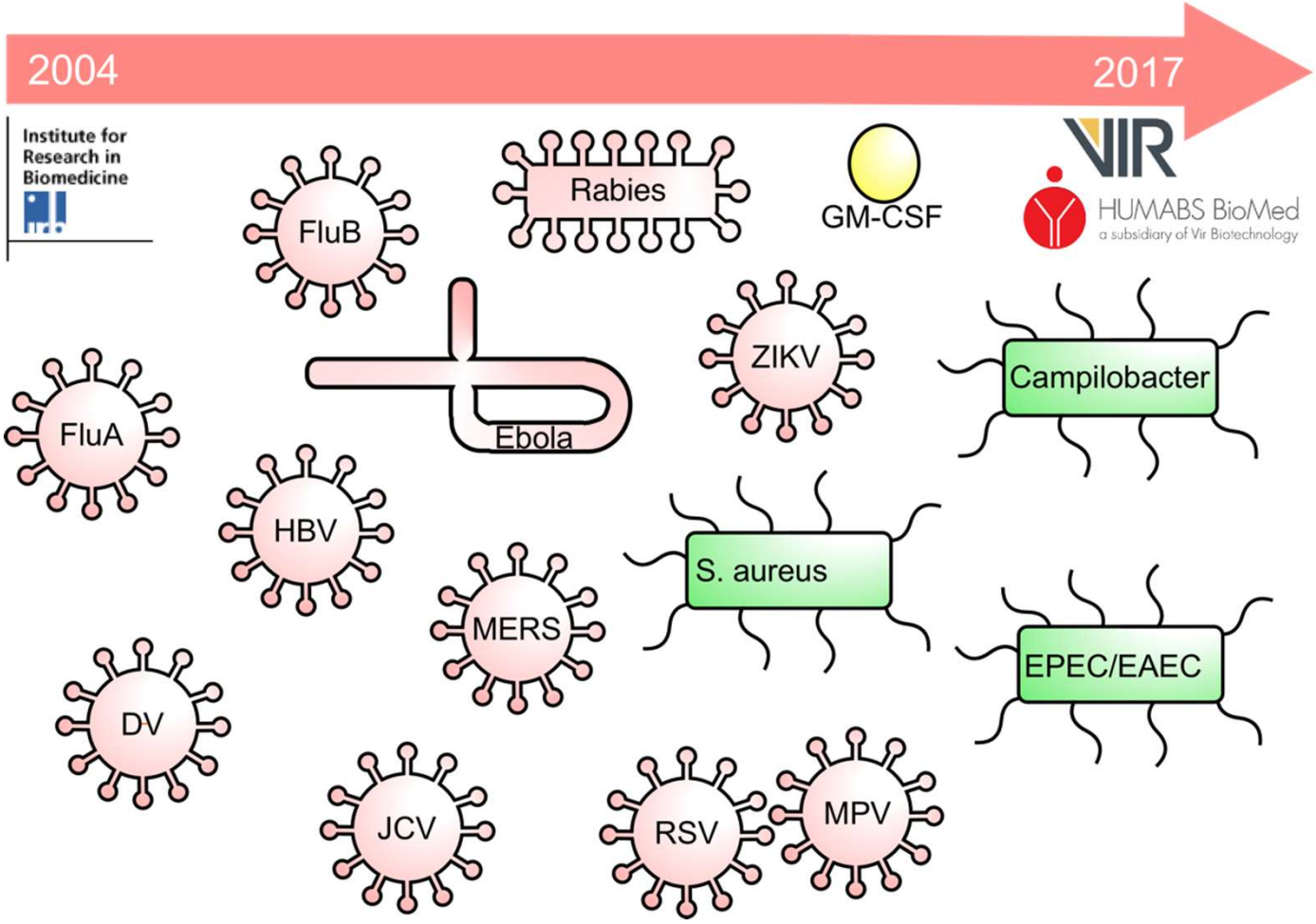
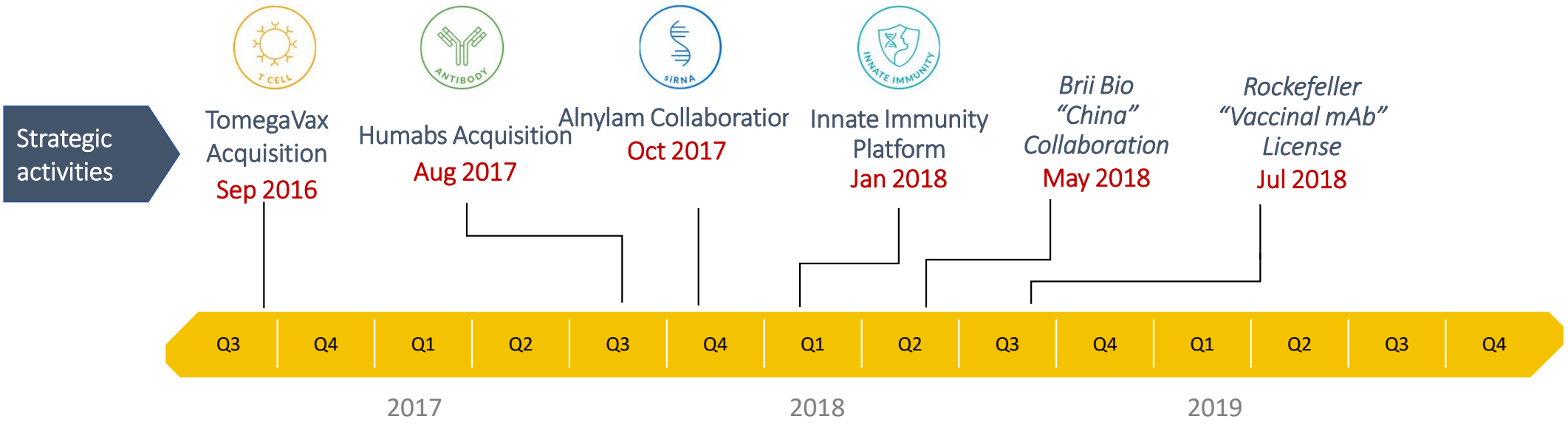
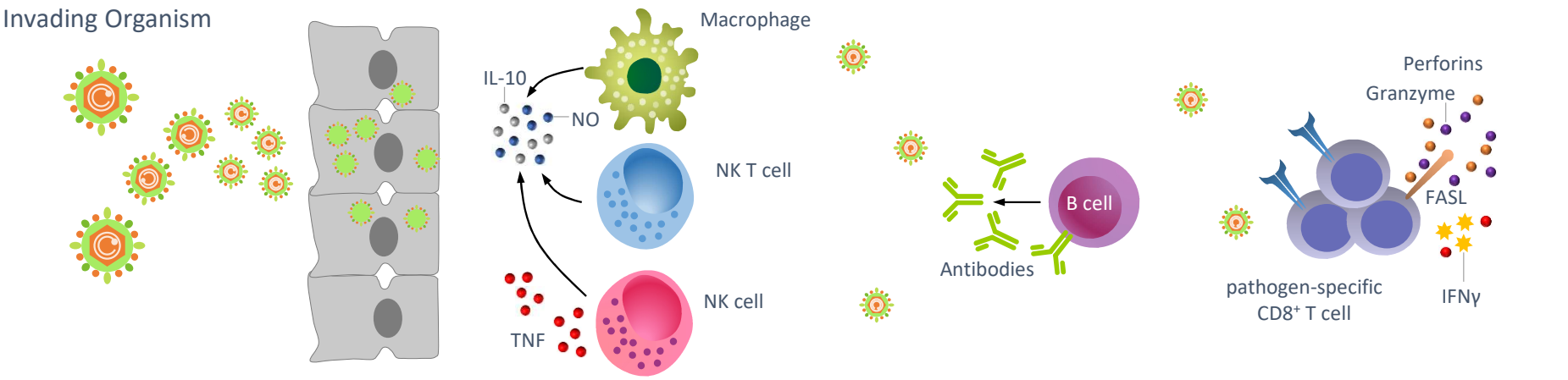


Vir Acquisitions, Licenses, and Collaborations



Vir Platforms: Augmenting the Components of the Immune System



siRNA

- Immunomodulation
- Direct Antiviral

Innate Immunity

- Potency
- Selectivity

Antibodies

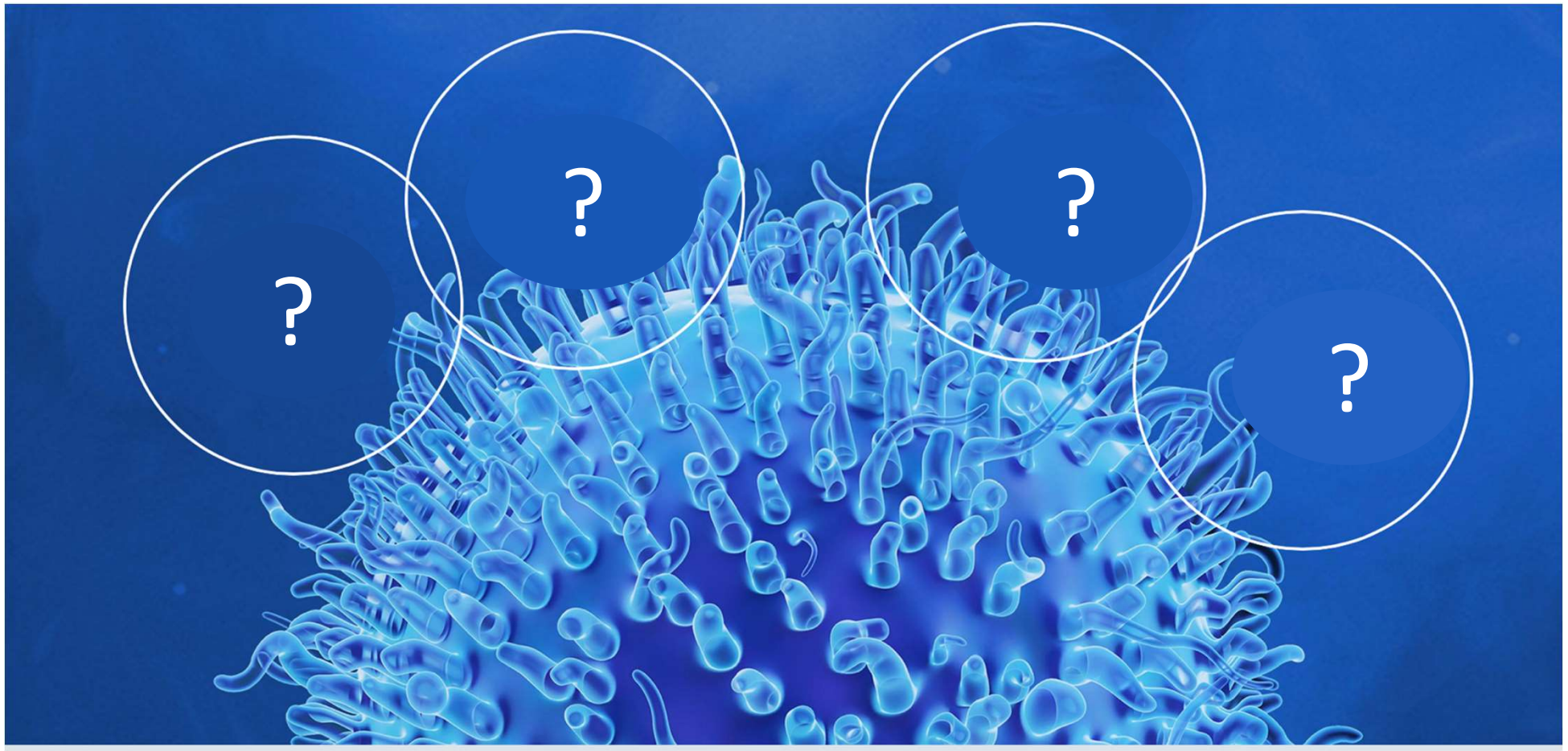
- Coverage
- Vaccinal effect
- Half-life

T cells

- Potency and type
- Universality
- Antigen coverage

NK: Natural killer; INF: Interferon; FASL: FAS Ligand; TNF: Tumor Necrosing Factor; NO: Nitric Oxide; IL: Interleukin

Which disease?



Clear Unmet Need

1,700,000,000 People worldwide latently infected with **TB**

290,000,000 People worldwide living with **chronic HBV**

37,000,000 People worldwide infected with **HIV**

2,000,000 U.S. **antibiotic resistance** cases annually

240,000 U.S. hospitalizations for **RSV** annually

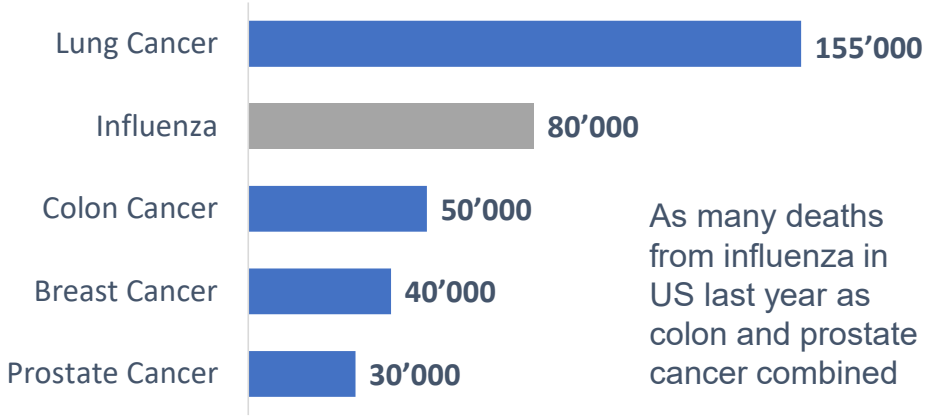
80,000 U.S. deaths from **influenza** in 2017/2018

Statistics are from: World Health Organization, U.S. Center for Disease Control, Razavi et al, Lancet 2018. RSV: Respiratory Syncytial Virus

Infectious Disease Unmet Need: Influenza

Despite a seasonal influenza vaccine, in 2017/18 in the US:

ESTIMATED DEATHS



As many deaths from influenza in US last year as colon and prostate cancer combined

ESTIMATED INFLUENZA DISEASE BURDEN

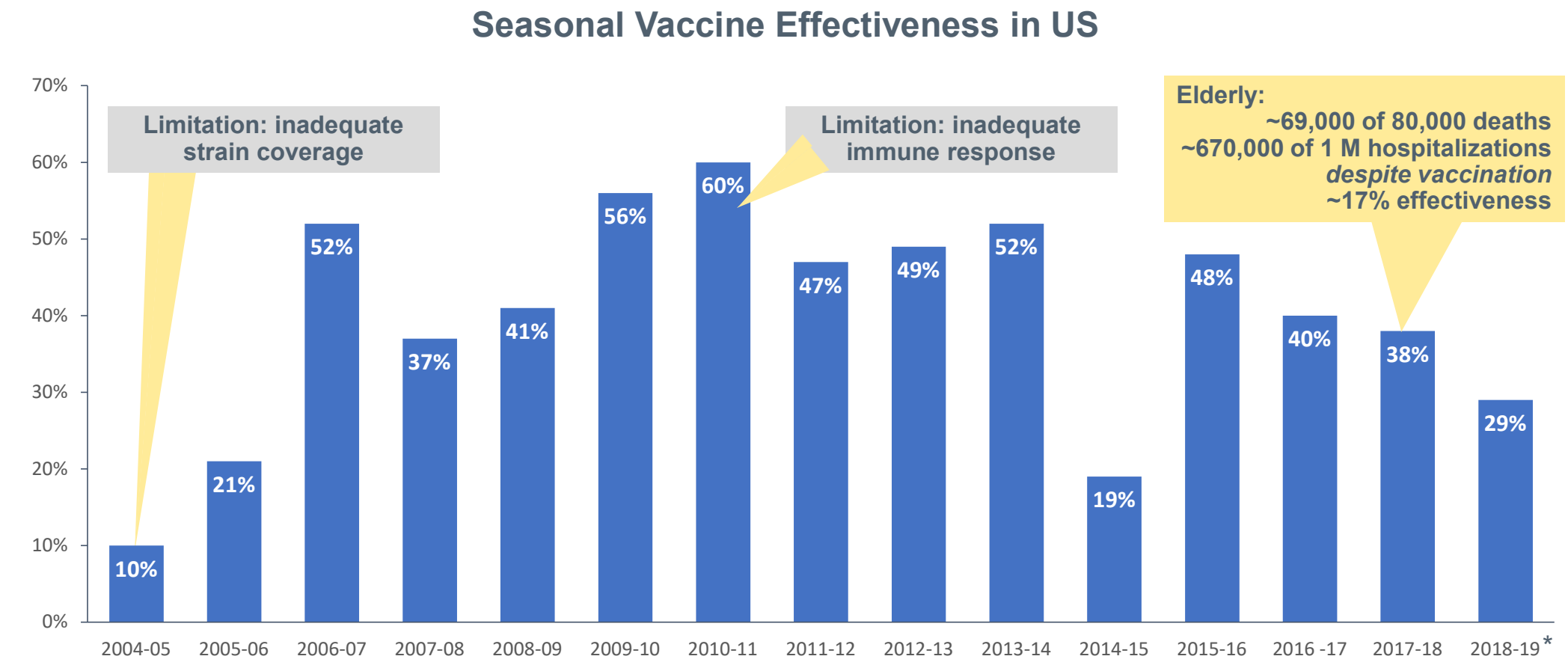
48 M
influenza illnesses

1 M
influenza-related hospitalizations

$87 B
annual influenza-related economic burden
(Molinari et al, 2007)

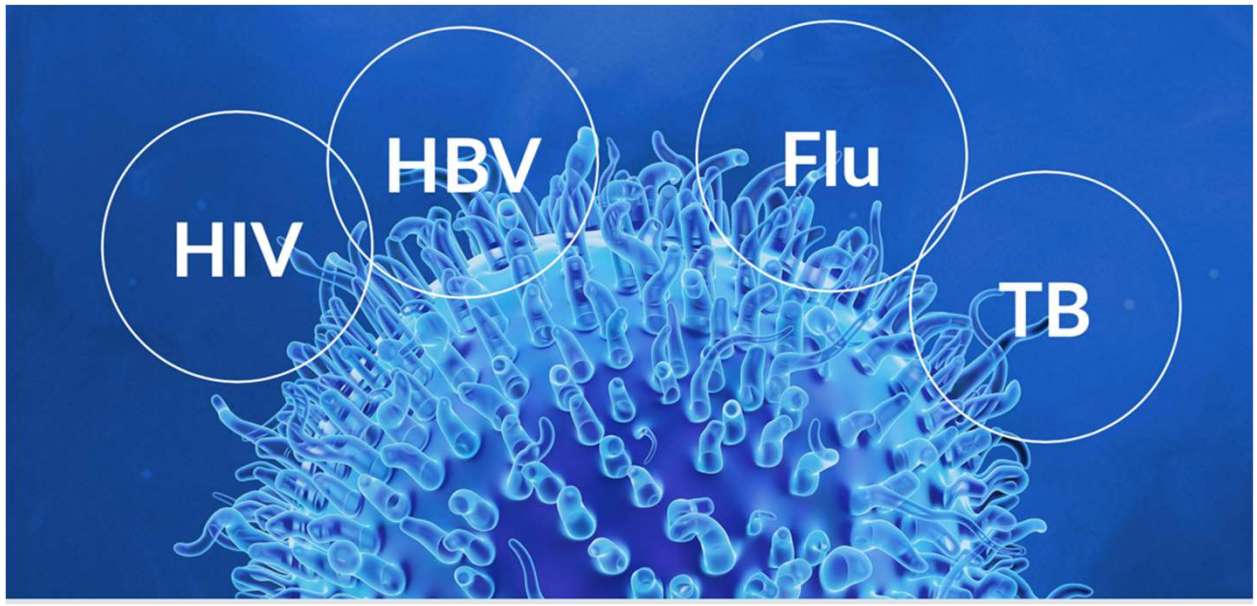
Statistics from US Centers for Disease Control and Prevention and National Institutes of Health.

Influenza Vaccines Have Limited Effectiveness



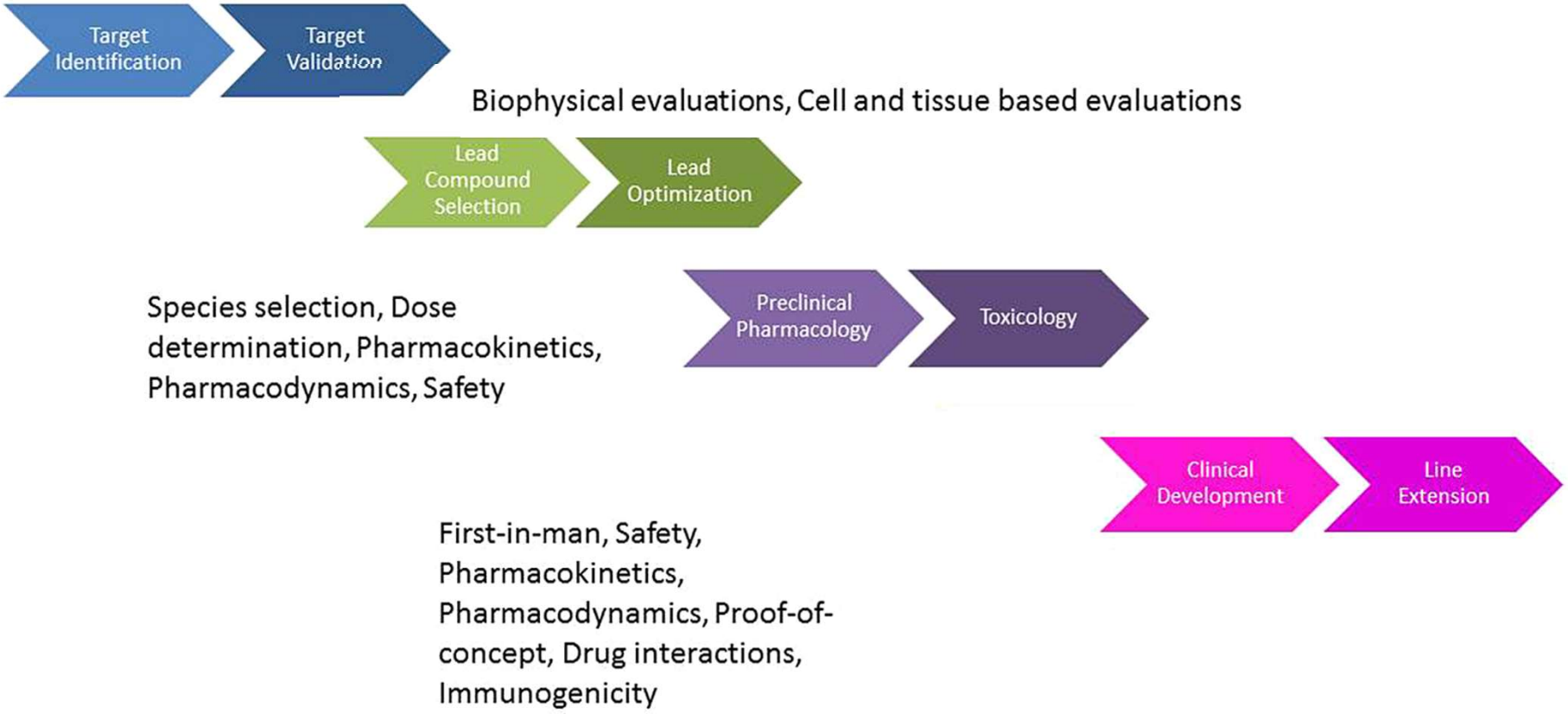
**Initial estimate.*

Statistics from Centers for Disease Control and Prevention.



		Platform	Pre-clinical	Phase 1	Phase 2	Phase 3	
HBV	VIR-2218	siRNA					Alnylam Collaborator
	VIR-3434	Antibody					
Influenza A	VIR-2482	Antibody					
HIV	VIR-1111	T Cell					Bill & Melinda Gates Foundation Collaborator
TB	VIR-2020	T Cell					Bill & Melinda Gates Foundation Collaborator

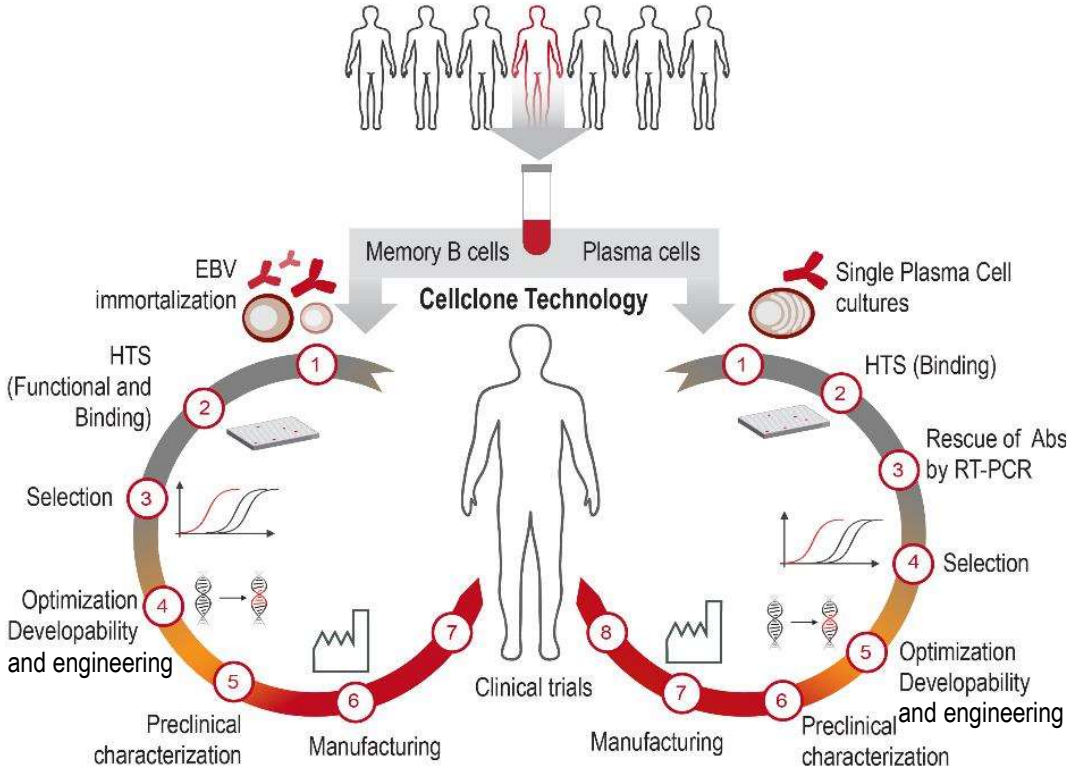
The pathway to drug development



Mould DR et al. BioDrugs 2016

Discovery: *Cellclone* Technology

- Discovery platforms and know-how for rapid lead generation of best-in-class anti-infective human monoclonal therapeutic antibodies from human responders



- **Validated** - Generation of best-in-class antibodies with high affinity, stability and expression levels for economic manufacturing
- **High-quality leads** - Cloning memory B and plasma cells from individuals who have had a strong immune response to pathogens or who survived severe infection
- **Rapid** - "target-agnostic" assays for screening allow for identification of functional antibodies with drug-like properties, thus accelerating the process to clinical development

Traggiai et al., Nat. Med. 2004; Corti et al., Science 2011; Corti et al., Nature 2013

Cellclone technology – MAB114

2007

Ebola survivor from **1995 Kikwit epidemic** identified who maintained circulating mAbs against the virus more than 10 years after infection

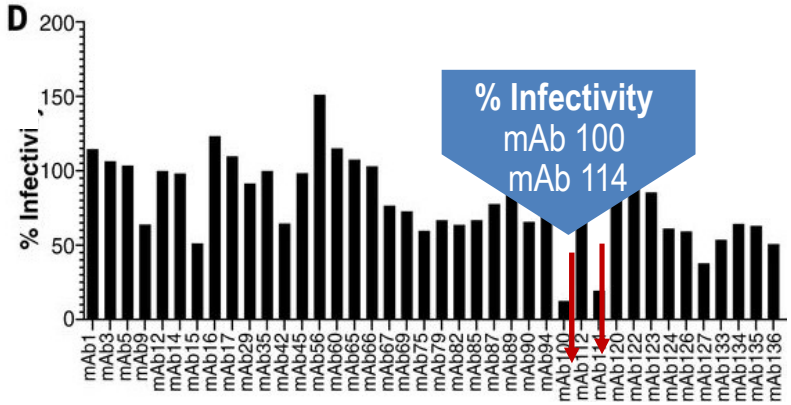
Cloned and screened **>10,000 memory B-cells** from survivor. Just **2 mAbs** blocked infectivity

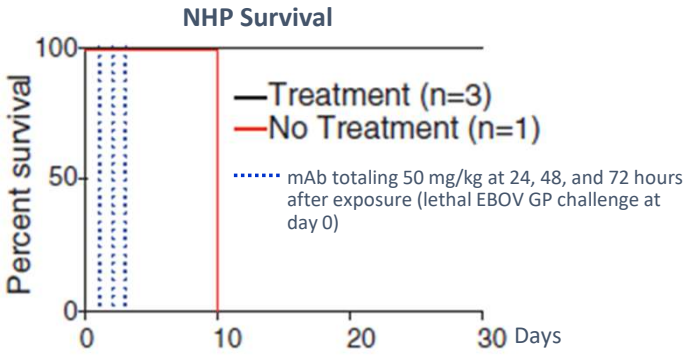
2014-2016

Tested mAb114 in Non-Human Primates (NHP)

Demonstrated mAb114 is an effective monotherapy even 5 days after infection

*mAb114 was being developed by NIH in 2018 and was licensed to Ridgeback Biotherapeutics LP in 2018 for further development with NIH





D. Corti et al. Science 2016

***Isolation and Characterization of mAb114 Collaboration**: IRB; Humabs BioMed SA; National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center (VRC); National Institute of Biomedical Research (INRB) in the DRC; U.S. Army Medical Research Institute of Infectious Diseases*

Ebola virus - 2019

NIH National Institutes of Health
Turning Discovery Into Health

Wednesday, May 23, 2018

NIH begins testing Ebola treatment in early-stage trial

Scientists developed monoclonal antibody from Ebola survivor.

THE AFRICA REPORT

Posted on Tuesday, 21 August 2018 13:08

Congo's experimental mAb114 Ebola treatment appears successful: authorities

By **Reuters**

STAT

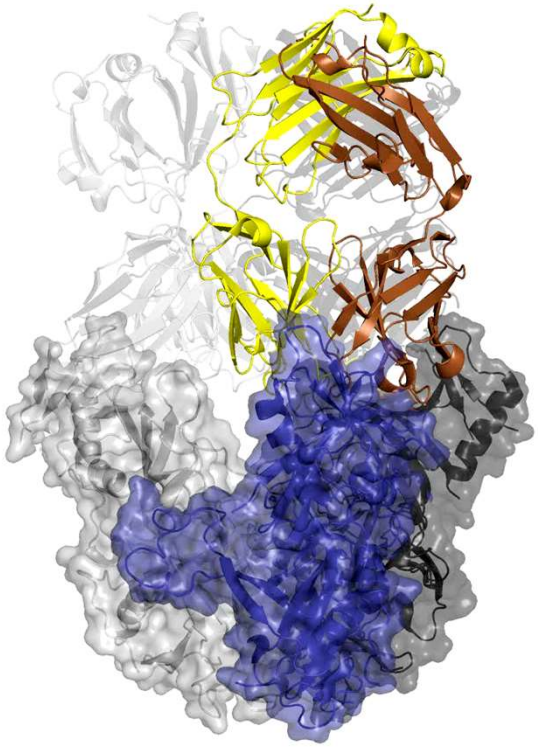
A pivotal day in world's response to Ebola nears: the launch of a clinical trial

By HELEN BRANSWELL @HelenBranswell / NOVEMBER 12, 2018

BBC

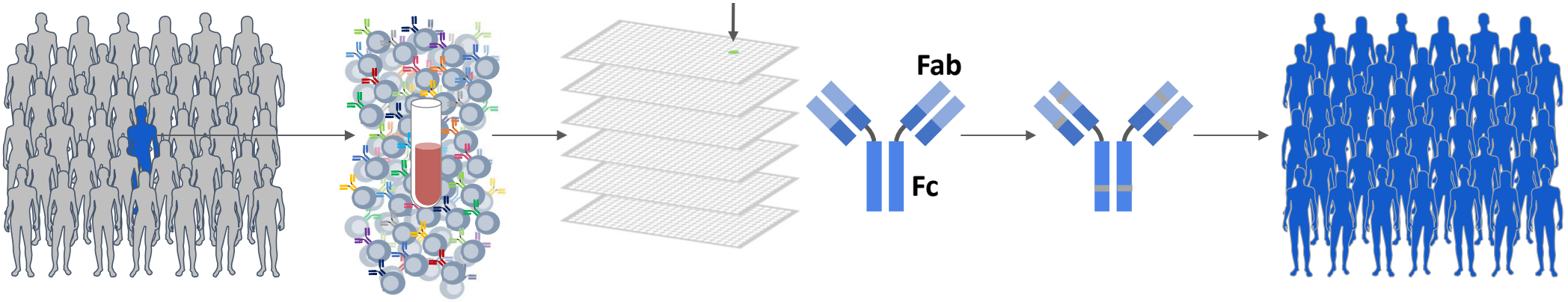
Ebola drugs show '90% survival rate' in breakthrough trial

Ebola may soon be a "preventable and treatable" disease after a trial of two drugs showed significantly improved survival rates, scientists have said.



**Certain of the data presented here was developed by NIH and/or Ridgeback Biotherapeutics LP without the involvement of Humabs Biomed or Vir

Antibody Platform: Potential to Create Optimal Antibodies for Infection



Identify individuals with the best possible fully human antibodies

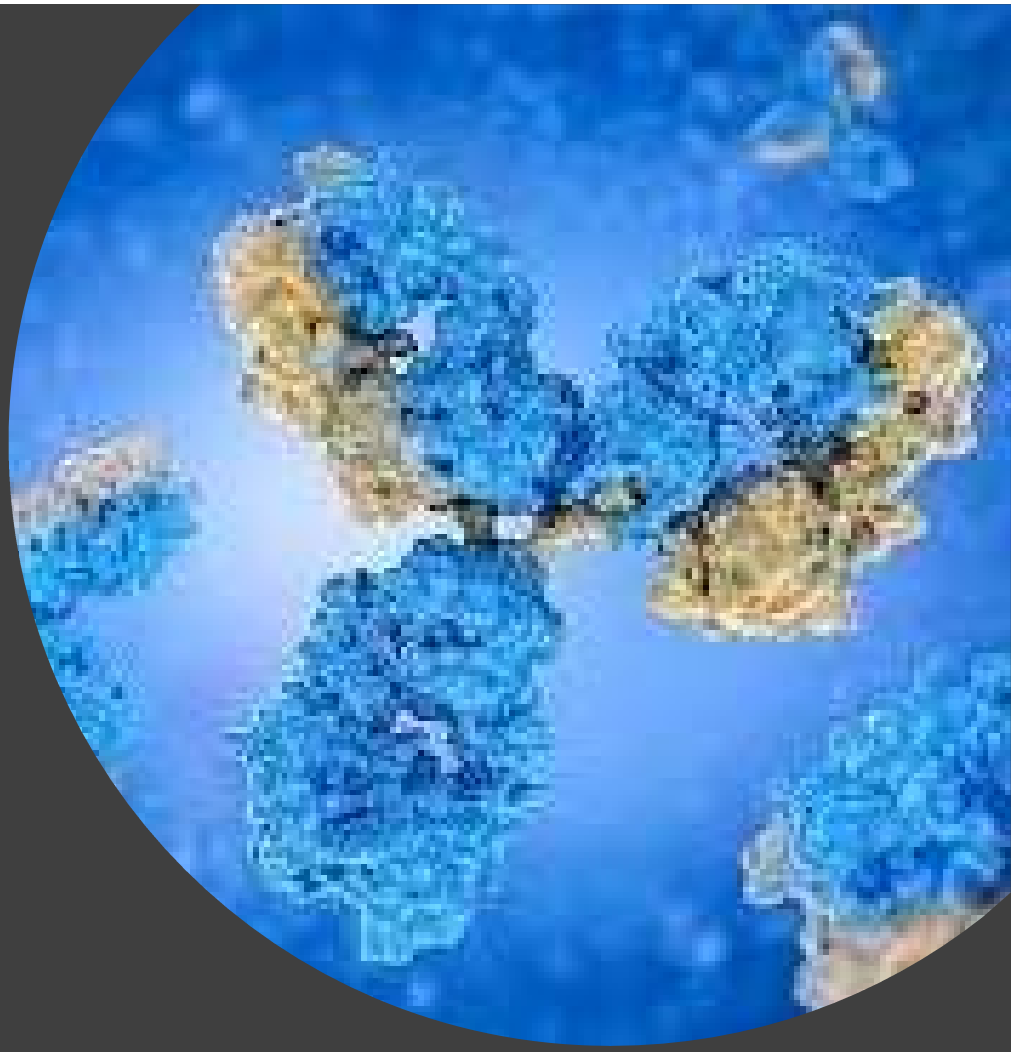
Screen 100s of millions of B cells to identify the rare antibodies with optimal characteristics

Engineer Fab and Fc regions to enhance efficacy

Transfer protective immunity

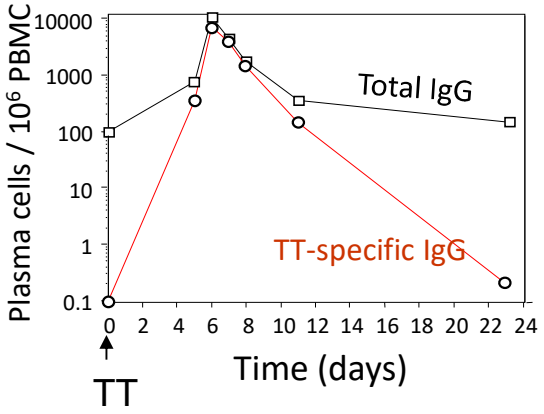
Antibodies

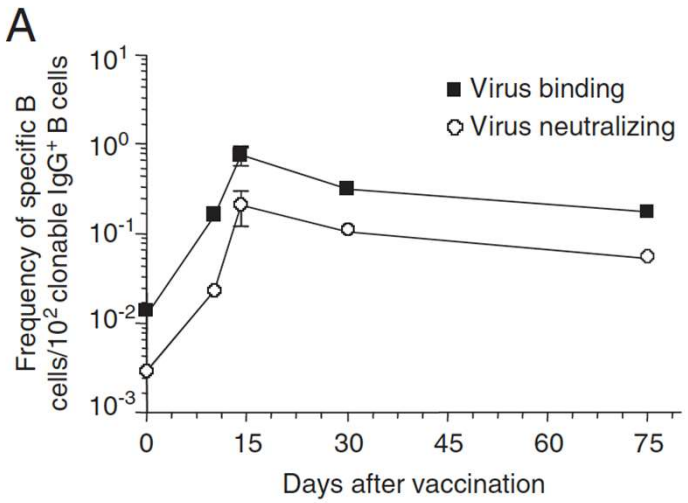
- KEY CONCEPTS



SOURCES OF ANTIBODIES: MEMORY B CELLS vs PLASMA CELLS

- Pathogens and vaccines expand antigen-specific B cells that proliferate and generate plasma cells and memory B cells
- **Plasma cells** highly enriched for a given antigenic specificity are readily accessible in peripheral blood one week after antigenic boost
- **Memory B cells** are present in peripheral blood for the lifetime of an individual

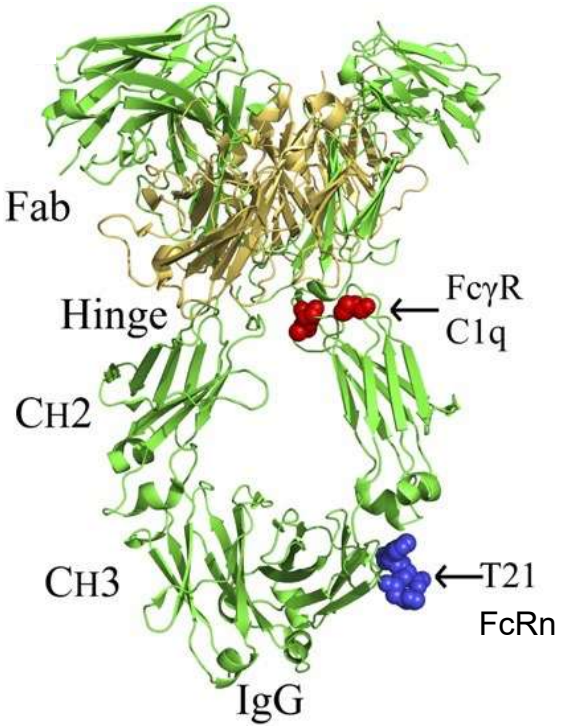




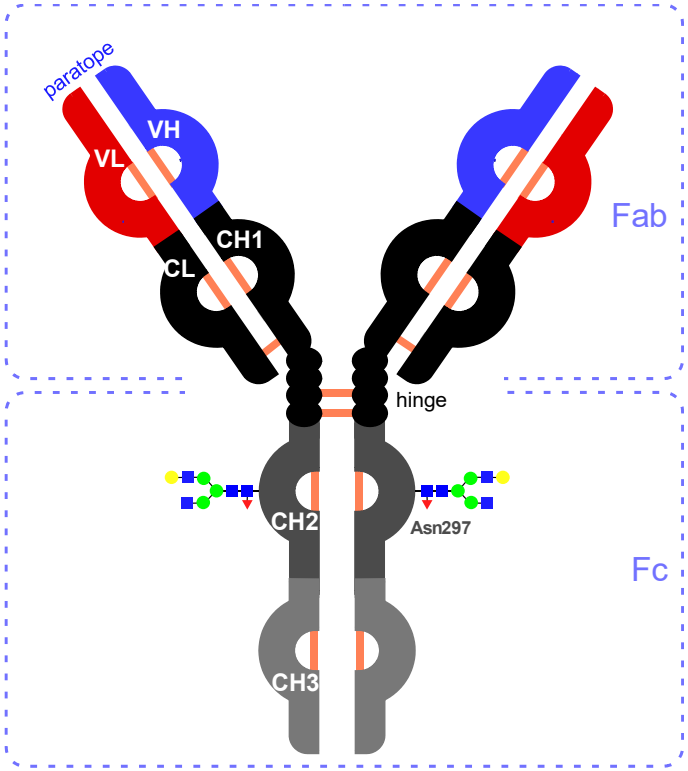
KEY CONCEPTS

The antibody molecule

- **Specificity**
- **Effector function**
- **Safety**
- **Long half life**



www.jbc.org



binding to antigen

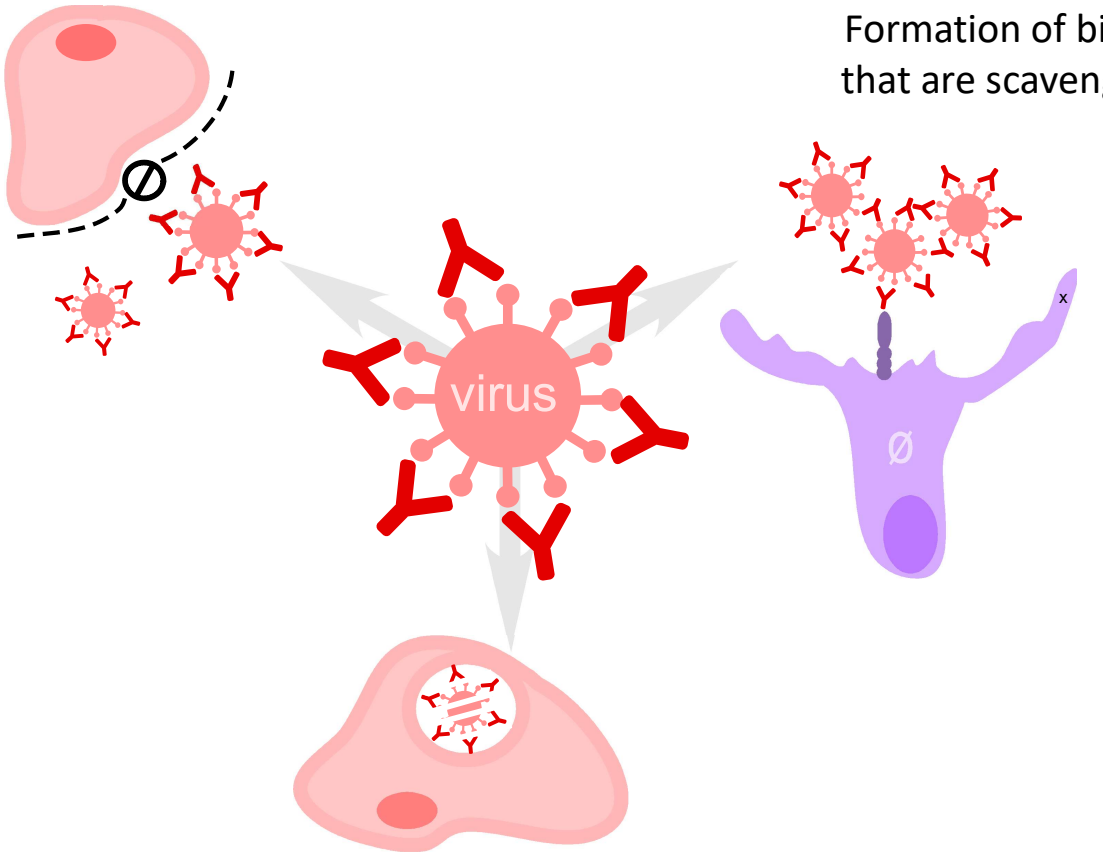
binding to receptors

KEY CONCEPTS

Antiviral activities of antibodies

Opsonized virus cannot infect new cells

Formation of big immunocomplexes that are scavenged by macrophages.



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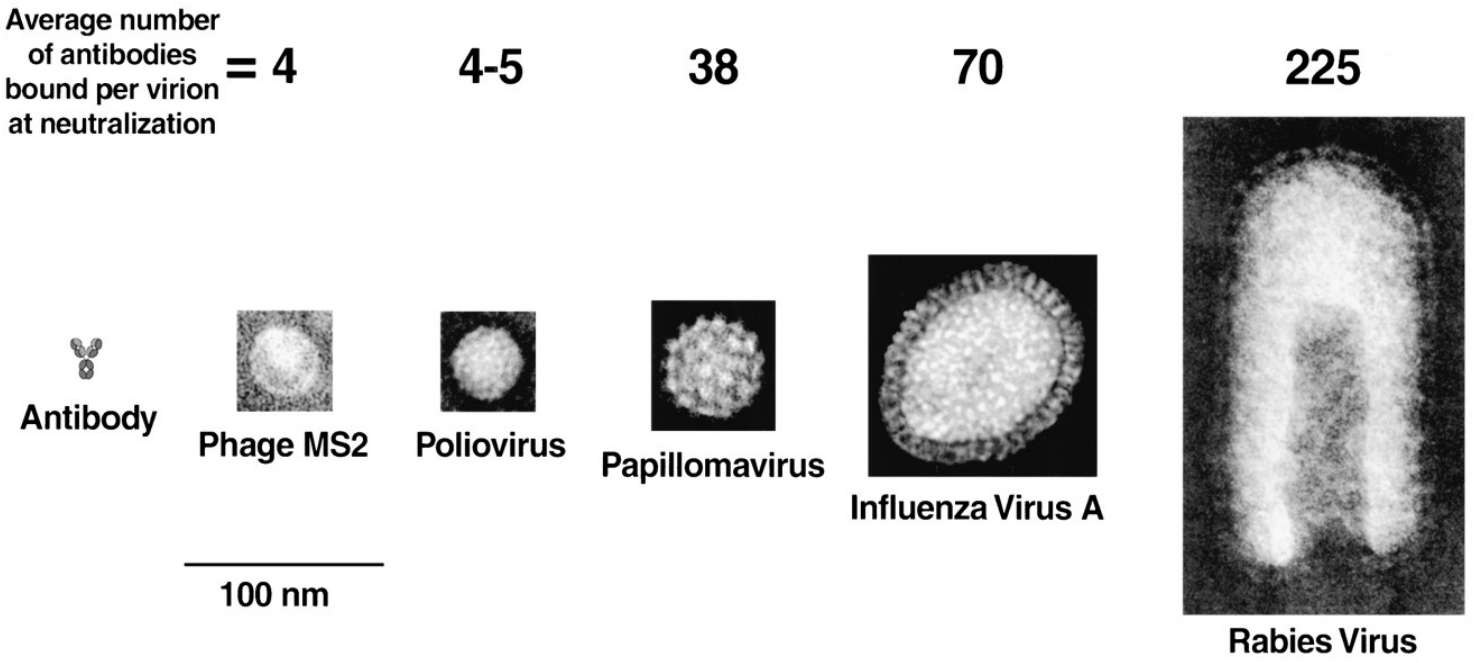
The antibody bound to the virus is engulfed by the infected cells and addressed to the intracellular degradation pathway.

KEY CONCEPTS

Antiviral activities of antibodies: neutralization

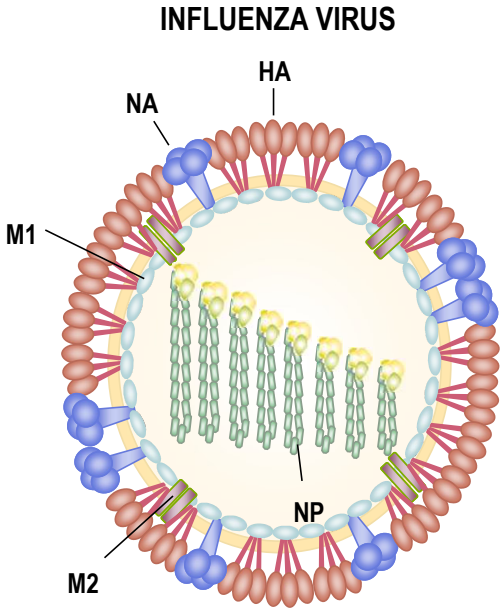


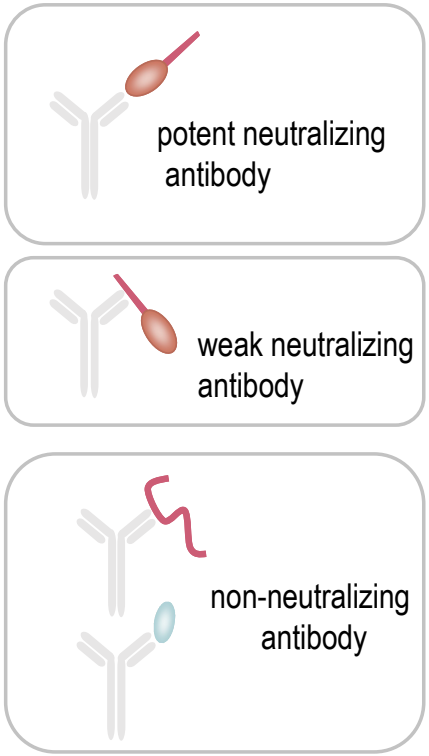
Some reports indicate that for Poliovirus one Ab may be sufficient

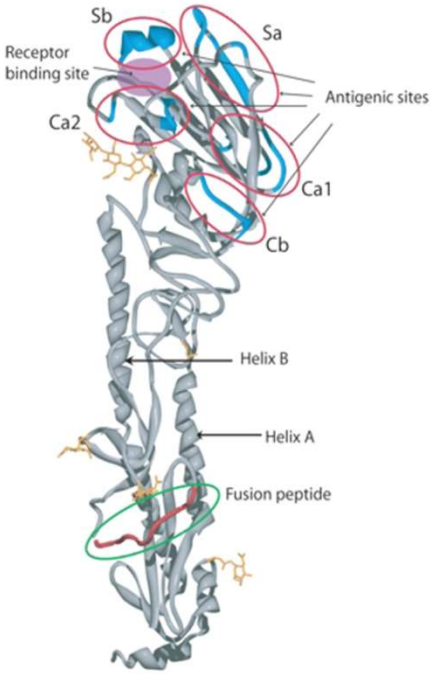
Burton Adv Immunol 2005

KEY CONCEPTS

Antiviral activities of antibodies: neutralization







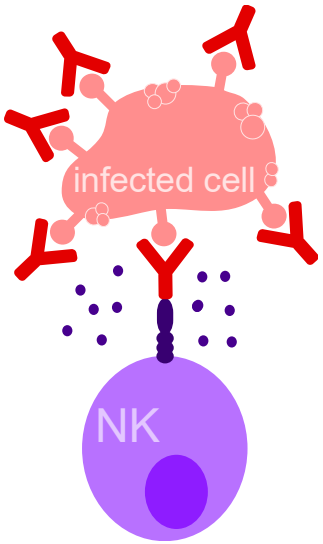
Neutralizing efficiency depends on:

- affinity, avidity and kinetics
- accessibility of the epitope on infectious virus

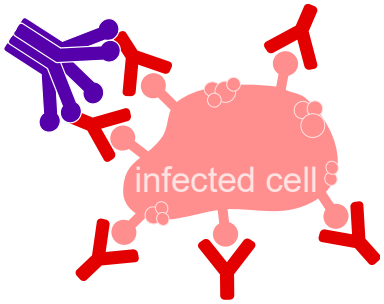
Burton Adv Immunol 2005

KEY CONCEPTS

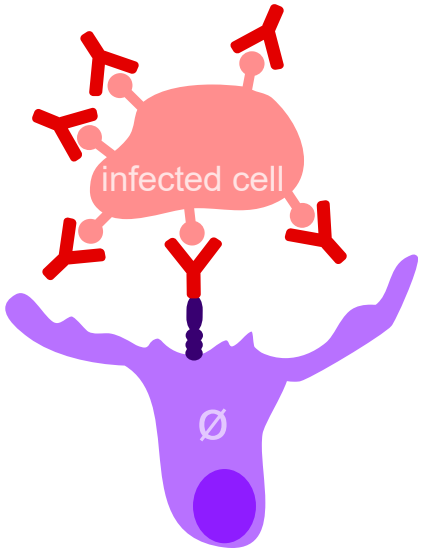
Effector Functions of antibodies



Antibody-dependent cell-mediated cytotoxicity (ADCC)



Complement-dependent cytotoxicity (CDC)



Antibody-dependent cellular phagocytosis

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KEY CONCEPTS

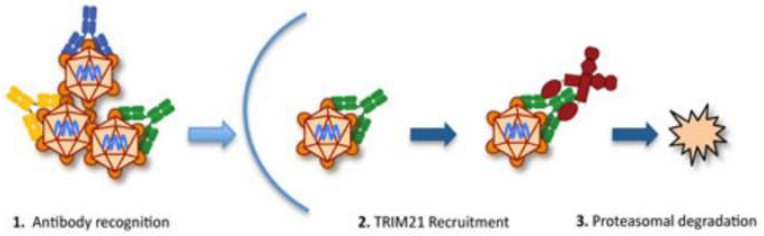
Antibodies mediate intracellular immunity through TRIM21, a cytosolic FcR

Non enveloped viruses such as polioma can be neutralized by a single IgG molecule

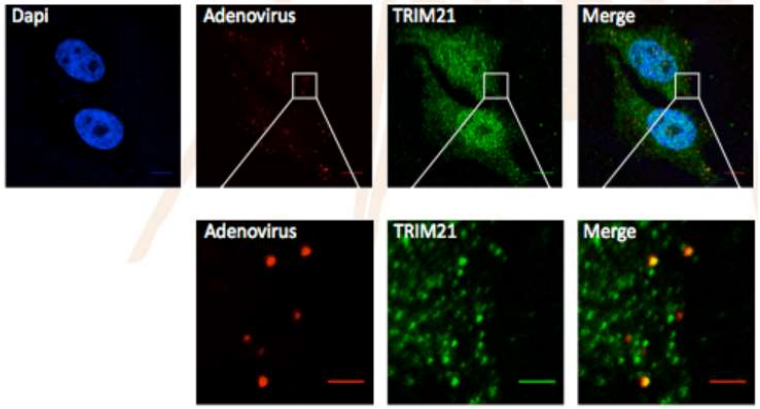
The virus penetrates into the cytosol with the IgG antibody bound

The antibody recruits TRIM21, a high affinity cytosolic IgG and IgM FcR with E3 ligase activity

TRIM21 catalyzes ubiquitination leading to proteosomal degradation of the complex



Intracellular antibody immmunity

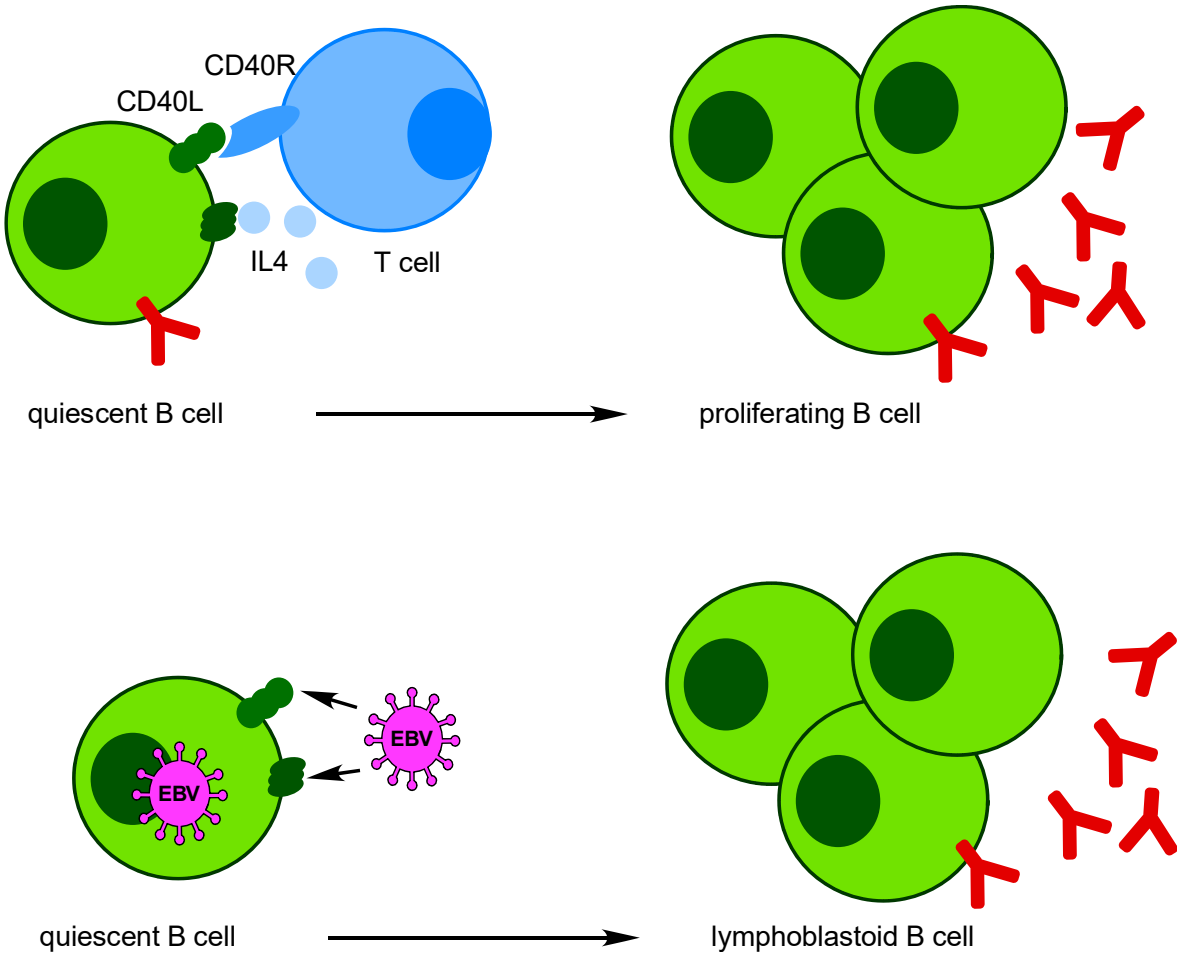


Antibody-coated adenovirus is targeted by TRIM21 inside cells

Mallery et al PNAS 2010

KEY CONCEPTS

Production of antibodies



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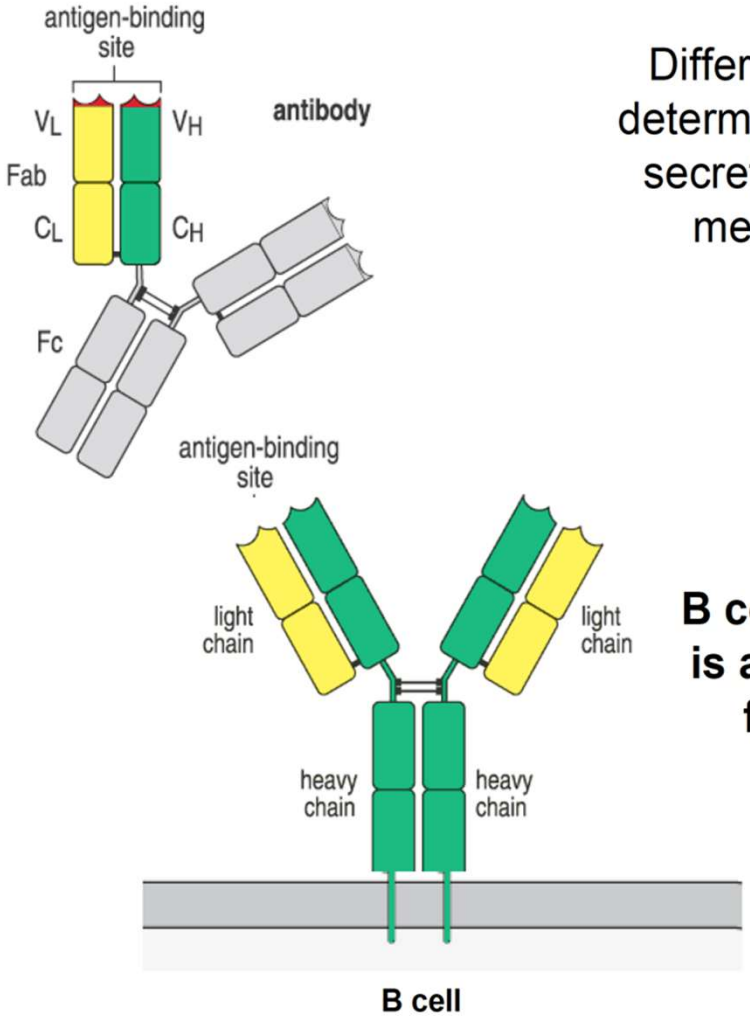
KEY CONCEPTS

BCR
B Cell receptor



Differential RNA splicing determines if an antibody is secreted or remains as a membrane receptor

B cell antigen receptor is a membrane bound form of antibody

KEY CONCEPTS

Genetic source of variability

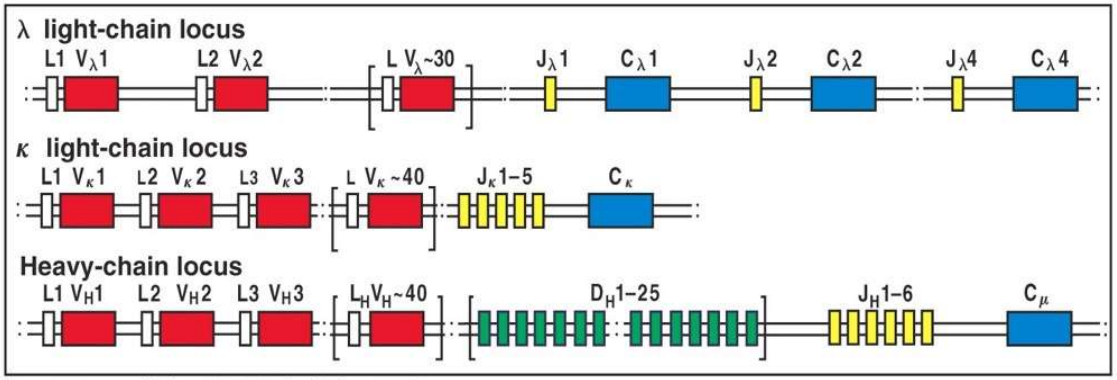


Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)

Number of functional gene segments in human immunoglobulin loci

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	40
Diversity (D)	0	0	25
Joining (J)	5	4	6

Figure 4-3 Immunobiology, 6/e. (© Garland Science 2005)

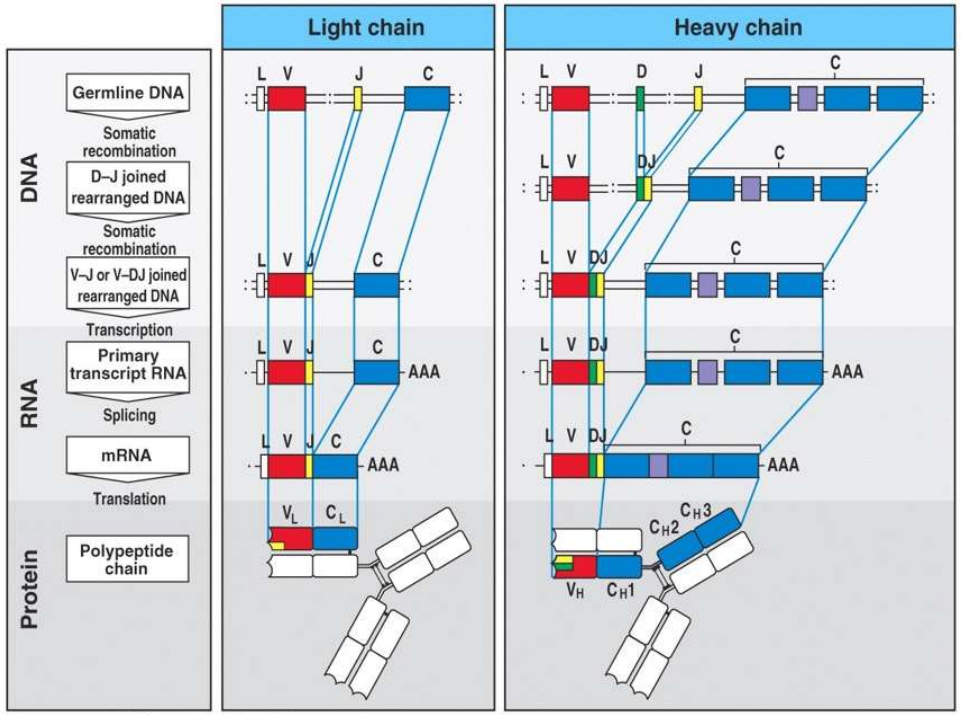


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

KEY CONCEPTS

Antibody CDRs

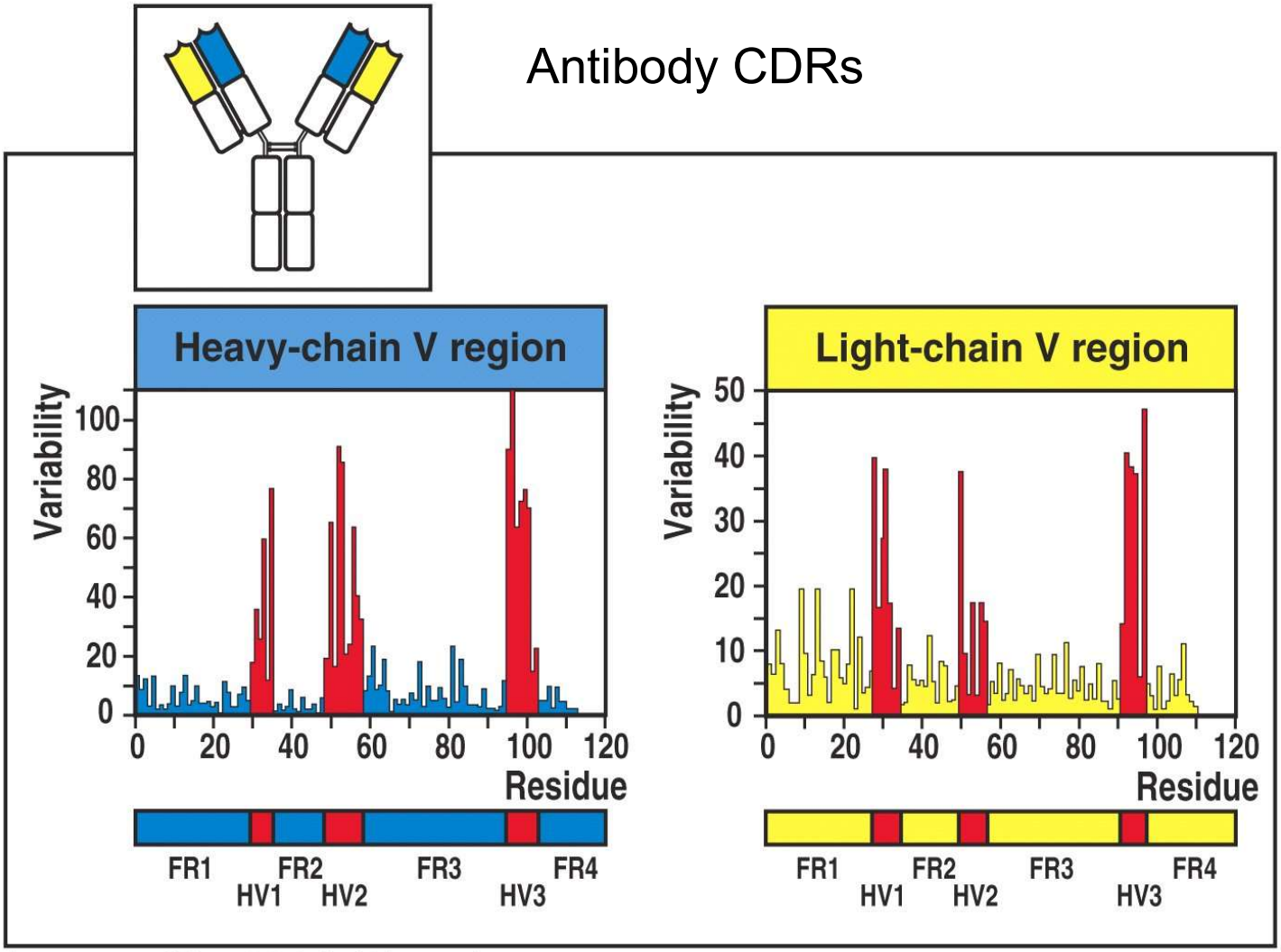
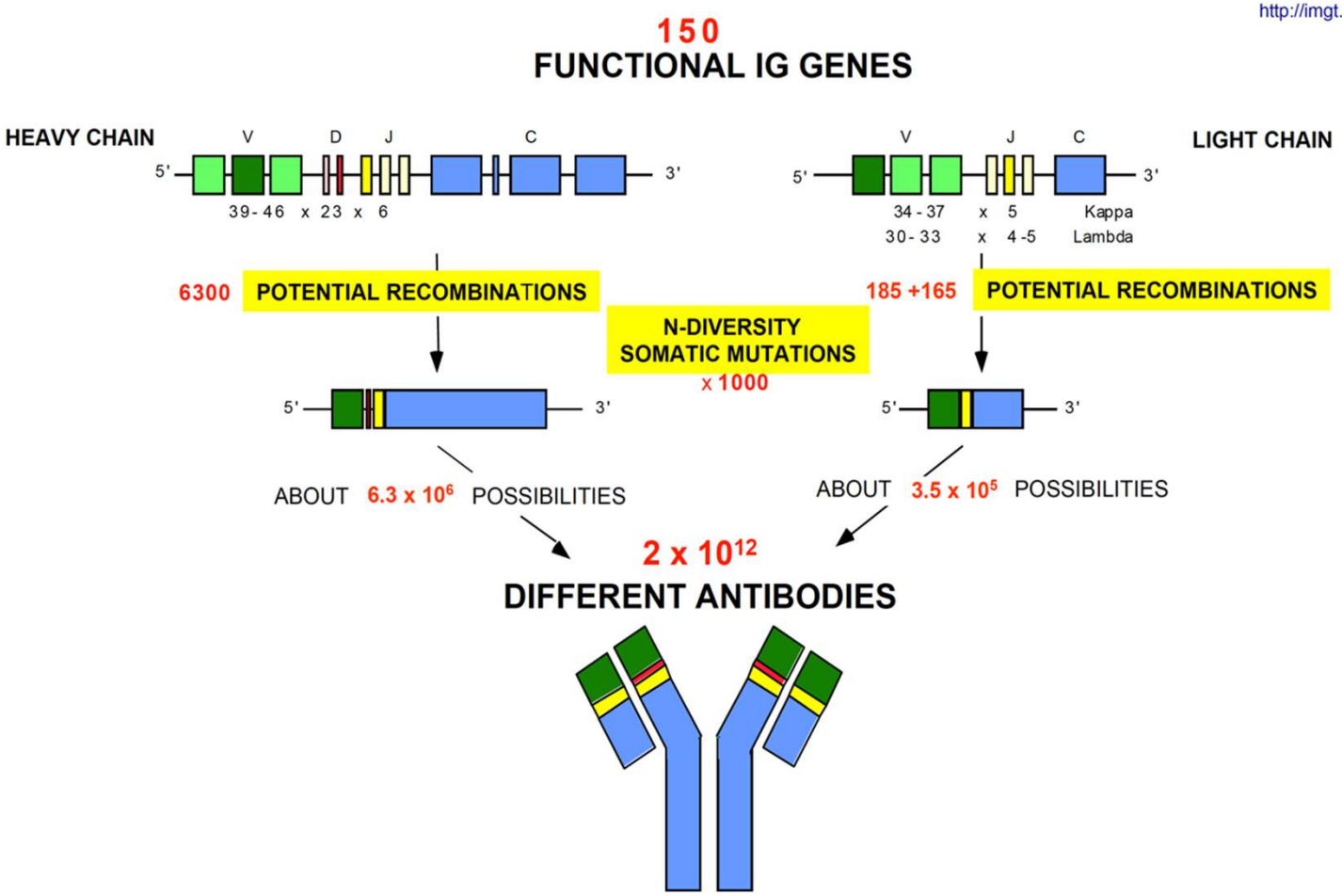


Figure 3-6 Immunobiology, 6/e. (© Garland Science 2005)

KEY CONCEPTS

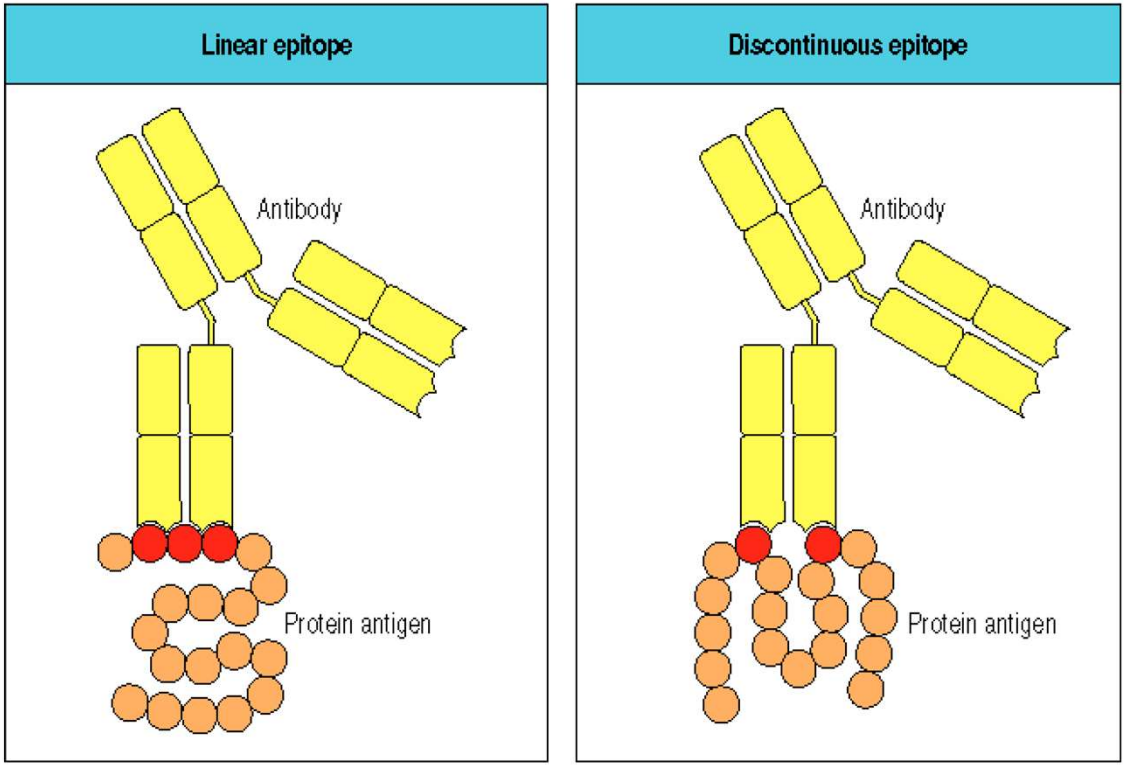
Immunoglobulin (IG) synthesis

http://imgt.cines.fr



KEY CONCEPTS

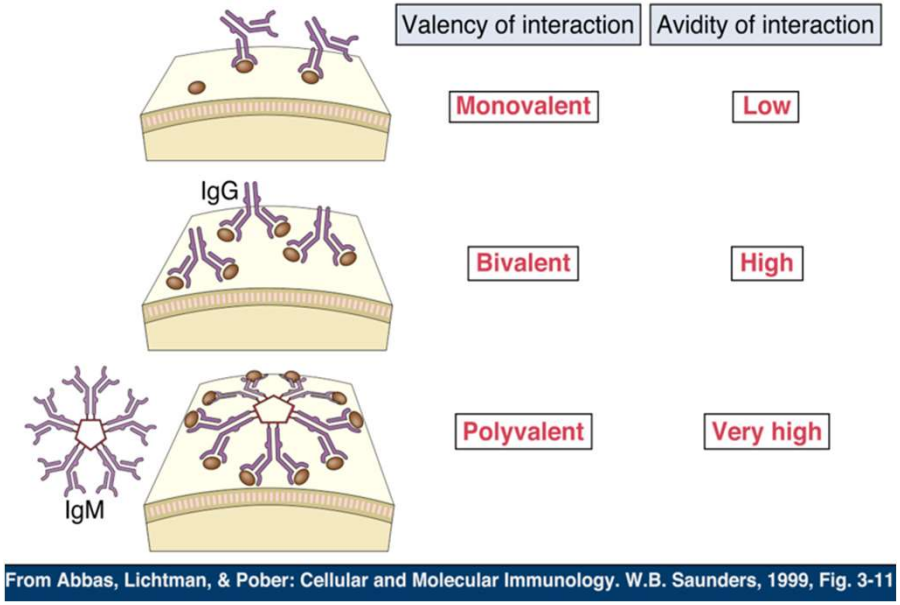
Antibody epitopes



Immunobiology, 6/e. (© Garland Science 2005)

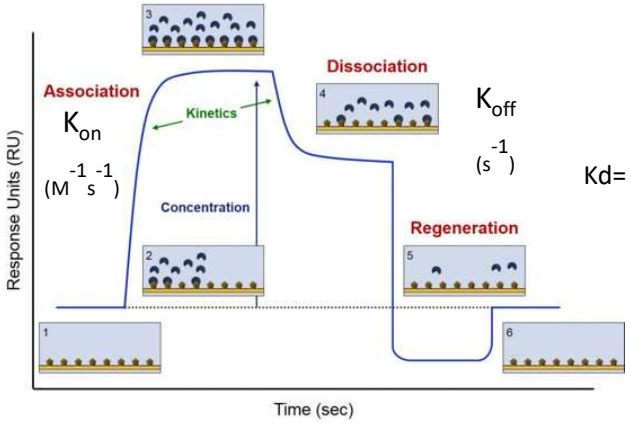
KEY CONCEPTS

Antibody avidity



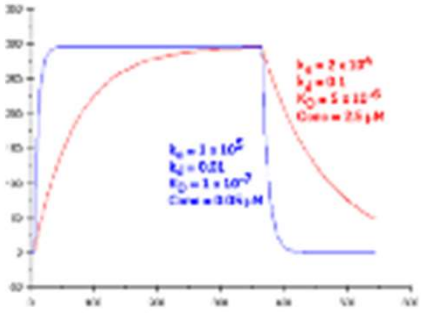
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 3-11

Antibody affinity



Kd= affinity constant

https://shared-resources.dhvi.duke.edu/dhvi-core-facilities/dhvi-bia-core



GE BiacoreTM Assay Handbook

KEY CONCEPTS

Antibody classes

	Immunoglobulin class or subclass								
	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgE
Heavy chain	μ	δ	γ_1	γ_2	γ_3	γ_4	α_1	α_2	ϵ
Molecular weight (kDa)	970	184	146	146	165	146	160	160	188
Serum level (mean adult mg ml^{-1})	1.5	0.03	9	3	1	0.5	2.0	0.5	$5x10^{-5}$
Half-life in serum (days)	10	3	21	20	7	21	6	6	2

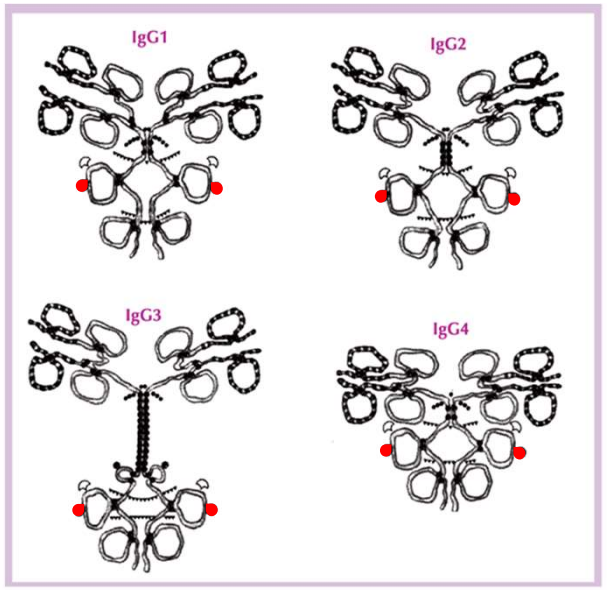
Figure 2-28 The Immune System, 2/e (© Garland Science 2005)

KEY CONCEPTS

IgG subclasses

	IgG1	IgG2	IgG3	IgG4
Functional form *in vivo*	Monomeric bivalent	Dimeric tetravalent[a]	Monomeric bivalent	Half-Ig monovalent
Biological role in host response	Protein antigens	Carbohydrate antigens	Protein antigens	Response to chronic stimulation, anti-inflammatory
Percentage of all IgG in humans[b]	60%	25%	10%	5%
Half-life (range in days)[c]	36.3 ± 9.2 (17.6–56.2)	37.1 ± 13.9 (22.9–62.5)	7-9	15.6 ± 4.5 (7.1–24.7)
Allotypes[d]	4	1	13	0
FcRn[e]	+	+	+	+
Hinge length (number of amino acids)	15	12	62	12
Potential (actual) inter-heavy chain disulfide bonds in hinge region	2 (2)	4 (4?[f])	11 (11)	2 (2)
Effector functions				
C1[e]	++	–	+++	–
FcgRI[e]	+++	–	+++	++
FcgRII[e]	+	±	+	?
FcgRIIIa/b[e]	+	–	+	±

[a]Homodimers of IgG2 are tetravalent for a given antigen, but heterodimers are also expected. [b]From ref. 2. [c]From ref. 22. [d]From ref. 23. [e]From ref. 2. [f]Discussed in ref. 12.



- Different IgG subclasses have different effector functions, stability and halflifes
- IgG2 can form covalent dimers in vivo (tetravalent)
- IgG4 (very poor effector activities) can undergo H+L chains exchange with another IgG4 to form a bispecific monomeric antibody

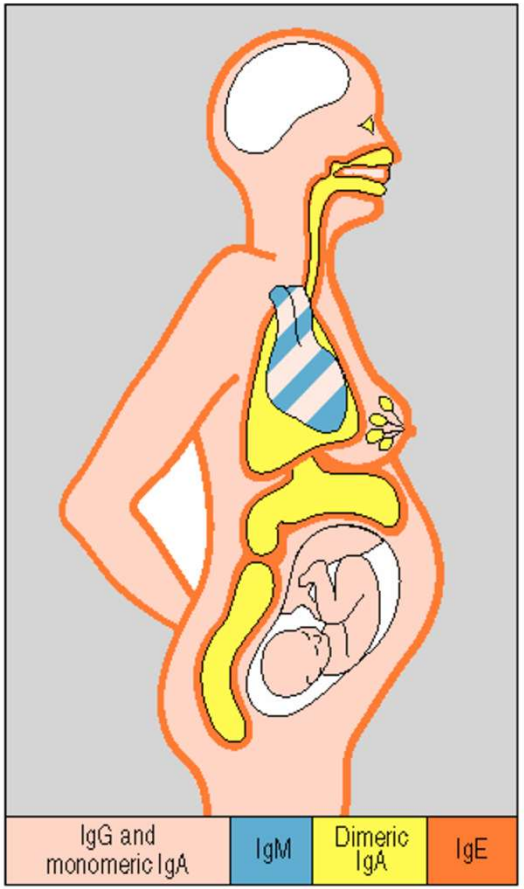
KEY CONCEPTS

Antibody classes have distinct and overlapping functions

Function	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activation of complement system	+++	–	++	+	+++	–	+	–

Figure 2-29 part 1 of 2 The Immune System, 2/e (© Garland Science 2005)

KEY CONCEPTS



Different antibody isotypes are found in different parts of the body

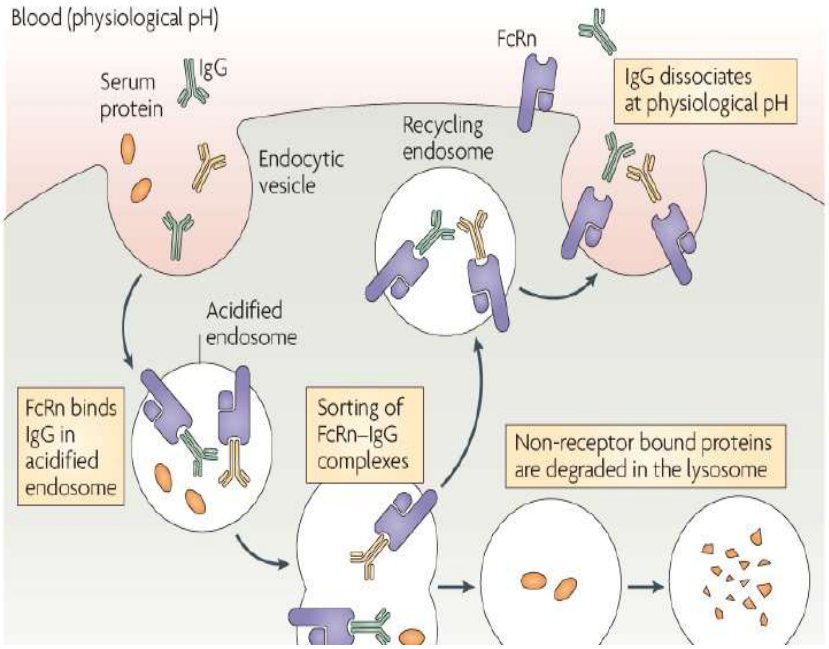
Property	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.03	9	3	1	0.5	2.1	5x10^{-5}

Figure 2-29 part 2 of 2 The Immune System, 2/e (© Garland Science 2005)

KEY CONCEPTS

Natural receptors for IgG

The role of neonatal FcR in antibody transport and persistence



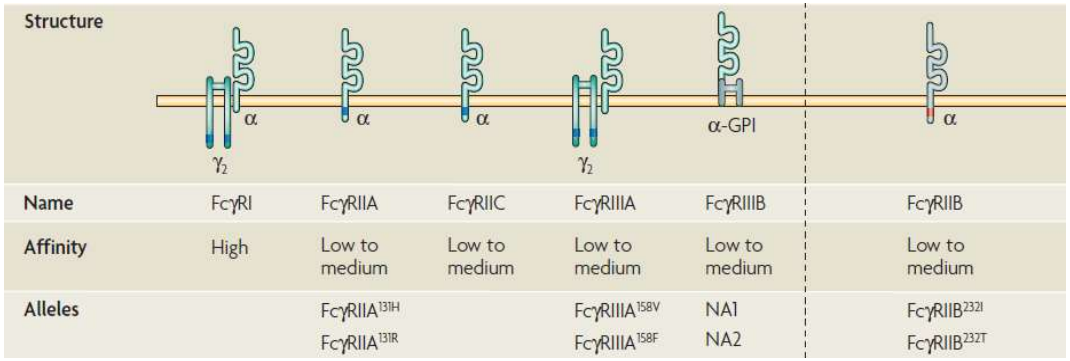
Roopenian & Akilesh Nat Rev Immunol 2007

KEY CONCEPTS

The role of Fc gamma R in antibody effector functions

Receptor	Gene	Expression	Function
FcγRI	CD64	MΦ, DC, neutrophil	activation
FcγRIIA	CD32a	MΦ, DC, neutrophil	activation
FcγRIIB	CD32b	MΦ, DC, neutrophil, B cell	inhibition
FcγRIIC	CD32c	MΦ, NK, neutrophil	activation
FcγRIIIA	CD16a	MΦ, DC, NK	activation
FcγRIIIB	CD16b	neutrophil	activation

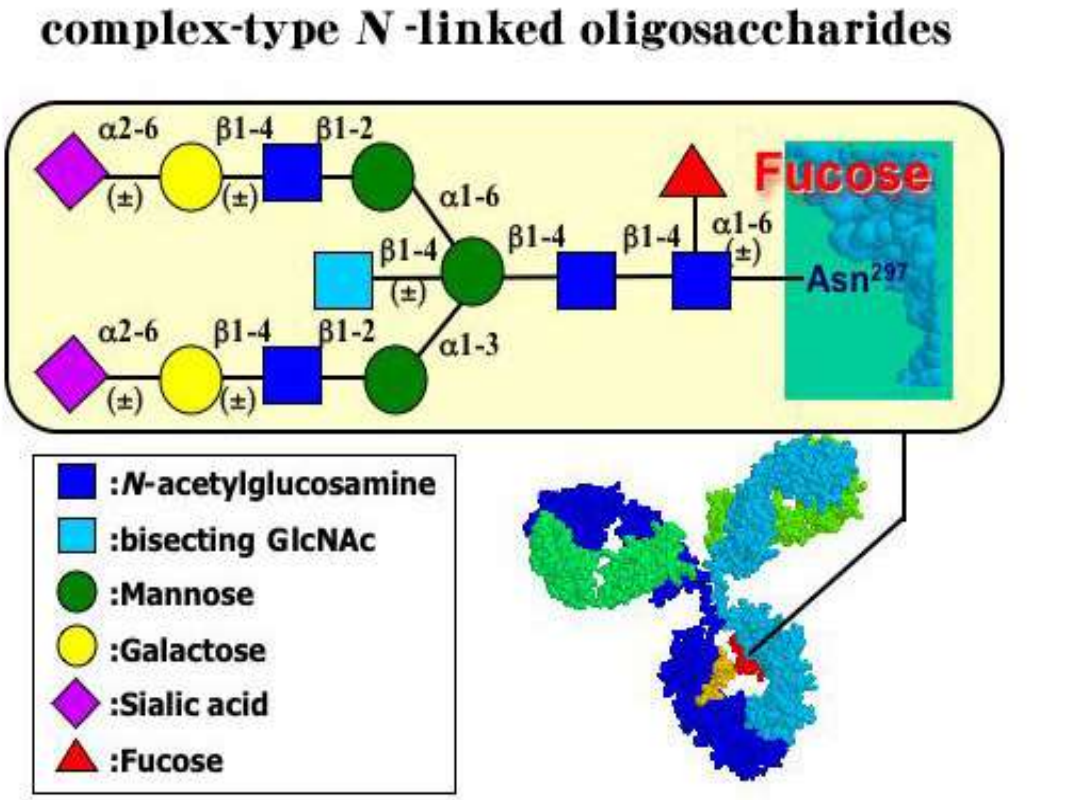
FcRs form a bridge between humoral and cell-based immunity



Nature Reviews Immunology

IgG afucosylation enhances ADCC

ADCC enhancement by non-fucosylated IgG1s is caused by a subtle conformational change in Fc and interactions formed between the carbohydrate at FcγRIII Asn-162 and regions of the Fc that are only accessible when the Fc N-glycans lack fucose residues



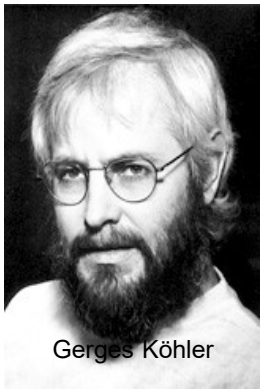
Afucosylation can be achieved by:

- KO of genes involved in the fucosylation (e.g. *FUT8*$^{-}$ in CHO-K1SV Potelligent line)→ complex type (no effect on C1q binding)
- Use of inhibitors of glycoenzymes (kifunensin and swainsonine inhibit α-mannosidase I or II, respectively) → hybrid or high-mannose glycan types (lower CDC)

KEY CONCEPTS

https://drug-dev.com/monoclonal-antibodies-the-development-of-therapeutic-monoclonal-antibody-products-a-comprehensive-guide-to-cmc-activities-from-clone-to-clinic/

Monoclonal antibodies



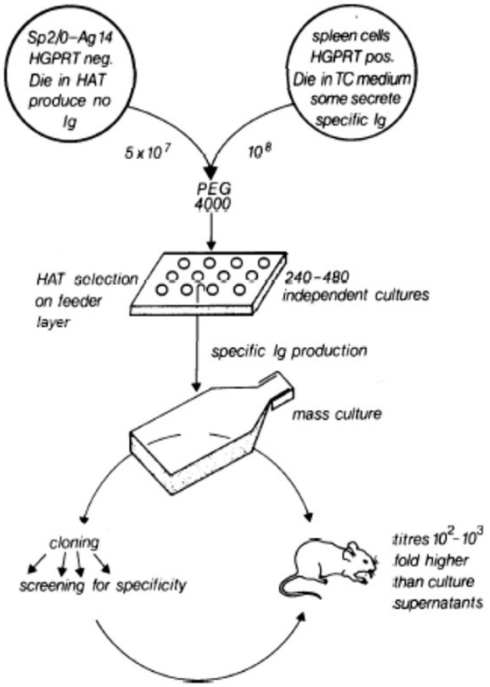
Gerges Köhler

Cesar Milstein

Nature Vol. 256 August 7 1975

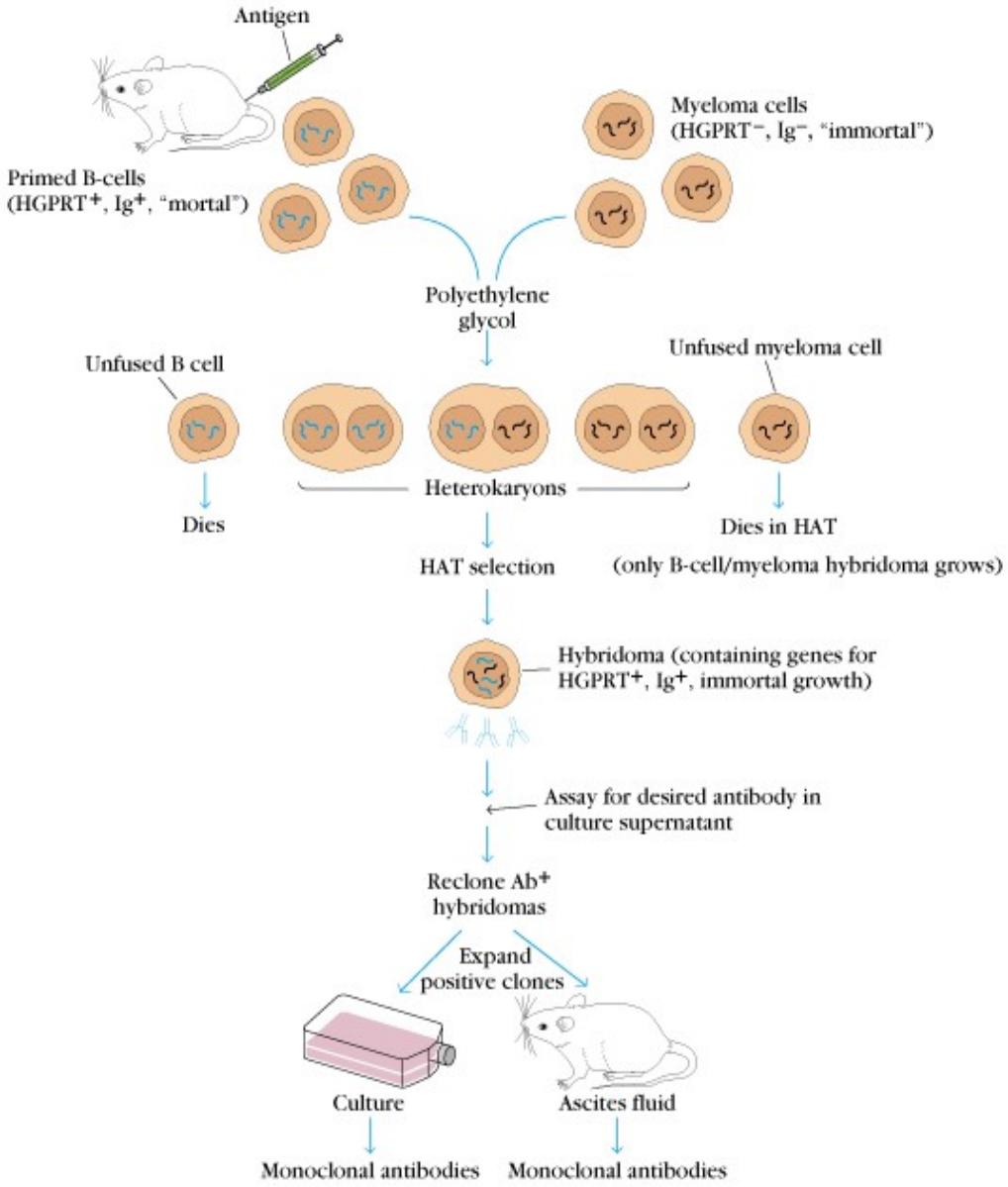
Continuous cultures of fused cells secreting antibody of predefined specificity

THE manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest. There are at present a considerable number of permanent cultures of myeloma cells[1,2] and screening procedures have been used to reveal antibody activity in some of them. This, however, is not a satisfactory source of monoclonal antibodies of predefined specificity. We describe here the derivation of a number of tissue culture cell lines which secrete anti-sheep red blood cell (SRBC) antibodies. The cell lines are made by fusion of a mouse myeloma and mouse spleen cells from an immunised donor. To understand the expression and interactions of the Ig chains from the parental lines, fusion experiments between two known mouse myeloma lines were carried out.



Hybridoma technology

- In the 1970's the B-cell cancer myeloma was known, and it was understood that these cancerous B-cells all produce a single type of antibody.
- Hybridoma
  - To be produce by using polyethylene glycol (PEG) to fuse cells
  - The myeloma cells: immortal growth properties
  - The B cells: to contribute the genetic information for synthesis of specific antibody
  - Selected by using HAT medium (hypoxanthine, aminoprotein, and thymidine)
    - Myeloma cells are unable to grow
    - B cells are able to survive, but can not live for extended periods



Making use of monoclonal antibodies in diagnostics and research

Diagnostics

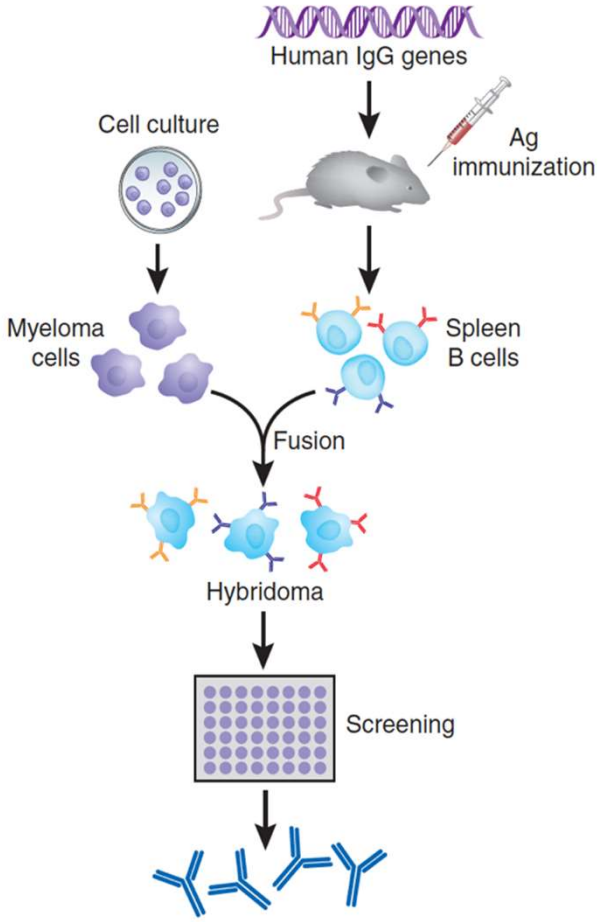
- Several diagnostic procedures that use monoclonal antibodies are now available
- A monoclonal antibody can be used to **detect pregnancy** only 14 days after conception.
- Other monoclonal antibodies allow rapid diagnosis of hepatitis, influenza, herpes, streptococcal, and Chlamydia infections.
- Radiolabeled monoclonal antibodies can also be **used in vivo detecting** or locating
- They can be used to detect for the presence and quantity of this substance, for instance in a Western blot test (to detect a substance in a solution) or an immunofluorescence test.
- Monoclonal antibodies can also be used to purify a substance with techniques called immunoprecipitation and affinity chromatography.

Heterohybridomas

- The use of human myelomas to make human hybridomas have met with limited success. Production of human–human hybridomas has been largely hindered by a **lack of good human fusion partners**.
- Human– mouse heterohybridomas against an immunising antigen can be generated by fusing human peripheral lymphocytes with a murine myeloma cell line (P3653) by PEG-mediated fusion (Jessup et al. JIM 2000) or PEG electrofusion (Buchacher et al 1994, AIDS). The efficiency ranges between 10^{-4} to 10^{-5}
- EBV-B cells have also been fused with human/mouse heteromyelomas (SHM-D33) to make heterohybridomas (Gorny et al, PNAS 1991)

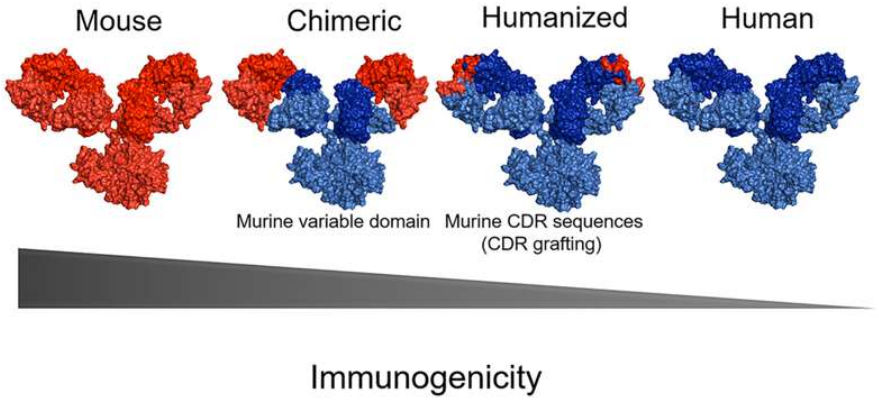
Human immunoglobulin transgenic mice

- Mice in which a human mini-immunoglobulin gene locus has been **knocked-in** to replace the mouse immunoglobulin gene locus
- The immune response in transgenic mice is sometimes less robust than in strains that are used to generate mouse mAbs
- Alternatively mice can be **adoptively transfered with human peripheral blood lymphocytes** into normal strains of mice or rats which are irradiated



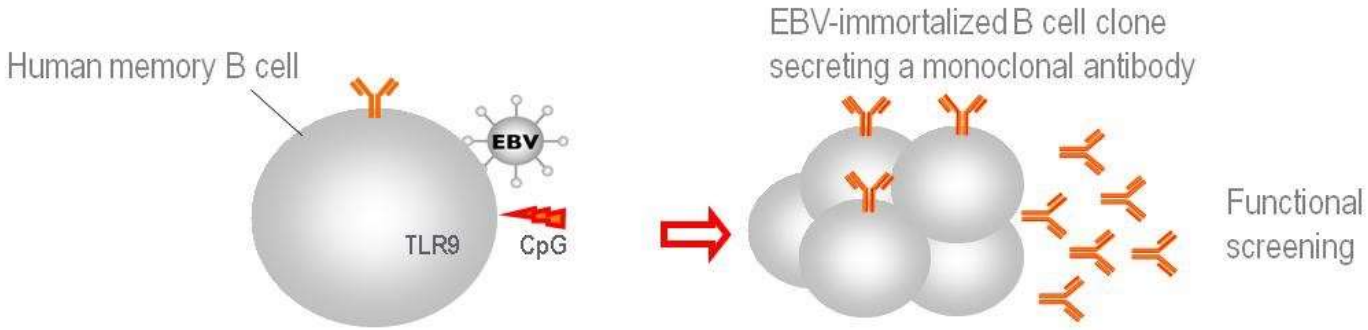
Humanization of murine mAbs

- ‘humanization’ of murine mAbs is still used, particularly when the murine mAbs have been extensively characterized and have desirable antiviral properties and/or superior fully human mAbs are hard to obtain.
- Grafting the human constant region is used in the so-called **chimeric** antibodies
- **Humanized antibodies**: Grafting complementarity determining region (CDR) residues together with minimal key framework residues from variable regions of a rodent donor mAb onto acceptor human antibody frameworks,
- Grafting specificity determining residues and is supported by advances in genetic engineering and three-dimensional modeling of protein structures.
- The fact that nearly half of all US Food and Drug Administration (FDA)-approved therapeutic mAbs are humanized antibodies testifies to their **safety and tolerance by humans.**



Human do it better

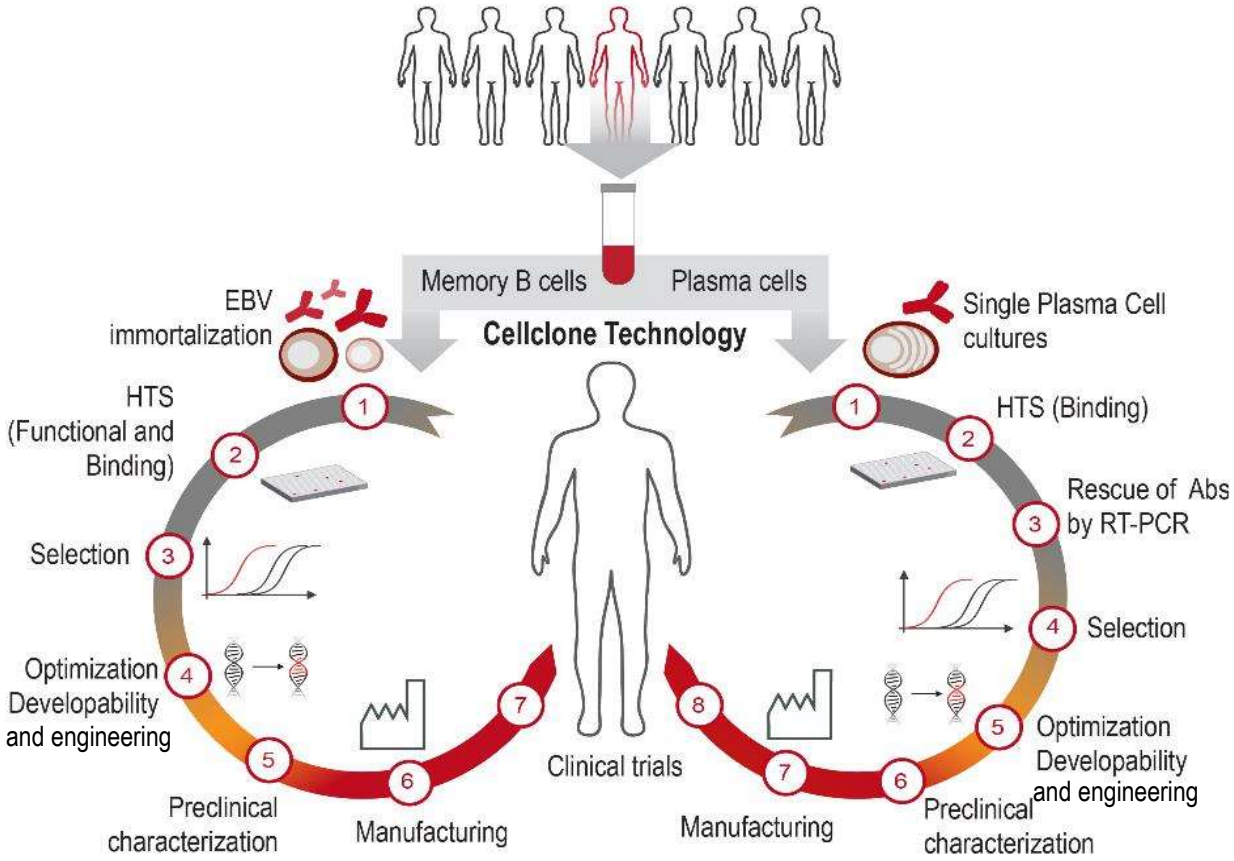
Improved EBV-immortalization protocol



- Human memory B cells are immortalized in monoclonal conditions using Epstein Barr Virus (EBV) and CpG oligonucleotides (a TLR9 agonist).
- Supernatants of immortalized B cell clones containing monoclonal antibodies (5-100 µg/ml) are screened using multiple parallel assays (binding or functional)
- Selected clones are expanded for production of antibodies and VH/VL genes are sequenced
- The interrogations are performed in 384 microwell plates. The cloning efficiency of memory B cells ranges from 20 to 50%. Thousands of memory B cells can be screened in a single experiment from 10-30 ml of blood (fresh or cryopreserved)

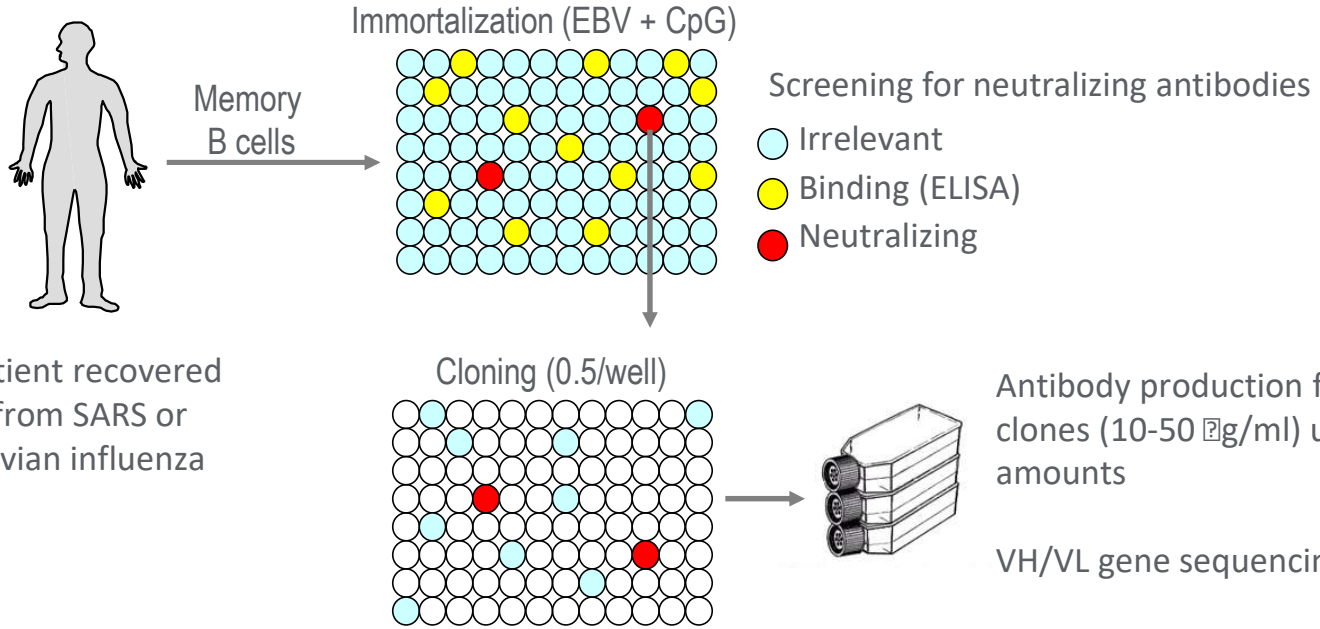
Traggiai et al. Nature Medicine 2004

Cellclone Technologies



Nature Medicine 2004; Science 2011; Nature 2013

Finding potent and broadly neutralizing antibodies



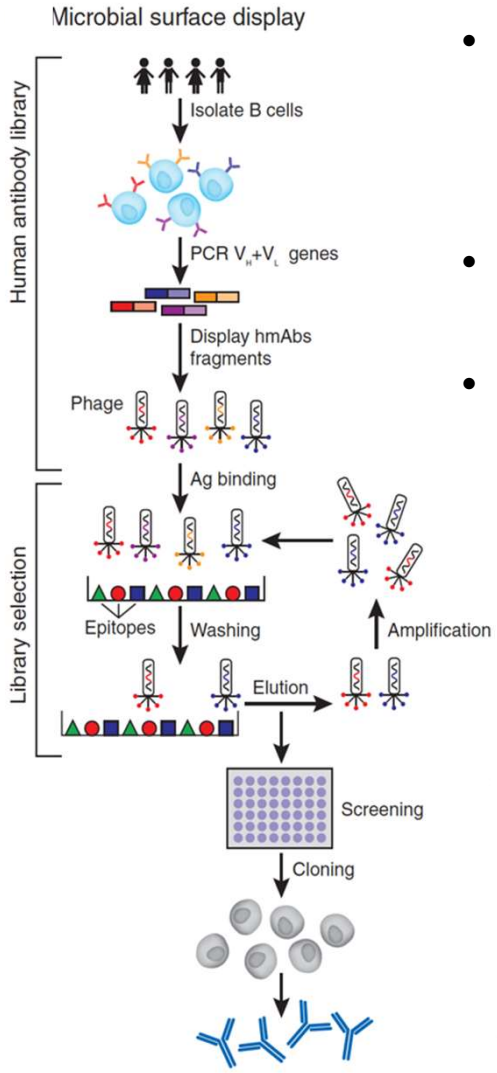
Other techniques to interrogate human memory B cells

- Retrovirus mediated gene transfer for the introduction of the genes **Bcl-6 and Bcl-xL** which prevent growth arrest and apoptosis of the B cells.
- The proliferating immortalized B cells express surface immunoglobulin and secrete antibody.
- Sorting antigen-binding B cells followed by single-cell RT-PCR and expression (Wu et al., Science 2010)
- Polyclonal stimulation of B cells with a cocktail of stimula followed by screening and rescue of selected clones by RT-PCR (Walker et al, Science 2009; Walker et al., Nature 2011)

Display libraries

- Display technologies in which different microorganisms—including **phage, yeast, bacteria and viruses** are used to display repertoires of single-chain variable antibody fragments (scFvs), antigen-binding fragments (Fabs) on their surfaces
- Different display technologies suggests that antibody folding efficiency, post-translational modification and epitope accessibility may all influence the outcome of selection
- The method of presentation of **antigen as bait for panning** may affect the number and distribution of available neutralizing epitopes.

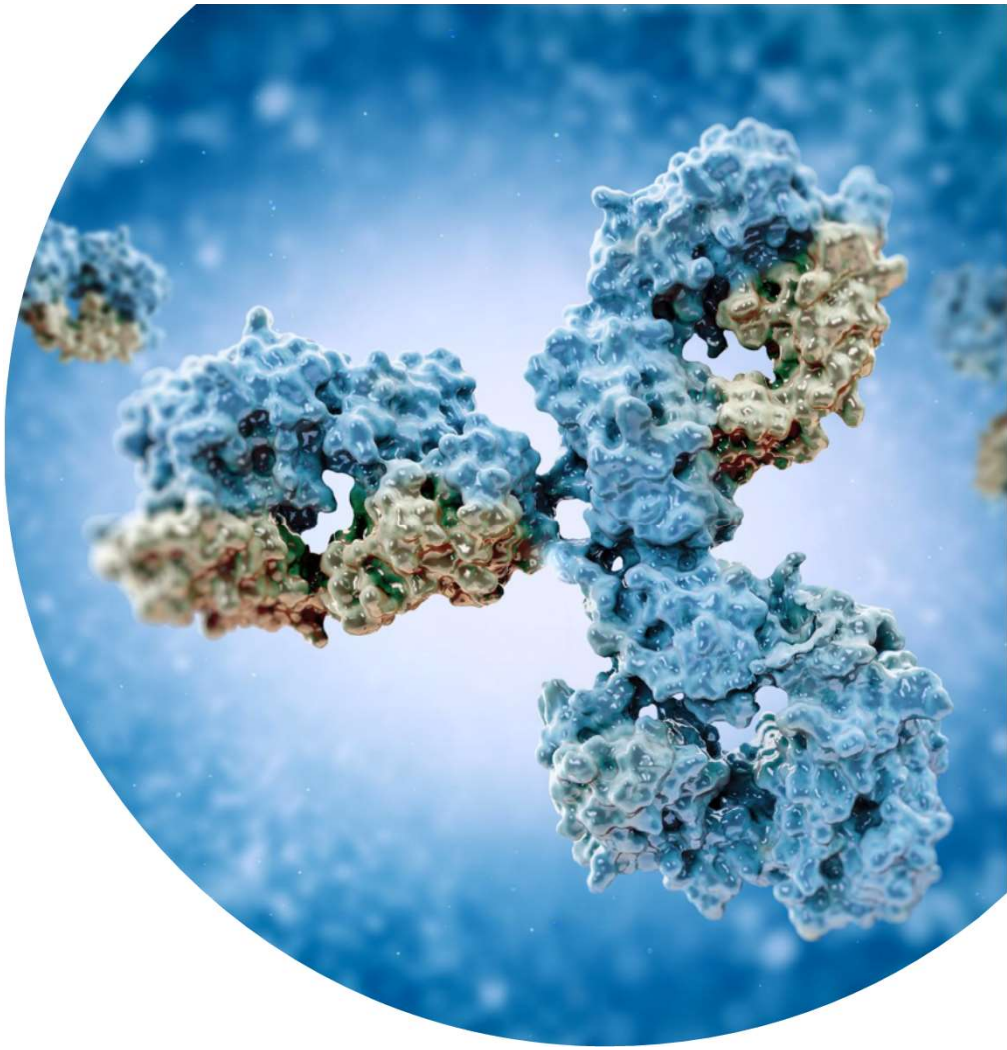
Phage Display libraries



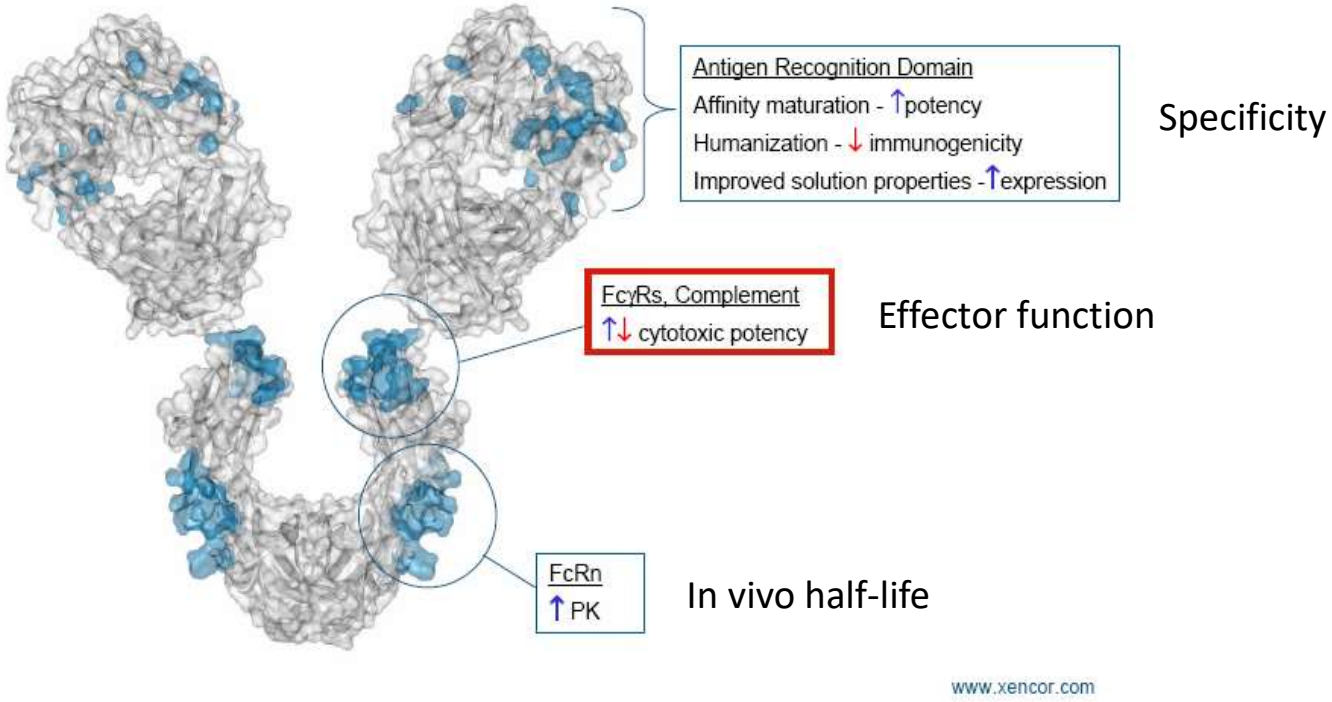
- Diverse human immunoglobulin-variable-region gene segments (as scFv or Fab fragments) are amplified from human B cells of **immune or non-immune sources** to construct the antibody library.
- The library is then cloned for display on the surface of the phage.
- Three steps are included in this technique:
  - antibody library construction and display onto the phage surface
  - **selection by panning** the library against antigen (Ag) targets,
  - screening for desired specificity. Antibodies that do not bind are washed away and the binders are eluted and amplified by infection of *Escherichia coli.*
  - Several rounds of such selection, desired specificity can be screened using ELISA or FACS if a cell-membrane bound protein is the target.
  - The genes of antibody variable regions can be cloned into whole human IgG expression vectors to produce fully human mAbs

Antibody Engineering

- A key passage for drug development

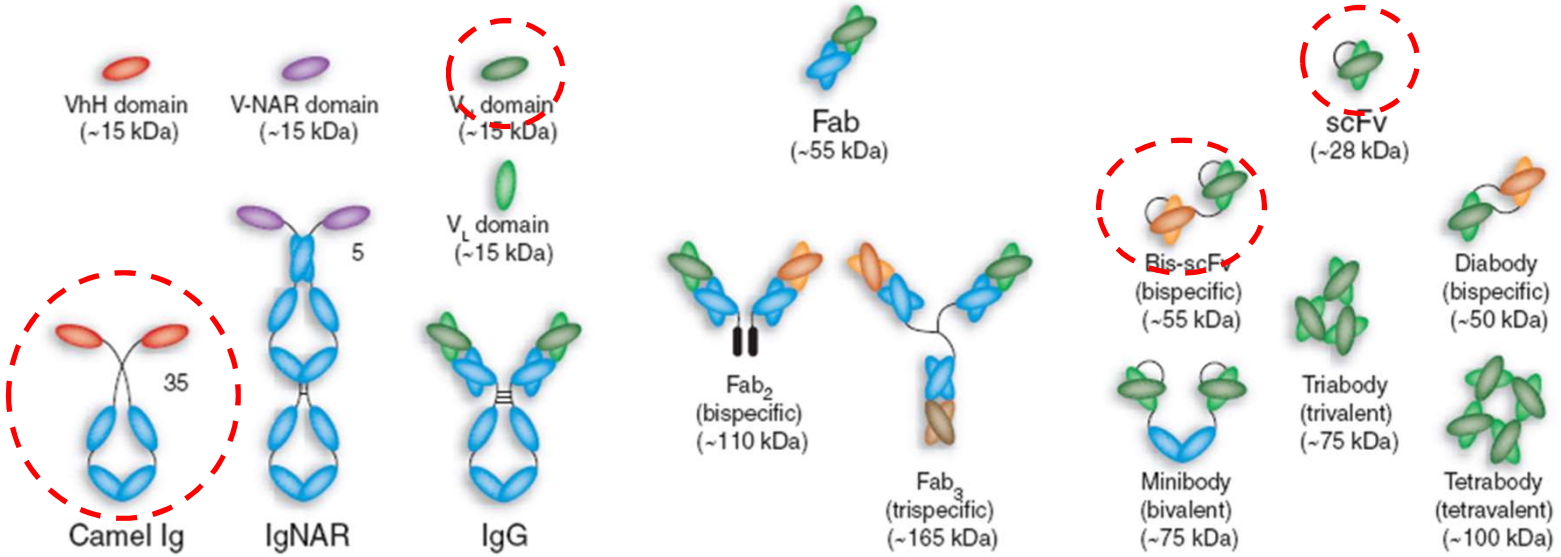


Antibody engineering: improve specificity and effector function



There are many different avenues that are and have been explored for improving antibodies for therapeutics

Engineering monoclonal antibodies



Antibody conjugates with:

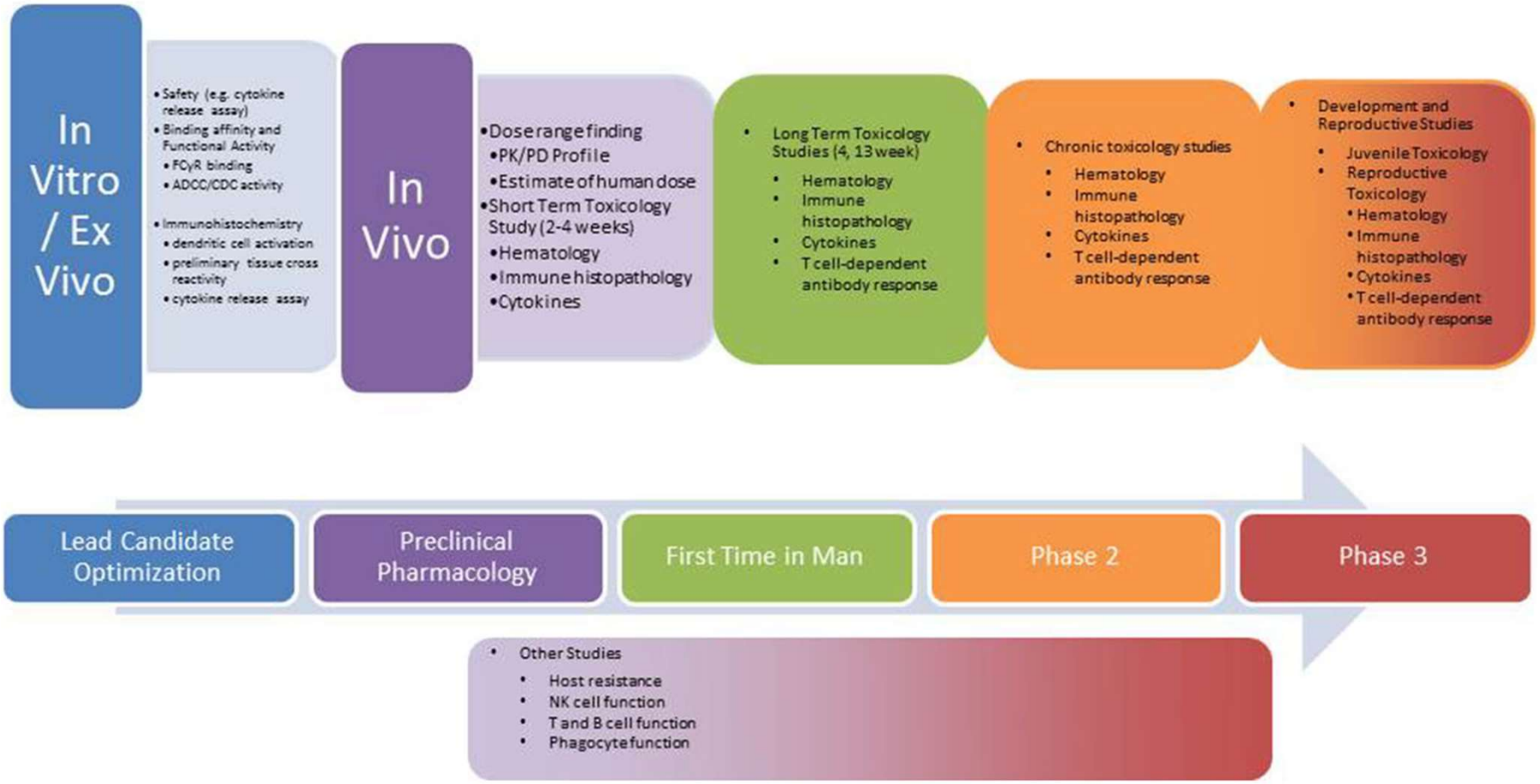
- Antigens
- Drugs
- Radionucleids
- Toxins

Additional scaffolds:

- Leucine Rich Repeats (Lampreys)
- Ankyrin Repeats (A. Plückthun, U Zürich)

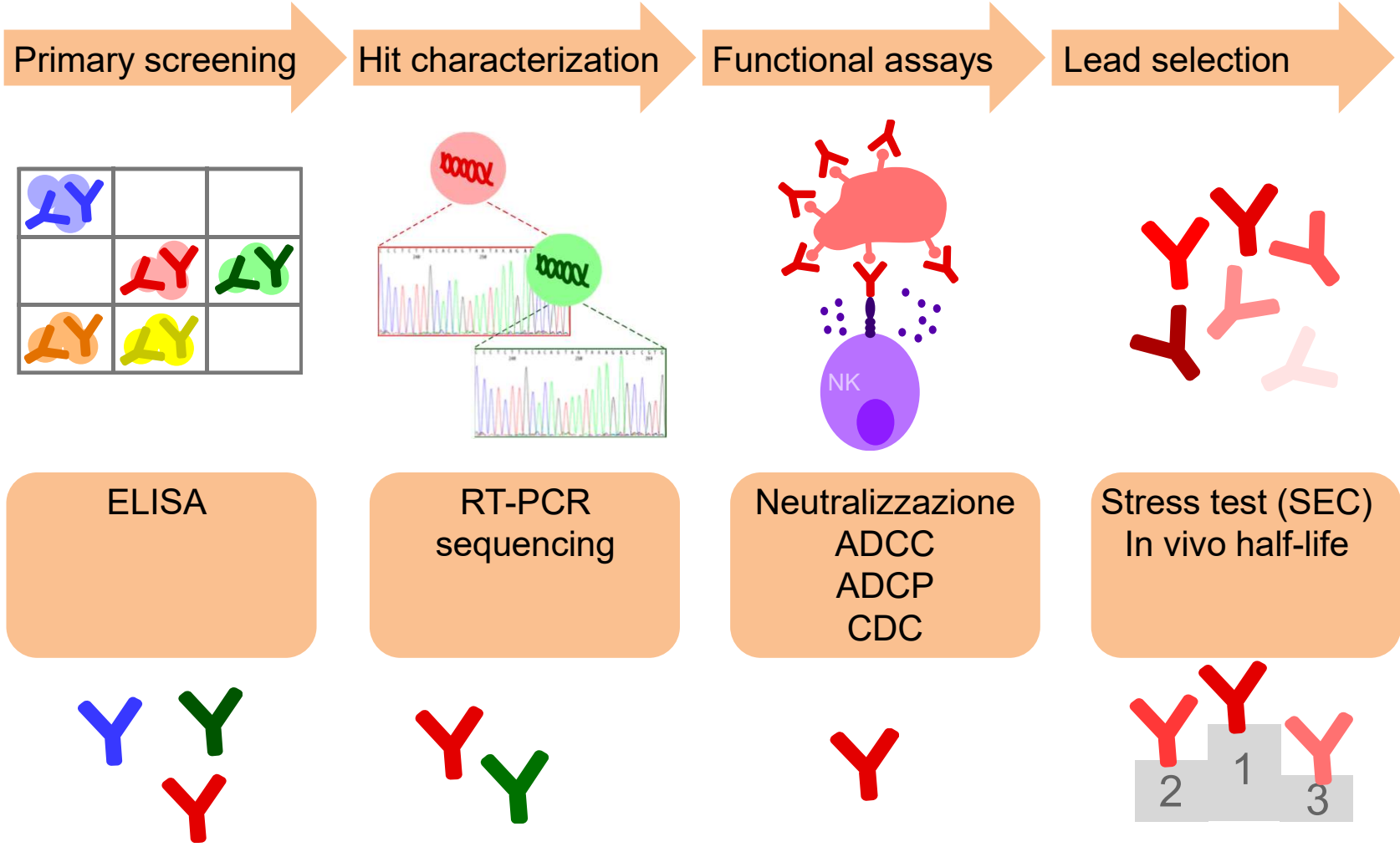
Researchgate.net

From discovery to clinical



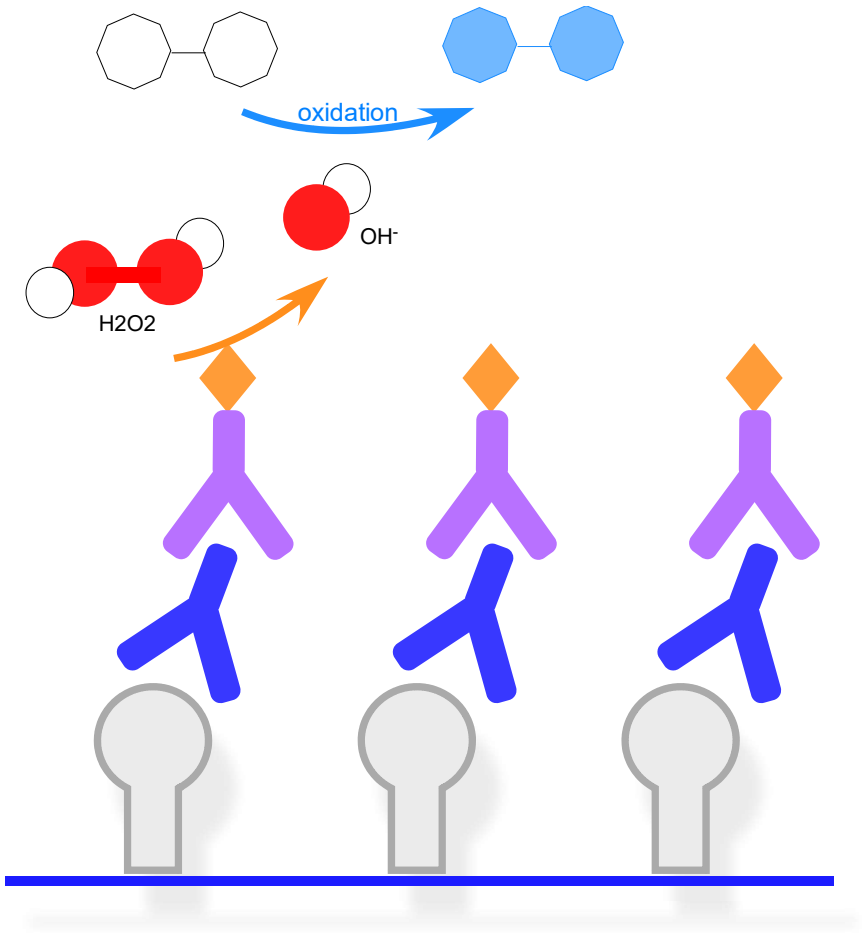
Mould DR et al. BioDrugs 2016

Drug pre-development activities



E. Cameroni Humabs

Enzyme-linked immunosorbent assay (ELISA)



Enzyme (es. HRP)

Conjugated secondary antibody

mAb (human IgG1)

Antigen (viral protein)

Support (plastic)

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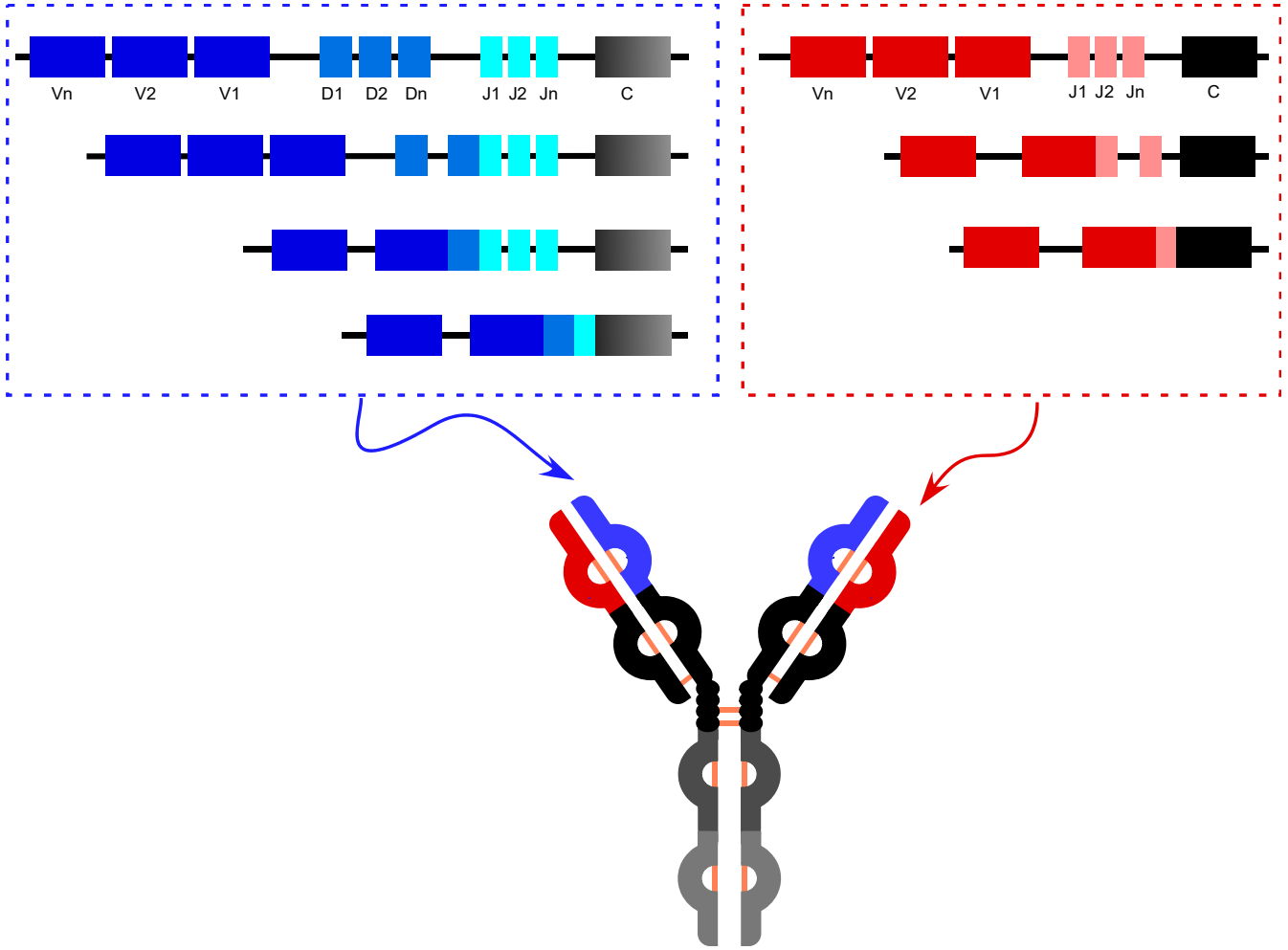
Sequencing of variable regions



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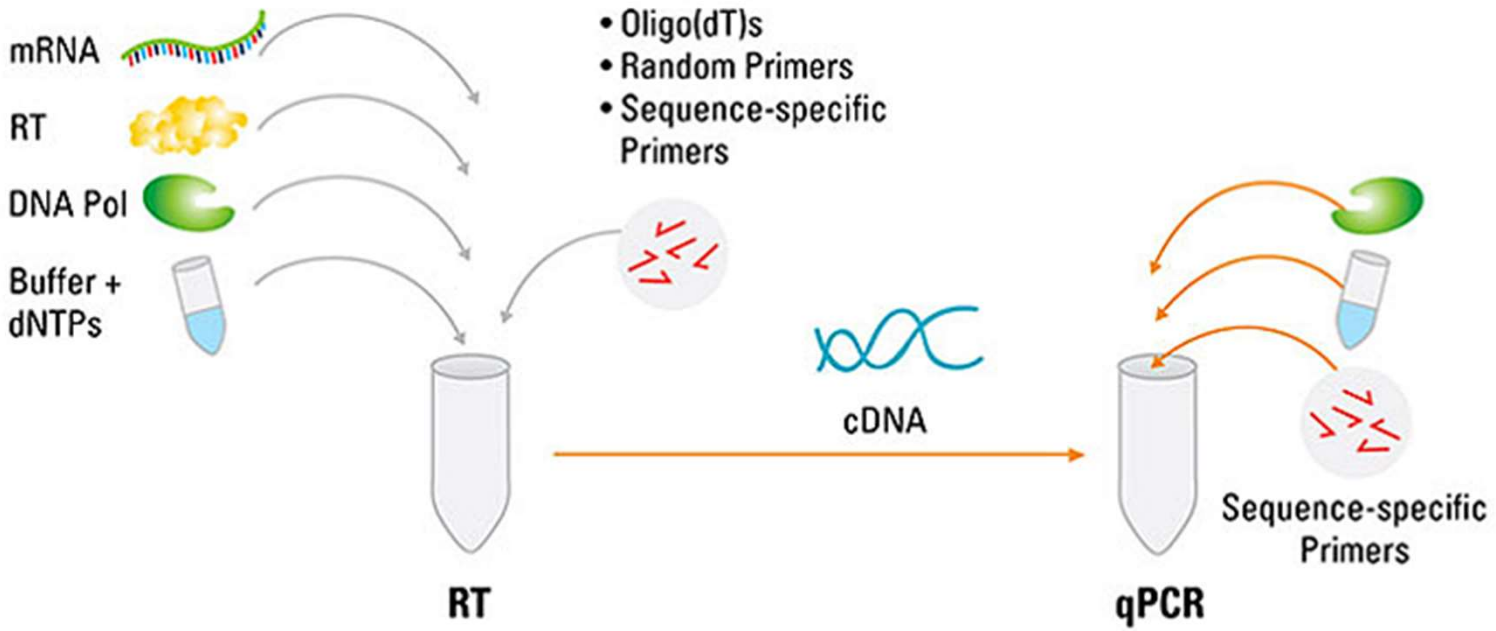
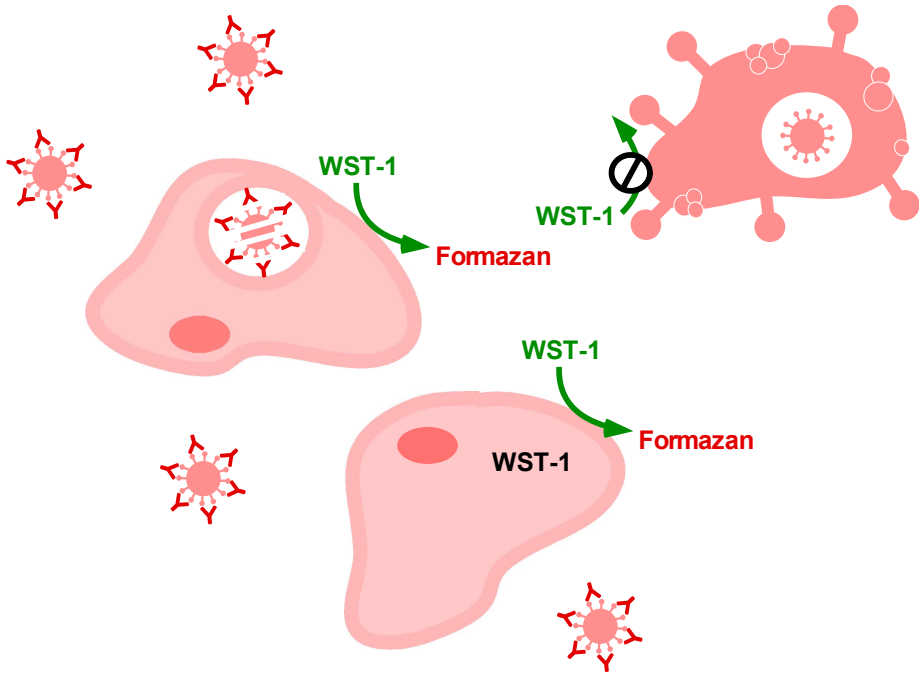
Reverse-transcription e PCR amplification of variable regions



https://www.thermofisher.com/ch/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/basic-principles-rt-qpcr.html

Neutralization assay

- ✓ Permissive cells
- ✓ Virus
- ✓ Antibody
- ✓ Viability stain



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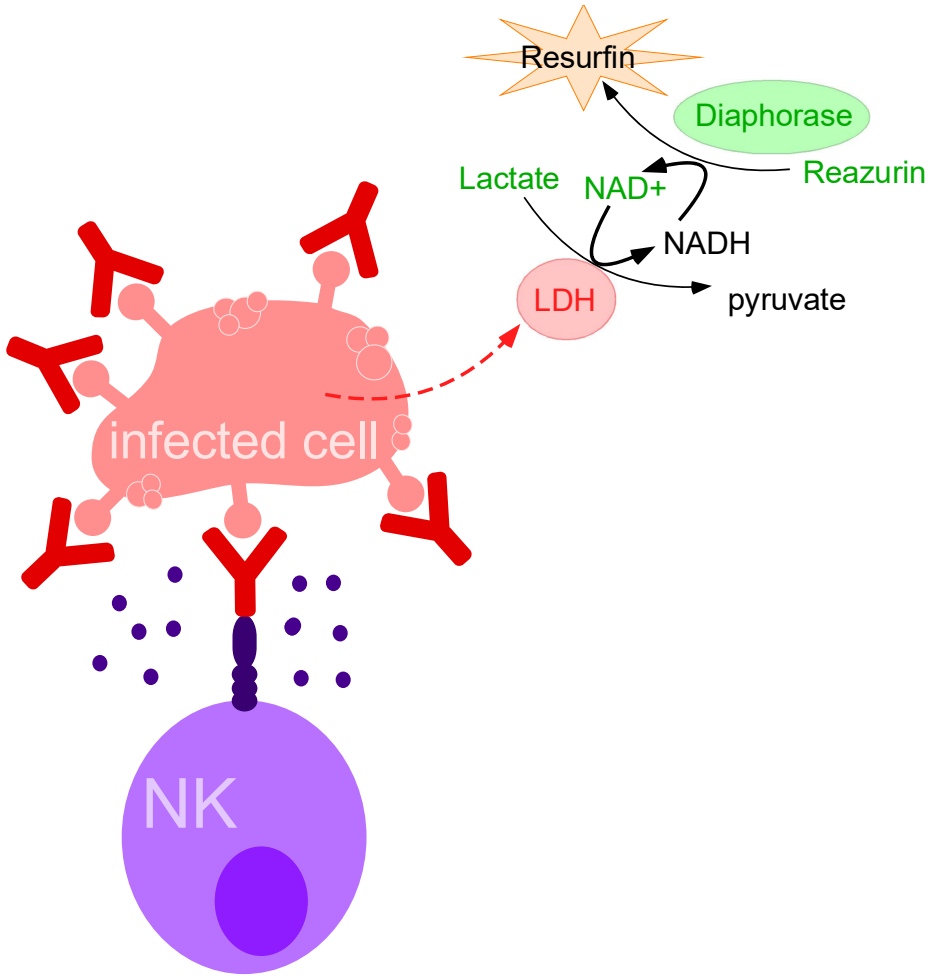
Antibody-dependent cell-cytotoxicity

- ✓ Effector cells (NK)
- ✓ Target cells (infected)
- ✓ Antibody

- ✓ Lactate
- ✓ NAD+
- ✓ Reazurin
- ✓ Diaphorase



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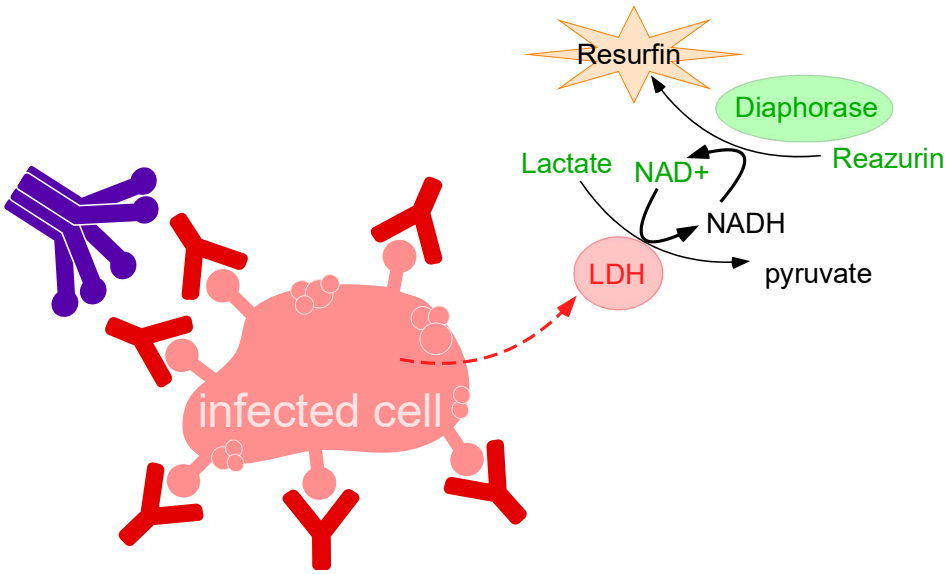
Complement-dependent cytolysis

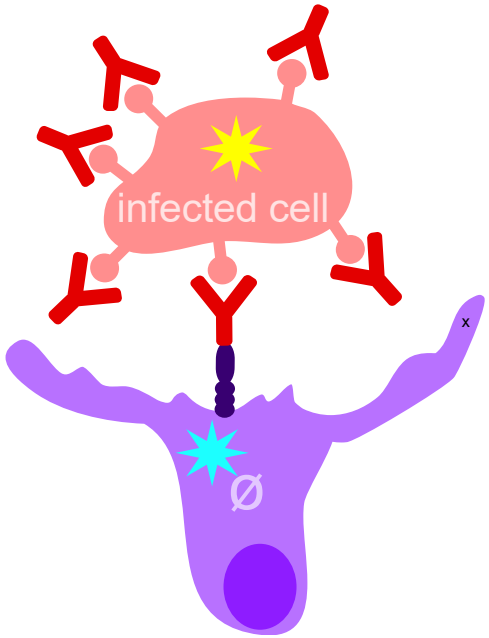
- ✓ Complement complex (e.g. from rabbit)
- ✓ Target cells (infected)
- ✓ Antibody

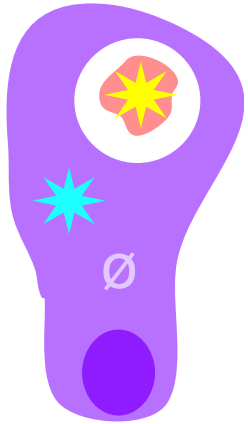
- ✓ Lactate
- ✓ NAD+
- ✓ Reazurin
- ✓ Diaphorase



Antibody-dependent cell phagocytosis

- ✓ Fluorescent effector cells (macrophages)
- ✓ Fluorescent target cells (infected)
- ✓ Antibody





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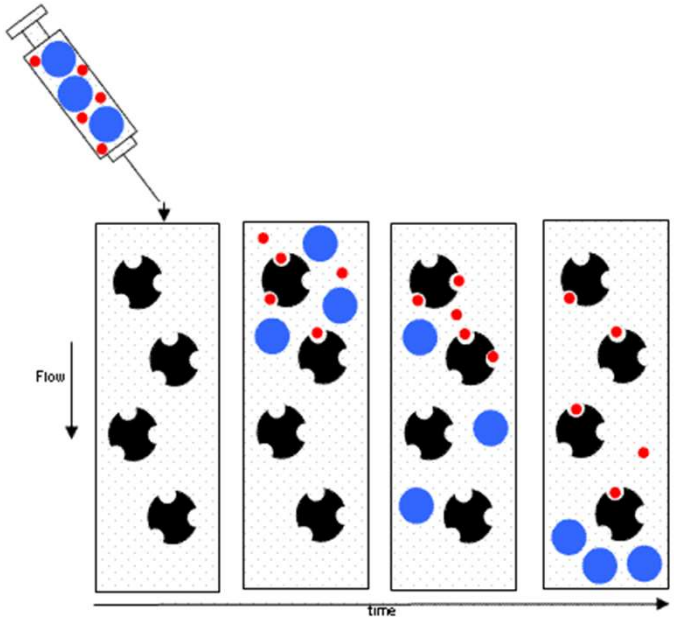
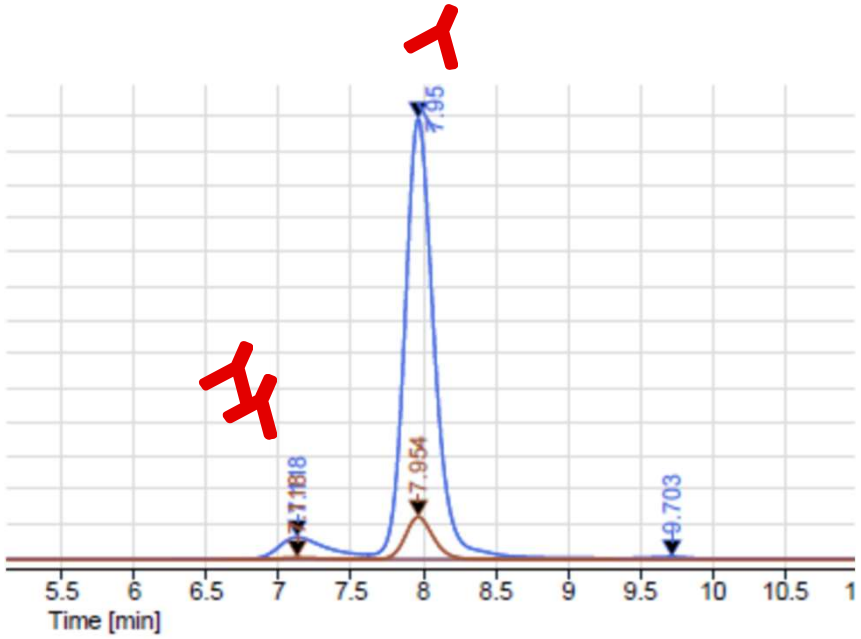
Antibody developability assessment

- Purification, formulation, conservation issues:
  - pH
  - High concentrations
  - Freeze/thaw
  - Heat shock
  - Light sensitivity

- Compatible Half-life
  - HuFcRn mice
  - NHP

Size exclusion chromatography and accelerated stress tests





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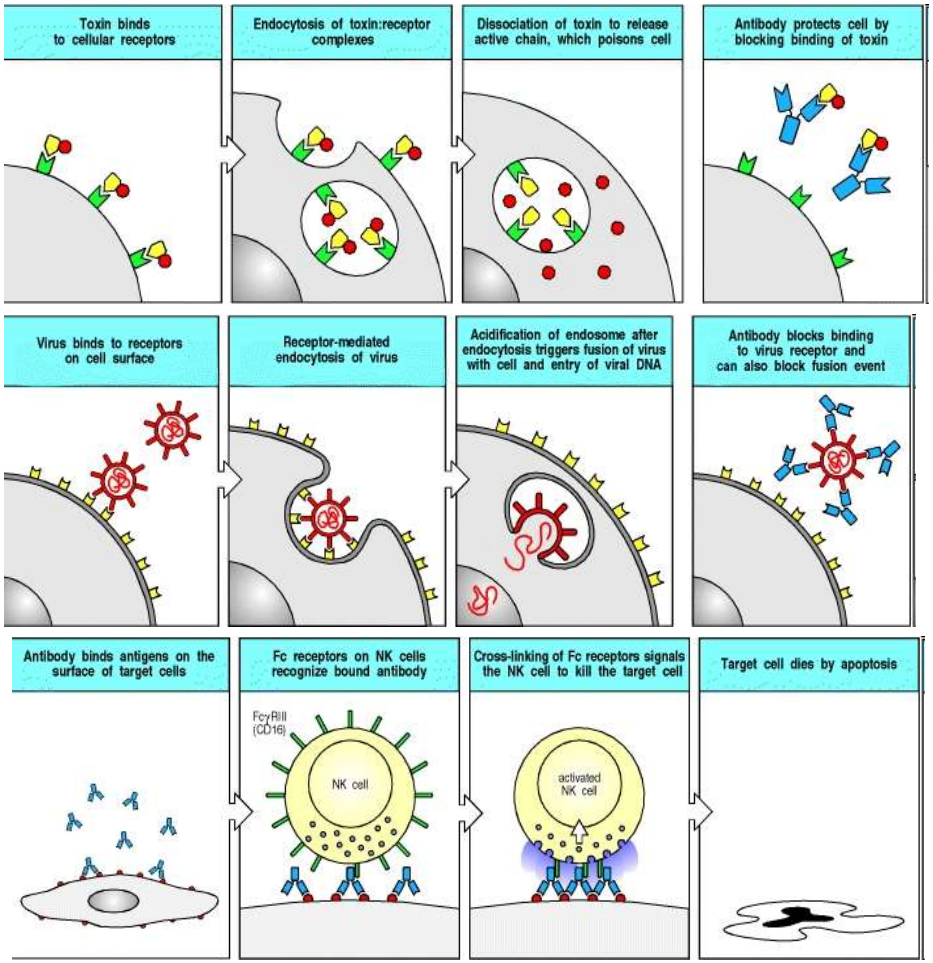
Some mechanisms of action of therapeutic antibodies

1. Neutralization by binding to viruses, toxins or cytokines (RSV…)

2. Killing of target cells by recruiting NK cells (ADCC), phagocytes or complement (CD20)

3. Blocking molecular interactions (CTLA4, PD1L)

4. Stimulation of target cells (CD3, CD28)

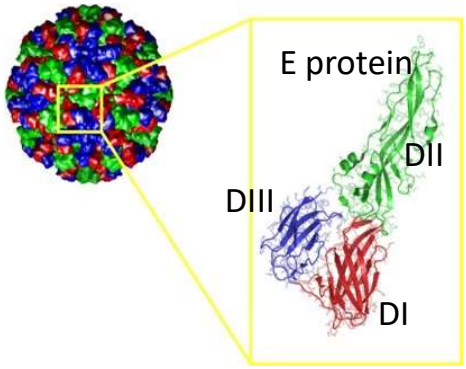


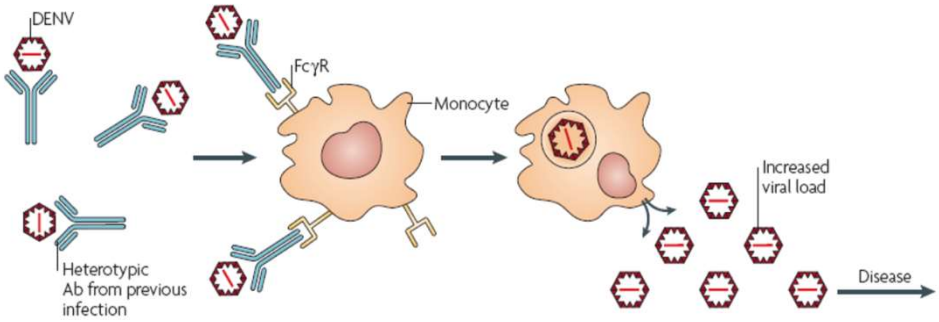
Slideshare.net

EXAMPLE 1

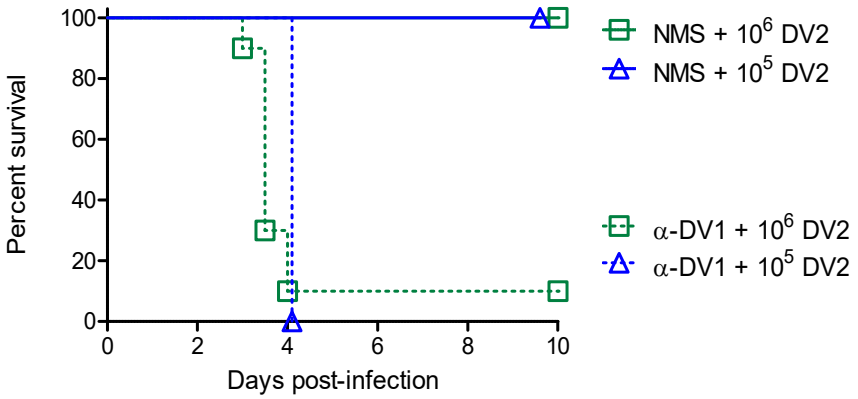
Dengue virus

serotypes, neutralization and enhancement





- Four serotypes (DENV-1, DENV-2, DENV-3, DENV-4)
- Antibodies can enhance virus entry through FcγR
- Immune donors are protected from homologous virus, but are at risk of developing **dengue hemorragic fever** when infected by a different serotype
- Mice receiving **and anti-DENV1 serum** die when infected with **DENV2** (Balsitis et al *PLoS pathogens* 2010)

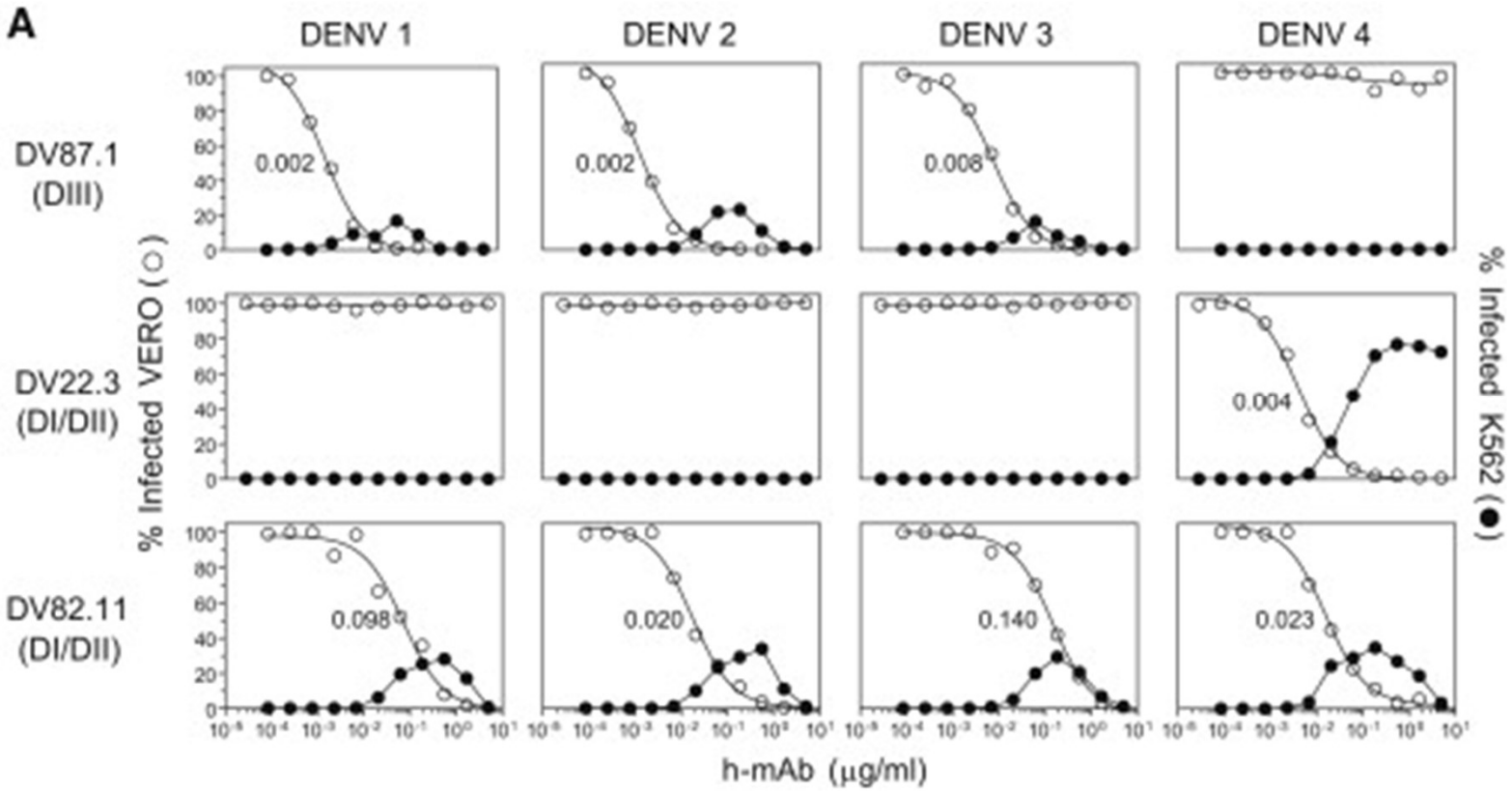


Balsitis et al PLoS pathogens 2010

Characteristics of mAbs for passive vaccination in Dengue

- Should neutralize all four DENV serotypes
- Should recognize at least two epitopes on each virus (to avoid selection of escape mutants)
- Should not enhance infection

DENV-specific antibodies: neutralization and enhancement



Beltramello et al. *Cell Host & Microbe* 2010

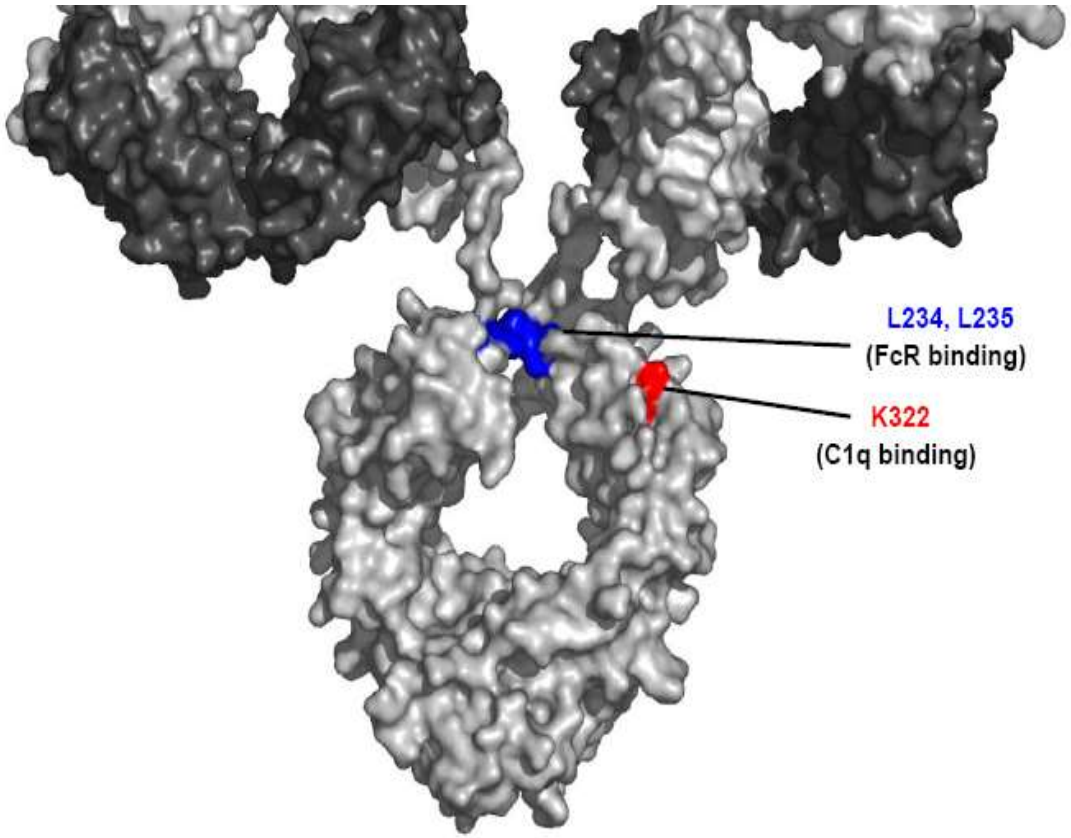
Two types of DENV specific antibodies

1. Antibodies to DIII have potent neutralizing activity but neutralize only a few DENVs
2. Antibodies to DI/DII are less potent but crossreact with all DENVs
3. All antibodies enhance infection at subneutralizing concentrations

mAb	Specificity	Neutralization (EC_{50} mg/ml)				Enhancement (log10 mg/ml)			
		DV1	DV2	DV3	DV4	DV1	DV2	DV3	DV4
DV 3.7	DIII	—	0.003	—	—	—	-3 , 1	—	—
DV 470.12	DIII	0.002	—	—	—	-3 , 1	—	—	—
DV 63.1	DIII	0.006	—	0.006	—	-4,-1	—	-3 , 0	—
DV 1.1	DI/DII	0.209	0.066	0.262	0.183	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 4.4	DI/DII	0.248	0.214	0.167	0.76	-2 , 1	-2 , 1	-1 , 1	-2 , 1
DV 5.1	DI/DII	0.156	0.103	0.092	0.069	-2 , 1	-2 , 1	-2 , 1	-3 , 1
DV 6.1	DI/DII	0.382	0.296	0.351	0.17	-1 , 1	-2 , 1	-1 , 1	-2 , 1
DV 8.1	DI/DII	0.103	0.022	0.031	0.024	-3 , 2	-2 , 1	-3 , 1	-3 , 0
DV 16.5	DI/DII	0.248	0.089	0.219	0.101	-2 , 1	-2 , 1	-2 , 1	-2 , 1

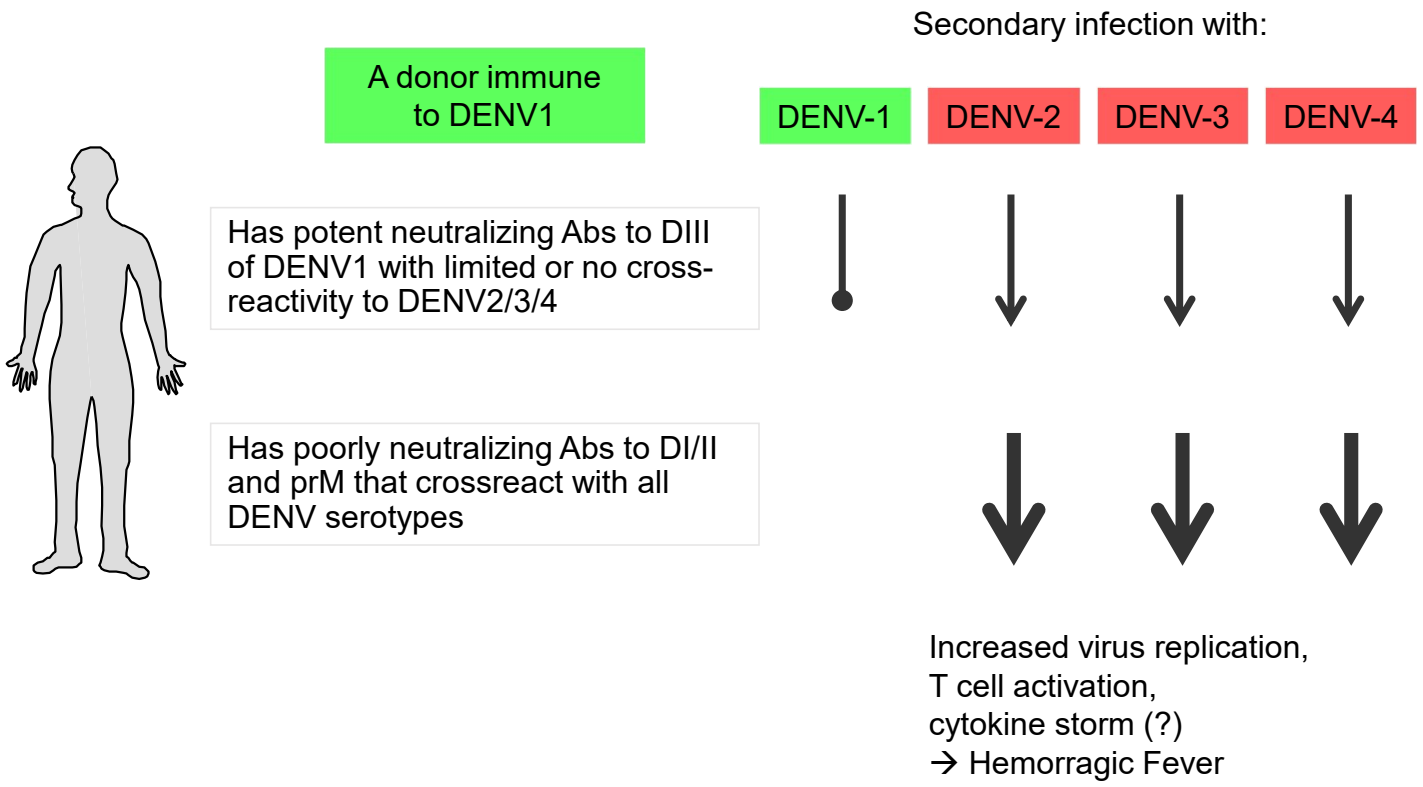
Beltramello et al. *Cell Host & Microbe* 2010

Two L To A substitutions in the CH2 Domain (LALA) abolish FcR Binding



Parren et al. J. Vir 2001
Hessel et al. *Nature* 2007

A model for protection and pathology in DENV infection

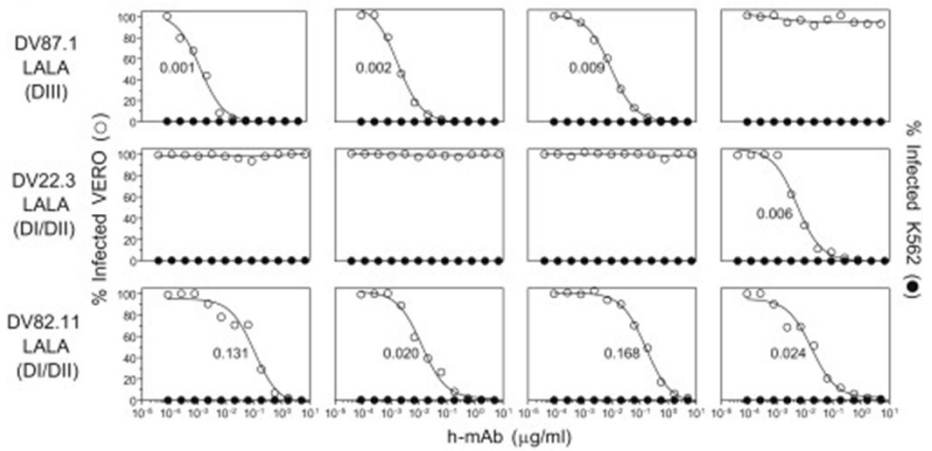


DIII antibodies mediate protection, DI/II antibodies pathology

Beltramello et al. *Cell Host & Microbe* 2010

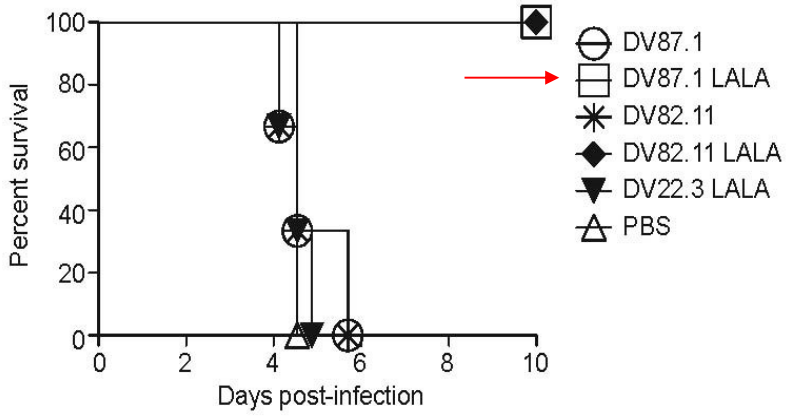
A mutant antibody protects from lethal DENV infection

A LALA mutant antibody carrying Leu to Ala substitutions at residues 234 and 235 does not cause infection enhancement



AG129 mice lacking IFNIR injected with:

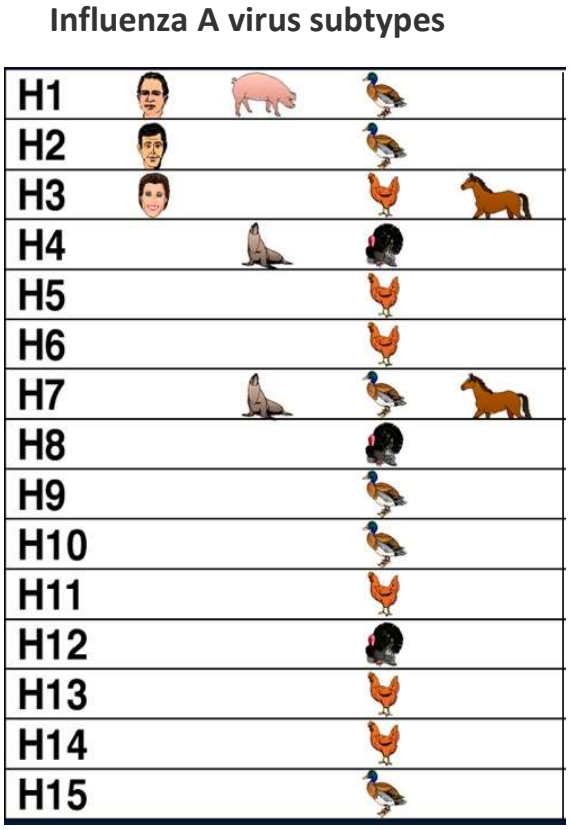
- Day -1: mice receive serum with infection enhancing activity (anti-DENV1) i.p.
- Day 0 : 10^5 DENV2 i.v.
- Day +1: LALA-mAb i.p.

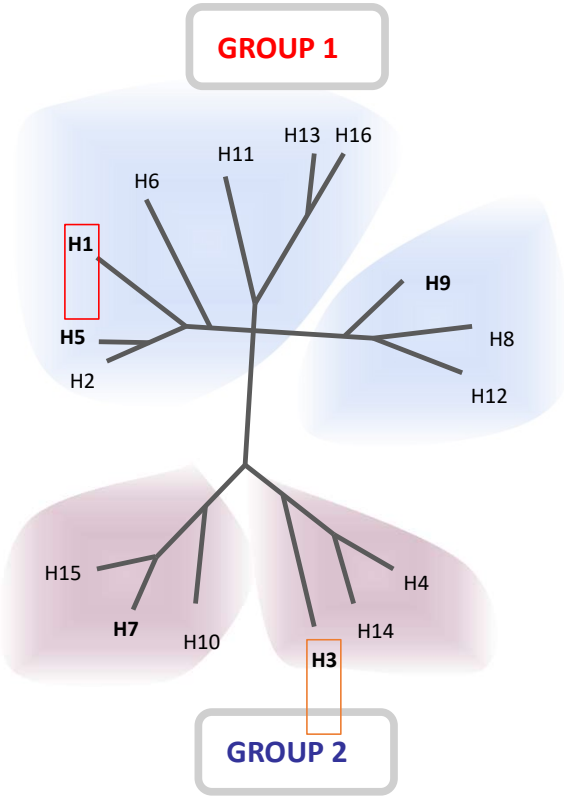


Beltramello et al. *Cell Host & Microbe* 2010

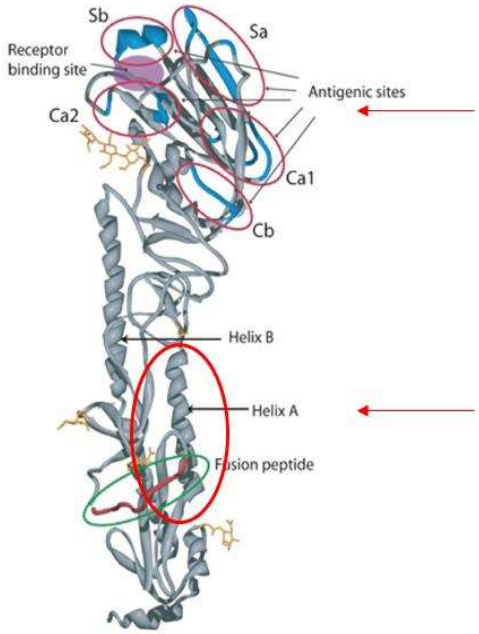
EXAMPLE 2

The recurrent problem of seasonal influenza and the unpredictability of pandemic viruses



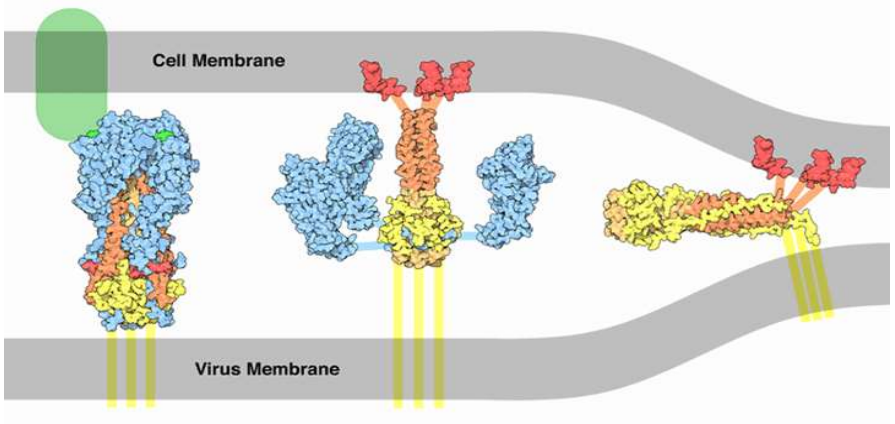


Two types of influenza neutralizing antibodies

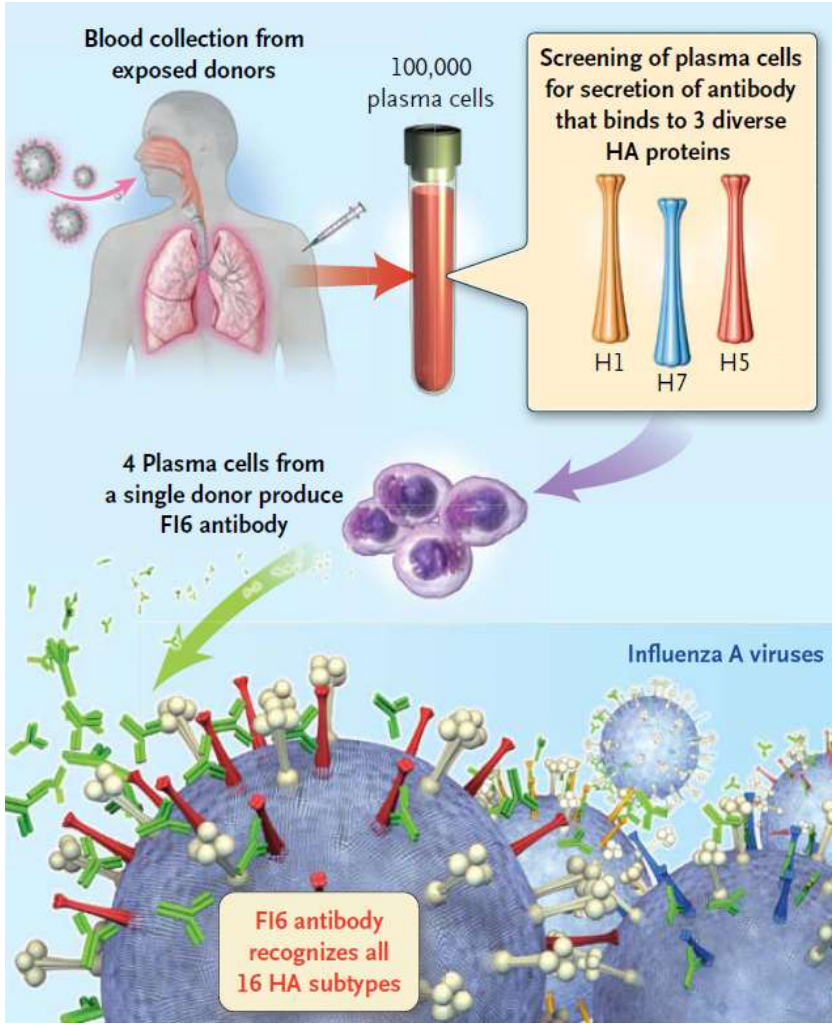


Classic antibodies recognize immunodominant epitopes in the globular head. They neutralize only a few related isolates within a given subtype and select escape mutants.

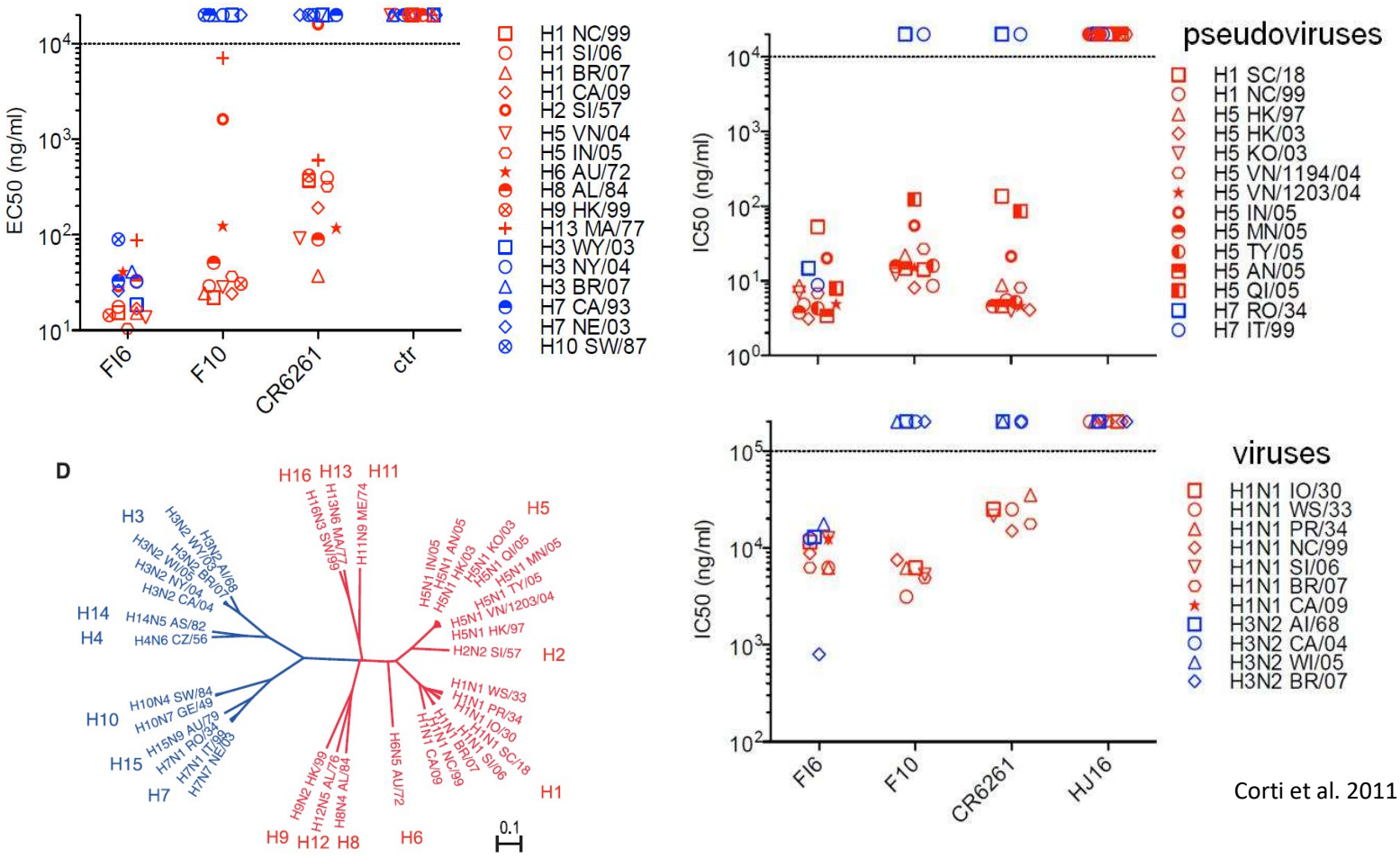
Antibodies that bind to the stem region neutralize several HA subtypes but only within Group 1.
They were isolated from mice (Okuno et al 1993), phage libraries (Throsby et al. 2008; Sui et al. 2009), human memory B cells (Corti et al. 2010).



Isolation of Group 1 and Group 2 specific mAb

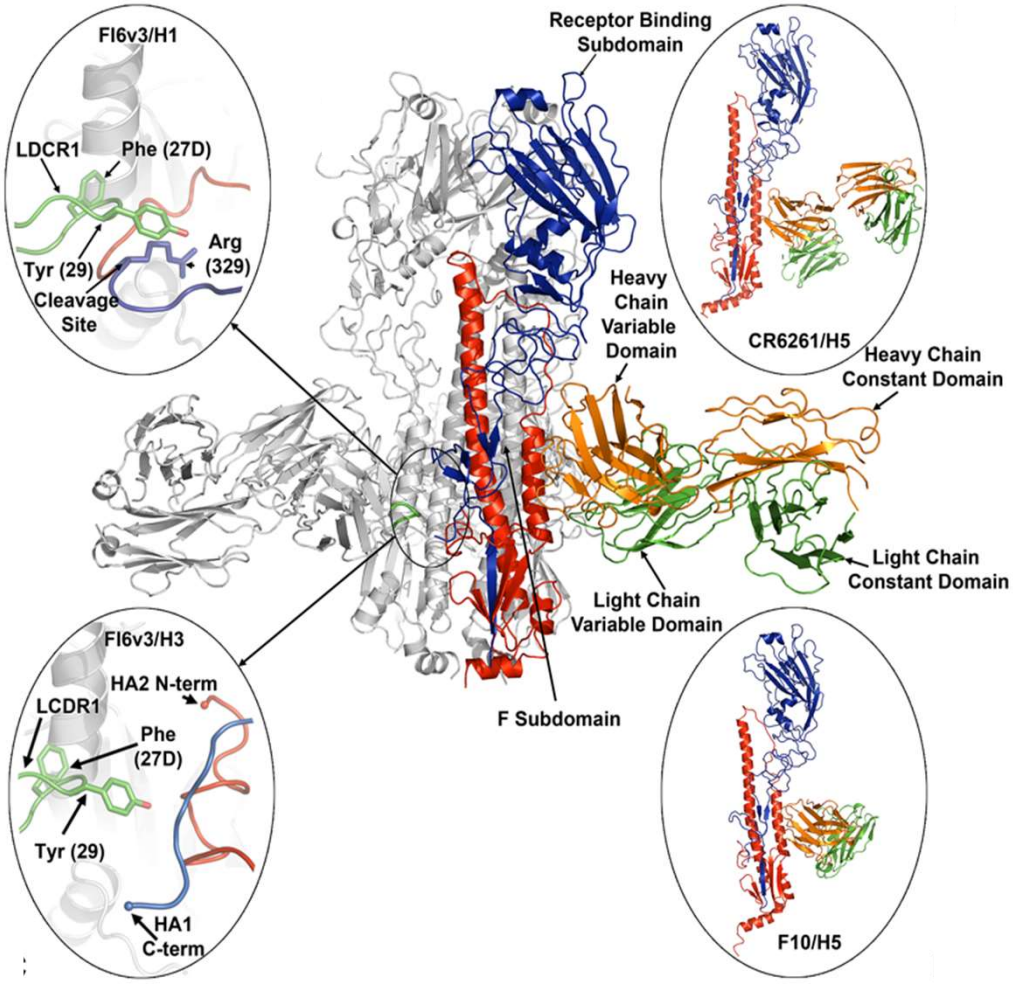


Isolation of Group 1 and Group 2 specific mAb



Corti et al. 2011

FI6 in complex with uncleaved H1 HA
A/California/04/09

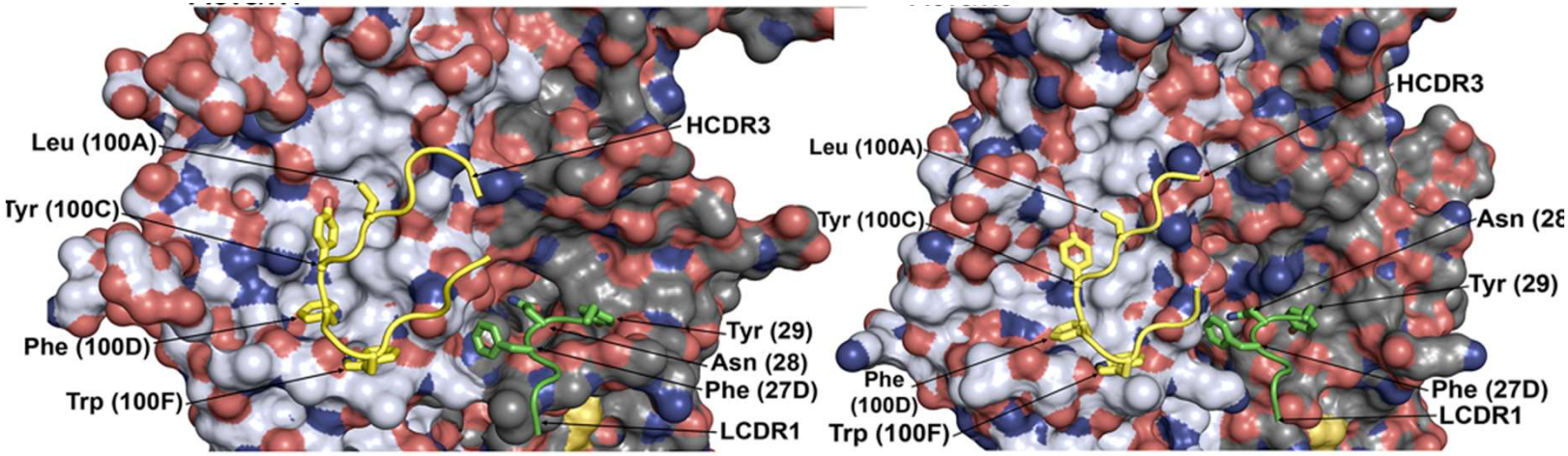


FI6 in complex with cleaved
H3 HA A/Hong Kong/1/68

Corti et al Science 2011

FI6 contacts a large surface area of two HA monomers

FI6v3/H1 (uncleaved) **FI6v3/H3 (cleaved)**



- HCDR3 (yellow) makes the most important contact with helix A
- LCDR1 (green) makes contacts with the fusion peptide region of the adjacent HA molecule

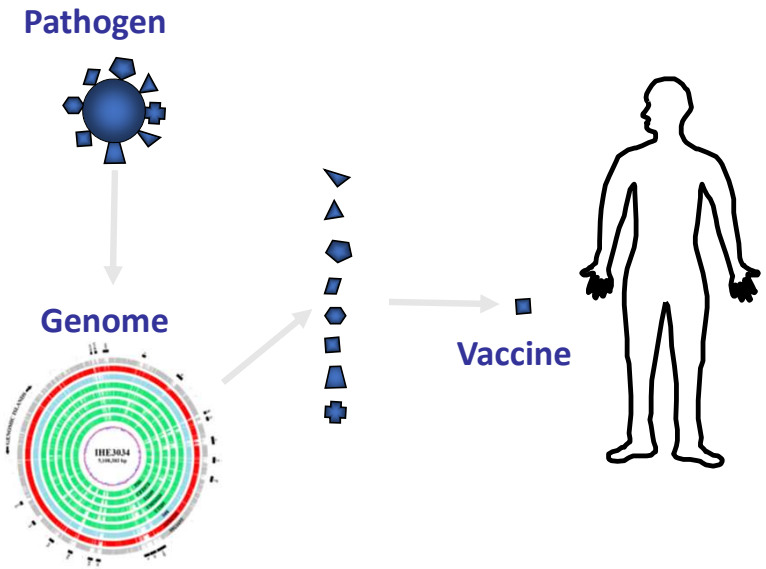
Corti et al. 2011

A pan-influenza A neutralizing antibody

- FI6 binds HAs of all 16 subtypes
- Group 1 reactivity is germline-encoded;
  Group 2 reactivity was acquired through somatic mutations
- FI6 protects mice from H1and H3 and ferrets from H5 viruses
- FI6 binds to virions and infected cells, neutralizes infectious virus, inhibits HA cleavage, prevents viral spread and targets infected cells for killing by NK cells
- The crystal structures reveal an accessible quaternary epitope conserved in Group 1 and Group 2 hemagglutinins, cleaved and uncleaved
- FI6 does not select escape mutants

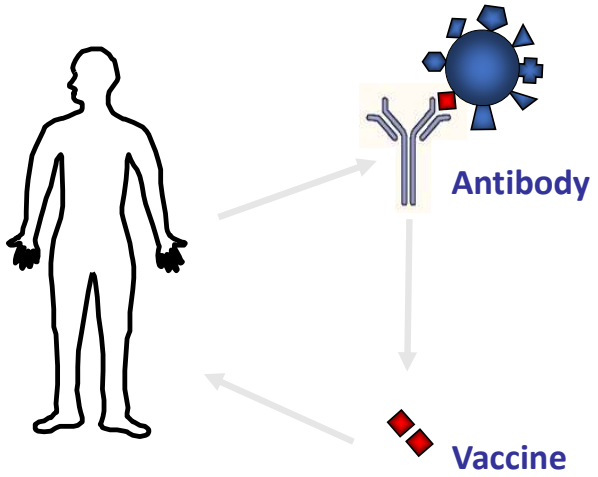
Novel approaches to vaccine design

Reverse Vaccinology



1) Analysis of the genomic sequence of the pathogen
2) Expression of candidate genes and test all the recombinant proteins in animals for their capacity to induce neutralizing antibodies
3) Selection of antigens capable of inducing neutralizing antibodies

Analytic vaccinology



1) Isolation of neutralizing antibodies
2) Identification of the antigens recognized
3) Expression of the selected antigens in order to preserve antibody binding

Concepts from:
Rappuoli 2011, Nat Rev. Immunoogy
Lanzavecchia et al 2016 Curr Op Immunol

mAb nomenclature

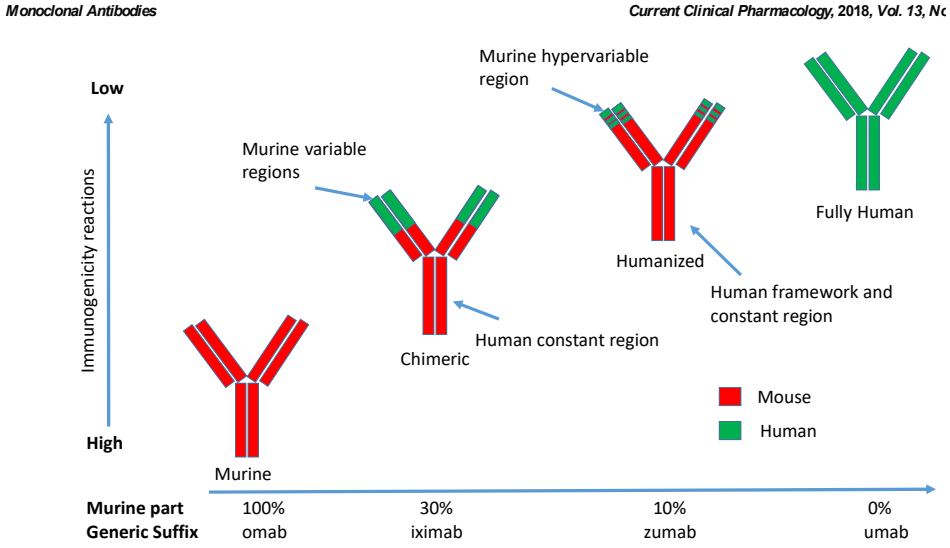


Fig. (1). Humanization of monoclonal antibodies.

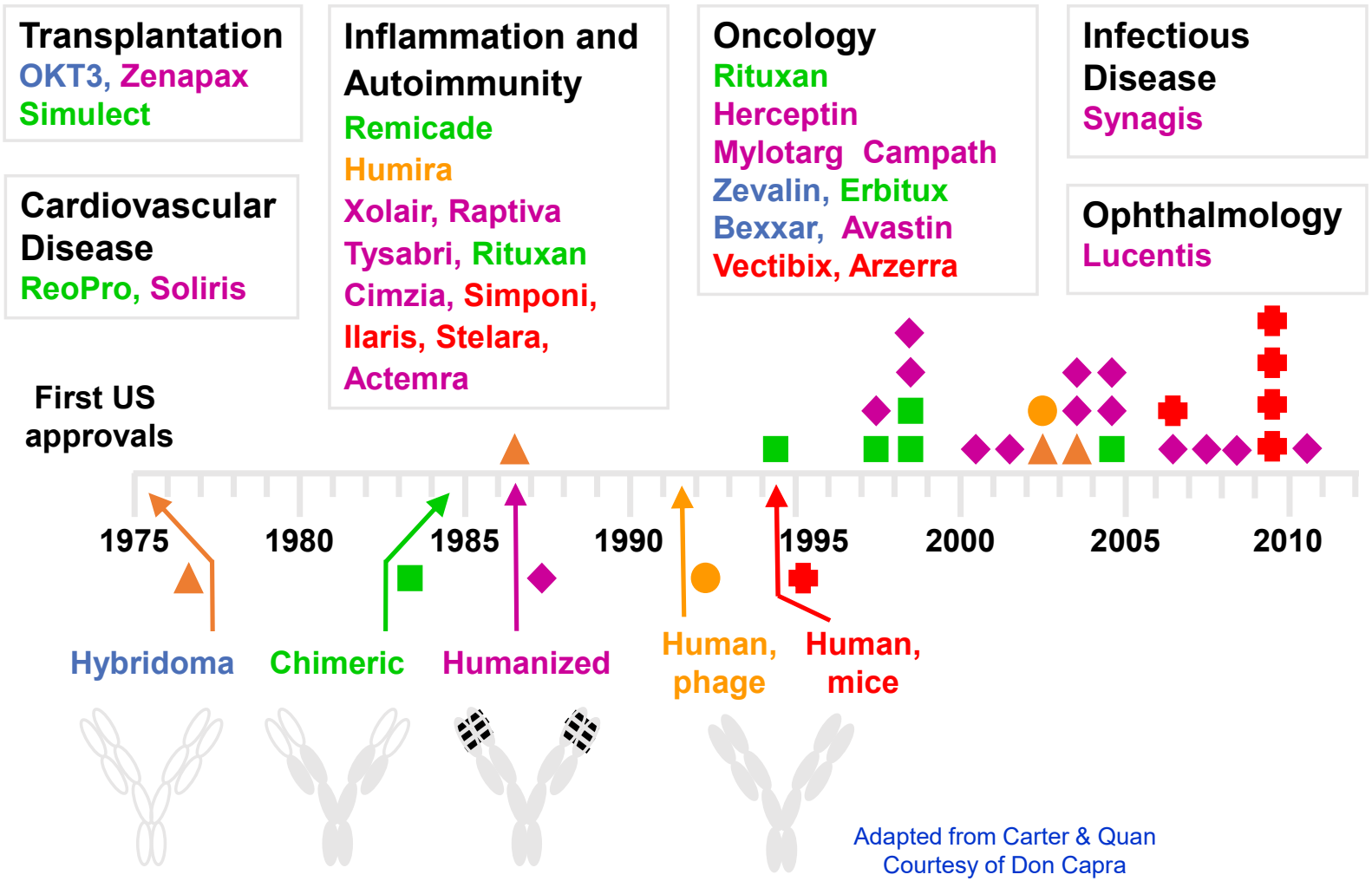
Table 1

Naming scheme of antibodies according to the international nonproprietary naming (INN) program by the WHO before 2014 and current (2018) revisions excluding the species substem B

Pre-2014 INN:					
1. Prefix		2. Target substem A		3. Species substem B	4. Universal stem
random	-b(a)-	bacterial	-a-	rat	-mab
	-am(i)-	serum amyloid protein (SAP)/amyloidosis (pre-substem)	-axo-	rat-mouse (pre-substem)	
			-e-	hamster	
	-c(i)-	cardiovascular	-i-	primate	
	-f(u)-	fungal	-o-	mouse	
	-gr(o)-	skeletal muscle mass related growth factors and receptors (pre-substem)	-u-	human	
			-vet-	veterinary use (pre-substem)	
	-k(i)-	interleukin	-xi-	chimeric	
	-l(i)-	immunomodulating	-xizu-	chimeric-humanized	
	-N(e)-	neural	-zu-	humanized	
	-s(o)-	bone			
	-tox(a)-	toxin			
	-t(u)-	tumour			
	-v(i)-	viral			

Post-2017 INN			
1. Prefix	2. Target substem (currently under revision)		3. Universal stem
random	-ba-	bacterial	-mab
	-ami-	serum amyloid protein (SAP)/amyloidosis (pre-substem)	
	-ci-	cardiovascular	
	-fung-	fungal	
	-gros-	skeletal muscle mass related growth factors and receptors (pre-substem)	
	-ki-	interleukin	
	-li-	immunomodulating	
	-ne-	neural	
	-os-	bone	
	-toxa-	toxin	
	-ta-	tumour	
	-vet-	veterinary use (pre-stem)	
	-vi-	viral	

Monoclonal antibodies on the market



Nomenclature of monoclonal antibodies

Murine: momab

Muromomab-CD3, Orthoclone, CD3
Ibritumomab, Zevalin, CD20-radio
Tositumomab, Bexxar, CD20-radio

Chimeric: ximab

Basiliximab, Simulect, CD25
Abciximab, ReoPro, platelets
Infliximab, Remicade, TNF
Rituximab, Mabthera, CD20

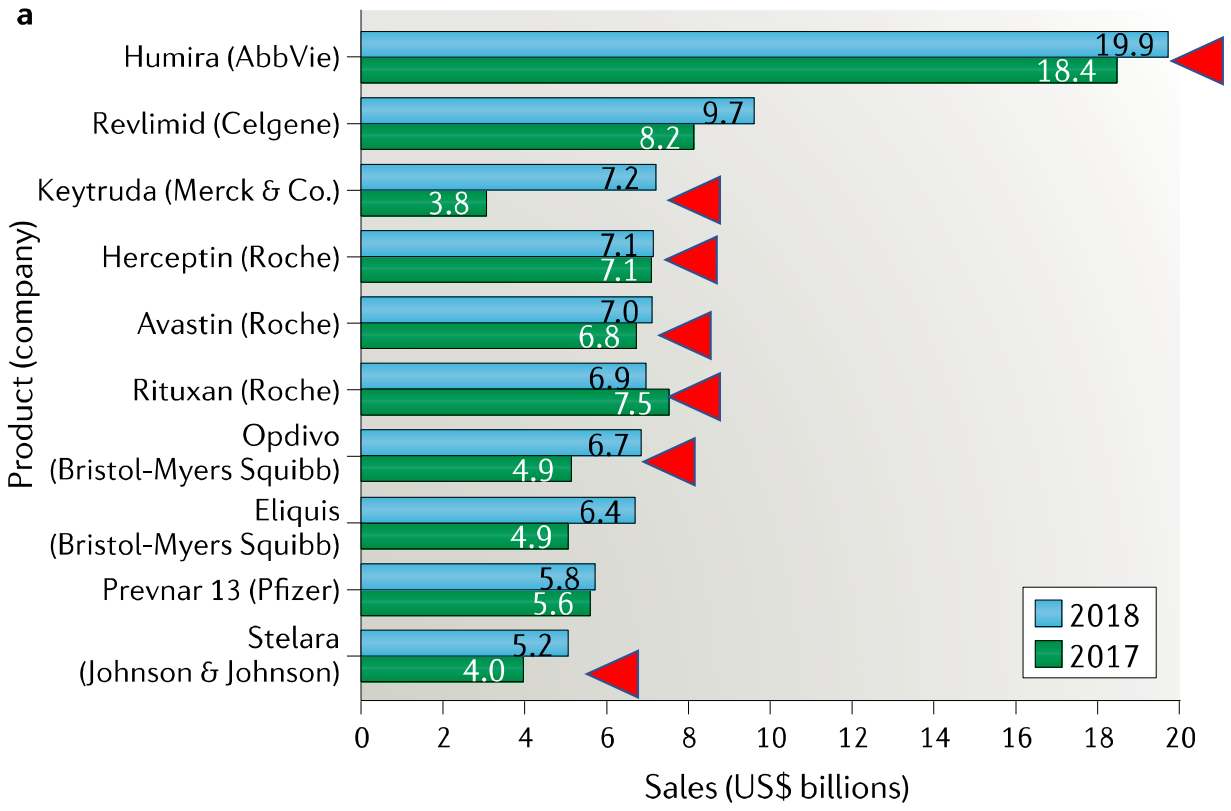
Humanized: zumab

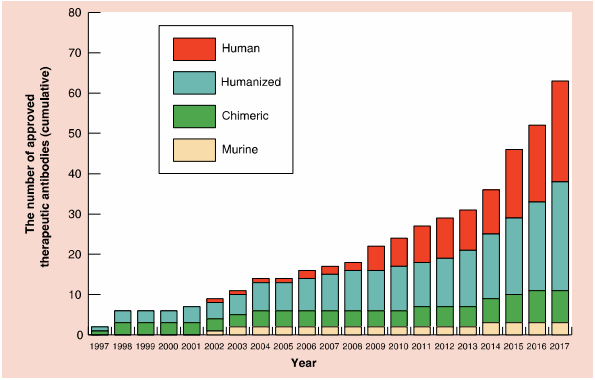
Trastuzumab, Herceptin, HER2
Daclizumab, Zenapax, CD25
Eculizumab, Soliris, C5
Omalizumab, Xolair, IgE
Efalizumab, Raptiva, CD11a
Natalizumab, Tysabri, $\alpha 4\beta 1$
Certolizumab, Cimzia, TNF (PEG)
Tocilizumab, Aktemra, IL-6
Trastuzumab, Herceptin, HER2
Gemtuzumab, Mylotarg, CD33-drug
Alemtuzumab, Campath, CD52
Bevacizumab, Avastin, VEGF-A
Ranibizumab, Lucentis, VEGF-A
Palivizumab, Synagis, RSV

Human: umab

Adalimumab, Humira, TNF
Golimumab, Simponi, TNF
Canakinumab, Ilaris, IL-1β
Ustekinumab, Stelara, IL-23
Panitumumab, Vectibix, EGF-R
Ofatumumab, Arzerra, CD20

Antibodies as therapeutics





Immunotherapy (2019) 11(2), 119–127

Table 1. Antibody therapies in clinical trials for the prevention and treatment of viral infections

Virus	MAb, sponsor	Notes	Reference
HCMV	**CSJ148 (LJP538 and LJP539)**, Novartis	**CSJ148** is a combination of two anti-HCMV human mAbs that bind to and inhibit the function of viral HCMV gB (LJP538) and pentameric gH complex (LJP539). The two antibodies were isolated from EBV immortalized B cells. CSJ148 is in Phase 2 clinical trials (NCT02268526)	52
	RG7667 (MCMV5322A and MCMV3068A), Genentech	**RG7667** is a combination of two mAbs that bind to glycoprotein complexes gH/gL (MCMV5322A) and pentameric gH complex (MCMV3068A). **MCMV5322A** is a human immunoglobulin antibody (IgG1κ) and is an affinity-matured version of MSL-109. **MCMV3068A** was isolated from an hybridoma screen in mice and subsequently humanized with an IgG1λ framework. Phase 2 clinical trials were completed for RG7667 (NCT01753167)	49
	TCN-202, Theraclone Sciences	**TCN-202** is a human mAb that targets AD-2, a linear, conserved, poorly immunogenic epitope on the N-terminal domain of HCMV gB. This mAb effectively neutralized infection and was observed to be well tolerated and non-immunogenic in initial clinical trials. However, development was discontinued after a Phase 1 adverse event (NCT01594437)	51
	MSL-109/Serivumab, NCRR, NIAID, Facet Biotech, Johns Hopkings Bloomberg School of Public Health, Sandoz Inc.	**MSL-109** is a human monoclonal IgG isolated from a HCMV seropositive individual that recognizes the viral glycoprotein H (gH). A Phase 2/3 trial of MSL 109 was completed (NCT00000836)	57
Influenza	**MHAA4549A (earlier known as 39.29)**, Genentech, Inc.	**MHAA4549A** is a human immunoglobulin G1 (IgG1) mAb that binds to a highly conserved epitope on the stalk of Group 1 and Group 2 influenza A hemagglutinins and blocks the hemagglutinin-mediated membrane fusion in the endosome, neutralizing all known human influenza A strains. MHAA4549A was cloned from a single human plasmablast cell isolated from an influenza vaccinated donor MHAA4549A is in Phase 2 clinical trials (NCT02623322)	62
	VIS410, Visterra, Inc.	**VIS410** targets a conserved epitope in the stem of influenza A hemagglutinin (HA). It was engineered using structural information on antibody–antigen interfaces. VIS410 is in Phase 2 clinical trials (NCT02989194)	151
	CR6261, Crucell Holland BV and the National Institute of Allergy and Infectious Diseases (NIAID)	Isolated from a healthy, vaccinated individual using phage display selection on recombinant H5 HA, mAb **CR6261** targets a highly conserved helical region in the membrane-proximal stem of HA1/HA2 from 1918 H1N1 influenza and H5N1 influenza. It uses the Ig VH1–69 germline segment. CR6261 is in Phase 2 clinical trials (NCT02371668)	70
	CR8020, Crucell Holland BV and Retroscreen Virology Ltd.	**CR8020** is a broadly neutralizing influenza hemagglutinin stem-specific human mAb. CR8020 was isolated from a B cell of a donor vaccinated against influenza. A Phase 2 clinical trial was completed for CR8020 (NCT01938352)	74
	TCN-032, Theraclone Sciences	One of a panel of mAbs derived from memory B cells of healthy human subjects, **TCN-032** targets an epitope in the ectodomain of the influenza matrix 2 protein M2e. This epitope, first identified with the isolation of the panel of mAbs including **TCN-032**, is highly conserved in influenza A viruses. A Phase 2 clinical study was initiated for TCN-032 (NCT01719874)	76
HIV	**VRC01**, NIAID	**VRC01** is a broadly neutralizing antibody targeting the CD4-binding site of HIV gp120. It was isolated from memory B cells of infected individuals using a targeted flow cytometry-based approach. VRC01 is in multiple Phase 2 clinical trials (NCT02664415, NCT02568215, NCT02716675)	40
	3BNC117, Rockefeller University, Weill Medical College of Cornell University, Brigham and Women's Hospital, and the University of Cologne	3BNC117 is a broad and potent neutralizing antibody against the CD4-binding site of the HIV-1 Env protein. 3BNC117 is being tested in multiple Phase 2 clinical trials (NCT02446847, NCT02588586. NCT02850016)	92
	10-1074, Rockefeller University and the University of Cologne	Several antibodies including **10-1074** were isolated from B cells from a clade A-infected African donor using YU-2 gp140 trimers as bait. 10-1074 is a broadly neutralizing antibody (bnAb) that targets the V3-glycan supersite of HIV gp120. 10-1074 is currently in multiple Phase 1 clinical trials in combination with 3BNC117 (NCT02825797, NCT02511990, NCT02825797)	152
	4E10, 2F5, 2G12, Rockefeller University	**4E10, 2F5, 2G12** are broadly and potently neutralizing mAb specific for gp41. A Phase 1/2 clinical trial was completed for well-suppressed HAART-treated individuals during acute and early HIV-1 infection (NCT00219986)	153
	PRO 140, Amarex Clinical Research, CytoDyn, Inc., National Institute on	**PRO 140** is an antibody used to treat HIV targeting host CCR5 receptors. Pro 140 is in multiple Phase 2/3 trials (NCT02859961, NCT02355184,	99

Table 1 continued

Virus	MAb, sponsor	Notes	Reference
	Drug Abuse (NIDA), and Drexel University	NCT02483078,NCT02990858, NCT02438345, NCT02737306, NCT01272258)	
	Ibalizumab (TNX-355), Genentech	This anti-CD4 mAb is in Phase 3 trials (NCT02707861)	154
RSV	**Synagis (Palivizumab; MEDI-493)**, MedImmune	Approved for prophylaxis in infants at high risk for RSV, Synagis is a humanized mAb of IgG1 isoform. It targets RSV glycoprotein F	103
	(Numax, MEDI-524), MedImmune	**Motavizumab** has completed Phase 3 trials for prophylaxis in infants at high risk for RSV. It is an affinity matured version of Palivizumab (NCT00129766)	106
	Motavizumab—YTE (MEDI-557), MedImmune	**Motavizumab—YTE** is an Fc-modified, half-life extended derivative of motavizumab, with amino-acid substitutions M252Y/S254T/T256E. This mAb has completed Phase 1 trials (NCT01562938, NCT01475305, NCT00578682, NCT01455402)	107
	MEDI8897, MedImmune	**MEDI8897** is an anti-RSV antibody isolated a human B cell with significantly higher potency than palivimab and an Fc-modification to extend half-life. This mAb is in Phase 2 trials (NCT02878330)	108
	REGN2222, Regeneron Pharmaceuticals	**REGN2222** is an IgGmAb targeting the RSV-F protein. This mAb is in Phase 3 trials for prevention of RSV in pre-term infants with lower respiratory tract infection (NCT02325791)	155
	ALX-0171, Ablynx	**ALX-0171** is in Phase 2 trials for infants hospitalized for respiratory syncytial virus lower respiratory tract infection (RSV LRTI). It is a single-domain camelid-derived antibody, or nanobody	156
Ebola	**ZMapp**, National Institute of Allergy and Infectious Diseases (NIAID)	**ZMapp** is an optimized combination of three antibodies. It was given special approval for compassionate use during the 2014/2015 Ebola outbreak and is in Phase 1 trials (NCT02389192)	113, 115, 157
Rabies	**CL184 (CR57 and CR4098)**, Crucell	**CL184** is a mAb cocktail consisting of **CR57 and CR4098**, and **CL184** was evaluated as a replacement for human rabies immunoglobulin (HRIG) (NCT00708084, NCT00656097, NCT0122838)	123
	RAB-1 (17C7), Serum Institute of India and MassBiologics	This antibody was developed using transgenic HuMab-Mouse (Medarex) and it has been tested in multiple clinical trials in India ((CTRI/2009/091/000465 and CTRI/2012/05/002709)	130, 131

HUMABS BioMed
a subsidiary of Vir Biotechnology