Monoclonal Antibody: A New Era of Medicine

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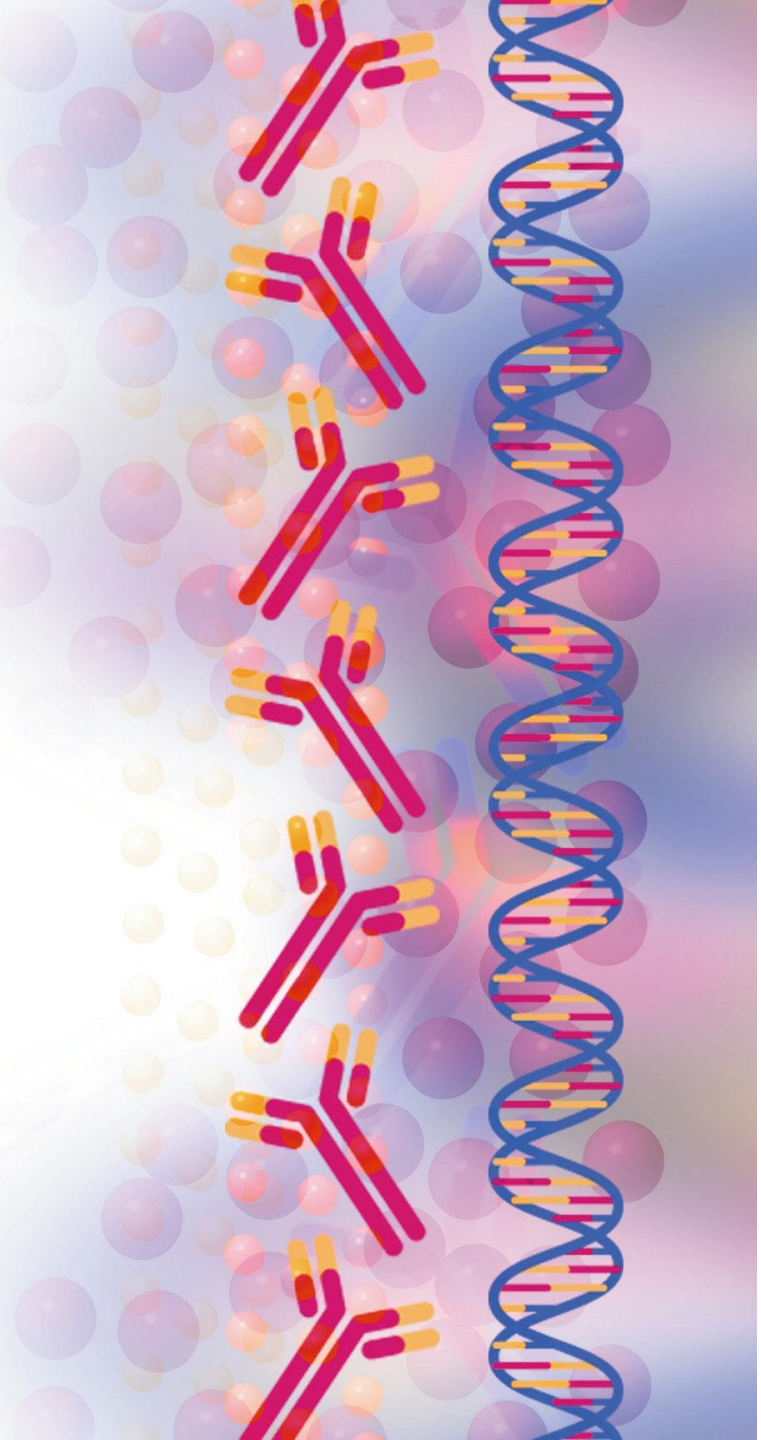


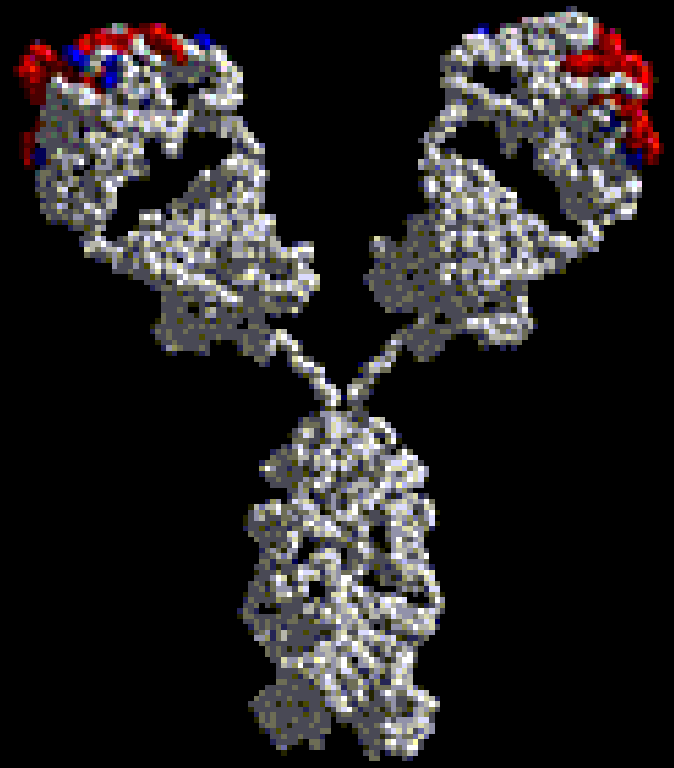

SERUM
Behring
Allopa
Phenol

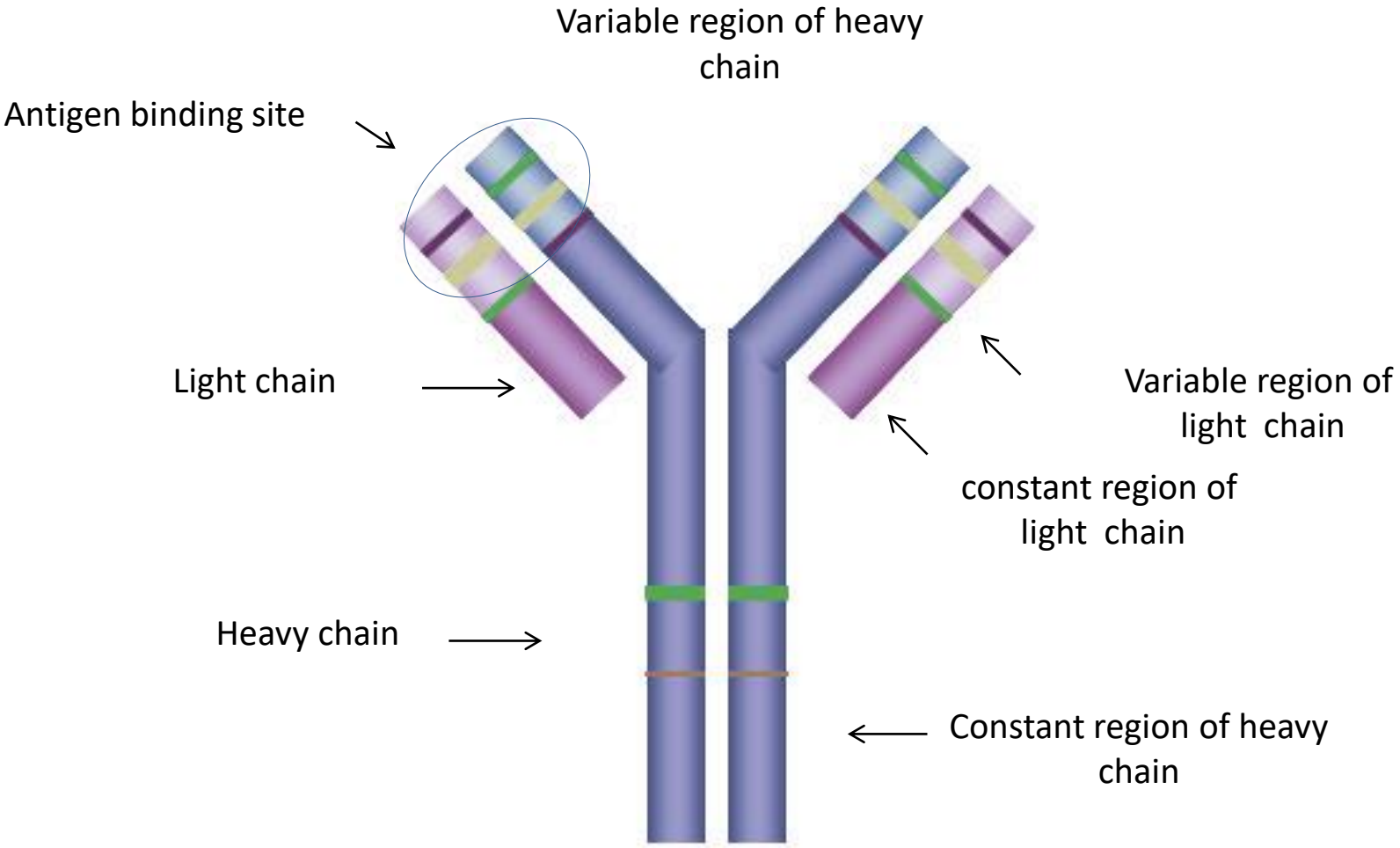
Immunological Basis

- Antigens are **recognized** by the body's immune system as **invaders**.

- Our **natural defenses** against these infectious agents are **antibodies.**

Antibody is the major player in biopharmaceutical bussiness.




Variable region of heavy chain
Antigen binding site
Light chain
Variable region of light chain
constant region of light chain
Heavy chain
Constant region of heavy chain

Modes of Antibody Therapy

Passive

- Infectious diseases

  diphtheria, measles, rubella, hepatitis, tetanus, rabies

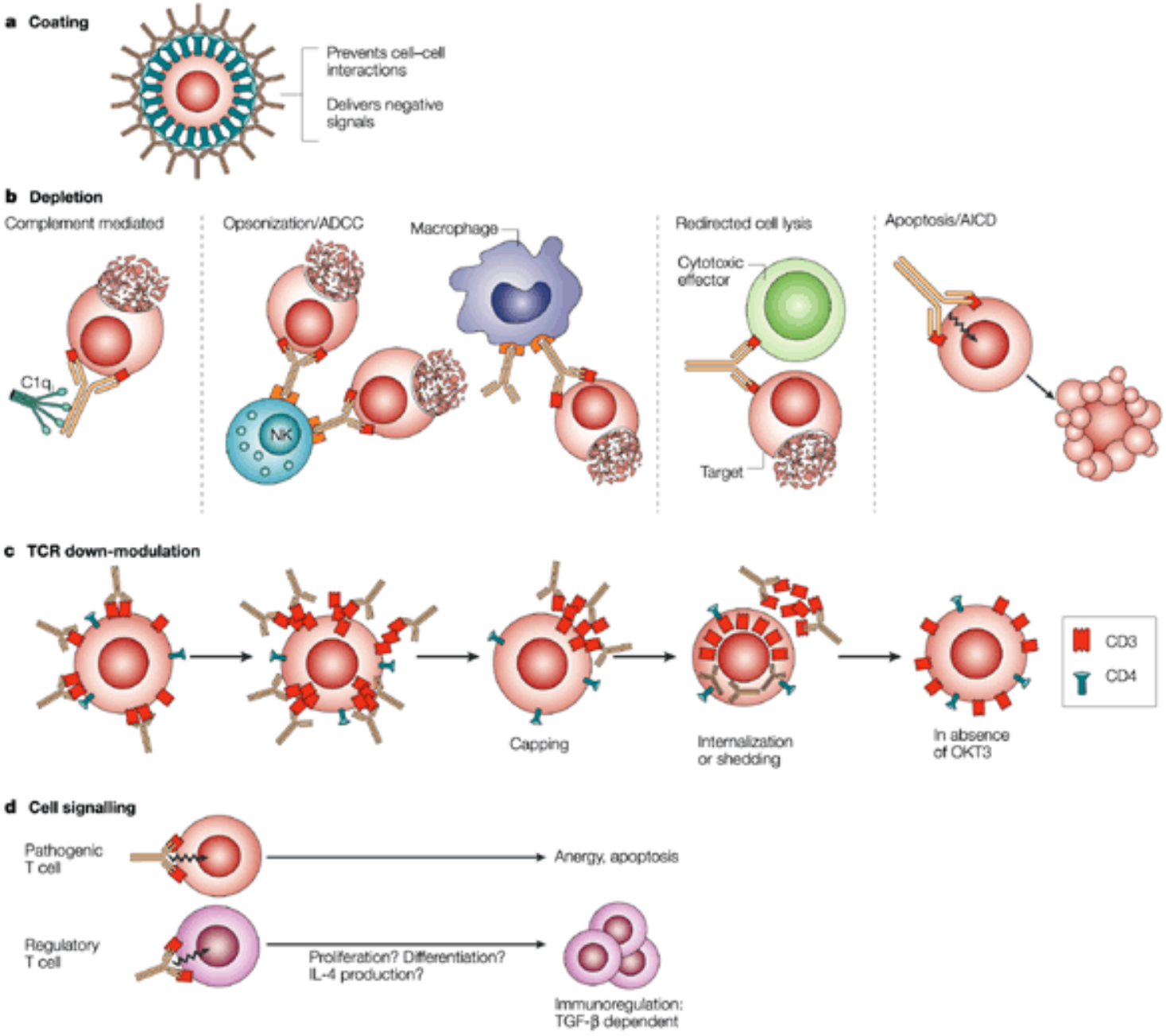
- Anti-venoms

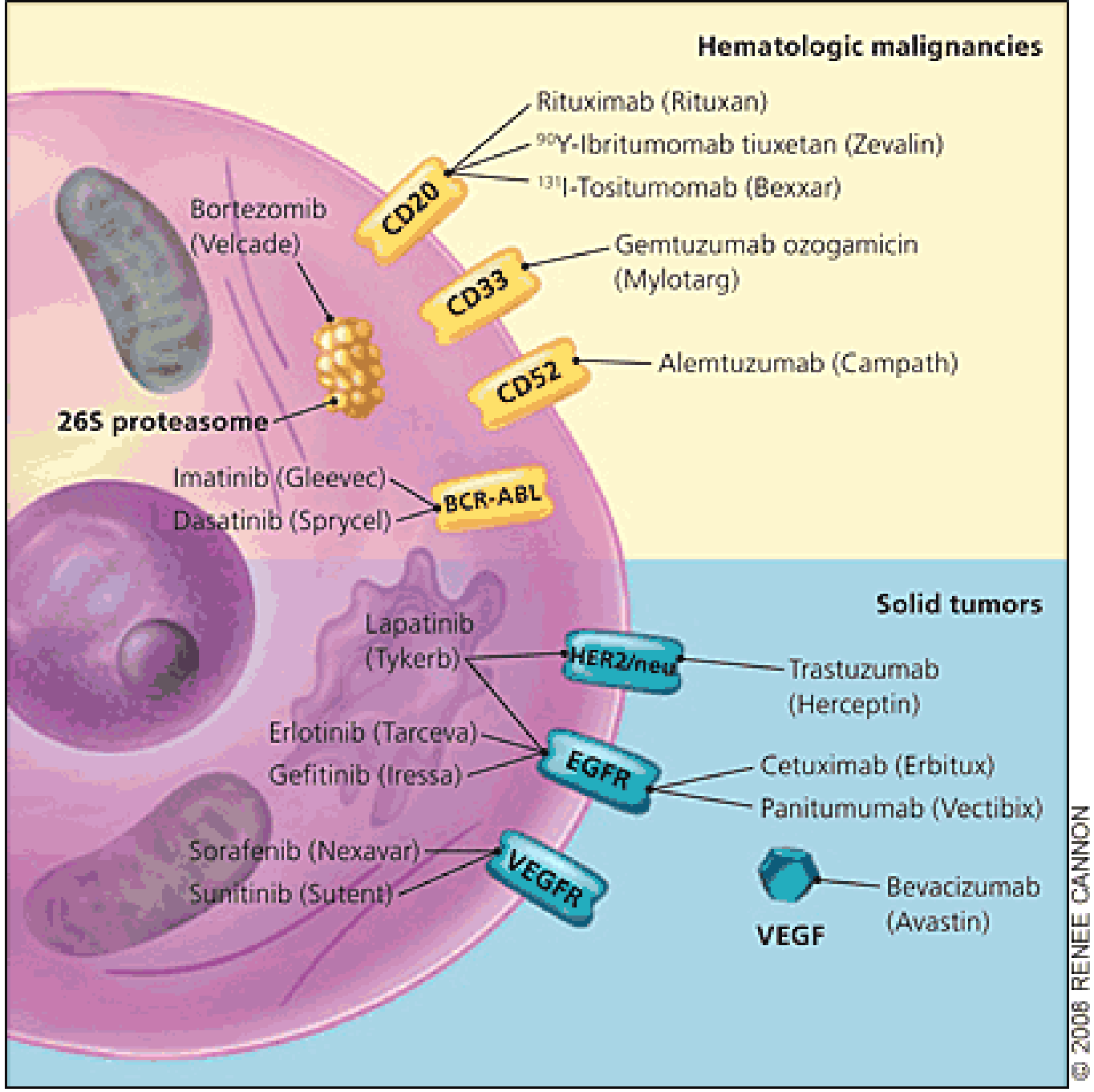
Active

- Cancer
- Cardiovascular diseases
- Autoimmune diseases and transplantation

Therapeutic Categories of MAb

- **Oncology**
  - Cell depletion (Rituxan™, Campath™, Mylotarg™, Zevalin™, Bexxar™)
  - Blocking receptor (Herceptin™)
  - Attacking vasculature (Avastin™, Erbitux™)
- **Immunology & Transplantation**
  - TNF-alpha (Humira™, Enbrel™)
  - Depletion of T cell (Orthoclone OKT3™, Zenapax™, Simulect™)
- **Anti-infective (Synagis™)**


a Coating
Prevents cell–cell interactions
Delivers negative signals
b Depletion
Complement mediated
C1q
Opsonization/ADCC
Macrophage
NK
Redirected cell lysis
Cytotoxic effector
Target
Apoptosis/AICD
c TCR down-modulation
Capping
Internalization or shedding
In absence of OKT3
CD3
CD4
d Cell signalling
Pathogenic T cell
Anergy, apoptosis
Regulatory T cell
Proliferation? Differentiation? IL-4 production?
Immunoregulation: TGF-β dependent



http://www.aafp.org/afp//AFPprinte

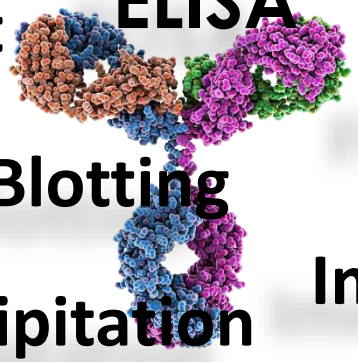
Location of DNA binding sites

Chromatin Immunoprecipitation

Isolate cell populations

ELISA

Dot Blot



Flow Cytometry

Presence of a substance in a sample

Western Blotting

Immunofluorescence

Immunoprecipitation

Analyze cells or subcellular localization

Immunohistochemistry

Radioimmunoassay

Measure the amount of a substance by a radioactive label

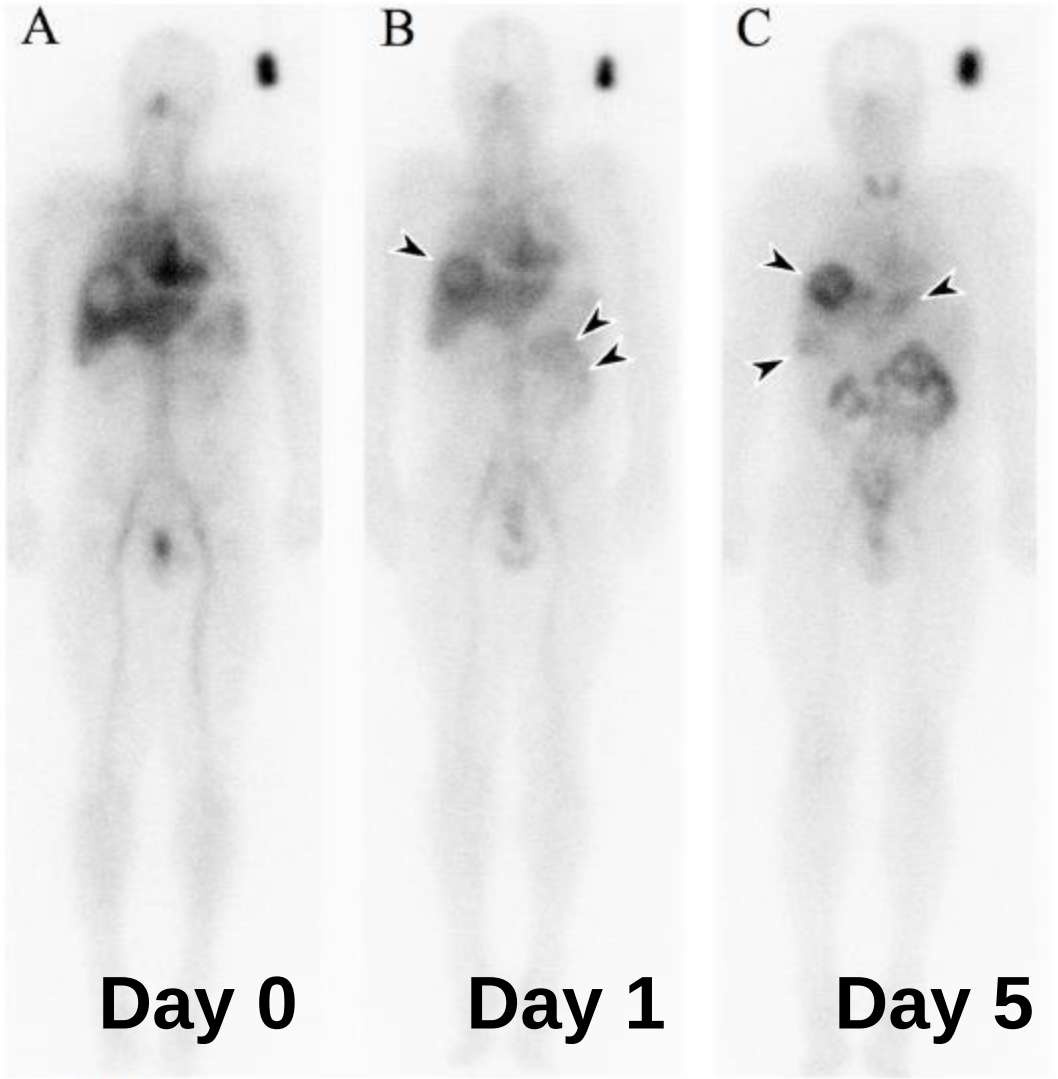
Localize antigens in cells or in a tissue

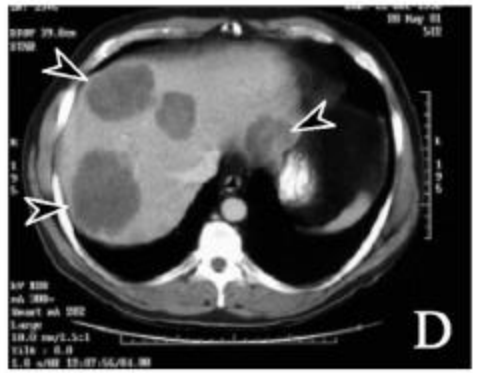
Mechanisms of tumor cell killing by antibodies

Cancer Immunity (1 May 2012) Vol. 12, p. 14

Direct tumor cell killing
• cell surface receptor agonist activity (leading to apoptosis) • cell surface receptor antagonist activity (inhibit signaling, reduce proliferation, induce apoptosis) • cell surface enzyme neutralization (leading to signaling abrogation) • conjugated antibody, delivery of payload (drug, toxin, radio-isotope, leading to cell death)
Immune-mediated tumor cell killing
• induction of phagocytosis • complement activation • antibody-dependent cell-mediated cytotoxicity (ADCC) • target gene-modified T cells • activate T cells (through inhibition of T cell inhibitory receptors, such as CTLA-4, or antibody-mediated cross presentation of antigen to dendritic cells)
Vascular and stromal ablation
• vessel receptor antagonism or ligand trap • stromal cell inhibition • conjugated antibody, delivery of payload

Biodistribution of 131I-huA33 in a patient with metastatic colorectal carcinoma





Monoclonal antibodies currently FDA-approved in oncology

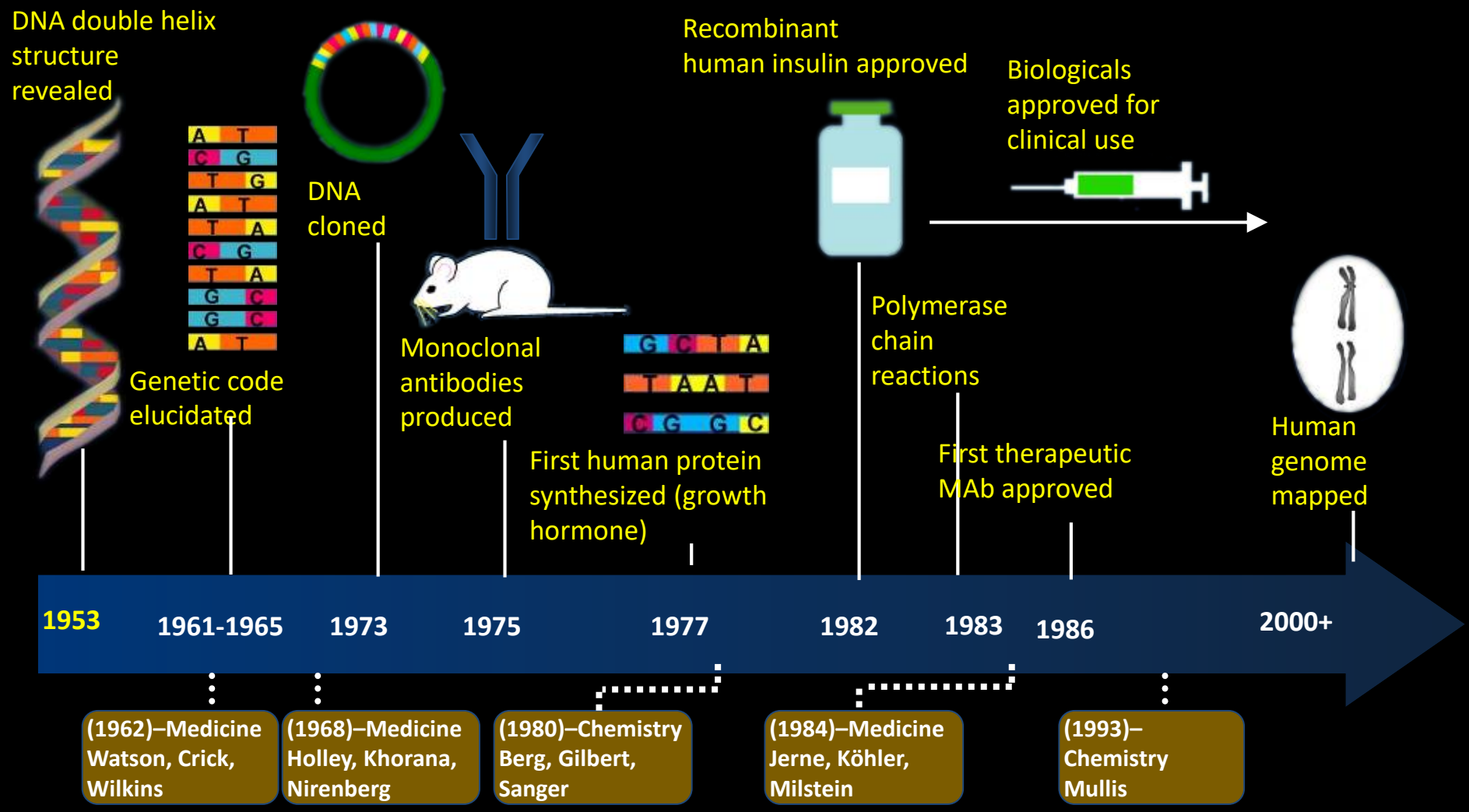
Antibody	Target	FDA-Approved indication	Mechanism of action
Trastuzumab (Herceptin®) humanized IgG1	HER2 (ErbB2)	HER2-positive breast cancer, as single agent or in combination with chemotherapy for (i) adjuvant or (ii) palliative treatment; HER2-positive gastric or gastroesophageal junction carcinoma, as first-line treatment in combination with cisplatin and capecitabine/5-FU	Inhibition of HER2 signaling; ADCC
Bevacizumab (Avastin®) humanized IgG1	VEGF	For the palliative treatment of colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, or renal cell carcinoma	Inhibition of VEGF signaling
Cetuximab (Erbitux®)* chimeric human/murine IgG1	EGFR (ErbB1)	In combination with radiation therapy for the initial treatment of locally or regionally advanced squamous cell cancer of the head and neck (SCCHN); As a single agent for SCCHN patients with whom prior platinum-based therapy has failed; Palliative treatment of pre-treated metastatic EGFR-positive colorectal cancer	Inhibition of EGFR signaling; ADCC
Panitumumab (Vectibix®)* human IgG2	EGFR (ErbB1)	As a single agent for the treatment of pre-treated EGFR-expressing, metastatic colorectal carcinoma	Inhibition of EGFR signaling
Ipilimumab (Yervoy®) IgG1	CTLA-4	For the treatment of unresectable or metastatic melanoma	Inhibition of CTLA-4 signaling
Rituximab (Rituxan® and Mabthera®) chimeric human/murine IgG1	CD20	For the treatment of CD20-positive B cell non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL), and for maintenance therapy for untreated follicular CD20-positive NHL	ADCC; direct induction of apoptosis; CDC
Alemtuzumab (Campath®) humanized IgG1	CD52	As a single agent for the treatment of B cell CLL	Direct induction of apoptosis; CDC
Ofatumumab (Arzerra®) human IgG1	CD20	Treatment of patients with CLL refractory to fludarabine and alemtuzumab	ADCC; CDC
Gemtuzumab ozogamicin (Mylotarg®) humanized IgG4	CD33	For the treatment of patients with CD33-positive acute myeloid leukemia in first relapse who are 60 years of age or older and who are not considered candidates for other cytotoxic chemotherapy (withdrawn from use in June 2010)	Delivery of toxic payload, calicheamicin toxin
Brentuximab vedotin (Adcetris®) chimeric IgG1	CD30	For the treatment of relapsed or refractory Hodgkin lymphoma and systemic anaplastic lymphoma	Delivery of toxic payload, auristatin toxin
^{90}Y-Ibritumomab Tiuxetan (Zevalin®) murine IgG1	CD20	Treatment of relapsed or refractory, low-grade, or follicular B cell NHL; Previously untreated follicular NHL in patients who achieve a partial or complete response to first-line chemotherapy	Delivery of the radio-isotope yttrium-90
^{131}I-Tositumomab (Bexxar®) murine IgG2	CD20	Treatment of patients with CD20 antigen-expressing relapsed or refractory low-grade, follicular, or transformed NHL	Delivery of the radio-isotope iodine-131; ADCC; direct induction of apoptosis

*Not recommended in colorectal cancer patients whose tumors express mutated KRas

Antibodies vs. Conventional drugs

- **Large size**
  - Unique biological activities
  - Restricted tissue distribution
  - Longer half life
- **Common structure**
  - Generic methodology
  - Idiosyncrasies
- **Potentially immunogenic**
  - Anti-idiotype therapy
  - Anti-globulin responses

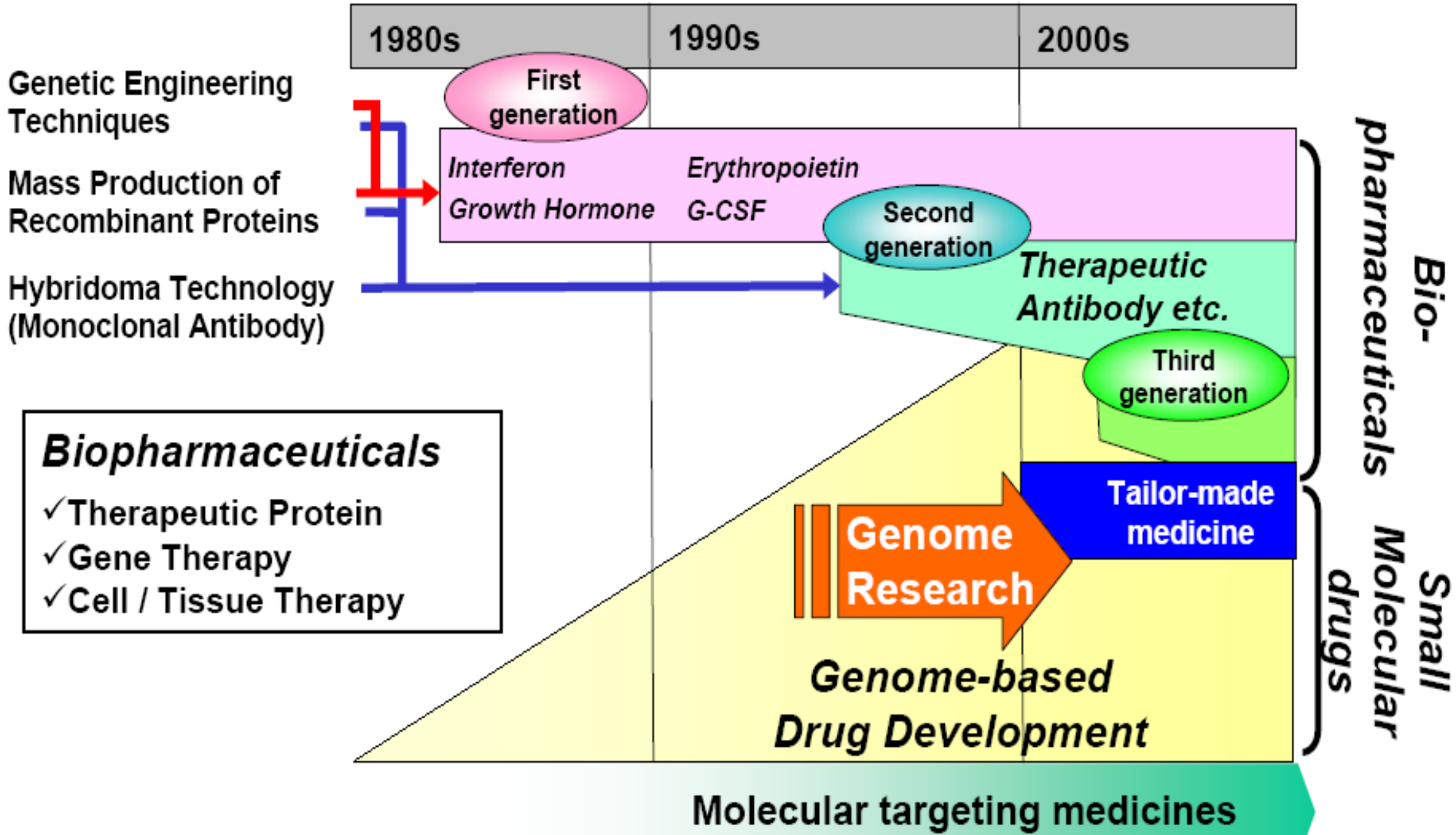
Evolution of Biotechnology

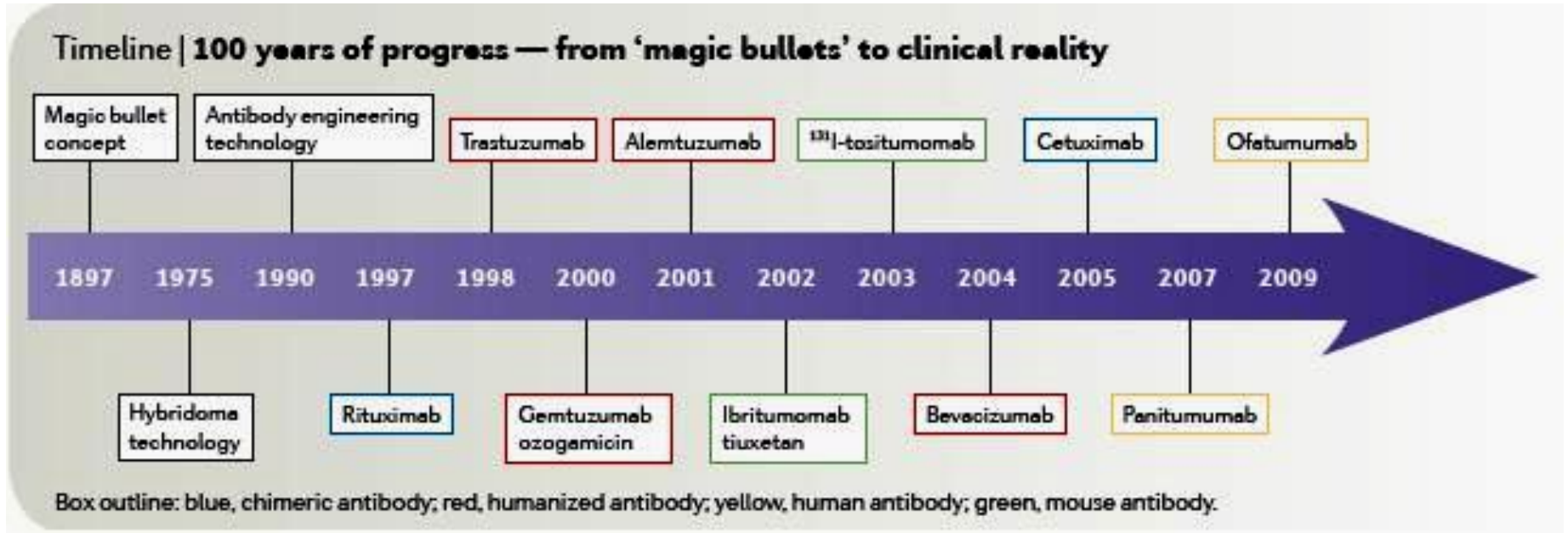


Historical Events in Biotechnology. Available at: www.biotechinstitute.org/what_is/timeline.html. Accessed August 10, 2007.
All Nobel Laureates. Available at: www./nobelprize.org. Accessed August 10, 2007

Development of Genomic/Bio-Pharmaceuticals

Bio-Pharmaceuticals: pharmaceuticals produced using bio-technology

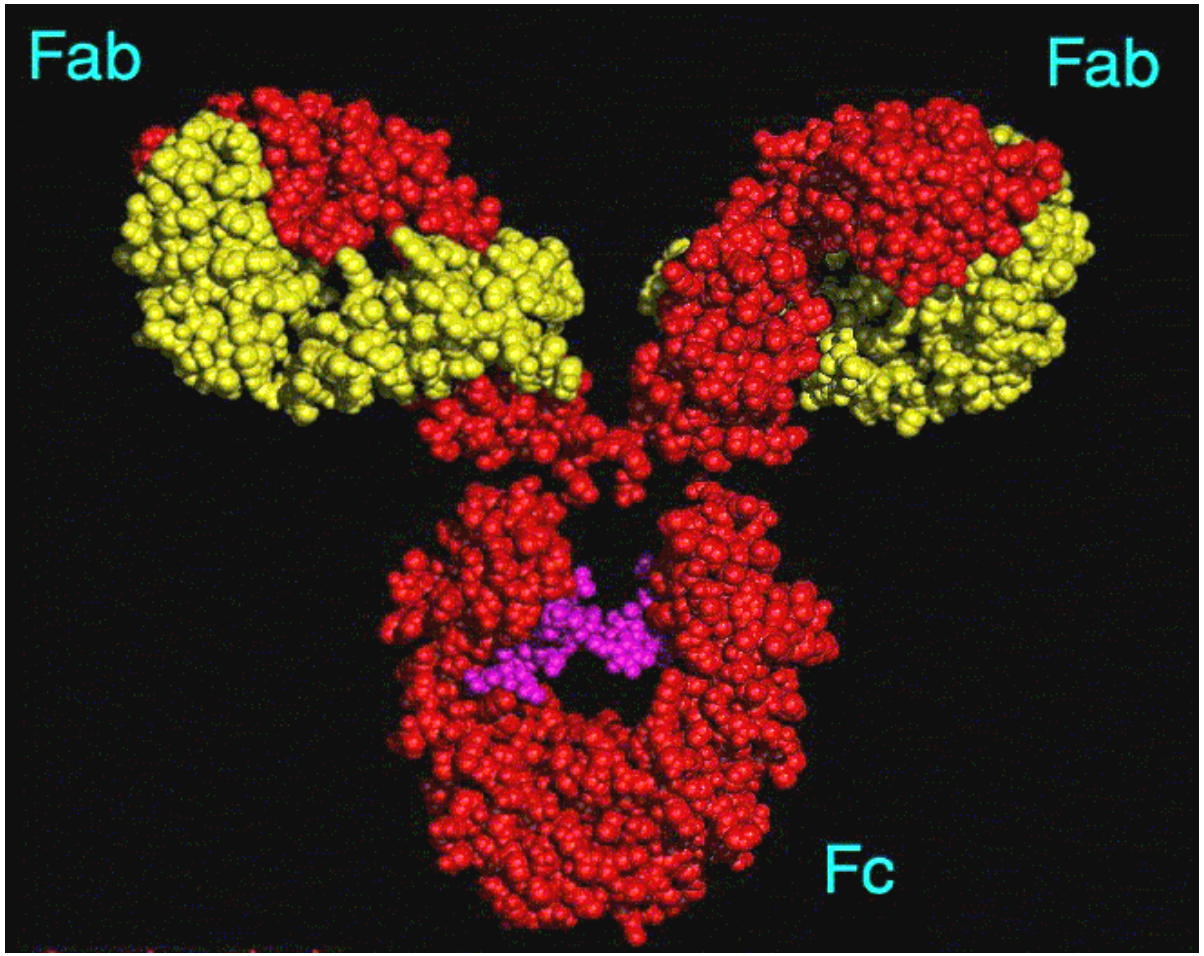



Timeline | 100 years of progress — from 'magic bullets' to clinical reality
Magic bullet concept
Antibody engineering technology
Trastuzumab
Alemtuzumab
131I-tositumomab
Cetuximab
Ofatumumab
1897
1975
1990
1997
1998
2000
2001
2002
2003
2004
2005
2007
2009
Hybridoma technology
Rituximab
Gemtuzumab ozogamicin
Ibritumomab tiuxetan
Bevacizumab
Panitumumab
Box outline: blue, chimeric antibody; red, humanized antibody; yellow, human antibody; green, mouse antibody.

First monoclonal therapeutic

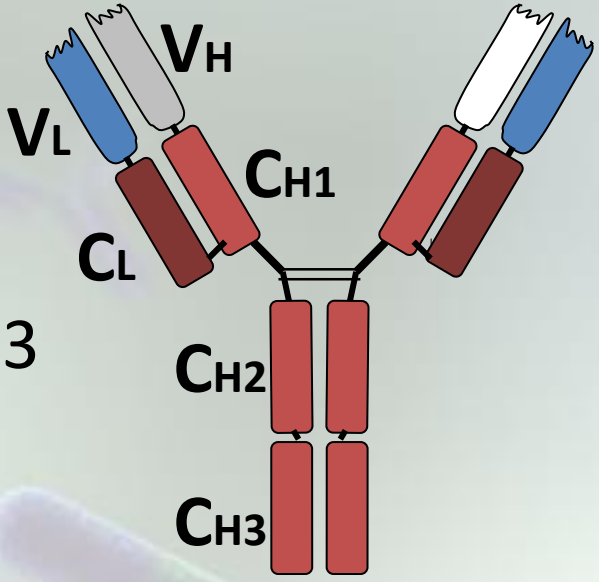
- T cells responsible for transplant rejection.
- Monoclonal target: T cells. Eliminates all T cells.
- Muromonab CD-3 (OKT3): (CD3 is a component of the T cell receptor).
- Immunosuppressive therapy.

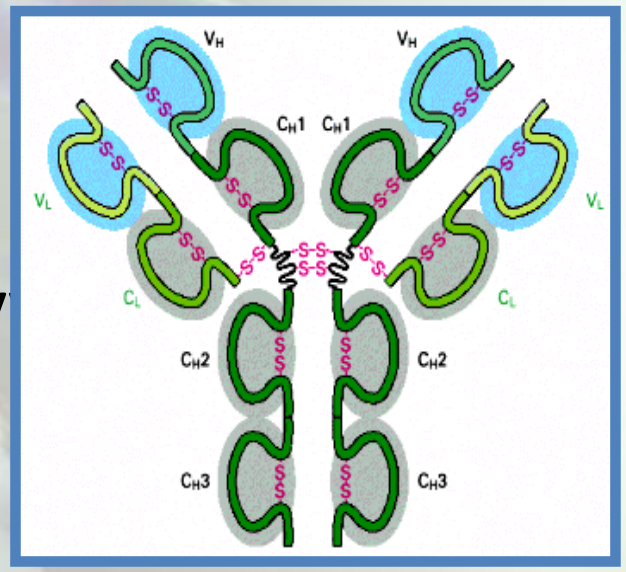
Antibody (Immunoglobulin; IgG) Structure & Functions



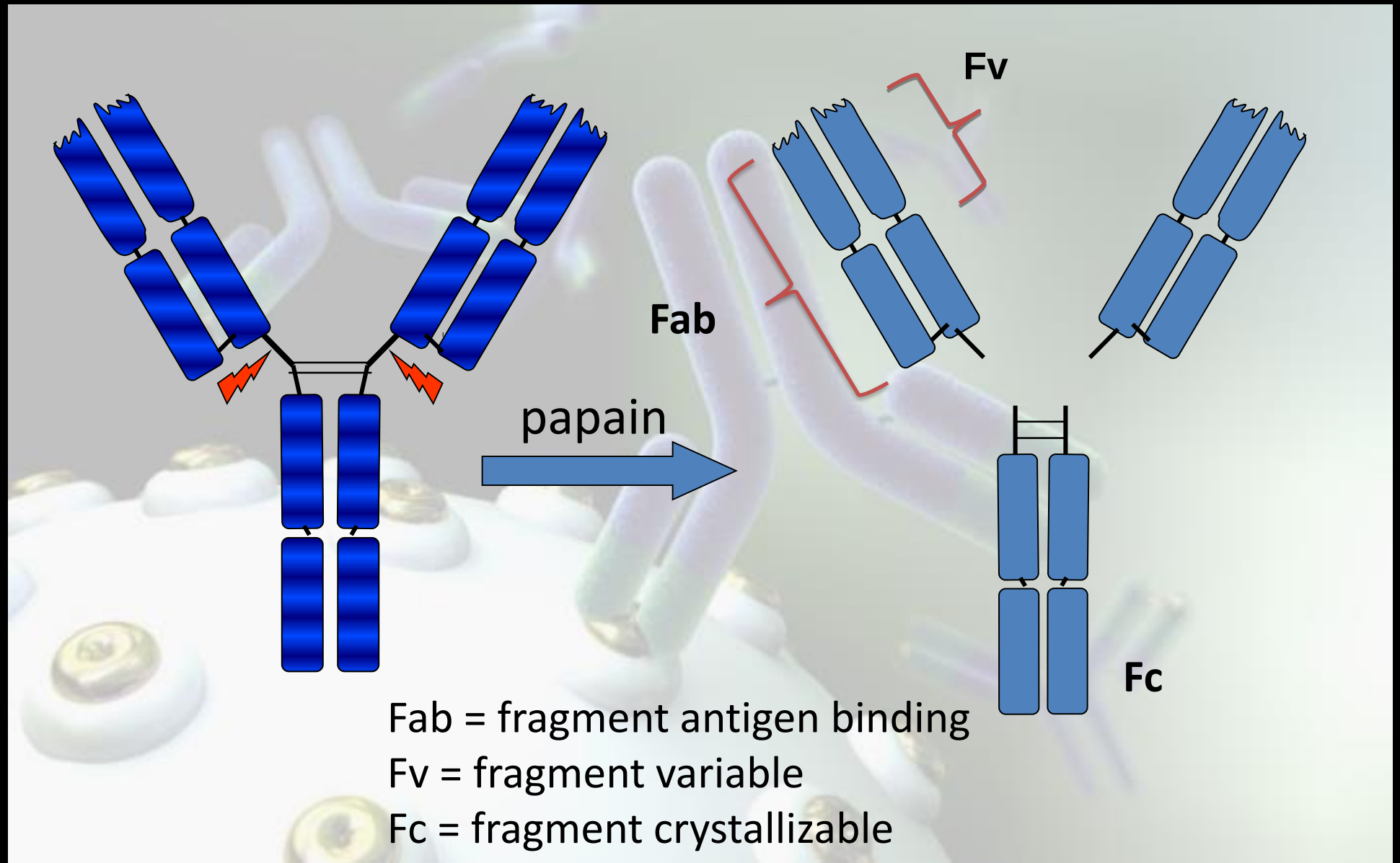
Structural features of IgG

- Y-shaped molecules
- Composed of a total of 4 protein chains
  - 2 heavy chains with 1 variable and 3 constant domains
  - 2 light chains with 1 variable and 1 constant domain
- Stem (Fc) of Y = 2x2 heavy chain constant domains
- Each arm (Fab) of Y = 1 variable and 1 constant domain from heav chain and 1 entire light chain.

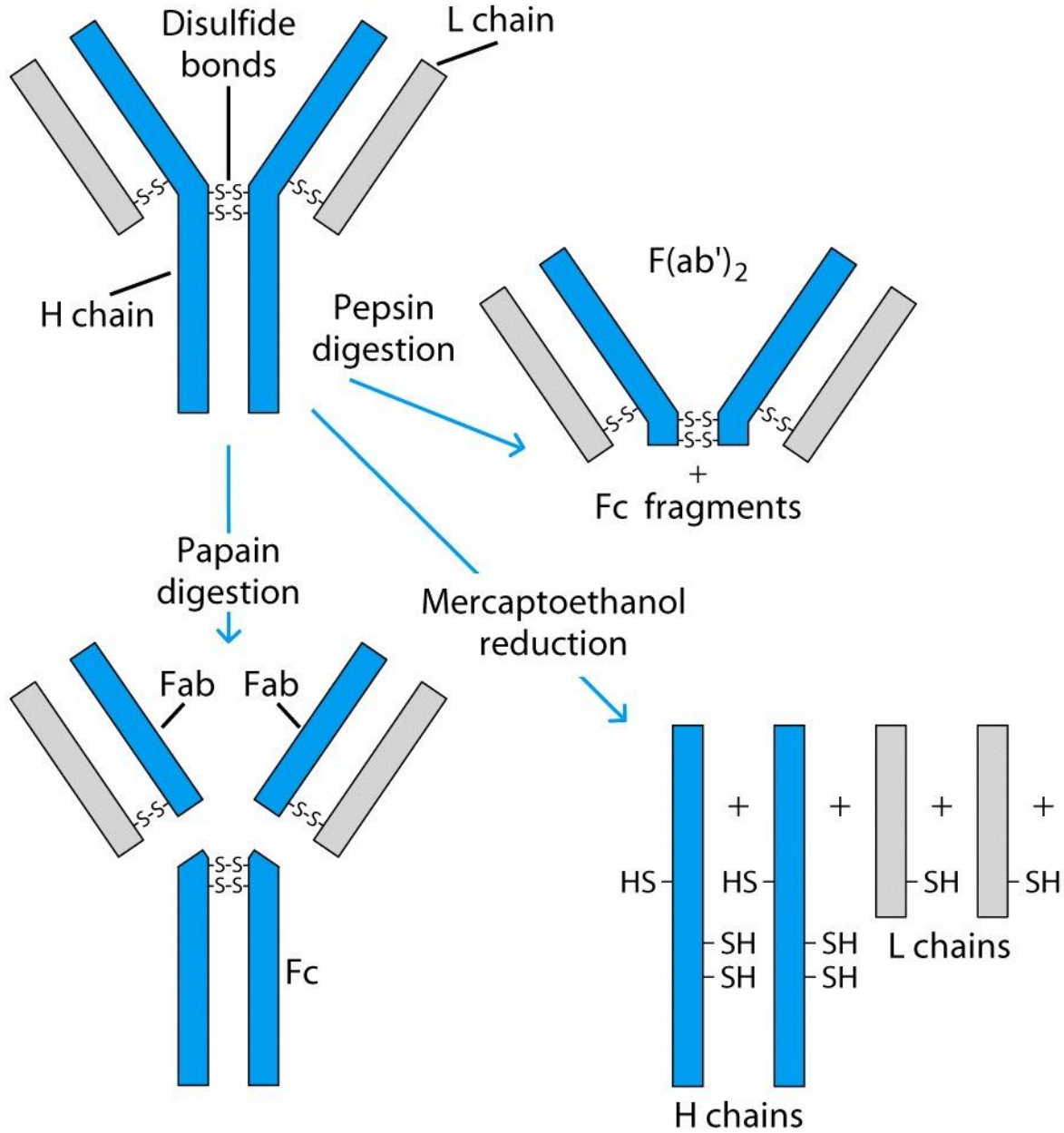




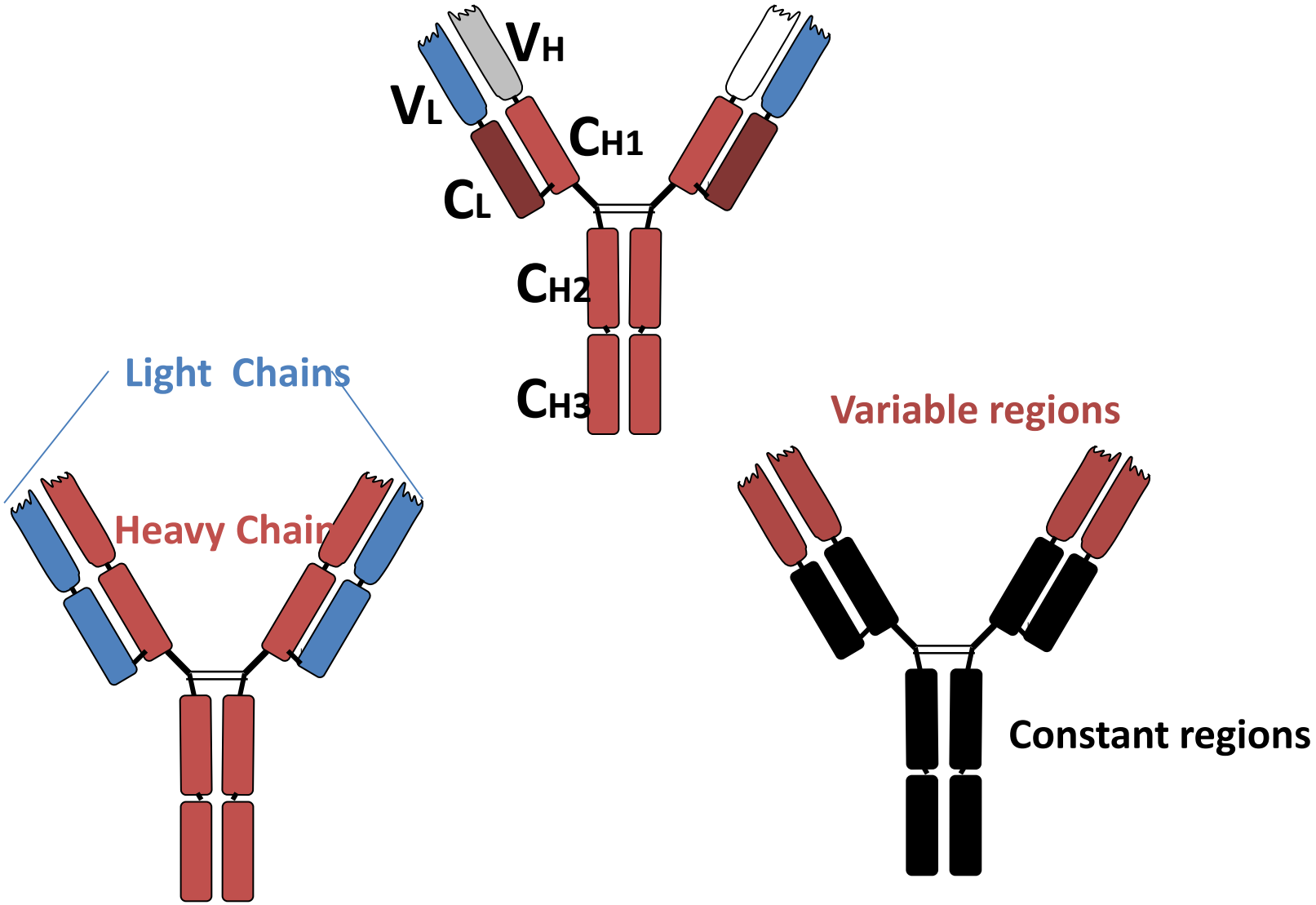
Antibody Structure



Fab = fragment antigen binding
Fv = fragment variable
Fc = fragment crystallizable


Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
+
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
HS
SH
SH
SH
SH
SH
SH
L chains
H chains

Antibody Domain Structure

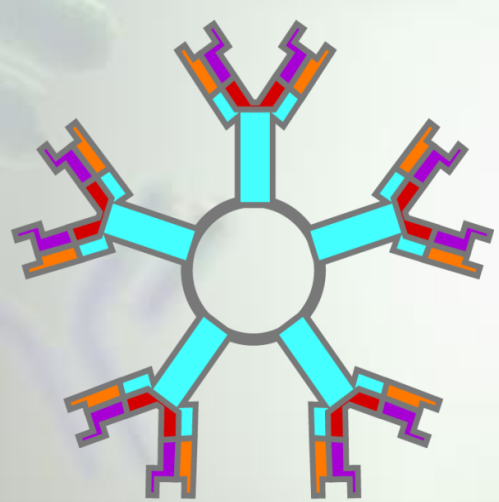


Antibodies

- **Five classes based on type of heavy chain**
  - IgA
  - IgD
  - IgE
  - IgG – derived from B-cells, most abundant Ig
  - IgM
- **IgG has two primary functions**
  - Bind foreign antigens
  - Eliminate or inactivate antigen

IgG, IgD, IgE, and IgA

sIgA



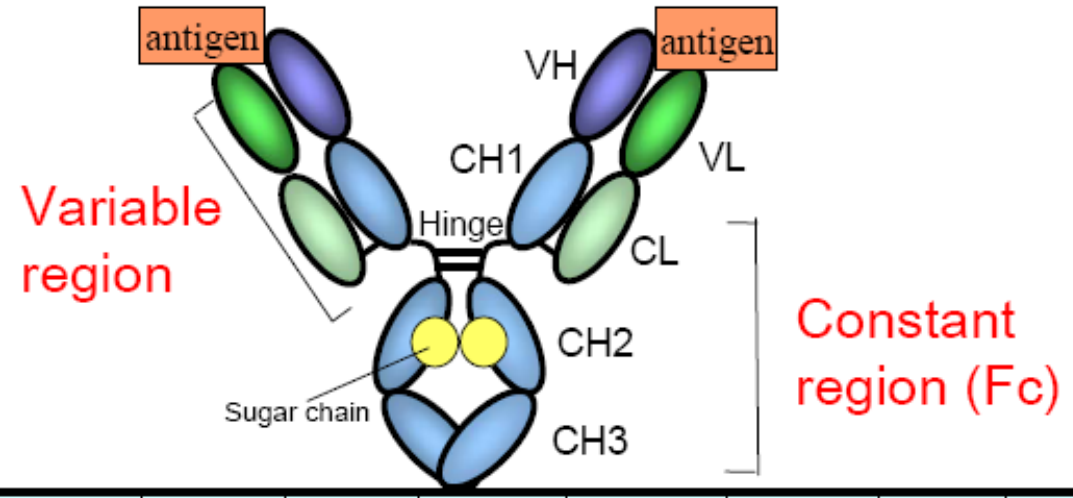
IgM

Artwork by Jeanne Kelly. ©2004.

Characteristic of Human Antibody by Class

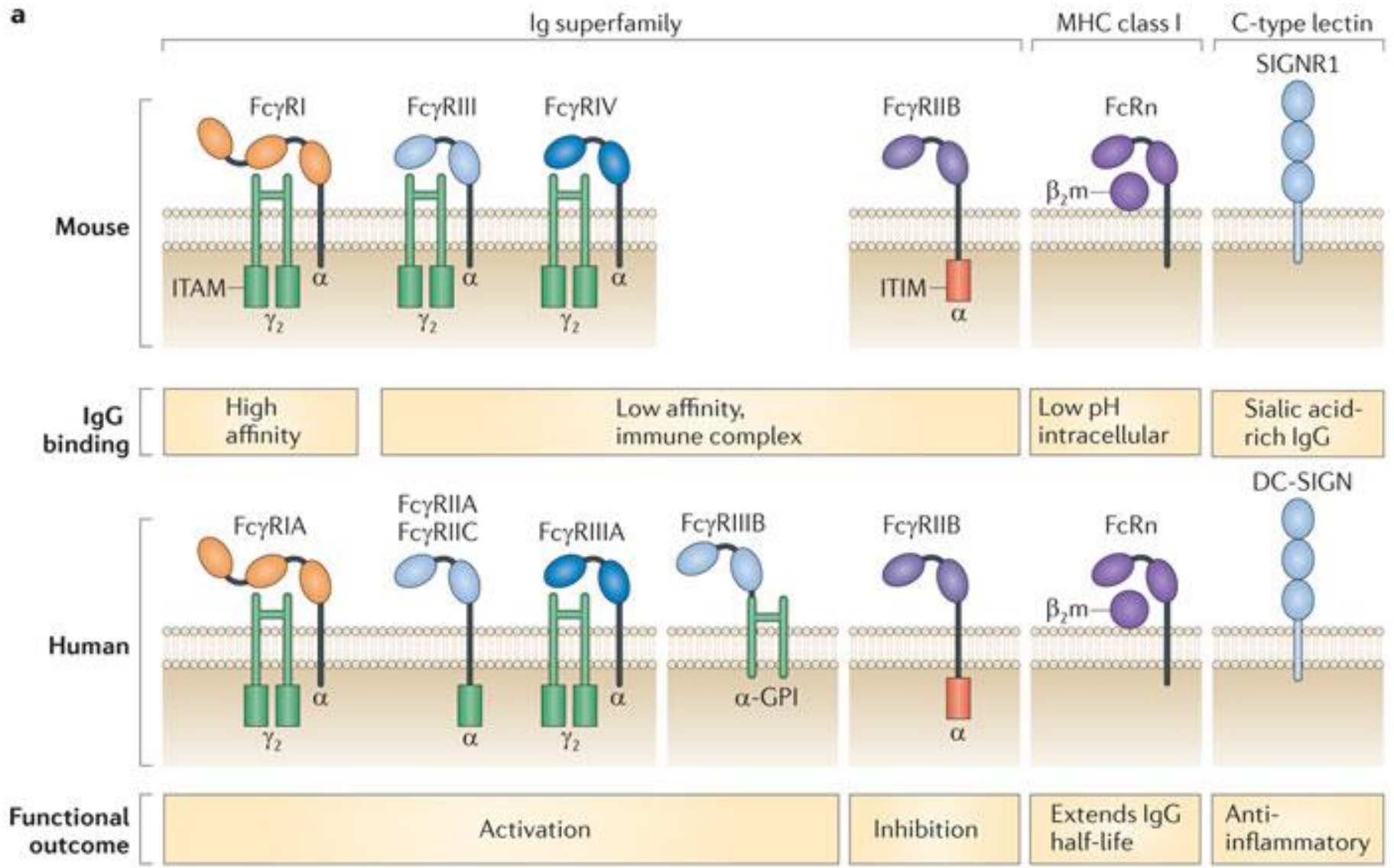
Structure of Immunoglobulin IgG1

Most of the therapeutic antibodies are IgG1



Class/Subclass		IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	sIgA	IgD	IgE
H chain		γ1	γ2	γ3	γ4	μ	α1	α2	α1/α2	δ	ε
Molecular weight (kDa)		146	146	170	146	970	160	160	385	184	188
Serum concentration (mg/ml)		9	3	1	0.5	1.5	3	0.5	0.05	0.03	0.00005
Half-life (day)		21	20	7	21	10	6	6	7	3	2
Complement binding		++	+	+++	−	+++	−	−	−	−	−
Fc receptor binding	FcγR	I, II, III	(IIa)	I, II, III	I	−	−	−	−	−	−
	FcαR	−	−	−	−	−	+++	+++	+++	−	−
	FcεR	−	−	−	−	−	−	−	−	−	I, II
Placenta permeability		+	+	+	+	−	−	−	−	−	−

The family of mouse and human FcγRs.



Characteristics of Antibody

- **Natural scaffold**
- **Neutralization** (extreme specific binding properties)
  - High affinity → Low concentration needed
  - Less cross reactivity → Less side effect --
  - 2 binding site per molecule → High avidity
- **Long plasma half-life** (IgG ~ 21 days)
  - Less frequent administration needed
- **Activate consequence immune response via FcR**
  - Complement acitvation
  - Antibody-dependent cellular cytotoxicity (ADCC)
- **Induce apoptosis**

How do antibodies work?

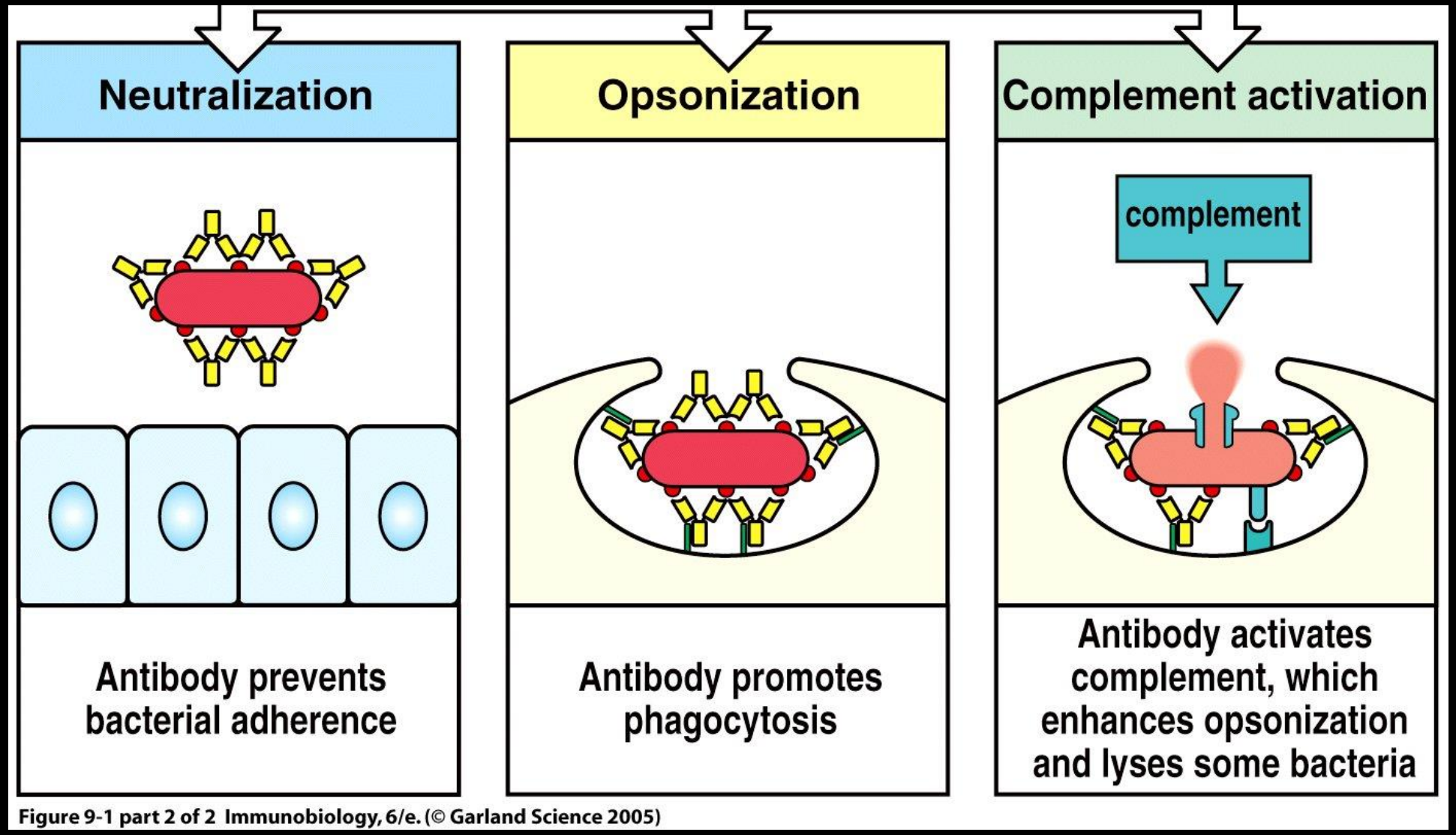
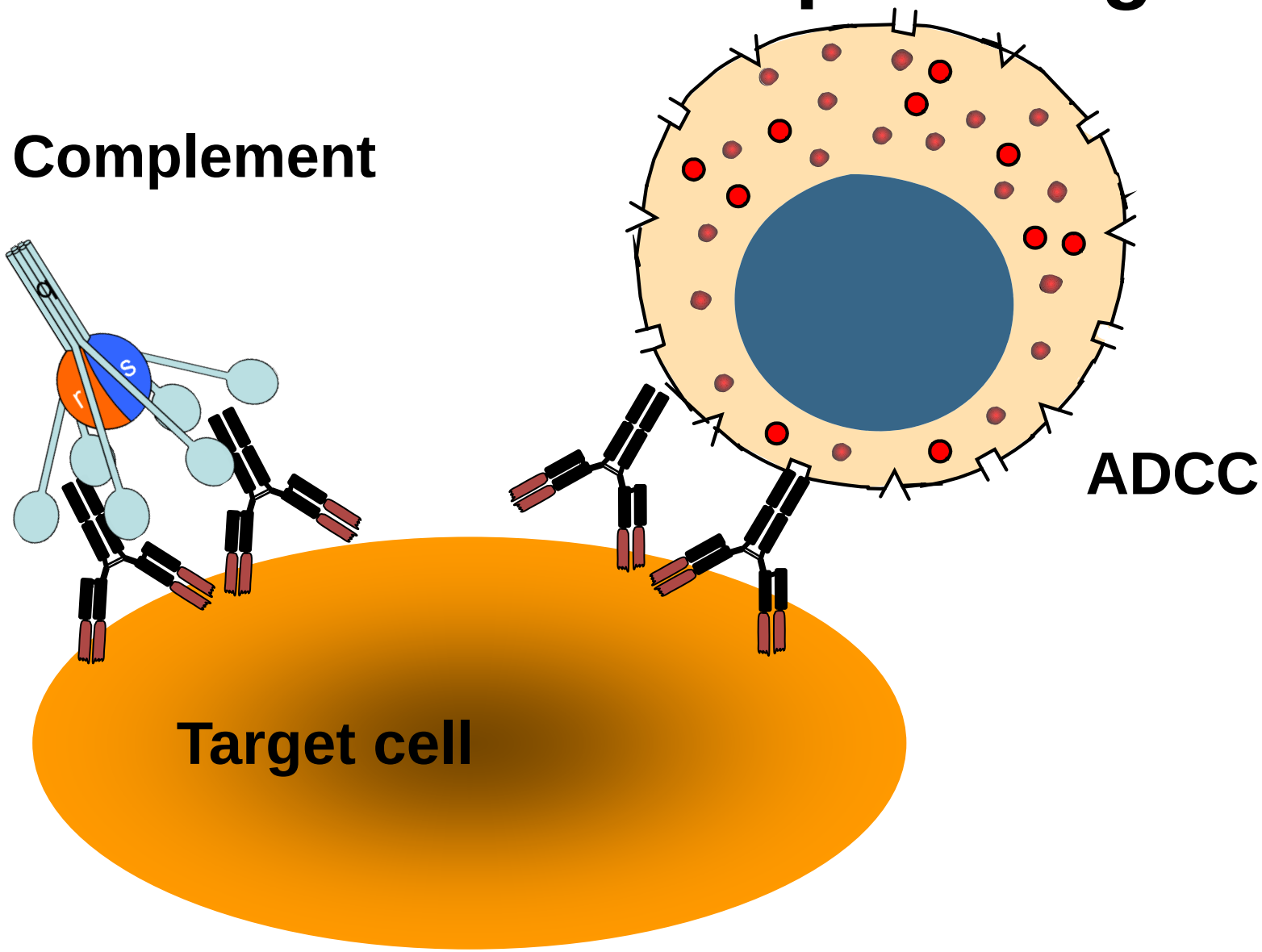
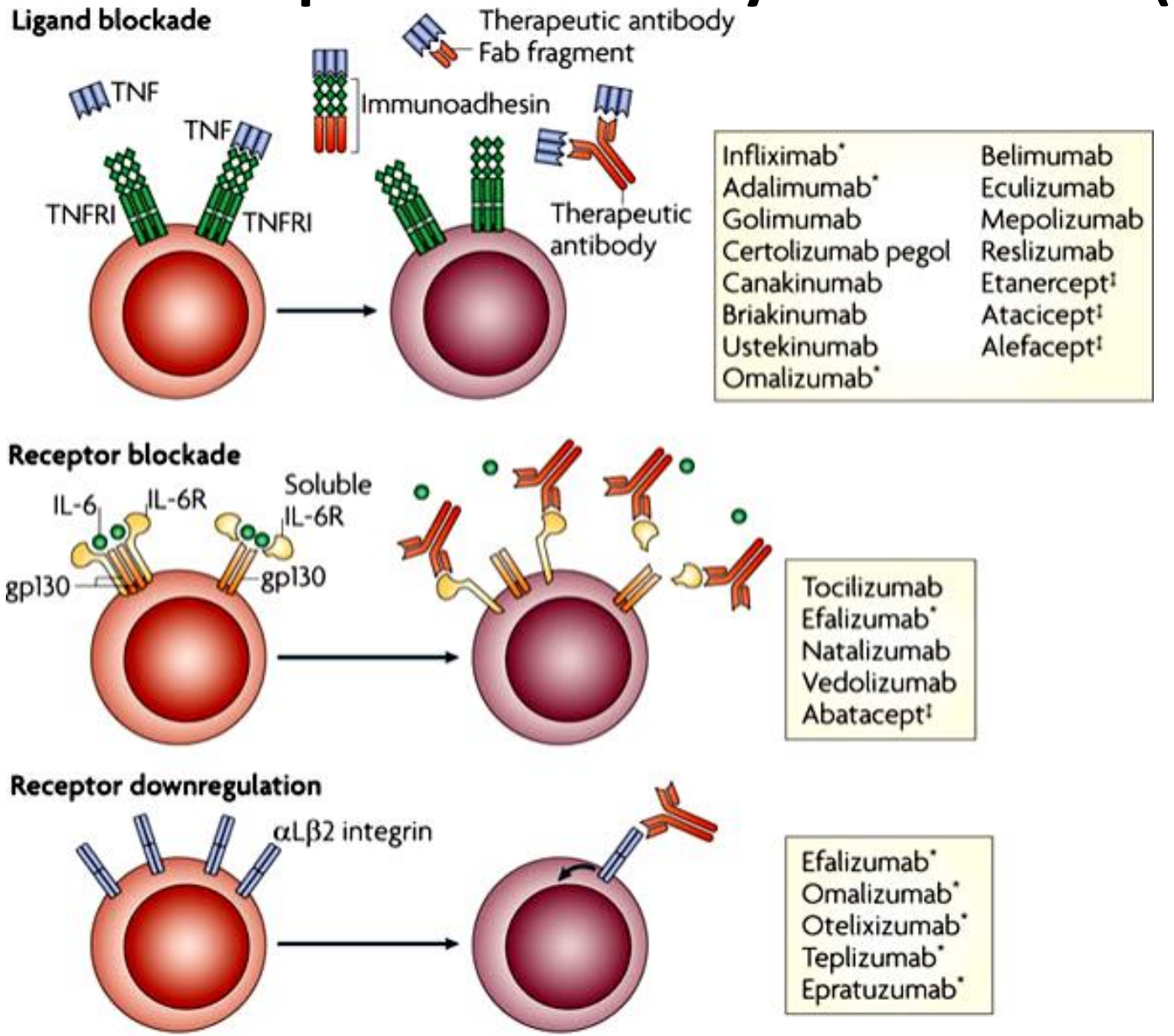


Figure 9-1 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

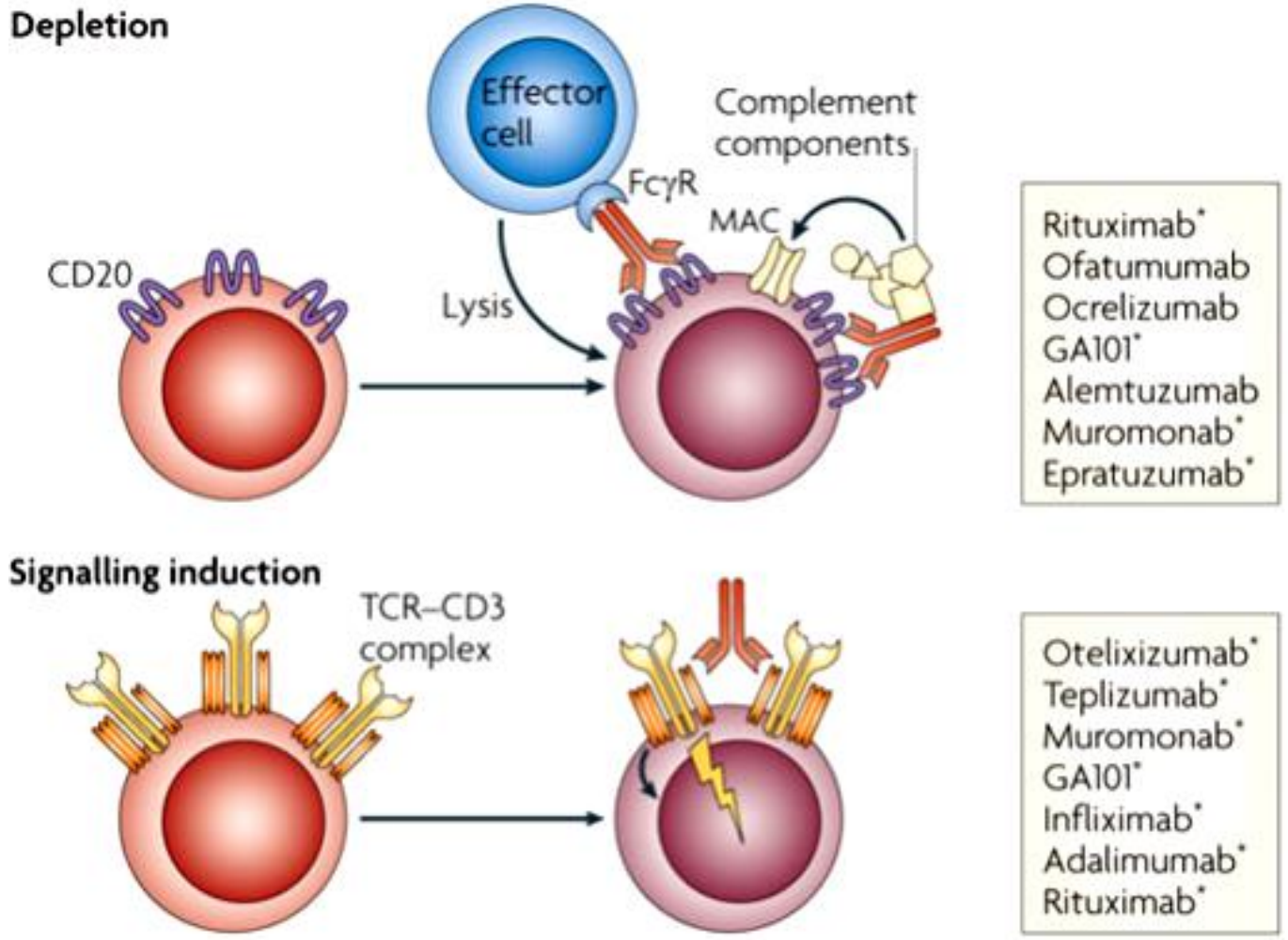
Antibodies – Nature's pro-drugs



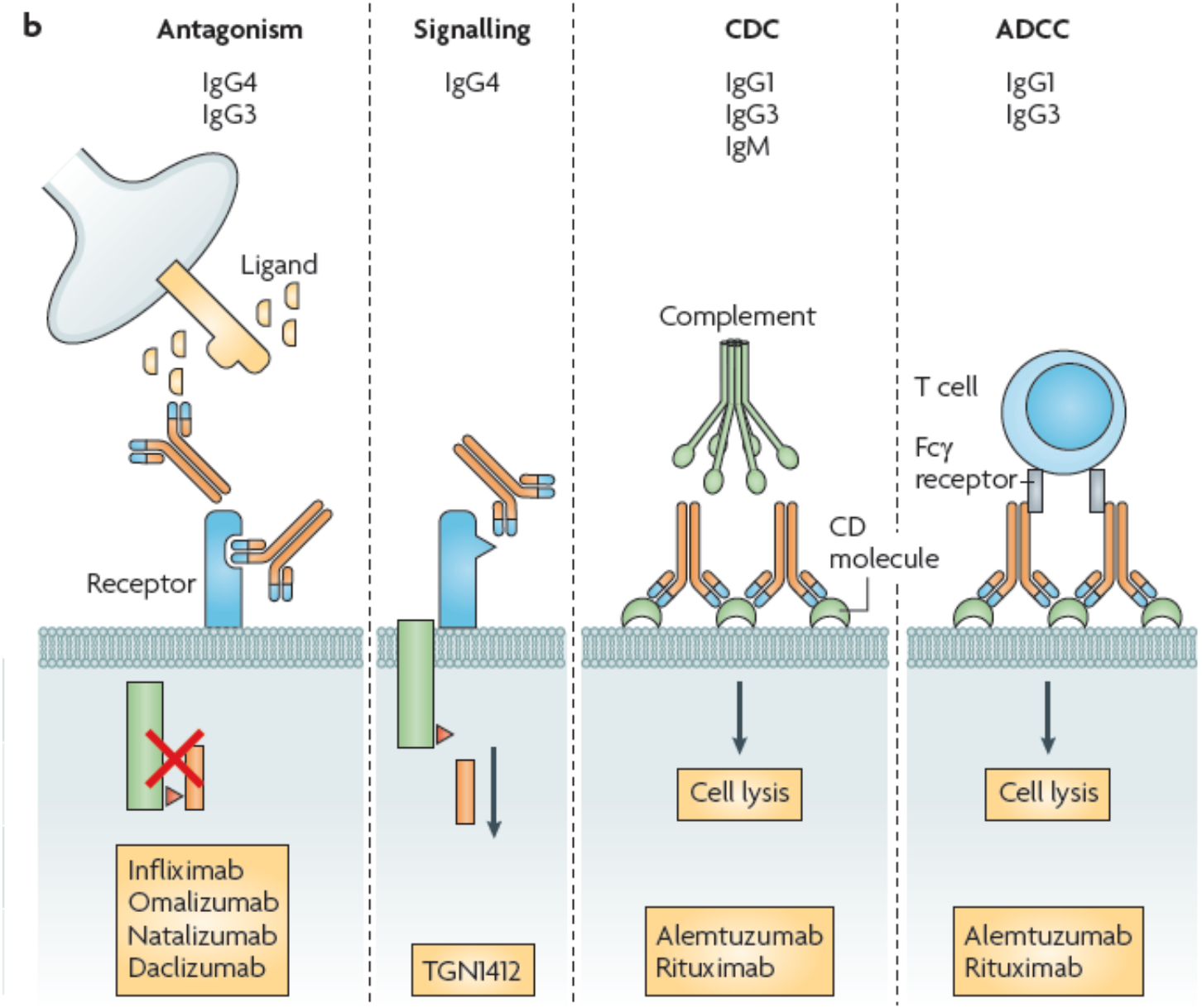
Mode of Therapeutic Antibody Mechanism (1)



Mode of Therapeutic Antibody Mechanism (2)



Functions of mAbs



Poly- and Mono- Clonal Antibodies

- **Polyclonal antibody**
  - Antigens possess multiple epitopes
  - Serum antibodies are heterogeneous,
    - To increase immune protection in vivo
    - To reduces the efficacy of antiserum for various in vitro uses
  - To response facilitates the localization, phagocytosis, and complement-mediated lysis of antigen
  - To have clear advantages for the organism in vivo

- **Monoclonal antibody**
  - Derived from a single clone, specific for a single epitope
  - For most research, diagnostic, and therapeutic purposes

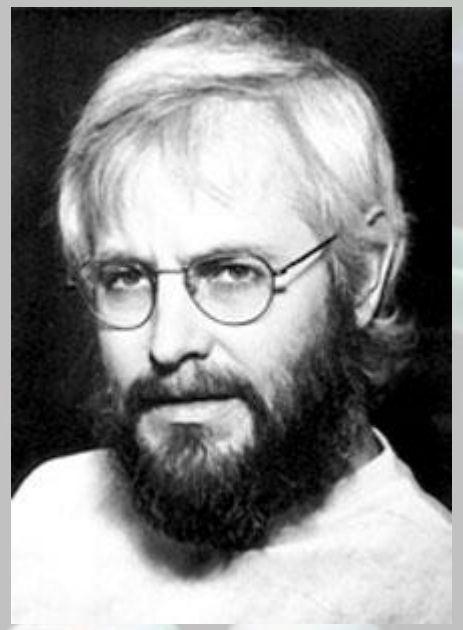
Antibody Production

- **Classical Method** (preparation for antiserum)
  - Immunization of laboratories animal with antigen (both in pure form or complex form)
  - Determination of titre after 1-3 immunizations
  - Antiserum is collected
  - *Problem ::*
    - contains undesired substances,
    - provides a very small amount of usable antibody.

Problems with polyclonal antisera

- **Lack of Reproducibility**
  Each batch could be different
- **Complex Mixture**
  Multi-specific antibodies
  Potential contaminants (eg viruses)
- **Immunogenicity**
  Animal proteins induce immune response; eg. HAMA (Human anti-murine antibody)
  Loss of efficacy and serum sickness

The Nobel Prize in Physiology/Medicine 1984



Georges J.F. Köhler

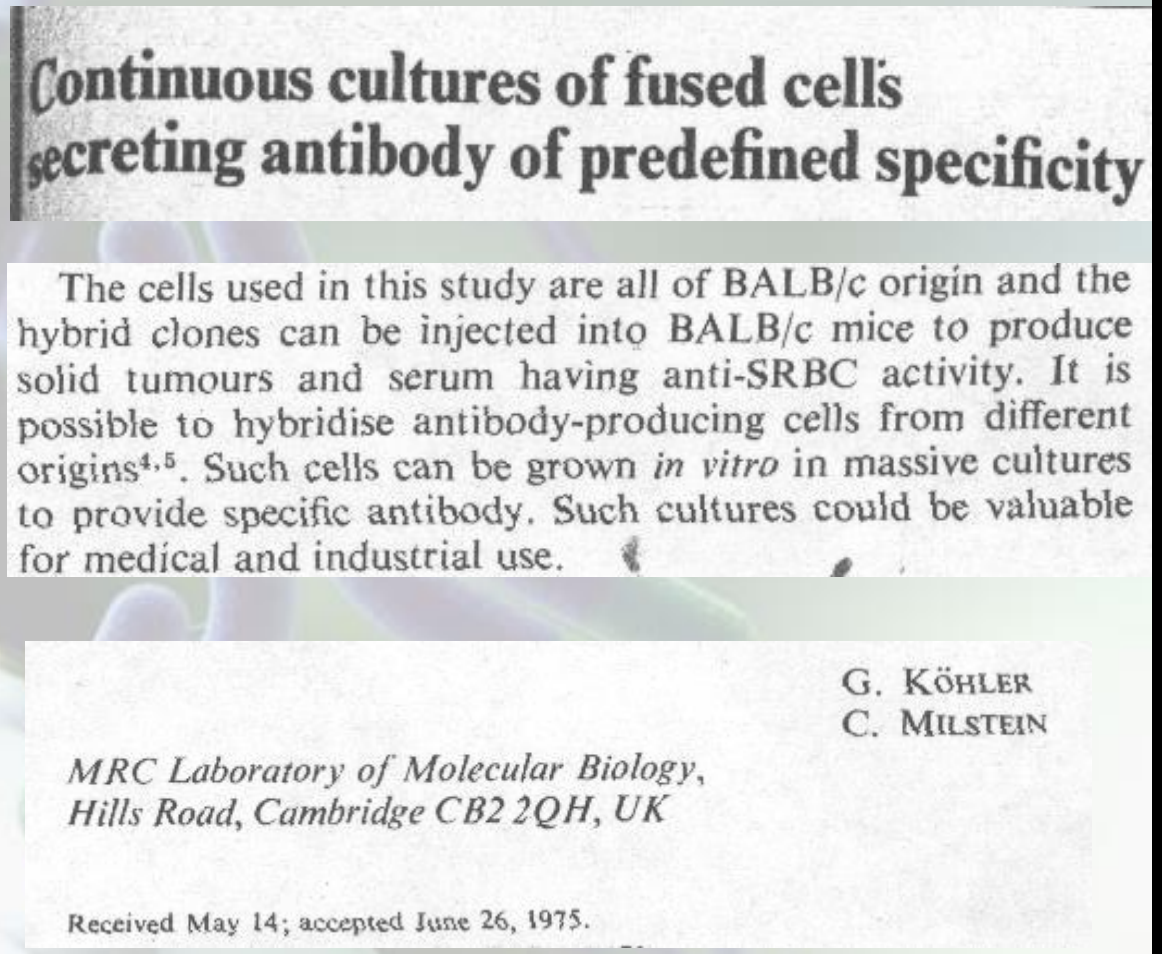
César Milstein

Niels K. Jerne

"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"

Køhler and Milstein (1975)

- Monoclonal antibody technology
- Immortal cells producing a single antibody of defined specificity in unlimited amounts



Continuous cultures of fused cells secreting antibody of predefined specificity

The cells used in this study are all of BALB/c origin and the hybrid clones can be injected into BALB/c mice to produce solid tumours and serum having anti-SRBC activity. It is possible to hybridise antibody-producing cells from different origins[4,5]. Such cells can be grown *in vitro* in massive cultures to provide specific antibody. Such cultures could be valuable for medical and industrial use.

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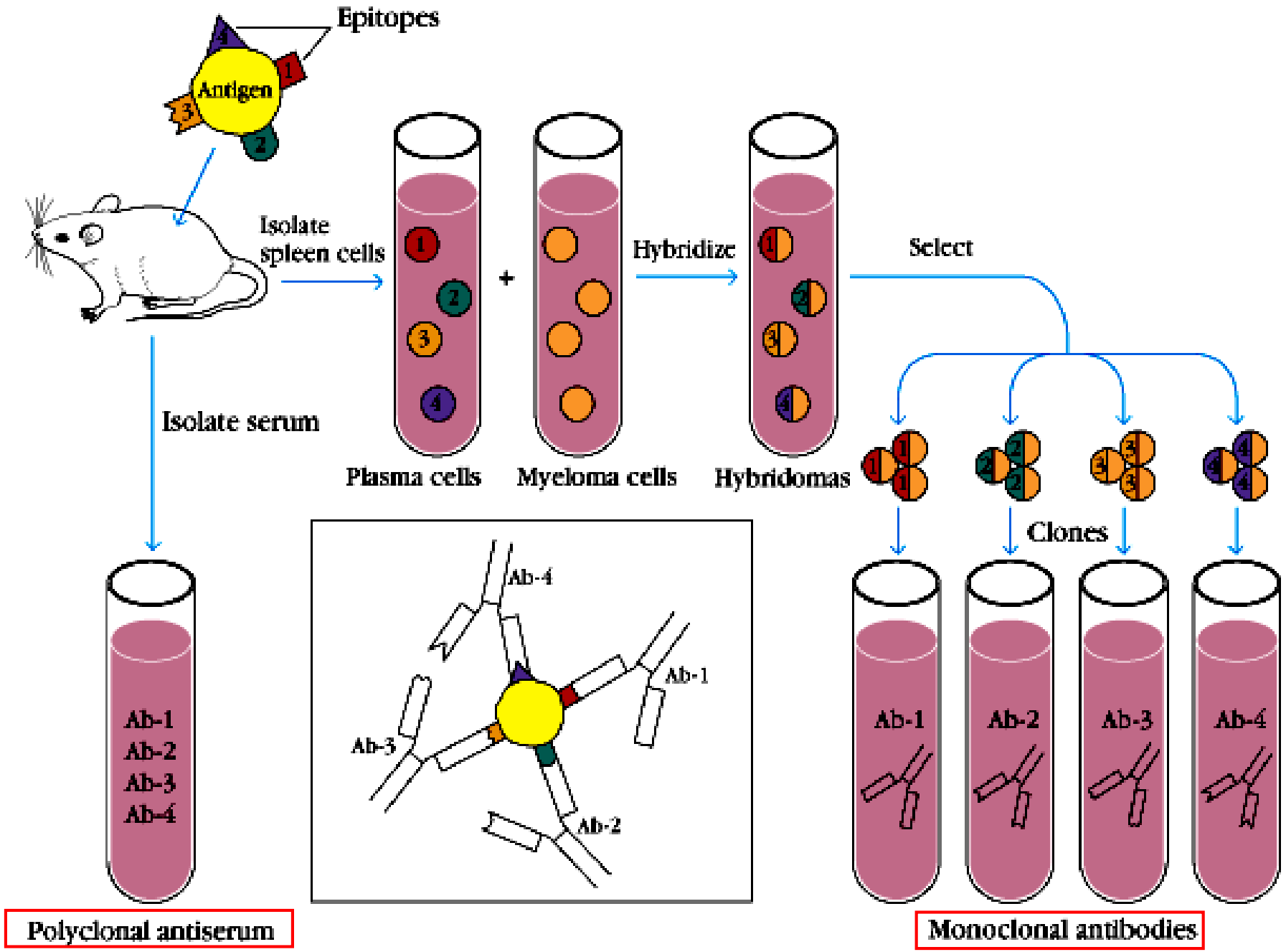
Received May 14; accepted June 26, 1975.

***Nature. 1975 Aug 7;256(5517):495-7*.**

Antibody Production

- **Monoclonal antibody technology**
  - produce large amounts of pure antibodies by cells (hybridoma) that grow continually in cell culture
  - antibodies are called monoclonal because they come from only one type of cell,
  - Hybridoma = myeloma + plasma cells
  - Immortal cells with antibody producing properties


Epitopes
Antigen
Isolate spleen cells
Hybridize
Select
Isolate serum
Plasma cells
Myeloma cells
Hybridomas
Clones
Ab-1
Ab-2
Ab-3
Ab-4
Polyclonal antiserum
Monoclonal antibodies

Production of monoclonals via hybridoma technology

Ascites production

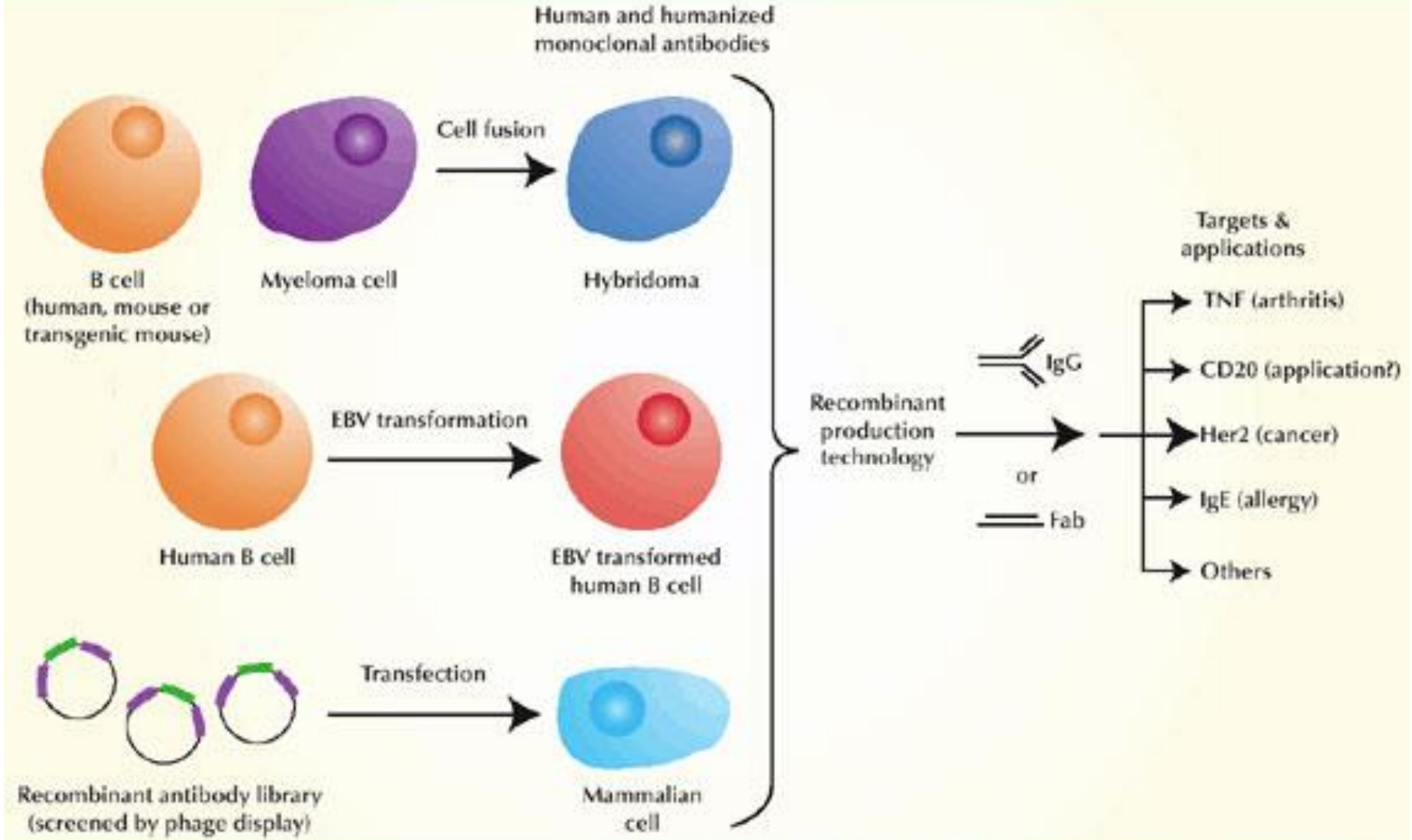
- Injecting hydridoma cells into the peritoneal cavity of mice
- Up to 15 mg/ml of antibody yield
- Early monoclonal antibody preparations e.g OKT-3
- Contaminated by mouse proteins

Direct animal cell culture

- Fermentation volumes in excess of 1000 liters can be used, which yield 100 g or more final product
- Fermentation yields antibody concentration of 0.1-0.5 mg/ml

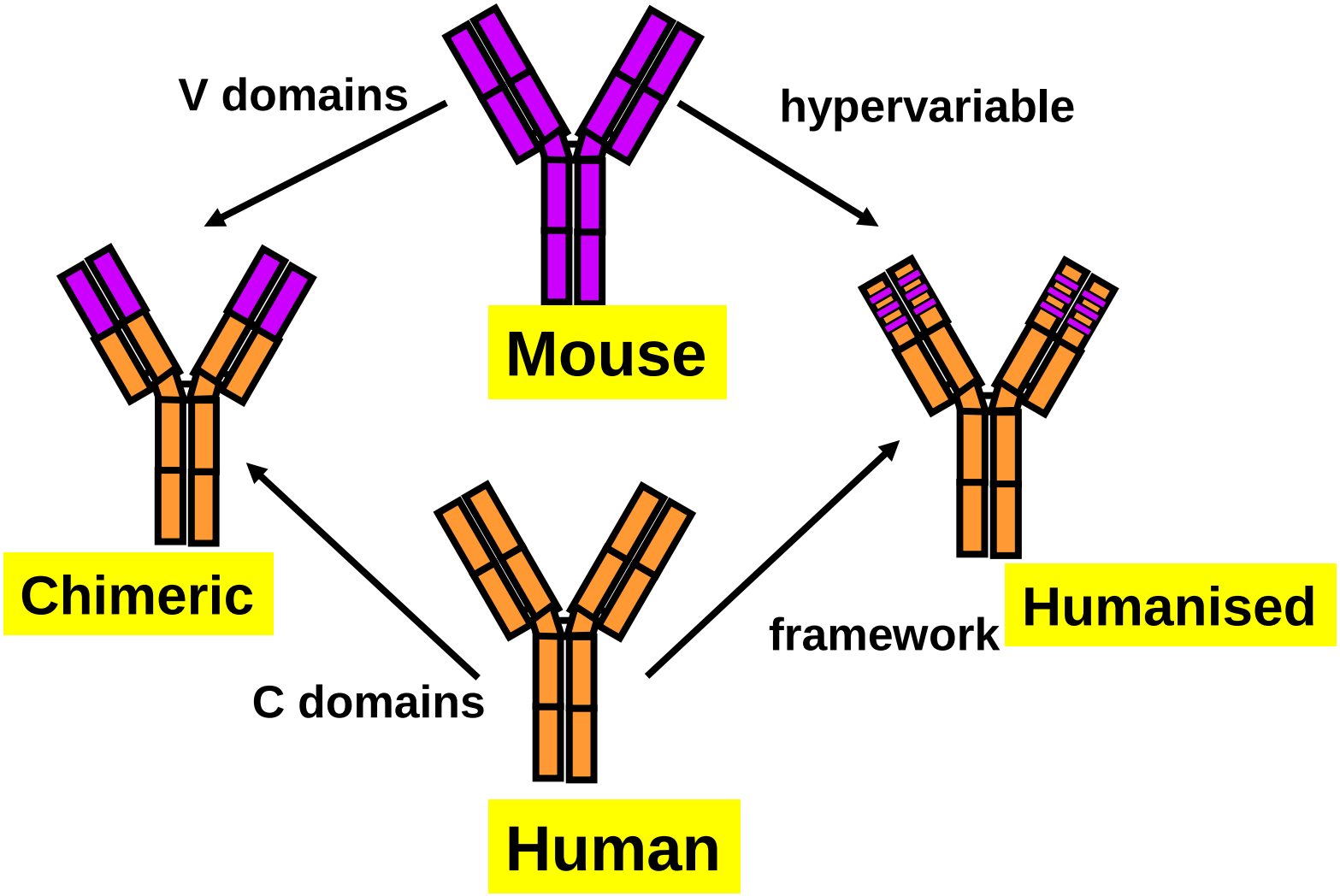
Monoclonal Technology Evolution

- Fusion of Mouse lymphocyte and myeloma (Kohler & Milstein, 1975; never patented) → therapeutic use in 1980s
- Chimera Antibody (chimeric vs. humanized)
- Fully human Antibody



Source: Nat Med © 2004 Nature Publishing Group

Format of Immunoglobulins



Genetic engineering of antibodies

- ***Reduce immunogenicity***

  Chimeric, Humanised, Phage, Transgenic

- ***Modify effector function***

  Aglycosyl, non-FcR binding

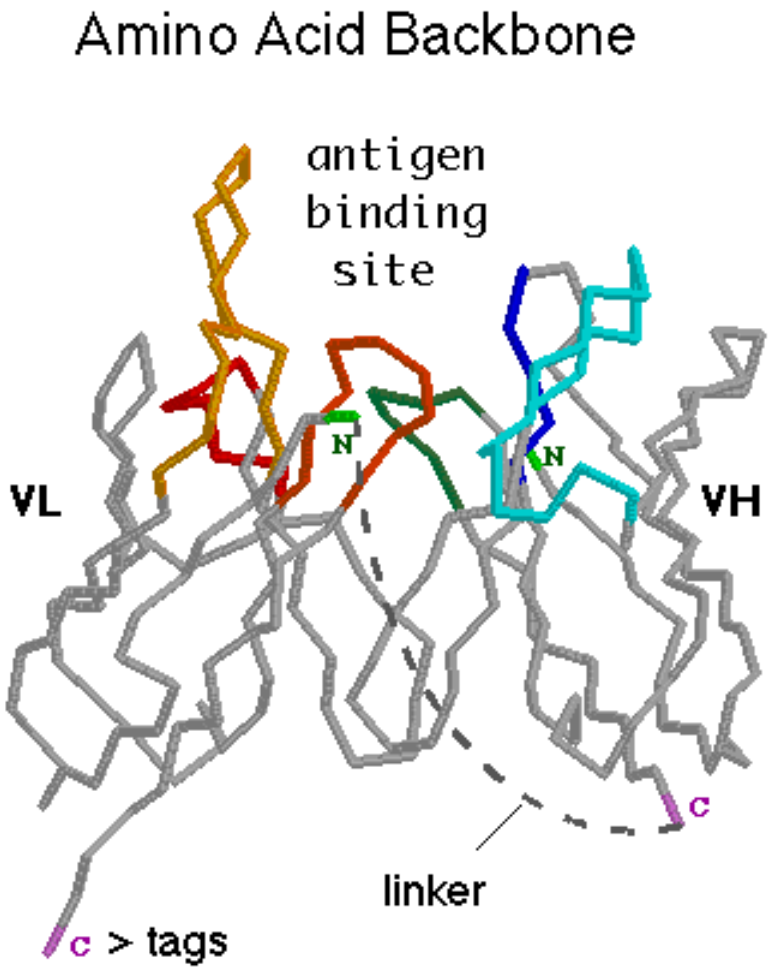
- ***Create fragments of complexes***

  Hybrids, Bispecific

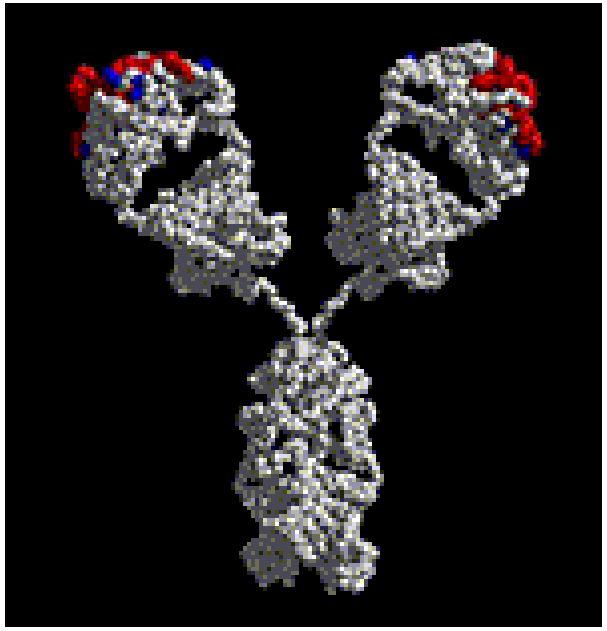
- ***Improve stability***

  Human IgG4

Humanised Antibodies

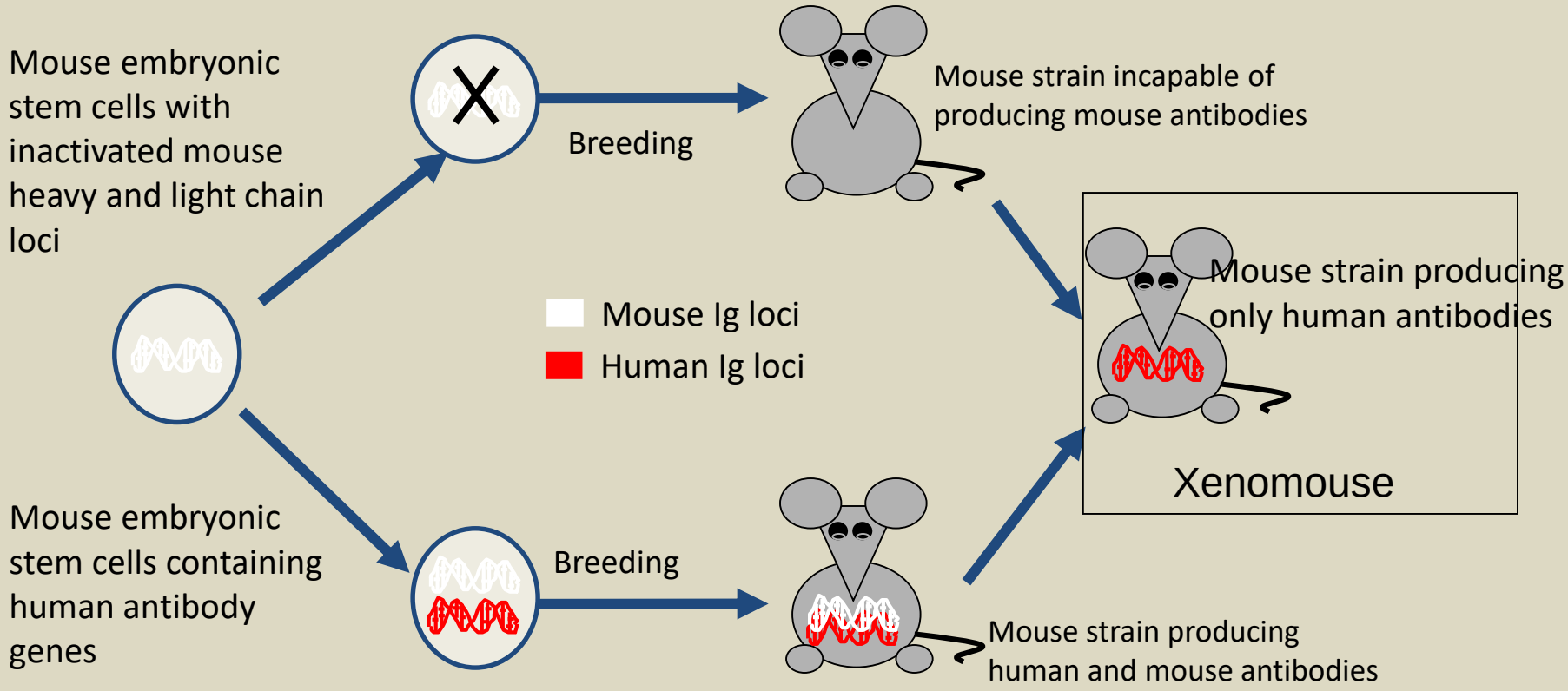


Allows specificity
Allows effector functions
Less immunogenic



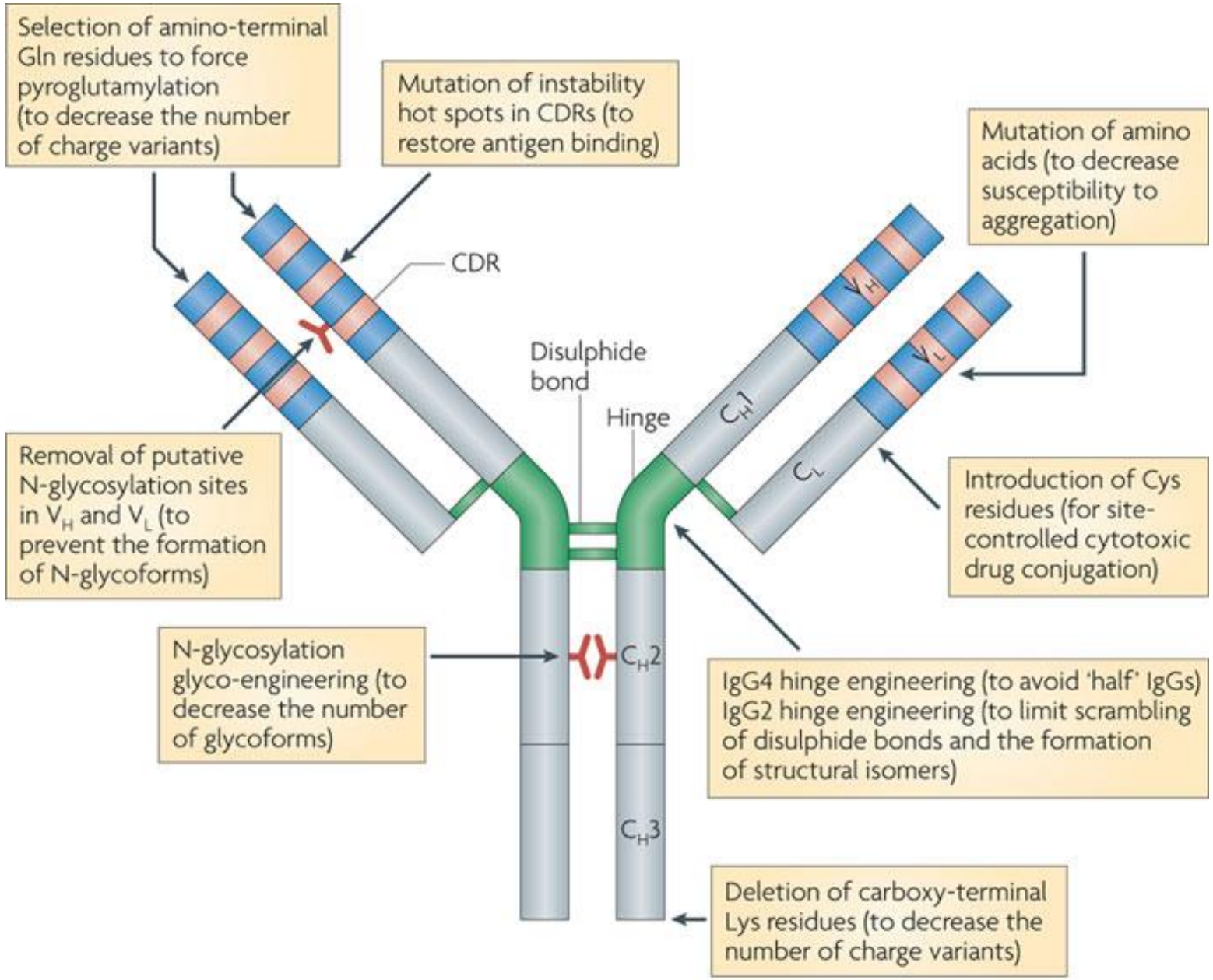
Creation of Fully Human mAbs From Transgenic Mice

Replace murine immunoglobulin (Ig)G genes with human IgG genes

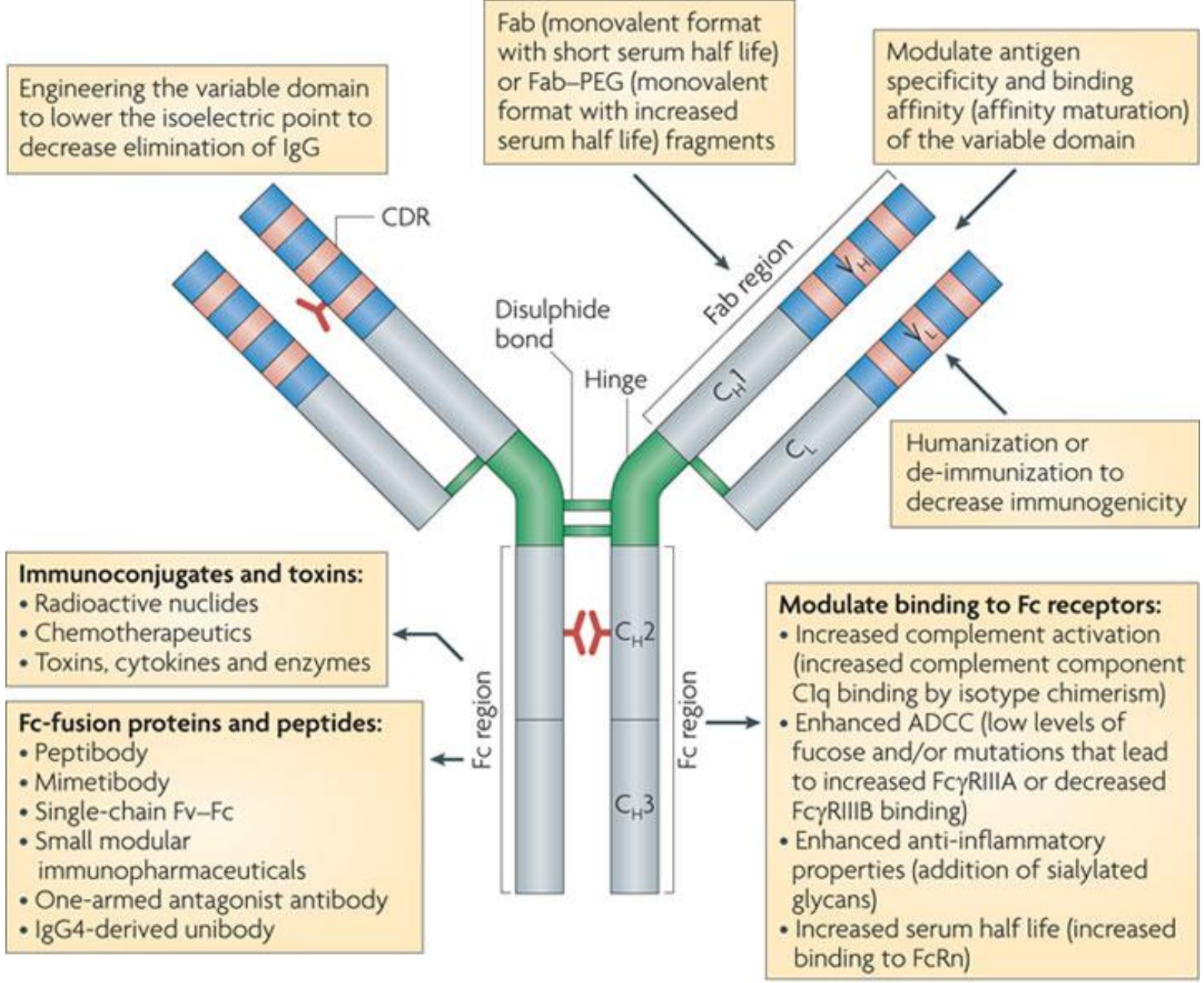


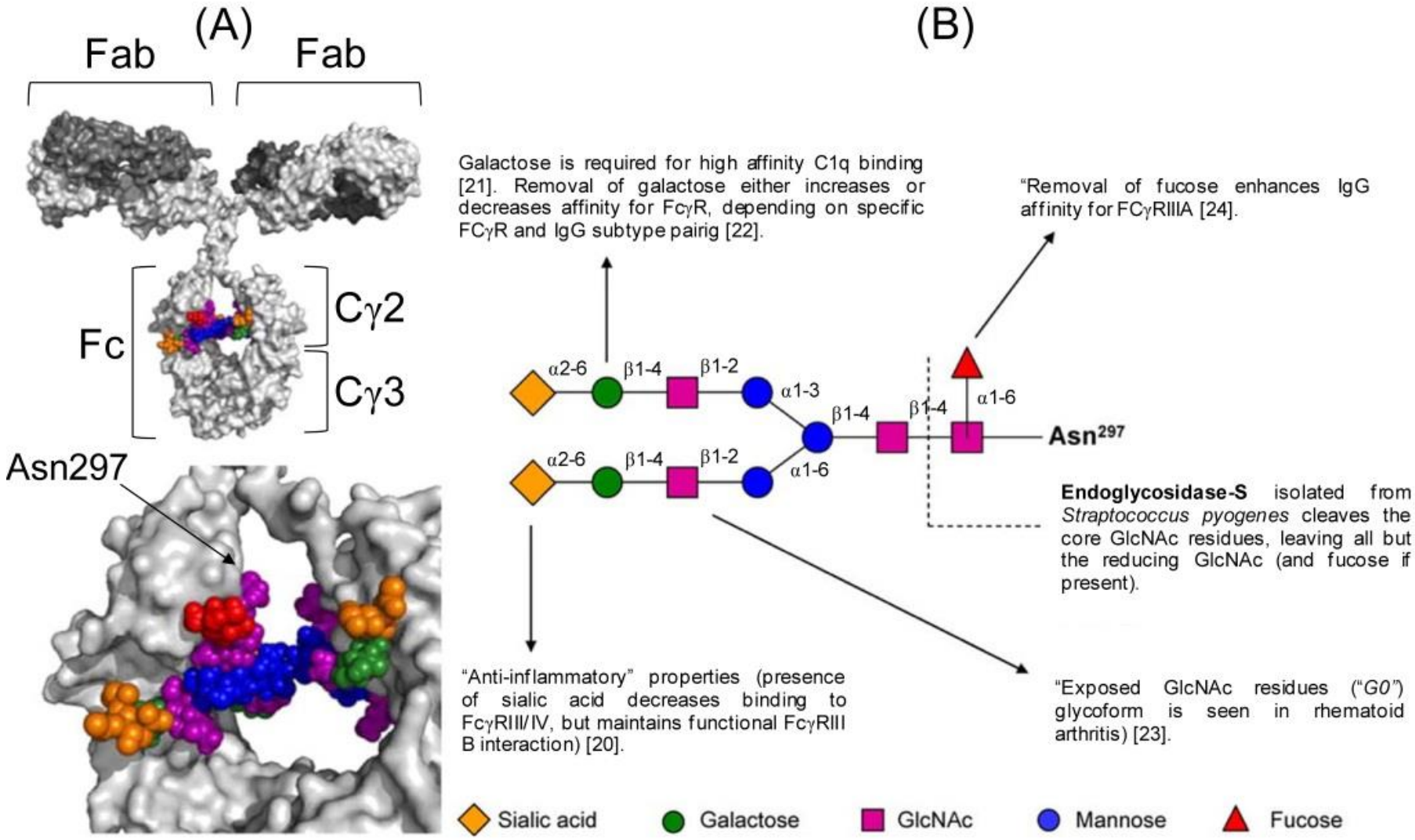
Weiner LM. *J Immunother*. 2006;29:1-9.

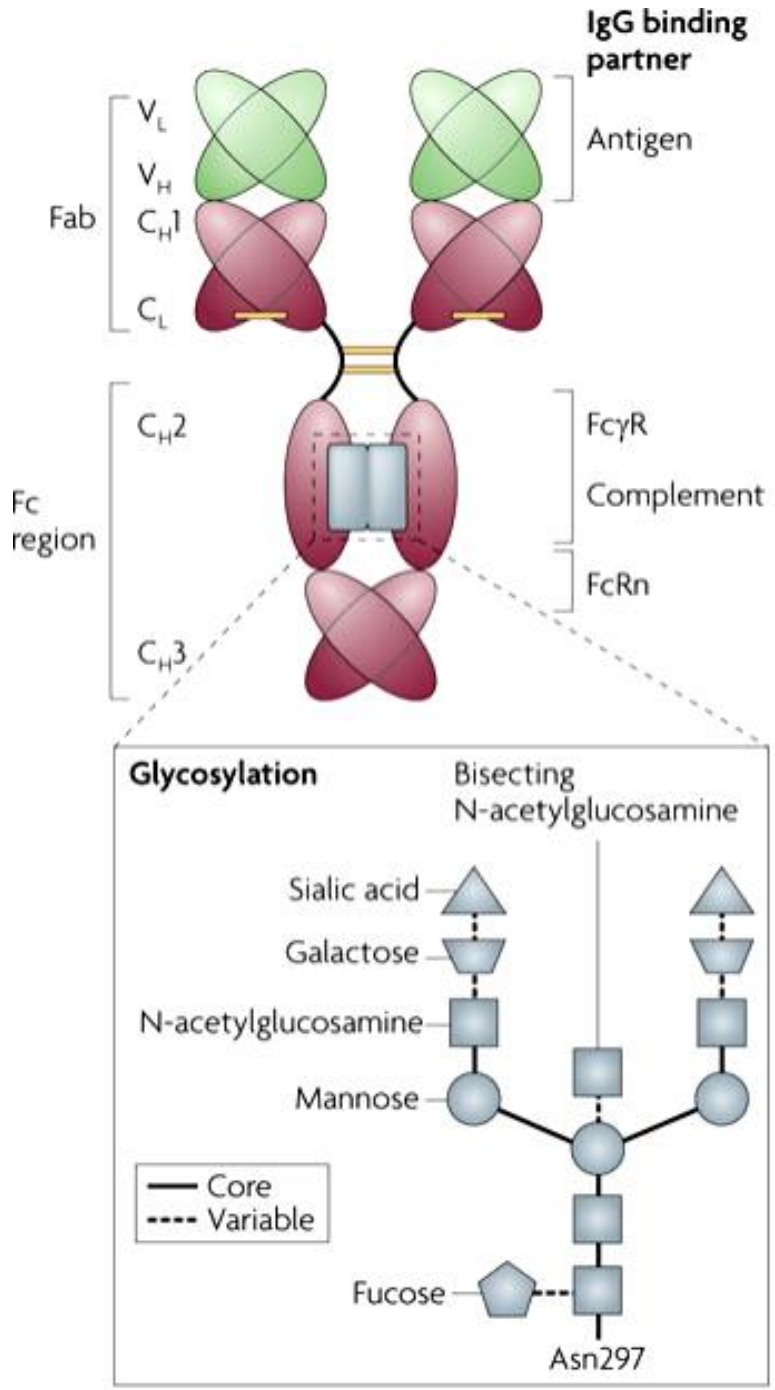
Yang X-D, et al. *Crit Rev Oncol Hematol*. 2001;38:17-23.



Nature Reviews | Immunology


Engineering the variable domain to lower the isoelectric point to decrease elimination of IgG
Fab (monovalent format with short serum half life) or Fab–PEG (monovalent format with increased serum half life) fragments
Modulate antigen specificity and binding affinity (affinity maturation) of the variable domain
CDR
Disulphide bond
Hinge
Fab region
V_H
V_L
C_H1
C_L
Humanization or de-immunization to decrease immunogenicity
Immunoconjugates and toxins:
• Radioactive nuclides
• Chemotherapeutics
• Toxins, cytokines and enzymes
Fc-fusion proteins and peptides:
• Peptibody
• Mimetibody
• Single-chain Fv–Fc
• Small modular immunopharmaceuticals
• One-armed antagonist antibody
• IgG4-derived unibody
Fc region
C_H2
C_H3
Fc region
Modulate binding to Fc receptors:
• Increased complement activation (increased complement component C1q binding by isotype chimerism)
• Enhanced ADCC (low levels of fucose and/or mutations that lead to increased FcγRIIIA or decreased FcγRIIIB binding)
• Enhanced anti-inflammatory properties (addition of sialylated glycans)
• Increased serum half life (increased binding to FcRn)


(A)
Fab
Fab
Fc
Cγ2
Cγ3
Asn297
(B)
Galactose is required for high affinity C1q binding [21]. Removal of galactose either increases or decreases affinity for FcγR, depending on specific FCγR and IgG subtype pairig [22].
"Removal of fucose enhances IgG affinity for FCγRIIIA [24].
α2-6
β1-4
β1-2
α1-3
β1-4
β1-4
α1-6
α2-6
β1-4
β1-2
α1-6
Asn297
Endoglycosidase-S isolated from Straptococcus pyogenes cleaves the core GlcNAc residues, leaving all but the reducing GlcNAc (and fucose if present).
"Anti-inflammatory" properties (presence of sialic acid decreases binding to FcγRIII/IV, but maintains functional FcγRIII B interaction) [20].
"Exposed GlcNAc residues ("G0") glycoform is seen in rhematoid arthritis) [23].
Sialic acid
Galactose
GlcNAc
Mannose
Fucose

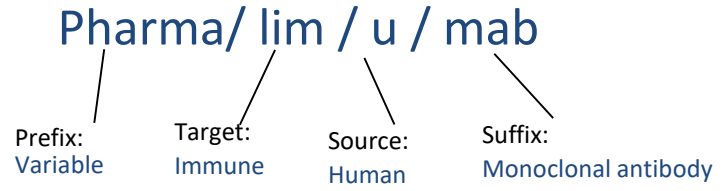


Protein strategies for modifying interactions	Potential impact of modifying interaction
Mutate V domain sequences using display libraries and/or rationale design	Altered binding affinity or specificity
Mutate Fc sequence using display libraries and/or rationale design; select IgG isotype	↑ or ↓ ADCC ↑ or ↓ ADCP ↑ or ↓ CDC
Mutate Fc sequence using display libraries and/or rationale design	↑ or ↓ half-life
Antibody fragment lacking Fc	↓ Half-life, ↓ CDC, ↓ ADCC and ↓ ADCP
Glycosylation strategies for modifying FcγR and complement interactions	
Aglycosylation	↓ ADCC, ↓ ADCP and ↓ CDC
Bisecting N-acetylglucosamine	↑ ADCC
Non-fucosylation	↑ ADCC

Monoclonal Antibody (mAb) Nomenclature

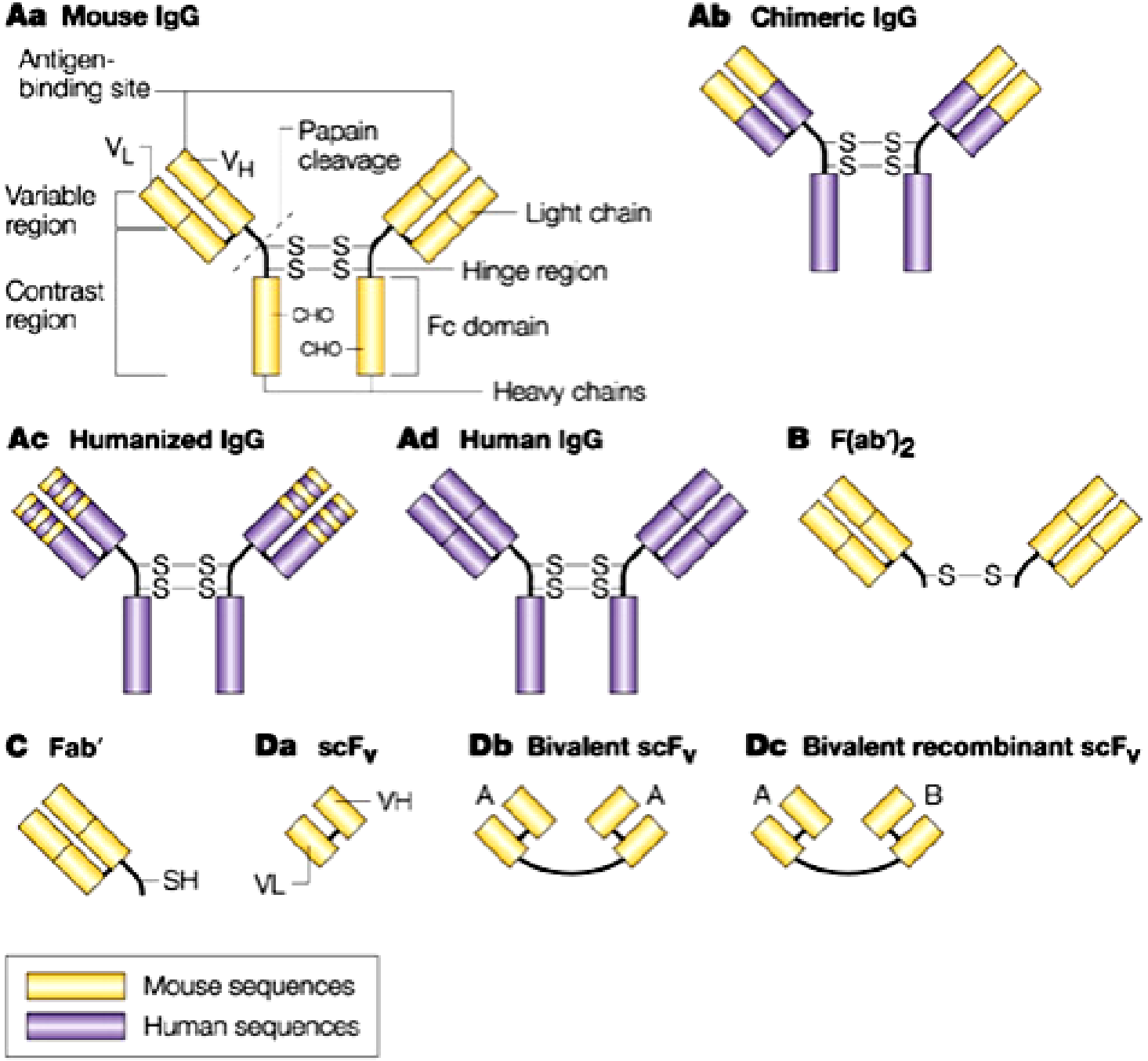
Prefix	Target or Disease State		Source		Suffix
varies	*-o(s)-*	bone	*-u-*	human	*-mab*
	-vi(r)-	viral	*-o-*	mouse	
	-ba(c)-	bacterial	*-a-*	rat	
	-li(m)-	immune	*-e-*	Hamster	
	-le(s)-	infectious lesions	*-i-*	Primate	
	-ci(r)-	cardiovascular	*-xi-*	Chimeric	
	-mu(l)-	musculoskeletal	*-zu-*	Humanized	
	-ki(n)-	interleukins	*-axo-*	rat/murine hybrid	
	-co(l)-	colonic tumor			
	-me(l)-	melanoma			
	-ma(r)-	mammary tumor			
	-go(t)-	testicular tumor			
	-go(v)-	ovarian tumor			
	-pr(o)-	prostate tumor			
	-tu(m)-	miscellaneous tumor			
	-ne(r)-	nervous system			
	-tox(a)-	toxin as target			
	-fu(ng)-	fungal			

Example:

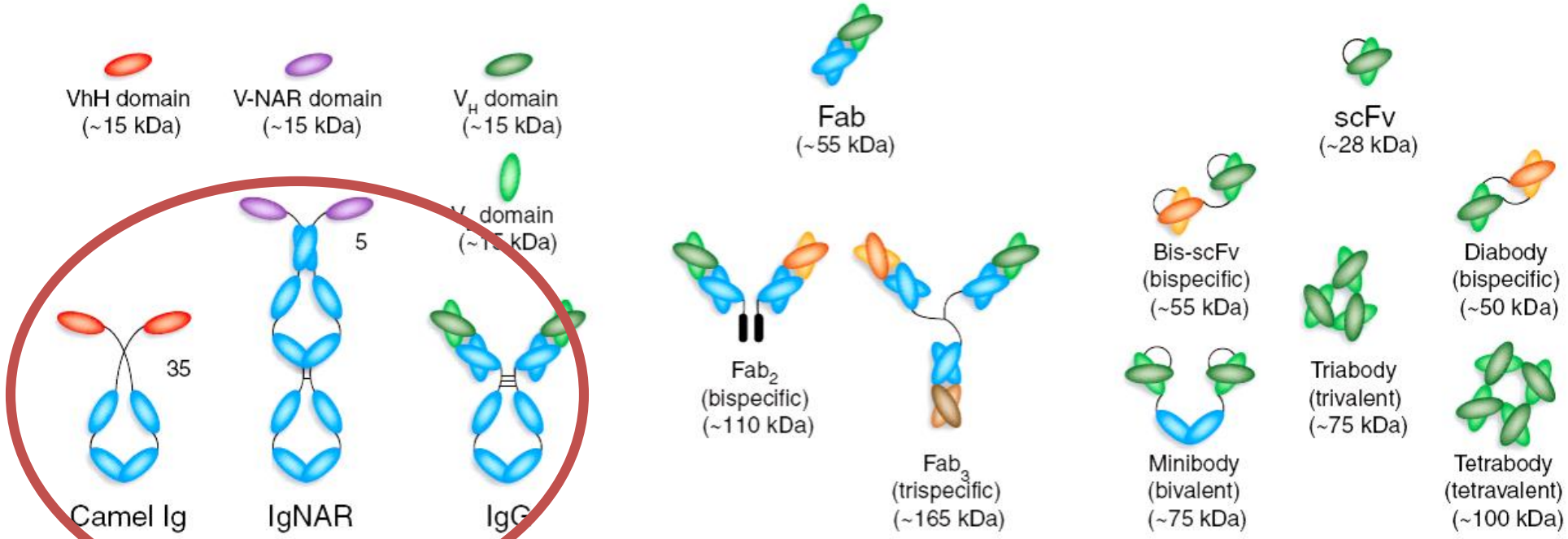


Available at: www.ama-assn.org/ama/pub/category/13280.html. Accessed August 10, 2007.

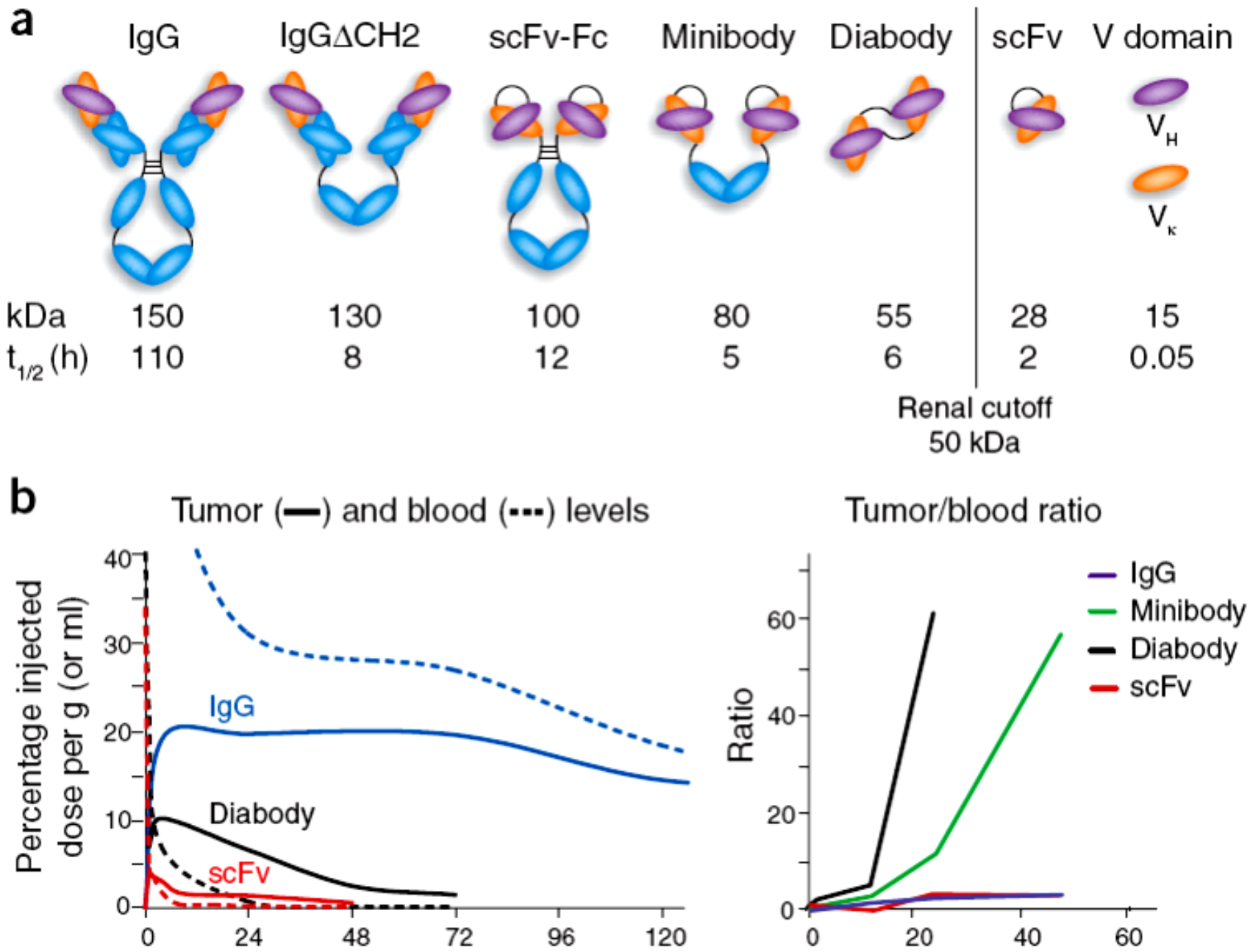
Antibody Engineering


Aa Mouse IgG
Antigen-binding site
VL
VH
Papain cleavage
Variable region
Light chain
Hinge region
Contrast region
CHO
CHO
Fc domain
Heavy chains
Ab Chimeric IgG
Ac Humanized IgG
Ad Human IgG
B F(ab')2
C Fab'
SH
Da scFv
VH
VL
Db Bivalent scFv
A
A
Dc Bivalent recombinant scFv
A
B
Mouse sequences
Human sequences

Different Antibody Format: Natural & Engineered

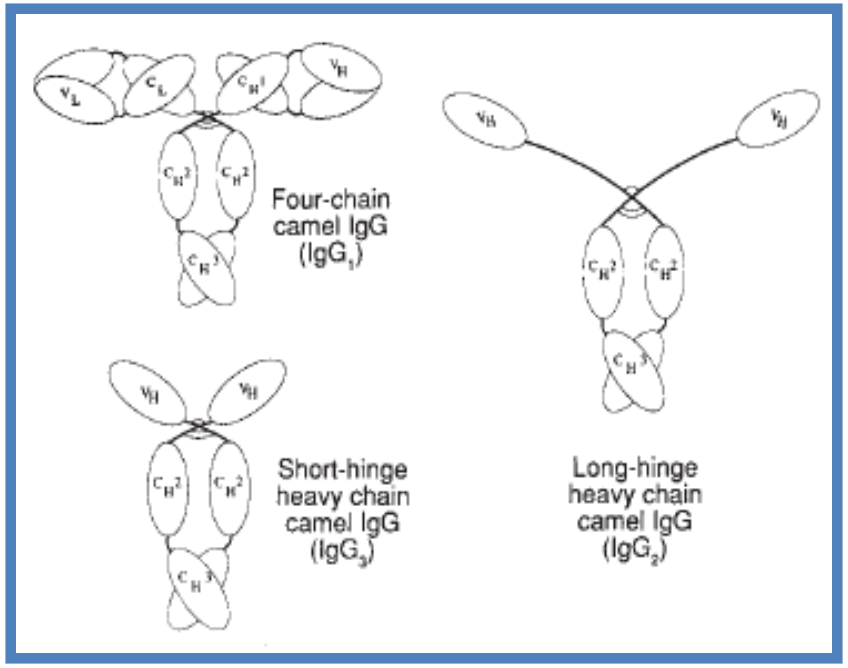


Natural

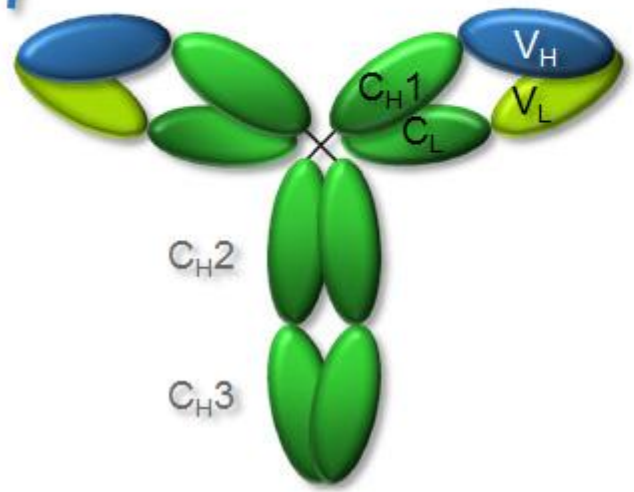

a
IgG
IgGΔCH2
scFv-Fc
Minibody
Diabody
scFv
V domain
V_H
V_κ
kDa 150 130 100 80 55 28 15
$t_{1/2}$ (h) 110 8 12 5 6 2 0.05
Renal cutoff
50 kDa
b
Tumor (—) and blood (---) levels
Percentage injected dose per g (or ml)
IgG
Diabody
scFv
0 24 48 72 96 120
Tumor/blood ratio
Ratio
IgG
Minibody
Diabody
scFv
0 20 40 60

Nanobodies

- 1989 - Raymond Hamers
- Discovered in camels
- Completely lack the light chain!
- Same antigen affinity as their four-chain counterparts
- Structure makes them more resistant to heat and pH
  - May lead to development of oral nanobody pills

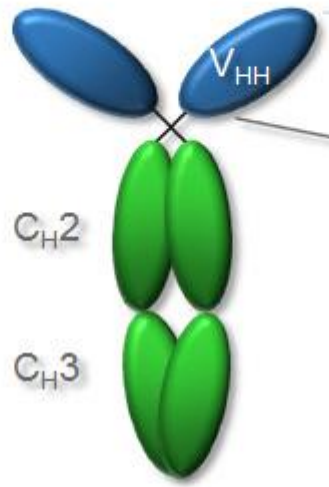


Camelidae family has both forms



Conventional antibody

- Heavy and light chains
- Both chains required for antigen binding and stability
- Large size and relatively low formatting flexibility
- Administered through injection



Heavy-chain antibody

- Only heavy chains
- Full antigen binding capacity and very stable

Ablynx' s Nanobody®

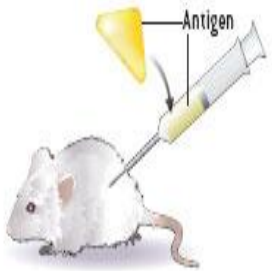
- Small (1/10 size of a mAb)
- Flexible formatting
- Highly potent, robust and stable
- Broad target applicability
- Multiple administration routes
- Ease of manufacture
- Speed of discovery

CONSTRUCTING ANTIBODIES AND NANOBODIES

Creating an effective nanobody takes less time and money than a therapeutic antibody requires, according to scientists at Ablynx. In both cases, the immune system of a live animal performs the initial "design" of a protein that can latch onto the target molecule. Geneticists then tweak the DNA encoding that protein to add the properties desired in a medicine.

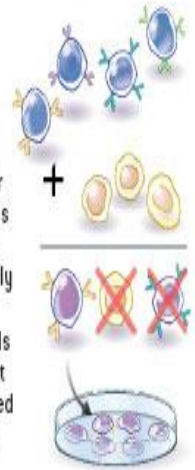
CONVENTIONAL MONOCLONAL ANTIBODIES

1 Immunization



Researchers inject a mouse with the target molecule. B cells of its immune system generate antibodies that recognize this antigen

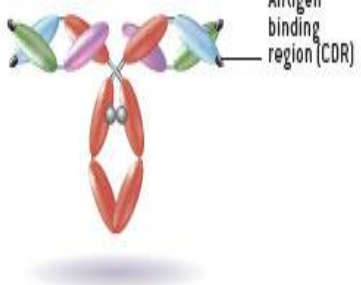
2 Fusion, Selection and Expansion



Mixing B cells (*blue*) with myeloma cancer cells (*orange*) creates hybridomas (*purple*) that divide indefinitely

Those hybridoma cells that make the correct antibody are identified and grown in culture

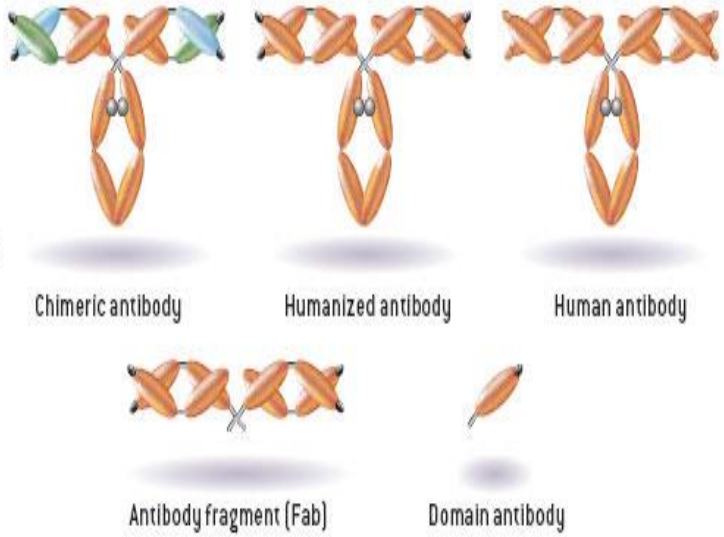
3 Harvesting Antibodies



Mouse antibody

The culture secretes copies of the antibody, which are then purified and tested

4 Humanization



Genetic engineers can replace pieces of the mouse antibody with human segments (*orange*) and can also trim the antibody to create fragments of various sizes

ABLYNX NANOBODIES

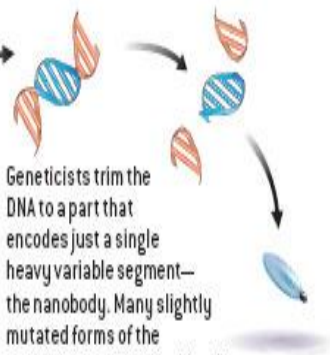
1 Immunization

A llama or camel is immunized and produces both normal (*left*) and heavy-chain-only (*right*) antibodies against the target

2 Isolation and Cloning

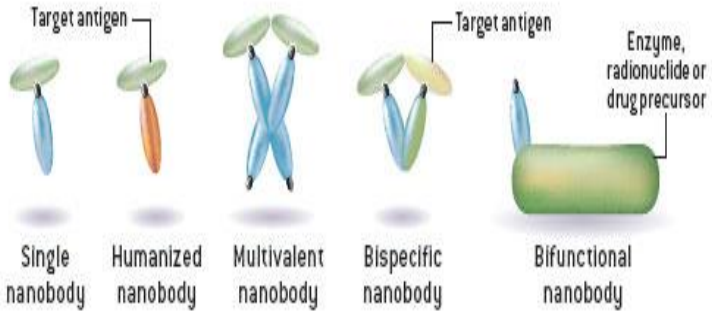
From a blood sample, biologists identify cells that produce a heavy-chain-only antibody with high affinity for the target. They then obtain the DNA sequence for the genes that code for the antibody

3 Genetic Engineering



Geneticists trim the DNA to a part that encodes just a single heavy variable segment—the nanobody. Many slightly mutated forms of the nanobody are tested to identify the one that is most medically useful

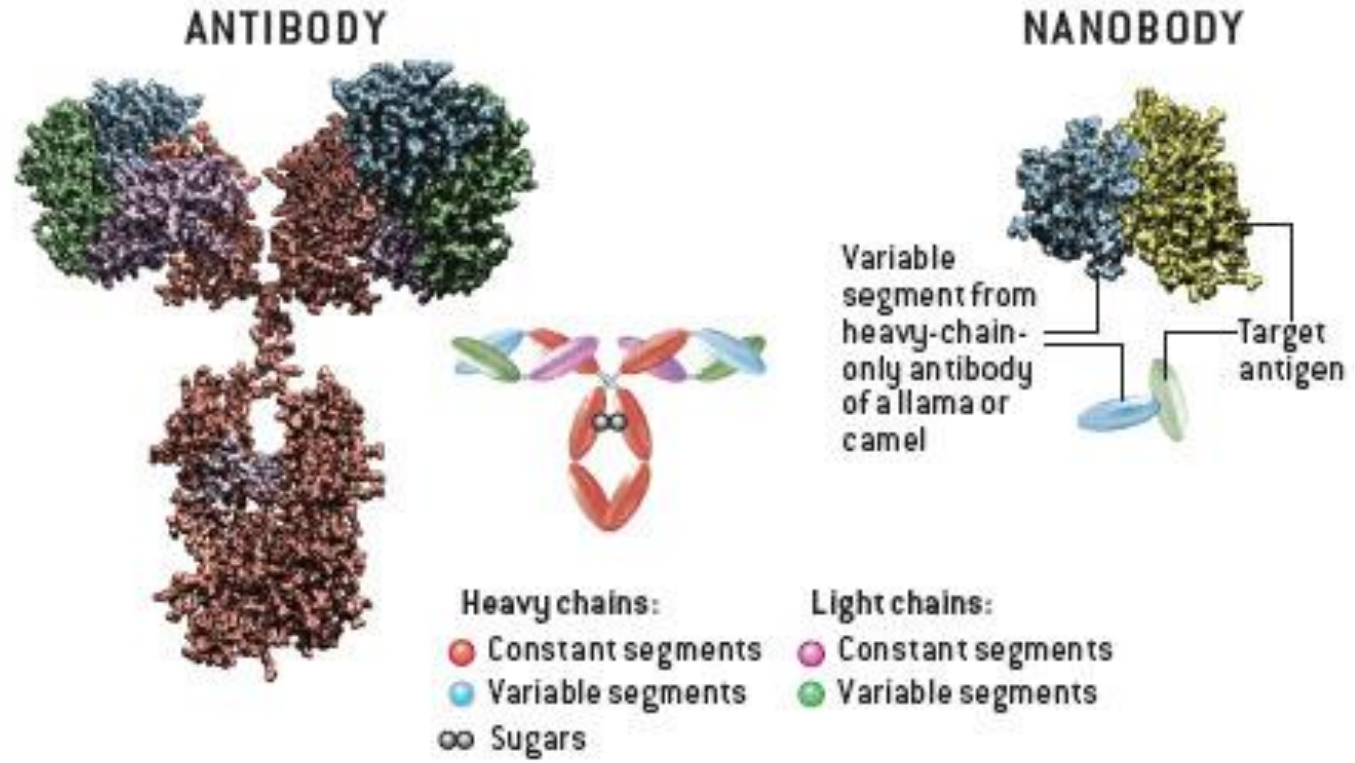
4 Construction of Nanobody Medicine



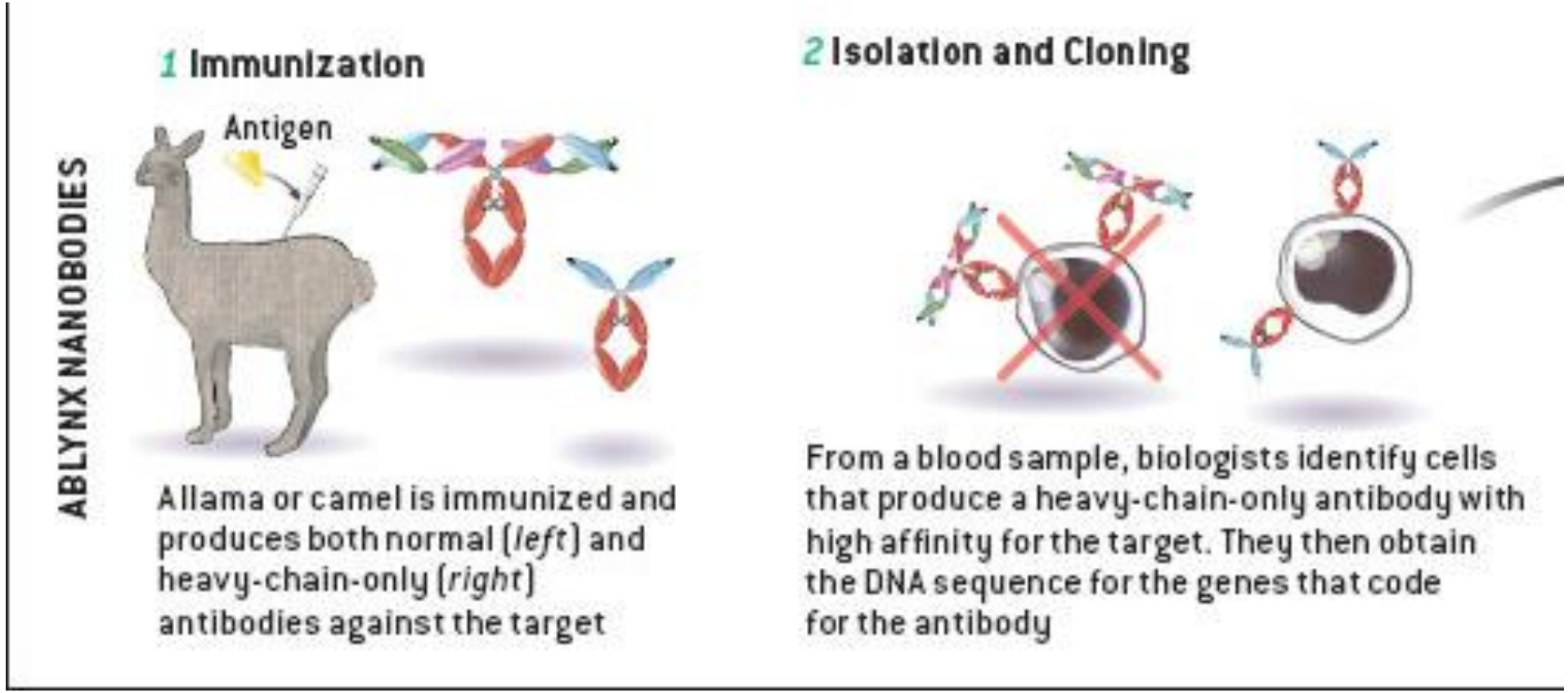
Nanobody genes can be spliced with genes for other nanobodies or other biochemicals to create medicines that are then produced in bacteria, fungi or yeast cultures

ANATOMY OF AN ANTIBODY

The millions of kinds of human antibodies all share the same basic structure: two larger (or heavy) protein chains linked with two smaller (or light) chains. The pair of variable segments at the tips of the arms are unique for each model of antibody and determine the target to which it will bind. A nanobody is the variable part of a camel antibody that lacks light chains; it is about one tenth the size of an antibody.

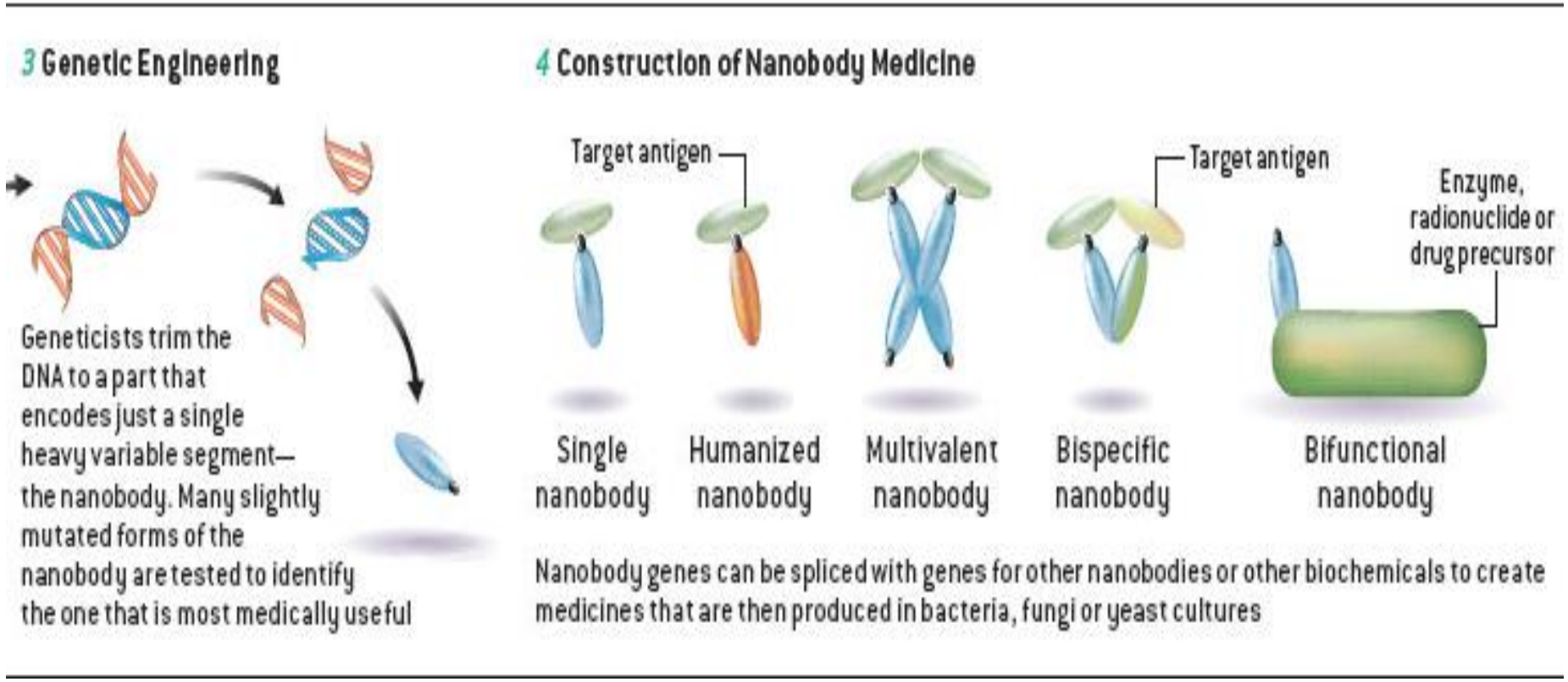


Construction of Nanobodies (1)



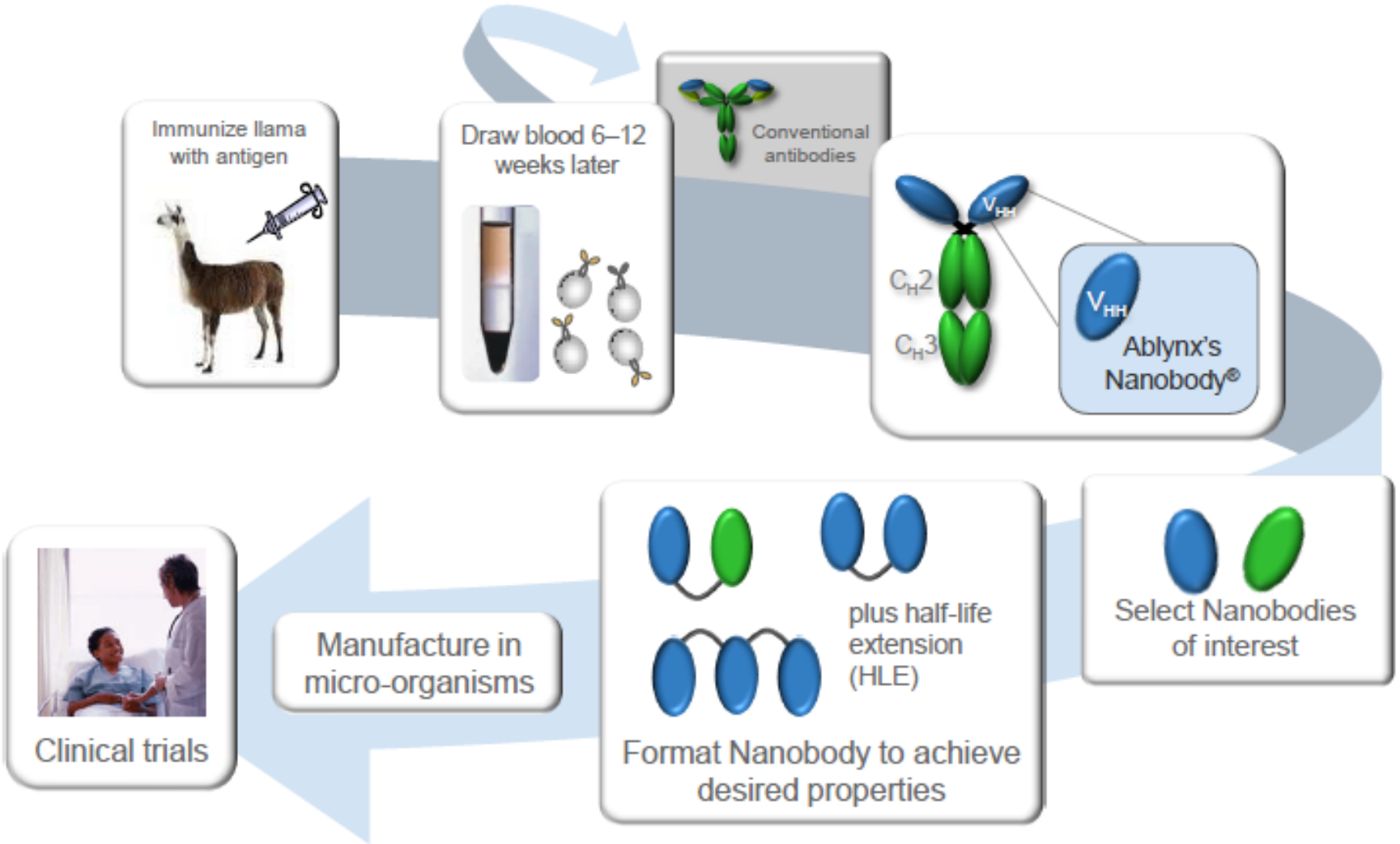
Nanobodies
W. Wayt Gibbs
Scientific American **293**, 78 - 83 (2005)

Construction of Nanobodies (2)

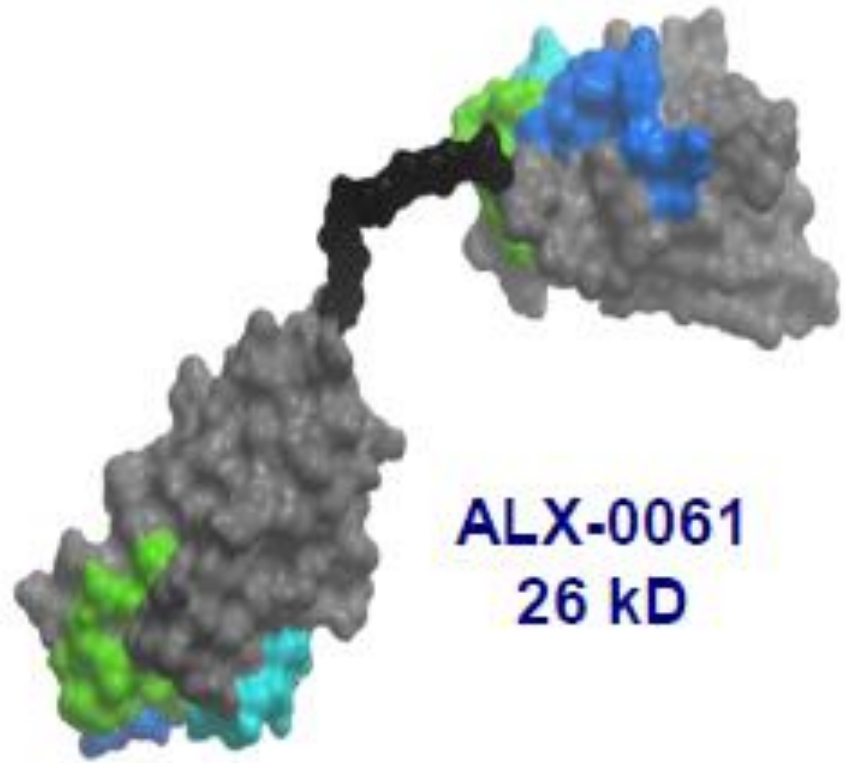


Nanobodies
W. Wayt Gibbs
Scientific American **293**, 78 - 83 (2005)

Nanobody discovery process



anti-HSA Nanobody



ALX-0061
26 kD

Anti IL6-R Nanobody

Designer antibodies for human therapy (1)

Design goal	Strategies	Potential benefits
Immunogenicity		
Minimize risk	Minimize non-human sequence (chimerization or humanization of mouse antibody, use of human monoclonal antibody, use of human germline sequences); design to remove T-cell epitopes; minimize presence of aggregated or misfolded antibody (purification, formulation, engineering of variable domains for improved stability or folding kinetics); minimize presence of contaminants (purification)	Increased efficacy; improved safety; more efficient effector functions; longer terminal half-life

Designer antibodies for human therapy (2)

Design goal	Strategies	Potential benefits
Antigen-binding specificity		
Improve selectivity for antigen	Select from display libraries; screen antibody panel; use structure-based design	Prerequisite for targeted therapy
Increase species crossreactivity	Select from display libraries; screen antibody panel; use structure-based design	Facilitates preclinical development (efficacy testing, toxicology)
Antigen-binding affinity		
Increase	Select from display libraries; use structure-based design	Increased efficacy; reduced dose or frequency of administration; increased potency of ADCC
Decrease	Select from display libraries; use structure-based design	Increased localization to tumours; more homogeneous distribution in tumours

Designer antibodies for human therapy (3)

Design goal	Strategies	Potential benefits
Biological activities associated with variable domains		
Isolate antibodies with potent activities	Screen antibody panel	Increased efficacy; reduced dose or frequency of administration
Improve activities of existing antibodies	Induce affinity maturation of existing antibody then screen for potency	Increased efficacy; reduced dose or frequency of administration
Effector functions		
Improve or tailor	Screen human IgG panel; engineer Fc region (point mutations, glycan modifications); increase antigen-binding affinity	Increased efficacy
Avoid or abolish	Screen human antibody panel; select IgG2 or IgG4 as isotype; use IgG with point mutations in Fc region; use IgG that lacks disulphide bonds in hinge region; use IgG with an aglycosylated Fc region; use antibody fragments	Reduced adverse events

Designer antibodies for human therapy (4)

Design goal	Strategies	Potential benefits
Pharmacokinetics		
Reduce plasma half-life	Use antibody fragments; use IgG with impaired affinity for FcRn	Less whole-body exposure to antibody; improved target-to-non-target ratios
Increase plasma half-life	Use IgG with increased affinity for FcRn (at pH 6.0); modify antibody fragments (PEGylation, binding to molecules with a long half-life such as IgG and serum albumin)	Increased plasma concentrations might improve localization to target; increased efficacy; reduced dose or frequency of administration
Internalization		
Decrease efficiency	Screen antibody panel; choose target antigen accordingly	Efficient effector functions
Increase efficiency	Select antibodies that can internalize from display libraries; screen antibody panel with drug-conjugated crosslinking antibody	Improved efficacy for antibody?drug conjugate or immunoliposomes

Designer antibodies for human therapy (5)

Design goal	Strategies	Potential benefits
Chemical, proteolytic and thermodynamic stability		
Increase	Design to remove 'problem sites'; select from display libraries; use structure-based design	Maintains potency; longer shelf life, improved expression yields; longer terminal half-life in vivo; reduced risk of immunogenicity; improvedin vivo localization to tumour
Other biophysical properties: solubility and folding kinetics		
Increase or improve	Select from display libraries; use structure-based design	Improved expression yields; reduced risk of immunogenicity
ADCC, antibody-dependent cell-mediated cytotoxicity; PEG, polyethylene glycol.		

Antibodies for cell depletion

Natural mechanisms (natural)

Complement

ADCC

Direct Induction of apoptosis

Cytotoxic T cells

Artificial mechanisms (conjugated)

Conventional drugs

Immune activators

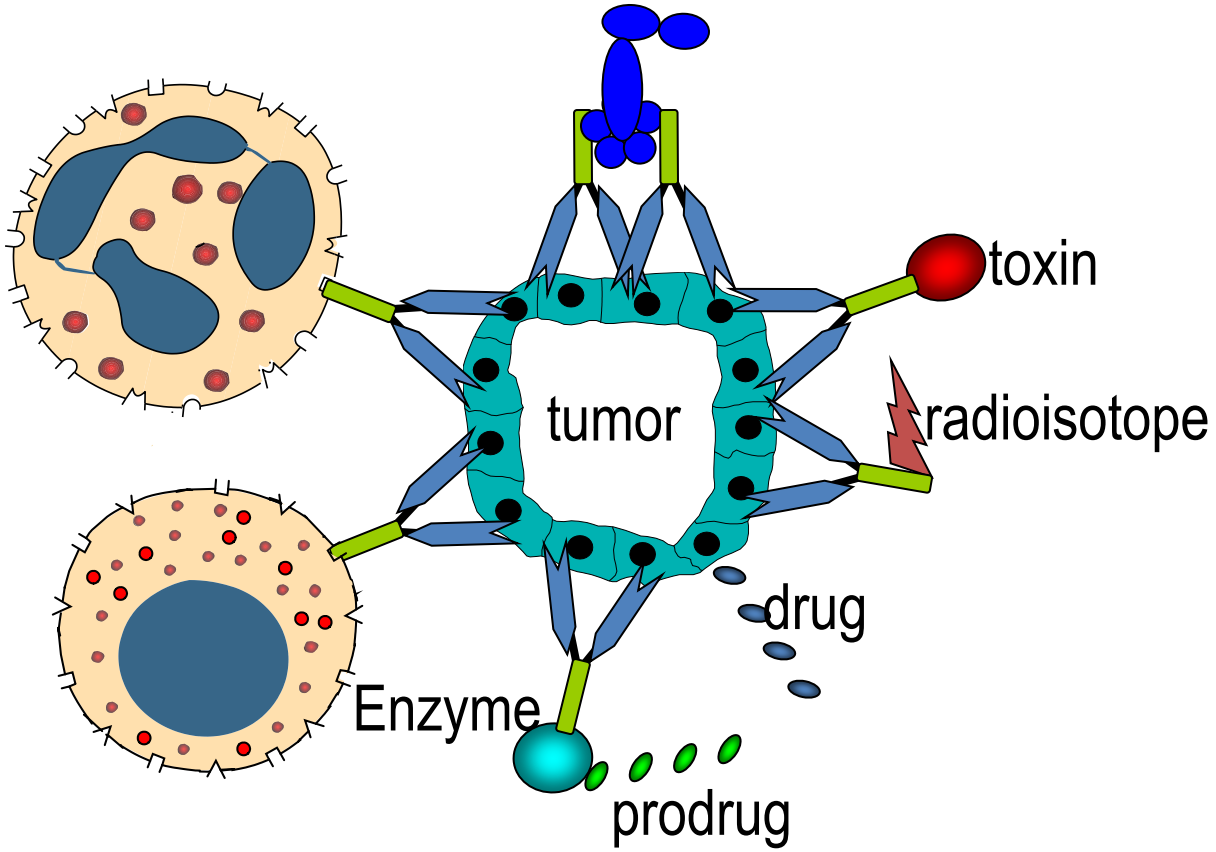
Radioisotopes

Toxins

Enzyme/pro-drug ("ADEPT")

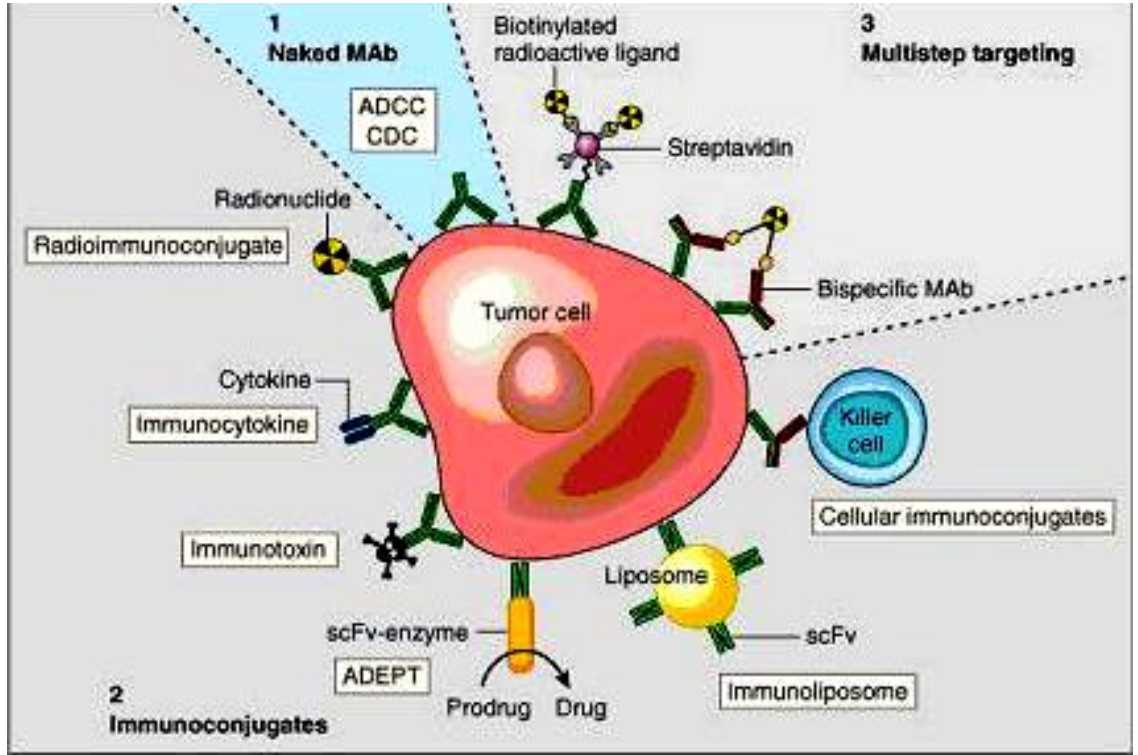
Viruses, genes etc

Use of modified monoclonal antibodies

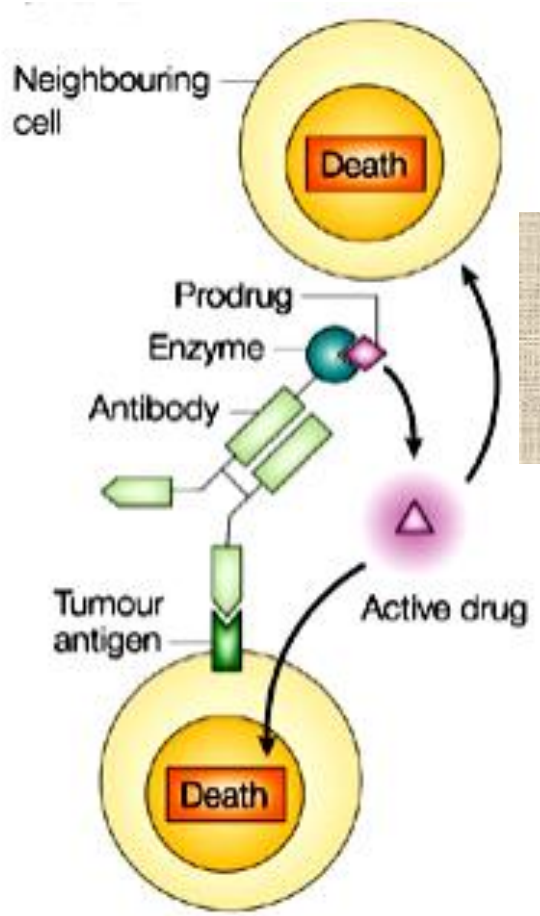


Conjugated/Modification of Antibody

Antibody	Conjugate / modification	Application
anti-CD20	Covalently attached tiuxetan (yttrium-90 chelator)	Radiotherapy for non-Hodgkin lymphoma (Zevalin)
(various)	Covalently attached PEG	Increased serum half life
(various)	Mutant Fc domain with higher affinity for Fc receptors	Increased serum half life
anti-ganglioside GD_2	IL-2 fusion	Recruit T-cells to tumor
anti-HER2	Anchored to a liposome containing doxorubicin	In animal model of breat cancer, worked better than anti-HER2 or doxorubicin alone.
(various)	Enzyme fusion	Prodrug activation (esp. for cancer)



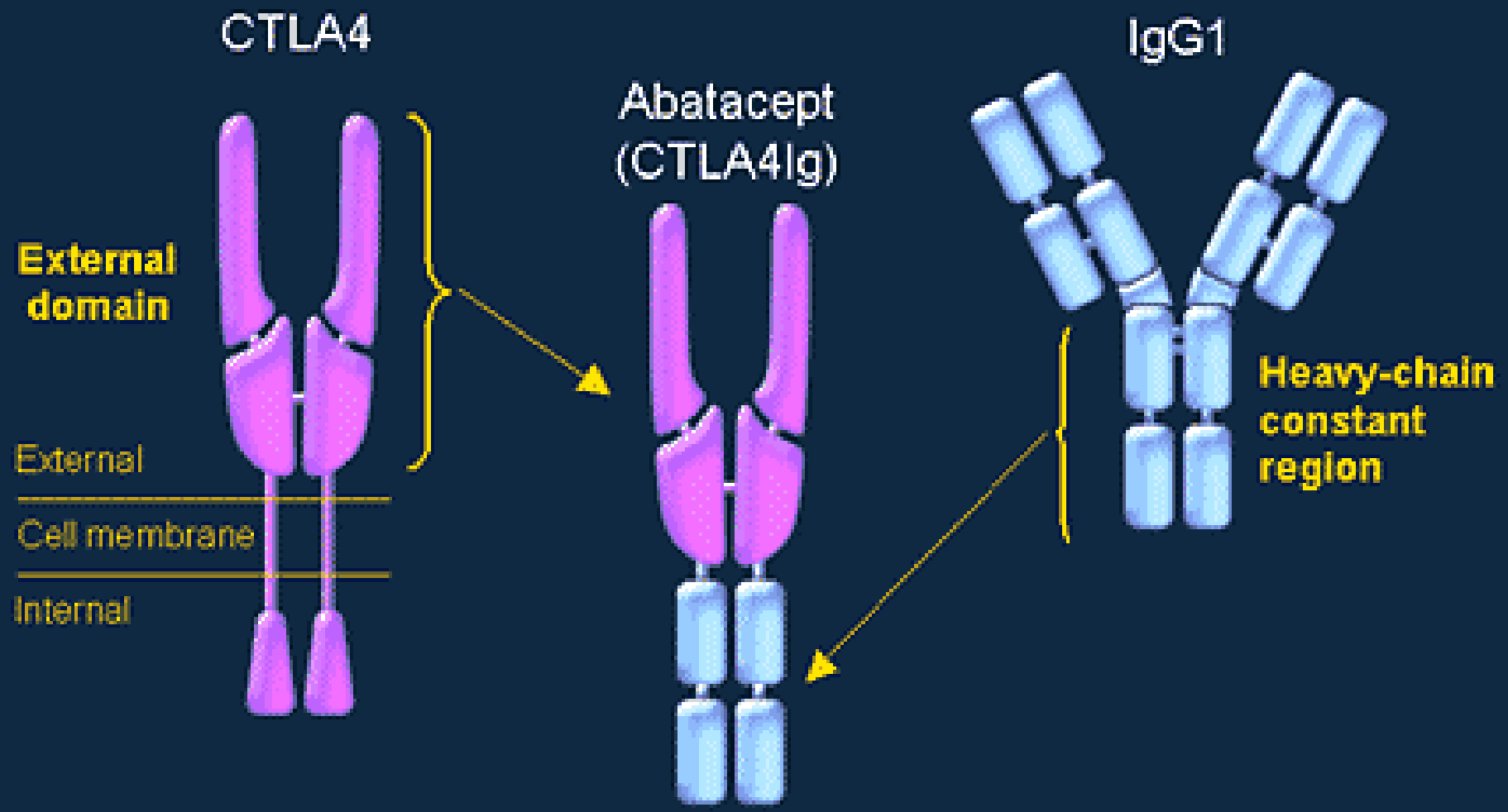
Antibody-directed Enzyme Prodrug Therapy (ADEPT)



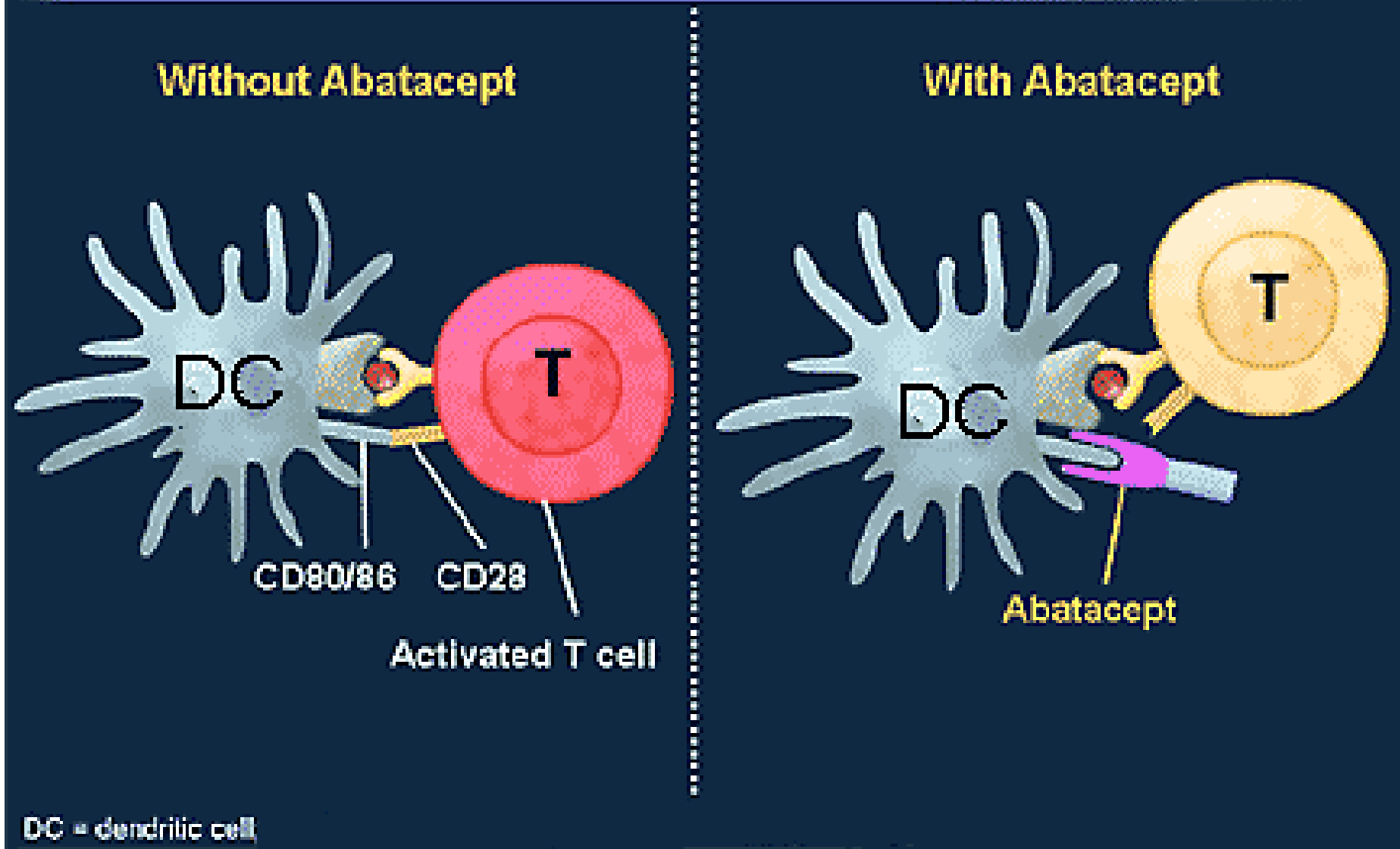
Enzyme	Prodrug	Drug
Carboxypeptidase A	Methotrexate alanine	Methotrexate
beta - Glucuronidase	Epirubicin glucoronide	Epirubicin

Nature Reviews Cancer **2**, 143-148 (February 2002)

A Human Immunoglobulin Receptor Fusion Protein



Abatacept Selectively Modulates T Cell Activation



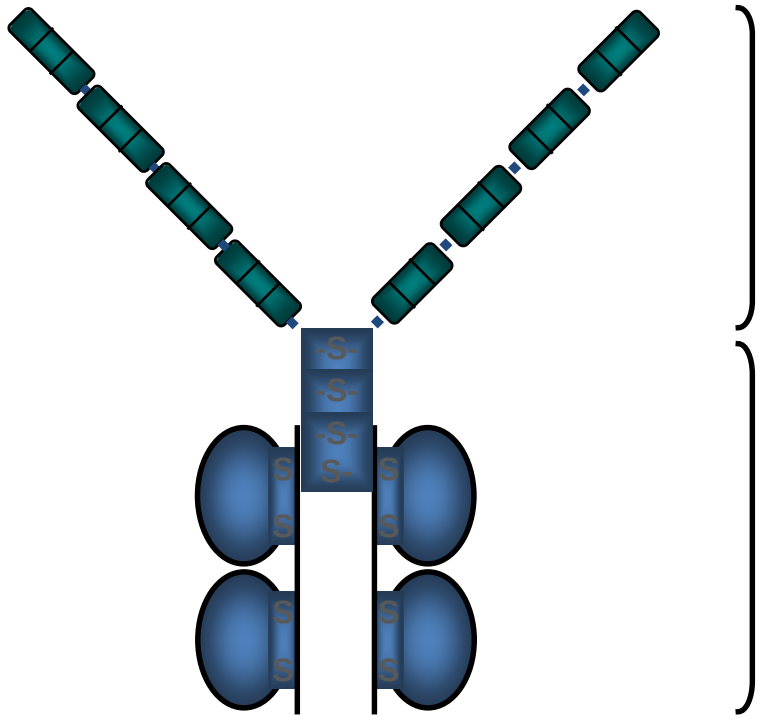
DC = dendritic cell

Etanercept (Enbrel®)

- biologic modifier
- recombinant human tumor necrosis factor receptor fusion protein
- binds & inactivates soluble TNF
- subcutaneously, once or twice a week
- retards erosive disease

Etanercept (Enbrel®)

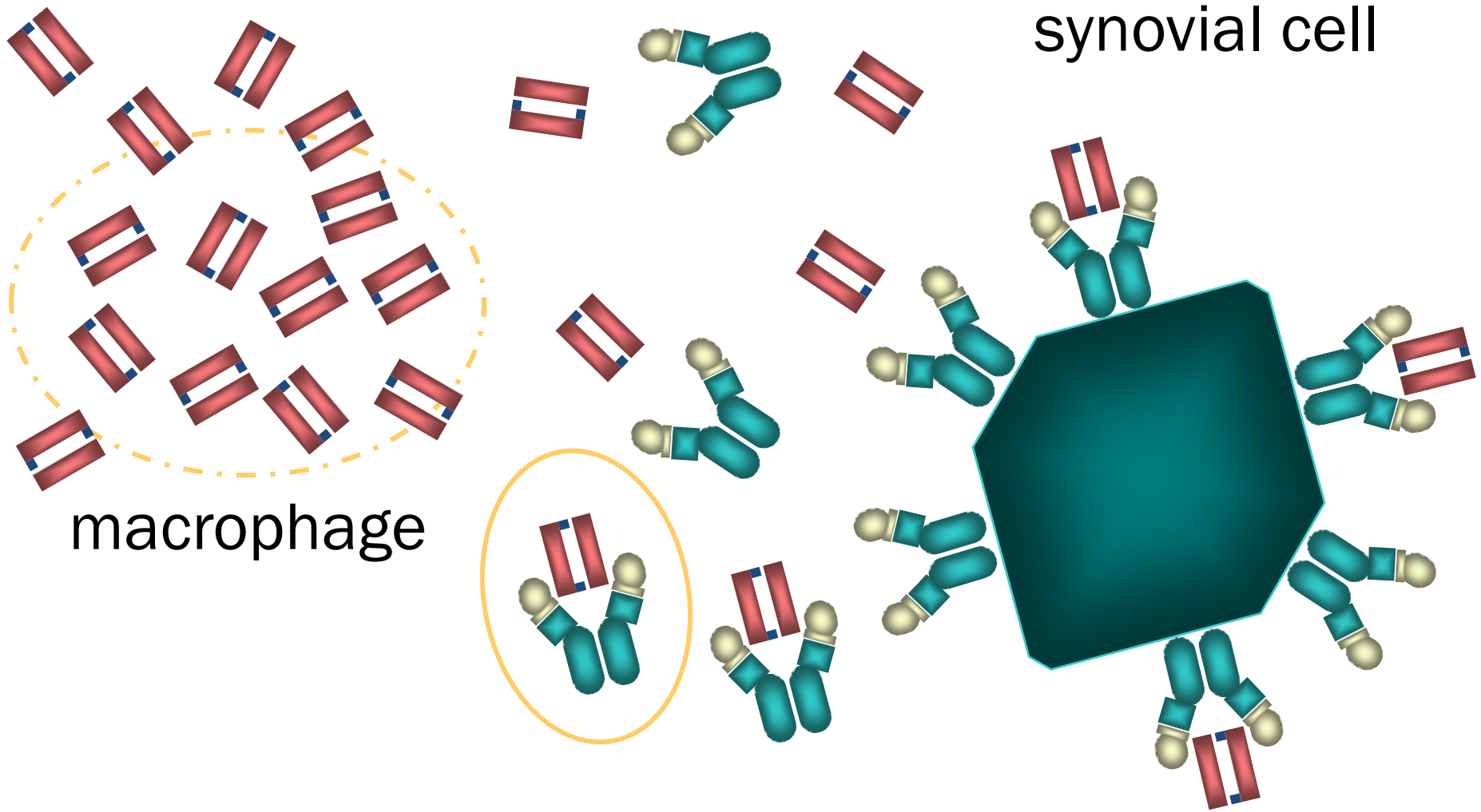
soluble TNF receptor fusion protein



extracellular human TNF-receptor p75 monomer

human IgG1 Fc domain

Soluble TNF receptor binding



CHO cells : Platform for Mab Production in Large Scale

- Safety for use in humans
- Similar glycan structure with natural human mAb
- Ease of transfection
- Presence of a powerful gene amplification system
- Adaptation to growth in suspension and serum-free medium
- Ability to grow at high densities

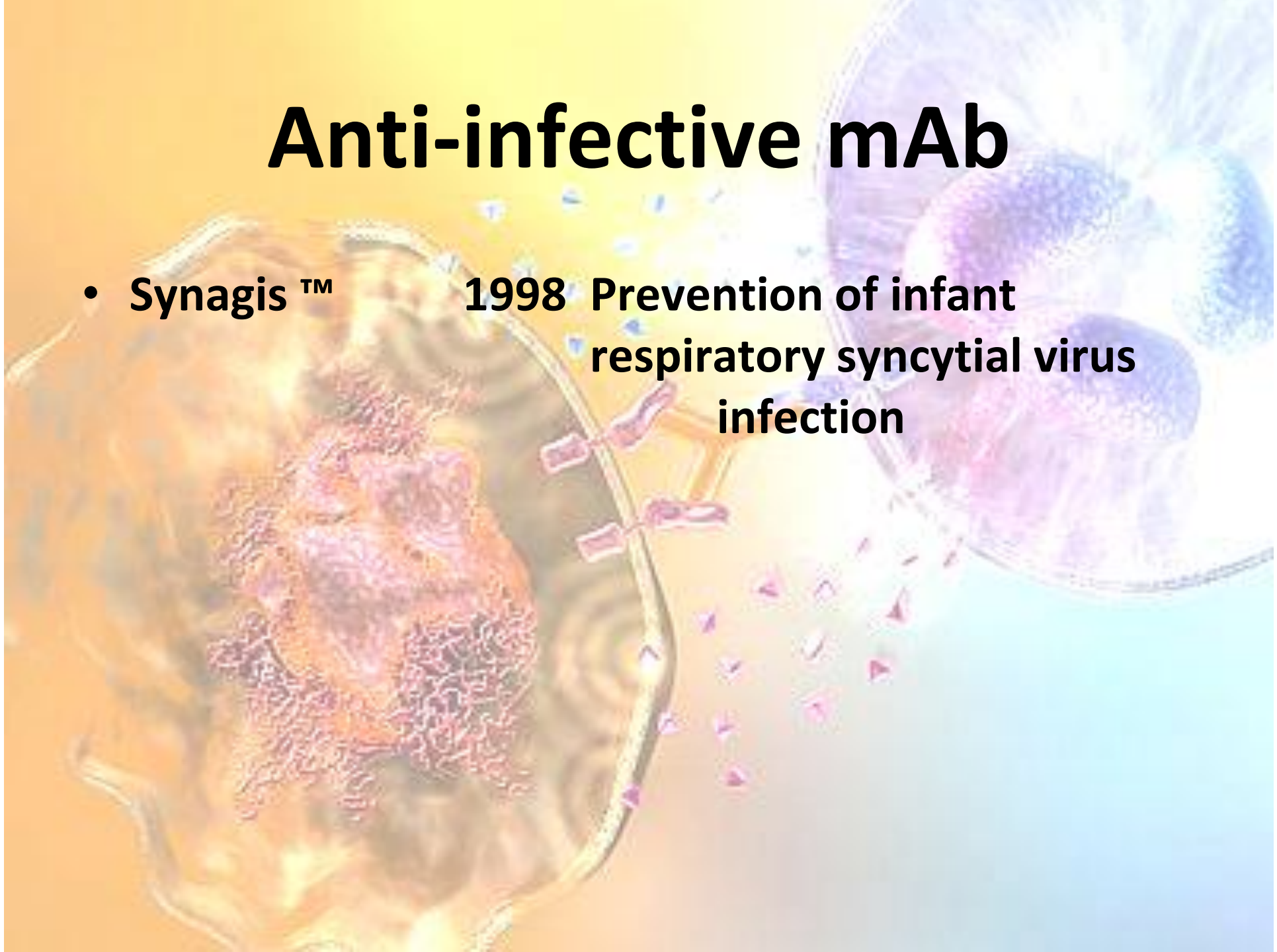
Some Examples of Approved Conjugated/Modified Mabs

- **Radiolabeled antibodies:**
  - 2002 Ibritumomab tiuxetan (Zevalin) Y-90 or In-111 + anti-CD20 (NHL)
  - 2003 Tositumomab-I-131 (Bexxar) – anti-CD20 (NHL)
  - OncoScint (for colorectal and ovarian cancer, CEA) and ProstaScint (for prostate cancer)
- **Immunotoxins**
  - 2000 Gemtuzumab ozogamicin (Mylotarg). ozogamicin- anti-CD33 (AML)
- **Fc conjugated peptides**
  - 1998 Etanercept (Enbrel) IgG1-Fc/p75 exodomain of TNFR (RA)
- **Pegylated**
  - 2008 Certolizumab pegol (Cimzia) PEG- Fab anti-TNF-alpha

Immunology/Transplantation mAbs

Orthoclone™	1986	Transplant rejection
Zenapax ™	1997	Transplant rejection
Simulect ™	1998	Transplant rejection
Remicade ™	1998	Crohn's disease
Humira ™	2002	Rheumatoid arthritis
Xolair ™	2003	Allergy-related asthma
Raptiva ™	2003	Psoriasis
Tysabri ™	2004	Multiple sclerosis
Soliris ™	2007	Paroxysmal nocturnal hemoglobinuria

Anti-infective mAb



- **Synagis ™ 1998 Prevention of infant respiratory syncytial virus infection**

Human mAb (trade name; company name)	Description	Indication of first US approval	FDA designations	Date of first US (EU) approval
Adalimumab (Humira; Abbott)	TNF-specific, IgG1κ	Rheumatoid arthritis	S	31 Dec 2002 (8 Sep 2003)
Panitumumab (Vectibix; Amgen)	EGFR-specific, IgG2κ	Colorectal cancer	P, FT, AA	27 Sep 2006 (3 Dec 2007)
Golimumab (Simponi; Centocor)	TNF-specific, IgG1	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis	S	24 Apr 2009 (1 Oct 2009)
Canakinumab (Ilaris; Novartis)	IL-1β-specific, IgG1κ	Cryopyrin-associated periodic syndromes	P, O	18 Jun 2009 (23 Oct 2009)
Ustekinumab (Stelara; Johnson & Johnson)	IL-12/IL-23 p40-specific, IgG1	Plaque psoriasis	S	25 Sep 2009 (16 Jan 2009)
Ofatumumab (Arzerra; Genmab)	CD20-specific, IgG1	Chronic lymphocytic leukaemia	P, FT	26 Oct 2009 (19 Apr 2010)
Denosumab (Prolia; Amgen)	RANKL-specific, IgG2	Treatment of postmenopausal osteoporosis‡	S	1 Jun 2010 (26 May 2010)
Raxibacumab	PA-specific, IgG1	Inhalation anthrax	P, FT, O	Under review by the FDA
Belimumab	B lymphocyte stimulator-specific, IgG1	Systemic lupus erythematosus	P, FT	Under review by the FDA and the EMA
Ipilimumab	CTLA4-specific, IgG1	Metastatic melanoma	P, FT, O	Under review by the FDA and the EMA

Human mAbs approved or under FDA review

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AA, accelerated approval; CTLA, cytotoxic T lymphocyte-associated antigen; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; EU, European Union; FDA, US Food and Drug Administration; FT, FDA fast track drug; Ig, immunoglobulin; IL, interleukin; mAb, monoclonal antibody; O, FDA orphan drug; P, priority review; PA, *Bacillus anthracis* protective antigen; RANKL, receptor for activation of nuclear factor-κB ligand; S, standard review; TNF, tumour necrosis factor. *As of June 2010. ‡Also approved in Europe for the treatment of bone loss in patients with prostate cancer undergoing hormone ablation therapy.

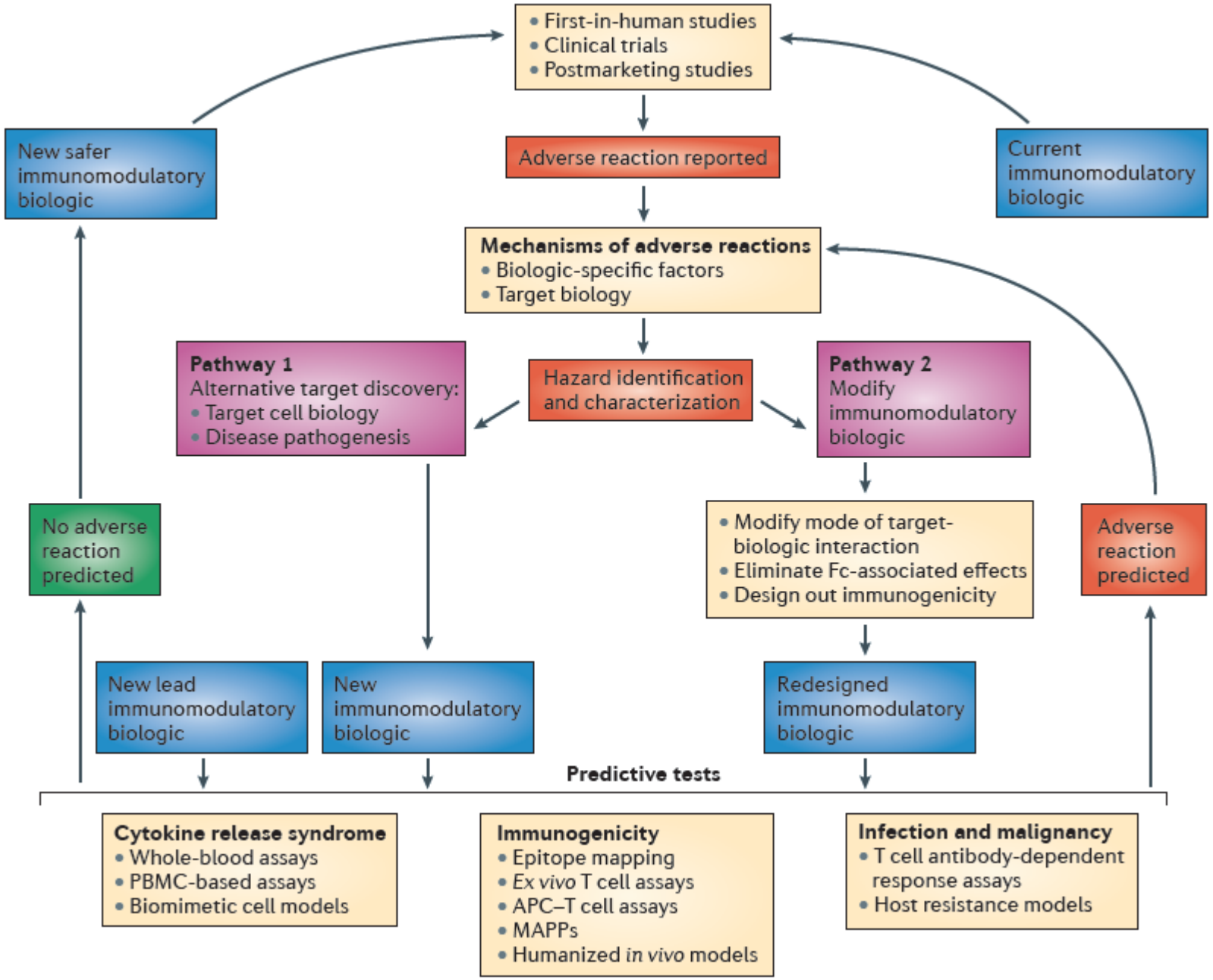
Optimal Therapeutic Antibody Selection

- **Target Selection**
  - Disease target(s)
  - Biochemical pathways
  - Molecular target in pathway
    - Expression level and distribution
- **Format selection**
  - Half-life
  - Effector function
  - Binding affinity
  - Potential problems in sequence
- **Screening and epitope selection**
  - Block interaction b/w ligand-receptor
  - Mediate effect after binding
    - Cross-link to intracelluar signal
    - Deliver toxic payload
    - Use effector function to kill
- **Antibody generation**
  - Rodent immunization
  - Phage-display library

SWOT diagram for therapeutic Mabs and Fc fusion proteins

Major Strengths:	Major Weaknesses:
• Targets that cannot be addressed with small molecules (e.g., protein–protein interactions) • Half-life leads to less frequent dosing • Efficacy • ADCC/CDC (i.e., immune system functionality)	• Parenteral delivery (IV, SC) • Limitation to extracellular and cell-surface targets • Cost, and cost of goods driving the pricing • Immunogenicity and injection site reactions
Major Opportunities:	**Major Threats:**
• Delivery improvements (e.g., transdermal, oral, intranasal) • Modified Fc; fine-tuning immune system functionality • Extended and/or tunable T1/2 • Multispecificity (e.g., ability to engage multiple targets while retaining long T1/2 • Novel scaffolds, approaches • Tissue targeting, e.g., ability to cross BBB	• Safety concerns in a post-Tegenero TGN-1412 era • Small molecules functioning in same pathways as biologic • Third party payer restrictions on reimbursement • Follow-on-biologics • Perception of a limited number of high quality targets leading to intense competition on certain "hot" targets (e.g., TNF-α, CD20)

Strohl, W. 2008: Therapeutic Monoclonal Antibodies: Past, Present, and Future

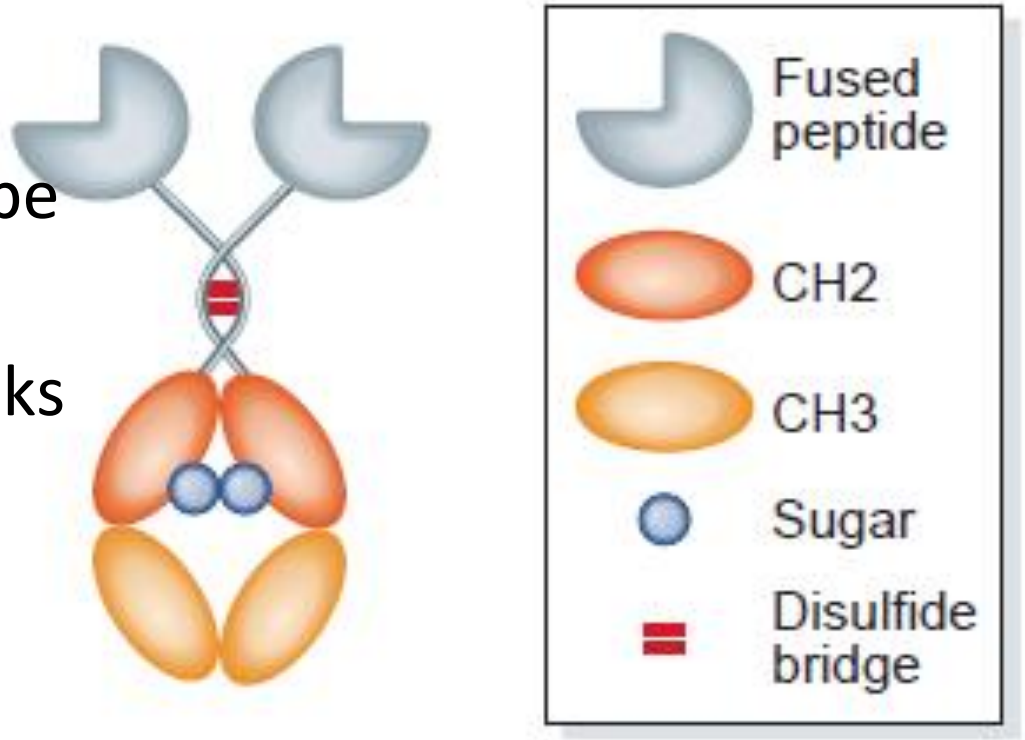

• First-in-human studies
• Clinical trials
• Postmarketing studies
Adverse reaction reported
Mechanisms of adverse reactions
• Biologic-specific factors
• Target biology
Hazard identification and characterization
Pathway 1
Alternative target discovery:
• Target cell biology
• Disease pathogenesis
Pathway 2
Modify immunomodulatory biologic
New safer immunomodulatory biologic
Current immunomodulatory biologic
No adverse reaction predicted
• Modify mode of target-biologic interaction
• Eliminate Fc-associated effects
• Design out immunogenicity
Adverse reaction predicted
New lead immunomodulatory biologic
New immunomodulatory biologic
Redesigned immunomodulatory biologic
Predictive tests
Cytokine release syndrome
• Whole-blood assays
• PBMC-based assays
• Biomimetic cell models
Immunogenicity
• Epitope mapping
• Ex vivo T cell assays
• APC–T cell assays
• MAPPs
• Humanized in vivo models
Infection and malignancy
• T cell antibody-dependent response assays
• Host resistance models

Future trends

- **Opportunities in major therapeutic categories**
  - Anticancer therapeutics
  - Immunological agents
  - Anti-infective agents
- **Increase in marketing approvals if success rates are consistent with previous rates**
- **Human mAbs**
- **Designed protein scaffolds/domains**

Fc Fusion Protein As drugs

- Fc scaffold + Fused peptide
- Immune effector function can be conserved or omitted
- Natural scaffold + building blocks



Ranking of Fc effector function potentials for therapeutic Abs

Therapeutic Ab type	Class I: *cell-bound antigen stable upon Ab binding. MOA involving Fc effector function (ADCC, CDC, ADCP)*	Class II: *cell-bound antigen. MOA not involving Fc effector function*	Class III: *soluble antigen. MOA not involving Fc effector function (blocking)*
IgG1 and IgG3	High	Moderate	Low
IgG1 and IgG3 with Fc mutations to enhance Fc functionality	High	Not applicable	Not applicable
Afucosylated IgG1	High	Not applicable	Not applicable
IgG1 and IgG3 with Fc mutations to reduce Fc functionality, or aglycosylated IgG1 and IgG3	Not applicable	Low	Low
IgG2 and IgG4; IgG2 and IgG4 with Fc mutations to reduce Fc functionality, or aglycosylated IgG2 and IgG4	Not applicable	Low	Low

Ab, antibody; ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity; Fc, crystallizable fragment; IgG, immunoglobulin G; MOA, mechanism of action.

Nature Reviews Drug Discovery **10**, 101-111 (February 2011)

Fc fusion proteins

Table 1. Key Fc-fusion proteins and monoclonal antibodies (mAbs) in the clinic

Trade name (generic name)	Description	Indication of first FDA approval	Stage	Company
Fc-fusion				
Nulojix (belatacept)	CTLA-4 fused to the Fc of human IgG1	Organ rejection	FDA Approved (2011)	Bristol-Meyers Squibb
Eylea (aflibercept)	VEGFR1/VEGFR2 fused to the Fc of human IgG1	Age related macular degeneration	FDA Approved (2011)	Regeneron Pharmaceuticals
Arcalyst (rilonacept)	IL-1R fused to the Fc of human IgG1	Cryopyrin-associated periodic syndromes	FDA Approved (2008)	Regeneron Pharmaceuticals
NPlate (romiplostim)	Thrombopoietin-binding peptide fused to the Fc of human IgG1	Thrombocytopenia in chronic immune thrombocytopenic purpura patients	FDA Approved (2008)	Amgen/Pfizer
Orencia (abatacept)	Mutated CTLA-4 fused to the Fc of human IgG1	Rheumatoid arthritis	FDA Approved (2005)	Bristol-Meyers Squib
Amevive (alefacept)	LFA-3 fused to the Fc of human IgG1	Psoriasis and transplant rejection	FDA Approved (2003)	Astellas Pharma
Enbrel (etanercept)	TNFR fused to the Fc of human IgG1	Rheumatoid arthritis	FDA Approved (1998)	Amgen/Pfizer

Name	Scaffold or format	Developer or licensee	Parent protein structure	Clinical trial phase	Disease	Target
Ecallantide (Kalbitor/ DX88)	Kunitz domain	Dyax	Human lipoprotein-associated coagulation inhibitor (LACI)	FDA approved (December 2009)	Hereditary angioedema	Kallikrein inhibitor
TRU-015	SMIP	Trubion/Pfizer	Various origin and length	Phase IIb	NHL	CD20
Dom-0200/ART621	Domain antibody	Domantis (now GlaxoSmithKline)/ Cephalon	V_H or V_L antibody domain; 100–130 amino acids	Phase II	Rheumatoid arthritis and psoriasis	TNF
MT103	BiTE	Micromet	scFv–scFv; 200–260 amino acids	Phase II	ALL	CD19 and CD3
				Phase I	NHL	
Angiocept (BMS-844203/ CT-322)	Adnectin	Adnexus (owned by Bristol-Myers Squibb)	10th FN3 domain of fibronectin; 94 amino acids	Phase II	Colorectal cancer, NSCLC and glioblastoma	VEGFR2
ALX-0081	Nanobody	Ablynx	VHH; ~100 amino acids	Phase II	ACS and TTP	vWF
ESBA105	Stable scFv	ESBATech/Alcon	scFv with hyperstable properties	Phase II	Uveitis	TNF
AMG-220 (C326)	Avimer	Avidia (owned by Amgen)	Domain A of LDL receptor; a repeating motif of ~35 amino acids	Phase I	Crohn's disease	IL-6
MT110	BiTE	Micromet	scFv–scFv; ~500 amino acids	Phase I	Lung and gastric cancers	EPCAM and CD3
ABY-002	Affibody	Affibody	Z domain of protein A from *Staphylococcus aureus*; 58 amino acids	Phase I	Breast cancer imaging	HER2
MP0112	DARPin	Molecular Partners	Ankyrin repeat proteins; 67 amino acids plus a repeating motif of 33 amino acids	Phase I	Ophthalmological diseases	VEGF
PRS-050 (Angiocal)	Anticalin	Pieris	Lipocalin; 160–180 amino acids	Phase I starts early 2010	Solid tumours	VEGF

ACS, acute coronary syndrome; ALL, acute lymphoblastic lymphoma; BiTE, bispecific T cell engager; DARPin, designed ankyrin repeat protein; EPCAM, epithelial cell adhesion molecule; FDA, United States Food and Drug Administration; HER2, human epidermal growth factor receptor 2; IL, interleukin; LDL, low-density lipoprotein; NHL, non Hodgkin's lymphoma; NSCLC, non-small-cell lung carcinoma; R, receptor; scFv: single-chain variable domain antibody fragment; SMIP, small modular immunopharmaceutical; TNF, tumour necrosis factor; TTP, thrombotic thrombocytopenic purpura; VEGF, vascular endothelial growth factor; V_H, heavy chain variable domain; VHH, heavy chain variable domain (in camelids); V_L, light chain variable domain; vWF, von Willebrand factor.

Attraction of mAbs

- Expansion of therapeutics pipeline
- High(er) approval success rates
- Established development and approval pathways
- Established production methods
- Competitive research and development times
- Potentially large markets

Global focus on mAb therapeutics

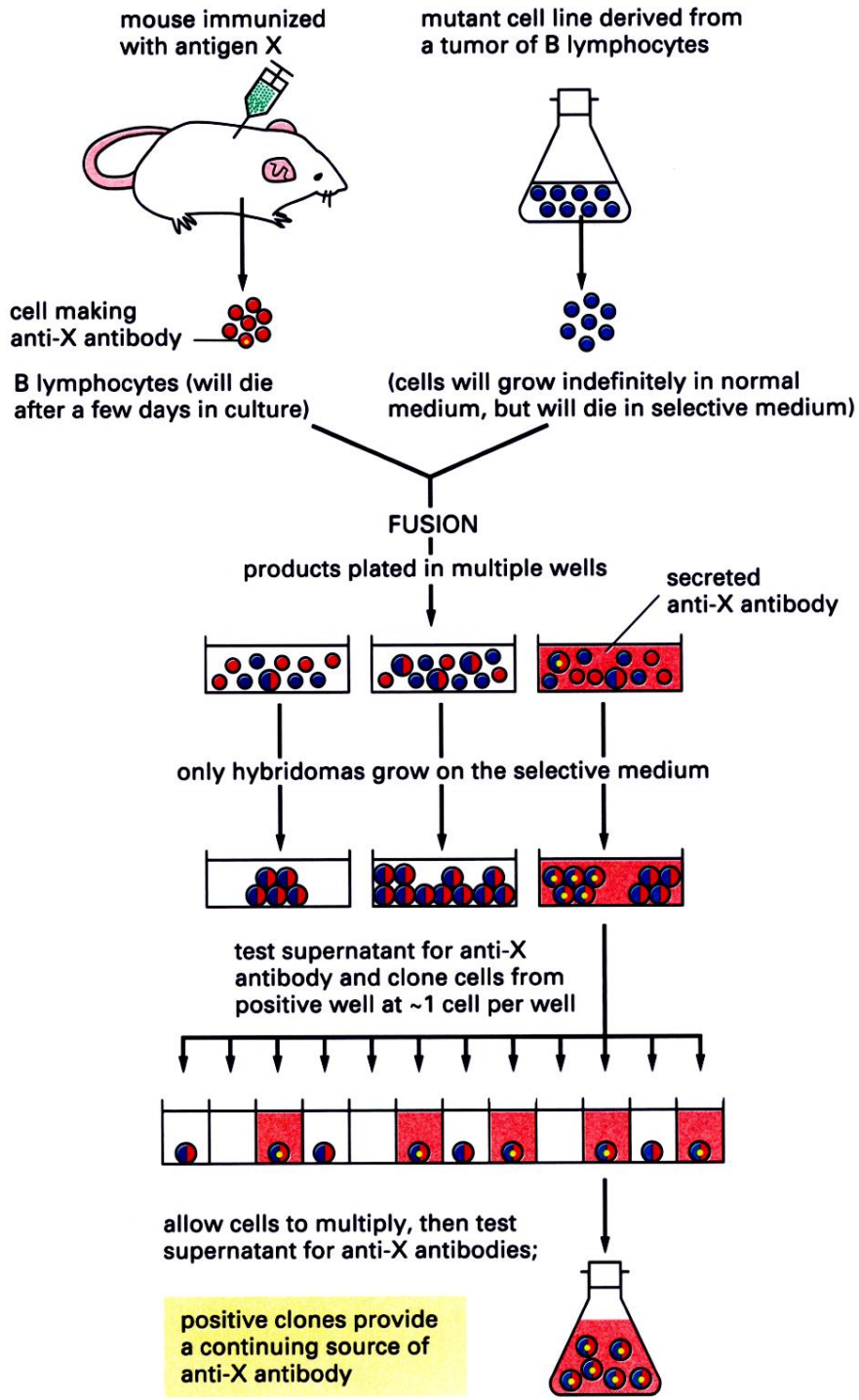
- **Acquisitions by major pharmaceutical firms**
  - Merck acquisition of Abmaxis, GlycoFi
  - GSK acquisition of Domantis
  - Eisai acquisition of Morphotek
  - AstraZeneca acquisition of CAT, MedImmune
- **Development in Asia**
  - First marketing approvals in China
  - “Generic” mAbs in India and S. Korea

Next generation mAbs

- **Fragments, e.g. Fab, single chains**
  - Smaller, easier/less costly to manufacture
  - But, shorter circulating half-life, no effector functions
  - Approved Fabs: Reopro (1994) and Lucentis (2006)

- **Modified versions**
  - Enhance ADCC/CDC functions
  - Modify pharmacokinetic properties – pegylation
  - Modify affinity and specificity – glycosylation, Fc region engineering


Questions??



MBOC, 3rd edition, pg. 188

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