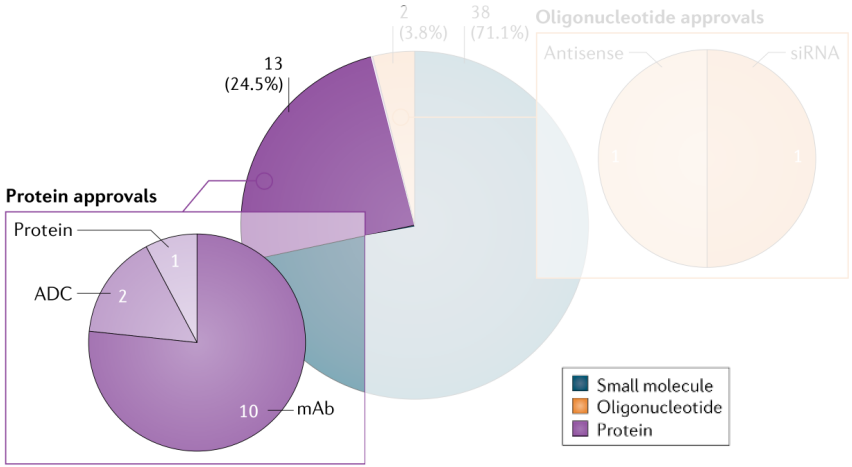
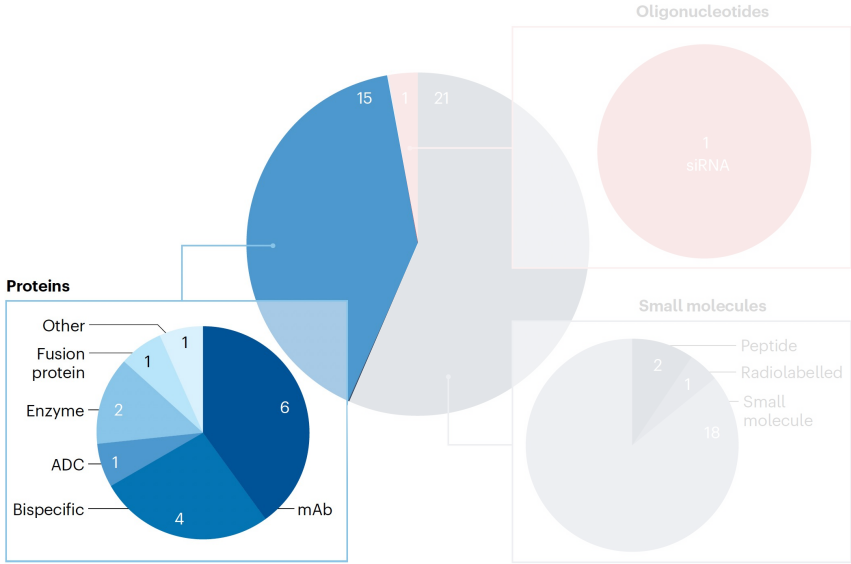

Antibody
Engineering

FDA approvals: antibodies on the rise



2020

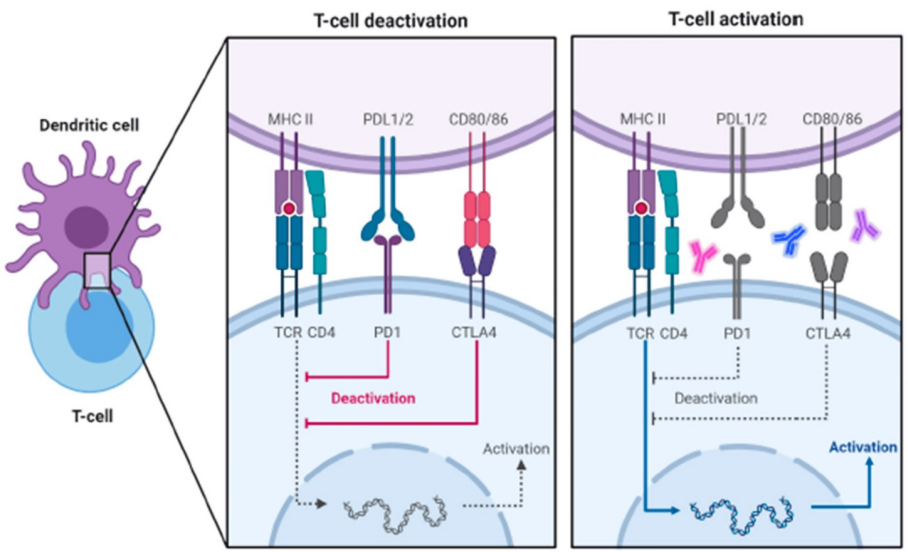


2022

Nature Reviews Drug Discovery **20**, 85-90 (2021)

Nature Reviews Drug Discovery **22**, 83-88 (2023)

Immune checkpoint inhibitors



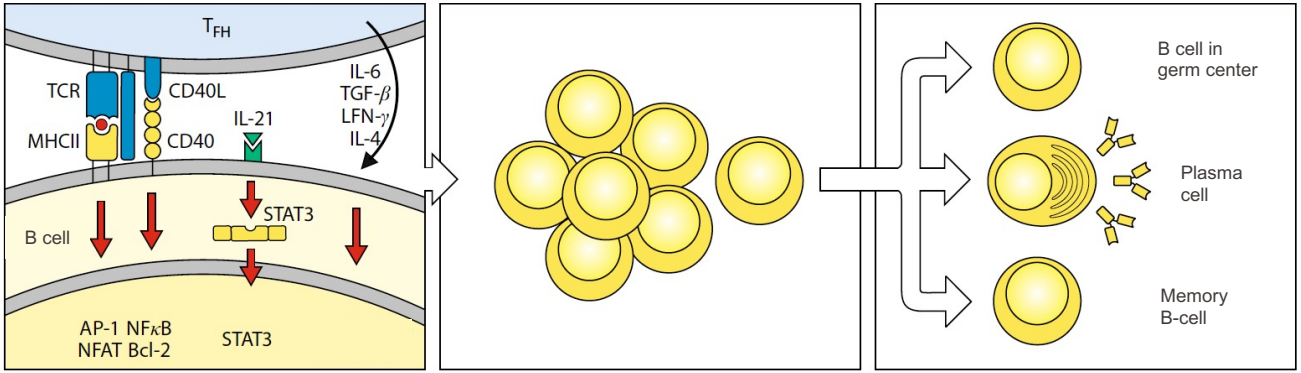
Anti-CTLA-4 Inhibitors:
Ipilimumab
Tremelimumab

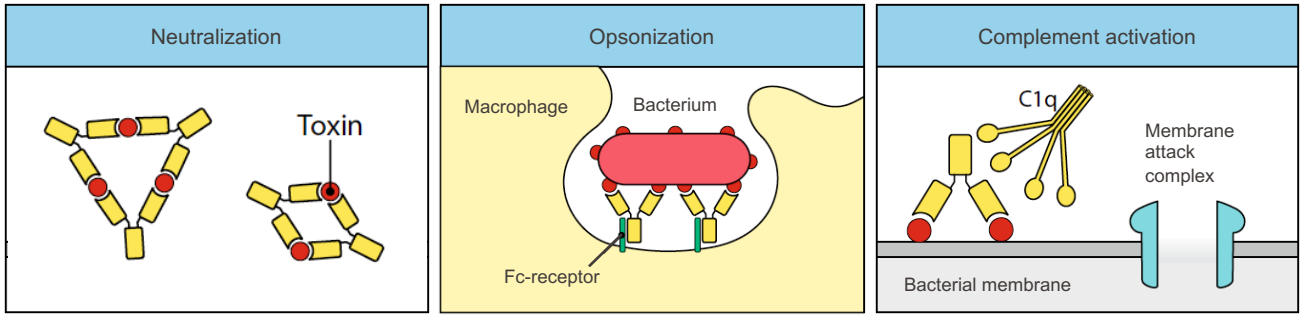
Anti-PDL1 Inhibitors:
Durvalumab
Avelumab
Atezolizumab

Anti-PD1 Inhibitors:
Nivolumab
Pembrolizumab
Tislelizumab
Sintilimab
Camrelizumab
Toripalimab
Spartalizumab
Penpulimab

Cancers **2021**, *13*(8), 1949

The humoral adaptive immune response

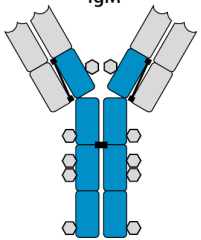




Murphy, K., & Weaver, C. (2018) Janeway Immunologie. 9th Edition (2018). Springer Berlin Heidelberg.

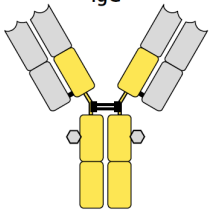
IgG is the most common antibody class

IgM



- First soluble immunoglobulin during immune reaction
- Usually low affinity
- Pentamer
  → avidity
  → complement activation

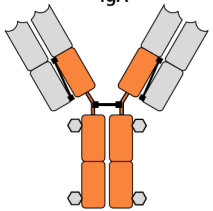
IgG



- Most prevalent human antibody
- Mediates immunity in extracellular space
- Requires class switch (IgM → IgG)

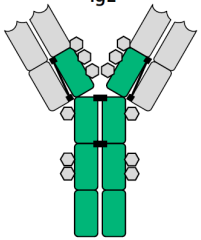
Therapeutic antibody format

IgA



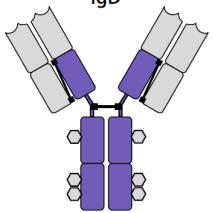
- Secreted antibody
- Mucosa-associated class (e.g. digestive tract)
- Can be monomer, dimer, or trimer

IgE



- Only low abundance
- Protection against parasites
- Associated with allergies

IgD



Funciton unclear

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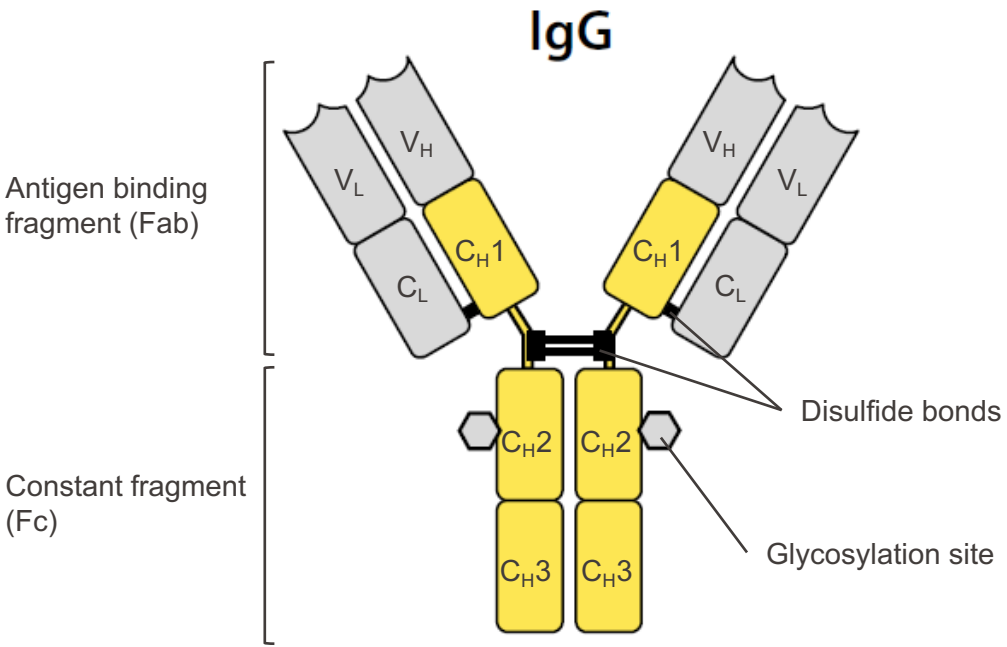
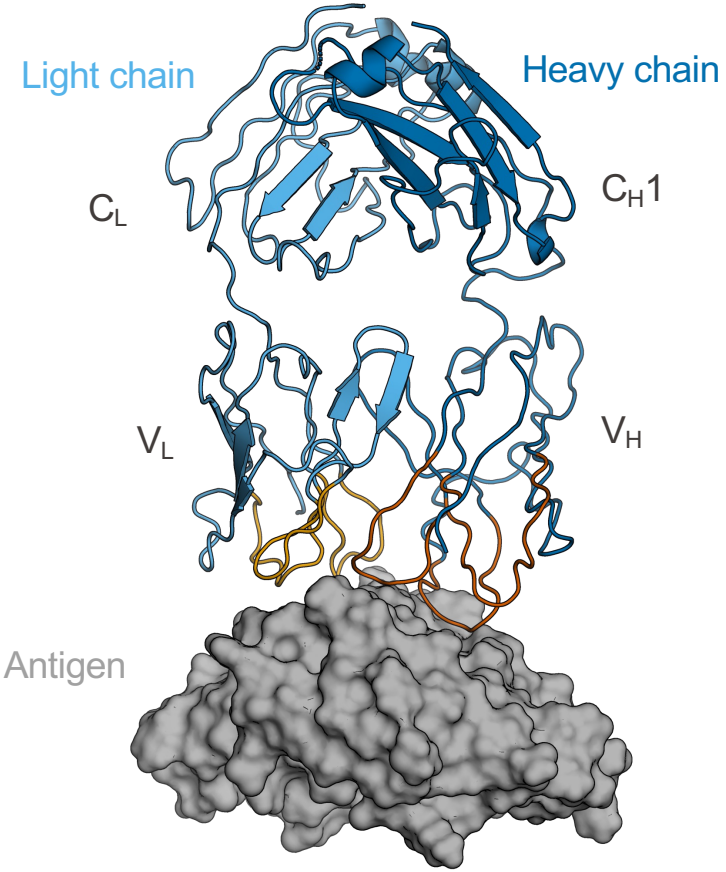
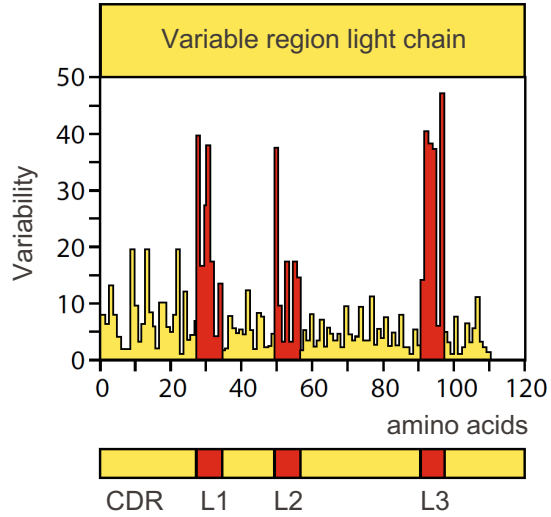
The structure of IgG

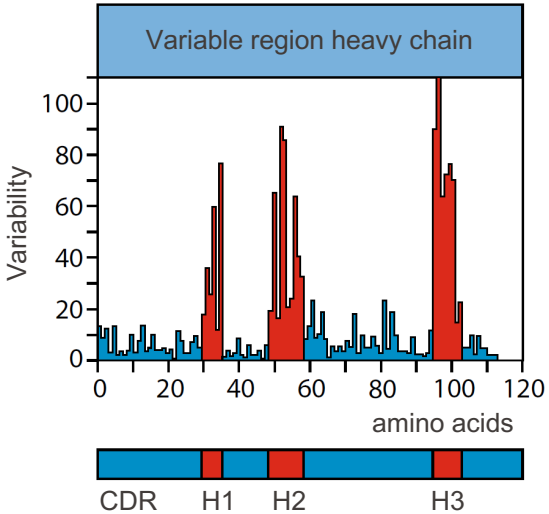


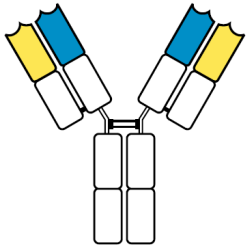
- IgG is a Y-shaped protein
- 2 light (L) and 2 heavy (H) chains
- Antibodies can be cleaved into antigen binding and constant fragments
- Constant (C) and variable (V) domains
- Stabilized by disulfide bnds
- Glycosylation on the Fc part

Fab: antigen binding









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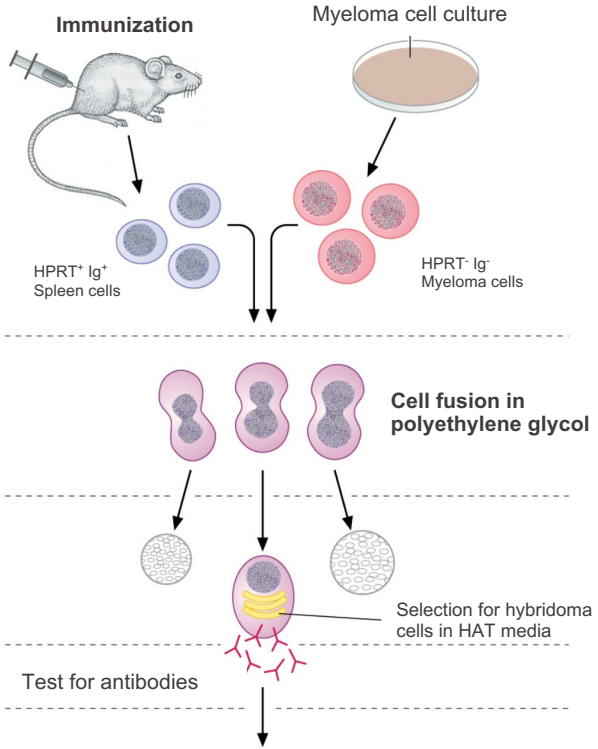
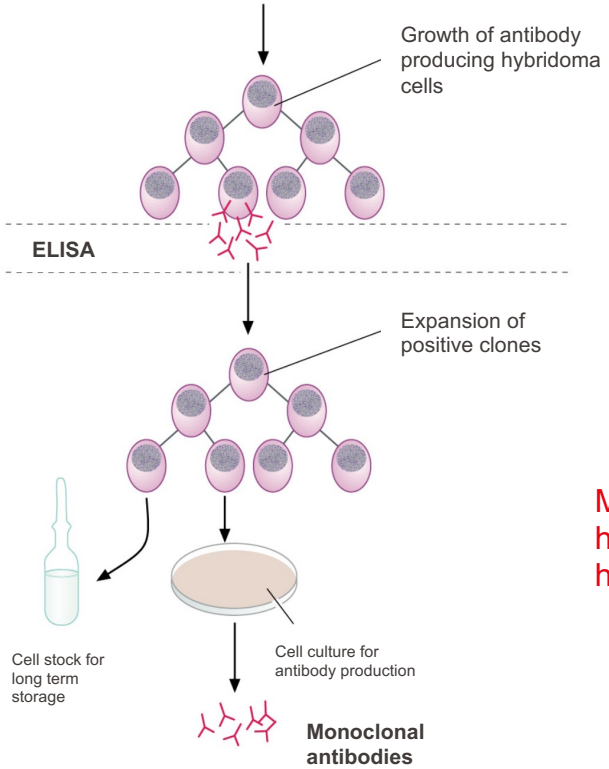
Monoclonal antibodies: Hybridoma technology

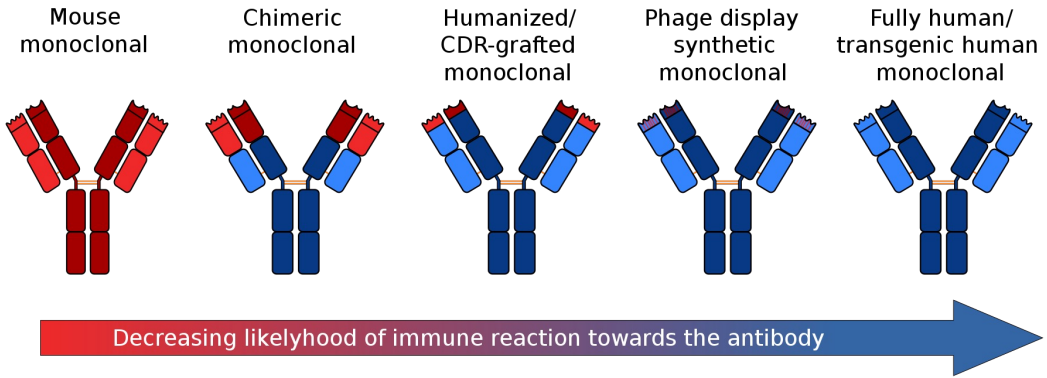




Murine antibodies are highly immunogenic to humans!

Köhler & Milstein, *Nature*, (1975), 495-497, 256(5517)

Humanization of therapeutic antibodies



- Chimeric: fusion of variable domains (e.g. from mouse antibody) with constant domains from human antibody
- Humanized: grafting (transferring) CDRs from mouse antibody to a human antibody
  - Selection of a human antibody closely matching the structure/sequence of the mouse antibody
  - Often results in reduced affinity (non-matching framework regions / vernier zones)
- Synthetic mAb: guided selection
- Humanization methods may be less important with the emergence of single B-cell sequencing for identification of fully human antibodies

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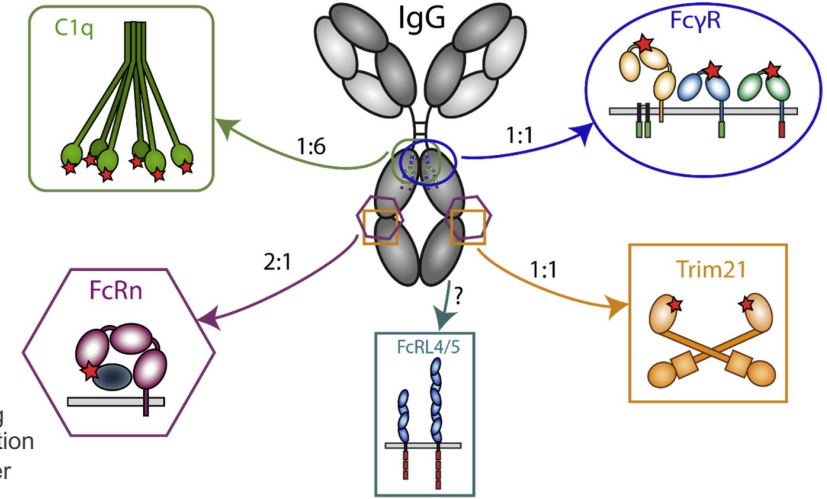
Beyond Antigen binding: Effector functions

CDC:
Complement dependent cytotoxicity
- Complement activation through binding of C1q
- Membrane attack complex lyses target cell (e.g. bacteria)



Fc receptors:
- Antibody dependent cellular cytotoxicity
- Antibody dependent cellular phagocytosis

Neonatal Fc receptor:
- Endosomal recycling → prolonged circulation
- Perinatal IgG transfer
- Transcytosis

ADIN:
Antibody dependent intracellular neutralization
- Recognition of internalized IgG
- Intracellular bacteria / virus
- Trim21 = E3 ubiquitin ligase → Ab mediated proteolysis

Fc like receptors:
- Present on B-, NK-, and T-cell (subsets)
- Regulation of humoral immune response?

Antibodies, (2019), 30, 8(2)

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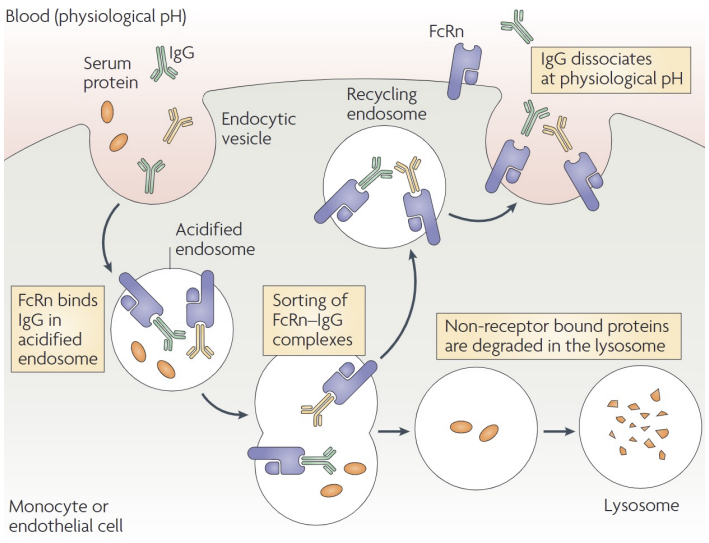
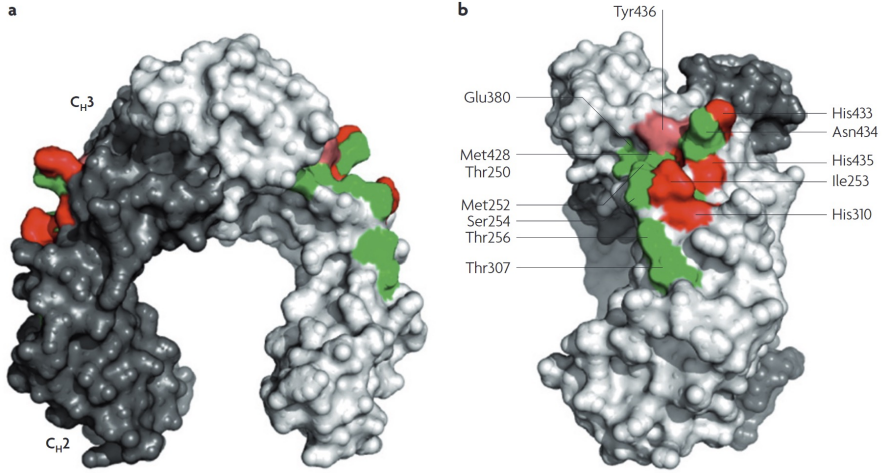
Prolonged circulation: Endosomal Recycling



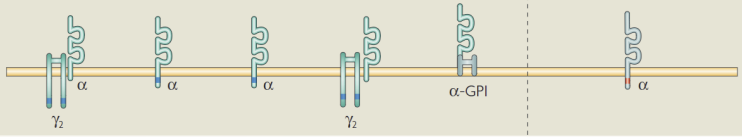


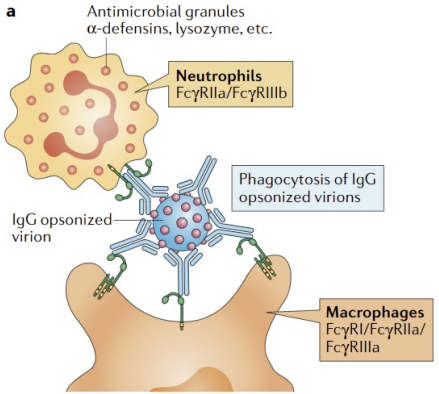
pH dependent binding of IgG Fc is mediated through histidine residues 310 and 435 located the C_H2 domain

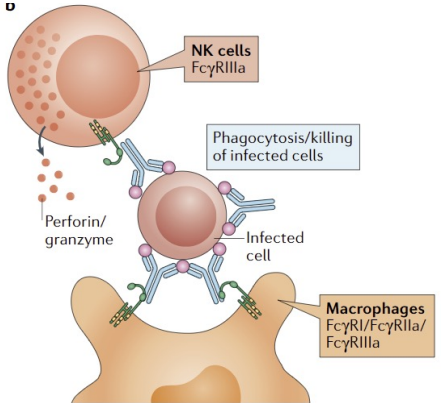
→ Most (but not all) antibodies, which can bind to FcRn have an increased half-life in blood plasma

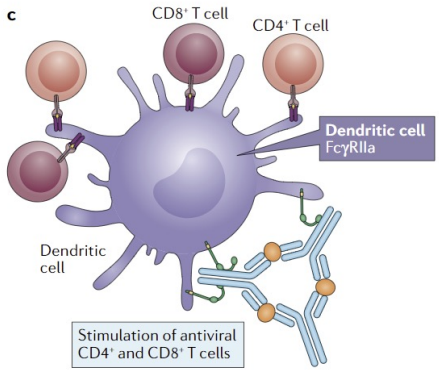
Nature Reviews Immunology, (2007), 715-725, 7(9)

Fc Receptors mediate effector funcitons

	Activating Fc receptors					Inhibitory Fc receptor
Human						
Structure						
Name	FcγRI	FcγRIIA	FcγRIIC	FcγRIIIA	FcγRIIIB	FcγRIIB
Affinity	High	Low to medium	Low to medium	Low to medium	Low to medium	Low to medium
Alleles		$Fc\gamma RIIA^{131H}$ $Fc\gamma RIIA^{131R}$		$Fc\gamma RIIIA^{158V}$ $Fc\gamma RIIIA^{158F}$	NA1 NA2	$Fc\gamma RIIB^{232I}$ $Fc\gamma RIIB^{232T}$







Nature Reviews Immunology, (2008), 34-47, 8(1). &. *Nature Reviews Immunology*, (2020), 633-643, 20(10)

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Choosing the right scaffold: IgG subtype

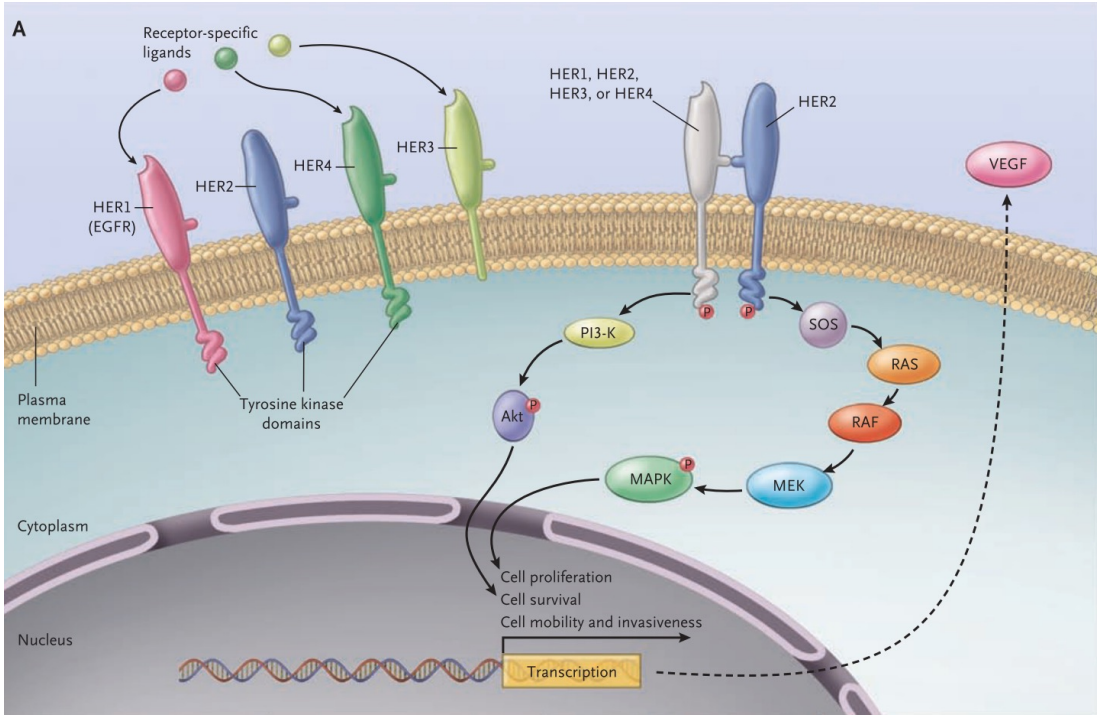
- Subtype of IgG will affect half-life of the therapeutic antibody
- Additional cysteines may cause tertavalent antibodies (IgG2)
- Chain exchange with in-vivo antibodies may lead to heterogenous antibodies (IgG4)
- Therpeutically relevant antibody subtypes: IgG1 and IgG3
- Subtype can affect effector function efficacy (e.g. IgG1 has lower CDC)

Table 1 Key features of the four IgG isotypes

	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)	28.6 ± 10.4 (13.0–50.2)	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.

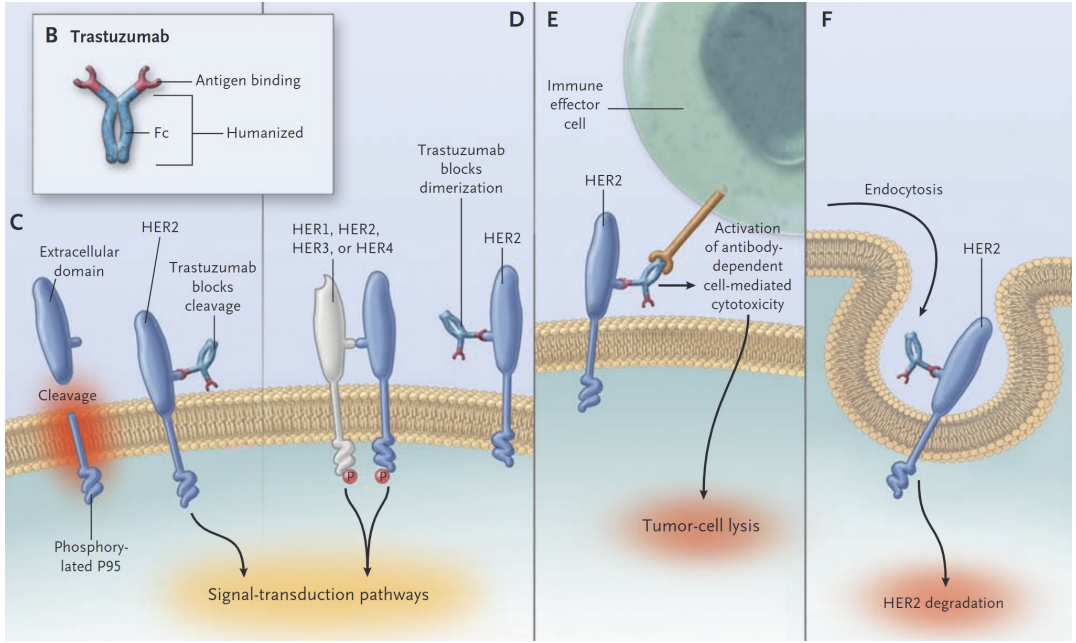
Breast cancer: HER2 overxpression



- HER2 overexpression in 20-30% of invasive breast cancer
- Overexpression results in ligand independent homo- and heterodimerization of EGFR → increased cell proliferation
- Downstream effect of HER2 signalling: VEGF expression → Increased angiogenesis

New England Journal of Medicine, (2007), 39-51, 357(1)

Trastuzumab (Herceptin)



- Humanized through CDR grafting
- First approved humanized antibody (Genentech)
- Plasma half-life ~5.8 days
- Only 1 case of HAHA (human-anti-humanized-antibody) response in 903 patients

Mechanism of Trastuzumab

- Prevents shedding of HER2
- Blocks dimerization
- Induction of ADCC
- Clustering induces receptor internalization via endocytosis

New England Journal of Medicine, (2007), 39-51, 357(1)

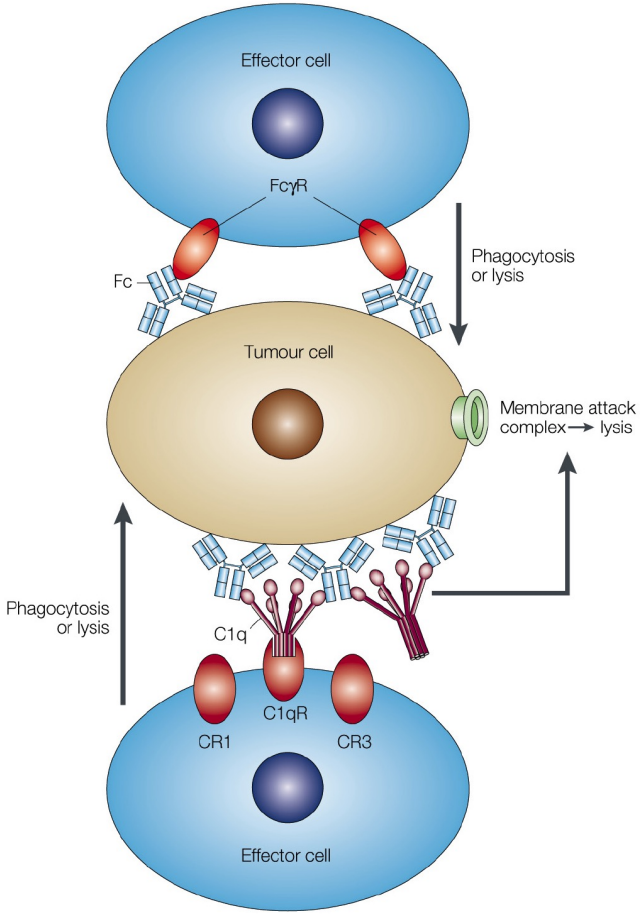
Low success of mAbs in Tumor Therapy

- Deplenished immune cell population in patients after chemotherpay / radiation
- Immunosuppressive microtumor environment

→ reduced ADCC

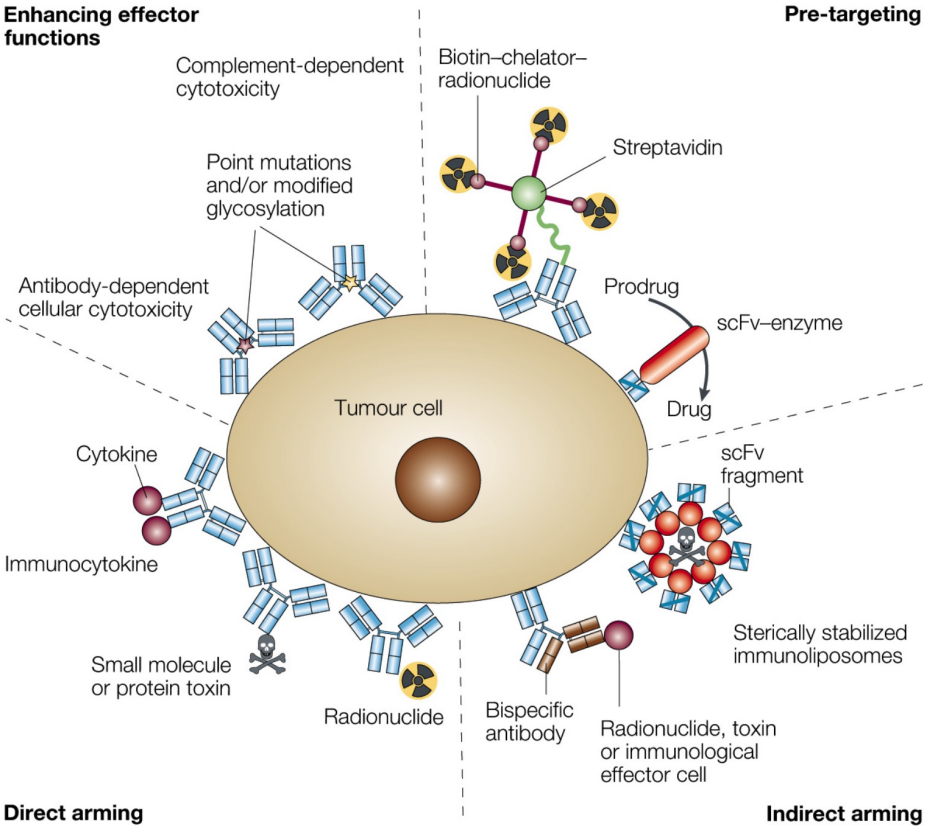
- Expression of inhibitory proteins (CD46, CD55, CD59) on tumor cells

→ reduced CDC

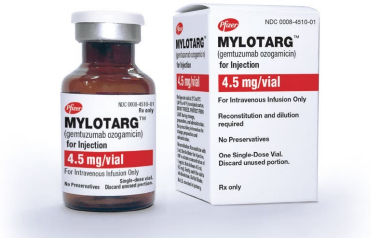


How can we enhance efficacy of natural antibodies?

Therpeutic antibodies: Armed Antibodies



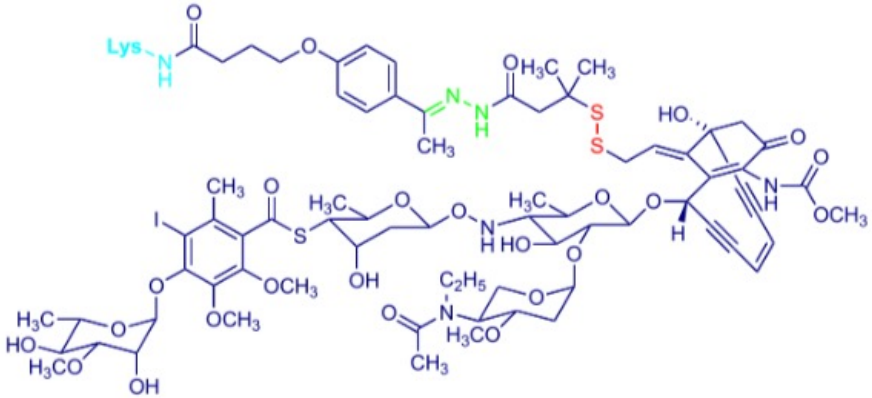
Mylotarg: Toxin armed mAb



- mAb humanized from mouse
- Target: CD33 (acute myeloid leukemia)

- Antibody conjugated with Calicheamicin (bacterial toxin)
- NHS coupling to lysine (on average 2-3 toxin molecules per mAb)
- After internalization of the ADC Calicheamicin breaks DNA → Apoptosis

Antibody Engineering of armed antibodies involves intensive linker chemistry!



Biologically instable linker:
1) Disulfide bridge: reduction upon internalization
2) Hydrazone: hydrolysis under acidic conditions

Development of a radioactive mAb

- Disease: B-cell non-Hodgkin's lymphoma
- Target: CD20 (B-cells)

Ibritumomab: murine mAb targeting CD20

Do we need to humanize the mouse antibody?

→ **No, we will eradicate all immune cells with this antibody – so there can not be a immune response!**

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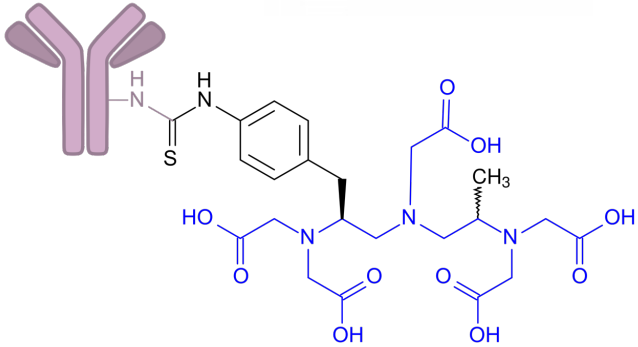
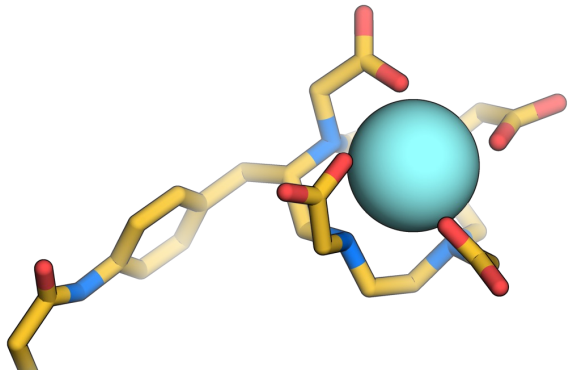
Zevalin:
^{90}Yttrium Ibritumomab Tiuxetan

- Coupling of a chelating agent (Tiuxetan, DTPA derivate) to Ibritumomab
- Tiuxetan can bind multiple isotopes:
  - $^{111}In^{3+}$ (diagnosis)
  - $^{90}Y^{3+}$ (therapy, $t_{1/2}$ = 64 h)
- $^{90}Y^{3+}$ will penetrate solid tissue (range of about 200 cells)
- Most successful therapeutic antibody
- Comparison to non-armed antibody equivalent:
  80/56% overall response, 30/16% complete remission

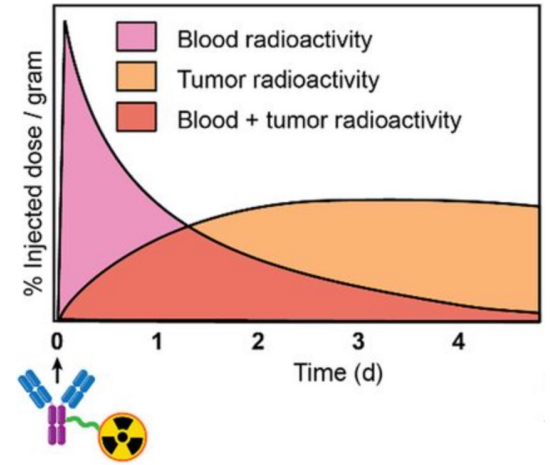


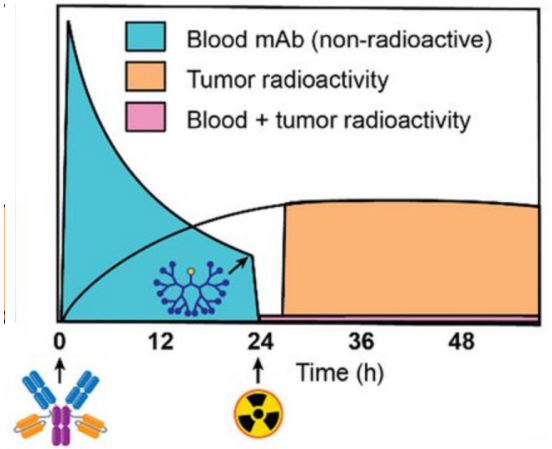


Pretargeting: Reducing Side Effects

- Strategy to reduce the exposure of healthy tissue to toxic or radioactive compounds of armed antibodies
- Administration of targeting antibody without toxin or radionuclide
- Independent administration of low molecular weight toxin / readionuclide leads to accumulation at the target site

- Pretargeting antibody is required to remain on the surface of the cell (Endocytosis!)
- Difficult pharmacokinetics (two-component system)





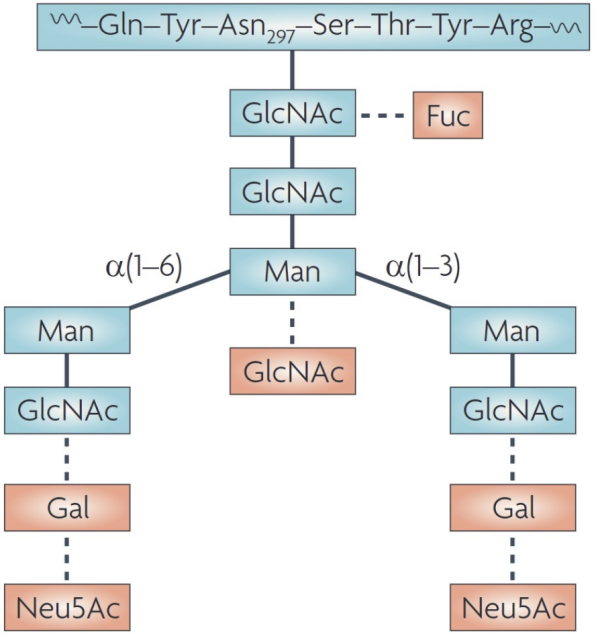
New England Journal of Medicine, (2007), 39-51, 357(1)

Glyco-Engineering: Enhancing natural effector functions

- IgG carries glycolylation at Asn297 in the Fc part
- Glycosylation is of complex diantennary type
- 20% of IgG glycosylated, about 128-512 glycoforms!
- Glycosylation is dependent on the enzyme repertoire of the producing cell

- terminal α-2,6-linked sialic acid
  → anti-inflammatory activity
- CHO, NS0, Sp2/0 cells: >90% fucosylated IgG
  → decreased ADCC

- Roche GlycoMAB: Cell line for recombinant expression of non-fucosylated antibodies



mAbs, (2012), 419-425, 4(4) & Nature Reviews Drug Discovery, (2009), 226-234, 8(3)