Monoclonal antibodies

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Disclosures – Sattva Neelapu, M.D.

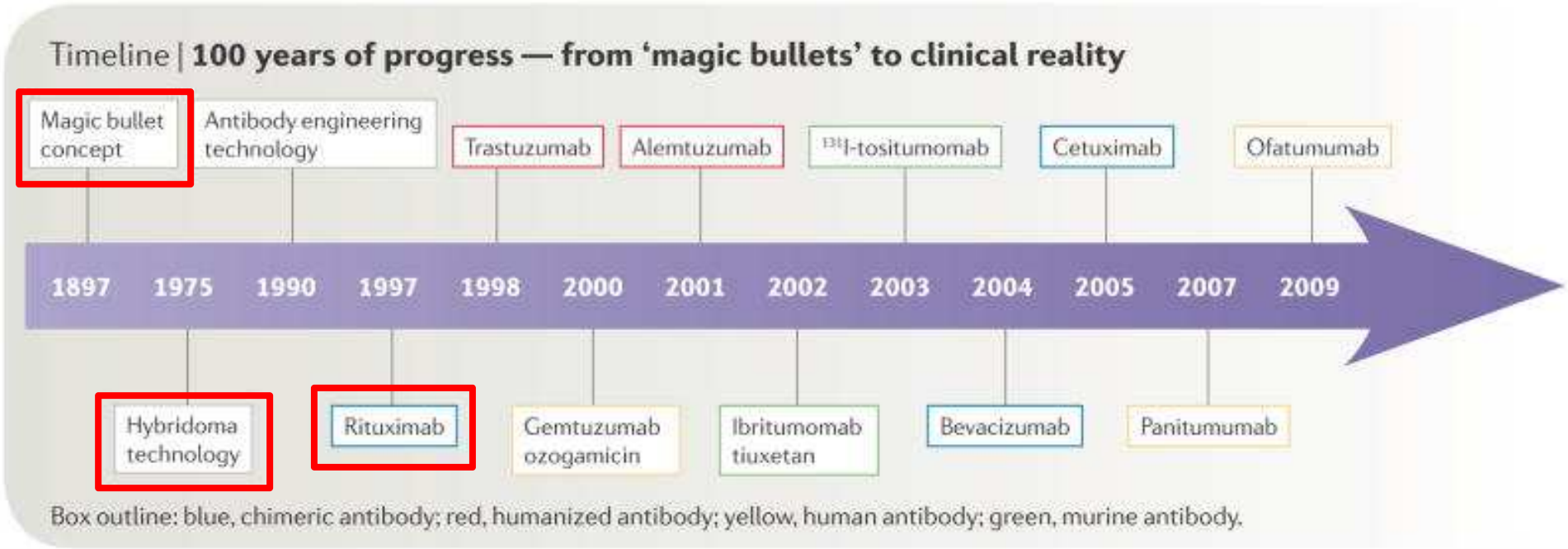
The following relationships exist related to this presentation:

- Received research support from BMS and Merck
- Consultant for Celgene

Outline

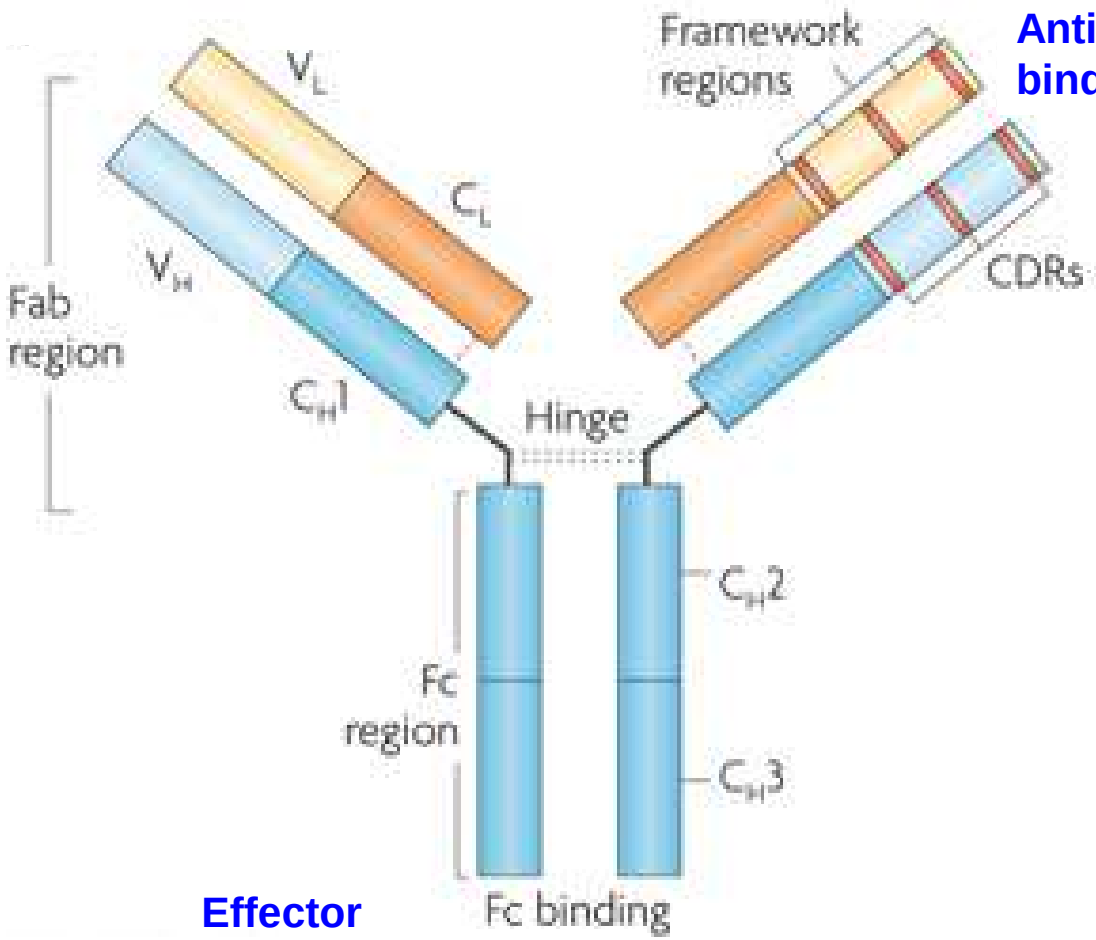
- Antibody structure and IgG subtypes
- Types of monoclonal antibodies (Mab)
- Methods of Mab production
- Mechanisms of action of Mab
- Role of Fc gamma receptors
- Improving the efficacy of Mab

Therapeutic monoclonal antibodies



Weiner LM, et al. *Nature Reviews Immunology* 2010;10(5):317-327.

Antibody structure



Fab – Fragment antigen-binding

Fc – Fragment crystallisable

V – Variable region

C – Constant region

H – Heavy Chain

L – Light chain

FWR – Framework regions

CDR – Complementarity determining region

Hansel et al, *Nature Rev Drug Discovery*, 2010: 9:325

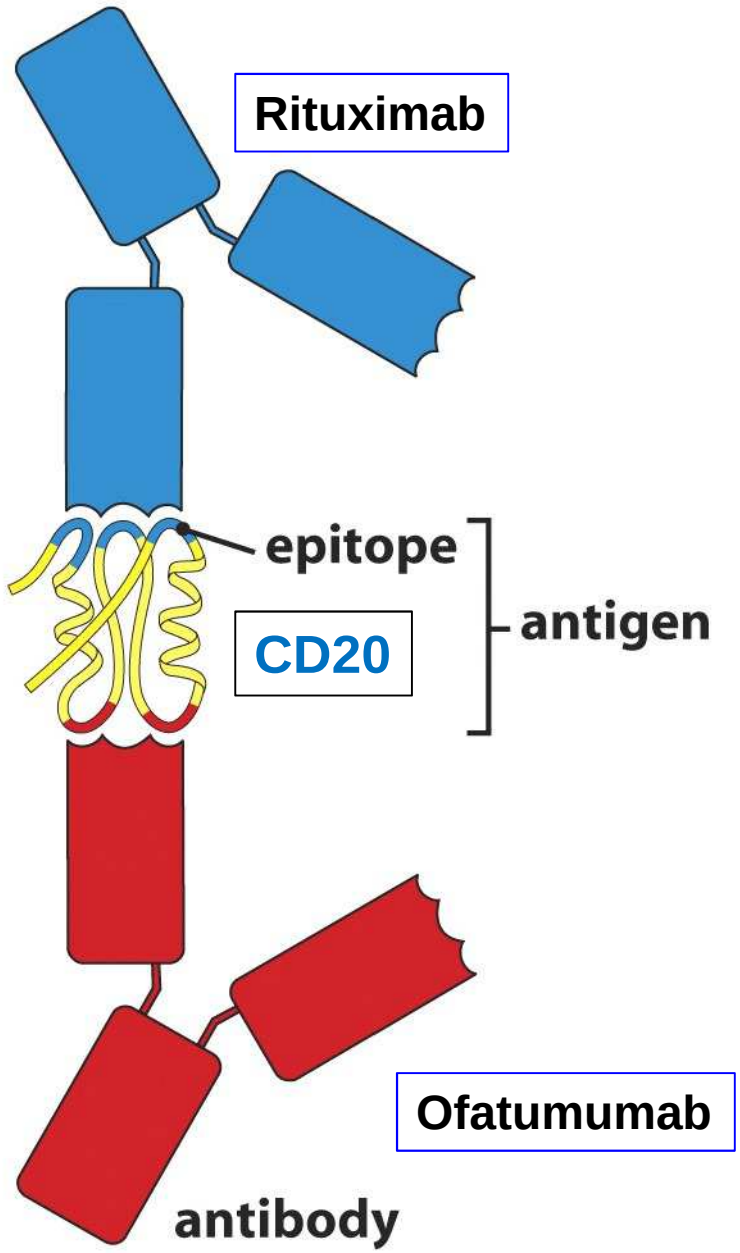
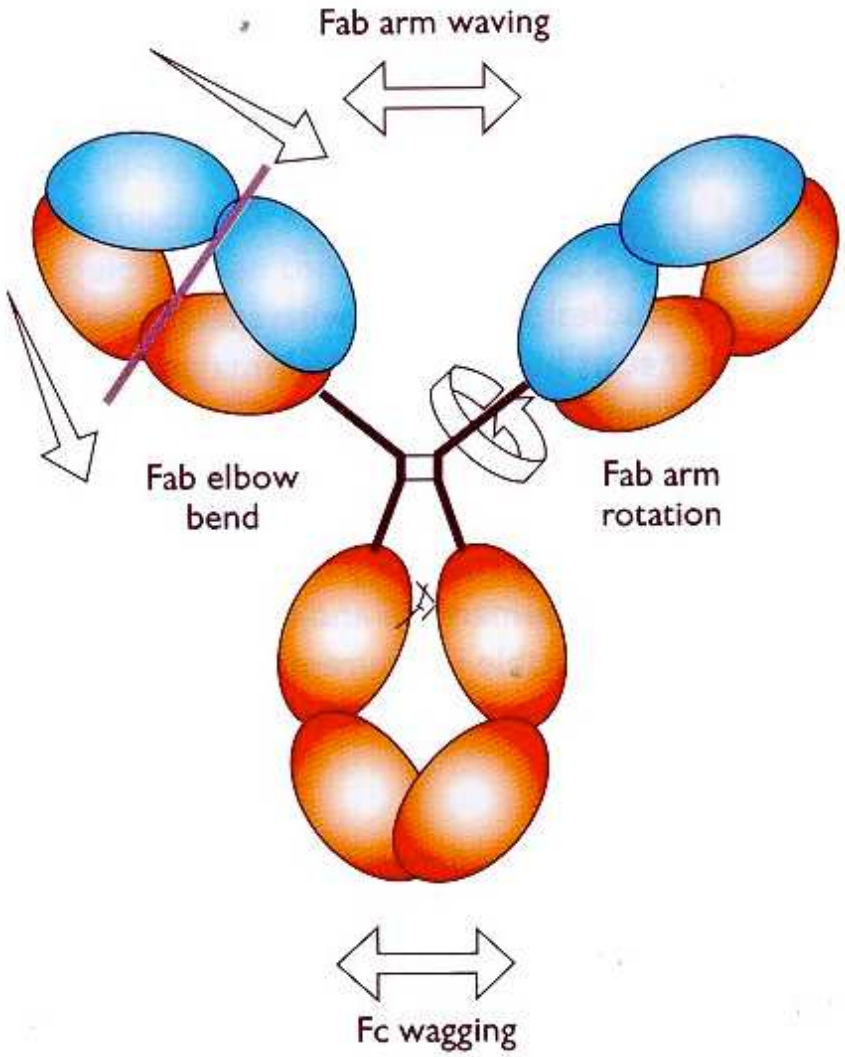


Figure 1.15 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Flexibility of human IgG



Antigen-binding

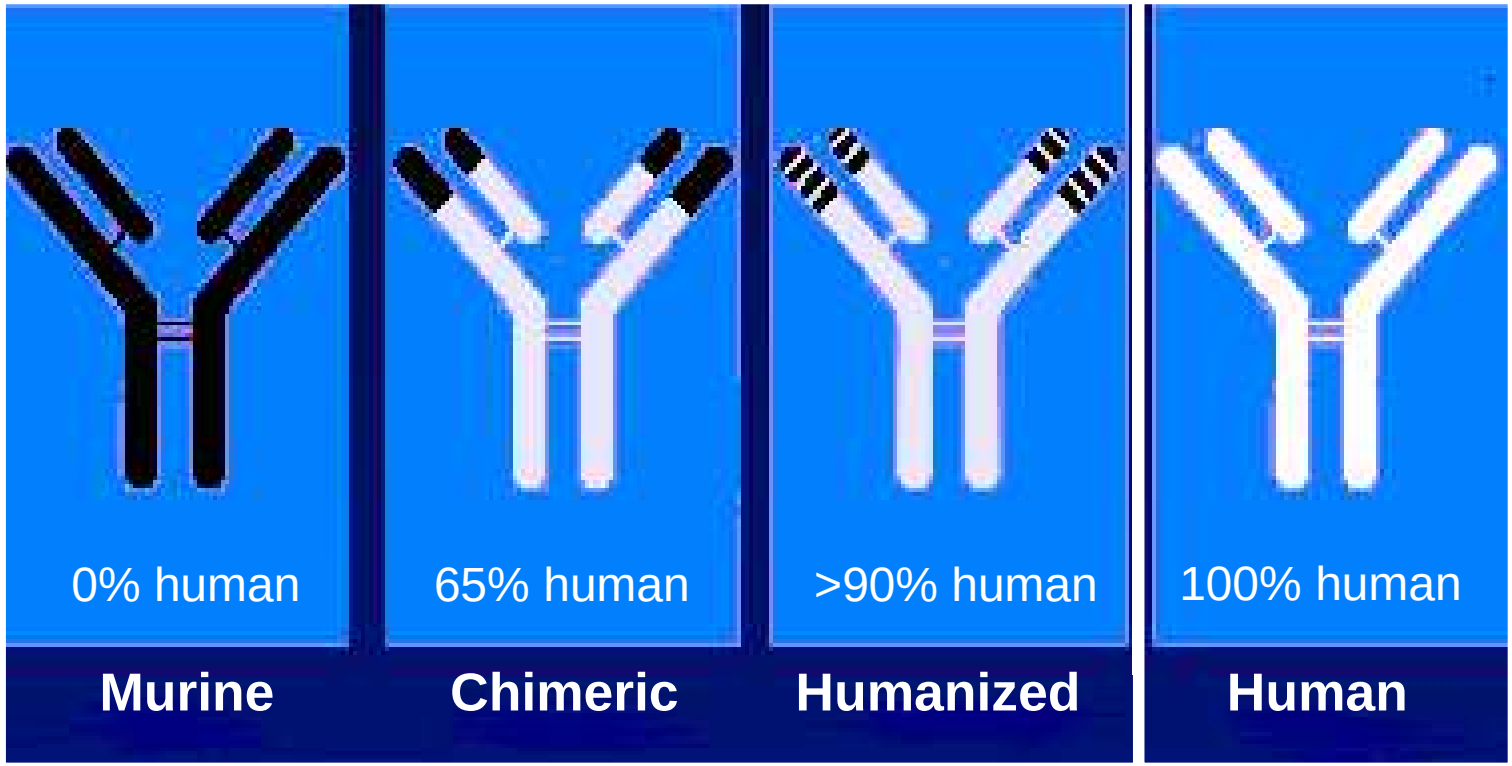
Effector function

Immunology Today, 1995

Properties of human IgG subclasses

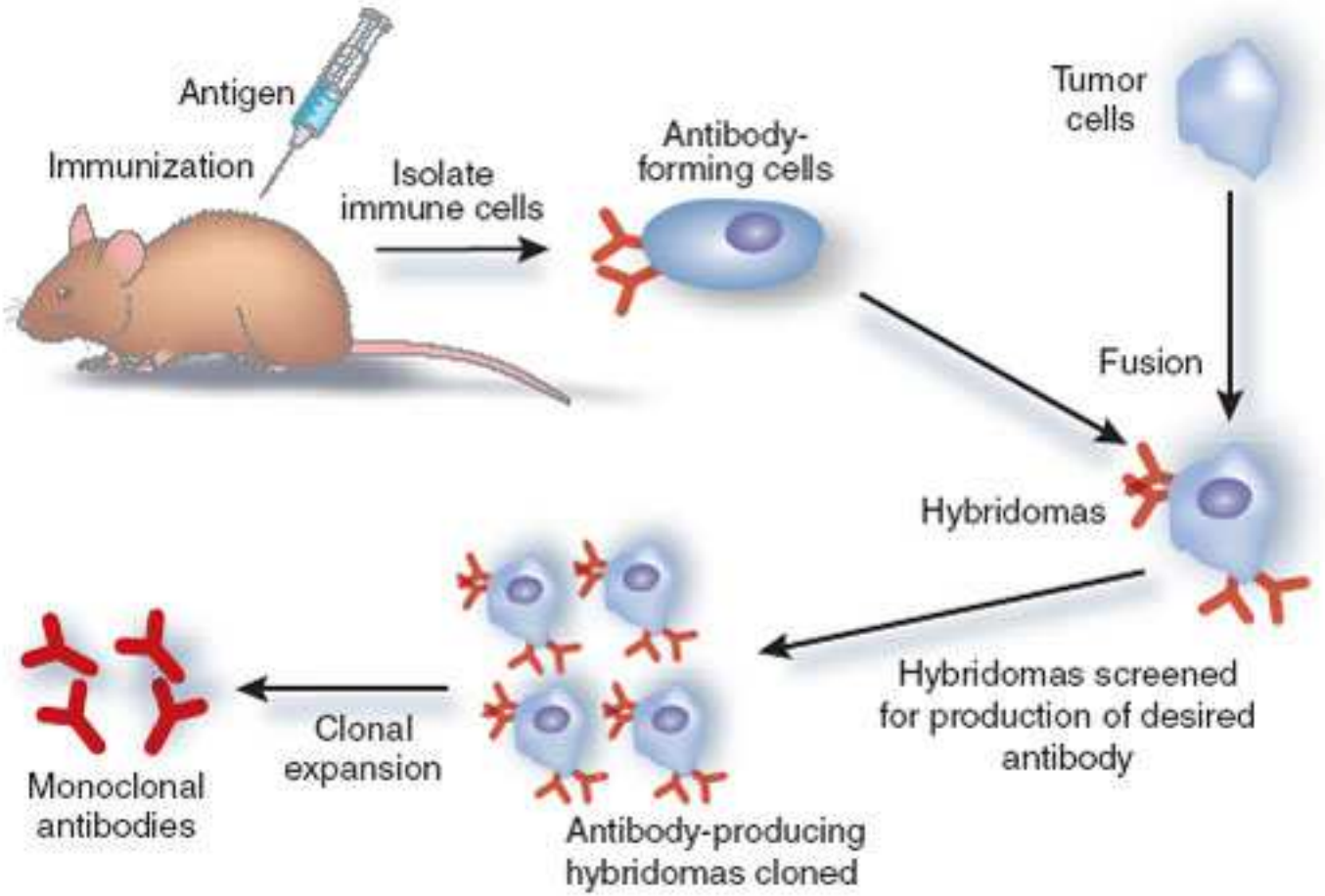
Property	IgG1	IgG2	IgG3	IgG4
Molecular mass (kD)	146	146	170	146
Amino acids in hinge	15	12	62	12
Disulfide bonds in hinge	2	4	11	2
Susceptibility for proteolytic enzymes	High	Low	Very high	Low
Half-life (days)	21	21	7	21
Antibody response	Proteins	Polysacch-aride	Proteins	Allergen
Complement activation	High	Low	Very high	None
Binding to Fc Receptors	High	Very low	High	Low

Types of monoclonal antibodies in clinic



Generic suffix	-**o**mab	-**xi**mab	-**zu**mab	-**u**mab
Examples	Ibriitum**o**mab Tositum**o**mab	Ritu**xi**mab Cetu**xi**mab	Obinutu**zu**mab Trastu**zu**mab	Ofatum**u**mab Ipilim**u**mab

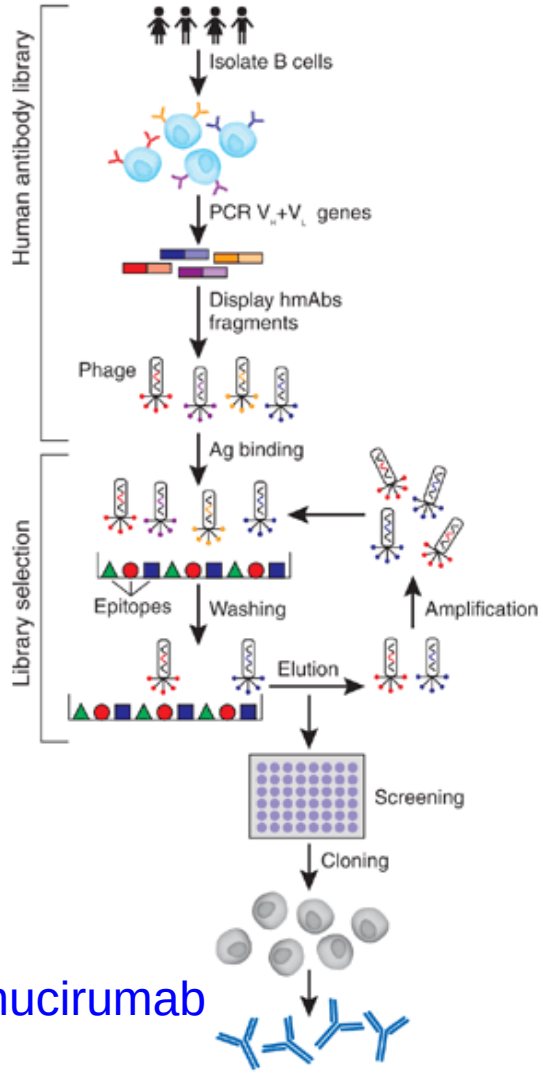
Mouse Mab production – Hybridoma approach



Michnick and Sidhu, Nature Chemical Biology **4**, 326 - 329 (2008)

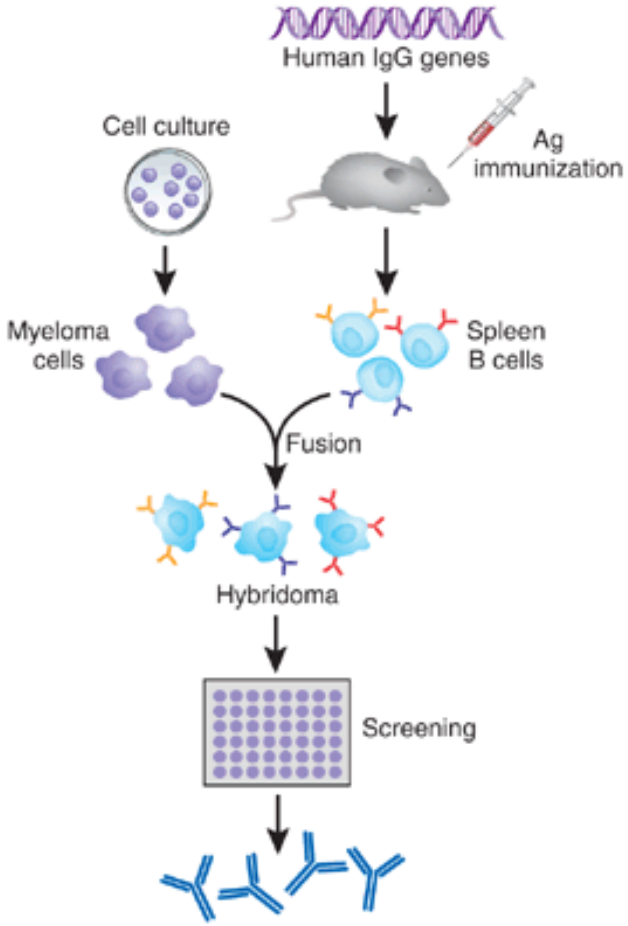
Human Mab production

Human phage Ab library



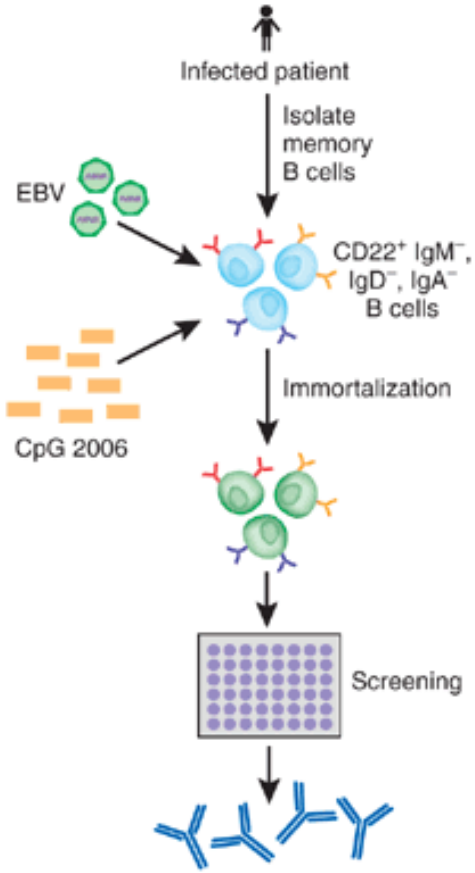
Ramucirumab

Transgenic mouse



Ipilimumab

Human memory B-cell immortalization



Abs to viral Ags

Marasco WA, Sui J. *Nat Biotechnol*. 2007 Dec;25(12):1421-34

Mechanisms of action of Mab

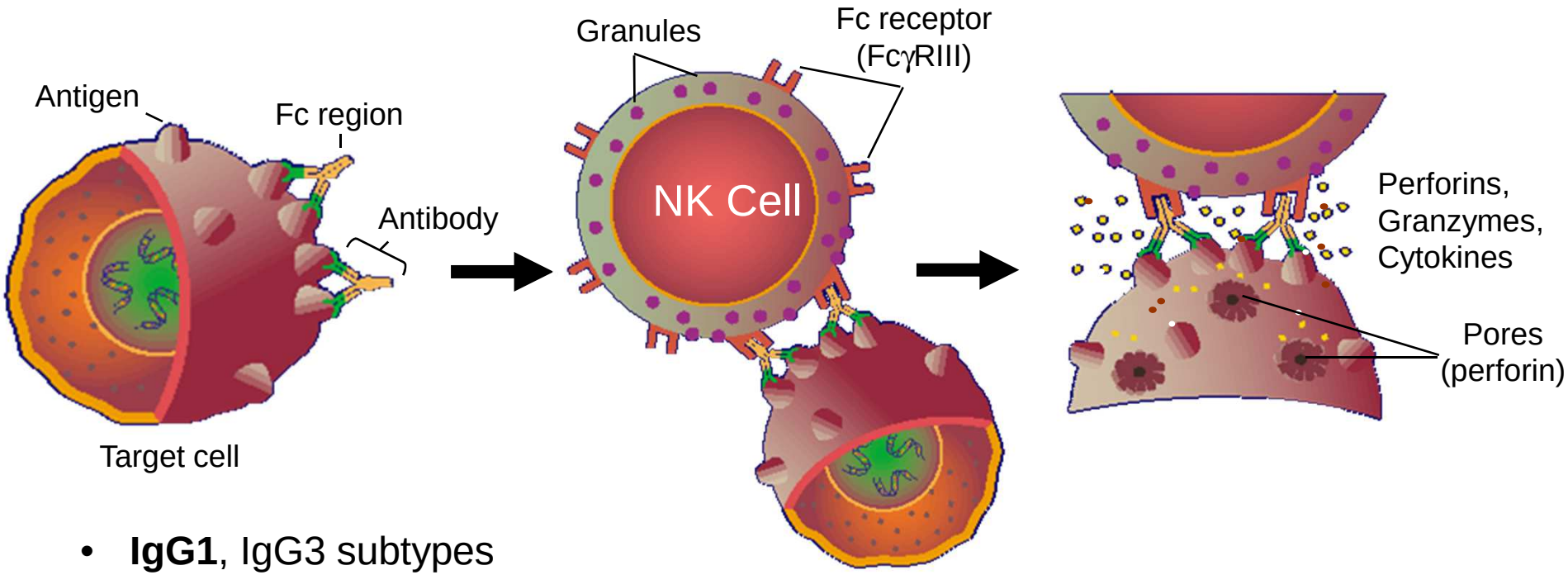
FC dependent

- Antibody-dependent cell-mediated cytotoxicity (ADCC)
- Antibody-dependent cellular phagocytosis (ADCP)
- Complement-dependent cytotoxicity (CDC)

Fc independent

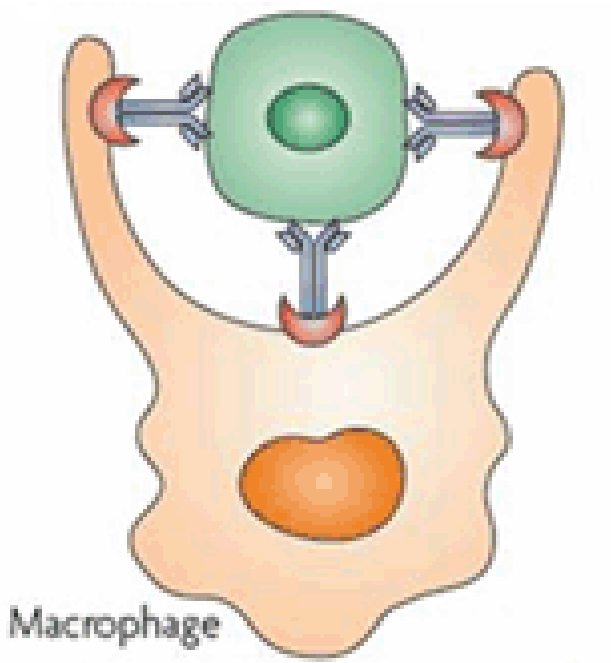
- Direct apoptosis
- Agonistic – induce signaling
- Antagonistic – block receptor-ligand interaction

ADCC



- **IgG1**, IgG3 subtypes
- Mediated by NK cells, macrophages, neutrophils
- Binding to Fc gamma receptor (FcγR) requires Fc glycosylation
  - Increase ADCC by modifying Fc glycosylation
  - Decrease ADCC by Fc deglycosylation
- Eg: Rituximab, trastuzumab

ADCP

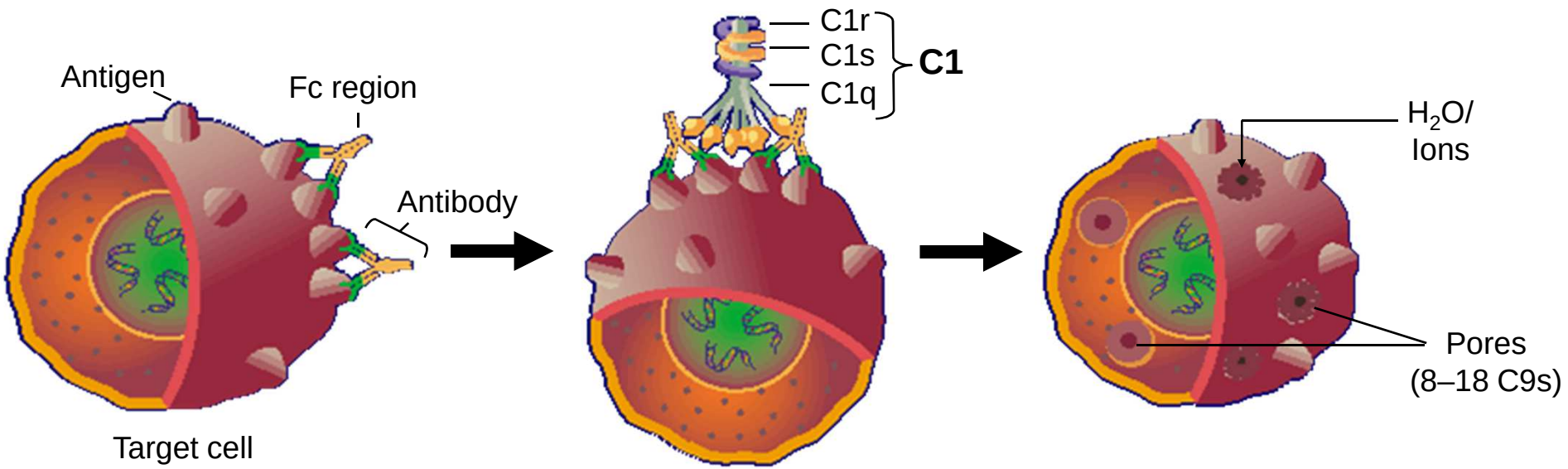


Fc-dependent phagocytosis and lysosomal degradation of tumour cell

- **IgG1**, IgG3 subtypes
- Mediated by monocytes / macrophages
- Binding to FcγR requires Fc glycosylation
  - Increase ADCP by modifying Fc glycosylation
  - Decrease ADCP by Fc deglycosylation
- Eg: Rituximab

Weiner LM, et al. *Nature Reviews Immunology* 2010;10(5):317-327.

CDC

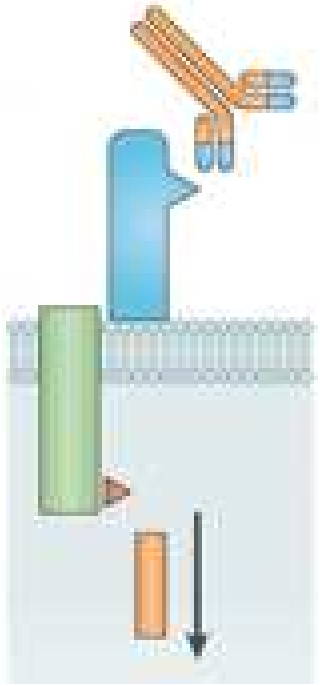


- **IgG1**, IgG3 subtypes
- Requires antibody cross-linking
- C1Q polymorphisms in humans affect efficacy
- Increase CDC by slowing dissociation from the traget
- Eg: Ofatumumab has greater CDC activity than rituximab

Agonist (Signaling)

Signaling

IgG4



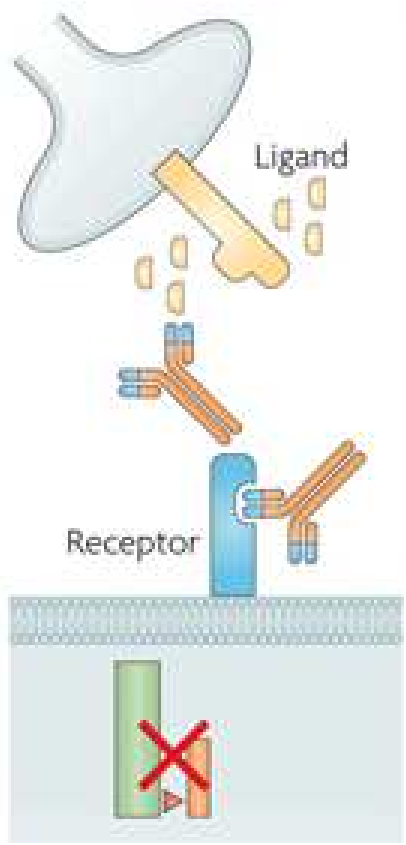
- **IgG4** subtype
- Fc function not desirable (usually use IgG4)
- Eliminate ADCC by decreasing Fc glycosylation
- Increasingly used in cancer immunotherapy
- Eg: Urelumab – agonistic antibody for 4-1BB (CD137)

Hansel et al, *Nature Rev Drug Discovery*, 2010: 9:325

Antagonist (Blocking)

Blocking

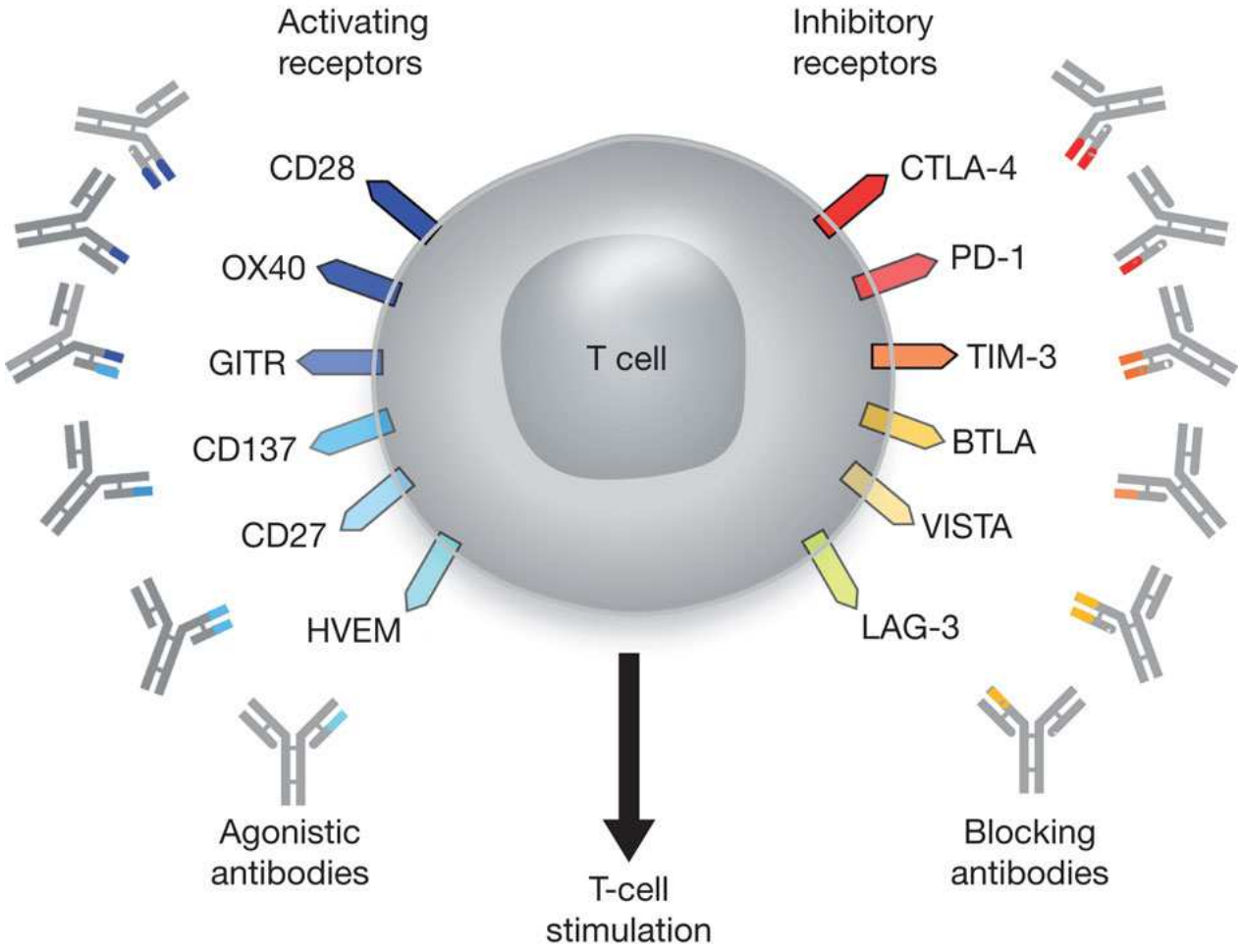
IgG4



- **IgG4** subtype
- Can be used to block receptor or the ligand (cell surface like PD-L1 or soluble like TNF-α)
- Fc function not desirable (usually use IgG4)
- Eliminate ADCC by decreasing Fc glycosylation
- Eg: Nivolumab and pembrolizumab block PD-1
- Eg: Panitumumab blocks EGFR

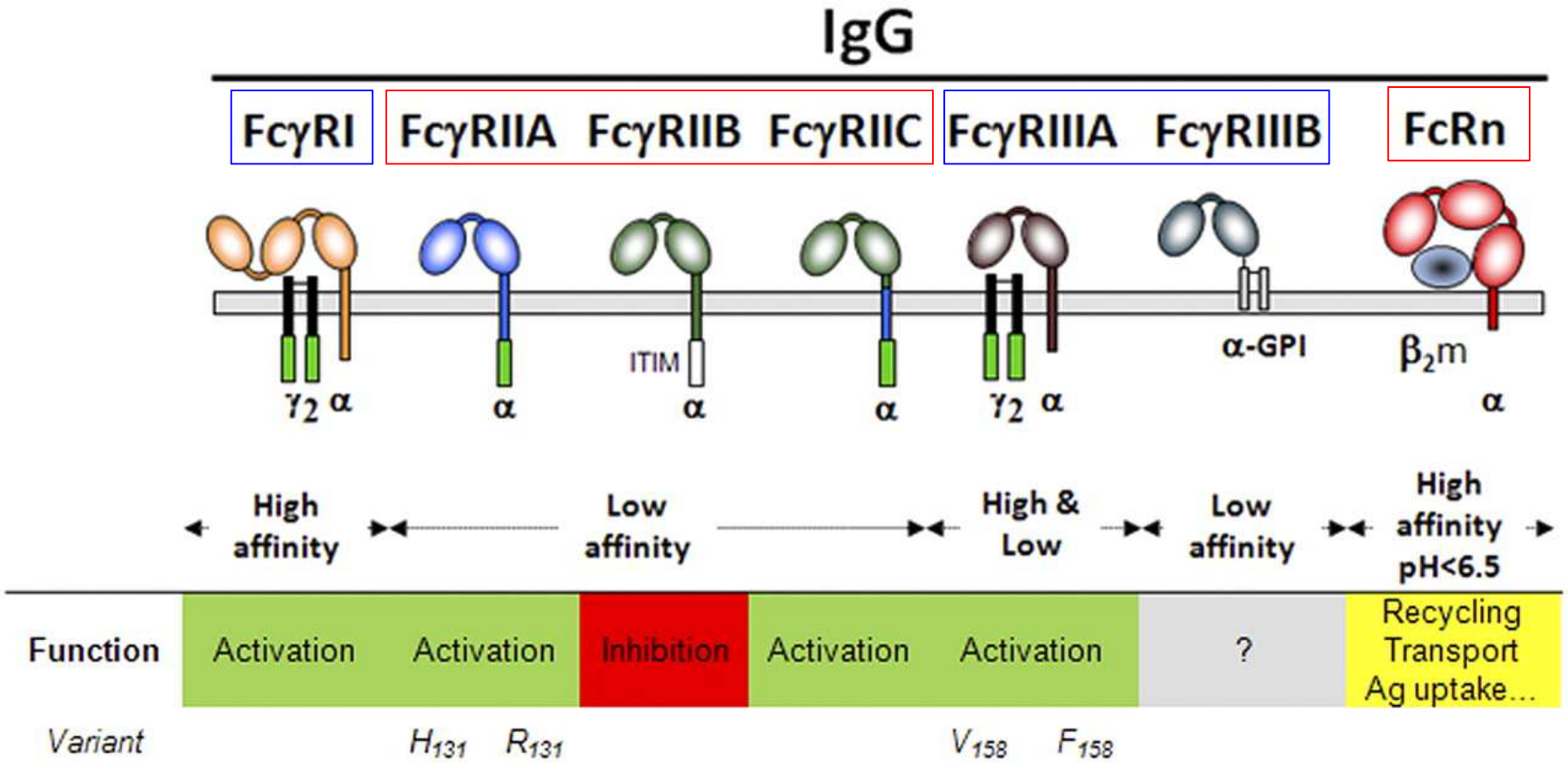
Hansel et al, *Nature Rev Drug Discovery*, 2010: 9:325

Agonist and Antagonist Mabs in development



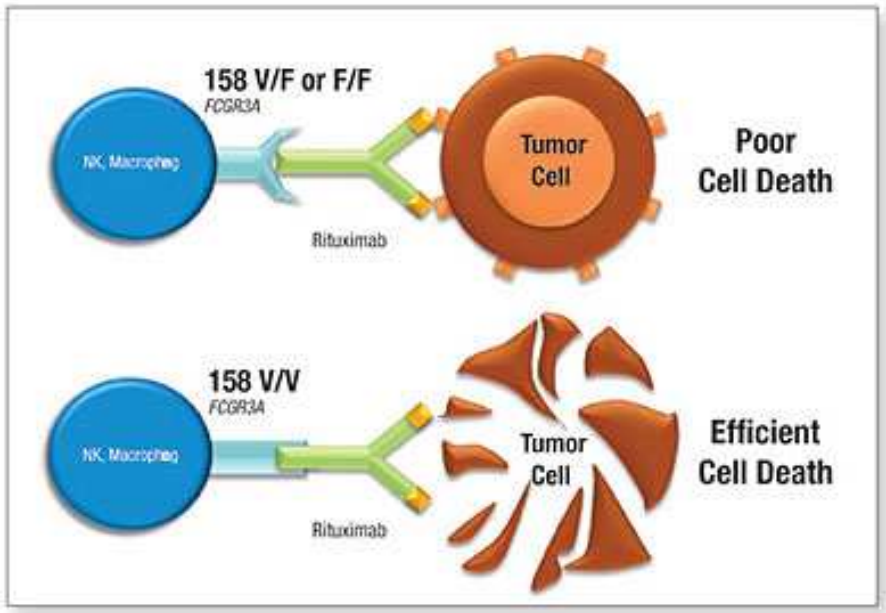
Mellman et al, *Nature*, 2011

Human IgG Fc receptors

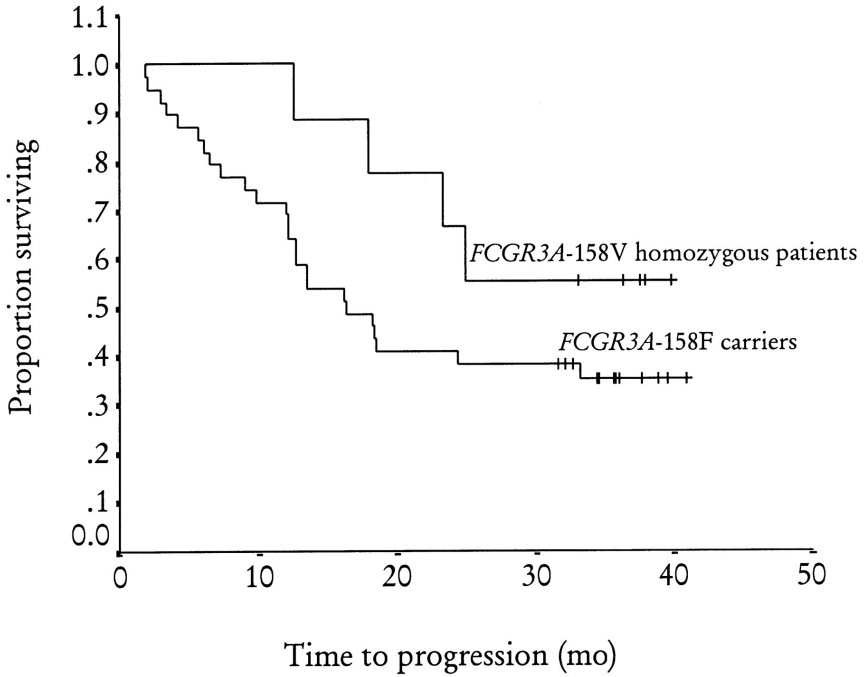


Bruhns P *Blood* 2012;119:5640-5649

Impact of *FCGR3A* polymorphism



Dall'Ozzo S, et al. Cancer Research, 2004; 64:4664-4669.



Cartron G et al. *Blood* 2002;99:754-758

Improving efficacy of Mab

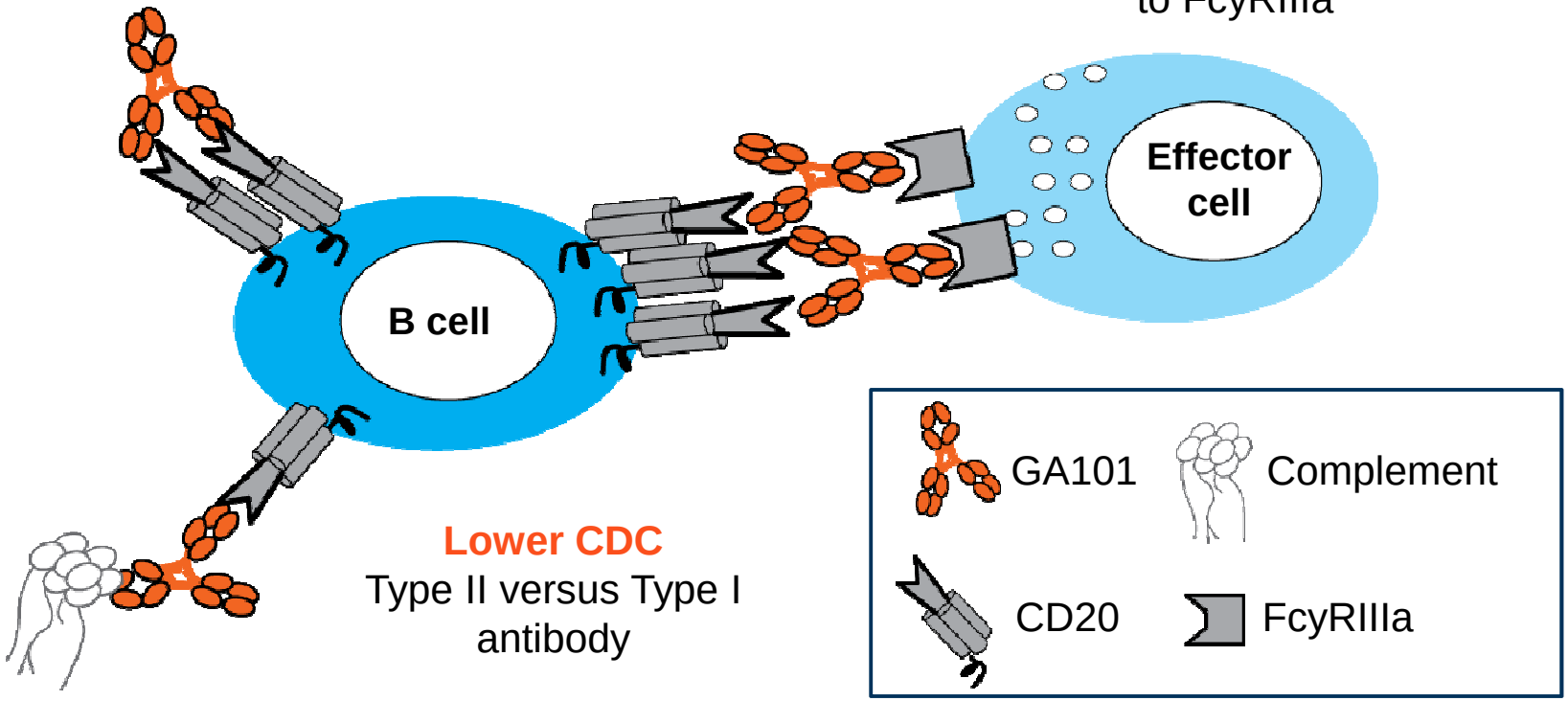
- Increase ADCC and ADCP by glycosylation
- Direct arming of Mab
  - Radioactive isotope (Radioimmunotherapy)
  - Small molecule (Antibody drug conjugates - ADC)
  - Cytokine (Immunocytokine)
- Indirect arming of Mab
  - Bispecific antibodies (BiSpecific T-cell Engager – BiTE)
  - Chimeric antigen receptors (CAR T cells)
  - Immunoliposomes

Glycoengineered Mab – Obinutuzumab (GA101)

Anti-CD20 Mab

Increased Direct Cell Death
Type II versus Type I antibody

Enhanced ADCC
Glycoengineering for increased affinity to FcγRIIIa

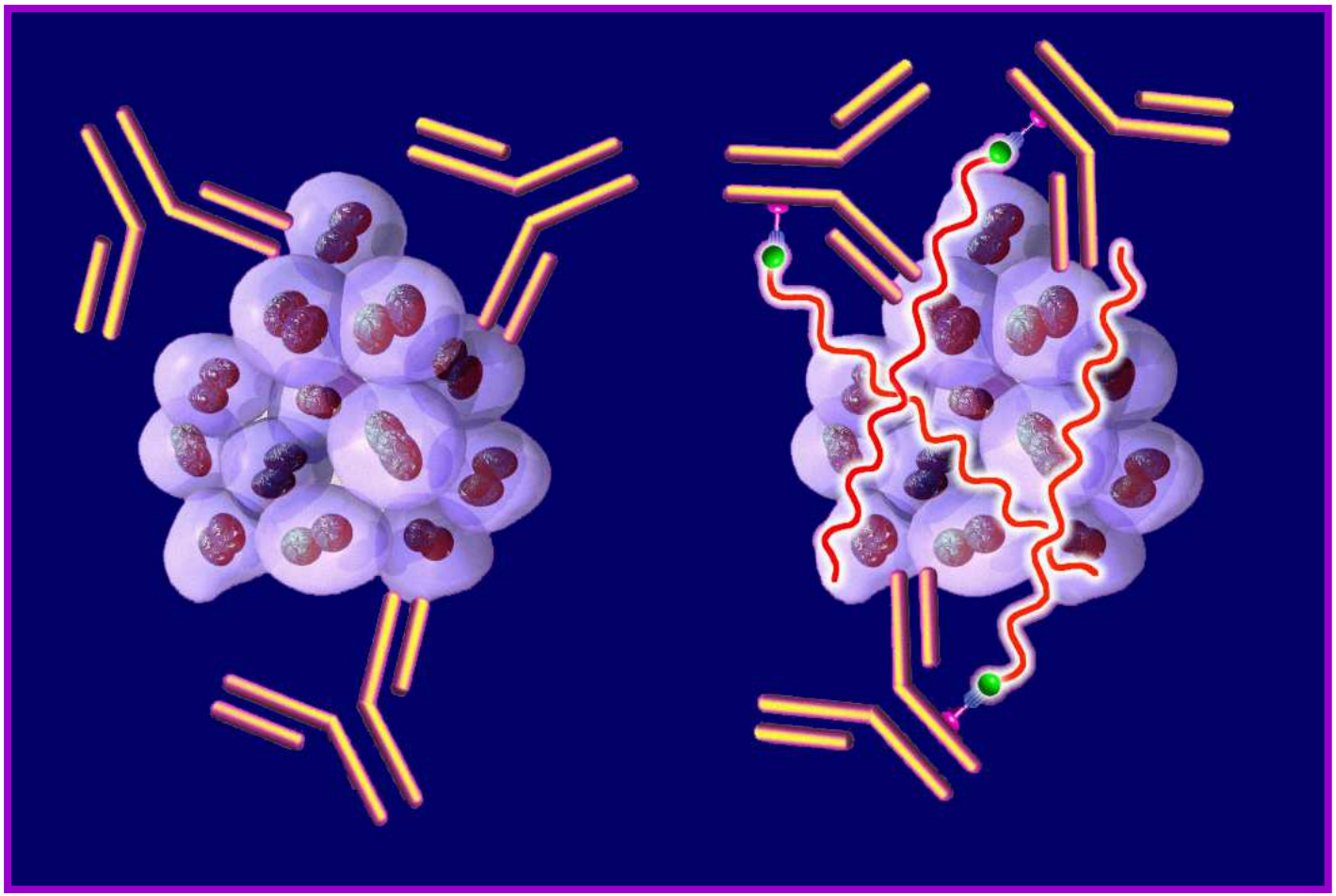


Lower CDC
Type II versus Type I antibody

Goede V et al. *Proc ASCO* 2013; Abstract 7004

Radiolabeled Mabs produce a Crossfire Effect

Naked Mab **Radiolabeled Mab**



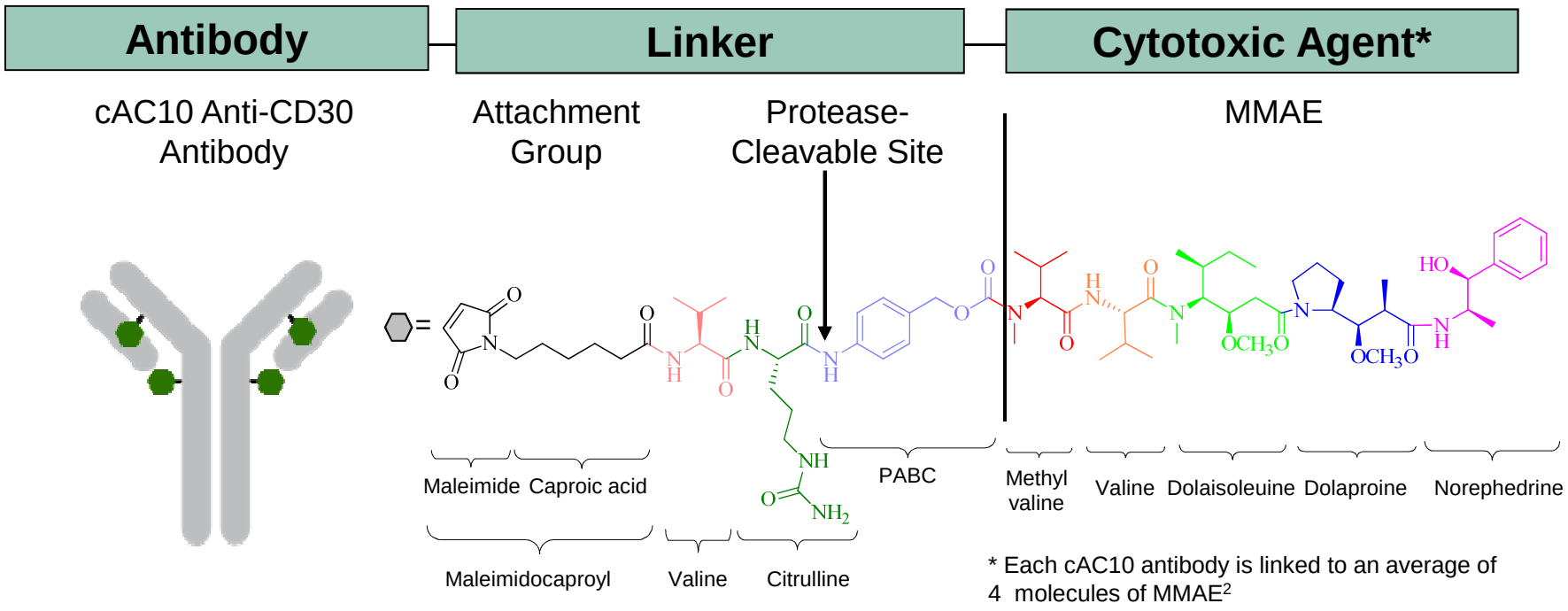
Examples:

- Yttrium 90 ibritumomab tiuxetan
- Iodine 131 tositumomab

Illidge et al. *Br J Haematol*. 2000;108:679

ADC – Brentuximab vedotin

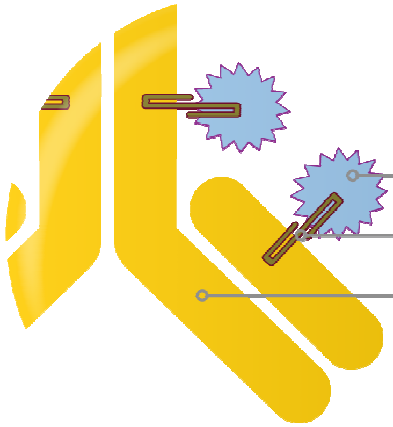
Brentuximab vedotin – designed to deliver antimicrotubule agent to CD30+ cells



* Each cAC10 antibody is linked to an average of 4 molecules of MMAE[2]

1. Doronina SO et al. *Nature Biotechnology.* 2003; 12(7):778-784.
2. Okeley et al. *Cliin Cancer Res.* 2010; 16(3):889-897

ADC – Mechanism of action

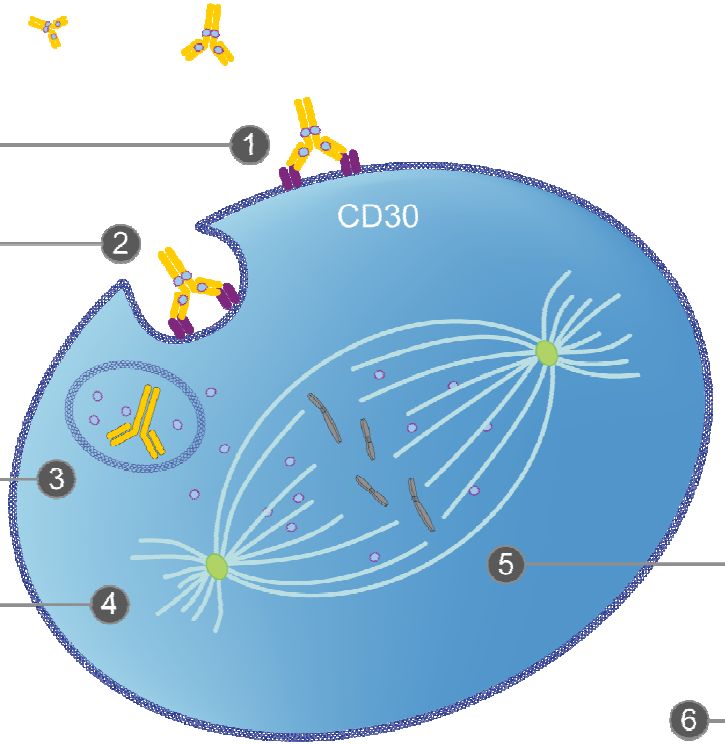


Brentuximab vedotin (SGN-35) ADC

monomethyl auristatin E (MMAE), potent antimicrotubule agent

protease-cleavable linker

anti-CD30 monoclonal antibody



1 ADC binds to CD30

2 ADC-CD30 complex is internalized and traffics to lysosome

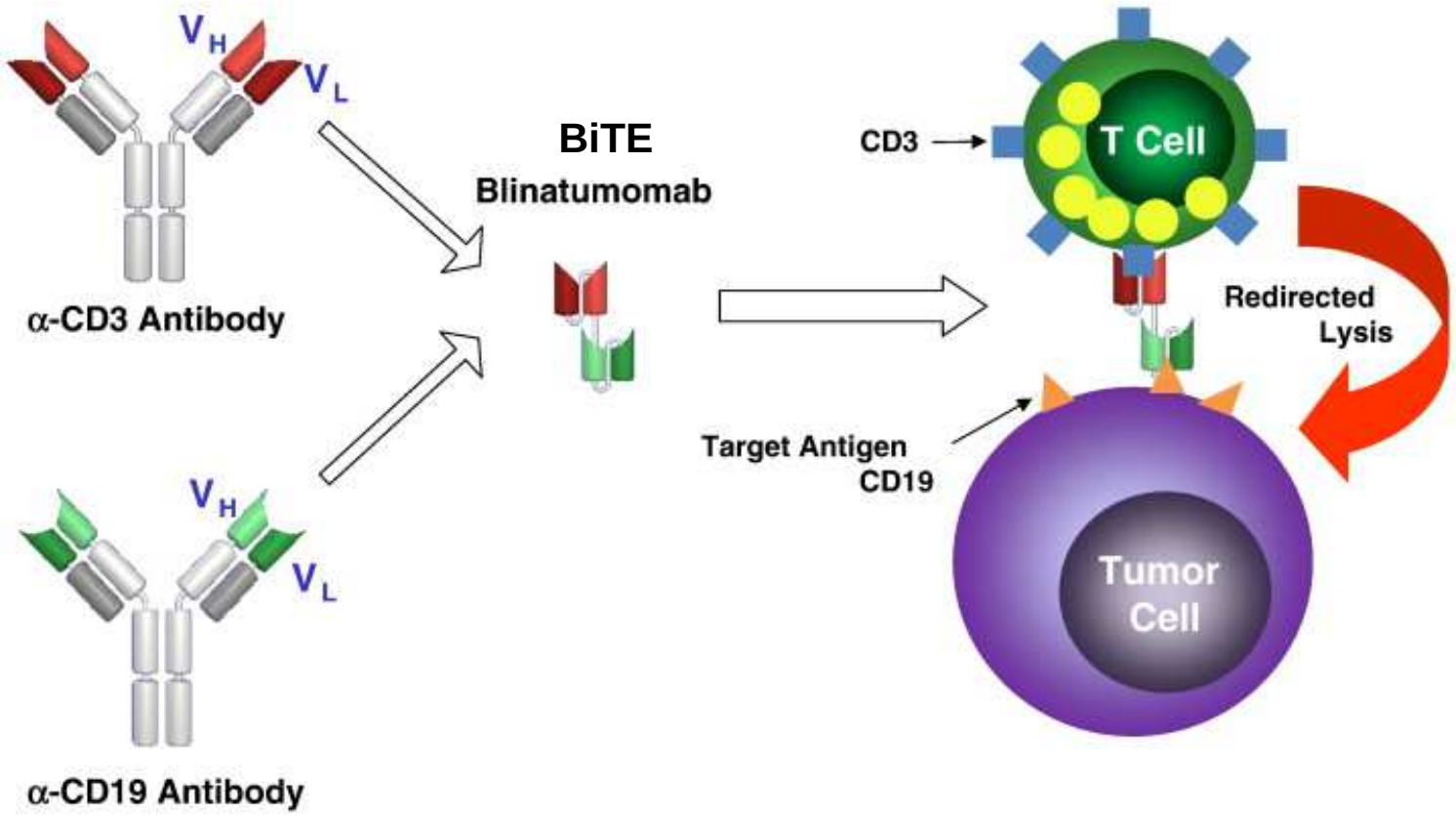
3 MMAE is released

4 MMAE disrupts microtubule network

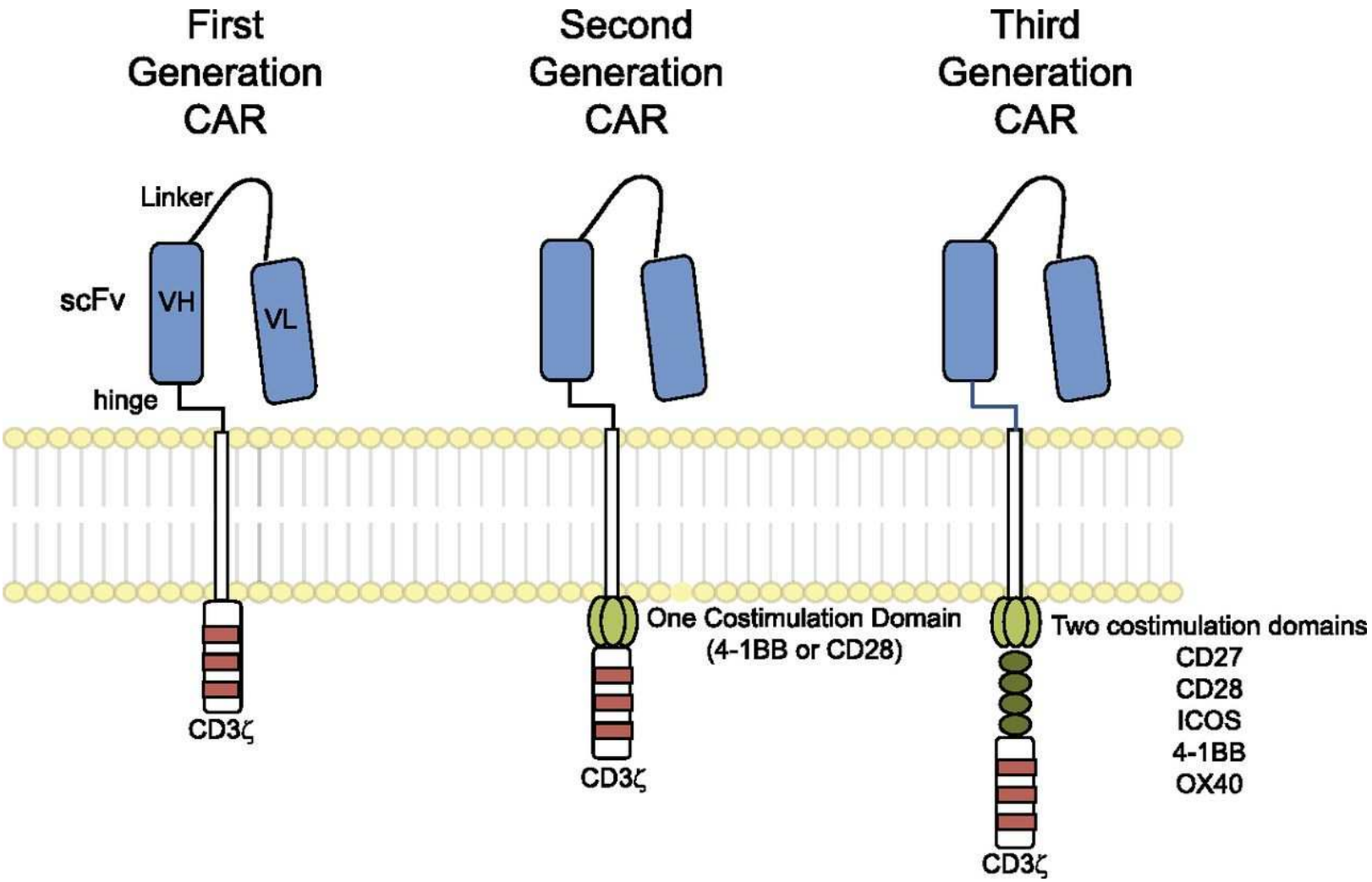
5 G2/M cell cycle arrest

6 Apoptosis

Bispecific T-cell Engager (BiTE) - Blinatumomab



CAR – scFV-CD28-CD3ζ transduced T cells



Maus M V et al. Blood 2014;123:2625-2635

Summary

- Mab have been shown to be highly effective in various cancers
- Humanized and fully human Mab are less immunogenic
- Engineering Fc enhances ADCC of Mab
- Direct or indirect arming of Mab further improves efficacy

Recommended Reading

1. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJ. The safety and side effects of monoclonal antibodies. *Nat Rev Drug Discov*. 2010 Apr;9(4):325-38.
2. Weiner LM, Surana R, Wang S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. *Nat Rev Immunol*. 2010 May;10(5):317-27.
3. Sliwkowski MX, Mellman I. Antibody therapeutics in cancer. *Science*. 2013 Sep 13;341(6151):1192-8.

Question 1

You have identified a novel cell surface molecule (LCR1) that is exclusively expressed on lung cancer cells. You have also determined that LCR1 acts as a receptor for a lung cancer growth factor (LCGF). To target this novel pathway, you would develop a human:

A. IgG4 antibody against LCR1 optimized for blocking interaction with LCGF

B. IgG1 antibody against LCGF

C. IgG3 antibody optimized for CDC against LCR1

D. B or C

Question 2

You have developed an IgG1 antibody against LCR1. Testing in clinical trials showed that the antibody is safe but has only modest efficacy. To improve the efficacy of the antibody, you might:

A. Glycosylate the Fab

B. Glycosylate the Fc

C. Generate an antibody-drug conjugate (ADC)

D. B or C