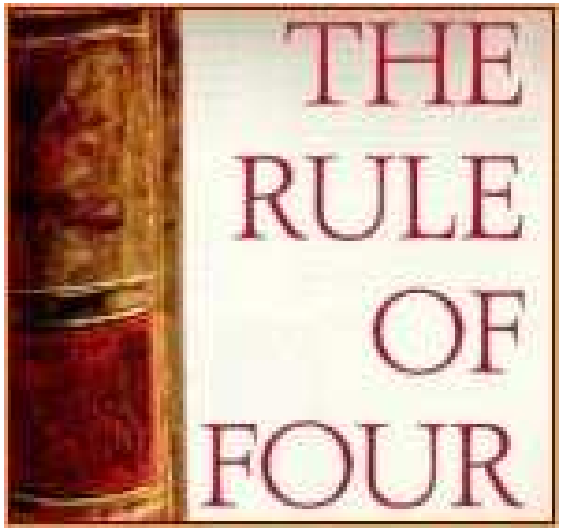
Introduction to Monoclonal Antibodies:

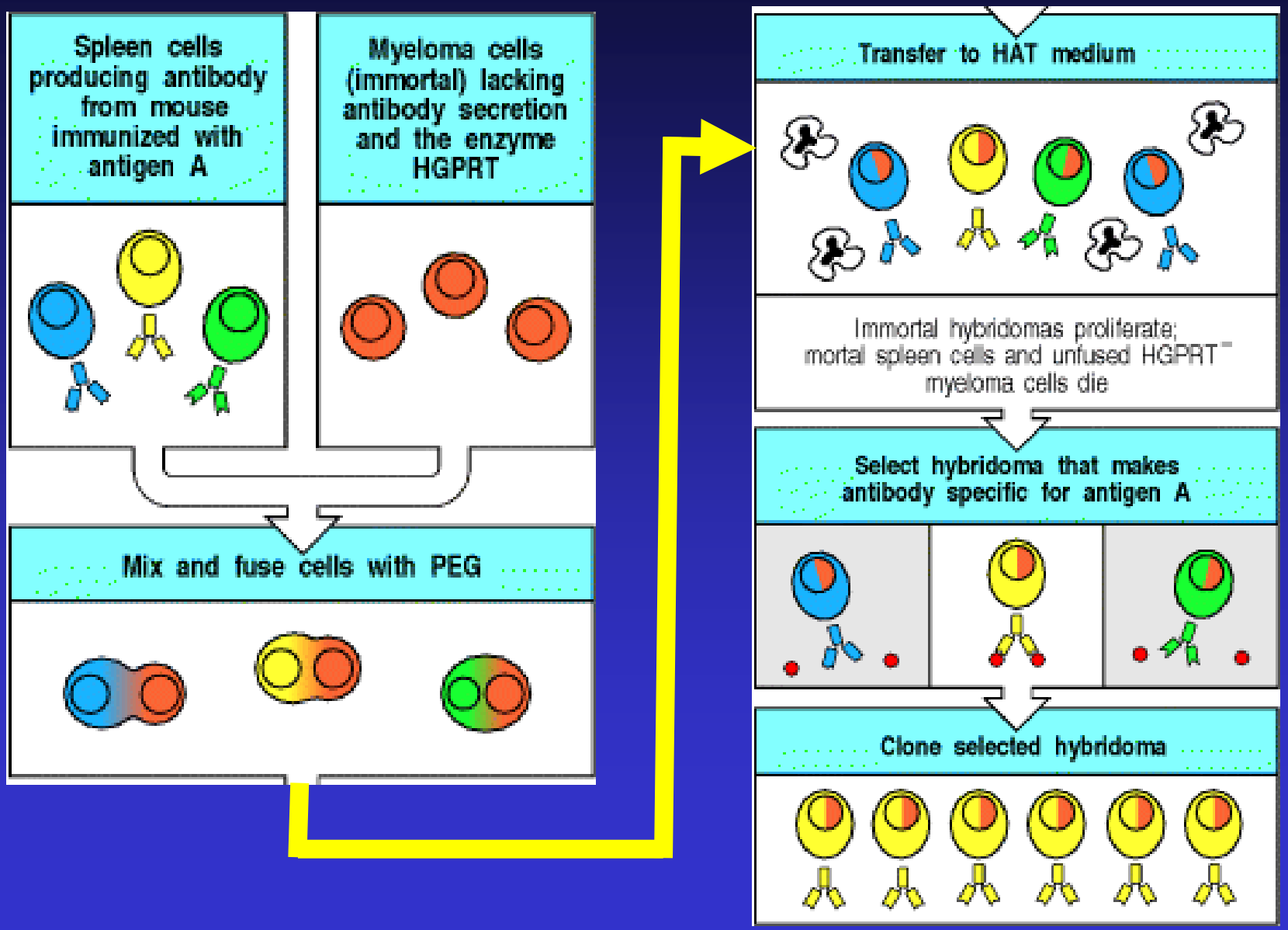


Charles G. Drake M.D. / Ph.D.
Associate Professor: Medical Oncology, Immunology and Urology
Johns Hopkins Kimmel Cancer Center

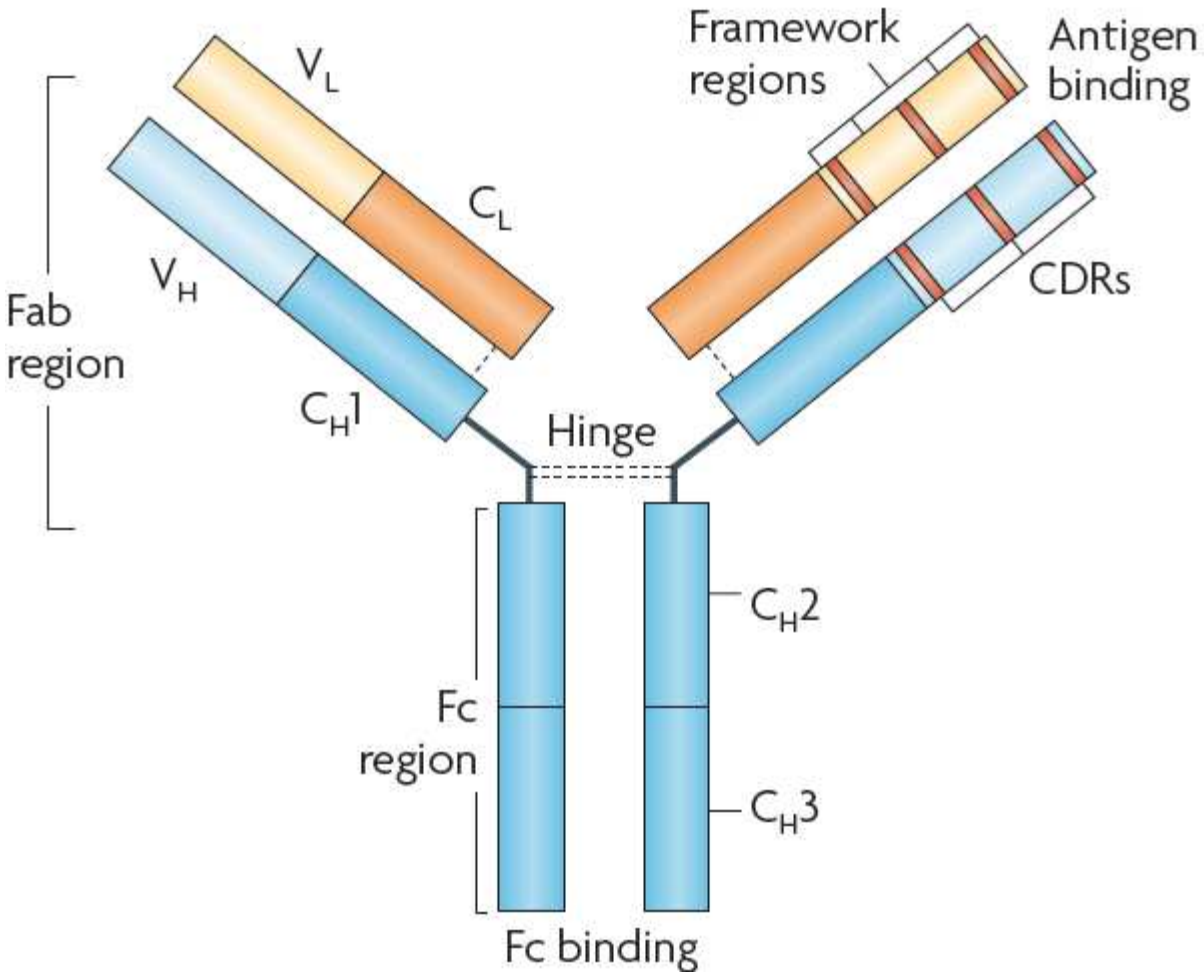
Learning Objectives

- Understand the FOUR basic Monoclonal Antibody (Mab) Types in the Clinic
- List the FOUR Major Mechanisms of Action of Mab
- Know the Differences Between the FOUR FcGamma Types
- Describe the FOUR Fc Gamma Receptors (FcgR)
- Introduce FOUR Modified Antibody Technologies
- Discuss FOUR Examples of Mab With Clinical Relevance

Where do monoclonal antibodies come from?

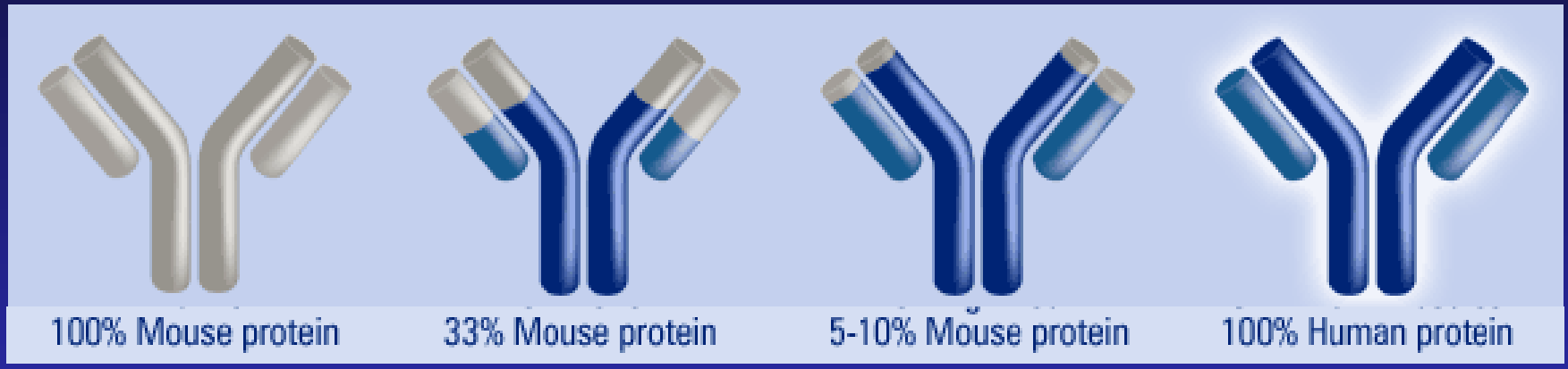


Antibody Structure



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

4 Kinds of Monoclonal Antibodies



Mouse	Chimeric	Humanized	Human
“o”	“xi”	“zu”	“u”
Mur**O**monab	Ritu**XI**mab	Trastu**ZU**mab	Ipilim**U**mab

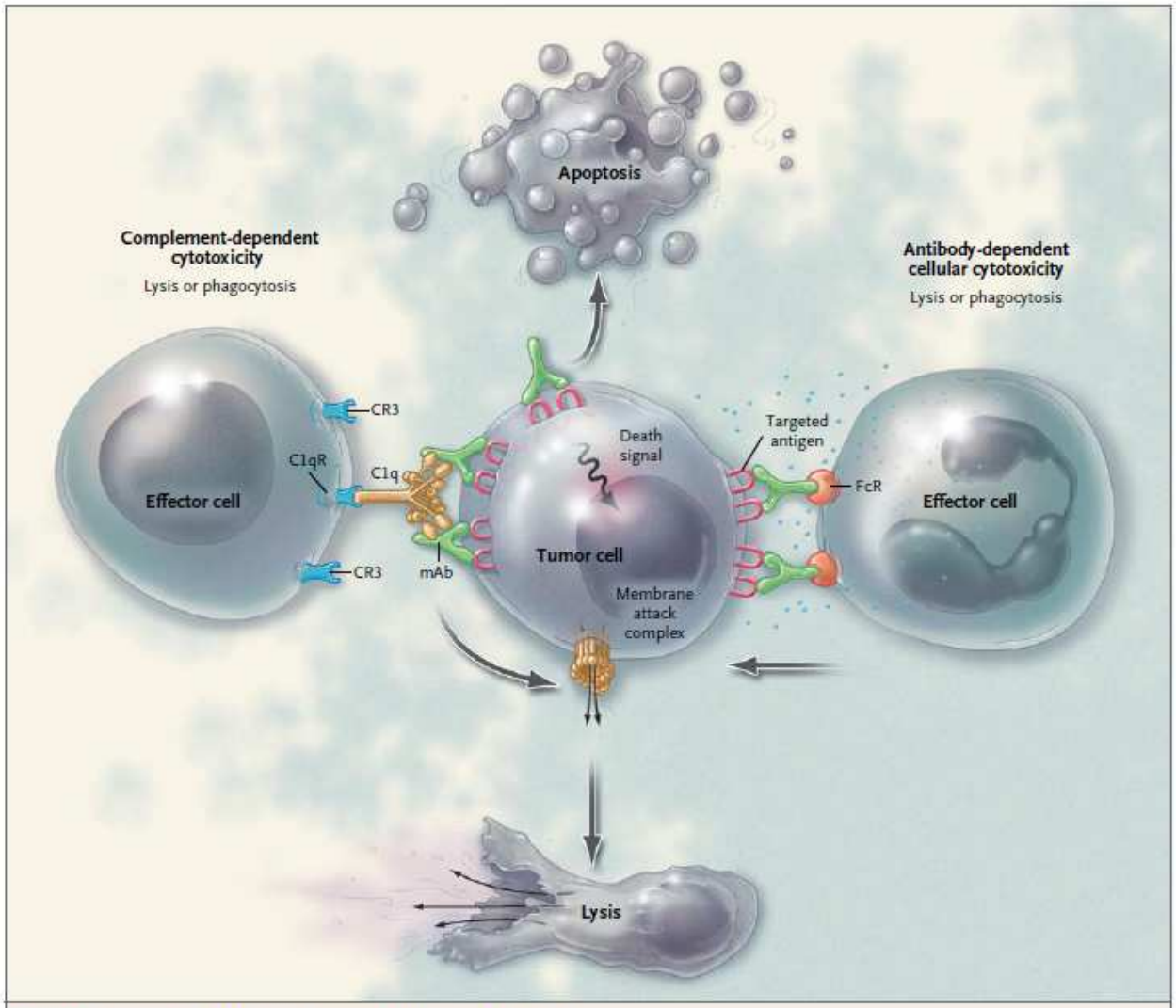


Figure 2. Potential Mechanisms of Action of Monoclonal Antibodies.

Monoclonal antibodies have several potential mechanisms of action, including antibody-dependent cellular cytotoxicity, which involves recruitment of effector cells, mediated by Fcγ receptors; complement-dependent cytotoxicity; and induction of apoptosis. FcR denotes Fc receptor, and mAb monoclonal antibody.

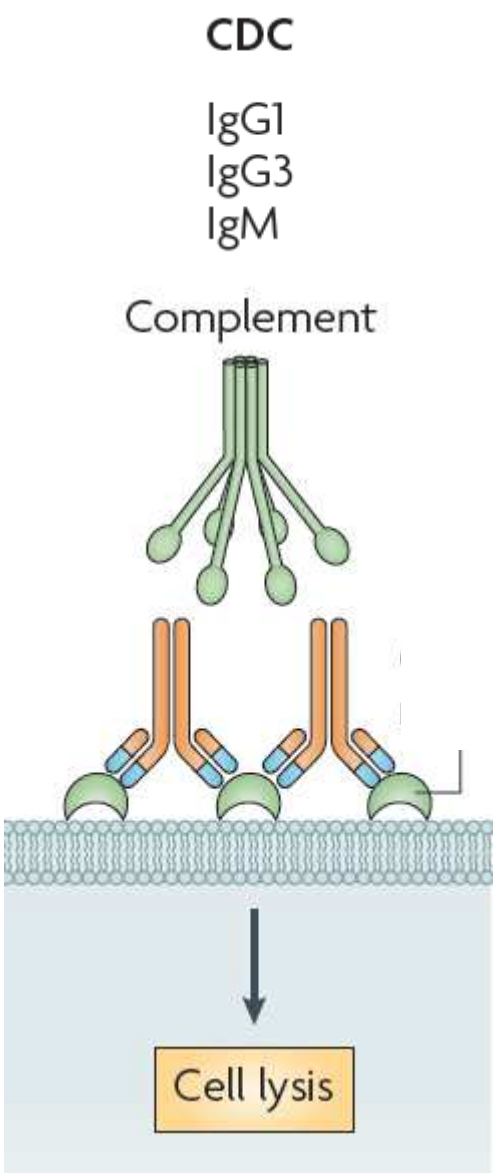
1)CDC

2)Antagonist

3)ADCC

4)Agonist

Rotschild et al, *NEJM* 2012

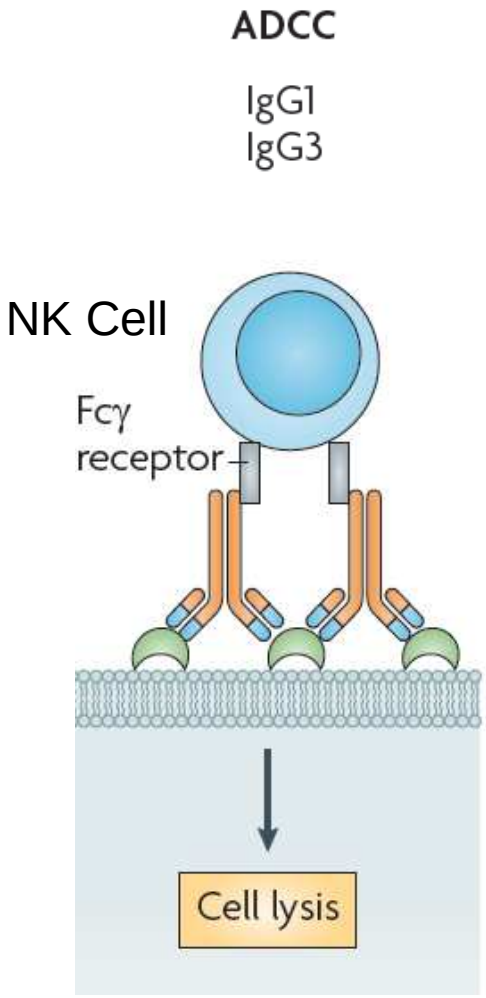


Complement Dependent Cytotoxicity (CDC)

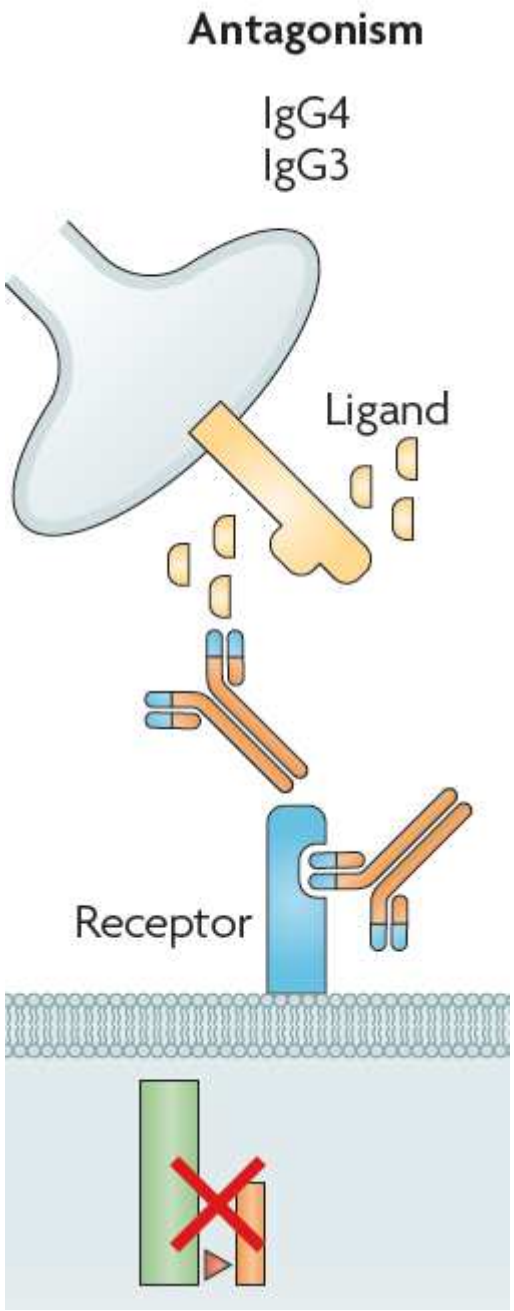
a) Requires antibody cross-linking / proximity
b) Differential effects in humans with polymorphisms in C1Q
c) Monoclonal antibodies rarely engineered to function via CDC

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

Antibody Dependent Cellular Cytotoxicity (ADCC)



a) Mediated by Natural Killer (NK) Cells, Macrophages or Neutrophils
b) Killing requires binding to Fc Gamma Receptor(s)
    a) Binding to Fc Gamma Receptors requires glycosylation
    b) Increase ADCC by modifying glycosylation of Fc
    c) Decrease ADCC using antibodies that lack glycosylation



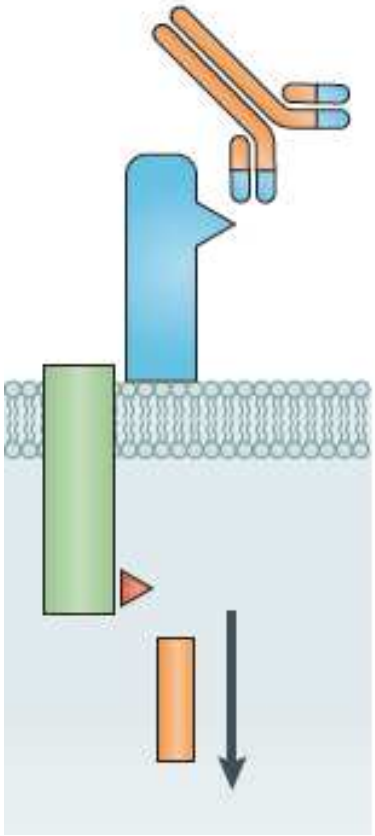
Antagonist (blocking)

a) Can block EITHER a receptor OR a ligand

b) Ligand may be soluble (like TNFα)

   a) Fc function not desirable, usually use IgG4

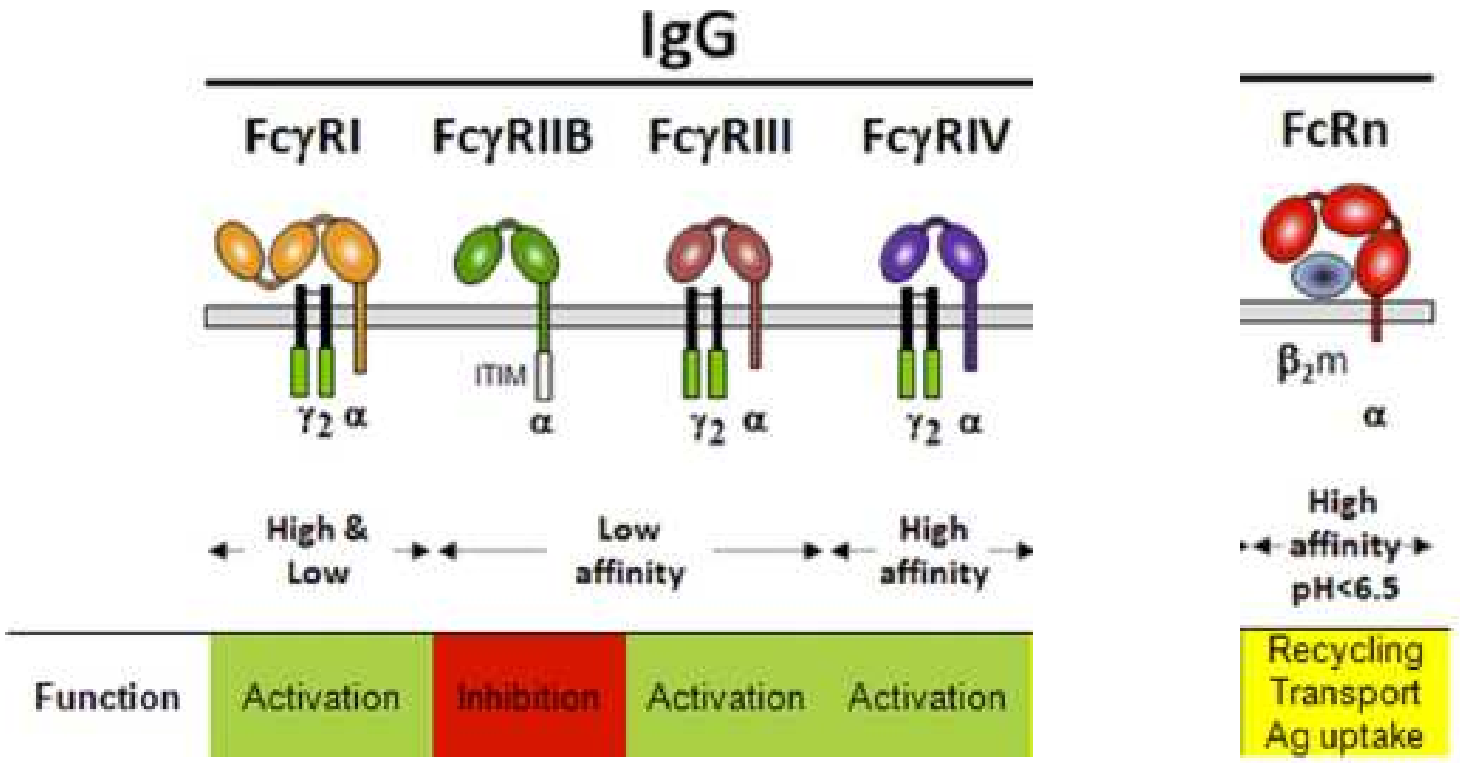
   b) Can eliminate ADC from IgG4 by decreasing Fc glycosylation



Agonist (Signalling)

a) Activating antibodies not so uncommon in cancer immunology
b) Examples include OX40, 41BB, CD40 etc.

FOUR Major Fc Gamma Receptors (Human)



Bruhns 2012 Blood 119:5640

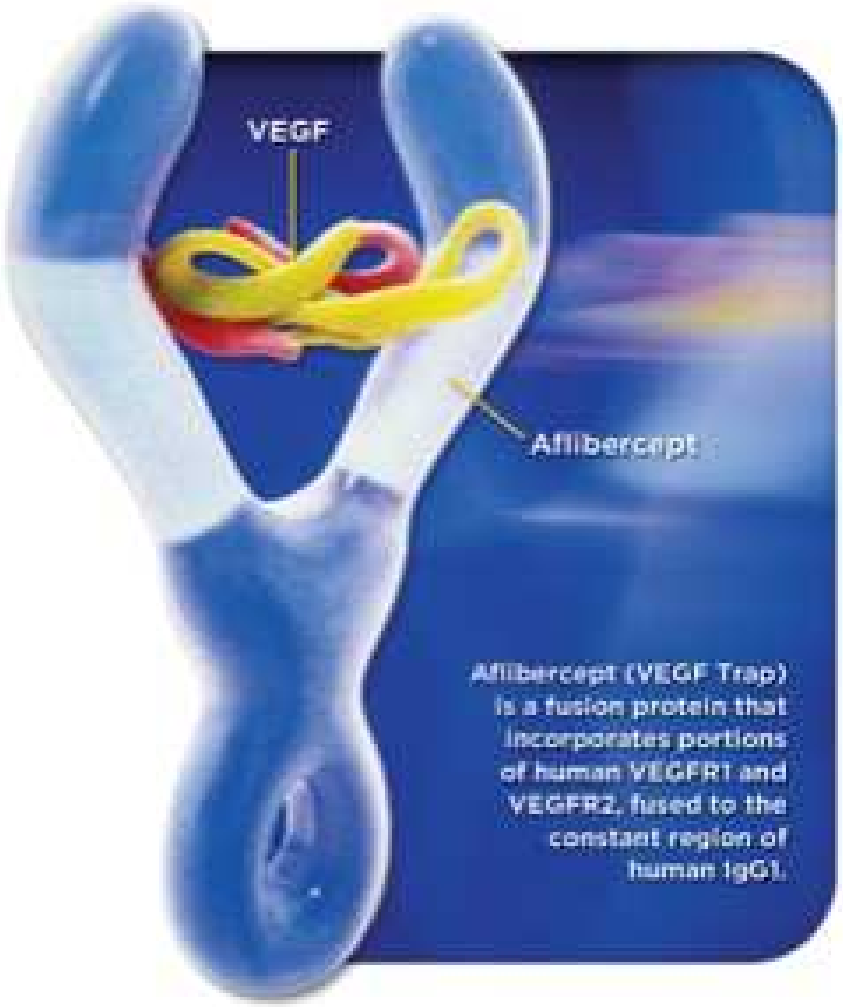
FOUR Considerations Regarding Mab Half-Life

- IgG3 = Short, hard to use
- IgG4 = Modify Hinge Region to Increase Half-Life
- Bind more strongly to recycling receptor FcRN = more recycling
  - LONGER half life
- Bind less strongly to FcRN = SHORTER half life

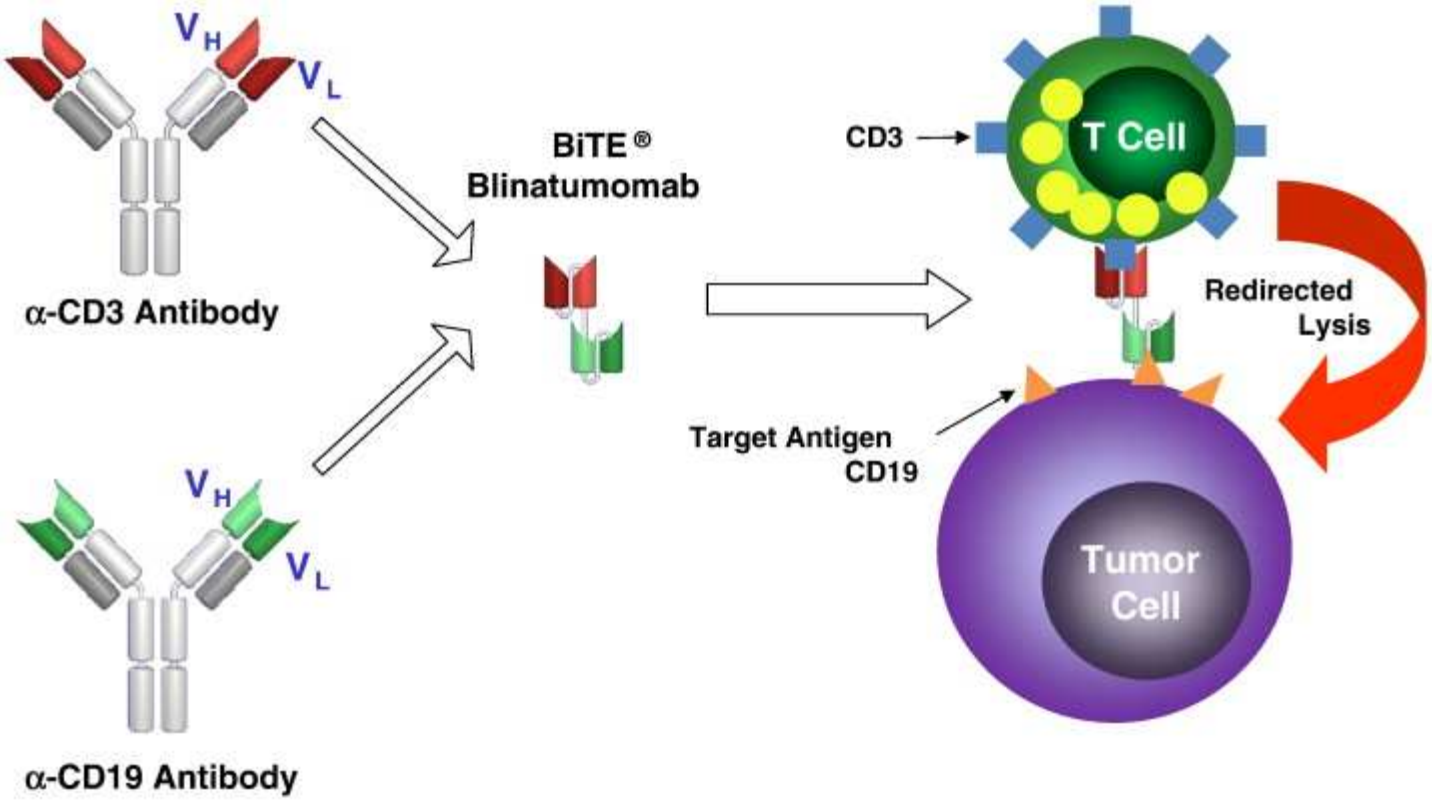
Modified Antibody Technologies

- TRAP molecules
- Single Chain, Dual Specificity, BiSpecific T-Cell Engager (BiTE)
- Chimeric Antigen Receptors
- ADC (Antibody Drug Conjugates)

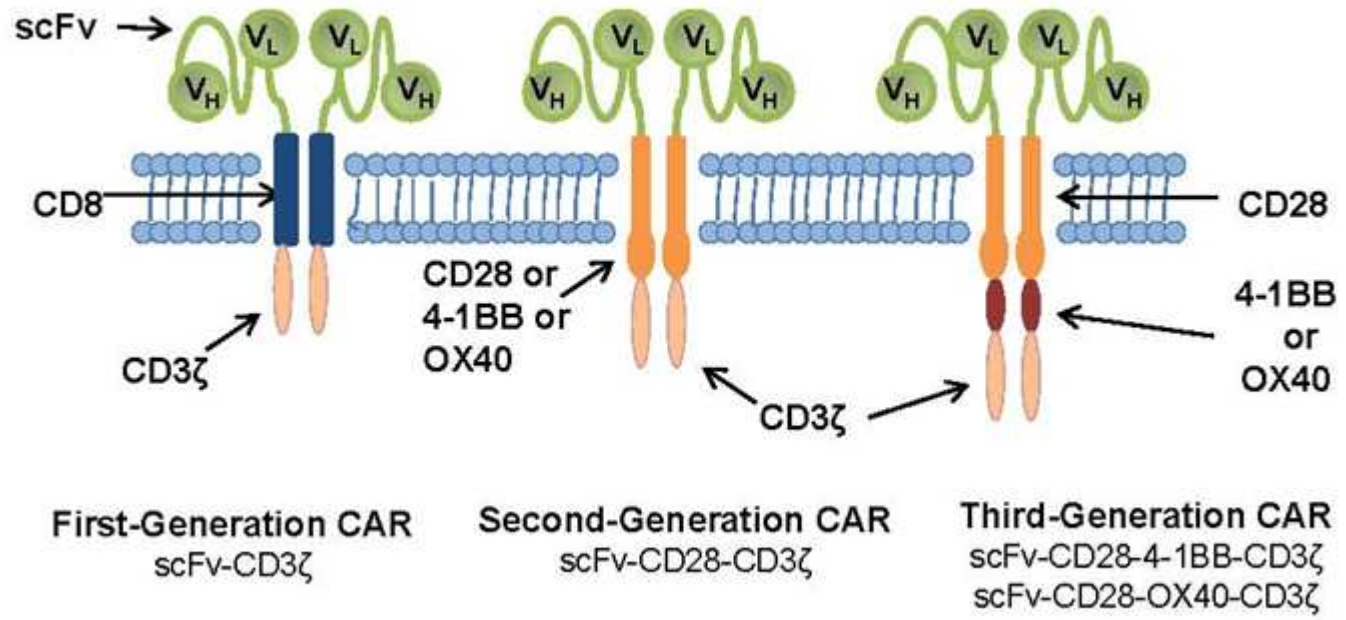
TRAP Molecules (Aflibercept)



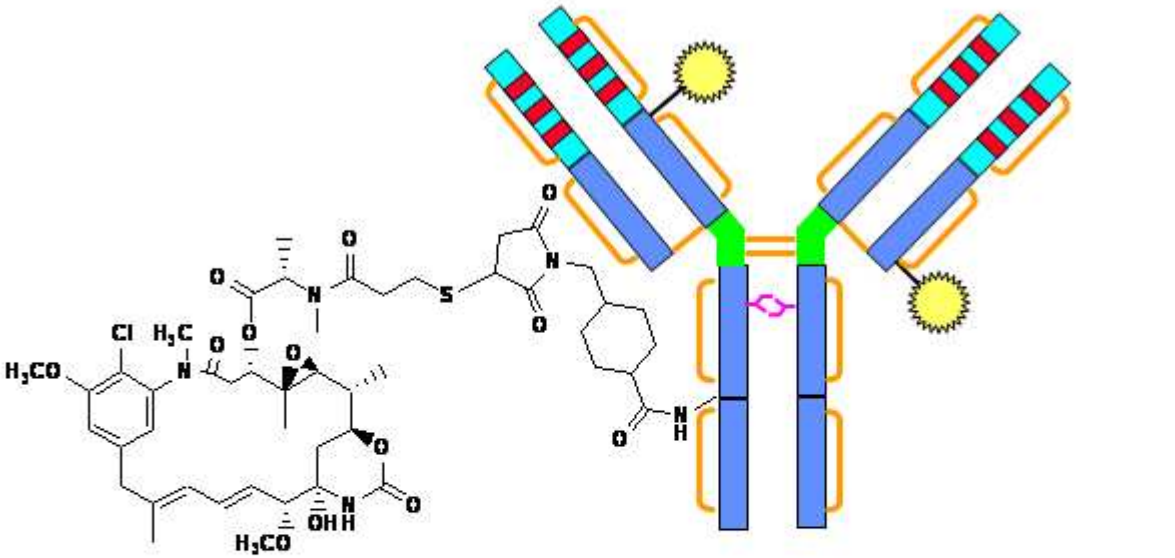
Single-Chain Dual Specificity (BiTE)



Chimeric Antigen Receptor



Antibody Drug Congugates (ADC)



Four Examples

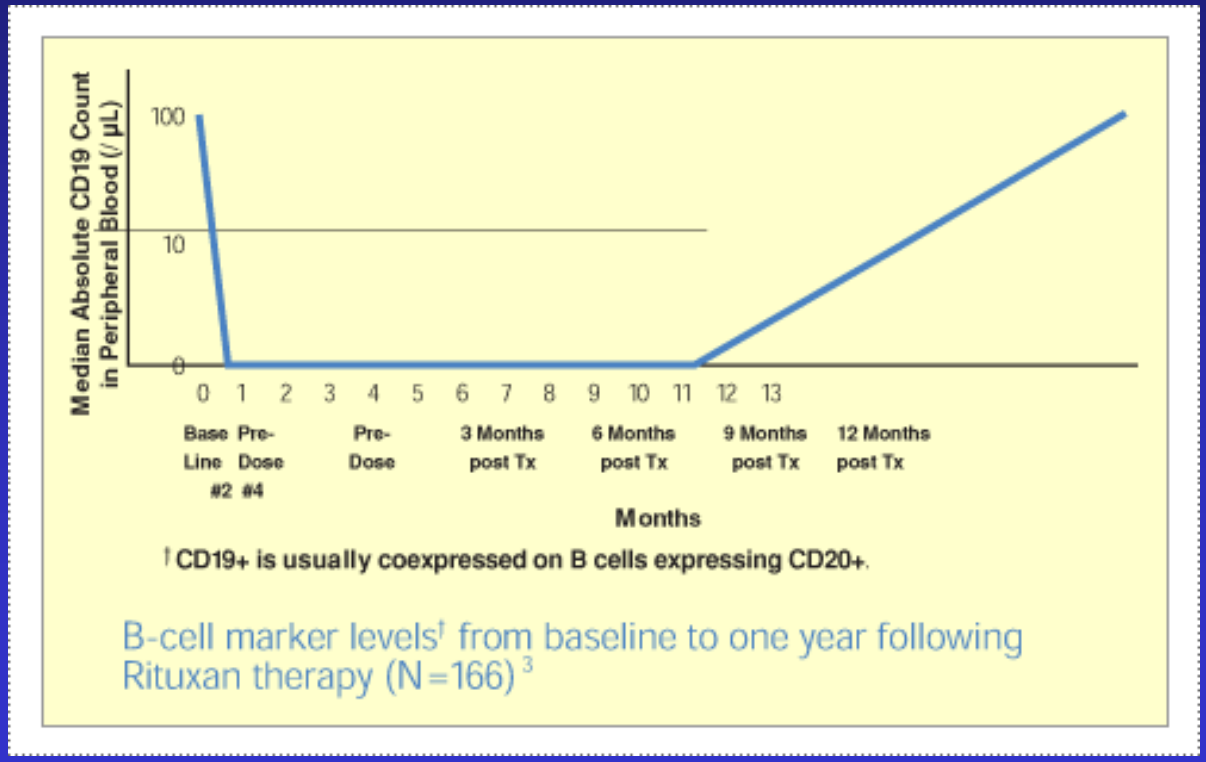
- Rituximab
- Trastuzumab
- Urelumab
- Lambrolizumab

Rituximab (Rituxan)

"xi" = Chimeric

First Monoclonal Antibody Approve to Treat Cancer (1997)

IgG1 (ADCC)



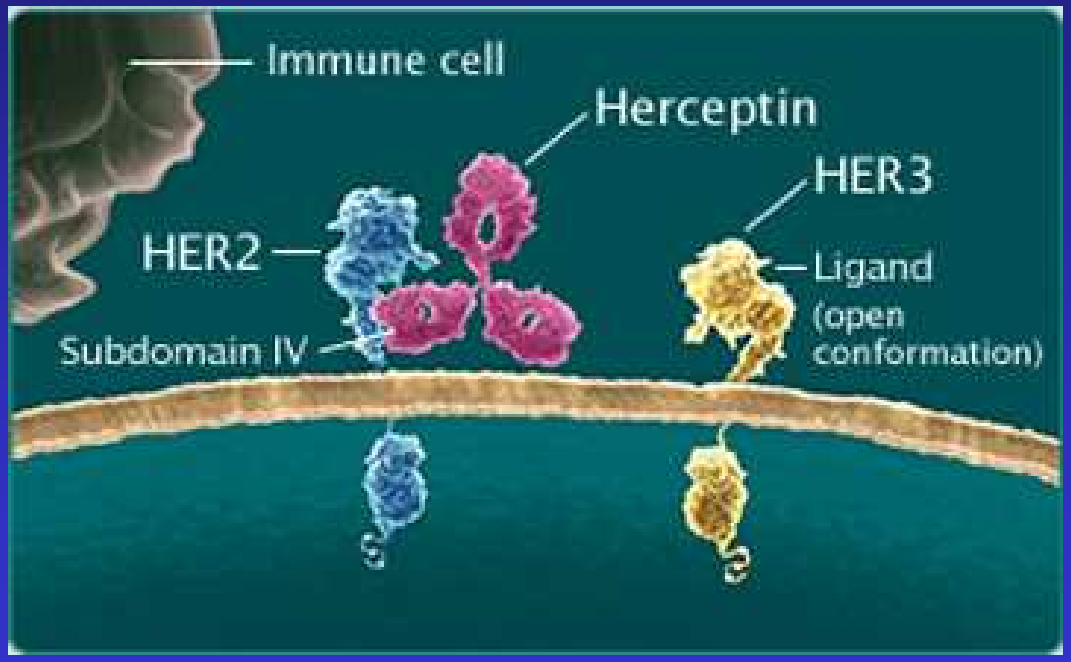
B-cell marker levels† from baseline to one year following Rituxan therapy (N=166)[3]

Trastuzumab (Herceptin)

“zu” = Humanized

IgG1

MOA = prevent dimerization / ADCC



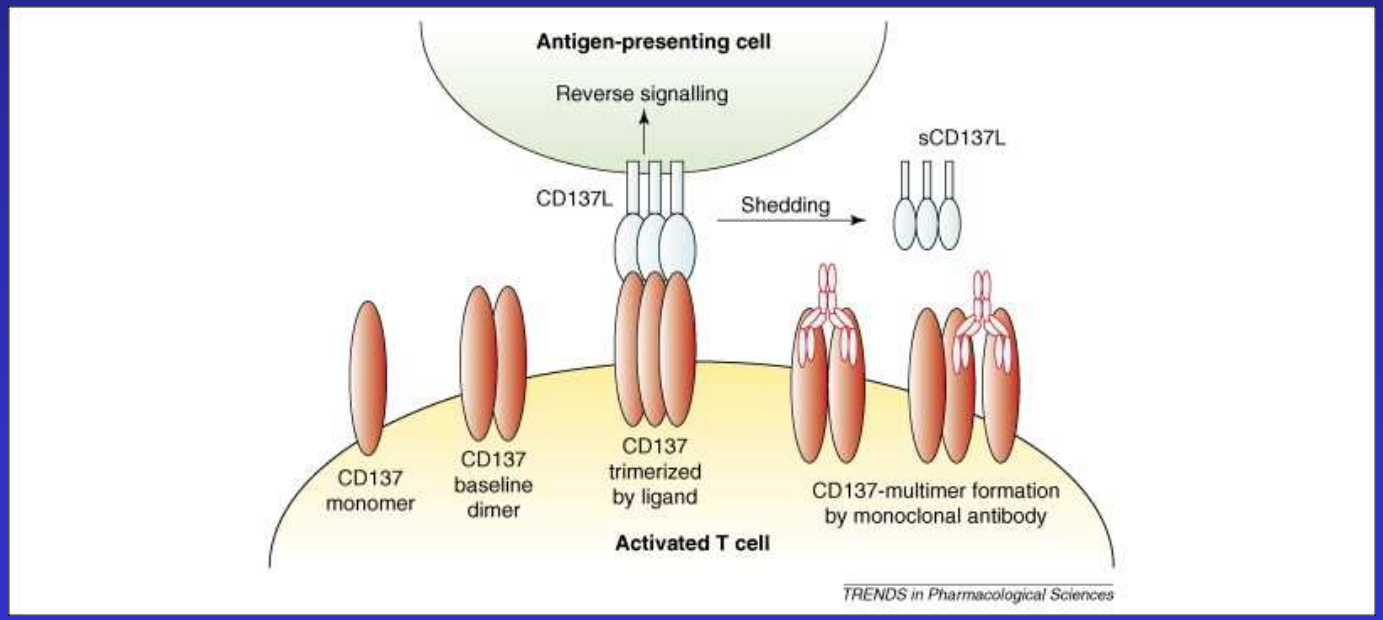
Urelumab (Anti-4-1BB)

“u” = Fully Human

IgG4

Agonist

In Phase I



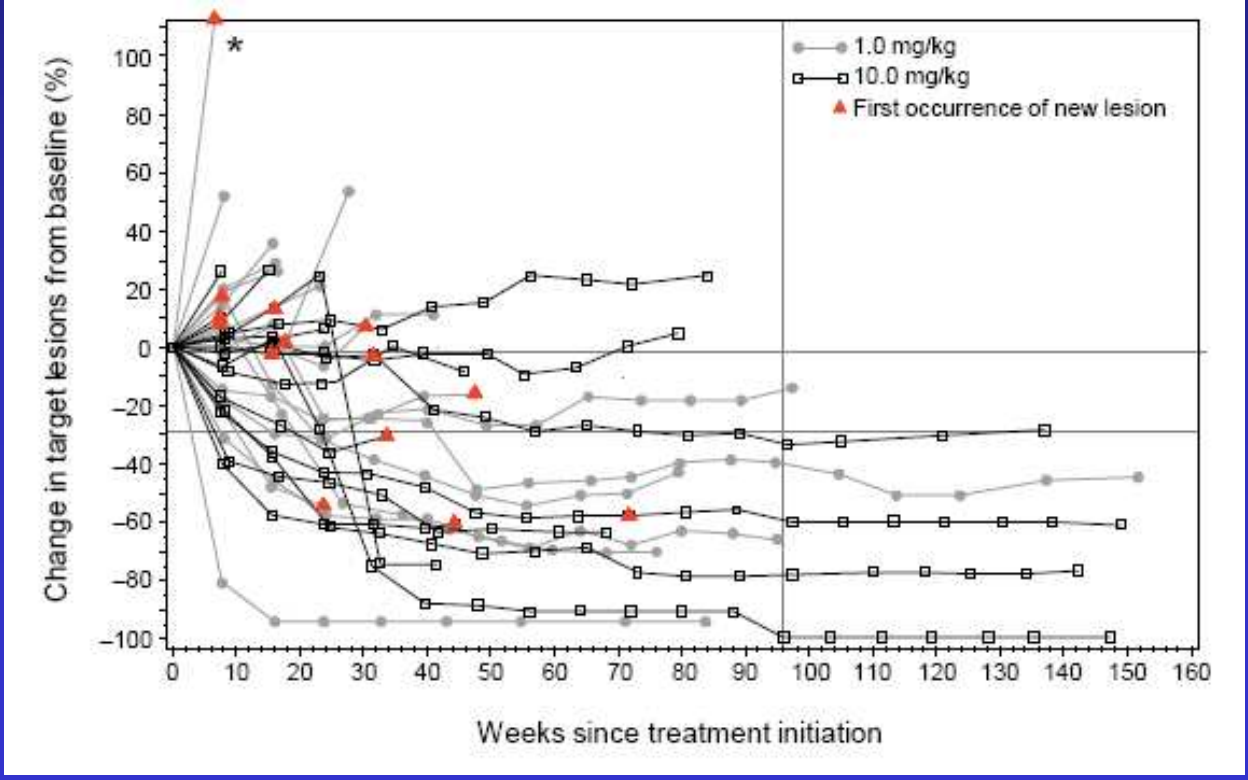
Nivolumab (Anti-PD-1)

"u" = Fully Human

IgG4 with modified hinge region

Antagonist

In Phase III in RCC, Mel and NSCLC



Summary

- Monoclonal Antibodies = Drugs
- Prominent in Cancer Immunotherapy
- Novel Technologies In Development
- Engineered Modifications to Fc Region affect multiple properties

Recommended Reading

1. Sliwkowski,M.X. and I.Mellman. 2013. Antibody therapeutics in cancer. *Science* 341:1192-1198.

2. Nimmerjahn,F. and J.V.Ravetch. 2012. Translating basic mechanisms of IgG effector activity into next generation cancer therapies. *Cancer Immun.* 12:13.

3. Hansel,T.T., H.Kropshofer, T.Singer, J.A.Mitchell, and A.J.George. 2010. The safety and side effects of monoclonal antibodies. *Nat.Rev.Drug Discov.* 9:325-338.

Q1. While employed at a small Bethesda biotech, you use RNAseq to identify a novel cell surface molecule (BT1) that appears to you be exclusively expressed on big toe cancer cells. Seeking to treat cancer, you call your antibody engineering division and have them start developing a human:

A. IgG4 antibody because you want to block signaling through BT1

B. IgG1 antibody because you want to kill all cells expressing BT1

C. IgG3 antibody optimized for CDCC

D. High affinity antibody of any type, which you will later use to generate an antibody-drug conjugate (ADC)

E. B or D

Q2. Your splendid engineering group generates a lovely IgG4 antibody with nice affinity to BT1, which you rapidly take to the clinic. Unfortunately, Phase I pharmokinetics data show that the antibody of that particular IgG4 is unfavorable, with a half-life of only 8 days *in vivo*. In order to increase half life they might:

A. Substitute the natural hinge region with a modified version

B. Make Fc modifications to increase binding to the recycling receptor FcRN

C. Decrease binding to he recycling receptor

D. Change approaches and generate a bi-specific antibody instead

E. A or B