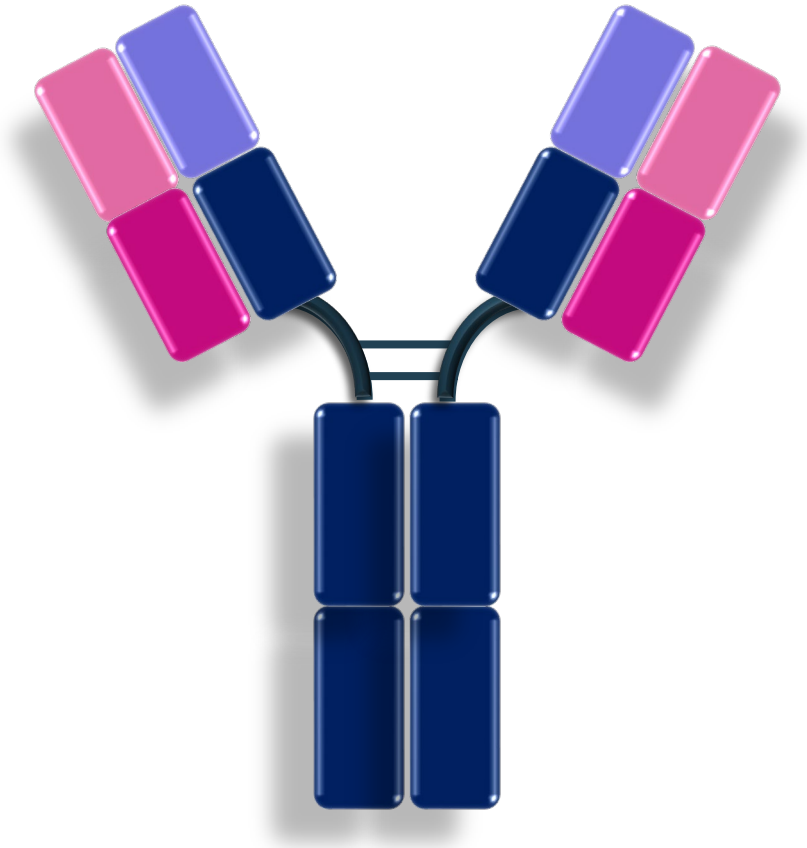

Antibody Fc Engineering: Designing Antibodies for Cancer, Covid-19, and Beyond

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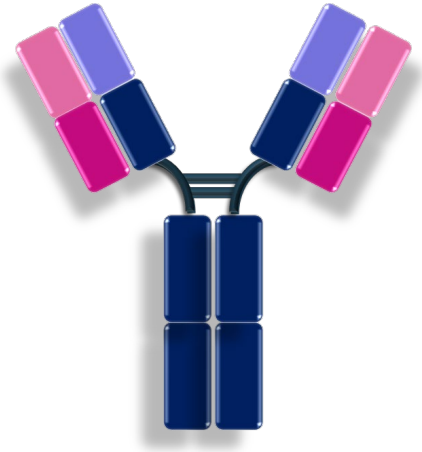
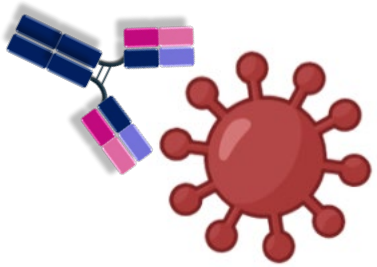
Monoclonal Antibodies

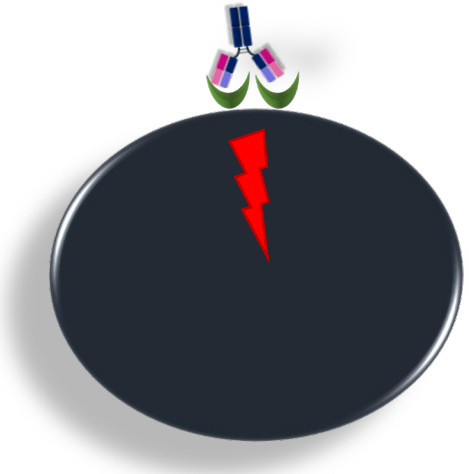
- Monoclonal antibodies (mAbs) are one of the most clinically successful therapeutic formats across a diverse range of diseases
  - 5 of the top 10 selling drugs of 2020 were mAbs
- Over 100 mAbs approved in the US/EU and over 600 in various stages of clinical development
- Majority of approved mAbs are for cancer indications, many also developed for autoimmune and inflammatory diseases and infectious diseases

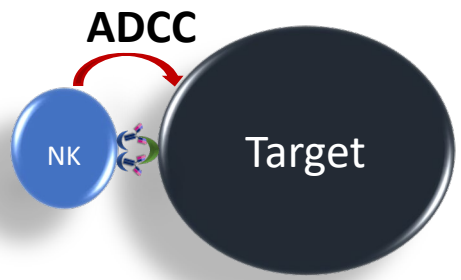


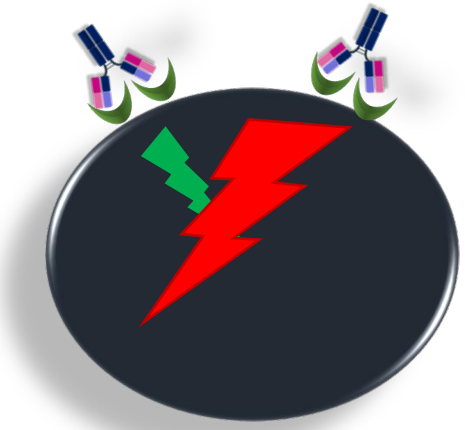
- Well-tolerated
- Highly specific for target
- Engages receptors on immune cells to induce effector functions

Antibody Functions









Blocking

- Infectious diseases (SARS-CoV-2)
- Checkpoint inhibitors
- Inflammatory targets (IL-6, C5a)

Agonism

- Co-stimulatory molecules for immunity (CD28)
- Induction of apoptosis (CD40)

Effector Cell Activation

- Killing of target cells by ADCC (HER2 on cancer cells)
- Killing of target cells by phagocytosis (CD38 in blood cancers)
- Release of pro-inflammatory mediators

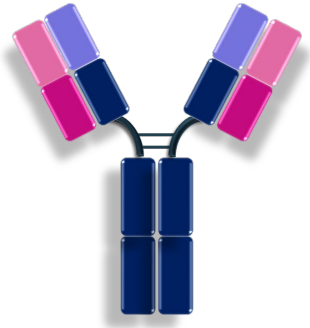
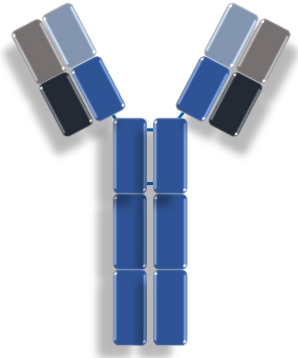
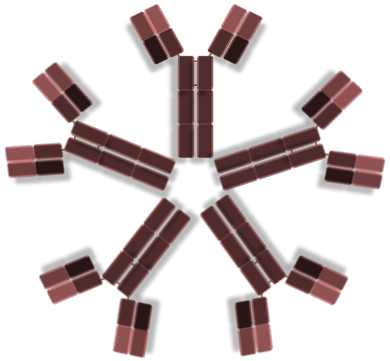
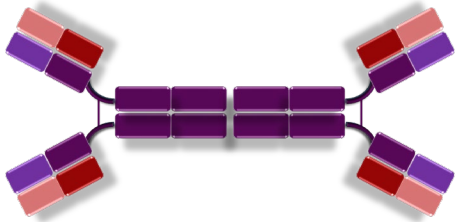
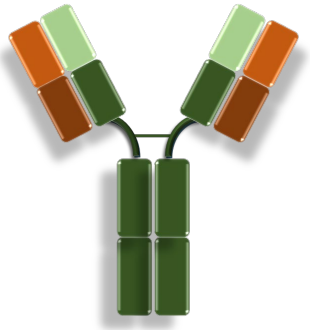
Inhibitory Signalling

- Inhibition of BCR signalling (anti-CD19 on B cells)

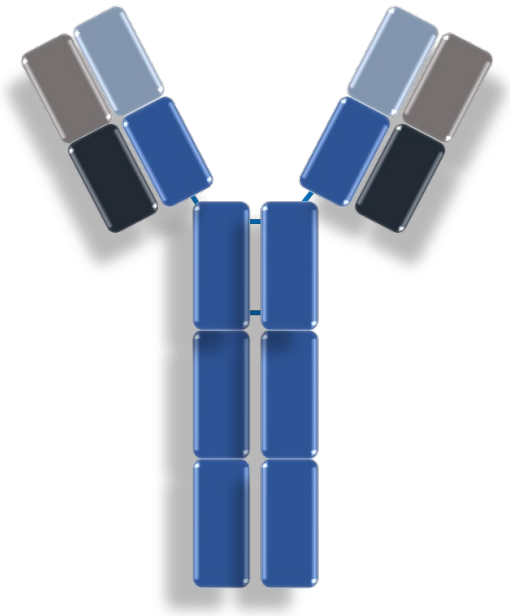
Fc-independent

Fc-dependent

Choosing the Antibody Backbone

	 **IgG**	 **IgE**	 **IgM**	 **IgA**	 **IgD**
Therapeutic Use	Most common for therapeutics	In clinical trials	In clinical trials	Not yet in clinical trials	Not used for therapeutics
Secreted Structure	Monomer	Monomer	Pentamer/hexamer	Monomer/Dimer	Monomer
Complement Fixation	Yes	No	Yes	Only alternative	No
Serum Half-Life	Long (21 days)	Short (2-3 days)	Short (5 days)	Short (6 days)	Short (3 days)
Pro-inflammatory Effector Functions	Yes	Yes	Poor	Yes	No
Inhibitory Receptor	Yes	No	No	ITAMi signalling by FcαRI	No

Choosing the Antibody Backbone

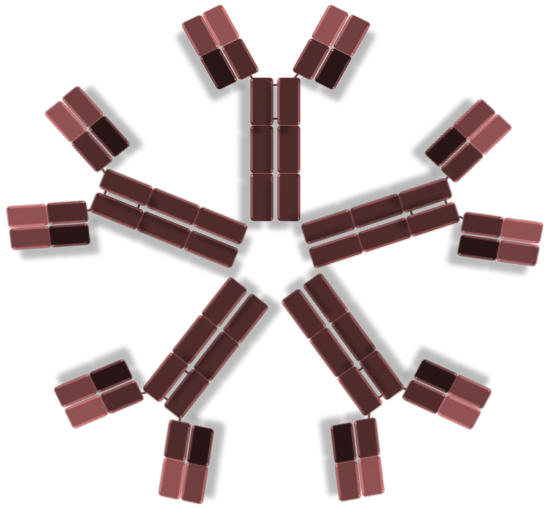


IgE

- Commonly associated with allergic/hypersensitivity responses
- Short serum half-life, but prolonged tissue half-life (up to 2 weeks)
- Able to induce strong pro-inflammatory effector functions through FcεRI on mast cells, basophils, monocytes and macrophages
- No known inhibitory receptor
- Phase I clinical trial of an IgE-based antibody therapeutic for cancer, well tolerated in most individuals

Chauhan J *et al.* (2020) *Antibodies*

Choosing the Antibody Backbone

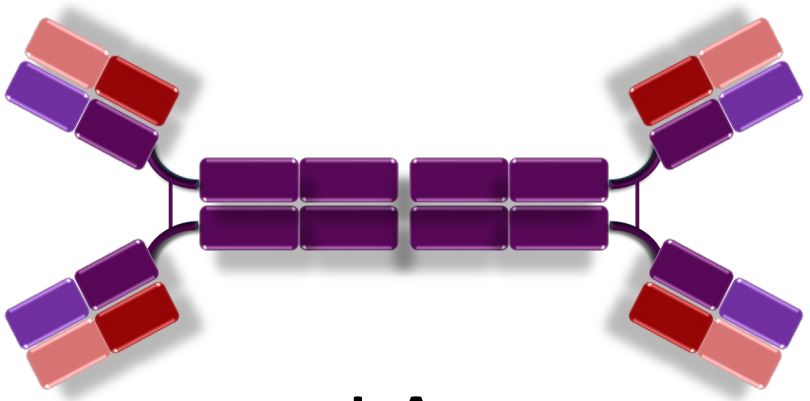


IgM

- Forms pentamers and hexamers
- Stronger avidity binding to targets
- Poor induction of cellular-based inflammatory activation
- Can potently activate the complement pathway
- Around 20 IgM-based antibodies have been tested in clinical trials, mostly against lipopolysaccharides, glycolipids, and glycans (poorly immunogenic targets)
- IgM-based therapeutics also being developed against SARS-CoV-2, has higher neutralisation of viral target compared to IgG

Keyt BA *et al.* (2020) *Antibodies*
Pisil Y *et al.* (2021) *Pathogens*
Ku Z *et al.* (2021) *Nature*

Choosing the Antibody Backbone

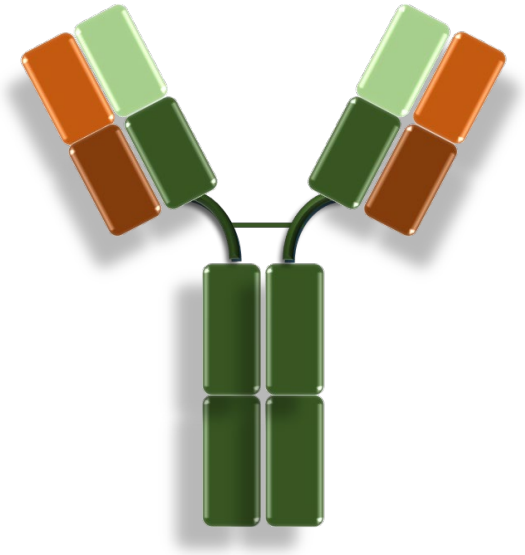


IgA

- Forms monomers and dimers
- Two subtypes (IgA1 and IgA2)
- Can induce pro-inflammatory effector functions by neutrophils, monocytes and macrophages through receptor FcαRI
- Short serum half-life
- *In vitro* studies have demonstrated greater neutrophil-mediated cancer cell killing compared to IgG, however no IgA in clinical studies yet

Brandsma AM *et al.* (2019) *Front. Immunol.*
Breedveld A & van Egmond M (2019) *Front. Immunol.*

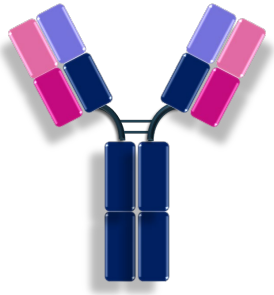
Choosing the Antibody Backbone

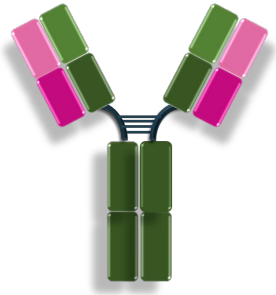


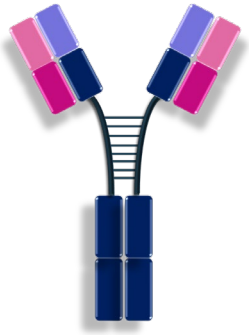
IgD

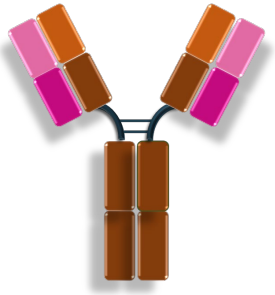
- Short serum half-life
- Function is poorly understood – no induction of complement or immune cell activation

IgG Antibody Subclasses









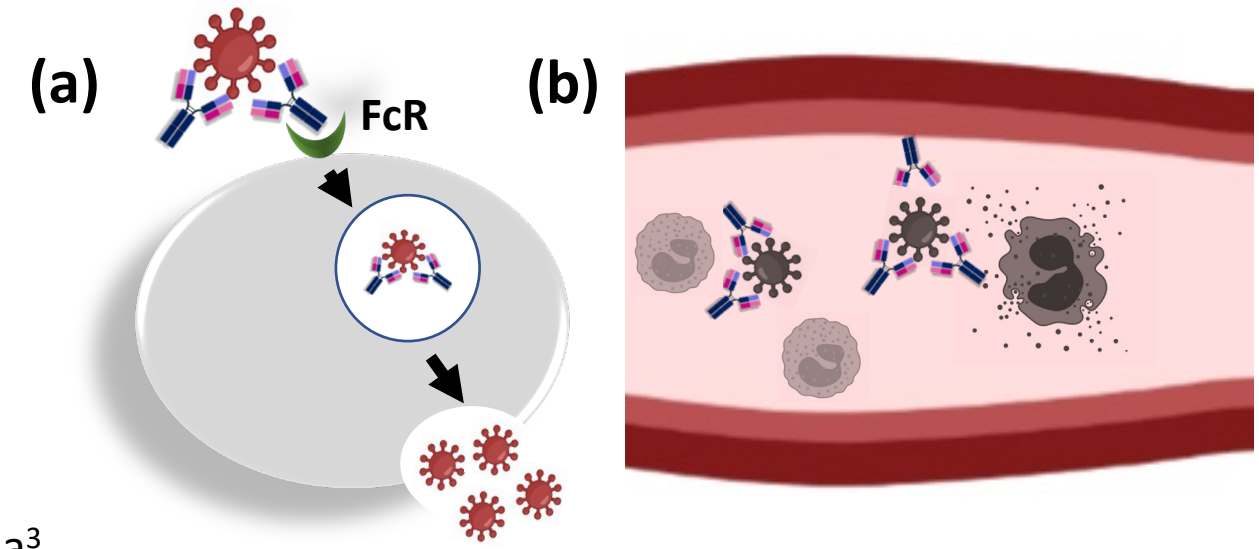
	IgG1	IgG2	IgG3	IgG4
Half-life	21 days	21 days	7-21 days (allotype-dependent)	21 days
Binding to Fc Receptors	All	Limited (only one polymorphic form of FcγRIIa)	All	Limited (only FcγRI and inhibitory FcγRIIb)
Complement binding	++	+	++	-
Hinge Length (amino acids)	15	12	62* (allotype dependent)	12
Therapeutic Use	**Fc-dependent functions**	**Fc-independent functions**	None	**Fc-independent functions**

Fc Engineering for Blocking Antibodies

1. Removing Effector Functions
2. Improving Half-Life

Removal of Effector Functions

- Removal of unwanted effector functions can improve safety
- Some anti-SARS-CoV-2 antibodies in late-stage trials have removed effector functions to avoid potential antibody-dependent enhancement (ADE)
- ADE has been demonstrated *in vitro* for influenza[1], HIV[2], Ebola[3], and Dengue virus[4]
- Also, for targets on immune cells i.e. checkpoint inhibitors, removal of effector functions can prevent immune cell depletion or undesired activation



Antibody-dependent Enhancement of Viral Infections
(a) Increased viral infection of immune cells (i.e. macrophages) by internalisation via FcR
(b) Increased airway inflammation by recruitment and activation of immune cells

[1]Ochiai H *et al.* (1992) *J Med Virol*
[2]Robinson WE *et al.* (1988) *Lancet*
[3]Takada A *et al.* (2003) *J Virol*
[4]Dejnirattisai W *et al.* (2010) *Science*

Figure adapted from Lee WS *et al.* (2020) *Nature Microbiology*

Common Ways to Remove Effector Functions

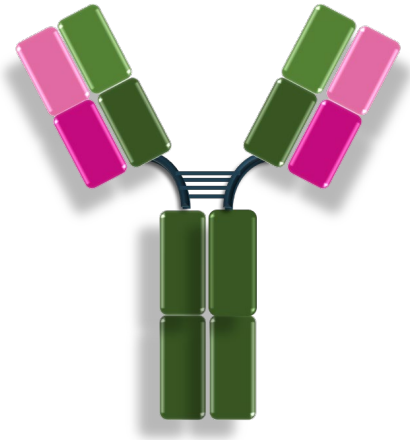
Change subclass from IgG1 to IgG2 or IgG4

- Many mAbs in clinic have used this approach, and mostly works well
  - Anti-PD-1 mAbs pembrolizumab, nivolumab, cemiplimab are IgG4
  - Anti-CTLA-4 mAb tremelimumab is IgG2

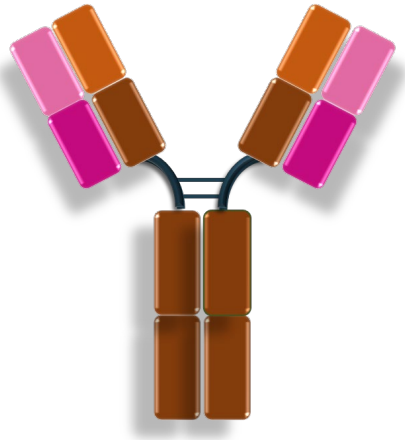
- Despite having a more restricted FcR binding profile than IgG1, IgG2 and IgG4 can still induce unwanted effector functions

- Anti-PD-1 IgG4 antibody induced activation of the receptor instead of blocking *in vitro*, and this function was ablated when the Fc binding capacity was removed[1]

- Anti-CD28 IgG4 mAb able to induce CD28 clustering and signalling by antibody scaffolding by FcγRIIb[2]



IgG2



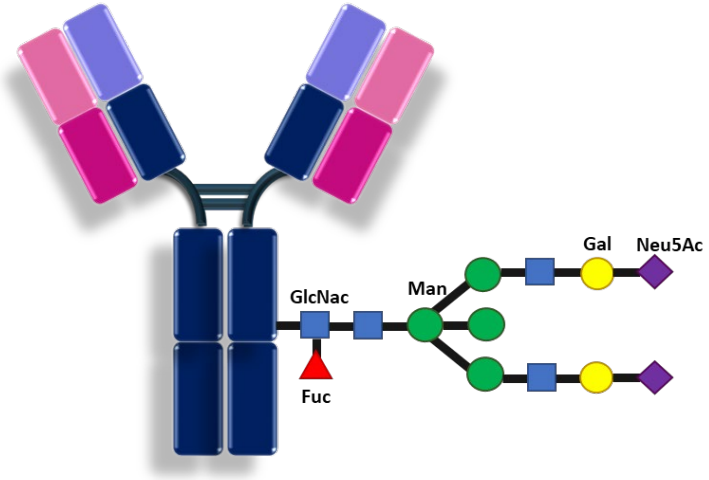
IgG4

[1]Zhang T *et al.* (2018) *Cancer Immunology*
[2]Suntharalingam G *et al.* (2006) *N Engl J Med*

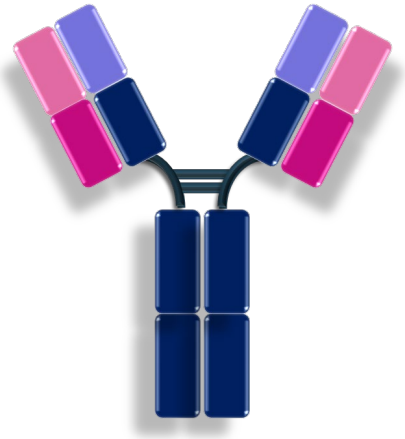
Common Ways to Remove Effector Functions

Removal of the Glycan

- Removing the N-linked glycan at position 297 of the Fc (often by mutation of the N residue to A or Q) can prevent binding to FcR[1]
- One approved antibody with $N^{297}A$ – atezolizumab anti-PD-L1
- May reduce antibody thermostability[2]



Glycosylated IgG1



Aglycosylated IgG1

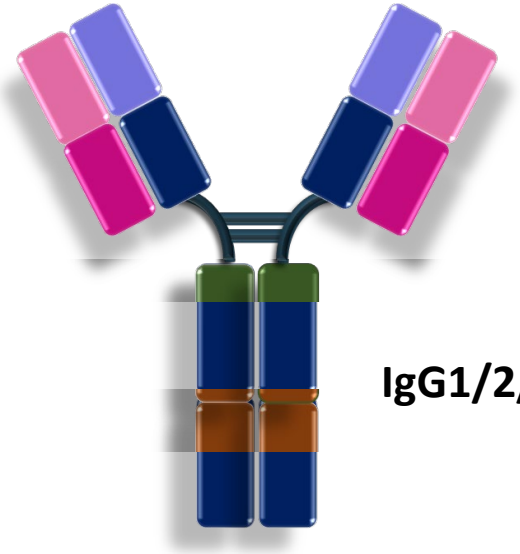
[1]Lux A *et al.* (2013) *J Immunol*
[2]Alsenaidy MA *et al.* (2013) *J Pharm Sci*

Common Ways to Remove Effector Functions

Amino acid modifications

- Hybrid antibodies
  - Common strategy to swap hinge and FG loop regions (binding to FcR regions) of IgG2 and/or IgG4
  - Some SARS-CoV-2 mAbs in development use this approach
  - More effective at removing effector functions than IgG2 or IgG4 full length backbones

- Two amino acid modifications in hinge – $L^{234}A/L^{235}A$ (LALA)
  - >100 fold reduced binding to FcRs[1]
  - Two approved mAbs use this technique
    - Spesolimab (anti-IL-36R) and Teplizumab (anti-CD3)



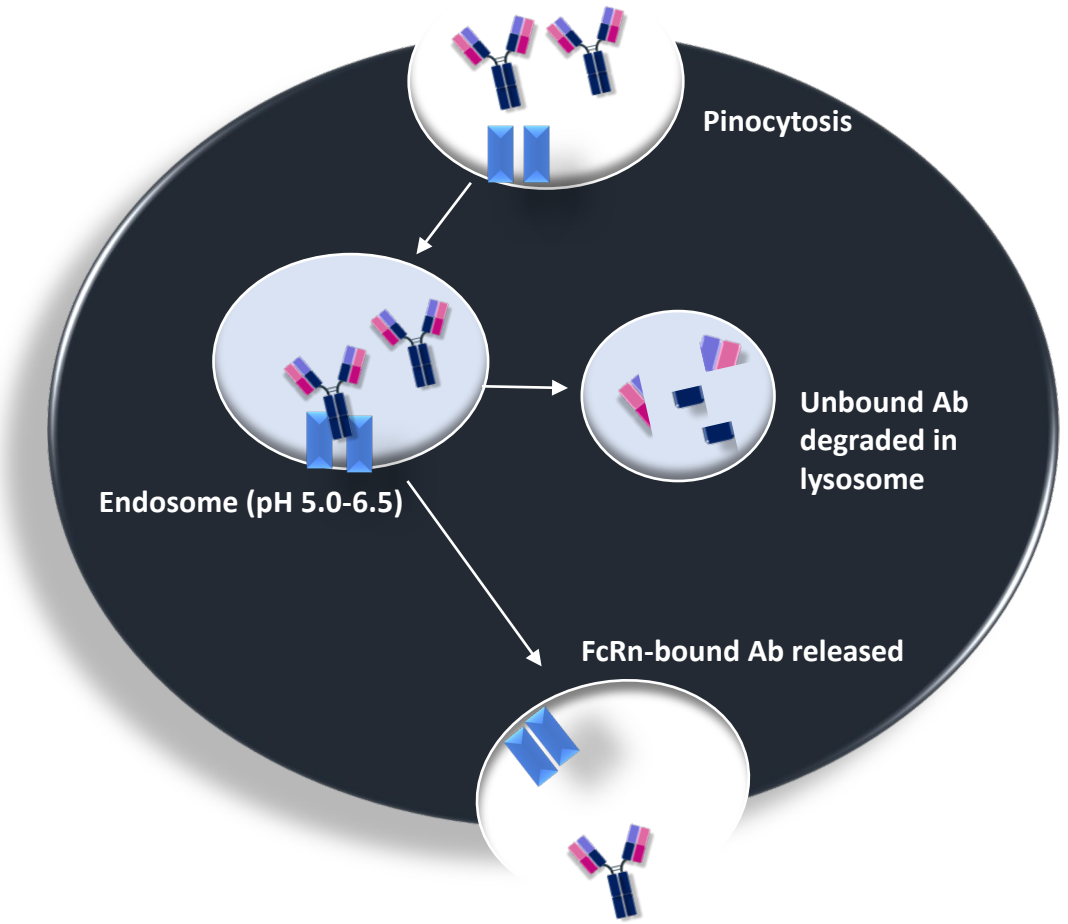
IgG1/2/4 hybrid

IgG1-LALA

[1]Xu D *et al.* (2000) *Cell Immunol*

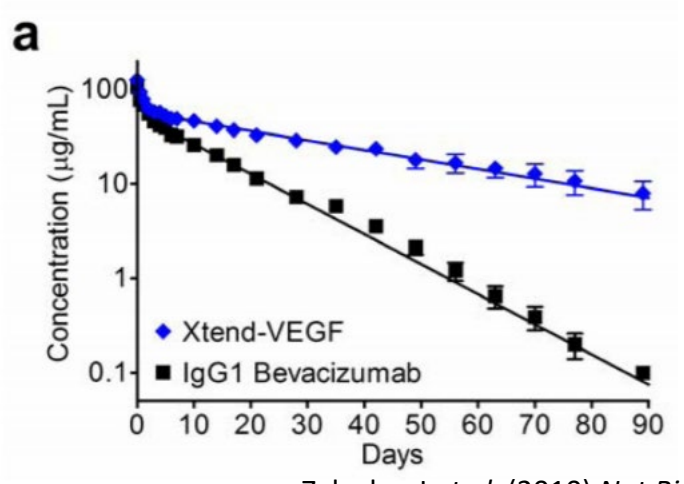
Half-Life Extension

- Increased half-life of mAbs may increase efficacy, decrease dosing frequency, and thus lower cost
- May also afford longer-term protection (> 3 months) against infectious diseases for high-risk individuals (e.g. SARS-CoV-2, HIV)
- Half-life of IgG determined by its interaction with FcRn
- FcRn binds IgG only at pH 5.0-6.5 (endosomal compartments) and not at physiological pH 7.4
- To improve half-life, efforts have concentrated on improving the affinity to FcRn at pH 5.0-6.5

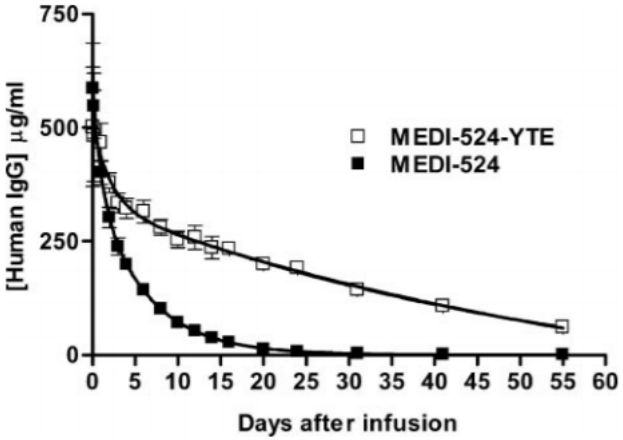


Half-Life Extension

- Two common half-life extension strategies:
  - $M^{428}L/N^{434}S$ (LS/Xtend)
    - 11-fold higher FcRn binding affinity at pH 6.0[1]
    - Ravulizumab (anti-C5a) approved for paroxysmal nocturnal hemoglobinuria, now in trials for SARS-CoV-2
      - Targets same epitope as eculizumab, but half-life of ~52 days compared to ~11 days[2]
      - Eculizumab required treatment every 2 weeks, Ravulizumab every 8 weeks
  - $M^{252}Y/S^{254}T/T^{256}E$ (YTE)
    - YTE improves half-life to up to 80-112 days in humans in anti-RSV mAb clinical trial[3]
    - Also decreases effector functions (i.e. ADCC)[4]
    - Levilimab (anti-IL-6R) approved for use in SARS-CoV-2 in Russia, originally developed for RA



Zalevksy J *et al.* (2010) *Nat Biotechnol*



Dall'Acqua WF *et al.* (2006) *J Biol Chem*

[1]Zalevksy J *et al.* (2010) *Nat Biotechnol*
[2]Rondeau E *et al.* (2020) *Kidney Int.*
[3]Yu XQ *et al.* (2017) *Antimicrob Agents Chemother.*
[4]Dall'Acqua WF *et al.* (2006) *J Biol Chem*

Fc Engineering for Improved ADCC

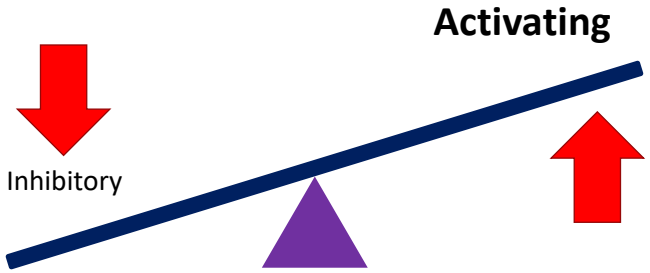
1. Amino Acid Modification
2. Glyco-Modification

Amino Acid Modification

- Many effector cells co-express the inhibitory FcγRIIb along with the activating FcγRs
- To increase pro-inflammatory effector functions, want to skew the activating/inhibitory ratio by increasing affinity to activating FcγRs and/or decreasing affinity to inhibitory FcγRIIb

IgG1-WT

- Able to induce activating signals but also binds inhibitory receptor FcγRIIb



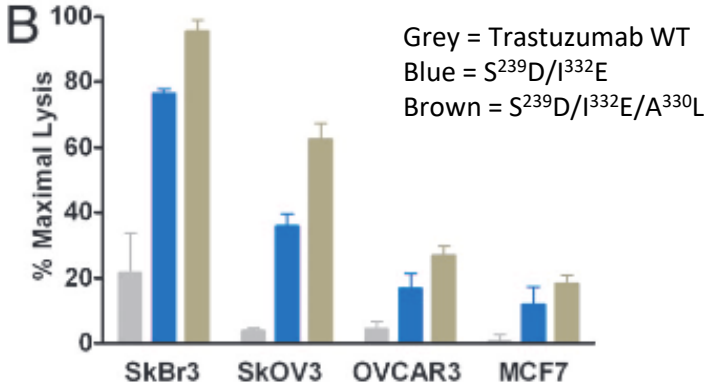
Modified IgG1

- Increased binding to activating receptors/decreased binding to inhibitory receptor

- Key regions and residues for binding to different FcR identified via crystallography and mutagenesis structure/function analyses

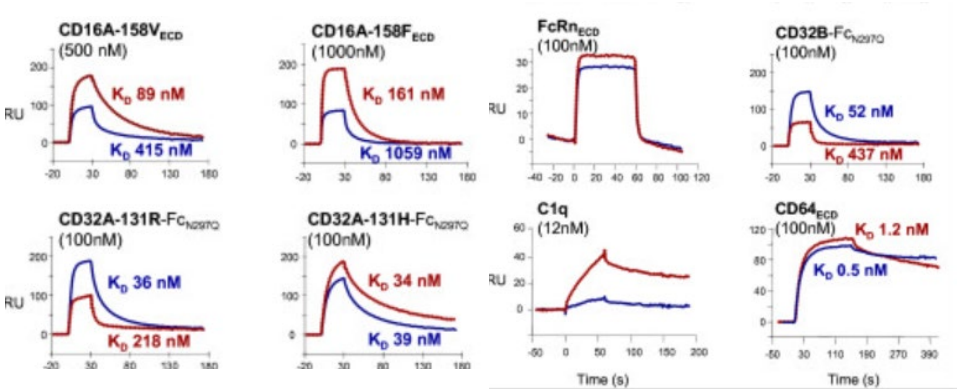
Amino Acid Modification

- Common Strategies to Improve ADCC:
  - $S^{239}D/I^{332}E$ and $S^{239}D/I^{332}E/A^{300}L$
    - Significant increase in binding to FcγRIIIa but also to FcγRIIb[1]
    - ADCC enhanced, modest improvement to ADCP, only $S^{239}D/I^{332}E$ retains CDC[2]
    - Anti-CD19 mAb tafasitamab (incorporating $S^{239}D/I^{332}E$) approved for DLBCL (non-Hodgkin lymphoma) therapy



Lazar G *et al.* (2006) *PNAS*

  - $L^{235}V/F^{243}L/R^{292}P/Y^{300}L/P^{396}L$
    - Enhanced binding to FcγRIIIa and decreased FcγRIIb binding[3,4]
    - Anti-HER2 mAb margetuximab approved for metastatic HER2+ breast cancer – increased progression-free survival compared to non-modified trastuzumab[4,5]

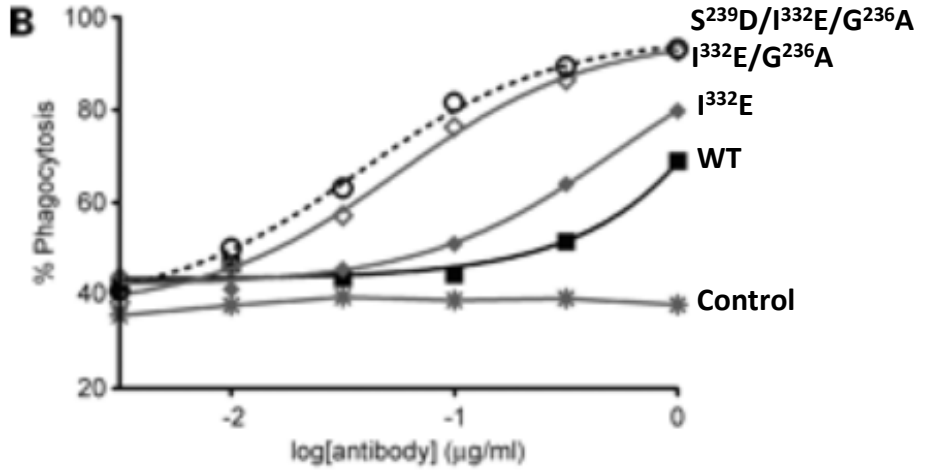


[4]Nordstrom JL *et al.* (2011) *Breast Cancer Res*

[1]Lazar G *et al.* (2006) *PNAS*
[2]Horton HM *et al.* (2008) *Cancer Research*
[3]Mimoto F *et al.* (2013) *Mabs*
[4]Nordstrom JL *et al.* (2011) *Breast Cancer Res*
[5]Rugo HS *et al.* (2021)

Amino Acid Modification

- Strategies to Improve ADCP:
  - **$G^{236}A$**
    - The $G^{236}A$ mutation selectively increases binding to FcγRIIa, but decreases affinity to FcγRI
    - Combined with $I^{332}E$ alone or $S^{239}D/I^{332}E$ restores FcγRI binding and increases FcγRIIIa binding[1]
    - Enhances macrophage-mediated ADCP

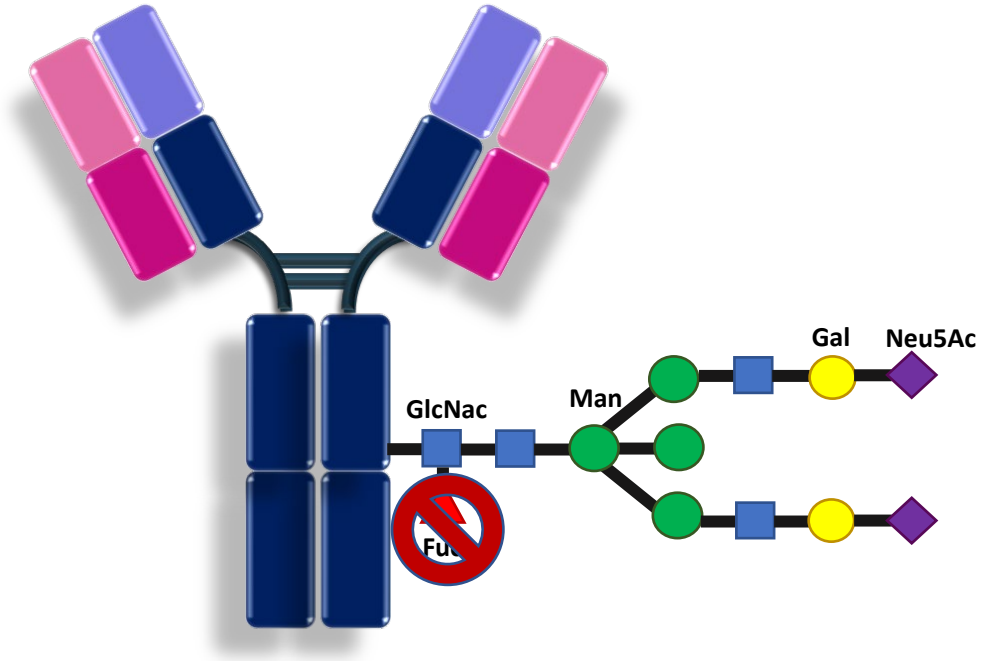


[1]Richards JO *et al.* (2008) *Mol Cancer Therapies*

[1]Richards JO *et al.* (2008) *Mol Cancer Therapies*

Glyco-Modification

- N-linked glycan in the Fc region important for binding to FcR
- Removal of the fucose residue increases binding to FcγRIIIa
  - Potent activating receptor expressed on NK cells and others, induces ADCC
- At least 6 approved/late stage clinical trials designed to be afucosylated to increase activating effector functions
  - Obinutuzumab nearly doubles progression-free survival in CLL patients compared to rituximab[1]
- Viable strategy for SARS-CoV-2? Probably not.
  - Low fucosylation in HIV associated with elite controllers, however associated with enhanced pathology in dengue
  - Recent study demonstrated increase in afucosylated antibodies in critically ill SARS-CoV-2 patients compared to mild symptoms, may be increasing inflammation and exacerbating infection[2]



[1]Goede V *et al.* (2015) *Leukemia*
[2]Laren MD *et al.* (2021) *Science*

Fc Engineering to Enhance FcγRIIb Affinity

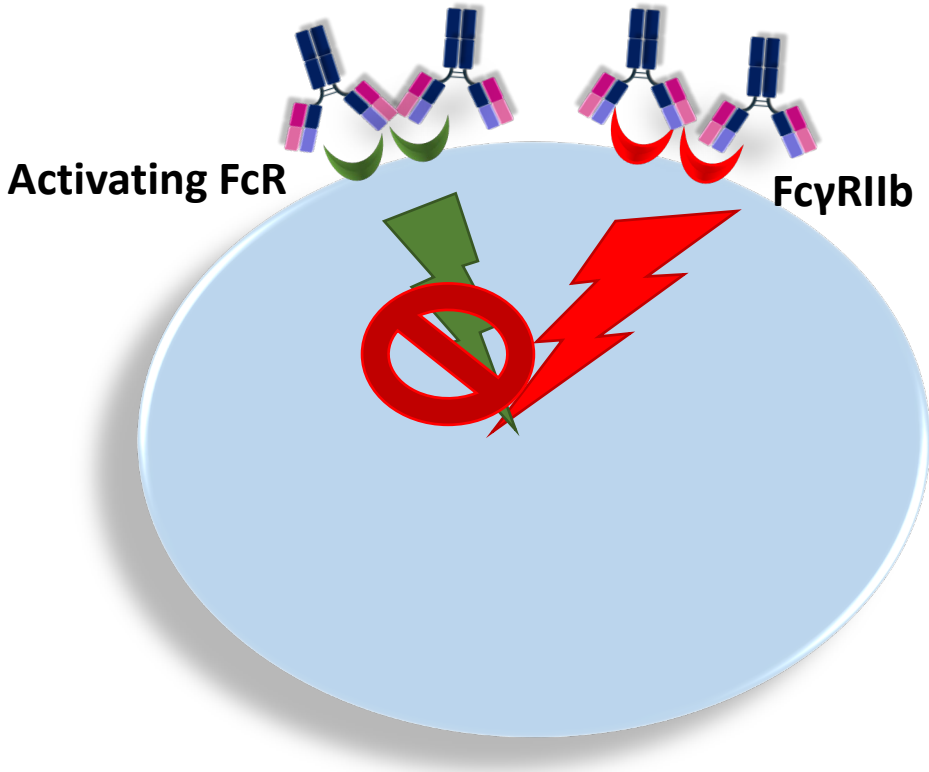
1. Allergy and Autoimmunity
2. Scaffolding

Allergy and Autoimmunity Therapeutics

- FcγRIIb is an inhibitory receptor
  - Can dampen and restrict activating signals – i.e. BCR, other FcR

- Increasing affinity for FcγRIIb can induce powerful suppression of activating signals
  - $S^{267}E/L^{328}F$ (SELF)
    - Increases FcγRIIb affinity by around 400-fold but also increases FcγRIIa-R^{131} allele affinity[1]
    - Obexelimab is anti-CD19 in clinical trials, suppresses BCR signalling and proliferation in autoimmunity
    - XmAb7195 is SELF modified version of the anti-IgE omalizumab, more efficient removal of IgE and also inhibits B cell IgE production

  - $P^{238}D/E^{228}D/G^{237}D/H^{268}D/P^{271}G/A^{330}R$ (V12)
    - Increases FcγRIIb affinity without increasing FcγRIIa interaction[2]



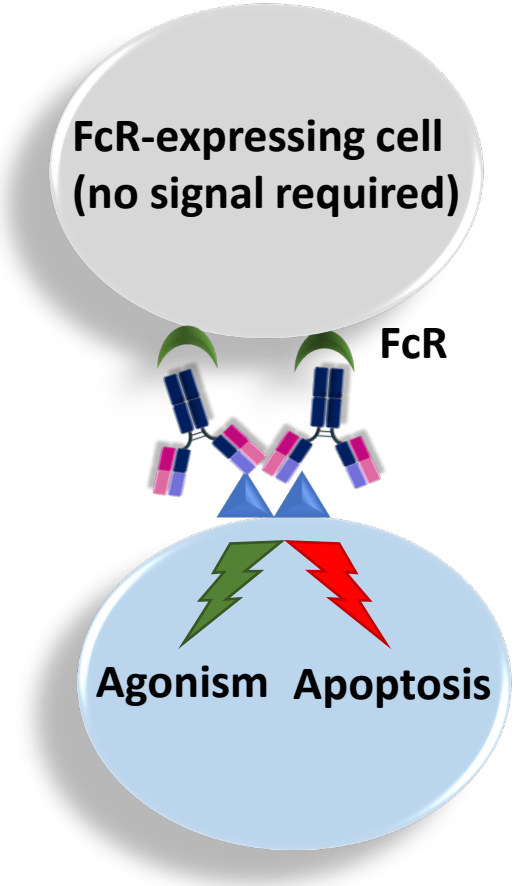
[1]Chu SY *et al.* (2008) *Mol Immunol*
[2]MimotoF *et al.* (2013) *Protein Eng Des Sel*

Scaffolding

- Passive cross-linking of mAb on opsonised target cell
  - FcR signalling not required, but binding to FcR used as a scaffold to help cluster and cross-link target antigen

- Can enhance antitumour immunity by engaging costimulatory molecules on antigen-presenting cells or T cells
  - i.e. CD40, OX40
- Promote apoptosis of target cells by engaging death receptors
  - i.e. Fas, DR4, DR5

- FcγRIIb very effective at scaffolding
  - mAbs developed to enhance FcγRIIb binding greatly improve agonistic function
  - Anti-DR5 mAb with FcγRIIb-increasing mutations induced greater apoptosis and decreased tumour growth[1]
  - Enhanced agonism in anti-OX40 and anti-CD137[2]



[1]Li F *et al.* (2012) *PNAS*
[2]Zhang D *et al.* (2016) *J Biol Chem*

Indirect Engineering for Improved Function

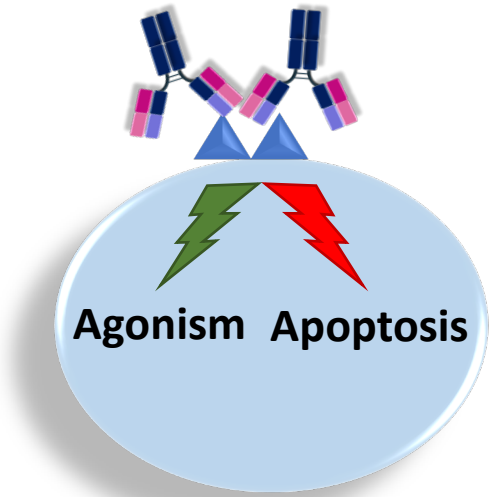
1. Hinge Modification for Enhanced Agonism

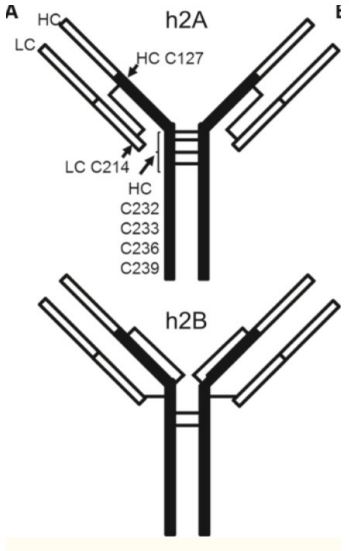
Hinge Modification for Enhanced Agonism

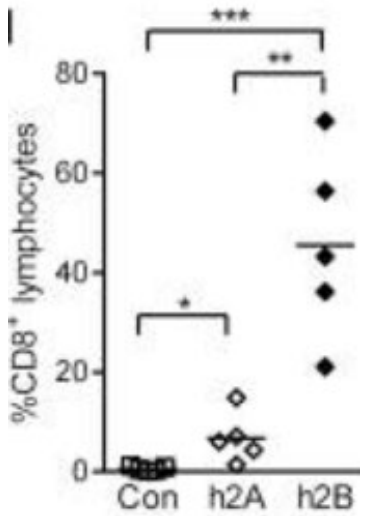
- Passive cross-linking of mAb on opsonised target cell
  - Agents that use FcγRIIb as a scaffolder are limited by the availability of the receptor
  - Alternative is to develop “super-agonists”

- IgG2 has a unique hinge configuration compared to other IgG subclasses
  - Four disulphide bonds, can be in different configurations
  - IgG2-A is more flexible, while IgG2-B is more compact and inflexible[1]
  - Can mutate the cysteine residues to “lock” the antibody into the IgG2-B conformation

- IgG2-B based anti-CD40 mAb was able to induce agonism independently of Fc receptor binding[2]





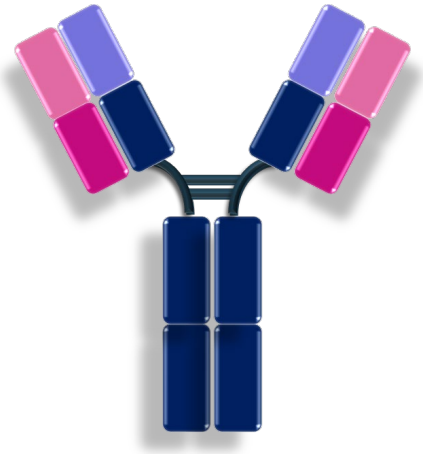


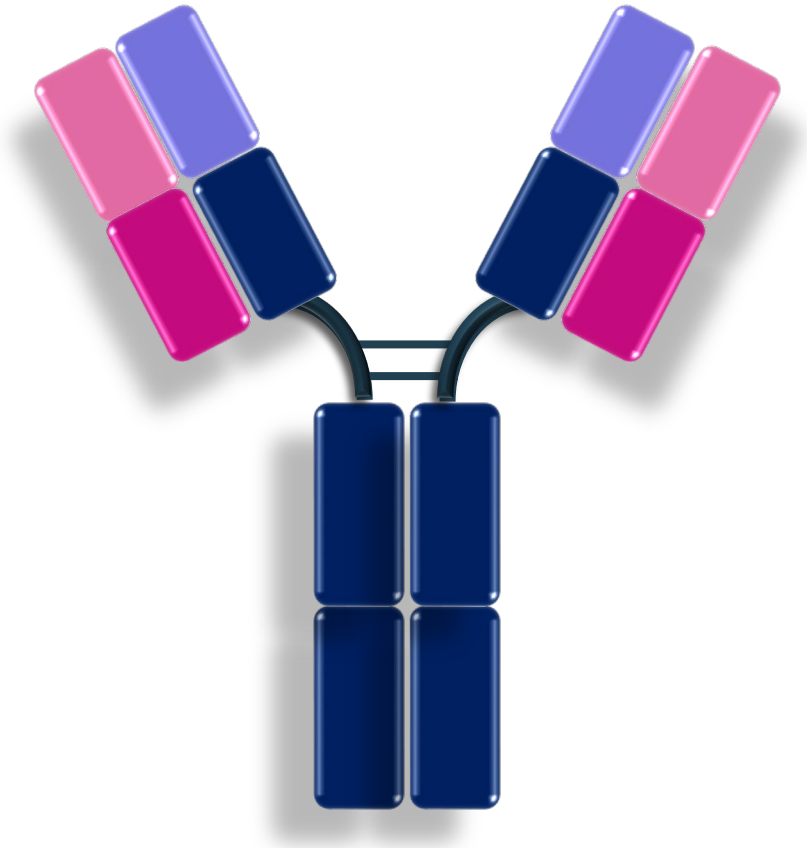
[1]Dillon TM *et al.* (2008) *J Biol Chem*
[2]White AL *et al.* (2015) *Cancer Cell*

[2]White AL *et al.* (2015) *Cancer Cell*

Summary

- Monoclonal antibodies are potent therapeutics in many chronic or otherwise uncurable diseases
- Still extensive unmet clinical need as well as considerable room for improvement in many existing therapeutics
- Understanding the structure/function relationship of antibodies and their receptors have allowed for fine tuning of the antibodies for specific functions
- Widely applicable – lessons learnt from therapeutics designed for one disease can inform the engineering strategy for another unrelated disease
- Improves therapeutics for emerging diseases right out of the gate – i.e. SARS-CoV-2 antibodies engineered with longer half-life for greater efficacy and/or removal of effector functions for greater safety
- **To create the optimum therapeutic, need expertise from multiple sectors**
  - **Antibody experts – understand the structure/function relationship of antibodies and their receptors to create novel modifications for specific functions**
  - **Disease experts – understand how the disease functions and what is required**





Questions?

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