Understanding Mechanisms of Action and Structure-Function Relationships: A Regulator's Perspective

Marjorie Shapiro, Ph.D.
Office of Biotechnology Products/CDER

Bioassays 2018
April 17, 2018

Disclaimer

- The views presented today do not represent official FDA policy, but rather represent my opinion based on my experience as a reviewer of monoclonal antibody and related products at the FDA.

Outline

- OBP regulated products
- In vitro mechanisms of action, in vivo complexities
- Structure function relationships
  - TNF antagonists
  - mAbs with effector functions
- Extra slides
  - Links to biosimilar AC materials
  - mAb glycan references

OBP regulated products MOAs: All need to bind something!

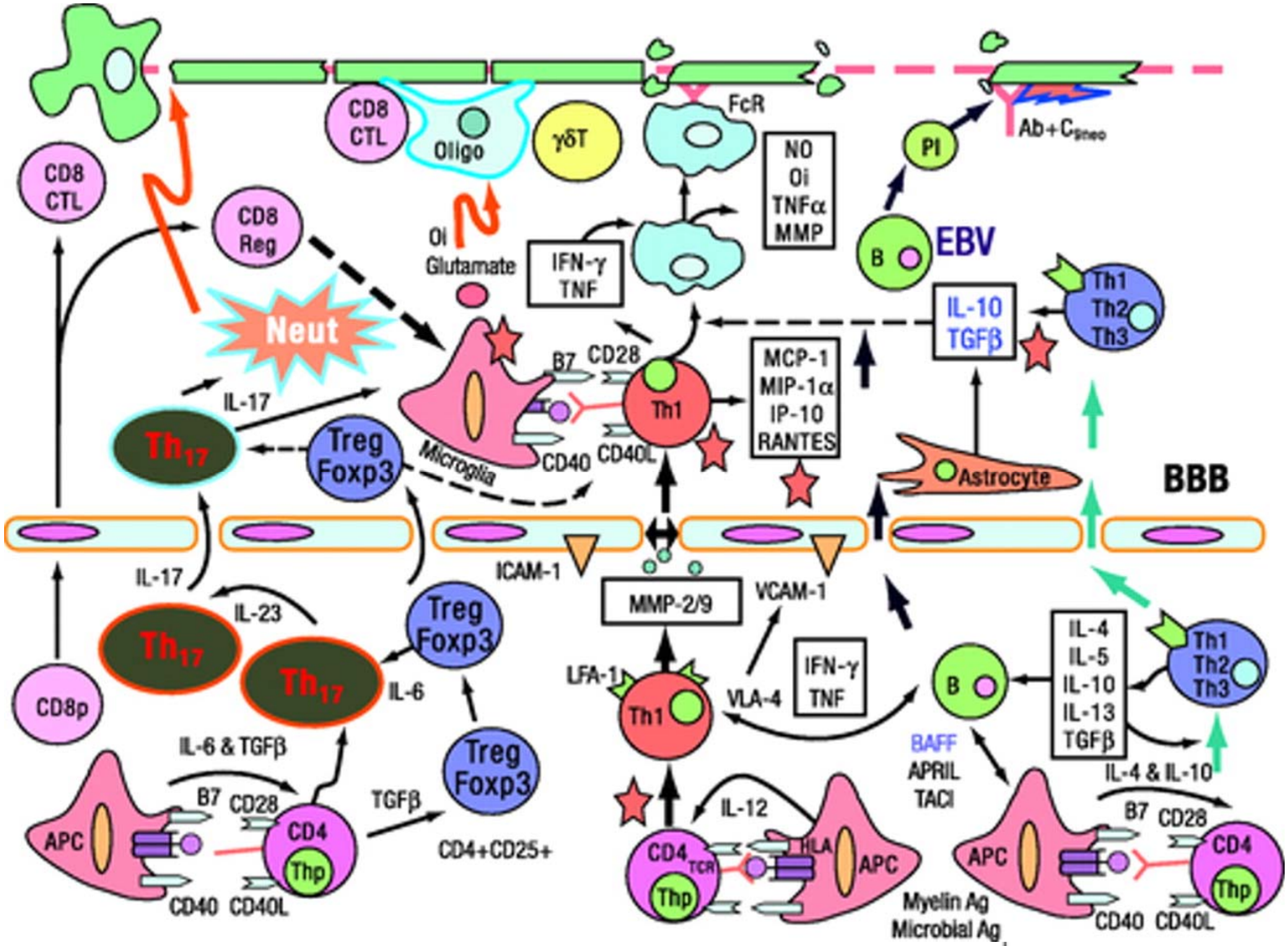
- Enzymes bind their substrates
- Cytokines, growth factors, hormones bind their receptors
- Soluble receptors bind their ligands
  - Soluble and/or membrane bound
- mAbs bind their antigens
  - Soluble and/or membrane bound

- Some enzymes only need to find their substrate, but ERTs need to bind specific mannose-6-phosphate receptors for uptake into lysosome
- Cytokines, growth factors, hormones may have more than one receptor
- Soluble receptors may have a different structure/bind ligand differently than membrane bound form
- mAbs may have Fc-effector functions
  - Multiple effector functions per indication
  - Different (predominant) effector function per indication

What happens after binding?

- Although binding something is a fundamental MOA for all OBP regulated products, we don't always understand how a product works in patients in any given indication
  - Daclizumab (anti-CD25)
    - For prophylaxis of transplantation rejection, blocks IL2 from binding it's receptor and inhibits T cell activation
    - In MS, led to expansion of CD56 bright NK cells which negatively inhibit T cell survival
  - Interferon betas were first approved in 1990s and although we know they have anti-proliferative and anti-viral activity, we still don't have a good understanding of why it works in MS

Figure 1 The immunopathogenesis of the MS lesion and potential IFNβ action sites
Overview of the components of the immune system that are involved in pathogenesis in MS



Suhayl Dhib-Jalbut, and Steven Marks Neurology 2010;74:S17-S24

AMERICAN ACADEMY OF NEUROLOGY

Definition of potency and how to measure it

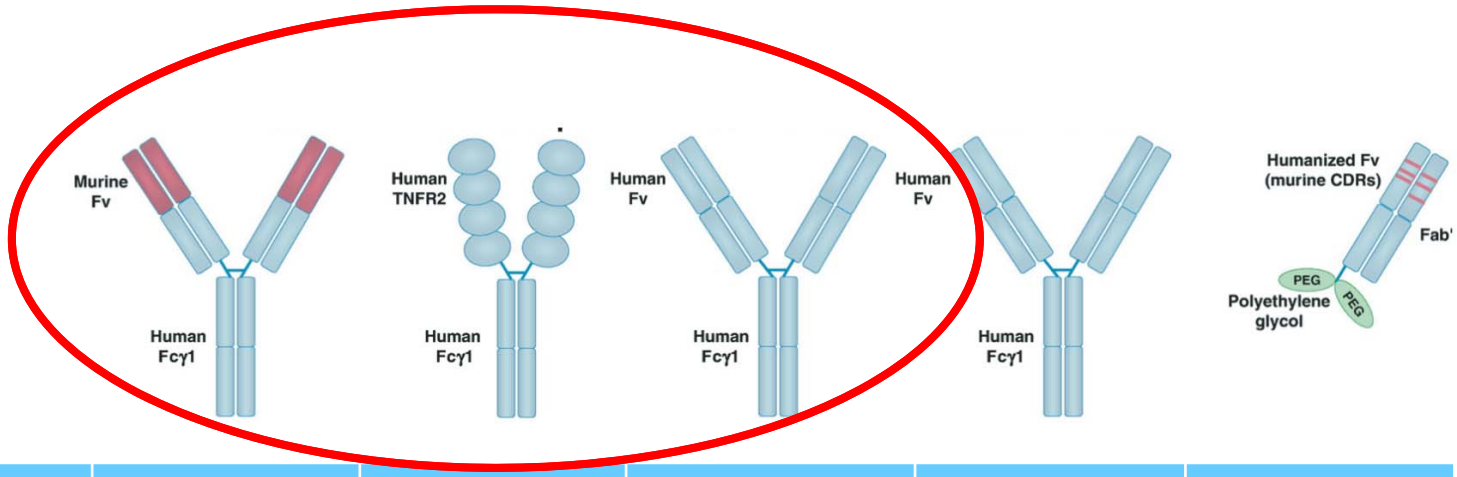
- The word potency is interpreted to mean the specific ability or capacity of the product (…laboratory tests or adequately controlled clinical data..) to effect a given result. 21 CFR 600.3(s)
- “Often, for complex molecules, the physicochemical information may be extensive but unable to confirm the higher-order structure which, however, can be inferred from the biological activity.” ICH Q6B
- Just as we continue to learn about new in vivo pathways by which our products work, we continue to learn new things about specific quality attributes that can affect in vitro potency and possibly in vivo mechanisms.

Structure-Function Relationship Case Studies

- TNF-antagonists
  - Biosimilar etanercept
  - Biosimilar infliximab
  - Biosimilar adalimumab

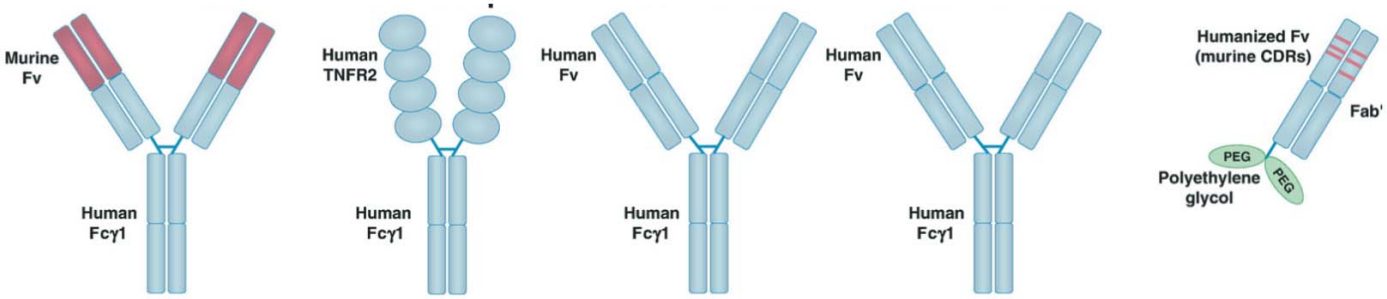
- MAbs with effector functions

Approved TNF Antagonists



Clinical Indication	Infliximab	Etanercept	Adalimumab	Golimumab	Certolizumab
Class	IgG1	TNFR2	IgG1	IgG1	IgG1 / Fab
Origin	Chimeric mouse	Fc Fusion	Human Phage	Human	PEG
Molecular Weight	150	150	150	150	95
Specificity	TNF-α	TNF-α + LT-α LT-α2β1	TNF-α	TNF-α	TNF-α
	Trimer Monomer	Trimer	Trimer Monomer	Trimer Monomer	Trimer

Approved Indications



Clinical Indication	Infliximab	Etanercept	Adalimumab	Golimumab	Certolizumab
Rheumatoid Arthritis	X	X	X	X	X
Juvenile Idiopathic Arthritis		X	X		
Ankylosing Spondylitis	X	X	X	X	X
Crohn's Disease	X		X		X
Pediatric Crohn's Disease	X		X		
Ulcerative Colitis	X		X	X	
Pediatric Ulcerative Colitis	X				
Plaque Psoriasis	X	X	X		
Pediatric Plaque Psoriasis		X			
Psoriatic Arthritis	X	X	X	X	X
Hidradenitis Suppurativa			X		
Uveitis			X		

TNF Antagonist Potential MOAs

MOA	RA	AS	PsA	PsO	CD Pediatric CD	UC Pediatric UC	Statistical approach
Blocking TNFR1 and TNFR2 activity via binding and neutralization of s/tmTNF							
	Yes	Yes	Yes	Yes	Likely	Likely	equivalence
Reverse (outside-to-inside) signaling via tmTNF:							
Apoptosis of lamina propria activated T cells	-	-	-	-	Likely	Likely	
Suppression of cytokine secretion	-	-	-	-	Likely	Likely	
Mechanisms involving the Fc region of the antibody:							
Induction of CDC on tmTNF-expressing target cells (via C1q binding)	-	-	-	-	Plausible	Plausible	QR
Induction of ADCC on tmTNF-expressing target cells (via FcγRIIIa binding expressed on effector cells)	-	-	-	-	Plausible	Plausible	QR
Induction of regulatory MΦ in mucosal healing	-	-	-	-	Plausible	Plausible	

Biosimilar Etanercept: Advisory Committee July 13, 2016

http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisAdvisoryCommittee/ucm481975.htm

Also see Lamanna et al. 2017. Scientific Reports 7 Article number 3951 doi:10.1038/s41598-017-04320-5

Etanercept Structure

- Reference Product : Enbrel®
- TNFR2 : Fc fusion
- 3 N-linked and 10 O-linked glycans
- Molecular weight: 150 kilodaltons
- 13 intrachain disulfide bonds (11 in TNFR2, 2 in Fc) and 3 interchain disulfide bonds (Fc hinge)
- Possesses heterogeneity typical of mammalian cell culture-derived mAbs and fusion proteins

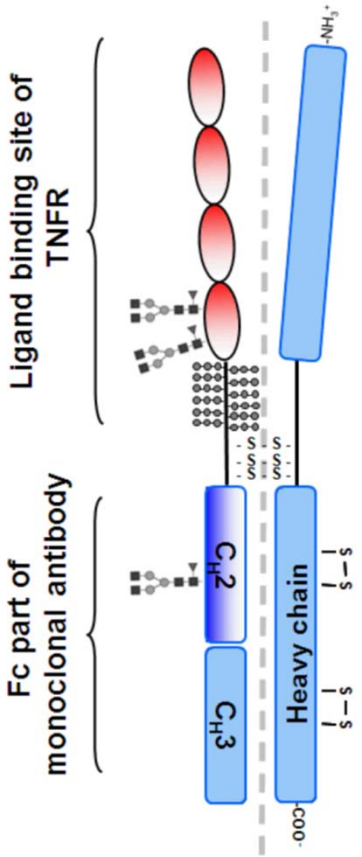
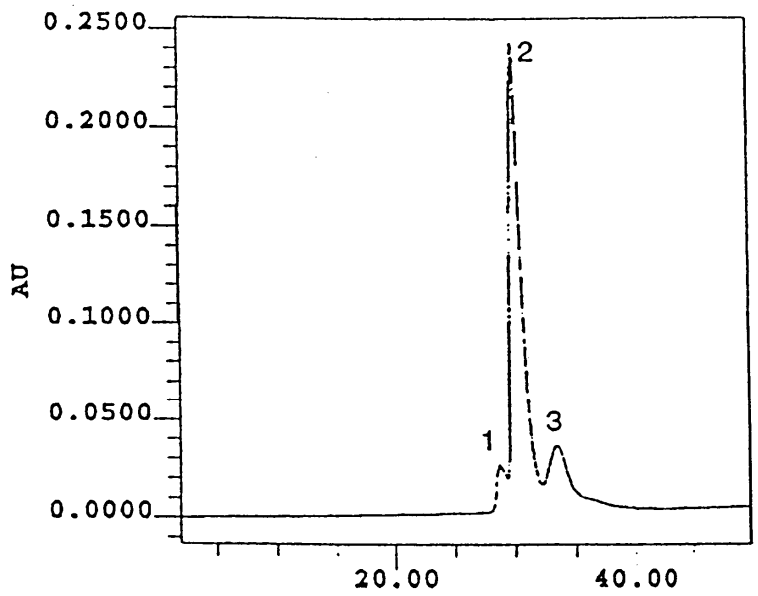
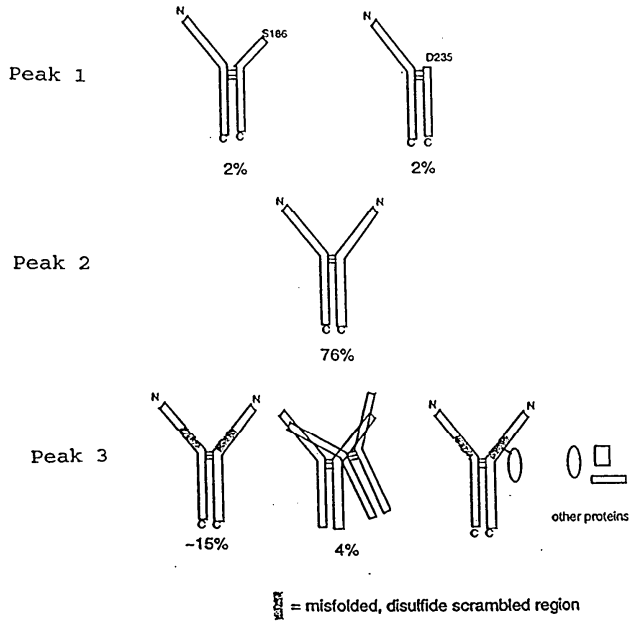


Figure taken from the Sandoz 351(k) BLA submission

Enbrel Product Related Impurities

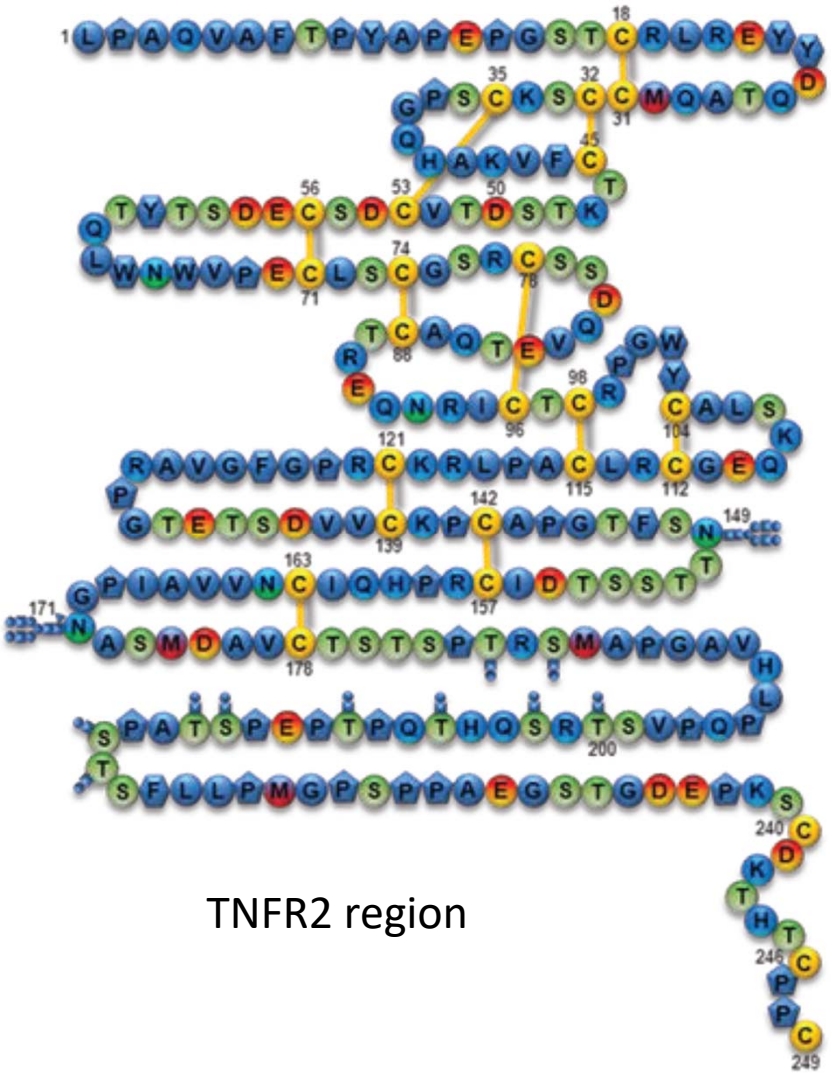




- Published data and patent submissions
- HIC: Peak #3 (compared to peak #2 has reduced potency 18%, reduced binding 13%)
- Misfolded (wrongly bridged disulfide bonds)
- The peak can be identified by HIC or RP-HPLC
- Can be removed/reduced during purification

Patent: Method for producing recombinant proteins US 7294481 B1 (Immunex)
Goswami S, et al., Antibodies 2 452-500 (2013)

Disulfide Bonds



TNFR2 region

TNFR2:Fc disulfide bonds

13 intramolecular
(11 TNFR2, 2 Fc region)

3 intermolecular (Fc region)

All disulfide bonds were identified in both GP2015, US-Enbrel and EU- Enbrel by non-reducing peptide mapping

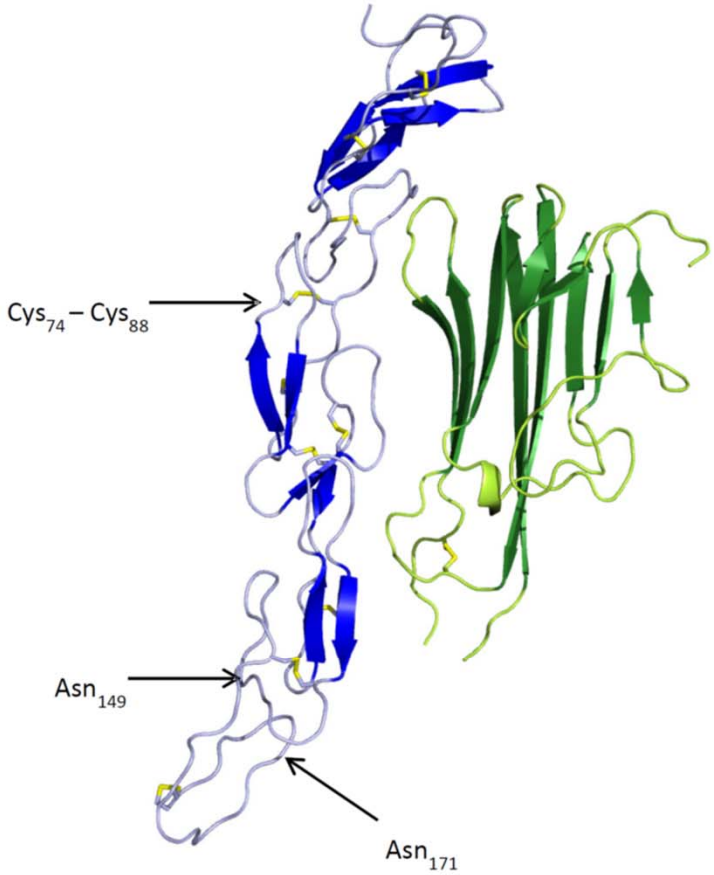
Etanercept contains some misfolded protein due to wrongly bridged variants (WBV)

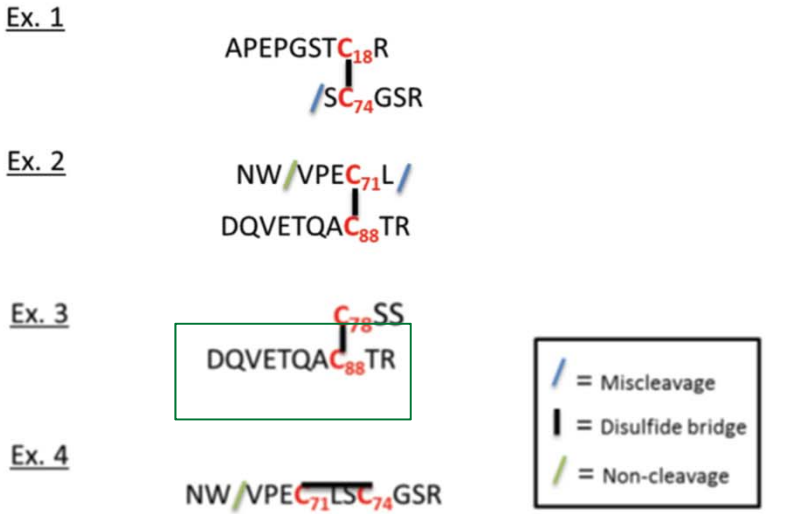
Figure taken from the Sandoz 351(k) BLA submission

TNFR2 binding site and WBV

U.S. FOOD & DRUG ADMINISTRATION

Figure 6-13 X-ray structure of GP2015 co-crystallized with TNF-alpha





In Figure 6-14, the binding regions of the TNFR2 (Etanercept is a fusion protein consisting of TNFR2 and an Fc-part of an IgG1 antibody) to TNF-α are assigned.

Figure taken from the Sandoz 351(k) BLA submission

Differences in Levels of Hydrophobic Variant by Reverse Phase Chromatography

Product	# of lots	Sample mean, %	Sample standard deviation, %	Min, %	Max, %
GP2015	19	10.73	0.62	9.6	11.8
US-licensed Enbrel	21	16.16	1.91	10.2	17.4
EU-approved Enbrel	26	17.54	2.01	12.3	19.8

Structure –function relationship: TNF neutralization assay did not meet equivalence criteria However, the difference in the level of WBV did not affect TNF binding.

Source: FDA analysis of the Sandoz 351(k) BLA submission

Relationship Between WBV and Potency

- The T7 peptide can be used as a surrogate for misfolded etanercept
- There is an inverse relationship between % T7 peptide and potency
- Differences in WBV between GP2015 and US-Enbrel affect bioassay results
- Requested that Sandoz explore the possibility that WBV can correctly refold

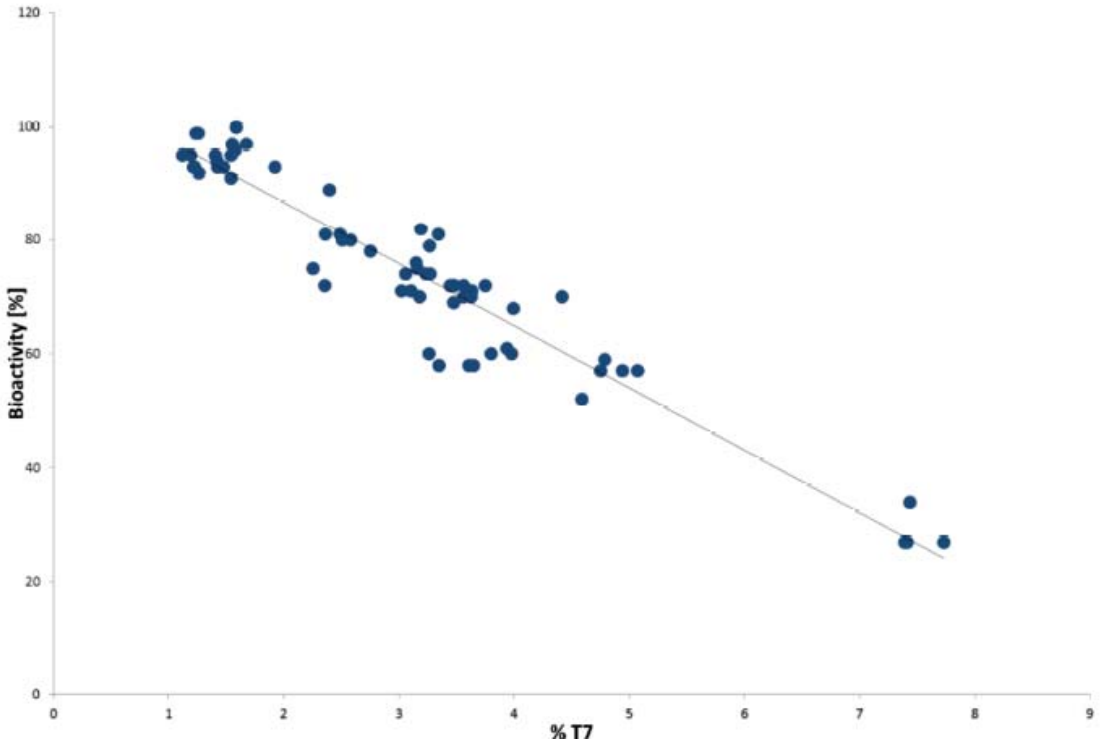


Figure taken from the Sandoz 351(k) BLA submission

Restoration of *in vitro* Potency Under Redox Conditions

- Using redox conditions for the TNF-α reporter gene assay
  - There is a decrease in the % T7 peptide and an increase in the % potency

Sample	Control		Redox Incubation	
	T7 (% rel to standard peptide)	Potency (%)	T7 (% rel to standard peptide)	Potency (%)
GP2015 DS	1.0	99	1.2	103
GP2015 Process Intermediate 1	3.4	76	1.6	98
GP2015 Process Intermediate 2	5.5	58	2.0	93
DP2015 DP 1	1.2	98	1.5	103
DP2015 DP 2	1.8	97	1.3	101
DP2015 DP 3	1.2	100	1.7	98
Enbrel/US 1	2.6	89	1.7	107
Enbrel/US 2	2.5	85	1.8	98
Enbrel/US 3	2.8	81	1.8	96
Enbrel/US 4	2.5	85	1.8	95
Enbrel/EU 1	2.3	92	1.6	100

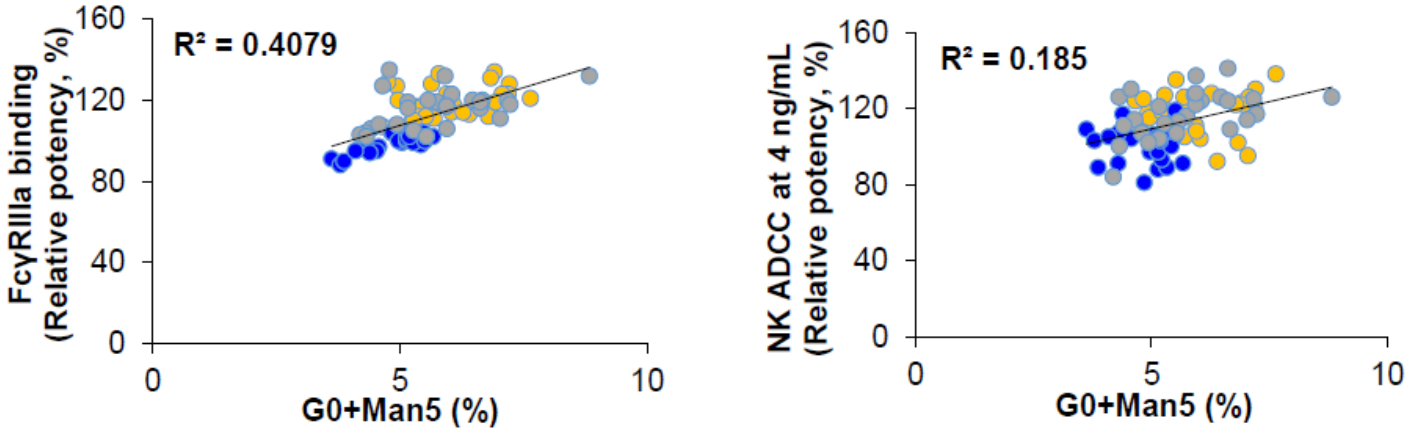
Figure taken from the Sandoz 351(k) BLA submission

Biosimilar Infliximab: Advisory Committee February 9, 2016

https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisAdvisoryCommittee/ucm510292.htm

Glycan Analysis and FcγRIIIa binding

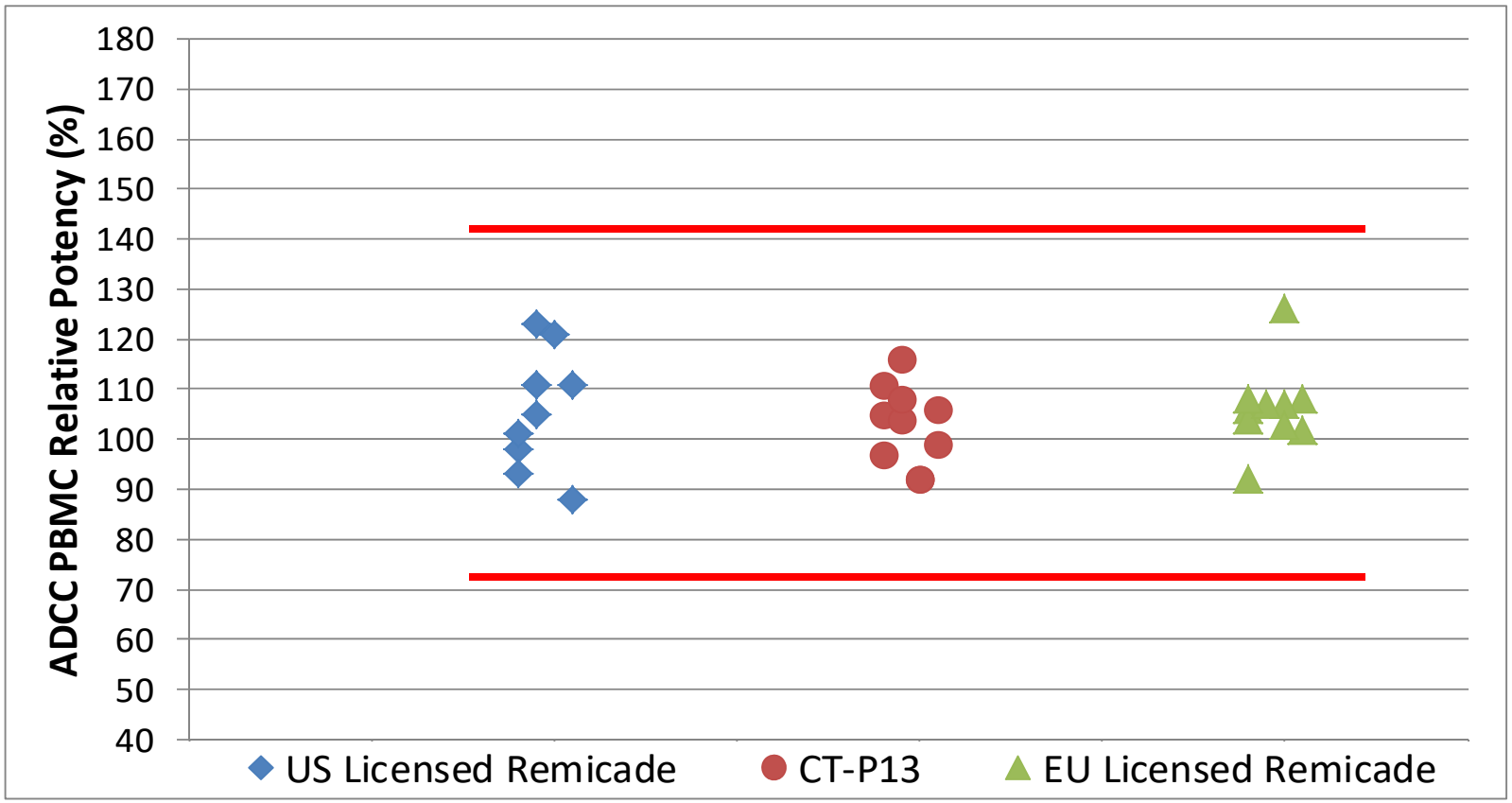
- Compared to US-licensed Remicade, the biosimilar product had slightly
  - Lower G0 (1.1 ± 0.1% vs 2.2 ± 0.2%)
  - Lower Man5 (4.5 ± 0.3% vs 5.1 ± 0.9%)
  - Lower binding to FcγRIIIa



CT-P13 lots are shown in blue; US Remicade lots are shown in yellow; EU Remicade lots are shown in grey

Source: Celltrion Advisory Committee briefing package

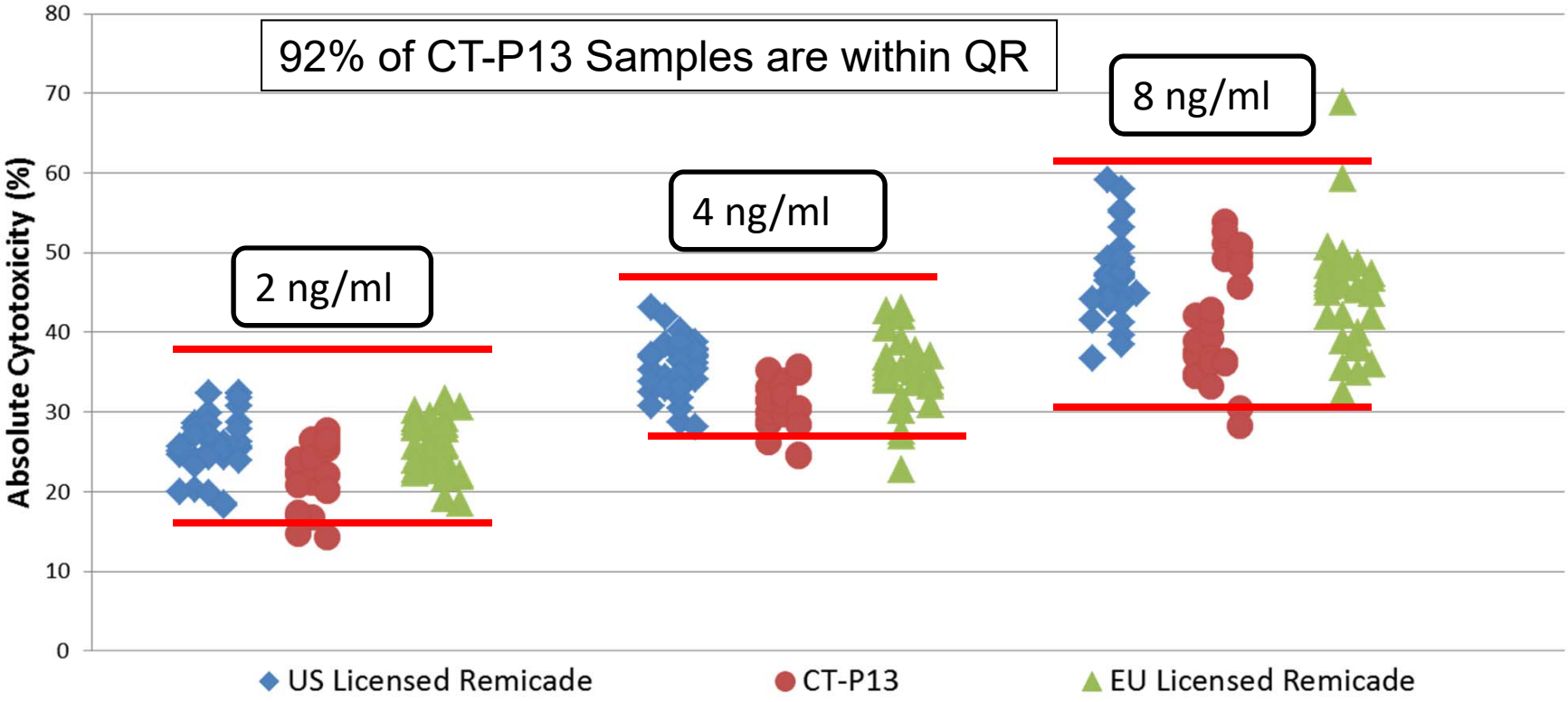
QR Analysis: PBMC ADCC



- ADCC assay uses
  - Transfected transmembrane TNF-α Jurkat cells as target cells
  - PBMC from healthy donor as effector cells

Source: FDA analysis of the Celltrion 351(k) BLA submission

QR Analysis: NK-ADCC Cytotoxicity



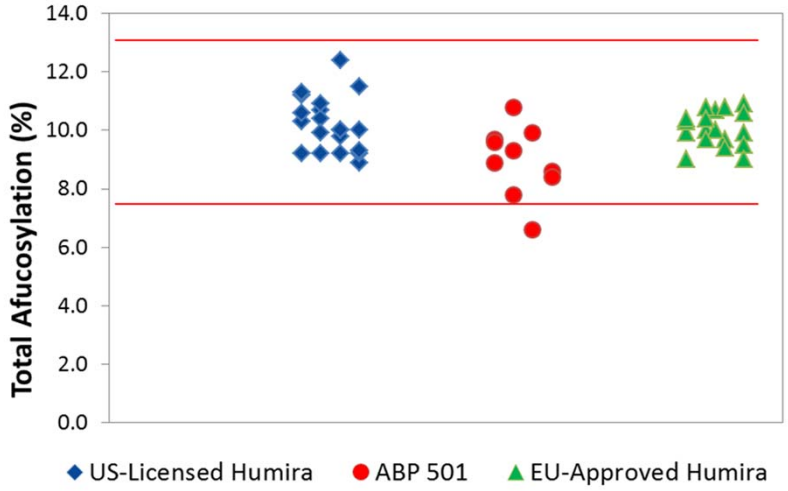
- NK-ADCC assay:
  - Transfected transmembrane TNF-α Jurkat cells used as target cells
  - NK cells purified from peripheral blood used as effector cells

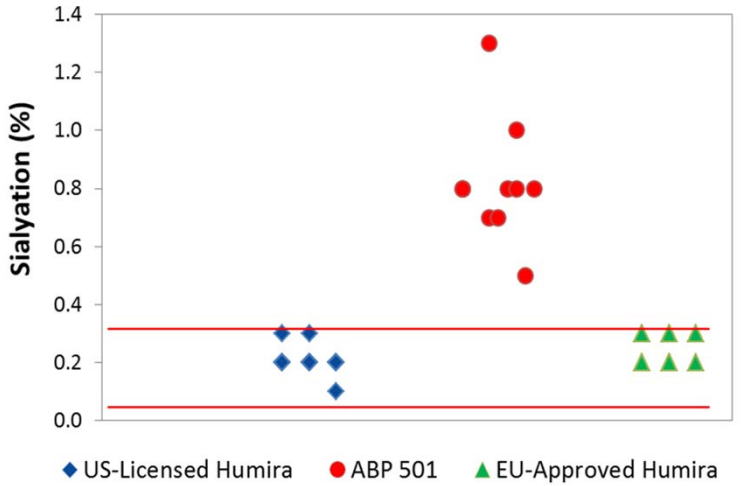
Source: FDA analysis of the Celltrion 351(k) BLA submission

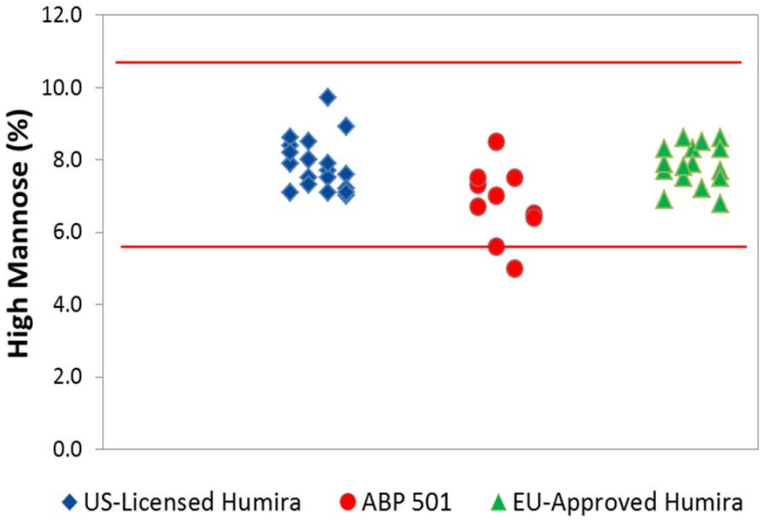
Biosimilar Adalimumab: Advisory Committee July 12, 2016

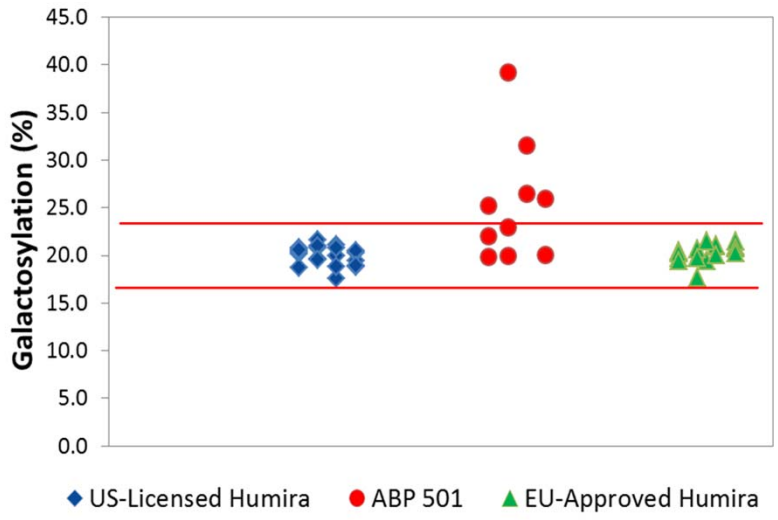
https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisAdvisoryCommittee/ucm510292.htm

Glycan Analysis









Source: FDA analysis of the Amgen 351(k) BLA submission

Summary: Glycan Profile

- Chromatographic profile is visually similar with no new peaks observed
- ABP 501 has a slightly different glycosylation pattern
  - Lower levels of high mannose
  - Lower levels of afucosylation
  - Higher levels of galactosylation
  - Higher levels of sialyation
- Slight differences are mitigated by:
  - Similar FcγRIIIa binding
  - Similar PK profiles
  - Similar ADCC activity
  - Similar CDC activity
- Slight change in levels of glycans do not preclude a determination of high similarity

Structure function relationship between glycan structures and Fc-effector function

Evolving understanding of impact of Fc glycan on mAb function

Boyd 1995 Alemtuzumab
Deglycosylation abolishes CDC/ADCC
Degalactosylation reduces, but does not abolish CDC, no effect on ADCC
Desialyation no effect on CDC/ADCC

Kanda 2006 Rituximab
Afucosylated complex, hybrid and high mannose glycans had higher binding to both FcγRIIIA variants and higher ADCC activity.

Yu 2012 (anti-B cell)
mAb with only high mannose forms has greater ADCC and FcγRIII binding than control mAb, but not as high as 100% afuc version. There was also a decrease in CDC activity

Shields 2002, Shinkawa 2003, Okazaki 2004
Anti-Her2, anti-IgE, anti-IL5R, anti-CD20
Afucosylation improves binding to FcγRIII and enhances ADCC

Houde 2010, Kiyoshi, 2018
Hyper gal (G2) affects CH2 domain conformation (more rigid), increases binding to FcγRIII

Ferrara 2006 and 2011, Shibata-Koyama 2009
Interactions between FcγRIII glycan and Fc glycan

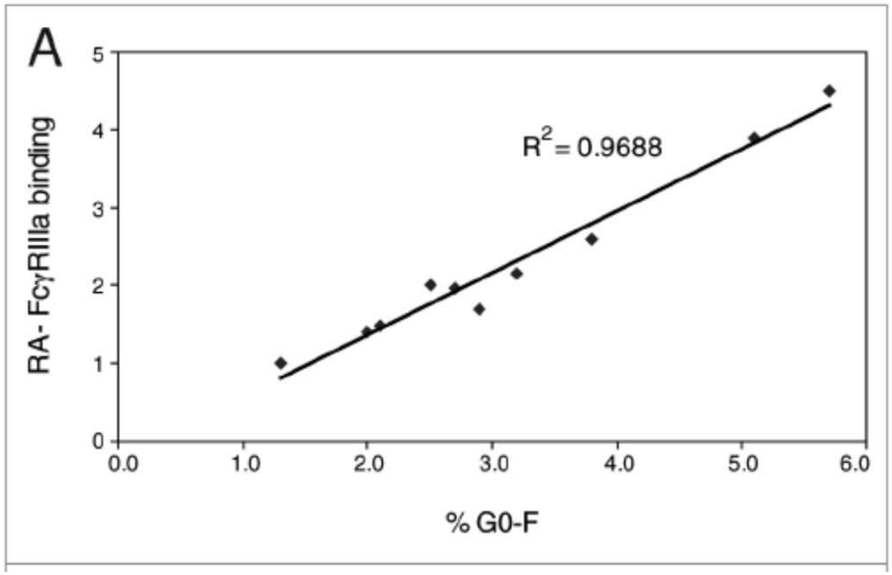
Hodoniczky 2005
Rituximab, trastuzumab
Degalactosylation reduces, but does not abolish CDC, no effect on ADCC
Bisecting GlcNac enhances ADCC

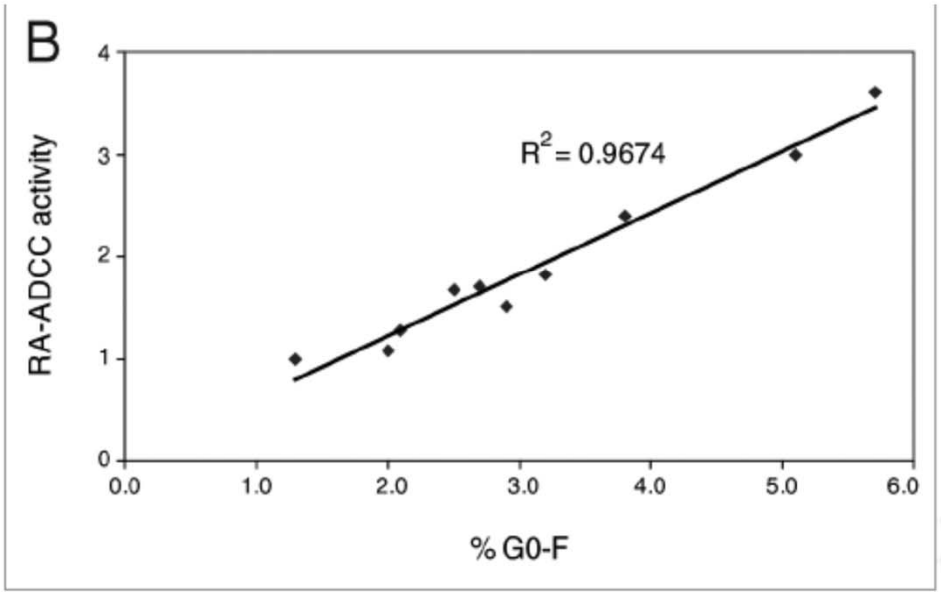
Chung 2012 anti-CD20
Differences in FcγRIII binding and ADCC activity between 0-10% afuc glycans

Shatz 2015 anti-CD20
Only 1 afuc glycan per mAb has as good ADCC activity as a fully afuc mAb

Scallon 2007, higher levels of sialylation associated with reduced ADCC
Lin 2015 rituximab Homogeneous disialyated (G2) afuc mAb has enhanced FcγRIII binding and ADCC

Relationship between afucosylated glycans, FcγRIIIa –F158 binding and ADCC



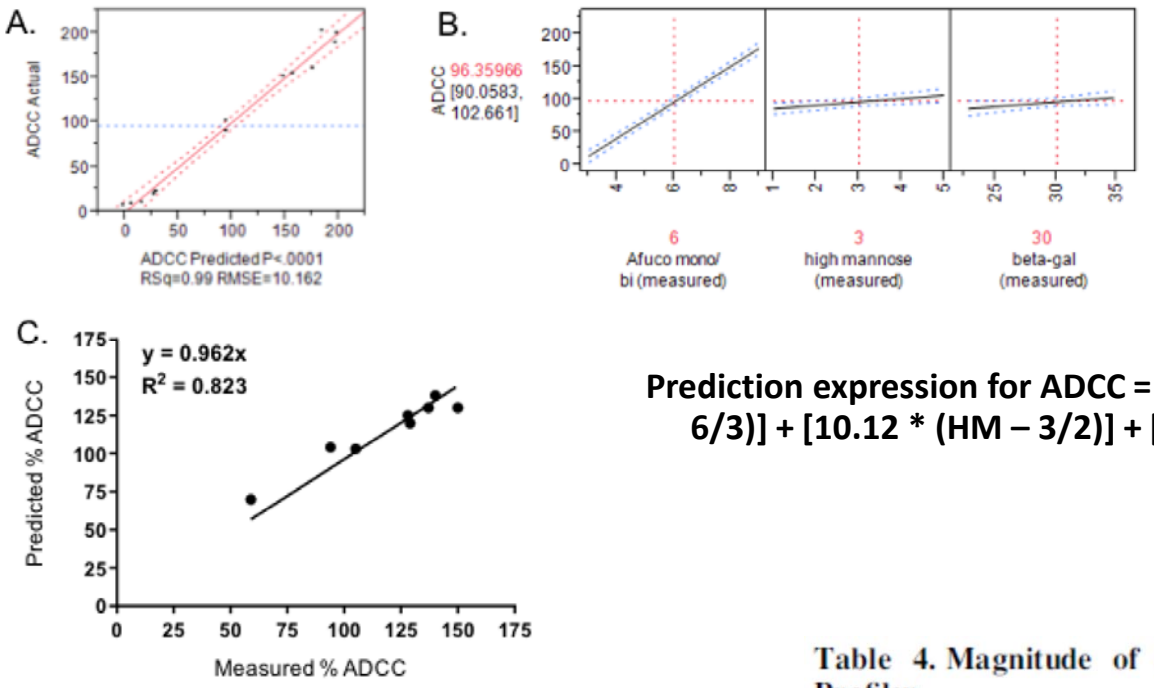


Chung et al 2012 Figure 9
RA = relative activity (relative to fucosylated mAb)

More recently…..

- Studies examined the potential interactions of different glycan structures – afucosylated, high mannose, hypergalactosylated (G2) and sialylated glycoforms
  - Thomann et al., 2016 – interactions between afuc and gal
  - Pace et al., 2016 – interactions among afuc, gal and high mannose
  - Dekkers et al., 2017 - interactions among afuc, gal, high mannose, bisecting GlcNAc and sialic acid
  - Li et al., 2017 - interactions between afuc, gal + sialic acid
- There is a consistent hierarchy in the effect of afuc, high mannose and G2 on binding to FcγRIII and ADCC activity
  - **Afucosylated glycans >>> high mannose glycans >> galactosylated glycans**
- Fucosylation status influences effects of sialic acid
  - See increased ADCC with afucosylated structures
  - In the presence of core fucosylation, sialylation decreases ADCC

Effect of glycans on ADCC



Prediction expression for ADCC = 54.65 + [82.35 * (Afuco mono/bi – 6/3)] + [10.12 * (HM – 3/2)] + [1.39 * β-gal].

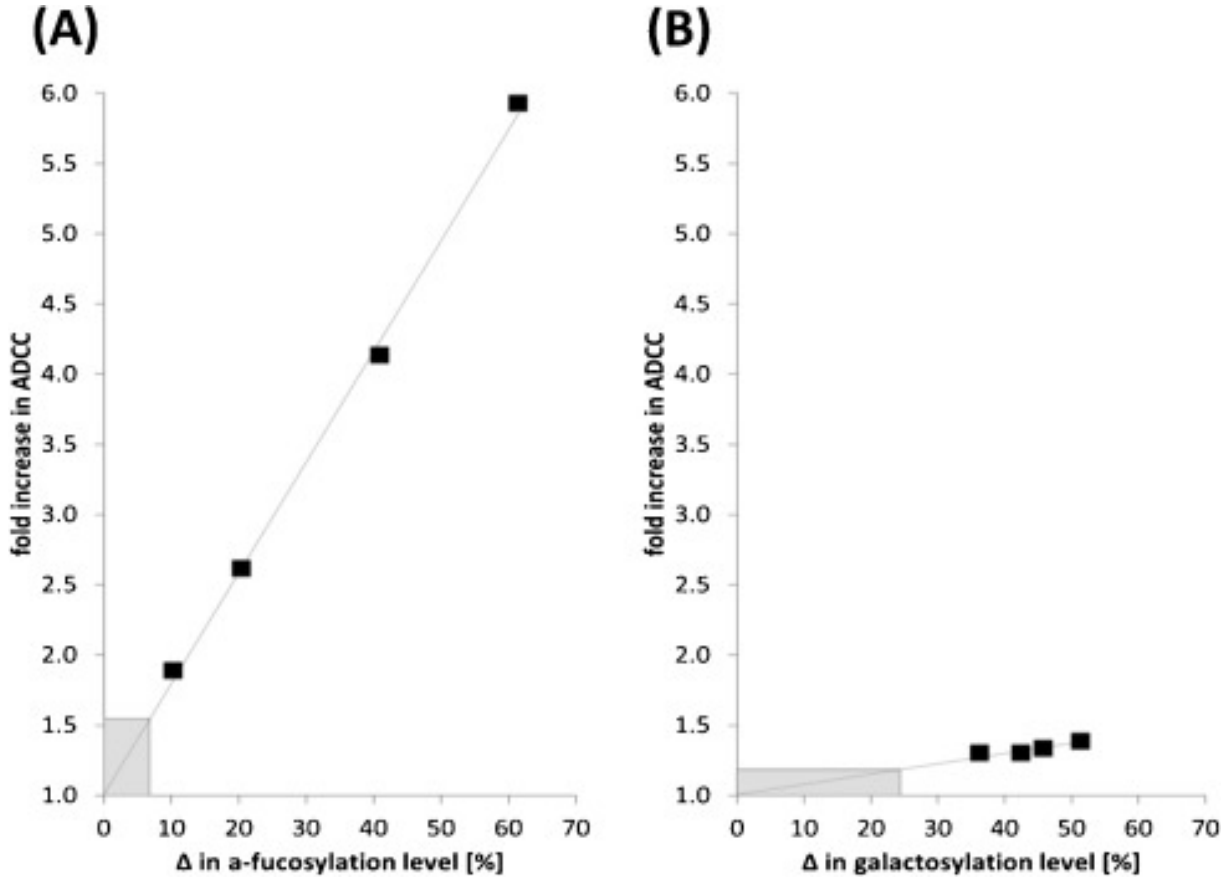
Table 4. Magnitude of Effect of Glycan Input Based on Model Profiler

	Response*			
Glycan	ADCC	FcγRIIIa (158V)	FcγRIIIa (158F)	CDC
% Afuco M/B	27.5	15.8	16.7	–
% HM	5.1	–	–	–
% β-gal	1.4	1.4	2.4	0.8

*Effect of a 1% change in glycan level on the response.

Pace et al, Figure 3 and Table 4

Effect of Glycans on ADCC



Thomann et al, Figure 4

Evolution of control strategies for mAbs with effector function

- Potency assays were historically CDC
  - ADCC with PBMCs not QC friendly
- Starting to see ADCC methods for release
  - Some sponsors now have NK cell lines
  - Reporter gene assays are commercially available or developed in house
    - Not a direct measure of ADCC, but rather binding to CD16 on effector cell
    - Demonstrate the method capability against a true ADCC assay
- Consider both CDC and ADCC methods for the control strategy
- ADCP may be more important for some mAbs or in some indications
  - Still in early days but have seen some

Evolution of control strategies for mAbs with effector function

- Some older products have release specs for some glycan species (generally galactose)
- Starting to see more granular release criteria for
  - Total afucosylated species (Σ afuc + high mannose)
  - Afucosylated species
  - High mannose species
  - Other glycans of concern if warranted (α-gal, NGNA)
  - For some mAbs with Fc-effector potential, but other mechanisms are considered more important, glycans still in the control strategy, but not as concerned with levels of afucosylation
- Under some circumstances may include FcγRIIIa binding

Acknowledgements

- Peter Adams
- Kurt Brorson
- Joel Welch

Links to FDA Advisory Committee Meetings

- **Arthritis AC**
  - Infliximab 2/9/2016
  - Adalimumab 7/12/2017
  - Etanercept 7/13/2017
- https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisAdvisoryCommittee/ucm481975.htm

- **Oncologic Drugs AC**
  - Filgrastim 1/7/2015
- https://wayback.archive-it.org/7993/20170403224015/https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/ucm426351.htm
  - Erythropoietin 5/25/2017
  - Trastuzumab 7/13/2017
  - Bevacizumab 7/13/2017
- https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/ucm547155.htm

MAb Glycan References

- P.N. Boyd et al. Molecular Immunology 1995 V32 p1311
- R.L. Shields et al. JBC 2002 v277 p26733
- T. Shinkawa et al. JBC 2003 v278 p3466
- A. Okazaki et al. JMB 2004 v336 [1239
- J Hodoniczky et al. Biotech Prog 2005 v21 p1644
- Y. Kanda et al. Glycobiology 2006 v17 p104
- C. Ferrara et al. JBC 2006 v281 p5032
- B. Scallon et al. Molecular Immunology 2007 v44 p1524
- M. Shibata-Koyoma et al. Glycobiology 2009 v19 p126
- D. Houde et al. Molecular and Cellular Dynamics 2010 v9 p1716
- C. Ferrara et al. PNAS 2011 v108 p12669
- M. Yu et al. mAbs 2012 v4 p475
- S. Chung et al. mAbs 2012 v4 p326
- W. Shatz et al. mAbs 2015 v5 p872
- C. W. Lin et al. PNAS 2011 v112 p10616
- M. Thomann et al. Molecular Immunology 2016 v73 p69
- D. Pace et al. Biotech Prog 2016 v32 p1181
- G. Dekkers et al. Frontiers in Immunology 2017 https://doi.org/10.3389/fimmu.2017.00877
- M. Kiyoshi et al. Scientific Reports 2018 v8 p3955