Immunogenicity of recombinant biologics:
Post-translational and chemical modifications

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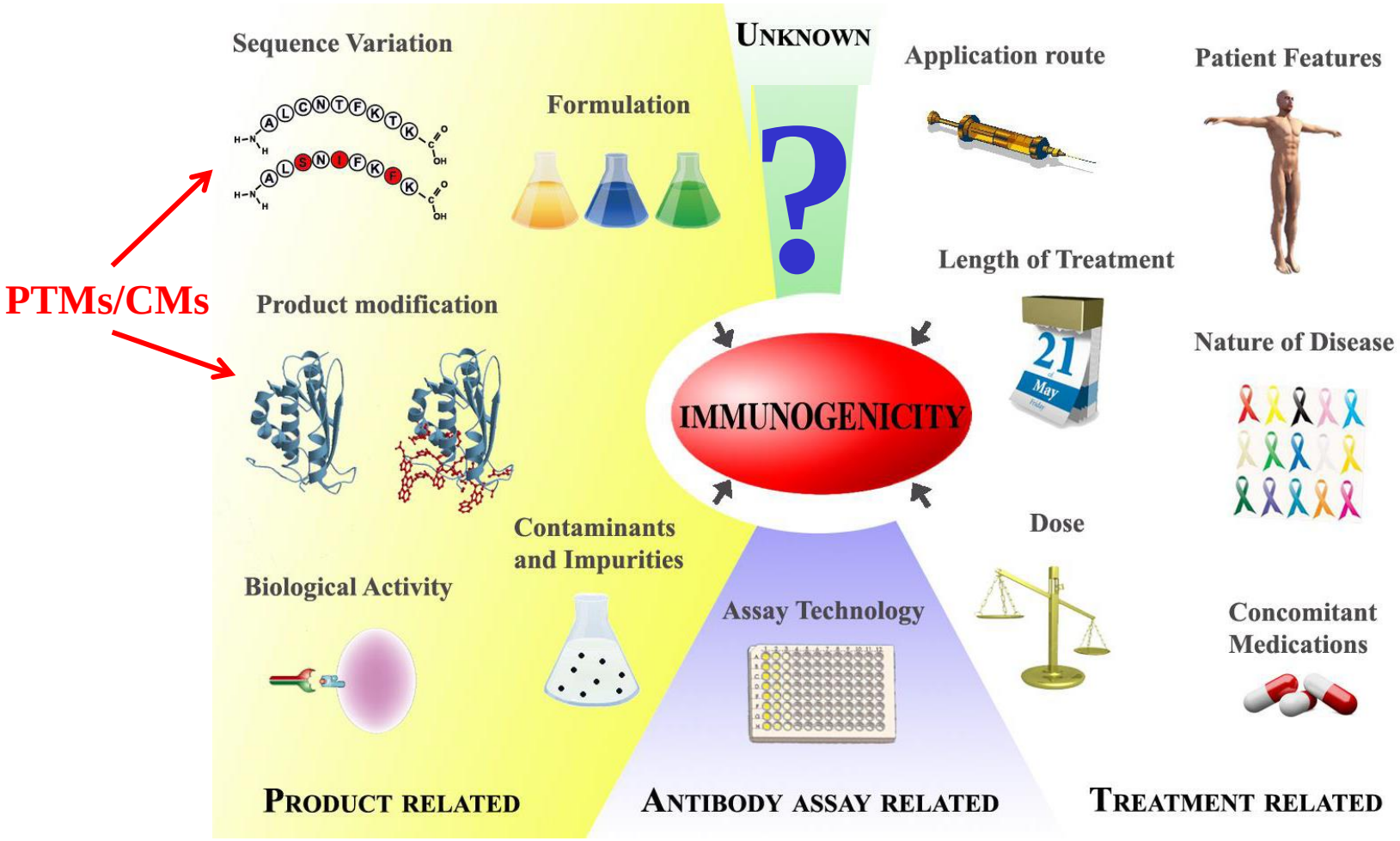
Parameters that may impact immunogenicity



http://www.epivax.com/wp-content/uploads/2013/05/Dr.-Sugiyama_Pfizer_EpiVax-Immunogenicity-Seminar_May2013.pdf

Post-translational and chemical modifications

Protein heterogeneity

Chemical modifications

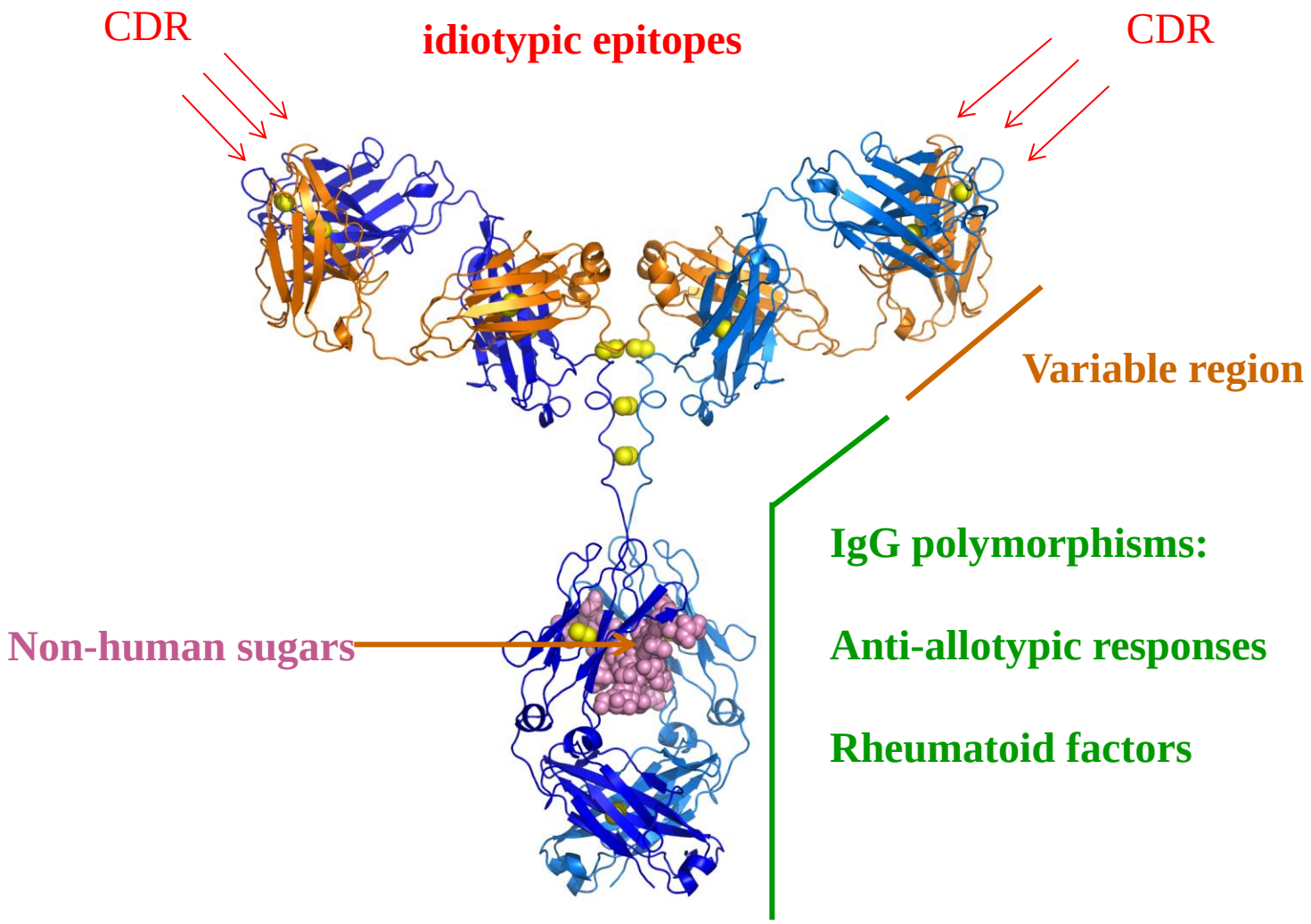
Immune complexes & aggregates

Tolerance/immunosuppression

Quality by design:

DNA sequence:	***Replication fidelity***
Nucleus:	***Transcription fidelity***
ER:	***Translation fidelity***
	Co- & post-translational modifications
Golgi:	***Post-translational modifications***
Culture medium:	***Chemical modifications***
Downstream processing:	***Chemical modifications***
Formulation:	***Chemical modifications***
Storage (shelf life)	***Chemical modifications***
Delivery	***IV, SC, IP, topically, orally***

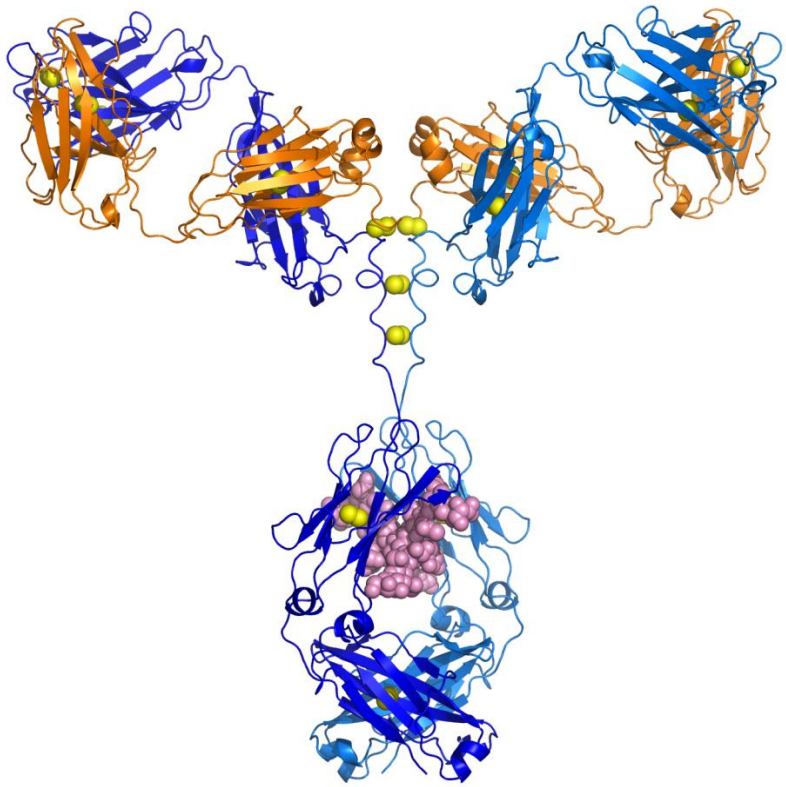
Potential epitopes present in native IgG therapeutics



Jefferis, R. & Lefranc, M-P. mAbs 1:1-7 (2009)

Post-translational and chemical modifications

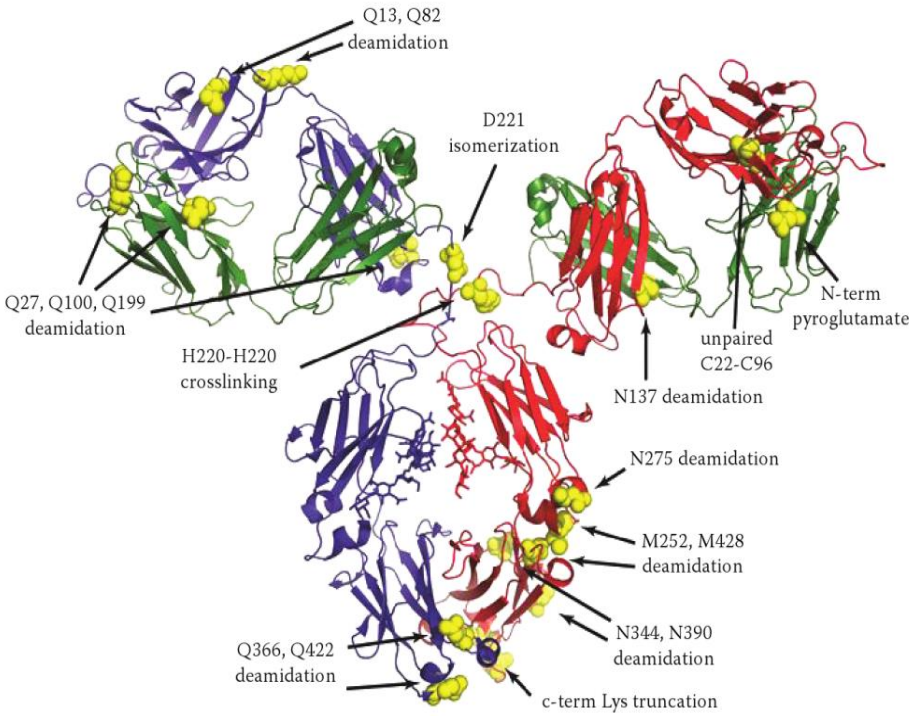
Potential heterogeneity of IgG1: 10^8 molecular forms possible



- **Glycosylation**
- **Deamidation**
- **Deimination**
- **Met oxidation in the Fc**
- **C-terminal Lys processing**
- **Hinge-region fragmentation**
- **Glycation of Lys residues**
- **Pyro-Glu**

Kozlowski, S. et al. Adv. Drug Delivery Reviews 58:707–72 (2006)
Harris RJ. Dev Biol (Basel) 122:117-27 (2005)

Developability of Biotherapeutics:
Computational Approaches



Liu H. et al., mAbs 6:1145-1154 (2014)

Haberger M. et al., MAbs. 6:327–339 (2014)

Kumar S. & Singh SK. Ed. Taylor & Francis Nov 2015

Antibody therapeutics: Mechanisms of action (MoA)

ADCC – antibody dependent cellular cytotoxicity

CDC – complement mediated cellular cytotoxicity

ADCP – antibody dependent phagocytosis

ADA – antibody dependent apoptosis

MBL - lectin pathway of CDC activation

MR – uptake by antigen presenting cells

ADCVI – antibody dependent cell mediated virus inhibition

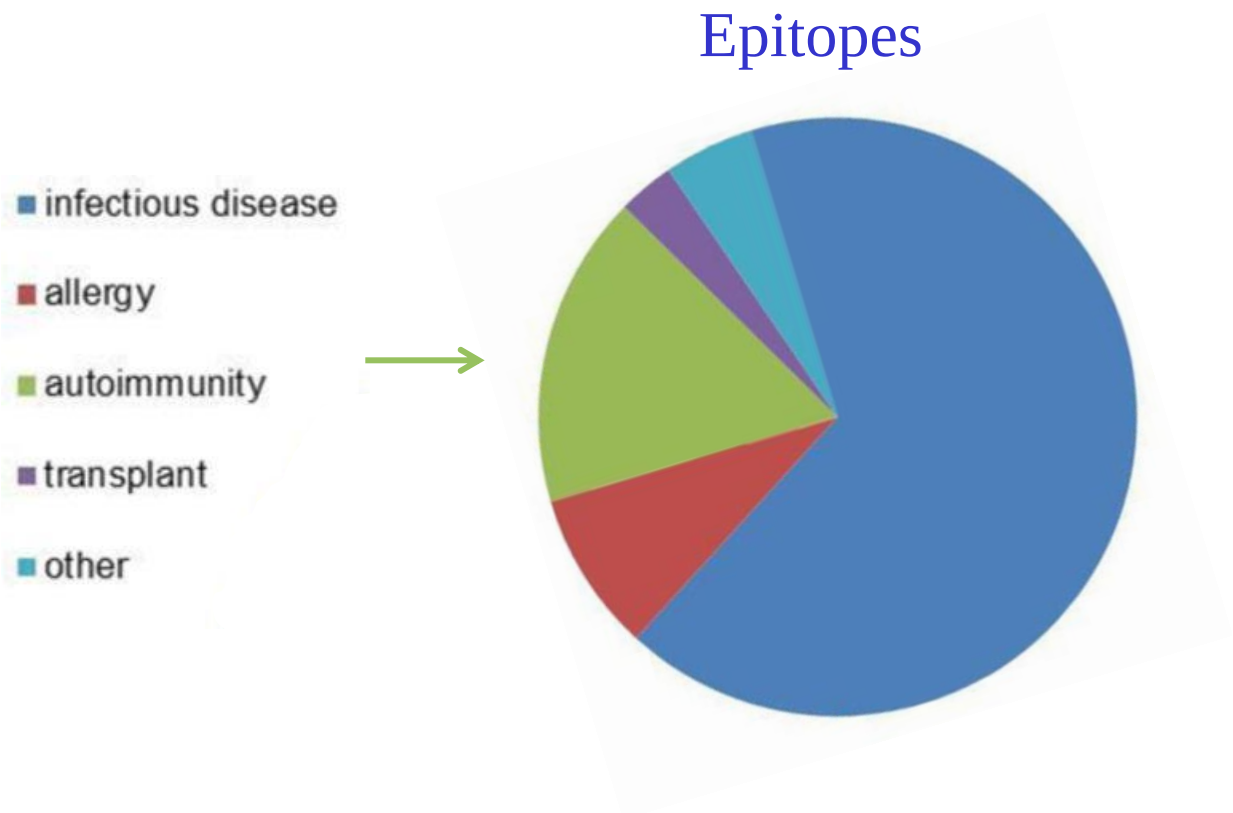
Altered “self”/loss of tolerance

Autoantibodies: the cause or consequence of disease?

Disease	Antigen	Modification
Rheum. Arthritis.	IgG	rheumatoid factor
	fibrinogen	deimination
	filaggrin	deimination
Coeliac	α-gliadin	deamidation
SLE	α-crystallin	phosphorylation
	SnRNP	isoasp generation
AI enceph	MBP	deimination
	MBP	acetylation

PAD4: peptidylarginine deiminase 4

The immune epitope database (IEDB) 3.0.



Vita R. et al., Nucleic Acids Res. 2015 Jan;43:D405-12

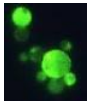
Glycoprotein production vehicles:

Mammalian: CHO, Sp2/0; NSO; Per.C6; HEK 293 etc

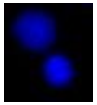


Transgenics: goat; sheep; cows; rabbits; pigs etc

Aves: chickens (eggs)



Yeasts: Pichia pastoris; Saccharomyces cerevisiae



Insect cells: Sf9 (baculovirus infected)

Plants: tobacco; corn; tomato; potato; moss

Bacteria: Escherichia coli; Bacillus subtilis

Post-translational and chemical modifications

Protein heterogeneity

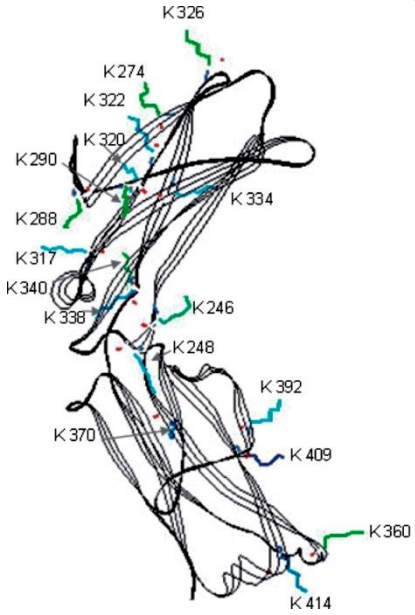
Chemical modifications

Immune complexes & aggregates

Tolerance/immunosuppression

Glycation:

Non-enzymatic adducts of lysine residues with glucose or sucrose



Glucose in media and sucrose in formulation buffer

Loss in complement-fixing activity was observed at glycation levels comparable to those found in diabetics.

IgG bearing 42-49 Glc residues bound FcγRIIIa, FcRn & SpA as for wild type

IgG bearing 67 % Glc express immunogenic epitopes

Ahmad S. et al., Life Sci. 90:980–987 (2012)

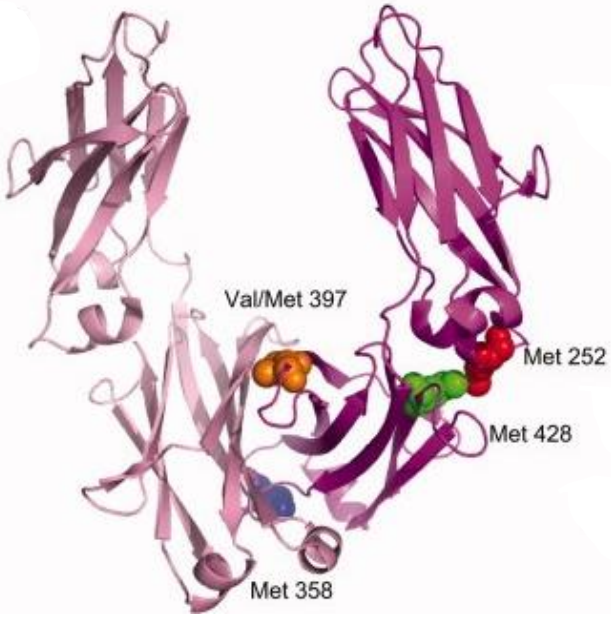
Goetze AM et al., Glycobiol. 22:221-234 (2012)

Gadgil HS. et al., J Pharm Sci. 96:2607-2621 (2007)

Dolhofer R. et al. Biol Chem Hoppe Seyler. 366:361-6 (1985)

Methionine oxidation

Oxidation of Met 252 & 428 impacts FcRn & SpA binding



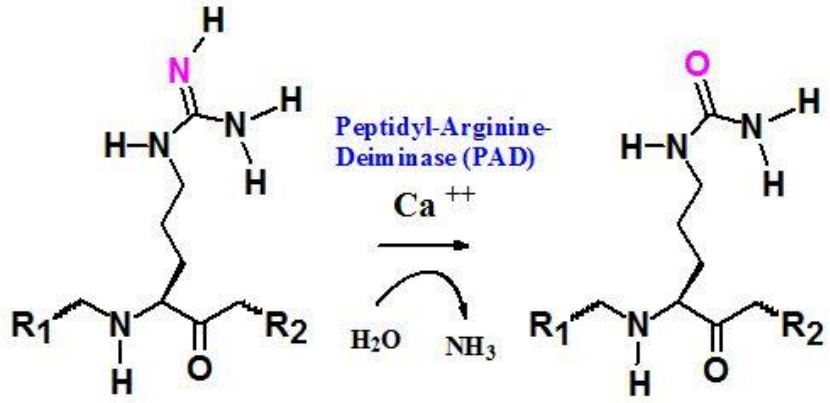
Serum IgG bears 2 – 5 % oxidised Met

Serum IgG half-life influenced when > 80 % Met 252 is oxidised

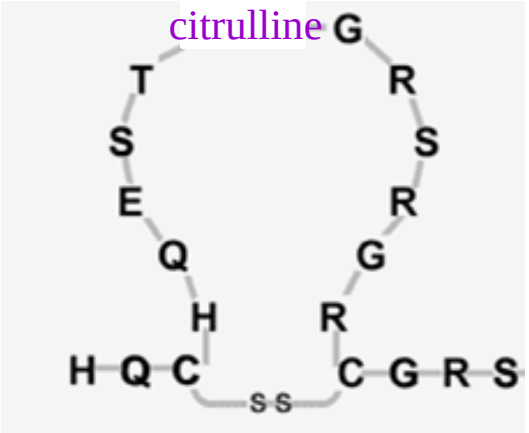
Pan H. et al., Protein Sci. 18: 424–433 (2009).

Deimination of arginine to citrulline

L-arginine



L-citrulline



Shelef MA. et al., Arth Rheumatol. 66:1482-1491 (2014)

Deamidation:

Hydrolysis of asparagine or glutamine to aspartic or glutamic acid

Fc deamidation:

pH 7.5: principally at Asn 389 and Asn 394; pH 5.3: Asn 325

Zhang YT. et al., J Chrom. B Analyt Tech Biomed Life Sci. 965:65-71 (2014)

IgG1 & IgG2 *in vitro* & *in vivo:* Asn residues 325 & 384 are susceptible to deamidation

23 % of Asn residues 384 are deamidated to Asp in normal serum IgG; therefore it may be considered a “self” structure

Diepold K. et al., PLoS One 7:e30295 (2012)

Harris R. et al., J Chromatog B, 752:233–245 (2001)

Deamidation:

Fab (CDR) deamidation

Succinimide formation of Asn 55 (CDR 2) led to loss of activity:
Asn 55 is a highly conserved residue in IgG1 mAbs

Yan B., et al., J Pharm Sci. 98:3509-3521 (2009)

Haverick M., et al., Mabs 6:852-858 (2014)

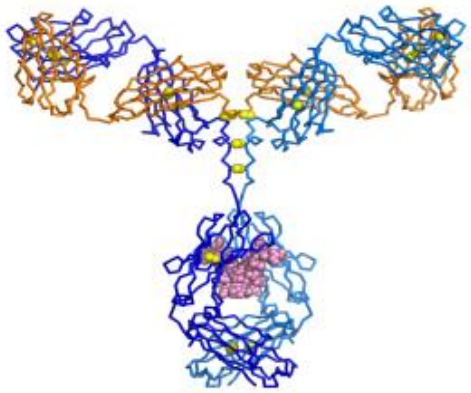
Herceptin (Trastuzumab)

Light chain: Asn 30 in CDR1 > some loss of potency

Heavy chain: Asn 55 > in CDR2;
Asp 102 > isoAsp in CDR3: loss of potency

Harris R. et al., J Chromatog B, 752:233–245 (2001)

Case study: Analysis of proposed Herceptin biosimilar



Conclusion:

This "biosimilar" cannot be approved due to sequence differences between it and the innovator product

Xei H et al., mAbs 2:1-16. (2010)

Note added in proof

The level of deamidation reported for the “biosimilar” antibody are **much higher** than previously reported for the innovator antibody.

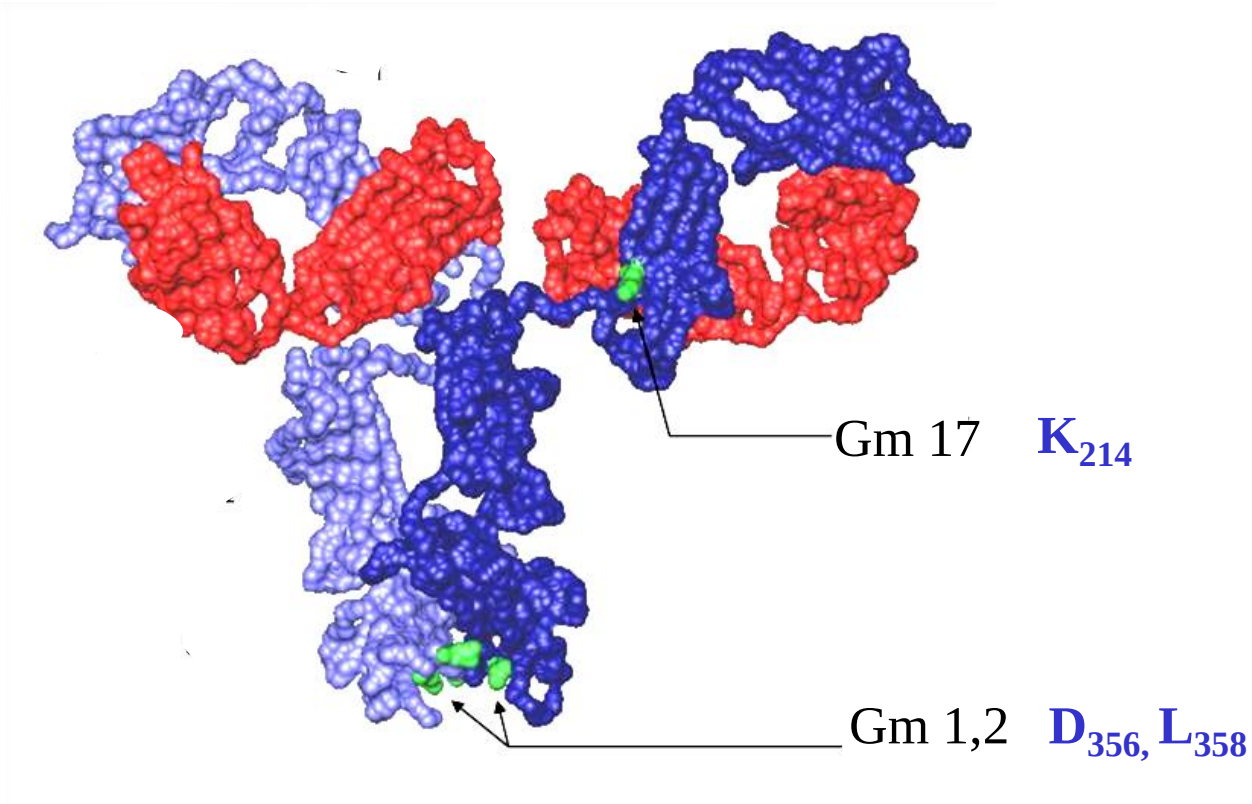
“We believe that the high levels reported for certain peptides may not be reflective of the true level of deamidation in the sample prior to digestion.”

Xie H., Mazeo JR et al., mAbs 2:1-16. (2010)

Harris R. et al., J Chromatog B, 752:233–245 (2001)

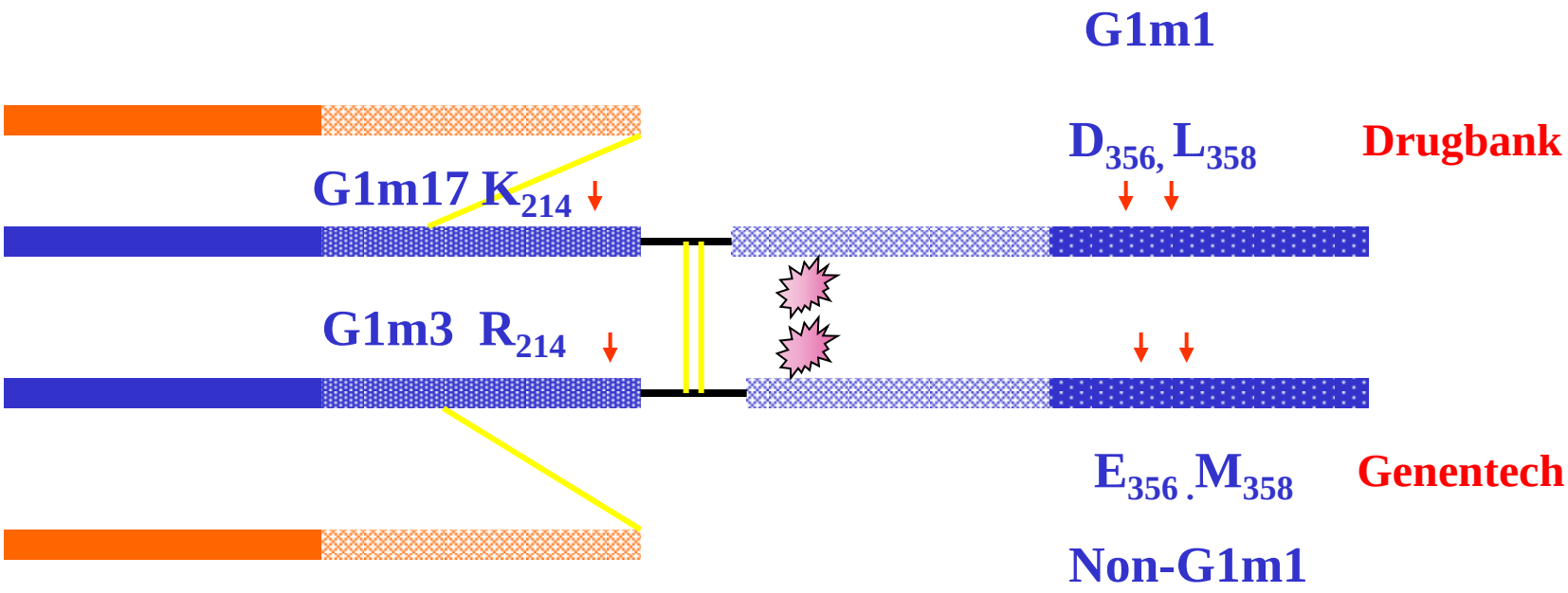
Human IgG polymorphisms:

Possible implications for immunogenicity of antibody therapeutics.



Jefferis R. & Lefranc M-P. mAbs 1:1-7 (2009)

Allotype & sequence of Herceptin



The mutations $D_{356,}$ L_{358} > E_{356} .M_{358} were introduced . “This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.”

Jefferis, R. & Lefranc, M-P. mAbs 1:1-7 (2009)
Carter, P. et al., PNAS 89:4285 (1992)

Epitope characterisation of pre-existing and developing antibodies to an aglycosylated mAb of G1m(1,17) allotype

AMG-x first in human (FIH) study was a randomized, placebo-controlled, double-blind, ascending single-dose study to evaluate the safety, tolerability and pharmacokinetics in healthy subjects

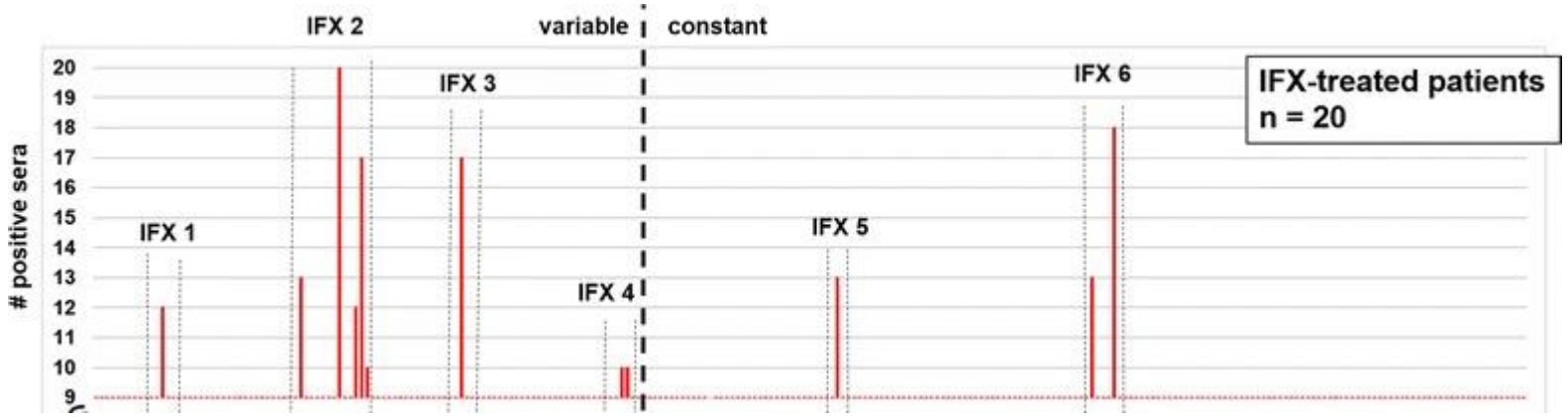
2/94 patients experienced Grade 2 adverse reaction

Each had pre-existing anti- G1m(1) antibody and were homozygous for G1m(3)

Dose: 20 mg SC and 5mg IV

Tatarewicz SM. et al, J Immunol Meth. 382:93-100 (2012)

B cell epitopes on infliximab identified by oligopeptide microarray with unprocessed patient sera.



IFX 5: LSSVVTVPSS**SL**GT**Q**TY**I**C̲NVNHKPSNTKVDK**K**VEP G1m(17)

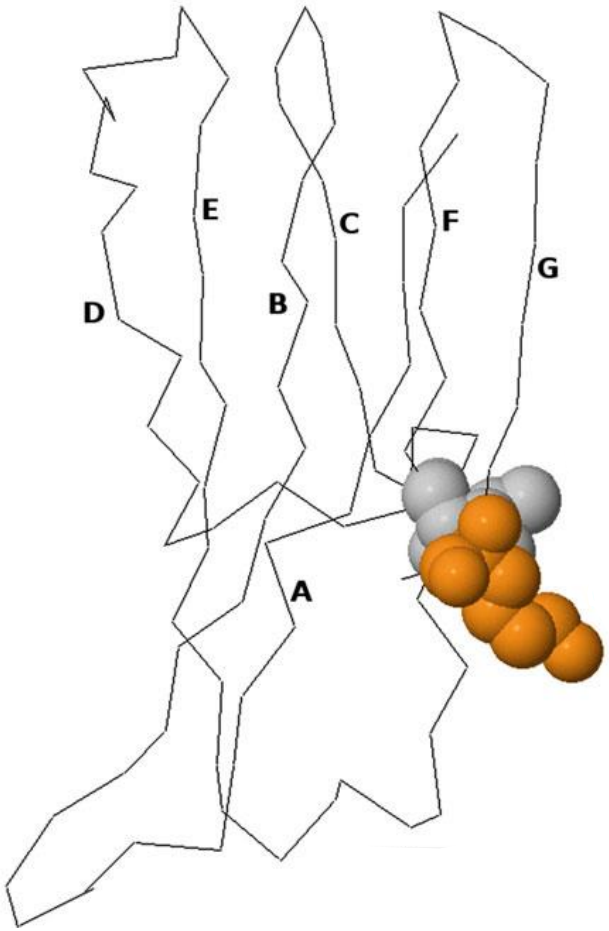
IFX 5: LSSVVTVPSS**SL**GT**Q**TY**I**C̲NVNHKPSNTKVDK**R**VEP G1m (3)

IFX 6: ^{270}DPEV**K**FNWYVDGVEVHNAKTKPREEQY**NST**Y^{300} IgG1

^{270}DPEV**Q**FNWYVDGVEVHNAKTKPREEQY**NST**Y^{300} IgG2,3,4

Homann A et al. J Transl Med. 13:339 (2015)

IgG1 allotypes and iso-allotypes



Allotopes

G1m(17): I,199; **K,214**

G1m(3): I,199; **R,214**

non-G1m(17) epitope

nG1m(17): I,199; **R,214**

nG1m(17): T,199; **R,214**

nG1m(17): T199; **R,214**

Lefranc MP, Lefranc G. Methods Mol Biol. 882:635-680 (2012)

Herceptin

Arvinte T. et al., MAbs. 5:491-500 (2013)

…..add it to an infusion bag containing 250 mL of 0.9% Sodium Chloride Injection, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** Gently invert the bag to mix the solution.

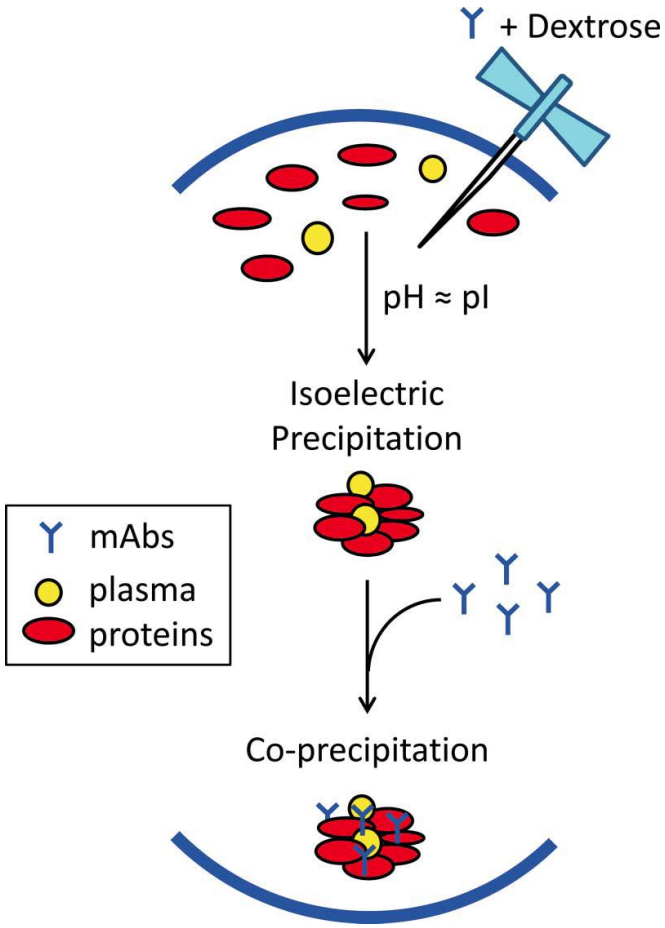
http://www.gene.com/download/pdf/herceptin_prescribing.pdf

Avastin

Withdraw necessary amount of Avastin and dilute in a total volume of 100 mL of 0.9% Sodium Chloride Injection, USP.. **DO NOT ADMINISTER OR MIX WITH DEXTROSE SOLUTION**

http://www.gene.com/download/pdf/avastin_prescribing.pdf

Isoelectric precipitation of plasma proteins:



C3/C4, factor H, fibronectin, and apolipoprotein B-100, whose pI values are proximate to the pH of product formulation (in the range of 6.0 to 6.2).

Herceptin becomes co-precipitated, possibly through interactions with complement proteins, resulting in insoluble protein aggregates containing both mAb and plasma proteins.

Luo S. et al., mAbs 7:1094-103. (2015)

Arvinte T. et al., MAbs. 5:491-500 (2013)

Post-translational and chemical modifications

Protein heterogeneity

Chemical modifications

Immune complexes & aggregates

Tolerance/immunosuppression

Mechanisms of action (MoA) od immune complexes

ADCC – antibody dependent cellular cytotoxicity

CDC – complement mediated cellular cytotoxicity

ADCP – antibody dependent phagocytosis

ADA – antibody dependent apoptosis

MBL - lectin pathway of CDC activation

MR – uptake by antigen presenting cells

ADCVI – antibody dependent cell mediated virus inhibition

Guidance for Industry:
Immunogenicity assessment for therapeutic protein products.
"Product Aggregates and Measurement of Aggregates"

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatory information/guidances/ucm338856.pdf (2014)

Therapeutic protein aggregation: mechanisms, design & control

Roberts CJ. Trends Biotechnol. 32:372-80 (2014)

Workshop on predictive science of the immunogenicity aspects of particles in biopharmaceutical products

Marzal E. et al., J Pharm Sci. 101:3555-9 (2012)

Managing uncertainty: Risk pertaining to product quality attributes and immunogenicity.

Rosenberg AS, et al., J Pharm Sci. 101:3560-7 (2012)

Aggregates in mAbs and therapeutic proteins: A regulatory perspective

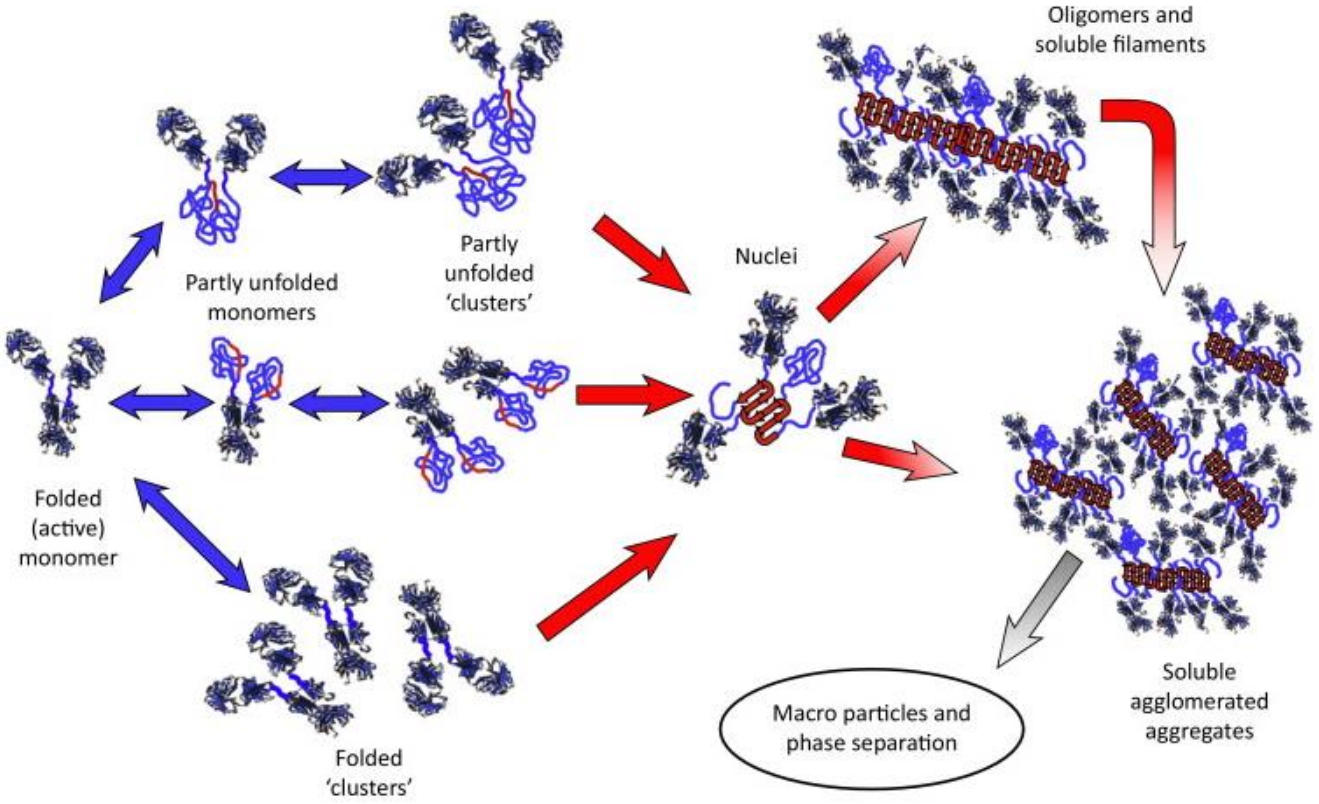
An important part of **protein aggregation** studies is evaluating the biological activity of the **aggregate**.

Differences between **monomeric** and **aggregated protein** can profoundly influence the potency of a **protein-based** drug.

There is no consensus on the maximum allowable limit for protein - based pharmaceutical **aggregates**.

Cordoba-Rodriguez, RV. BioPharm Internat. 21:44-53 (2008)

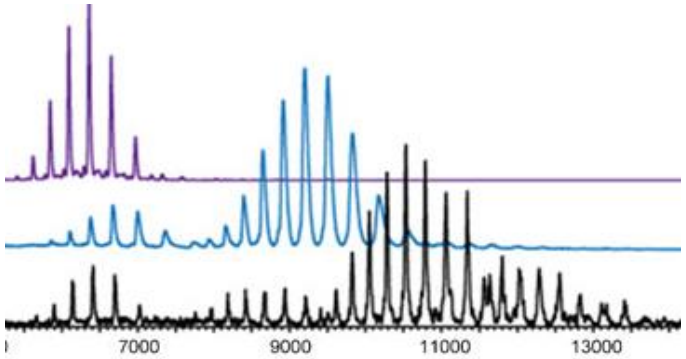
Therapeutic protein aggregation: mechanisms & control



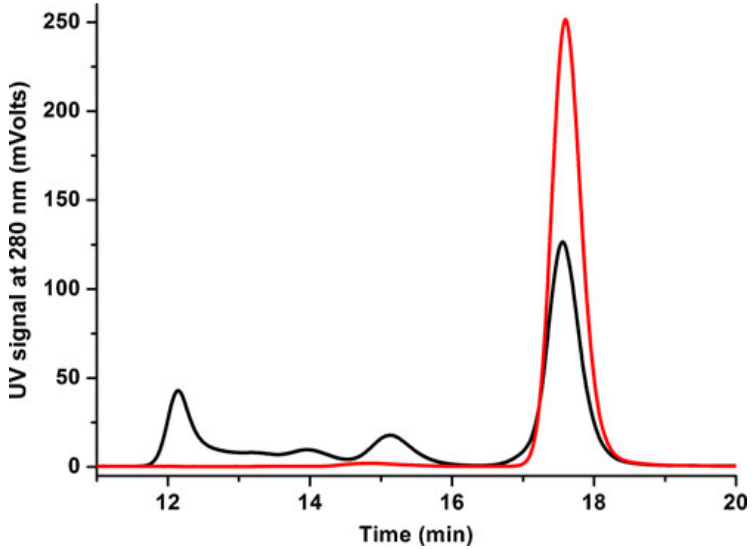
Roberts CJ. Curr Opin Biotechnol. 30:211-217 (2014)

Characterisation of aggregates by mass spectrometry (MS) and size exclusion chromatography (SEC)

MS



SEC

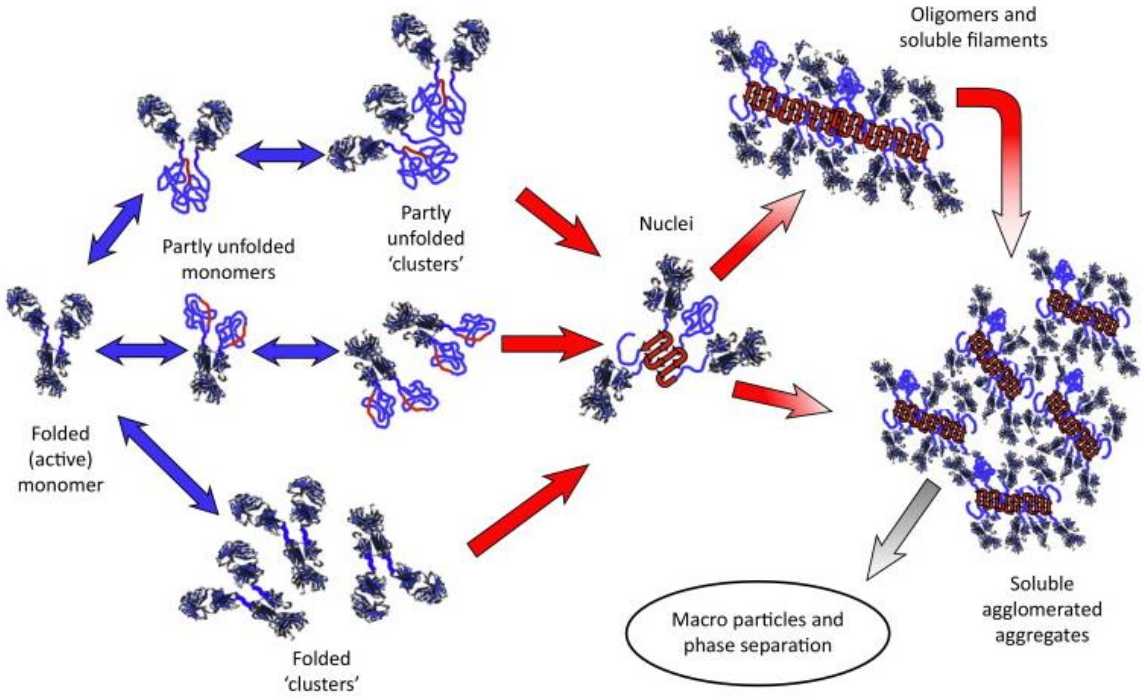


Bruhns P. et al. Blood. 113:3716-25 (2009)

Kukrer B. et al., Pharm Res 27:2197–2204 (2010)

Heat aggregated IgG1 & IgG3 bind and activate all FcγR

FcγRI: IgG1,3,4 **FcγRII:** IgG1,2,3 **FcγRIII:** IgG1,3,4



Bruhns P. et al. Blood. 113:3716-25 (2009)

Jefferis R. Arch Biochem Biophys 526:159-166 (2012)

The immunogenicity of biopharmaceuticals:

Administration of monomeric antibody results in the formation of immune complexes

Immune complexes and aggregated IgG may bind and activate FcγR and complement

THEREFORE

IC and aggregated IgG may be similarly immunogenic

Post-translational and chemical modifications

Protein heterogeneity

Chemical modifications

Immune complexes & aggregates

Tolerance/immunosuppression

Induction of tolerance:

High zone: Single injection of aggregate free protein

Low zone: Repeated low doses of aggregate free protein

Gonzalez S et al. Self/Nonself 2:19-25 (2011)

Mitchison NA Proc R Soc Lond B Biol Sci 161: 275–92 (1964)

Low dose tolerance: Allergen specific immunotherapy

Repeated exposure to low doses of aggregate free protein

Peanut allergy breakthrough

Anagnostuo K et al. Lancet 383(9925):1297-304 (2014)

Anagnostuo K & Clark A Annu Rev Med Aug 26 (2015)

Induction of high zone tolerance to Campath-1H

Campath/Alemtuzumab:

A humanised form of the original rat CAMPATH-1G

74 % of patient develop anti-drug antibody (ADA)

CD52 is highly expressed on normal and malignant B & T cells; monocytes, macrophages eosinophils, basophils, dendritic cells, male reproductive tract.

ADCC/CDC ~ correlates to level of expression of CD52

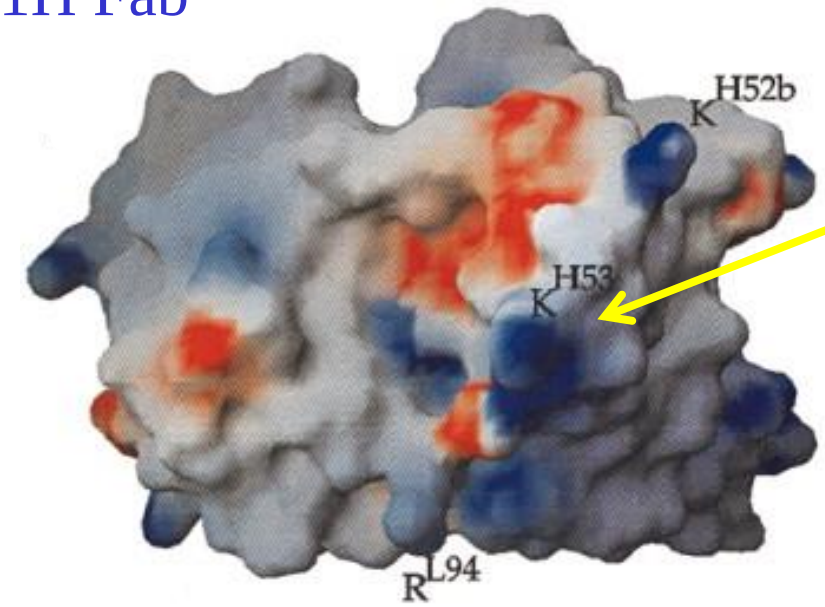
Rao SP et al., PLoS ONE 7: e39416 (2012)

Somerfield J, et al. Immunol. 185:763-8 (2010)

Van Walle et al, Expert Opin. Biol. Ther., 7:405-418 (2007)

Induction of high zone tolerance to Campath-1H

Campath-1H Fab



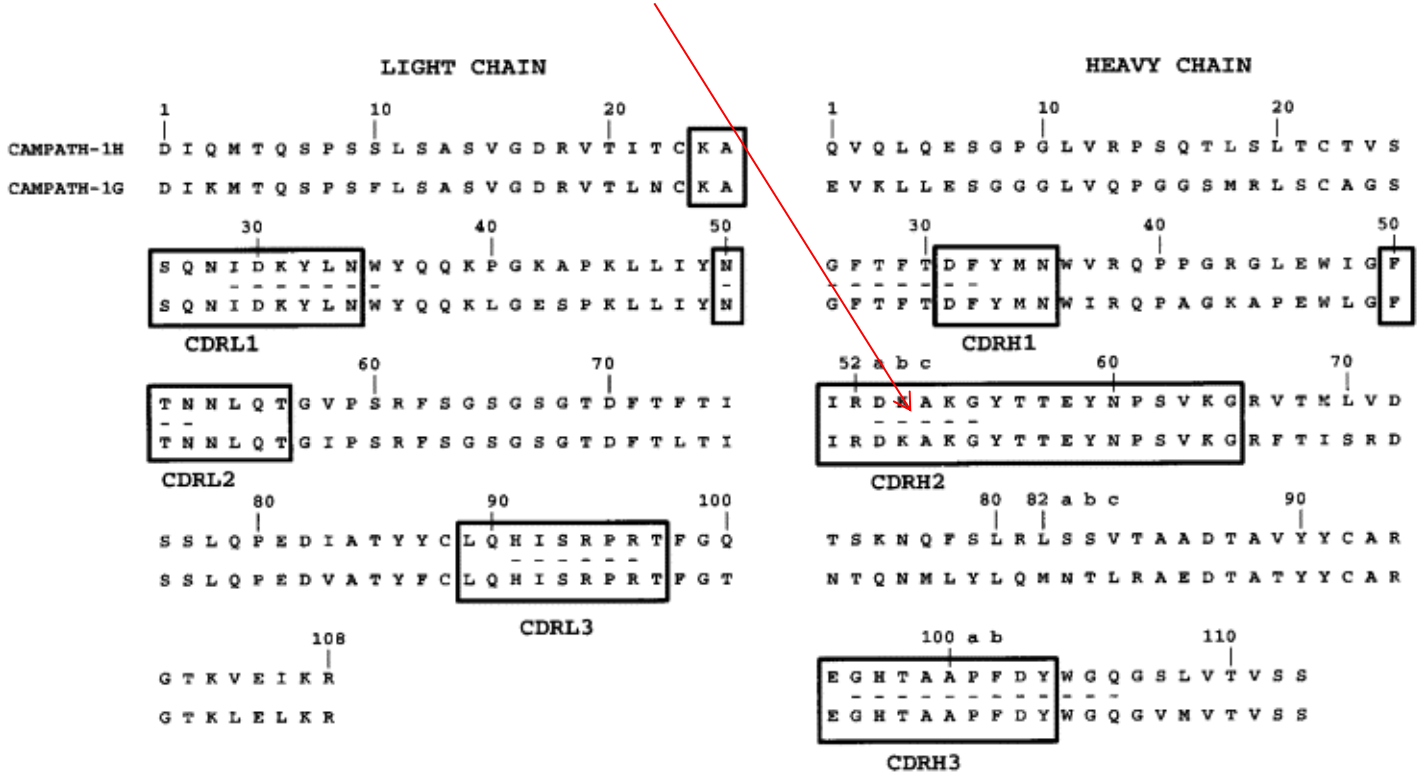
Lysine 53
Aspartic acid 53

SM3: a K/D non-CD52 binding variant was generated

Cheetam GM et al. J M B 284:85-99 (1999)

Sequence of rat Campath-1G & humanised Campath-1H

lysine$_{53}$ > aspartic acid$_{53}$ in the heavy chain

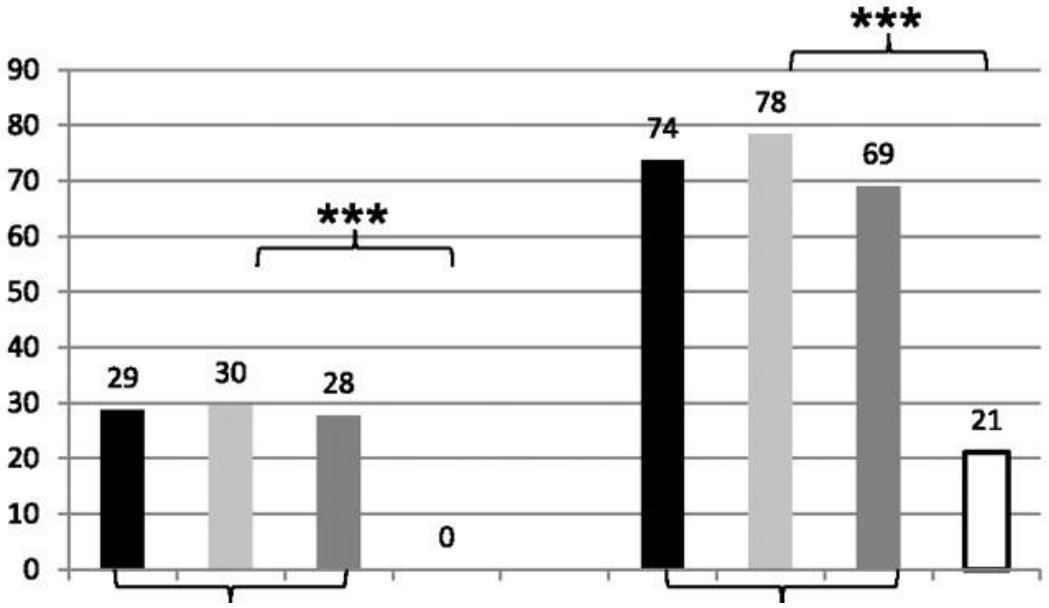


61 CDR residues of SM3 are the same as the original rat Campath-1G

Cheetham GM et al. J M B 284:85-99 (1999)

A strategy to reduce the immunogenicity of Campath-1H

Induce tolerance to SM3: K/D heavy chain CDR2 variant



Pooled alemtuzumab data

alemtuzumab 12mg/day

alemtuzumab 24mg/day

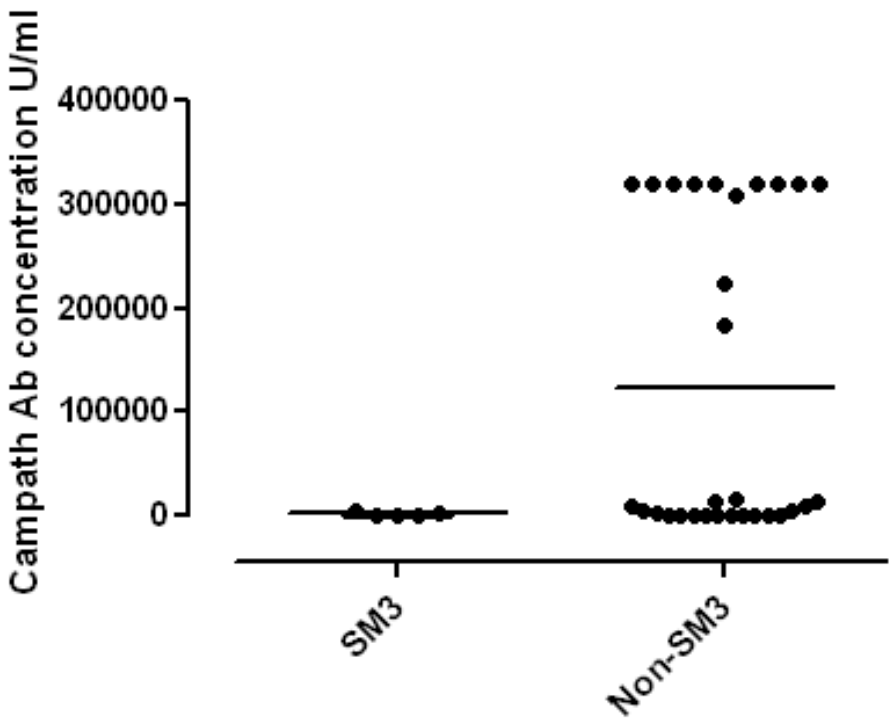
Alemtuzumab + SM3

N = 212 – SM3 **N = 20 + SM3** **N = 197 – SM3** **N = 19 + SM3**

month 1 post alemtuzumab month 13 post alemtuzumab

Somerfield J, et al. Immunol. 185:763-8 (2010)

Anti-alemtuzumab concentrations after 3rd cycle



http://www.e-i-p.eu/wp-content/uploads/2013/02/04_Alasdair_Coles_presentation.pdf

Induction of high zone tolerance to alemtuzumab

CAMPATH-1H (alemtuzumab) anti-CD52:

74 % of patient receiving alemtuzumab developed anti-drug antibody (ADA)

21% of patients developed ADA following exposure to SM3

Somerfield J, et al. Immunol. 185:763-8 (2010)

Immune complexes formed by MAbs

An important part of **immune complex** studies is evaluating the biological activity of the **complexes**.

Differences between **monomeric mAb** and **immune complexes** may influence the potency of a **mAb based drug.**

There has been little study of the **immune complexes** formed on administration of therapeutic mAbs

Jefferis R. mAbs 3 503-4 (2011)

Jefferis R. J. Immunol Research. 2016 In press

Jefferis R ***The immunogenicity of protein aggregates and immune complexes***. J. Immunological Research. 2016 In press

Jefferis R. ***Isotype & glycoform selection for antibody therapeutics***. Arch Biochem Biophys 526:159-166 (2012)

Jefferis R. ***Aggregation, immune complexes and immunogenicity***. mAbs 3: 503-4 (2011)

Jefferis, R. & Lefranc, M-P. ***Human Immunoglobulin Allotypes: Possible implications for immunogenicity***. mAbs 1:1-7 (2009)