Federal Institute for Vaccines and Biomedicines

Paul-Ehrlich-Institut

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Quality attributes impacting immunogenicity of therapeutic proteins

Workshop on immunogenicity assessment of biotechnology-derived therapeutic proteins
9th March 2016 (EMA, Room 3A)

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Disclaimer

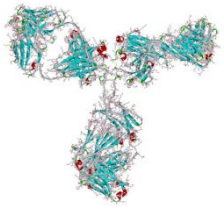
The view expressed in the following is the ones of the presenter and does not necessary express the view of either the CHMP, BWP, EDQM or the Paul-Ehrlich-Institut (including other sections)

Outline

Case studies: Impact of QA on immunogenicity

Immunogenicity studies-Why?

Proteins



Patients

Non immunogenic

No effect

Immunogenic (induce antibodies)

Anaphylactic reactions hypersensitivity

Neutralise biological effects and compromise further therapy

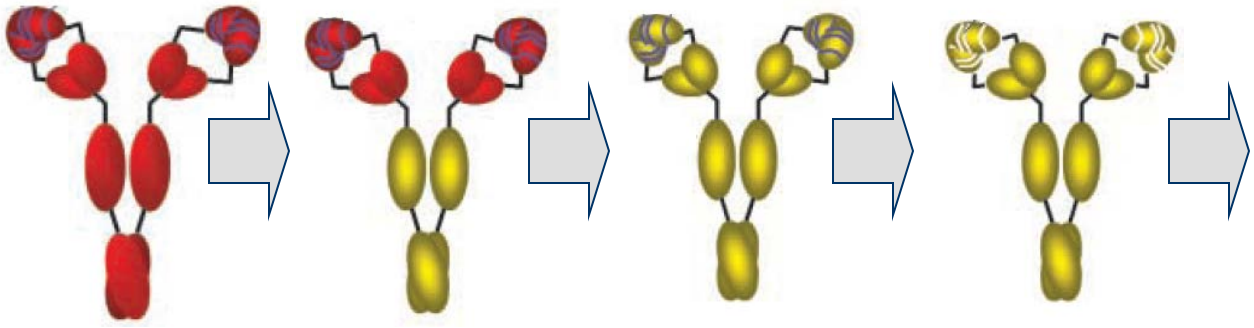
Alter PK/PD

Cross-react with native protein and induce adverse symptomes

„Humanisation“ of therapeutic proteins

Evolution of monoclonal antibodies („-mab“):

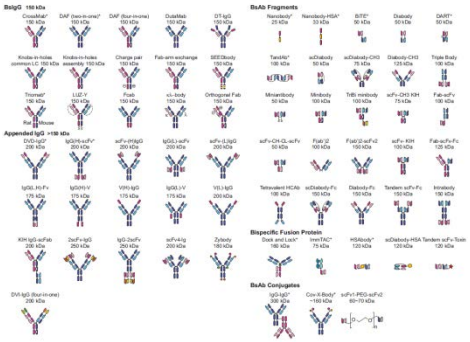
= murine
= human

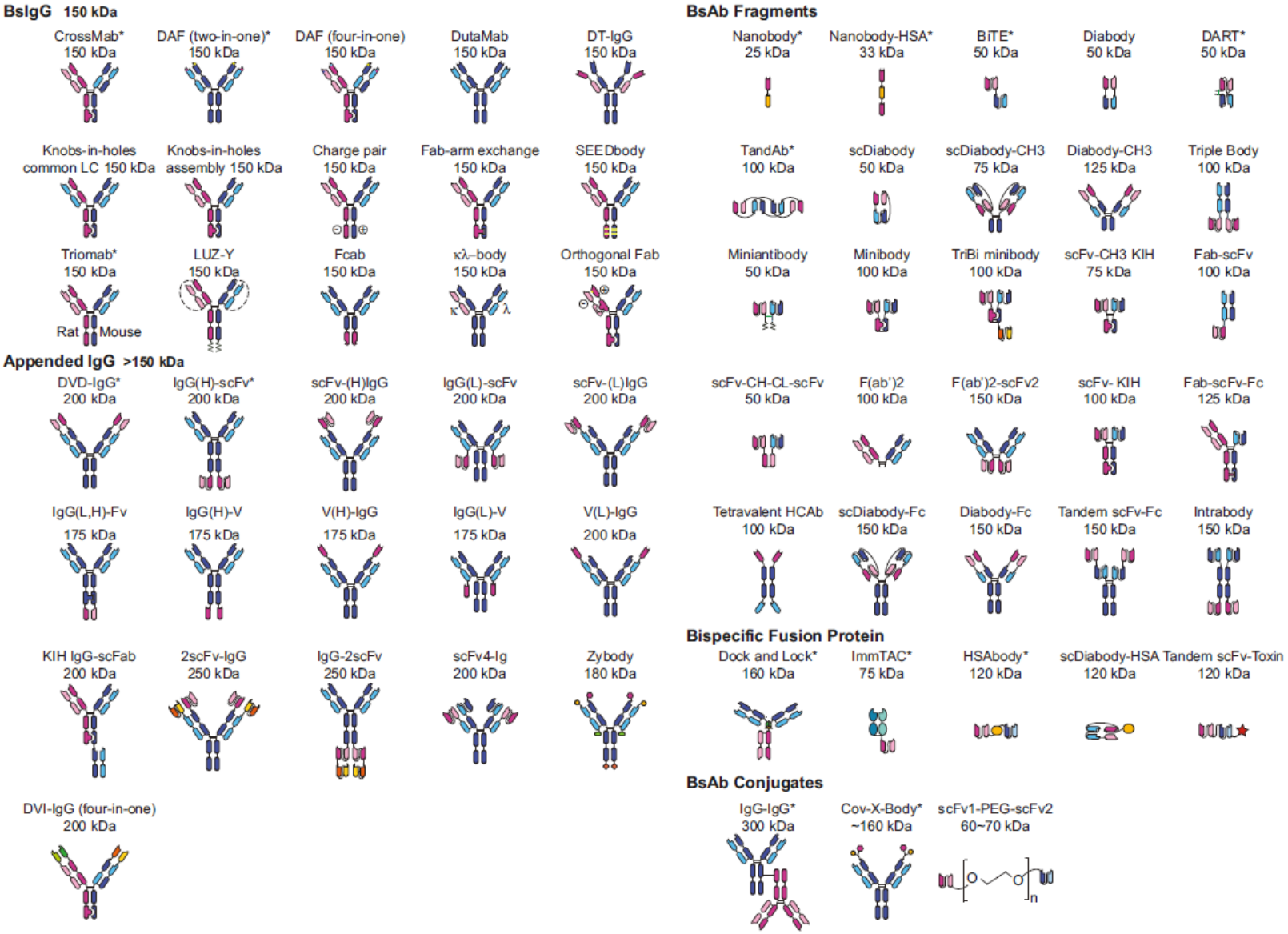


Murine mAb	Chimaeric mAb	Humanized mAb	Fully Human mAb
„-omab“	„-iximab“	„-zumab“	„-umab“
Arcitumomab (CEA-Scan®) (1996)	Infliximab (Remicade®) (1999)	Trastuzumab (Herceptin®) (2000)	Adalimumab (Humira®) (2003)

Immunogenicity

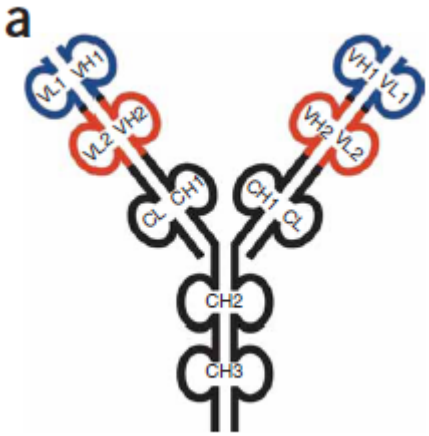
New constructs
- bispecific antibodies
- diabodies
- single chain fragments
- engineered Fc mAbs
- conjugated mAbs
- ...





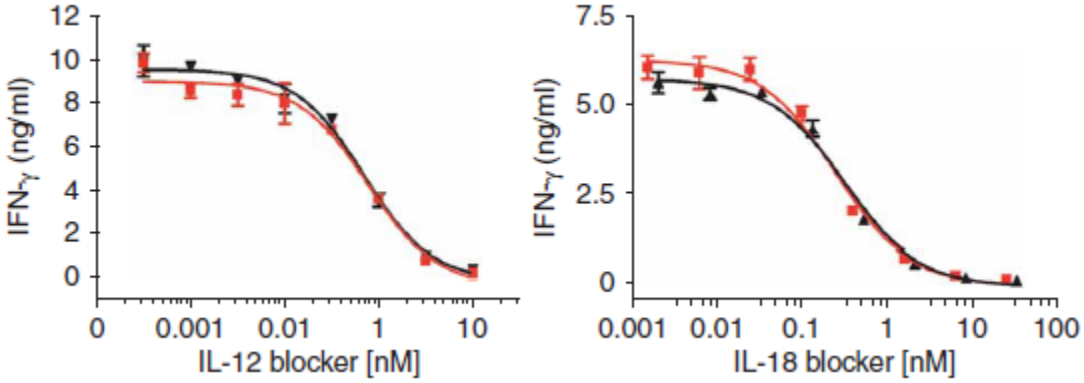
From C Siess et al, Alternative molecular formats and therapeutic applications for bispecific antibodies, Molecular Immunology 67 (2015) 95–106

New formats increased immunogenicity?



Design, generation and characterization of an anti–IL-12/IL-18 dual-variable-domain immunoglobulin DVD-Ig

- Impression that there are more reactions in cynomolgus monkeys than typical mAb
- Immune complex formation?
- manifestation in the clinic?



from Wu et al., NATURE BIOTECHNOLOGYVOLUME 25 NUMBER 11 NOVEMBER 2007

INN terminology - only „-mab“

Table 1. Analysis of the sequences of 14 approved 'fully human' antibodies using DomainGapAlign. Values in bold do not meet the ≥ 90% threshold and also overlap with the range of values found with humanized antibodies (see Table 2)

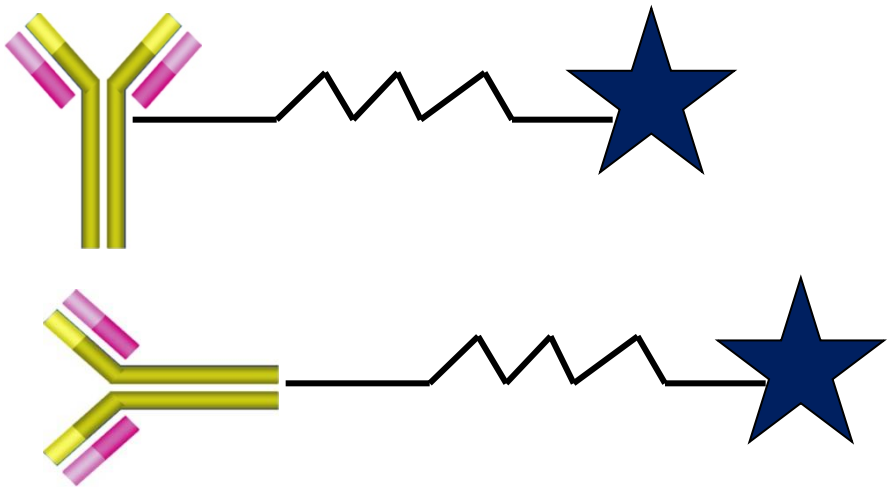
	Variable Heavy	Variable Light	
	% Human Identity	% Human Identity	Current WHO INN Designation
Panitumumab[a]	**89.9**	95.8	Human
Adalimumab[b]	93.9	95.8	Human
Canakinumab[a]	93.9	98.9	Human
Raxibacumab[b]	99.0	100.0	Human
Ipilimumab[a]	94.9	97.9	Human
Belimumab[b]	**86.7**	97.9	Human
Denosumab[a]	98.0	95.8	Human
Nivolumab[a]	91.8	98.9	Human
Secukinumab[a]	92.9		
Ramucirumab[b]	99.0		
Ustekinumab[a]	**87.8**		
Ofatumumab[a]	97.0		
Golimumab[a]	94.9		
Alirocumab[a]	**89.8**		
Evolocumab[a]	93.9		

Table 2. Sequence analysis of 16 approved humanized antibodies based upon the current WHO INN definitions using DomainGapAlign. Values in italics indicate criteria for humanized designation passed. Values in bold indicate criteria failed. Assumes decimals are rounded to the nearest whole number.

	Variable Heavy		Variable Light		
	% Mouse Identity	% Human Identity	% Mouse Identity	% Human Identity	New WHO INN Designation
Pembrolizumab[a]	72.4	**79.6**	79.8	*85.1*	Mixed
Vedolizumab[a]	81.6	*84.7*	85.0	*85.0*[e]	Humanized
Trastuzumab[b]	71.4	**81.6**	75.8	*86.3*[e]	Mixed
Obinutuzumab[c]	77.6	*84.7*	90.0	87.0[f]	Humanized
Pertuzumab[b]	72.4	**78.8**	77.9	**84.2**[e]	Chimeric
Tocilizumab[a]	77.3	*84.8*	83.2	*89.5*[e]	Humanized
Certolizumab[b]	70.6	**77.6**	77.9	*85.3*	Mixed
Natalizumab[a]	79.6	**83.7**	86.2	**80.9**	Chimeric
Ranibizumab[b]	69.4	**75.8**	80.0	*87.4*[e]	Mixed
Bevacizumab[b]	71.4	**76.8**	81.1	*88.4*[e]	Mixed
Eculizumab[a]	72.4	**83.7**	81.1	**84.2**[e]	Chimeric
Efalizumab[b]	68.4	**76.5**	83.2	*89.5*[e]	Mixed
Omalizumab[b]	69.7	**78.6**	77.8	*86.9*[e]	Mixed
Alemtuzumab[a,d]	62.0	**73.7**	88.4	*86.3*[f]	Mixed
Palivizumab[a]	78.8	*87.9*	68.5	**81.9**	Mixed
Daclizumab[a]	81.6	**82.7**	74.2	**84.0**	Chimeric

From TD Jones, mAbs 8:1, 1--9; January 2016; Published with license by Taylor & Francis Group, LLC

Antibody-Drug-Conjugates(ADC)



antibody

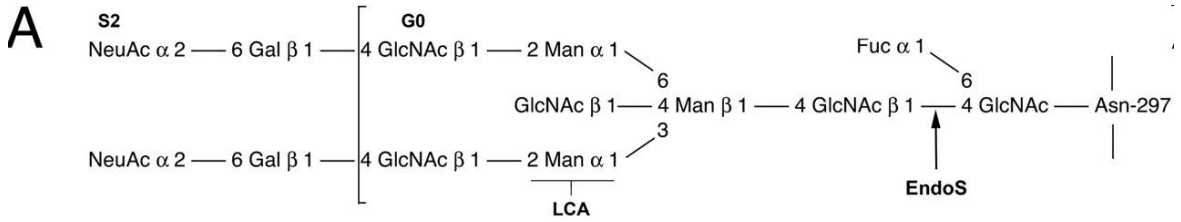
Attachment group

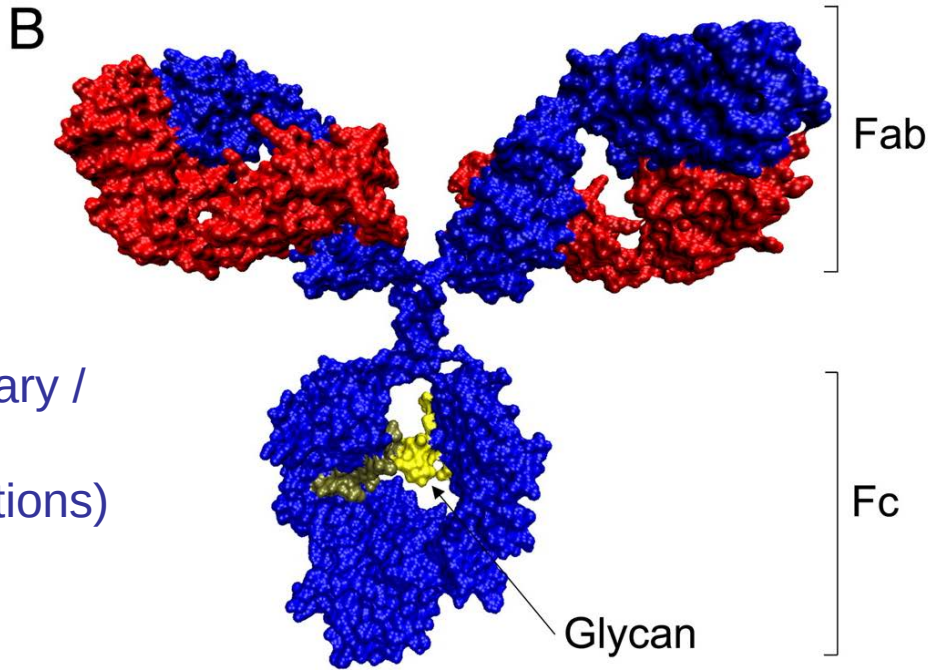
Protease-cleavable linker

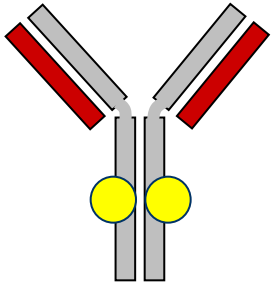
Cytotoxic drug

- antibody-drug conjugate (ADC) composed monoclonal antibody covalently linked, via an enzyme-cleavable linker, to the cytotoxic drug

Biotechnological products are highly complex molecules







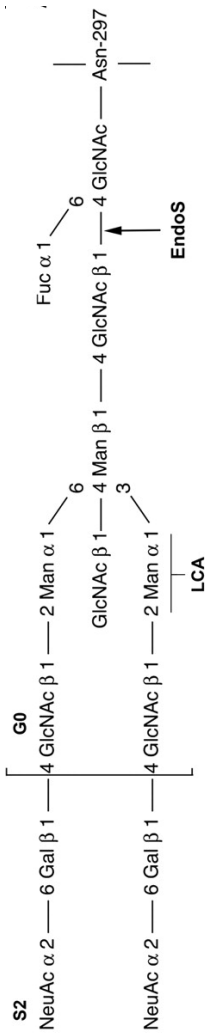
- high molecular weight

- complexity
  (primary / secondary / tertiary / quartery structure;
  post-translational modifications)

- heterogeneity

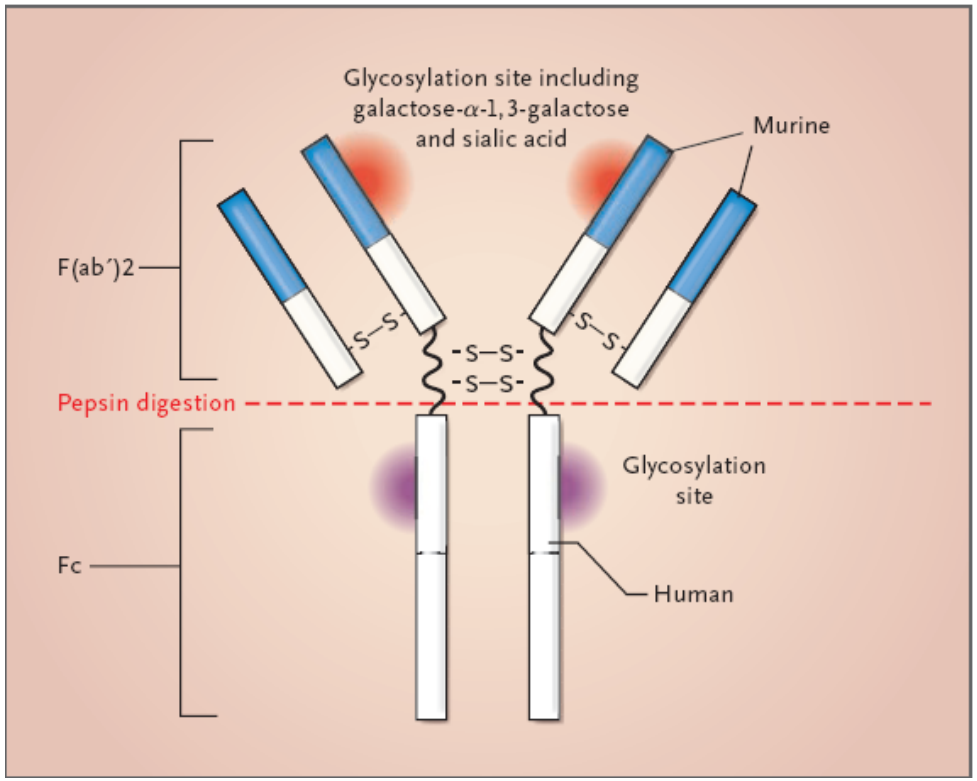
- process- and product-related impurities

Effects induced by Carbohydrate to IgG Function



Without Glycan	→	No ADCC activity
Without core Fucose	→	increases ADCC activity
Bisecting GlcNAc	→	increases ADCC activity
GlcNAc/Mannose (G0)	→	Ligand for C-typ Lectin (Mannose binding Lectin) and Complement activation
α1,3Galactose	→	higher antigenicity
N-Glycoylneuramine acid	→	higher antigenicity
Galactose	→	FcRn binding, transplacental transport, increased CDC activity
NeuNAc (Sialic acid)	→	decreases ADCC activity, anti-inflammatory

Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose



- Among 76 cetuximab-treated subjects, 25 had a hypersensitivity reaction to the drug
- The antibodies were specific for galactose-α-1,3-galactose.

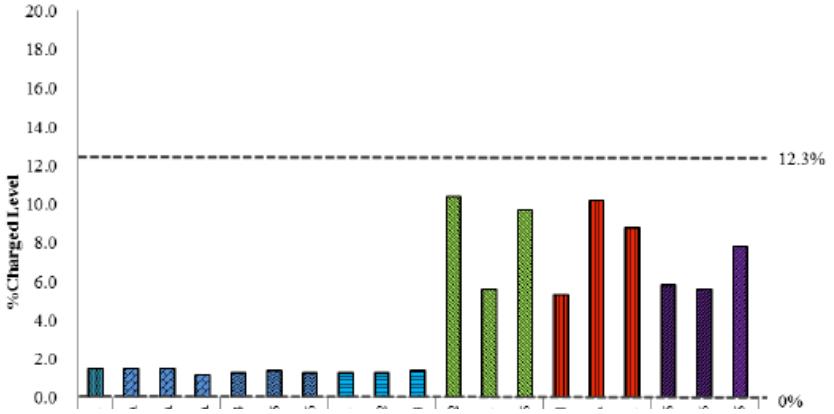
from Chung et al., N Engl J Med 2008;358:1109-17.

NANA vs NGNA

Difference in charged variants 1.5% vs 8% in biosimilar and reference product respectively

N-acetylneuraminic acid (NANA) forms were observed as a charged glycosylated form in biosimilar,

N-glycolylneuraminic acid (NGNA) forms were the only charged glycosylated form observed in reference product



N-glycan on antibodies expressed in murine cell lines (SP2/0) may contain significant quantities of terminal N-glycolylneuraminic acid (NGNA) in place of terminal N-acetylneuraminic acid (NANA)

Single use equipment

- Enables speed to market (R&D and Operations)
- Reduces capital investment and depreciation
- Reduced Start Up Time and Capital Investment
- Cost Savings
- Provides flexibility and geographic mobility
  - Validation
- Increases productivity and reduces cost
- Flexible Scaling to Meet Product Demand
- Enhances compliance
  - ➢ standardization and Process Analytical Technology – PAT
  - ➢ RTRT

Leachables/extractables

Extractables:
Chemical compounds that migrate from any product contact material when exposed to an appropriate solvent under exaggerated conditions of time and temperature.

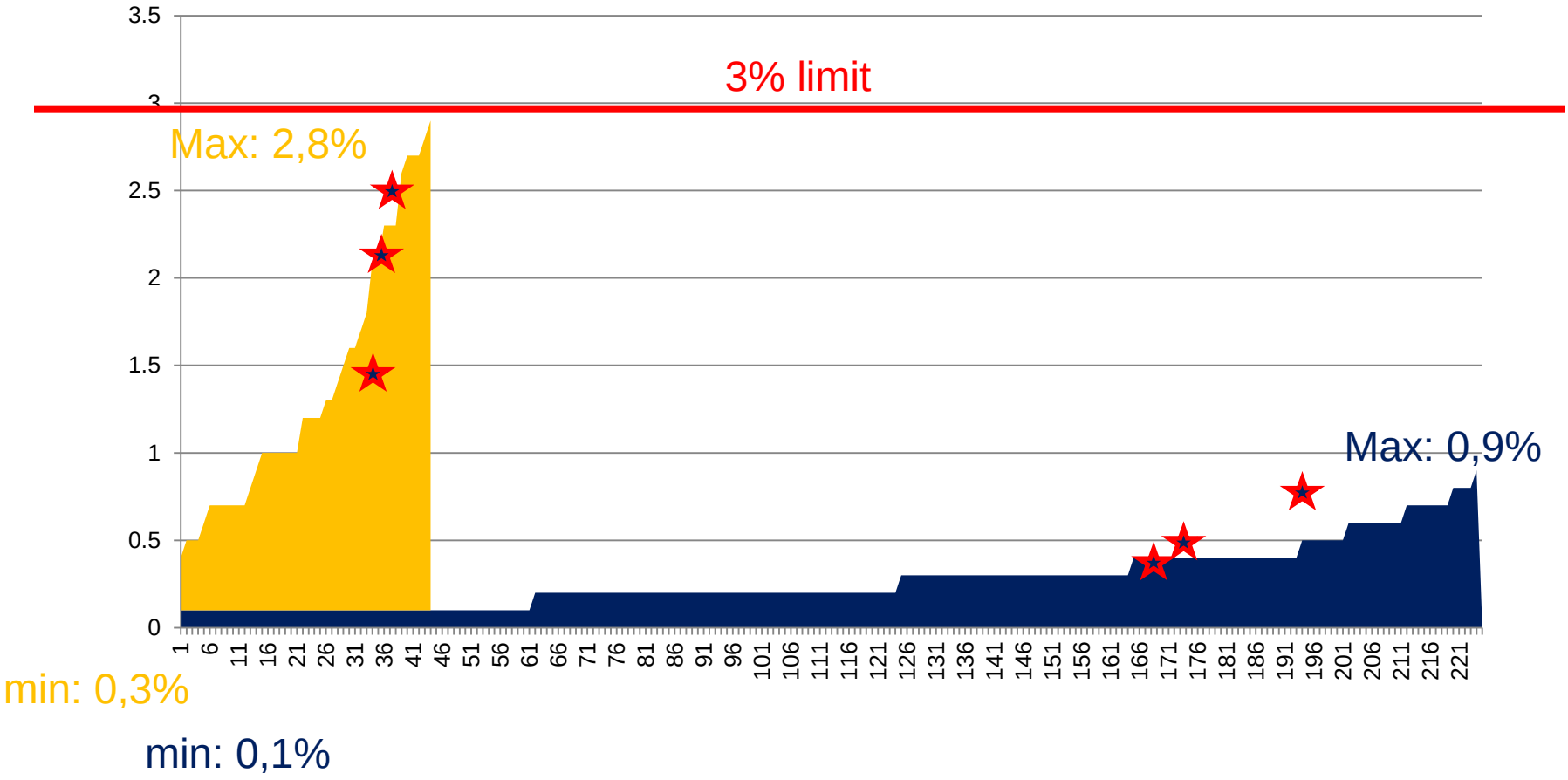
Leachables:
Chemical compounds, typically a subset of extractables, that migrate into the drug formulation from any product contact material, — including elastomeric, plastic, glass, stainless steel or coating components — as a result of direct contact with the drug formulation under normal process conditions or accelerated storage conditions and are found in the final drug product.

Product related impurities Aggregates

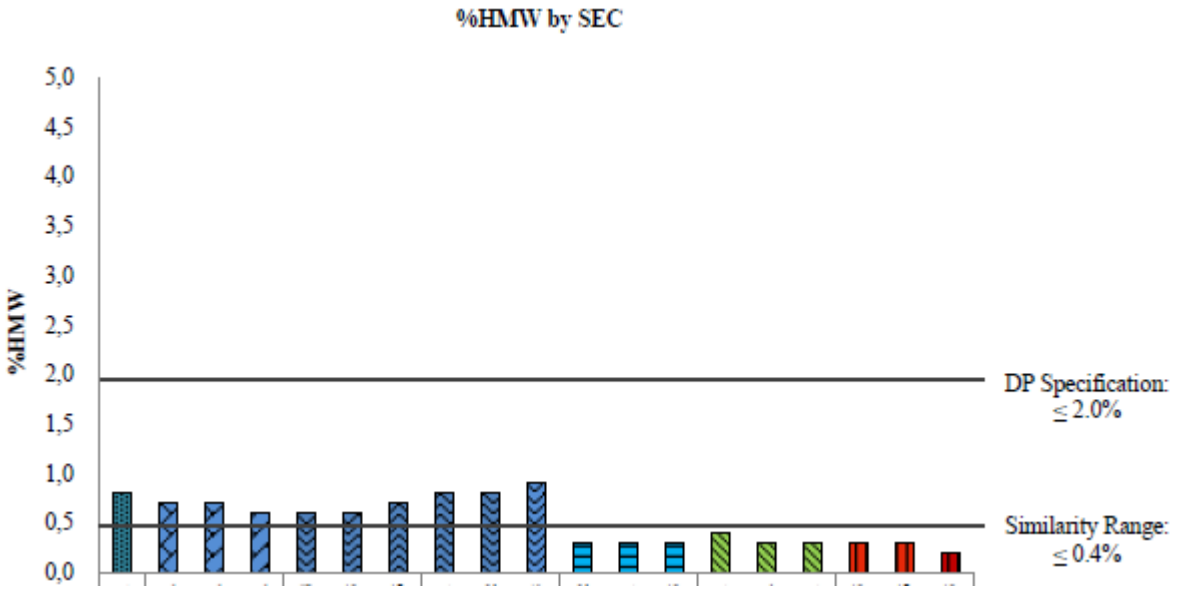
- The capacity of protein aggregates to enhance immune responses to the monomeric form of the protein
- Clear connection between protein aggregates and antibody mediated adverse events in treatment with early therapeutic protein products
- little is known about the nature of the aggregate species responsible for such effects.

Polymer content ADR

- Polymer content of new process batches (yellow)
- Polymer content of old process batches are low (blue)



% HMW species



Although the relative percentage of HMW is low (< 1.0%) in both Biosimilar and Originator the %HMW level of the biosimilar was out of the similarity range.
A negative impact on the patient safety cannot be excluded.

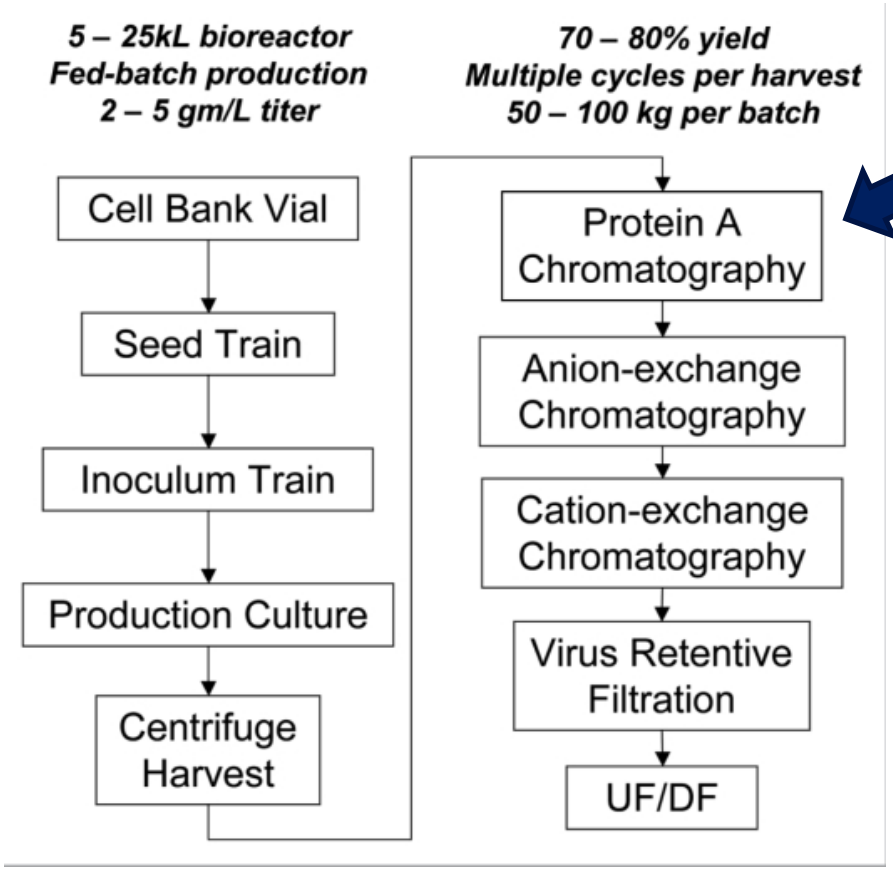
HCP assay

Validation parameter	Assay 1 generic	Assay 2 process specific
Accuracy Precision Specificity	comparable	comparable
LOD LOQ	49 ng/ml 100 ng/ml	0.2-1.2 ng/ml 1.25 ng/ml
Working Range	100-4000 ng/ml	1.25-25 ng/ml

- Specification: NMT 1200 ng/mg
- Batch results: 450-600 ng/mg

Investigating interactions between phospholipase B-Like 2 and antibodies during Protein A chromatography.

Tran B, Grosskopf V, Wang X, Yang J, Walker D Jr, Yu C, McDonald P.



Co-elution of PLBL2 during Protein A chromatography is highly dependent on the individual antibody and PLBL2 concentration in the chromatographic load.

Thank You!