Recombinant therapeutic proteins/biologicals and immune responses

Daan J.A. Crommelin, prof. emeritus, Utrecht Institute for Pharmaceutical Sciences, The Netherlands

16 May 2021

Immune response as part of the desired pharmacological effect

- Mabs as checkpoint inhibitors in cancer therapy
- Interferon beta in multiple sclerosis treatment
- Mabs in the treatment of auto-immune diseases
- Vaccines

Immune response as an unwanted effect

- Anti-drug antibodies (ADA)

How do biological products differ from conventional, low molecular weight drugs?

In contrast to most drugs that are chemically synthesized and their structure is known, most biologics are ***complex mixtures that are not easily fully characterized***……..

https://www.fda.gov/about-fda/center-biologics-evaluation-and-research-cber/what-are-biologics-questions-and-answers

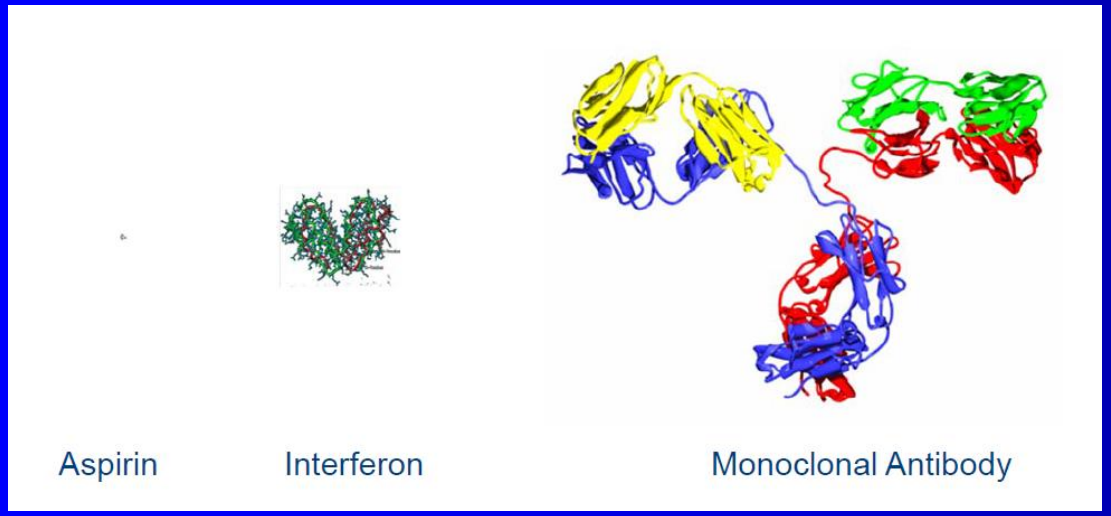
How do Biologicals compare to small, low molecular weight drugs?

	SMALL MOLECULE DRUGS	BIOLOGICALS
Molecular weight	Low (<500)	High (range 5-900 kDa)
Structure	Simple, well-defined	Complex, heterogeneous, defined by manufacturing pro
Manufacturing	Chemical synthesis	Produced in living cells or organisms
Stability	Stable	Generally unstable, sensitive to external conditions
Immunogenicity	**Mostly non-immunogenic**	**Mostly immunogenic**
Copy characteristics	Identical copies can be made	to generate identical copy versions is a challenge……

Adapted from GaBI Online – Generics and Biosimilars Initiative www.gabionline.net/Biosimilars/Research/Small-molecule-versus-biological-drugs, based on Declerck and Schellekens.

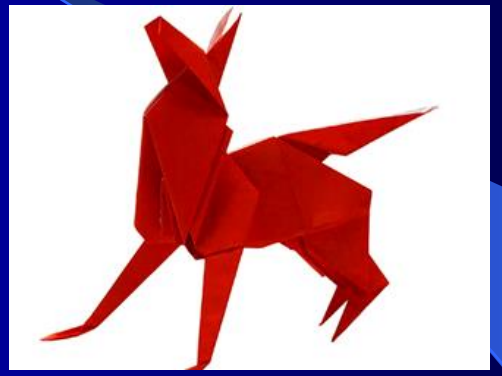
Small molecules versus proteins: size difference

Proteins are ….. Big!

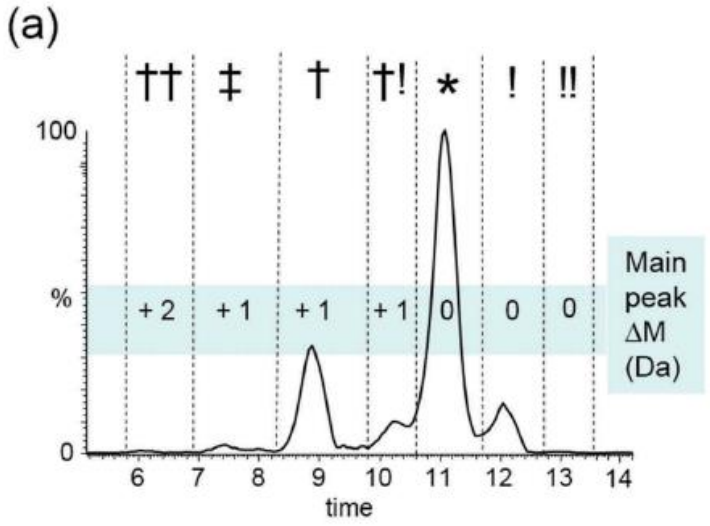


Mw around 150 around 20,000 around 150,000

Proteins are ‘vulnerable’



Therapeutic protein products are often heterogeneous mixtures of different molecules



Isoform Modification		ΔM (Da)
††	2 x Deamidation LC-N30	+ 2
‡	IsoAspartate HC-N55	+ 1
†	Deamidation LC-N30	+ 1
†!	Deamidation LC-N30 IsoAspartate HC-D102	+ 1
*	Relatively unmodified	0
!	IsoAspartate HC-D102	0
!!	2 x IsoAspartate HC-D102	0

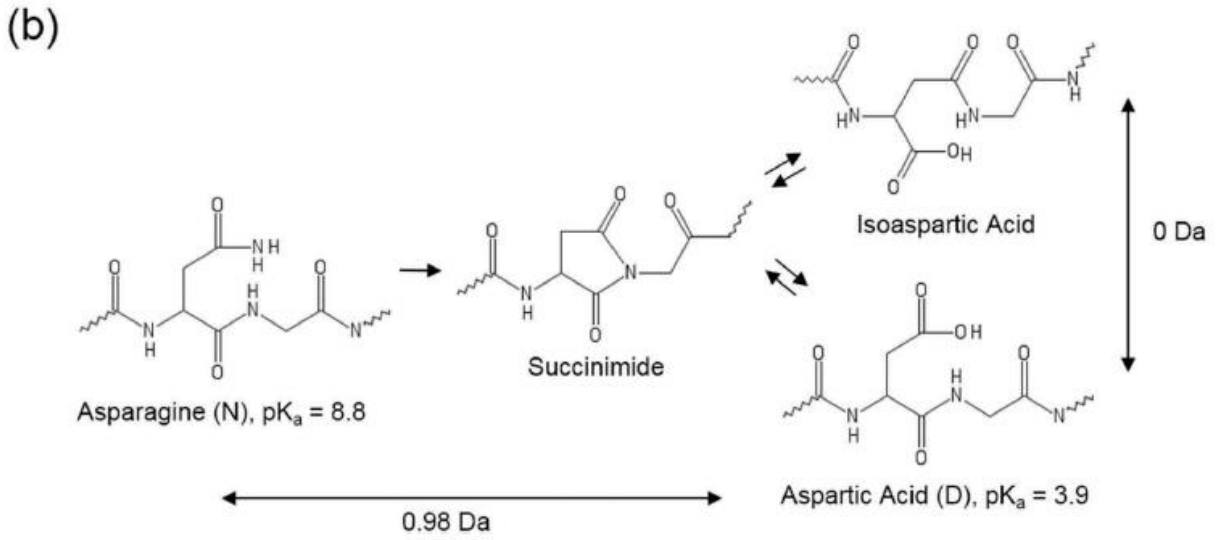


Figure 2. Trastuzumab charge heterogeneity is highly influenced by asparagine deamidation and aspartic acid isomerization. (a) The chromatogram resulting from our CVMS method is highly similar to the trastuzumab charge variant profile previously reported by Harris et al. showing amino acid site-specific charge variant peak assignments based on fractionation and peptide mapping data.[23] Delta masses are plotted for the seven major peaks. (b) Pathway for asparagine deamidation and aspartic acid isomerization. Deamidation of asparagine to aspartic acid results in a mass difference of +0.98 Da and changes the local pKa from basic (8.8) to acidic (3.9) and results in earlier elution by cation exchange separation. Isomerization of aspartic acid results in zero mass change and does not directly result in any predictable change to pI.

Charge variants Herceptin, trastuzumab

Aspirin contains between 99.5 and 100.5 percent acetyl salicylic acid. USPNF

Bailey et al., 2018
https://doi.org/10.1080/19420862.2018.1521131

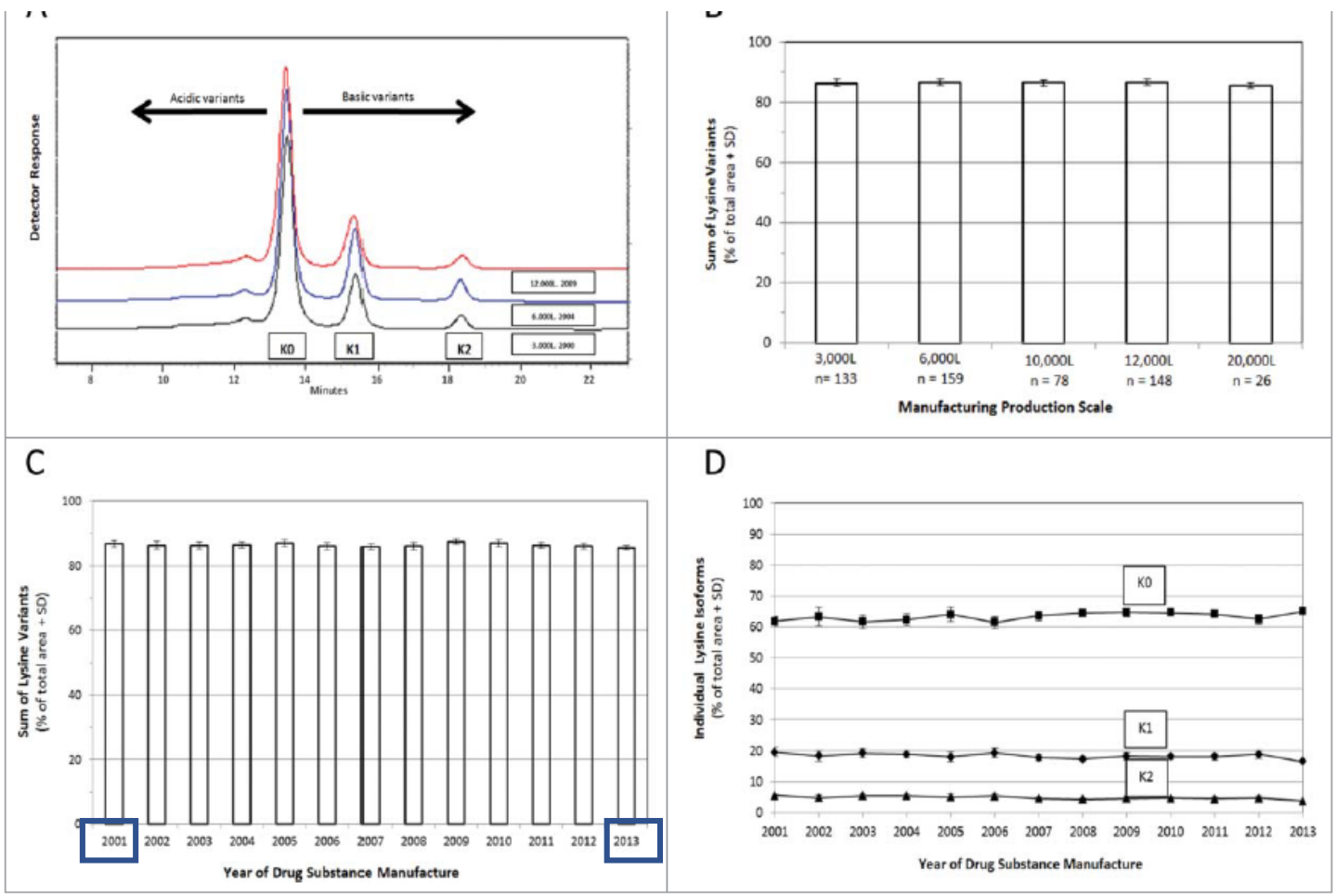


Figure 1. Lysine Profiles of Humira®. Chromatograms of representative batches are displayed in **A**; 3,000L (Black; 2000), 6,000L (Blue; 2004), 12,000L (Red; 2009). WCX-HPLC was performed on batches of Humira that derived from scale-up production (3,000 to 20,000 liters, **B**) and through each year 2001 to 2013 (**C & D**). The chromatograms illustrate the relative retention time and relative peak areas. The relative amount of the 3 C-terminal lysine isoforms (K0, K1, K2) was calculated from the chromatograms as a percent of total area. The mean sum of lysines of multiple batches per data point is presented with standard deviation (n = 544 batches for **B** and 525 total batches included for **C and D**). The number of drug substance batches evaluated per data point is displayed in **B**. For each year 2001 to 2013 (**C and D**), the number of batches included in each data point is 13, 38, 50, 44, 54, 40, 37, 34, 24, 34, 57, 52, 48, respectively. The mean of individual lysine species (K0 [square], K1 [diamond] & K2 [triangle]) is presented with standard deviation (**D**).

Consistency of quality attributes for the glycosylated monoclonal antibody Humira (adalimumab).

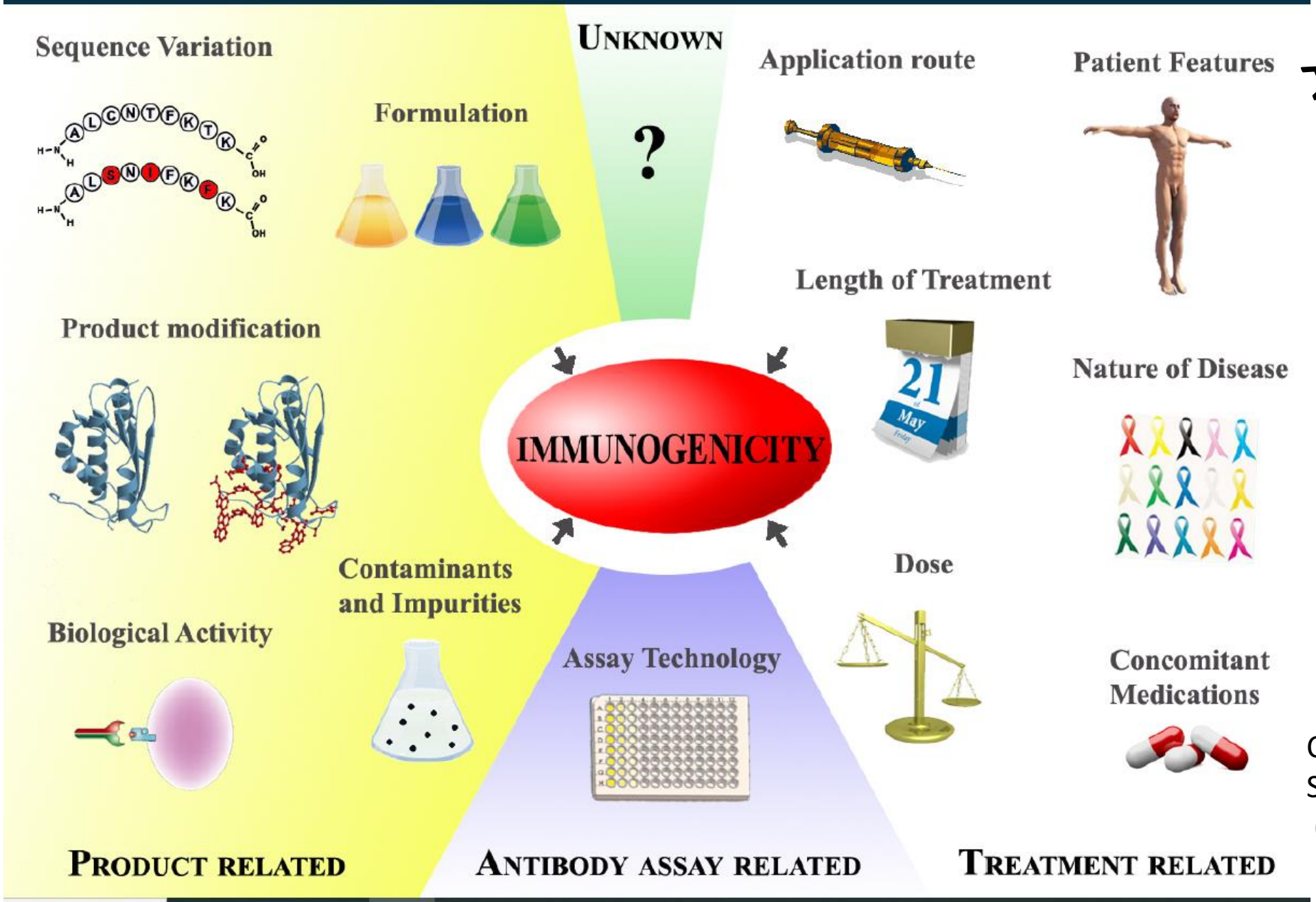
Full control over the manufacturing process!

Tebbey et al., 2015
http://dx.doi.org/10.1080/19420862.2015.1073429

Conclusion: diverse……

but batch to batch consistency can be ensured

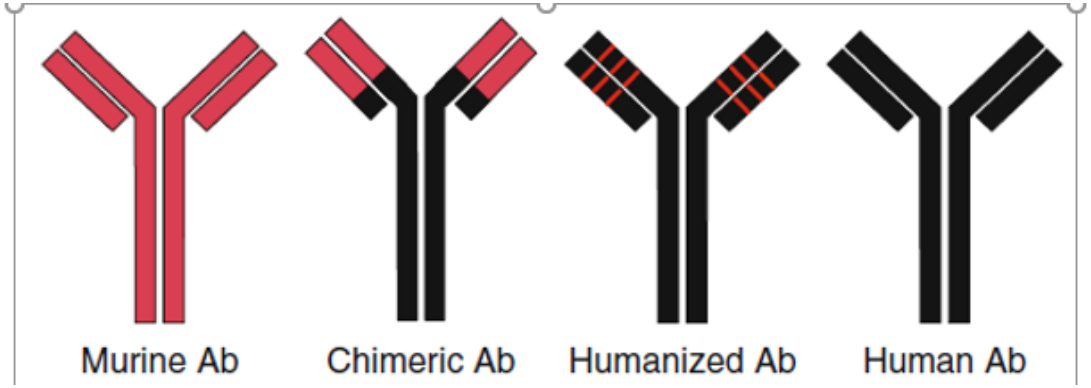
Factors influencing protein immunogenicity



ADA: antidrug antibodies

Courtesy of Jiskoot/ Schellekens (originally from 2002)

Examples of registered monoclonal antibodies and incidence of antibody formation reported in package insert

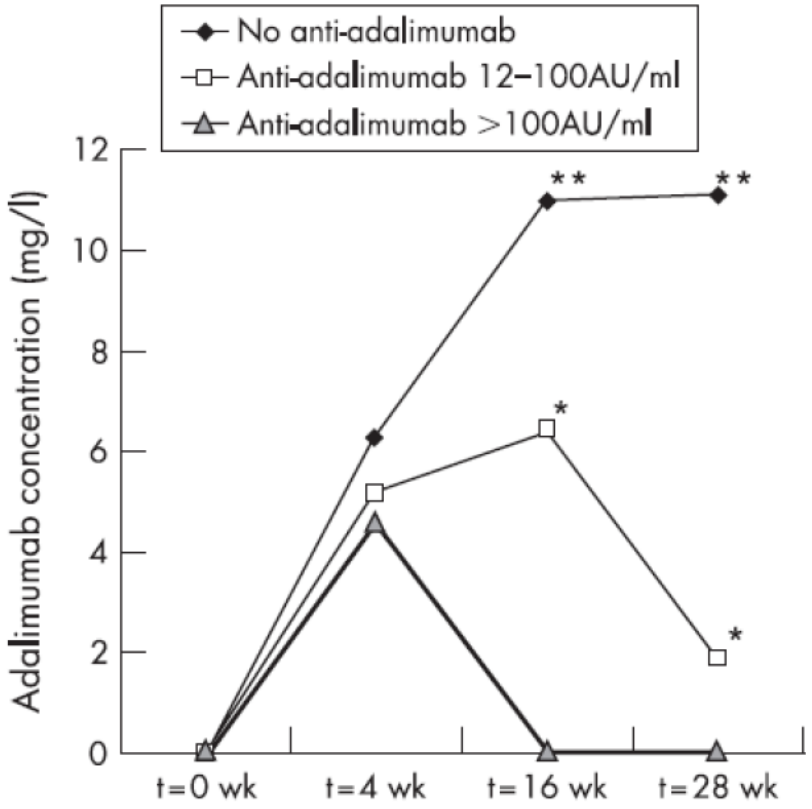


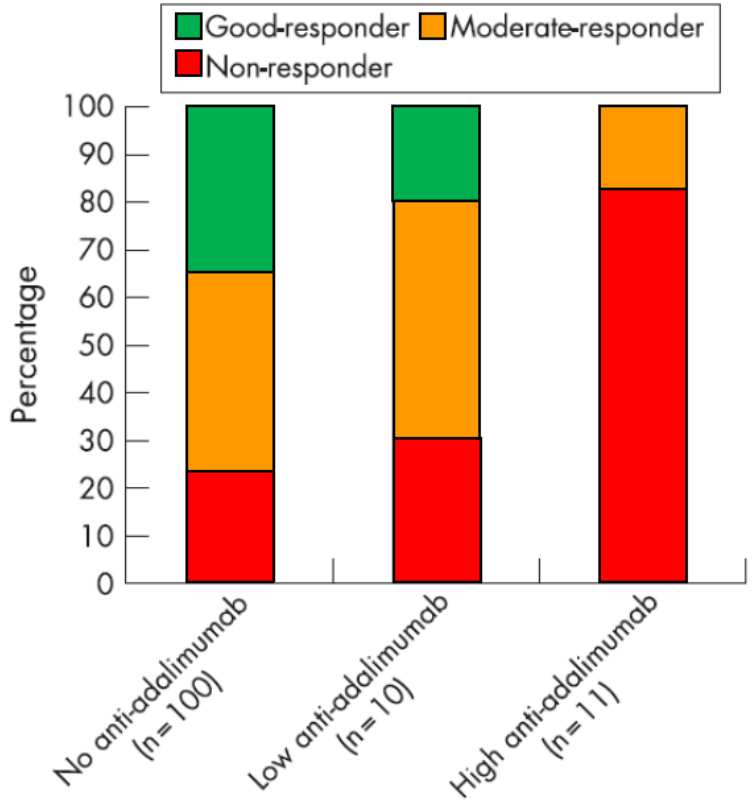
Trade name	Generic name	Type of MAb	Incidence antibody formation (%)
Humira	adalimumab	Human IgG1	12
Remicade	infliximab	Chimeric IgG1	24
Reopro	abciximab	Chimeric Fab	6
Herceptin	trastuzumab	Humanised IgG1	1

What is the effect of ADA (anti-drug-antibodies)?

- Pharmacokinetic profile changes
- Therapeutic efficacy changes

Anti-drug antibody levels in patients receiving adalimumab (Humira) negatively correlate with drug concentration in plasma and therapeutic effect





Bartelds et al.,
Ann. Rheum. Dis. 66:921-926 (2007)

Median serum *trough* adalimumab concentrations (mg/l) over time in patients with anti-adalimumab antibody concentrations of 12–100 AU/ml and .100 AU/ml compared with patients without antiadalimumab antibodies. A

Table 1 | **Clinical consequences of antibodies**

Consequence of antibody	Biopharmaceutical	References
Loss of efficacy	Insulin	5
	Streptokinase	6
	Staphylokinase	7
	ADA	63
	Salmon calcitonin	9
	Factor VIII	3
	IFN-α2	14,26
	IFN-β	15
	IL-2	23
	GnRH	64
	Denileukin diftitox	65
	HCG	66
	GM-CSF/IL-3	67
Enhancement of efficacy	Growth hormone	2
Neutralization of native protein	MDGF	45
	EPO	13,43

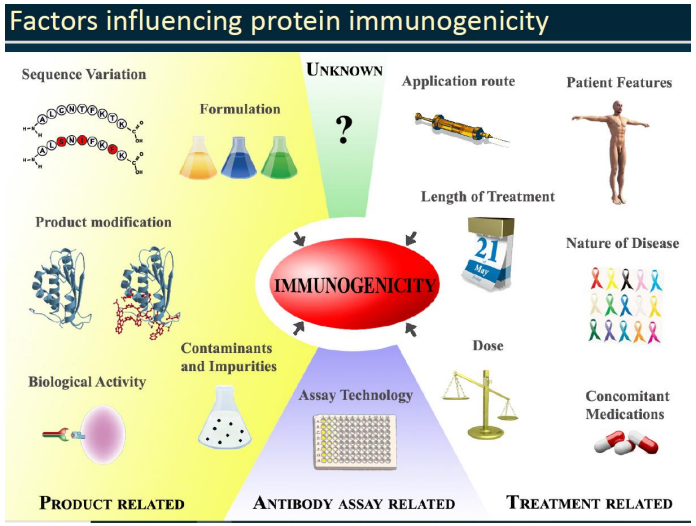
ADA, adenosine deamidase; EPO, erythropoietin; GM-CSF, granulocyte–macrophage colony-stimulating factor; GnRH, gonadotropin-releasing hormone; HCG, human chorionic gonadotropin; IFN-α2, interferon-α2; IL-2, interleukin-2; MDGF, megakaryocyte-derived growth factor.

Schellekens, Nature Reviews I Drug Discovery 2002

Immunogenicity of biologicals

Factors that will be discussed below:

- Structural properties
- Assays
- Formulation
- Other.....only mentioned
- Handling



Structural properties

- Degree of “non-self”: biopharmaceuticals of bacterial and plant origin (streptokinase, staphylokinase, asparaginase)

- Glycosylation
  - Protection of antigenic sites (GM-CSF)
  - Influence on solubility (Interferon beta)

Factors influencing immunogenicity

Assays/

FDA Guidance Document....

GUIDANCE DOCUMENT

Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection

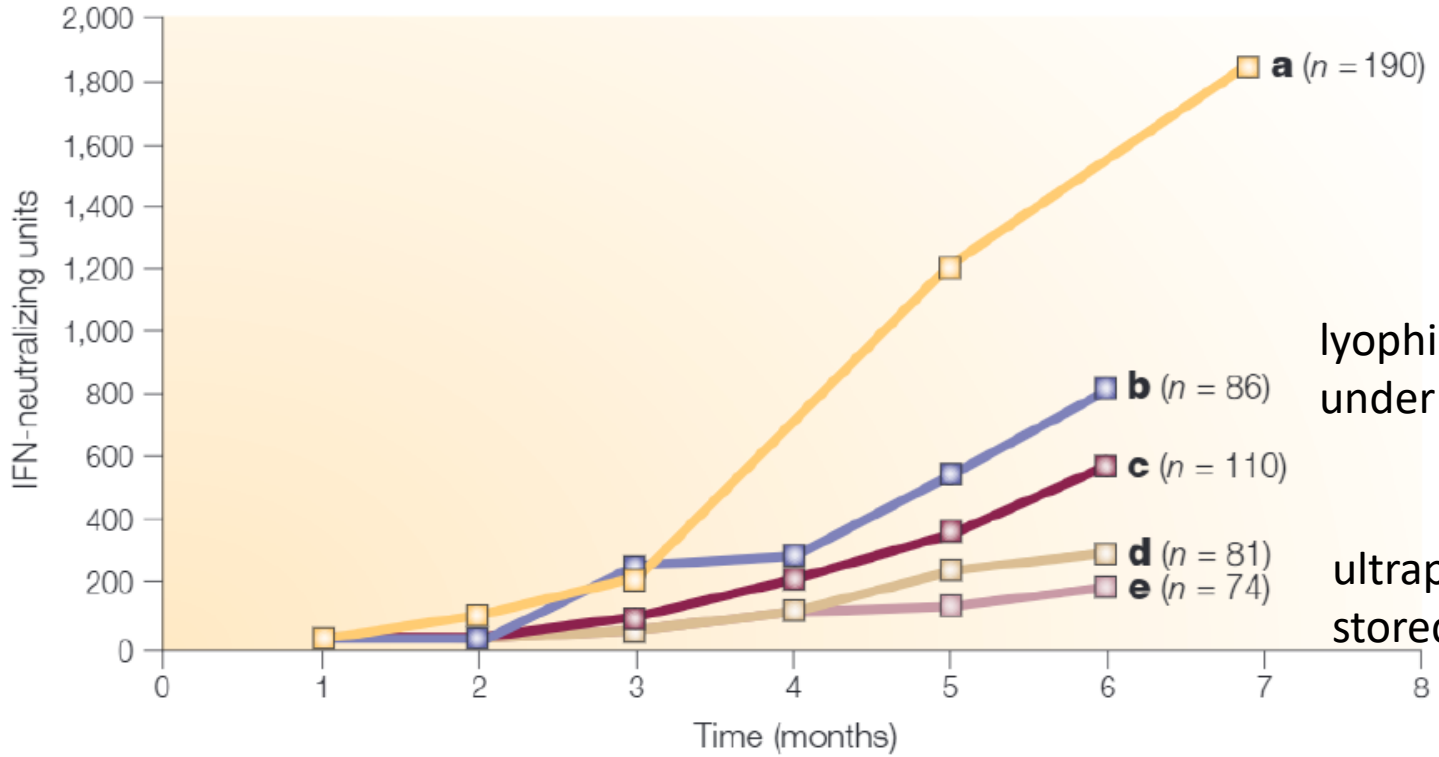
FEBRUARY 2019

…. ‘Specifically, this document includes guidance regarding the development and validation of screening assays, confirmatory assays, titration assays, and neutralization assays.’……

2019

Factors influencing immunogenicity

Formulation: the interferon alpha 2 and epo case



lyophilized powder stored at room temperature;

lyophilized powder stored under refrigeration

ultrapure lyophilized powder stored under refrigeration

Figure 2 | **Immunogenicity of different human IFN-α2A preparations.** The immunogenicity of human interferon-α2A (IFN-α2A) is highly dependent on the formulation and storage conditions, as shown here by the mean-population antibody titre in patients treated with different IFN preparations: **a** | lyophilized powder stored at room temperature; **b** | lyophilized powder stored under refrigeration; **c** | human serum albumin (HSA)-containing liquid stored under refrigeration; **d** | ultrapure liquid formulation (HSA-free) stored under refrigeration; and **e** | ultrapure lyophilized powder stored under refrigeration. IFN-neutralizing units; arbitrary unit of neutralizing activity; *n*, number of patients. Reproduced with permission from REF. 26 © (1997) Mary Ann Liebert, Inc.

Ryff, 1997

HPLC analysis of a highly immunogenic batch of Interferon (IFN)-alfa2A

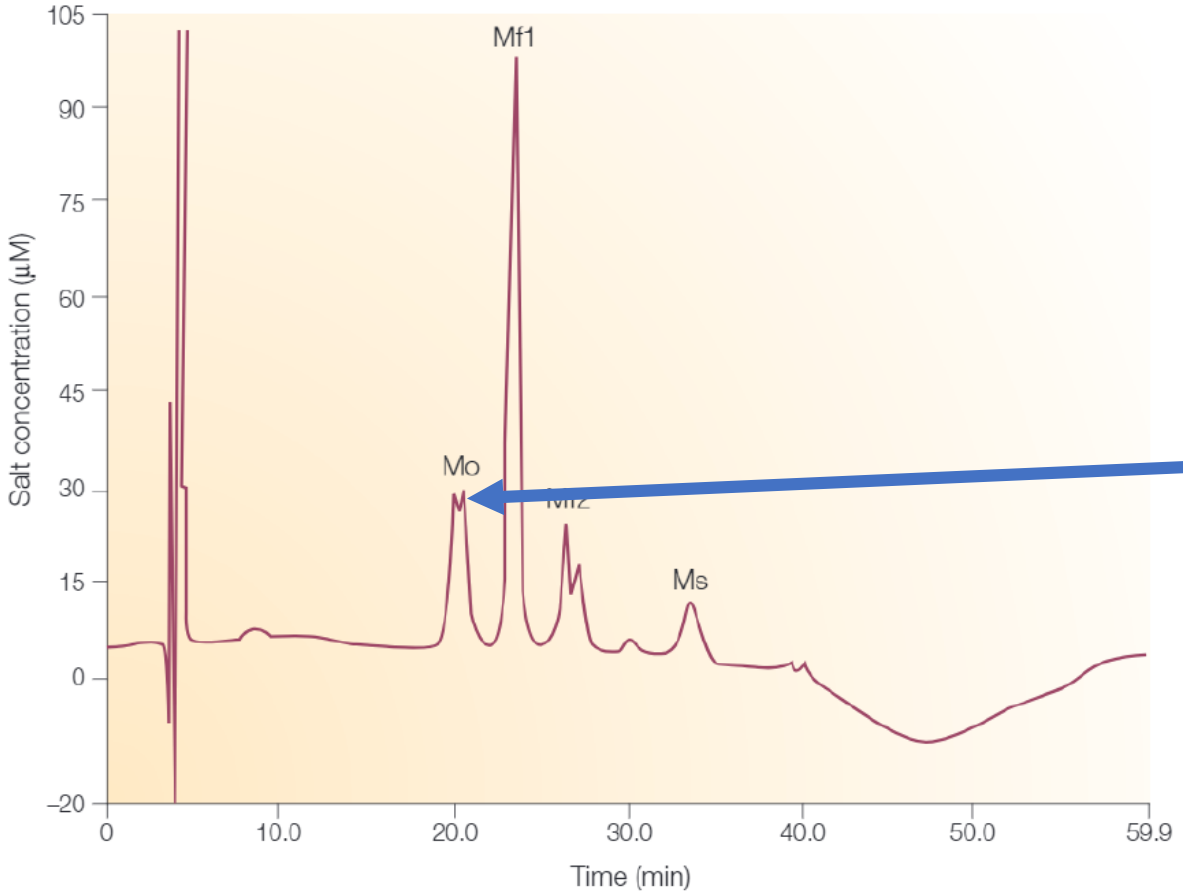


Figure 3 | **RP-HPLC of a highly immunogenic batch of interferon (IFN)-α2A.** The chart shows that this sample contains high levels of the oxidized form (Mo) of IFN-α2A. This oxidized form is more immunogenic than the non-oxidized form (Mf1), and it also contributes to the formation of aggregates, which greatly enhance immunogenicity. Mf2 is the acetylated form, and Ms is the form with only a single disulphide bridge. RP-HPLC, reversed-phase high-performance liquid chromatography. Reproduced with permission from REF. 28 © (1997) Mary Ann Liebert, Inc.

This oxidized form is more immunogenic than the non-oxidized form (Mf1), and it also contributes to the formation of aggregates, which greatly enhance immunogenicity

Ryff, 1997

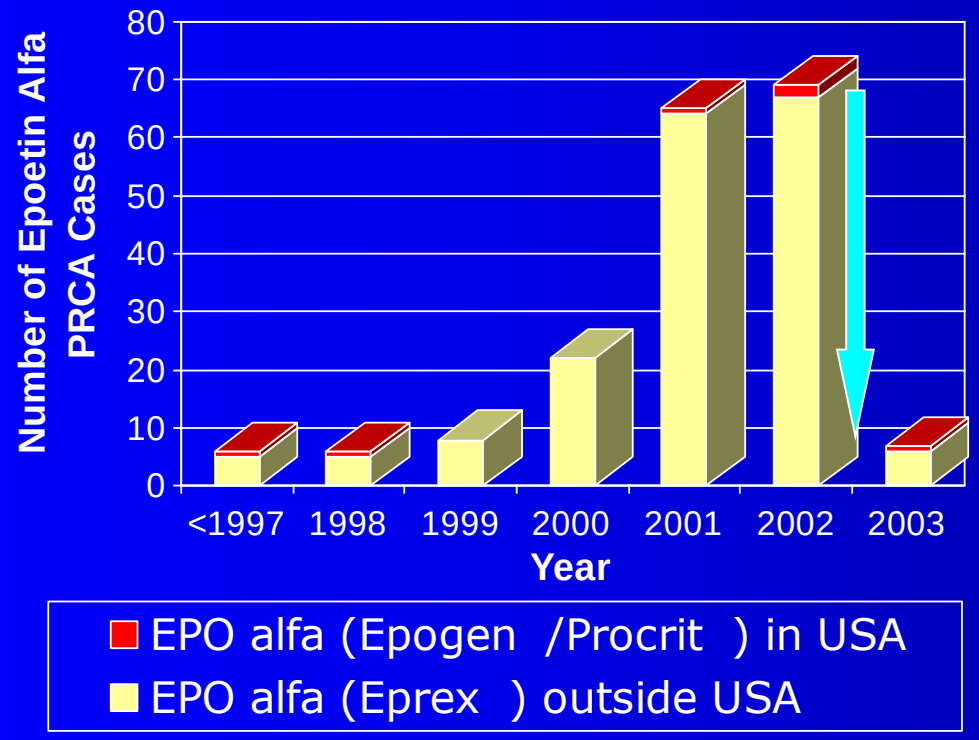
Main Stabilizers Used in Epoetin Formulations

Epogen®/Procrit® *(US)*	**Eprex®** *(pre 1998)*	**Eprex®** *(post 1998)*
HSA	HSA	Polysorbate 80
		Glycine

Because of BSE, mad cow disease" HSA had to be removed

Anti-epoetin antibody-related pure red cell aplasia cases (PRCA)

Removal of human serum albumin stabilizer
from epoetin alfa (outside USA)



Courtesy of Schellekens et al.

Other factors influencing immunogenicity

- Route of administration
  - S.c. > i.m. > i.v.
  - Type of disease
- Genetic background of patients
  - MHC?
- Unknown factors.... See later.

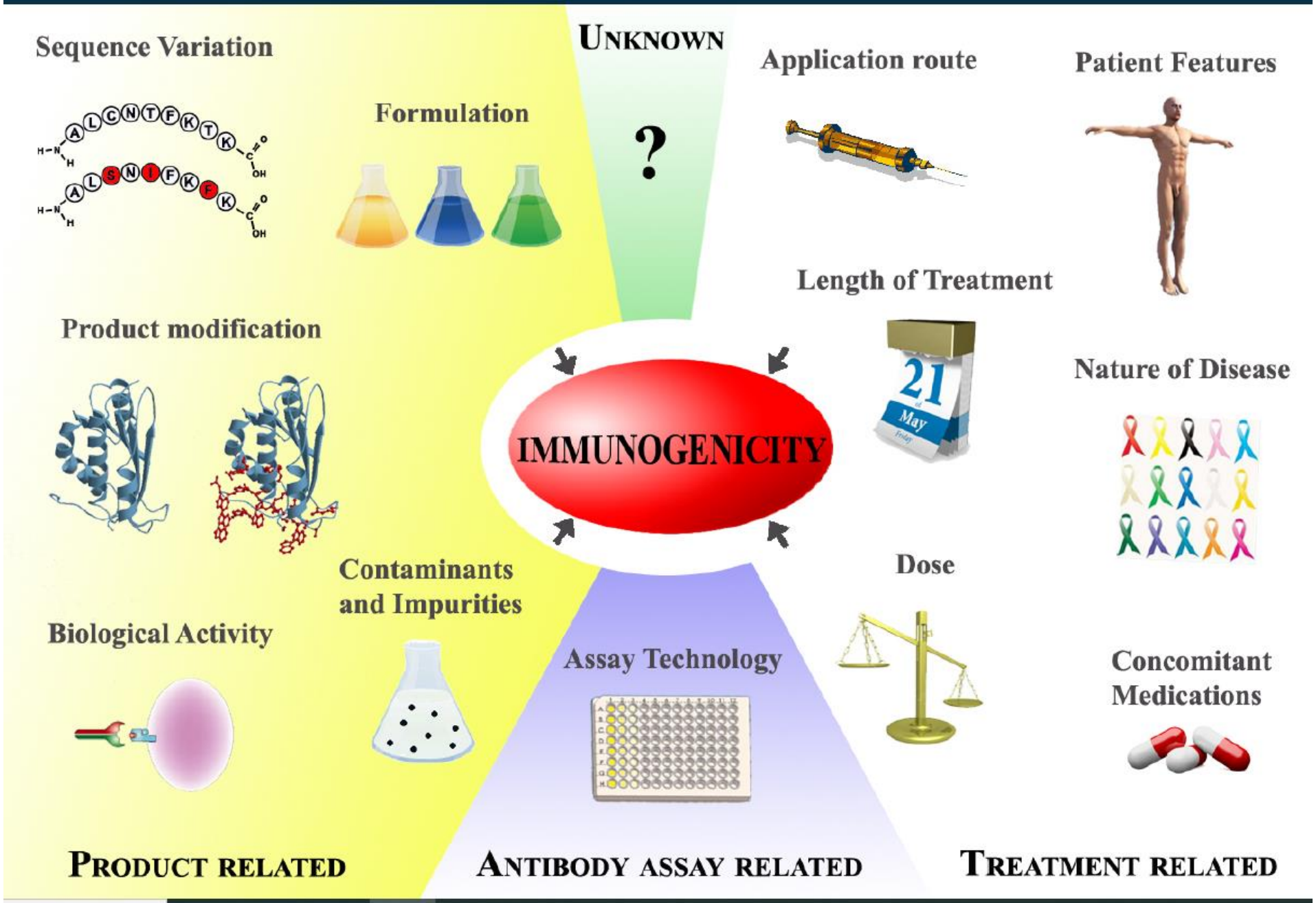
So, THE immunogenicity of a protein does not exist

Product	Characteristics	Indication	Immunogenicity (%)
Rituxan/rituximab	chimeric/ CD20	NHL	0
Rituxan/rituximab	chimeric / CD20	SLE	65
Rituxan/rituximab	chimeric / CD20	PSS	27

Prediction of immunogenicity

- Purity of the product
- Epitope analysis
- Reaction with patient sera
- Animal experiments
  - ‘Conventional’ animals (relative immunogenicity)
  - Non-human primates
  - Immune tolerant transgenic mice

Factors influencing protein immunogenicity



HANDLING!
Aggregate formation

Courtesy of Jiskoot/ Schellekens

Pharmaceutical Research, Vol. 21, No. 6, June 2004 (© 2004)

Review Article

Structure-Immunogenicity Relationships of Therapeutic Proteins

Suzanne Hermeling,[1,2,3] Daan J. A. Crommelin,[1] Huub Schellekens,[2] and Wim Jiskoot[1]

***The AAPS Journal* 2006; 8 (3) Article 59 (http://www.aapsj.org).**

Themed Issue: Proceedings of the 2005 AAPS Biotec Open Forum on Aggregation of Protein Therapeutics
Guest Editor - Steve Shire

Effects of Protein Aggregates: An Immunologic Perspective

Submitted: March 3, 2006; Accepted: May 24, 2006; Published: August 4, 2006

Amy S. Rosenberg[1]

Review

Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins

Miranda M.C. van Beers and Muriel Bardor

Biotechnol. J. 2012, 7

(over-)simplified summary:

protein aggregates are immunogenic

Proteins and interfaces…… aggregate formation……. Immunogenicity up

W

From Li, 2019: aggregate formation…

Shaking…..adherence to water–air interfaces
Freeze-thaw, e.g water-ice interfaces

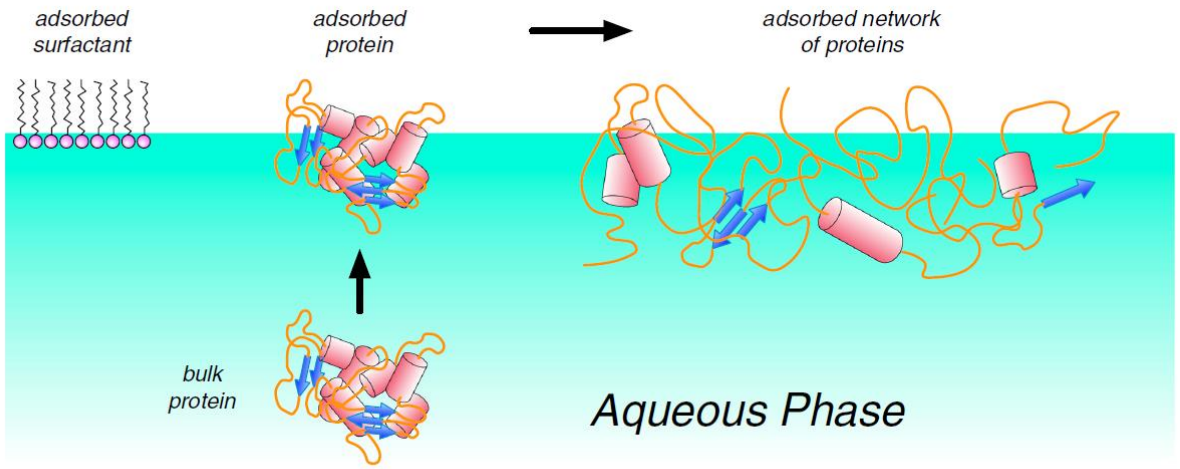


Fig. 1. Protein interfacial behavior. Proteins from the bulk solution can adsorb to the interface leading to an adsorbed network of proteins. Surfactants can mitigate this adsorption. Modified from Morris *et al.* (1)

Examples…. from Herceptin, insert….
'SWIRL the vial gently to aid reconstitution. DO NOT SHAKE
Following reconstitution….DO NOT FREEZE.'

Formulation excipients – examples

Excipient class	Function	Examples
Buffers	pH control, tonicity	Histidine, phosphate, acetate, citrate, succinate
Salts	Tonicity, stabilization, viscosity reduction	Sodium chloride
Sugars[a], polyols	Tonicity, stabilization, cryoprotection, lyoprotection[b], bulking agent[b], reconstitution improvement[b]	Sucrose, trehalose, mannitol, sorbitol
Surfactants	Adsorption prevention, solubilization, stabilization, reconstitution improvement[b]	Polysorbate 20, polysorbate 80, poloxamer 188
Amino acids	Stabilization, viscosity reduction, tonicity, pH control, bulking agent[b]	Arginine, glycine, histidine, lysine, proline
Anti-oxidants	Oxidation prevention	Methionine, sodium edetate
Preservatives[c]	Bacterial growth prevention	m-cresol, benzyl alcohol, phenol

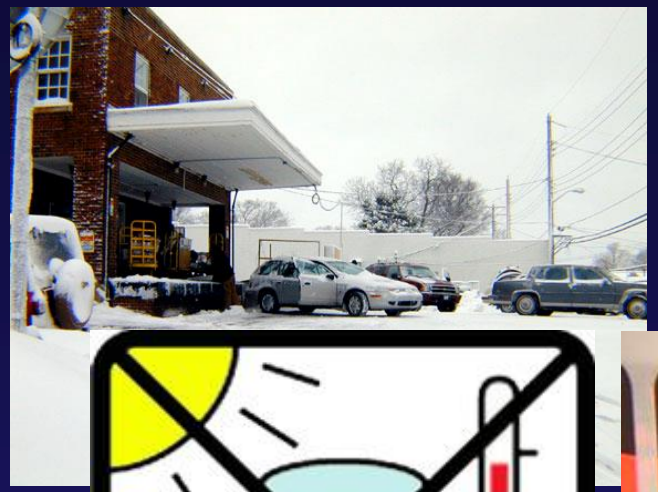
Adapted from Weinbuch et al. (2018)
[a]Only non-reducing sugars
[b]For freeze-dried products
[c]Multi-dose containers

Table 5.6 ■ Common excipients in protein drug products

Crommelin, D.J.A., Hawe, A., and Jiskoot, W. (2019) Formulation of biologicals including biopharmaceutical considerations. In: Pharmaceutical Biotechnology, 5th edition (D.J.A. Crommelin, R.D. Sindelar, and B. Meibohm, Eds.), Springer Nature Switzerland AG, pp. 83-103

How can we protect biologicals from aggregating through formulation design?

Recommendations for storage and handling of biopharmaceuticals in hospitals



Improper storage and handling can affect the integrity of a biopharmaceutical product that is administered to a patient. Proper storage and handling involves not only maintaining the cold chain, but also avoiding shaking and exposure to light, and using good sterile technique. Ensuring cold-chain management as part of medication integrity is thus of the utmost importance. Therefore, the International Working Group formulated the following recommendations.

biopharmaceuticals

- All manufacturers of biopharmaceuticals should produce product-specific information tools on the proper storage and handling of their products
- Professional organizations should be proactive in education of newly qualified pharmacists and continuing education of established pharmacists

Distribution

- The time a biopharmaceutical product remains outside the refrigerator must be kept as short as possible
- Tempreature-sensitive biopharmaceuticals should be transported between the hospital pharmacy and the ward in insulated transit packs
- Validated, insulated carry packs should be available for all patients who need to transport biopharmaceuticals
- Biopharmaceuticals should be protected from shaking and exposure to light.
- Prior to injection, biopharmaceuticals

- Policies and procedures should be made available for all situations in the hospital in which the cold chain is broken
- Hospital refrigerators outside the pharmacy should at least be equipped with a minimum-maximum thermometer. These thermometers should be read at least once daily
- Patients receiving long-term treatment with biopharmaceuticals should be encouraged to have high-quality refrigerators
- All healthcare professionals and patients involved in storing biopharmaceuticals must be aware of the need for good stock control and rotation

Standards

- Policies and procedures defining roles and responsibilities are needed for all stages of cold-chain management
- Routine audits and quality assurance are necessary to ensure cold-chain integrity from the hospital warehouse to the patient
- All facilities and transport systems must

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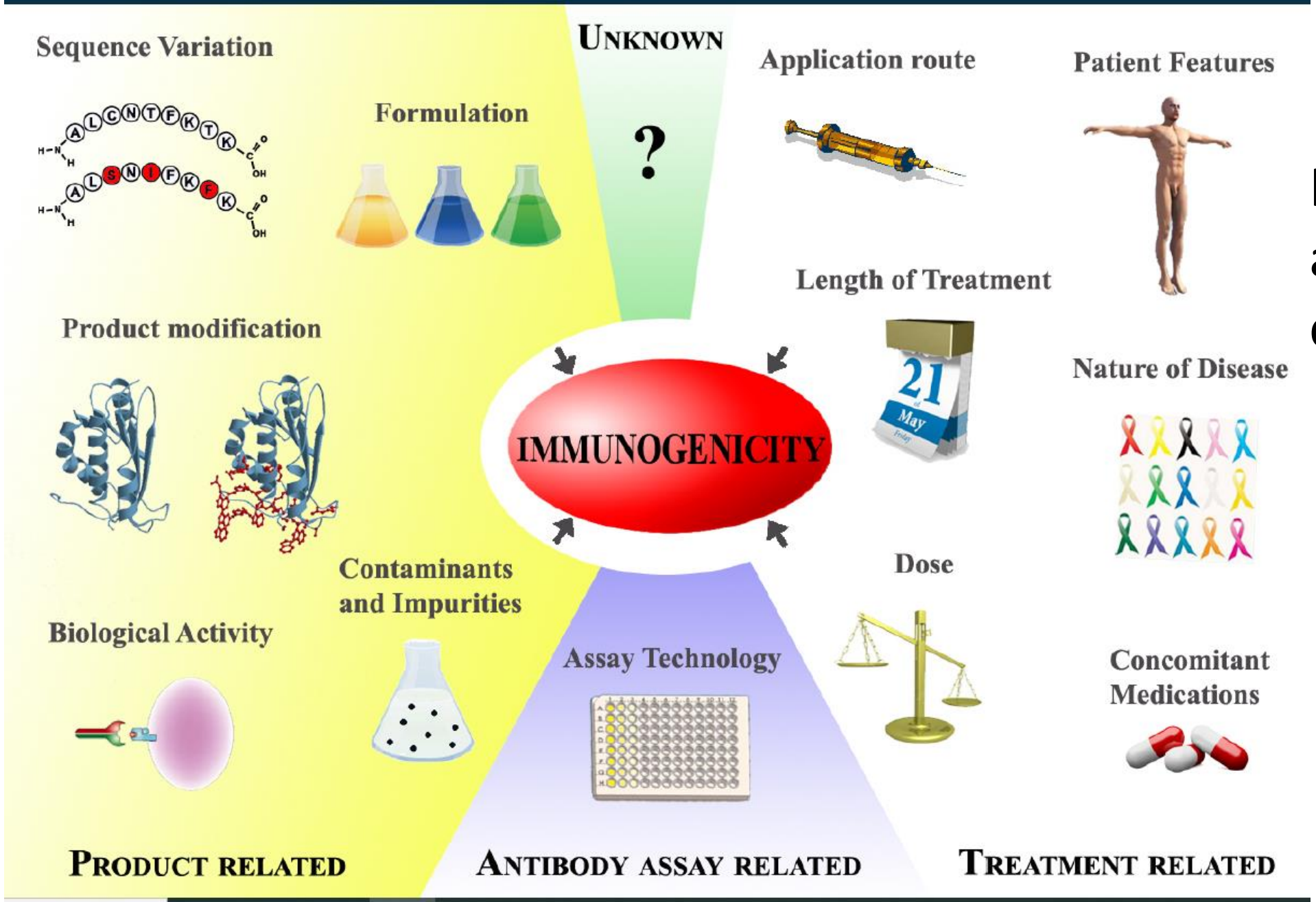
Teresa Bermejo, Hospital Severo Ochoa, Leganes Madrid, Spain
Marco Bissig, Ospedale Civico Lugano, Lugano, Switzerland
Daan Crommelin, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
Jaak Damiaans, Virga Jesse Ziekenhuis, Hasselt, Belgium
William Gouveia, Tufts New England Medical Center, Boston, USA
Kenneth Johansson, Norrlands Universitetssjukhus, Umeå, Sweden
Allan Karr, University College Hospitals London Trust, London, UK
Tom Paton, Sunnybrook and Women's College Health Science Centre, Toronto, Canada
Suzie Pearce, St George's Hospital, London, UK
Richard Plumridge, Pharmaceutical Healthcare Consulting, Churchlands, and Consultant, Hollywood Private Hospital, Nedlands Western Australia
Huub Schellekens, Central Laboratory Animal Institute, Utrecht University, Utrecht, the Netherlands
Giovanna Scroccaro, Ospedale Policlinico G.B. Rossi, Verona, Italy
Roger Tredree, St George's Hospital, London, UK

Daan Crommelin, Utrecht Institute for Pharmaceutical Sciences, Utrecht

Factors influencing protein immunogenicity



Immunogenicity: a multifaceted challenge!

Courtesy of Jiskoot/ Schellekens