

In Memoriam professor Wim Jiskoot
(1961-2021)



Papers Jiskoot/Schellekens as authors

PubMed_Timeline_Results_by_Year

Search query: jiskoot schellekens		
Year		Count
	2018	1
	2016	1
	2014	2
	2013	3
	2012	3
	2011	4
	2010	5
	2009	3
	2008	1
	2006	4
	2005	2
	2004	1
	2003	1

Tribute to Wim Jiskoot

- Role of aggregates in the immunogenicity of biopharmaceuticals
- The use of immune tolerant transgenic animals in immunogenicity studies
- Breaking of tolerance as main mechanism in immunogenicity of human homologues
- What caused Eprex associated PRCA
- Biosimilars and beyond

The road to aggregates

The interferon alpha case

The Assumption

- During the clinical trials with interferon alpha 2 in patients it became clear that about 20-30% of the patients produced antibodies after 6-12 months of treatment
- We started to test an interferon alpha B/D, an unnatural hybrid molecule. The prediction was: it would be immunogenic in patients

The results

- Interferon alpha B/D proved to be relatively highly immunogenic in rhesus monkeys
- A clinical trial was performed in Chronic Hep C. Treatment of 6 months: no antibodies

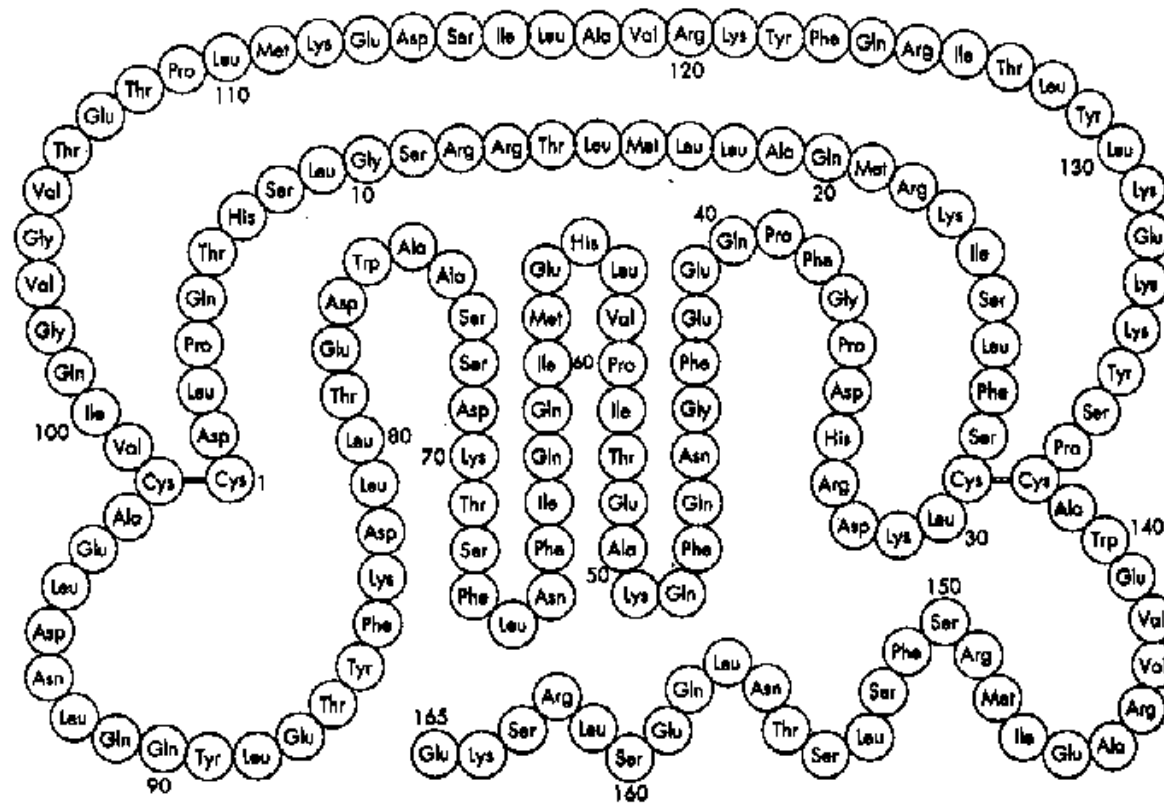
Why is a natural IFN alpha 2 immunogenic and a non-natural IFN alpha non-immunogenic?

Another part of the puzzle
the Roferon case

Antibodies and type of interferon alpha 2

Type of interferon	% Antibodies
r-IFN alpha 2a	20
r- IFN alpha 2b	7

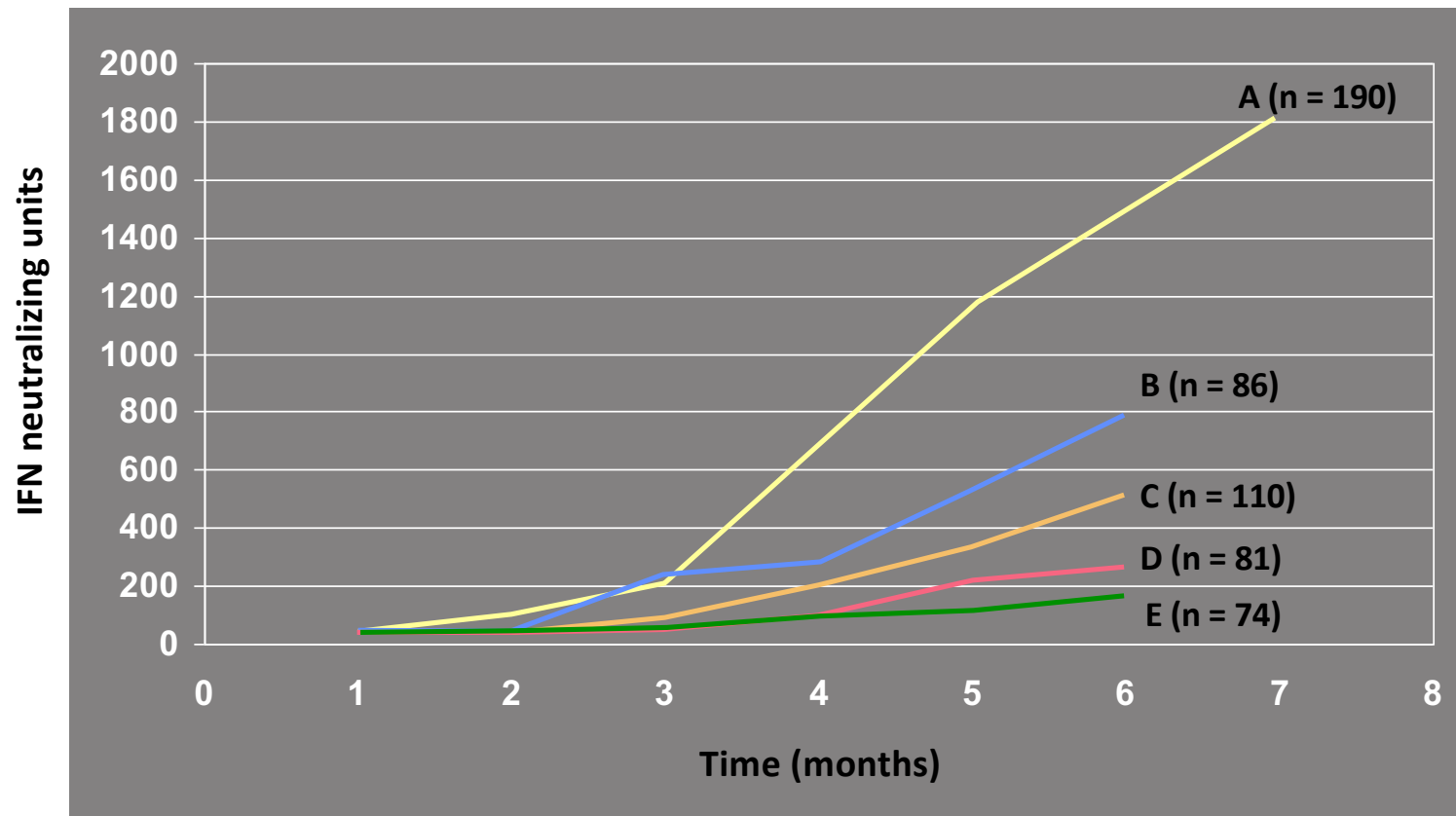
Natural Hu IFN alpha-2

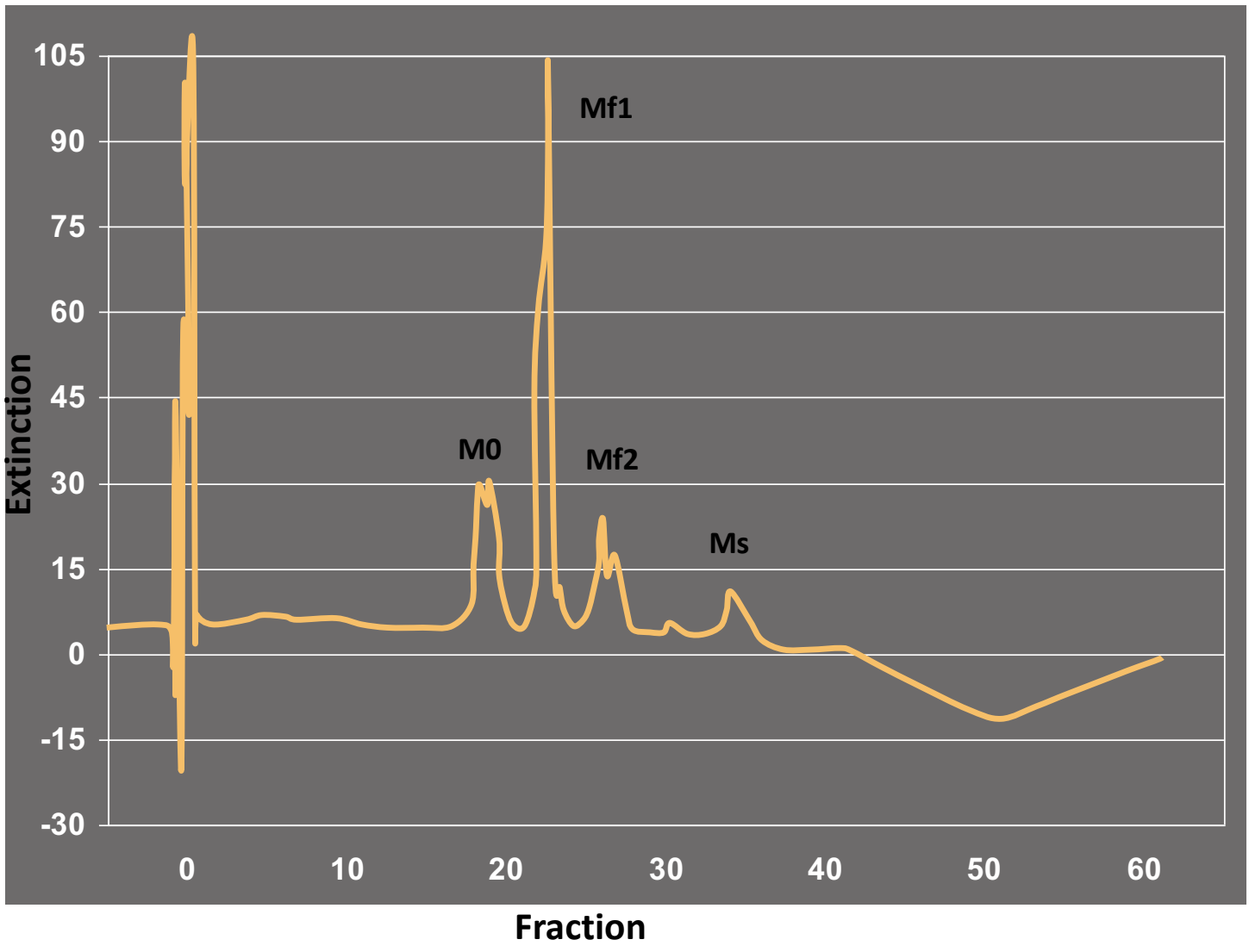


Two main IFN alpha-2 preparations

Generic name	Commercial name	Aa position 23	Natural allele
Hu IFN alpha-2a	Roferon	Lys	No
Hu IFN alpha-2b	Intron	Arg	Yes

Immunogenicity of different IFN alpha-2a formulations





Roferon immunogenicity

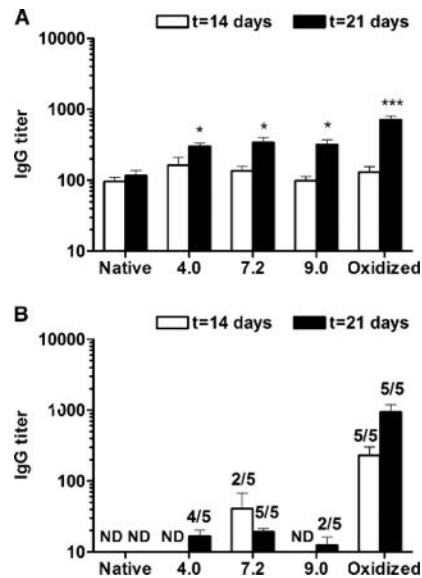
- Highest incidence of immunogenicity: freeze-dried HSA containing formulation stored at room temperature
- Storage at room temperature led to oxidation of IFN alpha 2 molecule
- Oxidated molecules lead to aggregates of interferon alpha 2 and HSA
- Aggregates break tolerance in IFN alpha 2b transgenic mice

First funding of immunogenicity studies

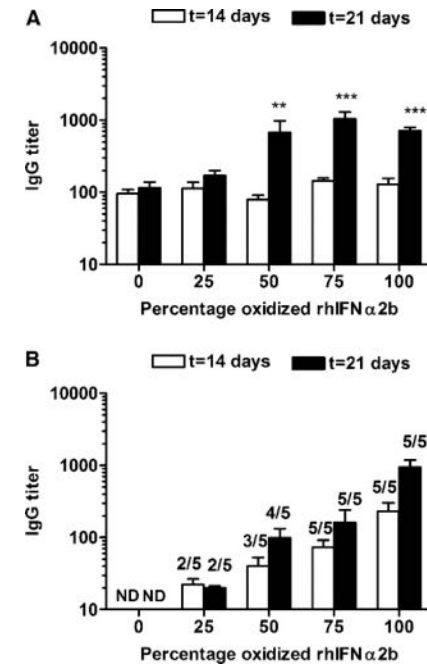
- EU concerted action: funding for meetings and coordination of studies of immunogenicity of biopharmaceuticals
- EU consortium developing new freeze- drying technology needed a biological read-out for level of aggregates

Antibody response to aggregated human interferon alpha2b in wild-type and transgenic immune tolerant mice depends on type and level of aggregation

Suzanne Hermeling¹, Huub Schellekens, Coen Maas, Martijn F B G Gebbink, Daan J A Crommelin, Wim Jiskoot



A: wildtype mice B: immune tolerant transgenic animals

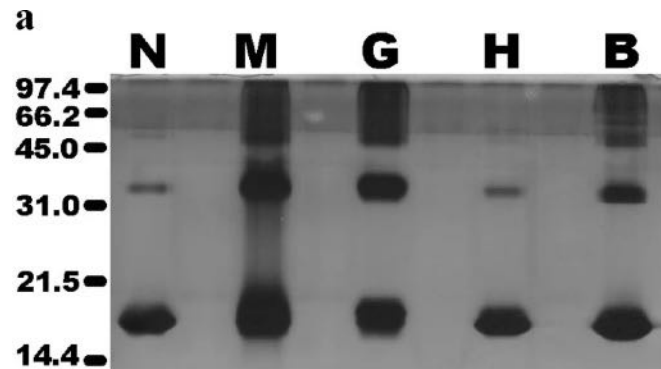


Structural characterization and immunogenicity in wild-type and immune tolerant mice of degraded recombinant human interferon alpha2b

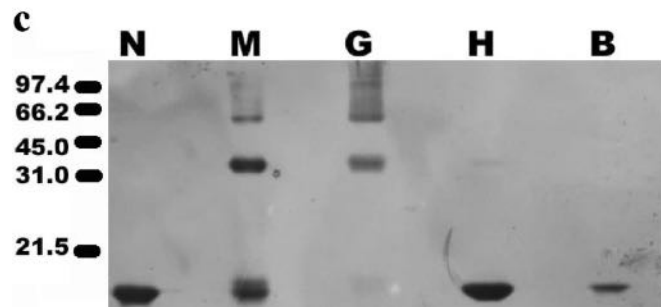
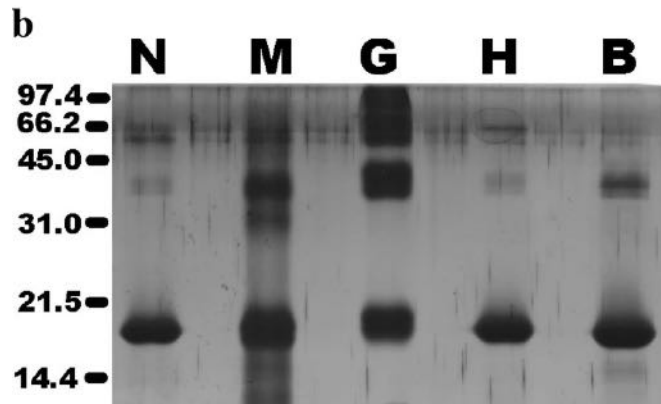
Suzanne Hermeling ¹, Liliana Aranha, J Mirjam A Damen, Monique Slijper, Huub Schellekens, Daan J A Crommelin, Wim Jiskoot

	Soluble fraction ^a				Insoluble fraction (%) ^b	Size (µm) ^c	Oxidation ^d	Denaturation ^e	Destruction of epitopes ^g
	Monomers (%)	Dimers (%)	Trimers (%)	Larger aggregates (%)					
Native rhIFNα2b	98	2	0	0	0	-	+	-	-
Oxidized rhIFNα2b									
By H ₂ O ₂	99	1	0	0	0	-	+++	-	-
By metal catalysis	43	16	6	17	19	0.7	+++	+	-
Aggregated rhIFNα2b									
By glutaraldehyde	27	20	15	33	5	-	-	+	+
By boiling	0	0	0	0	100	1-3	-	+++	+

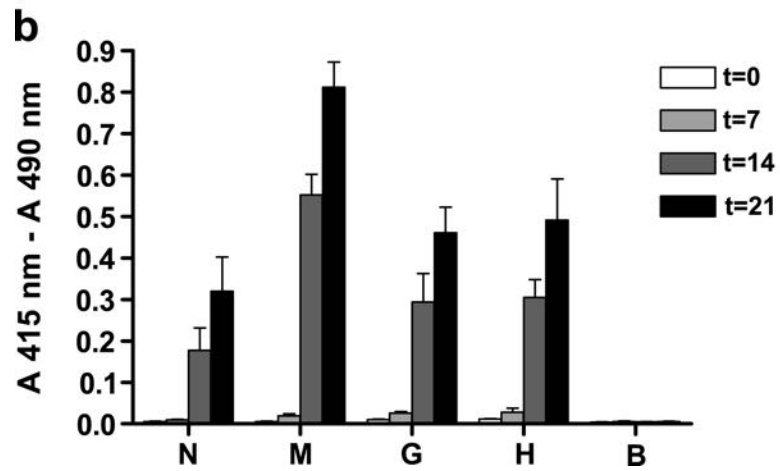
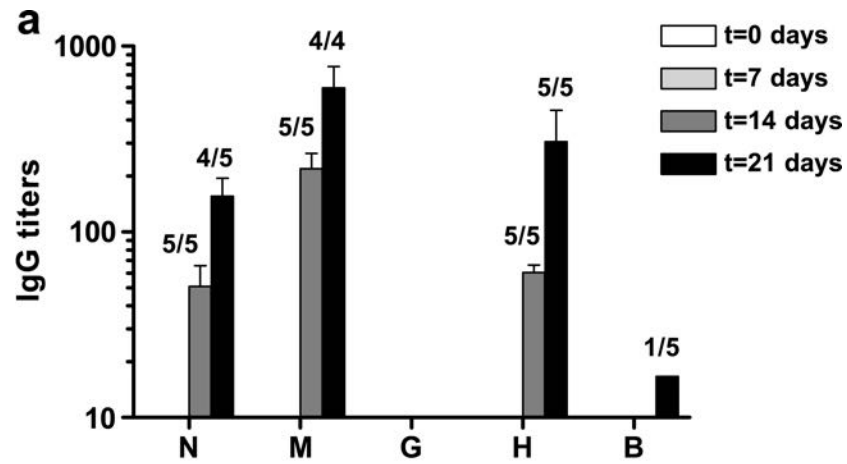
- Not detectable; + mild; +++ extensive



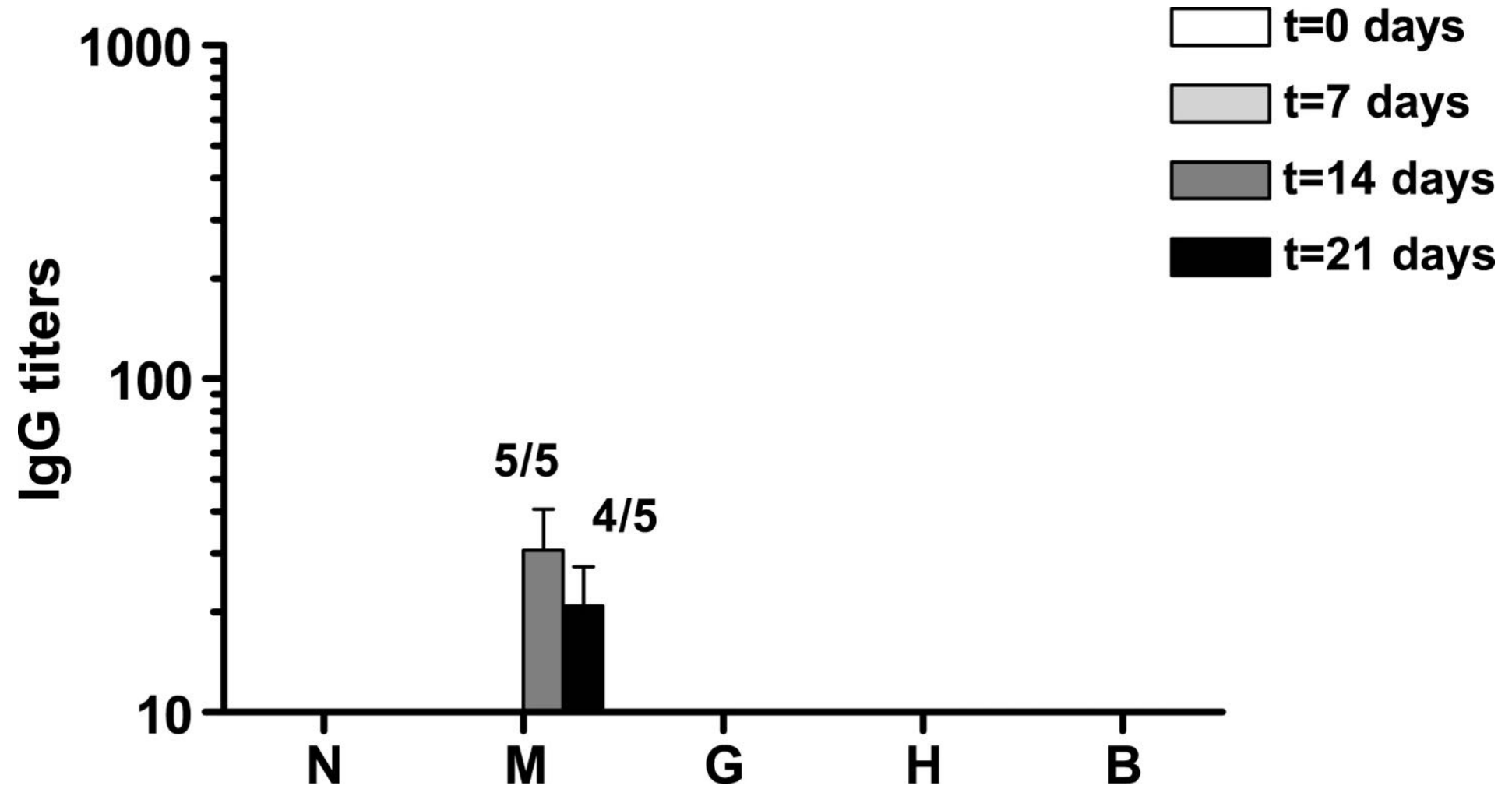
N: native interferon alpha 2
M: oxidized by metal catalysis
G: glutaraldehyde treated
H: H₂O₂ oxidized
B: boiled



Wildtype mice



Transgenic immune- tolerant mice

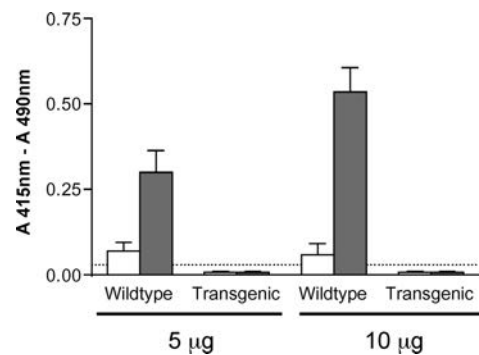


Human interferon beta
transgenic mice

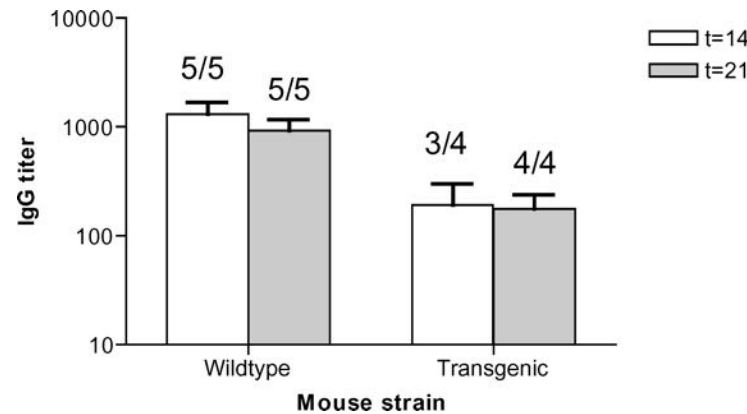
> Pharm Res. 2005 Jun;22(6):847-51. doi: 10.1007/s11095-005-4578-z. Epub 2005 Jun 8.

Development of a transgenic mouse model immune tolerant for human interferon Beta

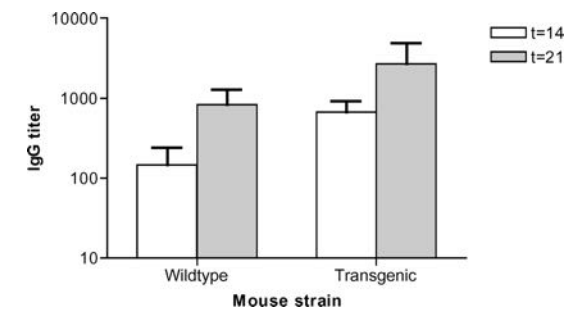
Suzanne Hermeling¹, Wim Jiskoot, Daan Crommelin, Claus Bornaes, Huub Schellekens



Treated with IFN beta 1a



Treated with IFN beta 1b

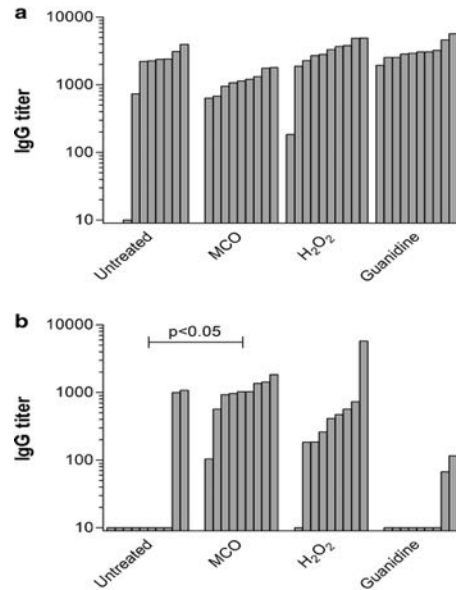
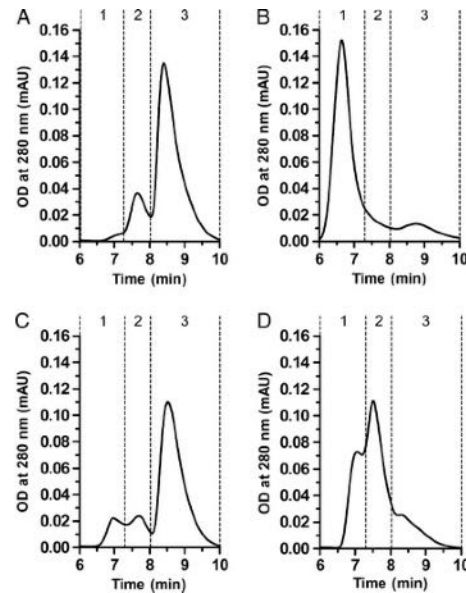


Albumin control

> Pharm Res. 2011 Oct;28(10):2393-402. doi: 10.1007/s11095-011-0451-4. Epub 2011 May 5.

Oxidized and aggregated recombinant human interferon beta is immunogenic in human interferon beta transgenic mice

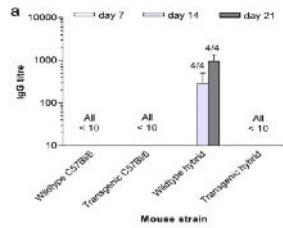
Miranda M C van Beers ¹, Melody Sauerborn, Francesca Gilli, Vera Brinks, Huub Schellekens, Wim Jiskoot



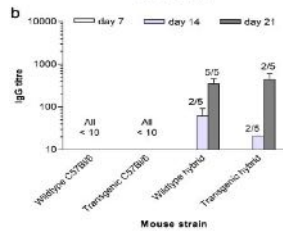
Hybrid transgenic immune tolerant mouse model for assessing the breaking of B cell tolerance by human interferon beta

Miranda M C van Beers ¹, Melody Sauerborn, Francesca Gilli, Suzanne Hermeling, Vera Brinks, Huub Schellekens, Wim Jiskoot

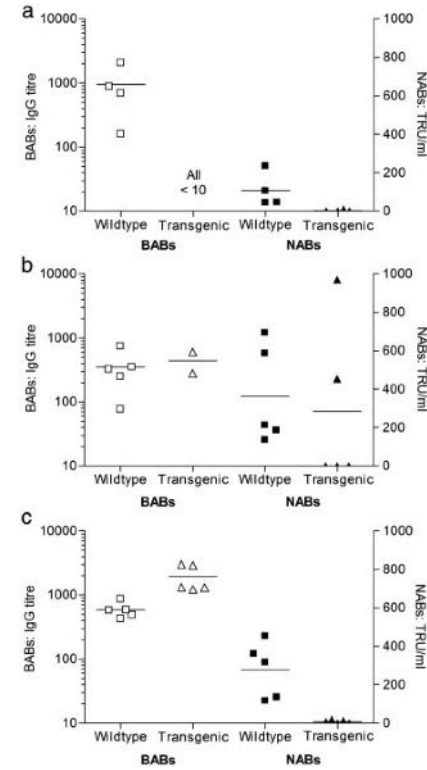
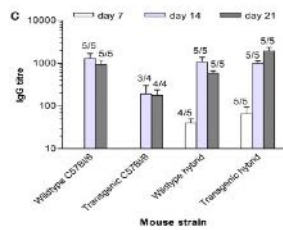
Rebif



Avonex



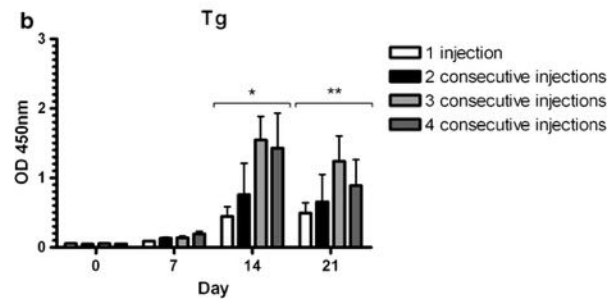
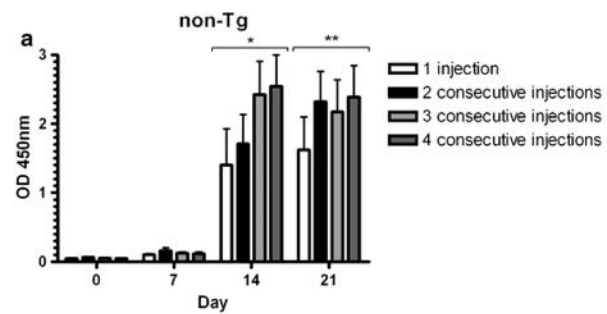
Betaseron



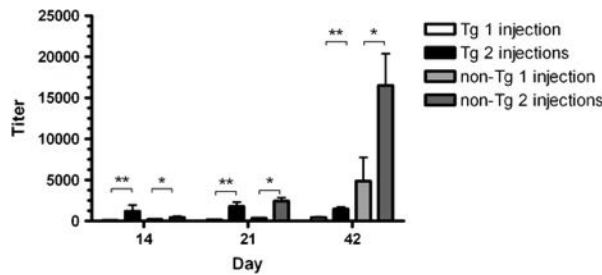
Effect of Treatment Regimen on the Immunogenicity of Human Interferon Beta in Immune Tolerant Mice

Grzegorz Kijanka , Wim Jiskoot, Huub Schellekens & Vera Brinks

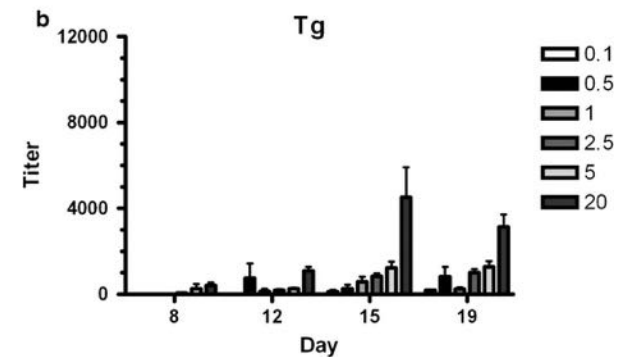
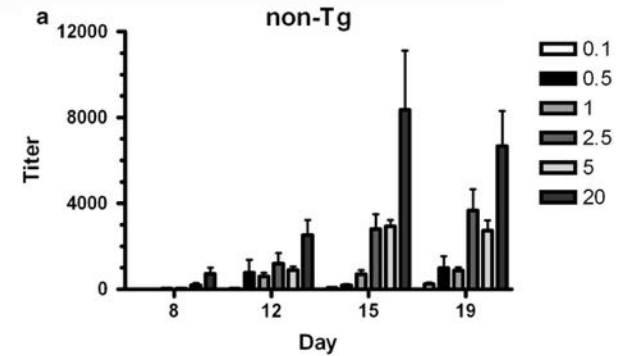
Pharmaceutical Research **30**, 1553–1560 (2013) | [Cite this article](#)



Consecutive injections



Number of injections



Different doses

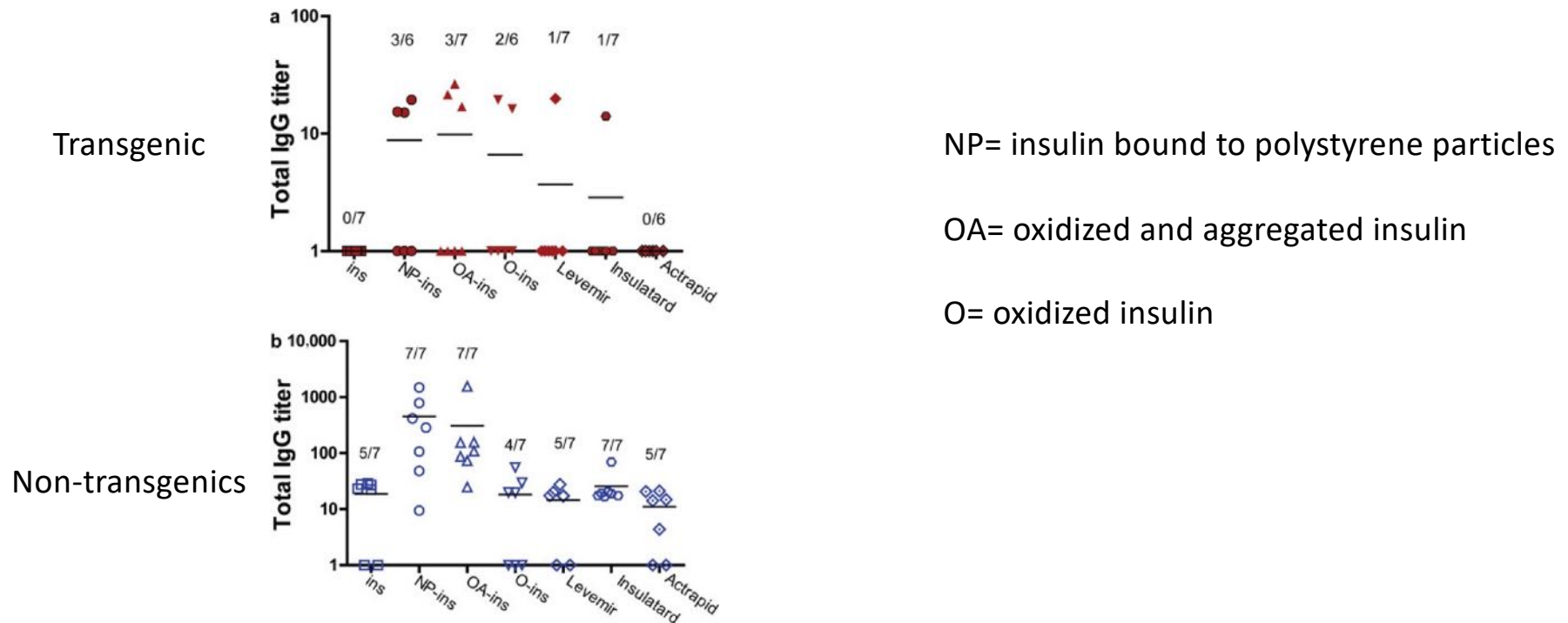
Other immune tolerant transgenic mice

- Human insuline
- Human Ig

> J Pharm Sci. 2014 May;103(5):1367-74. doi: 10.1002/jps.23935. Epub 2014 Mar 11.

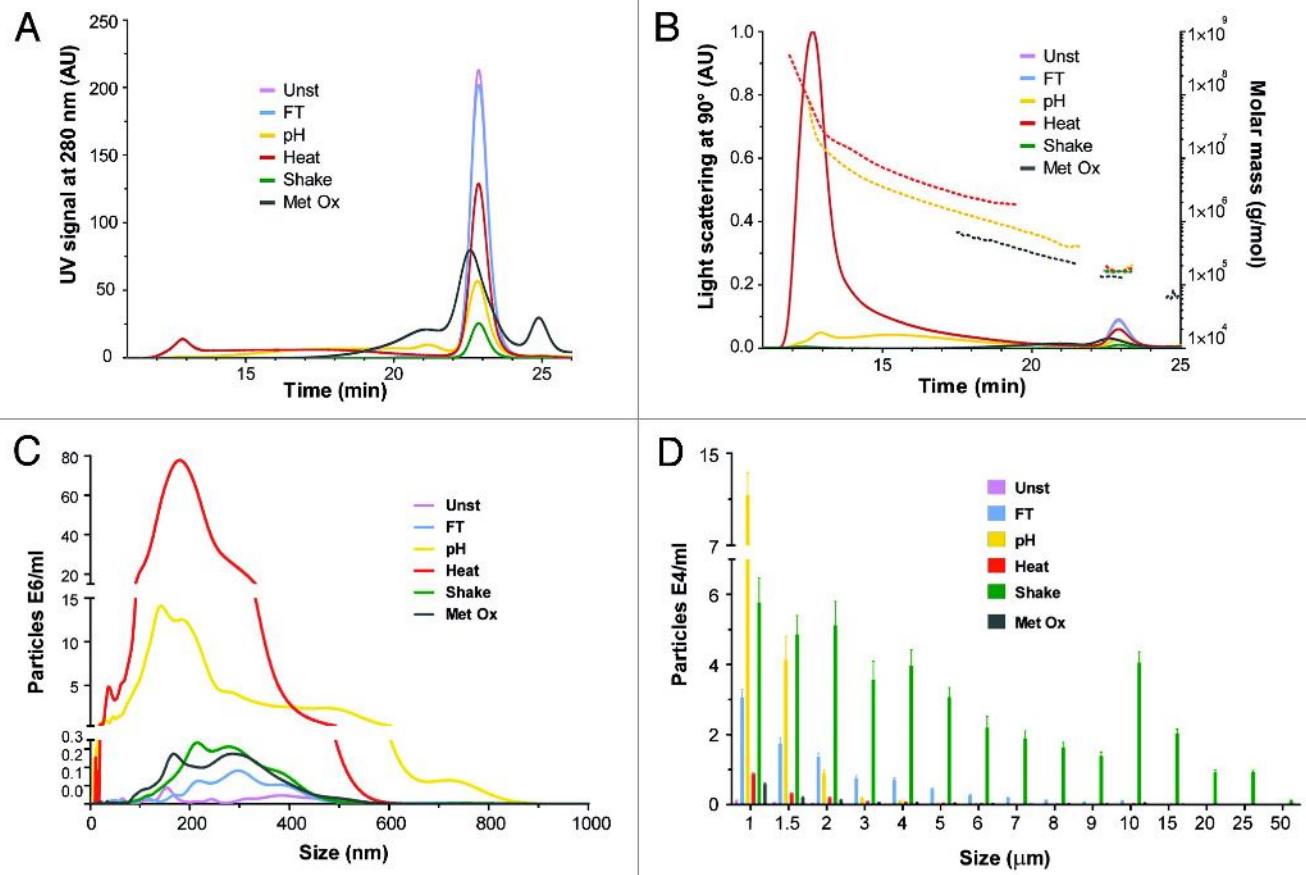
Development of a transgenic mouse model to study the immunogenicity of recombinant human insulin

Riccardo Torosantucci ¹, Vera Brinks, Grzegorz Kijanka, Liem Andhyk Halim, Melody Sauerborn, Huub Schellekens, Wim Jiskoot

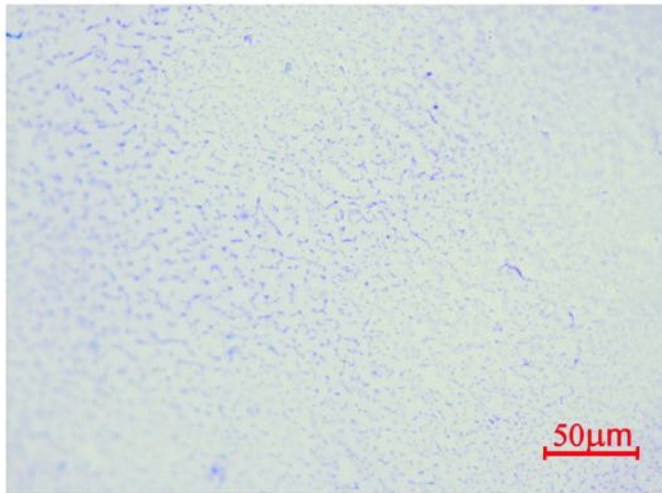


Immunogenicity of different stressed IgG monoclonal antibody formulations in immune tolerant transgenic mice

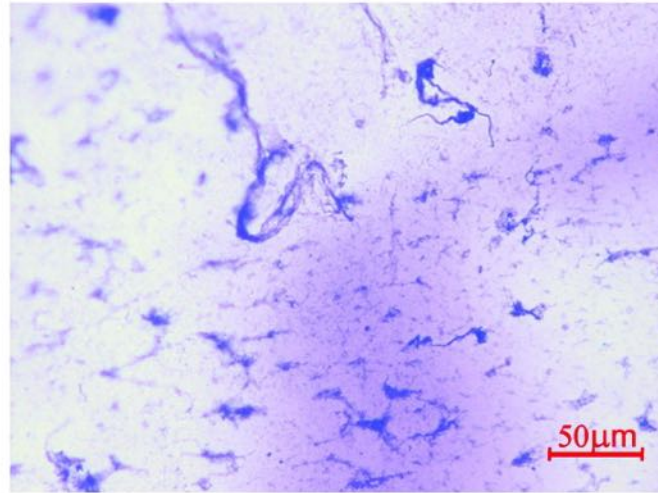
Vasco Filipe ¹, Wim Jiskoot, Abdul Hafid Basmeh, Andhyk Halim, Huub Schellekens, Vera Brinks



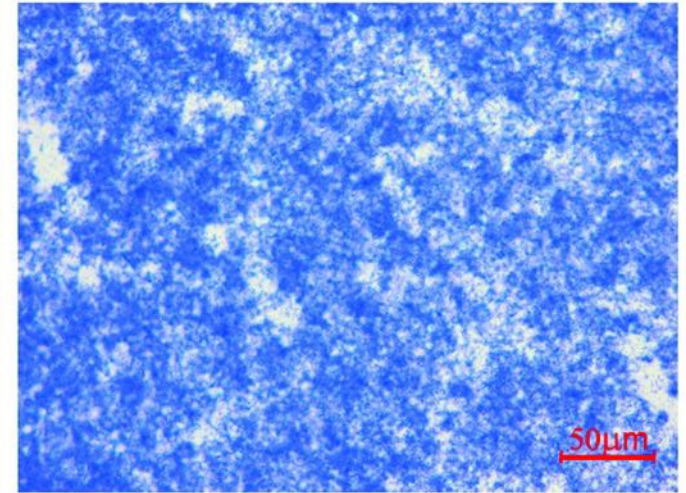
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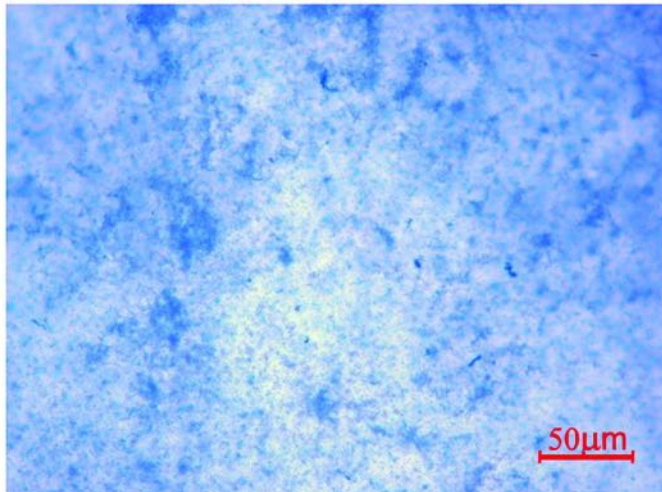
FT



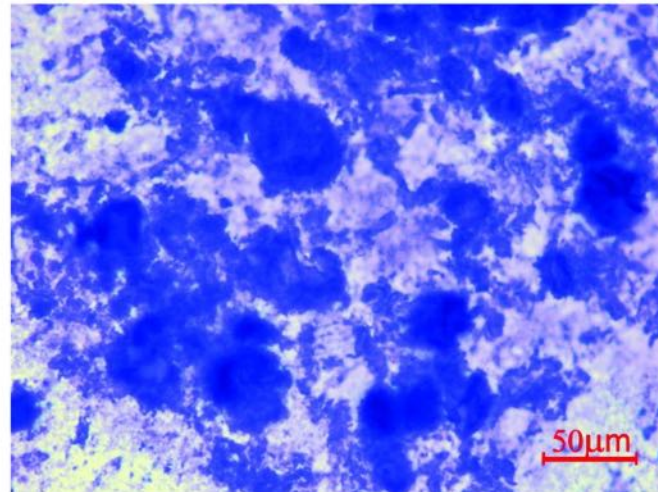
pH



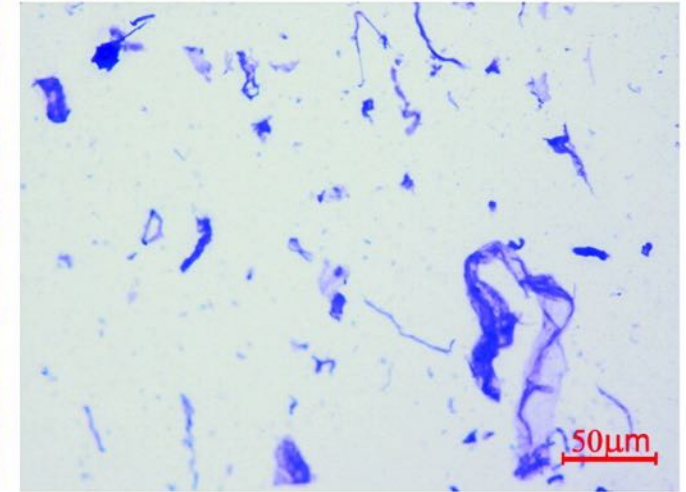
Heat



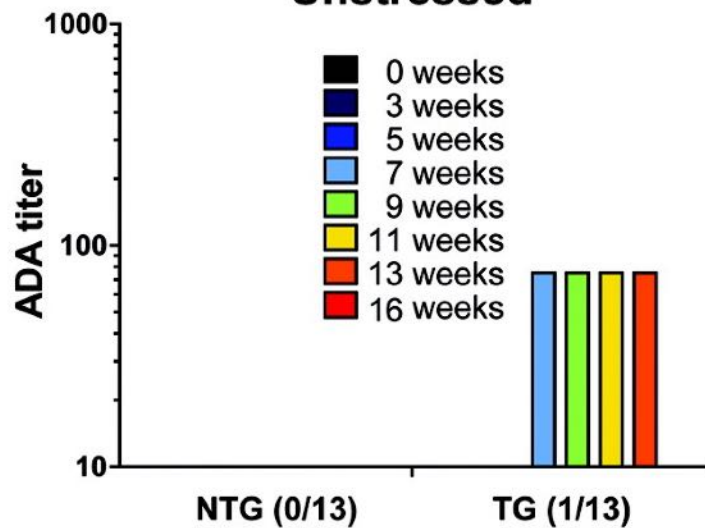
Shake*



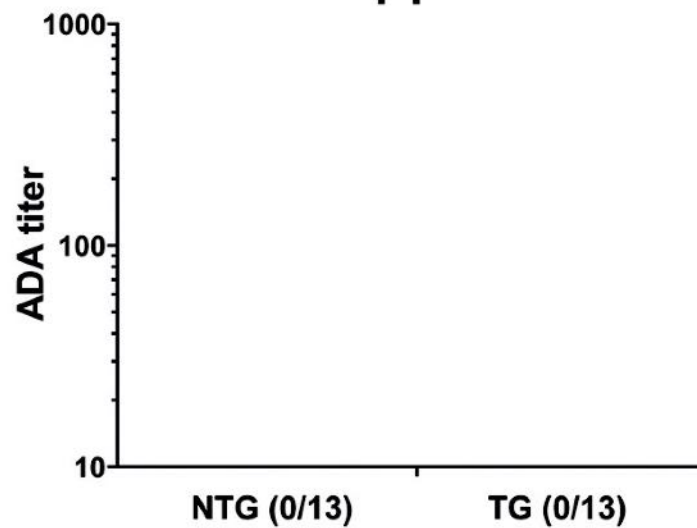
Met ox



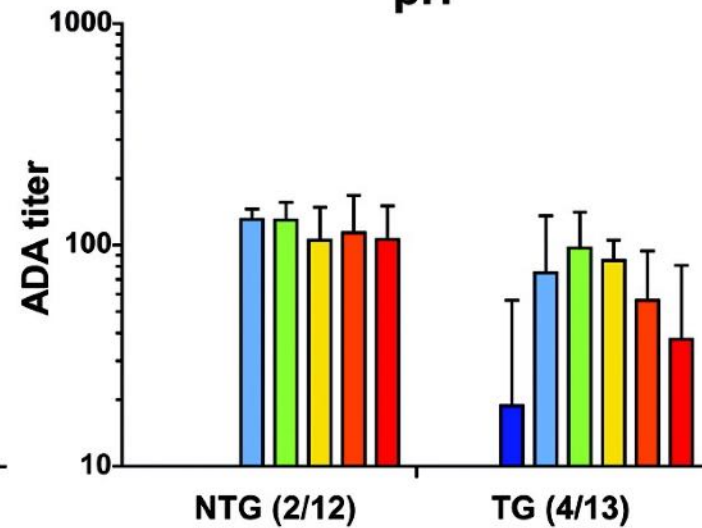
Unstressed



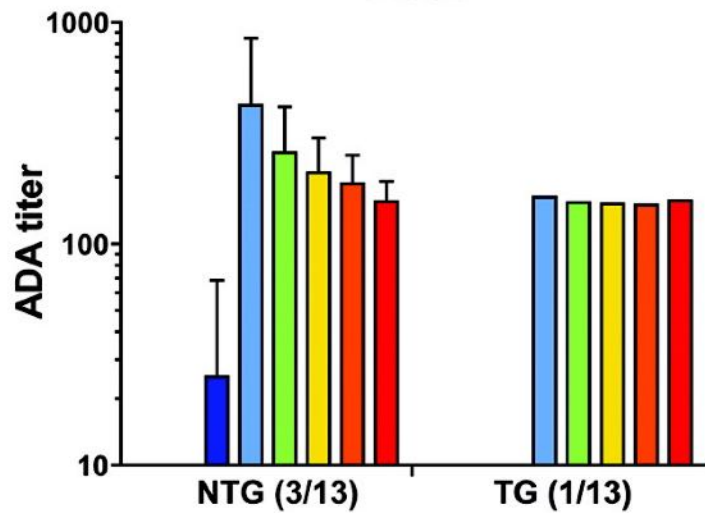
FT



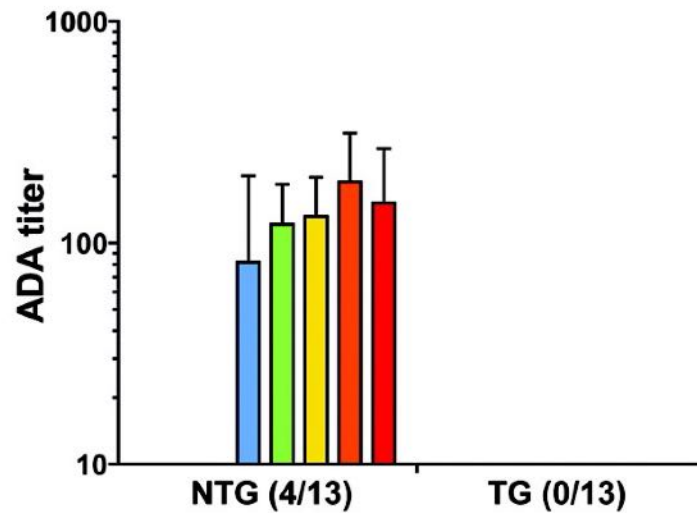
pH



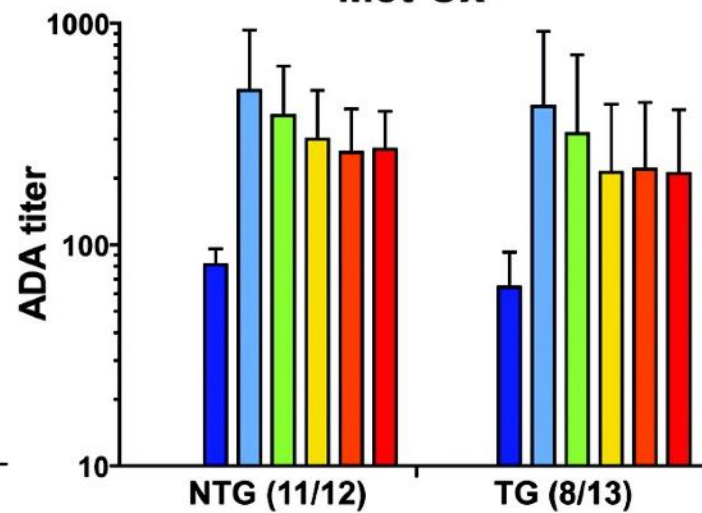
Heat



Shake



Met Ox



History of the medical use proteins

- Proteins of animal origin (e.g. equine antisera, porcine/bovine insulin): foreign proteins
- Human derived proteins (e.g.growth hormone, factor VIII): no immune tolerance
- Recombinant human proteins(e.g.insulin, interferons, GM-CSF): ??

Conclusion 1: Nearly all biopharmaceuticals induce antibodies

- Conclusion 2: There are two mechanisms
- Reaction to neo-antigens (foreign proteins)
 - Breakdown of immune tolerance

Types of immune reaction against biopharmaceuticals

Reaction to foreign proteins

Type of product	Products of microbial or animal origin
Characteristics of antibody production	Fast, often after a single injection, neutralising antibodies, long duration
Cause	The presence of foreign antigens

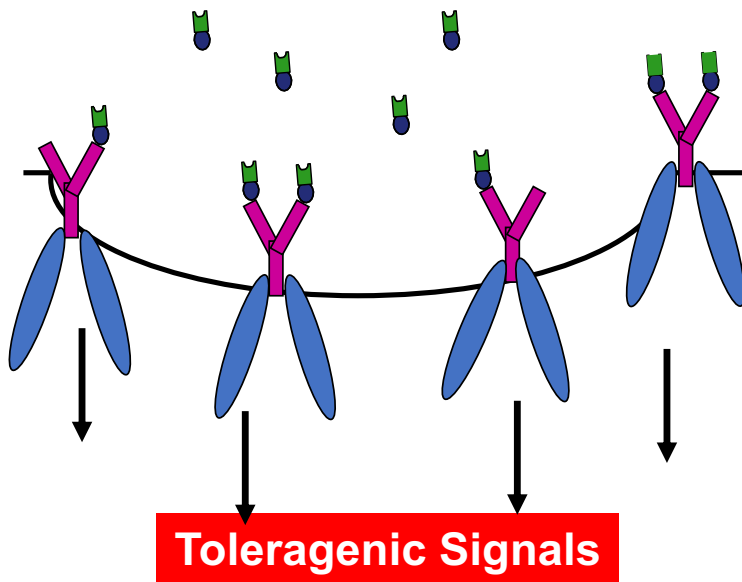
Types of immune reaction against biopharmaceuticals

Breaking of self-tolerance

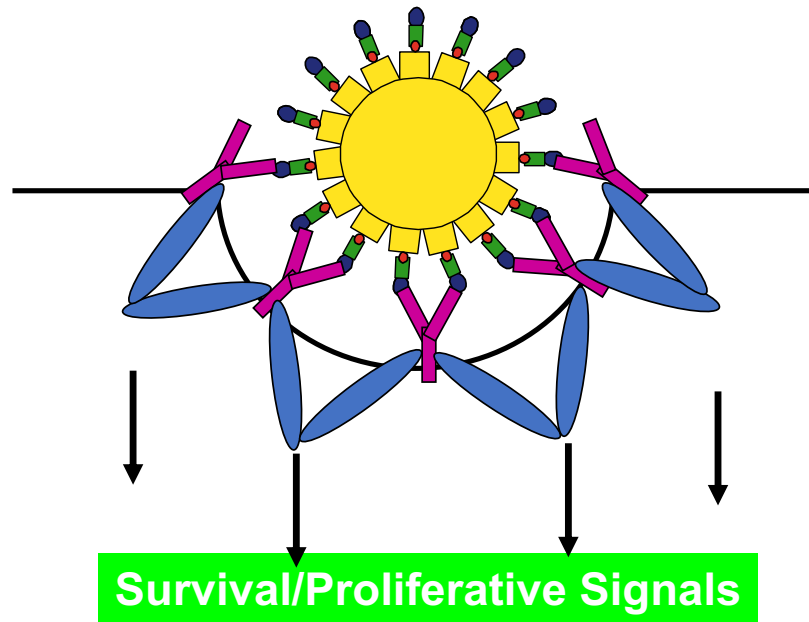
Type of product	Human homologues
Characteristics of antibody production	Slow, after long treatment, binding antibodies, disappear after treatment
Cause	Mainly impurities and aggregates

Fate of Auto-Reactive B Cells After Encountering Conjugated VLPs

Monomeric BCR/self-Ag Complexes



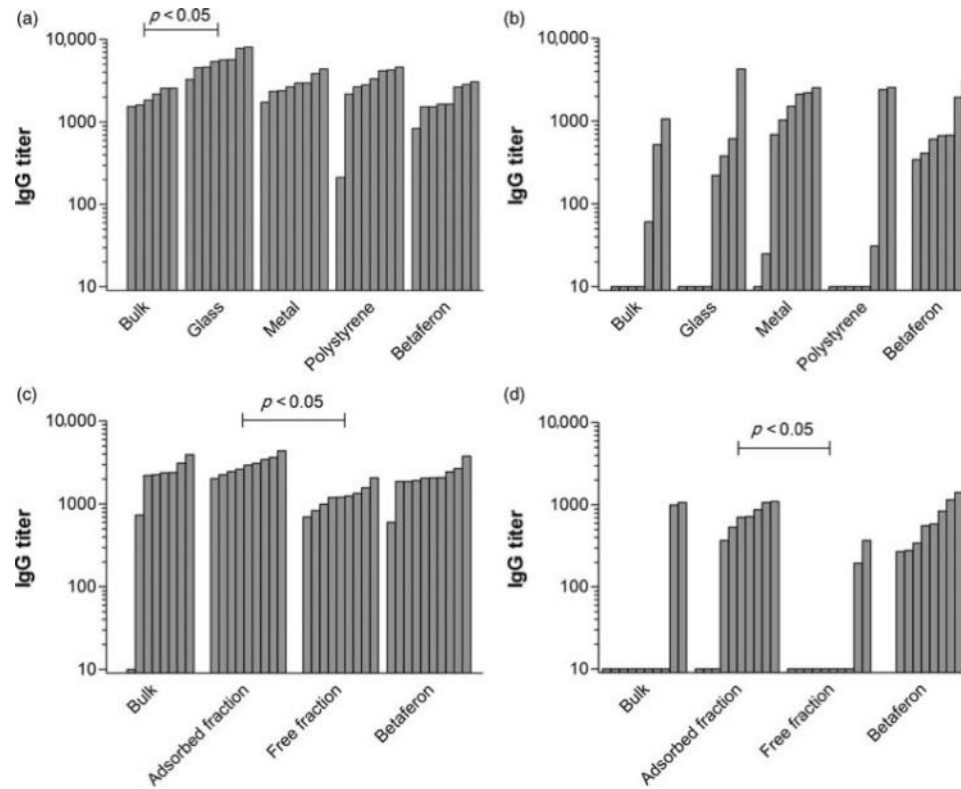
Oligomerization of BCR/self-Ag Signaling Complexes



Q's: Qualitative or Quantitative differences in signaling?
Involve initial activation of B cells or reactivation of anergic B cells?

Immunogenicity of recombinant human interferon beta interacting with particles of glass, metal, and polystyrene

Miranda M C Van Beers¹, Francesca Gilli, Huub Schellekens, Theodore W Randolph, Wim Jiskoot



> J Clin Immunol. 2013 Jan;33(1):255-63. doi: 10.1007/s10875-012-9783-z. Epub 2012 Sep 4.

Antibody response against Betaferon® in immune tolerant mice: involvement of marginal zone B-cells and CD4⁺ T-cells and apparent lack of immunological memory

Melody Sauerborn ¹, Miranda M C van Beers, Wim Jiskoot, Grzegorz M Kijanka, Louis Boon, Huub Schellekens, Vera Brinks

Results

Inactivation of MZ B-cells at the start of Betaferon® treatment drastically lowered ADA levels, suggesting a Tind immune response. However, persistent depletion of CD4⁺ T-cells before and during Betaferon® treatment abolished the ADA response in almost all mice.

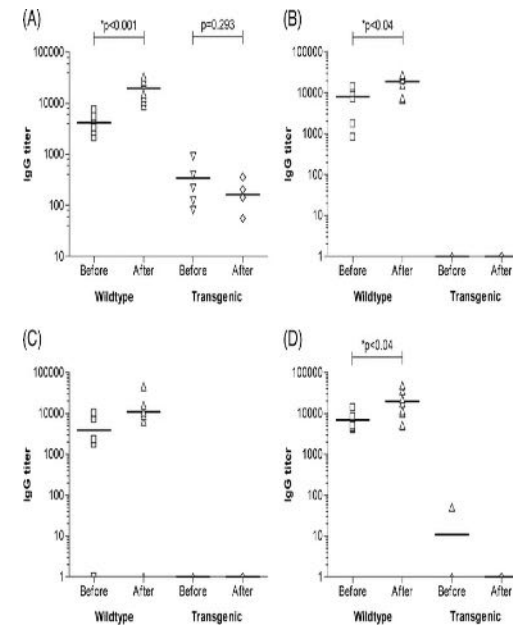
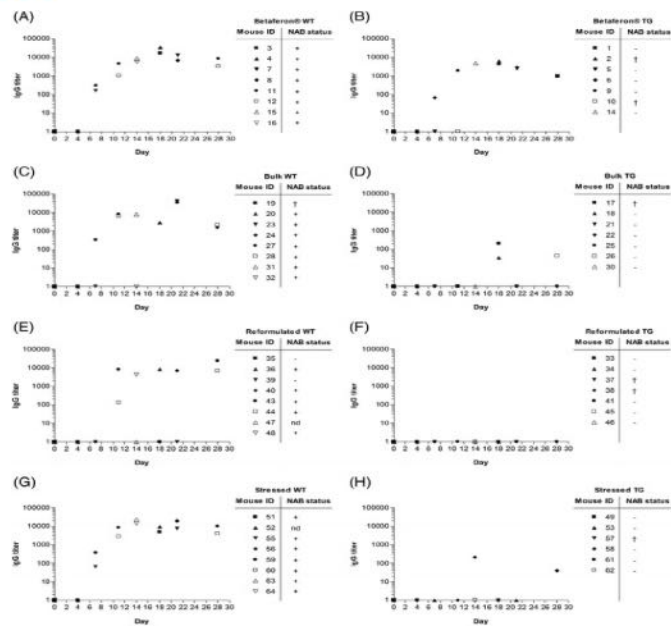
Conclusion

The immune response against rhIFN β in immune tolerant mice is neither a T-cell independent nor a classical T-cell dependent immune response. Further studies are needed to confirm absence of immunological memory (cells).

> Pharm Res. 2010 Sep;27(9):1812-24. doi: 10.1007/s11095-010-0172-0. Epub 2010 May 25.

Aggregated recombinant human interferon Beta induces antibodies but no memory in immune-tolerant transgenic mice

Miranda M C van Beers ¹, Melody Sauerborn, Francesca Gilli, Vera Brinks, Huub Schellekens, Wim Jiskoot



Pure red cell aplasia associated with EPO treatment

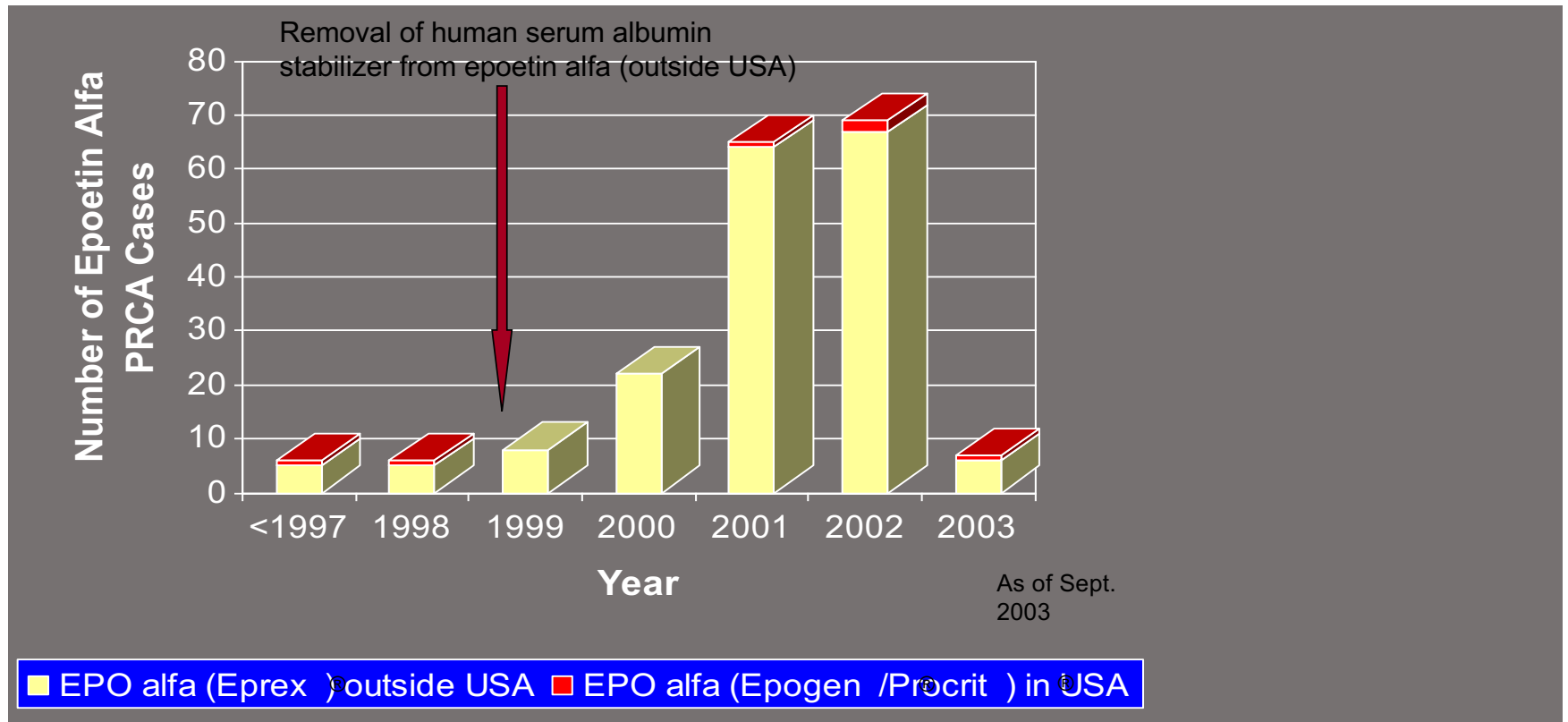
Immunogenicity became an important issue
in therapeutic protein development

Pure red cell aplasia associated with anti-EPO antibodies

Nicole Casadevall

- 1996 PRCA case with natural antibodies
- 2002 13 cases with antibodies associated with epoetin treatment

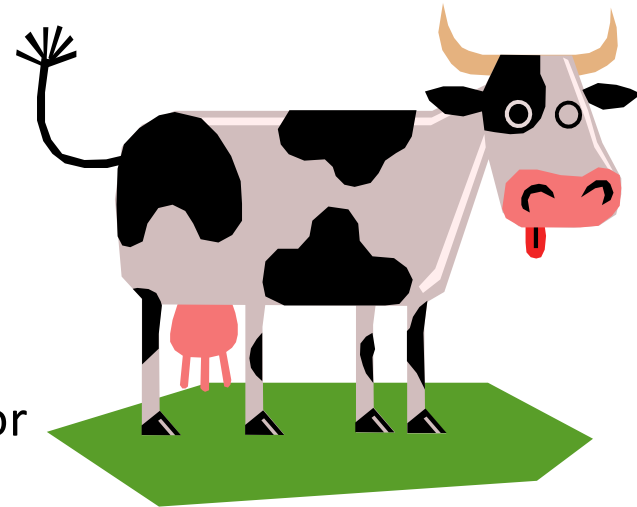
Epoetin Alfa PRCA Cases



Johnson & Johnson Pharmaceutical Research & Development LLC. Summary of PRCA case reports. Available at: http://www.jnj.com/news/jnj_news/1021024_095632.htm. Accessed December 15, 2003.

Product formulation

- Recent concern over use of HSA in Europe because of potential transmission of infectious viruses or BSE prions
- In 1998, HSA was replaced with polysorbate 80 in prefilled syringes of Eprex[®] distributed ex-US



The theory of an adjuvant effect of the leachates is based on experiments in mice immunized with ovalbumin¹. However, ovalbumin is a foreign protein and acts as a vaccine, invoking an immune response that is fundamentally different from that associated with activation of autoreactive B cells. In addition, an effect was only seen with concentrations of leachates above that in a single syringe, with no evidence an adjuvant effect of compounds leached from the rubber syringe stoppers by polysorbate 80 (refs. [1-4](#)). In contrast, we contend that the research presented by the company has many flaws and the mystery of Eprex-induced PRCA is far from solved.

With the imminent advent of the era of biosimilar epoetins, understanding what caused the Eprex-associated PRCA—where a minor change in formulation led to very serious adverse events—is imperative for patient safety. The results of all research concerning this issue should be published in such a way that detailed evaluation and independent confirmation is possible.

Eprex-associated pure red cell aplasia and leachates

[Huub Schellekens](#) & [Wim Jiskoot](#)

[Nature Biotechnology](#) **24**, 613–614 (2006) | [Cite this article](#)

Micelle-Associated Protein in Epoetin Formulations: A Risk Factor for Immunogenicity?

Suzanne Hermeling,^{1,2,3} Huub Schellekens,²
Daan J. A. Crommelin,¹ and Wim Jiskoot¹

Received 1 May 2003; accepted 1 August 2003

Micelle-Associated Protein in Epoetin Formulations

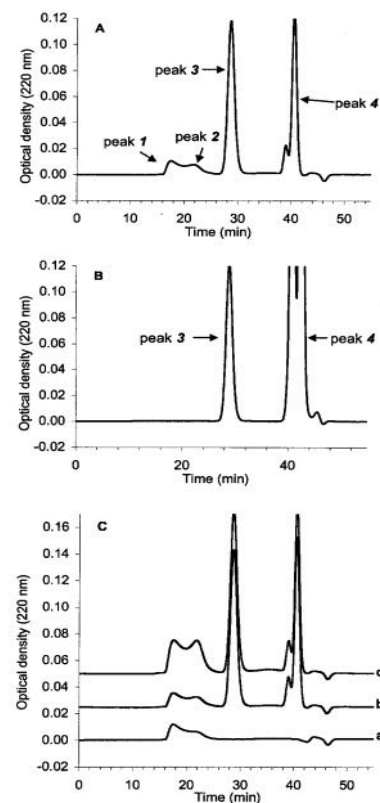
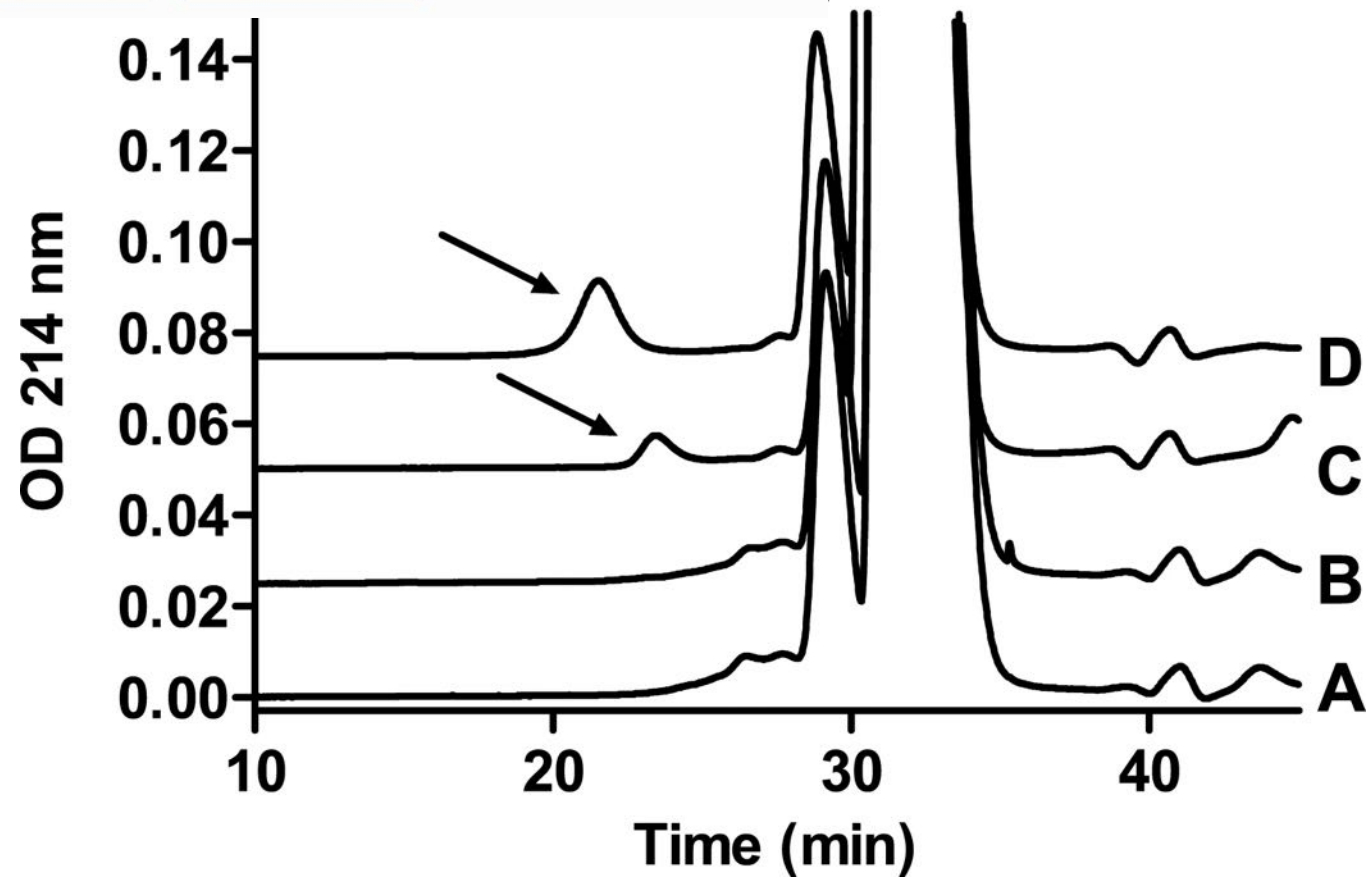


Fig. 1. GPC profiles obtained with a mobile phase consisting of 50 mM sodium phosphate buffer, pH 6.8; 300 mM NaCl. (A) Eprex®. (B) NeoRecormon®. Peak 1 has an apparent molecular weight of 670 kDa, peak 2 has an apparent molecular weight of 255 kDa and peak 3 has an apparent molecular weight of 55 kDa; peak 4 contains low-molecular-weight material. (C) Tween 80, 0.03% (w/v) (a), Eprex® (b), and Eprex® spiked with 0.03% (w/v) Tween 80 (c). Different baseline offsets are represented for sake of clarity.

Reaction to the paper: Interaction of Polysorbate 80 with Erythropoietin: A Case Study in Protein–Surfactant Interactions

[Suzanne Hermeling](#), [Wim Jiskoot](#), [Daan J. A. Crommelin](#) & [Huub Schellekens](#)



> [J Immunotoxicol.](#) 2006 Sep 1;3(3):123-30. doi: 10.1080/15476910600845567.

Erythropoietin-Associated PRCA: Still an Unsolved Mystery

Huub Schellekens ¹, Wim Jiskoot

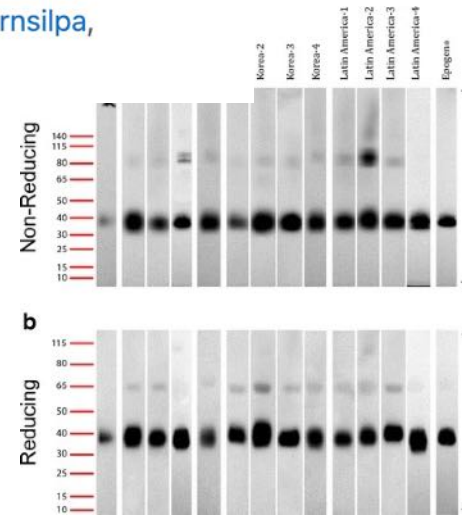
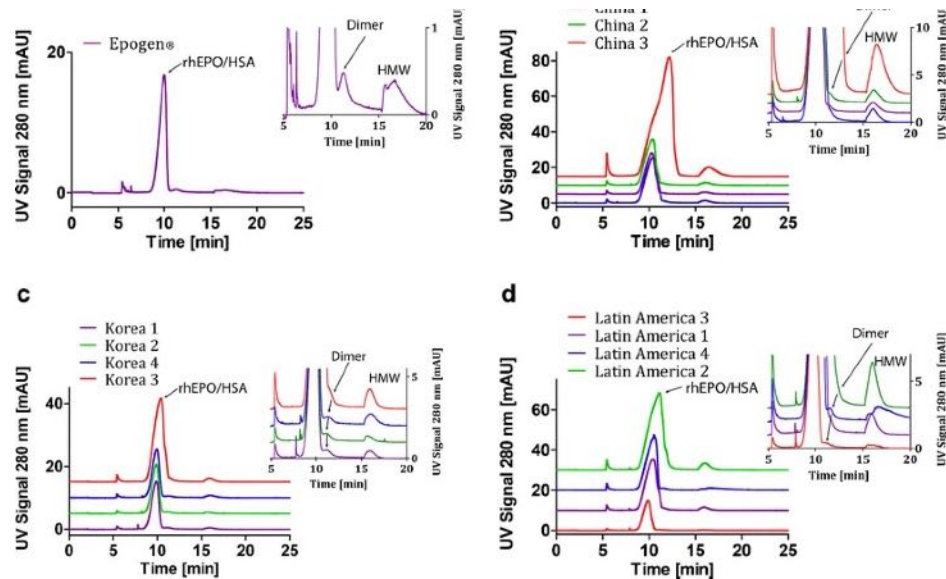
Mishandling

Several elements contribute to mishandling but the final consequence leading to PRCA is the formation of immunogenic aggregates of rhEPO. The biological rationale is feasible, in a similar way as the micelles hypothesis with enhanced B-cell activation upon aggregation of epitopes (Chackerian et al., 2002; Schellekens 2003). Experimental evidence from other therapeutic proteins supports this rationale (Braun et al., 1997; Schellekens, 2002) while several lines of evidence from rhEPO, including its epidemiology and pathology, are consistent with it.

> Pharm Res. 2014 May;31(5):1210-8. doi: 10.1007/s11095-013-1243-9. Epub 2013 Nov 21.

How bio-questionable are the different recombinant human erythropoietin copy products in Thailand?

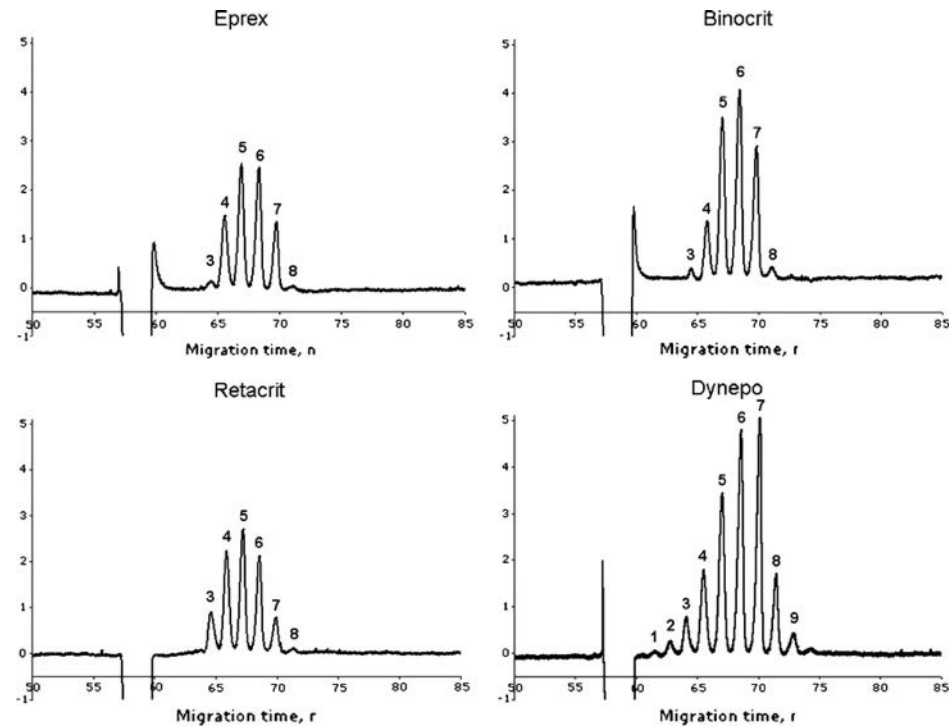
Liem Andhyk Halim¹, Vera Brinks, Wim Jiskoot, Stefan Romeijn, Kearkiat Praditpornsilpa, Anuchai Assawamakin, Huub Schellekens



> Pharm Res. 2011 Feb;28(2):386-93. doi: 10.1007/s11095-010-0288-2. Epub 2010 Oct 1.

Quality of original and biosimilar epoetin products

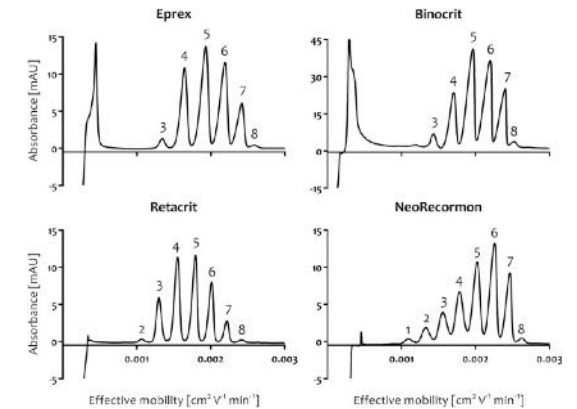
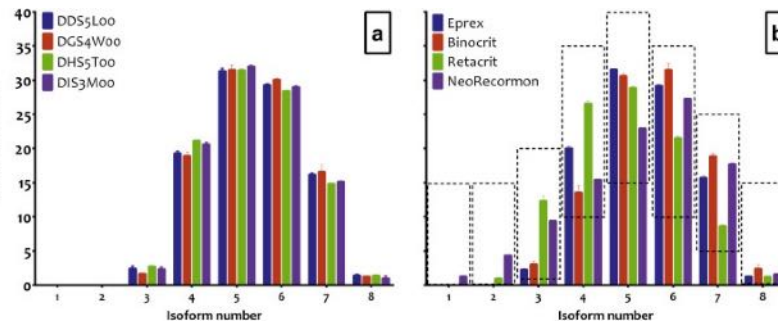
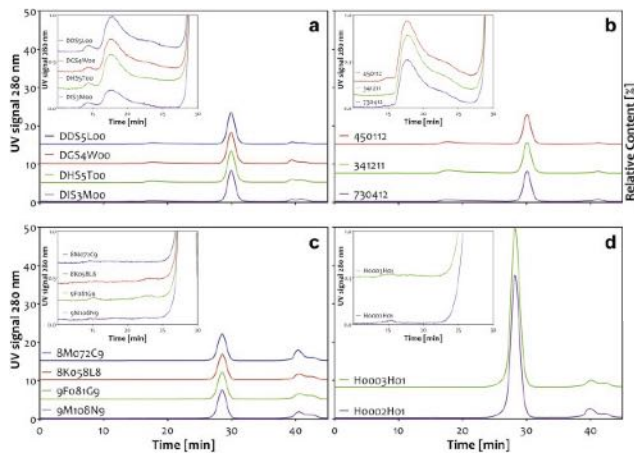
Vera Brinks ¹, Andrea Hawe, Abdul H H Basmeh, Liliana Joachin-Rodriguez, Rob Haselberg, Govert W Somsen, Wim Jiskoot, Huub Schellekens



> J Pharm Sci. 2016 Feb;105(2):542-550. doi: 10.1016/j.xphs.2015.10.019. Epub 2016 Jan 9.

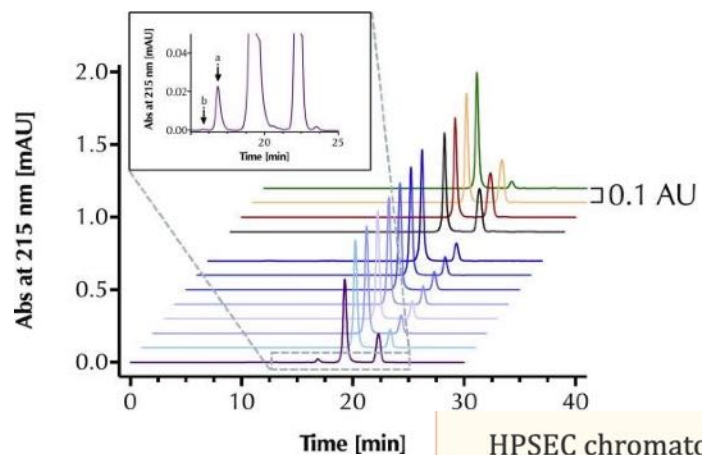
Quality and Batch-to-Batch Consistency of Original and Biosimilar Epoetin Products

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Quality Comparison of Biosimilar and Copy Filgrastim Products with the Innovator Product

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HPSEC chromatograms of (top to bottom) Biocilin (green), Tevagrastim (pink), Zarzio (brown), and Neupogen batch 1042036A (black) followed by seven batches of expired Neupogen (several shades of blue) and PDgrastim (purple). The inset is the zoom of PDgrastim's chromatogram. Peak (a) is filgrastim dimer and peak (b) is filgrastim oligomer.

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Wim Jiskoot

Enschede, 5 januari 1961

Nieuwe Wetering, 22 augustus 2021

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'Heb het goed samen!'

Het afscheid vindt plaats in besloten kring.

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