

Why patients make antibodies to therapeutic proteins?

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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): ??

Most biopharmaceuticals induce antibodies

Two mechanisms

- Reaction to neo-antigens
- 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

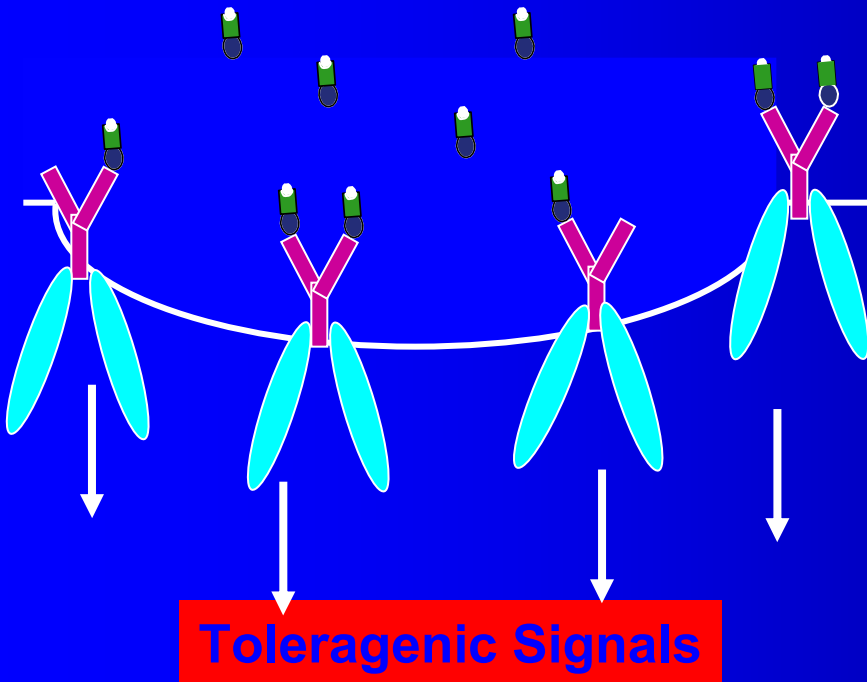
Type of product	Human homologues
Characteristics of antibody production	Slow, after long treatment, binding antibodies, disappear after treatment
Cause	Mainly impurities and aggregates

Mechanisms to break B cell tolerance

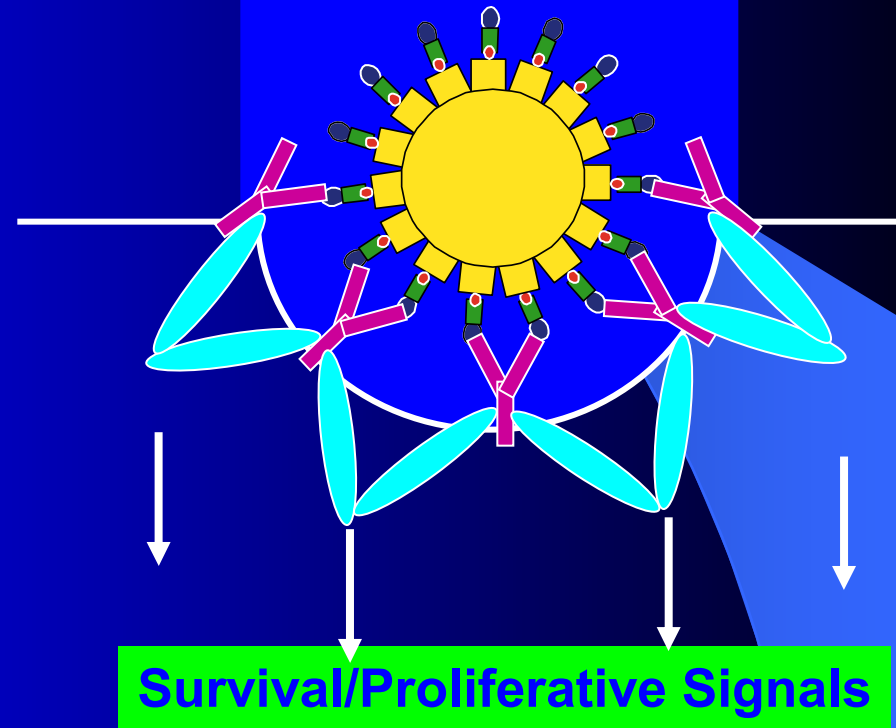
- Present self antigen with second signal (danger signals: endotoxins, denatured proteins) (T-cell dependent)
- Present self antigens in array form such as viral capsids (T cell independent ?)

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 biopharmaceuticals

Causes



Factors influencing immunogenicity

Structural properties

Sequence variation

Glycosylation

Other factors

Assays

Contaminants and impurities

Formulation

Downstream processing

Route of application

Dose and length of treatment

Patient characteristics

Unknown factors

Structural properties

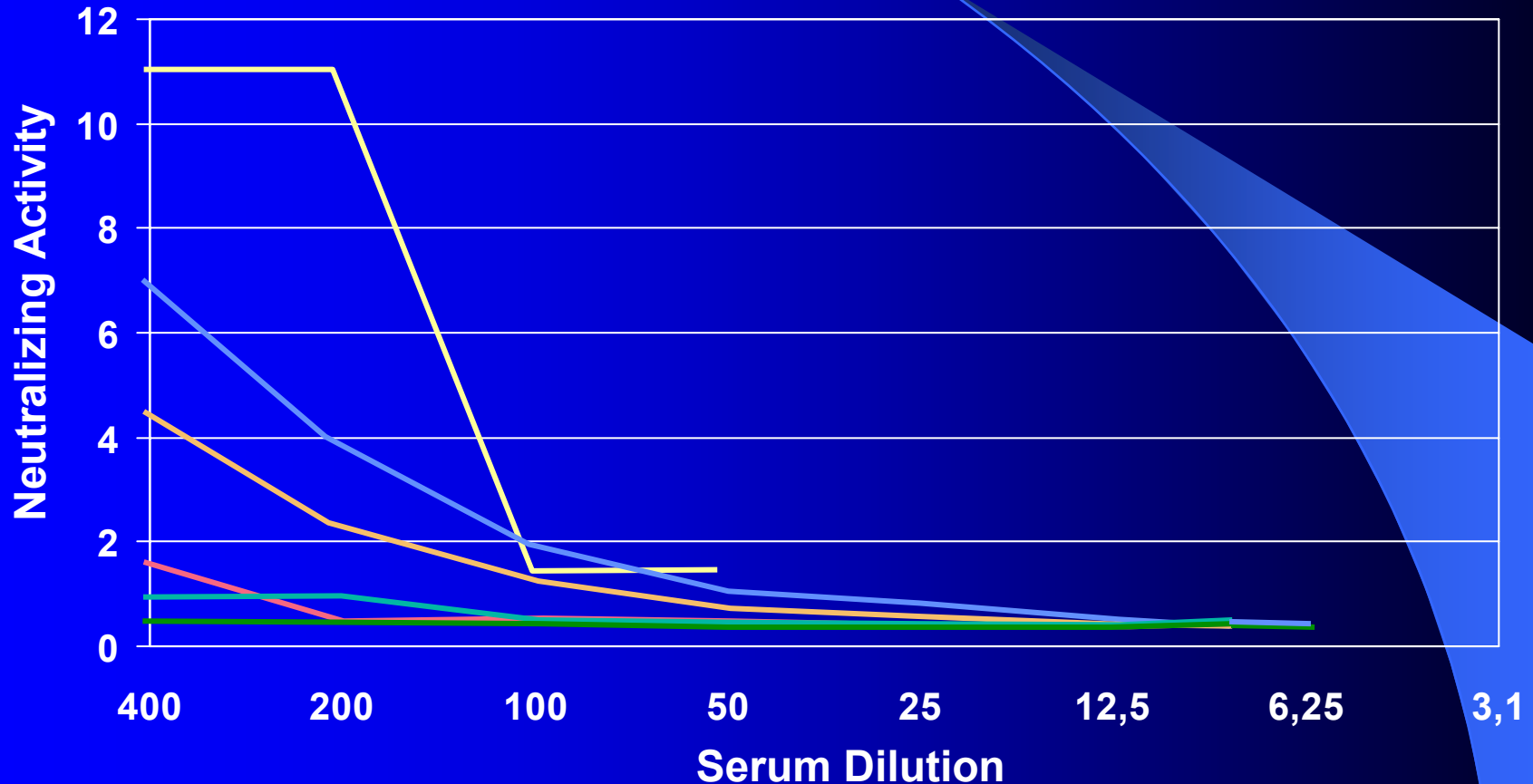
- Degree of “non-self”: biopharmaceuticals of bacterial and plant origin (Streptokinase, staphylokinase, asparaginase)
- Glycosylation
 - Protection of antigenic sites (GM-CSF)
 - Influence on solubility (Interferon beta)

Factors influencing immunogenicity

Assays



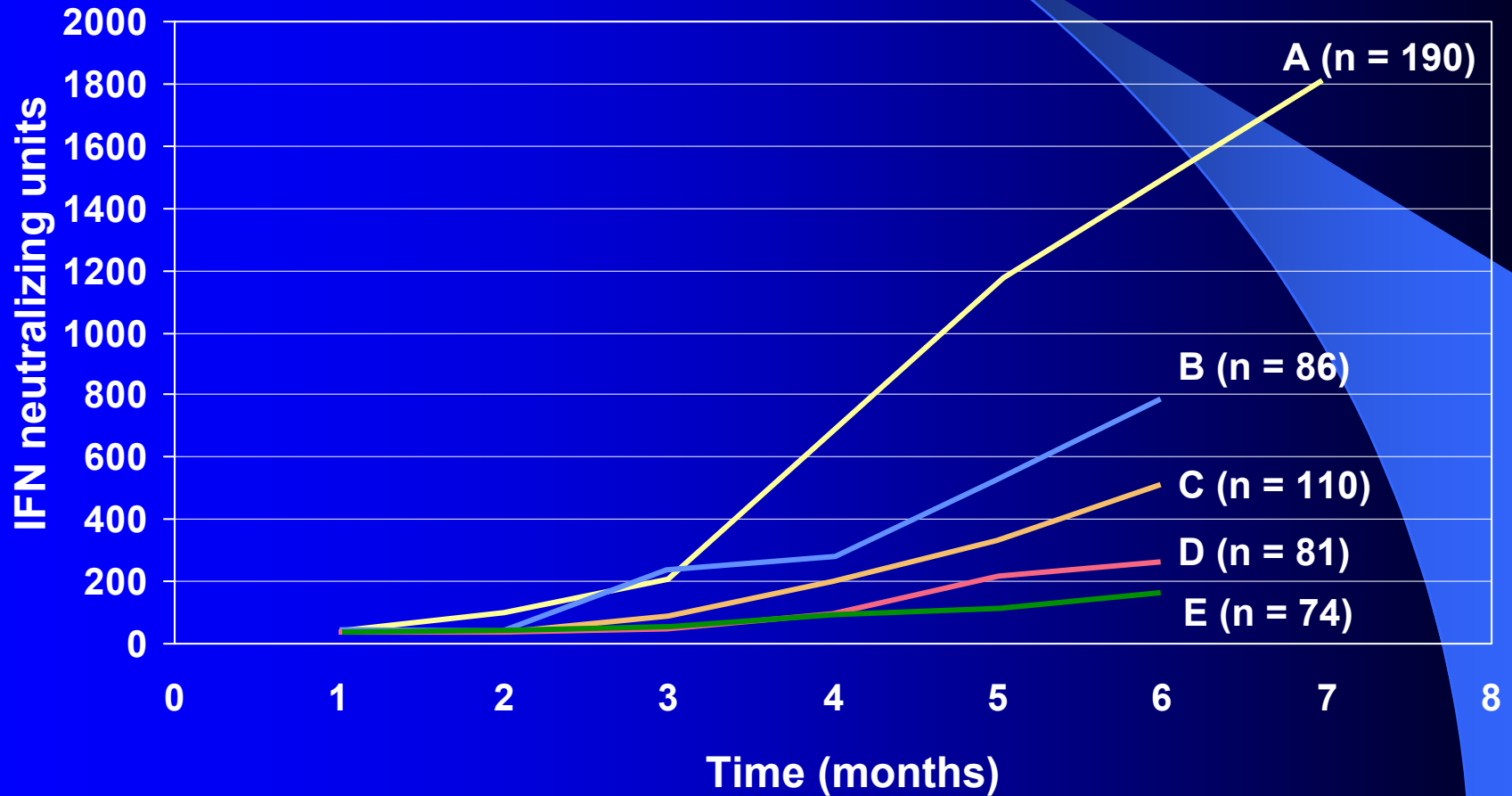
Neutralizing antibodies standard serum in different laboratories

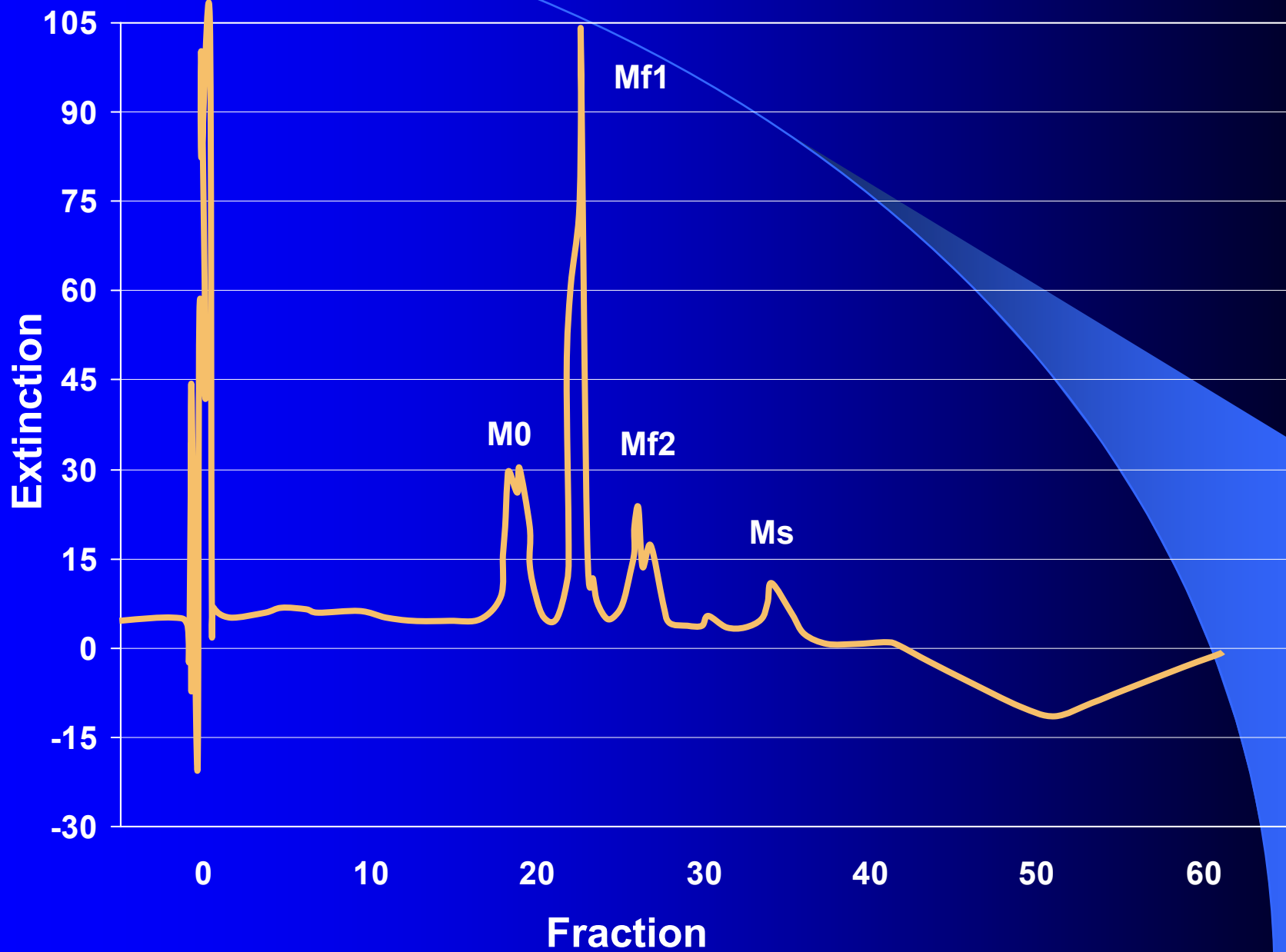


Factors influencing immunogenicity

Formulation: the interferon alpha 2 case

Antigenicity of different IFN alpha-2a formulations





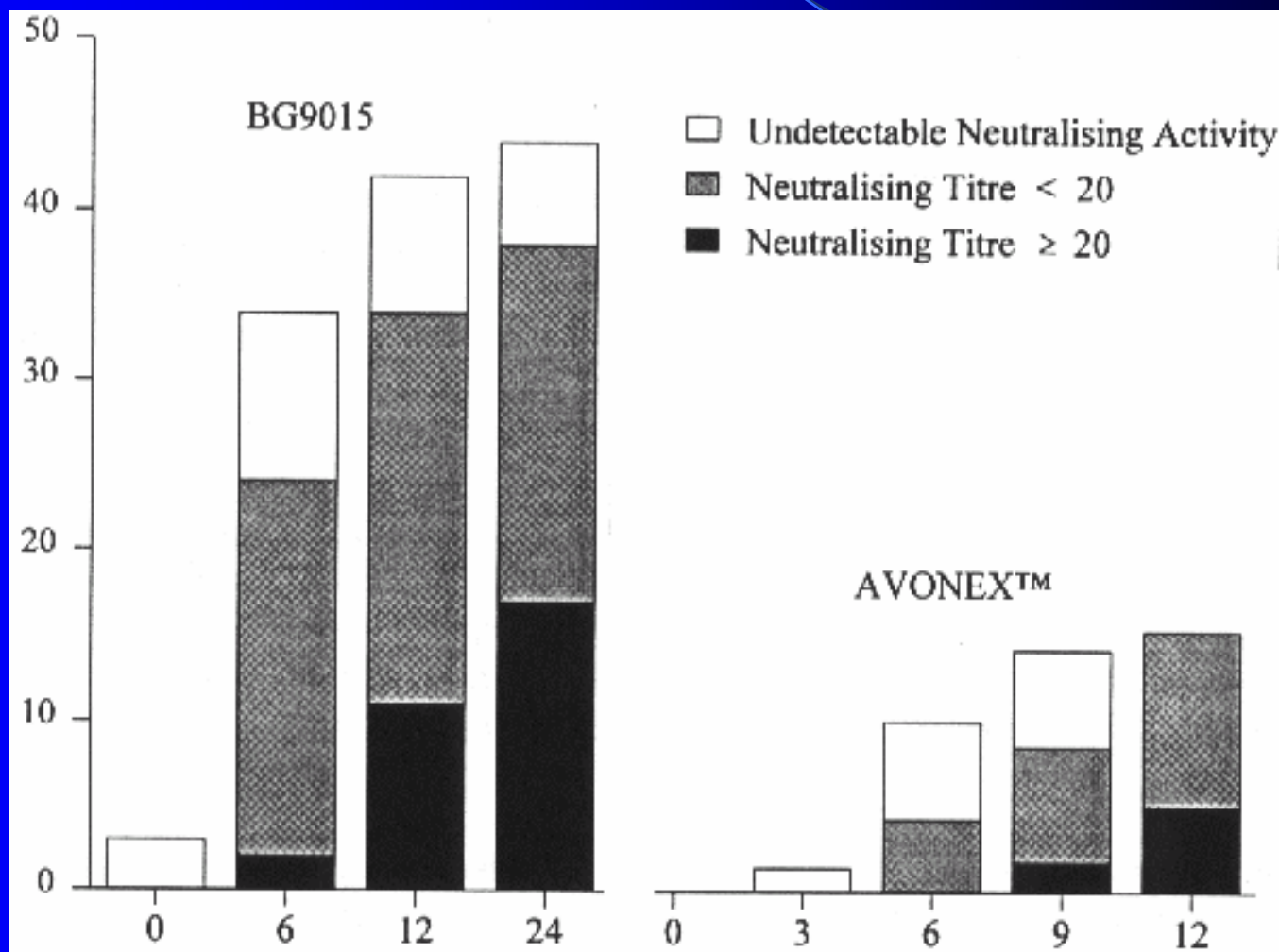
Other factors influencing immunogenicity

- Downstream processing
 - Viral inactivation factor VIII
- Impurities and contaminants
 - Insulin
 - Growth hormone
- Duration of treatment
 - Avonex/Rebif versus Betaseron

Other factors influencing immunogenicity

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

Antigenicity of identical Hu IFN beta produced at different sites



Consequences of antibodies

Loss of efficacy

Insulin

Streptokinase

Staphylokinase

ADA

Salmon calcitonin

Factor VIII

Interferon alpha 2

Interferon beta

IL-2

GnRH

TNFR55/IgG1

Denileukin diftitox

HCG

GM-CSF/IL3

Enhancement of efficacy

Growth hormone

Neutralization of native protein

MDGF

EPO

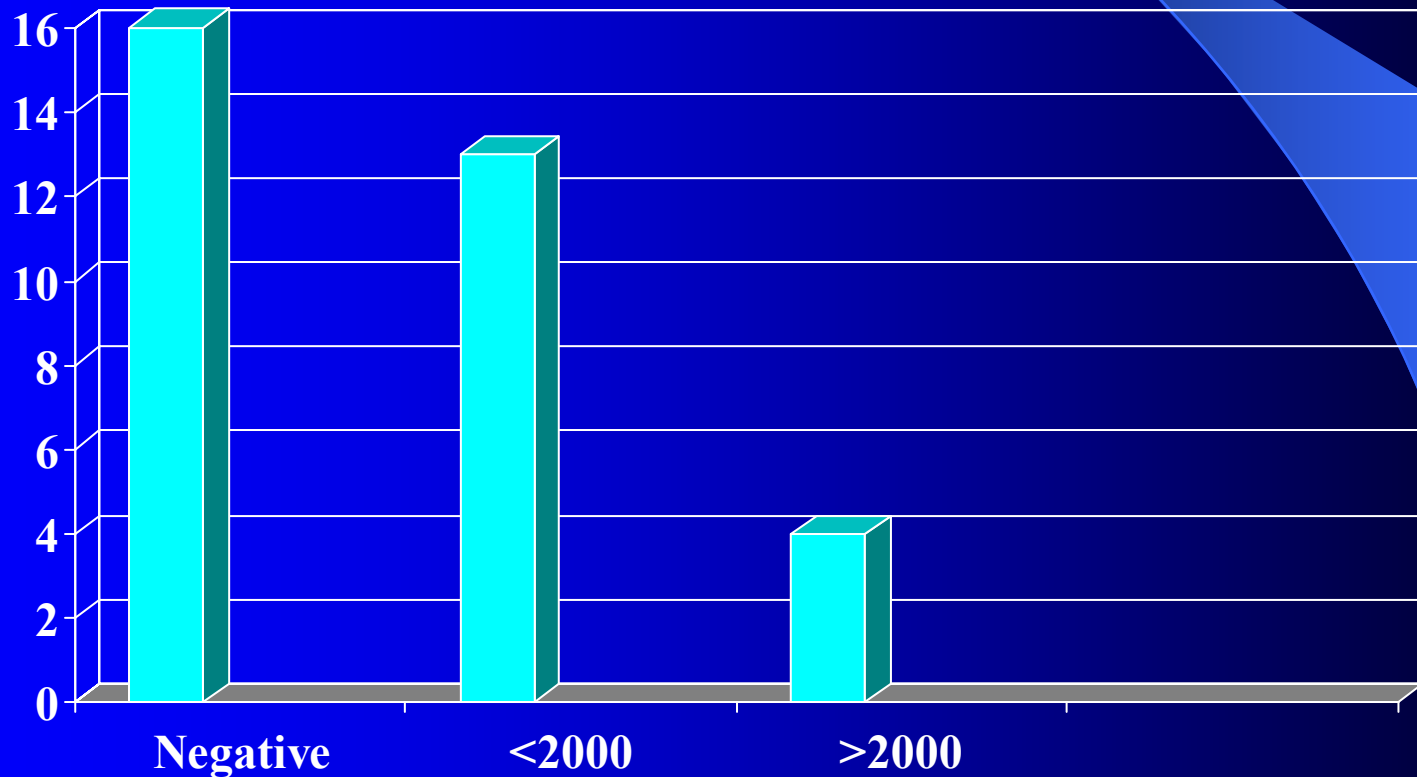
General immune effects

Allergy

Anaphylaxis

Serum sickness, etc

Relation between sustained response and antibody level in IFN alpha-2a treated HCV patients



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AMGEN DISCONTINUES DEVELOPMENT OF MGDF

FOR IMMEDIATE RELEASE

THOUSAND OAKS, Calif., September 11, 1998 -- Amgen (NASDAQ:AMGN) today reported that it has discontinued development of its megakaryocyte growth and development factor (PEG-rHuMGDF) due to evidence of neutralizing antibodies in a few patients participating in cancer clinical trials and in additional people in platelet donor clinical trials.

Amgen is a global biotechnology company that discovers, develops, manufactures and markets cost-effective human therapeutics based on advances in cellular and molecular biology.

CONTACT: Amgen, Thousand Oaks
 David Kaye, 805/447-6692 (media)
 Denise Powell, 805/447-4346 (investors)

EDITOR'S NOTE: An electronic version of this news release may be accessed via our web site at www.Amgen.com. Visit the Corporate Center and click on Amgen News. Journalists and media representatives may sign up to receive all news releases electronically at time of announcement by filling out a short form in the Amgen News section of the web site.

Eprex associated PRCA

Watch your formulation

Characteristics of PRCA

- Also known as erythroblastopenia, or red cell agenesis
- Rare disease
- Serious disease
- Patients become transfusion dependent

Nicole Casadevall and coworkers 2002

Upsurge of PRCA is caused by
antibodies induced by EPO

HAR (2) 22 years

Right renal agenesis

HD – 6/1998

Epo α (1/6/98) 3 000 – 2 000 IU>x2/sem

Hb 11-12g/dl

Anemia (11/98) 8g/dl – (low reticulocytes)

Bone marrow examination PRCA

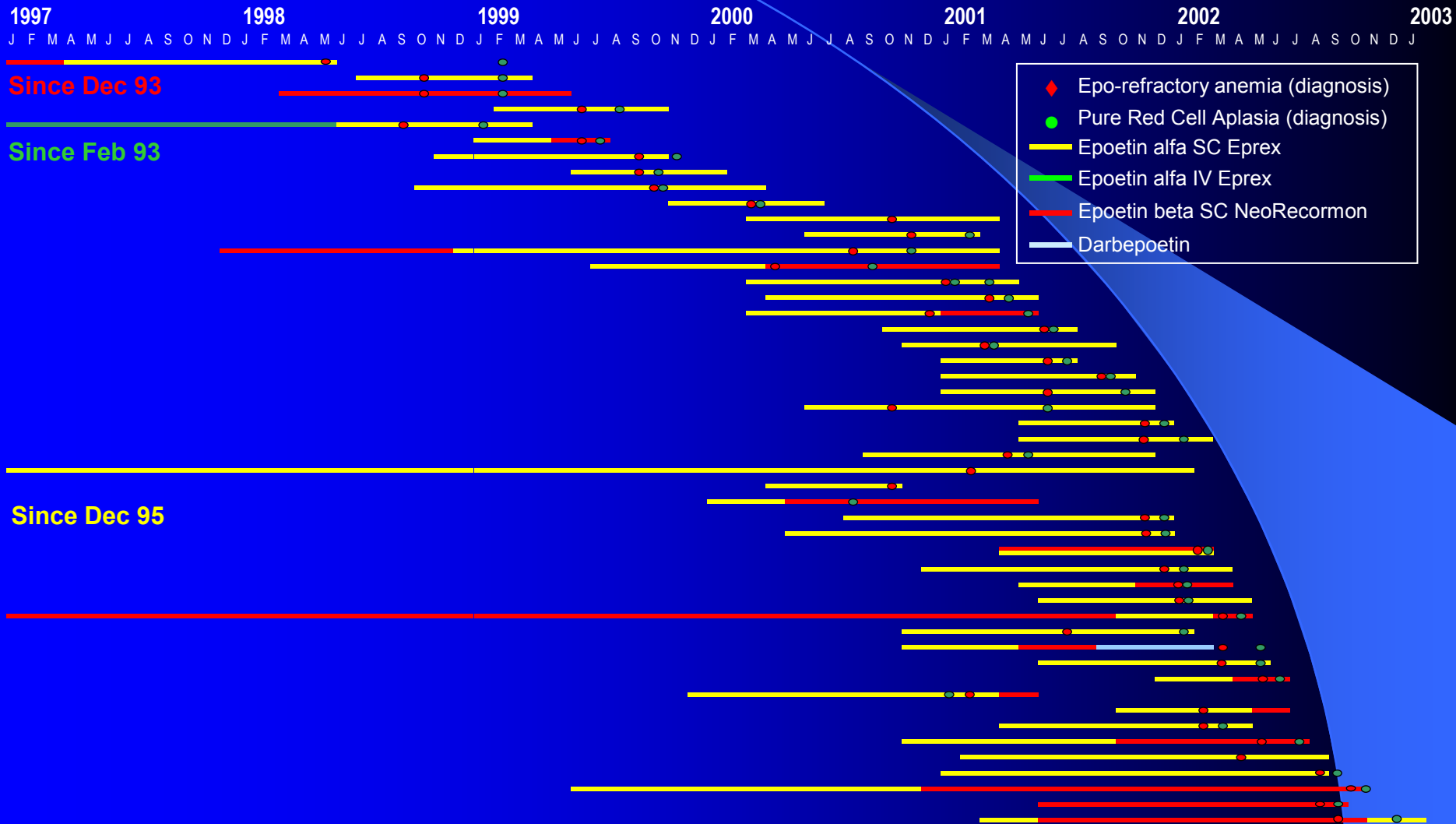
Transfused +++ (\approx 4 units/month)

Treated by

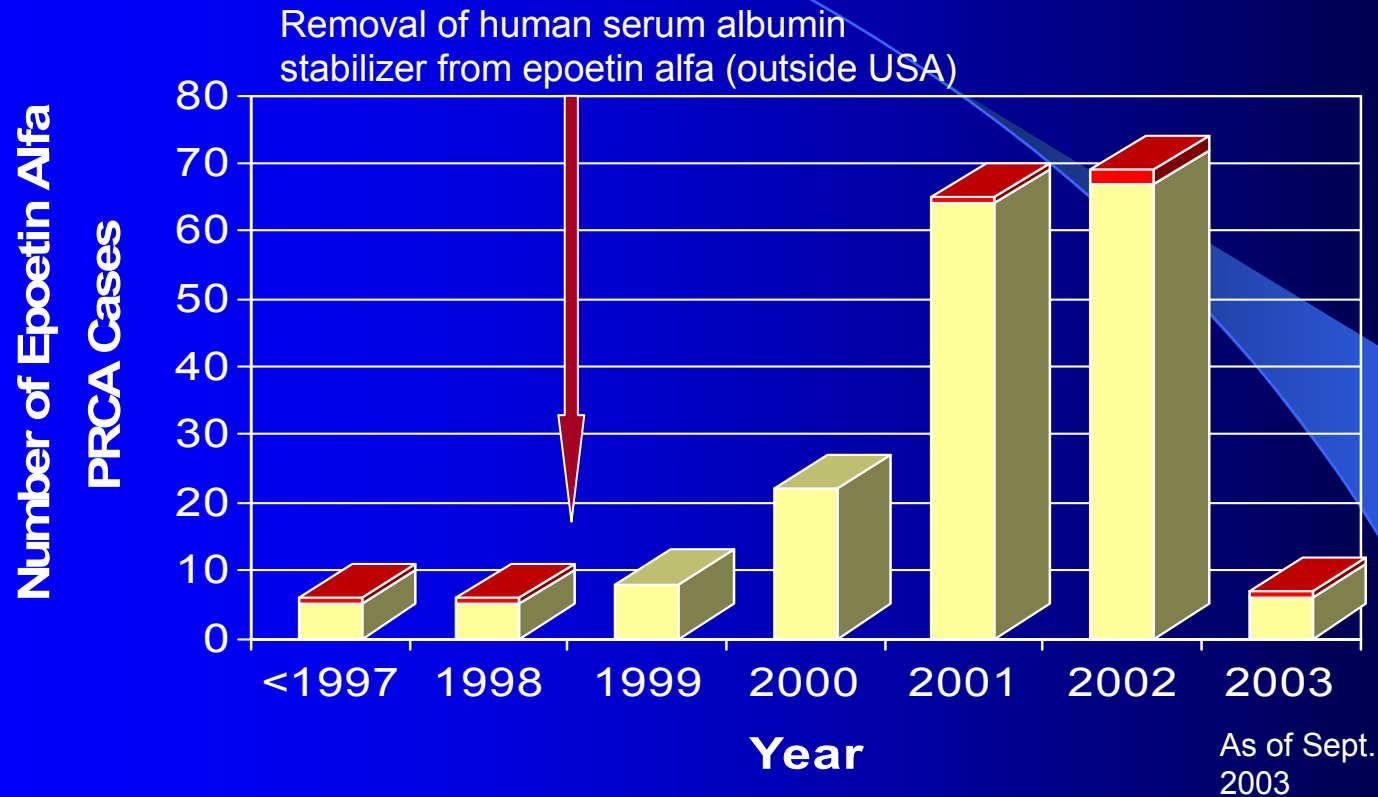
- IV Ig
- plasmapheresis
- corticosteroids (40mg/d)

Stop transfusions 8/99

Time course of individual PRCA cases



Epoetin Alfa PRCA Cases



■ EPO alfa (Eprex®) outside USA ■ EPO alfa (Epogen®/Procrit®) in USA

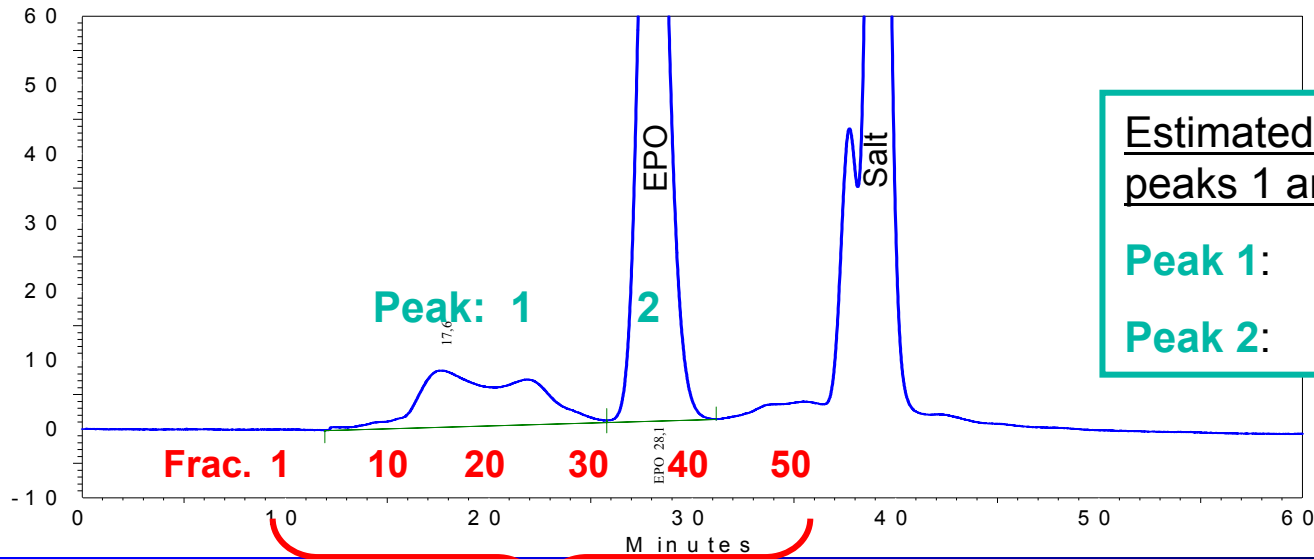
Johnson & Johnson Pharmaceutical Research & Development LLC. Summary of PRCA case reports. Available at: http://www.jnj.com/news/jnj_news/1021024_095632.htm. Accessed December 15, 2003.

Main stabilizers used in the epoetin formulations

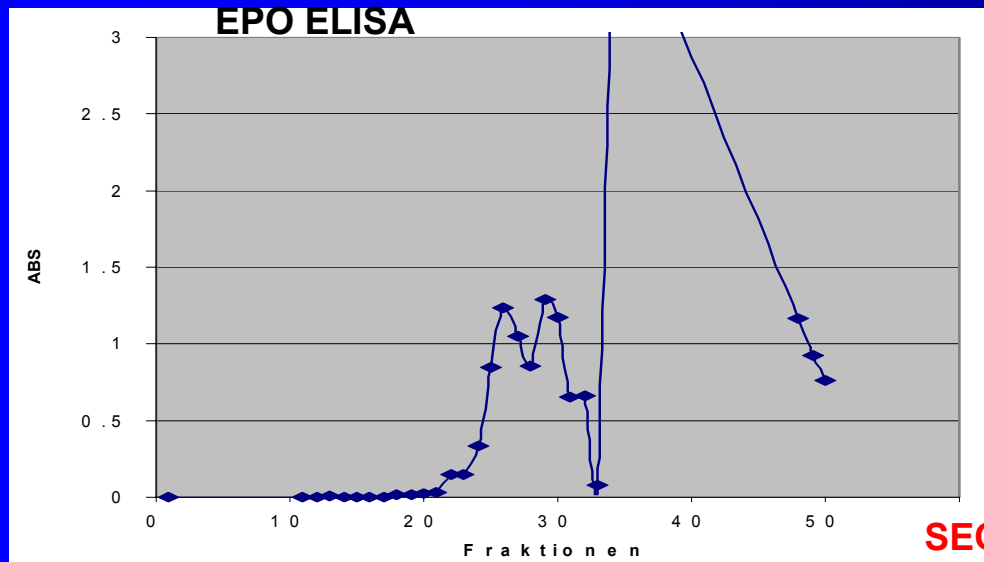
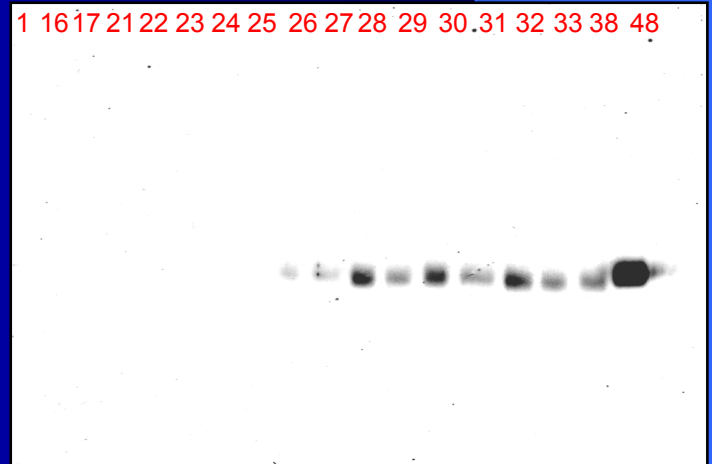
Epogen[®]/Procrit[®] <i>(US)</i>	Eprex[®] <i>(pre 1998)</i>	Eprex[®] <i>(post 1998)</i>	NeoRecormon[®] <i>(1990 launch)</i>
HSA	HSA	Polysorbate 80 Glycine	Polysorbate 20 Glycine Complex of 5 other amino acids Calcium chloride Urea

Erypo

SEC → Western Blot / ELISA



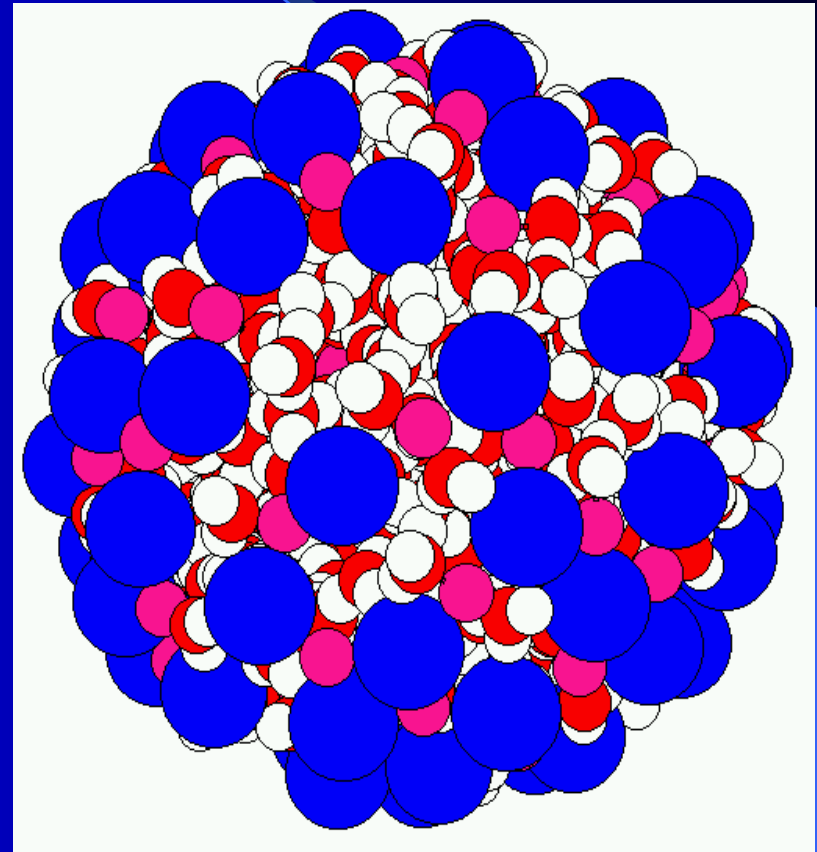
SEC Fraction:



SEC Fraction

What is the role of micelles?

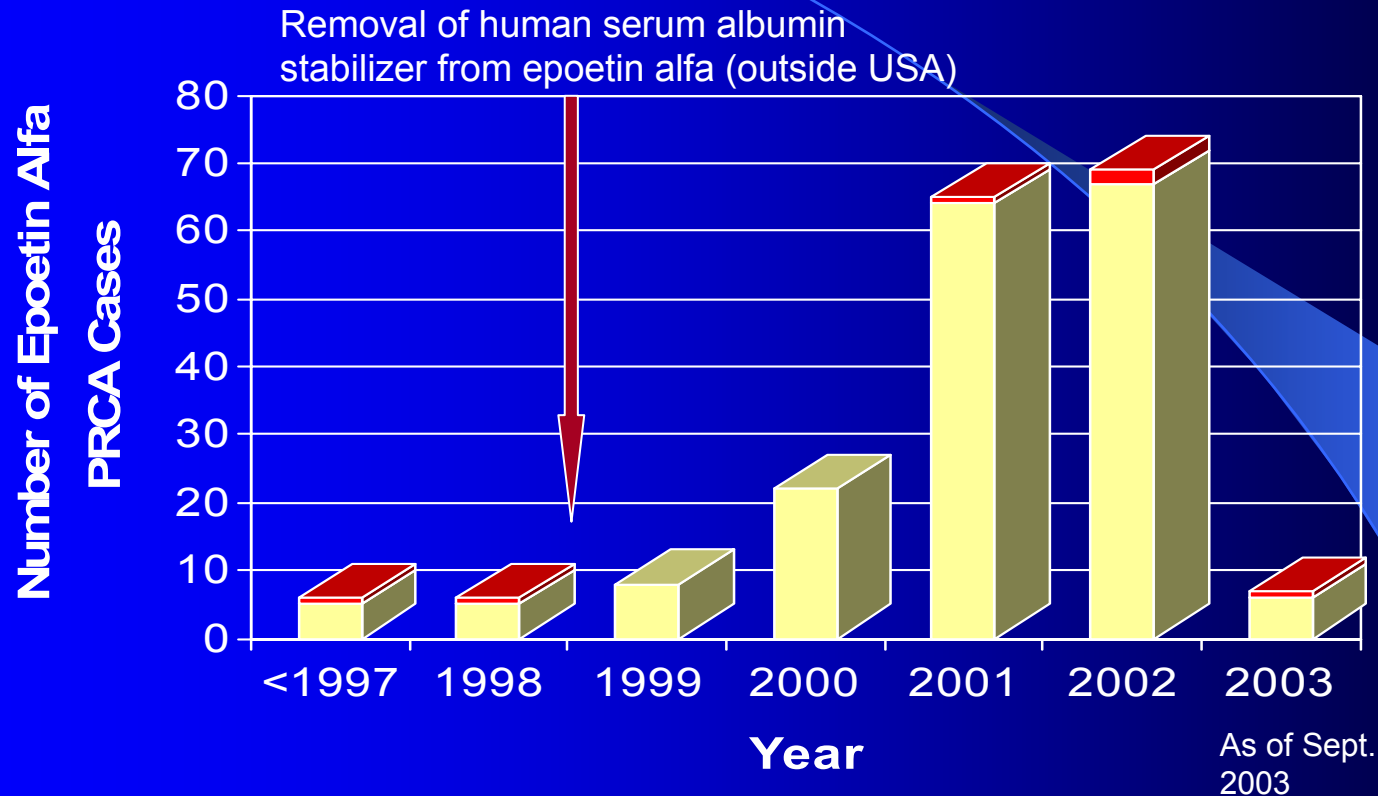
- Effects of the micelles will be investigated in animal models



Factors potentially contributing to the immunogenicity of Eprex[®]

- Formation of micelles associated with Epo (Hermeling et al, 2003)
- Silicon droplets in the prefilled syringes
- Leachates from rubber stoppers
- Polysorbate 80 interaction with Epo
- Mishandling

Epoetin Alfa PRCA Cases



■ EPO alfa (Eprex®) outside USA ■ EPO alfa (Epogen®/Procrit®) in USA

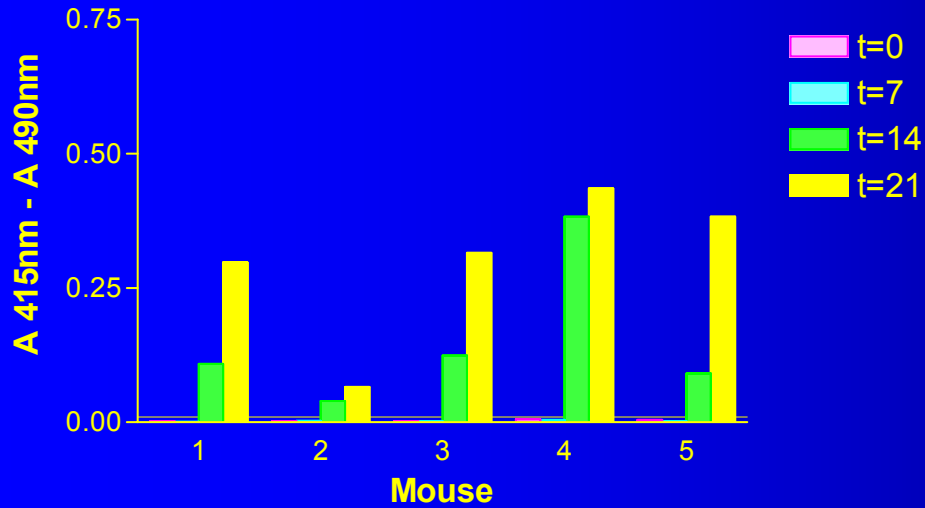
Johnson & Johnson Pharmaceutical Research & Development LLC. Summary of PRCA case reports. Available at: http://www.jnj.com/news/jnj_news/1021024_095632.htm. Accessed December 15, 2003.

Prediction of immunogenicity

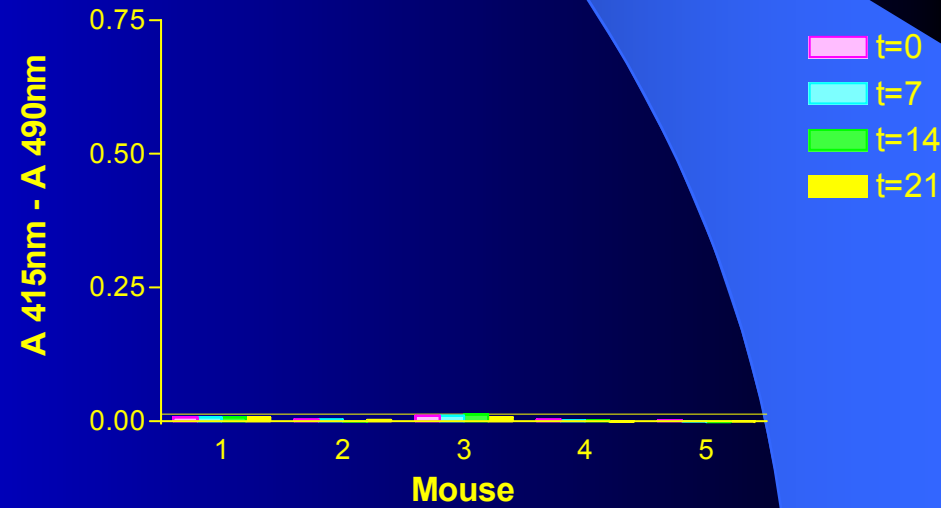
- Purity of the product
- Epitope analysis
- Reaction with patient sera
- Animal experiments
 - Conventional animals (relative immunogenicity)
 - Non-human primates
 - Immune tolerant transgenic mice

Daily i.p.

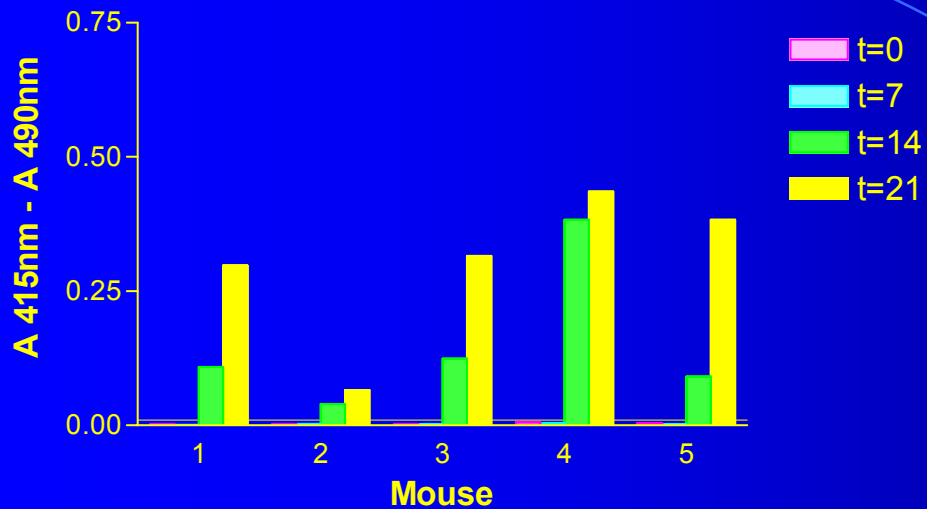
5 ug Avonex
Wildtype (C57Bl/6)



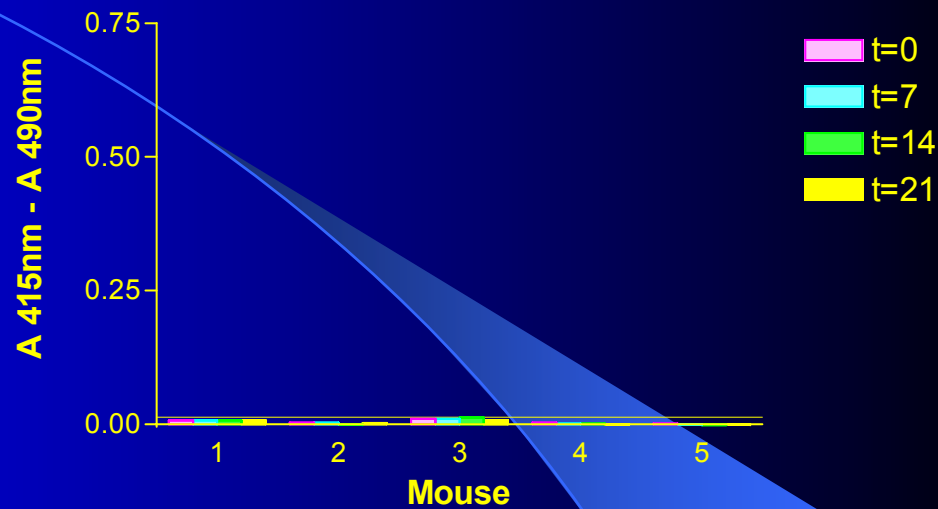
5 ug Avonex
Transgenic immune-tolerant



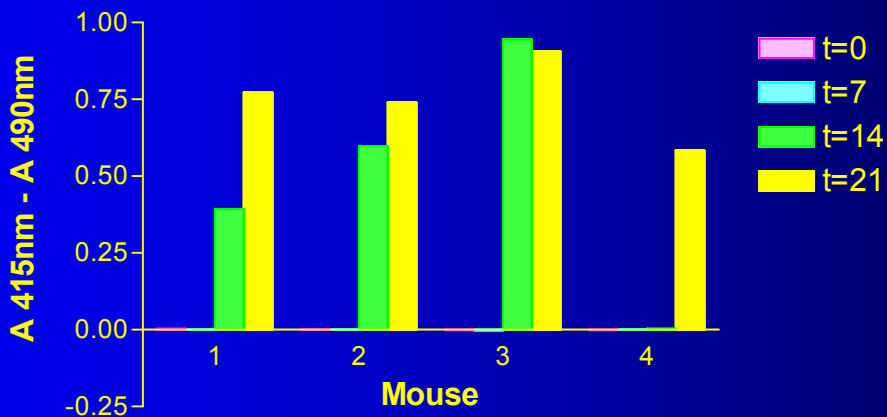
**5 ug Avonex
Wildtype (C57Bl/6)**



**5 ug Avonex
Transgenic immune-tolerant**



**5 ug Betaseron daily i.p.
Transgenic immune-tolerant**



Conclusion

- The majority of biopharmaceuticals is immunogenic
- The clinical consequences are mostly benign, but may be severe
- Formulation important factor influencing immunogenicity