Safety of biopharmaceuticals immunogenicity

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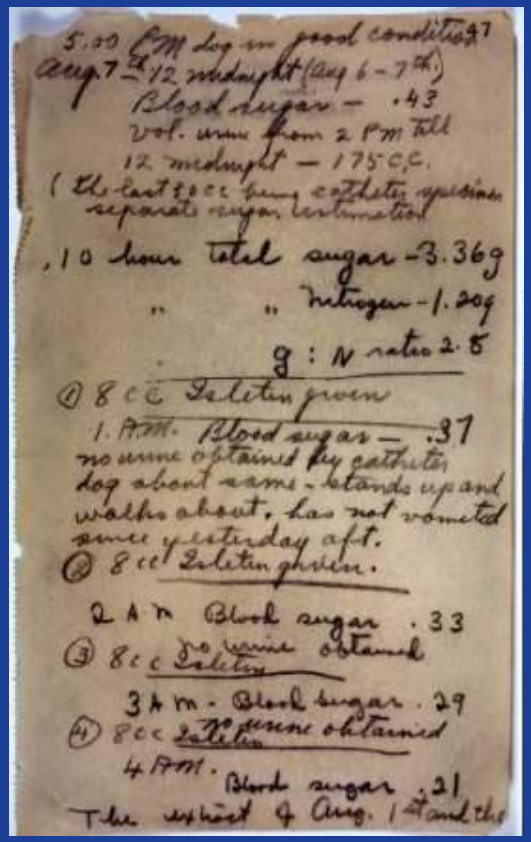
Utrecht University

The history of the use of proteins in medicine

An example of an animal derived biotech product: diphteria antitoxin

Production of diphtheria antitoxin by inoculating horses required great care to maintain purity and avoid contamination
Courtesy of National Archives and Records Administration

The most used animal derived biologic: porcine/bovine insulin



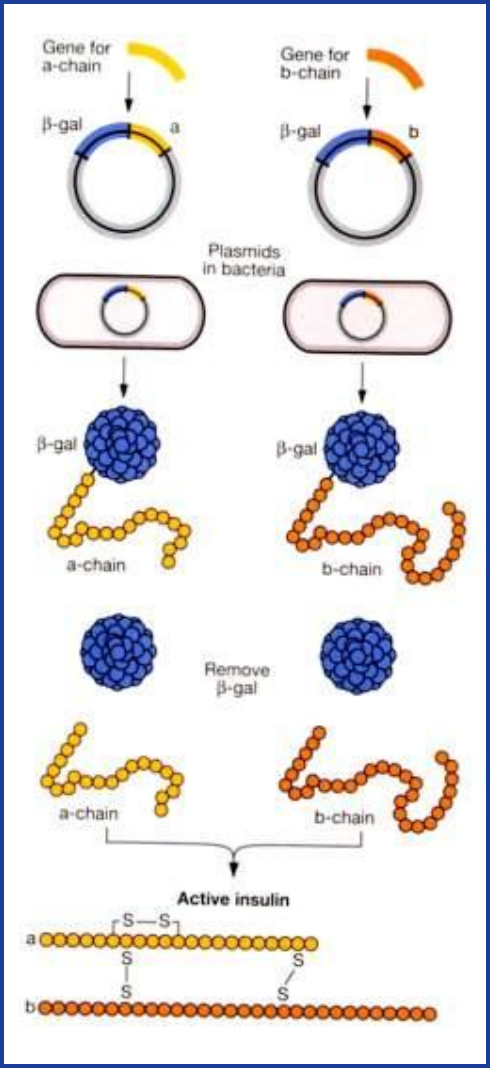
A human protein from natural source: human growth hormone

The first phase of protein drugs

- Based on
  - Recombinant DNA technology
  - Hybridoma technology
- Copies of natural products

DNA, deoxyribonucleic acid

Bacteria making insulin



First r-DNA derived human protein drug: human insulin (1982)

First generation biopharmaceuticals

- Insulin
- Growth hormone
- Interferon alfa
- Interferon beta
- Interferon gamma
- G-CSF
- GM-CSF
- EPO
- FSH
- HBV vaccine
- Monoclonal antibodies (MAb)

G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; EPO, erythropoietin; FSH, follicle stimulating hormone; HBV, hepatitis B virus

Failed biopharmaceuticals

- TNF
- IL-1,2 etc
- MDGF
- Centoxin
- TNFR-Ig

TNF, tumour necrosis factor; IL, interleukin; MDGF, macrophage-derived growth factor; TNFR-Ig; tumour necrosis factor receptor I protein

Problems with biopharmaceuticals

- Specificity
- Immunogenicity
- Parts of complicated network
- Unknown mode of action
- Unfavourable pharmacokinetics

Second generation biopharmaceuticals

- Sequence variants
- Variants of post translational modification
- Hybrid molecules
- Unnatural modification
- New forms of administration

Immunogenicity of therapeutic proteins

A key issue

European Association of Hospital Pharmacists

History of the medical use proteins

- Proteins of animal origin (eg equine antisera, porcine/bovine insulin): foreign proteins
- Human derived proteins (eg growth hormone, factor VIII): no immune tolerance
- Recombinant human proteins (eg 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

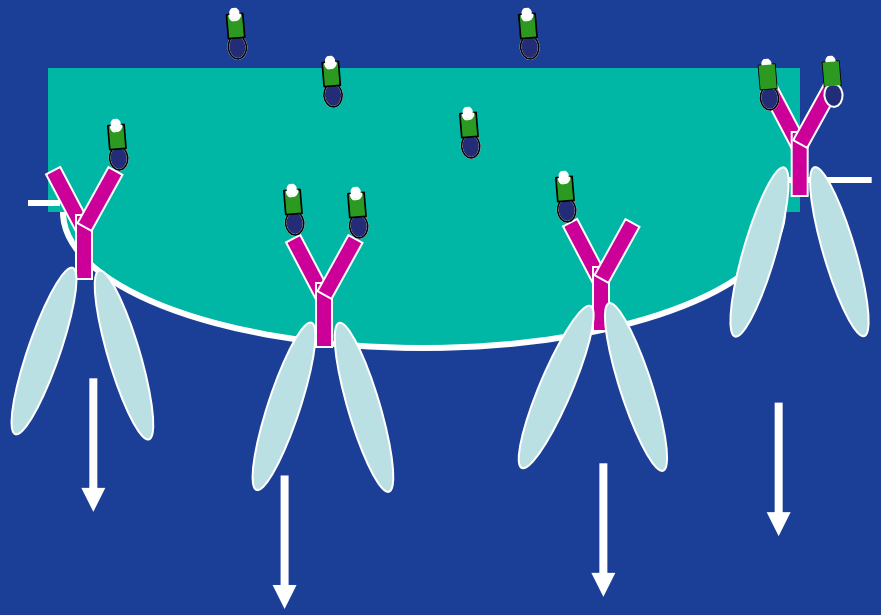
Types of immune reaction against biopharmaceuticals

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

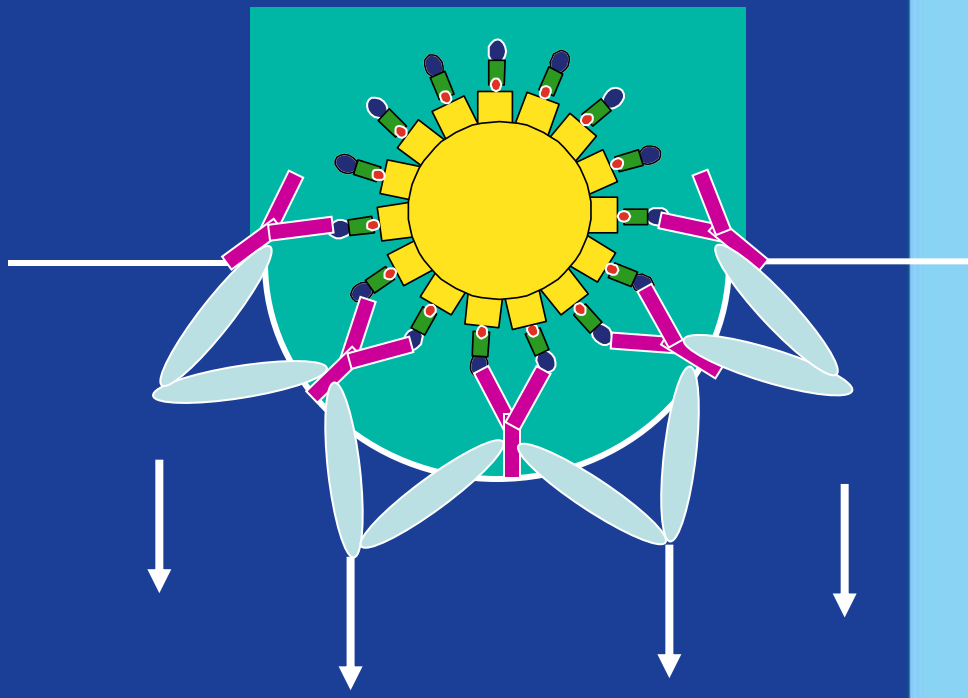
Fate of auto-reactive B cells after encountering conjugated VLPs

Monomeric BCR/self-Ag complexes



Toleragenic signals

Oligomerization of BCR/self-Ag signaling complexes



Survival/Proliferative signals

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

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

eurahp
European Academy of Hospital Pharmacy

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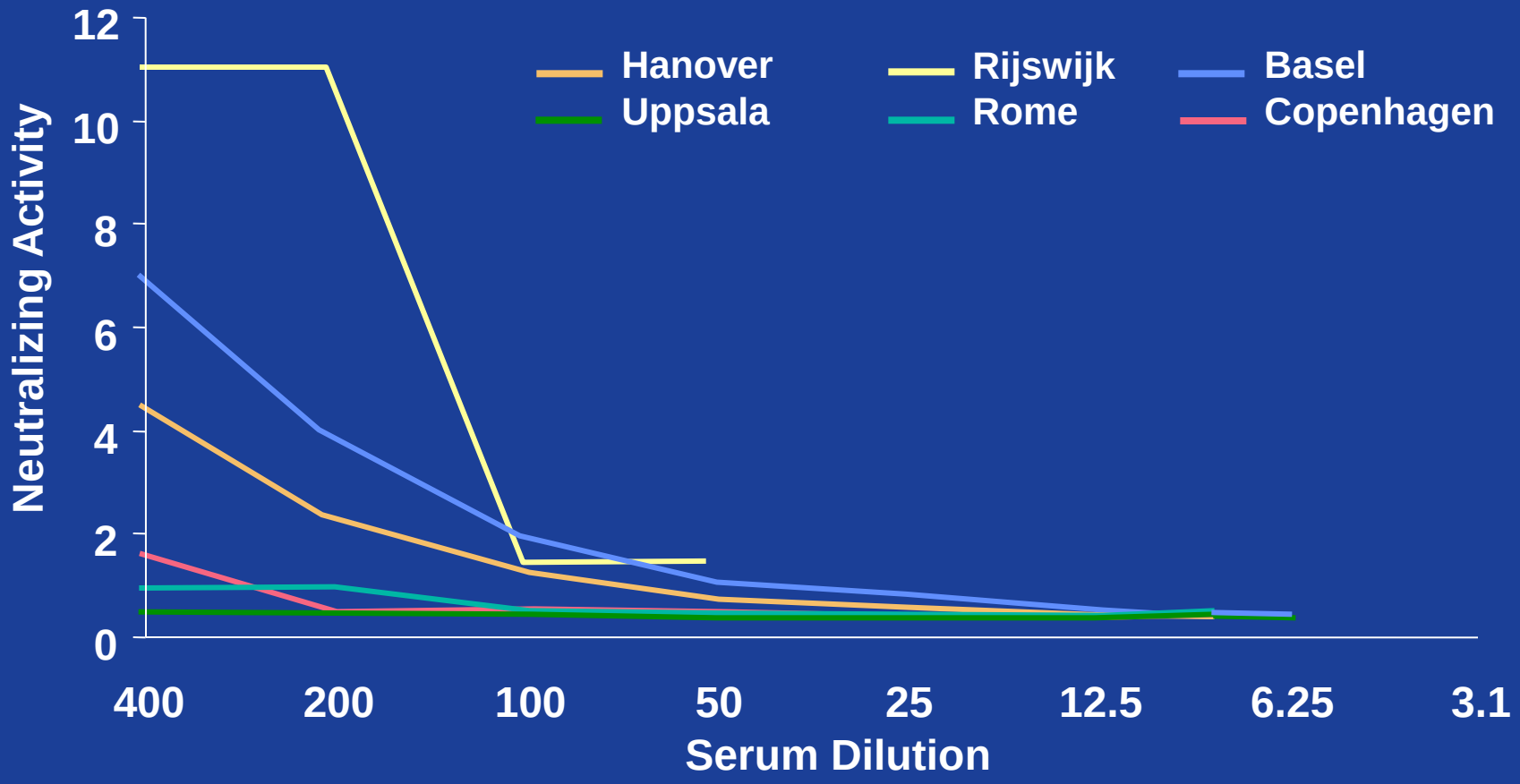
of Hospital Pharmacists

Neutralising antibodies standard serum in different laboratories



Factors influencing immunogenicity

Formulation: the interferon alpha 2 case

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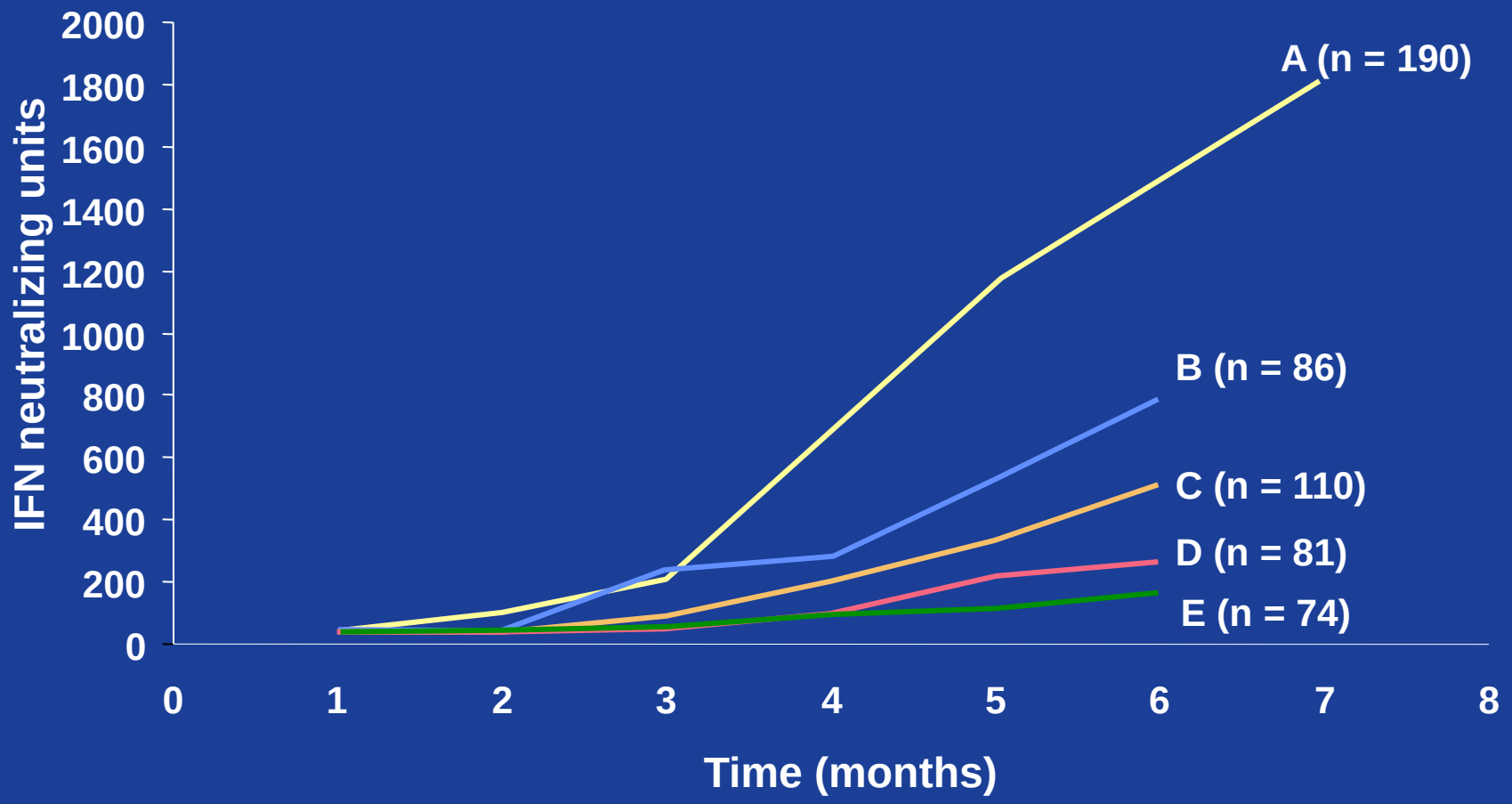
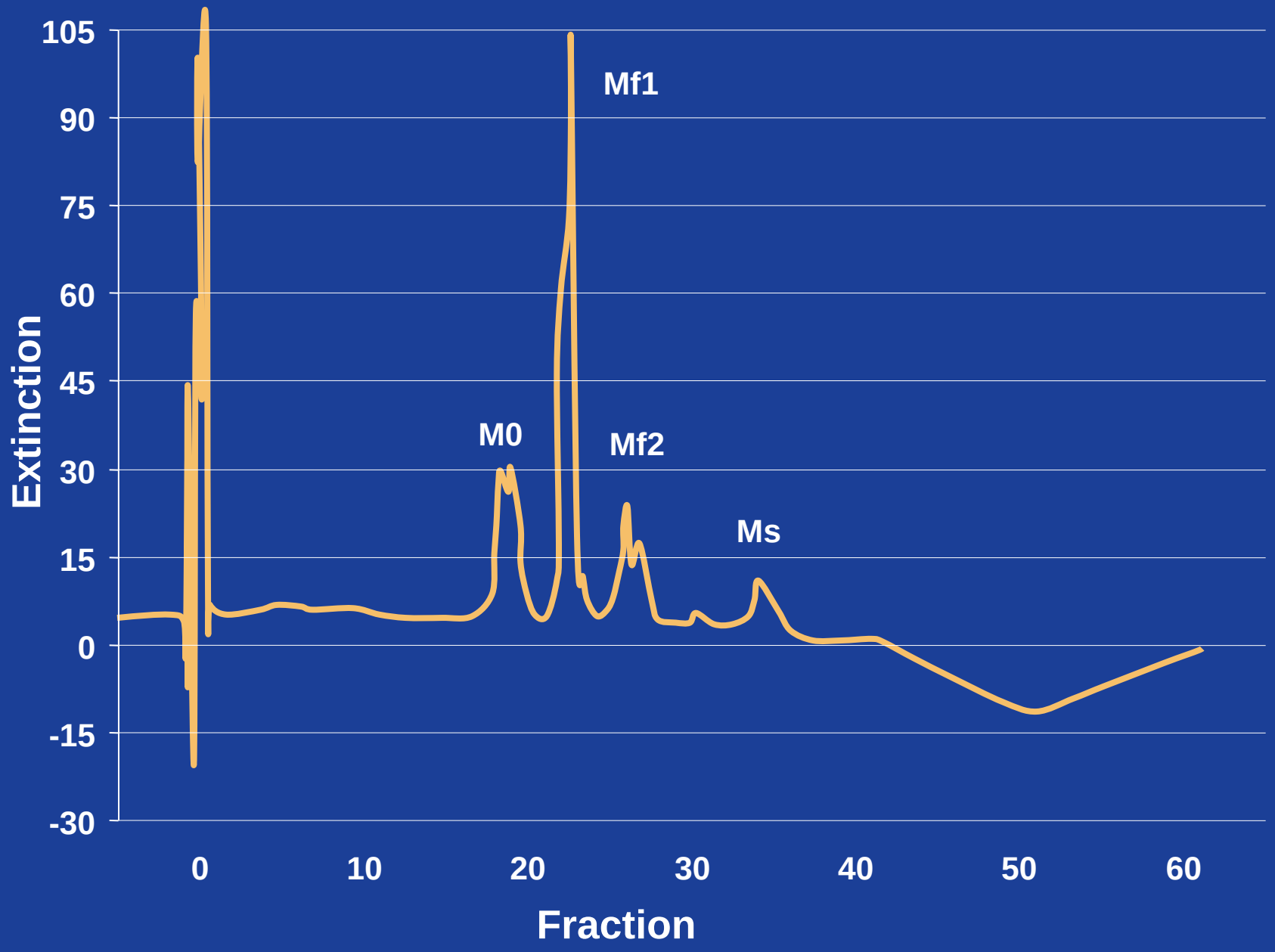
Two main IFN alpha-2 preparations

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

Hu IFN, human interferon

Antigenicity of different IFN alpha-2a formulations




105
90
75
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45
30
15
0
-15
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Extinction
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Fraction
Mf1
M0
Mf2
Ms

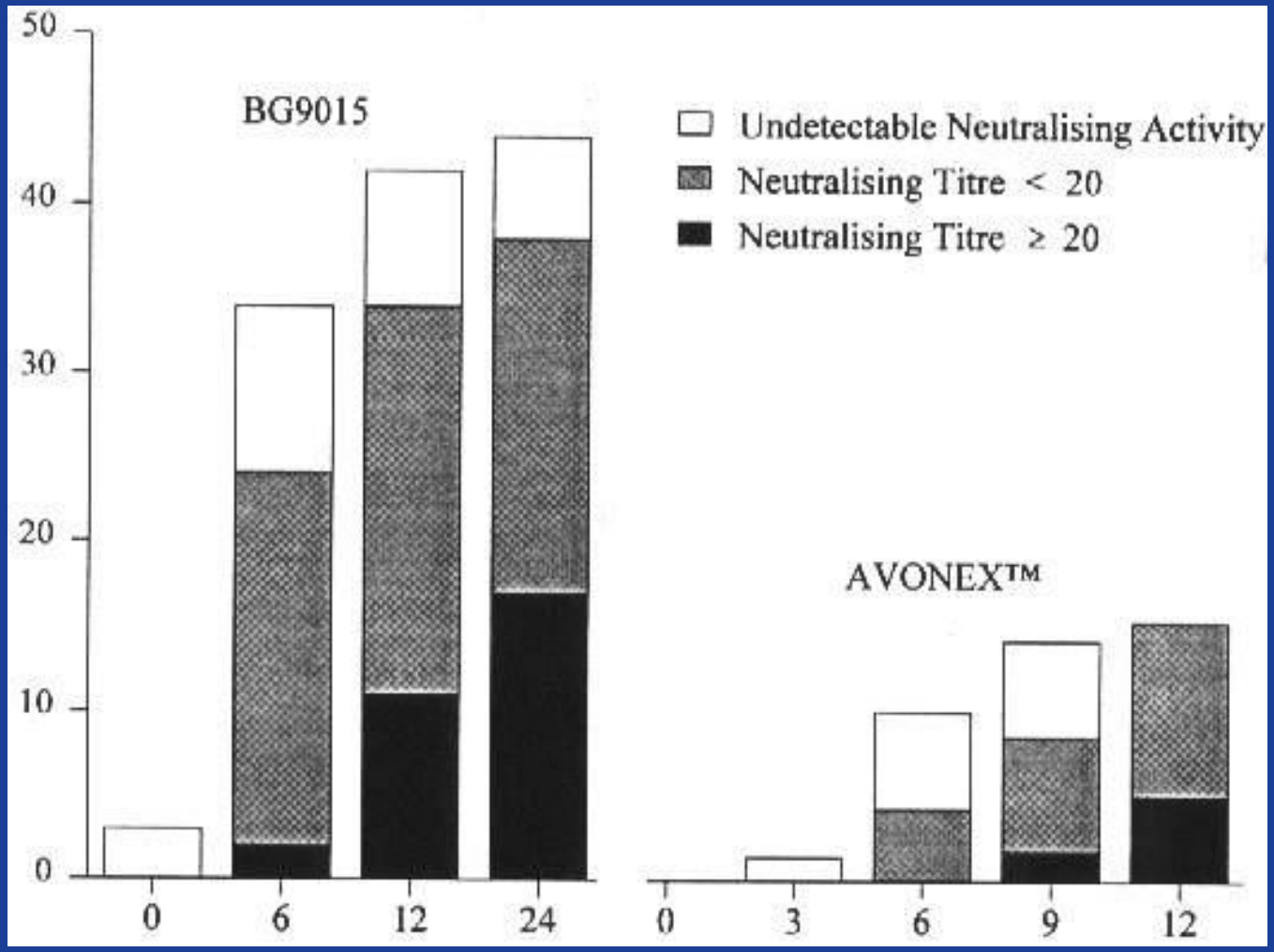
Other factors influencing immunogenicity

- 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
  - SC>IM>IV>local
- Type of disease
- Genetic background of patients
  - MHC?
  - Haemophilia
- 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

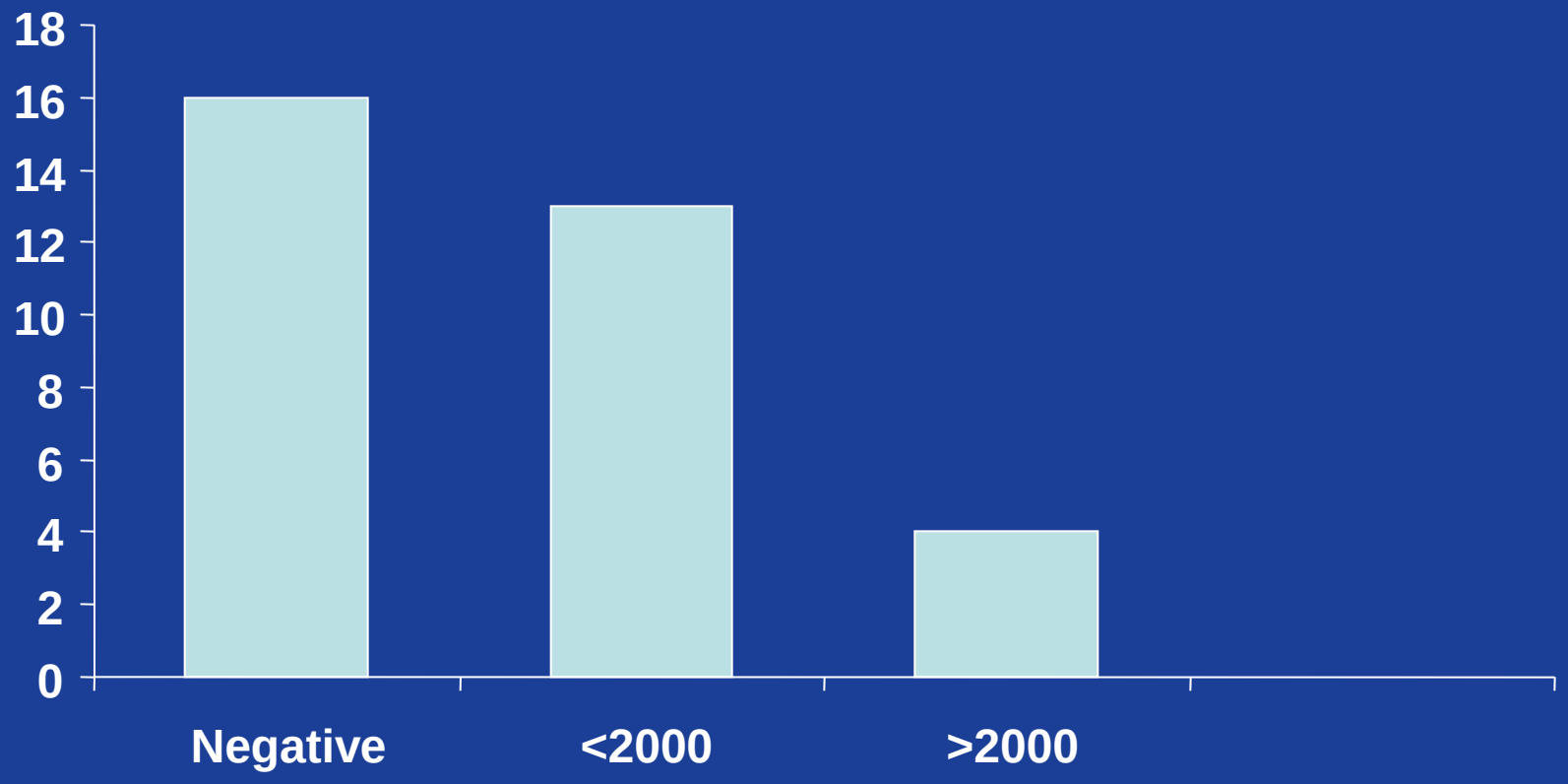
Allergy

Anaphylaxis

Serum sickness, etc

HCG, Human chorionic gonadotropin; ADA, adenoside deaminase; GnRH, gonadotropin-releasing hormone

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



HCV, hepatitus C virus

Consequences of antibodies

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Serum sickness, etc

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)

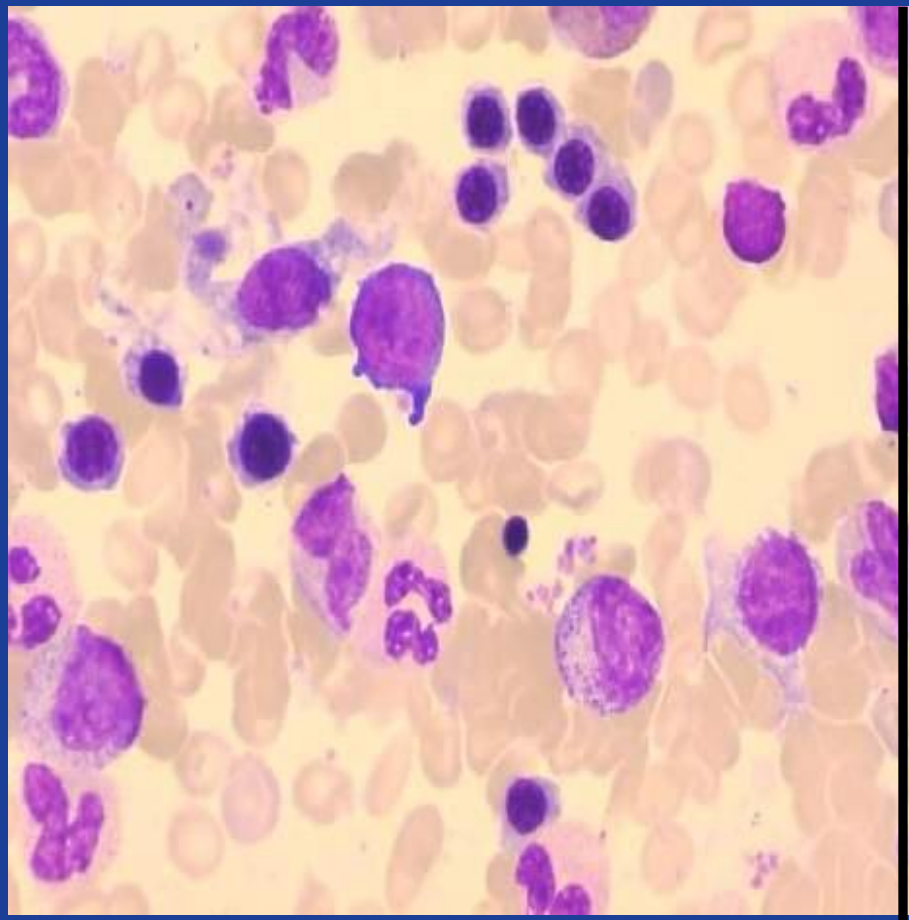
EDITOR'S NOTE: An electronic version of this news release may be accessed via our web site at **www.Amgen.com**. Visit the Corporate Center and click on Amgen News. Journalists and media representatives may sign up to receive all news releases electronically at time of announcement by filling out a short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

Prediction of immunogenicity

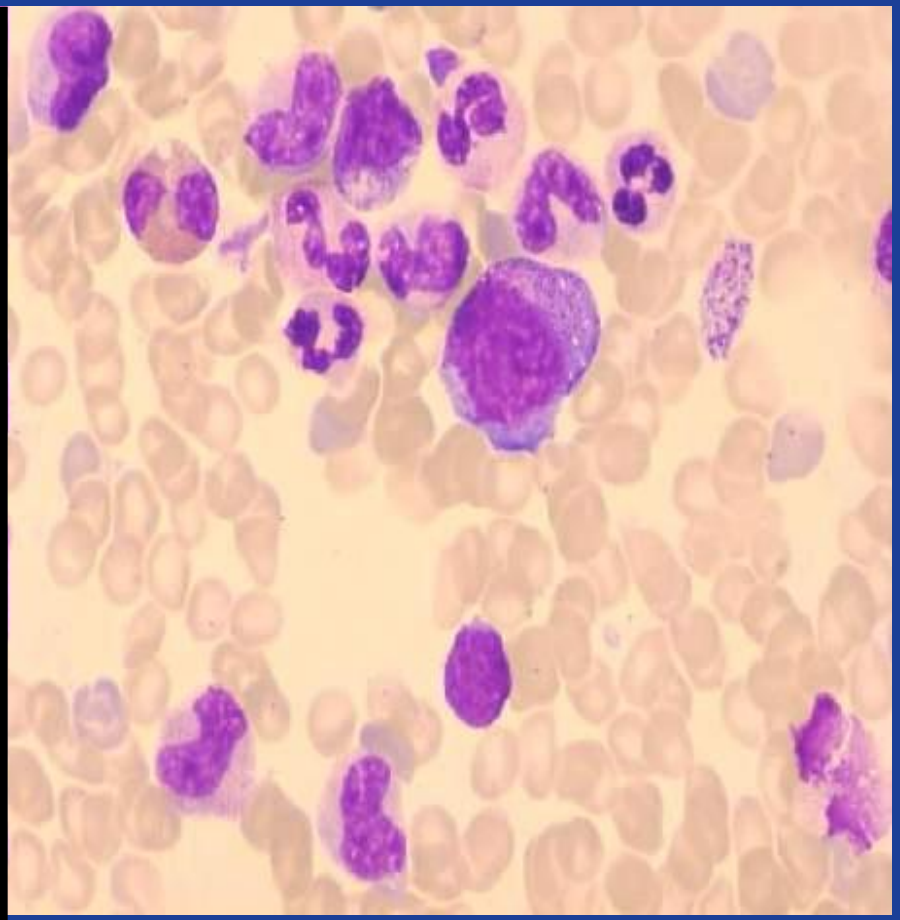
- Quality of the product
- Sequence analysis
- Reactivity with antibodies
- Animal studies
  - Conventional animals
  - Non-human primates
  - Transgenic immune tolerant mice

What caused Eprex associated PRCA?

Bone marrow smear



Normal bone marrow



PRCA bone marrow

PRCA, pure red cell aplasia

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

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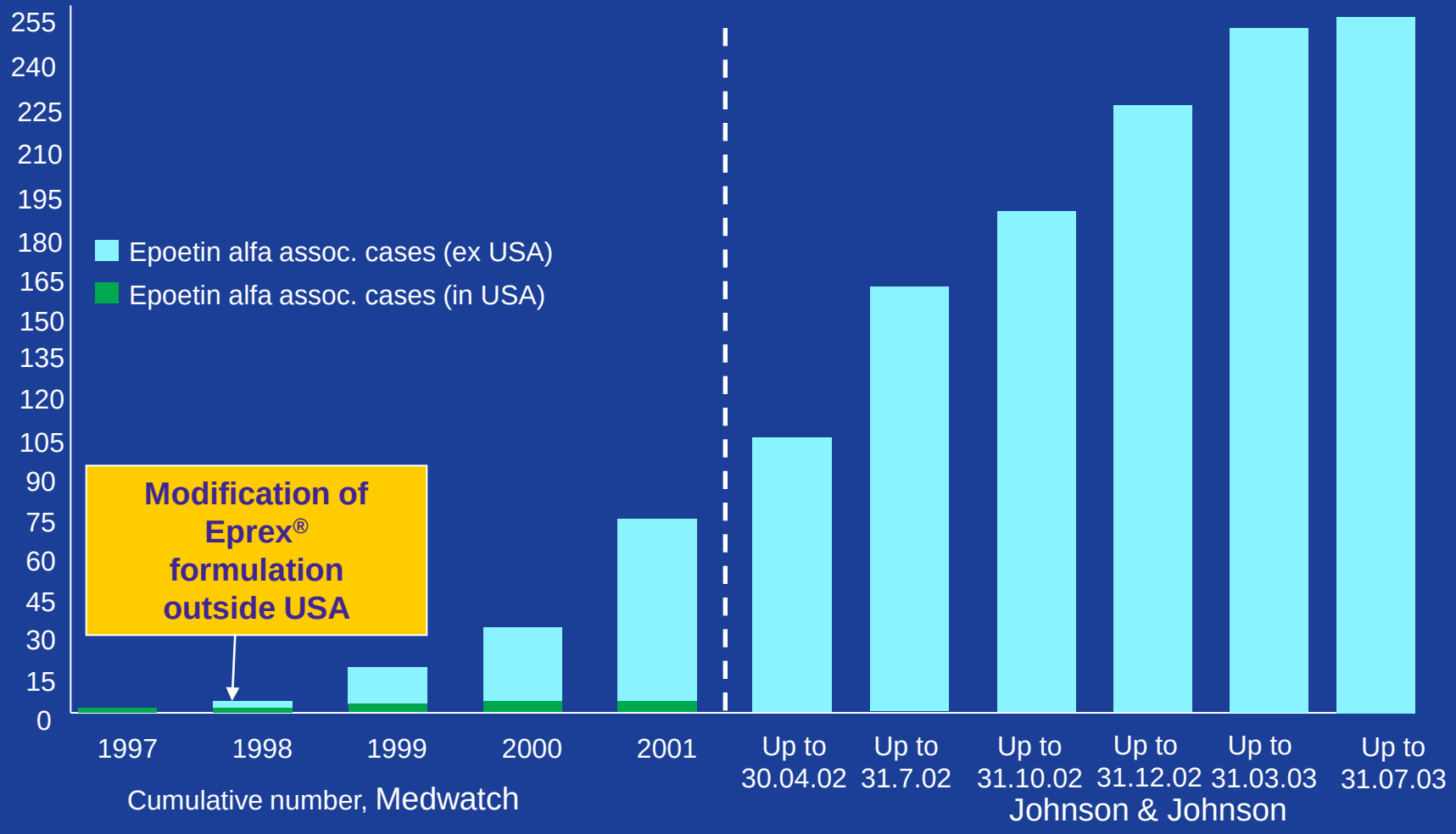
Why was Eprex implicated?

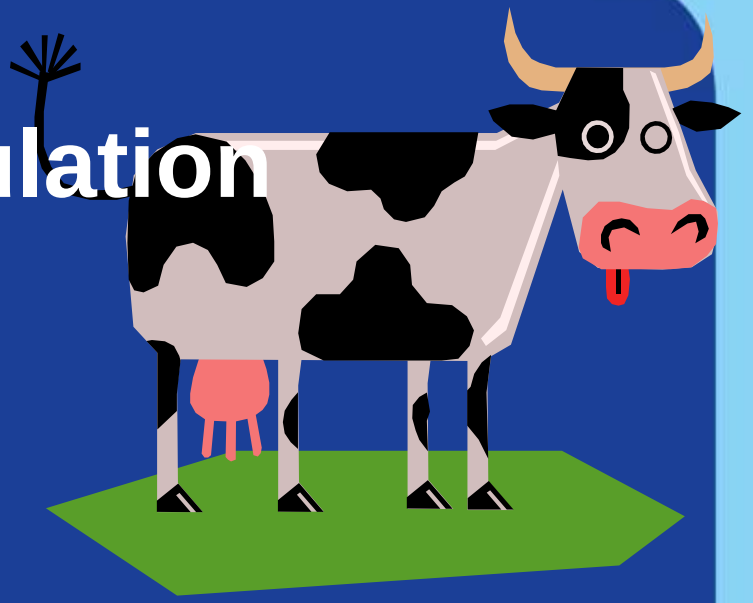
- High association between Eprex and PRCA
- Geographic distribution
- Association with formulation change

PRCA cases reported by the FDA and Johnson & Johnson



1. Gershon et al. *N Engl J Med* 2002;346:1584–1585; 2. Ortho Biotech *Dear Healthcare Professional letter* 17 July 2002; 3. Johnson & Johnson Statement. 10 Oct 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

BSE, Bovine spongiform encephalopathy

Main stabilizers used in the epoetin formulations

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

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
- Mishandling

Mishandling

- Mishandling with a slightly less stable product may explain all features of PRCA
  - Biological rationale
  - Fits with data concerning other product
  - Fits the pathogenesis
  - Fits with the epidemiological data

Conclusion

- The mystery of Eprex® associated PRCA has not been solved, but aggregates are the most likely explanation
- Immunogenicity is an issue with all therapeutic proteins