Immunogenicity of Biopharmaceuticals

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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

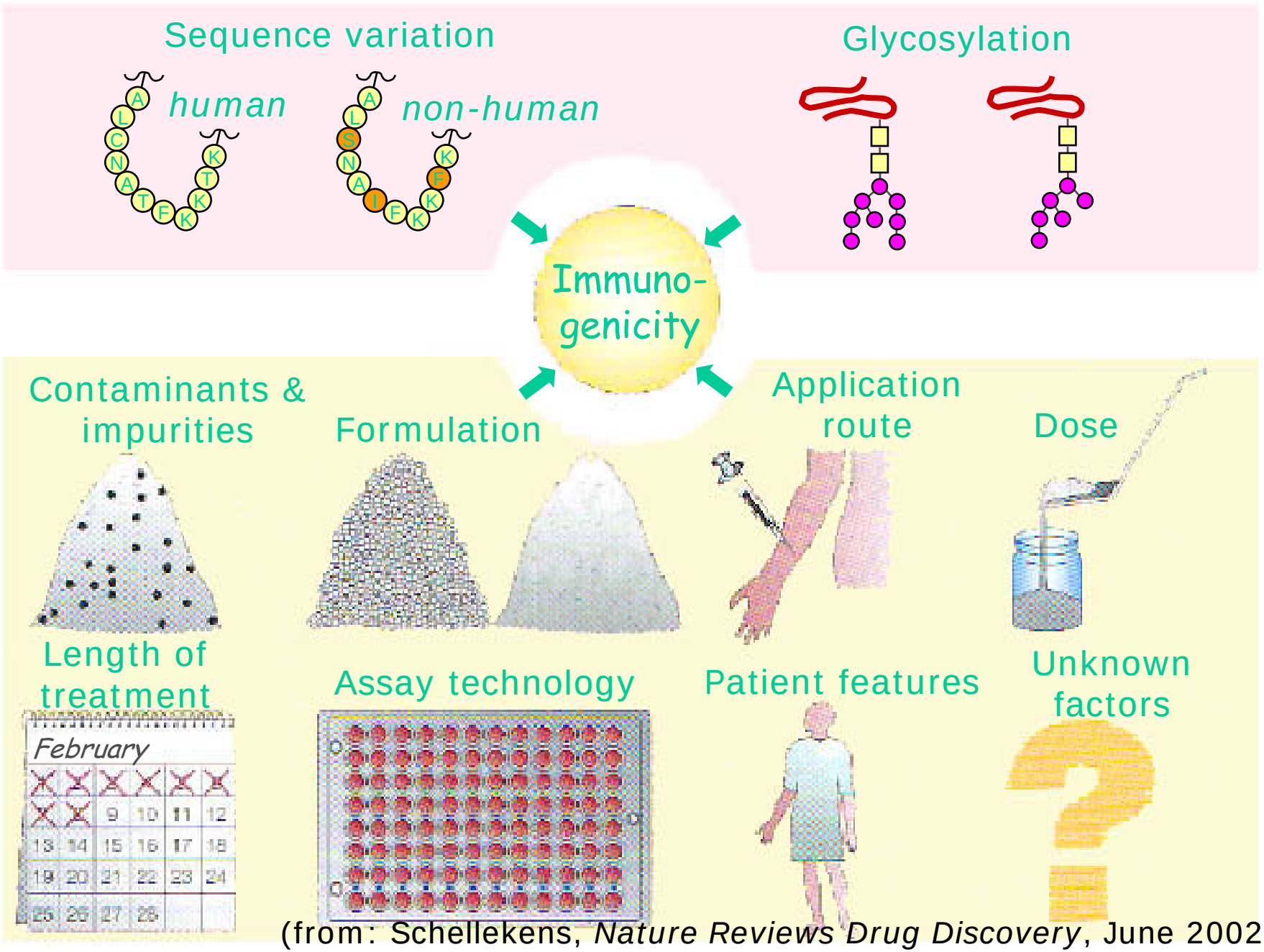
Reaction to foreign proteins

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



(from: Schellekens, *Nature Reviews Drug Discovery*, June 2002)

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

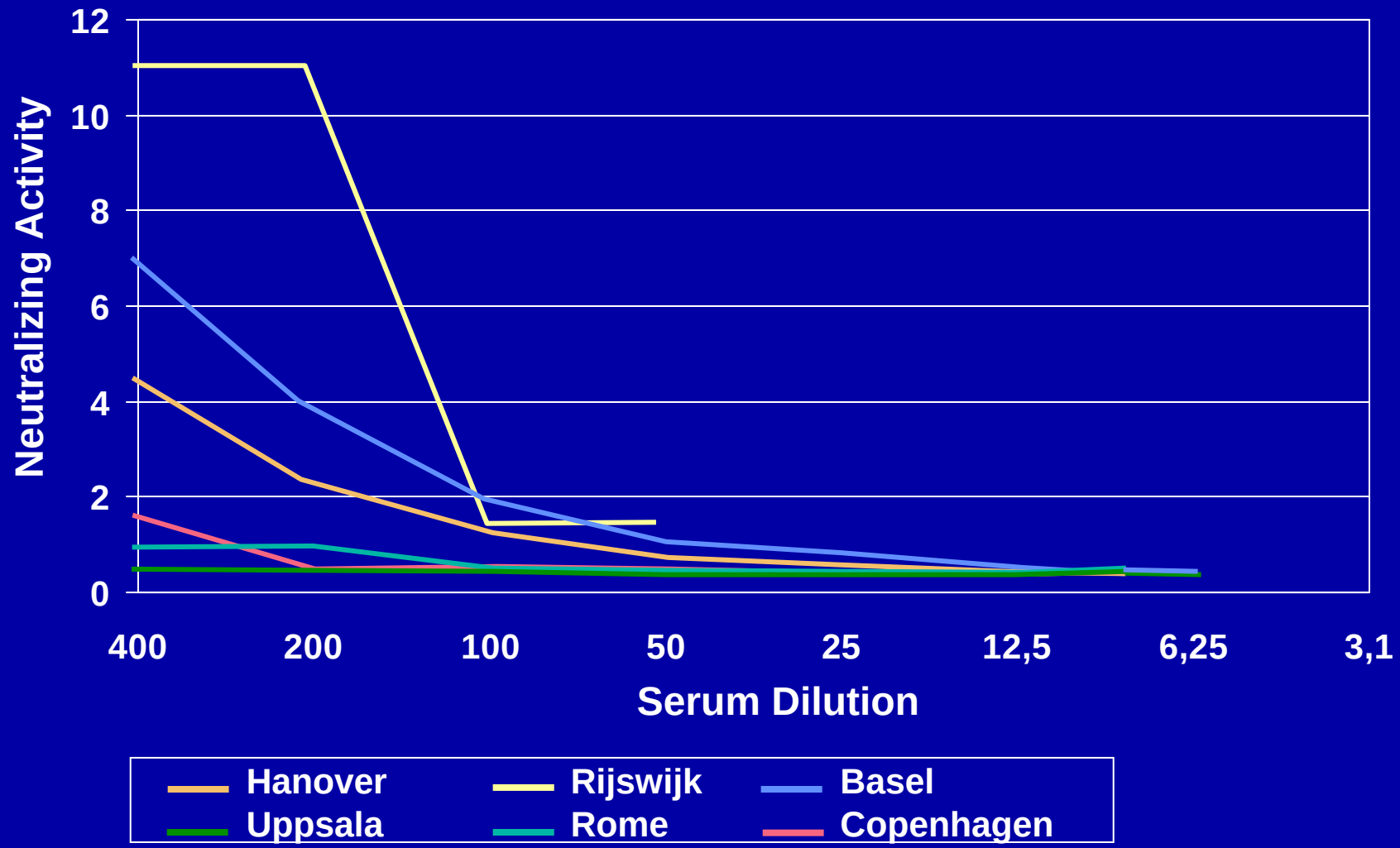
Structural properties

- 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



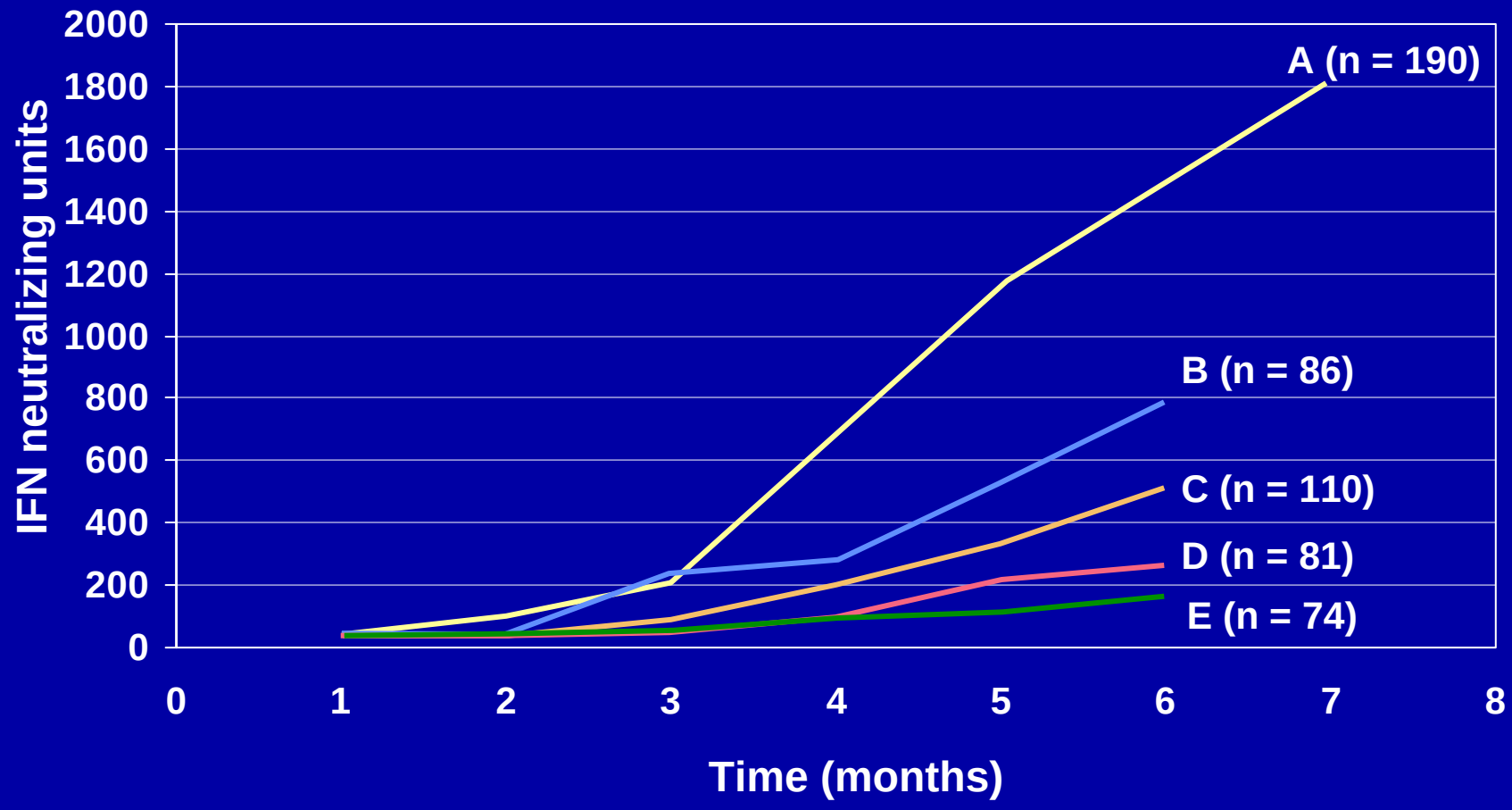
Factors influencing immunogenicity

Formulation: the interferon alpha 2 case

Two main IFN alpha-2 preparations

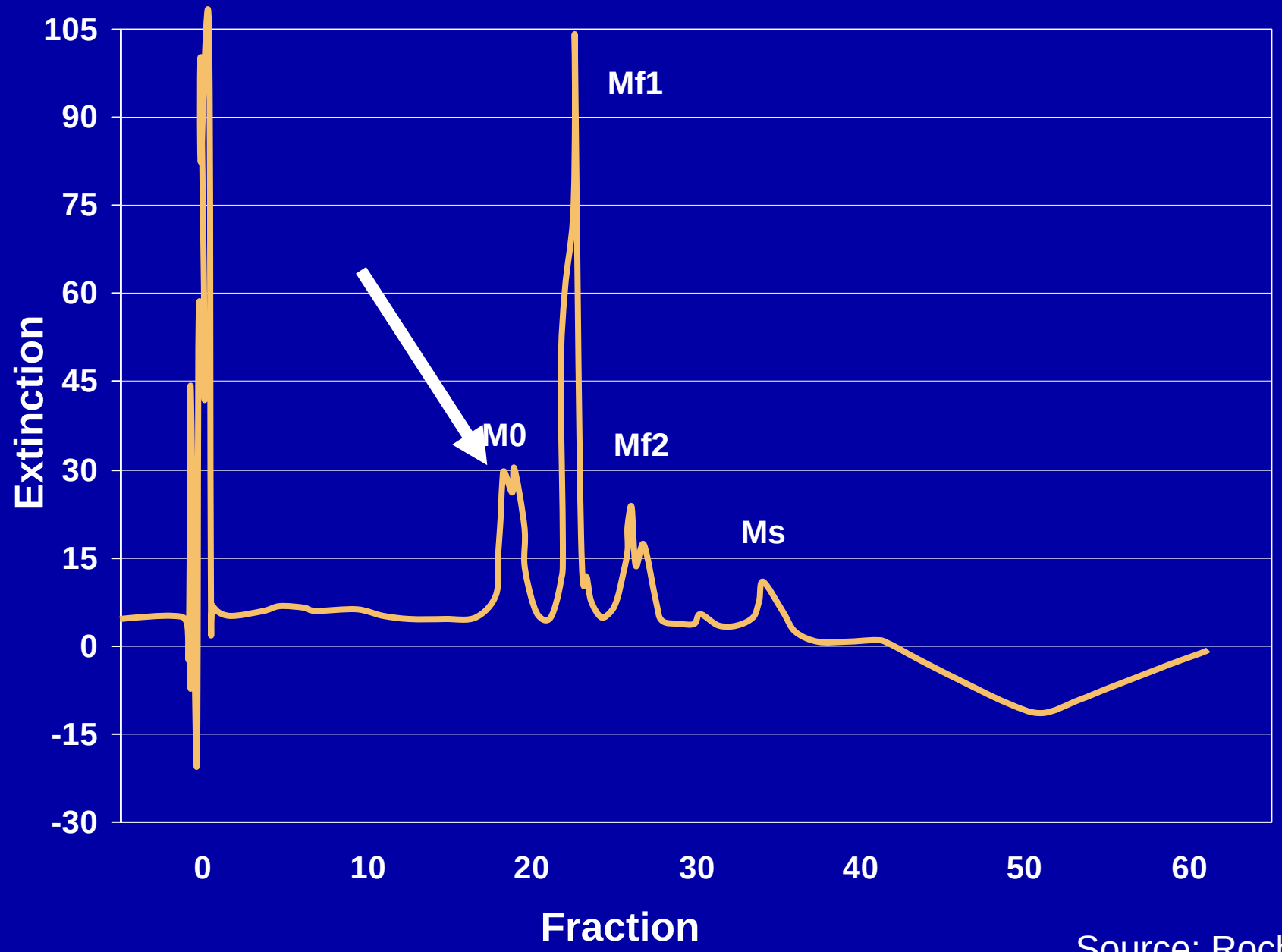
Generic name	Commercial name	Aa position 23	Natural alelle
Hu IFN alpha-2a	Roferon	Lys	No
Hu IFN alpha-2b	Intron	Arg	Yes

Antigenicity of different IFN alpha-2a formulations



Source: Roche

RP-HPLC of interferon-alfa formulations



Source: Roche

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

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

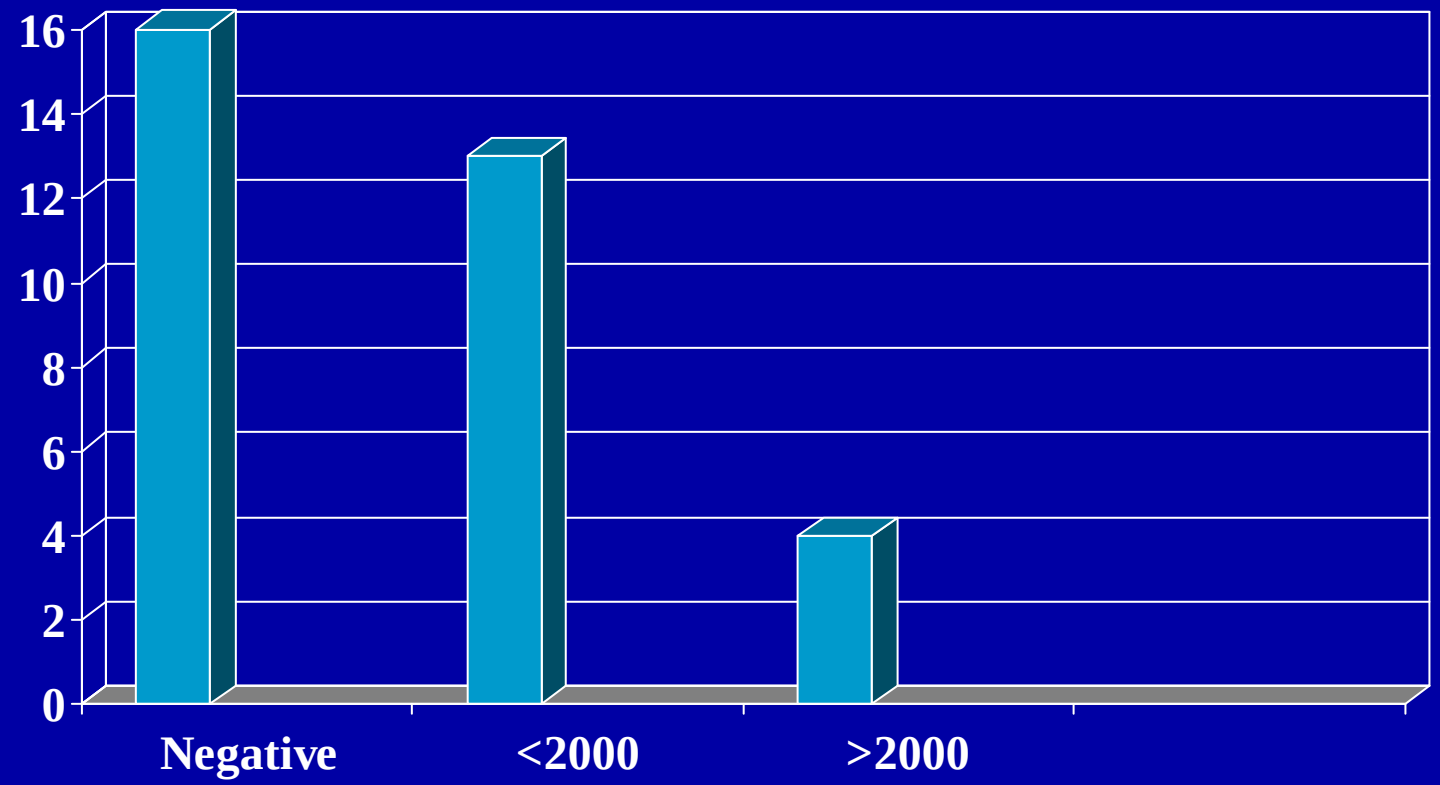
General immune effects

Allergy

Anaphylaxis

Serum sickness, etc

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



EPREX-induced PRCA cases (2001-2003)

HSA	Coated stoppers	Ab(+) PRCA cases	SC Exposure (pt-yrs)	Incidence rate (per 100,000 pt-yrs)
+	-	2	42,305	4.7 (0.57 – 17.1)
-	-	116	308,232	46.1 (38.8 – 54.3)
-	+	1	36,608	2.6 (0.07 – 14.4)

Adapted from Boven et al, Kidney Int 2005; 67: 2346

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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.

Prediction of immunogenicity

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

Previous use of immune tolerant transgenic mice

1. Human insulin transgenics
2. Human tPA transgenic
3. Human IFN alpha 2 transgenics
4. Human growth hormone transgenics

Human insulin transgenic immune tolerant mice

- Hu insulin transgenes in C57BL/6 backgrounds
- Immunisations with 3.5 nmol insulin/mouse ip in complete Freunds at day 0 and at day 21without adjuvant. Blood sampled day 30

Ottesen et al. Diabetologia 1994

Antibody response to recombinant human insulin in Hu insulin transgenic mice

	Transgenic mice	Wild type mice
Antibody positive	1/27	11/15

Ottesen et al. Diabetologia 1994

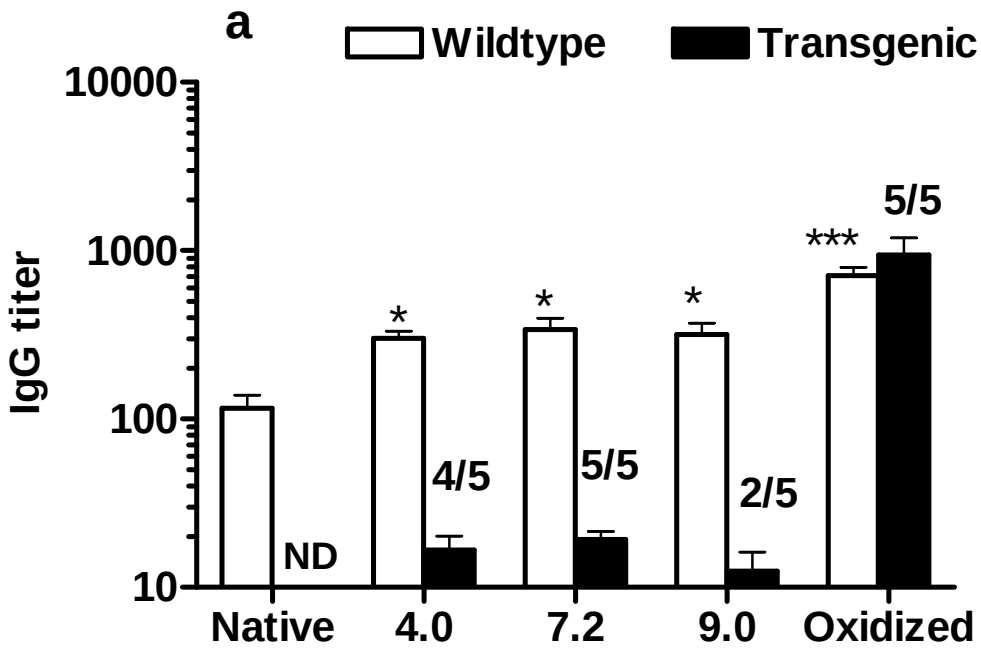
Transgenic immune tolerant mice to test immunogenicity of non-structural factors

The Hu IFN alpha 2 transgenics

Hu IFN alpha 2 immune tolerant mice

- Obtained from Roche
- Were used to evaluate the increased immunogenicity of freeze dried HSA containing formulation of Hu IFN alpha 2a
- Current use: to study immunogenicity of modified Hu IFN alpha 2b preparations

The immunogenicity of HuIFN alpha 2 stored at different pH



Hermeling et al.

Conclusions about transgenic immune tolerant mice

- It is the aggregates!
  - Native epitopes
  - Very sensitive (<1µg)
  - Not too big
- Beware of the mouse strain
- Always test antibodies to final product
- The immune reaction in wild type mice is different from breaking B cell tolerance