Immunogenicity of Therapeutic Proteins and the Problem of EPO associated PRCA

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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

	Classical reaction to neo-antigens	Breakdown of immune tolerance
Properties of product	Biomolecular plant origin	Human homologue
Characteristics of antibody formation	Fast; often after single injection; high incidence; neutralising antibodies; long duration.	Slow; after prolonged treatment; mainly binding antibodies; low incidence; disappear after stopping treatment, sometimes during treatment
Cause of immunogenicity	Presence of non-self antigens	Impurities and presence of aggregates
Predictive models	Conventional animals	Transgenic immune tolerant animals
Consequences	Loss of efficacy in majority of cases	In majority of patients no consequences

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)

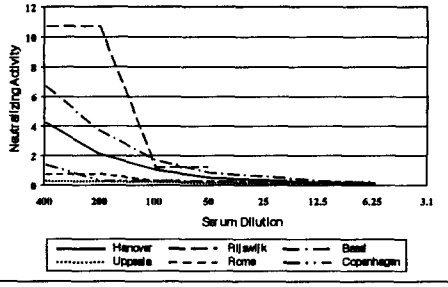
Factors influencing immunogenicity

Assays

Types of antibodies

- Binding antibodies (tested by ELISA, RIA etc)
- Neutralizing antibodies (tested by bioassays)

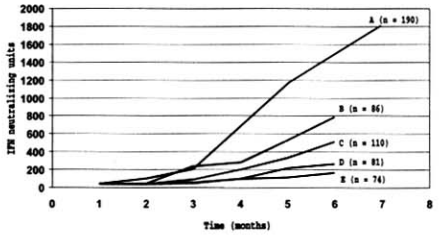
Neutralizing antibodies standard serum in different laboratories

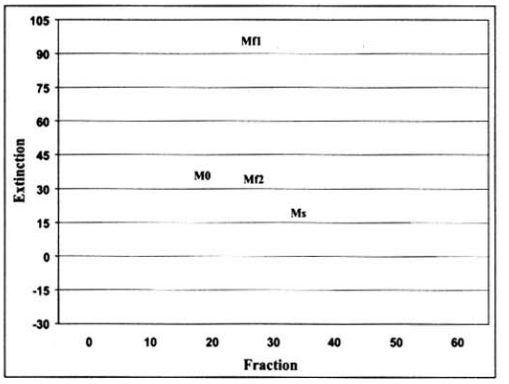


Factors influencing immunogenicity

Formulation: the interferon alpha 2 case

Antigenicity of different IFN alpha-2a formulations





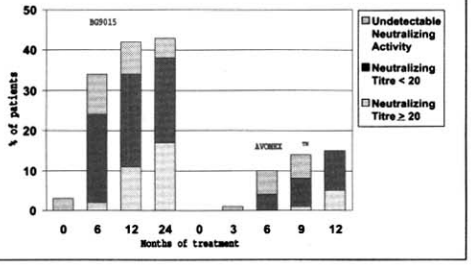
Other factors influencing immunogenicity

- 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

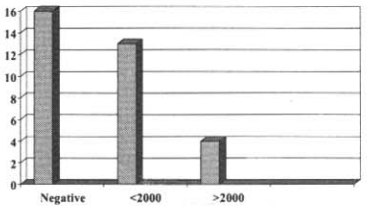
General immune effects

Allergy
Anaphylaxis
Serum sickness, etc

Relation between relaps and anti-IFN antibodies in HCV patients

	% antibodies
Overall	40
Relapsers	89

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



Neutralizing antibodies and the annual relapse rate in the IFN beta-1b trial

Time on study(months)		1,6 MU		8,0 MU	
	Placebo	NAB-	NAB+	NAB-	NAB+
0-6	1.68	1.41	0.94	0.99	1.24
7-12	1.21	1.31	0.08	0.92	0.76
13-18	1.18	1.06	1.11	0.72	0.89
19-24	1.17	1.26	0.61*	0.63	1.39*
25-30	0.92	0.75	1.16	0.53	1.19*
31-36	0.93	0.67	0.70	0.46	0.96
3-36	1.08	0.98	0.88	0.56	1.08*
9-36	1.02	0.93	0.81	0.50	1.16*

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AMGEN DISCONTINUES DEVELOPMENT OF MGDF

FOR IMMEDIATE RELEASE

THOUSAND OAKS, Calif., September 11, 1996 – 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, 805/447-0992 (media)
Denise Powell, 805/447-4346 (investors)

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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.

Pure red cell aplasia associated with EPO treatment

Data from Nicole Casadevall

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)

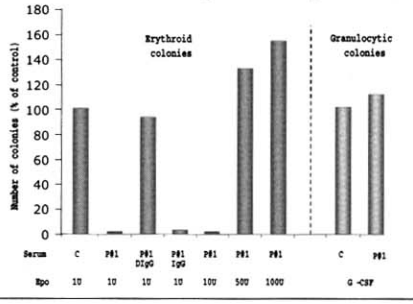
Bone marrow examination PRCA

Transfused +++ (≈ 4 units/month)

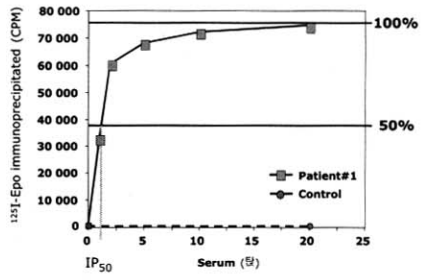
Treated by - IV Ig
- plasmapheresis
- corticosteroids (40mg/d)

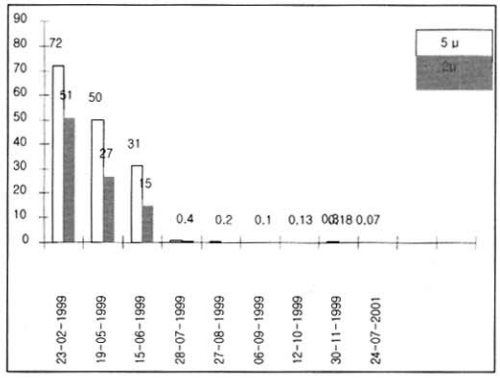
Stop transfusions 8/99

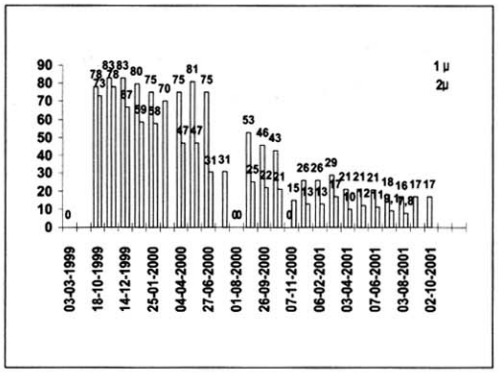
Neutralizing antibodies: Inhibition of CFU -E formation by serum of patient 1



Binding of ^{125}I-Epo by serum of patient 1



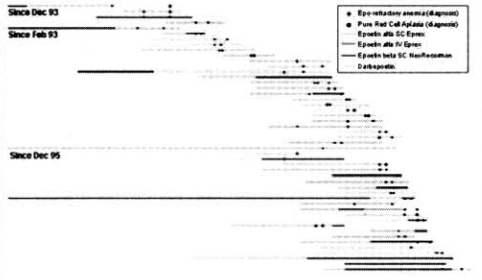




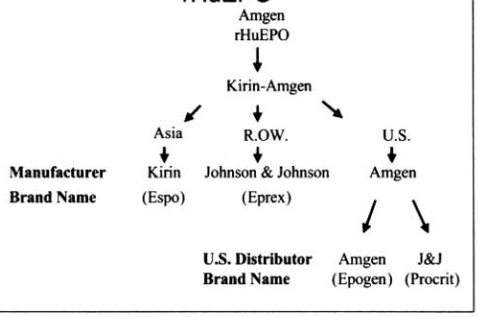
PRCA with rHu-Epo

- **Since 1998**
  - 113 antibody positive cases in the world
    - 111 in renal patients treated by subcutaneous route
      - 2 treated by Epo β exclusively
      - 12 mixed cases (exposed to both α and β)
      - Other cases after α exposure only
    - 2 in MDS patients
      - 1 treated by Epo α (SC)
      - 1 treated by Epo β (SC)

Time course of individual PRCA cases



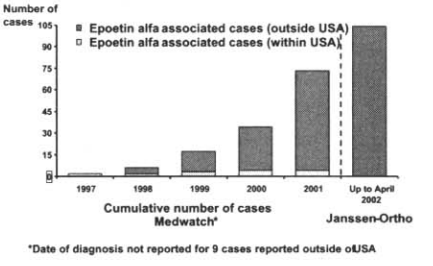
Amgen의 Licensing greements for rHuEPO



60 antibody cases with PRCA

- France	32
- UK	12
- Australia	5
- Spain	3
- Canada	3
- Switzerland	2
- Italia	2
- Austria	1

PRCA cases reported by the FDA and Janssen-Ortho Inc (Canada)



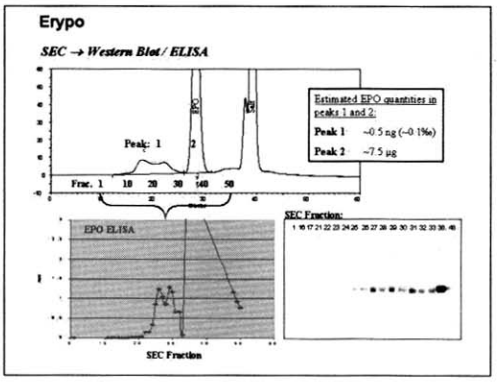
*Date of diagnosis not reported for 9 cases reported outside oUSA

Cause of the epidemic of pure red cell aplasia

- In all cases antibodies to EPO have been established to be the cause of the pure red cell aplasia
- The most like explanation is that a subtle change has occurred in the immunogenicity caused by a change in production or formulation of Eprex

Questions regarding the pure red cell aplasia

- What preceeds PRCA?
- What is the exact cause?
- How to prevent further cases? (I.v. treatment?)



Conclusion

- The majority of biopharmaceuticals is immunogenic
- The clinical consequences are mostly benign, but may be severe