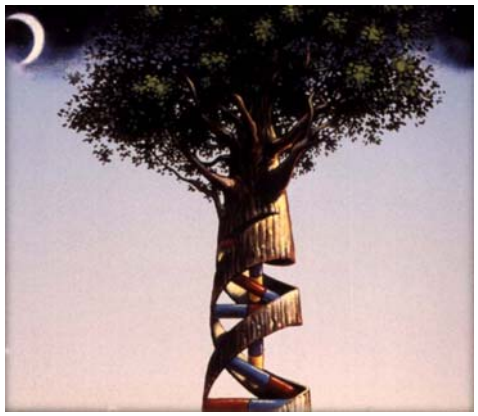
About Pipelines and Shifting paradigms: biopharmaceuticals versus low molecular weight drugs

- Prof. dr. Daan.J.A. Crommelin,
- Scientific Director TI Pharma, a Public Private Partnership, Leiden
- and Dept. Pharmaceutics, UIPS, UU

- *Alicante, October 2008*

‘Every protein has a life of its own’

(anonymous Ph.D. student)



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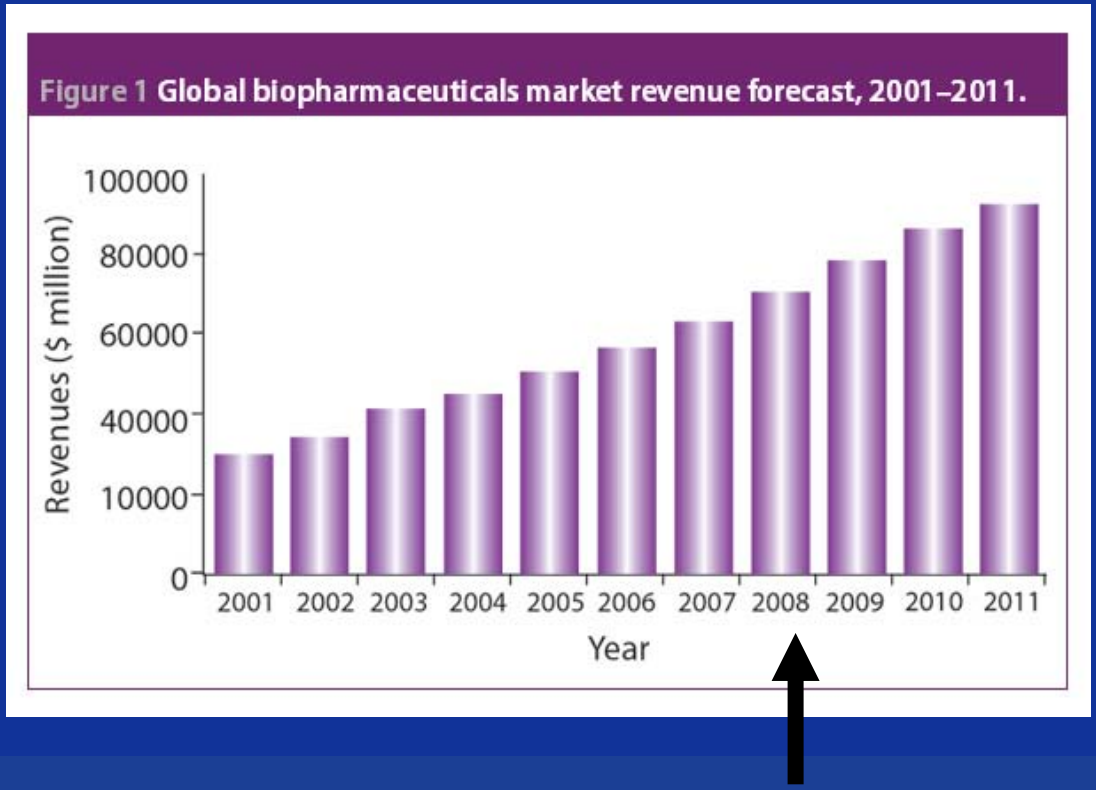
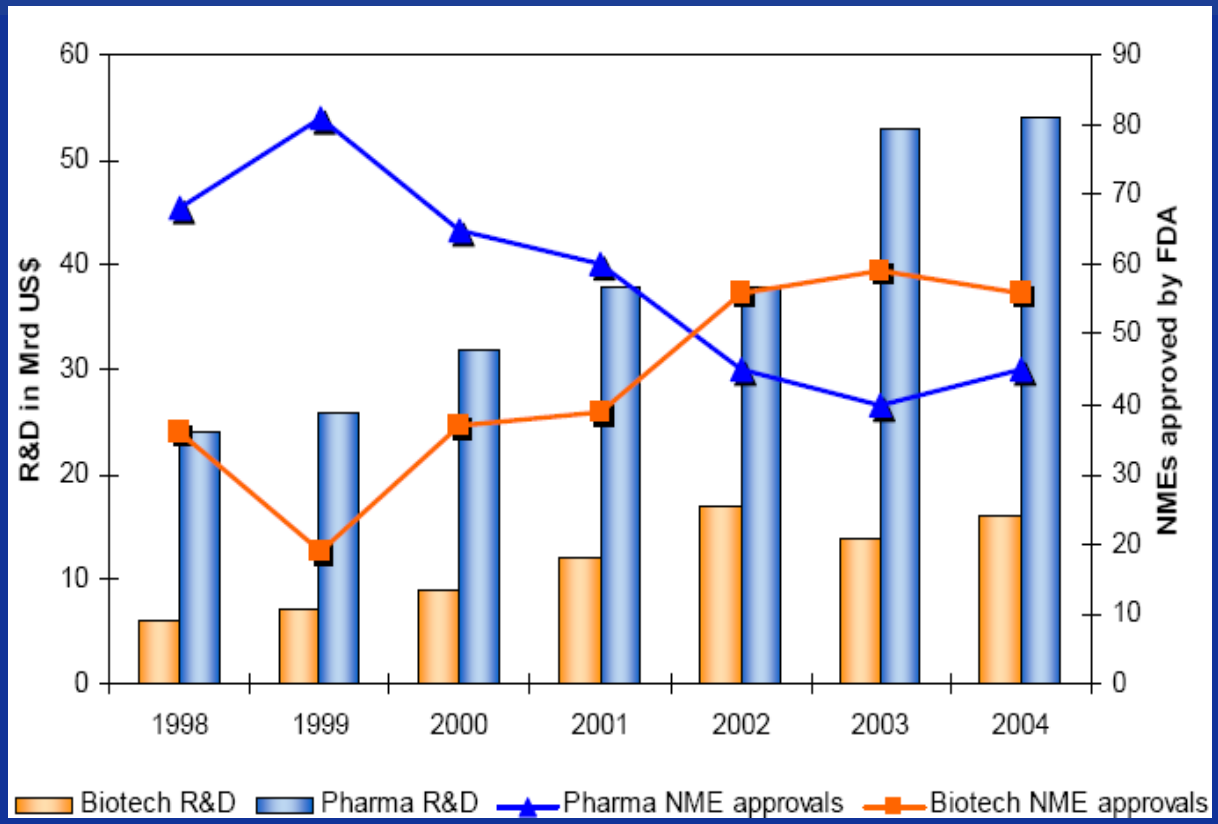
With the highest growth rates within the entire pharma market, biopharmaceuticals will reach > US$ 92 billion revenues in 2011

Most biopharmaceutical proteins have small markets, but high value
< 10 kg/yr, > US$10,000/g



Knaeplein, 2007

The Rise of Biopharma…



The number of Biotech approvals surpassed the small molecule approvals in 2002 (US)

Source: BioGeneriX

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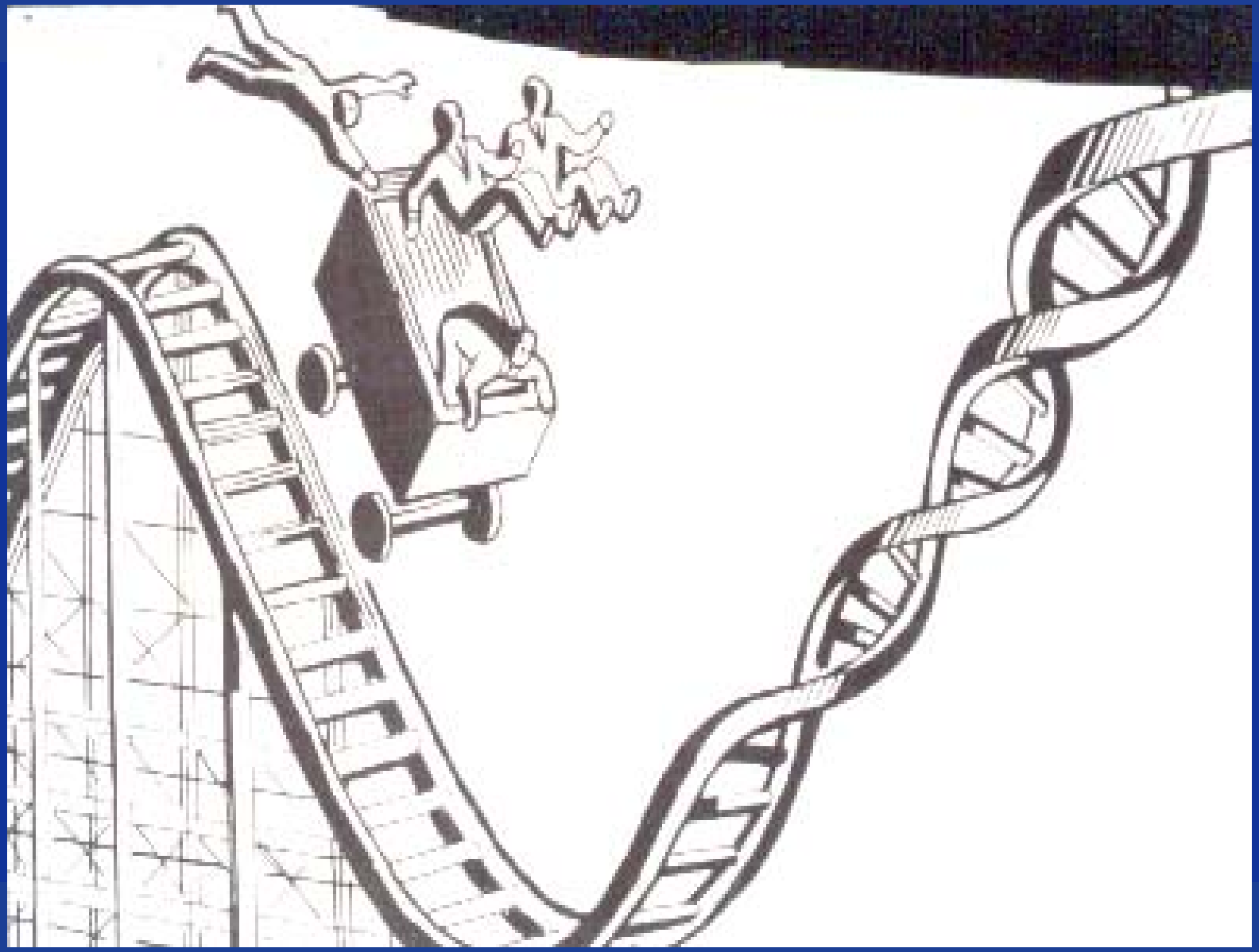
…Biopharma in Perspective

- The first biotech therapy to earn FDA approval was recombinant human insulin (Genentech & Eli Lilly) in 1982.
- Since then, as of Oct 2006, more than 250 drugs & vaccines for nearly 400 indications developed by biotech companies have been approved by FDA (inc. small-molecules and tissue-engineered products). http://bio.org/speeches/pubs/er/approveddrugs.asp
- More than 400 biotech drugs & vaccines are currently in clinical trials targeting more than 200 diseases

Courtesy Özceada

TI PHARMA



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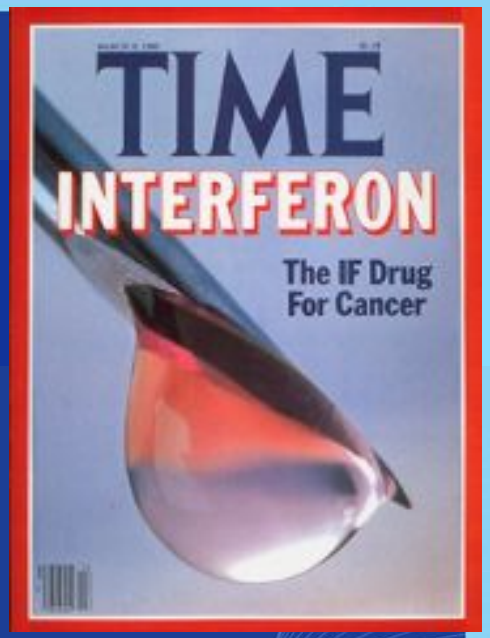
Dorr, 2002

Weimar W, Lameijer LD, Edy VG, Schellekens H.

Prophylactic use of interferon
in renal allograft recipients.
Transplant Proc. 1979 Mar;11(1):69-70. No abstract available.
PMID: 377705 [PubMed - indexed for MEDLINE]



Interferons

Drugs desperately looking for a disease….

5 billion dollars!

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Biopharmaceuticals = pharmaceutical biotech products = biologicals

- Medical aspects:
  - indications for serious diseases; meeting unmet medical needs
- Economical aspects
  - relatively small, but fast growing
- Pharmaceutical aspects:
  - delicate complex molecules
  - potent molecules (?)
  - delivery issues

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(Liver) storage diseases: orphan diseases

Enzymes	number of patients	treatment per year/keuros	total/y/product (x 1000)
Cerezyme® (Imiglucerase)	27000	476	12852000
Fabrazyme® (Agalsidase beta)	1200	182	218400
Replagal® (Agalsidase alfa)	1200	189	226800
Aldurazyme® (Laronidase)	1100	473	520300
Myozyme® (alglucosidase)	4500	351	1579500
Elaprase® (Idurosulfatase)	1000	234	234000
Naglazyme® (Galsulfase)	400	100	40000
Elaprase® (idurosulfatase)	1000	2340	2340000
Grand total	37400		18011000

18 billion euros!

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Adapted from Theo Dingermann

Present Arsenal

Examples of the types of product on the market:

- Hormones, growth factors, enzymes
  - Fertility hormones
  - Human insulin
  - Enzymes
  - Human growth factors (G-CSF, haematopoietic growth factors)
- Cytokines
  - Interleukins
  - Interferons
- Vaccines & antigens
  - Hepatitis B antigen
  - Cholera vaccine
- Antisense
  - Fomivirsen
- Cell therapy
  - Carticel, Epicel

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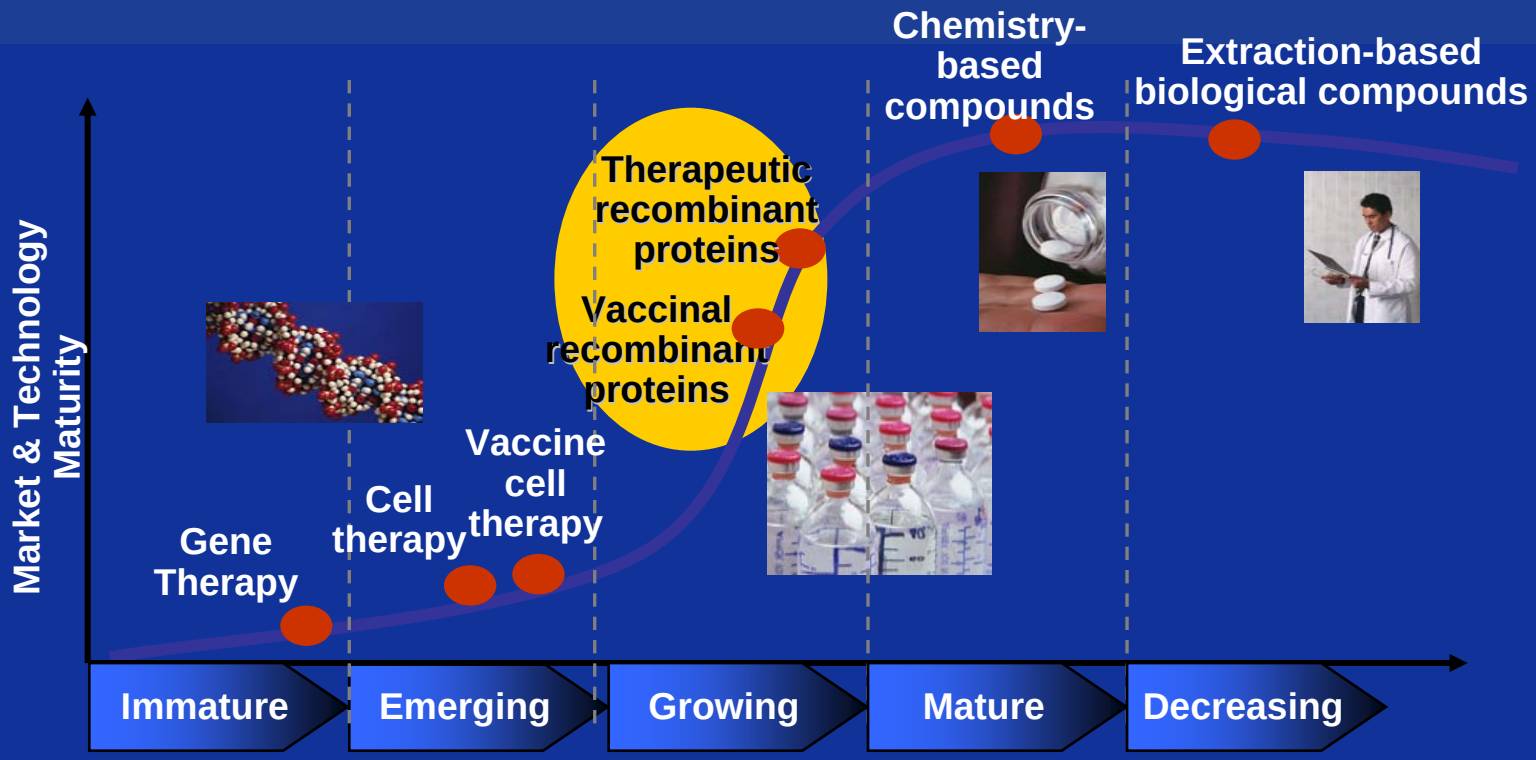
Technology Evolution in Pharma Industry



- Non validated technologies
- First products entering development phases
- No products in market

- Validated technologies
- More products in development
- Few products in the market

- Mastering technologies
- Ongoing optimization
- Many products in development and in the market

- Fully mastered and opmized technologies
- Many products in development and in the market

- Common technologies
- Manufacturing alternatives required
- Decreasing number of products in development and, eventually, in the market

Source: Paulo Barbanti

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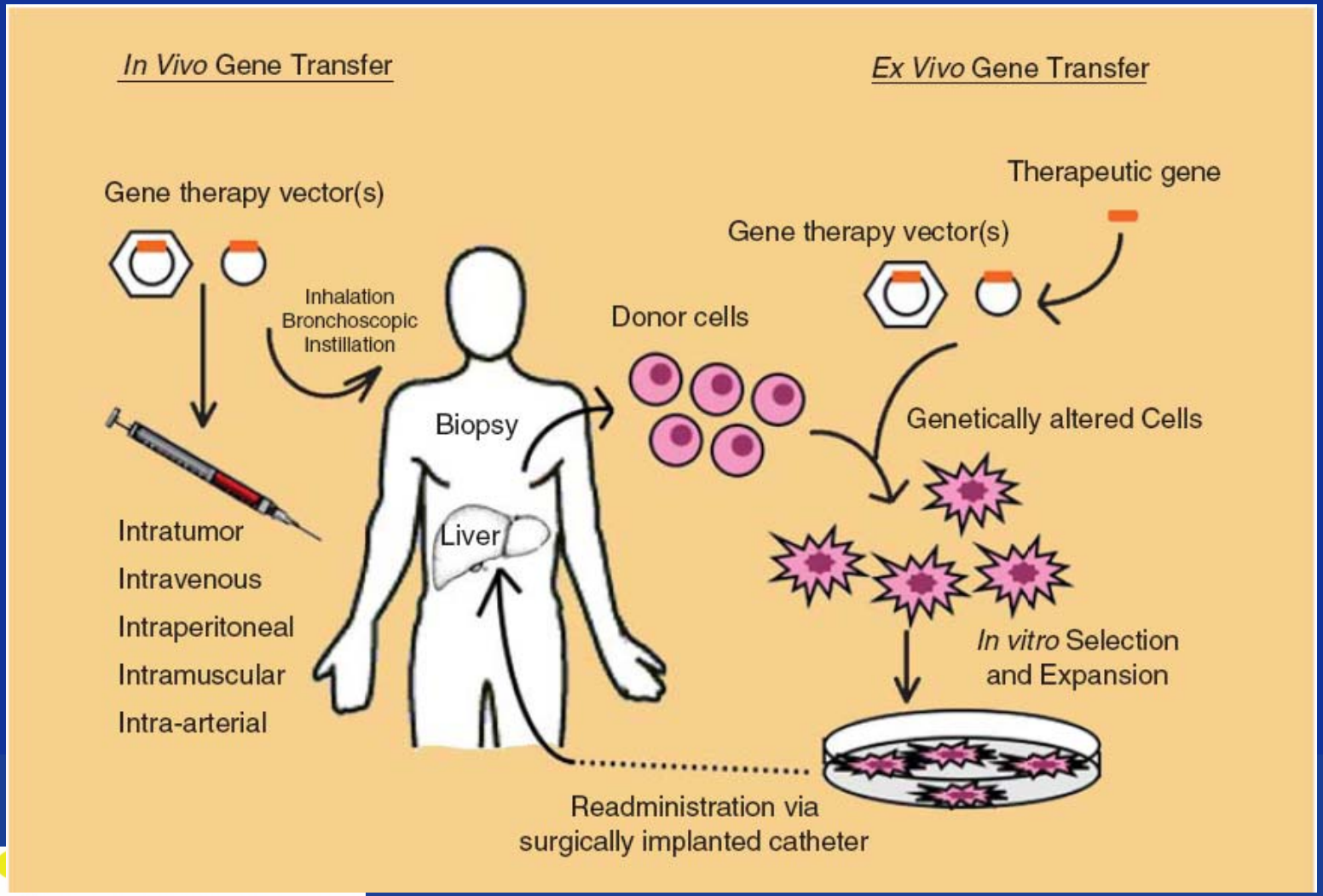
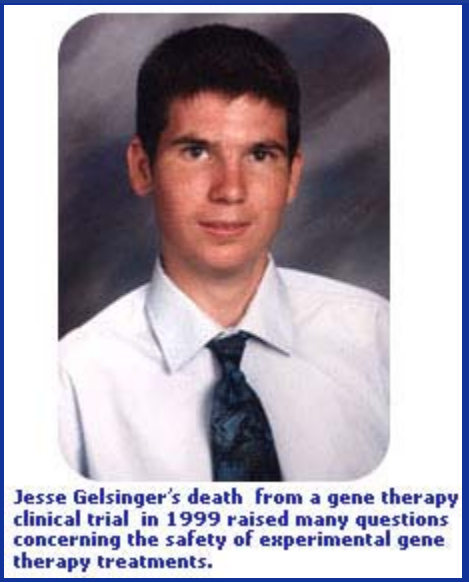
Gene Therapy: viruses as delivery system….



Plusses and minuses……



Jesse Gelsinger's death from a gene therapy clinical trial in 1999 raised many questions concerning the safety of experimental gene therapy treatments.

Joly Mohr, July 2007

Figure 2 The first gene therapy product is approved. On October 16, 2003, China's SFDA approved an adenovirus-based product, Gendicine, for treatment of head and neck cancer. The product was commercially available in January 2004 through the company SiBiono GeneTech. *Source*: SiBiono GeneTech press release.

.In 2002 and 2003, it was reported that three of nine children in France who had been cured of severe combined immunodeficiency disease (SCID) with gene therapy had developed cancer two to three years later. Children born with this disorder will die in the first year of life unless they can find a matching blood marrow donor, which is hard to do.

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Where biopharmaceuticals differ from low molecular weight drugs

- Molecular weight
- Complexity of structure
- Characterization
  - Structure and physico-chemical properties
  - Protein purity
  - Biological activity
- Stability (shelf life/life on the shelf)
- Immunogenicity
- Needle focused

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Crommelin DJA, et al. Int J Pharm 2003;266:3-16.

Molecular weight

Product	Molecular weight (kDa)	Number of amino acids
Paracetamol	0.151	N/A
Calcitonin	4.5	32
Epoetin-α	30.4	165
Factor VIII	264.0	2,332

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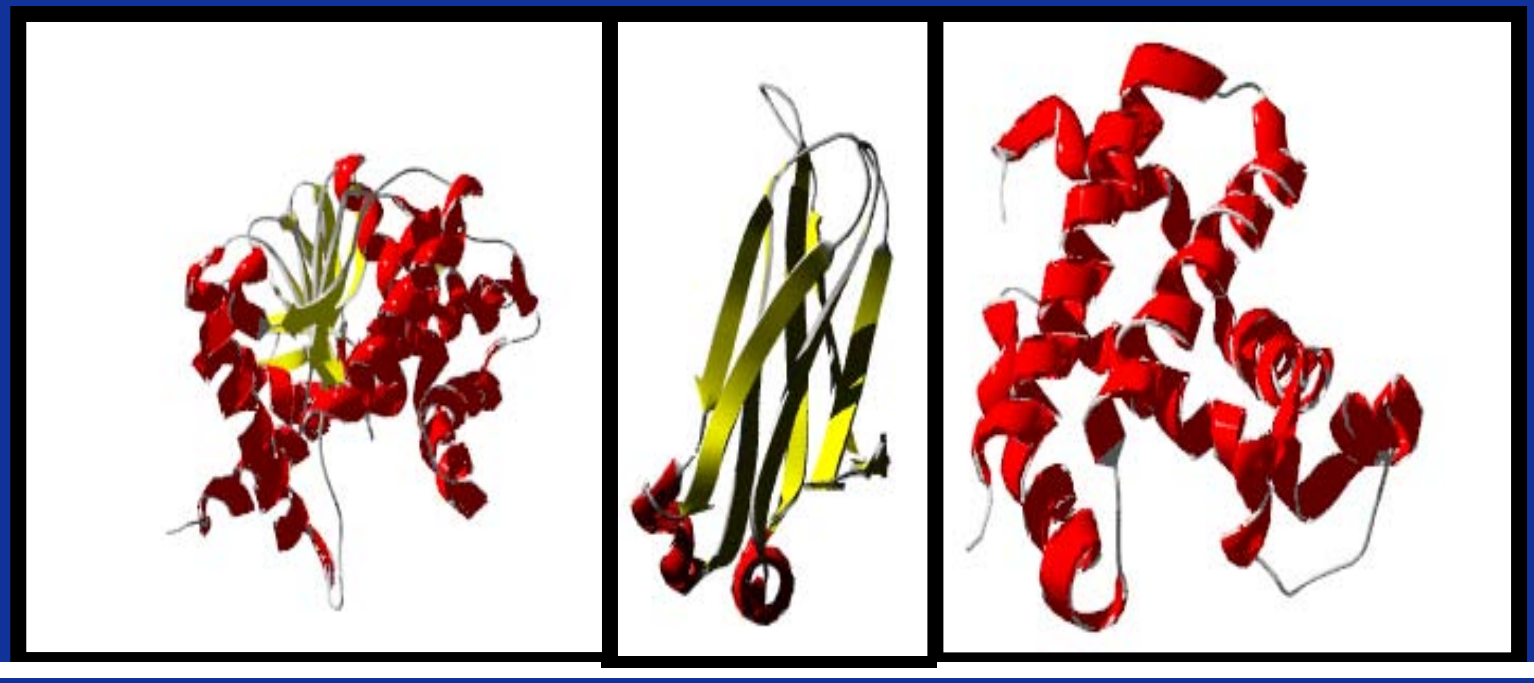
Protein Conformations



Lactate Dehydrogenase
Mixed α/β

Immunoglobulin
Fold: β

Hemoglobin B
Chain: α

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The conformational state of a protein

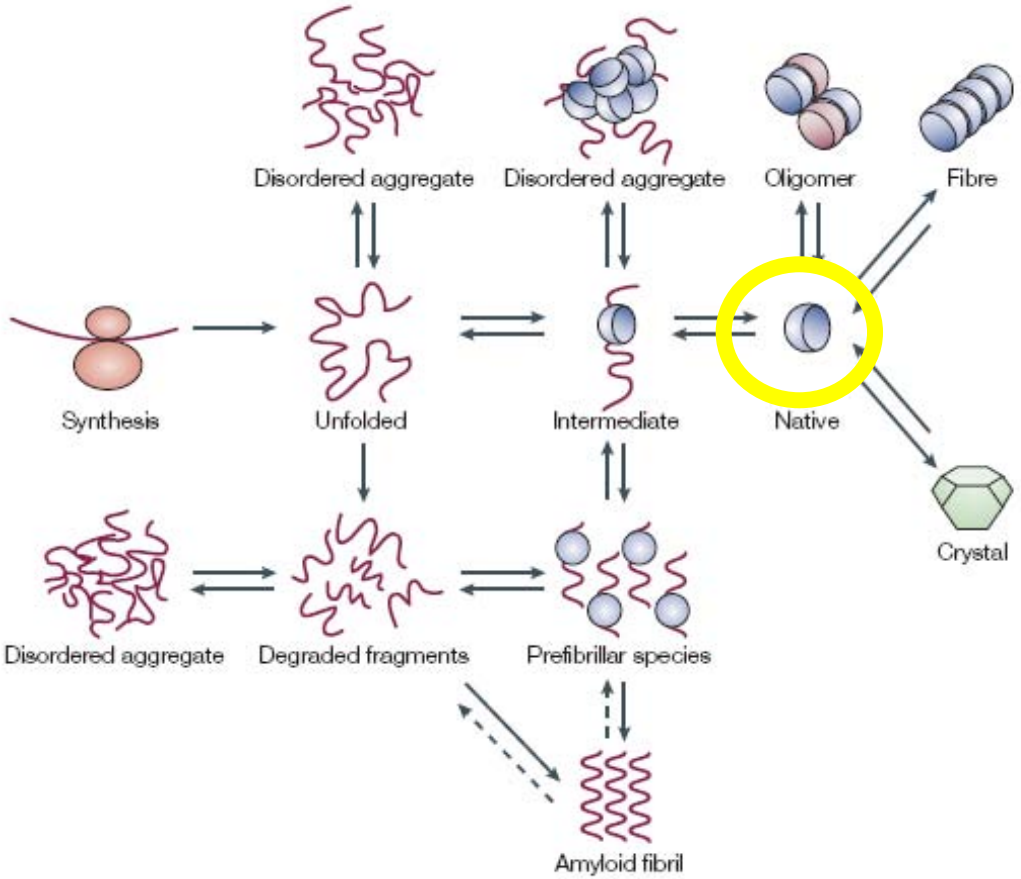

Figure 5 | **The many conformational choices for a polypeptide chain.** Adapted, with permission, from REF. 138 © (2003) Macmillan Magazines Ltd.

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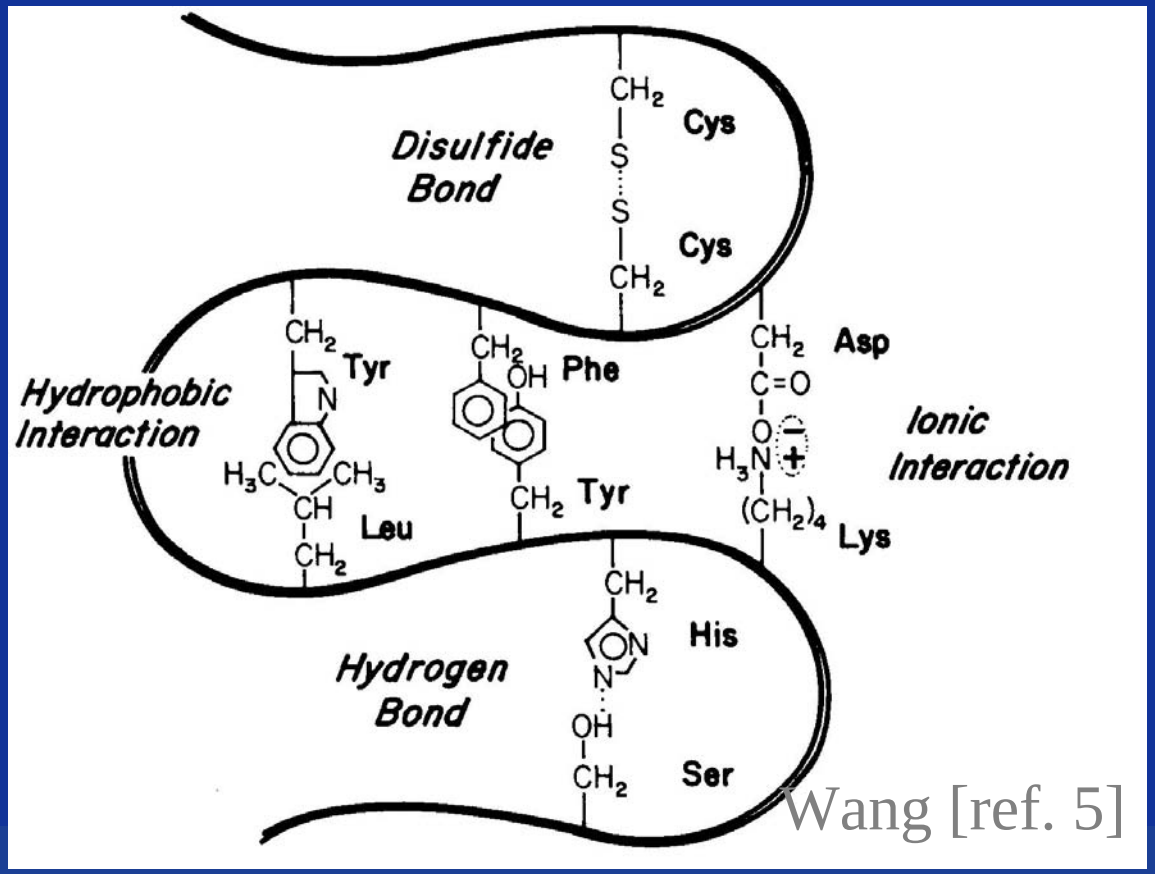
Interactions in Proteins



Wang [ref. 5]

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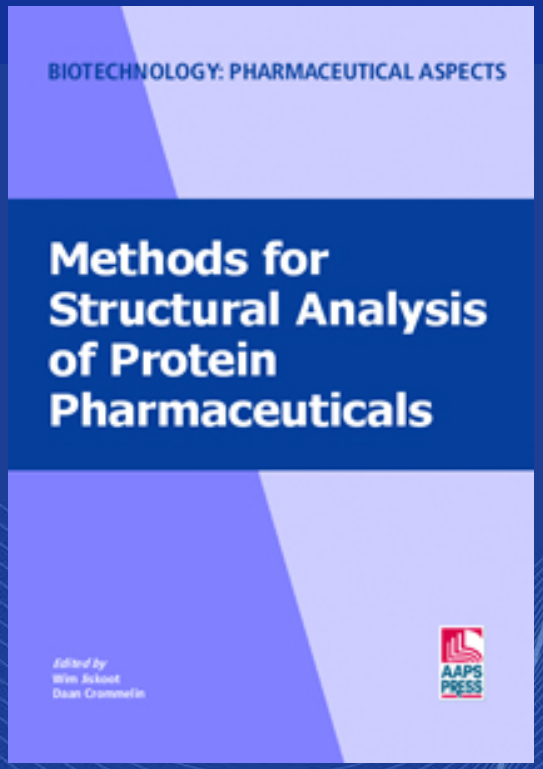
Edited by
Jiskoot and Crommelin
AAPS press 2005



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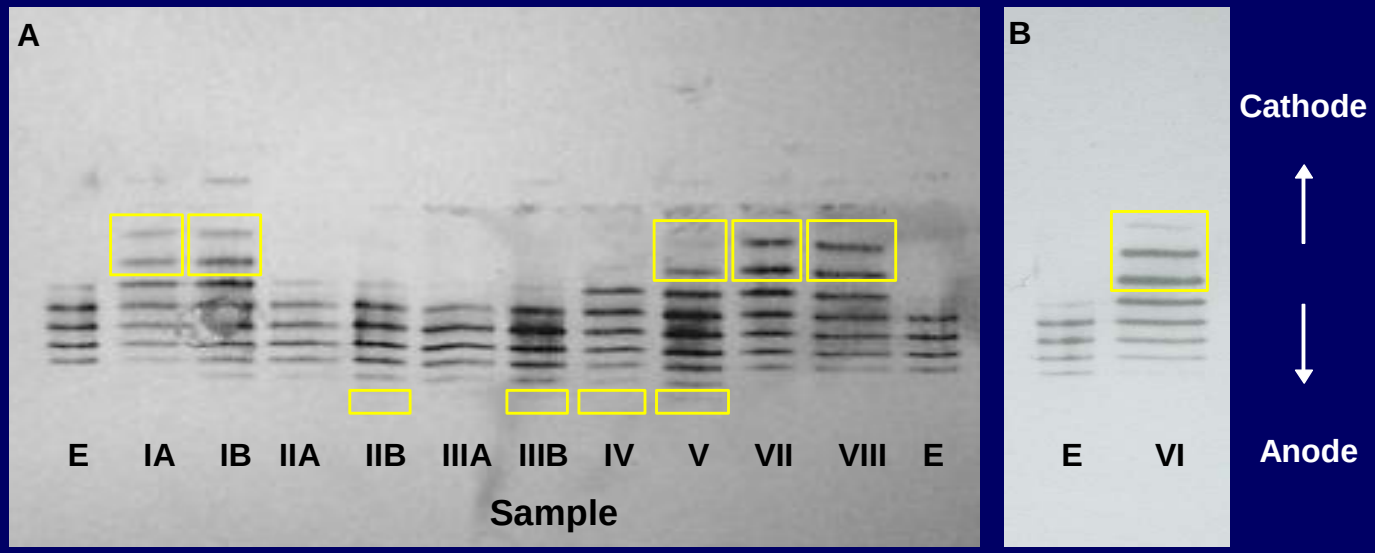
Epo: isoform distribution (IEF) of epo products

Isoform distribution



Isoform patterns: deviations displayed by 9 of the 11 samples (including additional basic and acidic isoforms, and increased bar intensity) compared with the EPREX® standard (E)

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Stability issues

Chemical instability

- Disulfide Exchange
- Deamidation
- Oxidation
- Proteolysis

Physical instability

- Denaturation
- Aggregation
- Precipitation
- Adsorption

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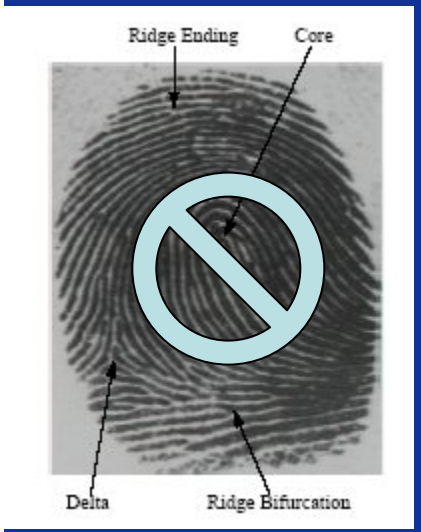
Bottom line: complete characterization: mission impossible

Table 3
(Analytical) techniques for monitoring protein structure

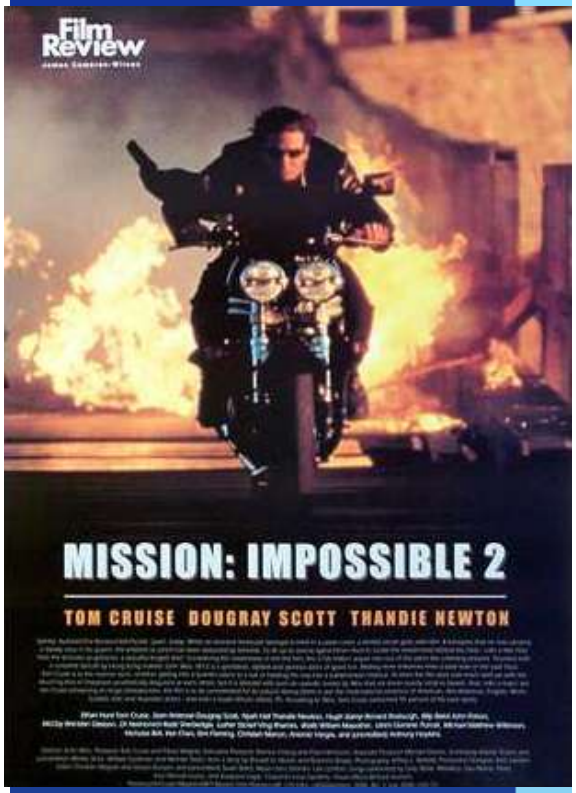
- UV absorption
- Circular dichroism spectroscopy
- Fourier transform IR
- Fluorescence spectroscopy
- NMR spectroscopy
- Calorimetric approaches
- Bio-assays
  - Immunochemical assays
    - ELISA
    - Immunoprecipitation
    - Biosensor (SPR, QCM)
  - Potency testing
    - In cell lines
    - In animals
- Chromatographic techniques
    - RP-HPLC
    - SEC-HPLC
    - Hydrophobic interaction HPLC
    - Ion-exchange HPLC
    - Peptide mapping
- Electrophoretic techniques
    - SDS–PAGE
    - IEF
    - CZE
- Field flow fractionaction
- Ultracentifugation
- Static and dynamic light scattering
- Electron microscopy
- X-ray techniques
- Mass spectrometry

The quality is in the process



International Journal of Pharmaceutics

2003, November, 266, 3-16



Characterization: purification

Biopharmaceutical purification means:

- **The product should only contain the desired protein**
  - **Biopharmaceuticals are produced by living host cells that are also naturally producing many other proteins, as well as sugars, fatty acids, etc**
- **All contaminants need to be excluded**
  - **Any trace of viruses, prions, and endotoxins needs to be removed**

Purification is a complex process critical to the performance of the biopharmaceutical

Crommelin DJA, et al. Int J Pharm 2003;266:3-16.

Characterization: biological activity

- The biological activity (i.e. efficacy and safety) of a biopharmaceutical depends on:
  - 3D structure
  - degree of glycosylation and location of glycosylation sites
  - isoform profile
- These characteristics are unlikely (for the larger proteins) to be the same for a biosimilar and the original biopharmaceutical

It is highly unlikely that a biosimilar is identical to the original biopharmaceutical

Crommelin DJA, et al. Int J Pharm 2003;266:3-16.

Potency tests……

European Pharmacopoeia

- **Insulin** — **no in vivo potency**
- **Human growth hormone** — **no in vivo potentcy**

USP

- **Insulin** — **in vivo potency**
- **Human growth hormone** — **in vivo potency**

Changing a formulation…….

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

PRCA = pure red cell aplasia…… by immunogenic epo

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Biopharmaceutical development

Species specificity…..

- Activity non-species specific….human insulin, human growth hormone, erythropoietins, G-CSF, number of enzymes
- Activity species specific… interferons, monoclonal antibodies, GM-CSF

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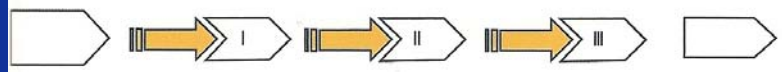
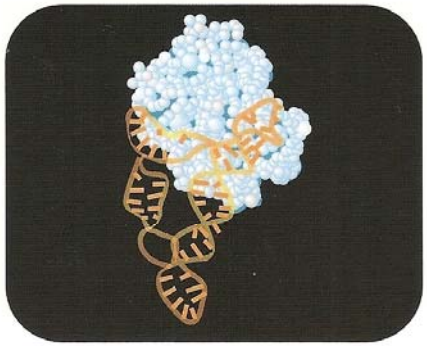
Afterword by Anthony D. Dayan



PRECLINICAL SAFETY EVALUATION OF BIOPHARMACEUTICALS

A Science-Based Approach to Facilitating Clinical Trials



Edited by

Joy A. Cavagnaro

WILEY

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TABLE 7.4 Preclinical support to phase 1

		Drug				Biologic		
Phase 1		Time (Mo)	Material (g)	Cost ($K)		Time (Mo)	Material (g)	Cost ($K)
Assay validation	PK (LC/MS/MS)	4	1	15	PK (ELISA)	10	1	30
	PD (LBI; FACS)	6		50	PD (LBI; FACS)	6		50
					Immunogenicity (ELISA)	10		30
					Tissue cross-reactivity (IHC)	2		20
Cross-reactivity	Receptor screening	2	1	15	Tissue cross-reactivity	2	1	80
Genetic toxicology	Mutagenicity (Ames)	2.5	2	6				
	Chromosomal aberration in vitro (CHO)	2.5	2	22				
	Chromosomal aberration in vivo (RMN)	2.5	2	30				
Safety pharmacology	Cardiovascular, monkey	2.5	25	150				
	Respiratory, rat	2.5	5	38				
	CNS, rat	2.5	5	20				
General toxicology	1 mo (+2 wk rec) rat	4	250	200	Single-dose (+1 mo rec) monkey	4	3	150
	1 mo (+2 wk rec) monkey	4	1000	600	1 mo (+2 mo rec) monkey	7	30	600
Studies		10	—	1146		17	—	960
Material		2	**1293**	40		6	**35**	1600
Total		**10**	—	**1186**		**17**	—	**2560**

Note: CHO = Chinese hamster ovary cells, ELISA = enzyme-linked immunosorbent assay, FACS = fluorescent-activated cell sorting or flow cytometry; IHC = immunohistochemistry, LBI = ligand binding inhibition, rec = recovery, RMN = rat micronucleus.

TABLE 7.5 Preclinical support to phase 2

Phase 2		Drug				Biologic		
		Time (Mo)	Material (g)	Cost ($K)		Time (Mo)	Material (g)	Cost ($K)
General toxicology	3 mo (+1 mo rec) rat	7	700	253				
	3 mo (+1 mo rec) monkey	7	3000	650	3 mo (+2 mo rec) monkey	9	100	650
Reproductive and developmental toxicology	Segment 2 RF, rat	2.5	40	43				
	Segment 2 RF, rabbit	2.5	500	57				
	Segment 2, rat	3.5	100	128				
	Segment 2, rabbit	3.5	500	185	Segment 2, monkey (1 mo)	12	50	950
	Segment 1, rat, male	6	50	193	Segment 1, monkey, male (3 mo)	12	120	710
	Segment 1, rat, female	6	50	119	Segment 1, monkey, female (3 mo)	12	120	742
Studies		7	—	1628		12	—	3052
Material		2	**4940**	125		5	**390**	1200
Total		**9**	—	**1753**		**17**	—	**4252**

Note: Rec = recovery, RF = range-finder.

TABLE 7.6 Preclinical support to phase 3 and registration

Phase 3		Drug Time (Mo)	Drug Material (g)	Drug Cost ($K)		Biologic Time (Mo)	Biologic Material (g)	Biologic Cost ($K)
Reproductive and developmental toxicology	Segment 3, rat	9	250	350	Segment 3, monkey (3 mo rec + 3 mo)	18	120	1500
Chronic toxicology	6 mo (+2 mo rec) rat	11	1250	355				
	12 mo (+2 mo rec) monkey	20	3600	770	9 mo (+3 mo rec) monkey	16	400	750
Studies		20	—	1475		18	—	2250
material		2.5	**5100**	125		5	**520**	1800
Total		22.5	—	**1600**		**23**	—	**4050**
Registration								
Carcinogenicity	2 yr rat	36	3000	1700				
	5 d/1 mo RF, Tg mouse	4	10	225				
	6 mo Tg mouse	18	15	900				
Studies		36	—	2825		18	—	2250
Material		2	**3025**	75		5	**520**	1800
Total		38		**2900**		**23**	—	**4050**

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CHAPTER 9

Selection of Relevant Species

MEENA SUBRAMANYAM, PhD, NICOLA RINALDI, PhD,
ELISABETH MERTSCHING, PhD, and DAVID HUTTO, PhD, DVM

Contents

9.1 INTRODUCTION

The goal of biopharmaceutical development is to maximize therapeutic benefit while minimizing the risk of treatment-related toxicity. To mimic putative interpatient treatment differences in test article responsiveness, it is important

Preclinical Safety Evaluation of Biopharmaceuticals: A Science-Based Approach to Facilitating Clinical Trials, edited by Joy A. Cavagnaro

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TABLE 9.2 Cross-species tissue cross-reactivity study of a monoclonal antibody

Tissue	Cell Type	Human	Cynomolgus Monkey	Mouse
Urinary bladder	Urothelium	2 (3–4)	3 (3)	NS
Ureter	Urothelium	2 (3–4)	3 (2–4)	NA
Tonsil	Mucosal epithelium	3 (2–3)	3 (2–3)	NA
Uterus-cervix	Mucosa	3 (1–3)	1 (2)	NA
Eye	Corneal epithelium	1 (1)	NS	1 (1)[a]
Breast	Glandular epithelium	3 (1–3)	3 (1–3)	NS
Fallopian tube	Tubular epithelium	2 (1–2)	1 (3)	NS
Kidney	Tubular epithelium–cortex	3 (3)	1 (3)[b]	3 (1–2)
Lung	Alveolar epithelium	3 (2–3)	2 (3)	NS
Pancreas	Ductular epithelium	3 (2)	2 (1–2)	2 (1)
Prostate	Glandular epithelium	2 (1–2)	1 (2)	3 (2)[c]
Thyroid	Follicular epithelium	3 (2–3)	2 (2)	NS
Uterus-endometrium	Endometrial mucosa	2 (2)	NS	3 (1–3)[d]
Adrenal	Cortical epithelium	3 (1–2)	2 (1)	NS
Pituitary	Adenohypophysis epithelium	3 (2)	NS	NS
Liver	Sinusoidal mesenchymal cells	3 (2–4)	3 (2–3)	NS

Immunogenicity of Biopharmaceuticals

Huub Schellekens, Daan Crommelin* and Wim Jiskoot

Dept. Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, *UIPS*

Scientific Director of the Dutch Top Institute Pharma*, Leiden

Co-founder of OctoPlus, Leiden*


Immunogenicity for Biologics 2006
Conference: 25th & 26th January 2006
Comparability and Immunogenicity of Biologicals
Europe's leading event on technological, clinical and regulatory issues
Papers are now available for this past event, which took place: 14 -
June 2005, Hilton Vienna Danube, Vienna, Austria
East Coast
Immunogenicity Testing for Biotherapeutics
October 18 - Octo
The Boston Park
MA
Barnett International's 3rd Annual
IMMUNOGENICITY TESTING FOR THERAPEUTICS
Strategies for Development of Immun
Regulatory Expectations and
September 29-30
BARNETT INTERNATIONAL'S ASSESSING THE IMPACT OF
PROTEIN AGGREGATION
Consequences of Aggregation
Immunology, Quali
June
Pre-conference Workshop • Tuesday 24th January 2006
ection and Characterisation of Immunogenicity of Therapeutic
"The induction of
neutralizing antibodies
can have a dramatic
effect on the potency
xt-Generation
ein Therapeutics
PRE-CONFERENCE SEMINAR:
Introduction to Immunogenicity
7 November 2005,
Radisson SAS Basel, Switzerland
09.00 Registration and Morning Coffee
09.30 Chairman's Opening Remarks
Regulatory Perspective
09.40 Antibodies Against Biotech Drugs: Cli
Regulatory Issues

Selected papers (2004) on the immunogenicity of recombinant human interferon beta

Antonio Bertolotto
Florian Deisenhammer
Paolo Gallo
Per Sölberg Sørensen

Immunogenicity of interferon beta: differences among products

Neutralizing antibodies reduce the efficacy of βIFN during treatment of multiple sclerosis

S. Malucchi, MD; A. Sala, PhD; F. Gilli, PhD; R. Bottero, MD; A. Di Sapio, MD; M. Capobianco, MD; and A. Bertolotto, MD

Neutralizing antibodies against IFN-β in multiple sclerosis: antagonization of IFN-β mediated suppression of MMPs

Francesca Gilli,[1] Antonio Bertolotto,[1] Arianna Sala,[1] Francine Hoffmann,[2] Marco Capobianco,[1] Simona Malucchi,[1] Tracy Glass,[2] Ludwig Kappos,[3] Raija L.P. Lindberg[2,*] and David Leppert[2,3,*]

Hans-Peter Hartung
Huub Schellekens
Frederick E. Munschauer III

Neutralizing antibodies to interferon beta in patients with multiple sclerosis: scientific background and clinical implications

In the EU ca. 100 million euro/year is spent on useless IFN-β therapy

History of the medical use of 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

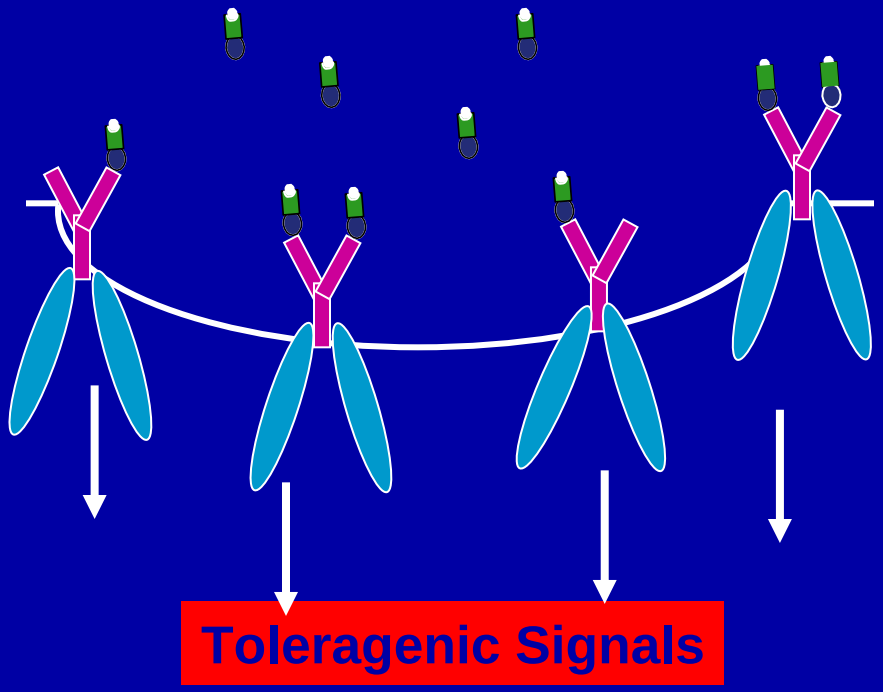
Types of immune reaction against biopharmaceuticals

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

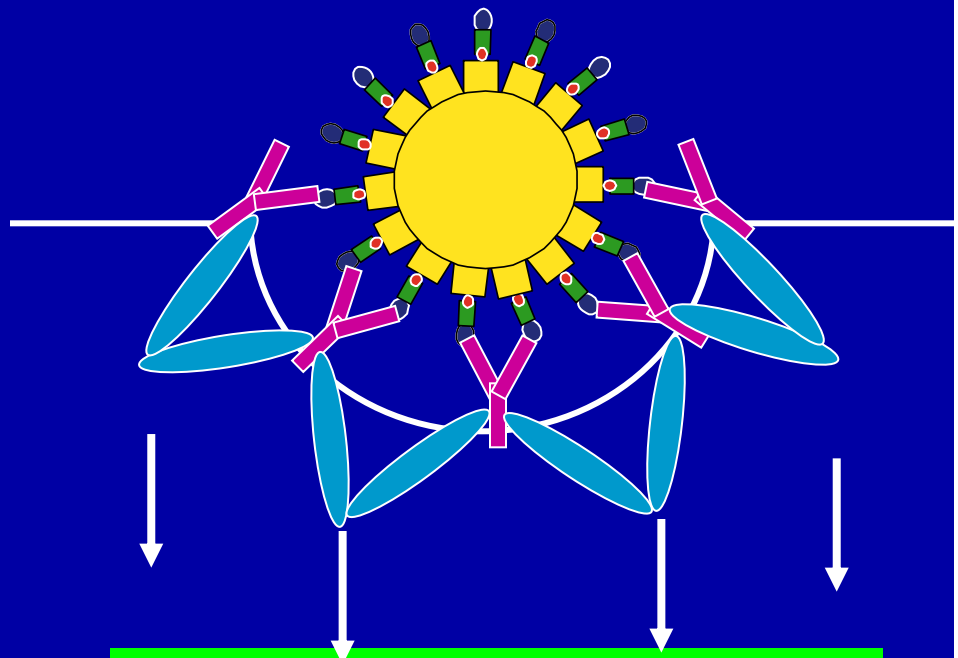
Fate of Auto-Reactive B Cells After Encountering Self-Antigen (complexes)

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?

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

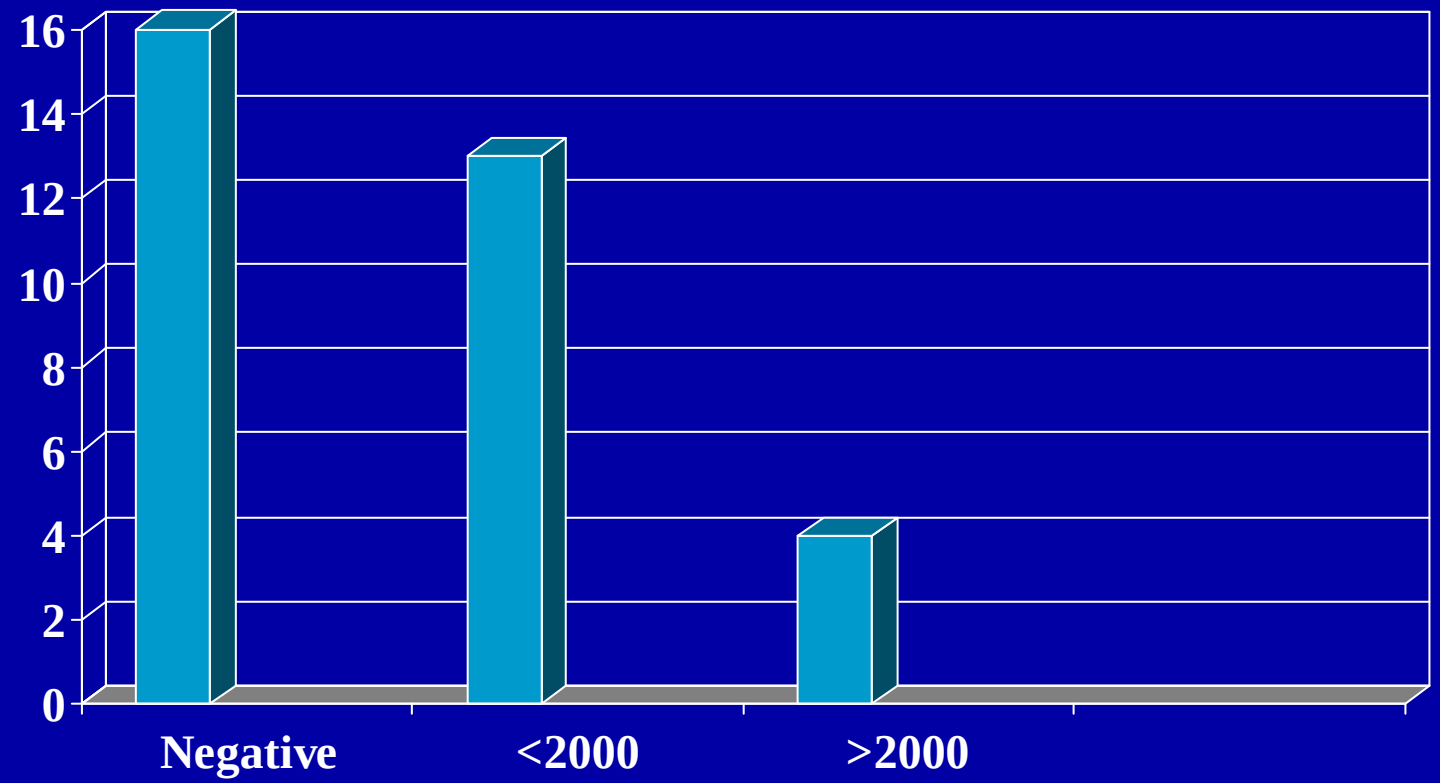
General immune effects

Allergy

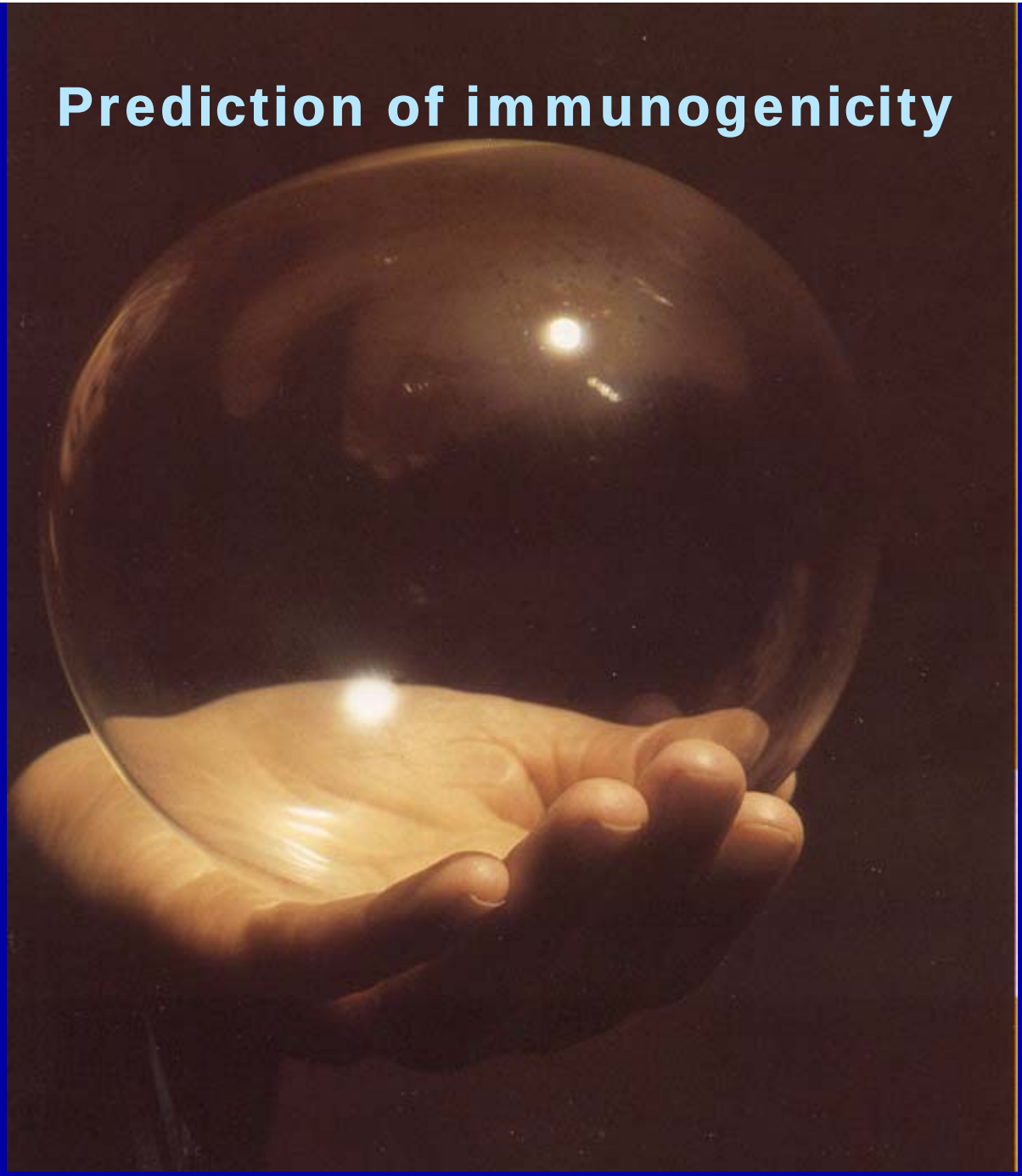
Anaphylaxis

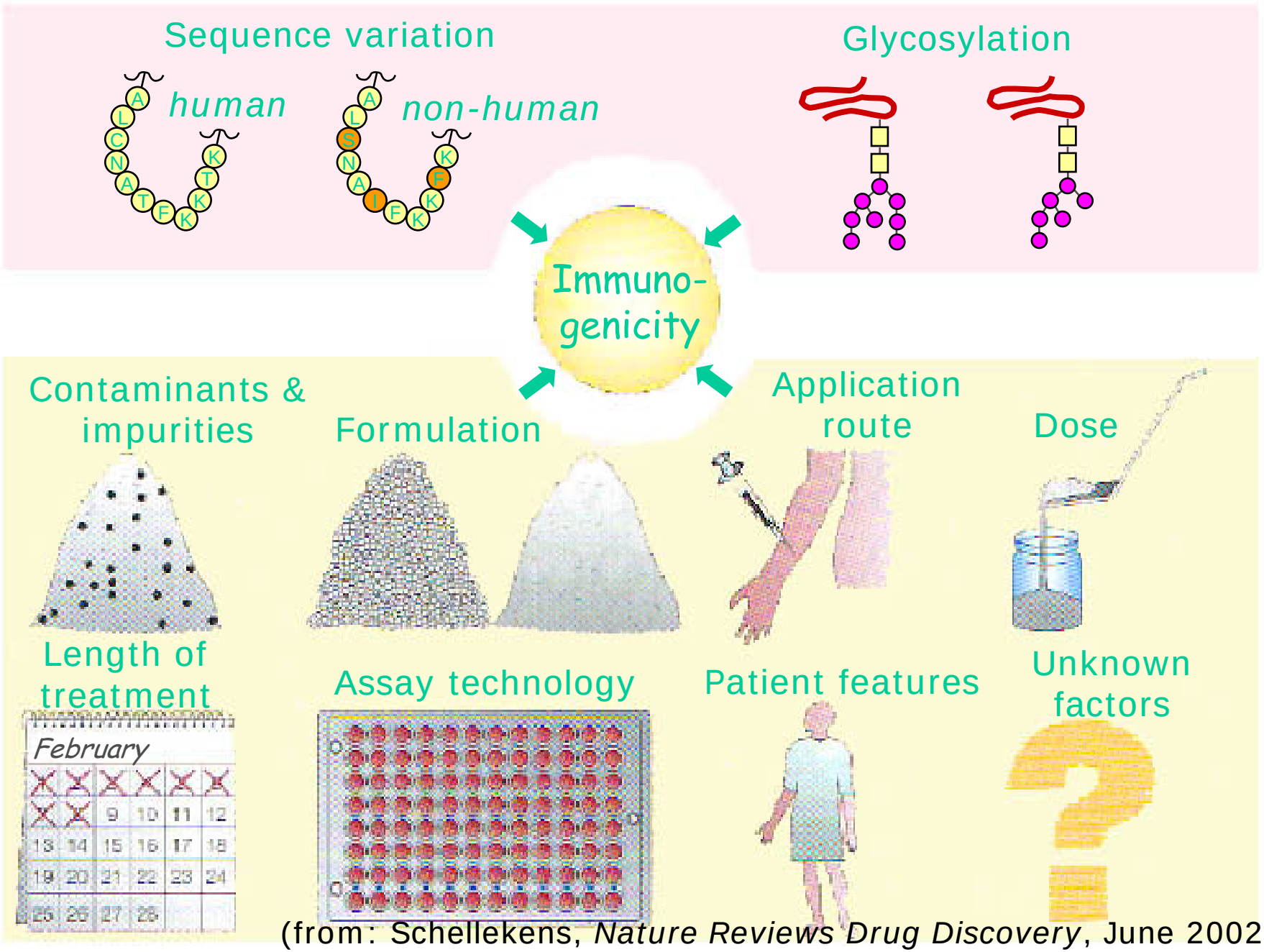
Serum sickness, etc

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



Prediction of immunogenicity





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

Prediction of immunogenicity

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

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

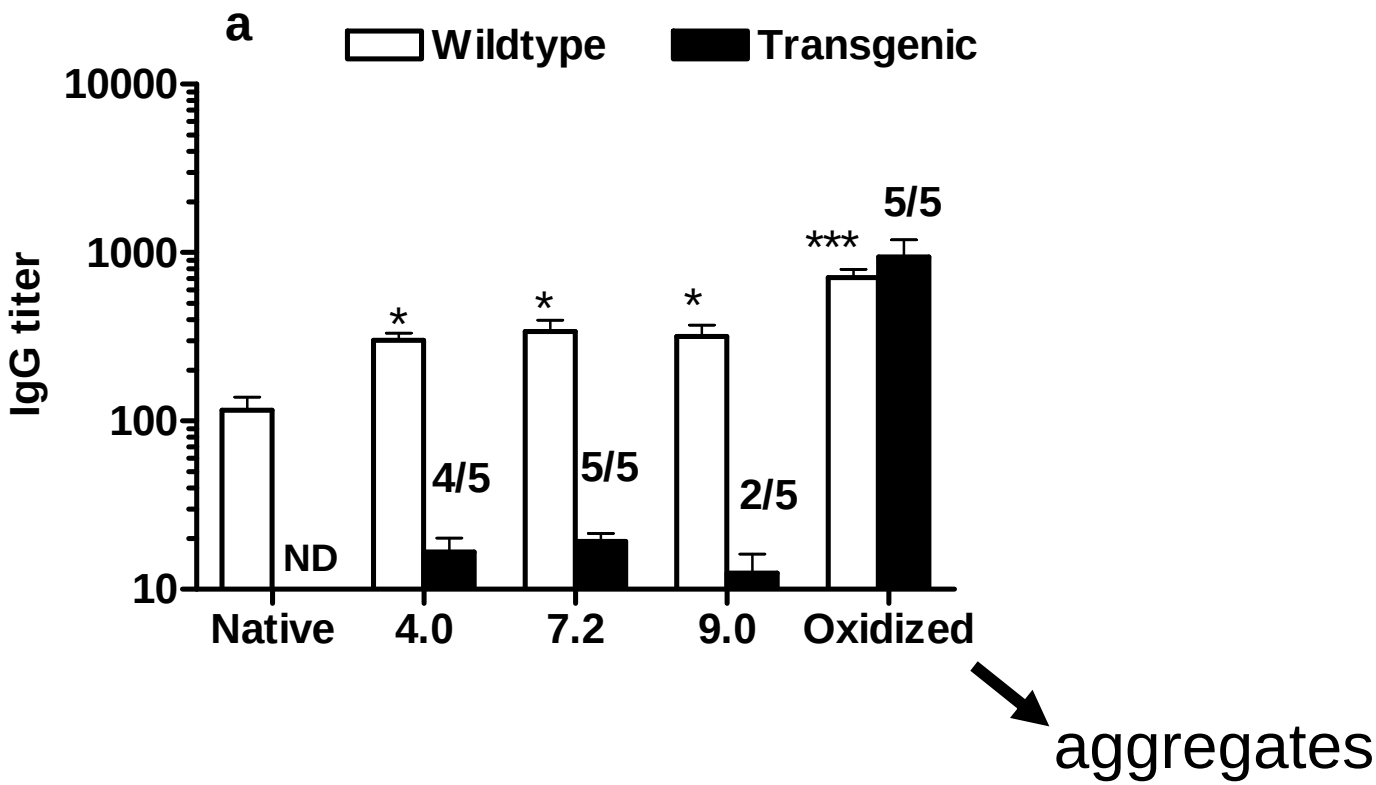
The Hu IFN alpha 2 transgenics

Hermeling, Crommelin, Jiskoot, Schellekens

Hu IFN alpha 2 immune tolerant mice

- Current use: to study immunogenicity of modified Hu IFN alpha 2b preparations
- Obtained from Roche

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 (<1 µg)
  - Aggregates should be 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

EU: Innovative Medicines Initiative

- EU Commission and EFPIA have established a Strategic Research Agenda (SRA), the 'roadmap' for the Innovative Medicines Initiative (IMI) – FP7

- Recommendations to address the key 'bottlenecks' in four areas of the biomedical R&D process:
  - (Predicting) drug safety
  - (Predicting) drug efficacy
  - Knowledge management
  - Education and Training

- Call for project proposals came out April 2008

- Inmmunogenicity of biopharmaceuticals on top…..

- More info: www.imi-europe.org


IMI | The Innovative Medicines Initiative - Windows Internet Explorer
http://www.imi-europe.org/
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Welcome to Innovative Medicines Initiative Online
The Innovative Medicines Initiative is a proposed partnership between the European Commission and the European Federation of Pharmaceutical Industry and Associations (EFPIA).
For more, please take a look at the new IMI Flyer or at the new IMI Overview Presentation
More...
IMI Objectives
The vision of IMI is to create Biomedical Research & Development leadership for Europe to benefit patients and society.
More...
Why IMI Matters to You
IMI will drive the creation of a vibrant and dynamic scientific environment and ensure a strong European biomedical science base.
More...
Done
Internet
100%
start
Postvak IN - Microsof...
IMI | The Innovative ...
GMD 2007
Microsoft Excel
Microsoft PowerPoint ...
16:58

Present Arsenal

Examples of the types of product on the market:

- Homones, growth factors, enzymes
  - Fertility hormones
  - Human insulin
  - Enzymes
  - Human growth factors (G-CSF, haematopoietic growth factors)
- Cytokines
  - Interleukins
  - Interferons
- Vaccines & antigens
  - Hepatitis B antigen
  - Cholera vaccine
- Antisense
  - Fomivirsen
- Cell therapy
  - Carticel, Epicel

Endogenous products

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Welcome to the kingdom of the needle?

- Are we stuck to the needle?

Get rid of the protein…..
Insulin-alternatives:
small is beautiful…

- Vaccines
  - Diamyd, Diapep277
- Thiazolinedione-derivatives
  - PPAR agonists e.g. netoglitazone, balaglitazone, rosiglitazone
- DPP4- inhibitors,
  - e.g. sitagliptine, vildagliptin, saxaglitptin, alogliptine
- GLP-1 analogues
  - e.g., liraglutide
- Metaglidasen
- Succinobucol
- Managlinat dialanetil
- Solabegron
- BGP15

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Further paradigm shifts at the horizon

- New production approaches
  - Transgenic animals, transgenic plants
- siRNA, gene therapy
- Stem cell therapy
- Modified proteins
  - IgG fragments
  - Fusion proteins

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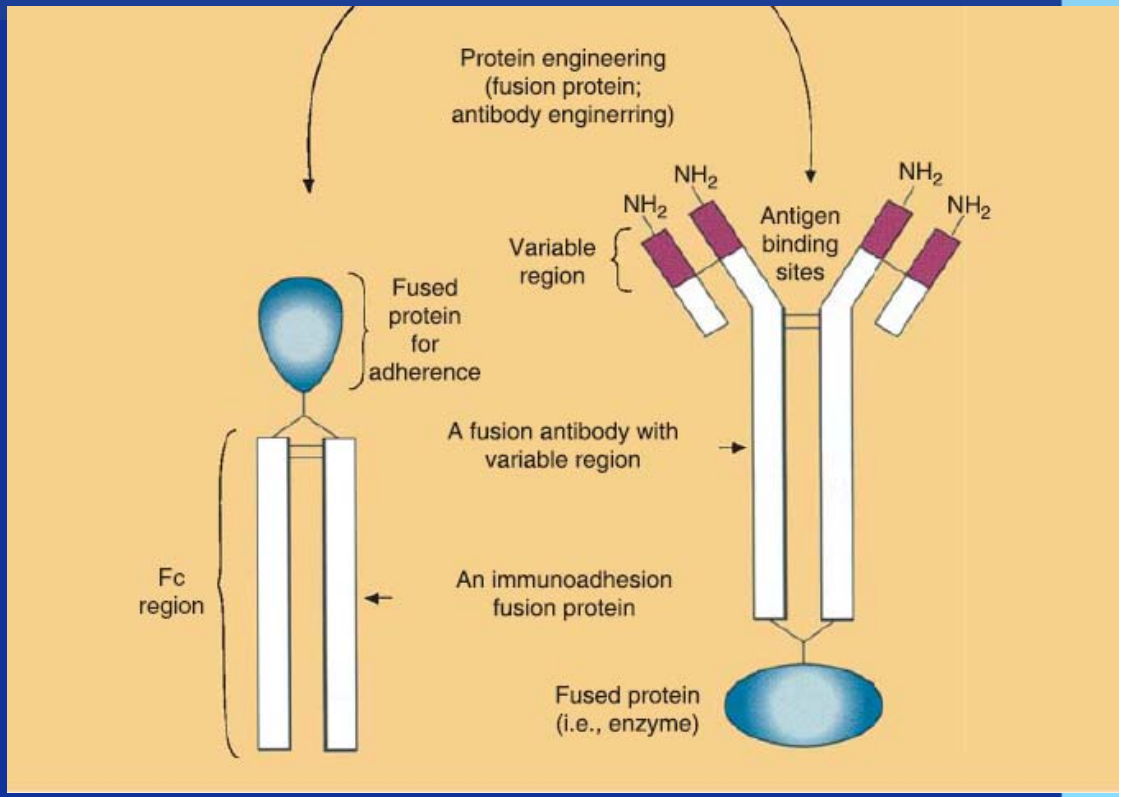
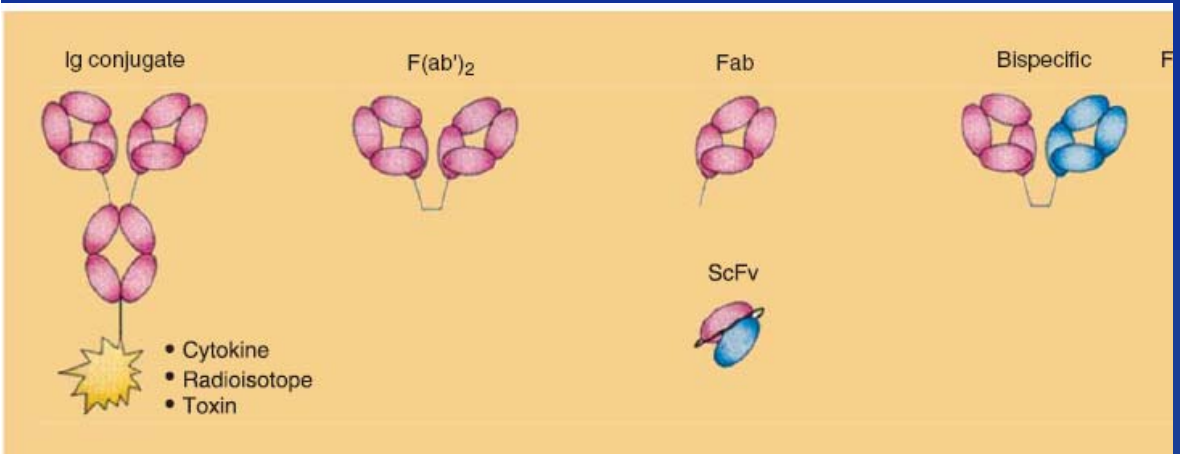
Modified proteins

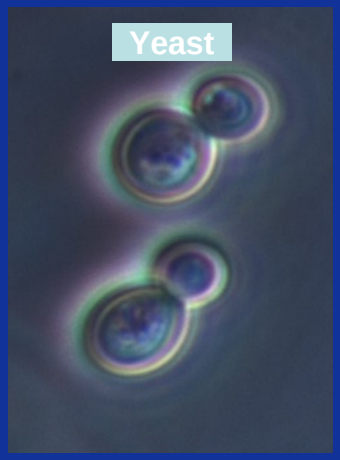
Challenging Mother nature



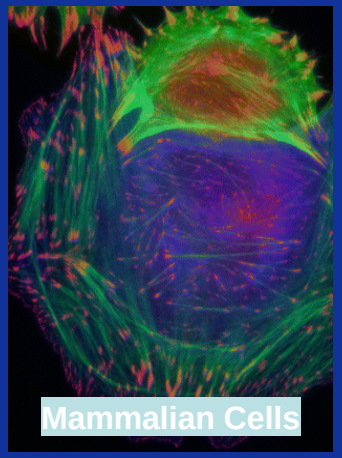


Five expression technologies for protein production

Sheep, goat, cow



Saccharomyces



CHO

Tobacco, moss

Escherichia coli

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knaeplein

BIOLEX

FOR IMMEDIATE RELEASE
February 16, 2005

BIOLEX AND OCTOPLUS ANNOUNCE JOINT DEVELOPMENT OF LOCTERON™: A NOVEL, CONTROLLED RELEASE FORMULATION OF ALFA INTERFERON

Clinical Trials to Commence in 2005

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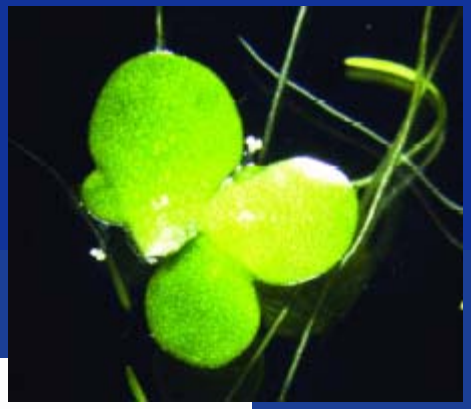
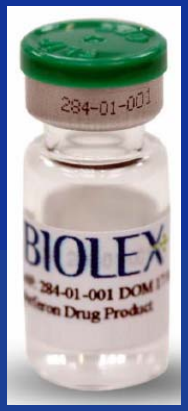
Biolex Interferon alpha

- Biolex' LEX™ system
  - Aquatic higher plant, Lemna
  - Secretes recombinant protein (e.g. IFNa2b)
  - Fast, inexpensive process
  - High expression levels
  - Highly scalable





www.biolex.com

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Immunogenicity in humans of an edible vaccine for hepatitis B, Y. Thanavala et al. (2005), PNAS, 102, 3379-82

A double-blind placebo-controlled clinical trial evaluated the immunogenicity of hepatitis B surface antigen (HBsAg) expressed in *potatoes* and delivered orally to previously vaccinated individuals. The potatoes accumulated HBsAg at 8.5 gg of potato tuber, and doses of 100 g of tuber were administered by ingestion. The correlate of protection for hepatitis B virus, a nonenteric pathogen, is blood serum antibody titers against HBsAg. After volunteers ate uncooked potatoes, serum anti-HBsAg titers increased in 10 of 16 volunteers (62.5%) who ate three doses of potatoes; in 9 of 17 volunteers (52.9%) who ate two doses of transgenic potatoes; and in none of the volunteers who ate nontransgenic potatoes. These results were achieved without the coadministration of a mucosal adjuvant or the need for buffering stomach pH. We conclude that a plant-derived orally delivered vaccine for prevention of hepatitis B virus should be considered as a viable component of a global immunization program.

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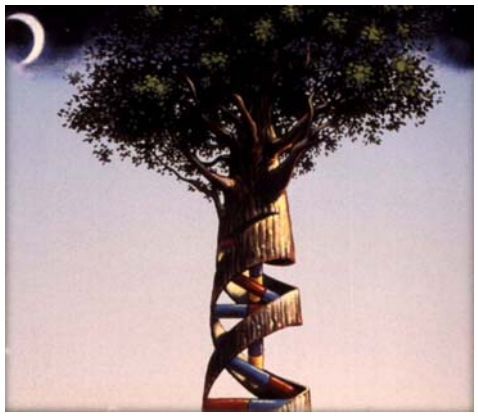
‘Every protein has a life of its own’

(anonymous Ph.D. student)



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