Biopharmaceuticals and Shifting Paradigms

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European Association of Hospital Pharmacists

Statement of Conflict

- See CV
- Advisory boards of several Biotech Companies
- Advisor to Venture Capital companies
- President of EUFEPS, Chair BPS/FIP

PHARMACEUTICAL BIOTECHNOLOGY

FUNDAMENTALS AND APPLICATIONS

Third Edition

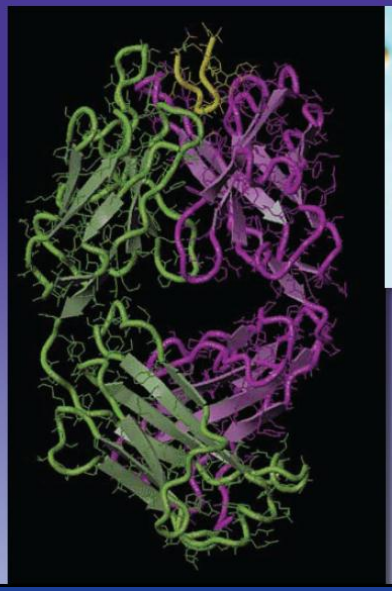
Crommelin
Sindelar
Meibohm

informa healthcare

Third Edition

PHARMACEUTICAL BIOTECHNOLOGY

FUNDAMENTALS AND APPLICATIONS



Edited by

Daan J. A. Crommelin
Robert D. Sindelar
Bernd Meibohm

informa healthcare

Learning Objectives and Outcomes

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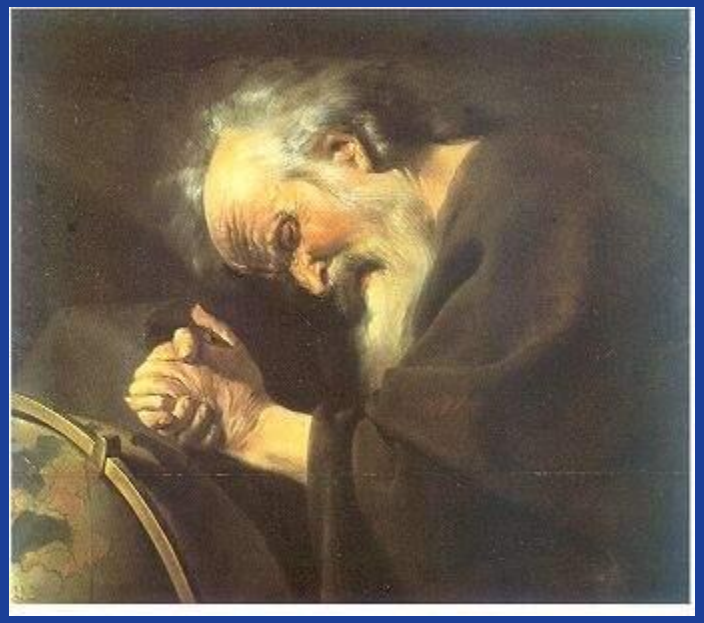


Where biopharmaceuticals differ from low molecular weight drugs

- Molecular weight
- Complexity of structure
- Characterization
  - Structure and physico-chemical properties
  - Protein purity
  - Biological activity
- Stability
- Immunogenicity
- Needle focused

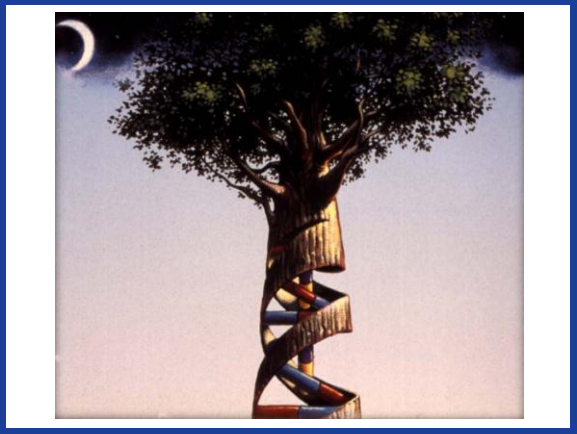
Crommelin DJA, et al. Int J Pharm 2003;266:3-16.

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

‘Every protein has a life of its own’

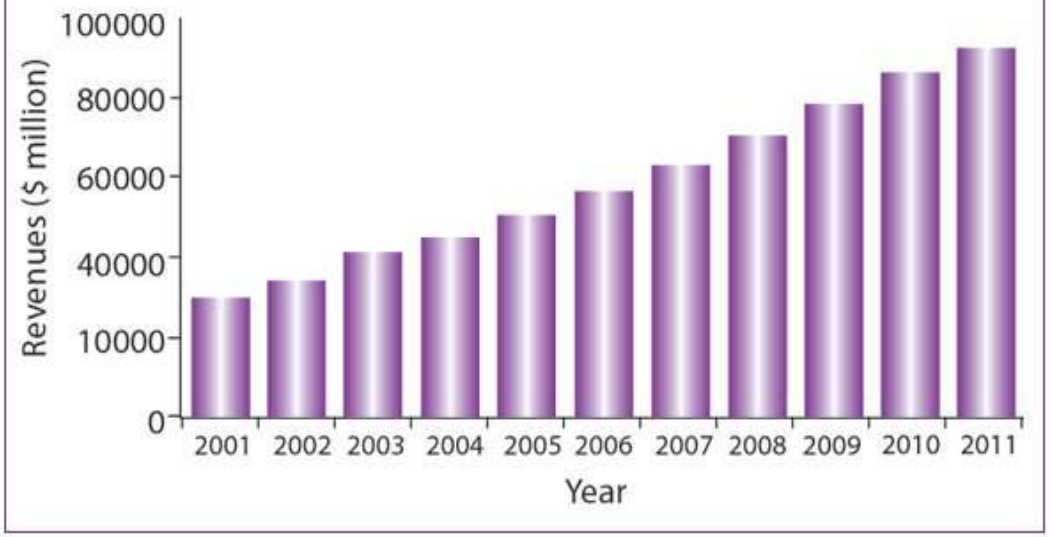
(anonymous Ph.D. student)



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

Figure 1 **Global biopharmaceuticals market revenue forecast, 2001–2011.**



Biopharmaceuticals will outperform the total pharmaceutical market

- With over ***1/3 of ALL pipeline products*** the market forecast is US$ 92 billion in 2011

Knaeplein, 2007

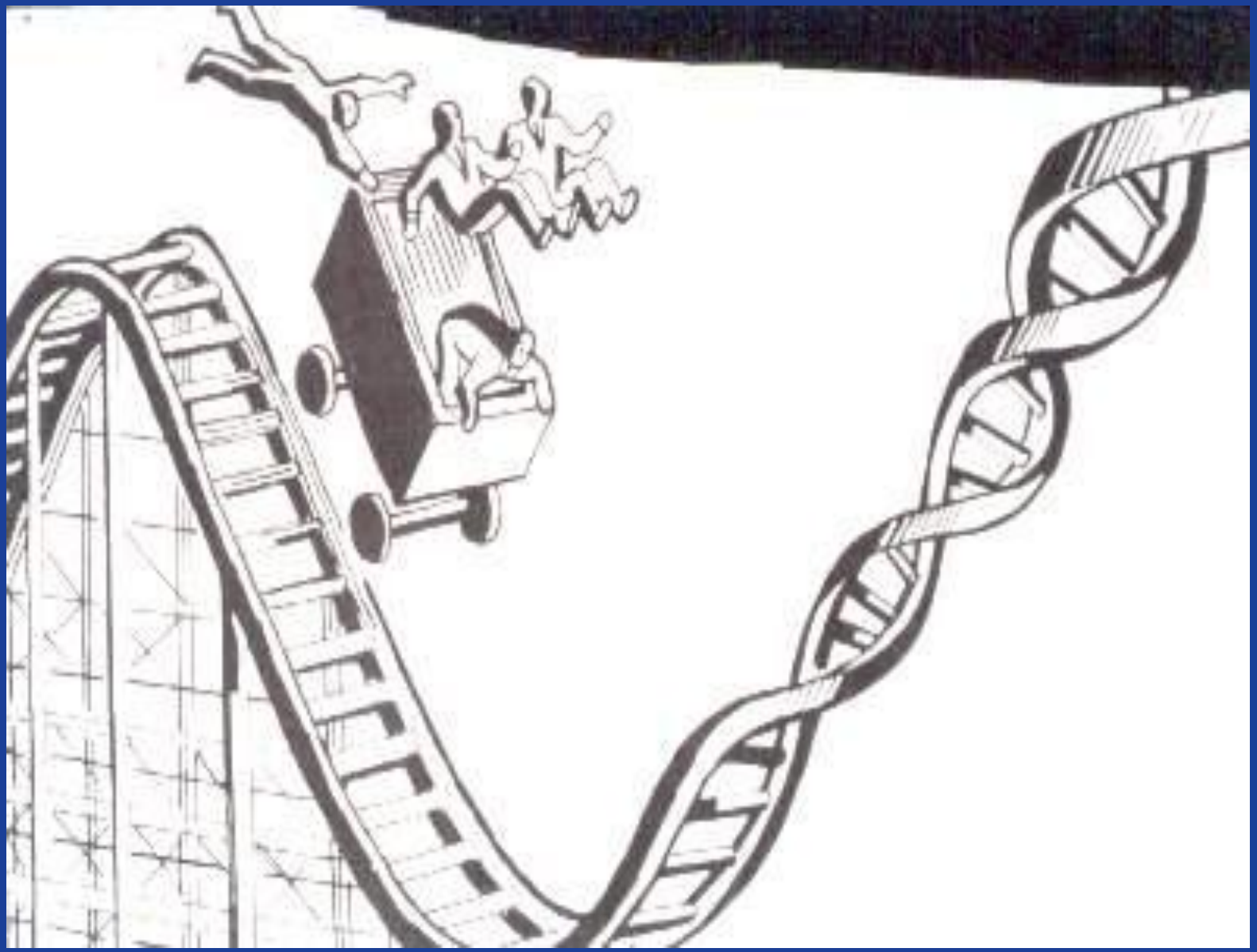
…Biopharma in Perspective

- 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

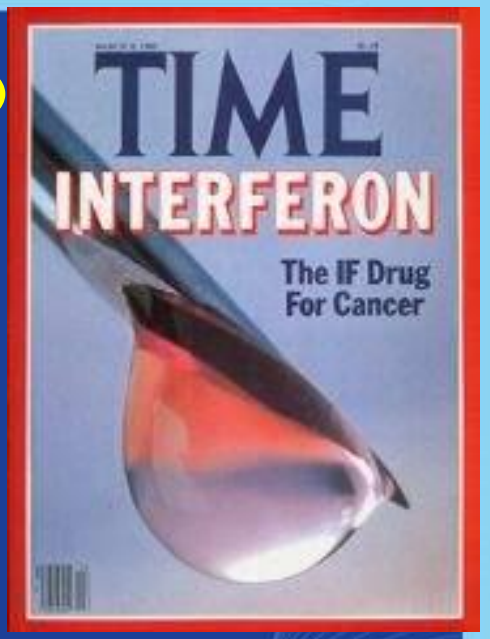
Courtesy Özceada



Dorr, 2002

Weimar W, Lameijer LD, Eldy 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]

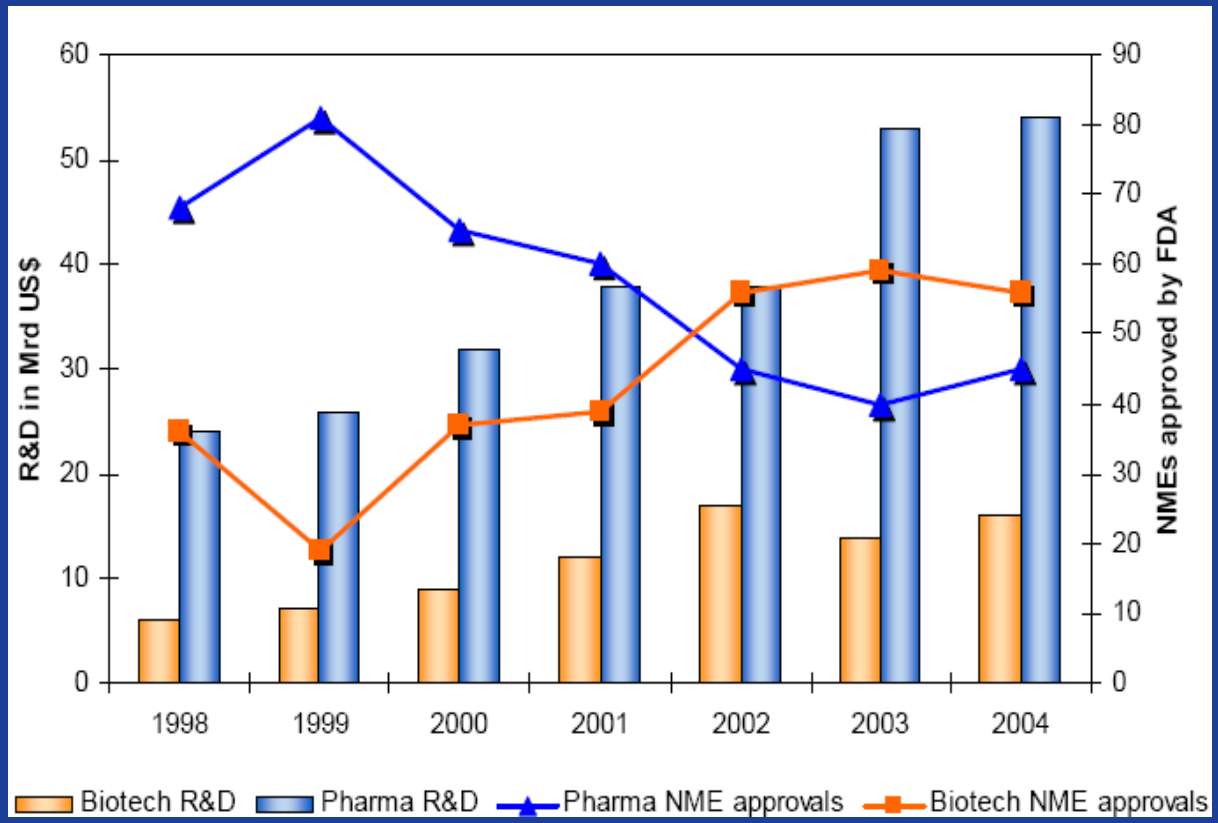


Interferons

Drugs desperately looking for a disease….

5 billion dollars!

The Rise of Biopharma…



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

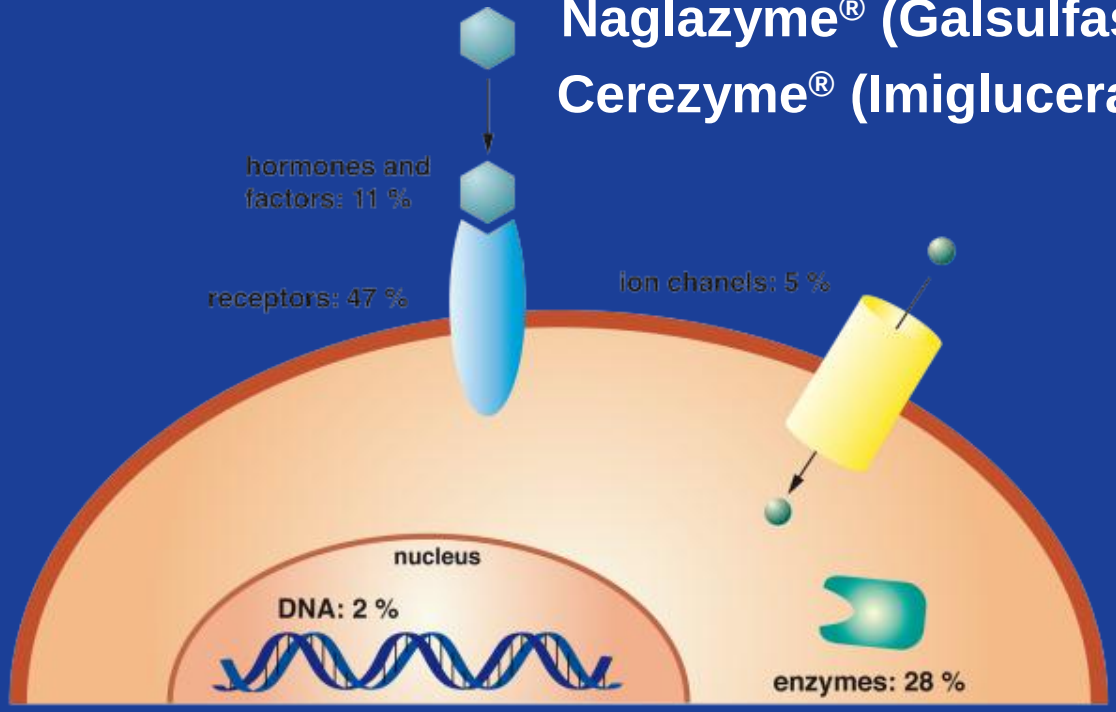
Source: BioGeneriX

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

Number Patients in EU/price in M€ per patient

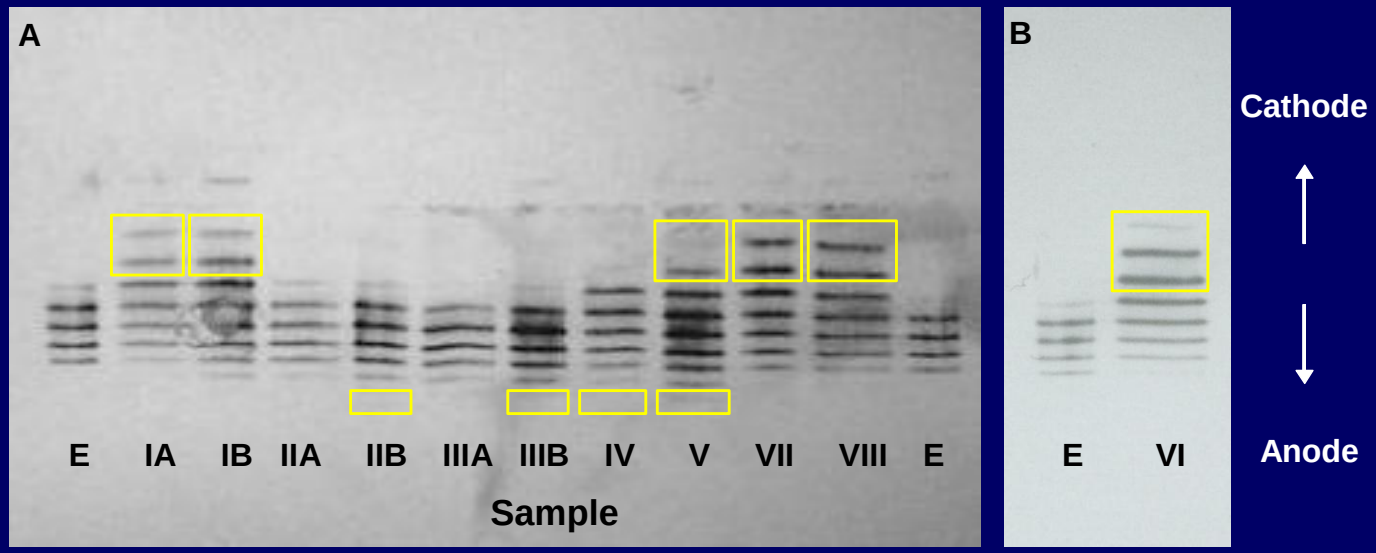
Fabrazyme® (Agalsidase beta) 1,200/0.182
Replagal® (Agalsidase alfa) 1,200/0.189
Aldurazyme® (Laronidase) 1,100/0.473
Myozyme® (Alglucosidase alfa) 4,500/0.351
Elaprase® (Idurosulfatase) 1,000/2.34
Naglazyme® (Galsulfase) 400/1.00
Cerezyme® (Imiglucerase) 27,000/0.476???



Theo Dingermann

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)

Bottom line: complete characterization: mission impossible

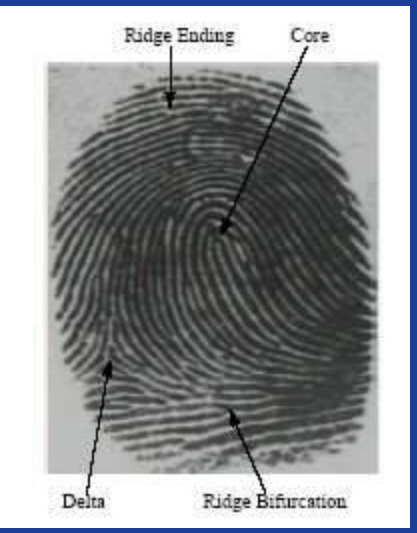
Table 3
(Analytical) techniques for monitoring protein structure

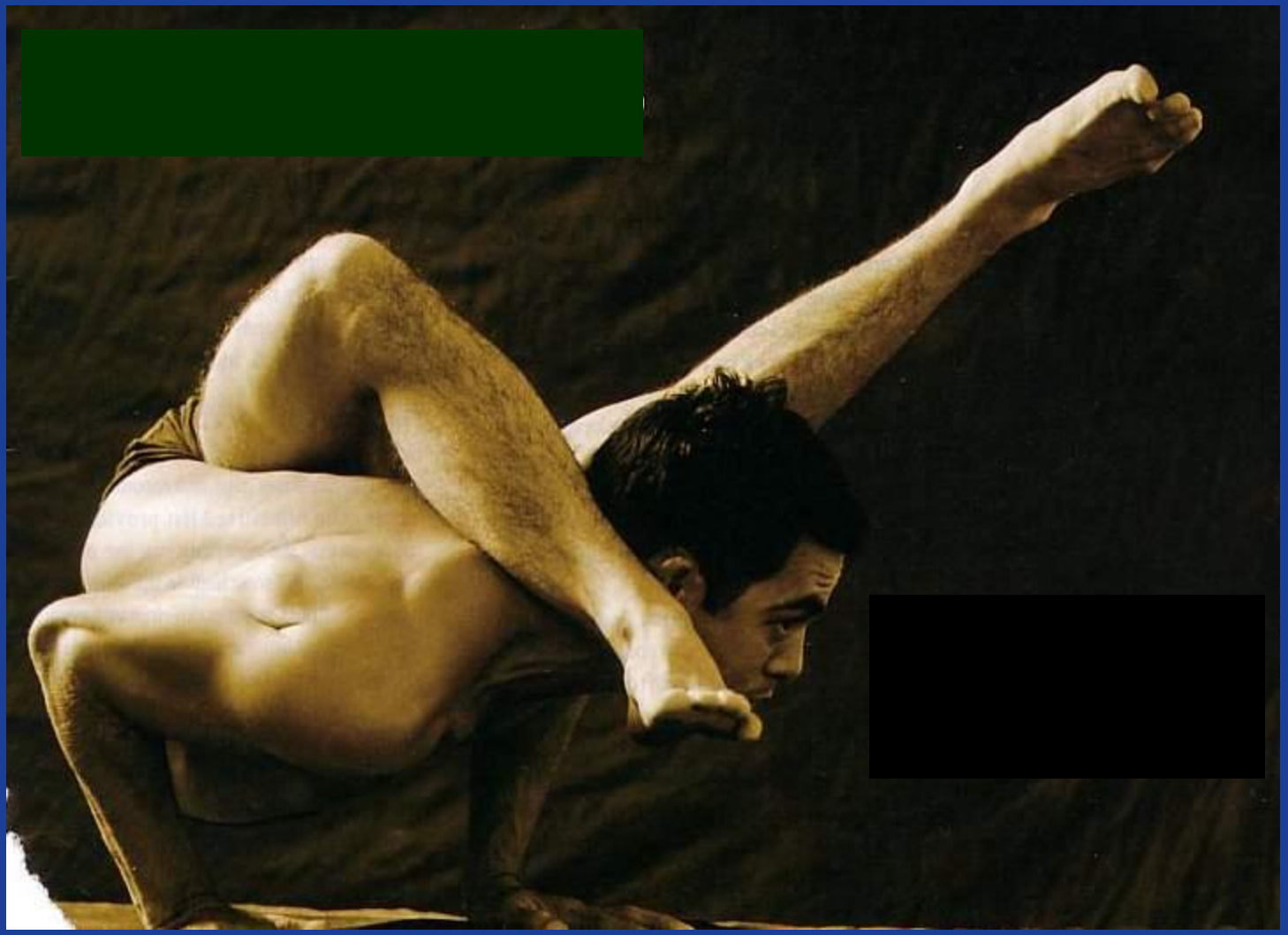
- UV absorption
- Circular dichroism spectroscopy
- Fourier transform IR
- Fluorescence spectroscopy
- NMR spectroscopy
- Calorimetric approaches
- Bio-assays
  - Immunochemical assays
    - ELISA
    - Immunoprecipitation
    - Biosensor (SPR, BCM)
  - Potency testing
    - In cell lines
    - In animals
- 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





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

Antisense oligonucleotides
Mechanism of action

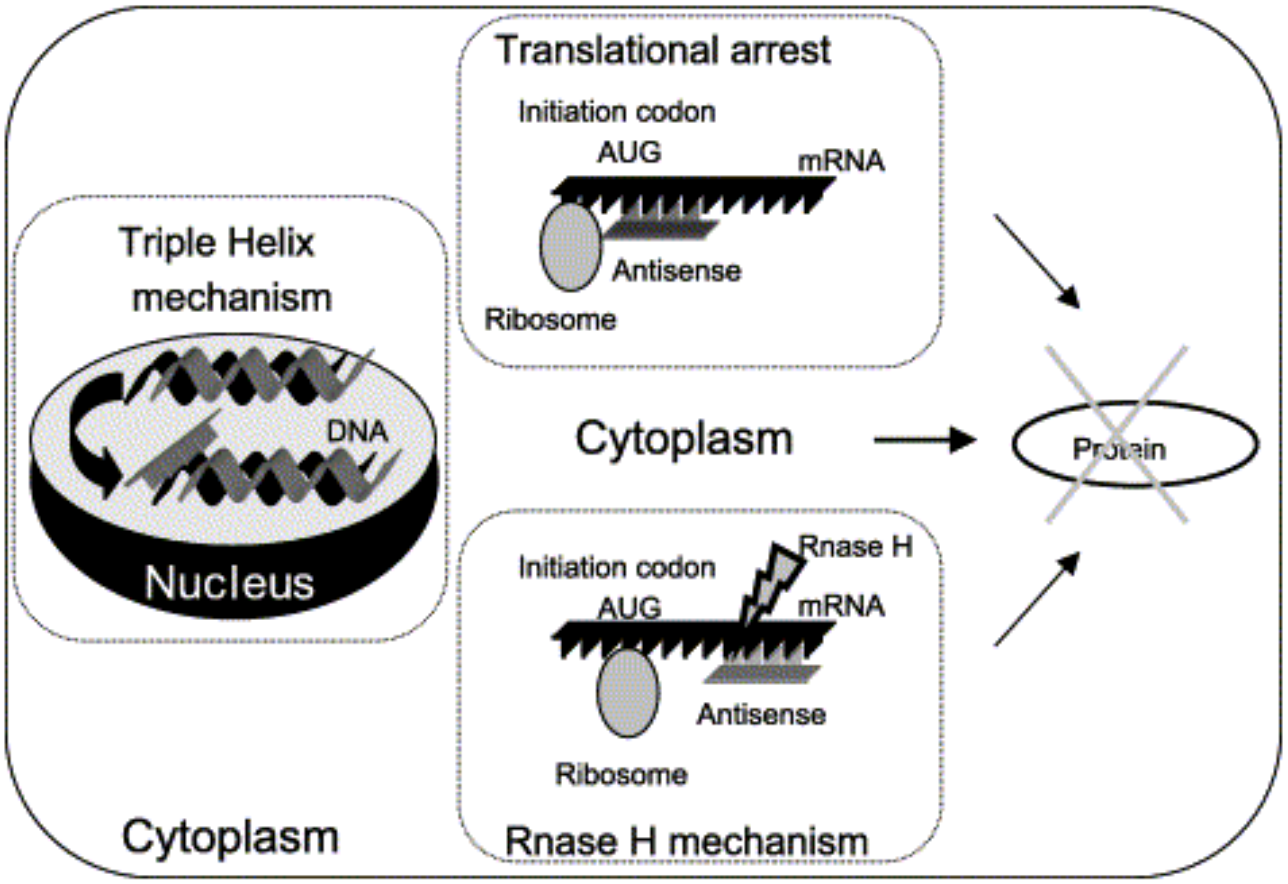
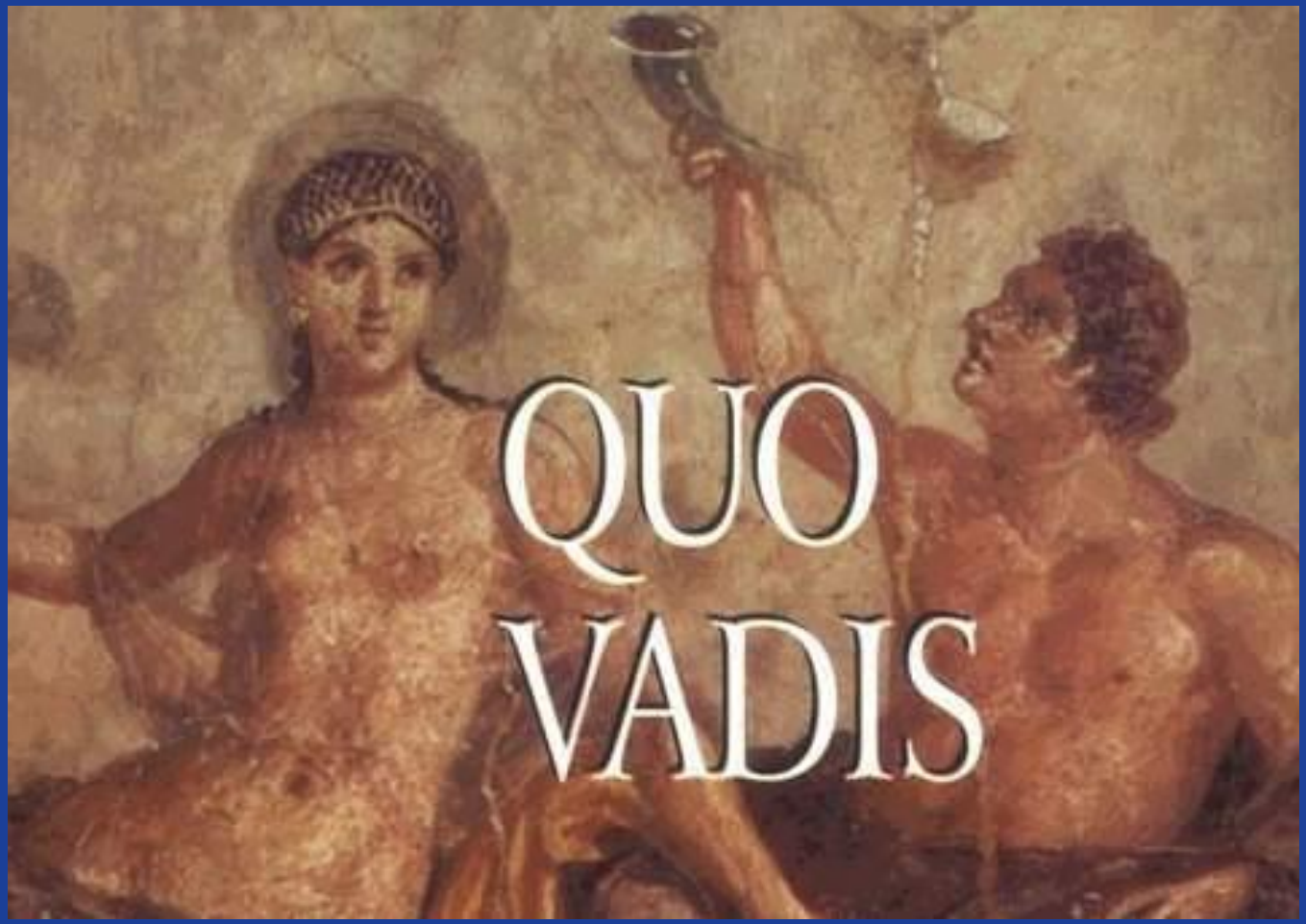


Fig. 1. Main mechanisms of action of antisense oligonucleotides.

Couvreur, Advanced Drug Delivery Reviews
Volume 56, Issue 7, 23 April 2004, Pages 931-946



Henryk Sienkiewicz

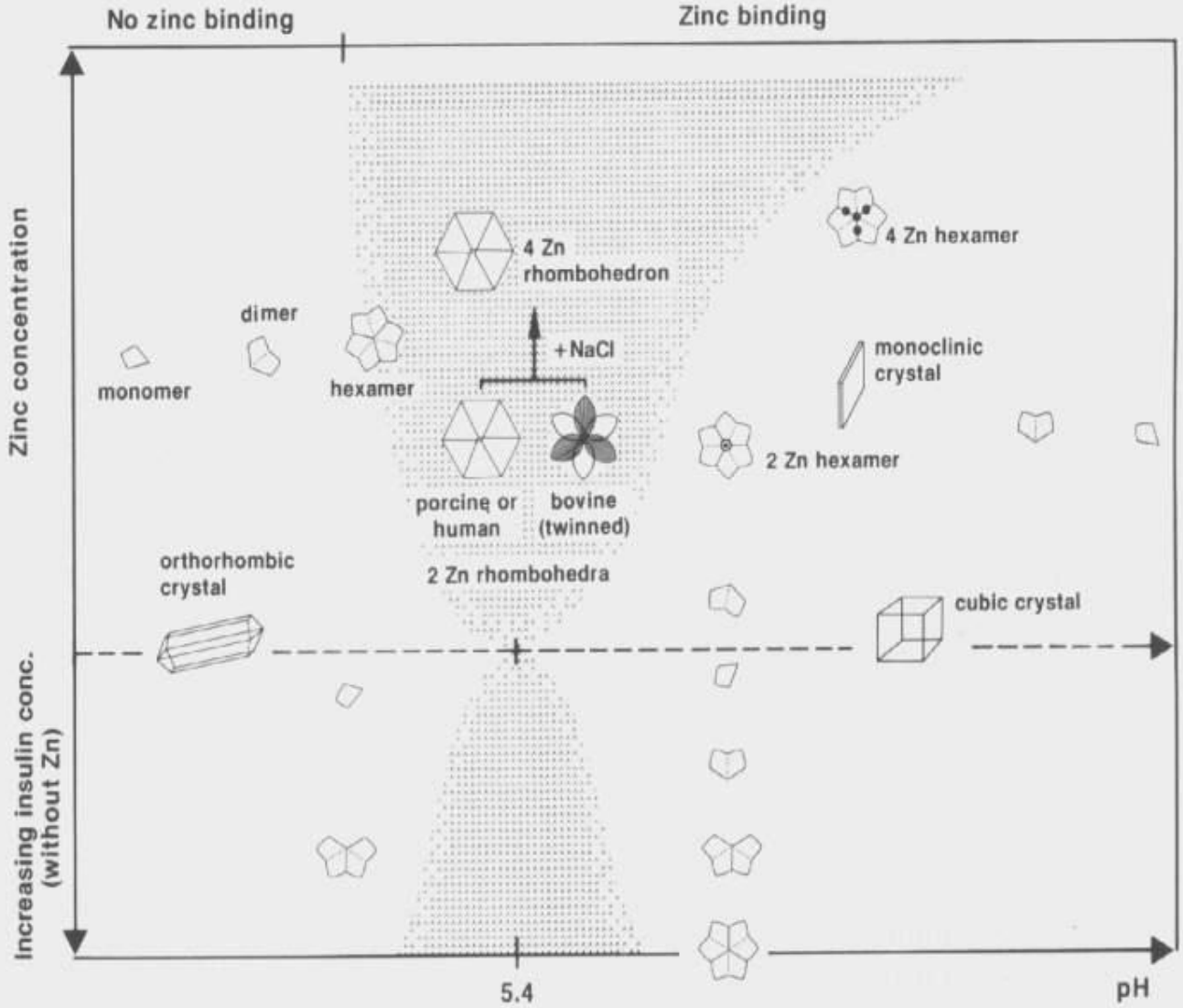


Fig. 8. Schematic diagram of the association and crystallization behaviour of insulin. The shaded area represents the insulin precipitation zone

Brange, 1987

Get rid of the protein…..
Insulin-altertives: 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

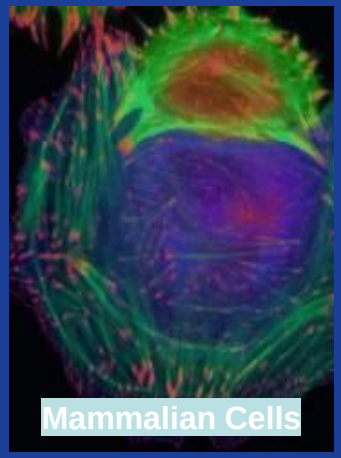
Further paradigm shifts at the horizon

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

Five expression technologies for protein production

Sheep, goat, cow

Saccharomyces



CHO

Tobacco, moss

Escherichia coli

knaeplein

Biopharmaceuticals from Plants

Next step: biopharmaceuticals produced in …..

Duck Weed

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

Biolex Interferon alpha

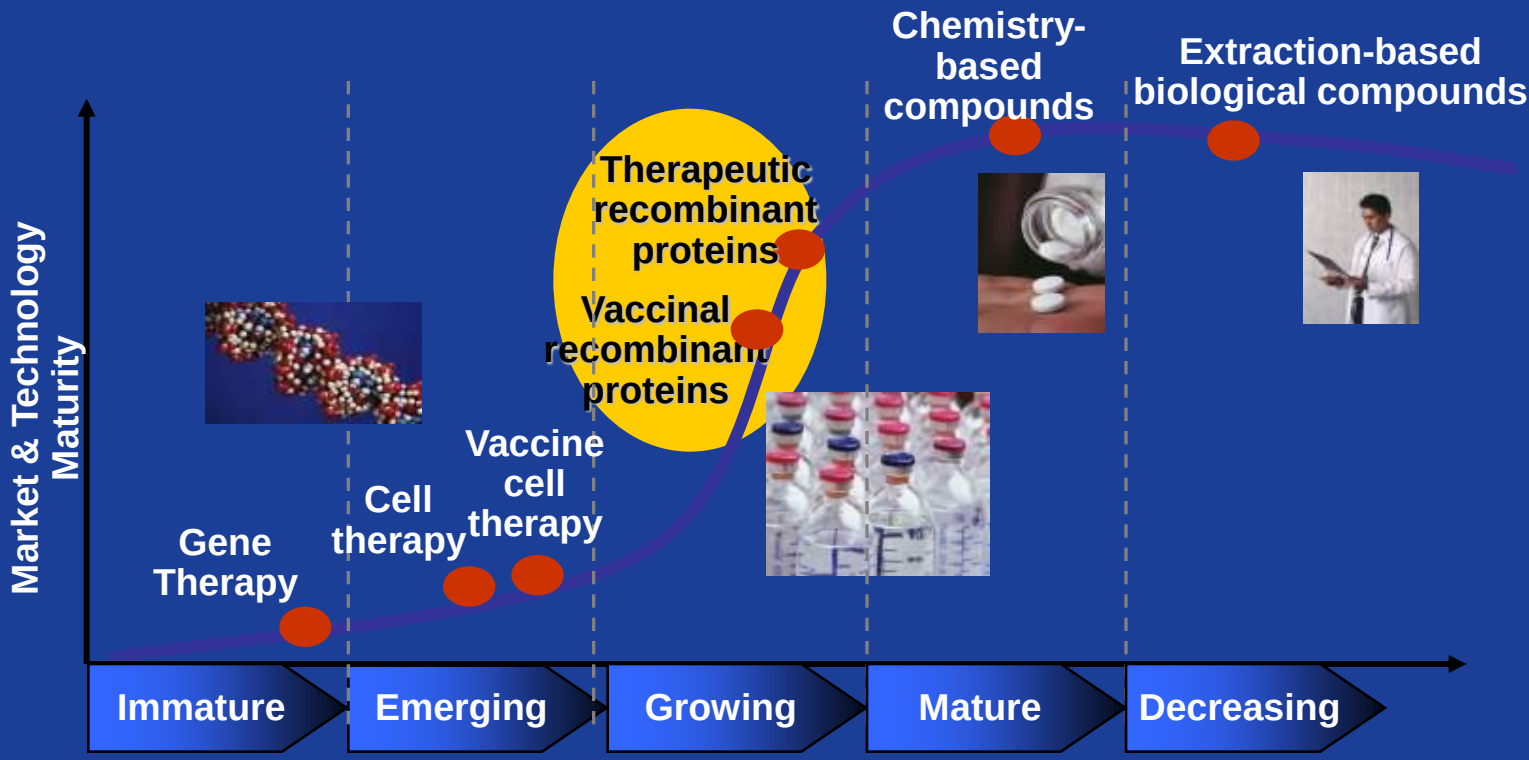
- Biolex' LEX™ system
  - Aquatic higher plant, Lemna
  - Secretes recombinant protein (e.g. IFNa2b)
  - Fast, inexpensive process
  - High expression levels
  - Highly scalable

www.biolex.com

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.

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

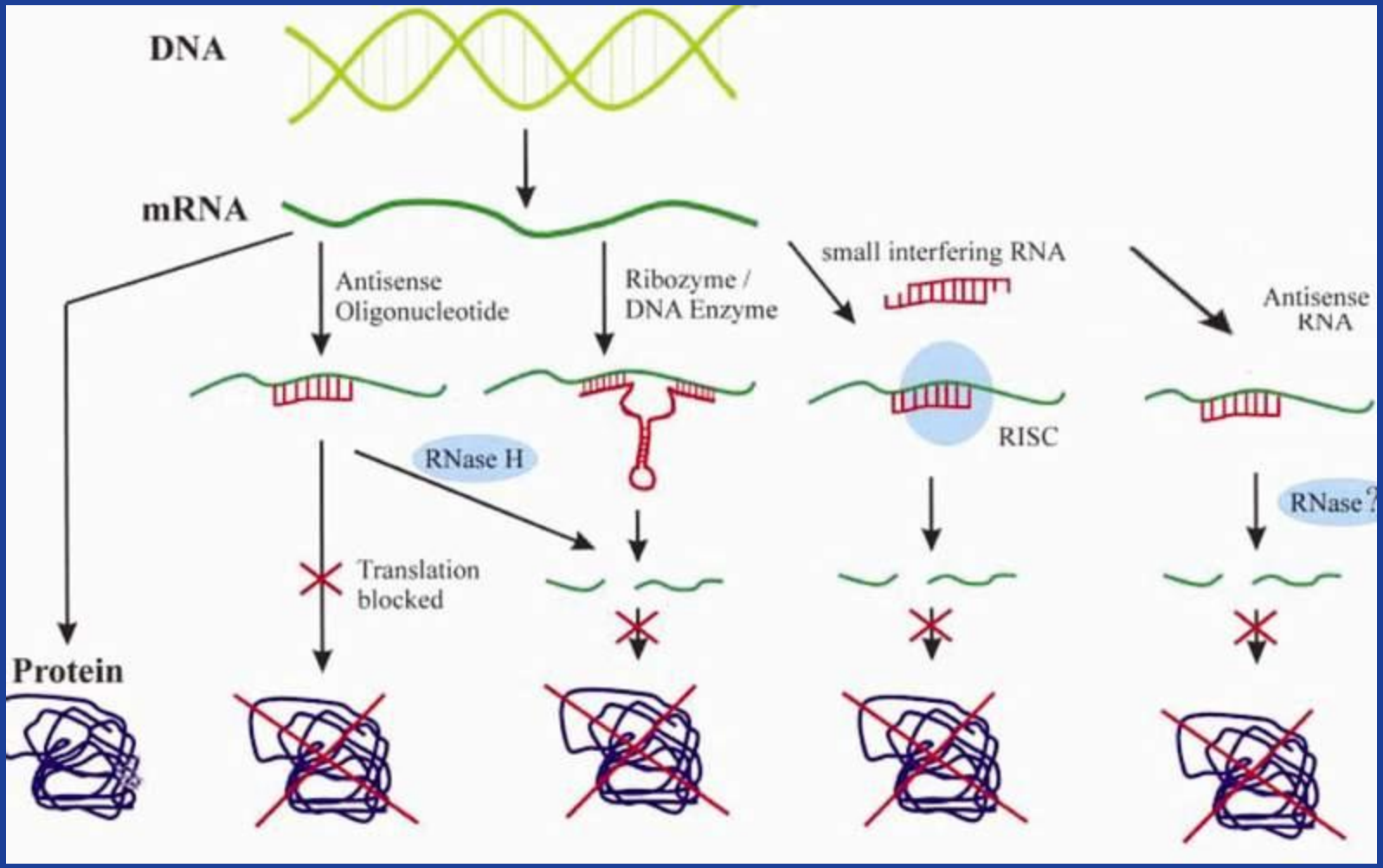
- Mastering technologies
- Ongoing optimization
- Many products in development and in the market

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

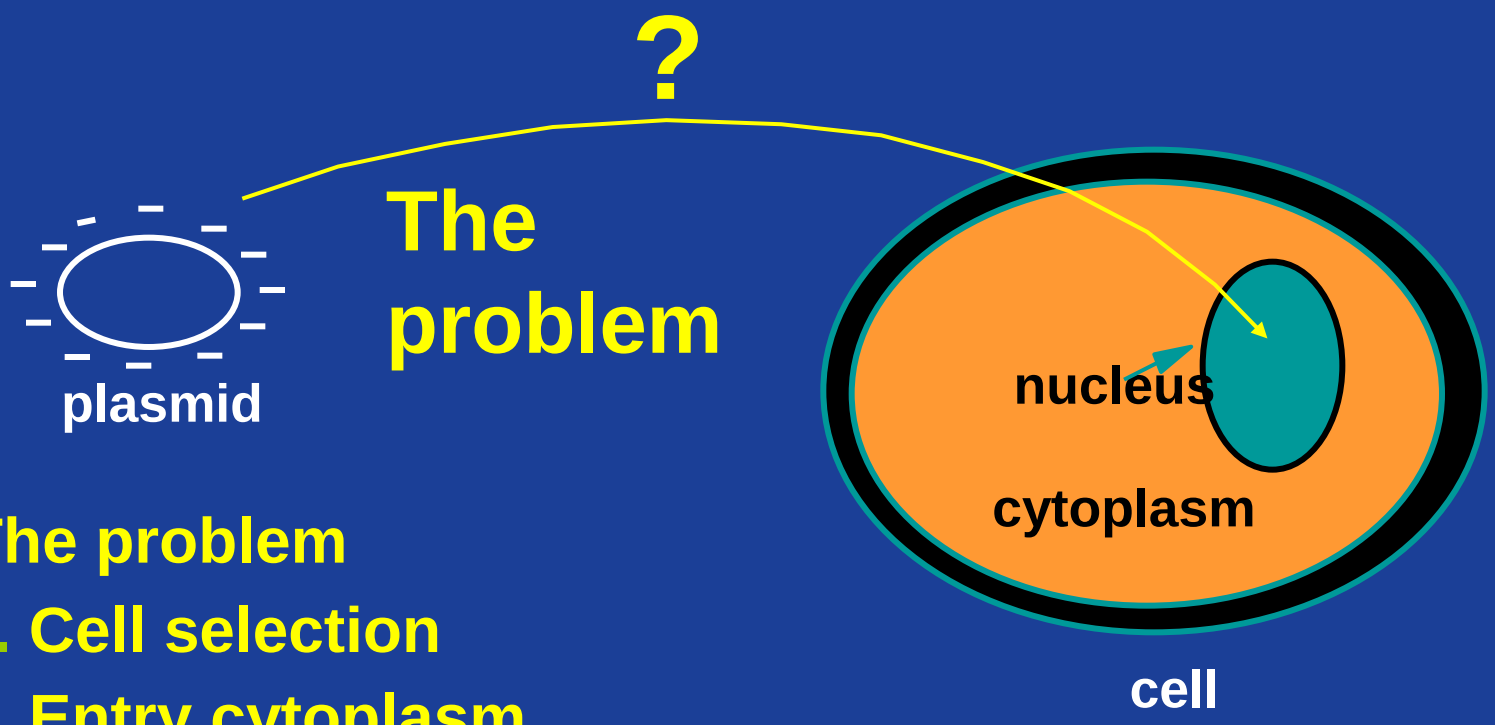
- Common technologies
- Manufacturing alternatives required
- Decreasing number of products in development and, eventually, in the market

Source: Paulo Barbanti

Interference with protein expression



The problem…



The problem

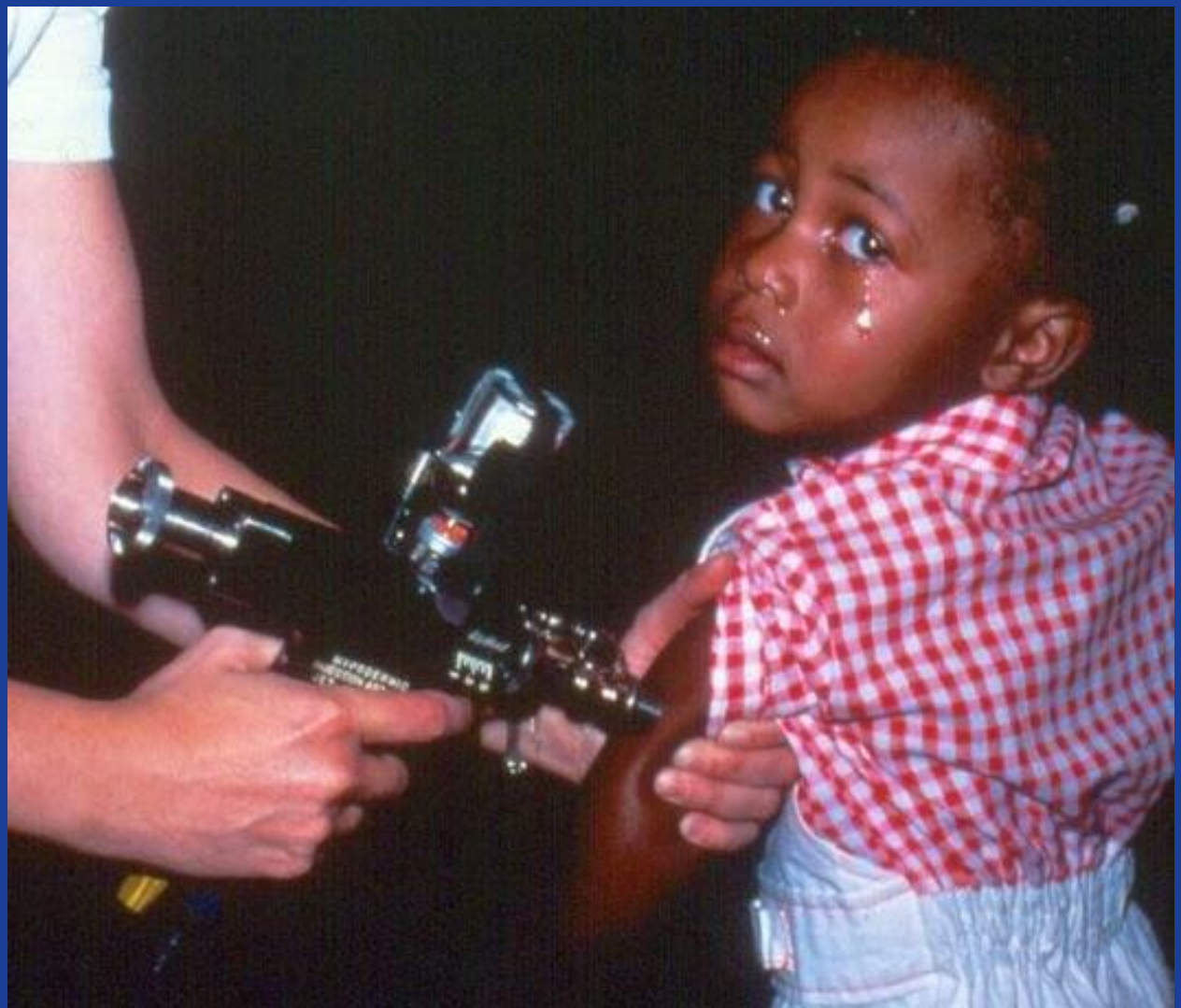
1. **Cell selection**
2. **Entry cytoplasm**
3. **Entry nucleus**

Welcome to the kingdom of the needle?

- Are we stuck to the needle?

Gas-driven intradermal injection

A quest for improved delivery of parenterally applied drugs



Insulin Uptake via the Pulmonary Route

- Bioavailability around 10 %
  - **loss in device**
  - **loss through disposition in non-lung tissue**
  - **absorption loss**

  - **Patton, J.S., Adv. Drug Delivery Research 35, 1999, 235-247**

U.S. Food and Drug Administration

U.S. Department of Health and Human Services

FDA Home Page | Search FDA Site | FDA A-Z Index | Contact FDA

This is a revised version of this press release, originally issued Jan. 27, 2006.
The release was revised to clarify recommendations for baseline tests.

FDA News

FOR IMMEDIATE RELEASE
P06-13
January 27, 2006

Media Inquiries:
Laura Alvey, 301-827-6242
Consumer Inquiries:
888-INFO-FDA

FDA Approves First Ever Inhaled Insulin Combination Product for Treatment of Diabetes

There is a new, potential alternative for many of the more than 5 million Americans who take insulin injections, with the Food and Drug Administration's approval today of the first ever inhaled insulin. Exubera, an inhaled powder form of recombinant human insulin (rDNA) for the treatment of adult patients with type 1 and type 2 diabetes, is the first new insulin delivery option introduced since the discovery of insulin in the 1920s.

"Until today, patients with diabetes who need insulin to manage their disease had only one way to treat their condition," said Dr. Steven Galson, Director, Center for Drug Evaluation and Research, FDA. "It is our hope that the availability of inhaled insulin will offer patients more options to better control their blood sugars."

Diabetes is a disease that affects the amount of insulin and sugar in your body. Exubera is a human form of insulin and as such, lowers

Exubera

Monogenetic Diseases

How to cure?

Cystic fibrosis
Hurler syndrome
Hunter syndrome
Huntington's chorea
canavan disease
Gaucher disease
Wiskott-Aldrich syndrome
Leber congenital amaurosis
SCID
Duchenne muscular dystrophy
Chronic granulomatous disease
Familial hypocholesterolaemia
Purine nucleoside phosphorylase deficiency
OTC deficiency
Leukocyte adherence deficiency
Amyotrophic lateral sclerosis
Junctional epidermolysis bullosa
Hemophilia A and B
Fanconi's anemia
Gyrate atrophy
RPE 65 defects
Fabry disease
Mucopolysaccharidosis type IV
Lipoprotein-lipase deficiency
Late infantile neuronal ceroid lipofuscinosis

Source: From O'Connor, 2006 and Edelstein, 2004.

Table 3 Monogenetic Diseases Treated by Gene Transfer in the Clinic

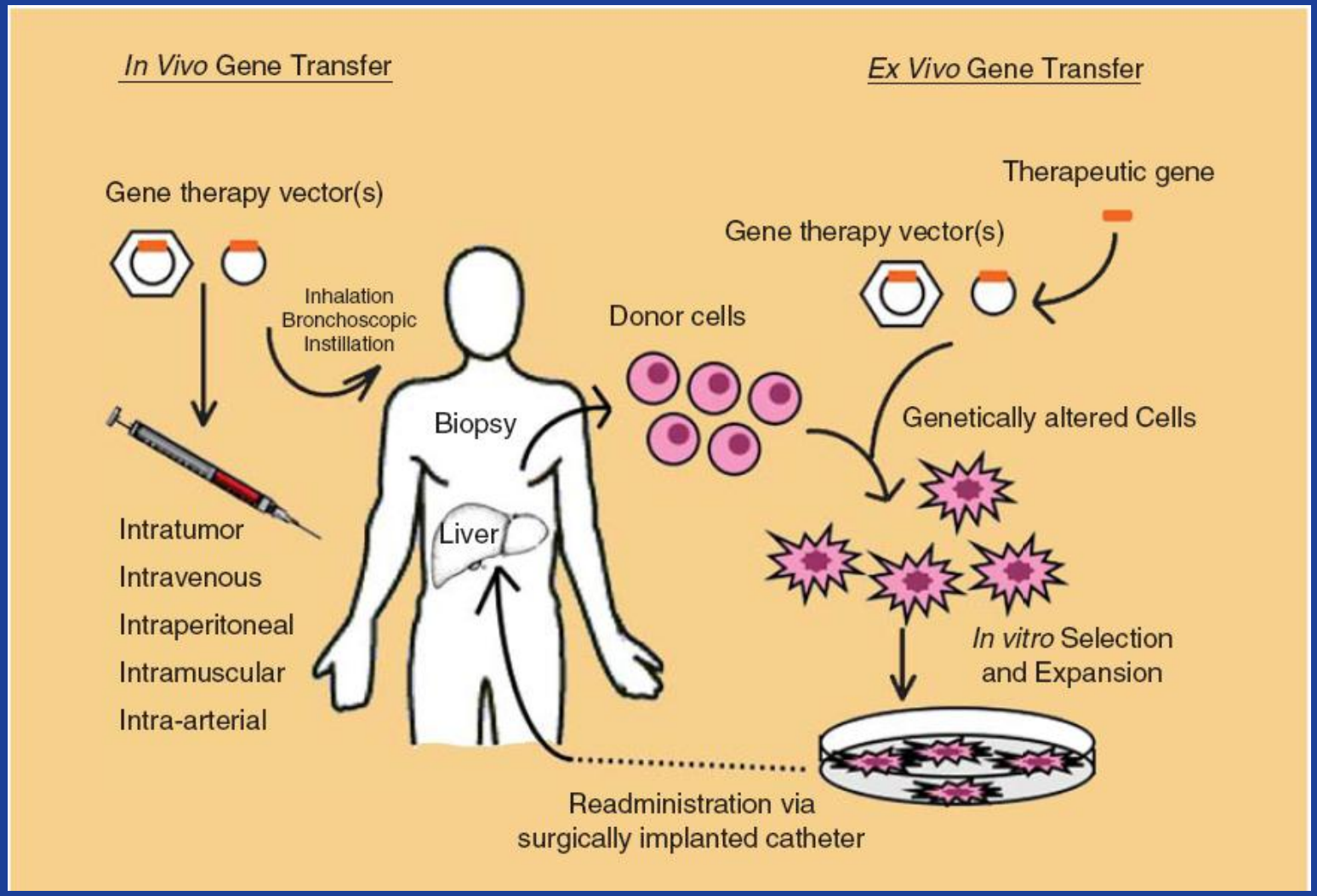
Disease	Gene therapy clinical trials	
	Number	Percentage
Cancer	842	67.0
Vascular diseases	113	9.0
Monogenetic diseases	104	8.6
Infectious disease	81	6.4
Gene marking	50	4.2
Healthy volunteers	21	1.7
Other diseases[a]	47	3.7

[a] Grouped in this category are treatments for: inflammatory bowel disease, rheumatoid arthritis, chronic renal disease, carpal tunnel syndrome, Alzheimer's disease, diabetic neuropathy, Parkinson's disease, erectile dysfunction, retinitis pigmentosa and glaucoma.

Source: From Wiley, 2006 and Edelstein, 2004.

Table 1 ■ Summary of Current Gene Therapy Clinical Trials by Indication

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.

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.

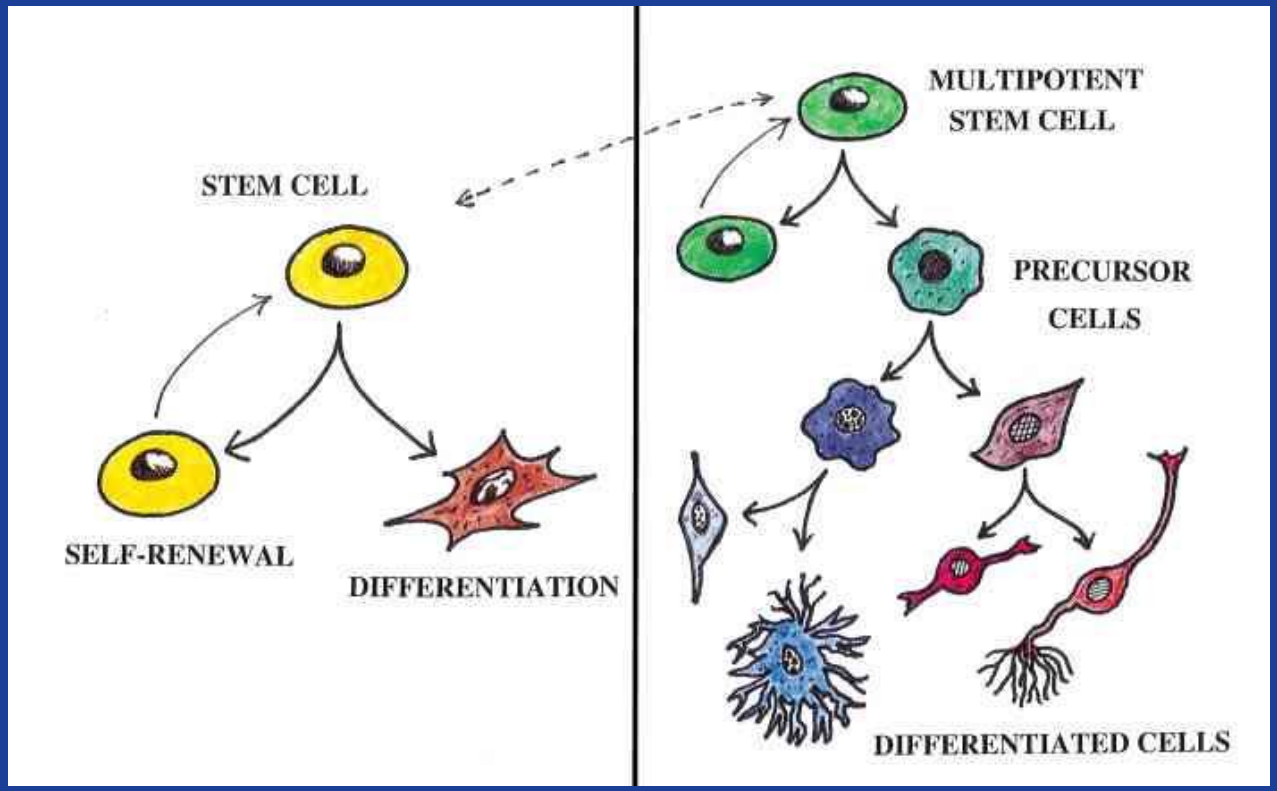
.In 2002 and 2003, it was reported that three of nine children in France who had been cured of severe combined immunodeficiency disease (SCID) with gene therapy had developed cancer two to three years later. Children born with this disorder will die in the first year of life unless they can find a matching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

Joly Mohr, July 2007

Stem cell therapies……

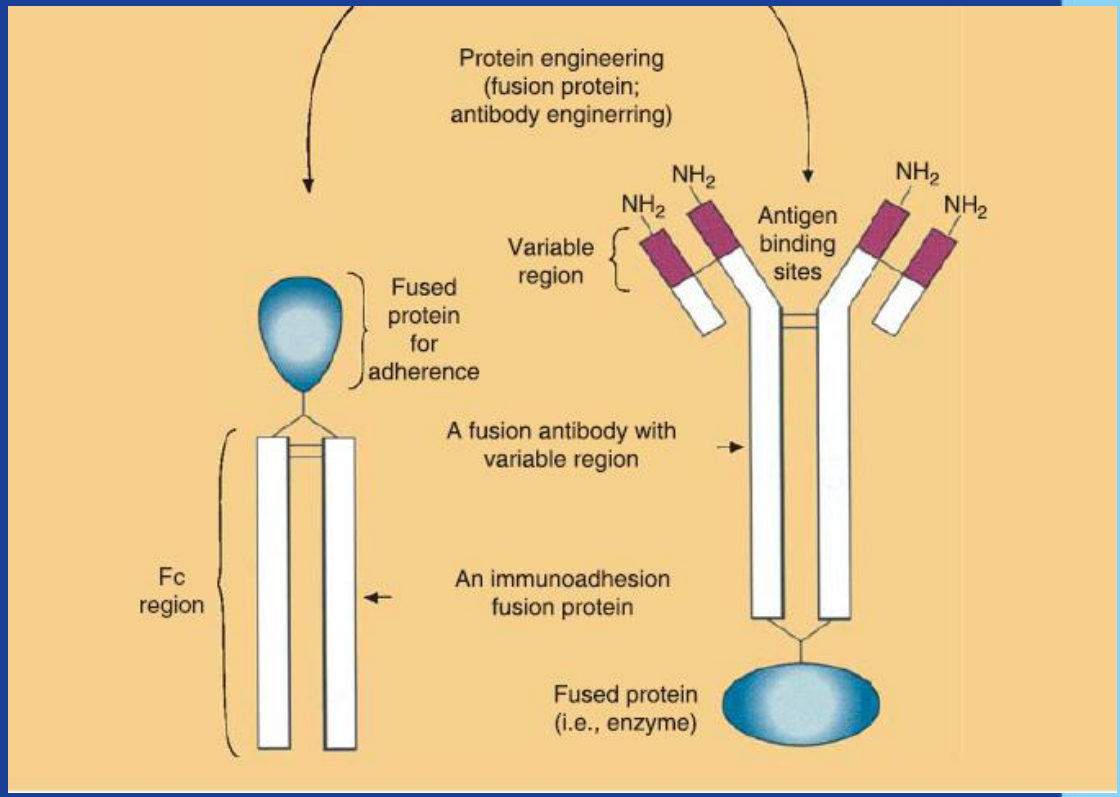
Adult stem cells

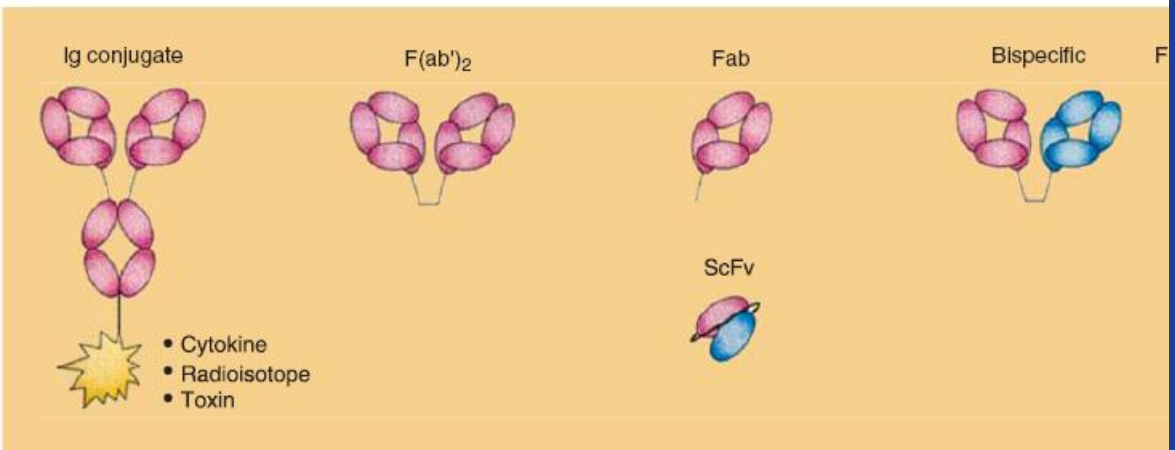
Embryonic stem cells



Therapy: Cancer, Type I diabetes, spinal cord injuries and muscle injury

Modified proteins



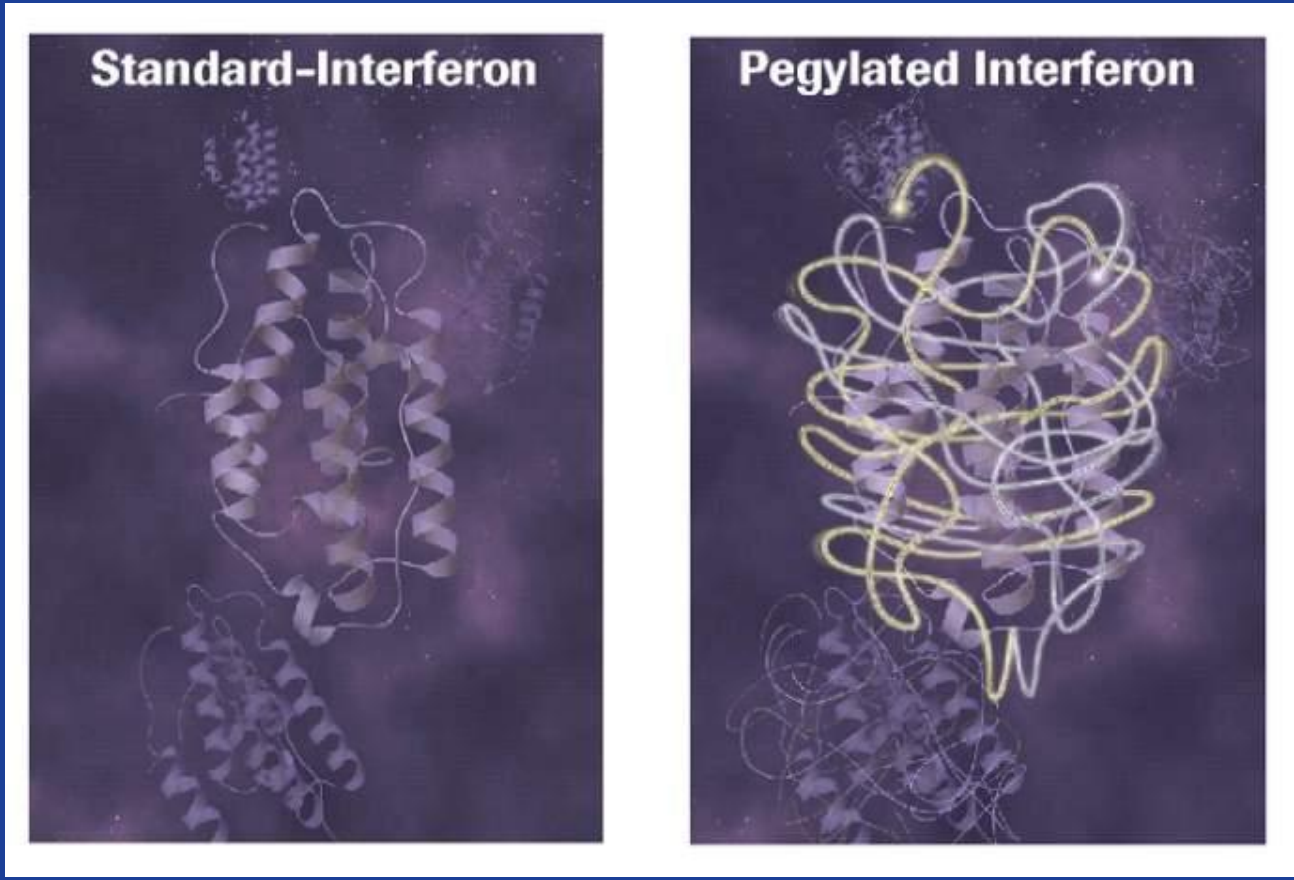


Chapter 7 and 15

PEGylation:

- Masking uptake-receptor sites
- Reducing clearance by glomerular filtration
- Reducing immunogenicity (?)

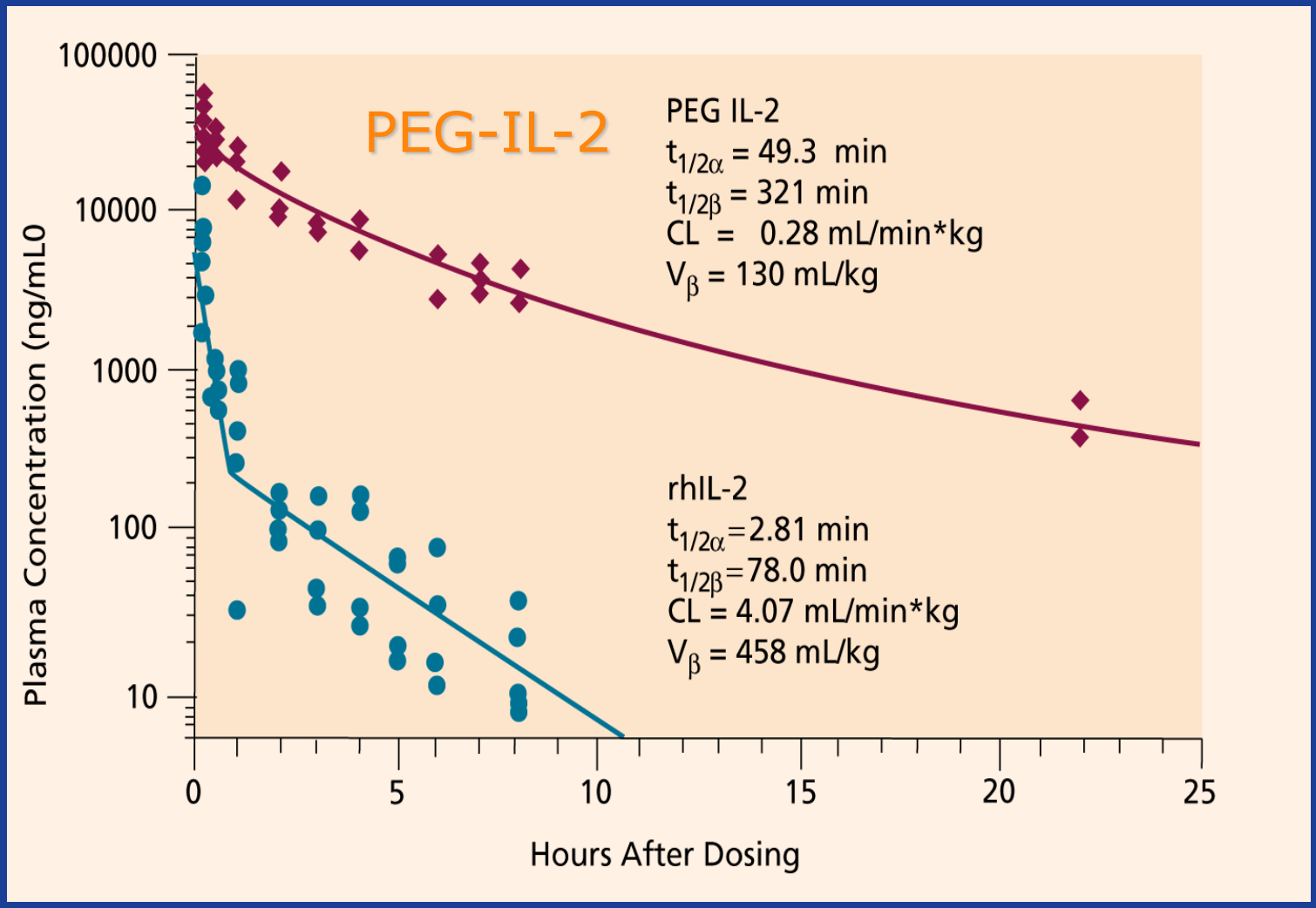
Strategies for improved protein delivery



protein

http://www.roche.com/pages/facets/10/pegasyse.htm

Pharmacokinetics of recombinant human interleukin-2 (rhIL2) and its PEGylated form (PEG IL-2) in rats after IV bolus administration of 0.25 mg/kg.



The data were described by a linear two-compartmental pharmacokinetic model.

Braekman 1997

Aranesp™ is an erythropoiesis stimulating protein closely related to erythropoietin that is produced in Chinese hamster ovary (CHO) cells by recombinant DNA technology. Aranesp™ is a 165-amino acid protein that differs from recombinant human erythropoietin in containing 5 N-linked oligosaccharide chains, whereas recombinant human erythropoietin contains 3.[1]

Table 2. Comparison of pharmacokinetic parameters for intravenous darbepoetin alfa and recombinant human erythropoietin*

Parameter	Darbepoetin alfa (n = 11)	rHu-EPO (n = 10)
Terminal half-life (hr)	25.3 ± 2.2	8.5 ± 2.4
Clearance (mL/h per kg)	1.6 ± 0.3	4.0 ± 0.3
$AUC_{(0-96\ h)}$ (ng·h per mL)	291.0 ± 7.6	131.9 ± 8.3
V_d (mL/kg)	52.4 ± 2.0	48.7 ± 2.1

*Adapted from reference 8. Results are given as mean ± standard error of the mean. rHu-EPO indicates recombinant human erythropoietin; AUC, area under the serum concentration–time curve; V_d, volume of distribution at steady state.

Darbepoetin alfa (Aranesp)

JOHN POWELL, RPH, BCOP, AND CHERYLE GURK-TURNER, RPH

dagen: op stations in Italië in de jaren dertig bracht een bode de telefoon naar de reizigers toe. (Uit 'The Ericsso
van John Meurling en Richard Jeans, 2000).

In Aeosop's fable,
the thirsty crow knows that
in order to get a drink from
the pitcher, he must force
the water to rise, one stone
at a time