UNIVERSITÀ DEGLI STUDI DI MILANO

RED BIOTECH

Informazione, salute e biotecnologie

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Farmaci biotecnologici - Pro e Contro

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Biotech Therapeutics – from theory…

The EMA view

Medicinal products derived from biotechnology
include those produced by the following biotechnological processes:

- recombinant DNA technology;
- monoclonal antibody methods;
- controlled expression of genes coding for biologically active proteins in prokaryotes and eukariotes

The FDA view

"Any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, applicable to the prevention, treatment, or cure of a disease or condition of human beings."

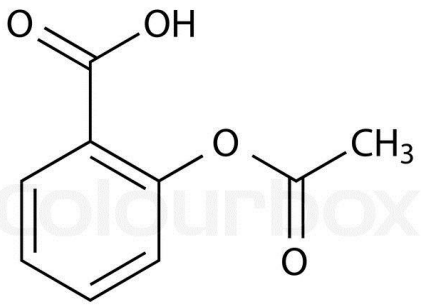
The process is the product

Biotech Therapeutics – …to practice

- Recombinant proteins
  - Hormones and derivatives
  - Interferon and cytokines
  - Factors that influence hematopoietic cells and blood coagulation
  - Enzymes and derivatives
  - Recombinant proteins for vaccines
- Monoclonal antibodies
- Oligonucleotides and vectors for gene therapy

Biotech Therapeutics vs small molecules

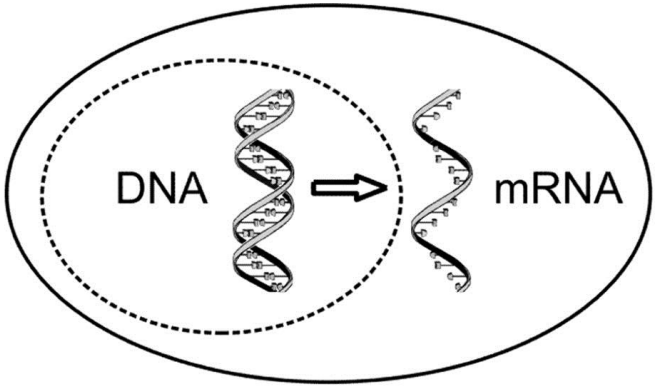
Alteplase – rhtPA MW: 75.000 D



Acetylsalicylic acid

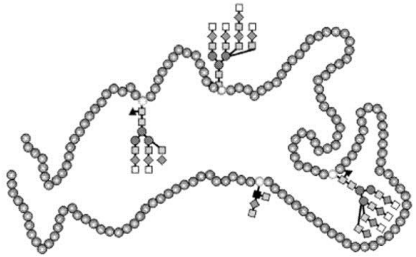
MW: 180 D

Biotech Therapeutics – expression systems



Bacteria or yeast

Mammalian cells



recombinant proteins

non-glycosylated

e.g.:
growth hormone, calcitonin, insulin, albumin

complex, glycosylated

e.g.:
erythropoietin, factor VIII, tPa

Biotech Therapeutics – expression systems

- Taliglucerase is the first plant cell-expressed biotherapeutic approved for human use
- It is produced in a closed sterile culture system (ProCellEx® expression system)
- Cells and media are not of human/animal sources
- Root carrot cells transformed with *Agrobacterium tumefaciens* carrying plasmid vector harboring β-glucosidase cDNA and kanamycin resistance gene
- Plastic bioreactors are disposable, sterile and recyclable
- Scaled-up horizontally
- The protein is stored within neutral vacuoles where is protected from degradation and α-mannosyl residues exposed
- Extraction by detergen solubilization and purification by chromatography

G.A. Grabowski et al. / Molecular Genetics and Metabolism 112 (2014) 1–8

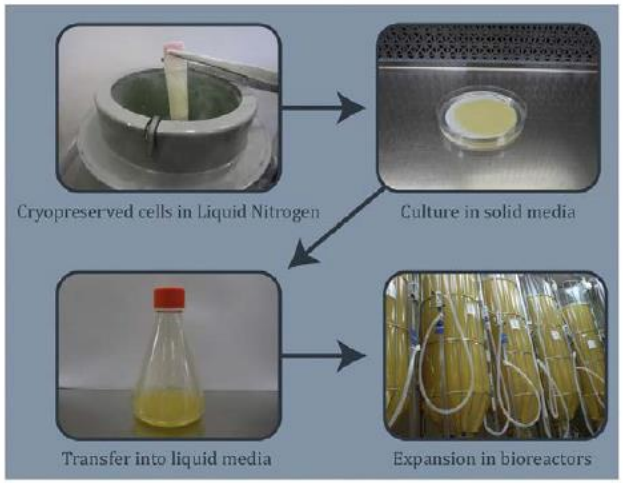
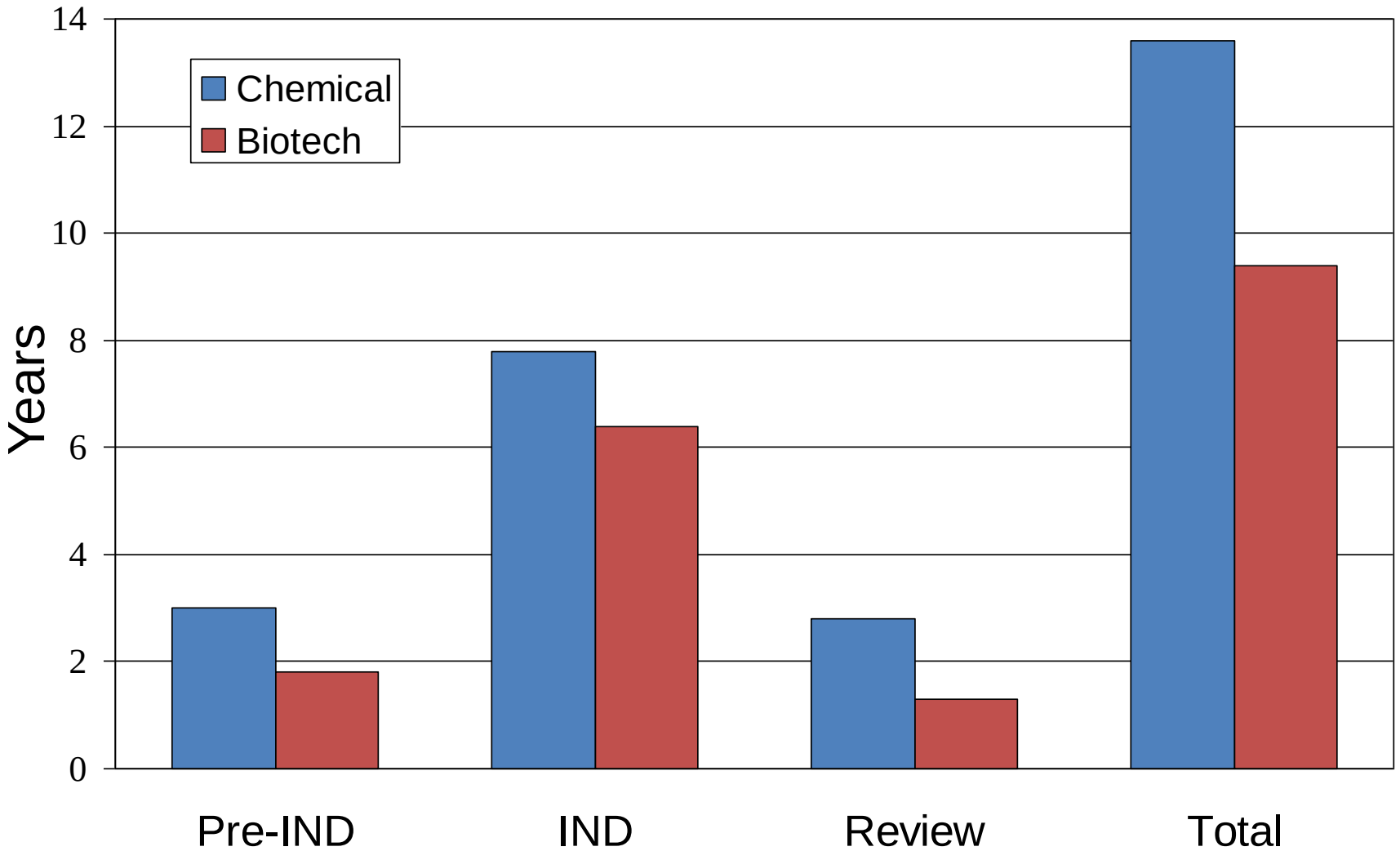


Fig. 1. Diagram of taliglucerase alfa production.
Photographs courtesy of Protalix Biotherapeutics, Carmiel, Israel.

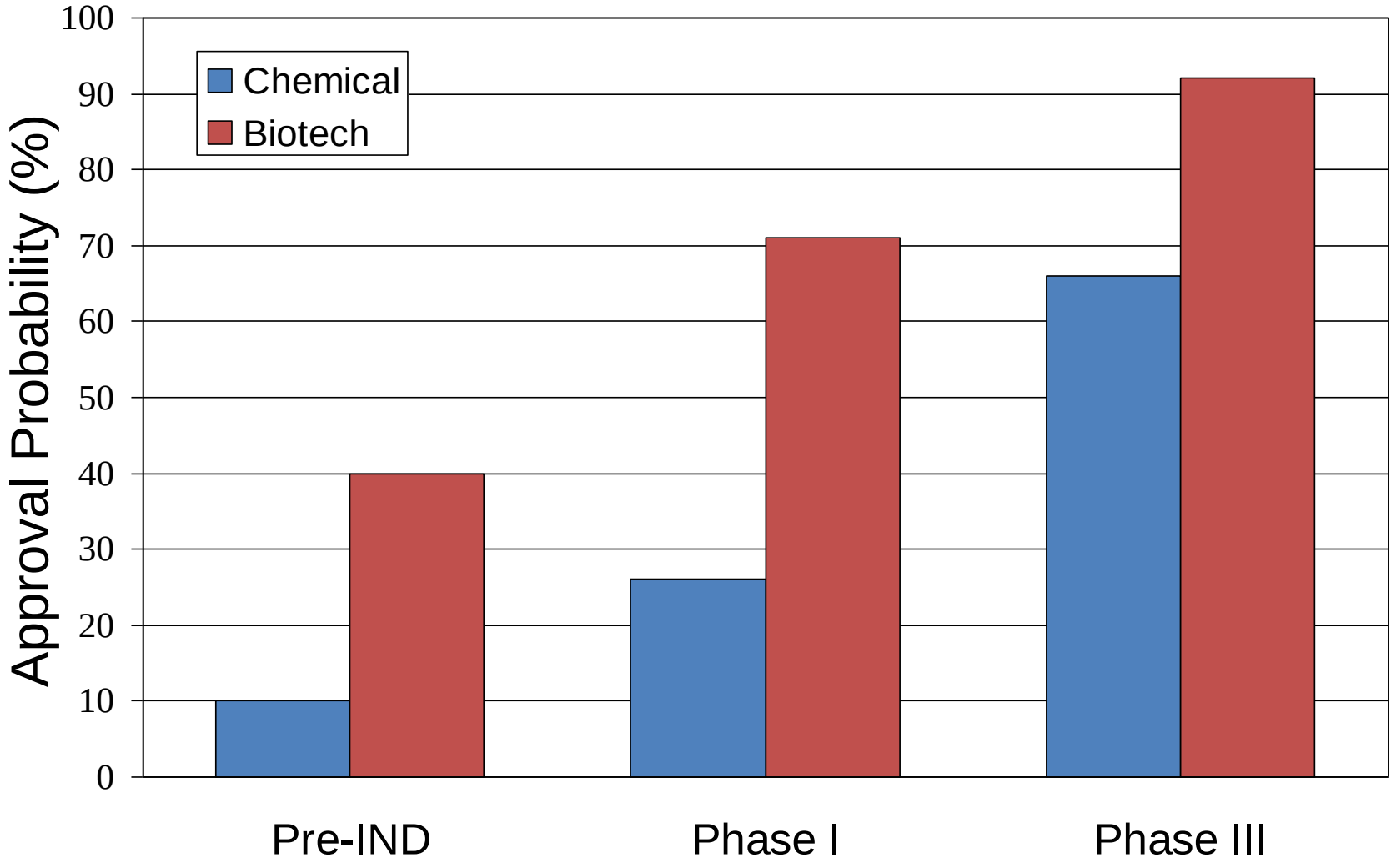
Grabowski GA et al, Mol Genet Metab 2014;112:1

These genetically modified carrot cells are growing inside plastic bags and producing drugs for a rare human genetic disorder.

Biotech Therapeutics vs small molecules



Biotech Therapeutics vs small molecules



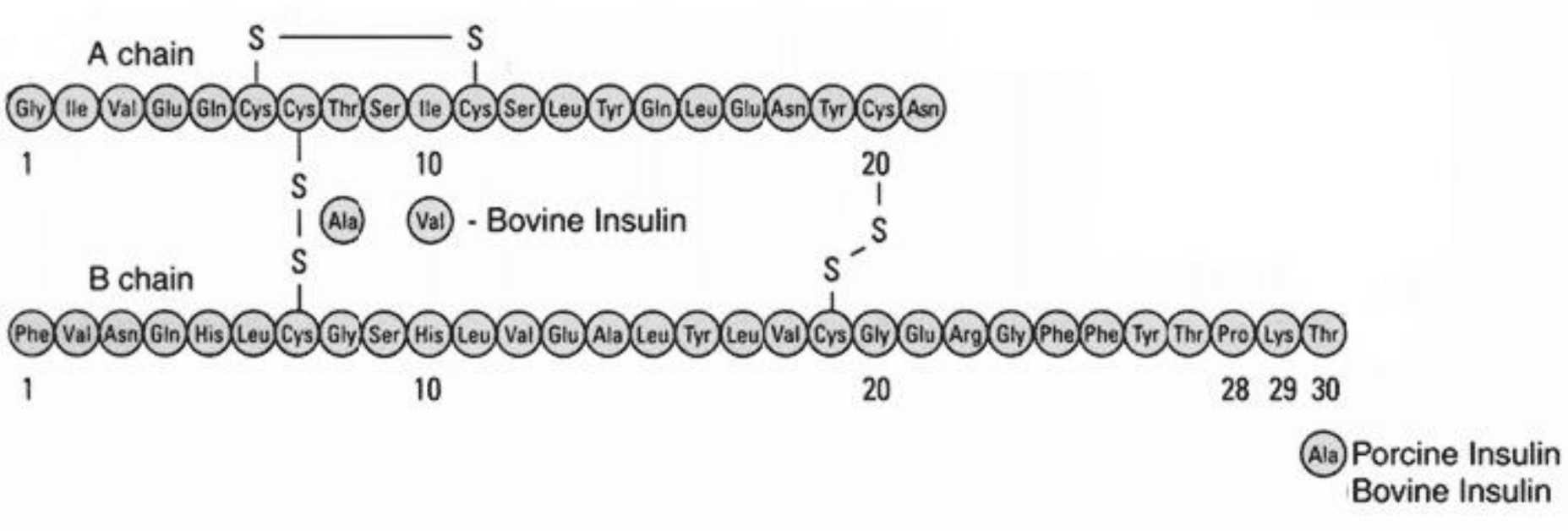
World's Top-selling Drugs 2006

			Sales ($ bil)
Lipitor	Atorvastatin	High Cholesterol	13.6
Nexium	Esomeprazole	Gastric Ulcer	6.7
Seretide	Fluticasone	Asthma	6.3
Plavix	Clopidogrel	Thrombosis	5.8
Norvasc	Amlodipine	High Blood Pressure	5.0
Aranesp	Darbepoetin	Anemia	5.0
Zyprexa	Olanzapine	Psychosis	4.7
Risperdal	Risperidone	Psychosis	4.6
Enbrel	Etanercept	Rheumatoid Arthritis	4.5
Effexor	Venlafaxine	Depression	4.0

World's Top-selling Drugs 2013

			Sales ($ bil)
Humira	Adalimumab	Rheumatoid Arthritis	11.2
Enbrel	Etanercept	Rheumatoid Arthritis	8.8
Remicade	Infliximab	Rheumatoid Arthritis	8.4
Advair/Seretide	Fluticasone	Asthma	8.3
Lantus	Insulin	Diabetes	7.6
MabThera/Rituxan	Rituximab	Cancer	7.5
Avastin	Bevacizumab	Cancer	6.7
Herceptin	Trastuzumab	Cancer	6.6
Crestor	Rosuvastatin	High Cholesterol	6.0
Abilify	Aripiprazolo	Mental disorders	5.5

First Biotech Therapeutic



Why a recombinant insulin? Supply, safety

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 1, pp. 106–110, January 1979
Biochemistry

Expression in *Escherichia coli* of chemically synthesized genes for human insulin

(plasmid construction/*lac* operon/fused proteins/radioimmunoassay/peptide purification)

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*Division of Molecular Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080; and ‡Division of Biology, City of Hope National Medical Center, Duarte, California 91010

Communicated by Ernest Beutler, October 3, 1978

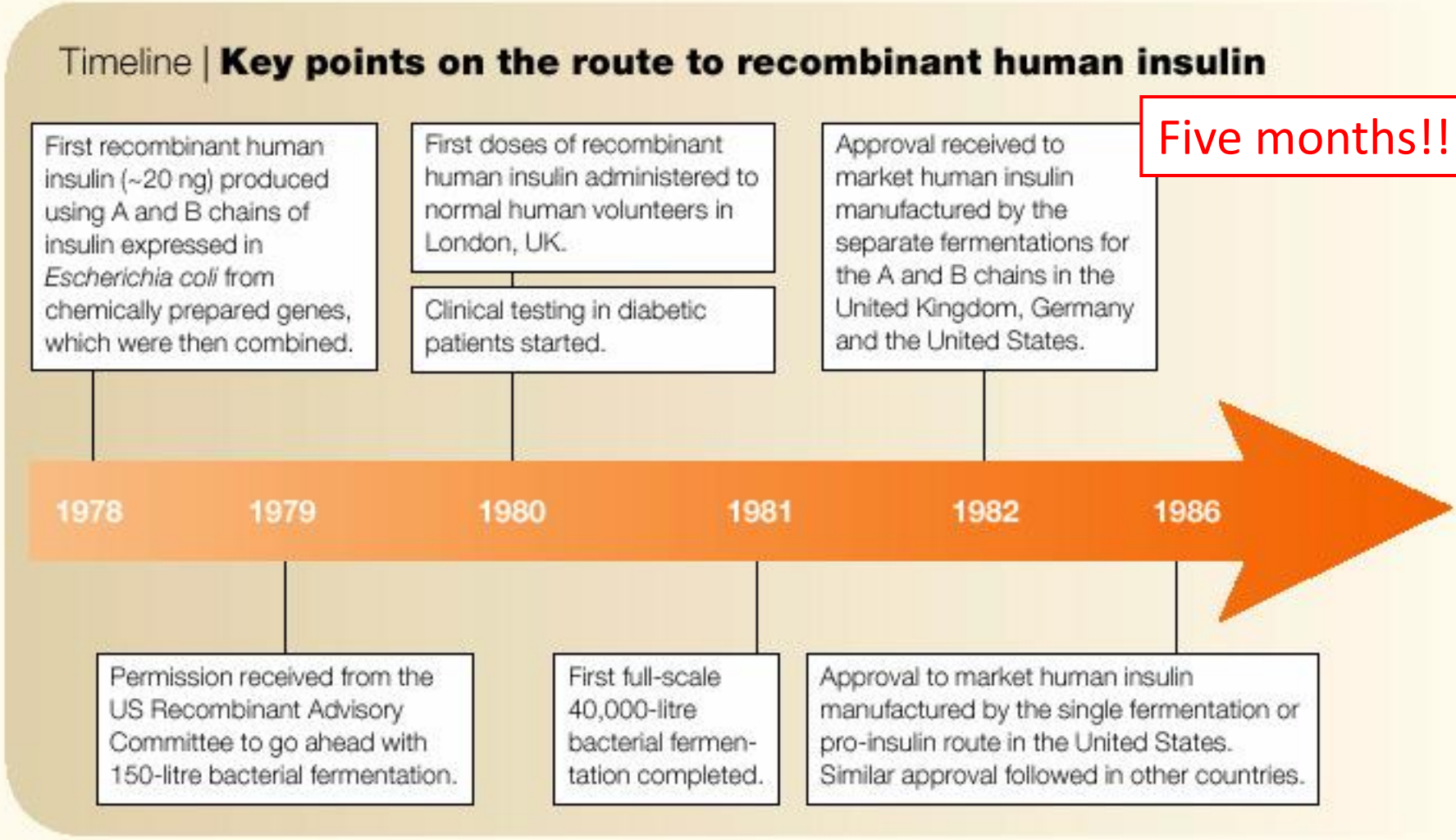
ABSTRACT **Synthetic genes for human insulin A and B chains were cloned separately in plasmid pBR322. The cloned synthetic genes were then fused to an *Escherichia coli* β-galactosidase gene to provide efficient transcription and translation and a stable precursor protein. The insulin peptides were cleaved from β-galactosidase, detected by radioimmunoassay, and purified. Complete purification of the A chain and partial purification of the B chain were achieved. These products were mixed, reduced, and reoxidized. The presence of insulin was detected by radioimmunoassay.**

Recently improved methods of DNA chemical synthesis,

Enzymes and DNA Preparations. T4 DNA ligase and T4 polynucleotide kinase were purified as described (6). Restriction endonuclease *Eco*RI was purified by the procedure of Greene *et al.* (7); *Hin*dIII was purified by a method developed by D. Goeddel (unpublished). Restriction endonuclease *Bam*HI was purchased from Bethesda Research (Rockville, MD); *E. coli* alkaline phosphatase was purchased from Worthington.

Plasmids, including pBR322 (8), were isolated by a published procedure (9) with some modifications. The chemical synthesis of the deoxyoligonucleotides (figure 1 of ref. 4) has been de-

First Biotech Therapeutic



First Biotech Therapeutic

PERSPECTIVES

TIMELINE

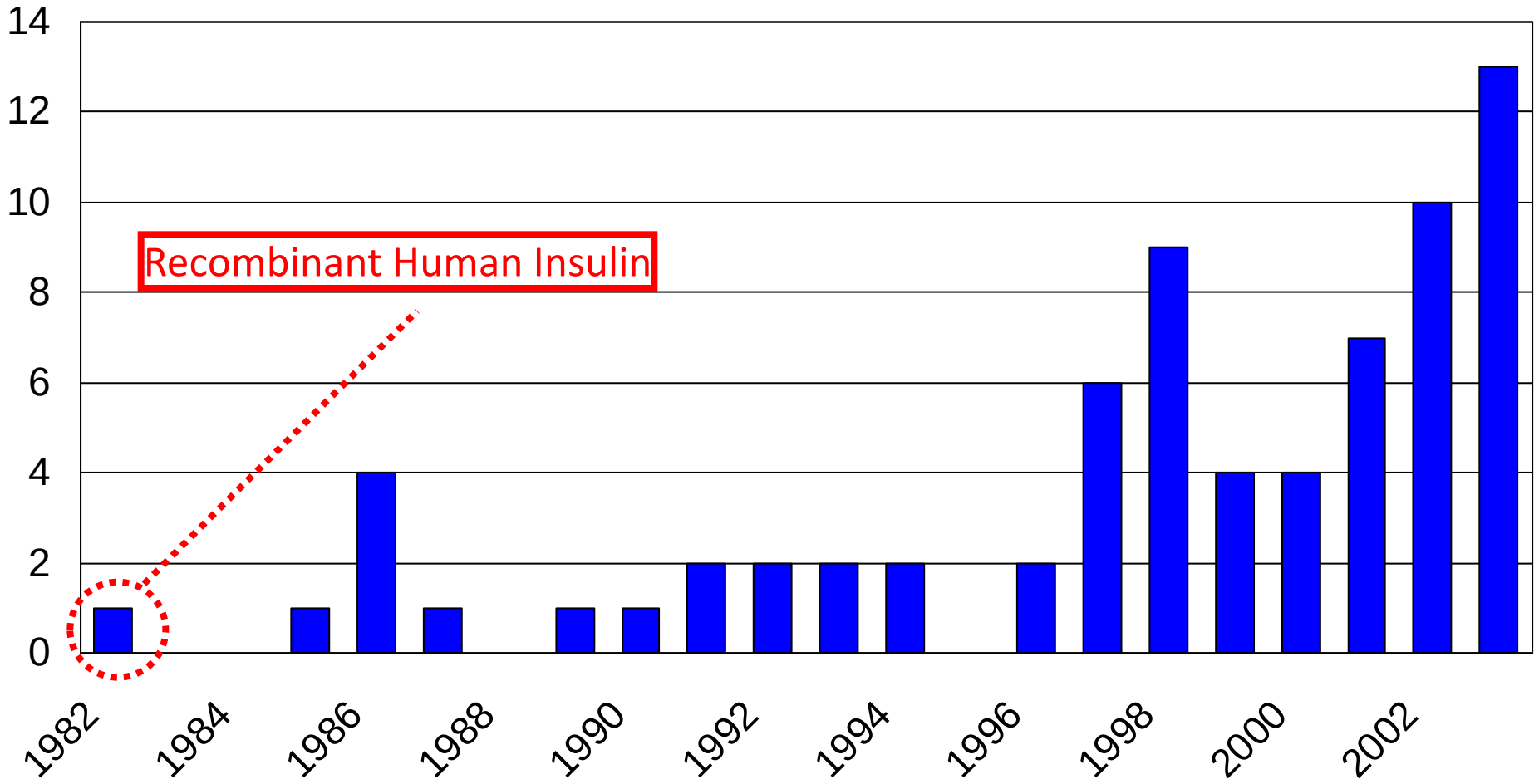
The trials and tribulations of producing the first genetically engineered drug

Irving S. Johnson

"One opponent of the technology actually had a paper published in a reputable journal suggesting that children living in the area around a Lilly production facility might become infected with *Escherichia coli* producing insulin and die of hypoglycaemia."

Nature Reviews Drug Discovery 2:747, 2003

Biotech Therapeutics – FDA approved



Enzyme Replacement Therapy

Disease	Defective/deficient enzyme	Primary substrate accumulation	Enzyme replacement therapy	Time of approval by FDA
Gaucher disease	β-Glucosidase (glucocerebrosidase)	Glucosylceramide	Alglucerase (Ceredase®)[a]	April 1991
			Imiglucerase (Cerezyme®)	May 1994
			Velaglucerase alfa (VPRIV®)	February 2010
			Taliglucerase alfa (Elelyso™)	May 2012
Fabry disease	α-Galactosidase A	Globotriaosylceramide	Agalsidase beta (Fabrazyme®)	April 2003
Pompe disease	Acid α-glucosidase	Glycogen	Alglucosidase alfa (Myozyme®)	April 2006
			Alglucosidase alfa (Lumizyme®)	May 2010
MPS I (Hurler, Hurler–Scheie, or Scheie syndrome)	α-l-Iduronidase	Dermatan sulfate and heparan sulfate	Laronidase (Aldurazyme®)	April 2003
MPS II (Hunter syndrome)	Iduronate-2-sulfatase	Dermatan sulfate and heparan sulfate	Idursulfase (Elaprase®)	July 2006
MPS IVa (Morquio A)	N-acetylgalactosamine-6 sulfatase (GALNS)	Keratan sulfate and chondroitin-6-sulfate	Elosulfase alfa (Vimizim®)	February 2014
MPS VI (Maroteaux-Lamy syndrome)	N-acetylgalactosamine 4-sulfatase (arylsulfatase B)	Dermatan sulfate	Galsulfase (Naglazyme™)	May 2005

Kishnani PS et al, Mol Genet Metab 2016;117:66

mAbs Evolution

DISCOVERY MILESTONES

- 1890: Serum therapy used as treatment against diphtheria and tetanus
- 1901: Side-chain theory predicted substances, which are today called antibodies; First idea of a "magic bullet"
- 1908
- 1961: Discovery of the chemical structure of antibodies
- 1972: Development of hybridoma technology
- 1975
- 1984: Production of the 1st monoclonal antibody
- 1986
- 2011: > 25 Ab-based therapies approved in the EU ~ 500 in development

NOBEL PRIZE


Emil Adolf von Behring


Paul Ehrlich

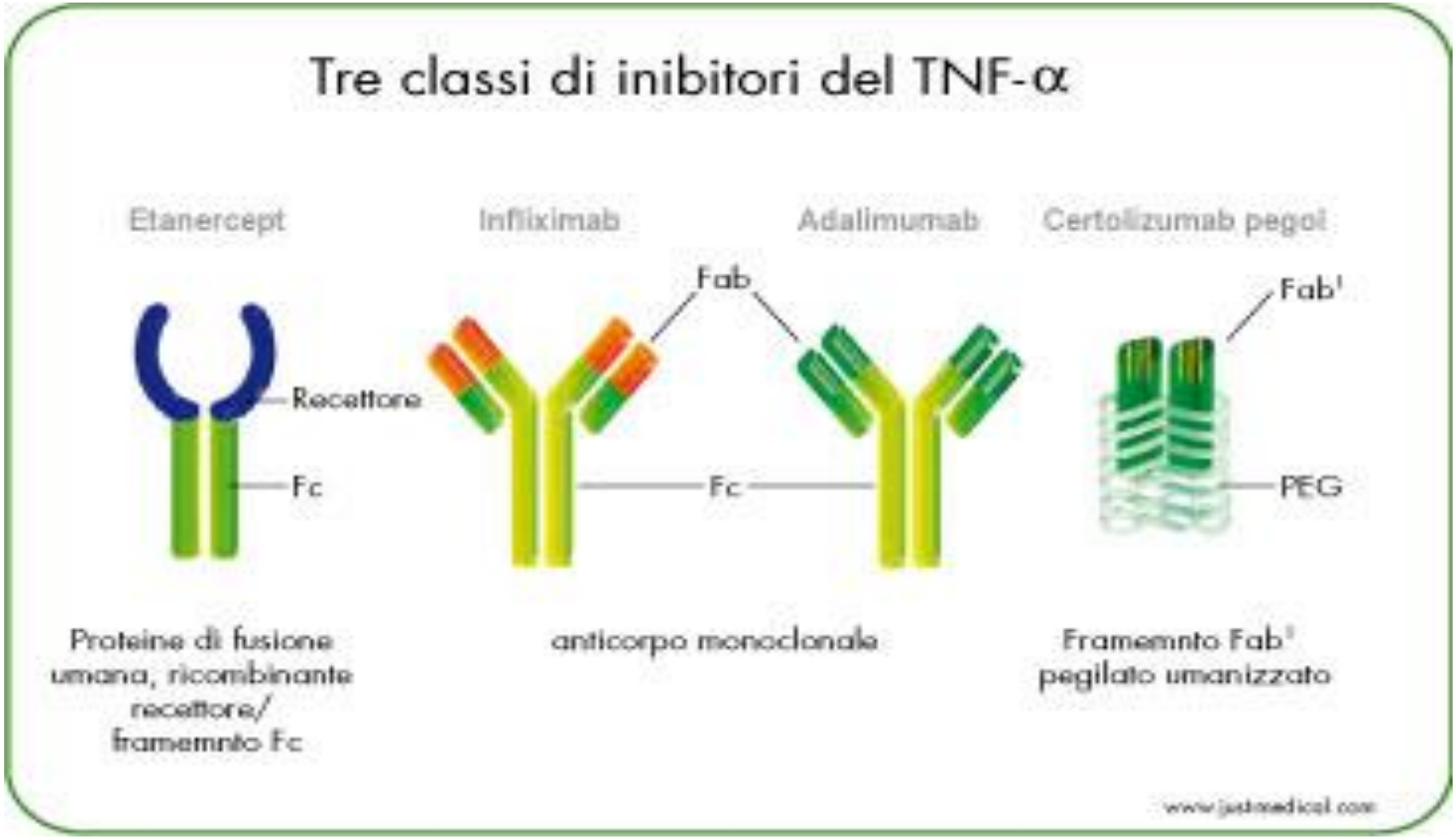

Gerald Edelman


Rodney Porter

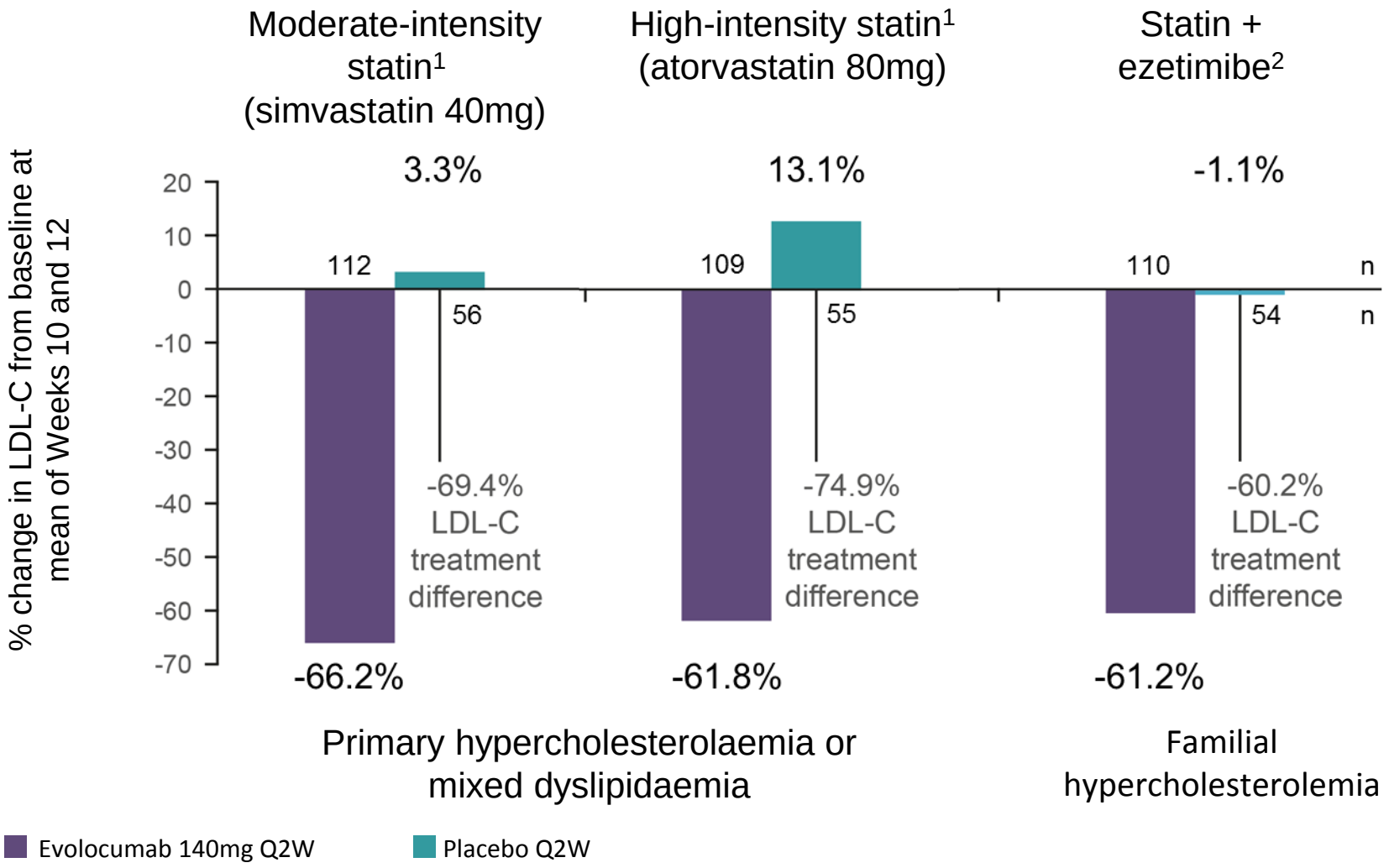

Georges Köhler


César Milstein

Anti TNF-a in rheumatoid arthritis

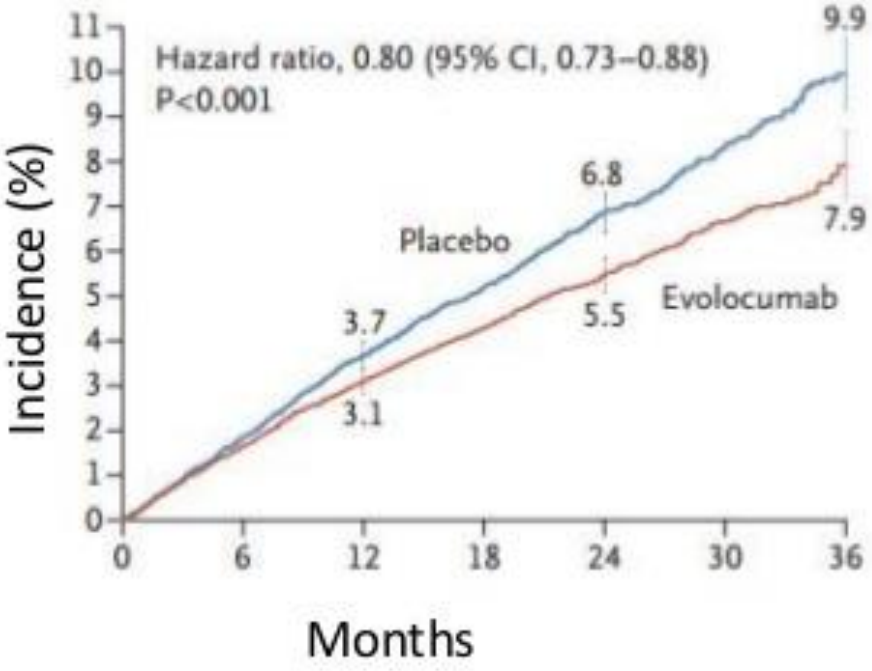


Evolocumab and cardiovascular prevention

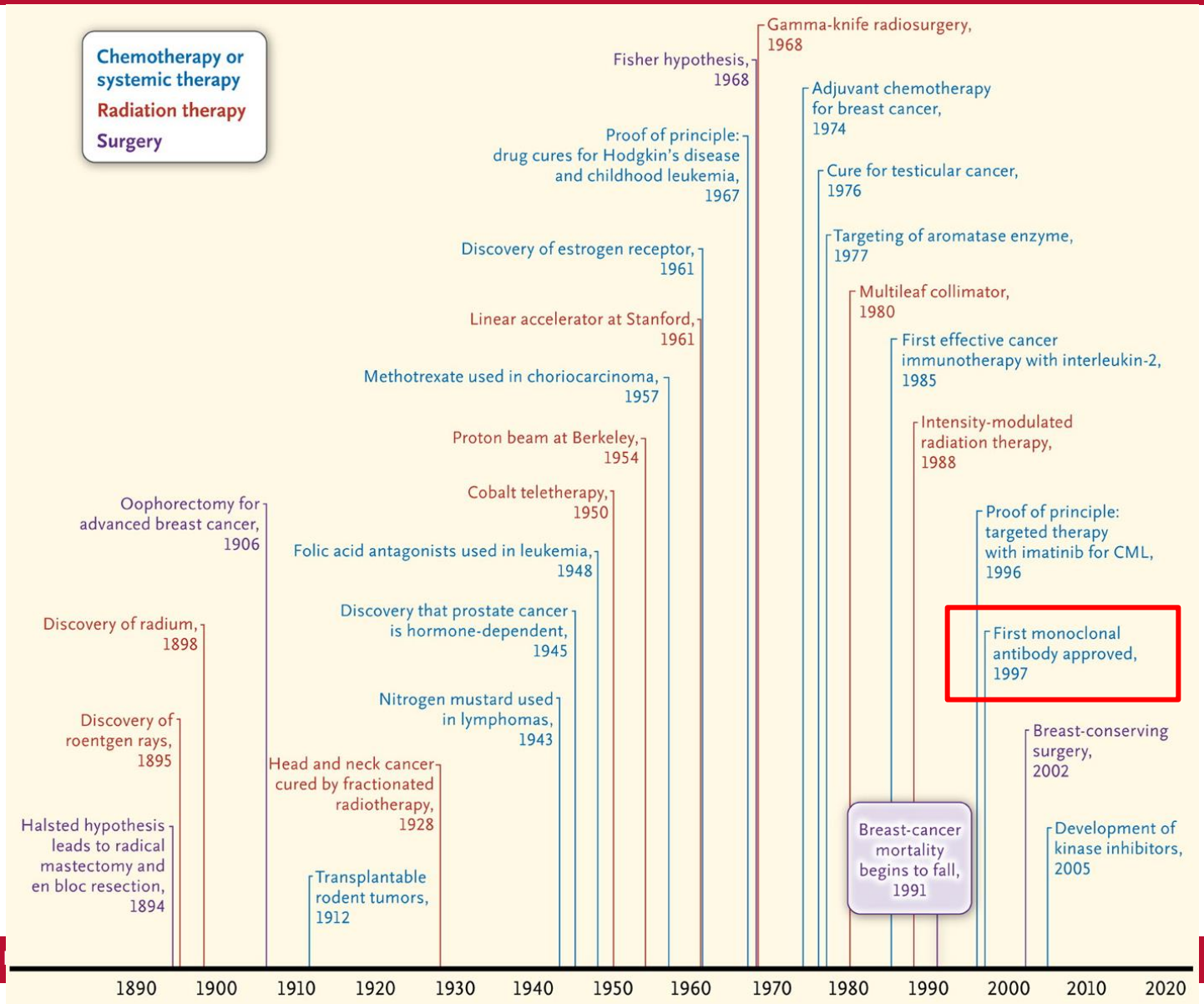


FOURIER Trial Results

- **Primary outcome of MCE**
  - ↓nonfatal MI, stroke, coronary revascularization by 20%
  - NNT=75 over 2 yrs
  - Mean LDL:0.78*
- **Secondary Outcomes**
  - No ↓ overall or CV mortality
  - CV death low (< 2%) in both grps
  - **SE:** injection-site reactions (2%)

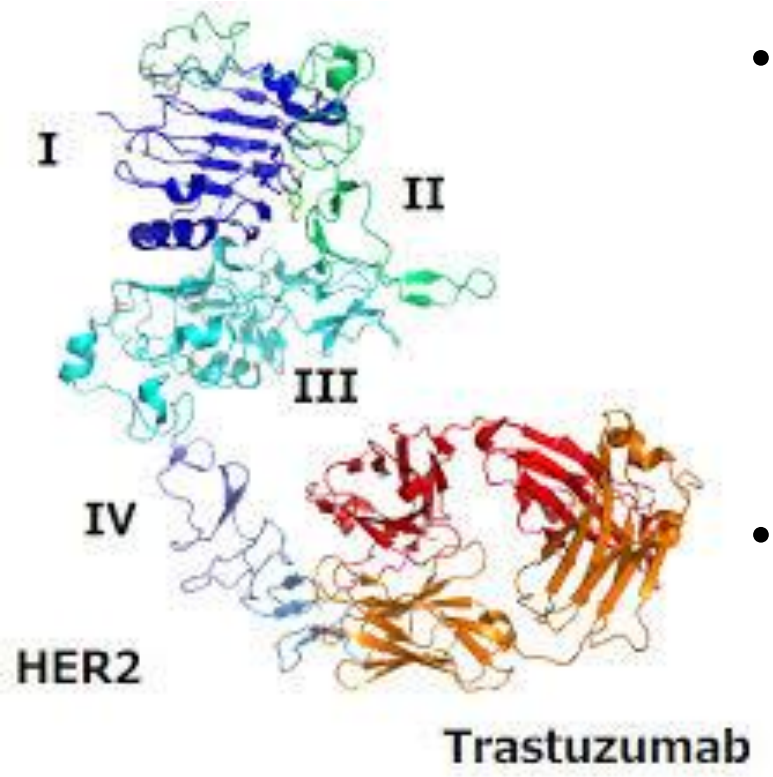


Milestones in Cancer Therapy



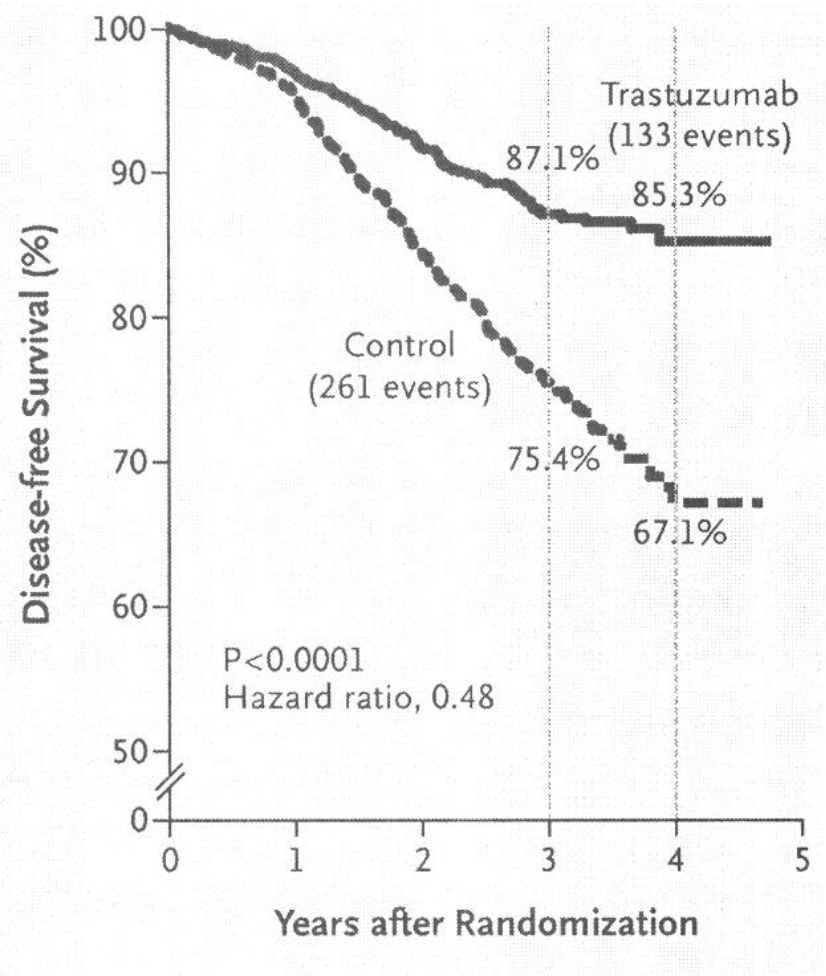
NEJM 366:2207,2012

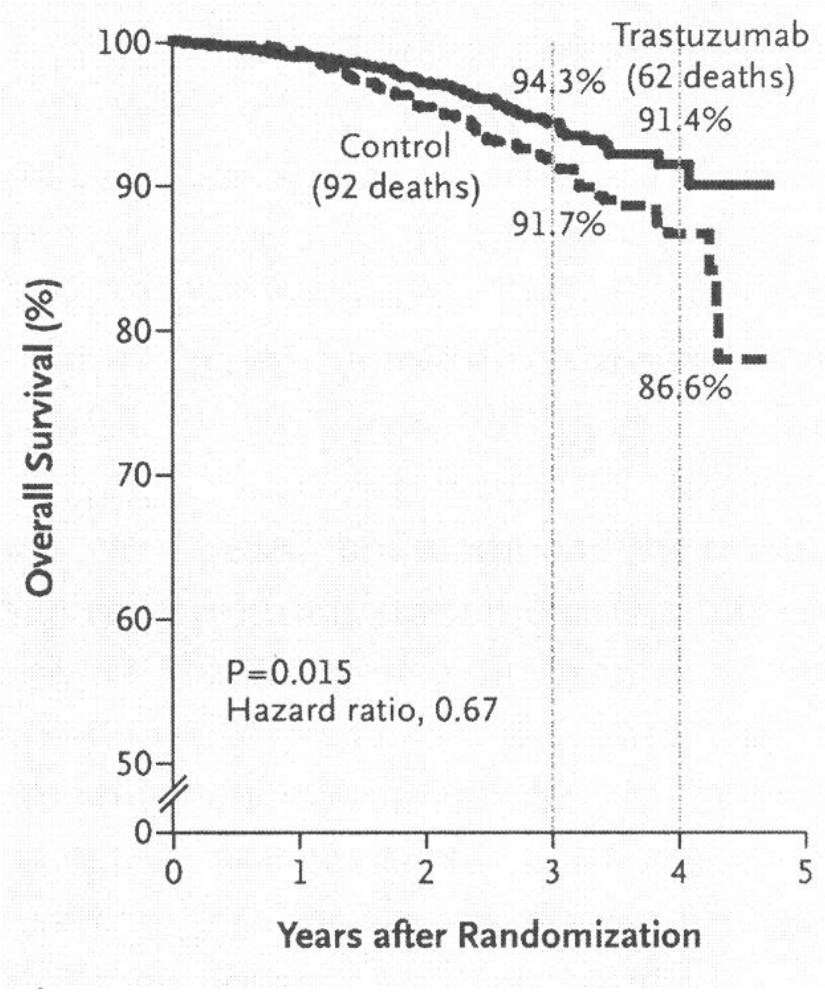
Trastuzumab



- Trastuzumab
  - therapeutic humanised monoclonal antibody specifically designed to target HER2
  - active in HER2-positive breast cancer patients
- HER2 positivity is the criterion to select patients for Trastuzumab therapy
  - strong overexpression of the HER2 protein on the cell surface
  - HER2 gene amplification

Trastuzumab in HER2+ Breast Cancer





NEJM 353:1673,2005

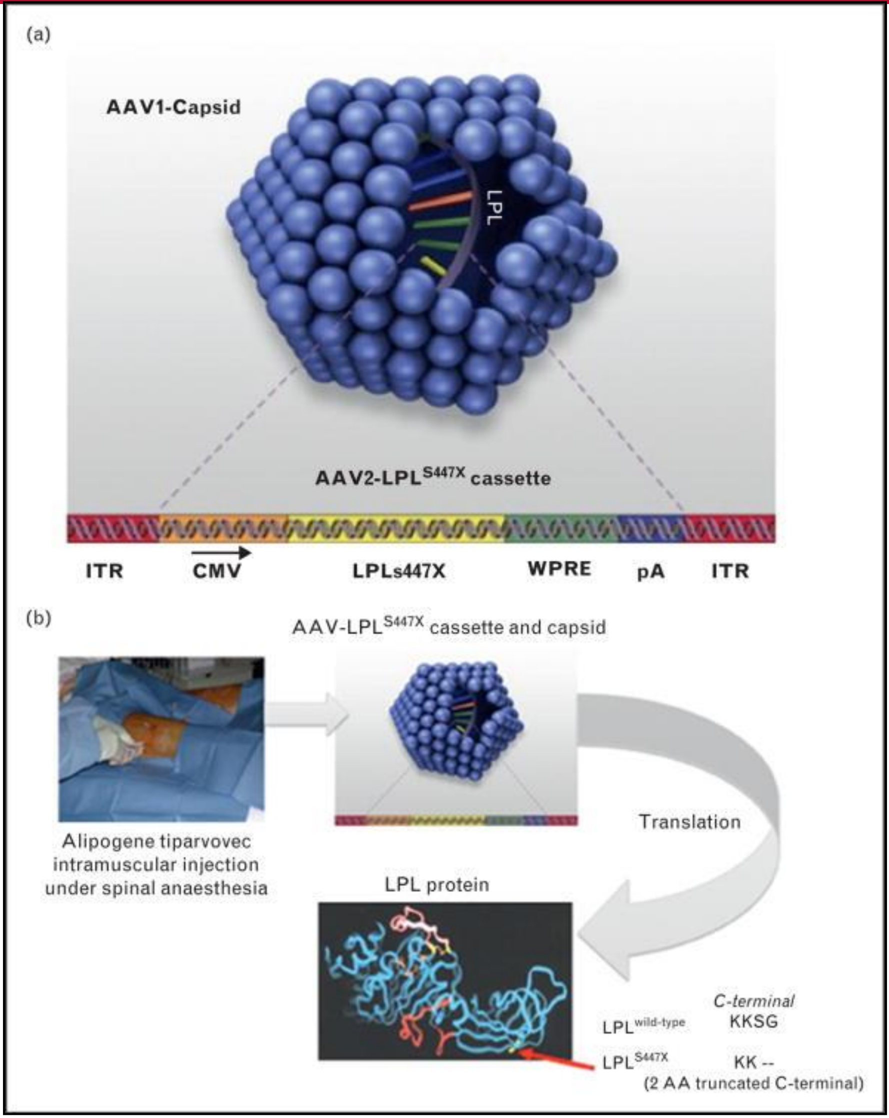
Gene therapy

Alipogene tiparvovec

July 2012 - EMA approved the first gene therapy

Cost: €1.000.000
Only 1 patient treated

August 2017: decision to not apply for the license to be renewed at expiration (25 October 2017)

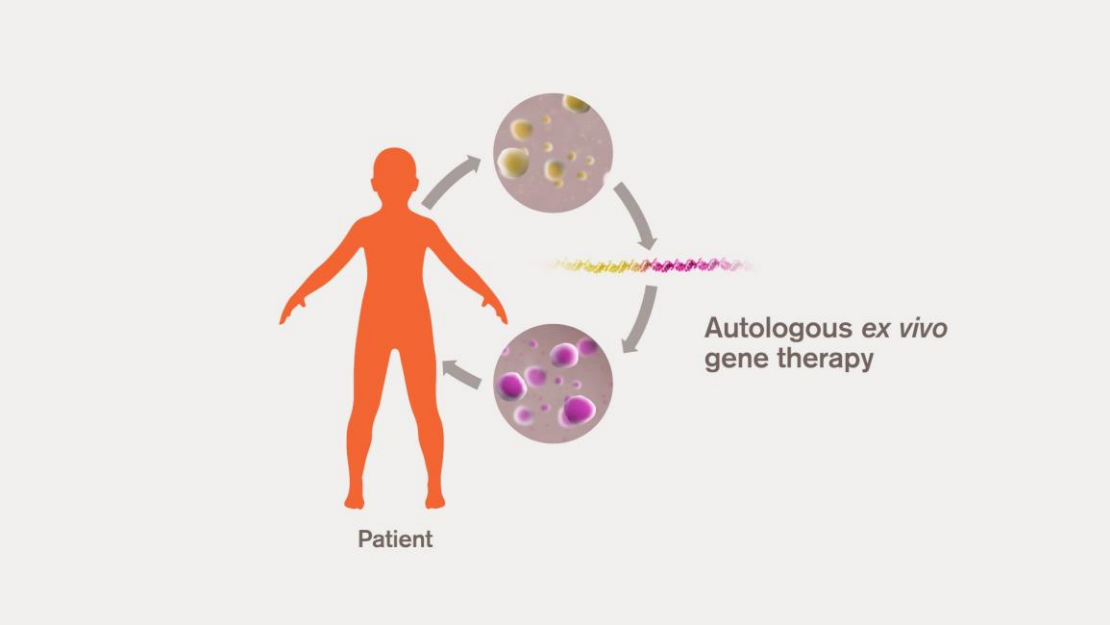


Current Opinion in Lipidology. 23(4):310-320, August 2012.

Current Opinion in Lipidology. 23(4):310-320, August 2012.

Gene therapy

May 2016 - EMA approval for Strimvelis, an ex-vivo stem cell gene therapy for a very rare disease called ADA-SCID



Cost: €594,000

Advanced Therapy Medicinal Products

	Product	Company	Therapeutic area	Active substance
2016	Strimvelis	GSK-(Telethon)	Severe Combined Immunodeficiency	Autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human adenosine deaminase (ADA) cDNA sequence from human haematopoietic stem/progenitor (CD34+) cells
2016	Zalmoxis	MolMed	GVH Disease Hematopoietic Stem Cell Transplantation	Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)
2015	Holoclar	Chiesi	Corneal Diseases Stemm cell transplantation	Ex vivo expanded autologous human corneal epithelial cells containing stem cells
2015	Imlygic	Amgen	Unresectable metastatic melanoma	An oncolytic virus derived from HSV-1 modified to replicate within tumours and to produce the immune stimulatory protein human GM-CSF, which promotes a systemic anti-tumour immune response and an effector T-cell response

Orphan Drugs

Drug	Disease	Annual Cost
Eculizumab (Soliris)	Paroxysmal nocturnal hemoglobinuria	$409,500
Idursulfase (Elaprase)	Mucopolysaccharidosis II (Hunter syndrome)	$375,000
Galsulfase (Naglazyme)	Mucopolysaccharidosis VI (Maroteaux-Larry syndrome)	$365,000
C1 esterase inhibitor [human] (Cinryze)	Hereditary angioedema	$350,000
Alglucosidase alfa (Myozyme)	Pompe disease	$300,000

- The 5 most expensive drugs in the world are orphan drugs
- 25-25 millions rare disease patients in the US
- Is the bill affordable?

JACC 62:2185,2013

Biosimilars

With the possible exception of small peptides, the concept of generics cannot be extrapolated to biopharmaceuticals product

Biosimilar
Follow-on biologic

“a medicine which is similar to a biological medicine that has already been authorized”