The background features a light purple-to-blue gradient. Scattered across the surface are numerous realistic water droplets of various sizes, some with highlights and shadows. A faint, glowing DNA double helix is visible in the center, serving as a subtle backdrop for the text.

# BIOLOGICAL THERAPIES

## PROTEIN THERAPEUTICS

KATALIN OLASZ

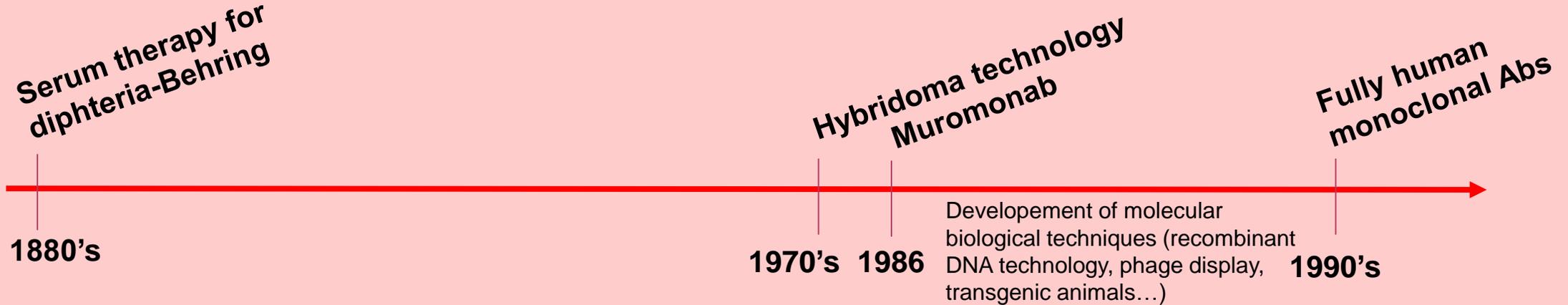
*2026. 02. 19.*

# HISTORICAL BACKGROUND

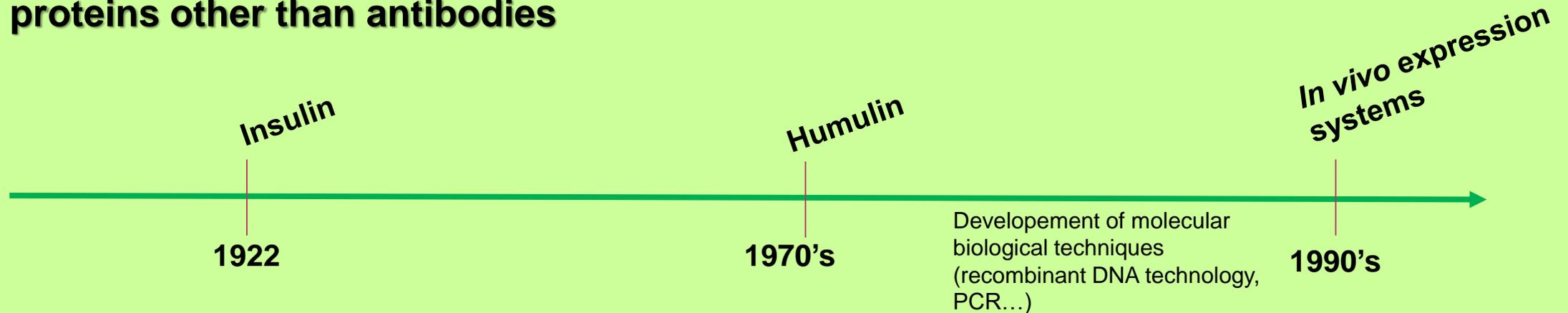
- **Proteins**, e.g., albumin from egg whites, blood serum albumin, fibrin, and wheat gluten, were recognized **in the eighteenth century as biological molecules with distinct properties** mostly by their ability to coagulate under treatments with heat or acid
- The term “**protein**” to describe these molecules was proposed in 1838 by **Jöns Jakob Berzelius**

# HISTORICAL BACKGROUND

## Paradigm changes for therapeutic antibodies



## Paradigm changes for therapeutic proteins other than antibodies



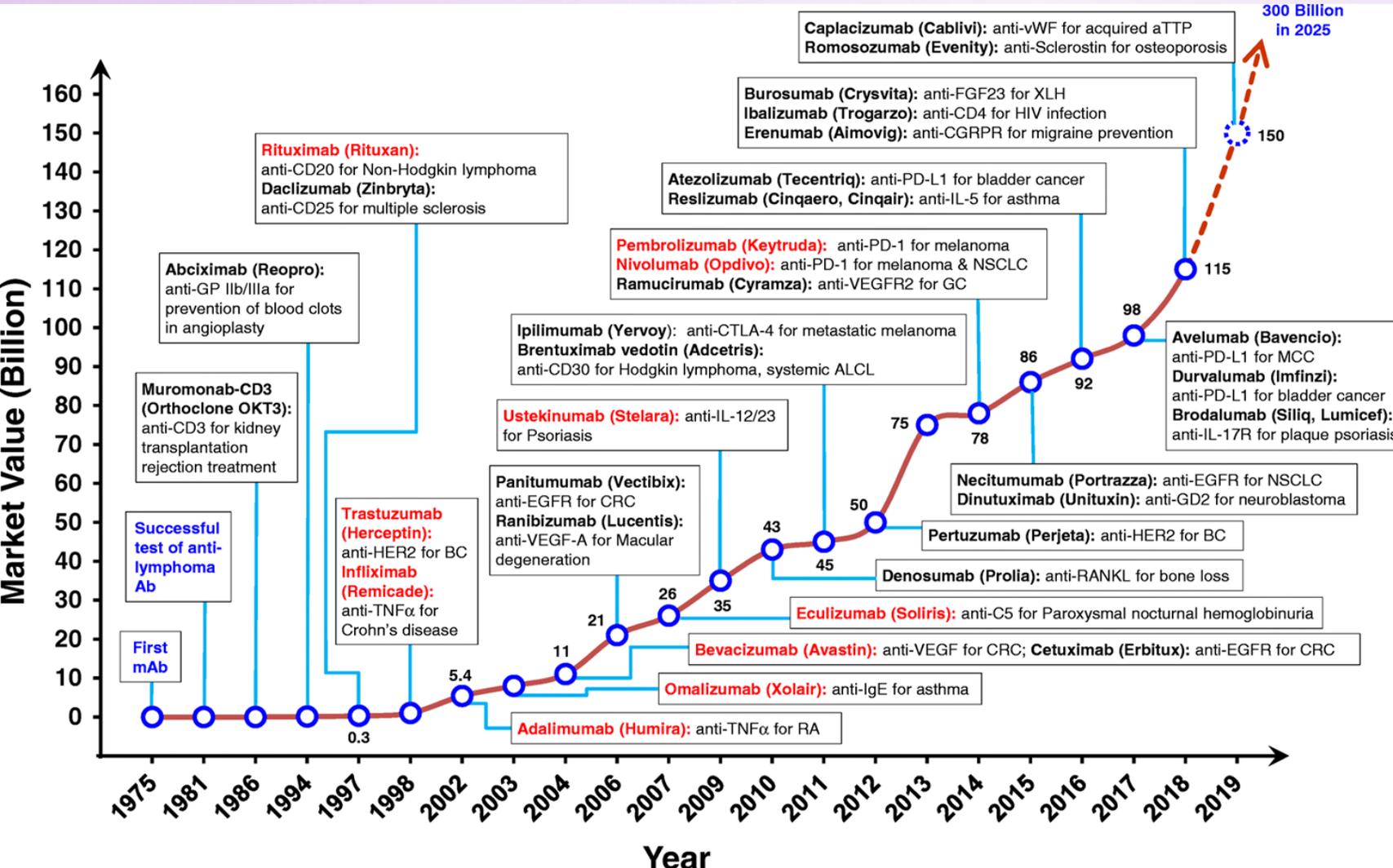
- **Protein therapeutics** have **increased** dramatically in **number and frequency of use** since the introduction of the first recombinant protein therapeutic — Humulin (human insulin) — about 50 years ago
- Protein therapeutics already have a significant role **in almost every field of medicine**, but this role is still only in its infancy
- **Around 200 protein therapeutics** are used currently

# PROTEINS

Proteins have the most dynamic and diverse roles of any macromolecule in the body:

- Catalyse biochemical reactions
- Form receptors and channels in membranes
- Provide intracellular and extracellular scaffolding support
- Transport molecules within a cell or from one organ to another
- ~20,000 different genes in the human genome. But because of alternative splicing of genes and post-translational modification of proteins (e.g., cleavage, phosphorylation, acylation, glycosylation), the number of functionally distinct proteins is likely to be much higher

# PROTEIN THERAPEUTICS



- Protein-based therapeutics are highly successful in clinic
- More than 100 genuine and similar number of modified therapeutic proteins are approved for clinical use in the European Union and the USA with 2010 sales of US \$108 bln; monoclonal antibodies (mAbs) accounted for almost half (48%) of the sales
- Market reports in 2025: the market hit the 304-305 billion USD in 2025!!!!

# CLASSIFICATION BASED ON THEIR PHARMACOLOGICAL ACTIVITY

- I. Replacing a protein that is deficient or abnormal
- II. Augmenting an existing pathway
- III. Providing a novel function or activity
- IV. Interfering with a molecule or organism
- V. Delivering other compounds or proteins, such as a radionuclide, cytotoxic drug, or effector proteins

# CLASSIFICATION BASED ON THEIR MOLECULAR TYPES AND MECHANISM OF ACTIVITY

## Molecular type

1. Antibody-based drugs
2. Fc fusion proteins
3. Anticoagulants
4. Blood factors
5. Bone morphogenetic proteins
6. Engineered protein scaffolds
7. Enzymes
8. Growth factors
9. Hormones
10. Interferons
11. Interleukins
12. Thrombolytics

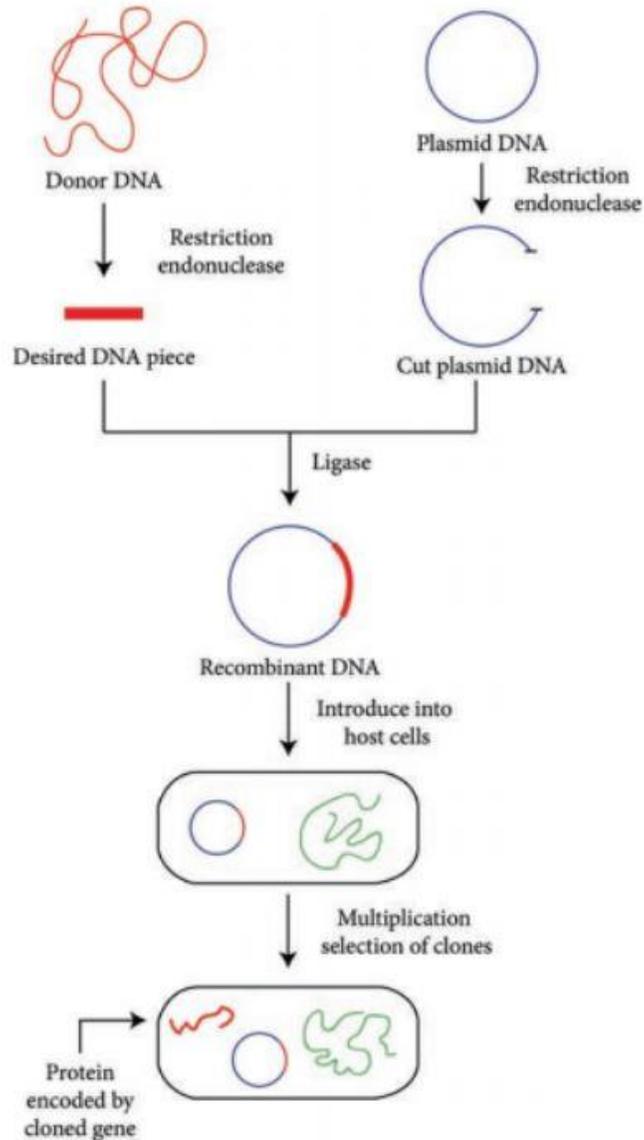
## Molecular mechanism of activity

1. Binding non-covalently to target (**73** genuine unmodified proteins including **29 mAbs**)
2. Effecting covalent bonds (**21** enzymes)
3. Exerting activity without specific interactions (serum albumin)

**Most selling therapeutic protein group!!**

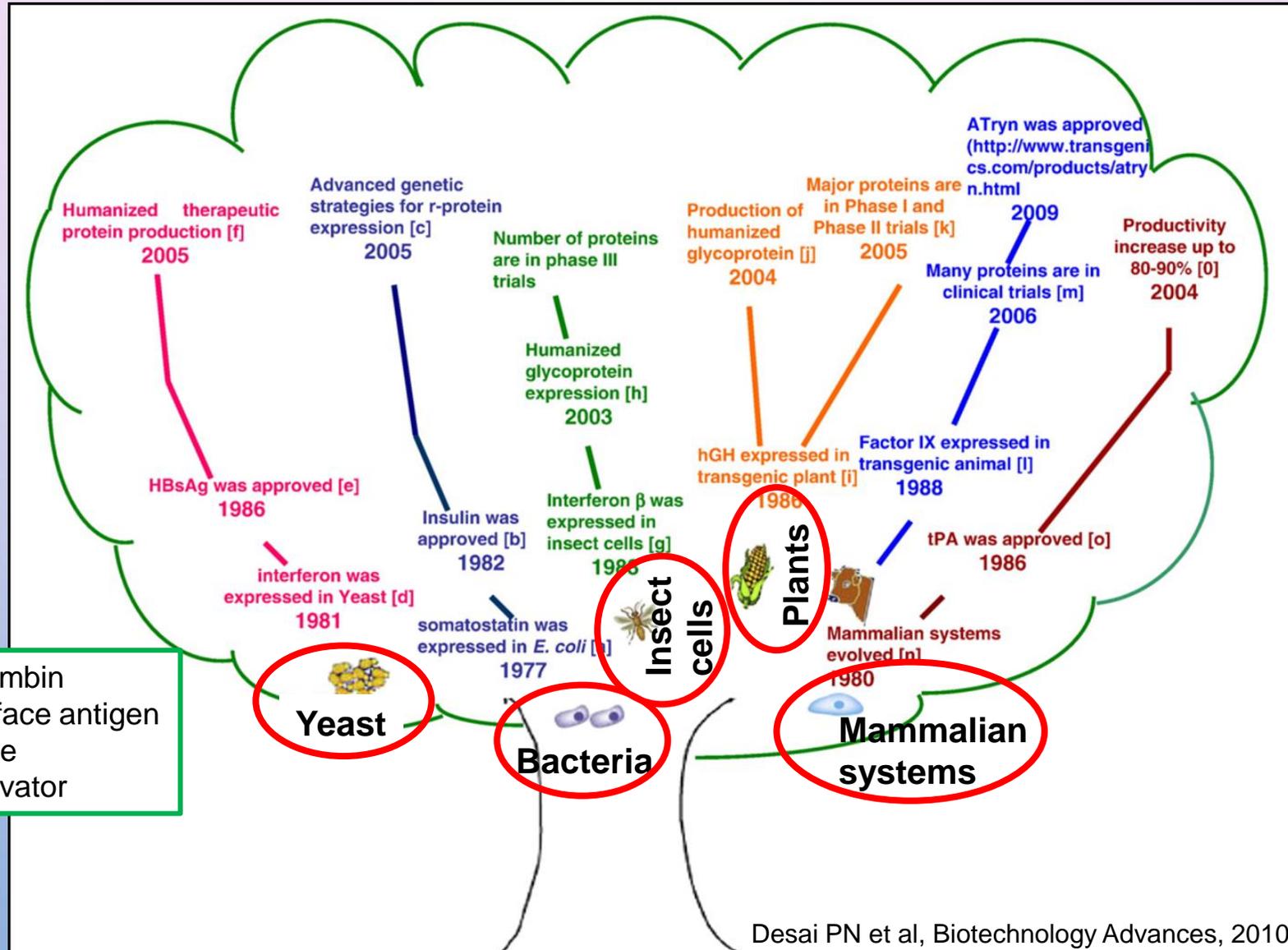
If group of **polyclonal antibodies** (either nonspecific pooled human immunoglobulin (Ig) or specific Ig) are included as therapeutic proteins, then the total number of genuine therapeutic proteins exceeds 100.

# PRINCIPLES OF RECOMBINANT DNA TECHNOLOGY



1. Isolation of DNA from the source (Donor)
2. Generation of DNA fragments and selection of the desired piece of DNA
3. Insertion of the selected DNA into a cloning vector (Example: a plasmid) to create a recombinant DNA or chimeric DNA.
4. Introduction of the recombinant vectors into host cells (Example: bacteria)
5. Multiplication and selection of clones containing the recombinant molecules
6. Expression of the gene to produce the desired product.

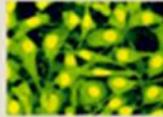
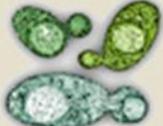
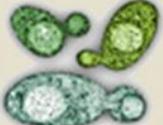
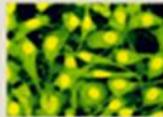
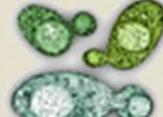
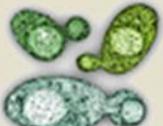
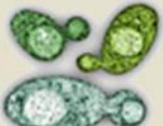
# MILESTONES IN ADVANCES OF VARIOUS EXPRESSION SYSTEMS



ATryn, Anticoagulant antithrombin  
 HBsAg, Hepatitis B virus surface antigen  
 hGH, Human growth hormone  
 tPA, Tissue plasminogen activator

# IN VIVO EXPRESSION SYSTEMS

- First recombinant human drug, insulin, was synthesized using recombinant *E. coli*
- The majority of the recombinant proteins are produced by using mammalian (Chinese Hamster Ovary cells) and microbial expression systems (*E. coli* and *Saccharomyces cerevisiae*) with more than 50% of biopharmaceuticals being produced by microbial factories.

	LOW			HIGH
<b>SPEED</b>	 MAMMALIAN	 BEVS/INSECT CELL	 YEAST	 BACTERIA
<b>COST</b>	 BACTERIA	 YEAST	 BEVS/INSECT CELL	 MAMMALIAN
<b>TYPICAL YIELD</b>	 MAMMALIAN	 BEVS/INSECT CELL	 BACTERIA	 YEAST
<b>POST – TRANSLATION MODIFICATION</b>	 BACTERIA	 YEAST	 BEVS/INSECT CELL	 MAMMALIAN
<b>FDA APPROVAL</b>	 BEVS/INSECT CELL	 YEAST	 BACTERIA	 MAMMALIAN

# CLASSIFICATION BASED ON THEIR PHARMACOLOGICAL ACTIVITY

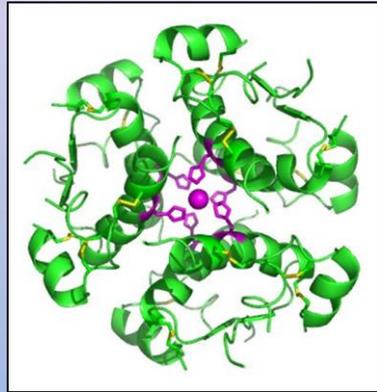
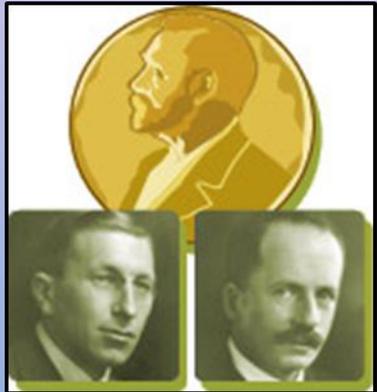
## **I. Replacing a protein that is deficient or abnormal**

- II. Augmenting an existing pathway
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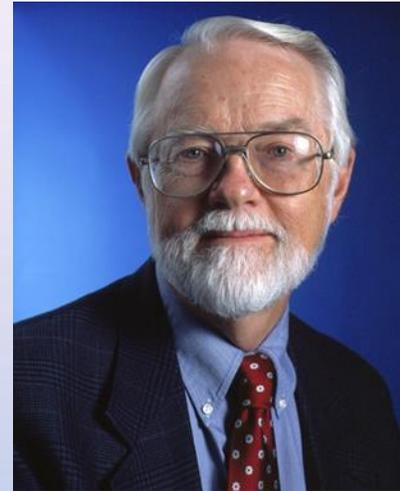
# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

## I/1. Endocrine disorders (hormone deficiency)

**Insulin:** A peptide hormone, produced by beta cells of the pancreas. Central to regulating carbohydrate and fat metabolism in the body.



Frederick G. **Banting** and John **Macleod** were awarded the **Nobel Prize** in Physiology or Medicine in 1923 "for the **discovery of insulin.**"



1977 - The **first genetically engineered, synthetic "human" insulin** was produced in laboratory by **Arthur D. Riggs** and K. Itakura at City of Hope and H. Boyer at Genentech.

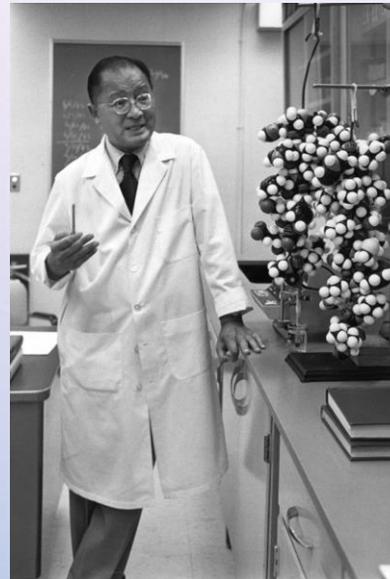


# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

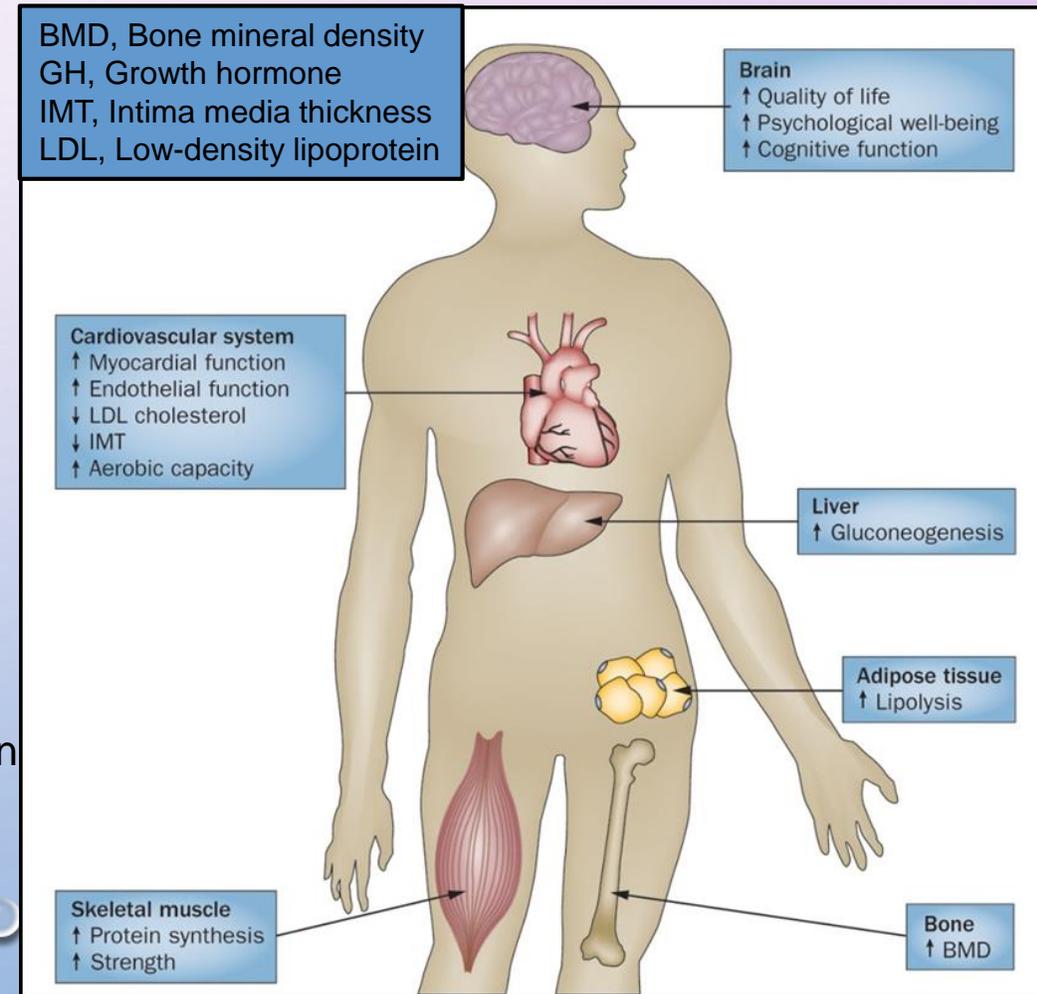
## I/1. Endocrine disorders (hormone deficiency)

### Growth hormone (GH) or somatotropin:

- secreted by pituitary gland
- peptide hormone that stimulates growth, cell reproduction, and cell regeneration
- can be used by children's growth disorders and adult growth hormone deficiency
- „*Performance enhancement*” in sports: improve the athletic performance of professional male athletes → athletic societies ban the use of GH



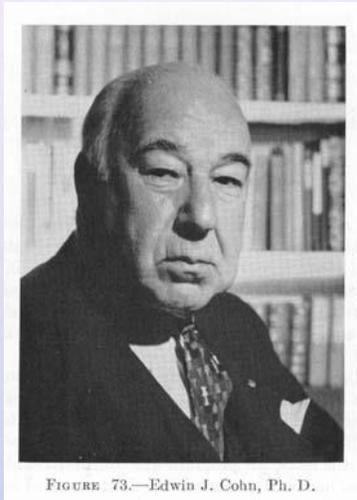
**Choh Hao Li:** discovered the human growth hormone (1966), (recombinant production in E. Coli from 1985)



# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

## I/2. Haemeostasis and thrombosis

Dr. Edwin Cohn: in 1940's developed the fractionation process"



Plasma fractionation: modifying the

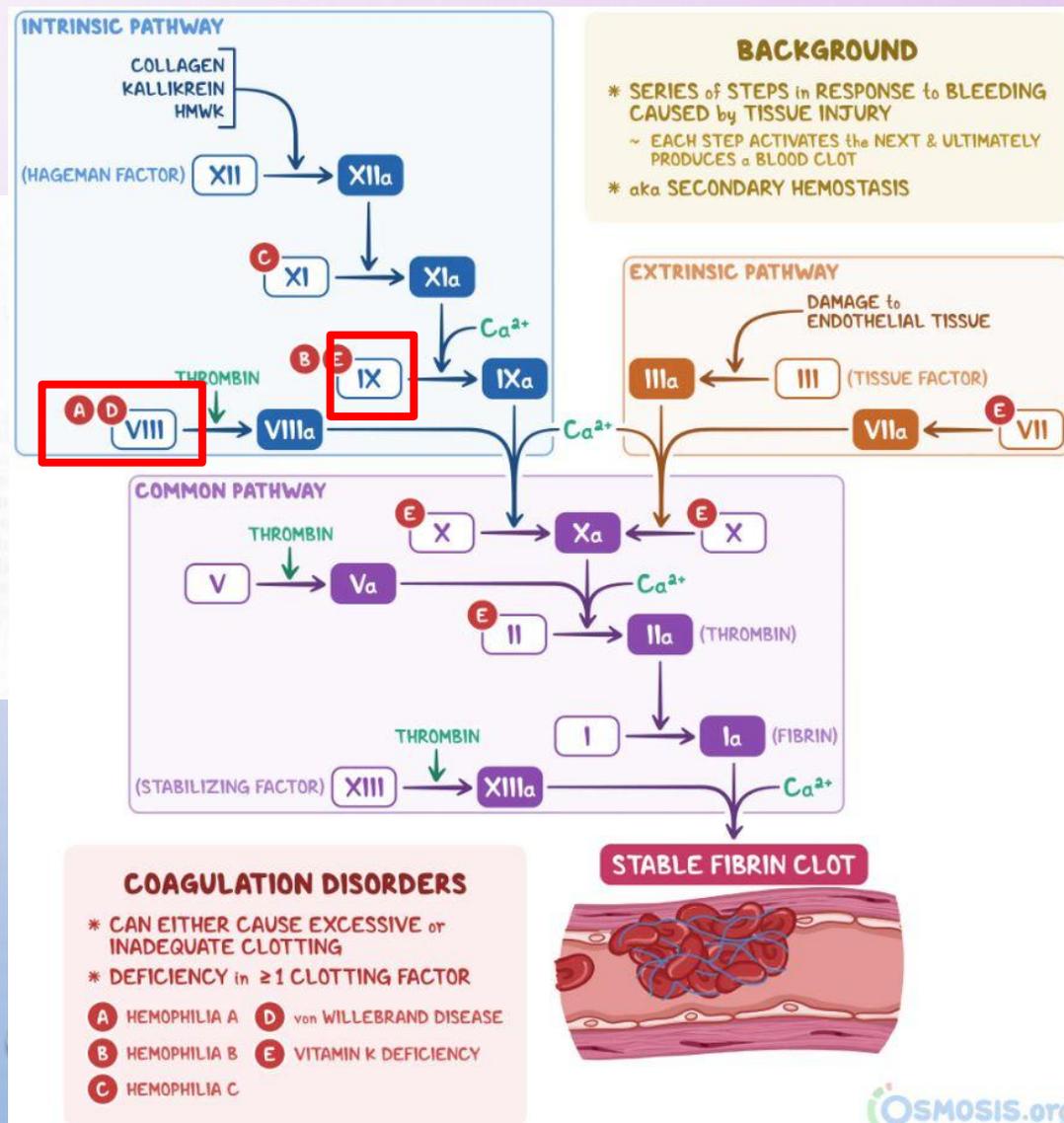
- pH of the plasma
- ethanol concentration of the plasma
- temperature of the plasma

precipitation

“cryoprecipitate”

factor VIII and IX

five “fractions” (IVIG, albumin, A1AT, antithrombin III)



# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

## I/3. Metabolic enzyme deficiencies

**Lactose intolerance:** occurs in people who lack the enzyme they need to break down lactose, the sugar in milk. It causes digestive distress when you eat dairy products. (A food intolerance is different from a food allergy!)

**Lactase** enzyme helps to digest lactose.



**Exocrine pancreatic insufficiency (EPI):** is a condition which occurs when the pancreas does not make enough of a specific enzyme the body uses to digest food in the small intestine. People with EPI don't have enough pancreatic (digestive) enzymes to break down foods and absorb nutrients. It can lead to malnutrition. Pancreatic enzyme replacement therapy can help

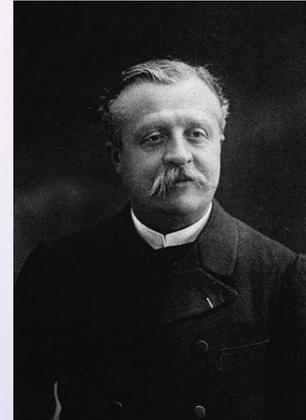


# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

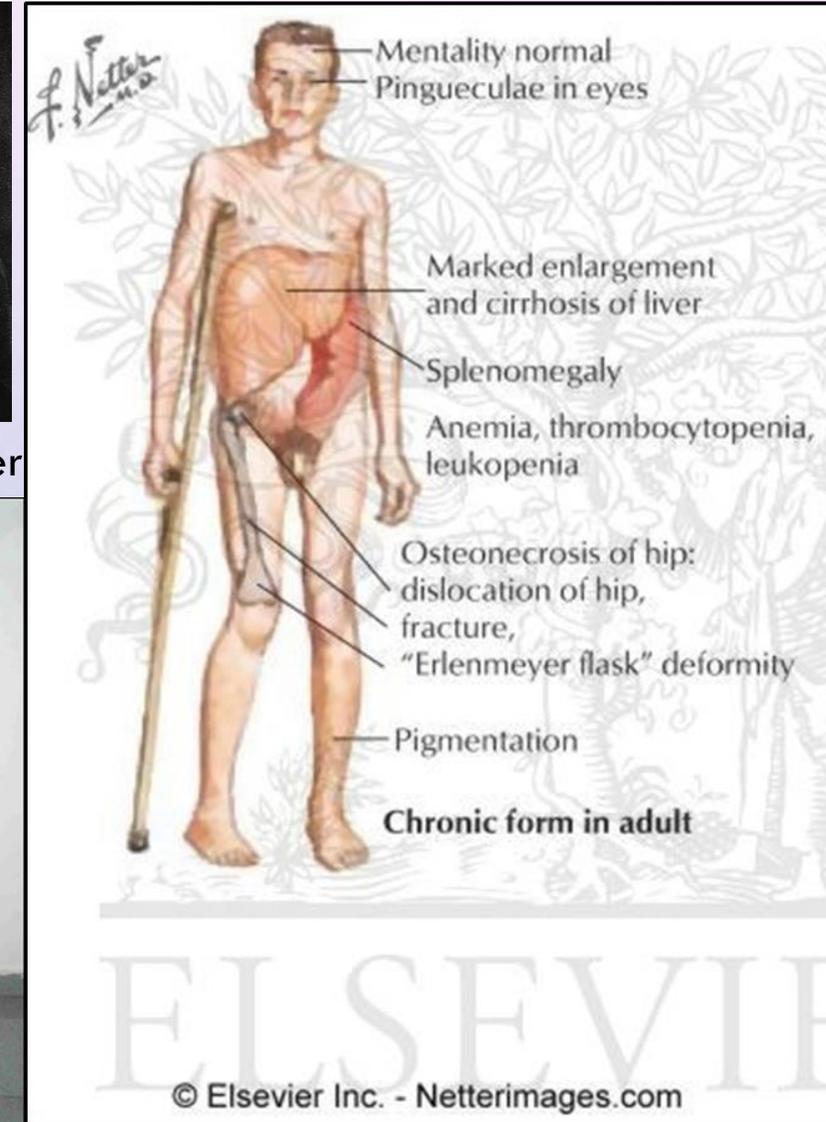
## I/4. Metabolic enzyme deficiencies

### Gaucher's disease:

- the most common of the **lysosomal storage diseases**. It is a form of sphingolipidosis, as it involves dysfunctional metabolism of sphingolipids.
- caused by a **recessive mutation** in the GBA (glucocerebrosidase) gene located on chromosome 1
- was described by a french physician **Philippe Gaucher**, who originally described it in 1882



Philippe Gaucher



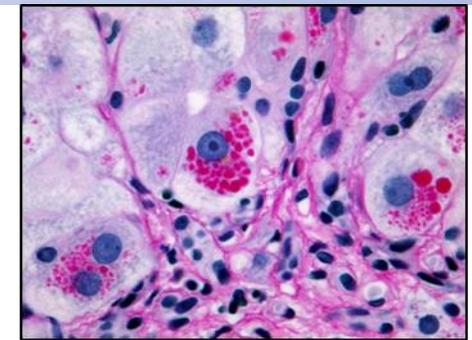
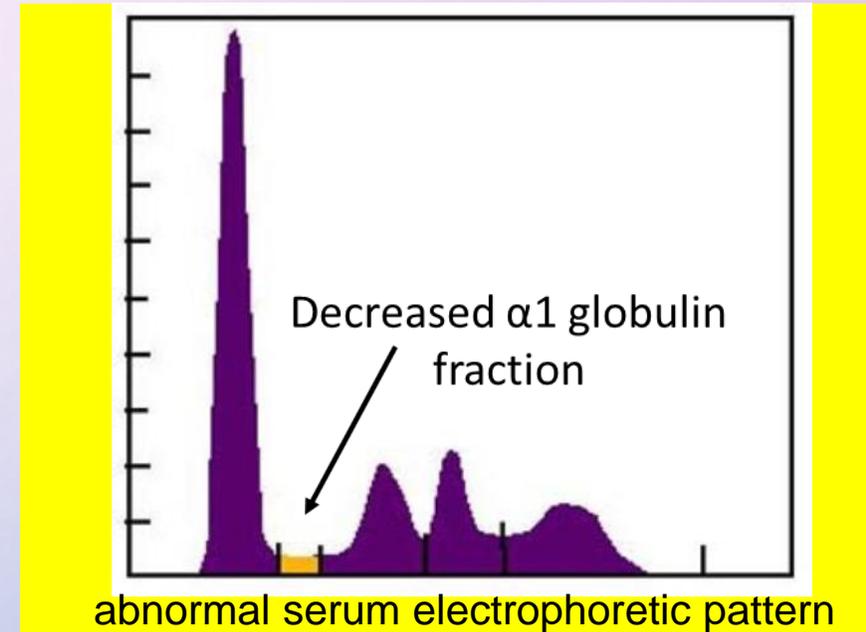
# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

## I/5. Pulmonary and gastrointestinal disorders

### $\alpha$ -1-antitrypsin deficiency:

- Genetic disorder
- Liver cells are unable to secrete  $\alpha$ 1-antitrypsin which accumulates in their cytoplasm
- The level of  $\alpha$ 1-antitrypsin greatly decreases in the blood which will lead to complications

- 
- **Liver damage** (because of A1AT deposition)
  - **Damage of the lungs** (inflammatory reactions will cause serious tissue damage without the inhibitory effects of A1AT)
  - **Chronic pancreatitis** (because of the absence of A1AT)



Accumulated A1AT can be seen as PAS-positive granules inside hepatocytes.

# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

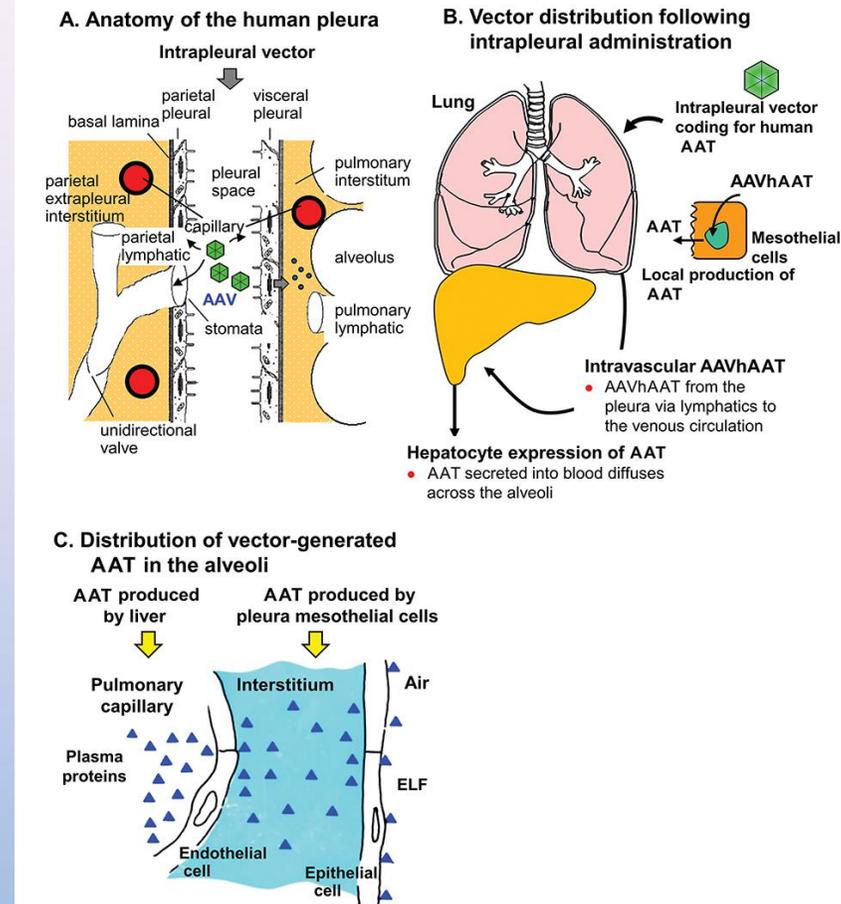
**Current therapy for  $\alpha$ -1-antitrypsin deficiency:** supplement the levels of plasma AAT (plasma fractionation)

Weekly intravenous infusions of 60 mg/kg of AAT purified from pooled human plasma.

Problems of patient compliance and risks of allergic reactions, viral contamination, or limitations in available supply.

## Gene Therapy for Alpha-1 Antitrypsin Deficiency

Figure 2. Intrapleural Administration of an Adeno-Associated Virus Vector Coding for Alpha-1 Antitrypsin



A. Anatomy of the lung pleura. B. Vector distribution following intrapleural administration, combining local lung delivery via vector transduction of mesothelial cells lining the pleura, and systemic delivery via vector leaking to the systemic venous system and then primarily to liver hepatocytes. C. Delivery to the alveoli of AAT produced by AAV gene therapy to the pleura. The endothelial junctions are relatively loose, such that the levels of AAT (MW 52 kDa) in the interstitium are 60% of that in plasma. The epithelial junctions are tight, resulting in ELF AAT levels 5% to 10% of plasma. The locally (mesothelial cell) expressed AAT is delivered directly to the alveolar interstitium, while the liver (hepatocyte) expressed AAT diffuses from plasma to the interstitium, and then to alveolar ELF.

AAV=adeno-associated virus; ELF=epithelial lining fluid; AAT=alpha-1 antitrypsin

<https://journal.copdfoundation.org/jcopdf/id/1202/Intrapleural-Gene-Therapy-for-Alpha-1-Antitrypsin-Deficiency-Related-Lung-Disease>

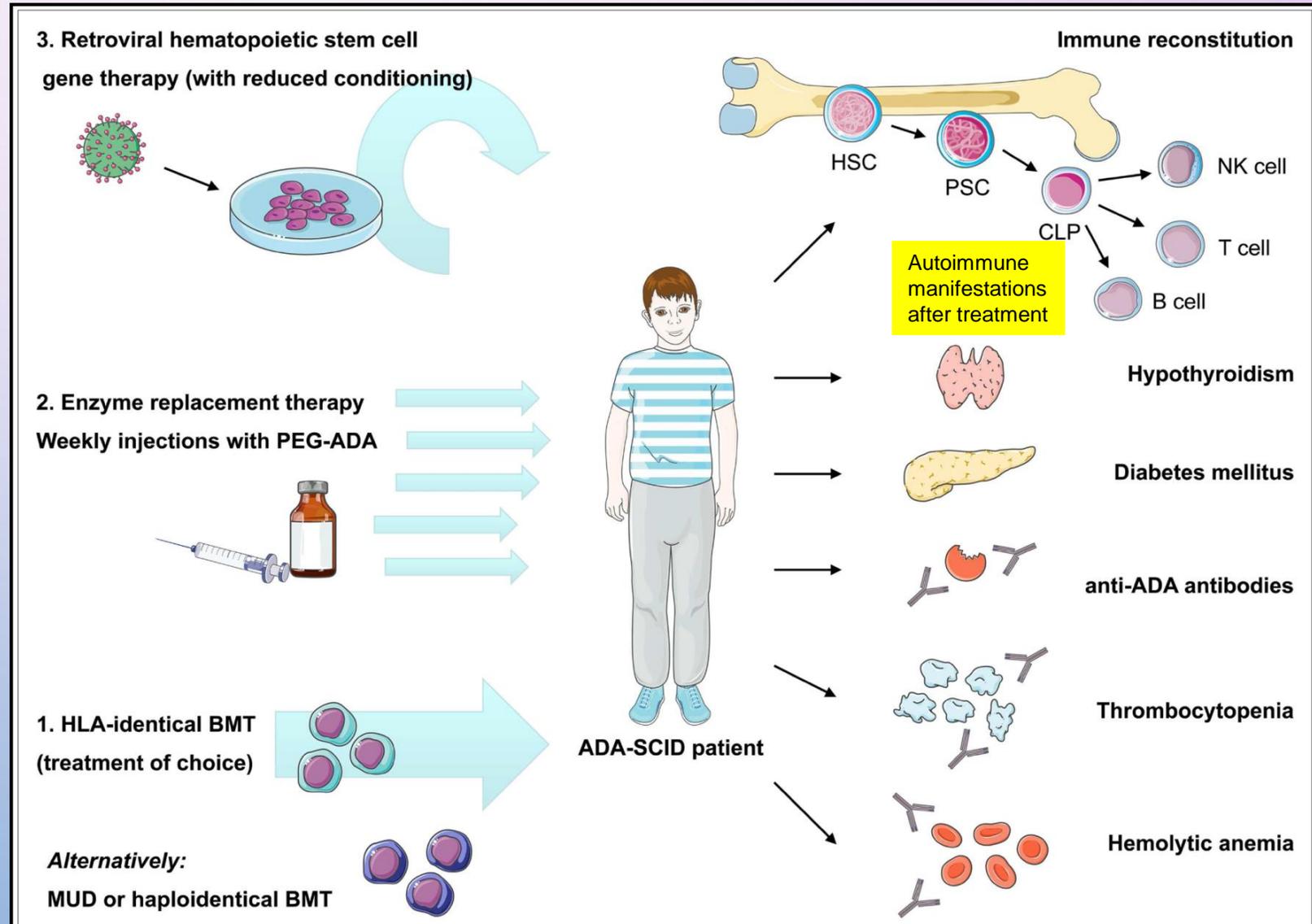
# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

## I/6. Immunodeficiencies

### ADA or adenosine deaminase

- is an enzyme involved in purine metabolism.
- has a role in the development and maintenance of the immune system.
- deficiency is one cause of **severe combined immunodeficiency (SCID)**

(MUD, Matched unrelated donor)



# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

## I/7. Immunodeficiencies

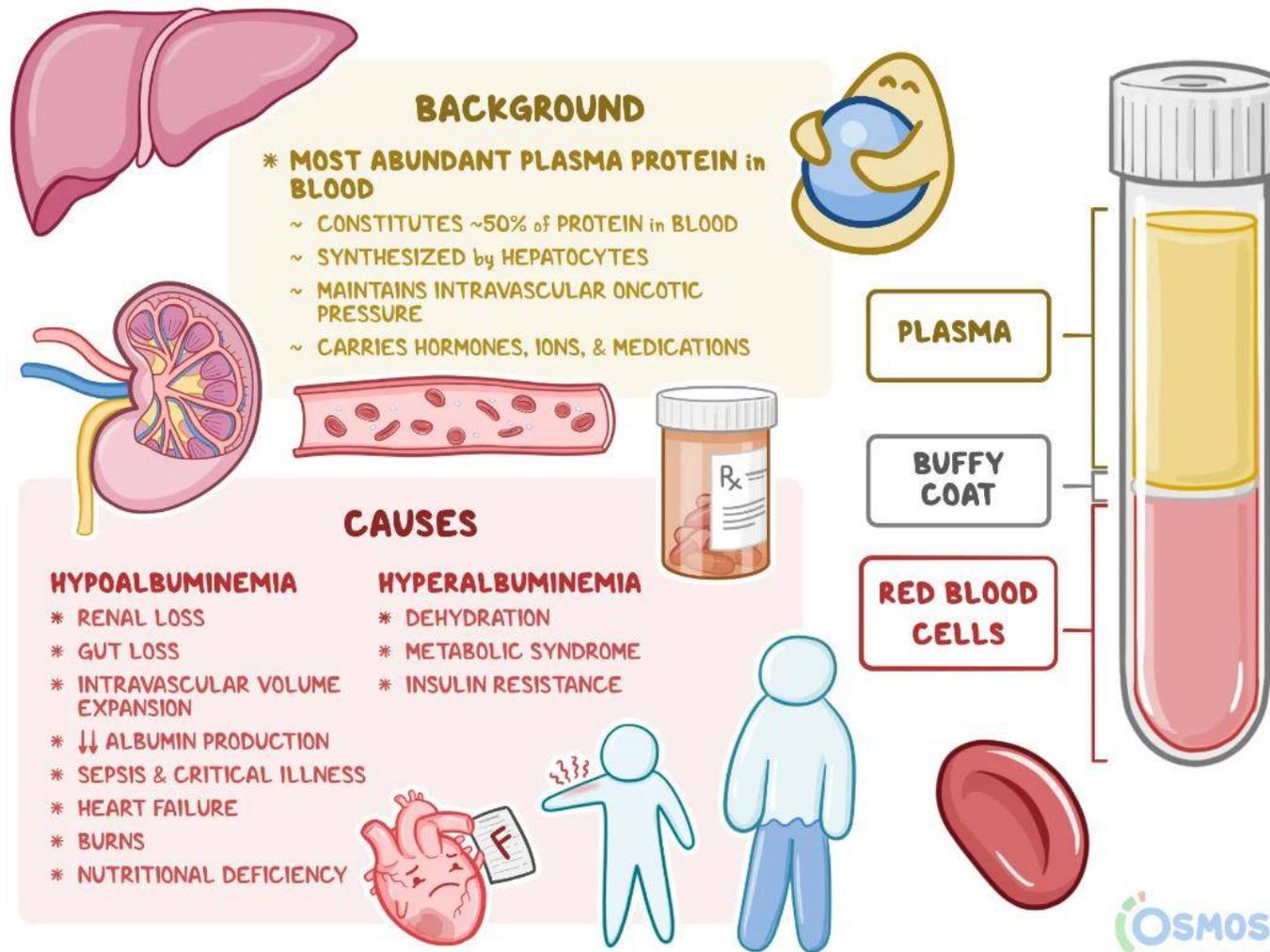
### Primary immunodeficiencies:

- Chronic Granulomatous Disease (CGD)
- Common Variable Immunodeficiency (CVID)
- Congenital Neutropenia Syndromes
- Severe Combined Immunodeficiency (SCID)
- X-Linked Agammaglobulinemia (XLA)
- Hyper-Immunoglobulin E Syndromes (HIES)



# I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

## 1/8. Other Albumin:



# CLASSIFICATION BASED ON THEIR PHARMACOLOGICAL ACTIVITY

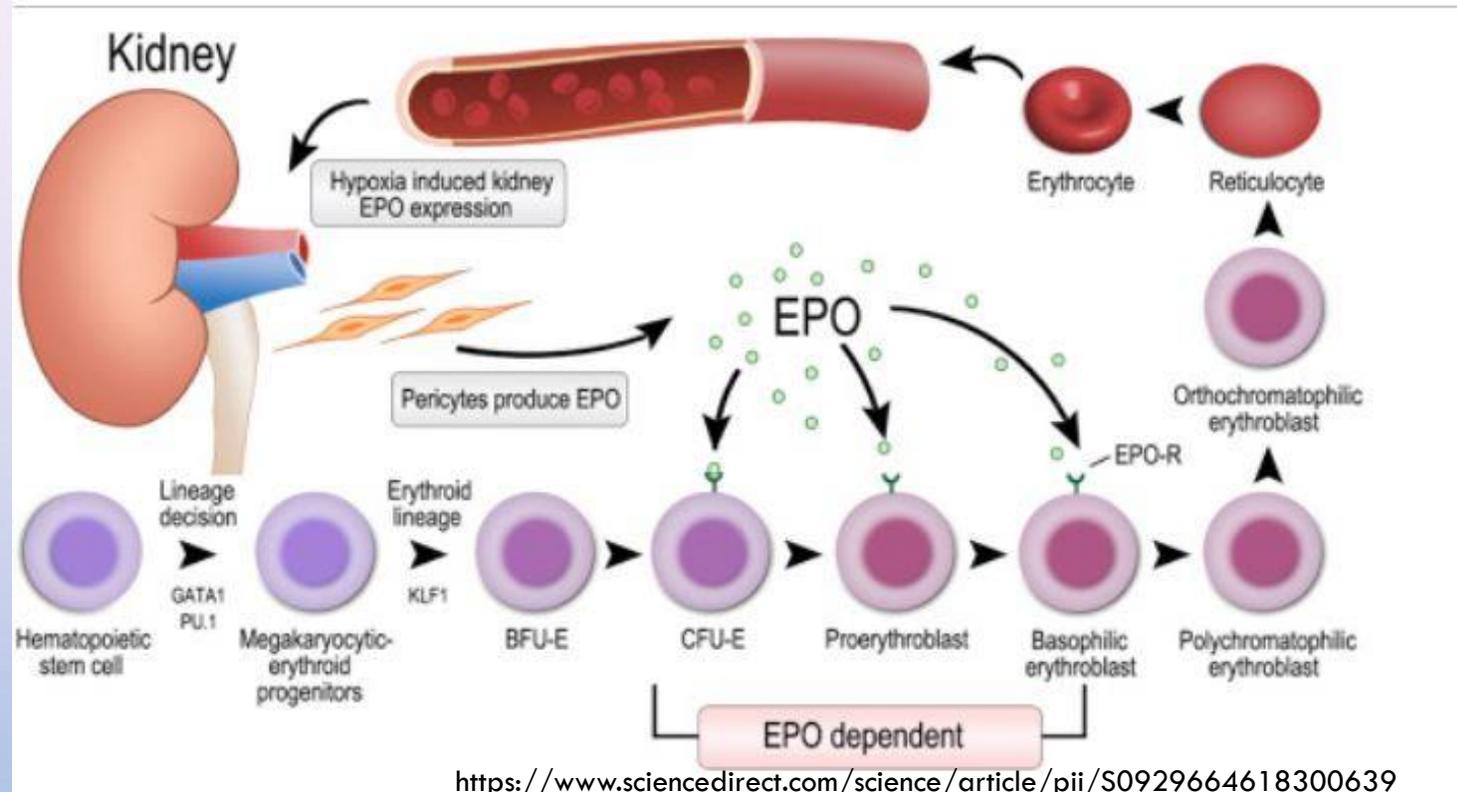
- I. Replacing a protein that is deficient or abnormal
- II. Augmenting an existing pathway**
- III. Providing a novel function or activity
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- V. Delivering other compounds or proteins, such as a radionuclide, cytotoxic drug, or effector proteins

## II. AUGMENTING AN EXISTING PATHWAY

### II/1. Hematopoiesis

#### Erythropoietin:

- A glycoprotein hormone, the master regulator of **erythropoiesis**.
- Anemia is a common complication and contributes to increased morbidity and mortality in **chronic kidney disease (CKD)** patients
- **recombinant human erythropoietin (rhEPO)**, is produced by recombinant DNA technology, and are collectively called **erythropoiesis-stimulating agents (ESA)**



## II. AUGMENTING AN EXISTING PATHWAY

### Epogen

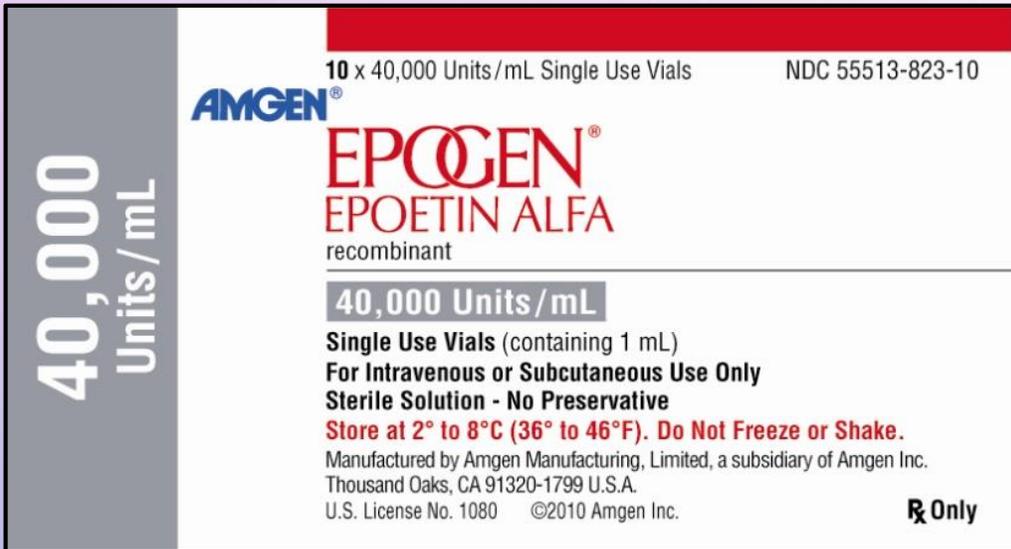
Stimulates erythropoiesis.

Anemia of chronic disease,  
myelodysplasia,  
anemia due to renal failure or  
chemotherapy,  
preoperative preparation.

### Neupogen

Stimulates neutrophil proliferation,  
differentiation and migration.

Neutropenia in AIDS or post-chemotherapy  
or bone-marrow transplantation, severe  
chronic neutropenia.



<https://dailymed.nlm.nih.gov/dailymed/fda/image.cfm?id=138205&name=epogen-7.jpg>



<http://dailymed.nlm.nih.gov/dailymed/archives/image.cfm?archiveid=132986&type=img&name=neupogen-21.jpg>

# II. AUGMENTING AN EXISTING PATHWAY

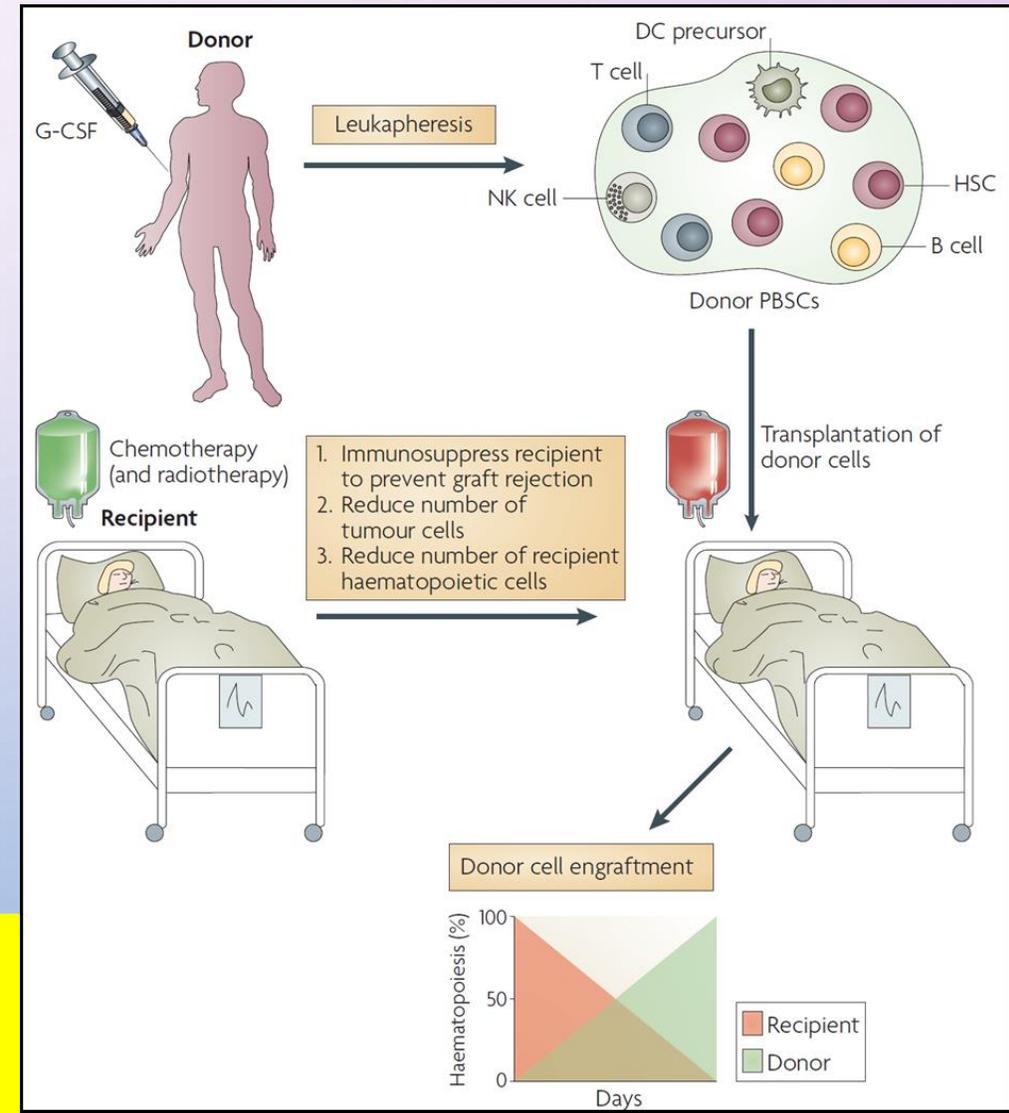
Schlomchik WG, Nature Rev Immunol, 2007

## II/1. Hematopoiesis

- Allogeneic peripheral blood hematopoietic stem cell transplantation

1. Acute leukemia
2. Aplastic anemia
3. Chronic leukemia
4. Immune deficiencies
5. Multiple myeloma
6. Hodgkin's lymphoma
7. Non-Hodgkin's lymphoma

**DC**, Dendritic cell  
**G-CSF**, Granulocyte colony-stimulating factor  
**HSC**, Hematopoietic stem cell  
**PBSC**, Peripheral-blood stem cells



# II. AUGMENTING AN EXISTING PATHWAY

## II/2. Fertility

Assisted reproduction

Augments ovulation



Estradiol ↑  
Follicular dev. ↑

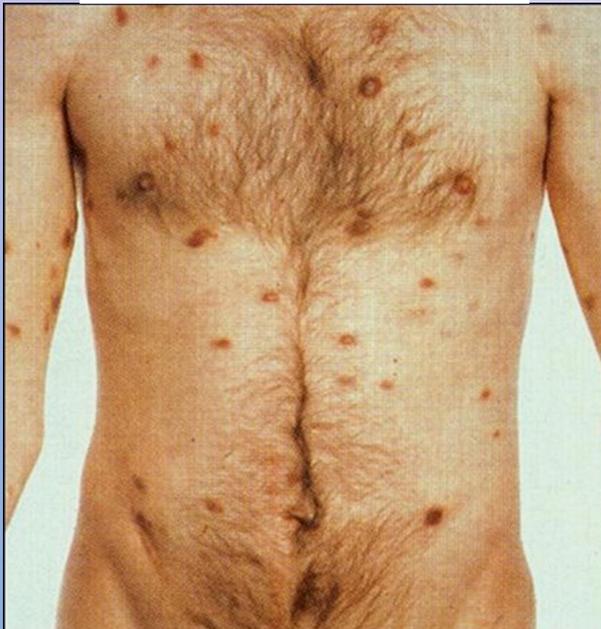
Infertility because of luteinising hormone deficiency

# II. AUGMENTING AN EXISTING PATHWAY

## II/3. Immunoregulation

- to enhance anti-viral immune functions
- treatment of 'active' relapsing MS

### Kaposi's sarcoma



<i>Immunoregulation</i>			
Type I alpha-interferon, interferon alfacon 1, consensus interferon <sup>173-178</sup>	Infergen	Mechanism unknown; immunoregulator	Chronic hepatitis C infection
Interferon- $\alpha$ 2a (IFN $\alpha$ 2a) <sup>179-183</sup>	Roferon-A	Mechanism unknown; immunoregulator	Hairy cell leukaemia, chronic myelogenous leukaemia, Kaposi's sarcoma, chronic hepatitis C infection
PegInterferon- $\alpha$ 2a <sup>184-186</sup>	Pegasys	Recombinant interferon- $\alpha$ 2a conjugated to polyethylene glycol (PEG) to increase half-life	Adults with chronic hepatitis C who have compensated liver disease and who have not been previously treated with IFN $\alpha$ ; used alone or in combination with ribavirin
Interferon- $\alpha$ 2b (IFN $\alpha$ 2b) <sup>187-189</sup>	Intron A	Mechanism unknown; immunoregulator	Hepatitis B, melanoma, Kaposi's sarcoma, follicular lymphoma, hairy-cell leukaemia, condylomata acuminata, hepatitis C
PegInterferon- $\alpha$ 2b <sup>190</sup>	Peg-Intron	Recombinant interferon- $\alpha$ 2b conjugated to polyethylene glycol (PEG) to increase half-life	Adults with chronic hepatitis C who have compensated liver disease and who have not been treated previously with IFN $\alpha$
†Interferon- $\alpha$ n3 (IFN $\alpha$ n3) <sup>191,192</sup>	Alferon N	Mechanism unknown; nonrecombinant human IFN $\alpha$ -n3 purified from pooled human leukocytes	Condylomata acuminata (genital warts caused by human papillomavirus)
Interferon- $\beta$ 1a (rIFN- $\beta$ ) <sup>178,193-196</sup>	Avonex, Rebif	Mechanism unknown; antiviral and immunoregulator	Multiple sclerosis
Interferon- $\beta$ 1b (rIFN- $\beta$ ) <sup>197-199</sup>	Betaseron	Mechanism unknown; antiviral and immunoregulator	Multiple sclerosis
Interferon- $\gamma$ 1b (IFN $\gamma$ ) <sup>200-204</sup>	Actimmune	Increases inflammatory and antimicrobial response	Chronic granulomatous disease, severe osteopetrosis
Aldesleukin <sup>205-208</sup> (interleukin 2 (IL2), epidermal thymocyte activating factor; ETAF)	Proleukin	Stimulates T and B cells, natural killer cells, and lymphokine-activated killer cells	Metastatic renal cell cancer, melanoma

# CLASSIFICATION BASED ON THEIR PHARMACOLOGICAL ACTIVITY

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# III. PROVIDING A NOVEL FUNCTION OR ACTIVITY

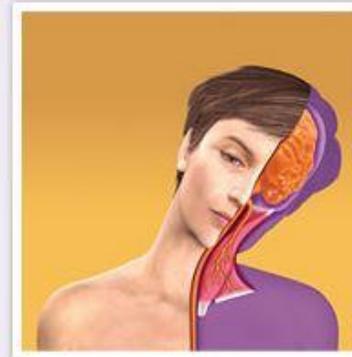
## III/1. Ezymatic degradation of macromolecules

### Botulinum toxin type A and B:

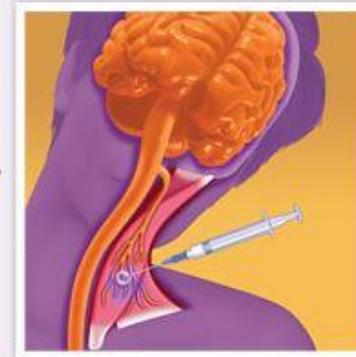
- produced by *Clostridium botulinum*
- cleaves SNAP25
- disrupt SNARE complex
- prevent acetylcholine release
- causing paralysis

Cosmetic use

Clinical use



Muscle contractions associated with Cervical Dystonia are thought to be caused by nerve signals sent from the brain to the affected muscles, telling them to contract or spasm.<sup>2</sup>



BOTOX® works in the muscle where it is injected to block signals that tell the muscle to contract.<sup>1</sup>



As a result, muscle contractions may be reduced.<sup>1</sup>

<https://www.ctspinedoc.com/botox-for-chronic-migraines-cervical-dystonia/>



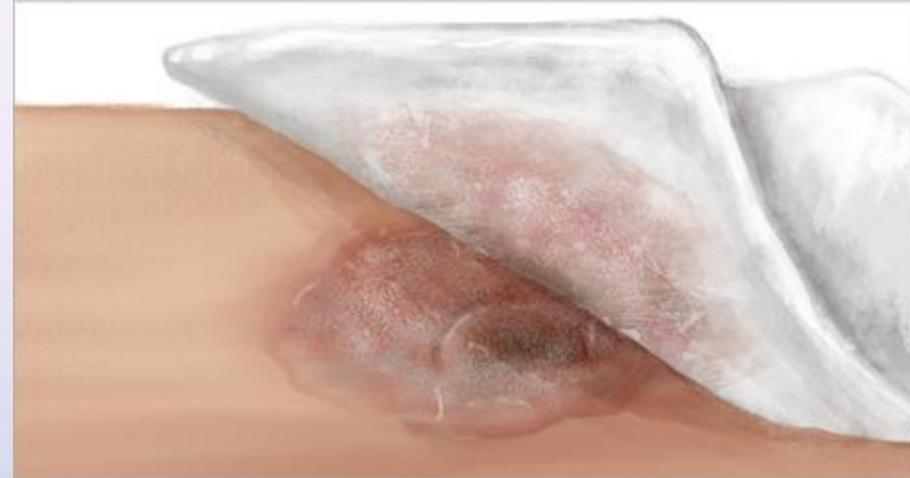
Excessive sweating  
Reduction of facial wrinkles

# III. PROVIDING A NOVEL FUNCTION OR ACTIVITY

## III/1. Ezymatic degradation of macromolecules

### Collagenase:

- obtained from fermentation by *Clostridium hystoliticum*
- digests collagen in necrotic wounds – wound debridement – CCO (clostridial collagenase ointment) -> removal of necrotic plug + promoting epithelialization



<https://santyl.com/hcp/application>

### Papain:

- protease from *Carica Papaya* fruit
- debridement of necrotic tissues

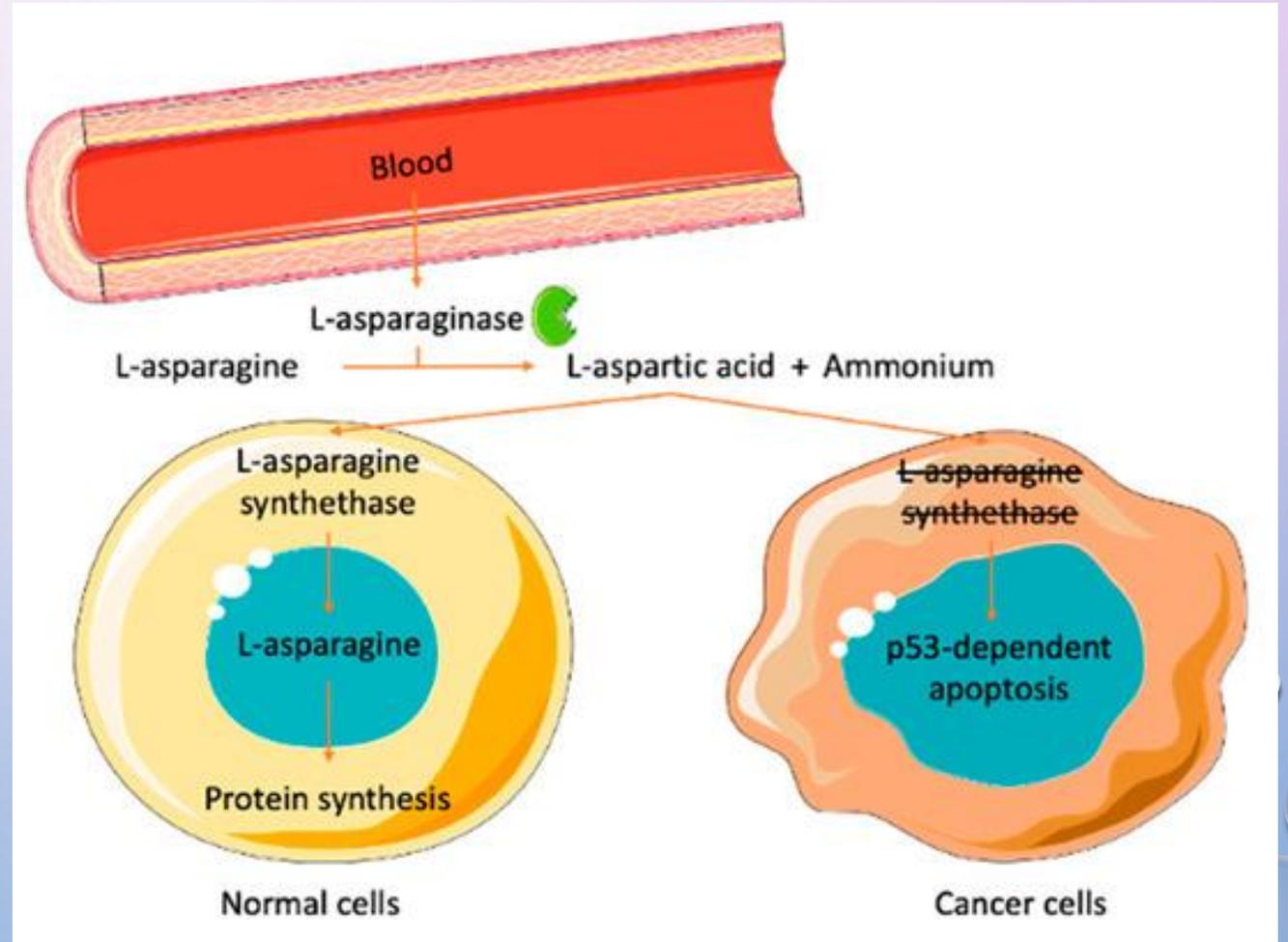


# III. PROVIDING A NOVEL FUNCTION OR ACTIVITY

## III/2. Ezymatic degradation of small-molecule metabolites

### L-Asparaginase:

- purified from *E. coli*
- removes available asparagine from serum
- can be used in **acute lymphocytic leukaemia**, which requires exogenous asparagine for proliferation



# III. PROVIDING A NOVEL FUNCTION OR ACTIVITY

## III/3. Haemostasis and thrombosis

### Recombinant hirudin:

- thrombin inhibitor from salivary gland of medical leech *Hirudo medicinalis*
- can be used in heparin induced thrombocytopenia

### Synthetic hirudin analogue

- binds on circulating and clot-bound thrombin
- can reduce blood-clotting risk in coronary angioplasty and heparin induced thrombocytopenia

Direct thrombin inhibitors

## HIT: Subtypes

### • HIT Type 1

- Occurs within 1-2 days after heparin administration
- Transient thrombocytopenia occurs
- Thrombocytopenia recovers even with continuous heparin administration
- **NO** associated increased thrombotic risk

### • HIT Type 2

- *Clinically significant*
- Antibodies against heparin-PF4 complex
- Also known as heparin-induced thrombocytopenia and thrombosis

# III. PROVIDING A NOVEL FUNCTION OR ACTIVITY

## III/3. Haemostasis and thrombosis

### Recombinant hirudin:

- thrombin inhibitor from salivary gland of medical leech *Hirudo medicinalis*
- can be used in heparin induced thrombocytopenia

### Synthetic hirudin analogue

- binds on circulating and clot-bound thrombin
- can reduce blood-clotting risk in coronary angioplasty and heparin induced thrombocytopenia

## HIT-Type 2: Clinical features

- *Venous and Arterial Thromboses*
  - Up to half of HIT cases will have thromboses
- **Venous Thromboses:**
  - *Deep vein thrombosis, pulmonary embolism*
- **Arterial Thromboses:**
  - *Myocardial infarction, stroke*
- **Heparin-Induced Skin Necrosis**
  - May occur when *low-molecular weight heparin* used

### Signs & Symptoms of Thrombocytopenia

- Mucosal bleeding
- Epistaxis
- Easy bruising
- Petechiae, purpura, ecchymoses



# CLASSIFICATION BASED ON THEIR PHARMACOLOGICAL ACTIVITY

- I. Replacing a protein that is deficient or abnormal
- II. Augmenting an existing pathway
- III. Providing a novel function or activity
- IV. Interfering with a molecule or organism → mAb therapies**
- V. Delivering other compounds or proteins, such as a radionuclide, cytotoxic drug, or effector proteins → vaccines**

**Details of monoclonal antibody therapies and vaccines will be discussed in separated lectures during the course!**