Medical Biotechnology 2024'

Biological therapies

Lecture 3-4th

Protein therapeutics

A proteins are large molecules comprised of a long chain of amino acids that is folded into a three-dimensional shape. The specific amino acid sequence and 3-D shape determines the biological function of the protein.

Therapeutic proteins can be used to replace a protein that is abnormal or deficient in a particular disease. They can also augment the body's supply of a beneficial protein to help reduce the impact of disease or chemotherapy. Genetically engineered proteins can closely resemble the natural proteins they replace, or they can be enhanced by adding sugars or other molecules that extend the protein's duration of activity.

Historical background

- Proteins are large biomolecules and macromolecules that comprise one or more long chains of amino acid residues.
- Proteins, e.g., albumin from egg whites, blood serum albumin, fibrin, and wheat gluten, were recognized in XIXth 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**

Proteins

Proteins are the basic molecular components of the living organisms have the most dynamic and diverse roles of any macromolecule in the body with catalyze 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, participate in immune functions or perform different storage functions.

~20,000 different genes in the human genome code different proteins, 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

Biological function of proteins

- **Digestive Enzymes:** catabolize nutrients into constituent monomeric units. E.g. pepsin and amylase.
- **Structural Proteins:** they form components of certain structures. E. g. keratin, tubulin, myosin and collagen
- **Hormonal Functions:** hormones are paramount for regulating body functions. E.g. insulin
- **Transportation:** transporting substances throughout the body. E.g. hemoglobin
- **Defense and Protection:** part of the immune system and protect the body from pathogens. E.g. immunoglobulins
- **Storage Functions:** provide nourishment for development of embryo – such as albumin, or the egg white.

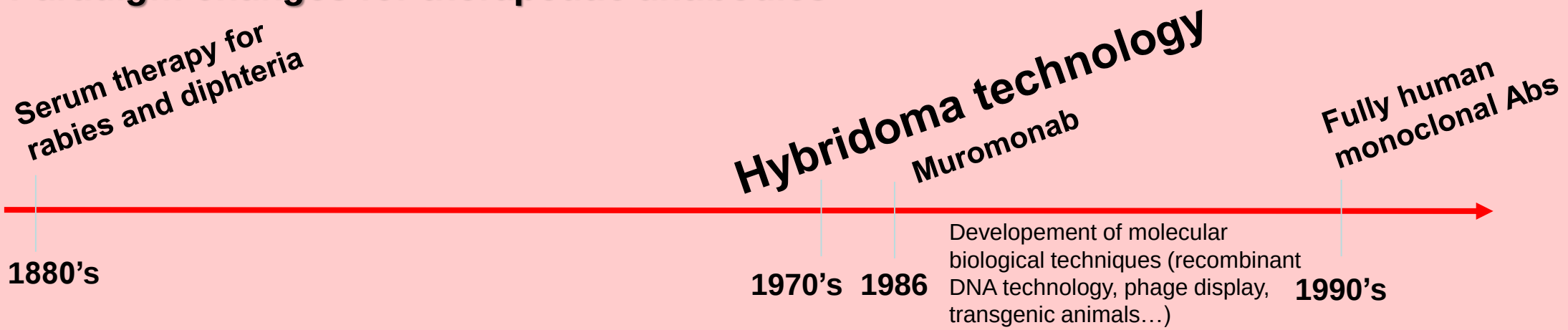
Protein therapeutics

Non-recombinant protein drugs are as old as contemporary medicine itself if one takes Jenner's attempts at vaccination as prima voce. In the meantime many more injectable protein based medicinal preparations have been used and these include:- blood transfusion, plasma and plasma-derived products, IVIg, Factor VIII, organ extracts, tissues isolated hormones, vaccines, preparations for vaccination and many more.

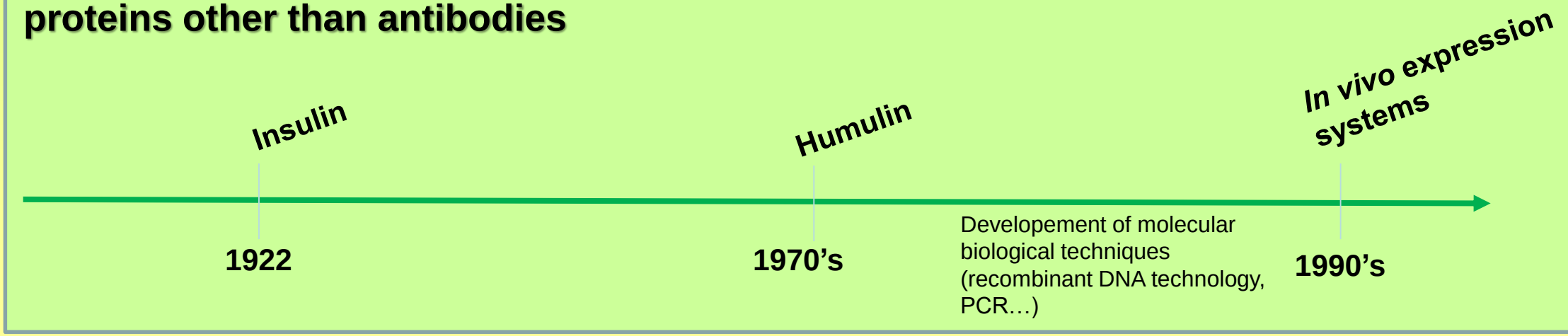
Even today the level of protein drug development is still very significant both recombinant and conventionally derived. Therapeutic monoclonal antibodies, recombinant cytokines or hormones have pivotal role in pharmaceutical developments and market.

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

FIELDS OF PROTEIN THERAPIES 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

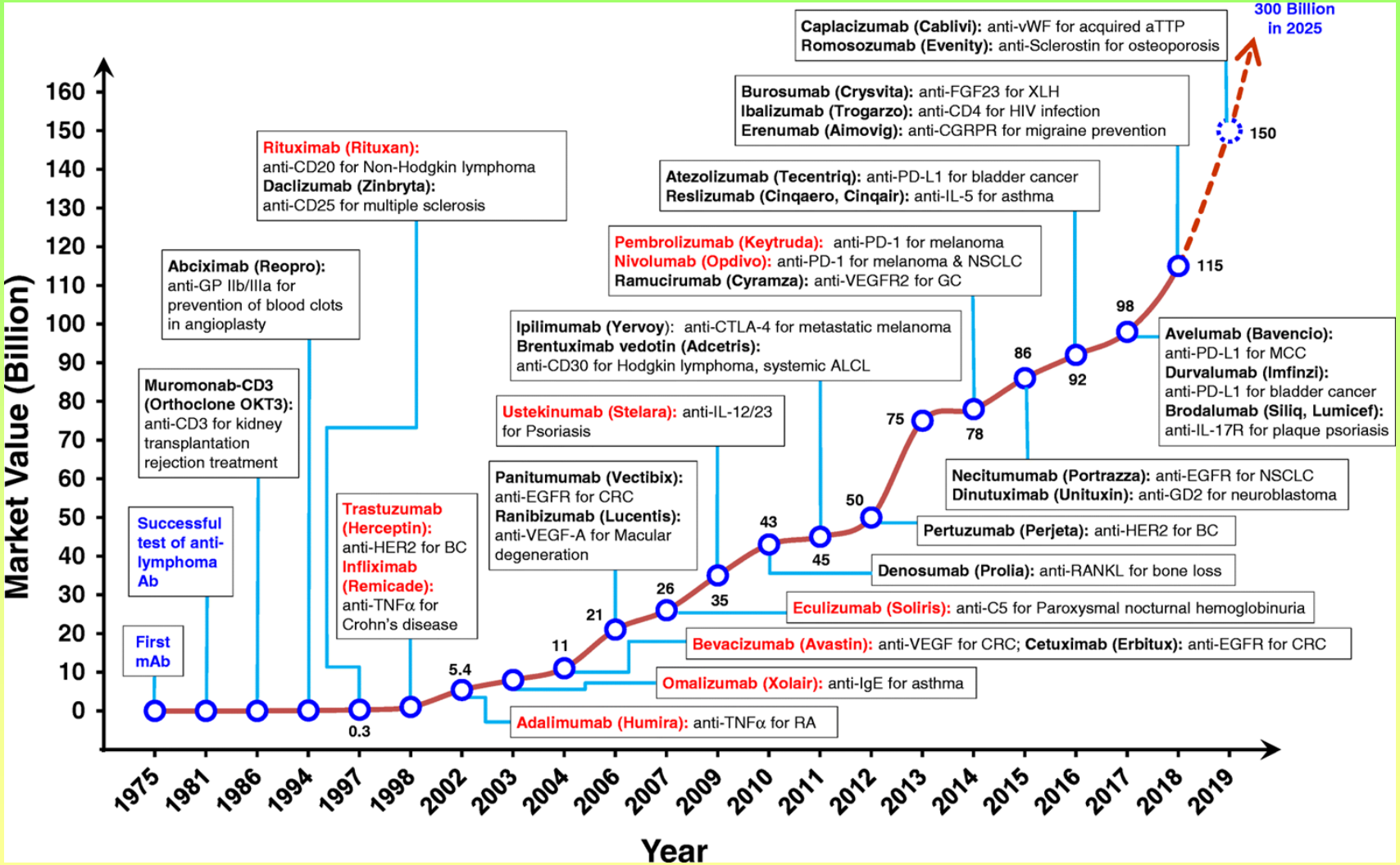
Molecular type
1. Antibody-based drugs
2. Fc fusion proteins
3. Anticoagulants
4. Blood factors
5. Bone morphogenetic proteins
6. Engineered protein scaffolds
7. Enzymes
8. Growth factors
9. Hormones
10. Interferons
11. Interleukins
12. Thrombolytics

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!!

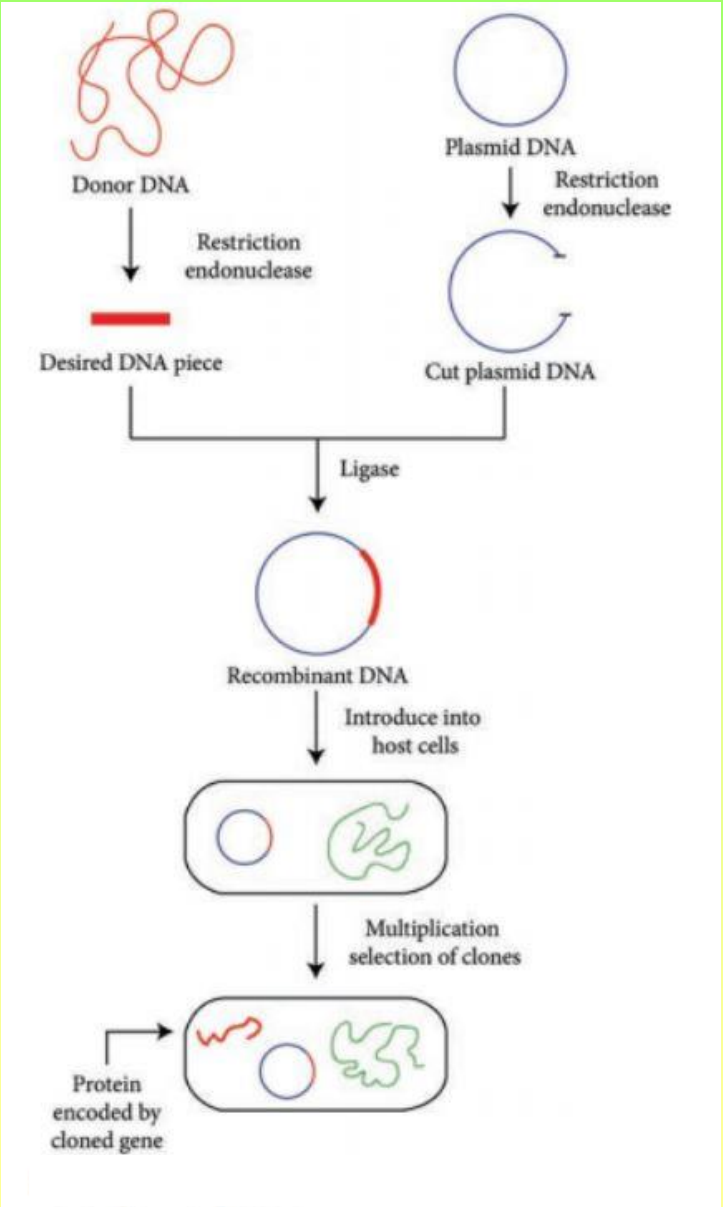
If group of **polyclonal antibodies** (either nonspecific pooled human immunoglobulin (Ig) or specific Ig) are included as therapeutic proteins, then the total number of genuine therapeutic proteins exceeds 100.

Changing of market value of monoclonal antibodies



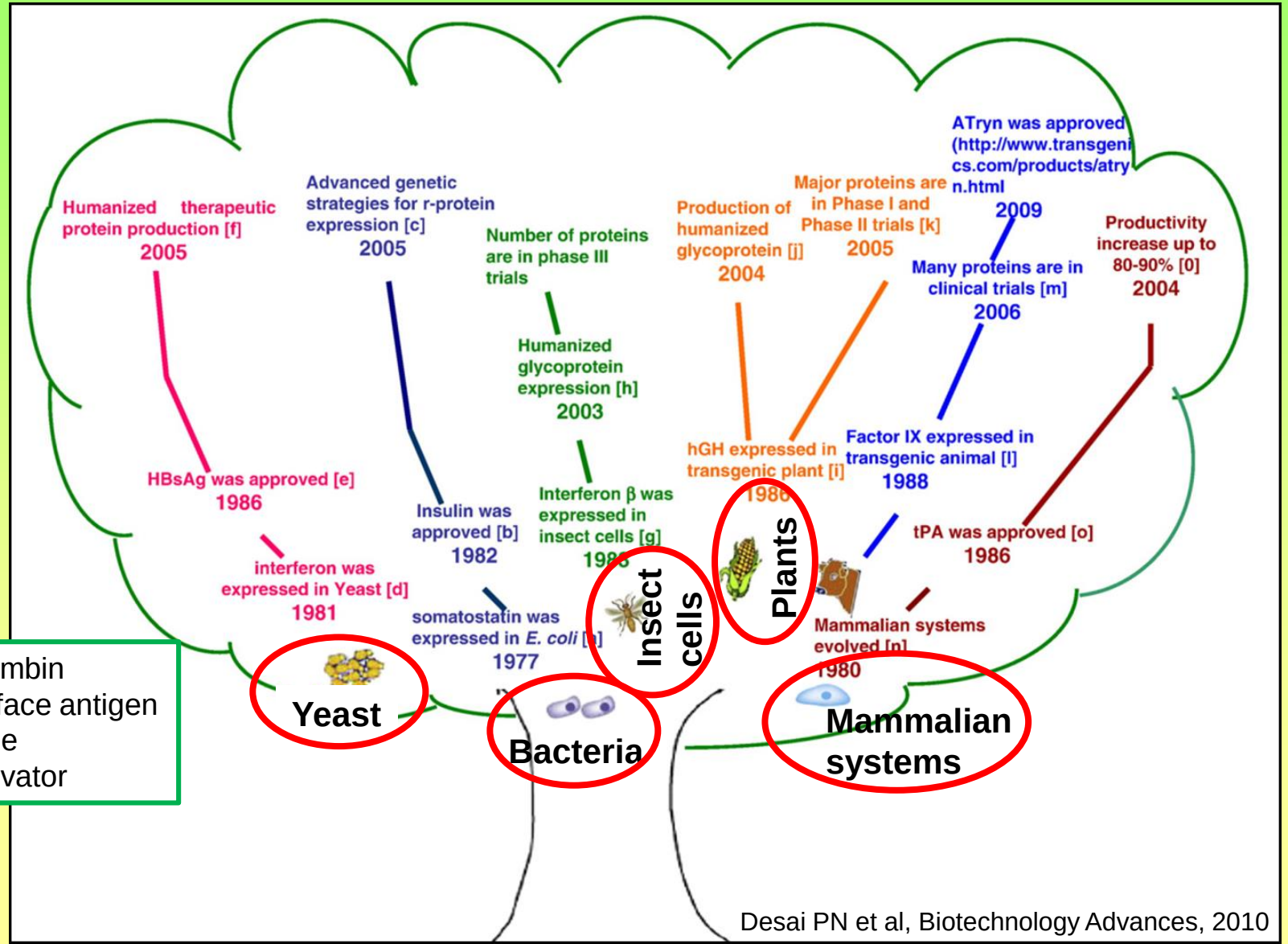
- Protein-based therapeutics are **highly successful in clinic and in market**
- **More than 100 genuine and similar number of modified therapeutic proteins** are approved for clinical use **in the European Union and the USA** with 2010 sales of US **$108 bln**; monoclonal antibodies (mAbs) accounted for almost half (48%) of the sales

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



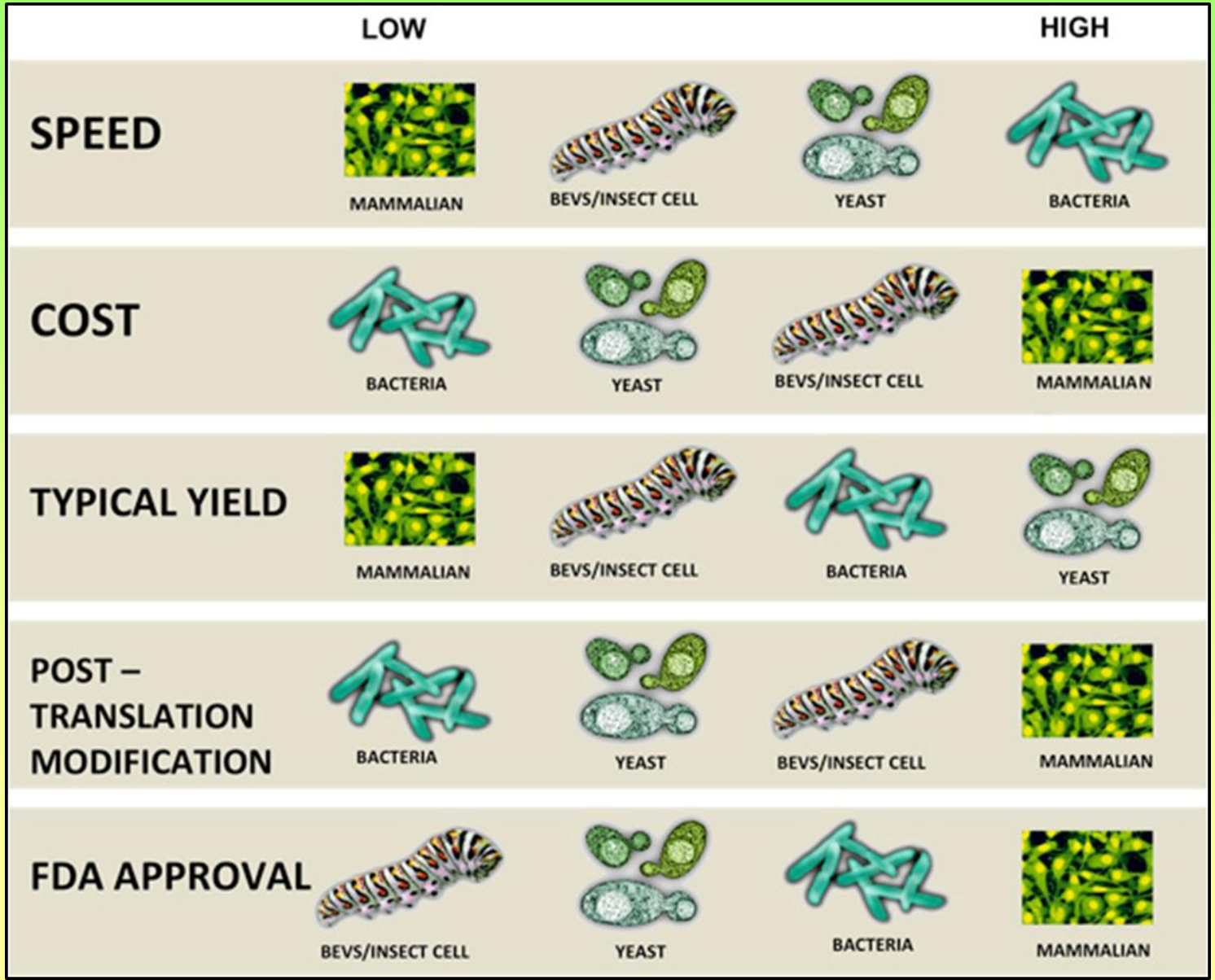
Desai PN et al, Biotechnology Advances, 2010

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.



http://pef.aibn.uq.edu.au/wordpress/wp-content/blogs.dir/1/files/2013/03/HighLow.jpg

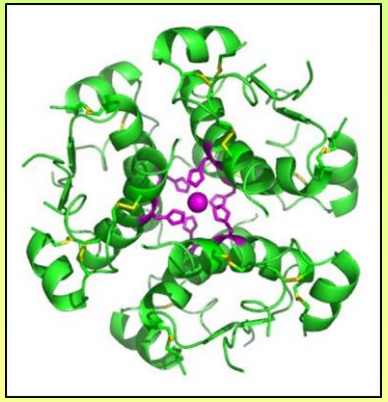
FIELDS OF PROTEIN THERAPIES BASED ON THEIR PHARMACOLOGICAL ACTIVITY

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

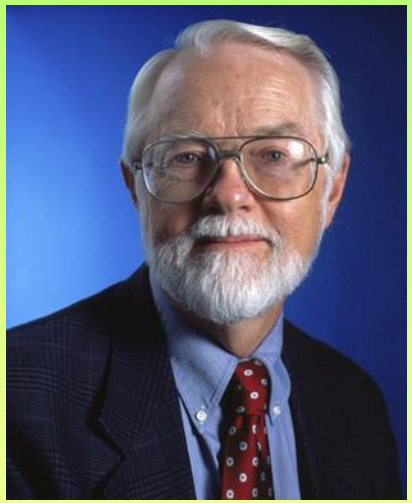
I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

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.

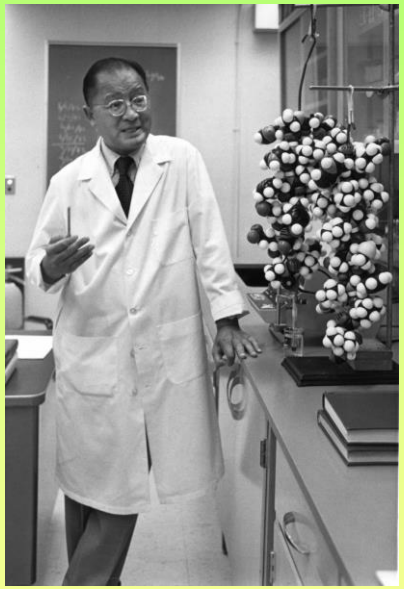
Venetianer P., Alföldi L. and Ferenczy L.: recombinant human insulin for industrial production (1984)

I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

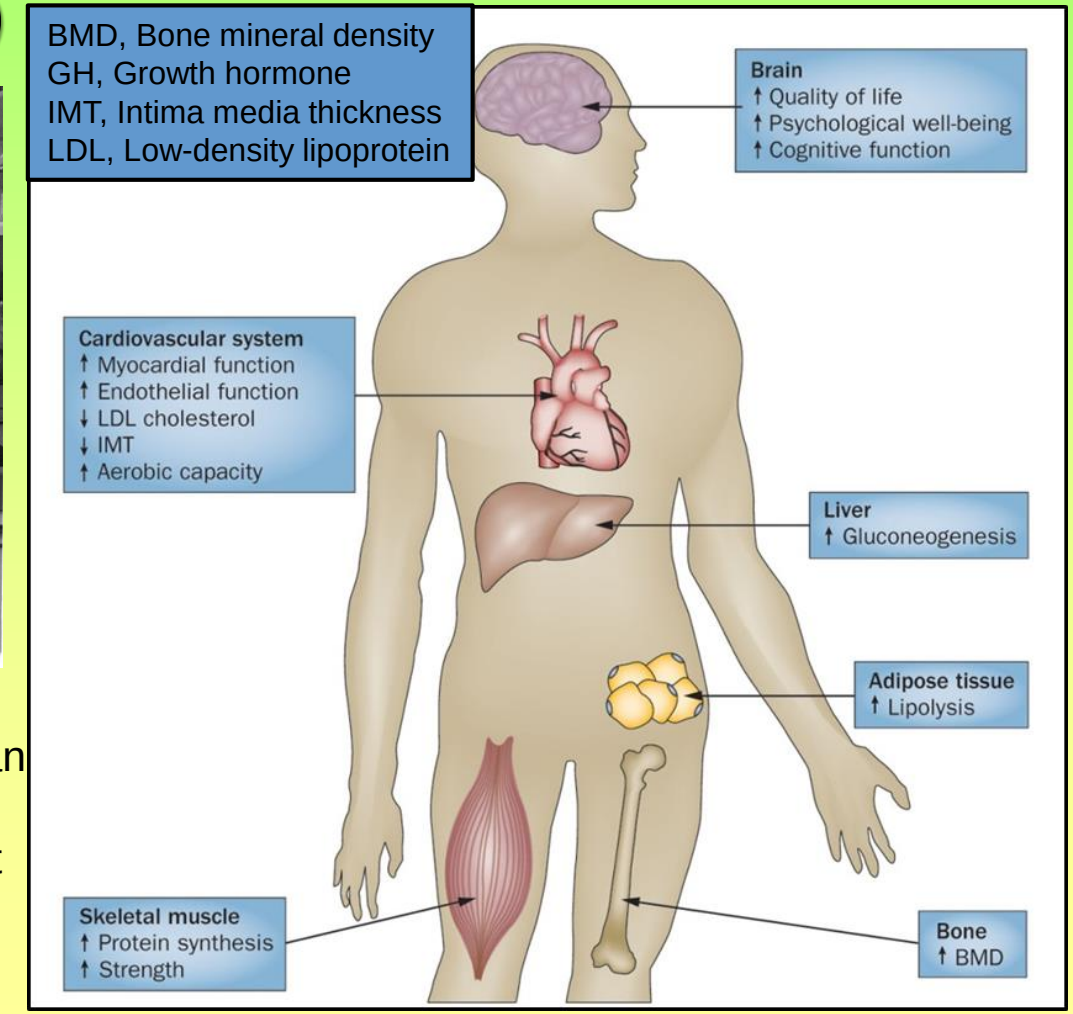
I/1. Endocrine disorders (hormone deficiency)

Growth hormon (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"

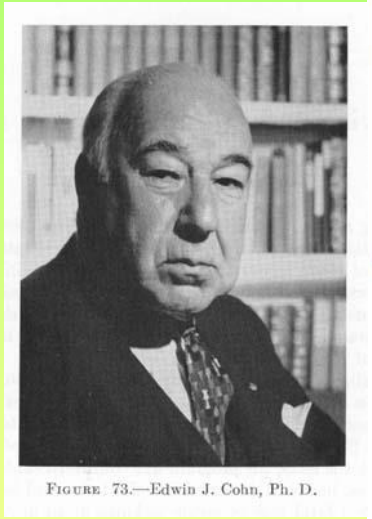


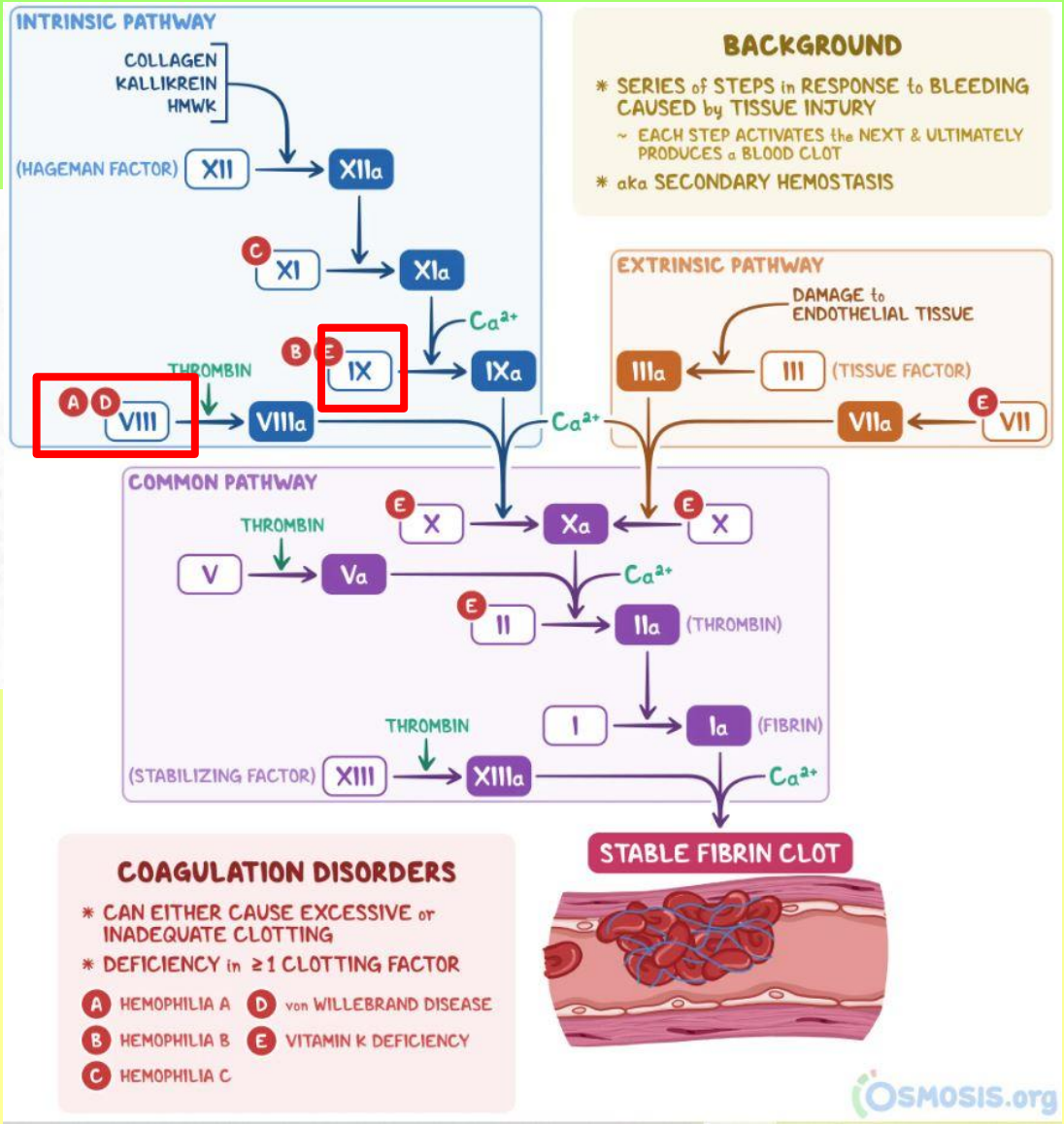
FIGURE 73.—Edwin J. Cohn, Ph. D.

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)

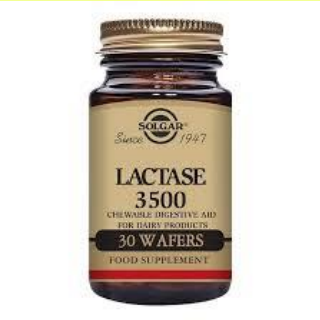


I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

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 digest lactose.



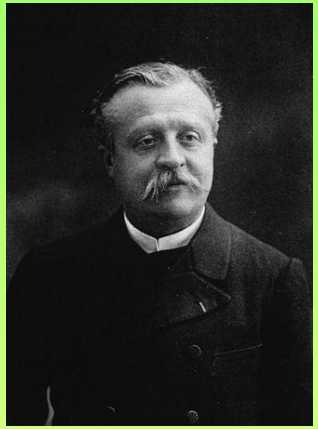
Exocrine pancreatic insufficiency (EPI): is a condition which occurs when the pancreas does not make enough of a specific enzyme the body uses to digest food in the small intestine. People with EPI don't have enough pancreatic (digestive) enzymes to break down foods and absorb nutrients. It can lead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

I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

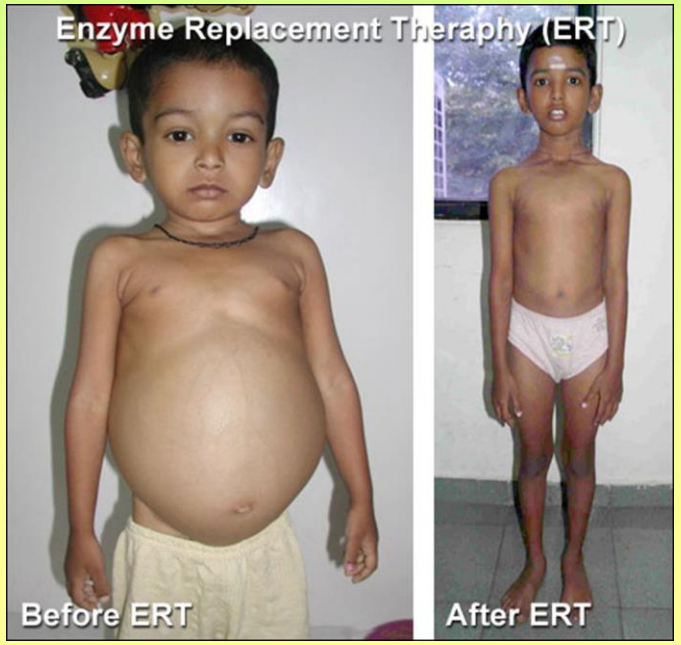
I/3. Metabolic enzyme deficiencies

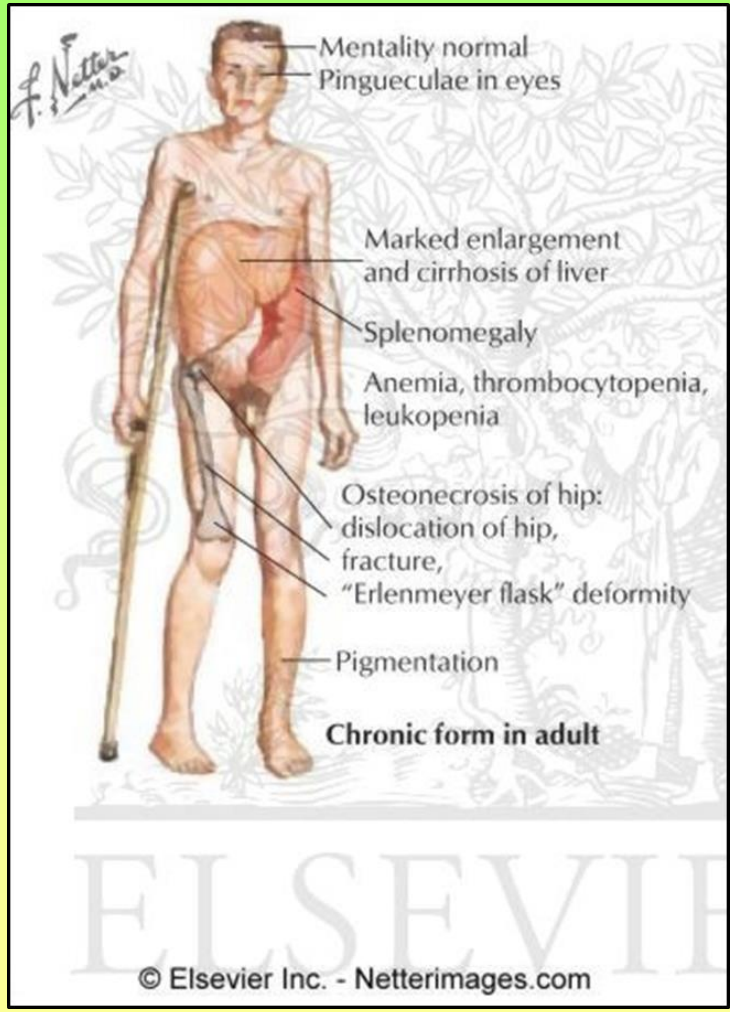
Gaucher's disease:

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



Philippe Gaucher



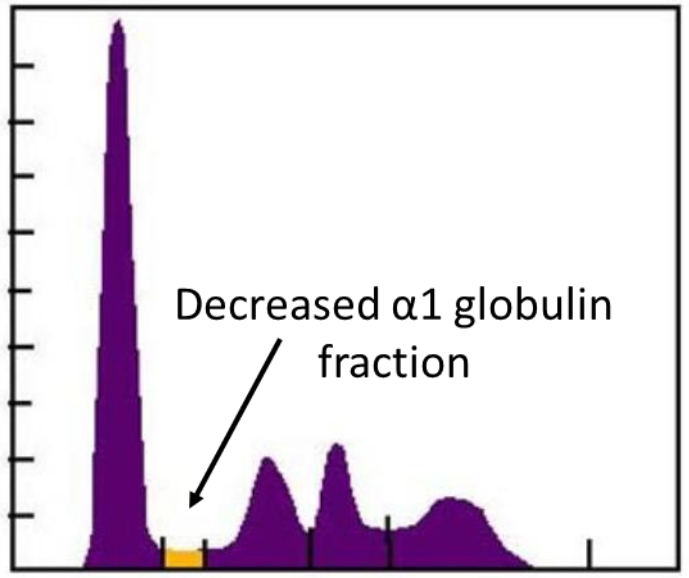


I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

I/3. Pulmonary and gastrointestinal disorders

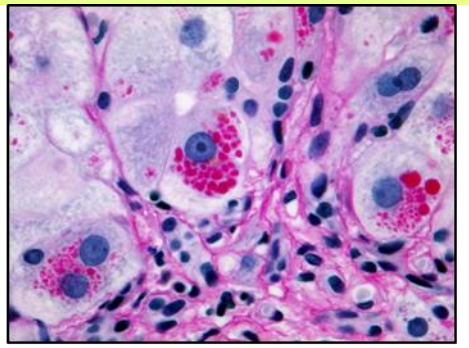
α-1-antitrypsin deficiency:

- Genetic disorder
- Liver cells are unable to secrete α1-antitrypsin which accumulates in their cytoplasm
- The level of α1-antitrypsin greatly decreases in the blood which will lead to complications



abnormal serum electrophoretic pattern

- **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 A1AT absence)


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

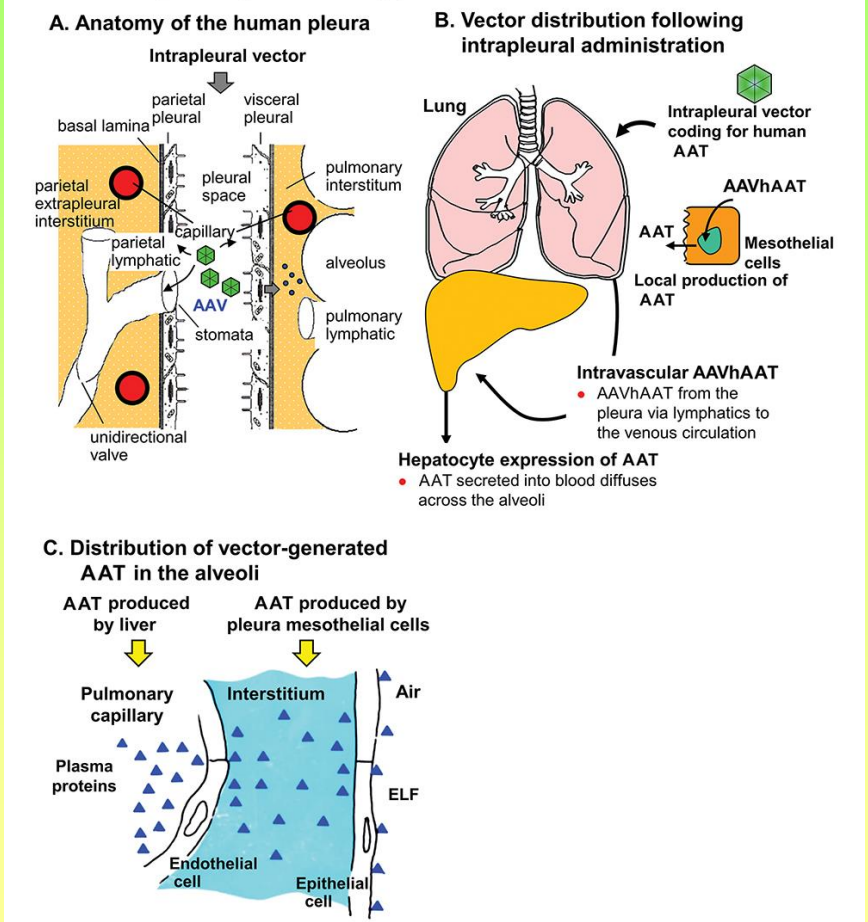
I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

Current therapy for α-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.

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

Gene Therapy for Alpha-1 Antitrypsin Deficiency

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

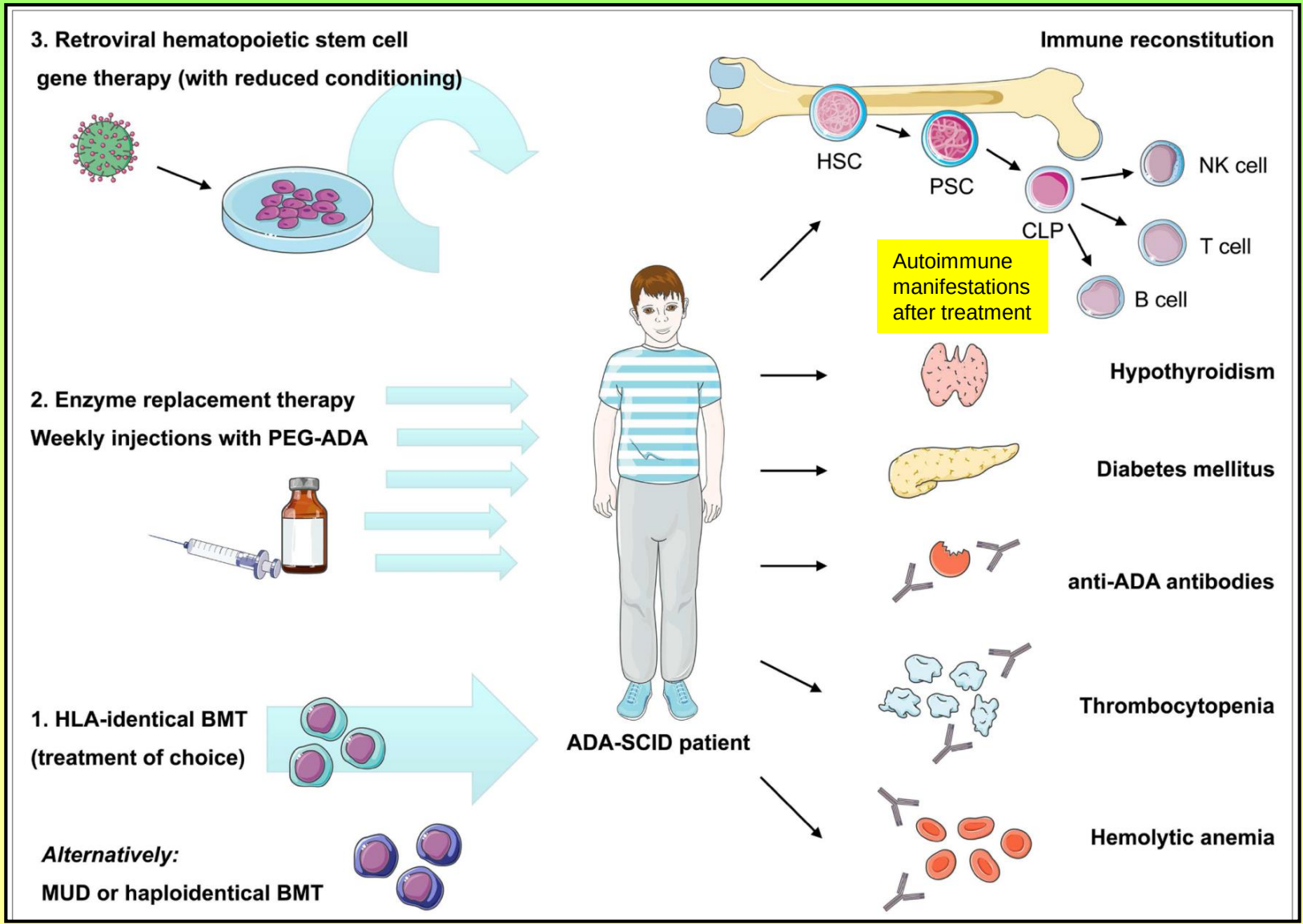
I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

I/4. 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/4. Immunodeficiencies

Primary immunodeficiencies:

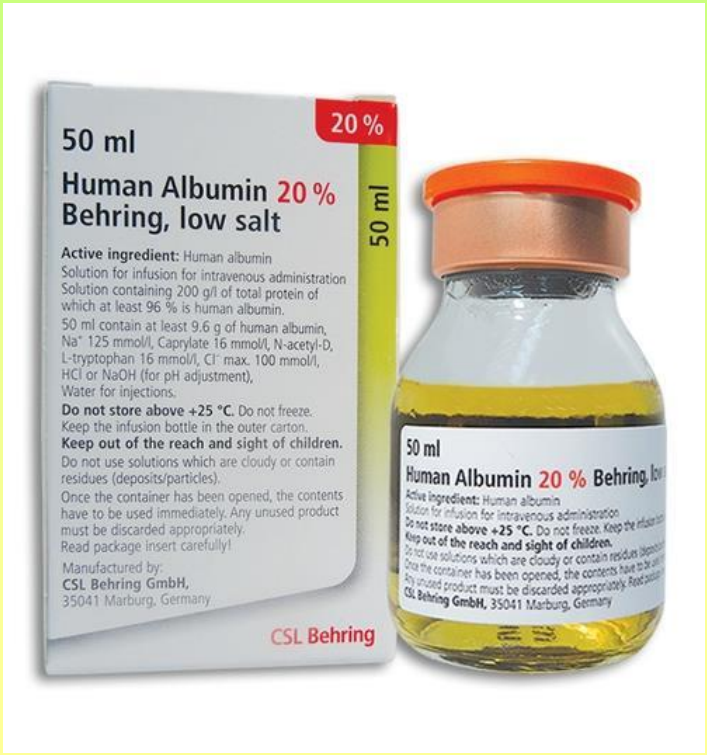
- Chronic Granulomatous Disease (CGD)
- Common Variable Immunodeficiency (CVID)
- Congenital Neutropenia Syndromes
- Severe Combined Immunodeficiency (SCID)
- X-Linked Agammaglobulinemia (XLA)
- Hyper-Immunoglobulin E Syndromes (HIES)

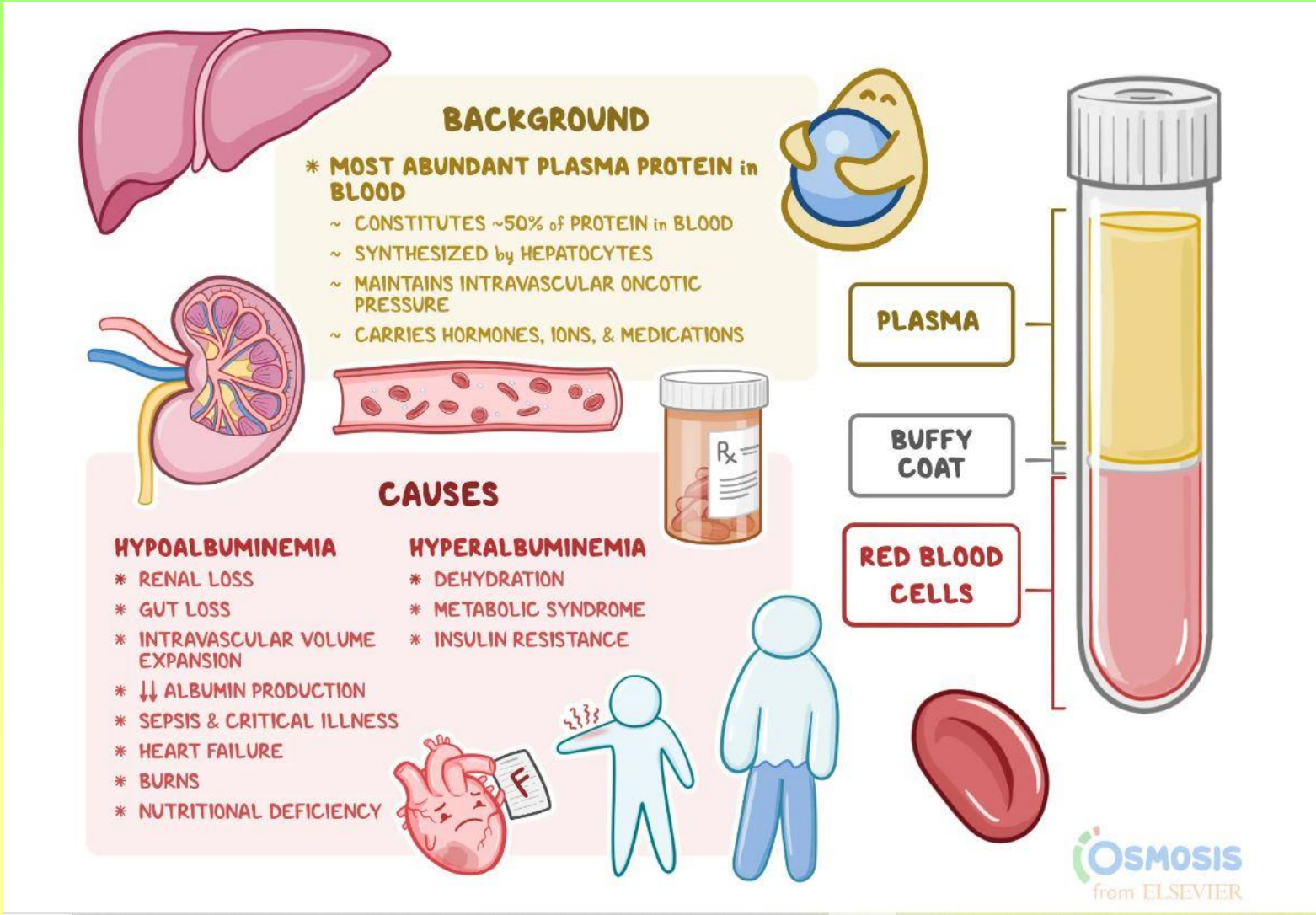
IVIg therapy: in different diseases successfully applied since 1940

I. REPLACING A PROTEIN THAT IS DEFICIENT OR ABNORMAL

I/5. Other

Albumin:





FIELDS OF PROTEIN THERAPIES BASED ON THEIR PHARMACOLOGICAL ACTIVITY

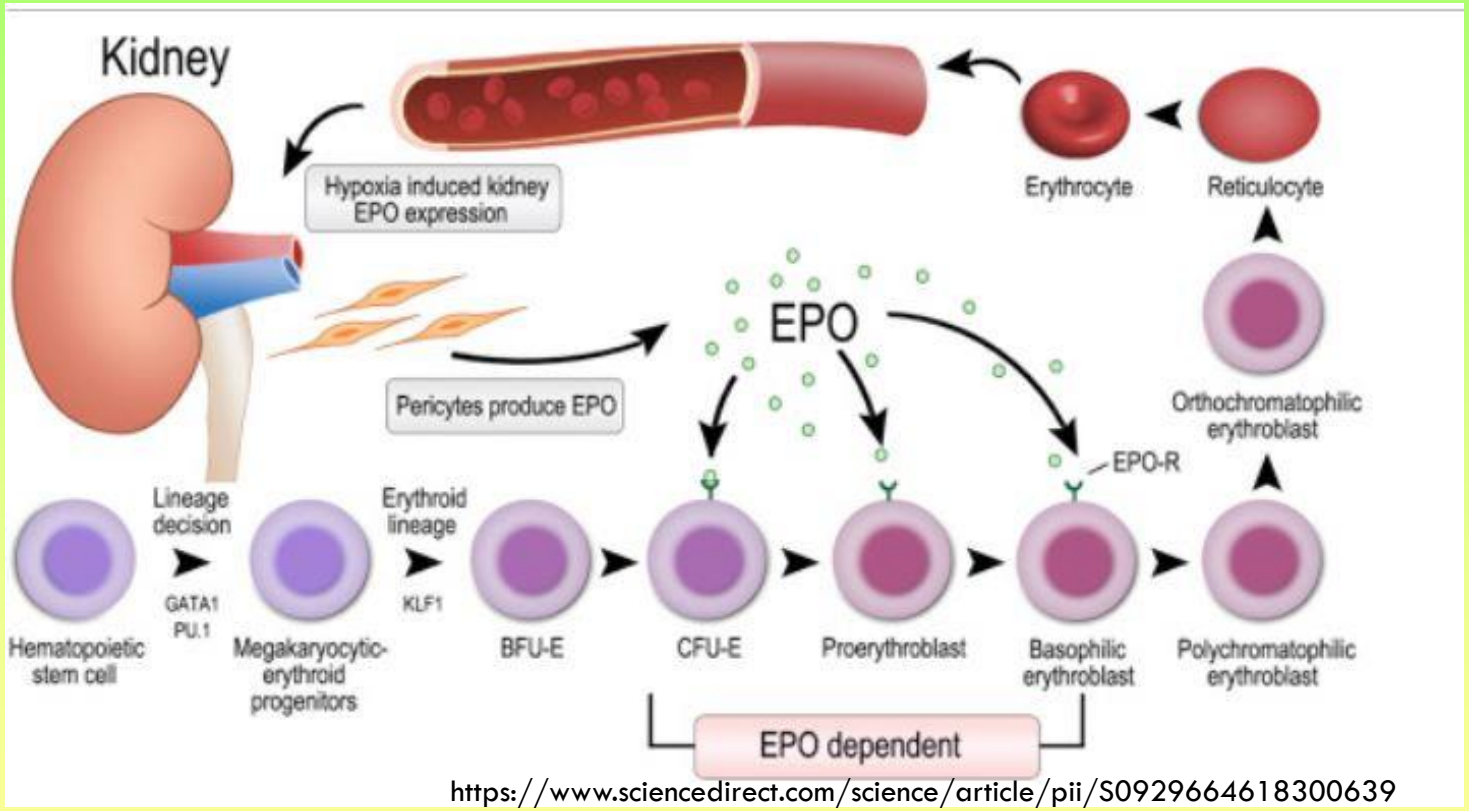
I. Replacing a protein that is deficient or abnormal
II. Augmenting an existing pathway
III. Providing a novel function or activity
IV. Interfering with a molecule or organism
V. Delivering other compounds or proteins, such as a radionuclide, cytotoxic drug, or effector proteins

II. AUGMENTING AN EXISTING PATHWAY

II/1. Hematopoiesis

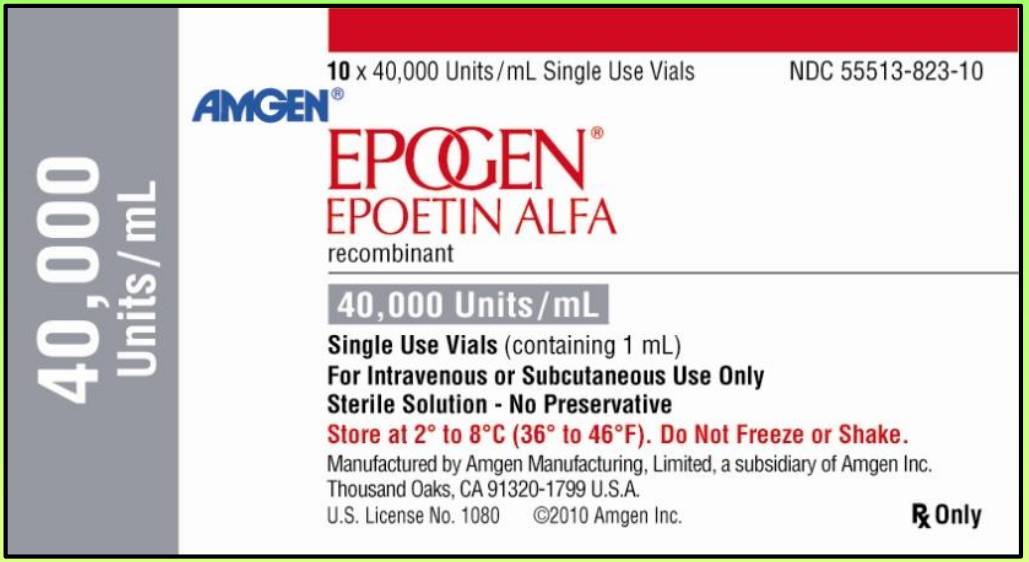
Erythropoietin:

- 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)**



https://www.sciencedirect.com/science/article/pii/S0929664618300639

II. AUGMENTING AN EXISTING PATHWAY



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

Epogen

Stimulates erythropoiesis.

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

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

Neupogen

Stimulates neutrophil proliferation, differentiation and migration.

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

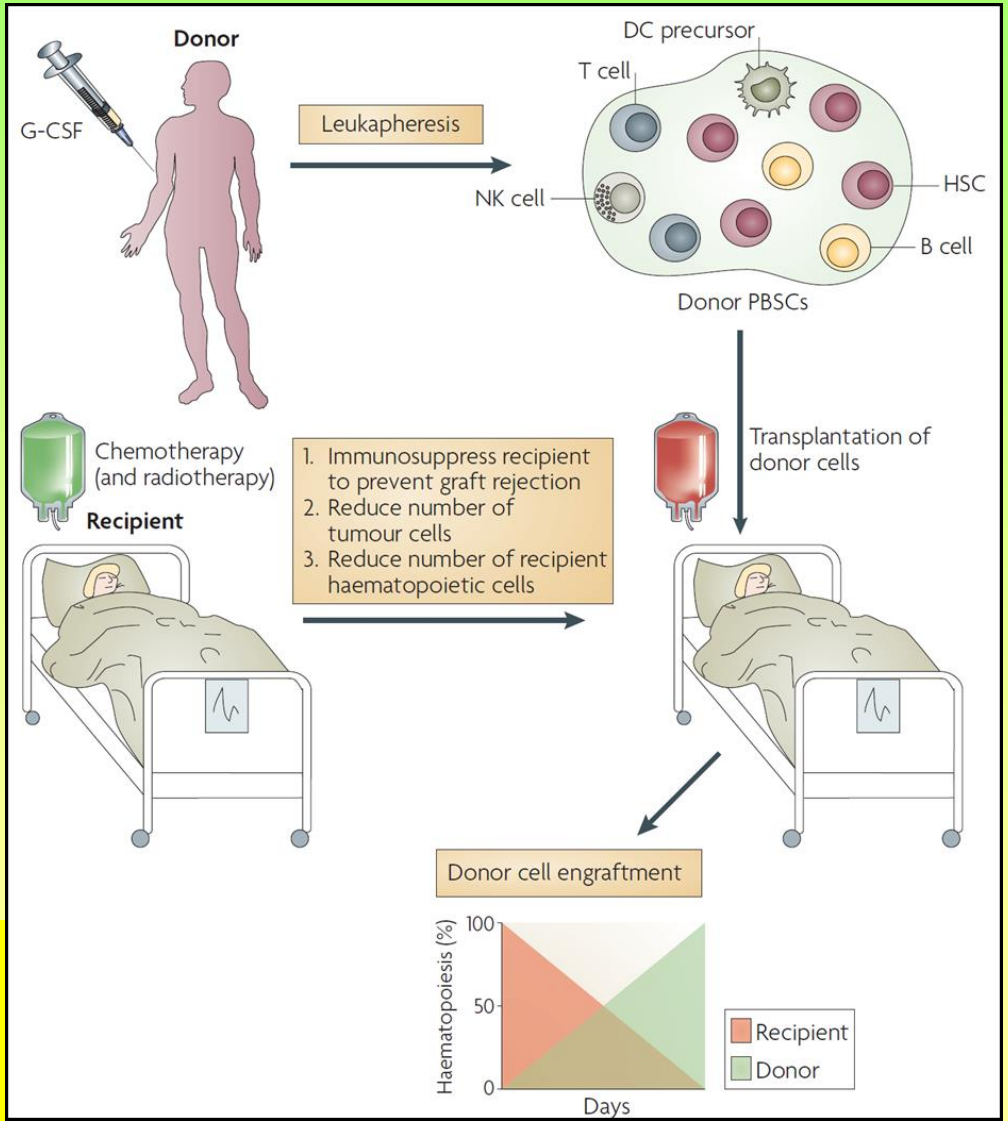
II. AUGMENTING AN EXISTING PATHWAY

II/1. Hematopoiesis

- **<u>Allogeneic peripheral blood hematopoietic stem cell transplantation</u>**

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

Schlomchik WG, Nature Rev Immunol, 2007



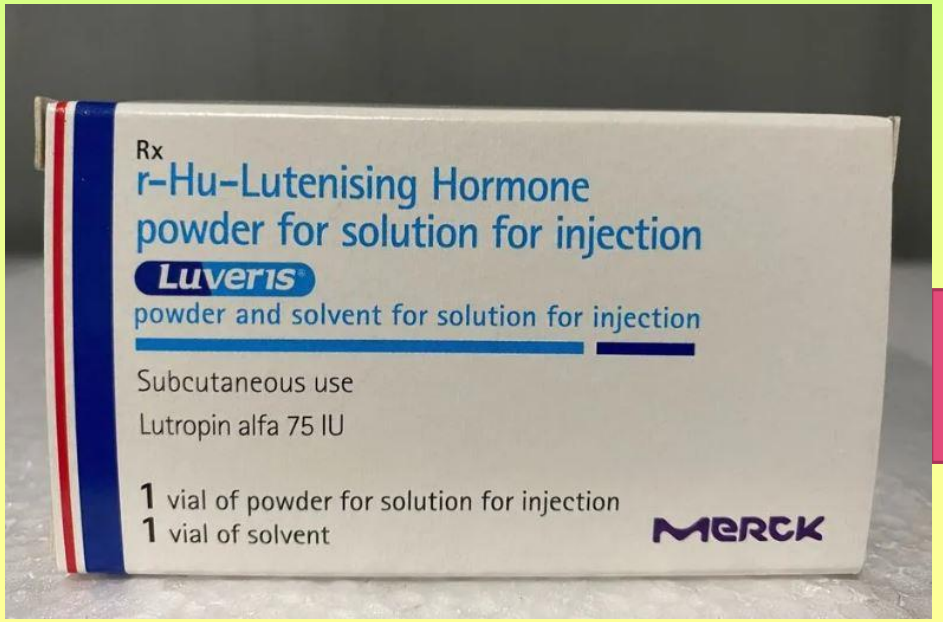
DC, Dendritic cell
G-CSF, Granulocyte colony-stimulating factor
HSC, Hematopoietic stem cell
PBSC, Peripheral-blood stem cells

II. AUGMENTING AN EXISTING PATHWAY

II/2. Fertility

Assisted reproduction

Augments ovulation



Estradiol↑
Follicular dev.↑

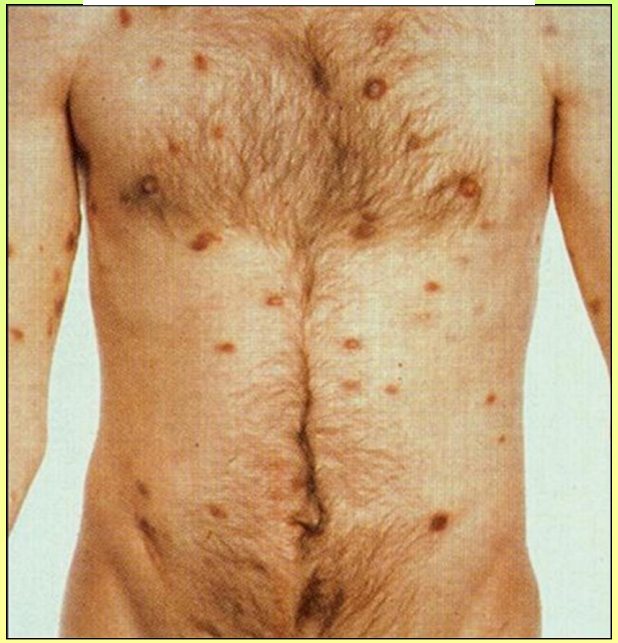
Infertility because of luteinizing hormone deficiency

II. AUGMENTING AN EXISTING PATHWAY

II/3. Immunoregulation

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

Kaposi's sarcoma



from Robinson WE Jr, University of California Irvine, 2011

Immunoregulation			
Type I alpha-interferon, interferon alfacon 1, consensus interferon[173–178]	Infergen	Mechanism unknown; immunoregulator	Chronic hepatitis C infection
Interferon-α2a (IFNα2a)[179–183]	Roferon-A	Mechanism unknown; immunoregulator	Hairy cell leukaemia, chronic myelogenous leukaemia, Kaposi's sarcoma, chronic hepatitis C infection
PegInterferon-α2a[184–186]	Pegasys	Recombinant interferon-α2a 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 IFNα; used alone or in combination with ribavirin
Interferon-α2b (IFNα2b)[187–189]	Intron A	Mechanism unknown; immunoregulator	Hepatitis B, melanoma, Kaposi's sarcoma, follicular lymphoma, hairy-cell leukaemia, condylomata acuminata, hepatitis C
PegInterferon-α2b[190]	Peg-Intron	Recombinant interferon-α2b 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 IFNα
‡Interferon-αn3 (IFNαn3)[191,192]	Alferon N	Mechanism unknown; nonrecombinant human IFNα-n3 purified from pooled human leukocytes	Condylomata acuminata (genital warts caused by human papillomavirus)
Interferon-β1a (rIFN-β)[178,193–196]	Avonex, Rebif	Mechanism unknown; antiviral and immunoregulator	Multiple sclerosis
Interferon-β1b (rIFN-β)[197–199]	Betaseron	Mechanism unknown; antiviral and immunoregulator	Multiple sclerosis
Interferon-γ1b (IFNγ)[200–204]	Actimmune	Increases inflammatory and antimicrobial response	Chronic granulomatous disease, severe osteopetrosis
Aldesleukin[205–208] (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

Leader B et al, Nature Rev Drug Disc, 2008

FIELDS OF PROTEIN THERAPIES 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

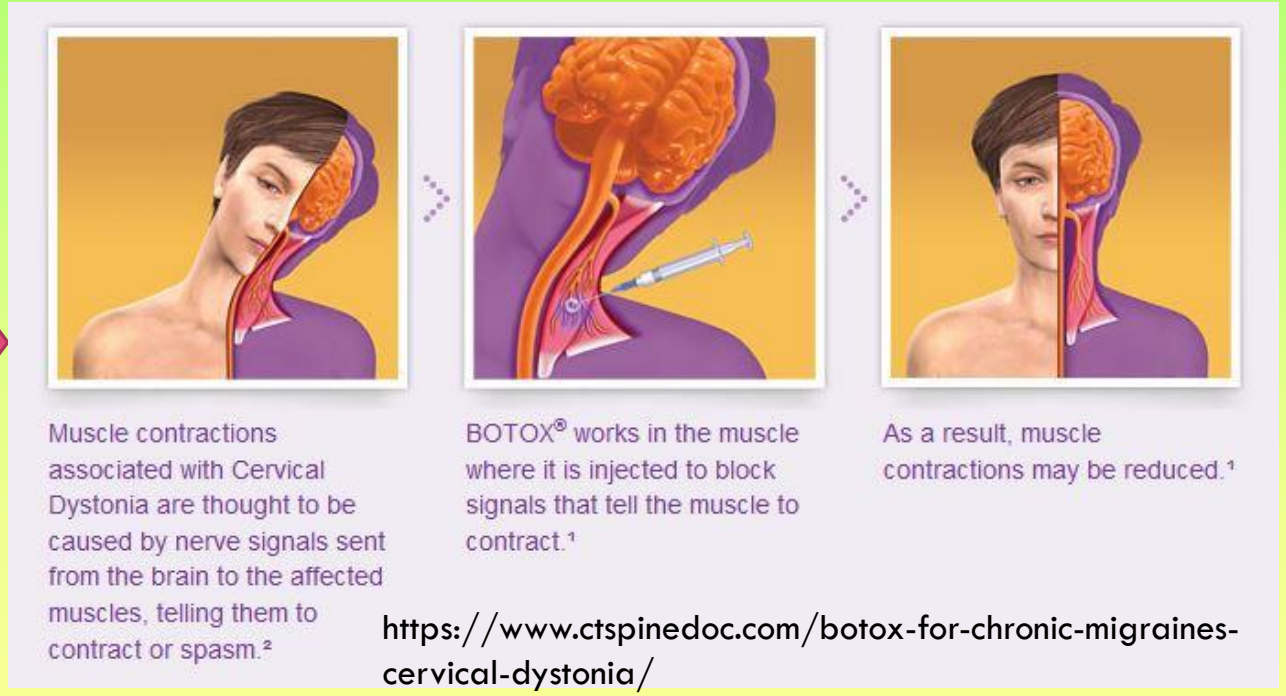
III. PROVIDING A NOVEL FUNCTION OR ACTIVITY

III/1. Ezymatic degradation of macromolecules

Botulinum toxin type A and B:

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

Clinical use



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

Cosmetic use

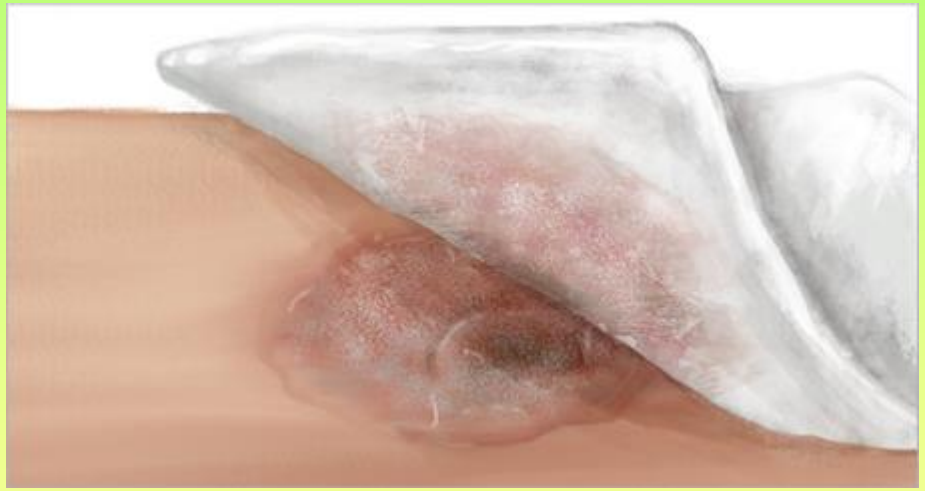
Excessive sweating
Reduction of facial wrinkles

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



https://santyl.com/hcp/application

Papain:

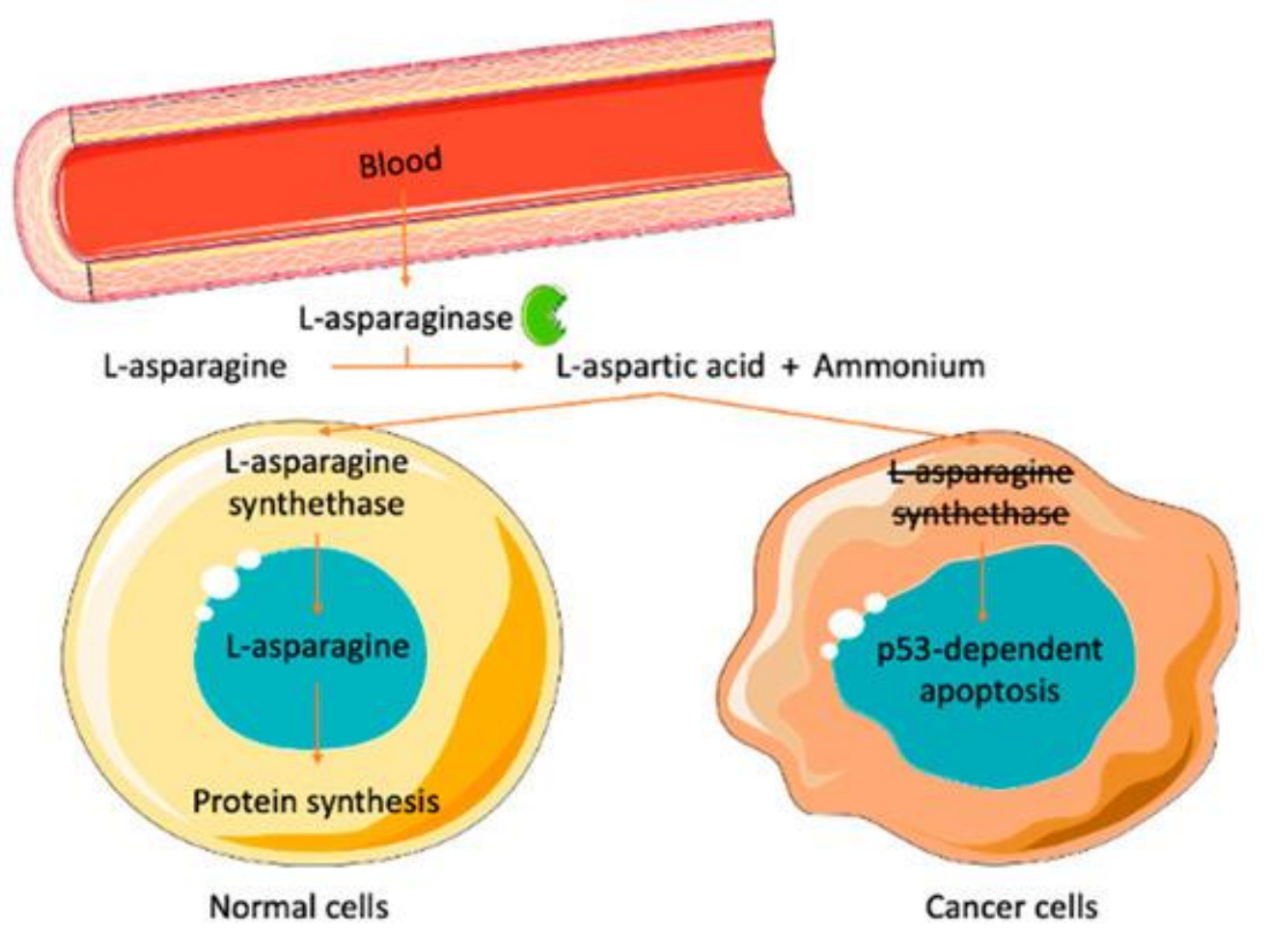
- protease from *Carica Papaya* fruit
- debridement of necrotic tissues

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



https://www.mdpi.com/1420-3049/25/24/5827

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

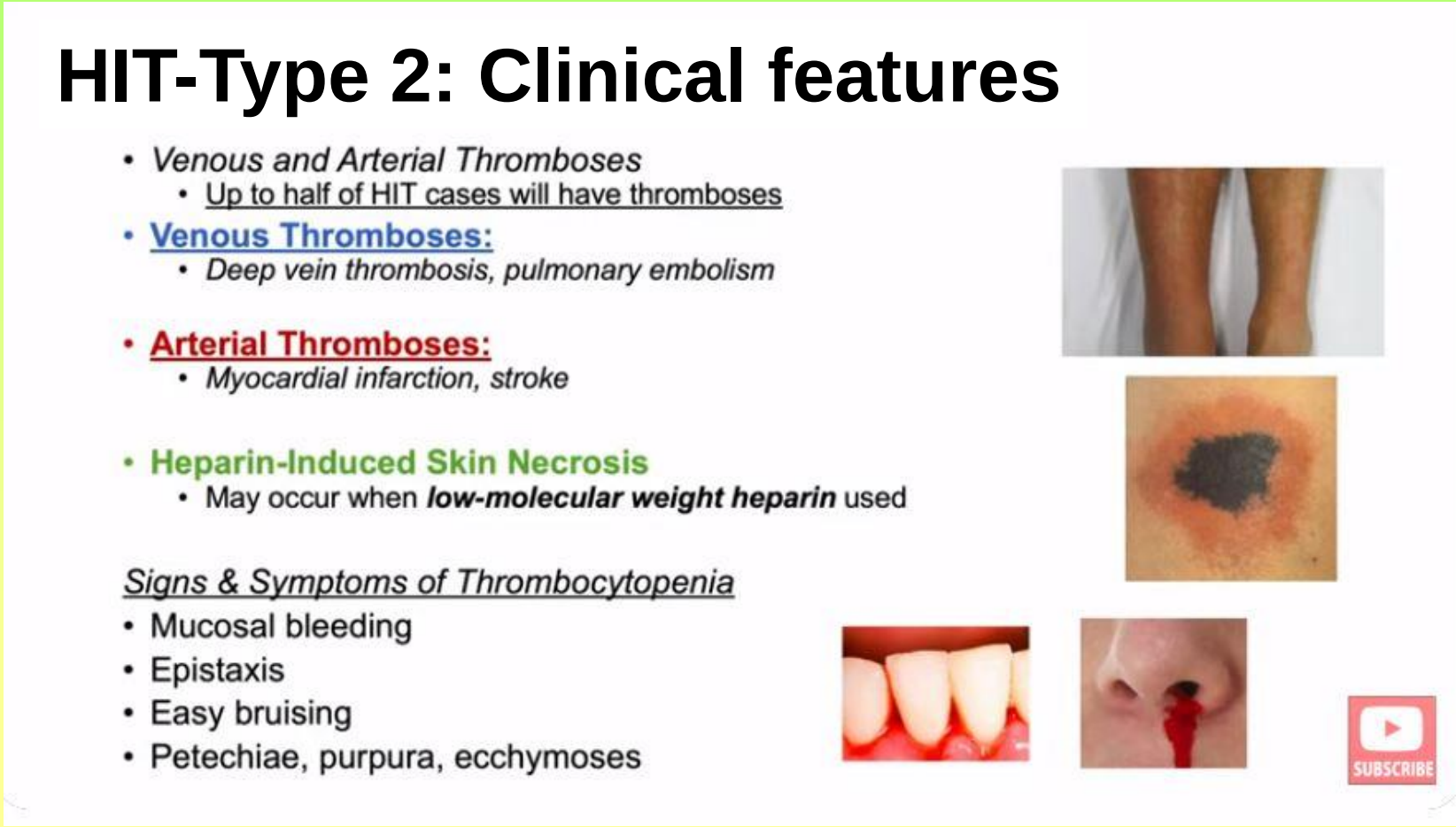
III/3. Haemostasis and thrombosis

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



FIELDS OF PROTEIN THERAPIES 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