

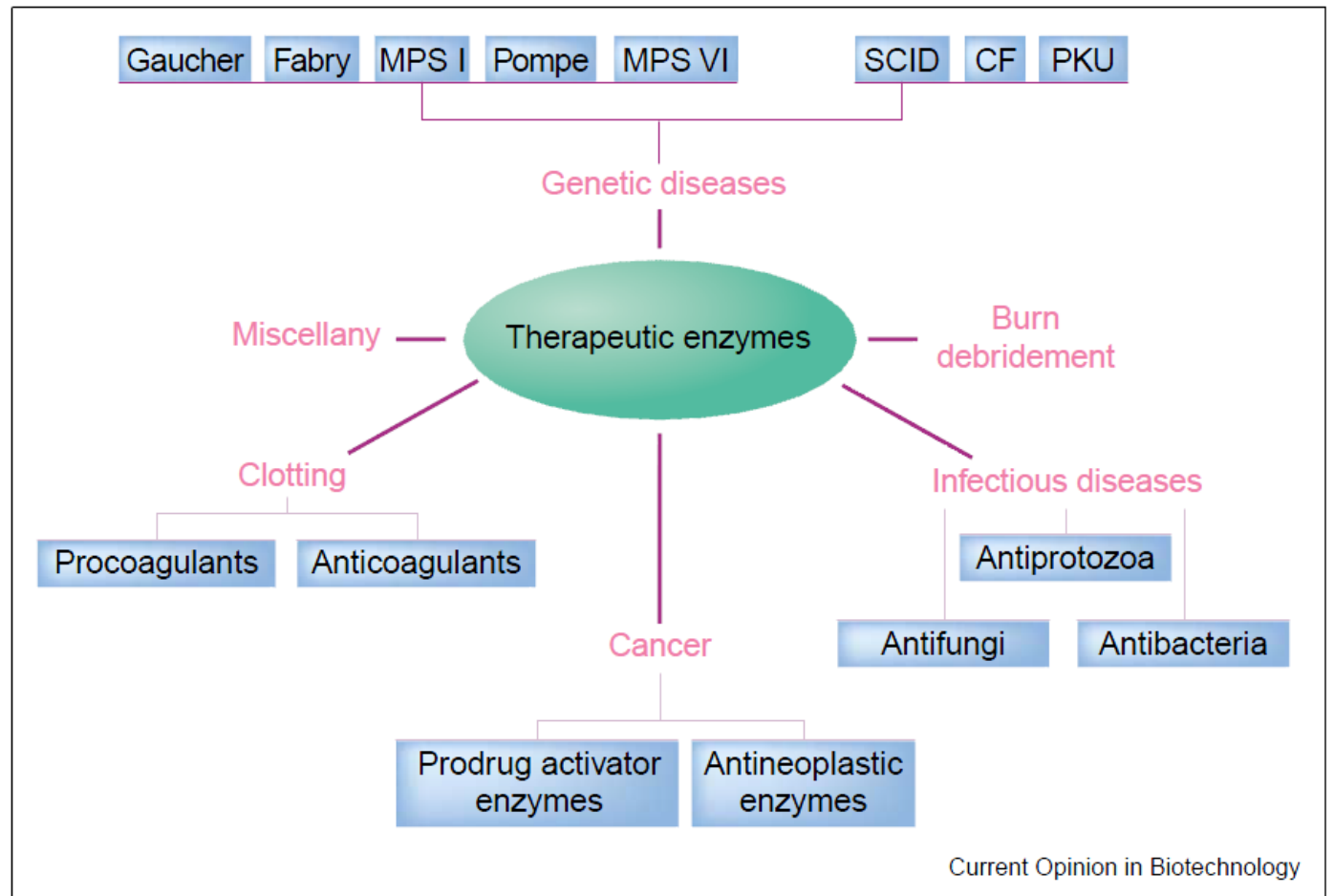
# Therapeutic Use of Enzymes

BBL433

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# Therapeutic Enzymes

- Therapeutic enzymes have a broad variety of specific uses
  - Oncolytics
  - Anticoagulants
  - Thrombolytics
  - Replacements for metabolic deficiencies
    - Digestive aids
    - Metabolic storage disorders, etc
  - Miscellaneous enzymes of diverse function



Enzymes	Therapeutic Use	Basis	Additional Information
<b>Prolactazyme</b>	Lactose Intolerance	Prolactazyme is a proenzyme that produces lactase in stomach.	About 75% of the world's population is intolerant to lactose in adulthood. It occurs due to lack of lactase in digestive system.
<b>Beta-Lactamase</b>	Penicillin Allergy	Penicillin is converted to penicillioate	Learn more about penicillin allergy <a href="#">here</a>
<b>Aglucerase</b>	Gaucher's Disease type I	Enzyme replacement therapy	This disease is characterized by the lack of enzyme glucocerebrosidase.
<b>Streptokinase</b>	Heart Attacks (Myocardial Infarction)	Used as "clot blusters" to dissolve clots in the arteries of heart wall. Plasminogen is converted to plasmin which is fibrinolytic.	Administered i.v. to patients as soon as possible after the onset of a heart attack

<b>Asparaginase</b>	Acute Childhood Leukemia	Decreased level of serum asparagine and inhibition of asparagine dependent multiplication of tumor cells.	Tumor cells cannot synthesize L-asparagine due to lack of aspartate-ammonia ligase.
<b>Collagenase</b>	Skin ulcers	Causes collagen hydrolysis	Break up and remove dead skin and tissue
<b>DNase</b>	Cystic Fibrosis (CF)	DNase hydrolyses extracellular DNA responsible for Cystic Fibrosis.	DNA present in the mucous, which arises from dead WBCs and bacterial cells, serves to cross link the mucous, changing it from a fluid gel to a semi-solid.
<b>Lysozyme</b>	Antibiotic Therapy	Causes Bacterial cell wall hydrolysis	
<b>Ribonuclease</b>	Antiviral Therapy	Causes RNA hydrolysis	
<b>Trypsin</b>	Inflammation	Causes Protein hydrolysis	
<b>Uricase</b>	Gout	Converts Urate to allantoin	
<b>Enzyme inhibitors</b>	To increase the efficacy of drugs	Against resistant bacteria	Example: Beta lactamase inhibitor

## Leukemia

- Leukemia is a cancer of the marrow and blood.

---The four major types:

- Acute Myeloid Leukemia
- Chronic myeloid leukemia
- Acute Lymphoblastic Leukemia
- Chronic lymphocytic leukemia.

- Acute leukemia

---A rapidly progressing disease that produces cells (blasts) that are not fully developed.

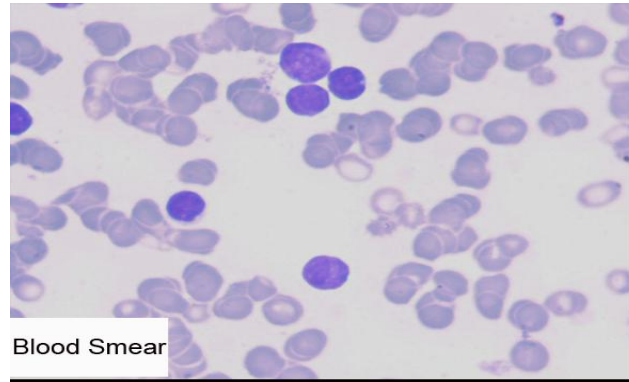
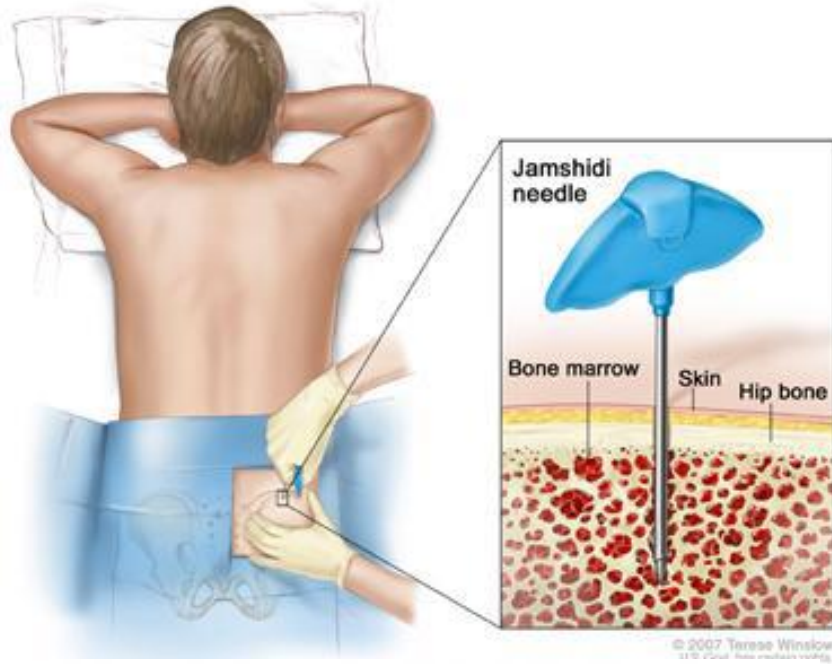
Most common childhood cancer

Demographics:

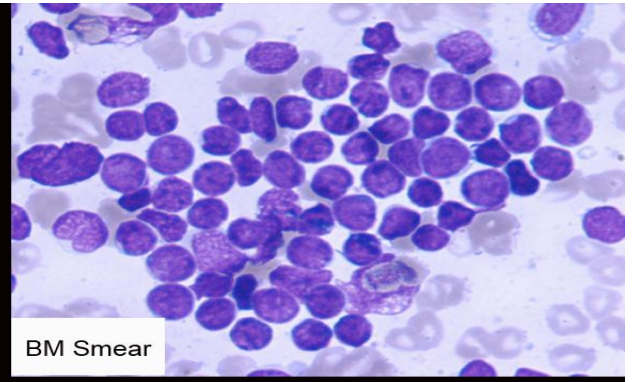
---Males more commonly than females

---Whites more than blacks

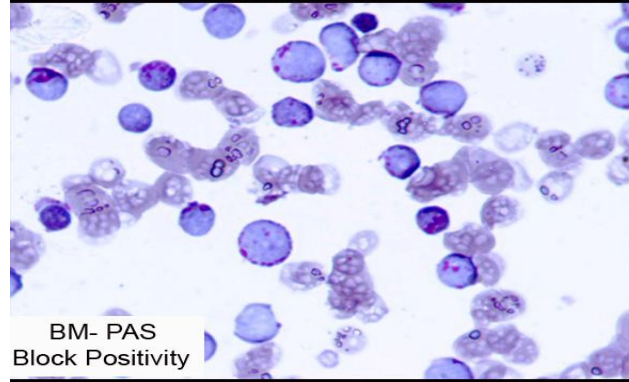
---More commonly in patients with Down Syndrome



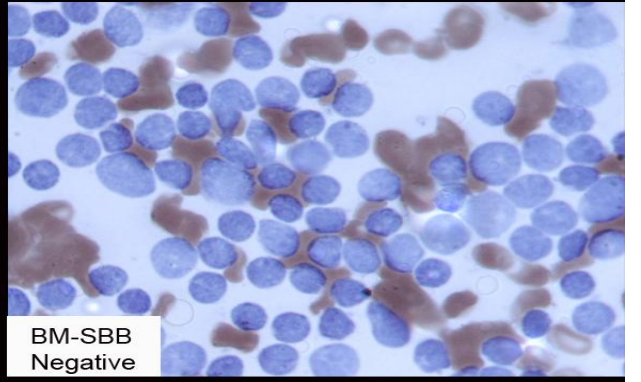
Blood Smear



BM Smear

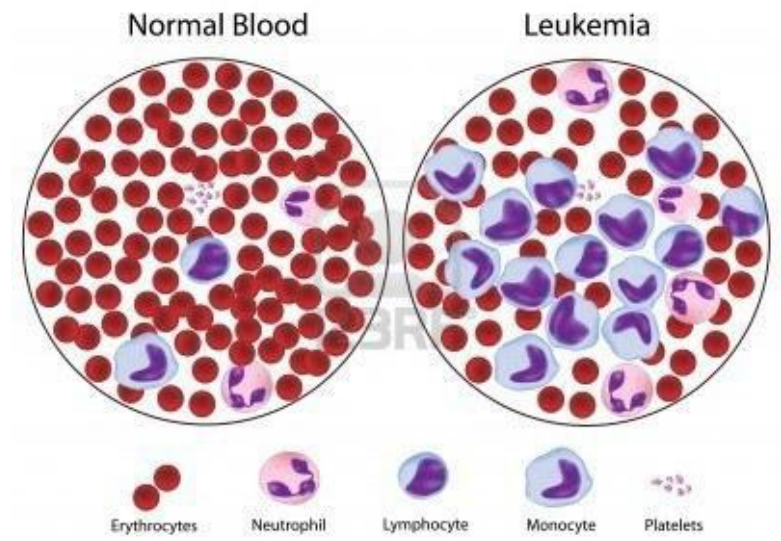


BM- PAS Block Positivity



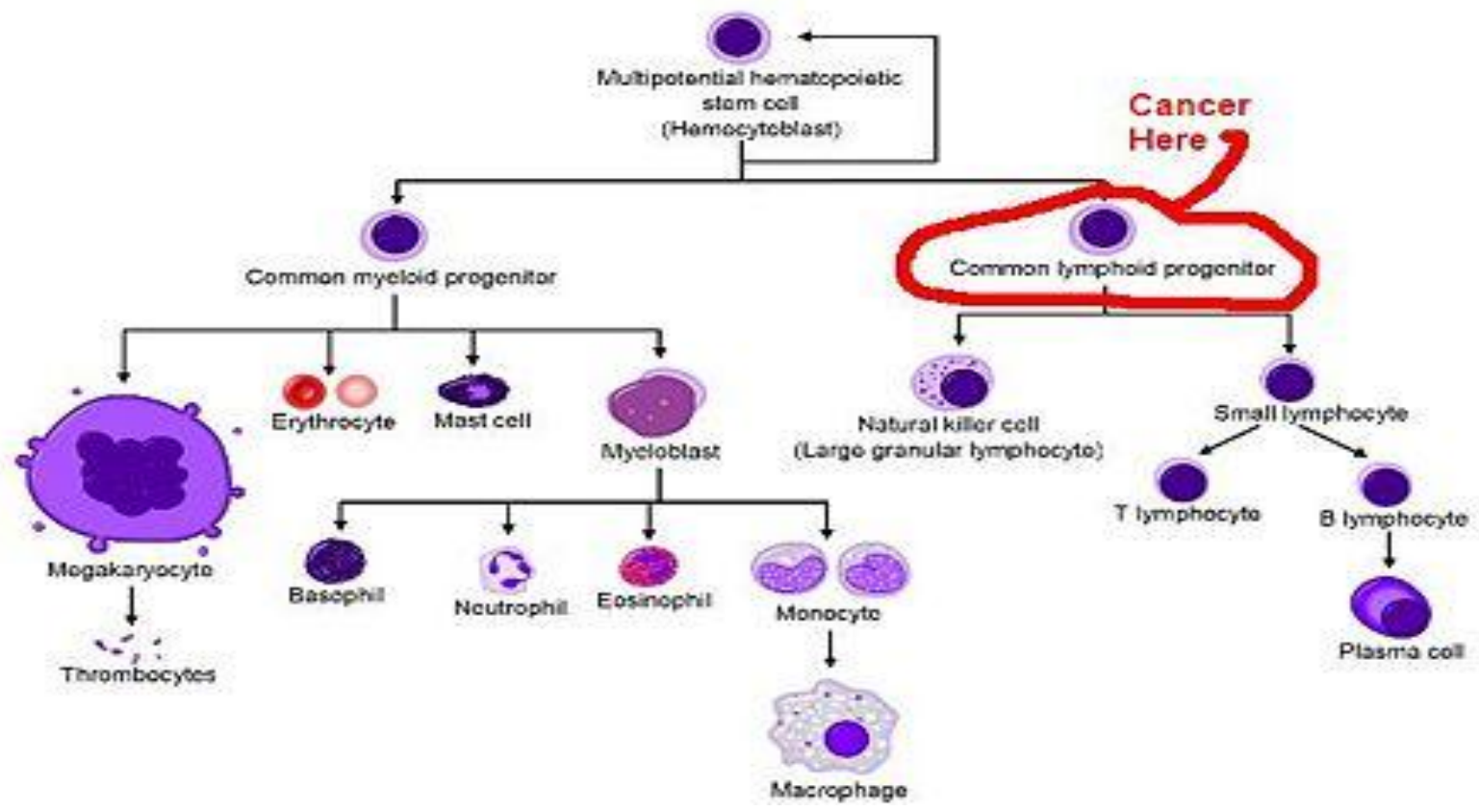
BM-SBB Negative

**Acute Lymphoblastic Leukemia (ALL-L1)**



**Bone marrow biopsy**

- >25% lymphoblast in the bone marrow
- Lumbar puncture
- CSF cytology
- Imaging/scans

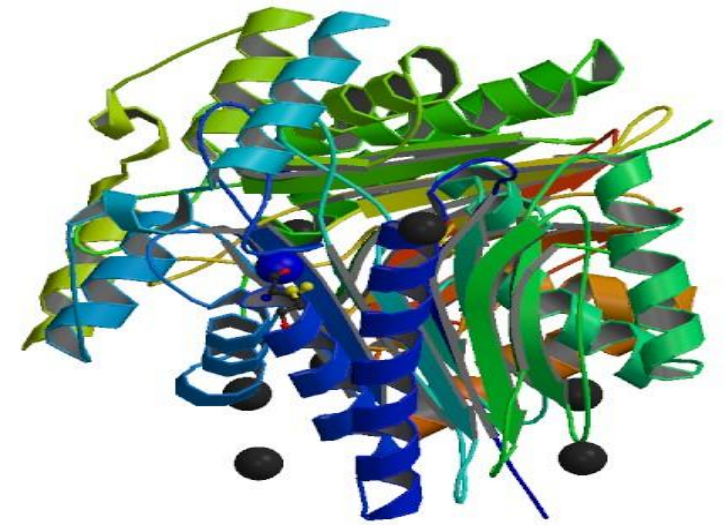


## Pegasparaginase (Oncaspar) for ALL

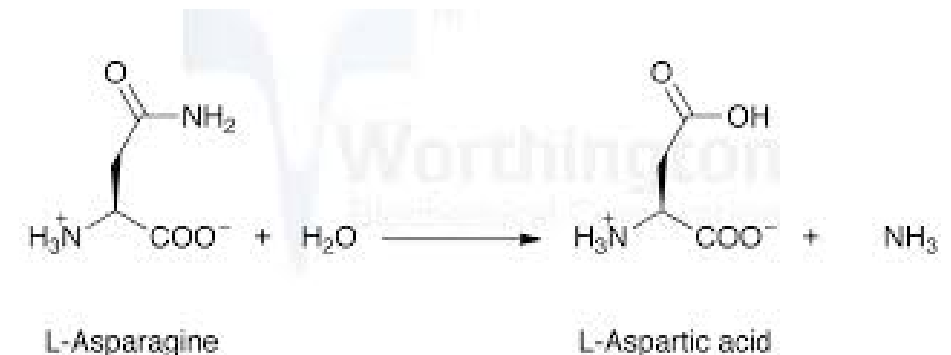
PEGylated L-asparaginase for the treatment of ALL in patients who are hypersensitive to the native unmodified form of L-asparaginase (obtained from *Escherichia coli* and *Erwinia chrysanthemi*). The drug was recently approved for front line use by FDA in 2007.

The malignant cells are dependent on an exogenous source of asparagine for survival.

Normal cells, however, are able to synthesize asparagine and thus are affected less by the rapid depletion produced by treatment with the enzyme asparaginase. Oncaspar exploits a metabolic defect in asparagine synthesis of some malignant cells.

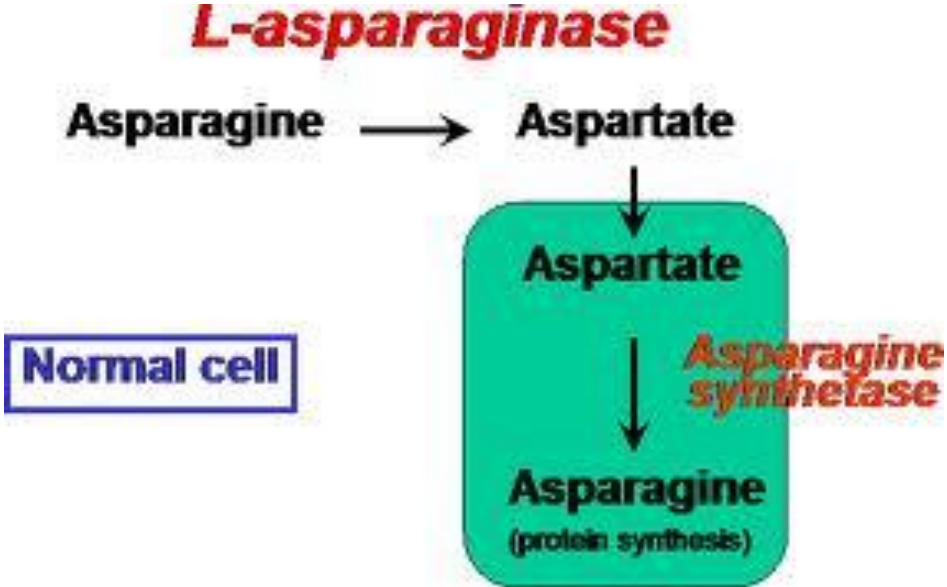


### Asparaginase

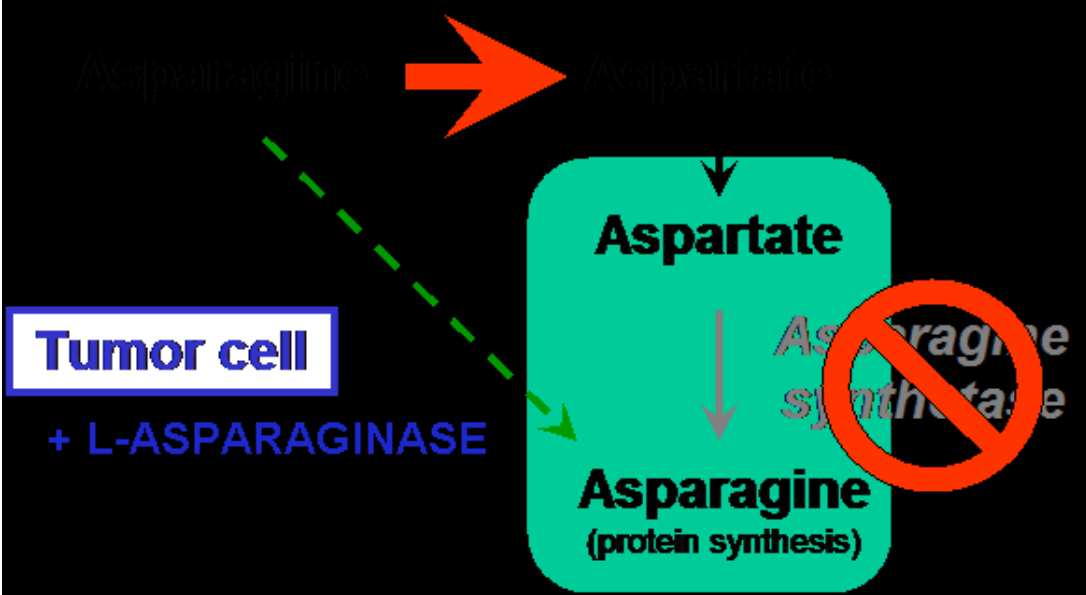




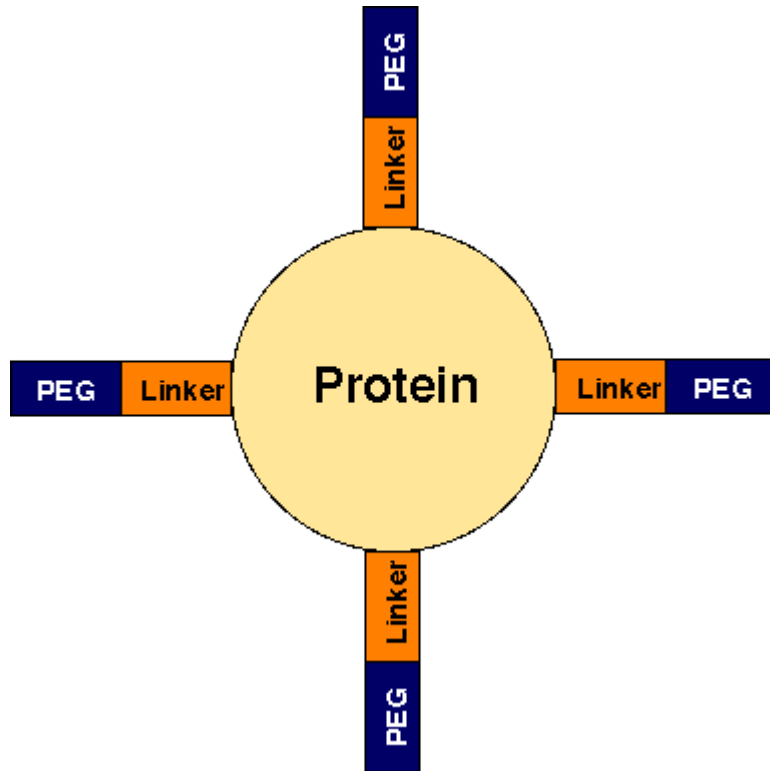
L-asparaginase in Normal Cells



L-asparaginase in Tumor Cells

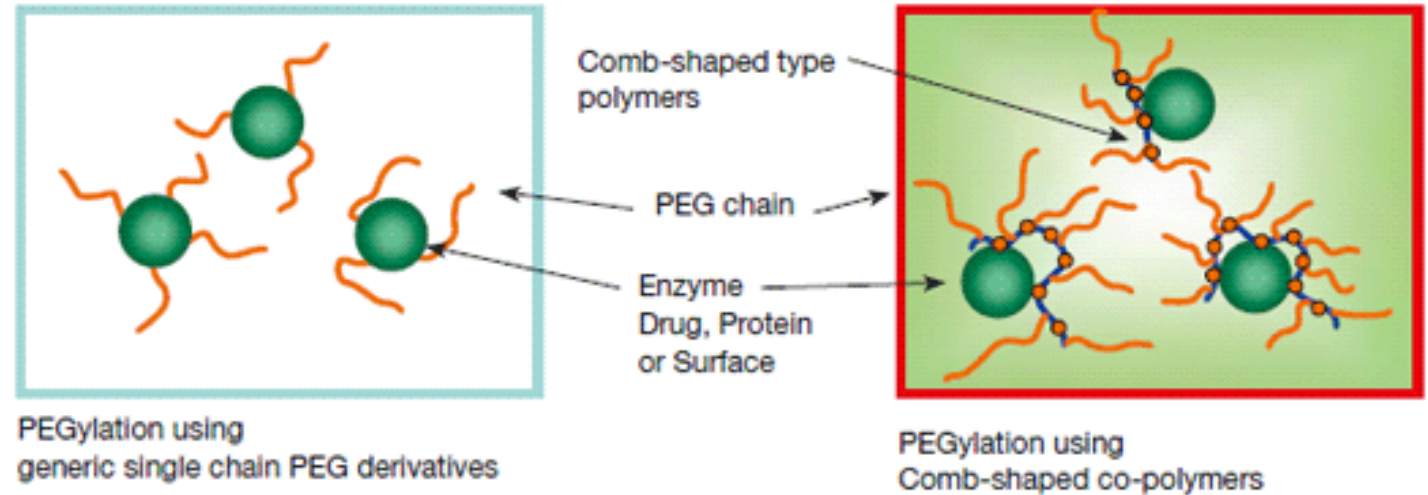


## PEGylation, successful approach to drug delivery



The molecular weight of a molecule increases which impart several significant pharmacological advantages over the unmodified form, such as

- Improved drug solubility
- Reduced dosage frequency, without diminished efficacy with potentially reduced toxicity.



# L-Asparaginase – Pharmacokinetics

- Extended circulating life
- Increased drug stability
- Enhanced protection from proteolytic degradation

-----PEGylated drugs have the following commercial advantages also:

- Opportunities for new delivery formats and dosing regimens
- Extended patent life of previously approved drugs

## Absorption

Not given orally because of intestinal degradation  
Intramuscular administration results in 50% lower peak blood levels than IV route

## Distribution

Primarily to intravascular space

Minimal blood-brain penetration

CSF levels are 1% of plasma concentration but depletion of plasma asparagine levels leads to an antileukemic effect in CNS

## Metabolism

Not known

# Half-lives of different L Asparaginase preparations Intramuscular

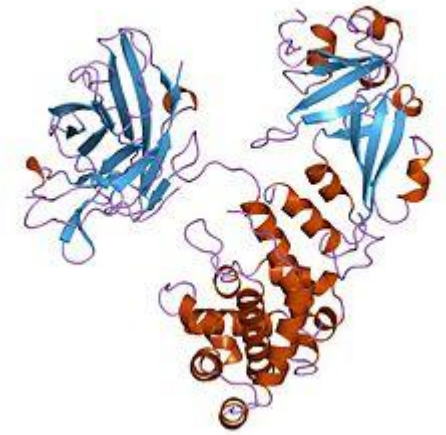
- Elimination
- **Asselin *et al* half-life (hours) Dose:25,000 IU i.m**
  - T  $\frac{1}{2}$  is variable with dose, disease status, renal or hepatic function, age, or gender
    - Depends on preparation
- PEG-Asparaginase(Oncaspar<sup>®</sup>) 137.5  $\pm$  77.8 hours
- *E.coli* L-Asparaginase (Crasnitin<sup>®</sup>/Elspar<sup>®</sup>) 29.8  $\pm$  4.1 hours
- *E. chrysanthemi* L-Aspa. (Erwinase<sup>®</sup>) 15.6  $\pm$  3.1 hours

## L-Asparaginase – Impaired Protein Synthesis

- Decreased production of insulin
  - Resultant hyperglycemia secondary to hypoinsulinemia
  - Hyperglycemia usually transient and resolves upon discontinuation
    - Fatal diabetic ketoacidosis has occurred
  - Patients with diabetes mellitus at increased risk of adverse reactions due to alteration in insulin production or pancreatic insults
    - Blood sugar should be closely monitored
- Decreased production of albumin
  - Hypoalbuminemia can be severe resulting in peripheral edema or ascites
    - Patients with limited hepatic synthetic function may be unable to tolerate the effects of L-asparaginase

# Other oncolytic enzymes

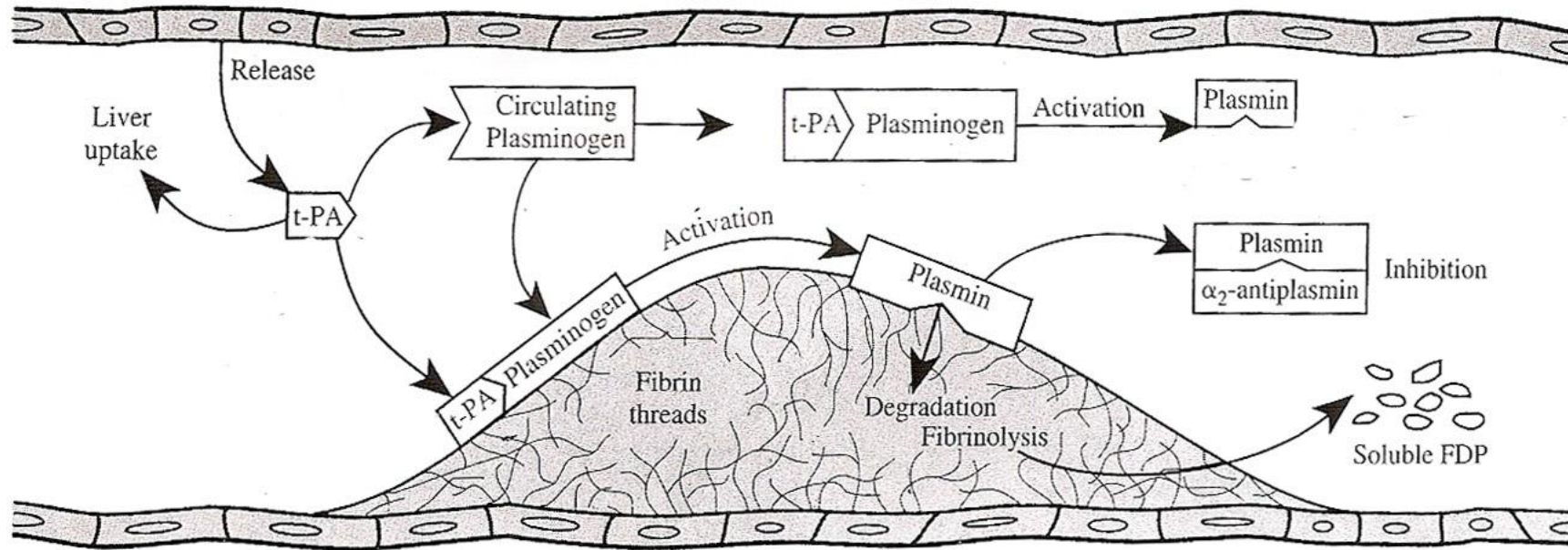
- **Diphtheria toxin** (an oncolytic enzyme still in the experimental stage), catalyzes transfer of the adenosine diphosphate ribose (ADP-ribose) moiety of nicotinamide adenine dinucleotide (NAD) to elongation factor 2
- This enzyme halts protein synthesis
- The protein synthesis in tumor cells is 100 to 10,000 time more sensitive to this toxin than the analogous process in normal cells
- Enzymes that degrade macromolecules: neuraminidase, ribonuclease, and a diverse group of proteases
  - **Neuraminidase** removes sialic acid residues from the surface of (neoplastic) cells, thereby altering their immunogenicity, and rendering them sensitive to immune response
  - 2000 -- The FDA has approved the Orphan Drug application of Wobe-Mugos as an adjunct therapy for multiple myeloma. Wobe-Mugos (vitamins + proteolytic enzymes), used successfully in Europe in conjunction with chemotherapy since 1977



Diphtheria toxin is an exotoxin secreted by *Corynebacterium diphtheriae*, the pathogenic bacterium that causes diphtheria.

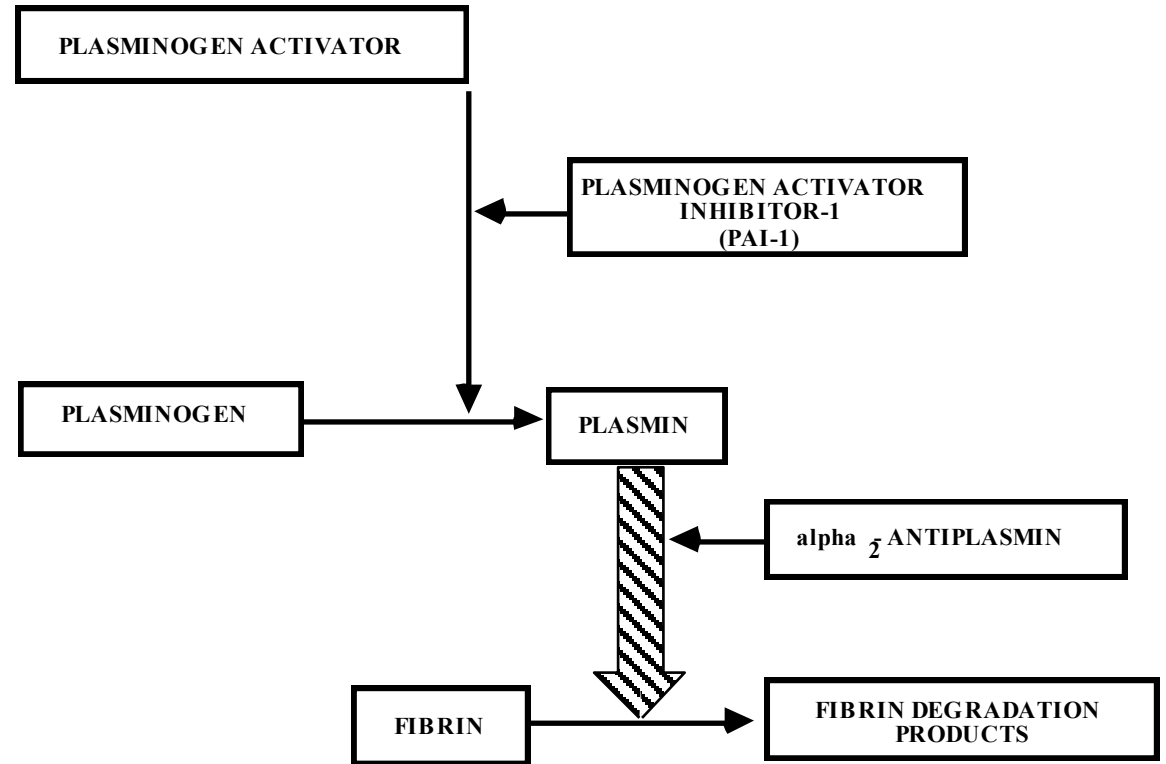
**Denileukin diftitox** (trade name Ontak) is an antineoplastic agent, an engineered protein combining Interleukin-2 and Diphtheria toxin.

# Fibrinolysis



## Thrombolytic Therapy

- Streptokinase
- Tissue Plasminogen Activator (rt-PA)
- Urokinase
- Retavase
- Tenecteplase, TNK-tPA ( TNKase™)





## Thrombolytic Drugs

### Streptokinase

It is a [bacterial protein](#) produced by group C (*beta*)-hemolytic streptococci

*Mechanism:* It binds to plasminogen producing an "activator complex" that lyses free plasminogen to the proteolytic enzyme plasmin

Plasmin degrades fibrin clots as well as fibrinogen and other plasma proteins (non-fibrin specific)

☐ Pharmacokinetics:

- The  $t_{1/2}$  of the activator complex is about 23 minutes
- The complex is inactivated by anti-streptococcal antibodies & by hepatic clearance

### **SIDE-EFFECTS:**

- Bleeding due to activation of circulating plasminogen
- Hypersensitivity: It is antigenic & can produce allergic reactions like rashes & fever (possibly via already present Streptococcal antibodies)

# Decreasing Protease Sensitivity

- **Streptokinase (Sk)** is produced by pathogenic strains of *streptococcus* and is a **blood clot-dissolving protease**.
- Sk complex with **plasminogen**→**plasmin**→ **degrades fibrin**. Plasmin→ also degrades Sk.
- For heart attack patients medical personnel has to administer Sk ASAP and in **30-90 min infusions**.
- Therefore a **long-lived** Sk is necessary.
- Plasmin cleaves peptide bonds after **Lys and Arg residues**.

# Streptokinase

- Plasmin cleaves Sk at **Lys 59 and 386** and the 328 peptide has only **16% activity** as the native molecule.
- To make Sk less susceptible, Lys at 59 and 386 were **changed to Glu** by site directed mutagenesis.
- Glu was chosen to replace Lys because the length of the side chain was similar and Glu does not have a +ve charge.
- Both single and double mutant retained their activity.
- Furthermore the half life of all three mutant increase and the double mutant was **21 fold more protease resistant** 3<sup>rd</sup> ed.

# Debriding agents

- Debriding agents effectively clean open wounds by removal of foreign matter and any surrounding dead tissue
- Trypsin, papain and collagenase (all proteolytic enzymes) have often be used
  - Trypsin: from mammalian pancreas, hydrolyse peptide bonds involving arg and lys
  - Papain: from the leaves and the unripe fruit of the papaya tree, hydrolyse peptide bonds involving basic amino acids (e.g. lys, arg, his)
  - Collagenase: from culture extracts of various animal cells or normally from various *Clostridium* species (pathogenic)

# Anti-inflammatory agents

- Administration of some enzymes is shown to be effective in the reduction of various inflammatory responses
  - Chymotrypsin: chymotrypsinogen (the zymogen form produced in pancreas) is converted to active form in small intestine
  - Bromelains: plant proteases purified from the stem or the fruit of pineapple
- Their anti-inflammatory action is not known in detail. Probably their ability to degrade protein-based inflammatory mediators play a role in their action

# Enzymes as digestive aids

- Most digestive aid preparations are based on depolymerases responsible from breakdown of polysaccharides, proteins and lipids
- Such preparations may include
  - a single enzyme or
  - multiple enzymes
- $\alpha$ -amylase: hydrolyse  $\alpha$ 1-4 glycosidic bonds
  - Amylase from *B. subtilis* or species of *Aspergillus* have various industrial applications
  - Oral amylase administration is used to aid digestion
- Lactase: hydrolysis of the lactose
  - In many geographical regions, adults has greatly reduced lactase activity

# Enzymes as digestive aids

- Various proteolytic enzymes, e.g. papain, pepsin
- Pancreatin: a preparation extracted from pancreas containing various enzymes
  - Used in deficiencies related with secretion of pancreatic enzymes (e.g. chronic pancreatitis, pancreatic carcinomas, cystic fibrosis)
- One problem associated with oral administration is gastric inactivation
  - Co-administration of inhibitors of gastric acid secretion
  - Enteric coated tablet or capsules
  - Use of microbial proteases, amylases and lipases

# Nuclease treatment of cistic fibrosis

- Cistic fibrosis (CF) is one of the most commonly occurring genetic diseases (1 in 2500 in northern Europe)
- Underlying cause is identified to the malfunction of ion transport
- Major clinical symptom is the production of viscous mucus in the respiratory track
- Change in lung physiology  $\Rightarrow$  bacterial infections  $\Rightarrow$  immune response  $\Rightarrow$  bacterial destruction  $\Rightarrow$  liberation of DNA  $\Rightarrow$  highly viscous mucus
- Therapy:
  - Percussion therapy is used to help the ejection of mucus
  - Bovine DNase treatment was approved in USA in 1950s but prolonged usage caused adverse reactions
  - DNase I produced by expression of cDNA in CHO cell lines (**Pulmozyme**) has been approved for medical use



# Enzyme-replacement therapy (ERT)

Brady and Schiffmann, The Lancet Neurology, 2004

- Metabolic storage disorders → insufficient activity of housekeeping enzymes
  - Gaucher's disease (\$40 000–320 000/year)
    - Glucocerebrosidase absence (glycolipid accumulation in cells, especially in macrophages)
    - Enzyme from human placentae
    - Recombinant enzyme in CHO cell line (Cerezyme, 1994)
  - Fabry's disease, in which the heart, kidney, gastrointestinal tract, and peripheral nerves are damaged (\$160 000/year)
  - Pompe's disease, in which the heart, skeletal muscles, and brain are involved
  - Hurler's disease and Maroteaux-Lamy syndrome in which the eyes, liver, joints, and skeleton are usually affected

