Deleterious consequences of defective purine metabolism

- **Gout (excess accumulation of uric acid)**

  -Overproduction of purines

- **Lesch-Nyhan syndrome**

  -Overproduction of purines

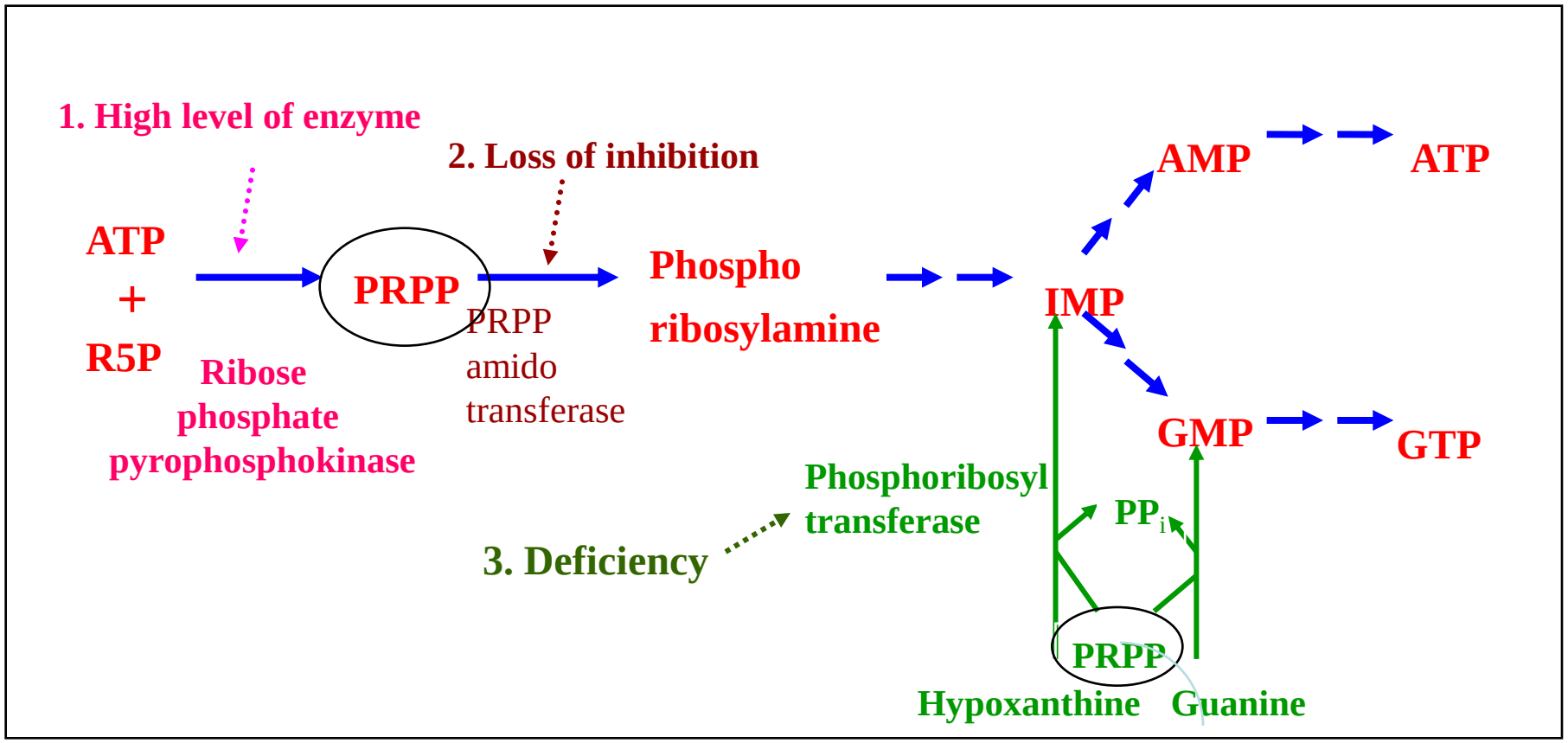
- **Immunodeficiency**

  Severe Combined Immunodeficiency disease (SCID).

  The disease is caused by the defects in ADA (adenosine deaminase).

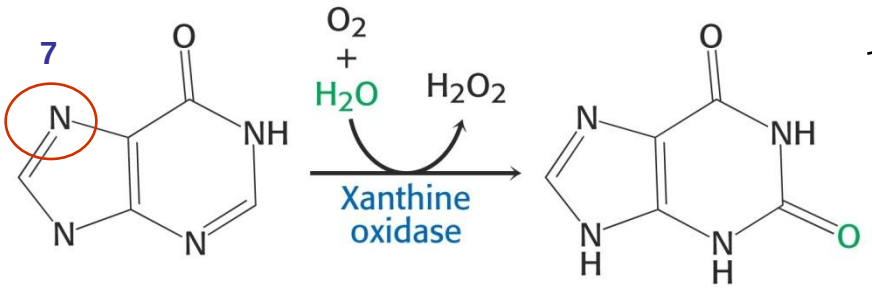
Causes of Purine Overproduction

1. Deficiency of phosphoribosyltransferase in purine salvage pathway.
2. High activity of PRPP synthesis, not sensitive to feedback inhibition.
3. Misfunction of PRPP amidotransferase.



Gout

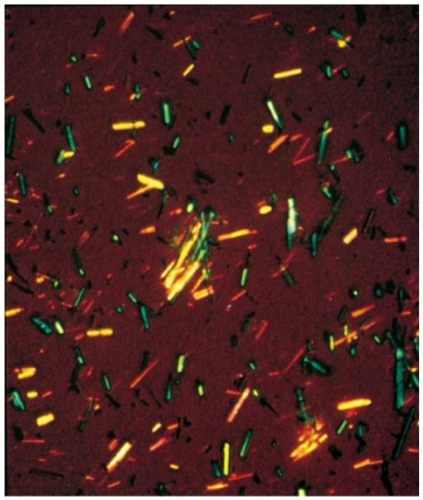
- **Precipitation and deposition of uric acid (in form of sodium urate) in joints, kidney and ureter**
- **Causes arthritic pain and kidney stones**



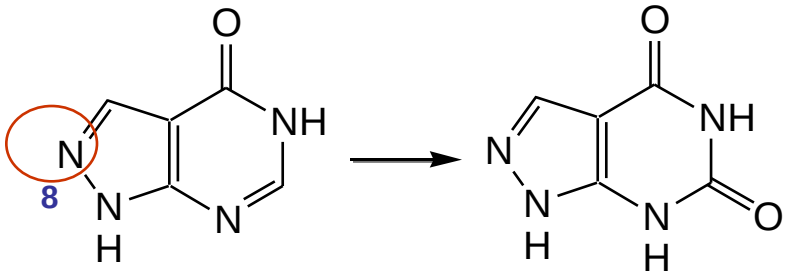
More soluble

Hypoxanthine **Xanthine**

Micrograph of sodium urate crystals



Suicide Inhibitor



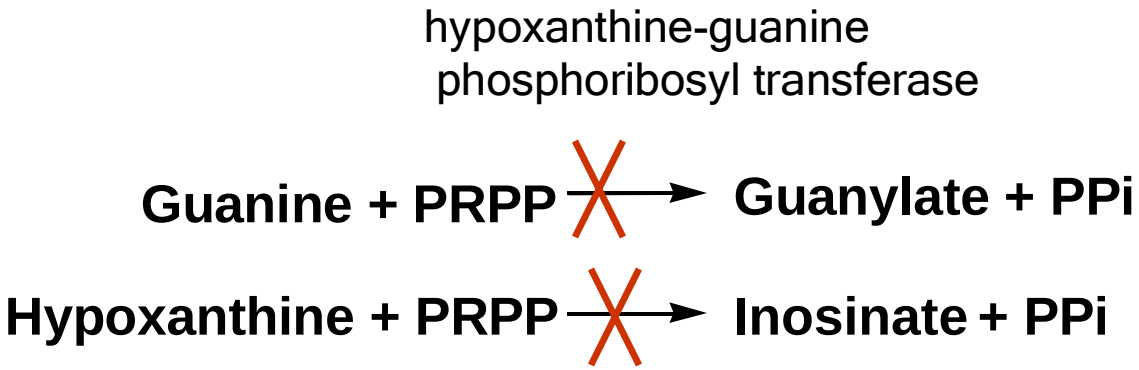
Allopurinol **Alloxanthine**

Also known as oxypurinol

Lesch-Nyhan Syndrome

- **Caused by a severe deficiency in HGPRT activity**
- **Symptoms are gouty arthritis due to uric acid accumulation and severe neurological malfunctions including mental retardation, aggressiveness, and self-mutilation**
- **Sex-linked trait occurring mostly in males**

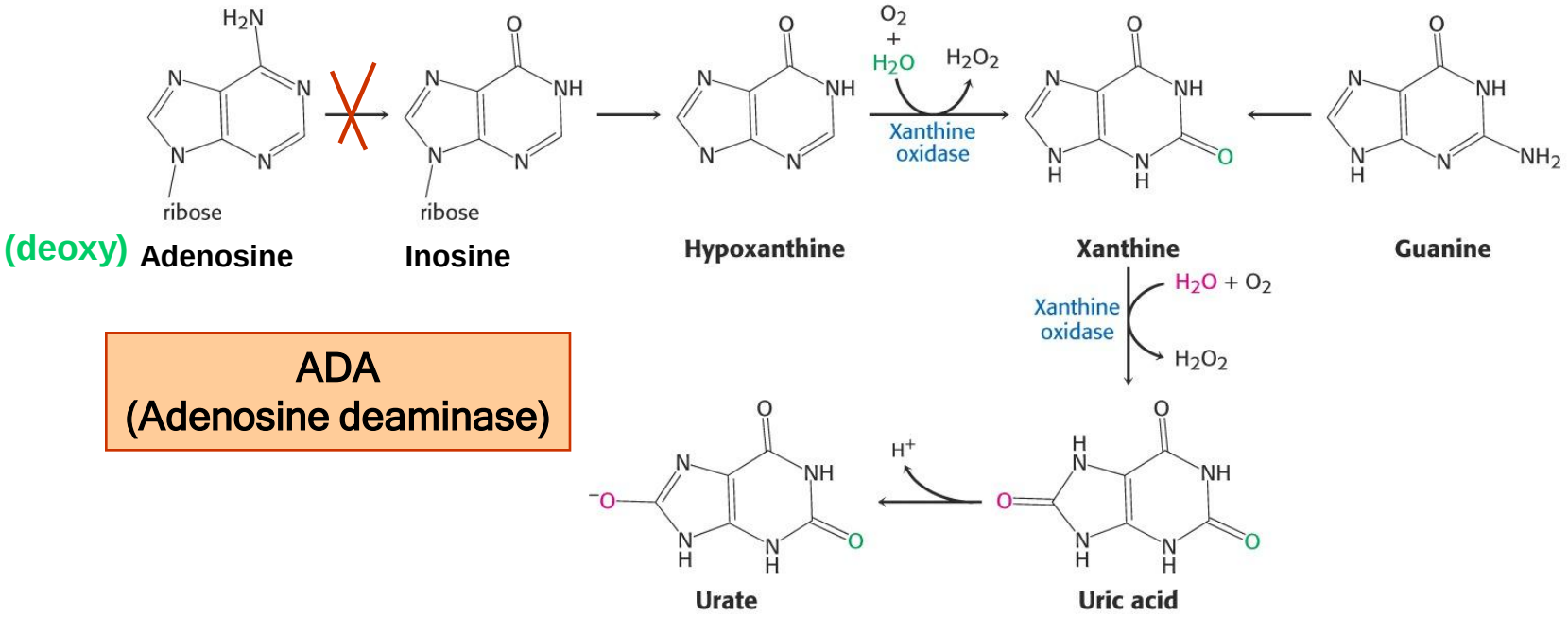
Lack of HGPRT activity in Lesch-Nyhan Syndrome causes a buildup of PRPP, which activates the synthesis of purine nucleotides



- **Excessive uric acid forms as a degradation product of purine nucleotides**
- **Basis of neurological aberrations is unknown**

Immunodeficiency

Severe Combined Immunodeficiency disease (SCID).

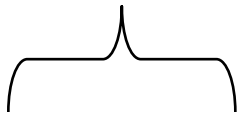


ADA
(Adenosine deaminase)

- **Defects in ADA causes accumulation of deoxyadenosine**
- **deoxyadenosine is converted into dAMP* → →dATP**
- **dATP inhibits the synthesis of deoxyribonucleotides by ribonucleotide reductase, causing problems with the immune system (death of lymphocytes, immunodeficiency disease)**

*lymphocytes have high levels of adenosine kinase

Enzyme sites on Ribonucleotide reductase

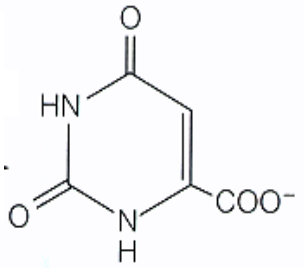


A/H site	S site	Catalyses reduction of	Inhibits reduction of	Result
ATP	ATP	CDP/UDP	None	↑[dCDP, dUDP]*
ATP	dTTP	GDP	CDP/UDP	↑[dGDP] ↓[dCDP, dUDP]
ATP	dGTP	ADP	CDP/UDP	↑[dADP] ↓[dCDP, dUDP]
dATP	Any	None	ADP/GDP/CDP/ UDP	OFF

Defects in *de novo* pyrimidine biosynthesis lead to clinical disease

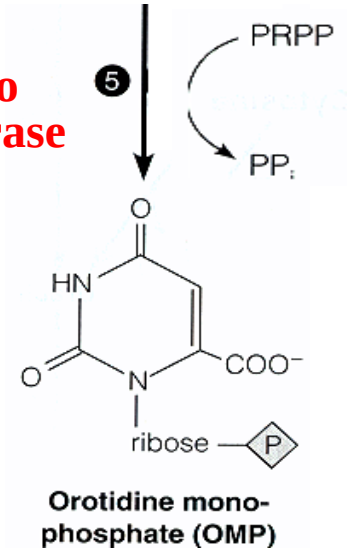
- **Orotic aciduria**
  - Genetic
  - Characterised by orotic acid (orotate) excretion
  - Symptoms: anemia*, growth retardation
  - Causes: a defect in phosphoribosyl transferase (step 5) or orotidine decarboxylase (step 6) **
  - No UMP produced (therefore no dTMP and no CTP)

Step 5



Orotate

Orotate-phospho Ribosyl transferase

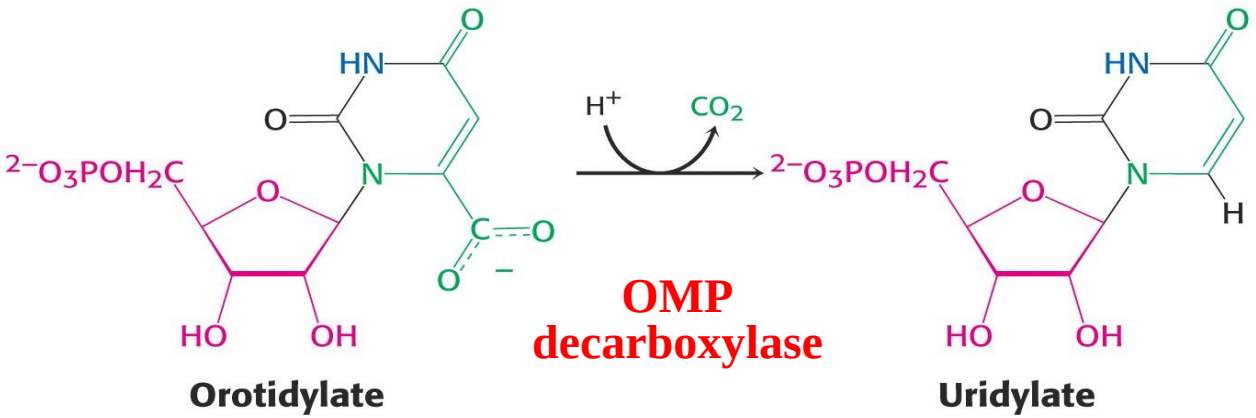


Orotidine mono-phosphate (OMP)

* Arises due to inhibition of DNA synthesis

** Multienzyme complex: UMP synthase

Step 6



Treatment:

a) patients are fed uridine (nucleoside)

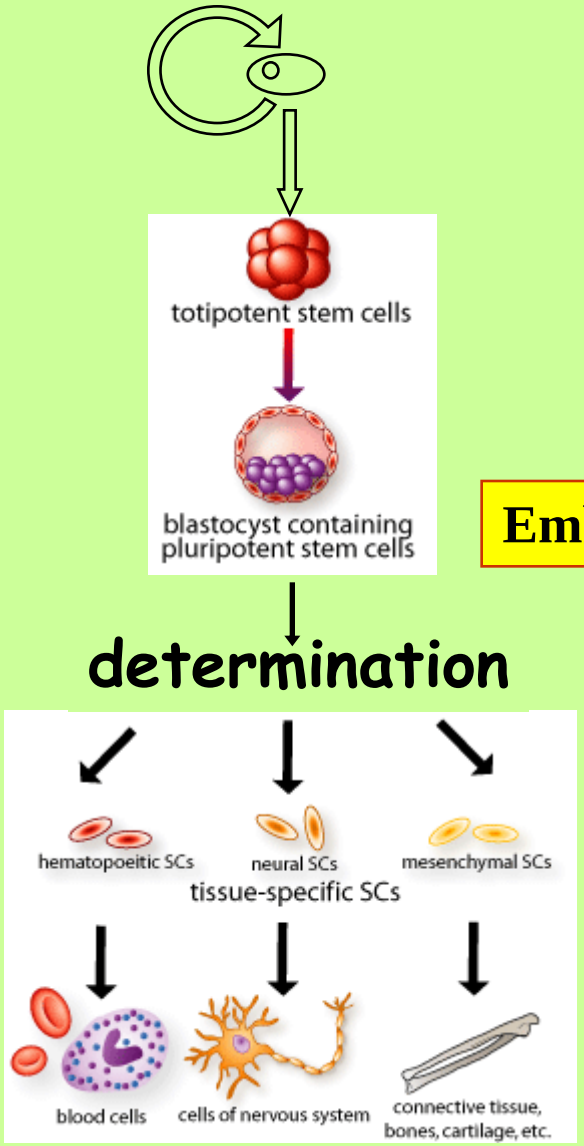
-converted to → UMP → UDP → UTP

- production of dTMP and CTP facilitated

b) Direct administration of CMP or UMP

UMP/UTP inhibits carbamoyl phosphate synthase II preventing the biosynthesis and accumulation of orotic acid

Treatment of primary immunodeficiency diseases



Embryonic stem cell

Adult stem cell

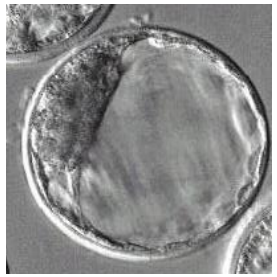
Gene therapy

Transplantation
Tissue repair, regeneration, reconstitution

Stem cell : unspecialised cell capable of self renewing AND generating specialized cells

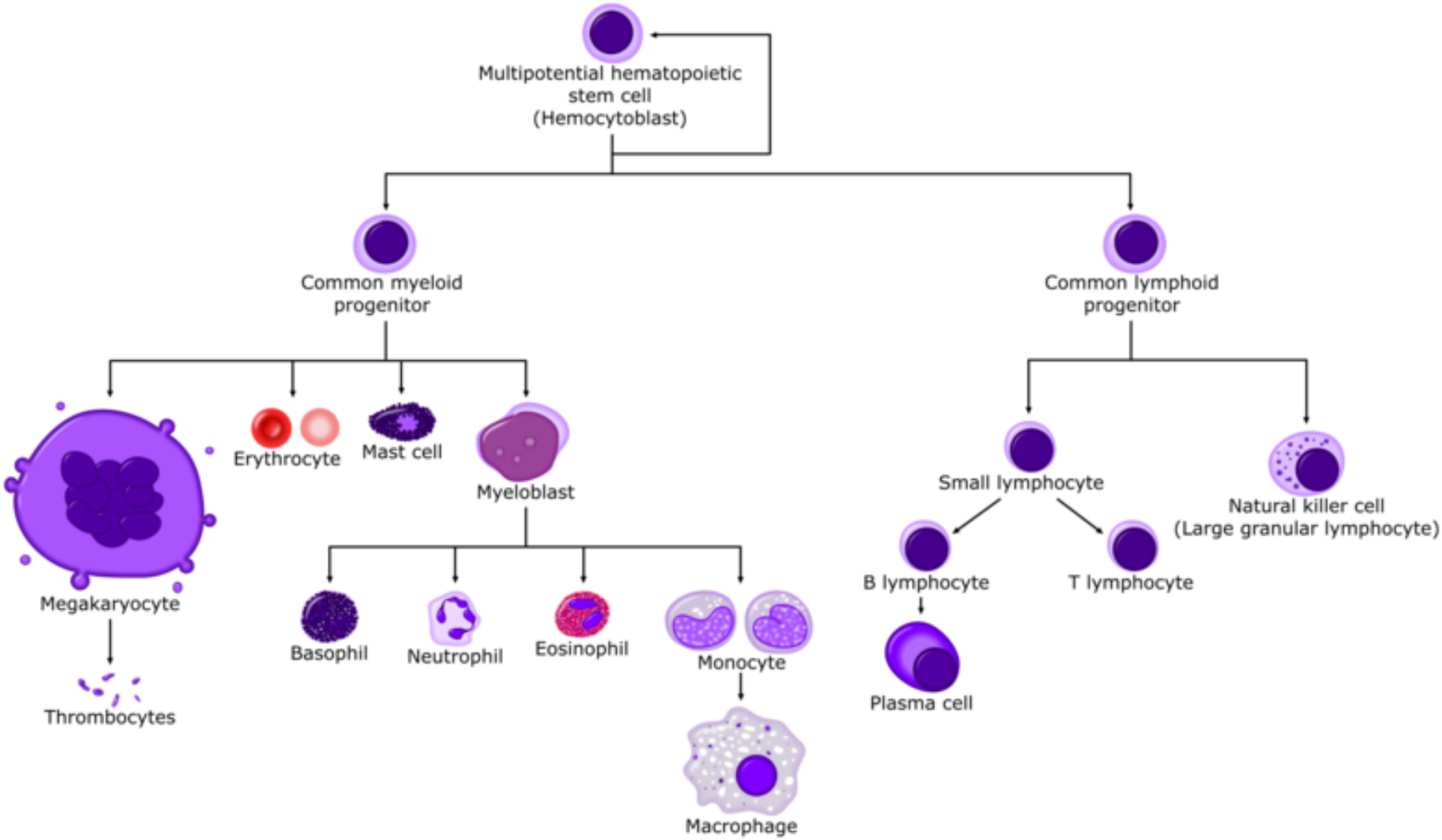
Stem cell types

- Embryonic
  - Pluripotent (can differentiate into all embryonic tissue)
  - Ethical issues

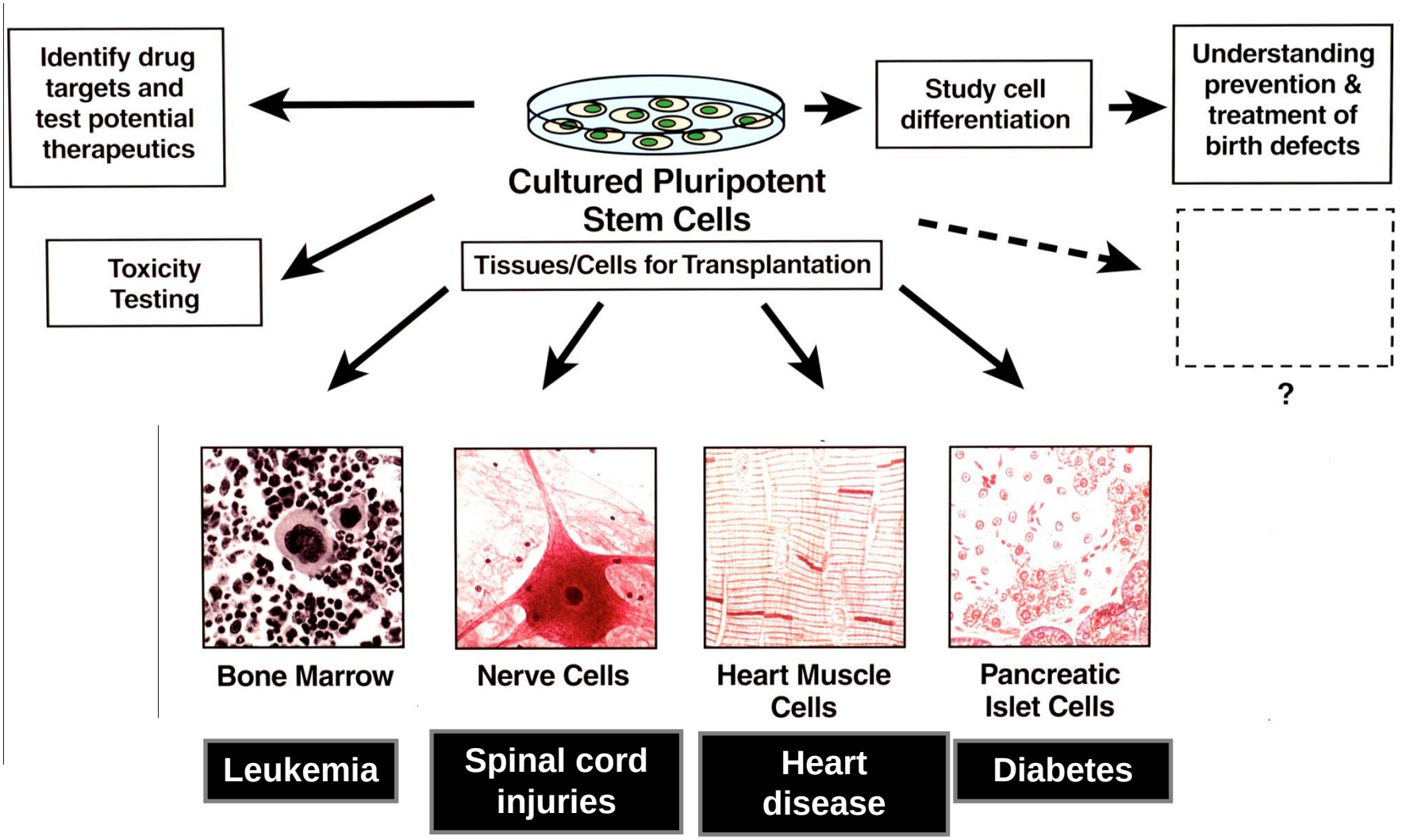


- Adult
  - Multipotent (tissue specific; restricted differentiation potential)
  - Autologous use

- Umbilical Cord Blood
  - Contains primitive hematopoietic progenitor cells
  - Limited yield


Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Thrombocytes
Erythrocyte
Mast cell
Myeloblast
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Small lymphocyte
B lymphocyte
Plasma cell
T lymphocyte
Natural killer cell
(Large granular lymphocyte)

The *Promise* of Stem Cell Research



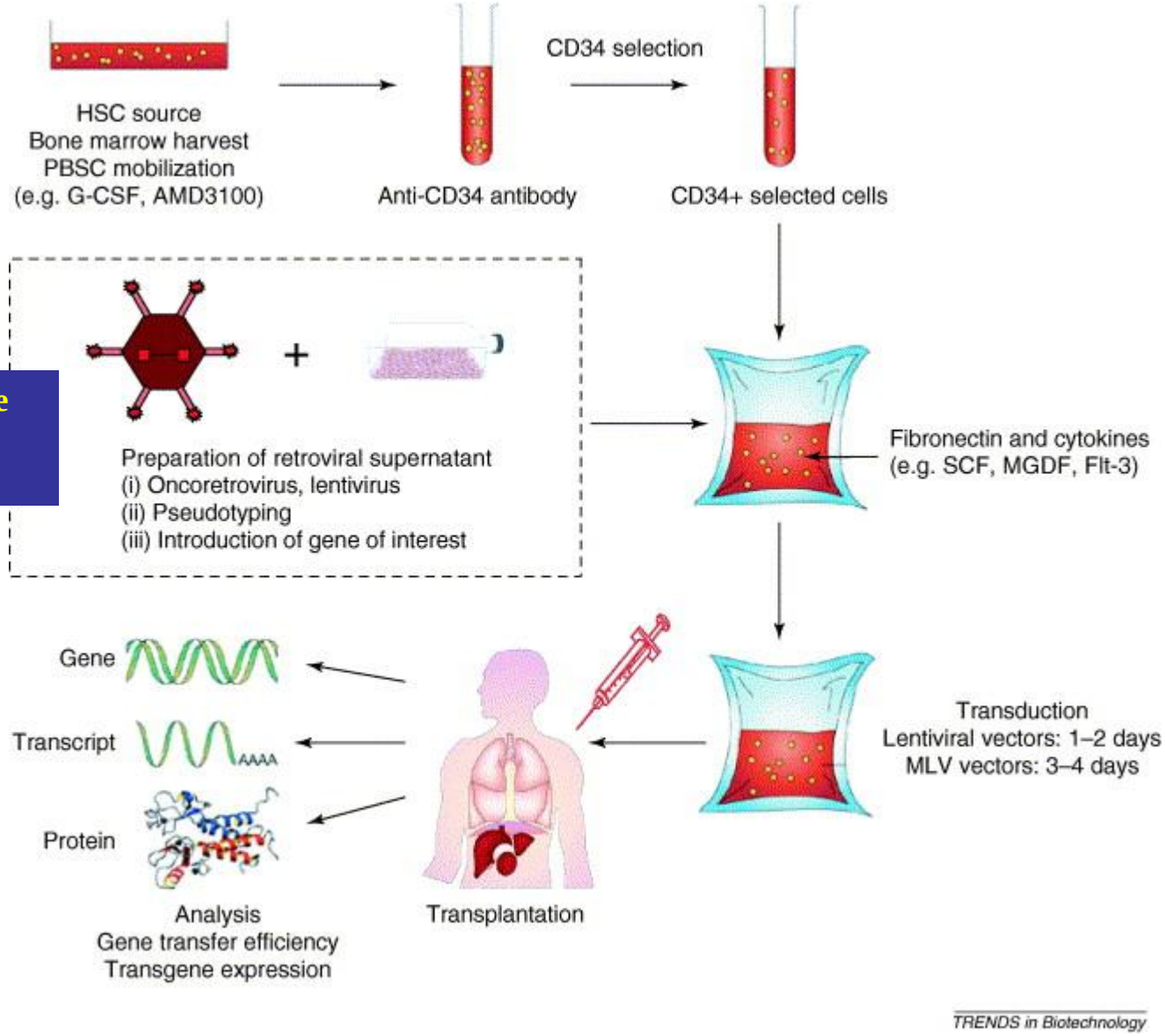
How Gene Therapy Works

- A vector (usually a virus) delivers the therapeutic gene into a patient's target cell
- The target cells become infected with the viral vector
- The vector's genetic material is inserted into the target cell
- Functional proteins are created from the therapeutic gene causing the cell to return to a normal state

Viruses

- Replicate by inserting their DNA into a host cell
- Gene therapy can use this to insert genes that encode for a desired protein to create the desired trait

Gene therapy



eg. Adenosine deaminase (SCID)

Taken from Ferguson et al. (2005) TRENDS in Biotechnology, Vol 23

Gene therapy

- Gene therapy:
  - a technique for correcting defective genes that are responsible for disease development
- Two strategies:
  - Direct infusion
  - Cell based (Genetic modification *in vitro)*
- Second strategy allows more control
- Can use cells or stem cells
  - Which one is better? Why?

The First Gene Therapy

- Performed on September 14th, 1990

  - Ashanti DeSilva was treated for SCID
  - Doctors removed her white blood cells, inserted the missing gene into the WBC, and then put them back into her blood stream.
  - This strengthened her immune system
  - Only worked for a few months
  - Why? What is lifespan of a lymphocyte?

Stem cell-based gene therapy

- Focus on cancer, HIV & single-gene diseases
  - Why HIV?
- Hematopoietic stem cells (HSC) are delivery cell of choice
  - Accessible from bone marrow, peripheral or umbilical cord blood
  - Easily identified and manipulated
  - Can be re-infused intravenously
  - HSC give rise to many cell types
  - HSC home to bone marrow, lymph and spleen

First HSC gene therapy trial

- Late 1990's
  - 11 X-SCID boys (IL-2 receptor mutation)
  - Received autologous CD34+ bone marrow cells containing correct gene
  - No prior chemo or radiotherapy
  - Rapid and extensive T and B cell reconstitution
  - All circulating T cells contained the vector

- BUT, January 2003, 3 of the children developed leukemia-like condition
  - One problem with gene therapy is that one does not have control over where the gene will be inserted into the genome.

Leukaemia in gene therapy patients primarily caused by insertional mutagenesis

LMO-2 is a gene involved in T cell development, so if it is turned on at the wrong time by a vector inserting close to it, it can cause blood cells to divide rapidly and lead to cancer

Unsuccessful Gene therapies

- Jesse Gelsinger, a gene therapy patient who lacked ornithine transcarbomylase (OTC) activity, died in 1999.

Within hours after doctors shot the normal OTC gene attached to a therapeutic virus into his liver, Jesse developed a high fever due to a severe immune response to adenovirus carrier

His immune system began raging out of control, his blood began clotting, his liver hemorrhaged and a flood of white blood cells shut down his lungs.

Other gene therapy candidates

- Myoblasts
  - Fuse with existing myofibres
  - Well supplied with blood and nerves
  - Myoblasts secreting erythropoietin (hormone produced by the kidney – stimulates RBC production) reversed anemia in mice with renal failure

Other gene therapy candidates

- Neural stem cells
  - Engineered to produce cytosine deaminase
    - Cytosine Deaminase converts nontoxic precursor 5-Fluorocytosine to toxic 5-Fluorouracil
  - Injected into brains of mice with human-derived gliomas + 5-FC
  - Neural stem cells “found” the glioma cells and converted 5-FC to 5FU at site
    - 80% reduction in tumor size

Purine metabolism: summary

- Nucleotides have many important functions in a cell.

- Two major sources of nucleotides are salvage pathway and de novo biosynthesis

- Purine nucleotides are biodegraded by nucleotidases, nucleotide phosphorylases, deaminases, and xanthine oxidase/dehydrogenase (oxidation).

- Uric acid is the final product of purine biodegradation in mammals

- Defective purine metabolism leads to clinical disease.

Pyrimidine metabolism: take home message

1. Pyrimidines are synthesized by de novo & salvage pathways.

2. Pyrimidine nucleotides are biodegraded by nucleotidases,nucleotide phosphorylases, deaminases, and dihydrouracil dehydrogenase (reduction).

3. β-Alanine is a product of pyrimidine biodegradation in mammals

4. The mammalian enzymes are multifunctional (e.g. CAD, UMP synthase) and form multienzyme complexes to increase efficiency.

5. Defective pyrimidine metabolism leads to clinical disease