Part II

Drugs acting on enzyme

(Antimetabolites)
ANTIMETABOLITES

- Antimetabolite are structurally related to normal compounds within the cell.

- Antimetabolite generally interfere with the availability of normal purine or pyrimidine nucleotide precursors either
  - by inhibiting their synthesis or
  - by competing with them in DNA or RNA synthesis.

- Antimetabolites are S phase-specific drugs that are structural analogues of essential metabolites and that interfere with DNA synthesis.

- Myelosuppression is the dose-limiting toxicity for all drugs in this class.
ANTIMETABOLITES

► Pyrimidine Antagonists
  ▪ Methotrexate, Fluorouracil, Floxuridine, Tegafur

► Purine Antagonists
  ▪ Mercaptopurine, Thioguanine

► DNA Polymerase/ DNA Chain Elongation Inhibitors
  ▪ Cytarabine, Gemcitabine, Fludarabine, Cladribine, Clofarabine

► Miscellaneous Antimetabolite
  ▪ Hydroxyurea
Antimetabolites

Pyrimidine Antagonists: deoxythymidine monophosphate (dTMP) Synthesis Inhibitors

- **Indirect Inhibitors:** Dihydrofolate reductase (DHFR) inhibitors
  - Methotrexate
- **Direct Inhibitors:** Thymidylate synthase inhibitors
  - Fluorouracil, Floxuridine, Tegafur
Antimetabolites

Pyrimidine Antagonists: Dihydrofolate reductase (DHFR) inhibitors

Methotrexate (MTX)

- The structures of MTX and folic acid are similar.
- MTX is actively transported into mammalian cells and **inhibits dihydrofolate reductase (DHFR)**, the enzyme that normally converts dietary folate to the tetrahydrofolate (THF) form required for thymidine and purine synthesis.

**Adverse Effects:**
- MTX is myelosuppressive, producing severe leukopenia, bone marrow aplasia, and thrombocytopenia.
- This agent may produce severe gastrointestinal disturbances.
- Renal toxicity may occur because of precipitation (crystalluria) of the 7-OH metabolite of MTX.
Methotrexate: Mechanism of Action

- The structures of MTX, folic acid and DHF are similar.
- MTX inhibits dihydrofolate reductase (DHFR) and prevents conversion of THF to cofactor $N^5,N^{10}$-THF.
- Depletion of cofactor ($N^5,N^{10}$-THF) effects thymidylate synthase and lowered synthesis of dTMP (DNA synthesis).
Methotrexate: Mechanism of Action

Methotrexate inhibits dihydrofolate reductase (DHFR), which prevents the conversion of dihydrofolate to tetrahydrofolate. This disrupts the folate cycle and affects DNA synthesis and cell division.
Pyrimidine Antagonists: Thymidylate synthase inhibitors

5-Fluorouracil

- 5-Fluorouracil (5-FU) act as a prodrug which is converted to the fluorinated analogue of 2’-deoxyuridylic acid monophosphate (5-FdUMP)
- 5-FdUMP competes with deoxyuridine monophosphate (dUMP) for the enzyme thymidylate synthetase
- 5-FdUMP inhibits thymidylate synthetases and prevents the synthesis of dTMP, a major building block of DNA.
5-Fluorouracil: Mechanism of Action

![5-Fluorouracil](image)

FdUMP

FdUMP → dUMP → dTMP

(dTMP: major building block of DNA)

Folic acid

NADPH + H⁺ → 7,8-Dihydrofolate (DHF) → NADP⁺

7,8-Dihydrofolate reductase

DHFR

N5, N10-Methylene H₄ folate (N₅,N₁₀ THF)

Glycine

Serine

PLP

H₄ folate (THF)

Serine hydroxymethyltransferase

thymidylate synthase
5-Fluorouracil (5-FU)

Adverse Effects:
• Fluorouracil may cause nausea and vomiting, myelosuppression, and oral and gastrointestinal ulceration.
• With fluorouracil, myelosuppression is more problematic after bolus injections, whereas mucosal damage is dose-limiting with continuous infusions.

Tegafur
• It’s a 3-tetrahydrofuranyl derivative of 5-FU
• It is a prodrug slowly metabolized to 5-FU
Antimetabolites

Purine Antagonists:

- All thiopurines: azathioprine, 6-mercaptopurine, 6-thioguanine are prodrugs
- Azathioprine is converted to 6-mercaptopurine (6-MP) by non-enzymatic activation in red blood cells
Mechanism of Action of Azathioprine, 6-Mercaptopurine, 6-Thioguanine

HGPRT = Hypoxanthine–guanine phosphoribosyltransferase

TPMT = Thiopurine S-methyltransferase

XO = Xanthine oxidase

HGPRT = Hypoxanthine–guanine phosphoribosyltransferase

5-phosphoribosylpyrophosphate

5-phosphoribosylamine

TIMP = Thio inosine monophosphate

TGMP = Thio guanosine monophosphate
Mechanism of Action of Azathioprine, 6-Mercaptopurine, 6-Thioguanine

- **Azathioprine** is converted to 6-mercaptopurine (6-MP) by non-enzymatic activation in red blood cells.
- The enzyme **hypoxanthine–guanine phosphoribosyltransferase (HGPRT)** convert (activate) 6-MP into **thio inosine monophosphate (TIMP)** and 6-TG into **thio guanosine monophosphate (TGMP)**.

- In **catabolic reactions**, **thiopurine S-methyltransferase (TPMT)** inactivates 6-MP and 6-TG by S-methylation and form **Me-6-MP** and **Me-6-TG**
  - Xanthine oxidase (XO) converts 6-MP to **6-thiouric acid**.

- **TIMP** and **TGMP** are also TPMT substrates.
- **TIMP and Methylated TIMP (meTIMP), but not meTGMP**, is an effective inhibitor of de novo purine biosynthesis by preventing the first step conversion of 5-phosphoribosyl pyrophosphate in to 5-phosphoribosylamine.

- TIMP that escapes catabolism is further metabolized by inosine monophosphate dehydrogenase (IMPDH) and guanine monophosphate synthetase (GMPS) to **TGMP**.

- **TGMP** is converted into **thioGTP** and **thio-dGTP** (by deoxynucleoside kinases and reductase) that incorporates into RNA and DNA leading to cell death.
6-MP & Allopurinol:

- 6-mercaptopurine is rapidly metabolized in the liver by xanthene oxidase (XO) enzyme into the inactive metabolite (6-thiouric acid) which are excreted in the urine.
- So when 6-mercaptopurine is co-administered with allopurinol (xanthine oxidase inhibitor) its **half-life will be increased**.
- **Allopurinol** is used frequently to treat/prevent **hyperuricemia** caused by many anticancer drugs.
- **If Allopurinol is used with 6-MP then the dose of 6-MP is reduced by more than 75%**

**Indications:**

- Mercaptopurine is used primarily for the maintenance of remission in patients with acute lymphocytic leukemia and is given in combination with MTX for this purpose.

**Adverse Effects:**

- Well tolerated.
- **Myelosuppression** is generally mild with thioguanine.
- Long-term mercaptopurine use may cause **hepatotoxicity**.
DNA Polymerase/ DNA Chain Elongation Inhibitors:

Cytarabine

• DNA Polymerase catalyse the synthesis of DNA using the four DNA building blocks (dATP, dGTP, dCTP, dTTP)

• Cytarabine is an analogue of 2’-deoxycytidine

• Cytarabine act as a prodrug.

• In cell, Cytarabine is phosphorylated to triphosphate (ara-CTP) which act as a competitive inhibitor.

• In addition, ara-CTP can act a substrate for DNA polymerases and become incorporated into the growing DNA chain leading to chain termination or prevent replication of the modified DNA causing inhibition of DNA synthesis

• Formulation: Cytarabine is available as a water soluble sterile powder for intravenous, intrathecal and subcutaneous use.

• Indications: ALL (Acute lymphoblastic leukemia), AML (Acute myelogenous leukemia), chronic myelocytic leukemia, meningeal leukemia.

• Adverse Effects: High doses can damage the liver, heart, and cause bone marrow depression.

• Metabolism: metabolized to an inactive product, arabinofuranosyluracil.
DNA Polymerase/ DNA Chain Elongation Inhibitors

Cytarabine  Gemcitabine  Fludarabine phosphate

Cladribine  Clofarabine
Miscellaneous Antimetabolites

**Hydroxyurea**

- Prevent DNA synthesis and DNA repair by inhibiting ribonucleotide reductase.

- Orally bioavailable

![Hydroxyurea and Pentostatin molecules](image)