I. ANTIMETABOLITES

Antimetabolites are structurally related to normal cellular components. They generally interfere with the availability of normal purine or pyrimidine nucleotide precursors by inhibiting their synthesis or by competing them in DNA or RNA synthesis. Their maximum cytotoxic effects are S-phase, and therefore cell-cycle specific.

A- Antifolates (Methotrexate, Pemetrexed, and Pralatrexate)

The vitamin folic acid (a water-soluble vitamin of the B complex) plays a central role in a variety of metabolic reactions involving the transfer of one-carbon units and is essential for cell replication. Folic acid is obtained mainly from dietary sources and from that produced by intestinal flora.

1. Mechanism of action: MTX is structurally related to folic acid and acts as an antagonist of the vitamin by inhibiting mammalian dihydrofolate reductase (DHFR), the enzyme that converts folic acid to its active, coenzyme form, tetrahydrofolatic acid (FH4).

![Fig. 4 mechanism of action of MTX](image-url)
2. **Therapeutic uses:** MTX, usually in combination with other drugs, is effective against acute lymphocytic leukemia, Burkitt lymphoma in children, breast cancer, bladder cancer, and head and neck carcinomas. In addition, low-dose MTX is effective as a single agent against certain inflammatory diseases, such as severe psoriasis and rheumatoid arthritis, as well as Crohn disease. All patients receiving MTX require close monitoring for possible toxic effects.

3. **Resistance:** Nonproliferating cells are resistant to MTX, probably because of a relative lack of DHFR, thymidylate synthase, and/or the glutamylating enzyme. Resistance can also occur from a reduced influx of MTX, apparently caused by a change in the carrier-mediated transport responsible for pumping the drug into the cell.

4. **Pharmacokinetics:** Methotrexate is readily absorbed from GIT at doses less than 25 mg/m², but larger doses are absorbed incompletely and are routinely administered intravenously. Because MTX does not easily penetrate the blood–brain barrier, it can be administered intrathecally to destroy neoplastic cells that are thriving in the sanctuary of the CNS. High concentrations of the drug are found in the intestinal epithelium, liver, and kidney, as well as in ascites and pleural effusions. MTX is also distributed to the skin. High doses of MTX undergo hydroxylation at the 7 position and become 7-hydroxymethotrexate. Most of the drug and its metabolite excreted via urine, and some of the drug and its metabolite through feces.

5. **Adverse side effects:** Nausia, vomiting, diarrhea, stomatitis, rash, alopecia myelosuppression, high-dose: renal damage, IT(intra thecal): neurologic toxicities.
B- Purine analogues (6-Mercaptopurine, 6-thioguanine, Fludarabine, Cladaribe)

6-Mercaptopurine
6-MP and 6-thioguanine were the first purine analogs to prove beneficial for treating neoplastic disease.

1. Mechanism of action:
a. Nucleotide formation: To exert its antileukemic effect, 6-MP must penetrate target cells and be converted to the nucleotide analog, 6-MP ribose phosphate (better known as 6-thioinosinic acid or TIMP). The addition of the ribose phosphate is catalyzed by the salvage pathway enzyme, hypoxanthine–guanine phosphoribosyltransferase (HGPRT).

b. Inhibition of purine synthesis: A number of metabolic processes involving purine biosynthesis and interconversions are affected by the nucleotide analog, TIMP. Similar to nucleotide monophosphates, TIMP can inhibit the first step of de novo purine ring biosynthesis (catalyzed by glutamine phosphoribosyl pyrophosphate amidotransferase). TIMP also blocks the formation of adenosine monophosphate and xanthinuric acid from inosinic acid.

c. Incorporation into nucleic acids: TIMP is converted to thioguanine monophosphate, which after phosphorylation to di- and triphosphates can be incorporated into RNA. The deoxyribonucleotide analogs that are also formed are incorporated into DNA. This results in nonfunctional RNA and DNA.
2. Resistance: Resistance is associated with
1) an inability to biotransform 6-MP to the corresponding nucleotide because of decreased levels of HGPRT.
2) increased dephosphorylation.
3) increased metabolism of the drug to thiouric acid or other metabolites.

3. Pharmacokinetics: Oral absorption is erratic and incomplete. Once it enters the blood circulation, the drug is widely distributed throughout the body, except for the cerebrospinal fluid (CSF). The bioavailability of 6-MP can be reduced by first-pass metabolism in the liver. 6-MP is converted in the liver to the 6-methylmercaptopurine derivative or to thiouric acid (an inactive metabolite). [Note:The latter reaction is catalyzed by xanthine oxidase.] The parent drug and its metabolites are excreted by the kidney.

4. Therapeutic uses: 6-MP is used principally in the maintenance of remission in acute lymphoblastic leukemia.
C- Pyrimidine analogues (5-Flourouracil, Capecitabine, Cytarabine, Azacitidine, Gemicitabine)

5-Flourouracil

5-Flourouracil (5-FU), a pyrimidine analog, has a stable fluorine atom in place of a hydrogen atom at position 5 of the uracil ring. The fluorine interferes with the conversion of deoxyuridylic acid to thymidylic acid, thus depriving the cell of thymidine, one of the essential precursors for DNA synthesis. 5-FU is employed primarily in the treatment of slowly growing solid tumors (for example, colorectal, breast, ovarian, pancreatic, and gastric carcinomas). When applied topically, 5-FU is also effective for the treatment of superficial basal cell carcinomas.

![Figure 5: Clinical structures of 5-FU and uracil.](image)

1. **Mechanism of action:** 5-FU itself is devoid of antineoplastic activity. It enters the cell through a carrier-mediated transport system and is converted to the corresponding deoxynucleotide (5-fluorodeoxyuridine monophosphate, which competes with deoxyuridine monophosphate for thymidylate synthase, thus inhibiting its action. DNA synthesis decreases
due to lack of thymidine, leading to imbalanced cell growth and “thymidine-less death” of rapidly dividing cells. 5-FU is also incorporated into RNA, and low levels have been detected in DNA. In the latter case, a glycosylase excises the 5-FU, damaging the DNA. 5-FU produces the anticancer effect in the S-phase of the cell cycle.

**5-FU**

Mechanism of the cytotoxic action of 5-FU

- 5-FU is converted to 5-FdUMP, which competes with deoxyuridine monophosphate (dUMP) for the enzyme thymidylate synthetase.
- 5-FU = 5-fluorouracil
- 5-FUR = 5-fluorouridine
- 5-FUMP = 5-fluorouridine monophosphate
- 5-FUDP = 5-fluorouridine diphosphate
- 5-FUTP = 5-fluorouridine triphosphate
- dUMP = deoxyuridine monophosphate
- dTMP = deoxythymidine monophosphate
- 5-FdUMP = 5-fluoro(deoxy)uridine monophosphate.

Fig.6: Mechanism of action of 5-FU
2. **Resistance**: Resistance is encountered when the cells have lost their ability to convert 5-FU into its active form (5-FdUMP) or when they have altered or increased thymidylate synthase levels.

3. **Pharmacokinetics**: Because of its severe toxicity to the GI tract, 5-FU is given IV or, in the case of skin cancer, topically. The drug penetrates well into all tissues, including the CNS. 5-FU is rapidly metabolized in the liver, lung, and kidney. It is eventually converted to fluoro-β-alanine, which is removed in the urine.

4. **Therapeutic uses**

   5-FU is employed primarily in the treatment of slowly growing solid tumors (for example, colorectal, breast, ovarian, pancreatic, and gastric carcinomas). When applied topically, 5-FU is also effective for the treatment of superficial basal cell carcinomas.
II. ALKYLATED AGENTS
(Cyclophosphamide, Ifosfamide, Carmustine (BCNU), Lomustine (CCNU), Dacarbazine, Temozolomide, Melphalan, Chlorambucil, Busulfan)

Alkylation agents exert their cytotoxic effects by covalently binding to nucleophilic groups on various cell constituents. Alkylation of DNA is probably the crucial cytotoxic reaction that is lethal to the tumor cells. Alkylation agents do not discriminate between cycling and resting cells, even though they are most toxic for rapidly dividing cells. They are used in combination with other agents to treat a wide variety of lymphatic and solid cancers. In addition to being cytotoxic, all are mutagenic and carcinogenic and can lead to secondary malignancies such as acute leukemia.

Cyclophosphamide
This drug is very closely related mustard agents that shares most of the same primary mechanisms and toxicities. It is cytotoxic only after generation of its alkylating species, which are produced through hydroxylation by cytochrome P450 (CYP450). This agent has a broad clinical spectrum, being used either singly or as part of a regimen in the treatment of a wide variety of neoplastic diseases, such as non-Hodgkin lymphoma, sarcoma, and breast cancer.
1. **Mechanism of action:**

   Cyclophosphamide is the most commonly used alkylating agent. Cyclophosphamide firstly biotransformed to hydroxylated intermediate primarily in the liver by the CYP450 system. The hydroxylated intermediate then undergoes breakdown to form the active compounds, phosphoramide mustard and acrolein. Reaction of the phosphoramide mustard with DNA is considered to be the cytotoxic step. The parent drug and its metabolites are primarily excreted in urine.

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Fig 7: Cyclophosphamide metabolism
2. Pharmacokinetics: Cyclophosphamide is available in oral or IV preparations. Cyclophosphamide is metabolized in the liver to active and inactive metabolites, and minimal amounts are excreted in the urine as unchanged drug.

3. Resistance: Resistance results from increased DNA repair, decreased drug permeability, and reaction of the drug with thiols (for example, glutathione). Cross-resistance does not always occur.

4. Adverse effects: A unique toxicity of both drugs is hemorrhagic cystitis, which can lead to fibrosis of the bladder.
III. TYROSINE KINASE INHIBITORS  
(Imatinib, dasatinib, nilotinib, Erlotinib, Sorafenib, and sunitinib)

The tyrosine kinases are a family of enzymes that are involved in several important processes within a cell, including signal transduction and cell division. Tyrosine kinases are enzymes that catalyze the transfer of a phosphate group from adenosine triphosphate to target proteins.

Imatinib mesylate is used for the treatment of chronic myelogenous leukemia (CML) as well as GI stromal tumors. It acts as a signal transduction inhibitor, used specifically to inhibit tumor tyrosine kinase activity. A deregulated BCR-ABL kinase is present in the leukemia cells of almost every patient with CML. In the case of GI stromal tumors, an unregulated expression of tyrosine kinase is associated with a growth factor. The ability of imatinib to occupy the “kinase pocket” prevents the phosphorylation of tyrosine on the substrate molecule and, hence, inhibits subsequent steps that lead to cell proliferation.

Fig 9: Mechanism of action of imatinib
IV. **STEROID HORMONES AND THEIR ANTAGONISTS**

(Prednisone, Tamoxifen, Anastrozole, Letrozole, Leuprolide, Goserelin, Triptorelin, Flutamide, Nilutamide, Bicalutamide)

Tumors that are steroid hormone sensitive may be either:

1) Hormone responsive, in which the tumor regresses following treatment with a specific hormone; or
2) hormone dependent, in which removal of a hormonal stimulus causes tumor regression; or
3) both. Removal of hormonal stimuli from hormone-dependent tumors can be accomplished by surgery.

**Tamoxifen** is an estrogen antagonist with some estrogenic activity, and it is classified as a selective estrogen receptor modulator (SERM). It is used for first-line therapy in the treatment of estrogen receptor–positive breast cancer. It also finds use prophylactically in reducing breast cancer occurrence in women who are at high risk.

1- **Mechanism of action:** Tamoxifen binds to estrogen receptors in the breast tissue. The mechanism of action of tamoxifen is complex. Clearly, its principal mechanism of action is mediated by its binding to the estrogen receptor and the blocking of the proliferative actions of estrogen on mammary epithelium. That is, complex (tamoxifen + ER) fails to induce estrogen-responsive genes, and RNA synthesis does not ensue. The result is a depletion (down-regulation) of estrogen receptors, and the growth-promoting effects of the natural hormone and other growth factors are suppressed.
2-Pharmacokinetics: Tamoxifen is effective after oral administration. It is partially metabolized by the liver. Some metabolites possess antagonist activity, whereas others have agonist activity. Unchanged drug and metabolites are excreted predominantly through the bile into the feces. Tamoxifen is an inhibitor of CYP3A4 and P-glycoprotein.

3-Adverse effects: Side effects caused by tamoxifen include hot flashes, nausea, vomiting, skin rash, and vaginal bleeding and discharge (due to estrogenic activity of the drug and some of its metabolites). Hypercalcemia may occur, requiring cessation of the drug. Tamoxifen can also lead to increased pain if the tumor has metastasized to bone. Tamoxifen has the potential to cause endometrial cancer. Other toxicities include thromboembolism and effects on vision.