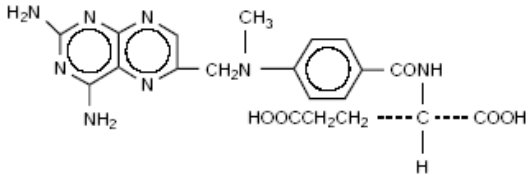
Lecture 3: Antimetabolites – cell cycle specific (S-phase)

All the antimetabolites mimic endogenous molecules. They "trick" enzymes involved in the synthesis of DNA, and instead of metabolizing the proper endogenous substrates the enzymes metabolize the antimetabolites. In many cases, the target enzymes are irreversibly inhibited.

1. Folate analogs

Methotrexate (Trexall, formerly Amethopterin):



Uses: Metastatic breast cancer, epidermoid head and neck cancer, lung cancer (especially squamous cell), pancreatic cancer, and in combination with other agents for late stage non-Hodgkin's lymphoma. Can be administered IV, IM, or PO.

Mechanism: Mimics the vitamin folic acid (dihydrofolic acid more specifically) and mainly inhibits the enzyme dihydrofolate reductase (DHFR) within the folate cycle (see Figure 1). Other enzymes in the folate cycle can also be inhibited but less so.

Toxicities: Myelosuppression, liver toxicity, renal toxicity. These can be unexpectedly severe so patients must be monitored. Some penetration into CNS with consequent toxicities.

ADME: Following administration, the agent exploits the folic acid uptake mechanism and is actively taken up into cells by the reduced folate carrier (RFC1). Once inside cells, the molecule is polyglutamated by folyl polyglutamate synthase (FPGS) which adds multiple negative charges and traps the agent inside cells. Amazingly, little other metabolism occurs and the polyglutamates will be removed prior to its excretion by the kidneys.

Note(s): Intentional high doses, or overdoses of methotrexate can be corrected by administration of leucovorin (see Figure 2). Leucovorin bypasses the enzymatic step inhibited by methotrexate and supplies the folate cycle with an intermediate that possesses a carbon moiety at the correct oxidation state for further metabolism in the cycle. This is called "leucovorin rescue". Finally, methotrexate is also used to treat psoriasis and severe rheumatoid arthritis.

Figure 1. Folate cycle and inhibition by methotrexate.

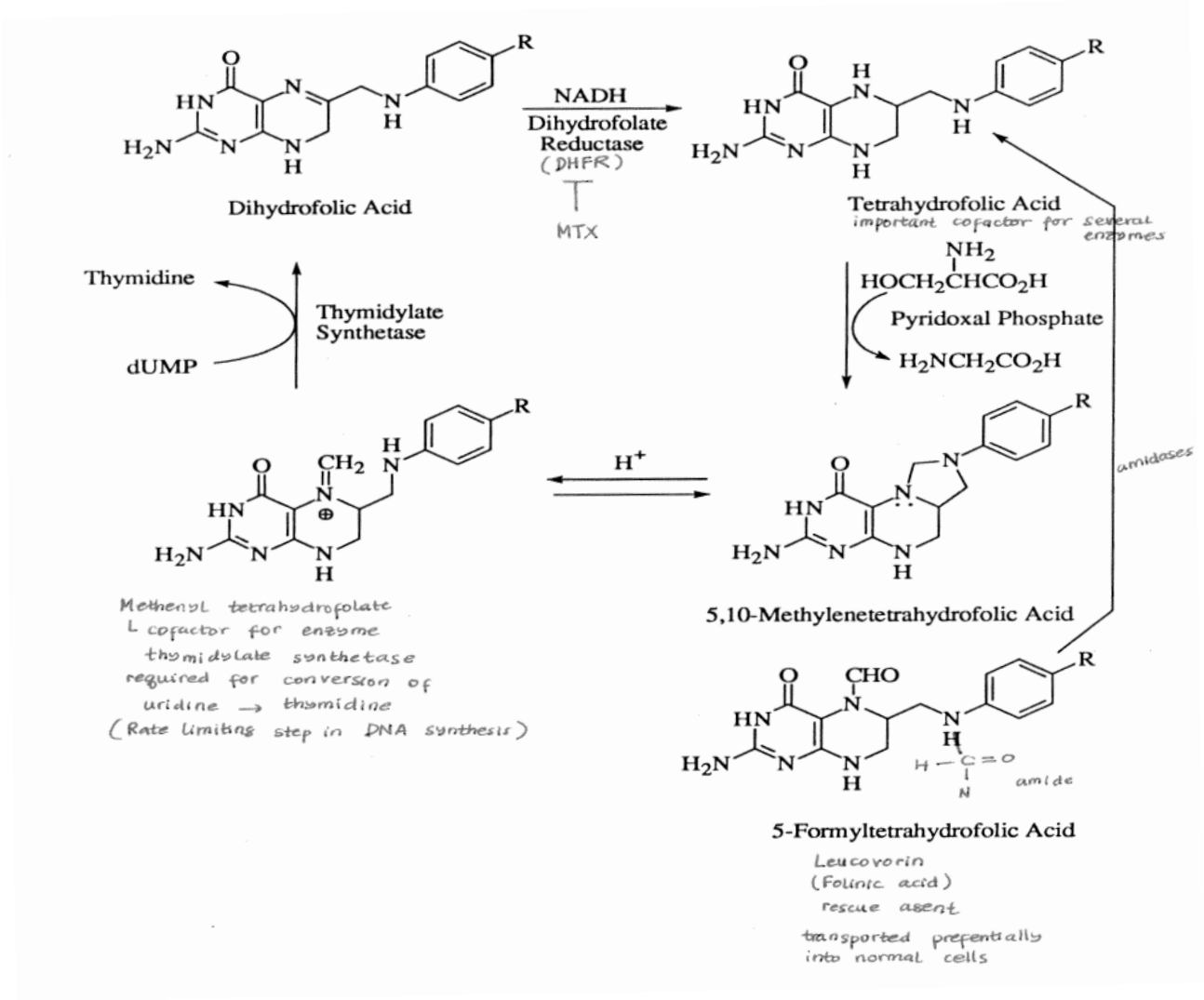
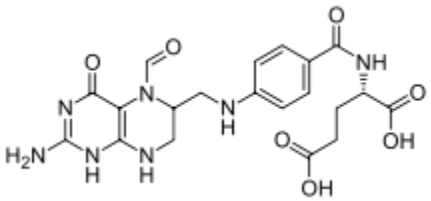
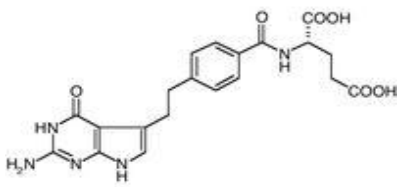


Figure 2: Structure of leucovorin.



Pemetrexed (Alimta):



Uses: Non-small cell lung cancer (non-squamous) in combination with cisplatin or gemcitabine commonly; also for mesothelioma in combination with cisplatin. Administered IV only.

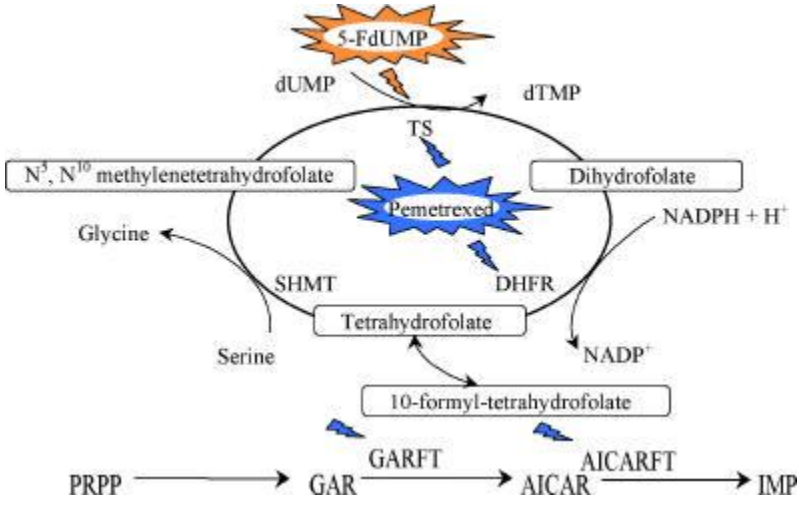
Mechanism: Similar to methotrexate but inhibits folate cycle at 3 steps including DHFR, TS and GARFT (glycinamide ribonucleotide formyl transferase). Premetrexed actually inhibits TS more effectively than does methotrexate (see Figure 3). GARFT is also inhibited by pemetrexed and this enzyme is involved in early steps of purine biosynthesis and utilizes 10-formyl tetrahydrofolate as a cofactor (see bottom of Figure 3 and figure in text).

Toxicities: Myelosuppression, liver toxicity, renal toxicity; less CNS penetration compared to methotrexate.

ADME: Uptake and polyglutamation similar to that of methotrexate but even better substrate for polyglutamation by FPGS so better trapping of drug within cells. Elimination t1/2 is about 4 hrs and like methotrexate is excreted renally without polyglutamates attached.

Note(s): This agent starting to overtake methotrexate in oncology.

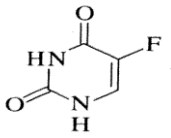
Figure 3. Inhibition of folate cycle by pemetrexed.



Pralatrexate (a newer folate antimetabolite) is also discussed in the text but this agent is not yet approved. The agent appears to have superior tumor uptake and therefore potentially greater efficacy the methotrexate and pemetrexed, but clinical trial will need to prove it.

2. Pyrimidine analogs

5-Fluorouracil (5-FU):



Uses: Metastatic breast cancer, colorectal cancer; for the latter 5-FU is component of FOL**F**OX (folinic acid/5-fluorouracil/oxaliplatin) and FOL**F**IRI (folinic acid/5-fluorouracil/irinotecan) regimens. While both 5-FU and irinotecan have anticancer activity in this regimen, the folinic acid servers to potentiate the activity of 5-FU by stimulating the turnover of TS, the target enzyme of 5-FU. Administered IV only.

Mechanism: A prodrug and mimic of uracil; it specifically inhibits thymidylate synthetase (TS), see Figure 4 below.

Toxicities: Myelosuppression, mucositis, diarrhea.

ADME: Upon administration, most 5-FU is rapidly ribosylated to 5-FUMP (Figure 4); the elimination t1/2 from plasma is bout 10-15 minutes; about 20% of the administered dose can be excreted unchanged in the urine.

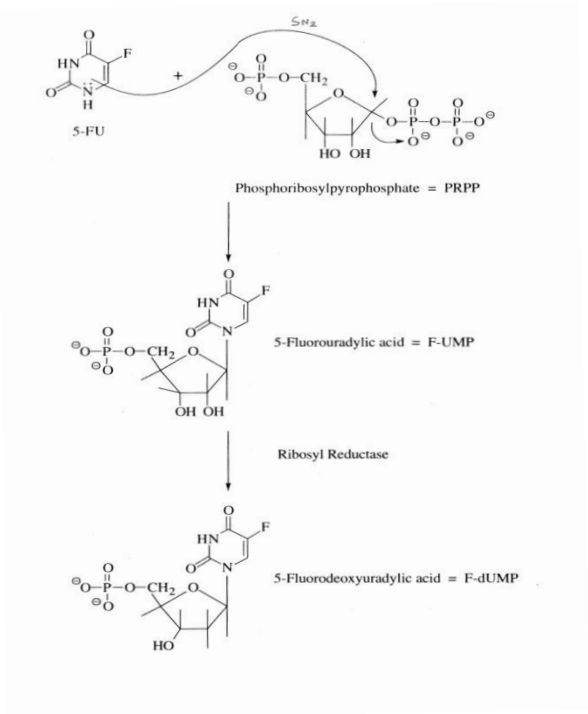
Note(s): One route of metabolic catabolism is by dihydropyrimidine dehydrogenase (DPD). Deficiency in the activity of this enzyme can lead to elevated levels of 5-FU and consequent toxicities. The DPD enzyme is polymorphic and it is more common in African Americans than Caucasians. In addition, the deficiency is more common in female African Americans than male African Americans.

There is a genetic test that is commercially available for determining DPD status for patients.

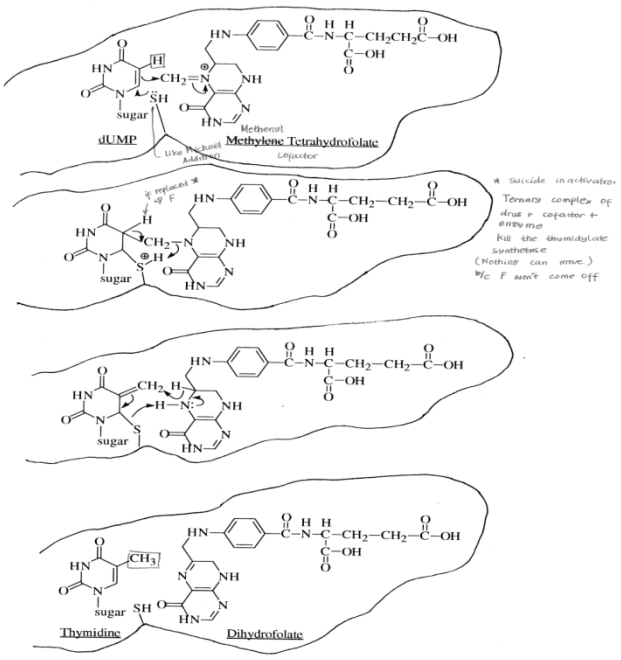
Floxuridine is discussed in the text. This molecule is one step removed from 5-FdUMP. It has some advantages over 5-FU. Used for liver metastases from GI cancers. Given slowly by intra-arterial infusion which limits formation of 5-FU and its toxicities. This circumvents the DPD polymorphism as well.

Figure 4. Inhibition of thymidylate synthetase (TS) by 5-FU.

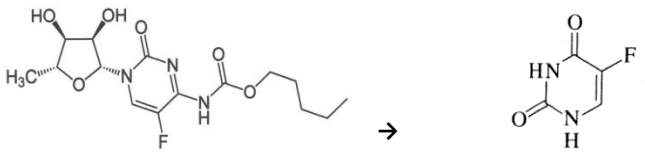
Initial steps:



Final steps (including inhibitory step):



Capecitabine (Xeloda):



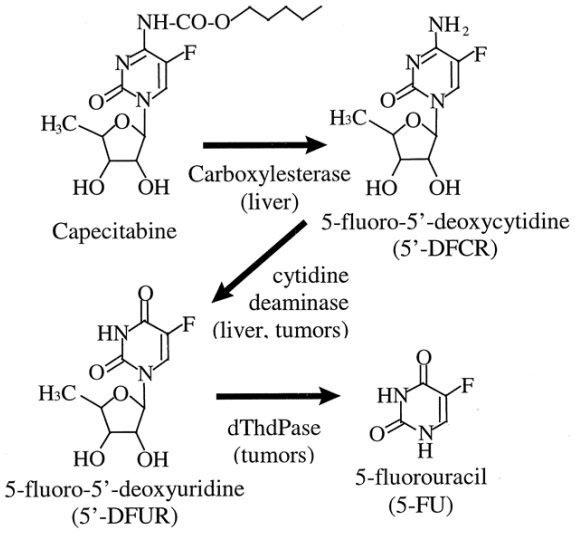
Uses: Metastatic breast cancer, colorectal cancer, pancreatic cancer, gastric cancer. Administered PO.

Mechanism: Prodrug of 5-FU; mimic of uracil and specifically inhibits thymidylate synthetase (TS); capecitabine is actually broken all the way down to 5-FU. However, the catabolic breakdown exploits the fact that critical enzymes are preferentially located in tumors, especially thymidine phosphorylase (see Figure 5).

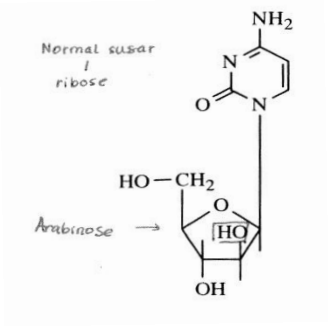
Toxicities: Toxicities like 5-FU but also and-foot syndrome (blistering of hands and feet) because prodrug increases 5-FU levels in skin of hands and feet.

ADME: Following oral administration, capecitabine is broken down rapidly; elimination t1/2 of capecitabine is about 45 min. Nearly all the dose of capecitabine is excreted in the urine, mainly as metabolites. Capecitabine can inhibit CYP2C9 and a clinically relevant interaction can occur with warfarin and phenytoin.

Figure 5. Conversion of capecitabine to 5-FU.



Cytarabine (AraC):



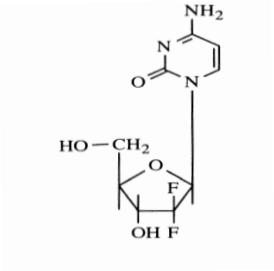
Uses: Various leukemias (acute lymphocytic leukemia, non-lymphocytic leukemia, chronic myelocytic leukemia, meningeal leukemia). Commonly given as part of a combination. Administered IV, and even intrathecally for meningeal leukemia.

Mechanism: Inhibitor of DNA polymerase; gets incorporated in DNA efficiently but then prevents continued elongation of DNA strands. While the base is normal (cytidine), the sugar moiety is abnormal; the arabinose sugar cannot be reduced to the deoxy form by ribonucleotide reductase.

Toxicities: Myelosuppression, mucositis, AraC syndrome (fever, myalgia, bone pain).

ADME: Biphasic kinetics with elimination t1/2 of about 4 hrs; rapidly converted to the uracil type metabolite by cytidine deaminase; over 80% of the administered dose (mainly metabolites) is excreted in the urine.

Gemcitabine (Gemzar):



Uses: Pancreatic cancer, non-small cell lung cancer in combination with cisplatin, breast cancer in combination with paclitaxel, ovarian cancer in combination with carboplatin. Administered IV only.

Mechanism: Dual mechanism: (1) inhibits ribonucleotide reductase – the enzyme that converts ribonucleotides into deoxy-ribonucleotides, and (2) dGTP competes with dCTP for incorporation into DNA.

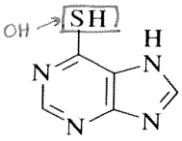
Toxicities: Myelosuppression, paresthesias (numbness or tingling), and severe rash.

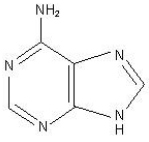
ADME: Qualitatively similar to cytarabine but elimination t1/2 is substantially longer (about 20 hrs) because the catabolic enzyme (cytidine deaminase) is inhibited by gemcitabine; like cytarabine excretion of most of the dose (as metabolites) is renal. Interestingly, the elimination of gemcitabine is both gender and age dependent. The t1/2 increases with age and is longer in women than men.

Note(s): Has demonstrated utility in many forms of cancer (in combination with other agents) due to synergistic effects.

3. Purine analogs

6-Mercaptopurine (Purinethol):





Adenine

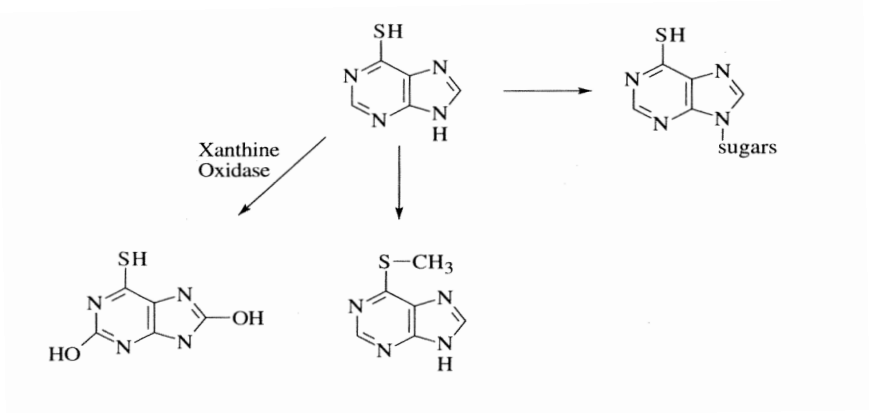
Uses: Acute lymphocytic and myelogenous leukemia. Administered PO.

Mechanism: Mimic of hypoxanthine, the precursor to adenine and guanine; inhibitor of de novo purine synthesis. Specifically competes with hypoxanthine and guanine for the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRTase).

Toxicities: Myelosuppression, hyperuricemia*, hepatotoxicity with high or chronic use.

ADME: Elimination t1/2 is about 45 min in adults; approximately 50% of administered dose is excreted in the urine. If patients have inherited or low thiopurine S-methyltransferase (TPMT) activity, they are at increased risk for severe toxicity (see Figure 6). For homozygous deficient patients, dose reductions are usually required but the optimal dose has not been established. For heterozygous TPMT patients, they typical can tolerate the recommended dose. Genotypic and phenotypic tests for TPMT status are available. *Renal effects can be reduced by hydration, alkalinization, and use of a xanthine oxidase inhibitor such as allopurinol. However, a dose reduction (1/2 to 1/3) of 6-mercaptopurine is required if allopurinol is used.

Figure 6. Ribosylation of 6-mercaptopurine and the role of TPMT in its elimination:



Thioguanine (Tabloid):

Uses: Acute non-lymphocytic leukemia. Administered PO.

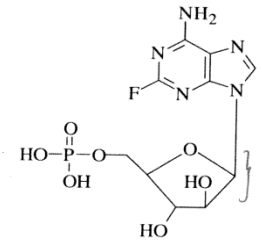
Mechanism: Being the thiol analog of hypoxanthine, thioguanine competes with hypoxanthine and guanine for the hypoxanthine-guanine phosphoribosyltransferase (HGPRTase). Thus, it is converted to 6-thioguanylic acid (TGMP) which allows it to compete at several points with the synthesis of guanine nucleotides.

Toxicities: Myelosuppression mainly, N/V; hepatotoxicity with high or chronic use.

ADME: Like mercaptopurine, elimination from the plasma is rapid; elimination t1/2 is estimated at about 80 min.

Note(s): The issue of TPMT status in patients is the same as for 6-mercaptopurine. Also, there is strong cross-resistance of cancer cells for thioguanine and 6-mercaptopurine.

Fludarabine (Fludara, Oforta):



Uses: Chronic lymphocytic leukemia. Administered IV and PO.

Mechanism: Both the base and the sugar are chemically modified relative to normal adenosine. The phosphate form of this drug is dephosphorylated extracellularly. Once inside cells it is re-phosphorylated by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. The triphospho form of the drug inhibits DNA polymerase, ribonucleotide reductase, and DNA primase.

Toxicities: Myelosuppression mainly, hemolytic anemia; CNS toxicities at high dose.

ADME: Elimination t1/2 of about 20 hrs: Fludarabine has high renal excretion so use caution in patients with renal impairment and adjust dose as necessary.

Note(s): Presence of phosphate group in the molecule is for solubility purposes; phosphate is rapidly cleaved in the plasma and then re-attached once inside cells.

Cladribine and **Clofaribine** are mentioned in the text. Both are used in types of leukemias. In addition to myelosuppression, cladribine can cause hemolytic anemia and CNS toxicities at high dose. Clofaribine can cause tumor lysis syndrome (TLS) due to rapid death of leukemia cells. TLS can lead to respiratory and cardiac toxicities due to excessive cytokine release.