I. Pemetrexed (Alimta®) is a relatively new folic acid analog, similar to the antimetabolite antitumor agent methotrexate, and is used to treat some lung cancers.

![Pemetrexed and Methotrexate structures](image)

1. Pemetrexed is available as a disodium salt for reconstitution for intravenous administration.
   a. (3 pts) Why is the salt form needed? To ensure that it will completely dissolve in the aqueous i.v. solution.
   b. (4 pts) To the right of the R structure above, draw the disodium salt.

2. (5 pts) Only the L-glutamate structure (glutamic acid amide) is active for both drugs. Draw this isomer perspective, below.

   ![L-glutamate structure](image)

3. (3 pts) Show on the structure of Pemetrexed above, a cytochrome P450 benzylic oxidation metabolite.

   ![Metabolite structure](image)

4. (4 pts) Show below, the structure of an acyl glucuronide metabolite of Pemetrexed.

   ![Metabolite structure](image)
5. (8 pts) Describe in words the mechanism of action of Pemetrexed and Methotrexate, and where they act in the cell cycle.

**CCS for S phase**

Both drugs inhibit synthesis of tetrahydrofolate acid by inhibiting the enzyme dihydrofolate reductase. Tetrahydrofolate is required for the synthesis of DNA, RNA, and some amino acids.

6. (6 pts) Give two reasons why NSAIDs should not be administered to patients who are on chemotherapy regimens containing Pemetrexed or Methotrexate.

1) NSAIDs inhibit PG synthesis → ↓ platelet activity which can augument bleeding problems caused by BMD ↓ platelet count.

2) NSAIDs are acidic drugs that may acidify the urine enough to ↓ solubility of metabolites of these drugs →↑ in urine and kidney damage.

II. Capecitabine (Xeloda) is a prodrug of 5-fluorouracil (5-FU) with the partial structure shown below.

1. (6 pts) Amide hydrolysis is one step in the conversion of Capecitabine to 5-FU. Next to the structure above, show a mechanism for general base-catalyzed hydrolysis of the amide structure.

   See above.

2. (3 pts) Briefly explain why this prodrug was developed.

   To increase the lipid solubility of 5-FU for oral administration and to penetrate tumors better.

3. (6 pts) One mechanism of action of 5-FU relies on its activity as an active-site directed enzyme inhibitor. Complete the reaction below (arrow convention) to show how 5-FU inhibits the enzyme thymidylate synthetase by forming a stable ternary complex of 5-FU, enzyme, and the cofactor methylene tetrahydrofolate.
III. Chemotherapy regimens for a variety of cancers are continuously evolving in attempts to increase efficacy and decrease toxicities.

1. (4 pts) Colorectal cancer is the third most common cancer in adult men and women, and 5-FU + Leucovorin has been one of the primary therapies. Why is leucovorin a part of this therapy?
   
   To maximize the amount of methotrexate available in order to maximize the amount of thymidylate synthetase irreversibly inactivated as the tetramer complex shown in question I.3. (last page).

2. (5 pts) Cetuximab (Erbitux®) is a newer agent now used to treat ~ 80% of cases of metastatic colon cancer. Describe this drug's mechanism of action and toxicities.
   
   Cetuximab is a monoclonal antibody that binds to the epidermal growth factor receptor (EGFR) thereby inhibiting tumor angiogenesis and invasion of tissues.

   Toxicities:
   - allergic rash(es) + N/V/D

3. Non-small cell lung cancer (NSCLC) is one of the lung cancers associated with cigarette smoking, and first-line chemotherapy treatment is often with the DNA-metalating agent, Cisplatin (Platinol®), and the antimitotic, Taxol (Paclitaxel).
   
   a. (8 pts) Describe where these two agents act in the cell cycle, their mechanisms of action, and major toxicities.

   cisplatin: ccNS
   Mod: Forms DNA interstrand cross-links by nucleophilic displacement of chloride
   Tux: Kidney damage
   N/V: Acoustic neuroma
   Drug Interact: Inhibits cYP3A4, other P450's

   Taxol: G2/M phases
   Mod: Binds to polymerized tubulin
   Tux: Malignant peripheral neuropathy, hypersensitivity

   b. (4 pts) Based on this information, would you consider this a good regimen, and if so, why?
   
   Yes, this is a good regimen because all phases of the cell cycle are covered, and there is no overlap in major toxicities, plus they act by different mechanisms.

   c. (6 pts) Erlotinib (Tarceva) is a newer targeted agent used to treat NSCLC. Describe its mechanism of action, toxicities, and drug-interactions.

   MoA: Inhibits phosphorylation of tyrosine kinases, especially the EGFR tyrosine kinase → inhibition of angiogenesis and tumor invasion.

   Tux: Interstitial lung disease, rash, N/V/D

   Drug Interact: Inhibits cYP3A4, other P450's → ↑ warfarin conc.
   Also is metabolized by cYP3A4 → induces cYP3A4

   d. (5 pts) A second-line therapy for NSCLC is a combination of Mitomycin, Ifex and Cisplatin (MIC). Explain why the DNA cross-linking agent, Mitomycin, might be a helpful addition based on both cell cycle coverage and mechanism of action.

   Mitomycin is a ccNS agent that x-links DNA by reductive alkylation. It penetrates solid tumors whose inner cells are less well oxygenated and have good reductase activity to activate the drug to its DNA x-linking agent.
IV. Ifex (ifosfamide) is a prodrug of a nitrogen mustard alkylating agent that is a component of the MIC regimen used to treat NSCLC (see last page). Below is a partial scheme for activation of ifosfamide to its DNA cross-linking metabolite.

\[
\begin{align*}
\text{A} & \xrightarrow{\text{P450}} \text{B} \\
\text{R-\(\text{N-P(O)}\text{O-R}\)} & \xrightarrow{\text{H}^+} \text{B} \\
\text{Cl-CH₂-CH₂} & \xrightarrow{\text{H}^+} \text{B:} \\
\text{HN-N-P(O)} & + \text{CH₂=CH₂} \\
\text{O} & \xrightarrow{\text{H}^+} \text{B:} \\
\text{Cl-CH₂-CH₂} & \xrightarrow{\text{H}^+} \text{B:} \\
\text{HN-CH₂} & \xrightarrow{\text{H}^+} \text{B:} \\
\text{Cl-CH₂-CH₂} & \xrightarrow{\text{H}^+} \text{B:} \\
\text{HN-CH₂} & \xrightarrow{\text{H}^+} \text{B:} \\
\end{align*}
\]

1. (3 pts) Show how mechanistically (arrow convention) how B undergoes N-dealkylation to C.

2. (4 pts) Show how C eliminates D to give E.

3. (4 pts) Show how E forms an aziridinium ion F and given the structure of F.

4. (2 pts) Show how DNA can react with F to give G.

5. (7 pts) Mesna is a chemoprotectant given with ifosfamide to protect against hemorrhagic cystitis caused by acrolein (D above). Show mechanistically how Mesna reacts with acrolein. (Show both the intermediate formed plus its further reaction to given the final product of this Michael addition reaction.)

\[
\begin{align*}
\text{Na}\text{O}\text{SO-CH₂-CH₂-S-H} & \xrightarrow{\text{B:}} \text{R-S-CH₂-CH₂-C=CH} \\
\text{R-\(\text{S-CH₂-CH₂-C=CH}\)} & \xrightarrow{\text{B:}} \text{R-\(\text{S-CH₂-CH₂-C=CH}\)} \\
\end{align*}
\]