ONC 7 – Med Chem of Topoisomerase and Microtubule Inhibitors and Crosslinking Agents

Today, we will cover three major anti-cancer drug strategies

1. **Interfering with DNA processing, replication and transcription**
   Rationale: Cancer cells replicate their DNA more frequently and must transcribe DNA into *m*RNA more frequently to keep up with protein production. Blocking DNA unwinding and interfering with transcription will disproportionately affect cancer cells.

2. **Messing with Microtubule dynamics**
   Rationale: The formation and break-down of microtubules is critically important during cell division and replication. Because cancer cells undergo replication more frequently than healthy cells, they will be more sensitive to disruptions in microtubule dynamics.

3. **Switching off signaling pathways (Inhibiting Protein kinases)**
   Rationale: Cancer cells are self-sufficient in growth signaling – they are constantly switched on, rapidly growing and proliferating. By blocking the signals that tell cancer cells to grow, one can induce cell death and slow the growth and progression of disease. In contrasts, most healthy cells are usually switched off and can survive if a single signaling pathway is blocked.

1. Cancer Cells replicate much *more frequently* therefore any disruption will **disproportionately affect** them
2. Microtubules are important during **cell division and replication** (anaphase, positioning, etc) therefore same disproportionate effect.
3. Many types of cancers have **constitutively active** signal pathways → abnormal growth

Describe the MoA for commonly used Oncology Drugs

Generally, **Alkylation** refers to the *transfer* of an alkane group from one molecule → another molecule. In biology however, this definition is slightly expanded to **covalent modification of DNA** (adding on more than just alkyl groups). Alkylation of DNA physiologically is important for *normal cell function* such as;

- **Methylation** of Cytosine will *impair transcription*
- **Hypermethylation** of Promoter Regions can cause *silencing* of Tumor Suppressor Proteins → Cancer
- **Hypomethylation** of Promoter Regions can cause *overexpression* of Oncogenes → Cancer
    - Methylation in general impairs transcription so some scenarios we will have too much or too little of what we want

Nitrogen Mustards

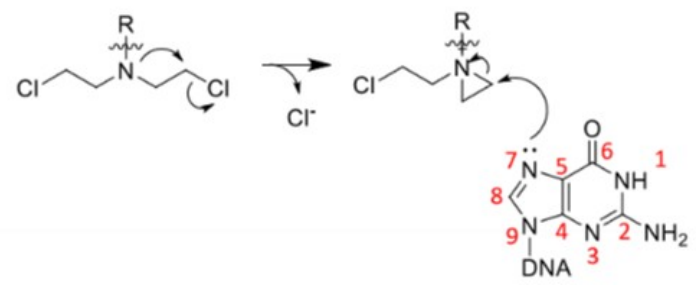
Earlier cancer therapies that utilized the use of Alkylation to **damage DNA** involved the use of various **mustard gases**.

Initial *intramolecular S_N2 nucleophilic reaction* to create a **highly reactive, 3 membered ring compound**

→ Alkylation of DNA

Sulfur > Nitrogen in reactivity

An important general note about Nitrogen Mustards is that the **intramolecular S_N2 reaction** is *much faster* than the **intermolecular interaction with DNA**. Therefore the reactive, 3-membered ring compound will always form first then alkylate DNA.



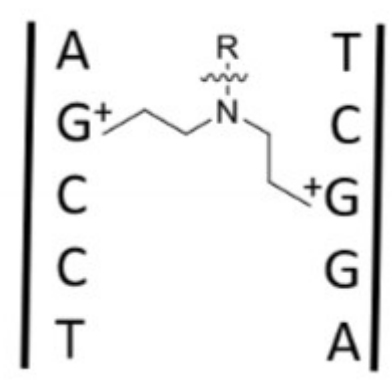
<u>Reason for N7 Preference</u>

N7 does not participate in Watson-Crick base pairing (no H-Bonds to other nucleotide)

Electrons in N7 are in an sp^2 hybrid orbital (aromatic) and not contributing to aromaticity

N7 is more sterically accessible than other nucleophilic centers

When these reactive species alkylate DNA, they tend to create **inter-strand crosslinking** between the two strands in dsDNA.



Note that the crosslink is between *Guanine-Guanine*

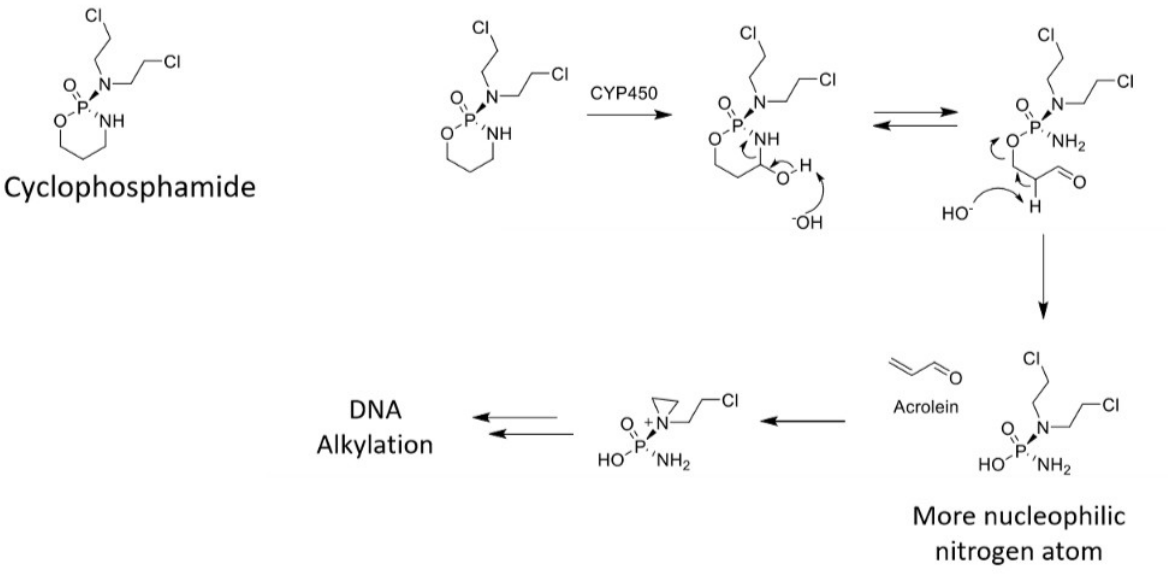
Damages DNA by impairing un-supercoiling (required for replication and other processes) and blocks processing of individual DNA strands.

This damage will be repaired through DNA repair processes (**Base Excision Repair**)

Older agents were found to be more toxic and displayed little selectivity for tumor cells over healthy cells.

- Newer agents introduced an **Aryl Group** to decrease the nucleophilicity of the nitrogen atom → ↑ $t_{1/2}$ (slower activation step into reactive species)

Cyclophosphamide is an example of a Nitrogen Mustard **Prodrug**;



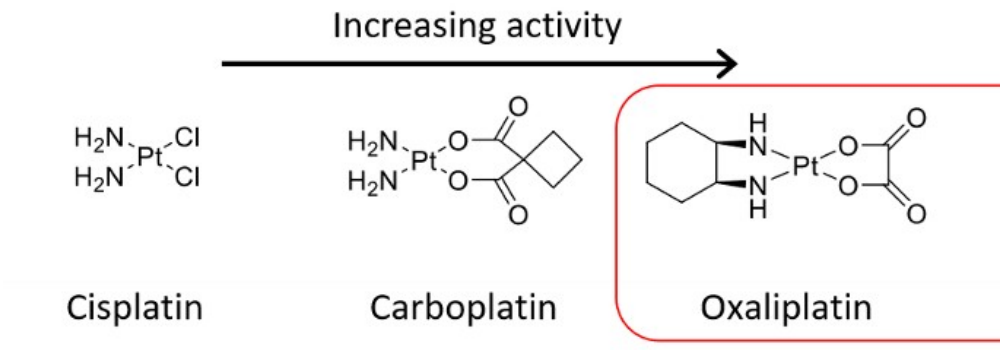
CYP450 is what activates the prodrug

Need to break ring before leaving group can leave

Platinum-Based Alkylating Agents

Platinum Drugs are useful alkylation agents as they form strong **Platinum → Nitrogen Bonds** especially at N7 for all the same reasons as above!

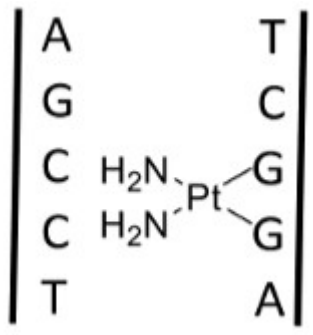
As described in the Pharmacology lecture, activation of these drugs also are dependent on **leaving groups** (similar to Nitrogen Mustards) through *hydrolysis*.



Larger leaving groups tend to dissociate more slowly allowing for;

- ↓ Adverse Effects (slower → safer)
- Increased Solubility
- Decreased Cross-Resistance

A **cis** configuration is important to form **intrastrand cross-links**



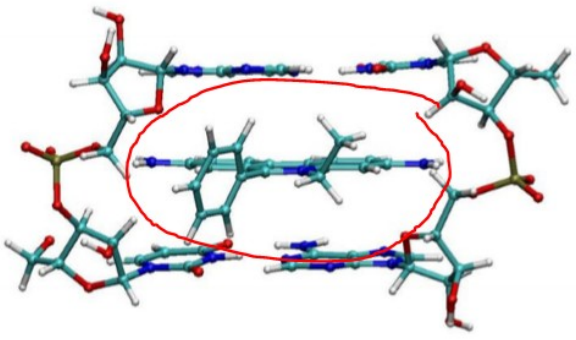
The binding of these "2 arms" of Platinum-Based agents → **intrastrand crosslinkage** which will "kink" the DNA

DNA will *bend* towards the major groove → **denaturation** due to unstable hydrogen base pairing between bases

This altered structure will **not allow transcription** to occur anymore as elements cannot properly interact!

Intercalators

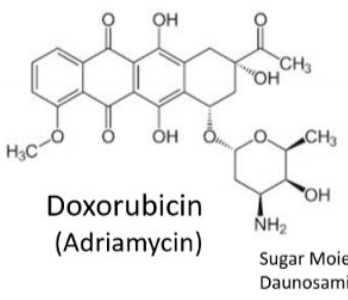
Intercalation is the *reversible insertion* of a molecule (or ion) into materials with layered structures such as DNA.

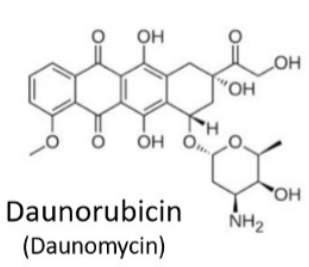


Insertion between *sequential bases* that binds tightly to **disrupt DNA Replication and Transcription** (portion that sticks out from planar portion).

Molecules that are *Planar* and *Aromatic* can do this intercalation reversibly and non-covalently (other methods of attachment).

Doxorubicin and **Daunorubicin** are intercalating agents however they also have other MoA.





Planar and Aromatic to allow for intercalation (with portion that sticks out too)

<u>Other MoA</u>

Bind and inhibit **Topoisomerase II** (↓ unwinding → can't replicate or read)

Form Iron Complexes → **free radicals** that cause oxidative damage (but also leads to *cardiotoxicity*)

Doxorubicin is also a **Topoisomerase II inhibitor**

Topoisomerase Inhibitors

Topoisomerases help break apart DNA to allow for *unwinding* and *untangling* so that the DNA strands can actually be read by enzymes for processes such as Replication/Transcription (otherwise they are usually in "storage format").

Supercoiling – "old school" phone cord
- Coiling, coiled-coil, supercoil brings tension and can mis-coil

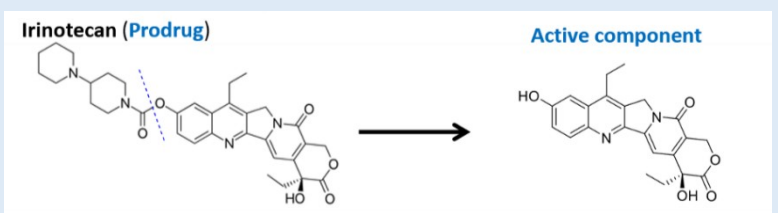
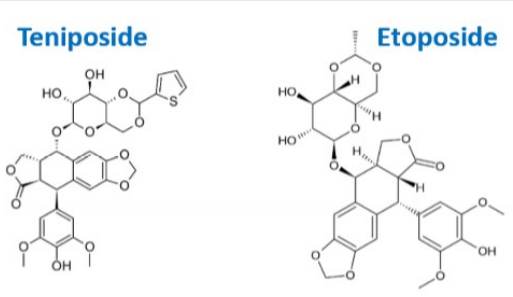
Knotting – headphones in your pocket
- Random movement in long chains leads to formation of knots

Catenation – cooked spaghetti
- Non-covalent interactions stimulate formation of higher order structures

In cancerous cells, due to their **↑ proliferation and gene expression**, Topoisomerase inhibitors will affect cancer cells **more** than healthy cells since they have higher activity of Topoisomerase (more winding/unwinding).

These inhibitors will **block the reattachment step** → DNA damage

- Need to break DNA to release the tension first so that you can uncoil/unwind to actually read codons.

Enzyme	*Notes*
Topoisomerase I	Enzyme that will **cleave DNA Backbone** to relieve pressure and allow for uncoiling/unwinding • Will use the energy stored in the strain instead of using ATP or Cofactors Will *stay connected* to DNA as it unwinds through a covalent bond between a Tyrosine residue and PO_4^- on backbone <u>Topoisomerase I Inhibitors</u>  Typically **planar** with a lactone ring that is metabolized for *inactivation* Will bind to the **cleavage site** that Topoisomerase I has activity at and will mimic base pairing → **intercalation** to prevent reattachment after cleavage.
Topoisomerase II	 Enzyme that will **cleave dsDNA** to allow another dsDNA strand to pass through • Is a **Homo-Dimer** (2 units) that will bind with a **4 base stagger** using Tyrosine Residues • Will require ATP and Metal Activation for activity <u>Topoisomerase II Inhibitors</u> Based off of an older natural product (Microtubule Inhibitor)

	Will also **block the reattachment step** preventing reformation Since Topoisomerase II is a homo-dimer, will need 2 drug molecules to inhibit

Summary:

Topo I:
- Induces a **single strand break** in the DNA backbone (cleaves the O-P bond)
- Forms a **hinge**, to unwind and allow DNA coil to relax
- Religates (sticks back together) cleaved fragments to form double stranded DNA
- A **single molecule of Topo I** can efficiently execute the single strand break

Topo II:
- Induces a **double strand break** in DNA, allowing a second intact DNA strand to pass through the break and then religates
- **Two molecules of Topo II** are required to induce a double strand break (one to cleave and religate each strand)

Topisomerase inhibitors **block the religation step**
to induce DNA damage response and cell death

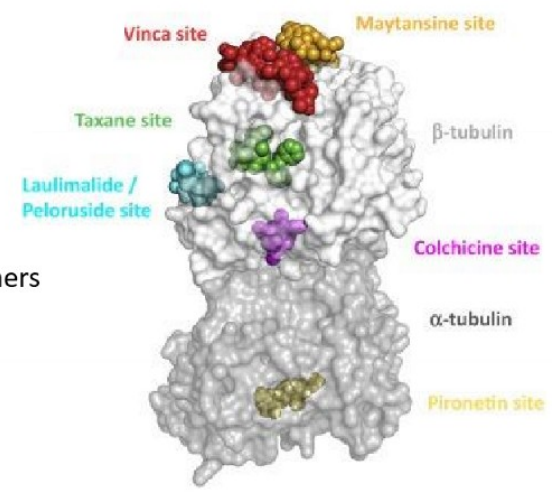
Tubulin Inhibitors

The **Mitotic Spindle** is a required apparatus for cell division (anaphase) and therefore cell proliferation.

- **Microtubules** are tubulin polymers that make up the Mitotic Spindle and their synthesis relies on the hydrolysis of **GTP → GDP**
  - α tubulin is **stable**
  - β tubulin is **unstable** and is the one that is hydrolyzed
  - **Microtubule Collapse** occurs when GTP hydrolysis (of β tubulin) has completely reached the (+) end from the (-) end

There are several binding pockets on Tubulin that have been targeted by drugs.

Destabilizers
1. **Vinca site**: Vincristine and vinblastine, introduces a wedge causing curved polymers
2. **Colchicine site**: Colchicine and Combretastatin*, impair α-β interactions
3. **Maytansine site**: Maytansine, Rhizoxin*, blocks polymerization



Stabilizers
4. **Taxane site**: Paclitaxel, Docetaxel, impair depolymerization of polymers (stabilize)

Slowing down Microtubule Dynamics → slower cell proliferation and growth (cancer)

Kinase Inhibitors

Tyrosine Kinases are enzymes that transfer phosphate groups from ATP → Tyrosine.

- The ATP binding site is similar across all types of Tyrosine Kinases → difficulty with *selectivity*

To bind to the ATP binding site, Tyrosine Kinase Inhibitors will need to **mimic ATP (specifically Adenosine)**

- Often have a *purine* or *purine mimetic* that binds that is similar in structure to Adenosine
- Will try to impart *selectivity* by having a portion reach into the **substrate binding pocket** (can tailor to specific substrates)