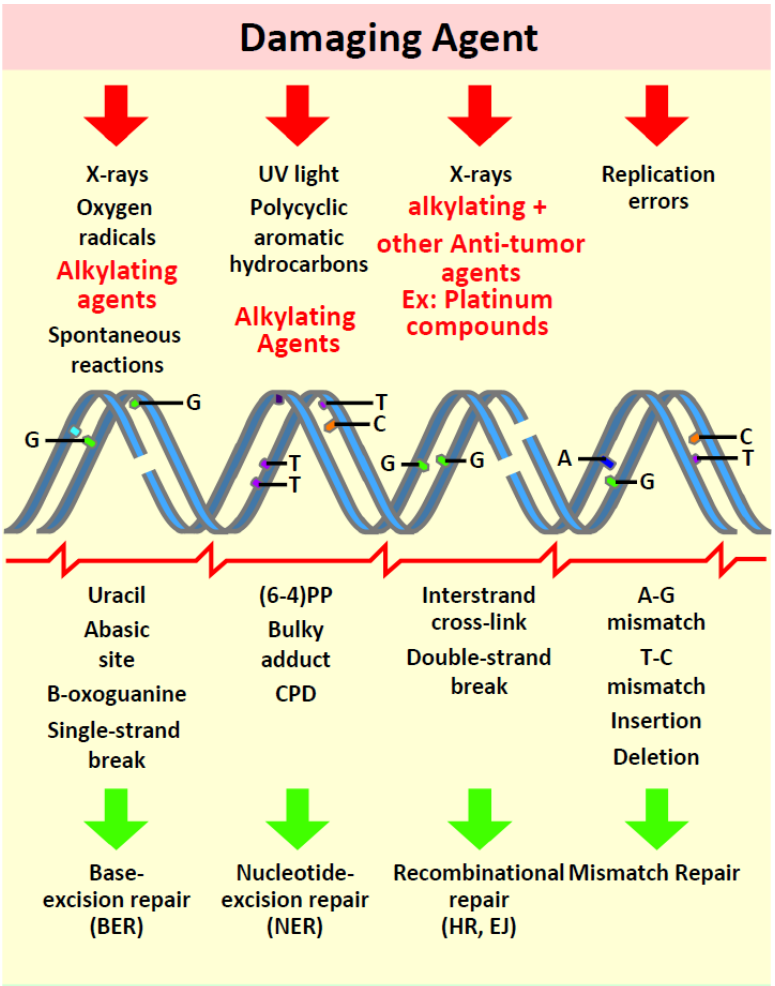
ONC 6 – Pharmacology of Genotoxic Agents

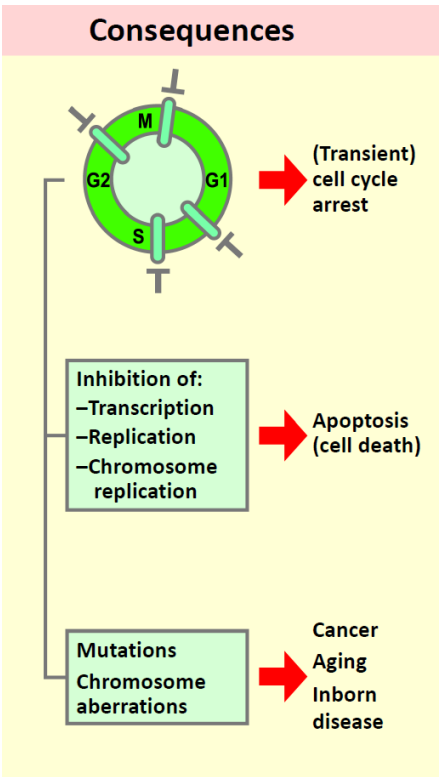
List the cellular responses to DNA damages

Cells have a variety of mechanisms in which they typically handle **DNA damage**. These mechanisms are typically used to correct either breaks in DNA strands or errors created throughout replication.



Type of Break	*Repair Mechanism*
Single Strand Break	**Base-Excision Repair** Proteins and enzymes come in and use the *intact complement strand* as a template to replace the *single base* that is missing.
Bulky Adducts or UV Attributed Malformations *eg. Pyrimidine Dimer Formation*	**Nucleotide-Excision Repair** Proteins and enzymes come in and use the *intact complement strand* as a template to replace a *portion* of the strand that is missing
Double Strand Break	**Homologous Recombination** This shit is ***wild***. The **unbroken, chromosome pair** that is a duplicate of the broken strand is used as a template to fix the broken portions of the double stranded DNA. This occurs at G2 and before cell division because we need the other copy to use as a template before the splitting of the cell (and other copy leaves). **Non-Homologous Recombination (**aka End Joining**)** No clue pictures looked too wild who cares
Replication Errors	**Mismatch Repair** Errors of bases is recognized. These are errors such as *insertion*, *deletion*, etc.

Some possible consequences of the above examples of DNA damage include;



Cell Cycle Arrest

Prior to any of the above repair mechanisms, the cell cycle needs to stop (cells need to stop dividing further/growing) before any repair can be attempted.

Apoptosis

p53 mediated Apoptosis helps prevent the propagation of harmful, hereditary mistakes in DNA → **Cancer**

Mutations and Chromosome Aberrations

When DNA is not repaired and Apoptosis is not induced, then this can lead to **Cancer**. **Aging** is a normal process and mutations can be gained across one's lifespan (somatic).

Explain how DNA damaging agents work in anticancer chemotherapy

There are 3 classes of DNA-damaging agents (*Genotoxic*) used in Cancer Chemotherapy.

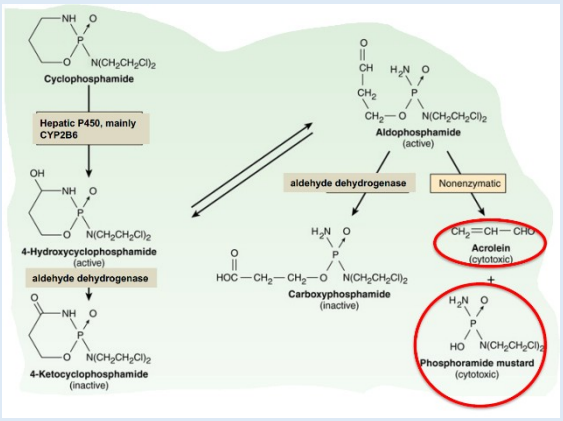
- **Alkylating Agents**
- **Platinum Compounds**
- **Anthracycline Antibiotics**

Alkylating Agents

Alkylating Agents exert their genotoxic effect by **covalently binding** to *nucleophilic sites* in DNA. This leads to *crosslinking* and a deformation of the DNA structure which is recognized by mechanisms and destroyed.

- Inter-Strand covalent bond → **double strand break**
- Intra-Strand covalent bond → **single strand break**

Cyclophosphamide is a common alkylating agent used in cancer chemotherapy.

Parameter	*Notes*
Efficacy	Mechanism as described above Requires *CYP2B* as it is a **pro-drug** that needs to be activated.  **CYP2B6** will *activate* the drug **Aldehyde Dehydrogenase** will inactivate the drug *Non-enzymatic* pathway results in Acrolein which is described below
Safety	A non-enzymatic pathway results in the formation of **Acrolein** which accumulates in the bladder and can lead to *hemorrhagic cystitis* as it destroys tissue there. **Mesna** is always co-administered as it will *covalently bind* to Acrolein. Major Toxicities include; • Myelosuppression – makes sense, it kills cells • Hemorrhagic Cystitis (see above) • **Cardiotoxicity** with high doses • **High-Moderate Emetogenicity**
Resistance	↑ Metabolism by **Glutathione Transferase** which is responsible for handling highly reactive compounds (radicals, etc) ↑ Metabolism by **Aldehyde Dehydrogenase** which is the main enzyme responsible for inactivation ↑ DNA Repair mechanisms to this **cross-linking** of DNA strands → ↓ Tumor Cell Death

Ifosfamide is an *analog* of Cyclophosphamide with slight differences in PK (enzymes, etc).

- *CYP3A4* instead of CYP2B6 for activation but clearance/elimination quite similar.
- Myelosuppression – Same, makes sense
- Hemorrhagic Cystitis (see above)
- **Encephalopathy**
- **Low-Moderate Emetogenicity**

Platinum Compounds

Platinum compounds act similarly to alkylating agents in that they exert their cytotoxic effect by also **crosslinking DNA molecules** through a similar interaction with *nucleophilic centers*.

Cisplatin is the most efficacious in the treatment of testicular and ovarian cancer.

Parameter	*Notes*
Efficacy	Not activated by CYP enzymes, rather activated by **hydrolysis**. Similar to Alkylating agents, the cross-linking (either inter or intrastrand) results in deformation of the DNA and recognition by various processes for destruction Have 2 arms (Cl^- allowing for attachment at 2 sites)
Safety	Most toxic out of the platinum compounds discussed in this lecture Major toxicities include; • Myelosuppression – not a surprise • **Nephrotoxicity** • **Irreversible Ototoxicity** • High emetogenicity – all platinum compounds are at least medium
Resistance	↑ Metabolism by **Glutathione Conjugation** or other sulfhydryls conjugation (see above point about reactive species) ↓ Absorption/Distribution to target tissues or ↑ Efflux ↑ DNA repair capacity

Carboplatin is also a pro-drug platinum compound that is activated through hydrolysis but is *less reactive* and *less toxic* than Cisplatin (the epoxide causes the leaving groups to depart more slowly)

- ↓ Leaving group rate → ↓ reactivity → ↓ toxic (Cytotoxicity and Nephrotoxicity), both in E and S
- Major Toxicities include;
    - Myelosuppression – same
    - ↓ **Nephrotoxicity and Ototoxicity** than Cisplatin

Oxaliplatin is another platinum compound that is even ↓ reactive (even more bulky leaving groups)

- Major toxicities include;
    - Myelosuppression
    - **Peripheral Sensory Neuropathy** (Neurotoxicity)

Anthracycline Antibiotics

Anthracycline Antibiotics, derived from *Streptococcus peucetius* have various mechanisms of Cytotoxicity.

Doxorubicin is the prototypical anthracycline antibiotic.

Parameter	*Notes*
Efficacy	Mechanisms of Action; 1. Intercalate DNA – similar to all the above agents 2. Tripartite complex with **Topoisomerase II** → double stranded breaks 3. Generation of **free radicals** → Oxidative Stress
Safety	Anthracycline antibiotics; **delayed onset cardiac toxicities → congestive heart failure** • **Dexrazoxane** is an iron chelating agent that can help prevent or reduce these cardiac toxicities • Due to these toxicities associated with long term use, patients have a lifetime "cap" of Doxorubicin (max amount they can take, then have to d/c) • Some **Liposomal** formulations have been found to ↓ Cardiac Toxicities All anthracycline antibiotics are high-moderate in emetogenicity
Resistance	↑ **P-Glycoprotein** → ↑ efflux of the drug ↓ **Topoisomerase II** activities → if the cell relies on this enzyme less, than our MoA sucks ↑ Metabolism by **Glutathione Peroxidase** (see above about reactive species)

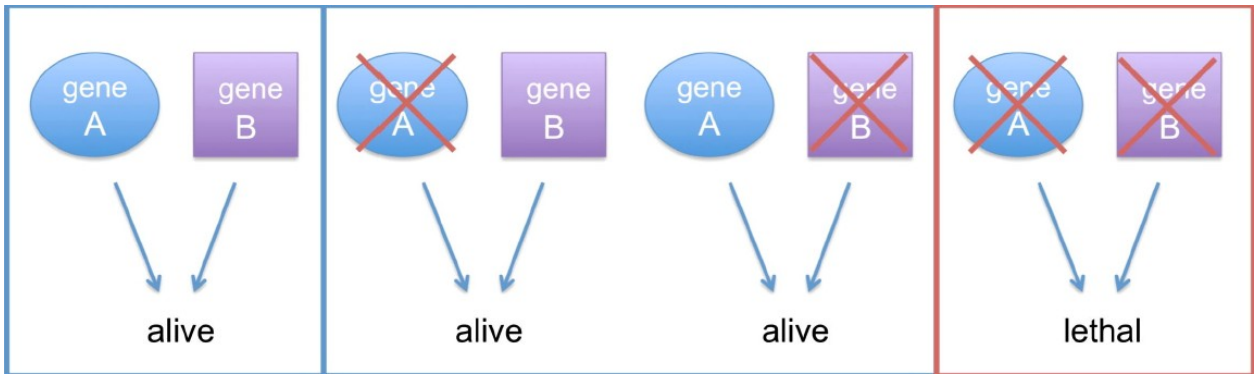
Epirubicin is a *synthetic* Doxorubicin analog approved for the use in breast cancer.

- ↓ **Cardiotoxicities** at equivalent doses
- Similar in Myelosuppression

Understand the concept of synthetic lethality and the use of PARP inhibitors in BRCA-defective cancers

Synthetic Lethality is an interesting approach to therapy that capitalizes on the idea that;

- Since in cancer, through all these mutations some genes are already **turned off/missing**
- These genes may share a *relationship* with *another gene* such that when **both are turned off** the cell dies
- The only difficulty now is to determine which genes share these relationships with our cancer mutations



Olaparib and **Niraparib** are examples of PARP inhibitors.

Olaparib	Niraparib
• PO • Metalized by CYP3A4 – increased interaction profiles • Major toxicities: – myelosuppression – Rare pneumonitis (1%) but could be fatal – low-moderate emetogenic	• PO • Metabolized by carboxylesterase • Major toxicities: – myelosuppression – CVS: cardiac toxicity and hypertensive crisis – moderate emetogenic

Secondary malignancy: myelodyplastic syndrome and acute myeloid leukemia

Resistance mechanisms (for both agents):

- increase expressions of P-glycoprotein
- reactivation of homologous recombination repair
- increase DNA replication fork protection

BRCA Mutations – Clinical Example

BRCA Mutations (associated with Breast Cancer) share a *synthetic lethality* relationship with **PARP Inhibitors**.

FOLLOW MY NUMBERS FOR EACH TEXT BOX, THIS IS A BUSY SLIDE

1. PARP-Mediated Repair

In a cancerous tumor cell, when a **single stranded DNA break occurs** (i.e. if we use an alkylating or similar agent) then **PARP** comes in and fixes these ssDNA breaks through **Base Excision Repair**.

This leads to survival of the tumor cell.

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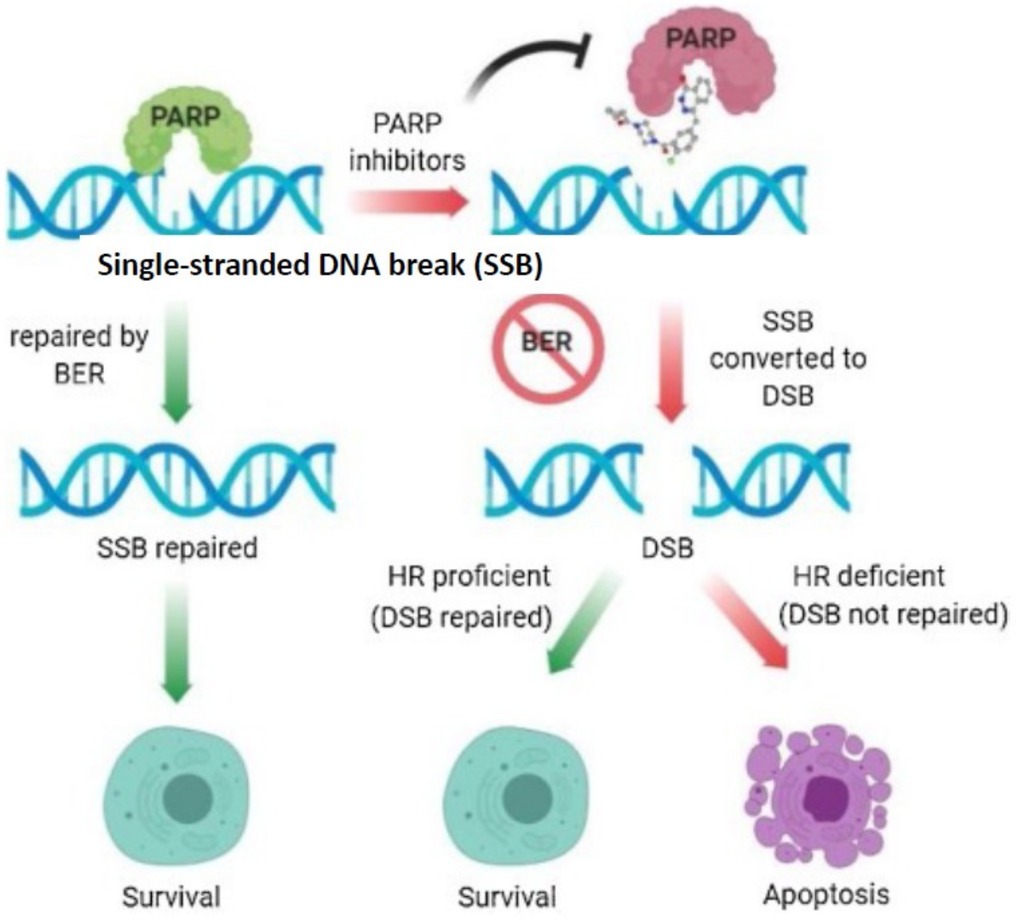

2. PARP Inhibition

If we administer something that will **inhibit PARP**, than Base Excision Repair cannot occur.

3. SSB conversion to DSB

When DNA tries to replicate a strand containing a single stranded break (because we inhibited PARP!), the single stranded break **turns into a double stranded break**

4. BRCA Mediated HR

The repair mechanism for a double stranded break (see LO #1) in this case is **Homologous Repair** which is **BRCA DEPENDENT**.

In cancer however, this DNA repair mechanism is **lost** because of BRCA mutations resulting in **cancerous growth**

(If this DNA repair mechanism was working, we wouldn't have a cancerous cell!)

5. Synthetic Lethality

PARP Inhibition + **BRCA Mutation** (from the cancer) → **Apoptosis** because the cell cannot

- Repair through BER (PARP)
- Repair through HR (BRCA)