

Therapeutic targeting and patient selection for cancers with homologous recombination defects

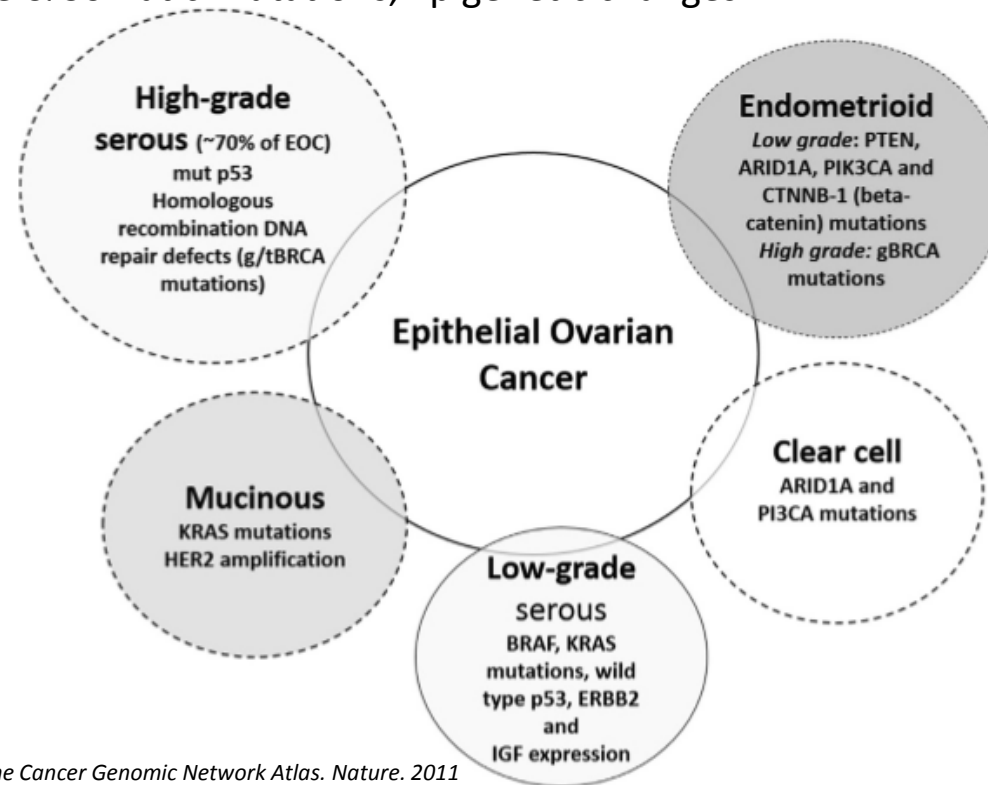
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Epithelial Ovarian Cancer

- **Standard Current Treatment: Surgery with De-bulking + Platinum-Taxane based Chemotherapy**
 - No significant improvement in OS in past 20 yrs
 - Clinically well recognized that histology subtypes within EOC have varying RR to Platinum-Taxane
- **Genetic Analysis Suggests that EOC are 5 distinct diseases**
 - High Grade Serous OC: Up to 51% with deficiencies in Homologous Repair Pathways*
 - Germline & Somatic Mutations, Epigenetic changes



* *Integrated Genomic Analysis of Ovarian Cancer. The Cancer Genomic Network Atlas. Nature. 2011*
Image from: Homologous Recombination Deficient in Ovarian Cancer. Eu J of Cancer. 2016

DNA Damage Response Types

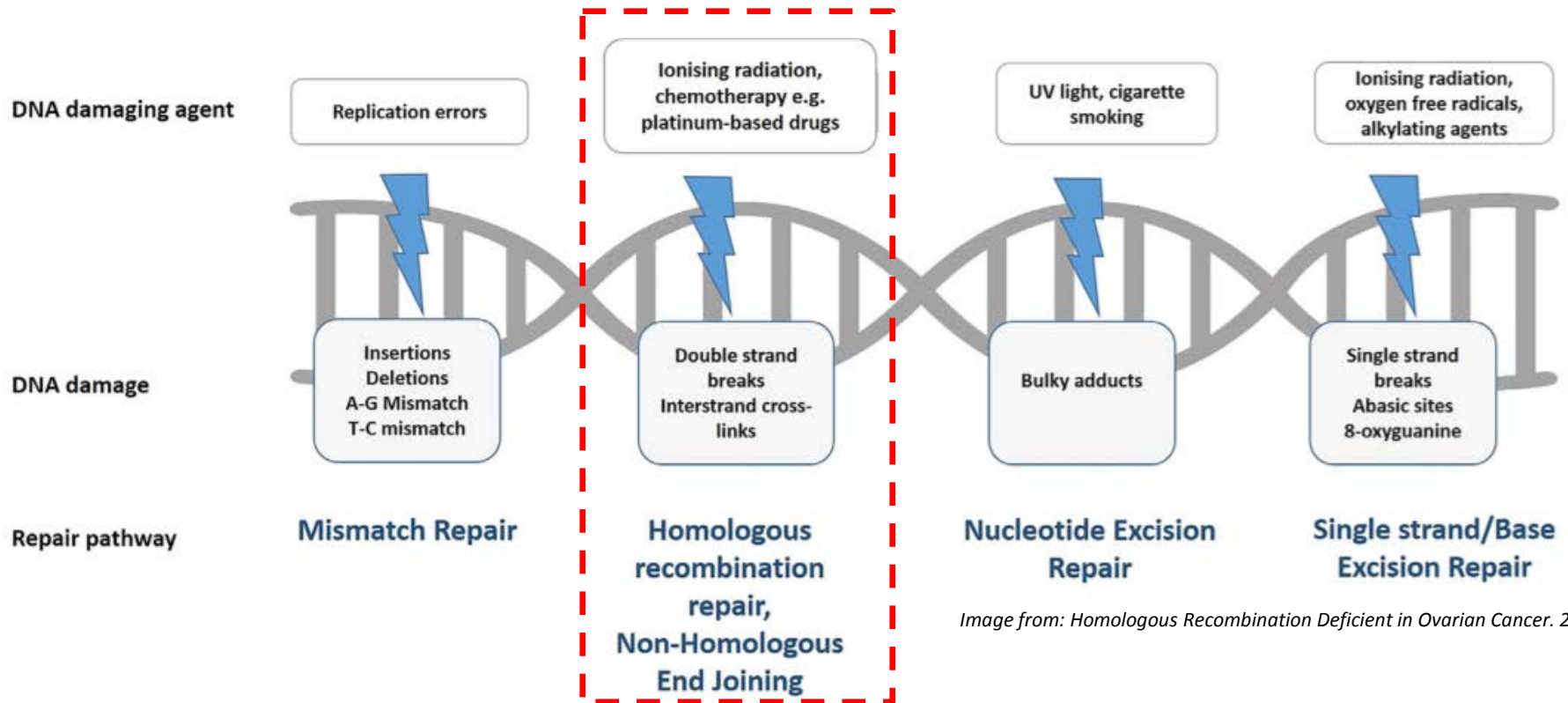
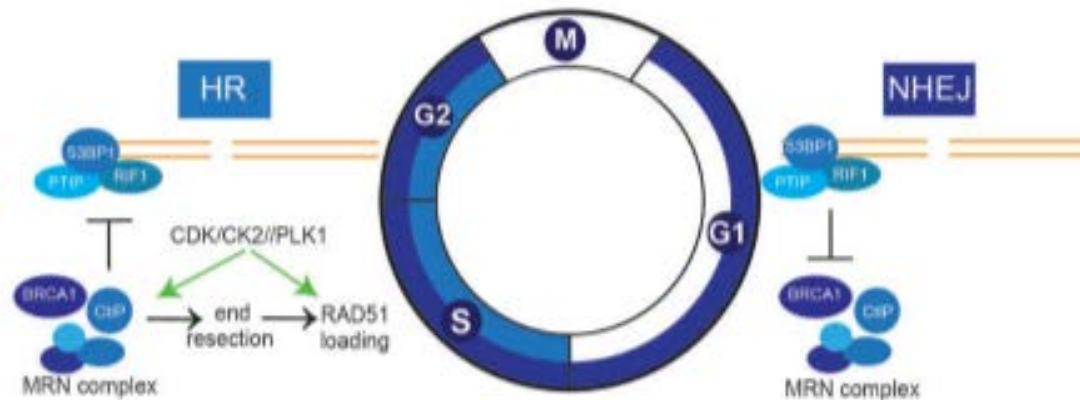


Image from: Homologous Recombination Deficient in Ovarian Cancer. 2016

- **DNA Damage is a critical step of oncogenesis**
 - 450 Genes involved in DNA repair
 - Repair be categorized into 5 distinct pathways
- **DNA Double Stranded Breaks (DSB) are most lethal of all types**
 - Can arise from unrepaired SSB, DNA cross-links or stalled replication forks
- **Platinum and Topoisomerase Inhibitors Exert Cytotoxic Effects though DNA replication**

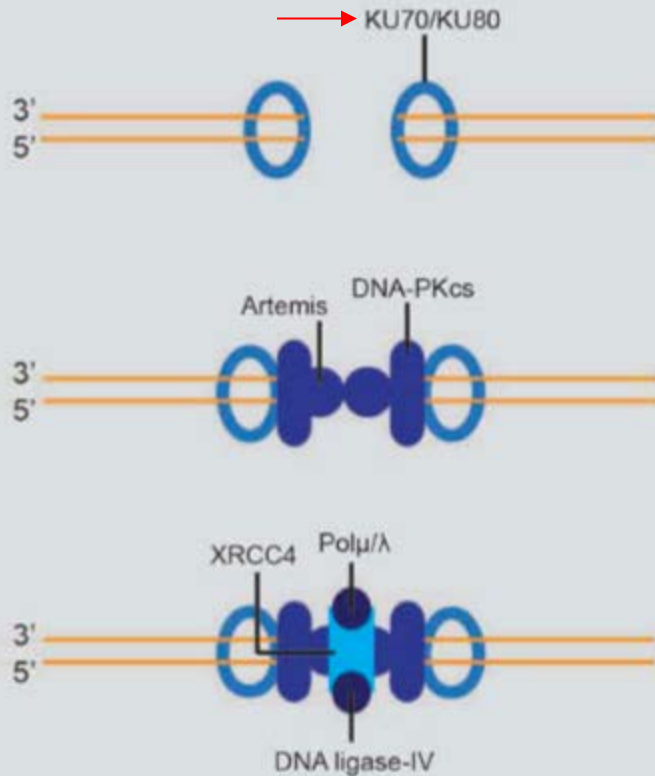
Repair of DNA Double-Stranded Breaks

- **Governed by Two Fundamentally Different Pathways:**
 - Non-Homologous End-Joining (NHEJ)
 - Homologous Recombination
- **The choice depends largely on the cell cycle phase**
 - NHEJ can be active in any cell cycle
 - HR is active only in S and G2 Cell Cycles



Non-Homologous End-Joining DNA Repair

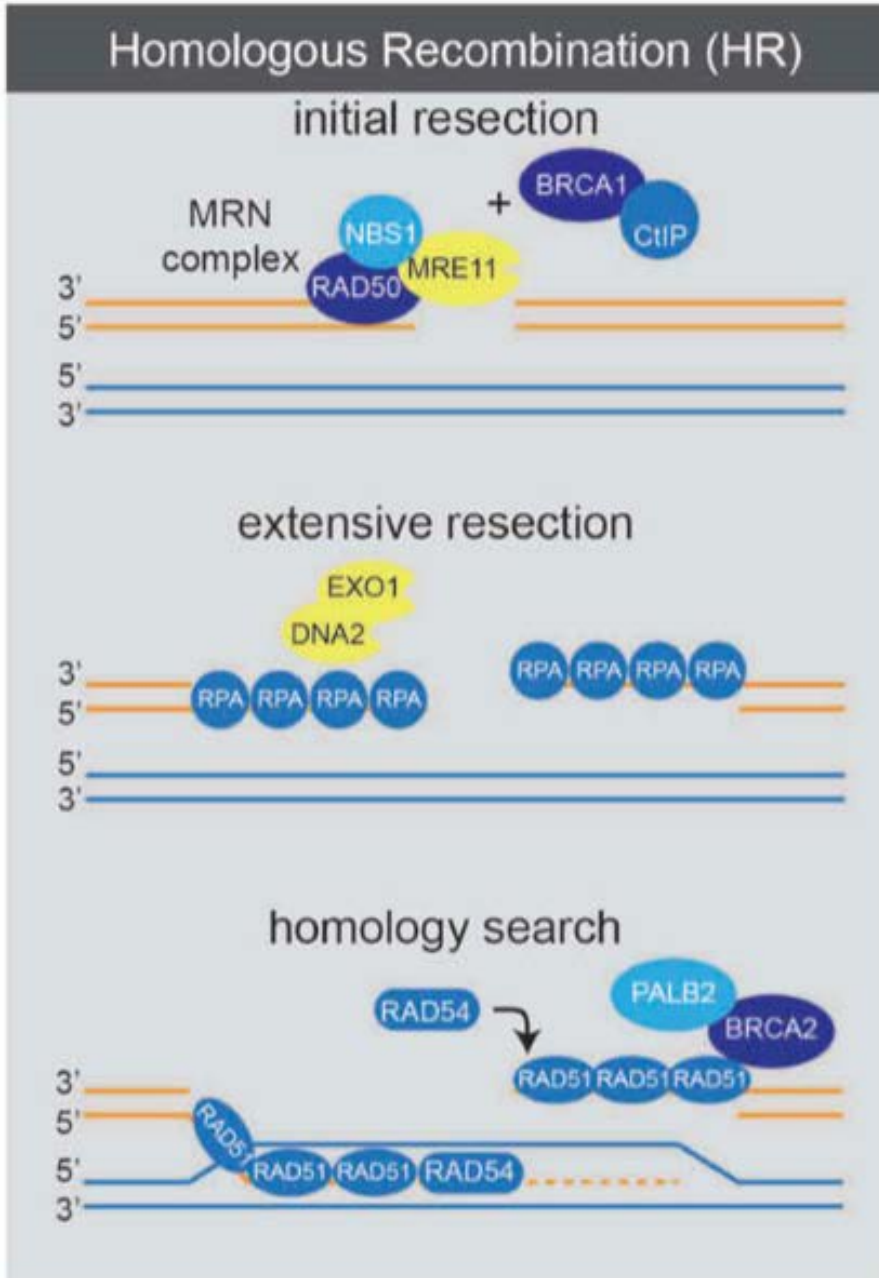
Non-homologous End-Joining (NHEJ)



1. Ku70/80 recognize to DSB
2. Ku70, Ku80 activate DNA Kinase & Artemis
3. XRCC4:DNA Ligase-IV complex is recruited and completes end-to-end joining

Sequence independent because the ends may have damage – direct ligation is error prone and can induce mutations

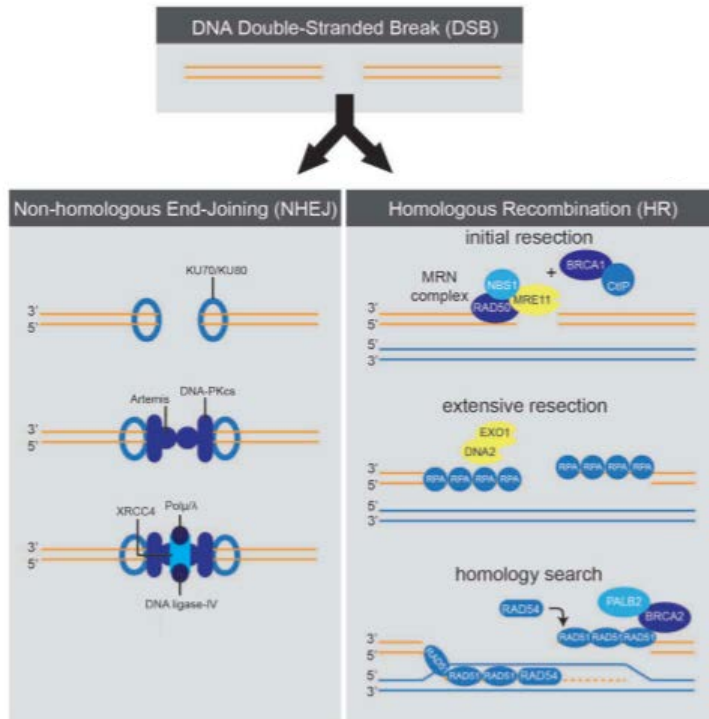
Homologous Recombination DNA Repair



HR repair uses a DNA template.
The use of a template makes HR conservative and **error-free compared to NHEJ**

1. DSB is recognized by MRN complex
 2. MRN promotes recruitment of ATM to site
 3. ATM phosphorylates MRN complex
 4. **Creation of ssDNA overhangs initiated by MRN complex in conjunction with BRC1/CTIP. MRE11 has endonuclease activity ~200-300 nucleotides from DSB site**
 5. ssDNA is stabilized by RPA
 6. BRCA2 is recruited by PALB2 & BRCA1
 7. PALB2/BRCA2 recruits RAD51
RAD51 replaces RPA
- RAD51 searches for sequence homology on the sister chromatid.
- Key RAD51 paralogs for RAD51 recruitment:
RAD51B-D, XRCC2, XRCC3
8. RAD51 initiates strand exchange.

Key Differences in Double-Stranded DNA Repair



Cells deficient in HR are dependent on alternative DSB repair pathways

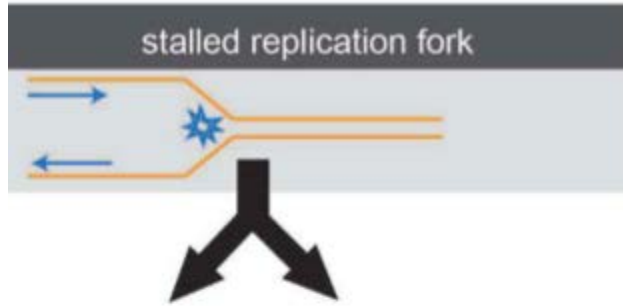
NHEJ is error prone leading to mutagenic DNA junctions

The switch from HR to NHEJ is regulated by several proteins

- CTIP is required for HR and predominantly in S/G2
- 53BP1 and RIF1 negatively regulate ATM and promotes NHEJ.
- 53BP1 interferes w/ BRCA1 function and DNA-end resection.

Studies have shown an altered abundance of 53BP1 lead to NHEJ DNA Repair, genomic instability and sensitivity to PARPi.

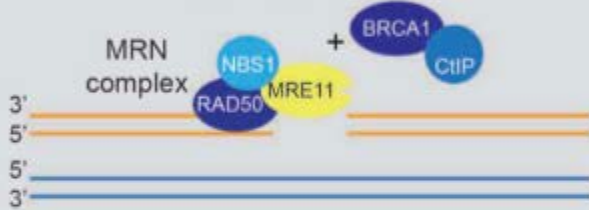
Replication Fork Stability



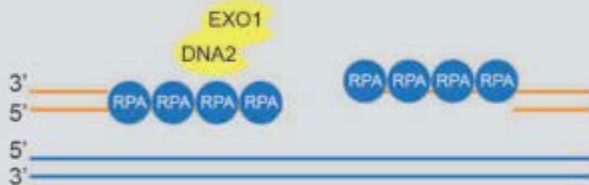
DNA replication occurs via semi-conservative replication where each strand acts as a template for a new DNA strand.

Homologous Recombination (HR)

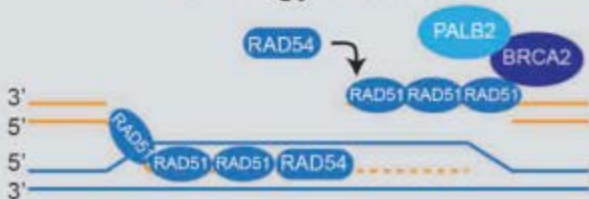
initial resection



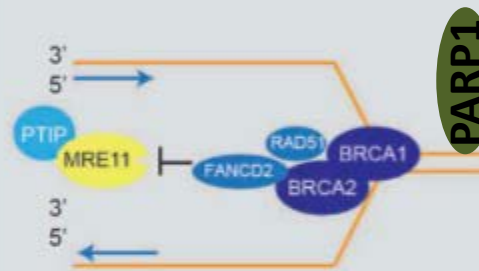
extensive resection



homology search



Replication fork protection



If replication presents stalls, the replication fork is highly vulnerability to collapse and genomic instability via NHEJ repair

HR proteins: BRCA1/2, RAD51 paralogs are **critical** for protection and stability of stalled replication forks

Prevent degradation at the forks and enzymatic activity of MRE11

Trapping of PARP/DNA complex by PARPi induced toxicity plays a critical role at replication forks

Key Homologous Recombination Gene Mutations

Germline BRCA1/2 Predominantly linked to development Breast & OC

- **BRCA1 65% BC, 40% OC lifetime risk**
 - Breast Cancer: Triple Negative
 - OC: High Grade Serous
- **BRCA2: 50% BC, 15% OC lifetime risk**
 - Breast Ca: Low Grade ER+
 - OC: High Grade Serous
- Pancreatic, Prostate, Endometrial Cancer

- **Two Hit Hypothesis: Mutation Carriers requires the loss of the remaining WT allele through somatic mutations to become HR defective**
 - **Homozygous BRCA1/2 found not compatible with life / Early Pregnancy Loss**

Somatic and epigenetic BRCA1/2 mutations also commonly occur in Ovarian Cancer

Other Key HR Genes (“BRCA-ness Mutations”)

PALB2, RAD51 Paralogs, ATM

Studies have shown CHEK2 gene mutation is involved in BRCA1 phosphorylation

Paradoxical Role of Tumorigenesis with Homologous Recombination Deficiency

BRCA1/2 genes:

- Tumor suppressive function
- BRCA1/2 Mutations Lead to HR Deficiency

HR Deficiency → Secondary mutations → DDR upregulation of TP53 → Apoptosis

Some evidence p53 mutation may be *required* for survival of HR deficient cells

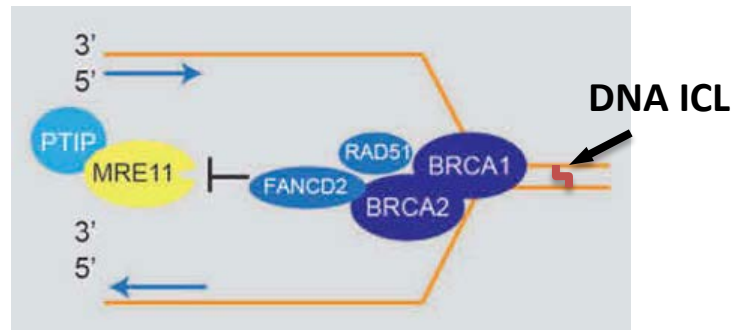
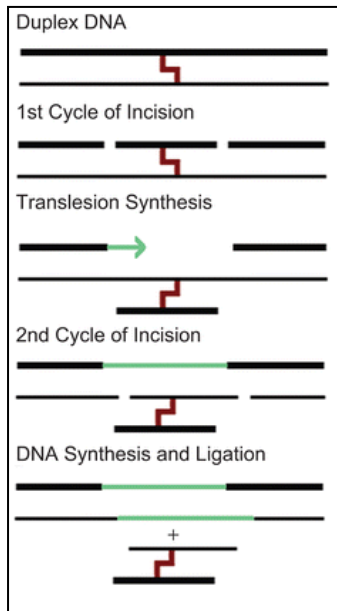
BRCA1 Deficient mouse model with introduction of TP53 mutation resulted in significantly increased tumorigenesis

*TCGA study TP53 mutations in 96% of High Grade Serous Ovarian Cancers**

DNA Crosslinking Agents

Inter and Intra-strand crosslinks (ICL) interfere with DNA replication

- **Several cytotoxic drugs induce ICLs including platinum compounds**
- Prevents separation of DNA strands, leading to **stalled replication forks**
- **HR proteins are critical for protection of stalled replication forks**
- Repair of ICLs and re-start of RF depends of BRCA-Fanconi Anemia (FA) genes



Improved clinical response rate for BRCA1/2 deficient patients to platinum-based chemotherapy

Platinum Therapy → ICL – Collapse RF → Error prone NHEJ DNA repair

Breast Cancers often have BRCA mutations as well

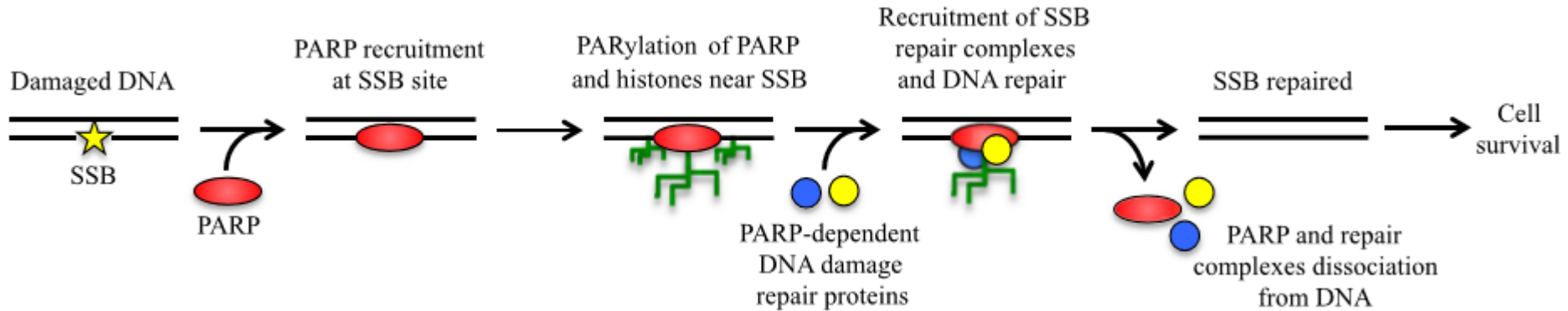
Ex: Stage III Breast Ca pt showed improved OS with Cyclophosphamide/Thiotepa/Carboplatin vs 5-FU/Epirubicin/Cyclophosphamide

Key Points

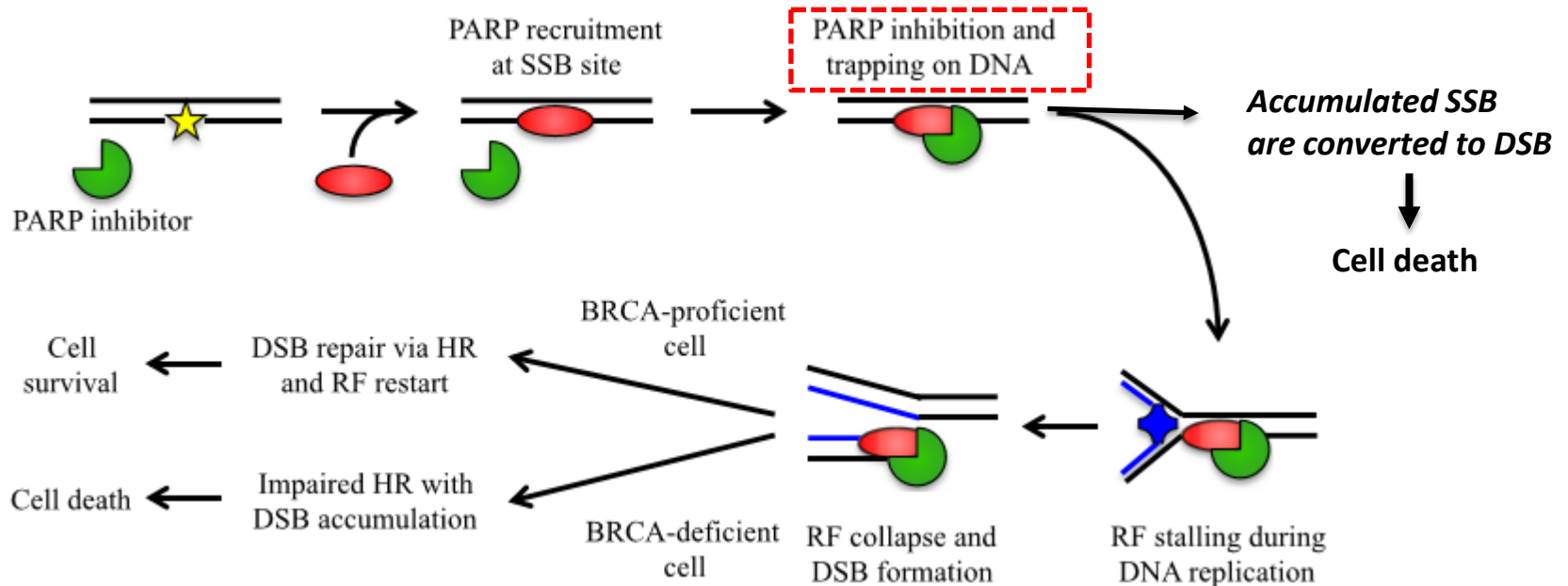
- **Homologous Recombination is critical for**
 - Efficient and **error free repair** of DNA Double Stranded Breaks
 - Stability at replication forks
- **Loss of Homologous Recombination functionality leads to NHEJ repair**
 - Error prone → **Genomic instability**
- **Agents that introduce DNA ICLs requires**
 - Nucleotide excision **and** HR related proteins for efficient DNA repair

PARP Mechanism and Inhibition

- PARP1/2 are enzymes involved in base excision repair (BER) for Single Stranded Breaks
- PARP1 is important for repair of collapsed RF

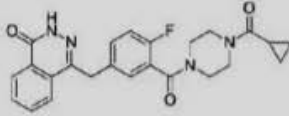
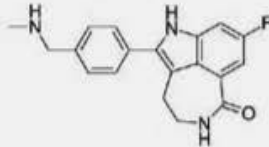
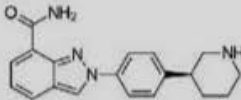
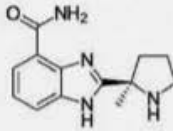
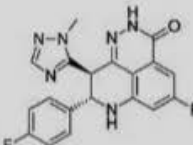


Key Mechanism of PARP Inhibition:



PARP Inhibitors in Clinical Development

FDA Approved

PARP inhibitor	trade name	status	structure	Ki	relative trapping capacity
olaparib/AZD-2281	Lynparza	2014: FDA/EMA approved		PARP1: 5 nM PARP2: 1 nM	+++
rucaparib/AG-014699	Rubraca	2016: FDA approved		PARP1: 1.4 nM	+++
niraparib/MK-4827	Zejula	2017: FDA approved		PARP1: 3.2 nM PARP2: 4.0 nM	++++
veliparib/ABT-888		2016: FDA Orphan Drug Designation		PARP1: 5.2 nM PARP2: 2.9 nM	+
talazoparib/BMN-673		phase 3 testing		PARP1: 1.2 nM PARP2: 0.9 nM	+++++

Cytotoxicity of different PARP Inhibitor's is related to their trapping potential
- BMN-673 has 100x higher trapping than Olaparib

Potential Strategies to Increase Sensitivity to PARPi

1. Anti-angiogenic + PARPi

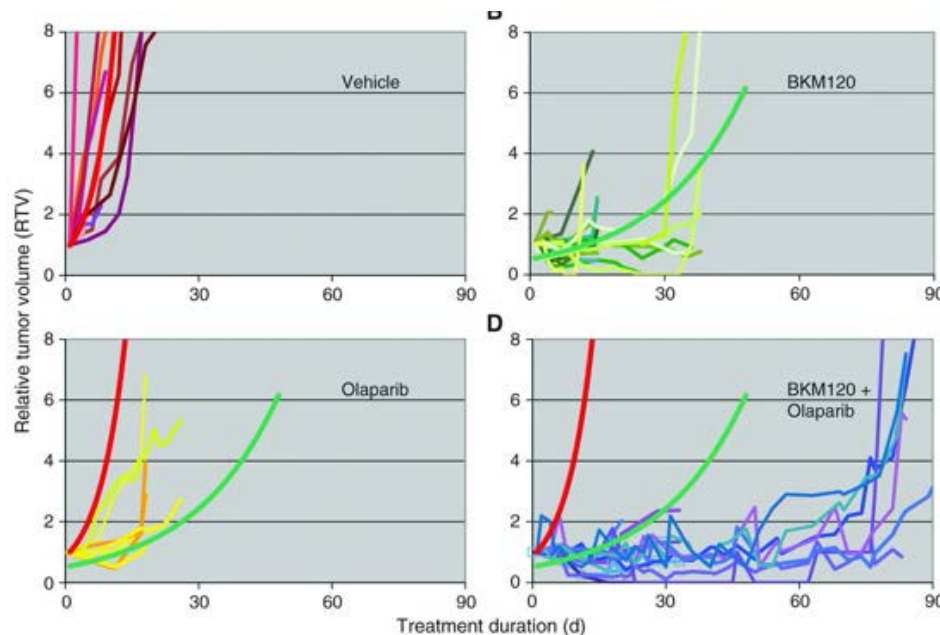
Hypoxic conditions appears to downregulate RAD51 in several cancer cell lines as well as decreased expression of BRCA1/2 in OC cells

Olaparib + Cediranib → Improved PFS w/ platinum sensitive recurrent OC
(Phase II Trial: PFS of 17.7 vs. 9.0 mos compared to olaparib monotherapy)

2. PI3K Inhibitor + PARPi

PI3Ks (phosphoinositide 3-kinases) – isoform PI3K β important for DSB sensing and recruits NBS1 – a subunit of MRN complex.

PI3K inhibition (via BKM120) resulted in increased DNA damage increased sensitivity to PARPi in BRCA1 deficient breast cancer*



PDX mice model

*Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. 2012

Proposed Mechanisms of Chemo-resistance to PARPi

HR defect	PARP resistance mechanism	Refs
<p><i>BRCA1/2</i> mutation</p>	<p>second mutation (intragenic)</p>	<p>142 143 157</p>
<p><i>BRCA1</i> mutation</p>	<p>second mutation (other genes)</p>	<p>36 37 149 150 151 152</p>
<p><i>BRCA2</i> mutation</p>	<p>second mutation (other genes)</p>	<p>47 153 154 155</p>
<p><i>BRCA1</i> promotor hypermethylation</p>	<p>promotor translocation</p>	<p>144 145</p>

Second somatic mutation
restoring *BRCA1/2* function


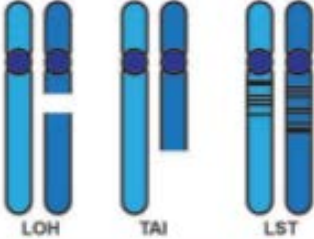
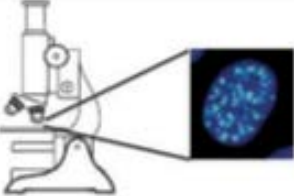
Loss of function of 53BP1,
RIF, etc → decreased PARP
sensitivity and partially
Restores HR
(mouse models)

Loss of function of PAXIP1
(PTIP) inhibits recruitment
of MRE11 in *BRCA* deficient
cells by inhibiting degradation
at RF

Re-arrangement of *BRCA*
locus to translocate a new
promoter region (not
hypermethylated).
Demonstrated in PDXs

Selecting Patients for PARP Inhibitor Treatment

PARPi Success is Based Largely on Exploiting Homologous Recombination Deficiency

	PROS	CONS
BRCA1/2 mutation analysis 	<ul style="list-style-type: none"> -easy and fast -can be performed on archived material 	<ul style="list-style-type: none"> -no information on other HR genes -secondary mutations (e.g. <i>TP53BP1</i>) remain undetected - 'variants of unknown significance (VUS)' complicate analysis
Genomic 'scar' analysis (eg: aCGH, SNP array) 	<ul style="list-style-type: none"> -tests consequences of HR deficiency -also detects 'BRCA-like' mutations -can be performed on archived material -provides additional genome-wide data - performance likely increases with increasing numbers of samples 	<ul style="list-style-type: none"> -historic scar may not represent functional HR defect
Functional HR read-out 	<ul style="list-style-type: none"> - allows detection of functional HR defect - includes all causes of HR deficiency 	<ul style="list-style-type: none"> -requires 'wet' lab work -requires fresh tumor material -requires vulnerable reagents (antibodies)

Comparative genomic hybridization (CGH) test

- Uses a set of BRCA-mutated tumors as well as control tumors

Myriad – “My Choice” – HRD Panel providing a

HRD score as well as BRCA deficiency.

Clinical trials suggest it is capable of identifying 2X patients that could benefit from PARPi compared to mutational status alone.

Rad51 foci correlated as a marker for HRD

Rad51 loading is an essential last step of HR and can be visualized as RAD51 foci formation.

RAD51 foci read out can be for HR-deficiency

RAD51 only present in cells in S/G2 cycle and can lead to false negatives

Conclusions

Defective HR leads to genomic instability and oncogenesis, tumor progression

HR deficiency has an inherent sensitivity to DNA damaging agents

Current patient selection is largely based on BRCA1/2 mutational status, however this alone can miss somatic HR deficiencies as well as germline BRCA1/2 deficient patients can have secondary somatic mutations restoring HR proficiency and thus not responding to targeted therapies.

Function Genomic Studies such as RAD51 may offer a greater potential for capturing patients who will respond to HRD targeted therapies

Clinical Trial:

Response to PARP Inhibitor Predicted by the RAD51 Assay (REPAIR)

- Using an Ex-Vivo assay for RAD51 to Predict Response to Verapariib

Recruiting Platinum sensitive high grade serous ovarian cancer or BRCA-mutated (non-)breast and (non-)ovarian cancer will be included prior to the RAD51 assay and treated with veliparib irrespective of the assay result.

Start: August 2017

Lynparza™ (olaparib)**Rubraca™ (rucparib)****Zejula™ (niraparib)**

Drug Class	PARP Inhibitor		
Mechanism of Action	Inhibits PARP1,2,3* which are enzymes involved in DNA transcription, cell cycle regulation, and DNA repair. Causes double strand breaks in DNA and prevents repair, which leads to cell death in BRCA1/2 deficient tumor cells. *niraparib inhibits PARP-1 and PARP-2		
Approval Date	Dec 19, 2014	Dec 19, 2016	March 27, 2017
Approved Indication	<p>Monotherapy in patients with deleterious or suspected deleterious germline <i>BRCA</i>-mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy.</p> <p>Maintenance after CR/PR in plat-sensitive recurrent setting</p>	<p>Monotherapy in patients with deleterious <i>BRCA</i> mutation (germline and/or somatic) associated advanced ovarian cancer who have been treated with two or more chemotherapies.</p>	<p>Maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy</p>
Dose	400mg PO BID 300mg PO BID (<i>maint</i>)	600mg PO BID	300 mg PO daily
Dosage Form	50mg capsules (Do not open, swallow whole)	200 and 300mg tablets	100 mg capsules
Duration	Continue until disease progression or unacceptable toxicity		
Absorption	Rapid, peak concentrations achieved in 1-3 hours	Rapid, peak concentrations achieved at 1.9 hours	Rapid, peak concentrations achieved within 3 hours
Distribution	167 ± 196 L; Protein binding: 82%	113 to 262 L; Protein binding: 70%	1220 ± 1114 L; Protein binding: 83%
Metabolism	Primarily hepatic via CYP3A4 (Verify no significant drug interactions)	Primarily hepatic via CYP2D6	Primarily by carboxylesterases to an inactive metabolite
Elimination	Half-life 11.9± 4.8 hours	Half-life 17 to 19 hours	Half-life 36 hours
Dosage Adjustments	Renal dose adjustment (<i>C_{cr}</i> 31-50ml/min):300mg BID Adjustments for adverse reactions provided in package insert (Pharmacist may assist with dose adjustments)	Adjustments for adverse reactions provided in package insert (Pharmacist may assist with dose adjustments)	Adjustments for adverse reactions provided in package insert (Pharmacist may assist with dose adjustments)
Pearl Notes	N/V reported more frequently when administered in the fasted state.		
Monitoring	CBC at baseline and monthly thereafter, or as clinically indicated, renal function	CBC at baseline and monthly thereafter, or as clinically indicated	CBC at baseline and monthly for next 11 months, or as clinically indicated. Monitor blood pressure and heart rate monthly for the first year and periodically thereafter.
Warnings/Precaution	Secondary Malignancy: MDS/AML		

	<u>Lynparza™ (olaparib)</u>	<u>Rubraca™ (rucaparib)</u>	<u>Zejula™ (niraparib)</u>
Side Effects	Peripheral edema(10-20%), fatigue (67%), nausea (64-75%), abdominal pain (43%), vomiting (32-43%), diarrhea (28-31%), dyspepsia (25%), decreased hemoglobin (85%-90%; grades 3/4: 8-15%), increased MCV (57-85%), decreased absolute lymphocyte count (56%; grades 3/4: 17%), anemia (25-34%; grades 3/4: 4-18%), decreased neutrophils (25-32%; grades 3/4: 7-8%), decreased platelet count (26-30%; grades 3/4: 3-6%), musculoskeletal pain (21-32%), myalgia (22-25%), increased serum creatinine (26-30%), and upper respiratory tract infection (26-43%)	Fatigue (77%), nausea (77%), vomiting (46%), constipation (40%), photosensitivity (10%), decreased appetite (39%), <u>dysgeusia</u> (39%), diarrhea (34%), abdominal pain (32%), decreased hemoglobin (67%, grades 3 to 4: 23%), decreased absolute lymphocyte count (45%, grades 3 to 4: 7%), anemia (44%, grades 3 to 4: 25%), thrombocytopenia (21%, grades 3 to 4: 5%), neutropenia (15%), increased serum ALT (74%), increased serum AST (73%), and increased serum creatinine (92%).	Thrombocytopenia (61%), anemia (50%), neutropenia (30%), leukopenia (17%), palpitations (10%), nausea (74%), constipation (40%), vomiting (34%), abdominal pain/distention (33%), mucositis/stomatitis (20%), diarrhea (20%), dyspepsia (18%), dry mouth (10%), fatigue/asthenia (57%), decreased appetite (25%), urinary tract infection (13%), AST/ALT elevation (10%), myalgia (19%), back pain (18%), arthralgia (13%), headache (26%), dizziness (18%), <u>dysgeusia</u> (10%), insomnia (27%), anxiety (11%), <u>nasopharyngitis</u> (23%), dyspnea (20%), cough (16%), rash (21%), and hypertension (20%)