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

- 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

Image from: Homologous Recombination Deficient in Ovarian Cancer. 2016
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

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
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.
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 insatiability 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.

If replication presents stalls, the replication fork is highly vulnerable 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 $\rightarrow$ Secondary mutations $\rightarrow$ DDR upregulation of TP53 $\rightarrow$ 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 on 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 inhibition and trapping on DNA
- Accumulated SSB are converted to DSB
- Cell death

Livraghi. BMC Medicine. 2015
### PARP Inhibitors in Clinical Development

<table>
<thead>
<tr>
<th>PARP inhibitor</th>
<th>trade name</th>
<th>status</th>
<th>structure</th>
<th>Ki</th>
<th>relative trapping capacity</th>
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<tbody>
<tr>
<td>olaparib/AZD-2281</td>
<td>Lynparza</td>
<td>2014: FDA/EMA approved</td>
<td><img src="image1" alt="structure" /></td>
<td>PARP1: 5 nM, PARP2: 1 nM</td>
<td>+++</td>
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<td>rucaparib/AG-014699</td>
<td>Rubraca</td>
<td>2016: FDA approved</td>
<td><img src="image2" alt="structure" /></td>
<td>PARP1: 1.4 nM</td>
<td>+++</td>
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<tr>
<td>niraparib/MK-4827</td>
<td>Zejula</td>
<td>2017: FDA approved</td>
<td><img src="image3" alt="structure" /></td>
<td>PARP1: 3.2 nM, PARP2: 4.0 nM</td>
<td>++++</td>
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<tr>
<td>veliparib/ABT-888</td>
<td></td>
<td>2016: FDA Orphan Drug Designation</td>
<td><img src="image4" alt="structure" /></td>
<td>PARP1: 5.2 nM, PARP2: 2.9 nM</td>
<td>+</td>
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<tr>
<td>talazoparib/BMN-673</td>
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<td>phase 3 testing</td>
<td><img src="image5" alt="structure" /></td>
<td>PARP1: 1.2 nM, PARP2: 0.9 nM</td>
<td>++++</td>
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</table>

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 (phosphinositide 3-kinases) – isoform PI3Kβ important for DSB sensing and recruits NBS1 – a subunit of MRN complex.

PI3K inhibition (via BMK120) resulted in increased DNA damage increased sensitivity to PARPi in BRCA1 deficient breast cancer*

*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

- **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

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 Veraparib

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
<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Lynparza™ (olaparib)</th>
<th>Rubraca™ (rucparib)</th>
<th>Zejula™ (niraparib)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism of Action</td>
<td>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</td>
<td>Monotherapy in patients with deleterious or suspected deleterious germline BRCA-mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy. Maintenance after CR/PR in plat-sensitive recurrent setting</td>
<td>Monotherapy in patients with deleterious BRCA mutation (germline and/or somatic) associated advanced ovarian cancer who have been treated with two or more chemotherapies. 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</td>
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<tr>
<td>Approval Date</td>
<td>Dec 19, 2014</td>
<td>Dec 19, 2016</td>
<td>March 27, 2017</td>
</tr>
<tr>
<td>Approved Indication</td>
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<td></td>
</tr>
</tbody>
</table>
| Dose          | 400mg PO BID  
300mg PO BID (maint) | 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 (CrCl 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) |
<p>| 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 | |</p>
<table>
<thead>
<tr>
<th>Side Effects</th>
<th>Lynparza™ (olaparib)</th>
<th>Rubraca™ (rucaparib)</th>
<th>Zejula™ (niraparib)</th>
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<tr>
<td>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%)</td>
<td>Fatigue (77%), nausea (77%), vomiting (46%), constipation (40%), photosensitivity (10%), decreased appetite (39%), dysgeusia (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%).</td>
<td>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%), dysgeusia (10%), insomnia (27%), anxiety (11%), nasopharyngitis (23%), dyspnea (20%), cough (16%), rash (21%), and hypertension (20%)</td>
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