PARP Inhibitor Resistance and Acquired Vulnerability in Ovarian Cancer

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Speaker Disclosure

Consultant: Lilly Oncology, EMD Serono, Intellia Therapeutics, Sierra Oncology, Formation Biologics

Consultant/Stock Holder: Ideaya, Inc
What are the cellular functions of BRCA1 and BRCA2?
Functions of BRCA1 and BRCA2 in Homologous Recombination Repair and in Protection of Stalled replication forks

BRCA1
Promotes End Resection

BRCA2
Loads RAD51

BRCA1/FANCS
PALB2/FANCN
BRCA2/FANCD1

Resection
-Mre11
-WRN
-Dna2
-BLM
-MUS81

HR Repair
Synthesis-dependent strand annealing
Second-end capture
Double Holliday-junction formation
Resolution
Non-crossover
Crossover

Fork Protection

Modified from Lee Zou
How do PARP inhibitors work?
Mechanisms of Sensitivity and Resistance to PARP Inhibitors

Rationale:
- 50% of Serous OC have HR Deficiency
- PARP inhibitors approved for recurrent OC
- BRCA1/2 mutations are neither necessary nor sufficient for response to PARPi
- Need for functionally distinguishing pathogenic mutations in HR genes
- Need to extend the use of PARPi

Konstantinopoulos, Ceccaldi, Shapiro, D’Andrea
Cancer Discovery 2015
How do PARP inhibitors kill HR deficient tumor cells?

Inhibition of BER

Trapping of PARP-DNA complex
A third mechanism: PARP inhibitors kill HR deficient tumor cells by blocking POLθ-mediated Alternative End-Joining

BRCA1 or BRCA2 Deficient tumors

Synthetic Lethality between HR and Polθ-mediated alt-EJ

\[ \text{Fancd2}^{-/-} \]
HR deficiency
Mild phenotype

\[ \text{Polθ}^{-/-} \]
Alt-EJ deficiency
Mild phenotype

Double Knockout
Embryonic lethal
PARP1 is required for POL$\theta$ recruitment to sites of DNA repair

Alt-End-joining Assay

Pol$\theta$ Foci Assay
What function of POLQ should be targeted in cancers?

- should we target the function of POLQ that regulates HR repair (the ATPase) or that regulates the end-joining process (the polymerase)?
POLQ has strong anti-recombinase activity
(POLQ inhibition increases RAD51 foci formation)

Conclusion: PolQ mediates DNA repair by Alt-EJ but also inhibits homologous recombination
PARP1/POLQ Pathway is Upregulated and Essential in HR-Deficient Cells

Wild Type Cells

DSB → End resection → HR → PARP1 → DNA synthesis/ligation → S phase → Accurate genome stability

HR-Deficient Cells

DSB → End resection → Alt-EJ → RAD51 → Toxic RAD51 filaments

HR- and POLQ-Deficient Cells

DSB → End resection → Alt-EJ → PARP1 → POLQ → DNA synthesis/ligation → S phase → Build up of toxic RAD51 intermediates → Inhibition of compensatory DNA repair mechanism

Cell Death
Increased RAD51
Toxic RAD51 filaments
Knockdown of RAD51 rescues the growth of BRCA2(-/-)PolQ(-/-) DKO cells

RAD51 toxicity is the mechanism of Synthetic Lethality in tumors with HR deficiency and POLQ knockdown
Small Molecule Inhibitor Screen To Identify Inhibitors of the ATPase of Polymerase Theta
BRCA1 (-/-) cells generated by CRISPR are hypersensitive to PARP inhibitor
Polθ–ATPase inhibitor selectively kills \textit{BRCA1}-deficient-RPE1 cells but not \textit{BRCA1}-complemented cells

**Classic Colonies on 100 dishes**

<table>
<thead>
<tr>
<th>Polθ inhibitor, µM</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>5</th>
<th>2</th>
<th>5</th>
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<td>RPE1\textunderscore P53\textasciitilde\textunderscore BRCA1\textasciitilde</td>
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</table>

\(\text{POL}\theta\text{ Inhibitor} \)
What are the Mechanisms of PARP Inhibitor Resistance?
What are the known mechanisms of PARPi Resistance? Restoration of HR proficiency

1. Somatic reversion or restoration of ORF
   - Mutant protein → Functional protein
   - cfDNA

2. Epigenetic reversion of BRCA1 promoter hypermethylation
   - Reduced expression → Normal expression
   - Hypomorphic allele

3. Hypomorphic BRCA1 or BRCA2 allele
   - Mutant protein
   - Exon splicing (BRCA1\(^{\Delta 11}\))

References:
New Mechanisms of PARP Inhibitor Resistance: Stabilization for the DNA Replication Fork

ATR inhibition disrupts rewired homologous recombination and fork protection pathways in PARP inhibitor-resistant BRCA-deficient cancer cells

EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation
Nature Cell Biology, 2017 (October 16)
PARPi resistant tumor cells hyperactivate the ATR/CHK1 Pathway to Stabilize their Replication Forks
The PTIP/MRE11 pathway is required for Replication Fork Restart and Normal Replication Velocity

Chaudhuri et al., 2016
Knockdown of the PTIP/MRE11 pathway enhances Replication Fork Stability

Chaudhuri et al., 2016
Low EZH2 is associated with poor survival of patients with BRCA2(-/-) tumors

Ovarian carcinoma TCGA:

- BRCA1/2 wild-type
- BRCA1-mutated
- BRCA2-mutated

Disease Free Survival

Time (months)

ns

ns

P = 0.002

EZH2 low
EZH2 high
Two Parallel Methyltransferase-mediated pathways regulating Replication Fork Restart


Rondinelli et al., 2017, Nature Cell Biology, in press

EZH2 → MUS81

MLL3-4/PTIP → MRE11

Fork Restart

Knockdown of either pathway results in Fork Stabilization and PARP inhibitor resistance
Knockdown or Inhibition of EZH2 induces resistance to PARP inhibitors in BRCA2-deficient cells
EZH2 knockdown or inhibition increases fork stability in BRCA2(-/-) cells

Fork stability:

- IdU 30 min
- CldU 30 min
- HU 4mM 2,5 h

**Fork stability (relative to Scr)**

VU423 (BRCA2(-/-)) + siRNA: Scr, GSK126, EZH2, PTIP

HU: hydroxyurea
EZH2 knockdown or inhibition increases fork stability in BRCA2(-/-) cells

Fork stability:
- IdU 30 min
- CldU 30 min
- HU 4mM 2,5 h

Fork stability (relative to Scr):
- **

VU423 (BRCA2(-/-)) + siRNA:
- Scr
- GSK126
- EZH2
- PTIP

HU: hydroxyurea

No effect on BRCA1 (-/-) cells
EZH2 knockdown or inhibition does not rescue HR repair in BRCA2(-/-) cells
So how does knockdown of EZH2 Stabilize the replication fork and confer PARP inhibitor resistance?
So how does knockdown of EZH2 stabilize the replication fork and confer PARP inhibitor resistance?

Answer: by limiting nuclease digestion of the fork by the nuclease MUS81
Mus81 localization to the Replication Fork is dependent on EZH2 expression
Two Parallel Methyltransferase-mediated pathways regulating Replication Fork Restart

Rondinelli et al., 2017, Nature Cell Biology, in press

EZH2 → MUS81

MLL3-4/PTIP → MRE11

Fork Restart


Knockdown of either pathway results in Fork Stabilization and PARP inhibitor resistance
Summary of the EZH2/MUS81 Pathway:

• EZH2 is overexpressed in rapidly growing solid tumors, contributing to replication fork restart and velocity

• EZH2 localizes to stalled forks and recruits MUS81 in a Histone methylation-dependent manner and enhances fork restart

• BRCA2(-/-) Tumor Cells cannot tolerate MUS81 recruitment and therefore knockdown EZH2 and MUS81

• BRCA2(-/-) Tumor Cells with reduced EZH2 and MUS81 expression grow slowly but are PARPi resistant

PARP inhibitor resistant tumors are rapidly emerging in the clinic.
PARP inhibitor resistant tumors are rapidly emerging in the clinic

So, how do we kill PARP inhibitor resistant tumors?
Ovarian Cancer PDXs for the study of PARPi Resistance and new therapeutic options.

<table>
<thead>
<tr>
<th>PDX ID</th>
<th>subtype</th>
<th>chemotherapy</th>
<th>BRCA germline</th>
<th>PARPi</th>
<th>Rad51_foci</th>
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<td>BRCA1 187del AG</td>
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</table>

IHC for RAD51

No RAD51 Foci

Positive RAD51 Foci

Reduced tumor growth in mice exposed to the combination of CHK1 inhibitor and olaparib
Generation of Ovarian Cancer Organoid Cultures
(Susan F. Smith Center for Women’s Cancers)

1. Harvest tissue/obtain tissue biopsy
2. Dissociate tissue into functional units
3. Enrich for stem cells
4. Niche factors
   - R-spondin
   - WNT3A
   - Retinoic acid
   - GSK3β inhibitors
   - TGF-β inhibitors
5. Culture media
6. ECM factors
   - Collagen
   - Entactin
   - Fibronectin
   - Lamin
7. 7-10 days

Stem cells
Differentiated cells

Spherical/cyst-like
Branching
Budding
Ovarian Tumors from Newly-Diagnosed BRCA1 (+/-) Carriers exhibit tumor heterogeneity.

Fresh Ovarian Tumor Cells on Day 1

Ovarian Tumor Organoids on Day 8

PARP inhibitor Resistance of Primary Ovarian Tumor Organoids

Sarah Hill, M.D., Ph.D.
Ovarian Tumor Organoids strongly resemble the Tumor of Origin

DF-17-39 Rectosigmoid Parent Tumor

DF-17-39 Rectosigmoid Organoid
Analysis of Ovarian Tumor Organoids from a BRCA1 Carrier with Acquired Olaparib Resistance

PARPi Resistant

IC50 for Olaparib

IC50 for CHK1 inhibitor

CHK1i Sensitive

New Clinical Trial: Combination of Olaparib plus CHK1i for High Grade Serous Ovarian Cancer
CHK1 inhibitor causes DNA damage in BRCA1 deficient ovarian tumor organoids

Organoids (DF-17-A39 Recto Sigmoid) were exposed to CHK1i for 14hrs and cell lysates were analyzed by western blots.
BRCA1 mutant PARP resistant HGSC organoids are resistant to olaparib but not other DDR agents.
Ovarian Tumor Organoids Differ in their intrinsic Replication Fork Stability

#103 stable forks  #104 unstable forks
Organoids with Unstable Forks are More Carboplatin Sensitive

#103 stable forks
#104 unstable forks
Rapid Analysis of Short Term Organoids for DNA repair capacity and drug sensitivity

Therapeutic Sensitivity

Rad51 foci formation

Paired WES or targeted sequencing

Fiber Assay: measure of fork stability

CHK1 activity

Prexasertib (nM)

0 40

- pKAP1
- pCHK1 (S345)
- CHK1
- γH2AX
- Vinculin

DF-17-A39 Rectosigmoid nodule

DF-17-A39 Transverse colon nodule

0 0.0005 0.005 0.05 0.5 1

0 20 40 60 80 100

% Untreated

uM Prexasertib
Goal is a CLIA certified organoid test combining functional assays and therapeutic sensitivity for personalized treatment plans.
Treatment for BRCA1/2 Deficient Tumors with de Novo or Acquired PARP Inhibitor Resistance

Agents that inhibit HR

HR proficient

HR deficient

Platinum or PARP Inhibitor

Combinations under preclinical and clinical investigation include PARP Inhibitor + Inhibitor of:

- CDK1 (phosphorylates BRCA1)
- CDK12 (HR gene transcription)
- PI3K
- AKT
- Proteasome
- HDAC (HR gene expression)
- HSP90 (HR protein stability)
- CHK1 (phosphorylates RAD51)
- ATR (upstream in DDR)
- VEGFR (hypoxia reduces BRCA expression)

Monotherapies for PARP Resistant Tumors:
- ATR inhibitor
- CHK1 inhibitor

So, how does one predict whether a breast Cancer will respond to a PARP inhibitor?
Evaluation of Mutational Signatures ("genomic scars")
provides a history of DNA Repair Abnormalities
in a Breast Tumor

A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer

Nature Genetics, 2017
Evaluation of Mutational Signatures ("genomic scars")
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in a Breast Tumor

A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer

Paz Polak1-3,21, Jaegil Kim1,21, Lior Z Braunstein1,4,21, Grace Tiao1, Rosa Karlic5, Daniel Rosebrock1, Dimitri Livitz1, Kirsten Kübler1-3, Kent W Moulw3,6, Nicholas J Haradhvala1,2, Atanas Kamburov1-3, Yosef E Maruvka1-3, Ignaty Leshchiner1, Eric S Lander1,7,8, Todd R Golub1,3,9, Aviad Zick10, Alexandre Orthwein11, Michael S Lawrence1-3, Rajbir N Batra12-14, Carlos Caldas12-14, Daniel A Haber2,3, Peter W Laird15, Hui Shen15, Leif W Ellisen2,3, Alan D’Andrea16,17, Stephen J Chanock18, William D Foulkes19,21 & Gad Getz1-3,20,21

Nature Genetics, 2017

But we need functional tests…..
RAD51 Foci by immunohistochemistry provide a functional test for the status of HR Repair

BRCA2 (+/-) Carrier with Breast Cancer (Patient #1)

Biopsy: 2013

Primary Tumor
Pre-Olaparib
BRCA2(-/-)

Biopsy: 2015

Metastatic Tumor
Post-Olaparib
ctDNA+ for BRCA2 reversion

Restoration of RAD51 Foci following resistance to olaparib and BRCA2 reversion
No evidence of restoration of RAD51 Foci following resistance to cisplatin
SU2C Ovarian Cancer Team

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Karen Lu, M.D.
Overall Conclusions:

- PARP inhibitors kill tumors which have a functional defect in the Fanconi Anemia/BRCA pathway.
- BRCA1/2 deficient tumors become resistant to PARP inhibitors by restoring HR repair and/or by stabilizing the RFs.
- There are multiple mechanisms for PARP resistance. The specific mechanisms may determine the best drug combination.
- Functional studies on growing tumor cells (freshly isolated viable cells or organoids) are required to assess currently-existing DNA repair defects.
- PARP inhibitors have elucidated novel DNA repair pathways.
Acknowledgments

D’Andrea Lab
Lisa Moreau
Raphael Ceccaldi
Kah Suan Lim
Kent Mouw
Beatrice Rondinelli
David Kozono
Prabha Sarangi
Connor Clairmont
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Mu-Yan Cai
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Shawn Johnson
Violeta Serra
Adrienne Waks
Nikhil Wagle
Geoffrey Shapiro

EZH2 Project
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Hatice Yücel
Alexandra Duarte
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Jos Jonkers
Sven Rottenberg

DFCI SU2C OC Dream Team
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