Disclosure

• Consultant/advisory role: Loxo Oncology
DNA Damage and Replication Stress Response Pathways

Double Strand Breaks (DSBs)

Stressed fork

Sensors

Transducers

Effectors

DNA repair
DNA replication
Telomere maintenance
Chromatin structure
Transcription

Cell cycle arrest

Apoptosis Senescence
The checkpoint pathways

**Yeast**
- DSBs, replication stress
  - PIKKs
    - Mec1-Ddc2, Tel1
  - Mediators
    - Rad9
      - Mrc1
        - Tof1
          - Csm3
  - CHKs
    - Chk1, Rad53
  - Effectors
    - Pds1, Cdc20...

**Human**
- DSBs
- DSBs, replication stress
  - ATM
  - ATR-ATRIP
    - Brca1
      - Claspin
    - Timeless
    - Tipin
  - Chk2
  - Chk1
    - p53, Brca1, Nbs1, FANCD2, Cdc25s, RPA...
A model of ATR activation in response to DNA damage and replication stress

Adapted from Zou & Elledge 2003 Science
Is the ATR checkpoint a good target for cancer therapy?
ATR is required for cancer cells to survive genomic instability
ATR is required for cancer cells to survive genomic instability.
Is the ATR checkpoint a good target for cancer therapy?

Inhibition of the ATR checkpoint may be beneficial to therapy in specific contexts.
ATR inhibition could be therapeutically beneficial in specific contexts.
What other cancer-specific vulnerabilities can be targeted by ATR inhibition?
PARP inhibitors selectively kill BRCA1/2-deficient cells

Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy

Hannah Farmer1,2, Nuara McCabe1,2, Christopher J. Lord2, Andrew N. J. Tutt3, Damian A. Johnson1, Tobias B. Richardson3, Manuela Santarosa3, Krystyna J. Dillon1, Ian Hickson4, Charlotte Knights4, Niall M. B. Martin1, Stephen P. Jackson4,5, Graeme C. M. Smith4 & Alan Ashworth1,2

Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase

Helen E. Bryant1, Niklas Schultz1, Huw D. Thomas1, Kayan M. Parker1, Dan Flower1, Elena Lopez1, Suzanne Kyle1, Mark Meuth1, Nicola J. Curtin2 & Thomas Hellday1,2

Nature 2005
How do PARP inhibitors selectively kill BRCA1/2-deficient cells?
FAD-approved PARP inhibitors are used for treatments of BRCA-deficient ovarian cancer

Olaparib (AstraZeneca)
Approved in 2015 for advanced ovarian cancer with BRCA mutations
Approved in 2017 for maintenance therapy of ovarian cancer

Rucaparib (Clovis)
Approved in 2016 for advanced ovarian cancer with BRCA mutations

Niraparib (Tesaro)
Approved in 2017 for maintenance therapy of ovarian cancer with or without BRCA mutations
Resistance to PARPi is a clinical challenge

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Population</th>
<th>BRCA Status</th>
<th>Study Arms</th>
<th>Primary Objective</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Audeh et al.⁸</td>
<td>Recurrent HGSOc, n = 57</td>
<td>Mutated</td>
<td>Olaparib 400 mg twice daily vs 100 mg twice daily</td>
<td>ORR</td>
<td>33% (95% CI, 20-51) olaparib 400 mg vs 13% (95% CI, 4-31) olaparib 100 mg</td>
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<tr>
<td>Kaye et al.⁹</td>
<td>Recurrent platinum-sensitive HGSOc, n = 97</td>
<td>Mutated</td>
<td>Olaparib 200 mg twice daily vs 400 mg twice daily vs PLD (50 mg/m² q 28 day)</td>
<td>PFS</td>
<td>6.5 months vs 8.8 months vs 7.1 months</td>
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<td>No significant difference in PFS</td>
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<tr>
<td>Gelmon et al.¹⁴</td>
<td>Recurrent HGSOc and TNBC, n = 91</td>
<td>Mutated and wild-type</td>
<td>Olaparib 400 mg twice daily</td>
<td>ORR</td>
<td>ORR not achieved in breast cancer cohort</td>
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<td>4.1% (95% CI, 22-64) BRCA1/2 -mutated HGSOc</td>
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<td>24% (95% CI, 14-38) in BRCA wild-type</td>
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<td>Liu et al.¹⁵</td>
<td>Recurrent platinum-sensitive HGSOc, n = 90</td>
<td>Mutated, wild-type, or unknown</td>
<td>Olaparib 400 mg twice daily vs olaparib 200 mg twice daily plus cediranib 30 mg daily</td>
<td>PFS</td>
<td>9.0 months vs 17.7 months (HR = 0.42; 95% CI, 0.23-0.76; P = .005)</td>
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<tr>
<td>Oza et al.¹⁷</td>
<td>Recurrent platinum-sensitive HGSOc, n = 162</td>
<td>Mutated, wild-type, or unknown</td>
<td>Olaparib 200 mg twice daily (days 1-10), paclitaxel (175 mg/m², day 1) and carboplatin (AUC 4 mg/mL per minute, day 1); then olaparib at 400 mg twice daily until disease progression vs paclitaxel (175 mg/m²) and carboplatin (AUC 6 mg/mL/minute)</td>
<td>PFS</td>
<td>12.2 months vs 9.6 months (HR = 0.51; 95% CI, 0.34-0.77; P = .0012)</td>
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<td>No OS difference</td>
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<tr>
<td>Ledermann et al.²¹</td>
<td>Recurrent platinum-sensitive HGSOc, n = 265</td>
<td>Mutated, wild-type, or unknown</td>
<td>Olaparib 400 mg twice daily following completion of platinum-based chemotherapy vs placebo</td>
<td>PFS</td>
<td>8.4 months vs 4.8 months (HR = 0.35; 95% CI, 0.25-0.49; P &lt; .001)</td>
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<td>No OS difference</td>
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AUC indicates area under the curve; HGSOc, high-grade serous ovarian cancer; HR, hazard ratio; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PLD, pegylated liposomal doxorubicin; TNBC, triple-negative breast cancer.
Can we overcome the PARPi resistance in BRCA-deficient cells?
Functions of BRCA1/2 in the DNA damage response

Homologous recombination (HR)

Protection of stalled replication forks

Schlacher et al. 2011 Cell
Many ways to acquire PARPi resistance

Loss of drug or drug targets
- Restoration of BRCA1/2 reading frames
- Loss of PARP1
- Up regulation of efflux pump

Restoration of HR
- Loss of 53BP1, RIF1, REV7, Artemis (increased resection)
- Loss of KU (NHEJ)

Restoration of fork protection
- Loss of PTIP, MLL3/4, CHD4
- Loss of PARP1
- Overexpression of RADX
Do PARPi-resistant BRCA-deficient cancer cells have a common vulnerability that can be targeted?
Development of BRCA1-deficient cell lines that are resistant to PARPi
PARPi resistance is not caused by loss of PARP1 or up regulation of efflux pump
PARPi resistance is not caused by restoration of BRCA1
Multiple proteins implicated in PARPi resistance are altered in PARPi-resistant cell lines

- Restore HR?
- Restore fork protection?
The ATR checkpoint pathway is transcriptionally up regulated in PARPi-resistant lines
ATRi preferentially kills PARPi-resistant BRCA1-deficient cells
ATRi and PARPi are more synergistic in PARPi-resistant BRCA1-deficient cells than in BRCA1-proficient cells.
ATRi broadly overcomes PARPi resistance in BRCA1-deficient cancer cell lines
ATRi prevents the emergence of PARPi resistance in BRCA1-deficient cancer cells
How does ATRi overcome the PARPi resistance in BRCA1-deficient cancer cells?
Rad51 focus formation is partially restored in some but not all PARPi-resistant BRCA1-deficient cells

Homologous recombination (HR)

The activity to form Rad51 foci is either maintained or partially restored in PARPi-resistant cells
PARPi-resistant cells partially bypass BRCA2 but not PALB2 and BRCA2 for Rad51 focus formation.

BRCA1-deficient cancer cells partially bypass BRCA1 but not PALB2.

PARPi-resistant, BRCA1-deficient cancer cells remain dependent on PALB2 and BRCA2.
PARPi-resistant cells rely on PAL2 and BRCA2 for survival in PARPi
ATRi blocks Rad51 focus formation when BRCA1 is bypassed by 53BP1 loss

ATR is required for HR even when BRCA1 is bypassed
ATRi blocks Rad51 focus formation in PARPi-resistant, BRCA1-deficient cancer cells.

ATR is required for the residual HR in PARPi-resistant, BRCA1-deficient cancer cells.
ATRi blocks BRCA2 localization to DSBs in PARPi-resistant BRCA1-deficient cancer cells
ATR is required for BRCA1-independent recruitment of PALB2 and BRCA2

BRCA1-deficient cancer cells (PARPi sensitive or resistant)

Partial bypass of BRCA1

PALB2 and BRCA2 remain indispensable

ATR is required for PALB2-BRCA2 recruitment

The residual HR activity is ATR-dependent and required for the resistant cells to survive in PARPi
Rad51 focus formation is partially restored in some but not all PARPi-resistant BRCA1-deficient cells

Restoration of HR is not an obligated requirement for PARPi resistance?
Is the function of BRCA1 in fork protection restored in PARPi-resistant cell lines?

The residual HR activity in PARPi-resistant cells is necessary but not sufficient for resistance.

What else is driving PARPi resistance?
DNA fiber assay to monitor degradation of stalled replication forks

Sequential labeling of newly synthesized DNA

Stalled forks are protected (BRCA1/2-deficient cells)

Stalled forks are unprotected (BRCA1/2-deficient cells)

CIdU  IdU

HU

Schlacher et al. 2011 Cell
PARPi-resistant cells regain the protection of stalled forks in the absence of BRCA1
ATRi reactivates Mre11-mediated fork degradation in PARPi-resistant cells
Stable association of Rad51 with stalled forks is required for protection against Mre11

Resection
-Mre11
-Exo1?
-Dna2?
-BLM/WRN?

Rad51

ATR?
ATRi blocks the stable association of Rad51 with chromatin and stalled forks
ATRi blocks the stable association of Rad51 with chromatin and stalled forks
ATRi blocks the stable association of Rad51 with chromatin and stalled forks
ATRi reactivates fork degradation in PARPi-resistant BRCA2-deficient cancer cells
ATRi overcomes PARPi resistance by blocking BRCA1-independent Rad51 loading at DSBs and stalled forks.
Why are PARPi-resistant BRCA-deficient cells more sensitive to ATRi than BRCA-proficient cells?

- Efficiency of Rad51 loading/stabilization

<table>
<thead>
<tr>
<th></th>
<th>high PARPi sensitivity</th>
<th>low PARPi sensitivity</th>
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<tbody>
<tr>
<td>BRCA-proficient</td>
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<td>BRCA-deficient, PARPi-sensitive (e.g. UWB1)</td>
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<td>BRCA-deficient, acquired PARPi resistance</td>
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</table>

A threshold for significant PARPi sensitivity
What other cancer-specific vulnerabilities can be targeted by ATR inhibition?
Cells under high replication stress are sensitive to ATR inhibition

ATR Prohibits Replication Catastrophe by Preventing Global Exhaustion of RPA

Distinct but Concerted Roles of ATR, DNA-PK, and Chk1 in Countering Replication Stress during S Phase
What causes replication stress in cancer cells?
APOBEC Family of Cytosine Deaminases

Holmes et al. 2007 TIBS

Swanton et al. 2015 Cancer Discovery
APOBEC-Signature Mutations Are Prevalent in a Subset of Cancers

Mutational Processes Molding the Genomes of 21 Breast Cancers

LETTER

APOBEC3B is an enzymatic source of mutation in breast cancer

Breast, Bladder, Head & Neck, Lung Cancers...
APOBEC3A/B are Mutation Drivers in Multiple Cancer Types

An APOBEC3A hypermutation signature is distinguishable from the signature of background mutagenesis by APOBEC3B in human cancers

Kin Chan¹, Steven A Roberts¹,², Leszek J Klimczak³, Joan F Sterling¹, Natalie Saini¹, Ewa P Malc⁴, Jaegil Kim⁵, David J Kwiatkowski⁵,⁶, David C Fargo³, Piotr A Mieczkowski⁴, Gad Getz⁵,⁷ & Dmitry A Gordenin¹
Mutational Strand Asymmetries in Cancer Genomes Reveal Mechanisms of DNA Damage and Repair


APOBEC-induced mutations in human cancers are strongly enriched on the lagging DNA strand during replication

Vladimir B. Seplyarskiy, Ruslan A. Soldatov, Konstantin Y. Popadin, Stylianos E. Antonarakis, Georgii A. Bazykin, and Sergey I. Nikolaev

APOBEC3A and APOBEC3B Preferentially Deaminate the Lagging Strand Template during DNA Replication

James I. Hoopes, Luis M. Cortez, Tony M. Mertz, Ewa P. Malc, Piotr A. Mieczkowski, and Steven A. Roberts
APOBEC3A/B May Act During DNA Replication

Haradhvala et al. Cell 2016
Do APOBEC3A/B induce DNA replication stress?
Inducible Expression of APOBEC3A Activates ATR But Not ATM
The Activation of ATR by APOBEC3A Is Dependent on UNG2

C

APOBEC3A

U

UNG2

Abasic site (AP site)
APOBEC3A Expressing Cells are Sensitive to ATRi
APOBEC3A Expressing Cells are **Uniquely** Sensitive to ATRi
APOBEC3A Expressing Cells are **Uniquely** Sensitive to ATRi

![Graphs showing cell survival vs. concentration of ATRi, ATMi, and DNA-PKi](images)
Why is APOBEC-induced replication stress unique?

Why are APOBEC-expressing cells sensitive to ATRi?
ATRi induces DSBs during DNA replication in APOBEC3A expressing cells
ATRi induces replication catastrophe in APOBEC3A expressing cells

ssDNA

Replication catastrophe
ATR Counteracts APOBEC-induced Replication Stress
ATR Counteracts APOBEC-induced Replication Stress

APOBEC3A → ATR → ssDNA → Replication catastrophe
ATR Counteracts APOBEC-induced Replication Stress

What is driving ssDNA accumulation and replication catastrophe after ATRi treatment?
UNG2 is required for the ATRi-induced replication catastrophe in APOBEC3A expressing cells.
ATR inhibition leads to accumulation of AP sites at replication forks in A3A expressing cells
AP sites may impede DNA polymerases and lead to ssDNA accumulation

Zhao et al. 2004 NAR
A ssDNA and APOBEC driven feed-forward loop that generates AP sites and ssDNA
A ssDNA and APOBEC driven feed-forward loop that generates AP sites and ssDNA
Is the endogenous APOBEC activity in cancer cells sufficient to induce replication stress?
An in vitro assay to measure APOBEC3A/B activity in cancer cells

Burns et al. 2013 Nature
APOBEC3A/B activity varies in different cancer cell lines
APOBEC3A/B-dependent basal Chk1 phosphorylation in cancer cells
Endogenous APOBEC activity in cancer cells is sufficient to render cells susceptible to ATRi
The unique replication stress imposed by APOBECs renders cancer cells susceptible to ATR inhibition.
The unique replication stress imposed by APOBECs renders cancer cells susceptible to ATR inhibition.
Thanks to...

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