Extending the utility of PARP inhibitors

Gordon Mills
Knight Cancer Institute
POTENTIAL CONFLICT OF INTEREST DISCLOSURES

• Financial Relationships
  - SAB/Consultant: AstraZeneca, Catena Pharmaceuticals, Critical Outcome Technologies, ImmunoMET, Ionis, Medimmune, Nuevolution, Pfizer, Precision Medicine, Signalchem Lifesciences, Symphogen, Takeda/Millennium Pharmaceuticals, Tarveda,
  - Stock/ Options/Financial: Catena Pharmaceuticals, ImmunoMet, SignalChem, Spindle Top Ventures, Tarveda
  - Licensed Technology: HRD assay to Myriad Genetics
  - Sponsored Research: Abbvie, Adelson Medical Research Foundation, AstraZeneca, Breast Cancer Research Foundation, Critical Outcomes Technology, Illumina, Ionis, Immunomet, Karus Therapeutics, Komen Research Foundation, Pfizer, Nanostring, Takeda/Millennium Pharmaceuticals, Tesaro

I will discuss off label use and/or investigational use of drugs
ADP ribosylation required for PARP to leave DNA
Trapped PARP creates “toxic” double strand breaks
Can PARP activity be extended beyond HRD
PARP inhibitor responses are transient
Ariel 2 Rucaparib Ian McNeish Lancet:
LOH high is HRD assay performed by Foundation Med

Conclusion: Germline BRCA1/2 is strongest predictor of benefit
HRD positivity identifies an additional population with significant benefit
A population of patients without HRD show modest benefit
Categorizing Predictive Biomarkers of Response for PARP inhibitors

**PARPness**
- Deleterious gene variants or RNA/protein expression differences (e.g., SLFN11, E-Cadherin) not directly related to HRR deficiency that still engender PARP sensitivity.

**HRDness**
- Increased genomic instability and reliance on error-prone DDR
- Loss of HRR efficiency

**BRCAness**
- Deleterious variants or post-translational loss of non-BRCA DDR genes (e.g., \textit{ATM}), or select non-DDR genes (e.g., \textit{ARID1A}); Hypoxia; Oncometabolites (e.g., 2-hydroxyglutarate).

**Molecular phenocopy of tumors with BRCA1/2 deleterious mutations.** Can arise from epigenetic or post-translational loss of BRCA, or through mutations/expression changes in other genes that impact HRR through the BRCA pathway.

**BRCA1/2 mutations**

- Recurrent Platin Sensitivity Bowtell
Subpopulations of tumors are HRD
Cut off 42 (Myriad) and 31 (based on BRCA1/2)
**Classes of PARP inhibitor resistance**

**PARP Inhibitor Resistance**

- **Restoration of HR Activity**
  - DIRECT
    - Reversion-to-WT mutations in HR genes and hypomorphic mutants (e.g. BRCA1/2, RAD51)
  - INDIRECT
    - Promoter demethylation of HR genes

- **Mitigation of Replication Stress**
  - Fork Stability
    - Decreased proliferation with increased fork protections via mechanisms such as EZH2 loss, MLL3/4 loss, and/or increased dependence on ATR/CHK1.
  - Cell Cycle Control
    - Decreased proliferation with increased fork protections via mechanisms such as EZH2 loss, MLL3/4 loss, and/or increased dependence on ATR/CHK1.

- **Resistance by Multiple Mechanisms**
  - Mechanisms inherent to PARP, such as mutations in catalytic or drug binding domain.
  - Mechanisms that hinder PARylation and release PARP from DNA, such as PARG-mediated.
  - P glycoprotein /MDR/ABC drug efflux transporters.
  - Biomarkers of resistance of unknown mechanism include loss of SLFN11 and loss of EMT signature.

**Reconstitution of Rad51 foci**
- Healing of BRCA1/2, PALB2, Rad51C, Rad51D
- Demethylation of BRCA1/2 promoter
- Upregulated hypomorphic mutant BRCA1/2 alleles
- Loss of shield complex: 53BP1, RIF1, Rev7 (MAD2L2), FAM35A and C20orf196 complex

**Loss of MLL3/4 (PTIP and MUS81 effectors)**
- Loss of EZH2 (MRE11 nuclease effector), Protects BRCA2 and not BRCA1
- Decreased proliferation BRCA2 and Rad51 but not BRCA1 play a role in replication fork protection

**PARP loss**
- PARP mutations:
  - PARG reverses ADP ribosylation of PARP and releases PARP from DNA
  - P glycoprotein/MDR/ABC transporters overexpression and fusions
  - SLFN11 loss
  - EMT
Rational combinatorial therapy will be required to fulfill the promise of targeted therapy

Systems are robust to individual perturbations but are susceptible to multiple perturbations Yossi Yarden and Arthur Lander

Mathematical modeling indicates that by chance during phylogeny many/most molecules in cell/organism will be blocked by mutation or environmental stress

Thus response to single targeted therapy is expected to be short and transient as observed!
Rational combinatorial therapy will be required to fulfill the promise of targeted therapy

Systems are robust to individual perturbations but are susceptible to multiple perturbations Yossi Yarden and Arthur Lander

Cells adapt by using an alternative pathway

Chance that both the original target and the adaptive response will be “hit” randomly (mutation or environmental stress) is vanishingly low

Adaptation can occur at the protein level which is best assessed by post translational modification
Rational combinatorial therapy will be required to fulfill the promise of targeted therapy

Systems are robust to individual perturbations but are susceptible to multiple perturbations. Yossi Yarden and Arthur Lander

Rational drug combinations will be required to convert transient responses into durable responses
A PLATFORM TO FACILITATE TARGETING ADAPTIVE RESISTANCE TO INCREASE UTILITY OF TARGETED THERAPEUTICS

Cells in 2D, 3D, in vivo, or patient tumors

Add drug

Early time points: target engagement
Medium time points: adaptive responses
Late time points: genomic resistance

Harvest cells for Omic analysis
DNA, RNA, protein, metabolomics
HUMAN PROTEOMICS ATLAS: RPPA

Quantitative high throughput multiplexed inexpensive ELISA

416 validated antibodies

Dot blot: less sensitive to degradation

Requires high quality validated antibodies and robotics

No Spatial orientation: combined tumor and stromal signature

Tcpaportal.org
Search Cancer Proteome Atlas

TCGA and internal patient samples (>10,000) with extensive DNA, RNA, miRNA, and clinical data

Cell lines with RNASeq and drug data
1200 cell lines

Broad Cancer Cell Line Encyclopedia
144,000 samples in total
Rank-Sum Analysis of AZD2281 and BMN673

5 representative cell lines were treated with 2 doses for 72 and 96 hours in 2D and 3D cultures. Lysates were collected and analyzed by RPPA for 191 antibodies. High levels are represented in Red. >50,000 data points.

Data is ratio of treated to untreated
Samples are ordered based on adding all antibody scores
Only significant changes presented

Yiling Lu Xiaohua Chen
Rank-Sum Analysis of AZD2281 and BMN673

5 representative cell lines were treated with 2 doses for 72 and 96 hours in 2D and 3D cultures. Lysates were collected and analyzed by RPPA for 191 antibodies. High levels are represented in Red.

Yiling Lu Xiaohua Chen
OCTOPUS – PARP/PI3K pathway combinations

Shannon Westin

SU2C: Olaparib and BKM120: Olaparib and BYL719
30-35% RR for OC: Not dependent on BRCA1/2 status
(Lotus AND PAKT AKTi and taxol)
OCTOPUS – PARP/PI3K pathway combinations

110 patients accrued
RR ~ 30% for OC, 50% for EC for AZD5363
Prolonged responses over 2 years
Rank-Sum Analysis of AZD2281 and BMN673

5 representative cell lines were treated with 2 doses for 72 and 96 hours in 2D and 3D cultures. Lysates were collected and analyzed by RPPA for 191 antibodies. High levels are represented in Red.
RAS pathway activation induces replication stress

RAS pathway activation increases HR

RAS pathway activation is indicative of PARP resistance

PARP resistant cells acquire RAS mutations and increased signaling

Inhibiting MEK or ERK increases PARP activity in RAS mutant or PARP resistant cell lines

Chaoyang Sun
Dong Zhang
Yong Fang

KRAS
OVCAR8

KRAS HPDE
Pancreas
SOLAR study: selumetinib and olaparib in RAS activated tumors

Original observation 4/8/2015
CRC Approved, IRB 3/1/17
FDA no Objection
SIV May 30 2017
First in human Nov 2017

DOSE EXPANSION
N=60

Endometrial Tumors with RAS Pathway Activation
N=15

Ovarian Tumors with RAS Pathway Activation
N=15

Ovarian Tumors with Progression on Prior PARP Inhibitor Treatment
N=15

Solid Tumors with RAS Pathway Activation
N=15

Shannon Westin
Funda Meric-Bernstam
Rational Strategy for Combination Therapies

Blocking critical signaling nodes “rewires” signaling pathways

Rewired networks contribute to cellular resistance to targeted therapeutics

Induced signaling events represent “vulnerabilities” that can be exploited leading to synthetic lethality

Adaptive responses can be restricted to specific tumor subpopulations

Combinatorial Adaptive Resistance Therapy CART
Combinations with PARPi

- PI3K/AKT/mTOR inhibitors
- MEK ERK inhibitors
- DNA damage checkpoint inhibitors
- Immune checkpoint inhibitors
- BET inhibitors
- Anti-apoptotic inhibitors
- Angiogenesis inhibitors
- HSP90 inhibitors
- HDAC inhibitors
- Azacytidine
- HER2 inhibitors
- Chemotherapy/radiation to induce double strand breaks
Adaptive responses to PARP inhibitors could be used to select rational combinations.

**PI3K pathway**
- DNA damage repair checkpoint
- RAS/MAPK
- Apoptotic pathway
- STING/Immune
- EMT
- Predictor of response
REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem

Limited systems information

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA
REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem

Adaptation to Therapeutic stress

PARPi

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Perturbed Tumor Ecosystem

PARPi

Limited systems information

Assess Adaptation to PARPi

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REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Target the adaptive response PD1/PDL1

Resting Tumor Ecosystem

Perturbed Tumor Ecosystem

Assess Adaptation to PARPi

Immune cell infiltration

PARPi

Limited systems information

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

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Combination selected based on tumor evolution

Adaptation to Therapeutic stress
Adaptation to Therapeutic stress

Resting Tumor Ecosystem

Perturbed Tumor Ecosystem

PARPi

Immune cell infiltration

PI3K pathway

mTOR/AKT

Target the adaptive response

PD1/PDL1

Assess Adaptation to PARPi

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Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

Combination selected based on tumor evolution

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

Target the adaptive response

PD1/PDL1
REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

- **Resting Tumor Ecosystem**
  - Limited systems information
  - Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

- **Perturbed Tumor Ecosystem**
  - Adaptation to Therapeutic stress
  - PARPi

- **Assess Adaptation to PARPi**
  - Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

- **Immune cell infiltration**
  - PD1/PDL1

- **PI3K pathway**
  - mTOR/AKT

- **RAS/MEK pathway**
  - MEK/MAPK

- **Combination selected based on tumor evolution**

Target the adaptive response
REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem → Adaptation to Therapeutic stress → PARPi → Limited systems information

Perturbed Tumor Ecosystem → Immune cell infiltration → PI3K pathway → Target the adaptive response PD1/PDL1

Assess Adaptation to PARPi → PI3K pathway → mTOR/AKT

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

DNA damage checkpoint → RAS/MEK pathway → MEK/MAPK

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

Combination selected based on tumor evolution → Wee1/ATR
REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem

Adaptation to Therapeutic stress

Perturbed Tumor Ecosystem

Immune cell infiltration

Assess Adaptation to PARPi

PI3K pathway

DNA damage checkpoint

Reconstituted HR

Apoptotic balance

Combination selected based on tumor evolution

Limited systems information

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

Target the adaptive response

PD1/PDL1

mTOR/AKT

MEK/MAPK

Wee1/ATR

BRD4

BCL2/MCL1
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