A Pathway-Based Approach to Targeted Therapy in Cancer: Rationalizing Outcomes with Molecular Pharmacology

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Clinical Assistant Professor,
University of Florida College of Pharmacy

Faculty Disclosure

• Nathan Seligson declares an existence of a financial interest in any amount related to the content of this activity.
  • Project funding: GlaxoSmithKline
  • Open data collaboration: Foundation Medicine Inc.

• Advisory Board members and other individuals, not previously disclosed, who may review, propose recommendations, and/or edit the content of PharmCon CE activities declare no existence of a financial interest in any amount related to the content of this activity.
Learning Objectives

At the conclusion of this activity, participants should be better able to:

1. Define the action of molecular pathways in oncology
2. Recognize common oncogenic pathways and their regulatory consequences
3. Connect common therapeutic targets with upstream and downstream regulators
4. Integrate molecular system connections with rational clinical outcomes

Overview

- Cancer as a disease of dysregulated pathways
  - Review of cancer biology
  - Incorporating clinical pathway based pharmacology
  - Differentiating agents by pharmacologic properties

- A pathway based approach
  - Poly (ADP-ribose) polymerase [PARP] inhibitors
  - Cyclin-dependent kinase 4/6 [CDK4/6] inhibitors
### Purpose Of This Lecture

<table>
<thead>
<tr>
<th>What We Will Cover</th>
<th>What We Will NOT Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>• How to think about cancer as a disease of dysregulated pathways</td>
<td>• Which patients should receive what drug</td>
</tr>
<tr>
<td>• How pathways can be applied to clinical practice</td>
<td>• What drugs are “better” than others</td>
</tr>
</tbody>
</table>

Not intended to be a comprehensive review of clinical genomic-pharmacology
Cancer As A Disease Of Dysregulated Pathways

- Review of cancer biology
- Incorporating clinical pathway based pharmacology
- Differentiating agents by pharmacologic properties

Cancer Has a Single Intent

Development of Cancer Requires Multiple Biologic Changes

Balancing Act of Oncogenic and Suppressive Signals
Cellular Signaling Pathways


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Cellular Signaling Pathways

“Up” And “Down” Stream
Good, Better, Best?

Good, Better, Best?

mTOR inhibitor

mTOR

mTOR inhibitor

mTOR

© 2020 PharmCon

Good, Better, Best?

mTOR inhibitor

mTOR

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Precision Medicine

Give the right drug to the right patient at the right dose at the right time

Incorporating Clinical Pathway Based Pharmacology

Rational Understanding of:

- Adverse Events
- Predictive Biomarkers

Optimize therapy selection

- Identify ideal combination therapy
- Review off-label, targeted therapy strategy
Pathway-Based Pharmacology

**BRAF/MEK Inhibition**

- Understanding upstream and downstream regulators
  - Predictive of response or resistance
- Understanding combination therapy
- Off-label therapy selection


---

**VEGF Inhibition**

- Known/unknown off-target effects
- Predicting adverse events
- Optimal therapy selection

Differentiating Agents by Pharmacologic Properties

Distinct intra-class biochemical properties define their clinical action

Combining genomic and pharmacology opens opportunity for better understanding of drugs

BRAF Inhibitors And Cutaneous Squamous Cell Carcinoma

- BRAF inhibitors induce a “paradoxical ERK activation” in BRAF wild-type cells
  - ~82% of paradoxical oncogenesis
- Results in variable rates of cutaneous squamous cell carcinoma (cuSCC)

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BRAF Inhibitors And Cutaneous Squamous Cell Carcinoma

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### BRAF Inhibitors and Cutaneous Squamous Cell Carcinoma

<table>
<thead>
<tr>
<th>BRAF Inhibitor</th>
<th>Clinical Induction Rate (%)</th>
<th>Paradox Index (EC&lt;sub&gt;50&lt;/sub&gt;/IC&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>ERK induction/Melanoma Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vemurafenib</td>
<td>22</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Dabrafenib</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Encorafenib</td>
<td>3.7</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>PLX8394</td>
<td>- (In development)</td>
<td>- (No ERK Induction)</td>
<td></td>
</tr>
</tbody>
</table>

#### Paradox Index

#### Cell Growth of BRAF wild-type cells

![Graph showing cell growth inhibition](image)
### Summary – Cancer as a Disease of Dysregulated Pathways

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<th>Understanding pathways</th>
<th>Full understanding of mechanism of action</th>
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<td></td>
<td>Rational for predictive biomarkers</td>
</tr>
<tr>
<td></td>
<td>Predicting adverse events within class</td>
</tr>
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</table>

### Poly (ADP-ribose) polymerase Inhibitors

- Review of DNA repair pathways
- Genomic predictors of response
- Differentiating agents by pharmacologic properties
Poly (ADP-ribose) polymerase Inhibitors

- Review of DNA repair pathways
- Genomic predictors of response
- Differentiating agents by pharmacologic properties

Review of DNA Repair Pathways

Oxidative respiration
Replication
UV exposure

Ionizing radiation
Chemotherapy

DNA damage
Review of DNA Repair Pathways

DNA damage

- Oxidative respiration
- Replication
- UV exposure
- Ionizing radiation
- Chemotherapy

Single-strand breaks (SSB)

- Base excision repair (BER)
- Nucleotide excision repair (NER)
- Mismatch repair (MMR)
Review of DNA Repair Pathways

Oxidative respiration
Replication
UV exposure

DNA damage

Ionizing radiation
Chemotherapy

Single-strand breaks (SSB)

Double-strand breaks (DSB)

• Base excision repair (BER)
• Nucleotide excision repair (NER)
• Mismatch repair (MMR)

Ionizing radiation
Chemotherapy

Oxidative respiration
Replication
UV exposure

DNA damage

Single-strand breaks (SSB)

Double-strand breaks (DSB)

• Base excision repair (BER)
• Nucleotide excision repair (NER)
• Mismatch repair (MMR)

• Homologous recombination (HR)
• Non-homologous end joining (NHEJ)
Review of DNA Repair Pathways

DNA damage
- Oxidative respiration
- Replication
- UV exposure
- Ionizing radiation
- Chemotherapy

Single-strand breaks (SSB)
- Base excision repair (BER)
- Nucleotide excision repair (NER)
- Mismatch repair (MMR)

Double-strand breaks (DSB)
- Homologous recombination (HR)
- Non-homologous end joining (NHEJ)

Left unrepaired

Oxidative respiration
Replication
UV exposure
Ionizing radiation
Chemotherapy

Single-strand Break Repair

Base excision repair (BER)
- Removal/replacement of single base residue
- Damage by: reactive oxygen species, hydrolytic reactions, and methylation
- PARP dependent

Nucleotide excision repair (NER)
- Repairs pyrimidine dimers and bulky lesions
- Damage by: UV radiation and platinum agents

Mismatch repair (MMR)
- Removes mismatches and small insertion/deletion loops
- Damage by: faulty replication

Double-strand Break Repair

Homologous recombination (HR)
- High fidelity DSB DNA repair
- Utilizes the sister chromatid as template (homologous)
- BRCA1/2 dependent

Non-homologous end joining (NHEJ)
- Low fidelity DSB DNA repair
- No template (non-homologous)

PARP-1’s Role in Homologous Recombination Repair
- PARP1 binds damaged DNA
- Produce linear branched PAR chains
- Recruits BER proteins to reseal SSBs

What Is So Important About the Homologous Recombination Pathway?

A Normal Cells
Base-excision repair
PARP1
BRCA
Homologous recombination
Repair

B Cells with BRCA Mutation
Base-excision repair
PARP1
BRCA
Homologous recombination
Repair

Normal Cellular Stress

<table>
<thead>
<tr>
<th>PARP1</th>
<th>HR Pathway</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>Cell Survives</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
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</tr>
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47

48
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<td>→ Cell Survives</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>→ Cell Survives</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>→ Cell Survives</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>→ Cell Death</td>
<td></td>
</tr>
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</table>

Referred To As Synthetic Lethality
Poly (ADP-ribose) polymerase Inhibitors

- Review of DNA repair pathways
- Genomic predictors of response
- Differentiating agents by pharmacologic properties

Overview of Homologous Recombination Repair

A. Double-strand DNA break – recognition and assembly of repair proteins
   - ATM, ATM: Kinase recognizes double-strand DNA break
   - CHEK2, BRCA1, HJX: Phosphorylated
   - BRCA1 acts as scaffold, organizes repair proteins
   - RAD51, BRF1: Interact with BRCA1

B. End Resection
   - Mre11, RAD51, NBS1: MRN complex excises DNA

C. RAD51 loading
   - BRF, BRF: Bound 3' overhang of single-stranded DNA
   - BRCA2, LEDD1, RAD51, PALB2: onto RPA-coated DNA

D. Strand invasion – RAD51 nucleoprotein filament invades homologous DNA

High fidelity DSB repair
70-150 related proteins
BRCA1/2 central to function
Genomic variants = dysfunctional HR
Genomic Markers of PARP Inhibitor Sensitivity

- Many genes being investigated
- Most evidence:
  - BRCA1, BRCA2
- Emerging evidence:
  - ATR, BRIP1, BAP1, PALB2, RAD51 (family)
- Genomic phenotypes
  - Loss of heterozygosity (LOH)
  - Homologous recombination deficiency (HRD)
- Clinical phenotypes
  - Platinum response

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1, BRCA2</td>
<td>Breast and ovarian cancer susceptibility genes</td>
</tr>
<tr>
<td>ATR</td>
<td>Homologous recombination deficiency</td>
</tr>
<tr>
<td>BRIP1, BAP1, PALB2, RAD51</td>
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- Clinical phenotypes
  - Platinum response

PARP Inhibitors for Combination Therapy

- Typically do not rely on synthetic lethality
- Still in development
- High adverse event rate

Translational impact

- Monotherapy
  - Synthetic lethality with defects in homologous recombination
- Combination therapy
  - Potentiation of chemotherapy or radiation therapy

Cancer therapy

- DNA repair
  - Single-stranded repair
  - Double-stranded repair
- Translational impact
  - Representative clinical trials

1. NCT00516733 (all tumors) (Feng et al., 2009)
2. NCT00664234 (BRCA1-mutant breast cancer) (Tuntel et al., 2010)
3. NCT00753455 (ovarian cancer) (Ledermann J et al., 2012)

Typically do not rely on synthetic lethality

- Monotherapy
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Cancer therapy

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Poly (ADP-ribose) polymerase Inhibitors

• Review of DNA repair pathways
• Genomic predictors of response
• Differentiating agents by pharmacologic properties

Mechanisms of PARP Inhibition

• Two mechanisms of action:
  • Catalytic inhibitors of PARP
  • Trapping PARP at damaged DNA sites
• Trapped PARP–DNA complexes more cytotoxic than unrepaired SSBs caused by PARP inactivation

1. What PARP inhibitors would you expect to be most potent?
2. What PARP inhibitors would you pair with cytotoxic combination therapy?
### Comparative Toxicity Profiles of PARP Inhibitors

<table>
<thead>
<tr>
<th>Drug type</th>
<th>Rucaparib</th>
<th>Veliparib</th>
<th>Niraparib</th>
<th>Olaparib</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (95% CI)</td>
<td>RR (95% CI)</td>
<td>RR (95% CI)</td>
<td>RR (95% CI)</td>
<td>RR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Any type</td>
<td>3.79 (2.66-5.40)</td>
<td>2.1 (1.12-3.90)</td>
<td>3.50 (2.89-4.24)</td>
<td>1.40 (1.24-1.58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Anemia</td>
<td>3.56 (4.98-254.04)</td>
<td>1.76 (1.43-2.16)</td>
<td>27.04 (10.71-68.26)</td>
<td>15.00 (7.18-31.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>20.91 (1.26-348.18)</td>
<td>5.70 (3.76-8.64)</td>
<td>94.48 (23.35-382.54)</td>
<td>1.63 (0.53-5.04)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>3.74 (1.58-9.79)</td>
<td>83 (1.25-2.14)</td>
<td>33 (0.69-20.76)</td>
<td>1.96 (1.20-3.29)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

RRs, risk ratios; AEs, adverse events.
*Difference in the RR of different PARP inhibitors.
†RR of thrombocytopenia caused by Olaparib treatment was not significant.

---

### Table 3 Grade 3–4 toxicity profiles of PARP inhibitors

<table>
<thead>
<tr>
<th>Grade 3–4 adverse events</th>
<th>Olaparib (n=299)</th>
<th>Talazoparib (n=71)</th>
<th>Rucaparib (n=204)</th>
<th>Veliparib (n=50)</th>
<th>Niraparib (n=367)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>0%</td>
<td>0%</td>
<td>4%</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2%</td>
<td>Not reported</td>
<td>2%</td>
<td>0%</td>
<td>2%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6%</td>
<td>Not reported</td>
<td>9%</td>
<td>Not reported</td>
<td>8%</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6%</td>
<td>Not reported</td>
<td>2%</td>
<td>Not reported</td>
<td>1%</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Not reported</td>
<td>18%</td>
<td>2%</td>
<td>2%</td>
<td>34%</td>
</tr>
<tr>
<td>Anemia</td>
<td>17%</td>
<td>23%</td>
<td>22%</td>
<td>0%</td>
<td>23%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>Not reported</td>
<td>10%</td>
<td>7%</td>
<td>2%</td>
<td>20%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>8%</td>
</tr>
<tr>
<td>Increased AST/ALT</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>8%</td>
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**Abbreviations:** AST, aspartate transaminase; ALT, alanine aminotransferase; PARP, poly(ADP-ribose) polymerase.

* Robson. NEJM. 2017.
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Olaparib vs Niraparib - Tumor Exposure

- Tumor exposure of niraparib is higher than plasma exposure
  - 3.3 times greater than plasma exposure

**Olaparib vs Niraparib - Tumor Exposure**

- Tumor exposure of niraparib is higher than plasma exposure
  - 3.3 times greater than plasma exposure
- At MTD, induces more potent tumor growth inhibition than olaparib in some BRCA wild-type tumor models

---

**Bone Marrow Exposure**

- Similar potency to CD34+ cells
  - niraparib IC$_{50} = 1.2$ μM
  - olaparib IC$_{50} = 1.6$ μM
- Volume of distribution
  - niraparib ~1,220L
  - olaparib ~170L
Summary – PARP Inhibitors

Understanding pathways related to PARP inhibition:

- Full understanding of mechanism of action
- Rational for predictive biomarkers
- Potency/adverse events within class

Cyclin-dependent kinase 4/6 [CDK4/6] inhibitors

- Review of Rb pathway
- Genomic predictors of response
- Differentiating agents by pharmacologic properties
Cyclin-dependent kinase 4/6 [CDK4/6] inhibitors

- Review of Rb pathway
- Genomic predictors of response
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Rb Pathway in Cancer

**RB1** (the retinoblastoma gene, originally identified in retinoblastoma)

Note: *RB1* is gene name of Rb protein

Acts as a tumor suppressor gene

Central role in cell proliferation regulation

Rb pathway because it is consistently altered in cancer cells

This pathway can be dysregulated by alteration in a number of genes
Review of Rb Pathway

- Rb regulates cell cycle
  - Rb (unphosphorylated) binds to E2F and inhibits cell cycle progression
  - Rb (phosphorylated) disassociates with E2F and cell cycle can progress
- The CDK4/6 + cyclin D1 complex regulates primary phosphorylation of Rb
- Secondary phosphorylation of Rb by CDK2/Cyclin E complex
- Multiple upstream regulatory pathways activate CDK4/6
- Negative regulators of CDK4/6 include: p16, p15
  - AKA: INK4 locus genes
  - AKA: CDKN2A, CDKN2B


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Review of Rb Pathway

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71
Review of Rb Pathway

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Cyclin-dependent kinase 4/6 [CDK4/6] inhibitors

- Review of Rb pathway
- Genomic predictors of response
- Differentiating agents by pharmacologic properties

Reminder: Up vs Down Stream
Reminder: Up vs Down Stream

Activator → Target → Downstream Activator

Reminder: Up vs Down Stream

Activator
Inhibitor → Target → Downstream Activator
Reminder: Up vs Down Stream

Activator → Target → Downstream Activator

Inhibitor

Reminder: Up vs Down Stream

Activator → Target → Downstream Activator

Inhibitor
Reminder: Up vs Down Stream

Activator → Target → Downstream Activator

Activator
Inhibitor

Reminder: Up vs Down Stream

Activator
Inhibitor → Target → Downstream Activator
Predictors of Response

- Cyclin D1, \((CCND1)\) amplification
- p16 \((CDKN2A)\) amplification
- Phosphorylation status of CDK4
- Phosphorylation status of p27

* Schoninger. MCT. 2020.
Predictors of Response

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• Phosphorylation status of CDK4
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Schoninger. MCT. 2020.

Predictors of Resistance

• Loss of Rb
• Cyclin E–CDK2
• CDK6 amplification + loss of the FAT1 tumor suppressor

Schoninger. MCT. 2020.
Predictors of Resistance

- Loss of Rb
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Schoninger. MCT. 2020.
Predictors of Resistance

- Loss of Rb
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Cyclin-dependent kinase 4/6 [CDK4/6] inhibitors

- Review of RB1 pathway
- Genomic predictors of response
- Differentiating agents by pharmacologic properties
FDA Approved CDK4/6 Inhibitors

**Palbociclib, ribociclib, abemaciclib**
- FDA approval in metastatic breast cancer

**Dosing**
- 21 days on/7 days off treatment
  - Palbociclib, ribociclib
  - Continuous dosing
  - Abemaciclib

---

Grade 3/4 Adverse Event of CDK4/6 Inhibitors

![Graph showing percentage of patients experiencing grade 3/4 adverse events for CDK4/6 inhibitors](image-url)

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Grade 3/4 Adverse Event of CDK4/6 Inhibitors
Grade 3/4 Adverse Event of CDK4/6 Inhibitors

Kinome Selectivity of CDK4/6 Inhibitors
Preference for CDK4

Table 1: IC\textsubscript{50} Values of CDK4/6 Inhibitors

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<thead>
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Kim, Oncotarget, 2018. © 2020 PharmCon
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Unique Activity of Abemaciclib

Table 1 CDK4/6 inhibitors' selectivity

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<td>CDK2/cyclin A/E</td>
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<td>&gt;10,000</td>
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</tr>
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Notes: Data are presented as average of independent determinations ± SD. Data from Gelbert et al and Tripathy et al. For number of repeats and other information, please see cited references.

Abbreviation: NR, not reported.

Critical role in hematopoietic stem cell differentiation

## Unique Activity of Abemaciclib

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- **Associated with gastrointestinal toxicity**
- **Critical role in hematopoetic stem cell differentiation**

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### Footnotes:

Summary – CDK4/6 Inhibitors

Understanding Rb pathway: Rational for predictive biomarkers

Adverse events within class

Understanding dosing rational

Key Takeaways

- Molecular pathways drive cancer and clinical phenotypes
- Understanding these pathways provides opportunity for tailored therapy for patients
- Integrating molecular pathways and therapeutic targets rationalize clinical outcomes
- Genomics and pharmacology go hand in hand
Thank You