Clinical Applications of Comprehensive Genomic Profiling in Gynecologic Malignancies

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Disclosure

- Honoraria: Pfizer, Astellas, BMS, Novartis, Roche, Astra-Zeneca, MSD
- Scientific Advisory Board: MSD, BMS, AstraZeneca, Astellas, Janssen, Novartis, Roche, Pfizer
- Research Grant: Janssen, BMS, Astra-Zeneca, Pfizer, MSD
Ovarian Cancer is a Heterogenous Disease with Distinct Histological Subtypes

- Majority (~90%) of ovarian cancer cases are of epithelial origin
  - 10% are of stromal, germ cell, and small cell histology
- Epithelial ovarian cancer is heterogenous with 4 distinct histological subtypes:
  - Serous
  - Endometrioid
  - Clear cell
  - Mucinous
- Histological subtypes and tumor grade have prognostic importance

• Subjective interpretation of light microscopic features and expert gynecologic pathology review was the dominant method of categorizing patients into histologically defined diagnostic categories (i.e., serous vs. nonserous ovarian carcinoma)

• Genomic mutations are objective data that can complement this diagnostic process and support accurate tumor classification, especially in the setting of challenging or rare diagnoses

• Also, many recurrent genomic alterations (GA) in gynecologic malignancies can provide insights into:
  • Tumor biology
  • Prognostic information
  • Potential targeted therapy options while limiting cumulative chemotherapeutic toxicities


A Tale of Two Approaches: Thoracic versus Gynecologic Oncology
Selected Examples of Cancer Genome Sequencing and Anti-Cancer Drug Selection

<table>
<thead>
<tr>
<th>Genetic Event</th>
<th>Disease</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>KRAS</em> Mutation</td>
<td>CRC</td>
<td>Cetuximab/Panitumumab (contraindicated by KRAS mutation)</td>
</tr>
<tr>
<td><em>BRAF</em> Mutation</td>
<td>Melanoma</td>
<td>Vemurafenib/Dabrafenib</td>
</tr>
<tr>
<td><em>EGFR</em> Mutation</td>
<td>NSCLC</td>
<td>Gefitinib/Erlotinib/Afatinib</td>
</tr>
<tr>
<td><em>EML4-ALK</em> Translocation</td>
<td>NSCLC</td>
<td>Crizotinib</td>
</tr>
<tr>
<td><em>KIT</em> Mutation</td>
<td>GIST/melanoma</td>
<td>Imatinib/Sunitinib/Regorafenib/Pazopanib</td>
</tr>
<tr>
<td><em>BCR-ABL</em> Translocation</td>
<td>CML</td>
<td>Imatinib/Dasatinib/Nilotinib/Bosutinib</td>
</tr>
<tr>
<td><em>PML-RARA</em> Translocation t(15;17)</td>
<td>APL</td>
<td>ATRA</td>
</tr>
<tr>
<td><em>HER2</em> Gene Amplification*</td>
<td>Breast and Upper GI Cancer</td>
<td>Trastuzumab/Lapatinib</td>
</tr>
<tr>
<td><em>ROS1</em> Fusion</td>
<td>NSCLC</td>
<td>Cabozantinib (investigational)</td>
</tr>
<tr>
<td><em>RET</em> Fusion</td>
<td>NSCLC</td>
<td>Cabozantinib (investigational)</td>
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</tbody>
</table>
Personalized approach improves cancer treatment outcomes

Genomics-matched targeted therapy = BEST OUTCOME
Targeted therapy w/o mutation matching = WORST OUTCOME

(Ref: Schwaederle et al., JCO 2015)
Availability of Molecular Diagnostics is Refining our Understanding of Lung Adenocarcinoma Dramatically

Over half of lung tumors have known alterations


* therapies are not approved in lung
Thoracic Oncology Reaps the Rewards of Personalized Cancer Treatment

Identifying genomic alteration and treating with targeted therapy increased survival in lung cancer dramatically

Kris et al., JAMA 2014
A Tale of Two Approaches

Comparatively modest improvements in gynecologic malignancies...
Can precision diagnostics usher in the molecular age of Ovarian Cancer?

Can precision diagnostics usher in the molecular age of Ovarian Cancer?

Help us recognize molecular heterogeneity in other gynecologic malignancies?

http://www.cancer.gov/types/uterine/hp/endometrial-treatment-pdq#section/_9;
TCGA, Nature 2013;
Talhouk et al., British J CA 2015
Help us recognize molecular heterogeneity in other gynecologic malignancies?

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Next-generation sequencing (NGS) overcomes the limitations of traditional approaches.
Four types of ways genes can be altered

- Normal
- Copy number alterations
- Substitutions
- Insertions and deletions
- Rearrangements
All 4 classes of alterations important

- **Trastuzumab in HER2 amplified breast cancer**

- **Gefitinib in *EGFR*-exon 19 del NSCLC**

- **Vemurafenib in *BRAF V600E* mut melanoma**

- **Crizotinib in *ALK*-rearranged Uterine STUMP**
  - Subbiah et al., 2015, *J Hem Onc*
The evolution of molecular testing

Impact on clinical management

Evolution of molecular profiling methodology

Traditional molecular testing approaches
- First-generation sequencing
- Next-generation sequencing
- NGS-based hotspot testing
- Hybrid capture
- FISH: fluorescence in situ hybridisation; IHC: immunohistochemistry; NGS: next-generation sequencing; PCR: polymerase chain reaction; RNA: ribonucleic acid; WES: whole exome sequencing; WGS: whole genome sequencing.

Routine single-marker molecular test

The most common type of molecular testing

Routine single marker molecular tests such as IHC, PCR and FISH that have been used for decades and will continue to play an important role in cancer diagnosis.

FISH: fluorescence in situ hybridization; IHC: Immunohistochemistry; PCR: Polymerase chain reaction
Diagnostic challenge: traditional molecular testing limitations

- Only a limited number of alterations screened at once
- Potentially misses some types of mutations
- Tissue samples exhausted
- Need to know ahead of time what to look for
Current diagnostic approaches work well when you know what your target is

Current diagnostic approaches are specific – the target gene and class of alteration must be known, which means some alterations can be missed.

<table>
<thead>
<tr>
<th>DETECTS</th>
<th>CAN MISS</th>
</tr>
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<tbody>
<tr>
<td><strong>FISH</strong></td>
<td></td>
</tr>
<tr>
<td>✓ Copy number alterations</td>
<td>✗ Small insertions/deletions</td>
</tr>
<tr>
<td>✓ Some rearrangements such as translocations, gene deletions or amplifications (limited to a single abnormality or gene)</td>
<td>✗ Rearrangements not known prior to testing</td>
</tr>
<tr>
<td><strong>IHC</strong></td>
<td></td>
</tr>
<tr>
<td>✓ Protein expression (e.g. mutation, deletion, translocation, amplification) pre-determined by clinicians for a particular tumour type</td>
<td>✗ Any protein expression not known prior to testing</td>
</tr>
<tr>
<td><strong>RT-PCR</strong></td>
<td></td>
</tr>
<tr>
<td>✓ Small insertions/deletions</td>
<td>✗ Large insertions/deletions</td>
</tr>
<tr>
<td>✓ Base substitutions</td>
<td>✗ Rearrangements</td>
</tr>
<tr>
<td>✓ Pre-determined rearrangements</td>
<td>✗ Copy alterations and chromosomal anomalies not known prior to testing</td>
</tr>
</tbody>
</table>

FISH, Fluorescence in situ hybridisation; IHC, Immunohistochemistry; RT-PCR, Reverse Transcriptase Polymerase Chain Reaction.
Diagnostic Challenge: Many clinical cancer specimens are small needle biopsies, FNAs and cell blocks

Formalin fixation and subsequent storage can damage nucleic acids

Percutaneous needle biopsy of lung nodules under CT fluoroscopic guidance

Sample preparation needs be optimized to maximize accuracy and isolate sufficient material for diagnostic testing from tiny specimens
Foundation Medicine®
The most comprehensive genomic test available

Foundation Medicine’s comprehensive genomic profiling approach of testing all of the known clinically relevant cancer genes for all classes of alterations (not only the hot spot)
FMI’s Current Service Offerings
Comprehensive Dx and Molecular Information

**Applies next-generation sequencing**
to identify genomic alterations across 315 cancer-related genes known to be drivers of solid tumors plus select introns of 28 genes.

**Designed to analyze and interpret DNA sequence information of 405 genes** and RNA sequence (cDNA) information of 265 commonly rearranged genes in hematologic malignancies.

**A liquid biopsy Assay for Circulating Tumor DNA**, interrogating all known classes of genomic alteration across 62 genes. Provides validated, blood-based profiling when tissue biopsy may not be feasible.

**Microsatellite instability (MSI)**
**Tumor mutational burden (TMB)**

A single solution for simultaneous assessment of MSI and TMB biomarkers – previously separate and time- and labor-intensive tests. Will provide additional and relevant genomic clues as to which patients may benefit the most from certain immunotherapies.
FMI’s Current Service Offerings
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How Does FoundationOne Work?

A process that follows standard operating processes

**Pre-Analytic Process (Pre-Sequencing)**

1) DNA/RNA extraction
   - Extensive optimization

2) LC, Hybrid Capture
   - Extensive optimization

**Post-Analytic Process (Post-Sequencing)**

3) Analysis pipeline
   - Advanced computational biology

4) Clinical report
   - Resource intensive

Powered by 20+ bioinformaticians and genomic scientists who optimize state-of-the-art algorithms to report the most clinically relevant information for a patient.
NGS-based bioinformatics analyses

Expert review
Results are reviewed by scientific and clinical experts to further personalise therapy and potentially improve patient outcomes. Clinical and scientific expert review of NGS results has been shown to aid clinical decision making.\(^2\)

FMI report
Curated, quality controlled report is generated to help physicians identify targeted treatment options.\(^3\)

Tertiary analyses\(^1\)
- Annotation of variants
- Contextualisation of variant information
- Validation

The total process time (from receipt of the sample to report generation) takes on average 11 days.\(^4\)

FMI: Foundation Medicine, Inc.; NGS: next-generation sequencing.
FoundationOne validated in top-tier reviewed journal

Impact of comprehensive diagnostics
Opens up more treatment possibilities for patients

Patients who may benefit from anti-HER2 using current standard testing

**Impact of comprehensive diagnostics**

Opens up more treatment possibilities for patients

**Patients who may benefit from anti-HER2 using current standard testing**

ERBB2 amplification in breast, gastric, and gastroesophageal cancers accounted for only 30% of these alterations.

Impact of comprehensive diagnostics
Opens up more treatment possibilities for patients

Impact of comprehensive diagnostics

Opens up more treatment possibilities for patients

Patients who may benefit from Herceptin using a comprehensive profiling test

Compared with current clinical standards, comprehensive genomic profiling of a more diverse set of tumor types may identify ~3.5 times the number of patients who may benefit from ERBB2-targeted therapy.

Genomic Alterations Under Investigation in Gynecologic Cancers
Genomic Alterations Under Investigation in Gynecologic Cancers

BRCA ½
Current standards for BRCA 1/2 testing recommend universal testing for all ovarian cancer patients

• National oncology societies (NCCN, SGO, ASCO) universally recommend testing all women with ovarian cancer, regardless of age or family history\(^1-3\)

• Missed opportunities for therapeutic interventions
  - in clinical practice, genetic testing is mostly driven by family history and age
  - Screening based on family history, histology, or age alone may miss a significant % of patients with BRCA1/2 mutations\(^4,5\)
  - Non-BRCA mutations and HRD status are not assessed
  - Current genetic testing uses targeted gene sequencing and not multigene panels

ASCO: American Society of Clinical Oncology; HRD: homologous recombination deficiency; NCCN: National Comprehensive Cancer Network; SGO: Society of Gynecologic Oncology

Current standards for BRCA 1/2 testing recommend universal testing for all ovarian cancer patients in Brazil
Germline vs Somatic mutations
## Results: BRCA testing

<table>
<thead>
<tr>
<th>tBRCA</th>
<th>Mutated</th>
<th>Wild type*</th>
<th>Not available</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>gBRCA</td>
<td>Mutated</td>
<td>71</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Wild type*</td>
<td>18</td>
<td>73</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Not available</td>
<td>22</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td><strong>TOTAL</strong></td>
<td><strong>265</strong></td>
<td></td>
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</table>

- 136 (51%, BLACK) patients had a known deleterious BRCAm (BRCAm dataset)
- 118 (45%, WHITE/PINK) patients were BRCA1/2 wild type for this analysis
- 11 (4%, GREY) patients had neither a tumour nor a germline result available

➢ The number of patients with a known BRCAm status increased from 97 (37%) to 254 (96%) out of 265

*Wild-type group includes patients with no known BRCAm or a mutation of unknown significance (a non-deleterious mutation)

### Results: BRCA testing

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<tr>
<td>Mutated</td>
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<td>3</td>
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<td>18</td>
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<td>22</td>
<td></td>
<td>11/256 (4%)</td>
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- 144/165 (87%) informative cases (both germline and tumor available) were consistent

*Wild-type group includes patients with no known BRCAm or a mutation of unknown significance (a non-deleterious mutation)

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- 21 informative cases showed discrepant somatic alterations relative to the germline.
  - 3/74 (4%) revertant
  - 18/89 (20%) somatic gain

*Wild-type group includes patients with no known BRCAm or a mutation of unknown significance (a non-deleterious mutation)

PARPi and “Synthetic Lethality”

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

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

Nature 2005
Phase I: Olaparib

Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from BRCA Mutation Carriers

Peter C. Fong, M.D., David S. Boss, M.Sc., Timothy A. Yap, M.D., Andrew Tutt, M.D., Ph.D., Peijun Wu, Ph.D., Marja Mergui-Roelvink, M.D., Peter Mortimer, Ph.D., Helen Swaidsland, B.Sc., Alan Lau, Ph.D., Mark J. O'Connor, Ph.D., Alan Ashworth, Ph.D., James Carmichael, M.D., Stan B. Kaye, M.D., Jan H.M. Schellens, M.D., Ph.D., and Johann S. de Bono, M.D., Ph.D.

ABSTRACT

The inhibition of poly(ADP-ribose) polymerase (PARP) is a potential synthetic lethal therapeutic strategy for the treatment of cancers with BRCA1 or BRCA2 DNA-repair defects, including those arising in carriers of a BRCA1 or BRCA2 mutation. We conducted a clinical evaluation of olaparib (AZD2281),
**BRCA1/2 reversion mutations**

*Resistance to PARP Inhibitors*

- Reversion mutations (revGA) restore BRCA function and can cause resistance to treatment with platinum-based therapies or PARP inhibitors.

- Found in many tumor types, but particularly prevalent in:
  - Breast carcinomas and Ovarian carcinomas\(^1\)
  - Prostate adenocarcinomas\(^2\)
  - Pancreatobiliary tumors\(^3\)

- The type of mutation that causes a reversion cannot be predicted – an unbiased testing approach is required.

- Breast and Ovarian/Peritoneal/Fallopian Tube carcinomas: 4.2% of BRCA-mutated tumors and 0.5% of all samples harbored a potential BRCA revGA:
  - more common in ovarian carcinomas

- These revGA can be acquired during treatment with PARPi or Pt therapies, and could contribute to drug resistance and disease progression.

- The acquisition of revGA over time can be observed through testing of serially acquired samples.

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1. Mayor P, et al. ASCO 2017; Abstract 5551
2. Daniel S, et al. ASCO 2017; Abstract 5024
# Frequency of BRCA Reversion Alterations

As Determined by Foundation Medicine

<table>
<thead>
<tr>
<th>Disease</th>
<th>Total Cases (% BRCA+)</th>
<th>Alteration Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Overlapping Indel</td>
</tr>
<tr>
<td>Breast Carcinoma</td>
<td>21 (2.1%)</td>
<td>10</td>
</tr>
<tr>
<td>Peritoneal Serous Ovarian Serous*</td>
<td>25 (4.4%)</td>
<td>11</td>
</tr>
<tr>
<td>Prostate aCA*</td>
<td>7 (3.2%)</td>
<td>4</td>
</tr>
<tr>
<td>Pancreatic aCA</td>
<td>12 (0.2%)</td>
<td>5</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>6 (0.9%)</td>
<td>1</td>
</tr>
<tr>
<td>CRC</td>
<td>2 (0.6%)</td>
<td>--</td>
</tr>
<tr>
<td>Stomach aCA</td>
<td>1 (1.9%)</td>
<td>1</td>
</tr>
<tr>
<td>Bladder aCA</td>
<td>1 (1.0%)</td>
<td>1</td>
</tr>
<tr>
<td>Myeloma</td>
<td>1 (n/a)</td>
<td>--</td>
</tr>
<tr>
<td>CUP</td>
<td>1 (1.0%)</td>
<td>1</td>
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<tr>
<td>Total</td>
<td>77 (1.6%)</td>
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<th>Exon Loss</th>
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Location of the BRCA2 mutation and survival
Patients with BRCA1-RING mutation have same survival than those without BRCA1m.
Genomic Alterations Under Investigation in Gynecologic Cancers
Other Mutations Rather than BRCA ½
Non-BRCA HR mutations in study

- The specific HRRm identified in the 21 tumour samples were: *BRIP1* (n=5), *CDK12* (n=2), *RAD54L* (n=2), *RAD51B* (n=2), *RAD54L* rearr (n=1), *ATM* rearr (n=1), *FANCA/CDK12* (n=1), *FANCA* rearr (n=1), *FANCD2* (n=1), *FANCI* rearr (n=1), *FANCL* (n=1), *RAD51C* (n=1), *RAD52 del* (n=1), *XRCC3* rearr (n=1).

![Graphs showing progression-free survival for BRCAwt HRRm and HRRwt](image)
Could genomic alterations help select patients for NACT vs. PDS?

- **CCNE1 amplification:**
  - Present 20% of HGSOC
  - Associated with primary chemotherapy resistance
    
  [Patch et al, Nature 2015]
  - Preferentially directed towards PDS?

- **BRCA mutations** or alterations in HR genes:
  - Present in almost half of HGSOC
  - Associated with platinum sensitivity
  - Likely to achieve good tumor shrinkage with NACT

---

**RR to 2nd line platinum in BRCA M+/WT OC**

<table>
<thead>
<tr>
<th></th>
<th>RR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA-mutated</td>
<td>92%</td>
<td>0.004</td>
</tr>
<tr>
<td>BRCA WT</td>
<td>41%</td>
<td></td>
</tr>
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</table>

[Tan et al, 2009, JCO]
Genomic Alterations Under Investigation in Gynecologic Cancers
More Refined Classification and Diagnosis
Diagnostic Application of Comprehensive Genomic Profiling in Ovarian Cancer

Fig. 1. Histological and molecular sub-types of epithelial ovarian cancer (EOC). g, germline, t, tumour.
Diagnostic Applications of Genomic Profiling in Gynecologic Malignancies

- **FOXL2** mutations are diagnostic for adult granulosa cell tumors (aGCTs)
  - Study of 336 histologically classified demonstrated 19% of tumors were misdiagnosed based on the absence of a FOXL2 mutation

- **SMARCA4** mutations are associated with the clinically aggressive ovarian small cell carcinoma, hypercalcemic type

- **DICER1** mutations are associated with the more indolent Sertoli–Leydig cell tumors

- In serous ovarian tumors, the presence of KRAS and BRAF, and absence of TP53 alterations support the diagnosis of a low grade serous ovarian carcinoma (LGSC)
Other biomarkers of interest in Gynecologic Malignancies

- TMB
- MSI
- LOH
Other biomarkers of interest in Gynecologic Malignancies

TMB

MSI
In addition to genomic targets, other biomarkers such as TMB and MSI are being discovered that help us understand more about tumour profiles.

- **Tumour mutational burden (TMB)** is defined as the overall quantity of mutations in a cancer genome.
  - Higher TMB levels may help to predict response to cancer immunotherapies.

- **Microsatellite instability (MSI)** may occur as a result of DNA mismatch repair.
  - MSI may help in prediction of patient response to immunotherapy where conventional therapy has failed.

References in notes:
- Rizvi NA et al., Science 2015
- Rosenberg JE et al., Lancet 2016
- Snyder et al., NEJM 2014
- Frampton et al., Ann Onclo 2016: Abstract 520
FDA approves first cancer treatment for any solid tumor with MSI-High or dMMR
FDA approves first cancer treatment for any solid tumor with MSI-High or dMMR

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

FDA approves first cancer treatment for any solid tumor with MSI-High or dMMR

2 patients with endometrial cancer
FDA approves first cancer treatment for any solid tumor with MSI-High or dMMR

First FDA Approval Agnostic of Cancer Site — When a Biomarker Defines the Indication
Steven Lemery, M.D., M.H.S., Patricia Keegan, M.D., and Richard Pazdur, M.D.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>No. of Tumors</th>
<th>Patients with a Response</th>
<th>Range of Response Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>90</td>
<td>32 (36)</td>
<td>1.6+ to 22.7+</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>14</td>
<td>5 (36)</td>
<td>4.2+ to 17.3+</td>
</tr>
<tr>
<td>Biliary cancer</td>
<td>11</td>
<td>3 (27)</td>
<td>11.6+ to 19.6+</td>
</tr>
<tr>
<td>Gastric or gastroesophageal junction</td>
<td>9</td>
<td>5 (56)</td>
<td>5.8+ to 22.1+</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>6</td>
<td>5 (83)</td>
<td>2.6+ to 9.2+</td>
</tr>
<tr>
<td>Small-intestine cancer</td>
<td>8</td>
<td>3 (38)</td>
<td>1.9+ to 9.1+</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>2</td>
<td>2 (100)</td>
<td>7.6 to 15.9</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>2</td>
<td>1 (50)</td>
<td>9.8+</td>
</tr>
<tr>
<td>Other cancers</td>
<td>7</td>
<td>3 (43)</td>
<td>7.5+ to 18.2+</td>
</tr>
</tbody>
</table>
Other biomarkers of interest in Gynecologic Malignancies

LOH
Levine D. The Cancer Genome Atlas, Molecular profiling of serous ovarian cancer, 2011

The Homologous Recombination (HR) Phenotype in Sporadic Ovarian Cancer

- **BRCA1**
  - Germline: 8%
  - Somatic: 3%
  - Methylation: 11%

- **BRCA2**
  - Germline: 6%
  - Somatic: 3%

- **EMSY**
  - Amplification: 6%

- **PTEN**
  - Loss: 5%

- **CCNE1**
  - Amplification: 15%

- **MMR**
  - Germline: 2%

- **Other HRD**: 7%

- **Other**: 34%

- **Not HR deficient**: HR Deficient (HRD)
Causes of Loss of Heterozygosity (LOH)

- Homologous recombination (HR) is the primary high-fidelity DNA repair pathway for double strand breaks
- Causes of HR-deficiency (HRD) are diverse
- HRD cells rely on alternative, error-prone DNA repair pathway resulting in accumulation of genomic LOH scar
- HRD cells are susceptible to synthetic lethality by PARPi
- Cells with intact HR need alternative treatment strategies

<table>
<thead>
<tr>
<th>BRCA1</th>
<th>BRCA2</th>
<th>PALB2</th>
<th>RAD51</th>
<th>etc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous gene deletion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsense and frameshift mutation (germline and somatic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epigenetic gene silencing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA-mediated gene silencing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other mechanisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-HRD associated tumors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutation directed genomic profiling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Elvin, J.A. Abstract #5512 – oral presentation at ASCO 2017
HRD causes genome-wide loss of heterozygosity (LOH) that can be measured by comprehensive genomic profiling based on NGS.

**Hypothesis 1:**
Ovarian cancer patients with high genomic LOH suggesting BRCA-like signature will respond to PARPi.

**Hypothesis 2:**
Ovarian cancer patients who are “biomarker negative” (ie, with low genomic LOH) will not respond to PARPi.

*mut=mutation; NGS=next-generation sequencing; wt=wild type.*
BRCA ½ & LOH status per tumor site origin

<table>
<thead>
<tr>
<th>Tissue of Origin</th>
<th>Count</th>
<th>% BRCA\text{mut}</th>
<th>% BRCA\text{wt}/LOH-High</th>
<th>%BRCA\text{wt}/LOH-Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumors</td>
<td>4114</td>
<td>17.2%</td>
<td>24.2%</td>
<td>58.7%</td>
</tr>
<tr>
<td>Ovary</td>
<td>3674</td>
<td>17.1%</td>
<td>24.3%</td>
<td>58.6%</td>
</tr>
<tr>
<td>Fallopian Tube</td>
<td>236</td>
<td>18.2%</td>
<td>25.8%</td>
<td>55.9%</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>204</td>
<td>10.3%</td>
<td>19.6%</td>
<td>63.2%</td>
</tr>
</tbody>
</table>

- Note: 2.6% of samples had a VUS in BRCA1 or BRCA2 across categories (except for Fallopian tube samples where VUS rate was 1.3%)

- 48% of ovarian cancer with difficult to classify histology is either BRCA mut or LOH High
Comprehensive genomic profiling (CGP) with loss of heterozygosity (LOH) identifies therapeutically relevant subsets of ovarian cancer (OC).

Julia Andrea Elvin, Yuting He, James Sun, Kunle Odunsi, James Brian Szender, Kathleen N. Moore, Laurie M. Gay, Garrett Michael Frampton, Jo-Anne Vergilio, James Suh, Shakti Ramkissoon, Eric Allan Severson, Suggan Daniel, Kevin K. Lin, June YiJuan Hou, Camille Catherine Gunderson, Laura L. Holman, Phil Stephens, Mitch Raponi, Jeffrey S. Ross

Foundation Medicine, Cambridge, MA; Roswell Park Cancer Institute, Buffalo, NY; University of Oklahoma Health Sciences Center, Oklahoma City, OK; Clovis Oncology, San Francisco, CA; Albert Einstein Coll of Medcn, Montefiore Medc Ctr, Bronx, NY; Albany Medical College, Albany, NY
Methods

- ≥50 ng of DNA extracted from FFPE archival tumor tissue obtained during routine clinical care.
- Analyzed for genomic alterations in 315 genes by hybrid-capture, next-generation sequencing (FoundationOne)
  - Base substitutions
  - Small indels
  - Copy number alterations
  - Rearrangements
- Proprietary algorithms simultaneously evaluated global genomic metrics:
  - Loss of heterozygosity (LOH; LOH-High > 16% of genome, LOH-Low < 16% of genome)\textsuperscript{1,2}.
  - Microsatellite instability (MSI),
  - Tumor mutation burden (TMB; TMB-High ≥ 10 muts/Mb)

Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817)


<table>
<thead>
<tr>
<th>Sample Characteristics</th>
<th>Count</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Samples</td>
<td>4114</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Tissue of Origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>3674</td>
<td>89%</td>
</tr>
<tr>
<td>Fallopian Tube</td>
<td>236</td>
<td>6%</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>204</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Histologic Subtypes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous (High grade &amp; NOS)</td>
<td>2770</td>
<td>67%</td>
</tr>
<tr>
<td>Epithelial NOS</td>
<td>807</td>
<td>20%</td>
</tr>
<tr>
<td>Non-Serous</td>
<td>537</td>
<td>13%</td>
</tr>
<tr>
<td>Clear cell</td>
<td>192</td>
<td>2%</td>
</tr>
<tr>
<td>Carcinosarcoma</td>
<td>147</td>
<td>4%</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>95</td>
<td>1%</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>21</td>
<td>5%</td>
</tr>
<tr>
<td>Mucinous</td>
<td>38</td>
<td>1%</td>
</tr>
<tr>
<td><em>Low grade serous</em></td>
<td>32</td>
<td>1%</td>
</tr>
<tr>
<td>Small cell</td>
<td>12</td>
<td>0%</td>
</tr>
</tbody>
</table>

[NOS – not otherwise specified; * low grade serous carcinoma is grouped with non-serous histologies to reflect clinical behavior]
Generating a LOH score

Distinguishing HRD versus other mechanisms of genomic instability

- Segments not under LOH are unshaded
- Focal LOH contributes to score (pink shading)
- Non-focal LOH are excluded from score (green shading)
Clinically validating LOH-High threshold score with ARIEL2 clinical data

Elvin, J.A. Abstract #5512 – oral presentation at ASCO 2017
**ARIEL3 RUCAPARIB: INVESTIGATOR-ASSESSED PFS IN PATIENTS WITH BRCA WILD-TYPE OC**

**LOH high**

<table>
<thead>
<tr>
<th></th>
<th>Median (months)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rucaparib (n=106)</td>
<td>9.7</td>
<td>7.9–13.1</td>
</tr>
<tr>
<td>Placebo (n=52)</td>
<td>5.4</td>
<td>4.1–5.7</td>
</tr>
</tbody>
</table>

**HR, 0.44; 95% CI, 0.29–0.66; P<0.0001**

**LOH low**

<table>
<thead>
<tr>
<th></th>
<th>Median (months)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rucaparib (n=107)</td>
<td>6.7</td>
<td>5.4–9.1</td>
</tr>
<tr>
<td>Placebo (n=54)</td>
<td>5.4</td>
<td>5.3–7.4</td>
</tr>
</tbody>
</table>

**HR, 0.58; 95% CI, 0.40–0.85; P=0.0049**

Visit cutoff date: 15 April 2017.
NOVA: Niraparib PFS in Subgroups of Non-gBRCAmut Cohort

### HRD-positive

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PFS Median (95% CI) (Months)</th>
<th>Hazard Ratio (95% CI) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niraparib (N=71)</td>
<td>9.3 (5.8, 15.4)</td>
<td>0.38 (0.231, 0.628) p=0.0001</td>
</tr>
<tr>
<td>Placebo (N=44)</td>
<td>3.7 (3.3, 5.6)</td>
<td></td>
</tr>
</tbody>
</table>

### HRD-negative

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PFS Median (95% CI) (Months)</th>
<th>Hazard Ratio (95% CI) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niraparib (N=92)</td>
<td>6.9 (5.6, 9.6)</td>
<td>0.58 (0.361, 0.922) p=0.0226</td>
</tr>
<tr>
<td>Placebo (N=42)</td>
<td>3.8 (3.7, 5.6)</td>
<td></td>
</tr>
</tbody>
</table>
From the Lab to Clinical Practice in Ovarian Cancer
CGP can be a valuable tool to integrate into decision-making and clinical trial design

- 82% of ovarian cancers have GA suggesting a mechanism of potential benefit for novel treatment approaches not captured by traditional histologic definitions
From the Lab to Clinical Practice in Ovarian Cancer

Recurrent Ovarian Cancer
**Patients**
- Platinum-sensitive high-grade serous ovarian cancer
- ≥2 previous platinum regimens
- Maintained PR or CR following last platinum regimen

**Olaparib**
400mg bid, orally
(n=136)

**Placebo**
(n=129)

**Primary endpoint**
PFS by RECIST

**Secondary endpoints**
TTP by CA-125 (GCIG criteria) or RECIST, OS, safety

82 sites in 16 countries

**Study 19: Progression-free survival**

- **Randomized treatment**
  - Placebo
  - Olaparib 400 mg bid

- **At risk (n)**
  - Olaparib: 136, 104, 51, 23, 6, 0, 0
  - Placebo: 129, 72, 23, 7, 1, 0, 0

- **No. of events: Total patients (%)**
  - Olaparib: 60:136 (44.1)
  - Placebo: 93:129 (72.1)

- **Median PFS (months)**
  - Olaparib: 8.4
  - Placebo: 4.8

- **Hazard ratio 0.35 (95% CI, 0.25–0.49)**
  - *P* < 0.00001

Study 19: PFS by BRCAm status

- **82% reduction in risk of disease progression or death with olaparib**

<table>
<thead>
<tr>
<th>BRCAm (n=136)</th>
<th>Olaparib</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events: total pts (%)</td>
<td>26:74 (35.1)</td>
<td>46:62 (74.2)</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>11.2</td>
<td>4.3</td>
</tr>
<tr>
<td>HR = 0.18</td>
<td>95% CI (0.11, 0.31); P &lt; 0.00001</td>
<td></td>
</tr>
</tbody>
</table>

- **Number at risk**
  - Olaparib BRCAm: 74, 59, 33, 14, 4, 0
  - Placebo BRCAm: 62, 35, 13, 2, 0, 0

Presented by: Jonathan Ledermann et al at ASCO 2013
Study 19: PFS by BRCAm status

<table>
<thead>
<tr>
<th>BRCAm (n=136)</th>
<th>BRCAwt (n=118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events: total pts (%)</td>
<td>Olaparib</td>
</tr>
<tr>
<td>26:74 (35.1)</td>
<td>46:62 (74.2)</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>11.2</td>
</tr>
</tbody>
</table>

HR=0.18
95% CI (0.11, 0.31); P<0.00001

HR=0.53
95% CI (0.33, 0.84); P=0.007

Number at risk

<table>
<thead>
<tr>
<th>Olaparib BRCAm</th>
<th>Placebo BRCAm</th>
<th>Olaparib BRCAwt</th>
<th>Placebo BRCAwt</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td>59</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>62</td>
<td>35</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>57</td>
<td>44</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>61</td>
<td>35</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

BRCAm, wild type (includes patients with no known BRCAm or a mutation of unknown significance)

Presented by: Jonathan Ledermann et al at ASCO 2013
Study 19 – ASCO 2016

Overall population*

Overall study population (N=265)

Olaparib (n=136)  Placebo (n=129)

Deaths, n (%)  94 (69)  109 (84)

Median OS, months  29.8  27.8

HR=0.73
95% CI 0.55–0.96
Nominal P=0.02483

Maturity: 77%
Criterion for statistical significance not met (P<0.0095)

Asco 2016, oral presentation.
Sem diferença entre mutações somáticas BRCA e mutações germinativas, contudo as conclusões são limitadas devido à amostra limitada n = 20)
- Phase II, n=298, various tumor types
- Ovarian cancer: 64%
- Previous treatments: median 4
- 76.7% BRCA1; 22.8% BRCA2

Kaufman, JCO, 2014
<table>
<thead>
<tr>
<th>Response</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor response rate</td>
<td>60</td>
<td>31.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>24.6 to 38.1</td>
<td></td>
</tr>
<tr>
<td>CR*</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>PR*</td>
<td>54</td>
<td>28</td>
</tr>
<tr>
<td>Stable disease ≥ 8 weeks</td>
<td>78</td>
<td>40</td>
</tr>
<tr>
<td>95% CI</td>
<td>33.4 to 47.7</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>64</td>
<td>33</td>
</tr>
<tr>
<td>Unconfirmed PR</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>PD†</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td>95% CI</td>
<td>15.7 to 27.7</td>
<td></td>
</tr>
<tr>
<td>RECIST progression</td>
<td>33</td>
<td>17</td>
</tr>
<tr>
<td>Early death‡</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>No follow-up assessments</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Stable disease &lt; 8 weeks</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
# PARPi maintenance in BRCA and HRD subgroups

## BRCA mutated

<table>
<thead>
<tr>
<th>Drug</th>
<th>Population</th>
<th>PFS Maintenance</th>
<th>PFS Placebo</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niraparib</td>
<td>germline or somatic</td>
<td>21.0</td>
<td>5.5</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.9</td>
<td>11.0</td>
<td>0.27</td>
</tr>
<tr>
<td>Olaparib</td>
<td>germline or somatic</td>
<td>19.1</td>
<td>5.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Rucaparib</td>
<td>germline or somatic</td>
<td>16.6</td>
<td>5.4</td>
<td>0.23</td>
</tr>
<tr>
<td>Olaparib*</td>
<td>germline or somatic</td>
<td>11.2</td>
<td>4.3</td>
<td>0.18</td>
</tr>
</tbody>
</table>

## BRCA wild-type

<table>
<thead>
<tr>
<th>Drug</th>
<th>Population</th>
<th>PFS Maintenance</th>
<th>PFS Placebo</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niraparib</td>
<td>No germline or somatic</td>
<td>9.3</td>
<td>3.9</td>
<td>0.45</td>
</tr>
<tr>
<td>Olaparib*</td>
<td>BRCAwt</td>
<td>7.4</td>
<td>5.5</td>
<td>0.54</td>
</tr>
<tr>
<td>Olaparib</td>
<td>HRD pos</td>
<td>9.3</td>
<td>3.7</td>
<td>0.38</td>
</tr>
<tr>
<td>Rucaparib</td>
<td>LOH high</td>
<td>11.1</td>
<td>5.6</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*Phase 2 trial
**Includes pts with somatic BRCA mutation*
Combinations with Immune Checkpoint Inhibitors
TOPACIO: Niraparib & Pembrolizumab

Percentage change in lesion size in (A) Recurrent Ovarian (B) TNBC

From the Lab to Clinical Practice in Ovarian Cancer

Newly Diagnosed Ovarian Cancer
First-Line Maintenance in Ovarian Cancer

**SOLO-1** in BRCA\textsuperscript{mut}

**PRIMA:** Niraparib in ovarian cancer

**SOLO-1**
- **St III-IV Ov**
- BRCA mutation
- HG serous or endometrioid
- PR/CR & > 6 cycles

**Primary endpoint:**
- PFS

**Secondary:**
- OS
- PFS2
- QoL

**Stratification factors:**
- Use of NACT: yes or no
- Best tumor response: CR or PR
- HRD status: pos or neg/hrd

**Patients with sBRCA or dBRCA and will be stratified as HRDpos**

**PRIMA**

**High Risk patients: Stage IV; suboptimal Stage III**

**Response to chemotherapy**

**Post-treatment follow-up (every 12 weeks)**

**Primary Endpoint**
- PFS in HRDpos patients; hierarchical analysis for all patients regardless of HRD status

**Secondary:**
- OS, Patient Reported Outcomes (PRO’s), Time to First Subsequent Treatment, PFS2, safety and tolerability of study therapy
Obrigado

Os conceitos emitidos são de responsabilidade do autor e não refletem necessariamente a opinião de Produtos Roche Químicos e Farmacêuticos S.A. - BR/NONC/0818/0023