Ovarian Cancer in the Genomics Era

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Ovarian Cancer

- Most lethal gynecologic malignancy in the US
- >16,000 deaths/yr
- 5th most common cancer death for women
- 70% diagnosed with advanced disease
- <35% of advanced stage patients alive at 5 years
# Ovarian Cancer

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Incidence</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Confined to ovaries</td>
<td>20%</td>
<td>90%</td>
</tr>
<tr>
<td>II</td>
<td>Confined to pelvis</td>
<td>5%</td>
<td>65%</td>
</tr>
<tr>
<td>III</td>
<td>Spread IP or nodes</td>
<td>58%</td>
<td>45%</td>
</tr>
<tr>
<td>IV</td>
<td>Distant metastases</td>
<td>17%</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>
Ovarian Cancer: Prognostic Factors

- Stage
- Extent of Cytoreduction
- Histology and Grade
- Performance Status
- p53 Status
- Vital Organ Function
- Physiologic Age

- Platinum+Taxane Primary Therapy
- Intraperitoneal therapy
- Information from Second-Look Surgery
- Genotype BRCA1/2
- VEGF Production
Treatment for Newly Diagnosed Ovarian Cancer

- Complete surgical staging
- Optimal reductive surgery
- Chemotherapy
- Clinical Trials
The State of Treatment for Newly Diagnosed Ovarian Cancer

- **Complete surgical staging**
  - Full assessment of abdomen and pelvis
  - Random biopsy of visually negative areas
  - Lymph node dissection (except Stage I)

- **Optimal reductive surgery**
- **Chemotherapy**
- **Clinical Trials**
Surgical staging of ovarian cancer

PROCEDURES REQUIRED FOR SURGICAL STAGING OF OVARIAN CANCER

- Scraping of the underside of the right diaphragm
- Removal of the para-aortic lymph nodes
- Liver
- Removal of the pelvic lymph nodes
- Removal of the omentum
- Ovarian tumor
- Uterus
- Cervix
- Vagina
The State of Treatment for Newly Diagnosed Ovarian Cancer

- Complete surgical staging
- Optimal reductive surgery
  - Stage I, II - Complete removal of all disease
  - Stage III, IV - Residual disease < 1 cm
- Chemotherapy
- Clinical Trials
Ovarian cancer survival curve

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**Optimal Cytoreduction**

- Proportion surviving
  - Time since initial surgery (years)

- Curves for different tumor sizes:
  - 0 cm
  - 0-1 cm
  - 1-2 cm
  - >2 cm
The State of Treatment for Newly Diagnosed Ovarian Cancer

- Complete surgical staging
- Optimal reductive surgery
- **Chemotherapy**
  - Platinum = cisplatin or carboplatin
    AND
  - Taxane = paclitaxel or docetaxel
  - *Intraperitoneal if Stage III, optimal reduction*

- **Clinical Trials**
GOG 172: The new standard of care

Median survival = 65.6 mo
Intraperitoneal therapy

Median survival = 49.7 mo
Intravenous therapy

P=0.03

Armstrong, NEJM 2006
# Ovarian Cancer

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Tissue of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous-80%</td>
<td>Fallopian tube?</td>
</tr>
<tr>
<td>Endometrioid-10%</td>
<td>serous</td>
</tr>
<tr>
<td>Clear cell-5%</td>
<td>Endometriosis?</td>
</tr>
<tr>
<td>Mucinous-3%</td>
<td>Endometrioid and clear cell</td>
</tr>
<tr>
<td>Other-2%</td>
<td>Mullerian epithelium</td>
</tr>
<tr>
<td></td>
<td>Extra-uterine</td>
</tr>
</tbody>
</table>
Integrated genomic analyses of ovarian carcinoma

The Cancer Genome Atlas Research Network*

Nature 474: 609-615
Background
The Cancer Genome Atlas (TCGA) Clinically annotated HGS-OvCa samples Identify molecular abnormalities that influence pathophysiology, affect outcome and constitute therapeutic targets.
Microarray analyses: 489 HGS-OvCa tumours, mRNA expression, microRNA (miRNA) expression, DNA copy number and DNA promoter methylation for and
Whole exome DNA sequence: 316 samples.
Methods
Sample inclusion criteria
Newly diagnosed patients
ovarian serous adenocarcinoma
no prior treatment
regardless of surgical stage or histologic grade
Each frozen tumor specimen had to have a companion normal tissue specimen, which could be adjacent normal tissue, peripheral lymphocytes, or previously extracted germline DNA.
Methods
Clinical data collection
Clinical data can be accessed and downloaded from the TCGA Data Portal
Demographics, histopathologic information, treatment details, outcome parameters
Mutated genes in HGS-OvCa

<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of mutations</th>
<th>No. validated</th>
<th>No. unvalidated</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>302</td>
<td>294</td>
<td>8</td>
</tr>
<tr>
<td>BRCA1</td>
<td>11</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>CSMD3</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>NF1</td>
<td>13</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>CDK12</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>FAT3</td>
<td>19</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>GABRA6</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>BRCA2</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>RB1</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Validated mutations are those that have been confirmed with an independent assay. Most of them are validated using a second independent whole-genome-amplification sample from the same tumour. Unvalidated mutations have not been independently confirmed but have a high likelihood to be true mutations. An extra 25 mutations in TP53 were observed by hand curation.
Gene and miRNA patterns

Gene and miRNA patterns:

Molecular subtype and outcome prediction
Altered pathways in HGS-OvCa

**RB and PI3K/RAS signalling**

**RB signalling**
- 67% of cases altered
- **CDKN2A**
  - 32%
  - Downregulated 30%, deleted 2%
- **CCNE1**
  - 20%
  - Amplified
- **CCND1**
  - 4%
  - Amplified
- **CCND2**
  - 15%
  - Upregulated
- **RB1**
  - 10%
  - Deleted 8%, mutated 2%

**Cell cycle progression**

**PI3K/RAS signalling**
- 45% of cases altered
- **PTEN**
  - 7%
  - Deleted 7%, mut. <1%
- **NF1**
  - 12%
  - Deleted 8%, mut. 4%
- **PIK3CA**
  - 18%
  - Amplified, mut. <1%
- **KRAS**
  - 11%
  - Amplified, mut. <1%
- **AKT1**
  - 3%
  - Amplified
- **AKT2**
  - 6%
  - Amplified
- **BRAF**
  - 0.5%
  - Mutated

**Proliferation/survival**
Altered pathways in HGS-OvCa

**b** NOTCH signalling

22% of cases altered

- **JAG1**: 2% Amplified
- **JAG2**: 3% Amplified

**NOTCH3**: 11% Amplified/mutated

**MAML1**: 2% amplified/mutated
**MAML2**: 4% amplified/mutated
**MAML3**: 2% mutated

Proliferation

Inhibition

Activation

Percentage of cases (%)

<table>
<thead>
<tr>
<th>50</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivated</td>
<td>Activated</td>
</tr>
</tbody>
</table>


Altered pathways in HGS-OvCa

**c. HR alterations**

BRCA altered cases, $N = 103$ (33%)

BRCA1

BRCA2

Germline mutation | Somatic mutation | Epigenetic silencing via hypermethylation

DNA damage Sensors

<table>
<thead>
<tr>
<th>ATM</th>
<th>ATR</th>
<th>BRCA1</th>
<th>EMSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutated</td>
<td>&lt;1%</td>
<td>23%</td>
<td>Amplified, mutated</td>
</tr>
</tbody>
</table>

HR pathway 51% of cases altered

FA core complex

<table>
<thead>
<tr>
<th>FANCD2</th>
<th>BRCA2</th>
<th>RAD51C</th>
<th>PTEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1%</td>
<td>Mutated</td>
<td>11%</td>
<td>7%</td>
</tr>
<tr>
<td>Mutated</td>
<td></td>
<td>Mutated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

HR-mediated repair

Log-rank test $P = 0.0008602$
Altered pathways in HGS-OvCa

- FOXM1 signalling
  - PLK1
  - CCNB1
  - AURKB
- 84% of cases altered
  - TP53
  - ATM
- Cell cycle progression
  - BIRC5
  - CDC25B
- DNA repair
  - BRCA1
  - BRCA2
  - BRCC
  - ATR
  - RAD51
TCGA findings
TCGA: large-scale integrative view of aberrations in HGS-OvCa
Mutational spectrum “surprisingly simple”
TP53 predominated = 96%
BRCA1 and BRCA2 =22%
Seven other significantly mutated genes = 2–6%
HGS-OvCa is distinct from other histological subtypes
Clear-cell: few TP53; recurrent ARID1A, PIK3CA mutations
Endometrioid: frequent CTNNB1, ARID1A and PIK3CA; fewer TP53
Mucinous: prevalent KRAS mutations
TCGA findings
“Remarkable degree of genomic disarray”
“Striking contrast to previous TCGA findings in glioblastoma”
Mutations and promoter methylation in putative DNA repair genes (HR) may explain the high prevalence of SCNAs.
TCGA – what next?

therapeutic approaches?

50% with HR defects: PARP inhibitors

commonly deregulated pathways: RB, RAS/PI3K, FOXM1, NOTCH, provide opportunities for therapeutic treatment

Inhibitors exist for 22 genes in regions of recurrent amplification

aberrant genes or networks: targeted therapies selected to be effective ...
The State of Treatment for Newly Diagnosed Ovarian Cancer

- Complete surgical staging
- Optimal reductive surgery
- Chemotherapy

*Clinical Trials: targeted therapies*
PARP inhibition: BRCA mutant cancers

SSB: cellular metabolism, environmental exposures

Replicating cells

Normal cell
Repair by Homologous Recombination
Survival

Cancer cell with BRCA deficiency
No effective repair (No HR pathway)
PARP inhibitor

- Olaparib (AZD2281)
  - novel, orally active PARP inhibitor
  - synthetic lethality in homozygous BRCA-mut cells
Phase I/Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer

- **Results:** 45 enrolled patients
  - 37 ovarian cancer
  - 8 breast cancer

  - Phase 1 dose escalation = 30 patients
  - Phase 1b expansion = 15 patients

  - MTD = Carboplatin AUC5 on day 1 + Olaparib 400mg twice daily on days 1-7, every 21 days

Phase I/1b study of Olaparib and Carboplatin
# Phase I/Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer

## Table 4. Clinical response (n = 42)*

<table>
<thead>
<tr>
<th>Best response</th>
<th>Ovarian cancer (n = 34)†</th>
<th>Breast cancer (n = 8)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median duration in months (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
<td>1 (12.5)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>PR</td>
<td>15 (44.1)</td>
<td>6 (75)</td>
<td>21 (50.0)</td>
</tr>
<tr>
<td>PR ≥ 4 mo</td>
<td>13 (38.2)</td>
<td>1 (12.5)</td>
<td>14 (33.3)</td>
</tr>
<tr>
<td>PD</td>
<td>6 (17.6)</td>
<td>0</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>15/34 (44.1)</td>
<td>7/8 (87.5)</td>
<td>22 (52.4)</td>
</tr>
<tr>
<td>Clinical benefit rate</td>
<td>28/34 (82.3)</td>
<td>8/8 (100)†</td>
<td>36 (85.7)</td>
</tr>
</tbody>
</table>

* CR = complete response; PD = progression of disease; PR = partial response; SD = stable disease.
† One patient with ovarian cancer was not censored for the response analysis because study treatment was discontinued after one cycle because of intercurrent illness, although computed tomography assessment at 6 weeks showed stable disease.
‡ One CR in triple-negative breast cancer, six PRs in triple-negative breast cancer (n = 3) and estrogen receptor/progesterone receptor (ER/PR)–positive breast cancer (n = 3), and 1 SD in ER/PR positive breast cancer were observed.
Phase I/Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer

**Conclusions**

Oral olaparib is well tolerated in combination with carboplatin. Highly active in advanced, chemotherapy-refractory BRCA-deficient cancer. Greater activity seen at the higher concentrations. Positive proof of the concept of the activity and tolerability of genetically defined targeted therapy with olaparib in BRCA-deficient cancers.
Gene expression - subgroups

TCGA. Nature 474: 609-615 (2011)
Gene expression - immunoreactive
NF-κB signaling

NF-κB signaling

TNFα → TNFR1 → Cell membrane

- TRAF2
- TAK1
- cIAP
- IKKβ
- IKKγ
- IKKα
- IKKε
- proteasome
- P → IκBα → Nucleus
- p50, p65 → NF-κB activity → NF-κB target genes

TNF, tumor necrosis factor
IAP, inhibitor of apoptosis protein
IKK, IκB kinase
IκB, Inhibitor of NF-κB
NF-κB, nuclear factor κB

NF-κB target genes
survival, proliferation
Expression of NF-κB transcription factors and kinases is associated with poor overall survival


Hernandez...Annunziata. Cancer Res 2010
NF-κB signaling

**Diagram Overview:**

1. **Cell Membrane:** TNFα binds to TNFR1 on the cell membrane.
2. **IKKβ Inhibitor:** Acts on IKKβ.
3. **IKK Complex:** IKKβ, IKKγ, and IKKα form a complex.
4. **IKK Activation:** IKK complex phosphorylates IκBα, targeting it for degradation.
5. **IκBα Degradation:** Leads to NF-κB activation.
6. **NF-κB Translocation:** NF-κB translocates to the nucleus.
7. **NF-κB Activity:** NF-κB regulates target genes, including survival and proliferation.
Defining the Ovarian cancer-specific IKKβ gene signature

Hernandez... Annunziata. Cancer Res 2010
IKKβ gene signature is coordinately expressed in primary ovarian cancers

Hernandez... Annunziata. Cancer Res 2010
High IKKβ activity associates with worse survival in ovarian cancer

Ovarian cancer patients (n=185)

IKKβ signature expression
- low
- high

(compared to median)

p=0.02
IKKβ inhibition diminishes pro-cancer functions

- Viability
- Invasion
- Adhesion
- Cytokine secretion
- Anchorage-independent survival
Apoptosis

Intrinsic pathway
(DNA damage, virus, stress)

Extrinsic pathway
SMAC mimetic

- Second mitochondrial activator of caspases
- Normal cells:
  - SMAC released from mitochondria
  - Inhibits cIAP 1
  - Removes blockade to activated caspase function
- Cancer cells
  - Apoptosis may be dysregulated due to low SMAC or upstream blockade e.g. overexpression of IAPs
SMAC mimetic blocks NF-κB signaling
SMAC mimetic
SMAC mimetic and IKKβ inhibition induces both apoptosis and necroptosis.
Phase 2 clinical trial: SMAC mimetic for platinum-resistant ovarian cancer

- SMAC mimetic administered on days 1, 8, 15 of 28-day cycle
- 18g needle biopsy prior to start, and at cycle 2 day 15
- CT scan restaging every 2 cycles

Bunch, Noonan…Annunziata. ASCO Annual Meeting, 2014
### Correlative studies

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>NUMBER COLLECTED</th>
<th>ANALYSIS</th>
<th>MARKERS</th>
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<tbody>
<tr>
<td>Frozen tumor</td>
<td>11 pre, 7 on</td>
<td>Capillary western</td>
<td>cIAP1, cIAP2, caspase 3, caspase 8, PARP, NFkB-p65, NFkB-p100/p52,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ProteinSimple)</td>
<td>IkBa, cFLIP, RIP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug levels</td>
<td>--</td>
</tr>
<tr>
<td>Fixed tumor</td>
<td>11 pre, 7 on</td>
<td>IHC</td>
<td>TNF, TRAIL, CD3, CD19, CD56, CD68</td>
</tr>
<tr>
<td>Plasma</td>
<td>11 (x6) cycle 1</td>
<td>Drug levels</td>
<td>--</td>
</tr>
<tr>
<td>Plasma</td>
<td>11 (x2) pre/on</td>
<td>Cytokines</td>
<td>TNF, TRAIL, IL-6, IL-8</td>
</tr>
<tr>
<td>PBMC</td>
<td>11 pre, 8 on</td>
<td>Capillary western</td>
<td>cIAP1, cIAP2, caspase 3, caspase 8, PARP, NFkB-p65, NFkB-p100/p52,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ProteinSimple)</td>
<td>IkBa, cFLIP, RIP</td>
</tr>
<tr>
<td>Whole blood</td>
<td>11 pre, 9 on</td>
<td>T, B, NK cell counts</td>
<td>CD3, CD4, CD8, CD19, CD56, CD16</td>
</tr>
</tbody>
</table>

Pre, prior to treatment; SM, SMAC mimetic; IHC, immunohistochemistry; PBMC, peripheral blood mononuclear cells; PK, pharmacokinetics

SMAC mimetic depletes cIAP1

Bunch, Noonan...Annunziata. ASCO Annual Meeting, 2014
SMAC mimetic de-activates NF-κB in PBMC (but not tumors)
Clinical activity of SMAC mimetics

- Stable disease x 4 months (LCL-161)
- Stable disease x 7 months (AT-406)
- Complete response x 2 (GDC-0917)
  - Ovarian cancer, BRCA1m
  - MALT lymphoma
- Stable disease x 6 months (TL32711)
- Stable disease x 8 months (HGS 1029)
Novel combinations to enhance SMAC mimetic effect on NF-κB

Step 1: Generate single agent results.

Step 2: Generate 6X6 matrix data to uncover potential synergies.

Step 3: Expand good combinations to 10X10 blocks to confirm synergistic combinations and perform self-crosses to provide context for activities.

Synergy: CI < 1 and beta parameter < 0.5
Novel combinations to enhance SMAC mimetic effect on Nf-κB
SMAC mimetic enhances docetaxel
Women’s cancer team

Stan Lipkowitz, MD, PhD
Jung-Min Lee, MD
Ciara O’Sullivan, MD
Anne Noonan, MD
Elise C. Kohn, MD
Nicole Houston, RN
Irene Ekwede, RN
MOS Fellows and Nursing Staff

Translational scientists:
Lidia Hernandez, MS
Marianne Kim, PhD
Carrie House, PhD
Kristen Bunch, MD