ESMO Preceptorship on Ovarian Cancer

A clinician guide to cancer genomics

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Presenter Disclosures

No disclosure
# Ovarian cancer genomics

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Frequently mutated genes</th>
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<tbody>
<tr>
<td>High-grade serous carcinoma</td>
<td>TP53, BRCA1, BRCA2</td>
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<tr>
<td>Low-grade serous carcinoma</td>
<td>BRAF, KRAS</td>
</tr>
<tr>
<td>Endometrioid carcinoma</td>
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<tr>
<td>- High-grade</td>
<td>BRCA1, BRCA2, TP53 (serous ca?)</td>
</tr>
<tr>
<td>- Low-grade</td>
<td>PTEN, PIK3CA, KRAS, BRAF</td>
</tr>
<tr>
<td>Clear cell / endometrioid carcinoma</td>
<td>ARID1A, PIK3CA, PTEN, MSI</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>KRAS, HER2 (amplification, 15-20%)</td>
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<td>Other types</td>
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ARID1A mutations in clear cell and endometrioid cancer

Loss of BAF 250a expression and ARID1A mutation

Wiegand, NEJM, 2010
ARID1A mutations in clear cell and endometrioid cancer

Analysis of OCC and associated endometriosis

Wiegand, NEJM, 2010
Clear cell and endometrioid cancer

- ARID1A mutated or lost in
  46% clear cell, 30% endometrioid, <1% serous (Wiegand, 2010)

- Unknown oncogenic mechanism

- Therapeutic utility?
  Loss of ARID1A expression sensitizes cancer cells to PIK3 and AKT-inhibitors (Samartzis, 2014)

- Diagnostic utility?
Integrated genomic analysis of ovarian cancer

The Cancer Genomic Atlas (TCGA)

- Clinically annotated HGS-OvCa samples identify molecular abnormalities that influence pathophysiology, affect outcome, and constitute therapeutic target.

- Microarray analysis*: 489 samples

- Whole-exome DNA sequence: 316 samples

* mRNA expression, microRNA expression, DNA copy number, DNA promoter methylation

TCGA, Nature 2011
The Cancer Genomic Atlas (TCGA)

Inclusion criteria

New diagnosis of HGS OvCa

No prior treatment

Companion normal tissue specimen

  adjacent normal tissue

  peripheral lymphocytes

  previously extracted germline DNA
The Cancer Genomic Atlas (TCGA)

Results

22 therapeutic target genes amplified in at least 10% of cases (including MECOM, MAPK1, CCNE1, KRAS)

Alterations in cancer associated pathways:
RB1 67%, HR 50%, PI3K /KRAS 45%, Notch 22%

Opportunities for therapeutic treatments?
Genomics in ovarian cancer

• Actionable mutation: DNA change that, if detected in a patient's tumor, would be expected (or predicted) to affect a patient's response to treatment.

• Premises
  the tumor is dependent from the target
  the target is inhibited by the therapy

• Examples
  BRCA mut and PARPi in OvCa
  TP53 mut and AZD1755 in NSCL
**Clinical Trial Design**

**UMBRELLA TRIAL**
- Histology-based clinical trial evaluating different aberrations

**BASKET TRIAL**
- Histology-independent aberrations specific clinical trial

Bedard, Nat., 2013
To understand the interaction between genotype and phenotype on a genome-wide scale.

To understand the functional impact (protein and pathway activation) of some mutations.
Liquid biopsy

Analysis of tumor material obtained in minimally invasive or non-invasive manner through the sampling of blood or other body fluids
Liquid biopsy

Genetic alterations detectable in cell free circulating tumor DNA.

Diaz, JCO, 2014
Potential advantages in the use of ctDNA over tissue

• Minimally invasive - Often close to 100 % samples acquired
• Serial real-time blood samples can be taken for patients on therapy
• ctDNA can potentially reflect real time status of the tumor
• If ctDNA is representative of tumor, it could potentially be used to :
  - assess tumor burden and disease prognosis
  - support patient selection for therapy
Potential disadvantages in the use of ctDNA over tissue

- Need sophisticated and expensive equipment
- Needs high sensitivity and specificity
- Not all tumors may release ctDNA (blood-brain barrier).
## Applications of Liquid Biopsy

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<tr>
<td>Assessment of molecular heterogeneity of overall disease</td>
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<tr>
<td>Monitoring of tumor dynamics</td>
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<tr>
<td>Identification of genetic determinants for targeted therapy</td>
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<tr>
<td>Evaluation of early treatment response</td>
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<tr>
<td>Monitoring of minimal residual disease</td>
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<tr>
<td>Assessment of evolution of resistance in real time</td>
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Technologies for profiling circulating tumor DNA

Next generation sequencing
Quantitative PCR
(BEAMing), TamSeq
Droplet digital PCR

Sensitivity

2%
1%
0.01 – 0.02%
0.005%

Wide approach (exome, gene panels)
Candidate approach (one target at a time)
Cell-free circulating tumor DNA levels in different cancer types

NGS analysis on liquid biopsy in a patient clinically defined with «BRCAness» phenotype.
## Molecular testing in ovarian carcinoma

<table>
<thead>
<tr>
<th>Molecular testing</th>
<th>Histological type</th>
<th>Purpose</th>
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<tbody>
<tr>
<td>BRCA1/2 mutation analysis</td>
<td>High-grade serous carcinoma (High-grade endometrioid carcinoma)</td>
<td>Response to PARP inhibitors</td>
</tr>
<tr>
<td>Microsatellite instability (MSI) analysis</td>
<td>Non-serous carcinoma types</td>
<td>Screening for Lynch syndrome</td>
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Homologous recombination (HR) gene mutations in serous carcinoma

- 390 carcinomas of ovary, fallopian tube, and peritoneum
- Sequence analysis of 13 homologous recombination (HR) genes (BRCA1, BRCA2, ATM, BARD1, BRIP1, CHEK1, CHEK2, FAM175A, MRE11A, NBN, PALB2, RAD51C, RAD51C)
- 31% of tumors carry mutations in at least one HR gene (24% germ line, 9% somatic)

BRCA1/BRCA2: 71%
Detection of homologous recombination (HR) deficiency

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<th>Method</th>
<th>Principle</th>
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<tr>
<td>Sequencing (NGS)</td>
<td>Mutation detection in homologous recombination genes (BRCA1, BRCA2, ATM, BRIP1, CHEK2, etc.)</td>
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<tr>
<td>Array comparative genomic hybridisation (aCGH)</td>
<td>Genomic signature: copy number alterations of a defined set of genomic loci</td>
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</table>
| Myriad HRDTM assay                     | NGS-based genome-wide SNP analysis  
3 quantitative parameters of genomic instability:  
• Loss-of-heterozygosity (LOH) score  
• Telomeric allelic imbalance (TAI) score  
• Large-scale state transitions (LST) score  
“Genomic scars”                           |
| Microarray-based gene expression analysis | Gene expression profile (44-gene DNA damage response deficiency signature, DDRDS)                                                       |
Molecular testing for Lynch Syndrome

- autosomal dominant familiar cancer risk syndrome
- due to a germline mutation in one of MMR genes in the tumor (MLH1, MSH2, PMS2, MSH6)
- 42-54 % risk of endometrial and 6 – 12 % risk of OvCa
Lynch syndrome-associated OvCa

- 2% of all OvCa carcinomas
- 10-15% of hereditary OvCa carcinomas
- Lifetime risk: 3-11 % (depends to the type of germline mutation, e.g. MSH2 > > MLH1)
- Histology: non-serous (clear cell, endometrioid, undifferentiated)
- No morphological criteria indicative of association with Lynch syndrome
**Revised Bethesda guidelines for Lynch syndrome (2003)**

Tumors from individuals should be tested for MSI in the following situations:

1. Colorectal cancer diagnosed in a patient who is <50 years of age.
2. Presence of synchronous, metachronous colorectal, or other Lynch syndrome-related tumors\(^1\), regardless of age.
3. Colorectal cancer with the MSI-high histology\(^2\) diagnosed in a patient who is <60 years of age.
4. Colorectal cancer diagnosed in one or more first-degree relatives with an Lynch syndrome-related tumor, with one of the cancers being diagnosed under age 50 years.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with Lynch syndrome-related tumors, regardless of age.

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1 Lynch syndrome-related tumors include colorectal, endometrial, gastric, ovarian, pancreatic, ureteral and renal pelvis, biliary tract, and brain (usually glioblastoma as observed in Turcot syndrome) tumors, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.

2 Presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern. MSI.

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Microsatellite instability (MSI)

Condition of genetic hypermutability due to defect of DNA mismatch repair (MMR) accumulation of errors with short DNA repeat sequencies.
Microsatellite instability testing

Dissection of tumor cells and non-tumor cells

<table>
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<tr>
<th>Instability</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>No Marker</td>
<td>No MSI</td>
</tr>
<tr>
<td>1 Marker</td>
<td>Low-grade MSI</td>
</tr>
<tr>
<td>≥ 2 Marker</td>
<td>High-grade MSI</td>
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Microsatellites of the Bethesda panel (BAT25, BAT26, D5S346, D2S123, D17S250)
Microsatellite length change (gel/capillary electrophoresis)
Microsatellite instability testing

Immunohistochemistry

To detect the presence / absence of the protein products of the MMR genes (MLH1, MSH2, MSH6 and PMS2)

83% sensitivity; 89% specificity
## Prevention and screening strategies for Lynch syndrome

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<th>Screening</th>
<th>Prevention/risk reduction</th>
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<tr>
<td>MLH1, MSH2, MSH6, EPCAM and PMS2 mutations</td>
<td>1) Consider risk-reducing hysterectomy and RRSO after completion of childbearing</td>
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<tr>
<td>1) Annual colonoscopy from age 20-25</td>
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<tr>
<td>2) Annual neurological examination for screening of CNS tumours may be considered</td>
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<tr>
<td>3) Annual endometrial ultrasound + biopsies from age 30-35 may be considered</td>
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A clinician guide to cancer genomics

Conclusions

Main clinical applications

Molecular diagnostic: type of molecular signature

Molecular testing

Functional genomics and identification of actionable mutations

Liquid biopsies: future developments