Genomic/morphological classification of endometrial carcinoma

Case presentation

- 49 year old woman with vaginal bleeding
- Underwent endometrial biopsy reported as “serous carcinoma”
- Hysterectomy, salpingo-oophorectomy, omentectomy and lymphadenectomy performed
- Findings: 2 cm sessile tumor confined to endometrium; no extra-uterine disease
Harnessing TCGA data for diagnosis

Aberrant p53?

- No
- Yes

EMC

PTEN
ARID1A
DNA MMR

Lost
Retained

EMC or clear cell
SC

Immunophenotype

- p53: overexpressed (aberrant)
- p16: overexpressed (aberrant)
- PTEN: loss of expression (aberrant)
- ARID1A: retained expression
- DNA mismatch repair: loss of MSH6 expression (aberrant)
**ARID 1A**

- Tumor suppressor gene
- Encodes BAF250a
  - Involved in chromatin remodeling (SWI-SNF)
- Mutated in human cancers
  - Endometriosis assoc OVCA
  - Endometrial cancer
- Anti-BAF250a antibody

Differential diagnosis

- Serous carcinoma
- “High-grade endometrial carcinoma”

Differential diagnosis

• Serous carcinoma
• “High-grade endometrial carcinoma”

POLE mutational analysis performed

POLE exonuclease domain mutation present

POLE and MMR functions

DNA mismatch repair system
POLE mutated carcinoma, resembling serous carcinoma, with excellent prognosis

Prototypical high grade endometrial carcinoma (EMC)
Molecular signatures and immunophenotype

• Endometrioid
  – Mutations: *PTEN, PIK3CA, ARID 1A, DNA MMR, KRAS, CTNNB1, TP53*
  – IHC: aberrant PTEN, ARID 1A, DNA MMR, β-catenin, p53


Molecular signatures and immunophenotype

• Serous
  – Mutations: *TP53, PPP2R1A, PIK3CA*
  – IHC: aberrant p53, high p16

Molecular signatures and immunophenotype

- Clear cell carcinoma
  - Mutations: PIK3CA, ARID 1A, TP53, DNA MMR
  - IHC: HNF-1β, aberrant ARID 1A, p53, DNA MMR

High grade EMC: Problem

- Many (30-50%) high grade EMCs are not prototypic
- Examples:
  - “endometrioid vs serous carcinoma”
  - “endometrioid carcinoma vs clear cell carcinoma”
  - “serous carcinoma vs clear cell carcinoma”
High-grade EMC: poor diagnostic reproducibility

- Vancouver General Hospital case series
  - Only 64% of cases diagnosed uniformly (n=59; 3 pathologists)
  - Problem: FIGO G3 endometrioid


Background:
Endometrial carcinoma TCGA subgroups

TCGA study: Integrated Genomic Characterization of Endometrial Carcinoma
Nature. 2013 May 2;497(7447):67-73

Background:
Endometrial carcinoma TCGA subgroups

TCGA study: Integrated Genomic Characterization of Endometrial Carcinoma
Nature. 2013 May 2;497(7447):67-73
POLE morphology


POLE morphology


MSI-H morphology

MSI-H morphology


Background:
FIGO G3 EMC is genomically promiscuous

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG EMC</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>HG EMC</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Serous</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>

TCGA study: Integrated Genomic Characterization of Endometrial Carcinoma
Nature. 2013 May 2;497(7447):67-73

Background:
FIGO G3 EMC is genomically promiscuous

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG EMC</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>HG EMC</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>1/4</td>
</tr>
<tr>
<td>Serous</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3/4</td>
</tr>
</tbody>
</table>

Clinical outcomes
Highly favorable  Intermediate  Intermediate  Poor

TCGA study: Integrated Genomic Characterization of Endometrial Carcinoma
Nature. 2013 May 2;497(7447):67-73
Background: Clinical applications

• Model TCGA Series
  — Stratified (N=143) endometrial carcinomas into 4 TCGA molecular groups
  — POLE exonuclease domain mutations
  — Mismatch repair protein IHC
  — p53 IHC


Background: Clinical applications

• Independent associations with clinical outcomes:
  — Molecular classification
  — Clinical risk group

• No independent association with clinical outcomes:
  — Histotype


Pilot project:
Significance of aberrant p53 expression in FIGO G3 EMC

<table>
<thead>
<tr>
<th>Recurrence</th>
<th>Total Normal Expression (p53)</th>
<th>Total Abnormal Expression (p53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>15 (68%)</td>
<td>7 (32%)</td>
</tr>
<tr>
<td>No</td>
<td>40 (95%)</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>

Levine D, et al. SGO abstract 2015
Objectives

• To apply the simplified TCGA-based classifier (PROMISE) to explore genomic promiscuity of FIGO G3 EMCs

• To determine whether PROMISE stratifies FIGO G3 EMCs into different clinical risk categories

Methods

• IRB approval

• 192 FIGO grade 3 endometrioid carcinomas (FIGO G3 EMC) from VGH and MSKCC
  – 123 cases (64%) from VGH, selected from the study presented just previously
  – 69 cases (36%) from MSKCC, selected by identifying recurrent cases with subsequent 2:1 matching for non-recurrent cases
Demographic data

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Entire cohort</th>
<th>VGH cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 years (0-12)</td>
<td>4 years (0-6)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Entire cohort</th>
<th>VGH cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>65 years (33-96)</td>
<td>68 years (38-96)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>Entire cohort</th>
<th>VGH cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>40%</td>
<td>37%</td>
</tr>
<tr>
<td>IB</td>
<td>30%</td>
<td>31%</td>
</tr>
<tr>
<td>II</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>IIIA</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>IIIB</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>IIIC</td>
<td>13%</td>
<td>11%</td>
</tr>
<tr>
<td>IV</td>
<td>6%</td>
<td>6%</td>
</tr>
</tbody>
</table>

Results

• Simplified TCGA classifier (PROMISE)
  – Stratified (N=192) endometrial carcinomas into 4 TCGA molecular groups
    – POLE exonuclease domain mutations
    –Mismatch repair protein IHC
    – p53 IHC

Demographics stratified by PROMISE cluster

<table>
<thead>
<tr>
<th>TCGA cluster</th>
<th>Age (median, n=183)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=23; 12%)</td>
<td>58 yrs (38-92)</td>
</tr>
<tr>
<td>2 (n=78; 41%)</td>
<td>64 yrs (33-89)</td>
</tr>
<tr>
<td>3 (n=55; 29%)</td>
<td>66 yrs (36-95)</td>
</tr>
<tr>
<td>4 (n=36; 19%)</td>
<td>69 yrs (49-92)</td>
</tr>
</tbody>
</table>
### Demographics stratified by PROMISE cluster

<table>
<thead>
<tr>
<th>TCGA cluster</th>
<th>Age (median, n=183)</th>
<th>Stage (n=186)</th>
<th>Stage (n=186)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=23; 12%)</td>
<td>58 yrs (38-92)</td>
<td>I/II 95%</td>
<td>III 5%</td>
</tr>
<tr>
<td></td>
<td>65% &lt; 60 yrs</td>
<td></td>
<td>IV 0%</td>
</tr>
<tr>
<td>2 (n=78; 41%)</td>
<td>64 yrs (33-89)</td>
<td>I/II 77%</td>
<td>III 19%</td>
</tr>
<tr>
<td></td>
<td>29% &lt;60 yrs</td>
<td></td>
<td>IV 4%</td>
</tr>
<tr>
<td>3 (n=55; 29%)</td>
<td>66 yrs (36-95)</td>
<td>I/II 74%</td>
<td>III 17%</td>
</tr>
<tr>
<td></td>
<td>36% &lt;60 yrs</td>
<td></td>
<td>IV 9%</td>
</tr>
<tr>
<td>4 (n=36; 19%)</td>
<td>69 yrs (49-92)</td>
<td>I/II 82%</td>
<td>III 12%</td>
</tr>
</tbody>
</table>

#### Univariable Cox regression

<table>
<thead>
<tr>
<th>Classifier</th>
<th># of events / n</th>
<th>Hazard Ratio (95% CI)</th>
<th>LRT P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMR IHC abn</td>
<td>57 / 192</td>
<td>0.85 (0.46-1.58)</td>
<td>0.039</td>
</tr>
<tr>
<td>POLE EDM</td>
<td></td>
<td>0.27 (0.05-0.84)</td>
<td></td>
</tr>
<tr>
<td>p53 abn</td>
<td></td>
<td>1.50 (0.74-2.95)</td>
<td></td>
</tr>
<tr>
<td>DSS</td>
<td>44 / 184</td>
<td>0.67 (0.33-1.36)</td>
<td>0.264</td>
</tr>
<tr>
<td>MMR IHC abn</td>
<td>0.67 (0.33-1.36)</td>
<td>0.264</td>
<td></td>
</tr>
<tr>
<td>POLE EDM</td>
<td></td>
<td>0.24 (0.05-0.74)</td>
<td></td>
</tr>
<tr>
<td>p53 abn</td>
<td></td>
<td>1.48 (0.70-2.72)</td>
<td></td>
</tr>
</tbody>
</table>
Progression Free Survival

Disease Specific Survival

Study Design

- Inclusion criteria:
  - grade 3 Endometrioid Endometrial Cancer all stages with clinical follow-up

- International collaboration:
  - N cases (%)
  - Total: 430 (100)
  - Vancouver, Canada: 126 (29.1)
  - Leiden, The Netherlands: 113 (26.3)
  - New York, USA: 69 (16.2)
  - Utero, Spain: 50 (11.7)
  - Boston, USA: 31 (7.4)
  - Birmingham, UK: 21 (5.0)

- TCGA allocation:
  - POLE mutant: pathogenic mutation in exons 9-14 (Sanger or NGS)
  - MMR/LOH: loss of expression by IHC (MSH2, MSH6, MLH1, MSSH)
  - Abnormal p53: "mutant-like" expression by IHC
  - MMR/lo/SS-p53 abnormal

- Statistics:
  - OS, PFS using KM methods (Log rank Test)
  - Uni-multivariate analysis (Cox proportional hazard models)
Molecular heterogeneity among grade 3 EEC

Total cohort gr 3 EEC
N=405

Stage IA
N=131
18.2%

Stage IB
N=114
10.4%

Stage II
N=90
2.2%

Stage II-IV
N=90
42%

Frequency of POLE mutations is relatively high in grade 3 EEC, and there is a significant association with early stage.

Molecular heterogeneity among grade 3 EEC

<table>
<thead>
<tr>
<th>POLE</th>
<th>MMRd</th>
<th>NSMP</th>
<th>TP53</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGO</td>
<td>Stage IA</td>
<td>35</td>
<td>18.2</td>
<td>62</td>
<td>36.9</td>
</tr>
<tr>
<td>2009</td>
<td>Stage III</td>
<td>14</td>
<td>10.4</td>
<td>63</td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td>Stage II-IV</td>
<td>2</td>
<td>1.2</td>
<td>45</td>
<td>58.0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>12.1</td>
<td>170</td>
<td>42.0</td>
<td>111</td>
</tr>
</tbody>
</table>

Age, years mean (range): 66 ± 42.93, 57 ± 27.93, 66 ± 33.82, 66 ± 36.94

- High frequency of POLE mutations and MMRdeficiency in grade 3 EEC
- POLE mutations are significantly associated with stage I disease, whereas MMRdeficiency is more frequent in higher stages.

Stratifying grade 3 EEC using TCGA classifiers

Overall Survival

Progression Free Survival

Grade 3 Endometrioid Endometrial Cancer is a heterogeneous disease in which the TCGA classifiers identify subgroups with distinct clinical outcome.
### Multivariate Analysis Grade 3 EEC All Stages (N=405)

<table>
<thead>
<tr>
<th></th>
<th>Overall Survival</th>
<th></th>
<th>Progression Free Survival</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>p-value</td>
<td>HR</td>
</tr>
<tr>
<td>POLE</td>
<td>0.43</td>
<td>0.20-0.91</td>
<td>0.028</td>
<td>0.14</td>
</tr>
<tr>
<td>MMRd</td>
<td>0.71</td>
<td>0.49-1.04</td>
<td>0.082</td>
<td>0.62</td>
</tr>
<tr>
<td>NSMP</td>
<td>1.45</td>
<td>0.95-2.18</td>
<td>0.101</td>
<td>1.87</td>
</tr>
<tr>
<td>PS3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.04</td>
</tr>
<tr>
<td>FIGO IA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>IB</td>
<td>1.17</td>
<td>0.79-1.75</td>
<td>0.63</td>
<td>1.68</td>
</tr>
<tr>
<td>HRv</td>
<td>3.72</td>
<td>2.52-5.50</td>
<td>&lt;0.001</td>
<td>4.04</td>
</tr>
<tr>
<td>age</td>
<td>1.04</td>
<td>1.00-1.09</td>
<td>&lt;0.001</td>
<td>1.00</td>
</tr>
</tbody>
</table>

- POLE and MMRd remain a independent prognostic factors for better OS and PFS in a multivariate analysis.
- PS3 and FIGO stage remains a independent prognostic factor for worse OS and PFS in a multivariate analysis.

### PFS Grade 3 EEC Stage IA and IB Using TCGA Class

- FIGO Stage I Grade 3 EEC is not a homogenous disease, and TCGA classifiers can help identify patients with distinct disease outcomes.

### Multivariate Analysis Stage I Grade 3 EEC (N=315)

<table>
<thead>
<tr>
<th></th>
<th>Overall Survival</th>
<th></th>
<th>Progression Free Survival</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>p-value</td>
<td>HR</td>
</tr>
<tr>
<td>POLE</td>
<td>0.38</td>
<td>0.17-0.85</td>
<td>0.018</td>
<td>0.18</td>
</tr>
<tr>
<td>MMRd</td>
<td>0.49</td>
<td>0.30-0.80</td>
<td>0.004</td>
<td>0.61</td>
</tr>
<tr>
<td>NSMP</td>
<td>1.28</td>
<td>0.78-2.13</td>
<td>0.333</td>
<td>2.42</td>
</tr>
<tr>
<td>PS3</td>
<td>1.13</td>
<td>0.75-1.69</td>
<td>0.56</td>
<td>1.76</td>
</tr>
<tr>
<td>FIGO IA</td>
<td>1.10</td>
<td>0.75-1.62</td>
<td>0.333</td>
<td>1.76</td>
</tr>
<tr>
<td>FIGO IB</td>
<td>1.06</td>
<td>1.04-1.10</td>
<td>&lt;0.001</td>
<td>1.00</td>
</tr>
<tr>
<td>age</td>
<td>1.06</td>
<td>1.04-1.09</td>
<td>&lt;0.001</td>
<td>1.00</td>
</tr>
</tbody>
</table>

- POLE mutations remains a independent prognostic factor for better OS and PFS in a multivariate analysis.
- Abnormal p53-IHC remains a independent prognostic factor for worse OS and PFS in a multivariate analysis.
In Grade 3 Endometrioid Endometrial Cancer:

- Stage remains an important prognostic factor
- Molecular classification is feasible with straightforward techniques such as IHC and hotspot sequencing of POLE
- POLE mutations are frequent and associated with early stage
- POLE is a prognostic factor for better and p53 for worse OS and PFS

---

Summary

- FIGO G3 EMCs comprise a mixture of different molecular subtypes
- FIGO G3 EMCs do not constitute a homogeneous clinicopathological entity
- PROMISE classifier can be performed using available technology
- PROMISE classification segregates FIGO G3 EMCs into clinically baseline, indolent and aggressive categories

---

Recent developments

- Genotypic heterogeneity underlies diagnostic difficulties
- Application of PROMISE classifier results in superior interobserver agreement
- PROMISE classifier can be used in biopsy material the results of which are concordant with tumor in hysterectomy
Recent developments

• PROMISE-like classifiers or NGS applied to:
  – Clear cell carcinoma
    • POLE and MMR groups are prognostically favorable
  – Un/de-differentiated carcinoma
    • POLE and MMR groups may be prognostically favorable
  – Carcinosarcoma
    • Rare POLE and MMR tumors identified
• Emerging evidence that POLE and MMR tumors are chemo/radiosensitive
  – Immunotherapy?

Unresolved issues

• Necessity of distinguishing histotypes within cluster 4
• How the PROMISE classifier should be used in concert with clinical risk group stratification and thereby affect clinical management
• Heterogeneity within the CN-L group
  – CTNNB1 mutation
  – ESR1 mutation
  – L1CAM expression
Some inter-institutional heterogeneity

Endometrial carcinoma TCGA subgroups: what's important?

Background:
Prevalence of TP53 mutations in genomic subtypes of endometrial carcinoma

Background:
Many serous-like FIGO grade 3 endometrioid carcinomas are histologically endometrioid


Background:
Some serous-like FIGO grade 3 endometrioid carcinomas have endometrioid-type mutations


Background:
Clinical applications