Applications of Molecular Diagnostics in Current Practice: Gynaecological Pathology

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Introduction

Molecular pathology: basis for modern disease classification (WHO 2014), and therefore patient management
Molecular diagnostics in gynaecological pathology

• Diagnosis
  – IHC correlates of many molecular abnormalities guide clinical practice
  – Tumour-specific genomic events (mutation/translocation/other)

• Genetic testing for heritable cancer syndromes
  – Correct diagnosis of tumour
  – Awareness of association with heritable syndrome

• Targeted therapy

For all molecular tests: Optimal tumour sampling for molecular testing
Outline

• Lower Genital Tract neoplasia:
  • HPV testing
  • Role of p16 immunostaining
  • HPV ISH

• Uterine neoplasia
  • Classification of endometrial carcinoma
  • Lynch Syndrome testing
  • Endometrial stromal sarcoma

• Ovarian neoplasia
  • Ovarian cancer histotypes
  • Non-epithelial tumours

• Gestational trophoblastic disease

• Vulval (non-squamous) neoplasia
  • Melanoma
  • Non-epithelial tumours

• Hereditary tumour syndromes
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Cervical Screening: HPV testing

• Current Applications:
  • Triage of low grade cytological abnormality
  • Test of Cure for all grades of CIN and CGIN

• Imminent Application:
  • HPV primary testing
Role of p16 in diagnosis of HPV-associated neoplasia

Figure 3  Stimulation of cell-cycle progression by high-risk HPV types

Doorbar J Clin Sci 2006;110:525-41
• BLOCK staining = continuous strong nuclear or nuclear + cytoplasmic staining of the basal layer with upward extension involving at least 1/3rd the epithelial thickness (1/3rd arbitrary but ↑ specificity)
• (Does not need to be full thickness)
• All other patterns are negative
Indications for p16 IHC in LGT

• To confirm a diagnosis of high grade CIN (or other –IN)
  – HG -IN vs mimics (including florid low grade –IN)
  – All –IN2

• To distinguish between HPV-mediated and non-HPV cancers
Biomarkers in CIN: LAST Project (WG4)

- Evidence for recommending usage in routine practice is ONLY available for p16 at present
- Pro-ExC and Ki67 can be used as adjuncts (clearer nuclear stains) but up to local practice/individual preference
- p16 recommendations applicable to HPV-related lesions at all sites
Recommendations for p16 in CIN (LAST project, WG4)

1. HG CIN vs mimic: strong and diffuse block-positive p16 → HGCIN

2. To help clarify a diagnosis of CIN2: negative or non-block-positive staining → LGCIN or non-HPV associated pathology (p16 should not be used if H&E DD is between LGCIN and negative, as CIN1 can be p16 +/-; p16 IHC is not recommended if diagnosis is “obvious” CIN1)

3. Adjudication in professional disagreement when DD includes HG CIN

4. Should not be used as routine adjunct in biopsies with morphologic interpretations of negative, -IN1 or -IN3

5. Recommended as adjunct for biopsies ≤ -IN1 at HIGH RISK for missed HG disease:
   - previous cytology of high grade dyskaryosis
   - borderline suspicious for HG
   - glandular abnormality
   - borderline with HPV +ve

Other Indications

• HPV-mediated vs HPV-independent carcinoma
  – Cervical adenocarcinoma (rarely SCC) vs Gastric type and others
  – Vulval SCC: Major implications for prognosis; important to classify as HPV-related or HPV-independent

• Cervical vs endometrial carcinoma
  • Use panel: ER, vimentin, CEA, MMR
  • High grade EC are p16 positive
Vulval Squamous cell carcinoma: Two pathways

- HPV-dependent
  - p16 positive
  - Preceded by uVIN
  - Younger patients
  - Better outcome; conservative surgery

- HPV-independent
  - p53 mutation most cases (IHC difficult)
  - Background LS
  - Older patients
  - Poor outcomes; wide margins recommended
p16 and Outcome in VSCC

In multivariable analysis, prognostic effect independent of age and stage. McAlpine *Histopathol* 2017
Survival by p16 Status and Surgical Era

Disease specific survival

Current practice
Radical *en bloc*
Current practice
HPV CISH

- Can be used as adjunct to p16
- Two patterns:
  - Integrated: coarse dot positivity in nuclei
  - Episomal: fine granular cloud-like staining
  - Beware non-specific precipitation: rod-like
- SCC staining is generally stronger while that in adenocarcinoma tends to be focal and fainter, often episomal pattern
- Alternative is HPV testing by PCR: more sensitive but less specific than ISH
HPV CISH
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• Hereditary tumour syndromes
Uterine neoplasia: Endometrial carcinoma

• Type 1: Endometrioid, oestrogen driven, favourable prognosis

• Type 2: Serous, ?clear cell, carcinosarcoma, oestrogen-independent, aggressive clinical course
Endometrial cancer: Molecular groups

TCGA, Nature 2013
Endometrial cancer: Molecular groups

• 4 distinct groups:
  – POLE
  – MSI
  – Copy-number low (endometrioid)
  – Copy-number high (serous-like)

TCGA, Nature 2013
Endometrial carcinoma: Molecular classification strategies being developed

Model: MMR IHC/POLE mut/p53 IHC

(n=143)

MMR MSH-high

MMR MSS

MMR IHC abn (n=41)

POLE mutated (n=102)

POLE wild type (n=88)

p53 IHC (1+)

p53 IHC (0 or 2+)

p53 wt (n=63)

p53 abn (n=25)

p53 missing

Unclassifiable (n=2)

Molecular Classification of EC

- Not yet incorporated into clinical practice
- Universal MMR IHC recommended +/- MSI (PCR-based, commercially available)
- POLE exonuclease domain mutation testing not commercially available but can be done on NGS platform (research)
- POLE/ultramutated cases do very well with conventional Rx (?need adjuvant RX at all)
- p53 IHC serves as good surrogate for TP53 mutation
- So with MMR and p53 IHC we can classify for current clinical usage
- Others in pipeline: CTNNB1 mutation, L1CAM expression, others
Lynch Syndrome testing in EC: Background

• An estimated 175,000 people in the UK have LS

• <5% are known to have LS; UNDER-RECOGNISED!

• Lifetime risk for CRC and EC is approximately 60%

• Autosomal dominant; caused by mutation in 1 of 4 DNA mismatch repair (MMR) genes: MLH1, MSH2, MSH6 or PMS2 (also EPCAM in CRC)
Lynch Syndrome testing in EC: Background

- February 2017, NICE guidance on CRC: ALL patients tested
- Reasons:
  - Surveillance/prevention of second malignancy
  - Extent of surgery: partial vs subtotal colectomy
  - Low risk of metastatic cancer
  - Prediction of chemosensitivity (5FU)
  - Screening and surveillance of family members (aspirin, colonoscopy)
Lynch Syndrome in Gynaecological Malignancies

<table>
<thead>
<tr>
<th>Group</th>
<th>ICD-9</th>
<th>Organ</th>
<th>Females</th>
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<tr>
<td>Urinary tract cancers</td>
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<tr>
<td></td>
<td>189</td>
<td>Kidney/ureter</td>
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Moller et al, Gut 2017
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Moller et al, Gut 2017
2-5% EC is due to LS

LS mutations in EC

Win et al, JNCI 2013
Incidence of EC by age and mutation in LS

Median age: 46
Range: 25-68

Moller et al, Gut 2017
Risk of further malignancy

• Over 50% of LS patients who develop cancer will have EC as their FIRST (sentinel) malignancy

• 55% women with LS who have EC go on to develop another malignancy

• 15% go on to have two or more malignancies
Risk of malignancy after EC in LS

<table>
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<th>Cancers following endometrial cancer*</th>
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<th>31</th>
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<tr>
<td>Vulva</td>
<td>1</td>
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</tr>
<tr>
<td>Head and neck (ill-defined tumor)</td>
<td>1</td>
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Win et al, JNCI 2013
Reasons to screen

• Risk of subsequent malignancy

• Screening of family members

**Surveillance and preventative strategies:**

• Reduction in incidence

• SUBSTANTIAL reduction in mortality
Why screen: summary

• Surveillance for subsequent cancers saves lives
• Screening of family members (and if + surveillance/preventative measures)
• MSI tumours have better outcome than most other molecular groups
• More responsive to RT
• Eligible for immune checkpoint inhibitor Rx
• ?candidates for progesterone Rx (not primarily oestrogen driven)
Who should be screened?

Limitations of targeted screening
- Only 30-40% of all patients have a suspicious family history of LS-related cancers
- Using 50 years of age as a cut off, misses half of women of LS (particularly MSH6)
- Screening with EC histologic features is only 70% sensitive and 40% specific; most cases have endometrioid morphology with no distinct features

Who should be screened?
- All new diagnoses of EC
- (All endometrioid and clear cell ovarian carcinomas)
How to screen?

• Molecular testing is recommended:
  
  • Immunohistochemistry for mismatch repair proteins (MMR IHC)
    • MLH1 promoter methylation testing to exclude sporadic cases
  
  • Microsatellite instability testing (MSI)

• Screen positive patients should be offered germline mutation testing
**MMR IHC**

**Table 1. Genetic defect in one of the 4 MMR genes and the corresponding IHC staining patterns expected.**

<table>
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<th>IHC staining pattern</th>
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<tr>
<td><strong>MLH1</strong></td>
<td>MLH1 $-/+$, $^b$ PMS2 $-$</td>
</tr>
<tr>
<td><strong>PMS2</strong></td>
<td>MLH1 $+/-$, PMS2 $-$</td>
</tr>
<tr>
<td><strong>MSH2</strong></td>
<td>MSH2 $-$, MSH6 $-$</td>
</tr>
<tr>
<td><strong>MSH6</strong></td>
<td>MSH2 $+$, MSH6 $-$</td>
</tr>
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$^a$ Germ line mutation—or, in the case of MLH1, gene silencing by somatic promoter hypermethylation in addition to germ line mutation—leads to loss of the protein.

$^b$ Occasionally, interpretation of IHC analysis can be problematic; abnormal methylation or some MLH1 mutations may produce false-normal MLH1 IHC staining [Umar et al. (28)].

Tafe et al, Clin Chem 2014
Mismatch repair gene expression

MLH1  MSH2
PMS2  MSH6
MMR IHC: difficulties

- Fixation affects IHC detection (use BIOPSIES)
- Staining protocol should be standardised with appropriate QC
- Unusual patterns (RARE!):
  - False positive, with missense mutations, non-functional protein with retained antigenicity
  - Dot-like nuclear staining (may be very strong; protocol-dependent)
  - Cytoplasmic expression, eg with mutation affecting nuclear transportation
  - Heterogeneous staining, subclonal loss
**MMR IHC**

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Tafe et al, Clin Chem 2014
MLH1 Methylation Testing

- NOT all tumors with loss of MMR proteins are due to Lynch Syndrome
- The majority of MLH1 loss (80% of cases) is due to MLH1 promoter hypermethylation (sporadic, non-hereditary)
- (BRAF testing does NOT distinguish sporadic EC cases as in CRC)
MLH1 Methylation Testing

- Methylation of the MLH1 promoter silences gene transcription
- Multiple techniques available to detect this in the lab

http://mmg-233-2014-genetics-genomics.wikia.com
MSI testing
Screening algorithms

• Neither test is 100% sensitive

• MMR IHC considered superior to MSI as first test:
  • MSH6 is sometimes MSS
  • Directs genetic testing
  • More readily available
  • Cheaper

• Algorithm adopted depends on local resources
Screening algorithms

• MMR IHC on all (➔ MLH1 promoter methylation testing if MLH1 and PMS2 loss; if negative) ➔ Germline testing

• MSI on all ➔ MMR IHC (➔ MLH1 promoter methylation testing if MLH1 and PMS2 loss; is negative) ➔ Germline testing

• MSI and MMR IHC normal BUT strong clinical suspicion: Consider Germline testing
Conclusions (likely upcoming recommendations)

• MMR IHC/MSI testing is recommended for all new diagnoses of EC (and selected OC)
• Identifies women at 10% or (substantially greater) risk of LS thereby reaching threshold for genetic counselling/testing
• Identifies a molecular group of EC with prognostic and predictive implications
p53 IHC

• Surrogate for underlying \textit{TP53} mutation
• Identifies serous-like (CN-H) EC
• Also tubo-ovarian HGSC, DVIN and related VSCC (algorithms for reporting being developed), LMS
• Standardisation of protocol is essential
• Classically “all or none”
• Recent identification of further patterns
Interpretation of p53 immunohistochemistry

- No TP53 mutation

Wild type pattern

- Nonsynonymous = missense
  - p53 overexpression

Abnormal

- Stopgain
  - Stopgain Indel
  - Splicing
  - p53 complete absence

- p53 cytoplasmic

### p53 immunohistochemistry pattern and interpretation

<table>
<thead>
<tr>
<th>Pattern</th>
<th>p53 IHC Interpretation</th>
<th>TP53 mutation type</th>
<th>% in HGSC</th>
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<tbody>
<tr>
<td><strong>TP53 MUTATION ABSENT</strong></td>
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<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>Normal</td>
<td>No mutation</td>
<td>0</td>
</tr>
<tr>
<td><strong>TP53 MUTATION PRESENT</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Overexpression</td>
<td>Abnormal</td>
<td>Non-synonymous (missense); also in-frame deletion, splicing</td>
<td>66%</td>
</tr>
<tr>
<td>Complete absence/ null</td>
<td>Abnormal</td>
<td>Indels, stopgains, splicing mutations</td>
<td>25%</td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>Abnormal</td>
<td>Indels and stopgains with disruption of the nuclear localization domain</td>
<td>4%</td>
</tr>
<tr>
<td>Wild type</td>
<td>Normal*</td>
<td>Truncating mutation</td>
<td>5%</td>
</tr>
</tbody>
</table>

HGSC- high-grade serous carcinoma
Technical and interpretive performance characteristics of p53 immunostaining: British Association of Gynaecological Pathologists (BAGP), United Kingdom National External Quality Assessment Service (UKNEQAS) and Canadian Immunohistochemistry Quality Control (CIQC) collaborative project
p53 IHC Results

• 38 labs
• 32 UK, 5 mainland Europe, 1 Asia
• 98 interpretation results: 88 pathologists, 10 BMS
• 31 labs returned slides for central review (87 participants)
• Total 87 interpretation responses x 42 cores = 3654 results for interpretation analysis
## p53 Interpretation Summary

<table>
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<tr>
<th>Participant Result</th>
<th>Review result</th>
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<td>561</td>
<td>2044</td>
<td>614</td>
<td>3654</td>
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**Overall concordance (excluding NA): 2717/2974 (91.3%)**
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Single commonest reason for discrepancy: WEAK staining
Optimal On-slide Control for p53: TONSIL
Conclusions from p53 IHC staining project

- **VERY GOOD** performance characteristics of p53 IHC
  - High agreement in diagnostic interpretation (91.3%)
  - Results probably underestimate accuracy in practice
  - **Aim to achieve 95% agreement**
- Recognition of all patterns ([www.thebagp.org](http://www.thebagp.org) - resources)
- Room for improvement in technical protocols:
  - Use of good control
  - Set to show strong staining
Uterine neoplasia: Endometrial stromal sarcoma

Molecular classification defines prognostically distinct groups of endometrial sarcoma

- **WHO 2003 Classification**
  - LGESS
  - Undifferentiated endometrial sarcoma (UES)

- **WHO 2014 Classification**
  - LGESS w JAZF1-SUZ12
  - LGESS fusion-neg
  - HGESS w YWHAE-NUTM2
  - UUS (UES) fusion-neg

Log rank test $P<0.001$

Slide courtesy of Dr CH Lee
Classical low grade ESS

- Simple karyotype
- Chromosomal translocations → genetic fusions of genes/proteins in chromatin remodelling (i.e. polycomb complex proteins, histone acetyl/methyltransferase)
  - $t(7;17)(p15;q21) \rightarrow \text{JAZF1-SUZ12}$
  - 6p21 rearranged → JAZF1-PHF1, EPC1-PHF1*
  - $t(1;6)(p34;p21) \rightarrow \text{MEAF6-PHF1*}$
  - $t(X;17) \rightarrow \text{MBTD1-CXORF67}$
  - $t(X;22) \rightarrow \text{ZC3H7B-BCOR*}$
High grade ESS

- Myopermeative tumor with LVI (similar to LGESS)
- High grade round cell area (present in nearly all cases)
  - Monomorphic nuclear features but with greater nuclear atypia compared to LGESS
    - Larger nuclei with more irregular contour
    - > 10MF/10HPF (average 21)
    - Tumor necrosis present

Lee et al, AJSP 2012;36:641-53
YWHAE-HGESS - high-grade area consistently shows diffuse strong cyclin D1 nuclear positivity (Low-grade variable)

Lee et al, AJSP. 2012;36(10):1562-70.
WHO 2014

UUS*
- Complex karyotype
- Post-menopausal
- Poor prognosis
  (no effective treatment)

HG ESS
- YWHAE-NUTM2
- Pre- and post-menopausal
- Intermediate prognosis
  (adjuvant radiation/chemotherapy strategy if stage 2 or higher)

LG ESS
- JAZF1-SUZ12
- JAZF1-PHF1
- EPC1-PHF1
- MEAF6-PHF1
- ZC3H7B-BCOR
- MBTD1-CXORF67
- Peri-menopausal
- Good prognosis
  (anti-estrogenic therapy)

* Very rare dedifferentiated JAZF1-SUZ12 ESS

Complex karyotype
Post-menopausal
Poor prognosis
(No effective treatment)

Intermediate prognosis
(Adjuvant radiation/chemotherapy strategy if stage 2 or higher)

Good prognosis
(Anti-estrogenic therapy)
Outline

• Cervical neoplasia:
  – HPV testing
  – Role of p16 immunostaining

• Uterine neoplasia
  – Classification of endometrial carcinoma
  – Endometrial stromal sarcoma

• Ovarian neoplasia
  – Ovarian cancer histotypes
  – Non-epithelial tumours

• Gestational trophoblastic disease

• Vulval neoplasia
  – Squamous cell carcinoma
  – Melanoma
  – Non-epithelial tumours

• Hereditary tumour syndromes
Ovarian neoplasia

2014: Epithelial ovarian cancers
- 5 separate major histotypes
- Distinct molecular profiles
- Different origins
- Different prognosis and response to treatment
- CAN BE RELIABLY SEPARATED HISTOLOGICALLY
- Limited role for diagnostic molecular testing in the majority of cases
- MOST IMPORTANT: identification of histotypes relevant for familial genetic testing

Epithelial ovarian carcinoma subtypes

Kurman and Shih. Hum Pathol. 2011 Jul; 42(7): 918–931.
High Grade Serous Carcinoma

• Commonest subtype
• Tubal origin: Serous Tubal Intraepithelial Carcinoma (STIC) at fimbrial end of tube
• p53 mutation is early and ubiquitous event
• 10-15% have germline BRCA1/BRCA2 mutation
• Many HGSC show ‘BRCA-ness’: SET pattern; higher mitotic indices; increased TIL; necrosis - 100% sensitivity, 57% specificity
High Grade Serous Carcinoma

• Genetic counselling

• Treatment: Platinum-based chemotherapy; PARP inhibitors in those with HRD
Some molecular defects that can lead to BRCAness.

<table>
<thead>
<tr>
<th>Defective mechanism</th>
<th>% in ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>BRCA1/2</em> germline mutation</td>
<td>10–15</td>
</tr>
<tr>
<td><em>BRCA1/2</em> somatic mutation</td>
<td>5–10</td>
</tr>
<tr>
<td><em>BRCA</em> promoter methylation</td>
<td>5–30</td>
</tr>
<tr>
<td><em>EMSY</em> amplification</td>
<td>20</td>
</tr>
<tr>
<td>Fanconi anemia complex defects</td>
<td>21</td>
</tr>
<tr>
<td><em>PTEN</em> focal deletion/mutation</td>
<td>7</td>
</tr>
<tr>
<td><em>Rad51C</em> hypermethylation</td>
<td>3</td>
</tr>
<tr>
<td><em>ATM/ATR</em> mutation</td>
<td>2</td>
</tr>
</tbody>
</table>

Georgios Rigakos, and Evangelia Razis The Oncologist  
2012;17:956-962
HGSC and PARP inhibitors

- DNA is unstable and breakages occur commonly.
- Homologous recombination repair is one mechanism for repairing DS-DNA breaks.
- HR defects result from BRCA1/2 mutation and other mechanisms.
- PARP inhibitors result in accumulation of DNA breaks making the tumour cells non-viable.
Non-HGSC subtypes

The Histomorphology of Lynch Syndrome–associated Ovarian Carcinomas
Toward a Subtype-specific Screening Strategy

Michael Herman Chui, MD,* Paul Ryan, MD,† Jordan Radigan, MD,* Sarah E. Ferguson, MD,‡§
Aaron Pollett, MD,*∥ Melyssa Aronson, MSc,¶ Kara Semotiuk, MSc,¶ Spring Holter, MSc,¶
Keiyan Sy, MD,* Janice S. Kwon, MD,# Anita Soma, MD,** Naveena Singh, MD,††
Steven Gallinger, MD,‡‡ Patricia Shaw, MD,*§§ Jocelyne Arseneau, MD,∥∥
William D. Foulkes, MD,∥∥ C. Blake Gilks, MD,¶¶ and Blaise A. Clarke, MD*§§

• 20% non-HGSC show MMR defect
• ALL endometrioid or clear cell
• Supports reflex testing of all non-HGSC for Lynch syndrome by MMR-IHC or MSI
Non-HGSC subtypes: Mucinous carcinoma

Research article

**HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy**

Jessica N McAlpine*¹, Kimberly C Wiegand², Russell Vang³, Bridgett M Ronnett³, Anna Adamiak⁴, Martin Köbel⁴, Steve E Kalloger², Kenneth D Swenerton⁵, David G Huntsman²,⁴, C Blake Gilks²,⁴ and Dianne M Miller¹

- <4% ovarian Ca are mucinous
- Around 20% show HER2 amplification by IHC/FISH/CISH
- No prognostic significance
- Dramatic response to Herceptin

BMC Cancer 2009, 9:433
Non-epithelial ovarian neoplasms: AGCT and FOXL2

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Mutation of FOXL2 in Granulosa-Cell Tumors of the Ovary

Sohrab P. Shah, Ph.D., Martin Köbel, M.D., Janine Senz, B.Sc.,
Ryan D. Morin, M.Sc., Blaize A. Clarke, M.B., B.Ch., Kimberly C. Wiegand, B.Sc.,
Gillian Leung, B.Sc., Abdalnasser Zayed, B.Sc., Erika Mehl, B.M.L.Sc.,
Steve E. Kalloger, B.Sc., Mark Sun, B.Sc., Ryan Giuliani, Erika Yorida, B.M.L.Sc.,
Steven Jones, Ph.D., Richard Varhol, M.Sc., Kenneth D. Swenerton, M.D.,
Dianne Miller, M.D., Philip B. Clement, M.D., Colleen Crane, B.Tech.,
Jason Madore, M.Sc., Diane Provencher, M.D., Peter Leung, Ph.D.,
Anna DeFazio, Ph.D., Jaswinder Khattra, M.Sc., Gulisa Tushashvili, M.D., Ph.D.,
Yongjun Zhao, M.Sc., D.V.M., Thomas Zeng, M.Sc., J.N. Mark Glover, Ph.D.,
Barbara Vanderhyden, Ph.D., Chengquan Zhao, M.D.,
Christine A. Parkinson, Ph.D., M.R.C.P., Mercedes Jimenez-Linan, Ph.D.,
David D.L. Bowtell, Ph.D., Anne-Marie Mes-Masson, Ph.D.,
James D. Brenton, M.D., F.R.C.P., Samuel A. Aparicio, B.M., B.Ch.,
Niki Boyd, Ph.D., Martin Hirst, Ph.D., C. Blake Gilks, M.D., Marco Marra, Ph.D.,
and David G. Huntsman, M.D.

Adult-type Granulosa Cell Tumour

- Missense point mutation, 402C→G (C134W) in FOXL2 gene
- Encodes a transcription factor critical for granulosa-cell development
- Present in 86/89 AGCT (97%)
- Absent in other SCST and other ovarian tumours
- Diagnostic role in challenging cases
- FOXL2 mutation ≠ FOXL2 IHC expression
Other ovarian neoplasms: OSSCHT and SMARCA4 mutation

Germline and somatic SMARCA4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type

Leora Witkowski1–3,26, Jian Carrot-Zhang3,4,26, Steffen Albrecht5, Somayyeh Fahiminiya3,4, Nancy Hamel1,6, Eva Tomiak7, David Grynspan8, Emmanouil Saloustros9, Javad Nadaf3,4, Barbara Rivera1,3, Catherine Gilpin7, Ester Castellsague1,3, Rachel Silva-Smith1,2, François Plourde1,2, Mona Wu1,3, Avi Sasin3, Madeleine Arseneault3,4, Rouzan G Karabakhtsian10,25, Elizabeth A Reilly10, Frederick R Ueland10, Anna Margiolaki9, Kitty Pavlakis11, Sharon M Castellino12, Janez Lamovec13, Helen J Mackay14, Lawrence M Roth15, Thomas M Ulbright15, Tracey A Bender15, Vassilis Georgoulias9, Michel Longy16, Andrew Berchuck17, Marc Tischkowitz18, Inga Nagel19, Reiner Siebert19, Colin J R Stewart20, Jocelyne Arseneau21, W Glenn McCluggage22, Blaise A Clarke23, Yasser Riazalhosseini3,4, Martin Hasselblatt24, Jacek Majewski3,4 & William D Foulkes1–3,6

Nature Genetics 46, 438–443 (2014)
Ovarian small cell carcinoma of hypercalcemic type

- most common undifferentiated ovarian malignancy in women <40
- *SMARCA4* mutation is the major cause of SCCOHT, familial and sporadic
- loss of SMARCA4 (BRG1) protein in 38/40 tumors
- Implications for genetic counseling and new treatment approaches
Outline

• Cervical neoplasia:
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• Hereditary tumour syndromes
Gestational trophoblastic disease: p57

- $p57^{\text{Kip2}}$ is an inhibitor of G1 cyclin complexes; antiproliferative
- imprinted gene, expressed from maternal but not paternal allele during intrauterine development

**IN MOLAR PREGNANCY:**
- $p57$ NEGATIVE = complete hydatidiform mole (rare $p57$ positive complete moles reported)
- $p57$ POSITIVE = partial hydatidiform mole
- NOT USED for partial hydatidiform mole vs hydropic abortion (HER2 FISH can be used (not completely specific) as trisomy = triploidy

Intervillous trophoblast is p57 POSITIVE in all POCs

Villous cytотrophoblast POSITIVE in non-molar and PHM
CHM: Villous cytотrophoblast NEGATIVE

!Intervillous trophoblast will be POSITIVE!
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• Vulval (non-squamous) neoplasia
  – Melanoma
  – Non-epithelial tumours

• Hereditary tumour syndromes
Vulval and vaginal melanoma

Comparison of molecular abnormalities in vulvar and vaginal melanomas

Sebastian Aulmann¹,⁷, Hans P Sinn¹,⁷, Roland Penzel¹, C Blake Gilks², Sarah Schott³, Jessica C Hassel⁴, Dietmar Schmidt⁵, Friedrich Kommoss⁵, Peter Schirmacher¹ and Stefan Kommoss⁶

Modern Pathology (2014) 27, 1386–1393
Vulval and vaginal melanoma

- <5% of melanomas in women
- 65 cases
- No case contained BRAF mutation
- NRAS mutations and KIT amplifications detected in approximately 12% tumors of vulva and vagina
- KIT mutations present in 18% vulval, but not in any vaginal melanomas
Aggressive/deep angiomyxoma
Aggressive Angiomyxoma

- Translocations involving HMGA2 in approximately 50% of cases
- Molecular testing rarely used in practice
- IHC has utility, especially for margin assessment
- HMGA2 abnormalities also seen in other tumors (e.g. leiomyomas)
Outline

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• Hereditary tumour syndromes
### Hereditary Cancer Syndromes (other than BRCA and Lynch)

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Gynaecological tumours</th>
<th>Associated tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peutz-Jeghers Syndrome</td>
<td>STK11/LKB1</td>
<td>Ovary: SCST (5-15% risk: SCTAT, Sertoli cell tumours)</td>
<td>Hamartomatous GI polyps</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cervix: Adenoma malignum (10% risk)</td>
<td>Breast, GI, lung, pancreas, testis cancers</td>
</tr>
<tr>
<td>Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)</td>
<td>Fumarase hydratase</td>
<td>Uterus: Leiomyomas (prominent nucleoli, perinuclear halos, young patients (most patients))</td>
<td>Renal cell carcinoma (15% risk)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cutaneous leiomyomas</td>
</tr>
<tr>
<td>Gorlin Syndrome (neviod basal cell Syndrome)</td>
<td>PTCH</td>
<td>Ovary: Fibromas, bilateral and calcified (2-25% risk)</td>
<td>Basal cell carcinomas</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Odontogenic keratocysts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medulloblastomas</td>
</tr>
<tr>
<td>Cowden Syndrome (PTEN hamartoma tumour Syndrome)</td>
<td>PTEN</td>
<td>Uterus: Leiomyomas Endometrial carcinoma (5-19% risk)</td>
<td>Hamartomas of GI tract, and multiple other sites</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Breast (25-50% risk) and Thyroid(3-10% risk) carcinomas</td>
</tr>
</tbody>
</table>
Summary of molecular diagnostics in gynaecological pathology

- DIAGNOSIS: IHC correlates of many molecular abnormalities guide clinical practice:
  - p16/HPV-ISH: cervix/vulval neoplasia
  - p53: Uterine serous carcinoma, HG serous tubo-ovarian carcinoma, VIN, smooth muscle tumours
  - MMR gene proteins: EC with MSI
  - p57 in molar pregnancy
  - Cyclin D1 in ESS
Summary of molecular diagnostics in gynaecological pathology

• Tumour-specific mutations: diagnosis
  – AGCT: FOXL2
  – HG ESS: YWHAE-NUTM2 fusion
  – Other uterine mesenchymal lesions
  – OSSCHT: SMARCA4
  – Deep angiomyxoma: HMGA2 mutation
Summary of molecular diagnostics in gynaecological pathology

• Tumour typing guides referral for genetic counselling/testing:
  – HGSC: BRCA1/2
  – All endometrial carcinomas: Lynch syndrome
  – All non-HGSC ovarian carcinomas: Lynch syndrome
  – Other tumours and specific syndromes
Summary of molecular diagnostics in gynaecological pathology

• Targeted therapy:
  – PARP inhibitors in HR defect HGSC
  – Trastuzumab in mucinous ovarian carcinomas
  – (BRAF mutation absent in vulvovaginal melanomas)
Other targeted therapies in Gynaecological Ca

• Anti-angiogenic agents (Bevacizumab)
• Immune checkpoint inhibitors: potential role in Ca’s with high neoantigen load (high mutation rate): HGSC, POLE and MSI EC
• MEK inhibitors in LGSC
Thank you