Applications of Molecular Diagnostics in Current Practice: Gynaecological Pathology

Naveena Singh
Barts Health NHS Trust
London
Introduction

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

• Diagnosis
  – Genomic abnormalities (IHC surrogates) 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
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HPV testing in Cervical Screening

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

• Imminent Application:
  • HPV primary testing
p16 IHC in HPV-mediated anogenital neoplasia

• 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
What is p16?

• 16 kDa protein encoded by CDKN2A, within the INK4/ARF tumour suppressor locus on Ch’9 (9p21.3)
• Inhibits cyclin-dependent kinases (CDK4 and CDK6) which are required to phosphorylate RB
• Inhibits G1/S checkpoint traversal

• **CELL CYCLE BLOCKADE**: Blocks inappropriate cell division
• Marker of **AGING/cell SENESCENCE** due to other stressors
p16 in Cancer

• COMPLEX

• Classical role is to maintain state of cell cycle arrest: ie it is a TUMOUR SUPPRESSOR

• Other roles: apoptosis, invasion, angiogenesis

• INACTIVATED in about 50% of all human cancers (variety of mechanisms)

• OVEREXPRESSED in some tumours (mechanism best understood in HPV)
What is $p16^{INK4a}$ ($p16$)?
What does p16 expression signify in HPV neoplasia?

• Marker of E7 mediated inactivation of Rb protein
• (Rb suppression is likely mechanism of overexpression in other tumours)
• Occurs as a response to uncontrolled proliferation

Therefore a **diagnostic** marker of **transforming HRHPV infection**
*(necessary but not sufficient* for progression to cancer)*
What does p16 **NOT** signify?

- **Not present in ALL HRHPV infection**
  - SPECIFIC for hrHPV but not seen in ALL cases
  - HRHPV spectrum: silent-productive-transforming/abortive
  - Not seen in lrHPV

- **Not diagnostic of high grade CIN**
  - About 50% of CIN1 is also p16 positive

- **Does NOT indicate likelihood of progression**
  - Many conflicting papers but overall p16 (in CIN1) does NOT have predictive value for progression
  - >90% CIN1 and up to 2/3rds CIN2 in young women will spontaneously regress after biopsy
  - (CIN1 overcalled; CIN2 poorly reproducible)
p16 IHC interpretation

• In squamous epithelium:

**ABNORMAL (aka block positive)** p16 expression is a sensitive and specific diagnostic marker of a transforming HRHPV infection

  • Diffuse and strong nuclear and cytoplasmic
  • Basal and parabasal layers with upward extension
  • At least lower one-third of epithelium
  • At least 6 cells across
  • All other patterns are not specific
p16 Interpretation

• Do you use p16 to grade CIN?
  • NO; 50 % of CIN1 is p16 positive

• Does the thickness of stained epithelium matter?
  • NO; the extent of p16 staining within the squamous epithelium does not necessarily correlate with CIN grade
Patterns of normal/reactive p16 expression
p16 IHC interpretation

• In glandular epithelium:

  *Diffuse and strong positive* p16 expression is a sensitive and specific diagnostic marker of a transforming HRHPV infection *in the right context*
Current recommendations for p16 usage in HPV-mediated Precancerous lesions

The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions:
Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology

Teresa M. Darragh, MD; Terence J. Colgan, MD; J. Thomas Cox, MD; Debra S. Heller, MD; Michael R. Henry, MD; Ronald D. Luff, MD; Timothy McCallmont, MD; Ritu Nayar, MD; Joel M. Palefsky, MD; Mark H. Stoler, MD; Edward J. Wilkinson, MD; Richard J. Zaino, MD; David C. Wilbur, MD; for members of the LAST Project Work Groups

• Co-sponsored by the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology
• 5 working groups; surgical pathologists, gynecologic pathologists, dermatopathologists, and medical and surgical specialists including gynecologists, gynecologic oncologists, dermatologists, infectious disease specialists and surgeons
• WG4 focused on biomarkers: Reviewed 265 full papers and extracted data from 72 with full data; 53 on p16
• Consensus conference included invited specialists from 35 organisations; all recommendations voted upon
Recommendations for use of p16 (LAST project, WG4)

1. DD between HG CIN and a 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

3. As an adjudication tool in cases of professional disagreement when DD includes HG CIN

4. p16 should **not** be used as routine adjunct in biopsies with morphologic interpretations of negative, -IN1 or –IN3

   4a. p16 is recommended as an adjunct for biopsies interpreted as ≤ -IN1 that are at HIGH RISK for missed HG disease: previous cytology of high grade dyskaryosis/borderline suspicious for HG/glandular abnormality/borderline with HPV +ve

Current recommendations for p16 in anogenital IN

• Useful and specific **diagnostic** marker of transforming HRHPV infection
• NOT a *predictive* or *prognostic* marker
• Any identified p16-positive area must meet H&E morphologic criteria for a high-grade lesion to be interpreted as such
• Not suitable for determining management in CIN1 cases; not recommended in CIN1
• Useful to triage morphological “CIN2” (and “CIN1-2”)
• LAST criteria recommended
• Usage should be in approx 20% biopsies (prob <10 in practice)
• Concurrent routine Ki67 is not recommended
BAGP-UK NEQAS p16 QC Project

- 94% concordance in p16 IHC interpretation

- 5/35 laboratories (14%) showed sub optimal technical results affecting final interpretation in >5% cases: usually weak stain interpreted as normal
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
HPV vs non-HPV cancers

• HPV-mediated vs HPV-independent cervical adenocarcinoma: major prognostic implications

• 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
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
HPV E6/E7 RNA In Situ Hybridization Signal Patterns as Biomarkers of Three-Tier Cervical Intraepithelial Neoplasia Grade

Mark F. Evans¹, Zhihua Peng¹, Kelli M. Clark¹, Christine S.-C. Adamson¹, Xiao-Jun Ma³, Xingyong Wu³, Hongwei Wang³, Yuling Luo³, Kumarasen Cooper¹,²

¹Department of Pathology, University of Vermont, Burlington, Vermont, United States of America; ²Department of Pathology and Laboratory Medicine, Fletcher Allen Health Care, Burlington, Vermont, United States of America; ³Advanced Cell Diagnostics, Inc., Hayward, California, United States of America

Stratification of HPV-induced cervical pathology using the virally encoded molecular marker E4 in combination with p16 or MCM

Heather Griffin¹,², Yasmina Soneji², Romy Van Baars³, Rupali Arora⁴, David Jenkins³, Miekel van de Sandt³, Zhonglin Wu², Wim Quint³, Robert Jach³, Krzysztof Okon³, Hubert Huras³, Albert Singer⁴ and John Doorbar¹,²

¹Department of Pathology, University of Cambridge, Cambridge, UK; ²National Institute for Medical Research, London, UK; ³DDL Diagnostic Laboratory, Rijswijk, The Netherlands; ⁴University College Hospital, London, UK and ⁵Department of Gynecology and Oncology, Jagiellonian University College, Krakow, Poland
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For all molecular tests: Optimal tumour sampling for molecular testing
p53 IHC as a surrogate for *TP53* mutation

- Reacts to cell stress and arrests cell cycle – repair OR causes apoptosis
- ‘Guardian of the genome’
- TRANSCRIPTIONAL ACTIVATION OF A RANGE OF GENES
- “Tumour suppressor gene”
p53 and cancer

- Most human cancer types show p53 defects
- Most commonly mutated gene in human cancers
- Germline: Li-Fraumeni syndrome
- >600 known mutations; anywhere on the gene (Ch’ 17); also post translational (HPV)
Ubiquitous (99%) as driver mutation in tubo-ovarian high-grade serous carcinoma (HGSC)

(Kurman and Shih. Am J Pathol 2016, 186: 733e747)
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 MSI-high
  - MMR MSS
    - MMR IHC abn (n=41)
      - POLE (n=12)
        - POLE mutated
        - p53 HIC (+)
          - p53 wt (n=63)
        - p53 HIC (0 or 2+)
          - p53 abn (n=25)
      - POLE wild type
        - p53 IHC (+)
          - p53 missing
            - Unclassifiable (n=2)

Molecular Classification of EC

• Not yet incorporated into clinical practice
• Universal MMR IHC/MSI recommended
• POLE exonuclease domain mutation testing not commercially available
• 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
p53 IHC

- Surrogate for underlying \textit{TP53} mutation
- Identifies serous-like (CN-H) EC
- Identifies tubo-ovarian HGSC; p53 abn + WT1 pos
- Also 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

- **Stopgain**
  - Indel
  - Splicing
  - p53 complete absence

- **Stopgain**
  - Indel
  - Splicing
  - p53 cytoplasmic

**Abnormal**

# 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>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TP53 MUTATION ABSENT</strong></td>
<td></td>
<td></td>
<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>
<td></td>
<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>
<thead>
<tr>
<th>Participant Result</th>
<th>Review result</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>OE</td>
<td>CA</td>
<td>WT</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>oe</td>
<td>385</td>
<td>2</td>
<td>9</td>
<td>11</td>
<td>407</td>
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<tr>
<td>ca</td>
<td>4</td>
<td>474</td>
<td>102</td>
<td>320</td>
<td>900</td>
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<tr>
<td>cy</td>
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<td>28</td>
<td>5</td>
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</tr>
<tr>
<td>wt</td>
<td>36</td>
<td>73</td>
<td>1858</td>
<td>139</td>
<td>2106</td>
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</tr>
<tr>
<td>na</td>
<td>7</td>
<td>12</td>
<td>47</td>
<td>139</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>435</td>
<td>561</td>
<td>2044</td>
<td>614</td>
<td>3654</td>
<td></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
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Mismatch Repair Defects (MMR): Background

- MMR proteins recognise and repair errors in DNA sequence which occur when DNA is replicated during cell division
- MMRd leads to error-prone DNA replication and accumulation of mutations resulting in cancer
- Most error-prone are **microsatellites**: areas of 1-6 nucleotide repeats, replicated 5-50 times
- Occur in different parts of different genes; thousands are present within the genome
- In MMRd tumours, errors in copying microsatellite sequences cause them to vary in length; this is known as MSI
- MSI results in a hypermutated state: x10 mutations seen in MSS tumours
Mechanisms of MMRd

INHERITED (Lynch Syndrome, LS)
  – Autosomal dominant; 1/250 of population
  – Mutation in 1 of 4 DNA mismatch repair (MMR) genes:
    MLH1, MSH2, MSH6 or PMS2 (also EPCAM in CRC)
  – LS patients second allele is lost as a somatic event

SPORADIC (acquired, may or may not be driver)
  – MLH1 promoter methylation
  – Somatic mutation
Identification of mismatch repair defects in gynaecological cancer

Reasons:

• Screening for Lynch Syndrome
• Molecular classification of EC
• Selection for immune modulatory treatment (checkpoint inhibitors)
Lynch Syndrome testing in EC

• An estimated 175000 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)
2-5% EC is due to LS

LS mutations in EC

Win et al, JNCI 2013

- Percentage
  - MLH1 24%
  - MSH2 57%
  - MSH6 17%
  - PMS2 2%
Risk of further malignancy

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

In one study:
- 55% women with LS who have EC went on to develop another malignancy
- 15% went on to have two or more malignancies
Why screen: summary

• Surveillance for subsequent cancers saves lives
• Screening of family members (and if + surveillance/preventative measures)
• Molecular grouping has better prognostic value than morphological histotyping
• 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.\

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<th>IHC staining pattern</th>
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<td>MLH1</td>
<td>MLH1 −/+(^{b}), PMS2 −</td>
</tr>
<tr>
<td>PMS2</td>
<td>MLH1 +/−, PMS2 −</td>
</tr>
<tr>
<td>MSH2</td>
<td>MSH2 −, MSH6 −</td>
</tr>
<tr>
<td>MSH6</td>
<td>MSH2 +, MSH6 −</td>
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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)].
Mismatch repair gene expression
MMR IHC: difficulties

• Fixation affects IHC detection (use BIOPSIES)
• Staining protocol should be standardised with appropriate QC
• Unusual patterns (RARE!):
  • Weak expression, eg with missense mutations
  • False positive, 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
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</tr>
<tr>
<td>\textit{PMS2}</td>
<td>\textit{MLH1} (+/-, \textit{PMS2} (-)</td>
</tr>
<tr>
<td>\textit{MSH2}</td>
<td>\textit{MSH2} (-, \textit{MSH6} (-)</td>
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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 on MMR testing in gyn cancers

• MMR IHC/MSI testing is recommended for all new diagnoses of EC (and selected OC)
• Identifies women at risk of LS appropriate for genetic counselling/testing
• Identifies a molecular group of EC with prognostic and predictive implications
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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

![Graphs showing survival analysis](image)

Log rank test $P<0.001$
Classical low grade ESS

• Simple karyotype

• Chromosomal translocations $\rightarrow$ genetic fusions of genes/proteins in chromatin remodelling (i.e. polycomb complex proteins, histone acetyl/methyltransferase)
  
  • $t(7;17)(p15;q21) \rightarrow JAZF1-SUZ12$

  • $6p21$ rearranged $\rightarrow$ $JAZF1-PHF1$, $EPC1-PHF1^*$

  • $t(1;6)(p34;p21) \rightarrow MEAF6-PHF1^*$

  • $t(X;17) \rightarrow MBTD1-CXORF67$

  • $t(X;22) \rightarrow ZC3H7B-BCOR^*$
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-estrogentic therapy)

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*Very rare dedifferentiated JAZF1-SUZ12 ESS*
Mutation of FOXL2 in Granulosa-Cell Tumors of the Ovary

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 Saskin3, 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 Georgoulas9, 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

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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
Molecular diagnostics in gynaecological pathology

• Diagnosis
  – Genomic abnormalities (IHC surrogates) 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
Screening for BRCA1/2 and LS

- All HGSC eligible for reflex germline BRCA1 and BRCA2 testing

- Universal screening for Lynch Syndrome in endometrial carcinoma - recently approved for NICE diagnostic assessment programme
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Gynaecological tumours</th>
<th>Associated tumours</th>
</tr>
</thead>
<tbody>
<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) 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 Odontogenic keratocysts 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 Breast (25-50% risk) and Thyroid(3-10% risk) carcinomas</td>
</tr>
<tr>
<td>DICER1 mutation</td>
<td>DICER1</td>
<td>Sertoli-Leydig cell tumours, intermediate and poorly diff</td>
<td>Embryonal rhabdomyosarcoma</td>
</tr>
</tbody>
</table>
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For all molecular tests: Optimal tumour sampling for molecular testing
HGSC and PARP inhibitors – another driver for BRCA testing

• 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: poly(adenosine diphosphate [ADP]-ribose) polymerase – required for repair of single strand DNA breaks
• PARP inhibitors result in accumulation of DNA breaks making the tumour cells non-viable
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 McAlpine¹, 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
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