THE UNIVERSITY *of* EDINBURGH

Molecular Gynaecological Pathology

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igmm | MRC Medical Research Council | CANCER RESEARCH UK

Outline

- What is Molecular Pathology?
- Lower Genital Tract
  - HPV infection
  - HPV testing
- Endometrium
  - Molecular changes
  - Molecular classification
  - Stromal tumours
- Ovary, Fallopian tube and Peritoneum
  - Origins and types of epithelial tumour
  - Non-epithelial tumours
- Hereditary Gynaecological Tumours

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What is Molecular Pathology?

The identification of diagnostically and therapeutically relevant molecular abnormalities in clinical samples.

The identification of diagnostically and therapeutically relevant molecular abnormalities in patients.

The molecular investigation of disease processes.

What is Molecular Pathology?

- Molecular abnormalities
  - Global approaches – usually untargeted and discovery driven
  - Targeted approaches – often used to reduce complexity or for validation
  - Specific approaches – defined targets with specific contextual meanings

- -omics usually refers to global approaches
  - Genomics, epigenomics, transcriptomics, proteomics, metabolomics etc

Molecular Pathology

The Investigation of Disease at a Molecular Level

- Understanding
- Diagnosis
- Prognosis
- Prediction

Molecular Pathology

- Diagnostic Histopathology
  - Surrogate markers e.g. p16
  - 'Genogenic' immunohistochemistry
    - Identification of specific mutations e.g. *TP53*, *BRAF*
    - Identification of products of translocation e.g. t(2:5)
    - Identification of therapeutic targets e.g. HER2

      Gown AM Diagnostic Histopathology 2002; 8: 193-200
  - In situ hybridisation
    - FISH/CISH e.g. HER2, translocations, viruses
- Ancillary Molecular Testing
  - PCR-based methods – DNA/RNA
  - 'omics' technology

Beyond the Microscope

- Ancillary Molecular Testing
  - PCR-based methods – DNA/RNA
  - ‘omics’ technology
- Non/Pauci-cellular Samples
  - ‘The liquid biopsy’
  - Cell-free DNA
- Molecular Imaging
  - Label-free spectroscopy
  - Tomography
  - Probe-based imaging

Questions to Ask

- Is there a robust method for detecting the molecule(s) of interest?
- Do I have the right sample?
- Is the method technically feasible?
- Will the result answer my question?
- (Is there an immunohistochemical approach that provides the same information?)

What Techniques Are Available?

- Blotting techniques
  - DNA (e.g. Southern)
  - RNA (e.g. Northern)
- PCR-based approaches
  - DNA vs RNA (RT-PCR)
  - Quantitative vs Semi-quantitative
  - Verification of product
    - Restriction digestion
    - Hybridisation
    - Sequencing
- *In situ* hybridisation (FISH)
- Next generation sequencing

What Samples Can Be Used?

- Cytological samples
  - Almost any technique (sample size permitting)
- Fresh / frozen tissue
  - Almost any technique (sample size permitting)
  - Good quality nucleic acids
- Paraffin-embedded material
  - In situ hybridisation
  - PCR/RT-PCR but product size must be small

What Makes a Good Test?

- Sensitivity / Specificity
  - Diagnostic sensitivity
  - Analytical sensitivity
- Predictive Value
- Cost
- Practical Applicability
  - Methodology
  - Interpretation
- Relevance to the Problem

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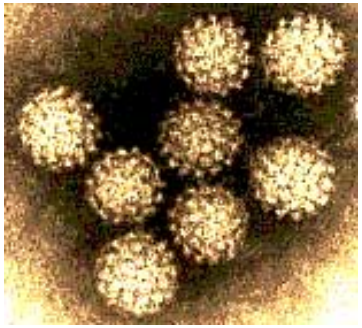
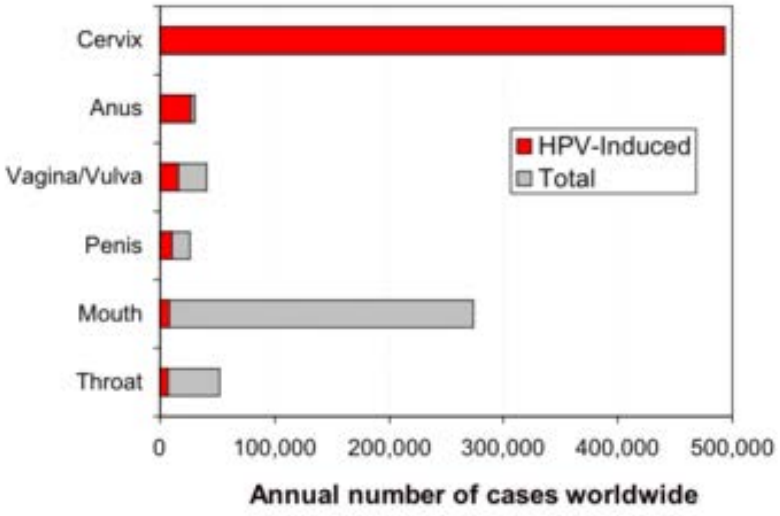
Human Papillomavirus Infection and Anogenital Disease



- HPV infection is present in 99.7% of invasive cervical carcinomas

- Mucosal HPV infection can also cause vulval and vaginal pre-cancerous lesions and genital warts



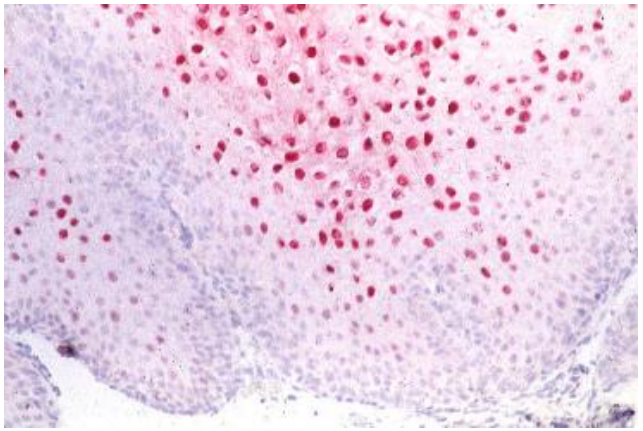
HPV testing

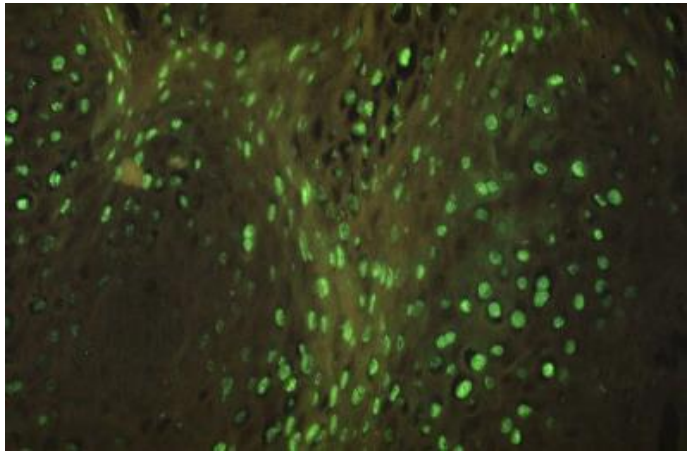
- Histopathology
  - SIL/AIS
  - Invasive disease
  - Metastatic disease
- Cytology
  - Population screening
  - Specific groups

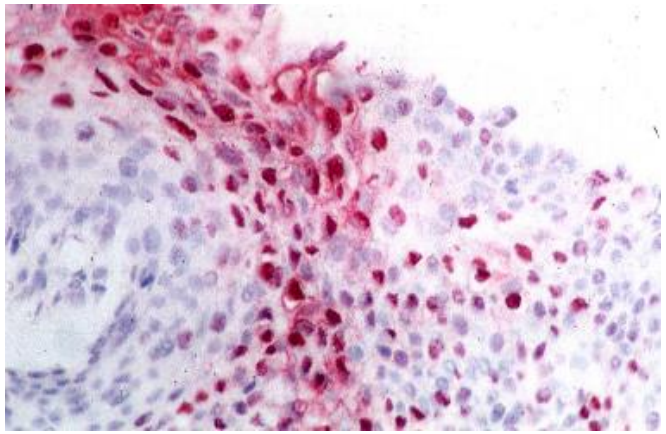
Does Presence = Relevant Infection?

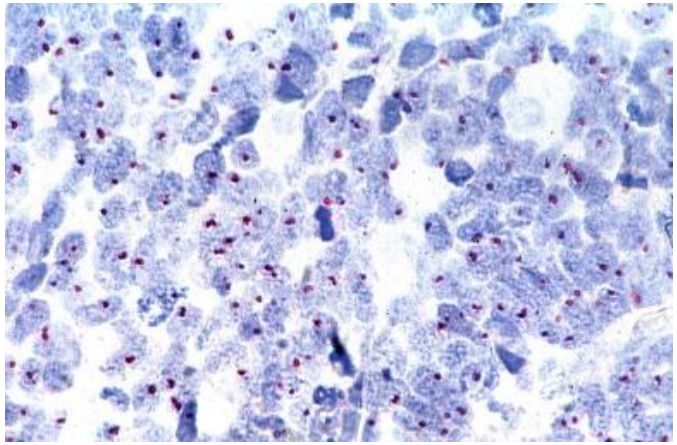
- Sensitivity of molecular techniques is a problem
- Non-morphological techniques do not identify origin of sequence
- Relevance depends on application
  - Ubiquitous vs unusual/rare organisms

HPV ISH on Tissues

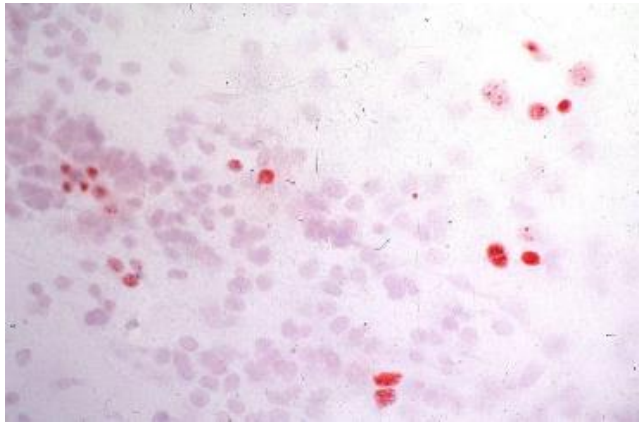


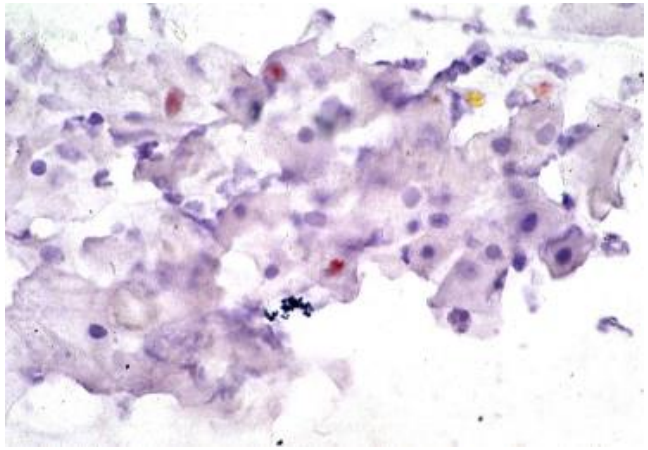


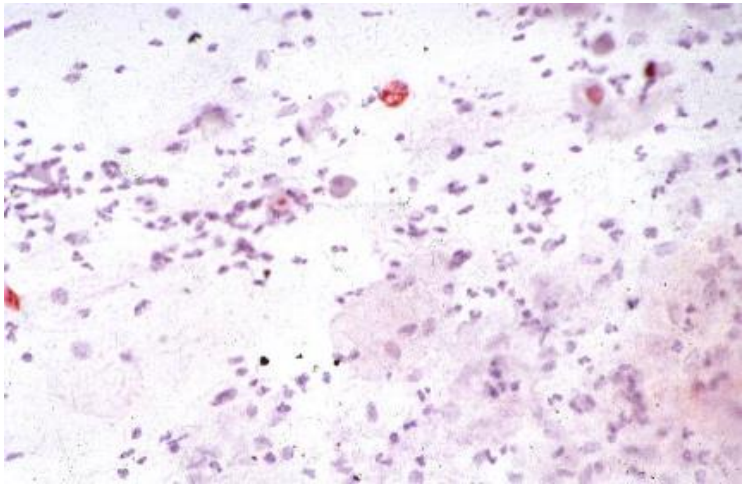




HPV ISH on Cervical Smears







Assessment of Intra-epithelial Disease

- Reactive vs Neoplastic
- HPV infection vs SIL
- Grading of SIL
- Risk of lesion progression
  - Low to high grade SIL
  - High grade SIL to invasive

Invasive and Metastatic Disease

- Invasive Disease
  - Prognostic assessment of the primary
- Metastatic Disease
  - Detection of metastases
  - Identification of primary site

HPV Testing in Cervical Screening

- Population screening
  - Adjunct to cytology
  - Replacement for cytology
  - Initial screening modality with reflex cytology
- Specific Groups
  - Low grade abnormalities
  - Immunosuppressed patients
- Follow-up after treatment
  - ‘Test of cure’

Testing the Test

		Disease: Present	Disease: Absent
Test	Positive	a	b
	Negative	c	d

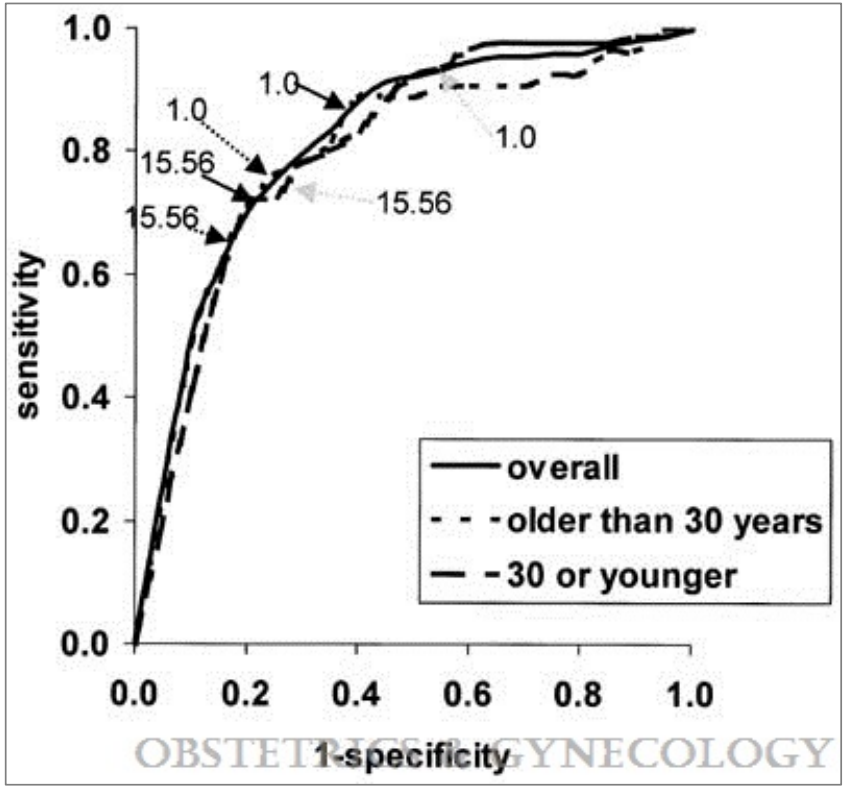
Sensitivity = a / a+c

Specificity = d / b+d

Positive Predictive Value = a / a+b

Negative Predictive Value = d / c+d

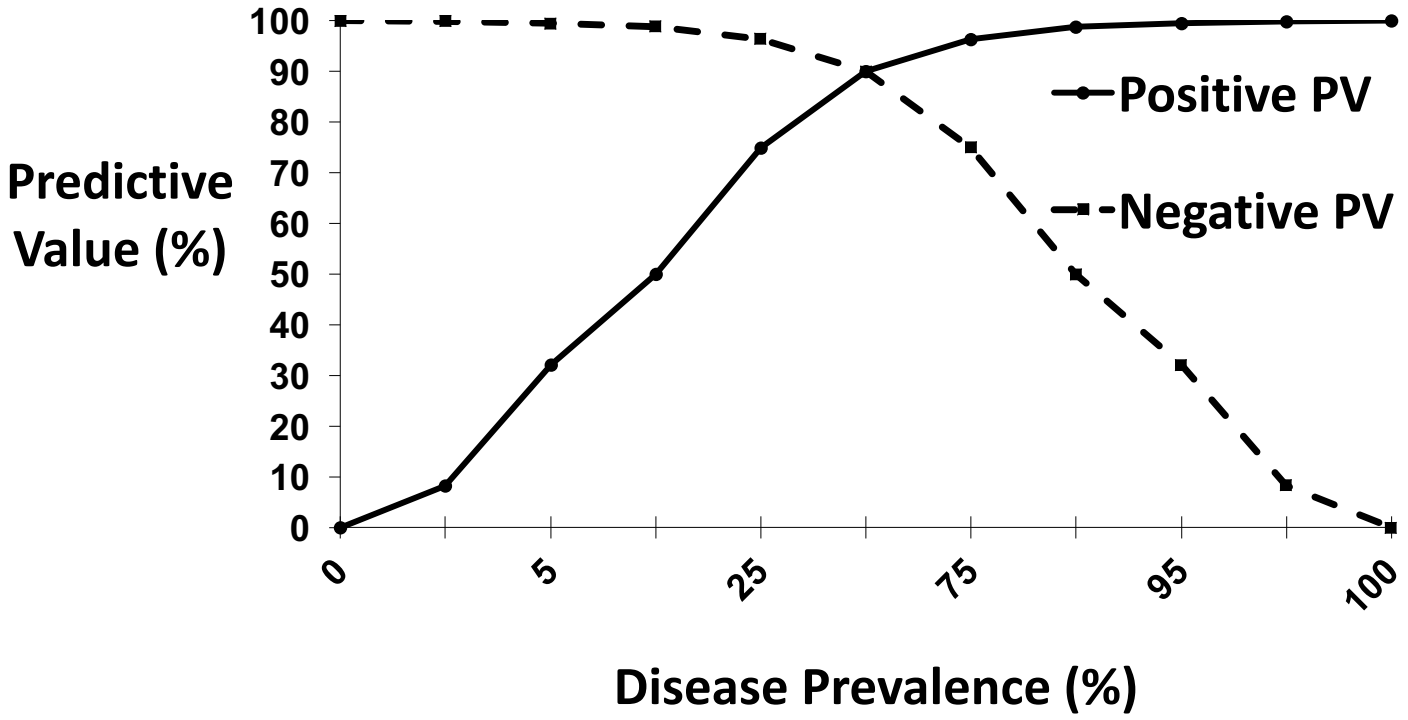
Receiver Operating Characteristic (ROC) Curve Analysis of Hybrid Capture 2



Optimum balance between sensitivity and specificity at '15.56 relative light units' (AUC 0.82)

Howard et al. Obstet Gynecol 2002; 100: 972-980

Clinical Prediction

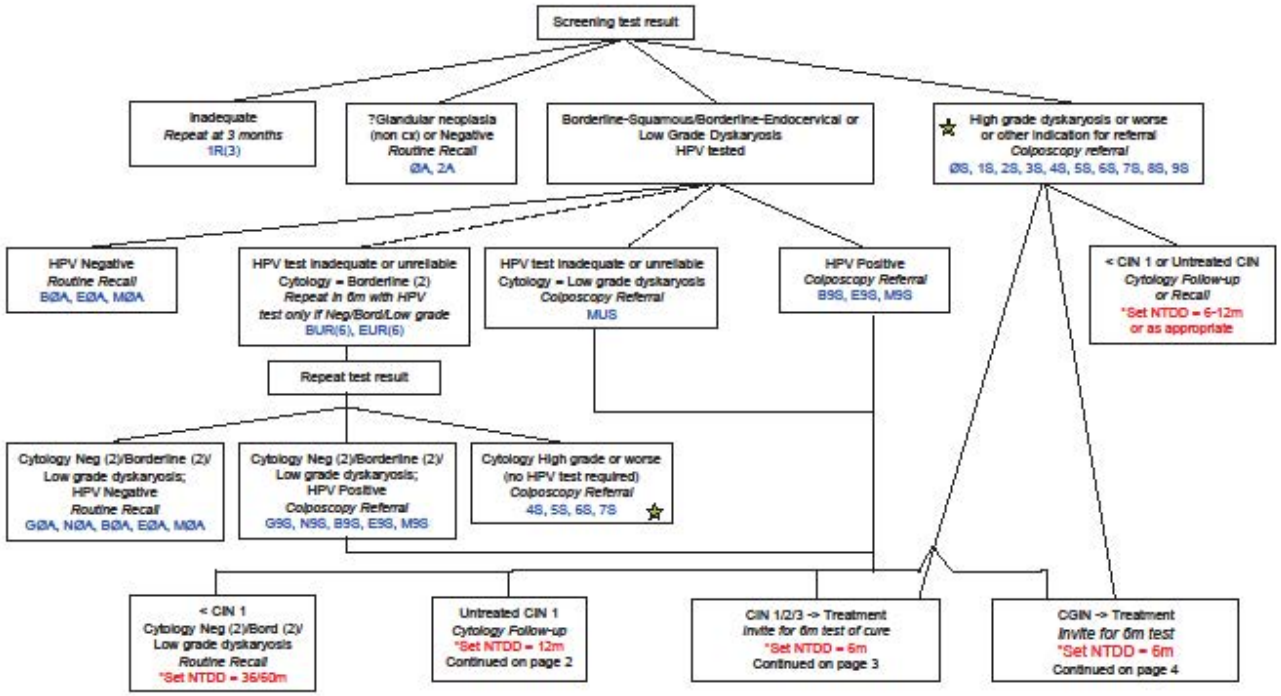


Sensitivity = Specificity = 90%

Public Health England

NHS Cancer Screening Programmes

NHS Cervical Screening Programme
Screening Protocol Algorithm for HPV Triage and TOC



http://www.cancerscreening.nhs.uk/cervical/hpv-triage-test-flowchart-201407.pdf

HPV Typing

- Cervical screening
  - Test of cure
  - Triage of low grade abnormalities
  - Primary screening
- Tumour diagnosis
  - HPV-related vs non-HPV-related primary tumours
  - Metastases

WHO Classification of Tumours of the Cervix

Epithelial tumours	
Squamous cell tumours and precursors	
Squamous intraepithelial lesions	
Low-grade squamous intraepithelial lesion	8077/0
High-grade squamous intraepithelial lesion	8077/2
Squamous cell carcinoma, NOS	8070/3
Keratinizing	8071/3
Non-keratinizing	8072/3
Papillary	8052/3
Basaloid	8083/3
Warty	8051/3
Verrucous	8051/3
Squamotransitional	8120/3
Lymphoepithelioma-like	8082/3
Benign squamous cell lesions	
Squamous metaplasia	
Condyloma acuminatum	
Squamous papilloma	8052/0
Transitional metaplasia	
Glandular tumours and precursors	
Adenocarcinoma in situ	8140/2
Adenocarcinoma	8140/3
Endocervical adenocarcinoma, usual type	8140/3
Mucinous carcinoma, NOS	8480/3
Gastric type	8482/3
Intestinal type	8144/3
Signet-ring cell type	8490/3
Villoglandular carcinoma	8263/3
Endometrioid carcinoma	8380/3
Clear cell carcinoma	8310/3
Serous carcinoma	8441/3
Mesonephric carcinoma	9110/3
Adenocarcinoma admixed with neuroendocrine carcinoma	8574/3

Other epithelial tumours	
Adenosquamous carcinoma	8560/3
Glassy cell carcinoma	8015/3
Adenoid basal carcinoma	8098/3
Adenoid cystic carcinoma	8200/3
Undifferentiated carcinoma	8020/3
Neuroendocrine tumours	
Low-grade neuroendocrine tumour	
Carcinoid tumour	8240/3
Atypical carcinoid tumour	8249/3
High-grade neuroendocrine carcinoma	
Small cell neuroendocrine carcinoma	8041/3
Large cell neuroendocrine carcinoma	8013/3

Cervical Epithelial Lesions

Squamous cell tumours and precursors

- Squamous intraepithelial lesions
  - Low-grade squamous intraepithelial lesion (HPV only, CIN 1)
  - High grade squamous intraepithelial lesion (CIN 2, CIN 3)
- Squamous cell carcinoma (keratinising, non-keratinising etc)

Glandular tumours and precursors

- Adenocarcinoma in situ (High grade CGIN)
- Adenocarcinoma
  - Endocervical adenocarcinoma, usual type
  - Mucinous carcinoma, NOS
    - Gastric type (including adenoma malignum / minimal deviation adenocarcinoma)
    - Intestinal type
    - Signet-ring cell type
  - Villoglandular adenocarcinoma
  - Endometrioid adenocarcinoma
  - Clear cell adenocarcinoma
  - Serous adenocarcinoma
  - Mesonephric adenocarcinoma
  - Adenocarcinoma admixed with neuroendocrine carcinoma

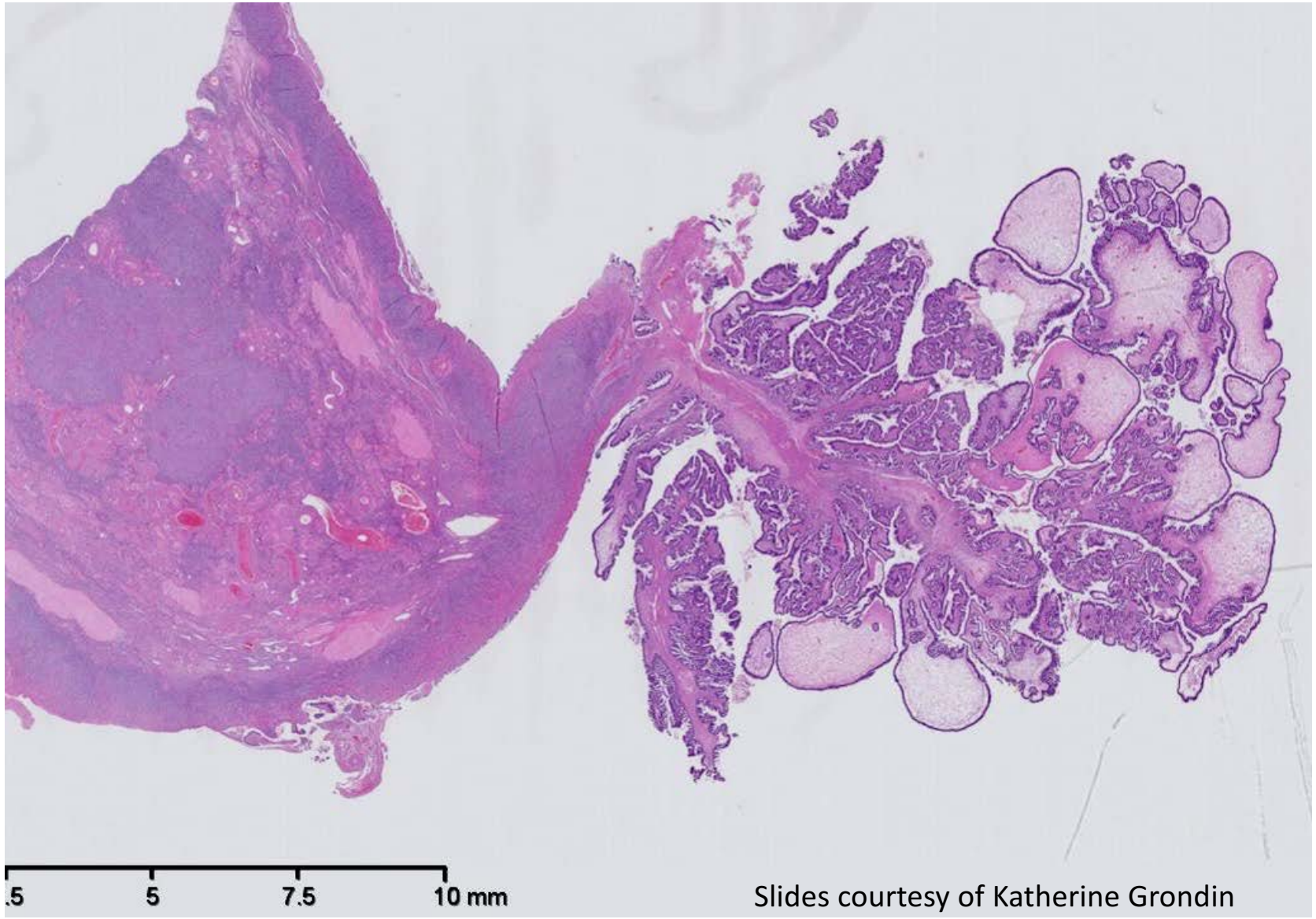
Red = associated with high-risk HPV
Blue = not associated with high-risk HPV
Black = uncertain

Park et al, Am J Surg Pathol 2011; 35: 633-636

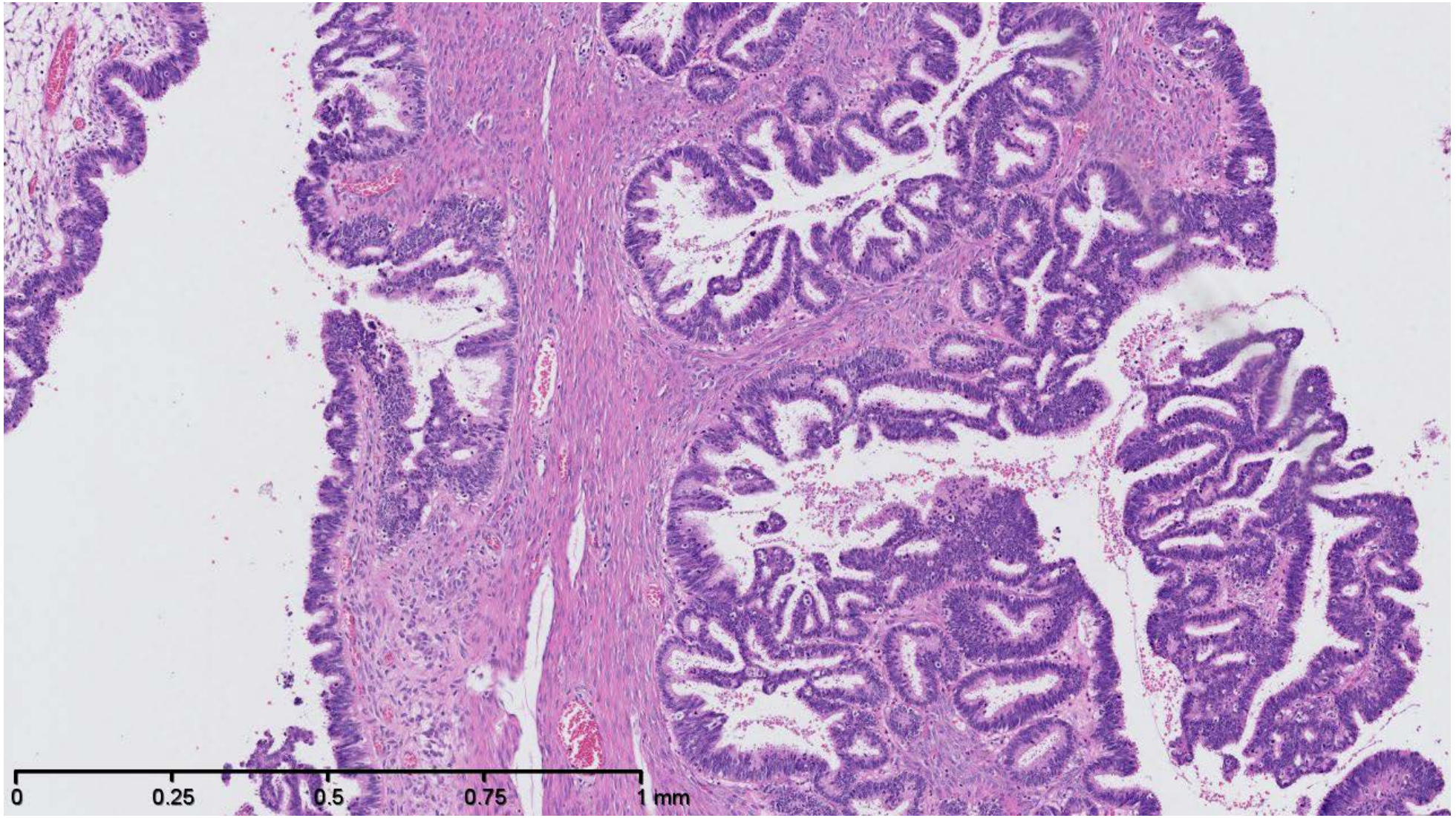
Metastatic Endocervical Adenocarcinoma

- May produce mucinous or ’endometrioid’ ovarian metastases
- Strong diffuse p16 positivity may help to identify the primary site
- HPV typing may be useful in difficult cases

Vang et al. Am J Surg Pathol. 2007; 31:653–63



Slides courtesy of Katherine Grondin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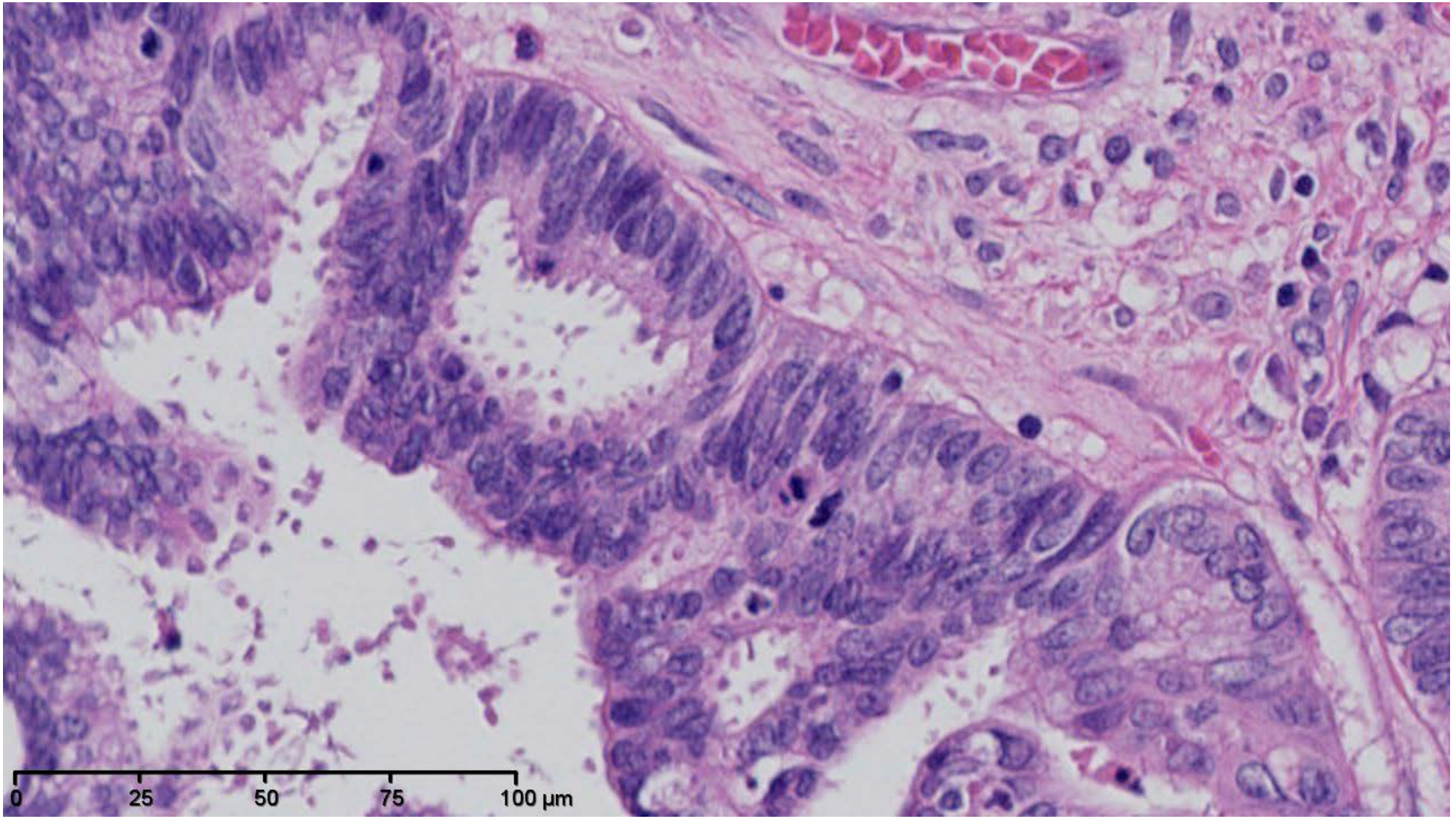
0.25
0.5
0.75
1 mm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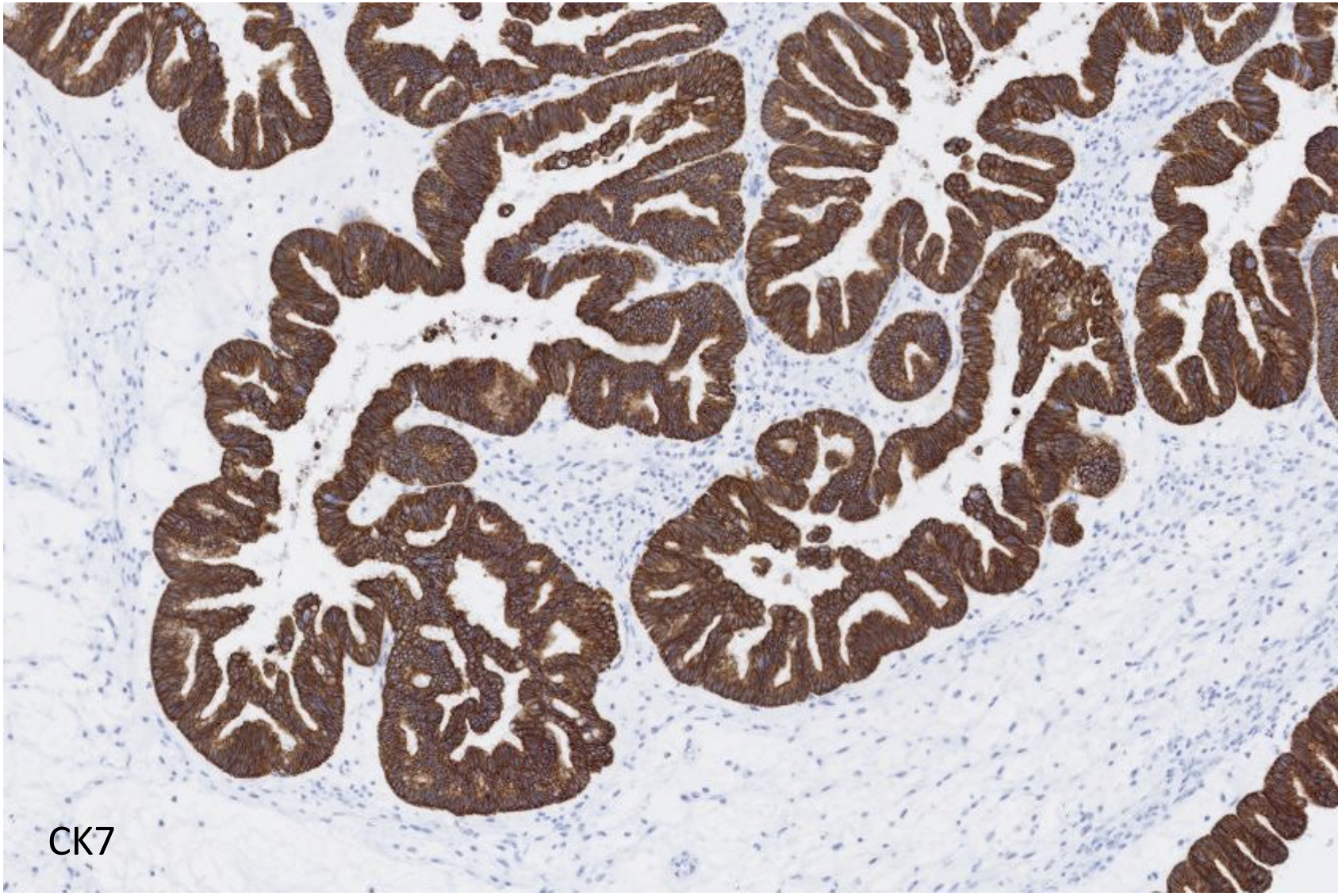
100 µm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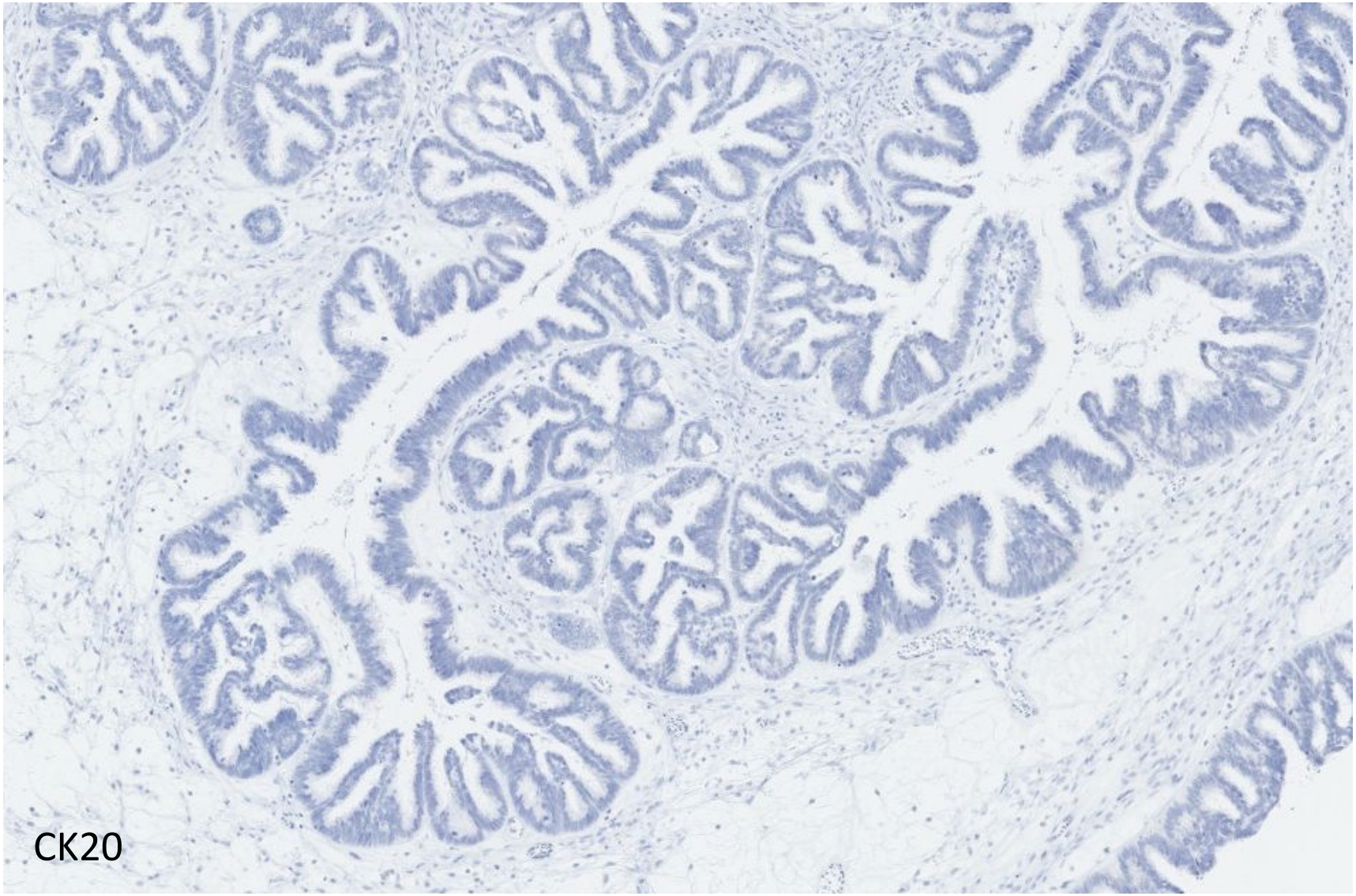
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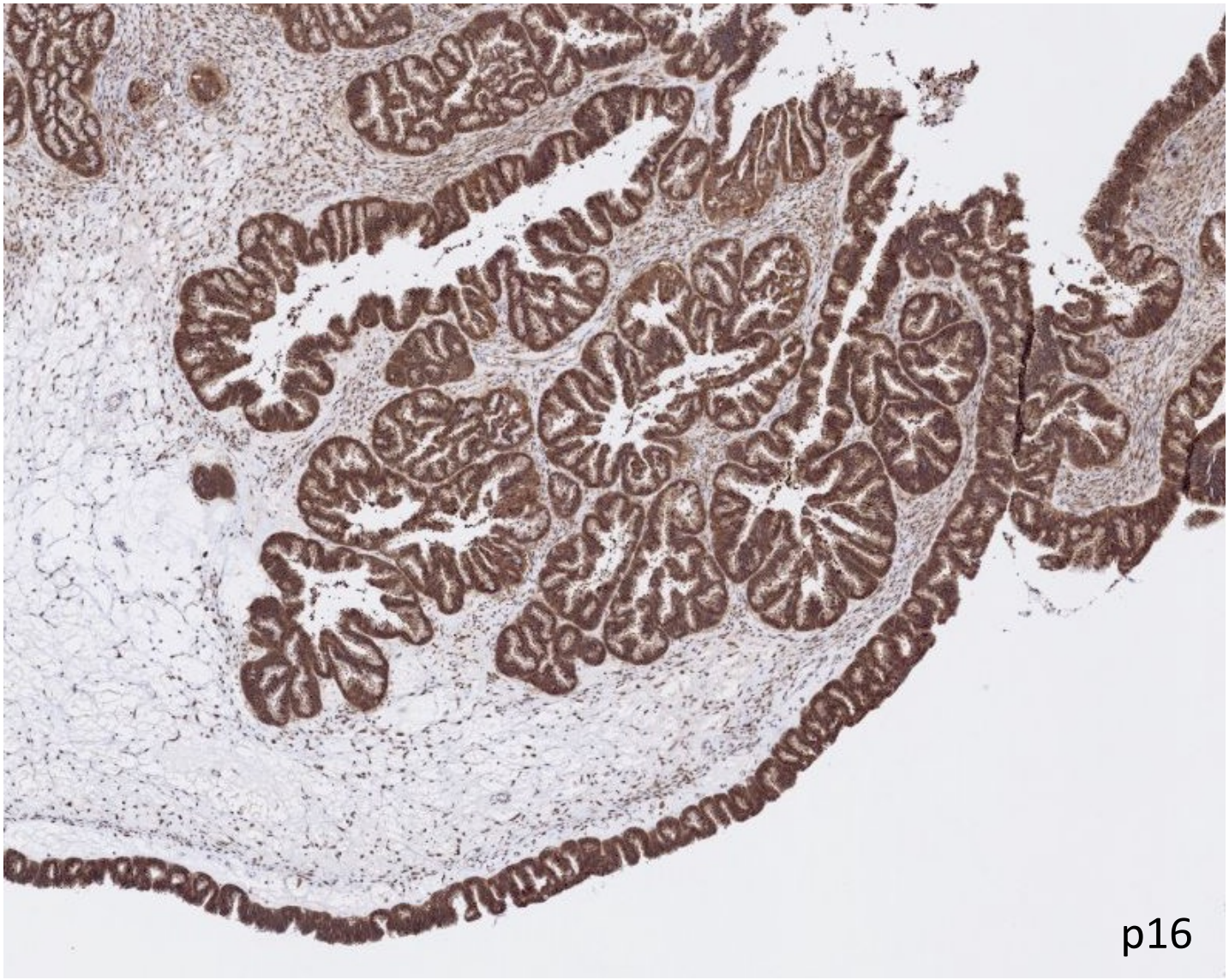

p16

WHO Classification of tumours of the vulva

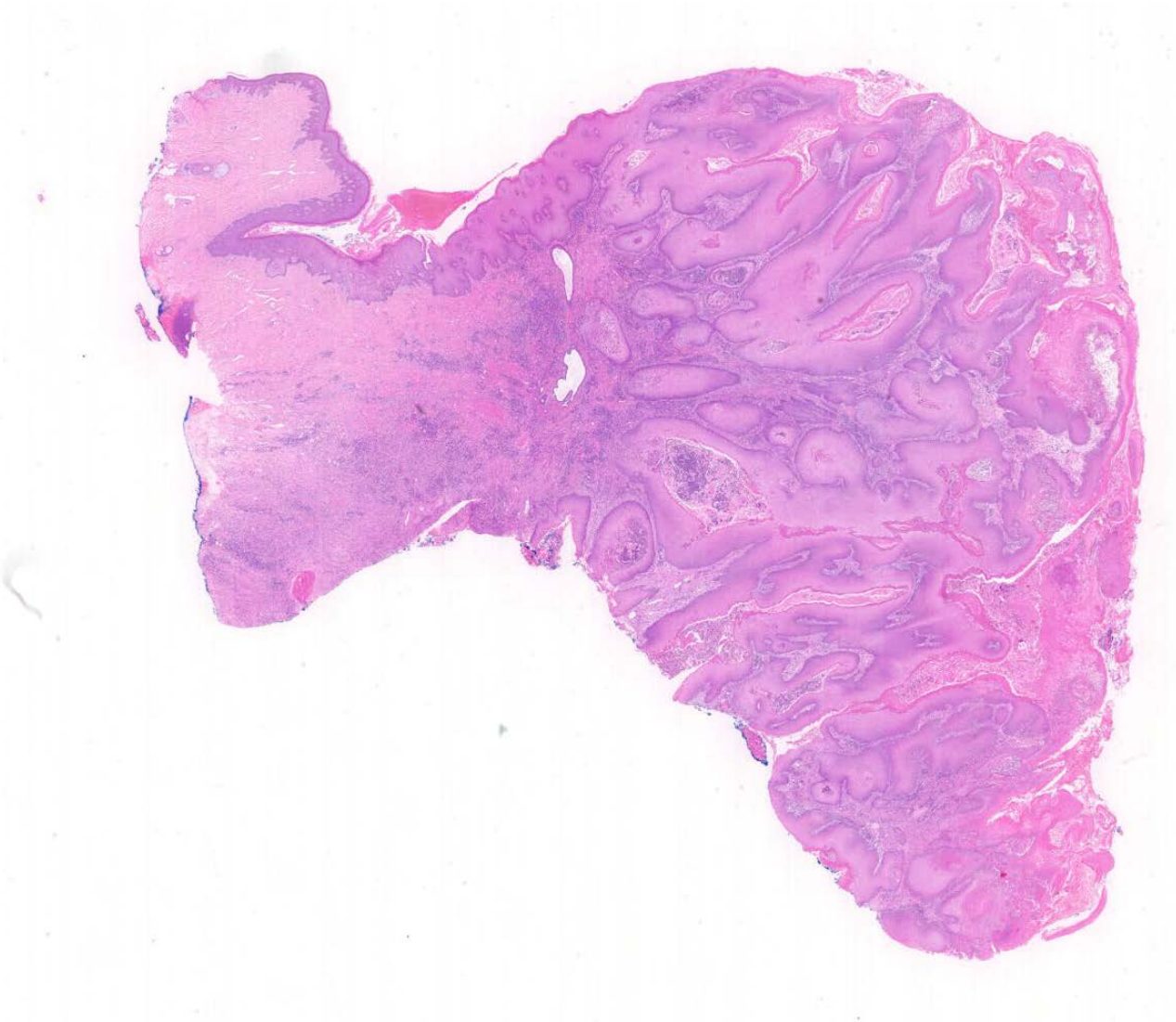
Epithelial tumours	
Squamous cell tumours and precursors	
Squamous intraepithelial lesions	
Low-grade squamous intraepithelial lesion	8077/0
High-grade squamous intraepithelial lesion	8077/2
Differentiated-type vulvar intraepithelial neoplasia	8071/2
Squamous cell carcinoma	8070/3
Keratinizing	8071/3
Non-keratinizing	8072/3
Basaloid	8083/3
Warty	8051/3
Verrucous	8051/3
Basal cell carcinoma	8090/3
Benign squamous lesions	
Condyloma acuminatum	
Vestibular papilloma	8052/0
Seborrheic keratosis	
Keratoacanthoma	
Glandular tumours	
Paget disease	8542/3
Tumours arising from Bartholin and other specialized anogenital glands	
Bartholin gland carcinomas	
Adenocarcinoma	8140/3
Squamous cell carcinoma	8070/3
Adenosquamous carcinoma	8560/3
Adenoid cystic carcinoma	8200/3
Transitional cell carcinoma	8120/3

Neuroectodermal tumour	
Ewing sarcoma	9364/3
Soft tissue tumours	
Benign tumours	
Lipoma	8850/0
Fibroepithelial stromal polyp	
Superficial angiomyxoma	8841/0
Superficial myofibroblastoma	8825/0
Cellular angiofibroma	9160/0
Angiomyofibroblastoma	8826/0
Aggressive angiomyxoma	8841/0
Leiomyoma	8890/0
Granular cell tumour	9580/0
Other benign tumours	
Malignant tumours	
Rhabdomyosarcoma	
Embryonal	8910/3
Alveolar	8920/3
Leiomyosarcoma	8890/3
Epithelioid sarcoma	8804/3
Alveolar soft part sarcoma	9581/3
Other sarcomas	
Liposarcoma	8850/3
Malignant peripheral nerve sheath tumour	9540/3
Kaposi sarcoma	9140/3
Fibrosarcoma	8810/3
Dermatofibrosarcoma protuberans	8832/1

Verrucous Carcinoma of the Vulva

- *Verrucous carcinoma* is warty appearing, highly differentiated, variably keratinized and invades in the form of bulbous pegs with a pushing border. There is minimal atypia, abundant eosinophilic cytoplasm, normal mitotic figures and no increased p53 or p16 staining. Using these criteria, lesions with prominent koilocytotic atypia and HPV positivity are better classified as giant condyloma

WHO, 2014



Verrucous Carcinoma of the Vulva

- Difficult diagnosis
- p16 immunostaining helpful if positive as indicates HPV-driven carcinoma
- p16 does not distinguish between giant condyloma and verrucous carcinoma
- HPV typing can help to identify giant condyloma (HPV 6, 11 positive)
- In most cases, diagnosis is morphological

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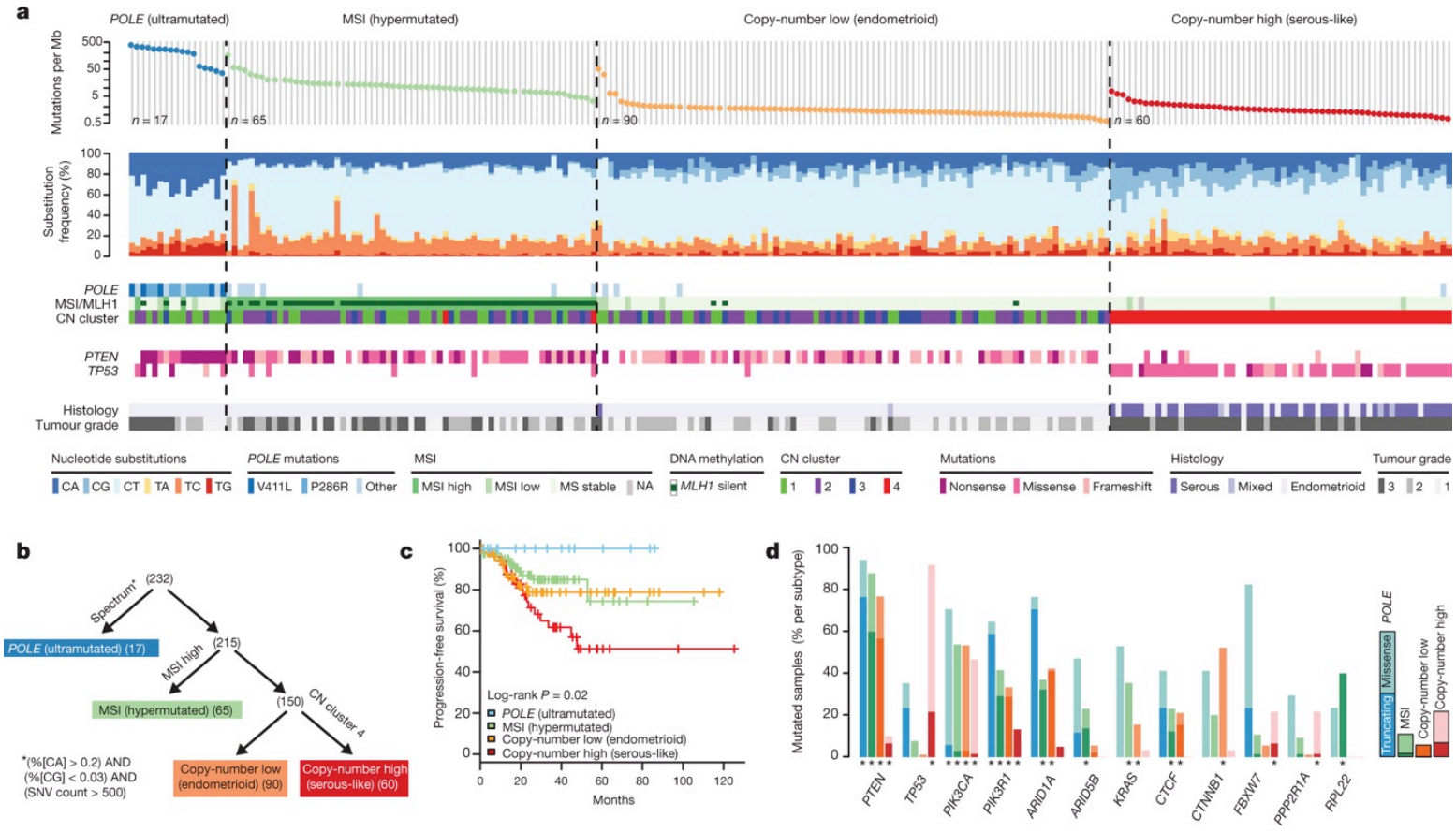
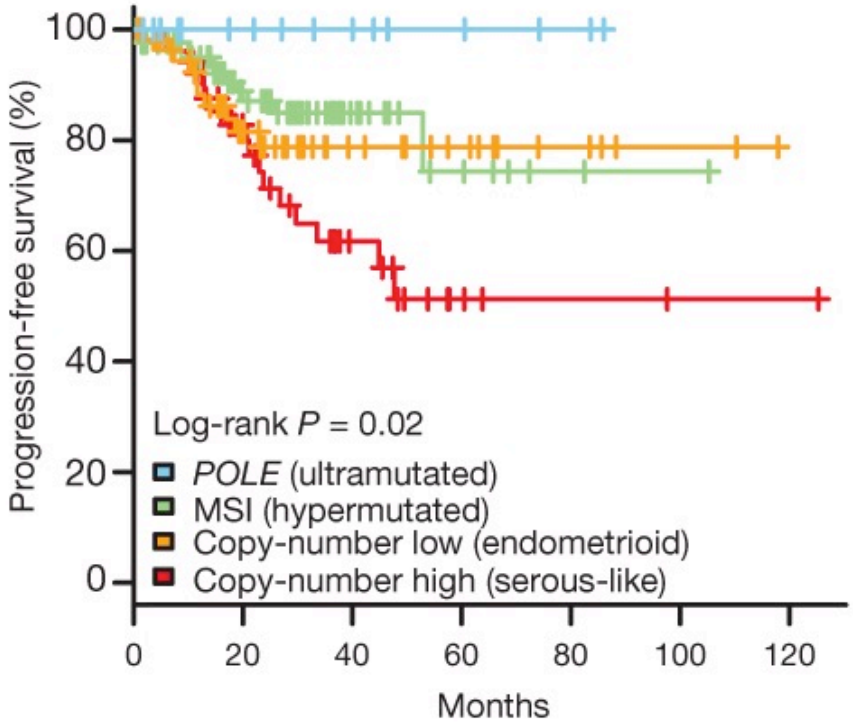
Mutation Spectra Across Endometrial Carcinomas



Getz et al Nature 2013; 497: 67-73

Mutation Spectra Across Endometrial Carcinomas



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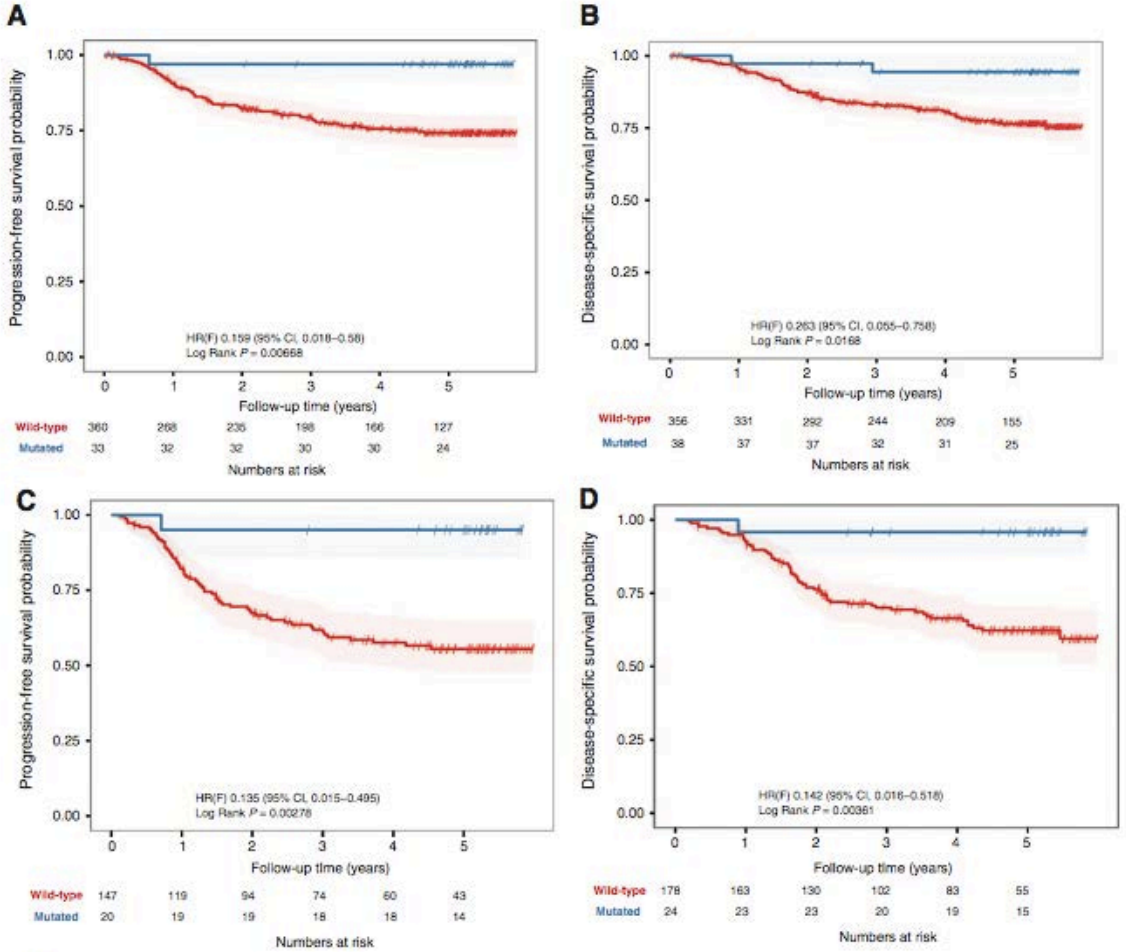
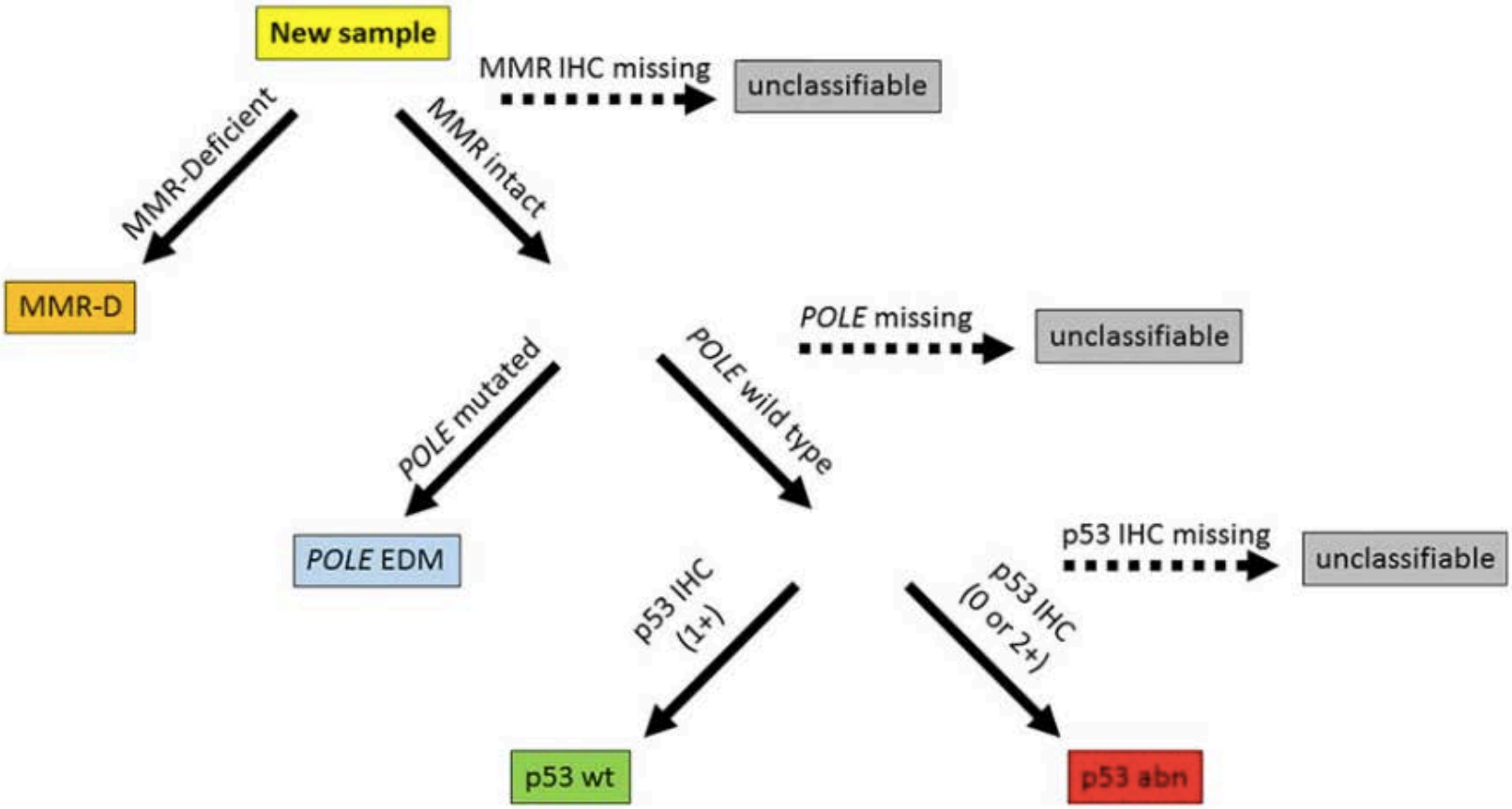


Figure 1.
Kaplan–Meier survival curves for *POLE*-mutated and *POLE* wild-type endometrial carcinomas. A, PFS for the full endometrial carcinoma cohort. B, DSS for the whole endometrial carcinoma cohort. C, PFS for grade 3 endometrial carcinomas only. D, DSS for grade 3 endometrial carcinoma cohort only. Blue lines, *POLE*-mutated cases; red lines, *POLE* wild-type cases. *P* values were obtained by a two-sided log-rank test. F, Firth correction.

McConechy MK et al Clin Cancer Res 2016;22:2865–73.

Prospective Molecular Risk Classifier for Endometrial Cancer



Talhouk A et al Cancer 2017;123:802–13

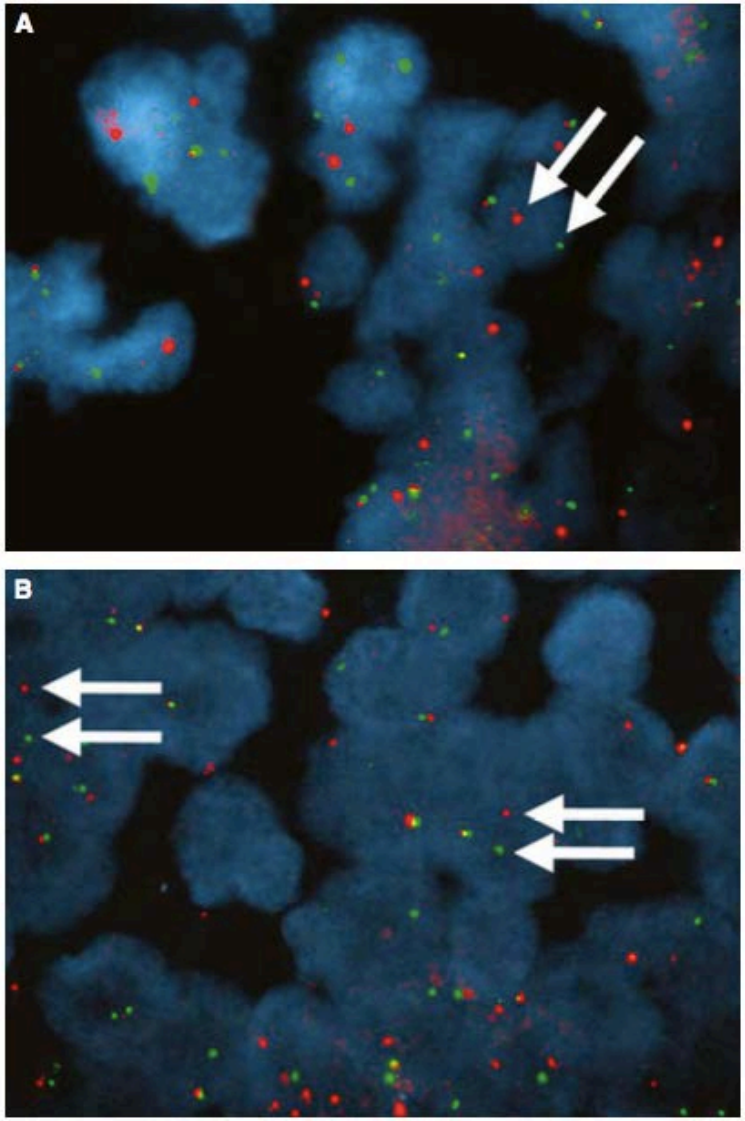
Translocations in Endometrial Stromal Tumours

- t(7;17)(p15;q21) leads to fusion of *JAZF1* and *SUZ12*
- Present in 92% of ESNs and 70% of low-grade ESSs

  Chiang & Oliva Adv Anat Pathol 2011; 42: 609-617
- t(10;17)(q22;p13) *YWHAE-NUTM2 (FAM22)* fusion identifies most high-grade endometrial stromal sarcoma – cyclin D1 positive

  Lee et al Am J Surg Pathol 2012; 36: 641-653

  Lee et al Am J Surg Pathol 2012; 36: 1562-1570
- Undifferentiated uterine sarcoma
  - No specific pattern
  - Diagnosis of exclusion



JAZF1

YWHAE

Testing Methods

- FISH – break apart probes
- RT-PCR – fusion transcripts
- NGS of FFPE tissue can detect fusions involving *JAZF1* or *YWHAE*

Li et al Histopathology 2016;69:551–9

Stewart et al Histopathology. 2014; 65: 473–82.

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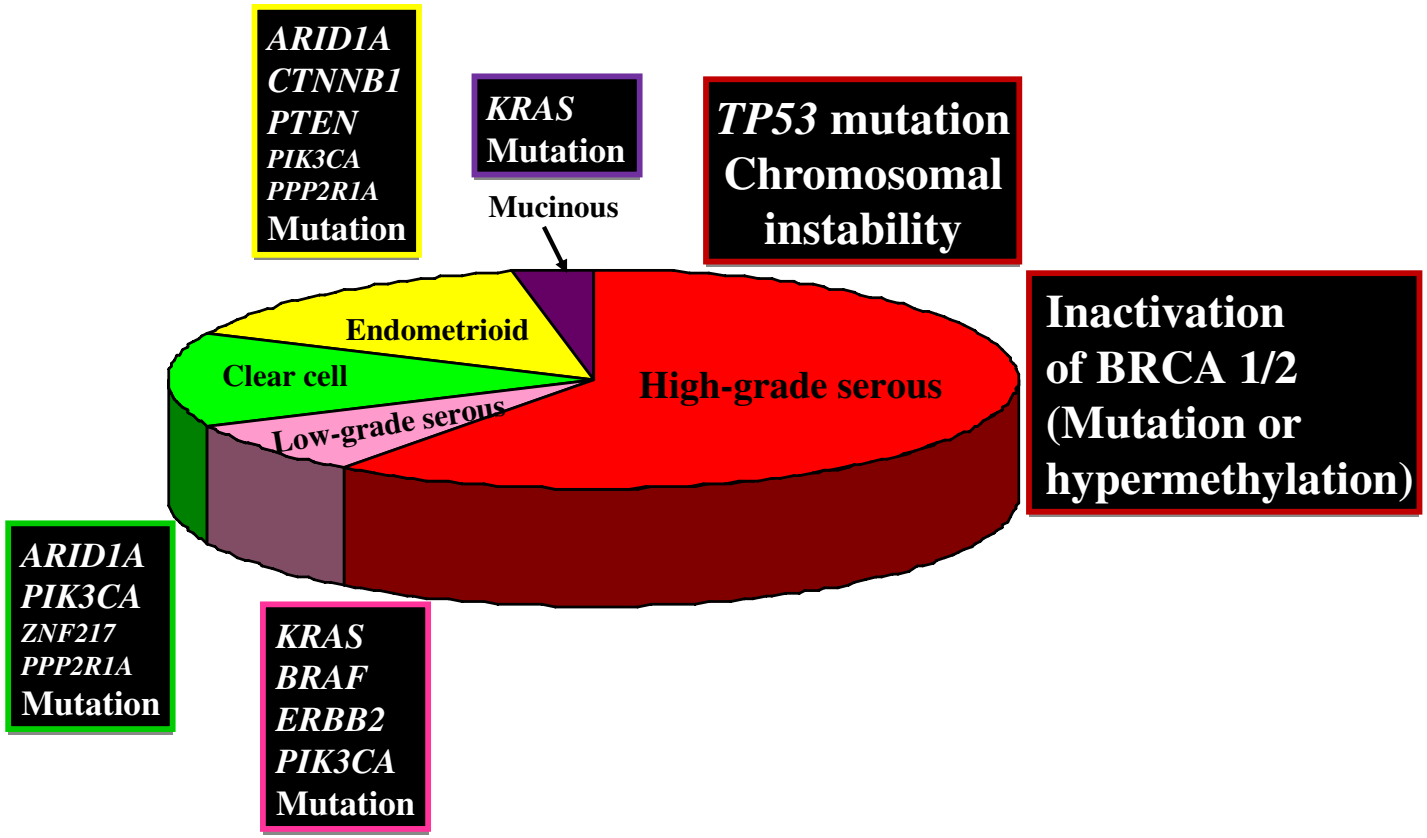
Ovarian tumours
(Tumours involving the ovary)

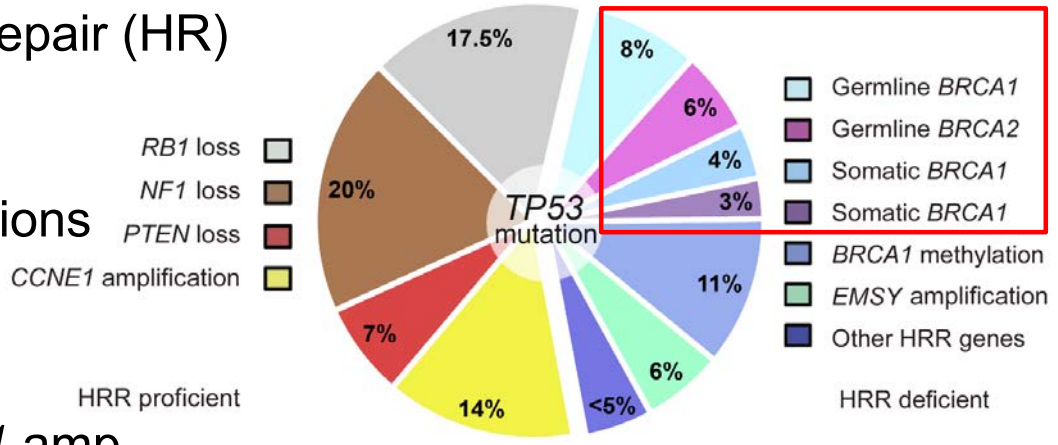
	Cell of origin	Type	Proportion (%)
Primary			
Epithelial	**Not entirely clear. The different histological types have different origins and arise through different molecular pathways**	**High-grade serous** **Low-grade serous** **Endometrioid/clear cell** **Mucinous** **Seromucinous** **Brenner** **Carcinosarcoma** **Undifferentiated**	**65–70**
Germ cell tumours	Germ cells	Teratoma Dysgerminoma Yolk sac tumour Embryonal carcinoma	15–20
Sex cord/stromal tumours	Ovarian sex cords and stroma	Granulosa cell tumours Thecoma/fibroma Sertoli–Leydig tumours	5–10
Miscellaneous	Various	e.g. Lymphoma	
Secondary			
Metastases	-	-	5-10



Kurman and Shih Hum Pathol 2011; 42: 918-931

Genomic Features of High-grade Serous Ovarian Carcinoma

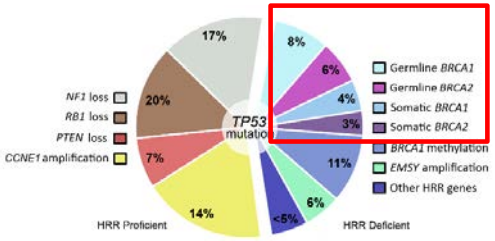
- Around half HGS OC have identifiable molecular changes in homologous recombination DNA repair (HR) genes
- ~ 20% have germline or somatic *BRCA* mutations
- Non-BRCA HR hits, including *EMSY* amp.
- HR proficient tumours: *NF1/RB1* loss, *CCNE1* amp.
- *BRCA*m OC patients represent a clinically and molecularly distinct subgroup of OC
    - "BRCAness" phenotype

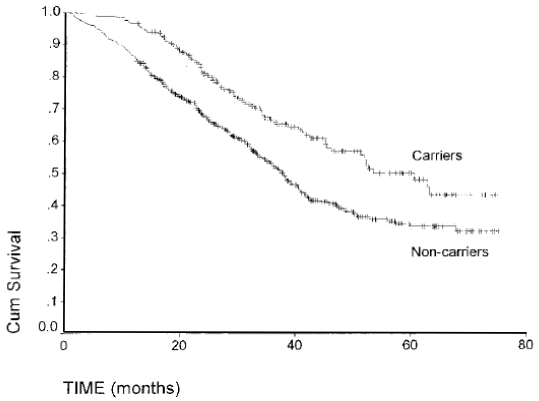


Robb Hollis

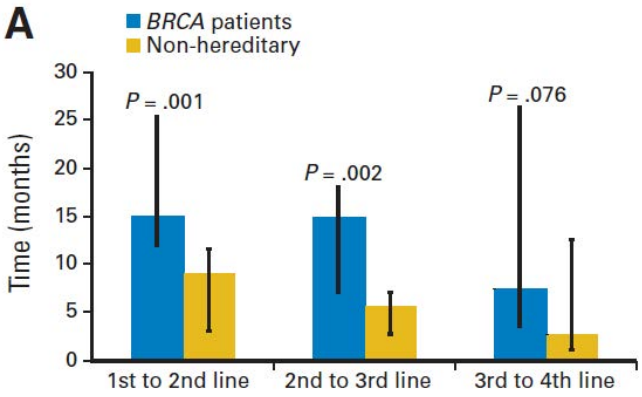
TCGA investigators. Nature. 2011;474:609-15.
Hollis RL, Gourley C. Cancer Biol Med. 2016;13:236-47
Patch AM et al. Nature. 2015;521:489-94

BRCA Mutant Ovarian Carcinoma - “BRCAness”

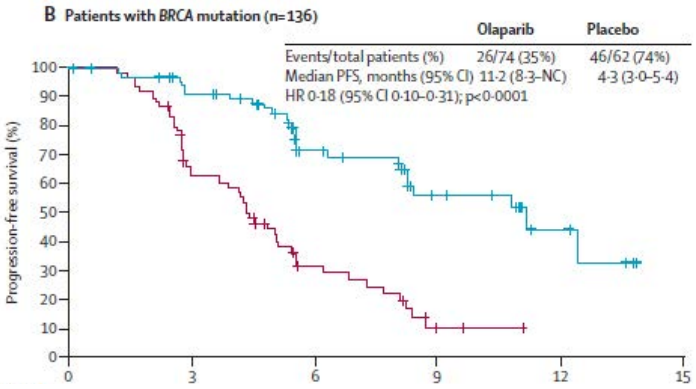




- Superior survival



- Superior response rate to multiple lines of platinum and prolonged platinum-free interval



- Sensitivity to PARP inhibitors

Ledermann J et al. Lancet Oncol 2014;15:852-61.
Tan DS et al. J Clin Oncol 2008;26:5530-6.
Ben David Y et al. J Clin Oncol 2002;20:463-6.

Ovarian Epithelial Tumours

Origin	Fallopian Tube		Endometriosis			Unclear	
	High–Grade Serous	Low–Grade Seorus	Endometrioid	Clear Cell	Seromucinous	Mucinous	Brenner
Borderline /AP							
Grade 1				?			
Grade 2							
Grade 3			Rare				

Summary

- Epithelial ovarian tumours rarely arise from the ovary
- Most, if not all, high-grade serous carcinomas take origin from the Fallopian tube
- Most endometrioid, clear cell and seromucinous carcinomas arise from ovarian endometriosis
- These differences correlate with the clinical behaviour of these tumour types
- Refinements in classification are leading to more homogeneous recruitment of patients to clinical trials
- Stratification of patients between and within morphological categories using molecular data has therapeutic implications e.g.
  - PARP inhibitors
  - Lynch syndrome

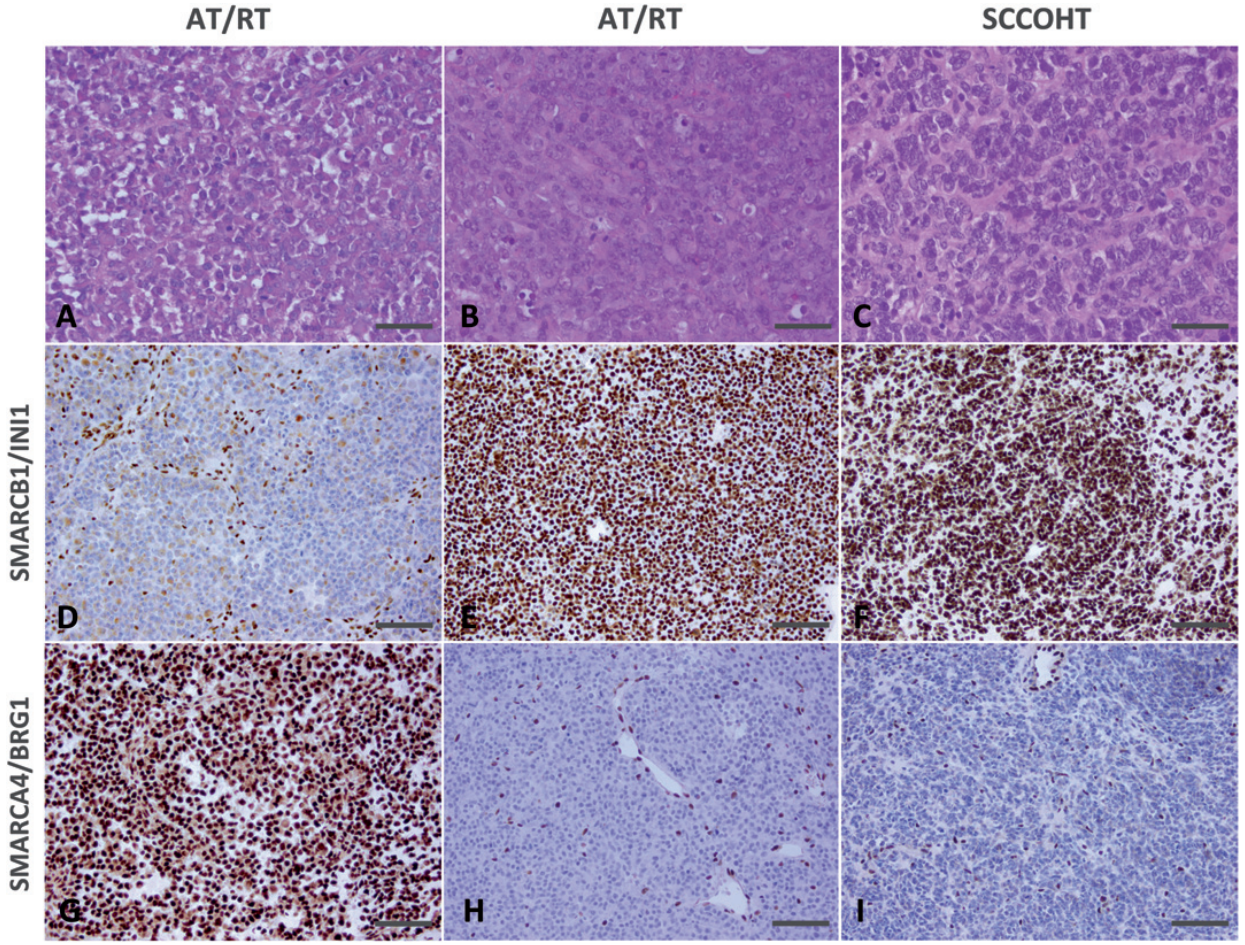
Non-epithelial Tumours

Table 1. Summary of clinicopathological features of adult granulosa cell tumour (AGCT), Sertoli–Leydig cell tumour (SLCT), and small-cell carcinoma of the ovary, hypercalcaemic type (SCCOHT)

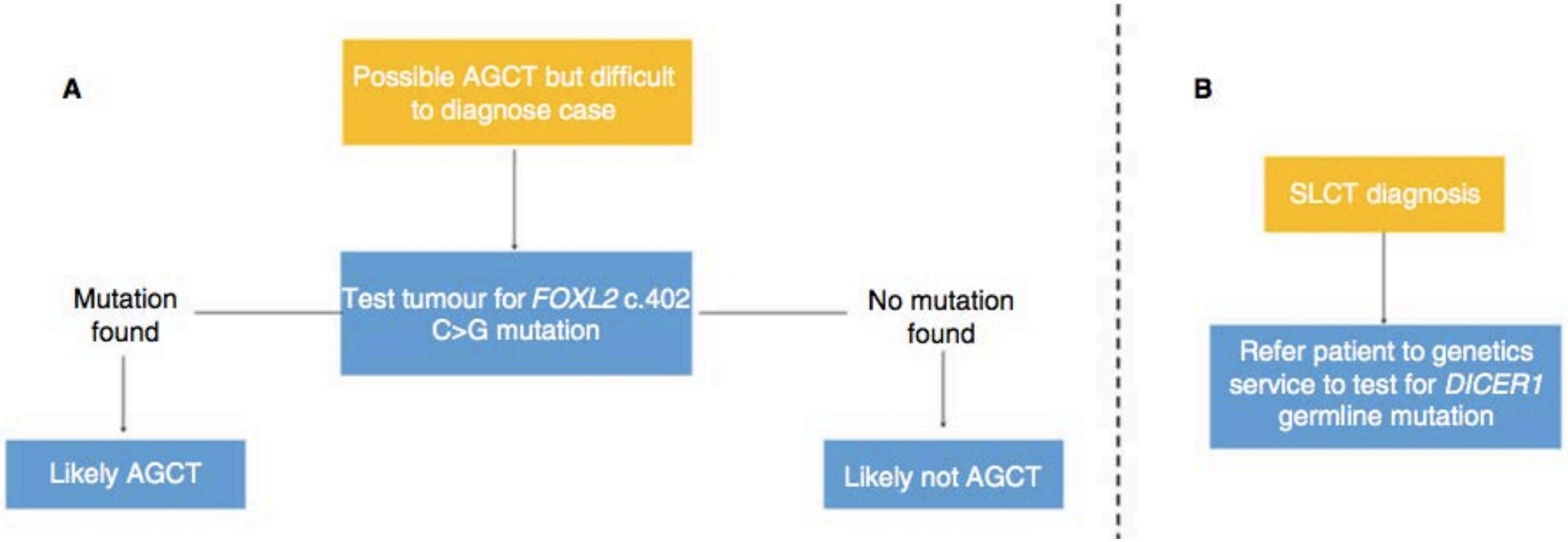
	AGCT	SLCT	SCCOHT
Mean age at diagnosis (years)	50	25	24
Percentage of all ovarian malignancies	2–4	<1	<1
Five-year survival (%)	60–80	70–100	Stage I: 55 Stage II: 40 Stage III: 29 Stage IV: 0
Somatic genetics	*FOXL2* c.402C>G mutation in >90% of cases	*DICER1* mutations in up to 60% of cases	*SMARCA4* mutations in 98% of cases
Germline genetics	None known	*DICER1* mutations—frequency unknown	*SMARCA4* mutations in ~43% of cases, including all cases diagnosed under the age of 15 years

Witkowski L et al Histopathology 2016 69:903–13

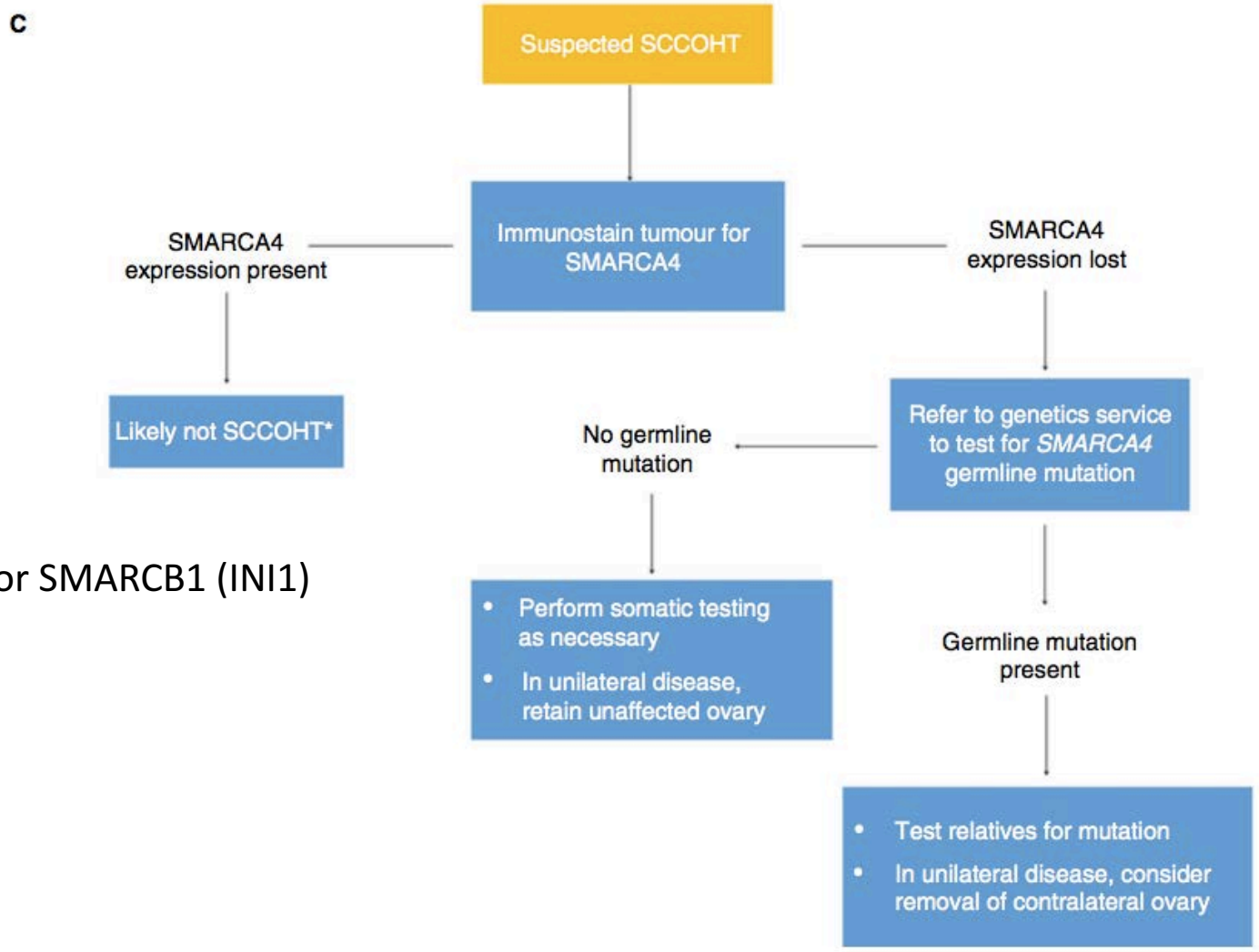
BRG1 Loss in Small Cell Carcinoma, Hypercalcaemic Type



Foulkes et al J Pathol 2014; 233: 209 - 214



Witkowski L et al Histopathology 2016 69:903–13



*Stain for SMARCB1 (INI1)

Witkowski L et al Histopathology 2016 69:903–13

Outline

- What is Molecular Pathology?
- Lower Genital Tract
  - HPV infection
  - HPV testing
- Endometrium
  - Molecular changes
  - Molecular classification
  - Stromal tumours
- Ovary, Fallopian tube and Peritoneum
  - Origins and types of epithelial tumour
  - Non-epithelial tumours
- Hereditary Gynaecological Tumours

Hereditary Gynaecological Tumours

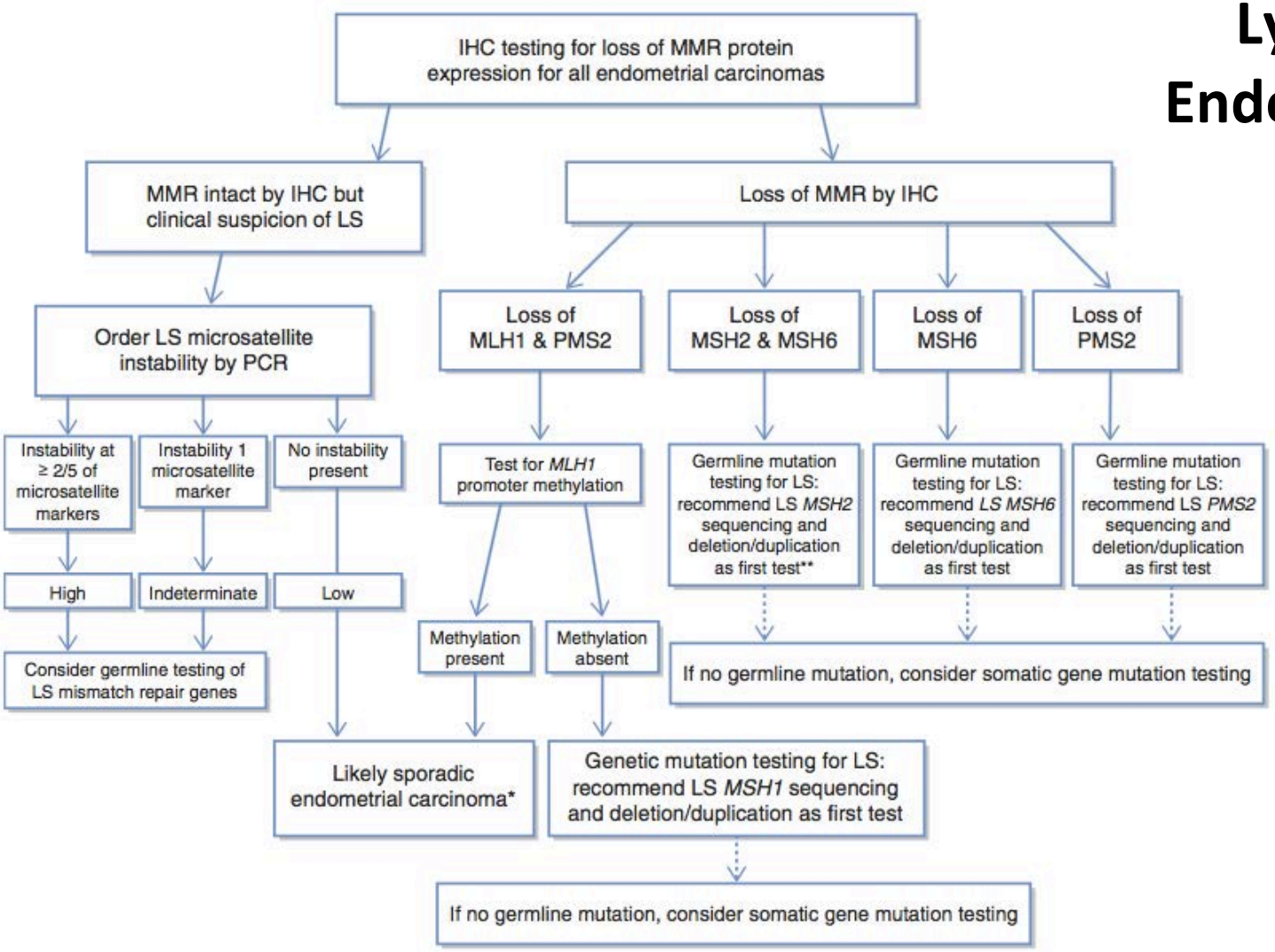
- Breast-ovarian cancer syndrome
- Site-specific ovarian cancer syndrome
- Lynch syndrome
- Other syndromes
  - Small cell carcinoma, hypercalcaemic type
  - DICER1 syndrome
  - Peutz-Jeghers
  - Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)
  - Gorlin syndrome
  - Cowden syndrome

Folkins & Longacre. Histopathology 2013; 62: 2-30

Lynch Syndrome

Colorectum	25-50%
Endometrium	25-70%
Ureter and renal pelvis	10%
Ovary	10%
Stomach	10%
Small bowel	5%
Brain (usually glioblastoma)	4%
Skin (sebaceous adenoma/carcinoma)	4%
Biliary tract	2%
Pancreas	2%

Lynch Syndrome – Endometrial Carcinoma



Mills & Longacre Am J Surg Pathol 2016;40:e35–44

Lynch Syndrome – Endometrial Carcinoma

- Universal vs age-dependent screening
- Unusual staining patterns
- Methylation testing
  - *BRAF* mutation not a surrogate for *MLH1* promoter hypermethylation, unlike in the colon
- Somatic mutation testing?

Mills & Longacre Am J Surg Pathol 2016;40:e35–44

Lynch Syndrome – Ovarian Carcinoma

- 2-4% of ovarian carcinomas
- Occur at younger age
- 85% clear cell
- 10% endometrioid
- Associated particularly with *MSH2* and *MSH6* mutations

Rambau PF et al Histopathology 2016;69:288–97

The Histomorphology of Lynch Syndrome–associated Ovarian Carcinomas

Toward a Subtype-specific Screening Strategy

TABLE 1. Patient Demographics

Characteristic	Total (n = 20)	Gene Mutated		
		MLH1 (n = 5)	*MSH2* (n = 13)	*MSH6* (n = 2)
Age (y)				
Median	43	43	43	38
Range	25-69	42-45	32-69	25-52
Sentinel OC (n [%])	13 (65)	4 (80)	7 (54)	2 (100)
Index case (n [%])	15 (75)	4 (80)	9 (69)	2 (100)
Other tumors (n [%])				
Endometrial/synchronous	9 (45)/6	3 (60)/1	5 (38)/5	1 (50)/0
Colorectal	8 (40)	2 (40)	6 (46)	0 (0)
Other	5 (25)	1 (20)	4 (31)	0 (0)

- MMR deficiency identified in 10/48 consecutive non-serous ovarian carcinomas
- All were of endometrioid or clear cell type
- ‘Given the widespread availability of MMR-IHC, reflex testing for MMR deficiency is recommended for non-serous OCs, particularly of endometrioid or clear cell type’.

Chui et al, Am J Surg Pathol 2014; 38: 1173-1181

Summary

- **Molecular Testing**
  - There must be a clear clinical question
  - Test performance must be established
  - Quality control must be ensured

- **Lower Genital Tract**
  - HPV typing is useful in some (uncommon) situations in histopathology
  - Use is more established in cytopathology
    - Primary HPV testing
    - Reflex testing of low grade abnormalities
    - Follow-up of treated disease (‘test of cure’)

Summary

- **Endometrium**
  - Improved molecular understanding suggests a diagnostic algorithm for endometrial carcinomas, involving *POLE* mutation testing
  - Endometrial stromal tumours have characteristic translocations
- **Ovary, Fallopian tube and Peritoneum**
  - The different types of epithelial ovarian carcinoma have different anatomical and molecular origins
  - Identification of specific molecular abnormalities may indicate type (e.g. *TP53* mutation) and possibly behaviour (e.g. *BRAF* mutation)
  - Some (rare) ovarian tumours have defining mutations e.g. *FOXL2*, *SMARCA4*
- **Hereditary Gynaecological Tumours**
  - The possibility of hereditary predisposition should be considered in relevant situations
  - Lynch syndrome screening is likely to be adopted in the near future