Molecular Gynaecological Pathology

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Outline

- What is Molecular Pathology?
- Lower Genital Tract
  - HPV infection
  - p16 immunostaining
- Endometrium
  - Molecular changes
  - Molecular classification
  - Stromal tumours
- Ovary, Fallopian tube and Peritoneum
  - Origins and types of epithelial tumour
  - Non-epithelial tumours
  - Patterns of genomic complexity
- Hereditary Gynaecological Tumours

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

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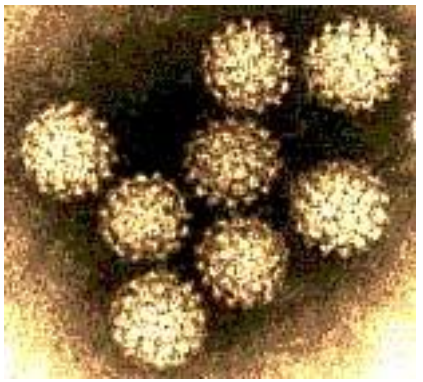
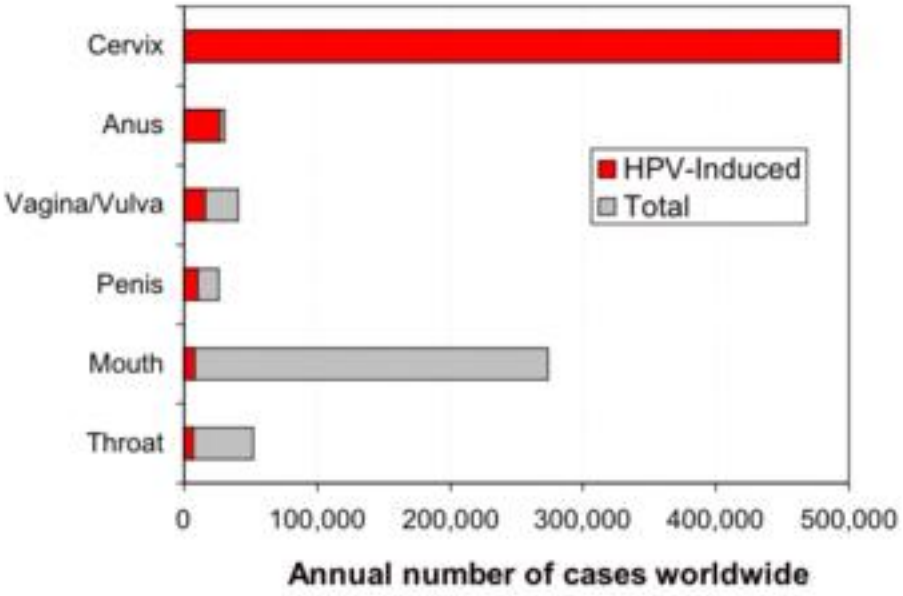
Human Papillomavirus Infection and Anogenital Disease

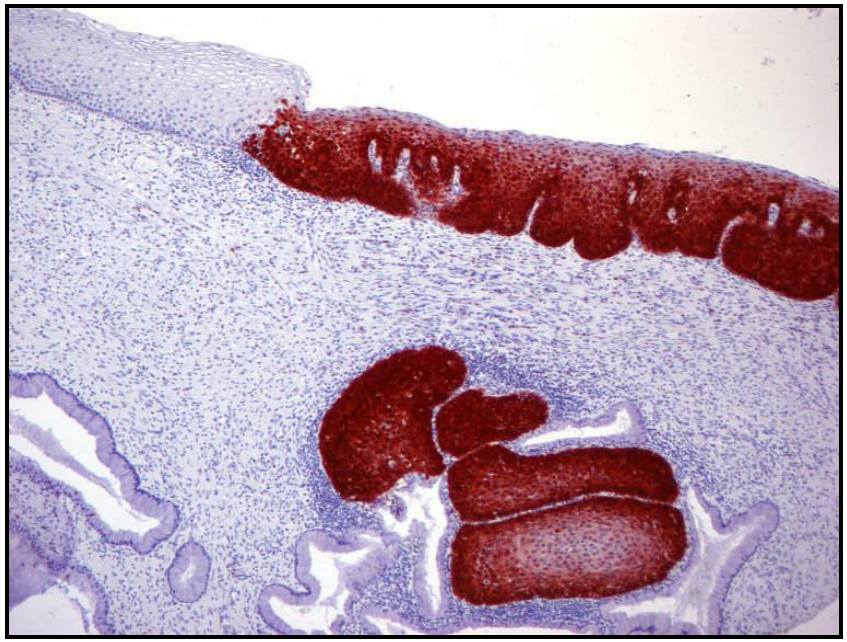


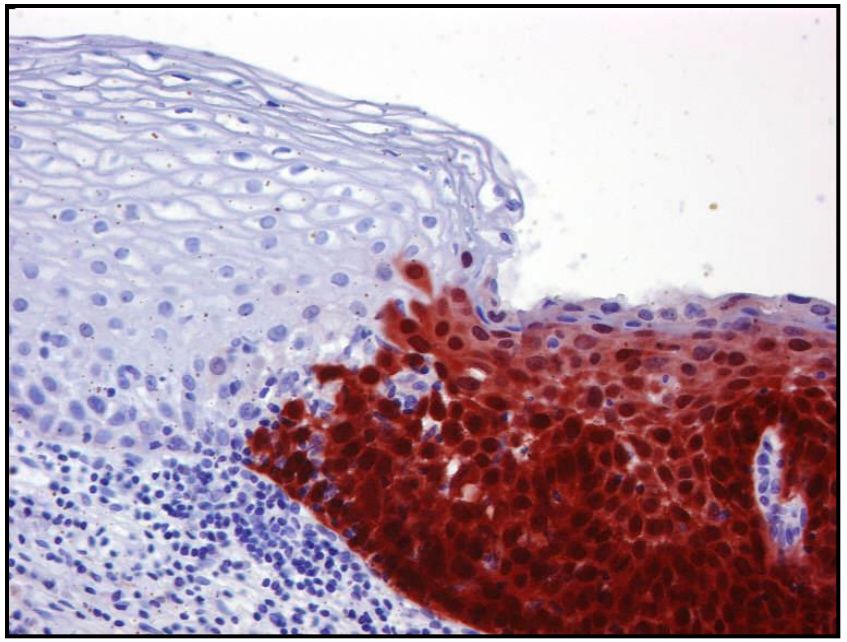
- HPV infection is present in 99.7% of invasive cervical carcinomas

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

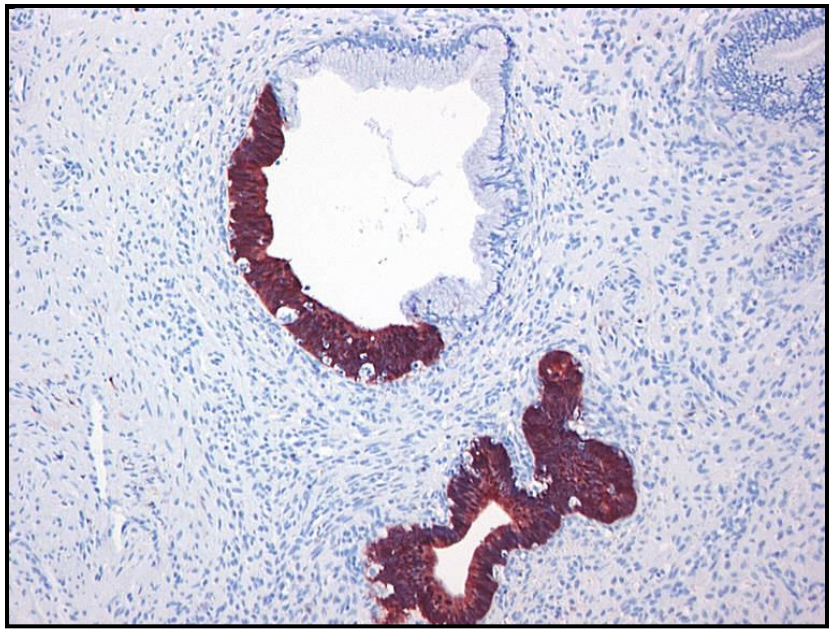


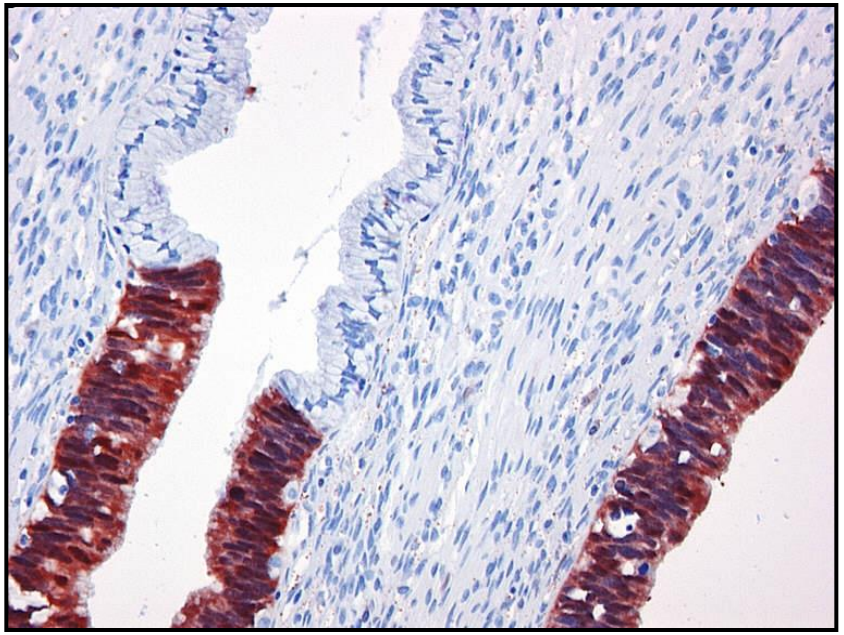
$p16^{INK4A}$ in Squamous Lesions





$p16^{INK4A}$ in Glandular Lesions





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

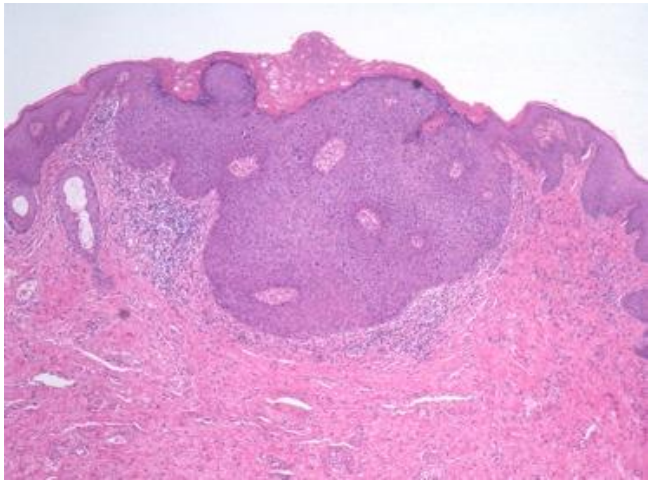
- Adenocarcinoma in situ (High grade CGIN)
- Adenocarcinoma
  - Endocervical adenocarcinoma, usual type
  - Mucinous carcinoma, NOS
    - Gastric type (including adenoma malignum / minimal deviation adenocarcinoma)
    - Intestinal type
    - Signet-ring cell type
  - Villoglandular adenocarcinoma
 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

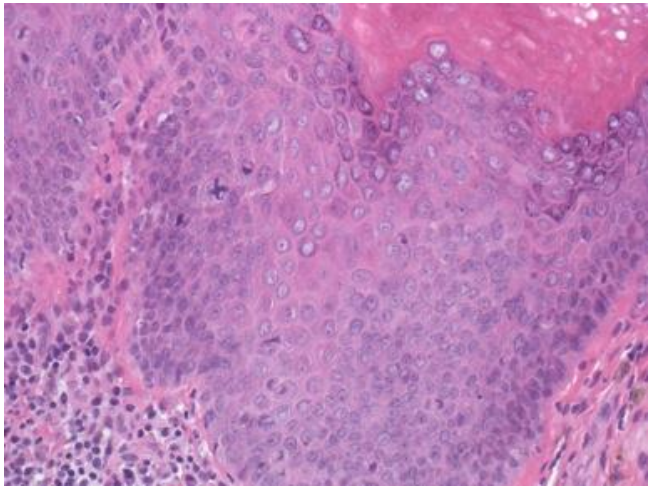
Park et al, Am J Surg Pathol 2011; 35: 633-636

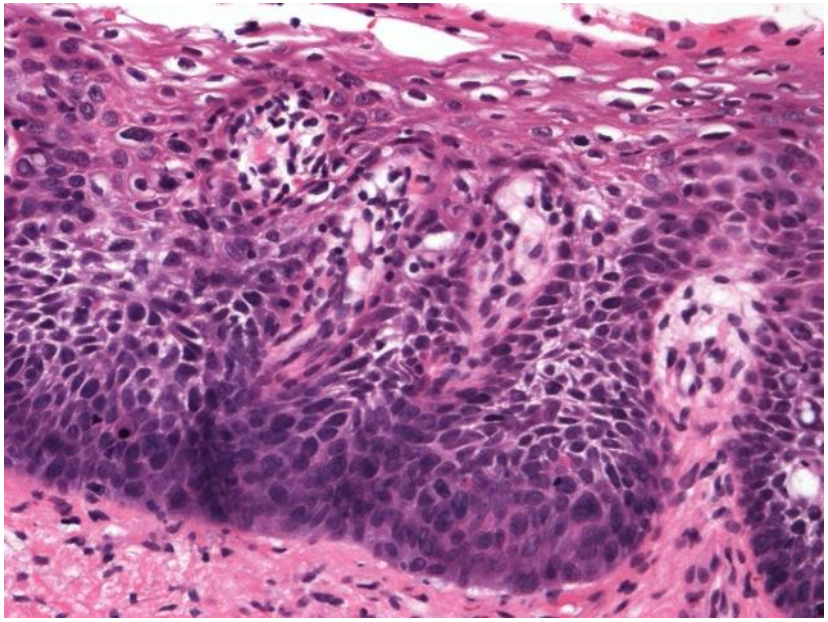
Two Pathways to Vulval Neoplasia

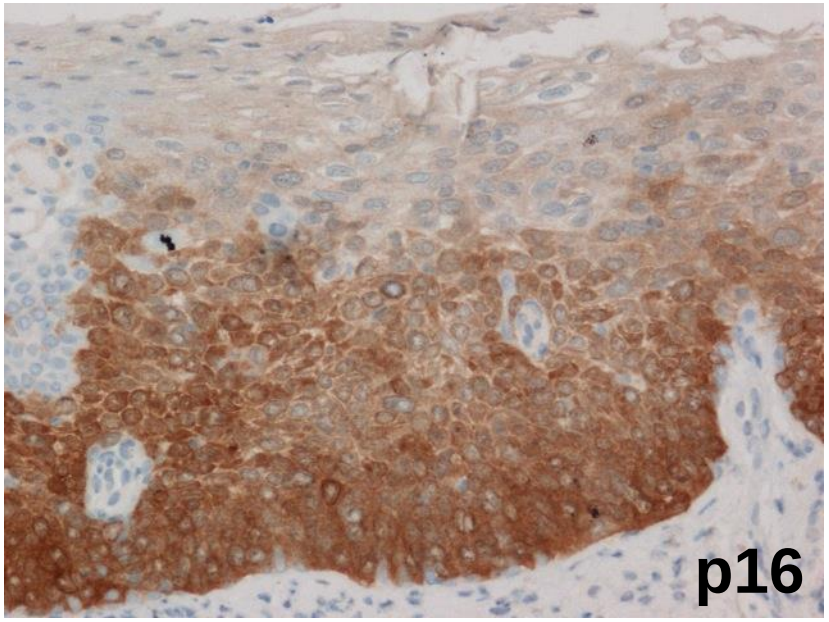
HPV-related

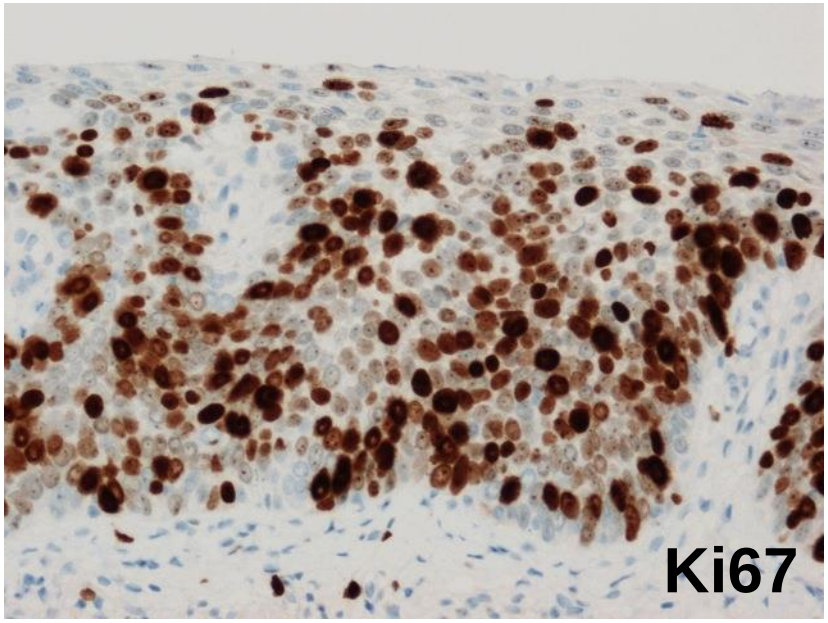
- Young women
- Warty/basaloid (undifferentiated) vulvar intraepithelial neoplasia (VIN)
- Warty/basaloid carcinoma
- Associated with other intraepithelial lesions
- Same HPV types as CIN
- Predominance of HPV 16
- Mechanisms probably similar
- p16 is a surrogate marker

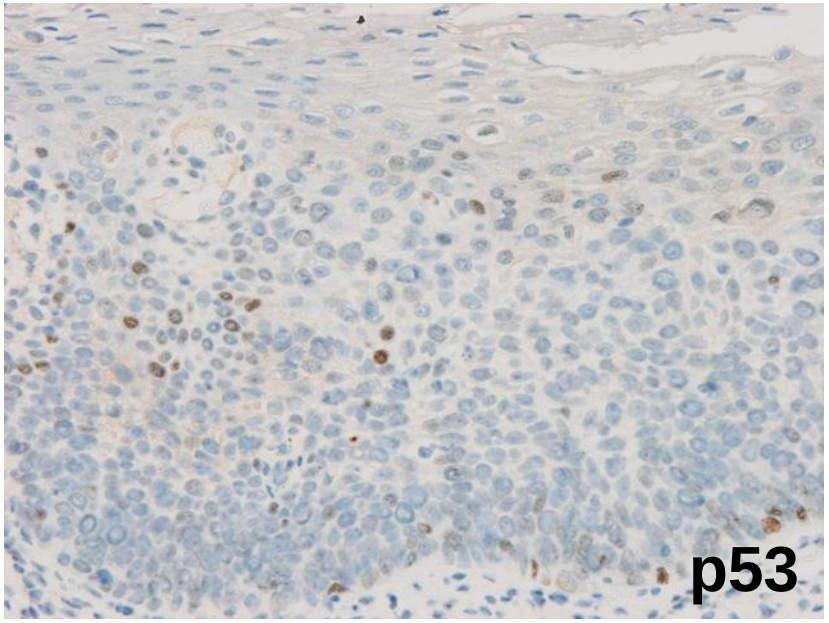






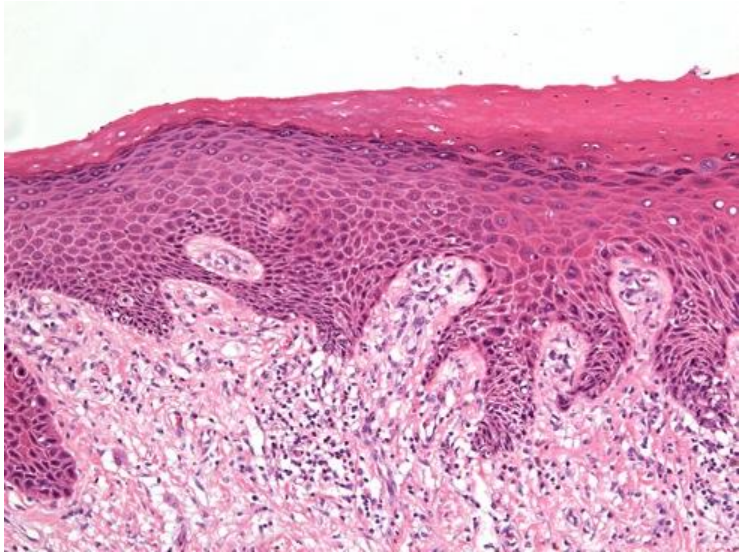

p16


Ki67


p53

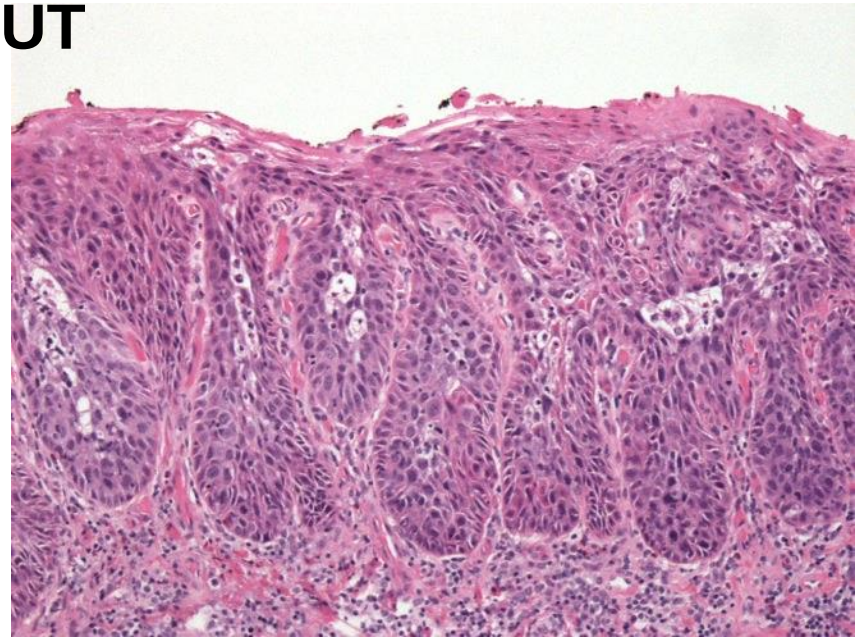
Two Pathways to Vulval Neoplasia

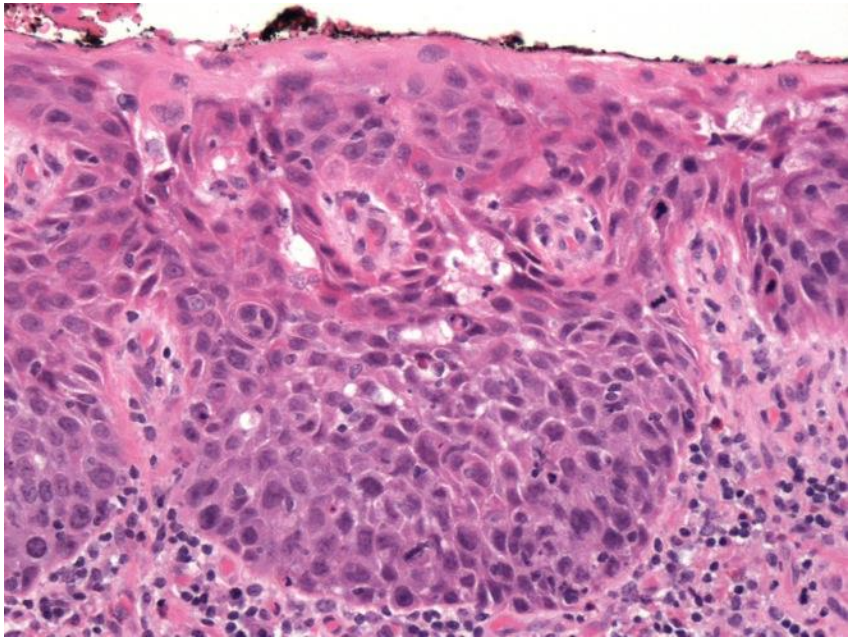
Non-HPV-related

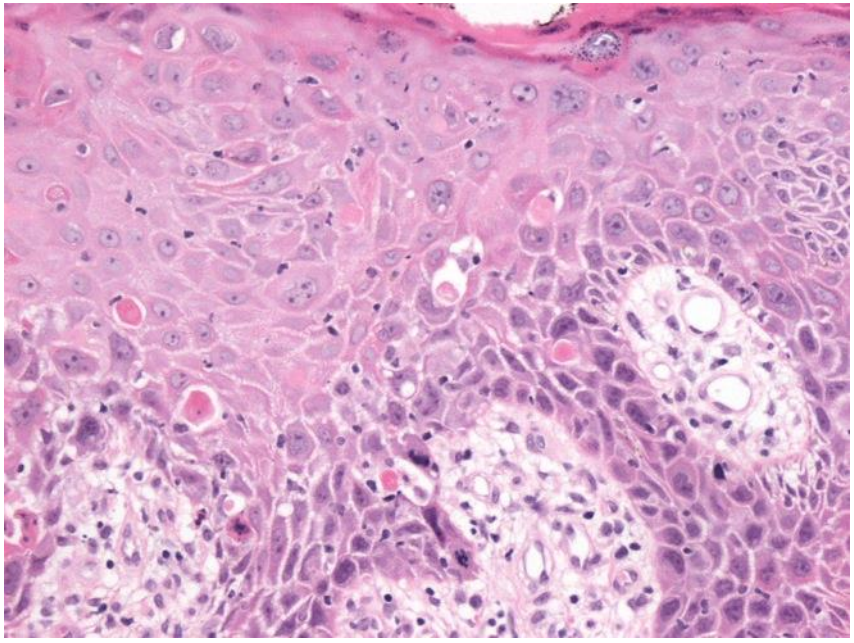


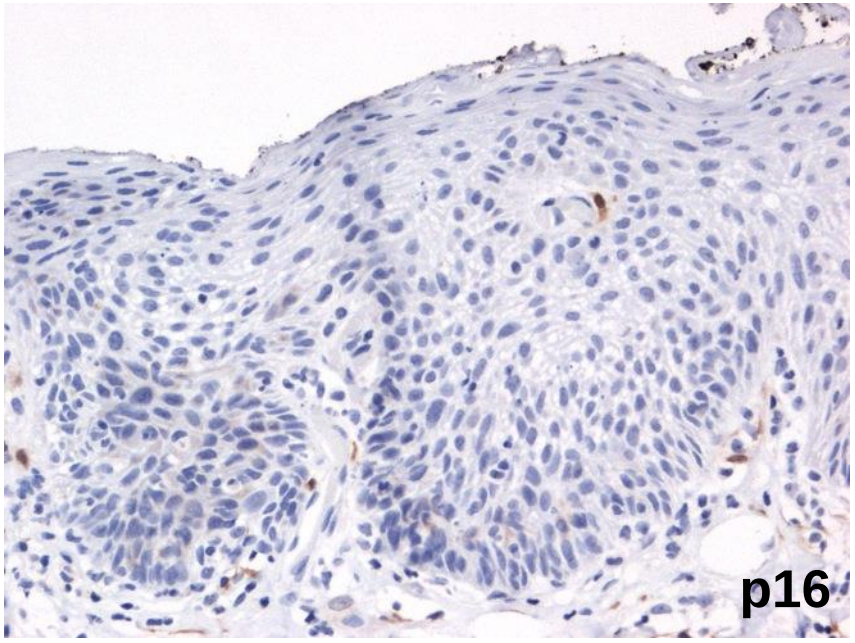
- Older women
- Associated with lichen sclerosus
- Differentiated (simplex type) VIN
- Often well differentiated squamous cell carcinoma but clinically aggressive
- p16 negative
- ? p53 mutation important (Pinto et al, Mod Pathol 2010; 23: 404-412)

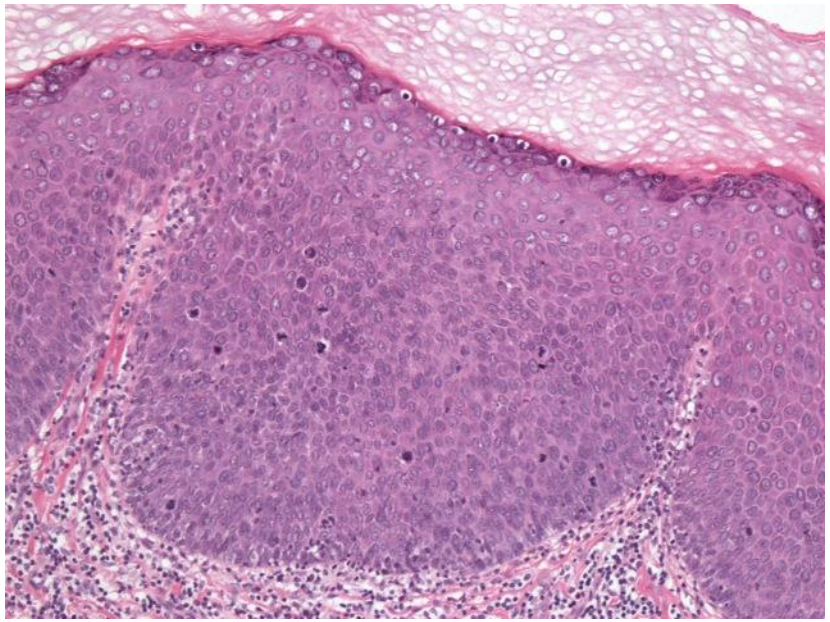
BUT

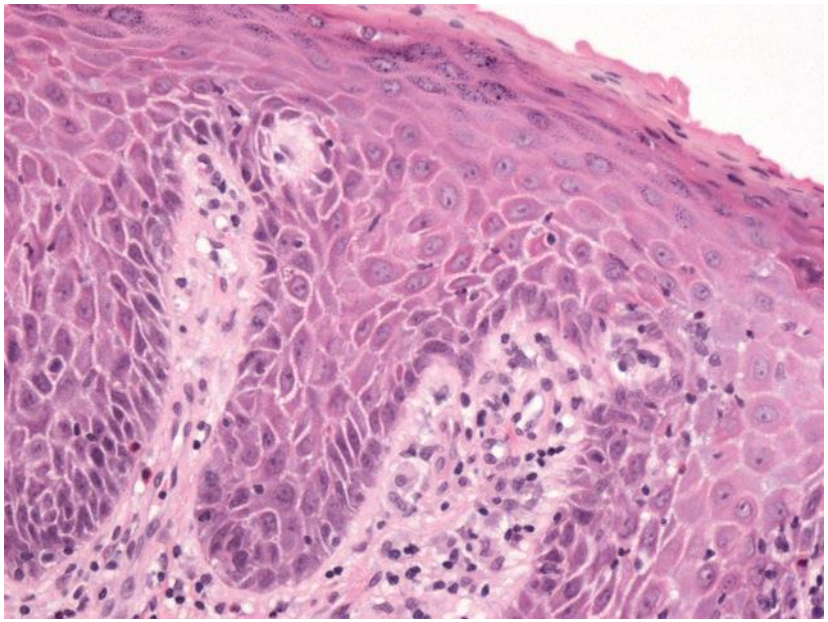


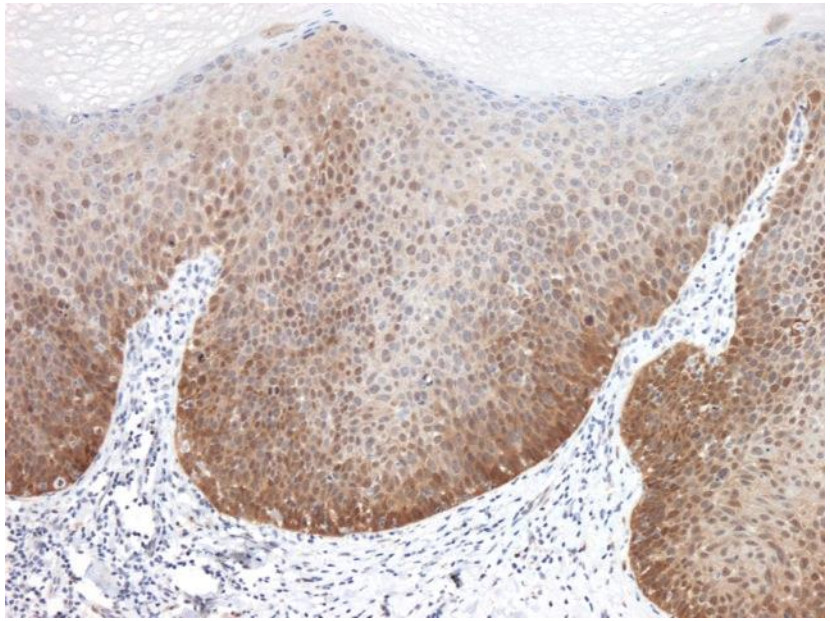


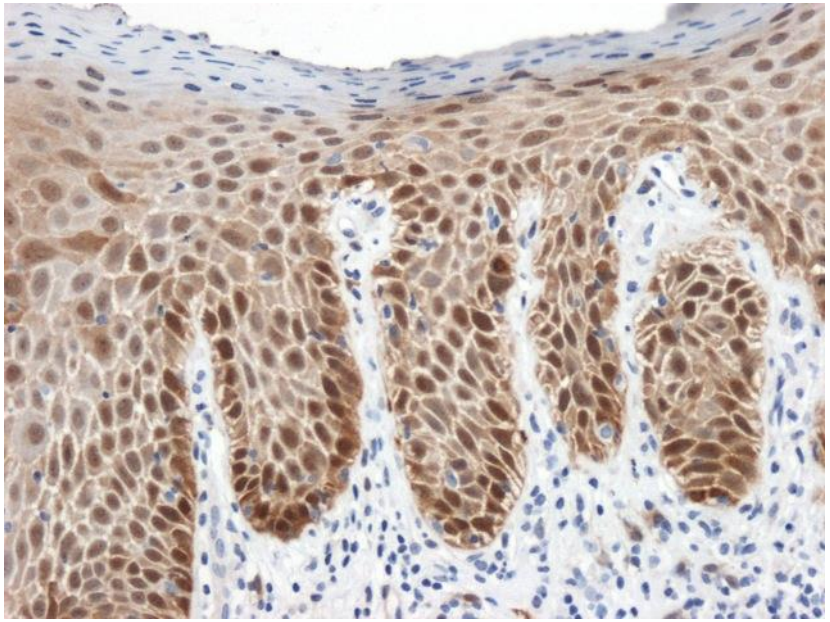












p16 as a Surrogate Marker of High-Risk HPV Infection

- In lower anogenital squamous intraepithelial lesions
  - Discrimination between high-grade SIL and mimics
  - Triage of 'CIN 2'
  - Not for diagnosis of low-grade SIL
  - Only 'block-type' positivity should be considered positive

Darragh et al Int J Gynecol Pathol 2013; 32: 76-11

- In lower genital tract tumours
  - Strong diffuse p16 positivity supports an HPV-associated aetiology
  - Endometrioid endometrial adenocarcinomas can be diffusely positive
  - Serous carcinomas are typically diffusely positive
  - Context is important and p16 should be used as part of a panel

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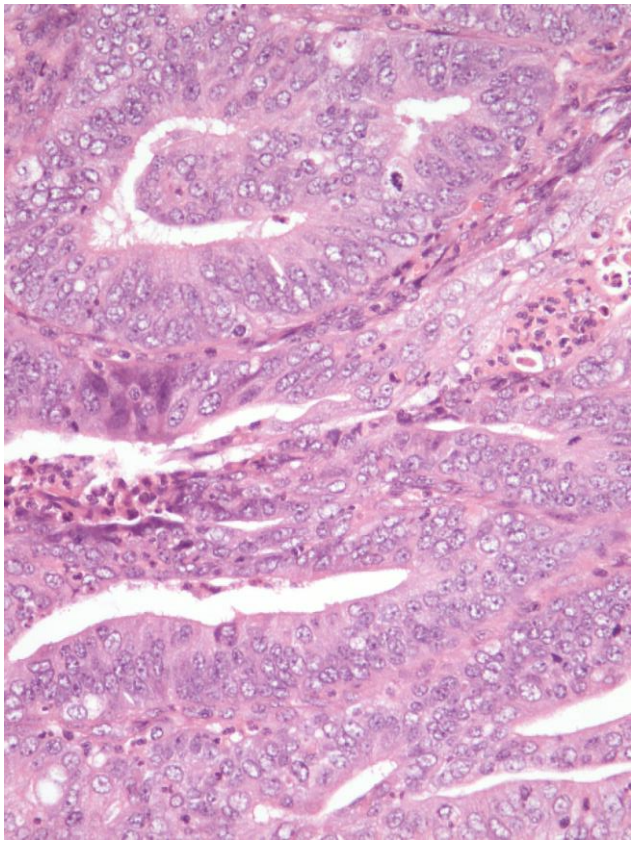
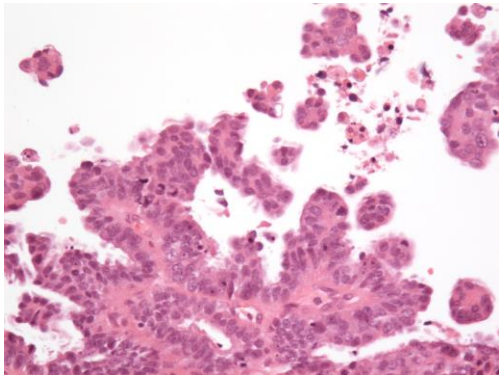
Endometrial Carcinoma

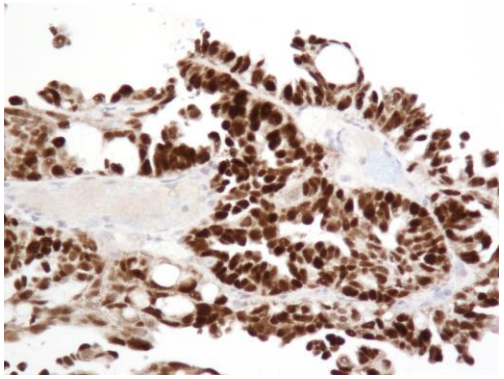
‘Type I’ tumours

- Endometrioid and mucinous phenotypes
- *PTEN, CTNNB1, KRAS, PIK3CA* mutations
- *PTEN* loss and mutation identifiable in morphologically normal proliferative glands
- Microsatellite instability
  - Germline mutation of MMR genes
  - Promoter hypermethylation esp *hMLH1*



Endometrial Carcinoma



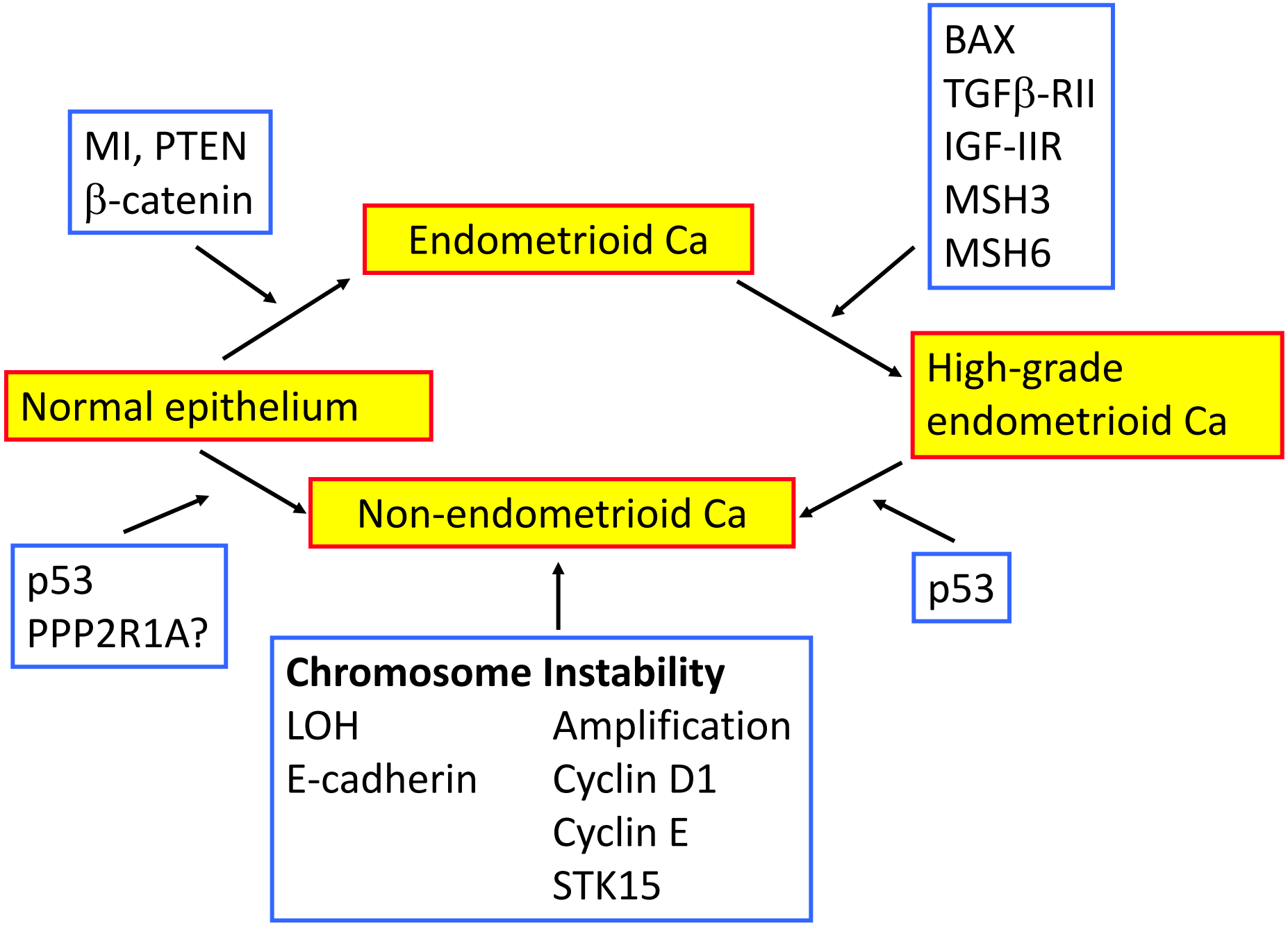


- 'Type II' tumours
  - Serous and ? clear cell phenotypes
  - *p53* mutation and overexpression
  - Inactivation of *p16* and *E-cadherin*
  - *PPP2R1A* mutation in 41% of serous

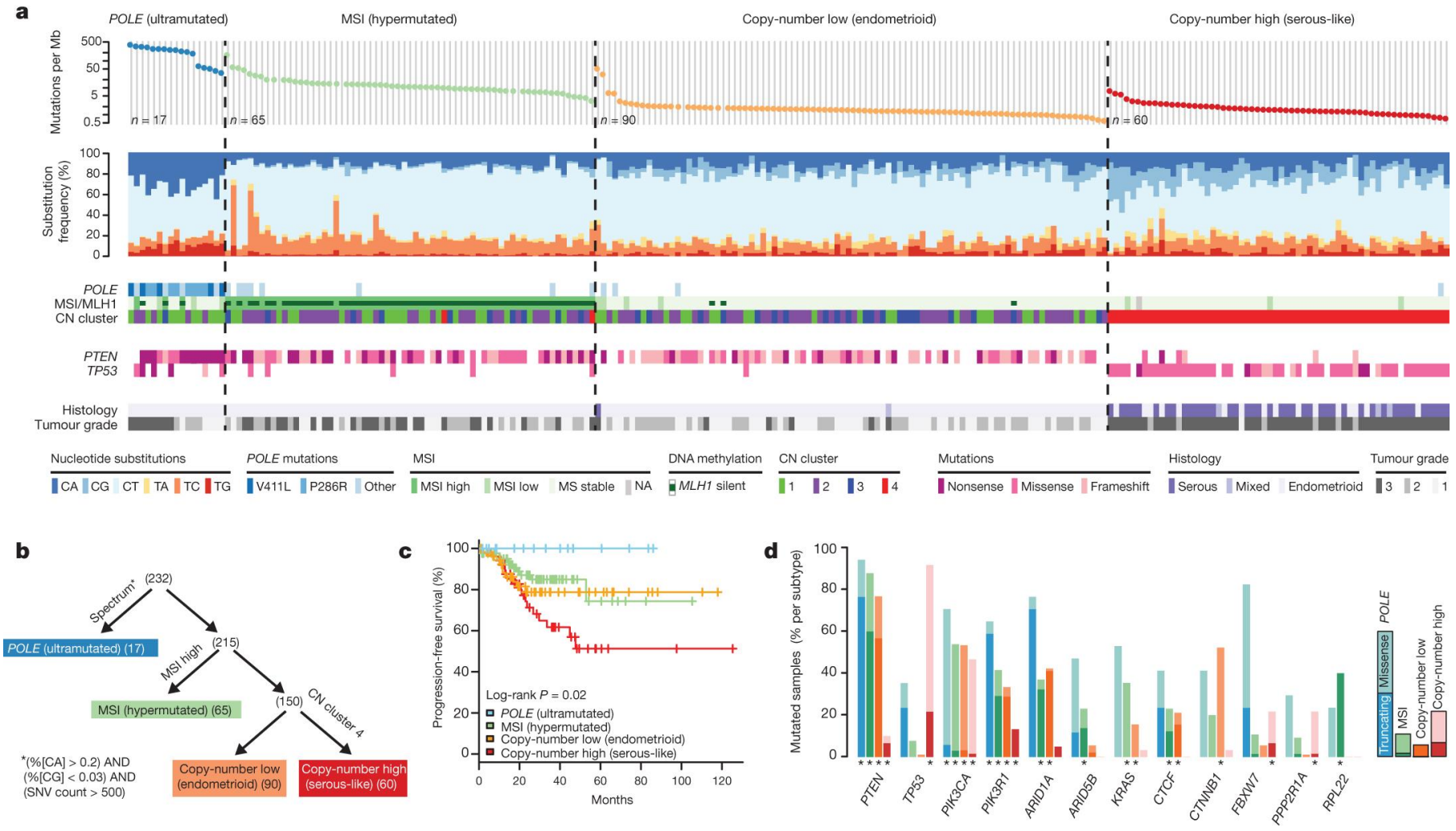
McConechy et al J Pathol 2011; 223: 567-573

- Ambiguous and mixed tumours
  - Overlapping morphological and molecular features
  - More frequently MSI-high
  - ? Dedifferentiation by acquisition of *p53* mutation

Soslow RA. Histopathology 2013; 62: 89-110


MI, PTEN
β-catenin
Endometrioid Ca
BAX
TGFβ-RII
IGF-IIR
MSH3
MSH6
Normal epithelium
High-grade endometrioid Ca
Non-endometrioid Ca
p53
PPP2R1A?
p53
Chromosome Instability
LOH
E-cadherin
Amplification
Cyclin D1
Cyclin E
STK15

Mutation Spectra Across Endometrial Carcinomas



Getz et al Nature 2013; 497: 67-73

Diagnostic Algorithm?

- Tumours associated with *POLE* mutation
  - 65% microsatellite stable, 35% p53 mutant
  - Often high grade and morphologically ambiguous

    Hussein et al Mod Pathol 2014; doi: 10.1038/modpathol.2014.145
  - Excellent outcome

    Meng et al Gynecol Oncol 2014; 134: 15-19
- Microsatellite unstable tumours
  - MMR protein immunohistochemistry
- Serous-like tumours
  - *TP53* mutation
- Endometrioid tumours
  - None of the above

Translocations in Endometrial Stromal Tumours

- Recurrent translocations present in endometrial stromal nodules and sarcomas
- 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-FAM22* fusion identifies high-grade endometrial stromal sarcoma

  Lee et al Am J Surg Pathol 2012; 36: 641-653
- Undifferentiated uterine sarcoma
  - No specific pattern

Endometrial Stromal Tumors: The New WHO Classification

Christopher M. J. Conklin, MD, FRCPC and Teri A. Longacre, MD†*

TABLE 3. Molecular Translocations Identified in Endometrial Stromal Tumors, Named Sarcomas, and Other Tumors in Differential Diagnosis

ESN	LG-ESS	HG-ESS*	UUS	LMS	LM	AS	CS	UTROSCT
JAZF1-SUZ12[29,73–77] (formerly *JAZF1-JJAZ1*)	*JAZF1-SUZ12*[29,73–77]	*YWHAE-FAM22*[10,52,78]	Complex karyotype[7,79]	—	—	—	—	—
PHF1-JAZF1[74,80]	*MEAF6-PHF1*[81]		*JAZF1-SUZ12*[29,73] (rare)					
EPC1-PHF1[74,80]								

*As defined by the *YWHAE-FAM22* translocation.

AS indicates adenosarcoma; CS, carcinosarcoma; ESN, endometrial stromal nodule; HG-ESS, high-grade endometrial stromal sarcoma; LG-ESS, low-grade endometrial stromal sarcoma; LM, leiomyoma; LMS, leiomyosarcoma; UTROSCT, uterine tumor resembling ovarian sec cord tumor; UUS, undifferentiated uterine sarcoma.

Conklin & Longacre Adv Anat Pathol 2014; 21: 383-393

WHO Classification of tumours of the uterine corpus

Epithelial tumours and precursors	
Precursors	
Hyperplasia without atypia	
Atypical hyperplasia / Endometrioid intraepithelial neoplasia	8380/2*
Endometrial carcinomas	
Endometrioid carcinoma	8380/3
Squamous differentiation	8570/3
Villoglandular	8263/3
Secretory	8382/3
Mucinous carcinoma	8480/3
Serous endometrial intraepithelial carcinoma	8441/2*
Serous carcinoma	8441/3
Clear cell carcinoma	8310/3
Neuroendocrine tumours	
Low-grade neuroendocrine tumour	
Carcinoid tumour	8240/3
High-grade neuroendocrine carcinoma	
Small cell neuroendocrine carcinoma	8041/3
Large cell neuroendocrine carcinoma	8013/3
Mixed cell adenocarcinoma	8323/3
Undifferentiated carcinoma	8020/3
Dedifferentiated carcinoma	
Dissecting (cotyledonoid) leiomyoma	8890/0
Diffuse leiomyomatosis	8890/1
Intravenous leiomyomatosis	8890/1
Metastasizing leiomyoma	8898/1
Smooth-muscle tumour of uncertain malignant potential	8897/1
Leiomyosarcoma	8890/3
Epithelioid leiomyosarcoma	8891/3
Myxoid leiomyosarcoma	8896/3
Endometrial stromal and related tumours	
Endometrial stromal nodule	8930/0
Low-grade endometrial stromal sarcoma	8931/3
High-grade endometrial stromal sarcoma	8930/3
Undifferentiated uterine sarcoma	8805/3
Uterine tumour resembling ovarian sex cord tumour	8590/1
Miscellaneous mesenchymal tumours	
Rhabdomyosarcoma	8900/3
Perivascular epithelioid cell tumour	
Benign	8714/0
Malignant	8714/3
Others	

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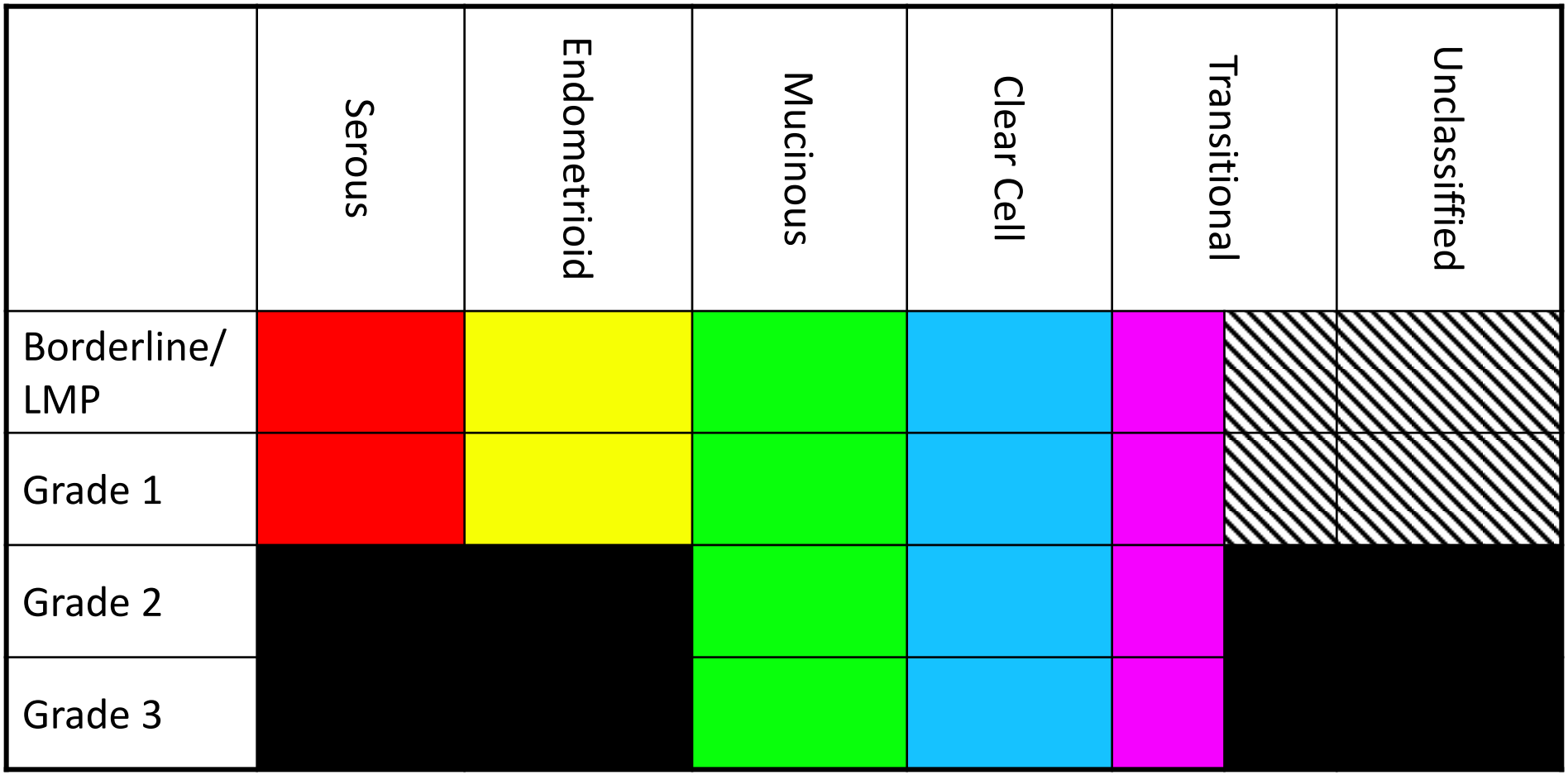
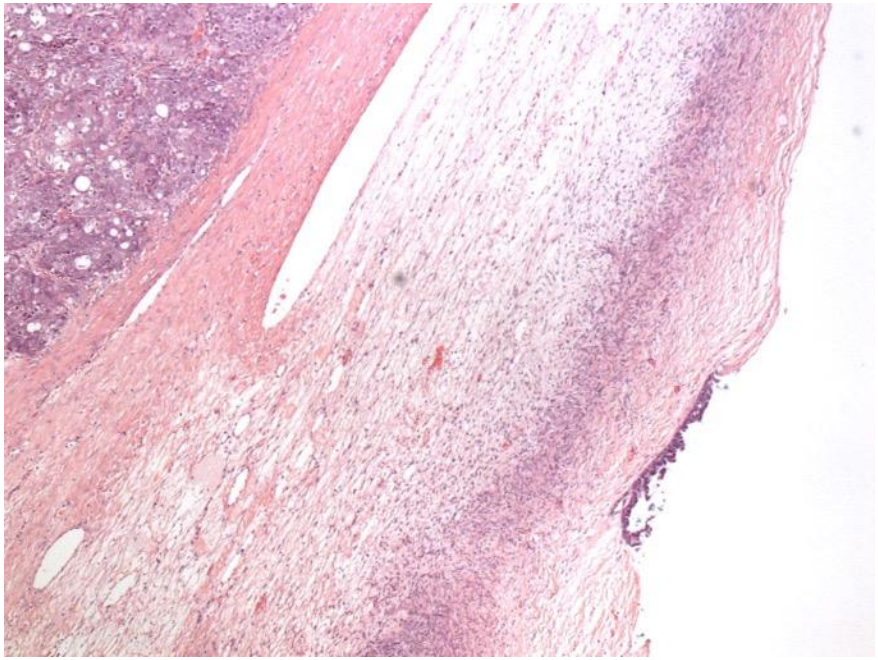
Ovarian Epithelial Tumours

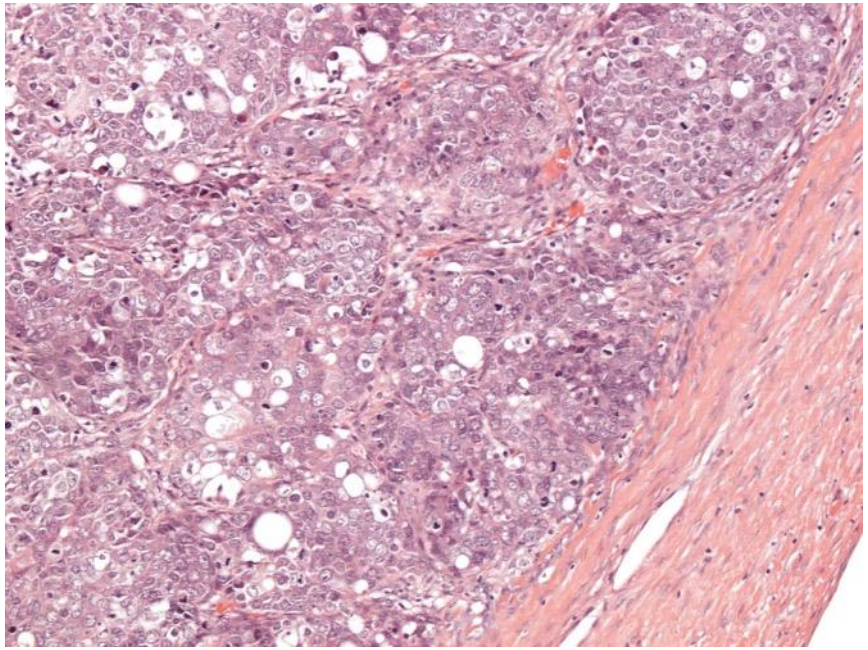


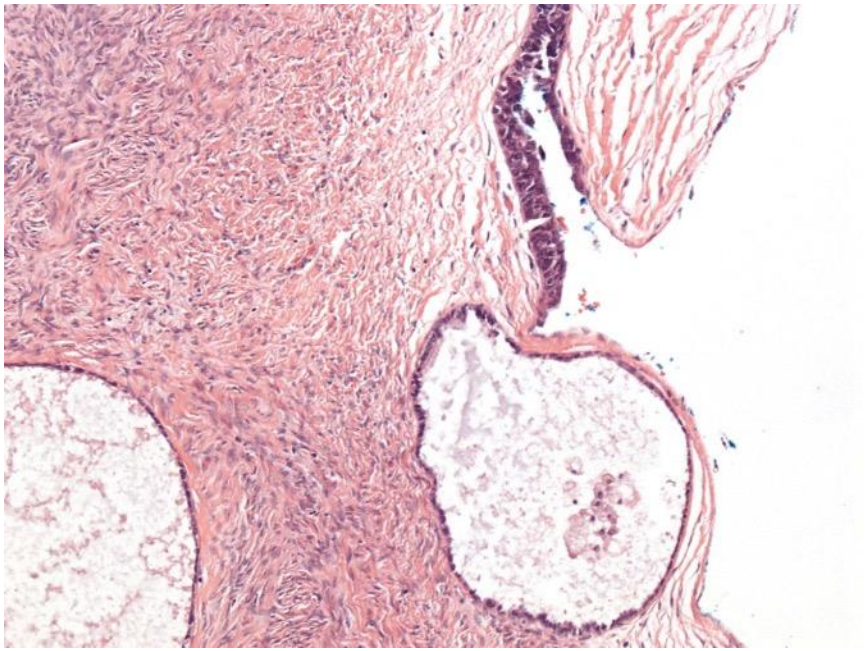
Modified from Gilks CB. Int J Gynecol Pathol 2004; 23: 200-205

High-Grade Serous, Endometrioid and Unclassified Tumours

- Loss of BRCA1/BRCA2 function
  - Germline/somatic mutation; loss of heterozygosity
  - Promoter hypermethylation
  - Amplification of EMSY
- Unable to repair dsDNA breaks
  - Complex karyotypes
- *TP53* mutation common in high-grade serous carcinoma (almost 100%)
  - Ahmed et al J Pathol 2010; 221: 49-56
- WT1 immunopositive and p53 aberrant (diffuse or absent)
- Most of tubal origin?



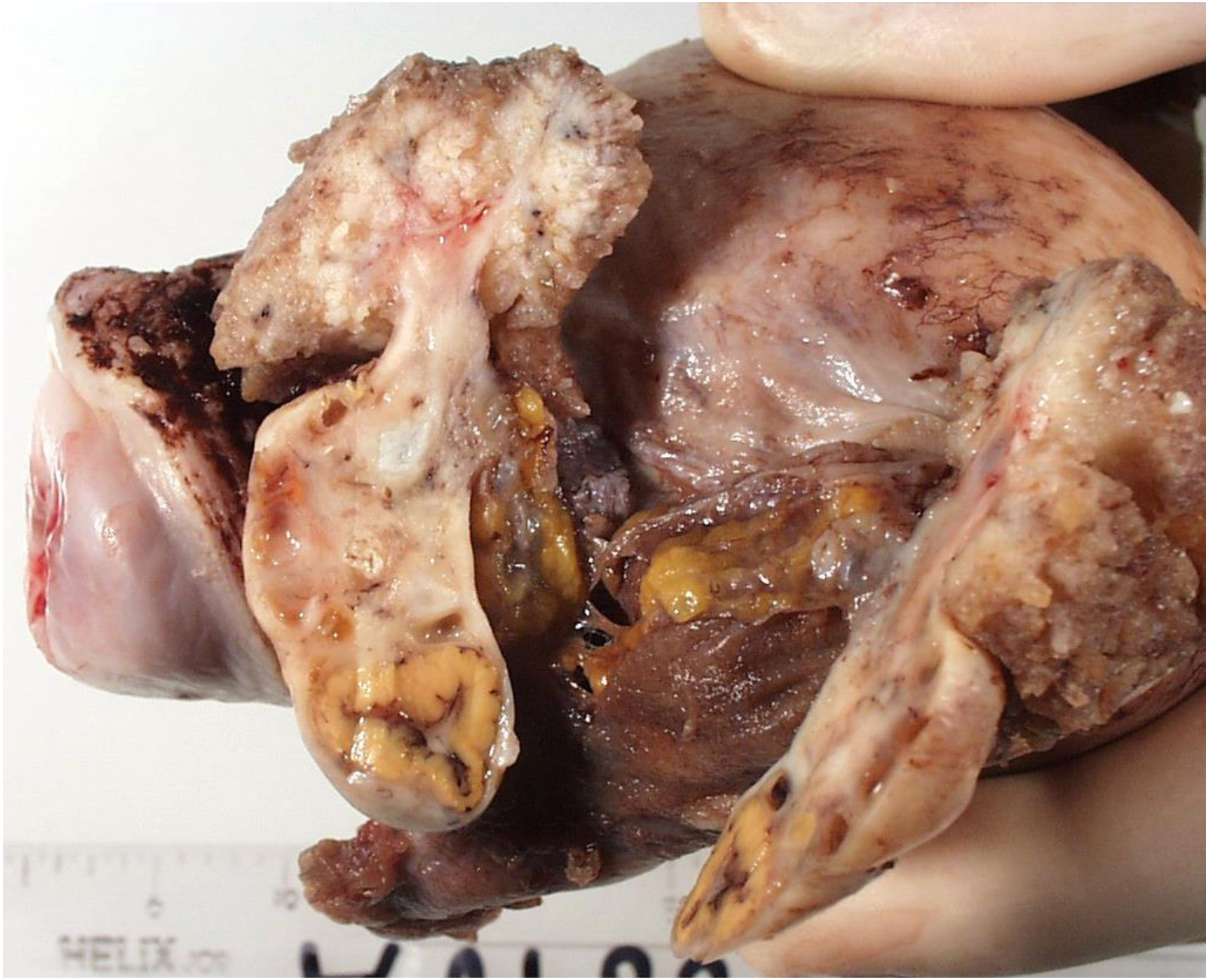


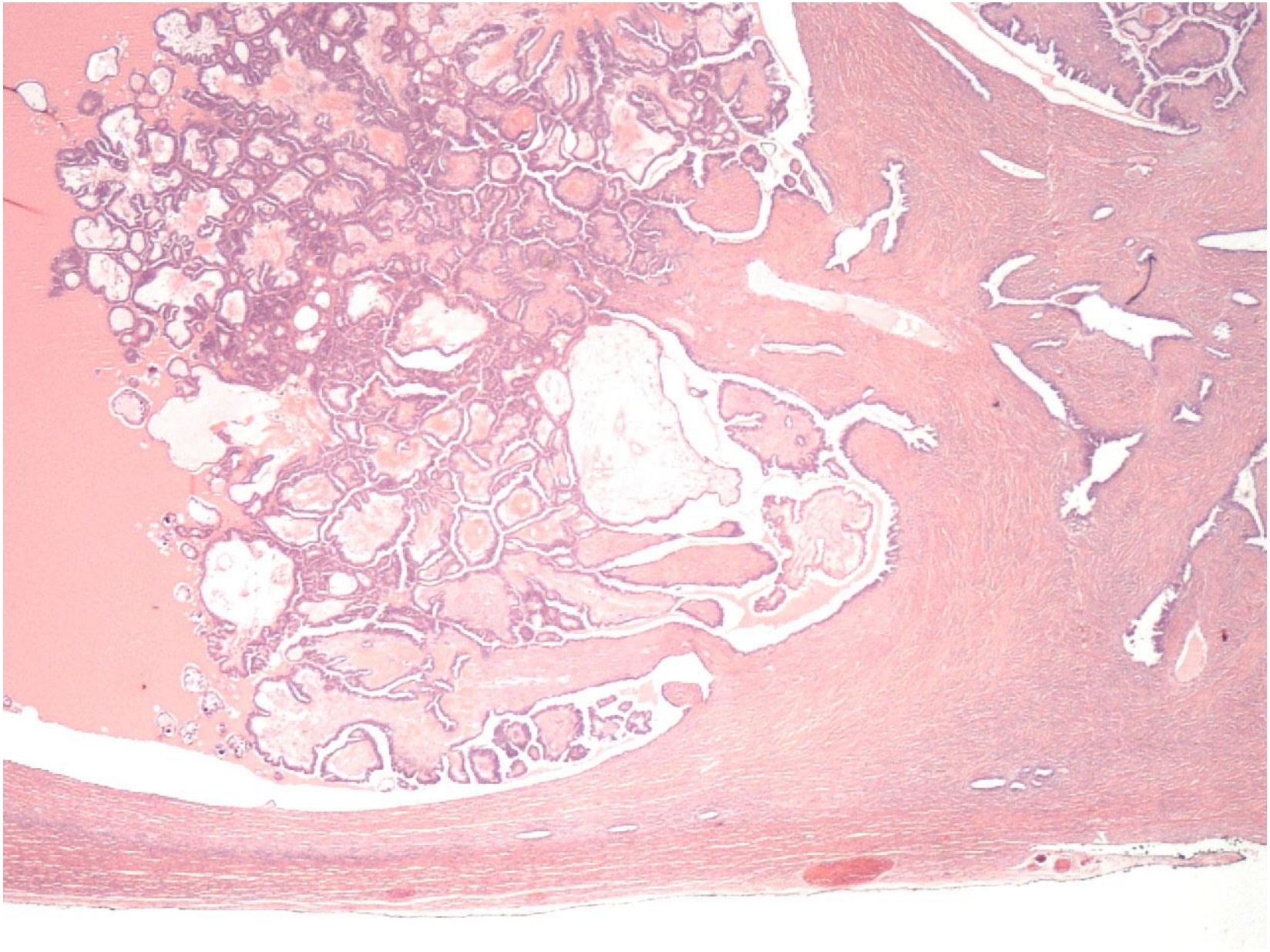


High Grade Serous Carcinoma

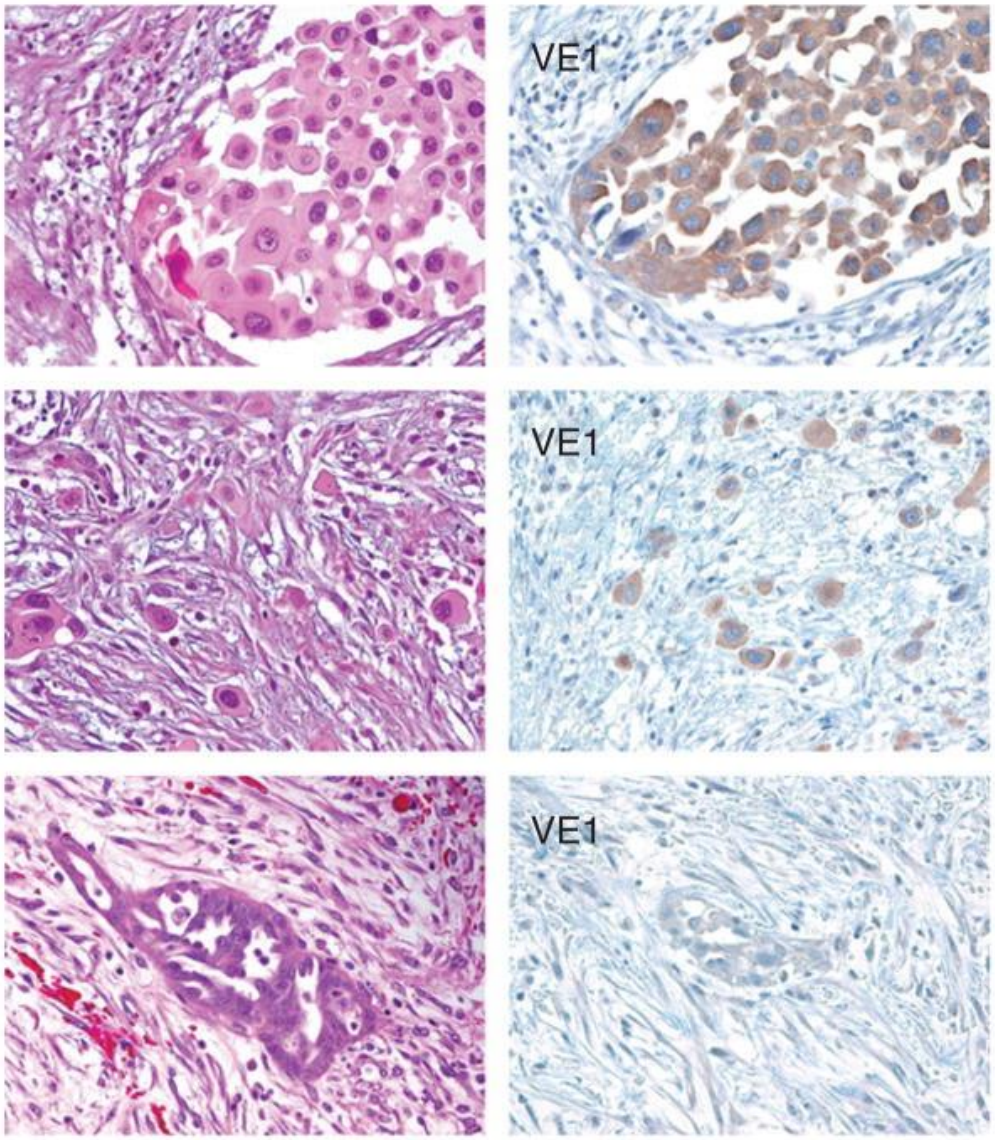
Low-grade Serous Tumours

- *BRAF* and *KRAS* mutation common in borderline and invasive tumours (60-65%)
- *p53* mutation uncommon (<10%) and often diploid
- Fewer karyotypic and other molecular abnormalities than high-grade tumours
- Diagnosis
  - Two-tier grading system based on nuclear atypia alone
    - Malpica A et al Am J Surg Pathol 2007; 31: 1168-74
- Treatment
  - Differences in chemosensitivity
    - Santillan A et al Int J Gynecol Cancer 2007; 17: 601-606


HELIX



Immunohistochemical Detection of *BRAF* V600E Mutation



Ardighieri et al J Pathol 2014; 232: 16-22

Low-grade serous tumours

- *KRAS* mutation in serous borderline tumours associated with recurrent low-grade serous carcinoma

  Tsang et al J Pathol 2013; 231: 449-456

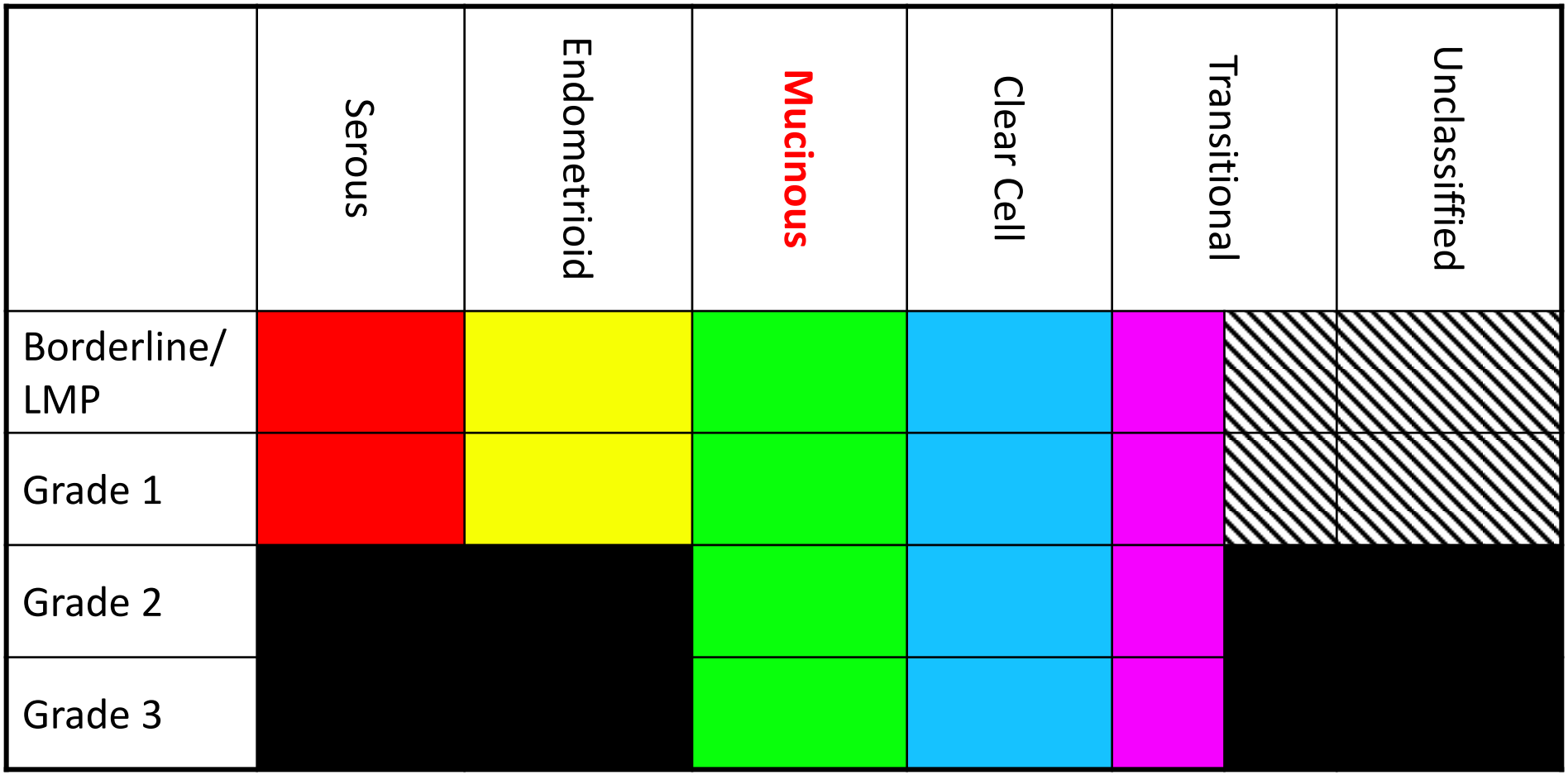
- *BRAF* V600E mutation associated with senescent phenotype in serous borderline tumours

  Zeppernick et al Am J Surg Pathol 2014; 38: 1603-11

- *NRAS* mutation restricted to invasive component in serous carcinomas with adjacent borderline regions

  Emmanuel et al Clin Cancer Res 2014; Epub Oct 14, 2014

Ovarian Epithelial Tumours



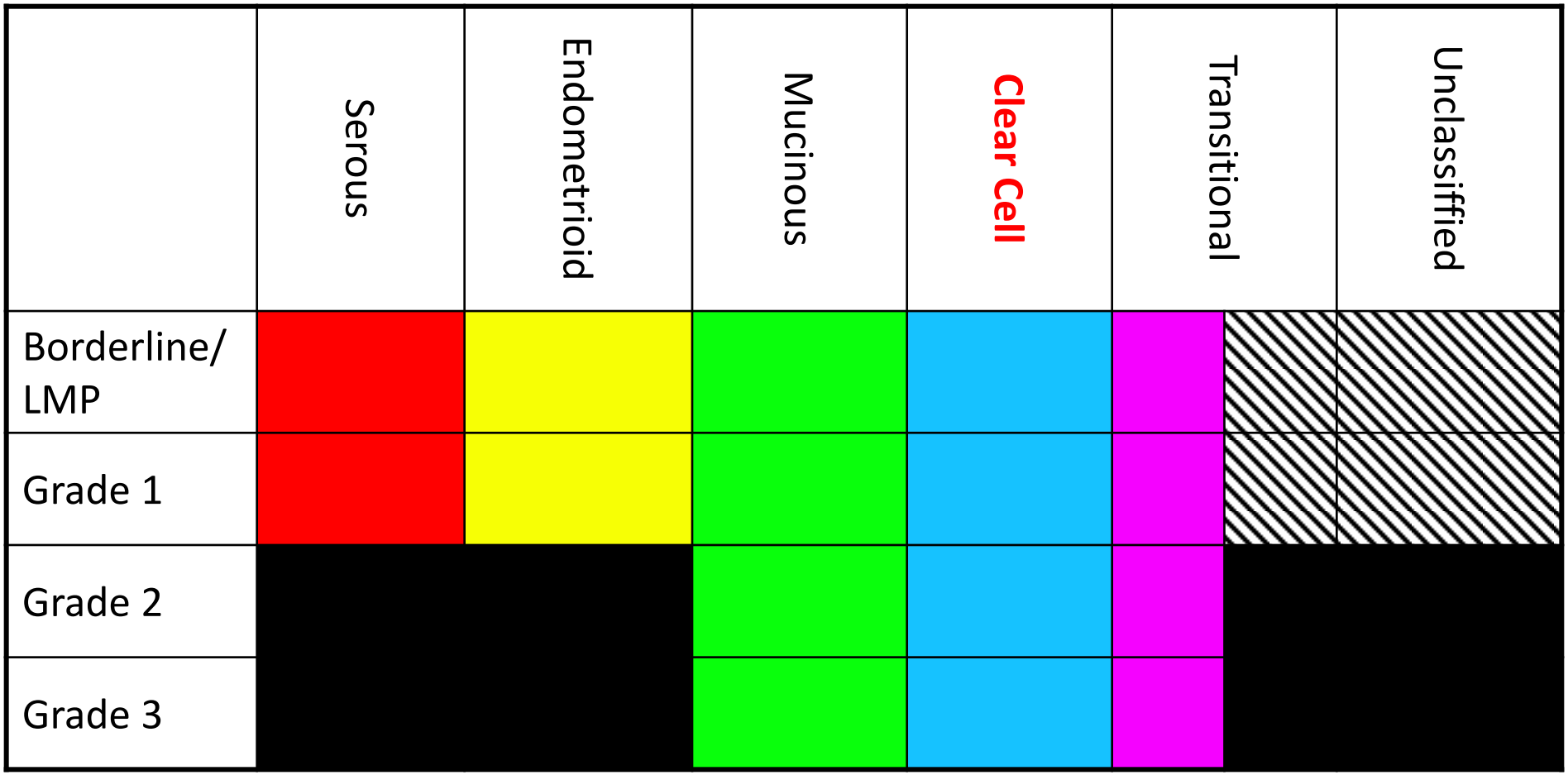
Modified from Gilks CB. Int J Gynecol Pathol 2004; 23: 200-205

Mucinous Tumours

- Borderline tumours, microinvasive and invasive carcinomas
- *KRAS* but not *BRAF* mutations common
- True primary tumours are uncommon (6 of 220 ovarian carcinomas - Seidman et al. Int J Gynecol Pathol 2004; 23: 41-4)
- *HER2* amplification in approx 20% of primary mucinous carcinomas (clinical significance unclear)

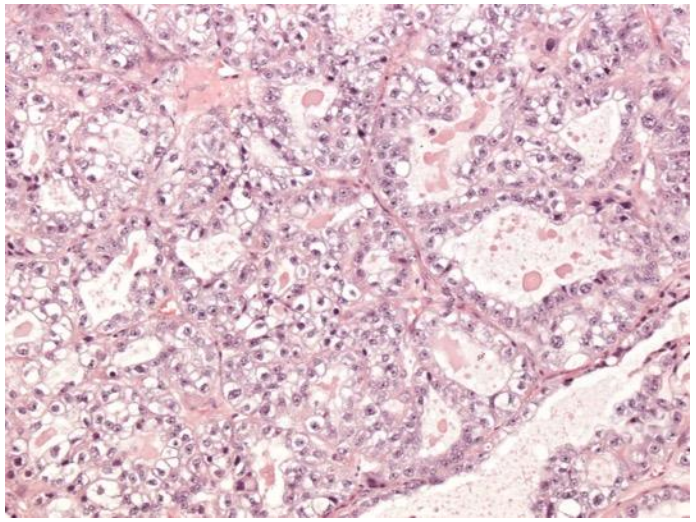
  Anglesio et al J Pathol 2013; 229: 111-120
- Must rigorously exclude metastases

Ovarian Epithelial Tumours



Modified from Gilks CB. Int J Gynecol Pathol 2004; 23: 200-205

Clear Cell Carcinoma



- Associated with endometriosis
- Also associated with Lynch syndrome
- Not clear if can separate low and high grade groups
- Some evidence that tumours associated with endometriosis less aggressive than those associated with clear cell adenofibroma

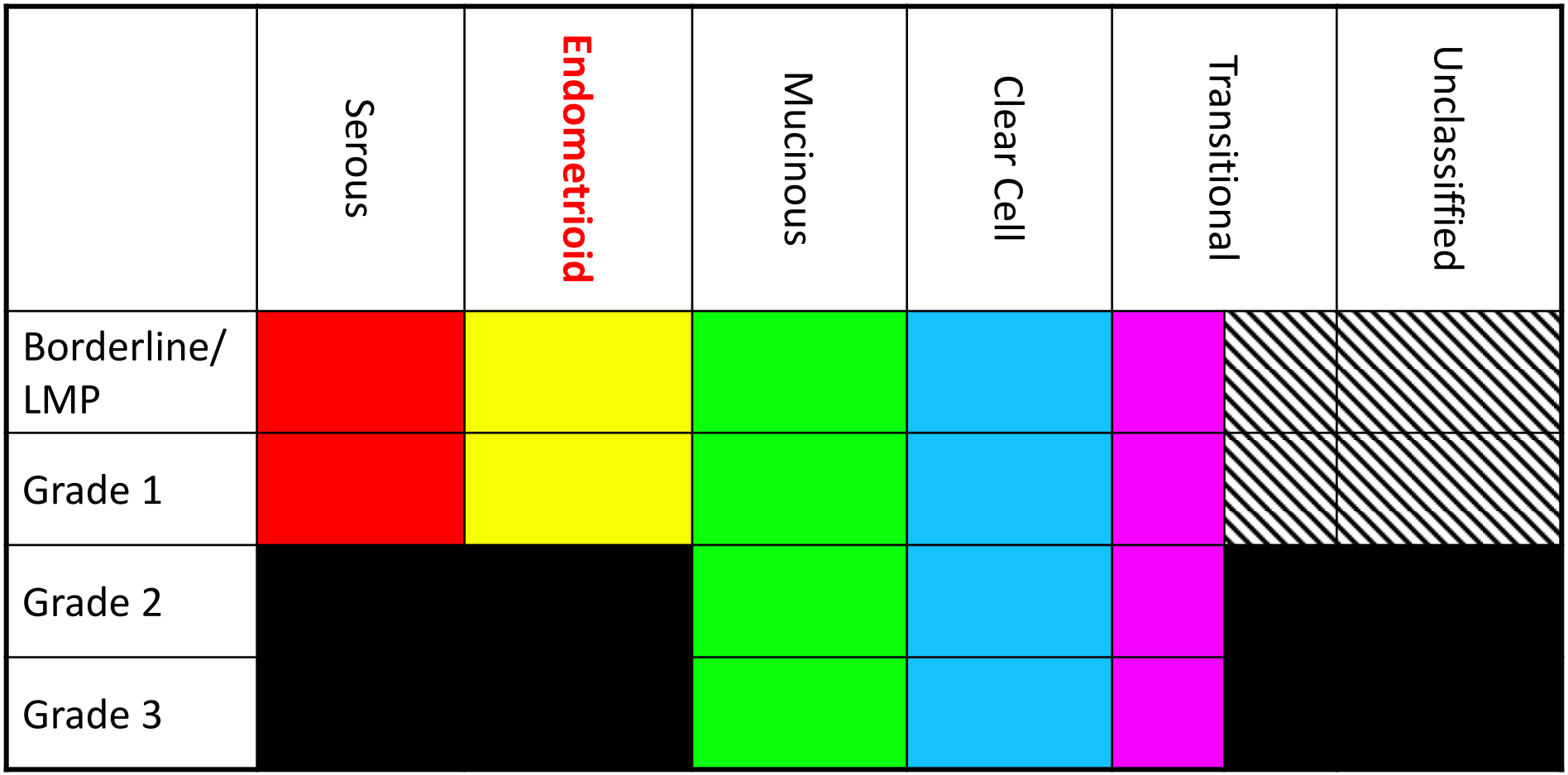
  Veras et al. *Am J Surg Pathol* 2009; 33: 844-853

- Inactivating mutation of *ARID1A* in approx 50%, activating mutation of *PIK3CA* in approx 50%, deletion of *PTEN in* approx 20%

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

  Lowery et al Int J Gynecol Cancer 2012; 22: 9-14

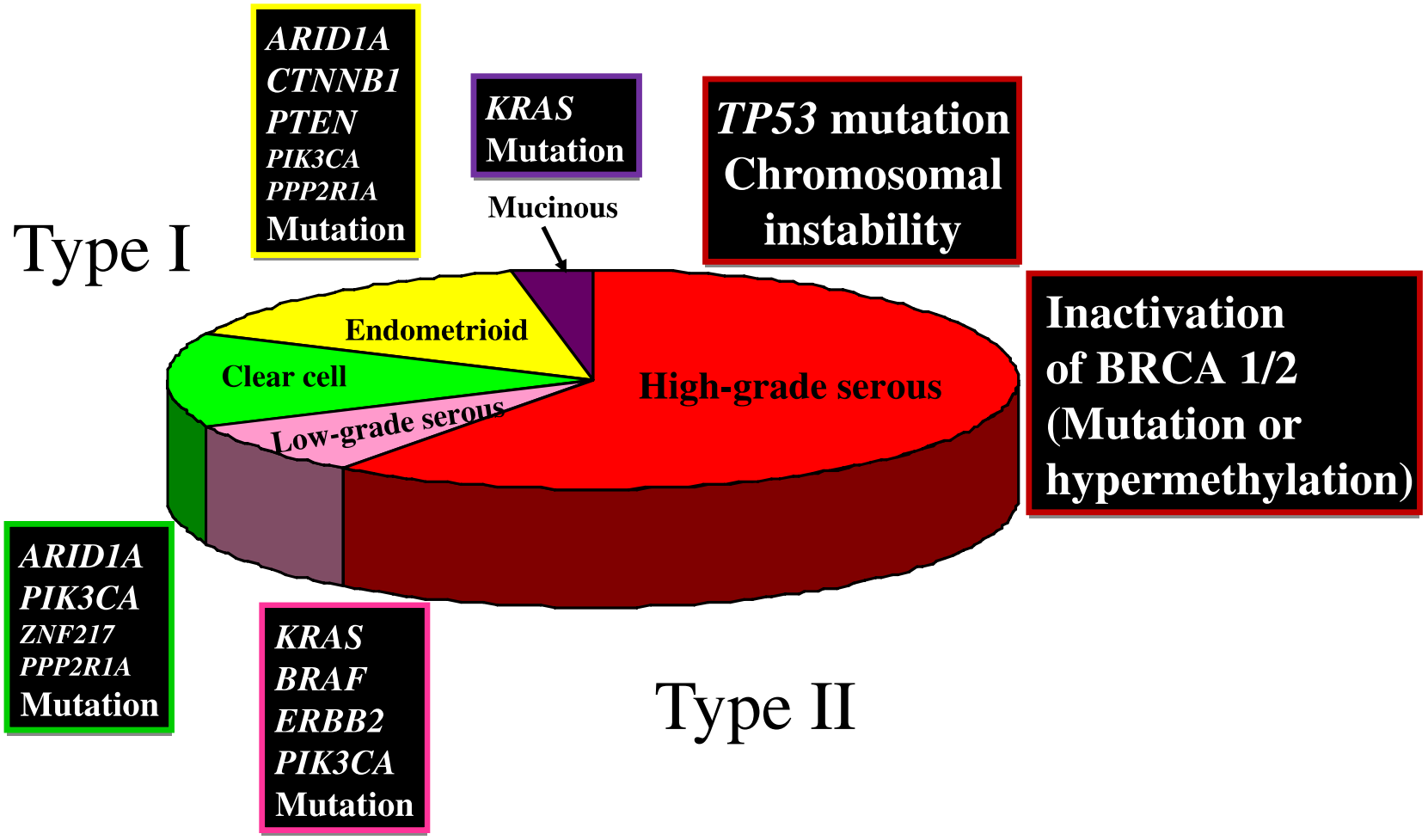
Ovarian Surface Epithelial Tumours



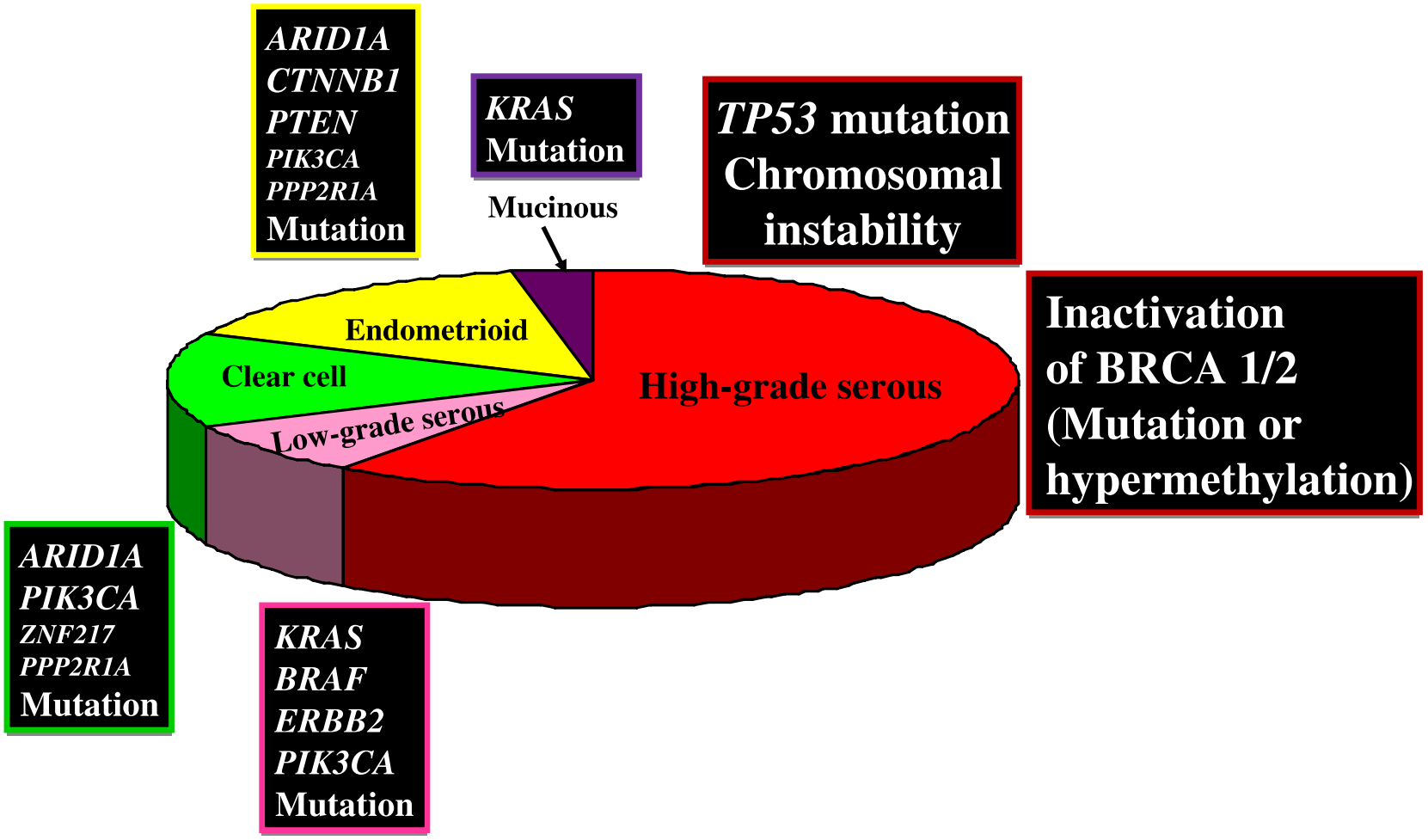
Modified from Gilks CB. Int J Gynecol Pathol 2004; 23: 200-205

Low-Grade Endometrioid Tumours

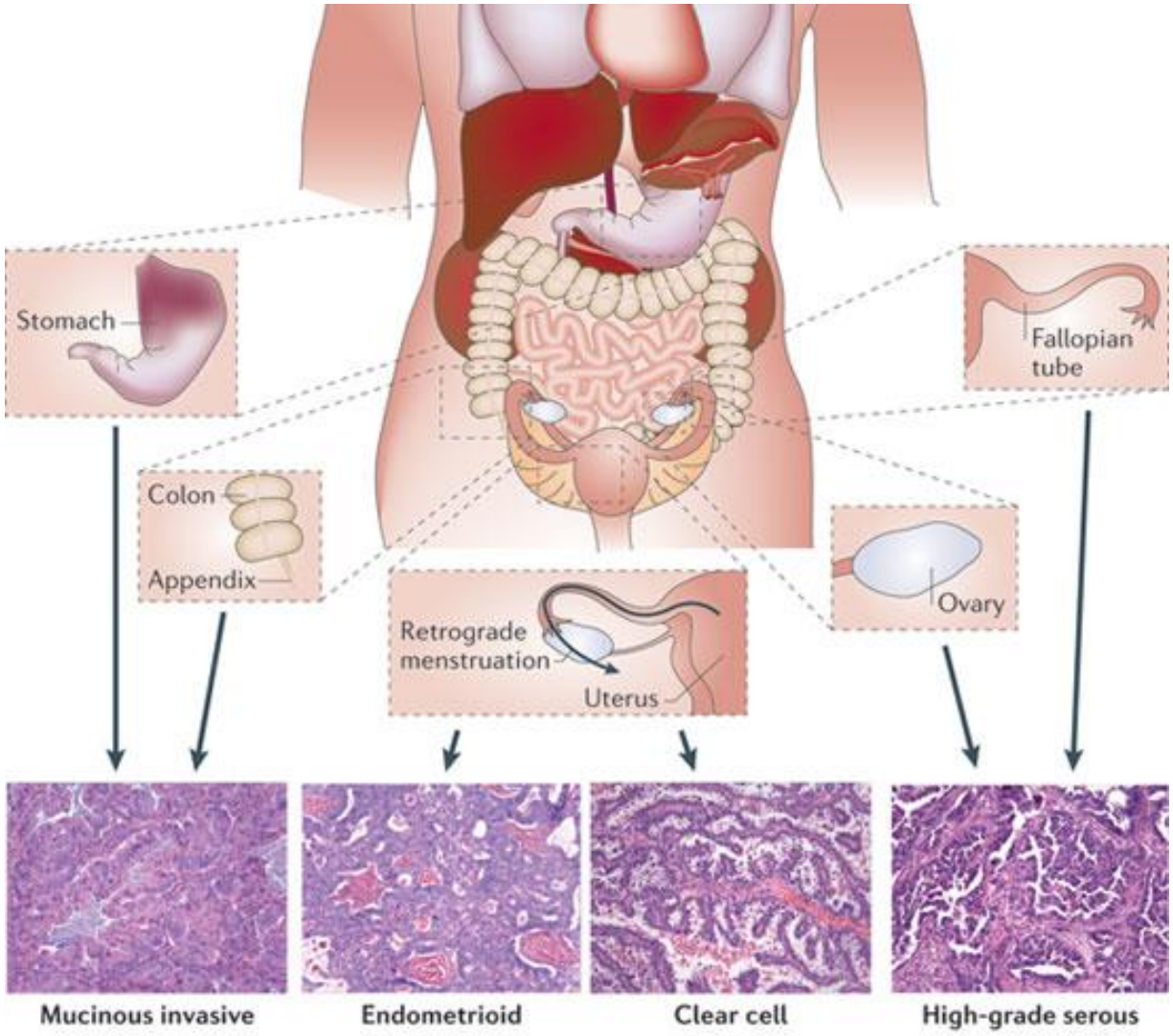
- Association with endometriosis and endometrioid hyperplasia
- Also associated with Lynch syndrome
- Borderline endometrioid tumours
  - Borderline adenofibroma
  - Atypical hyperplasia in endometriosis
- *Beta-catenin* mutation common (16 - 54%)
  - occurs in endometriosis and tumours
- *ARID1A* mutation in approx 50%
- *PTEN* mutation in approx 20%
- Boundary with high-grade tumours?
  - WT1 and p53 useful



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



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



Nature Reviews | Cancer

FOXL2 in ovarian sex cord-stromal tumours

- C134W *FOXL2* mutation identified in 4 index adult-type granulosa cell tumours
- Present in 86/89 (97%) aGCTs, 3/14 thecomas, 1/10 jGCTs
- Absent in 49 other sex cord stromal tumours and 329 other ovarian and breast tumours
  - Shah et al NEJM 2009; 360: 2719-2729

FOXL2 in ovarian sex cord-stromal tumours

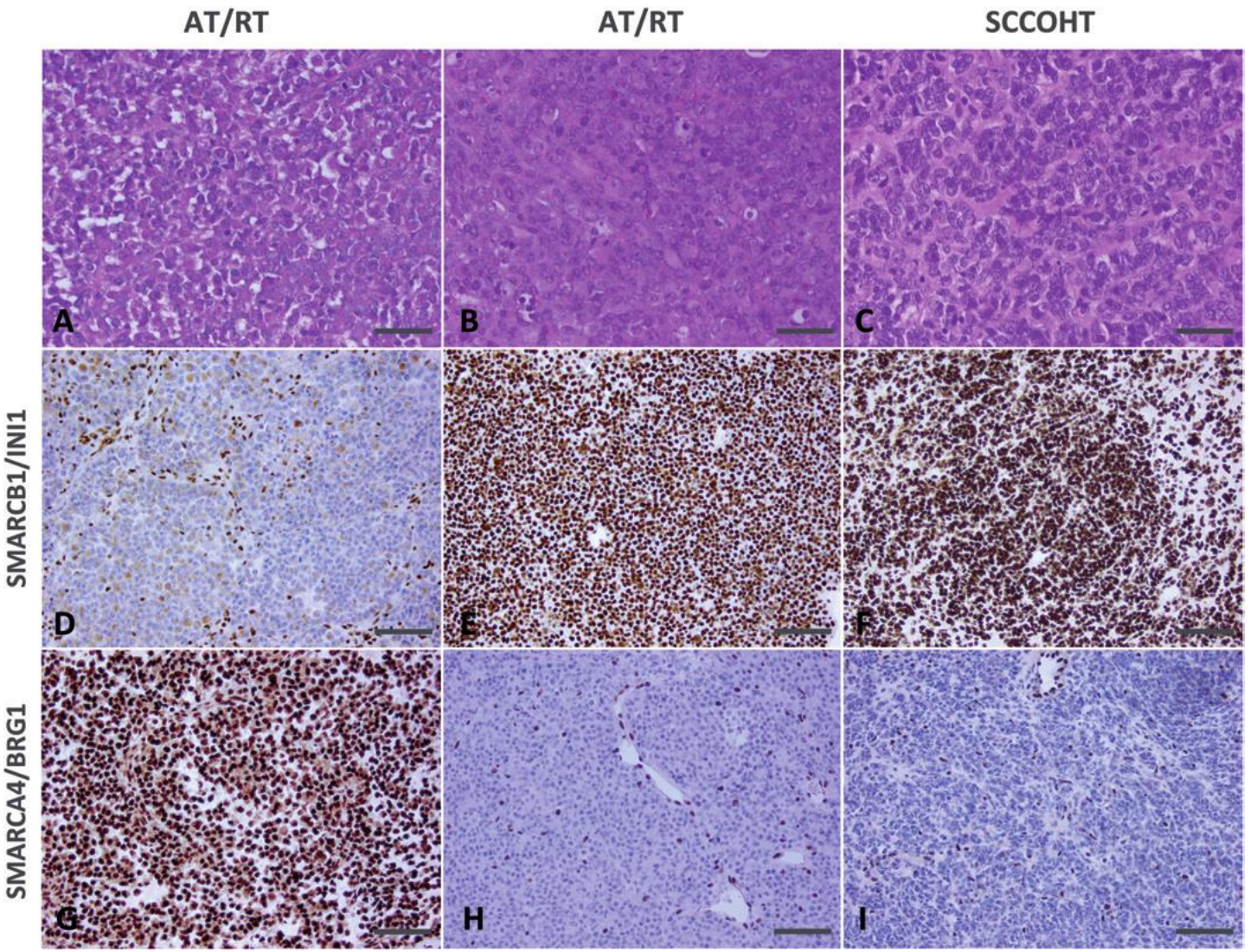
- C134W mutation in 53/56 aGCTs, 2/6 thecomas but none of remaining 1281 tumours from a range of sites
  - Kim et al J Pathol 2010; 221: 147-152
- Mutation present in 18/20 aGCT and 0/3 jGCTs
  - Kim et al Histopathology 2010; 56: 408-410
- Mutation present in 52/56 aGCTs; ?3/4 negative cases mis-diagnosed
  - Jamieson et al Mod Pathol 2010; 23: 1477-1485
- FOXL2 immunohistochemistry sensitive (80%) and specific (99%) marker of SCSTs but not aGCT specifically
  - Al-Agha et al Am J Surg Pathol 2011; 35: 484-494
- Mutation testing useful in ambiguous cases
  - Kommoss et al Histopathology 2014; 64: 380-388

Small Cell Carcinoma of Hypercalcaemic Type

- *SMARCA4* mutation redefines this tumour as a rhabdoid tumour
- Identified by whole-exome sequencing
- Mutation may be germline
- Leads to loss of expression of BRG1

Witkowski et al Nat Genet 2014; 46: 438-443

BRG1 Loss in Small Cell Carcinoma, Hypercalcaemic Type



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

Possible Therapeutic Approaches

	High Complexity Cancer	Moderate Complexity Cancer	Low Complexity Cancer
Ovarian tumour example	High-grade serous carcinoma	Clear cell carcinoma	Granulosa cell tumour
Mutational spectrum	Defining mutations unlikely	Mutations in specific pathways, in common with other cancers	Defining mutations often present
Inter- and intratumoural heterogeneity	Profound	Unknown	Minimal
Therapeutic approach	Personalised	Stratified	Generic

Concept courtesy of David Huntsman

Outline

- What is Molecular Pathology?
- Lower Genital Tract
  - HPV infection
  - p16 immunostaining
- Endometrium
  - Molecular changes
  - Molecular classification
  - Stromal tumours
- Ovary, Fallopian tube and Peritoneum
  - Origins and types of epithelial tumour
  - Non-epithelial tumours
  - Patterns of genomic complexity
- Hereditary Gynaecological Tumours

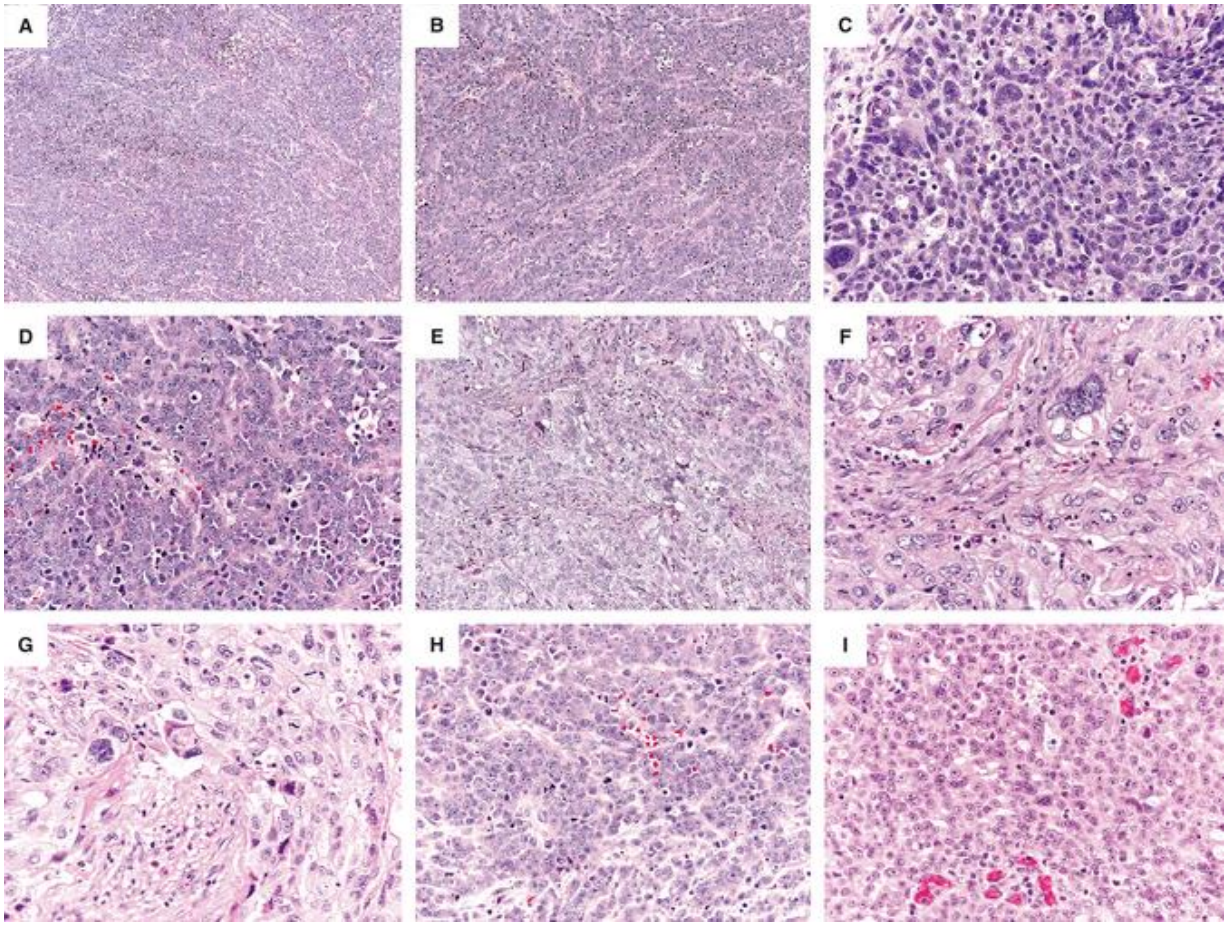
Hereditary Gynaecological Tumours

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

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

BRCA 1 and 2 Mutation

High-grade serous and undifferentiated carcinomas



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 – Ovarian Carcinomas

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

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

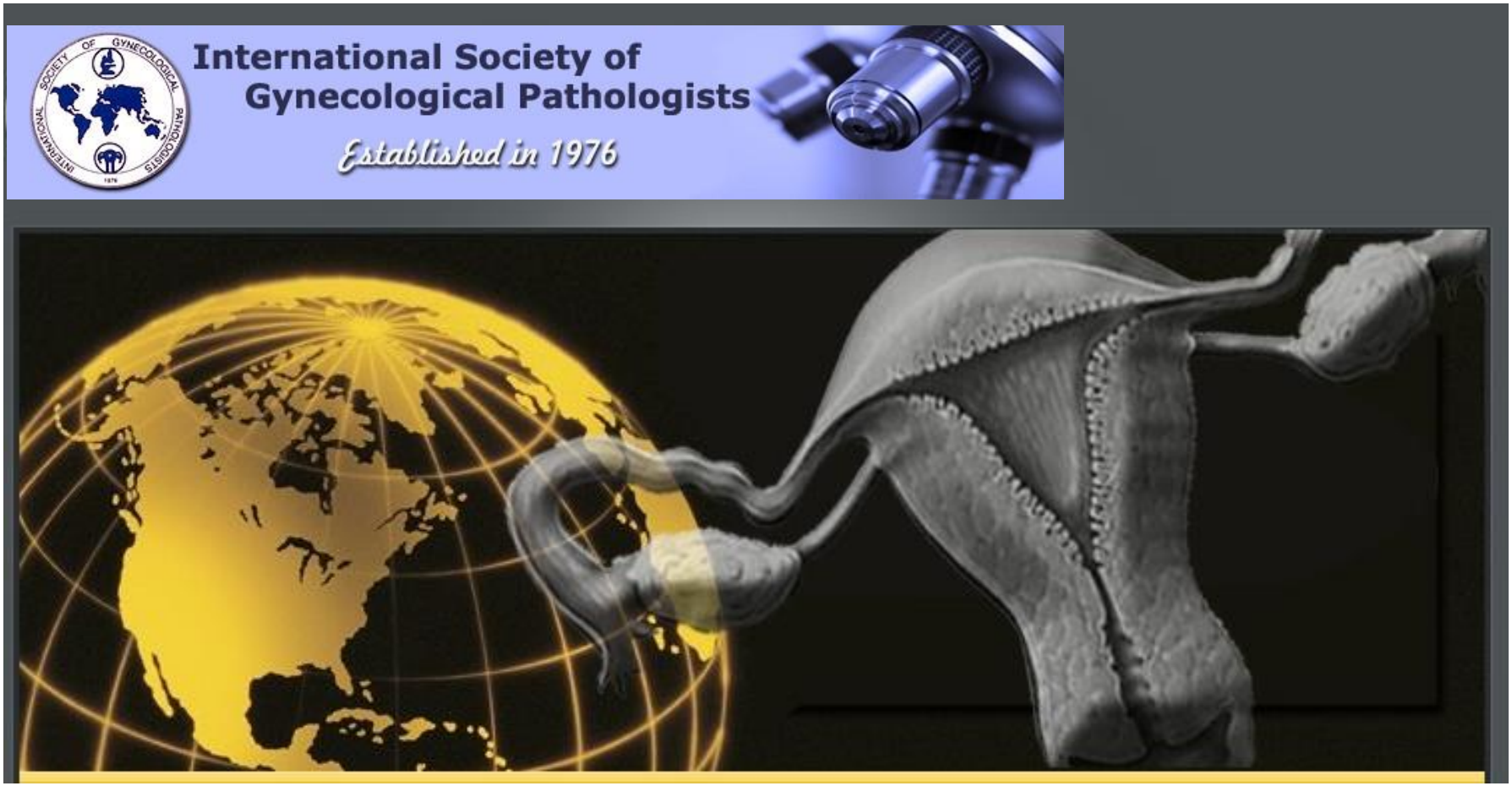
Other Syndromes

Syndrome	Gene	Gynaecological Tumours	Associated Tumours
Peutz-Jeghers Syndrome	*STK11/LKB1*	Ovary – sex cord stromal tumours (5-15% risk) Cervix – adenoma malignum	Hamartomatous GI polyps Breast, GI, lung, pancreas, testis cancers
Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)	*Fumarate hydratase*	Uterus – leiomyomas with prominent nucleoli and perinuclear halos	Renal cell carcinoma (15% risk) Cutaneous leiomyomas
Gorlin syndrome (nevoid basal cell syndrome)	*PTCH*	Ovary – fibromas, bilateral and calcified (2-25% risk)	Basal cell carcinomas Odontogenic keratocysts Medulloblastomas
Cowden syndrome	*PTEN*	Uterus – leiomyomas, endometrial carcinoma (5-20% risk)	Hamartomas of GI tract, skin etc Breast (25-50% risk) and thyroid (3-10% risk) carcinomas

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

Summary

- Lower Genital Tract
  - p16 is a useful surrogate marker of high-risk HPV infection
  - Staining pattern and context are important
- Endometrium
  - Improved molecular understanding may lead to a diagnostic algorithm for endometrial carcinomas, involving p53 and MMR protein immunostaining
  - 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. p53, WT1) and possibly behaviour (e.g. *BRAF*)
  - Some (rare) ovarian tumours have defining mutations e.g. *FOXL2*, *SMARCA4*
- Hereditary Gynaecological Tumours
  - Patients with high-grade serous carcinoma should have *BRCA* gene testing
  - MMR immunohistochemistry should be performed on ovarian endometrioid and clear cell carcinomas



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