The many uses of p53 IHC in Gynecological Pathology

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Martin Köbel reported no relevant financial relationships

PLEASE TURN OFF YOUR CELL PHONES

Fixed the p53 IHC assay using tubo-ovarian carcinoma

The Journal of Pathology: Clinical Research
J Path: Clin Res October 2016; **2:** 247–258
Published online 9 June 2016 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/cjp2.53

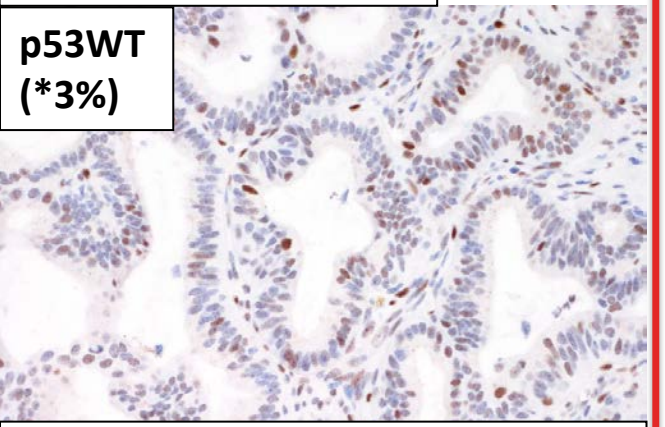
Original Article

Optimized p53 immunohistochemistry is an accurate predictor of *TP53* mutation in ovarian carcinoma

Martin Köbel,[1†] Anna M Piskorz,[2†] Sandra Lee,[1] Shuhong Lui,[1] Cecile LePage,[3,4] Francesco Marass,[2] Nitzan Rosenfeld,[2] Anne-Marie Mes Masson[3,4] and James D Brenton[2*]

The normal Wild-type pattern

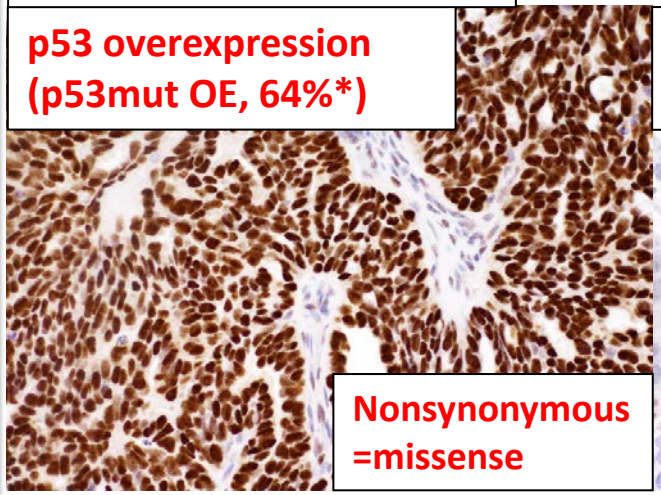
p53WT (*3%)



No *TP53* mutation (rare exceptions)

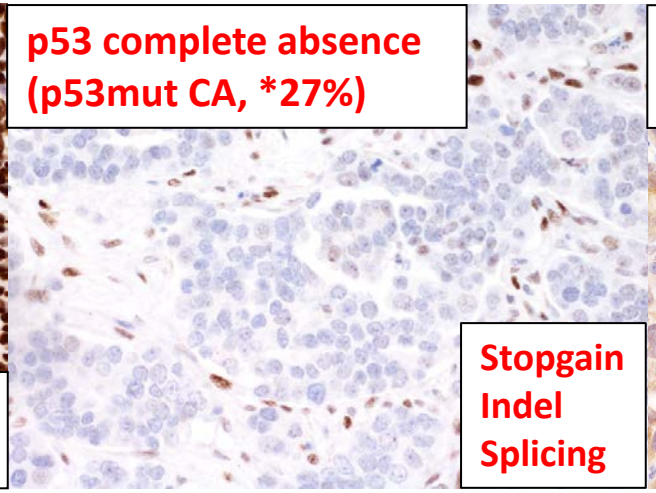
3 Mutant Patterns:

p53 overexpression (p53mut OE, 64%*)



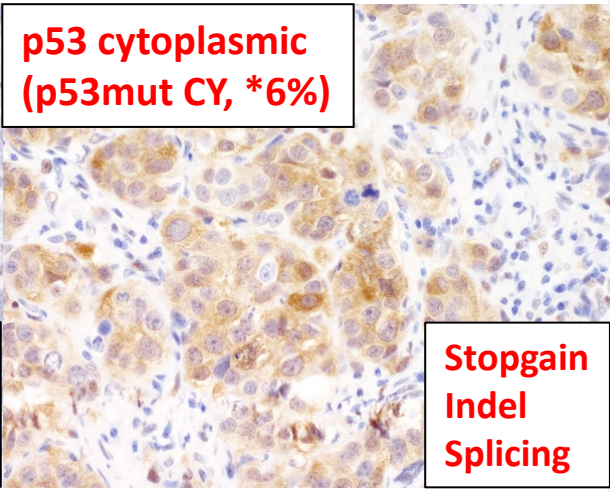
Nonsynonymous =missense

p53 complete absence (p53mut CA, *27%)



Stopgain Indel Splicing

p53 cytoplasmic (p53mut CY, *6%)



Stopgain Indel Splicing

Köbel et al. J Pathol Clin Res PMID: 27840695; *distribution in HGSC
https://www.bgcs.org.uk/wp-content/uploads/2019/06/BAGP-UKNEQAS-project-p53-interpretation-guide-2016.pdf

Optimized Sequencing and optimized IHC

A

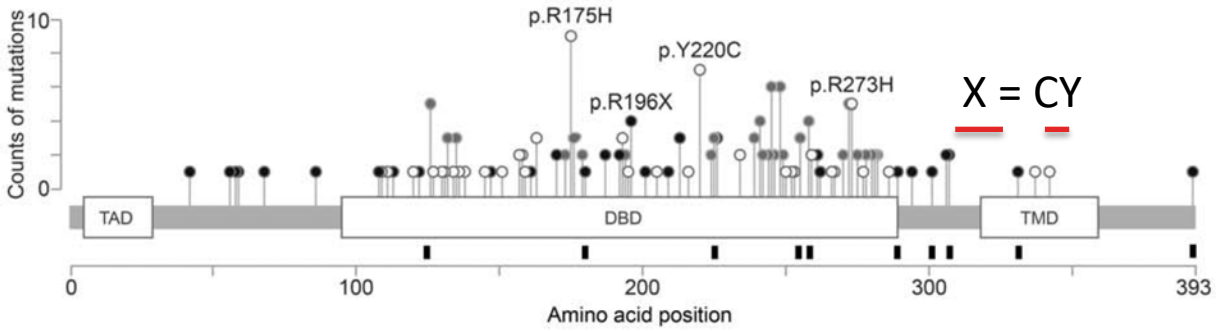
DO7

N-terminus
5’ end

C-terminus
3’ end

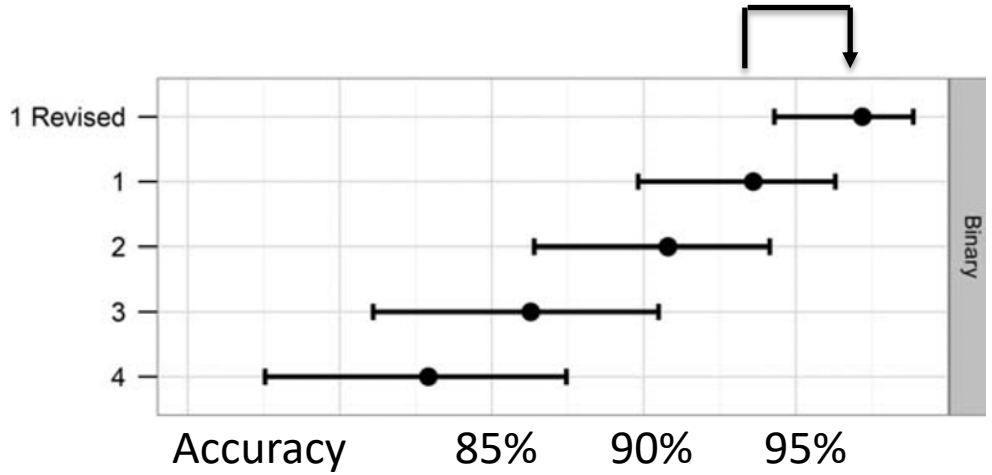
X<213 = CA vs X>245+WT

X = CY



B

Full section



C

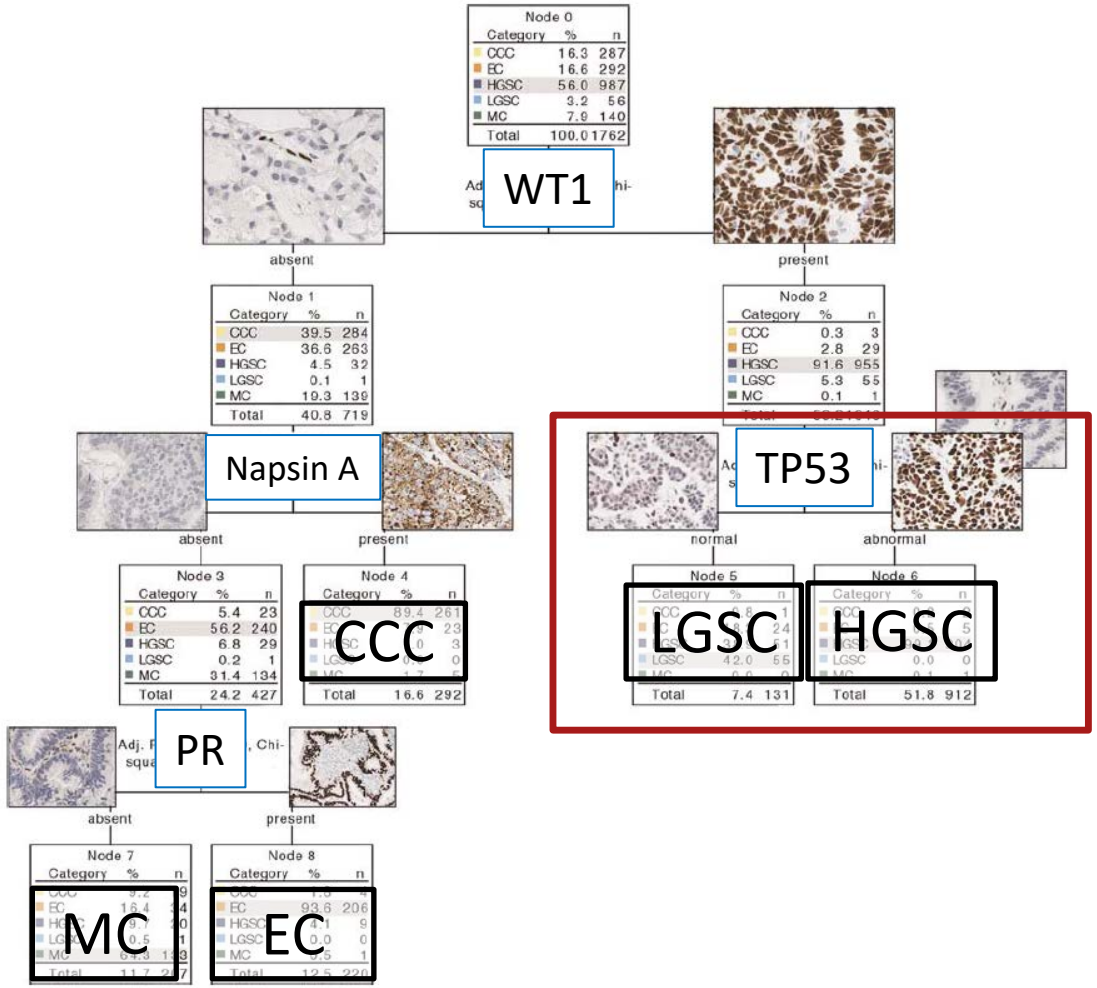
IHC	Nonsynonymous	Indel	Stopgain	Splicing	NDM	Total
OE	115	2	2	2	0	121
CA	0	16	13	12	0	41
CY	0	2	2	0	0	4
WT	0	4	0	3	76	83
Total	115	24	17	17	76	249

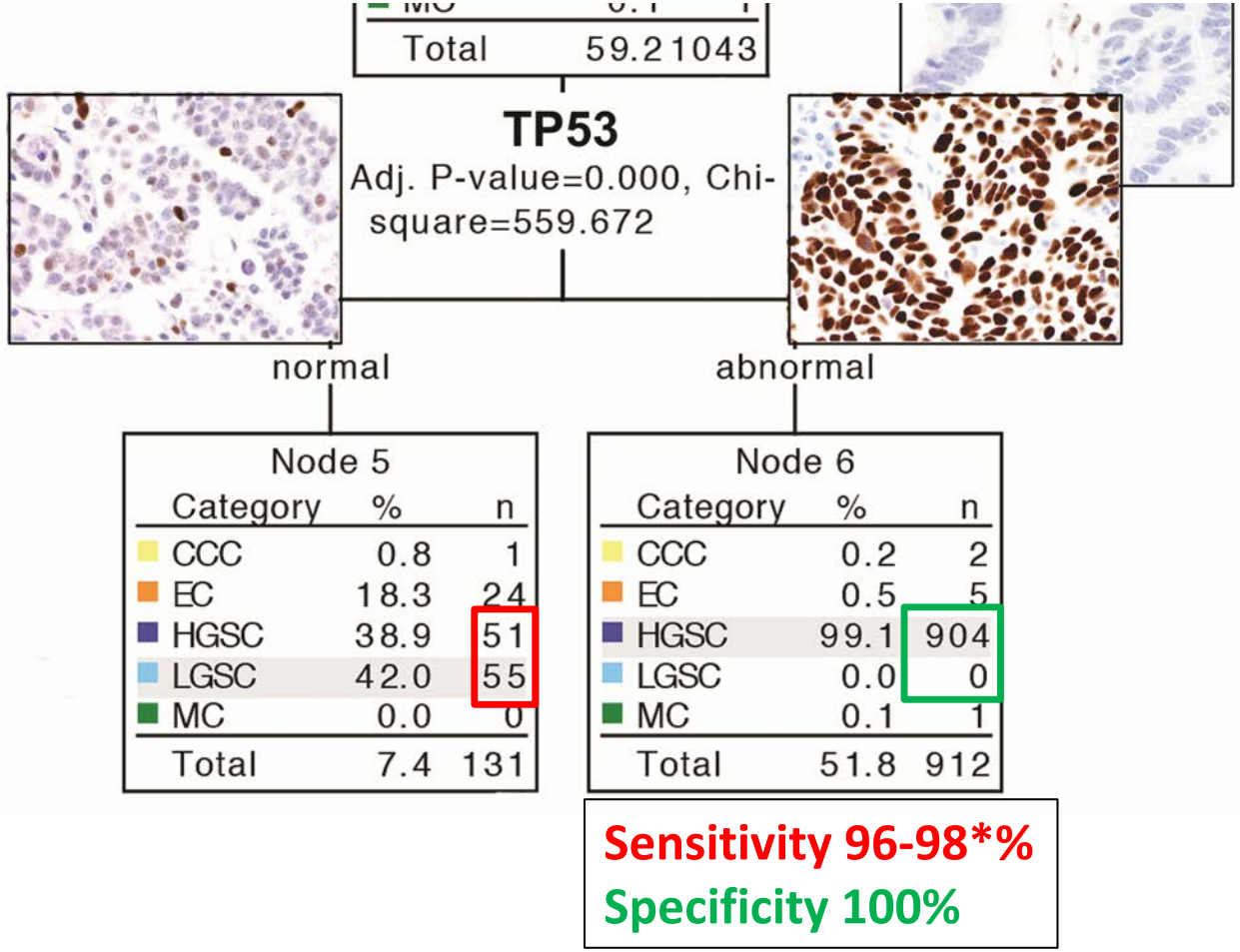
Sensitivity 96%
Specificity 100%

Köbel et al. J Pathol Clin Res PMID: 27840695

The many uses of p53 IHC: 1. HGSC versus LGSC

1. Distinction of tubo-ovarian high-grade serous from ovarian/peritoneal low-grade serous carcinoma





Köbel et al. Int J Gynecol Pathol PMID: 26974996, 29901523*

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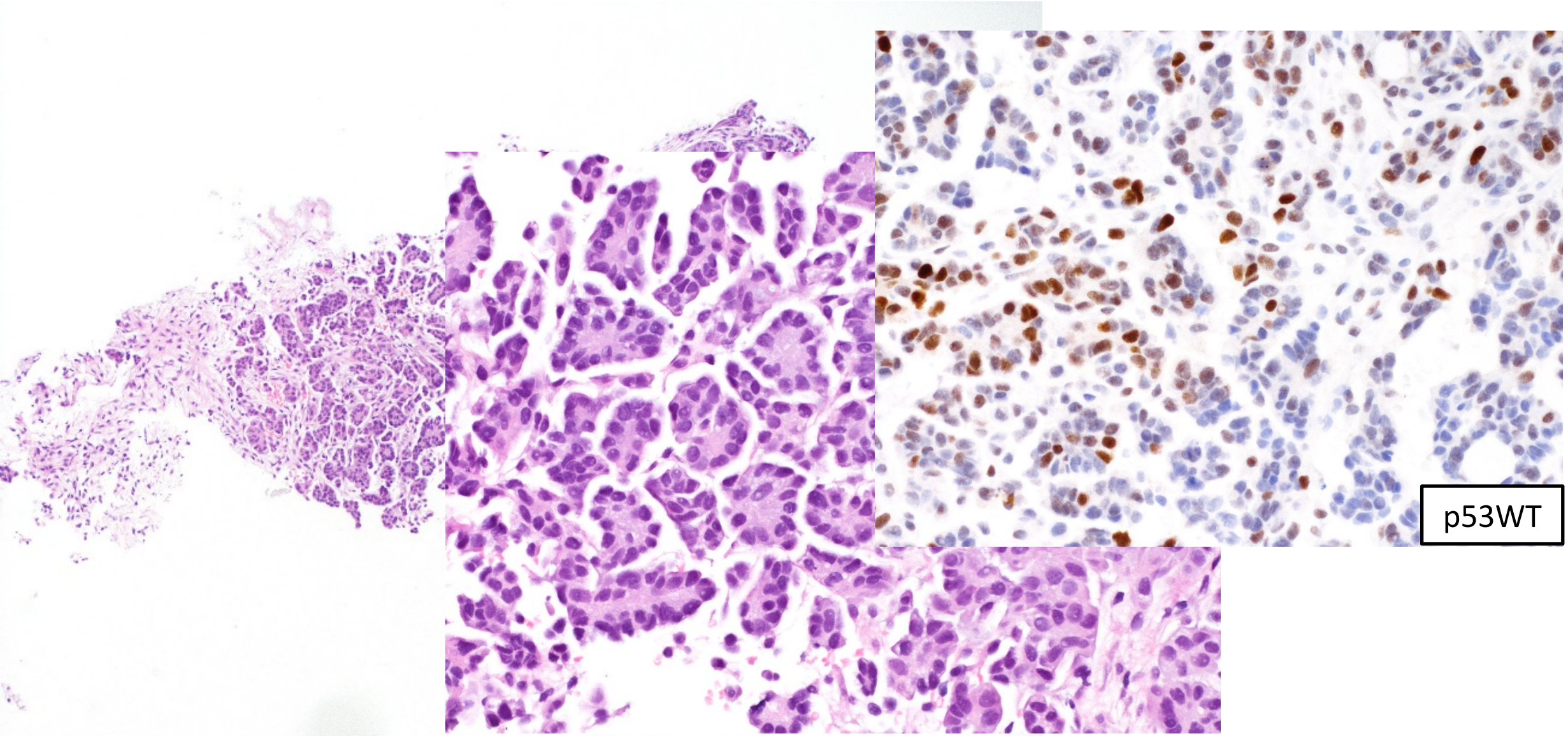
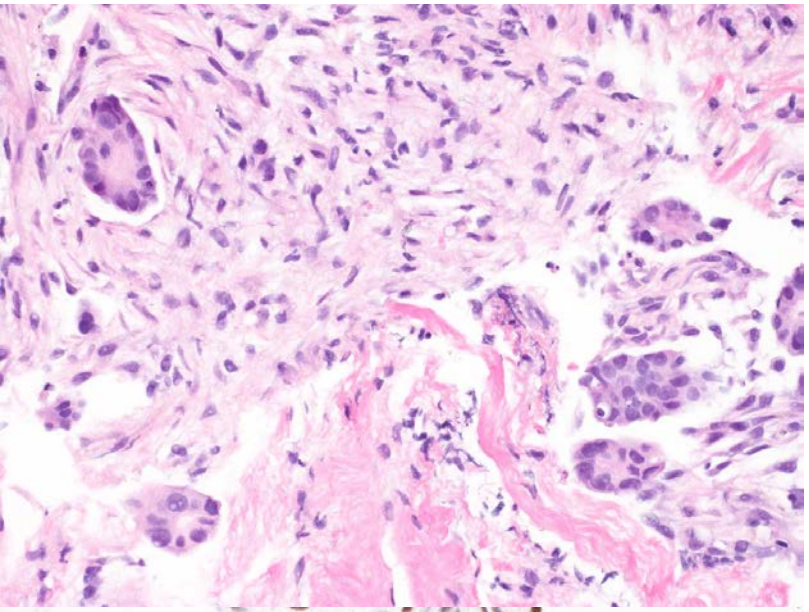
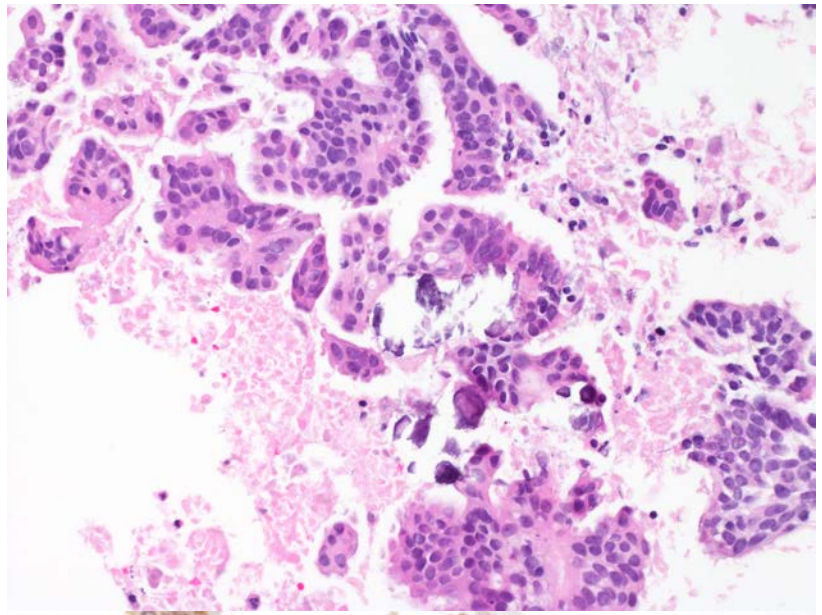
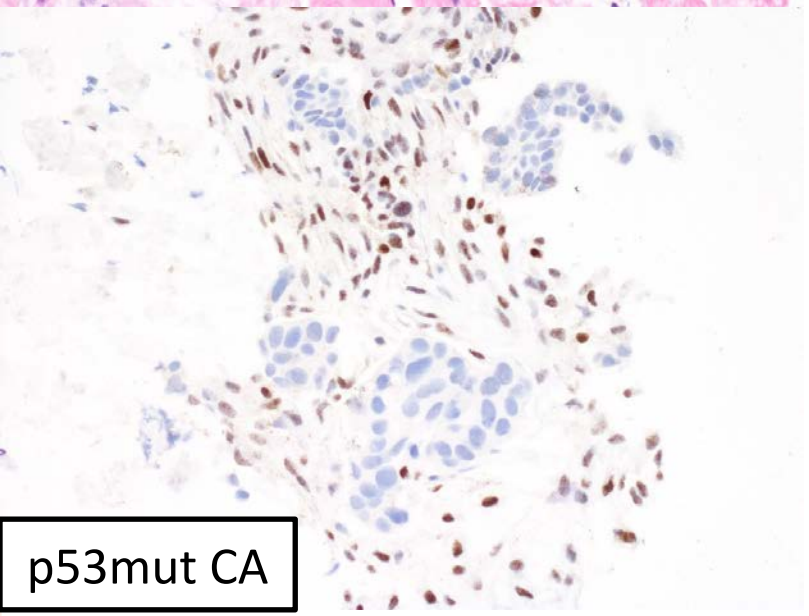
1.a) Confirmation of LGSC



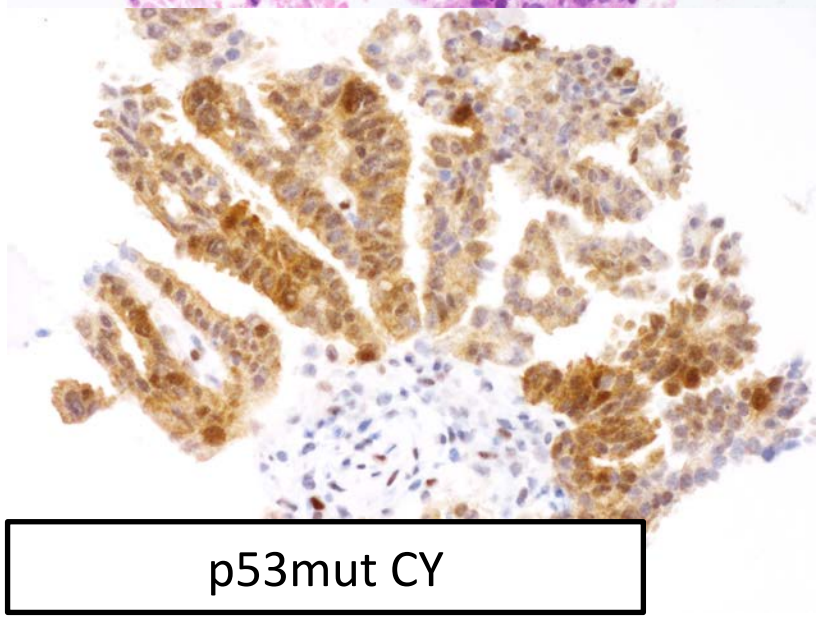
1.b) The danger of underreliance on p53 IHC







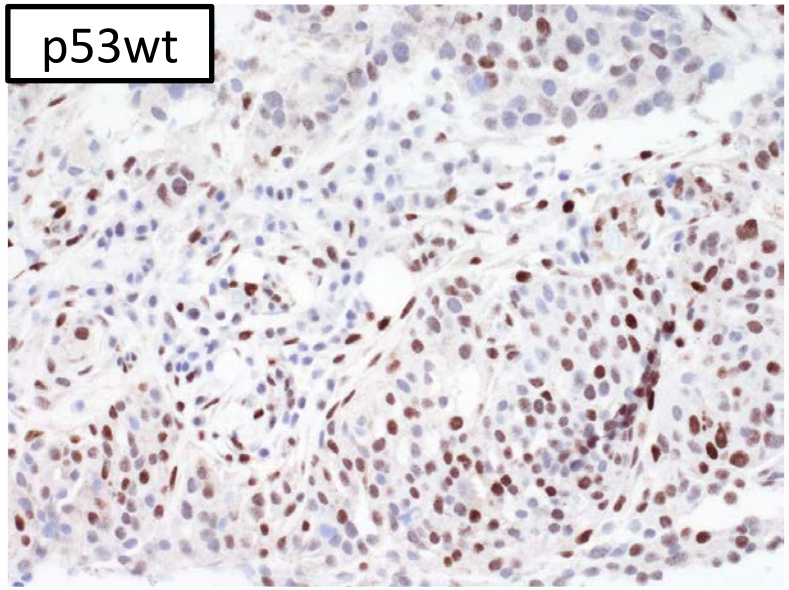
p53mut CA

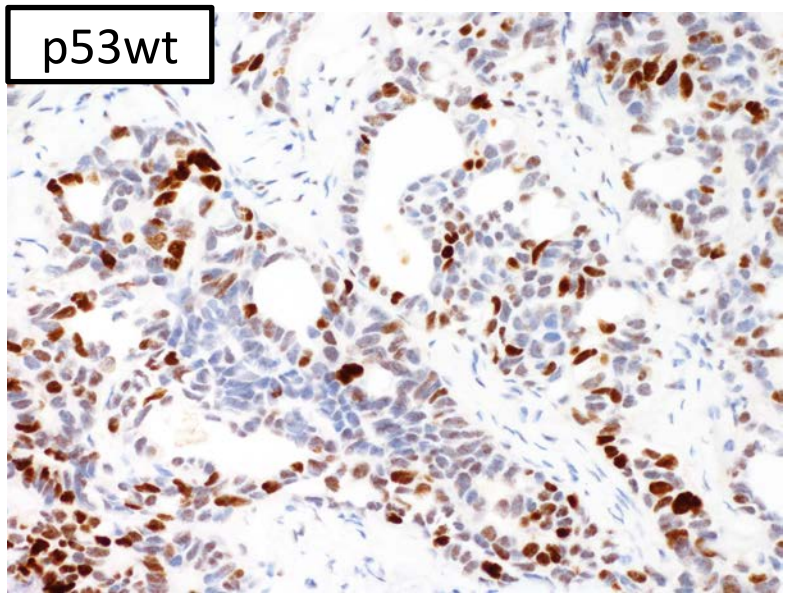


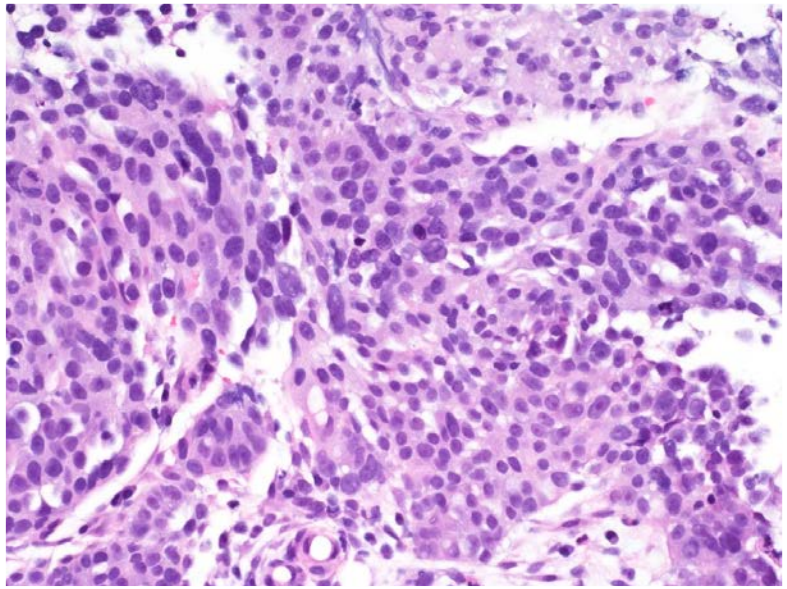
p53mut CY

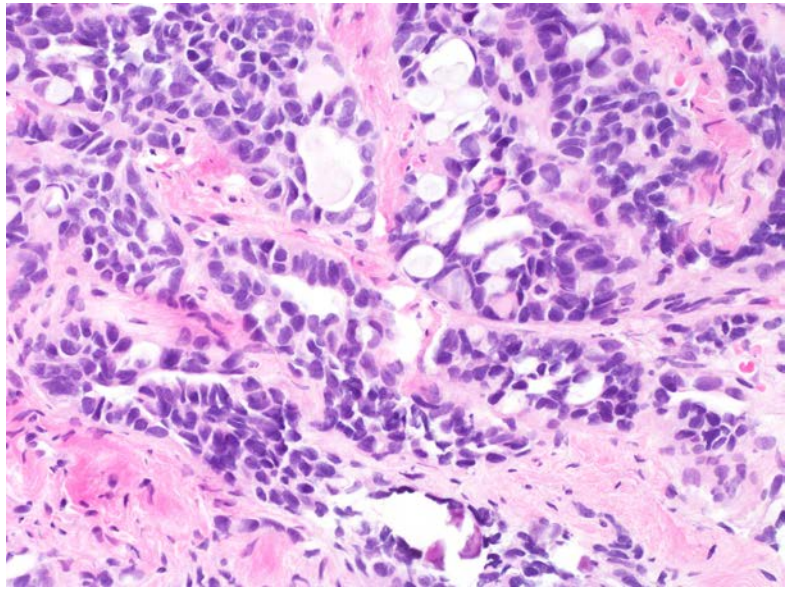
- Some HGSC can mimic LGSC on preoperative biopsy: micropapillary architecture and moderate nuclear atypia
- Confirm with p53 IHC

1.c) Avoid the sensitivity trap





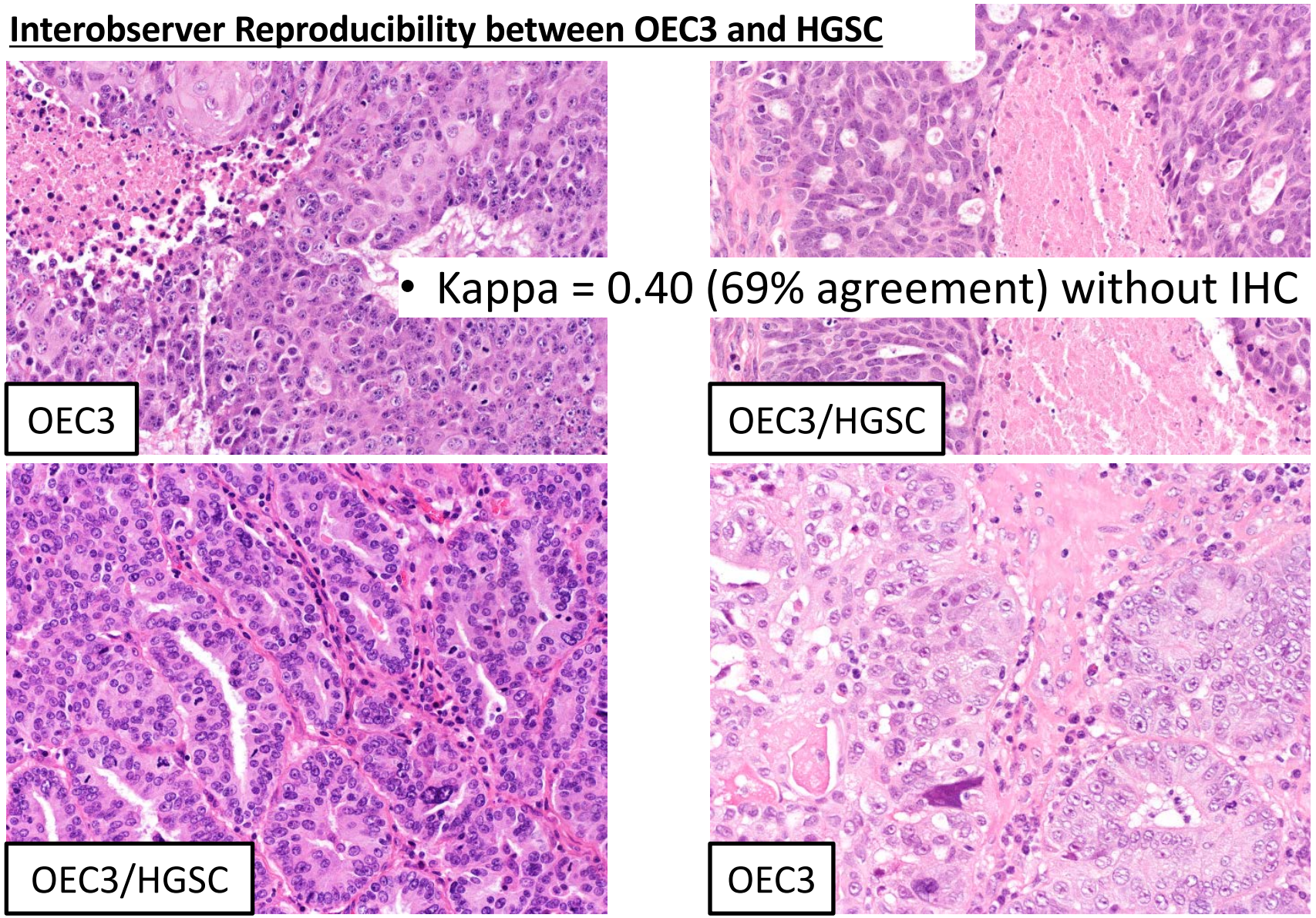




- ~2% of tubo-ovarian carcinoma harbor a *TP53* mutation that results in expression of a non functional protein in wild type pattern.
- These mutation can either be C-terminal truncating or splice site mutation.
- A tumor with p53wt can still be a tubo-ovarian high-grade serous carcinoma – morphological correlation is required.

2. Differential diagnosis of ovarian endometroid grade 3 versus HGSC

Interobserver Reproducibility between OEC3 and HGSC



- Kappa = 0.40 (69% agreement) without IHC

Assem et al AJSP
PMID: 29309296

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Fist line ancillary markers assessing tubo-ovarian arcinoma

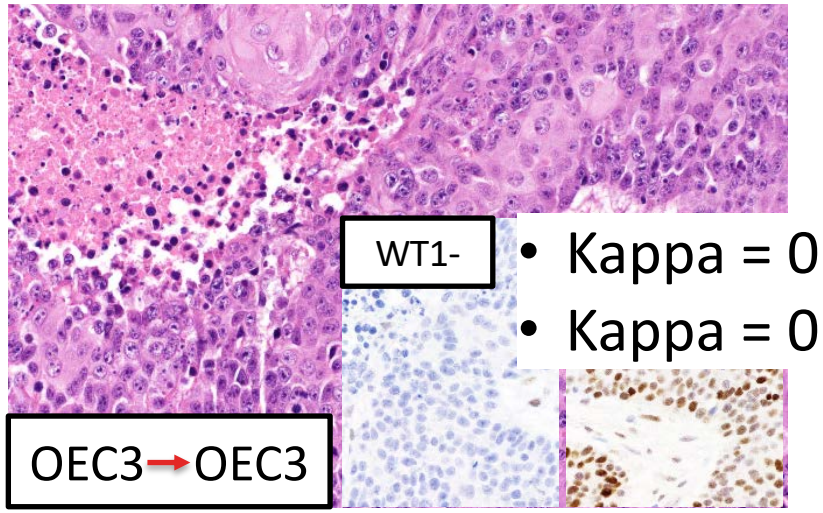
Histotype	PAX8, present	WT1, present	TP53, mutant-type	Napsin A, present	PR, present
HGSC	95%	97%	94-98%	1%	37-42%
LGSC	87-100%	98-100%	**0**	0	59-60%
EC	**82%**	10-14%	14-15%	3-8%	81-85%
CCC	95%	**1%**	11-12%	92%	5-7%
MC	39-47%	**0-1%**	61-66%	0-3%	**0-4%**

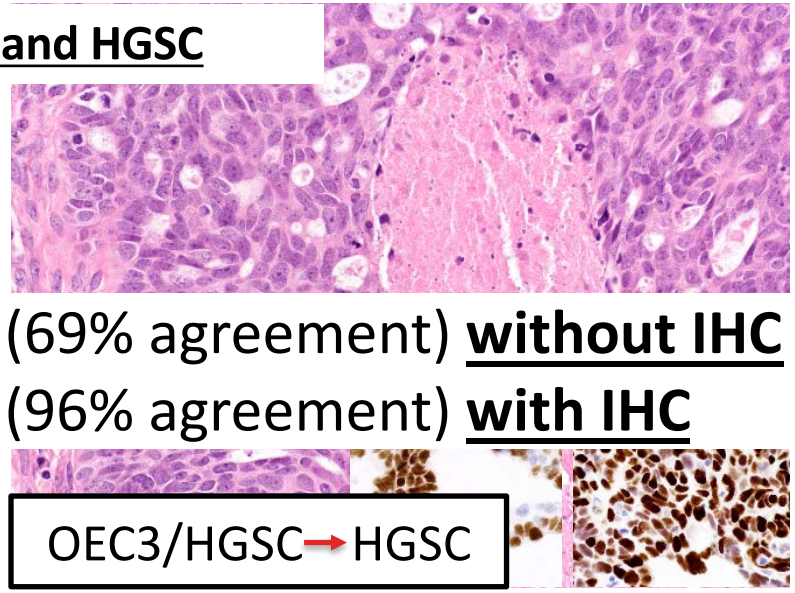
WHO 2020

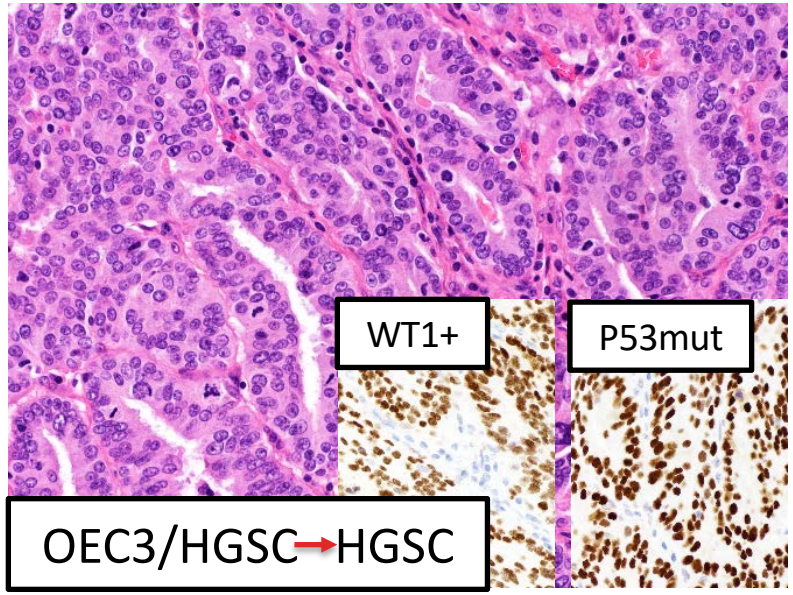
2. Differential diagnosis of ovarian endometroid grade 3 versus HGSC

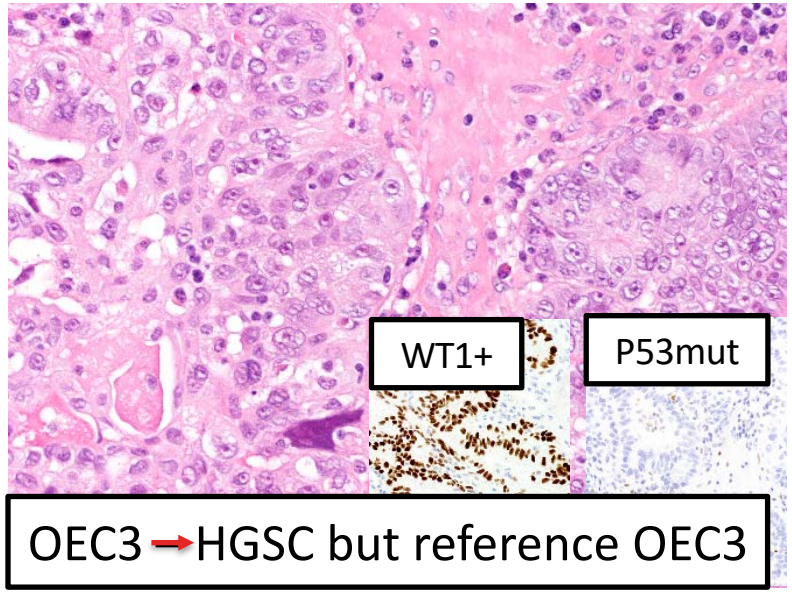
Interobserver Reproducibility between OEC3 and HGSC

- Kappa = 0.40 (69% agreement) **without IHC**
- Kappa = 0.92 (96% agreement) **with IHC**





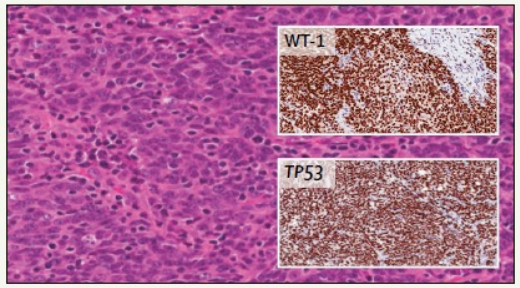




Assem et al AJSP
PMID: 29309296

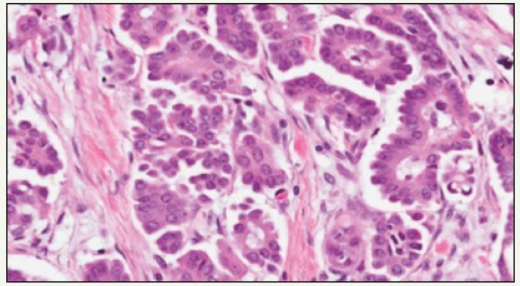
2.b) WT1/p53 to confirm HGSC: rare pitfalls

HGSOC

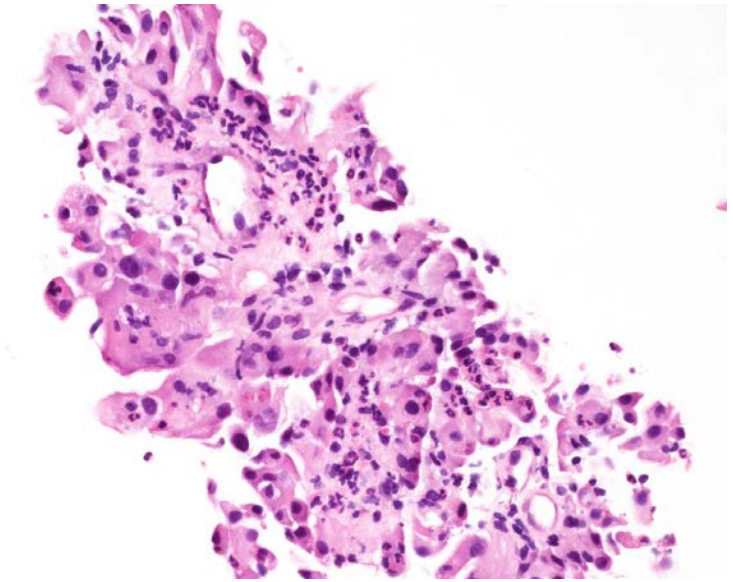


- Highly aggressive tumours
- Papillary or solid growth pattern
- Tumour cells with atypical, large irregular nuclei
- High proliferative rate
- Initial chemosensitivity with subsequent acquisition of increasing resistance
- Key targets: *TP53*, *BRCA1* and 2, and *HRR* genes

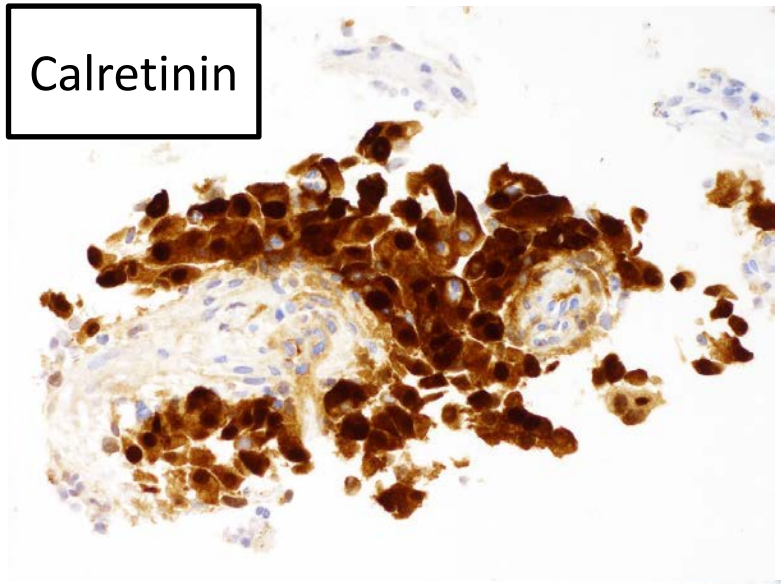
LGSOC



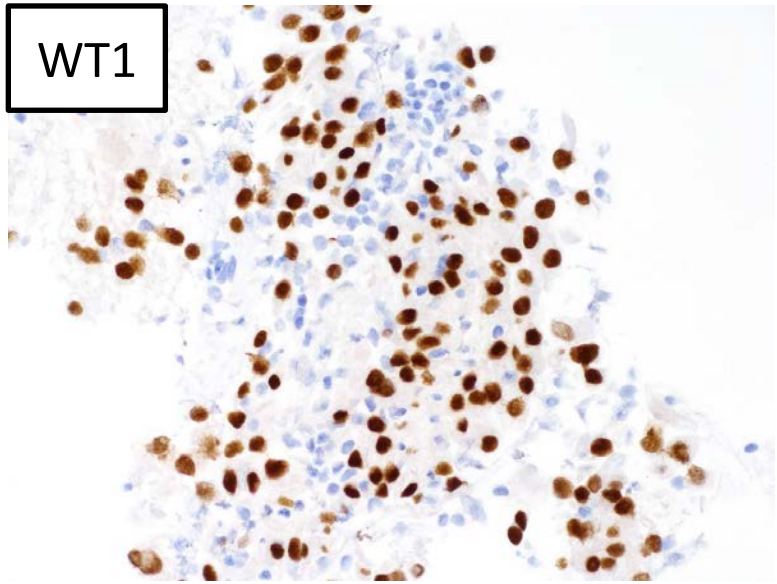
- Indolent behaviour
- Micro-papillary pattern
- Tumour cells with small uniform nuclei
- Low proliferative rate
- Relative chemoresistance
- Key targets: *BRAF*, *KRAS*, *NRAS*, and *PIK3CA*



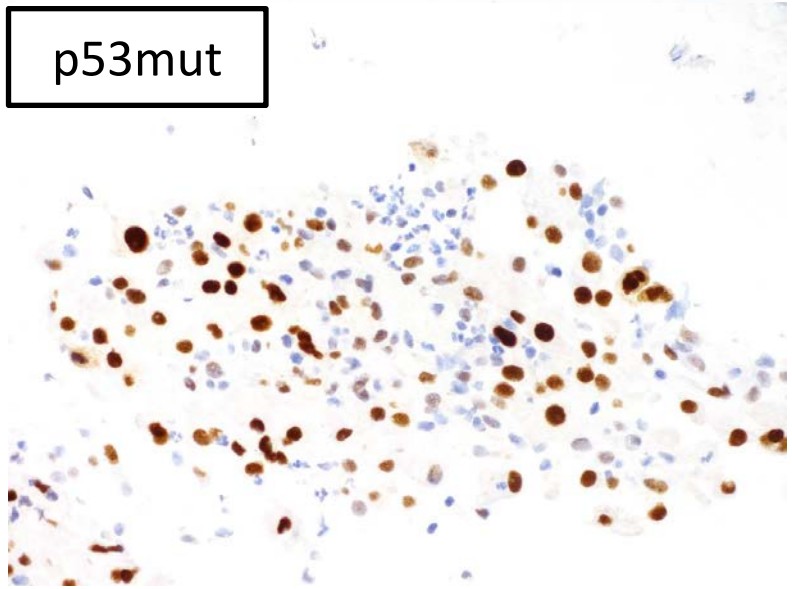
Calretinin



WT1

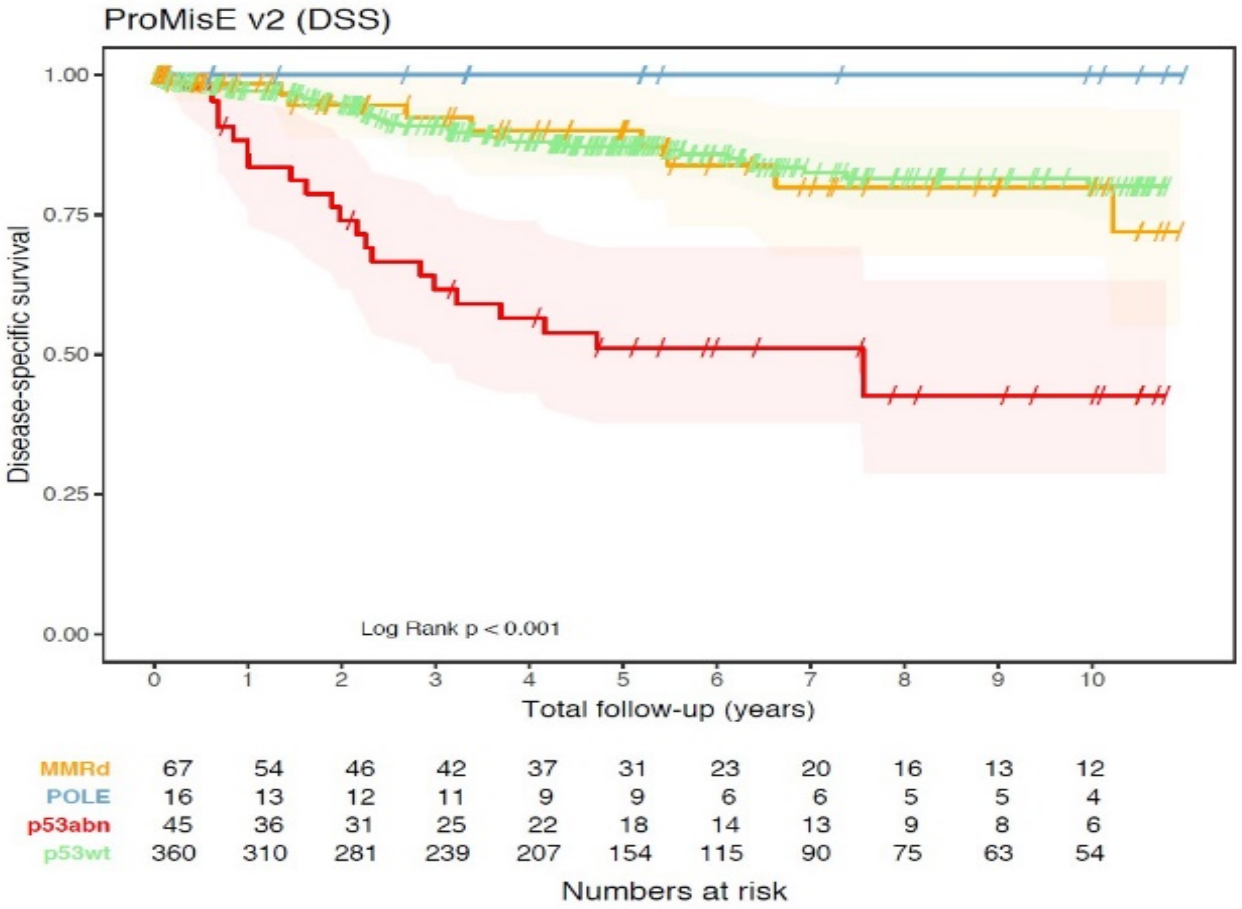


p53mut



Lancet 2019 PMID: 30910306

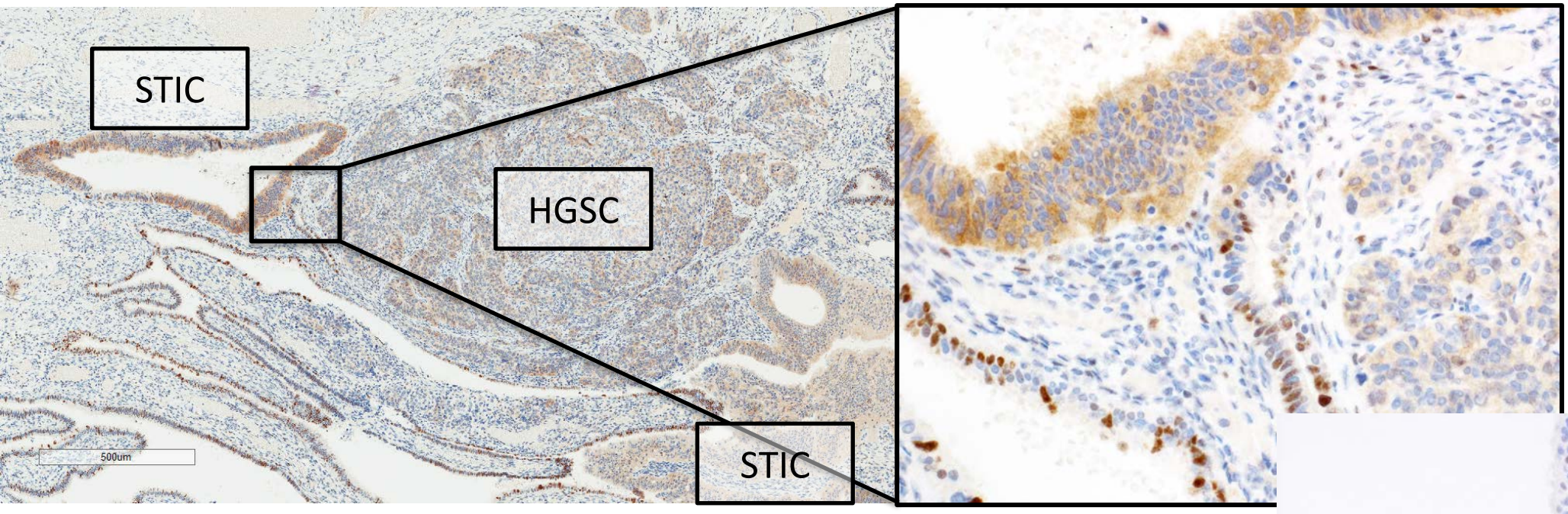
3. Molecular subtype of ovarian endometrioid carcinoma

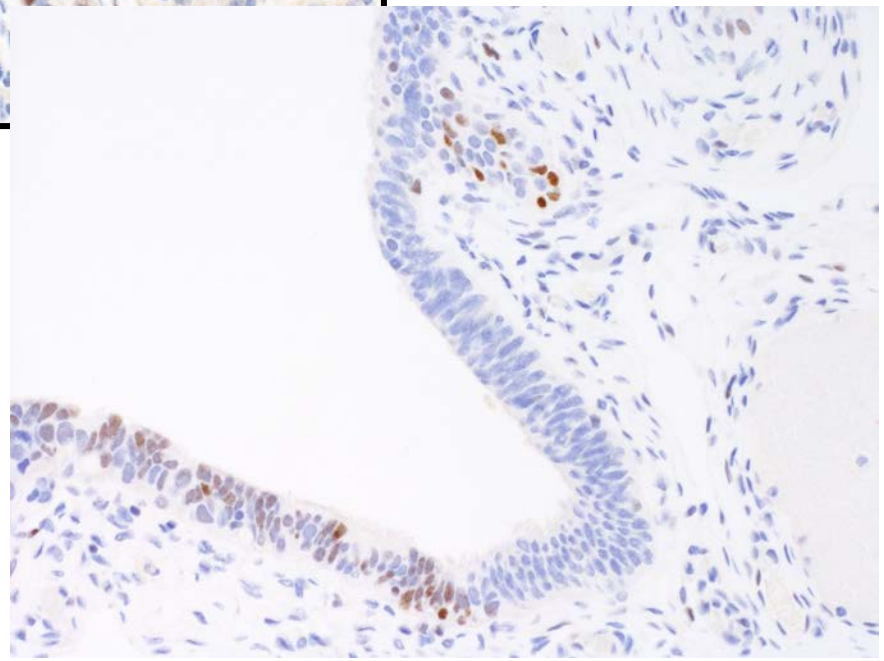


	Ovarian endometrioid carcinoma N=511	Endometrial carcinoma N=920
POLE	17 (3.3%)	84 (9.1%)
MMRd	70 (13.7%)	232 (25.2%)
NSMP	375 (73.4%)	430 (46.7%)
P53 mut	49 (9.6%)	166 (18.0%)

Brett et al. USCAP 2019#

3. Confirming precursor lesion of HGSC

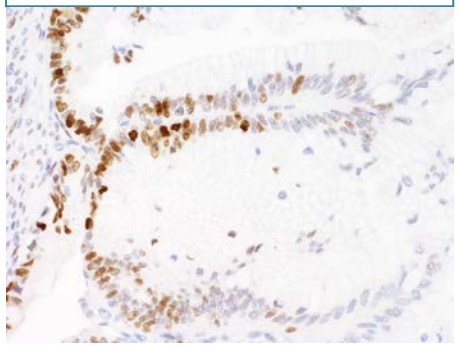




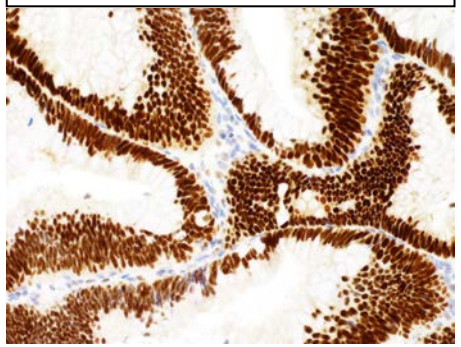
Uncommon patterns can only be appreciated with optimized immunohistochemistry

3. p53 IHC in ovarian mucinous tumors

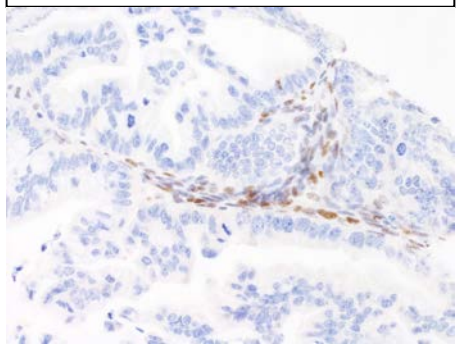
Wild-type pattern



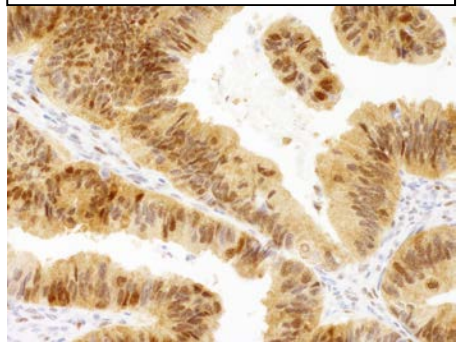
p53mut OE



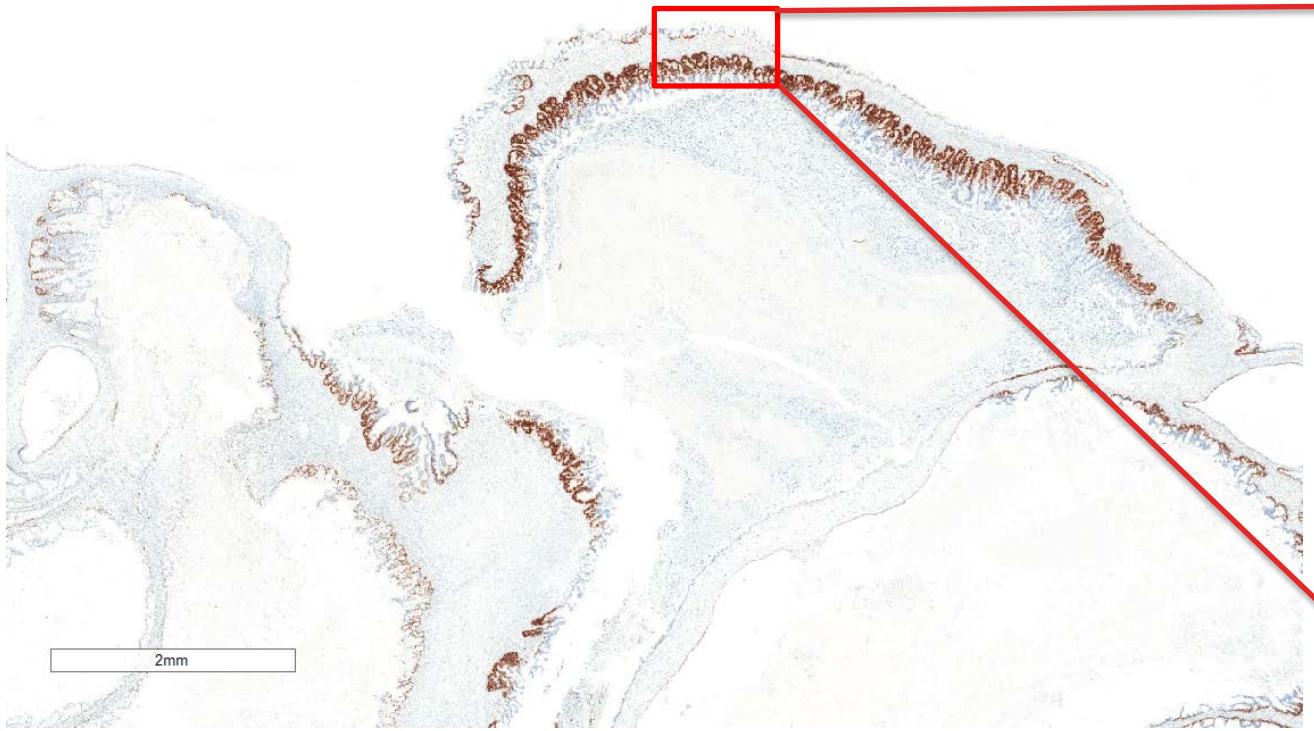
p53mut CA



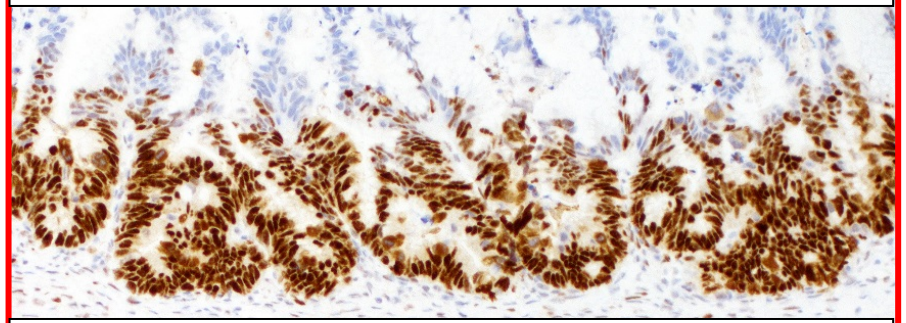
p53mut CY



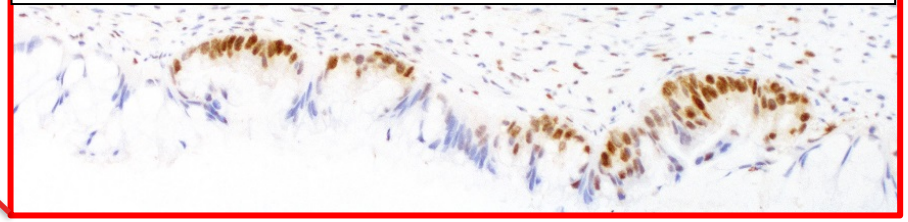
The 4 patterns of p53 IHC also exist in ovarian mucinous tumors but …



p53mut OE with terminal differentiation



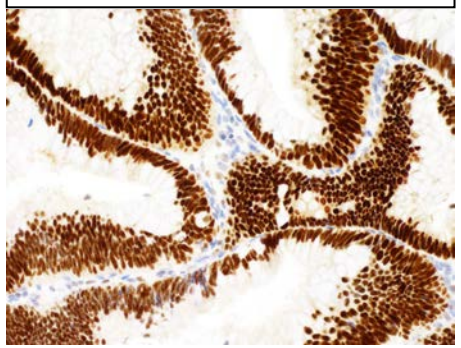
Below wild type = subclonal

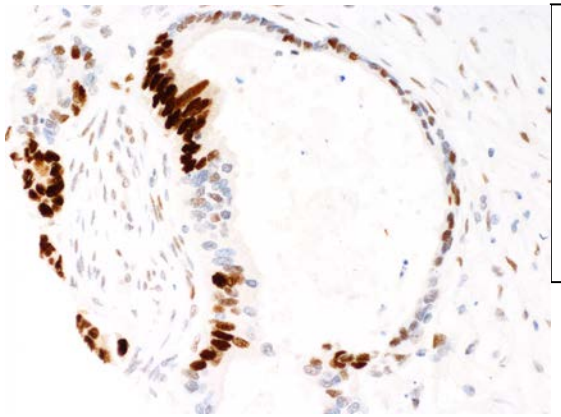


Kang et al. Abstract#1450 Poster#136 Monday 1:00-4:40PM

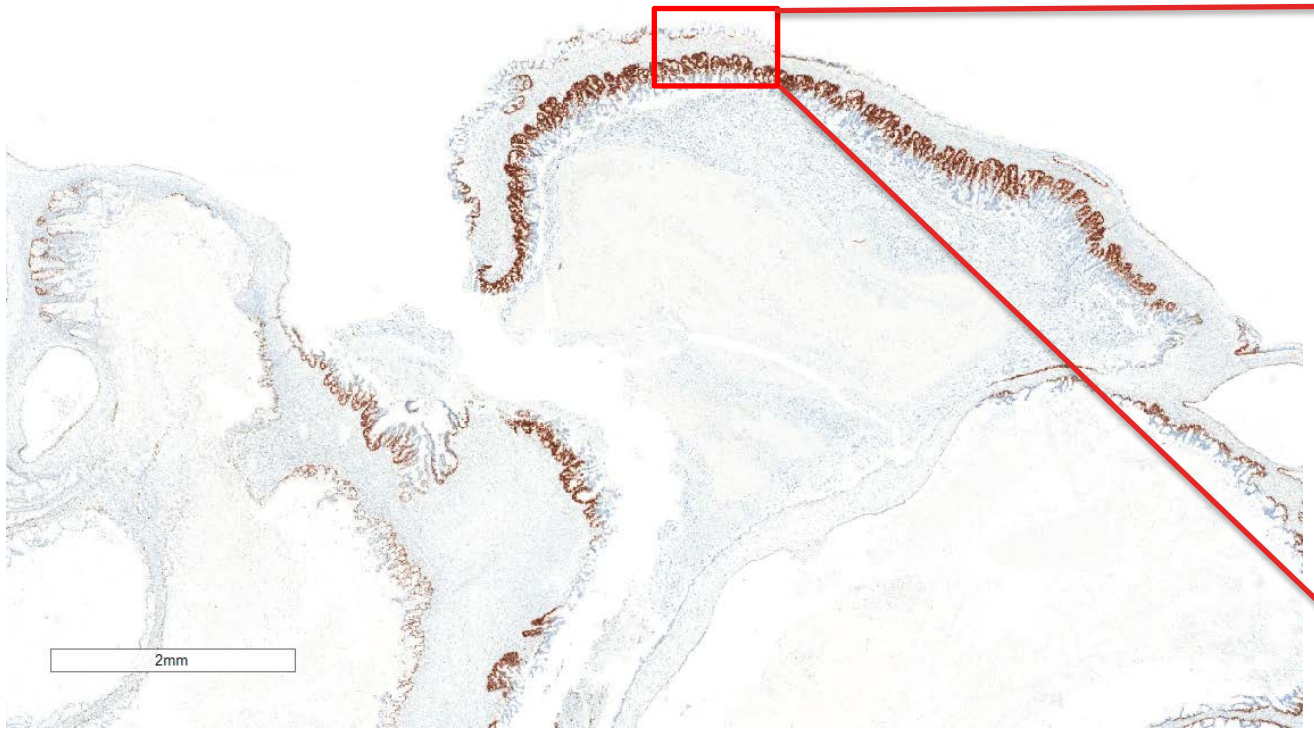
3. p53mut OE cut-off in ovarian mucinous tumors

p53mut OE

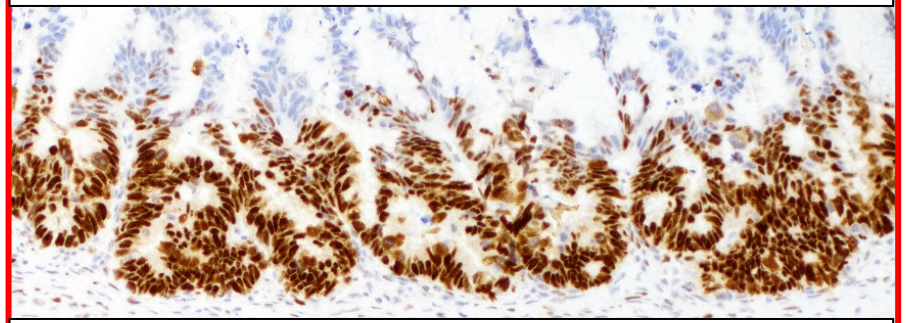




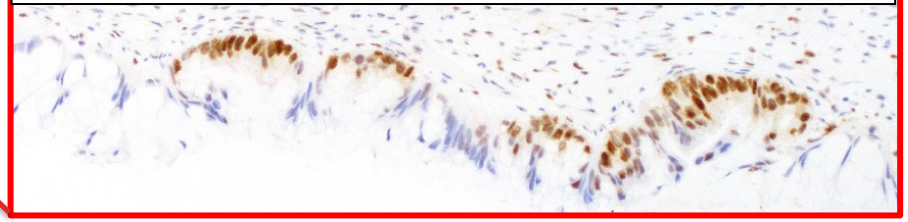
12 consecutive strongly staining cells ("p53 signature") – lowest threshold for p53mut OE in mucinous tumors



p53mut OE with terminal differentiation

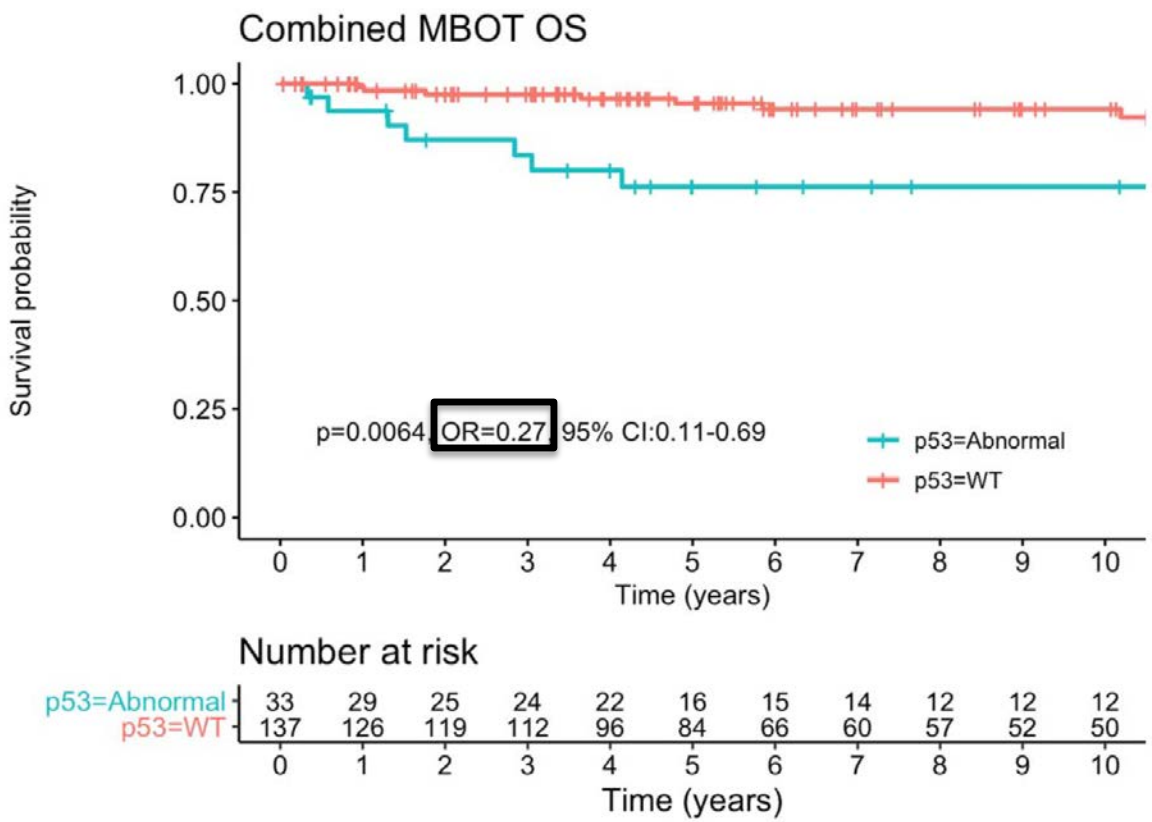


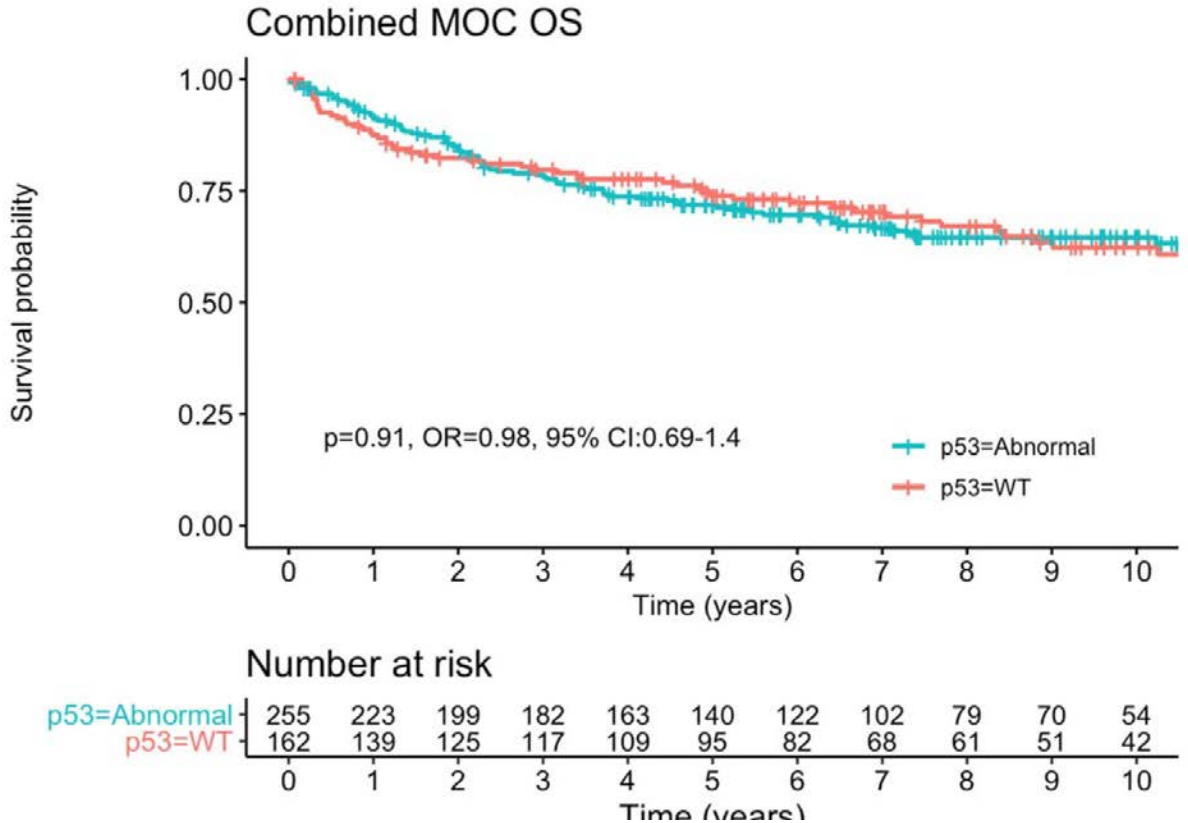
Below wild type = subclonal



Kang et al. Abstract#1450 Poster#136 Monday 1:00-4:40PM

3. Prognostic implications in MBOT





Kang et al. Abstract#1450 Poster#136 Monday 1:00-4:40PM

4. Endometrial carcinoma histotypes/molecular subtype as per WHO

Histotype/Grade:	**Molecular subtype:**
1. Endometrial endometrioid carcinoma, grade 1 (50%)	1. NSMP (32-59%)
2. Endometrial endometrioid carcinoma, grade 2 (15%)	2. MMRd (26-33%)
3. Endometrial endometrioid carcinoma, grade 3 (13%)	3. POLEmut (6-13%)
	4. p53mut (9-22%)

4. Dedifferentiated/undifferentiated (from endometrioid, <1%)

5. Endometrial serous carcinoma (10%)
6. Clear cell carcinoma (3%)
7. Carcinosarcoma (7%)
8. Neuroendocrine carcinoma (rare)

Histotype/grade frequency from Alberta Cancer Registry; Molecular subtype frequency from PORTEC1/2 HIR & PORTEC 3 HR

4. Debate on how to integrate molecular subtype into conventional

Journal of Pathology
J Pathol 2018; **245:** 249–250
Published online 16 April 2018 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/path.5068

LETTER TO THE EDITOR

Letter in response to: McAlpine J, Leon-Castillo A, Bosse T. The rise of a novel classification system for endometrial carcinoma; integration of molecular subclasses. J Pathol 2018; 244: 538–549

Received 16 February 2018; Accepted 1 March 2018

Implementation or integration of molecular subclasses?

We have difficulty supporting their proposal because we don't interpret the cited evidence in the same way.

Köbel & Nelson J Pathol 2018 Pubmed ID 29512840

4. Endometrial carcinoma histotypes as per WHO

Histotype/Grade:

1. Endometrial endometrioid carcinoma, grade 1 (0%)
2. Endometrial endometrioid carcinoma, grade 2 (low)
3. Endometrial endometrioid carcinoma, grade 3 (25%)
4. Dedifferentiated/undifferentiated (from endometrioid, 0%)

5. Endometrial serous carcinoma (100%)
6. Clear cell carcinoma (59%)
7. Carcinosarcoma (90%)
8. Neuroendocrine carcinoma (Common)

Molecular subtype:

1. NSMP (32-59%)
2. MMRd (26-33%)
3. POLEmut (6-13%)
4. p53mut (9-22%)

Baniak 2019 PMID: 30585826; McConechy 2015 PMID: 27499902

4. Endometrial carcinoma histotypes as per WHO

Histotype/Grade:

1. Endometrial endometrioid carcinoma, grade 1 (25%)
2. Endometrial endometrioid carcinoma, grade 2 (25%)
3. Endometrial endometrioid carcinoma, grade 3 (45%)
4. Dedifferentiated/undifferentiated (from endometrioid, 53%)
5. Endometrial serous carcinoma (0%)
6. Clear cell carcinoma (0%)
7. Carcinosarcoma (from endometrioid, 4%)
8. Neuroendocrine carcinoma (44%)

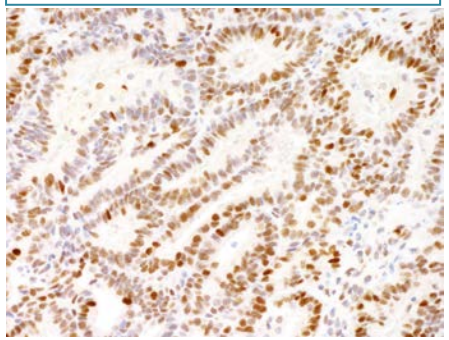
Molecular subtype:

1. NSMP (32-59%)
2. MMRd (26-33%)
3. POLEmut (6-13%)
4. p53mut (9-22%)

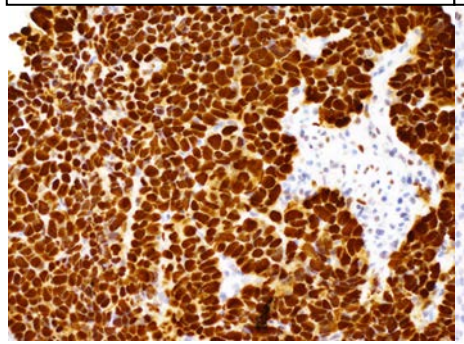
Chen 2017 PMID: 27167671; Köbel 2018 PMID: 28863077; Baniak 2019 PMID: 30585826; Cherniack 2017 PMID: 28292439, Pocrnich 2016 PMID: 26945341

Endometrial carcinoma: high interassay & observer IHC agreement

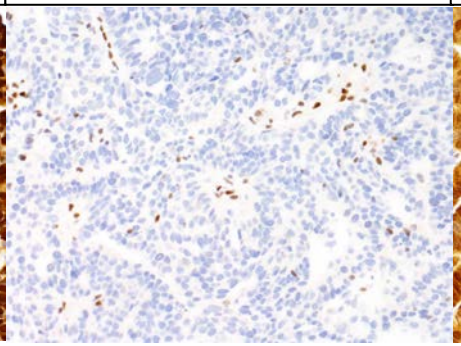
Wild-type pattern



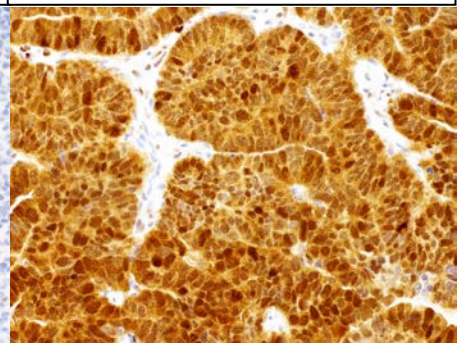
Mut-OE



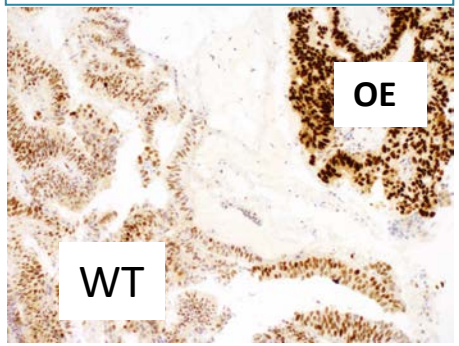
Mut-CA



Mut-CY



Subclonal

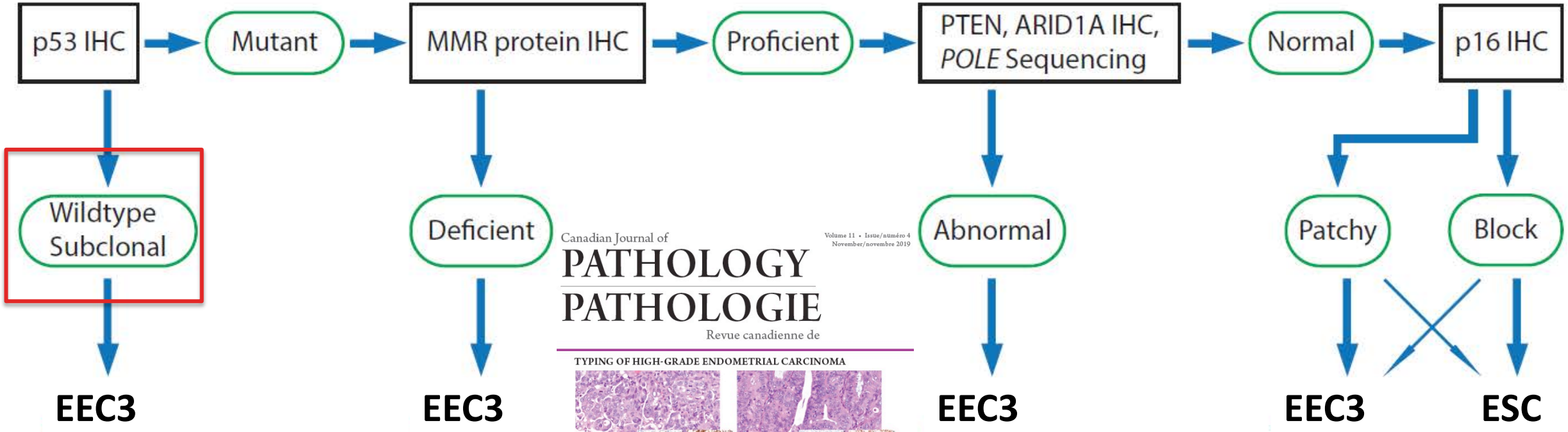


		CENTRAL p53 IHC					
		WT	Mut-OE	Mut-CA	Mut-CY	Subclonal	TOTAL
LOCAL p53 IHC	WT	**67**	1	0	1	3	72
	Mut-OE	2	**68**	1	1	0	72
	Mut-CA	1	0	**14**	1	0	16
	Mut-CY	0	0	0	**1**	0	1
	Subclonal	0	0	0	0	**3**	3
	TOTAL	70	69	15	4	6	164

		TP53 mutation	
		Present	Absent
IHC	**Mut**	85	4
	WT	2	32
Sensitivity: 97.70% (95% CI 91.94% to 99.7%)			
Specificity: 88.89% (95% CI 73.94% to 96.89%)			
Accuracy*: 95.12% (95% CI 89.68% to 98.19%)			

Singh et al. J Pathol PMID: 31829441; *excl. subclonal, MMRd, POLEmut

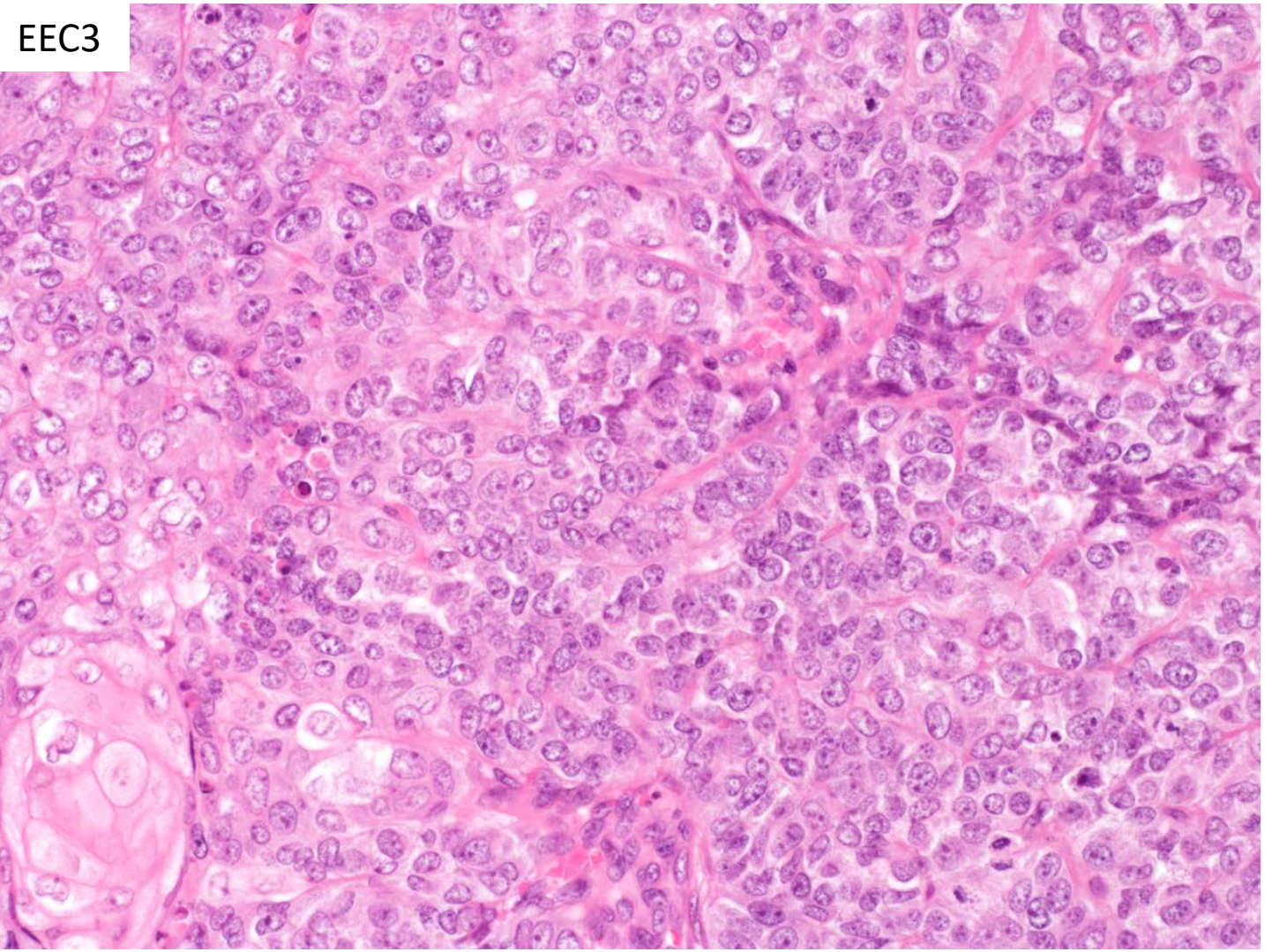
4. Histotype distinction of endometrial endometrioid carcinoma grade 3 (EEC3) from serous (ESC)



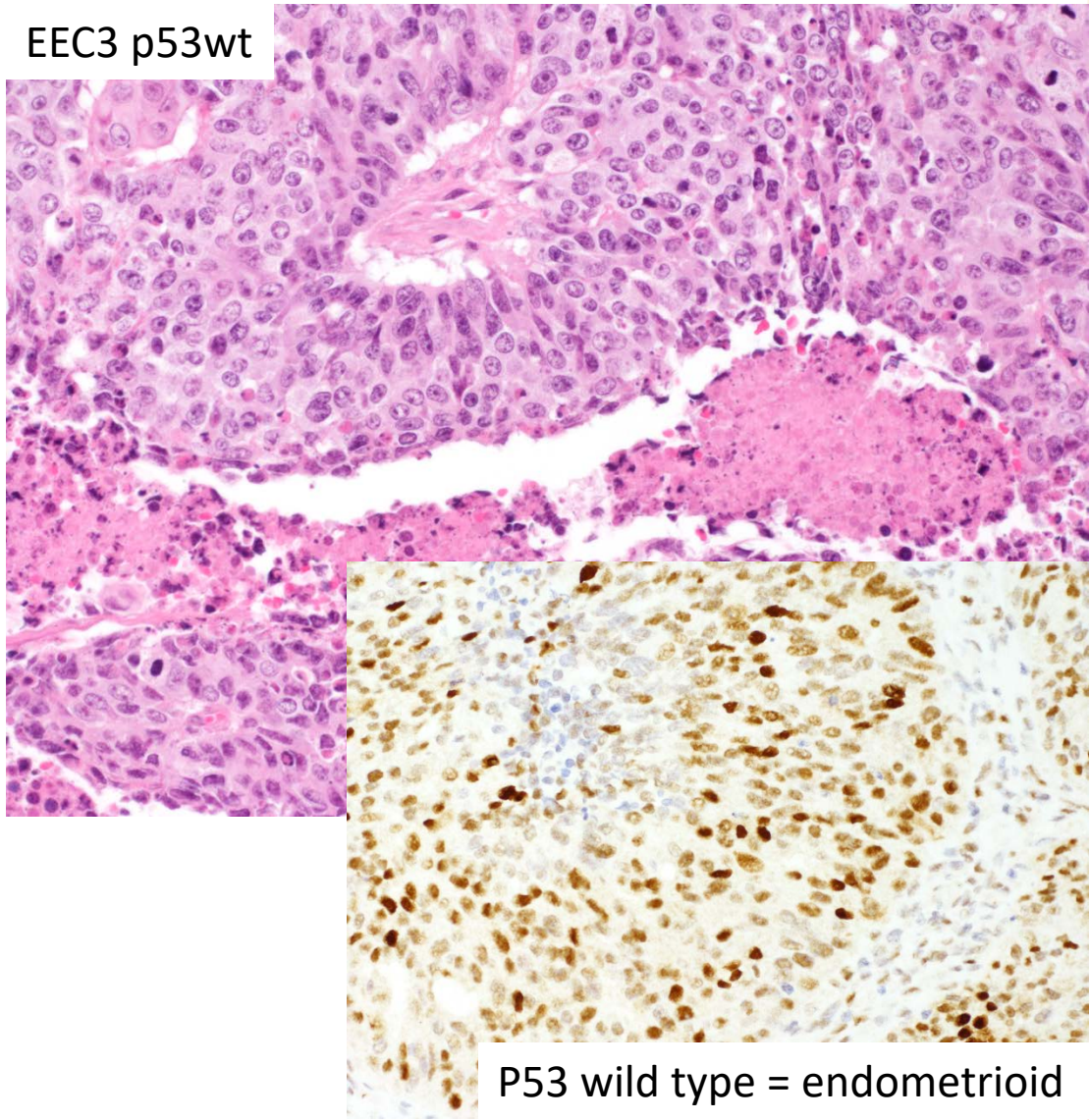
Brett et al. Can J Pathol 2019 Nov:11(4):44-58

4. p53 wild type ~ endometrioid

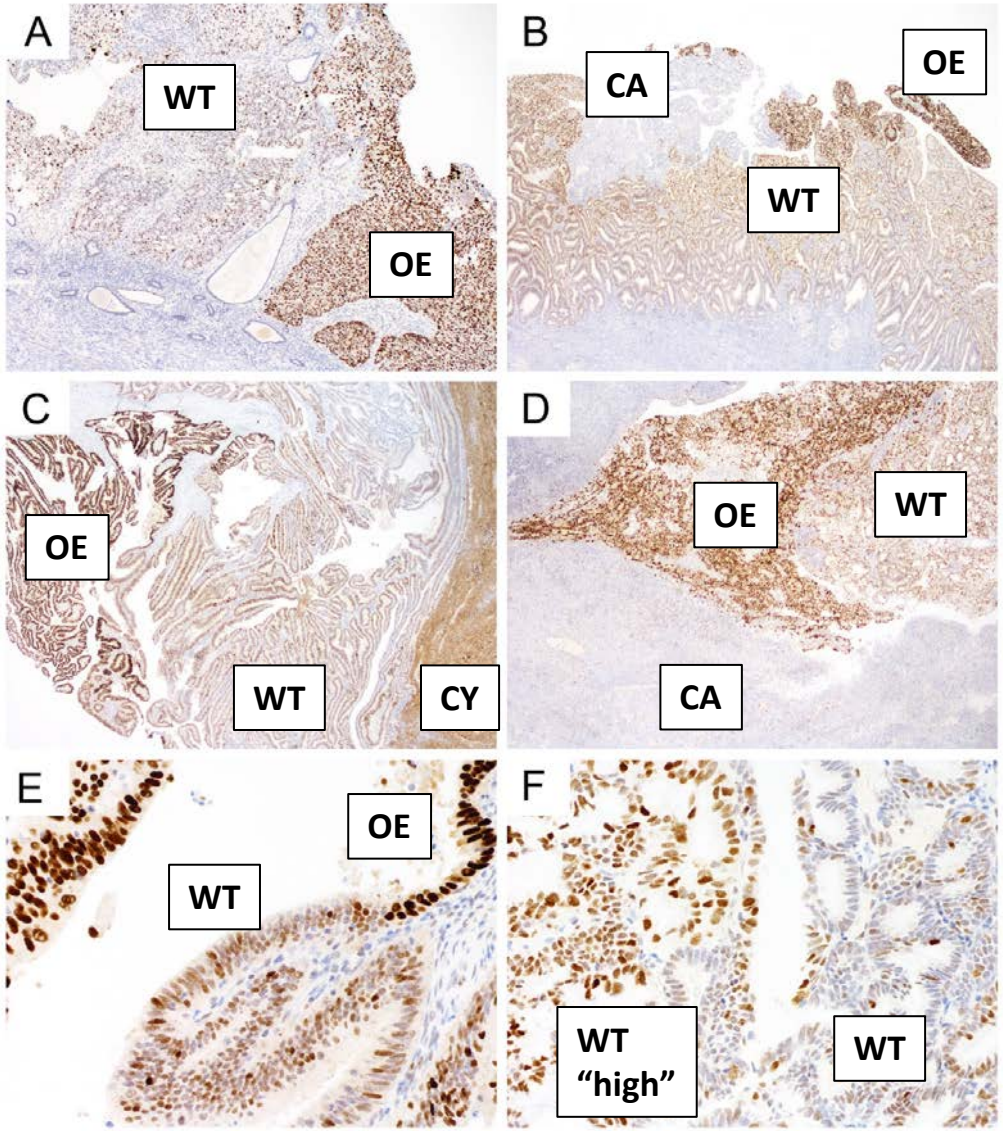
EEC3



EEC3 p53wt



P53 wild type = endometrioid



P53 subclonal

Definition: combination of wild type pattern (WT) with mutant patterns (OE, CA, CY)

Frequency: ~5%

Interpretation:
Confirms endometrioid histotype
Majority associated with MMRd or POLEmut

Köbel et al. Int J Gynecol Pathol 2019 PMID: 29517499

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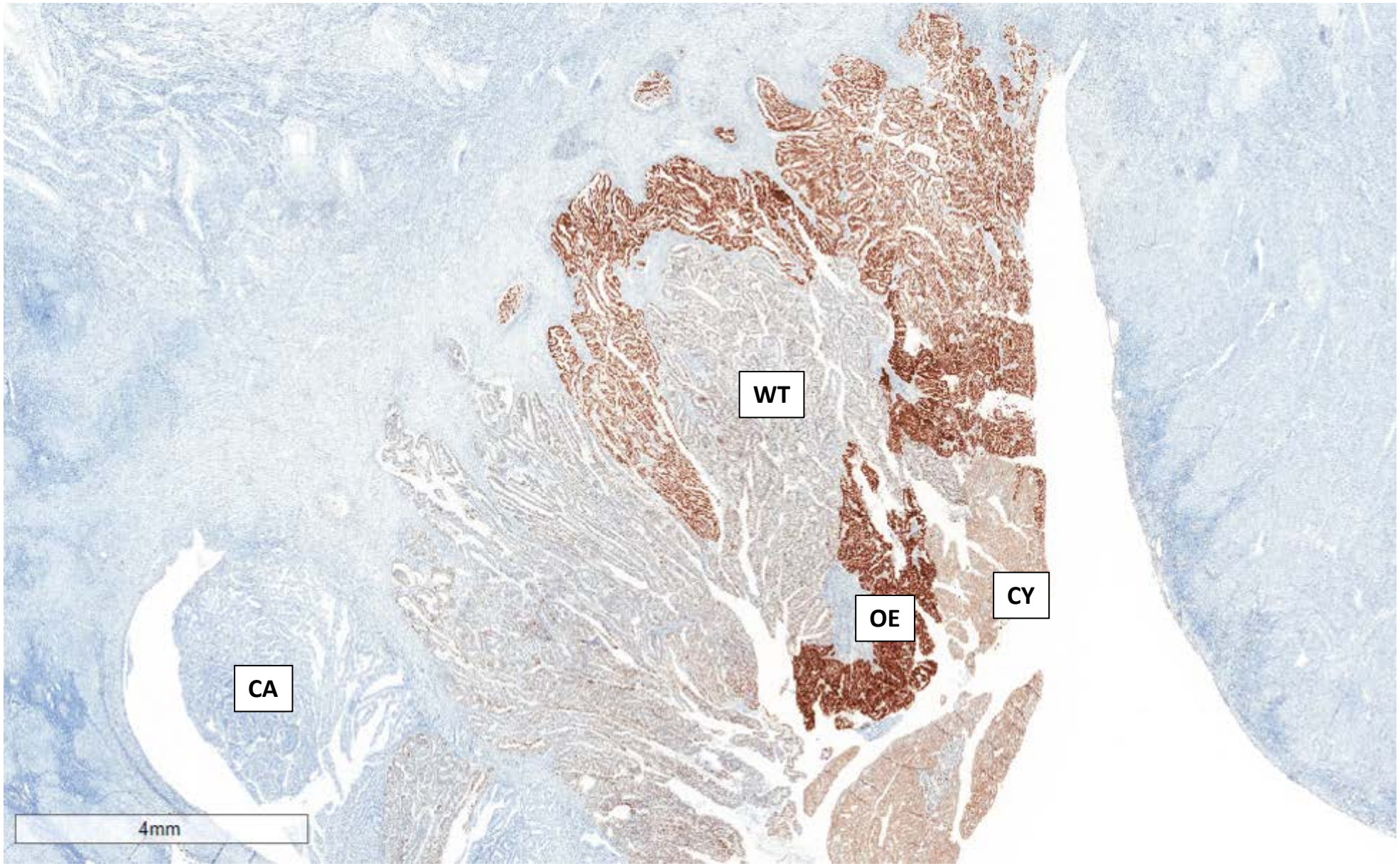
4. EEC, subclonal p53 showing all 4 patterns



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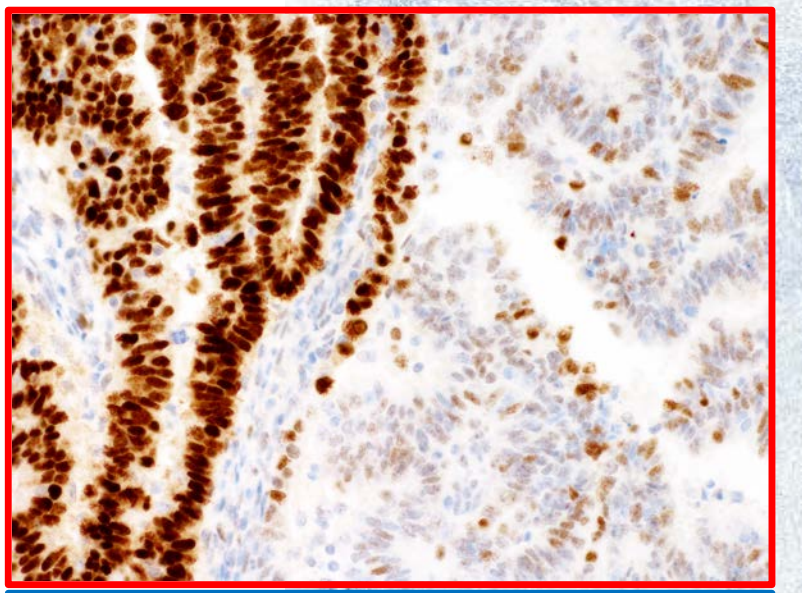
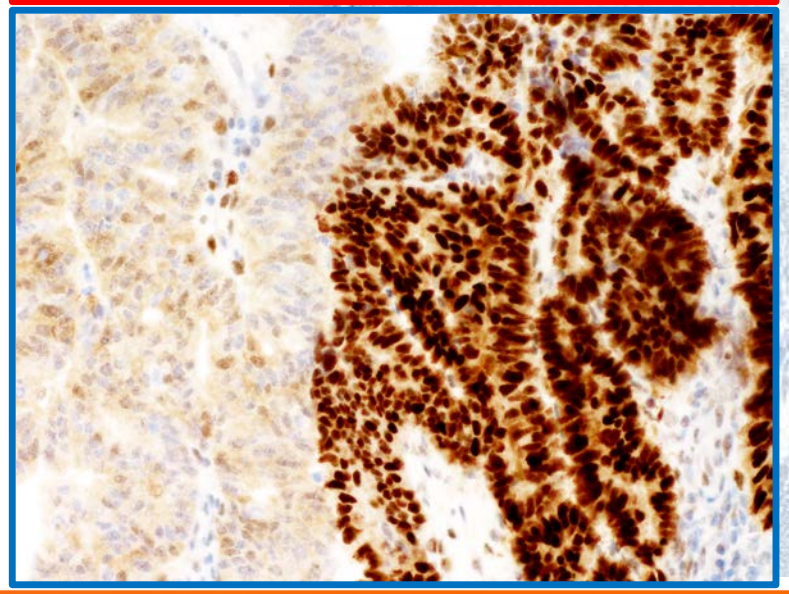
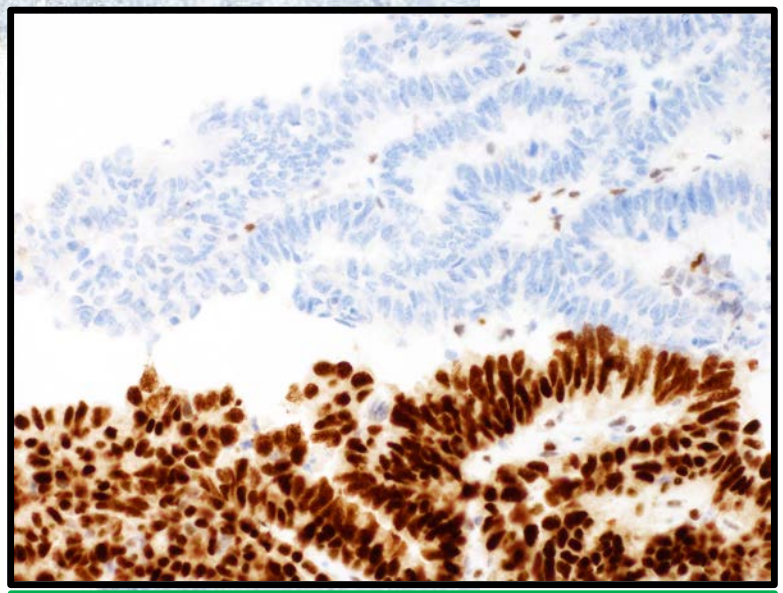
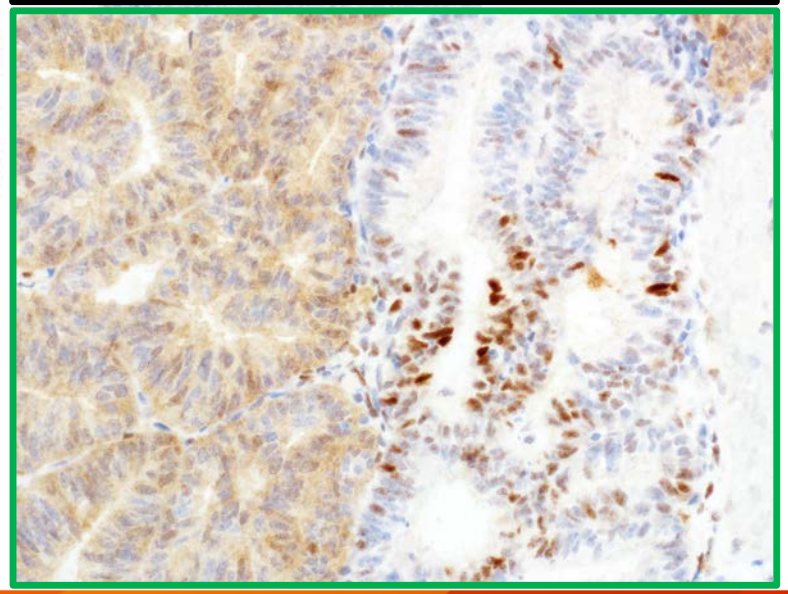
4. EEC, subclonal p53 showing all 4 patterns









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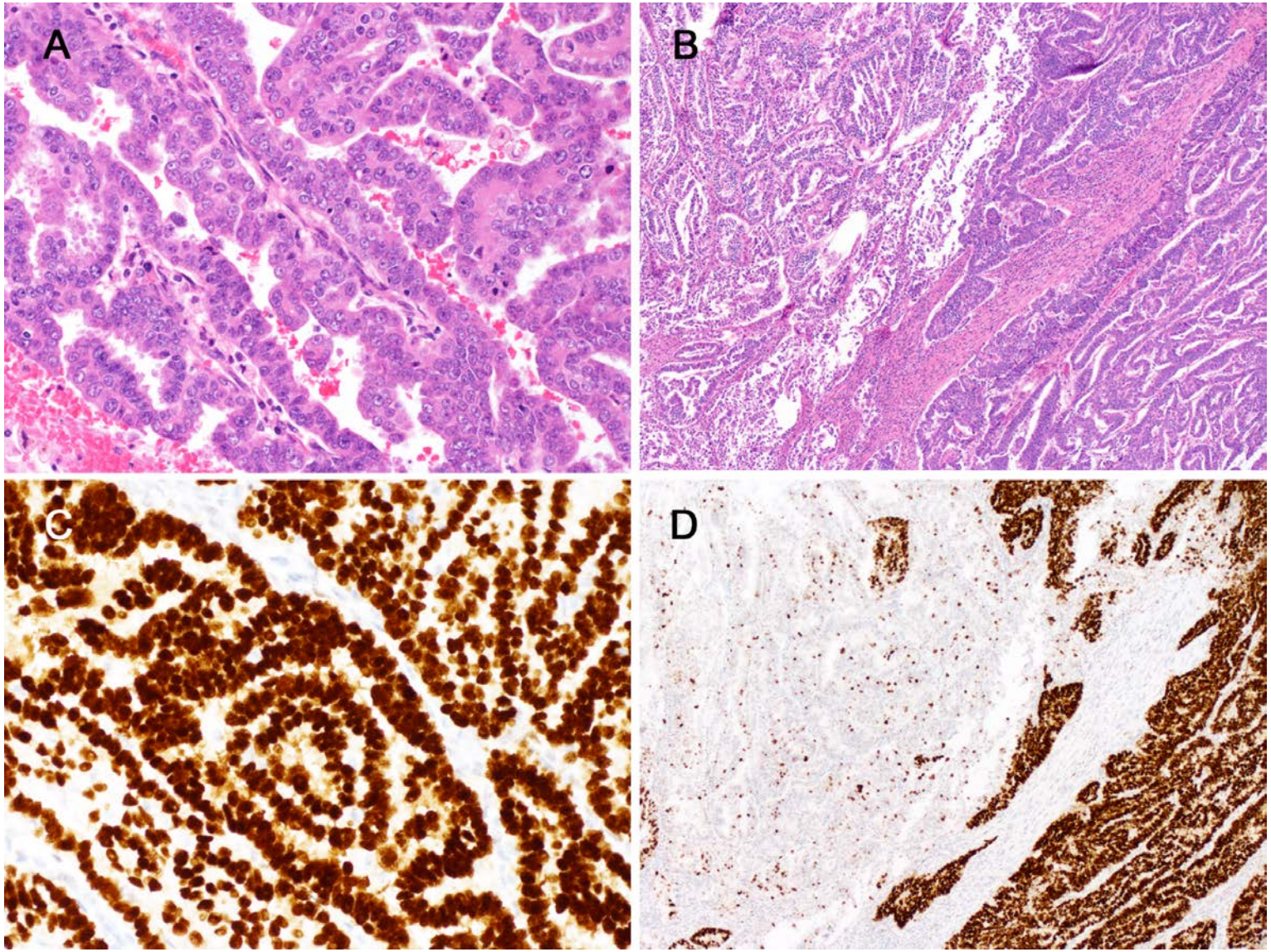
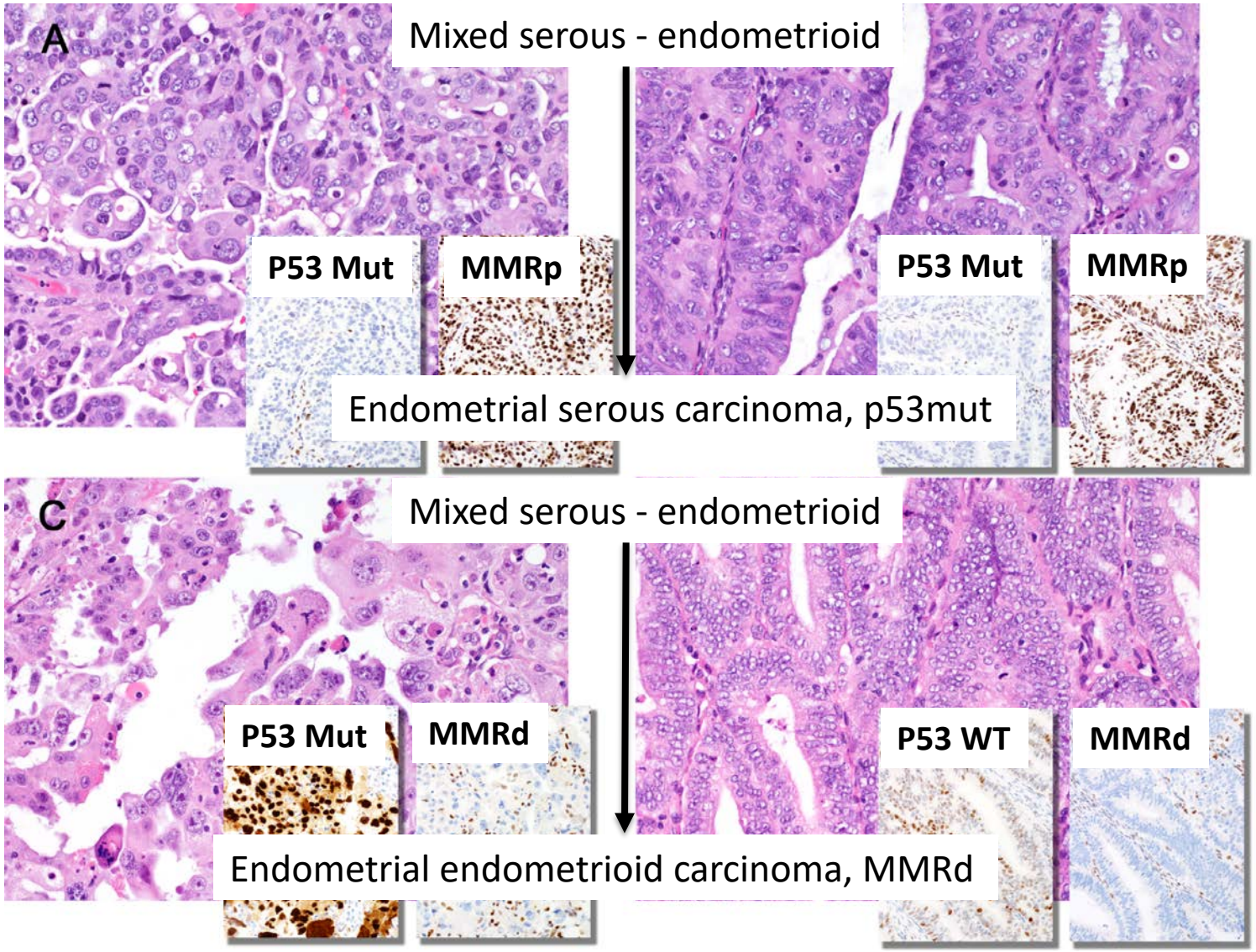
4. ESC with poor fixation mimicking subclonal



Brett et al. Can J Pathol 2019 Nov:11(4):44-58

4. Subclonal ~ mixed morphology

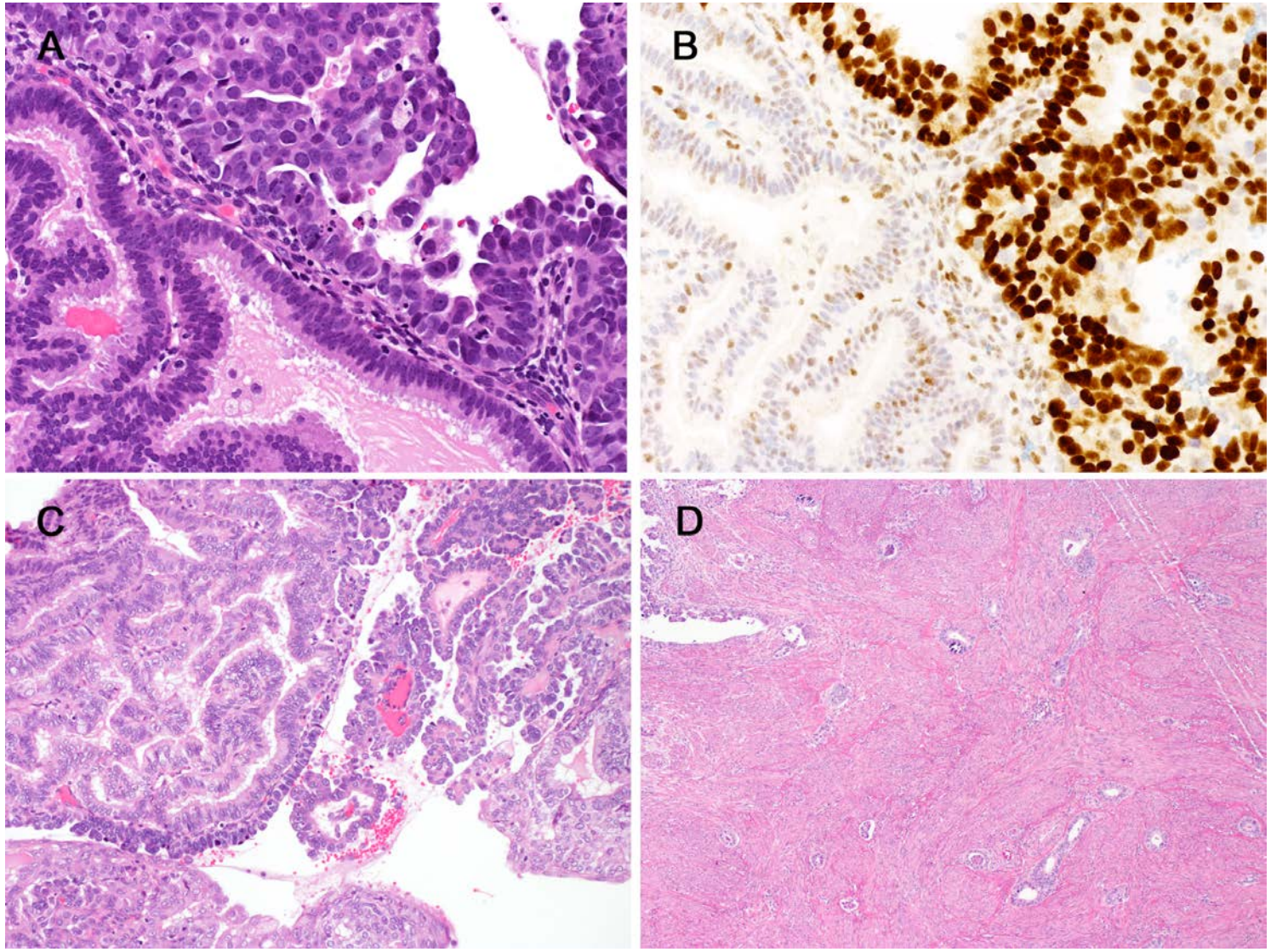


Mixed endometrial carcinoma can be assigned to a single histotype

Represent intratumoral heterogeneity (morphological mimicry) probably due to acquisition of non-founder ('passenger?') mutations

Brett et al. Can J Pathol 2019 Nov:11(4):44-58, Köbel et al. AJSP 2016 PMID: 26492180, Köbel et al. Int J Gynecol Pathol 2017 28114191

4. Subclonal MMRp, no POLE mutation



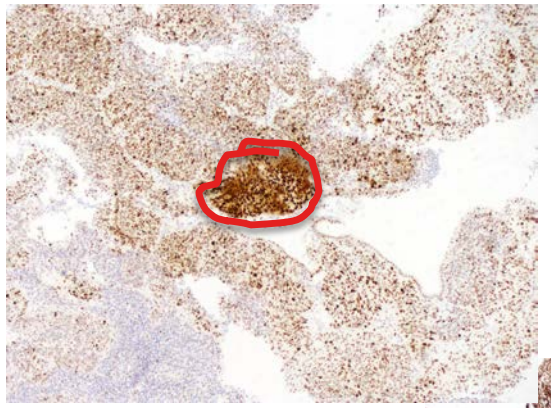
1. Exclude **collision tumor** of ESC with EEC1 (left)

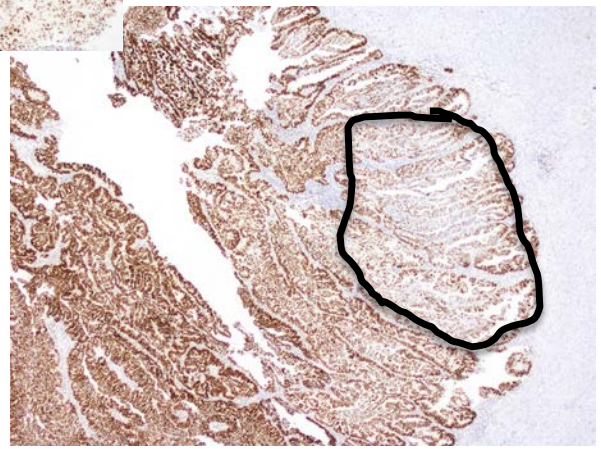
Single tumor MMRp, no POLE mutation:

Uncommon (~2%) but prognostic uncertain

What amount of subclonal p53 is relevant?

Example below 2% OE versus 5% WT?





Brett et al. Can J Pathol 2019 Nov:11(4):44-58

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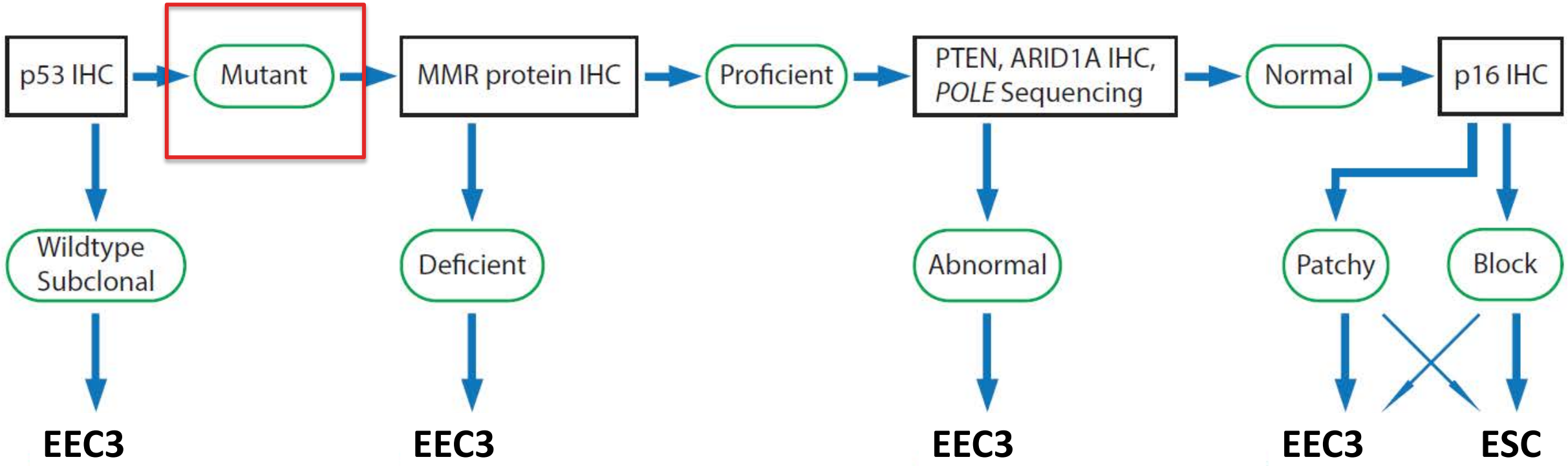
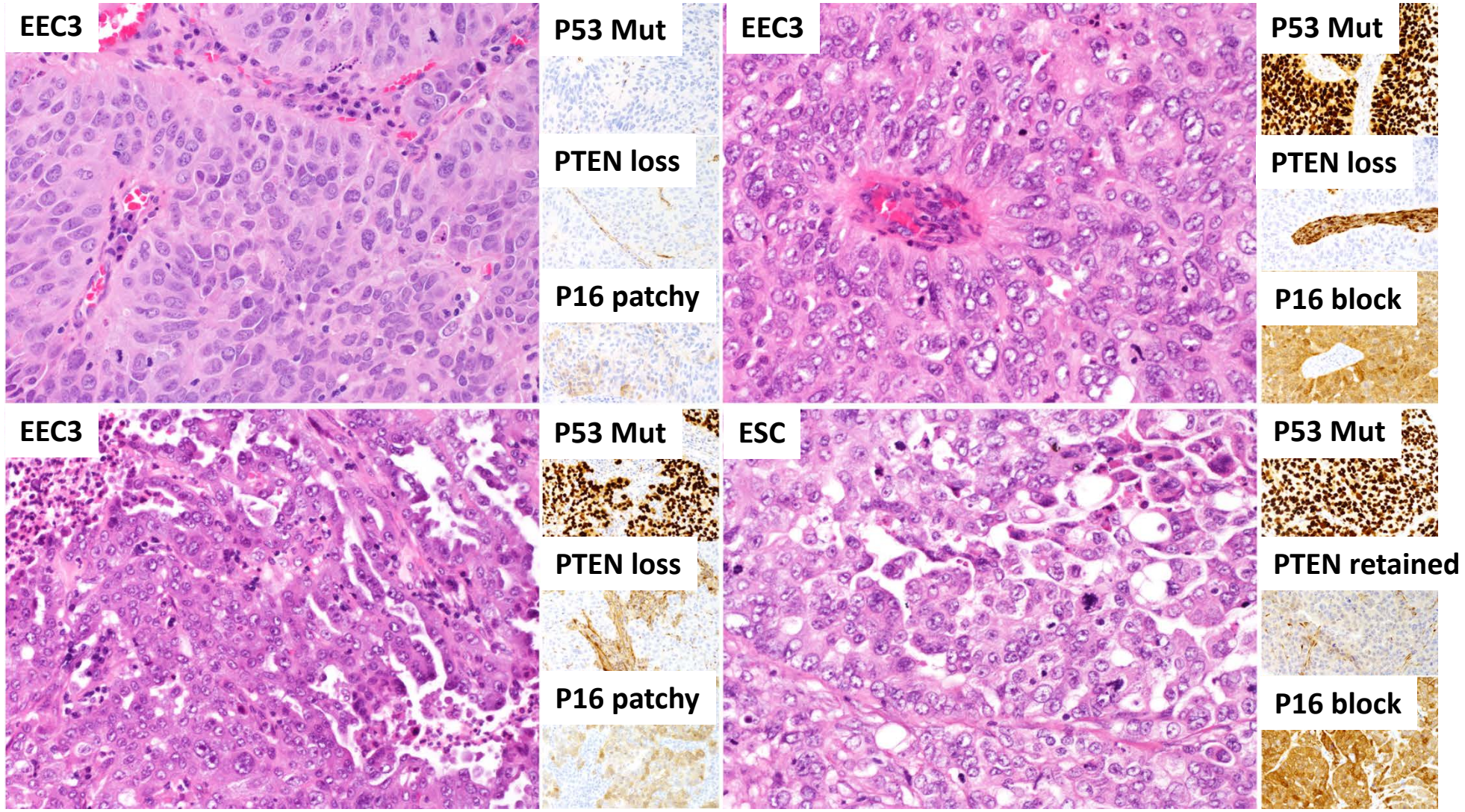
4. Histotype distinction of endometrial endometrioid carcinoma grade 3 (EEC3) from serous (ESC)



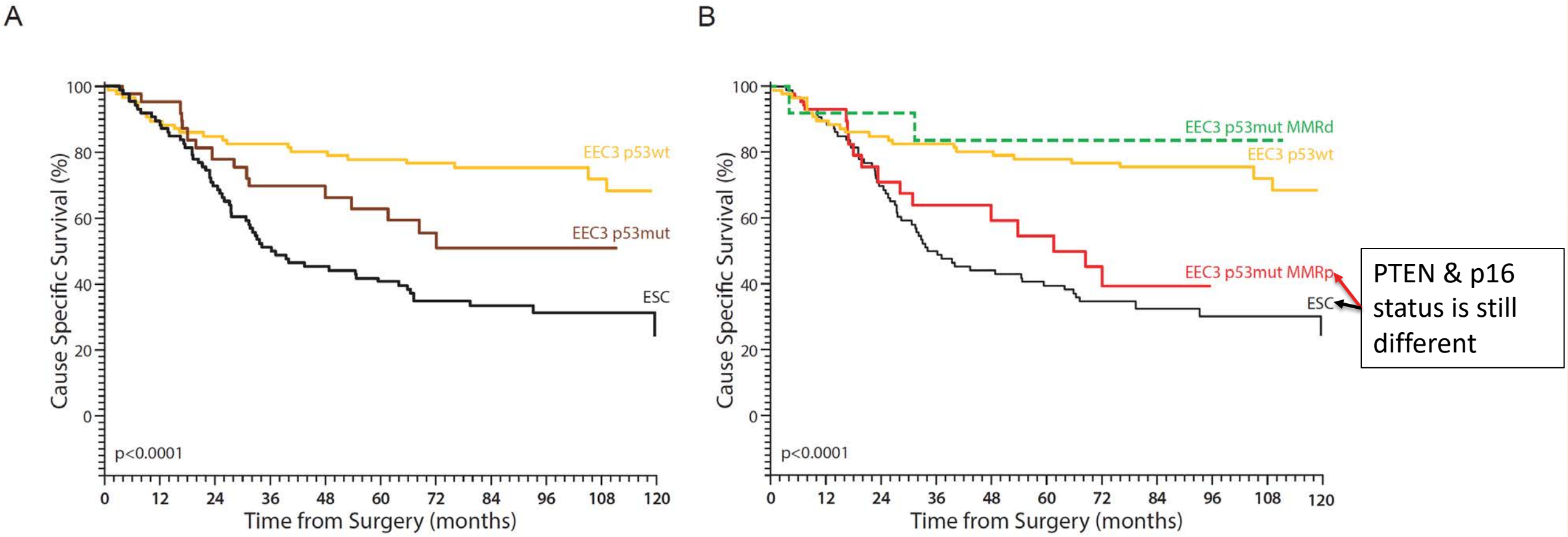
Brett et al. Can J Pathol 2019 Nov:11(4):44-58

4. Histotyping in p53mut, MMRp cases



Brett et al. Can J Pathol 2019 Nov:11(4):44-58

4. Subclonal p53 often associated with MMRd (“double classifier”)



EEC3 MMRd and p53mut (double classifier) can be classified as MMRd

Leon Castillo et al. J Pathol 2020 31829447; Brett et al. Int J Gynecol Pathol in press

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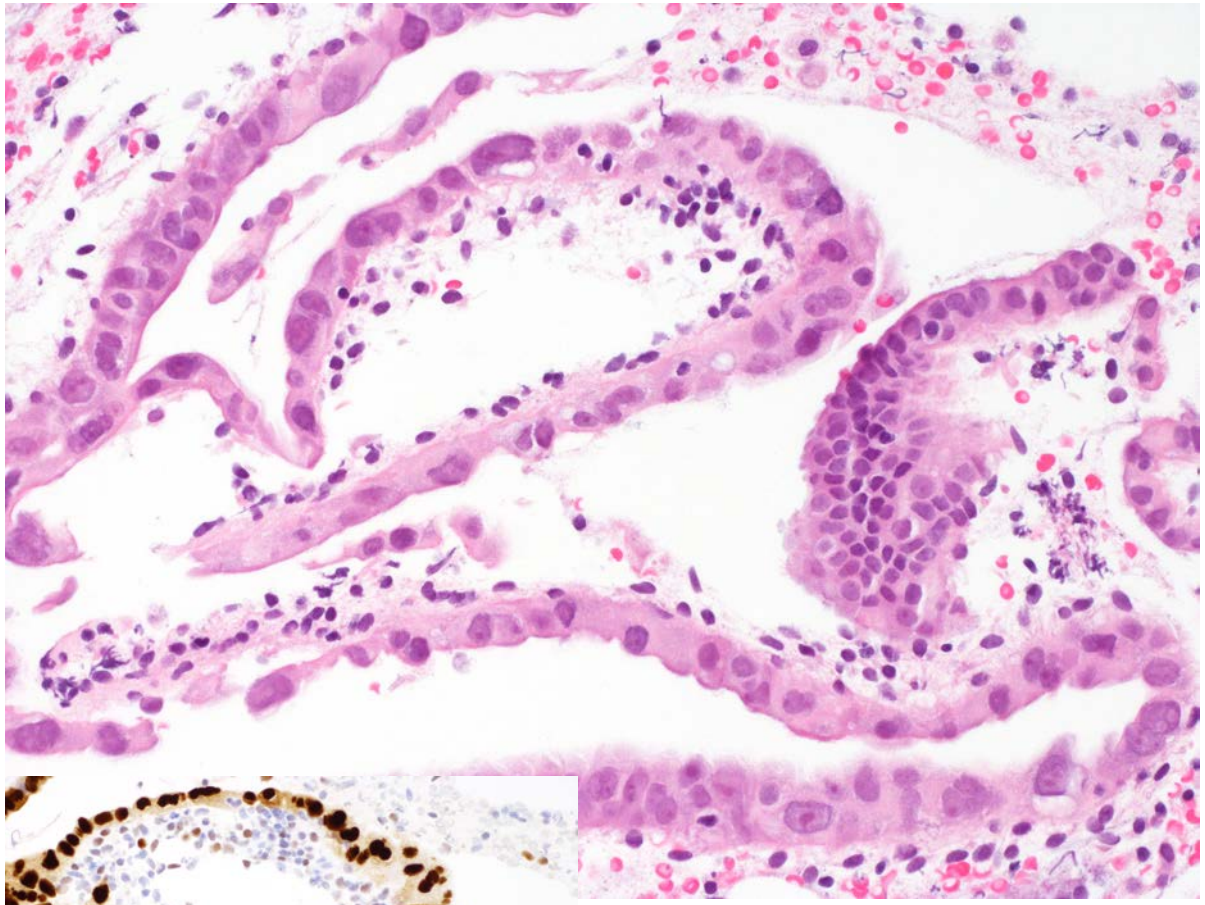
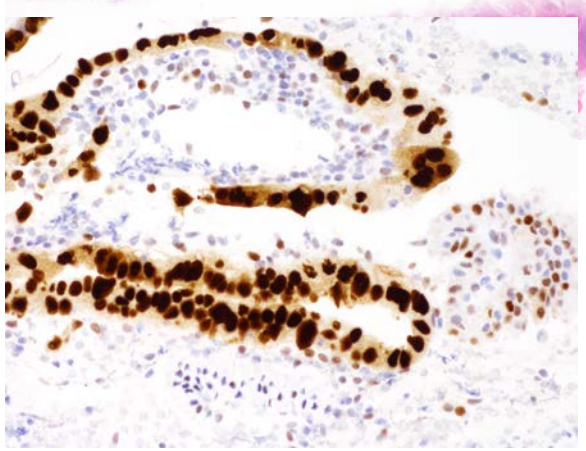
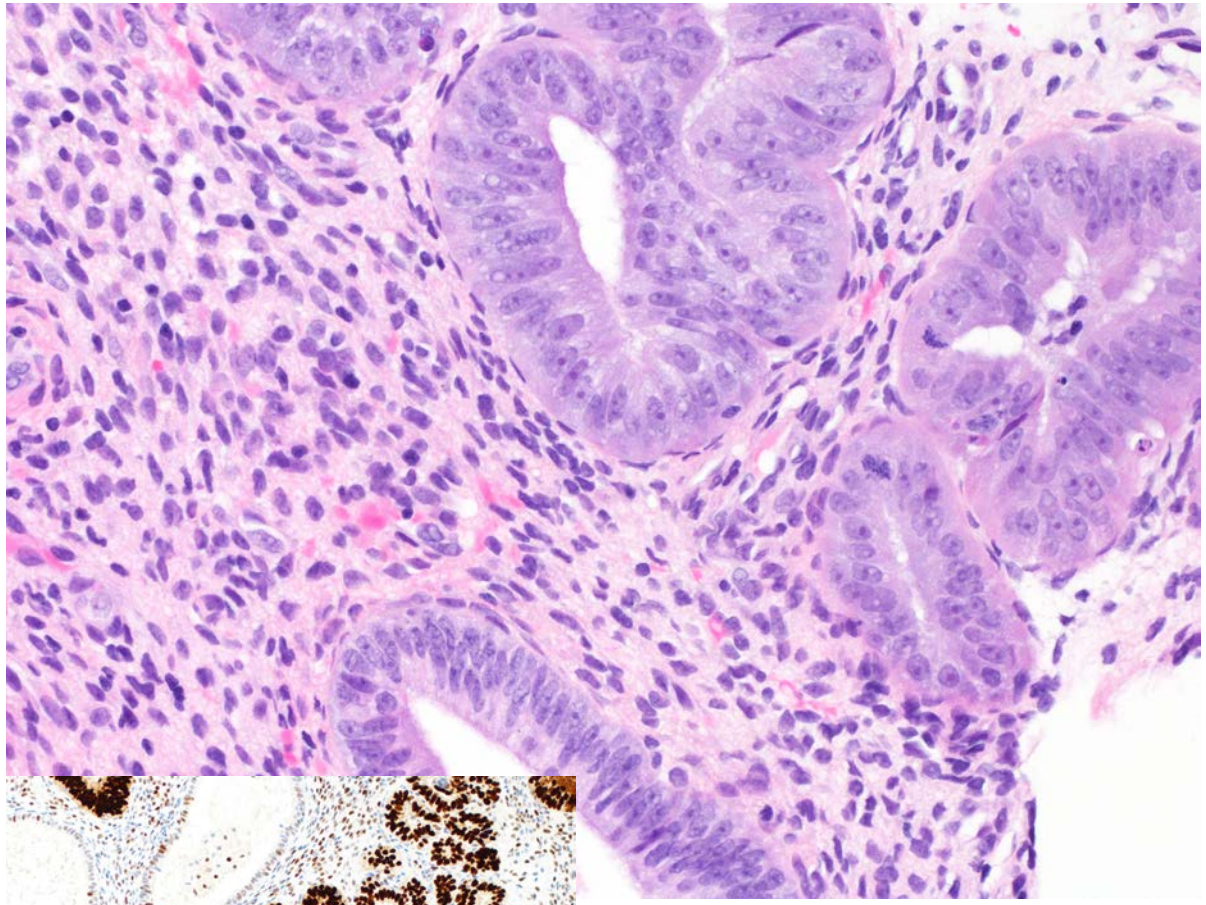
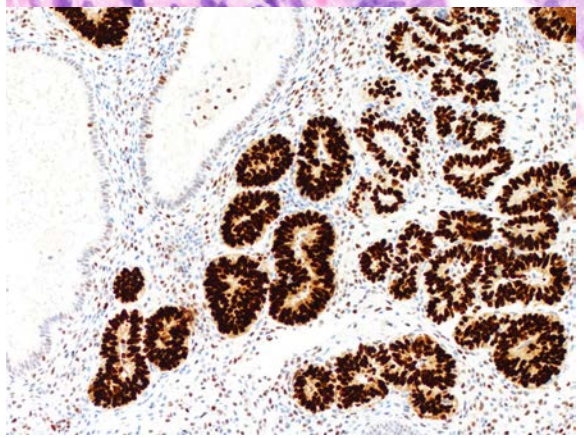
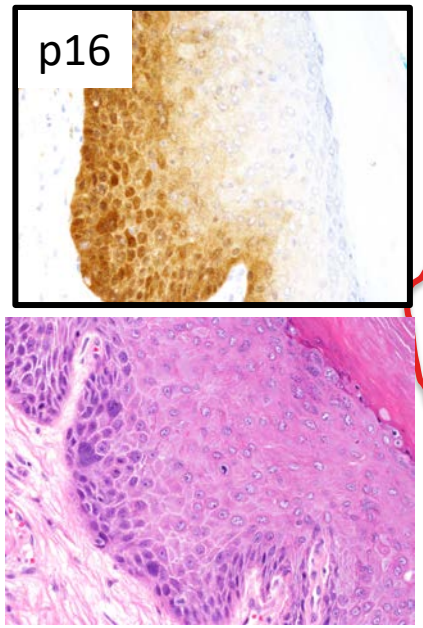
5. Confirming serous precursor lesion: SEIC/MUSC

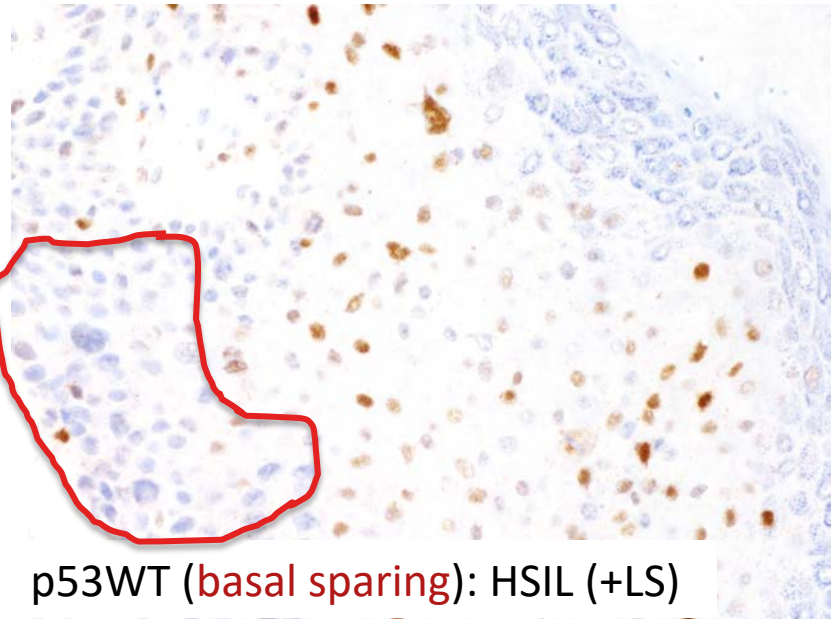




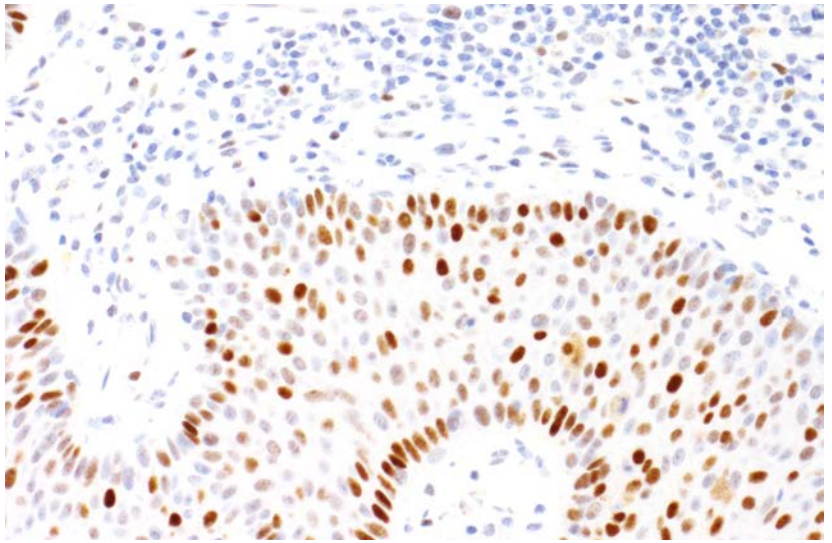


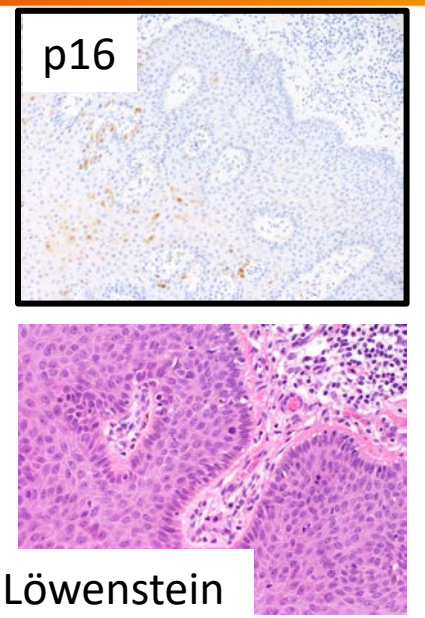




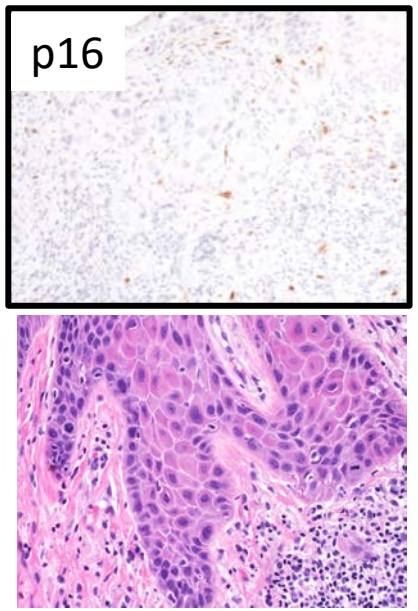


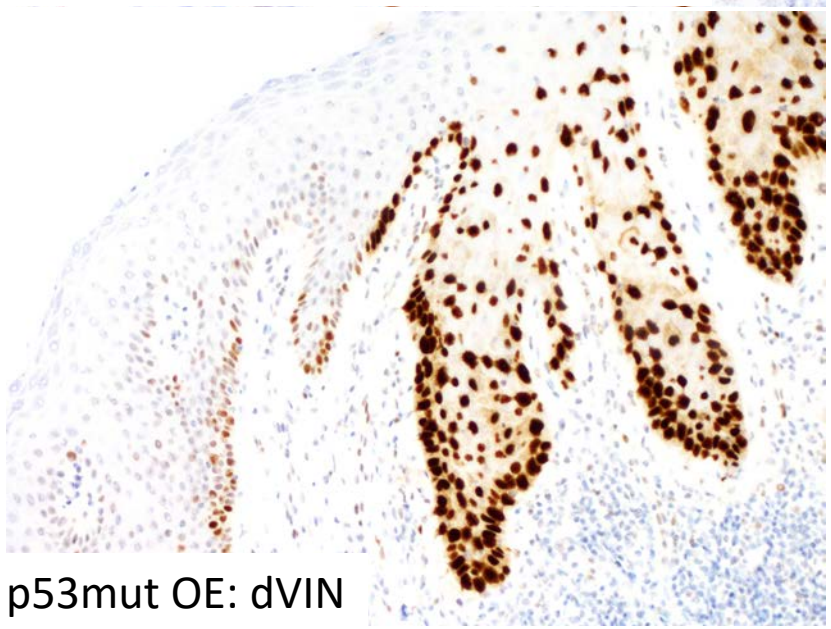
p53WT (basal sparing): HSIL (+LS)



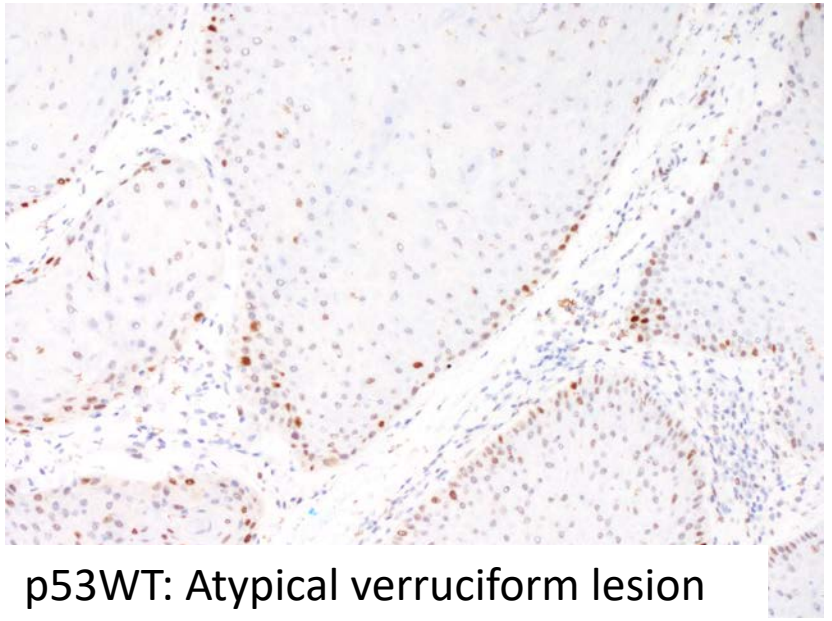


p53 wild type: Giant condyloma Buschke Löwenstein

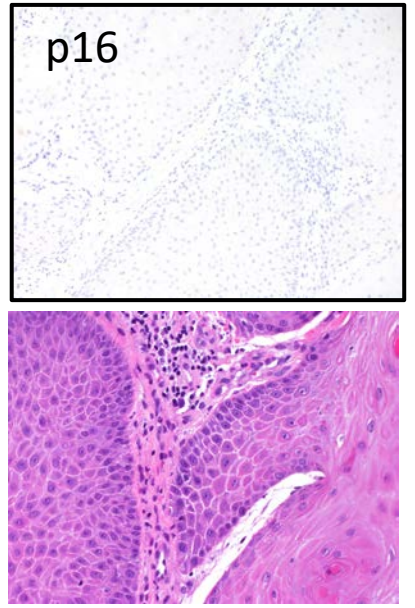




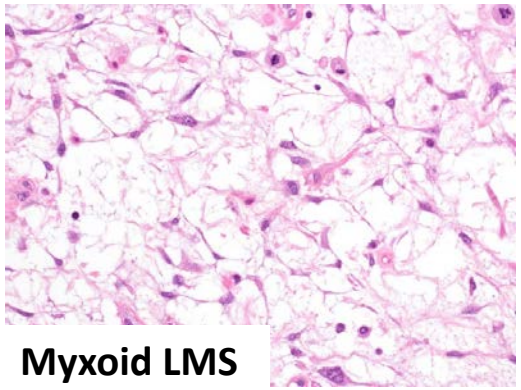
p53mut OE: dVIN

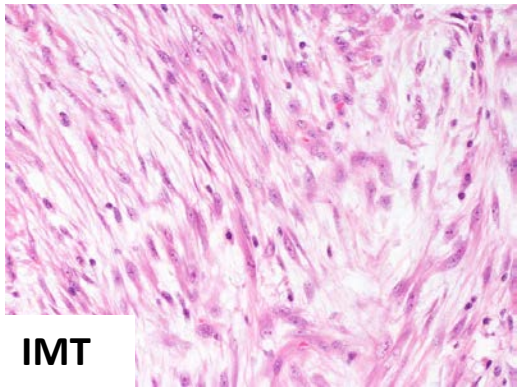


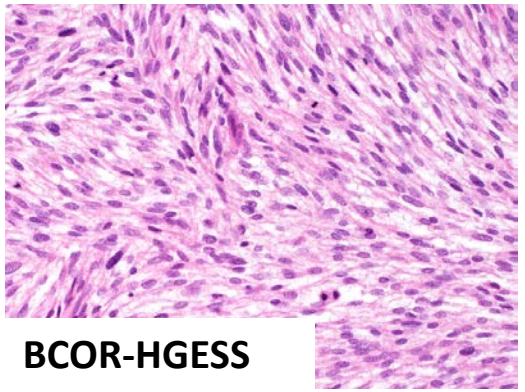
p53WT: Atypical verruciform lesion

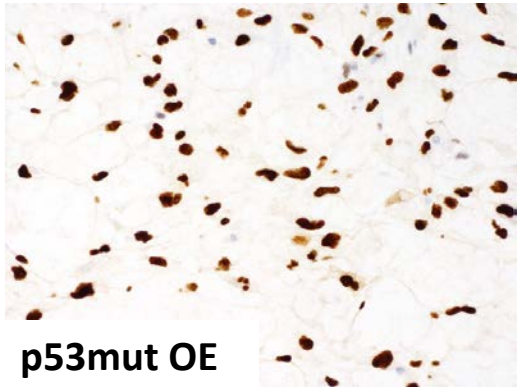


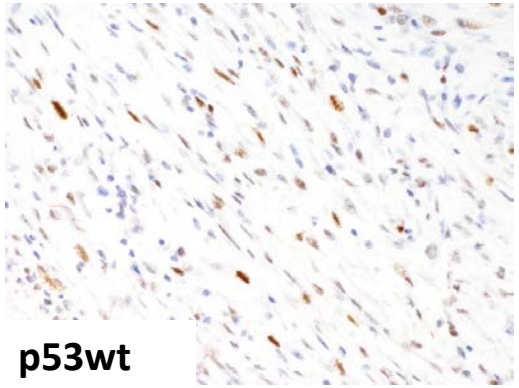
8. Triaging of uterine mesenchymal neoplasms to translocation testing

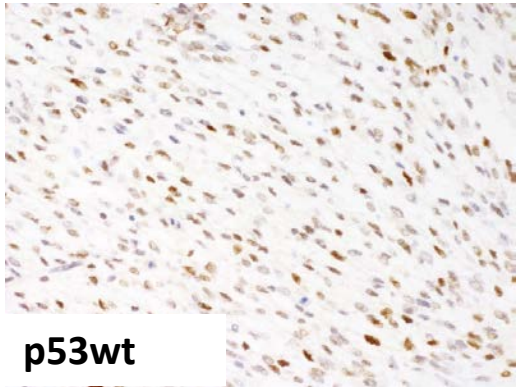

Myxoid LMS

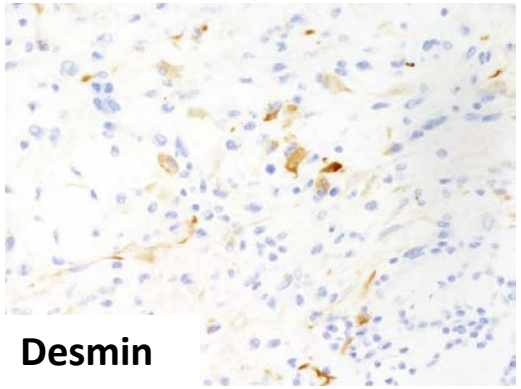

IMT

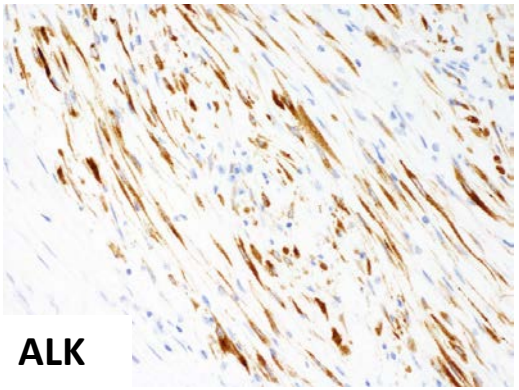

BCOR-HGESS

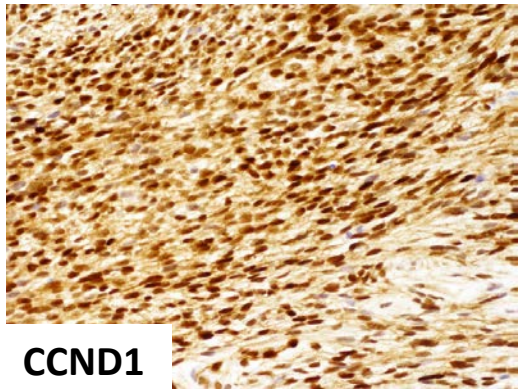

p53mut OE


p53wt


p53wt


Desmin


ALK


CCND1

P53 wild type cases may be referred to molecular translocation testing

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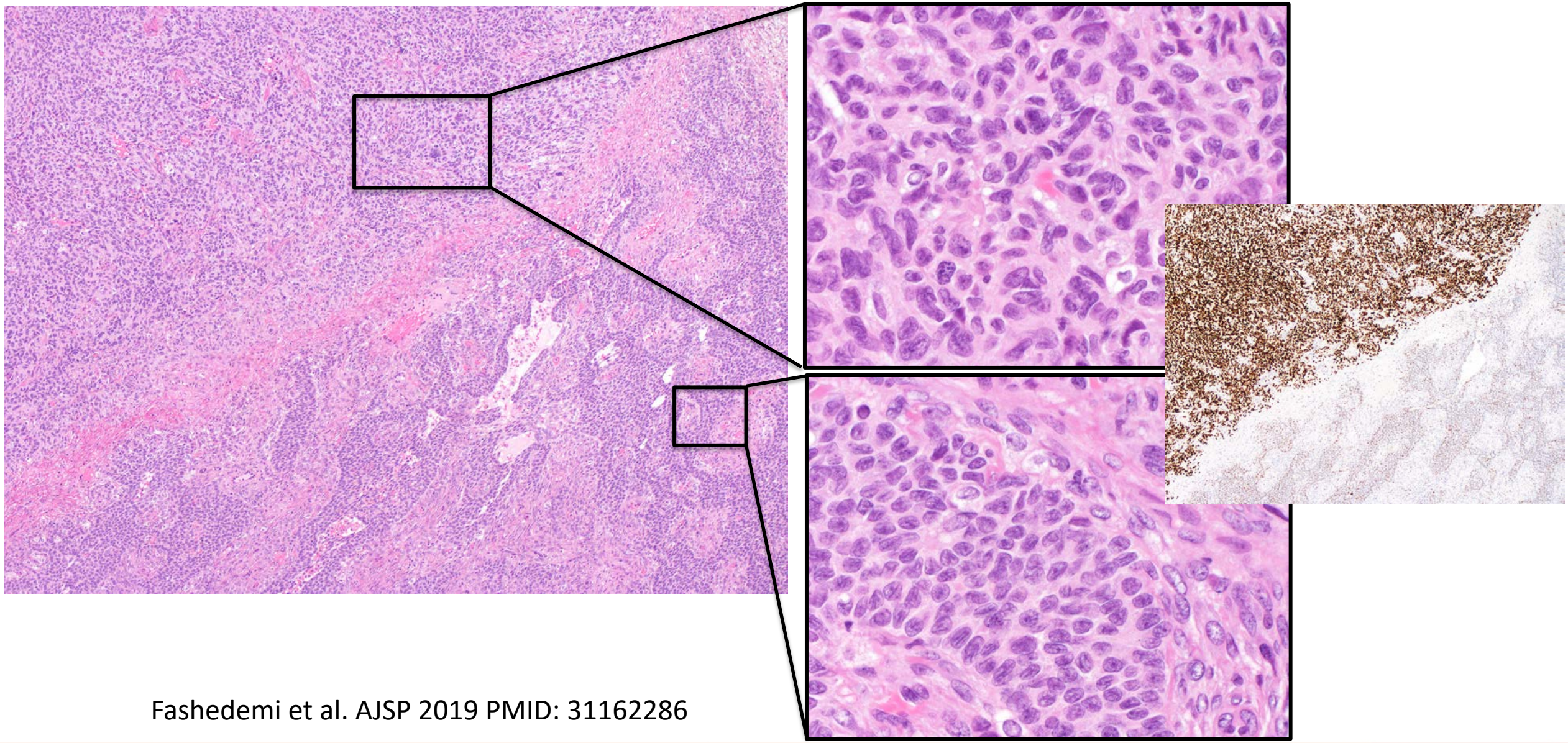
Extra: AGCT with high-grade transformation



Fashedemi et al. AJSP 2019 PMID: 31162286

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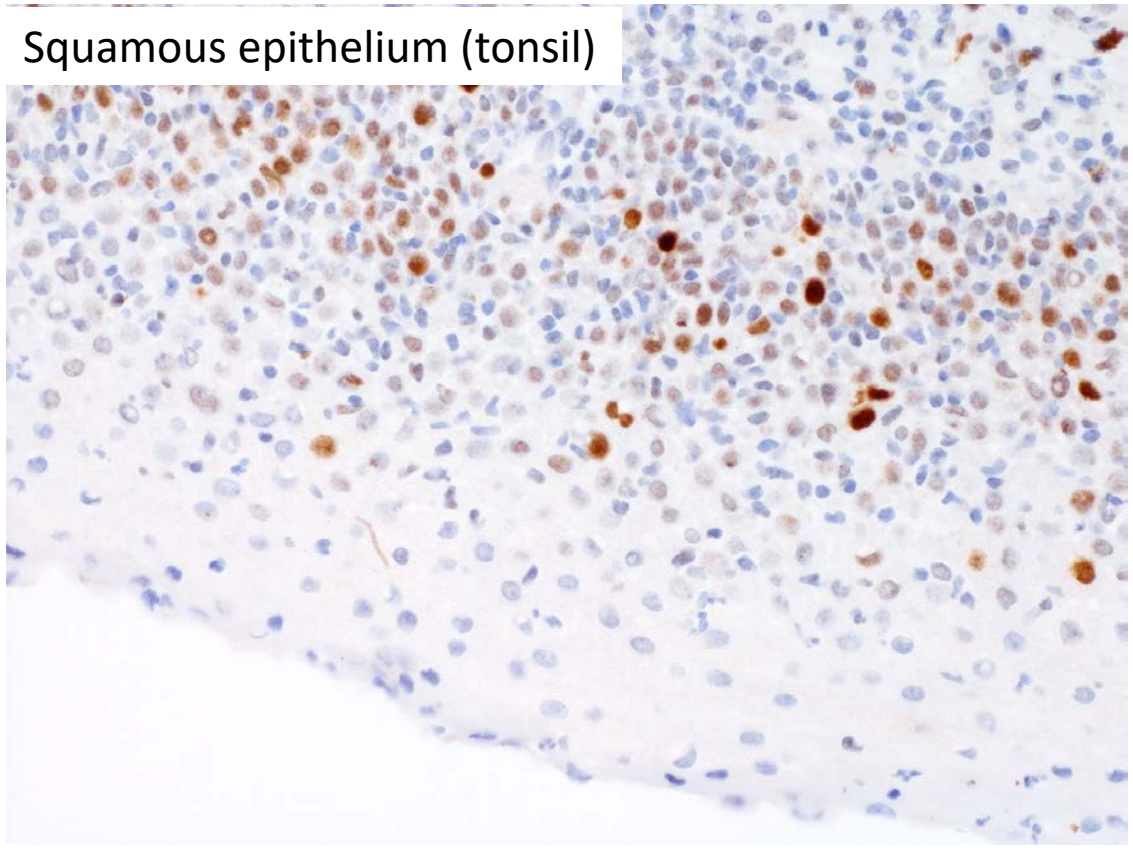
Conclusion

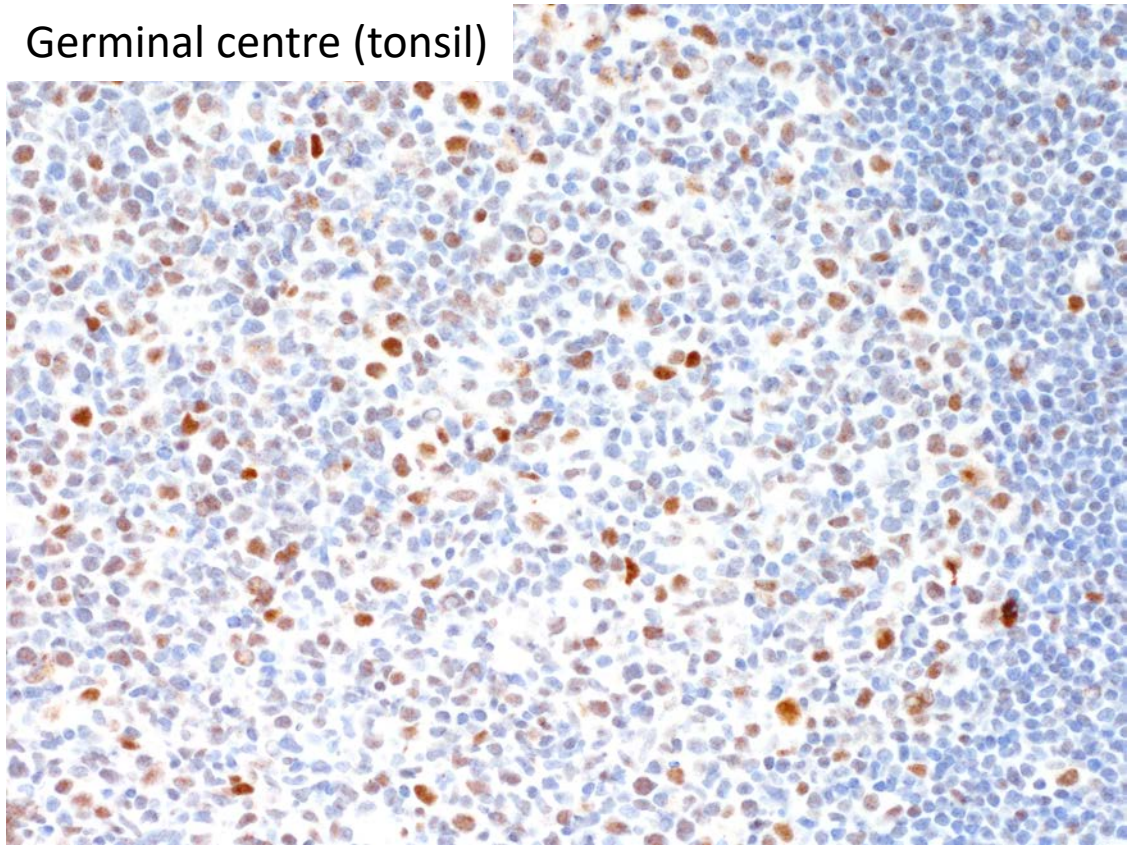
1. Distinction of LGSC from HGSC
2. Distinction of OEC from HGSC
    a) Assigning molecular subtype of OEC
3. Confirmation of HGSC precursors
4. Assessing risk in MBOT
5. Distinction of EEC3 from ESC
    a) Assigning molecular subtype of EEC
6. Confirming of ESC precursors
7. Prognostication in squamous vulvar lesions
8. Triaging of uterine mesenchymal neoplasms to translocation testing

Controls, Controls, Controls

Squamous epithelium (tonsil)



Germinal centre (tonsil)



DO7 clone on Dako Omins

Detailed protocol: Singh et al. J Pathol PMID: 31829441

Email: mkoebel@ucalgary.ca

https://www.nordiqc.org/

https://www.cpqa.ca/

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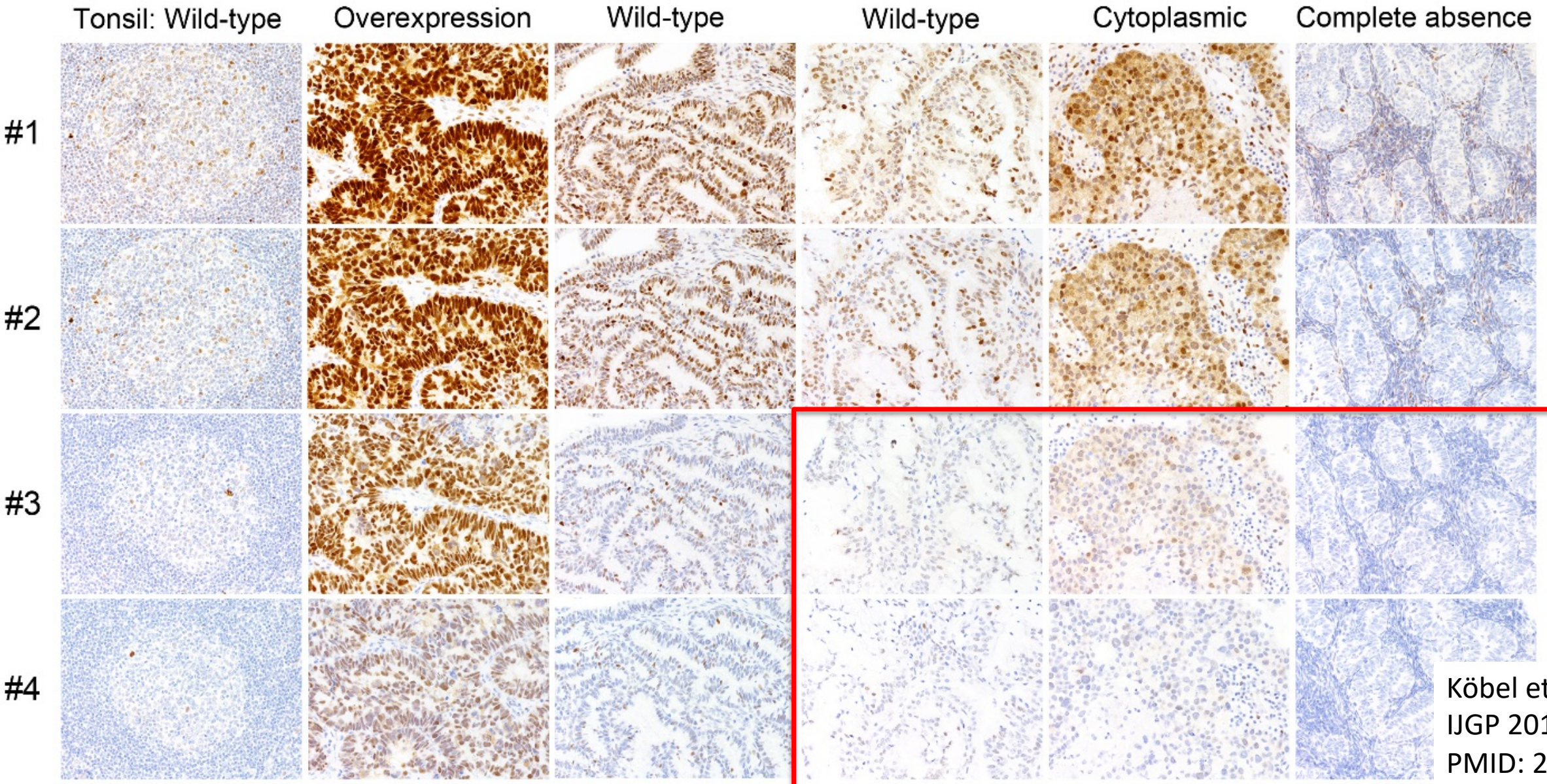
Right Protocol



Köbel et al.
IJGP 2019
PMID: 29517499

THANK YOU!