# MMR, MSI and POLE Testing in Endometrial Carcinoma

PRESENTED BY
**Naveena Singh**

# Important Information Regarding CME/SAMs

The **Online CME/Evaluations/SAMs claim** process will only be available on the USCAP website until **September 30, 2020**

**No claims can be processed after that date!**

After **September 30, 2020** you will NOT be able to obtain any CME or SAMs credits for attending this meeting.

# Disclosure of Relevant Financial Relationships

The faculty, committee members, and staff who are in position to control the content of this activity are required to disclose to USCAP and to learners any relevant financial relationship(s) of the individual or spouse/partner that have occurred within the last 12 months with any commercial interest(s) whose products or services are related to the CME content. USCAP has reviewed all disclosures and resolved or managed all identified conflicts of interest, as applicable.

**Naveena Singh** reported no relevant financial relationships.

# PLEASE TURN OFF YOUR CELL PHONES

# Acknowledgements


- Tjalling Bosse and the TransPORTEC team

- C Blake Gilks


**PORTEC-1: 1990-1997, 714 patients: Pelvic EBRT gives better local control than no Adjuvant Rx in high-intermediate risk EC**

**PORTEC-2: 2002-2006, 427 patients: VBT is as effective for vaginal control with lower toxicity compared to EBRT in high-intermediate risk EC**

**PORTEC-3: 2006-2013, 686 patients: Significantly improved FFS and OS with Chemoradiotherapy vs RT alone in high-risk EC**

**PORTEC-4A: Ongoing; risk stratification according to molecular classification**


**COMBINED DATA ON IMPACT OF ADJUVANT RX ON >1200 MOLECULARLY CLASSIFIED PATIENTS RANDOMISED TO DIFFERENT ADJUVANT TREATMENT ARMS**

PORTEC-1: 1990-1997, 714 patients: Pelvic EBRT gives better local control than no Adjuvant Rx in EC

427 patients: VBT is as trol with lower toxicity gh-intermediate risk EC

686 patients: Significantly with Chemoradiotherapy EC

risk stratification according to molecular classification

# Introduction: Why test for POLE and MMRd?

ENDOMETRIAL CARCINOMA:

- Commonest gynaecological malignancy in affluent societies
- Rising incidence
- Low but ***unchanged*** mortality for decades

# Current basis for treatment decisions

- Risk prediction algorithms (ESMO-ESGO/NCCN)

- Stratify into Low/Intermediate/High-Intermediate/High Risk based on:
  - Clinical: age; co-morbidities; fertility
  - Pathological: FIGO stage; **tumour type**, grade, LVSI

# Current Classification of EC (WHO 2014): Morphology-based

- Endometrioid carcinoma and variants
- Mucinous carcinoma
- Serous endometrial intraepithelial carcinoma
- Serous carcinoma
- Clear cell carcinoma
- Carcinoid tumour
- Small cell neuroendocrine carcinoma
- Large cell neuroendocrine carcinoma
- Mixed cell adenocarcinoma
- Undifferentiated carcinoma; Dedifferentiated carcinoma

Type I vs Type II

# Current Risk Stratification

- LOW: G1/2 EEC, FIGO IA; no LVSI
- INTERMEDIATE: G1/2, FIGO IB, no LVSI
- HIGH-INTERMEDIATE: G1/2 with LVSI; G3 EEC IA
- HIGH: G3 EEC IB; all non-EEC, any stage, all stage II+

# Risk stratification: Current

- LOW: G1/2 EEC, FIGO IA; no LVSI
- INTERMEDIATE: G1/2, FIGO IB, no LVSI

---

- HIGH-INTERMEDIATE: G1/2 with LVSI; G3 EEC IA
- HIGH: G3 EEC IB; all non-EEC, any stage, all stage II+

# Problems

- Histotype diagnosis in EC shows high inter-observer variation (especially in high grade EC)

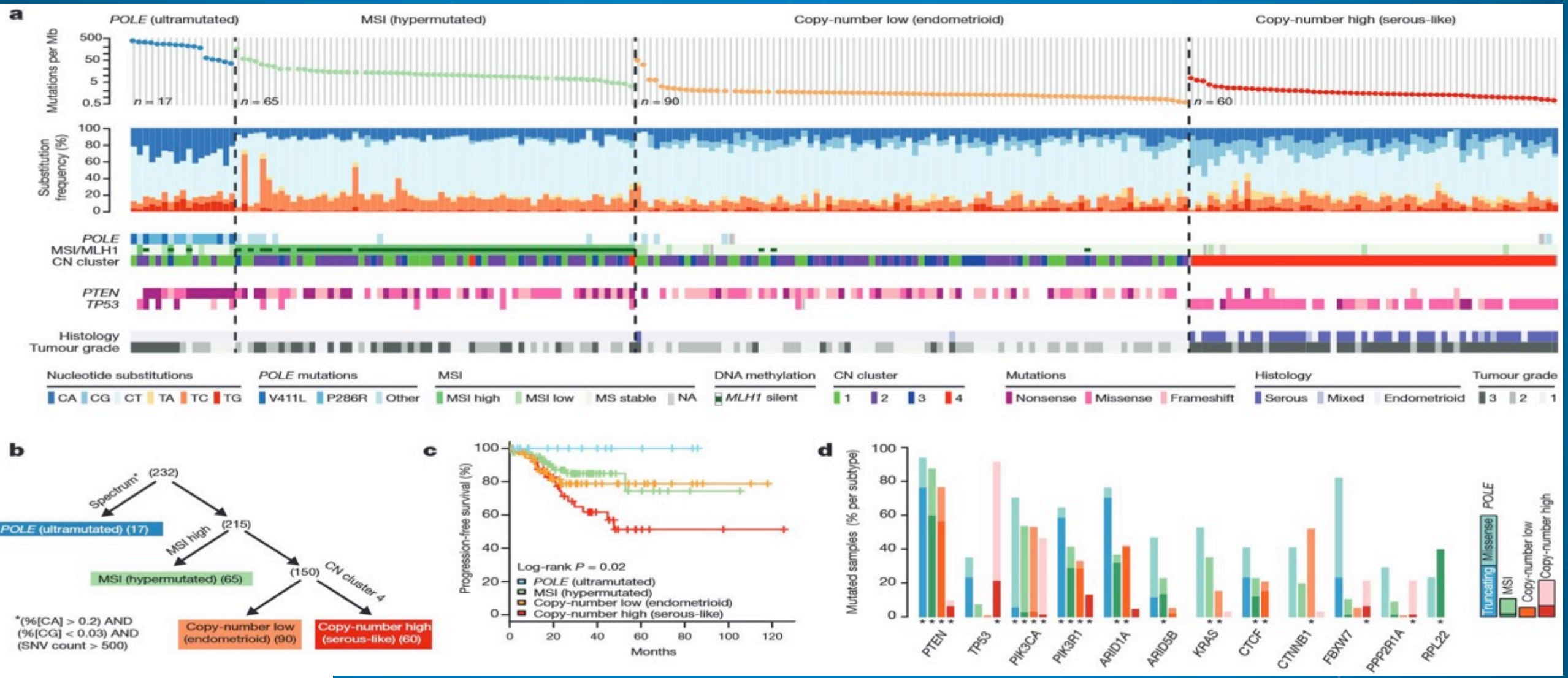
- Histotype diagnosis in EC does not consistently predict clinical outcome

**Prognostic separation of histotypes is therefore unreliable and inaccurate**

# Attributes of a meaningful histopathological diagnosis:

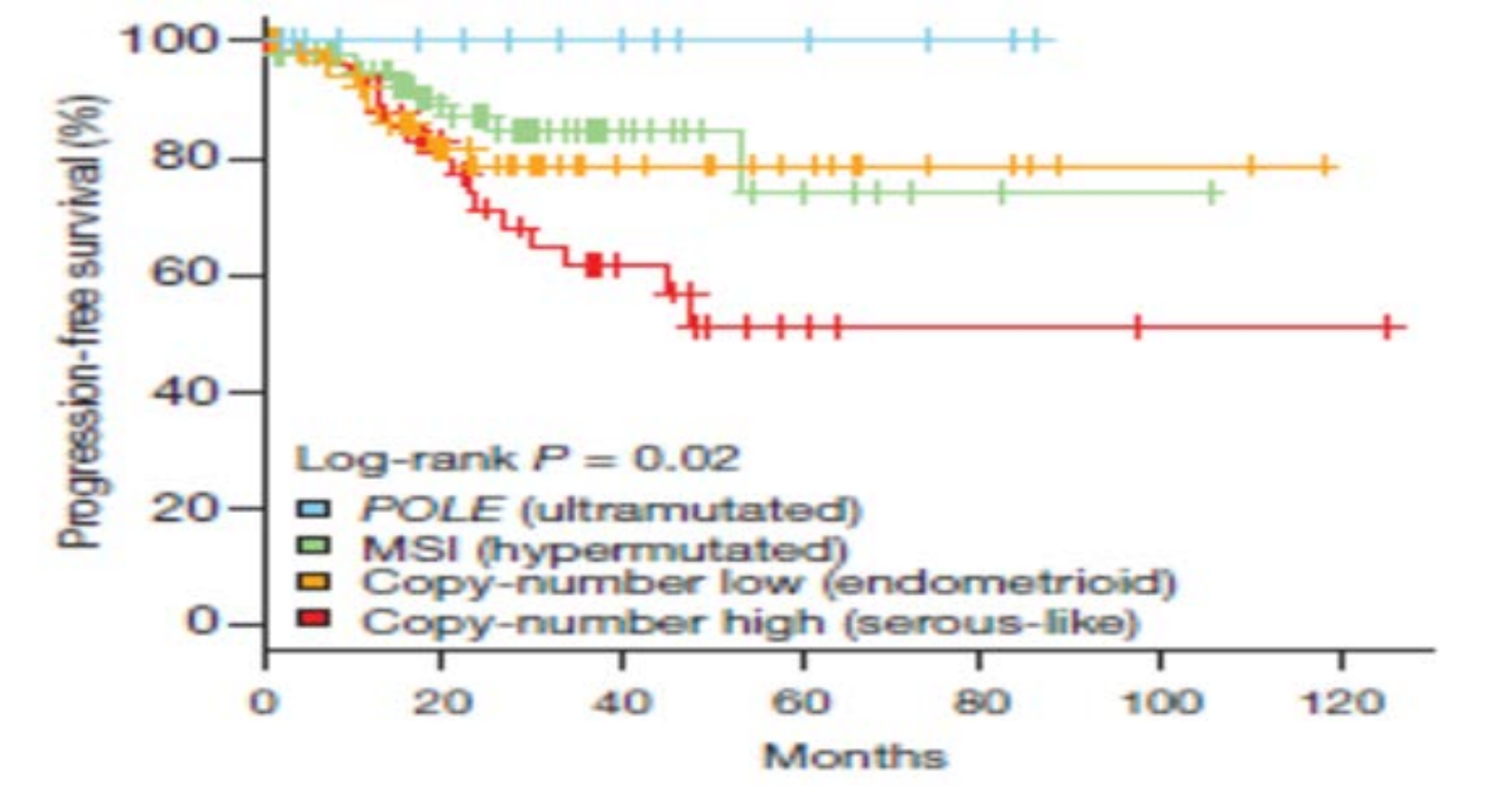
- Understandable by clinicians (and patients)
- Reproducible i.e. objective
- Clinically relevant
- Sensitive and specific

# The Cancer Genome Atlas (TCGA): Endometrial Carcinoma




Nature 2013; 497(7447): 67–73.

# Molecular Classification of EC

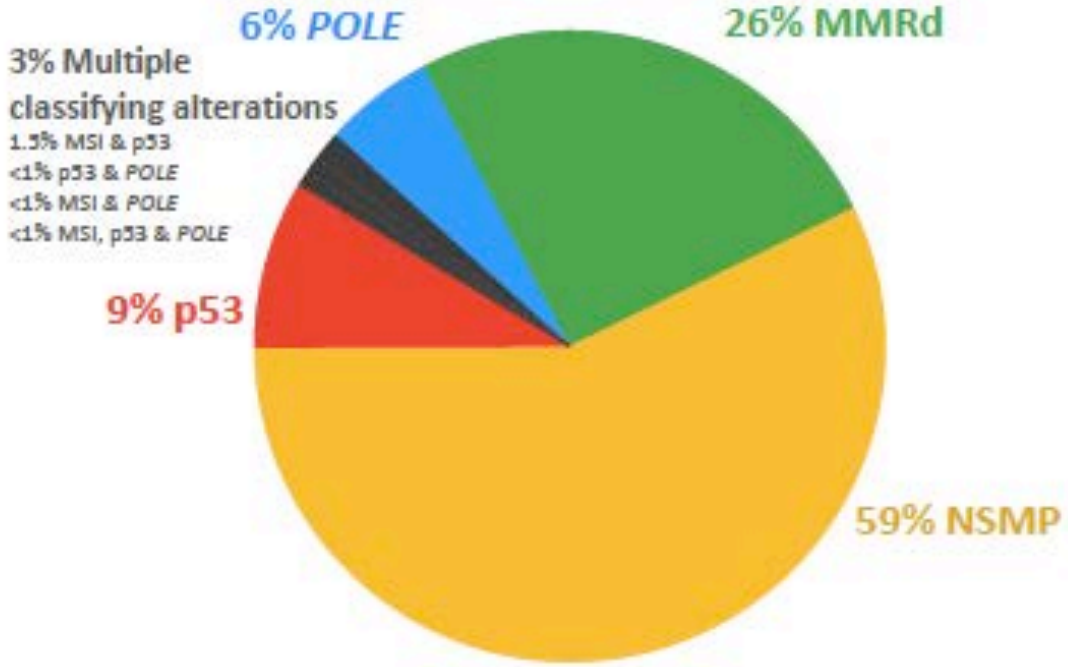



Molecular classification of EC has clear prognostic implications

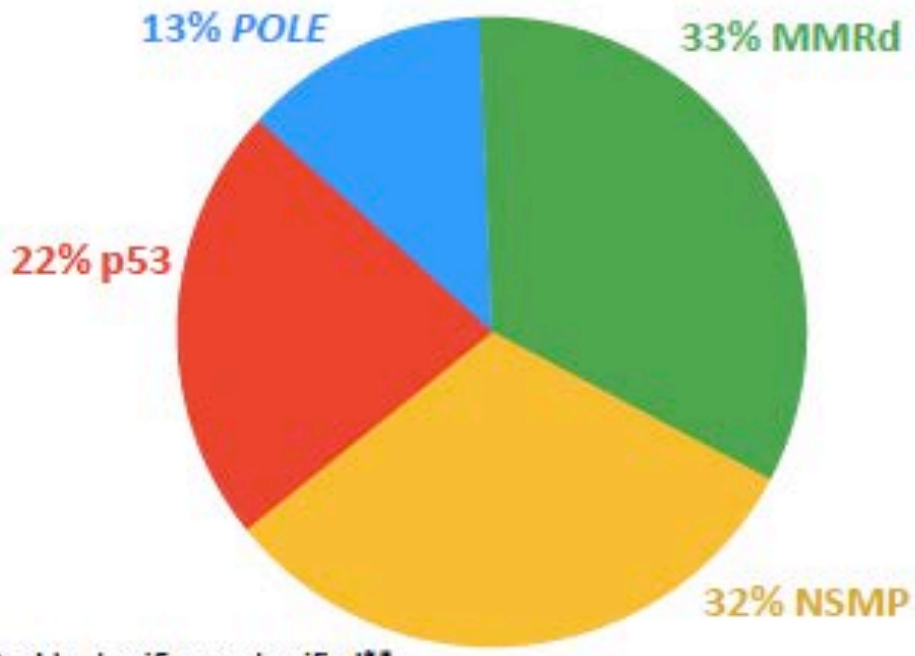
# PORTEC-3 molecular analysis

## Molecular subgroups

**PORTEC-1 and-2 HIR-EC trial cohorts N=861**




**PORTEC-3 HR-EC trial cohort N=410**



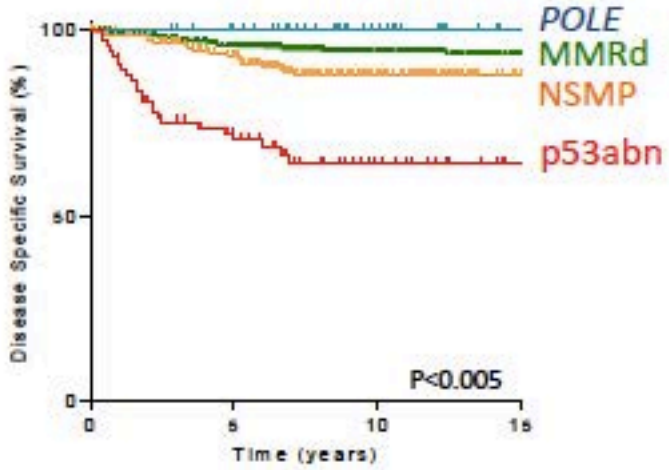

Double classifiers reclassified**

Stelloo et al, Clinical Cancer Research 2016; Leon-Castillo et al, ESMO 2019; **Leon-Castillo et al, J Pathol in press

# PORTEC-3 molecular analysis

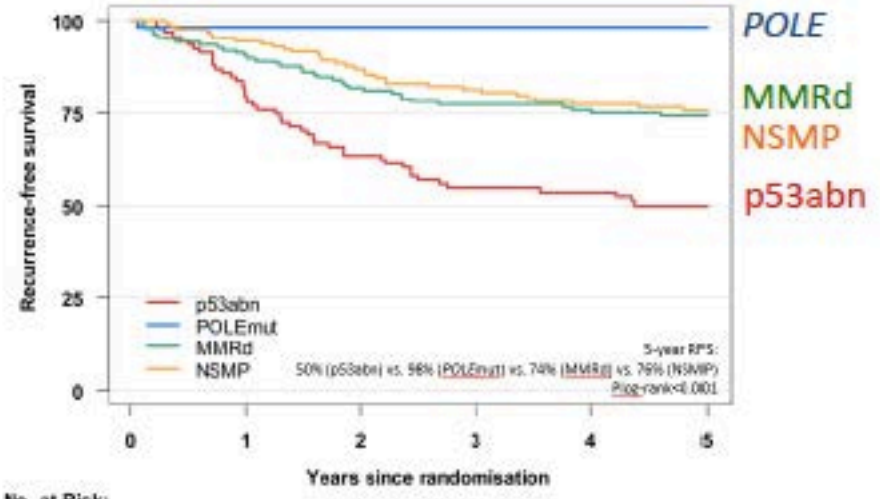
## Prognostic significance of TCGA groups

N=834 (PORTEC-1&2)




High-intermediate risk EC

N=410 (PORTEC-3)

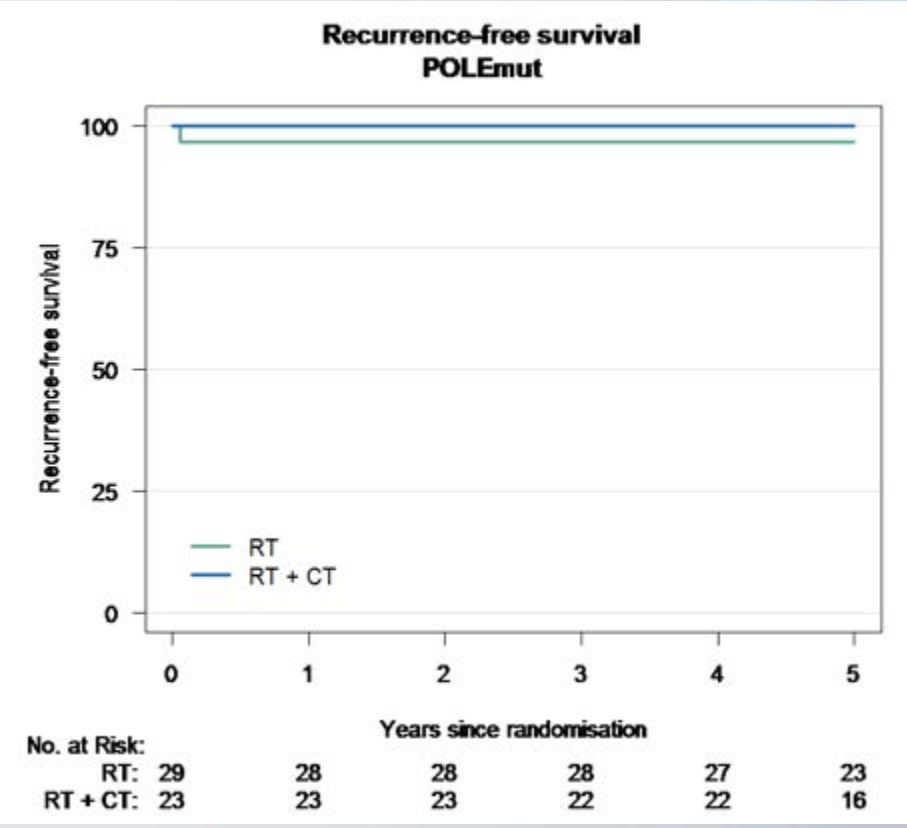



| No. at Risk: | | | | | | |
|---|---|---|---|---|---|---|
| p53abn: | 92 | 71 | 57 | 49 | 44 | 32 |
| POLEmut: | 52 | 51 | 51 | 50 | 49 | 39 |
| MMRd: | 137 | 124 | 112 | 102 | 96 | 73 |
| NSMP: | 129 | 122 | 112 | 104 | 93 | 68 |

Stelloo et al, Clinical Cancer Research 2016; Leon-Castillo et al, ESMO 2019

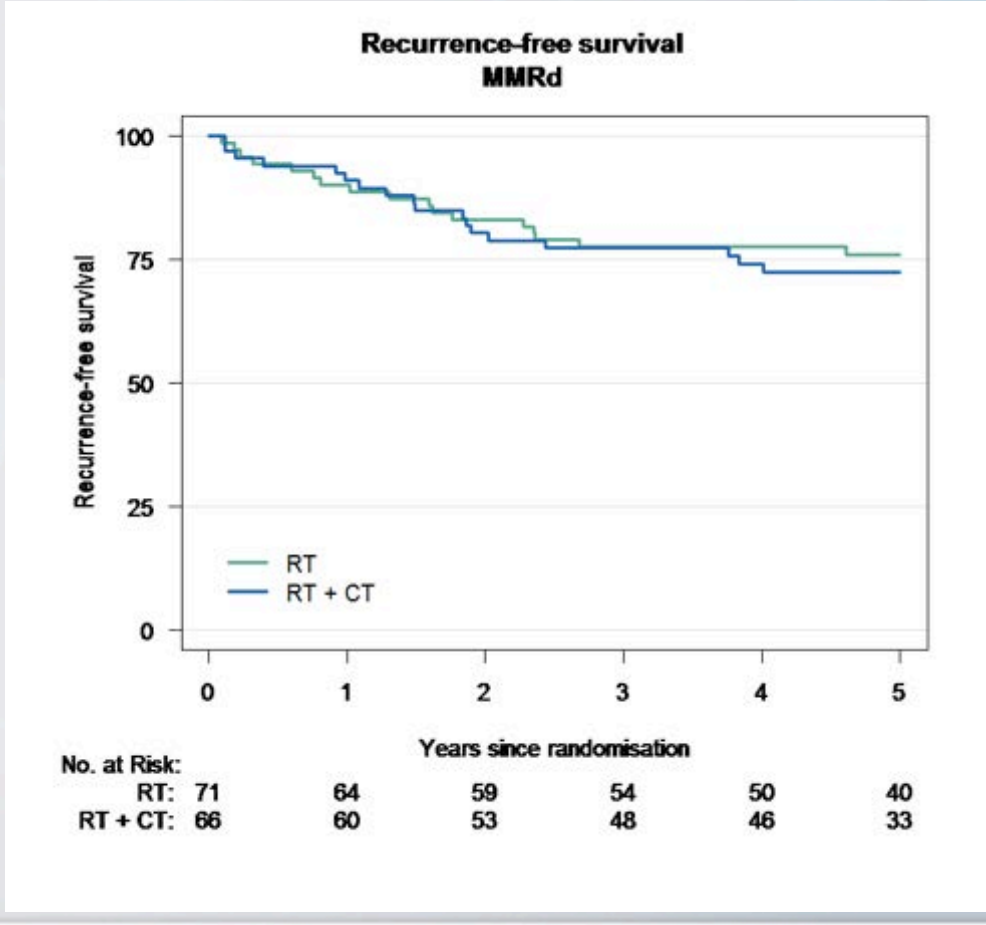
# POLEmut EC

- 10% of endometrioid EC
- Relatively young, low stage, high tumour grade, scattered tumour giant cells, prominent lymphocytic infiltrate
- High mutational burden (>100 mut/MB)
- **Classified as HIGH RISK by current algorithms**
- **EXCEPTIONALLY GOOD PROGNOSIS**
- Implications: Treatment de-escalation: No RT for low-stage; omit chemo for high stage



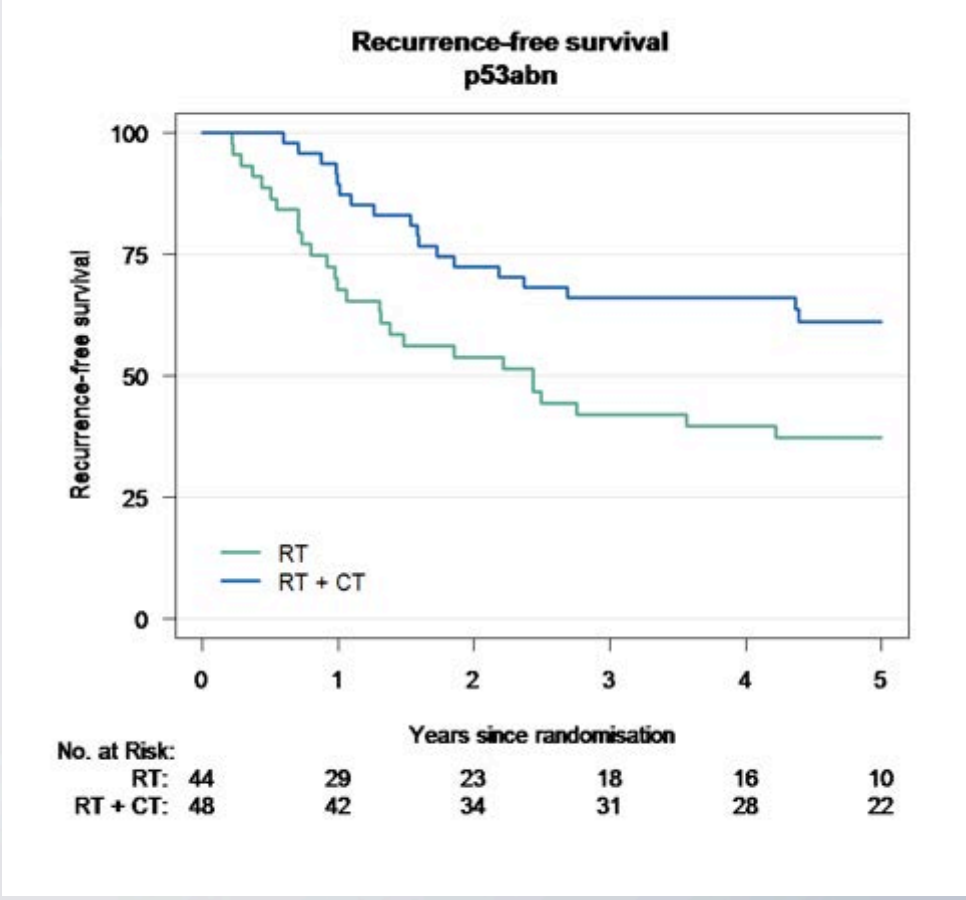
# MMRd EC

- 25-30% of EC
- Majority sporadic (MLH1 promoter methylation); about 3% LS
- Like *POLE*mut these are higher grade, endometrioid, with large numbers of TIL's
- Higher prevalence of substantial LVSI
- Good response to RT (including just VBT in absence of unfavourable risk factors); additional chemotherapy does NOT improve prognosis; immune checkpoint inhib Rx in recurrent cases



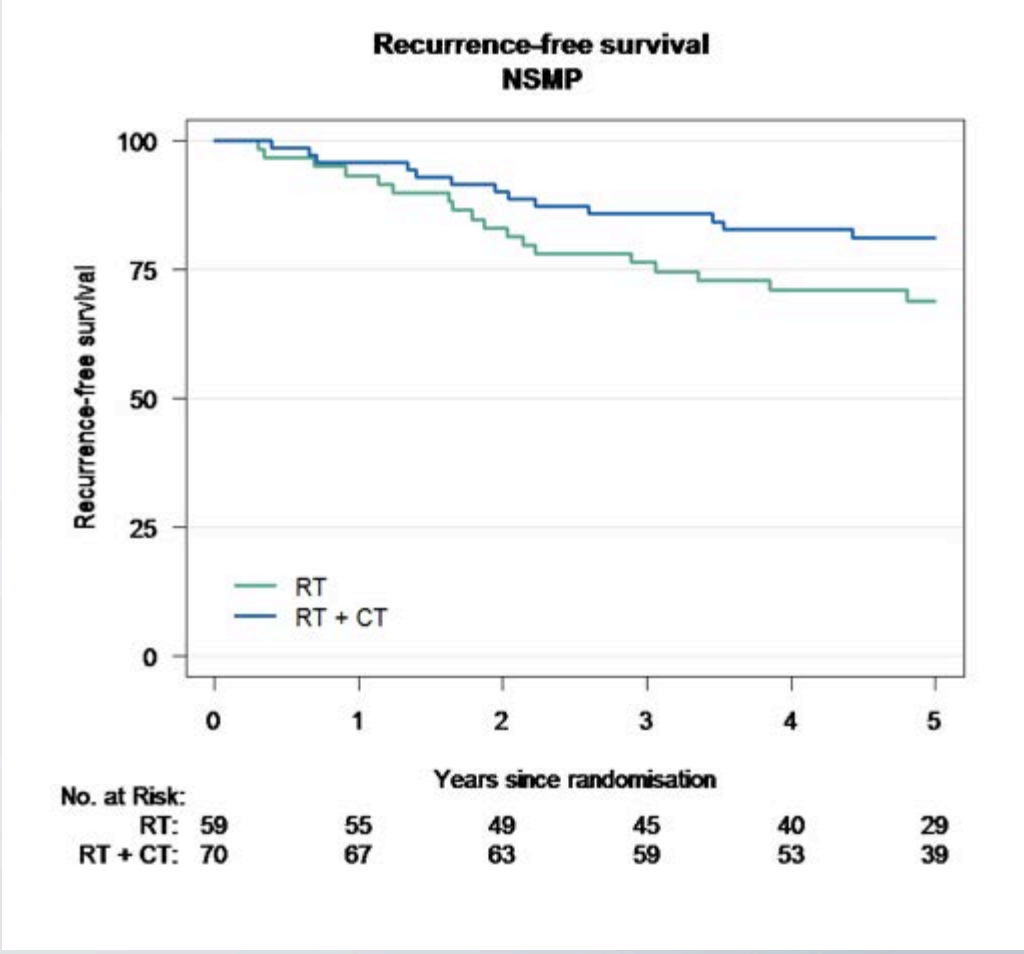
# p53abn (CNH/serous-like) EC

- Diagnosis is easy and reproducible once *POLE*mut and MMRd are excluded
- Significant improvement in survival with chemotherapy
- Targeting HER2 and HRD are being explored



# NSMP EC

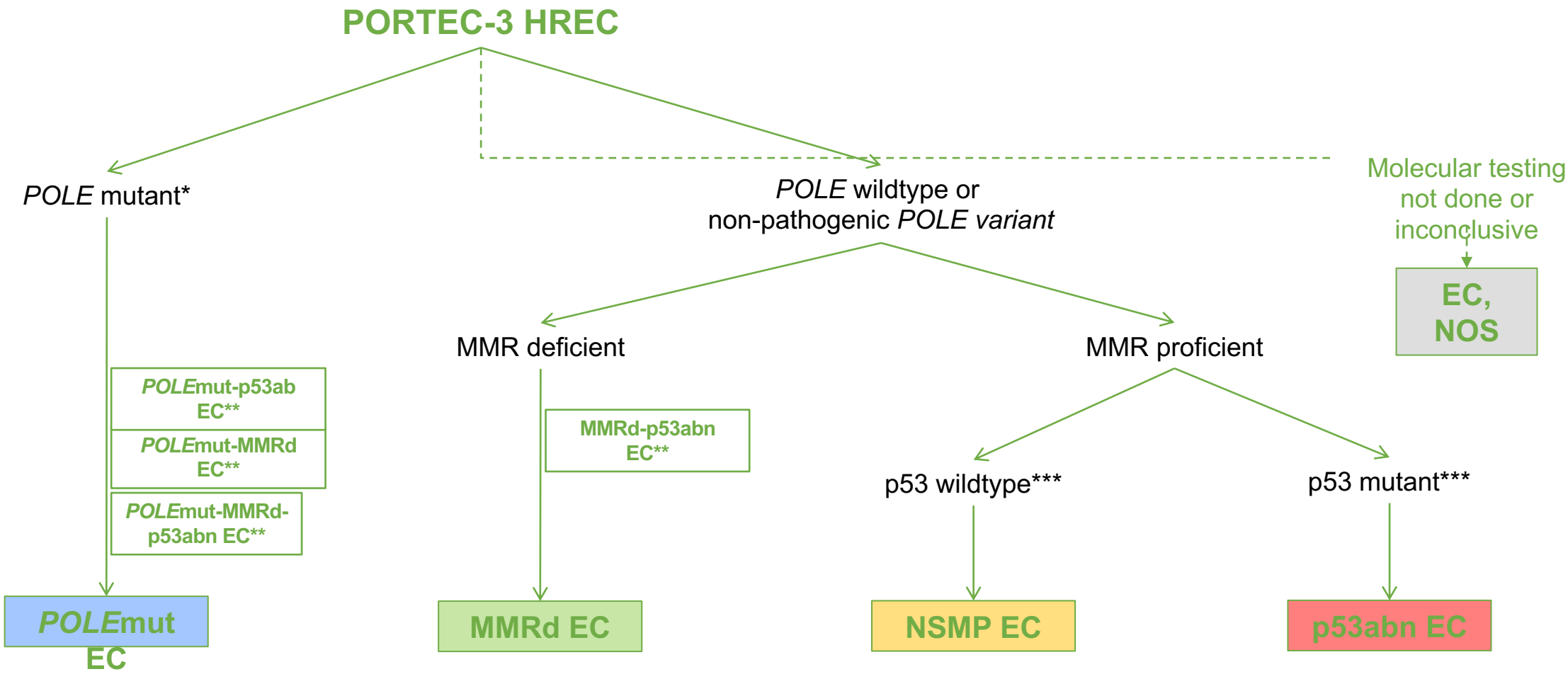
- Classic Type 1
- Oestrogen-driven
- Amenable to conservative treatment
- Stage-dependent prognosis
- Largest group; requires further prognostic sub-grouping (beta-catenin; L1CAM)



# Four Molecular Subtypes of EC

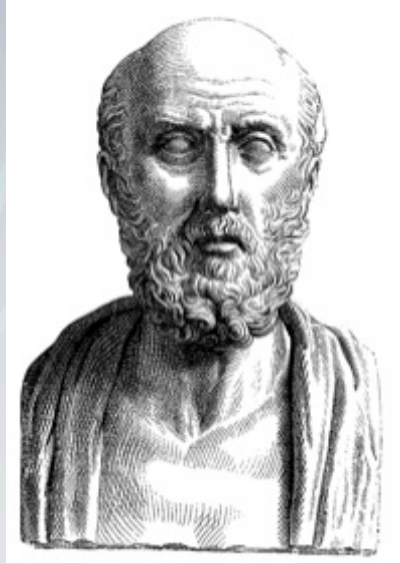
- Just like ovarian cancer histotypes these are essentially NON-OVERLAPPING
- About 3% of cases appear to fall into multiple groups
  - Not all *POLE* mutations are pathogenic
  - POLE, TP53 mutations and MMR defects can be secondary
- In order of frequency: MMRd+p53; POLE+p53; MMRd+POLE; MMRd+POLE+p53

# Endometrial Carcinoma Molecular classification



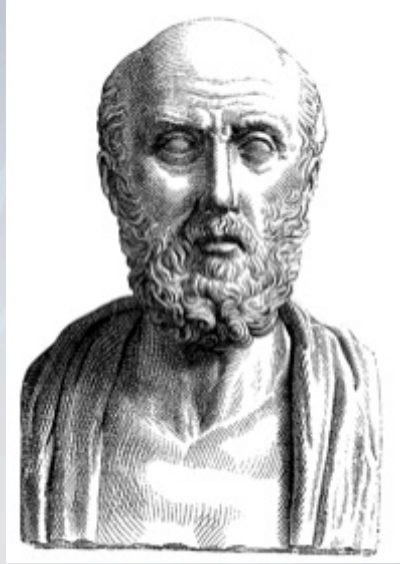

Adapted from Vermij et al, *Histopathology* 2020
*Pathogenic *POLE* exonuclease domain mutations (EDM) as per León-Castillo et al, *J Pathol* 2019
**León-Castillo et al, *J Pathol* 2019
****p53 IHC is as a excellent surrogate marker for mutational status (Singh et al, J Pathol 2019)*

# Four Molecular Subtypes of EC



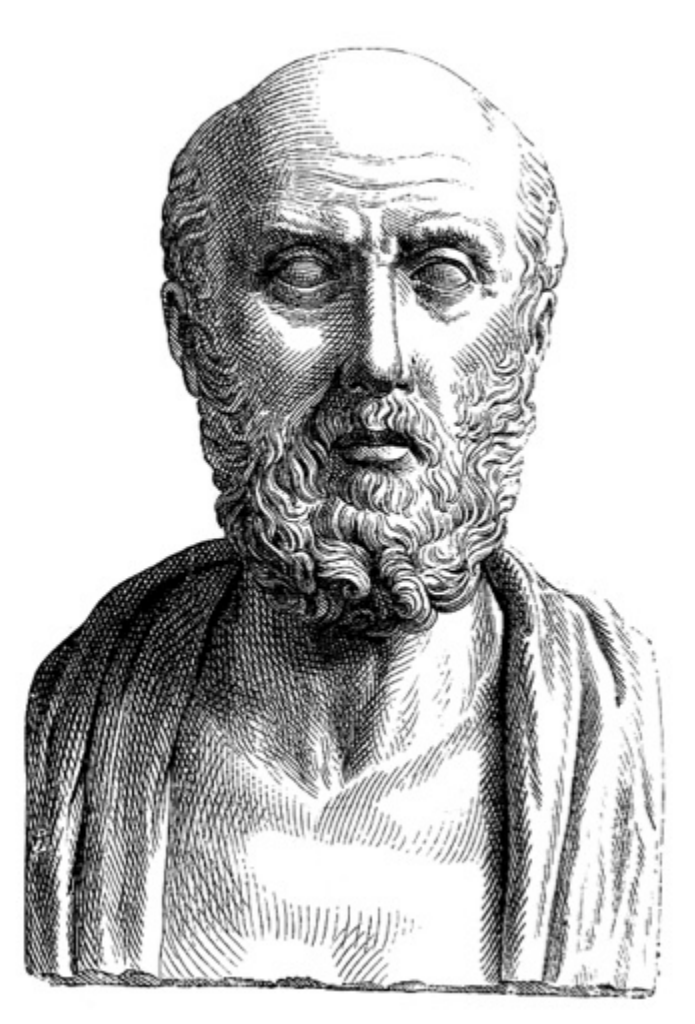
- Different clinical settings (e.g. age, BMI), reflecting differences in pathogenesis
- Different genetic risk factors/associations with hereditary cancer susceptibility syndromes
- Different precursor lesions (wrt morphology & latency)
- Different prognoses (with prognostic information independent of/additive to clinical risk stratification)
- Excellent inter-observer/inter-lab diagnostic reproducibility
- Can be diagnosed accurately based on biopsy (thus can be used for planning of definitive treatment)
- **Predictive of response to treatment (Pt-taxane CT, RT, immune, hormonal)**

# Molecular classification of EC



- By current classification:
  - 6/7 HIR EC patients receive unnecessary adjuvant RT
  - 7% EC patients suffer from potentially preventable recurrence/death
  - Only c20% HR EC (true ‘serous-like’) benefit from platinum-based chemotherapy

**MORPHOLOGY ALONE DOES NOT DISTINGUISH BETWEEN THESE CATEGORIES (POLEmut, MMRd and p53abn variably appear endometrioid/non-endometrioid)**



Hippocrates, the father of modern medicine

**If we are to:**

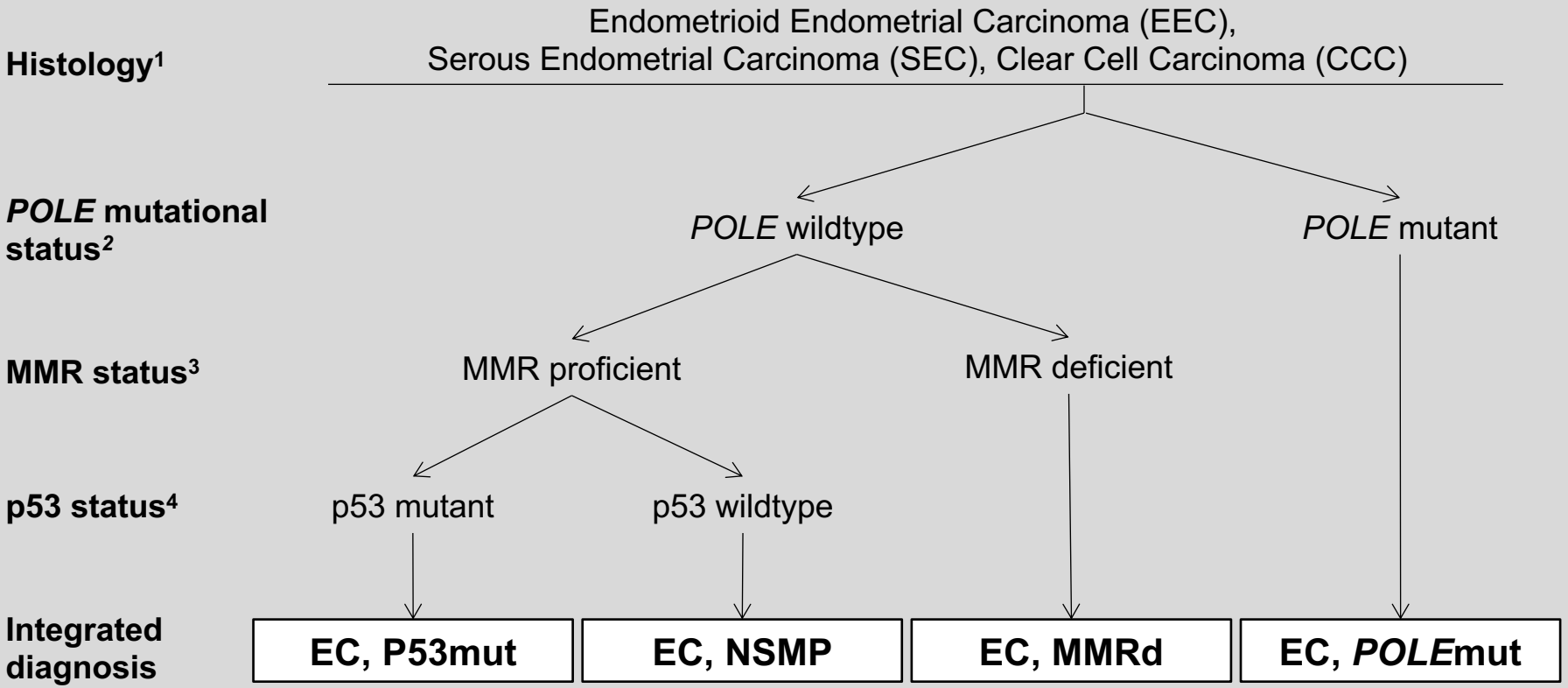
- **Apply our knowledge to the care of our patients**

**AND**

- **Do no harm**

# POLE and MMRd TESTING MUST BE INCORPORATED INTO ROUTINE DIAGNOSIS

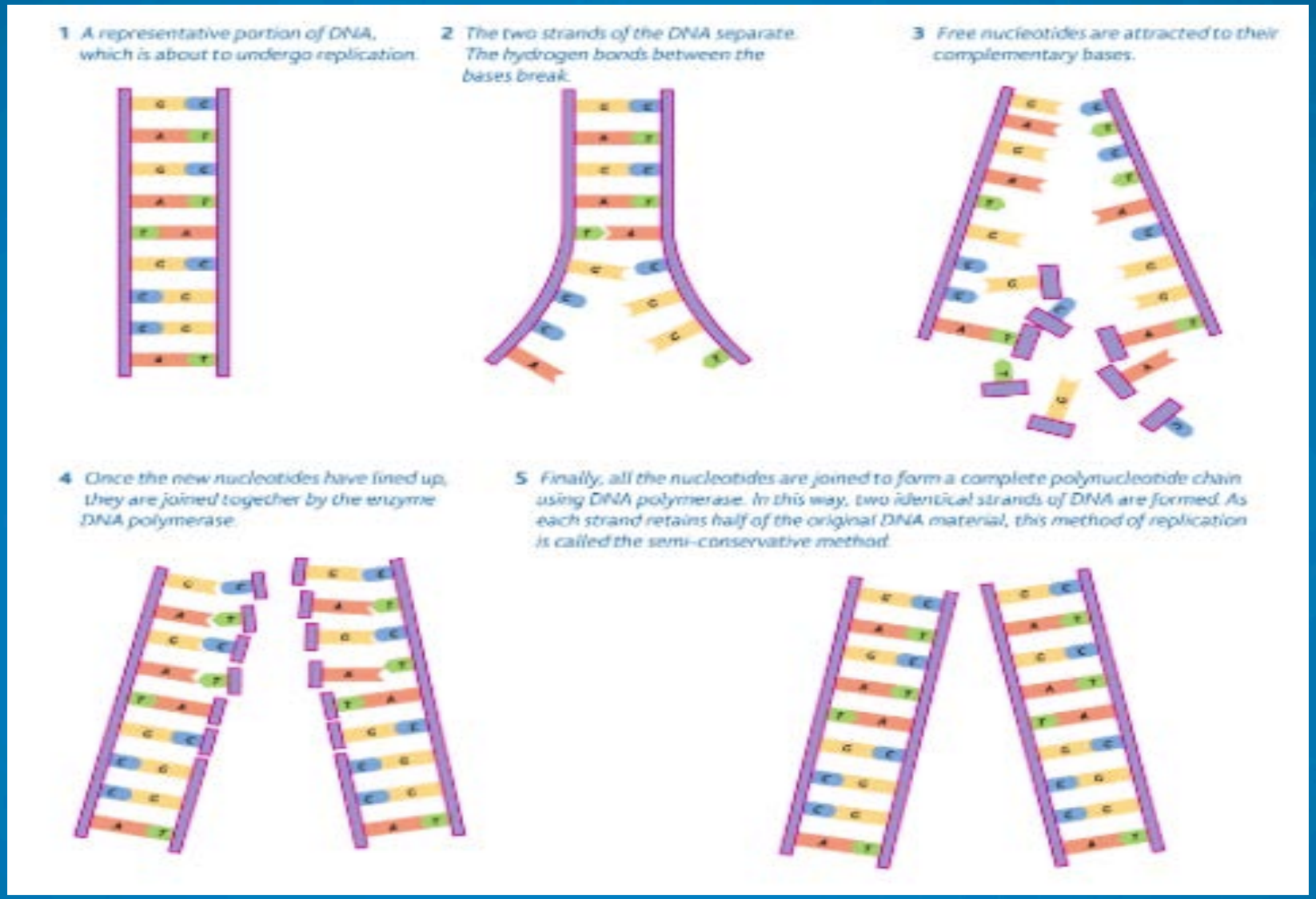
# PATHOLOGISTS MUST FACILITATE THIS CHANGE




*[1]This approach is particularly valuable in high-grade endometrial carcinomas*
*[2]POLE mutant includes the 5 pathogenic variants P286R, V411L, S297F, A456P, and S459F (Leon et al., Journal of Path 2019)*
*[3]MMR deficiency is defined by loss of one or more MMR-proteins (MLH1, PMS2, MSH2 and MSH6)*
*[4]P53 IHC is as a excellent surrogate marker for mutational status (Singh et al, Journal of Path 2019)*

## Integrated “histo-molecular” endometrial cancer classification

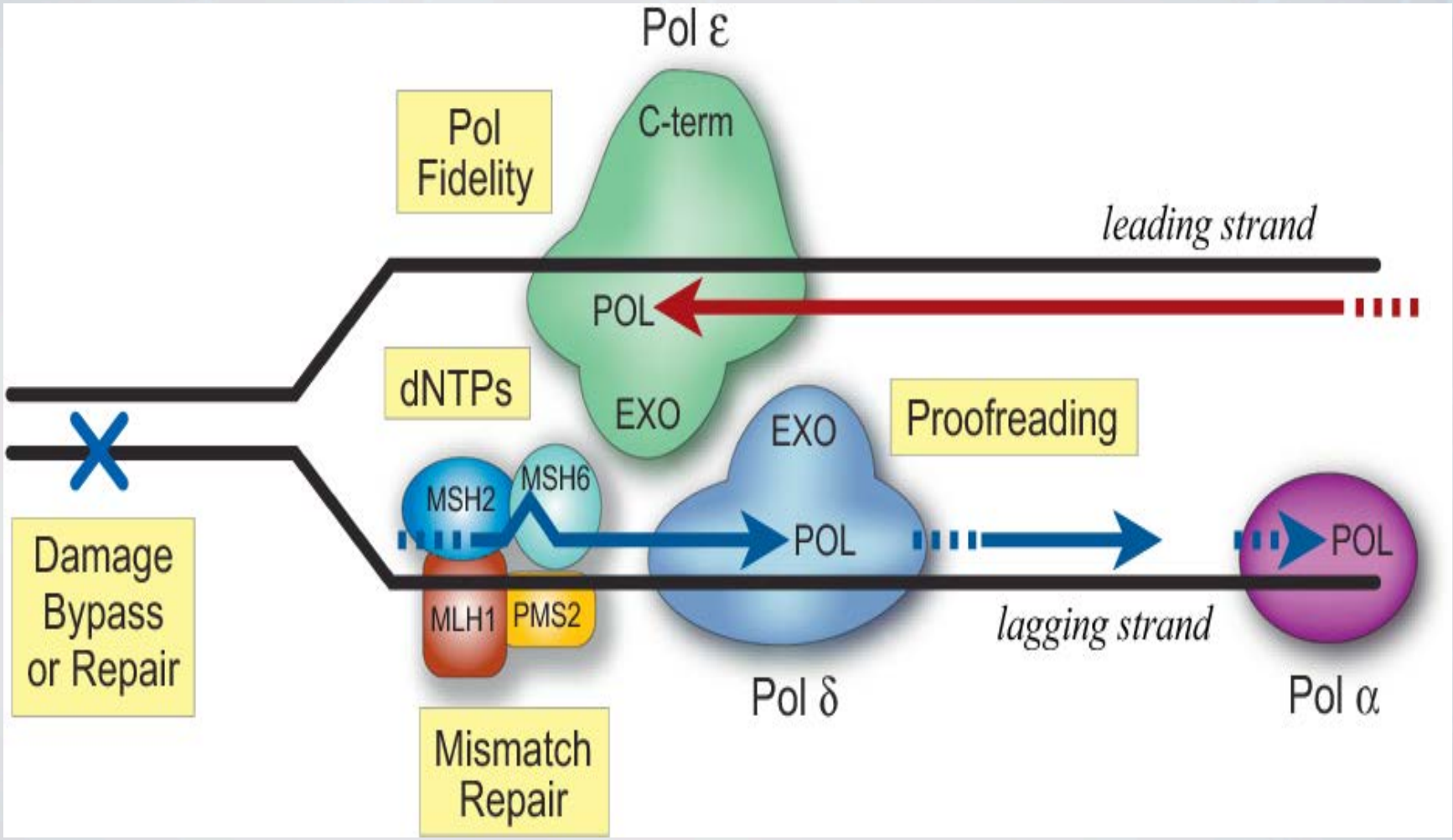
# What are POLE and MMR proteins?




BEFORE A CELL DIVIDES IT HAS TO REPLICATE ITS DNA

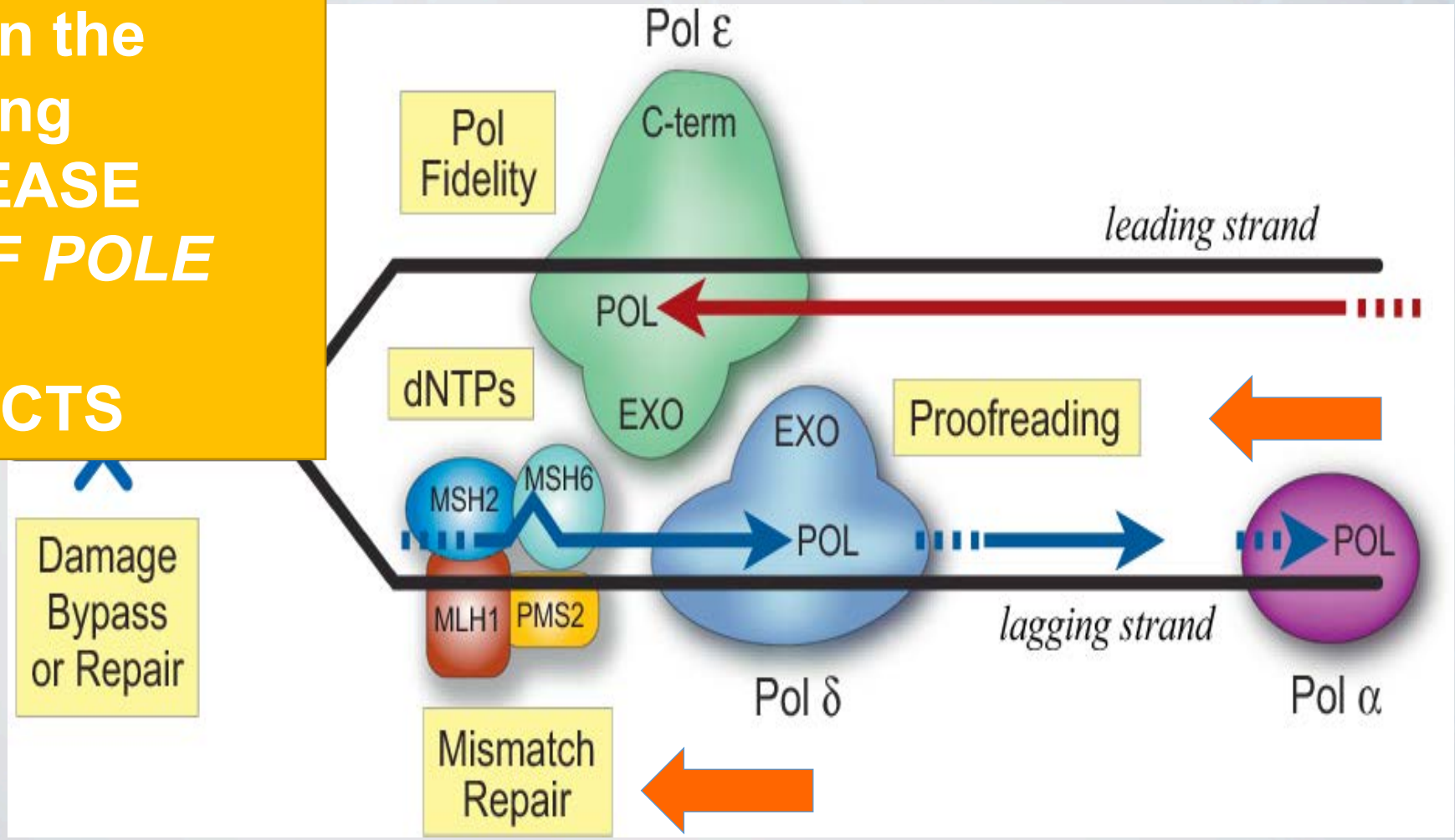
# 3 processes in series (ie one after the other) prevent replication errors

- Accurate nucleotide selection (POLYMERASE site of DNA polymerase delta and epsilon)
- Accurate proof-reading of the growing DNA strand (EXONUCLEASE domain of POLD and POLE)
- Detection and repair of incorrect base insertions by the mismatch repair system


Pol ε
Pol Fidelity
C-term
leading strand
POL
dNTPs
EXO
EXO
Proofreading
MSH2
MSH6
POL
POL
Damage Bypass or Repair
MLH1
PMS2
lagging strand
Pol δ
Pol α
Mismatch Repair

**The 2 most important mechanisms in EC are:**

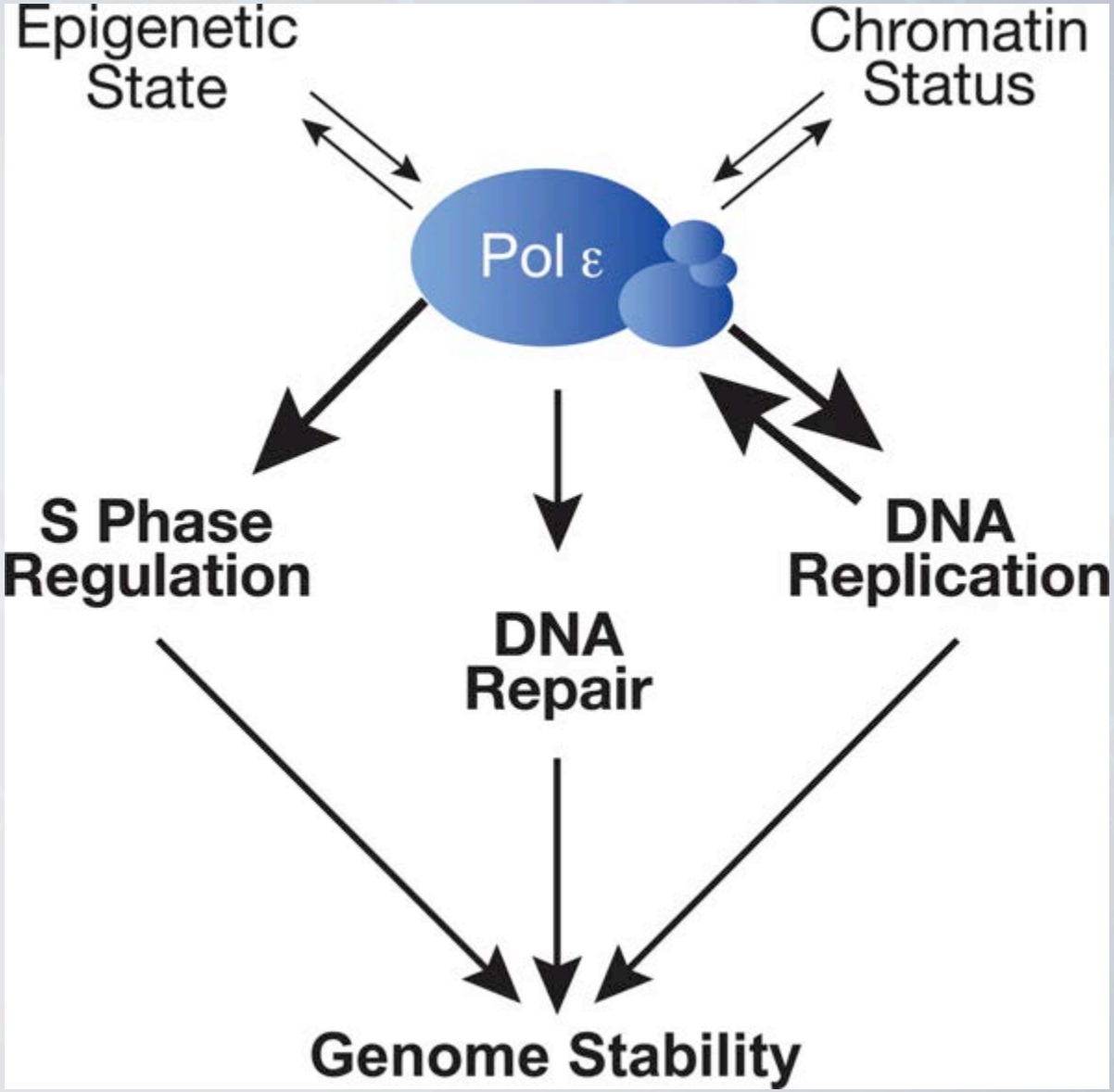
- **Mutations in the proof-reading EXONUCLEASE DOMAIN OF *POLE***
- **MMR DEFECTS**



# POLE testing in EC

- First: a few more points ….

1. POLE functions are more far-reaching than depicted – effect of EDM are therefore more than loss of proof-reading alone



# 2. POLE mutations occur in other cancers

-but the clinical effects vary

-POLEmut EC are unique

# 3. Effects of pathogenic *POLE* extranuclease domain mutations in EC

- Highest rate of mutations of any human cancer
- Excellent prognosis, 3 possible explanations:

  - ~~Better response to adjuvant treatment~~

  - Immunogenicity; neoantigens, immune infiltrates, expression of immune checkpoint molecules

  - Error catastrophe

# 3. Effects of pathogenic *POLE* extranuclease domain mutations in EC

- Highest rate of mutations of any human cancer
- Excellent prognosis:
  - ~~Better response to adjuvant treatment~~; ***no need for adjuvant treatment, ie conventional RT/CTRT***

  —
  - Immunogenicity; neoantigens, immune infiltrates, expression of immune checkpoint molecules: ***specific therapies: immune checkpoint inhibitors***
  - Error catastrophe: ***specific therapies: nucleoside analogs***

# POLEmut EC

- 10% of endometrioid EC
- Majority are due to mutations in one of 5 hotspots; a few others now characterised
- Relatively young, low stage, high tumour grade, scattered tumour giant cells, prominent lymphocytic infiltrate
- High mutational burden (>100 mut/MB)
- **Classified as HIGH RISK by current algorithms**
- **EXCEPTIONALLY GOOD PROGNOSIS**
- Implications: Treatment de-escalation: No RT for low-stage; omit chemo for high stage

# *POLE* Testing in Clinical Practice

- NO IMMUNOHISTOCHEMICAL SURROGATE
- NOT ALL VARIANTS ARE PATHOGENIC
- GUIDELINES ON INTERPRETATION ONLY RECENTLY PUT FORWARD

# *POLE* Testing in Clinical Practice

- Sequencing of POLE ‘hotspots’: Exons 9,13 and 14 OR exons 9-14
- Sanger vs NGS depending on throughput/access
- Role of pathologist:
  - Identify suitable tissue: whole section/marked area
  - Indicate tumour nuclear content accurately
  - Provide as sections mounted on slides or rolls or cores

# *POLE* Testing in Clinical Practice: Pathogenic Variants

| PROTEIN CHANGE | NUCLEOTIDE SUBSTITUTION |
|---|---|
| **P286R** | **c.857C>G** |
| **V411L** | **c.1231G>T/C** |
| **S297F** | **c.890C>T** |
| **S459F** | **c.1376C>T** |
| **A456P** | **c.1366G>C** |
| F367S | c.1100T>C |
| L424I | c.1270C>A |
| M295R | c.884T>G |
| P436R | c.1307C>G |
| M444K | c.1331T>A |
| D368Y | c.1102G>T |

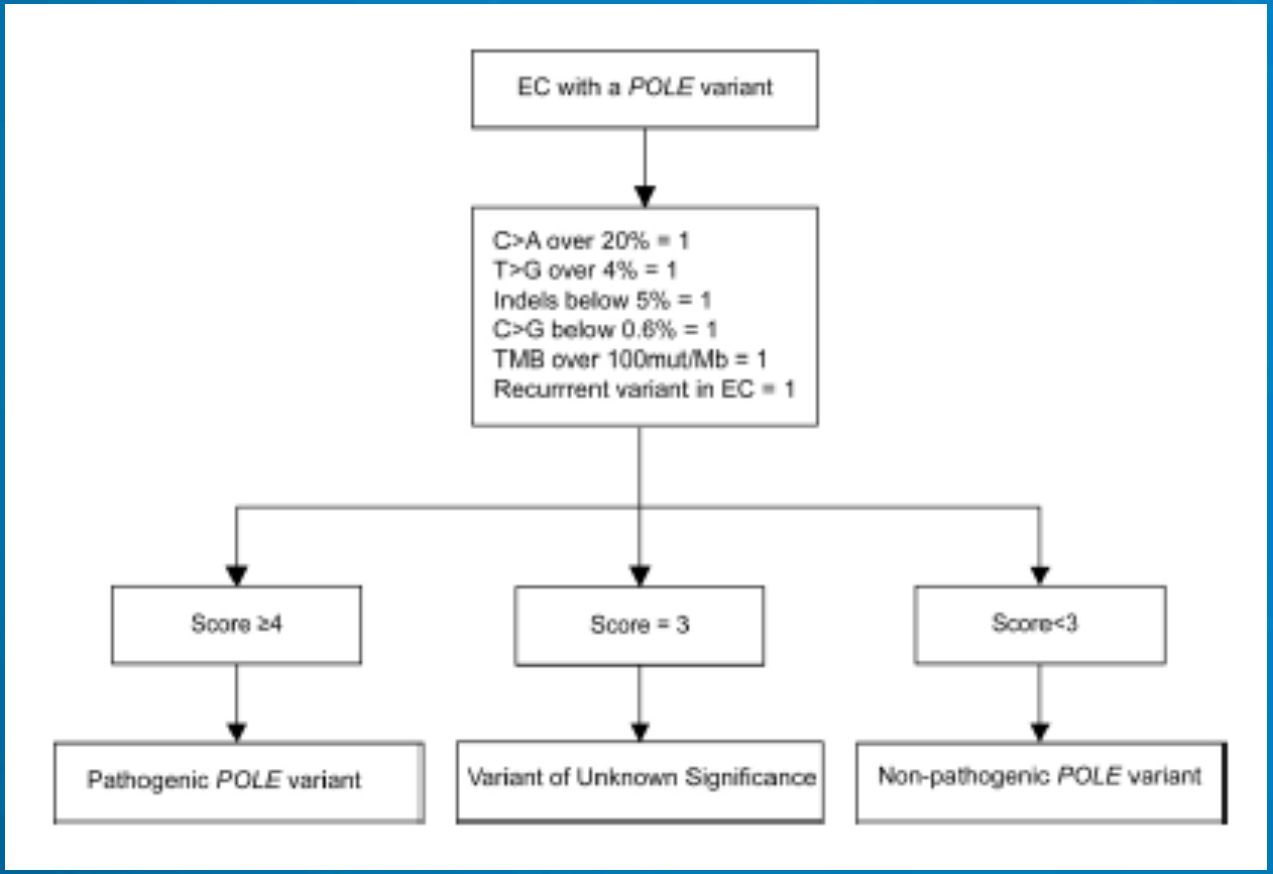
- **EC with of any of these 11 variants should be classified as *POLE*mut**
- **About 2/3rds of all *POLE*mut EC show either P286R or V411L**
- **About 80% *POLE*mut EC show 1 of the top 5 variants (in bold)**

Léon-Castillo et al.
Interpretation of somatic *POLE* mutations in endometrial carcinoma.
J Pathol 2019.
doi:10.1002/path.5372

# *POLE* Testing in Clinical Practice: Non pathogenic variants and VUS

- *POLE* mutations have been reported in 7.7% EC in cohorts other than TCGA
- <10% of these remain to be categorised as pathogenic or not (0.7% of all non-TCGA EC till now)
- Important not to misclassify

# *POLE* Testing in Clinical Practice: Non pathogenic variants and VUS




Léon-Castillo et al.
Interpretation of somatic *POLE* mutations in endometrial carcinoma.
J Pathol 2019.
doi:10.1002/path.5372

**Table 6.** Recommendations for the interpretation of somatic *POLE* mutations in EC. Recommendations to classify EC with *POLE* mutations with (A) POLE-score available, or (B) POLE-score absent.

A

| *POLE* mutation | Predicted pathogenicity | MSI/MMR status | Treatment recommendation |
|---|---|---|---|
| Exonuclease domain mutation | Pathogenic | MSS/MMRp | *POLE*mut EC |
| POLE-score ≥4 | Pathogenic | MSI/MMRd | *POLE*mut EC[1] |
| Exonuclease domain mutation | Non-pathogenic | MSS/MMRp | *POLE*wt EC |
| POLE-score <4 | Non-pathogenic | MSI/MMRd | MMRd EC |
| Non-exonuclease domain mutation | – | MSS/MMRp | NSMP/p53abn EC[2] |
| | – | MSI/MMRd | MMRd EC |

If tumours-only sequencing is performed, detection of L424V variant should prompt consideration of germline testing [37, 38]

B

| *POLE* mutation | Predicted pathogenicity | MSI/MMR status | Treatment recommendation |
|---|---|---|---|
| Exonuclease domain mutation predicted to be pathogenic by ≥4 *in silico* prediction tools | VUS | MSS/MMRp | WES or NSMP/p53abn EC[2,3] |
| | VUS | MSI/MMRd | WES or MMRd EC[3] |
| Exonuclease domain mutation predicted to be NON-pathogenic by >1 *in silico* prediction tool | Non-pathogenic | MSS/MMRp | NSMP/p53abn EC[2] |
| | Non-pathogenic | MSI/MMRd | MMRd EC |
| Non-exonuclease domain mutation | – | MSS/MMRp | NSMP/p53abn EC[2] |
| | – | MSI/MMRd | MMRd EC |

If tumours-only sequencing is performed, detection of L424V variant should prompt consideration of germline testing [39, 40]

1 Treat as *POLE*mut EC (based on genomic alteration) independently of MMR status (insufficient data to suggest otherwise)
2 p53-IHC should be performed to exclude a p53abn EC
3 Treat conservatively i.e. as MMRd/NSMP or send for WES

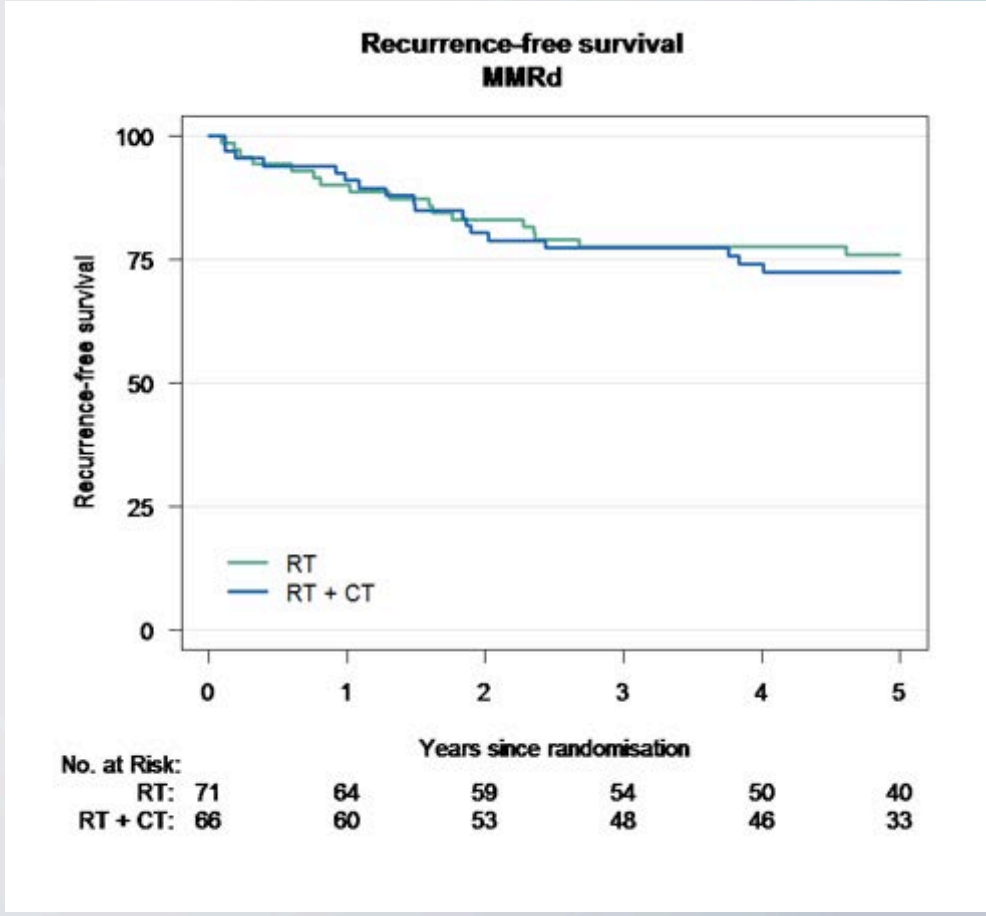
VUS: Variant of Unknown Significance. NSMP: No Specific Molecular Profile.

Léon-Castillo et al.
Interpretation of somatic *POLE* mutations in endometrial carcinoma.
J Pathol 2019.
doi:10.1002/path.5372

# MMRd Testing in EC

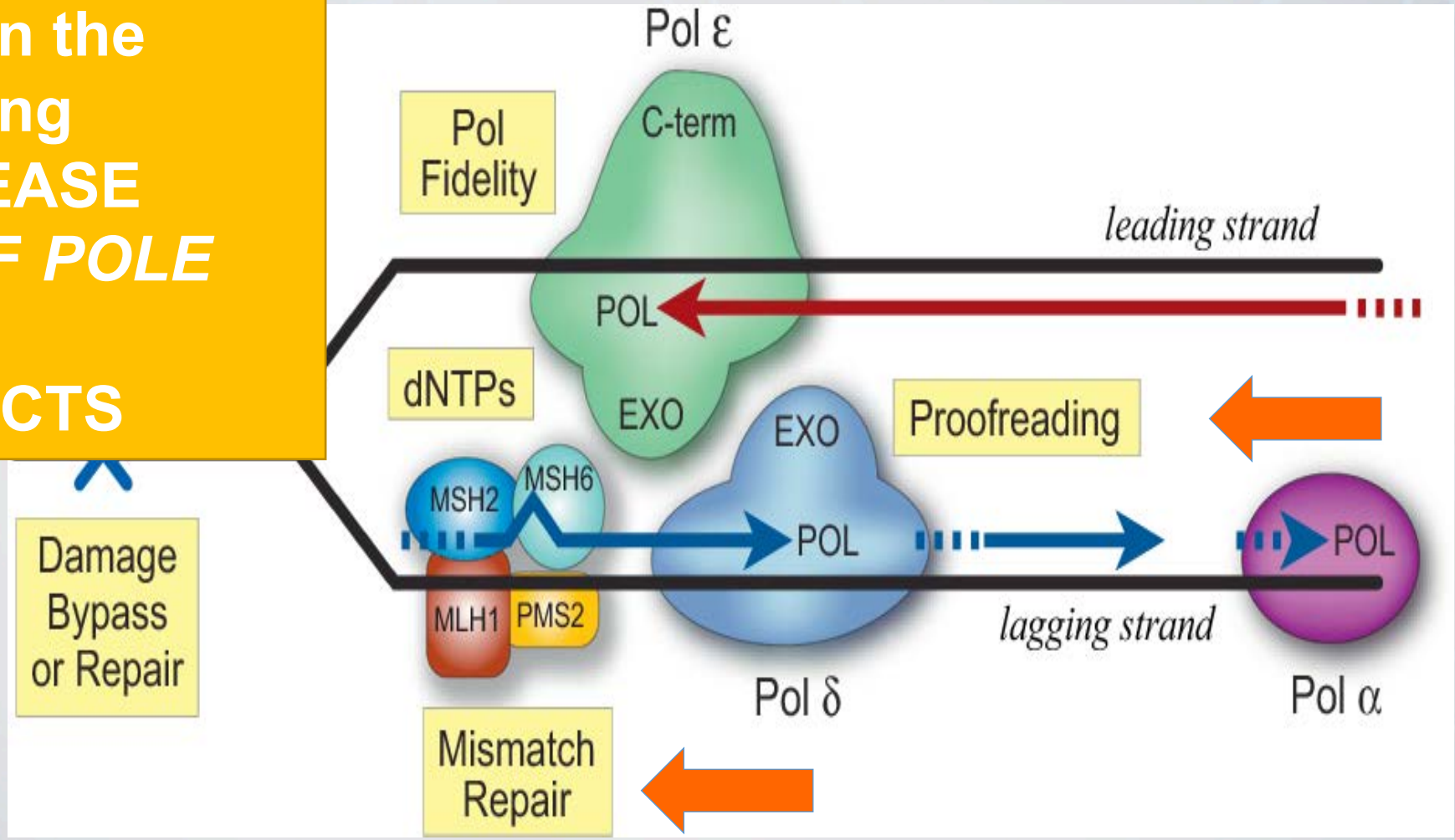
# MMRd EC

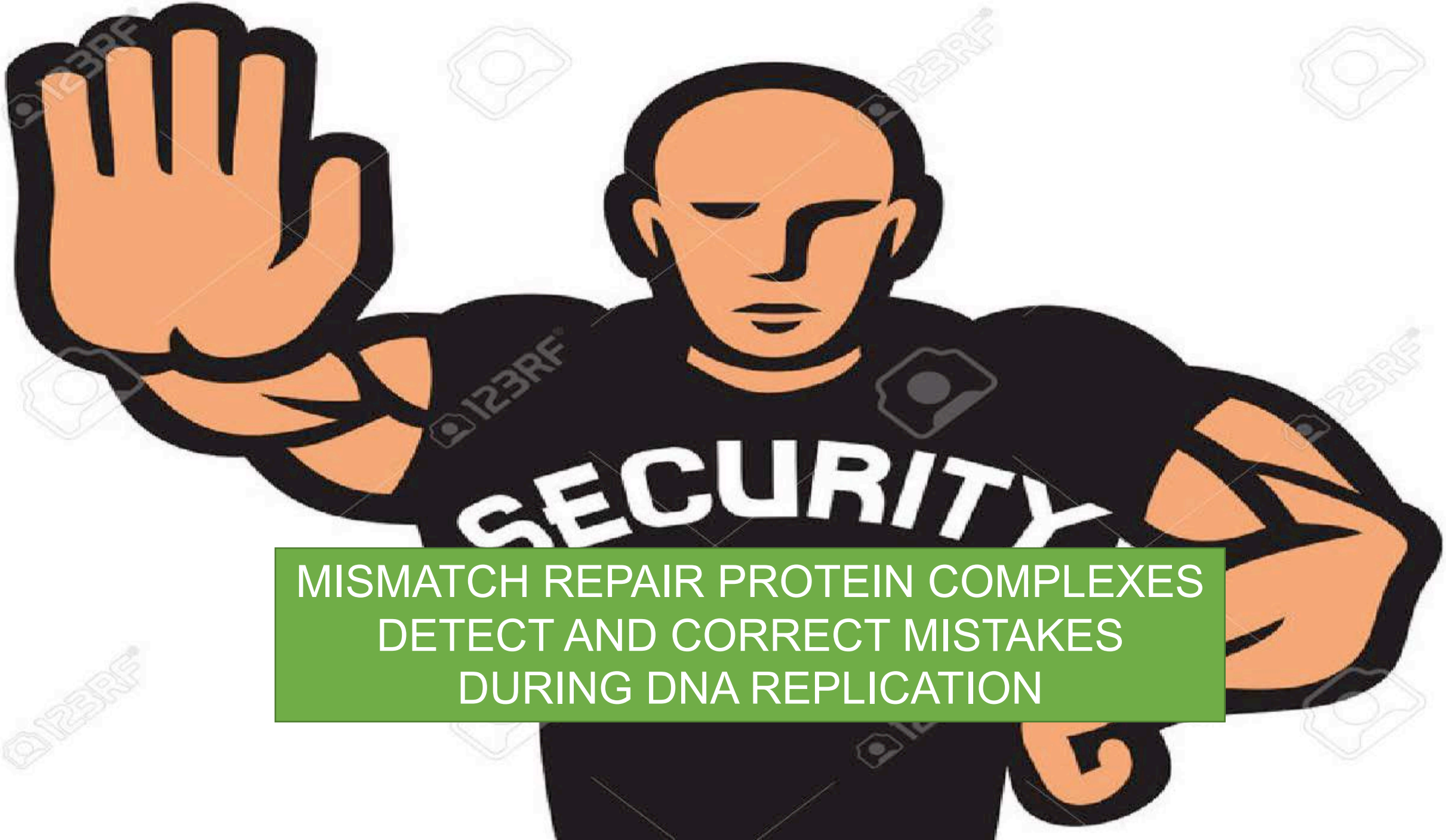
- 25-30% of EC
- Majority sporadic (MLH1 promoter methylation); about 3% LS
- Like *POLE*mut these are higher grade, endometrioid, with large numbers of TIL's
- Higher prevalence of substantial LVSI
- Good response to RT (including just VBT in absence of unfavourable risk factors); additional chemotherapy does NOT improve prognosis; immune checkpoint inhib Rx in recurrent cases



**The 2 most important mechanisms in EC are:**

- **Mutations in the proof-reading EXONUCLEASE DOMAIN OF *POLE***
- **MMR DEFECTS**






MISMATCH REPAIR PROTEIN COMPLEXES DETECT AND CORRECT MISTAKES DURING DNA REPLICATION


MLH1


PMS2

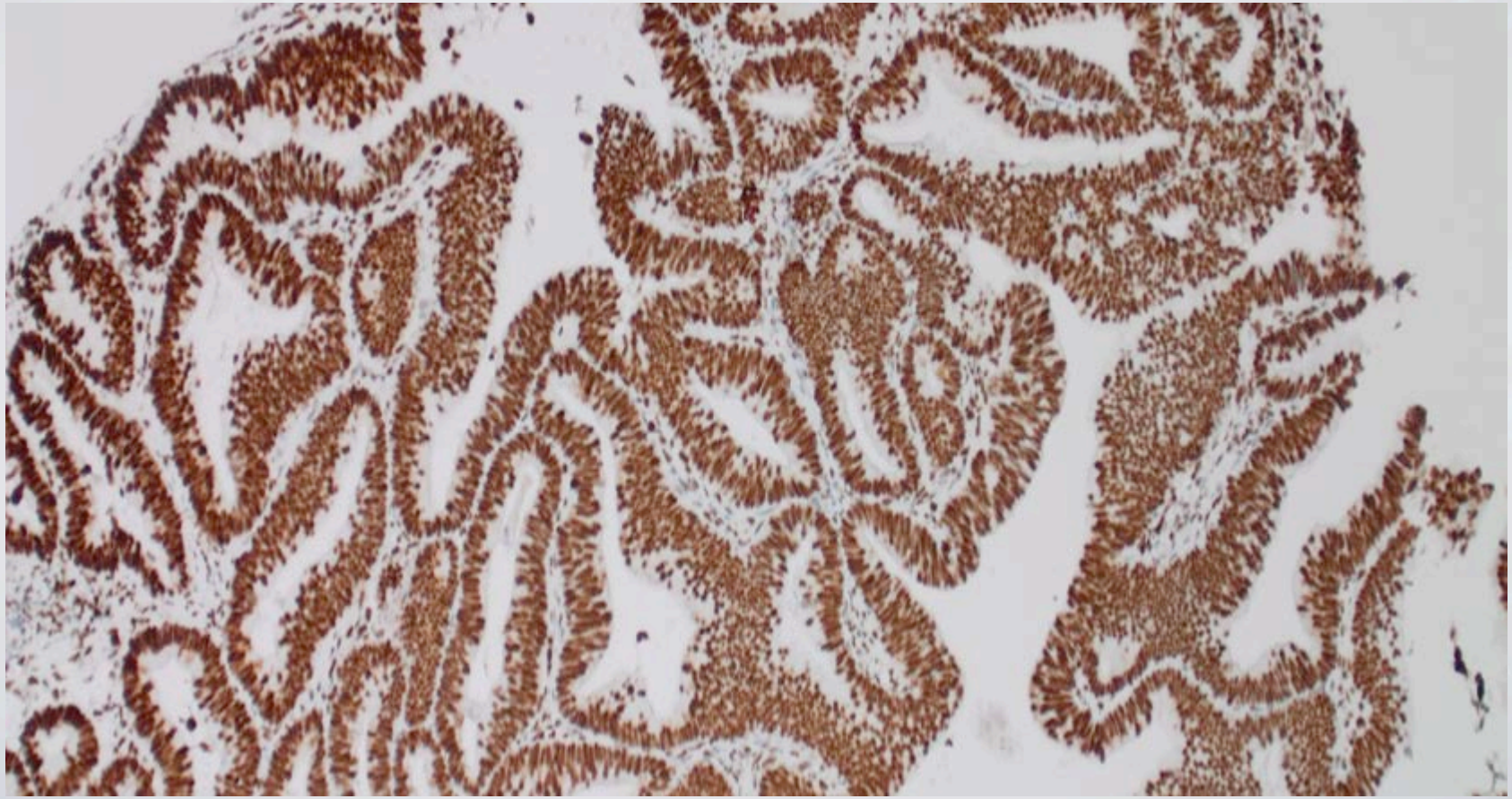

MSH2


MSH6

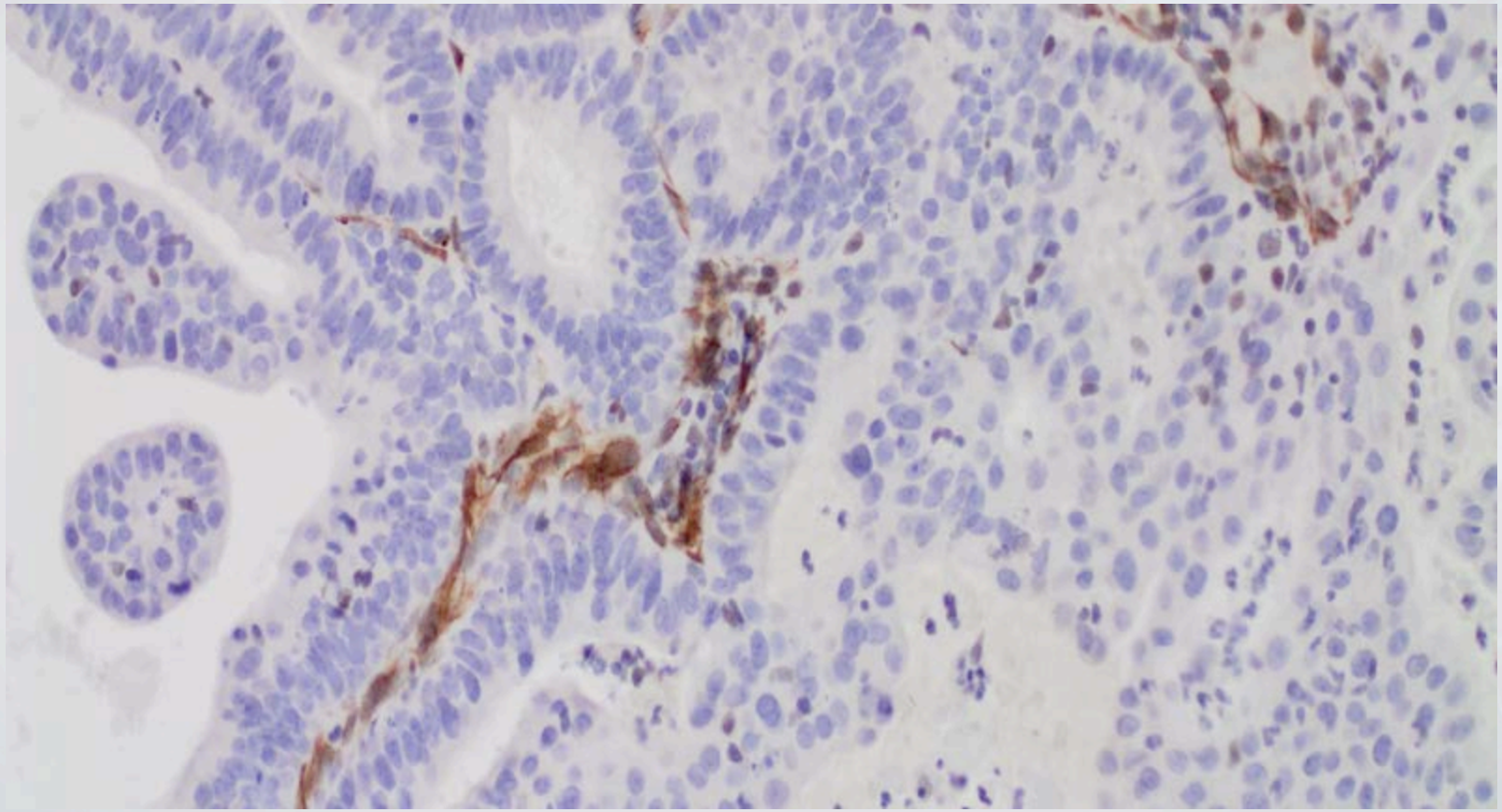
ABSENCE/LOSS OF FUNCTION OF ONE OF THESE MMR PROTEINS = MISMATCH REPAIR DEFECT (MMRd)

WE CAN DETECT THIS SIMPLY BY LOOKING FOR ABSENCE/PRESENCE OF THESE PROTEINS IN THE CELL (by immunohistochemistry, IHC)

# MMR IHC: Normal expression

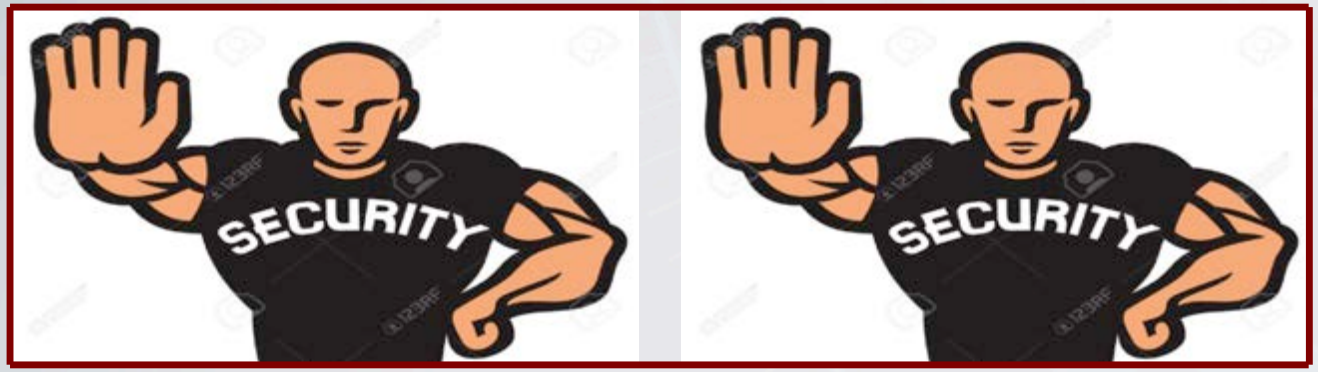


# MMR IHC: Loss of expression



# Problems with MMR IHC: 2 markers or 4?

# THE 4 MAJOR MMR PROTEINS EXIST IN STABLE FORM AS PAIRS (HETERODIMERS)




MLH1 + PMS2


MSH2 + MSH6

# THE 4 MAJOR MMR PROTEINS ARE NOT EQUALS IN REGARD TO STABILITY


PMS2

MLH1

IF MLH1 IS ABSENT, PMS2 IS ALWAYS ABSENT


MSH6

MSH2

IF MSH2 IS ABSENT, MSH6 IS ALWAYS ABSENT

# THE 4 MAJOR MMR PROTEINS ARE NOT EQUALS IN REGARD TO STABILITY


PMS2

MLH1


MSH6

MSH6

IF MLH1 IS ABSENT, PMS2 IS ALWAYS ABSENT

PMS2 AND MSH 6 CAN BE ABSENT WITHOUT AFFECTING THE EXPRESSION OF THE OTHER PROTEIN IN THE PAIR

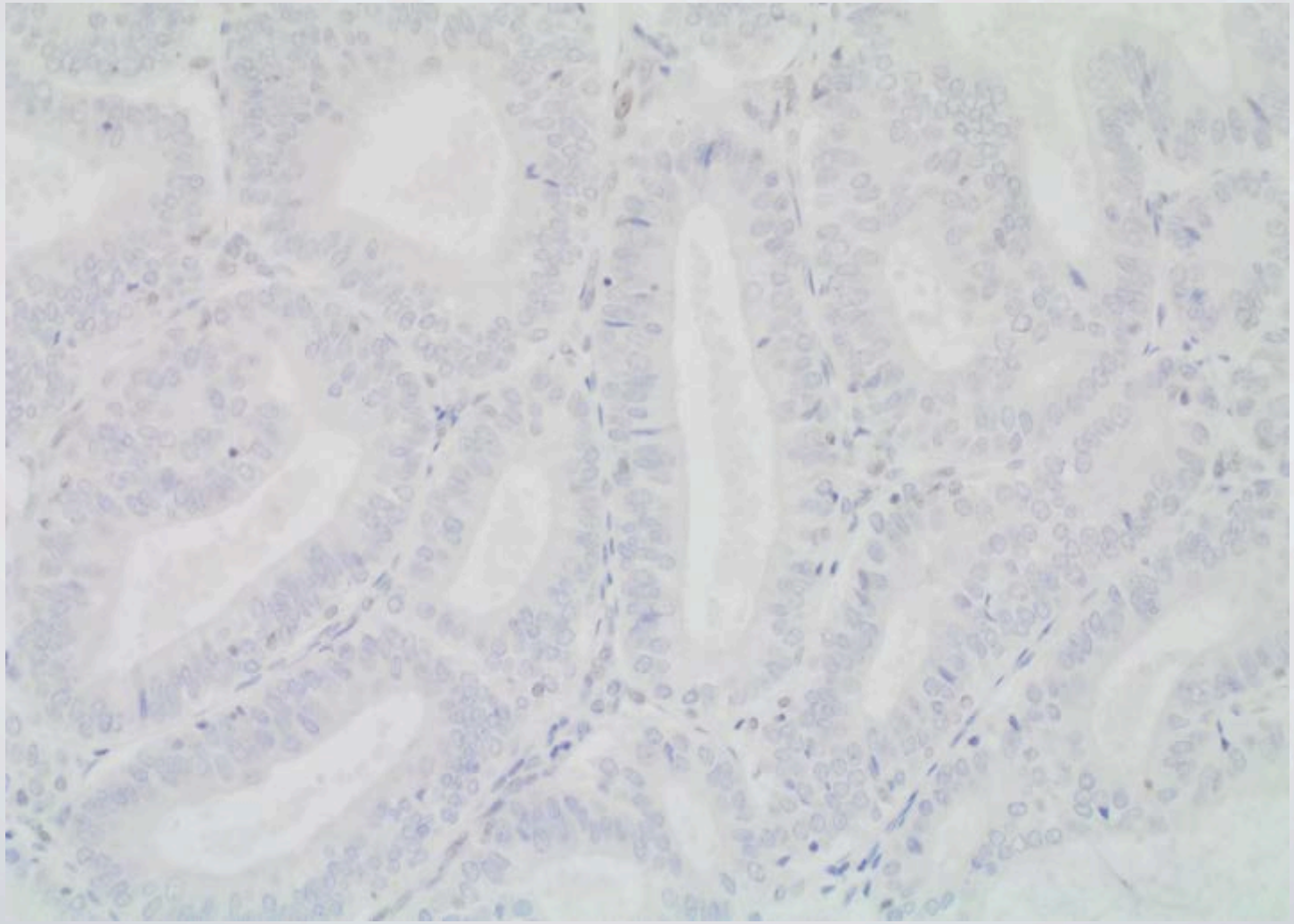
IF MSH2 IS ABSENT, MSH6 IS ALWAYS ABSENT

# MMR IHC (typical combinations)

| **MMR Defect** (retained MMR IHC does not exclude MMRd) | **MMR IHC Pattern** |
|---|---|
| MLH1 promoter methylation | MLH1 loss + PMS2 loss |
| MLH1 gene defect | MLH1 loss + PMS2 loss |
| PMS2 gene defect | Isolated PMS2 loss |
| MSH2 gene defect | MSH2 loss + MSH6 loss |
| MSH6 gene defect | Isolated MSH6 loss |

# MMR IHC: Problems in interpretation

# MMR IHC, Problems & pitfalls: POOR FIXATION

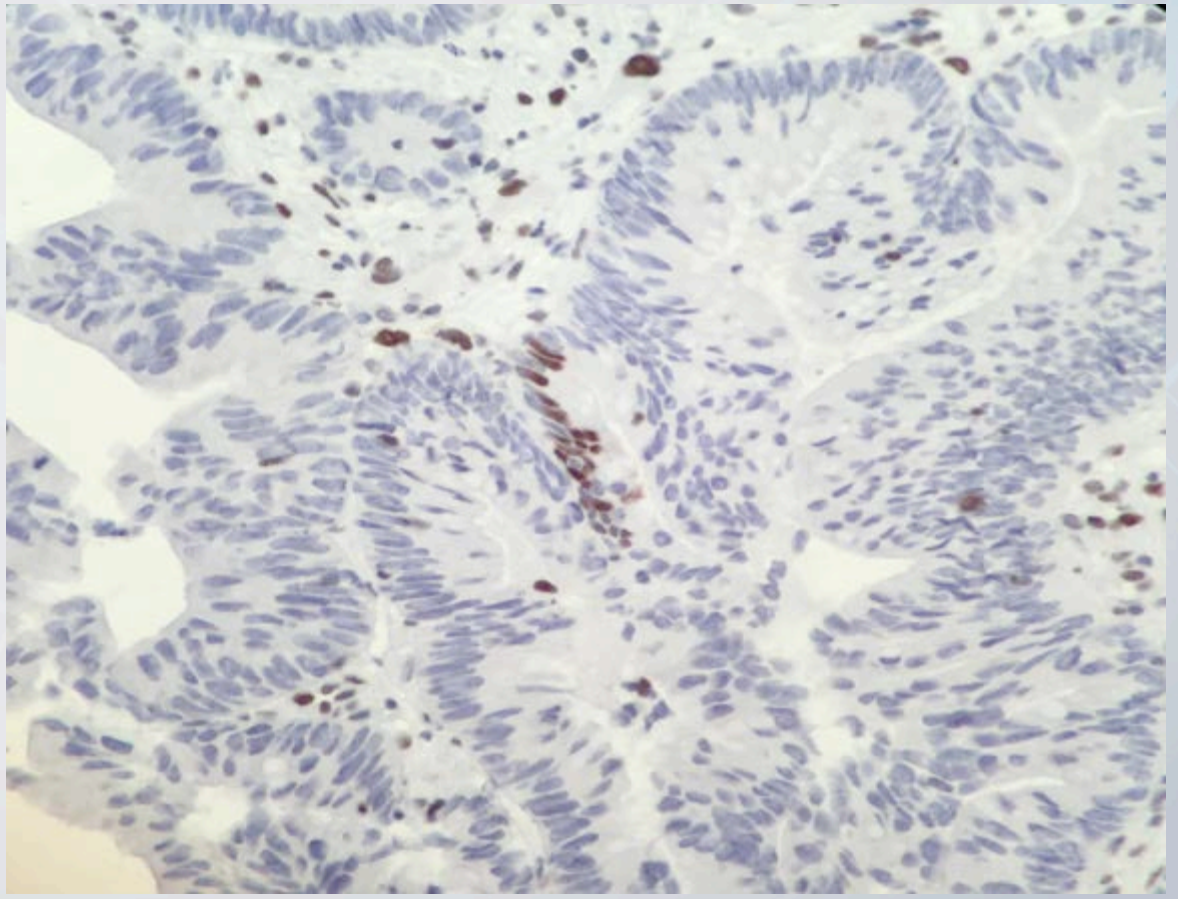


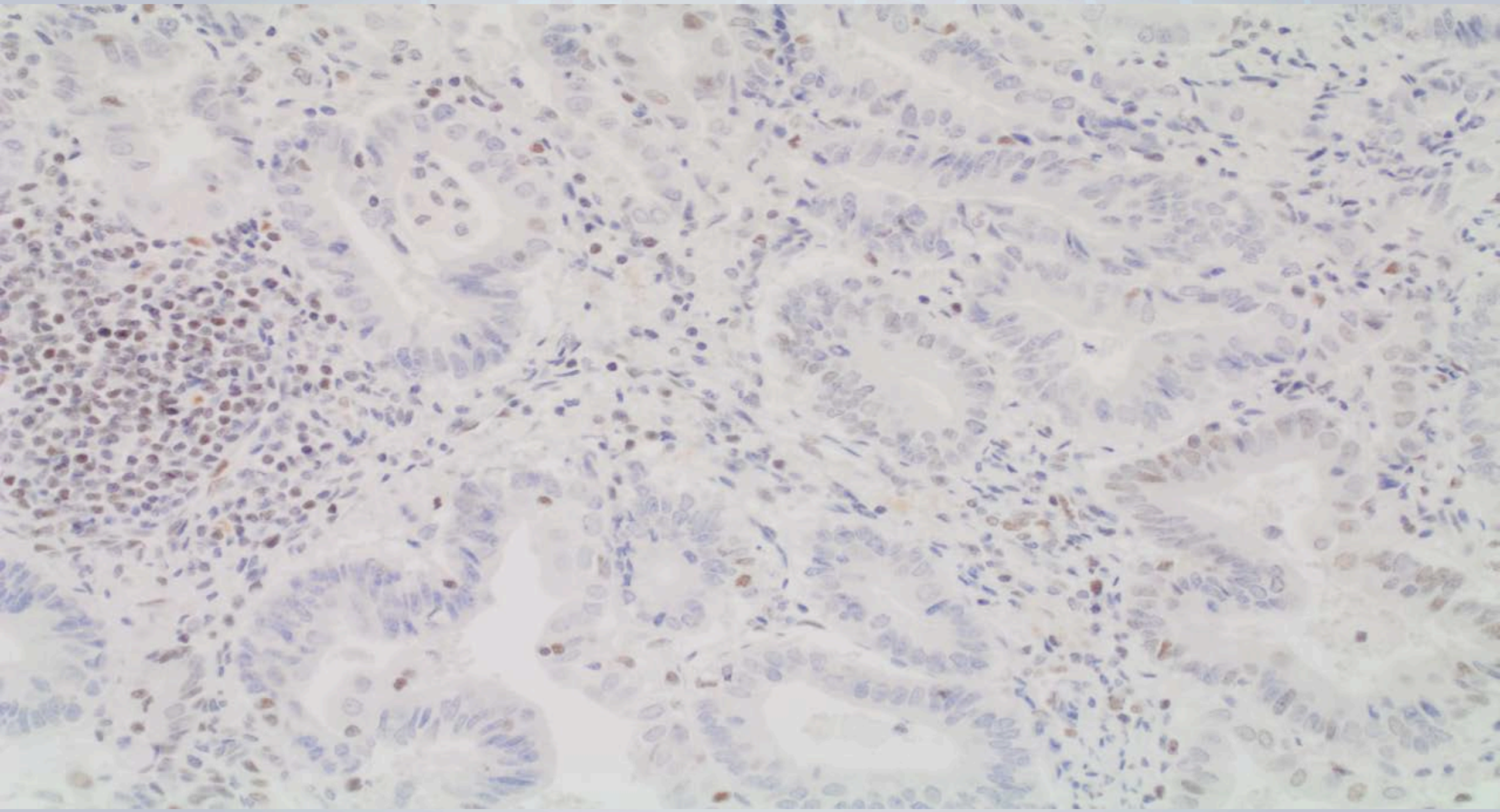
# MMR IHC, Problems & pitfalls: POOR FIXATION

- **Fixation** affects IHC detection (use BIOPSIES)
- Staining protocol should be standardised with appropriate QC (external proficiency testing, eg UKNEQAS)
- Due regard to presence of internal control
- Other reasons for loss of expression: neoadjuvant chemo; freezing of tissue

# MMR IHC, Problems & pitfalls: WEAK/FOCAL EXPRESSION

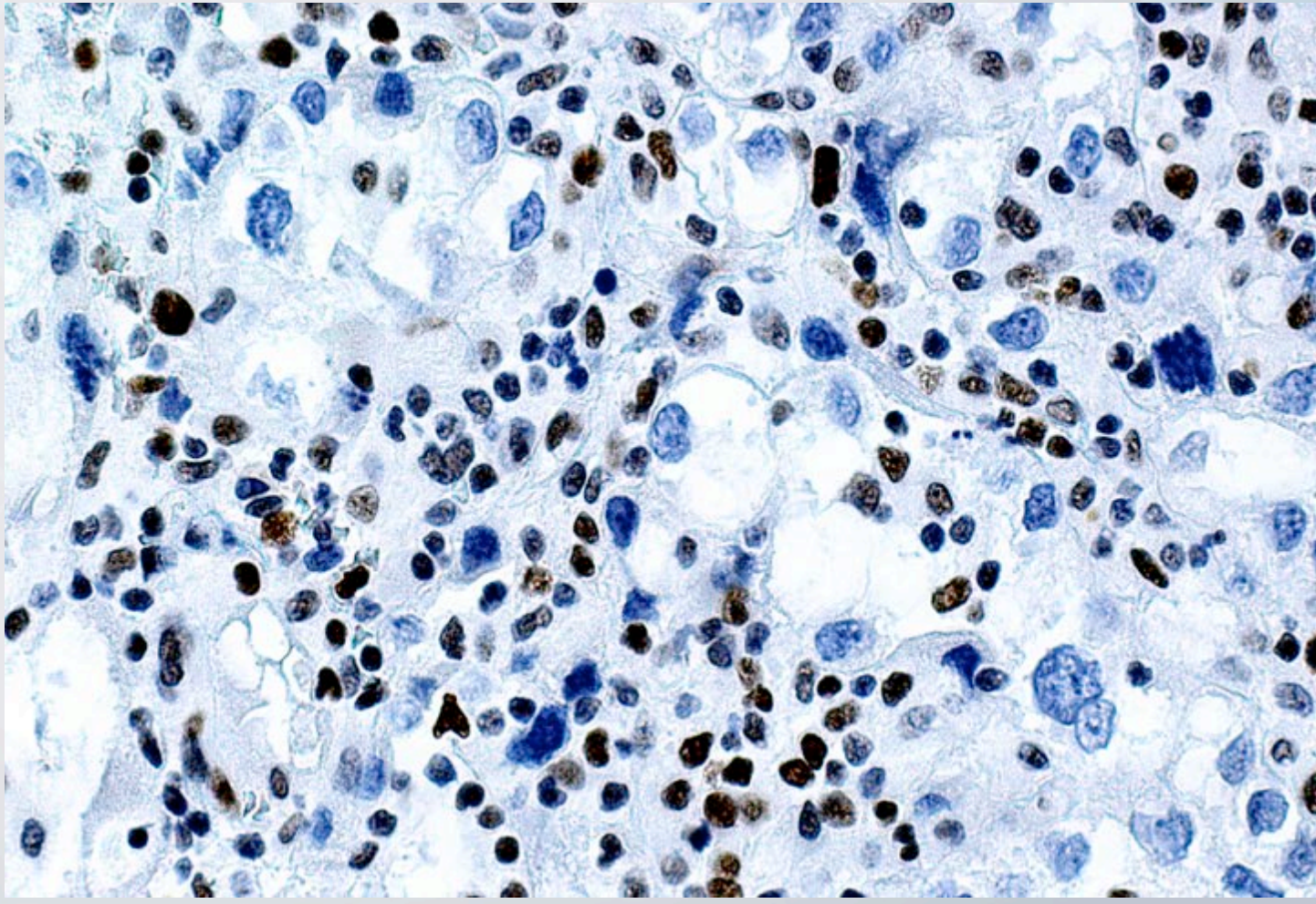
- Standard teaching: ‘any positivity’ is reported as retained expression
- Some missense mutations can result in weak/focal expression
- Very weak staining/very focal expression (in comparison to internal control) best regarded as ‘loss’



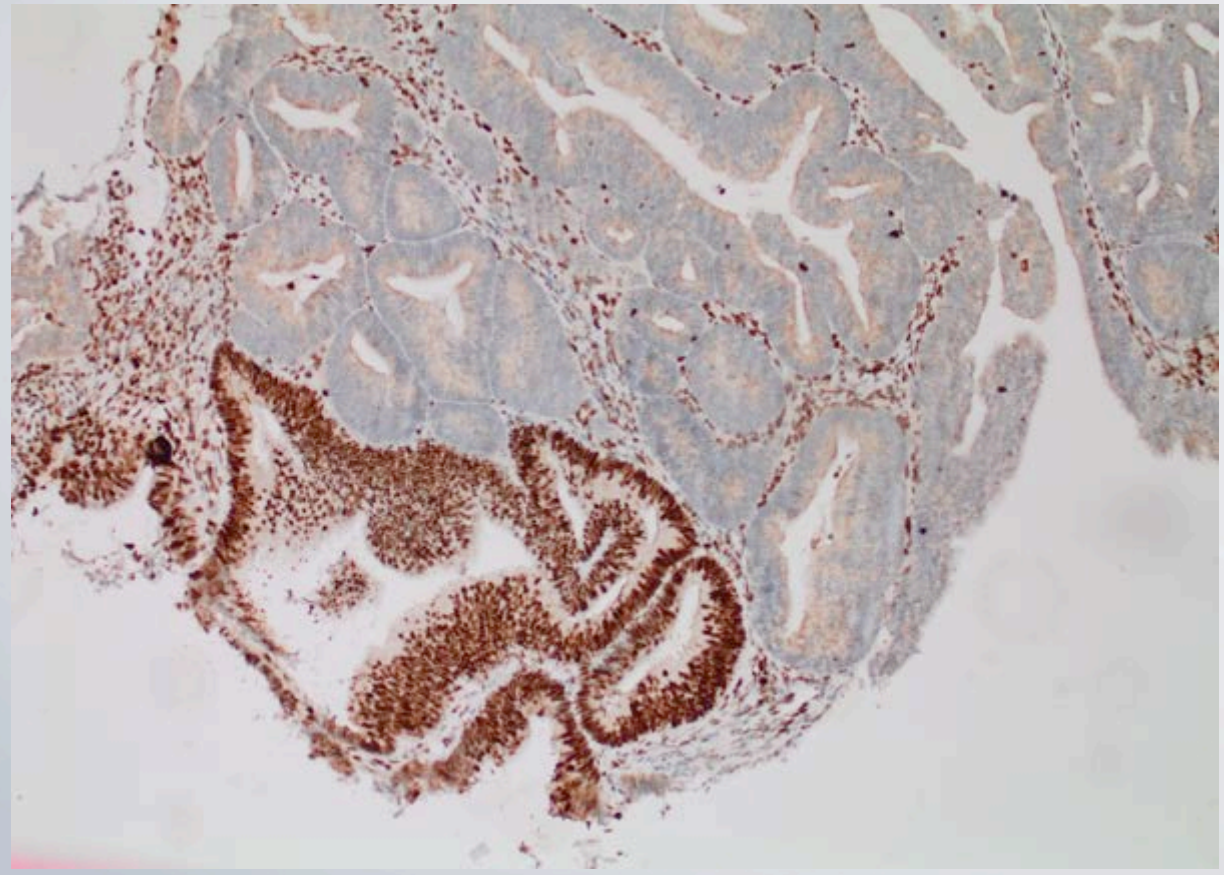


# MMR IHC, Problems & pitfalls: Lymphocytes/stromal cells

- MMRd EC typically have large numbers of TIL's
- Do not interpret these or stromal cells as positive



# MMR IHC, Problems and pitfalls: HETEROGENEOUS loss (subclonal pattern)

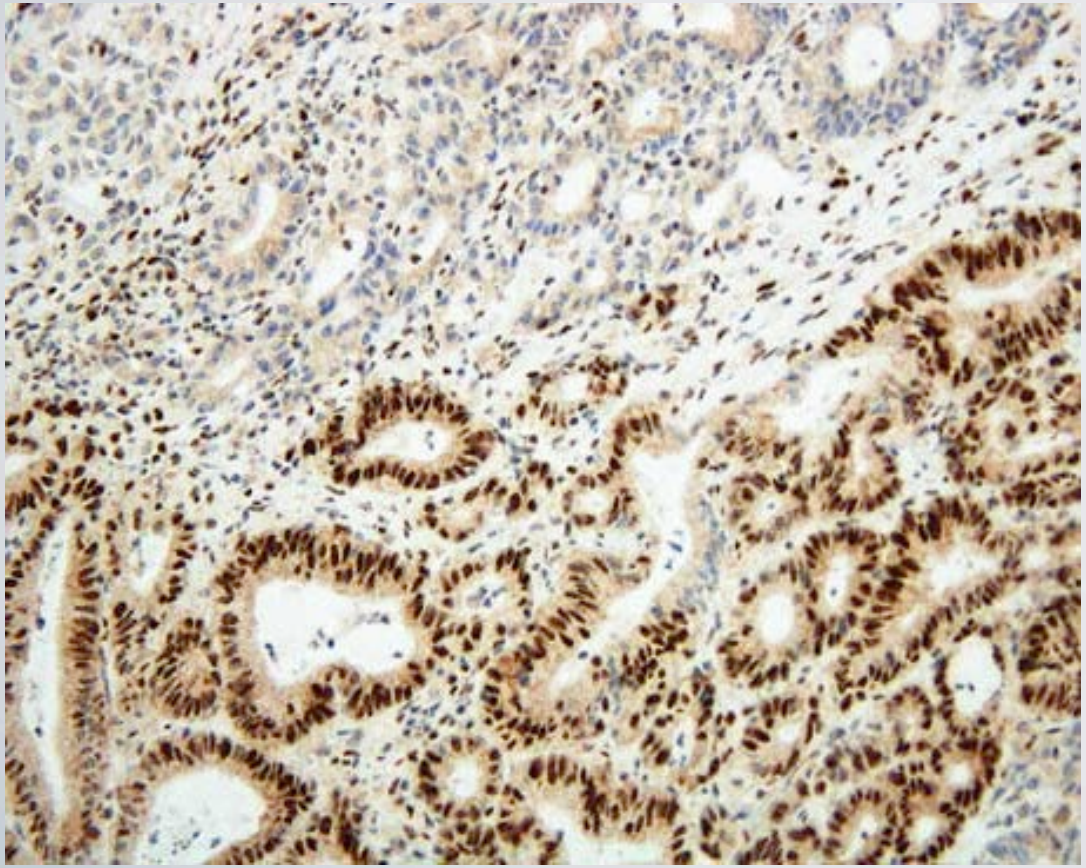


- Exclude fixation artefact (internal control positive)
- Results from actual tumour heterogeneity as subclones with survival advantage are gradually propagated
- Most commonly seen with epigenetic MLH1 defect

# Heterogeneous staining of MLH1 +/- PMS2:

- Results from ***sporadic*** MLH1 promoter methylation
- (Rare cases of germline MLH1 promoter methylation show uniform staining)
- (MLH1 promoter methylation may occur sporadically in any EC, ie including those arising in LS)
- Functional loss precedes protein loss (MSI+/IHC normal)
- Extent of methylation corresponds to MSI and protein expression
- Associated PMS2 loss is also generally heterogeneous, BUT may be complete and present as isolated PMS2 loss

# MMR IHC, Problems and pitfalls: HETEROGENEOUS loss of MSH6



- MSH6 gene has a mutation-prone microsatellite: exon 5, polycytosine tract (C8); can undergo frame shift mutation in cases with MSI
- This can result in subclonal MSH6 loss in MSI due to ANY CAUSE – ie sporadic/germline
- Subclonal MSH6 loss therefore (almost always) indicates absence of MSH6 germline defect

Graham et al, AJSP 2015; 39:1370

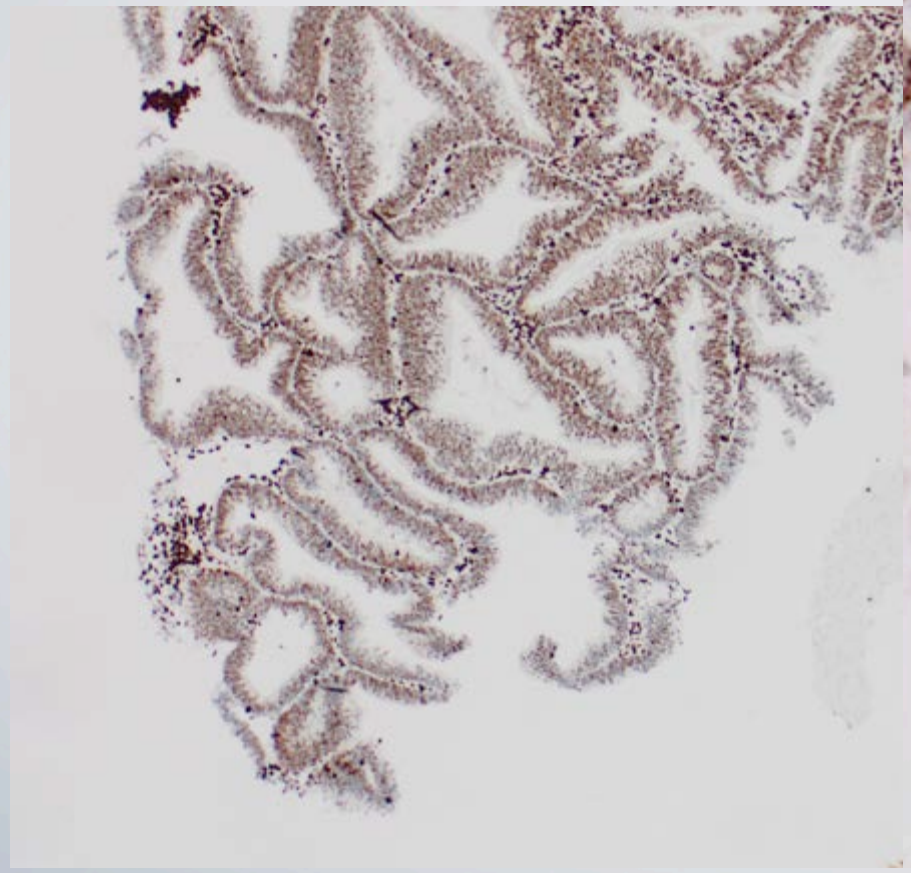
# MMR IHC, Problems and pitfalls: HETEROGENEOUS patterns recommendations

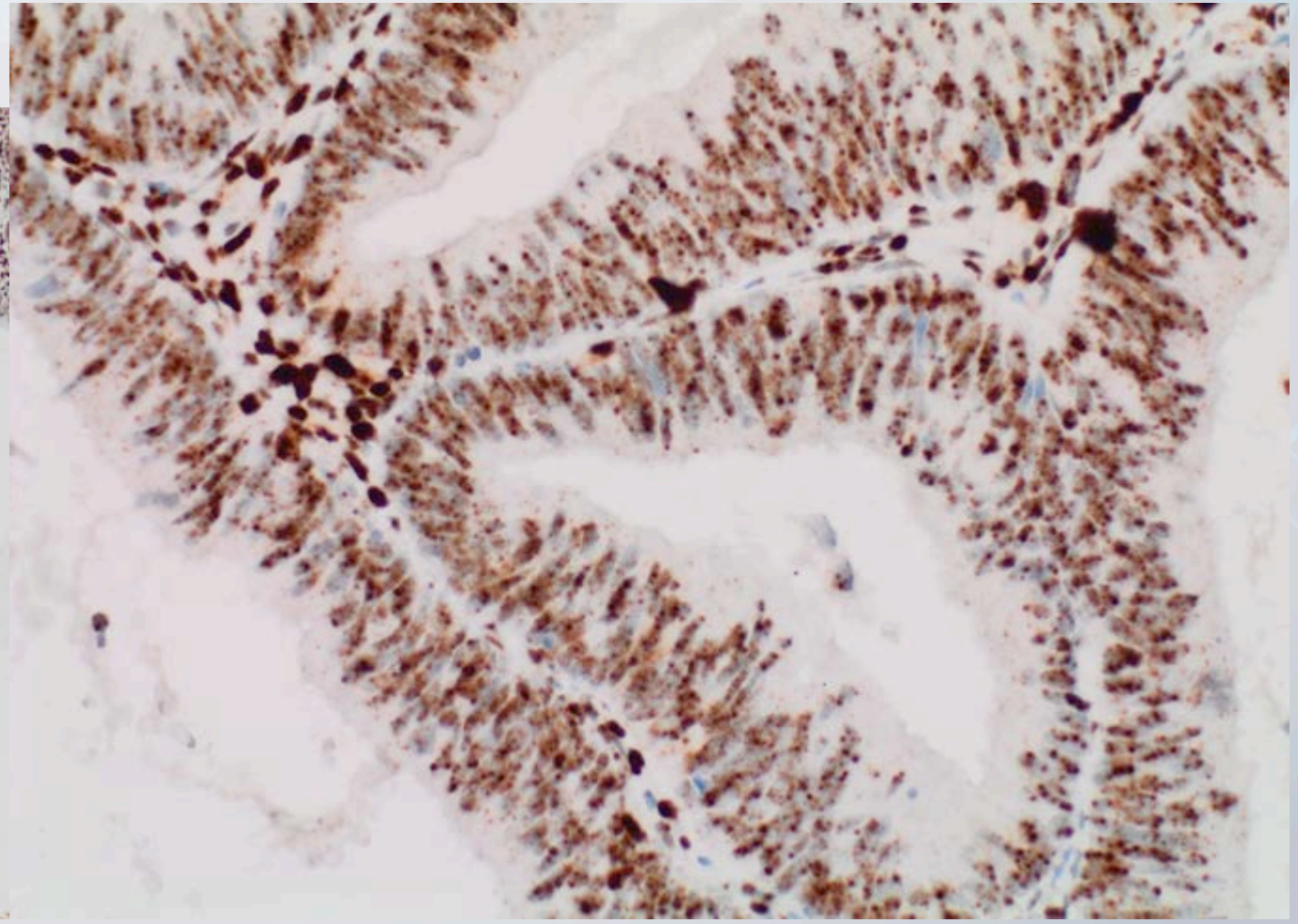
- 10% cut off has been suggested
- Some cases may represent germline defects
- Report as MMRd (subclonal MMR loss) as biology currently poorly understood

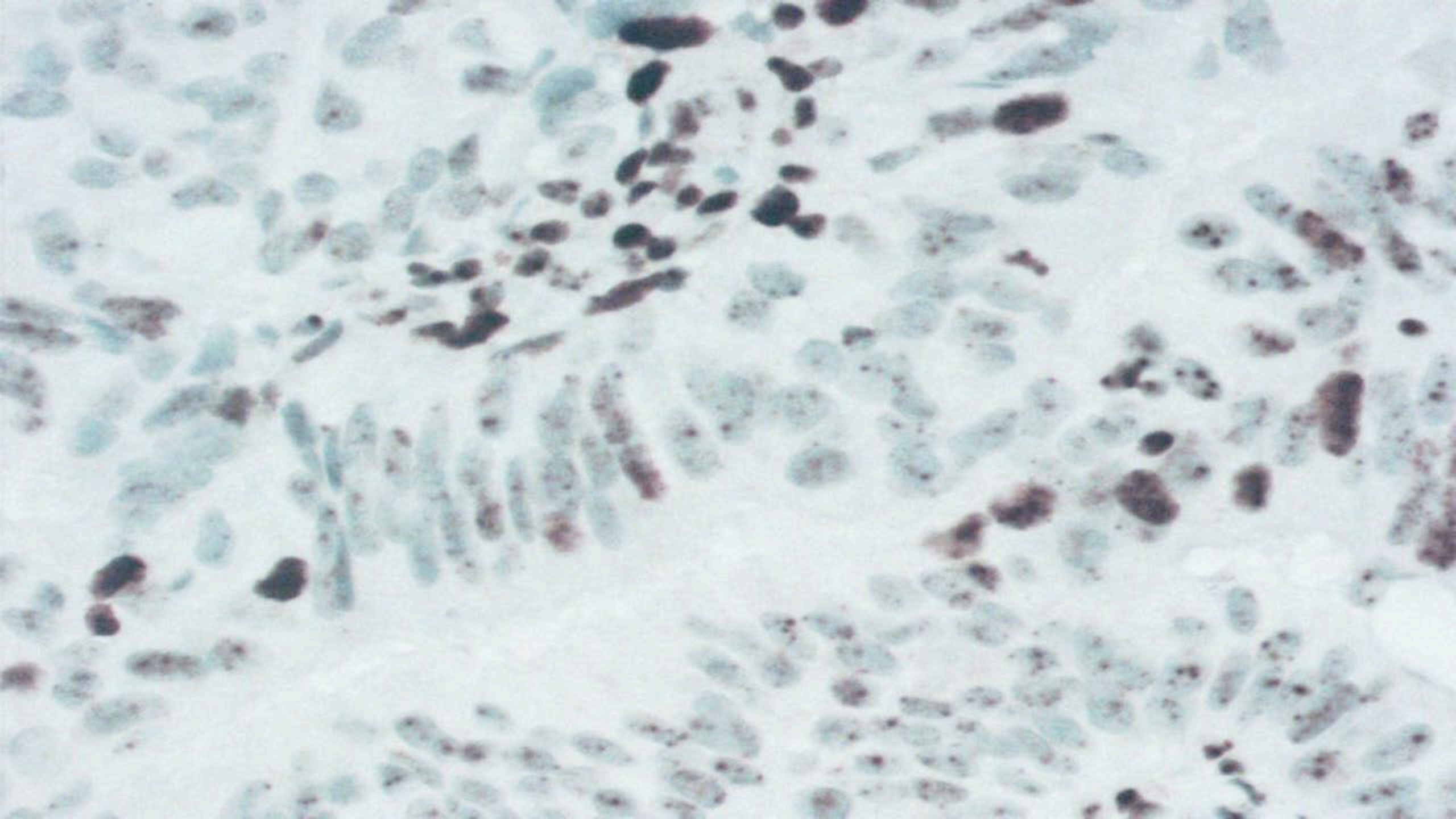
# MMR IHC, Problems and pitfalls: FALSE NEGATIVE RESULT

- Non-functional protein with retained antigenicity (most commonly MLH1)
- MSI +/- Germline testing if strong family history

# MMR IHC, Problems and pitfalls: DOT-LIKE STAINING



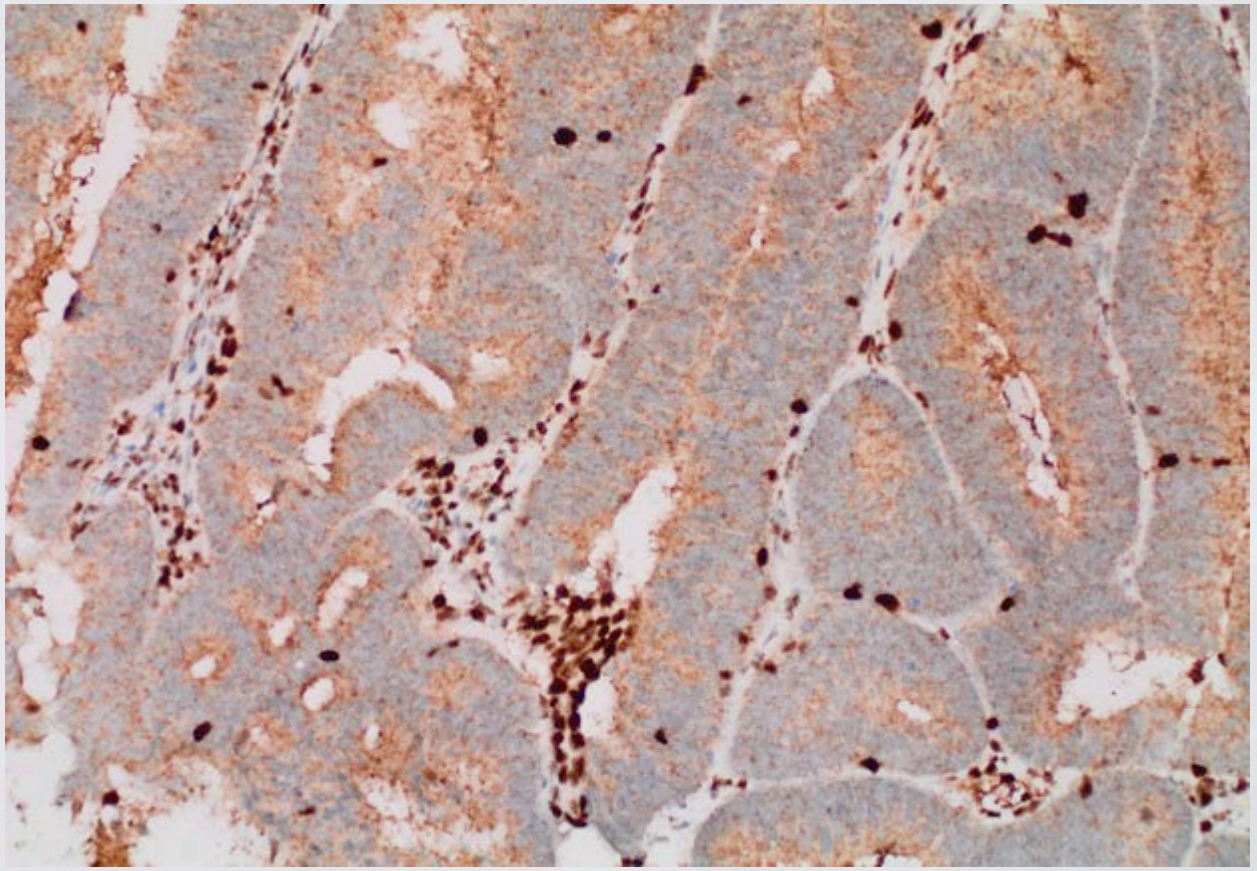




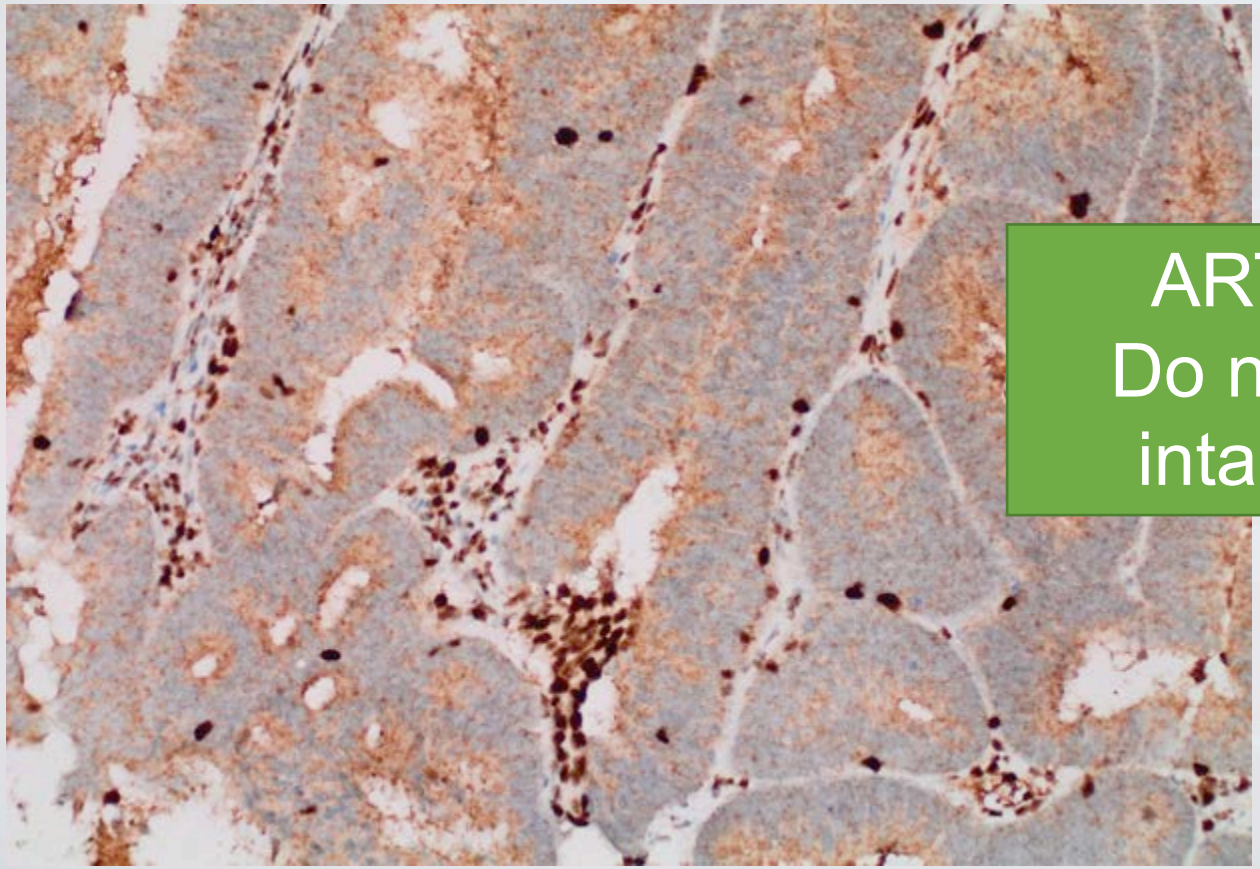
# MMR IHC, Problems and pitfalls: DOT-LIKE STAINING

ARTEFACTUAL
Do not interpret as intact expression

# MMR IHC, Problems and pitfalls: CYTOPLASMIC STAINING



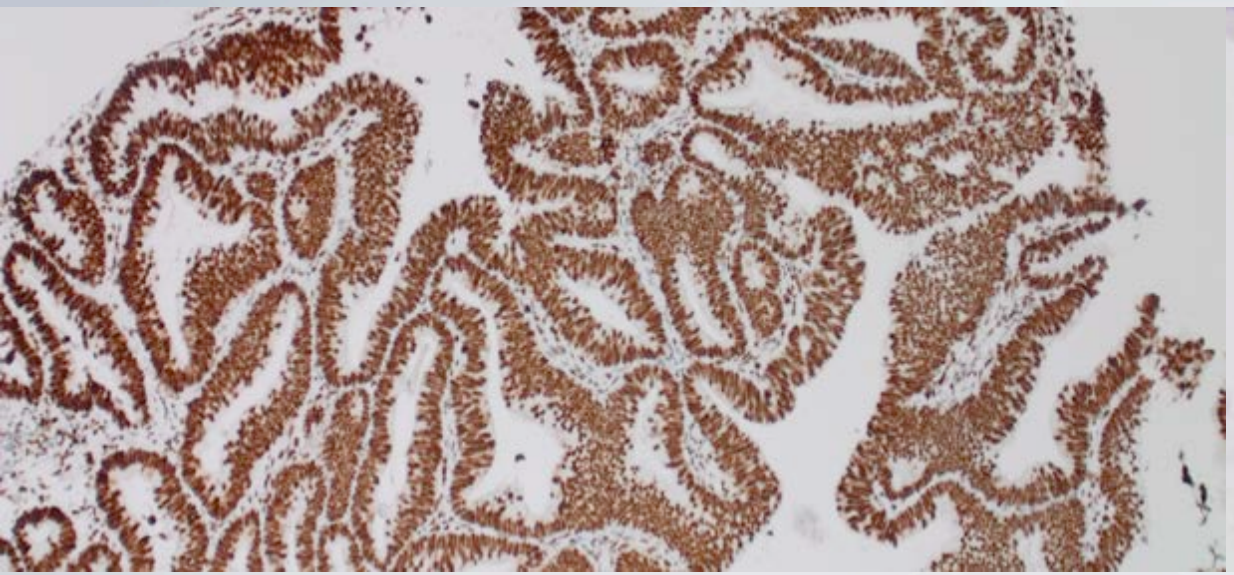
# MMR IHC, Problems and pitfalls: CYTOPLASMIC STAINING

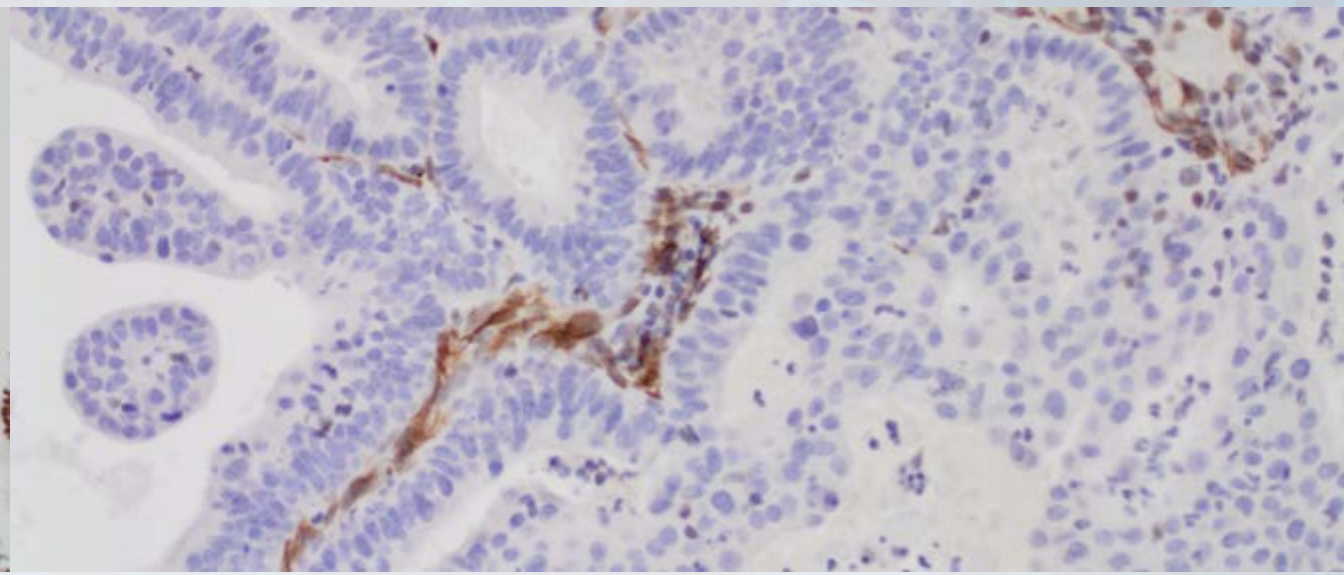


ARTEFACTUAL
Do not interpret as intact expression

# MMR IHC: Rare combinations

- Loss of 2 discordant proteins or 3 or all 4
  - MLH1 promoter methylation as sporadic event in MMRd due to any cause
  - MSH6 loss as added mutation in MMRd due to any cause
  - Multiple MMR gene mutations in POLEmut EC; may be subclonal loss
  - Other somatic mutations





# How should we report MMR IHC results?

# Results: Normal

- All 4 proteins tested, or
- Only PMS2 and MSH6 tested

**There is no immunohistochemical evidence of a mismatch repair deficiency*.**

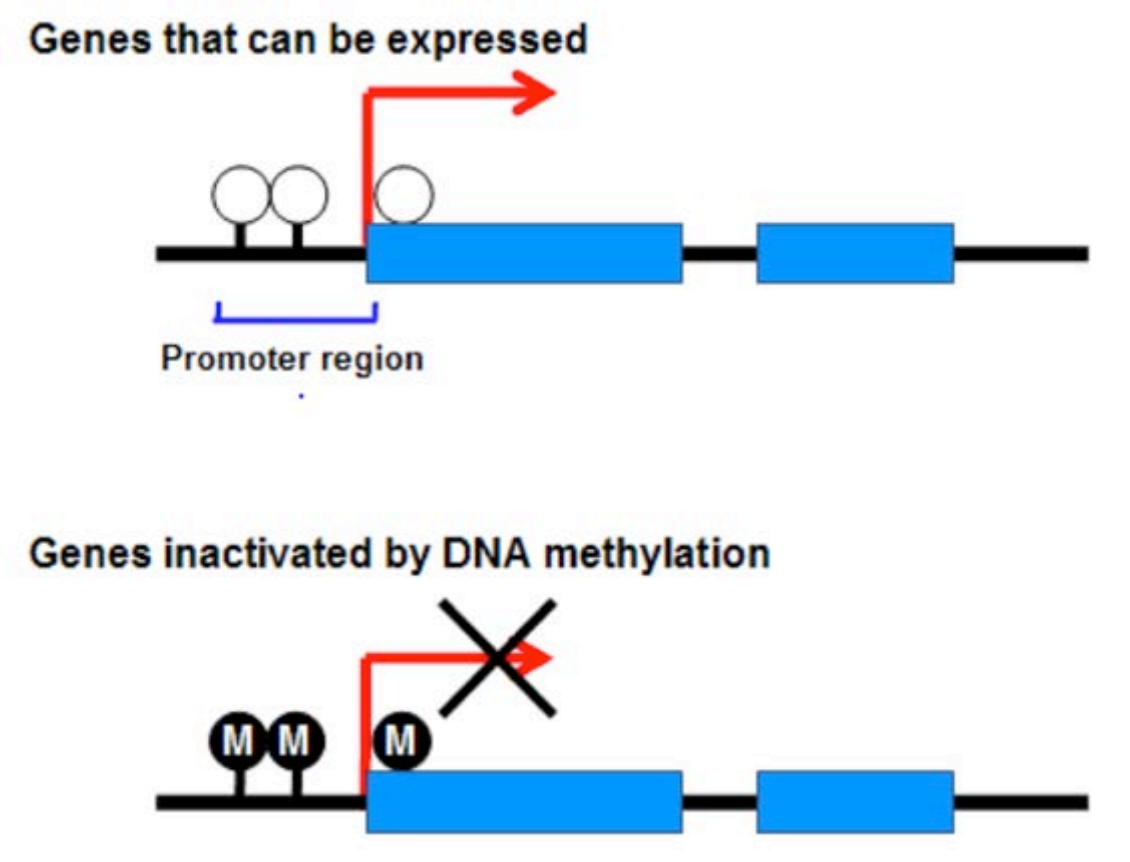
***Referral to Clinical Genetics services should be considered despite this result in the presence of a strong family/clinical history.**

# Results: Abnormal (1)

- MLH1 and PMS2 loss

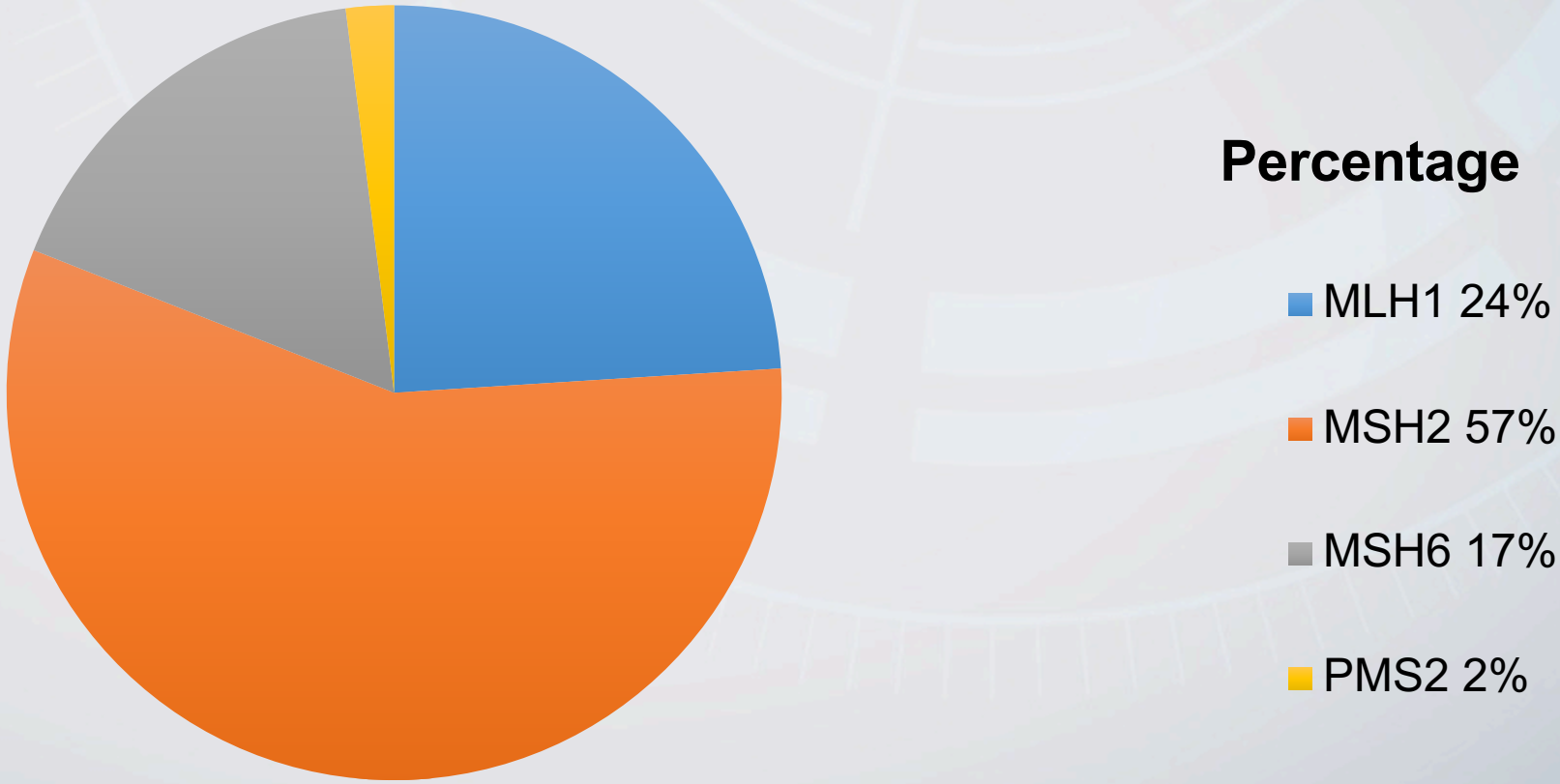
**This pattern is likely to be sporadic, although it is possible that this mismatch repair deficiency is due to Lynch or related syndromes. Testing for *MLH1* Promoter hypermethylation is recommended**

# MLH1 promoter methylation




- >80% of MLH1 loss is due to promoter methylation
- Methylation of the MLH1 promoter silences gene transcription
- Multiple techniques available to detect this in the lab

# LS associated EC




Win et al, JNCI 2013

# Results: Abnormal (2)

- MSH2 and MSH6 loss
- MSH6 loss
- PMS2 loss
- MLH1 and PMS2 loss **without** *MLH1* promoter methylation

**This mismatch repair deficiency is associated with Lynch and related syndromes. This patient should be referred to Clinical Genetics services.**

# Results: Abnormal (2)

- MSH2 and M
- MSH6 loss
- PMS2 loss
- MLH1 and PM ethylation

**This mismatch with Lynch and related sy referred to Clinical Genet**

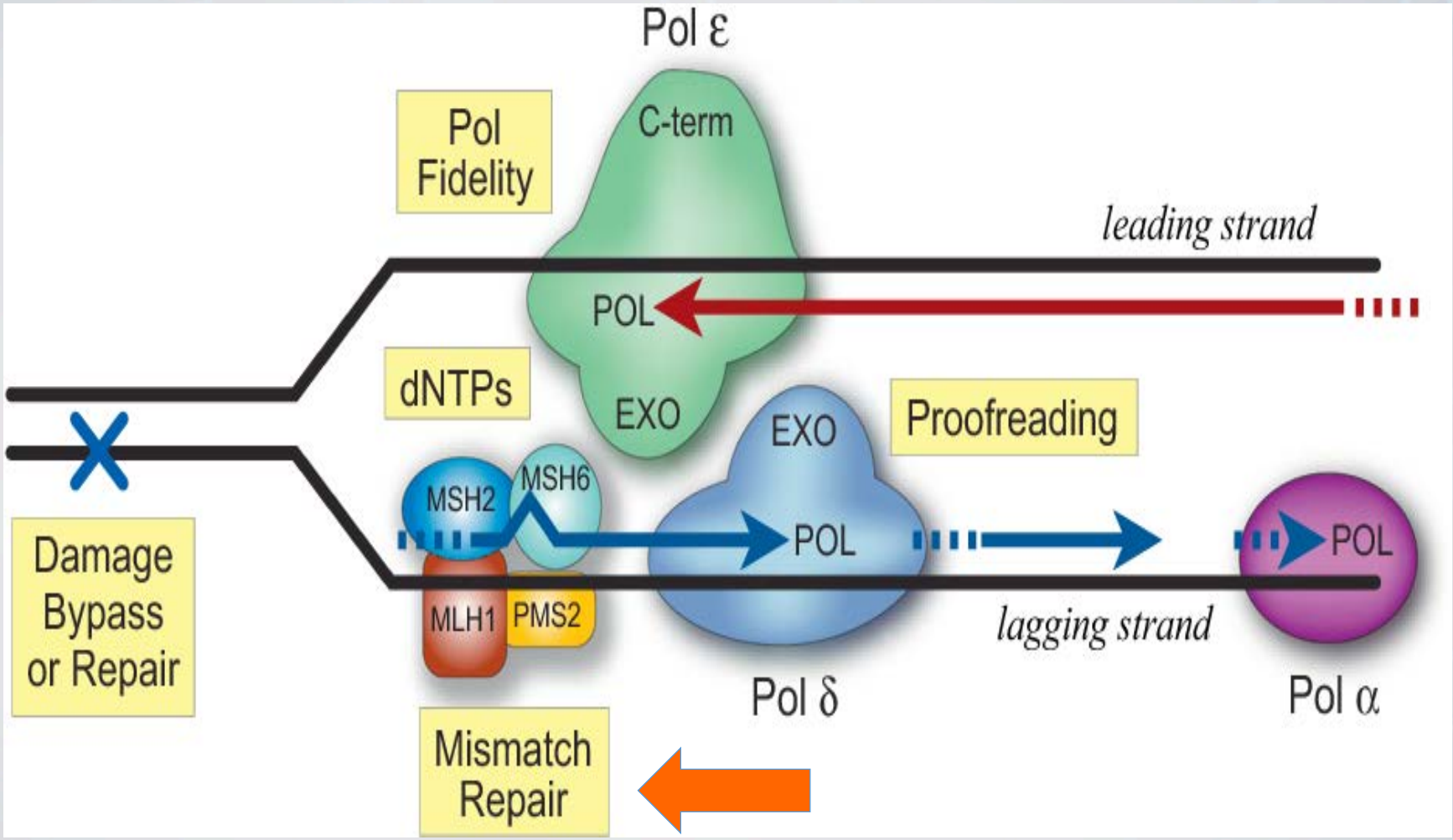
<50% OF THESE WILL TURN OUT TO BE LS

THE REMAINDER ARE SPORADIC

PRECLUDES NECESSITY FOR CONSENT PRIOR TO THIS STEP

# MSI testing in EC

What is microsatellite instability?


Pol ε
Pol Fidelity
C-term
leading strand
POL
dNTPs
EXO
EXO
Proofreading
MSH2
MSH6
POL
POL
Damage Bypass or Repair
MLH1
PMS2
lagging strand
Pol δ
Pol α
Mismatch Repair

# Types of *mismatches*

- Two types of *mismatches* may occur despite normal POLE function:
  - base-base mismatches
  - insertion-deletion (indel) errors at repetitive sequences
- MMR system is a post-replication mechanism to detect and correct all mismatches

# Types of *mismatches*

- Two types of *mismatches* may occur despite normal POLE function:
  - base-base mismatches
  - ***insertion-deletion (indel) errors at repetitive sequences - particularly resistant to detection by the proof-reading function of DNA polymerases.***
- MMR system is a post-replication mechanism to detect and correct all mismatches

# MICROSATELLITE INSTABILITY


- MISMATCHES IN THE PRESENCE OF MMRd CAN OCCUR ANYWHERE, BUT
- MOST PRONE ARE ***MICROSATELLITES*** OR ***SHORT TANDEM REPEATS***
- THESE ARE SEGMENTS OF DNA IN WHICH 1-6 NUCEOTIDE BASES ARE REPEATED, 5-50 TIMES
- **AS A RESULT WE CAN DETECT MMRd THROUGH TESTING FOR MICROSATELLITES (MICROSATELLITE INSTABILITY, MSI)**

# MICROSATELLITE INSTABILITY

Normal BAT25 $(A)_{25}$:

TACGAAAAAAAAAAAAAAAAAAAAAAAAATGACT

MSI BAT25:

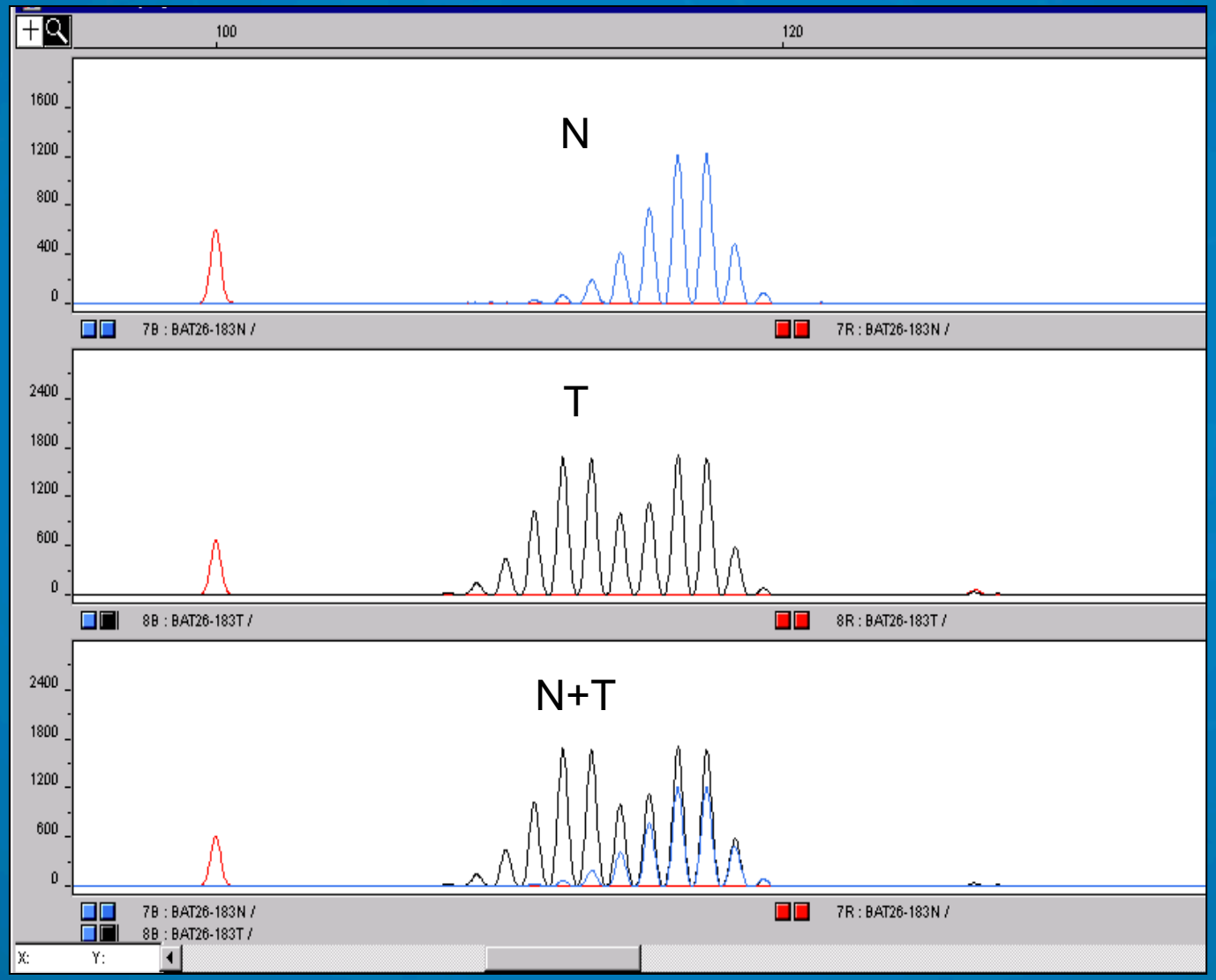
- TACGAAAAAAAAAAAAAAAAAAAAATGACT $(A)_{22}$

- TACGAAAAAAAAAAAAAAAAAAAAAAAAATGACT $(A)_{27}$

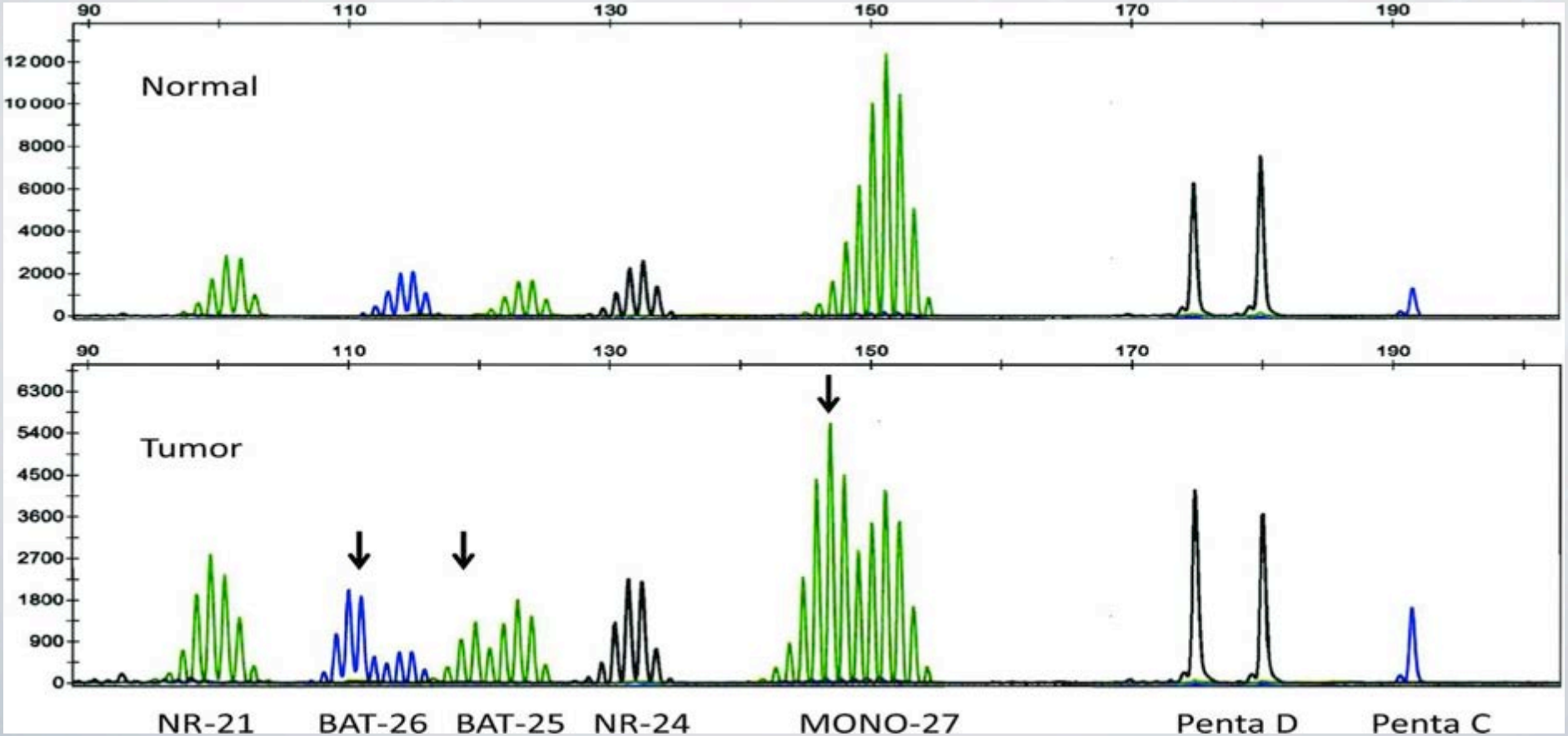
# MSI Testing

- Bethesda Panel: BAT-25, BAT-26, D5S346, D2S123, D17S250
- Promega panel: BAT-25, BAT-26, NR-21, NR-24, MONO 27, Penta C, Penta D

# BAT26


100
120
1600
1200
800
400
0
N
7B : BAT26-183N /
7R : BAT26-183N /
2400
1800
1200
600
0
T
8B : BAT26-183T /
8R : BAT26-183T /
2400
1800
1200
600
0
N+T
7B : BAT26-183N /
8B : BAT26-183T /
7R : BAT26-183N /
X:
Y:

# MSI testing


Normal
Tumor
90
110
130
150
170
190
12 000
10 000
8000
6000
4000
2000
0
6300
5400
4500
3600
2700
1800
900
0
NR-21
BAT-26
BAT-25
NR-24
MONO-27
Penta D
Penta C

# MSI Reporting

- MSS: 0 markers show instability
- MSI-L: 1 marker/<30% of markers show instability
- MSI-H: 2 or more/>30% of markers show instability

# Pitfalls in MSI Reporting

- Platforms with assay sensitivity for EC: > mononucleotide markers = greater sensitivity

- Minimal microsatellite shift resulting in FALSE NEGATIVE results

# Newer platforms: Idylla™

- Tumour-specific
- 7 microsatellites:  ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, and SULF2
- Fully automated, compact desktop system
- Single FFPE section
- 150 minute TAT
- No normal tissue required
- Readout based on melting curve analysis
- Accurate results

# MMR IHC/MSI/Both?

- Neither test is 100% sensitive; some cases missed by both
- High concordance (94%)
- MMR IHC considered superior to MSI as first test:
  - MSH6 is sometimes MSS
  - (Directs genetic testing)
  - Lower cost
  - Widely available
- Algorithm adopted depends on local resources

# MMRd testing algorithms for LS

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

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

# MMRd testing by NGS

- In presence of abnormal MMR IHC or strong family history, other tests can be performed via NGS:
  - Direct germline/somatic MMR mutation testing
  - Tumor mutational burden; %age of indel mutations
- Testing strategies likely to evolve rapidly

# Conclusions

- POLE and MMR/MSI testing are vital for accurate histo-molecular classification of EC
- Likely to become mainstream over the coming years
- Pathologists must become familiar with testing strategies and their pitfalls

# THANK YOU!