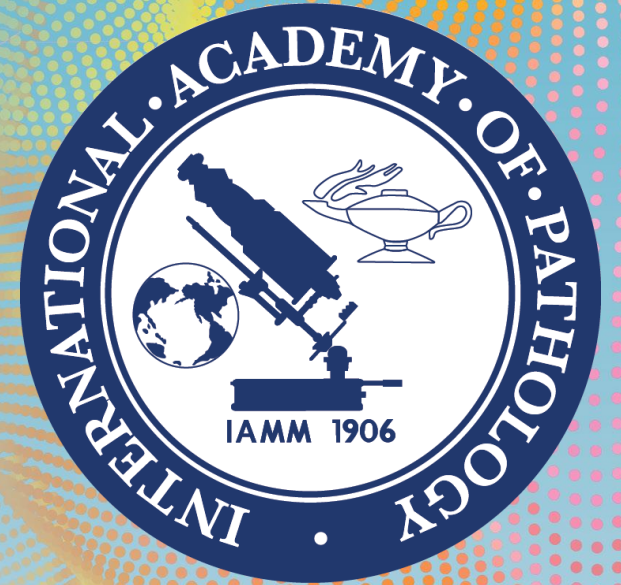


A practical approach to molecular analysis of endometrial carcinoma

Dr Jonathan Clark

Austin

HEALTH | Pathology



Disclosure of Relevant Financial Relationships

No relevant financial relationships

Background

TCGA endometrial carcinoma study

4 molecular subgroups

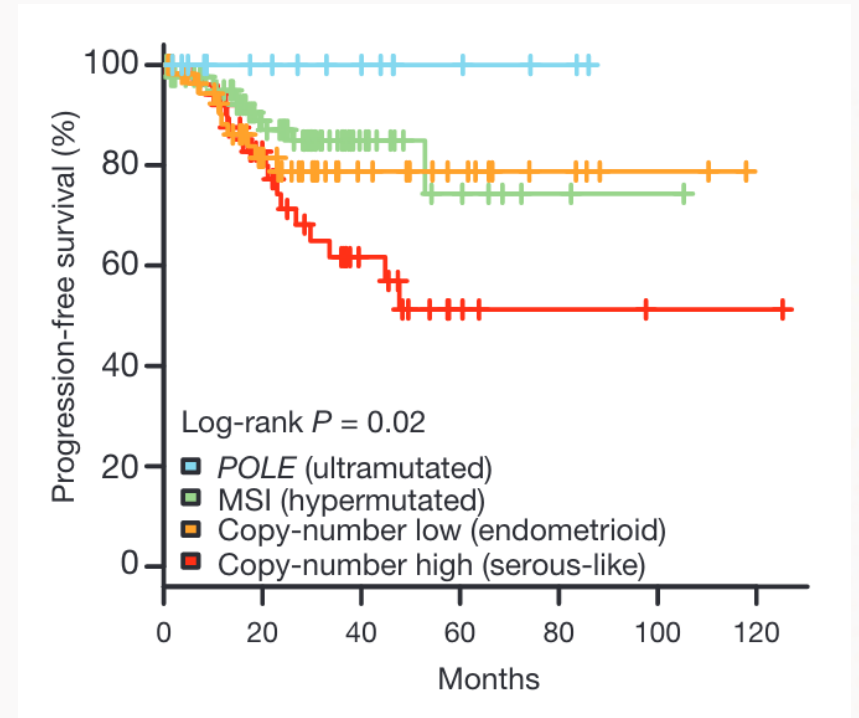
POLE mutant

MSI

Copy number low

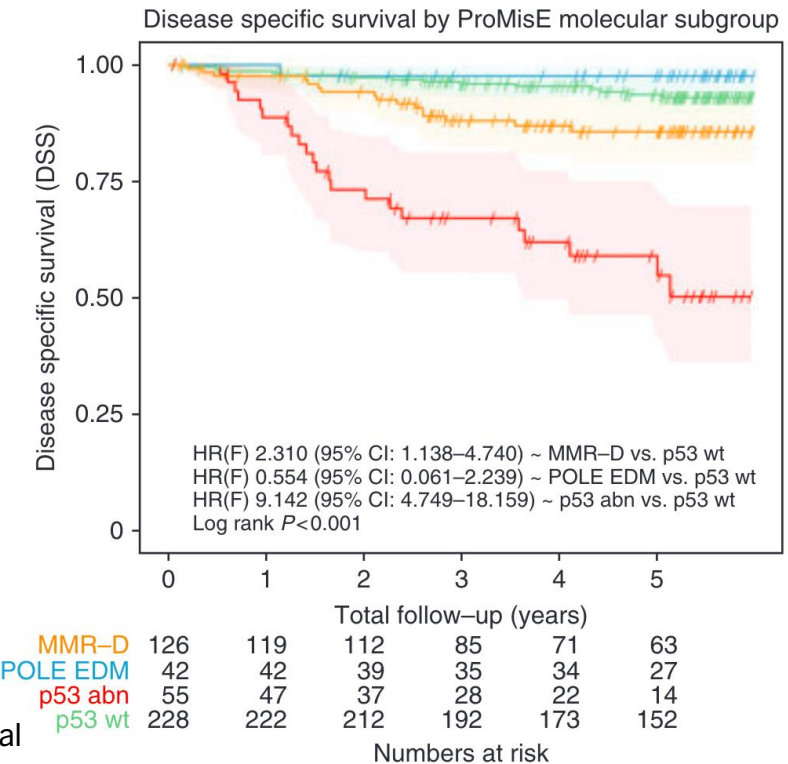
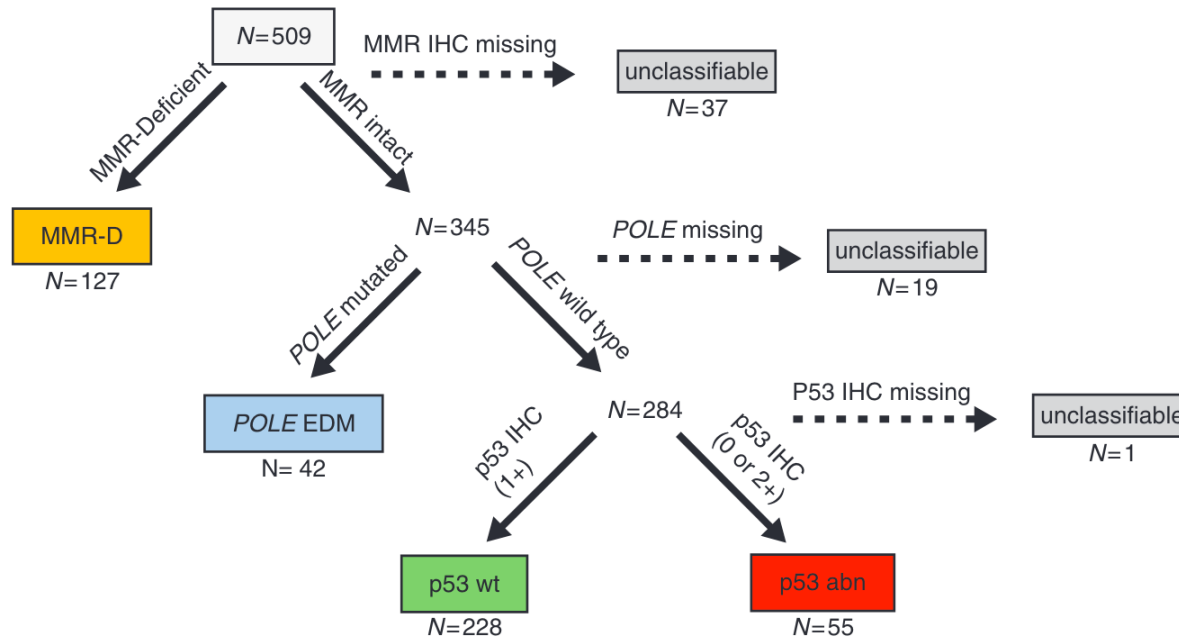
Copy number high (*TP53* mutant)

Her2 amplification – therapeutic target



Levine, 2013 “Integrated Genomic Characterization of Endometrial Carcinoma.”

Proactive Molecular Risk Classifier in Endometrial Carcinoma (ProMisE)



Kommos et al., 2018 “Final Validation of the ProMisE Molecular Classifier for Endometrial Carcinoma in a Large Population-Based Case Series.”

FIGO 2023 staging and molecular status

BEREK ET AL.

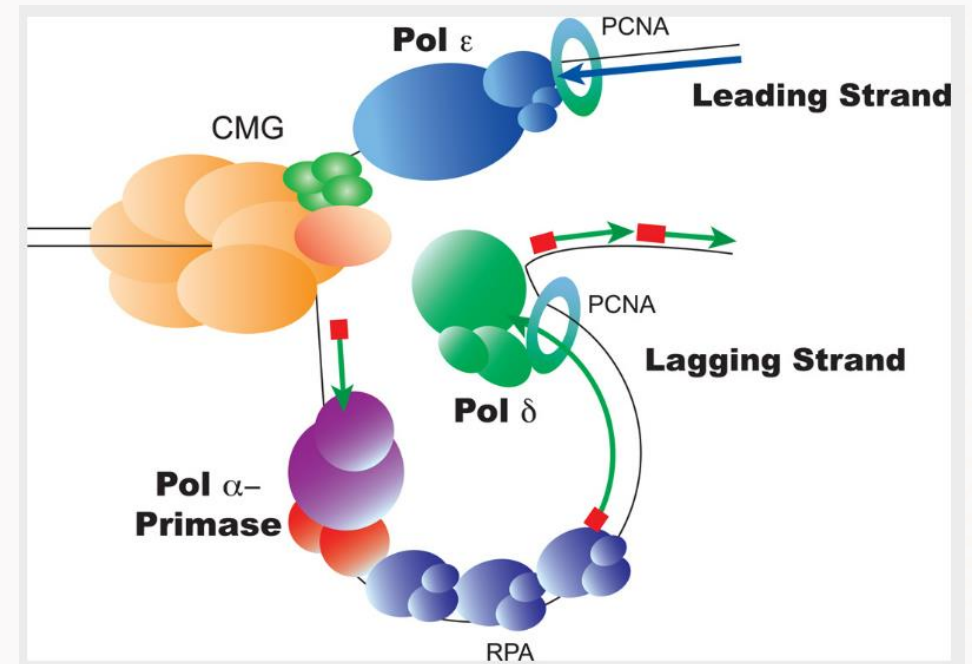
TABLE 2 FIGO endometrial cancer stage with molecular classification.^a

Stage designation	Molecular findings in patients with early endometrial cancer (Stages I and II after surgical staging)
Stage IA _m _{POLEmut}	POLEmut endometrial carcinoma, confined to the uterine corpus or with cervical extension, regardless of the degree of LVSI or histological type
Stage IIC _m _{p53abn}	p53abn endometrial carcinoma confined to the uterine corpus with any myometrial invasion, with or without cervical invasion, and regardless of the degree of LVSI or histological type

Berek JS, Matias-Guiu X, Creutzberg C, et al. FIGO staging of endometrial cancer: 2023. *Int J Gynecol Obstet.* 2023; 162: 383-394. doi:[10.1002/ijgo.14923](https://doi.org/10.1002/ijgo.14923)

What is *POLE*?

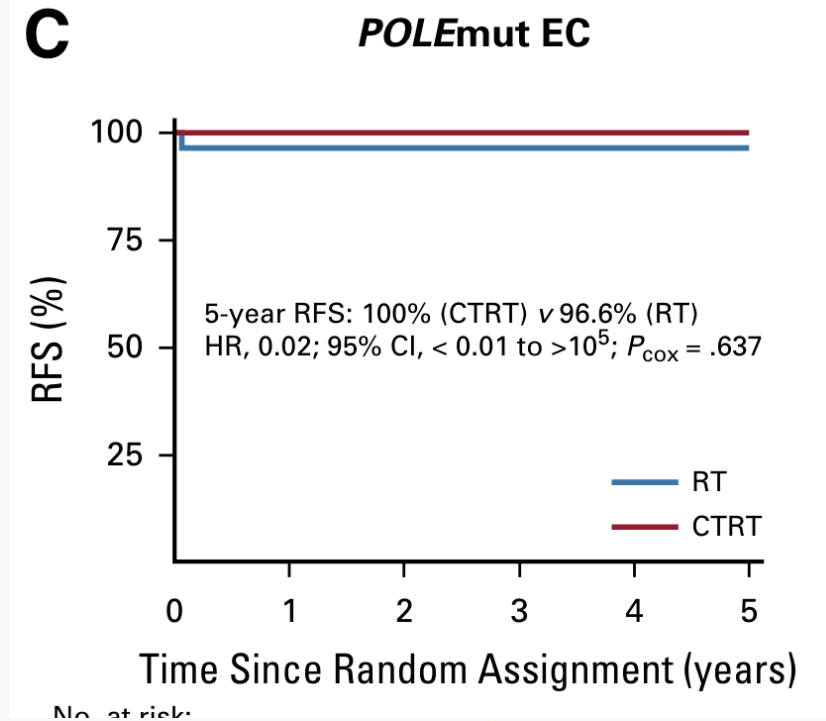
- Gene encoding polymerase epsilon
- Enzyme involved in DNA replication and proofreading/error correction
- Functionally significant mutations in the exonuclease domain impair error correction
- Ultramutated tumour (>100 mutations per megabase)
- Tumour may be more susceptible to immune system targeting



Pursell ZF, Kunkel TA. DNA polymerase epsilon: a polymerase of unusual size (and complexity). *Progress in Nucleic Acid Research and Molecular Biology*. 2008 ;82:101-145. DOI: 10.1016/s0079-6603(08)00004-4. PMID: 18929140; PMCID: PMC3694787.

POLE – why test?

- Significantly better prognosis of *POLE*mut endometrial carcinoma
- Potential for de-escalation of adjuvant therapy to avoid side effects
- Pending additional research (eg. PORTEC 4a trial, RAINBO)
- Testing recommended by NCCN, WHO and ESGO



León-Castillo et al., “Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer.”

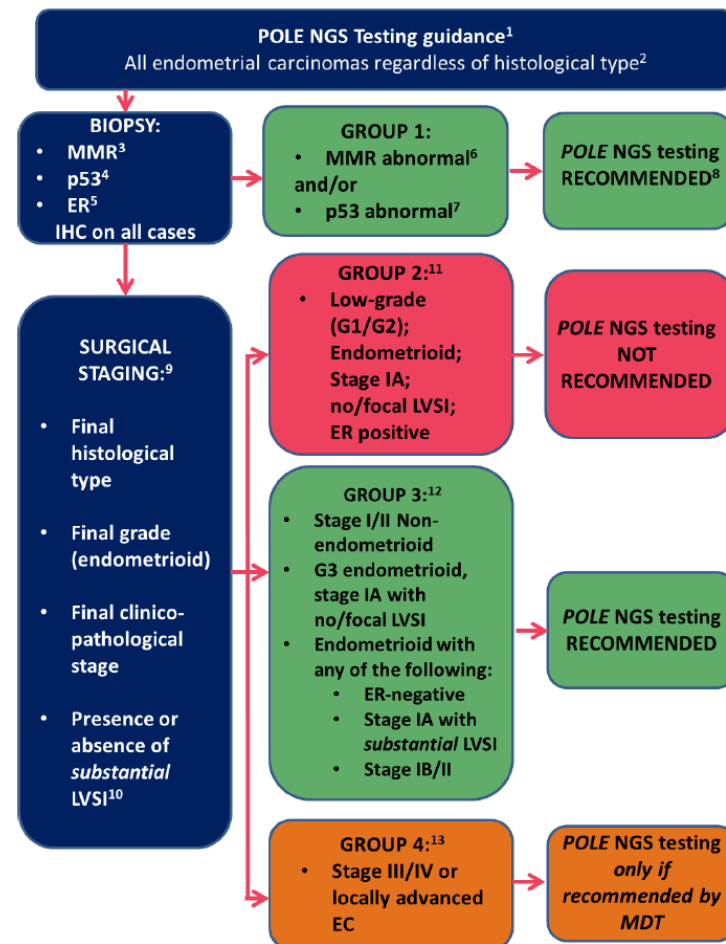
POLE testing guidelines

ESGO

Recommendations

- Molecular classification is encouraged in all endometrial carcinomas, especially high-grade tumors (IV, B).
- POLE mutation analysis may be omitted in low-risk and intermediate-risk endometrial carcinoma with low-grade histology (IV, C).

Concin et al., 2021, "ESGO/ESTRO/ESP Guidelines for the Management of Patients with Endometrial Carcinoma."



POLE – how to test

NGS

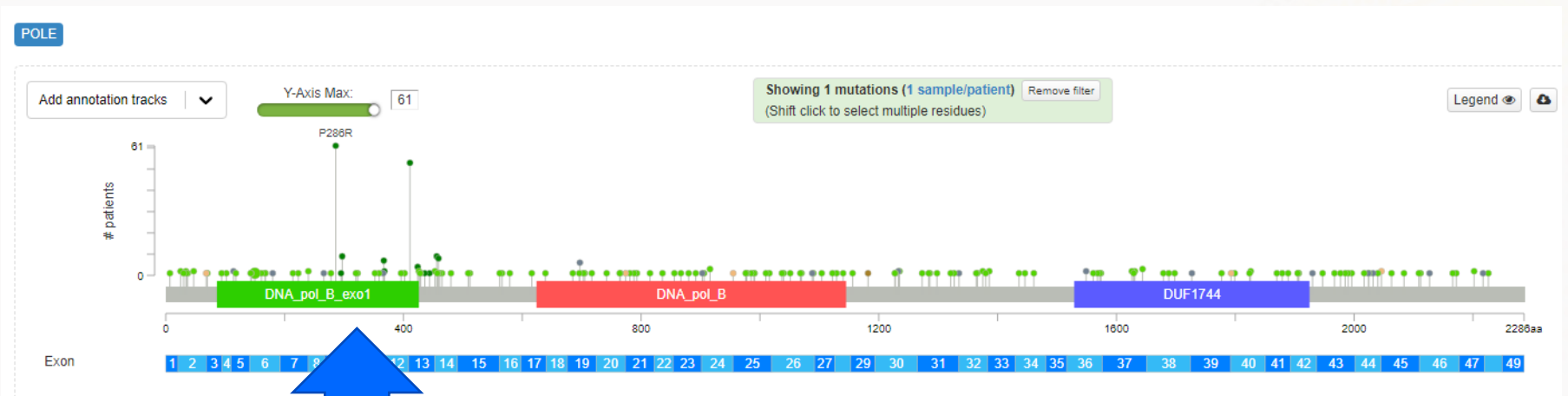
Hot spots (exons 9, 11, 13, 14),
or sequence whole exonuclease
domain



POLE – interpretation of results

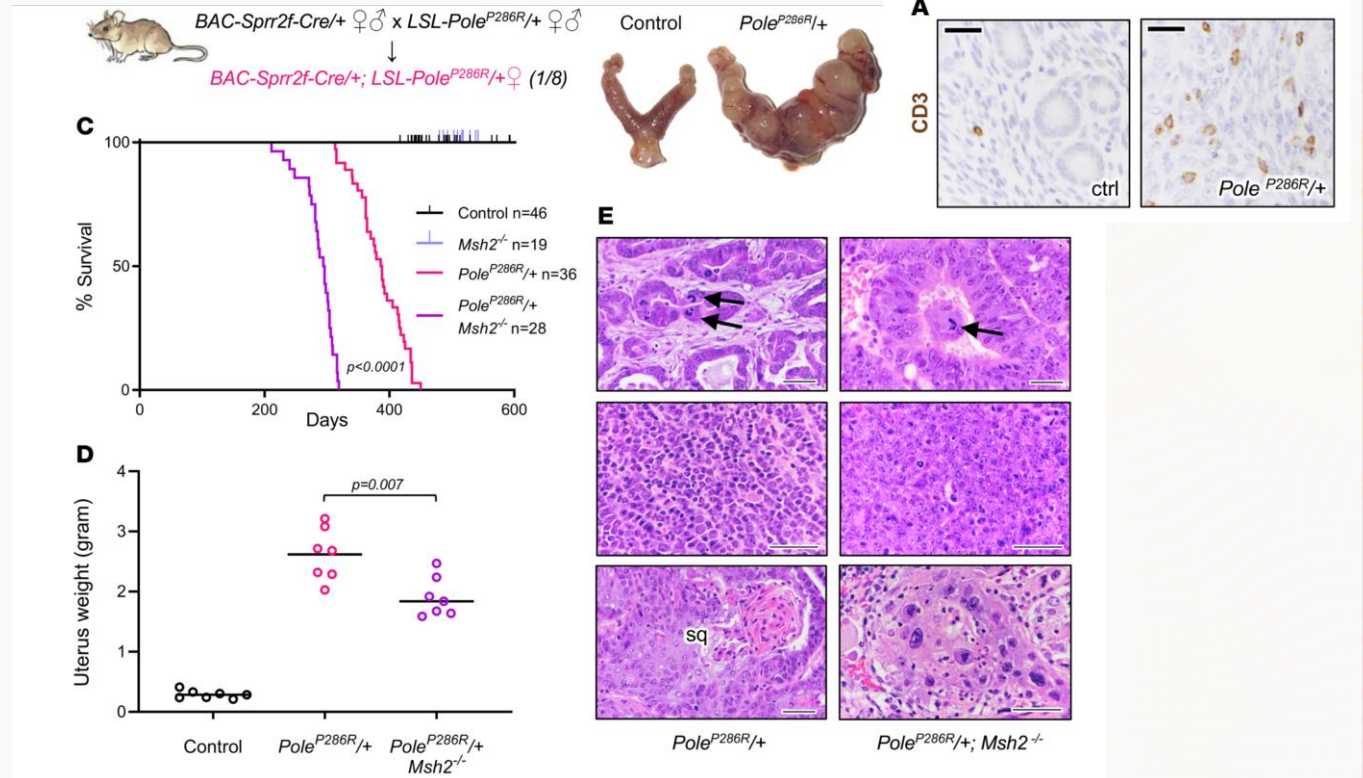
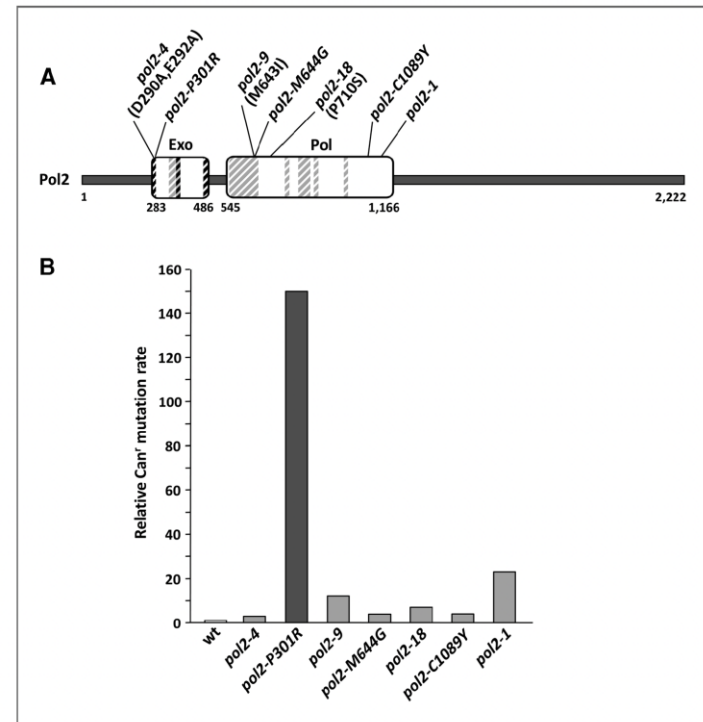
Hotspot mutations (likely to be oncogenic based on varying functional evidence)

POLE P286R
POLE V411L/M
POLE S297F
POLE F367S
POLE S459F
POLE L424V/I
POLE A456P
POLE M295R
POLE P436R
POLE M444K
POLE D368Y



<https://www.cbioportal.org/>

POLE functional studies (P286R mutation)



Kane and Shcherbakova, 2014 “A Common Cancer-Associated DNA Polymerase ϵ Mutation Causes an Exceptionally Strong Mutator Phenotype, Indicating Fidelity Defects Distinct from Loss of Proofreading.”

Li et al., 2020 “A PoleP286R Mouse Model of Endometrial Cancer Recapitulates High Mutational Burden and Immunotherapy Response.”

Pathogenicity of genetic variants

POLE score based on TCGA whole exome sequencing data

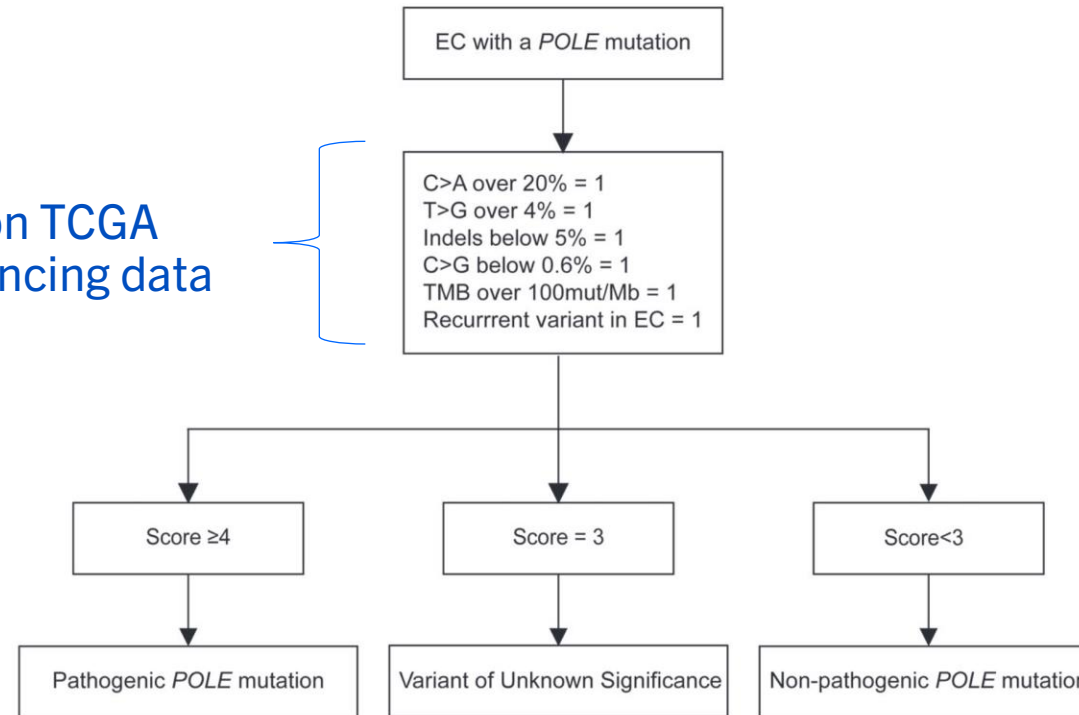
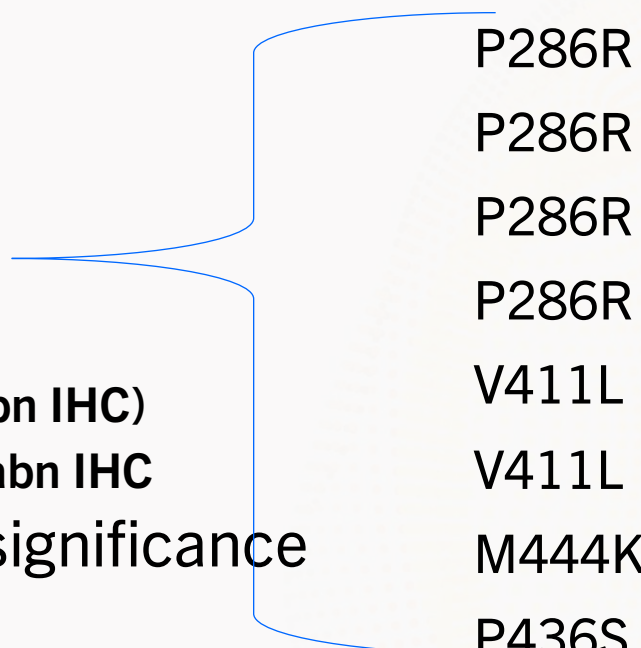


Figure 2. *POLE* genomic alteration score (POLE-score). Diagnostic scoring system based on mutation type proportion and TMB of the five hotspot *POLE* mutations, as well as the variant recurrence.

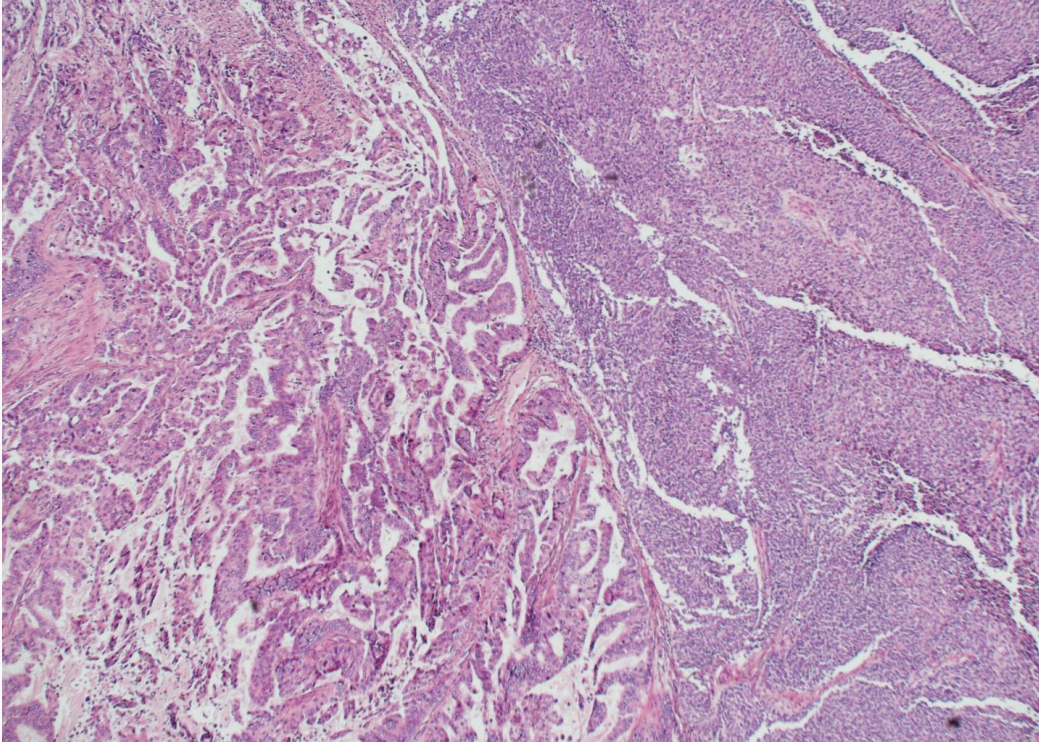
León-Castillo et al., 2020 “Interpretation of Somatic *POLE* Mutations in Endometrial Carcinoma.”

NGS testing of *POLE* in endometrial carcinoma at Austin Pathology

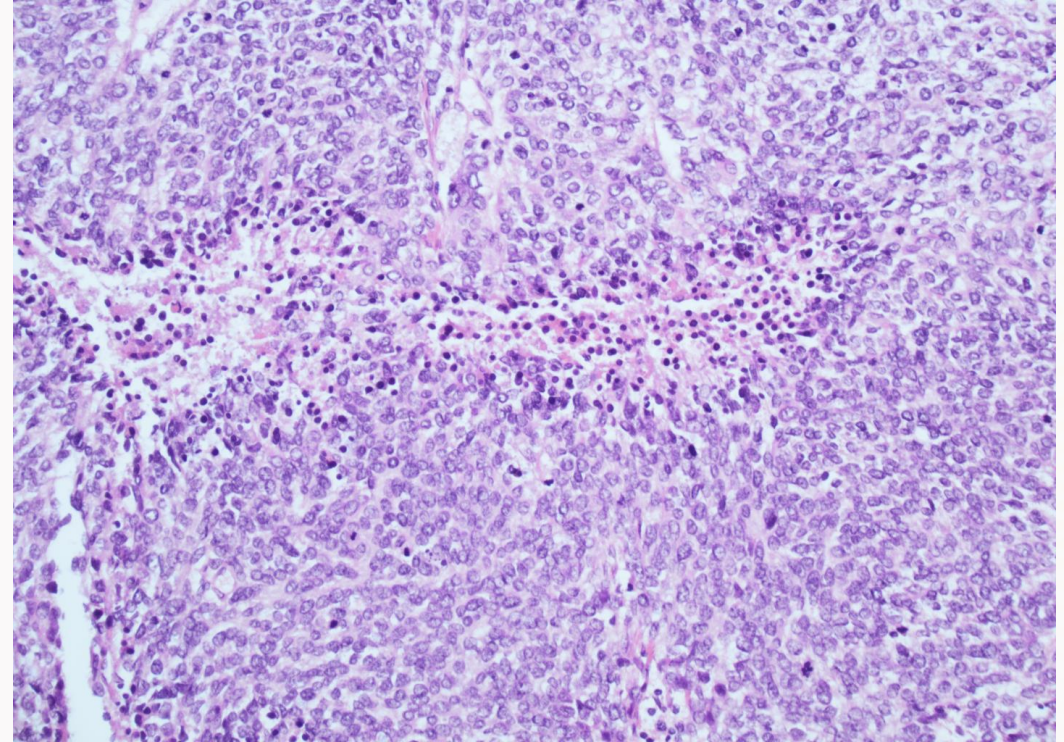
- Custom amplicon-based NGS panel (*POLE*, *TP53*, *PIK3CA*, *ERBB2*)
 - 104 samples tested over 3 years
 - 11 samples with *POLE* variants
 - 8 pathogenic or likely pathogenic (7.7%)
 - all high grade histology
 - 5/8 had concurrent *TP53* mutations (4 with p53abn IHC)
 - 2/8 previously called serous carcinoma, with p53abn IHC
 - 3 exonuclease domain variants of uncertain significance
- 
- | |
|-------|
| P286R |
| P286R |
| P286R |
| P286R |
| V411L |
| V411L |
| M444K |
| P436S |

POLE mutant example

Endometrioid carcinoma FIGO grade 3

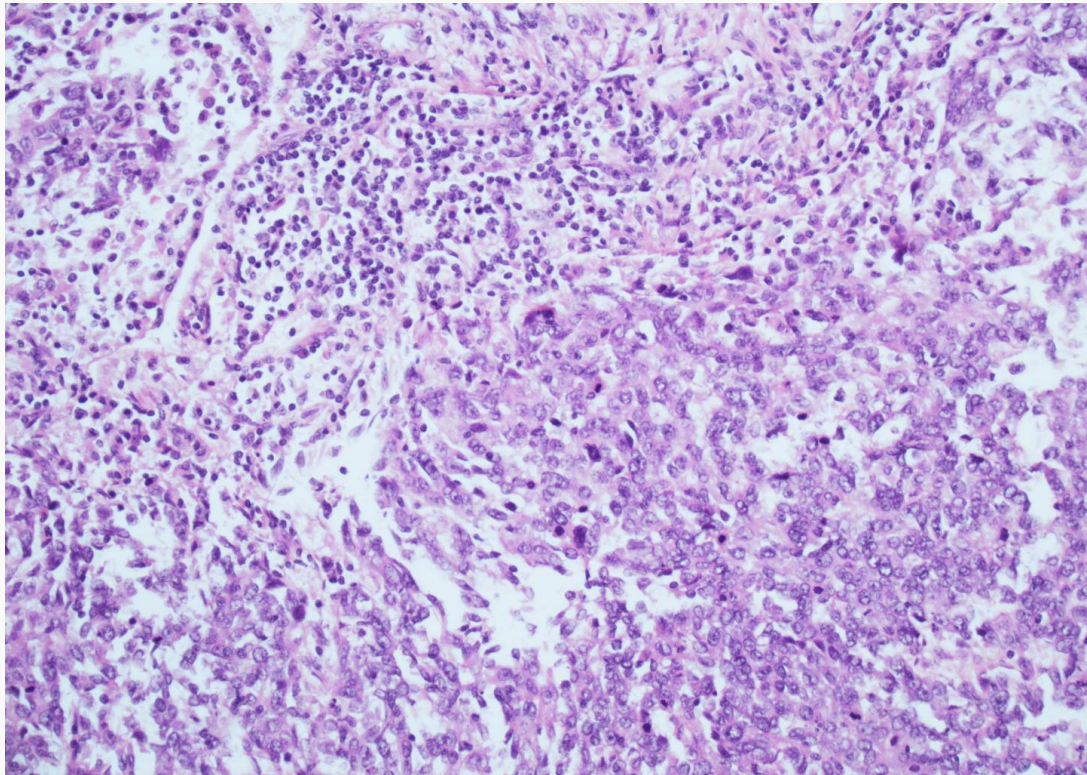


Solid morphology with necrosis

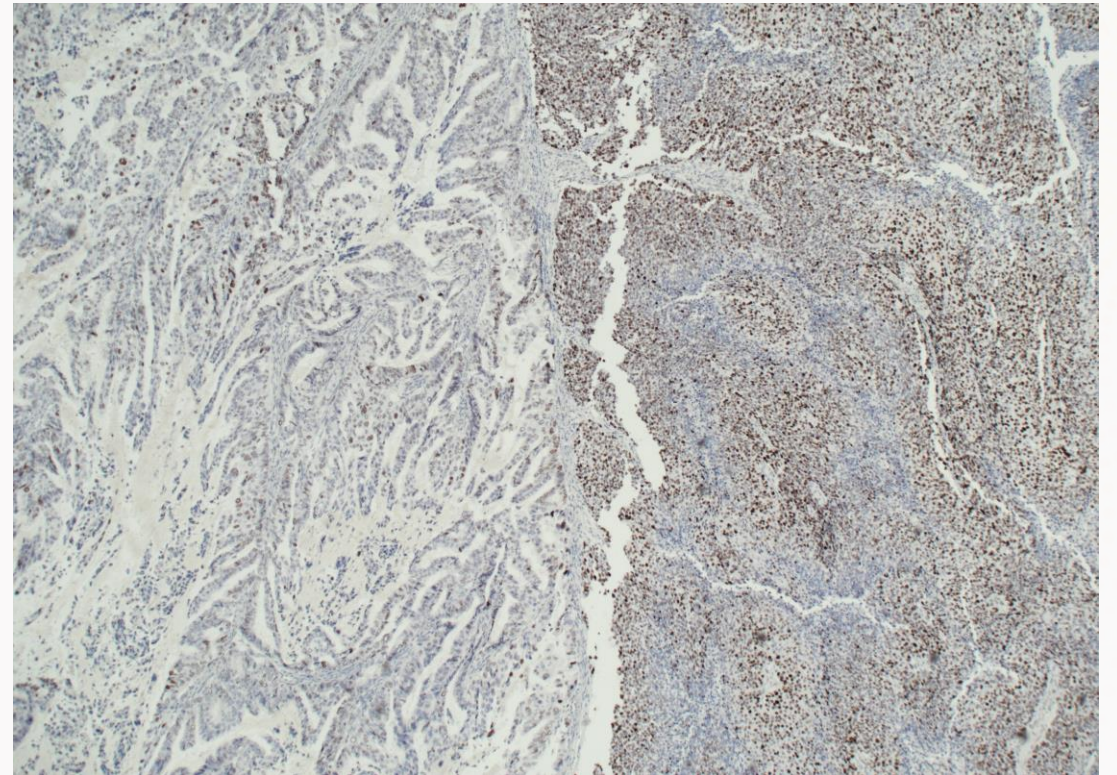


***POLE* mutant example**

Lymphoid infiltrates



P53 immunohistochemistry



POLE mutant example

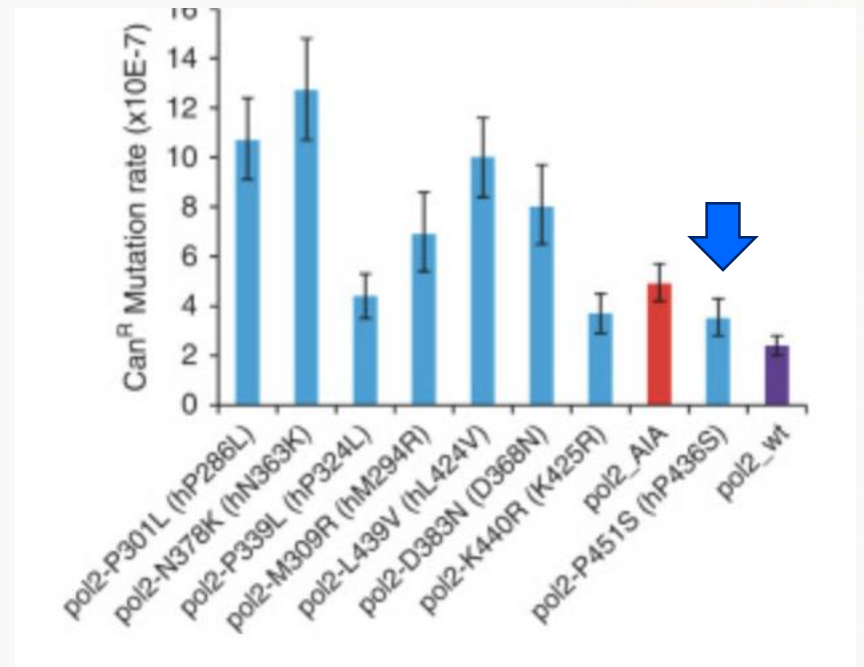
Actions	Classification	Report	Artifact	Symbol	HGVSp	HGVSc	Genomic Location	Ref/Alt Allele	Exon	Depth	AO	AF	UDP
	Tier II	<input checked="" type="checkbox"/>		TP53	p.Pro278Ser	c.832C>T	chr17:7577106	G / A	8/11	252	94	0.3730	130
		<input type="checkbox"/>	<input type="checkbox"/>	GNAS	p.Arg228Cys	c.682C>T	chr20:57484598	C / T	9/13	540	29	0.0537	143
		<input type="checkbox"/>	<input type="checkbox"/>	BRAF	p.Arg509Ter	c.1525C>T	chr7:140476881	G / A	13/18	458	83	0.1812	149
		<input type="checkbox"/>	<input type="checkbox"/>	MAP2K1	p.Arg260Met	c.779G>T	chr15:66777413	G / T	7/11	605	23	0.0380	164
	Tier III	<input type="checkbox"/>	<input type="checkbox"/>	PIK3CA	p.Lys986Asn	c.2958G>T	chr3:178951903	G / T	21/21	756	19	0.0251	169
	Tier III	<input type="checkbox"/>	<input type="checkbox"/>	TP53		c.-29+5G>A	chr17:7590690	C / T		538	119	0.2212	179
		<input type="checkbox"/>	<input type="checkbox"/>	POLD1	p.Pro300=	c.900G>A	chr19:50905928	G / A	8/27	681	144	0.2115	213
	Tier IV	<input type="checkbox"/>	<input type="checkbox"/>	TP53	p.Gln5His	c.15G>T	chr17:7579898	C / A	2/11	724	94	0.1298	244
	Tier II	<input checked="" type="checkbox"/>	<input type="checkbox"/>	PIK3CA	p.Glu545Asp	c.1635G>T	chr3:178936093	G / T	10/21	883	371	0.4202	254
		<input type="checkbox"/>	<input type="checkbox"/>	HRAS	p.Ala59Thr	c.175G>A	chr11:533881	C / T	3/6	888	385	0.4336	265
		<input type="checkbox"/>	<input type="checkbox"/>	ALK	p.Ala1126Thr	c.3376G>A	chr2:29445457	C / T	21/29	1051	403	0.3834	292
		<input checked="" type="checkbox"/>		POLE	p.Pro436Ser	c.1306C>T	chr12:133250214	G / A	13/49	815	356	0.4368	296
		<input type="checkbox"/>	<input type="checkbox"/>	KRAS	p.Gly12Ala	c.35G>C	chr12:25398284	C / G	2/6	1050	471	0.4486	336
		<input type="checkbox"/>	<input type="checkbox"/>	TERT			chr5:1295228	G / T		1438	603	0.4193	356
		<input type="checkbox"/>	<input type="checkbox"/>	MAP2K1	p.Val127Met	c.379G>A	chr15:66729171	G / A	3/11	1161	454	0.3910	361

POLE mutant example (P436S)

Campbell, Brittany B., Nicholas Light, David Fabrizio, Matthew Zatzman, Fabio Fuligni, Richard de Borja, Scott Davidson, et al. "Comprehensive Analysis of Hypermutation in Human Cancer." *Cell* 171, no. 5 (November 16, 2017): 1042-1056.e10.

Hamzaoui et al. "Genetic, Structural, and Functional Characterization of POLE Polymerase Proofreading Variants Allows Cancer Risk Prediction." *Genetics in Medicine* 22, no. 9 (September 1, 2020): 1533–41.

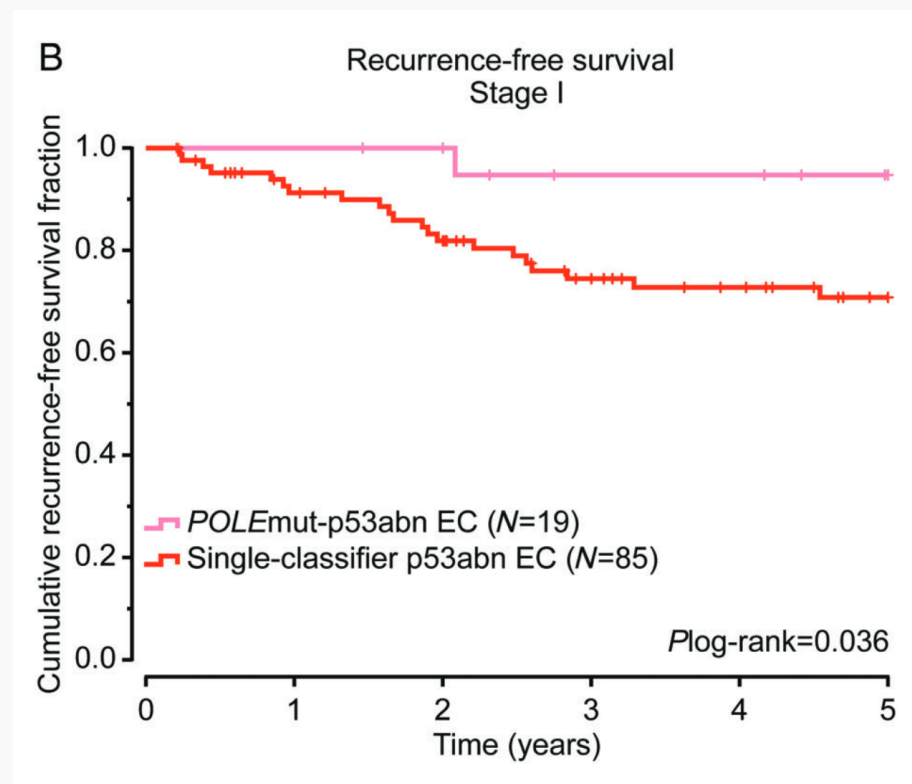
	A	B	C	D	E	F	G
1	Specimen ID	Tumor Type	Gene	Driver Mutation	Mutation Burden	Stage	Status
41	39	Uterus endometrial adenocarcinoma (NOS)	POLE	S459F	102.7	Adult	Known
42	40	Duodenum adenocarcinoma	POLE	S459F	26.9	Adult	Known
43	41	Brain glioblastoma (GBM)	POLE	S297F		Adult	Known
44	42	Brain glioblastoma	POLE	S297F		Pediatric	Known
45	43	Skin melanoma	POLE	S297F		Adult	Known
46	44	Uterus endometrial adenocarcinoma (NOS)	POLE	S297F	39.6	Adult	Known
47	45	Colorectal Carcinoma	POLE	P436S	433	Adult	New
48	46	Brain glioblastoma (GBM)	POLE	P436S	333.3	Adult	New
49	47	Brain glioblastoma	POLE	P436S	318.06	Pediatric	New
50	48	Brain glioblastoma	POLE	P436S	302	Pediatric	New
51	49	Osteochondroma	POLE	P436S	195	Pediatric	New
52	50	Colon adenocarcinoma (CRC)	POLE	P436R	493.7	Adult	New
53	51	Brain glioblastoma	POLE	P436H	541.36	Pediatric	New
54	52	Brain glioblastoma	POLE	P436H	532	Pediatric	New
55	53	Brain glioblastoma	POLE	P436H	409.18	Pediatric	New
56	54	Brain glioblastoma	POLE	P436H	359	Pediatric	New
57	55	Skin basal cell carcinoma	POLE	P286S	58.6	Adult	New
58	56	Unknown primary melanoma	POLE	P286S	42.3	Adult	New
59	57	Uterus endometrial adenocarcinoma (NOS)	POLE	P286R	493.7	Adult	Known
60	58	Colon adenocarcinoma (CRC)	POLE	P286R	479.3	Adult	Known
61	59	Brain glioblastoma (GBM)	POLE	P286R	450.5	Adult	Known
62	60	Colon adenocarcinoma (CRC)	POLE	P286R	394.6	Adult	Known



*POLE*mut-p53abn multiple classifier

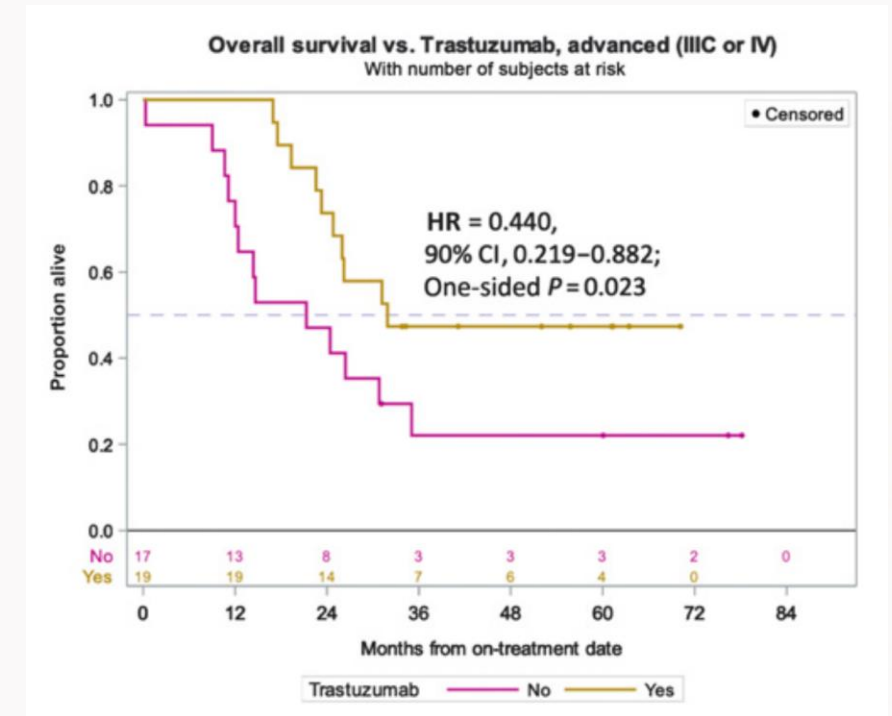
León-Castillo, et al. “Clinicopathological and Molecular Characterisation of ‘Multiple-classifier’ Endometrial Carcinomas.” *The Journal of Pathology* 250, no. 3 (March 2020): 312–22.

POLE mutation + p53 abnormal = *POLE* mutant subgroup



Her2/ERBB2 status

- Her2/ERBB2 amplified in 25% (18-42%) of serous or serous-like carcinomas
- Her2/ERBB2 amplified associated with poorer prognosis
- PFS improved for trastuzumab (added to chemo) in patients with advanced/recurrent Her2 positive uterine serous carcinoma (Fader et al 2018, PMID:29584549)
- NCCN 2024 recommendations for Her2 IHC:
 - All serous and carcinosarcomas
 - All p53 abnormal carcinomas regardless of histology
 - Reflex to FISH for equivocal IHC



Fader et al., 2020

Assessment of Her2

Buza, Natalia. "HER2 Testing in Endometrial Serous Carcinoma: Time for Standardized Pathology Practice to Meet the Clinical Demand." *Archives of Pathology & Laboratory Medicine* 145, no. 6 (July 9, 2020): 687–91.

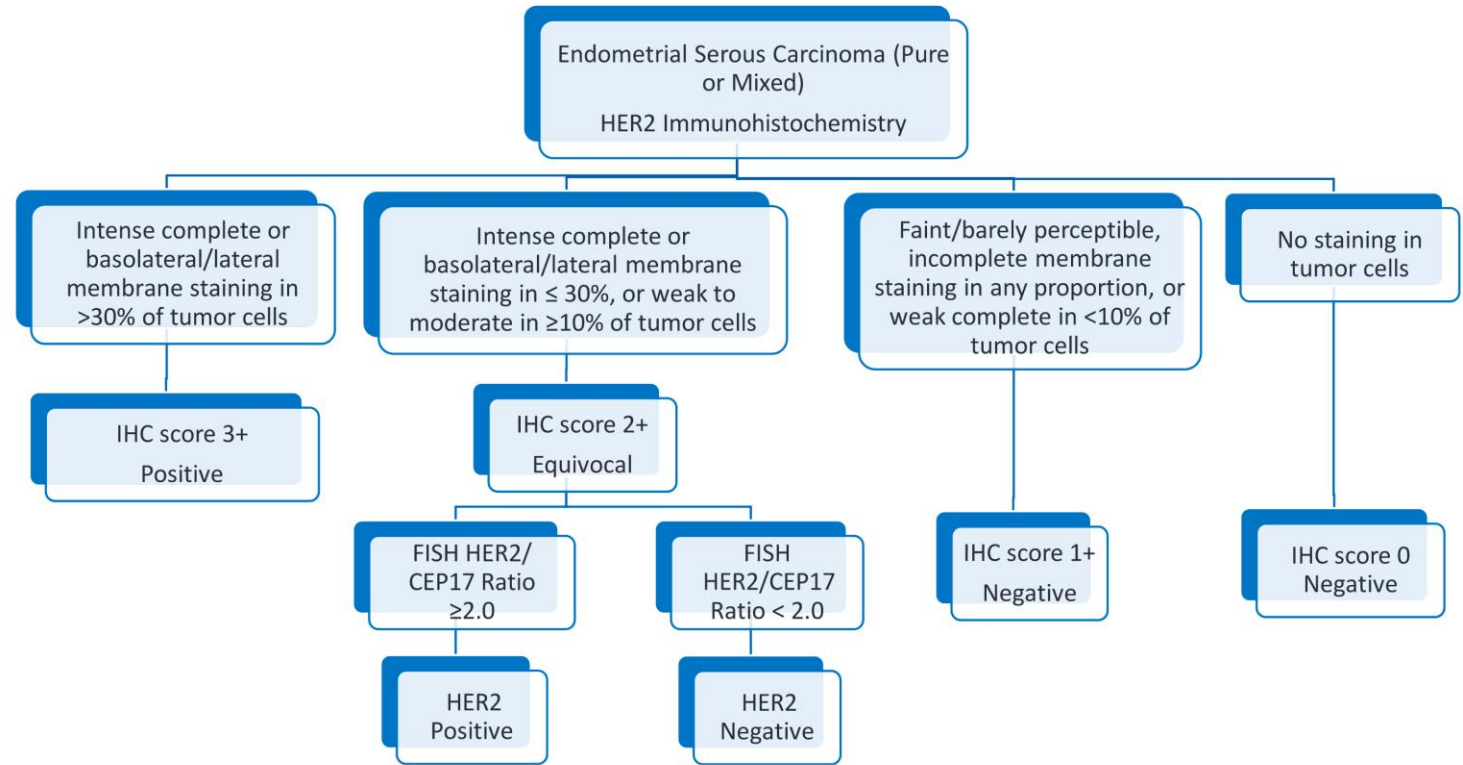
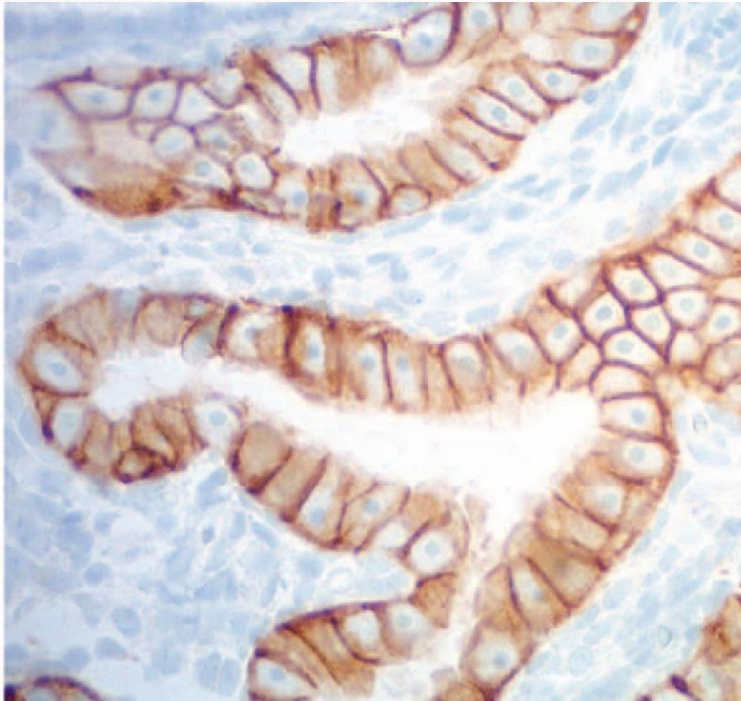


Figure 3. Proposed human epidermal growth factor receptor 2 (HER2) testing algorithm for endometrial serous carcinoma based on the clinical trial patient enrollment criteria. Abbreviations: FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.

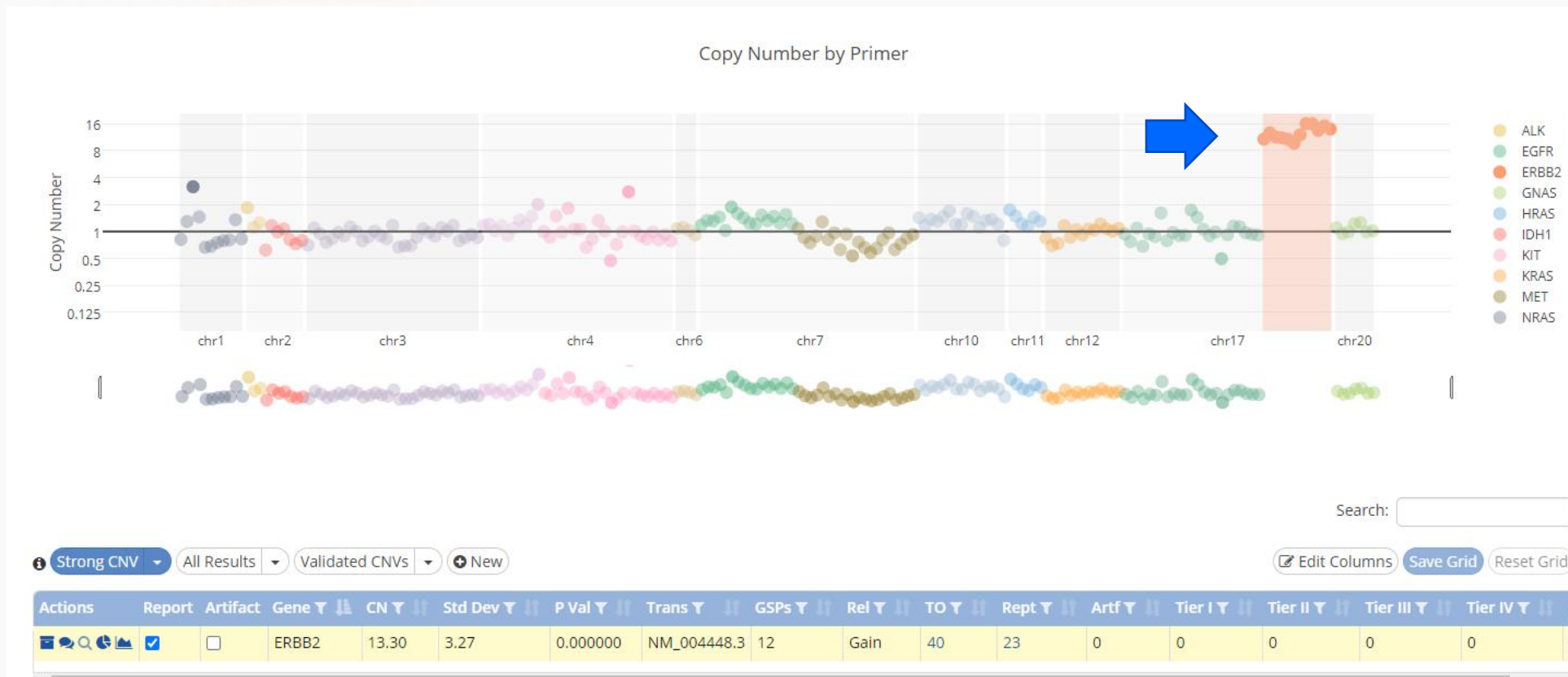
Klc et al., 2022 "HER2 in Uterine Serous Carcinoma." NGS has a 91.6% concordance with ISH

ERBB2/Her2 gains on NGS vs Histo

Tumour purity of sample	NGS relative copy number	Her2:CEP17 ratio (SISH)	Her2 IHC
25%	8.29	7.53	3+
50%	5.2	6.06	3+
40%	10.72	5.19	3+
25%	2.15	6.21	3+
70%	13.2	13.04	3+
80%	1.64	1.45	2+

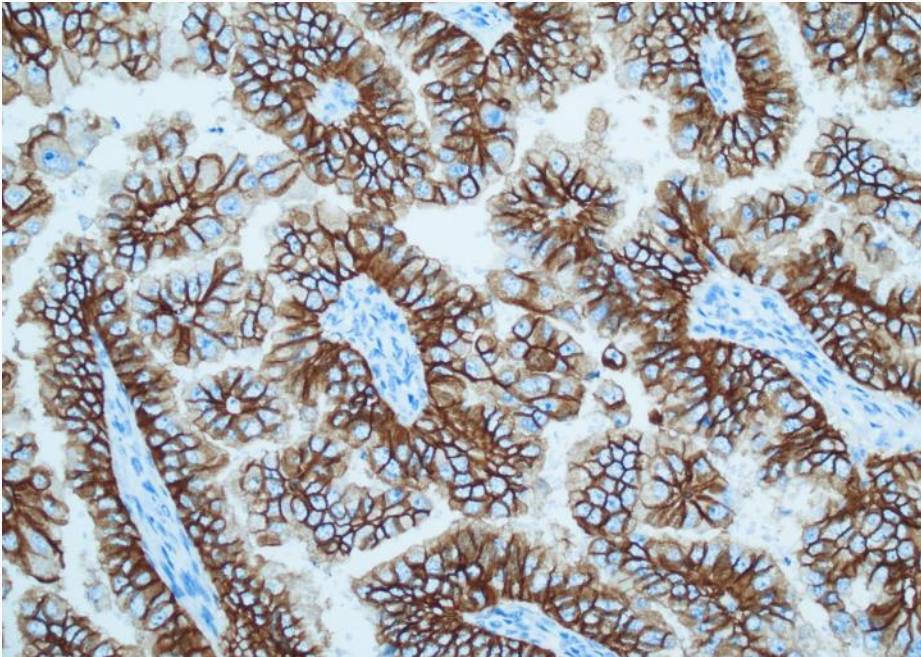


Her2/*ERBB2* amplification example



Her2/*ERBB2* amplification example

Her2 Immunohistochemistry (3+)



HER2 SISH +



Ancillary testing at Austin Pathology

- Immunoprofile (MMR, p53, ER, PR, other)
- If MLH1 deficient we check for MLH-1 promoter methylation (excludes germline mutation)
- Her2 IHC (+/-SISH) on majority of serous, carcinosarcomas and p53 abnormal carcinomas – particularly advanced or metastatic disease
- NGS panel (*POLE*, *TP53*, *PIK3CA*, *ERBB2*) – particularly high grade histology with early stage, p53abn

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Dr Alison Skene

A/Prof Kerry Ireland-Jenkin

A/Prof David Williams

Dr Julie Lokan

Dr Chris Hogan

Austin

HEALTH | Pathology

Key References

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- Fader, et al. “Randomized Phase II Trial of Carboplatin–Paclitaxel Compared with Carboplatin–Paclitaxel–Trastuzumab in Advanced (Stage III–IV) or Recurrent Uterine Serous Carcinomas That Overexpress Her2/Neu (NCT01367002): Updated Overall Survival Analysis.” *Clinical Cancer Research* 26, no. 15 (August 3, 2020): 3928–35. <https://doi.org/10.1158/1078-0432.CCR-20-0953>.
- Kommoss, et al. “Final Validation of the ProMisE Molecular Classifier for Endometrial Carcinoma in a Large Population-Based Case Series.” *Annals of Oncology* 29, no. 5 (May 1, 2018): 1180–88. <https://doi.org/10.1093/annonc/mdy058>.
- Klc, et al. “HER2 in Uterine Serous Carcinoma: Testing Platforms and Implications for Targeted Therapy.” *Gynecologic Oncology* 167, no. 2 (November 2022): 289–94. <https://doi.org/10.1016/j.ygyno.2022.09.006>.
- Levine, Douglas A. “Integrated Genomic Characterization of Endometrial Carcinoma.” *Nature* 497, no. 7447 (May 2013): 67–73. <https://doi.org/10.1038/nature12113>.
- León-Castillo, Alicia, Heidi Britton, Melissa K McConechy, Jessica N McAlpine, Remi Nout, Stefan Kommoss, Sara Y Brucker, et al. “Interpretation of Somatic *POLE* Mutations in Endometrial Carcinoma.” *The Journal of Pathology* 250, no. 3 (March 2020): 323–35. <https://doi.org/10.1002/path.5372>.