Targeted Next-Generation Sequencing Panel Reveals Differences in Mutational Patterns between Endometrial Cancer Molecular Classifier Subgroups

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Background

Four molecular subgroups of endometrial carcinomas (ECs) are currently recognized based on prognostic classification derived from The Cancer Genome Atlas (TCGA) study using multi-omic analysis:

- Hypermutated (Mismatch repair deficient, MMRd)
- Ultramutated (*POLE* mutated)
- Copy number high (p53 abnormal)
- Copy number low (No specific molecular profile, NSMP)

These subgroups can be recapitulated by a targeted testing approach using the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) stratification. The National Comprehensive Cancer Network (NCCN) guidelines currently encourage the use of ancillary studies to detect aberrancies in *POLE*, *TP53*, and MMR/MSI. Here, we assign ProMisE molecular subgroups using combined immunohistochemistry (IHC) and next generation sequencing (NGS). We demonstrate the ability to ascribe defined molecular spectra to each subtype. Furthermore, we demonstrate preliminary data to suggest *KRAS* may be associated with more advanced disease.

Methods

From November 2020 to December 2021, 188 EC specimens across all stages and histotypes underwent molecular subtyping using a combination of p53 and MMR IHC and NGS with a customized Archer VariantPlex assay designed to include *POLE* (Fig. 1, Table 1). Unadjusted mutational load was derived by counting the absolute number of confirmed mutations across roughly 45Kb of targeted sequence from 56 genes. Variant allele frequency (VAF) of *TP53* mutations was used as a surrogate measure for loss of heterozygosity (LOH). Statistical analysis of mutational load was performed using the Kruskal-Wallis test with post hoc multiple comparisons correction. PI3K pathway alterations were assessed using the Chi-square test. Lymphovascular invasion (LVI) and *KRAS* status was analyzed using Fisher's exact test. All statistical analyses were performed with GraphPad Prism version 9.4.1.

Figure 1: Workflow for Establishing Molecular Subgroups

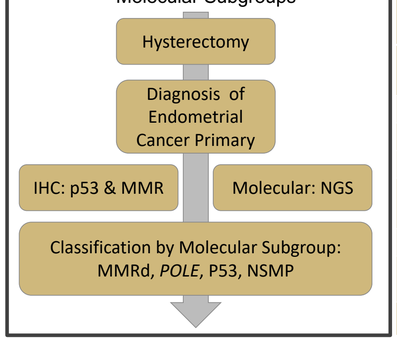


Table 1. Breakdown of endometrial cancers by histotype

Histotype	Number of Specimens
Endometrioid	149
Serous	17
Carcinosarcoma	15
Undifferentiated/ Dedifferentiated	4
Clear cell	2
Mesonephric-like adenocarcinoma	1
Total	**188**

Table 2. Distribution of Molecular Subgroups by Endometrial Cancer Histotype

Histotype	**MMRd**	***POLE***	**p53**	**NSMP**	Total (histotype)
Endometrioid	46 (24.8%)	10 (4%)	10 (6.7%)	84 (56.4%)	149
Serous	n/a	n/a	17 (100%)	n/a	17
Carcinosarcoma	1 (6.7%)	n/a	12 (80.0%)	1 (6.7%)	15
Undifferentiated/ Dedifferentiated	4 (100%)	n/a	n/a	n/a	4
Clear cell	n/a	n/a	1 (50%)	1 (50%)	2
Mesonephric-like adenocarcinoma	n/a	n/a	n/a	1 (100%)	1
Total (Molecular subtype)	51 (27.1%)	10 (5.3%)	40 (21.3%)	87 (46.3%)	188

Dual classifiers comprised 17.6% (9/51) of MMRd and 40% (4/10) of *POLE* tumors (Table 2). For all analyses, MMRd-p53 and *POLE*-p53 tumors were incorporated into the MMRd and *POLE* molecular subgroups, respectively.

Results

The *POLE* (ultramutated) subgroup had a greater average mutational load than the MMRd (hypermutated) subgroup (P=0.0097) (Fig. 2). Similarly, the molecular load of MMRd subgroups was greater than p53 and NSMP (P=0.001 and P=0.0064, respectively). No difference was observed between p53 and NSMP. When evaluated by histotype, endometrioid tumors had more mutations than serous tumors (P=0.0043) (Fig. 3).

Table 3. Molecular subgroups harbor PI3K pathway activation: *PTEN*, *PIK3CA*, *PIK3R1*, &/or *ATK1* mutations*

Molecular subgroup	One gene	Two genes	Three genes	Four genes	PI3K Pathway Activation
MMRd	9	35	4	1	**96.1%**
POLE	2	6	2	0	**100%**
p53	18	10	0	0	**70%**
NSMP	14	64	5	0	**95.4%**
No. altered tumors	43	115	11	1	170/188 (90.4%)

*No statistically significant differences in activating mutations was identified among the four molecular subgroups (P=0.6373).

Table 4. The p53 molecular subgroup harbors fewer kinase and B-catenin activating mutations

Molecular subgroup	***KRAS***	***FGFR2***	***ERBB2***	***BRAF***	***CTNNB1***	**Activating Mutations***
MMRd	16 (31.4%)	11 (21.6%)	5 (9.8%)	0 (0%)	3 (5.9%)	**56.9%**
POLE	3 (30%)	2 (20%)	0 (0%)	3 (30%)	2 (20%)	**70%**
p53	5 (12.5%)	2 (5%)	0 (0%)	0 (0%)	2 (5%)	**22.5%**
NSMP	24 (27.6%)	11 (12.6%)	1 (1.1%)	3 (3.4%)	27 (31%)	**67.8%**

*Percentages represent the proportion of tumors with one or more activating mutations. 16% of tumors with activating mutations harbored mutations in more than one gene. Only single gene activation was observed in the p53 subgroup.

Figure 2. *POLE* and MMRd molecular subgroups have higher mutational loads

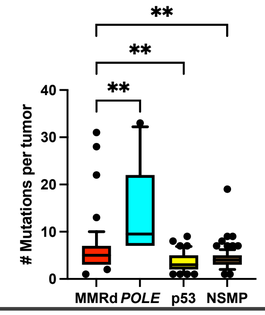
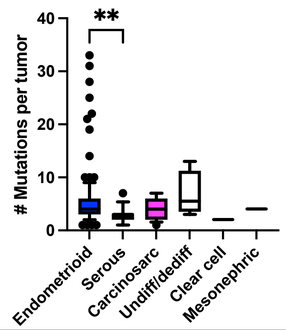


Figure 3. Endometrioid tumors have higher mutational loads than Serous tumors



Results

Table 5. p53-classified tumors may preferentially undergo LOH

Molecular subgroup	# tumors w *TP53* mut	*TP53* VAF % Average (min, max)	Percent cases with *TP53* VAF >50%
MMRd	9	29.7% (6.6%, 72.5%)	11.1%
POLE	4	26.5% (14%, 32.2%)	0%
p53	40	47.3% (5.5%, 94.4%)	42.5%

KRAS status was associated with lymphovascular invasion (P=0.0012) (Fig. 4). *KRAS* mutations were observed in 46 (32.4%) of EC tumors with no significant difference in frequency among groups (P=0.1882) (Fig. 5).

Figure 4. *KRAS* mutation status positively associates with lymphovascular invasion

Figure 5. No difference in *KRAS* status among molecular subgroups

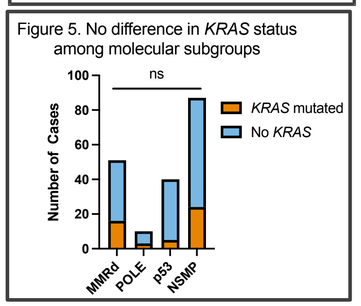


Table 6. G12D & G12V are the most frequent *KRAS* variants

KRAS variant	Number of occurrences	% *KRAS* mutations
G12D	13	28.3%
G12V	12	26.1%
G13D	7	15.2%
G12C	4	8.7%
G12A	3	6.5%
G13C	3	6.5%
Q61H	2	4.3%
A59G	1	2.2%
E63K	1	2.2%
Total	46	100%

Conclusions

- ProMisE classification can be feasibly incorporated into standard clinical workflow.
- NGS may identify clinicopathologic trends and potentially targetable alterations.
- p53 VAFs may represent a combination of higher LOH in the p53 subgroup and subclonality in the MMRd and *POLE* subgroups. Dual classifier tumors may represent *TP53* 'passenger' events rather than genomic instability.
- *KRAS* mutations may be associated more advanced disease, as demonstrated by positive association with LVI and lymph node (data not shown) status.
- Limitations of this study include small sample size for many of the tumor histotypes and molecular subgroups.

References

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