Development of a single-test molecular classifier for endometrial carcinoma using an amplicon-based gene panel and next generation sequencing technology

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Objective

Molecular classification of endometrial carcinoma (EC) is now recommended by the WHO[1],ESGO/ESTRO/ESP and NCCN guidelines. The pragmatic molecular classification tool**, ProMisE (Proactive Molecular Risk Classifier for Endometrial Cancer**)[2], identifies four molecular subtypes based on next generation sequencing (NGS) for the detection of somatic pathogenic *POLE* mutations, and immunohistochemistry for mismatch repair and p53 proteins. ProMisE provides valuable prognostic and predictive information to direct care[3], however, multiple molecular results are often received from different time periods and/or from different centers which can cause delays. Therefore, we developed a classifier that relies on a single DNA-based NGS test with the goal of recapitulating the prognostic value of ProMisE by producing concordant results.

Methods

- FFPE tumor DNA extracted from 164 ECs were sequenced using the clinically validated Imagia Canexia Health Find It™ amplicon-based NGS 38 gene panel that detects targeted hotspots in single nucleotide variants (SNVs), indels (insertions and deletions), gene amplification and microsatellite instability (MSI) using 21 MSI loci. The panel was sequenced on an Illumina MiSeq™.
- The ProMisE NGS panel assesses for *POLE* and *TP53* mutations and microsatellite instability (MSI)
- ProMisE NGS somatic results were compared to the original ProMisE molecular classification assessing *POLE* mutations, and IHC for p53 and MMR
- Molecular subtypes assigned by both classifiers were assessed for concordance metrics and Kaplan-Meier survival curves

SNVs & Indels (37 genes)						CNVs (9 genes)		MSI
AKT1	*ALK*	*AR*	*BRAF*	*CTNNB1*	*DICER1*	*CCNE1*	*EGFR*	21 loci
DDR2	*EGFR*	*ERBB2*	*ESR1*	*FGFR1*	*FGFR2*	*ERBB2*	*FGFR1*	
FGFR3	*FOXL2*	*GNA11*	*GNAQ*	*GNAS*	*HRAS*	*FGFR2*	*KIT*	
IDH1	*IDH2*	*KIT*	*KRAS*	*MAP2K1*	*MAP2K2*	*KRAS*	*MET*	
MET	*NRAS*	*NTRK1*	*NTRK3*	*PDGFRA*	*PIK3CA*	*PIK3CA*		
POLE	*PTCH1*	*PTEN*	*RET*	*ROS1*	*STK11*			
*TP53**								

Figure 1. Imagia Canexia Health Find It™ gene content with targeted hotspots. CNVs detect amplification only, T*P53** covers all exons.

ProMisE NGS molecular subtype assignment

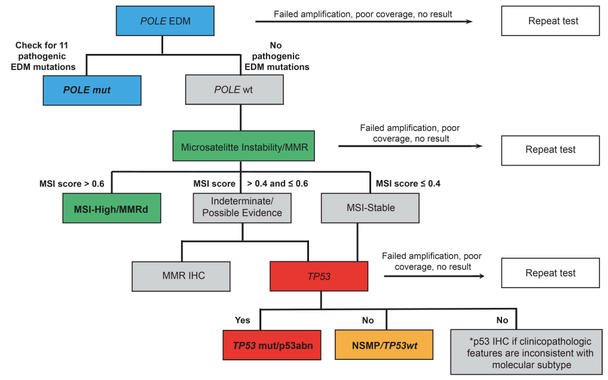
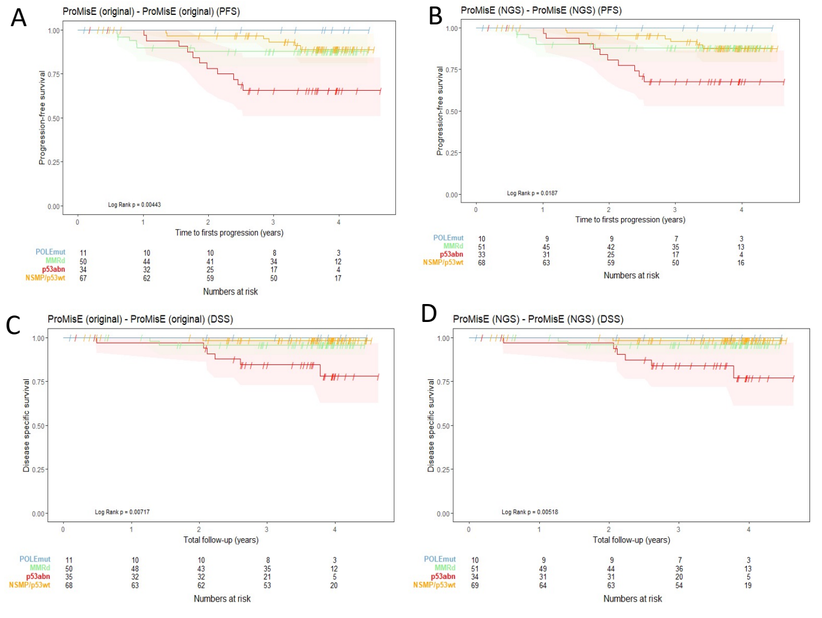


Figure 2. Classification tree for ProMISE NGS assigning molecular subtype. The order of molecular assignment is important for cases with multiple features such as a MSI-high/MMRd sample with *TP53* mutations. EDM: exonuclease domain mutations



ProMisE NGS maintains similar survival curves

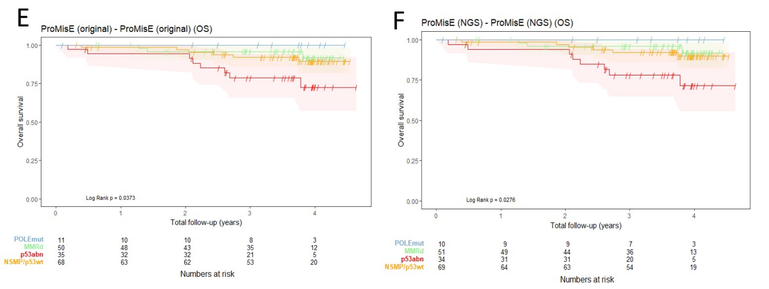


Figure 3. Kaplan-Meier survival curves for the original ProMisE and ProMisE NGS A-B. Progression-free survival (PFS), C-D. Disease-free survival (DSS), E-F. Overall survival (OS).

Summary of Results

- ProMisE NGS results showed 10 (6.1%) *POLE*mut, 51 (31.1%) MSI/MMRd, 34 (20.7%) *TP53*mut/p53abn ECs, and 69 (42.1%) NSMP
- 159/164 (97%) of cases were concordant with kappa statistic of 0.96 and overall accuracy of 0.97
- 15/15 (100%) were concordant between biopsy and hysterectomy specimens
- Two cases initially did not pass coverage QC, but passed upon repeat testing
- ProMisE NGS Kaplan-Meier survival analysis showed statistical differences between molecular subtype groups and recapitulates similar curves compared to the original ProMisE classifier
- 5 discordant cases were reviewed.
- 2 false negative *TP53*mut/p53abn by NGS likely due to large indels
- 1 NSMP with a *TP53* mutation. Sequencing normal indicated a germline SNP
- 1 NSMP shown as MSI-High by NGS. Orthogonal testing confirmed as MSI-High
- 1 rare *POLE*mut (p.P436R) was missed by ProMisE NGS due to primer coverage.

The ProMisE NGS molecular classifier is feasible and shows similar results to the original endometrial molecular classifier. Reliable molecular results can be obtained at diagnosis from a single test to provide important prognostic information to clinicians and for all patients with endometrial cancer

References

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3. Jamieson A, Huvila J, Leung S, et al. Gynecologic Oncology Molecular subtype strati fi ed outcomes according to adjuvant therapy in endometrial cancer. *Gynecol Oncol*. 2023;170:282-289. doi:10.1016/j.ygyno.2023.01.025