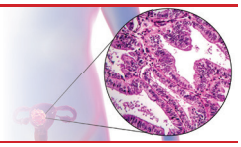
THE PATHCARE NEWS

MOLECULAR SUBTYPING OF ENDOMETRIAL CARCINOMA



The Cancer Genome Atlas (TCGA) classifies endometrial cancer into four molecular subtypes: **(1) POLE ultra-mutated, (2) Microsatellite unstable, (3) Abnormal p53 expression (p53 Abn) and (4) No specific molecular profile (NSMP),** forming the basis of the Proactive Molecular Risk Classifier for Endometrial Cancer[1,2] (ProMisE). Molecular subtyping can assist with predicting treatment outcomes and prognosis of early endometrial cancer and is recommended by the National Comprehensive Cancer Network (NCCN) guidelines[3].

Molecular Subtypes

- **POLE ultra-mutated:** Inactivating *POLE* mutations in the exonuclease domain occur in 5-15% of endometrial cancers, and are associated with high tumour mutation burden (TMB >100 mutations/Mb), younger age groups and lower body mass index (BMI). POLE-mutated endometrial tumours are most often endometrioid cancers with high grade and adverse histopathologic features. However, despite the tumour's aggressive histologic appearance, *POLE* mutations confer a favourable overall prognosis.

  Testing for POLE/ POLD1 will assist with:

  - **Prognostication:**
    - These tumours have an excellent prognosis despite high-grade histologic features.
  - **Guiding Treatment Decisions:**
    - POLE-mutated endometrial carcinoma may not benefit from adjuvant chemotherapy, sparing unnecessary side effects in patients[4,5].
    - Immunotherapy (e.g., immune checkpoint inhibitors) shows promise in POLE-mutated endometrial carcinomas due to high tumour mutational burden (TMB)[6].

- **MMRd/MSI-H:** The group of MMRd (Mismatch Repair-deficient) / MSI-H (microsatellite instability-high/ microsatellite unstable) carcinomas accounts for 23–36% of endometrial cancers. Only 2% of this group are associated with Lynch syndrome. The tumour mutation burden is considerably high but not as high as the POLE ultra-mutated group. *PTEN* and *PIK3CA* mutations are also commonly associated with this group. Significant lymphovascular space invasion is more often present in MMRd/MSI-H tumours, and these tumours are associated with intermediate outcomes.

- **P53 Abn:** The p53 Abn subtype typically harbours *TP53* mutations and represents the most aggressive subtype. These tumours are predominantly composed of the serous carcinoma histologic types but may also include high grade endometrioid carcinomas. These tumours have a poor overall prognosis.
  - p53 mutations may also occur as subclones in the MMRd/MSI-H as well as the POLE/POLD1 subtypes. These are regarded as secondary mutations and do not affect the prognosis of these subtypes.
  - The significance of a subclonal p53 mutation in the NSMP subtype is uncertain with regards to prognosis. Some authors regard <10% p53 Abn as subclonal and ≥10% as p53 Abn with poorer prognosis[7].

- **NSMP/ p53 wild-type:** The group of endometrial cancers does not exhibit any of the previously described features and is classified as p53 wild-type or no specific molecular profile (NSMP). This subgroup is found to be heterogeneous since it covers the remaining tumours. These tumours are predominantly of endometrioid histologic type and express oestrogen and progesterone receptors. The majority of endometrial cancers (30–60%) fall into this subtype. Patients with p53 wild-type tumours generally show a poorer progression free survival than patients in the POLE ultra-mutated and MMRd/MSI-H groups.

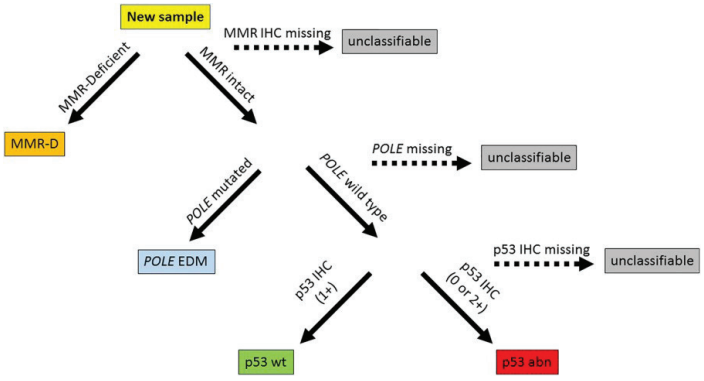


Figure 1 A stepwise approach for molecular subtyping using Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), considering prohibitive costs and limited tissue for testing (illustration from Talhouk et al. 2017)

Available Tests to Assist with Molecular Subtyping:

- **POLE/POLD1 Mutation Assay** performed on the Biocartis Idylla™ System tests for **17 *POLE* mutations** in the exonuclease domain across 4 exons: Exon 9: P286H, P286L, P286R, P286S, M295R, S297F; Exon 11: F367S, F367V, D368Y; Exon 13: V411L (c.1231 G>C and c.1231 G>T), L424I, L424V, P436R, M444K; Exon 14: A456P, S459F. Additionally, the assay detects **1 *POLD1* mutation** S478N in exon 12. This is the first stand alone test for *POLE/POLD1* to be available in South Africa. This is not part of a larger NGS panel, making it **more cost effective and providing a shorter turnaround time.**
- **MMR deficiency (MMRd)** testing by immunohistochemistry (IHC) is used to detect the loss of MMR protein expression. In case of no abnormality detected on IHC, **MSI PCR** can also be requested to determine microsatellite instability (MSI-High or MSI-stable) based on the number of altered lengths of microsatellite repeats.
- **p53 IHC** to evaluate the presence of mutant type staining pattern (overexpression, null type or cytoplasmic staining of the p53 protein). Alternatively, *TP53* mutations causing abnormal p53 expression could be detected by various methods such as next generation sequencing (NGS) and -panels containing the *TP53* gene.

Biocartis Idylla™ System and NGS gene-panels

The Biocartis Idylla™ System is fully automated, covering the entire process from sample-to-result. The system enables the delivery of results within 48 hours of sample receipt at the PathCare molecular laboratory. Various single gene PCR assays are offered by PathCare via the use of the Biocartis Idylla™ System, including POLE/POLD1 Mutation Assay and MSI PCR, which is relevant for endometrial cancer molecular subtyping.

NGS gene-panels can assist with further prognostication and identifying molecular targets to guide targeted therapies.

		Molecular markers recommended by NCCN[3]						
		POLE mutations	dMMR/MSI	p53 aberrations	HER2 aberrations	ER/PR	NTRK fusions	Tumour mutation burden (TMB)
		To complement morphologic assessment regardless of histologic tumour type.			For all p53 aberrant carcinomas regardless of histology.	Stage III, IV and recurrent disease.	For metastatic or recurrent carcinomas.	For metastatic or recurrent carcinomas.
Available assays	IDYLLA POLE/POLD1 PCR	✓						
	MMR IHC		✓ (dMMR)					
	MSI PCR		✓ (MSI)					
	p53 IHC			✓				
	HER2 IHC/FISH				✓			
	ER/PR IHC					✓		
	Oncomine Focus NGS assay				✓		✓	
	Oncomine Comprehensive NGS assay	✓	✓ (MSI)	✓ (TP53 mutations)	✓	✓	✓	✓

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