Managing Difficulties of Microsatellite Instability Testing in Endometrial Cancer-Limitations and Advantages of Four Different PCR-Based Approaches

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STUDY AIM

The aim of the study is to examine four different molecular approaches for MSI testing.

STUDY DESIGN

In this retrospective study, two objectives were assessed (1) comparison of four different molecular technologies using a testing cohort of 25 previously characterized cases (with IHC) and (2) validation of the Idylla™ MSI Assay for detecting MSI status in EC using a validation cohort of 100 previously characterized cases (with IHC) . The four technologies evaluated were an in-house Bethesda, Promega, Idylla™ System and NGS (see Table 1).

RESULTS

COMPARISON OF FOUR DIFFERENT MOLECULAR ASSAYS

Testing cohort 1 (n=25) is composed of 11 ECs with dMMR, 1 with pMMR and 13 with uncertain MMR status.
This cohort was enriched for challenging cases to reveal potential complexities of the systems.

Testing cohort N=25	Overall concordance vs. IHC	Root-cause discordant results / recommendations	Reanalysis
Bethesda, in-house	92%	Discordant results were associated with small additional peak profiles and adapted through expertise in interpretation (second molecular biologist).	100%
Promega v1.2.	80%	Discordant results were associated with small additional peak profiles and adapted through expertise in interpretation (second molecular biologist).	100%
Idylla™	**88%**	**Discordant results were associated with testing preconditions and adapted through use of higher input, either tumor cells and/or higher DNA input.**	**100%**
NGS GeneRead v2	80%	Discordant results were associated with small overall nucleotide shifts and adapted through changing the cut-offs of the algorithm.	100%

Recommendations for Idylla™

Following the manufacturer's instructions, i.e., using at least 25 mm^2 of a 10 µm tissue slice with at least 20% tumor cell content, turned out to not be enough for all EC samples. Therefore, they recommend a higher amount of tumor tissue (50 mm^2, 10 µm slice) with **≥ 40% tumor cell content**, or a minimum of **200 ng extracted DNA.**

100% RESCUE OF UNCERTAIN MMR CASES

IHC still has its limitation due to inconclusive staining results in some of the samples, using PCR-based technologies; MSI status of all 28 samples with uncertain MMR status could be clarified.

SMALLER NUCLEOTIDE SHIFTS IN EC COMPARED TO CRC

Complex MSI profiles of EC were reasoned by either small additional peaks of only 1 or 2 nucleotides or a small overall nucleotide shift, as demonstrated in Figure 1.

VALIDATION IDYLLA™ ON AN EXTENSIVE EC COHORT (N=100) (68 dMMR AND 32 pMMR)

Specificity of **100%**

Sensitivity of **92.6%***

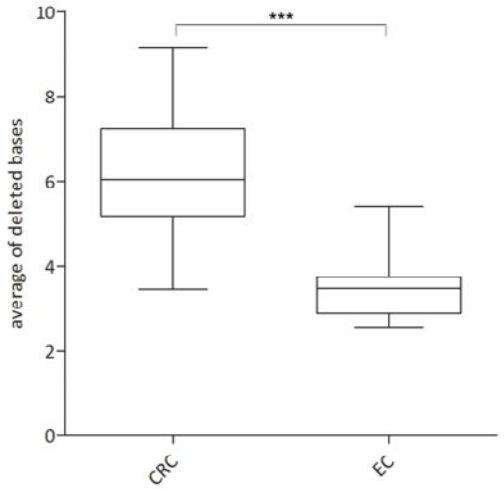
*Siemanowski et al. showed that samples with poor DNA quality or low tumor cellularity led to discordant negatives in MSI status.

Idylla™ provides fast and reliable results if an overall tumor area of more than 50 mm^2 is available and the tumor cell content is higher than 40%. Borderline samples should be tested with an alternative method.
The authors propose a screening strategy for EC as follows: "IHC to detect dMMR as initial analysis should be performed in all EC patients. The loss of expression in only one marker could be related to either MSS or MSI-H in EC. For that reason, follow up testing with a PCR-based method is recommended. Additionally, blurred staining results must be verified using PCR."

Table 1. Materials and methods.

Bethesda In-house	Promega V1.2.	NGS	Idylla™	IHC
BAT25, BAT26, D5S346, D2S123, D17S250	BAT25, BAT26, NR21, NR24, MONO27	MiSeq (illumina)	**ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2**	Ventana Clones MLH1, MSH2, MSH6, PMS2
Fragment length analysis	Capillary electrophoresis	Sequencing	**High resolution melting**	Antibody staining
Extracted DNA	Extracted DNA	Extracted DNA	**Directly FFPE**	Directly FFPE
Interpretation of profiles by expert	Interpretation of profiles by expert	Thresholds defined for MSI-H and MSS	**Threshold defined by fully automated decision tree**	Interpretation of staining patterns by expert
Report MSI-H MSI-L MSS	Report MSI-H MSS	Report Microsatellite instability status	**Report** **MSI-H** **MSS**	Staining 2/4: dMMR 4/4: pMMR 3/4 or irregular: uncertain
Reanalysis by a second molecular biologist	Reanalysis by a second molecular biologist	Reanalysis by adapted cut-off	**Reanalysis with adapted input**	Reference value
TAT 3 working days	TAT 3 working days	TAT 7 working days	**TAT** **short**	TAT short
Paired normal tissue needed	Paired normal tissue needed	No paired normal tissue needed	**No paired normal tissue needed**	Control needed

Figure 1. Statistical analysis of the average of deleted bases in colorectal cancer (CRC) and endome-trial cancer (EC). Box plot graph was obtained via the GraphPad Prism software and *p*-value was calculated using the unpaired *t*-test with a significance level of $p < 0.05$. Statistical analysis revealed a significant divergence of the average of deleted bases between CRC and EC (p-value ≤ 0.0001). Level of significance is indicated by ***



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