Multi-Center Comparison of the Fully Automated Idylla™ Microsatellite Instability Assay with Routine Molecular Methods and Immunohistochemistry on Formalin-Fixed Paraffin-Embedded Tissue of Colorectal Cancer

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Background

Microsatellite instability (MSI) is present in 15-20% of primary colorectal cancers (CRC). Determination of MSI status is performed to screen for Lynch Syndrome, guide adjuvant chemotherapy, determine prognosis, and as a predictive test for use with checkpoint blockade inhibitors. Routine methods for MSI status are based on immunohistochemical (IHC) expression of MisMatch Repair (MMR) proteins and molecular testing of poly-A microsatellites by Polymerase Chain Reaction (PCR) (1).
The Idylla™ MSI Assay is a highly automated molecular method (including automated result interpretation), using seven novel MSI biomarkers to detect the microsatellite instability status of formalin-fixed paraffin-embedded tissue (FFPET) (2). The assay does not need matched normal tissue, and provides an automated workflow with short turnaround time.

Methods

1,301 archival CRC FFPE tissue sections from 44 clinical sites (worldwide) were screened for MSI by routine methods (IHC and PCR) and by Idylla™ MSI Assay. More than 75% of sections came from primary tumors, slices thickness were between 5 and 10 microns in 95% of cases, between 1 and 5 cuts were included for the analysis in 98% of cases and 76% of them contained more than 20% tumor burden.
Expression analysis by IHC of the marker genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* in FFPE tissue material was performed using routine standard protocols and equipment, including commercial antibodies from Ventana (Roche Diagnostics) and Dako (Agilent), and systems from Ventana and Leica Biosystems.
PCR analysis was performed with the PCR-based Promega MSI Analysis System and other markers: NR-21, NR-22, NR-24, NR-27, BAT-25, BAT-26, BAT-40, D2S123, D5S346, D10S197, D13S153, D17S250, D18S58, D18S69, CAT25, HSP110, TGFbetaRII, and MYCL1.
The Idylla™ MSI Assay was performed on FFPET sections from the same block used for routine methods. 5-10 µm sections with at least 20% neoplastic cells were loaded into Idylla™ MSI cartridges (figure 1). The Idylla™ MSI Assay automates the entire process of determining repeat length of seven monomorphic homopolymer biomarkers located in the ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A & SULF2 genes by PCR followed by melting curve analysis.
Statistical analysis: The agreement between the Idylla™ MSI Assay and the comparator methods (IHC or PCR-based tests on MSI panels) was evaluated based on point estimates for Overall, Positive, and Negative Percent Diagnostic agreement together with 95% one-sided Wilson-score confidence intervals.

Results

The MSI mutations detected with the Idylla™ MSI Assay were equally distributed over the seven biomarkers (table 1), and 84.48% of the microsatellite-instable-high (MSI-H) samples had >5 mutated biomarkers, while 98.25% of the microsatellite-stable (MSS) samples had zero mutated biomarkers (table 2).
The concordance level between the Idylla™ MSI Assay and immunohistochemistry was 96.56% (1,038/1,075). Compared to routine molecular methods, the concordance level was 98.03% (796/812) (table 3).
The failure rate of the Idylla™ MSI Assay (0.23%; 3/1.301) was lower than that of reference immunohistochemistry (4.37%; 47/1,075) or molecular assays (0.86; 7/812). Routine method failure rates might be an underestimation as the current analysis was done retrospectively on samples with known routine results.

Conclusion

The Idylla™ MSI Assay showed high concordances with IHC and molecular testing, with a simple workflow and short turnaround time (less than 150 minutes). It required limited amount of tumor tissue (no matched normal tissue), had a lower failure rate, and was less dependent on poor pre-analytical conditions in comparison with other methods.

Table 1: Idylla™ MSI Assay calls per biomarker.
MSS: Microsatellite Stable; MSI-H: Microsatellite Instable-High

	ACVR2A	BTBD7	DIDO1	MRE11	RYR3	SEC31A	SULF2
Overall							
Mutant	579	514	576	505	411	373	457
Wild type	718	782	725	780	887	926	840
Invalid	4	5	0	16	3	2	4
Total	1,301	1,301	1,301	1,301	1,301	1,301	1,301
MSI-H samples							
Mutant	575	513	570	504	411	373	457
Wild type	37	99	42	107	201	239	154
Invalid	0	0	0	1	0	0	1
Total	612	612	612	612	612	612	612
MSS samples							
Mutant	4	1	6	1	0	0	0
Wild type	680	683	680	672	685	686	685
Invalid	2	2	0	13	1	0	1
Total	686	686	686	686	686	686	686
Invalid samples							
Mutant	0	0	0	0	0	0	0
Wild type	1	0	3	1	1	1	1
Invalid	2	3	0	2	2	2	2
Total	3	3	3	3	3	3	3

Table 2: Number of Idylla™ MSI Assay "mutant" markers.

MSI status	#	# mutant markers	#	% of MSI-H
MSS	686	0	674	
		1	12	
MSI-H	612	2	15	2.45
		3	28	4.58
		4	52	8.50
		5	156	25.49
		6	226	36.93
		7	135	22.06

Figure 1: The Idylla™ MSI Assay cartridge

Table 3: Concordance analysis. **dMMR/pMMR: deficient or proficient MisMatch Repair**

PCR / Idylla™	MSI-H	MSS	Invalid	Doubtful	Total
MSI-H	381	4	0	2	387
MSS	12	408	1	2	423
Invalid	0	0	2	0	2
Total	393	412	3	4	812

IHC / Idylla™	MSI-H (dMMR)	MSS (pMMR)	Invalid	Doubtful	Total
MSI-H	501	12	1	10	524
MSS	25	487	6	30	548
Invalid	2	1	0	0	3
Total	528	500	7	40	1,075

1. Richman S. Deficient mismatch repair: Read all about it (Review). Int J Oncol 2015; 47: 1189-1202 .
2. Zhao et al. Mismatch repair deficiency endows tumors with a unique mutation signature and sensitivity to DNA double-strand breaks. Elife. 2014 Aug 1;3:e02725.

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