Evaluation of a Fully Automated System for Use in Somatic Mutation Testing in Colorectal Cancer: a Prospective Study with Comparison to Next-Generation Sequencing

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The authors have no conflicts of interest that relate to the content of this abstract

BACKGROUND

- Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the United States among both men and women.
- The management of CRC has been aided by the advancement and increased usage of molecular testing.
- Mutational analysis of *KRAS* and *NRAS* is warranted for CRC patients considered for anti-EGFR therapy. *BRAF* codon 600 testing is also recommended in CRC for prognostic stratification.
- The American Society of Clinical Oncology (ASCO) recently expanded their recommendations on molecular testing for CRC patients considered for anti-epidermal growth factor receptor (anti-EGFR) therapy to include *KRAS* and *NRAS* codons 12 and 13 of exon 2, codons 59 and 61 of exon 3, and codons 117 and 146 of exon 4 (so-called "expanded" or "extended" *RAS* testing).
- The Idylla™ system (Biocartis, Mechelen, Belgium) is a fully automated platform that utilizes a single-use cartridge containing all necessary reagents and steps for sample processing and real-time PCR-based mutation amplification and detection.
- In this prospective study, we evaluated the Idylla™ system against next generation sequencing using colorectal cancer tissue specimens.

DESIGN & METHODS

- Seventy-five CRC tissue specimens which were ordered for next-generation sequencing (NGS) between January 2018 and December 2018 were also prospectively tested on the Idylla™ system using the KRAS and NRAS-BRAF-EGFR S492R cartridges (Research Use Only), which test for actionable mutations in *KRAS*, *NRAS* and *BRAF*.
- Two 10 µm formalin-fixed paraffin-embedded (FFPE) tissue sections (one for each cartridge) were used for each run on the Idylla™ and all cases met the system's minimum tumor requirement of 10%.
- NGS testing was performed using the Ion AmpliSeq 50-gene Cancer Hotspot Panel v2 (Thermo Fisher Scientific). NGS results and turnaround time of the testing process were compared to those obtained by the Idylla™ system.

Figure 1. The Idylla™ system

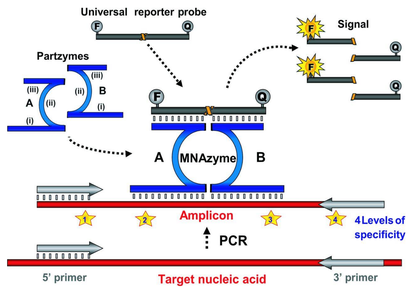

Figure 2. PlexPrime®/PlexZyme® Technology

KRAS Mutation Assay (RUO)		
Exon	**Codon**	**Mutation**
2	12	G12C, G12R, G12S, G12A, G12D, G12V
	13	G13D
3	59	A59T, A59E, A59G
	61	Q61K, Q61L, Q61R, Q61H
4	117	K117N
	146	A146P, A146T, A146V

NRAS-BRAF-EGFR S492R Mutation Assay (RUO)			
Gene	**Exon**	**Codon**	**Mutation**
NRAS	**2**	12	G12C, G12S, G12A, G12D, G12V
		13	G13D, G13R, G13V
	3	59	A59T
		61	Q61K , Q61L, Q61R, Q61H
	4	117	K117N
		146	A146T, A146V
BRAF	**15**	600	V600E, V600D, V600K, V600R
EGFR	**12**	492	S492R

Tables 1 & 2. Mutations detectable by the Idylla™ KRAS and NRAS-BRAF-EGFR S492R Mutation Assays

RESULTS

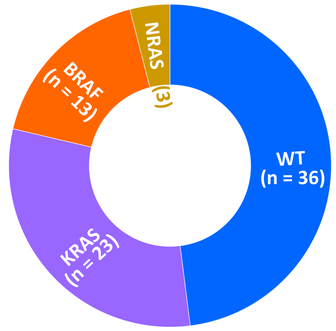

Figure 3. Distribution of *KRAS, BRAF, and NRAS* mutations among study samples

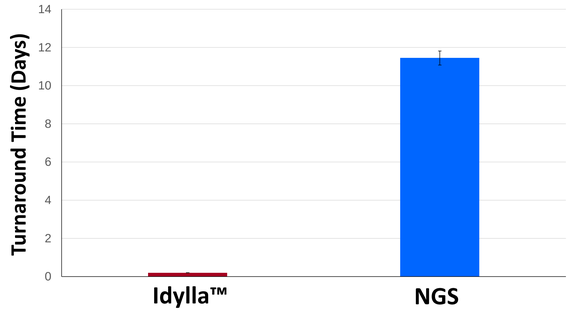

Figure 4 . Comparison of Idylla™ and NGS average testing turnaround time

Gene Mutated	Number of samples	Idylla™	NGS
BRAF	10	*BRAF* V600E/D	*BRAF* V600E
	1	*BRAF* V600E/D	*BRAF* V600E, *KRAS* T20M*
	1	No mutation detected	*BRAF* D594N*
	1	No mutation detected	*BRAF* K601E*
KRAS	3	*KRAS* G12A	*KRAS* G12A
	1	*KRAS* G12C	*KRAS* G12C
	7	*KRAS* G12D	*KRAS* G12D
	1	*KRAS* G12D, *NRAS* G12A/V¶	*KRAS* G12D
	1	*KRAS* G12S	*KRAS* G12S
	1	*KRAS* G12S, *NRAS* G12A/V¶	*KRAS* G12S
	4	*KRAS* G12V	*KRAS* G12V
	3	*KRAS* G13D	*KRAS* G13D
	1	*KRAS* Q61K	*KRAS* Q61K
	1	*KRAS* A146P/T/V	*KRAS* A146T
NRAS	1	*NRAS* G12D	*NRAS* G12D
	1	*NRAS* Q61K	*NRAS* Q61K
	1	*NRAS* Q61R	*NRAS* Q61R
None (WT)	36	No mutation detected in *BRAF, KRAS,* or *NRAS*	No mutation detected in *BRAF, KRAS,* or *NRAS*

Table 3. Idylla™ results compared with NGS
*Mutations not included in the list of mutations detectable by the Idylla™
¶ Additional *NRAS* mutation detected by the Idylla™ but not by NGS

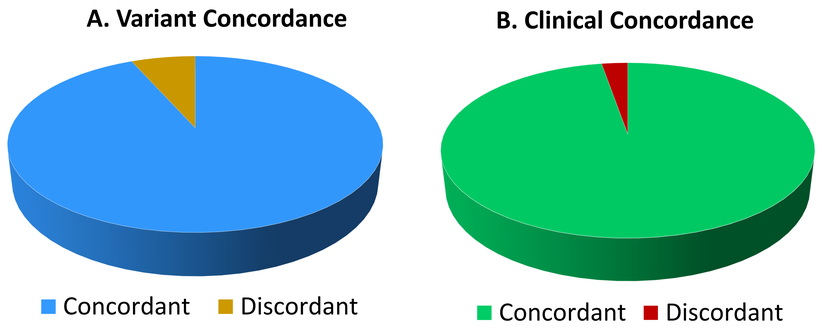

Figure 5. Variant (A: 93%, 70/75) and clinical (B: 97%, 73/75) concordance between Idylla™ and NGS results

CONCLUSIONS

- The Idylla™ system offers rapid and reliable testing of clinically actionable mutations in colorectal cancer specimens directly from FFPE tissue sections.
- This prospective study demonstrates high concordance between the Idylla™ system and NGS.
- The Idylla™ system is ideal for centers that lack highly trained molecular staff or infrastructure and can complement NGS in larger centers by providing rapid turnaround time.