Rapid clinical mutational testing of *KRAS*, *BRAF* and *EGFR*: a prospective comparative analysis of the Idylla technique with high-throughput next-generation sequencing

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Received 13 May 2019
Revised 24 June 2019
Accepted 25 June 2019
Published Online First 11 July 2019

To cite: Van Haele M, Vander Borght S, Ceulemans A, *et al*. *J Clin Pathol* 2020;**73**:35–41.

ABSTRACT

Aims Precision medicine therapy is remodelling the diagnostic landscape of cancer. The success of these new therapies is often based on the presence or absence of a specific mutation in a tumour. The Idylla platform is designed to determine the mutational status of a tumour as quickly and accurately as possible, as a rapid, accurate diagnosis is of the utmost importance for the treatment of patients. This is the first complete prospective study to investigate the robustness of the Idylla platform for *EGFR*, *KRAS* and *BRAF* mutations in non-small cell lung cancer, metastatic colorectal cancer and metastatic melanoma, respectively.

Methods We compared prospectively the Idylla platform with the results we obtained from parallel high-throughput next-generation sequencing, which is the current gold standard for mutational testing. Furthermore, we evaluated the benefits and disadvantages of the Idylla platform in clinical practice. Additionally, we reviewed all the published Idylla performance articles.

Results There was an overall agreement of 100%, 94% and 94% between the next-generation panel and the Idylla *BRAF*, *KRAS* and *EGFR* mutation test. Two interesting discordant findings among 48 cases were observed and will be discussed together with the advantages and shortcoming of both techniques.

Conclusion Our observations demonstrate that the Idylla cartridge for the *EGFR*, *KRAS* and *BRAF* mutations is highly accurate, rapid and has a limited hands-on time compared with next-generation sequencing.

INTRODUCTION

Molecular testing is currently included in the standard of care to determine the treatment of different advanced cancers. This rapidly evolving specialty within the medical field has become an important part of the pathological report of the patient.[1] The notable technological advances of molecular testing require much expertise and investments in current clinical laboratories. Therefore, it is important to critically assess and compare the performance of molecular testing platforms.

The Idylla (Biocartis, Mechelen, Belgium) is a fully automated real-time PCR-based system that is applicable to a wide range of clinical settings. The Idylla platform uses a small amount of formalin-fixed paraffin-embedded (FFPE) tissue in individual disposable cartridges. These closed-system cartridges contain all of the reagents that are necessary for a reliable reaction, resulting in minimal hands-on time and a comprehensive presentation of the results. We analysed all peer-reviewed literature on the use of the Idylla platform in all tumours in which mutational status is required for treatment of the patient (table 1).[2–25]

Lung cancer is the foremost leading cause of cancer death worldwide.[26] Multiple studies have shown that a mutation in the epidermal growth factor receptor (*EGFR*) plays an important role in the regulation of the tumour. EGFR inhibitors, such as tyrosine kinase inhibitors (TKIs), are an interesting therapeutic option for patients with advanced non-small cell lung cancer (NSCLC).[27 28] The incidence of *EGFR* mutations in lung cancer differs between Caucasian (range: 7%–36%) and Asian patients (range: 20%–76%).[29] These mutations occur within the *EGFR* kinase domain (exons 18–21), leading to hyperactivity of the prosurvival signal pathways and therefore increased sensitivity to EGFR-TKIs. Most of the mutations are exon 19 deletions or a specific exon 21 p.(Leu858Arg) point mutation, representing 45% and 40% of the *EGFR*-mutated NSCLCs, respectively. However, it should be noted that not all mutations lead to increased sensitivity to EGFR-TKIs.[30] Mutations such as an exon 20 insertion, present in up to 9.2% of the *EGFR* mutations, induce a decreased EGFR-TKI sensitivity.[31]

Colorectal cancer (CRC) is the third most common cause of cancer worldwide.[26] Antibody-mediated inhibition of EGFR is a therapeutic option in the treatment of advanced CRC. Only patients bearing no hotspot mutation in *KRAS* and *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61) or exon 4 (codons 117 and 146) benefit from receiving anti-EGFR therapy.[32–34] The detection of these mutations is therefore included in international guidelines.[35 36]

The prevalence of skin cancer, particularly melanoma, has continued to increase over the years. Researchers have performed intensive investigations into the role of ultraviolet exposure as a cause, but genetic predisposition also seems to play an important role in the pathogenesis of this tumour.[37 38] With the discovery of different

Table 1 Overview of studies on Idylla performance analysis testing

Reference	Year	Type of cancer	Input	Gene	Samples (n)	Comparison	Concordance	Idylla cartridge
Janku *et al*[2]	2015	CRC, melanoma and others	FFPE tissue	*BRAF*	60	Real-time PCR; NGS	97% (real-time PCR); 100% (NGS)	Idylla BRAF mutation test
					100	PCR-based method; mass spectrometry; NGS	96%	Idylla BRAF mutation test
Melchior *et al*[3]	2015	Melanoma	FFPE tissue	*BRAF*	139	Real-time PCR; Sanger or pyrosequencing	97.84%	Idylla BRAF mutation test
Colling *et al*[4]	2016	CRC	FFPE tissue	*BRAF*	100	Real-time PCR	98.97%	Idylla BRAF mutation test
Janku *et al*[5]	2016	CRC, melanoma and others	Cell-free DNA vs FFPE	*BRAF*	160	PCR-based method; mass spectrometry; NGS	88%	Idylla BRAF mutation test
Schiefer *et al*[6]	2016	Melanoma, papillary thyroid cancer and others	FFPE tissue	*BRAF*	419	Sanger or pyrosequencing	96.2%–97.5%	Idylla BRAF mutation test
Yeo *et al*[7]	2016	Papillary thyroid cancer and nodular hyperplasia	FFPE tissue	*BRAF*	110	Peptide nucleic acid clamping; real-time PCR; pyrosequencing	0.974 (Cohen's kappa)	Idylla BRAF mutation test
De Luca *et al*[8]	2016	NSCLC	Cytological samples	*EGFR*	74	(Real-time) PCR	100%	Idylla EGFR mutation assay
de Biase *et al*[9]	2016	Pancreatic cancer	Cytological samples	*KRAS*	52	NGS; Sanger sequencing; allele-specific locked nucleic acid PCR	Undisclosed	Idylla KRAS mutation test
Solassol *et al*[10]	2016	CRC	FFPE	*KRAS*	374	Sanger sequencing; ddPCR; HRM; pyrosequencing; NGS; real-time PCR; others	98.9% (overall)	Idylla KRAS mutation assay
Harlé *et al*[11]	2016	Melanoma	FFPE	*BRAF*	59	HRM, real-time PCR, NGS, IHC	Undisclosed	Idylla BRAF mutation test
Bisschop *et al*[12]	2017	Melanoma	FFPE tissue	*BRAF*	37	HRM; Sanger sequencing; IHC; NGS	97.3% (overall)	Idylla BRAF mutation test
Barel *et al*[13]	2017	Melanoma	FFPE	*BRAF/NRAS*	36	NGS; IHC	97.2% (overall)	Idylla NRAS-BRAF-EGFRS492R mutation assay
Johnston *et al*[14]	2018	CRC	FFPE tissue	*BRAF/NRAS*	242	Mass spectrometry	99.59% (NRAS); 100% (BRAF)	Idylla NRAS-BRAF mutation test
Colling *et al*[15]	2017	CRC	FFPE tissue	*BRAF/NRAS/ KRAS*	43	NGS; IHC	BRAF: 90% (IHC), BRAF: 100% (NGS); KRAS: 100% (NGS); NRAS: 100% (NGS)	Idylla NRAS-BRAF-EGFR492R mutation assay; Idylla KRAS mutation test
Ilie *et al*[16]	2017	Lung adenocarcinoma	FFPE tissue	*EGFR*	55	Pyrosequencing	95%	Idylla EGFR mutation assay
Lambros *et al*[17]	2017	NSCLC	FFPE tissue	*EGFR/KRAS*	18	ddPCR; NGS	Undisclosed	Idylla EGFR and KRAS mutation assays
Thomas De Montpréville *et al*[18]	2017	NSCLC	FFPE and fresh samples	*EGFR/KRAS*	93	NGS	Undisclosed	Idylla EGFR and KRAS mutation tests
Weyn *et al*[19]	2017	mCRC	FFPE tissue	*KRAS*	182	Real-time PCR	96.7%	Idylla KRAS mutation test
De Luca *et al*[20]	2017	Pancreatic cancer and mCRC	Cytological samples	*KRAS*	18	Real-time PCR	100%	Idylla KRAS mutation test
Sherwood *et al*[21]	2017	NSCLC	KRAS-mutant cell lines	*KRAS*	N/A	12 distinct techniques	N/A	Idylla KRAS mutation test
Harle *et al*[22]	2018	Melanoma	FFPE tissue	*BRAF*	37	Real-time PCR	Undisclosed	Idylla BRAF mutation assay
Prieto-Potin *et al*[23]	2018	mCRC	FFPE tissue	*BRAF/NRAS*	418	NGS; mass spectrometry; Sanger sequencing; pyrosequencing; ddPCR	99.51% (BRAF overall) 99.27% (NRAS overall)	Idylla NRAS-BRAF mutation test
De Luca *et al*[24]	2018	NSCLC	Cytological samples	*EGFR*	68	NGS	100%	Idylla EGFR mutation test
Ghigna *et al*[25]	2018	NSCLC	FFPE	*EGFR/KRAS*	43	NGS; Sanger sequencing	Undisclosed	Idylla EGFR and KRAS mutation tests

CRC, colorectal carcinoma; ddPCR, digital droplet PCR; FFPE, formalin-fixed paraffin-embedded; HRM, high-resolution melting; IHC, immunohistochemistry; mCRC, metastatic colorectal cancer; N/A, not applicable; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer.

melanoma molecular targets, BRAF has become one of the most interesting therapeutic targets. BRAF is a serine-threonine protein kinase that plays an extensive and decisive role in the mitogen-activated protein kinase pathway, which controls cell survival and proliferation. Approximately 50% of the melanomas bare a *BRAF* mutation, mostly detected in codon 600 (>90%). Among these, 90% are single nucleotide base mutations resulting in the replacement of a valine (Val) by a glutamic acid (Glu, E).

Less common are the *BRAF* p.(Val600Lys), *BRAF* p.(Val600Arg), *BRAF* p.(Val600Glu2) and *BRAF* p.(Val600Asp) mutations. This frequency makes the mutation of *BRAF* Val600 an interesting therapeutic target and a BRAF inhibitor a promising optional treatment for patients harbouring these mutations.[39] Since all described tumours have a rapidly progressing nature, delays in the process of diagnosis and the detection of the biomarkers can lead to delayed treatment and could have a negative outcome on the prognosis of the patient. Reliable and rapid genetic testing with high sensitivity and specificity is therefore required. Currently, significant effort has been invested in the development of fast and cost-efficient techniques. Certainly, healthcare budgets are becoming more oriented towards evidence-based cost-effectiveness. Therefore, it is important to thoroughly investigate the speed, accuracy, usability and implementation of the techniques in the clinic.

This prospective study was designed to evaluate the performance of Idylla in comparison with next-generation sequencing (NGS) mutation analysis, which is currently the gold standard in most centres offering this kind of high-level expertise. We examined *EGFR*, *BRAF* and *KRAS* mutations in NSCLC, metastatic melanoma and metastatic colorectal cancer (mCRC), respectively, since these mutations are the most frequently requested by clinicians to determine the effectiveness of certain therapies. Furthermore, we aimed to determine the clinical use of Idylla by assessing turnaround time, detection limit and implementation in the clinic.

MATERIALS AND METHODS

All cases were selected from clinical patients at the University Hospitals of Leuven, Belgium over a 6-month period in 2017. These clinical cases, surgical and endoscopically obtained biopsies, were randomly included based on the following criteria. First, an expert pathologist diagnosed melanoma, NSCLC or mCRC with the aid of morphological and immunohistochemical testing. Second, mutational analysis was required to establish a personalised treatment plan for the patient. Exclusion criteria were lack of sufficient tissue for molecular testing (see below for each platform) after performing immunohistochemical tests. Every sample was analysed in parallel with the mutation-specific Idylla cartridge and NGS. If the results showed a discrepancy, we retested this sample and used a third validation method to establish a reliable result. This retesting was executed on the Cobas 4800 (Roche, Basel, Switzerland) at an accredited third-party laboratory to establish a reliable outcome. Testing was performed until we reached 48 cases (NSCLC, n=17; mCRC, n=18; melanoma, n=13). Forty-five samples were fixed on 10% neutral buffered formalin (4% formaldehyde). Three samples of NSCLC tumours were cytological samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration, which were fixed in CytoRich Red.

A literature search was performed using MEDLINE/PubMed and EMBASE using the term 'Idylla'. All peer-reviewed articles that implemented a performance analysis of the Idylla platform were included until February 2019.

Idylla platform

Mutation analysis for each gene was performed on the fully automated Idylla platform using the appropriate cartridges, following the manufacturer's protocol. One slide was stained with H&E to check if the inclusion criteria were met. The next slide was a non-deparaffinised and non-stained FFPE 5 µm section of tissue that was cut and placed on a non-coated slide. Idylla requires a tumour cell percentage of at least 50% for *BRAF* and 10% for *EGFR* and *KRAS*. The 5 µm FFPE tissue section required an area between 50 and 600 mm² or one section of 10 µm between 25 and 300 mm² to meet the Idylla tumour surface requirement. For all 48 cases, no microdissection or macrodissection was necessary. The tissue section was placed between pieces of filter paper and wetted with nuclease-free water. Then, it was placed inside the appropriate Idylla *EGFR* Mutation test, Idylla *KRAS* Mutation test or Idylla *BRAF* mutation test cartridge corresponding to the correct mutation–tumour association. Subsequently, the cartridge was loaded into the Idylla instrument for testing. The Idylla platform is a fully integrated system that allows to process the FFPE tissue sample using a high-intensity focused ultrasound followed by fluorescent-based PCR amplification. The Idylla platform analyses the obtained data and translates them into an easy-to-use message for the clinician whether the mutation or group of mutations was detected.

MiSeq/NextSeq Illumina NGS

Six or twelve consecutive 4 µm sections were prepared from the FFPE tissue samples to meet the required 50 ng, depending on the size of the available material. The first and last sections were stained with H&E and used as a control with a required tumour cell percentage of at least 10%. After deparaffinisation with xylene and alcohol, the tumour foci in the marked areas were manually macrodissected. The tissue was digested overnight with proteinase K. DNA extraction was performed using the Maxwell16 System (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions, and DNA was quantified using the Qubit 2.0 fluorometer (Life Technologies, Carlsbad, California, USA). Analysis for hotspot mutations was performed with the TruSight Tumor26 kit (Illumina, San Diego, California, USA), which enables the detection of mutations in 26 genes (*AKT1*, *ALK*, *APC*, *BRAF*, *CDH1*, *CTNNB1*, *EGFR*, *ERBB2*, *FBXW7*, *FGFR2*, *FOXL2*, *GNAQ*, *GNAS*, *KIT*, *KRAS*, *MAP2K1*, *MET*, *MSH6*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *SMAD4*, *SRC*, *STK11*, *TP53*). The TruSight technology is based on extension and ligation-based amplicon library preparation specific for each of the two strands of DNA. The two independent libraries were combined and sequenced on a MiSeq/NextSeq instrument (Illumina) by paired-end sequencing (2×121 bp) with a minimum read depth of at least 1000× coverage, according to the manufacturer's instructions. The paired-end reads were mapped against the Genome Reference Consortium Human Build 19 (GRCh37). Data analysis was performed using an in-house developed bioinformatics pipeline that begins with the FASTQ files and incorporates BWA for alignment, GATK for variant calling and Annovar for variant annotation.[40–42]

Statistical analysis

The overall percentage of agreement, positive and negative percentage of agreement, and κ Cohen's coefficient were calculated to test the Idylla platform against the reference standard test (NGS). The analysis was performed using GraphPad Prism V.8.00 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS

We prospectively tested the mutational status of 45 formalin-fixed and three CytoRich Red-fixed paraffin-embedded tumours of patients diagnosed with a malignancy. No mutation from our panel was found in 5 out of 13 tested patients with melanoma, 8 out of 18 tested patients with mCRC and 11 out of 17 patients

Table 2 Results of the comparative analysis of the Idylla platform vs TruSight Tumor26 NGS

Cancer type	Idylla	TruSight Tumor26 NGS	Discordances
Melanoma (*BRAF*)			
Mutants (n)	8 of 13	8 of 13	0
Type of mutation	Val600Glu (n=7) Val600Arg (n=1)	Val600Glu (n=7) Val600Arg (n=1)	
Idylla performance analysis	100% overall percentage of agreement (κ=1.00; 95% CI 1.0) PPA: 100% (95% CI 1.0); NPA: 100% (95% CI 1.0)		
Metastatic colon carcinoma (*KRAS*)			
Mutants (n)	9 of 18	8 of 18	1*
Type of mutation	Gly12Asp (n=2) Gly12Ala (n=1) Gly12Val (n=1) Gly13Asp (n=1) Gly12Cys (n=1) Gln61Arg/Leu (n=1) Ala146Thr/Val/Pro (n=1) Gln61His (n=1)*	Gly12Asp (n=2) Gly12Ala (n=1) Gly12Val (n=1) Gly13Asp (n=1) Gly12Cys (n=1) Gln61Arg (n=1) Ala146Thr (n=1)	
Idylla performance analysis	94% agreement (κ=0.883; 95% CI 0.661 to 1.0) PPA: 100% (95% CI 1.0); NPA: 90% (95% CI 0.80 to 1.0)		
Non-small cell lung cancer (*EGFR*)			
Mutants (n)	5 of 17	6 of 17	1†
Type of mutation	ins_exon 20 (n=1) del_exon 19 (n=1) del_Thr790Met (n=1) Gly719X (n=1) Leu861Gln (n=1)	ins_exon 20 (n=1) del_exon 19 (n=1) del_Thr790Met (n=1) Gly719Ala (n=1) Leu861Gln (n=1) Uncommon mutation (n=1)†	
Idylla performance analysis	94% agreement (κ=0.866; 95% CI 0.614 to 1.0) PPA: 83% (95% CI 0.65 to 1.0); NPA: 100% (95% CI 1.0)		

*KRAS mutation analysis performed on Cobas 4800 by a blinded third party.
†c.2217_2234dup, p.(Ile740_Lys745dup) is not included in the Idylla panel.
κ, Cohen's kappa coefficient; NPA, negative percentage of agreement; PPA, positive percentage of agreement.

with NSCLC using Idylla. These cases were categorised as wild type (table 2).

In the melanoma group, we found seven cases showing a c.1799T>A p.(Val600Glu) mutation and one case that harboured a c.1798_1799delinsAG p.(Val600Arg) mutation. There were no discordant results among the melanoma group. However, in one case, Idylla displayed a no-mutation result with the remark that the threshold was not reached and a mutation below <5% could be missed. This sample had a tumour cell percentage >90% and a tissue surface area 52.2 mm², which was just within the inclusion limit. Thus, an overall agreement of 100% and a perfect κ coefficient of 1.0 (95% CI 1.0) was found between the Idylla *BRAF* mutation test and the NGS panel.

In the mCRC group, 10 cases showed a *KRAS* mutation (including codons 12, 13, 61 and 146). In contrast, one mutation was detected by Idylla but not by TruSight 26 NGS. In this case, Idylla noted a *KRAS* c.183A>T p.(Gln61His) mutation. The amplification profiles in the Idylla Explore software V.1.2 showed a delta Cq of 10.12. In concordance with NGS, the variant was not detected in the Cobas *KRAS* IVD assay on the Cobas 4800 platform using a consecutively cut extract from this sample. These findings showed an overall agreement of 94% and an almost perfect κ coefficient of 0.883 (95% CI 0.661 to 1.0) between the Idylla *KRAS* mutation test and the NGS panel.

In the NSCLC group, 6 out of 17 cases showed an *EGFR* mutation including a deletion in exon 19, insertion in exon 20, deletion in exon 19 in c.2369C<T p.(Thr790Met), c.2156G>T p.(Gly719Ala), c.2582T>A p.(Leu861Gln) and a duplication in exon 19. The latter rare mutation c.2217_2234dup p.(Ile740_Lys745dup) detected with the TruSight26 NGS panel was not detected by Idylla. An overall agreement of 94% was found with an almost perfect κ coefficient of 0.866 (95% CI 0.614 to 1.0) between the Idylla *EGFR* mutation test and the NGS panel. The duplication in exon 19 is not included in the screening list of the Idylla platform and is therefore not considered a real false negative since this mutation was by design not included in this study. It is also important to note that three of these lung samples were cytological samples, which were taken and processed as Cellient blocks. Despite the small amount of tissue, both platforms were able to process the tissue of our Cellient blocks and detected a mutation (T790M) in one case and two no-mutations in the other two samples.

To investigate the practical use of the Idylla platform, we compared its technical characteristics with the NGS method (table 3). The number of mutations that can be detected by NGS is predetermined by specific exonic regions per gene (n=26)

Table 3 Comparison of technology platform usability characteristics: Idylla cartridge assay vs TruSight Tumor26 NGS

	TruSight Tumor26 NGS	Idylla BRAF	Idylla EGFR	Idylla KRAS
Turnaround time	3 days	90 min	150 min	120 min
Hands-on time	4 hours*	5 min	5 min	5 min
Mutations (n)	Hotspot regions (26 genes); small insertions/deletions, SNVs	7 SNV	51 SNV/del/ins	21 SNV
CE-IVD	No (RUO)	Yes	Yes	Yes
Upfront extraction	Yes	No	No	No
Minimal input: FFPE	50 ng ≥10% tumour cells	1×5 µm FFPE section between 50 and 600 mm²† and ≥50% tumour cells‡	1×5 µm FFPE section and ≥10% tumour cells‡	1×5 µm FFPE section between 50 and 600 mm²† and ≥10% tumour cells‡
Data processing	Open source	Closed source	Closed source	Closed source
Required expertise	High	Low	Low	Low
Output	VCF file	Mutation present: yes/no+specific or group of mutations	Mutation present: yes/no+specific or group of mutations	Mutation present: yes/no+specific or group of mutations

*These are estimates based on the turnaround time at the University Hospitals of Leuven.
†Alternatively, a 1×10 µm FFPE section between 25 and 300 mm².
‡If less microdissection is necessary.
FFPE, formalin-fixed paraffin-embedded; RUO, research use only;SNV, single nucleotide variant; VCF, variant call format.

including hotspot variants, while the Idylla cartridges target a known mutation (*BRAF*, 7; *EGFR*, 51; *KRAS*, 21). However, turnaround time and hands-on time are the main advantages of the Idylla platform when compared with the time-consuming and labour-consuming NGS method. In addition, the Idylla platform has a CE-IVD accreditation for the *BRAF*, *KRAS* and *EGFR* mutation cartridge, while there is no accreditation for NGS. Both methods require a certain tumour cell percentage. However, a complete comparison cannot be made because Idylla requires a minimal tissue surface and NGS a minimal input of 50 ng. The analysis of the mutation detection is automatically performed by the Idylla platform and an answer regarding the presence of a mutation is generated. The NGS platform requires separate bioinformatic tools, a highly trained person performing data analysis and interpretation of the results. The Idylla software is a black box for which the source code is not available. In contrast, the NGS results can be analysed by open-source software that has a steep learning curve.

DISCUSSION

Newly discovered precision medicine therapies demand genetic mutational testing with punctual performance in a very short time-frame. To date, most centres with mutational testing expertise use a deep-genome sequencing approach. These methods were introduced to the clinic after extensive use in the research field. Consequently, the centres had to obtain expensive equipment and experienced technical staff to perform mutation testing in a validated clinical setting. Despite the yearly progress in the field of NGS, there is still a relatively long turnaround time of 3 days (table 3). Fast detection without loss of performance is of utmost importance for a select group of patients to start with the appropriate therapy. Multiple groups have published validation studies over the last few years (table 1), which stress the high concordance of Idylla in different settings. Unfortunately, these studies had a retrospective or only a partially prospective design and often included various reference methods as comparison. Therefore, we designed a prospective study to test the performance of each Idylla mutation cartridge in each specific tumour relative to a currently available gold standard method (NGS; massive parallel sequencing). A shortcoming of this prospective study is the absence of the Idylla *NRAS* cartridge, which has an important clinical relevance. However, this cartridge has been tested in other studies (table 1) and showed excellent results.[14 15]

The Idylla *EGFR* mutation test revealed one conflicting result compared with the NGS test from a total of 17 NSCLC tissue samples. The NGS identified an insertion of 18 base pairs in exon 19, which is seen in almost 1% of the NSCLCs. He *et al*[30] studied a cohort of patients with *EGFR* exon 19 insertions and summarised that exon 19 insertions are a new family of TKI-sensitising *EGFR* mutations although further follow-up is necessary to study the long-term prognosis of these patients. The patient with this rare mutation that was included in our investigation was treated with an EGFR-TKI. This treatment resulted in a partial response after 5 and 11 months (unpublished data). Mutation-specific techniques such as Idylla are focused on the detection of currently relevant clinical mutations and are hence not able to detect rare, less prevalent, but potentially interesting mutations for the future. Nevertheless, the rapid Idylla *EGFR* mutation test has proven to be very sensitive for the detection of *EGFR* hotspot mutations in FFPE tissue and cell blocks. To the best of our knowledge, only six other studies have investigated the performance of the rapid *EGFR* Idylla mutation test.[8 16–18 24 25] In this study, we obtained an overall 94% agreement for the NSCLC cases between NGS and Idylla, which is comparable with the other recent publications. One of these studies showed the *EGFR* Idylla mutation test to be highly sensitive for cytological samples of NSCLC.[8] Together with our cytological results, these findings suggest the implementation of rapid *EGFR* testing in a clinical setting looks promising for cytological samples.

Furthermore, we detected one discordant result in the mCRC subgroup. Idylla test identified a *KRAS* p.(Gln61His), c.(183A>T) mutation whereas the NGS platform did not. A third party retested the sample and could not detect the mutation. All slides were taken consecutively, and a tumour area of 263 mm^2 and the tumour cell percentage of 40% indicated that tumour heterogeneity would not cause this discrepancy. Thus, it can be due to a false-positive result in the Idylla test or a sensitivity problem with TruSight tumour NGS and Cobas *KRAS* kit. However, the result of 94% overall agreement for the mCRC cases observed in this study is in accordance with other performance studies, which demonstrates the high sensitivity of this rapid *KRAS* mutation test.[9 20] Interestingly, a study of Johnston *et al*[14] included samples with a high amount of necrosis or mucinosis. This finding led us to suggest that the Idylla platform could be helpful in these difficult samples. Therefore, future studies should test clinically challenging samples (eg, necrosis, mucinosis and pigmented melanomas) on the Idylla platform more extensively.

The Idylla *BRAF* mutation test showed 100% overall agreement with our NGS mutational testing of the melanoma tissue samples. The results of our test are comparable with previous studies.[2 3] As mentioned, the Idylla *BRAF* mutation test currently includes seven clinically relevant mutations in exon 15 of the *BRAF* oncogene and is highly sensitive on the Idylla platform. Currently, up to 13 studies have investigated *BRAF* mutation detection by Idylla compared with a reference method.[2–7 11–15 22 23] A study by Bisschop *et al*[12] tested different available fast *BRAF* mutational testing methods that are currently used in the clinical setting for diagnostic purposes. They found that the Idylla *BRAF* mutation test was the most suitable and rapid testing method for the patient. Another study performed a cost/benefit analysis of the Idylla mutation test.[43] They calculated that up to four additional mutations per 100 samples would be detected if Idylla was used as a first-line testing method followed by additional second-line NGS.

The strength of the Idylla platform is the significantly shorter overall assay turnaround time and the limited hands-on time required for FFPE tissue handling using a closed fully integrated cartridge format, which also minimises costs and the risk of contamination. In addition, there is no need for experienced technicians and advanced bioinformatic data processing due to the integration of processing, data analysis and reporting in one platform. This implies that the Idylla platform could be implemented in non-expert centres where no sequencing machines or expert technicians are currently available due to low output. On the other hand, a broader range of mutations is detectable by NGS. After all, it is indisputable that other mutations or a combination of mutations will become important in the near future. Therefore, in its current state, we expect that the Idylla platform will be less suitable when new evidence appears in which the presence or co-occurrence of different variants will influence the outcome of certain therapies. This disparity will result in the need for retesting when state-of-the-art therapies based on newer tumour genetic fingerprints are available. A recent example is seen in the field of immune therapy where a combination of different mutations guides the clinician towards a more accurate treatment decision.[44] Furthermore, from the point of

view of research-based strategies, a complete mutation analysis panel without the repeated testing cost and reuse of material in an open-source data processing programme is preferred. Therefore, in our opinion, a rapid test such as Idylla has value in the clinical setting for confirmation of doubtful NGS results close to or just below the detection limit, in case of scarce tissue material such as cytological samples and in rare cases of high clinical urgency. Thus, it seems that Idylla is dividing the landscape of genetic testing towards two different endpoints. On the one hand, we have to choose between a rapid approach that requires low expertise and on the other hand a more thorough but slower approach. However, the cost:benefit ratio for the practical and financial implementation of such a scenario is an open question that needs to be investigated and answered separately by every healthcare system.

In conclusion, the Idylla *BRAF*, *EGFR* and *KRAS* mutation tests showed a very high overall agreement with the TruSight Tumor26 NGS kit for all clinically relevant mutations in this prospective study. Our data and previous studies confirm Idylla as a robust and fast platform that has proven to be an interesting technique. Idylla platform has been able to resolve the shortcomings of other techniques. Therefore, we recognise its utility in case-specific situations such as cytological, high-urgency and low-input samples.

Take home messages

- The Idylla platform was prospectively compared with the gold standard test (high-throughput next-generation sequencing).
- The Idylla *BRAF*, *EGFR* and *KRAS* mutation tests showed a high concordance compared with next-generation sequencing.
- The Idylla cartridges contain mutations that are currently relevant in the clinic.

Handling editor Runjan Chetty.

Acknowledgements The authors are grateful for the technical support of Eef Allegaert. We would like to thank Gwen van Knippenberg for reviewing the manuscript. Biocartis provided the Idylla platform and the mutation test cartridges.

Contributors MVH and SVB conceived and designed the study; SVB, MVH, AC and SM performed the experiments; MVH and MW analysed the literature; MVH and SVB wrote the manuscript; Biocartis provided the cartridges; BW and XS supervised the study.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethical Committee of the University Hospitals of Leuven.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplementary information.

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