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ORIGINAL ARTICLE

Drastic Reduction of Turnaround Time After Implementation of a Fully Automated Assay for RAS-BRAF Mutations in Colorectal Cancer: A Pilot Prospective Study in Real-life Conditions

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Abstract

In some situations, there is a need for rapid mutation tests for guiding clinical decisions and starting targeted therapies with minimal delays. In this study we evaluated the turnaround time before and after the implementation of a fully automated multiplex assay for *KRAS* and *NRAS/BRAF* mutation tests (Idylla™ platform, Biocartis) in metastatic colorectal cancer. The objective of this project was to compare the turnaround times in 2017–2018 with the fully automated multiplex assay to the 2016 results with previous methods. Centers with a number of tests for metastatic colorectal cancer > 100 yearly and a usual turnaround time ≥ 3 weeks for mutation detection were selected. Results of 505 *KRAS* tests and 369 *NRAS/BRAF* tests were transmitted by 10 centers. The mean turnaround time from test prescription to reception of results was reduced from 25.8 days in 2016 to 4.5 days in 2017–2018. In conclusion, this pilot project shows that the Idylla™ platform for testing *KRAS* and *NRAS/BRAF* mutations allows an optimized turnaround time from test prescription to reception of results.

Keywords Metastatic colorectal cancer · Mutation analysis · KRAS mutation · BRAF mutation

Introduction

Colorectal cancer is the 2nd most common cancer in Europe and the 4th most common worldwide [1, 2]. For many years, palliative treatment of colorectal cancer rested on chemotherapy. Recently, the combination of chemotherapy and targeted therapies with human vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR) monoclonal antibodies improved treatment response and patient survival. Thus, median overall survival in patients with metastatic disease increased from 8 to 12 months to 21–24 months with panitumumab, a monoclonal antibody that targets EGFR [3].

EGFR and its downstream signaling pathway are involved in the development of several tumors including colorectal cancer [4]. The growth of cancer cells is driven by the activation of signaling pathways including the RAS-RAF-BRAF-MAPK and the PI3P-Akt pathways [5]. The monoclonal antibodies panitumumab or cetuximab are efficient on colorectal cancer by disrupting the EGFR signal. A chief condition for the efficacy of these targeted therapies is the absence of mutation in the EGFR signaling pathways [6]. Thus, the presence

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of mutations in *KRAS* exon 2 (codon 12 and 13) was shown to reduce strongly the efficacy of panitumumab or cetuximab [7, 8]. Therefore, the exon 2 mutation was used a biomarker to select patients who could benefit from the targeted therapy.

Other mutations causing resistance to anti-EGFR therapy have been evidenced: mutation in *KRAS* exon 3 (codons 59 and 61) and exon 4 (codons 117 and 146) and in *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61) and exon 4 (codons 117 and 146) [9–11]. Therefore, an extensive analysis of *RAS* mutations is now necessary to select patients eligible to therapies targeting EGFR. Mutations in *KRAS* (exons 2, 3 and 4) have been reported in 40–45% of patients and *NRAS* (exons 2, 3 and 4) in 5–10% [12–14]. The *BRAF* V600E mutation is a prognostic factor of outcome and is observed in 4–18% of patients with colon cancer (this mutation is not used to guide anti-EGFR therapy) [13–16].

Guidelines from the European Society for medical Oncology (ESMO) recommend the genotyping of tumor tissue from primary or metastatic tissue both for KRAS exon 2 and non-exon 2 mutations [17]. A recent study reported that KRAS genotyping results were available within 15 days for 82% of tests in Europe, 51% in Latin America and 98% in Asia [18]. For patients with rapidly progressing disease, shorter turnaround times are needed and systems that perform automatically the time-consuming procedures could be of great value. In France, RAS testing circuit involves several actors: health centers for the management of patients, anatomic pathology laboratories for the preparation of tissue samples and national molecular genetics platforms for genotyping. A recent national French survey reported a median turnaround time for molecular tests (EGFR, RAS, BRAF) of 18 days [19].

In this pilot project we evaluated the turnaround time before and after the implementation of the fully automated multiplex Idylla™ KRAS Mutation Test and Idylla™ NRAS-BRAF Mutation Test in metastatic colorectal cancer.

Materials and Methods

This pilot project was performed in metropolitan France and overseas departments. The objective was to compare the turnaround time in 2017–2018 (with fully automated multiplex assay) compared to the 2016 results (previous methods).

The Idylla™ KRAS Mutation Test (Biocartis, Mechelen, Belgium) detects 21 KRAS mutations: 7 mutations in codons 12 and 13 (exon 2), 9 mutations in codons 59 and 61 (exon 3) and 5 mutations in codons 117 and 146 (exon 4). The Idylla™ NRAS-BRAF Mutation Test detects 23 mutations: 8 NRAS mutations in codons 12 and 13 (exon 2), 6 NRAS mutations in codons 59 and 61 (exon 3), 4 NRAS mutations in codons 117 and 146 (exon 4) and 5 BRAF mutations in codon 600 (exon 15).

The cartridges contain reagents necessary to perform sample preparation, real-time PCR amplification and detection starting from formalin-fixed paraffin-embedded tissue sections directly inserted in the cartridges. All operations for deparaffinization, DNA extraction and PCR are performed automatically in the single-use cartridge. Manufacturer specifications and previous studies reported that 2 h approximately were necessary to perform a mutation test using the Idylla™ cartridges [20].

Table 1 Overall turnaround time for obtaining RAS mutation status in patients with colorectal cancer

Centers	Number of KRAS tests in 2017–2018	Number of NRAS/BRAF tests in 2017–2018	Mean turnaround times (days) [a]	
			2016 [b] (before Idylla)	2017–2018 (after Idylla)
#1	63	32	25.4	5.0
#2	40	16	26.2	3.4
#3	108	108	NC	7.8
#4	3	0	36.8	15.7
#5	110	110	21.0	3.2
#6	8	8	NC	4.8
#7	11	11	NC	3.7
#8	34	16	24.4	5.1
#9	95	56	21.0	2.9
#10	33	12	25.8	1.9
All	**505**	**369**	**25.8**	**4.5**

NC, not calculated

[a] From test prescription to reception of results by oncologist

[b] Calculated for at least 30 tests performed in 2016 before the implementation of the automated Idylla™ system

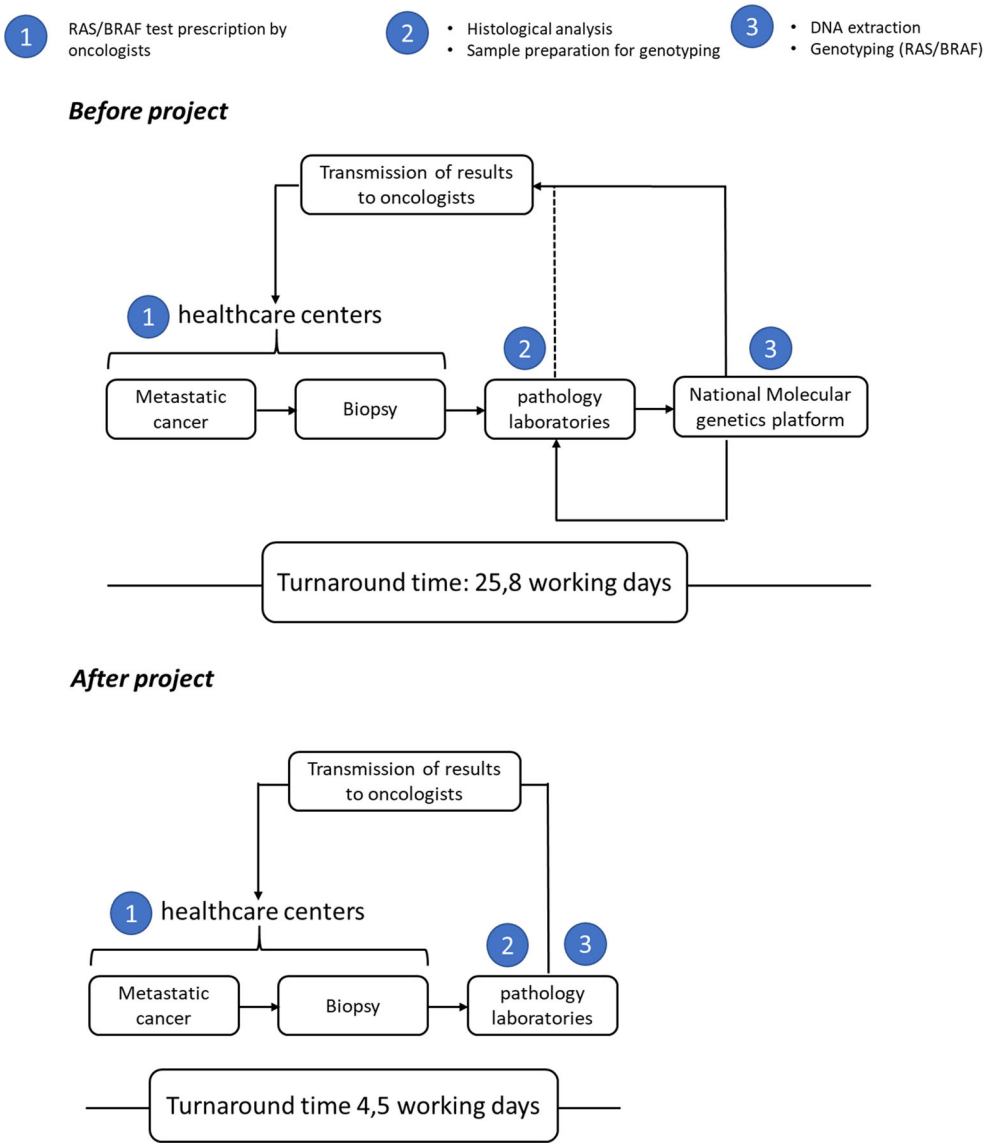

Fig. 1 Successive steps and turnaround time from test prescription to reception of results by prescribing physician before and after project implementation

Centers with a number of tests for patients with metastatic colorectal cancer > 100 yearly and a usual turnaround time ≥ 3 weeks for mutation detection were selected. Since the Idylla™ tests are not yet reimbursed by social security, the Idylla™ system was installed in centers that were included in the pilot project and Idylla™ cartridges were provided by Amgen free of charge.

A qualitative survey was conducted in participating pathologists and oncologists in order to evaluate the impact of this molecular testing platform in their daily clinical practice.

Results

Twelve centers (one public and 11 privates) were selected and 10 of them transmitted results for 505 KRAS tests and 369 NRAS/BRAF tests. The average time from test prescription to reception of results was reduced from 25.8 days in 2016 to 4.5 days in 2017–2018 after implementation of the Idylla™ platform (Table 1).

Ten out the 12 participating pathologists and oncologists completed the survey questionnaire. The satisfaction rates of pathologists on the molecular test circuit increased from 60% (satisfied) to 100% (satisfied plus very satisfied) after the implementation of the project; 80% of pathologists considered that this project had a very positive impact on the organization of the RAS/BRAF testing in their laboratory; 100% of them judged satisfactory (satisfied plus very satisfied) the Idylla™ technology, as well as the RAS testing turnaround time after the implementation of the project.

Regarding oncologists, the satisfaction rates (i.e., very satisfied plus satisfied) on RAS testing turnaround time increased from only 30–100%. Oncologists highlighted that the long turnaround time impacted the therapeutic strategy for the

patients (e.g., synchronous metastatic patients, symptomatic patients, patients who need cytoreduction) and led to a delay in the introduction of the targeted therapy (often introduced in the second chemotherapy cycle) or the administration of anti-VEGF regardless of the molecular status. The implementation of the project within the centers had, according to 100% of physicians, a positive impact on the patient's care (80% very positive and 20% positive). They underlined that patients received more quickly the targeted therapy suited to their Idylla™ RAS status.

Discussion

Our results in real life show that the installation of an automated molecular diagnostic tool resulted in drastic reduction of the mean turnaround time from test prescription to reception of results by the oncologist who prescribed the test (Fig. 1). The specificity of this method and the comparisons with other routine methods have been reported in previous studies for metastatic colorectal cancer [21, 22]. The shortening of turnaround times with automated assay for RAS mutations installed in decentralized anatomic pathology laboratories meets the need for rapid and reliable tests for guiding clinical decisions and starting the most beneficial cancer therapies. A recent survey from the French National Cancer Institute (INCa) showed that the median turnaround time from test prescription to reception of results by the clinician for EGFR, RAS and BRAF molecular tests was 18 days: it was < 1 month for 86% of patients, 1–2 months for 12% and > 2 months for 2% [19]. This turnaround time included 11 days (median) for the management of the sample and the realization of the test itself by the molecular genetic platform. In a study performed in Australia including nine participating laboratories, the major factor that contributed to a long turnaround time was the time needed to retrieve archived tumor block, particularly when they were obtained from sources external to the testing site [23]. Thus, in almost 30% of cases more than 2 weeks elapsed before sample was received by the center that performed the test. Then a result was obtained within 2 weeks in 85% of cases. Overall, the median turnaround time (from ordering of the test to report of the results) was 17 days and in 20% of cases this time was more than 4 weeks for a KRAS test; in only 10% of cases, the results were available within one week.

According to the recommendations of the INCa, the turnaround time should be 2–3 weeks from the prescription date to the transmission of the results to the clinician; therefore, the results of the test should be transmitted with a maximum of 7–10 days after the reception of the sample [24].

For this project, we selected centers where the routine turnaround times exceeded three weeks: the mean turnaround time of the different centers varied from 21.0 to 36.8 days (Table 1). After the implementation of Idylla™ RAS testing in anatomic pathology laboratories, this turnaround time was strongly reduced with times varying from 1.9 to 15.7 days. Generally, mean values of turnaround time did not exceed one week; only one center had turnaround time near two weeks. Overall, these results indicate that a turnaround time below 2 weeks is an objective that can be easily achieved and in most cases this time could be one week.

Another survey of the INCa evidenced that a mutation test had not been performed in 4.8% of patients with colorectal cancer although they were at the metastatic stage [19]. These results raise the question of an equitable access to targeted therapies. One of the objectives of the French Cancer Plan for 2014–2019 was that all cancer patients in metropolitan France and overseas departments would benefit from access to genetic molecular tests regardless their localization or medical structure where they are managed [25]. The Idylla™ Mutation Assay meets these requirements since it is a fully-automated and one-step solution. Therefore, specialized infrastructure for molecular testing and highly qualified staff are not necessary. Frequently, laboratories do not perform in-house assays for molecular testing and send samples to external specialized centers where samples are often tested in batch in order to optimize costs. This step is avoided with the automatic Idylla™ system which is implemented in cancer centers where patients are managed.

In conclusion, this pilot project shows that the Idylla™ platform for testing KRAS and NRAS/BRAF mutations allows optimizing turnaround time from test prescription to reception of results.

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Compliance with Ethical Standards

Conflict of Interest Stéphane ROSSAT: No conflict of interest.
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