Cross-platform comparison of next-generation sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for detecting KRAS/NRAS/BRAF/PIK3CA mutations in cfDNA from metastatic colorectal cancer patients

Xiaojing Xu¹ | Fei Huang² | Minlu Cao² | Xinning Chen² | Hao Wang² © | Huiqin Jiang² | Yiyi Yu¹ | Minna Shen² | Yihui Yang² | Beili Wang² © | Tianshu Liu¹ | Wei Guo²,³,⁴ ©

¹Department of Medical Oncology, Zhongshan Hospital, Fudan University, Shanghai, China
²Department of Laboratory Medicine, Zhongshan Hospital, Fudan University, Shanghai, China
³Department of Laboratory Medicine, Xiamen Branch, Zhongshan Hospital, Fudan University, Xiamen, China
⁴Department of Laboratory Medicine, Wusong Branch, Zhongshan Hospital, Fudan University, Shanghai, China

Correspondence
Wei Guo, Department of Laboratory Medicine, Zhongshan Hospital, 180 Feng Lin Road, Shanghai 200032, China. Email: guo.wei@zs-hospital.sh.cn
Tianshu Liu, Department of Medical Oncology, Zhongshan Hospital, Fudan University, 180 Feng Lin Road, Shanghai 200032, China. Email: liu.tianshu@zs-hospital.sh.cn
Beili Wang, Department of Laboratory Medicine, Zhongshan Hospital, Fudan University, 180 Feng Lin Road, Shanghai 200032, China. Email: wang.beili1@zs-hospital.sh.cn

Funding information
This study was supported by grants from the National Natural Science Foundation of China (81972000, 81772263, 81902139, 81772511, 81602038, 81502003), the the Constructing Project of Clinical Key Disciplines in Shanghai (shslcxdzk03302), the Shanghai Medical Key Specialty (ZK2019B28), the Key Medical and Health Projects of Xiamen (YDX2019350200002), the Specialized Fund for the clinical researches of Zhongshan Hospital affiliated Fudan University (2018ZSLC05), the Science

Abstract

Background: Examining tumor KRAS/NRAS/BRAF/PIK3CA status in metastatic colorectal cancer (mCRC) is essential for treatment selection and prognosis evaluation. Cell-free DNA (cfDNA) in plasma is a feasible source for tumor gene analysis.

Methods: In this study, we recruited mCRC patients and analyzed their KRAS/NRAS/BRAF/PIK3CA status in cfDNA using two platforms, next-generation sequencing (NGS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). The performance between the two platforms and the concordance rate between cfDNA and tissue were analyzed. The relationship between cfDNA-related variables and clinical variables was also assessed. Tumor mutations in cfDNA from patients receiving continuous treatments were monitored in the follow-ups.

Results: Next-generation sequencing and MALDI-TOF had similar specificity (100.0% vs. 99.3%) and negative predictive value (99.9% vs. 99.4%), whereas NGS had higher sensitivity (97.1% vs. 85.3% of MALDI-TOF) and positive predictive value (100% vs. 82.9% of MALDI-TOF). The overall concordance rate of NGS and MALDI-TOF was 98.6%. For the reportable types of mutations in both cfDNA and tissue, the concordance rate was 96.1%. Among 28 tissue-positive patients, the allele frequencies of tumor mutations in cfDNA were higher in patients with primary tumor burden ($p = 0.0141$). Both CEA and CA 19-9 were positively correlated with cfDNA
1 | INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide. The mutations of driver genes, including KRAS, NRAS, BRAF and PIK3CA, contribute to tumorigenesis of CRC. It has been reported that mutation frequencies of KRAS, NRAS, BRAF and PIK3CA in CRC are 35.7%–45.4%, 3.9%–6.3%, 3.1%–7.1%, and 3.5%–16.1%, respectively. Meanwhile, these mutations may suppress the response to targeted treatments for metastatic colorectal cancer (mCRC), like epidermal growth factor receptor (EGFR) targeted monoclonal antibodies (mAbs), cetuximab and panitumumab. Therefore, it is critically essential to assess the mutation status of these genes in patients.

Tumor tissue is a suitable source for mutation detection in cancer patients. However, it is not always available during cancer management, due to the difficulty and invasion of tissue biopsy. An alternative approach is liquid biopsy including cell-free DNA (cfDNA) and RNA. A fraction of cfDNA originates from tumors in cancer patients, referred as circulating tumor DNA (ctDNA), and may carry the same genetic mutations as those of a primary tumor. ctDNA has shown a prognostic value comparable to tissue biopsy in the management of non-small cell lung cancer (NSCLC) and CRC. A number of platforms are available for cfDNA analysis and classified into two major groups, single-spot detection and broad-coverage profiling. Initially, only a few driver gene mutations with a prognostic value were known and single-spot detection platform, including droplet digital polymerase chain reaction (ddPCR) and amplification refractory mutation system (ARMS), was sufficient for clinical practice. Gradually an increasing number of genes and mutations have been found of clinical importance, raising the demand for broad-coverage profiling. Nowadays there are already some commercially available kits for broad-coverage profiling of cfDNA on NGS or MALDI-TOF platform.

Here, in this study, we recruited mCRC patients and performed a prospective study to evaluate the capacities of two platforms, NGS and MALDI-TOF, in detecting tumor mutations from plasma cfDNA. We also compared the cfDNA results with tissue results to assess their concordance. Furthermore, we analyzed the correlation of cfDNA-related variables with clinical variables and monitored them in the follow-ups. The findings in our study may help better understand the potential clinical value of cfDNA analysis and determine the suitable platform in clinical practice.

2 | MATERIALS AND METHODS

2.1 | Patient enrollment

This prospective study recruited mCRC patients receiving treatments at the department of medical oncology, Zhongshan Hospital from June 2016 to December 2017 (Figure 1). All patients enrolled should be pathologically confirmed at stage IV, with known KRAS, NRAS, BRAF and PIK3CA status of the tissue, which was tested by ARMS according to routine procedures. In addition, the patients should successfully complete the cfDNA analysis by both NGS and MALDI-TOF. This study was conducted in accordance with the Declaration of Helsinki principles and was approved by the Institutional Review Board of Zhongshan Hospital. All subjects gave written informed consent.

2.2 | Sample collection and DNA extraction

The sample collection and DNA extraction were described previously. Briefly, a total of 20 ml of venous blood was collected from each patient and the plasma was separated. cfDNA from 8.0 ml of plasma was extracted, quantified and qualified using QIAamp Circulating Nucleic Acid Kit (QIAGEN, 55114), Qubit fluorometer 3.0 (Life Technologies, Grand Island, NY) and 2100 bioanalyzer (Technologies, Palo Alto, CA). Extracted cfDNA was stored at −80°C until use.

2.3 | Mutation profiling by NGS

Plasma cfDNA was subjected to amplicon-based Firefly CRC panel (Accu-Kit CRC01, AccuraGen, Shanghai, China), comprising a total of 216 hotspots in exon 2, 3, and 4 of KRAS, exon 2 and 3 of NRAS, exon 9 and 20 of PIK3CA and exon 15 of BRAF, to construct the DNA library (Table S1). DNA libraries were sequenced on an Illumina
MiSeq Dx (Illumina, San Diego, CA, USA) and sequencing data were analyzed using CometScope software (AccuraGen, Shanghai, China). The limit of detection of Firefly NGS was at an allele frequency of 0.2% for 20 ng cfDNA as previous reported.\(^1\)

### 2.4 | Mutation profiling by MALDI-TOF

Plasma cfDNA analysis was also carried out by MALDI-TOF using the UltraSEEK Panel (Agena Bioscience, San Diego, CA), comprising a total of 97 hotspots in exon 2, 3, and 4 of KRAS and NRAS, exon 9 and 20 of PIK3CA, exon 14 and 15 of BRAF and exon 12 of EGFR (Table S2). Briefly, UltraSEEK analysis consisted of standard multiplex PCR, a mutation-specific single-base extension reaction using chain terminators labeled with a moiety, and characterization using MALDI-TOF. The mutational genotypes were identified using the supporting Typer software and automated UltraSEEK Report software.

### 2.5 | Validation of discordant results using ddPCR

The results for the overlapped hotspot mutations between NGS and MALDI-TOF underwent a comparative analysis (Table S3). Confirmatory tests for the discrepancies between NGS and MALDI-TOF were performed on a QX200 ddPCR system using commercial ddPCR kits (BIO-RAD, Hercules, CA, USA). The results were analyzed using Quantasoft v1.7 software. The limit of detection of ddPCR was at an allele frequency of 0.1% for 10 ng cfDNA.

### 2.6 | Comparison between NGS and MALDI-TOF on cfDNA analysis

Hotspot mutations confirmed by at least two of the three platforms (NGS, MALDI-TOF and ddPCR) were defined as true. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by comparing the results of either platform (NGS and MALDI-TOF) with true results.

### 2.7 | Comparison between plasma cfDNA and tissue on the tumor mutations

The true results of cfDNA were compared with the tissue results (Table S4). The concordance rate among results reported in both cfDNA and tissue was calculated. The data from tissue, cfDNA and the Cancer Genome Atlas (TCGA) database were used to assess the proportions of mCRC patients carrying different mutated genes.

### 2.8 | Correlation between cfDNA-related variables and clinical variables

For the patients with mutations in tumor tissue, the allele frequencies of tumor mutations in cfDNA were compared between patient groups with and without tumor burden at primary site. The correlation between plasma cfDNA concentration and primary tumor burden, and serum biomarkers including carcinoembryonic antigen (CEA), carbohydrate antigen (CA 19–9) and carbohydrate antigen 125 (CA 125), were also evaluated.

### 2.9 | Follow-ups of patients receiving continuous treatments

For patients that had tumor mutations in tissue and received continuous treatments at Zhongshan Hospital, their cfDNA (by NGS) and serum biomarker (CEA) were tested in the follow-ups and their treatment responses were monitored using computed tomography (CT) scan.

### 2.10 | Statistical analysis

All the analyses were performed on GraphPad Prism software (Version 8.0.1, San Diego, CA). The Mann-Whitney U test and Fisher’s exact test were used for non-Gaussian and categorical
variables, respectively. Spearman correlation coefficient was calculated to evaluate the correlation of two continuous variables. A p value < 0.05 was considered as statistically significant.

3 | RESULTS

3.1 | Performance of NGS and MALDI-TOF on cfDNA analysis

In 60 mCRC patients, 14 types of gene mutations were identified in at least one patient by either platform (Figure 2A, Table 1 and Table S5). In all the 840 reportable results (14 results per patient, 60 patients), NGS reported one false negative result, whereas MALDI-TOF reported 6 false positive results and 5 false negative results. For the remaining 828 ones, both NGS and MALDI-TOF reported 28 true positive and 800 true negative results. Compared to cfDNA true results, NGS and MALDI-TOF had similar specificity (100.0% vs. 99.3%) and NPV (99.9% vs. 99.4%), whereas NGS had higher sensitivity (97.1% vs. 85.3% of MALDI-TOF) and PPV (100% vs. 82.9% of MALDI-TOF) (Figure 2B). Among 34 cfDNA-positive results and 806 cfDNA-negative results, the overall concordance rate of the two platforms was 98.6%, whereas the concordance rate was significantly lower in cfDNA-positive ones (Figure 2C). All these results suggested that NGS platform had a slight advantage over MALDI-TOF on broad-coverage profiling.

3.2 | Concordance of cfDNA and tissue on tumor mutations

6 types of mutations were reportable, by both cfDNA profiling and tissue ARMS, and identified in a least one patient by either sample source (Figure 3A). Among 360 reportable results, 21 ones were reported positive and 325 ones were reported negative in both cfDNA and tissue, with a concordance rate of 96.1% (Figure 3B). In addition, 7 results were positive only in tissue and 7 others were positive only in cfDNA. In patients with primary tumor burdens at blood sampling, the cfDNA and tissue had a higher concordance rate (97.9% of P0 M0 vs. 94.9% of P1 M0 + P1 M1) (Figure 3B). The tumor mutations in CRC patients mostly occur in KRAS gene, which was consistent in TCGA database, patient tissue and patient cfDNA (Figure 3C). The increased diversities in cfDNA broad-coverage profiling results and TCGA database indicated the importance of broad-coverage profiling in tumor mutation detection (Figure 3C).

3.3 | Relationship between cfDNA-related variables and clinical variables

Profiling of cfDNA is to examine the DNA released into blood stream by tumor. The tumor burden and proteins released by tumor also reflect the tumor status, having internal relationship with cfDNA. Among 28 tissue-positive patients, the allele frequencies of tumor mutations in cfDNA were significantly higher in patients with primary tumor burden (Figure 4A). However, among all the patients, the cfDNA concentrations in plasma were comparable between patients with and without primary tumor burden (p = 0.2367), indicating that the amount of DNA released by tumor was limited (Figure 4B). Considering the size of tumor varied among patients, the serum biomarkers were also analyzed. Both CEA and CA 19-9 were positively correlated with cfDNA concentration (r = 0.3278 and r = 0.3992, respectively), whereas CA 125 was not correlated (Figure 4C–E).

3.4 | Monitoring treatment response using tumor mutations in cfDNA

Patient CRC2-39 underwent surgery and a KRAS exon 4 mutation was detected in the tissue. Then he received three cycles of

---

**FIGURE 2** Tumor mutations detected by NGS and MALDI-TOF in cfDNA. (A) A heatmap showing the tumor mutations detected by NGS and MALDI-TOF. 14 types of mutations were analyzed in 60 patients. (B) The performance of NGS and MALDI-TOF in detecting mutations. Sensitivity, specificity, PPV and NPV were evaluated according to the true cfDNA results. (C) The concordance between NGS and MALDI-TOF in detecting mutations. The 840 reportable results were divided into two groups according to the true cfDNA results.
A rapid increase of mutant allele frequency (KRAS K117N and PIK3CA E545K) during treatment indicated regimen failure, which was also confirmed by an observation of progressive disease by CT. Subsequently, seven cycles of bevacizumab + FOLFIRI were carried out. The gradually decreased allele frequency and an

| Characteristic   | All (n = 60) | Positive (n = 29) | Negative (n = 31) | p     |
|-----------------|-------------|-------------------|-------------------|-------|
| Age (years)     |             |                   |                   |       |
| Median (IQR)    | 59.5 (51.3–66.8) | 59.0 (51.0–65.5) | 60.0 (52.0–69.0) | 0.5489|
| Sex             |             |                   |                   |       |
| Male, n (%)     | 44 (73.3)   | 16 (55.2)         | 28 (90.3)         | 0.0031|
| Female, n (%)   | 16 (26.7)   | 13 (44.8)         | 3 (9.7)           |       |
| Primary site    |             |                   |                   |       |
| Left, n (%)     | 49 (81.7)   | 22 (75.9)         | 27 (87.1)         | 0.3271|
| Right, n (%)    | 11 (18.3)   | 7 (24.1)          | 4 (12.9)          |       |
| Metastatic sites|             |                   |                   |       |
| Liver, n (%)    | 30 (50.0)   | 13 (44.8)         | 17 (54.8)         | 0.0385|
| Lung, n (%)     | 2 (3.3)     | 0 (0.0)           | 2 (6.5)           |       |
| Both, n (%)     | 9 (15.0)    | 8 (27.6)          | 1 (3.2)           |       |
| Others, n (%)   | 19 (31.7)   | 8 (27.6)          | 11 (35.5)         |       |
| Tissue ARMS     |             |                   |                   |       |
| Positive, n (%) | 28 (46.7)   | 24 (82.8)         | 4 (12.9)          | <0.0001|
| Negative, n (%) | 32 (53.3)   | 5 (17.2)          | 27 (87.1)         |       |
| Tumor load      |             |                   |                   |       |
| POPO², n (%)    | 24 (40)     | 14 (48.3)         | 10 (32.2)         | 0.1439|
| P1PO³, n (%)    | 33 (55)     | 15 (51.7)         | 18 (58.1)         |       |
| P1M1¹, n (%)    | 3 (5)       | 0 (0.0)           | 3 (9.7)           |       |
| CEA (ng/ml)     |             |                   |                   |       |
| Median (IQR)    | 48.2 (8.5–270.1) | 95.7 (19.7–288.1) | 21.0 (6.3–224.4) | 0.1253|
| <5, n (%)       | 9 (15.0)    | 2 (6.9)           | 7 (22.6)          | 0.1478|
| ≥5, n (%)       | 51 (85.0)   | 27 (93.1)         | 24 (77.4)         |       |
| CA 19-9 (U/ml)  |             |                   |                   |       |
| Median (IQR)    | 38.4 (12.2–762.1) | 207.8 (12.6–7788.5) | 28.0 (11.5–89.9) | 0.0146|
| <37, n (%)      | 29 (48.3)   | 9 (31.0)          | 20 (64.5)         | 0.0115|
| ≥37, n (%)      | 31 (51.7)   | 20 (69.0)         | 11 (35.5)         |       |
| CA 125 (U/ml)   |             |                   |                   |       |
| Median (IQR)    | 20.6 (10.6–43.0) | 24.4 (14.7–43.1) | 16.2 (9.9–43.1)  | 0.1644|
| <35, n (%)      | 41 (68.3)   | 19 (65.5)         | 22 (71.0)         | 0.7828|
| ≥35, n (%)      | 19 (31.7)   | 10 (34.5)         | 9 (29.0)          |       |

Abbreviations: CA 125, carbohydrate antigen 125; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; IQR, interquartile range; n, number of patients.

¹Tumors arising from the splenic flexure, descending, sigmoid, or rectosigmoid colon.
²Tumors arising from the cecum, ascending, hepatic flexure, or transverse colon.
³Liver only with or without lymph node.
⁴Lung only with or without lymph node.
⁵Both liver and lung with or without lymph node.
⁶Pelvis, peritoneum, bone, breast, omentum or abdominal.
⁷Neither primary nor metastatic lesions resected at blood sampling.
⁸Primary lesion resected, metastatic lesions not resected at blood sampling.
⁹Both primary and metastatic lesions resected at blood sampling.
observation of stable disease by CT scan showed the effectiveness of new therapeutic regimen.

Patient CRC2-23 was diagnosed as colon cancer with liver metastasis. A KRAS exon 4 mutation was identified in tissue biopsy. Four cycles of mFOLFOX6 were administered since November 2017 and a decrease of mutant allele frequency (KRAS A146T) was observed (Figure 5B and Table S6). After stable disease was confirmed by CT scan, the primary tumor and liver metastasis were removed. After surgery, plasma KRAS A146T mutation was undetectable. Although the patient received subsequent adjuvant chemotherapy, a fluctuation of the tumor mutations in cfDNA occurred and imaging showed liver relapse and lung progression in May 2017.

Patient CRC2-25 underwent colonoscopy in November 2017, which showed colon malignant tumor. Positron emission tomography/
CT suggested multiple liver metastases and implantation metastases at the abdomen. A KRAS exon 3 mutation was detected in the surgical sample. The mutant allele frequency (KRAS Q61R) increased during mFOLFOX6 treatment and a CT scan showed progression of liver metastases by the end of four-cycle mFOLFOX6 treatment (Figure 5C and Table S6). However, allele frequency reduced after another 4 cycles of bevacizumab + FOLFIRI and CT scan suggested stable disease. However, subsequent 3 cycles of treatments ended up with a rise of allele frequency and CT images indicating progressive disease.

4 | DISCUSSION

The effectiveness of targeted drugs is raising the awareness about the importance of molecular typing of tumor.6,9 Considering the tumor accumulate mutations all the time, the demand of molecular typing is always urgent during cancer management. Liquid biopsy, especially ctDNA analysis, meets the real-time examining requirement and attracts the attention of clinicians.10,12 Several platforms and assays have been developed to analyze tumor mutations in cfDNA.18 Among these platforms, NGS and MALDI-TOF offer cost-effective broad-coverage profiling.

In this prospective study, we compared two commercially available assays, amplicon-based Firefly CRC panel and UltraSEEK Panel, on corresponding measuring systems, Illumina NGS and Agena MALDI-TOF, in detecting tumor mutations in cfDNA from mCRC patients. Although the overall concordance rate was high, the MALDI-TOF assay showed its shortage in accurately detecting the complicated hotspots and newly discovered mutations (Figure 2). NGS interrogates more targets (>200 hotspots) within one assay than MALDI-TOF. The commercial NGS assay reports the exact nucleotide sequence with high sensitivity (~0.1%) and accuracy. However, MALDI-TOF reports only the existence of a mutation signal and the capacity to distinguish the signal from noise is limited. The commercial MALDI-TOF assay has to amplify the mutation signal in addition, raising strict requirements for primer design. This may explain the limitation the MALDI-TOF assay suffered in this study. ddPCR considered as gold standard, is useful for detection of specific known variants, at very low level (~0.1%), mainly for validation and serial monitoring (Table 2).
Since the tumor releases its DNA into plasma, cfDNA may reflect the mutational status of tumor tissue. At single-reportable-result level, cfDNA and tissue had a high concordance rate (Figure 3). Considering the purpose of cfDNA analysis is to report the positive incidence of tumor mutation, the concordance rate at single-patient level was also analyzed in tissue-positive patients. 23 of 28 patients were also cfDNA-positive, with a concordance rate of 82.1%, suggesting that cfDNA is a promising source to monitor tumor mutations in cancer management of mCRC. Moreover, the comparison with TCGA database indicates that, for both cfDNA and tissue, broad-coverage profiling is a better option in characterizing tumor mutation status (Figure 3). These findings are consistent with pre-knowledge on cfDNA.16,17

The data in this manuscript are available.

ACKNOWLEDGEMENTS
The author thanks all participants for their contributions.

CONFLICT OF INTEREST
The authors declare that no conflicts of interests exist.

AUTHOR CONTRIBUTIONS
All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. W.G., T.L. and B.W. made the concept. W.G., T.L., B.W., X.X., F.H. and H.W. designed the experiments. X.X. and Y. Yu. collected clinical samples. X.X., F.H., H.W., X.C., H.J., M.C. Y.Y and M.S performed experiments, analyzed data and interpreted the results. F.H. and H.W. drafted the manuscript. H.W. and B.W. edited the manuscript.

DATA AVAILABILITY STATEMENT
The data in this manuscript are available.

ORCID
Hao Wang https://orcid.org/0000-0001-6881-9977
Beili Wang https://orcid.org/0000-0001-5167-170X
Wei Guo https://orcid.org/0000-0003-4406-1094

REFERENCES
1. Wang B, Wu S, Huang F, et al. Analytical and clinical validation of a novel amplicon-based NGS assay for the evaluation of circulating tumor DNA in metastatic colorectal cancer patients. Clin Chem Lab Med. 2019;57(10):1501-1510.
2. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467-1480.
3. Benson AB, Venook AP, Al-Hawary MM, et al. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(3):329-359.
4. McCubrey JA, Steelman LS, Abrams SL, et al. Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. Adv Enzyme Regul. 2006;46:249-279.
5. Cremolini C, Rossini D, Dell’Aquila E, et al. Rechallenge for patients with RAS and BRAF wild-type metastatic colorectal cancer with acquired resistance to first-line Cetuximab and Irinotecan: a phase 2 single-arm clinical trial. JAMA Oncol. 2019;5(3):343-350.
6. Guo F, Gong H, Zhao HH, et al. Mutation status and prognostic values of KRAS, NRAS, BRAF and PIK3CA in 353 Chinese colorectal cancer patients. Sci Rep. 2018;8(1):6076.
7. Martini G, Ciardiello D, Vitiello PP, et al. Resistance to anti-epidermal growth factor receptor in metastatic colorectal cancer: what does still need to be addressed. Cancer Treat Rev. 2020;86:102023.
8. Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 2012;486(7404):532-536.
9. Ibrahim AT, Fawzy MS, Abu AlSel BT, Toraih EA. Prognostic value of BRAF/MIR-17 signature and B-Raf protein expression in patients with colorectal cancer: a pilot study. J Clin Lab Anal. 2021;35(3):e23679.
10. Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American society of clinical oncology and college of American pathologists joint review. J Clin Oncol. 2018;36(16):1631-1641.
11. Yoshino T, Arnold D, Taniguchi H, et al. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS. Ann Oncol. 2018;29(1):44-70.
12. Oliveira KCS, Ramos IB, Silva JMC, et al. Current perspectives on circulating tumor DNA, precision medicine, and personalized clinical management of cancer. Mol Cancer Res. 2020;18(4):517-528.
13. Shi L, Tao C, Tang Y, Xia Y, Li X, Wang X. Hypoxia-induced hsa_circ_0000826 is linked to liver metastasis of colorectal cancer. J Clin Lab Anal. 2020;34(9):e23405.
14. Dasari A, Morris VK, Allegra CJ, et al. ctDNA applications and integration in colorectal cancer: an NCI Colon and Rectal-Anal Task Forces whitepaper. Nat Rev Clin Oncol. 2020;17(12):757-770.
15. Zhu L, Zhang S, Xun Y, et al. Comparison of the amplification refractory mutation system, super amplification refractory mutation system, and droplet digital PCR for T790 M mutation detection in non-small cell lung cancer after failure of tyrosine kinase inhibitor treatment. Pathol Oncol Res. 2018;24(4):843-851.
16. Xu T, Kang X, You X, et al. Cross-platform comparison of four leading technologies for detecting EGFR mutations in circulating tumor DNA from non-small cell lung carcinoma patient plasma. Theranostics. 2017;7(6):1437-1446.
17. Belloumi Y, Janning M, Mohme M, et al. Discovery of targetable genetic alterations in NSCLC patients with different metastatic patterns using a MassARRAY-based circulating tumor DNA assay. Cells. 2020;9(11):2337.
18. Weber S, Spiegel B, Perakis SO, et al. Technical evaluation of commercial mutation analysis platforms and reference materials for liquid biopsy profiling. Cancers (Basel). 2020;12(6):1588.
19. Esposito R, Frezzetti D, Maiello MR, et al. Next generation sequencing-based profiling of cell free DNA in patients with advanced non-small cell lung cancer: advantages and pitfalls. Cancers (Basel). 2020;12(12):3804.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Xu X, Huang F, Cao M, et al. Cross-platform comparison of next-generation sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for detecting KRAS/NRAS/BRAF/PIK3CA mutations in cfDNA from metastatic colorectal cancer patients. J Clin Lab Anal. 2021;35:e23818. https://doi.org/10.1002/jcla.23818