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Combined Analysis of Concordance between Liquid and Tumor Tissue Biopsies for *RAS* Mutations in Colorectal Cancer with a Single Metastasis Site: The METABEAM Study

Yoshinori Kagawa[1], Elena Elez[2], Jesús García-Foncillas[3], Hideaki Bando[4], Hiroya Taniguchi[5], Ana Vivancos[6], Kiwamu Akagi[7], Ariadna García[2], Tadamichi Denda[8], Javier Ros[2], Tomohiro Nishina[9], Iosune Baraibar[2], Yoshito Komatsu[10], Davide Ciardiello[2,11], Eiji Oki[12], Toshihiro Kudo[13], Takeshi Kato[14], Takeharu Yamanaka[15], Josep Tabernero[2], and Takayuki Yoshino[5]

ABSTRACT

Purpose: OncoBEAM™ is a circulating tumor DNA (ctDNA) test that uses the BEAMing digital PCR technology. We clarified the association between the baseline tumor burden and discordance in the *RAS* status by metastatic sites in patients with a single metastatic site.

Experimental Design: Data from previous Spanish and Japanese studies investigating the concordance of the *RAS* status between OncoBEAM™ and tissue biopsy in 221 patients with metastatic colorectal cancer (mCRC) were used. We collected data from patients with liver, peritoneal, or lung metastases and evaluated the concordance rates according to the metastatic site and the association between the concordance rate and tumor burden.

Results: Patients had metastases in the liver ($n = 151$), peritoneum ($n = 25$), or lung ($n = 45$) with concordance rates of 91% (95% confidence interval, 85%–95%), 88% (68%–97%), and 64% (49%–78%), respectively. Factors associated with concordance included the baseline longest diameter and lesion number ($P = 0.004$), and sample collection interval ($P = 0.036$). Concordance rates ≥90% were observed in the following groups: liver metastases alone, regardless of the baseline longest diameter and lesion number; peritoneal metastases alone in patients with a baseline longest diameter ≥20 mm; and lung metastases alone in patients with a baseline longest diameter ≥20 mm and/or number of lesions ≥10.

Conclusions: Plasma ctDNA-based liquid biopsy in patients with mCRC may be useful depending on the metastatic site. The maximum diameter and lesion number should be carefully considered when determining patients' *RAS* status with only peritoneal or lung metastases.

[1]Department of Surgery, Kansai Rosai Hospital, Amagasaki, Hyogo, Japan. [2]Department of Medical Oncology, Vall d'Hebron University Hospital (HUVH), Vall d'Hebron Institute of Oncology (VHIO), IOB-Quiron, UVic-UCC, Barcelona, Spain. [3]Department of Oncology, Fundacion Jimenez Diaz University Hospital, Autonomous University, Madrid, Spain. [4]Department of Clinical Oncology, Aichi Cancer Center Hospital, Nagoya, Aichi, Japan. [5]Department of Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan. [6]Cancer Genomics Group, Vall d'Hebron Institute of Oncology, Barcelona, Spain. [7]Division of Molecular Diagnosis and Cancer Prevention, Saitama Cancer Center, Ina, Saitama, Japan. [8]Division of Gastroenterology, Chiba Cancer Center, Chuo, Chiba, Japan. [9]Department of Gastrointestinal Medical Oncology, National Hospital Organization Shikoku Cancer Center, Matsuyama, Ehime, Japan. [10]Department of Cancer Chemotherapy, Hokkaido University Hospital Cancer Center, Sapporo, Hokkaido, Japan. [11]Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy. [12]Department of Surgery and Science, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan. [13]Department of Frontier Science for Cancer and Chemotherapy, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan. [14]Department of Surgery, National Hospital Organization Osaka National Hospital, Chuo, Osaka, Japan. [15]Department of Biostatistics, Yokohama City University School of Medicine, Yokohama, Kanagawa, Japan.

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Corresponding Author: Takayuki Yoshino, Department of Gastrointestinal Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. Phone: 814-7133-1111; Fax: 814-7131-9960; E-mail: tyoshino@east.ncc.go.jp

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Introduction

Colorectal cancer is the third most common cause of cancer-related deaths, and the fourth most diagnosed cancer worldwide (1). Substantial progress has been made in treating metastatic colorectal cancer (mCRC) through the development of anti-VEGF and anti-EGFR mAbs (2, 3). However, several studies have reported that patients with mCRC with tumors harboring *RAS* mutations are unlikely to benefit from anti-EGFR antibodies (2, 3); therefore, such therapies are not recommended (4).

Almost all patients with mCRC have low, but detectable levels of cell-free circulating tumor DNA (ctDNA), in which tumor mutations can be characterized (5). Liquid biopsy is highly sensitive for detecting mutations present at low frequencies.

The OncoBEAM™ RAS CRC Kit (Sysmex Corporation), which uses highly sensitive BEAMing digital PCR technology (6–8), is an *in vitro* diagnostic tool that has been CE-marked (conformity with health, safety, and environmental protection standards for products sold within the European Economic Area) and approved by the Pharmaceuticals and Medical Devices Agency in Japan for detecting plasma *RAS* mutations in patients with mCRC. This kit has been widely used for patients whose tissues are difficult to collect and who are planned to be treated with anti-EGFR antibody rechallenge. A comparison between the use of BEAMing technology to determine the *RAS* mutational status in plasma ctDNA and the reference method of tumor tissue DNA in previous clinical trials revealed concordance rates between 86% and 93% in all patients with mCRC regardless of the metastatic sites (9–13).

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Translational Relevance

The BEAMing technology with the OncoBEAM™ RAS CRC Kit can detect the plasma *RAS* status in patients with metastatic colorectal cancer (mCRC). However, the concordance rate in circulating tumor DNA (ctDNA) and tumor tissue *RAS* status varies by metastatic site. We conducted the first international collaboration study on the concordance rate of liquid and tumor biopsy *RAS* status by metastatic site. The utility of OncoBEAM may depend on tumor burden and/or the metastatic site. There is no need to consider the cutoff for patients with only liver metastases. The baseline longest diameter and number of lesions are associated with the concordance rate. Peritoneal metastases alone with a lesion diameter <20 mm, lung metastases alone with a lesion diameter <20 mm, and <10 lesions are discordant for ctDNA testing. Careful consideration should be given to patients with mCRC with lung or peritoneal metastases only when using liquid biopsy.

Previous studies of small numbers of patients suggested that the concordance rates in ctDNA and tissue *RAS* mutational status varied by metastatic site (9, 10). An adequate concordance between the plasma and tissue *RAS* mutational status was observed in patients with mCRC with liver metastases alone (9, 10). Although they were thought to be associated with a significant discordance in patients with only lung metastases, the baseline longest diameter and number of lesions in the lungs were significantly associated with concordance (9). Moreover, a range of concordance rates depending on the metastatic site was suggested in previous studies (9, 10).

We integrated individual patient data from cases evaluated in previous studies (9–11) wherein the *RAS* status in ctDNA using the OncoBEAM™ RAS CRC Kit was examined. Through a combined analysis, we investigated the clinical factors associated with concordance between the plasma and tissue *RAS* mutational status in patients with mCRC with liver, peritoneal, or lung metastases only to identify individuals eligible for liquid biopsy analysis.

Materials and Methods

Study design and patients

Individual patient data from one Japanese (9) and two Spanish studies (refs, 10, 11; $n = 662$), which examined the concordance rates between plasma and tissue *RAS* mutational status using the OncoBEAM™ RAS CRC Kit and tissue reference method, respectively, were integrated. Patients who met the following criteria were selected ($n = 261$, CONSORT diagram; Supplementary Fig. S1): (i) patients with pathologically confirmed metastatic colorectal adenocarcinoma; (ii) patients who were chemo naïve or confirmed to have a progressive disease without having initiated subsequent treatment; (iii) patients with no prior treatment with anti-EGFR mAb or regorafenib; (iv) patients with only liver metastases, peritoneal metastases, or lung metastases; and (v) patients with no tumor removal before blood sample collection. The interval between plasma collection and CT was ≤3 months.

Tumor burden data for *post-hoc* analysis were obtained by investigating the baseline longest diameter of the largest lesion and number of tumor lesions on CT within 3 months before or after ctDNA sample collection. Scatterplots of concordant and discordant cases based on the tumor burden data were used to confirm the significance of the cutoffs. This combined study was approved by the ethics and review committees at each institution, and all patients provided written informed consent. All procedures related to the study were performed in accordance with the Helsinki Declaration and the Ethical Guidelines for Medical Health Research Involving Human Subjects in Japan and Spain.

Study outcomes

This study's primary outcome was to investigate the clinical factors associated with concordance between the plasma and tissue *RAS* mutational status. The secondary outcome was to determine the optimal cutoffs for clinical factors to maintain a sufficient concordance greater than 90% at each metastatic site.

Statistical analysis

Continuous variables were described using medians and ranges, whereas categorical variables were presented as percentages. The concordance between the plasma and tissue *RAS* mutational status in the overall cohort, *RAS*-mutant concordance based on tissue *RAS*-mutant cohort, and *RAS* wild-type concordance based on tissue *RAS* wild-type cohort were calculated with 95% confidence interval (CI) and tested using Fisher's exact test.

Factors associated with concordance were calculated using univariate and multivariate logistic regression models. Multivariate analysis was performed for factors with $P < 0.1$ based on univariate analysis. Variables were entered directly if $P < 0.1$ in the univariate analysis. Statistical analyses were performed using R version 3.6.1.

Results

Of the 261 patients with mCRC who were initially enrolled (CONSORT diagram; Supplementary Fig. S1), 40 were excluded because of missing measurements for the baseline longest diameter or number of lesions ($n = 15$) or due to an interval of more than 3 months between plasma collection and CT ($n = 25$). The full analysis set comprised of 221 patients who had liver metastases alone ($n = 151$), peritoneal metastases alone ($n = 25$), and lung metastases alone ($n = 45$). Patient characteristics categorized by metastatic sites are shown in Supplementary Table S1. The cutoffs for the baseline longest diameter and number of lesions were provisionally set to 20 mm and 10 lesions, respectively, based on a previous report (9).

The overall concordance rate in liver metastases alone was 91% (95% Cl, 85%–95%), whereas those of peritoneal and lung metastases alone were 88% (95% CI, 68%–97%) and 64% (95% CI, 49%–78%), respectively. Similar trends were observed in the European and Asian subsets (**Fig. 1**). The concordance rate of lung metastases alone was relatively low compared with that of liver or peritoneal metastases alone. The mean of mutation allele fraction (MAF) of liver, peritoneum, and lung was 6.8%, 7.2%, and 2.6%, respectively. The MAF was relatively low in lung metastases alone, as shown in Supplementary Fig. S2.

RAS mutations were detected in 39% and 47% of plasma and tissue samples, respectively. The overall concordance between plasma- and tissue-based analyses was 85% (188/221; 95% CI, 80%–89%), with 76% (79/104; 95% CI, 66%–84%) *RAS*-mutant concordance based on tissue *RAS*-mutant cohort and 93% (109/117; 95% CI, 87%–97%) *RAS* wild-type concordance based on tissue *RAS* wild-type cohort. The *RAS*-mutant concordance was significantly lower than the *RAS* wild-type concordance ($P < 0.001$). Almost no difference in the concordance rate for each codon of *RAS* was observed (data not shown).

Univariate/multivariate analysis of the clinical findings between concordant and discordant cases was conducted. The analysis for

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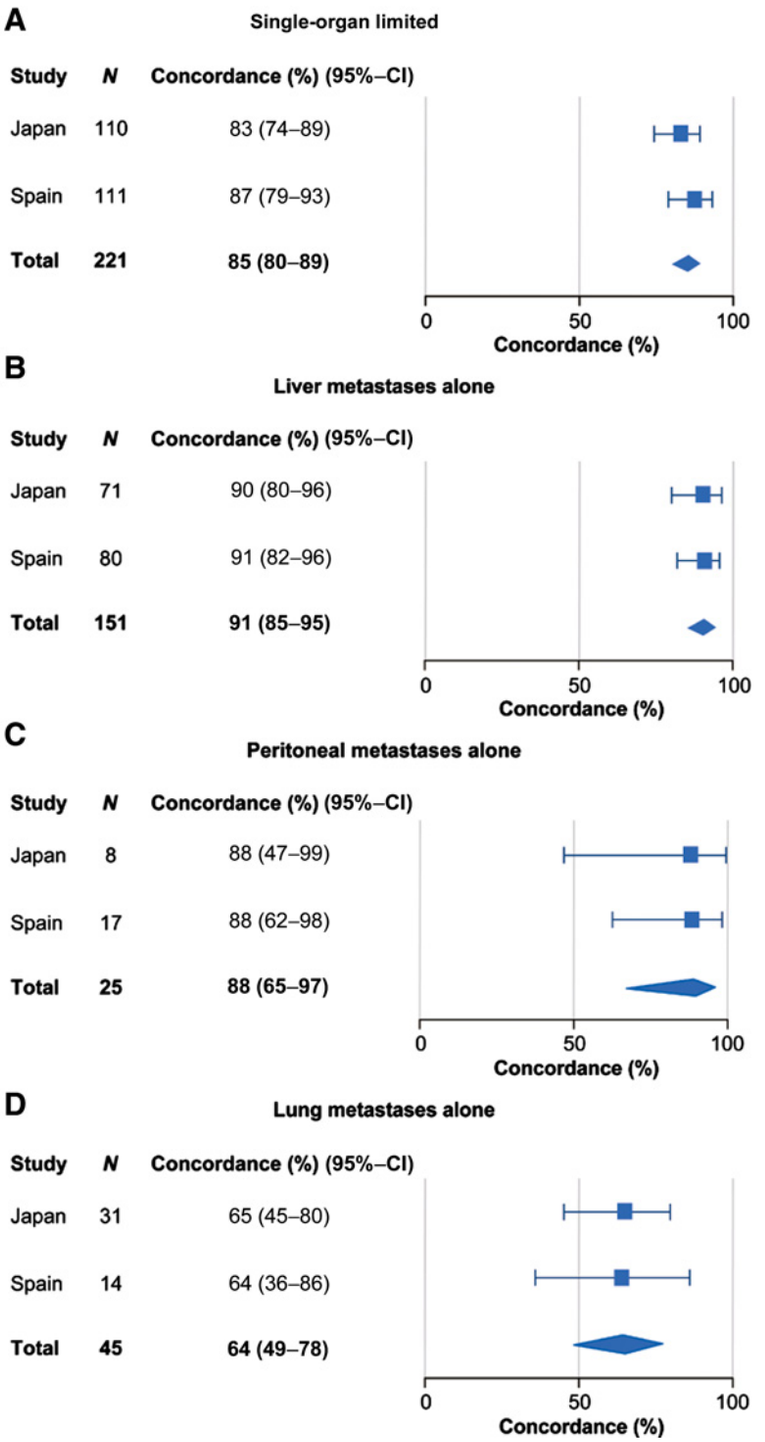

Figure 1.

Combined analysis of concordance in the *RAS* mutational status between plasma- and tumor tissue–based analyses for individual metastatic sites: single-organ limited (**A**), liver metastases alone (**B**), peritoneal metastases alone (**C**), and lung metastases alone (**D**).

identifying factors associated with discordance is described in Supplementary Table S2. On multivariate analysis, the most significant factors associated with concordance were the baseline longest diameter of the lesion and the number of lesions ($P = 0.004$; **Table 1**). Both factors are related to tumor burden and are evaluable factors in clinical practice, thus, these values were adopted as cutoffs to select concordant cases in this study.

Furthermore, univariate and multivariate analyses of the clinical findings by tissue *RAS* mutational status between concordant and discordant cases were conducted (Supplementary Tables S3 and S4). In the tissue *RAS*-mutant cohort, the analysis for identifying factors associated with discordance is described in Supplementary Table S3. In multivariate analysis of the tissue *RAS*-mutant cohort, the significant factors associated with the *RAS*-mutant concordance

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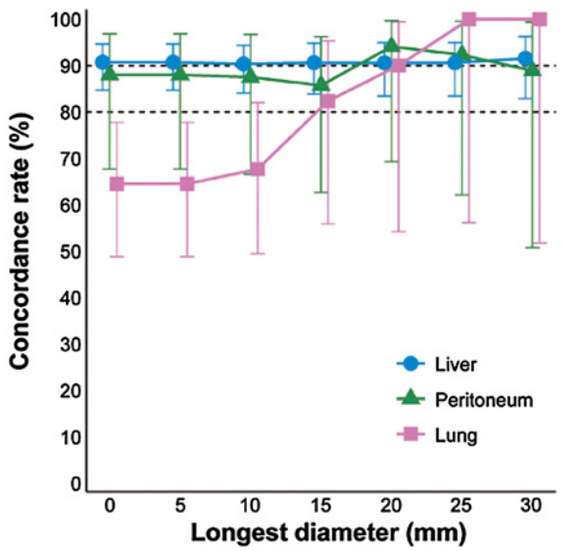
Table 1. Univariate and multivariate analyses of clinical findings between concordant and discordant cases in the overall cohort.

		Univariate analysis		Multivariate analysis	
	Categorical variables[a]	OR (95% CI)	*P*[b]	OR (95% CI)	*P*[b]
Age		1.0 (0.6–1.7)	0.869	NA	NA
Sex	Male/female	1.4 (0.6–3.3)	0.383	NA	NA
Previous chemotherapy	Yes/no	2.4 (1.1–5.2)	0.032	0.7 (0.2–2.0)	0.500
Location of primary tumor[c]	Left/right	2.9 (1.0–8.6)	0.059	2.0 (0.6–6.8)	0.261
Metastatic site	Liver/other	0.3 (0.1–0.6)	<0.001	0.9 (0.2–3.9)	0.897
	Peritoneum/other	0.8 (0.2–2.7)	0.663	NA	NA
	Lung/other	5.2 (2.3–11.4)	<0.001	1.8 (0.4–8.1)	0.443
Collection interval (tissue vs. plasma)		2.2 (1.5–3.3)	<0.001	1.9 (1.0–3.3)	0.036
Source of tissue samples	Metastasis/primary	1.9 (0.7–5.7)	0.237	NA	NA
Baseline longest diameter and number of lesions	<20 mm and <10/≥20 mm or ≥10	5.0 (2.3–10.9)	<0.001	3.8 (1.5–9.5)	0.004
Resection of primary tumor	Yes/no	3.8 (1.1–13.1)	0.032	1.4 (0.3–5.6)	0.633
CEA	<5 ng/mL/≥5 ng/mL	1.6 (0.7–3.6)	0.296	NA	NA

Abbreviations: CEA, carcinoembryonic antigen; NA, not available; OR, odds ratio.
[a]The second category for each variable was used as reference in the model to calculate the OR.
[b]*P* values were derived using Fisher's exact test for categorical variables and the Kruskal–Wallis test for continuous variables.
[c]Left includes splenic flexure to rectum; right includes caecum to transverse colon.

were collection interval ($P = 0.004$), baseline longest diameter, and number of lesions ($P = 0.005$), as it was with the overall cohort. On the other hand, there was no significant factor in univariate analysis of the tissue *RAS* wild-type cohort (Supplementary Table S4).

The concordance rate in the liver metastases alone was more than 90%, with any cutoff value used (**Fig. 2**). Using the baseline longest diameter of lesion ≥20 mm as a cutoff, the concordance rate of peritoneal metastases alone was 94%, providing a sufficient concordance and indication for OncoBEAM™ testing, with a coverage of 68%, that is, this cutoff covered 68% of patients with liver metastases. The reliability of using the cutoff of a baseline longest diameter ≥20 mm for patients with mCRC with peritoneal metastases alone was reflected



Longest diameter		0	5	10	15	20	25	30
Concordance (%)	Liver	91	91	90	91	91	91	92
	Peritoneum	88	88	88	86	94	92	89
	Lung	64	64	68	82	90	100	100
Coverage (%)	Liver	100	100	96	85	77	65	55
	Peritoneum	100	100	96	84	68	52	36
	Lung	100	100	76	38	22	16	13

Figure 2.
Concordance and coverage with an optimal cutoff of the baseline longest diameter of lesions alone. Coverage refers to the percentage of patients having the specific metastatic lesions (liver/peritoneum/lung) covered or included in the categories specified by lesion size, as defined by CT.

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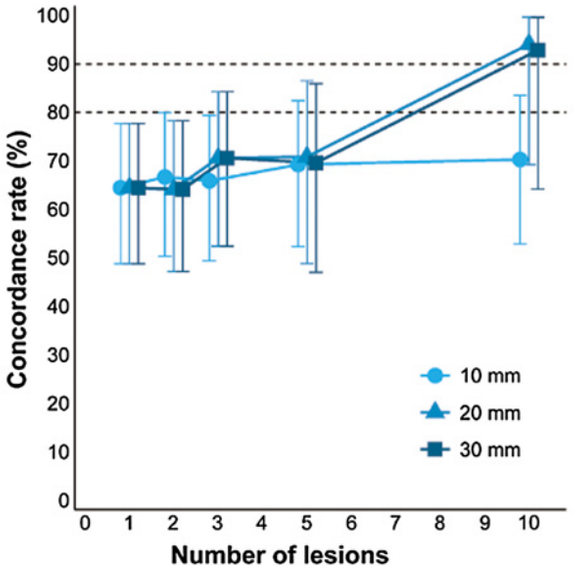

Figure 3.
Concordance and coverage with optimal cutoffs of the baseline longest diameter of lesions and number of lesions in lung metastases alone. The concordance rate of lung metastases increased to 94%, with an improved coverage rate of 38%, when using the cutoff of baseline longest diameter of ≥20 mm or ≥10 lesions. Coverage refers to the percentage of patients having the lung metastatic lesions covered or included in the categories specified by lesion size and numbers, as defined by CT.

Number of lesions		1	2	3	5	10
Concordance (%)	10 mm	64	67	66	69	70
	20 mm	64	64	71	71	94
	30 mm	64	64	71	70	93
Coverage (%)	10 mm	100	93	91	87	82
	20 mm	100	87	76	53	38
	30 mm	100	87	76	51	31

by a high *RAS*-mutant concordance of 86% (95% CI, 42%–99%), along with 100% (95% CI, 66%–100%) *RAS* wild-type concordance. Using 20 mm as the cutoff value for the baseline longest diameter in peritoneal metastases alone, 67% (2/3) of discordant cases were below the cutoff value, and 73% (16/22) of concordant cases were above the cutoff (Supplementary Fig. S3).

The concordance rate for lung metastases alone using the cutoff of the baseline longest diameter ≥20 mm alone was 90%, but the coverage was poor (22%; **Fig. 2**). Similarly, when the number of lesions ≥10 alone was used as a cutoff, the concordance rate for lung metastases alone was 89%, with poor coverage of 20% (Supplementary Fig. S4). However, the concordance rate of lung metastases alone increased to 94%, with improved coverage of 38% when using the cutoff of a baseline longest diameter ≥20 mm and ≥10 lesions (**Fig. 3**), with 88% *RAS*-mutant concordance. Most discordant cases (94%, 15/16) occurred in the baseline longest diameter of lung lesions <20 mm and <10 lesions (**Fig. 4**, as shown in the black square). Concordance within and without the cutoffs were 46% (95% CI, 28%–66%) and 94% (95% CI, 69%–100%), respectively, showing a significant difference ($P < 0.001$).

Discussion

This is the first combined analysis of individual patient data from Europe and Asia on the concordance of *RAS* mutational status by metastatic site between plasma ctDNA and tumor tissue DNA testing (14). There were no differences between the European and Asian data. We found that the concordance rates differed by the metastatic site. Although there is currently no clear explanation for this result, the effects may have been organ specific. Therefore, it is necessary to investigate the clinical factors associated with high concordance in each metastatic site so that patients with these clinical factors can be selected for OncoBEAM™-based detection of their *RAS* mutational status.

In our multivariate statistical model, the most significant concordance factors were the baseline longest diameter and the number of lesions ($P = 0.004$), followed by the sample collection interval ($P = 0.036$). Tumor burden (i.e., baseline longest diameter and number of lesions) impacts the amount of ctDNA released into plasma (15). Thus, an increasing amount of ctDNA results in increased concordance rates. The baseline longest diameter and number of lesions can be easily measured and implemented in clinical practice. In addition, the sample collection interval has been associated with discordance, in which longer intervals between plasma and tissue collections may contribute to clonal evolution (9, 16).

For patients with liver metastases alone, the concordance rate was high in Europe and Asia. As such, the lesion diameter and number are not necessary to select patients for testing using the OncoBEAM™ RAS CRC Kit. The median MAF was higher in liver metastases than in peritoneal and lung metastases (9, 10, 12, 17). Bachet and colleagues reported that primary tumor resection and the presence of liver metastases were significantly associated with the presence of ctDNA, although liver metastases appeared to be the key factor (18). Elez and colleagues observed a high MAF burden in liver metastases, which was

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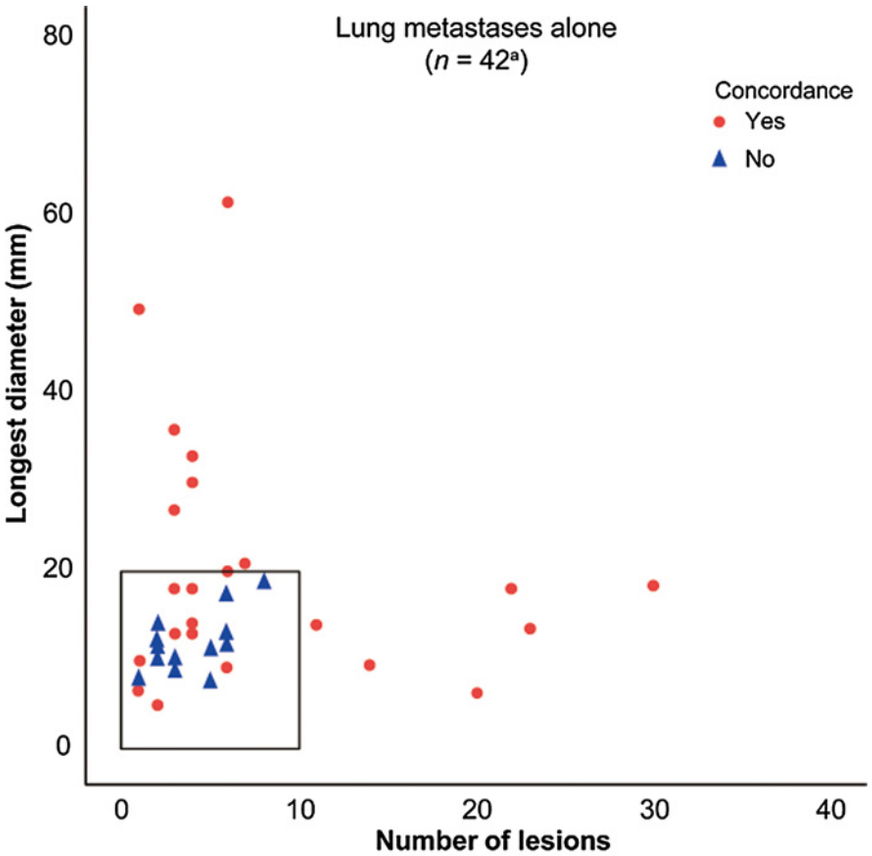

Figure 4.
Scatterplot of concordant and discordant cases based on baseline longest diameter and number of lesions to confirm the optimal cut-offs in lung metastases alone. [a], Excluded three cases where the exact number of lesions was unknown, for example, >30. Two of three cases were concordant and one case was discordant. [b], *P* value was derived using Fisher's exact test.

	Longest diameter <20 mm and number <10	Longest diameter ≥20 mm or number ≥10
Concordance (*n* = 29)	13 (45%: 13/29)	16 (55%: 16/29)
Discordance (*n* = 16)	15 (94%: 15/16)	1 (6%: 1/16)

	Longest diameter <20 mm and number <10	Longest diameter ≥20 mm or number ≥10	*P*[b]
Concordance(%) (95% CI)	46 (28–66)	94 (69–100)	0.001
RAS MT concordance(%) (95% CI)	30 (14–54)	88 (47–99)	0.011
RAS WT concordance(%) (95% CI)	88 (47–99)	100 (63–100)	0.471

not related to the volume and number of lesions in liver metastases (17). ctDNA is thought to be easily released into the circulation regardless of the tumor size or lesion number in the liver.

In previous studies, patients with peritoneal metastases alone showed a low MAF burden (12, 17). MAF from patients with only peritoneal metastases was lower than in patients with peritoneal metastases and at least one other metastatic site (12). The discordance was associated with the presence of peritoneal metastases (18). However, our combined study revealed considerable high concordance (88%) without setting a cutoff value. This may be because we enrolled cases in which lesions could be detected by CT; a total of 68% (17/25) of

peritoneal metastases with the longest lesion diameter ≥20 mm were included. In addition, sufficient concordance and good coverage were observed when we selected cases with the *RAS* wild-type concordance longest diameter ≥20 mm, with high *RAS*-mutant concordance, and *RAS* wild-type concordance. In peritoneal metastases alone, the maximum size may be related to the amount of ctDNA release.

In contrast, the concordance in patients with lung metastases alone was extremely low in Europe and Asia (9, 10), likely because of the low MAF (9, 10, 12, 17). Like previous studies, this study's results also showed that the MAF with lung metastases tended to be lower than for other metastatic sites (Supplementary Fig. S2). The release of ctDNA from lung metastases is thought to be extremely low, thus, tumor burden plays a more important role in lung metastases. As such, the lesion size and number must be determined to select appropriate patients with lung metastases alone for OncoBEAM™ testing, that is, a closer evaluation of the tumor burden is required. These two factors were also selected as cutoffs for patients with lung metastases alone in the Japanese study (9). Under the cutoff value, a liquid biopsy may not detect *RAS* mutations in lung metastases. In such cases, the tissue-based analysis would be the preferred choice.

In a *post-hoc* analysis, we reviewed the size and number of metastatic tumors of baseline CT in each case. This is the first international collaborative study to evaluate the relationship between the tumor burden and concordance by metastatic site between plasma- and tissue-based *RAS* mutational analysis. This study reveals the impact of the tumor burden and organ specificity on concordance rates.

In our study, the presence of previous chemotherapy was significantly associated with a higher discordance than its absence for the overall cohort ($P = 0.045$; Supplementary Table S2). In univariate analysis of clinical findings between concordance and discordance in overall and tissue *RAS*-mutant cohorts, previous chemotherapy was indicated to be one of the significant factors, as described in **Table 1** and Supplementary Table S3 ($P = 0.032$ and $P = 0.019$, respectively); however, it was not a significant factor in multivariate analysis for overall and *RAS*-mutant cohorts ($P = 0.500$ and $P = 0.214$, respectively). This study included 50 patients who received chemotherapy between tissue and plasma collection. The mutational state was changed in 12 cases, including 10 cases (one case in the liver, three in the peritoneum, and six in the lung) that changed from mutant to wild-type, suggesting below limit-of-detection level (called NeoRAS; ref. 19). All of the 10 cases had tumors with sizes <20 mm, and each case had <10 lesions, indicating a low tumor burden. The collection interval between tissue and plasma sampling is also a significant factor in the multivariate analysis for the overall and tissue *RAS*-mutant cohorts, as shown in **Table 1** and the Supplementary Table S3 ($P = 0.036$ and $P = 0.004$, respectively), indicating that the tumor volume reduction as a result of chemotherapy may affect the discordance of the tissue *RAS* mutant. Only two cases (one case each in the lung and the liver) changed from wild-type to mutant during chemotherapy. These might be due to clonal evolution or tumor heterogeneity, even in cases that did not receive targeting agents, such as an anti-EGFR antibody and/or regorafenib.

In conclusion, the OncoBEAM™ RAS CRC Kit's utility in patients with mCRC may depend on the metastatic site. Although no further selection is required for patients with liver metastases alone, careful attention should be given to patients with peritoneal metastases alone with lesion size <20 mm, as well as those with lung metastases alone with lesion size <20 mm and <10 metastatic lesions when determining the *RAS* mutational status using the ctDNA-based assay.

Authors' Disclosures

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Authors' Contributions

Y. Kagawa: Writing–original draft, project administration, writing–review and editing. **E. Elez:** Writing–review and editing. **J. García-Foncillas:** Writing–review and editing. **H. Bando:** Project administration, writing–review and editing. **H. Taniguchi:** Writing–review and editing. **A. Vivancos:** Writing–review and editing. **K. Akagi:** Writing–review and editing. **A. García:** Writing–review and editing. **T. Denda:** Writing–review and editing. **J. Ros:** Writing–review and editing. **T. Nishina:** Writing–review and editing. **I. Baraibar:** Writing–review and editing. **Y. Komatsu:** Writing–review and editing. **D. Ciardiello:** Writing–review and editing. **E. Oki:** Writing–review and editing. **T. Kudo:** Writing–review and editing. **T. Kato:** Writing–review and editing. **T. Yamanaka:** Writing–review and editing. **J. Tabernero:** Writing–review and editing. **T. Yoshino:** Project administration, writing–review and editing.

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References

1. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol 2019; 14:89–103.
2. Bokemeyer C, Köhne CH, Ciardiello F, Lenz H-J, Heinemann V, Klinkhardt U, et al. FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. Eur J Cancer 2015;51:1243–52.
3. Van Cutsem E, Lenz H-J, Köhne C-H, Heinemann V, Tejpar S, Melezínek I, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol 2015;33:692–700.
4. Yoshino T, Arnold D, Taniguchi H, Pentheroudakis G, Yamazaki K, Xu R-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;29:44–70.
5. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
6. Diehl F, Li M, He Y, Kinzler KW, Vogelstein B, Dressman D. BEAMing: single-molecule PCR on microparticles in water-in-oil emulsions. Nat Methods 2006;3: 551–9.
7. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008;14:985–90.
8. Vivancos A, Aranda E, Benavides M, Élez E, Gómez-España MA, Toldeano M, et al. Comparison of the clinical e of the Idylla platform and the OncoBEAM RAS CRC assay for KRAS mutation detection in liquid biopsy samples. Sci Rep 2019; 9:8976.
9. Bando H, Kagawa Y, Kato T, Akagi K, Denda T, Nishina T, et al. A multicentre, prospective study of plasma circulating tumour DNA test for detecting RAS mutation in patients with metastatic colorectal cancer. Br J Cancer 2019;120: 982–6.
10. García-Foncillas J, Tabernero J, Élez E, Aranda E, Benavides M, Camps C, et al. Prospective multicenter real-world RAS mutation comparison between Onco-BEAM-based liquid biopsy and tissue analysis in metastatic colorectal cancer. Br J Cancer 2018;119:1464–70.
11. Grasselli J, Elez E, Caratù G, Matito J, Santos C, Macarulla T, et al. Concordance of blood- and tumor-based detection of RAS mutations to guide anti-EGFR therapy in metastatic colorectal cancer. Ann Oncol 2017;28:1294–301.
12. Vidal J, Muinelo L, Dalmases A, Jones F, Edelstein D, Iglesias M, et al. Plasma ctDNA RAS mutation analysis for the diagnosis and treatment monitoring of metastatic colorectal cancer patients. Ann Oncol 2017;28:1325–32.
13. Schmiegel W, Scott RJ, Dooley S, Lewis W, Medlrum CJ, Pockney P, et al. Blood-based detection of RAS mutations to guide anti-EGFR therapy in colorectal cancer patients: concordance of results from circulating tumor DNA and tissue-based RAS testing. Mol Oncol 2017;11:208–19.
14. Galvano A, Taverna S, Badalamenti G, Incorvaia L, Castiglia M, Barraco N, et al. Detection of RAS mutations in circulating tumor DNA: a new weapon in an old war against colorectal cancer. A systematic review of literature and meta-analysis. Ther Adv Med Oncol 2019;11:1–15.
15. Thierry AR, Mouliere F, Gongora C, Ollier J, Robert B, Ychou M, et al. Origin and quantification of circulating DNA in mice with human colorectal cancer xenografts. Nucleic Acid Res 2010;38:6159–75.
16. Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med 2015;21:795–801.
17. Elez E, Chianese C, Sanz-García E, Martinelli E, Noguerido A, Mancuso FM, et al. Impact of circulating tumor DNA mutant allele fraction on prognosis in RAS-mutant metastatic colorectal cancer. Mol Oncol 2019;13:1827–35.
18. Bachet JB, Bouché O, Taieb J, Dubreuil O, Garcia ML, Meurisse A, et al. RAS mutation analysis in circulating tumor DNA from patients with metastatic colorectal cancer: the AGEO RASANC prospective multicenter study. Ann Oncol 2018;29:1211–9.
19. Henry J, Willis J, Parseghian CM, Raghav KPS, Johnson B, Dasari A, et al. NeoRAS: incidence of RAS reversion from RAS mutated to RAS wild type. J Clin Oncol 2020;38:180.

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