Abstract

Detection of RAS and BRAF mutations is essential to determine the optimal treatment strategy for metastatic colorectal cancer (CRC). We prospectively evaluated the MEBGEN RASKET-B KIT (RASKET-B), a novel multiplex kit, simultaneously detecting 48 types of RAS mutations and the BRAF V600E mutation using Luminex xMAP technology. The aim was to obtain market approval for RASKET-B as an in vitro diagnostic (IVD) option in Japan. Genomic DNA was extracted from 302 formalin-fixed paraffin-embedded tissues obtained from CRC patients. The
Introduction

RAS (KRAS and NRAS) mutations are present in approximately 50% to 55% of colorectal cancer (CRC) cases. The clinical significance of the detection of RAS mutations has been previously established as a required test prior to the initiation of anti-epidermal growth factor receptor (EGFR) antibody therapy to predict the efficacy in metastatic CRC [1–5]. Prospective-retrospective biomarker analyses in randomized clinical trials have consistently demonstrated that anti-EGFR antibodies, cetuximab and panitumumab, are unlikely to benefit patients with KRAS exon 3 and 4 and NRAS exons 2, 3, and 4, mutations, in addition to those with a KRAS exon 2 mutation [6–8]. Moreover, recent results from clinical trials revealed that overall survival is possibly better when patients are treated with anti-EGFR therapy as a first-line treatment than when treated with bevacizumab in the RAS wild-type population [9,10]. This suggests that RAS mutation status has a large impact on the treatment decision in patients with metastatic CRC.

Many studies have reported that the BRAF V600E mutation is detected in approximately 5%–12% of metastatic CRC patients. RAS and BRAF V600E mutations are almost mutually exclusive [11]. Unlike RAS mutations, the predictive value of BRAF mutations for anti-EGFR mAb efficacy is less certain. On the other hand, the BRAF V600E mutation leads to a poor prognosis or rapid progression, regardless of treatment in metastatic CRC [12,13]. Recently, the possibility was reported that triplet chemotherapy combining 5-fluorouracil, oxaliplatin, and irinotecan (FOLFOXIRI) with bevacizumab is more effective than other chemotherapies for patients with the BRAF V600E mutation [14,15], and both European Society for Medical Oncology (ESMO) consensus guidelines and pan-Asian adapted ESMO consensus guidelines recommend FOLFOXIRI plus bevacizumab as the preferred choice for these patients [16,17]. Therefore, the BRAF mutation status should be assessed before starting the first-line chemotherapy. The latest edition of the Japanese Society of Medical Oncology Clinical Guidelines: Molecular Testing for Colorectal Cancer Treatment states that proper testing for BRAF V600E mutation and mismatch repair deficiency is necessary in addition to testing for RAS mutation [18].

We previously reported that the MEBGEN RASKET KIT (RASKET) is useful for rapid detection of 48 types of mutations in codons 12, 13, 59, 61, 117, and 146 of KRAS and NRAS using PCRR-SSO (PCR-reverse sequence specific oligonucleotide) technology [19]. The RASKET clinical validation study confirmed the precise detection of RAS mutations, with a concordance rate (CR) of 98.4% between the RASKET KIT and direct sequencing in RAS mutations (UMIN000011781). The RASKET KIT was approved in Japan as an in vitro diagnostic (IVD) and has become widely used in daily practice and is recognized as an RAS testing platform in Japan.

As mentioned above, the detection of RAS and BRAF mutations is an essential step for decision-making regarding therapeutic approaches and predicting resistance to EGFR-targeted therapy. The PCR-SSO and xMAP technologies allow multiplex molecular testing in a single well. It would be clinically beneficial to develop a new kit for the simultaneous detection of BRAF V600E mutations and RAS gene mutations. In this study, we evaluated the newly developed MEBGEN RASKET-B KIT (RASKET-B) to detect 48 different RAS amino acid mutations and the BRAF V600E mutation in CRC patients. This study was performed as a registration trial for regulatory approval of the kit in Japan.

Material and Methods

Patients and Tumor Samples

The RASKET-B study used the identical cohort and the DNA sample sets that were used in the RASKET study (Study ID: UMIN000011784) [19]. Briefly, the eligibility criteria for patients were 1) histologically confirmed adenocarcinoma of colorectal origin, 2) age ≥20 years at the time of informed consent, and 3) patients’ written consent for participation in the study. Patients with insufficient amounts of formalin-fixed paraffin-embedded (FFPE) tissues, those with an undetermined RAS status by the RASKET kit in the previous RASKET study, and those who withdrew consent were excluded from the RASKET-B study. One central pathologist assigned for the study microscopically confirmed cancer in each patient, classified the tumor into the appropriate histologic type, calculated the tumor area ratio and tumor cell ratio, and then marked the tumor area on the prepared hematoxylin and eosin–stained slides for manual microdissection (MMD).

Study Design

All specimens were anonymized. Only the participating affiliations were able to access patients’ information using a correspondence table, which was only available at each study site to eliminate any
disclosure to outsiders. Sample anonymity was the task of one employee of G&G Science Co., Ltd., who was not involved in the study.

The set of extracted DNA from the FFPE specimens was sent to three different reference laboratories (G&G Science Co., Ltd.; Health Sciences Research Institute, Inc.; and SRL Inc.), where independent assays were performed with RASKET-B, direct sequencing (DS), and pyrosequencing (PYRO), respectively. All samples were deidentified and blinded to the tissue genotype and clinical characteristics of each patient.

The primary endpoints in this study were the CR between results from RASKET-B and RASKET for \( RAS \) mutations, and the CR between RASKET-B and DS for \( BRAF \) mutations. The results of \( RAS \) mutations by the RASKET study [19] were used for comparison with those obtained by RASKET-B. As the secondary endpoints, we determined the CR between results from RASKET-B and DS for the \( RAS \) gene, and results from RASKET-B and PYRO for the \( BRAF \) gene. In addition, the accuracy of genotyping was evaluated by comparing data between RASKET-B and DS. The original and revised protocols were approved by the ethical committees in each of the participating affiliations. The study was conducted in accordance with the Declaration of Helsinki and ethical guidelines for clinical research.

Direct Sequencing

After the pathological confirmation of cancer in each patient, 10-μm-thick sections were processed by MMD. DNA extraction was performed with QIAamp DNA FFPE Tissue Kit (Qiagen, Venlo, Netherlands) according to the manufacturer’s protocol and as previously reported [19,20]. Briefly, each extracted DNA was amplified using six sets of primers to amplify exon 2, exon 3, and exon 4 in \( KRAS \) and \( NRAS \), and exon 15 in \( BRAF \). The mutations in these regions were detected using the BigDye Terminator Cycle Sequencing Kit (Thermo Fischer Scientific, Waltham, MA).

Pyrosequencing

DNA samples were analyzed for codon 600 in exon 15 of \( BRAF \) with the Therascreen \( BRAF \) Pyro Kit (Qiagen) as described by the manufacturer’s protocol. The PYRO method was performed without MMD. For one patient with a discrepancy between RASKET-B and PYRO, MMD was additionally performed for PYRO to carefully confirm the existence of \( BRAF \)-mutated tumor cells.

Assay with MEBGEN RASKET-B KIT

Extracted DNA samples were diluted to a concentration of 10–20 ng/μl with sterile TE buffer (1 mmol/l Tris–HCL [pH = 8.0], 0.1 mmol/l EDTA). Assays with the RASKET-B kit (MBL, Nagoya, Japan) were performed according to the manufacturer’s protocol. The PYRO method was performed without MMD. For one patient with a discrepancy between RASKET-B and PYRO, MMD was additionally performed for PYRO to carefully confirm the existence of \( BRAF \)-mutated tumor cells.

Table 1. Patient Characterization in the Blinded Clinical Evaluation Study for the MEBGEN RASKET-B KIT

| Age, years (minimum-maximum) | 64 (26-89) |
| Male | 178 |
| Female | 124 |
| Right-sided colon | 62 |
| Recurrent | 98 |
| Stage 0-I | 142 |
| Stage II | 33 |
| Stage III | 48 |
| Stage IV | 125 |
| Well to moderately differentiated adenocarcinoma | 273 |
| Poorly differentiated or mucinous | 29 |
| Tumor area ratio (%) | 50 (5-100) |
| Tumor cell ratio (%) | 50 (5-90) |

* Descending colon and sigmoid colon.
† Recurrent (Rx, and Rb). Rx upper rectum (above peritoneal reflection), Rb lower rectum (below peritoneal reflection).
‡ Descending colon and sigmoid colon.
at the lower limit of the 95% confidence interval. We then determined the number of specimens for the RASKET-B study needed to exceed 278. Patients' demographic and disease characteristics were reported as standard statistics. Statistical analysis was carried out using StatFlex (Artech Co. Ltd., Osaka, Japan). The RASKET-B study is registered with UMIN22742.

Results

Patients

Tissues were obtained from 309 consenting patients with histologically confirmed CRC. Insufficient amounts of FFPE were available in four patients, and RAS gene status in three patients was not reportable using RASKET in the previous RASKET study. Therefore, 302 patients were eligible for the primary endpoint analysis (Figure 1) in the RASKET-B study. Patient data are provided in Table 1.

Frequency of RAS and BRAF Mutations in MEBGEN RASKET-B KIT

Among the 302 samples, RASKET-B detected 142 RAS mutations [113 (37.4%) KRAS exon 2, 5 (1.7%) KRAS exon 3, 10 (3.3%) KRAS exon 4, 6 (2.0%) NRAS exon 2, 8 (2.6%) NRAS exon 3] and 18 (6.0%) BRAF exon 15 mutations (Table 2). All mutations detected from the recruited patients were mutually exclusive. Both RAS and BRAF mutation rates were statistically higher in colon cancer on the right side than on the left side. Especially, the frequency of BRAF mutations among patients with RAS wild type was six-fold higher on the right side than on the left side (Table 3). Among patients with ascending colon cancer, 25% (9/36) had BRAF V600E mutations, a higher frequency than the other tumor locations (Figure 2). In terms of histologic types, the frequency of BRAF V600E mutations was significantly higher in patients with poorly differentiated or mucinous colon cancer. Also, the frequency of BRAF V600E mutations in females was not significant but tended to be higher than in males ($P = .0745$, Table 3).

Concordance of RAS and BRAF Status

In the primary endpoint analysis, the CR between results concerning RAS mutation status obtained from RASKET-B and RASKET was 100% (302/302) (95% CI: 98.8%-100%) (Table 4A). Concerning the BRAF mutation status, the CR between results obtained from RASKET-B and DS was also 100% (302/302) (95% CI: 98.8%-100%) (Table 4B).

In the secondary endpoint analyses of all samples, the CR between RASKET-B and DS was 97.4% (294/302) (95% CI: 94.9%-98.9%) in RAS (Table 5A). Among the eight samples with conflicting results between RASKET-B and DS, six samples were positive with RAS mutations (two cases with KRAS G12D, two cases with KRAS G13D, one case with KRAS G12R, and one case with KRAS Q61H) in RASKET-B but negative in DS. Genotypic results of these samples in RASKET-B were consistent with RASKET and with results from the TaqMan Mutation Detection Assay, which is a more sensitive method (cutoff levels 0.1%-1%). The other two samples were negative with RAS mutations in RASKET-B and positive with DS. One of the samples had two mutations in KRAS codon 11 and codon 12 (G12C). The other sample had a KRAS mutation in A59E, which was not covered by RASKET-B (Table 6).

Table 2. Frequency of Breakdown of RAS and BRAF Mutations Detected in Colorectal Cancer Patients

| Mutation Status                  | No. of Cases | Proportion Among 302 Cases |
|----------------------------------|--------------|----------------------------|
| **WT RAS or BRAF**               | 142          | 47.0%                      |
| **KRAS exon 2 mutant**           | 113          | 37.4%                      |
| p.G12S                           | 5            | 1.7%                       |
| p.G12C                           | 8            | 2.6%                       |
| p.G12R                           | 4            | 1.3%                       |
| p.G12D                           | 44           | 14.6%                      |
| p.G12V                           | 23           | 7.6%                       |
| p.G12A                           | 6            | 2.0%                       |
| p.G12A, p.G12R                   | 1            | 0.3%                       |
| p.G13D                           | 20           | 6.6%                       |
| p.G12D, p.G13D                   | 2            | 0.7%                       |
| Other KRAS exon 2 mutant*        | 0            | 0.0%                       |
| **KRAS exon 3 mutant**           | 6            | 2.0%                       |
| p.A59E                           | 1            | 0.3%                       |
| p.Q61K                           | 5            | 1.7%                       |
| Other KRAS exon 3 mutant*        | 0            | 0.0%                       |
| **KRAS exon 4 mutant**           | 10           | 3.3%                       |
| p.K117N                          | 2            | 0.7%                       |
| p.A146T                          | 6            | 2.0%                       |
| p.A146P                          | 1            | 0.3%                       |
| p.A146V                          | 1            | 0.3%                       |
| **NRAS exon 2 mutant**           | 6            | 2.0%                       |
| p.G12D                           | 4            | 1.3%                       |
| p.G12V                           | 2            | 0.7%                       |
| Other NRAS exon 2 mutants        | 0            | 0.0%                       |
| **NRAS exon 3 mutant**           | 8            | 2.6%                       |
| p.Q61K                           | 2            | 0.7%                       |
| p.Q61L                           | 5            | 1.7%                       |
| p.Q61R                           | 1            | 0.3%                       |
| Other NRAS exon 3 mutants        | 0            | 0.0%                       |
| **NRAS exon 4 mutant**           | 0            | 0.0%                       |
| **BRAF V600E mutant**            | 18           | 6.0%                       |

†KRAS p.G13S, p.G13R, p.G13V, and p.G13A.
‡KRAS p.A59T, p.A59G, p.Q61E, p.Q61L, and p.Q61R.
§NRAS p.G12S, p.G12C, p.G12R, and p.G12A.
¶NRAS p.A59T, p.A59G, p.Q61E, p.Q61L, and p.Q61R.
**KRAS p.A59T, p.A59G, p.Q61K, p.Q61E, p.Q61L, and p.Q61R.

Table 3. Correlation Between Tumor Location Side and Mutation Status, Sex, or Tissue Type

|                | n         | RAS          | | | BRAF          | | | Braf mutant/wt Wild Type |
|----------------|-----------|--------------|---|---|--------------|---|---|-------------------------|
|                | Wild Type | Mutant       | P Value | Wild Type | Mutant       | P Value | Wild Type |
| Age, median (minimum-maximum) | 160 | 142 (47.0%) | P = .275 | 284 | 18 (6.0%) | P = .0077 |
| Sex (%)        | 6 (33-89) | 63 (26-84) | 77 (43.3%) | 70 (46-89) | 113 | 11 (8.9%) | 7/77 (9.1%) |
| Male           | 178 | 101 | 65 | 124 | 59 | 65 | 113 | 11 (8.9%) |
| Female         | 273 | 144 | 129 (47.3%) | 10 (3%) | 263 | 10 (3.7%) | 10/144 (6.9%) |
| Histologic types (%) | 246 | 134 | 106 (44.2%) | 232 | 8 (3.3%) | 8/134 (6.0%) |
| Well to moderately differentiated adenocarcinoma | 227 | 124 | 129 (47.3%) | 10 (3%) | 263 | 10 (3.7%) | 10/144 (6.9%) |
| Poorly differentiated or Mucinous | 29 | 16 | 13 (44.8%) | 10 (3.3%) | 21 | 8 (38.1%) | 8/16 (50%) |
| Primary tumor location (%) | 62 | 26 | 36 (58.1%) | 10 (16.1%) | 52 | 10 (16.1%) | 10/126 (8.5%) |

Each parameter was analyzed via the Fisher's test, except age, which was analyzed via the Student's t test.
For secondary endpoint analysis of \textit{BRAF} V600E mutation detection, the result of RASKET-B was compared with PYRO. The CR was 99.7% (301/302) (95% CI: 98.2%-100%) (Table 5B). One sample was positive with \textit{BRAF} V600E by RASKET-B and negative by PYRO without MMD. We also performed PYRO assays with MMD and then confirmed the detection of a mutation in \textit{BRAF}. The percentage of \textit{BRAF} mutant alleles in the sample transcript was 11.2%.

Genotyping Performance in RASKET-B

One hundred and fifty-seven specimens with positive \textit{RAS} or \textit{BRAF} mutation results by both the RASKET-B and DS were included. The concordance of each genotype for the overall population assessed by RASKET-B and DS was 100% (157/157) (95% CI: 98.3%-100%) (data not shown).

Table 4A. Consistency of \textit{RAS} Mutation Status (Primary Endpoint Analysis)

|          | RASKET |          |      |          |      |
|----------|--------|----------|------|----------|------|
|          | Positive | Negative | Total |          |      |
| RASKET-B |         |          |      |          |      |
| Positive | 142     | 0        | 142  |          |      |
| Negative | 0       | 160      | 160  |          |      |
| Total    | 142     | 160      | 302  |          |      |
| Overall agreement percentage | 100% (95% CI, 98.8%-100%) |
| Positive agreement percentage | 100% (95% CI, 98.8%-100%) |
| Negative agreement percentage | 100% (95% CI, 98.8%-100%) |

Table 4B. Consistency of \textit{BRAF} V600E Mutation Status (Primary Endpoint Analysis)

|          | DS |          |      |          |      |
|----------|----|----------|------|----------|------|
|          | Positive | Negative | Total |          |      |
| RASKET-B |         |          |      |          |      |
| Positive | 18    | 0        | 18   |          |      |
| Negative | 0     | 284      | 284  |          |      |
| Total    | 18    | 284      | 302  |          |      |
| Overall agreement percentage | 100% (95% CI, 98.8%-100%) |
| Positive agreement percentage | 100% (95% CI, 98.8%-100%) |
| Negative agreement percentage | 100% (95% CI, 98.8%-100%) |

Table 5A. Consistency Between RASKET-B and DS in \textit{RAS} Gene Mutations (Secondary Endpoint-1 Analysis)

|          | DS |          |      |          |      |
|----------|----|----------|------|----------|------|
|          | Positive | Negative | Total |          |      |
| RASKET-B |         |          |      |          |      |
| Positive | 136   | 6        | 142  |          |      |
| Negative | 2     | 158      | 160  |          |      |
| Total    | 138   | 164      | 302  |          |      |
| Overall agreement percentage | 97.4% (95% CI, 94.9%-98.9%) |
| Positive agreement percentage | 95.8% (95% CI, 92.1%-99.5%) |
| Negative agreement percentage | 98.8% (95% CI, 96.4%-100%) |

Table 5B. Consistency Between RASKET-B and PYRO in \textit{BRAF} Gene Mutations (Secondary Endpoint-1 Analysis)

|          | PYRO |          |      |          |      |
|----------|------|----------|------|----------|------|
|          | Positive | Negative | Total |          |      |
| RASKET-B |         |          |      |          |      |
| Positive | 17    | 1        | 18   |          |      |
| Negative | 0     | 284      | 284  |          |      |
| Total    | 18    | 284      | 302  |          |      |
| Overall agreement percentage | 99.7% (95% CI, 98.2%-100%) |
| Positive agreement percentage | 94.4% (95% CI, 80.2%-100%) |
| Negative agreement percentage | 100% (95% CI, 98.8%-100%) |
Discussion

This study is the first to demonstrate the clinical usefulness of the RASKET-B, which can simultaneously detect RAS and BRAF mutations using the PCR-rSSO and xMAP technologies. For RAS genes, we compared the clinical significance of the RASKET-B to RASKET (previously confirmed and approved in Japan[19]) and DS with MMD. The overall CRs of RAS gene detections were 100% and 97.4%, respectively. For the BRAF gene, the results from the RASKET-B were compared to DS with MMD and PYRO; CRs were 100% and 99.7%, respectively. The CRs satisfied the predefined criteria. Based on these results, the RASKET-B was approved by the Ministry of Health, Labour, and Welfare of Japan as an IVD kit for simultaneous determination of both RAS and BRAF mutation status in FFPE and fresh frozen tissues of CRC patients on 05 December 2017.

The presently detected frequency of RAS mutations obtained using the RASKET-B agrees with those reported in several previous studies[2,3,6,7,22], while the frequency of BRAF mutations was slightly lower than that in Western countries[23–26]. The detection limit of RASKET-B was approximately 1%-5% (Supplementary Table 1, A, B, and C), which is identical to RASKET [19]. This detection sensitivity is similar to that of other allele-specific PCR-based technologies, which suggests that the discrepancy of the BRAF mutation rate was not due to sensitivity differences. The frequency of BRAF mutations in Asian countries is approximately 5%[12,27–31], which is consistent with the present study. The BRAF frequency was higher in the right-sided CRC and in females, which is consistent with a previous report[32].

In this study, we observed several inconsistencies between the results of the RASKET-B and the reference assays. Six specimens with positive results in the RASKET-B and negative results in DS were confirmed to be RAS mutation positive via the TaqMan method. The discrepancy may be mainly caused by the sensitivity difference: the detection limit of RASKET-B is higher than the DS (>10%). In fact, five of six cases included a smaller amount of mutant DNA. For the BRAF gene, one sample showed discrepant results between RASKET-B and PYRO, possibly due to its small tumor ratio (tumor cell ratio 70%, tumor area ratio 15%). MMD additionally performed with PYRO showed a positive result.

Conversely, among the two specimens with a negative result in RASKET-B and a positive result in DS, one sample had a KRAS A59E mutation that was not reported in the PRIME study [3]. The other false-negative sample had a double mutation in KRAS codon 11 and codon 12 (G12C). Based on the assay principle, PCR amplifications including codon 11 mutations cannot hybridize to the detection beads for codons 12 and 13 because the codons are adjacent. However, this would have little impact in clinical practice due to the very rare frequency[1,3,7,8]. Thus, this kit can provide clinically appropriate detection of RAS and BRAF mutations.

The Luminox xMAP technology is widely applied for multiplex molecular testing, such as tissue and virus genotyping, which requires differential detections from a number of similar sequences[33,34]. Additionally, for the amplification of multiple genes or their regions, any possible cross-reactions should be minimized to provide appropriate assays. The RASKET-B allows the simultaneous PCR in the same well of nine regions (four regions in each of KRAS and NRAS genes and one region in the BRAF gene) with few cross-reactions. The turnaround time for detection of both RAS and BRAF gene mutations is approximately 4.5 hours, regardless of the number of samples (<96). Thus, the RASKET-B can potentially solve unmet medical needs of clinicians and reference laboratories, as it can be designed for the rapid, high-throughput, and multiplex detection of all RAS and BRAF mutations.

There were some limitations in this study. First, all samples were obtained through surgery and not biopsies. Also, there may be a bias in that only samples with available RAS mutation data were recruited in this study. Poor quality DNA extracted from FFPE tissues could possibly lead to an inaccurate result. However, a sensitivity of 1%-5% in the RASKET-B would be enough to provide RAS/BRAF mutation status in biopsy samples and in clinical practice. Another limitation was that the RASKET-B was designed to detect only V600E mutations in the BRAF gene. This is because the clinical significance of other BRAF mutations still remains unclear in CRC. Even so, the RASKET-B could provide results of RAS and BRAF (V600E) mutations simultaneously with lower cost and a shorter turnaround time compared to other methods, such as next-generation sequencing.

In conclusion, clinical evaluation of the MEBGEN RASKET-B KIT met the predefined primary and secondary endpoints and displayed a high CR with existing RAS and BRAF assays. The RASKET-B provides rapid and precise detection of RAS and BRAF mutations from FFPE tissue from CRC patients.

Conflict of Interest

The study was designed under the responsibility of MBL and was funded by MBL, Japan. The RASKET-B and RASKET were provided by MBL. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neo.2018.10.004.

References

[1] Amado RG, Wolff M, Peeters M, van Cersum E, Siena S, Freeman DJ, Juan T, Silkorski R, Suggs S, and Radinsky R, et al (2008). Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26(10), 1626–1634. http://dx.doi.org/10.1200/JCO.2007.14.7116 [Available from].

[2] Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donata S, Ludwig H, Schuch G, and Stroh C, et al. (2009). Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of KRAS p.G12D WT 1.78% (KRAS p.G12D)

RASKET-B | DS | Percentage of Mutant DNA in TaqMan Detection Assays
--- | --- | ---
KRAS p.G12D WT | 2.81% (KRAS p.G12D) |
KRAS p.G12D WT | 1.78% (KRAS p.G12D) |
KRAS p.G12D WT | 0.31% (KRAS p.G12D) |
KRAS p.G13D WT | 0.41% (KRAS p.G13D) |
KRAS p.G13D WT | 2.03% (KRAS p.G13D) |
KRAS p.Q61H WT | 38.9% (KRAS p.Q61H) |
WT | KRAS p.A11A Not tested |
WT | KRAS p.G12C Not tested |
results of the RASKET-B study

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receiving fluoropyrimidine-based adjuvant chemotherapy. *BMC Cancer* **11**, 344. [Available from](http://dx.doi.org/10.1186/1471-2407-11-344).

[29] Nakanishi R, Harada J, Tuul M, Zhao Y, Ando K, Saeki H, Oki E, Ohga T, Kitao H, and Kakeji Y, et al (2013). Prognostic relevance of KRAS and BRAF mutations in Japanese patients with colorectal cancer. *Int J Clin Oncol* **18**(6), 1042–1048. [Available from](http://dx.doi.org/10.1007/s10147-012-0501-x).

[30] Kawazoe A, Shitara K, Fukuoka S, Kuboki Y, Bando H, Okamoto W, Kojima T, Fuse N, Yamanaka T, and Doi T, et al (2015). A retrospective observational study of clinicopathological features of KRAS, NRAS, BRAF and PIK3CA mutations in Japanese patients with metastatic colorectal cancer. *BMC Cancer* **15**, 258. [Available from](http://dx.doi.org/10.1186/s12885-015-1276-z).

[31] Fujiyoshi K, Yamamoto G, Takenoya T, Takahashi A, Arai Y, Yamada M, Kabuta M, Yamaguchi K, Akagi Y, and Nishimura Y, et al (2017). Metastatic pattern of stage IV colorectal cancer with high-frequency microsatellite instability as a prognostic factor. *Anticancer Res* **37**(1), 239–247.

[32] Gonsalves WI, Mahoney MR, Sargent DJ, Nelson GD, Alberts SR, Sinicrope FA, Goldberg RM, Limburg PJ, Thibodeau SN, and Grothey A, et al (2014). Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. *J Natl Cancer Inst* **106**(7). [Available from](http://dx.doi.org/10.1093/jnci/dju106).

[33] Itoh Y, Mizuki N, Shimada T, Azuma F, Iizuka M, Kashiwase K, Kikkawa E, Kulkki JK, Satake M, and Inoko H (2005). High-throughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. *Immunogenetics* **57**, 717–729. [Available from](http://dx.doi.org/10.1007/s00251-005-0048-3).

[34] Ozaki S, Kato K, Abe Y, Hara H, Kubota H, Kubushiro K, Kawahara E, and Inoue M (2014). Analytical performance of newly developed multiplex human papillomavirus genotyping assay using Luminex xMAP technology (Mehgen HPV Kit). *J Virol Methods* **204**, 73–80. [Available from](http://dx.doi.org/10.1016/j.viromet.2014.04.010).