**BRAFV600E Mutation Analysis in Patients with Metastatic Colorectal Cancer (mCRC) in Daily Clinical Practice: Correlations with Clinical Characteristics, and Its Impact on Patients’ Outcome**

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**Abstract**

**Background:** To prospectively evaluate the usefulness of the BRAFV600E mutation detection in daily clinical practice in patients with metastatic Colorectal Cancer (mCRC).

**Patients and Methods:** 504 mCRC patients treated with systemic chemotherapy ± biologics were analyzed.

**Results:** A statistically significant higher incidence of the BRAF mutation was observed in patients with ECOG-PS 2 (p=0.001), multiple metastatic sites (p=0.002), > 65 years old (p=0.004), primary tumors located in the colon (p<0.001), high-grade tumors (p=0.001) and in those with mucinous features (p=0.037). Patients with BRAFV600E mutated tumors had a statistically significantly reduced progression-free survival (PFS) compared to wild-type (wt) ones (4.1 and 11.6 months, respectively; p<0.001) and overall survival (OS) (14.0 vs. 34.6 months, respectively; p<0.001). In the multivariate analysis the BRAFV600E mutation emerged as an independent factor associated with reduced PFS (HR: 4.1, 95% CI 2.7–6.2; p<0.001) and OS (HR: 5.9, 95% CI 3.7–9.5; p<0.001). Among the 273 patients treated with salvage cetuximab or panitumumab, the BRAFV600E mutation was correlated with reduced PFS (2.2 vs. 6.0 months; p<0.0001) and OS (4.3 vs. 17.4 months; p<0.0001).

**Conclusions:** The presence of BRAFV600E-mutation in mCRC characterizes a subgroup of patients with distinct biologic, clinical and pathological features and is associated with very poor patients’ prognosis.

**Introduction**

Mutations in the BRAF oncogene have been found in approximately 8% of human cancers, including 50-60% of melanomas, 30-70% of thyroid cancers, 30% of serous low-grade ovarian cancers and 10% of CRCs[1]. The most common oncogenic mutation accounting for more than 95% of the mutations in BRAF found in CRCs is the single substitution missense mutation V600E, which is located within the kinase domain of the gene[2]. This amino acid change results in constitutive activation of the BRAF kinase and promotes cell transformation[1,3]. Mutations in other codons of the BRAF gene in colon cancer are extremely rare, counting for <5% of all mutations in the gene [2].

Several studies have reported that the existence of a BRAF mutation in a primary CRC tumor marks patients who carry an especially poor prognosis, regardless of treatment type administration. Its presence has been associated with decreased survival in early-operable stages treated with adjuvant chemotherapy[4], similarly, in the metastatic disease...
setting patients do not seem to respond to any of the existing chemotherapy regimens and their outcome resembles that of untreated patients[5-8]. In CRC, BRAF mutations are reported to occur more frequently in cases characterized by the presence of a defective DNA mismatch repair (dMMR) system resulting in microsatellite instability (MSI)[9-11]; this seems to be due to hMLH1 promoter hypermethylation (sporadic CRC) and not to germ-line alterations (hereditary CRC)[12,13]. As it has been previously reported, the BRAF mutation retains its prognostic value both in MSI-high and in microsatellite stable (MSS) tumors[4-6,14]; the latter being also confirmed by the recently published BRAF signature[15].

Besides its prognostic implications, several retrospective studies have attributed a predictive role to the BRAFV600E mutation due to the observed lack of benefit related to treatment with anti-EGFR moAbs. This was, initially, first documented by Di Nicolantonio et al[3], and Souglakos et al[8], but over the years, this was further confirmed by subsequent studies[7,16,17]. Furthermore, this mutation’s adverse prognostic significance was confirmed in the post-hoc subgroup analysis in two first line phase III randomized trials [CAIRO2 and CRYSTAL][18,19]. Despite the fact that, the above mentioned data require further validation in prospective randomized trials, they support the notion that the natural history and response to treatment to various chemotherapeutic regimens of BRAF-mutant CRC tumors differ markedly from the BRAF wild type tumors. Apparently, a mutant BRAF does not simply substitute for KRAS activation in a linear signaling pathway; most likely it confers distinct characteristics with ominous consequences, something which justifies its utilization in patient selection and stratification in future clinical trials[8].

In order to evaluate the usefulness of the BRAFV600E mutation detection in daily clinical practice, to investigate its correlation with the various clinico-pathological characteristics, as well as, its prognostic and predictive impact, we sought to conduct this study in a prospective database of CRC patients treated for metastatic disease.

**Patients and Methods**

**Patient population**

The study was approved by the Ethics Committee/ Institutional review board of the University Hospital of Heraklion and all patients gave their written informed consent for the use of the tissue material for translational research. Since 1/1/2007 until 31/12/2012, we prospectively analyzed for BRAF V600E all patients with newly diagnosed mCRC at the Department of Medical Oncology, University Hospital of Heraklion (Crete, Greece). Five hundred and four consecutive patients, with histologically confirmed mCRC and available tumor material for molecular analysis, who were treated with at least one cycle of systemic chemotherapy with or without the addition of bevacizumab, cetuximab or panitumumab were enrolled. Patients’ evaluation was performed at baseline and every four cycles of chemotherapy. Disease status was coded, without the knowledge of the laboratory analysis.

**Tissue selection and DNA extraction**

Formalin-fixed, paraffin-embedded (FFPE) tumor sections were reviewed by a pathologist (MT) to confirm the diagnosis and define tumor-enriched areas for dissection. Ten serial sections of 5μm thickness were stained with nuclear fast red (Sigma-Aldrich, St Louis, MO, USA) and scrape dissection under a binocular microscope was performed for samples with ≥ 80% tumor cells; for samples with < 80% malignant cells, microdissection with the piezoelectric Eppendorf microdissector (Eppendorf, Hamburg, Germany) was performed. DNA extraction was performed using the MasterPure™ Complete DNA and RNA Purification Kit according to the manufacturer’s instructions (Epicentre Biotechnologies, Madison, WI, USA) and the isolated cancer cells were lysed in buffer containing Proteinase K at 60 °C for 72 h.[11]

**KRAS mutational analysis**

KRAS mutational analysis was performed by Sanger sequencing after PCR amplification of KRAS exon 2. PCR conditions and primers sets used have been previously reported[8].

**BRAF mutational analysis**

The V600E BRAF mutation was detected by real-time PCR using the allelic discrimination method as previously described[11,20]. In brief, tumor cells’ DNA was amplified with the use of a set of primers and two hydrolysis probes in the ABI PRISM 7900T Sequence Detection System (AB; Applied Biosystems, Forest City; CA; USA). The two hydrolysis probes were labeled at 5’ with VIC and FAM fluorophores reporters for the wt and the mutant allele, respectively. The SDS 2.3 software was used for the analysis of the results.

**Study Design**

The aim of this study was to evaluate the usefulness of the BRAFV600E detection in the daily clinical practice and to correlate its existence with clinical and pathological characteristics, as well as treatment outcome in order to define possible prognostic and/or predictive implementations in a prospective database of patients with mCRC. All available biopsies of the primary tumor with more than 100 cells per section were included in the analysis. Associations between BRAF and baseline characteristics were assessed using the Fisher’s exact test for categorical variables or logistic regression for continuous variables. Progression Free Survival (PFS) and overall survival (OS) were measured from the date of diagnosis of metastatic disease to the first radiographic documentation of disease progression or death, respectively. Kaplan–Meier curves were used to describe the proportion of patients who remained free of events over the follow-up period. Associations between prognostic factors and PFS or OS were examined using Cox proportional hazards regression models. All reported p-values are two-sided and not adjusted for multiple testing.
Results

Patients’ characteristics and disease features

The characteristics of the enrolled patients were typical for metastatic CRC and are summarized in Table 1. In brief, the median patients’ age was 64 year (range: 21-89), 59% were men and their PS (ECOG) was 0-1 (90%); the primary tumor was located in the rectum in 28% of the patients and in 40% of the cases was undifferentiated (high grade) (Table 1). Twenty-seven per cent of the patients had one metastatic site and 64 (13%) underwent a metastasectomy with curative intent after the administration of systemic treatment. The BRAF V600E mutation was detected in 41 (8.2%) patients and in all cases was mutually exclusive with KRAS mutations which were detected in 217 (43%) of the total study population.

Systemic treatment and patients’ outcome

The median time from initial diagnosis to diagnosis of metastatic disease was 21.6 months (95% CI 17.6–24.2) for patients with early-stage disease (stage I-III) and the median interval from the diagnosis of metastatic disease to treatment initiation 0.6 months (95% CI 0.4–1.0). The median follow up time was 30.4 months (range, 2.6-72.9 months) and at the time of analysis 329 (65%) patients were deceased, mainly from disease progression (n=322; 98%); five (1%) deaths were treatment-related and two (0.4%) were due to reasons unrelated to disease or treatment. The median PFS was 10.5 months (95% CI: 8.9-12.4) and the median OS 29.9 months (95% CI: 26.8-34.5). All patients were treated with 5-FU-based first-line chemotherapy and in 96% of the cases the patients received an oxaliplatin or irinotecan combination (Table 2).
Table 2. Systemic treatment.

| REGIMENS                        | N     | %  |
|---------------------------------|-------|----|
| Oxaliplatin-based 1st line       | 174   | 34 |
| Irinotecan-based 1st line        | 195   | 39 |
| FOLFOXIRI                       | 115   | 23 |
| Fluoropyrimidins monotherapy     | 18    | 4  |
| Bevacizumab + chemotherapy 1st line | 239  | 48 |
| Cetuximab or Panitumumab        | 74    | 15 |
| Cetuximab or Panitumumab Salvage treatment | 273   | 54 |

Table 3. Correlation of BRAF V600E mutation with clinical and pathological characteristics.

| Feature No (%)                  | BRAF V600E | p value |
|---------------------------------|------------|---------|
| Total                           | Wild Type  | Mutant  |
| Age ≤ 65 years                  | 270 (54)   | 254 (49.4) | 16 (5.9) | 0.004 |
| > 65 years                      | 232 (46)   | 207 (89.2) | 25 (10.8) |       |
| Tumor Differentiation           |            |         |         |
| Low grade                      | 303 (60)   | 290 (95.7) | 13 (4.3) | 0.001 |
| High grade                     | 199 (40)   | 171 (86.9) | 28 (14.1) |       |
| Tumor Location                  |            |         |         |
| Colon                           | 361 (72)   | 324 (96.8) | 17 (6.2) | <0.001 |
| Rectum                          | 141 (28)   | 137 (97.2) | 4 (2.8)  |       |
| Mucinous                        | Yes        | 98 (20)  | 84 (86)  | 14 (14)  | 0.0037 |
| No                              | 404 (80)   | 377 (94.6) | 27 (5.4) |       |
| ECO PS§                         | 0-1        | 453 (90) | 432 (95.2) | 21 (4.6) | <0.001 |
|                                | 2          | 49 (10)  | 29 (59)  | 20 (41)  |       |
| Number of metastatic sites      | 1          | 138 (27) | 133 (96.4) | 5 (3.6)  | 0.002  |
|                                | >1         | 364 (73) | 328 (90.1) | 36 (9.9) |       |
| 2nd line treatments             | Yes        | 489      | 449 (90.1) | 40 (9.1) | 0.314  |
|                                | No         | 25       | 23 (86.7)  | 2 (13.3) |       |
| Metastasectomy                  | Yes        | 65 (13)  | 64 (98.5)  | 1 (1.5)  | <0.001 |
|                                | No         | 439 (87) | 398 (91)   | 41 (9)   |       |

Figure 1. Progression Free Survival in 1st systemic treatment according to BRAF V600E mutation in 504 patients with metastatic Colorectal Cancer (A) and Median Overall Survival according to BRAF V600E mutation in 504 patients with metastatic Colorectal Cancer (B).

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Correlations of BRAF mutation with clinico-pathological features and patients' Progression Free and Overall Survival

The detection of the BRAF V600E mutation has been correlated with specific clinical characteristics and pathological features (Table 3). More precisely, the BRAF V600E mutation was detected in 10.8% and 5.9% (p=0.004) of the patients older and younger than 65 years old, respectively. Also, high grade tumors presented a higher frequency of the BRAF V600E mutation (14.1%) in comparison with low grade tumors (4.3%; p=0.001). In addition, a higher incidence of the BRAF V600E mutation was found in tumors located in the colon (10.2%) than in the rectum (2.8%; p=0.001), as well as in tumors with mucinous histology (14%) compared to those with non-mucinous features (5.4%; p=0.037). Finally, the BRAF V600E mutation was more frequently detected in patients with ECOG PS 2 (41%) compared to those with PS 0-1 (4.6%; p<0.001) and in patients with multiple metastasis (9.9%) compared to those with one metastatic site (3.6%; p=0.002) (Table 3). There was no significant correlation between the BRAF V600E mutation status and the gender (p=0.412). Only one patient (1.5%) with BRAF V600E mutation underwent a metastasectomy in comparison with 63 (13%) patients with WT BRAF tumors (p<0.001).

Univariate analysis revealed significant association of several clinical and pathological features with PFS and/or mOS. Indeed, patients with BRAF V600E mutated primary tumors presented significantly lower PFS (4.1 vs. 11.6 months; HR: 2.67, 95% CI: 1.54-2.83; p<0.001) in comparison with those with WT BRAF wild type primary tumors (Table 1 and Figure 1A); this finding was independent of the type of the administered first line treatment (all p values > 0.05). Similarly, the PFS was significantly lower in patients with high grade tumors (12.5 vs. 16 months; HR: 1.81, 95% CI: 1.40-2.34; p<0.001) and ECOG PS 2 (7.8 vs. 11.9 months; HR: 1.81, 95% CI: 1.54-2.83; p<0.001) in comparison with those with low grade tumors and ECOG PS 0-1, respectively (Table 1). In addition, patients with both KRAS/BRAF V600E WT tumors present significantly higher PFS (13.3 vs. 9.6 months; HR: 1.89, 95% CI: 1.65-2.16; p=0.034) in comparison with those with any mutation in KRAS or BRAF V600E (Table 1) In contrast, patients who underwent a metastasectomy of a metastasis with curative intent presented significantly higher PFS (22.3 vs. 9.8 months; HR: 0.29, 95% CI: 0.09-0.42; p<0.001) (Table 1). There was no significant association between PFS and age, gender, tumor location, mucinous histology, number of metastatic sites and KRAS mutations (Table 1).

Univariate analysis also showed that patients with BRAF V600E mutated primary tumors had significantly lower
BRAF Mutations, Prediction, Metastatic CRC

Table 4. Multivariate analysis.

|                      | Hazard Ratio | 95% CI* | p value |
|----------------------|--------------|---------|---------|
| **Progression-Free Survival** |              |         |         |
| BRAF (mutant vs. WT*) | 4.1          | (2.7-6.2) | <0.001 |
| Tumor Grade (High vs. Low) | 2.3          | (1.4 - 3.1) | 0.008 |
| Metastatectomy (yes vs. no) | 0.4          | (0.26-0.8) | 0.003 |
| ECOG PS (2 vs. 0-1) | 1.6          | (1.2-2.1) | 0.034 |
| **Overall Survival** |              |         |         |
| BRAF (mutant vs. WT*) | 5.9          | (3.7-9.5) | <0.001 |
| Tumor Grade (High vs. Low) | 2.8          | (1.8 - 4.1) | 0.003 |
| Metastatectomy (yes vs. no) | 0.6          | (0.4 - 0.9) | 0.028 |
| ECOG PS (2 vs. 0-1) | 1.7          | (1.4-2.3) | 0.021 |

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Predictive significance of BRAFV600E mutation in treatment with anti-EGFR monoclonal antibodies

Seventy-four (25%) patients, with KRAS wt primary tumors, received an anti-EGFR monoclonal antibody in combination with chemotherapy as first line treatment. BRAF mutations were detected in 6 (8.1%) patients. Although the median PFS and OS were arithmetically lower in patients with BRAFV600E mutations compared to patients with wild type BRAFV600E status (4.2 vs. 11.1 months and 14.3 vs. 35.0 months, respectively) these differences were not statistically different probably because of the small sample size. On the other hand, 273 patients with KRAS wt primary tumors were treated with an anti-EGFR mAb as second (84 patients, 31%) or subsequent line of treatment (189 patients, 69%). Patients with BRAFV600E mutant tumors (22 patients, 8%), presented significantly shortened PFS (2.2 vs. 6.0 months, p<0.0001) and mOS (4.3 vs. 17.4 months, p<0.0001) compared with those with BRAFV600E wt tumors (Figure 2A and B), stratified for the line of treatment. Another 13 patients with BRAFV600E mutation were not treated with anti-EGFR mAbs, in the 2nd or higher treatment lines. Eleven of them received 2nd line combination chemotherapy and the median PFS was 2.4 months while the median OS was 4.8, comparable with those observed in patients with BRAFV600E mutations treated with anti-EGFR mAbs.

Discussion

In this present study we evaluated the impact of BRAFV600E testing for mCRC patients in daily clinical practice. To the best of our knowledge this is the largest prospective series of patients ever reported in the literature providing valuable data regarding epidemiological patterns, as well as, the impact of the BRAFV600E mutation status on patients’ outcome. Indeed the incidence of the mutation is significantly higher in patients with ECOG PS 2 (41%) compared to those with ECOG PS 0-1 (46%: p<0.001), but also in patients with undifferentiated tumors (14.1%), multi-metastatic disease (9.9%) and advanced age (10.8%) in comparison to those with differentiated tumors (4.3%: p=0.001), disease confined in one metastatic site (3.6%; p=0.002) and aged ≤ 65 years (5.9%; p=0.004). On the other hand, the incidence of the BRAFV600E mutation was very low in patients with metastatic rectal cancer (2.8%). These data indicate that the BRAFV600E mutation is correlated with
other known detrimental clinical prognostic factors whose may influence the patients' outcome. On the other hand, one may argue that the aggressive biological behavior of tumors harboring a BRAFV600E is responsible for the presence of these clinical factors such as rapidly progressive multimeatstastic disease and poor performance status or low probability of a secondary metastasectomy. Our study proposes that the assessment of BRAFV600E mutation is a step forward into “personalized treatment” since may modify the treatment intent (palliative or curative) and by that may influence the treatment strategy.

Also, the current study, confirms in a prospectively analyzed patients’ cohort the adverse prognostic significance of the BRAFV600E mutation which has been previously reported in retrospective studies[3,6,8,11,14]. In fact, patients with BRAFV600E mutation in their primary tumor had significantly lower median PFS (4.1 vs. 11.6 months; HR: 4.07, 95% CI: 2.66-6.20; p<0.001) and OS (14.0 vs. 34.6 months; HR: 5.43, 95% CI: 3.60-8.18; p<0.001; Figure 1A-B), while the BRAFV600E mutation was revealed as the strongest independent factor for decreased PFS (HR: 4.1, 95% CI: 2.7-6.2; p<0.001) and OS (HR: 5.9, 95% CI: 3.7-9.5; p<0.001; Table 4). These findings are in agreement with previous retrospective studies from our group[8,11] and others[3,6,14,18] regarding the adverse prognostic significance of the BRAFV600E mutation in CRC.

We also observed, in accordance with previous reports[3,7,8,16,18], that patients with BRAFV600E gain a limited if any benefit from the treatment anti-EGFR mAbs. In fact, patients with BRAFV600E mutant tumors treated with an anti-EGFR mAb in the second or subsequent line, presented significantly decreased median PFS (2.2 vs. 6.0 months, p<0.0001) and mOS (4.3 vs. 17.4 months, p<0.0001) compared to those with BRAFV600E wt tumors (Figure 2A and B). The same finding has been observed both in retrospective studies in series of patients[3,7,8,16,21], as well as, in randomized clinical trials[18]. On the other hand, the investigators in the CRYS tal study reported a minor benefit from the addition of cetuximab to chemotherapy (FOLFIRI) in patients with BRAFV600E mCRC, but this finding remains questionable since an interaction test is not provided[19]. However, the adverse prognostic significance of the BRAFV600E mutation is clearly demonstrated even in this retrospective analysis of the CRYS tal trial[19]. Our data as well as those from the studies previously mentioned suggest that the anti-EGFR moAbs are not capable to reverse the adverse prognosis of the BRAFV600E mutation. The current study is not capable to answer the questions where the BRAFV600E mutation has a predictive value for the treatment with anti-EGFR moAbs or if the patients with BRAFV600E mutation should be treated or not with anti-EGFR mAbs. This question should be probable addressed in a prospective manner either with a combination of BRAF and anti-EGFR inhibitor using an adaptive model or in a randomize trial using the BRAFV600E mutation as stratification factor.

Nevertheless, the analysis of mutations in RAS/RAF pathway has been proven significantly important for the management of patients with mCRC. In the present study patients with double WT type tumors present significantly higher PFS (HR: 1.89; p=0.034) and median OS (HR: 2.28; p=0.016) in comparison with those with a mutation in either of KRAS or BRAF genes. In addition, recently reported studies emphasize the importance of KRAS mutations outside hotspots in codon 12 and 13, as well as, of the NRAS mutations, especially in patients treated with Panitumumab[22]. All the data emphasize the importance of the testing for RAS/RAF family in mCRC in order to design the optimal treatment strategy in the daily clinical practice.

From the biological point of view our results support the concept that CRC with BRAFV600E is a distinct subset of the disease with specific biological characteristics. Indeed, the BRAFV600E mutation in CRC is correlated with MSI-H status and cyclin D1 overexpression, and characterizes a subgroup of patients with poor prognosis[9-11]. In addition, the distinct natural history and unresponsiveness of BRAF-mutant tumors to the commonly used chemotherapeutic regimens implies that BRAFV600E mutation does not simply substitute for KRAS activation in a linear signaling pathway but likely confers additional or distinct properties. For example, in cell cultures, the V600E mutation increases BRAF activity independent of KRAS and shows lower transforming activity[1], while inhibition of MEK with small molecules prevents tumor growth in BRAF-mutant tumor xenografts but not in KRAS-mutant counterparts[23]. These dissimilarities may in part explain the differences regarding the prognostic value of activating KRAS and BRAF mutations.

Finally, the analysis of the BRAFV600E in the daily clinical practice is feasible since it may be performed from the same DNA used for the analysis of the KRAS mutations which is mandatory for all patients with mCRC[24]. The analysis of the BRAFV600E using the allelic discrimination method is sensitive (sensitivity > 95%)[11,20], inexpensive [20] and provides the results within two hours.

In summary, the BRAFV600E mutation identifies a subgroup of mCRC patients with distinct biological behavior, clinical characteristics and pathological features. These patients often present metastatic disease in multiple sites, have poor PS and a poor prognosis, being resistant to all currently available treatment options. The analysis of the BRAFV600E mutation in the daily clinical practice may be a step forward in the concept of “personalized” management of patients with mCRC, since new agents targeting this specific mutation are urgently warranted.

**Author Contributions**

Conceived and designed the experiments: ZS DM ES VG JS. Performed the experiments: ZS MT MS CP AV AK IM KM JS. Analyzed the data: ZS JS. Contributed reagents/materials/analysis tools: ZS MT MS CP AV AK IM KM JS. Wrote the manuscript: ZS VG JS.
References

1. Davies H, Bignell GR, Cox C, Stephens P, Edkins S et al. (2002) Mutations of the BRAF gene in human cancer. Nature 417: 949-954. doi:10.1038/nature00766. PubMed: 12068308.

2. http: and www.sanger.ac.uk/genetics/CGP/cosmic/, COSMIC database.

3. 13 A.D. Ref type: internet communication

4. Di Nicolantonio F, Martini M, Molinaro F, Sartore-Bianchi A, Arena S et al. (2008) Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 26: 5705-5712. doi:10.1200/JCO.2008.18.0786. PubMed: 19001320.

5. French AJ, Sargent DJ, Burgart LJ, Foster NR, Kabat BF et al. (2008) Prognostic significance of defective mismatch repair and BRAF V600E mutations in patients with colon cancer. Clin Cancer Res 14: 3408-3415. doi:10.1186/1078-0432.CCR-07-1489. PubMed: 18519771.

6. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA et al. (2009) CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 58: 90-96. doi:10.1136/gut.2008.155473. PubMed: 18832519.

7. Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR et al. (2006) Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 65: 6063-6069. doi:10.1158/0008-5472.CAN-05-0404. PubMed: 16024606.

8. Saridaki Z, Tzardi M, Papadaki C, Atlantaki M, Pega F, et al. (2011) Impact of KRAS, BRAF, PIK3CA mutations, PTEN, AREG, EREG expression and skin rash in >2 line cetuximab-based therapy of colorectal cancer patients. PLOS ONE 6: e15980.

9. Souglakos J, Philips J, Wang R, Marwah S, Silver M et al. (2009) Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer 101: 465-472. doi:10.1038/sj.bjc.6605164. PubMed: 19603024.

10. Oliveira C, Pinto M, Duval A, Brennetot C, Domingo E et al. (2003) BRAF mutations, microsatellite instability status and mismatch repair deficiency. Oncogene 22: 9192-9196. doi:10.1038/ sj.onc.1207061. PubMed: 14668801.

11. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B et al. (2002) Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature 418: 934. doi:10.1038/141934a. PubMed: 12198537.

12. Saridaki Z, Papadatos-Pastos D, Tzardi M, Mavroudis D, Bairaktari E et al. (2010) BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients’ outcome. Br J Cancer 102: 1762-1768. doi:10.1038/sj.bjc.6605994. PubMed: 20485284.

13. Domingo E, Laiho P, Ollikainen M, Pinto M, Wang L et al. (2004) BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet 41: 684-688. doi:10.1136/jmg.2004.020651. PubMed: 15342696.

14. Wang L, Cunningham JM, Winters JL, Guenther JC, French AJ et al. (2003) BRAF mutations in colon cancer are not likely attributable to defective DNA mismatch repair. Cancer Res 63: 5209-5212. PubMed: 14500346.

15. Roth AD, Teijpar S, Delorenzi M, Yan P, Fiocca R et al. (2010) Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol 28: 466-474. doi:10.1200/JCO.2009.23.3452. PubMed: 20008640.

16. Popovici V, Budinska E, Teijpar S, Weinrich S, Estrella H et al. (2012) Identification of a poor-prognosis BRAF-mutant-like population of patients with colon cancer. J Clin Oncol 30: 1288-1295. doi:10.1200/JCO.2011.39.5814. PubMed: 22393095.

17. De Roock W, Claes B, Bersacchi D, De Schutter J, Biesmans B et al. (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 11: 753-762. doi:10.1016/S1470-2045(10)70130-3. PubMed: 20619739.

18. Loupakis F, Ruzzo A, Creminelli C, Vincenzi B, Salvatore L et al. (2009) KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer 101: 715-721. doi:10.1038/sj.bjc.6605177. PubMed: 19603018.

19. Tol J, Nagtegaal ID, Punt CJ (2009) BRAF mutation in metastatic colorectal cancer. N Engl J Med 361: 98-99. doi:10.1056/NEJMct0904160. PubMed: 19571295.

20. Van Cutsem E, Kühne CH, Láng I, Folprecht G, Nowacki MP et al. (2011) Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol 29: 2011-2019. doi:10.1200/JCO.2010.33.5091. PubMed: 21902544.

21. Beniloch S, Payá A, Alenda C, Bessa X, Andreu M et al. (2006) Detection of BRAF V600E mutation in colorectal cancer: comparison of automatic sequencing and real-time chemistry methodology. J Mol Diagn 8: 540-543. doi:10.2353/jmldx.2006.060070. PubMed: 17065421.

22. Pentheroudakis G, Koutoula V, De Roock W, Kouvatseas G, Papakostas P et al. (2013) Biomarkers of benefit from cetuximab-based therapy in metastatic colorectal cancer: interaction of EGFR ligand expression with RAS/RAF, PIK3CA genotypes. BMC Cancer 13: 49. doi:10.1186/1471-2407-13-49. PubMed: 23374602.

23. Oliner KS, Douillard J-Y, Siena S, Tabernero J, Burkes R et al. (2013) Analysis of KRAS/NRAS and BRAF mutations in the phase III PRIME study of panitumumab (pmab) plus FOLFIRI versus FOLFIRI as first-line treatment (tx) for metastatic colorectal cancer (mCRC). J Clin Oncol 31 suppl, Abstr: 3511.

24. Solt DB, Garraway LA, Pratilas CA, Sawai A, Getz G et al. (2006) BRAF mutation predicts sensitivity to MEK inhibition. Nature 438: 358-362. doi:10.1038/nature04304. PubMed: 16273091.

25. Scbmoll HJ, Van Cutsem E, Stein A, Valentini V, Gilmelius B et al. (2012) ESMO Consensus Guidelines for management of patients with colon and rectal cancer: a personalized approach to clinical decision making. Ann Oncol 23: 2479-2516. doi:10.1093/annonc/mds236. PubMed: 23012255.