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Impact of BRAF Mutation and Microsatellite Instability on the Pattern of Metastatic Spread and Prognosis in Metastatic Colorectal Cancer

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Abstract

Background—It is hypothesized that BRAF mutant cancers represent a discrete subset of metastatic colorectal cancer (CRC) defined by poorer survival. This study investigates whether BRAF mutant CRC is further defined by a distinct pattern of metastatic spread and explores the impact of BRAF mutation and microsatellite instability (MSI) on prognosis in metastatic CRC.

Methods—Using prospective clinical data and molecular analyses from two major centers (Royal Melbourne Hospital and MD Anderson Cancer Centre) patients with known BRAF mutation status were analyzed for clinical characteristics, survival and metastatic sites.

Results—We identified 524 metastatic CRC patients where BRAF mutation status was known, 57 (11%) were BRAF mutant tumors. BRAF mutant tumors were significantly associated with right-sided primary tumor, MSI and poorer survival (median 10.4mo v 34.7mo, p<0.001). A distinct pattern of metastatic spread was observed in BRAF mutant tumors, namely higher rates of peritoneal metastases (46% v 24%, p=0.001), distant lymph node metastases (53% v 38%, p=0.008) and lower rates of lung metastases (35% v 49%, p=0.049). In additional survival analyses, MSI tumors had significantly poorer survival compared to micro-satellite stable tumors (22.1mo v 11.1 mo, p=0.017), but this difference was not evident in the BRAF mutant population.

Conclusions—The pattern of metastatic spread observed in this study further defines BRAF mutant CRC as a discrete disease subset. We demonstrate that, unlikely early stage disease, MSI is associated with poorer survival in metastatic CRC, although this is driven by its association with BRAF mutation.

Keywords

BRAF; MSI; CRC; Metastases; Survival

Background

Colorectal cancer (CRC) is the third most common cancer and most frequent cause of cancer death worldwide[1]. Over the past decade, there has been considerable progress in the systemic treatment of CRC. Median survival for metastatic CRC has improved from 10 months to over 20 months with the development of new chemotherapeutic (oxaliplatin and irinotecan) and molecularly targeted agents (bevacizumab, cetuximab and panitumumab)[2]. Significant advances in molecular biology have driven rational development of molecularly targeted therapies in CRC through an improved understanding of the genetic/epigenetic changes and signal transduction pathways involved. This understanding together with ongoing identification of predictive and prognostic factors is leading to an increasing appreciation of the molecular diversity that exists in CRC.

BRAF is a protein kinase downstream of RAS in the RAS-RAF-MEK-ERK kinase pathway. Mutations in BRAF are present in approximately 10% of patients with metastatic CRC[3], and may be a significant predictor of resistance to EGFR targeted treatments[4-7]. BRAF mutant CRC is reported to have associations with female gender, right-sided primary tumors, older age and microsatellite instability (MSI)[3]. Metastatic BRAF mutant tumors also possess a significantly poorer overall survival when compared to wild-type tumors[3,8,9]. Although

resistance to EGFR targeted therapies may contribute to the poor outcomes observed in BRAF mutant CRC, this would only explain a small portion of observed survival differences. Several studies have suggested BRAF mutant tumors are resistant to standard chemotherapy[9], whilst others have suggested the association between BRAF mutant tumors and treatment resistance is minimal and non significant[10]. It remains unclear why BRAF mutant CRC has such a poor prognosis.

The association between BRAF mutant CRC and microsatellite Instability (MSI) is well reported. MSI is related to a deficient mismatch repair system, due to either germline mutations as part of Lynch syndrome (2-5% of CRC) or hypermethylation of the MLH1 promoter (12-15% of CRC)[11]. Tumors with MSI tend to be more proximal and are reported to have significantly improved survival in early stage disease[12], however, the prevalence of MSI in metastatic CRC is low and there is limited data regarding its prognostic impact in this setting.

Similar to her2/neu over-expression in metastatic breast cancer, BRAF mutant metastatic CRC appears to be a discrete disease subtype with a distinct patient population and significantly poorer survival. Her2/neu over-expressing breast cancer is further defined by the availability of effective targeted treatments[13] and a distinct pattern of metastatic spread, namely an increased incidence of CNS metastases[14]. Agents targeting BRAF mutations in CRC are currently in early development and differences in metastatic sites in this population have not been previously reported. Our study investigates whether BRAF mutant CRC has a distinct pattern of metastatic spread, further defining this discrete disease subtype. In addition, we explore the impact of MSI in the metastatic setting, in particular the relationship with BRAF and the effect on overall survival; although it is well reported that MSI is a good prognostic factor in early stage CRC[12], it is not clear whether this remains true in metastatic disease. To date, our study is the largest series exploring the impact of both BRAF mutation and MSI in metastatic CRC.

Methods

Patient Population

This study involved collaboration between two sites, The Royal Melbourne Hospital (RMH) in Australia and The University of Texas M.D. Anderson Cancer Centre (MDACC) in the United States. Patients with known BRAF mutation status from both sites were identified for retrospective analysis in this study.

The RMH patients were identified using an Australian prospective multi-site, multi-disciplinary comprehensive CRC database (BioGrid Australia), whilst the MDACC patients were identified from a similar institutional database. 316 patients from RMH and 208 patients from MDACC with metastatic CRC and known BRAF mutation status were identified and selected for analysis.

From the 316 RMH patients, 265 (84%) had BRAF mutation testing for the purposes of a retrospective study that identified poorer survival in BRAF mutant metastatic CRC and an association between BRAF mutation and older age, female gender and right sided

primaries[3]. Patients selected for that study (n=600) were representative of the general population, with a median age of 70, and included all stages of CRC. Two hundred and sixty five of those patients had developed metastatic disease and were included in the current analysis. The remaining 51 RMH patients and all 208 MDACC patients had BRAF mutation testing for the purposes of enrollment onto clinical trials, including a phase I trial using a BRAF targeting agent[15].

Data collection

The selected patients were analyzed for specific clinical and pathological characteristics, including age, sex, primary site, metastatectomy, BRAF mutation status, MSI, survival and sites of metastatic disease. Primary site was divided into right-sided and left-sided tumors (right-sided defined as tumors arising anywhere from caecum to transverse colon and left-sided defined as tumors arising anywhere from splenic flexure to anorectal junction). At both hospitals BRAF mutation testing was performed using a mutation-specific real-time polymerase chain reaction assay. MSI was identified by either DNA testing (using the recommended National Cancer Institute panel of microsatellite markers, where high level MSI was defined as the presence of two or more foci showing instability) or immuno-histochemistry (defined as hMSH2, hMLH1, hMSH6, or PMS2 loss). Tumors with high level MSI are referred to as MSI throughout this manuscript. Tumors with low level MSI or micro-satellite stable tumors are grouped together and referred to as MSS, as extensive data indicates the two molecular phenotypes are biologically similar[16]. Recorded metastatic sites are representative of metastases that have developed over the course of the patient's illness, and include liver, lung, peritoneal, distant lymph nodes (LN) and brain. Metastatic sites were identified by each site's database and confirmed by review of medical records and/or imaging to improve accuracy.

Statistical Analysis

The selected patients were divided into a population-based cohort (consisting of 265 patients from RMH tested for BRAF mutations for a population-based study) and trial-screened cohort (consisting of 51 patients from RMH and 208 patients from MDACC tested for BRAF for enrolment onto clinical trials) for comparison in the analyses. Associations between BRAF mutant tumors and clinical characteristics (sex, primary site, MSI status, metastatectomy) and sites of metastatic disease were analyzed using Fisher's exact test. The same was performed for MSI tumors. Survival rates were estimated by the Kaplan-Meier method and compared by log-rank test. All patients were included in the univariate survival analysis. Multivariate survival analysis including all significant variables in the univariate analsyis, used cox proportional hazard models to adjust for potential confounding factors. Given a high number of incomplete data for MSI, the multivariate analysis was performed on 350 patients where complete data (including MSI status) was available. All reported P values are two sided and P values less than 0.05 are considered statistically significant. The software program SAS/STAT® was used for the multivariate analysis, whilst GraphPad Prism® was used for the univariate analysis.

Results

Overall

We identified 524 metastatic CRC patients with known BRAF mutation status. The proportion of BRAF mutant tumors was consistent across the population-based and trial-screened cohorts, approximately 11% of metastatic CRC. In total, 57 (11%) patients had BRAF mutant tumors and 467 (89%) had BRAF wild-type tumors. Only two BRAF mutant tumors had non-V600E mutations (one each of G593D and Q609X) both arising from the population-based cohort at RMH and both were included as BRAF mutants in our analysis. MSI status was available on 350 (67%) patients, of which 40 (11%) were MSI tumors.

BRAF mutant tumors

Differences in clinical characteristics between BRAF mutant and BRAF wild-type tumors are displayed in **Table 1**. Within the population-based cohort, BRAF mutant CRC was significantly more likely to occur in females (76% v 44%, p=0.0001), however, this was not observed in the trial-screened cohort (32% v 43%, p=0.314). Although the median age of the population-based cohort was considerably older, there is no significant difference in age between BRAF mutant and wild-type tumors in either cohort.

The association between BRAF mutant tumors and right-sided primary tumors was statistically significant in each cohort. Overall, 68% of BRAF mutant tumors had right-sided primaries compared to 35% for wild-type tumors (p<0.001). A higher proportion of metastatectomy in the wild-type population was observed at both sites, although this was only statistically significant in the trial-screened cohort. Micro-satellite instability was significantly more common in BRAF mutant tumors (29% v 9%, p<0.001).

Differences in sites of metastatic disease between BRAF mutant and wild-type tumors are displayed in **Table 2**. BRAF mutant tumors had significantly lower rates of lung metastases (35% v 49%, p=0.049), higher rates of peritoneal metastases (46% v 24%, p=0.001) and higher rates of distant LN metastases (53% v 38%, p=0.044). There were no significant differences in the rates of liver metastases and CNS metastases in BRAF mutant tumors compared to wild-type.

MSI tumors

Differences in clinical characteristics between MSI and MSS tumors are displayed in **Table 3**. Overall, MSI tumors occurred in a significantly older population (median age 72 v 64, p=0.022). A significantly increased proportion of right-sided primaries was also observed in MSI tumors (68% v 37%, p<0.001). Additionally, in MSI tumors, BRAF mutations were significantly more common (30% v 10%, p<0.001), whilst metastatectomy was significantly less common (10% v 26%, p=0.02).

As displayed in **Table 4,** the only significant difference in metastatic spread between MSI and MSS tumors, was a lower rate of liver metastases (50% v 71%, p=0.010) in MSI tumors. There were no significant differences in rates of lung, peritoneal, distant LN and brain metastases between MSI and MSS tumors.

Although MSI data was incomplete and available in only 350 (67%) patients, the clinical characteristics of the MSI tested population is representative of the overall study population, as outlined in **Table 5**, albeit with a slight under representation of the trial-screened cohort and subsequent poorer overall survival.

Survival analysis

Table 6 outlines the results of the univariate survival analysis. In our series, BRAF mutant CRC was confirmed to have a poorer prognosis with median overall survival of 10.4 months versus 34.7 months for BRAF wild-type tumors (p<0.001) **(Figure 1).** These results are consistent across both cohorts **(Figure 1b and c)**. The trial-screened cohort had a significantly better overall survival compared to the population-based cohort (median overall survival not reached versus 11.0 months, p<0.001). An analysis of the trial-screened cohort demonstrates that BRAF mutation testing was performed at a median of 12.1 months after diagnosis of metastatic disease. For BRAF mutant tumors, the median time to testing was 4.7 months and for BRAF wild-type tumors, 13.4 months. A significant survival difference was also seen between the MDACC population (all trial-screened) and the overall RMH population (median overall survival not reached versus 17.9 months, p<0.001), although there was no difference between the RMH trial-screened population and the MDACC population **(Figure 1d).**

Other clinical factors significantly associated with poorer survival included female gender (26.8 mo v 33.9 mo, p=0.041), right-sided primary tumors (17.3 mo v 39.5 mo, p<0.001) and absence of lung metastases (22.1 mo v 40.2 mo, p<0.001).

MSI tumors were observed to have significantly poorer survival compared to MSS tumors (11.1 months versus 22.1 months, p<0.001). In a bivariate analysis where MSI status was stratified by BRAF status there was no significant survival differences between MSI and MSS tumors in BRAF mutant tumors, however, in BRAF wild-type tumors, significantly poorer survival for MSI remained **(Figure 2b,2c).** In the reverse analysis where BRAF mutant and wild-type tumors are stratified by MSI status, we demonstrated poorer survival for BRAF mutant tumors regardless of MSI status **(Figure 2d,2e)**.

A multivariate survival analysis was performed on 350 patients with complete data available. This demonstrated profoundly poorer survival for BRAF mutant tumors (HR 10.662, p<0.001). **Table 7** outlines all significant hazard ratios from the multivariate analysis. Poorer survival was also noted in right sided primary tumors (HR 1.359, p=0.042), whilst better survival was noted in the metastatectomy population (HR 0.218, p<0.001). Other significant prognostic factors in the univariate survival analysis – MSI, female gender, absence of lung metastases – were not significant in the multivariate analysis.

Discussion

BRAF mutations are becoming increasingly relevant in the clinical setting. They are present in approximately 10% of CRC, are a significant negative prognostic factor in advanced disease and may predict resistance to EGFR inhibitors[3,17]. BRAF mutation testing is recommended for metastatic CRC in the latest National Comprehensive Cancer Network

guidelines. BRAF mutant tumors are also associated with clinical characteristics such as female gender, older age and right-sided primary tumors[3,18]. Given the recent development of agents targeting BRAF in metastatic CRC, a greater understanding of the impact of BRAF mutations in metastatic disease is increasingly relevant. Similarly, MSI has been extensively studied in early stage CRC, but rarely analyzed in the metastatic setting. Prior reports have demonstrated a strong association between BRAF mutant tumors and MSI[19], but few have analyzed the impact on survival. To date, our study of 524 patients with metastatic CRC is the largest analysis of BRAF and MSI in the metastatic setting. We analyzed the impact of MSI and BRAF on survival and pattern of metastatic spread. Whilst our study confirms poorer survival in BRAF mutant tumors[3,18,20] , it also reports, for the first time, a distinct pattern of metastatic spread in both BRAF mutant CRC and MSI tumors[21,22] . We also describe poorer survival in metastatic CRC exhibiting MSI and demonstrate this is explained by a strong association between BRAF mutations and MSI.

Overall, several statistically significant differences in clinical characteristics and pattern of metastatic spread were seen in BRAF mutant tumors. We observed significant associations between BRAF mutant CRC and right-sided primaries, MSI and poorer overall survival, all consistent with previous reports[3,18,20]. The significantly lower rate of metastatectomy in BRAF mutant tumors is not unexpected, given the poorer survival observed in these patients and the higher rates of metastatic disease to sites that are not typically amenable to resection, such as the peritoneum and lymph nodes. Our study is the first to investigate and report a distinct pattern of metastatic spread in BRAF mutant CRC, demonstrating a significantly increased rate of peritoneal and distant LN metastases and a significantly decreased rate of lung metastases in BRAF mutant tumors compared to BRAF wild-type tumors. It has long been known that right-sided and left-sided tumors have different patterns of spread that have been attributed to tumor biology[23]. Given the strong association between right sided tumors and BRAF mutations, the results of this study suggest that BRAF mutations may be the genetic alteration that is responsible for differences in metastatic spread previously observed in right versus left tumors. Although our study does not confirm peritoneal metastases as a poor prognostic factor, this may be due to small numbers and should not detract from existing reports that identify peritoneal metastases as an independent and significant poor prognostic factor[21,24]. Subsequently, the strong association between BRAF mutant tumors and peritoneal metastases observed in this study may partially explain the poorer outcomes in these tumors.

In addition to our major findings, our study adds to the growing body of literature confirming BRAF mutations as a significant poor prognostic factor in metastatic CRC. We report a median overall survival of 10.4 months for BRAF mutant tumors compared to 34.7 months for BRAF wild-type tumors ($p<0.001$). Moreover, the multivariate analysis confirms BRAF mutant tumors as an independent poor prognostic factor with a hazard ratio of 10.662 ($p<0.001$). Both univariate and multivariate analyses also confirmed that metastatectomy is associated with significantly improved survival (HR 0.218, $p<0.001$). This is expected, given that metastatectomy is often performed as a potentially curative procedure. The multivariate analysis also demonstrates right-sided primary tumors to be an independent poor prognostic factor (HR 1.359, $p=0.042$). This has previously been described in both early stage and metastatic CRC[2526]. The significantly poorer survival observed in female

gender and absence of lung metastases in the univariate analysis was not observed in the multivariate survival analysis as it is likely these survival differences were confounded by strong associations with BRAF mutant tumors.

Microsatellite instability is uncommon in metastatic CRC, with rates as low as 4% reported[11]. Subsequently, few studies have analyzed its impact in metastatic disease. Although only 67% (350) of patients had MSI status determined in our study, this subset appears representative of the overall study population. Slightly poorer survival in the MSI-tested population is explained by an under-representation of the better prognosis trial-screened cohort. In addition, as seen in **Table 5** overall survival of the MSI tested population is similar to the overall study population when stratified by cohort (population-based and trial-screened). Our study confirmed associations between MSI tumors and both right-sided primary tumors and older age. We also report, for the first time, a significantly reduced rate of liver metastases in MSI tumors compared to MSS tumors. A study of the impact of mucinous histology on CRC survival reported a reduced rate of liver metastases in mucinous histology metastatic CRC[24]. As MSI is known to be associated with mucinous histology, our finding of a reduced rate of liver metastases in MSI tumors is not entirely unexpected. However, it remains unclear whether this unique difference in metastatic spread is related to mucinous histology, MSI or both. Our study also confirms the close relationship between MSI and BRAF mutant CRC, further reflected in the significantly decreased proportion of metastatectomy and poorer survival in MSI tumors. In early stage CRC, MSI is a good prognostic factor, associated with significantly better survival than MSS tumors[12]. Multiple reports have also demonstrated it is predictive for non-response to 5FU chemotherapy in stage B disease[25]. Previous studies of MSI in metastatic CRC have reported no significant difference in survival or response to treatment[11,26,27]. Our study is the first to demonstrate a significant survival advantage for MSS disease compared to MSI disease in the metastatic setting. Microsatellite instability in early stage tumors is a mixture of germ line mutations and epigenetic loss of mismatch repair genes. In the metastatic setting, the majority of patients have epigenetic loss as the mechanism of MSI, which is associated with BRAF mutations[19]. Indeed, in our population, BRAF mutant tumors are more prevalent in the MSI population (29%) than the MSS population (9%). Importantly, after stratifying for BRAF, the survival difference between MSS and MSI is no longer evident in the BRAF mutant population, and MSI is not a significant factor in the multivariate survival model that includes BRAF. In the reverse analysis, significantly poorer survival is observed in BRAF mutants in both the MSI and MSS population. Previously, this has only been observed in the MSS population[3,28] , although most of these studies only analyzed patients with early stage disease. It is increasingly clear that in the metastatic setting, BRAF mutations are a very strong negative prognostic indicator.

The major limitation of our study is patient selection. BRAF mutation testing is relatively novel and not routine at either of the sites participating in this study. Subsequently, we examined and reported on only patients that had been chosen for BRAF mutation testing. Furthermore, the study is a combined analysis of patients from two different hospitals (RMH, an Australian site, and MDACC, an American site) and two very different groups of patients (population-based and trial-screened cohorts). A significant survival difference was

noted between hospitals; however, this appears to be solely driven by the higher proportion of clinical trial patients in the MDACC group. In fact, within the trial-screened cohort, there is no difference in survival between MDACC and RMH. The trial-screened cohort consists of patients where BRAF testing is performed for the sole purpose of enrollment onto clinical trials[15]. Consequently, given that these studies were for patients with refractory CRC, it is likely that the excellent survival outcomes are a result of conditional survival, as only patients that had progressed through multiple lines of treatment and yet still maintained a very good performance status were considered for trial prescreening. Our findings are consistent with these assumptions; the trial-screened cohort is younger and has significantly better survival and had BRAF mutation testing at a median of 12.1 months after diagnosis of metastatic disease. Furthermore, it would be intriguing to consider that the only modest response rates seen in patients treated on the phase Ib trial of PLX4032 in metastatic CRC could in part be explained by the heterogeneity we have observed in our trial-screened/ population-based populations. This needs to be explored further. For the most part, the clinical characteristics and survival differences observed in both cohorts are consistent with previous reports and subsequently, despite the potential problems with patient selection, we believe the differences observed in BRAF mutants within our study population are representative of real differences in BRAF mutant metastatic CRC.

Conclusions

To our knowledge, this study is the largest analysis of BRAF and MSI in metastatic CRC. Reported for the first time, we demonstrate significantly poorer survival associated with MSI in the metastatic setting, which is accounted for by the strong association between MSI and BRAF mutation. In addition, a significantly lower proportion of liver metastases in MSI tumors is observed, whilst BRAF mutant tumors are associated with a distinct pattern of metastatic spread that includes frequent peritoneal and distant lymph node involvement. This adds further strength to the hypothesis that BRAF mutant CRC is a unique subset of CRC, defined by significantly poorer prognosis, strong association with MSI and, a distinct pattern of metastatic spread. Further intervention studies specific to this unique subset of patients are warranted.

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References

1. Weitz J, Koch M, Debus J, et al. Colorectal cancer. The Lancet. 2005; 365(9454):153–165.
2. Kopetz S, Chang GJ, Overman MJ, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. Journal of Clinical Oncology. 2009; 27(22):3677–83. [PubMed: 19470929]
3. Tie J, Gibbs P, Lipton L, et al. Optimizing targeted therapeutic development: Analysis of a colorectal cancer patient population with the BRAFV600E mutation. International journal of cancer. 2010

4. Van Cutsem E, Köhne C-H, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. The New England Journal of Medicine. 2009; 360(14):1408–17. [PubMed: 19339720]
5. Laurent-Puig P, Cayre A, Manceau G, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. Journal of Clinical Oncology. 2009; 27(35):5924–30. [PubMed: 19884556]
6. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. Journal of Clinical Oncology. 2008; 26(35):5705–12. [PubMed: 19001320]
7. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Research. 2007; 67(6):2643–8. [PubMed: 17363584]
8. Tol J, Nagtegaal ID, Punt CJA. BRAF mutation in metastatic colorectal cancer. The New England Journal of Medicine. 2009; 361(1):98–9. [PubMed: 19571295]
9. Souglakos J, Philips J, Wang R, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. British Journal of Cancer. 2009; 101(3):465–72. [PubMed: 19603024]
10. Richman SD, Seymour MT, Chambers P, et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. Journal of Clinical Oncology. 2009; 27(35):5931–7. [PubMed: 19884549]
11. Müller CI, Schulmann K, Reinacher-Schick a, et al. Predictive and prognostic value of microsatellite instability in patients with advanced colorectal cancer treated with a fluoropyrimidine and oxaliplatin containing first-line chemotherapy. A report of the AIO Colorectal Study Group. International journal of colorectal disease. 2008; 23(11):1033–9. [PubMed: 18594845]
12. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. Journal of Clinical Oncology. 2005; 23(3):609–18. [PubMed: 15659508]
13. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. The New England journal of medicine. 2001; 344(11):783–92. [PubMed: 11248153]
14. Leyland-Jones B. Human epidermal growth factor receptor 2-positive breast cancer and central nervous system metastases. Journal of Clinical Oncology. 2009; 27(31):5278–86. [PubMed: 19770385]
15. Kopetz S, Desai J, Chan E, et al. PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors. Journal of Clinical Oncology. 2010; 28(15s)(suppl) abstract 3534.
16. Sargent DJ, Marsoni S, Monges G, et al. Defective Mismatch Repair As a Predictive Marker for Lack of Efficacy of Fluorouracil-Based Adjuvant Therapy in Colon Cancer. Journal of Clinical Oncology. 2010; 28(20)
17. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. Journal of Clinical Oncology. 2008; 26(35):5705–12. [PubMed: 19001320]
18. Li WQ, Kawakami K, Ruszkiewicz A, et al. BRAF mutations are associated with distinctive clinical, pathological and molecular features of colorectal cancer independently of microsatellite instability status. Molecular Cancer. 2006; 5:2. [PubMed: 16403224]
19. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 2009; 58(1):90–6. [PubMed: 18832519]
20. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Research. 2005; 65(14):6063–9. [PubMed: 16024606]
21. Assersohn L, Norman A, Cunningham D, et al. Influence of metastatic site as an additional predictor for response and outcome in advanced colorectal carcinoma. British Journal of Cancer. 1999; 79(11-12):1800–5. [PubMed: 10206296]

22. Wang L, Cunningham JM, Winters JL, et al. Advances in Brief BRAF Mutations in Colon Cancer Are Not Likely Attributable to Defective DNA Mismatch Repair 1. Cancer Research. 2003; 63:5209–5212. [PubMed: 14500346]

23. Benedix F, Kube R, Meyer F, et al. Comparison of 17,641 patients with right- and left-sided colon cancer: differences in epidemiology, perioperative course, histology, and survival. Diseases of the colon and rectum. 2010; 53(1):57–64. [PubMed: 20010352]

24. Catalano V, Loupakis F, Graziano F, et al. Mucinous histology predicts for poor response rate and overall survival of patients with colorectal cancer and treated with first-line oxaliplatin- and/or irinotecan-based chemotherapy. British Journal of Cancer. 2009; 100(6):881–7. [PubMed: 19259089]

25. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. The New England Journal of Medicine. 2003; 349(3):247–57. [PubMed: 12867608]

26. Chen WS, Chen JY, Liu JM, et al. Microsatellite instability in sporadic-colon-cancer patients with and without liver metastases. International Journal of Cancer. 1997; 74(4):470–4.

27. Des Guetz G, Uzzan B, Nicolas P, Schischmanoff O, Morere J-F. Microsatellite instability: a predictive marker in metastatic colorectal cancer? Targeted Oncology. 2009; 4(1):57–62. [PubMed: 19343302]

28. Roth AD, Tejpar S, Delorenzi M, et al. 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. Journal of Clinical Oncology. 2010; 28(3):466–74. [PubMed: 20008640]

Condensed Abstract

A large retrospective study of patients from Royal Melbourne Hospital (Australia) and M.D. Anderson Cancer Centre (USA) analyzed the impact of BRAF and MSI in metastatic CRC. The major findings include a distinct pattern of metastatic spread in BRAF mutant tumors (increased rates of peritoneal and distant lymph node metastases and reduced rates of lung metastases) and significantly poorer survival observed in MSI tumors, although this survival difference is not evident in the BRAF mutant population, further confirming BRAF mutation as a significant poor prognostic factor in metastatic CRC.

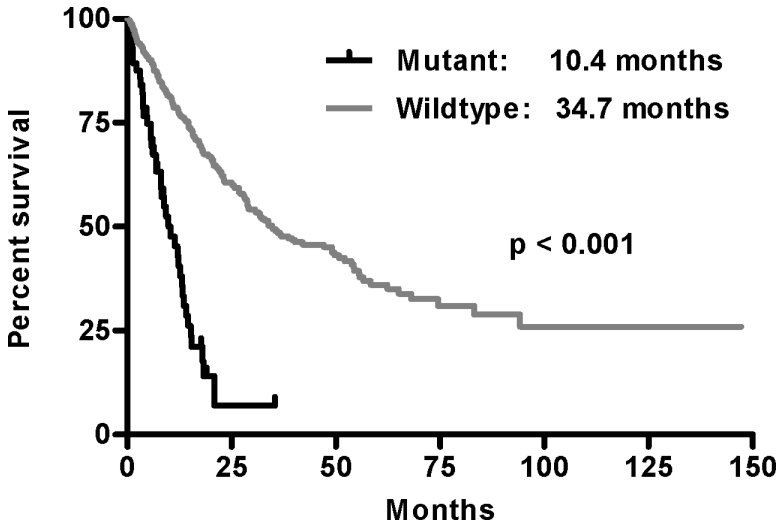

Figure 1a.
Overall Survival: BRAF mutant tumors versus BRAF wild-type tumors

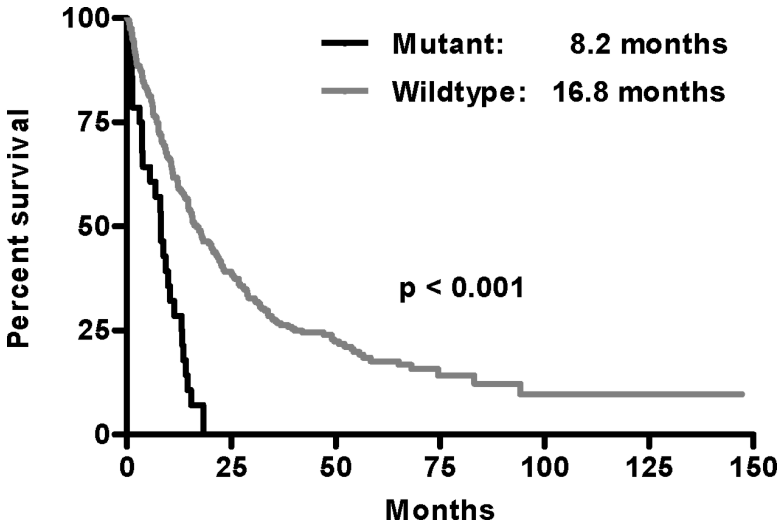

Figure 1b.

Overall Survival: BRAF mutant tumors versus BRAF wild-type tumors in population-based cohort

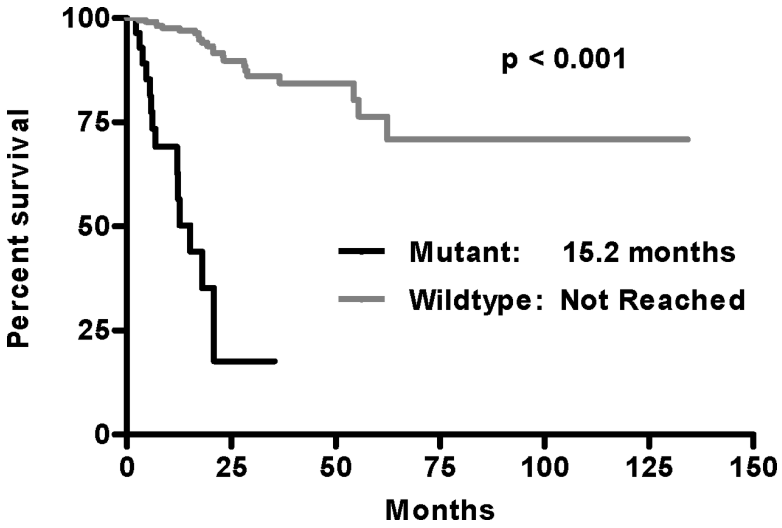

Figure 1c.

Overall Survival: BRAF mutant tumors versus BRAF wild-type tumors in trial-screened cohort

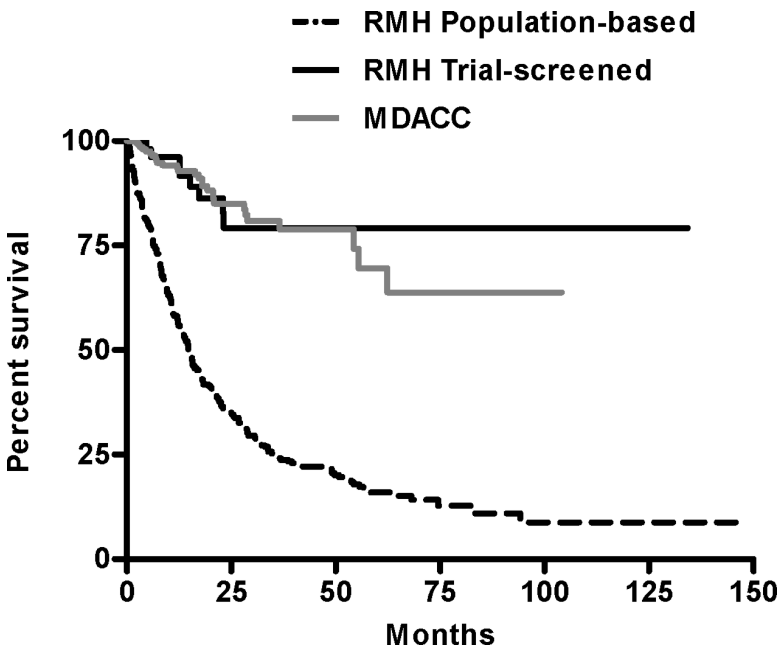

Figure 1d.
Overall survival: RMH versus MDACC

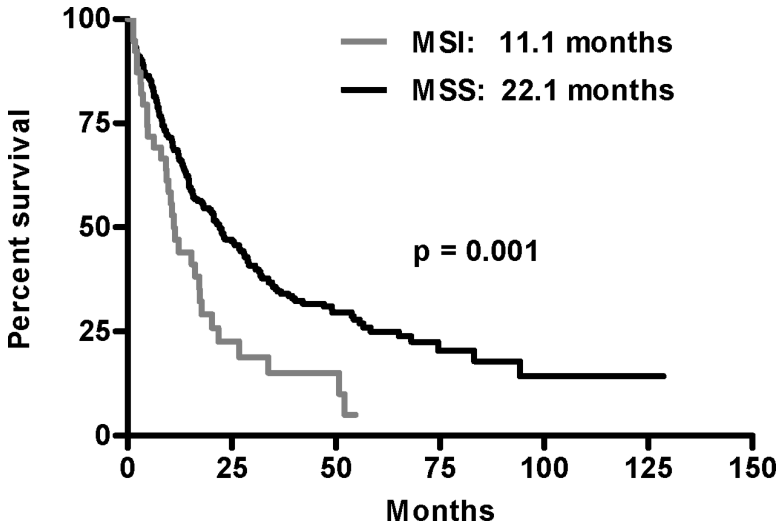

Figure 2a.
Overall Survival: MSS tumors versus MSI tumors

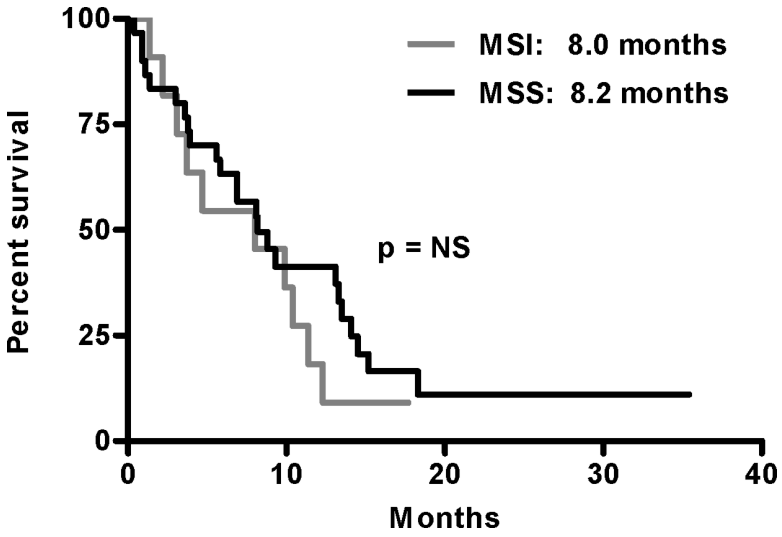

Figure 2b.
Overall Survival: MSS versus MSI in BRAF mutant tumors

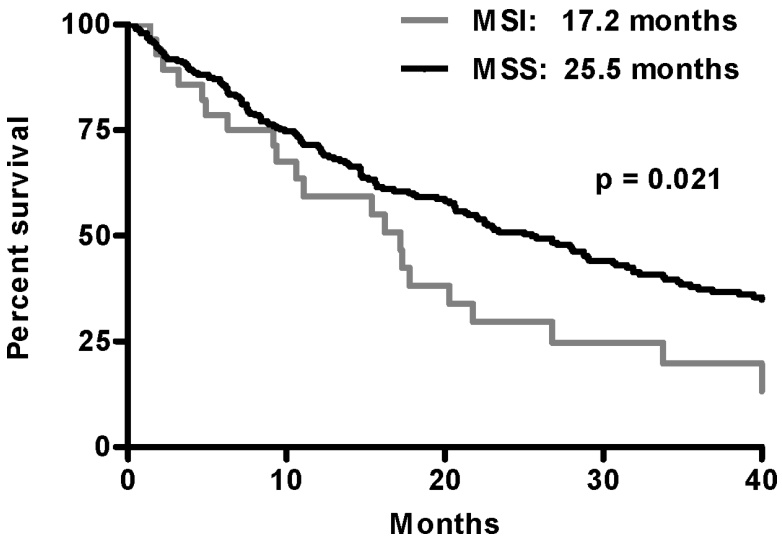

Figure 2c.
Overall Survival: MSS versus MSI in BRAF wild-type tumors

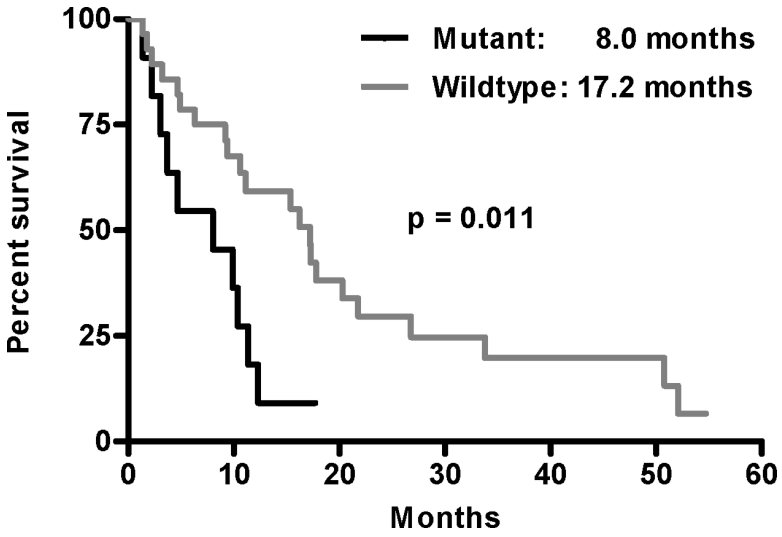

Figure 2d.
Overall Survival: BRAF mutant versus BRAF wild-type in MSI tumors

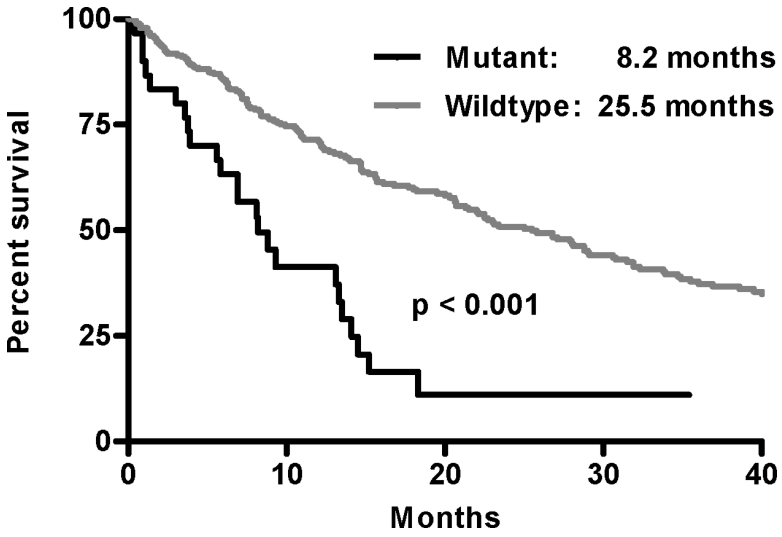

Figure 2e.
Overall Survival: BRAF mutant versus wild-type in MSS tumors

Table 1

Patient Characteristics in BRAF mutant and wild-type tumors

	Population-based (n=264)			Trial-screened (n=260)			Both (n=524)		
	Mutant (n=29)	**WT (n=235)**	**P**	**Mutant (n=31)**	**WT (n=232)**	**P**	**Mutant (n=57)**	**WT (n=467)**	**P**
Total	29 (11%)	235 (89%)	-	28 (11%)	232 (89%)	-	57 (11%)	467 (89%)	-
Median Age	74	70	*0.370*	58.5	57	*0.234*	66	63	*0.174*
Female	22 (76%)	104 (44%)	*0.001*	9 (32%)	100 (43%)	*0.314*	31 (54%)	204 (44%)	*0.158*
Male	7 (24%)	131 (56%)		19 (68%)	132 (57%)		26 (46%)	263 (56%)	
Right Primary	2 (76%)	87 (37%)	*<0.001*	17 (61%)	75 (32%)	*0.006*	39 (68%)	162 (35%)	*<0.001*
Left Primary	7 (24%)	148 (63%)		11 (39%)	157 (68%)		18 (32)	305 (65%)	
MSI*	8 (29%)	24 (11%)	*0.015*	4 (29%)	4 (5%)	*0.013*	12 (29%)	28 (9%)	*<0.001*
MSS*	20 (71%)	198 (89%)		10 (71%)	82 (95%)		30 (71%)	280 (91%)	
Metastatectomy	6 (21%)	52 (22%)	1.000	2 (7%)	82 (35%)	0.002	8 (14%)	134 (29%)	*0.018*
No Metastatectomy	23 (79%)	183 (78%)		26 (93%)	150 (65%)		49 (86%)	333 (71%)	

Mutant = BRAF mutant tumor

WT = BRAF wild-type tumor

* MSI status available on 350 (67%) patients

Table 2

Pattern of metastatic spread in BRAF mutant and wild-type tumors

	Population-based (n=264)			Trial-screened (n=260)			Both (n=524)		
Metastatic Site	**Mutant (n=29)**	**WT (n=235)**	**P**	**Mutant (n=31)**	**WT (n=232)**	**P**	**Mutant (n=57)**	**WT (n=467)**	**P**
Liver	18 (62%)	170 (72%)	*0.279*	18 (64%)	168 (72%)	*0.380*	36 (63%)	338 (72%)	*0.163*
Lung	7 (24%)	97 (41%)	*0.106*	13 (46%)	134 (58%)	*0.314*	20 (35%)	231 (49%)	*0.049*
Peritoneal	13 (45%)	58 (25%)	*0.027*	13 (46%)	55 (24%)	*0.021*	26 (46%)	113 (24%)	*0.001*
Distant LN	15 (52%)	62 (26%)	*0.008*	15 (54%)	115 (50%)	*0.842*	30 (53%)	177 (38%)	*0.044*
Brain	4 (14%)	15 (6%)	*0.141*	1 (4%)	7 (3%)	*0.603*	5 (9%)	22 (5%)	*0.200*

Mutant = BRAF mutant tumor

WT = BRAF wild-type tumor

LN = Lymph Node

Table 3

Patient Characteristics in MSI and MSS tumors

	Population-based (n=250)			Trial-screened (n=100)			Both (n=350)		
	MSI (n=32)	**MSS (n=218)**	**P**	**MSI (n=8)**	**MSS (n=92)**	**P**	**MSI (n=40)**	**MSS (n=310)**	**P**
Total	32 (13%)	218 (87%)	-	8 (8%)	92 (92%)	-	40 (11%)	310 (89%)	-
Median Age	75	69	*0.013*	47	52	*0.265*	72	64	*0.022*
Female	18 (56%)	103 (47%)	*0.352*	6 (75%)	37 (40%)	*0.072*	24 (60%)	140 (45%)	*0.092*
Male	14 (44%)	115 (53%)		2 (25%)	55 (60%)		16 (40%)	170 (55%)	
Right Primary	19 (59%)	84 (39%)	*0.030*	8 (100%)	30 (33%)	*<0.001*	27 (68%)	114 (37%)	*<0.001*
Left Primary	13 (41%)	134 (61%)		0 (0%)	62 (67%)		13 (33%)	196 (63%)	
BRAF Mutation	8 (25%)	20 (9%)	*0.010*	4 (50%)	10 (11%)	*0.013*	12 (30%)	30 (10%)	*<0.001*
BRAF Wild-type	24 (75%)	198 (91%)		4 (50%)	82 (89%)		28 (70%)	280 (90%)	
Metastatectomy	3 (9%)	52 (24%)	0.070	1 (13%)	29 (32%)	0.429	4 (10%)	81 (26%)	*0.02*
No Metastatectomy	29 (91%)	166 (76%)		7 (88%)	63 (68%)		36 (90%)	229 (74%)	

Table 4

Pattern of metastatic spread in MSI and MSS tumors

	Population-based (n=250)			Trial-screened (n=100)			Both (n=350)		
Metastatic Site	**MSI (n=32)**	**MSS (n=218)**	**P**	**MSI (n=8)**	**MSS (n=92)**	**P**	**MSI (n=40)**	**MSS (n=310)**	**P**
Liver	18 (56%)	156 (72%)	*0.099*	2 (25%)	63 (68%)	*0.020*	20 (50%)	219 (71%)	*0.010*
Lung	12 (38%)	86 (39%)	*1.000*	3 (38%)	45 (49%)	*0.717*	15 (38%)	131 (42%)	*0.613*
Peritoneal	9 (28%)	60 (28%)	*1.000*	3 (38%)	23 (25%)	*0.425*	12 (30%)	83 (27%)	*0.706*
Distant LN	11 (34%)	65 (30%)	*0.681*	8 (100%)	41 (45%)	*0.002*	19 (48%)	106 (34%)	*0.115*
Brain	2 (6%)	17 (8%)	*1.000*	0 (0%)	3 (3%)	*1.000*	2 (5%)	20 (6%)	*1.000*

LN = Lymph Node

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Table 5

Characteristics of MSI tested population

	MSI tested population (n=350)	Entire study population (n=524)	P
Trial screened	100 (29%)	260 (50%)	*<0.001*
Median Age	65	63	*0.452*
Female	164 (47%)	235 (45%)	*0.579*
Right Primary	141 (40%)	201 (38%)	*0.572*
BRAF mutation	42 (12%)	57 (11%)	*0.663*
Metastatectomy	85 (24%)	142 (27%)	*0.387*
mOS (months)			
Overall	20.6	29.1	*<0.001*
Population-based	14.7	14.7	*0.945*
Trial-screened	NR	NR	*0.590*

NR = Not Reached

Table 6

Univariate Survival Analysis (n=524)

	mOS (months)	P
Patient Cohort		
Population based	14.7	*<0.001*
Trial screened	NR	
BRAF status		
Mutant	10.4	*<0.001*
Wildtype	34.7	
MSI status*		
MSS	22.1	*0.001*
MSI	11.1	
Sex		
Male	33.9	*0.041*
Female	26.8	
Primary Site		
Right	17.3	*<0.001*
Left	39.5	
Metastatectomy		
Yes	NR	*<0.001*
No	19.3	
Liver Metastases		
Yes	30.6	*0.195*
No	28.8	
Lung Metastases		
Yes	40.2	*<0.001*
No	22.1	
Peritoneal Metastases		
Yes	20.7	*0.575*
No	33.0	
Lymph Node Metastases		
Yes	32.3	*0.127*
No	28.1	
Brain Metastases		
Yes	16.1	*0.270*
No	31.5	

mOS = Median Overall Survival

*MSI status available on 350 (67%) patients for survival analysis

Table 7

Multivariate Survival Analysis

	n=350 (67%)	
	HR	**P**
BRAF Mutant	10.662	*<0.001*
Metastatectomy	0.218	*<0.001*
Right sided primary	1.359	*0.042*

HR = Hazard Ratio