BRAF^{V600E} as a Therapeutic Target in Colorectal Cancer

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Abstract

Colorectal cancer is a major cause of cancer death in developed countries. Increasing knowledge of the underlying signalling pathways and molecular defects involved in carcinogenesis has led to the development of a multitude of novel target-based therapeutics. The Ras/Raf/MEK/ERK mitogen-activated protein kinase (MAPK) signalling pathway plays a critical role in colorectal cancer progression. Therefore, targeting components of this pathway has been the subject of intense therapeutic effort. BRAF, a principal effector of Ras in this signalling cascade, was found to be mutated in a variety of cancers, including a small percentage of colorectal cancers. This article summarises the rationale for targeting BRAF^{V600E} in colorectal cancer, a potential strategy to enrich the colorectal cancer population for the BRAF^{V600E} mutation, and the prognostic and predictive role of BRAF^{V600E} mutations in metastatic colorectal cancer.

Keywords

Colorectal cancer, BRAF, prognostic biomarker, predictive biomarker, mitogen-activated protein kinase (MAPK) signalling pathway, therapeutic target, BRAF inhibitor

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Colorectal cancer is the third most common cancer worldwide and the second leading cause of cancer deaths in the developed world,[1,2] with an estimated 149,000 new cases and 50,000 deaths each year in the US.[3] Considerable progress has been achieved in the systemic treatment of colorectal cancer over the last decade with the incorporation of new chemotherapeutic (oxaliplatin and irinotecan) and biologic agents (bevacizumab, cetuximab and panitumumab) to the backbone of fluorouracil (5-FU)-based therapy. This has resulted in an improvement in median survival from 10–12 months to over 20 months for patients with metastatic colorectal cancer.[4] Advances in molecular biology have also allowed a more in-depth understanding of the genetic/epigenetic perturbations and the signal transduction pathways involved in colorectal cancer, leading to an appreciation of the molecular diversity in colorectal cancer. Nonetheless, until recently, colorectal cancer was treated as a single disease with a 'one size fits all' approach. Using *KRAS* mutation status to select patients against anti-epidermal growth factor receptor (EGFR) monoclonal antibody (MoAb) therapy represents the first step towards personalising treatment in colorectal cancer, where treatment is guided by a tumour's molecular profile.[5–8]

The Ras/Raf/MEK/ERK mitogen-activated protein kinase (MAPK) pathway is a critical signal transduction pathway involved in many cancers, including colorectal cancer, and has been the target of therapeutic intervention in recent years.[9] However, therapeutic efforts against Ras have thus far been unsuccessful in the clinic. This has led to interest in Raf kinase, in particular the *BRAF* oncogene, which is immediately downstream of Ras in the MAPK cascade. A specific BRAF^{V600E} inhibitor has now entered into early-phase clinical development in melanoma and colorectal cancer expressing the BRAF^{V600E} mutation.[10] However, the therapeutic development of BRAF inhibitors in colorectal cancer will be challenging due to the low frequency of BRAF^{V600E} mutations (approximately 10%) in this disease. This article will highlight the role of the MAPK pathway in colorectal cancer and BRAF^{V600E} as a molecular target. We will also address the potential of enriching a colorectal cancer patient population for BRAF^{V600E} mutations based on clinical features and the influence of BRAF^{V600E} mutations on patient outcome.

Mitogen-activated Protein Kinase Signalling, *BRAF* Mutations and Colorectal Cancer

The MAPK signalling cascade functions immediately downstream of cell surface receptors and cytoplasmic signalling proteins, relaying extracellular signals to transcriptional regulation of fundamental cellular functions such as growth, survival, proliferation, differentiation and migration.[11–13] This pathway is deregulated in about 30% of all human cancers and can be activated by mutations in oncogenes such as *KRAS* and *BRAF*, or activation of upstream receptor tyrosine kinases such as EGFR (see *Figure 1*). The 'oncogenic addiction' of the MAPK pathway in colorectal cancer is reflected by the frequent perturbation of this pathway in this malignancy, with EGFR overexpression reported in about 50% of cases[14,15] and activating mutations in *KRAS* and *BRAF* reported in 30–50 and 10% of cases, respectively.[16-19] The oncogenic role of *KRAS* is well described in the stepwise model of colorectal carcinogenesis and will not be discussed in this article.[20–22]

The BRAF serine/threonine kinase is a member of the Raf kinase family consisting of A-Raf, B-Raf and C-Raf or Raf-1.[23] Although all three Raf isoforms share considerable sequence homology and exhibit the same substrate specificity (MEK1 and MEK2), they differ in their biological functions and regulations, which have not been fully elucidated.[24] Raf remains the best characterised activator of MEK, with BRAF being the most potent MEK activator of the Raf isoforms. The Raf family of genes were first identified as potent retrovirus oncogenes in 1984,[25,26] and subsequent high-throughput genomic sequencing has identified activating mutations in BRAF as the predominant genetic alterations in human cancers.[27,28] Our work and that of others have found that *KRAS* and *BRAF* mutations almost never occur in the same tumour, suggesting not only that *BRAF* is the principal effector of *KRAS* in the MAPK pathway, but also that they may be equivalent in their tumorigenic effects.[16,18,19,29,30]

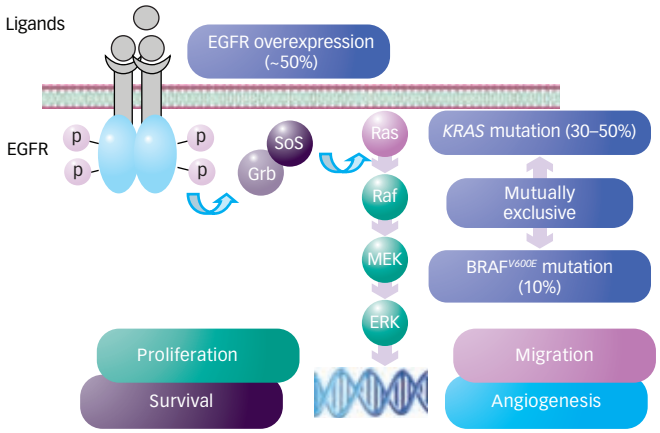
The discovery of BRAF mutations in various human cancers has stimulated further intensive research of this gene. The most common mutation in *BRAF*, accounting for up to 90% of all BRAF mutations in human cancers, is a thymidine-to-adenine transversion at nucleotide 1799 in the kinase domain of the protein resulting in a V600E amino acid (valine-to-glutamate) exchange.[31,32] This mutation leads to constitutive activation of the MAPK signalling cascade with the mutated protein demonstrating greatly elevated kinase activity, and potently transforms rodent fibroblasts and other cell types.[9,27] Furthermore, while mutant BRAF^{V600E} cells were shown to be dependent on continued BRAF activity for their tumorigenic growth,[33-35] they do not require Ras function for proliferation.[27] Interestingly, BRAF^{V600E} mutations were found to be associated with colorectal cancers exhibiting the microsatellite instability (MSI) phenotype and CpG island methylator phenotype (CIMP).[16,29,36] Subsequent studies have shown that BRAF^{V600E} mutations occur mainly in tumours with hypermethylation of hMLH1 promoter causing transcriptional silencing of the hMLH1 gene and hence defective DNA mismatch repair (dMMR) and the MSI phenotype.[17,37] By contrast, BRAF^{V600E} mutations rarely occur in tumours with dMMR attributable to the presence of germ-line mutations, indicating that BRAF^{V600E} mutations are not a direct result of defective DNA mismatch repair.[37] The complex relationships between BRAF, MSI and CIMP are the subject of ongoing investigation and are beyond the scope of this article.

Targeting BRAF^{V600E} in Colorectal Cancer

The unfulfilled promise of targeting KRAS in cancer has shifted the focus of therapeutic development to its downstream effector, BRAF.[32] Moreover, KRAS is central to a complicated network of signal transduction pathways, including the phosphatidylinositol 3-kinase (PI3K) pathway, characterised by cross-talks and feedback loops.[32] BRAF, on the other hand, would appear to be a 'purer' target with a relatively unidirectional MEK–ERK effector pathway. The finding that tumour cells and xenografts harbouring the BRAF^{V600E} mutation were extremely sensitive to MEK inhibition compared with those without this mutation or those bearing the Ras mutation further highlights the dependency of BRAF^{V600E} mutant cancer cells on MEK–ERK signalling.[38] Consistent with this is the pre-clinical work with a specific BRAF^{V600E} inhibitor, PLX4032, which demonstrated the selective sensitivity of melanoma and colorectal cancer cell-lines and xenografts harbouring BRAF^{V600E} mutation to BRAF^{V600E} inhibition compared with BRAF wild-type cells or xenografts.[10]

The first 'proof-of-concept' clinical data validating BRAF^{V600E} mutation as a therapeutic target came from the preliminary result

Figure 1: Oncogenic Signalling of the ERK–Mitogen-activated Protein Kinase Pathway in Colorectal Cancer



Epidermal growth factor receptor (EGFR) overexpression and mutation in KRAS *and* BRAF^{V600E} *result in constitutive activation of this signalling cascade in colorectal cancer, ultimately affecting nuclear targets involved in regulating cell proliferation, differentiation, survival, migration and angiogenesis.* KRAS *and* BRAF^{V600E} *mutation rarely co-exist in the same tumour, suggesting that Raf is the principal effector of Ras in tumorigenesis.*

of the phase I study of PLX4032 in advanced melanoma that was presented at the 45th American Society of Clinical Oncology (ASCO) annual meeting.[10] Notably, patients in the initial cohorts were not stratified by BRAF^{V600E} mutational status; of the 21 melanoma patients treated at the ≥240mg twice daily dose level (minimum target dose for tumour regression), 16 carried the BRAF^{V600E} mutation and five did not. The efficacy data were extremely promising with nine partial responses, all seen in tumours carrying the BRAF^{V600E} mutation. The interim progression-free survival (PFS) for patients with BRAF-mutated melanoma was six months, with many patients still on treatment, while all five patients with BRAF wild-type melanomas had progressive disease. The safety data were encouraging, with the majority of adverse events being mild and transient. The most common side effects were rash, fatigue and photosensitivity. Cutaneous squamous cell carcinoma following chronic dosing was also observed. Initial data suggesting a favourable therapeutic index and selectivity of this BRAF^{V600E} inhibitor therefore provide an appealing therapeutic strategy for BRAF^{V600E}-mutated cancers. This study is currently being expanded to include advanced melanoma and colorectal cancer harbouring the BRAF^{V600E} mutation.

Unlike melanoma, where BRAF^{V600E} mutations are frequently seen (43–66%), the prevalence of BRAF^{V600E} mutations in colorectal cancer is considerably lower, at about 10%. This poses a unique set of challenges in the clinical development of BRAF inhibitors in the colorectal cancer patient population, as large numbers of patients will need to be screened to select for patients who will be carrying this mutation. Therefore, being able to enrich the population screened by selecting patients with clinical features associated with the BRAF^{V600E} mutation may assist in drug development and allow for more efficient utilisation of resources.

Defining Colorectal Patient Subgroups with Higher Frequencies of BRAF^{V600E} mutation

We designed a study specifically to address this matter.[18] Five hundred and twenty-five colorectal cancer cases were identified from a prospectively collected clinical database and analysed for BRAF^{V600E}, KRAS mutational and MSI status. Patients were selected

Table 1: Clinical Features Define Colorectal Cancer Patient Subsets with Higher Frequencies of BRAF^{V600E} Mutation

Patient Subgroup	Frequency of BRAF^{V600E} Mutation (%)
All patients	10
Right colon	22
Right colon + female	31
Right colon + female + >70 years of age	37
Left colon or rectum + male + <70 years of age	0

based on pre-defined clinical features previously suggested to be associated with BRAF^{V600E} mutation,[29,39,40] such as age (<70 versus >70 years), gender (male versus female), tumour site (right colon versus left colon versus rectum) and disease stage (stage A–C versus D). The frequencies of BRAF^{V600E}, KRAS mutation and MSI were 10, 33 and 15%, respectively.

A multivariate analysis demonstrated that BRAF^{V600E} mutation was independently associated with increasing age (p=0.04), female gender (p=0.0005) and right-sided tumour location (p<0.0001), but not with stage. The frequency of BRAF^{V600E} mutation in the >80 years of age group was more than double that of the <70 years of age group (17.2 versus 7.6%). This mutation was three times more common in females (15%) than males (5%), and was rarely found in left-sided colon cancer (4%) and rectal cancer (2%). The prevalence of this mutation in right-sided colon cancer was 22% in our cohort.

Perhaps more clinically relevant was the finding that specific subgroups of patients with a higher or lower prevalence of BRAF^{V600E} mutation can be defined by these clinical features (see *Table 1*). We found a BRAF^{V600E} mutation frequency of 37% in older female patients with a right-sided colon cancer while no BRAF^{V600E} mutation was found in younger male patients with a left-sided colon cancer or rectal cancer. This information may be useful in guiding clinical trial design, tailoring to the patient population who are most likely to have BRAF^{V600E} mutations, such as the elderly population.

BRAF^{V600E} as a Prognostic and Predictive Biomarker in Metastatic Colorectal Cancer

There is accumulating evidence suggesting that BRAF^{V600E} is a negative prognostic biomarker for patient outcome and a negative predictive marker for response or outcome to anti-EGFR MoAb therapies in metastatic colorectal cancer (see *Table 2*).[18,30,41,42] A prognostic biomarker helps distinguish a patient population in terms of the outcome of interest (e.g. survival or recurrence) in the absence of treatment or non-targeted treatment (i.e. standard chemotherapy without anti-EGFR MoAb or BRAF inhibitor). The prognostic role of a biomarker is best examined by using data from an untreated patient cohort or from patients who are treated with standard treatment only.[43] On the other hand, a predictive biomarker should be able to predict the differential effect of a specific treatment (usually a targeted therapy) on the outcome of interest (e.g. response, survival, toxicity) in the population.

Thus far, findings from two studies where treatment information was available have shed light on the prognostic impact of BRAF^{V600E} in metastatic colorectal cancer. In a cohort of 165 patients with metastatic colorectal cancer naïve to anti-EGFR therapies (74 treated with standard chemotherapy, 86 untreated, five treatment status unknown), we found that BRAF^{V600E} was associated with an significantly inferior overall survival where median survival was 7.3 months in BRAF^{V600E} mutant tumours compared with 13.7 months in BRAF wild-type tumours (p=0.003). BRAF^{V600E} remains independently associated with increased mortality in a multivariate analysis adjusting for other known prognostic factors (hazard ratio [HR] 1.89; p=0.04) and in the subgroup analysis of patients treated with palliative chemotherapy (HR 2.9; p=0.006).

This is consistent with the recent findings by Tol et al.,[30] who performed a retrospective analysis of BRAF^{V600E} status in 516 tumours from a randomised controlled trial,[44] in which 260 patients received chemotherapy and bevacizumab alone and chemotherapy, bevacizumab and cetuximab was administered to 259 patients. The authors found that patients with BRAF^{V600E}-mutated tumours experienced a significantly shorter median PFS and overall survival than patients with *BRAF* wild-type tumours, regardless of their exposure status to cetuximab treatment. This is in contrast to the findings with *KRAS* mutational status, where *KRAS* mutations were predictive of a poorer outcome exclusively in patients who were treated with cetuximab,[44,45] suggesting that *KRAS* is a predictive biomarker for anti-EGFR MoAb rather than a prognostic biomarker in metastatic colorectal cancer.

As BRAF kinase lies downstream of EGFR and KRAS in the MAPK pathway, it is not surprising that tumours harbouring BRAF^{V600E} mutation with persistent activation of this signalling cascade will be resistant to any targeted therapy against EGFR. To this end, several retrospective analyses involving patients who have been treated with anti-EGFR MoAb have supported the role of BRAF^{V600E} as a negative predictive biomarker for these targeted treatments.[30,41,42] Findings from the study by Tol et al. have been referred to above. Di Nicolantonio et al. examined the *KRAS* and BRAF^{V600E} mutational status in 113 tumours from patients treated with either cetuximab or panitumumab. Individuals with BRAF^{V600E}-mutated tumours were found to have a significantly shorter PFS (p=0.01) and overall survival (p<0.0001) compared with patients with BRAF wild-type tumours, independent of their *KRAS* status. Souglakos et al. analysed 168 patients with metastatic colorectal cancer treated with 5-FU-based first-line chemotherapy; 60% of patients from this cohort received cetuximab as part of their first-line or salvage regimen. Similar to the previous study, patients with BRAF^{V600E}-mutated tumours experienced a shorter first-line PFS (4.3 versus 12.5 months; p<0.0001) and overall survival (10.9 versus 40.5 months; p<0.0001) compared with their wild-type counterparts. Unlike other studies, this cohort included patients who underwent metastatectomy (18%), which could account for the long overall survival in the *BRAF* wild-type group (40.5 months) compared with the other studies.

Implications and Future Challenges

We are venturing into an exciting era in cancer treatment with the development of multiple novel agents targeting signal transduction pathways coupled with an ever-growing knowledge of the critical molecular aberrations and signalling pathways driving malignant cell progression. Previous work has provided compelling evidence that the BRAF^{V600E} mutation is a major driver in various types of cancer, including a proportion of colorectal cancers. Initial efficacy results from an early-phase trial of the specific BRAF^{V600E} inhibitor in melanoma confirmed the validity of BRAF^{V600E} as a therapeutic target as well as a predictive molecular biomarker for response to BRAF

Table 2: BRAF^{V600E} and Outcome in Metastatic Colorectal Cancer Treated with Systemic Chemotherapy ± Anti-epidermal Growth Factor Receptor Monoclonal Antibodies

Study Details				Median Overall Survival (months)			Median First-line PFS (months)		
Study	Clinical Trial	Anti-EGFR MoAb	Number	BRAF WT	BRAF Mut	p	BRAF WT	BRAF Mut	p
Tie et al.	No	No	74	22.2	11	0.006	N/A		N/A
Tol et al.	Yes	No	260	24.6	15	0.002	12.2	5.9	0.003
		Yes	259	21.5	15.2	0.001	10.4	6.6	0.01
Souglakos et al.[a]	No	Yes[b]	168	40.5	10.9	<0.0001	12.5	4.3	<0.0001
Di Nicolantonio et al.	±[c]	Yes	113	N/A		0.01	N/A		<0.0001

a. 18% of patients in this study underwent metastatectomy. b. 60% of patients in this study were treated with cetuximab; 5% of patients received first-line cetuximab. c. 57% of patients in this study were part of a clinical trial; the remainder received anti-epidermal growth factor receptor (EGFR) monoclonal antibody (MoAb) as per label indication.
N/A = not available; PFS = progression-free survival.

inhibition. Nevertheless, history informs us that promising pre-clinical development of a targeted agent may fail to translate into practical benefit due to lack of patient stratification in clinical trials.[46]

The successful development of trastuzumab in breast cancer overexpressing human epidermal growth factor receptor 2 (HER2) offers a clear example of the importance of enriching the study population with patients who are most likely to respond based on their molecular profile. Not only will an enrichment strategy speed drug development and eventual approval, but it will also allow for a more efficient use of the limited patient populations available for early-phase clinical trials. More importantly, it would help avoid the early rejection of a novel agent with clinical activity in only a small proportion of tumours. Additionally, in an era in which cost-effectiveness is becoming increasingly relevant, defining patient populations most likely to benefit from a given agent during its development will likely assist greatly in gaining eventual approval and reimbursement.

The clinical development of BRAF inhibitors in colorectal cancer carrying the BRAF^{V600E} mutation, the enriched population, will be challenging due to the low BRAF^{V600E} mutation rate in this tumour type. Our group investigated a strategy to enrich the colorectal cancer population with BRAF^{V600E} mutation by selecting patients with clinical features associated with this mutation. In addition to reducing the cost of screening, understanding this clinico-molecular correlation will also allow for more informed clinical development of BRAF inhibitors. For example, we found that older patients (>70 years of age), and especially the very elderly (>80 years of age), have a higher prevalence of BRAF^{V600E} mutation compared with younger patients. The elderly may be a subset of patients who would derive the most benefit from BRAF^{V600E}-targeted therapy, especially in light of the encouraging preliminary safety data. Furthermore, the consistent finding of BRAF^{V600E} mutation as a negative prognostic biomarker for survival outcome in patients treated with conventional cytotoxic chemotherapy with or without anti-EGFR MoAb may justify the development of novel targeted agents, such as the BRAF inhibitor, in the first- or second-line setting in these patients.

Many challenges lie ahead in the development of agents targeting BRAF^{V600E} and other target-based therapies. The clinical application of BRAF inhibitors will undoubtedly involve their use in combination with other agents, be it cytotoxic chemotherapy or other signal transduction inhibitors.

Robust pre-clinical analyses with well-validated models will be required to better inform clinical trial design. As is evident from experience with conventional cytotoxic treatment and other targeted drugs, resistance will likely develop. Understanding the mechanisms of resistance, whether they are intrinsic to BRAF or due to the development of escape pathways in related kinases, will be invaluable in helping the rational development of other inhibitors of the same target with a different mechanism of action, or drugs targeting the escape signalling pathway. To this end, exploration of *in vivo* biology with pharmacodynamic markers (such as pre- and post-treatment biopsies) in patients treated with these agents could be highly informative and should be strongly encouraged when possible. ■

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Jayesh Desai is a Medical Oncologist at the Royal Melbourne Hospital and Peter MacCallum Cancer Institute in Melbourne, and Senior Clinic Research Fellow in the Ludwig Colon Cancer Initiative (LCCI) programme at the Ludwig Institute for Cancer Research. He is also an Associate Director for Cancer Trials Australia (CTA) and chairs the Phase I Drug Development Group. Dr Desai's clinical and research interests are in rationally developing new anticancer therapeutics, with a particular emphasis on colorectal cancer, and in exploring predictive markers of response to these agents.

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