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BRAF Mutations as Predictive Biomarker for Response to Anti-EGFR Monoclonal Antibodies

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Key Words. BRAF • RAS • Cetuximab • Panitumumab • Predictive biomarker • Anti-epidermal growth factor receptor

Abstract

Recently, the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO) recommended that patients with epidermal growth factor receptor (EGFR)-expressing metastatic colorectal cancer could be treated with anti-EGFR monoclonal antibodies (mAbs) cetuximab and panitumumab only in absence of Rat-Sarcoma (*RAS*) mutations. In addition to the previously established biomarker Kirsten rat sarcoma viral oncogene homolog (*KRAS*) exon 2, cumulative evidence also shows that patients whose tumors harbor *KRAS* exons 3 or 4 and neuroblastoma rat-sarcoma viral oncogene homolog (NRAS) exons 2, 3, and 4 mutations are found unlikely to benefit from anti-EGFR treatment.

In line with the resistance of *RAS* mutated (mt) tumors, treatment response in *BRAF*mt tumors may also be altered given their important role in the EGFR signaling pathway. However, *BRAF* is not recommended as predictive biomarker yet because the evidence for the impact of *BRAF* mutations on treatment outcome is considered insufficient.

This article summarizes the evidence for the impact of *BRAF* mutations on treatment outcome of anti-EGFR mAbs. Based on a review of literature, eight meta-analyses were included that consistently show that patients with *BRAF* mutations have a lack of treatment benefit of anti-EGFR mAbs. After discussing the quality and quantity of available evidence, we conclude that evidence is stronger than suggested by ESMO and ASCO. Additionally, we highlight that the quality of evidence for *BRAF* is even higher than for extended *RAS* as a biomarker. We therefore advise ESMO and ASCO to reconsider *BRAF* status as a predictive biomarker for response. ***The Oncologist*** 2017;22:864–872

Implications for Practice: In metastatic colorectal cancer (mCRC), therapy with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab is indicated in absence of *RAS* mutations. Cumulative evidence shows that patients with *BRAF* mutations, who comprise 10% of the mCRC population, do not benefit from anti-EGFR-antibody treatment. Although guidelines state that evidence for *BRAF* as a predictive marker is insufficient, we highlight that the quality and quantity of evidence is higher than suggested. We therefore encourage the use of *BRAF* as a predictive marker in order to exclude patients from therapy for whom limited treatment benefit is expected.

Introduction

The RAS-RAF-MEK-ERK (MAPK) pathway plays a pivotal role in the regulation of cell proliferation, survival, and differentiation. Constitutive activation of this pathway is frequently observed in human cancers and is associated with high rates of cancer cell proliferation. Within the MAPK pathway, RAS, RAF, MEK, and ERK are key proteins in signal transduction. In tumor cells, the MAPK pathway is often constitutively activated by gain-of-function mutations in one of the signaling proteins including but not limited to RAS and RAF. In colorectal cancer (CRC), activation of the MAPK pathway is often a result of mutations in the RAS family protein Kirsten rat sarcoma viral oncogene homolog (*KRAS*), which is found in 40% of the patients [3, 4]. Mutations occur most frequently in exon 2 (36%) and less frequently in exons 3 (2%) and 4 (2%). In addition to KRAS, neuroblastoma rat sarcoma viral oncogene homolog (*NRAS*) mutations occur in about 3% [3, 4]. KRAS and NRAS are very closely related, although their biological roles are slightly different. Whereas functional KRAS is essential for cell survival, NRAS is not required. Therefore, KRAS gain-of-function mutations may have a larger impact on tumor growth and proliferation compared with NRAS mutations [5].

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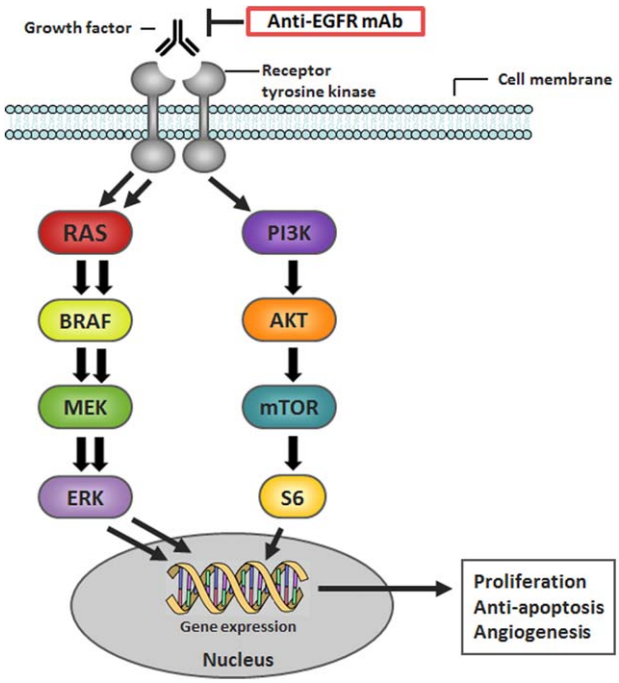

Figure 1. Schematic overview of the MAPK signaling pathway. Adapted with permission from van Geel et al. [66].

Abbreviations: EGFR, epidermal growth factor receptor; mAb, monoclonal antibody; MAPK, RAS-RAF-MEK-ERK.

The first effector protein of RAS is RAF, comprising c-RAF1, BRAF, and ARAF. Of these, BRAF has the most important biological function and is also most frequently mutated [6]. BRAF and RAS mutations are mutually exclusive, which highlights their functional importance [7]. Gain-of-function mutations in exon 15 result in the BRAFV600E variant in about 10% of the CRC population and induce constitutive MAPK-pathway activation [5, 8]. Other mutations that occur less frequently include the variants G469V (<0.1%), D594G (<0.3%), and K601E (unknown frequency) [6]. All mutations lead to constitutive activation of downstream proteins within the MAPK-pathway independent of upstream activation signals, yet the p.V600E variant is the strongest activator [8, 9]. BRAF mutations in CRC occur most frequently in tumors originating from the appendix and the ascending and transverse colon, defined as right-sided tumors [10–12].

In up to 90% of colorectal tumors, epidermal growth factor receptor (EGFR) is overexpressed, which renders EGFR an attractive drug target [13–16]. Upon binding of its ligands, including epidermal growth factor, betacellulin, epiregulin, and neuregulins, cell proliferation and growth are induced primarily through the MAPK and PI3K/AKT signaling pathways (Fig. 1) [17].

Cetuximab (Erbitux®) [14, 18]) and panitumumab (Vectibix®) [15, 19]) are anti-EGFR monoclonal antibodies (mAbs) that exert their antitumor effect through inhibition of EGFR signaling. Both drugs are registered for the treatment of EGFR-expressing metastatic CRC (mCRC) after failure of first- and/or second-line therapies.

In line with the biological mechanism, several trials showed that the effects of anti-EGFR treatment are decreased when mutations downstream of EGFR are present that cause MAPK-pathway activation independent of EGFR signaling such as mutations in KRAS (in 40%) and BRAF (in 10%) [1, 3–5, 20]. It is now generally accepted that mutations in *KRAS* exon 2 diminish treatment response when anti-EGFR mAbs are given as a single agent or combined with chemotherapy [1, 14, 15, 21]. More recently, several retrospective analyses showed that not only *KRAS* exon 2 mutations but also *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 mutations are predictive biomarkers [1, 2, 7, 22–27]. Treatment guidelines for metastatic CRC now recommend upfront *RAS* testing before start of anti-EGFR mAb therapy [1, 2] in order to exclude patients with mutated *RAS* from therapy with these agents.

BRAF mutations could have comparable effects on anti-EGFR mAb treatment response as *RAS* mutations. BRAFV600E gain-of-function mutations comprise 80%–96% of all *BRAF* mutations and occur in about 10% of CRC patients [4, 6, 28]. Although several meta-analyses indicate that *BRAF* status may be a predictive biomarker for treatment efficacy [4, 29–32], the use of *BRAF* status as a predictive biomarker is not recommended yet because evidence is considered less convincing than the evidence for *RAS* mutations [1, 2].

This manuscript describes the evidence that is available for *BRAF* mutations as a predictive biomarker for response to anti-EGFR mAbs in mCRC. We will discuss the load and quality of clinical evidence for the impact of *BRAF* mutations on anti-EGFR mAb treatment outcomes and argue why this can be considered convincing enough to include *BRAF* mutation status in the panel of upfront mutation tests in anti-EGFR mAb therapy.

Evidence for *BRAF* Mutations as a Predictive Biomarker

A PubMed search was performed to collect meta-analyses that included data of *BRAF* mutated (mt) patients and *BRAF* wild-type (wt) patients and survival outcome of treatment with the anti-EGFR mAbs cetuximab or panitumumab using the following terms: (molecular testing OR mutation) AND (BRAF OR RAF) AND survival AND EGFR AND "colorectal cancer" AND meta-analysis (full methods available in the supplemental online Appendix 1). Eight meta-analyses were identified that report on the overall response rate (ORR), progression-free survival (PFS), or overall survival (OS) of *BRAF*mt patients treated with anti-EGFR mAbs cetuximab or panitumumab as single agents or combined with chemotherapy. Four of these were considered high-quality reviews, and only these will be extensively discussed in this section [4, 29–31]. The results of all meta-analyses are summarized in Table 1.

De Roock et al. [4] comprehensively analyzed the relationship between different pathway mutations and treatment response and survival in mCRC patients treated with cetuximab combined with chemotherapy. The authors collected tumor samples and clinical data from 11 European investigators who had published data on cetuximab-treated mCRC patients. Finally, 761 tumor samples were analyzed for *BRAF* status (screened for the mutations p.D594G, p.V600E, p.V600M, and p.K601E). In 36 patients, a BRAF mutation was found, being mostly p.V600E ($n = 35$) and one p.D594G. In a selection of patients without *KRAS* mutations, it was found that *BRAF*mt patients ($n = 24$) had a significantly lower ORR (8.3%) compared with *BRAF*wt patients ($n = 326$, ORR 38%; OR 0.15 [95% confidence interval {CI} 0.02–0.51]) and shorter PFS (hazard ratio {HR} 3.74 [95% CI 2.44–5.75]) and OS (HR 3.03 [95% CI

Table 1. Overview of meta-analyses, which show hazard ratios for PFS, OS, and odds ratios for ORR on anti-epidermal growth factor receptor monoclonal antibody therapy by BRAF status for the KRASwt group and (°) for the KRAS unselected group

Study (n/n *BRAF*mt) [Ref]	*BRAF*mt			*BRAF*wt			[x]*BRAF*mt/*BRAF*wt [y]*BRAF*wt/*BRAF*mt		
	PFS	OS	ORR	PFS	OS	ORR	PFS	OS	ORR
De Roock[x] (761/36) [4]							3.74 [2.44–5.75]	3.03 [1.98–4.63]	0.15 [0.02–0.51]
Pietrantonio (6,256/469) [31]	0.88 [0.67–1.14]	0.91 [0.62–1.34]	1.31 [0.83–2.08]						
Rowland[x] (3,186/351) [30]	0.86 [0.61–1.21]	0.97 [0.67–1.41]	n.d.	0.62 [0.50–0.77]	0.81 [0.70–0.95]	n.d.	1.39[a] [0.92–2.08]	1.19[a] [0.80–1.78]	n.d.
Therkildsen[x] (1,267/123) [29]							2.95 [1.89–4.61]	2.52 [1.39–4.56]	0.29 [0.16–0.54]
Yuan[y] (4,616/343) [32]							0.29 [0.19–0.43]	0.26 [0.20–0.36]	0.31 [0.18–0.53]
Xu[x] (2,875/246) [36]							2.41 [1.23–4.71]	2.74° [2.31–3.52]	0.26 [0.07–0.98]
Wang[x] (1,352/74) [37]							2.78 [1.62–4.76]	2.54 [1.93–3.32]	0.27 [0.10–0.70]
Cui[x] (1,245/126) [38]							n.d.	n.d.	0.43 [0.16–0.75]

Four meta-analyses included only or primarily RCTs [29–31, 38] three included retrospective and prospective studies [32, 36, 37] and one included only retrospective data [4].

[a]Based on re-calculation performed by the authors of this proposal.

Abbreviations: KRAS, Kirsten rat sarcoma viral oncogene homolog; mt, mutated; n, number of patients; n BRAFmt, number of patients with a BRAF mutation; n.d., not described; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; RCT, randomized controlled trial; wt, wild type.

1.98–4.63]). The association between disease control and *BRAF* status was significant in multivariate analysis (adjusted OR *BRAF*mt vs. *BRAF*wt 0.059; $p < .0001$), as was the association with *KRAS, NRAS*, and *PIK3CA* exon 20. Still, 2 out of 24 patients had a response to treatment despite *BRAF* mutations. The authors report that one of these had a p.D594G mutation that leads to weaker activation of the MAPK pathway compared with p.V600E mutations [33]. The other responder had a low copy number of *BRAFV600E*mt genes that may explain the sensitivity to cetuximab. The authors conclude that the response rate of 24.4% in an unselected population could be increased to 36.3% in a *KRAS*wt population and further to 38.4% in *KRAS*wt and *BRAF*wt patients [4]. Another 1.5% ORR improvement could be achieved by *NRAS* testing according to their results. This study highlighted the importance of *BRAF* in addition to *KRAS* status in treatment with anti-EGFR mAbs. Several meta-analyses have been performed to confirm the findings of De Roock et al.

Pietrantonio et al. [31] performed a meta-analysis of randomized controlled trials (RCTs) to examine the effect of anti-EGFR mAbs on PFS, OS, and ORR in *BRAF*mt/*KRAS*wt advanced CRC. Nine phase III trials and one phase II trial were included that compared anti-EGFR mAbs as monotherapy or added to chemotherapy with chemotherapy or best supportive care in advanced *KRAS*wt CRC. In total, these comprised 6,256 patients on first-line (six trials) and second-line treatment (two trials) or who were chemo-refractory (two trials). The authors show that patients with *BRAF*mt CRC ($n = 469$) do not have a significant benefit in PFS (HR PFS benefit 0.88 [95% CI 0.67–1.14]), OS (HR OS 0.91 [95% CI 0.62–1.34]), or ORR (OR 1.31 [95% CI 0.83–2.08]) from treatment with anti-EGFR mAbs. All mutations comprised p.V600E mutations except for 21 patients (13%) in the trial from Smith et al. who had the p.D594G mutation [34]. The response rate of patients with *BRAF*mt varied from 10.8% to 52.2% on anti-EGFR mAbs compared with 6.4% to 40% on chemotherapy. Based on these results, *BRAF*mt patients seem to have modest responses to anti-EGFR mAbs, yet, overall, a significant response rate and survival benefit is lacking in this population [31]. A drawback of this meta-analysis is that a comparison with *BRAF*wt patients has not been made.

Rowland et al. [30] reviewed RCTs that evaluated the effect of *BRAF* mutations on treatment benefit (OS and PFS) from anti-EGFR mAbs for *KRAS* exons 2 and 3 wt metastatic CRC mCRC. All of the included trials have also been reviewed by Pietrantonio et al. [31]. However, Rowland et al. excluded the trials by Tveit et al. [35] and Stintzing et al. [24], probably because the former did not provide data on PFS and OS and the latter had bevacizumab with FOLFIRI as control treatment instead, which did not meet the inclusion criteria. The review by Rowland et al. thus differs from Pietrantonio et al. by inclusion criteria but moreover by their statistical tests [30, 31].

Seven articles covering eight RCTs were included in which 3,168 *KRAS*wt patients were treated with cetuximab or panitumumab (four studies each) added to chemotherapy or with chemotherapy alone. About 11% of the tumors harbored a *BRAF* mutation ($n = 351$), of which 94% ($n = 330$) were p.V600E mutations and 6% ($n = 21$) were p.D594G mutations [34]. Rowland et al. not only reported outcomes for the *BRAF*mt subgroup but also compared *BRAF*mt patients with *BRAF*wt patients. Results show a lack of PFS benefit in the *BRAF*mt group (HR PFS benefit 0.86 [95% CI 0.61–1.21]), whereas patients with *BRAF*wt had significant benefit (HR PFS benefit 0.62 [95% CI 0.50–0.77]) from addition of anti-EGFR mAbs to chemotherapy. The interaction test (PFS HR *BRAF*mt/PFS HR *BRAF*wt) showed a close to significant difference ($p = .07$). For OS, *BRAF*mt patients (HR 0.97 [95% CI 0.67–1.41]) also had no

Table 2. Summary of the results from five randomized clinical trials which report on the effects of *KRAS* exon 2 mutations[a]

		KRAS exon 2 wild type		*KRAS* exon 2 mutation	
Study [Ref] (*n*)	**Treatment**	**PFS (months)**	**ORR**	**PFS (months)**	**ORR**
Van Cutsem [22] ($n = 540$)	Cetuximab + FOLFIRI	9.9	59.3	7.6	36.2
	vs. FOLFIRI	8.7	43.2	9.1	40.2
	HR/OR	0.68[b]	1.37[b]	1.07	0.9
Bokemeyer [13] ($n = 233$)	Cetuximab + FOLFOX	7.7	60.7	5.5	32.7
	vs. FOLFOX	7.2	37.0	8.6	48.9
	HR/OR	0.57[b]	2.54[b]	1.83[b]	0.51
Punt [67] ($n = 501$)	Cetuximab + CAPOX-B	10.5	n.d.	8.6	n.d.
	vs. CAPOX-B	10.7	n.d.	12.5	n.d.
	HR/OR	n.d.	n.d.	n.d.	n.d.
Amado [39] ($n = 427$)	Panitumumab	12.3	17	7.4	0
	vs. BSC	7.3	0	7.3	0
	HR/OR	0.45[b]	n.d.	0.99	n.d.
Karapetis [57] ($n = 394$)	Cetuximab	3.7	12.8	1.8	1.2
	vs. BSC	1.9	0	1.8	0
	HR/OR	0.4[b]	n.d.	0.99	n.d.

[a]Referred to in the American Society of Clinical Oncology's clinical opinion update 2009 [21].
[b]$p < .05$
Abbreviations: BSC, best supportive care; CAPOX, capecitabine, oxaliplatin; FOLFIRI, 5-fluorouracil, folinic acid, irinotecan; FOLFOX, 5-fluorouracil, folinic acid, oxaliplatin; HR, hazard ratio; KRAS, Kirsten rat sarcoma viral oncogene homolog; n.d., not described; OR, odds ratio; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

benefit, whereas *BRAF*wt patients (HR 0.81 [95% CI 0.70–0.95]) had significantly improved OS. The interaction test for OS was not significant ($p = .43$). For both PFS and OS, the difference between *BRAF*mt and *BRAF*wt patients was bigger in the second-line setting, showing a very strong trend towards significance (interaction test PFS $p = .05$; OS $p = .38$). The authors conclude that based on the nonsignificant interaction test values, the effect of *BRAF* mutations on PFS and OS cannot be confirmed [30].

However, it is of great importance to note that the interaction test for PFS was very close to significance. To allow proper interpretation of the *p* value (.07), CIs of the interaction test value should be taken into account. However, these were not provided by the authors. Based on our re-estimation, as described in the methods section, the 95% CI for the interaction on PFS should range from 0.92 to 2.08. This underscores that there is a high chance that anti-EGFR mAbs have a different effect on PFS in *BRAF*mt patients. To summarize, these results confirm a significant lack of PFS benefit of anti-EGFR mAbs treatment in *BRAF*mt patients, which is relevantly though not significantly different from the *BRAF*wt patients.

Another meta-analysis was performed by Therkildsen et al. [29], who reviewed the impact of alterations in *KRAS* other than exon 2, *NRAS, BRAF, PIK3CA*, and *PTEN* on clinical benefit of anti-EGFR treatment combined with chemotherapy in *KRAS* exon 2 wt patients in 21 RCTs and one nonrandomized trial. Among 1,267 patients treated with cetuximab or panitumumab in first to greater than fourth setting mostly, *BRAF* mutations were detected in 123 patients, all of which were p.V600E except for one p.K601E variant. *BRAF*mt patients were found to have significantly lower ORR (OR ORR 0.29 [95% CI 0.16–0.54]) and shorter PFS (HR 2.95 [95% CI 1.89–4.61]) and OS (HR 2.52 [95% CI 1.39–4.56]) compared with *BRAF*wt patients. The authors also report that the response rate can be increased from 37.6% on average in *KRAS*wt selected patients to 39% in *KRAS*wt/*BRAF*wt selected patients.

In our opinion, these meta-analyses provide high-quality clinical evidence for the lack of efficacy of anti-EGFR mAb treatment on response and survival endpoints for patients with *BRAF*mt tumors. Supporting evidence can be found in four meta-analyses that included survival endpoints based on retrospective studies, mainly [32, 36, 37], or that included response rate as an endpoint only (Table 1) [38].

Evidence for *BRAF* Compared with *RAS*

Initially, Erbitux® and Vectibix® were registered for patients with EGFR-expressing metastatic CRC only in presence of *KRAS* exon 2 wt. This was based on seven pivotal trials with cetuximab that show that the *KRAS* exon 2 mutated population had no benefit on primary endpoints ORR, PFS, or OS [14, 15, 39]

This is supported by a review of five RCTs that was performed by the American Society of Clinical Oncology (ASCO) [21]. The authors showed that all five trials, comprising 2,095 patients, consistently detected a lack of benefit from treatment with anti-EGFR mAbs in 720 patients with *KRAS* exon 2 mutations in terms of PFS and ORR (Table 2), whereas *KRAS* exon 2 wt patients did have significant benefit.

Table 3. Summary of the data from meta-analyses addressing the effect of *KRAS* exons 2, 3, and 4 mutations referred to in the American Society of Clinical Oncology's clinical opinion update 2015 [1]

		KRAS exons 2, 3, and 4 wild type		*KRAS* exons 2,3,4 mutation	
Study [Ref] (*n*)	**Treatment**	**HR PFS**	**HR OS**	**HR PFS**	**HR OS**
Adelstein [40] (*n* = 8,924)	P/C vs. SOC or P/C + SOC vs. SOC Interaction: *KRAS*wt/*KRAS*mt	0.80 [0.64–0.99] 0.71 [0.57–0.90]	n.d.	1.11 [0.97–1.27]	n.d.
Dahabreh [41] (n=1,945)	P/C +- SOC vs. BSC or SOC Interaction: *KRAS*mt/*KRAS*wt	n.d.	n.d.	n.d. 2.22 [1.74–2.55]	n.d. 1.30 [0.95–1.78]
Lin [42] (*n* = 5,325)	P/C +SOC vs. SOC	0.66 [0.53–0.82]	n.d.	1.07 [0.91–1.27]	n.d.
Loupakis [43] (*n* = 6,609)	P/C+ SOC vs. SOC	0.91 [0.84–0.99]	0.95 [0.87–1.04]	1.13 [1.03–1.25]	1.04 [0.95–1.13]
Petrelli [44] (*n* = 484)	P/C+ SOC vs. SOC	0.68 [0.53–0.87]	0.88 [0.65–1.20]	n.d.	n.d.
Petrelli [45] (*n* = 3,254)	P/C+ SOC vs. SOC	0.65 [0.51–0.83]	0.84 [0.73–0.98]	n.d.	n.d.
Qiu [46] (*n* = 2,188)	C + SOC vs. SOC Interaction: *KRAS*mt/*KRAS*wt	5.8 1.94 [1.62–2.33]	6.9 2.17 [1.72–2.74]	3.0	13.5
Vale [47] (*n* = 5,966)	P/C+ SOC vs. SOC Interaction: *KRAS*wt/*KRAS*mt	0.83 [0.76–0.90] 0.78 [0.68–0.89]	0.89 [0.82–0.97] 1.04 [0.95–1.15]	1.06 [0.96–1.17]	1.04 [0.95–1.15]
Zhang [48] (*n* = 2,912)	P/C+ SOC vs. SOC	0.64 [0.50–0.84]	0.84 [0.64–1.11]	1.37 [0.81–2.31]	1.03 [0.74–1.44]
Ibrahim [49] (*n* = 2,115)	P + SOC vs. SOC	0.58 [0.36–0.93]	0.90 [0.76–1.05]	n.d.	n.d.

All meta-analyses included RCTs only, except for Qui [46] and Dahabrabeh et al. [41] who included also retrospective and observational studies.
Abbreviations: BSC, best supportive care; C, cetuximab; HR, hazard ratio; KRAS, Kirsten rat sarcoma viral oncogene homolog; n, number of patients; n.d., not described; OS, overall survival; P, panitumumab; PFS, progression-free survival; RCT, randomized controlled trial; SOC, standard of care.

In October 2015, ASCO recommended to extend upfront testing of *KRAS* exon 2 with *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 [1]. In July 2016, the European Society for Medical Oncology (ESMO) supported this "extended *RAS*" testing in their consensus guideline on the management of metastatic CRC [2]. In Europe, these findings were incorporated in the product labels of Erbitux® and Vectibix®, which state that the benefit-risk ratio of treatment is negative for patients with *KRAS* or *NRAS* exons 2, 3, or 4 mutations [18, 19].

As supportive evidence for *KRAS* exons 2, 3, and 4 testing, ASCO referred to ten [40–49] meta-analyses and two [50, 51] health technology assessment reports that reviewed 137 primary studies with 19,543 patients. Table 3 summarizes findings of these ten meta-analyses. In all studies, a statistically significant PFS benefit in patients without *KRAS* exons 2, 3, or 4 mutations was found, whereas benefit was not significant in *KRAS* exons 2, 3, and 4 mutants. The effects of *KRAS* mutations on OS were less consistent. Five out of the 13 trials detected no statistically significant difference in OS between *KRAS*mt and *KRAS*wt patients treated with anti-EGFR mAbs. This is mainly due to lack of consistent OS benefit when adding anti-EGFR mAbs to standard of care in the overall and *KRAS*wt population. In *KRAS*wt patients, OS benefit was detected in only 6 out of 13 trials [45–47, 49–51].

In contrast to the evidence for *KRAS* exons 2, 3, and 4, only five articles report on the impact of *KRAS* exons 3 and 4 and *NRAS* mutations by itself (Table 3) [2, 7, 22, 25–27]. Although these five trials consistently show that patients with *RAS* mutations do not have significant treatment benefit, it should be noted that this is based on a small group with *RAS* mutations other than *KRAS* exon 2. The subgroup of *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 mutated patients comprises only 10%–20% of the study populations, which were 360 patients in total. To increase the power, most studies merge patients with any *RAS* mutation into one or two groups. This supports extended *RAS* testing but does not provide evidence on the effect of *KRAS* exons 3 and 4 and *NRAS* mutations by itself. In addition, the detected lack of OS benefit in the *RAS*mt subgroup is of limited value in three out of five trials because the *RAS*wt group did not have OS benefit either, and no significant interaction has been confirmed.

Despite these limitations, evidence was considered convincing enough by ASCO and ESMO to recommend upfront *KRAS* and *NRAS* exons 2, 3, and 4 mutation testing, so that only patients whose tumors do not harbor mutations in these exons will be given anti-EGFR mAb therapy [1, 2].

Discussion

Strong Evidence for Impact of *BRAF*mt on Anti-EGFR mAb Treatment Outcome

ASCO's and ESMO's most recent guidelines for the treatment of mCRC posit that there is currently insufficient evidence to recommend *BRAF* mutations as a biomarker for response to

Table 4. Overview of RCTs showing hazard ratios for PFS and OS stratified by *RAS* status[a]

Author [Ref]	Treatment	No *RAS* mutation		*KRAS* exon 2 mutation		*KRAS* exon 2 wild type, *KRAS* exons 3 and 4 or *NRAS* exons 2, 3, and 4 mutations		Any *RAS* mutation	
		PFS	OS	PFS	OS	PFS	OS	PFS	OS
Douillard [7] (n = 1,060)	P + FOLFOX4 vs. FOLFOX4	0.72 [0.58–0.90]	0.78 [0.62–0.99]	1.29 [1.04–1.62]	1.24 [0.98–1.57]	1.28 [0.79–2.07]	1.29 [0.79–2.1]	1.31 [1.07–1.6]	1.25 [1.02–1.55]
Van Cutsem [22] (n = 639)	C+ FOLFIRI vs. FOLFIRI	0.56 [0.41–0.76]	0.69 [0.54–0.88]	n.d.	n.d.	0.81 [0.39–1.67]	1.22 [0.69–2.16]	1.10 [0.85–1.42]	1.05 [0.86–1.28]
Schwartz-berg [27] (n = 285)	P + mFOLFOX6 vs. B + mFOLFOX6	0.65 [0.44–0.96]	0.63 [0.39–1.02]	n.d.	n.d.	1.39 [0.73–2.64]	0.41 [0.19–0.87]	n.d.	n.d.
Peeters [26] (n = 1,014)	P + FOLFIRI vs. FOLFIRI	0.70 [0.54–0.91]	0.81 [0.63–1.03]	0.85 [0.68–1.06]	0.94 [0.76–1.15]	0.89 [0.56–1.42]	0.83 [0.53–1.29]	0.86 [0.70–1.05]	0.91 [0.76–1.10]
Bokemeyer [25] (n = 118)	C + FOLFOX4 vs. FOLFOX4	0.53 [0.27–1.04]	0.94 [0.56–1.56]	n.d.	n.d.	0.77 [0.28–2.08]	1.09 [0.44–2.68]	1.54 [1.04–2.29]	1.29 [0.91–1.84]

[a]Referred to in the American Society of Clinical Oncology's clinical opinion updates of 2009 and 2015 [1, 21].

Abbreviations: B, bevacizumab; C, cetuximab; FOLFIRI, folinic acid + fluorouracil + irinotecan; FOLFOX, folinic acid + fluorouracil + oxaliplatin; KRAS, Kirsten rat sarcoma viral oncogene homolog; n, population evaluable for *RAS* status; n.d., not described; NRAS, neuroblastoma rat sarcoma viral oncogene homolog; OS, overall survival; P, panitumumab, PFS, progression-free survival; RAS, rat sarcoma viral oncogene homolog.

anti-EGFR therapy [52]. ESMO's guideline refers to three clinical trials [53–55] and two meta-analysis [30, 31], which show conflicting results. The authors of the guideline suggest that the evidence for *BRAF* mutations as a predictive biomarker for anti-EGFR therapy in later lines is accumulating, but the role in earlier treatment lines is uncertain [2]. It is therefore recommended that *BRAF* status is used as a prognostic marker or as a selection tool for clinical trials only. Based on our literature review, the evidence for the use of *BRAF* as a predictive marker may be stronger than suggested.

All eight meta-analyses that were reviewed, covering first-line and second-line settings (supplemental online Table 1), consistently show that *BRAF*mt patients do not have significant benefit from anti-EGFR mAbs in terms of ORR, PFS, and OS [30, 31], and when compared with *BRAF*wt patients, they have significantly less ORR, PFS, and OS benefit (Table 1) [4, 29, 32, 36, 37]. A significant interaction between *BRAF* and outcome has been confirmed in five meta-analyses [4, 29, 32, 36, 37]. Because both cetuximab and panitumumab were registered mainly based on PFS benefit [7, 18, 19, 22, 25–27], the detected lack of PFS benefit in *BRAF*mt patients should warrant the use in this population.

Only Rowland et al. reported a nonsignificant interaction between the *BRAF*wt and *BRAF*mt group on both PFS and OS, although the result for PFS was close to significant ($p = .07$). This finding should be interpreted in context with the power to detect significant differences. Results of Rowland et al. are based on a group of 3,096 patients. Although this is one of the three most extensive meta-analyses, it provides a power of 19% [56], whereas a sample size of 6,500 patients is required to detect a significant interaction effect with a power of 80% (calculation provided in methods section) [30].

Overall, the load of strong clinical evidence for *BRAF* mutations as a biomarker is based on a population of 628 patients with *BRAF* mutations [4, 29, 31]. In comparison, the evidence for *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 comes from only five studies with 360 patients with *RAS* mutations other than *KRAS* exon 2 [7, 22]. The lack of treatment benefit in this group guided ASCO's and ESMO's clinical opinion on extended *RAS* testing, while a significant interaction between *KRAS* exons 3 and 4/*NRAS* exons 2, 3, and 4 mutant and wt patients has not been confirmed. Based on this, the evidence for the impact of *BRAF* mutations on efficacy of anti-EGFR mAb treatment as a single agent or combined with chemotherapy can be considered stronger than for *KRAS* and *NRAS* mutations.

Biologically, it is possible that *BRAF*mt tumors respond to anti-EGFR treatment due to tumor heterogeneity, low copy numbers, and/or a varying potency of BRAF mutations to activate the MAPK pathway. As an example, the p.V600E mutation is a strong pathway activator, whereas the p.D594G is less activating and may still allow responses.

Risks of Using *BRAF* as a Predictive Marker

If upfront molecular testing of *BRAF* will be applied, it is expected that 10% of the mCRC population will be identified as *BRAF*mt and will be excluded from anti-EGFR mAb therapy. The

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most important risk of the use of *BRAF* as a predictive biomarker lies in withholding *BRAF*mt patients from a potentially effective treatment with anti-EGFR mAbs. In *BRAF*mt patients, response rates of 8.3% [4] to 18% [36] have been reported compared with 38% [4] to 42.4% [36] in *BRAF*wt patients. Although a direct effect of anti-EGFR mAbs cannot be ruled out, the responses may also be induced by backbone chemotherapy, which was administered in the majority of trials and which induced response rates of 13%–40% [31]. This is supported by the finding that responses rates in *BRAF*mt patients are higher when anti-EGFR mAbs are combined with chemotherapy (ORR 8.3%–18% [4, 36]) compared with monotherapy (ORR 1.2% [57]). As a comparison, it should also be noted that in patients with *KRAS* exon 2 mutations relevant response rates of 33%–36% [13, 54] have been observed on anti-EGFR treatment added to chemotherapy (Table 2) as well as incidental responses on monotherapy [18]. Yet, this did not hinder implementation of *KRAS* as a biomarker.

Biologically, it is possible that *BRAF*mt tumors respond to anti-EGFR treatment due to tumor heterogeneity, low copy numbers, and/or a varying potency of BRAF mutations to activate the MAPK pathway [8]. As an example, the p.V600E mutation is a strong pathway activator, whereas the p.D594G is less activating and may still allow responses [58, 59]. It is unlikely that this plays a big role in the study results as described in this review because the majority of patients had BRAFV600E mutations. However, it may explain specific cases of responders. Importantly, preclinical and clinical evidence shows that *BRAF* mutations may sensitize tumors to anti-EGFR treatment when combined with targeted agents such as BRAF and MEK inhibitors [60–62]. The potential of these combinations is currently studied in clinical trials [62, 63]. The use of *BRAF* as predictive marker as discussed in this review therefore only applies to anti-EGFR monotherapy or combined with chemotherapy.

Limitations

The robustness of evidence for *BRAF* mutations as a predictive biomarker is limited by some factors.

Firstly, compared with *KRAS*, *BRAF* mutations may have a less pronounced predictive effect. Whereas patients with *KRAS* exons 2, 3, and 4 mutations have 10%–30% higher risk of progression during treatment with anti-EGFR mAbs compared with control treatment (Tables 3 and 4), the effect of *BRAF* mutations seems smaller. This is based on PFS HRs with a wider CI in patients with *BRAF*mt on anti-EGFR therapy (Table 1) compared with patients with *KRAS* exons 2, 3, and 4 mutations (Table 3). However, it should be noted that the evidence for extended *RAS* testing was based on groups in which patients with *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 mutations were merged. The effect of these mutations are even more convincing if *KRAS* exon 2 mutations are also included (any *RAS* mutation, Table 4). Merging all *RAS* mutations is needed to improve the power of the study. However, when comparing the load of evidence for extended *RAS* with the load of evidence for *BRAF*, it should be taken into account that the group of *BRAF*mt patients is always analyzed separately, resulting in a less evident result. Merging this group with *RAS*mt patients would improve the power, but the relevance is questionable because of the different biology of the mutations.

Secondly, the impact of patient selection by *BRAF* status on OS remains uncertain. Because even in *RAS*wt and *BRAF*wt patients, the OS benefit of anti-EGFR mAbs is not consistently confirmed among different studies (Table 4); OS seems an unreliable endpoint to assess the predictive value of *BRAF* for outcomes on anti-EGFR therapy. Therefore, the effects on PFS should guide decision-making instead of effects on OS. In addition, uncertainties about the predictive value of *BRAF* may be a result of other predictive biomarkers beyond *KRAS* and *BRAF*. Recent evidence highlights the importance of primary sidedness on CRC prognosis and response [12]. Right- and left-sided tumors have a different biological origin, resulting in different molecular characteristics. While left-sided tumors are associated with EGFR overexpression, right-sided tumor more often carry *BRAF* mutations [10, 12] and are associated with poorer response and a shorter survival independent of treatment [11, 64]. Although sidedness has been identified as an independent biomarker [11], the association between sidedness, *KRAS* and *BRAF* status, and response to anti-EGFR mAbs is still to be clarified.

Moreover, cumulating evidence shows that in absence of a confirmed *BRAF* mutation, a similar gene expression profile can be present, referred to as *BRAF*-like tumors. *BRAF*-like tumors have comparable characteristics as *BRAF*mt tumors, leading to treatment resistance by constitutive MAPK-pathway activation independent of EGFR signaling. Future clinical validation studies should reveal whether the evidence for *BRAF* status as a predictive biomarker could become stronger by including *BRAF*-like gene signatures [65].

Future clinical validation studies should reveal whether the evidence for *BRAF* status as a predictive biomarker could become stronger by including *BRAF*-like gene signatures

Conclusion

Recent guidelines recommend upfront extended *RAS* testing in mCRC patients in order to exclude patients with *KRAS* exons 2, 3, and 4 and *NRAS* exons 2, 3, and 4 mutations from therapy with anti-EGFR mAbs. As outlined in this review, the evidence for *BRAF* testing is of an even higher level than the evidence for extended *RAS* testing. Across all studies, no ORR, PFS, or OS benefit could be detected in any *BRAF*mt subgroup. Moreover, significant interactions of *BRAF* status with treatment outcome have been observed. This review highlights that despite limitations in power and effect size, the current evidence should be enough to draw conclusions.

Based on consistent lack of benefit of anti-EGFR mAb therapy in *BRAF*mt patients, it is advised that anti-EGFR mAb therapy is excluded for these patients. The authors therefore encourage ASCO and ESMO to reconsider *BRAF* as a predictive biomarker, as this will help in selecting patients for whom maximum treatment benefit is expected.

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