Precision medicine in colorectal cancer: the molecular profile alters treatment strategies

Nguyen H. Tran, Ludmila L. Cavalcante, Sam J. Lubner, Daniel L. Mulkerin, Noelle K. LoConte, Linda Clipson, Kristina A. Matkowskyj and Dustin A. Deming

Abstract: When considering treatment options for patients with metastatic colorectal cancer (mCRC), molecular profiling has become a pivotal component in guiding clinical decisions. FOLFOX and FOLFIRI (fluorouracil, leucovorin plus oxaliplatin or irinotecan, respectively) are the standard base regimens used for the treatment of mCRC. Biologic agents, such as the epidermal growth factor receptor (EGFR) targeted therapies, cetuximab and panitumumab and the vascular endothelial growth factor monoclonal antibody, bevacizumab, are safe and effective in the first-line setting. The most efficacious use of these agents in terms of timing and selection of the right patient population continues to be debated. Here we review multiple investigations into the effectiveness of treatment options as a function of the mutations present in colon cancers. Early studies have reported that *KRAS* mutations at exon 2 predict resistance to EGFR targeted therapies. More recently the data have expanded to include *KRAS* mutations at exons 3 and 4 and *NRAS* mutations at exons 2, 3 and 4 as well as other biomarkers including *BRAF* and *PIK3CA*, leading to the evolution of the treatment of mCRC to a more precision-based approach. As our understanding of relevant biomarkers increases, and data from both molecular profiling and treatment response become more readily available, treatment options will become more precise and their outcomes more effective.

Keywords: BRAF, cetuximab, KRAS, NRAS, panitumumab, PIK3CA

Introduction

Colorectal cancer remains a leading cause of cancer death with more than 50,000 people dying each year in the United States alone [Siegel *et al.* 2014]. The antimetabolite 5-fluorouracil (5FU) has been the backbone of treatments for metastatic colorectal cancer (mCRC) for many years with addition of leucovorin (LV) in the 1990s. The past two decades have seen improvements in median survival from 10–14 months with 5FU/LV to 16–23 months with addition of oxaliplatin or irinotecan (FOLFOX or FOLFIRI) [Advanced Colorectal Cancer Meta-Analysis Project, 1992; de Gramont *et al.* 2000; Douillard *et al.* 2000; Saltz *et al.* 2000; Fuchs *et al.* 2007]. Current standard of care first-line treatments for mCRC include FOLFOX and FOLFIRI (capecitabine may be substituted for infusional 5FU). Since 2004, targeted therapies alone or in combination

with standard chemotherapies have provided more treatment options and better results. These include the human vascular endothelial growth factor (VEGF) monoclonal antibody, bevacizumab, and the epidermal growth-factor receptor (EGFR) monoclonal antibodies, cetuximab and panitumumab. Additional anti-angiogenic agents, including aflibercept and ramucirumab, have also been approved by the US Food and Drug Administration (FDA) for mCRC.

With the development of multiple pharmaceutical agents for mCRC come numerous questions regarding the most efficacious timing of agents and the patient populations most likely to benefit from these therapies. To investigate which biologic agent (bevacizumab *versus* cetuximab) should be given in the first-line metastatic setting with either FOLFOX or FOLFIRI, the phase III

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Correspondence to:

Dustin A. Deming, MD

Division of Hematology
and Oncology, Department
of Medicine, University of
Wisconsin–Madison School
of Medicine and Public
Health, 600 Highland
Avenue, K6/544, Madison,
WI 53792, USA
[ddeming@medicine.wisc.
edu](mailto:ddeming@medicine.wisc.edu)

Nguyen H. Tran, MD

Division of Hematology
and Oncology, Department
of Medicine, University of
Wisconsin–Madison School
of Medicine and Public
Health, Madison, WI, USA

Ludmila L. Cavalcante, MD

Sam J. Lubner, MD

Daniel L. Mulkerin, MD

Noelle K. LoConte, MD

Division of Hematology

and Oncology, Department

of Medicine, University of

Wisconsin–Madison School

of Medicine and Public

Health, and University of

Wisconsin Carbone Cancer

Center, Madison, WI, USA

Linda Clipson, BA

Department of Oncology,

University of Wisconsin–

Madison, Madison, WI, USA

Kristina A. Matkowskyj,

MD, PhD

University of Wisconsin

Carbone Cancer Center,

and Department of

Pathology and Laboratory

Medicine, University

of Wisconsin–Madison

School of Medicine and

Public Health, and William

S. Middleton Memorial

Veterans Hospital,

Madison, WI, USA

Table 1. Prevalence of KRAS/NRAS mutation.

Study	N	Prevalence of mutation						Total RAS**
		KRAS exon 2 (codon 12 or 13)	KRAS exon 3 (codon 61)	KRAS exon 2 (codon 12 or 13) or 3 (codon 61)	KRAS exon 4 (codon 146)	KRAS exon 3 (codon 61) or 4 (codon 146)	NRAS exon 2, 3, 4	
European consortium De Roock <i>et al.</i> [2010]	747	36.3%	2.1%	38.4%	2.0%	4.1%	2.6% ^{††}	43.0%*
OPUS Bokemeyer <i>et al.</i> 2011, Tejpar <i>et al.</i> [2014]	315	43.0%	3.4% ^{\$}	46.4%	5.3% ^{\$}	8.7%	7.2% ^{\$}	58.9%*
CRYSTAL Ciardiello <i>et al.</i> 2014, Van Cutsem <i>et al.</i> [2011]	1063	37.3%	2.1% ^{\$}	39.4%	3.5% ^{\$}	5.6%	4.5% ^{\$}	47.4%*
20050181 Peeters <i>et al.</i> [2014]	1083	44.9%	2.3% ^{\$}	47.2%	4.2% ^{\$}	6.5%	4.2% ^{\$}	55.6%*
PRIME Douillard <i>et al.</i> [2013]	1096	40.0%	2.3%**	42.3%	3.4%**	5.7%	4.5%**	52.0%*
PICCOLO Seymour <i>et al.</i> [2013]	400	NA	NA	NA	3.7% ^{†‡\$}	NA	6.8% ^{§§\$}	Insufficient data
20020408 Patterson <i>et al.</i> [2013]	427	43.1%	2.7% ^{\$}	45.8%	2.8% ^{\$}	5.5%	4.7% ^{\$}	53.3%*
PEAK Schwartzberg <i>et al.</i> [2014]	NA	NA	4% [#]	NA	7.6% [#]	11.6%	11.2% [#]	Insufficient data
COIN Maughan <i>et al.</i> [2011]	1316	NA	NA	43%	NA	NA	3.8% [§]	46.8%*
Chui <i>et al.</i> [2014]	190	35%	NA	NA	NA	5%	4.0%	44%
Shinozaki <i>et al.</i> [2014]	1001	38%	NA	NA	NA	4.6%	3.5%	46.1%

*Assuming distribution of un-evaluable sample in study is the same as the overall distribution of RAS mutants.
^{\$}Ex: KRAS exon 2 wt made up 56.8% of overall evaluable population and KRAS exon 3 mutation was 5.9% of KRAS exon 3 mutant made up 3.4% of the overall evaluable population.
[†]Denominator derived from KRAS exon 2, 3 (codon 12 or 13, codon 61) wild type minus patients with undetermined KRAS status
[‡]Did not examine exon 4
^{||}Does not include mutant KRAS exon 4 and NRAS exon 4
[#]Incidence of simultaneous mutations were negligible
[§]Percent derived from KRAS wild type exon 2 population
^{**}Denominator is 1060
^{§§}Denominator is 428
^{††}17/644
NA, not available.

multicenter prospective Cancer and Leukemia Group B (CALGB)/SWOG 80405 clinical trial was performed [Venook *et al.* 2014]. This study was initiated in 2004 with recently completed data involving 1137 *KRAS* wildtype (at codons 12 and 13) patients receiving chemotherapy (FOLFOX or FOLFIRI) and randomized to either cetuximab or bevacizumab. Preliminary results indicated no difference in overall survival (OS) or serious toxicity whether patients received chemotherapy/cetuximab or chemotherapy/bevacizumab. This study demonstrates, through one of the longest median OS rates in mCRC to date at ~29 months, that our ability to treat patients with this disease is continuing to improve.

Recent data, reviewed below, indicate predictive and prognostic benefits to extended-spectrum *RAS* testing along with *BRAF* and potentially *PIK3CA* mutation profiling. Further analysis from CALGB/SWOG 80405 and other similar studies with extended mutation profiling have yielded further information pertaining to other biomarkers along the EGFR pathway, including *KRAS*, *NRAS*, *BRAF* and *PIK3CA*. Mutations along these pathways have been shown to alter anti-EGFR therapies. The full mechanisms and biology remain unclear and current research is now focused on biomarkers involved in this process. Here we present an updated summary of these biomarkers and discussion of treatment strategies in mCRC.

KRAS/NRAS

KRAS and *NRAS* belong to the same *RAS* family of oncogenes. The most common *KRAS* mutations are found in exon 2 (codon 12 or 13) (see Table 1). Numerous studies have confirmed the presence of *KRAS* mutations at exon 2 as a predictor of resistance to anti-EGFR therapies [Lievre *et al.* 2006, 2008; Benvenuti *et al.* 2007; Di Fiore *et al.* 2007; Van Cutsem *et al.* 2009; De Roock *et al.* 2010; Bokemeyer *et al.* 2011; Douillard *et al.* 2013]. It is currently standard of care to test tumor samples for *KRAS* exon 2 mutations, as this has been demonstrated to be a cost-effective means to predict resistance to these therapies.

Among *KRAS* exon 2 wildtype patients, as many as 65% are resistant to EGFR monoclonal antibodies [Allegra *et al.* 2009], necessitating a further search for other biomarkers responsible for this resistance (see Table 2). In a retrospective

European consortium analysis, De Roock and colleagues analyzed tumor samples from a large cohort of patients with chemotherapy-refractory mCRC treated with cetuximab and chemotherapy [De Roock *et al.* 2010]; 40% of evaluable samples harbored *KRAS* mutations, most commonly at codons 12 or 13 (exon 2) with 2.1% at codon 61 (exon 3) and 2% at codon 146 (exon 4). Among those treated with cetuximab plus chemotherapy, *KRAS* mutation in any of these codons was shown to portend a highly significant lower response rate (RR), and shorter median progression-free survival (PFS) and OS. *NRAS* mutations were found in 2.6% of evaluable samples, mostly in codon 61, and were mutually exclusive of *KRAS* mutations. *NRAS* mutant cancers had a significantly lower RR when treated with chemotherapy and cetuximab; lower PFS and OS were not statistically significant perhaps owing to the low sample size of *NRAS* mutants.

In the OPUS clinical study, efficacy of cetuximab in combination with FOLFOX4 as first-line treatment for mCRC was assessed according to biomarkers status [Bokemeyer *et al.* 2011]. *KRAS* mutations were assessed at exon 2 at codons 12 or 13 with 93% mutational status known (315/337). When treated with FOLFOX4/cetuximab *versus* FOLFOX alone, the *KRAS* exon 2 wildtype population had a better RR and median PFS. Among the *KRAS* exon 2 mutant population, outcomes were reversed; adding cetuximab to FOLFOX4 resulted in worse RR and shorter PFS. OS was not significantly affected in either population. Further analysis of patients with *KRAS* exon 2 wildtype cancers in the OPUS study demonstrated that other *KRAS* and *NRAS* mutations led to resistance to anti-EGFR therapies [Tejpar *et al.* 2014]. Among those with extended spectrum *RAS* mutations (*KRAS* mutations at exons 2, 3 and 4, and *NRAS* mutations at exons 2, 3 and 4), there was no benefit to the addition of cetuximab to FOLFOX4 in RR, PFS or OS compared with FOLFOX4 alone. Interestingly, median OS was shorter in those treated with cetuximab when a *RAS* mutation was present (though not statistically significant).

A revisit of the CRYSTAL study to assess for other *RAS* mutations found similar results [Van Cutsem *et al.* 2015]. New *RAS* mutations (*KRAS* exons 3 and 4, and *NRAS* exons 2, 3 and 4) were further assessed in previous *KRAS* exon 2 wildtype cancers treated with FOLFIRI/cetuximab *versus* FOLFIRI alone. The presence of new

Table 2. RAS mutational status as a predictor of response and survival with anti-EGFR therapy.

Study	RAS status	n	Treatment	RR		PFS		OS	
				%	p value*	Median (months)	p value*	Median (months)	p value*
CALGB/SWOG 80405 ESMO [2014], Lenz <i>et al.</i> [2014], Venook <i>et al.</i> [2014] European consortium De Roock <i>et al.</i> [2010]	KRAS wt exon 2	559	(FOLFOX or FOLFIRI)/bevacizumab	NA	NA	10.84	0.55	29.0	0.34
	RAS wt at all loci	578	(FOLFOX or FOLFIRI)/cetuximab			10.45		29.9	
		256	(FOLFOX or FOLFIRI)/bevacizumab	53.6	<0.01	NA	NA	31.2	0.40
		270	(FOLFOX or FOLFIRI)/cetuximab	68.6		NA		32.0	
	KRAS mut exon 2, 3, 4	253	Chemotherapy/cetuximab	6.7	<0.0001	2.8	<0.0001	7.4	<0.0001
	KRAS wt exon 2, 3, 4	352		35.8		5.5		11.5	
	NRAS mut exon 2, 3, 4	13	Chemotherapy/cetuximab	7.7	0.013	3.5	0.055	8.8	0.051
	NRAS wt exon 2, 3, 4	289		38.1		6.5		11.5	
	KRAS wt exon 2	82	FOLFOX4/cetuximab	57	0.0027	8.3	0.0064	22.8	0.39
		97	FOLFOX4	34		7.2		18.5	
OPUS Bokemeyer <i>et al.</i> [2011], Tejpar <i>et al.</i> [2014]	KRAS mut exon 2	77	FOLFOX4/cetuximab	34	0.0290	5.5	0.015	13.4	0.20
		59	FOLFOX4	53		8.6		17.5	
	KRAS mut exon 3, 4	17	FOLFOX4/cetuximab	47.1	0.57	7.3	0.96	14.8	0.41
	NRAS mut exon 2, 3, 4	19	FOLFOX4	36.8		7.4		17.8	
	RAS mut at any exon	94	FOLFOX4/cetuximab	36.2	0.11	5.6	0.018	13.4	0.089
		78	FOLFOX4	48.7		7.8		17.8	
	RAS wt at all exon	36	FOLFOX4/cetuximab	61.1	0.008	12	0.018	20.7	0.50
		46	FOLFOX4	30.4		5.8		17.8	
	KRAS mut exon 3, 4	32	FOLFIRI/cetuximab	34.4	0.97	7.2	0.56	18.2	0.50
	NRAS mut exon 2, 3, 4	31	FOLFIRI	35.5		6.9		20.7	
CRYSTAL Van Cutsem <i>et al.</i> [2015] 20050181 Peeters <i>et al.</i> [2014]	RAS mut at any exon	246	FOLFIRI/cetuximab	31.7	0.40	7.4	0.47	16.4	0.64
		214	FOLFIRI	36		7.5		17.7	
	RAS wt at all loci	178	FOLFIRI/cetuximab	66.3	<0.0001	11.4	0.0002	28.4	0.0024
		189	FOLFIRI	38.6		8.4		20.2	
	RAS mut at any loci	299	FOLFIRI/panitumumab	NA	NA	4.8	0.14	11.8	0.34
		294	FOLFIRI			4.0		11.1	
	RAS wt at all loci	208	FOLFIRI/panitumumab	NA	NA	6.4	0.007	16.2	0.08
		213	FOLFIRI			4.6		13.9	

(Continued)

Table 2. (Continued)

Study	RAS status	n	Treatment	RR		PFS		OS	
				%	p value*	Median (months)	p value*	Median (months)	p value*
PRIME Douillard <i>et al.</i> [2013]	KRAS wt exon 2 with other RAS mut	51	FOLFOX4/panitumumab	NA	NA	7.3	0.33	17.1	0.12
		57	FOLFOX4			8.0		17.8	
	RAS mut at any loci	272	FOLFOX4/panitumumab	NA	NA	7.3	0.008	15.5	0.001
		276	FOLFOX4			8.7		18.7	
	RAS wt at all loci	259	FOLFOX4/panitumumab	NA	NA	10.1	0.004	25.8	0.009
PLANET Abad <i>et al.</i> [2014]		253	FOLFOX4			7.9		20.2	
	KRAS wt exon 2	38	FOLFOX4/panitumumab	73.7	NA	12.5	0.943	32.5	0.848
		39	FOLFIRI/panitumumab	66.7	NA	12.6		42.4	
	RAS mut in any foci	NA	FOLFOX4/panitumumab	50.0	NA	NA	NA	NA	NA
		NA	FOLFIRI/panitumumab	57.1	NA				
PEAK Schwartzberg <i>et al.</i> [2014]	RAS wt at all loci	NA	FOLFOX4/panitumumab	77.8	NA	12.8	0.621	39.0	0.935
		NA	FOLFIRI/panitumumab	73.1	NA	14.8		45.8	
	KRAS wt exon 2	142	mFOLFOX6/panitumumab	NA	NA	10.9	0.353	34.2	0.009
		143	mFOLFOX6/bevacizumab			10.1		24.3	
	RAS wt at all loci	88	mFOLFOX6/panitumumab	NA	NA	13	0.029	41.3	0.058
		82	mFOLFOX6/bevacizumab			9.5		28.9	

*Compared to row immediately below. EGFR, epidermal growth factor receptor; mut, mutation; NA, not available; OS, overall survival; PFS, progression-free survival; RR, relative risk; wt, wildtype.

RAS mutants made no difference in RR, PFS or OS. Similar results were found when all *RAS* mutations were combined. In contrast, the *RAS* wildtype population demonstrated highly significant improvements in RR, PFS and OS when treated with FOLFIRI/cetuximab compared with FOLFIRI alone.

The results of the CALGB/SWOG 80405 extended spectrum *RAS* testing have now been presented [ESMO, 2014; Venook *et al.* 2014]. In the *RAS* wildtype population, the median OS was 31.2 months in the chemotherapy plus bevacizumab arm and 32.0 months in the chemotherapy plus cetuximab arm (no significant difference). No difference in PFS was observed. A significant improvement in the RR was seen in the cetuximab arm for the *RAS* wildtype population (Table 2).

Multiple additional studies have also confirmed similar benefits in different patient populations [Douillard *et al.* 2013; Abad *et al.* 2014; Peeters *et al.* 2014; Schwartzberg *et al.* 2014].

Peeters and colleagues recently provided an update on *RAS* and *BRAF* status from study 20050181 investigating the addition of panitumumab to FOLFIRI [Peeters *et al.* 2014]. Among all *RAS* wildtype (*KRAS* at exons 2, 3 and 4, and *NRAS* at exons 2, 3 and 4) patients, benefits were observed in PFS when treated with FOLFIRI / panitumumab *versus* FOLFIRI alone as second-line treatment. Similar to results in other studies, the addition of an anti-EGFR therapy to standard chemotherapy provided no benefit in the presence of an extended-spectrum *RAS* mutation. In another study performed by Douillard and colleagues of the PRIME data [Douillard *et al.* 2013], *RAS* mutations were assessed in patients treated with FOLFOX4 with and without panitumumab. Among those with a *RAS* mutation other than at *KRAS* exon 2 (*KRAS* at exons 3 and 4, and *NRAS* at exons 2, 3 and 4) treated with FOLFOX4/panitumumab *versus* FOLFOX4 alone, there was no difference in PFS or OS. Even more compelling, complete *RAS* mutation analysis (all *KRAS* at exons 2, 3 and 4, and *NRAS* mutations at exons 2, 3 and 4) showed those treated with chemotherapy and an anti-EGFR therapy had a significantly shorter median PFS and OS. Having no *RAS* mutations treated with FOLFOX4/panitumumab conferred a longer median PFS and OS compared with FOLFOX4 alone.

BRAF

BRAF is an oncogene in the *RAF* gene family that encodes a serine-threonine protein kinase found in the RAS-RAF-MAPK cascade. Approximately 10% of colorectal cancer harbors a *BRAF* mutation, though this number is highly variable depending on the study population [Davies *et al.* 2002; Samowitz *et al.* 2005; Di Nicolantonio *et al.* 2008]. The most significant and prevalent mutation occurs at the kinase domain from a single substitution V600E. Numerous clinical studies have suggested the presence of this mutation as a predictor of resistance to anti-EGFR therapies [Di Nicolantonio *et al.* 2008; Laurent-Puig *et al.* 2009; De Roock *et al.* 2010; Bokemeyer *et al.* 2012] and a significant marker of poor prognosis [Di Nicolantonio *et al.* 2008; Laurent-Puig *et al.* 2009; Richman *et al.* 2009; Bokemeyer *et al.* 2012; Tveit *et al.* 2012] (see Table 3). In one study [De Roock *et al.* 2010], *BRAF* mutant tumors had a significantly lower RR compared with wildtype cancers when treated with an anti-EGFR therapy as well as shorter PFS and OS. Similarly, in a retrospective analysis of *RAS* and *BRAF* mutation status of PRIME data [Douillard *et al.* 2013], patients with neither *RAS* nor *BRAF* mutations showed significantly better OS and PFS when treated with FOLFOX4/panitumumab compared with FOLFOX4 alone. The presence of *BRAF* mutations in *RAS* wildtype patients resulted in a worse outcome. Treatment with anti-EGFR therapy did not significantly improve median PFS or OS. Having a *BRAF* V600E mutation portends a poor prognosis regardless of treatment group. In previous pooled data from the OPUS and CRYSTAL studies [Bokemeyer *et al.* 2012], a *BRAF* mutation led to overall decreased PFS and OS compared with wildtype tumors irrespective of treatment groups. FOLFIRI/panitumumab *versus* FOLFIRI alone was examined in the second-line setting by Peeters and colleagues [Peeters *et al.* 2014]. The presence of a *BRAF* mutation resulted in no significant differences in PFS or OS whether patients were treated with FOLFIRI/panitumumab or FOLFIRI alone, indicating that *BRAF* mutations may confer EGFR therapy resistance, although this study was not powered to definitively for this purpose.

PIK3CA

Phosphoinositide 3-kinase (PI3K) is a lipid kinase heterodimeric in nature consisting of regulatory and catalytic subunits. It is important for multiple

Table 3. *BRAF* mutational status as a predictor of response and survival with anti-EGFR therapy.

Study	* <i>BRAF</i> status exon 15 codon 600	<i>n</i>	Treatment	RR		PFS		OS	
				%	<i>p</i> value ^{\$}	Median (months)	<i>p</i> value ^{\$}	Median (months)	<i>p</i> value ^{\$}
Di Nicolantonio <i>et al.</i> [2008]	<i>BRAF</i> mut <i>BRAF</i> wt	11 68	(Cetuximab or panitumumab) ± chemotherapy	0 3	0.029	NR	0.011	NR	<0.0001
Laurent-Puig <i>et al.</i> [2009]	<i>BRAF</i> mut <i>BRAF</i> wt	5 110	Cetuximab ± chemotherapy	0 46.8	0.063	1.8 7.2	0.001	6.5 14.8	0.001
European consortium De Roock <i>et al.</i> [2010]	<i>BRAF</i> mut <i>BRAF</i> wt	24 326	Cetuximab ± chemotherapy	8.3 38.0	0.0012	1.8 6.0	<0.0001	6.0 12.5	<0.0001
Bokemeyer <i>et al.</i> [2012]	<i>BRAF</i> mut <i>BRAF</i> wt	32 38	Chemotherapy/cetuximab Chemotherapy	21.9 13.2	0.46	7.1 3.7	0.23	14.1 9.9	0.076
Tveit <i>et al.</i> [2012]	<i>BRAF</i> mut <i>BRAF</i> wt	349 381	Chemotherapy/cetuximab Chemotherapy	60.7 40.9	<0.0001	10.9 7.7	<0.0001	24.8 21.1	0.048
PRIME Douillard <i>et al.</i> [2013]	<i>BRAF</i> mut <i>BRAF</i> wt	55 402	NA FOLFOX4/panitumumab	20 50	<0.001	5.1 8.3	<0.001	9.5 22.0	<0.001
20050181 Peeters <i>et al.</i> [2014]	<i>BRAF</i> mut <i>BRAF</i> wt	24 29 228 218 22 23 186 190	FOLFOX4/panitumumab FOLFOX4 FOLFOX4/panitumumab FOLFOX4 FOLFIR/panitumumab FOLFIRI FOLFIR/panitumumab FOLFIRI	NA NA NA NA NA NA NA NA	NA NA NA NA NA NA NA NA	6.1 5.4 10.8 9.2 2.5 1.8 6.9 5.5	0.12 0.002 0.34 0.006	10.5 9.2 28.3 20.9 4.7 5.7 18.7 15.4	0.76 0.02 0.20 0.15

*All RAS wildtype. \$Compared to row immediately below.

Chemo, chemotherapy; EGFR, epidermal growth factor receptor; NA, not available; NR, not reached; mut, mutation; OS, overall survival; PFS, progression-free survival; RR, relative risk; wt, wildtype.

cellular processes including cell growth, proliferation, survival and apoptosis. PI3K is downstream of EGFR signaling and activation of this pathway might lead to resistance to anti-EGFR therapies. The *PIK3CA* gene encodes the catalytic subunit, p110a, and when mutated results in a constitutively active PI3K. *PIK3CA* mutations occur in 10–20% of colorectal cancers [Barault *et al.* 2008; Prenen *et al.* 2009; Sartore-Bianchi *et al.* 2009; De Roock *et al.* 2010]. Exons 9 and 20 are responsible for more than 80% of *PIK3CA* mutations in colorectal cancer [Samuels *et al.* 2004]. To date, the clinical data are still unclear regarding whether the presence of *PIK3CA* mutation is predictive of response to EGFR-directed therapies.

Sartore-Bianchi and colleagues examined 110 patients with mCRC treated with either panitumumab or cetuximab [Sartore-Bianchi *et al.* 2009]. Of patients carrying *PIK3CA* mutations (13.6%; 15/110), of which the majority (11/15) were located at exon 20, and 4 of 15 at exon 9, 0/15 patients with *PIK3CA* mutation responded to anti-EGFR therapies compared with wildtype ($p = 0.038$). Further, PFS was noted to be significantly lower ($p = 0.0035$). The authors concluded that *PIK3CA* mutations may be an independent predictor of resistance to anti-EGFR. Other studies have similar results [Perrone *et al.* 2009; Sood *et al.* 2012]. In contrast, Prenen and colleagues found no such association [Prenen *et al.* 2009].

PIK3CA and *KRAS* status were assessed in 200 chemotherapy-refractory mCRC patients subsequently treated with cetuximab as a monotherapy or in combination with irinotecan. A total of 23 (12%) of 200 carried *PIK3CA* mutations, of which the majority were found on exon 9. There were no differences in *PIK3CA* mutation status among responders and nonresponders (5/39 *versus* 18/160, $p = 0.781$). Furthermore, there were no differences in median PFS (24 *versus* 18 weeks; $p = 0.760$) and OS (45 *versus* 39 weeks; $p = 0.698$) when comparing mutant with wildtype tumors. In the European consortium, a similar prevalence of *PIK3CA* mutations, 14.5%, was found [De Roock *et al.* 2010]. *PIK3CA* mutations at exon 20 were associated with lack of response to cetuximab whereas mutations in exon 9 were not. This indicates the potential for varying clinical implications depending upon whether *PIK3CA* is mutated in the helical or kinase domain. This would be unexpected since mutations in either domain result in the constitutive activation of PI3K. In addition, most studies to date have

looked at exons 9 and 20 alone, as they account for the majority of the mutations. Other mutations at different sites may play a role. Clearly, further investigations are needed to clarify the role of *PIK3CA* mutations as predictive biomarker for the treatment of mCRC patients with anti-EGFR therapies.

Discussion

The treatment paradigm for mCRC is rapidly shifting to a more personalized or precision-based approach. Molecular biomarkers now play an increasingly important role in making decisions about targeted therapies. Mutational analysis of genes encoding proteins downstream of EGFR have allowed for the development of biomarkers predicting resistance to anti-EGFR therapies. These same mutations have not been predictive of benefit from anti-VEGF therapies. *KRAS* exon 2 testing for patients with mCRC was recommended by the European Society of Pathology in 2008 and the American Society for Clinical Oncology (ASCO) in 2009 [van Krieken *et al.* 2008; Allegra *et al.* 2009]. Data on *KRAS* mutations at exon 2 prompted changes by the FDA in 2009 to the approval of anti-EGFR therapies, recommending that it be used only in patients with mCRC without mutations at codons 12 or 13.

The data presented here indicate that any mutation of *KRAS* at exons 2, 3 and 4 or *NRAS* at exons 2, 3 and 4 confers a poor response to anti-EGFR therapy [De Roock *et al.* 2010; Bokemeyer *et al.* 2011; Douillard *et al.* 2013; Peeters *et al.* 2014; Schwartzberg *et al.* 2014; Tejpar *et al.* 2014; Van Cutsem *et al.* 2015]. Conversely, tumors that are wildtype at all loci when treated with anti-EGFR therapies demonstrated significant benefit with extended OS [De Roock *et al.* 2010; Bokemeyer *et al.* 2011; Douillard *et al.* 2013; Abad *et al.* 2014; Peeters *et al.* 2014; Schwartzberg *et al.* 2014; Tejpar *et al.* 2014; Van Cutsem *et al.* 2015]. In addition, *BRAF* mutations are an indicator of poor prognosis and appear to also lead to cetuximab and panitumumab resistance [Di Nicolantonio *et al.* 2008; Laurent-Puig *et al.* 2009; Richman *et al.* 2009; De Roock *et al.* 2010; Bokemeyer *et al.* 2012; Tveit *et al.* 2012; Douillard *et al.* 2013; Peeters *et al.* 2014]. These data have led the National Comprehensive Cancer Network (NCCN) to alter its guidelines, which now state that all patients with mCRC should have their tumors tested for *KRAS* (exons 2–4), *NRAS* (exons 2–4) and *BRAF* mutations. Anti-EGFR

Table 4. Clinical use of CRC mutational profiling.

Mutation	Anti-EGFR therapy
<i>KRAS</i> (exons 2, 3, 4)	Not indicated
<i>NRAS</i> (exons 2, 3, 4)	Not indicated
<i>BRAF</i> (V600E)	Not indicated
<i>PIK3CA</i>	Consider in second or greater line of therapy
All wildtype	Consider in first, second or greater line of therapy
EGFR, epidermal growth factor receptor.	

therapies should not be utilized for patients with *RAS* or *BRAF* mutations due to the lack of benefit in all lines of therapy outside of a clinical trial. There is not currently evidence that these mutations significantly alter the response to the approved anti-angiogenic agents bevacizumab, aflibercept, ramucirumab and regorafenib.

There are insufficient data regarding *PIK3CA* mutations to make any clear conclusion about their effect on response to anti-EGFR therapies. However, due to the concern that activation of the PI3K signaling cascade can result in continued proliferative signaling independent of inhibition of EGFR, continued efforts should be made to better understand the role of *PIK3CA* mutations in mCRC and its influence on treatment response.

The timing of when to incorporate EGFR-directed therapies for patients with wildtype *KRAS*, *NRAS* and *BRAF* is still being debated. With the currently available data, first-line treatment with anti-EGFR agents in combination FOLFOX or FOLFIRI should be considered for all patients with *KRAS*, *NRAS* and *BRAF* wildtype mCRCs (Table 4). The toxicities of anti-EGFR therapies will also need to be considered for this setting, since some patients do find the acneiform rash, fatigue, nausea and diarrhea that occur with these agents can have a negative impact on their quality of life. Until further information is available, the use of cetuximab or panitumumab for the treatment of *PIK3CA* mutant mCRCs might be best used in the treatment-refractory.

The advances in our understanding of how to utilize the mutation profile to tailor therapies for mCRC outlined in this review demonstrate the critical value molecular profiling plays in the interpretation of clinical trials. Concerted efforts are

required to acquire molecular information in conjunction with treatment response data in publically accessible databases. High-quality large-volume data sets will continue to become more important as each molecular subtype of cancer becomes less common. Further investigations are needed not only to look for other markers of resistance, but to also identify biomarkers predictive of treatment sensitivity. This is an exciting time in the treatment of many cancers, as routine DNA sequencing of patient samples has allowed for rapid advances in the realization of precision medicine.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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References

- Abad, A., Massuti, B., Gravalos, C., Escudero, P., Guillen-Ponce, C., Layos, L. *et al.* (2014) Panitumumab plus FOLFOX-4 or panitumumab plus FOLFIRI in subjects with wild-type *KRAS* (exon 2) colorectal cancer and multiple or unresectable liver-limited metastases: data from the randomized, phase II PLANET study. *Ann Oncol* 25: ii7–ii18.
- Advanced Colorectal Cancer Meta-Analysis Project (1992) Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. *J Clin Oncol* 10: 896–903.
- Allegra, C., Jessup, J., Somerfield, M., Hamilton, S., Hammond, E., Hayes, D. *et al.* (2009) American Society of Clinical Oncology provisional clinical opinion: testing for *KRAS* gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 27: 2091–2096.
- Barault, L., Veyrie, N., Jooste, V., Lecorre, D., Chapusot, C., Ferraz, J. *et al.* (2008) Mutations in the *RAS*-*MAPK*, *PI(3)K* (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer* 122: 2255–2259.
- Benvenuti, S., Sartore-Bianchi, A., Di Nicolantonio, F., Zanon, C., Moroni, M., Veronese, S. *et al.* (2007)

- Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res* 67: 2643–2648.
- Bokemeyer, C., Bondarenko, I., Hartmann, J., de Braud, F., Schuch, G., Zubel, A. *et al.* (2011) Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 22: 1535–1546.
- Bokemeyer, C., Van Cutsem, E., Rougier, P., Ciardiello, F., Heeger, S., Schlichting, M. *et al.* (2012) Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. *Eur J Cancer* 48: 1466–1475.
- Ciardiello, F., Lenz, H., Kohne, C., Heinemann, V., Tejpar, S., Esser, R. *et al.* (2014) Effect of KRAS and NRAS mutational status on first-line treatment with FOLFIRI plus cetuximab in patients with metastatic colorectal cancer (mCRC): New results from the CRYSTAL trial. *J Clin Oncol* 32(no 3 Suppl.): abstr LBA443.
- Chiu, J., Bedard, P., Roehrl, M., Serra, S., Kryzanowska, M., Knox, J. *et al.* (2014) Molecular profiling of patients (pts) with advanced colorectal cancer (CRC): Princess Margaret Cancer Centre experience. *J Clin Oncol* 32(no 15 Suppl.): abstr 3572.
- Davies, H., Bignell, G., Cox, C., Stephens, P., Edkins, S., Clegg, S. *et al.* (2002) Mutations of the BRAF gene in human cancer. *Nature* 417: 949–954.
- De Gramont, A., Figer, A., Seymour, M., Homerin, M., Hmissi, A., Cassidy, J. *et al.* (2000) Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 18: 2938–2947.
- De Roock, W., Claes, B., Bernasconi, D., De Schutter, J., Biesmans, B., Fountzilas, G. *et al.* (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 11: 753–762.
- Di Fiore, F., Blanchard, F., Charbonnier, F., Le Pessot, F., Lamy, A., Galais, M. *et al.* (2007) Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. *Br J Cancer* 96: 1166–1169.
- Di Nicolantonio, F., Martini, M., Molinari, F., Sartore-Bianchi, A., Arena, S., Saletti, P. *et al.* (2008) Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 26: 5705–5712.
- Douillard, J., Cunningham, D., Roth, A., Navarro, M., James, R., Karasek, P. *et al.* (2000) Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 355: 1041–1047.
- Douillard, J., Oliner, K., Siena, S., Tabernero, J., Burkes, R., Barugel, M. *et al.* (2013) Panitumumab–FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 369: 1023–1034.
- European Science for Medical Oncology (ESMO) (2014) *Optimal treatment strategy with anti-EGFR or anti-VEGF treatments in first-line RAS wild type metastatic colorectal cancer patients*. Available at: <http://www.esmo.org/Conferences/Past-Conferences/ESMO-2014-Congress/News-Articles/Results-From-the-CALGB-SWOG-80405-and-FIRE-3-AIO-KRK-0306-Studies-In-All-RAS-Wild-Type-Population> (accessed 18 June 2015).
- Fuchs, C., Marshall, J., Mitchell, E., Wierzbicki, R., Ganju, V., Jeffery, M. *et al.* (2007) Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. *J Clin Oncol* 25: 4779–4786.
- Laurent-Puig, P., Cayre, A., Manceau, G., Buc, E., Bachet, J., Lecomte, T. *et al.* (2009) Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol* 27: 5924–5930.
- Lenz, H., Niedzwiecki, D., Innocenti, F., Blanke, D., Mahony, M., O’Neil, B. *et al.* (2014) CALGB/SWOG 80405: Phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with expanded ras analyses untreated metastatic adenocarcinoma of the colon or rectum (mCRC). *Ann Oncol* 25(Suppl. 5): v1–v41.
- Lievre, A., Bachet, J., Boige, V., Cayre, A., Le Corre, D., Buc, E. *et al.* (2008) KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 26: 374–379.
- Lievre, A., Bachet, J., Le Corre, D., Boige, V., Landi, B., Emile, J. *et al.* (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 66: 3992–3995.
- Maughan, T., Adams, R., Smith, C., Meade, A., Seymour, M., Wilson, R. *et al.* (2011) Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 377: 2103–2114.
- Patterson, S., Peeters, M., Siena, S., Van Cutsem, E., Humblet, Y., Van Laethem, J. *et al.* (2013) Comprehensive analysis of KRAS and NRAS mutations as predictive biomarkers for single agent

- panitumumab (pmab) response in a randomized, phase III metastatic colorectal cancer (mCRC) study (20020408). *J Clin Oncol* 31(15 Suppl.): abstract 3617.
- Peeters, M., Oliner, K., Price, T., Cervantes, A., Sobrero, A., Ducreux, M. *et al.* (2014) KRAS/ NRAS and BRAF mutations in the 20050181 study of panitumumab plus FOLFIRI for the 2nd-line treatment of metastatic colorectal cancer: updated analysis. *Ann Oncol* 25(Suppl. 2): ii5–ii13.
- Perrone, F., Lampis, A., Orsenigo, M., Di Bartolomeo, M., Gevorgyan, A., Losa, M. *et al.* (2009) PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. *Ann Oncol* 20: 84–90.
- Prenen, H., De Schutter, J., Jacobs, B., De Roock, W., Biesmans, B., Claes, B. *et al.* (2009) PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. *Clin Cancer Res* 15: 3184–3188.
- Richman, S., Seymour, M., Chambers, P., Elliott, F., Daly, C., Meade, A. *et al.* (2009) 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. *J Clin Oncol* 27: 5931–5937.
- Saltz, L., Cox, J., Blanke, C., Rosen, L., Fehrenbacher, L., Moore, M. *et al.* (2000) Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 343: 905–914.
- Samowitz, W., Sweeney, C., Herrick, J., Albertsen, H., Levin, T., Murtaugh, M. *et al.* (2005) Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res* 65: 6063–6069.
- Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S. *et al.* (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304: 554.
- Sartore-Bianchi, A., Martini, M., Molinari, F., Veronese, S., Nichelatti, M., Artale, S. *et al.* (2009) PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res* 69: 1851–1857.
- Schwartzberg, L., Rivera, F., Karthaus, M., Fasola, G., Canon, J., Hecht, J. *et al.* (2014) PEAK: a randomized, multicenter phase II study of panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6 in patients with previously untreated, unresectable, wild-type KRAS exon 2 metastatic colorectal cancer. *J Clin Oncol* 32: 2240–2247.
- Seymour, M., Brown, S., Middleton, G., Maughan, T., Richman, S., Gwyther, S. *et al.* (2013) Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. *Lancet Oncol* 14: 749–759.
- Shinozaki, E., Miki, Y., Ueno, M., Igarashi, M., Suenaga, K. *et al.* (2014) Molecular profiling of EGFR pathway according to location of colorectal cancer (CRC): analysis of 1001 patients in single institute. *J Clin Oncol* 32(no 15 Suppl.): abstr 3597.
- Siegel, R., Ma, J., Zou, Z. and Jemal, A. (2014) Cancer statistics, 2014. *CA Cancer J Clin* 64: 9–29.
- Sood, A., McClain, D., Maitra, R., Basu-Mallick, A., Seetharam, R., Kaubisch, A. *et al.* (2012) PTEN gene expression and mutations in the PIK3CA gene as predictors of clinical benefit to anti-epidermal growth factor receptor antibody therapy in patients with KRAS wild-type metastatic colorectal cancer. *Clin Colorectal Cancer* 11: 143–150.
- Tejpar, S., Lenz, H., Kohne, C., Heinemann, V., Ciardiello, F., Esser, R. *et al.* (2014) Effect of KRAS and NRAS mutations on treatment outcomes in patients with metastatic colorectal cancer (mCRC) treated first-line with cetuximab plus FOLFOX4: new results from the OPUS study. *J Clin Oncol* 32(Suppl. 3): abstract LBA444.
- Tveit, K., Guren, T., Glimelius, B., Pfeiffer, P., Sorbye, H., Pyrhonen, S. *et al.* (2012) Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. *J Clin Oncol* 30: 1755–1762.
- Van Cutsem, E., Kohne, C., Hitre, E., Zaluski, J., Chang Chien, C., Makhson, A. *et al.* (2009) Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 360: 1408–1417.
- Van Cutsem, E., Lenz, H., Kohne, C., Heinemann, V., Tejpar, S., Melezinek, I. *et al.* (2015) Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol* 33: 692–700.
- van Krieken, J., Jung, A., Kirchner, T., Carneiro, F., Seruca, R., Bosman, F. *et al.* (2008) KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program. *Virchows Arch* 453: 417–431.
- Venook, A., Niedzwiecki, D., Lenz, H., Innocenti, F., Mahoney, M., O'Neil, B. *et al.* (2014) CALGB/ SWOG 80405: Phase III trial of irinotecan/5-FU/ leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with KRAS wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (mCRC). *J Clin Oncol* 32: abstract LBA3.