Review Article

Extended *RAS* testing in metastatic colorectal cancer—Refining the predictive molecular biomarkers

Humaid O. Al-Shamsi[1], Waleed Alhazzani[2], Robert A. Wolff[1]

[1]Department of Gastrointestinal Medical Oncology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, USA; [2]Department of Gastroenterology, Internal Medicine, McMaster University, Hamilton, Ontario, Canada

Correspondence to: Humaid O. Al-Shamsi, MD, FRCPC, FACP. Department of Gastrointestinal Medical Oncology, Department of Gastrointestinal Medical Oncology, Unit 426 1515 Holcombe Blvd, Houston, TX 77030, USA. Email: halshamsi@mdanderson.org.

Abstract: Mutations of exon 2 of *Kirsten rat sarcoma viral oncogene homologue* (*KRAS*) (exon 2 codons 12/13) lead to constitutive activation of the EGFR (epidermal growth factor receptor) mediated signal transduction pathway and been shown to be a negative predictive biomarker for EGFR-directed monoclonal antibodies among patients with colorectal cancer (CRC). As selection of patients is very important for administration of anti-EGFR therapy, this lone biomarker has proved to be insufficient for selecting the appropriate patients as more patients lacking exon 2 *KRAS* mutation were resistant to anti-EGFR therapy. The results of various randomized clinical trials have confirmed the presence of other *RAS* mutation including additional *RAS* mutations (*KRAS* exons 3/4 and *NRAS* exon 1/2/3/4). Extended *RAS* analysis should be considered before initiating anti-EGFR therapy to patients of metastatic CRC. This can help in proper selection of patients leading to tailored individualistic treatment, decreasing cost of treatment and the adverse effects related to use of monoclonal antibody therapy. The new evidence is supporting the need to make 'Extended *RAS*' analysis essential before start of treatment with anti-EGFR monoclonal antibody therapy. Prior to this the Extended *RAS* testing should be standardized.

Keywords: Extended *RAS* analysis; metastatic colorectal cancer (CRC); monoclonal antibody therapy

Submitted Dec 30, 2014. Accepted for publication Jan 26, 2015.
doi: 10.3978/j.issn.2078-6891.2015.016
View this article at: http://dx.doi.org/10.3978/j.issn.2078-6891.2015.016

Introduction

According to the American Cancer Society, the latest records of year 2012 showed that colorectal cancer is the third most commonly diagnosed cancer and the third leading cause of cancer death among both men and women in USA (1). The management of this widely prevalent cancer has also been evolving from being non-specific to being patient and target specific in the recent past. As a step towards targeted treatment, epidermal growth factor receptor (EGFR) was validated as a therapeutic target for chemotherapeutic agents (2).

Various randomized controlled trials (RCTs) proved the beneficial effects of anti-EGFR monoclonal antibodies as monotherapy as well as in combination therapy among patients with metastatic colorectal cancer (mCRC) in the last decade (3-7). Two anti-EGFR monoclonal antibodies (mAbs), cetuximab and panitumumab, were approved for use alone or with standard chemotherapy among patients with advanced CRC (8,9). As the mAbs are expensive and can be potentially toxic drugs, there was a need for proper selection of patients eligible for administration of the antibody based therapy. EGFR expression level was the first biomarker to be studied among patients likely to be prescribed anti-EGFR mAbs. But, no correlation could be established between the response to anti-EGFR mAbs and the EGFR expression levels (6,10). Later, an association between the occurrence of mutation of *KRAS* gene and the poor response with anti-EGFR mAbs was established (11). This was followed by the recommendation of testing of

mutational status of *KRAS* gene before initiation of therapy with anti-EGFR mAbs among patients of mCRC (12,13).

EGFR and RAS signaling pathway

EGFR, a tyrosine kinase receptor involved in signal transduction mechanism, is one of the important molecular targets for drug therapy (14). Binding of EGF or any other ligand to EGFR activates signal transduction via various pathways. These include the RAS-RAF-BRAF-MAPK (mitogen activated protein kinase) pathway or phosphatidylinositol 3-kinase (PI3K)-Akt or phospholipase Cγ pathway (15). *RAS* is the most important superfamily of proteins, which includes mainly *KRAS* and *NRAS* proteins. *KRAS* is a guanosine triphosphate cleaving enzyme (GTPase). The signaling through KRAS-RAF-BRAF-MAPK pathway controls gene transcription, cell proliferation, apoptosis, angiogenesis, invasion and migration (16-18).

Although EGFR is a molecular target for anti-EGFR mAbs and is also over expressed among approximately 80% of CRCs, it could not be established as a predictive biomarker in the management of CRC (16,19). Positive EGFR protein expression proved to be a poor biomarker for response with anti-EGFR mAbs (18). Thus, other effectors in the downstream signal transduction pathway were evaluated for their predictive value. It was observed that mutation in *KRAS*, *NRAS*, *BRAF* or *PI3KCA* genes result in constitutive activation of signaling pathway. Approximately 30-50% CRCs carry a mutation at codon 12 or 13 of exon 2 of the *KRAS* gene, followed by mutations of *NRAS*, *PI3KCA* and *BRAF* (20,21). These mutations are responsible for constitutive activation of EGFR downstream pathways which disrupt the normal signaling pathway independent of EGFR (15,18). Mutations in *BRAF* lead to uncontrolled *BRAF* activation independent of EGFR and *RAS* (17).

KRAS mutant status as a predictive biomarker

After the approval of cetuximab and panitumumab for use among patients with mCRC, various studies demonstrated that these drugs were effective among patients with *KRAS* exon 2 wild type tumors only and not among those with *KRAS* exon 2 mutant tumors (22,23). The median progression free survival (PFS) and overall survival (OS) significantly improved among the *KRAS* exon 2 wild type group with anti-EGFR antibody therapy when used either in monotherapy or combination therapy as compared to the basic support care group or standard chemotherapy regimen respectively (22,23). On the other hand, the *KRAS* exon 2 mutant group did not show any difference in efficacy with the addition of anti-EGFR mAbs as compared to the standard chemotherapy regimen (22-25). In addition, somewhat unexpected detrimental effects were observed in the mutant *KRAS* groups in the PRIME (panitumumab randomized trial in combination with chemotherapy for metastatic colorectal cancer to determine efficacy) and OPUS (oxaliplatin and cetuximab in first-line treatment of mCRC) studies (26,27). Both prospective and retrospective analysis of the clinical studies concluded that mutation of codon 12 or 13 of exon 2 of *KRAS* is a negative predictive biomarker for therapy with anti-EGFR antibody therapy (11,22-27).

This led to the recommendation for routine *KRAS* exon 2 mutational testing. The American Society of Clinical Oncology and National Comprehensive Cancer Network (NCCN) recommended that all patients with mCRC who are candidates for anti-EGFR antibody therapy should have their tumor tested for *KRAS* mutations. If *KRAS* mutation in codon 12 or 13 is detected, then patients with mCRC should not receive anti-EGFR antibody therapy as part of their treatment due to the predicted lack of response (12,13,28). This recommendation restricted the use of anti-EGFR mAbs to about 60% of all patients with *KRAS* wild type tumors (20). A meta-analysis of 45 clinical studies (29) concluded that *KRAS* mutations are predictive of survival, disease progression, and treatment failure in patients with advanced colorectal cancer treated with anti-EGFR antibodies. The benefits of anti-EGFR therapy were largely limited to *KRAS* wild type patients (29).

Unfortunately, not all patients with *KRAS* wild type status respond to anti-EGFR mAbs. The presence of *KRAS* mutations has low sensitivity and relatively high negative likelihood for determining non-responsiveness among the patients (30). One hypothesis to explain this could be the simultaneous or isolated presence of genetic aberrations of genes encoding the other downstream effectors of the EGFR mediated signal transduction pathway (31-34). This hypothesis was proven by the results of the following clinical studies which show that additional *RAS* mutation (*KRAS* exons 3 and 4 and *NRAS* exon 1, 2, 3, 4) analysis can help in further refining the treatment modalities.

Table 1 PRIME study, primary end points of (PFS and OS) efficacy results according to *RAS* mutation status

Variable	FOLFOX4 + panitumumab (months)	FOLFOX4 (months)	P value
PFS			
Extended *RAS* wild	10.1	7.9	0.004
No *KRAS* exon 2 (12+13) mutation	9.6	8.0	0.02
Non *KRAS* exon 2 (12+13) mutation but other *RAS* mutation present	7.3	8.0	0.040
Extended *RAS* mutation	7.3	8.7	0.001
OS			
Extended *RAS* wild	25.8	20.2	0.009
No *KRAS* exon 2 (12+13) mutation	23.8	19.4	0.03
Non *KRAS* exon 2 (12+13) mutation	17.1	17.8	0.01
Extended *RAS* mutation	15.3	18.7	0.001

Abbreviations: PFS, progression free survival; OS, overall survival.

Clinical evidence of presence of other genetic mutations in patients resistant to anti-EGFR therapy

In the era of personalized medicine various retrospective and prospective analyses are being conducted to search for more predictive biomarkers in the treatment protocol for various malignancies specially mCRC. As *KRAS* wild type status was not sufficient to ensure response to anti-EGFR mAbs, other predictive biomarkers (*KRAS*, *NRAS*, *BRAF* mutations, *PIK3CA* mutations and PTEN loss) from the signaling pathway were analyzed. Although the results are favorable for the predictive strength of some other genomic biomarkers, till now no recommendation has been made for extensive genotypic analysis before initiation of anti-EGFR antibody therapy (34-36).

A systematic review and meta-analysis by Yang *et al.* explored the association of *BRAF*, *PIK3CA* mutations and/or loss of PTEN expression with PFS, OS and objective response rate (ORR) among patients with *KRAS* wild type tumors treated with anti-EGFR mAbs were included. The authors concluded that *BRAF* mutations, *PIK3CA* mutations and loss of PTEN are promising biomarkers and can help in identifying the appropriate patients (37). In contrast, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (EWG) discouraged the testing of *BRAF*, *NRAS* or *PIK3CA*, and/or loss of expression of PTEN or AKT proteins for taking decisions regarding the administration of anti-EGFR antibody therapy among patients with mCRC (38).

These contradictory statements could not help in establishing the status of other biomarkers in the algorithm of mCRC management. Later, the retrospective analysis of PRIME study by Douillard *et al.* initiated the concept of Extended *RAS* analysis (39). The prospective-retrospective analysis of PRIME study assessed the efficacy and safety of panitumumab plus FOLFOX4 (oxaliplatin, fluorouracil and leucovorin) as compared with FOLFOX4 alone, according to *RAS* (*KRAS* or *NRAS*) or *BRAF* mutation status. Of the study population, 48% patients had tumors with non mutated *RAS* (no *KRAS* or *NRAS* mutations in exons 2, 3, or 4) and rest had mutations in *RAS* (any *KRAS* or *NRAS* mutations in exon 2, 3, or 4). The administration of panitumumab-FOLFOX4 led to a significant improvement in PFS and OS (*Table 1*). In the subgroup of patients without *RAS* mutations, there was a significant improvement in PFS (P=0.004) and OS (P=0.04) with panitumumab-FOLFOX4, as compared with FOLFOX4 alone (39). Another subset (17%), consisting of those patients with wild type *KRAS* tumors, but with mutations in other *RAS* exons [non *KRAS* exon 2 codon (12 and 13) mutation, *KRAS* exon 3 (at codon 61) and exon 4 (at codons 117 and 146); *NRAS* exon 2 (at codons 12 and 13), exon 3 (at codon 61), and exon 4 (at codons 117 and 146); and *BRAF* exon 15 (at codon 600)], showed a non-significantly shorter PFS and OS in the panitumumab-FOLFOX4 group than in the FOLFOX4-alone group (*Figure 1*). These results were similar to those observed in the subgroup of patients with *KRAS* mutations in exon 2 in tumors (*Table 1*). Another important observation of the study was that the treatment effects were different between the subgroups of patients without *RAS*

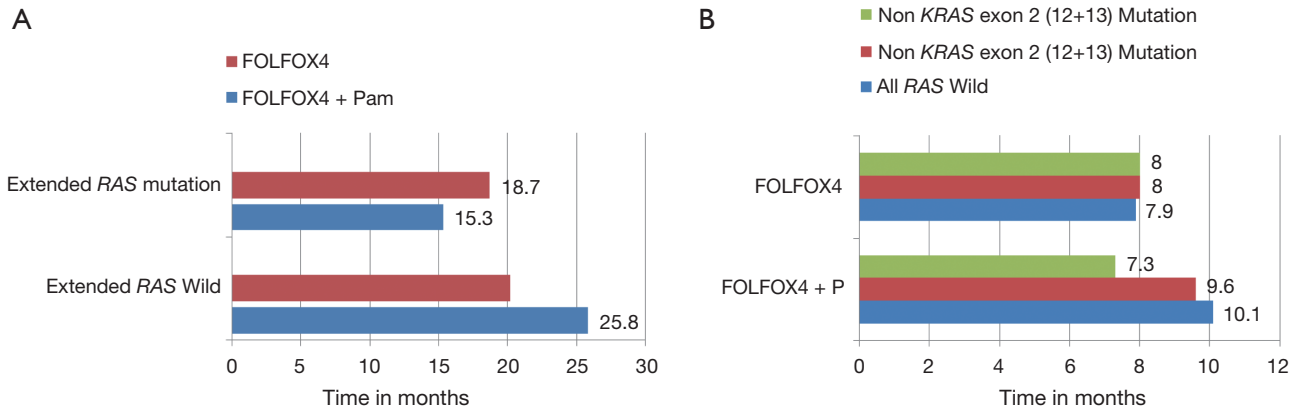

Figure 1 (A) PRIME study, in the wild *KRAS* exon 2 group FOLFOX + P improved progression free survival (PFS) when compared with FOLFOX only group (9.6 *vs.* 8 months respectively), the absolute magnitude of improvement of PFS is more pronounced when Extended *RAS* analysis is used to determine the RAS status with PFS 10.1 m in FOLFOX + P compared with 7.9 m in FOLFOX only group (P=0.004). The absolute improvement in PFS has increased from 1.6 months with wild *KRAS* exon 2 analysis to 2.2 months when Extended *KRAS* analysis is utilized; (B) PRIME study, clinically and statistically significant improvement in survival in FOLFOX and panitumumab in Extended *RAS* wild group when compared to FOLFOX alone, the presence of *RAS* mutation (any *KRAS* or *NRAS* mutations in exon 2, 3, or 4) in this population had detrimental effect on survival and did not drive any survival benefit from the addition of panitumumab in contrast to Extended *RAS* wild population where significant improvement in survival with FOLFOX and panitumumab comparing with FOLFOX only population (25.8 *vs.* 20.2 months respectively) (P=0.009).

mutations and those without *KRAS* mutations in exon 2 but with other *RAS* (*KRAS* or *NRAS* mutations in exons 2, 3, 4) mutations. This might suggest that *RAS* mutations, in addition to *KRAS* mutations in exon 2 codon (12 and 13), were negative predictive factors. The results suggest that presence of *RAS* mutations was a negative predictive factor. Further analysis showed that in the nonmutated *RAS* and nonmutated *BRAF* subgroup, panitumumab-FOLFOX4 was associated with a 1.6-month improvement in PFS and a 7.4-month improvement in OS, as compared with FOLFOX4 alone. Analysis of the prognostic effect of *BRAF* mutations showed that *BRAF* mutations were associated with reduced OS among patients without *KRAS* mutations in exon 2 and among those with *NRAS* mutations in exon 3. The safety profile for patients with *RAS* mutations was similar to that reported for patients with *KRAS* mutations in exon 2 (39).

Similarly, Soeda *et al.* while studying the response with cetuximab among irinotecan- and oxaliplatin-refractory Japanese patients with mCRC, found that the *KRAS*, *BRAF*, and *PIK3CA* wild type group had a better response rate and PFS than did the wild-type *KRAS* exon 2 subgroup (40). In the GERCOR efficacy, tolerance and translational molecular study, Andre *et al.* also studied *BRAF*, *NRAS* mutations and EGFR copy number in addition to the *KRAS* mutant status. Patients with *BRAF* mutations had a poorer prognosis and lower response rates to anti-EGFR antibody therapy as compared to other groups. Evidence for rare *KRAS*, *NRAS* and *PIK3CA* mutations was poor because of small number of patients in these groups. The response was highly dependent on the mutant status of the patients and thus recommended an extended genotyping including rare *KRAS* and *NRAS* mutants (41).

The PEAK [panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6] study also assessed the treatment effect with an extended *RAS* analysis including exons 2, 3, 4 of both *KRAS* and *NRAS* among patients with previously untreated, unresectable, wild type *KRAS* exon 2 mCRC. Patients with wild type *RAS* tumors had better PFS (P=0.029) and median OS (P=0.058) with anti-EGFR therapy. PFS was similar and OS was better in the panitumumab group among the patients with wild type *KRAS* exon 2 tumors (42).

New evidence was presented at the American Society of Clinical Oncology 2014 and European Cancer Congress 2013 (25,43). Peeters *et al.* assessed the effect of second line treatment of panitumumab plus FOLFIRI (continuous infusion fluorouracil, oxaliplatin, and irinotecan) *vs.* FOLFIRI based on *RAS* mutation status in the population of the earlier study conducted in 2010. Mutations detected included *KRAS* exon 3, 4 and *NRAS* exons 2, 3, 4 in patients

Table 2 FIRE 3 study, primary end points of (PFS and OS) efficacy results according to *RAS* mutation status

Variable	FOLFIRI + cetuximab (months)	FOLFIRI + bevacizumab (months)	P value
PFS			
KRAS exon 2 (12+13) wild	10.0	10.3	0.55
Extended *RAS* wild-type [Excluding all non *KRAS* exon 2 (12+13) mutation]*	10.4	10.2	0.54
OS			
KRAS exon 2 (12+13) wild	28.7	25.0	0.017
Extended *RAS* wild-type [Excluding all non *KRAS* exon 2 (12+13) mutation]*	33.1	25.6	0.011

*exon 3 (codon 61), and exon 4 (codon 146), and *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146). Abbreviations: PFS, progression free survival; OS, overall survival.

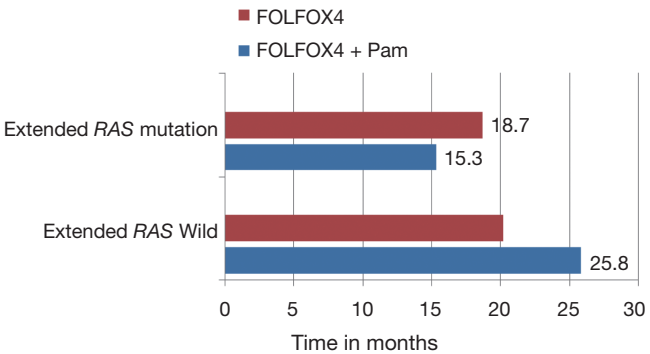

Figure 2 FIRE 3 study, clinically and statistically significant improvement in survival in FOLFIRI and cetuximab *KRAS* wild group in the preplanned analysis when compared to FOLFIRI and bevacizumab, the overall survival was more pronounced among Extended *RAS* wild type patients with the addition to cetuximab to FOLFIRI regimen as compared to addition of bevacizumab to FOLFIRI group.

with known *KRAS* wild type exon 2 mCRC. About 18% of the wild type *KRAS* patients had additional *RAS* mutations. The PFS and OS were better in the *RAS* wild type group as compared to *RAS* mutant group. Bokemeyer *et al.* studied *KRAS* exon 2 wild type patients from the OPUS study for 26 mutations (referred as *new RAS*) and additional *KRAS*, *NRAS* codons. New *RAS* mutations were present among 26% of patients. The patients from *RAS* wild type group showed significant improvement with addition of cetuximab to FOLFOX4 therapy. The distinctive observation of this study is that there was a trend towards worse outcome among patients with *RAS* mutation with the addition of cetuximab (26,44). Tejpar *et al.* (45) presented another set of results from the OPUS study about the patients which were tested for *KRAS* exons 3 and 4 and *NRAS* exons 2, 3 and 4. The tumor status was available for 31% of patients and there was benefit among *RAS* wild type population with addition of cetuximab to FOLFOX4. There was a less favorable outcome and no benefit among *RAS* mutant population with addition of cetuximab (45). Ciardiello *et al.* studied the *new RAS* mutations among *KRAS* wild type exon 2 tumors from CRYSTAL study patients and *RAS* mutations were present in 15% of the patients. There was a significant benefit in all end points among *RAS* wild type patients with the addition of cetuximab to FOLFIRI regimen. Also, there was no benefit among the *RAS* mutant group with the addition of cetuximab (46). Stintzing *et al.* evaluated the effect of mutations in exon 3 (codon 61), and exon 4 (codon 146), and *NRAS* exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) on the ORR, PFS and OS among the *KRAS* (exon 2) codon 12/13 wild type patients. The ORR and OS were increased among *RAS* wild type patients with the addition to cetuximab to FOLFIRI regimen as compared to addition of bevacizumab to FOLFIRI regimen (*Table 2*, *Figure 2*) (47).

A recent systematic review and meta-analysis on alterations in *KRAS* exons 3 and 4, *NRAS*, *BRAF* and *PIK3CA* and PTEN and the outcome with anti-EGFR antibody therapy suggests that mutations in *KRAS* exons 3 and 4 and *NRAS* predict resistance to anti-EGFR mAbs. The ORR was significantly poor among those with *KRAS* mutation in exon 3 and 4 (odds ratio 0.26). The PFS was also significantly shorter due to mutations in *KRAS* exons 3 and 4 and *NRAS* (48). Sorich *et al.* have included all the above mentioned clinical trials assessing the role of

anti-EGFR mAbs for tumors harboring *RAS* mutations. They divided the patients from various RCTs into three subgroups. First group was the "*KRAS* exon 2" mutant group; second consisted of "*new RAS* mutant" (wild-type for *KRAS* exon 2, but with a *KRAS* mutation in exons 3 or 4 and/or a *NRAS* mutation in exons 2, 3 or 4) patients and third consisted of "Extended *RAS* wild type" patients. Tumors without any *RAS* mutations (either *KRAS* exon 2 or *new RAS* mutations) had significantly superior response [PFS (P<0.001) and OS (P=0.008)] with anti-EGFR mAb treatment as compared to tumors with any of the *new RAS* mutations. There was no PFS and OS benefit with anti-EGFR mAbs for tumors with any *RAS* mutations (P>0.05) (49).

Discussion

Although in the initial years of use of anti-EGFR mAbs for mCRC, testing of *KRAS* exon 2 mutation helped in individualizing the treatment with anti-EGFR mAbs, yet, even after this analysis, a subset population of *KRAS* exon 2 wild type patients showed continues resistance to anti-EGFR agents. Since the isolation of *KRAS* mutant status as a lone negative predictor marker few years back to the present day scenario each and every step has been corroborated by evidence from clinical studies. The results of the above mentioned RCTs, systematic reviews and meta-analysis show that patients with tumors that are *KRAS* exon 2 wild-type (which includes both the "Extended *RAS* wild-type" and "*new RAS* mutant" subgroups) should not be considered to represent a single homogenous group for efficacy or resistance to anti-EGFR mAbs. The "Extended *RAS* wild-type" subgroup is distinct and has a significantly better response to anti-EGFR mAbs as compared to other patients. The response is indistinguishable among the *KRAS* exon 2 mutant patients and those with newly identified *RAS* mutations which include *KRAS* mutation in exons 3 or 4 and/or a *NRAS* mutation in exons 2, 3 or 4. Although the beneficial effects of anti-EGFR mAbs are explicit in the Extended *RAS* wild type group, results are still limited regarding the detrimental effects of anti-EGFR mAbs among *RAS* mutant groups (39,44). A broader analysis of mutant status can help in tailoring patient specific regimen and achieving maximum benefit. Thus based on the emerging benefit Extended *RAS* analysis, beyond *KRAS* exon 2, should be utilized in practice for predicting the benefit from the anti-EGFR mAbs among patients with mCRC.

Conclusions

The additional analysis of *KRAS* and *NRAS* genes as predictive markers can allow more accurate selection of patients who are more likely to benefit from anti-EGFR antibody therapy. Treatment with anti-EGFR mAbs should only be initiated after screening tumors for mutations in exon 2, 3 and 4 of both *KRAS* and *NRAS* genes. This will help in preventing unnecessary drug toxicity and associated expenses. Prior to the implantation of such recommendation there is a need to establish a standardized acceptable expanded *RAS* mutant status testing.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. American Cancer Society. Colorectal Cancer Facts & Figures 2011-2013. American Cancer Society, Atlanta. 2011. Available online: http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-028312.pdf. Accessed 19th September 2014.
2. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Cancer 2004;4:361-70.
3. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337-45.
4. Tabernero J, Van Cutsem E, Díaz-Rubio E, et al. Phase II trial of cetuximab in combination with fluorouracil, leucovorin, and oxaliplatin in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 2007;25:5225-32.
5. Saltz LB, Meropol NJ, Loehrer PJ Sr, et al. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004;22:1201-8.
6. Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007;25:1658-64.
7. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007;357:2040-8.
8. US FDA. FDA Approves Erbitux for Colorectal Cancer.

2004. Available online: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2004/ucm108244.htm. Accessed 29th August, 2014.
9. US FDA. FDA Approves a New Drug for Colorectal Cancer, Vectibix. 2006. Available online: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2006/ucm108745.htm. Accessed 29th August, 2014.
10. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005;23:1803-10.
11. Lièvre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992-5.
12. Engstrom PF, Arnoletti JP, Benson AB 3rd, et al. NCCN Clinical Practice Guidelines in Oncology: colon cancer. J Natl Compr Canc Netw 2009;7:778-831.
13. Engstrom PF, Arnoletti JP, Benson AB 3rd, et al. NCCN Clinical Practice Guidelines in Oncology: rectal cancer. J Natl Compr Canc Netw 2009;7:838-81.
14. Prenen H, Tejpar S, Van Cutsem E. New strategies for treatment of KRAS mutant metastatic colorectal cancer. Clin Cancer Res 2010;16:2921-6.
15. Wicki A, Herrmann R, Christofori G. Kras in metastatic colorectal cancer. Swiss Med Wkly 2010;140:w13112.
16. Yokota T. Are KRAS/BRAF Mutations Potent Prognostic and/or Predictive Biomarkers in colorectal cancers? Anti-Cancer Agents in Medicinal Chemistry 2012;12:163-71.
17. Domagała P, Hybiak J, Sulżyc-Bielicka V, et al. Kras mutation testing in colorectal cancer as an example of the pathologist's role in personalized targeted therapy: a practical approach. Pol J Pathol 2012;63:145-64.
18. Siena S, Sartre-Bianchi A, Di Nicolantonio F, et al. Biomarkers Predicting Clinical Outcome of Epidermal Growth Factor Receptor—Targeted Therapy in Metastatic Colorectal Cancer. J Natl Cancer Inst 2009;101:1308-24.
19. Tan C, Du X. KRAS mutation testing in metastatic colorectal cancer. World J Gastroenterol 2012;18:5171-80.
20. Bekaii-Saab T. Moving Forward With Expanding to an "All- RAS Mutational Analysis" in Metastatic Colorectal Cancer: Beyond KRAS mutations. J Natl Compr Canc Netw 2014;12:299-300.
21. Samowitz WS, Curtin K, Schaffer D, et al. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev 2000;9:1193-7.
22. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757-65.
23. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:1626-34.
24. Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408-17.
25. Peeters M, Price TJ, Cervantes A, et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared to FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol 2010;28:4706-13.
26. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 2009;27:663-71.
27. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluoruracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol 2010;28:4697-705.
28. Allegra CJ, Jessup JM, Somerfield MR, et al. 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 2009;27:2091-6.
29. Dahabreh IJ, Terasawa T, Castaldi PJ, et al. Systematic Review: Anti–Epidermal Growth Factor Receptor Treatment Effect Modification by KRAS Mutations in Advanced Colorectal Cancer. Ann Intern Med 2011;154:37-49.
30. Linardou H, Dahabreh I, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol 2008;9:962-72.
31. Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol 2010;28:1254-61.
32. Peeters M, Oliner KS, Parker A, et al. Massively parallel tumor multigene sequencing to evaluate response to panitumumab in a randomized phase 3 study of metastatic colorectal cancer. Clin Cancer Res 2013;19:1902-12.
33. Janakiraman M, Vakiani E, Zeng Z, et al. Genomic and biological characterization of exon 4 KRAS mutations in

human cancer. Cancer Res 2010;70:5901-11.
34. De Roock W, Claes B, Bernasconi D, et al. 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 2010;11:753-62.
35. Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer 2009;101:715-21.
36. Molinari F, Felicioni L, Buscarino M, et al. Increased detection sensitivity for KRAS mutations enhances the prediction of anti-EGFR monoclonal antibody resistance in metastatic colorectal cancer. Clin Cancer Res 2011;17:4901-14.
37. Yang ZY, Wu XY, Huang YF, et al. Promising biomarkers for predicting the outcomes of patients with KRAS wild-type metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: a systematic review with meta-analysis. Int J Cancer 2013;133:1914-25.
38. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: can testing of tumor tissue for mutations in EGFR pathway downstream effector genes in patients with metastatic colorectal cancer improve health outcomes by guiding decisions regarding anti-EGFR therapy? Genet Med 2013;15:517-27.
39. Douillard JY, Oliner KS, Siena S, et al. Panitumumab–FOLFOX4 Treatment and RAS Mutations in Colorectal Cancer. N Engl J Med 2013;369:1023-34.
40. Soeda H, Shimodaira H, Watanabe M, et al. Clinical usefulness of KRAS, BRAF, and PIK3CA mutations as predictive markers of cetuximab efficacy in irinotecan- and oxaliplatin-refractory Japanese patients with metastatic colorectal cancer. Int J Clin Oncol 2013;18:670-7.
41. André T, Blons H, Mabro M, et al. Panitumumab combined with irinotecan for patients with KRAS wild-type metastatic colorectal cancer refractory to standard chemotherapy: a GERCOR efficacy, tolerance, and translational molecular study. Annals of Oncology 2013;24:412-9.
42. Schwartzberg LS, Rivera F, Karthaus M, et al. 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 2014;32:2240-7.
43. Peeters M, Oliner KS, Price TJ, et al. Analysis of KRAS/NRAS mutations in phase 3 study 20050181 of panitumumab (pmab) plus FOLFIRI versus FOLFIRI for second-line treatment (tx) of metastatic colorectal cancer (mCRC). Available online: http://meetinglibrary.asco.org/content/122548-143. Accessed 20th August, 2014.
44. Bokemeyer C, Kohne CH, Ciardiello F, et al. Treatment outcome according to tumor RAS mutation status in OPUS study patients with metastatic colorectal cancer (mCRC) randomized to FOLFOX4 with/without cetuximab. Available online: http://meetinglibrary.asco.org/content/127861-144. Accessed 20th August, 2014.
45. Tejpar S, Lenz HJ, Köhne CH, et al. 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. Available online: http://meetinglibrary.asco.org/content/121584-143. Accessed 20th August, 2014.
46. Ciardiello F, Lenz HJ, Kohne CH, et al. 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. Available online: http://meetinglibrary.asco.org/content/121586-143. Accessed 20th August, 2014.
47. Heinemann V, von Weikersthal LF, Decker T, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. Lancet Oncol 2014;15:1065-75.
48. Therkildsen C, Bergmann TK, Henrichsen-Schnack T, et al. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. Acta Oncol 2014;53:852-64.
49. Sorich MJ, Wiese MD, Rowland A, et al. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Ann Oncol 2015;26:13-21.

Cite this article as: Al-Shamsi HO, Alhazzani W, Wolff RA. Extended *RAS* testing in metastatic colorectal cancer—Refining the predictive molecular biomarkers. J Gastrointest Oncol 2015;6(3):314-321. doi: 10.3978/j.issn.2078-6891.2015.016

