VOLUME 27 · NUMBER 35 · DECEMBER 10 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Analysis of *PTEN*, *BRAF*, and *EGFR* Status in Determining Benefit From Cetuximab Therapy in Wild-Type *KRAS* Metastatic Colon Cancer

Pierre Laurent-Puig, Anne Cayre, Gilles Manceau, Emmanuel Buc, Jean-Baptiste Bachet, Thierry Lecomte, Philippe Rougier, Astrid Lievre, Bruno Landi, Valérie Boige, Michel Ducreux, Marc Ychou, Fréderic Bibeau, Olivier Bouché, Julia Reid, Steven Stone, and Frédérique Penault-Llorca

See accompanying editorial on page 5866 and article on page 5931

From the Institut National de la Santé et de la Recherche Médicale (INSERM) Unité Mixte de Recherche (UMR) -S775 Molecular Basis of Xenobiotics Response; Université Paris Descartes; Assistance Publique–Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Hôpital Ambroise Paré, Paris; Centre Jean Perrin, Université d'Auvergne Equipe Associée EA4233 Centre Hospitalier Universitaire (CHU), Clermont-Ferrand; CHU, Tours; Institut Gustave Roussy, Villejuif; Centre Régional de Lutte contre le Cancer (CRLC) Val d'Aurelle Paul Lamarque, Montpellier; CHU Robert Debré, Reims, France; and Myriad Genetics, Salt Lake City, UT.

Submitted January 9, 2009; accepted June 25, 2009; published online ahead of print at www.jco.org on November 2, 2009.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Pierre Laurent-Puig, INSERM UMR-S775, 45 rue des Saints-Pères, Paris, France 75006; e-mail: pierre.laurent-puig@parisdescartes.fr.

The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2735-5924/$20.00

DOI: 10.1200/JCO.2008.21.6796

ABSTRACT

Purpose
The occurrence of *KRAS* mutation is predictive of nonresponse and shorter survival in patients treated by anti–epidermal growth factor receptor (anti-EGFR) antibody for metastatic colorectal cancer (mCRC), leading the European Medicine Agency to limit its use to patients with wild-type *KRAS* tumors. However, only half of these patients will benefit from treatment, suggesting the need to identify additional biomarkers for cetuximab-based treatment efficacy.

Patients and Methods
We retrospectively collected tumors from 173 patients with mCRC. All but one patient received a cetuximab-based regimen as second-line or greater therapy. *KRAS* and *BRAF* status were assessed by allelic discrimination. EGFR amplification was assessed by chromogenic in situ hybridization and fluorescent in situ hybridization, and the expression of PTEN was assessed by immunochemistry.

Results
In patients with *KRAS* wild-type tumors (n = 116), *BRAF* mutations (n = 5) were weakly associated with lack of response ($P = .063$) but were strongly associated with shorter progression-free survival ($P < .001$) and shorter overall survival (OS; $P < .001$). A high EGFR polysomy or an EGFR amplification was found in 17.7% of the patients and was associated with response ($P = .015$). PTEN null expression was found in 19.9% of the patients and was associated with shorter OS ($P = .013$). In multivariate analysis, *BRAF* mutation and PTEN expression status were associated with OS.

Conclusion
BRAF status, *EGFR* amplification, and cytoplasmic expression of PTEN were associated with outcome measures in *KRAS* wild-type patients treated with a cetuximab-based regimen. Subsequent studies in clinical trial cohorts will be required to confirm the clinical utility of these markers.

J Clin Oncol 27:5924-5930. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Cetuximab and panitumumab are monoclonal antibodies that target epidermal growth factor receptor (EGFR) and are used alone or in combination with fluoruracil, irinotecan, or oxaliplatin in metastatic colorectal cancer (mCRC).[1-3] Recently, several studies[4-9] have shown that activating mutations in KRAS protein, which partially transduces the activation signal from EGFR, abrogates the therapeutic effect of anti-EGFR therapy. This effect is seen in colorectal cancer patients regardless of line of treatment or which anti-EGFR antibody is used. These results have changed the way anti-EGFR drugs are prescribed. For example, the European Medicine Agency has restricted drug prescription to the set of patients with wild-type *KRAS* tumors.

However, selecting patients on the basis of tumor *KRAS* status is an imperfect process. While the test for nonresponse is highly specific (nearly 95% of the patients with mutations of *KRAS* fail to respond to anti-EGFR therapy), it is not sensitive. In fact, 40% to 60% of patients with wild-type *KRAS* fail to respond to the treatment.[10] This suggests that there are other important molecular determinants of response that have yet to be identified.

Based on the example of *KRAS*, these additional determinants may be components of the

signaling pathways activated by EGFR. Three candidates are the EGFR itself, the phosphatase and tensin homolog gene (*PTEN*), and the v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*).[11,12] These genes are the targets of genetic alterations in colon cancer,[12,13] and they have been associated with the efficacy of therapies directed against *EGFR* and/or members of *EGFR*-activated pathways.[14-25]

These data are compelling but not conclusive. Therefore, we have simultaneously ascertained *EGFR* amplification, *PTEN* protein expression, and mutations in *KRAS* and *BRAF* in a large patient cohort with mCRC. These molecular markers were then evaluated individually and in combination for their ability to predict efficacy of cetuximab treatment.

PATIENTS AND METHODS

We collected retrospective tumor samples from 173 patients with mCRC. Collection has been partially described.[9] Briefly, patients with mCRC were sequentially ascertained from six hospitals. Three patients received cetuximab only, 141 received a combination of irinotecan and cetuximab, and 28 received a combination of folfiri and cetuximab. All but two patients exhibited clear clinical evidence of having progressed on irinotecan-based therapies. One progressed on folfox-based chemotherapy and received a combination of folfox and cetuximab; one received cetuximab alone in first-line treatment. All but one patient received cetuximab-based therapy as a second-line or greater treatment for mCRC (13 in second-line, 78 in third-line, 49 in fourth-line, 20 in fifth-line, and 12 in sixth-line or greater). This study was approved by the Ile-de-France ethical committee number 2 (ID-RCB-AO1058-47/2008-135). The outcome measures were response based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria (54 patients were responders), progression-free survival (PFS), and overall survival (OS).

The seven most frequent mutations on codons 12 and 13 of *KRAS* were assessed as previously described.[9] *BRAF* V600E mutation detection was assessed by allelic discrimination using TaqMan probes following the same protocol as that for *KRAS* mutation. Probes and protocol are available on request.

EGFR In Situ Hybridization

Chromogenic in situ hybridization (CISH) experiments were performed for all the specimens by using the digoxigenylated ZytoDot SPEC EGFR Probe (CE-Marked), the ZytoDot pretreatment kit, and the ZytoDot CISH polymer detection kit, according to the supplier's protocol (Zytovision, Clinisciences, Montrouge, France). The DNA probe and sections were denatured at 95°C and hybridized at 37°C overnight using a HYBrite instrument (Vysis, Downers Grove, IL). An overlook was made with a ×40 dry objective to assess the heterogeneity of the staining on all tumor cells, and 60 nuclei were scored with a ×60 oil objective in at least four areas by two observers independently (A.C. and F.P.-L.). Only tumors with four or more copies of the *EGFR* gene (focally or in more than 40% of the cells) or with clusters were controlled by fluorescent in situ hybridization (FISH). All other tumors were considered to be FISH-negative. FISH experiments were performed using an EGFR-specific sequence probe (LSI EGFR) and control chromosome enumeration probe 7 (CEP7), according to the manufacturers' recommended protocol (Vysis-Abbott Molecular Diagnostics, Rungis, France) with some minor modifications. The DNA probes and tissue sections were denatured at 85°C for 5 minutes using a HYBrite instrument. An additional wash in distilled water was added before counterstaining and mounting with a solution of 4≪,6-diamidino-2-phenylindole dihydrochloride. As defined previously by Hirsch et al,[15] tumors with four or more copies of the *EGFR* gene in ≥ 40% of the cells (high polysomy) or tumors with *EGFR* gene amplification (gene-to-chromosome ratio ≥ 2, or presence of gene cluster, or ≥ 10 gene copies in ≥ 10% of the cells) were considered to be FISH-positive, whereas all other tumors were considered to be FISH-negative.

PTEN Expression

PTEN status was determined by immunohistochemistry (IHC) on 3-μm paraffin sections. Immunostaining was performed with a Benchmark XT immunostainer (Ventana, Illkirch, France). After deparaffinization, antigen retrieval was carried out for 60 minutes in CCl citrate buffer (pH = 8) at 95°C. PTEN antibody (AF847; R&D Systems, Lille, France) was incubated for 32 minutes at 37°C and the revelation was made with the Ultraview detection kit (Ventana). The primary antibody dilution varied between 1/200 to 1/400 according to the lot number. A PTEN IHC control slide (8106; Cell Signaling Technology, Ozyme, Saint Quentin en Yvelines, France) was used to optimized the dilution and run with each slide series. Sections were scored

Table 1. Univariate Analysis With Kaplan-Meier Estimates of Median

Variable	Patients With Mutated *KRAS* (n = 53)		Patients With Nonmutated *KRAS* (n = 116)				*P*
	No.	95% CI	No.	95% CI	No.	95% CI	
Responder	1		52				< .001*
Median PFS, weeks	10	8.0 to 12	30		25.9 to 34		< .001†
Median OS, months	8.4	6.4 to 10.7	14.4		12.0 to 17.9		< .001†
			Mutated *BRAF* (n = 5)		Nonmutated *BRAF* (n = 110)		
Responder			0		52		.063*
Median PFS, weeks			8.0	8 to NA	31.4	28 to 34.4	.001†
Median OS, months			6.5	5.9 to NA	14.8	13.0 to 19.6	.001†
			PTEN cytoplasmic null expression (n = 22)		*PTEN* cytoplasmic non-null expression (n = 89)		
Responder			10		41		1*
Median PFS, weeks			30.0	19.4 to 43	31.4	26.0 to 36	.275†
Median OS, months			11.8	9.1 to 17.9	16.2	13.9 to 20.7	.013†
			Nonamplified *EGFR* (n = 79)		Amplified *EGFR* (n = 17)		
Responder			29		12		.015*
Median PFS, weeks			28	19.9 to 34.0	34	29.0 to 65.6	.280†
Median OS, months			13.9	11.4 to 17.3	19.7	13.4 to NA	.180†

Abbreviations: PFS, progression-free survival; OS, overall survival; NA, not assessable.
*Fisher's exact test.
†Log-rank test.

semiquantitatively by light microscopy by two pathologists (A.C. and F.P.-L.). Normal endothelial cells were used as an internal positive control. Cytoplasmic and nuclear staining were specified. For each localization, the percentage of stained tumor cells was multiplied by the intensity (1, 2, or 3) and added to a score between 0 and 300. Tumors were then dichotomized into PTEN-negative (cytoplasmic score = 0) or PTEN-positive (cytoplasmic score = > 0) groups.

Statistical Analysis

Statistical analysis was conducted in S-PLUS 7.0 (Insightful, Seattle, WA) using the Cox proportional hazard model for time-to-event data and logistic regression or Fisher's exact test for binary outcomes. Median times-to-event and CIs were obtained from Kaplan-Meier plots and the log-rank test. All *P* values are two-sided.

RESULTS

We identified 53 (31.3%) activating mutations in codons 12 or 13 of *KRAS* gene from 169 patients. Genotyping data were unattainable for four samples. The mutations were tested for association with drug efficacy. In fact, the mutation status of 114 of these patients was previously reported and was shown to correlate with cetuximab response.[4] Not surprisingly, in the complete set of 169 patients, *KRAS* is a strong predictor of drug efficacy. *KRAS* mutations were associated with absence of response ($P < .001$), with a shorter period of PFS ($P < .001$) and shorter OS ($P < .001$; Table 1).

The V600E mutation in the *BRAF* gene was determined in the entire series. Five (3%) of 171 tumors were mutated; as expected, all were found in KRAS wild-type tumors. *BRAF* mutations were tested for association with treatment outcome. Since *KRAS* mutations are reproducibly associated with outcome after cetuximab therapy, we focused our analyses on patients with *KRAS* wild-type tumors. But to be complete, in the subgroup of patients with KRAS mutation, the other markers investigated in this study were not associated with outcome measures (Appendix Table A1, online only). In patients with *KRAS* wild-type tumors (n = 116), *BRAF* mutations were associated with lack of response ($P = .063$; Table 1). They were significantly associated with shorter PFS ($P < .001$) and shorter OS ($P < .001$; Table 1 and Fig 1). Interestingly, the PFS survival of these patients was similar to that of patients with KRAS mutations (Appendix Fig A1, online only).

EGFR amplification status was determined in 138 of 173 patients. The high dropout rate was due to a technical problem related to the fixation process and therefore is not expected to create patient ascertainment bias. *EGFR* high polysomy or amplification (FISH-positive phenotype) was found in 22 patients. Among them, 17 (77.3%) occurred in the 96 *KRAS* wild-type tumors (17.7%).

Restricting our analysis to *KRAS* wild-type tumors only, we tested for association between *EGFR* high polysomy or amplification and treatment outcome. In the *KRAS* wild-type subgroup, *EGFR* FISH-positive phenotype was associated with higher response rate (RR; 71%) than that in tumors with normal *EGFR* copy number (37%). This difference was significant ($P = .015$; Table 1). In addition, there was a trend toward longer PFS and OS in patients with FISH-positive phenotype, but the difference did not reach the threshold of significance (Table 1).

PTEN status was determined by IHC in 162 samples. The evaluation of PTEN cytoplasmic expression as a continuous trait did not demonstrate any significant association with response or PFS or OS. We defined PTEN status for each patient as a dichotomous trait of either expressing PTEN in the cytoplasmic compartment of the cell (ie, any positive tumor cells) or lacking PTEN expression. Thirty-one tumors (19%) were negative for PTEN expression in the tumor cell cytoplasmic compartment. Among them, 22 (19.8%) occurred in the group of 111 wild-type *KRAS* patients. In this patient subgroup, the lack of PTEN expression was not associated with response or PFS but was associated with a shorter OS ($P = .013$; Table 1 and Fig 2).

We performed a multivariate analysis of response and OS to determine the respective roles of EGFR, BRAF, and PTEN in determining treatment outcome for colon cancer patients with KRAS wild-type tumors. The multivariate analysis also included adjustments for other variables such as sex, age, number of previous chemotherapy regimens before the use of anti-EGFR therapy, and type of tumor material used for the determination of biomarker status (ie, primary or metastatic tumor). As a result, we found that EGFR and BRAF were significant predictors of response even after adjustment for all other covariates (Table 2). This association was also significant for EGFR in the subset of patients who were wild-type for both KRAS and BRAF (Table 2). Finally, we found that *PTEN* and *BRAF* were joint predictors of overall survival (Table 3).

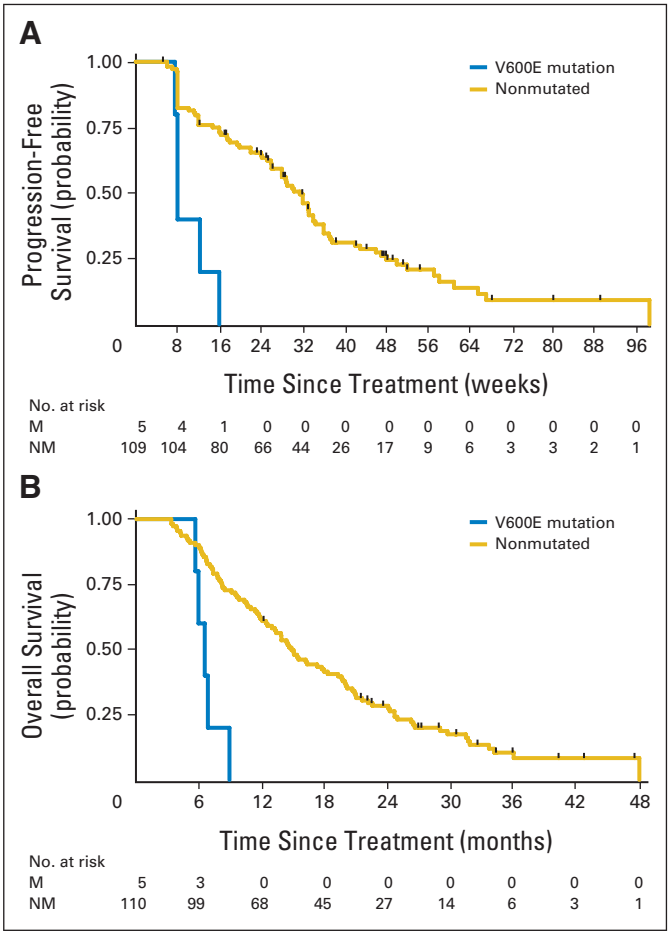

Fig 1. (A) Progression-free survival and (B) overall survival in wild-type *KRAS* patients according to *BRAF* status. M, mutated; NM, nonmutated.

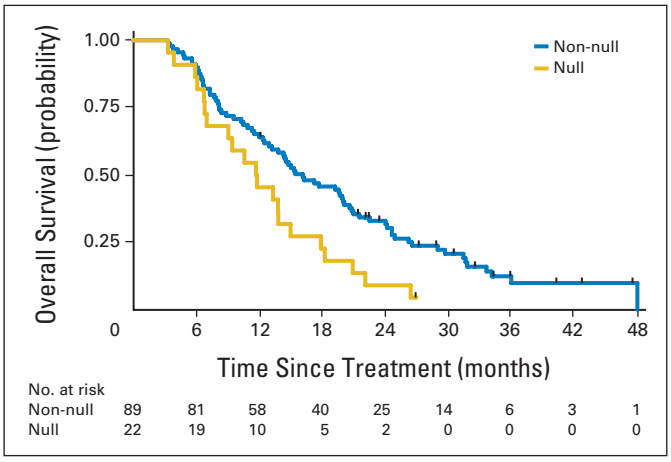

Fig 2. Overall survival in wild-type *KRAS* patients according to PTEN status.

Table 3. Multivariate Cox Proportional Hazard Model for Overall Survival

Variable	Categories	HR	95% CI	*P*
KRAS wild-type (n = 110)				
BRAF	Nonmutated	1.0		
	Mutated*	6.6	2.4 to 18.2	< .001
PTEN	Non-null expression	1.0		
	Null expression*	1.8	1.1 to 3.1	.023
KRAS and *BRAF* wild-type (n = 105)				
PTEN	Non-null expression	1.0		
	Null expression*	1.9	1.1 to 3.2	.026

NOTE. Adjusted for sex, age, tumor location (primary or metastatic), and number of previous chemotherapy regimens prior to use of anti–epidermal growth factor receptor therapy (≤ 2 or > 2).
Abbreviation: HR, hazard ratio.
*Correlates with shorter survival.

DISCUSSION

There is ample evidence that activating mutations in *KRAS* are strong predictors of resistance to cetuximab.[4-7,9] However, *KRAS* is not a perfect predictor since only 40% to 60% of patients with wild-type *KRAS* tumors respond to treatment. Here, we have provided evidence that in patients with wild-type KRAS, mutations in *BRAF* are associated with response, PFS, and OS; *EGFR* amplification is associated with response; and PTEN protein expression is associated with OS. The RRs and frequencies of all the genetic subclasses defined in this study are summarized in Figure 3. These data are based on a retrospective analysis of a nonclinical trial disease cohort and need to be validated in subsequent studies before they are used to inform clinical care. However, they clearly support the emerging view that a comprehensive assessment of genetic alterations in *EGFR* signaling pathways will enable an accurate prediction of patient benefit from anti-EGFR treatment.

This study adds to the growing evidence that *BRAF* status predicts benefit from anti-EGFR therapy in colorectal cancer.[24,25] In fact, the odds ratio of nonresponse increases from 42 in patients with mutated *KRAS* to 50 in patients with either *KRAS* or *BRAF* mutations. However, the clinical impact of *BRAF* gene testing depends on the prevalence of *BRAF* mutations in CRC. Here, the prevalence of *BRAF* mutations was 3%, which is lower than expected on the basis of previous population studies of microsatellite-stable mCRC.[26,27] Moreover, in a recent study[24] of mCRC patients treated with cetuximab, the mutation frequency was 9.7%, significantly higher than in

Table 2. Multivariate Logistic Regression Model for Response

Variable	Categories	OR	95% CI	*P*
KRAS wild-type (n = 96)				
EGFR	Nonamplified	1.0		
	Amplified*	4.8	1.4 to 15.7	.007
BRAF	Nonmutated*	1.0		
	Mutated	Infinity		.004
KRAS and *BRAF* wild type (n = 91)				
EGFR	Nonamplified	1.0		.002
	Amplified*	7.7	1.9 to 31.2	

NOTE. Adjusted for sex, age, tumor location (primary or metastatic), and number of previous chemotherapy regimens prior to use of anti–epidermal growth factor receptor therapy (≤ 2 or > 2).
Abbreviation: OR, odds ratio.
*Correlates with response.

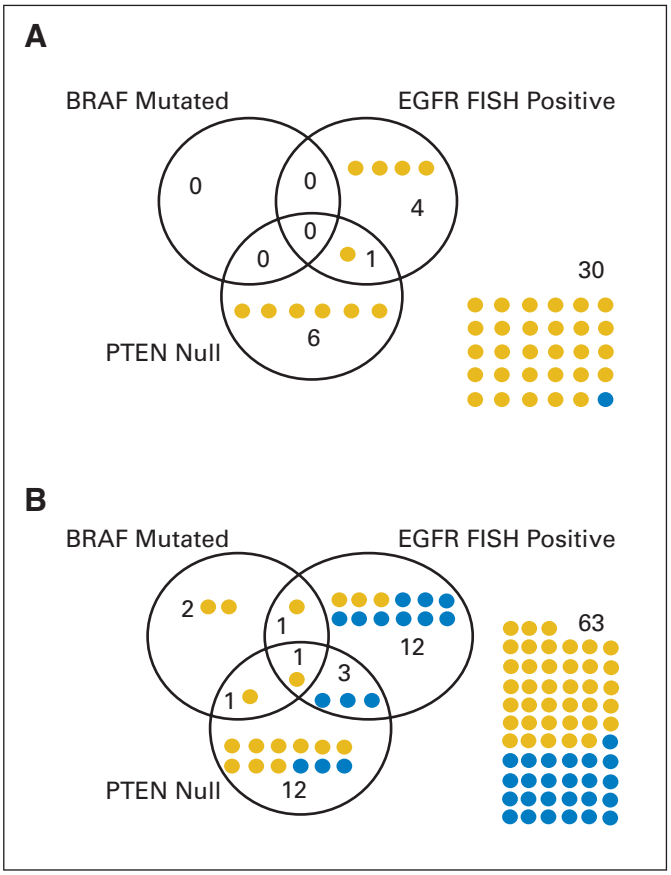

Fig 3. Venn diagram summarizing the number and response rate frequency for the genetic subsets analyzed in this study for (A) patients with *KRAS* mutations and (B) patients with *KRAS* wild-type tumors. There was no significant association between the variants (data not shown). Patients with missing data were excluded from the figure. (Blue dots) responders; (yellow dots) nonresponders. FISH, fluorescent in situ hybridization.

this study ($P = .02$). To eliminate concerns about potential artifacts, we regenotyped 92 of the 116 *KRAS* wild-type tumors by Sequenom genotyping technology (Sequenom, San Diego, CA; data not shown). No additional *BRAF* mutants were found. Perhaps the explanation for the low *BRAF* mutation frequency comes from the fact that BRAF mutations are thought to be negatively prognostic in microsatellite-stable tumors,[26] and unlike the patients in Di Nicolantonio et al[24], our patients generally survived through multiple rounds of chemotherapy before receiving cetuximab. For example, 30% of our cohort received two or more lines of therapy before cetuximab compared with 46% in the previous study ($P = .011$). Therefore, at least some patients with *BRAF* mutations may not have survived long enough to be recruited into this study. But this potential selection bias does not affect the conclusions of this and other studies[24], that BRAF status should be taken into account before considering anti-EGFR therapies for mCRC patients. The frequency of BRAF mutations is expected to be higher, and therefore more clinically relevant, in patients with tumors showing microsatellite instability or (assuming anti-EGFR therapies are eventually approved for adjuvant care) in patients with stage III CRC, in which up to 16% of tumors could carry somatic *BRAF* mutations.[26-28]

In CRC, the association of EGFR amplification with efficacy of anti-EGFR therapy has been controversial. Initially, it was reported that EGFR copy number as determined by FISH was associated with tumor response to cetuximab.[14] This association was confirmed and extended to panitumumab in subsequent studies.[8,16,22,29] However, there have been discrepant reports. For example, tumor response to anti-EGFR therapies was observed in colorectal tumors without an increase in EGFR copy number,[30] and EGFR copy number as determined by quantitative polymerase chain reaction was not associated with drug response in some recent studies.[5,31,32] These inconsistencies are due, in part, to difficulties in determining copy number by polymerase chain reaction in samples containing a mixture of somatic and tumor DNA and are also due to the lack of a standardized system for scoring and interpreting FISH data. These inconsistencies have led to the conclusion that this molecular marker would be difficult to include in clinical practice.[14,33] To address these issues, we measured EGFR copy number by CISH and FISH and applied the scoring algorithm of Hirsch (Patients and Methods) that has been demonstrated to be associated with response in lung cancer patients treated by cetuximab or EGFR–tyrosine kinase inhibitor–based chemotherapy.[15,34,35] This scoring algorithm is relatively simple to apply in clinical practice and therefore could be used to select patients who may benefit from anti-EGFR therapies. It also addresses potential tumor heterogeneity of EGFR amplifications and counts only true polysomic events by excluding cells that are actively dividing (S or M2 phase).

We compared the classification developed by Hirsh and used in this study to the performance of other previously published EGFR FISH thresholds associated with tumor response to cetuximab (ie, EGFR-to-chromosome probe intensity ratios of 2.47,[36] 2.83,[33] and 2.92[35]). Applying these published thresholds to our set of *KRAS* wild-type patients discriminated drug responders and nonresponders with an accuracy of 57.7%, 63.9%, and 64.9%, respectively, compared with 64.9% for the Hirsh scoring algorithm (data not shown). Some of the alternative thresholds were also associated with longer PFS and OS in our patient cohort (Table 4), which suggests that there is a biologic link between high *EGFR* copy number and response to anti-EGFR targeted therapies. Despite these data, we agree with Personeni et al[33] that clinical decisions based on EGFR copy number are not warranted as long as FISH technology and scoring are not standardized. Perhaps the scoring system used here and commonly used in lung cancer will help unify the field. Although EGFR status should not be used to exclude patients from treatment (negative predictive value = 0.62 in patients with KRAS and BRAF wild-type tumors), here we have identified a subgroup of patients (16.5% of the entire cohort) who are EGFR FISH-positive (and wild-type for KRAS and BRAF) with cetuximab RRs of approximately 80% (RR, 0.80; 95% CI, 0.51 to 0.95). If confirmed, this selection could identify patients for whom early and aggressive cetuximab-based therapy is clearly warranted.

Table 4. Performance of Reported *EGFR* Number of Copies Cutoff Point

Reference	*EGFR* Cutoff Point	PFS (weeks)* No.	95% CI	*P*†	OS (months)* No.	95% CI	*P*
Sartore-Bianchi et al[36]	> 2.47	32	28 to 36	< .01	15	13 to 20	.12 (NS)
	≤ 2.47	12	8 to 32		10	7 to 14	
Personeni et al[33]	> 2.83	33	29 to 36	< .05	16	13 to 22	< .05
	≤ 2.83	22	12 to 30		11	8 to 15	
Cappuzzo et al[35]	> 2.92	32	28 to 36	.23 NS	18	13 to 22	.08 (NS)
	≤ 2.92	25	15 to 33		12.5	9 to 15	

Abbreviations: PFS, progression-free survival; OS, overall survival; NS, not significant.
*Kaplan-Meier estimates of median for patients with *KRAS* wild-type tumors.
†Log-rank test.

We have also shown that PTEN expression was associated with OS in a cetuximab-treated cohort. This association does not seem to be related to response, which strongly supports the view that PTEN is prognostic (ie, loss of expression identifies a subset of tumors that are likely to have poor outcomes). Since this was a single-arm study, we cannot know if this effect is enhanced by cetuximab treatment or whether it is simply related to the natural history of PTEN-negative tumors. In any case, it seems clear that patients with KRAS wild-type/PTEN-negative tumors require more effective treatment options. mTOR inhibitors,[37] which target the AKT pathway downstream of PTEN, could be useful in this set of patients.

Four studies have provided evidence that PTEN status is associated with response in cetuximab-treated mCRC.[20-22,38] We did not confirm this result, and the reasons for the result are unclear. There are numerous differences between studies, including PTEN antibodies, IHC scoring algorithms, and whether PTEN expression was measured in primary or metastatic tumor tissue. Clearly, these differences could be significant and could contribute to discrepant results. To the contrary, patients with PTEN-negative tumors have poor outcomes regardless of response. Additional studies will be needed to determine the exact role PTEN status plays in influencing patient outcome.

Accurately predicting drug response is the eventual goal of personalized medicine. Somewhat surprisingly, that goal seems achievable for cetuximab. If key regulatory molecules in the signaling pathways downstream of EGFR are mutated so that these pathways gain EGFR-independent activity, then that patient is unlikely to benefit from cetuximab-based therapies. Unfortunately, the predictive algorithm may never be perfect, because the complete list of relevant

molecules may never be known. But as the list of approved therapeutics increases, clinicians will be forced to choose between nearly equally effective treatment regimes. The ability to inform that choice with the biomarkers described in this and similar studies should lead to dramatic improvements in patient care.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Julia Reid, Myriad Genetics (C); Steven Stone, Myriad Genetics (C) **Consultant or Advisory Role:** Pierre Laurent-Puig, Merck Lipha Santé France (C), Amgen France (C); Philippe Rougier, Pfizer (C), Merck Serono (C), sanofi-aventis (C); Michel Ducreux, Amgen (C); Marc Ychou, Merck Lipha Santé (C), Roche (C), Amgen (C), Bayer Pharmaceuticals (C); Fréderic Bibeau, Amgen (C), Merck Serono (C), PGxHealth (C); Frédérique Penault-Llorca, Merck Lipha Santé (C), Amgen (C) **Stock Ownership:** Julia Reid, Myriad Genetics; Steven Stone, Myriad Genetics **Honoraria:** Pierre Laurent-Puig, Merck Lipha Santé France, Amgen France; Philippe Rougier, Merck Serono, Amgen, sanofi-aventis; Astrid Lievre, Merck Lipha Santé, Roche; Bruno Landi, Novartis; Michel Ducreux, Amgen, Merck Serono; Marc Ychou, Merck Lipha Santé, Roche, Amgen, Bayer Pharmaceuticals; Fréderic Bibeau, Merck Serono, Amgen; Olivier Bouché, Merck Lipha Santé, Amgen; Frédérique Penault-Llorca, Merck Lipha Santé, Amgen **Research Funding:** Pierre Laurent-Puig, Merck Lipha Santé France, Amgen France, Myriad Genetics; Fréderic Bibeau, Merck Serono, Amgen, Roche, Pfizer **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Pierre Laurent-Puig, Astrid Lievre, Valérie Boige, Steven Stone, Frédérique Penault-Llorca
Financial support: Pierre Laurent-Puig, Frédérique Penault-Llorca
Administrative support: Pierre Laurent-Puig
Provision of study materials or patients: Pierre Laurent-Puig, Anne Cayre, Emmanuel Buc, Jean-Baptiste Bachet, Thierry Lecomte, Philippe Rougier, Astrid Lievre, Bruno Landi, Valérie Boige, Michel Ducreux, Marc Ychou, Fréderic Bibeau, Olivier Bouché, Frédérique Penault-Llorca
Collection and assembly of data: Pierre Laurent-Puig, Anne Cayre, Gilles Manceau, Emmanuel Buc, Jean-Baptiste Bachet, Thierry Lecomte, Philippe Rougier, Astrid Lievre, Bruno Landi, Valérie Boige, Michel Ducreux, Marc Ychou, Olivier Bouché, Frédérique Penault-Llorca
Data analysis and interpretation: Pierre Laurent-Puig, Anne Cayre, Gilles Manceau, Astrid Lievre, Michel Ducreux, Julia Reid, Steven Stone, Frédérique Penault-Llorca
Manuscript writing: Pierre Laurent-Puig, Anne Cayre, Astrid Lievre, Valérie Boige, Julia Reid, Steven Stone, Frédérique Penault-Llorca
Final approval of manuscript: Pierre Laurent-Puig, Anne Cayre, Gilles Manceau, Emmanuel Buc, Jean-Baptiste Bachet, Thierry Lecomte, Philippe Rougier, Astrid Lievre, Bruno Landi, Valérie Boige, Michel Ducreux, Marc Ychou, Fréderic Bibeau, Olivier Bouché, Julia Reid, Steven Stone, Frédérique Penault-Llorca

REFERENCES

1. 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 25:1658-1664, 2007

2. Cunningham D, Humblet Y, Siena S, et al: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351:337-345, 2004

3. Saltz LB, Cox JV, Blanke C, et al: Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 343:905-914, 2000

4. Di Fiore F, Blanchard F, Charbonnier F, et al: Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. Br J Cancer 96:1166-1169, 2007

5. Khambata-Ford S, Garrett CR, Meropol NJ, et al: Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 25:3230-3237, 2007

6. De Roock W, Piessevaux H, De Schutter J, et al: KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol 19:508-515, 2008

7. 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 26:1626-1634, 2008

8. Lièvre A, Bachet JB, Le Corre D, et al: KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66: 3992-3995, 2006

9. Lièvre A, Bachet JB, Boige V, et al: KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 26:374-379, 2008

10. Linardou H, Dahabreh IJ, 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 9:962-972, 2008

11. Sansal I, Sellers WR: The biology and clinical relevance of the PTEN tumor suppressor pathway. J Clin Oncol 22:2954-2963, 2004

12. Davies H, Bignell GR, Cox C, et al: Mutations of the BRAF gene in human cancer. Nature 417:949-954, 2002

13. Nassif NT, Lobo GP, Wu X, et al: PTEN mutations are common in sporadic microsatellite stable colorectal cancer. Oncogene 23:617-628, 2004

14. Moroni M, Veronese S, Benvenuti S, et al: Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: A cohort study. Lancet Oncol 6:279-286, 2005

15. Hirsch FR, Herbst RS, Olsen C, et al: Increased EGFR gene copy number detected by fluorescent in situ hybridization predicts outcome in non-small-cell lung cancer patients treated with cetuximab and chemotherapy. J Clin Oncol 26:3351-3357, 2008

16. Cappuzzo F, Varella-Garcia M, Finocchiaro G, et al: Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. Br J Cancer 99:83-89, 2008

17. Mellinghoff IK, Wang MY, Vivanco I, et al: Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 353:2012-2024, 2005

18. Nagata Y, Lan KH, Zhou X, et al: PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6:117-127, 2004

19. Berns K, Horlings HM, Hennessy BT, et al: A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12:395-402, 2007

20. Razis E, Briasoulis E, Vrettou E, et al: Potential value of PTEN in predicting cetuximab response in colorectal cancer: An exploratory study. BMC Cancer 8:234, 2008

21. Jhawer M, Goel S, Wilson AJ, et al: PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer Res 68:1953-1961, 2008

22. Frattini M, Saletti P, Romagnani E, et al: PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 97:1139-1145, 2007

23. Solit DB, Garraway LA, Pratilas CA, et al: BRAF mutation predicts sensitivity to MEK inhibition. Nature 439:358-362, 2006

24. Di Nicolantonio F, Martini M, Molinari F, et al: Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 26:5705-5712, 2008

25. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al: Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 67:2643-2648, 2007

26. Samowitz WS, Albertsen H, Sweeney C, et al: Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. J Natl Cancer Inst 98:1731-1738, 2006

27. Barault L, Veyrie N, Jooste V, et al: 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, 2008

28. Deng G, Bell I, Crawley S, et al: BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. Clin Cancer Res 10:191-195, 2004

29. Sartore-Bianchi A, Cerea G, Schiavetto I, et al: Anti-EGFR monoclonal antibodies in the treatment of non-small cell lung cancer. Ann Oncol 17:ii49-ii51, 2006 (suppl 2)

30. Italiano A, Follana P, Caroli FX, et al: Cetuximab shows activity in colorectal cancer patients with tumors for which FISH analysis does not detect an increase in EGFR gene copy number. Ann Surg Oncol 15:649-654, 2008

31. Lenz HJ, Van Cutsem E, Khambata-Ford S, et al: Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. J Clin Oncol 24:4914-4921, 2006

32. Vallböhmer D, Zhang W, Gordon M, et al: Molecular determinants of cetuximab efficacy. J Clin Oncol 23:3536-3544, 2005

33. Personeni N, Fieuws S, Piessevaux H, et al: Clinical usefulness of EGFR gene copy number as a predictive marker in colorectal cancer patients treated with cetuximab: A fluorescent in situ hybridization study. Clin Cancer Res 14:5869-5876, 2008

34. Hirsch FR, Varella-Garcia M, Cappuzzo F, et al: Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. Ann Oncol 18:752-760, 2007

35. Cappuzzo F, Finocchiaro G, Rossi E, et al: EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients. Ann Oncol 19:717-723, 2008

36. Sartore-Bianchi A, Moroni M, Veronese S, et al: Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. J Clin Oncol 25:3238-3245, 2007

37. Rowinsky EK: Targeting the molecular target of rapamycin (mTOR). Curr Opin Oncol 16:564-575, 2004

38. Perrone F, Lampis A, Orsenigo M, et al: PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. Ann Oncol 20:84-90, 2009

Glossary Terms

KRAS: The gene that encodes K-Ras, a protein that is a member of the small GTPase superfamily, in which a single amino acid substitution results in an activating mutation. Alternative splicing gives rise to variants encoding two isoforms that differ in the C-terminal region.

EGFR (epidermal growth factor receptor): Also known as HER-1, EGFR belongs to a family of receptors (HER-2, HER-3, HER-4 are other members of the family) and binds to the EGF, TGF-α, and other related proteins, leading to the generation of proliferative and survival signals within the cell. It also belongs to the larger family of tyrosine kinase receptors and is generally overexpressed in several solid tumors of epithelial origin.

PTEN (phosphatase and tensin homolog): PTEN is a tumor suppressor gene with a gamut of regulatory activities. The gene product is a multifunctional molecule. The predominant activity identified for PTEN is its lipid phosphatase activity that converts inositol trisphosphates into inositol bisphosphates, thus inhibiting survival and proliferative pathways that are activated by inositol trisphosphates. PTEN acts to maintain arrest in the G1 phase of the cell cycle and enable apoptosis through an AKT-dependent mechanism.

BRAF: BRAF is an isoform of RAF. Raf proteins (Raf-1, A-Raf, B-Raf) are intermediate to Ras and MAPK in the cellular proliferative pathway. Raf proteins are typically activated by Ras via phosphorylation, and activated Raf proteins in turn activate MAPK via phosphorylation. However, Raf proteins may also be independently activated by other kinases.

BRAF V600E: The V600E is the most common oncogenic mutation of BRAF in cancer. The V600E amino acid change results in constitutive activation of the BRAF kinase and promotes cell transformation.

FISH (fluorescent in situ hybridization): In situ hybridization is a sensitive method that is generally used to detect specific gene sequences in tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest. FISH uses a fluorescent probe to increase the sensitivity of in situ hybridization.

CISH (chromogenic in situ hybridization): CISH is a method to detect gene amplification and chromosomal translocations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest. In contrast to FISH (see definition), the DNA probe is detected using a simple enzymatic peroxidase reaction and visualized using a conventional microscope.

