# PIK3CA Mutations Are Not a Major Determinant of Resistance to the Epidermal Growth Factor Receptor Inhibitor Cetuximab in Metastatic Colorectal Cancer

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**Abstract**

**Purpose:** It has been reported that activating *KRAS* mutations negatively affect response to anti–epidermal growth factor receptor (EGFR) monoclonal antibodies in metastatic colorectal cancer. The mutation status of signaling molecules downstream of the EGFR target is thus crucial to predict clinical benefit to EGFR-targeted therapies. Other mechanisms of resistance to EGFR inhibitors could involve activating mutations of the other main EGFR effector pathway, i.e., the PI3K/PTEN/AKT pathway.

**Experimental Design:** We analyzed the *PIK3CA* and *KRAS* mutation status in a large group ($n$ = 200) of chemorefractory metastatic colorectal cancers treated with cetuximab (Erbitux) in monotherapy or in combination with irinotecan, and correlated the mutation status with outcome.

**Results:** Twenty-three (12%) of the 200 samples carried 1 of the *PIK3CA* mutations included in our assay. We found no correlation between the presence of a *PIK3CA* mutation and impaired response to cetuximab.

**Conclusions:** Our findings do not provide any evidence for a strong role of *PIK3CA* mutations as a single marker in determining response to cetuximab in chemorefractory metastatic colorectal cancer.

The identification of patients with colorectal cancer that respond to the epidermal growth factor receptor (EGFR) inhibitors cetuximab and panitumumab remains a major challenge.

It is well-established that activation of the EGFR pathway triggers a complex program of intracellular signals. The two major pathways activated by the EGFR are the KRAS/RAF/MEK/ERK 1/2 pathway, which controls gene transcription, cell-cycle progression, and cell proliferation, and the PI3K/PTEN/AKT pathway, which activates a cascade of antiapoptotic and prosurvival signals. It has been shown recently that KRAS, a small G-protein downstream of EGFR, can acquire activating mutations, which isolate the KRAS/RAF/MEK/ERK1/2 pathway from its receptor and thereby render EGFR inhibitors ineffective. As a result, activating *KRAS* mutations negatively affect response to anti-EGFR monoclonal antibodies in metastatic colorectal cancer (1–3). The mutation status of signaling molecules downstream of the EGFR target is thus crucial to predict clinical benefit to EGFR-targeted therapies.

Although patients without a *KRAS* mutation exhibit a much better response to EGFR inhibitors, not all of them respond to treatment. For instance, the response rate to cetuximab in advanced colorectal cancer patients with wild-type (WT) *KRAS* tumors was still limited to only 40% (3). Other mechanisms of resistance to EGFR inhibitors thus exist, and could for instance involve activating mutations of the other main EGFR effector pathway, i.e., the PI3K/PTEN/AKT pathway. Indeed, a recent report by Perrone et al. (4) investigated a cohort of 54 colorectal cancer tumors treated with cetuximab, and identified 4 mutations in the *PIK3CA* gene, which is the p110α catalytic subunit of PI3K, in patients not responding to cetuximab, thereby suggesting that mutations in *PIK3CA* cause resistance to cetuximab.

In the current study, we further investigated the *PIK3CA* and *KRAS* mutation status in a much larger group ($n$ = 200) of chemorefractory metastatic colorectal cancers treated with cetuximab (Erbitux) in monotherapy or in combination with irinotecan, and correlated the mutation status with outcome. Our aim was to investigate a possible role of *PIK3CA* mutations in resistance to cetuximab in *KRAS* WT patients.

## Patients and Methods

***Patients and treatment.*** Two hundred irinotecan-refractory patients with metastatic colorectal adenocarcinoma who received cetuximab (Erbitux; Merck Serono) in monotherapy or in combination with irinotecan in several clinical trials (salvage, Bond, Babel, Everest; $n$ = 197; refs. 5–7), or outside of a clinical trial in the same setting

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## Translational Relevance

The identification of patients with colorectal cancer that respond to the epidermal growth factor receptor (EGFR) inhibitors cetuximab and panitumumab remains a major challenge. It has been shown recently that activating *KRAS* mutations negatively affect response to anti-EGFR monoclonal antibodies in metastatic colorectal cancer. Although patients without a *KRAS* mutation exhibit a much better response to EGFR inhibitors, not all of them respond to treatment. Other mechanisms of resistance to EGFR inhibitors could involve activating mutations of the other main EGFR effector pathway, i.e., the PI3K/PTEN/AKT pathway. In the current study, we investigated the *PIK3CA* and *KRAS* mutation status in a large group ($n$ = 200) of chemorefractory metastatic colorectal cancer treated with cetuximab (Erbitux) in monotherapy or in combination with irinotecan, and correlated the mutation status with outcome. Our findings did not provide any evidence for a strong role of *PIK3CA* mutations as a single marker in determining response to cetuximab.

according to standard reimbursement ($n$ = 3), were screened (median age, 61 y; range, 26-89; 120 men). These 200 patients were randomly chosen according to the availability of formalin-fixed, paraffin-embedded tissue and availability of clinical data. Table 1 summarizes the number of patients in each trial and their type of treatment. Tumor assessment was done using the Response Evaluation Criteria in Solid Tumors criteria as planned in these trials. The patients showing a complete or partial response were classified as responders and those with stable disease or progressive disease as nonresponders. All the patients gave their informed consent to participate in these clinical trials.

***Samples and mutational analysis.*** The analysis was done using formalin-fixed, paraffin-embedded tissue of the primary colorectal cancer. Seven *KRAS* codon 12 and 13 mutations (p.G12V, p.G12D, p.G13D, p.G12C, p.G12A, p.G12S, and p.G12R) were detected by allele specific PCR, as reported previously (3). Activating mutations in *PIK3CA* tend to cluster in hotspots with ~80% accounted for by oncogenic substitution in exon 20 (H1047R) and exon 9 (E542K and E545K) that encode portions of the helical and kinase domains (8). Known *PIK3CA* mutations in these exons were evaluated using the Sequenom MALDI TOF MassArray system. Briefly, genotyping assays (primers for PCR amplification and the extension probe) were designed using the Sequenom MassARRAY Assay Design 3.0 software, applying default parameters. Multiplexed PCR was done with 0.2 units of Taq polymerase and 20 ng of genomic DNA, as described previously (9). Thermocycling was at 95°C for 15 min followed by 45 cycles of 95°C for 20 s, 56°C for 30 s, and 72°C for 1 min. Unincorporated deoxynucleotide triphosphates were deactivated using shrimp alkaline phosphatase, and primer extension was carried out using extension primers, dideoxynucleotide triphosphates, and Thermosequenase DNA polymerase. Reactions were cycled at 94°C for 2 min, followed by 44 cycles of 94°C for 5 s, 52°C for 5 s, and 80°C for 5 s. After the addition of a cation exchange resin to remove residual salt from the reactions, the purified primer extension reaction was loaded onto a matrix pad (3-hydroxypicoloinic acid) of a SpectroCHIP (Sequenom) and analyzed using a matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer. The mutations were assessed as follows: p.R88Q (c263G>A), p.N345K (c1035T>A), p.E542K (c1624G>A), p.E545K, A, G or V (c.1633G>A, c.1634A>C, G or T), p.Q546K (c.1636C>A), p.H1047Y, R or L (c.3139C>T and c.3140A>G or T). In case mutations were detected on the Sequenom, they were subsequently also confirmed by standard DNA sequencing.

***Statistical analysis.*** Sample size calculation was done using the following parameters: α, 0.05; power, 85%; global *PIK3CA* mutation rate, 10%; response rate in *KRAS* WT tumors, 40%; and prevalence of *KRAS* mutation, 40% (3). With these assumptions, a sample of 200 patients would be able to detect a decrease in response rate from 40% to 7% or lower. This calculation was done using pwr package for R (function: pwr.2p2n.test).

A two-sided Fisher's exact test was used to evaluate the association between *PIK3CA*, *KRAS* mutations, and response to cetuximab. The progression-free survival (PFS) and the overall survival (OS) were estimated by the Kaplan-Meier method, and the groups were compared with the log-rank test. All statistical tests were two-sided. A $P$ value of $<0.05$ was considered statistically significant. Statistical analyses were done using SPSS software version 16.0 (SPSS, Inc.).

## Results

***Mutation analysis.*** *KRAS* mutations were detected in 1 of 38 (2.6%) responders and 76 of 161 (47%) nonresponders. Twenty-three (12%) of the 200 samples carried 1 of the *PIK3CA* mutations included in our assay. These missense mutations were found in exon 1 [p.R88Q ($n$ = 1)], exon 9 [p.N345K ($n$ = 1), p.E542K ($n$ = 5), p.E545K ($n$ = 11), and p.Q546K ($n$ = 1)], and exon 20 [p.H1047R ($n$ = 3)]. One sample carried a mutation in both exon 9 (p.E545K) and 20 (p.H1047R). All mutations were confirmed by sequencing; however, it must be noted that for at least 8 samples (4%), the mutation may have been missed by sequencing if Sequenom data had not been available. One new mutation, A1565G, was identified by sequencing and was not present on the Sequenom assay. This patient also showed an E542K mutation and was thereby classified in the *PIK3CA* mutant group.

Nine of 77 (11.7%) *KRAS* mutants and 14 of 122 (11.5%) *KRAS* WT tumors harbored a *PIK3CA* mutation ($P$ = 1.00), thus indicating that *PIK3CA* mutations occurred independently of the *KRAS* mutation status.

***Correlation with response.*** Response was observed in 39 of 200 (19.5%) patients. As expected, almost all responders were in *KRAS* WT tumors [37 of 122 (30.3%)], whereas 1 response occurred in a *KRAS* mutant tumor [1 of 77 (1.3%)]. We found no correlation between the presence of a *PIK3CA* mutation and impaired response to cetuximab. *PIK3CA* mutations were detected in 5 of 39 (13%) of the responders and 18 of 160 (11%) of the nonresponders ($P$ = 0.781). In *KRAS* WT patients, the response rate is 32 of 108 (29.6%) in *PIK3CA* WT versus 5 of 14 (35.7%) in PIK3CA mutants (Fisher's Exact test, $P$ = 0.758; Table 2). Table 3 summarizes the distribution of all mutations in the different response groups.

**Table 1.** Overview of patients treated in the different clinical trials

| Clinical trial | Cetuximab monotherapy (no of patients) | Cetuximab + irinotecan (no of patients) | Total |
|---|---|---|---|
| BABEL | 0 | 48 | 48 |
| EVEREST[5] | 0 | 117 | 117 |
| SALVAGE[7] | 8 | 0 | 8 |
| BOND[6] | 8 | 16 | 24 |
| By label | 0 | 3 | 3 |
| Total | 16 | 184 | 200 |

**Table 2.** Summary of *PIK3CA* mutations in responders versus nonresponders

| Codon | Cases in responders | Cases in nonresponders |
|---|---|---|
| p.R88Q | 0 | 1 |
| p.N345K | 0 | 1 |
| p.E542K | 2 | 3 |
| p.E545K | 3 | 8 |
| p.Q546K | 0 | 1 |
| p.H1047R | 0 | 3 |
| p.E545K/p.H1047R | 0 | 1 |
| TOTAL | 5 | 18 |

The PFS was significantly better in *KRAS* WTs compared with mutants (median PFS 24.0, weeks [95% confidence interval (95% CI), 22.0-26.0] versus 12.0 weeks [95% CI, 8.6-15.4]; Log-rank test, $P < 0.0001$; hazard ratio, 0.56 [95% CI, 0.41-0.77)]. Similarly, the OS was significantly better in KRAS WTs compared with mutants [median OS, 45.0 weeks (95% CI, 35.9-54.1) versus 26.0 weeks (95% CI, 18.8-33.2); Log-rank test, $P < 0.0001$; hazard ratio, 0.50 (95% CI, 0.37-0.69)].

Median PFS (Fig. 1) and OS (Fig. 2) in *PIK3CA* mutant and WT patients were 24 weeks (95% CI, 17.3-30.7) versus 18 weeks (95% CI, 16.6-19.4), and 45 weeks (95% CI, 32.5-57.5) versus 39 weeks (95% CI, 35.4-42.6; $P = 0.760$ and $P = 0.698$, respectively).

Adding the therapy (monotherapy or combination) as a covariate did not affect the results for *PIK3CA*. The type of therapy (combination versus monotherapy) was an independent predictive factor for OS [hazard ratio, 0.54 (0.32-0.91); $P = 0.02$] but not for PFS.

## Discussion

Response prediction to the EGFR inhibitors remains a major challenge. The largest success till now has been to identify negative predictive markers such as KRAS that would render tumors EGFR independent and therefore not sensitive to EGFR inhibition (10).

Mutations in *KRAS* occur in almost 40% of the patients, and activating mutations of the gene that encodes the catalytic subunit of class 1A PI3K (*PIK3CA*) have also been identified in significant numbers of breast and other cancers (8, 11). As a consequence, *PIK3CA* and *KRAS* are the most commonly mutated oncogenes identified in human cancer (12, 13). However, both these oncogenic mutations play different roles in colorectal carcinogenesis: whereas *KRAS* mutations are involved in an earlier stage such as adenoma, *PIK3CA* mutations may be involved in a later stage such as invasive carcinoma.

A recent addition to the family of genes encoding resistance to EGFR inhibitors is *BRAF* (14). Indeed, a *BRAF* V600E mutation was detected in 11 patients of 80 *KRAS* WT patients, with none of these patients responding to EGFR inhibitors. Likewise, mutations in *PIK3CA* could also explain resistance to anti-EGFR treatment. Therefore, it was our aim to investigate the role of *PIK3CA* mutations in resistance to cetuximab in *KRAS* WT patients.

In our study, *KRAS* mutations were detected in 1 of 38 responders and 76 of 161 nonresponders ($P < 0.0005$), which is in line with previously published papers (1, 15, 16). Twelve percent of metastatic colorectal cancer patients carried one of the *PIK3CA* mutations that could be detected by our assay. The frequency and distribution of the various *PIK3CA* mutations detected in our colorectal cancer patients are also in agreement with previous studies (8, 17–22) and with frequencies reported in the Catalogue Of Somatic Mutations In Cancer database, with p.E542K, pE545K, and p.H1047R being most commonly mutated. Although we agree that several groups have reported a higher mutation frequency in *PIK3CA* (13), others have found mutation rates comparable with our results. For instance, Nosho et al. (18) sequenced exon 9 and 20 of *PIK3CA* and reported a mutation frequency of 15% in 590 primary colorectal cancer. Velho et al. (17) also screened exon 9 and 20 and found a *PIK3CA* mutation frequency of 13.6% ($n = 100$). Because the Sequenom assay was focused on the most frequent mutations (80%) in exon 9 and 20, these results are in fact comparable with those reported in the literature.

In colorectal cancer, deregulation of the PI3K signaling pathway is frequent (10-30%) and increases with stage (13, 17–23). Some studies reported that 8% to 24% of the tumors carried both *PIK3CA* and *KRAS* mutations, whereas other failed to report such frequencies (18–22). Our study did not show any association between the presence of a *KRAS* and a *PIK3CA* mutation.

Our data suggest that activating mutations of PI3K do not play a major role in causing resistance to the EGFR-inhibitor cetuximab because five patients with *PIK3CA* mutations showed an objective response to the drug and response rates

**Table 3.** Distribution of mutations throughout the response groups

| Mutation | Best response | | | | |
|---|---|---|---|---|---|
| *n* (row %) | CR | PR | SD | PD | Total |
| KRAS WT/PI3K WT | 1 (0.9) | 31 (29) | 55 (51) | 21 (19) | 108 (100) |
| KRAS WT/PI3K mutant | 0 | 5 (36) | 8 (57) | 1 (7) | 14 (100) |
| KRAS mutant/PI3K WT | 0 | 1 (1) | 42 (62) | 25 (37) | 68 (100) |
| KRAS mutant/PI3K mutant | 0 | 0 | 7 (78) | 2 (22) | 9 (100) |
| Total | 1 (0.5) | 37 (19) | 112 (56) | 49 (25) | 199* (100) |

NOTE: %, distribution of responses in each mutation group.
Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*KRAS data were not available for one of the patients.

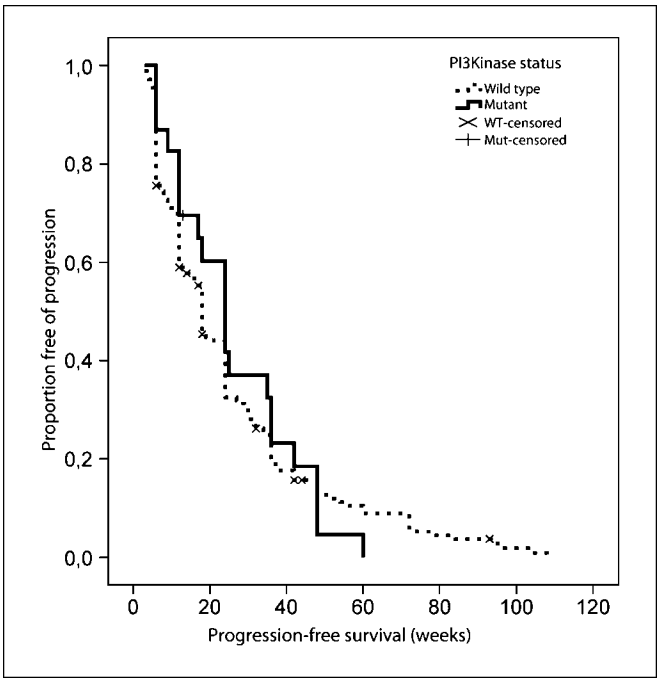


**Fig. 1.** Kaplan Meier PFS analysis in *PIK3CA* mutant versus WT patients.

were equal between *KRAS* WT without a PI3K mutation. There are several arguments that could explain why *PIK3CA* may not be major regulators of anti-EGFR resistance. First, as stated previously, *PIK3CA* is mutated in diverse human cancers, but the functional aspect of some of the mutations still has to be defined. In 2005, Samuels et al. (24) showed in a preclinical model that both *PIK3CA* E545K and H1047R mutations promote cell growth and invasion of human cancer cells. It is unclear whether other *PIK3CA* mutations have the same functional properties. Second, we only investigated 80% of known *PIK3CA* mutations that are published in literature for colorectal cancer. As shown recently in lung adenocarcinoma, genomic screening resulted in the detection of new somatic mutations (25). It is thus possible that we have missed other mutations in *PIK3CA* that may confer other effects. Third, it has been shown that the frequency of *PIK3CA* mutations increases in metastases. Because we only used primary tumors in our study, the additional incidence of *PIK3CA* mutations in metastatic disease could have been masked.

Finally, an alternate explanation for our results could be that as *PIK3CA* mutations are a negative prognostic factor (21), this poor prognosis could be reversed by treating those patients with cetuximab.

Sample size is important in these exploratory studies that aim to define the effect of a novel biomarker. A power analysis showed that with an expected response rate in unselected patients of 25%, our sample size (200) would have allowed to detect a significant drop in response rate to 4% or lower in *PIK3CA* mutants with a power of 85% at a significance level of 0.05. These shifts of response rates are in the range of those observed in the unselected versus *KRAS* selected population (26). However, because EGFR inhibitors are now restricted to *KRAS* WT patients, it is more relevant to evaluate the potential shift in response rate in the *KRAS* WT subgroup. Hence, with our WT cohort, we would be able to detect a drop in response rate to 7% or lower. In summary, this analysis shows that *PIK3CA* mutations do not have a negative predictive effect in the same order of magnitude as *KRAS* mutations (near 0% response rates). If they had an impact similar to KRAS, this would have been detected by our study. However, our study is not powered sufficiently to detect mild negative effects of *PIK3CA* mutations such as for example a reduction in response rates from 40% to 20% in *KRAS* WT. This would need a study with over 400 *KRAS* WT patients, and may be the focus of large international collaborative efforts in the future.

Theoretically *KRAS* and *PIK3CA* mutations could have the same effect, as they are one of the two (PI3K/Akt and KRAS/MAPK) effector pathways downstream of EGFR (27).

A negative predictive role for pi3K mutations has recently been shown in breast cancer (28) where *PIK3CA* mutations, present in 25% (14 of 55) of tumors, mediated resistance to the anti-HER2 monoclonal antibody trastuzumab (Herceptin) *in vitro* and *in vivo*. *PIK3CA* mutations contributed to an increased risk for progression ($P = 0.0052$); and a combined analysis of PTEN status and PIK3CA status (loss of PTEN and PI3K mutations being mutually exclusive events leading to a similar pathway deregulation) not only identified twice as many patients at increased risk for disease progression, but the combined analysis also reached statistical significance as a biomarker for prognosis after trastuzumab therapy.

The stronger single effect of *PIK3CA* mutations observed in breast cancer than in our series may be due to a different ERBB (HER) receptor being targeted in breast and colorectal cancer. Active receptors can stimulate the PI3K signaling pathway via binding of the p85 subunit of PI3K resulting in activation of AKT. Due to the presence of multiple binding sites for p85, ERBB3 is the most efficient activator of PI3K (29). ERBB3 has impaired kinase activity and therefore only becomes

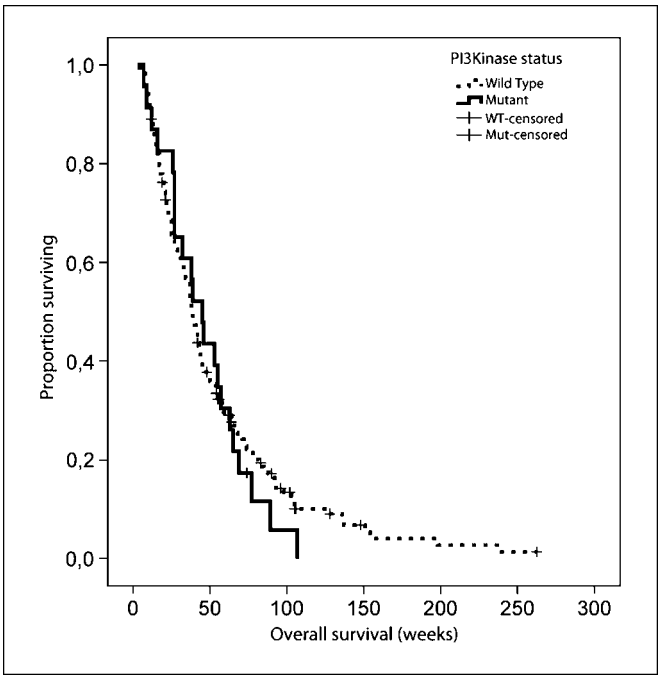


**Fig. 2.** Kaplan Meier OS analysis in *PIK3CA* mutant versus WT patients.

phosphorylated when it is dimerized with another ERBB receptor, with ERBB2 being its preferred partner (30). ERBB1 (EGFR) has no consensus sequence for the p85 subunit but can still couple to this pathway via GAB1 and Ras/raf/MEK/ERK1/2.

This could explain why the PI3K mutation status could play a more central role in breast cancer treated with HER2-targeting drugs, in contrast to colorectal cancer treated with the ERBB1 (EGFR)-targeting drugs such as cetuximab. Another likely explanation could be the fact that we assessed only a single marker and future studies should address the prognostic and predictive effect of a comprehensive analysis of the PI3K/PTEN/AKT axis in colorectal cancer. Indeed, for example methylation and, hence, silencing of the PTEN gene, resulting in activation of the PI3K/AKT pathway, is commonly seen in human cancers and can therefore also play an important role in response to EGFR inhibitors (31). To fully assess the role of the PI3K/PTEN axis in response prediction, these alterations should be taken in to account.

Finally, our study was only powered to detect large differences in response rate between the two PI3K groups, similar to those observed for *KRAS* mutations. Large populations need to be studied to detect more subtle effects.

In conclusion, our data do not provide any evidence for a strong role of *PIK3CA* mutations as a single marker in determining response to cetuximab in chemorefractory metastatic colorectal cancer.

## Disclosure of Potential Conflicts of Interest

The authors have received an unrestricted research grant from Merck Serono.

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