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CCR New Strategies

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New Strategies in Colorectal Cancer: Biomarkers of Response to Epidermal Growth Factor Receptor Monoclonal Antibodies and Potential Therapeutic Targets in Phosphoinositide 3-Kinase and Mitogen-Activated Protein Kinase Pathways

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Abstract

Initial experience with the epidermal growth factor receptor monoclonal antibodies (EGFR MoAb) in unselected patients with metastatic colorectal cancer (mCRC) showed that most of the treated patients did not derive therapeutic benefit. This outcome has driven the search for biomarkers for this population. Recent advances have further shown the heterogeneous nature of this disease with multiple interlinked pathways being implicated. Two such pathways downstream to the EGFR, mitogen-activated protein kinase (MAPK) and (phosphoinositide 3-kinase) PI3K, have gained increasing attention and become targets for development of novel biomarkers and therapeutic agents. Here, we highlight recent progress.

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Background

Epidermal growth factor receptor (EGFR) is a 170-KDa transmembrane growth factor receptor, which, after being bound by 1 of 10 different ligands, undergoes homo- or heterodimerization and triggers a series of signaling events via a receptor-linked tyrosine kinase, mainly through the RAS/RAF/MEK and the phosphoinositide 3-kinase (PI3K)/AKT pathways (1, 2). Currently, two EGFR monoclonal antibodies (MoAb) are U.S. Food and Drug Administration (FDA) approved for metastatic colorectal cancer (mCRC), and several are in development. Cetuximab is a chimeric mouse-human immunoglobulin G1 (IgG1) MoAb, whereas panitumumab is a fully human monoclonal IgG2 antibody. Initial EGFR MoAb trials that preselected patients on the basis of EGFR immunohistochemistry (IHC) failed to show a correlation with response to EGFR MoAb, and lead to an intense search for other predictive biomarkers downstream to EGFR (3).

KRAS is a GTPase that encodes the human cellular homolog of the transforming gene Kirsten rat sarcoma-2 virus immediately downstream to EGFR. Activating mutations of KRAS are an early component of colon cancer carcinogenesis, occurring in up to 58% of polyps > 1 cm (4–6). KRAS mutations in CRC occur with a prevalence of 30 to 40% with the most common mutations being in codons 12 and 13 of exon 2 (7–9). A randomized phase III study, evaluating panitumumab as third line therapy versus best supportive care in mCRC (n = 463, 184 with KRAS mutation), was the first large study confirming the negative predictive value of KRAS mutations. The response rate (RR) in KRAS wild-type (WT) versus mutant (MT) was 10% versus 0%, and progression free survival (PFS) 12.3 versus 7.4 weeks (10). These results were extended by the National Cancer Institute (NCIC) CO.17 trial evaluating third line cetuximab versus best supportive care in 394 patients (42% patients with KRAS mutations; ref. 11).

Several recently reported clinical trials have evaluated EGFR MoAbs in the first and second line settings in combination with cytotoxic chemotherapy. The CRYSTAL trial (540 out of 1,198 tested for KRAS mutation) evaluated FOLFIRI ± cetuximab, and the OPUS trial (233 out of 337 tested for KRAS mutation) evaluated FOLFOX ± cetuximab (12, 13). Both these trials in the first line setting confirmed the negative predictive value of KRAS mutation for benefit with EGFR MoAb. A recently updated meta-analysis of KRAS WT patients from these two studies further confirmed these findings with significant improvements in RR [odds ratio (OR) 2.16, $P < 0.0001$), PFS [hazard ratio (HR) 0.66, $P < 0.0001$], and overall survival [(OS); HR 0.81, $P = 0.006$; ref. 14]. In contrast, the large COIN trial evaluating first line fluoropyrimidine-based chemotherapy and oxaliplatin ± cetuximab did not show any benefit with the addition of cetuximab in the metastatic setting even in KRAS WT patients (15).

With regards to panitumumab, the PRIME trial evaluating FOLFOX4 ± panitumumab showed improvement in PFS (9.6 versus 8 months, $P = 0.02$) in the first line setting

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in the KRAS WT population (16). A similar reduction in PFS was seen in KRAS MT patients treated with panitumumab. The 20050181 ("181") study examined panitumumab in the second line setting (FOLFIRI ± panitumumab), and also showed improvement in PFS (5.9 versus 3.9 months, $P = 0.004$) with addition of panitumumab in KRAS WT patients (17). Overall, except for the COIN study, these studies showed improved outcomes in the KRAS WT patients with addition of EGF MoAbs to cytotoxic chemotherapy. The reasons for the contradictory findings of the COIN study, and the shorter median survival compared with other recent trials, are unclear.

Currently, it is recommended that mCRC patients being considered for EGFR MoAb therapy be tested for mutations in codons 12 and 13 in exon 2 of the *KRAS* gene (18). Although KRAS mutation testing seems to have a 100% negative predictive value for response to EGFR MoAb therapy, there is only an incremental improvement in RR, PFS, and (in some studies) OS with this selection. Thus, a significant portion of the KRAS WT patients still derives no benefit. Furthermore, most studies also indicate that KRAS is not a prognostic marker (3, 8, 19–22). Overall, these studies show a need for the search for better biomarkers for EGFR MoAb therapy.

On the Horizon

Although prior studies suggest that more than 95% of all KRAS mutations are limited to codons 12 and 13, a recent study revealed that 9 of 106 colon cancer samples (8.4%) had mutations outside of these hotspots, with 7 in codon 146. Another 2% had KRAS gene amplification. Furthermore, these genetic changes had a differential phenotypic expression *in vitro* with a mutation in codon 164 actually being equivalent to KRAS WT (23). Although these new findings may contribute to our understanding of EGFR MoAb resistance in KRAS WT patients, the majority seem to have aberrant signaling through other components of the MAPK pathway such as BRAF, MEK, and/or other pathways downstream to EGFR, especially PI3K (24). Recent advances provide considerable information on these pathways, which is being used to generate new predictive and/or prognostic information (Table 1), and novel therapeutic options (Table 2).

BRAF is a serine-threonine kinase belonging to the RAF family of proteins (BRAF, CRAF, and ARAF). KRAS activates BRAF, which in turn activates MEK (see Fig. 1; refs. 25, 26). Large population-based studies indicate that activating mutations of BRAF occur in 10 to 20% of CRC (27–29), and all these mutations are in the P-loop (exon 11) or activation segment (exon 15) of the kinase domain of the gene. The mutation in the kinase domain is a single substitution (V600E) accounting for nearly 90% of all the mutations (30). Interestingly, with very rare exceptions, KRAS and BRAF mutations are mutually exclusive, and BRAF mutations are far more frequent in colon than rectal cancers (9). BRAF mutations also seem to be associated with right-sided tumors with sporadic microsatellite instability (MSI) and CpG island methylator phenotype (9, 31, 32). A recent retrospective study showed high concordance in

Table 1. Predictive biomarkers for EGFR MoAb treatment in mCRC

Biomarker	Frequency of alteration in CRC	Most frequent alterations	Predictive value for EGFR MoAbs
KRAS	30-40% with recent studies suggesting several novel mutations outside of the known hotspots in codons 12 and 13 of exon 2	Activating mutation in codons 12 and 13 of exon 2	Negative predictor
BRAF	10-20%	Activating mutation V600E in exon 15	Unclear, but possibly little predictive value
PTEN	20-40%	Loss of protein expression measured by IHC	Unclear, secondary to lack of standardized IHC scoring
PIK3CA	15-25% with several mutations scattered outside of the known hotspots in exons 9 and 20	Mutations in exons 9 and 20, with constitutive activation of PI3K pathway	Unclear, but PI3K pathway activation by PTEN loss or PIK3CA mutations appears to be negative predictor
Amphiregulin, epiregulin	NA	Overexpression of ligands	Higher values likely positive predictor
FcR polymorphisms	About 40%	FcγRIIa-FcγRIIIa H131H and/or V158V polymorphism	Unclear but FcγRIIIa alone may have positive predictive value

Abbreviations: NA, not applicable, continuous variable.

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Table 2. Summary of selected drugs in clinical trials targeting MAPK and/or PI3K pathways

Class	Drugs	Trials	Comments
EGFR MoAb	Nimotuzumab (h-HR3), zalutumumab (HuMax-EGFR), necitmumab (IMC-11F8), matuzumab (EMD7200)	Phase I, II	Nimotuzumab and zalutumumab in trials in irinotecan resistant mCRC. Necitumumab in trials as first line with mFOLFOX6
RAF inhibitors	Sorafenib, PLX-4032, RAF265, XL281	Phase I-III	Sorafenib in several phase II-III trials in mCRC to look for benefit in relation to KRAS and BRAF mutations
MEK inhibitors	CI-1040, PD0325901, AZD6244, AS703026, GSK1120212, PD-325901, RO5126766, GDC-0973, TAK-733	Phase I, II	AZD6244 in phase II trial in patients with refractory mCRC
PI3K inhibitors	PI3K and mTOR inhibitors: BEZ235, BGT226, XL765, SF1126, PF-04691502 PI3K inhibitors: BKM120, XL147, GDC0941, PX-866, CAL-101, GSK1059615	Phase I, II	Several agents in phase II trials in patients with metastatic breast cancer
AKT inhibitors	perifosine (KRX-0401), MK2206, VQD-002 (API-2), XL418, GSK2141795, SR13668, GSK690693, nelfinavir	Phase I, II	Nelfinavir is an antiretroviral protease inhibitor shown to inhibit AKT
mTOR inhibitors	Rapamycin-sirolimus, temsirolimus, everolimus, AP23573, AZD8055, OSI-027, palomid 529	Phase I, II	Everolimus with bevacizumab in patients with refractory mCRC. Sirolimus in phase II trial with patients having germline mutations in PTEN

NOTE: Data are from http://clinicaltrials.gov.

the mutational status of BRAF and KRAS between primary and metastatic tumor biopsies, with less than 5% of the tumors acquiring a new mutation at metastasis (33). This finding suggests that KRAS and BRAF mutations can be analyzed with fairly high accuracy by sequencing specific hot spots from either the primary or the metastatic site.

Evidence suggests that BRAF has a significant negative prognostic value and possibly no predictive value for EGFR-targeting MoAb therapy. A retrospective analysis of mCRC patients who received therapy with EGFR MoAbs showed that 11 out of 113 (10%) tumors had a BRAF V600E mutation, and none of them responded to EGFR monotherapy ($P = 0.029$). Moreover, those with a mutation had significantly shorter PFS and OS (34). A retrospective analysis of 516 tumors from the phase III CAIRO2 trial, evaluating the role of two biological agents in the first line setting (CapOx + bevacizumab ± cetuximab), with 8.7% of the tumors having this mutation showed a negative prognostic value for BRAF V600E with significantly decreased OS (15.2 versus 21.5 months, $P = 0.01$), PFS (6.6 versus 10.4 months, $P = 0.001$), but no difference in rate of response to cetuximab (35). In another retrospective study of 173 refractory KRAS WT mCRC samples treated with cetuximab that analyzed for EGFR amplification, PTEN and BRAF status again showed that 5 out of 116 tumors had BRAF V600E, and this mutation was weakly associated with lack of response ($P = 0.063$), but strongly associated with shorter PFS and OS ($P = 0.001$; ref. 36). Data from Ogino and colleagues also showed that the 105 out of 649 patients (17%) who had BRAF V600E mutation had increased CRC-specific mortality (multivariate HR 1.97), confirming the negative prognostic value of BRAF mutation (32). A planned analysis of 1,404 patients from PETACC-3 [irinotecan versus irinotecan + infusional 5-fluorouracil (5-FU) in adjuvant setting for stages II and III CRC] showed that 7.9% of patients had BRAF mutations, and they had decreased OS [HR 1.66, 95% confidence interval (CI) 1.15-2.40, $P = 0.0069$]. This effect was more pronounced in patients with MSI-low and MSI-stable tumors (HR 2.19, 95% CI 1.43-3.37, $P = 0.0034$). Finally, a recent update on the CRYSTAL trial showed that 59 out of 625 evaluated patients (9%) had the BRAF V600E mutation. In this small group, no difference was found in OR, OS, and PFS when cetuximab was added to FOLFIRI. However, when compared with the patients with BRAF WT, these patients had lower median OS (10.3 versus 21.8 months on FOLFIRI and 14.1 versus 25.1 months on FOLFIRI + cetuximab) and median PFS (5.6 months versus 8.8 months on FOLFIRI and 8 months versus 10.9 months on FOLFIRI + cetuximab; ref. 19).

Apart from KRAS and BRAF, two other groups of molecules related to the EGFR pathway have also emerged as potential biomarker candidates: EGFR ligands and FcγR polymorphisms. EGFR is activated by a variety of ligands such as amphiregulin, epiregulin, EGF, and transforming growth factor-α. Using transcriptional profiling and reverse transcriptase-PCR results of 80 mCRC patients, Khambata-Fort and colleagues showed that elevated epiregulin and amphiregulin had a positive predictive value in determining response to cetuximab (37). Furthermore, patients with

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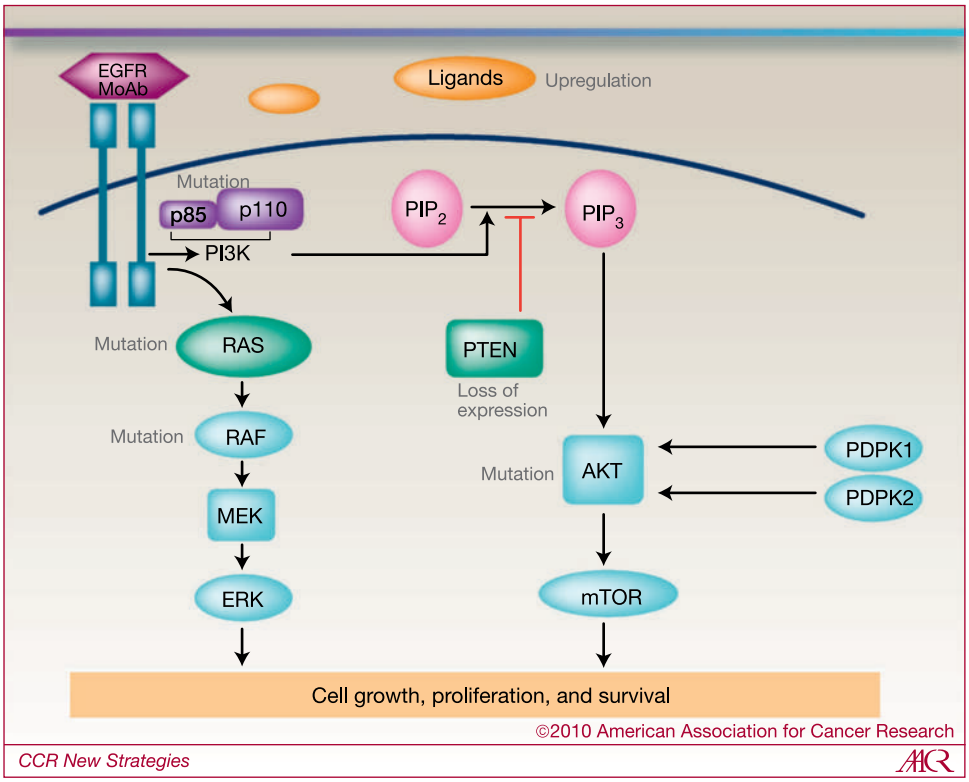

Fig. 1. EGFR signaling pathways. Ligand binding to EGFR leads to receptor dimerization. Subsequently, downstream pathways, including the MAPK pathway (through RAS) and the PI3K pathway (through p110 and subsequently PIP_3), are activated. In the PI3K pathway, PIP_3 activates AKT by facilitating its phosphorylation by PDPK1 and PDPK2 (mtorC2). AKT, in turn, activates mTOR. PTEN regulates activity of the PI3K pathway by converting PIP_3 back to PIP_2. In the MAPK pathway, RAS, RAF, MEK, and ERK are activated by sequential kinase activity. Both the pathways regulate multiple cellular processes vital for the malignant cell. Ligand binding and, therefore, downstream activation are blocked by EGFR MoAb. The most common aberrations leading to inappropriate activation of the pathways in cancer cells are also depicted.

higher levels of these ligands had a significantly better PFS (103 and 115 versus 57 days for high versus low levels of epiregulin and amphiregulin, respectively). These results were validated in two other recent studies. A retrospective analysis of 385 patients enrolled in the NCIC CO.17 trial showed that patients with both high epiregulin and KRAS WT had a significantly better response to cetuximab (38). A second study evaluated 220 mCRC samples from patients treated with irinotecan + cetuximab and showed that patients with KRAS WT and higher levels of epiregulin and amphiregulin had significantly better PFS, OS, and response to treatment (39). Finally, another study evaluated 95 mCRC patients treated with cetuximab + irinotecan and similarly showed significant prognostic and predictive values to higher levels of epiregulin and amphiregulin (40).

Cetuximab, being an IgG1 antibody, should be capable of initiating antibody-dependent cell-mediated cytotoxicity (ADCC) mediated via the Fc-receptor (FcR). Data from other MoAbs (trastuzumab in breast cancer, rituximab in lymphoma) suggest that polymorphisms in two receptor genes, *FcγRIIa-H131R* and *FcγRIIIa-V158F*, are clinically relevant. These polymorphisms were evaluated in normal colonic tissue from 69 irinotecan-resistant mCRC patients treated with irinotecan + cetuximab. In patients with KRAS WT, the subset with genotypes FcγRIIIa-158V/V and/or FcγRIIa-131H/H were found to have better PFS and OS as compared with carriers of the polymorphisms (41). However, further analysis suggested that there is a high degree of linkage disequilibrium between these two polymorphisms in the Caucasian population and only FcγRIIIa-158V/V has predictive value (42). If true, this finding would suggest better response with antibodies that can initiate ADCC; however, there is no difference in RRs between cetuximab and panitumumab. Moreover, if this effect were clinically significant, KRAS mutant tumors should also have some benefit with cetuximab that has not been observed so far.

Although attempts at developing drugs targeting KRAS (farnesyl inhibitors) have largely been unsuccessful, several BRAF inhibitors have been discovered. Sorafenib is an oral multikinase inhibitor that targets both WT BRAF and oncogenic BRAF V600E and has *in vitro* activity in CRC cell lines with this mutation (34, 43). However, sorafenib also inhibits multiple other tyrosine kinases, especially those involved in angiogenesis, and it is unclear how much effect sorafenib exerts through the MAPK pathway. Currently, several phase II-III clinical trials are evaluating the role of sorafenib in mCRC treatment in relation to KRAS and/or BRAF mutation status, which should help define the role of this agent in this setting. More selective RAF inhibitors are being studied and are in various stages of development (Table 2; refs. 44, 45). However, a series of recent studies has shown that BRAF V600E inhibitors may, in fact, cause paradoxical activation of the MAPK pathway in KRAS MT/BRAF WT or kinase dead tumors through formation of CRAF homo- or heterodimers (with BRAF), and subsequent interaction with the upstream mutated KRAS (46–48). The clinical implications of these findings in colorectal cancer treatment are yet unclear. Although BRAF is the best-studied kinase, at least five kinases phosphorylate MEK (ARAF, BRAF, CRAF, Tpl2,

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and Mos). This high level of redundancy at the level of activation of MEK would suggest that inhibition of this enzyme may be a more effective strategy to inhibit MAPK signaling than at KRAS or BRAF, and multiple agents have been developed toward this target (Table 2; ref. 45).

PI3K is an effector pathway of EGFR and multiple other receptors. This pathway has been reviewed extensively (49, 50), and will only be summarized briefly here. PI3Ks are divided into classes and subclasses of which class IA is the most extensively studied. The class IA PI3Ks are heterodimers consisting of a regulatory subunit, p85 that has three isoforms, p85α, p85β, and p55γ collectively called p85, and the p110 catalytic subunit with p110α, p110β (ubiquitous distribution), and p110γ (expressed mainly in leukocytes) isoforms. p85α has an inhibitory effect on the p110α catalytic subunit in the basal state, but upon activation by receptor tyrosine kinases or G-protein coupled receptors, it mediates localization of the enzyme to the membrane. The p110α catalytic subunit, upon activation, phosphorylates membrane phosphatidyl-inositol-, 4,5-triphosphate (PIP_2) to phosphatidyl-inositol-3, 4,5-triphosphate (PIP_3). PIP_3 is an important lipid second messenger that provides docking sites for multiple downstream components, including a putative 3-phosphoinositide-dependent protein kinase 1 (PDPK 1) and AKT. The cellular levels of PIP_3, and thus the activity of the PI3K pathway, are tightly regulated by the opposing lipid phosphatase activity of the tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN). AKT is a serine-threonine protein kinase expressed as three isoforms (AKT1, AKT2, and AKT3), and upon docking with PIP_3, undergoes phosphorylation at two sites by PDPK1 and PDPK2 to be activated. Once activated, it activates multiple downstream proteins important in protein synthesis [mammalian target of rapamycin (mTOR), S6K], metabolism (glycogen synthase3), and cell survival and proliferation (FOXO1, MDM2, etc). mTOR is also a serine-threonine protein kinase belonging to the PI3K superclass, with significant structural homology to the PI3Ks. mTOR exists in two distinct complexes (mTORC1, mTORC2), each with multiple subunits. Upon activation, mTORC1 targets ribosomal and translational components important in protein synthesis, whereas mTORC2 is thought to be the primary PDPK2 phosphorylating AKT.

Recent studies have shown convincing evidence that many components of the PI3K pathway are frequently altered in numerous human cancers. Germline mutations in the *PTEN* gene cause syndromes with predisposition to hamartomatous CRC (Cowden's syndrome; ref. 51). Although somatic mutations in the *PTEN* gene are rare in CRC, several retrospective studies have shown by using IHC that 20 to 40% of CRC samples have loss of PTEN expression (52, 53). In contrast to the *PTEN* gene, the *PIK3CA* gene encoding the p110α subunit of class IA PI3K is frequently mutated in CRC. Most studies, including the large population-based study by Nosho and colleagues (54), have only evaluated exons 9 and 20 of this gene and showed a mutation rate of 10 to 15% (52–56). A retrospective study that sequenced all the coding exons of this gene, in contrast, revealed that 74 of the 234 (31.6%) evaluated colorectal cancer samples had a mutation in the *PIK3CA* gene. Although this study suggests that a significant proportion of the mutations may occur outside exons 9 and 20, this study, like others on this gene, is not population based and thus may have suffered from a selection bias (57). Overall, on the basis of current data, it seems that the PIK3CA gene mutation frequency in CRC is probably between 15 and 25%, and that these mutations show a more scattered pattern than those in the KRAS and BRAF genes. The PIK3R1 gene encoding p85α has also been reported to be mutated in CRC (58).These mutations cause constitutive activation of the PI3K pathway in preclinical models (59, 60).

The data on PTEN status are more limited. Because PTEN can be mutated, deleted, or silenced, ascertainment of PTEN status is usually done at the protein level. A small retrospective study showed that none of 11 patients with PTEN loss had response to cetuximab, in contrast to 10 out of 16 with intact PTEN expression who had partial response ($P < 0.001$; ref. 61). Another retrospective study evaluated PTEN expression by IHC and KRAS mutation analysis by DNA sequencing in primary, metastatic, and paired (96, 59, and 53, respectively) samples from patients with irinotecan-resistant mCRC treated with cetuximab (53). The concordance rate between primary and metastatic tumors for PTEN was 60%, and PTEN loss in metastatic tumors significantly predicted lack of response to cetuximab (36% versus 5%, $P = 0.007$). Moreover, patients with PTEN null metastases had shorter PFS (3.3 versus 4.7 months, $P = 0.005$), which was even more significant in KRAS WT patients. However, in sharp contrast, the PTEN analysis of the primary tumor did not reveal any predictive or prognostic information. Although the relatively low concordance rate between primary and metastatic tumors for PTEN expression could be secondary to selection of clonal populations during metastasis, it could also be secondary to the subjective nature of IHC testing with significant method and observer variability. This consideration and the possible need to analyze PTEN from metastatic tumors may limit the role of PTEN as a biomarker in CRC. This study also analyzed AKT expression by IHC and failed to show any correlation with prognosis or response to cetuximab. Studies, however, have shown a role for mutations or amplification of AKT1 and amplification of AKT2 gene in aberrant activation of this pathway (62).

Another retrospective study analyzing KRAS, PTEN, and PIK3CA mutations in 110 mCRC patients treated with either of the EGFR MoAbs showed that none of the 15 patients with a PIK3CA mutation responded to treatment ($P = 0.038$). This effect was even stronger when limited to patients with KRAS WT ($P = 0.016$). Furthermore, patients with PIK3CA mutations had shorter PFS ($P = 0.0035$), which was again more significant when limited to KRAS WT tumors ($P = 0.0021$). PTEN loss was also associated with decreased OS ($P = 0.0048$; ref. 52). However, Prenen and colleagues, who retrospectively analyzed PIK3CA and KRAS mutation status in 200 mCRC patients treated with cetuximab ± irinotecan, provided conflicting data. No

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significant difference was found between PIK3CA WT and MT in response to cetuximab or prognosis in their study (56).

PIK3CA mutations were also analyzed as a prognostic marker in 450 patients with resected nonmetastatic CRC. This study showed that upon multivariate analysis controlling for other risk factors for recurrence, PIK3CA mutations predicted increased CRC specific mortality (multivariate HR 2.23; 95% CI, 1.21-4.11) However, this effect seemed to be limited to patients with KRAS WT (55). PIK3CA mutations were also shown to be associated with increased risk of local recurrence upon multivariate analysis in 19 out of 240 patients with resected rectal cancer who did not receive radiation (HR 3.4; 95% CI 1.2-9.2, $P = 0.017$; ref. 63).

Given the key role of the PI3K pathway in many cell processes vital for tumorigenesis, this pathway has been targeted recently for drug development. The first generation PI3K inhibitors (Wortmannin and LY294002) were nonselective for the different classes and had poor pharmacological properties (49, 50). Chemotypes more specific to PI3K class I, and further, more specific to different isoforms, are in various stages of development (Table 2). One of the p110α specific compounds, PI-103, was found to have the ability to inhibit mTOR as well, which is not surprising given the structural homology between PI3Ks and mTOR. This finding has led to the development of a new class of compounds with dual effects at two different sites in the pathway, with the prospect of more potent inhibition (49, 50).

Given the central role of AKT in the PI3K pathway and its potential to activate multiple downstream effector proteins, it promises to be an attractive anticancer therapeutic target and many such compounds are either in the preclinical or early phase trials. Perifosine is a synthetic alkylphospholipid similar to miltefosine, but without the latter's severe gastrointestinal side effects, and has been shown to have strong antineoplastic effects in preclinical and early clinical studies (64, 65). It is thought that this compound inhibits signaling through multiple pathways, including the MAPK, JNK, and PI3K (via AKT) pathways. Perifosine has been shown to have activity in combination with capecitabine (P-CAP) in heavily treated mCRC, especially in patients previously refractory to 5-FU in a small phase II trial that compared this combination to capecitabine with placebo (CAP). The overall median OS for P-CAP versus CAP was 24.3 versus 16.3 months ($P = 0.1348$), and the median time to progression and OS for refractory 5-FU patients (P-CAP versus CAP) were 18 versus 10.3 weeks ($P = 0.0188$) and 24.3 versus 11.7 months ($P = 0.0346$), respectively (66). In contrast to AKT inhibitors, mTOR inhibitors have been in clinical use for a considerable time, first as antifungals, later as immunosuppressives (sirolimus), and, finally, as antineoplastic agents in renal cell carcinoma (sirolimus, temsirolimus, and everolimus). However, mTOR inhibitors may have alternate effects by inhibiting either mTORC1 (protein synthesis), mTORC2 (PDPK2 activation of AKT), or both (49, 50). Multiple mTOR inhibitors are currently in development (Table 2). Preclinical studies have suggested a role for mTOR inhibitors in treatment of CRC, and this is being explored in phase I-II clinical trials with everolimus. A recent retrospective study suggested that the PI3K pathway could have a particularly high rate of derangement in hereditary nonpolyposis CRC (HNPCC), with 51 out of 58 (88%) of the analyzed tumors having alteration in at least one pathway component, raising the intriguing possibility of targeting this pathway in this small, but significant, subset of CRC (67). Finally, preclinical evidence suggests that the PI3K and MAPK pathways are strongly interlinked, and that the combination of MEK and PI3K inhibitors act synergistically to inhibit tumor cells with RAS mutations (68).

In summary, both MAPK and PI3K pathways are stimulated by EGFR, with important implications for EGFR MoAb therapy and future drug development. Current American Society of Clinical Oncology (ASCO) guidelines recommend testing only for KRAS mutations in codons 12 and 13, in patients being considered for EGFR MoAb therapy. Although the data reviewed here are mostly from retrospective studies and need to be confirmed before routine clinical use, it appears that BRAF has a negative prognostic value, and possibly little predictive value. In the case of the PI3K pathway, its activation by either loss of PTEN or mutation of PIK3CA appears to have significant negative predictive and prognostic effects that are more pronounced in KRAS WT tumors. The subjective nature of PTEN assessment, however, is a significant challenge. Finally, early data suggest that elevated levels of EGFR ligands appear to hold promise as positive predictive biomarkers. New drugs are being developed against numerous targets in these pathways, and many are in early clinical stages.

Several important issues need to be addressed during this process of developing novel drugs targeting these pathways. Treatment of patients with EGFR MoAb without KRAS preselection cost the U.S. health care system billions of dollars, and also caused toxicities in thousands of patients with no chance of benefit from 2004 to 2009. It would be far more economical to collect tumor samples and/or design biomarker-driven clinical trials during development of these targeted agents, prior to widespread use, without the knowledge gained from such studies. It is notable that the majority of biomarker studies with EGFR MoAbs originated outside of the United States, where, ironically, biomarker studies have been chronically underfunded despite massive sums spent on unselected patient populations. Finally, the ability of the cancer cell to develop drug resistance via new mutations or alternate signaling pathways also needs to be addressed by combination therapy, and, if possible, analysis of tumor tissue upon progression. Given the frequency of alterations in the PI3K and MAPK pathways in colorectal cancer, these agents represent a promising avenue of investigation.

Disclosure of Potential Conflicts of Interest

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References

1. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008;358:1160–74.
2. Shepard HM, Brdlik CM, Schreiber H. Signal integration: a framework for understanding the efficacy of therapeutics targeting the human EGFR family. J Clin Invest 2008;118:3574–81.
3. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. J Natl Cancer Inst 2009;101:1308–24.
4. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer 2003;3:459–65.
5. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67.
6. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525–32.
7. Brink M, de Goeij AF, Weijenberg MP, et al. K-ras oncogene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. Carcinogenesis 2003;24:703–10.
8. Samowitz WS, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML. 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.
9. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature 2002;418:934.
10. 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.
11. 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.
12. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–17.
13. 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.
14. Kohne C, Rougier P, Stroh C, Schlichting M, Bokemeyer C. Cetuximab with chemotherapy (CT) as first-line treatment for metastatic colorectal cancer (mCRC):A meta-analysis of the CRYSTAL and OPUS studies according to KRAS and BRAF mutation status [abstract]. Proceedings of the 2010 Gastrointestinal Cancers Symposium, Orlando (FL); 2010. Alexandria (VA): ASCO; 2010. Abstract nr 406.
15. Maughan T, Adams R, Smith C, et al. Oxaliplatin and fluoropyrimidine chemotherapy plus or minus cetuximab: the effect of infusional 5-FU or capecitabine on the outcomes of the MRC COIN trial in advanced colorectal cancer (ACRC) [abstract]. Proceedings of the 2010 Gastrointestinal Cancers Symposium, Orlando (FL); 2010. Alexandria (VA): ASCO; 2010. Abstract nr 402.
16. Siena S, Cassidy J, Tabernero J, et al. Randomized phase III study of panitumumab (pmab) with FOLFOX4 compared to FOLFOX4 alone as first-line treatment (tx) for metastatic colorectal cancer (mCRC): PRIME trial [abstract]. Proceedings of the 2010 Gastrointestinal Cancers Symposium, Orlando (FL); 2010. Alexandria (VA): ASCO; 2010. Abstract nr 283.
17. Peeters M, Price TJ, Hotko YS, et al. Randomized phase III study of panitumumab (pmab) with FOLFIRI versus FOLFIRI alone as second-line treatment (tx) in patients (pts) with metastatic colorectal cancer (mCRC):Patient-reported outcomes (PRO) [abstract]. Proceedings of the 2010 Gastrointestinal Cancers Symposium, Orlando (FL); 2010. Alexandria (VA): ASCO; 2010. Abstract nr 282.
18. 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.
19. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60–00 trial. J Clin Oncol 2010;28:466–74.
20. Zlobec I, Bihl MP, Schwarb H, Terracciano L, Lugli A. Clinicopathological and protein characterization of BRAF- and K-RAS-mutated colorectal cancer and implications for prognosis. Int J Cancer 2010;127:367–80.
21. Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. J Natl Cancer Inst 2005;97:981–9.
22. Ogino S, Meyerhardt JA, Irahara N, et al. KRAS mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89803. Clin Cancer Res 2009;15:7322–9.
23. Smith G, Bounds R, Wolf H, Steele RJ, Carey FA, Wolf CR. Activating K-Ras mutations outwith 'hotspot' codons in sporadic colorectal tumours - implications for personalised cancer medicine. Br J Cancer 2010;102:693–703.
24. Banck MS, Grothey A. Biomarkers of resistance to epidermal growth factor receptor monoclonal antibodies in patients with metastatic colorectal cancer. Clin Cancer Res 2009;15:7492–501.
25. Moodie SA, Willumsen BM, Weber MJ, Wolfman A. Complexes of Ras.GTP with Raf-1 and mitogen-activated protein kinase kinase. Science 1993;260:1658–61.
26. Catling AD, Schaeffer HJ, Reuter CW, Reddy GR, Weber MJ. A proline-rich sequence unique to MEK1 and MEK2 is required for raf binding and regulates MEK function. Mol Cell Biol 1995;15:5214–25.
27. English DR, Young JP, Simpson JA, et al. Ethnicity and risk for colorectal cancers showing somatic BRAF V600E mutation or CpG island methylator phenotype. Cancer Epidemiol Biomarkers Prev 2008;17:1774–80.
28. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 2005;65:6063–9.
29. de Vogel S, Weijenberg MP, Herman JG, et al. MGMT and MLH1 promoter methylation versus APC, KRAS and BRAF gene mutations in colorectal cancer: indications for distinct pathways and sequence of events. Ann Oncol 2009;20:1216–22.
30. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949–54.
31. Fransen K, Klintenas M, Osterstrom A, Dimberg J, Monstein HJ, Soderkvist P. Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas. Carcinogenesis 2004;25:527–33.
32. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 2009;58:90–6.
33. Italiano A, Hostein I, Soubeyran I, et al. KRAS and BRAF mutational status in primary colorectal tumors and related metastatic sites: biological and clinical implications. Ann Surg Oncol 2010;17:1429–34.
34. 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 2008;26:5705–12.
35. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. N Engl J Med 2009;361:98–9.
36. Laurent-Puig P, Cayre A, Manceau G, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. J Clin Oncol 2009;27:5924–30.
37. 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 2007;25:3230–7.
38. Jonker DJ, Karapetis C, Harbison C, et al. High epiregulin (EREG) gene expression plus K-ras wild-type (WT) status as predictors of cetuximab benefit in the treatment of advanced colorectal cancer (ACRC):Results from NCIC CTG CO.17-A phase III trial of cetuximab versus best supportive care (BSC). J Clin Oncol 2009;27:4016.
39. Prenen H, Jacobs B, De Roock W, et al. Use of amphiregulin and epiregulin mRNA expression in primary tumors to predict outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol 2009;27:4019.
40. Tejpar S, De Roock W, Biesmans B, et al. High amphiregulin and

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epiregulin expression in KRAS wild type colorectal primaries predicts response and survival befefit after treatment with cetuximab and irinotecan for metastatic disease [abstract]. Proceedings of ASCO Gastrointestinal Cancers Symposium, Orlando (FL); 2008. Alexandria (VA): ASCO; 2010. Abstract nr 411.

41. Bibeau F, Lopez-Crapez E, Di Fiore F, et al. Impact of Fc{γ}RIIa-Fc{γ} RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol 2009;27:1122–9.
42. Lejeune J, Thibault G, Ternant D, Cartron G, Watier H, Ohresser M. Evidence for linkage disequilibrium between Fcγ RIIIa-V158F and Fcγ RIIa-H131R polymorphisms in white patients, and for an Fcγ RIIIa-restricted influence on the response to therapeutic antibodies. J Clin Oncol 2008;26:5489–91, author reply 91–2.
43. Wilhelm SM, Carter C, Tang L, et al. BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099–109.
44. Hersey P, Bastholt L, Chiarion-Sileni V, et al. Small molecules and targeted therapies in distant metastatic disease. Ann Oncol 2009; 20 [Suppl 6]:vi35–40.
45. Wong KK. Recent developments in anti-cancer agents targeting the Ras/Raf/MEK/ERK pathway. Recent Pat Anticancer Drug Discov 2009;4:28–35.
46. Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. Nature 2010;464:427–30.
47. Hatzivassiliou G, Song K, Yen I, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature 2010;464:431–5.
48. Heidorn SJ, Milagre C, Whittaker S, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell 2010;140:209–21.
49. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550–62.
50. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 2009;8:627–44.
51. Wang ZJ, Taylor F, Churchman M, Norbury G, Tomlinson I. Genetic pathways of colorectal carcinogenesis rarely involve the PTEN and LKB1 genes outside the inherited hamartoma syndromes. Am J Pathol 1998;153:363–6.
52. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 2009;69:1851–7.
53. Loupakis F, Pollina L, Stasi I, et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J Clin Oncol 2009;27:2622–9.
54. Nosho K, Kawasaki T, Ohnishi M, et al. PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. Neoplasia 2008;10:534–41.
55. Ogino S, Nosho K, Kirkner GJ, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol 2009;27:1477–84.
56. Prenen H, De Schutter J, Jacobs B, et al. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clin Cancer Res 2009;15:3184–8.
57. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
58. Philp AJ, Campbell IG, Leet C, et al. The phosphatidylinositol 3′-kinase p85α gene is an oncogene in human ovarian and colon tumors. Cancer Res 2001;61:7426–9.
59. Miled N, Yan Y, Hon WC, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science 2007;317:239–42.
60. Huang CH, Mandelker D, Schmidt-Kittler O, et al. The structure of a human p110α/p85α complex elucidates the effects of oncogenic PI3Kα mutations. Science 2007;318:1744–8.
61. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 2007;97:1139–45.
62. van der Heijden MS, Bernards R. Inhibition of the PI3K pathway: hope we can believe in?. Clin Cancer Res 2010;16:3094–9.
63. He Y, Van't Veer LJ, Mikolajewska-Hanclich I, et al. PIK3CA mutations predict local recurrences in rectal cancer patients. Clin Cancer Res 2009;15:6956–62.
64. Hilgard P, Klenner T, Stekar J, Nossner G, Kutscher B, Engel J. D-21266, a new heterocyclic alkylphospholipid with antitumour activity. Eur J Cancer 1997;33:442–6.
65. Unger C, Berdel W, Hanauske AR, Sindermann H, Engel J, Mross K. First-time-in-man and pharmacokinetic study of weekly oral perifosine in patients with solid tumours. Eur J Cancer 2010;46:920–5.
66. Bendell J, Richards D, Vukelja S. Randomized phase II study of perifosine in combination with capecitabine (P-CAP) versus capecitabine plus placebo (CAP) in patients with second- or third-line metastatic colon cancer (mCRC):Updated results [abstract]. Proceedings of ASCO Gastrointestinal Cancers Symposium, Orlando (FL); 2008. Alexandria (VA): ASCO; 2010. Abstract nr 447.
67. Ekstrand AI, Jonsson M, Lindblom A, Borg A, Nilbert M. Frequent alterations of the PI3K/AKT/mTOR pathways in hereditary nonpolyposis colorectal cancer. Fam Cancer 2010;9:125–9.
68. Yu K, Toral-Barza L, Shi C, Zhang WG, Zask A. Response and determinants of cancer cell susceptibility to PI3K inhibitors: combined targeting of PI3K and Mek1 as an effective anticancer strategy. Cancer Biol Ther 2008;7:307–15.

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