Therapeutic targeting of the phosphatidylinositol 3-kinase signaling pathway: novel targeted therapies and advances in the treatment of colorectal cancer

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Abstract: Colorectal cancer (CRC) is one of the leading causes of cancer-related death in the USA, and more effective treatment of CRC is therefore needed. Advances in our understanding of the molecular pathogenesis of this malignancy have led to the development of novel molecule-targeted therapies. Among the most recent classes of targeted therapies being developed are inhibitors targeting the phosphatidylinositol 3-kinase (PI3K) signaling pathway. As one of the most frequently deregulated pathways in several human cancers, including CRC, aberrant PI3K signaling plays an important role in the growth, survival, motility and metabolism of cancer cells. Targeting this pathway therefore has considerable potential to lead to novel and more effective treatments for CRC. Preclinical and early clinical studies have revealed the potential efficacy of drugs that target PI3K signaling for the treatment of CRC. However, a major challenge that remains is to study these agents in phase III clinical trials to see whether these early successes translate into better patient outcomes. In this review we focus on providing an up-to-date assessment of our current understanding of PI3K signaling biology and its deregulation in the molecular pathogenesis of CRC. Advances in available agents and challenges in targeting the PI3K signaling pathway in CRC treatment will be discussed and placed in the context of the currently available therapies for CRC.

Keywords: AKT, colorectal cancer, PI3 kinase, signaling, therapy

Introduction

Over the past decade, remarkable progress has been made in the management of colorectal cancer (CRC). Consequently, the treatment of CRC has evolved into a multimodality therapeutic approach for advanced primary CRC and metastatic disease. Surgical resection of the primary tumor is the definitive treatment for CRC, but is only curative for localized disease. Unfortunately, up to one third of patients have locally advanced or metastatic forms of CRC at the time of diagnosis, which essentially precludes a cure by surgical treatment alone [Segal and Saltz, 2009]. Furthermore, even among patients who undergo apparently curative resection, many still develop recurrent CRC that then requires systemic treatment. The current conventional cytotoxic agents used for the systemic treatment of CRC are 5-fluorouracil (5-FU), which is a nucleotide analog that was developed in 1957;

irinotecan, which inhibits topoisomerase I; and oxaliplatin, which is a platinum-based compound and bulky DNA adduct [Wolpin and Mayer, 2008]. Although clearly shown to improve disease-free survival and overall survival in some patients, it is clear that conventional chemotherapies have a relatively modest effect on CRC compared with surgery alone [Saltz, 2010].

Advances in our understanding of cancer biology have ushered in an era of targeted therapies for many cancers, including CRC. Following the dramatic success of imatinib for the treatment of chronic myeloid leukemia, which demonstrated the potential impact of targeted therapy, great effort has been put into developing therapies that are directed against specific molecules and pathways that are altered in malignant cells. Under this premise, it is believed that targeted therapies will achieve anti-tumor efficacy with limited

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toxicity to normal tissues. Successful examples of this approach include bevacizumab, which inhibits angiogenesis by binding to vascular endothelial growth factor A, as well as cetuximab and panitumumab, which are monoclonal antibodies that block the epidermal growth factor receptor (EGFR) [Van Loon and Venook, 2011].

Recent studies have revealed a prominent role for the phosphatidylinositol-3-kinase (PI3K) pathway in promoting colon cancer cell growth and survival [Katso *et al.* 2001; Engelman *et al.* 2006; Yuan and Cantley, 2008; Zhao and Vogt, 2008], which suggests that the agents being developed to inhibit this pathway may be effective for the treatment of CRC. A recent phase II clinical trial in patients with metastatic CRC using perifosine (KRX-0401; Keryx Biopharmaceuticals, New York, NY, USA), which is an orally bioavailable alkylphospholipid signal transduction modulator that affects the PI3K/AKT (v-akt murine thymoma viral oncogene homologue kinase), c-Jun N-terminal kinase (JNK), and mitogen activated protein kinase (MAPK) signaling pathways, showed improved time to progression in the group receiving capecitabine and perifosine compared with capecitabine treatment alone [Bendell *et al.* 2011]. (On 2 April 2012 the results of the X-PECT [Xeloda® + Perifosine Evaluation in Colorectal cancer Treatment] phase III study were announced. In patients with refractory advanced colorectal cancer, perifosine + capecitabine did not improve overall survival compared with capecitabine alone.) Thus, in light of recent advances in our understanding of the biology of the PI3K pathway in CRC and of the results of ongoing clinical trials using agents that inhibit this pathway, in this review we focus on providing an up-to-date assessment of our current understanding of PI3K signaling biology and its deregulation in the molecular pathogenesis of CRC. The advances in available agents and challenges in specifically targeting PI3K/AKT in CRC treatment will be discussed and placed in the context of the currently available treatments.

The mutations and epigenetic alterations in colorectal cancer often deregulate common signaling pathways, including the PI3K/AKT pathway.

It is widely appreciated that CRCs arise and progress as a result of the accumulation of mutations and epigenetic alterations in colon epithelial cells, which is best described in the adenoma → cancer

sequence hypothesis [Fearon, 1995]. Sequencing of the colon cancer genome has revealed that there are hundreds to thousands of somatic mutations in the average colon cancer genome, but only a subset of these mutations occur repeatedly in CRCs [Sjoblom *et al.* 2006]. The high incidence of specific mutant genes has led to the concept that a subset of mutant genes may be selected for during the adenoma → cancer sequence and thus may contribute to CRC pathogenesis. These commonly occurring mutations have been called 'driver mutations' to reflect their potential role in causing the formation of CRC [Wood *et al.* 2007]. Furthermore, elucidation of the functions of these cancer genes has also made it clear that CRCs commonly deregulate key signaling pathways that are presumed to play a central role in the formation of the CRCs. These signaling pathways appear to be essential for maintaining homeostasis in normal colon epithelial cells, whereas deregulation of these signaling pathways leads to the hallmark behaviors of cancer that transform normal cells into CRC. In CRCs, the most common deregulated signaling pathways include the WNT/APC/CTNNB1, TGFB1/SMAD, RAS/RAF/MAPK, PI3K/AKT, and p53 pathways [Pritchard and Grady, 2011] (WNT, Wingless integrated in tumors; APC, Adenomatous Polyposis Coli; CTNNB1, gene name for Beta-catenin; TGFB1, transforming growth factor beta 1; MAPK, mitogen activated protein kinase). In this review, we will focus on the PI3K/AKT pathways because defects in these pathways appear to be important for the growth and survival of colon cancer cells, and because therapies that inhibit this pathway are under intense development, with many agents in early-phase clinical trials [Ihle *et al.* 2011; Kobayashi *et al.* 1999].

The PI3K signaling pathway and its role in intestinal development and homeostasis

The PI3K signaling pathway

The PI3Ks are a family of lipid and protein kinases that are divided into three classes (I, II, and III) based on their structures and substrate specificity [Cantley, 2002]. The class I PI3Ks are further divided into class IA and IB. Class IA PI3K is strongly expressed in CRC cell lines [Shao *et al.* 2004]. The activation of class IA PI3K typically results from the activation of receptor tyrosine kinases (RTKs) (e.g. EGFR activation), and this

class of PI3K has been extensively studied because of the role that RTKs play in cancer [Vivanco and Sawyers, 2002]. In light of this association with receptor tyrosine kinase pathway activation and the recent finding that up to 32% of colorectal cancers have mutations in the genes that encode this enzyme complex (e.g. *PIK3CA*), this article focuses specifically on the class IA PI3Ks, referring to them as PI3Ks unless otherwise specified [Samuels, 2004].

The molecular mechanisms of PI3K signaling and the coordinately regulated signaling networks have been discussed in detail and the interested reader is directed to previously published outstanding reviews on this topic [Engelman *et al.* 2006; Katso *et al.* 2001; Luo *et al.* 2003; Yuan and Cantley, 2008; Zhao and Vogt, 2008]. Briefly, class IA PI3Ks are heterodimers that are composed of a p85 regulatory subunit and a p110 catalytic subunit. There are three p85 isoforms, p85 α , p85 β , and p85 γ , and three p110 isoforms, p110 α , p110 β , and p110 γ . The p85 subunit inhibits the catalytic p110 subunit in the basal unstimulated state. Upon stimulation of the cells by growth factors, the p110 subunit directly associates with RTKs through the physical interaction of its SH2 domain with phosphotyrosine residues on the kinase receptor. In some cases, the p85 regulatory subunit can indirectly interact with RTKs through intermediate phosphoproteins (e.g. the insulin receptor substrates [IRSs]) (Figure 1) [Luo *et al.* 2005].

The primary consequence of PI3K activation is to catalyze the conversion of membrane-bound phosphatidylinositol-(4,5)-phosphate (PIP2) to phosphatidylinositol-(3,4,5)-phosphate (PIP3) (Figure 1). As a second messenger to activate downstream pathways, PIP3 works as a ligand to recruit pleckstrin homology (PH) domain-containing proteins (most notably the serine-threonine protein kinase AKT1 and phosphoinositide-dependent kinase 1 (PDK1)) to the inner surface of the cell membrane. Once positioned at the cell membrane, AKT1 is activated by PDK1 through phosphorylation of threonine (T) 308, which is in the activation loop of AKT1. The full repertoire of AKT functions is then achieved when it undergoes phosphorylation of serine (S) 473, which is in the hydrophobic motif domain, by the protein mechanistic target of rapamycin (serine/threonine kinase) complex 2 (mTORC2) or DNA polymerase kinase [Bozulic and Hemmings, 2009]. Once activated, AKT is poised to serve as a central

node for regulating a variety of cellular functions, including, but not limited to, proliferation, cell survival, metabolism, and angiogenesis [Manning and Cantley, 2007]. Such pleiotropic effects of AKT are achieved by its ability to phosphorylate a broad spectrum of substrates, all of which share the consensus sequence RXXXS/T for AKT phosphorylation. These substrates include glycogen synthase kinase 3 β (GSK3 β), forkhead box O1 (FOXO1), AKT1 substrate 1 (proline-rich) (AKT1S1), cyclin-dependent kinase inhibitor 1A (CDKN1A), BCL2-associated agonist of cell death (BAD), Yes-associated protein (YAP), endothelial nitric oxide synthase 3 (eNOS), and many others [Hers *et al.* 2011; Manning and Cantley, 2007]. It is not clear at this time how the specificity of these substrates is regulated, but this is likely to be an important issue as PI3K inhibitors become more widely used in the clinic.

Although AKT is a central effector of PI3K signaling and is responsible for many of the biological consequences of PI3K activation, it is important to acknowledge that there exist PI3K-dependent, AKT-independent pathways in cells (Figure 1). Some of the non-AKT effectors of PI3K signaling that have been identified are the serum- and glucocorticoid-inducible kinase (SGK) [Park *et al.* 1999], the Bruton tyrosine kinase (BTK) [Qiu and Kung, 2000], and regulators of the small guanosine triphosphate (GTP) binding proteins cell division cycle 42 (CDC42) and ras-related C3 botulinum toxin substrate 1 (RAC1) GTPases [Han *et al.* 1998; Welch *et al.* 1998]. SGK and BTK have attracted more attention recently because they can also mediate pro-survival and pro-growth signals induced by activated PI3K [Brunet *et al.* 2001b]. The roles and significance of these non-AKT effectors in cancer biology and in cancer therapies remain to be fully determined and are under active investigation.

Overall, there are a variety of pathways and downstream proteins that are regulated by PI3K/AKT and all of them have been shown to play a role in regulating specific biological activities in normal epithelial cells [Manning and Cantley, 2007]. The mTOR pathway is one of the pathways that deserves special mention because of the advanced state of development of inhibitors for this pathway [Faivre *et al.* 2006; Guertin and Sabatini, 2007; Sabatini, 2006]. This pathway includes the enzyme complexes mTORC1 and mTORC2. mTORC1 is emerging as one of the key downstream effectors of AKT in cancer [Manning and Cantley,

Figure 1. The class I phosphoinositide-3-kinase (PI3K) signaling pathway. Class IA PI3Ks consist of p110 α /p85, p110 β /p85, and p110 δ /p85. Upon growth factor or insulin stimulation, receptor tyrosine kinases activate class IA PI3Ks by direct interaction with the p85 subunit or indirectly through adaptor proteins associated with the receptors (e.g. insulin receptor substrate 1, IRS1). The activated PI3K converts phosphatidylinositol-4,5-bisphosphate (PIP₂) to phosphatidylinositol-3,4,5-trisphosphate (PIP₃), an important secondary messenger, at the membrane. Phosphatase and tensin homolog deleted (PTEN) functions as a lipid phosphatase to antagonize this conversion. When PTEN is lost or mutated, as is the case in many types of cancers, PIP₃ accumulates and recruits proteins containing pleckstrin homology (PH) domains to the cell membrane, including serine threonine protein kinase AKTs and phosphoinositide-dependent kinase 1 (PDK1). Once positioned at cell membrane, AKTs are activated by PDK1 at T308 and mTORC2 at S473. Activation of AKT promotes a broad spectrum of downstream events, including protein synthesis, proliferation, and glucose metabolism through phosphorylation of myriad cellular substrates, including mTORC1, GSK3 β , FOXO1, and BAD. There are also non-AKT-dependent effectors of PI3K signaling, such as RAC1, SGK and PKC. In another scenario, G-protein-coupled receptors (GPCRs), which are activated by chemokines or lysophosphatidic acid, activate class IB PI3K (p110 γ /P101) through the G $\beta\gamma$ subunit of trimeric G proteins. BAD, BCL2-associated antagonist of cell death; FOXO1, forkhead box O1 (also known as FKHR); GSK3, glycogen synthase kinase 3; mTOR, mammalian target of rapamycin; PKC, protein kinase C; RAC1, RAS-related C3 botulinum toxin substrate 1; SGK, serum and glucocorticoid-regulated kinase; S6K, ribosomal protein S6 kinase; 4E-BP1, eukaryotic translation initiation factor 4E binding protein 1.

2007]. Of importance, besides receiving signals from PI3K/AKT, mTORC1 also integrates inputs from many other sources, including growth factor signaling pathways (e.g. insulin growth factor), the energy state of the cell (adenosine monophosphate (AMP) levels), and nutrient and oxygen availability [Bjornsti and Houghton, 2004]. In light of the integrative nature of mTOR signaling and the existence of multiple feedback loops that interact with the PI3K signaling pathway, it is

anticipated that effective treatment of CRC may require the use of both AKT inhibitors and mTOR inhibitors.

Regulators of PI3K signaling

In normal cells, PI3K/AKT pathway activity is tightly regulated at multiple levels [Carracedo and Pandolfi, 2008]. As mentioned above, RTKs can act upstream of PI3K signaling to regulate its

activation [Cantley, 2002]. Upon nutrient and growth factor stimulation, RTKs can activate PI3K by directly or indirectly (e.g. via IRS) binding to the regulatory subunit p85. RTKs can also activate PI3K through interactions between RAS and the RAS-binding domain of the p110 component of PI3K [Kodaki *et al.* 1994; Rodriguez-Viciana *et al.* 1994].

One of the best studied negative regulators of PI3K signaling is a protein and lipid phosphatase called the phosphatase and tensin homolog (PTEN) (Figure 1) [Li *et al.* 1997; Steck *et al.* 1997]. The major function of PTEN is to antagonize PI3K activity by hydrolyzing the 3'-phosphate on PIP3 to generate PIP2, thus inhibiting PIP3-mediated downstream pathway activation [Machama and Dixon, 1998; Stambolic *et al.* 1998]. The lipid phosphatase function of PTEN is impaired when *PTEN* expression is inhibited or when *PTEN* is mutated, as is the case in many human cancers, including breast cancer, brain cancer, prostate cancer, and colorectal cancer [Li *et al.* 1997]. The consequence of PTEN loss is that PIP3 accumulates and leads to elevated activity of the AKT/mTORC pathway, which can enhance cell survival and cell proliferation [Salmena *et al.* 2008].

The activation of AKT can also be negatively regulated. AKT is activated by the serial phosphorylation of T308 and S473. The phosphorylation of AKT at the T308 site is removed by protein phosphatase 2A, whereas the PH domain leucine-rich repeat protein phosphatase (PHLPP) directly dephosphorylates S473 [Bayascas and Alessi, 2005; Gao *et al.* 2005]. Interestingly, the cellular level of PHLPP is regulated by mTOR-dependent protein translation [Liu *et al.* 2011]. In addition, AKT can stabilize its negative regulator PHLPP by phosphorylating and subsequently inactivating GSK3 β [Li *et al.* 2009]. Therefore, as a consequence of unintended effects on feedback loops, inhibition of mTOR and AKT activity could decrease PHLPP levels, thus potentially negating some of the antitumor effects of therapies directed at these pathways [Warfel and Newton, 2012].

In addition to the negative regulators of the PI3K pathway mentioned above, some of the downstream components of the pathway have also been identified as negative feedback regulators of PI3K signaling. The most prominent negative feedback loop is one triggered by mTORC1 signaling, which attenuates insulin-induced PI3K activation

via IRS1 degradation [Haruta *et al.* 2000]. This is a particularly important issue because inhibition of mTOR could cause the release of this feedback inhibition. Indeed, treatment of cancer patients with the mTORC1 inhibitor RAD001 led to AKT activation due to the release of mTOR-dependent negative regulation [O'Reilly *et al.* 2006], which may be a reason for the modest antitumor effect of mTORC inhibitors. Similar negative feedback loops also occur in the regulation of AKT, such that inhibition of AKT activation may actually upregulate AKT-independent PI3K signaling by relieving the feedback repression [Vasudevan *et al.* 2009].

PI3K signaling and intestinal epithelial cell biology

The normal role of Pten and PI3K signaling in the intestines is best understood through studies of mouse models in which *Pten* is deleted [Byun *et al.* 2011; Di Cristofano *et al.* 1998; Langlois *et al.* 2009; Marsh *et al.* 2008]. Homozygous deletion of *Pten* causes early embryonic lethality at embryonic day 9.5 in mice [Di Cristofano *et al.* 1998]. Using the Cre/loxP system, in which *Pten* was knocked out specifically in the intestinal epithelial cells, the role of *Pten* in intestinal homeostasis and epithelial cell morphogenesis has been investigated. Interestingly, one group of investigators found that *Pten* could regulate intestinal architecture and secretory cell commitment [Langlois *et al.* 2009], but other investigators have found that epithelial *Pten* is dispensable for intestinal homeostasis [Byun *et al.* 2011; Marsh *et al.* 2008]. Thus, the role of PI3K signaling in the intestines may have to do with responses to perturbations of the basal state of the intestines rather than with the constitutive regulation of intestinal epithelial cells.

Molecular mechanisms of PI3K/AKT pathway deregulation in CRC

Recent studies have shown that the PI3K/AKT signaling pathway is aberrantly activated in many cancer types, including CRC, and that activation of PI3K signaling promotes cancer formation through a variety of mechanisms, including the induction of cell proliferation and cancer cell survival. In light of the complexity of the PI3K signaling pathway network, it is widely believed that determining the specific mechanisms of PI3K pathway activation in CRC will be critical to the design and clinical implementation of the most effective therapeutic approaches to achieve the maximum clinical benefit from PI3K/AKT

Table 1. Genetic/epigenetic alterations in components of the PI3K/AKT signaling pathway in colorectal cancer.

Gene	Epi/genetic alterations	Incidence in colorectal cancer
<i>PIK3CA</i>	Mutations	15%
<i>PTEN</i>	Loss of heterozygosity	23–35%
	Epigenetic silencing	19% of MSI-high tumors
	Mutation	<5%
<i>AKT1</i>	E17K mutation	6%
<i>AKT2</i>	Kinase domain mutation	Rare (<1%)
	Amplification	Rare (<1%)
<i>PDK1</i>	Kinase domain mutation	Rare (<1%)

AKT, serine-threonine protein kinase; MSI, microsatellite instable; PDK1, phosphoinositide-dependent kinase 1; PIK3CA, phosphoinositide-3-kinase, catalytic, alpha subunit; PTEN, phosphatase and tensin homolog.

pathway inhibitors. It is highly likely that the most effective therapies for treating tumors that carry activating mutations in the PI3K/AKT pathway will not be the same as those that affect negative regulators of the pathway.

Receptor tyrosine kinase hyperactivation

Acting upstream of the PI3K/AKT pathway, RTK signaling activates PI3K/AKT through its interaction with the p85 regulatory subunit of PI3K. One of the best characterized RTKs is EGFR, which is a member of the ERBB family of receptor tyrosine kinases, consisting of EGFR (also called ERBB1), ERBB2, ERBB3, and ERBB4 [Gullick, 1998]. Overexpression of *EGFR*, the most common deregulated protein in cancers in the EGFR signaling pathway, correlates with poor prognosis in a variety of epithelial tumors, including breast, ovarian, head and neck, and lung cancer [Dassonville *et al.* 1993; Rusch *et al.* 1993; Tewari *et al.* 2000; Umekita *et al.* 2000]. Abnormal activation of the ERBB family members leads to hyperactivation of PI3K/AKT signaling, as well as increased activation of other signaling pathways implicated in tumorigenesis, including MAPK signaling [Yarden and Sliwkowski, 2001]. Interestingly, ERBB3, which binds amphiregulin and epiregulin, as well as epidermal growth factor (EGF), has the highest binding affinity for PI3K among the ERBB receptors, directly binding with the p85 regulatory subunit [Kim *et al.* 1994; Soltoff *et al.* 1994]. In addition to EGFR, the insulin growth factor receptor (IGFR) can also activate the PI3K/AKT pathway in CRC [Donovan and Kummar, 2008].

KRAS mutation

v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) is a component of multiple

RTK signaling pathways, including the MAPK and PI3K pathways [Downward, 2003]. After activation of RTKs such as EGFR, KRAS is activated and can signal through v-raf murine sarcoma viral oncogene homolog B1 (BRAF) to activate the MAPK pathway [Davies *et al.* 2002]. KRAS is constitutively activated in many types of cancers as a consequence of mutations in codons 12, 13, 61, and 146 [Edkins *et al.* 2006; Karapetis *et al.* 2008]. Mutations in *KRAS* or *BRAF*, which usually are mutually exclusive, occur in approximately 40%–45% of CRCs [Bos *et al.* 1987; Davies *et al.* 2002; Fransen *et al.* 2004; Rajagopalan *et al.* 2002; Russo *et al.* 2005]. Although *KRAS* has been shown to bind directly to the p110 subunit [Rodriguez-Viciano *et al.* 1994], whether mutant *KRAS* is sufficient to activate PI3K/AKT remains elusive. Emerging data indicate that mutant *KRAS* activates MAPK signaling directly, but it activates PI3K/AKT signaling in an indirect fashion via the IGFR pathway [Ebi *et al.* 2011].

Somatic genetic alterations in genes encoding members of the PI3K pathway are common in CRC

Mutations in genes that encode a number of different components of the PI3K/AKT pathway have been observed in up to 40% of CRCs (Table 1) [Parsons *et al.* 2005].

PTEN

Deletion of *PTEN* was the first genetic alteration identified to activate the PI3K/AKT pathway in cancer [Li *et al.* 1997; Steck *et al.* 1997]. In fact, *PTEN* is one of the most frequently mutated/deleted genes in human cancers [Salmena *et al.* 2008]. Even a minor impairment

in *PTEN* function can lead to cancer development, demonstrating the significance of dosage effects of this gene on the cancer formation process [Salmena *et al.* 2008]. This concept is illustrated by the finding in some patients with Cowden syndrome that they have germline mutations in *PTEN* that preserve partial lipid phosphatase function, but inactivate the protein phosphatase function. These patients have a modestly increased risk for colon cancer [Tan *et al.* 2012; Waite and Eng, 2002]. Immunohistochemical staining for the PTEN protein shows that PTEN is absent in up to 40% of CRCs [Zhou *et al.* 2002]. The loss of *PTEN* in CRC can occur through multiple mechanisms, including somatic mutations in poly(A)₆ tracts in exons 7 and 8 (19% of Microsatellite unstable (MSI) cases) [Shin *et al.* 2001] and allelic loss at chromosome 10q23 (23% of cases) [Frayling *et al.* 1997]. In addition, 19% of microsatellite-unstable CRCs display *PTEN* inactivation through promoter hypermethylation [Goel *et al.* 2004]. *PTEN* inactivation leads to constitutive activation of the downstream components of the oncogenic PI3K pathway, including AKT and mTOR kinases [Di Cristofano and Pandolfi, 2000], and *PTEN* is therefore considered an important CRC tumor suppressor gene [Salmena *et al.* 2008].

PIK3CA

Somatic-activating mutations in the catalytic subunit p110 α (encoded by *PIK3CA*) are the most common genetic alterations in the PI3K signaling pathway found in CRC (32%) [De Roock *et al.* 2010; Samuels *et al.* 2004]. Interestingly, they occur more frequently in advanced CRC than early polyps, suggesting that *PIK3CA* mutations are late events during CRC development [Samuels *et al.* 2004; Velho *et al.* 2008]. Most *PIK3CA* mutations in CRC (80%) occur in the kinase domain (E542K and E545K) or in the helical domain (H1047R) of p110 α [Simi *et al.* 2008]. *In vitro* and *in vivo* studies demonstrate that these tumor-associated *PIK3CA* mutations lead to the constitutive activation of the PI3K/AKT pathway and result in oncogenic transformation [Ikenoue, 2005; Ikenoue *et al.* 2005; Samuels *et al.* 2005].

Notably, several studies have reported that there are concurrent *KRAS* and *PIK3CA* mutations in CRC samples, indicating that simultaneous activation of the *KRAS* and PI3K pathways may be needed for the formation of some tumors [Barault

et al. 2008; Nosho *et al.* 2008]. As will be discussed later, the presence of a *KRAS* mutation is an important negative predictor for response to PI3K/AKT inhibition in *in vitro* studies.

PIK3CB

In contrast to *PIK3CA*, no tumor-associated mutations have been identified in genes encoding another class I p110 isoform. However, amplification and overexpression of *PIK3CB* has been observed in colon neoplasms and CRC cells [Benistant *et al.* 2000; Kang *et al.* 2006].

P85 α (encoded by *PIK3R1*)

Mutations in the inter-SH2 domain are found in fewer than 10% of CRCs [Philp *et al.* 2001], but can lead to constitutively active PI3K/AKT signaling.

AKT

Among the three isoforms of AKT (AKT1, AKT2, and AKT3), the somatic mutation E17K in the PH domain of *AKT1* was found in 6% (3 out of 51) of CRCs [Carpten *et al.* 2007]. This mutation allows AKT to bind to the plasma membrane even in the absence of PIP₃, leading to constitutive AKT activation. Mutations in *AKT2* have been observed infrequently in CRC [Parsons *et al.* 2005].

Rationale for targeting the PI3K/AKT pathway in CRC

Cancer cells, but not normal cells, appear to be frequently dependent on the PI3K pathway for proliferation and survival [Engelman *et al.* 2006; Katso, 2001; Katso *et al.* 2001; Yuan and Cantley, 2008; Zhao and Vogt, 2008]. Thus, inhibiting this pathway could have specific anti-tumor effects by either inhibiting tumor-cell proliferation or sensitizing the cancer cells to other cytotoxic drugs [Roy *et al.* 2002]. Furthermore, many components of the PI3K pathway are kinases, making them 'druggable' targets. Lastly, it has become increasingly apparent that in some cancers PI3K/AKT activation is a major molecular mechanism of acquired resistance to certain forms of therapy, including most notably anti-EGFR therapies [Berns *et al.* 2007]. *In vitro* and *in vivo* data demonstrate that targeting the PI3K/AKT pathway may be effective in treating CRC when used in combination with EGFR inhibitors [Lee *et al.* 2009]. These features of the PI3K

pathway have led to active programs in drug development in both academia and industry.

Advances in targeting PI3K/AKT signaling in CRC

Tumors with an aberrantly activated PI3K/AKT pathway may be addicted to PI3K signaling for growth, survival, and angiogenesis [Engelman *et al.* 2006; Katso *et al.* 2001; Yuan and Cantley, 2008; Zhao and Vogt, 2008]. Consistent with this concept, emerging preclinical data have revealed the potential efficacy of PI3K inhibition for the treatment of CRC [Fujishita *et al.* 2008; Hung *et al.* 2010; Roper *et al.* 2011]. As a result of these preclinical studies, several inhibitors of PI3K signaling pathway are in clinical development, with a number of agents being used in clinical trials for CRC treatment (Table 2).

Dual PI3K/mTOR inhibitors

Dual PI3K/mTOR inhibitors, such as NVP-BEZ235 (Novartis), XL765 (Exelixis), and SF-1126 (Semafore), have been developed based on the structural similarity between the p110 subunit of PI3K and mTOR [Garcia-Echeverria and Sellers, 2008]. They are predicted to target all the p110 isoforms, mTORC1 and mTORC2. Complete inhibition of all p110 isoforms as well as mTORC1/2 is expected to completely inhibit PI3K/AKT/mTORC1 signaling in cancers with *PIK3CA* mutations, *PIK3R1* mutations and loss of *PTEN*. In addition, since treatment with mTORC1 inhibitors as single agents often leads to feedback PI3K activation in cancers [O'Reilly *et al.* 2006], dual PI3K/mTOR inhibitors have the potential to be more effective therapies than agents that selectively inhibit the PI3K pathway because they

Table 2. Clinical advances in targeting PI3K/AKT signaling for colorectal-cancer treatment.

Monotherapy				
Inhibitor	Company	Target	Cancer type	Phase of clinical trial
SF1126	Semafore	PI3K and mTOR	Advanced solid tumors (including CRC)	I
GSK1059615	GSK	PI3K and mTOR	Solid tumors (including CRC), malignant breast cancer, lymphoma, endometrial cancer	I
XL147	Exelixis	Pan-PI3K	Solid tumors (including CRC), breast, lymphoma, endometrial, NSCLC, ovarian	I
GDC-0941	Genentech	Pan-PI3K	Locally advanced or metastatic solid tumors (including CRC)	I
OSI027	Astellas	mTORC1/2	Lymphoma, solid tumors	I
ZD8055	AstraZeneca	mTORC1/2	Solid tumors	I/II
Everolimus	Novartis	mTORC1	Metastatic CRC	II
Temsirolimus	Pfizer	mTORC1	CRC	II
BKM120	Novartis	Pan-PI3K	CRC, breast cancer, ovarian cancer, endometrium cancer	I
Combination therapy				
GDC-0941 + GDC0973 (MEK inhibitor)	Genentech	Pan-PI3k and MEK	Locally advanced or metastatic solid tumors	I
GDC-0941 + erlotinib (EGFR inhibitor)	Genentech	Pan-PI3K and EGFR	NSCLC, solid tumors	I
BEZ235 + MEK162 (MEK1/2 inhibitor)	Novartis	Class I PI3K and mTOR, MEK kinase	Breast cancer, CRC, pancreatic, melanoma, NSCLC	I/II

(Continued)

Table 2. (Continued)

Monotherapy				
Inhibitor	Company	Target	Cancer type	Phase of clinical trial
PX866 + cetuximab	Oncothyreon	Pan-PI3K and EGFR	Incurable metastatic CRC	I/II
BKM120 + everolimus	Novartis	Pan-PI3K and mTORC1	Advanced solid tumors	I
BKM120 + irinotecan	Novartis	Pan-PI3K	Advanced CRC	I
Perifosine + capecitabine	Keryx	AKT	Refractory advanced CRC	III
MK2206 + AZD6244 (MEK inhibitor)	Merck	AKT and MEK	Advanced CRC	II
Everolimus + irinotecan, cetuximab	Novartis	mTORC1, EGFR and DNA topoisomerase 1	Metastatic CRC	II
Everolimus + bevacizumab	Novartis	mTORC1 + angiogenesis	Advanced CRC	II
Everolimus + FOLFOX and bevacizumab	Novartis	mTORC1 + angiogenesis	CRC	I/II
Everolimus + OSI-906 (dual IGFR and IR tyrosine kinase inhibitor)	Novartis	mTORC1, IGFR/IR	Refractory metastatic CRC	I
Everolimus + panitumumab, irinotecan	Novartis, Amgen	mTORC1, EGFR and DNA topoisomerase 1	Advanced CRC	I/II
Everolimus + AV951 (VEGF inhibitor)	Novartis	mTORC1 and VEGF	Refractory, metastatic CRC	I/II
Ridaforolimus + cetuximab	Merck	mTORC and EGFR	CRC, advanced head and neck cancer, NSCLC	I
Temsirolimus + cetuximab	Pfizer	mTORC1 + EGFR	Cetuximab-refractory CRC	I
Temsirolimus + irinotecan		mTORC1 and DNA topoisomerase 1	Chemotherapy-refractory patients with <i>KRAS</i> -mutated metastatic CRC	II

Data were obtained from www.clinicaltrials.gov.
 AKT, serine threonine protein kinase; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FOLFOX, Oxaliplatin, 5-Fluorouracil [5-FU], Leucovorin; IGFR, insulin growth factor receptor; IR, insulin receptor; MEK, MAP kinase kinase; mTORC, mechanistic target of rapamycin [serine/threonine kinase]; NSCLC, non-small cell lung cancer; PI3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor.

overcome the feedback activation of PI3K by targeting both upstream and downstream elements of the PI3K signaling pathway. In addition to trials that are using these agents as monotherapy, a regimen that uses combined therapy with NVP-BEZ235 and the MEK inhibitor MK162 has entered phase I/II trials for treating advanced solid tumors, including CRC.

A key issue that will affect the application of these types of inhibitors is whether they will result in greater toxicity in patients due to complete inhibition of PI3K/AKT/mTORC1/2 signaling

in noncancerous cells. The hope is that that cancer cells will show preferential toxicity as a result of being addicted to oncogenic PI3K signaling. However, the need for PI3K pathway activation for these agents to be effective has been challenged by recent studies in mouse models. Results from *in vivo* mouse models demonstrated efficacy of NVP-BEZ235 in treating CRC with wild-type *PIK3CA* [Roper *et al.* 2011], suggesting wider applicability of dual PI3K/mTOR inhibitors in treating CRC. These results also suggest that the toxicity of these agents may apply to normal cells as well as cancer cells.

PI3K inhibitors

PI3K inhibitors can be divided into two classes: pan-PI3K inhibitors, which target all class IA PI3Ks, and isoform-specific PI3K inhibitors. PX866 and BKM120 are examples of pan-PI3K inhibitors that are being used in phase I and phase II clinical trials for the treatment of CRC, either as single agents or in combination with other targeted agents (e.g. cetuximab and MEK162) or irinotecan. (Please see the NCI Clinical Trials website (www.clinicaltrials.gov) for more information on these trials.) The use of isoform-specific agents is based on the concept that if one isoform of PI3K is found to be predominant in driving tumor formation, using isoform-specific PI3K inhibitors would have the benefit of reduced toxicity, increased potency, fewer off-target effects, and improved tolerability [Baba *et al.* 2011]. Thus, p110 α -specific inhibitors would be as effective in cancers with *PIK3CA* mutations as pan-PI3K inhibitors, but would have less toxicity. Recent studies have suggested an essential role for p110 β , but not p110 α , in mediating PI3K signaling in some PTEN-deficient cancers. These studies suggest that these subsets of cancers would potentially be more susceptible to p110 β -specific inhibitors than cancers with intact PTEN [Jia *et al.* 2008; Wee *et al.* 2008].

The different efficacies of PI3K inhibitors and dual PI3K/mTOR inhibitors are likely to depend on whether PI3K activity is the strongest input for mTORC signaling in the cell. In cancers with *PIK3CA* mutations or PTEN loss, PI3K inhibition alone could completely inactivate mTORC1 signaling. In this scenario, using PI3K inhibitors would be advantageous in that they would effectively downregulate mTORC1 signaling while avoiding toxicities resulting from inhibiting mTORC1/2 in normal cells. However, in cases in which mTORC1 activity is regulated by a variety of upstream pathways, such as in cancers with *KRAS* or *BRAF* mutations, using dual PI3K/mTOR inhibitors may achieve better antitumor effects. Notably, PI3K inhibitors are predicted not to be effective in cancers with *AKT1 E17K* mutations or *AKT1/2* gene amplifications.

AKT inhibitors

According to the current paradigm of targeted therapies being most effective in CRCs carrying mutations in the gene for the targeted enzyme, CRCs with *AKT1* mutations and *AKT1/2* amplification should be particularly sensitive to AKT

inhibitors. AKT inhibitors can be classified according to their mechanism of action. These classes include ATP-competitive inhibitors, lipid-based phosphatidylinositol analogues, and allosteric inhibitors [Liu *et al.* 2009]. Perifosine, a lipid-based phosphatidylinositol analog, is in the most advanced stage of clinical development. It targets the PH domain of AKT and interferes with the binding of the PH domain to phosphoinositides, thus preventing recruitment of AKT to the cell membrane, where it would normally be phosphorylated. Perifosine has displayed evidence of antitumor activity in both *in vitro* and *in vivo* studies [Kondapaka *et al.* 2003; Ruiter *et al.* 2003]. It exerts its antitumor activity, at least partly, through inhibition of AKT activation [Kondapaka *et al.* 2003]. In addition, it affects other signal pathways simultaneously, which could certainly contribute to its antineoplastic effect. For example, perifosine can affect jun N-terminal kinase (JNK) activation, which has been shown to play a role in perifosine-induced apoptosis in cancer cells [Hideshima *et al.* 2006; Rahmani *et al.* 2005]. At this time it is not known how much of its antineoplastic effect is derived from inhibiting PI3K/AKT signaling and how much from inhibiting other signaling pathways.

Other agents that inhibit AKT include AKT catalytic site inhibitors, which prevent AKT from phosphorylating its substrates. AKT catalytic site inhibitors do not inhibit the activation of AKT by phosphorylation, and may even enhance AKT activation as a result of loss of negative feedback regulation of PI3K [Han *et al.* 2007]. These agents are typically nonselective and inhibit all three AKT isoforms. GSK690693 is an example of this class of inhibitor. It inhibits AKT1, AKT2, and AKT3, as well as the AGC (cyclic AMP-dependent, cyclic GMP-dependent and protein kinase C) family and some non-AGC family kinases [Hers *et al.* 2011; Rhodes *et al.* 2008]. These agents are still largely in preclinical testing.

Importantly, the efficacy of AKT inhibitors might be diminished due to the prosurvival and pro-growth signals mediated through non-AKT effectors of the PI3K pathway, such as SGK and BTK [Brunet *et al.* 2001a]. Thus, it is not clear what role AKT inhibitors will ultimately have in the treatment of CRC, and it is likely that they will need to be used in combination with other targeted therapies to prevent unintended effects secondary to feedback loop deregulation.

mTOR inhibitors

mTOR inhibitors are the most clinically advanced class of PI3K pathway inhibitors currently in use. Interestingly, rapamycin (also known as sirolimus) is the prototypical mTOR inhibitor but was originally developed as an anti-fungal agent and later as an immunosuppressive agent for use in the setting of solid-organ transplantation [Liu *et al.* 2009]. Its anticancer effects and its role in the PI3K pathway have been appreciated more recently. This class of agents inhibits mTOR by associating with an intracellular receptor, FK506-binding protein 12 (FKBP12), which then binds to and inhibits mTORC1 [Liu *et al.* 2009]. Rapamycin and analogs of rapamycin (known as rapalogs), which include everolimus (RAD001/Afinitor; Novartis) and temsirolimus (CCI-779/Torisel; Wyeth), have been shown to have clinical activity for breast cancer, glioma, and renal cell cancer [Faivre *et al.* 2006; Liu *et al.* 2009]. These agents are currently being used in phase II studies for the treatment of CRC.

A newer class of mTOR inhibitors that inhibits both mTORC1 and mTORC2 is in development. mTOR inhibitors that target both mTORC1 and mTORC2 complexes, concomitantly inhibiting the phosphorylation of AKT at the S473 site by mTORC2, could also mitigate against some of the feedback activation of PI3K signaling that is induced by mTORC1 inhibition alone [Liu *et al.* 2009]. *In vitro* studies have demonstrated that these agents can achieve better antitumor effects than rapamycin or its analogs [Feldman *et al.* 2009]. However, phosphorylation of AKT at T308, which is less affected by these inhibitors, is able to activate some targets of AKT, raising questions about how well these drugs will perform in clinical trials [Jacinto *et al.* 2006]. Furthermore, treatment with these inhibitors could also lead to hyperactivation of AKT-independent effectors of PI3K signaling [Liu *et al.* 2009]. At this time, there are no clinical trials involving the treatment of CRC with these agents.

Opportunities and challenges in targeting PI3K/AKT in colorectal cancer

The ultimate success of targeting the PI3K/AKT pathway in CRC is likely to rely on advancing our current understanding of the role this pathway plays in the molecular pathogenesis of CRC and on the interactions of this signaling pathway with other pathways that are deregulated in

cancer. This knowledge will lead to the accurate identification of the patients who have the highest likelihood of benefiting from this class of drugs. The principles involved in the success of PI3K inhibitors, and of targeted therapies in general, for the treatment of CRC are discussed in the following sections.

Characterization of the entire spectrum of gene mutations and epigenetic alterations in the cancers of individual CRC patients in order to identify the most promising targets for combination targeted therapy regimens

There is substantial molecular heterogeneity among CRCs regarding the specific genes that are mutated in a specific tumor. The diverse mutations and epigenetic alterations occurring in CRCs can affect the response of CRCs to specific therapies. Notably, colon cancers carrying mutations in *PIK3CA* often have concurrent mutations in *KRAS* [Simi *et al.* 2008]. The presence of *KRAS* mutations might adversely affect the efficacy of selective PI3K inhibitors (see next section). In order to accurately determine how CRCs will respond to currently available therapies and new therapies, knowledge of the spectrum of alterations in a specific tumor will be needed. Notably, the great biological complexity of human cancers is in contrast to preclinical animal models, in which tumor formation is usually driven by a small number of known oncogenic events. The differences between human cancers and transgenic animal models pose challenges for translating results from preclinical models to clinical trials.

Identification of patients who have the potential to respond to specific targeted therapies using biomarkers that predict sensitivity or resistance to PI3K/AKT inhibitors

Initial studies indicated that activation of the PI3K pathway is one of the molecular consequences that drive mutant *KRAS*-induced tumorigenesis [Gupta *et al.* 2007; Yang *et al.* 2008]. Thus, cancers with *KRAS* mutations were predicted to be sensitive to single-agent PI3K inhibitors. However, emerging data suggest that this is not necessarily the case [Di Nicolantonio *et al.* 2010; Engelman *et al.* 2008; Ihle *et al.* 2009]. Single-agent PI3K inhibitors have failed to exert antitumor activity in cancers harboring *KRAS* mutations. In fact, the presence of *KRAS*

mutations might adversely affect the efficacy of single-agent PI3K inhibitors. Notably, colon cancers carrying mutations in *PIK3CA* often have concurrent mutations in the *KRAS* gene, which makes this an important clinical issue [Simi *et al.* 2008]. Furthermore, a recent study in mouse models of oncogenic *KRAS*-induced lung cancer demonstrated that only by combining PI3K and MEK inhibitors was a significant antitumor effect realized [Engelman *et al.* 2008]. This study suggests that combination therapies might be needed in the subset of CRC patients with cancers carrying both *PIK3CA* and *KRAS* mutations.

Regimen design: rational combination therapy

The presence of negative and positive feedback loops in cancer cells has posed challenges in targeting PI3K signaling for the treatment of CRC. As mentioned above, the release of negative feedback regulation induced by AKT and mTOR inhibitors can lead to unanticipated increased activity of PI3K and non-AKT regulated proteins. For instance, mTORC1 inhibition can lead to feedback activation of the MAPK pathway in a PI3K-dependent manner [Carracedo *et al.* 2008]. These observations provide a rationale for combining PI3K signaling inhibitors with other targeted therapies in order to overcome feedback activation of oncogenic signaling pathways, which should lead to improved efficacy of these agents.

Consistent with this concept is a promising strategy that simultaneously targets PI3K signaling and EGFR signaling. The presence of *PIK3CA* mutations and loss of PTEN have been associated with resistance to EGFR inhibitors [Engelman and Janne, 2008; Engelman and Settleman, 2008]. Furthermore, activation of the PI3K/AKT pathway by *PI3CA* mutation, *MET* amplification, or the insulin-like growth factor 1 receptor has been observed in cancer cells resistant to EGFR inhibitors [Engelman and Janne, 2008; Guix *et al.* 2008; Sequist *et al.* 2011]. However, these resistant cells become sensitive when EGFR inhibitors are combined with PI3K inhibitors [Guix *et al.* 2008]. This study suggests that combining PI3K signaling inhibitors with EGFR inhibitors will improve the efficacy of EGFR inhibitors and help to overcome the development of resistance to EGFR inhibitor therapy.

Another combination therapy approach involves using agents targeting PI3K pathways

in conjunction with conventional chemotherapeutic agents used to treat CRC. The PI3K/AKT pathway mediates major survival signals that protect cells from undergoing apoptosis. Thus, it is possible that in primary CRC conventional cytotoxic chemotherapeutic agents could induce PI3K/AKT activity in cancer cells [Goggins *et al.* 2006], which could confer chemoresistance [Huang and Hung, 2009]. Thus, inhibiting PI3K signaling has the potential to improve the sensitivity of CRCs to the cytotoxic effects of standard chemotherapy. These agents could be used as novel adjuvant treatments for selected CRCs [Foster, 2002]. A number of phase I trials have investigated the toxicity of combining the AKT inhibitor perifosine with traditional chemotherapeutic agents, such as docetaxel, paclitaxel, and gemcitabine [Cervera *et al.* 2006; Ebrahimi *et al.* 2006; Goggins *et al.* 2006; Weiss *et al.* 2006]. The preliminary results were promising and established the safety of these combinations. However, in the case of combining mTOR inhibitors with conventional chemotherapy, increased toxicities have been revealed and have led to the early discontinuation of these clinical trials. For example, combining CCI-779 with 5-FU and leucovorin in patients with advanced solid tumors resulted in overlapping mucocutaneous toxicities in a phase I clinical trial [Punt *et al.* 2003]. In another phase I clinical trial investigating the combination of weekly everolimus with gemcitabine, myelosuppression was reported in a majority of patients with advanced tumors [Pacey *et al.* 2004]. Therefore, investigators should give careful consideration to potential overlapping toxicities when combining pathway-targeted inhibitors and conventional chemotherapies.

Finally, as discussed above, mutant *KRAS* has the capacity to directly activate both the MEK/ERK and the PI3K/AKT signaling pathway, thus conferring resistance to therapies targeting receptor tyrosine kinases and leading to heterogeneous responses to MEK inhibitors. In fact, it appears that activation of the PI3K pathway is one of the mechanisms that underlies the resistance to MEK inhibitors in *KRAS* mutant cancers [Wee *et al.* 2009]. Therefore, inhibition of both the PI3K/AKT and the MEK/ERK pathway could be more effective than inhibition of either pathway alone in *KRAS* mutant cancers. Recently, promising results have emerged to show the efficacy of combining PI3K and MEK inhibitors in *KRAS* mutant cancers *in vivo* with limited toxicity

[Engelman *et al.* 2008; Halilovic *et al.* 2010; Sos *et al.* 2009].

Accurate monitoring of drug efficacy and toxicity

As more PI3K pathway inhibitors are being developed and entering the clinic, it would be helpful to develop less invasive approaches for monitoring drug efficacy and toxicity. One promising approach is to use circulating tumor cells (CTCs) as a source material for assessing the effects of these therapies. CTCs can be isolated from blood samples, obviating the need for invasive biopsies. Some studies have shown that CTCs at baseline and during treatment are prognostic markers for progression-free survival and overall survival [Cohen *et al.* 2009; Tol *et al.* 2009]. It will be interesting to see whether CTCs can be used to assess pathway activation or target inhibition by the assessment of downstream pathway targets through the use of immunohistochemistry- or flow cytometry-based assays. Another non-invasive approach is to develop imaging techniques to evaluate PI3K-pathway inhibitors in patients. Cancers with high levels of PI3K signaling are predicted to require high rates of glycolysis for their survival. A change in [¹⁸F]2-fluoro-2-deoxy-D-glucose (FDG) avidity might be a rapid marker of efficacy for PI3K inhibitors, as observed in mouse models [Engelman *et al.* 2008]. Therefore, FDG-positron emission tomography imaging might be useful to monitor the response to PI3K pathway inhibitors in CRC [Chowdhury *et al.* 2010]. Combining CTC-based assays with functional imaging has the potential to provide a less invasive and more accurate way to predict the clinical responses of tumors under treatment that could be useful not only in clinical studies but, ultimately, in the clinical management of patients.

Conclusion

The search for targeted therapies to treat advanced CRC has been under way since the initial discovery of mutated genes in CRC. The pace of the development and assessment of these therapies has accelerated as our understanding of the molecular pathology of CRC has evolved. The past several years in particular have witnessed the successful implementation of some of these agents (e.g. cetuximab) in clinical care and have created the hope that agents that inhibit other pathways, such as the PI3K pathway, will also be clinically effective. Whether the success of drugs that target the PI3Ks, AKT, or mTOR in preclinical studies

can be translated into patients' outcome will soon be determined. As we have learned from developing targeted therapies against EGFR, the success of therapeutic targeting of PI3K signaling is likely to rely on genotype-based patient selection, the optimal use of drug combinations, and the accurate monitoring of drug efficacy and toxicity. In addition, resistance will invariably occur as a result of selection for mutations in drug targets that arise in evolving cancer cells. Therefore, the need to identify complementary therapies to overcome resistance is anticipated if targeted therapies are to have a role in the treatment of patients with CRC. In conclusion, targeted therapies directed at PI3K signaling show promise to make a significant impact on CRC treatment, but their ultimate efficacy is likely to require further advances in our understanding of the molecular biology of CRC.

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Conflict of interest statement

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

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