4 Targeting the PI3K Signalling as a Therapeutic Strategy in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) remains one of the leading causes of cancer mortality worldwide. Regarded as a heterogeneous disease, a number of biomarkers have been proposed to help in the stratification of CRC patients and to enable the selection of the best therapy for each patient towards personalized therapy. However, although the molecular mechanisms underlying the development of CRC have been elucidated, the therapeutic strategies available for these patients are still quite limited. Thus, over the last few years, a multitude of novel targets and therapeutic strategies have emerged focusing on deregulated molecules and pathways that are implicated in cell growth and survival. Particularly relevant in CRC are the activating mutations in the oncogene *PIK3CA* that frequently occur in concomitancy with *KRAS* and *BRAF* mutations and that lead to deregulation of the major signalling pathways PI3K and MAPK, downstream of EGFR. This review focus on the importance of the PI3K signalling in CRC development, on the current knowledge of PI3K inhibition as a therapeutic approach in CRC and on the implications PI3K signalling molecules may have as potential biomarkers and as new targets for directed therapies in CRC patients.

Keywords

Colorectal cancer · PI3K signalling pathway · PI3K p110α · Targeted therapies · KRAS

4.1 Introduction

Colorectal cancer (CRC) is one of the most common cancer types, and despite intensive research, remains one of the leading causes of cancer mortality worldwide (Torre et al. 2015). It results from the accumulation of genetic and epigenetic alterations in oncogenes and tumour suppressor

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genes, leading to the transformation of the normal epithelia towards invasive carcinoma (Markowitz and Bertagnolli 2009). Although a large number of molecules have been shown to be altered in these patients, to date, the use of targeted therapies has been limited to anti-epidermal growth factor receptor (EGFR) and anti-vascular endothelial factor (VEGF) agents. Moreover, patients harbouring *KRAS* and *NRAS* mutations are currently excluded from anti-EGFR therapies, as these alterations were shown to cause resistance (Allegra et al. 2016). Therefore, it is urgent to unravel novel therapeutic approaches for CRC patients in an attempt to improve patient outcomes and overcome therapy resistance. While several inhibitors are already being tested in preclinical and clinical trials, the interplay of the signalling pathways has proved to be rather complex and no other agents have yet been approved for these patients. In this review, we focus on the key role of the phosphatidylinositol 3-kinase (PI3K) signalling pathway in CRC development, the latest developments in the field of PI3K targeted specific agents as well as on the implications of PI3K inhibition as an alternative therapeutic approach for CRC patients.

4.2 Molecular Aspects of CRC Progression

Colorectal carcinogenesis is characterized by the gradual accumulation of alterations, genetic and epigenetic, in specific oncogenes and tumour suppressor genes in a multistep manner (Markowitz and Bertagnolli 2009).

The classical model of colorectal cancer progression is the adenoma-carcinoma sequence, however this pathway oversimplifies the heterogeneity of CRC and is not able to explain the development of all types of CRC (Fearon and Vogelstein 1990; Walther et al. 2009). Due to the distinct molecular, clinical and pathological characteristics observed in CRC tumours, other mechanisms for CRC development have emerged, namely the serrated pathway (O'Brien et al. 2006). In each of these models, a unique progression pathway is associated with a distinct mutational spectra. While alterations in the genes *APC*, *KRAS* and *p53* are classic molecular alterations in the Vogelstein pathway, mutations in *BRAF* are typical of the serrated polyp pathway (Jass 2006; Velho et al. 2010; Vilar and Gruber 2010).

Moreover, these models are often associated with different types of genetic instability. Indeed, according to the type of genetic instability, CRC can be subdivided in different molecular subsets, microsatellite instability (MSI) and microsatellite stability (MSS), the latest characterized by having chromosomal instability (CIN) and observed in the majority of the cases (approximately 85%) (Vilar and Gruber 2010; Cunningham et al. 2010). In contrast, MSI is detected in about 15% of CRC patients and is characterized by a defective mismatch repair system through epigenetic silencing or germline mutations, leading to the accumulation of mutations across the genome mainly in repetitive sequences (microsatellites) (Cunningham et al. 2010). As previously highlighted, MSS and MSI are preferentially observed in the adenoma-carcinoma sequence and the serrated pathway, respectively (Velho et al. 2010; Vilar and Gruber 2010). Notably, while for most patients (about 70%) CRC occurs sporadically (MSI and MSS), in other cases CRC develops in a hereditary context being the most common form the hereditary non-polyposis CRC (HNPCRC) also termed Lynch syndrome (Tops et al. 2009).

Of particular importance in CRC, is the fact that MSI status can be used as a prognostic marker and predictor of therapeutic resistance in CRC patients. More specifically, MSI CRC tumours have been shown to be associated with a better prognosis than MSS tumours (Malesci et al. 2007; Gryfe et al. 2000). In addition, these subsets are known to respond differently to the available therapies and studies indicate that, in contrast to MSS, MSI tumours do not benefit from 5-fluouracil (5-FU) based adjuvant chemotherapies (Ribic et al. 2003).

Overall, to successfully design and develop novel targeted therapies, more studies are needed to clarify the value of specific biomarkers for predictive and prognostic purposes, including MSI

status, as well as to better understand the mechanisms underlying the development of CRC in the different molecular subsets.

4.3 The MAPK and PI3K Signalling Pathways and Their Deregulation in CRC

The mitogen activated protein kinase (MAPK) and PI3K are ubiquitous signalling pathways, downstream of EGFR, implicated in a variety of key biological processes including cell proliferation and survival, cell cycle regulation, differentiation, metabolism and apoptosis, among others (Sebolt-Leopold and Herrera 2004; Liu et al. 2009). Figure 4.1 illustrates, in a simplified manner, the classical MAPK and PI3K signalling pathways and their intervenient molecules.

Overall, these pathways are of major relevance in CRC as activating mutations in genes of these cascades are frequently detected in CRC patients, leading to the constitutive activation of the signalling pathway independently of a stimuli. Indeed, as determined by others and our group, a high frequency of mutations has been observed in *KRAS, BRAF* and *PIK3CA* (the gene coding for PI3K p110α, the catalytic subunit of PI3K) (De Roock et al. 2011; Lievre et al. 2010; Oliveira et al. 2004, 2007; Velho et al. 2005, 2008).

Briefly, as part of the RAS-RAF-MAPK cascade, KRAS is a member of the RAS superfamily of GTPases, along with N-RAS and H-RAS, all belonging to the larger class of regulatory GTP hydrolases (Pylayeva-Gupta et al. 2011). By switching between on and off states, GTP- and GDP-bound respectively, KRAS is important in controlling a complex network of signalling pathways by transducing signals from cell surface receptors namely EGFR to specific intracellular effectors (Sebolt-Leopold and Herrera 2004; Samatar and Poulikakos 2014). Upon stimulation, guanine nucleotide exchange factors (GEFs) promote the activation of RAS by stimulating GDP for GTP exchange; conversely, GTPase-activating proteins (GAPs) accelerate RAS-mediated GTP hydrolysis. In their active state, RAS proteins interact and activate their effectors and stimulate downstream signalling

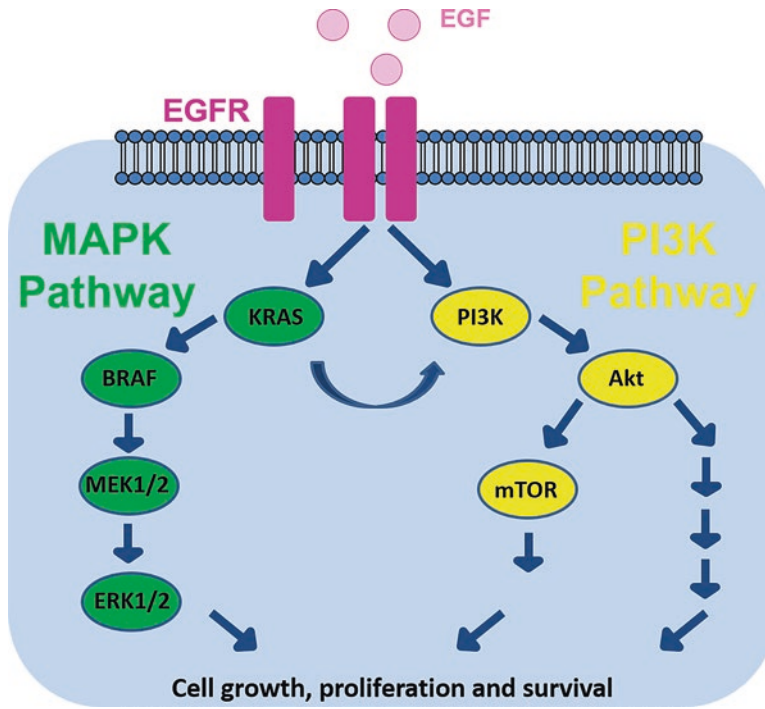

Fig. 4.1 Simplified representation of the MAPK and PI3K signalling pathways. *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor, *PI3K* phosphatidylinositol 3-kinase, *MAPK* mitogen activated protein kinase, *MEK1/2* MAPK kinase 1/2, *ERK1/2* extracellular signal-regulated kinase 1/2, *mTOR* mammalian target of rapamycin

pathways (Bos et al. 2007). More specifically, the classical RAS signal transduction pathway comprises sequential phosphorylations of the serine/threonine kinase RAF, MAPK kinase 1/2 (MEK1/2) and extracellular signal-regulated kinase 1/2 (ERK1/2), ultimately modulating other molecules and regulating the distinct biological functions (Sebolt-Leopold and Herrera 2004; Samatar and Poulikakos 2014). Importantly, RAS is also known to activate other molecules and signalling cascades namely the PI3K signalling pathway, with major implications in targeted therapies (Liu et al. 2009; Fernandes et al. 2013; Murillo et al. 2014; Gupta et al. 2007).

The B-RAF serine/threonine kinase, which belongs to the RAF kinase family of protein kinases together with A-RAF and C-RAF, is one of the best characterized RAS effectors. RAF phosphorylates MEK1/2, which in turn phosphorylates and activates ERK1/2 that will modulate downstream effectors (Sebolt-Leopold and Herrera 2004; Samatar and Poulikakos 2014).

On a separate and parallel signalling cascade, PI3Ks are a rather ubiquitous family of lipid kinases activated by receptor tyrosine kinases (RTK) or other molecules as G-proteins (Liu et al. 2009). PI3Ks are able to phosphorylate the 3′-hydroxyl group of phosphatidylinositol and phosphoinositides and these lipid products act as second messengers to trigger a multitude of signalling cascades with impact in key mechanisms as survival, differentiation and metabolism (Liu et al. 2009; Vanhaesebroeck et al. 2012). In terms of classification, PI3Ks are grouped into three classes (IA/IB, II and III), with distinct structures and substrate specificities but class IA have received much attention as they have been implicated in many human cancers. Class IA PI3Ks, able to phosphorylate phosphatidylinositol (4,5)-biphosphate (PIP2), converting it to phosphatidylinositol (3,4,5)-triphosphate (PIP3), are composed of a heterodimer of a p85 regulatory subunit (p85α, p85β, p55γ or splice variants) and a p110 catalytic subunit (p110α, p110β or p110δ) (Liu et al. 2009; Vanhaesebroeck et al. 2012). Notably, the different p110 and p85 isoforms seem to preferentially mediate specific signalling cascades, though with some redundancy as reviewed in (Hennessy et al. 2005). Moreover, while p110α and p110β are ubiquitously expressed, p110δ expression is mostly restricted to the immune system (Engelman et al. 2006; Liu et al. 2009). In CRC, the p110α subunit of PI3K, encoded by the *PIK3CA* gene, is of particular relevance as it is often mutated in these patients (De Roock et al. 2011). The p110 catalytic isoforms share high homology and have common specific domains namely the p85 binding domain (able to interact with the p85 subunit), a RAS binding domain (to mediate activation by RAS family members) and a kinase catalytic domain (to generate PIP3 and activate downstream targets) (Liu et al. 2009; Thorpe et al. 2015). Similarly, the common p85 subunit domains include a p110-binding domain also termed inter-Src homology 2 (iSH2), and SH2, SH3 and BCR homology (BHD) domains (Liu et al. 2009; Thorpe et al. 2015). Mechanistically, activation (dimerization and autophosphorylation) of the RTK, upon stimulation by growth factors, leads to the recruitment of class IA PI3K to the membrane where the regulatory p85 subunit will bind RTK phosphorylated motifs but also relieve the p85 inhibition of p110; the activated p110 is then able to generate PIP3, a second messenger that provides docking sites for specific proteins, i.e., PIP3 binds to specific domains, as the pleckstrin-homology (PH) domain, of downstream targets including Akt (also termed protein kinase B, PKB) and phosphoinositide-dependent kinase (PDK1); PDK1 is then able to phosphorylate Akt at Thr308 important to activate Akt (Liu et al. 2009; Thorpe et al. 2015). Remarkably, the activation of the serine/threonine-specific protein kinase Akt, one important downstream effector of PI3K, leads to phosphorylation and subsequent activation or inhibition of additional downstream molecules that will ultimately regulate other proteins modulating the many functions of the PI3K signalling cascade (Manning and Cantley 2007). Indeed, a panoply of Akt substrates have been identified including glycogen synthase kinase 3β (GSK-3β), forkhead box O (FoxO) transcription factors, mouse double minute 2 homologue (MDM2), Bcl-2 associated death promoter (Bad), tuberous sclerosis complex

2 (TSC2), proline-rich Akt substrate of 40 kDa (PRAS40) among others, that are involved in cell growth, metabolism, insulin signaling and survival (Manning and Cantley 2007; Manning and Toker 2017). In addition, an important downstream effector of Akt is the mechanistic target of rapamycin (mTOR), a serine/threonine protein kinase. Interestingly, the catalytic subunit mTOR can be found in two distinct complexes, named mTOR complex 1 (mTORC1, composed by mTOR, raptor, mLST8, PRAS40 and DEPTOR) and mTOR complex 2 (mTORC2, composed by mTOR, rictor, mLST8, DEPTOR, Sin1 and Protor) and while mTORC1 is activated by Akt and known to modulate protein synthesis by phosphorylating the key mTOR effectors elF4E Binding Protein (4EBP1) and p70S6 Kinase 1 (S6 K1), mTORC2 is able to phosphorylate Akt at Ser473 and activate it (Sarbassov et al. 2005, 2006; Liu et al. 2009; Manning and Toker 2017; Saxton and Sabatini 2017). Importantly, the phosphorylation of Akt at both Thr308 and Ser473 is required for full Akt activation (Manning and Toker 2017). In contrast to the above mentioned activation mechanisms, negative regulation of the PI3K pathway is also mediated by the tumour suppressor gene phosphatase and tensin homolog (*PTEN*) that removes the 3′ phosphate from PIP3 hampering the PI3K signalling (Cully et al. 2006). Noteworthy, in addition to the described regulatory mechanisms, the PI3K-Akt-mTOR signaling is also tightly controlled by a number of feedback loops and cross-talk with other signaling pathways as reviewed in (Manning and Toker 2017; Rozengurt et al. 2014). Indeed, an important negative feedback mediated by mTORC1 is known to inhibit the PI3K pathway through distinct mechanisms. In particular, mTORC1 and its downstream effector S6K are able to inhibit the PI3K/Akt signalling through phosphorylation, inhibition and degradation of the insulin receptor substrate I (IRS-I) (Harrington et al. 2004; Manning and Toker 2017). In addition, RTKs are also targets of negative feedback regulation by activated PI3K-Akt-mTOR (Zhang et al. 2007; Chandarlapaty et al. 2011; Manning and Toker 2017).

As previously mentioned, mutations in genes of the MAPK and PI3K pathways are frequent in CRC. Specifically, about 30–40% of CRC patients harbour a mutation in *KRAS* (mostly affecting codons 12 and 13 of exon 2), whereas mutations in the *KRAS* effector, *BRAF*, are detected in about 15% of CRC patients (typically V600E on exon 15) (De Roock et al. 2011; Velho et al. 2010). Notably, *KRAS* and *BRAF* oncogenes have been suggested to play different roles in the development and progression of CRC, as *KRAS* and *BRAF* mutations are rarely detected in the same tumour and are instead observed as alternative molecular modifications (Rajagopalan et al. 2002; Velho et al. 2010). Moreover, *KRAS* and *BRAF* mutation frequencies and patterns are distinct in MSI, MSS, sporadic and hereditary subsets of CRC, with mutations in *BRAF* mostly found in MSI sporadic CRC and *KRAS* in MSS and MSI sporadic and hereditary CRC (Oliveira et al. 2004, 2007; Velho et al. 2010; Domingo et al. 2004; Lubomierski et al. 2005). In addition to *KRAS* and *BRAF*, *PIK3CA* mutations are observed in approximately 15% of CRC patients and, in contrast, often arise concomitantly with *KRAS* or *BRAF* mutations (De Roock et al. 2011; Velho et al. 2005, 2008). These *PIK3CA* mutations are of the missense type and are mostly in hotspots involving exon 9 that corresponds to the helical domain of PI3K p100α, and exon 20 that corresponds to the kinase domain of PI3K p100α; two common examples are E545K and H1047R (De Roock et al. 2011; Liu et al. 2009). In contrast to these, alterations in other molecules of the PI3K pathway are rare, except for PTEN for which controversial information has been raised in terms of mutations and loss of expression (De Roock et al. 2011; Nassif et al. 2004).

In any case, aberrant activation of these molecules will have a major impact in cell behaviour with effects on proliferation, survival, invasion and therefore in the initiation and progression of CRC (Thorpe et al. 2015). Of particular importance, and essential to better understand the underlying signalling mechanisms, is the increasing evidence in support of RAS-RAF-MAPK and PI3K-Akt cross-talk resulting in a complex signalling network (Castellano and Downward

2011; Thorpe et al. 2015). However, the extent of such cross-talk and the implications for CRC therapy are still not clear.

4.4 Current Targeted Therapies for CRC and Their Limitations

To date, apart from the conventional therapeutic strategies, CRC is limited to two distinct types of targeted therapy. These include anti-angiogenic and anti-EGFR agents (Ciardiello and Tortora 2008; Welch et al. 2010; Weng et al. 2015). In particular, cetuximab and panitumumab, two anti-EGFR antibodies that bind the extracellular domain of EGFR, have received much attention and are currently approved for the treatment of patients with mCRC (Ciardiello and Tortora 2008). Regrettably, only a subgroup of mCRC patients can benefit from such therapies and only a small percentage will be sensitive (Ciardiello and Tortora 2008; Lievre et al. 2017). More specifically, *KRAS* mutations have been recognized as predictive biomarkers of resistance to anti-EGFR agents, though some controversy exist as to the type of *KRAS* mutation (De Roock et al. 2010; Allegra et al. 2016). Nonetheless, CRC patients harbouring somatic *KRAS* mutations are currently not eligible to cetuximab and panitumumab targeted therapies (Allegra et al. 2009, 2016). Such limitation has a major impact, as about 30–40% of CRC patients do harbour a *KRAS* mutation (De Roock et al. 2011; Velho et al. 2010). Importantly, not all mCRC patients with wild type *KRAS* respond to anti-EGFR agents, suggesting that other mechanisms of resistance are involved (Heinemann et al. 2016; Lievre et al. 2017; Price et al. 2016). Indeed, *NRAS* mutations were also shown to be associated with resistance to anti-EGFR agents, and recommendations are now to exclude these patients from EGFR targeted therapies (Allegra et al. 2016). However, these account for only about 2% of CRC patients, indicating that other molecules are involved (Irahara et al. 2010). Mutations in other genes downstream of EGFR, including *BRAF* and *PIK3CA*, have been associated with resistance to EGFR targeted therapies but inconsistent results have been obtained and additional evidence is required to clarify such controversy (De Roock et al. 2011; Mohamed et al. 2018; Lievre et al. 2010; Therkildsen et al. 2014). As above mentioned, mCRC patients can also be offered anti-angiogenic therapy using the anti-VEGF agent bevacizumab, namely in combination with chemotherapy regimens, which has proven some clinical efficacy (Welch et al. 2010).

Altogether, at present, not only the available targeted agents for mCRC patients are limited but also exclude a considerable proportion of patients. Therefore, there is an urgent need to develop novel therapeutic strategies for CRC patients, particularly for those with *KRAS* mutations.

4.5 Targeting the PI3K Signalling Pathway

The PI3K signalling pathway is a key signalling cascade implicated in many human cancers including CRC (Janku et al. 2018). It is well established that deregulation of the PI3K signalling can occur through activating mutations in the *PIK3CA* gene, but other important activation mechanisms are known to exist, namely through oncogenic KRAS, with strong implications for CRC patients (Thorpe et al. 2015; Castellano and Downward 2011). Thus, efforts have been made to advance in the development of novel targeted therapies directed to the many molecules of the PI3K signalling cascade. Attractive therapeutic targets include PI3K p110 isoforms, Akt and mTOR, and inhibitors can be isoform specific- or pan-PI3K inhibitors, dual PI3K/mTOR inhibitors, Akt inhibitors and mTORC1 and mTORC2 inhibitors.

A vast number of drugs have already been tested in preclinical assays, however, only a few have reached clinical studies for several cancer types, including CRC (Fig. 4.2). Furthermore, the available data on the clinical effects of PI3K inhibitors is still limited (Rodon et al. 2013; Janku et al. 2018). Notably, despite reports suggesting specific alterations to be predictive of responsiveness, as the presence of *PIK3CA*

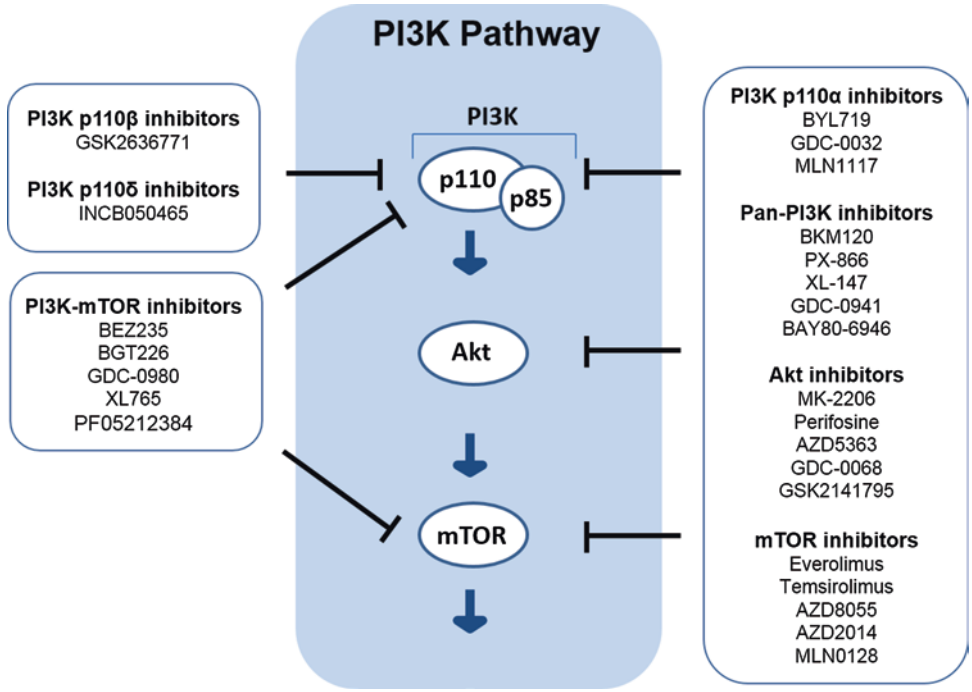

Fig. 4.2 Inhibitors of the PI3K signalling pathway used in clinical trials for several cancer types, including CRC

mutations for PI3K p110α specific inhibitors, reliable predictive biomarkers of therapeutic response or resistance are still awaited. To date, no PI3K signaling pathway inhibitor has yet been approved for CRC patients and only temsirolimus and everolimus (mTORC1 inhibitors) and copanlisib and idelalisib (PI3K inhibitors) have been approved for specific types of cancer as subsequently described in more detail (Janku et al. 2018). A brief overview of the current knowledge is described below.

4.5.1 PI3K Isoforms as Therapeutic Targets

As above mentioned, agents that target the PI3K are classified into pan-class I PI3K inhibitors targeting all class I PI3K isoforms, or into isoform specific-PI3K inhibitors, targeting specifically one p110 isoform (Thorpe et al. 2015; Janku et al. 2018).

Initially, many studies were performed in several cancer type models using the PI3K pan-inhibitors wortmannin and LY294002, but these were only tested in preclinical studies and did not reach clinical trials, in part due to selectivity and toxicity issues (Liu et al. 2009). In particular, the irreversible PI3K inhibitor wortmannin was shown to have antitumour activity in several human tumour cell lines, including a colon carcinoma cell line (Schultz et al. 1995). Moreover, LY294002, a reversible small molecule PI3K inhibitor, demonstrated a remarkable growth-inhibitory and apoptosis-inducing effect in colon cancer cell lines and, experiments using mouse xenografts revealed that LY294002 administration *in vivo* also resulted in suppression of tumour growth and induction of apoptosis (Semba et al. 2002).

In recent years, novel inhibitors were generated with improved characteristics, namely in terms of specificity, potency and stability while simultaneously minimizing toxicity. In most cases, these inhibitors are ATP competitive agents and many of them are now being evaluated in clinical trials in patients with solid tumours, including CRC, either as monotherapy or in

combination with other therapies (Thorpe et al. 2015). The list of inhibitors is vast and includes BKM120, PX-866, XL147, GDC0941, GSK1059615, BYL719, GDC0032, INK1117 (Janku et al. 2018). Some relevant studies focusing on CRC are briefly described.

BKM120 (buparlisib), a pan-PI3K inhibitor, when tested in a panel of 353 cell lines exhibited preferential inhibition of tumour cells harbouring *PIK3CA* mutations, in contrast to either *KRAS* or *PTEN* mutant models (Maira et al. 2012). In addition, BKM120 was shown to reduce cell proliferation in wild type and mutant *PI3KCA* CRC cells and treatment with cetuximab and BKM120 significantly reduced the growth of xenograft tumours originating from *PIK3CA* wild type and *KRAS* mutant cells compared with cetuximab alone (Hong et al. 2016). Noteworthy, the mechanisms underlying resistance to PI3K inhibition are known to involve other molecules. For instance, high nuclear β-catenin concentrations were shown to confer resistance to BKM120 in sphere cultures derived from patients with colon cancer (Tenbaum et al. 2012). Clinical studies have shown that BKM120 was well tolerated and had preliminary antitumour activity (Bendell et al. 2012; Rodon et al. 2014). However, a phase I trial of BKM120 plus mFOLFOX6 (5-FU/LV + oxaliplatin), in patients with refractory solid tumours, including CRC, resulted in increased toxicity compared to either therapy alone (McRee et al. 2015). At present, other clinical trials using BKM120 are under investigation, one in combination with panitumumab in *KRAS* wild type mCRC patients (NCT01591421) and another in combination with irinotecan in previously treated advanced CRC patients (NCT01304602). PX-866, an irreversible pan-PI3K inhibitor, has been shown to cause prolonged inhibition of PI3K signalling in human tumour xenografts, namely in colon tumour xenografts (Ihle et al. 2004). In clinical trials, it was well tolerated and was associated with prolonged stable disease in patients with advanced solid tumours, namely CRC (Hong et al. 2012). A multicenter phase I study of PX-866 and cetuximab in patients with mCRC or recurrent/metastatic squamous cell carcinoma of the head and neck has shown that PX-866 and cetuximab treatment was tolerated with signs of antitumour activity (Bowles et al. 2014). Subsequently, a randomized phase II study evaluated cetuximab with or without PX-866 in patients with *KRAS* wild type mCRC; however the addition of PX-866 to cetuximab did not improve progression free survival, objective response rate, or overall survival in patients with mCRC but instead the combination arm had greater toxicity (Bowles et al. 2016). In addition to these, other pan-PI3K inhibitors are being investigated in clinical trials for several tumour types and include XL-147 (pilaralisib), GDC-0941 (pictilisib), CH5132799, GSK1059615, SF1126 and ZSTK474 (Wheler et al. 2017; Sarker et al. 2015; Thorpe et al. 2015; Patnaik et al. 2016; Blagden et al. 2014; Janku et al. 2018). To date, copanlisib (BAY80-6946) is the only pan-PI3K inhibitor, with predominant activity against PI3K p110α and PI3K p110 δ isoforms, approved for relapsed lymphoma (Markham 2017).

In the last few years, isoform specific PI3K inhibitors have also been developed and of particular interest for CRC patients are the PI3K p110α inhibitors, as mutations in the gene that code for the PI3K p110α are frequently observed. BYL719 (alpelisib), a selective inhibitor of the PI3K p110α, was shown to have antitumour activity in preclinical studies and *PIK3CA* mutation was suggested to be a positive predictor of BYL719 sensitivity (Fritsch et al. 2014). Our group has also demonstrated the potential benefit of targeting PI3K p110α in CRC cells. Notably, not only cells with *PIK3CA* mutations were sensitive to PI3K p110α inhibition, but also cells with *KRAS* mutations (Fernandes et al. 2016). In particular, we have shown that the specific inhibition of PI3K p110α, by small interfering RNA (siRNA) or BYL719, had an impact in the viability of SW480 and HCT116 CRC cells harbouring mutations in *KRAS* and *KRAS/PIK3CA*, respectively (Fernandes et al. 2016). In addition, PI3K inhibition induced apoptosis in HCT116 cells and cell cycle arrest in SW480 cells suggesting that different mechanisms may be involved (Fernandes et al. 2016). Thus, specific inhibition of the p110α subunit of PI3K could provide an

alternative therapeutic approach for CRC patients, particularly those harbouring *KRAS* mutations, who are currently excluded from EGFR-targeted therapies. In addition to preclinical studies, data from clinical trials is now emerging. The results from the first in-human phase Ia study revealed that BYL719 was tolerable and encouraging preliminary activity was observed in patients with *PIK3CA*-altered solid tumours (Juric et al. 2018). Moreover, due to the limited efficacy of BRAF inhibitors as single agents in *BRAF* mutant CRC and since EGFR and PI3K activation have been associated with resistance, a clinical phase Ib study evaluated the selective RAF kinase inhibitor encorafenib plus cetuximab or encorafenib plus cetuximab and BYL719; the results demonstrate that the treatments were tolerable and provided promising clinical activity in *BRAF* mutant mCRC patients (van Geel et al. 2017). Taselisib (GDC-0032) and MLN1117 (TAK-117, INK1117) are other PI3K p110α inhibitors currently in clinical trials for several cancer types (Janku et al. 2018).

In addition to PI3K p110α inhibitors, other p110 isoforms have been targeted including PI3K p110β and PI3K p110δ, but these have been mostly used for other cancer types. Indeed, idelalisib (CAL-101), a selective PI3K p110δ inhibitor, has already been approved for the treatment of patients with haematological malignancies (Gopal et al. 2014; Yang et al. 2015). In addition, the PI3K p110β inhibitor GSK2636771 and the PI3K p110δ inhibitor INCB050465 are also in clinical trials for various cancer types including CRC.

4.5.2 PI3K/mTOR Axis as a Therapeutic Target

The p110 catalytic domain of PI3K is structurally similar to that of the mTOR and therefore a class of inhibitors has been developed that target both molecules (Takeda et al. 2016). Using this strategy, PI3K-Akt-mTOR activation should be more efficiently inhibited as feedback mechanisms could be prevented. A number of dual inhibitors have been evaluated both in preclinical and clinical settings including BEZ235, XL765, BGT226 and PKI587.

BEZ235 (dactolisib), a potent ATP-competitive dual PI3K-mTOR inhibitor, was shown to have antitumour activity *in vitro* and *in vivo* using human tumour cell lines and tumour xenografts (Maira et al. 2008). In addition, BEZ235 was shown to induce tumour regression in genetically engineered mouse models of *PIK3CA* wild type CRC (Roper et al. 2011). Also in preclinical studies, BEZ235 was able to inhibit the PI3K/mTOR axis and to have antiproliferative and antitumoural activity in cancer cells with both wild type and mutated *PIK3CA* (Serra et al. 2008). Regarding predictive biomarkers, *PIK3CA* mutations have been associated with antitumour activity in preclinical and clinical studies, as shown with the association of the *PIK3CA* mutation H1047R with response to PI3K/AKT/mTOR signalling pathway inhibitors in early-phase clinical trials (Janku et al. 2013). Importantly, other preclinical studies have shown that coexistent mutations in *PIK3CA* and *KRAS* in CRC cells conferred resistance to BEZ235 (Kim et al. 2013). In addition, alterations in distinct molecules have also been associated with resistance to PI3K signaling inhibitors as is the case of TRIB2 that was shown to confer *in vivo* resistance to BEZ235 treatment through activation of Akt (Hill et al. 2017). Nonetheless, additional data is awaited from clinical trials. BEZ235 has also been combined with the mTOR inhibitor everolimus. Indeed, a phase Ib study of BEZ235 combined with everolimus was evaluated in patients with advanced solid malignancies but the combination of BEZ235 and everolimus demonstrated limited efficacy and tolerance (Wise-Draper et al. 2017).

BGT226, another dual PI3K-mTOR inhibitor, has also been evaluated in a clinical trial in patients with advanced solid tumours, including patients with colon cancer. However, BGT226 was shown to have limited preliminary antitumour activity and inconsistent target inhibition (Markman et al. 2012). The first-in-human study of PF05212384 (PKI-587) in patients with advanced cancer demonstrated a manageable

safety profile and antitumour activity supporting further clinical development for patients with advanced solid malignancies (Shapiro et al. 2015).

Additional dual PI3K-mTOR inhibitors have been tested in clinical trials for many cancer types, but for some inhibitors, little success or toxicity issues were observed and no further studies were pursued (Janku et al. 2018; LoRusso 2016). More data on CRC is still awaited.

4.5.3 Akt and mTOR as Therapeutic Targets

As a key molecule in the PI3K signalling cascade, Akt has been appointed as a potential therapeutic target. Indeed, MK-2206, a potent allosteric inhibitor of all Akt isoforms, has already been evaluated in preclinical and clinical settings (Brown and Banerji 2017). For instance, in mice with established xenograft tumours, MK-2206 exhibited a significant deceleration of tumour progression and primary patient-derived tumour sphere growth was significantly inhibited by MK-2206 (Malkomes et al. 2016). In the clinic, the first-in-man clinical trial of MK-2206 demonstrated good tolerability with evidence of Akt signalling blockade in patients with advanced solid tumours that included CRC patients (Yap et al. 2011). Further clinical trials using MK-2206, alone or in combination, are ongoing in patients with advanced CRC namely a phase II study in patients with metastatic *KRAS* wild type and *PIK3CA* mutant (NCT01186705). Perifosine is another Akt inhibitor that, as MK-2206, targets the PH domain of Akt, thereby preventing its translocation to the plasma membrane and blocking its phosphorylation and activation (Gills and Dennis 2009; Brown and Banerji 2017). In a phase II trial in patients with mCRC, perifosine plus capecitabine showed promising clinical activity when compared with capecitabine alone (Bendell et al. 2011). Other inhibitors, including ATP competitive inhibitors of Akt, are being tested in patients with different cancer types and these include AZD5363, GDC-0068 and GSK2141795 (Janku et al. 2018; LoRusso 2016). Importantly, special attention should be taken when using these inhibitors alone as data indicates that PI3K may signal through both Akt-dependent and Akt-independent mechanisms. Indeed, an Akt-independent signalling downstream of *PIK3CA* mutations has been described in human cancer cells (Vasudevan et al. 2009).

Inhibitors targeting mTOR are also being evaluated in clinical trials for many cancer types. These inhibitors, which can be rapamycin analogs inhibiting mTORC1, or ATP-competitive inhibiting both mTORC1 and mTORC2, have been investigated in preclinical and clinical studies (Guertin and Sabatini 2009; Papadatos-Pastos et al. 2015). Indeed, temsirolimus, which is an mTORC1 inhibitor, has already been approved for advanced renal cancer and everolimus (RAD001), also an mTORC1 inhibitor, was approved for certain cancer types including advanced renal cancer and particular types of advanced breast cancer (Hudes et al. 2007; Baselga et al. 2012; Motzer et al. 2008; Janku et al. 2018). Temsirolimus and everolimus have also been evaluated in several clinical trials in mCRC patients, either alone or in combination with other therapeutic agents. For instance, in a phase II study in patients with refractory mCRC, the combination of tivozanib (a VEGFR inhibitor) and everolimus was shown to be well tolerated, with stable disease achieved in 50% of patients (Wolpin et al. 2013). In contrast, in a phase II study in patients with mCRC heavily pretreated, everolimus was well tolerated but did not confer meaningful efficacy (Ng et al. 2013). A phase I trial of everolimus in combination with 5-FU/LV, mFOLFOX6 and mFOLFOX6 plus panitumumab in patients with refractory solid tumours including CRC has shown that the further addition of panitumumab resulted in an unacceptable level of toxicity that cannot be recommended for further study (McRee et al. 2014). A phase II trial of temsirolimus, alone or in combination with irinotecan, in *KRAS* mutant mCRC revealed that treatment was well tolerated but had limited efficacy in chemotherapy resistant *KRAS*

mutant (Spindler et al. 2013). Nonetheless, plasma KRAS quantification was suggested as a strong predictor of outcome (Spindler et al. 2013). In addition to these, other mTOR inhibitors entered clinical trials including AZD8055, AZD2014 and MLN0128 (LoRusso 2016; Papadatos-Pastos et al. 2015). More studies and data are required evaluating mTOR inhibitors, namely in combination with other regimens.

4.5.4 PI3K Inhibition in Combination with MAPK Targeted Therapies

The available PI3K targeted therapies have shown limited success in CRC patients. Indeed, despite the development of many specific inhibitors, some of which already in clinical trials, none of these have yet been approved for the treatment of patients with CRC. Therefore, in an attempt to improve the response rates of these patients, combined targeted therapies have been proposed and investigated.

Notably, it is well established that the MAPK and PI3K signalling pathways are interconnected and inhibition of one signalling cascade could induce feedback loops and compensatory mechanisms, ultimately leading to resistance (Britten 2013). Moreover, as mutations in *KRAS* and *BRAF* are frequently observed in CRC patients, inhibition of both MAPK and PI3K pathways could be a more effective strategy. Thus, several studies have been performed to evaluate the combination of inhibitors targeting molecules of these pathways.

In preclinical models, inhibition of both the MAPK and PI3K signalling pathways have been reported to be synergistic in various cancer types (Temraz et al. 2015). For instance, although treatment with BEZ235 led to marked tumour regression in a mouse model of lung cancer with the *PIK3CA* H1047R mutation, in *KRAS* G12D mutant mice only BEZ235 combined with the MEK inhibitor ARRY-142886 induced tumour regression but not BEZ235 alone (Engelman et al. 2008). In CRC, the combination of a PI3K/mTOR (PF-04691502) and a MEK (PD-0325901) inhibitor demonstrated enhanced anti-proliferative effects against CRC cell lines and demonstrated enhanced reduction in tumour growth in patient-derived CRC tumour xenograft models, regardless of *KRAS* or *PI3K* mutational status (Pitts et al. 2014). In a panel of CRC cell lines, dual targeting of PI3K (GDC-0941) and MEK (AZD6244) induced synergistic growth inhibition but the combination of specific PI3K inhibitors, rather than dual mTOR/PI3K inhibitors, with MEK inhibitors resulted in greater synergy (Haagensen et al. 2012).The inhibition of MEK and PI3K/mTOR was shown to suppress tumour growth in patient-derived xenografts of *RAS*-mutant colorectal carcinomas, though it did not cause tumour regression (Migliardi et al. 2012). However, preclinical data also indicates that such therapeutic strategies have limitations namely related to toxicity issues and periodic rather than continuous inhibition has been suggested as an alternative strategy (Will et al. 2014). Indeed, rapid induction of apoptosis by PI3K inhibitors was reported to be dependent of the transient inhibition of RAS–ERK signalling (Will et al. 2014).

In a retrospective analysis, dual targeting of the PI3K and MAPK pathways was evaluated in patients with advanced cancers including CRC and treated with phase I study drugs; the results suggested that dual inhibition may potentially exhibit favourable efficacy compared with inhibition of either pathway, although with greater toxicity (Shimizu et al. 2012). In a biomarker-driven trial, no clinical benefit was observed in CRC patients treated with the Akt inhibitor MK-2206 and the MEK1/2 inhibitor selumetinib; instead, overlapping toxicities limited the ability to dose escalate to achieve exposures likely needed for clinical activity (Do et al. 2015). At present, many clinical trials are ongoing with MAPK and PI3K inhibitors for many cancer types including CRC but data is still awaited (Temraz et al. 2015; Tolcher et al. 2018).

4.6 The Importance of Bioinformatic Tools for Biomarker and Therapy Predictions

The number of studies evaluating specific inhibitors, both in preclinical and clinical settings, is enormous. Whether evaluating single and combination of drugs in cancer cell lines, performing *in vivo* experiments in animal models, or clinical trials in patients with advanced cancers, the amount of generated information is huge and rather under analysed.

Over the last few years, bioinformatic tools have gained much interest and proved powerful in unravelling some key aspects in cancer research, namely in the discovery of cancer biomarkers and evaluation of therapy responsiveness. Currently, it is well established that despite similarities in the mutational patterns among several cancer types, specific molecular alterations have context specific functional consequences with impact in therapy outcome. For instance, using a computational strategy for integrating (phospho) proteomic and mRNA sequencing data across tumour data sets, it was possible to link the dysregulation of upstream signalling pathways with altered transcriptional programs. More specifically, it was possible to associate *PIK3CA* activating mutations with altered activities of distinct sets of transcription factors and therefore this model could help to better predict which patients will benefit from targeted and combination therapies (Osmanbeyoglu et al. 2017). In a different study, a model is proposed to integrate oncogene and tumour suppressor activity in CRC cells and used to identify cancer drivers and compute patient-specific gene activity scores (Pavel et al. 2016). In this model, the integrative score improved prediction of drug sensitivity and the gene activity scores were also used to cluster CRC cell lines (Pavel et al. 2016). In addition to these, other studies focusing on bioimaging and bioinformatics have been developed. For instance, a novel method was proposed to characterize E-cadherin signature in gastric cancer cells in order to identify E-cadherin deregulation and functional impairment (Sanches et al. 2015). More specifically, this strategy included a bioimaging pipeline to quantify the expression level and characterize the distribution of the protein from *in situ* immunofluorescence images (Sanches et al. 2015). As for gastric cancer, bioimaging tools could be used in CRC models with potential implications in biomarker identification and therapy outcome predictions.

4.7 Conclusions and Future Perspectives

The ultimate goal in cancer therapy is to provide patients with treatments that will improve their overall survival and eventually manage cancer as a chronic disease. To achieve successful outcomes, personalized therapy will most probably be needed, i.e., select the best targeted therapy to each patient tumour characteristics. Although many drugs are being developed, there is still the urgent need to develop novel therapeutic strategies.

In recent years, inhibitors to the various molecules of the PI3K-Akt-mTOR cascade have emerged. In most cases, these inhibitors have shown a wide range of adverse effects and limited success. A deeper knowledge of the complex interplay between distinct signalling pathways, as well as a better understanding of feedback-loops disruption and the occurrence of compensatory mechanisms upon PI3K inhibition, will guide and improve the design of novel therapeutic strategies. The available clinical results have shown that dual MAPK and PI3K inhibition is possible, although toxicity has been an issue. Additional combination therapy regimens are being tested and should be considered.

A challenge in the treatment of CRC patients will be the identification of specific biomarkers predictive of therapy responsiveness, for which bioinformatics tools will be essential. Indeed, a proper patient stratification will most probably be a key issue for a successful outcome. Although many studies have been performed to identify such biomarkers, further studies are required. Overall, and despite ongoing clinical trials with some of these drugs, CRC patients are still await-

ing alternative therapies to be approved. More data on novel drugs, combination regimens and clinical trials is expected to shed light on CRC best targeted therapies.

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