Cancer Chemother Pharmacol (2013) 71:1395–1409
DOI 10.1007/s00280-013-2121-1

REVIEW ARTICLE

PI3K and MEK inhibitor combinations: examining the evidence in selected tumor types

Carolyn D. Britten

Received: 14 January 2013 / Accepted: 8 February 2013 / Published online: 27 February 2013

Abstract The PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways are two of the most frequently dysregulated kinase cascades in human cancer. Molecular alterations in these pathways are implicated in tumorigenesis and resistance to anticancer therapies. The PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways are known to interact with each other at several nodes, and mounting evidence suggests that dual blockade of both pathways may be required to achieve anticancer effects in certain contexts. This may include tumor types with a high frequency of RAS/RAF/MEK/ERK pathway activation, or situations in which dual pathway strategies may be required to overcome resistance to current targeted therapies. Several clinical studies are currently evaluating the combination of PI3K and MEK inhibitors in a variety of different cancers with certain types of molecular alterations. This review will summarize existing knowledge of the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways, the cross-talk between them, and the current generation of PI3K and MEK inhibitors that target them. The preclinical rationale for dual pathway inhibition will be discussed within the context of the major tumor types currently being explored in ongoing clinical trials, namely malignant melanoma with BRAF or NRAS mutations, and colorectal, ovarian, pancreatic, and basal-like breast cancers. The emerging clinical profile of PI3K and MEK inhibitor combinations, as reported in Phase I trials, will also be discussed.

C. D. Britten (✉)
Division of Hematology/Oncology, Department of Medicine, Hollings Cancer Center, Medical University of South Carolina, 96 Jonathan Lucas Street, Clinical Science Building, Suite 903, Charleston, SC 29425, USA
e-mail: britten@musc.edu

Keywords BRAF mutation · MEK inhibitor · NRAS mutation · PI3K inhibitor · PI3K/AKT/mTOR pathway · RAS/RAF/MEK/ERK pathway

Introduction

The PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways are two of the most frequently dysregulated kinase cascades in human cancer [1, 2]. Both pathways represent important signal transduction mechanisms that facilitate the proliferation and survival of cancers driven by growth factor receptors, such as human epidermal growth factor receptor 2 (HER2) or epidermal growth factor receptor (EGFR). The individual downstream components of these signaling cascades are also known to be frequently altered in cancer, either through somatic mutation or epigenetic modification, thus contributing to tumorigenesis and resistance to anticancer therapies [3]. Our understanding of both pathways and their role in cancer continues to grow, and agents that target specific components of these pathways have recently been approved for use in certain tumor types, with many more agents in clinical development.

The PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways are known to interact with each other at several nodes, and mounting evidence suggests that dual blockade of both pathways may be required to achieve anticancer effects in certain contexts. Several clinical studies are currently evaluating the particular combination of PI3K and MEK inhibitors in a variety of different cancers with certain types of molecular alterations. This review will summarize existing knowledge on the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways, the cross-talk between them, and the current generation of investigational agents that target them. The preclinical rationale for dual

pathway inhibition will then be discussed within the context of the major tumor types currently being explored in ongoing clinical trials.

The PI3K/AKT/mTOR pathway

The PI3K protein family comprises three different classes of lipid kinase, of which Class I_A has been most frequently implicated in human cancer [2, 4, 5]. Class I_A PI3Ks are heterodimers that consist of a regulatory and a catalytic subunit. Several different isoforms of the catalytic (p110α, p110β, and p110δ) and the regulatory [p85α, p55α, p50α (all splice variants of the same gene), p85β, and p55γ] subunits have been described. In particular, amplification or mutation of PIK3CA (which encodes the p110α catalytic subunit) has frequently been described in a broad range of human cancers.

The PI3K/AKT/mTOR kinase cascade is triggered by receptor tyrosine kinases (RTKs) and G-protein-coupled receptors (GPCRs) located at the cell surface (Fig. 1). When these receptors bind their extracellular ligands and become phosphorylated, they sequester the regulatory subunit of PI3K, leaving the catalytic subunit free to catalyze the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PIP_3 is an important propagator of intracellular signaling, and its production is antagonized by the tumor suppressor phosphatase and tensin homolog (PTEN), which has been found to be frequently silenced or inactivated in a range of cancers. PIP_3 directly binds the pleckstrin homology domains of various proteins, including phosphoinositide-dependent kinase 1 (PDK1) and AKT. PDK1 binds PIP_3 at the plasma membrane and phosphorylates AKT, which is then free to phosphorylate a multitude of targets in the nucleus and cytoplasm.

AKT promotes cell survival by phosphorylating MDM2 (a negative regulator of the p53 tumor suppressor) and by negatively regulating the pro-apoptotic Bcl-2 family members BAD and BAX, and forkhead transcription factors, such as FOXO. Activated AKT also negatively regulates tuberous sclerosis protein complex 1 (TSC1) and TSC2, which leads to activation of mTOR complex 1 (mTORC1), a key regulator of cellular growth and protein synthesis. mTORC1 stimulates S6 kinase activity, which has multiple substrates, including the ribosomal protein S6. mTORC1 also phosphorylates factor 4E-binding protein 1 (4E-BP1), releasing eukaryotic initiation factor 4E (eIF4E) to permit assembly of the cap-binding complex, initiating translation of specific mRNAs.

The RAS/RAF/MEK/ERK pathway

The RAS/RAF/MEK/ERK pathway is triggered by RTKs, GPCRs, and cytokine receptors [1, 6]. RAS, the key controller of the pathway, comprises a family of four small GTPases: HRAS, NRAS, and two forms of KRAS (KRAS4A and KRAS4B), which arise through alternative splicing. RAS proteins are recruited to ligand-bound receptors at the plasma membrane via activation of the Grb2/SOS coupling complex; however, this process is dependent on RAS undergoing post-translational modification, including farnesylation, geranylgeranylation, or palmitoylation. Activation of RAS, via the exchange of GDP for GTP, allows the recruitment of the serine/threonine RAF to the plasma membrane.

The RAF family of proteins comprises three members: ARAF, BRAF (which is frequently mutated in certain cancers), and CRAF (RAF-1). The regulatory control of these kinases is highly complex, involving dimerization, phosphorylation at multiple sites, and further interactions with other proteins. Activated RAF phosphorylates MEK, a dual-specificity tyrosine and serine/threonine kinase, which has the predominant role of activating the serine/threonine kinases ERK1 and ERK2. ERKs have over 160 different targets and directly phosphorylate cytoplasmic proteins, such as p90 ribosomal S6 kinase (RSK), and many transcription factors, including ETS-1, c-JUN, and c-MYC. Moreover, ERKs are known to have key roles in the control of cell cycle regulation, proliferation, and survival.

PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathway cross-talk

The PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways are known to interact at multiple points, resulting in cross-activation, cross-inhibition, and pathway convergence (Fig. 1) [7]. First, and most importantly, RAS is capable of directly activating PI3K through a mechanism that is independent of the p85 regulatory subunit [8]. Cross-activation between the two pathways also occurs through ERK, which phosphorylates TSC2 at sites distinct from those phosphorylated by AKT [9]. Phosphorylation by either ERK or AKT suppresses TSC2, thus promoting activation of mTORC1.

Under certain circumstances, cross-inhibition between the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways can occur; for example, AKT can phosphorylate the regulatory domain of RAF in the setting of strong insulin-like growth factor 1 (IGF1) stimulation, thus inhibiting RAF activity [10]. Similarities between certain pathway components can also result in pathway convergence. RSK, AKT, and S6K are all AGC kinases (named after the protein kinase A, G, and C families), and as such, they are sometimes capable of phosphorylating the same motif within the same protein [7]. This, and many other

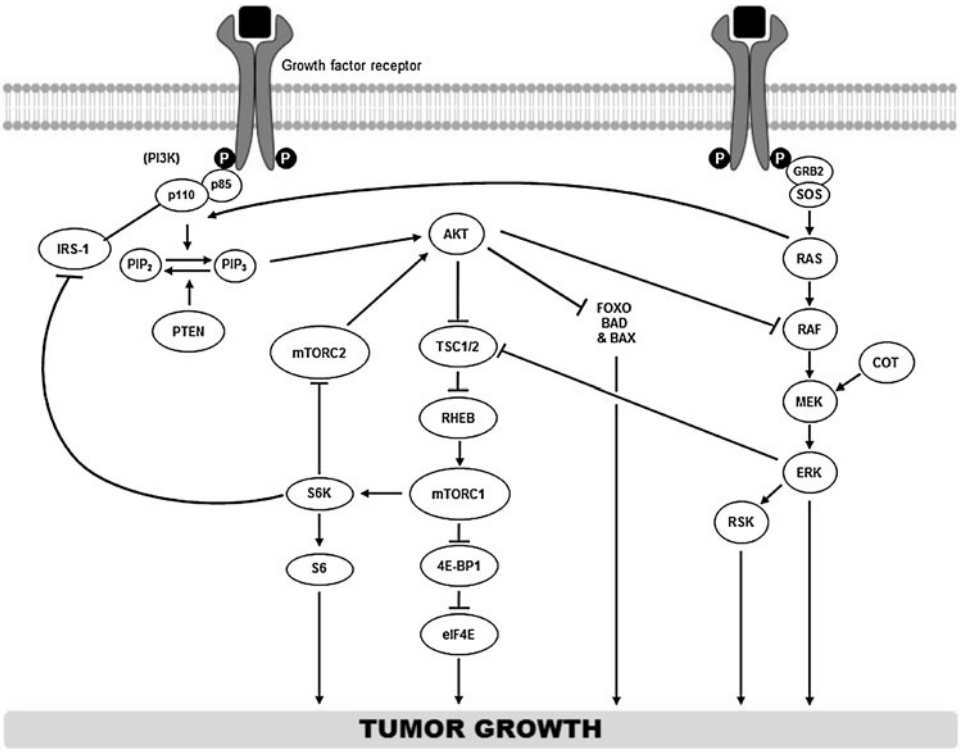

Fig. 1 PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways. *4E-BP1* factor 4E-binding protein 1, *AKT (PKB)* protein kinase B, *BAD* Bcl-2-associated death promoter, *BAX* Bcl-2-associated X protein, *COT* cancer Osaka thyroid, *eIF4E* eukaryotic initiation factor 4E, *ERK* extracellular signal-regulated kinase, *FOXO* forkhead box O, *GRB2* growth factor receptor-bound 2, *IGF* insulin-like growth factor, *IRS-1* insulin receptor substrate 1, *MEK* mitogen-activated protein kinase/ERK kinase, *mTORC* mammalian target of rapamycin complex, *PI3K* phosphatidylinositol 3-kinase, PIP_2 phosphatidylinositol 4,5-bisphosphate, PIP_3 phosphatidylinositol 3,4,5-trisphosphate, *PTEN* phosphatase and tensin homolog, *RAF* rapidly accelerated fibrosarcoma, *RHEB* Ras homolog enriched in brain, *RSK* ribosomal S6K, *RAS* rat sarcoma, *SOS* son of sevenless, *S6K* S6 kinase, *TSC* tuberous sclerosis protein complex

mechanisms of cooperation between the two pathways, is summarized elsewhere [7, 11].

The network of cross-talk between the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways is complex, and the relative importance of each interaction under physiologic conditions may vary from observations made in vitro. Pathway signaling and cross-talk may also vary across different tissue or tumor types, and can also depend on the relative strengths of different growth factors, and/or other pathway inputs. Data from preclinical experiments and retrospective studies suggest that tumors can utilize redundancy in signaling as a means of developing resistance to anticancer agents that target upstream RTKs or components of the pathways themselves.

Current-generation pathway inhibitors

The observation that the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways are frequently activated in cancer, and that components of these pathways are frequently mutated or altered, has driven the development of an expanding range of small-molecule inhibitors that target various nodes of both pathways.

PI3K/AKT/mTOR pathway inhibitors

Inhibitors of the PI3K/AKT/mTOR pathway include the first-generation agent rapamycin and second-generation agents everolimus and temsirolimus, all of which allosterically inhibit the mTORC1 complex by interacting with FK-binding protein 12 (FKBP12) [2, 12]. Although these second-generation agents have been proven effective in certain tumor types, it has been speculated that their broader efficacy may be limited by their inability to inhibit mTORC2 (an activator of AKT) and the resulting abrogation of negative feedback loops that occur when mTORC1 activity is inhibited [12, 13]. The current generation of PI3K inhibitors was designed with the hope of avoiding the issues of negative feedback seen with mTORC1 inhibitors, by targeting the pathway further upstream.

In contrast to mTORC1 inhibitors, which allosterically inhibit their target, current-generation PI3K inhibitors bind to the ATP-binding pocket of the catalytic domain of PI3K [13]. mTOR and PI3K share structural similarities, and as a consequence, many of the first PI3K inhibitors to be investigated have been dual inhibitors of Class IA PI3K and both

mTOR complexes [13]. It has been speculated that dual PI3K/mTOR inhibition could offer advantages over selective inhibition of PI3K or mTOR, by virtue of more complete blockade of the pathway [12]. However, the identification of more specific inhibitors has continued to be pursued due to the possibility of improved toxicity profiles, which could enable higher therapeutic doses to be given, especially when combined with other therapies [12, 13].

Advances in drug design led to the development of "pure" PI3K inhibitors, which selectively target the catalytic sites of Class I PI3K only [12, 13]. Isoform-specific PI3K inhibitors have also recently emerged, including agents that target p110α, the catalytic subunit isoform encoded by PIK3CA, which is most commonly implicated in solid cancers, and p110δ, an isoform of importance in chronic lymphocytic leukemia and indolent non-Hodgkin's lymphoma. PI3K inhibitors in current clinical development are outlined in Table 1A. While the clinical data are in evolution, common toxicities noted to date include gastrointestinal effects (diarrhea, nausea, vomiting, anorexia), rash, hyperglycemia, transaminase elevation, fatigue, and pneumonia [12, 13]. Reversible mood alteration has been observed with at least one agent (BKM120) and may be related to the effects of PI3K on neurocognitive dysfunction [14]. Properties of individual agents are reviewed in detail elsewhere [12, 13].

RAS/RAF/MEK/ERK pathway inhibitors

Early attempts at targeting the RAS/RAF/MEK/ERK pathway focused on inhibiting RAS by preventing its activation via farnesylation. Unfortunately, these farnesyltransferase inhibitors proved disappointing in the clinic, due to the realization that RAS could still be activated through geranylgeranylation [15]. The observation that BRAF is frequently mutated in several human cancers (namely melanoma, colorectal cancer, thyroid cancer, and low-grade serous ovarian cancer) led to the development of BRAF-specific inhibitors, and one such inhibitor, vemurafenib, is approved for use in late-stage melanoma with BRAF V600E mutation [16]. Inhibition of MEK provides another approach, with potential applications for malignancies with alterations in BRAF and NRAS.

MEK inhibitors in clinical development are outlined in Table 1B. Common toxicities include rash and/or dermatitis acneiform, diarrhea, peripheral edema, and fatigue [17, 18]. Ocular events, particularly blurred vision, central serous retinopathy, and retinal vein occlusion, are considered class effects [18]. Asymptomatic reversible ventricular dysfunction has also been observed in a small percentage of patients [17]. Properties of individual agents are available in clinical trial results cited throughout this review.

Rationale for dual pathway inhibition

Interaction between the parallel PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways may explain the modest single-agent activity of agents that target these pathways. Aside from the encouraging results with the PI3Kδ-specific inhibitor CAL-101 (now GS-1101) in indolent non-Hodgkin's disease [19], early-phase single-agent trials of PI3K inhibitors have frequently demonstrated cytostatic rather than cytotoxic clinical activity, with stable disease but few partial responses in patients with advanced solid tumors [2, 12–14, 20–24]. Regarding MEK inhibitors, although BRAF-mutant disease has demonstrated sensitivity [17], studies in more heterogeneous patient populations have only shown modest clinical efficacy [18, 25–28].

In tumors driven by activated or amplified RTKs (such as EGFR-mutant lung cancer or HER2-amplified breast cancer), oncogenic signaling can potentially take place via either or both pathways [29, 30]. Single-agent inhibition of PI3K or MEK in these tumor types has been shown to lead to compensatory increases in ERK or AKT activity, respectively [29–31]. Many Phase II/III clinical trials with PI3K, mTOR, or MEK inhibitors are currently examining inhibitors as combination therapy with receptor-targeted therapies [32]. However, different tumor types exhibit different mechanisms of resistance to receptor-targeted therapies, suggesting that the addition of PI3K or MEK inhibitors will not always be capable of reversing resistance to receptor-targeted therapies [33]. In these circumstances, combined PI3K and MEK inhibition may ensure the blockade of downstream proliferation, growth, and survival signals in tumors that develop resistance to RTK-directed therapy.

Not all cancers demonstrate expression or activation of a known receptor that can be therapeutically targeted. RAS is capable of signaling through both the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways, and activating mutations in RAS are generally recognized as one of the most frequently observed alterations in cancer, being found in up to approximately 30 % of all cases [34]. Preclinical studies regarding the use of *KRAS* mutations as a predictive marker for resistance to single-agent PI3K inhibitors have yielded mixed results [35–38], whereas in the clinic, at least one response with single-agent PI3K inhibitor therapy has been observed in a patient with KRAS-mutant triple-negative breast cancer [14]. Although MEK inhibitors have demonstrated preclinical activity in some RAS-mutant cancers, sensitivity to MEK inhibitors is likely to be dependent on additional factors, such as tumor type, or the presence of coexisting molecular alterations, such as *PIK3CA* mutation, PTEN loss, or the activation of compensatory pathways other than PI3K/AKT/mTOR [37, 39–42].

Table 1 PI3K and MEK inhibitors currently in clinical development

Agent	Company	ROA	Target	Phase of development
A. PI3K inhibitors				
BKM120	Novartis	Oral	Class I PI3K	Phase III
GS-1101	Gilead Sciences	Oral	Isoform specific (PI3Kδ)	Phase III
BAY 80-6946	Bayer	IV	Class I PI3K	Phase II
BEZ235	Novartis	Oral	PI3K/mTORC1/2	Phase II
GDC-0941	Genentech	Oral	Class I PI3K	Phase II
PF-04691502	Pfizer	Oral	PI3K/mTORC1/2	Phase II
PF-05212384	Pfizer	IV	PI3K/mTORC1/2	Phase II
PX-866	Oncothyreon	Oral	Class I PI3K	Phase II
BYL719	Novartis	Oral	Isoform specific (PI3Kα)	Phase II
GDC-0980	Genentech	Oral	PI3K/mTORC1/2	Phase II
SAR245408 (XL147)	Sanofi	Oral	Class I PI3K	Phase I/II
SAR245409 (XL765)	Sanofi	Oral	PI3K/mTORC1/2	Phase I/II
GSK2126458	GlaxoSmithKline	Oral	PI3K/mTORC1/2	Phase I
B. MEK inhibitors				
Trametinib (GSK1120212)	GlaxoSmithKline	Oral	MEK1/2	Phase III
Selumetinib (AZD6244)	AstraZeneca	Oral	MEK1/2	Phase II
GDC-0973 (XL518)	Genentech	Oral	MEK1/2	Phase I/II
BAY 86-9766	Bayer	Oral	MEK 1/2	Phase I/II
Pimasertib (AS703026/ MSC1936369B)	Merck	Oral	MEK 1/2	Phase I
PD325901	Pfizer	Oral	MEK1/2	Discontinued as a single agent but under evaluation in combination with PI3K inhibitors
CI-1040 (PD184352)	Pfizer	Oral	MEK1/2	Discontinued

IV intravenous, *ROA* route of administration

Source: ClinicalTrials.gov

Since RAS can potentially signal through both the PI3K/AKT/mTOR and RAF/MEK/ERK pathways, combined inhibition of both pathways may be necessary to affect a clinical response in certain tumor types or in tumors with alterations in both pathways. The following sections discuss preclinical evidence and the rationale for PI3K plus MEK inhibitor combinations in selected cancers with a high frequency of RAS/RAF/MEK pathway activation, or in which dual pathway strategies may be required to overcome resistance to current targeted therapies.

Rationale for PI3K plus MEK inhibitor combinations in selected cancers

Melanoma

BRAF-mutant melanoma

A total of 40–60 % of cutaneous melanomas harbor a BRAF mutation, and 90 % of these are BRAF V600E [16, 43, 44]. Vemurafenib demonstrates an impressive 80 % response rate in patients with BRAF V600E-positive melanoma [16, 43], and its introduction represented an important breakthrough in this disease. Although the activity of vemurafenib in patients with sensitizing mutations has been extremely encouraging, the duration of response is limited and tumors quickly develop resistance via additional molecular alterations in other pathway components [45].

Resistance to BRAF inhibitors Preclinical data and tumor genomic profiling suggest that resistance to BRAF inhibitors may arise through several mechanisms, including increased expression or aberrant splicing of BRAF [46, 47], increased expression of CRAF [48], increased expression of COT [49], activating mutations in NRAS [50, 51], activating mutations in MEK1 [52], and activation or altered expression of RTKs, such as platelet-derived growth factor receptor β (PDGFRβ) [51].

Current-generation MEK inhibitors have shown some promise in the treatment of BRAF-mutant melanoma [17, 53, 54]. When compared with chemotherapy, the MEK

inhibitor trametinib improved progression-free survival and overall survival in patients with V600E or V600K BRAF-mutant metastatic melanoma [17]. Furthermore, preclinical experiments with dabrafenib and trametinib suggest that BRAF and MEK inhibitor combinations may be capable of overcoming resistance to BRAF inhibitors caused by certain mutations in NRAS or MEK [55]. In addition, the combination of a MEK inhibitor with a BRAF inhibitor may prevent BRAF inhibitor-induced tumorigenesis in latent keratinocytes [56, 57]. A randomized Phase II trial in metastatic BRAF V600-mutant melanoma compared the BRAF inhibitor dabrafenib in combination with trametinib versus single-agent dabrafenib [58]. Combination therapy resulted in a significant improvement in progression-free survival (median 9.4 vs. 5.8 months for dabrafenib monotherapy) and a trend toward decreased incidence of cutaneous squamous cell carcinoma [58].

The heterogeneous range of resistance mechanisms observed in BRAF inhibitor-refractory cancer cells implies that additional inhibitor combinations beyond those of BRAF plus MEK inhibitors may be required to fully address the problem of BRAF inhibitor-refractory disease [50]. Activation of the PI3K/AKT/mTOR pathway, through RTK activation, alterations in PI3K pathway components, or alterations in RAS, represents one potentially important route of resistance and could limit the effectiveness of therapies solely targeting the RAS/RAF/MEK/ERK pathway.

Preclinical evidence with PI3K and MEK inhibitor combinations Evidence supporting the use of PI3K and MEK inhibitor combinations in vemurafenib-resistant BRAF-mutant melanoma comes from several preclinical experiments. *In vitro* experiments suggest that resistance to BRAF inhibitors may confer cross-resistance to MEK inhibitors [55, 59]. BRAF-mutant melanoma cell lines resistant to vemurafenib and the MEK inhibitor, selumetinib, were shown to be dependent upon the induction or persistence of AKT signaling for survival [59]. In these cell lines, the addition of an AKT inhibitor was able to overcome the resistance to single-agent MEK inhibition [59]. Similarly, in vitro generation of dabrafenib-resistant BRAF V600E cell lines, arising through MEK or NRAS mutations, was resistant to single-agent trametinib but showed enhanced cell growth inhibition when combined with the PI3K inhibitor GSK2126458 [55].

Vemurafenib-resistant BRAF V600E melanoma cell lines with PDGFRβ upregulation showed dual activation of ERK and AKT in vitro [60]. Although the combination of vemurafenib and the PI3K/mTOR inhibitor BEZ235 was capable of synergistically inhibiting growth, antiapoptotic effects were not observed. However, the combination of selumetinib and BEZ235 was found to consistently trigger apoptosis in all cell lines investigated [60]. Lastly, in BRAF-mutant melanoma cells chronically treated with the BRAF inhibitor SB-590885, resistance was found to occur through the upregulation of alternative RAF isoforms [61]. Single-agent MEK inhibition in these cells was found to have cytostatic rather than cytotoxic effects, and resistance was associated with increased IGF-1R and pAKT levels, which were also identified in a post-relapse tumor sample [61]. Combined treatment with selumetinib and an IGF-1R or PI3K inhibitor was able to overcome this resistance mechanism in vitro, leading to apoptosis [61].

NRAS-mutant melanoma

Around 15–20 % of cutaneous melanomas harbor a mutation in NRAS [62, 63], and these alterations are generally not found to coexist with BRAF mutations [62, 63]. There are currently no approved drugs that directly target altered NRAS, and the treatment of melanomas with these alterations represents an unmet medical need.

NRAS-mutant melanoma cell lines have been shown to be partially sensitive to MEK inhibition [64]. However, single-agent MEK inhibitors have shown disappointing activity against NRAS-mutant melanoma in the clinic. In a Phase I trial of the MEK inhibitor selumetinib in patients with advanced malignancies, one patient with uveal NRAS-mutant melanoma plus renal cancer had stabilization of disease for 88 weeks [65]. A randomized Phase II trial of single-agent selumetinib versus temozolomide showed no difference in progression-free survival in 200 unselected melanoma patients [66]. In this trial, 10 patients with NRAS-positive melanoma were treated with selumetinib, and none responded. Lastly, a Phase II study with the MEK inhibitor MEK162 demonstrated a response rate of 8 % among 24 patients with NRAS-mutant melanoma [53].

Since RAF has multiple downstream pathways, combination therapy with PI3K and MEK inhibitors may be more effective than single-agent strategies in treating RAS-mutant melanoma. As an example, genetically engineered mouse models of RAS-mutant melanoma demonstrated resistance to both selumetinib and the BEZ235 monotherapy; however, the combination of both agents was able to induce tumor regression and increase survival [67].

Colorectal cancer

The anti-EGFR antibodies cetuximab and panitumumab are active as single agents in chemorefractory metastatic colorectal cancer; however, these therapies are only effective in patients with KRAS wild-type tumors [68, 69]. KRAS mutations are observed in 35–40 % of colorectal cancers, while NRAS and HRAS mutations are less

common (1–3 %) [70]. In addition to causing primary resistance to anti-EGFR therapy, the emergence of KRAS mutations has also been associated with acquired resistance to these agents [71]. BRAF mutations occur in 5–15 % of colorectal cancers, but are mutually exclusive with KRAS mutations in this disease [70, 72, 73]. Although BRAF mutations do not appear to predict for resistance to cetuximab, they do predict for poor prognosis [72, 74].

Since perturbations in the RAS/RAF/MEK/ERK pathway are frequent in colorectal cancer, therapies targeted against this axis are urgently required. Unfortunately, efforts to treat advanced colorectal cancer with MEK inhibitors have thus far been disappointing. A Phase II trial with the first-generation MEK inhibitor CI-1040 identified an amenable safety profile, but very little antitumor activity in a heterogeneous patient population that included 20 patients with advanced colorectal cancer [26]. While CI-1040 may be an inferior drug compared with the next generation of MEK inhibitors, selumetinib (second-generation MEK inhibitor) has not performed well in unselected metastatic colorectal cancers either. A Phase II trial with selumetinib in patients with advanced colorectal cancer demonstrated similar efficacy to capecitabine in patients who had progressed after one or two prior lines of chemotherapy [27].

Experiments suggest that RAS/RAF wild-type cell lines are resistant to MEK inhibitors, whereas RAF-mutant, and to a lesser extent RAS-mutant, cell lines are sensitive [64]. In KRAS-mutant colorectal cancer cells, resistance to MEK inhibitors may arise through a variety of mechanisms, including amplification of the oncogene [75, 76], upregulation of the Wnt signaling pathway [77], or through coexisting mutations in PIK3CA, and/or activation of the PI3K pathway [39, 40, 78, 79]. Importantly, PIK3CA mutations occur in about 15 % of colorectal cancers [70, 80], and two large series with a combined number of over 1,200 patients demonstrated that 8–9 % of colorectal cancers harbor mutations in both PIK3CA and KRAS [70, 80].

Preclinical data indicate that combined MEK and PI3K inhibition will be required to instigate or maintain a pro-apoptotic effect in KRAS-mutant colon cancer with alterations in the PI3K pathway. The HCT116 cell line, which harbors mutations in both KRAS and PIK3CA, was resistant to the PI3K inhibitors WAY-266176 and WAY-266175; however, combined therapy with the MEK inhibitors CI-1040 or UO126 led to growth suppression and apoptosis [81]. In other investigations with the KRAS/PIK3CA-double-mutant HCT116 and DLD-1 cell lines, selective knockout of mutant PIK3CA conferred sensitivity to the MEK inhibitor PD0325901 [40, 78], whereas exogenous re-expression of mutant PIK3CA restored resistance [78]. In addition, murine xenografts of the HCT116 cell line were resistant to single-agent MEK inhibition (PD0325901); however, xenografts engineered with knockout of mutant PIK3CA demonstrated sensitivity [40, 78]. Similar increased antitumor activity has been observed with combined MEK and AKT inhibition in KRAS/PIK3CA-double-mutant HCT15 and BRAF/PIK3CA-double-mutant RKO colon cancer models [39, 78].

Recent evidence suggests that the mechanism of synergy between PI3K and MEK inhibitors in colorectal cancer cells can be modulated by mTOR inhibition [82]. The pan-PI3K inhibitor GDC-0941 demonstrated greater synergy than the dual PI3K/mTOR inhibitor BEZ235 when combined with the MEK inhibitors selumetinib and PD0325901 in BRAF/PIK3CA-double-mutant HT29 and KRAS/PIK3CA-double-mutant HCT116 colorectal cancer cell lines [82]. The addition of the mTORC1/2 inhibitor KU0063794 impaired the synergistic effect of GDC-0941 with MEK inhibitors. This suggests that different types of PI3K inhibitors should be evaluated as part of combination therapy with MEK inhibitors [82].

Despite the strong preclinical rationale for PI3K and MEK inhibitor combinations in colorectal cancer, some studies suggest that combined blockade of both pathways may not consistently lead to durable responses or regressions in this tumor type [83]. Migliardi et al. [83] recently reported on an analysis of 40 different patient-derived murine xenografts of metastatic colorectal cancer. In these models, the combination of BEZ235 and selumetinib demonstrated greater rates of disease stabilization than monotherapy (disease control rates were 70 % for combined therapy, and 27.5 and 42.5 % for selumetinib and BEZ235, respectively); however, substantial tumor regression was not observed [83]. These findings could suggest that PI3K and MEK inhibitor combinations may not be capable of durable responses in patients with KRAS-mutant colorectal cancer and that additional biomarkers may be required to identify those patients who will respond best [83].

Ovarian cancer

Ovarian cancer is a heterogeneous disease comprising different histologic subtypes that are characterized by frequent loco-regional dissemination to the ovary and related pelvic organs; as such, a considerable proportion of tumors classified as “ovarian cancer” may not arise from ovarian tissue [84]. The current model for epithelial ovarian carcinogenesis defines two broad categories, designated type I and type II [85]. Type I tumors are typically low-grade neoplasms that develop from borderline tumors. Type I tumors include low-grade serous carcinoma, mucinous carcinomas, endometrioid carcinomas, malignant Brenner tumors, and clear-cell carcinomas [86]. The type I molecular signature is characterized by frequent BRAF and KRAS mutations, and wild-type p53 and BRCA1/2 [85].

Type II tumors are typically high-grade tumors that have no recognizable precursor lesion. Type II tumors include high-grade serous carcinoma (the most prevalent epithelial ovarian cancer), malignant mixed mesodermal tumors (carcinosarcoma), and undifferentiated carcinomas [86]. The type II molecular signature is characterized by mutated p53, dysregulated BRCA1/2, and wild-type BRAF and KRAS [85].

Low-grade serous ovarian cancers are slow growing but very resistant to standard-of-care platinum therapies [85]. As the prototypical type I epithelial ovarian cancer, they are associated with a high rate of KRAS and BRAF mutation, implying that MEK inhibitors could be a relevant strategy for this specific subtype of tumor. In a Phase II trial of single-agent selumetinib in 52 patients with low-grade serous ovarian carcinoma, eight patients (15.4 %) had complete or partial responses, and 34 patients (65.4 %) had stable disease [87]. Although 41 % of evaluable patients had KRAS mutation, this was not found to predict response to MEK inhibition [87]. Due to the significant cross-talk between the RAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathways, the addition of a PI3K inhibitor could potentiate single-agent MEK inhibition in low-grade serous ovarian cancer.

Combined therapy with PI3K and MEK inhibitors could also play a role in the treatment of ovarian cancer subtypes that harbor abnormalities in the PI3K/AKT/mTOR pathway. Clear-cell carcinomas have a PIK3CA mutation rate of approximately 50 % and a PTEN deletion rate of about 20 %, whereas low-grade endometrioid carcinomas have a PIK3CA mutation rate of 20 % and PTEN mutation rate of 20 % [85]. PI3K and MEK inhibitors have shown synergy in preclinical experiments with ovarian cancer models harboring abnormalities in the PI3K/AKT/mTOR pathway. For example, in a KRAS-mutant/PTEN deletion mouse model of epithelial ovarian cancer, the PI3K/mTOR inhibitor, PF-04691502, did not induce tumor regression or long-term growth inhibition. However, the combination of PF-04691502 and PD0325901 instigated striking tumor regression and observable pro-apoptotic effects [88].

Type II tumors are not generally associated with baseline alterations in the PI3K/AKT/mTOR or RAS/RAF/MEK/ERK pathways, and for these high-grade tumors, other strategies are more likely to be successful.

Pancreatic cancer

Pancreatic cancer is characterized by an extremely high frequency of KRAS mutation (50–80 % of cases) [89, 90], and preclinical experiments have confirmed that KRAS expression is required for oncogenesis and tumor maintenance [37, 91]. In KRAS-driven mouse models of pancreatic cancer, signaling appears to predominantly occur via the RAS/RAF/MEK/ERK pathway, with pERK expression readily detectable and pAKT expression comparatively low [37]. Pancreatic cancer xenografts were sensitive to MEK inhibition with selumetinib and relatively resistant to PI3K inhibition with GDC-0941, whereas the combination of selumetinib and GDC-0941 demonstrated synergistic antitumor effects [37]. *In vitro* models of pancreatic cancer have demonstrated that dual inhibition of the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways leads to synergistic apoptotic effects through the activation of FOXO transcription factors [92].

The pivotal role of RAS in pancreatic cancer has led to clinical trials with MEK inhibitors in this disease. A single-agent Phase I trial of the MEK inhibitor CI-1040 enrolled 77 subjects with solid tumors and demonstrated one partial response in a subject with pancreatic cancer [93]. A subsequent Phase II trial with CI-1040 in subjects with colorectal, non-small cell lung, breast, or pancreatic cancer, without pre-defined molecular abnormalities, failed to demonstrate any responses [26]. Most recently, a Phase II trial of selumetinib versus capecitabine in patients with advanced pancreatic cancer identified only a marginal improvement in median survival (5.4 vs. 5.0 months, respectively) [28]. It has been hypothesized that the low efficacy observed in the Phase II trial of selumetinib could be due to the advanced stage of the patients enrolled, who would have received several rounds of prior therapies [28, 37]. Nevertheless, the findings of these trials suggest that single-agent MEK inhibitors will not be sufficient to improve current standards of pancreatic cancer care.

Basal-like breast cancer

Basal-like breast cancer is a molecular intrinsic subtype that exhibits a gene expression profile similar to the basal/myoepithelial layer of normal breast cells, as defined by microarray analysis [94]. A substantial proportion of basal-like breast cancers correspond with the "triple-negative" subtype and therefore do not express estrogen receptor, progesterone receptor, or HER2 [95]. These tumors are aggressive, highly proliferative, and not sensitive to treatment with conventional anti-HER2 or hormonal therapies [94, 95]. In the absence of targeted therapy, the identification of new therapeutic targets that can be exploited in basal-like or triple-negative breast cancer is an important research goal.

KRAS mutations are almost never observed in triple-negative breast cancer [96]; however, triple-negative breast cancer cell lines have been shown to exhibit gene expression profiles similar to those of KRAS-mutant cancers [97, 98]. Breast cancer cell lines with these gene expression profiles demonstrate upregulation of pMEK and pERK and are sensitive to MEK inhibition [97, 98]. In contrast, cell

Table 2 Ongoing clinical trials with PI3K and MEK inhibitor combinations

Trial	Sponsor	PI3K inhibitor	MEK inhibitor	Phase	Patient population
NCT01363232	Novartis	BKM120	MEK162	Phase I	Advanced solid cancers, including triple-negative breast cancer, pancreatic cancer, colorectal cancer, malignant melanoma, non-small cell lung cancer, and other cancers with *KRAS*, *BRAF*, and *NRAS* mutation
NCT01337765	Novartis	BEZ235	MEK162	Phase I	Advanced solid cancers, including triple-negative breast cancer, pancreatic cancer, colorectal cancer, malignant melanoma, non-small cell lung cancer, and other cancers with *KRAS*, *BRAF*, and *NRAS* mutation
NCT01390818	EMD Serono Sanofi	SAR245409	Pimasertib (MSC1936369B)	Phase I	Advanced solid tumors, including either of the following: (1) cancer with diagnosed alteration in one or more of the following genes: *PTEN, BRAF, KRAS, NRAS, PI3KCA, ErbB1, ErbB2, MET, RET, c-KIT, GNAQ, GNA11;* or (2) any of the following cancers: pancreatic, thyroid, colorectal, non-small cell lung, endometrial, renal, breast, ovarian carcinoma, or melanoma
NCT01347866	Pfizer	PF-04691502 or PF-05212384	PD0325901	Phase I	Advanced cancers demonstrating *KRAS* or *BRA*F mutation and patients with advanced colorectal cancer with evidence of *KRAS* mutation and no more than 1 prior regimen of systemic therapy
NCT01155453	Novartis	BKM120	Trametinib (GSK1120212)	Phase I	Advanced solid tumors, including expansion arms consisting of *RAS*- or *BRAF*-mutant advanced non-small cell lung cancer, ovarian cancer, or pancreatic cancer
NCT01392521	Bayer	BAY80-6946	BAY86-9766	Phase Ib	Advanced solid cancers
NCT01449058	Novartis	BYL719	MEK162	Phase II	Advanced colorectal cancer, esophageal cancer, pancreatic cancer, non-small cell lung cancer, or other advanced solid tumors with documented *RAS* or *BRAF* mutations
NCT00996892	Genentech	GDC-0941	GDC-0973	Phase I	Advanced solid cancers
NCT01248858	GSK	GSK2126458	Trametinib (GSK1120212)	Phase I	Advanced solid tumors

Source: ClinicalTrials.gov

lines with PTEN loss, or other mechanisms of PI3K activation, are resistant to single-agent MEK inhibition [97, 98]. In basal-type breast cancer cells, MEK inhibition has been shown to lead to activation of the PI3K pathway, thus limiting the efficacy of MEK inhibitors [98]. The combination of PI3K and MEK inhibition has been shown to have synergistic antitumor effects in these tumor cells, both in vitro and in vivo, regardless of PI3K pathway alterations [97–99]. Given the lack of therapeutic targets in triple-negative or basal-like breast cancer, PI3K plus MEK inhibitor combinations could be a rational therapeutic strategy for further investigation.

The emerging clinical profile of PI3K and MEK inhibitor combination therapy

Several trials evaluating PI3K plus MEK inhibitors are underway in patients with advanced solid tumors (Table 2), and preliminary data are now available for three Phase I trials [100–102]. The combination of GDC-0973 and GDC-0941 was evaluated in 78 patients with advanced solid tumors [100]. This combination was well tolerated with toxicities similar to those observed in single-agent Phase I trials. Dose-limiting toxicities (DLTs) were grade 3 lipase and grade 4 creatine phosphokinase (CPK) elevation. Common adverse events were rash, nausea, fatigue, vomiting, decreased appetite, dysgeusia, and elevated CPK. Partial responses were observed in three patients: one patient BRAF-mutant melanoma, one patient with BRAF-mutant pancreatic cancer, and one patient with KRAS-mutant endometrial cancer. Five patients had stable disease lasting more than 5 months.

The combination of trametinib and BKM120 was evaluated in 49 patients with advanced RAS- or BRAF-mutant cancers [101]. Combination treatment with these agents was also well tolerated. DLTs included gastrointestinal events, left ventricular ejection fraction decrease, and CPK increase. Common adverse events were dermatitis acneiform/rash, diarrhea, nausea, vomiting, CPK increase, decreased appetite, stomatitis, and hyperglycemia. Partial responses were observed in three patients with KRAS-

mutant ovarian cancer, two of whom maintained their responses for more than 9 months. Two patients with BRAF-mutant melanoma had stable disease.

In another combination study, the dual oral PI3K/mTOR inhibitor PF-04691502 was combined with either the MEK inhibitor PD-0325901 or irinotecan, while in a third arm, the intravenous PI3K/mTOR inhibitor PF-05212384 was combined with irinotecan [102]. In the PF-04691502/PD-0325901 combination arm, the maximum tolerated dose was exceeded in the first dosing cohort, with four of six evaluable patients developing DLTs including one patient with grade 3 diarrhea, nausea, and vomiting; one patient with grade 4 mucositis; one patient with grade 3 increased alkaline phosphatase; and one patient with grade 2 uveitis. Among the three treatment arms, PF-05212384 plus irinotecan showed the most promise because it was well tolerated, and one patient with KRAS wild-type metastatic colorectal cancer, previously treated with irinotecan, responded. A fourth arm combining PF-05212384 with PD-0325901 is now underway.

In the first two trials, PI3K and MEK inhibitor combinations were generally well tolerated, and therapeutically active dose levels were achievable in patients with advanced solid cancers. Regarding the third trial, data from the PF-05212384 plus PD-0325901 arm will be required before an assessment of the viability of PI3K inhibitor combinations with PD-0325901 can be made. Although hyperglycemia (an "on-target" side effect of PI3K inhibition) was reported in the BKM120/trametinib study, serous-like retinopathies (a class effect of MEK inhibition [103, 104]) were not reported in any of the three trials described above. This may have been due to improved patient selection and adverse event management, informed by previous clinical experience with MEK inhibitors. Data from these trials suggest that some tumors may be more likely to respond to therapy than others; however, these empirical observations should be treated with caution, due to the small sample sizes and lack of statistical analysis [100, 101].

Despite the common inclusion of colorectal cancer patients into early-phase clinical trials with PI3K inhibitors, MEK inhibitors, or PI3K/MEK inhibitor combinations, tumor shrinkage was rarely observed. A recent Phase I study attempted to use molecular profiling to optimize current targeted therapies for advanced chemorefractory colorectal cancer [105]. Patients with PIK3CA mutation or PTEN loss ($n = 32$) received a PI3K inhibitor, patients with KRAS or BRAF mutation ($n = 11$) received PI3 K and MEK inhibitors in combination, patients with wild-type KRAS received second-generation anti-EGFR monoclonal antibodies ($n = 11$), and patients with MET amplification ($n = 10$) received an mTOR inhibitor plus anti-IGFR1 monoclonal antibody (if low PTEN) or BRAF inhibitor (for BRAF-mutant tumors) [105]. Only one partial response was observed (PI3K inhibitor therapy; PIK3CA mutation) suggesting that current targeted agents may not be capable of effectively treating chemorefractory colorectal cancer, even if patients are stratified by tumor molecular status [105]. Additional targets or therapeutic combinations, potentially including chemotherapy, may therefore be required to advance the treatment of colorectal cancer.

Based on the preliminary reports of tumor shrinkage in trials of PI3K and MEK inhibitor combination therapy, advanced melanoma and low-grade serous ovarian cancer appear to be more sensitive. Patients with low-grade serous ovarian cancer have previously demonstrated an excellent rate of response to single-agent MEK inhibitor therapy [87]. Further studies are required to determine if the anti-tumor activity demonstrated with MEK inhibitors can be potentiated by the addition of PI3K inhibitors. Similarly, single-agent MEK inhibitors have also shown good responses in BRAF inhibitor-naïve malignant melanoma [17, 54]; however, the addition of PI3K inhibitors could still be required for patients who develop resistance. Additional studies with PI3K and MEK inhibitor combinations may be warranted in both of these tumor types.

Conclusion

The PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways are two of the most important signaling cascades in cancer, and both are frequently involved in resistance to current target therapies. Dual inhibition of both pathways may be needed to overcome certain resistance mechanisms or to treat cancers with driver mutations that cannot be directly targeted, such as mutational activation of RAS. At least two PI3K and MEK inhibitor combinations are well tolerated and can be administered at therapeutic doses; however, additional work is required to establish the tumor types that will respond best to therapy.

Acknowledgments Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals. Ben Holtom DPhil provided medical editorial assistance with this manuscript. The author retained full editorial control over the content of the manuscript and received no compensation from any party for this work. Carolyn Britten is appointed as the SmartState Endowed Chair in GI Malignancy Diagnostic & Therapeutic Trials in the South Carolina SmartState® Center for Gastrointestinal Cancer Diagnostics. Carolyn Britten has received research funding from Bayer HealthCare Pharmaceuticals, Genentech, Novartis Pharmaceuticals, and Pfizer. She has received honoraria from Bayer HealthCare Pharmaceuticals.

References

1. Santarpia L, Lippman SM, El-Naggar AK (2012) Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. Expert Opin Ther Targets 16:103–119

2. Liu P, Cheng H, Roberts TM, Zhao JJ (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 8:627–644
3. McCubrey JA, Steelman LS, Kempf CR, Chappell WH, Abrams SL, Stivala F, Malaponte G, Nicoletti F, Libra M, Basecke J, Maksimovic-Ivanic D, Mijatovic S, Montalto G, Cervello M, Cocco L, Martelli AM (2011) Therapeutic resistance resulting from mutations in Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR signaling pathways. J Cell Physiol 226:2762–2781
4. Courtney KD, Corcoran RB, Engelman JA (2010) The PI3K pathway as drug target in human cancer. J Clin Oncol 28:1075–1083
5. Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B (2010) The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol 11:329–341
6. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM, Franklin RA (2007) Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 1773:1263–1284
7. Mendoza MC, Er EE, Blenis J (2011) The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends Biochem Sci 36:320–328
8. Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, Waterfield MD, Downward J (1994) Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature 370:527–532
9. Ma L, Teruya-Feldstein J, Bonner P, Bernardi R, Franz DN, Witte D, Cordon-Cardo C, Pandolfi PP (2007) Identification of S664 TSC2 phosphorylation as a marker for extracellular signal-regulated kinase mediated mTOR activation in tuberous sclerosis and human cancer. Cancer Res 67:7106–7112
10. Zimmermann S, Moelling K (1999) Phosphorylation and regulation of Raf by Akt (protein kinase B). Science 286:1741–1744
11. Aksamitiene E, Kiyatkin A, Kholodenko BN (2012) Cross-talk between mitogenic Ras/MAPK and survival PI3K/Akt pathways: a fine balance. Biochem Soc Trans 40:139–146
12. Markman B, Dienstmann R, Tabernero J (2010) Targeting the PI3K/Akt/mTOR pathway—beyond rapalogs. Oncotarget 1:530–543
13. Ogita S, Lorusso P (2011) Targeting phosphatidylinositol 3 kinase (PI3K)-Akt beyond rapalogs. Target Oncol 6:103–117
14. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, De Buck SS, Ru QC, Peters M, Goldbrunner M, Baselga J (2012) Phase I, dose-escalation study of BKM120, an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol 30:282–290
15. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 3:11–22
16. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA, BRIM-3 Study Group (2011) Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364:2507–2516
17. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, Dummer R, Trefzer U, Larkin JM, Utikal J, Dreno B, Nyakas M, Middleton MR, Becker JC, Casey M, Sherman LJ, Wu FS, Ouellet D, Martin AM, Patel K, Schadendorf D, METRIC Study Group (2012) Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 367:107–114
18. Wang D, Boerner SA, Winkler JD, LoRusso PM (2007) Clinical experience of MEK inhibitors in cancer therapy. Biochim Biophys Acta 1773:1248–1255
19. Kahl B, Byrd JC, Flinn IW, Wagner-Johnston N, Spurgeon S, Benson DM, Furman RR, Brown JR, Coutre S, Lannuttie B, Giese NA, Ulrich RG, Webb HK, Peterman S, Holes L, Yu AS (2010) Clinical safety and activity in a phase 1 study of CAL-101, an isoform-selective inhibitor of phosphatidylinositol 3-kinase p110d, in patients with relapsed or refractory non-Hodgkin lymphoma. 52nd ASH annual meeting and exposition (abstract #1777)
20. Moreno Garcia V, Baird RD, Shah KJ, Basu B, Tunariu N, Blanco M, Cassier PA, Pedersen JV, Puglisi M, Sarker D, Papadatos-Pastos D, Omlin AG, Biondo A, Ware JA, Koeppen H, Levy GG, Mazina KE, De Bono JS (2011) A phase I study evaluating GDC-0941, an oral phosphoinositide-3 kinase (PI3K) inhibitor, in patients with advanced solid tumors or multiple myeloma. ASCO Meeting Abstracts 29:3021
21. Burris H, Rodon J, Sharma S, Herbst RS, Tabernero J, Infante JR, Silva A, Demanse D, Hackl W, Baselga J (2010) First-in-human phase I study of the oral PI3K inhibitor BEZ235 in patients (pts) with advanced solid tumors. ASCO Meeting Abstracts 28:3005
22. Lotze MT, Appleman LJ, Ramanathan RK, Tolcher AW, Beeram M, Papadopoulos KP, Rasco DW, Weiss GJ, Mountz JM, Toledo FGS, Alvarez RJ, Oborski MJ, Rajagopalan P, Jeffers M, Roth D, Dubowy RL, Patnaik A (2012) Phase I study of intravenous PI3K inhibitor BAY 80-6946: activity in patients (pts) with advanced solid tumors and non-Hodgkin lymphoma treated in MTD expansion cohorts. J Clin Oncol 30 (suppl; abstr 3019)
23. Millham R, Houk B, Borzillo G, LoRusso P, Marburg L, Britten C, Wainberg Z, Adjei A, Grace D (2011) First-in-patient study of PF-04691502, a small molecule intravenous dual inhibitor of PI3K and mTOR in patients with advanced cancer: Update on safety, efficacy, and pharmacology. In: Proceedings of the AACR-NCI-EORTC international conference: molecular targets and cancer therapeutics; 2011 Nov 12–16; San Francisco, CA Philadelphia (PA): AACR; Mol Cancer Ther 10(11 Suppl):Abstract nr B163
24. Millham R, Houk B, Borzillo G, Tabernero T, Bell-McGuinn K, Bendell J, Molina J, Kwakm E, Shapiro G (2011) First-in-patient study of PF-05212384, a small molecule intravenous dual inhibitor of PI3K and mTOR in patients with advanced cancer: Update on safety, efficacy, and pharmacology. In: Proceedings of the AACR-NCI-eortc international conference: molecular targets and cancer therapeutics; 2011 Nov 12–16; San Francisco, CA Philadelphia (PA): AACR; Mol Cancer Ther 10(11 Suppl):Abstract nr A167
25. Bekaii-Saab T, Phelps MA, Li X, Saji M, Goff L, Kauh JS, O'Neil BH, Balsom S, Balint C, Liersemann R, Vasko VV, Bloomston M, Marsh W, Doyle LA, Ellison G, Grever M, Ringel MD, Villalona-Calero MA (2011) Multi-institutional phase II study of selumetinib in patients with metastatic biliary cancers. J Clin Oncol 29:2357–2363
26. Rinehart J, Adjei AA, Lorusso PM, Waterhouse D, Hecht JR, Natale RB, Hamid O, Varterasian M, Asbury P, Kaldjian EP, Gulyas S, Mitchell DY, Herrera R, Sebolt-Leopold JS, Meyer MB (2004) Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. J Clin Oncol 22:4456–4462
27. Bennouna J, Lang I, Valladares-Ayerbes M, Boer K, Adenis A, Escudero P, Kim TY, Pover GM, Morris CD, Douillard JY (2011) A Phase II, open-label, randomised study to assess the efficacy and safety of the MEK1/2 inhibitor AZD6244 (ARRY-142886) versus capecitabine monotherapy in patients with

colorectal cancer who have failed one or two prior chemotherapeutic regimens. Invest New Drugs 29:1021–1028
28. Bodoky G, Timcheva C, Spigel DR, La Stella PJ, Ciuleanu TE, Pover G, Tebbutt NC (2012) A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. Invest New Drugs 30:1216–1223
29. Serra V, Scaltriti M, Prudkin L, Eichhorn PJ, Ibrahim YH, Chandarlapaty S, Markman B, Rodriguez O, Guzman M, Rodriguez S, Gili M, Russillo M, Parra JL, Singh S, Arribas J, Rosen N, Baselga J (2011) PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. Oncogene 30:2547–2557
30. Pratilas CA, Hanrahan AJ, Halilovic E, Persaud Y, Soh J, Chitale D, Shigematsu H, Yamamoto H, Sawai A, Janakiraman M, Taylor BS, Pao W, Toyooka S, Ladanyi M, Gazdar A, Rosen N, Solit DB (2008) Genetic predictors of MEK dependence in non-small cell lung cancer. Cancer Res 68:9375–9383
31. Turke AB, Song Y, Costa C, Cook R, Arteaga CL, Asara JM, Engelman JA (2012) MEK inhibition leads to PI3K/AKT activation by relieving a negative feedback on ERBB receptors. Cancer Res 72:3228–3237
32. National Institutes of Health (NIH) (2012) ClinicalTrials.gov. http://clinicaltrials.gov. Accessed 11 Apr 2012
33. Faber AC, Wong KK, Engelman JA (2010) Differences underlying EGFR and HER2 oncogene addiction. Cell Cycle 9:851–852
34. Fernandez-Medarde A, Santos E (2011) Ras in cancer and developmental diseases. Genes Cancer 2:344–358
35. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia-Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 14:1351–1356
36. Maira SM, Pecchi S, Huang A, Burger M, Knapp M, Sterker D, Schnell C, Guthy D, Nagel T, Wiesmann M, Brachmann S, Fritsch C, Dorsch M, Chene P, Shoemaker K, De Pover A, Menezes D, Martiny-Baron G, Fabbro D, Wilson CJ, Schlegel R, Hofmann F, Garcia-Echeverria C, Sellers WR, Voliva CF (2012) Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-Kinase inhibitor. Mol Cancer Ther 11:317–328
37. Hofmann I, Weiss A, Elain G, Schwaederle M, Sterker D, Romanet V, Schmelzle T, Lai A, Brachmann SM, Bentires-Alj M, Roberts TM, Sellers WR, Hofmann F, Maira SM (2012) K-RAS mutant pancreatic tumors show higher sensitivity to MEK than to PI3K inhibition in vivo. PLoS ONE 7:e44146
38. Ihle NT, Lemos R Jr, Wipf P, Yacoub A, Mitchell C, Siwak D, Mills GB, Dent P, Kirkpatrick DL, Powis G (2009) Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. Cancer Res 69:143–150
39. Jing J, Greshock J, Holbrook JD, Gilmartin A, Zhang X, McNeil E (2012) Comprehensive predictive biomarker analysis for MEK inhibitor GSK1120212. Mol Cancer Ther 11:720–729
40. Wee S, Jagani Z, Xiang KX, Loo A, Dorsch M, Yao YM, Sellers WR, Lengauer C (2009) PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. Cancer Res 69:4286–4293
41. Yoon YK, Kim HP, Han SW, Oh DY, Im SA, Bang YJ, Kim TY (2010) KRAS mutant lung cancer cells are differentially responsive to MEK inhibitor due to AKT or STAT3 activation: implication for combinatorial approach. Mol Carcinog 49:353–362
42. Dry JR, Pavey S, Pratilas CA, Harbron C, Runswick S, Hodgson D, Chresta C, McCormack R, Byrne N, Cockerill M, Graham A, Beran G, Cassidy A, Haggerty C, Brown H, Ellison G, Dering J, Taylor BS, Stark M, Bonazzi V, Ravishankar S, Packer L, Xing F, Solit DB, Finn RS, Rosen N, Hayward NK, French T, Smith PD (2010) Transcriptional pathway signatures predict MEK addiction and response to selumetinib (AZD6244). Cancer Res 70:2264–2273
43. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB (2010) Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 363:809–819
44. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA (2002) Mutations of the BRAF gene in human cancer. Nature 417:949–954
45. Corcoran RB, Settleman J, Engelman JA (2011) Potential therapeutic strategies to overcome acquired resistance to BRAF or MEK inhibitors in BRAF mutant cancers. Oncotarget 2:336–346
46. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA (2010) BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. Sci Signal 3:ra84
47. Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, Shi H, Atefi M, Titz B, Gabay MT, Salton M, Dahlman KB, Tadi M, Wargo JA, Flaherty KT, Kelley MC, Misteli T, Chapman PB, Sosman JA, Graeber TG, Ribas A, Lo RS, Rosen N, Solit DB (2011) RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature 480:387–390
48. Montagut C, Sharma SV, Shioda T, McDermott U, Ulman M, Ulkus LE, Dias-Santagata D, Stubbs H, Lee DY, Singh A, Drew L, Haber DA, Settleman J (2008) Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res 68:4853–4861
49. Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA, Emery CM, Stransky N, Cogdill AP, Barretina J, Caponigro G, Hieronymus H, Murray RR, Salehi-Ashtiani K, Hill DE, Vidal M, Zhao JJ, Yang X, Alkan O, Kim S, Harris JL, Wilson CJ, Myer VE, Finan PM, Root DE, Roberts TM, Golub T, Flaherty KT, Dummer R, Weber BL, Sellers WR, Schlegel R, Wargo JA, Hahn WC, Garraway LA (2010) COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature 468:968–972
50. Gowrishankar K, Snoyman S, Pupo GM, Becker TM, Kefford RF, Rizos H (2012) Acquired resistance to BRAF inhibition can confer cross-resistance to combined BRAF/MEK inhibition. J Invest Dermatol 132:1850–1859
51. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, Chen Z, Lee MK, Attar N, Sazegar H, Chodon T, Nelson SF, McArthur G, Sosman JA, Ribas A, Lo RS (2010) Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature 468:973–977
52. Wagle N, Emery C, Berger MF, Davis MJ, Sawyer A, Pochanard P, Kehoe SM, Johannessen CM, Macconaill LE, Hahn WC, Meyerson M, Garraway LA (2011) Dissecting therapeutic

resistance to RAF inhibition in melanoma by tumor genomic profiling. J Clin Oncol 29:3085–3096
53. Ascierto PA, Berking C, Agarwala SS, Schadendorf D, Van Herpen C, Queirolo P, Blank CU, Hauschild A, Beck JT, Zubel A, Niazi F, Wandel S, Dummer R (2012) Efficacy and safety of oral MEK162 in patients with locally advanced and unresectable or metastatic cutaneous melanoma harboring *BRAF*V600 or *NRAS* mutations. J Clin Oncol 30 (suppl; abstr 8511)
54. Falchook GS, Lewis KD, Infante JR, Gordon MS, Vogelzang NJ, De Marini DJ, Sun P, Moy C, Szabo SA, Roadcap LT, Peddareddigari VG, Lebowitz PF, Le NT, Burris HA 3rd, Messersmith WA, O'Dwyer PJ, Kim KB, Flaherty K, Bendell JC, Gonzalez R, Kurzrock R, Fecher LA (2012) Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. Lancet Oncol 13:782–789
55. Greger JG, Eastman SD, Zhang V, Bleam MR, Hughes AM, Smitheman KN, Dickerson SH, Laquerre SG, Liu L, Gilmer TM (2012) Combinations of BRAF, MEK, and PI3K/mTOR inhibitors overcome acquired resistance to the BRAF inhibitor GSK2118436 dabrafenib, mediated by NRAS or MEK mutations. Mol Cancer Ther 11:909–920
56. Oberholzer PA, Kee D, Dziunycz P, Sucker A, Kamsukom N, Jones R, Roden C, Chalk CJ, Ardlie K, Palescandolo E, Piris A, MacConaill LE, Robert C, Hofbauer GF, McArthur GA, Schadendorf D, Garraway LA (2012) RAS mutations are associated with the development of cutaneous squamous cell tumors in patients treated with RAF inhibitors. J Clin Oncol 30:316–321
57. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, Reis-Filho JS, Kong X, Koya RC, Flaherty KT, Chapman PB, Kim MJ, Hayward R, Martin M, Yang H, Wang Q, Hilton H, Hang JS, Noe J, Lambros M, Geyer F, Dhomen N, Niculescu-Duvaz I, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte NJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Kockx M, Andries L, Lestini B, Nolop KB, Lee RJ, Joe AK, Troy JL, Gonzalez R, Hutson TE, Puzanov I, Chmielowski B, Springer CJ, McArthur GA, Sosman JA, Lo RS, Ribas A, Marais R (2012) RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 366:207–215
58. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, Kudchadkar R, Burris HA 3rd, Falchook G, Algazi A, Lewis K, Long GV, Puzanov I, Lebowitz P, Singh A, Little S, Sun P, Allred A, Ouellet D, Kim KB, Patel K, Weber J (2012) Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 367:1694–1703
59. Atefi M, von Euw E, Attar N, Ng C, Chu C, Guo D, Nazarian R, Chmielowski B, Glaspy JA, Comin-Anduix B, Mischel PS, Lo RS, Ribas A (2011) Reversing melanoma cross-resistance to BRAF and MEK inhibitors by co-targeting the AKT/mTOR pathway. PLoS ONE 6:e28973
60. Shi H, Kong X, Ribas A, Lo RS (2011) Combinatorial treatments that overcome PDGFRbeta-driven resistance of melanoma cells to V600EB-RAF inhibition. Cancer Res 71:5067–5074
61. Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK, Wubbenhorst B, Xu X, Gimotty PA, Kee D, Santiago-Walker AE, Letrero R, D'Andrea K, Pushparajan A, Hayden JE, Brown KD, Laquerre S, McArthur GA, Sosman JA, Nathanson KL, Herlyn M (2010) Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell 18:683–695
62. Omholt K, Platz A, Kanter L, Ringborg U, Hansson J (2003) NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clin Cancer Res 9:6483–6488
63. Ellerhorst JA, Greene VR, Ekmekcioglu S, Warneke CL, Johnson MM, Cooke CP, Wang LE, Prieto VG, Gershenwald JE, Wei Q, Grimm EA (2011) Clinical correlates of NRAS and BRAF mutations in primary human melanoma. Clin Cancer Res 17:229–235
64. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N (2006) BRAF mutation predicts sensitivity to MEK inhibition. Nature 439:358–362
65. Adjei AA, Cohen RB, Franklin W, Morris C, Wilson D, Molina JR, Hanson LJ, Gore L, Chow L, Leong S, Maloney L, Gordon G, Simmons H, Marlow A, Litwiler K, Brown S, Poch G, Kane K, Haney J, Eckhardt SG (2008) Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol 26:2139–2146
66. Kirkwood JM, Bastholt L, Robert C, Sosman J, Larkin J, Hersey P, Middleton M, Cantarini M, Zazulina V, Kemsley K, Dummer R (2012) Phase II, open-label, randomized trial of the MEK1/2 inhibitor selumetinib as monotherapy versus temozolomide in patients with advanced melanoma. Clin Cancer Res 18:555–567
67. Roberts PJ, Usary J, Darr D, Dillon PM, Pfefferle AD, Whittle MC, Duncan JS, Johnson SM, Combest A, Jin J, Zamboni WC, Johnson GL, Perou CM, Sharpless NE (2012) Combined PI3K/mTOR and MEK inhibition provides broad anti-tumor activity in faithful murine cancer models. Clin Cancer Res 18:5290–5303
68. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, McAllister PK, Morton RF, Schilsky RL (2009) American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 27:2091–2096
69. De Roock W, De Vriendt V, Normanno N, Ciardiello F, Tejpar S (2011) KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 12:594–603
70. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 11:753–762
71. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, Valtorta E, Schiavo R, Buscarino M, Siravegna G, Bencardino K, Cercek A, Chen CT, Veronese S, Zanon C, Sartore-Bianchi A, Gambacorta M, Gallicchio M, Vakiani E, Boscaro V, Medico E, Weiser M, Siena S, Di Nicolantonio F, Solit D, Bardelli A (2012) Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 486:532–536
72. Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, Wolff RK, Slattery ML (2005) Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 65:6063–6069
73. Di Nicolantonio F, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A (2008) Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 26:5705–5712

74. Bokemeyer C, Cutsem EV, Rougier P, Ciardiello F, Heeger S, Schlichting M, Celik I, Kohne CH (2012) Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer 48:1466–1475
75. Wang Y, Van Becelaere K, Jiang P, Przybranowski S, Omer C, Sebolt-Leopold J (2005) A role for K-ras in conferring resistance to the MEK inhibitor, CI-1040. Neoplasia 7:336–347
76. Little AS, Balmanno K, Sale MJ, Newman S, Dry JR, Hampson M, Edwards PA, Smith PD, Cook SJ (2011) Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK1/2 inhibitors in colorectal cancer cells. Sci Signal ra4:17
77. Tentler JJ, Nallapareddy S, Tan AC, Spreafico A, Pitts TM, Morelli MP, Selby HM, Kachaeva MI, Flanigan SA, Kulikowski GN, Leong S, Arcaroli JJ, Messersmith WA, Eckhardt SG (2010) Identification of predictive markers of response to the MEK1/2 inhibitor selumetinib (AZD6244) in K-ras-mutated colorectal cancer. Mol Cancer Ther 9:3351–3362
78. Halilovic E, She QB, Ye Q, Pagliarini R, Sellers WR, Solit DB, Rosen N (2010) PIK3CA mutation uncouples tumor growth and cyclin D1 regulation from MEK/ERK and mutant KRAS signaling. Cancer Res 70:6804–6814
79. Balmanno K, Chell SD, Gillings AS, Hayat S, Cook SJ (2009) Intrinsic resistance to the MEK1/2 inhibitor AZD6244 (ARRY-142886) is associated with weak ERK1/2 signalling and/or strong PI3K signalling in colorectal cancer cell lines. Int J Cancer 125:2332–2341
80. Nosho K, Kawasaki T, Ohnishi M, Suemoto Y, Kirkner GJ, Zepf D, Yan L, Longtine JA, Fuchs CS, Ogino S (2008) PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. Neoplasia 10:534–541
81. Yu K, Toral-Barza L, Shi C, Zhang WG, Zask A (2008) Response and determinants of cancer cell susceptibility to PI3K inhibitors: combined targeting of PI3K and Mek1 as an effective anticancer strategy. Cancer Biol Ther 7:307–315
82. Haagensen EJ, Kyle S, Beale GS, Maxwell RJ, Newell DR (2012) The synergistic interaction of MEK and PI3K inhibitors is modulated by mTOR inhibition. Br J Cancer 106:1386–1394
83. Migliardi G, Sassi F, Torti D, Galimi F, Zanella ER, Buscarino M, Ribero D, Muratore A, Massucco P, Pisacane A, Risio M, Capussotti L, Marsoni S, Di Nicolantonio F, Bardelli A, Comoglio PM, Trusolino L, Bertotti A (2012) Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas. Clin Cancer Res 18:2515–2525
84. Vaughan S, Coward JI, Bast RC Jr, Berchuck A, Berek JS, Brenton JD, Coukos G, Crum CC, Drapkin R, Etemadmoghadam D, Friedlander M, Gabra H, Kaye SB, Lord CJ, Lengyel E, Levine DA, McNeish IA, Menon U, Mills GB, Nephew KP, Oza AM, Sood AK, Stronach EA, Walczak H, Bowtell DD, Balkwill FR (2011) Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer 11:719–725
85. Kurman RJ, Shih I (2011) Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer–shifting the paradigm. Hum Pathol 42:918–931
86. Shih I, Kurman RJ (2004) Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol 164:1511–1518
87. Farley J, Brady W, Birrer M, Lankes H, Coleman R, Morgan M, Mannel R, Yamada D, Mutch D, Rodgers W, Gershenson D (2012) A phase II trial of selumetinib in women with recurrent low-grade serous carcinoma of the ovary or peritoneum. AACR Meeting Abstracts:CT–05
88. Kinross KM, Brown DV, Kleinschmidt M, Jackson S, Christensen J, Cullinane C, Hicks RJ, Johnstone RW, McArthur GA (2011) In vivo activity of combined PI3K/mTOR and MEK inhibition in a Kras(G12D); Pten deletion mouse model of ovarian cancer. Mol Cancer Ther 10:1440–1449
89. Hruban RH, van Mansfeld AD, Offerhaus GJ, van Weering DH, Allison DC, Goodman SN, Kensler TW, Bose KK, Cameron JL, Bos JL (1993) K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. Am J Pathol 143:545–554
90. Kim ST, Lim do H, Jang KT, Lim T, Lee J, Choi YL, Jang HL, Yi JH, Baek KK, Park SH, Park YS, Lim HY, Kang WK, Park JO (2011) Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. Mol Cancer Ther 10:1993–1999
91. Collins MA, Bednar F, Zhang Y, Brisset JC, Galban S, Galban CJ, Rakshit S, Flannagan KS, Adsay NV, di Magliano M (2012) Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. J Clin Invest 122:639–653
92. Roy SK, Srivastava RK, Shankar S (2010) Inhibition of PI3K/AKT and MAPK/ERK pathways causes activation of FOXO transcription factor, leading to cell cycle arrest and apoptosis in pancreatic cancer. J Mol Signal 5:10
93. Lorusso PM, Adjei AA, Varterasian M, Gadgeel S, Reid J, Mitchell DY, Hanson L, DeLuca P, Bruzek L, Piens J, Asbury P, Van Becelaere K, Herrera R, Sebolt-Leopold J, Meyer MB (2005) Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. J Clin Oncol 23:5281–5293
94. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lonning PE, Borresen-Dale AL (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98:10869–10874
95. Pal SK, Childs BH, Pegram M (2011) Triple negative breast cancer: unmet medical needs. Breast Cancer Res Treat 125:627–636
96. Grob TJ, Heilenkotter U, Geist S, Paluchowski P, Wilke C, Jaenicke F, Quaas A, Wilczak W, Choschzick M, Sauter G, Lebeau A (2012) Rare oncogenic mutations of predictive markers for targeted therapy in triple-negative breast cancer. Breast Cancer Res Treat 134:561–567
97. Hoeflich KP, O'Brien C, Boyd Z, Cavet G, Guerrero S, Jung K, Januario T, Savage H, Punnoose E, Truong T, Zhou W, Berry L, Murray L, Amler L, Belvin M, Friedman LS, Lackner MR (2009) In vivo antitumor activity of MEK and phosphatidylinositol 3-kinase inhibitors in basal-like breast cancer models. Clin Cancer Res 15:4649–4664
98. Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, Hu Z, Knight Z, Feiler HS, Gascard P, Parvin B, Spellman PT, Shokat KM, Wyrobek AJ, Bissell MJ, McCormick F, Kuo WL, Mills GB, Gray JW, Korn WM (2009) Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res 69:565–572
99. Ibrahim YH, Serra V, Scaltriti M, Prudkin L, Anton P, Perez J, Balmana J, Baselga J (2011) Combined PI3K and MEK inhibition in patient-derived xenografts generated from recurrent triple negative breast cancer. In: Proceedings of the 102nd annual meeting of the American Association for Cancer Research:[abstract 648]

100. LoRusso P, Shapiro G, Pandya SS, Kwak EL, Jones C, Belvin M, Musib LC, de Crespigny A, McKenzie M, Gates MR, Chan IT, Bendell JC (2012) A first-in-human phase Ib study to evaluate the MEK inhibitor GDC-0973, combined with the pan-PI3K inhibitor GDC-0941, in patients with advanced solid tumors. ASCO Meeting Abstracts 30:2566
101. Bedard P, Tabernero J, Kurzrock R, Britten CD, Stathis A, Perez-Garcia JM, Zubel A, Le NT, Carter K, Bellew KM, Gallarati C, Niazi F, Demanse D, De Buck SS, Sessa C (2012) A phase lb, open-label, multicenter, dose-escalation study of the oral pan-PI3K inhibitor BKM120 in combination with the oral MEK1/2 inhibitor GSK1120212 in patients (pts) with selected advanced solid tumors. ASCO Meeting Abstracts 30:3003
102. Britten C, Wainberg Z, Tabernero J, Alsina Maqueda M, Leong S, Sessa C, Millham R, Gallo J, Siu L, Brana I (2012) A Multi-arm Phase 1 Dose Escalation Study of Safety, Pharmacokinetics, and Pharmacodynamics of the Dual PI3K/mTor Inhibitors PF-04691502 (oral) and PF-05212384 (IV) in Combination with the MEK Inhibitor PD-0325901 or Irinotecan in Patients with Advanced Cancer. EORTC-NCI-AACR 2012 Abstract 366
103. Infante JR, Fecher LA, Falchook GS, Nallapareddy S, Gordon MS, Becerra C, Demarini DJ, Cox DS, Xu Y, Morris SR, Peddareddigari VG, Le NT, Hart L, Bendell JC, Eckhardt G, Kurzrock R, Flaherty K, Burris HA 3rd, Messersmith WA (2012) Safety, pharmacokinetic, pharmacodynamic, and efficacy data for the oral MEK inhibitor trametinib: a phase 1 dose-escalation trial. Lancet Oncol 13:773–781
104. Renouf DJ, Velazquez-Martin JP, Simpson R, Siu LL, Bedard PL (2012) Ocular toxicity of targeted therapies. J Clin Oncol 30:3277–3286
105. Dienstmann R, Serpico D, Rodon J, Saura C, Macarulla T, Elez E, Alsina M, Capdevila J, Perez-Garcia J, Sanchez-Olle G, Aura C, Prudkin L, Landolfi S, Hernandez-Losa J, Vivancos A, Tabernero J (2012) Molecular profiling of patients with colorectal cancer and matched targeted therapy in phase I clinical trials. Mol Cancer Ther 11:2062–2071