Review

Molecular Cancer Therapeutics

Rational Approaches for Combination Therapy Strategies Targeting the MAP Kinase Pathway in Solid Tumors

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Abstract

Molecular characterization of oncogenic mutations within genes in the MAPK and PI3K/AKT/mTOR pathways has led to the rational development of targeted therapies. Combining BRAF and MEK inhibitors to target two steps in the MAPK pathway (vertical inhibition) is now standard of care in advanced-stage melanoma harboring *BRAF* V600 mutation. Encouraging results have been seen in several tumor types with the same mutation, including *BRAF* V600–mutant non–small cell lung cancer. Yet similar results in other tumors, such as colorectal cancer, have not been observed, highlighting the unique nature of different tumors. Furthermore, considerable cross talk occurs between signaling pathways, and cancer cells usually harbor multiple aberrations and/or develop compensatory mechanisms that drive resistance. Therefore, it is logical to target multiple pathways simultaneously (horizontal inhibition) by combining selective inhibitors or engineering multitargeted agents. Yet horizontal inhibition has proven to be a significant challenge, primarily due to dose-limiting toxicities. This review focuses on ongoing or completed clinical trials with combination targeted therapies for solid tumors and highlights the successes and ongoing challenges. Novel strategies to overcome these obstacles include new delivery technologies, combinations with emerging agents, and treatment schedule optimization. *Mol Cancer Ther; 17(1); 3–16. ©2018 AACR.*

Introduction

The MAPK and PI3K/AKT/mTOR cell signaling pathways play important roles in regulation of cell growth, proliferation, and survival. Mutations within these pathways are frequently implicated in the pathogenesis of solid tumors (1, 2). Indeed, the MAPK pathway is dysregulated in approximately one-third of human cancers (3), and the PI3K/AKT pathway has also been estimated to be dysregulated in a similar fraction (4). Characterization of these genetic alterations has led to rational development of inhibitors that have greatly improved treatment for some tumors (Table 1).

Targeting different steps in the same pathway (vertical inhibition) with BRAF plus MEK inhibitors has become standard of care for patients with advanced-stage melanoma harboring *BRAF* V600 mutations (5). However, variable patient responses, drug resistance, and disease progression continue to be challenges (6–8). In addition, although some other solid tumors such as colorectal cancer show the same *BRAF* mutation, in these cancers BRAF plus MEK inhibitors have not been observed to provide similar beneficial effects to those observed in melanoma (9). Clearly, a "one-size-fits-all" approach for treatment of *BRAF*-mutant tumors is not appropriate.

Because of the broad cross talk between the MAPK and PI3K/AKT pathways driven by parallel feedback mechanisms and common downstream targets (10), targeting of both pathways (horizontal inhibition) represents a promising strategy. Yet horizontal inhibition is a significant challenge, primarily due to development of dose-limiting toxicities (DLT) that prevent delivery of optimal therapeutic concentrations (11, 12). Therefore, a need remains for novel combinations.

Here we review ongoing or completed clinical trials with combination targeted therapies for solid tumors. We highlight some of the ongoing challenges while leveraging the extensive experience in melanoma. Insights gained in this tumor type may apply to other solid tumors.

MAPK and PI3K/AKT pathways

The MAPK and PI3K/AKT pathways share common inputs via receptor tyrosine kinases (RTK)/growth factor receptors (GFR) and RAS, and interact in an interconnected signaling network (Fig. 1; refs. 1, 13). The key activation step is the binding of a ligand to an RTK, resulting in the activation of RAS. RAF kinases act as RAS effectors and ERK activators in oncogenesis (14). Activated ERK also acts as a form of negative feedback, inhibiting the MAPK pathway (3). Oncogenic activation of the MAPK pathway can occur via multiple mechanisms, most of which include increased activity of RTKs, RAS, or RAF and result in constitutive activation of MEK and ERK (3, 13). Activating mutations in *KRAS* and *NRAS* occur at diverse rates in different cancer types (3). The three known

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Table 1. Targeted therapy in combination trials in cutaneous advanced melanoma (52, 102, 103)

Drug	Molecular target	PubChem ID
MAPK Pathway		
Dabrafenib (GSK2118436)	BRAF V600	44462760
Encorafenib (LGX818)	BRAF V600	50922675
Vemurafenib (PLX4032)	BRAF V600	42611257
Cobimetinib (GDC-0973, XL-518)	MEK	16222096
Binimetinib (MEK162)	MEK	10288191
PD-0325901	MEK	9826528
Pimasertib (MSC-1936369B, AS-703026)	MEK	44187362
Refametinib (BAY 86-9766)	MEK	44182295
Selumetinib (AZD6244)	MEK	10127622
Trametinib (GSK1120212)	MEK	11707110
WX-554	MEK	Not listed
RO5126766 (CH5126766)	Dual RAF/MEK	16719221
PI3K Pathway		
Buparlisib (BKM120)	Pan-PI3K	16654980
Copanlisib (BAY-80-6946)	Pan-PI3K	24989044
Pictilisib (GDC-0941)	Pan-PI3K	17755052
Sonolisib (PX-866)	Pan-PI3K	9849735
Alpelisib (BYL-719)	PI3K-α	56649450
SAR260301	PI3K-β	49854424
WX-037	PI3K[a]	Not listed
Everolimus (RAD001)	mTOR	6442177
Temsirolimus (CCI-779)	mTOR	6918289
Omipalisib (GSK-2126458) (104)	Dual PI3K/mTOR	25167777
Dactolisib (BEZ235)	Dual PI3K/mTOR	11977753
PF-04691502	Dual PI3K/mTOR	25033539
Voxtalisib (SAR245409)	Dual PI3K/mTOR	49867926
Afuresertib (GSK2110183)	AKT	46843057
Ipatasertib (GDC-0068)	AKT	74078320
MK-2206	AKT	24964624
Uprosertib (GSK2141795)	AKT	51042438
Multi-RTK and RAF		
Sorafenib (BAY43-9006)	RAF kinases and VEGF receptors	216239
RAF265	BRAF and VEGFR-2	11656518
Regorafenib (BAY 73-4506)	VEGFR-2 and -3, RET, KIT, PDGFR, RAF kinases	11167602
RTK/GFRs Pathways		
Cabozantinib (XL184)	RET, MET, VEGFR-1, -2, and -3, KIT, TRKB, FLT-3, AXL, TIE-2	25102847
Lenvatinib (E7080)	VEGF-1, -2, -3, FGFR-1, -2, -3, -4, PDGFR-α, KIT, RET	9823820
Pazopanib (GW786034B)	VEGFR-1, -2, -3, PDGFR-α, -β, c-KIT	10113978
Cetuximab	EGFR	85668777
Capmatinib (INC280)	HGFR (c-MET)	25145656
Golvatinib	HGFR (c-MET), VEGFR2	16118392
Onartuzumab (MetMAb)	HGFR (c-MET)	Not listed
Tivantinib (ARQ-197)	HGFR (c-MET)	11494412
Ganitumab (AMG 479)	IGF-1R	Not listed
Bevacizumab	VEGF	Not listed
MEHD7945A	Dual EGFR/HER3 (ErbB3)	Not listed
BGJ398 (NVP-BGJ398)	pan-FGFR	53235510
CDK Pathway		
Palbociclib (PD-0332991)	CDK4/6	5330286
Ribociclib (LEE011)	CDK4/6	44631912
Voruciclib (P1446A-05)	CDK4/6	67409219

Abbreviations: AKT, v-Akt murine thymoma viral oncogene/protein kinase-b; CDK, cyclin-dependent kinase; c-MET, hepatocyte receptor growth receptor (also HGFR); HGFR, hepatocyte growth factor receptor (also c-MET); PDGFR, platelet-derived growth factor receptor; TRKB, tropomyosin receptor kinase B.
[a]PI3K specificity has not been reported.

RAF kinase isoforms, CRAF, BRAF, and ARAF, each have distinct characteristics in tissue distribution, kinase activity, and regulation (14). The *BRAF* gene is mutated in up to 7% of all human malignancies (Table 2) (15).

Three classes of PI3Ks exist, each with a unique structure, cellular distribution, and set of substrates (2, 4). Class I PI3Ks are divided into classes 1A (activated by RTKs) and 1B (activated by G-protein–coupled receptors), with class 1A PI3Ks frequently implicated in oncogenesis. Mechanisms responsible for aberrant PI3K signaling include receptor amplification or mutations of RTKs, activating mutations in or amplification of the catalytic subunits of PI3Ks, and changes to downstream effectors or regulators. PI3K converts phosphatidylinositol bisphosphate (PIP2) to phosphatidylinositol trisphosphate (PIP3), allowing the activation of AKT and downstream mTOR. Negative regulation of this pathway by the PTEN occurs by dephosphorylating PIP2 and PIP3 (2). Activation of the PI3K/AKT pathway can also occur due to mutations to activated RAS, highlighting the cross talk with the MAPK pathway (Fig. 1; refs. 2, 4).

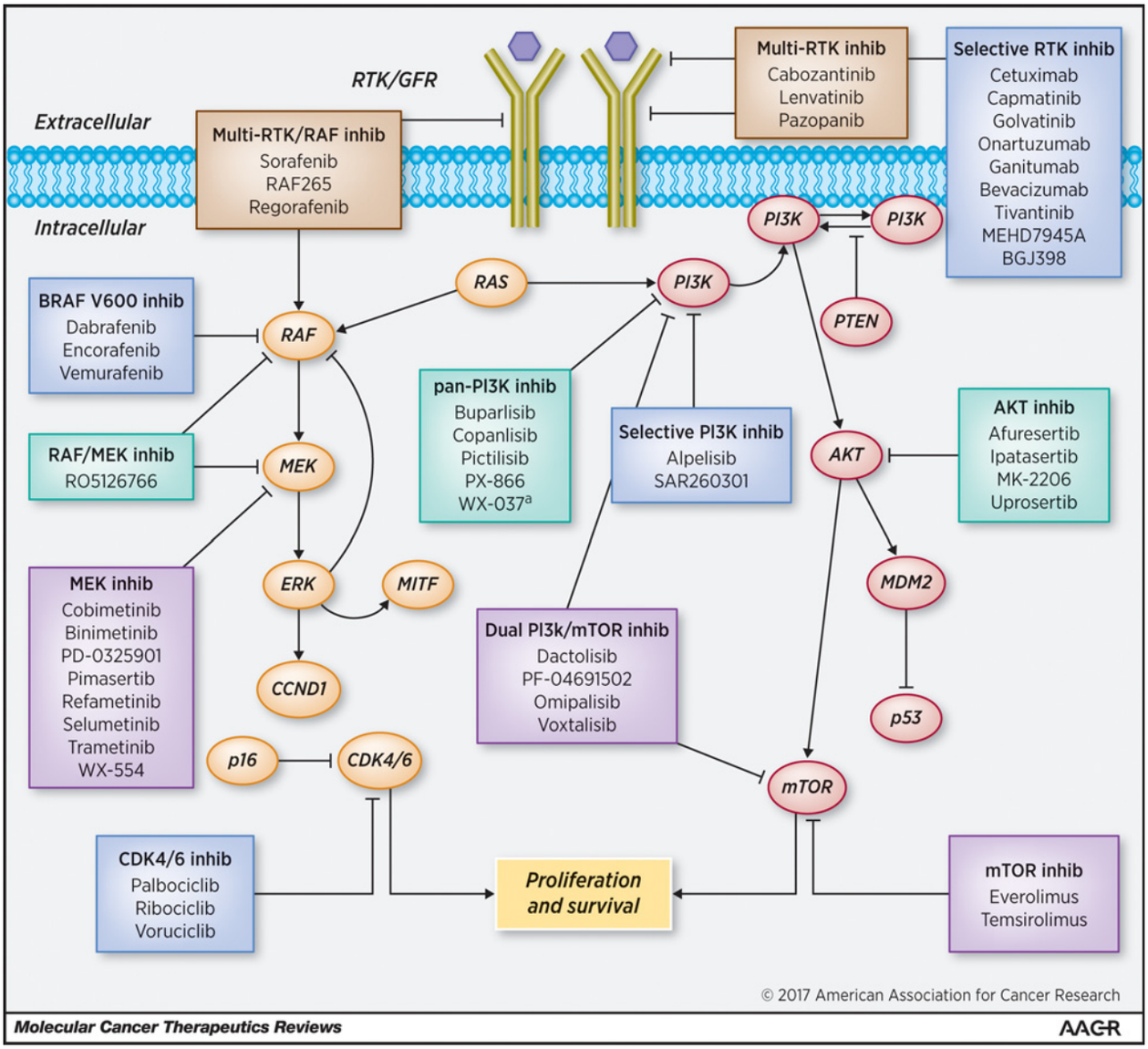

Figure 1.

Overview of the MAPK and PI3K/AKT pathways and targeted therapies. These pathways interact to form an interconnected signaling network via shared common inputs, including receptor tyrosine kinases (RTK)/growth factor receptors (GFR) and RAS. Binding of a ligand to an RTK is the key RAS activation step. In oncogenesis, RAF kinases act not only as RAS effectors, but also as ERK activators which serve as a form of negative feedback that inhibits the MAPK pathway. Increased activity of RTKs, RAS, and RAF are among the mechanisms implicated in the oncogenic activation of the MAPK pathway, resulting in constitutive activation of MEK and ERK. Regarding the PI3K/AKT pathway, AKT and downstream mTOR are activated by the conversion of phosphatidylinositol bisphosphate (PIP2) to phosphatidylinositol trisphosphate (PIP3) via PI3K. The PTEN acts as a negative regulator of this pathway by dephosphorylating PIP2 and PIP3. The PI3K/AKT pathway can also be activated by RAS mutants and acts as part of the cross-talk mechanism with the MAPK pathway. Both pathways can be activated by oncogenic RAS and likely serve a compensatory signaling function in cases where either pathway is inhibited. Abbreviations: CDK, cyclin-dependent kinase; GFR, growth factor receptor; inhib, inhibitor; RTK, receptor tyrosine kinase. [a]WX-037 PI3K specificity not reported.

Downstream of both pathways, the cyclin-dependent kinases (CDK) are involved in regulation of cell cycles, DNA replication, and cell division (16). The cyclin-D-CDK4/6-INK4-Rb pathway is frequently dysregulated in cancers through amplifications (*CCND1*, *CDK4*, *CDK6*) and loss of negative regulators (i.e., *p16INK4A*).

Although it is useful to think of these pathways in isolation, downstream interactions, feedback loops, and cross talk exist, such that therapeutic targeting of one pathway may result in activation of others as a compensatory mechanism.

Single-agent inhibition

Targeting MAPK pathway with single-agent BRAF inhibitors. The *BRAF* V600E point mutation is the most common *BRAF* mutation across all solid tumors and is responsible for substantial increase in kinase activity (3, 15). For example, in non–small cell lung

Table 2. Relative frequency of *BRAF* mutations by tumor type and frequency of mutation (adapted from Flaherty and McArthur [Cancer 2010]) (15)

Tumor type	Patients with *BRAF* mutation, %
Melanoma	30–70
Papillary thyroid cancer	40–70
Cisplatin-refractory testicular cancer	25
Cholangiocarcinoma	10–20
Colorectal cancer	5–20
Ovarian cancer	5–10
Prostate cancer	5–10
Glioblastoma, NSCLC, HNSCC, breast cancer, and pancreatic cancer	1–5
***BRAF* mutation**	**Percentage of all *BRAF* mutations**
V600E	97.3
V600K	1
K601E[a]	0.4
G469A[a]	0.4
D594G[a]	0.3
V600R	0.3
L597V[a]	0.2

[a]Most common amino acid change reported at these positions; percentage provided includes all amino acid changes reported for the position.

cancer (NSCLC), where *BRAF* mutations account for only 1% to 5% of mutations, the *BRAF* V600E mutation makes up approximately 50% of all *BRAF* mutations (17, 18). The *BRAF* gene was shown to be mutated at a high rate in melanoma (66%) and a lower rate in colorectal cancer (12%) and ovarian cancer (14%), providing an opportunity to develop selective targeted therapies for the mutated BRAF protein (19). Initial studies in melanoma were conducted with the first-generation oral multikinase inhibitor sorafenib (20, 21). Sorafenib inhibits the activity of CRAF, BRAF-wild type (WT) and BRAF mutant, as well as multiple RTKs implicated in tumor angiogenesis and progression (22). In melanoma, sorafenib had little or no antitumor activity, as a single agent or combined with chemotherapy (20, 21). However, positive results were obtained in metastatic renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), and differentiated thyroid carcinoma; and as a result, sorafenib was approved for use in these cancers (22). Possible reasons for the lack of clinical activity of sorafenib in melanoma include weak direct inhibitory effect on mutant BRAF at clinically achievable concentrations and/or compensatory mechanisms resulting in activation of additional signaling pathways (13, 20). Nevertheless, these results demonstrate the differences in pathophysiology across different tumors (20).

Dabrafenib and vemurafenib are highly selective inhibitors of the V600-mutant BRAF and have demonstrated rapid tumor regression in approximately half of patients with unresectable or advanced *BRAF* V600-mutant melanoma as well as improvements in progression-free survival (PFS), overall survival (OS), and overall response rate (ORR) compared with dacarbazine (23–25). Yet single-agent BRAF inhibitor activity was limited by high interpatient variability in response, with approximately 15% of patients demonstrating intrinsic resistance to treatment (26) and, in the majority of patients with an initial response, acquired resistance and disease progression (23, 24). BRAF inhibitors are also associated with certain unique adverse events (AEs; e.g., pyrexia, photosensitivity) and secondary hyperproliferative skin disorders due to paradoxical activation of wild-type BRAF in nonmelanoma cells (23, 24).

A basket trial recently evaluated vemurafenib in several *BRAF*-mutant cancers (27). Preliminary activity was observed in NSCLC, Erdheim–Chester disease, and Langerhans cell histiocytosis. In these cancers, histologic context was considered an important determinant of response in *BRAF* V600–mutated cancers. In a phase II study evaluating dabrafenib in patients with previously treated *BRAF* V600–positive advanced NSCLC, an ORR of 33% was observed, with a median duration of response of 9.6 months (28). Alternatively, in the 5% to 8% of patients with colorectal cancer who harbor the *BRAF* V600E mutation, clinical activity was not observed with vemurafenib treatment, with an ORR of only 5% (29).

Targeting MAPK pathway with single-agent MEK inhibitors. Clinical trials have also evaluated the single-agent MEK inhibitor trametinib in patients with advanced-stage *BRAF*-mutant melanoma (30–32). Trametinib resulted in improved clinical outcomes in patients with *BRAF* V600–mutant melanoma versus chemotherapy without paradoxical activation of the MAPK pathway in *BRAF*-WT cells (32). Yet while there are no head-to-head trials, the response rate associated with MEK inhibition appears to be inferior to that of BRAF inhibition (23, 24).

Binimetinib (MEK162), another MEK inhibitor, has been evaluated in patients with melanoma harboring *NRAS* or *BRAF* V600 mutations. Uniquely, binimetinib has shown some activity in patients with *NRAS*-mutated melanoma. In the phase III NEMO study comparing binimetinib versus dacarbazine, the ORR was 15% in patients treated with binimetinib versus 7% in patients treated with dacarbazine. Binimetinib significantly prolonged the median PFS versus dacarbazine [2.8 vs. 1.5 months; HR, 0.62 (95% confidence interval (CI), 0.47-0.80); $P < 0.001$; ref. 33). However, no significant difference was observed in OS in a preliminary analysis [11.0 vs. 10.1 months; HR, 1.00 (95% CI, 0.75–1.33); $P = 0.499$].

Targeting MAPK pathway at other kinases. Currently, no targeted therapies are approved for *RAS*-mutated tumors. Mutated versions of *RAS* are the most common oncogenes, reported at 16% to 30% of all human cancers (34). Activating *NRAS* mutations are observed in 15% to 25% of melanoma tumors and are associated with poor prognosis (35, 36). Different cancers show a tendency to arise from genetic aberrations in different *RAS* isoforms; melanoma tends to arise from *NRAS*, while colorectal cancer and lung cancer tend to arise from *KRAS* (34). For patients with *NRAS*-mutant melanoma, BRAF inhibition can drive paradoxical activation of MEK–ERK signaling, suggesting that current MAPK standard-of-care (SOC) combinations (BRAF inhibitors + MEK inhibitors) will provide no benefit (37). Therefore, inhibition of MEK alone has been a major focus, and, as mentioned previously, binimetinib has shown some preliminary activity but is not yet approved (33). In patients with *KRAS*-mutant colorectal cancer, two current SOC agents that specifically target this pathway, cetuximab and panitumumab, have demonstrated essentially no clinical activity (38, 39). In addition, as with MEK targeting, ERK targeting further downstream is also attractive. A phase I dose-escalation study was conducted with the ERK inhibitor ulixertinib in 27 patients with advanced solid tumors (40). One patient had a PR, while 7 patients experienced stable disease (SD) for $\geq$3 months.

Vertical pathway inhibition

Targeting MAPK pathway with combination BRAF and MEK inhibitors. In a preclinical mouse model, combination of BRAF and MEK inhibitors demonstrated enhanced inhibition of tumor xenograft growth as well as reduction in paradoxical activation of the MAPK pathway in *BRAF*-WT cells (41). The phase I/II study BRF113220 (NCT01072175) was the first to evaluate dabrafenib plus trametinib in patients with *BRAF* V600–mutant melanoma (42). When dabrafenib and trametinib were combined at their full monotherapy doses DLTs were considered infrequent. Results from large phase III studies with BRAF plus MEK inhibitors [dabrafenib plus trametinib, COMBI-d (NCT01584648) and COMBI-v (NCT01597908); vemurafenib plus cobimetinib, coBRIM (NCT01689519)] demonstrated higher ORRs and improvements in median PFS and OS compared with single-agent BRAF inhibitors, with a manageable safety profile (7, 43, 44). More specifically, the ORR and median PFS and OS in the COMBI-d study were 69%, 11.0 months, and 25.1 months, respectively, in the dabrafenib and trametinib group; versus 53%, 8.8 months, and 18.7 months, respectively, in the dabrafenib monotherapy group (43). Similar results were seen in the coBRIM study, including ORR and median PFS and OS of 70%, 12.3 months, and 22.3 months, respectively, in the cobimetinib and vemurafenib group; versus 50%, 7.2 months, and 17.4 months, respectively, in the vemurafenib monotherapy group (44). Hyperproliferative cutaneous events were less frequently observed with combination therapy versus monotherapy, suggesting a reduction in activation of *BRAF*-WT cells. However, an increase in some BRAF inhibitor–associated toxicities was observed. In particular, an increased incidence of pyrexia (25% with single-agent dabrafenib vs. 52% with dabrafenib plus trametinib) was observed (43). Likewise, increased incidences of photosensitivity and elevated values from liver function tests (LFT) were observed with vemurafenib plus cobimetinib compared with vemurafenib alone (44).

A phase III trial (COLUMBUS; NCT01909453) evaluating encorafenib plus binimetinib in patients with *BRAF* V600E/K–mutated melanoma recently reported data (45). The combination of encorafenib plus binimetinib significantly prolonged the median PFS versus vemurafenib monotherapy [14.9 vs. 7.3 months; HR, 0.54 (95% CI, 0.41–0.71); $P < 0.001$].

Although *BRAF* V600E mutations occur at a low rate in NSCLC (1%–5%; refs. 17, 18), the *BRAF* V600E–mutant patient population is significant due to the overall incidence of NSCLC, and these patients currently have limited therapeutic options (17, 46). In a cohort of 57 patients with previously treated *BRAF* V600E–mutant advanced NSCLC from a phase II trial (NCT01336634), dabrafenib plus trametinib demonstrated clinically meaningful antitumor activity, with an ORR of 63% and a median PFS of 9.7 months (47). An additional cohort is ongoing to evaluate dabrafenib plus trametinib in patients with previously untreated *BRAF* V600E–mutant advanced NSCLC. In a real world setting, patients with *BRAF*-mutant metastatic NSCLC had response rates of 23% and 5% in first- and second-line treatment, respectively. The majority of patients received SOC chemotherapy (48).

Targeting MAPK pathway with combination MEK and ERK inhibitors. ERK provides another downstream point of inhibition, and ERK inhibition may also reduce the chance for developing feedback resistance (49, 50). The binding of *BRAF* mutants with intermediate or low kinase activity to form heterodimers has been shown to result in CRAF activity, thus providing a mechanism for ERK hyperstimulation by *BRAF* mutants with reduced kinase activity (13, 14).

A number of ERK inhibitors are currently in development, including GDC-0994, SCH772984, and ulixertinib. GDC-0994, a highly selective ERK1/2 inhibitor, has demonstrated activity in *KRAS*-mutant and *BRAF*-mutant mouse models (51) and is being evaluated in combination with cobimetinib in a phase I trial in patients with locally advanced or metastatic solid tumors (NCT02457793; ref. 52). SCH772984 has shown promising results in a panel of melanoma cell lines, including cells with innate or acquired resistance to vemurafenib, those with *BRAF*/*NRAS* double mutations, *NRAS* mutations, and *RAS*-WT (53, 54).

Targeting MAPK pathway with dual RAF/MEK inhibitors

RO5126766 is a first-in-class, selective dual BRAF/CRAF and MEK inhibitor currently in early development for advanced solid tumors (55). Studies in tumor cell lines suggest that RO5126766 may be a good candidate for targeting *RAS*-mutated tumor cells (56). RO5126766 was evaluated in a phase I dose-escalation study in 52 heavily pretreated patients with advanced solid tumors, most with melanoma ($n = 21$), colorectal cancer ($n = 10$), or ovarian cancer ($n = 6$). Tumor shrinkage was observed in approximately 40% of patients across all tumor types, with 3 PRs [all in patients with *BRAF*- ($n = 2$) or *NRAS*- ($n = 1$) mutant melanoma; ref. 55)]. Seventeen of 39 analyzed tumors contained mutations in *BRAF, NRAS/HRAS*, or *PI3KCA*; 1 patient showed loss of PTEN staining, while PTEN was intact in the 3 patients who achieved a PR, indicating PTEN suppression of PI3K/AKT pathway activation. Common treatment-related toxicities across all dosing regimens were cutaneous, gastrointestinal, metabolic, and ocular reactions. No cases of cutaneous squamous cell carcinoma were reported.

Targeting MAPK pathway and upstream RTKs/GFRs

Overexpression of the EGFR in *BRAF*-mutated tumors is a common alteration associated with MAPK pathway inhibition and acquired resistance to BRAF inhibitors (57).

A recent study profiled 25,019 solid tumors using next-generation sequencing (58). Overexpression of EGFR in *BRAF*-mutated tumors was highest in colorectal cancer (88%), which is known to have a poor response to BRAF inhibitors, and lowest in melanoma (13%), which is known to have a good response. In *BRAF* V600–mutant colon tumor cell lines, blockade of EGFR by cetuximab or gefitinib was synergistic with BRAF inhibition, and in xenograft models erlotinib was synergistic with BRAF inhibition (57), suggesting that the use of combination BRAF plus RTK inhibitor may be a promising approach.

Various multi-RTK inhibitors (RAF265, sorafenib, regorafenib, cabozantinib, pazopanib, and lenvatinib), selective small-molecule RTK inhibitors (capmatinib, BGJ398, and MEHD7945A), and mAbs that bind to the extracellular domain of the RTK (onartuzumab, ganitumab) are in clinical trials in combination with BRAF or MEK inhibitors (Table 3). RAF265, sorafenib, and regorafenib also display activity against WT and mutant BRAF in addition to RTK inhibition.

Combinations of selective RTK inhibitors with MAPK pathway–targeted agents are also under way. While limited activity in colorectal cancer has been observed with targeted BRAF and MEK combination therapy (9), preclinical data suggest that combined inhibition of the MAPK pathway with upstream inhibition of

Table 3. Clinical trials of vertical inhibition of MAPK pathway and targeted therapy in advanced solid tumors

Molecular targets, therapeutic agents	Study[a]	Phase	Patient population	Trial status[a,b]
BRAF + MEK inhibitors				
Dabrafenib + trametinib	BRF113220 (NCT01072175) (42, 105)	1/2	*BRAF* V600–mutated unresectable or MM	Achieved primary outcome; ongoing pt follow-up
	COMBI-d (NCT01584648) (6, 43)	3	*BRAF* V600E/K–mutated unresectable or MM, stage IIIC or IV	Achieved primary outcome; ongoing pt follow-up
	COMBI-v (NCT01597908) (7, 106)	3	*BRAF* V600E/K–mutated unresectable or MM, stage IIIC or IV	Achieved primary outcome; ongoing pt follow-up
	NCT01978236	2	*BRAF* V600E/K–mutated melanoma with active brain metastases	Ongoing
	NCT02039947	2	*BRAF* V600–mutated melanoma with active brain metastases	Ongoing
	Neoadjuvant (NCT01972347) (107)	2	*BRAF* V600–mutated melanoma stage IIIB/C	Ongoing
	Neoadjuvant and adjuvant (NCT02231775) (107)	2	*BRAF* V600E/K–mutated clinical stage III or oligometastatic melanoma	Ongoing
	BRF113928 (NCT01336634)	2	*BRAF* V600E–mutant metastatic NSCLC	Ongoing
Vemurafenib + cobimetinib	BRIM-7 (NCT01271803) (108, 109)	1b	*BRAF* V600–mutated unresectable or MM, stage IIIC or IV	Achieved primary outcome; ongoing pt follow-up
	coBRIM (NCT01689519) (8, 44)	3	*BRAF* V600–mutated unresectable or MM, stage IIIC or IV	Achieved primary outcome; ongoing pt follow-up
	coBRIM-B (NCT02230306) (110)	2	*BRAF* V600–mutated melanoma with active brain metastases	Terminated
	NEO-VC (NCT02303951)	2	*BRAF* V600–mutated melanoma with limited metastasis of MM, stage IIIC or IV, in neoadjuvant setting	Ongoing
	NCT02036086	2	*BRAF* V600–mutated melanoma with palpable lymph node metastases, in neoadjuvant setting	Ongoing
	CONVERCE (NCT02537600)	2	*BRAF* V600–mutated stage IV melanoma with brain metastasis	Ongoing
Encorafenib + binimetinib	NCT01543698 (111, 112)	1b/2	*BRAF* V600–mutated unresectable or MM, stage IIIB to IV	Achieved primary outcome; ongoing pt follow-up
	COLUMBUS (NCT01909453) (45)	3	*BRAF*-mutated unresectable or MM, stage IIIB/C or IV	Achieved primary outcome; ongoing pt follow-up
MEK + ERK1/2 inhibitors				
Cobimetinib + GDC-0994	NCT02457793	1b	Locally advanced or metastatic solid tumors	Ongoing
Dual RAF/MEK inhibitor				
RO5126766	NCT00773526 (55)	1	Select advanced solid tumors	Completed
	NCT02407509	1	Select advanced solid tumors	Ongoing
BRAF + CDK4/6 inhibitors				
Vemurafenib + voruciclib	NCT01841463 (69)	1	*BRAF*-mutated unresectable or MM, stage III or IV	Suspended after enrollment of 9 patients (2 dose-escalation cohorts) for nonmedical reasons; MTD not reached
Encorafenib + ribociclib	NCT01777776	1b/2	*BRAF*-mutated unresectable or MM, stage III or IV	Terminated for nonmedical reasons
MEK + CDK4/6 inhibitors				
Trametinib + palbociclib	NCT02065063	1/2	*BRAF*-WT advanced solid tumors	Terminated
PD-0325901 + palbociclib	NCT02022982	1/2	*KRAS*-mutant NSCLC, solid tumors	Ongoing

(Continued on the following page)

Table 3. Clinical trials of vertical inhibition of MAPK pathway and targeted therapy in advanced solid tumors (Cont'd)

Molecular targets, therapeutic agents	Study[a]	Phase	Patient population	Trial status[a,b]
Binimetinib + ribociclib	NCT01781572 (70)	1b/2	*NRAS*-mutant MM	Ongoing
MAPK pathway inhibitor + growth factors and/or their receptors tyrosine kinases (GFR/RTK) inhibitors				
Multi-RTK inhibitors ± BRAF or MEK or BRAF + MEK inhibitors				
RAF265	NCT00304525	2	Locally advanced or metastatic melanoma	Completed
Binimetinib + RAF265	NCT01352273	1	*RAS* or *BRAF* V600E–mutated advanced solid tumors	Completed
Refametinib + regorafenib	NCT02168777	1b/2	Locally advanced or metastatic solid tumors	Terminated
Dabrafenib + pazopanib	NCT01713972 (113)	1	*BRAF*-mutated advanced malignant tumors	Ongoing
Vemurafenib + cabozantinib	NCT01835184	1	*BRAF* V600–mutated unresectable or metastatic solid tumor malignancy	Terminated
Trametinib + pazopanib	NCT01438554 (114)	1	Advanced solid tumors	On-going
BRAF/multi-kinase inhibitor/MEK inhibitor + selective RTK inhibitors				
Lenvatinib + golvatinib	NCT01433991	1/2	Unresectable stage III or IV melanoma after prior systemic therapy	Ongoing
Vemurafenib and/or cobimetinib + onartuzumab	NCT01974258	1b	*BRAF* V600–mutated unresectable or MM, stage IIIC or IV	Withdrawn prior to enrollment
Sorafenib + tivantinib	NCT00827177 (61)	1	Locally advanced, inoperable, or metastatic solid tumors, including melanoma	Completed
Sorafenib + bevacizumab	NCT00387751	2	Unresectable stage III or stage IV malignant melanoma	Completed
	NCT00632541	2	Metastatic breast cancer	Terminated
Vemurafenib + cobimetinib + bevacizumab	NCT01495988	2	*BRAF* V600–mutated unresectable or MM, stage IV	Terminated
Binimetinib + ganitumab	NCT01562899	1b/2	Selected advanced solid tumors	Terminated due to nonmedical reasons
Cobimetinib + MEHD7945A	NCT01986166	1	Locally advanced or metastatic cancer with mutant *KRAS*	Completed

Abbreviation: pt, patient.

[a]All studies listed are identified in clinicaltrials.gov. Trial status based on clinicaltrials.gov status, accessed January 25, 2017.

[b]Ongoing trials include trials currently recruiting and those not actively recruiting patients.

EGFR may be required to maximally inhibit growth of *BRAF*-mutated metastatic colorectal cancer (57). Despite controversy, EGFR mAbs are thought to have little benefit as monotherapy in *BRAF*-mutated metastatic colorectal cancer (59). In addition, as previously mentioned, treatment with vemurafenib monotherapy in patients with colorectal cancer who harbor the *BRAF* V600E mutation produced an ORR of only 5% (29). A phase I/II study of dabrafenib and trametinib plus an anti-EGFR mAb, panitumumab, was conducted in patients with *BRAF*-mutant colorectal cancer (NCT01750918; ref. 60). The confirmed ORR was 10% in patients treated with dabrafenib plus panitumumab (1 CR and 1 PR) and 21% in patients who received all 3 drugs (1 CR and 18 PRs), suggesting that targeting of EGFR may be able to overcome some resistance to MAPK pathway inhibition in metastatic colorectal cancer. The incidence of grade 3 or 4 dermatologic toxicity, for example, acneiform dermatitis in 10% and 18% of patients with the triplet and trametinib plus panitumumab combinations, respectively, necessitated dose reductions and interruptions/delays. Results from the triplet combination therapy were considered encouraging by the investigators, despite lower activity than that observed with dabrafenib plus trametinib in melanoma.

Several hepatocyte growth factor–receptor (HGFR or c-MET) inhibitors are being evaluated in combination with RAF and/or MEK inhibitors, including tivantinib, capmatinib, and golvatinib (52, 61). In a phase I study of tivantinib plus sorafenib, preliminary anticancer activity was observed in patients with melanoma [ORR, 26%; disease control rate (DCR), 63%], RCC (ORR, 15%; DCR, 90%), and HCC (ORR, 10%; DCR, 65%). In patients with melanoma, the median PFS was 5.4 months in patients with *NRAS* mutation and 3.3 months in patients with *NRAS*-WT or unknown *NRAS* status. The safety profile was considered manageable and predictable (61).

Bevacizumab is an inhibitor of VEGF-A that has demonstrated efficacy across a number of tumor types (62). A phase II study with bevacizumab and sorafenib (NCT00387751) was terminated after 14 of 21 patients were enrolled with no observed responses and increased toxicity (63). Another study with bevacizumab plus vemurafenib and cobimetinib (NCT01495988) in melanoma was terminated due to slow accrual and toxicity (52).

Other inhibitors of RTKs that are under investigation in combination with BRAF and/or MEK inhibitors include MEHD7945A; a dual EGFR/HER3 inhibitor, BGJ398, a pan-fibroblast growth factor receptor (FGFR) inhibitor, and the mAbs onartuzumab and ganitumab, which inhibit HGFR and insulin-like growth factor 1 receptor (IGF-1R), respectively. All are currently in phase Ib trials (52).

Targeting MAPK pathway and downstream inhibition of cyclin-dependent kinase pathway

The CDK4/6 pathway acts downstream of the MAPK pathway and has been reported to be altered or upregulated in 90% of melanomas (64). In a study by Shain and colleagues, homozygous loss of CDKN2A, a known tumor suppressor, was observed only in invasive melanoma samples and not at earlier histologic stages (65). Preclinical studies indicate that combination MEK plus CDK4/6 inhibition may lead to enhanced antitumor activity in *NRAS*-mutant melanoma (66). CDK4/6 inhibitors palbociclib (PD-0332991), voruciclib (P1446A-05), and ribociclib (LEE011) are currently being evaluated in combination with BRAF and MEK inhibitors in phase I/II studies. Ribociclib has received breakthrough therapy designation from the FDA for the treatment of hormone receptor–positive (HR+), human EGFR 2–negative ($HER2^-$) advanced breast cancer in combination with the aromatase inhibitor letrozole (67).

Palbociclib was evaluated in a phase I dose-finding study in combination with trametinib in patients with *BRAF*-WT solid tumors (68) (NCT02065063). Two patients (1 patient with melanoma and 1 with *NRAS*-mutant colorectal cancer) had a PR. The incidence of grade 3 and 4 AEs was 75% and 21%, respectively, and the study was terminated prior to expansion. Another study (NCT02022982) is evaluating palbociclib plus the MEK inhibitor PD-0325901 in patients with *KRAS*-mutated solid tumors, particularly NSCLC, and is still ongoing (52).

Preliminary data from a phase I trial with voruciclib and vemurafenib in advanced *BRAF*-mutant melanoma were reported for 9 enrolled patients; 3 patients were BRAF inhibitor naïve, and 6 patients had refractory disease (NCT01841463). The combination was reported to be well tolerated, and responses (1 CR and 2 PRs) were observed in all BRAF inhibitor–naïve patients. The MTDs were not reached, but the study was stopped for nonmedical reasons (69).

Two phase I/II trials are evaluating ribociclib and encorafenib in patients with *BRAF*-mutated melanoma (NCT01777776, completed) and ribociclib with binimetinib in patients with *NRAS*-mutated melanoma (NCT01781572, ongoing; ref. 52). Preliminary data for ribociclib and binimetinib demonstrated that 5 of 22 patients treated with the 28-day regimen had a confirmed PR, and the preliminary median PFS was estimated to be 6.7 months (70).

As seen, significant advances have been made with vertical inhibition of the MAPK pathway. Other combinations for targets upstream and downstream of BRAF and MEK are actively being pursued, and additional combinations are expected to bring further benefit to patients.

Horizontal inhibition of MAPK and PI3K/AKT pathways

MAPK and PI3K pathways can both be activated by oncogenic RAS and appear to provide compensatory signaling when one or the other is inhibited (1, 71, 72). Loss of PTEN and PI3K pathway activation is common in *BRAF*-mutant melanoma and is implicated as a mechanism of acquired resistance to BRAF and MEK inhibitors (Fig. 1; refs. 73, 74).

Numerous inhibitors that target various levels of the PI3K pathway are in development, including PI3K, AKT, and mTOR inhibitors; the mTOR inhibitors everolimus and temsirolimus have been shown to be effective in the treatment of certain solid tumors (52, 75, 76). Because there are numerous feedback loops in the PI3K pathway, inhibition most likely requires targeting multiple steps of the pathway. Clinical trials are evaluating vertical inhibition with combinations that target PI3K and mTOR as well as dual PI3K/mTOR inhibitors. Preclinical studies in tumor cell lines have suggested that this cotargeting strategy can result in synergistic inhibition and induction of apoptosis (13, 77). In addition, horizontal inhibition of MEK and PI3K effector pathways may be required to effectively inhibit *NRAS*-mutant melanoma (78).

Targeting MAPK pathway combined with PI3K inhibitors

Several pan-PI3K inhibitors, buparlisib, pictilisib, copanlisib, and PX-866, are currently in early-phase clinical trials in combination with MAPK pathway inhibitors in patients with advanced solid tumors, including melanoma (52). Buparlisib is being evaluated in combination with the BRAF inhibitor vemurafenib

(NCT01512251) and MEK inhibitors trametinib (NCT01155453) and binimetinib (NCT01363232; ref. 52). A preliminary report of buparlisib plus vemurafenib demonstrated limited activity in 8 patients, with 1 patient achieving a PR at week 8 and subsequently progressing with brain metastases at week 16. In addition, concerns were raised regarding the tolerability of the combination (79).

Preliminary results have also been presented from a phase I study of pictilisib with cobimetinib (NCT00996892; ref. 80). In 78 enrolled patients, DLTs included grade 3 elevations in lipase and grade 4 elevations in CPK; however, higher doses of pictilisib were tolerated when cobimetinib was given intermittently, and toxicities were reported to be similar to those observed in single-agent phase I trials. Signs of clinical activity were observed; 3 patients had a PR (1 patient with *BRAF*-mutated melanoma, 1 with *BRAF*-mutated pancreatic cancer, and 1 with *KRAS*-mutated endometrioid cancer), and 5 patients had an SD $\geq$ 5 months.

Copanlisib was evaluated in combination with the allosteric MEK inhibitor refametinib in a dose-escalation phase I trial in 49 patients with advanced solid tumors (NCT01392521; ref. 81). Interestingly, both the MTD and the recommended phase II dose for the combination were below the MTD of either compound alone. Preliminary signals of clinical activity were observed; 1 patient with endometrial cancer had a PR, and 9 patients had an SD. However, another phase I/II trial with PX-866 and vemurafenib was terminated early due to slow accrual (NCT01616199; ref. 52). Finally, the PI3K inhibitor WX-037 was being evaluated in combination with MEK inhibitor WX-554 (NCT01859351), but the trial was terminated early due to business reasons, and WX-037 development was discontinued (52).

Isoform-selective PI3K inhibitors may provide better tolerability than dual PI3K/mTOR inhibitors or pan-class I PI3K inhibitors; perhaps allowing for higher dosing and signal pathway inactivation (82, 83). Two selective PI3K isoform inhibitors, α-specific alpelisib (BYL719) and β-specific SAR260301, are being evaluated in combination therapy. Alpelisib plus binimetinib is being evaluated in patients with advanced solid tumors (NCT01449058); results for this trial are not yet available (52). Preliminary results from a phase II study comparing the triplet combination of alpelisib with BRAF inhibitor encorafenib and EGFR inhibitor cetuximab versus encorafenib plus cetuximab in patients with *BRAF*-mutant advanced colorectal cancer demonstrated promising clinical activity for both combinations (84). The median PFS was prolonged, although not significantly, with the triplet combination versus the doublet [5.4 vs. 4.2 months; HR, 0.69 (95% CI, 0.43–1.11); $P = 0.064$]; however, the triplet was associated with increased toxicity (grade 3/4 AEs triplet vs. doublet, 79% vs. 58%).

Targeting MAPK pathway combined with mTOR inhibitors

Everolimus plus MEK inhibitor trametinib was evaluated in a phase Ib trial (NCT00955773) in 67 patients with advanced solid tumors, including pancreatic cancer, colorectal cancer, and melanoma, among others (85). A recommended phase II dose and schedule of the combination with an acceptable tolerability and adequate drug exposure could not be identified; therefore, development of the combination was terminated.

Temsirolimus plus the MEK inhibitor pimasertib was investigated in a phase Ib trial in patients with advanced solid tumors (NCT01378377; ref. 52). Results from the study have not been published; however, the incidence of mucositis, a common DLT associated with single-agent mTOR inhibitor therapy, was evaluated in a retrospective analysis of 3 phase I clinical trials, including the pimasertib and temsirolimus combination trial (86). The overall incidence and severity of mucositis with the temsirolimus-based combination was significantly greater than with temsirolimus alone. Everolimus plus vemurafenib is currently being evaluated in a dose-escalation phase I trial in patients with *BRAF*-mutated cancer (NCT01596140; ref. 52). Of 20 evaluable patients with *BRAF*-mutant tumors, 4 patients had PR, and 9 had SD. Six of these patients (2 with PR, 4 with SD) had previously progressed with single-agent BRAF inhibitor therapy prior to the addition of everolimus. At the higher everolimus dose (10 mg once daily) with vemurafenib, the DLTs rash and fatigue occurred in 3 patients; no DLTs were reported at the lower everolimus dose (5 mg once daily; ref. 87).

Targeting MAPK pathway combined with dual PI3K/mTOR inhibitors

In preclinical melanoma models, combining MEK and dual PI3K/mTOR inhibitors was shown to have synergistic activity, and combination targeting of these signaling pathways was effective in models of *NRAS*-mutant melanoma (78). Dactolisib, omipalisib, voxtalisib, and PF-04691502, target both PI3K and mTOR and have entered clinical trials in combination with a MEK inhibitor (Table 4; ref. 52).

Dactolisib combined with binimetinib was evaluated in a dose-escalation phase I study in patients with advanced solid tumors, including those with *KRAS*, *NRAS*, or *BRAF* mutations (NCT01337765); results have not been reported (52). A phase I trial of omipalisib plus trametinib in patients with advanced solid tumors was terminated due to lack of tolerability and efficacy of the combination (NCT01248858). Voxtalisib plus pimasertib was evaluated in a phase Ib study in 60 patients with locally advanced or metastatic solid tumors (NCT01390818; ref. 88). Preliminary results suggested the combination was tolerated, and there were signs of clinical activity in 4 patients with PR [1 patient with *KRAS* colorectal cancer and 3 with low-grade ovarian cancer (1 of whom had *KRAS*-mutant/*PIK3CA*-mutant disease and 2 of whom had WT; ref. 88)]. An additional expansion phase is designed to enroll 4 cohorts: patients with dual *KRAS*/*PIK3CA*–mutated CRC, triple-negative breast cancer (TNBC), *KRAS*- or *NRAS*-mutated NSCLC, and *BRAF*-mutant melanoma.

Targeting MAPK pathway combined with AKT inhibitor

MEK and AKT inhibitor combinations have shown poor tolerability, with toxicities limiting administration. The AKT inhibitors afuresertib and uprosertib have been evaluated in dose-escalation phase I/II trials in combination with trametinib in patients with advanced solid tumors, including *BRAF*-mutant and WT or *KRAS*-mutant tumors (NCT01476137, NCT01941927, NCT01138085, and NCT01964924); an additional study evaluated the combination dabrafenib and trametinib plus uprosertib (NCT01902173). Poor tolerability and limited efficacy was observed with trametinib plus afuresertib in patients with advanced solid tumors (11). In patients with TNBC, trametinib with uprosertib also showed limited efficacy (89).

The AKT inhibitor MK-2206 plus the MEK inhibitor selumetinib was evaluated in a phase I study in patients with locally advanced or metastatic solid tumors, a high proportion of whom were *KRAS*-mutant positive (NCT01021748) (90), and a phase II study in patients with *BRAF* V600–mutant advanced melanoma

Table 4. Clinical trials of horizontal inhibition of MAPK and PI3K pathways and targeted therapy in advanced solid tumors

Molecular targets, therapeutic agents	Study[a]	Phase	Patient population	Trial status[a,b]
BRAF inhibitor + PI3K inhibitor				
Vemurafenib + buparlisib	NCT01512251 (115)	1/2	*BRAF* V600E/K–mutated unresectable or MM	Unknown
Vemurafenib + PX-866	NCT01616199	1/2	*BRAF*-mutated advanced cancer, including melanoma	Terminated due to slow accrual
Vemurafenib + SAR260301	NCT01673737	1	Advanced solid tumors and unresectable or metastatic *BRAF*-mutated melanoma	Completed
MEK inhibitor + PI3K inhibitor				
Trametinib + buparlisib	NCT01155453	1b	Selected advanced solid tumors	Completed
Binimetinib + buparlisib	NCT01363232	1b	Selected advanced solid tumors	On-going
Cobimetinib + pictilisib	NCT00996892 (80)	1b	Locally advanced or metastatic solid tumors	Terminated
Refametinib + copanlisib	NCT01392521 (81)	1b	Advanced solid tumors	Completed
Binimetinib + alpelisib	NCT01449058	1b/2	Selected advanced solid tumors	On-going
WX-554 + WX-037	NCT01859351	1	Advanced solid tumors	Terminated
BRAF inhibitor + mTOR inhibitor				
Vemurafenib + everolimus or temsirolimus	NCT01596140 (116)	1	*BRAF*-mutated advanced cancer, including melanoma	On-going
MEK inhibitor + mTOR inhibitor				
Trametinib + everolimus	NCT00955773 (85)	1b	Advanced solid tumors	Completed
Pimasertib + temsirolimus	NCT01378377 (86)	1	Advanced solid tumors	Completed
MEK inhibitor + Dual PI3K/mTOR inhibitor				
Binimetinib+ dactolisib	NCT01337765	1b	Selected advanced solid tumors	Completed
Trametinib + omipalisib	NCT01248858	1	Advanced solid tumors	Terminated
Pimasertib + voxtalisib	NCT01390818 (88)	1b	Locally advanced or metastatic solid tumors	Completed
PD-0325901 + PF-04691502	NCT01347866 (117)	1	Advanced solid tumor	Terminated due to internal portfolio review
MEK inhibitor + AKT inhibitor				
Trametinib + afuresertib	NCT01476137 (11)	1/2	Solid tumor malignancy, including melanoma and multiple myeloma	Completed
Trametinib + uprosertib	NCT01941927 (118)	2	*BRAF*-WT unresectable stage III or IV melanoma	Ongoing, not recruiting
	NCT01138085 (119)	1	Solid tumor with *KRAS or BRAF* mutation or none specified dependent on tumor type	Completed
	NCT01964924 (89)	2	Advanced TNBC	Ongoing
Selumetinib + MK-2206	NCT01021748 (90)	1	Locally advanced or metastatic solid tumors and expansion in *KRAS*-mutated solid tumors	Completed
	NCT01519427	2	*BRAF* V600–mutant unresectable, stage III or IV melanoma that had progressed after therapy on selective BRAF inhibitor	Terminated due to slow enrollment
Cobimetinib + ipatasertib	NCT01562275 (91)	1b	Locally advanced or metastatic solid tumors	Completed
BRAF + MEK + AKT inhibitors				
Dabrafenib + trametinib + uprosertib	NCT01902173	1/2	*BRAF* V600–mutated unresectable or metastatic solid tumors, including melanoma, stage IIIC or IV	Temporarily stopped for assessment

Abbreviation: WT, wild type.
[a]All studies listed identified in www.clinicaltrials.gov. Trial status based on www.clinicaltrials.gov status, accessed January 25, 2017.
[b]Ongoing studies includes those recruiting and those not recruiting patients.

(NCT01519427) that was terminated due to slow accrual (52). Rash and diarrhea limited the dosing, and deescalation of both agents was needed to improve tolerability of the combination. Heterogeneity of response was observed in patients with *KRAS*-mutant cancers (90).

The combination ipatasertib plus cobimetinib was evaluated in a phase I study in 47 patients with advanced solid tumors (NCT01562275; ref. 91). Antitumor activity was seen in 3 patients with PRs (in *KRAS*-mutant, PTEN-low ovarian cancer; *KRAS*-mutant mesonephric cervical cancer; and *KRAS*-mutant, PTEN-null endometrial cancer), and prolonged SD (> 6 months) was observed in both treatment arms in 4 patients, including >16 months in 1 patient with PTEN-null endometrial cancer. Nineteen patients with PTEN-low- endometrial cancer and TNBC were enrolled in part 2 of the study. Disease progression or death was reported in 16 and 3 patients, respectively (52).

Certainly, the rationale for horizontal targeting of complementary signaling pathways is solid and holds great promise. Finding the right combinations, doses, and schedules continues to be a challenge, and additional studies are needed. Mitigation strategies designed to reduce the increased toxicities observed with horizontal inhibition of the MAPK and PI3K pathways have so far been unsuccessful; however, tumor-selective delivery using new technologies may hold promise (92, 93).

Future directions

Agents targeting the MAPK pathway have led to significant benefit for patients with various tumor types. Immunotherapy with checkpoint inhibitors that target CTLA-4 and PD-1 is another therapeutic approach that has been successfully applied to the treatment of solid tumors (94). Thus, there is considerable interest in combining immunotherapy with targeted therapy (95, 96). The

triplet combination of targeted therapy (dabrafenib and trametinib) plus immunotherapy (pembrolizumab) is currently being evaluated in phase I/II trials in patients with *BRAF* V600–mutant advanced melanoma (KEYNOTE-022, NCT02130466; ref. 97). Preliminary data from 15 patients indicated a manageable toxicity profile, with 10 patients (67%) experiencing a grade 3 or 4 treatment-related AE and 4 patients (27%) discontinuing due to treatment-related AEs. The unconfirmed ORR was 60% (9 PR, 2 SD, and 3 PD).

Incorporation of nanoparticle systems may overcome toxicity associated with horizontal inhibition by targeting drug delivery to the tumor site at therapeutic levels while sparing the rest of the body from off-target toxicities (98, 99). Polymeric micelles and liposome nanoparticles have been found to preferentially accumulate in solid tumors. This phenomenon, termed the enhanced permeability and retention effect, is thought to be due to the abnormal tumor microenvironment, and researchers are looking for ways to further manipulate this effect to increase drug delivery (99). One study evaluating nanoparticle-mediated delivery of MEK inhibitor PD98059 in a melanoma mouse model showed significant tumor inhibition over vehicle (100). Another approach used encapsulated siRNA in nanoliposomes to target BRAF V600E and AKT3. Combined with low-frequency ultrasound, the nanoliposomal siRNA complex penetrated epidermal and dermal layers in reconstructed skin and decreased early or invasive cutaneous melanoma (101).

Despite the clinical success of combination strategies in MAPK pathway–driven solid tumors, a number of challenges remain moving forward. Perhaps the most substantial challenge is tolerability of combination regimens, particularly in efforts to target both the MAPK and PI3K/AKT pathways. The development of DLTs has often precluded delivery of optimal therapeutic concentrations (11, 12). In addition, a "one-size-fits-all" approach for treatment of solid tumors harboring MAPK alterations cannot be applied, because not all solid tumors containing the same *BRAF* mutation show the same response to BRAF plus MEK inhibitors (9). Further challenges with targeted agents in general include variable patient responses, drug resistance, and disease progression (6–8). Together, all of these areas will be a focus of intense research in the coming years and could provide enhanced patient benefit in the near future.

Conclusions

Advancements in the treatment of tumors with dysregulated MAPK pathway are a clear example of successes in translational research. Significant strides have been made with vertical inhibition using BRAF and MEK inhibitors in *BRAF* V600–mutant melanoma, and encouraging results have been seen in several tumor types, including *BRAF* V600–mutant NSCLC. Limited responses have been observed in other solid tumors, highlighting the unique nature of different tumors. Clearly, MAPK is a druggable pathway, and future efforts should continue to address the unmet need in tumor types with aberrant MAPK activation. In contrast, horizontal targeting of complementary signaling pathways that mediate resistance to MAPK targeting has been difficult, but development of novel agents and combinations is ongoing. The systemic toxicity of cancer therapies is a continual challenge, and novel strategies such as new delivery technologies, novel combinations with emerging agents, and treatment-schedule optimization can help to overcome these obstacles. Overall, targeting of the MAPK pathway is a clinical success, and continued research to understand the mechanisms of resistance, as well as to identify patients with other tumor types that may derive benefit from these agents, is of considerable interest.

Disclosure of Potential Conflicts of Interest

A.W. Tolcher is a member, Board of Directors at Symphogen and is a consultant/advisory board member for Akebia, Asana, Dicerna, Elekta, EMD Serono (Merck), Endocyte, Formation Biologics, Genmad, Heron, Ignyta, Janssen, Johnson & Johnson, Ascentage, Median, Mersana, Merus, Celldex, Nanobiotix, New B Innovation, OncoMed, Pierre Fabra, PharmaHealth, Rigontec, Astex, Symphogen, Upsher Smith, Zymeworks, Bayer, Bicycle Therapeutics, BioInvent, Blend Therapeutics, Boehringer-Ingelheim, and Celator. No potential conflicts of interest were disclosed by the other authors.

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