CHAPTER 3

The Therapeutic Promise of Anti-Cancer Drugs Against the Ras/Raf/MEK/ERK Pathway

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Abstract: The Ras/Raf/MEK/ERK mitogen-activated protein kinase (MAPK) pathway mediates cellular responses to different growth signals and is frequently deregulated in cancer. There are three Raf kinases-A-Raf, B-Raf, and C-Raf; however, only B-Raf is frequently mutated in various cancers. The most common B-Raf mutation involves a substitution of a glutamic acid residue to a valine moiety at codon 600. Subsequently, the MAPK pathway is constitutively activated, even in the absence of any growth signals. Although early attempts to target Ras have not yielded any viable drug candidates, many novel compounds inhibiting the activities of B-Raf and MEK have been developed and investigated in clinical trials in recent years and have shown promising result. The first MEK inhibitor (CI-1040) lacked efficacy in clinical trials, but its low toxicity encouraged the search for novel compounds-now there are over a hundred open clinical trials employing various B-Raf and MEK inhibitors. Several of these trials are now in Phase III. In this chapter, we will discuss new patents and patent applications related to inhibitors of the Ras/Raf/MEK/ERK pathway and some recent clinical trial results.

Keywords: ARQ736, AS703026, B-Raf inhibitors, dabrafenib, ERK, GDC-0879, heterocyclic compounds, imidazole derivatives, MEK inhibitors, MEK162, RAF265, refametinib, regorafenib, RO4987655, selumetinib, sorafenib, TAK-733, trametinib, vemurafenib, XL281.

INTRODUCTION

The Ras/Raf/MEK/ERK signaling pathway regulates the expression of a large number of proteins involved in the control of cell proliferation, differentiation, and apoptosis. In response to the binding of growth factors, cytokines, and hormones to cell surface receptors [1, 2], the level of Ras-guanosine triphosphate

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(GTP) increases in cells, which in turn promotes kinase activation. The GTP-bound forms of Ras directly bind and thus recruit cytosolic dimers of the Raf kinases to the plasma membrane. Once localized at the membrane, Raf is activated through phosphorylation by other kinases or by autophosphorylation [3]. Activated and membrane-associated Raf assembles a mitogen-activated protein kinase (MAPK) signaling complex that consists of two kinase classes: extracellular signal-regulated kinase (ERK) and MAPK/ERK (MEK) [4]. The MAPK cascade initiates with the phosphorylation and activation of MEK by Raf and, subsequently, the phosphorylation and activation of ERK by MEK. Activated ERK dissociates from the Ras/Raf/MEK/ERK complex and phosphorylates a number of cytoskeletal proteins, kinases, and transcription factors, such as nuclear factor NF-κB [5], AP-1, ETS-1, c-Jun, and c-Myc [2, 6, 7]. The functional consequences of substrate-level phosphorylation by ERK include changes in cellular motility and gene expression changes that promote proliferation, differentiation, cellular survival, immortalization, and angiogenesis [7, 8].

Aberrant activation of the Ras/Raf/MEK/ERK pathway is common and is observed in one third of all cancers [9]. Thus, therapeutic targeting of individual components of the Ras/Raf/MEK/ERK pathway has attracted much attention in the development of anti-cancer drugs. In addition, inhibitors targeting "active" protein kinases have demonstrated potential utility in anti-cancer drug activity, as is the case with B-Raf mutations. These mutations are especially prevalent in melanomas, thyroid cancers, and colorectal carcinomas, and are the focus of many clinical trials evaluating B-Raf inhibitors. Several drugs targeting B-Raf mutation or the Ras/Raf/MEK/ERK pathway are either in development or are currently in clinical trial and have shown promising results. The purpose of this chapter is to provide an update on the drugs currently in development that target the Ras/Raf/MEK/ERK pathway.

1. B-RAF ACTIVATING MUTATION

B-Raf is a serine/threonine kinase and a primary target of oncogenic Ras [10]. The identification of *B-Raf* gene mutations in various human cancers has stimulated numerous studies [11]. More than 80% of *B-Raf* mutations are single amino acid substitutions of glutamic acid for valine at codon 600 (previously

thought to be at 599) within the kinase domain. *B-Raf* mutations have been identified in a wide variety of human cancers, such as melanomas, ovarian borderline tumors, sporadic colorectal carcinomas, and thyroid carcinomas Table **1** [11-15]. The data were extracted from "The Catalogue of Somatic Mutations in Cancer" (COSMIC) database [16]. The B-Raf mutated protein, referred as B-Raf^{V600E}, has increased kinase activity compared to wild-type B-Raf [11]. B-Raf^{V600E} activates the downstream MEK/ERK signaling pathway independently of Ras-GTP and its expression is required to maintain the proliferative and oncogenic characteristics of B-Raf^{V600E}-expressing human tumor cell lines [14-16]. Recent evidence suggests that mutant B-Raf also causes chromosome instability via phosphorylation of Mps1; up-regulates GLUT-1 which enhances glucose metabolism in tumor cells; and causes DNA strand breakage and damage [17, 18]. Mutated B-Raf^{V600E} is also no longer repressed by SPRY2-an inhibitor of MAPK signaling in epithelial and fibroblast cell lines [19] which only binds to wild-type B-Raf to initiate the inhibition of MEK/ERK signaling, but will not bind to B-Raf^{V600E} [20]. Thus, specifically targeting the mutated B-Raf^{V600E} protein without inhibiting the wild-type B-Raf is one of the goals of anti-cancer drug development to improve anti-tumor cell activities while minimizing toxicity.

Table 1: Frequency of mutated B-Raf genes in various tumor locations

Primary Tumor Location	**Total Number of Samples**	**Number of Mutated Samples (%)**
Skin	10885	4550 (42%)
Thyroid	22408	8547 (38%)
Large Intestine	46321	5596 (12%)
Eye	526	2865 (12%)
Ovary	2022	236 (12%)
Small Intestine	137	10 (7%)
Biliary Tract	490	29 (6%)
Hematopoietic and Lymphoid Tissue	1645	107 (7%)
Prostate	1188	42 (4%)
Central Nervous System	1572	70 (4%)
Endometrium	848	30 (4%)
Soft Tissue	1191	30 (3%)
Breast	603	14 (2%)

Table 1: contd…

Lung	6261	135 (2%)
Upper Aerodigestive Tract	769	12 (2%)
Pancreas	563	10 (2%)
Oesophagus	203	3 (1%)
Cervix	451	5 (1%)
Liver	175	2 (1%)
Stomach	1128	11 (1%)

2. DRUGS TARGETING THE RAS/RAF/MEK/ERK PATHWAY CURRENTLY IN CLINICAL TRIAL

Several anti-cancer drugs targeting Raf or downstream MEK have been developed and are in various Phases of clinical trials (Table **2**), including PLX4032 Fig. (**1A**) (also known as Vemurafenib, RG7204, R7204 & RO5185426, specifically targets B-Raf^{V600E}), RAF265 Fig. (**1B**) (targets both Raf and vascular endothelial growth factor receptor [VEGFR]-2, sorafenib tosylate Fig. (**1C**) (targets multiple kinases), regorafenib (targets multiple kinases), XL281 (targets Raf kinases, structure not disclosed), and AZD6244 (Selumetinib) Fig. (**1D**) (specifically targets MEK).

Table 2: Clinical trials of B-Raf and downstream MEK inhibitors

NCT ID	Trial Name	Tumor Type	Drug Name (Target)	Trial Phase
Examples of Phase II Clinical Trials with Raf Inhibitors				
NCT01400451	Ph I/II Ipilimumab vemurafenib combo	Metastatic Melanoma	PLX4032 and Iplimumab (CTLA-4)	Phase I/II
NCT01512251	BKM120 combined with vemurafenib (PLX4032) in B-RafV600E/K mutant advanced melanoma	Metastatic Melanoma	PLX4032 and BKM120 (PI3K)	Phase I/II
NCT01286753	A study of RO5185426 (vemurafenib) in patients with metastatic or unresectable papillary thyroid cancer positive for the B-Raf V600 mutation	Papillary Thyroid Cancer	PLX4032	Phase II
NCT01378975	A study of vemurafenib in metastatic melanoma patients with brain metastases	Metastatic Melanoma	PLX4032	Phase II

Table 2: contd…

NCT01474551	Vemurafenib (R05185426) in poor performance status patients with unresectable locally advanced or metastatic melanoma harboring a V600EB-Raf mutation	Metastatic Melanoma	PLX4032	Phase II
NCT01495988	Trial of vemurafenib with or without bevacizumab in patients with stage IV B-RafV600 mutant melanoma	Metastatic Melanoma	PLX4032 with or without bevacizumab (VEGF)	Phase II
NCT01524978	A study of vemurafenib in patients with B-Raf V600 mutation-positive cancers	Solid tumors and multiple myeloma, except melanoma and papillary thyroid cancer	PLX4032	Phase II
NCT01352273	MEK162 and RAF265 in adult patients with advanced solid tumors harboring RAS or B-RafV600E mutations	Solid Tumors	RAF265 and MEK 162 (MEK1/2)	Phase I/II
NCT01336634	A Phase II study of the selective B-Raf kinase inhibitor GSK2118436 in subjects with advanced non-small cell lung cancer and B-Raf mutations	Non-Small Cell Lung Cancer	SB-590885	Phase II
Examples of Phase II Clinical Trials with MEK Inhibitors				
NCT01029418	AZD6244 and sorafenib in advanced hepatocellular carcinoma	Hepatocellular carcinoma	AZD6244 and Sorafenib (multiple kinase)	Phase I/II
NCT01242605	ABC-04 a study of cisplatin, gemcitabine and selumetinib in patients with advanced biliary tract cancer	Biliary tract cancer	AZD6244 and Cisplatin/Gemcitabine	Phase I/II
NCT01143402	Phase II randomized study of temozolomide vs. MEK inhibitor AZD6244 (selumetinib) in patients with metastatic uveal melanoma	Uveal melanoma	AZD6244 vs. Temozolomide	Phase II
NCT01160718	Fulvestrant with or without AZD6244 in treating patients with advanced breast cancer that progressed after aromatase inhibitor therapy	Breast cancer	AZD6244 with or without fulvestrant	Randomized Phase II
NCT01206140	Study of MEK inhibitor AZD6244 with vs. without temsirolimus in patients with metastatic, recurrent, or locally advanced unresectable soft tissue sarcomas	Sarcomas	AZD6244 and/or temsirolimus (mTOR)	Phase II

Table 2: contd…

NCT012 22689	Phase II study of MEK inhibitor AZD6244 and erlotinib hydrochloride for second-line treatment in patients with locally advanced or metastatic pancreatic adenocarcinoma	Pancreatic cancer	AZD6244 and erlotinib (EGFR)	Phase II
NCT012 39290	Phase II randomized study of selumetinib and erlotinib hydrochloride in patients with K-RAS wild type or mutant K-RAS advanced non-small cell lung cancer	Non-small cell lung cancer	AZD6244 and erlotinib (EGFR)	Rando mized Phase II
NCT012 56359	Docetaxel with or without AZD6244 in melanoma	Melanoma	AZD6244 with or without docetaxel	Phase II
NCT013 06045	Molecular profiling and targeted therapy for advanced non-small cell lung cancer, small cell lung cancer, and thymic malignancies	Lung and thymic cancer	AZD6244, erlotinib (EGFR), MK-2206 (Akt), lapatinib (ERBB2), or sunitinib (multiple kinase), based on molecular profiling of tumor	Phase II
NCT013 33475	MK-2206 and AZD6244 in Patients with advanced colorectal carcinoma	Colorectal carcinoma	AZD6244 and MK-2206 (Akt)	Phase II
NCT015 19427	Phase II randomized study of selumetinib with vs. without akt inhibitor MK2206 in patients with B-Raf V600E mutant stage III or IV melanoma who failed prior therapy with vemurafenib or dabrafenib	Melanoma	AZD6244 and/or MK2206 (Akt)	Rando mized Phase II
NCT005 51070	AZD6244 in treating woman with recurrent low-grade ovarian cancer	Ovarian cancer	AZD6244	Phase II
NCT015 53851	GSK1120212 in surgically resectable oral cavity squamous cell cancer	Oral squamous cell carcinoma	GSK1120212	Phase II
NCT013 62296	An open-label study of GSK1120212 compared with docetaxel in stage IV KRAS-mutant non-small cell lung cancer	Non-small cell lung cancer	GSK1120212 vs. docetaxel	Phase II

Table 2: contd…

NCT00920140	Open-label study to evaluate the safety, PK, and PD of MEK inhibitor GSK1120212 in subjects with relapsed or refractory leukemia's	Leukemia	GSK1120212	Phase II
NCT01251640	Combination with gemcitabine in advanced pancreatic cancer	Pancreatic cancer	BAY86-9766	Phase I/II
NCT01016483	Trial of gemcitabine with or without MSC1936369B in pancreatic cancer	Pancreatic cancer	AS703026 and/or gemcitabine	Phase I/II
NCT00957580	Trial of mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) inhibitor	Advanced hematologic malignancies	AS703026	Phase I/II
NCT01266967	A study of GSK2118436 in B-Raf mutant metastatic melanoma to the brain (Break MB)	Metastatic melanoma to the brain	SB-590885	Phase II
Examples of Phase III Clinical Trials with Sorafenib or Regorafenib				
NCT00111007	A treatment combination for patients with unresectable stage III or stage IV melanoma	Melanoma	Sorafenib tosylate with paclitaxel/carboplatin vs. paclitaxel/carboplatin	Phase III
NCT00492258	Phase III randomized study of sorafenib tosylate in patients with resected primary renal cell carcinoma at high- or intermediate-risk of relapse	Renal cell carcinoma	Sorafenib tosylate	Phase III
NCT00541021	Phase III randomized study of gemcitabine hydrochloride and sorafenib tosylate in patients with locally advanced or metastatic adenocarcinoma of the pancreas	Pancreatic cancer	Sorafenib tosylate and gemcitabine	Phase III
NCT01015833	Phase III randomized study of sorafenib tosylate with vs. without doxorubicin hydrochloride in patients with locally advanced or metastatic hepatocellular carcinoma	Hepatocellular carcinoma	Sorafenib tosylate and/or doxorubicin	Phase III
NCT01223027	Study of dovitinib vs. sorafenib in patients with metastatic renal cell carcinoma	Renal cell carcinoma	Sorafenib tosylate vs. dovitinib (VEGFR and FGFR)	Phase III
NCT01234337	Phase III trial comparing capecitabine in combination with sorafenib or placebo in the treatment of locally advanced or metastatic HER2-negative breast cancer	Metastatic breast cancer	Sorafenib tosylate and/or capecitabine	Phase III

Table 2: contd…

NCT01189903	Clinical evaluation - A Phase IIA proof of concept study of regorafenib (Bayer 73-4506) in biopsy-amenable asian colorectal cancer patients	Colorectal cancer	Regorafenib	Phase II/III
NCT01298570	Regorafenib+FOLFIRI vs. placebo+FOLFIRI as 2nd line Tx in K-RAS/B-Raf mutant metastatic colorectal cancer	Colorectal cancer	FOLFIRI (Leucovorin, 5-fluorouracil, irinotecan, and cetuximab) with or without regorafenib	Phase II

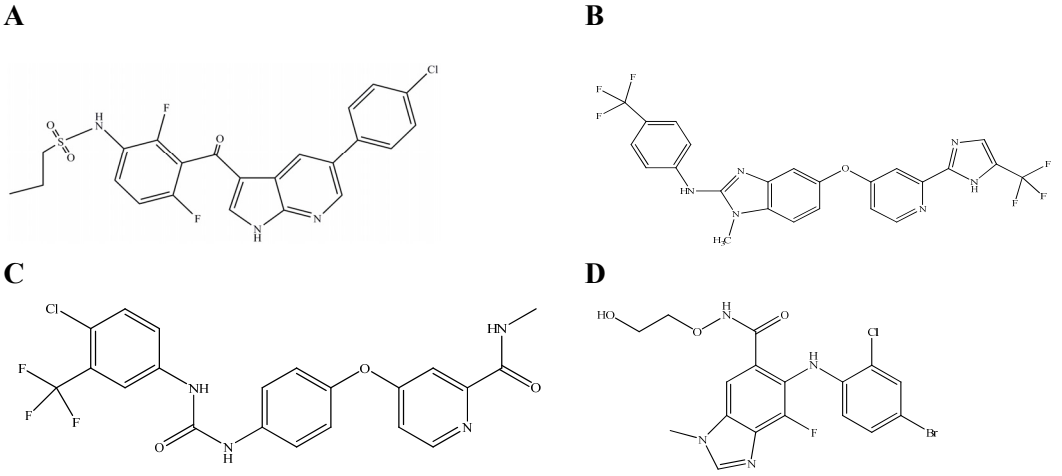


Figure 1: Chemical structures of (A) PLX4032, (B) RAF265, (C) Sorafenib, and (D) AZD6244.

PLX4032 (Vemurafenib, Plexxikon, Inc., Berkeley, CA) is a highly selective inhibitor of B-Raf kinase activity with an IC_{50} of 44nM against mutant B-Raf^{V600E}. The IC_{50} of PLX4032 is the lowest among a panel of 65 non-Raf kinases tested [21]. This small molecule was identified from Plexxikon's proprietary Scaffold-based Drug Discovery platform [22]. Most of the other kinases tested have a more than 100-fold higher IC_{50}, except Brk (also known as PTK6), which has an IC_{50} of 240nmol/L. PLX4032 specifically targets B-Raf^{V600E}; however, inhibition of tumor cell proliferation and MEK phosphorylation was only observed in colorectal tumor cell lines harboring B-Raf^{V600E} but not wild-type B-Raf [23]. Clinically, this compound has had profound effects on melanoma patients who previously had a very grim prognosis. Several Phase I-III clinical

trials have evaluated the efficacy of vemurafenib, especially in the setting of melanoma. In one hundred and thirty two patients with previously treated B-Raf^{V600E}-mutant metastatic melanoma enrolled in a multicenter Phase II trial of vemurafenib, overall response rate was 53% with a median duration of response of 6.7 months and median overall survival of 15.9 months [24]. A randomized Phase III trial also showed improved overall and progression-free survival in melanoma as a first-line agent when compared to dacarbazine in 675 patients with metastatic melanoma [25]. At 6 months, 84% of the vemurafenib group was still alive, compared to 64% of the dacarbazine group. This data led to FDA approval of vemurafenib for use in B-Raf^{V600E}-positive melanoma in 2011. While highly efficacious in melanoma, unfortunately, the same activity has not been observed in colorectal carcinoma. In a Phase I study of 21 patients with B-Raf^{V600E}-positive metastatic colorectal carcinoma, there was only one partial response and 4 minor responses [26]. Toxicity of vemurafenib is generally mild: The most common adverse events in the clinical trials were rash, nausea, diarrhea, arthralgia, photosensitivity, fatigue, and alopecia. However, paradoxical activation of the Ras pathway leads to formation of keratoacanthomas and squamous cell carcinomas in these patients-a significant side effect which requires routine screening [27].

RAF265 (CHIR-265; Novartis Pharmaceuticals, Basel, Switzerland), an orally bioavailable small molecule, is a potent inhibitor of Raf with a highly selective profile and is a derivative of benzazoles (Chiron, a subsidiary of Novartis) [28]. RAF265 binds and inhibits Raf kinases, which results in apoptosis and reduction in tumor proliferation. In addition, RAF265 inhibits VEGFR-2, thereby disrupting tumor angiogenesis [29]. A preclinical study found that RAF265 inhibits all three isoforms of Raf, as well as B-Raf^{V600E}, with high potency. RAF265's antitumor activity has also been seen in preclinical xenograft models [30]. In a Phase I first-in-human trial of RAF265 in 76 patients with advanced melanoma, overall response rate was 16% for patients with B-Raf mutations and 13% for wild-type/unknown mutations [31]. A major side effect was dose-limiting hematologic toxicity. Phase II trials are currently ongoing.

Sorafenib (BAY43-9006; Bayer Schering Pharma, Pittsburgh, PA) is a bi-aryl urea which was initially identified as an adenosine triphosphate competitive inhibitor of the C-Raf kinase (from now on referred to as the Raf1 kinase). *In vitro* biochemical

assays confirmed that sorafenib is a potent *in vitro* inhibitor of the Raf1 kinase (IC_{50} = 6nM) [32]. Sorafenib has also been shown to inhibit Raf1 and, thus, tumor cell proliferation and tumor growth in several human tumor xenograft models [33]. Subsequently, sorafenib was shown to have multikinase inhibition activities, which is likely responsible for sorafenib's clinical efficacy [32]. Sorafenib targets two kinase classes known to be involved in both tumor proliferation and angiogenesis [34]. These include the enzyme Raf kinase, a critical component of the Ras/Raf/MEK/ERK signaling pathway; and the VEGFR-2/platelet-derived growth factor receptor (PDGFR)-beta signaling cascade, thereby blocking tumor growth and angiogenesis. It also inhibits c-Kit and fibroblast growth factor receptor 1 (FGFR1) [35]. Sorafenib has been evaluated as a single-therapy agent and in combination with various chemotherapy drugs in a number of clinical trials [36-38]. In a study that compared sorafenib with placebo, treatment with sorafenib prolonged progression-free survival in patients with advanced clear-cell renal cell carcinoma for whom previous therapy had failed. Subsequently, sorafenib was approved by the U.S. Food and Drug Administration for the treatment of advanced renal cell carcinoma and advanced hepatocellular carcinoma and has since performed well in Phase III trials [39]. Over 200 clinical trials are currently ongoing, and a few studies are investigating the correlation between clinical response and B-Raf mutation status. Interestingly, the most clinically beneficial function of sorafenib arises from its anti-angiogenicity; the Raf inhibition seems less potent, as evidenced by Phase II and III trials in melanoma patients, where sorafenib had limited efficacy even when response was analyzed according to B-Raf mutation status [37, 40].

Regorafenib (BAY73-4506; Bayer Schering Pharma, Pittsburgh, PA) is a more potent derivative of sorafenib formed by adding a fluorine atom to the phenyl group. Similar to sorafenib, it inhibits multiple kinases, including VEGFR 1-3, TIE2, PDGFR, FGFR1, cKIT, RET, and B-Raf, with an IC_{50} ranging from 4 to 311nM [41]. In xenograft models, it inhibited tumor growth in glioblastoma, breast, renal cell, and colorectal carcinomas [41]. A recent Phase I trial demonstrated acceptable activity and toxicity in 53 patients with solid tumors, three of 47 patients achieved a response and the most common side effects were dermatologic manifestations, hypertension, and diarrhea [42]. In Phase II trials, regorafenib has performed well: In pretreated gastrointestinal stromal tumors (GIST), 19 of 22 patients were without

progression after 4 cycles [43]. In a Phase III trial in patients with metastatic colorectal cancer, a statistically significant benefit in progression-free survival (PFS) and overall survival (OS) was observed in the regorafenib group vs. supportive care, such that the control group was allowed crossover into regorafenib treatment [44]. Phase II and III trials are ongoing.

XL281 (Exelixis, San Francisco, CA) is an orally active small molecule with potential antineoplastic activity that specifically inhibits Raf kinases, including Raf1, B-Raf, and activated B-Raf^{V600E} [45]. XL281 has shown activity in tumor xenograft models [46]. A Phase I clinical trial concluded that XL281 had acceptable activity and toxicity in adult patients with solid tumors [47]; however, there are currently no open clinical trials evaluating this compound.

AZD6244 (ARRY-142886, Selumetinib; AstraZeneca, London, England) is an oral, highly selective allosteric inhibitor of MEK [48]. AZD6244 was the second MEK inhibitor to enter clinical trials after the first MEK inhibitor, CI-1040, demonstrated poor clinical efficacy. However, the encouraging safety profile of CI-1040 provided the momentum to search for more potent analogues [49]. AZD6244 is a benzimidazole derivative with reported nanomolar activity against the purified MEK1 enzyme [50]. Through a series of studies using preclinical cell cultures and animal models, it was shown that AZD6244 suppresses the growth of melanoma cells through the induction of cytostasis, but AZD6244 has a limited ability to induce apoptosis or block angiogenesis [51]. In a Phase I study in patients with advanced cancer, AZD6244 was well tolerated with demonstrable clinical activity [35], with rash being the most common dose-limiting toxicity. This prompted several Phase II studies which have now evaluated AZD6244. Overall, AZD6244 has displayed modest antineoplastic activity in iodine-refractory papillary thyroid cancer [52] and metastatic biliary cancer [53], while results in hepatocellular cancer patients were less robust [54]. In the papillary thyroid patient population, patients with B-Raf^{V600E} mutations had longer median PFS [52]. Phase II studies have also compared single-agent AZD6244 to other antineoplastics, and have found that AZD6244 is effective but not superior to standard chemotherapeutics. Compared to temozolomide in 200 advanced-stage melanoma patients [55], AZD2644 was active, but there was no difference in progression free survival between the two drugs. However, 5 of 6 partial responders to AZD6244 had B-Raf mutations. Compared to permextred in 84

heavily-pretreated patients with advanced non-small cell lung cancer, AZD6244 was again active, but there was no difference in disease progression between both groups [56]. Finally, in patients with advanced pancreatic cancer who had progressed through treatment with gemcitabine, there was no difference in overall survival in patients treated with AZD6244 compared to capecitabine [57]. Common side effects of AZD6244 in the above clinical trials included nausea, rash, peripheral edema, and diarrhea.

3. DERIVATIVES OF INITIAL LEAD COMPOUNDS IMPROVE SPECIFICITY AND POTENCY

Patents issued and patent applications published in recent years have identified numerous lead compounds that target Raf, MEK, or multiple tyrosine kinases [58-66]. Many of these compounds are derived from aromatic heterocyclic compounds, such as imidazole, quinazoline, phenethylamide, malonamide, and benzazoles Tables **3** and **4**. Using these compounds as the initial scaffolds for modifications, more potent inhibitors have been identified. For example, to improve the bioavailability and potency of CI-1040 [67], a derivative, PD0325901, was developed [68]. PD0325901 was derived by replacing the cyclopropylmethoxy group with a (*R*)-2,3-dihydroxypropoxy group and replacing the 2-chloro substituent with a 2-fluoro group on the second aromatic ring of CI-1040 Fig. (**2A**). Such small structural changes resulted in a more than 100-fold target potency as reflected by PD0325901's ability to inhibit both purified MEK, as well as cellular activation of MAPK, at concentrations in the nanomolar range. Moreover, the activity of PD0325901 has also been observed against a panel of B-Raf^{V600E} xenografts [69]. A Phase I trial investigated PD0325901 in 13 patients with metastatic melanoma, colorectal, or breast cancer at doses of 20 mg and 15 mg orally twice daily [70]. Although one patient had a complete response and 5 had stable disease, the study was terminated early due to an unexpected high incidence of musculoskeletal and neurological toxicity. However, another Phase I trial (NCT01347866) is examining a PI3K/mTOR inhibitor in combination with PD0325901 or irinotecan. Given its untoward neurologic side effects, modifications of PD0325901 have led to the development of two new more specific, stable compounds: RO4987655 (CH4987655; Hoffman-La Roche, Basel, Switzerland) and TAK-733 (Takeda Pharmaceutical, Osaka, Japan), both of which are less susceptible to hydrolyzation

than PD0325901. TAK-733 is a novel 5-phenylamino-8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione with a bicyclic pyridopyrimidinione core and a dihydroxypropane side chain. It is highly selective, performing in the nanomolar range with an IC_{50} of 3.2nM against MEK1/2, and antitumor activity in xenograft models of melanoma, non-small cell lung, breast, pancreatic, and colorectal carcinoma [71]. A Phase I study is underway evaluating TAK-733 in advanced nonhematologic malignancies (NCT00948467). RO4987655 was developed with a unique 3-oxo-[1,2]oxazinan-2-ylmethyl group at the 5-position, and inhibits MEK at an IC_{50} of 5nM. In xenograft models with colorectal, non-small cell lung, and pancreatic carcinomas, RO4987655 potently inhibited MEK activity [72].

Table 3: Recently issued patents

Patent Number	Title	Assignee
US7737152	6-Carboaryl-oxy-pyrazin-2-yl-carboaryl-amines and compositions comprising said compounds	The Wellcome Trust, Ltd.
US7772246	Pyrazole compounds as RAF inhibitors	Pfizer, Inc.
7795249	Certain pyrazoline derivatives with kinase inhibitory activity	Millenium Pharmaceuticals, Inc.
US7799827	Macrocyclic compounds useful as pharmaceuticals	Eisai Co., Ltd.
US7807672	Compounds that are ERK inhibitors	Schering Corporation
US7951819	Imidazo[4, 5-B]pyridin-2-one and oxazolo[4, 5-B] pyridin-2-one compounds and analogs thereof as cancer therapeutic compounds	Astex Therapeutics, Ltd.
US7968536	Heterocyclic compounds useful as RAF kinase inhibitors	Millenium Pharmaceuticals, Inc.
US7968554	Pyrazolo[3,4-d]pyrimidine derivatives	Hoffman La Roche, Inc.
US7994321	Substituted thieno[3,2-C]pyridine-7-carboxylic acid derivatives	Hoffman La Roche, Inc.
US8044049	Fused heterocyclic derivative and use thereof	Takeda Pharmaceutical Co., Ltd.
US8063066	MAPK/ERK kinase inhibitors	Takeda Pharmaceutical Co., Ltd.
US8076486	Heteroaryl-substituted arylaminopyridine derivatives as MEK inhibitors	Merck Sorono S.A.
US8110687	Bicyclic compounds with kinase inhibitory activity	Millenium Pharmaceuticals, Inc.

Table 3: contd…

US8119637	Substituted pyrrolo[2,3-b]pyrazines and methods for kinase modulation, and indications thereof	Plexxikon, Inc.
US8129394	Heteroaryl-substituted imidazole compounds and uses thereof	Novartis AG
US8129404	Compounds and uses thereof	Plexxikon, Inc.
US8143258	Benzothiazole compounds useful for Raf inhibition	Takeda Pharmaceutical Co., Ltd.
US8143271	Compounds and methods for kinase modulation, and indications thereof	Plexxikon, Inc.

Table 4: Recent patent applications.

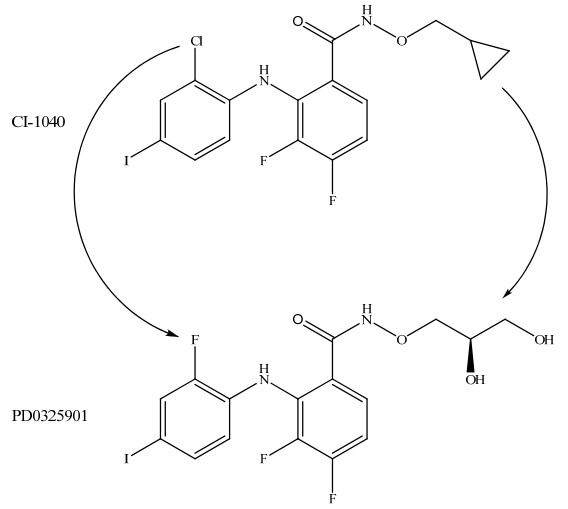
Patent Application Number	**Title**	**Target**	**Applicants**	**Reference**
US20110306625	Compounds and compositions as protein kinase inhibitors	B-Raf	Novartis, Inc.	Huang *et al.,* 2011 [122]
US20120053177	Compounds and methods for kinase modulation, and indications thereof	B-Raf	Plexxikon, Inc.	Ibrahim *et al.,* 2012 [58]
US20110003859	*N*- (6-Aminopyridin-3-yl) -3- (sulfonamido) benzamide derivatives as B-Raf inhibitors for the treatment of cancer	B-Raf	Array Bio Pharma, Inc.	2011 [61]
US2011051297	Substituted azaindoles	B-Raf	Concert Pharmaceuticals, Inc.	2011 [65]
US20110166191	3-(2-Amino-ethyl)-5-(3-cyclohexyl-propylidene)-thiazolidine-2,4-dione and its derivatives as multiple signaling pathway inhibitors and for the treatment of cancer	Dual Raf/PI3K	N/A	2011 [123]
WO2012036997	Fused pyrazole derivatives as novel ERK inhibitors	ERK	Schering Corporation	2011 [62]
WO2012030685	Indazole derivatives useful as ERK inhibitors	ERK	Schering Corporation	2011 [66]

Table 4: contd…

US20100240613	Pyrimidine compound and medical use thereof	MEK	Japan Tobacco, Inc.	Kawasaki *et al.,* 2010 [63]
US20110158971	Compositions comprising N3 alkylated benzimidazole derivatives as MEK inhibitors and methods of use thereof	MEK	Array Bio Pharma, Inc.	2011 [92]
US20100234435	Cycloalkylamino acid derivatives	Multiple kinases	Pfizer, Inc.	Bhattacharya *et al.,* 2010 [121]
US20110294806	Azaindole derivatives as kinase inhibitors	Multiple kinases	ARIAD Pharmaceuticals, Inc.	Qi *et al.,* 2011 [64]
US20110257165	Bicyclic pyrazoles as protein kinase inhibitors	Multiple kinases	Nerviano Medical Sciences S.R.I.	2011 [60]
US20100330069	Heterocyclic compounds and methods of use	Raf	TargeGen, Inc.	Wrasidlo *et al.,* 2010 [59]
US20100234394	Substituted benzimidazoles and methods of their use	Raf	Novartis, Inc.	Aikawa *et al.,* 2010 [90]
US20110118245	Raf kinase modulator compounds and methods of use thereof	Raf	Plexxikon, Inc.	2011 [119]
US20110257207	Raf inhibitors	Raf	Agennix USA, Inc.	2011 [120]
US20110110889	Raf inhibitor compounds and methods of use thereof	Raf	Array Bio Pharma, Inc.	2011 [124]

In continuing to improve specificity and circumvent undesirable neurologic side effects, BAY86-9766 (RDEA119, refametinib; Bayer Schering Pharma, Pittsburgh, PA) was developed. It binds noncompetitively to MEK1/2 adjacent to the Mg-ATP binding region and inhibits MEK1/2 activity at an IC_{50} of 19 and 47nM, respectively [73]. It inhibits colorectal carcinoma and melanoma tumor xenografts without neurologic penetration [73], and Phase I trials have confirmed safety. A Phase II trial evaluating refametinib in combination with gemcitabine in pancreatic cancer patients is ongoing (NCT01251640).

A



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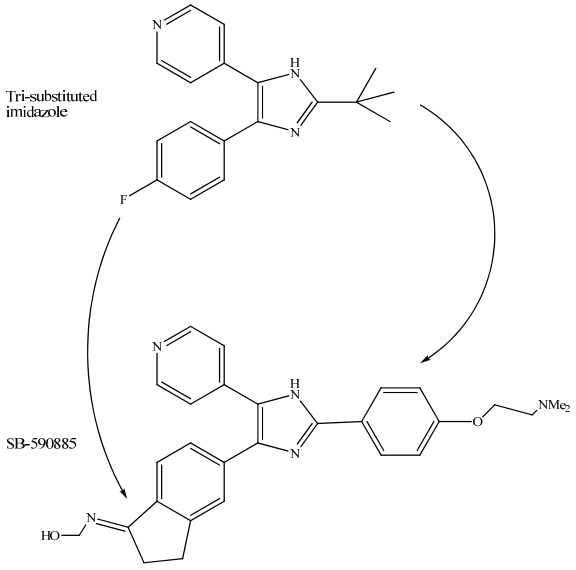

Figure 2: Derivatives of lead compounds with enhanced target potency. (A) PD0325901 is derived by replacing the cyclopropylmethoxy group with a (*R*)-2,3-dihydroxypropoxy group and replacing the 2-chloro substituent with a 2-fluoro group on the second aromatic ring of CI-1040. (B) SB-590885 is a triarylimidazole derivative with a 2,3-dihydro-1H-inden-1-one oxime substituent from a derivative of imidazole.

A derivative of imidazole was identified from the SmithKline Beecham compound bank as a submicromolar inhibitor of B-Raf [74, 75]. Structural

modification of imidazole resulted in the formation of GSK2118436 (SB-590885, dabrafenib; SmithKline Beecham, Middlesex, England), which is a triarylimidazole derivative with a 2,3-dihydro-1*H*-inden-1-one oxime substituent Fig. (**2B**). Is a potent and extremely selective inhibitor of the B-Raf kinase in the nanomolar range [76]. Unlike the multikinase inhibitor BAY43-9006, dabrafenib seems to target cells that express oncogenic B-Raf [77]. A Phase I trial displayed encouraging results in melanoma patients, with 24 of 32 patients with a partial response and 2 patients exhibiting a complete response. Median progression-free survival was over 7 months [78]. Perhaps most excitingly, preliminary results from a Phase II clinical trial (NCT01266967) in metastatic melanoma with brain involvement revealed a 90% response rate. Clinical trials are now examining this compound in the setting of lung and thyroid cancer, and B-Raf mutant-positive tumors.

Further modification of GSK2118436 has also resulted in the identification of a series of furan-based derivatives with enhanced central nervous system penetration and B-Raf inhibitory activity [79, 80]. Such B-Raf inhibitors may be of value in the treatment of specific types of pediatric gliomas (*e.g.*, low-grade astrocytomas), as MAPK pathway activation was discovered in low-grade astrocytomas as a result of *B-Raf* gene duplication [81]. GSK1120212 (Trametinib) was created with a pyrido-pyrimidin core and an N-phenylacetamide substituent. It inhibits MEK1/2 enzymatic activity at an IC_{50} of 10nM, with a particularly long circulating half-life and p-ERK suppression for over 24 hours. It also displayed potent activity in melanoma and colorectal cancer xenograft models [82], which has led to several Phase I trials confirming its safety and efficacy. The first Phase I trial investigated trametinib in 84 patients, and in 20 evaluable melanoma patients of known B-Raf status there were 5 partial responses, three of whom have stayed $\geq$ 30 weeks on study [83]. There are now several Phase II studies.

AS703026 (Merck KGaA, Darmstadt, Germany) is yet another imidazole derivative, with an *N*-substitution and the chemical formula (*N*-[(2*S*)-2,3-dihydroxypropyl]-3-[(2-fluoro-4-iodophenyl)amino] isonicotinamide hydrochloride). Preclinical testing yielded effective and specific MEK1/2 inhibition in multiple myeloma and colorectal tumors [84, 85], which prompted several clinical trials. A

Phase I trial in advanced solid tumors with AS703026 resulted in two confirmed partial responses prior to reaching the maximum tolerated dose [86]. Common side effects were asthenia, diarrhea, skin reactions, nausea, and vomiting. There are currently 6 Phase I or combination Phase I/II trials open evaluating the compound as a single agent or in combination with other antitumor drugs.

By using a structure-guided discovery approach [22], a small-molecule 7-azaindole was found to bind the ATP-binding site of kinases with weak affinity. Subsequently, a group of mono- and disubstituted 7-azaindoles with increased affinity was synthesized. Screening of these compounds revealed a set of compounds containing a difluoro-phenylsulfonamide substructural motif that demonstrated excellent potency for oncogenic B-Raf. Co-crystallization of these compounds with engineered forms of B-Raf^{V600E} and wild-type B-Raf provided co-crystal structures for subsequent optimization chemistry, which led to the discovery of propane-1-sulfonic acid [3-(5-chloro-1*H*-pyrrolo[2,3-*b*]pyridine-3-carbonyl)-2,4-difluoro-phenyl]-amide (PLX4720). PLX4720 inhibits B-Raf^{V600E} kinase activity *in vitro*, with an IC_{50} of 13 nM, which is 10-fold lower than the concentration needed to inhibit wild-type B-Raf. Furthermore, in B-Raf^{V600E}-dependent tumor xenograft models, oral PLX4720 significantly reduced tumor growth and even caused tumor regression, without evidence of toxicity [87]. PLX4032 is a structurally distinct analog of PLX4720. PLX3603, another derivative of PLX4032, is under evaluation in a Phase I clinical trial.

MEK162 (ARRY162, Novartis Pharmaceuticals, Basel, Switzerland) is another new oral MEK inhibitor, initially developed for rheumatoid arthritis, which functions through noncompetitive allosteric inhibition against MEK1/2 (IC_{50} 12nM) and ERK (IC_{50} 11nM), as well as IL-1, TNF, and IL-6, with activity against pancreatic, colorectal, and non-small cell lung carcinoma, as well as fibrosarcoma [88]. Phase I studies have been completed with tolerable efficacy and toxicity, and several Phase I/II studies are examining its efficacy in combination with other targeted agents (AMG479, an IGF-1R inhibitor, NCT01562899; RAF265, NCT01352273; BEZ235, a PIK/mTOR inhibitor, NCT01337765). Another Phase II trial is investigating MEK162 as a single agent in melanoma (NCT01320085).

ARQ 736 (ArQule, Woburn, Massachusetts) is an allosteric inhibitor which specifically targets B-Raf^{V600E} kinase and functions at nanomolar concentrations. It is under investigation in humans in NCT01225536, a Phase I dose escalation study with ARQ 736, in patients with advanced solid tumors harboring B-Raf and/or NRAS mutations.

Other recently-developed inhibitors currently in Phase I trials include GDC-0879 (B-Raf inhibitor; Genentech, San Francisco, CA), GDC-0623 (MEK1/2 inhibitor; Genentech, San Francisco), AZD8330 (MEK1/2 inhibitor; AstraZeneca, London, England), RO5126766 (dual Raf and MEK1/2 inhibitor; Hoffman La Roche, Basel, Switzerland), RO5212054 (B-Raf inhibitor, Hoffman La Roche, Basel, Switzerland), RO5068760 (MEK inhibitor, Hoffman La Roche, Basel, Switzerland), and PD318088 (MEK inhibitor, Pfizer, New York, New York).

4. CURRENT & FUTURE DEVELOPMENTS

Targeting the Ras/Raf/MEK/ERK cascade has provided novel opportunities for the development of new anti-cancer drugs that are less toxic than conventional chemotherapeutic drugs. Several promising compounds have been developed to inhibit the activities of B-Raf, MEK, or multiple kinases Fig. (**3**). Continued efforts aimed at structural optimization of the chemical scaffolds of compounds such as imidazole and quinazoline have resulted in more potent inhibitors in nanomolar or even subnanomolar ranges [89-92]. Multikinase inhibitors or B-Raf-specific inhibitors can have been developed from similar chemical scaffolds. For example, derivatives of quinazoline may have multiple tyrosine kinase [93] or B-Raf-specific [94] inhibition activities. (E)-α-benzylsulfonyl chalcone derivatives have also been identified as B-Raf inhibitors [95].

While MEK inhibitors harbor anticancer activity, they have generally not outperformed standard chemotherapy in Phase II and III trials. However, more specific compounds which preferentially target B-Raf mutations, such as SB-590885 and PLX4032, have had a profound effect on melanoma patients, extending lifespan in B-Raf^{V600E}-positive patients by an average of seven months [24]. Targeting specific oncogenic mutated kinases will theoretically allow specific inhibition or elimination of tumor cells, depending on the mutated

kinases, without introducing too much toxicity in the subjects, since the normal physiological function of wild-type kinases provides an essential role in normal regulation of cell growth.

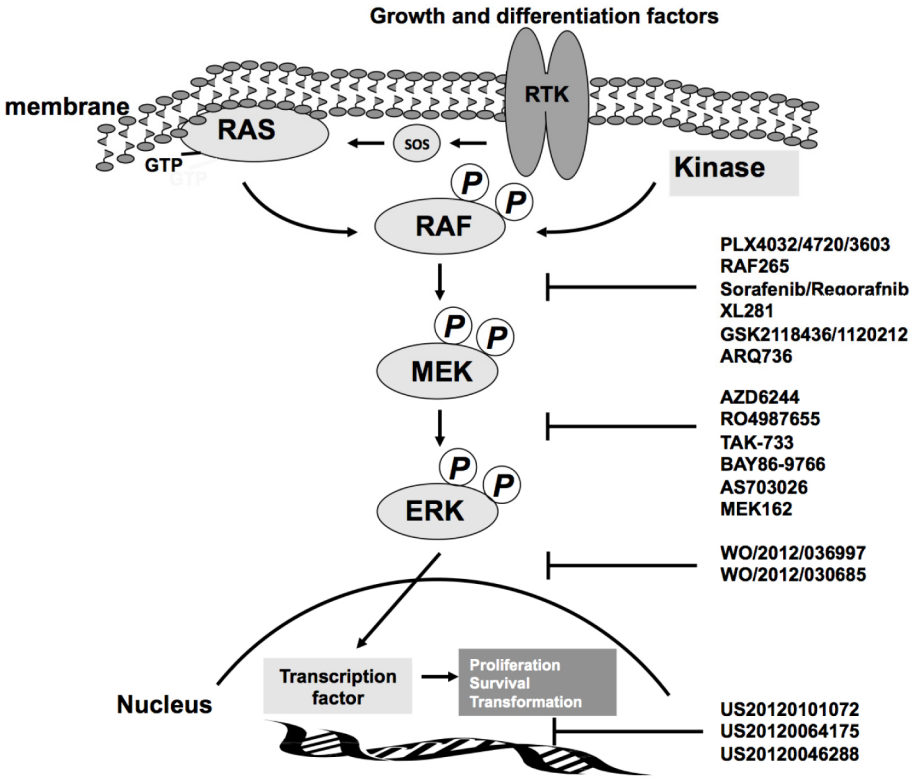

Figure 3: Intracellular signaling pathways that mediate ERK activation and are targeted by anti-cancer drugs currently in development.

However, the clinical success of sorafenib and other multikinase inhibitors has provoked a debate regarding the pros and cons of highly specific vs. broadly specific kinase inhibitors [96]. Indeed, drug resistance to B-Raf inhibitors has emerged, stemming from both intrinsic and extrinsic pathways. Resistance has been associated with increased Akt signaling in the presence of PTEN loss [97], as well as increased insulin-like growth factor-1 (IGF-1) signaling [98]. On the other hand, resistant mechanism to MEK inhibitors involves the amplification or up-regulation of B-Raf or KRAS has also been found [99]. Logically, attention has turned towards co-targeting multiple pathways in an effort to improve

efficacy. *In vitro,* increased Akt signaling and B-Raf inhibitor resistance were attenuated with the combination of a PI3K and B-Raf inhibitor [97]. Similarly, in the case of colorectal carcinoma, where B-Raf^{V600E} inhibition was less effective than in melanoma, Prahallad and colleagues [100] discovered that dual inhibition with PLX4032 and an EGFR inhibitor (cetuximab, gefitinib, or erlotinib), overcame B-Raf-mediated resistance in colorectal carcinoma cell lines. Co-targeting MEK, the IGF-1 receptor, and PI3K in combination with B-Raf inhibition has also overcome resistance preclinically [101]. Indeed, several patents combining MEK and B-Raf inhibition with other agents have recently emerged [102-105], as have clinical trials. Preliminary results from a Phase I/II trial combining the MEK 1/2 inhibitor GSK1120212 with the B-Raf inhibitor GSK2118436 in patients with stage IV previously-untreated melanoma revealed an objective response rate of 77%, and a decrease in the incidence of keratoacanthomas [106].

Multikinase inhibitors such as sorafenib and regorafenib are therefore attractive owing to their multitarget activity. Fortuitously, sorafenib was found to have activity against multiple protein kinases, yet the original goal in developing sorafenib was to identify a Raf inhibitor. It remains uncertain whether Raf inhibition alone provides adequate clinical efficacy. Therefore, future clinical trials of more specific and potent Raf kinase inhibitors are warranted. The clinical success of highly selective protein kinase inhibitors, in particular monoclonal antibody-based drugs (e.g., trastuzumab and bevacizumab) [107, 108], demonstrates that there is clinical value for both highly selective and multiple-target inhibitors.

Along the same lines, additional interest has developed in heat shock protein 90 (Hsp90) inhibitors in targeting B-Raf and attenuating resistance. Hsp90 is a "chaperone" molecule for proteins such as HER2, EGFR, mutant ER, HIF1α, Raf-1, AKT and mutant p53, the inhibition of which blocks several signaling pathways [109]. It also specifically binds mutant but not wild-type B-Raf, and early evidence suggests that Hsp90 inhibitors may overcome drug resistance [110]. While a clinical trial with the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) in melanoma patients yielded no objective clinical responses [111], novel non-benzoquinone small molecule Hsp90

inhibitors may prove more effective with their improved solubility and specificity [110]. Accordingly, several patents exploiting Hsp90 inhibition have surfaced in the past two years [112-114].

Patient selection also plays an important role in targeted therapeutics; patients with a B-Raf^{V600E} mutation are more likely to derive clinical benefit than those with wild-type B-Raf, therefore mutation analysis is an important component of screening prior to clinical trial entry. Additional new patents are aimed at determining which patients will respond to therapy [115, 116].

Important advances have been achieved in expanding our knowledge of how to make highly potent and selective MAPK pathway inhibitors. More drugs are currently in the pipeline, such as GDC-0879, GDC-0623, AZD8330, RO5126766, RO5212054, RO5068760, and PD318088. These drugs all promise increased potency and specificity, with less toxicity. The clinical oncology field will be anxious to evaluate these and other B-Raf and MEK inhibitors, and other MAPK inhibitors, including those targeting ERK, which were reported in recent patent applications [66, 117-124]. Although there is still a paucity of published data, the novel ERK inhibitors AEZS-131 (Æterna Zentaris, Frankfurt, Germany) and SCH772984 (Bayer Schering Pharma, Pittsburgh, PA) are currently in preclinical evaluation. Determining which cancer patients will receive the most benefit and with what regimens or combinations of inhibitors targeting multiple pathways to overcome drug resistance will be a challenge as we move forward to more individualized cancer therapies.

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CONFLICT OF INTEREST

The authors confirm that this chapter content has no conflict of interest.

DISCLOSURE

The chapter submitted for Patent eBook Series "Topics in Anti-Cancer Research", Volume 2 is an update of our article "Recent Developments in Anti-Cancer Agents Targeting the Ras/Raf/MEK/ERK Pathway", published in the journal 'Recent Patents on Anti-Cancer Drug Discovery', Volume 4, Number 1, January 2009, Page 28 to 35 with additional text and references.

ABBREVIATIONS

AP-1 = Activator protein 1 (JUN)

c-Myc = Cellular myelocytomastosis oncogene

COSMIC = Catalogue of somatic mutations in cancer

b.i.d. = "Bis in die" which Latin means twice a day

ERK = Extracellular signal-regulated kinase

ETS-1 = Erythroblastosis virus E26 oncogene homolog 1

GTP = Guanosine-5′-triphosphate

IC_{50} = Inhibition concentration which is required for 50% inhibition *in vitro*

MAPK = Mitogen-activated protein kinase

MEK = MAPK/ERK kinase 1

NF-κB = Nuclear factor-kappaB

nM = Nanomolar

PDGFR = Platelet-derivated growth factor receptor

Raf1 = Murine leukemia viral oncogene homolog 1

B-Raf = V-Raf murine sarcoma viral oncogene homolog B1

KRAS = V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog

SPRY2 = Sprouty homolog 2

VEGFR-2 = Vascular endothelial growth factor receptor-2

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