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TARGETING ONCOGENIC BRAF IN HUMAN CANCER

Christine Pratilas, **Feng Xing**, and **David Solit**
Memorial Sloan-Kettering Cancer Center, New York, NY 10065

Abstract

MAPK pathway activation is a frequent event in human cancer and is often the result of activating mutations in the BRAF and RAS oncogenes. BRAF missense kinase domain mutations, the vast majority of which are V600E, occur in approximately 8% of human tumors. These mutations, which are non-overlapping in distribution with RAS mutations, are observed most frequently in melanoma but also in tumors arising in the colon, thyroid, lung and other sites. Supporting its classification as an oncogene, ^{V600E}BRAF stimulates ERK signaling, induces proliferation and is capable of promoting transformation. Given the frequent occurrence of BRAF mutations in human cancer and the continued requirement for BRAF activity in the tumors in which it is mutated, efforts are underway to develop targeted inhibitors of BRAF and its downstream effectors. These agents offer the possibility of greater efficacy and less toxicity than the systemic therapies currently available for tumors driven by activating mutations in the MAPK pathway. Early clinical results with the BRAF-selective inhibitors PLX4032 and GSK2118436 suggest that this strategy will prove successful in a select group of patients whose tumors are driven by oncogenic BRAF.

INTRODUCTION

Over 25 years have passed since oncogenic RAS was first identified as the transforming factor in the Harvey and Kirsten strains of the Mouse Sarcoma Virus (Chang et al. 1982; Der et al. 1982; Malumbres and Barbacid 2003; Shimizu et al. 1983). Homologous mutations were later identified in a broad range of human cancers including tumors of the pancreas, colon and lung. RAS mutations, single amino acid missense mutations most commonly at residues G12, G13 or Q61, impair GTP hydrolysis and thus promote formation of constitutively activated GTP-bound RAS. RAS can also be activated in human tumors as a result of upstream activation of receptor tyrosine kinases or by loss of function of the NF-1 tumor suppressor. Activated RAS promotes transformation through its downstream effectors, the best studied of which include the RAF proteins, PI3'-kinases and RalGEFs. These downstream effectors contain a RAS-binding domain, which interacts with the core-effector domain of GTP-bound RAS. RAS binding induces effector activation through alterations in effector localization, intrinsic catalytic activity or by facilitating complex formation with other signaling components (Repasky et al. 2004).

Oncogenic RAS through activation of RAF proteins induces constitutive activation of the classical mitogen activated protein kinase (MAPK) cascade. The RAF proteins (B-RAF, C-RAF and A-RAF) are serine/threonine protein kinases that phosphorylate and thus activate mitogen-activated protein kinase (MAPK/ERK) kinase 1 and 2 (MEK1/MEK2), which in turn phosphorylate and activate extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) (Catling et al. 1995; Moodie et al. 1993). ERK regulates gene expression by phosphorylating several nuclear transcription factors (i.e. ets, elk, and myc) or indirectly by targeting other intracellular signaling molecules (p90-RSK and others).

SOMATIC BRAF MUTATIONS IN HUMAN TUMORS

Somatic point mutations in BRAF were first reported in 2002, and occur in approximately 8% of human tumors, most frequently in melanoma, colorectal and thyroid cancers (Davies et al. 2002; Gorden et al. 2003) **(Table 1)**. BRAF mutations are found, with rare exception, in a mutually exclusive pattern with RAS mutations, suggesting that these genetic alterations activate common downstream effectors of transformation. In tumors, BRAF mutations are found clustered within the P-loop (exon 11) and activation segment (exon 15) of the kinase domain. A single point mutation, a glutamic acid for valine substitution at residue 600 (V600E, initially designated V599E) within the activation segment of the kinase domain, accounts for approximately 90% of cases (Brose et al. 2002; Davies et al. 2002). Structural analysis of the V600E mutation suggests that it disrupts an interaction between the P-loop and the activation segment, which normally locks the kinase in the inactive conformation (Wan et al. 2004). In functional studies, the majority of BRAF mutations identified in human tumors exhibit elevated kinase activity compared to the wild-type protein (Wan et al. 2004). Several BRAF mutations, however, demonstrate reduced kinase activity *in vitro* (designated as low-activity mutants). These low-activity mutants activate ERK indirectly through the formation of C-RAF/B-RAF heterodimers (Wan et al. 2004).

The high frequency of BRAF mutations in human cancer suggests that BRAF functions as an oncogene in the tumors in which it is mutated. In cell culture studies, mutant forms of BRAF are capable of inducing transformation of NIH-3T3 cells (Davies et al. 2002). Expression of ^{V600E}BRAF in non-transformed melanocytes also promotes the ability of these cells to form tumors in nude mice (Wellbrock et al. 2004). Conversely, BRAF suppression by RNAi in ^{V600E}BRAF mutant models induces growth arrest and apoptosis and slows tumor growth in xenograft models (Hingorani et al. 2003; Hoeflich et al. 2006). Activating BRAF mutations are, however, present in the majority of melanocytic nevi, benign skin lesions that rarely progress to melanoma (Pollock et al. 2003). Furthermore, transfection of mutant BRAF into non-transformed human melanocytes has been shown to induce p16 expression, cell cycle arrest and senescence (Michaloglou et al. 2005). As most melanomas are deficient in p16, these data suggest that concurrent inactivation of p16 may be one of several alterations that cooperate with oncogenic BRAF to promote melananomagenesis (Bennett 2003; Gray-Schopfer et al. 2006; Sviderskaya et al. 2003). Similarly, BRAF mutations are common in colonic polyps suggesting that BRAF mutation in colorectal cancer is an early lesion that requires additional cooperative events to achieve transformation. Several candidate cooperative genetic alterations have been identified in melanoma, including *MITF* amplification and mutation and/or deletion of the tumor suppressor genes *PTEN, TP53* and *CDKN2A* (Dankort et al. 2009; Dankort et al. 2007; Garraway et al. 2005).

Studies in genetically engineered zebrafish and mouse models highlight the requirement for cooperative genetic events in ^{V600E}BRAF-driven melanomagenesis. In zebrafish, TP53 inactivation cooperates with ^{V600E}BRAF to induce melanocyte transformation (Patton et al. 2005). Zebrafish engineered to express ^{V600E}BRAF develop melanocytic nevi, whereas expression of ^{V600E}BRAF in TP53 deficient zebrafish results in formation of invasive tumors resembling those of human melanoma. Similarly, melanocyte-specific expression of ^{V600E}BRAF in mice results in melanocytic hyperplasia that fails to progress to invasive melanoma (Dankort et al. 2009). In the setting of a PTEN null background, melanocyte-specific ^{V600E}BRAF expression induces melanoma formation with 100% penetrance in genetically engineered mice. In melanocytes, loss of IGFBP7 may also allow for escape from BRAF mediated senescence (Wajapeyee et al. 2008). Analogous results have also been observed in lung tissue, where expression of mutant BRAF at physiological levels in mice was associated with the development of benign lung tumors that only rarely progressed to

invasive adenocarcinoma (Dankort et al. 2007). Loss of Ink4a/Arf and TP53 function, however, promoted cancer progression in this model (Dankort et al. 2007). In summary, these data suggest that multiple genetic changes likely cooperate with oncogenic BRAF to induce transformation and progression in human cancers. The extent to which lineage determines the complement of these additional genetic alterations remains unknown but may be critical. Whether any of these concurrent genetic alterations leads to a reduced requirement for continued BRAF and MEK activity for tumor maintenance also remains to be determined.

DISABLING PHYSIOLOGIC FEEDBACK AS A REQUIREMENT FOR RAS-MAPK ACTIVATION

Physiologic activation of RAS/RAF signaling is balanced by inhibitory regulators of the pathway which include the sprouty proteins, the MAP kinase phosphatases (DUSPs), KSR-1 and RKIP (Morrison and Cutler 1997), and by scaffolding proteins such as 14-3-3 which regulate RAF cellular localization and stability (Dougherty and Morrison 2004; Morrison 1994). Pathway activity is also regulated by cross-talk with parallel signaling pathways, such as by AKT phosphorylation of inhibitory sites on RAF (Zimmermann and Moelling 1999) and through PI3K-dependent feedback (Carracedo et al. 2008). Under physiologic conditions, activation of the MAPK pathway is balanced by inhibitory signals, which dampen or limit the duration of pathway activity. In tumor cells, this normal feedback regulation is often disabled through mutation or decreased expression of feedback regulators thus allowing for unhindered pathway activation.

Sprouty proteins, encoded by one of four SPRY genes (*SPRY 1-4*), negatively regulate RAS activity and may have direct inhibitory effects on RAF, by blocking its activation by protein kinase C-δ (PKCδ) (Kim and Bar-Sagi 2004; Sasaki et al. 2003; Yusoff et al. 2002). The inducible expression of *SPRY* family members by ERK activation follows the classic pattern of a negative feedback loop whereby the expression of negative feedback regulator is controlled by the signaling pathways that it ultimately regulates (Hanafusa et al. 2002). The idea that disruption of this negative feedback loop is a prerequisite for sustained pathway activation is supported by the observation that sprouty expression is suppressed in a variety of cancers. For example, inactivation or loss of sprouty expression has been reported in breast, hepatocellular, lung, and prostate cancers, suggesting that activation of the RAS/MAPK pathway mediated in part by disruption of its normal physiological feedback may play a role in the development of these cancers (Fong et al. 2006; Lee et al. 2008; Lo et al. 2004; Sutterluty et al. 2007).

In contrast, in melanoma, *SPRY2* expression is higher in cells harboring the ^{V600E}BRAF mutation compared to cells that are wild-type for BRAF (Tsavachidou et al. 2004). Using an unbiased approach to identify MEK-ERK dependent transcriptional targets in ^{V600E}BRAF melanoma cells, *SPRY2* transcription (along with other feedback regulators) was found to be profoundly and rapidly downregulated in response to MEK inhibition. Additionally, *SPRY2* expression is significantly higher in cells harboring a BRAF mutation compared to cells in which the MAPK pathway is activated by receptor tyrosine kinases (Pratilas et al. 2009). This seemingly paradoxical overexpression of feedback regulators of the pathway in the setting of high pathway activation in ^{V600E}BRAF tumors can be attributed to the inability of SPRY2 to inhibit ^{V600E}BRAF activity (Brady et al. 2009).

MAP kinase phosphatases (MKPs, or DUSPs) recognize dually phosphorylated proteins at threonine/tyrosine residues in a consensus –pTXpY- motif, found in several MAPK family members including ERK, SAPK/JNK, and p38 MAPK. Analogous to the loss of expression of sprouty proteins, loss of DUSP6 function may contribute to pancreatic cancer progression

(Furukawa et al. 2005; Furukawa et al. 2003) and MAPK pathway activation in endometrial cancer (Ogawa et al. 2005). In melanomas with ^{V600E}BRAF mutation, the upstream feedback mediated by sprouty proteins is disrupted as outlined above whereas the downstream feedback at the level of ERK, mediated by the MAP kinase phosphatases, remains intact. This pattern of feedback deregulation in ^{V600E}BRAF cancer cells leads to steady state levels of phosphorylated ERK that are not strikingly high despite high levels of MEK phosphorylation and ERK pathway output. Furthermore, these data provide a mechanistic basis for the lack of correlation between phosphorylated ERK expression and MAPK pathway output and suggest that levels of phosphorylated ERK should not be used as a predictive biomarker in clinical trials of RAF and MEK inhibitors.

MAPK pathway activity is regulated not only by transcriptional targets of ERK but also by direct phosphorylation events. CRAF activation is regulated by its phosphorylation at S338 and other activating sites (Chong et al. 2001). CRAF also contains six ERK-dependent phosphorylation sites through which its activity is negatively regulated (S29, S43, S289, S296, S301, and S642) (Dougherty et al. 2005). In support of this, CRAF activation of MEK has been observed following treatment with MEK inhibitors (Alessi et al. 1995; Friday et al. 2008; Pratilas et al. 2009), in the setting of impaired ERK activation by dominant negative kinase suppressor of RAS (KSR) (Therrien et al. 1996), and in cells overexpressing IMP (Matheny et al. 2004). Together, these findings suggest a critical role for ERK signaling in the attenuation of CRAF activity. They also suggest that the clinical activity of selective inhibitors of BRAF may be attenuated by relief of feedback inhibition of CRAF. Consistent with this possibility, overexpression of CRAF has been demonstrated as a mechanism of acquired resistance to the selective RAF inhibitor AZ628 (Montagut et al. 2008).

MEK KINASE INHIBITORS

Several strategies for inhibiting MAPK signaling are now being tested as cancer therapies **(Table 2)**. These include selective inhibitors the RAF and MEK kinases and inhibitors of Hsp90 chaperone function. We will focus first on selective inhibitors of the MEK kinases. CI-1040 (PD184352, Pfizer Oncology) was the first selective small molecule inhibitor of MEK to advance into clinical testing (Sebolt-Leopold et al. 1999). CI-1040 is non-ATP-competitive and inhibits MEK activation by binding to a pocket adjacent to the ATP binding site (Ohren et al. 2004). CI-1040 is highly selective for MEK1 and MEK2, with the only other known target being MEK5, whose inhibition occurs at a 100-fold greater concentration than that required to inhibit MEK1/2. Cell lines with BRAF mutation are selectively sensitive to CI-1040 (Solit et al. 2006). In BRAF mutant tumors, MEK inhibition results in downregulation of cyclin D1, upregulation of p27, hypophosphorylation of RB and growth arrest in G1 (Solit et al. 2006). MEK inhibition also induces differentiation and senescence of BRAF mutant tumors and apoptosis in some, but not all, ^{V600E}BRAF mutant models (Solit et al. 2006; Solit et al. 2007). Anti-tumor activity was observed in the phase I trial of CI-1040, with one patient demonstrating a partial response and 25% of patients treated exhibiting prolonged stable disease (Lorusso et al. 2005). Clinical activity was, however, disappointing in the phase 2 setting and therefore development of CI-1040 was halted in favor of a more potent second-generation compound (Rinehart et al. 2004). Notably, the clinical development of CI-1040 was initiated prior to the identification of BRAF mutations in human cancer and therefore this agent was not tested in tumor types, including melanoma, with the highest reported frequency of BRAF mutation.

PD0325901 (Pfizer Oncology) is a second-generation allosteric inhibitor of MEK1 and MEK2. PD0325901 is 50-100-fold more potent than CI-1040, exhibits improved oral bioavailability and increased metabolic stability (Brown et al. 2007; Sebolt-Leopold and Herrera 2004). BRAF mutant cell lines are also selectively sensitive to PD0325901 (Solit et

al. 2006). In pharmacodynamic studies, the drug reduced the expression of phosphorylated ERK by more than 70% relative to baseline in the tumors of seven out of nine patients tested. Three patients with melanoma had RECIST (Response Evaluation Criteria in Solid Tumors) responses with PD0325901 on the phase 1 clinical trial, but development of this agent beyond the phase 1 setting has not been pursued due to concerns over neurological toxicity (Wang et al. 2007).

AZD6244 (AstraZeneca) is also an ATP non-competitive, allosteric inhibitor of MEK1 and MEK2. AZD6244 recently completed phase 2 testing in melanoma, lung and colorectal cancers (Dummer et al. 2008; Lang et al. 2008; Tzekova et al. 2008). In a phase 2 trial of AZD6244 in patients with melanoma, 200 patients were randomized to AZD6244 (100 mg BID) or temozolamide (200 mg/m^2 for 5 days, q28days) (Dummer et al. 2008). Antitumor activity with AZD6244 was observed on the trial with partial responses in six patients, five of whom had tumors that expressed ^{V600E}BRAF. There was, however, no significant difference between the treatment arms for the primary endpoint of progression free survival. In the NSCLC trial, MEK inhibition with AZD6244 was compared with pemetrexed (Tzekova et al. 2008). Two partial responses were observed in both arms, with no difference between the agents in time to progression. Similarly, in colon cancer, AZD6244 was compared with capecitabine with no difference observed between the two arms in time to progression (Lang et al. 2008). In summary, the three randomized phase 2 trials of AZD6244 in melanoma, lung and colorectal cancer suggested that activity with this agent was comparable but not superior to disease-specific standard chemotherapy. In each case, enrichment for those patients most likely to respond to MEK inhibition as predicted by the preclinical data was not incorporated into the trial designs. On the basis of these clinical results and the preclinical data suggesting a correlation between BRAF mutation status and sensitivity to MEK inhibition, ongoing studies are testing the efficacy of AZD6244 in patients with mutational activation of the pathway (BRAF and RAS mutant only).

The importance of pretreatment stratification is highlighted by the promising results recently reported with the MEK inhibitor GSK1120212. GSK1120212 is a potent, non-ATP competitive MEK inhibitor (IC_{50}s for MEK1 and MEK2 of 0.7 and 0.9 nM, respectively) (Gilmartin et al. 2011). Preliminary results from the Phase 1 trial of GSK1120212 were reported by at the 2010 American Society of Clinical Oncology Annual meeting. In twenty patients with BRAF mutant melanoma, two achieved complete responses with an additional six patients demonstrating partial responses for a total response rate of 40% (Infante et al. 2010). The GSK1120212 trial is the only trial of a MEK inhibitor reported to date to have stratified patients based upon BRAF mutational status. It thus remains unknown whether the greater activity of this compound as compared to others in the class was the result of enrichment for patients whose tumors harbored a BRAF mutation or to the compound's specific pharmacologic properties.

RAF KINASE INHIBITORS

Sorafenib (BAY43-9006, Nexavar) was the first RAF kinase inhibitor to enter clinical testing. Sorafenib is now FDA approved for use in renal cell carcinoma and hepatocellular carcinoma. Although this compound was initially developed as a selective inhibitor of RAF, later studies revealed other targets of sorafenib, including VEGF receptor 2 and 3, PDGFR, Flt-3, c-KIT, and FGFR-1 (Wilhelm et al. 2004). Sorafenib has virtually no activity as a single-agent in melanoma, the tumor type with highest frequency of BRAF mutations (Eisen et al. 2006). Phase 2 trials combining sorafenib with chemotherapy showed early promise in melanoma but the activity of this combination regimen did not correlate with BRAF mutational status (Flaherty et al. 2008). Furthermore, a Phase 3 trial of sorafenib in combination with carboplatin and paclitaxel in patients with advanced melanoma failed to

meet its primary endpoint of improvement in progression-free survival (Hauschild et al. 2009). Overall, the data suggest that the primary basis for the anti-tumor activity of sorafenib in renal cancer is likely anti-angiogenic and that RAF inhibition contributes minimally, if at all, to its activity in patients with advanced cancer.

The limited activity of sorafenib in tumors with BRAF mutation prompted the development of second-generation RAF inhibitors with greater selectivity for BRAF and greater potency for the target *in vivo*. PLX4032 (RG7204/ vemurafenib) and its close analogue PLX4720 are selective RAF inhibitors developed by Plexxikon (Bollag et al. 2010; Tsai et al. 2008). These compounds were designed to bind to the active conformation of BRAF and show 3-fold higher selectivity for ^{V600E}BRAF versus wild-type BRAF, good oral bioavailability, and little toxicity in pre-clinical models. The compounds demonstrate potent antiproliferative effects, but in contrast to sorafenib and MEK inhibitors, do so only in BRAF mutant cell lines (Joseph et al. 2010). The BRAF mutant selective antitumor activity of PLX4032 is attributable to its mutant-selective inhibition of MAPK pathway activity. PLX4032 binds to all three RAF isoforms and exhibits only modest selectivity for mutant BRAF over wild-type BRAF. Despite binding to all three RAF isoforms as low nanomolar concentrations, the drug potently inhibits MAPK pathway activity in cells expressing ^{V600E}BRAF, whereas it induces a paradoxical activation of ERK in BRAF wild-type tumor and normal cells (Hatzivassiliou et al. 2010; Heidorn et al. 2010; Poulikakos et al. 2010). The basis for this paradoxical activation of ERK activity lies in the formation of RAF homo- and heterodimers, a process regulated by RAS (Poulikakos et al. 2010). In BRAF wild-type tumor and normal cells, the current model suggests that PLX4032 induces ERK signaling by transactivating RAF dimers in a process that is RAS-dependent. At low concentrations binding of PLX4032 to one protomer within a RAF dimer results in transactivation and thus activation of the non-drug bound RAF. At higher concentrations, PLX4032 binds to both protomers within such dimers, thus inhibiting RAF activation (Poulikakos et al. 2010).

In the Phase 1 trial of PLX4032, 81% of patients with ^{V600E}BRAF mutant tumors treated at the recommended Phase II dose achieved a RECIST response (Flaherty et al. 2010). The most common toxicities of PLX4032 included skin rash, arthralgia and fatigue. In addition, approximately one-third of patients developed squamous cell carcinomas (keratoacanthoma type) while on treatment (Flaherty et al. 2010). These toxicities have been attributed to the paradoxical activation of ERK induced by RAF inhibitors such as PLX4032 in non-tumor cells that are BRAF wild-type. The level of clinical activity observed with PLX4032 is significantly greater than that of the MEK1/2 inhibitors AZD6244 and GSK1120212, even when considering only the cohort of patients whose tumors express ^{V600E}BRAF. Consistent with the clinical profile of PLX4032, a second RAF inhibitor GSK2118436 (GlaxoSmithKline) also demonstrated a high response rate (63% by RESICT) in BRAF mutant patients in a recently reported Phase I trial (Kefford et al. 2010).

One possible basis for the greater antitumor activity of PLX4032 as compared to the MEK inhibitors is that the mutant selectivity for ERK pathway inhibition of the former may allow for more potent and durable pathway inhibition. Whereas the MEK inhibitor downregulates ERK activity in all cells including normal tissues, the RAF inhibitor PLX4032 inhibits ERK activity only in tumor cells expressing oncogenic BRAF (Joseph et al. 2010). This mutant selectivity for pathway inhibition likely confers a broader therapeutic index, which allows for greater MAPK pathway inhibition with PLX4032 and thus increase anti-tumor efficacy in patients whose tumors harbor a sensitive BRAF mutation. Supporting this hypothesis, pharmacokinetic data from the Phase I trial suggest that the half-life of PLX4032 is long (~40-50 hours) and steady state plasma levels of over 40 μM can be achievable in patients (Flaherty et al. 2010). Alternatively, RAF inhibition may be superior to MEK inhibition in

tumors with mutant BRAF due to inhibition of non-MEK effectors of RAF by the RAF inhibitor but not the MEK inhibitor. Though potential non-MEK effectors of CRAF have been reported, their biological significance remains controversial and their relevance in the setting of mutant BRAF unexplored. Additionally, differences in CNS penetration and thus a lower frequency of progression in brain may also have played a role in the greater activity observed with PLX4032. The high level of activity observed with PLX4032 also supports the contention that the lack of clinical activity observed with sorafenib in BRAF mutant tumors is attributable to its low potency against the ^{V600E}BRAF mutation.

The promising Phase I results with PLX4032 prompted the initiation of a randomized phase III study (BRIM3) comparing PLX4032 to dacarbazine in previously untreated patients with metastatic melanoma (clinicaltrials.gov identifier: NCT01006980). Eligibility for BRIM3 was restricted to treatment-naïve (no prior systemic anticancer therapy) patients with Stage IIIC and IV melanoma whose tumors were positive for the ^{V600E}BRAF mutation. The BRIM3 results have not yet been published but the study sponsors (Roche/Plexxikon) have announced that treatment with PLX4032 resulted in significant improvements in overall survival and progression free survival. Future studies will be needed to address the mechanistic basis for the heterogeneity of responses observed with PLX4032 within the ^{V600E}BRAF cohort and the molecular basis for acquired resistance in patients who initially respond to this approach (Johannessen et al. 2010; Nazarian et al. 2010; Poulikakos et al. 2011; Villanueva et al. 2010). Novel RAF inhibitors that potently suppress ERK activation in BRAF mutant cells but lack the paradoxical activation of ERK noted with PLX4032 in normal cells are also in development on the basis of the presumption that such agents would exhibit a broader therapeutic index (Bollag 2011).

FUTURE PERSPECTIVE

Activation of the MAP kinase pathway is a frequent event in human cancer and pathway activity is often the result of activating mutations in RAS and BRAF. Drugs that target RAF and its primary downstream effector MEK are in clinical development. Preliminary reports suggest that one such compound, the selective RAF inhibitor PLX4032, prolongs survival in patients with ^{V600E}BRAF mutant melanomas. As the activity of these agents correlates with the mechanism responsible for pathway activation (BRAF mutation, RAS mutation or receptor tyrosine kinase activation), prospective genotyping of patients to enrich for those most likely to respond will be critical in the future clinical development of selective inhibitors of this pathway.

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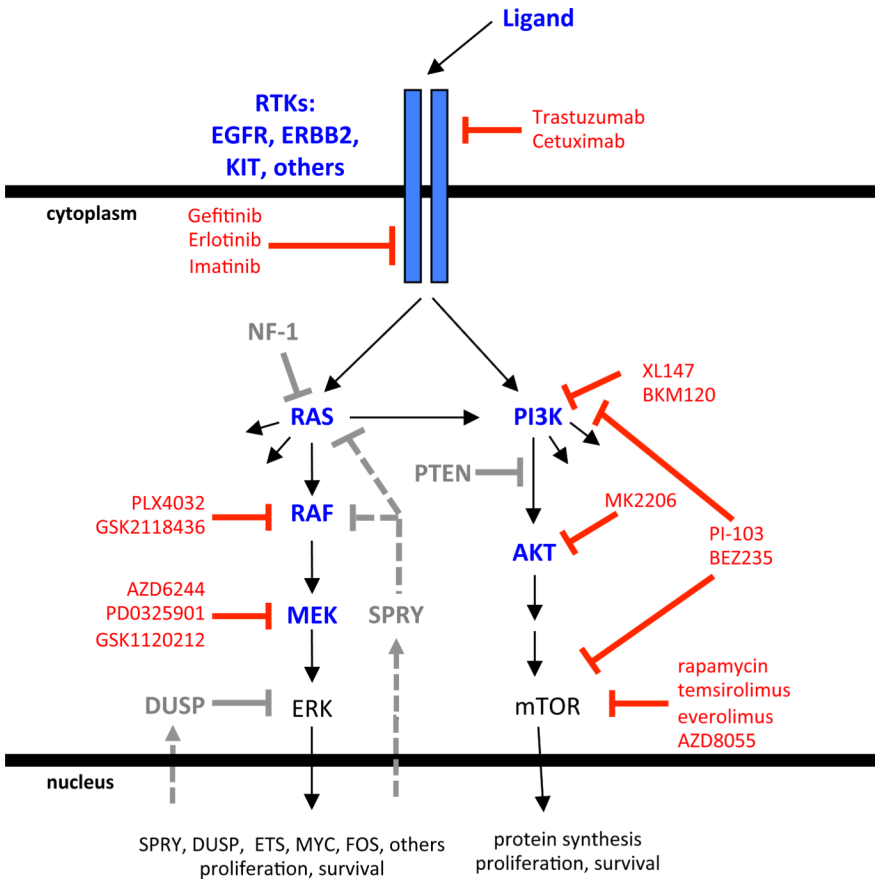

Figure 1. The MAP kinase signaling pathway

The MAP kinase pathway is activated in human tumors by the binding of ligand to receptor tyrosine kinases (RTKs), by constitutive activation of an RTK, by loss of NF1 function, or by mutations in RAS, BRAF and MEK1. Phosphorylation and thus activation of ERK regulates transcription of target genes which promote cell cylce progression and tumor survival. The ERK pathway contains a classical feedback loop in which the expression of feedback elements such as SPRY and DUSP family proteins are regulated by ERK (dashed grey lines). Selected agents that target the MAP kinase and PI3 kinase/AKT pathway are shown (red).

Table 1

Frequency of BRAF mutations in human cancer.

Melanoma	27-67%
Papillary Thyroid	36-69%
Colon Cancer	5-17%
Head and Neck	3-5%
Pancreatic Cancer	4-7%
Glioblastoma	3-6%
Lung Cancer	1-3%
Ovarian Cancer	0-27%
Gastric	0-11%
Cholangiocarcinoma	0-22%
Prostate	0-10%
Endometrial	0-21%

Table 2

RAF and MEK kinase inhibitors

RAF inhibitors		
Sorafenib (Nexavar)	Bayer	FDA approved
PLX4032/ RG7204	Plexxikon/Roche	Phase 3
XL281/BMS-908662	Exelixis/ BMS	Phase 1/2
RAF265	Novartis	Phase 1
GDC-0879	Genentech	Pre-clinical testing
GSK2118436	GlaxoSmithKline	Phase 3
ARQ680	Arqule	Phase 1
MEK inhibitors		
AZD6244	Array BioPharma/AstraZeneca	Phase 3
ARRY-704/AZD8330	Array BioPharma/AstraZeneca	Phase 1
PD0325901	Pfizer	Phase 1
CI-1040	Pfizer	Phase 2
XL518	Exelixis/Genentech	Phase 1
RDEA119	Ardea Biosciences	Phase 1
AS703026	Merck Serono	Phase 1
GSK1120212	GlaxoSmith Kline	Phase 1/2
CH5126766	Chugai/ Roche	Phase 1