



DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD

The RAS/Mitogen Activated Protein (MAP) Kinase Pathway in Melanoma Biology and Therapeutics

The Harvard community has made this article openly available.
[Please share](#) how this access benefits you. Your story matters.

Citation	Jarell, Abel D, Donald Lawrence, and Hensin Tsao. 2007. The RAS/mitogen activated protein (MAP) kinase pathway in melanoma biology and therapeutics. <i>Biologics : Targets & Therapy</i> 1(4): 407-414.
Accessed	February 19, 2015 7:43:03 AM EST
Citable Link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:8268110
Terms of Use	This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

(Article begins on next page)

The RAS/mitogen activated protein (MAP) kinase pathway in melanoma biology and therapeutics

Abel D Jarell¹
 Donald Lawrence²
 Hensin Tsao^{1,2,3}

¹Department of Dermatology; ²MGH Cancer Center, and ³Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Abstract: An effective treatment for metastatic melanoma remains one of the most elusive goals in all of oncology. Several generations of therapeutic trials have yet to yield any agents that can significantly prolong survival for widespread disease. Despite this disheartening history, our understanding of the biology and molecular genetics of melanoma hold the promise of a new era of molecular targets. One pathway that appears to be universally activated in and critically needed for melanoma growth is the Ras/mitogen activated protein (MAP) kinase signaling cascade. Since the enzymatic functions of the signaling partners are well characterized, this pathway offers many potential “druggable” candidates including Braf, Mek and Ras itself. In this review, we describe this pathway in the context of melanoma tumorigenesis and discuss some of the current relevant pharmacologic treatments and clinical trials.

Keywords: malignant melanoma, therapeutics, chemotherapy, RAS, MAP kinase

Introduction

The incidence of malignant melanoma in Western countries is increasing at an alarming rate and has become a major public health concern. In the United States the incidence has risen by 619% from 1950 to 2000 (Tsao, Atkins et al 2004). In 2007, there will be an estimated 59,940 cases of melanoma with 8,110 deaths (Jemal et al 2007); although this represents a slight drop in the incidence of melanoma, recalibration of the statistical methodology (Pickle et al 2007) in the U.S. may in fact account for the apparent decline. Fortunately, in spite of the aforementioned estimates, more people diagnosed with melanoma are surviving longer. This improved survival is essentially due to early detection and surgery. However, despite decades of intense investigation, an effective therapeutic regimen for advanced disease is still lacking.

With the large scale sequencing of the human genome and breakthroughs in cancer genetics among all malignancies, there is tremendous enthusiasm for compounds that inhibit specific cellular targets. Imatinib mesylate, which selectively inactivates a class of enzymes known as tyrosine kinases, is one of the best examples of translational medicine in recent history (Druker 2002). Given its potent and highly specific mechanism of action, imatinib mesylate has become the standard of treatment for patients with BCR-ABL-associated chronic myelogenous leukemia (CML) among other cancers with activated tyrosine kinases. It is important to note, however, that none of the success of imatinib mesylate could have been possible without the early recognition that CML is associated with the Philadelphia chromosome and is genetically driven by a single translocation that juxtaposes the BCR gene with the ABL tyrosine kinase. Thus, underlying genetic insight is now viewed as a prerequisite for drug development. With imatinib mesylate as a model, there is general excitement in oncologic therapeutics that a full molecular disclosure of affected genetic targets will, in time, lead to previously unanticipated approaches for all cancers.

Correspondence: Hensin Tsao
 Department of Dermatology/MGH,
 Bartlett 622; 48 Blossom Street, Boston,
 MA 02114, USA
 Tel +1 617 726 9569
 Fax +1 617 726 1206
 Email htsao@partners.org

In the post-genomic era, our understanding of the molecular biology of melanoma has also increased dramatically. Unlike CML however, advanced melanoma has yet to yield to molecular therapeutics.

Melanoma is a form of skin cancer that emanates from melanocytes, which are cells highly specialized in the formation and transfer of melanin pigment. In their normal function, melanocytes provide the pigment that gives rise to the color of the skin, hair and eyes. They originate embryologically from neural crest progenitors that ultimately migrate to the epidermis and hair follicles where they terminally differentiate and principally reside. Keratinocytes, which also reside in the epidermis and outnumber melanocytes by a ratio of about 10:1, release factors, which help promote the survival, differentiation, proliferation and motility of melanocytes (Gray-Schopfer, Wellbrock et al 2007). These growth factors bind to specific receptors at the cell surface and generate an intracellular message which ultimately leads to transcriptional changes and physiological responses, such as proliferation and survival. This entire cellular process is termed “signal transduction” and is mediated by a set of enzymes aimed at catalytically amplifying a small signal which emerges from the growth factor receptor. In the past few decades, much has been learned about growth signaling at the cellular and molecular levels and it has become clear that many of the participating molecules are in fact oncogenes in cancer, including melanoma. Recent insights into melanoma at the molecular level have demonstrated a stepwise progression of genetic hits that transforms a normal melanocyte or nevus into a primary and then metastatic melanoma (Bennett 2003). Multiple tumor-promoting events, including activation of oncogenes and inactivation of tumor suppressor genes, lead pigment cells through this transition.

One growth factor pathway that has garnered considerable attention in the last few years has been the RAS-BRAF-MAPK-ERK signaling cascade. Much of the attention surrounding this pathway in human melanoma focuses on the fact that in virtually all cases, there is an alteration at some level in the RAS signaling cascade (Haluska et al 2006). One component of this cascade, the BRAF protein kinase, is in fact the most frequently mutated oncogene in melanoma (Haluska et al 2006). Because of this, the BRAF molecule is a particularly appealing target for therapy, and indeed several biologic agents have been developed specifically for the purpose of affecting BRAF (Flaherty 2006). In this review our aim is to describe the current understanding of the most notable signaling cascade involved in melanoma, ie, the RAS/MAP kinase pathway, and to focus on the various

molecules as they have been identified or noted as potential therapeutic targets.

The RAS signaling pathway

RAS

Historically, the rat sarcoma (RAS) virus homologue was the first oncogene to be described in human cancer (Der et al 1982). In cancer, the most commonly mutated members of the RAS superfamily include *HRAS*, *KRAS* and *NRAS*. The RAS proteins are small (21 kilodalton) G-proteins that are active with bound GTP and inactive with bound GDP. Although the GTP can self-hydrolyze, there is a class of enzymes termed GAPs (GTPase activating proteins) that facilitate this hydrolysis and terminate RAS activity.

Oncogenic lesions introduce changes in the primary sequence of *RAS* so that the protein is constitutively active. By 1990, studies had identified *NRAS* mutations in a fraction of melanomas (Dicker et al 1990) thereby establishing a critical link between growth factor signaling and melanocytic tumors. Two decades later, the RAS pathway still remains one of the most investigated pathways in human cancer (Solit et al 2006), including melanoma, and our current understanding suggests that several possible mutations along this cascade lead to tumor-promoting physiology.

Although RAS proteins are frequently mutated in cancer, there is preferential targeting of specific family members in different tumor types. For melanomas, *NRAS* is mutated to a much greater extent than either *KRAS* or *HRAS* (Hocker and Tsao 2007; Tsao, Goel et al 2004). The basis for this specificity is currently unknown but recent data suggest that *NRAS* may have unique functions in melanocytes that involve another oncogene, *MYC* (Whitwam et al 2007). In a recent meta-analysis of all mutations reported in melanoma, *NRAS* was found to be mutated in 26.4% of all uncultured cutaneous melanoma specimens (N = 1064 screened) (Hocker and Tsao 2007). Interestingly, a set of three *NRAS* mutations, *NRAS*^{Gly12Asp}, *NRAS*^{Gln61Arg} and *NRAS*^{Gln61Lys}, accounted for 82% of the 255 substitutions at the *NRAS* locus.

RAF

A downstream effector of RAS is RAF (Figure 1) – a family of protein kinases which includes the sequentially homologous A-Raf, B-Raf and C-Raf proteins. RAF kinases are serine/threonine phosphotransferases that initiate the mitogenic cascade which eventually converges on the ERKs. The ERKs then modulate gene expression through phosphorylation of transcription factors such as Jun, Elk1, c-Ets1/2, Stat 1/3,

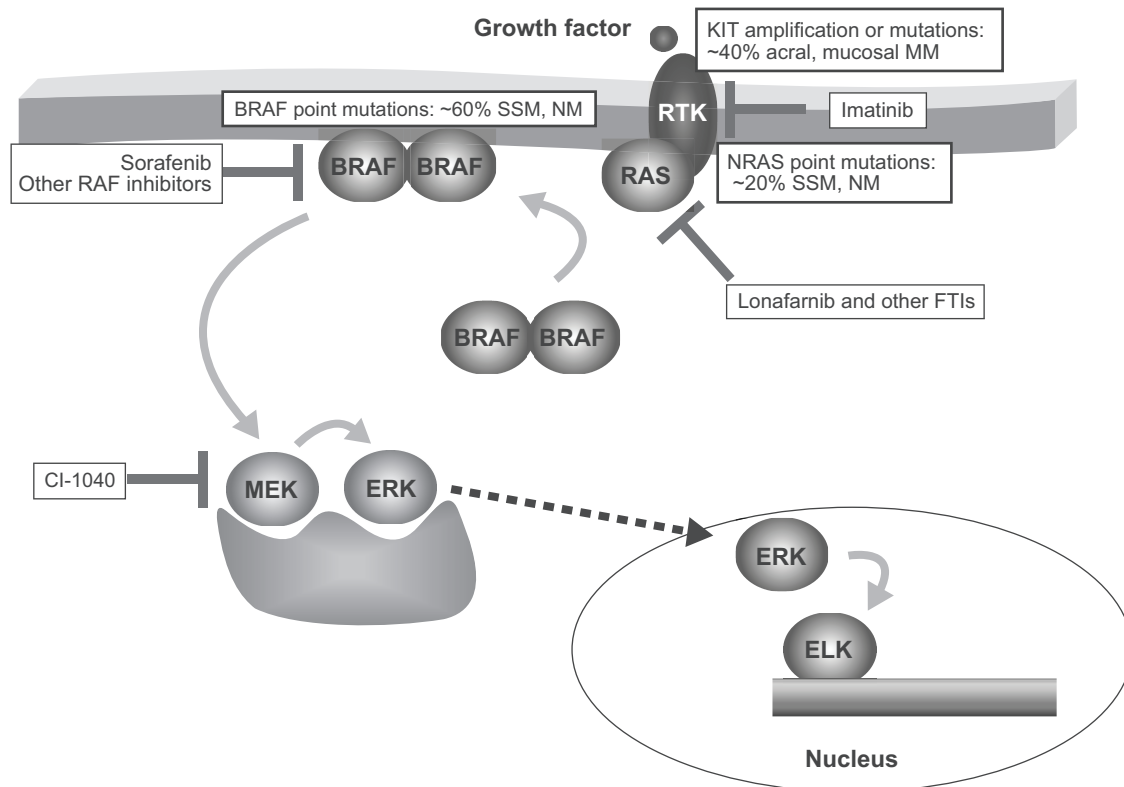


Figure 1 The RAS/MAP kinase signaling pathway. A growth factor binds to its cognate receptor tyrosine kinase (RTK), along with a transductive complex, which then activates RAS through an exchange of GTP for bound GDP. Active RAS then recruits inactive cytosolic BRAF to the membrane and activates BRAF. Active BRAF in turn phosphorylates MEK thereby activating this MAPK kinase to phosphorylate and activate the ERKs. Active ERK then translocates to the nucleus where it phosphorylates and regulates transcription factors, such as ELK. BRAF is mutated in about 60% of melanomas while NRAS is mutated in an additional 20%; the two events are functionally overlapping and thus genetically exclusive. The RTK, KIT, is mutated or amplified in about 40% of acral and mucosal melanomas. The various inhibitors are shown in red boxes.

Abbreviations: MM, malignant melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma.

or Myc. Not surprisingly, since both NRAS and BRAF are frequently activated in melanocytic tumors, ERKs has been shown to be phosphorylated, and therefore active, in up to 90% of human melanomas (Cohen et al 2002).

Through a large systematic genetic screen of mutations in cancer, Davies et al identified BRAF as a common oncogene in many cancers, particularly melanoma (Davies et al 2002). In our meta-analysis of 1336 uncultured cutaneous melanomas, we found that BRAF mutations were reported in 42.4% of the tumors (Hocker and Tsao 2007). Even more striking than NRAS, one mutation, the BRAF^{V600E} variant, accounts for nearly all of the reported changes in BRAF. This one mutation appears to release BRAF from an inactive conformation thereby activating the kinase (Wan et al 2004). It is important to note, however, that BRAF mutations are not sufficient to induce melanoma since many benign acquired nevi also harbor BRAF alterations (Uribe et al 2003). The emerging view of RAS-BRAF-MAPK signaling pathway is becoming increasingly intricate with multiple players acting at various levels, and it seems clear

that at least some aspects of tumorigenicity are conferred through this pathway.

The RAF kinase signaling molecules have been shown to be involved in a variety of cellular processes, such as growth, proliferation, survival, differentiation and transformation (Schreck and Rapp 2006). Despite the fact that much is known about the function of these molecules, there remains a substantial gap in the complete understanding. Some key findings suggest that RAF signaling is important in the activation of NF- κ B, and it is widely believed that the NF- κ B transcription factor is an important mediator of antiapoptotic, proliferative, metastatic, and proangiogenic effects, primarily through its induction of gene expression of proteins critical to these activities (Mayo et al 1997; Sosman and Puzanov 2006).

MAPK/ERK kinase (MAP kinase kinase)

The RAF kinases phosphorylate MEK thereby continuing the signaling stream. There have been no reports of MEK mutations in melanoma, although mutagenic activation of NRAS

or BRAF is probably sufficient to fully stimulate the MAP kinase signaling stream as in most tumors. The BRAF^{V600E} mutation directly leads to activation of MEK although other mutations in BRAF appear to indirectly activate ERK through CRAF and not MEK (Wan et al 2004).

Extracellular signal regulated kinase (ERKs)/MAP kinase

The ERKs were originally cloned by Melanie Cobb and colleagues in 1991 through traditional biochemical strategies (Boulton and Cobb 1991; Boulton et al 1991). The ERKs comprise a family of protein kinases at 42/44 kilodaltons and are themselves activated by phosphorylation. With the development of antibodies specific for phospho-ERKs, it has become possible to gauge the activity of ERKs, and therefore MAP kinase signaling, in tissues without relying on actual enzymatic assays.

In melanoma, ERK activity has been shown to increase from early- to advanced-stage disease (Satyamoorthy et al 2003). An attractive hypothesis is that NRAS or BRAF mutagenesis, which occurs upstream of ERK, is primarily responsible for the observed ERK activation. However, since BRAF mutations have been reported in nevi and yet ERKs are activated in only a minority of these specimens (Cohen et al 2002), other regulatory mechanisms must be in place to minimize unwanted MAP kinase stimulation. Notwithstanding, strong evidence supports the contention that oncogenic BRAF mutations, most prevalently BRAF^{V600E} (Brose et al 2002) eventuate in overactivation of MEK/ERK, which maintains the transformed phenotype in malignant melanoma (Eisen et al 2006).

Growth factor receptors

The general dogma of RAS-RAF-MEK signaling posits that along with multiple other molecules (more than 70, participating at both the nuclear and nonnuclear level) (Schreck and Rapp 2006), the mitogenic cascade follows a fairly orderly sequence beginning at the cell surface with receptor tyrosine kinases (RTKs). In melanocytes, it is thought that the primary growth factors involved in this pathway include stem-cell factor (SCF), hepatocyte growth factor (HGF), and fibroblast growth factor (Gray-Schopfer, Wellbrock et al 2007). The growth factors, in turn, through engagement of their specific RTKs, activate RAS, which subsequently triggers the cascade described above. This molecular game of “tag” serves to amplify the limited growth factor signal that is triggered at the extracellular membrane. For example, if one RAS molecule activates two RAF molecules, which

in turn stimulates two MEK molecules each, then there is a 4-fold amplification of the RAS-initiated signal.

There is a subset of melanomas, primarily those occurring on mucosal membranes, acral skin (palms of the hands, soles of the feet, and nail bed), and skin with chronic sun-induced damage in which BRAF and NRAS mutations are only infrequently seen. These melanomas more frequently have mutations in KIT, the RTK for SCF (Curtin et al 2006). In a study by Bastian and coworkers, examination of 102 primary melanomas revealed mutations and/or copy number increases of KIT in 39% of mucosal, 36% of acral, and 28% of melanomas on chronically sun-damaged skin, but not in any (0%) melanomas on skin without chronic sun damage; 79% of the KIT mutations in melanoma resulted in increased KIT protein levels. These results clearly show that KIT is a bona fide oncogene in melanoma. Interestingly, germline mutations in KIT (Tomita 1994) and the microphthalmia transcription factor (MITF) (Steingrimsson et al 2004) lead to disorders of pigment cell development while somatic activation of both KIT and MITF (Garraway, Widlund et al 2005) have been demonstrated in pigment cell tumors. These findings suggest that tumors, including melanomas, undergo a phenomenon of “lineage” addiction (Garraway, Weir et al 2005).

The KIT RTK has been shown to function in signal transduction in several cell types including mast cells, melanocytes, hematopoietic stem cells, germ cells, and Cajal cells (Hussein 2006). Since KIT mutations have been shown to play an important role in several human malignancies, notably breast cancer (Roussidis et al 2007), gastrointestinal stromal tumors (Fletcher and Rubin 2007), mastocytosis (Gotlib 2006), and others, vigorous efforts have unfolded at treatment targeting this factor.

RAS pathway therapeutic targets for melanoma

To date, the clinical trials using pharmacological agents aimed at the treatment of melanoma have been frankly discouraging. There are currently at least 18 drugs being studied at various phases for the targeted treatment of melanoma (Gray-Schopfer, Wellbrock et al 2007).

RAS inhibitors

Given the widespread prevalence of RAS mutations in cancer, this oncogene represents an ideal target for inhibition. However, the only agents that have been evaluated in clinical trials to alter RAS activity are the farnesyltransferase inhibitors (FTIs) (Flaherty 2006). At the molecular level,

it is believed that these FTIs impair the posttranslational modification of the RAS proteins, which consequently prevents their membrane localization, an event required for signaling activity (Johnston 2001).

These compounds have been studied extensively, and the diseases in which attempts have been made or are currently being studied include cancers as varied as leukemia, breast, pancreas, and glioma, as well as other diseases including progeria and neurofibromatosis (current search for “farnesyltransferase inhibitors” in Clinicaltrials.gov, 2007). So far there has been no clinical efficacy demonstrated. One FTI, SCH66336 (lonafarnib), inhibited melanoma growth *in vitro* although it has yet to be vigorously tested in melanoma trials (Smalley and Eisen 2003). However, the collective experience with lonafarnib in Phase III trials for other cancers has been generally disappointing (Morgillo and Lee 2006). Tipifarnib (R115777) is an oral nonpeptidomimetic FTI that also showed promise in early Phase I and II trials; however, subsequent Phase II and III trials of tipifarnib as monotherapy for breast, colorectal, lung (both non-small cell and small cell), brain, pancreatic and urothelial cancers, have all demonstrated tipifarnib’s lack of significant efficacy (Mesa 2006).

RAF inhibitors

Sorafenib (BAY 43-9006, Nexavar[®], Bayer Pharmaceuticals Corporation, West Haven, CT, USA) is an orally available multikinase inhibitor with effects on tumor-cell proliferation and angiogenesis (Escudier et al 2007). It was first identified as an inhibitor of RAF kinase (C-RAF > B-RAF) (Sosman and Puzanov 2006; Wilhelm et al 2004), but then later found to also inhibit vascular endothelial growth factor receptors (VEGFR) 1, 2, and 3; platelet-derived growth factor receptor β (PDGFR β); FMS-like tyrosine-kinase 3 (Flt-3); c-Kit protein (c-kit); and RET receptor tyrosine kinases (Carlomagno et al 2006; Escudier et al 2007; Wilhelm et al 2004). *In vitro* studies have shown that sorafenib induces cell cycle arrest and apoptosis in melanoma cell lines through MAPK activity inhibition (Gray-Schopfer, Karasarides et al 2007). Specific interference of the BRAF^{V600E} mRNA by RNAi also leads to melanoma cell death suggesting that these cells are “addicted” to the BRAF oncogene (Hingorani et al 2003).

Sorafenib has been granted FDA approval for treatment of advanced clear-cell renal cell carcinoma based on a randomized, placebo-controlled trial demonstrating prolongation of progression-free survival (Escudier et al 2007). Additionally, a phase II randomized trial was discontinued due to superior efficacy of sorafenib compared to placebo in metastatic

renal cell carcinoma (Ratain et al 2006). Both of these trials demonstrated only modest benefits, but given the fact that renal cell carcinoma has notoriously been recalcitrant to all treatment modalities, these studies represented significant breakthroughs. It should be noted that mutations of RAF are absent in renal cell carcinoma, and it is therefore likely that the activity of sorafenib in this disease is mediated by VEGF inhibition.

Clinical trials of sorafenib in melanoma were initiated with great hope and anticipation given its known and putative mechanisms of action (Beeram et al 2005; Karasarides et al 2004; Panka et al 2006; Rodriguez-Viciano et al 2005; Strumberg 2005; Wilhelm et al 2004). In a Phase II discontinuation trial of 37 metastatic melanoma patients (Eisen et al 2006), there was one objective response and six patients who had stable disease. In a phase I/II study, the combination of sorafenib with carboplatin and paclitaxel was associated with an objective response rate of 37%, substantially higher than the response rates historically reported with chemotherapy for melanoma (Flaherty et al 2004).

Based on these findings, several randomized trials of chemotherapy with or without sorafenib have been initiated. In a phase III study of 270 patients who had received prior chemotherapy for metastatic melanoma, the addition of sorafenib to carboplatin and paclitaxel failed to prolong progression-free survival. A similar trial in patients with no prior chemotherapy exposure is ongoing, as is a trial with the oral alkylating agent, temozolomide, in patients with brain metastases from melanoma (Amaravadi et al 2006). At present, the role of sorafenib in the treatment of advanced melanoma remains undefined, and it is not clear that BRAF mutational status predicts responsiveness to this agent. Reported adverse effects of sorafenib include hypertension (Veronese et al 2006), hand-foot syndrome, vasculitis (Chung et al 2006), rash, erythema multiforme (Macgregor et al 2007), diarrhea and fatigue (Hahn and Stadler 2006).

Given the broad range of cancers that exhibit mutations in BRAF, there is intense interest in RAF inhibition as cancer therapy. Outlined in Table 1 is a list of compounds that are currently under development as anti-RAF agents. Several of these agents inhibit BRAF more potently and selectively than sorafenib.

MEK inhibitors

Using small-molecule inhibitors of MEK and an integrated genetic and pharmacologic analysis, Solit et al found that mutation of BRAF predicts sensitivity to MEK inhibition when compared to either ‘wild-type’ cells or cells with a RAS

Table 1 Current table of RAF kinase inhibitors

Molecule	Developer	Study phase	References
sorafenib/BAY 43-9006/ Nexavar®	Bayer/Onyx	III	(Eisen et al 2006; Strumberg 2005; Wilhelm and Chien 2002; Wilhelm et al 2004)
AAL881	Novartis	Preclinical	(Ouyang et al 2006)
RAF-265	Novartis	I	(Amiri et al 2006; Stuart et al 2006; Tsai et al 2006)
Compound 2	GlaxoSmithKline	Preclinical	(Hall-Jackson et al 1999)
LBT613	Novartis	Preclinical	(Khire et al 2004)
L-779450	Merck	Preclinical	(Hall-Jackson et al 1999), (Heimbrook et al 1998)
Omega-carboxypyridyl	Bayer	Preclinical	(Lackey et al 2000)
PLX4032	Plexxikon	I	(Venetsanakos et al 2006)
SB-590885 (33)	GlaxoSmithKline	Preclinical	(Takle et al 2006)
X-6-(3 acetamidophenyl) pyrazines	CCT, Sutton, UK	Preclinical	(Niculescu-Duvaz et al 2006)
ZM 336372	AstraZeneca	Precinical	(Heimbrook et al 1998)

Adapted from Schreck and Rapp (2006) and Gray-Schopfer, Wellbrock et al (2007).

mutation (Solit et al 2006). This inhibition may be partially mediated by downregulation of cyclin D1 protein expression and the induction of G1 arrest.

One MEK inhibitor, CI-1040, has entered clinical trials. In a Phase I study of 77 patients with a variety of advanced cancers (Lorusso et al 2005), 1 partial response was achieved in a patient with pancreatic cancer and 19 patients (28%) achieved stable disease lasting a median of 5.5 months (range, 4 to 17 months) with CI-1040. Although both target suppression and antitumor activity were demonstrated in this phase I study, clinical response was limited.

Another potent and selective inhibitor of MEK, AZD6244, has demonstrated activity against BRAF-mutant melanoma both in vitro and in vivo. It has completed phase I testing and is currently being evaluated in a randomized phase II trial in advanced melanoma.

KIT inhibitors

The tyrosine kinase inhibitor, imatinib mesylate, potently inhibits KIT and has been approved for treatment of gastrointestinal stromal tumors (Schnadig and Blanke 2006) and chronic myelogenous leukemia, as described above. In two Phase II studies of 26 and 18 metastatic melanoma patients, respectively, imatinib mesylate showed little clinical efficacy (Ugurel et al 2005; Wyman et al 2006). However, based on the recognition that acral lentiginous and mucosal melanomas frequently harbor amplifications or mutations of KIT, a Phase II trial of imatinib mesylate (NIH Clinical Trial number NCT00424515) has been initiated for patients with these subtypes of melanoma.

Conclusion

The dramatic successes achieved with targeted therapies in some cancers, notably imatinib mesylate in chronic myelogenous leukemia and gastrointestinal stromal tumors, have not been observed in melanoma. However, there are myriad new agents targeting the MAP kinase pathways either in preclinical development or in clinical trials. Agents targeting BRAF and MEK will undoubtedly continue to undergo intensive evaluation in clinical trials for melanoma in the next few years.

While mutations in the MAP kinase signaling cascade are found in the majority of melanomas, numerous other dysregulated processes contribute to the malignant phenotype, including the PI3-kinase/AKT pathway, and regulators of apoptosis and cell cycle control. Given the complex and heterogeneous genetic properties of melanomas, a “personalized” treatment regimen will likely involve cocktails of highly targeted therapies aimed at various pathways.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health, American Cancer Society and Department of Defense (to HT).

References

- Phase III Trial of Nexavar in patients with advanced melanoma does not meet primary endpoint. Press Release published by Onyx Pharmaceuticals, Inc. Accessed 12/4/2006. http://www.onyx-pharm.com/wt/page/pr_1165242111.
- Amaravadi RK, Schuchter LM, Kramer A, et al. 2006. 2006 ASCO Annual Meeting Proceedings Part I. *Journal of Clinical Oncology*, 24(18S):8009.

- Amiri P, Aikawa M, Dove J, et al. 2006. CHIR-265 is a potent selective inhibitor of c-Raf/B-Raf/mutB-Raf that effectively inhibits proliferation and survival of cancer cell lines with Ras/Raf pathway mutations. 97th AACR annual meeting. Washington, DC.
- Beeram M, Patnaik A, Rowinsky EK. 2005. Raf: a strategic target for therapeutic development against cancer. *J Clin Oncol*, 23:6771–90.
- Bennett DC. 2003. Human melanocyte senescence and melanoma susceptibility genes. *Oncogene*, 22:3063–9.
- Boulton TG, Cobb MH. 1991. Identification of multiple extracellular signal-regulated kinases (ERKs) with antipeptide antibodies. *Cell Regul*, 2:357–71.
- Boulton TG, Nye SH, Robbins DJ, et al. 1991. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell*, 65:663–75.
- Brose MS, Volpe P, Feldman M, et al. 2002. BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res*, 62:6997–7000.
- Carlomagno F, Anaganti S, Guida T, et al. 2006. BAY 43-9006 inhibition of oncogenic RET mutants. *J Natl Cancer Inst*, 98:326–34.
- Chung NM, Gutierrez M, Turner ML. 2006. Leukocytoclastic vasculitis masquerading as hand-foot syndrome in a patient treated with sorafenib. *Arch Dermatol*, 142:1510–11.
- Cohen C, Zavala-Pompa A, Sequeira JH, et al. 2002. Mitogen-activated protein kinase activation is an early event in melanoma progression. *Clin Cancer Res*, 8:3728–33.
- Curtin JA, Busam K, Pinkel D, et al. 2006. Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol*, 24:4340–6.
- Davies H, Bignell GR, Cox C, et al. 2002. Mutations of the BRAF gene in human cancer. *Nature*, 417:949–54.
- Der CJ, Krontiris TG, Cooper GM. 1982. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proc Natl Acad Sci USA*, 79:3637–40.
- Dicker AP, Volkenandt M, Albino AP. 1990. Mutational analysis of human NRAS genes in malignant melanoma: rapid methods for oligonucleotide hybridization and manual and automated direct sequencing of products generated by the polymerase chain reaction. *Genes Chromosomes Cancer*, 1:257–69.
- Druker BJ. 2002. Perspectives on the development of a molecularly targeted agent. *Cancer Cell*, 1:31–6.
- Eisen T, Ahmad T, Flaherty KT, et al. 2006. Sorafenib in advanced melanoma: a Phase II randomised discontinuation trial analysis. *Br J Cancer*, 95:581–6.
- Escudier B, Eisen T, Stadler WM, et al. 2007. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med*, 356:125–34.
- Flaherty KT. 2006. Chemotherapy and targeted therapy combinations in advanced melanoma. *Clin Cancer Res*, 12:2366s–2370s.
- Flaherty KT, Brose M, Schuchter L, et al. 2004. 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). *Journal of Clinical Oncology*, 22(14S):7507.
- Fletcher JA, Rubin BP. 2007. KIT mutations in GIST. *Curr Opin Genet Dev*, 17:3–7.
- Garraway LA, Weir BA, Zhao X, et al. 2005. “Lineage addiction” in human cancer: lessons from integrated genomics. *Cold Spring Harb Symp Quant Biol*, 70:25–34.
- Garraway LA, Widlund HR, Rubin MA, et al. 2005. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature*, 436:117–22.
- Gotlib J. 2006. KIT mutations in mastocytosis and their potential as therapeutic targets. *Immunol Allergy Clin North Am*, 26:575–92.
- Gray-Schopfer V, Wellbrock C, Marais R. 2007. Melanoma biology and new targeted therapy. *Nature*, 445:851–7.
- Gray-Schopfer VC, Karasarides M, Hayward R, et al. 2007. Tumor necrosis factor- α blocks apoptosis in melanoma cells when BRAF signaling is inhibited. *Cancer Res*, 67:122–9.
- Hahn O, Stadler W. 2006. Sorafenib. *Curr Opin Oncol*, 18:615–21.
- Hall-Jackson CA, Eyers PA, Cohen P, et al. 1999. Paradoxical activation of Raf by a novel Raf inhibitor. *Chem Biol*, 6:559–68.
- Haluska FG, Tsao H, Wu H, et al. 2006. Genetic alterations in signaling pathways in melanoma. *Clin Cancer Res*, 12:2301s–2307s.
- Heimbrook D, Huber H, Stirdivant S, et al. 1998. Identification of potent, selective kinase inhibitors of Raf. *American Association of Cancer Research*, p. 558.
- Hingorani SR, Jacobetz MA, Robertson GP, et al. 2003. Suppression of BRAF(V599E) in human melanoma abrogates transformation. *Cancer Res*, 63:5198–202.
- Hocker T, Tsao H. 2007. Ultraviolet radiation and melanoma: a systematic review and analysis of reported sequence variants. *Hum Mutat*.
- Hussein MR. 2006. Expression of KIT receptor tyrosine kinase protein in normal human skin: Preliminary observations. *Cell Biol Int*.
- Jemal A, Siegel R, Ward E, et al. 2007. Cancer statistics, 2007. *CA Cancer J Clin*, 57:43–66.
- Johnston SR. 2001. Farnesyl transferase inhibitors: a novel targeted therapy for cancer. *Lancet Oncol*, 2:18–26.
- Karasarides M, Chioleches A, Hayward R, et al. 2004. B-RAF is a therapeutic target in melanoma. *Oncogene*, 23:6292–8.
- Khire UR, Bankston D, Barbosa J, et al. 2004. Omega-carboxypyridyl substituted ureas as Raf kinase inhibitors: SAR of the amide substituent. *Bioorg Med Chem Lett*, 14:783–6.
- Lackey K, Cory M, Davis R, et al. 2000. The discovery of potent cRaf1 kinase inhibitors. *Bioorg Med Chem Lett*, 10:223–6.
- Lorusso PM, Adjei AA, Varterasian M, et al. 2005. Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. *J Clin Oncol*, 23:5281–93.
- Macgregor JL, Silvers DN, Grossman ME, et al. 2007. Sorafenib-induced erythema multiforme. *J Am Acad Dermatol*.
- Mayo MW, Wang CY, Cogswell PC, et al. 1997. Requirement of NF- κ B activation to suppress p53-independent apoptosis induced by oncogenic Ras. *Science*, 278:1812–15.
- Mesa RA. 2006. Tipifarnib: farnesyl transferase inhibition at a crossroads. *Expert Rev Anticancer Ther*, 6:313–19.
- Morgillo F, Lee HY. 2006. Lonafarnib in cancer therapy. *Expert Opin Investig Drugs*, 15:709–19.
- Niculescu-Duvaz I, Roman E, Whittaker SR, et al. 2006. Novel inhibitors of B-RAF based on a disubstituted pyrazine scaffold. Generation of a nanomolar lead. *J Med Chem*, 49:407–16.
- Ouyang B, Knauf JA, Smith EP, et al. 2006. Inhibitors of Raf kinase activity block growth of thyroid cancer cells with RET/PTC or BRAF mutations in vitro and in vivo. *Clin Cancer Res*, 12:1785–93.
- Panka DJ, Wang W, Atkins MB, et al. 2006. The Raf inhibitor BAY 43-9006 (Sorafenib) induces caspase-independent apoptosis in melanoma cells. *Cancer Res*, 66:1611–19.
- Pickle LW, Hao Y, Jemal A, et al. 2007. A new method of estimating United States and state-level cancer incidence counts for the current calendar year. *CA Cancer J Clin*, 57:30–42.
- Ratain MJ, Eisen T, Stadler WM, et al. 2006. Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol*, 24:2505–12.
- Rodriguez-Viciano P, Tetsu O, Oda K, et al. 2005. Cancer targets in the Ras pathway. *Cold Spring Harb Symp Quant Biol*, 70:461–7.
- Roussidis AE, Theocharis AD, Tzanakakis GN, et al. 2007. The Importance of c-Kit and PDGF Receptors as Potential Targets for Molecular Therapy in Breast Cancer. *Curr Med Chem*, 14:735–43.
- Satyamoorthy K, Li G, Gerrero MR, et al. 2003. Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. *Cancer Res*, 63:756–9.
- Schnadig ID, Blanke CD. 2006. Gastrointestinal stromal tumors: imatinib and beyond. *Curr Treat Options Oncol*, 7:427–37.
- Schreck R, Rapp UR. 2006. Raf kinases: oncogenesis and drug discovery. *Int J Cancer*, 119:2261–71.
- Smalley KS, Eisen TG. 2003. Farnesyl transferase inhibitor SCH66336 is cytostatic, pro-apoptotic and enhances chemosensitivity to cisplatin in melanoma cells. *Int J Cancer*, 105:165–75.
- Solit DB, Garraway LA, Pratilas CA, et al. 2006. BRAF mutation predicts sensitivity to MEK inhibition. *Nature*, 439:358–62.

- Sosman JA, Puzanov I. 2006. Molecular targets in melanoma from angiogenesis to apoptosis. *Clin Cancer Res*, 12:2376s–2383s.
- Steingrimsson E, Copeland NG, Jenkins NA. 2004. Melanocytes and the microphthalmia transcription factor network. *Annu Rev Genet*, 38:365–411.
- Strumberg D. 2005. Preclinical and clinical development of the oral multikinase inhibitor sorafenib in cancer treatment. *Drugs Today (Barc)*, 41:773–84.
- Stuart D, Aardalen K, Lorenzana E, et al. 2006. Characterization of a novel Raf kinase inhibitor that causes target dependent tumor regression in human melanoma xenografts expressing mutant B-Raf. 97th AACR annual meeting. Washington, DC.
- Takle AK, Brown MJ, Davies S, et al. 2006. The identification of potent and selective imidazole-based inhibitors of B-Raf kinase. *Bioorg Med Chem Lett*, 16:378–81.
- Tomita Y. 1994. The molecular genetics of albinism and piebaldism. *Arch Dermatol*, 130:355–8.
- Tsai J, Zhang J, Bremer R, et al. 2006. Development of a novel inhibitor of oncogenic B-RAF. 97th AACR annual meeting. Washington, DC.
- Tsao H, Atkins MB, Sober AJ. 2004. Management of cutaneous melanoma. *N Engl J Med*, 351:998–1012.
- Tsao H, Goel V, Wu H, et al. 2004. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. *J Invest Dermatol*, 122:337–41.
- Ugurel S, Hildenbrand R, Zimpfer A, et al. 2005. Lack of clinical efficacy of imatinib in metastatic melanoma. *Br J Cancer*, 92:1398–405.
- Uribe P, Wistuba II, Gonzalez S. 2003. BRAF mutation: a frequent event in benign, atypical, and malignant melanocytic lesions of the skin. *Am J Dermatopathol*, 25:365–70.
- Venetsanakos E, Stuart D, Tan N, et al. 2006. CHIR-265, a novel inhibitor that targets B-Raf and VEGFR, shows efficacy in a broad range of preclinical models. 97th AACR annual meeting. Washington, DC.
- Veronese ML, Mosenkis A, Flaherty KT, et al. 2006. Mechanisms of hypertension associated with BAY 43-9006. *J Clin Oncol*, 24:1363–9.
- Wan PT, Garnett MJ, Roe SM, et al. 2004. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell*, 116:855–67.
- Whitwam T, Vanbrocklin MW, Russo ME, et al. 2007. Differential oncogenic potential of activated RAS isoforms in melanocytes. *Oncogene*.
- Wilhelm S, Chien DS. 2002. BAY 43-9006: preclinical data. *Curr Pharm Des*, 8:2255–7.
- Wilhelm SM, Carter C, Tang L, et al. 2004. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res*, 64:7099–109.
- Wyman K, Atkins MB, Prieto V, et al. 2006. Multicenter Phase II trial of high-dose imatinib mesylate in metastatic melanoma: significant toxicity with no clinical efficacy. *Cancer*, 106:2005–11.