Introduction

Malignant melanoma is the most fatal type of skin cancer. In 2013, approximately 76,690 new cases of invasive melanoma will be diagnosed and 9,480 deaths will occur. The incidence of melanoma among men and women is increasing. Because of the uniformly poor prognosis of this disease, efforts focusing on combining surgical management with systemic treatments are ongoing.

Significant progress has been made in the field of immunotherapy. Ipilimumab, a human monoclonal antibody that targets cytotoxic T-lymphocyte antigen 4 (CTLA-4), is the first agent to improve overall survival (OS) in patients with metastatic melanoma. Prior to this discovery, no therapy in a phase III randomized...
controlled trial improved OS in patients with advanced melanoma. Ipilimumab potentiates the immunological response to melanoma, both as monotherapy and in combination with other treatments. A CTLA-4 is an immune checkpoint molecule that downregulates T-cell signaling to inhibit the costimulatory CD28-B7 pathway, limiting T-cell responses and contributing to tolerance to self-antigens. By blocking this molecule, ipilimumab prevents the downregulation of T cells and increases the immune response against tumor antigens. In addition, programmed death 1 (PD-1) protein, an inhibitory receptor on activated T cells, and PD-1 ligand (PD-L1), which is selectively expressed on tumor cells, are thought to play critical roles in the ability of a tumor to overcome the immune system.

PD-1 is another immune-checkpoint molecule that mediates immunosuppression to cancer cells. Blockade of PD-1 binding to PD-L1 enhances the immune response. A phase I trial of nivolumab, a fully human immunoglobulin G4 (IgG4) anti-PD-1 antibody, demonstrated objective responses (either partial or complete) in 18% of patients with non–small cell lung cancer, 28% of patients with melanoma, and 27% of patients with renal cell cancer. PD-L1, the primary ligand of PD-1, is upregulated on solid tumors. PD-L1 impedes cytokine production and inhibits the cytolytic activity of PD-1–expressing, tumor-infiltrating CD4+ and CD8+ T cells.

A phase I trial of MDX-1105, a human PD-L1 monoclonal antibody, demonstrated durable and objective responses in 6% to 17% of patients with advanced cancers, including non–small cell lung cancer, melanoma, and renal cell cancer. Emerging knowledge of several mutations in melanoma has also led to dramatic clinical advances in the use of molecularly targeted therapy in patients with melanoma harboring these mutations. Vemurafenib, a small molecule inhibitor of mutant BRAF, was recently approved by the US Food and Drug Administration (FDA) for the treatment of melanoma harboring the BRAF V600E mutation. A significant impact was demonstrated on progression-free survival (PFS) and OS in a phase III trial of patients with melanoma and the BRAF V600E mutation.

In this review, we describe several signaling pathways in melanoma and discuss how mutations of BRAF, NRAS, KIT, GNAQ, and GNA11 cause aberrant signaling and malignant transformation.

**BRAF Mutations**

**Oncogenic Mechanism of Action**

V-raf murine sarcoma viral oncogene homolog B1 (BRAF) is a member of the rapidly accelerated fibrosarcoma (RAF) family of serine/threonine kinases, which also contains enzymes ARAF and CRAF (RAF1). It is part of the rat sarcoma (RAS)/RAF/mitogen-activated protein kinase (MAPK)/extracellular-signal–regulated kinase (ERK [MEK]) signal transduction pathway. The MAPK cascade receives activation signals from upstream tyrosine kinases, cytokines, and G-protein coupled receptors, leading to cell growth and differentiation (Fig 1). RAS, a G protein attached to the inner layer of the plasma membrane, is responsible for direct activation of BRAF. The other pathway kinases, including RAF, MEK, and ERK, are all located in the cytosol. In melanocytes, growth factors such as fibroblast growth factor and stem cell factor lead to temporary activation of the MAPK cascade, causing a modest mitogenic effect. However, cell proliferation can occur if robust and prolonged activation of ERK is present, which requires a combination of multiple growth factors.

A high frequency of activating mutations of the RAS/RAF/MEK/ERK pathway exists in melanoma. BRAF is the most commonly mutated gene in this malignancy, with a frequency ranging from 50% to 70%, depending on the distinct subset of melanoma examined (Table 1). More than 80% of BRAF mutations result from a substitution of valine to glutamic acid (Val600Glu, designated as BRAF V600E). The BRAF V600E mutation destabilizes the inactive conformation of the BRAF kinase, shifting the equilibrium to the constitutively active state. The BRAF V600E mutation then leads to continuous downstream signaling of the MAPK pathway and resultant ERK activation, causing a cell proliferation and melanoma survival advantage. Other BRAF mutations include V600K (1798 1799 GT > AA; 5% to 6%), followed by V600R (1798 1799 GT > AG; 1%), V600E2 (1799 1800 AG > AA; 0.7%), and V600D (1799 1800 AG > AT). Other rare mutations affecting different codons of the BRAF gene have also been described. The frequency of the BRAF V600K mutation is as high at 20% in some populations. In addition to melanoma, BRAF mutations have been found in many other cancers, including ovarian carcinoma, colorectal carcinoma, and papillary thyroid cancer.

Oncogenic BRAF V600E leads to the malignant transformation of human melanocytes in vitro and in vivo. The melanocyte expression of BRAF V600E leads to the spontaneous melanoma formation in transgenic mice. However, this finding is observed in conjunction with inactivation of the tumor suppressor phosphatase and tensin homolog (PTEN), suggesting that mutant BRAF may be an early initiator of melanoma formation and additional concomitant events are required for full malignant transformation.

Vredeveld et al recently demonstrated that PTEN depletion or increased protein kinase B (Akt) activation abrogated mutant BRAF V600E–induced senescence in fibroblasts and melanocytes, suggesting that activation of the phosphatidylinositol 3-kinase (PI3K) pathway contributes to the progression of nevi.
to melanomas. Clinically, oncogenic BRAF V600E is found in approximately 50% of melanomas that occur on skin lacking chronic sun-induced damage (CSD; also called non-CSD melanoma). BRAF mutations are less common in melanomas on skin with marked CSD (CSD melanoma), suggesting that the deleterious effects of ultraviolet light may be unrelated to the development of BRAF mutations.21,25,29

Constitutive activation of the MAPK cascade via mutant BRAF V600E leads to growth of melanoma cells through the upregulation of cyclin D1 expression and by the downregulation of p27 KIP1, a cell cycle inhibitor. BRAF V600E also serves to promote melanoma survival by regulating expression and function of many proapoptotic and antiapoptotic proteins such as those from the Bcl-2 family (Bmf, Bim, Bad) and Mcl-1.30-34 Melanoma migration and capability for invasion are also enhanced by mutant BRAF. Continuous activation of the MAPK cascade promotes the invasion and migration through alterations in the cytoskeletal organization, upregulation of matrix metalloprotease expression, and activation of migratory machinery.35,36 This occurs through the downregulation of the cyclic guanosine monophosphate (cGMP) phosphodiesterase PDE5A, which facilitates cell invasion and contractility by increasing intracellular cGMP levels leading to calcium release into the cytosol and myosin light chain phosphorylation.37 Melanoma migration and motility are enhanced by the effect of mutant BRAF on the expression of RND3/RhoE/Rho8, which serves to mediate crosstalk between the MAPK and Rho/Rock/LIM domain kinase (LIMK)/Cofilin pathways.38,39

Oncogenic BRAF may also play a role in the immunological response to melanoma. The inhibition of BRAF or MEK signaling in melanoma cells increases the expression of highly immunological differentiation antigens such as melanoma antigen (Melan-A) and MART-1, leading to enhanced T-cell recognition.40,41 This increased recognition of melanoma by the immune system suggests that a combination of BRAF inhibitors with immunotherapeutic agents such as

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Fig 1. — Major signaling pathways in melanoma and oncogenic mutation frequency. MEK1 and MEK2 are also known as MAP2K1 and MAP 2K2, respectively. ERK1 and ERK2 are also known as MAPK3 and MAPK1, respectively. Akt1 = protein kinase B, ERK = extracellular-signal-regulated kinase, GEFT = RAC/CDC42 exchange factor, GNAQ = guanine nucleotide-binding protein G(q), GPCR = G-protein coupled receptor, IκB = inhibitor of kappa B, LIMK = LIM domain kinase, MAPK = mitogen-activated protein kinase, MEK = MAPK/ERK kinase, MLC = myosin light chain, MPK1 = MAPK phosphatase 1, mTOR = mammalian target of rapamycin, NFκB = nuclear factor kappa light-chain enhancer of activated B cells, PAK = p21 protein (CDC42/RAC)-activated kinase, PI3K = phosphoinositide 3 kinase, PTEN = phosphatase and tensin homolog, RAC = RAS-related C3 botulinum toxin substrate, RAF = rapidly accelerated fibrosarcoma, RAS = rat sarcoma, RHO = RAS homolog, ROCK = Rho-associated, coiled-coil containing protein kinase, RTK = receptor tyrosine kinase (eg, type III transmembrane receptor tyrosine kinase [KIT], fibroblast growth factor receptor 1 [also known as FLT2], PDGF, VEGF), UM = uveal melanoma. Modified from Romano E, Schwartz GK, Chapman PB, et al. Treatment implications of the emerging molecular classification system for melanoma. Lancet Oncol. 2011;12(9):913-922. Reprinted from The Lancet with permission from Elsevier © 2011.
ipilimumab may be an effective therapeutic strategy against this type of cancer.\textsuperscript{42}

\textbf{Therapeutic Considerations}

Given the relevance of the MAPK pathway in melanoma, efforts have focused on the development of pathway inhibitors in the treatment of this disease. Mutant \textit{BRAF} demonstrates greater sensitivity to both \textit{RAF} and \textit{MEK} inhibitors compared with wild-type \textit{BRAF} or oncogenic \textit{NRAS} melanoma cell lines.\textsuperscript{43} Therefore, inhibitors of \textit{RAF} and \textit{MEK} are in various stages of development for the treatment of melanomas with \textit{BRAF} mutations.

Sorafenib, a small multitargeted kinase inhibitor of \textit{BRAF} and \textit{CRAF (RAF1)}, was one of the first small molecule inhibitors to be tested in melanoma. It is an oral biaryl urea with additional activity against vascular endothelial growth factor receptor 2 and 3 (VEGFR2/3), the receptor-type tyrosine-protein kinase FLT3, the type III transmembrane receptor tyrosine kinase (KIT), and platelet-derived growth factor receptor (PDGFR).\textsuperscript{44} Although encouraging effects were observed in early-phase trials, particularly in combination with chemotherapeutic agents such as carboplatin and paclitaxel, randomized trials failed to show clinical benefit in front-line and second-line settings.\textsuperscript{45}

The next generation of \textit{RAF} inhibitors offers the potential for increased efficacy against \textit{BRAF}-mutant melanoma. Vemurafenib is an adenosine triphosphate (ATP)-competitive selective \textit{RAF} kinase inhibitor of \textit{RAF1}, \textit{BRAF}, and oncogenic \textit{NRAS} V600E, with half maximal inhibitory concentration (IC\textsubscript{50}) values of 44 nmol/L, 110 nmol/L, and 44 nmol/L, respectively.\textsuperscript{46} In cell lines harboring mutant \textit{BRAF}, vemurafenib causes both gap 1 (G₁) cell-cycle arrest and apoptosis as well as regression in \textit{BRAF} V600E melanoma xenograft models. However, unexpected results were documented when small-molecule BRAF inhibitors were used to treat non–\textit{BRAF} V600E melanoma. In vitro, inhibition of \textit{BRAF} wild-type melanoma in the presence of an oncogenic \textit{RAS} mutation leads to the paradoxical activation of the MAPK pathway through homodimeric or heterodimeric complex formation of CRAF (RAF1) with wild-type \textit{BRAF} or kinase-dead \textit{BRAF}, resulting in potent ERK phosphorylation (Fig 2).\textsuperscript{47-50} This paradoxical activation of \textit{RAS} after the inhibition of \textit{BRAF} resulted in the increased capacity of melanoma cells to proliferate and invade via FAK activity in addition to the increased survival of mutant \textit{NRAS} cell lines.\textsuperscript{51} This suggests that the use of \textit{BRAF} inhibitors to treat patients with wild-type melanoma or melanoma harboring \textit{NRAS} mutations may lead to enhanced tumorigenicity.

Clinically, this paradoxical pathway activation in wild-type \textit{BRAF} cells may explain how \textit{RAF} inhibitors, such as sorafenib, vemurafenib, and dabrafenib, trigger the formation of hyperkeratotic lesions, which are frequently seen with their use. These lesions include squamous cell carcinoma, keratoacanthomas, verrucal keratosis, and plantar hyperkeratosis. Several studies have reported mutations in genes encoding \textit{RAS} proteins in 30% to 70% of cutaneous squamous cell carcinoma excised from patients treated with \textit{BRAF} inhibitors, further emphasizing how the paradoxical activation of the MAPK pathway in wild-type \textit{BRAF} cells (eg, keratinocytes) via \textit{RAF} inhibitors may lead to the development of cutaneous neoplasms.\textsuperscript{52-56}

The emergence of other types of tumors has also been attributed to the use of \textit{RAF} inhibitors. One study examined 22 cutaneous melanocytic lesions that had either emerged or considerably changed in morphology in 19 patients undergoing treatment with type I \textit{RAF} inhibitors for \textit{BRAF}-mutant melanoma.\textsuperscript{57} Of these lesions, 12 newly detected melanomas were found in 11 patients within 27 weeks of initiating a BRAF blockade. Ten new nevi were also seen in these patients, of which 9 were dysplastic. All melanocytic lesions were wild-type \textit{BRAF}. Molecular examination

\begin{table}[h]
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\hline
\textbf{Mutated Gene} & \textbf{Frequency in Cutaneous Melanoma (%)} \\
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\textit{BRAF} & 46 \\
\textit{CDKN2A} & 26 \\
\textit{NRAS} & 18 \\
\textit{PS3} & 16 \\
\textit{PTEN} & 15 \\
\textit{MEK (MAP2K1)} & 9 \\
\textit{KIT} & 8 \\
\textit{CTNNB1} & 4 \\
\textit{APC} & 4 \\
\textit{CDK4} & 1 \\
\textit{GNA11} & 1 (36\% of UM) \\
\textit{GNAQ} & 0.4 (36\% of UM) \\
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\end{tabular}
\caption{Frequencies of Selected Somatic Gene Mutations in Melanoma According to the COSMIC Database}
\end{table}

revealed an increased expression of cyclin D1 in these new primary melanomas compared with nevi ($P = .01$ and $P = .03$, respectively). In addition, a report of a patient with BRAF-mutant metastatic melanoma undergoing treatment vemurafenib revealed accelerated growth of a previously unsuspected RAS-mutant leukemia.  Exposure to RAF inhibition induced hyperactivation of ERK signaling and the proliferation of the leukemic cell population, an effect that was reversed on drug withdrawal. These findings support the concept that BRAF inhibitors induce growth and tumorigenesis in RAF wild-type cells. Increased surveillance for the emergence of new cutaneous and noncutaneous neoplasms in patients receiving RAF inhibitors may be appropriate moving forward.

The BRIM-3 study, a phase III clinical trial of vemurafenib, consisted of 675 patients with previously untreated BRAF V600E-mutant melanoma who were randomized to receive either vemurafenib (960 mg orally twice a day) or dacarbazine (1,000 mg/m$^2$ of body-surface area intravenously every 3 weeks).  The trial was halted early due to the clear efficacy of vemurafenib over dacarbazine. At a 6-month analysis, the OS rate was 84% for patients treated with vemurafenib compared with 64% for those treated with dacarbazine. Those treated with vemurafenib achieved an overall response rate of 48% and a median PFS of 5.3 months compared with a 5% response rate and a 1.6-month median PFS for dacarbazine. In 2011, the FDA approved vemurafenib for the treatment of metastatic BRAF V600E-mutant melanoma. Updated results of the BRIM-3 study were recently presented.  Median follow-up times on vemurafenib and dacarbazine were 10.5 and 8.4 months, respectively. The median OS was 13.2 months with vemurafenib compared with 9.6 months with dacarbazine. One-year OS rates were 55% for vemurafenib and 43% for dacarbazine, demonstrating that patients with BRAF V600E-mutant melanoma treated with vemurafenib continued to have improved OS rates.

Another BRAF inhibitor, dabrafenib, also demonstrated dramatic responses in BRAF V600E/K-mutant melanoma. Dabrafenib has a greater than 100-fold selectivity for BRAF V600E-mutant cell lines and demonstrates dose-dependent inhibition of MEK and ERK phosphorylation associated with tumor regression in xenograft models. The phase I study of this agent consisted of 184 patients, of which 156 had BRAF-mutant melanoma.  At the recommended phase II dose (150 mg twice daily), 18 of the 36 patients

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**Fig 2.** — BRAF-mutant and WT cell responses to RAF inhibition. (A) Normal activation of the wild-type cell RAS/RAF/MEK/ERK pathway promotes cell growth. Normally, RAS enzymes activate signaling when bound to guanosine triphosphate. (B) Mutations of the MAPK pathway, especially those of oncogenic BRAF V600E in melanoma, produce excessive activation and eventual tumor formation and growth. In melanoma cells that harbor BRAF V600E, RAS/GTP activation is low, and ERK signaling is predominantly activated by dimers of the mutant kinase. (C) In the presence of a BRAF inhibitor that blocks RAF activation, MEK/ERK signaling in BRAF V600E-mutant cells is suppressed, causing cell death and tumor shrinkage. (D) In RAS-mutant cells that contain wild-type BRAF, RAF inhibitors block phosphorylation of RAF monomers (BRAF or CRAF [RAF1]), forming homodimeric and heterodimeric complexes (BRAF/CRAF or CRAF/CRAF [RAF1]). In addition, kinase-dead BRAF, which cannot be phosphorylated, forms a dimeric complex with CRAF (RAF1). The formation of these dimers causes excessive downstream activation stimulated by oncogenic RAS, resulting in a paradoxical hyperactivation of MEK and ERK by the RAF inhibitor and subsequent tumor growth. BRAF = v-raf murine sarcoma viral oncogene homolog B1, ERK = extracellular-signal-regulated kinase, GTP = guanosine triphosphate, MAPK = mitogen-activated protein kinase, MEK = MAPK/ERK kinase, MLC = myosin light chain, RAF = rapidly accelerated fibrosarcoma, RAS = rat sarcoma, RTK = receptor tyrosine kinase, WT = wild type.
(69%) with BRAF-mutant melanoma demonstrated confirmed clinical responses. Of the 27 patients with BRAF V600E-mutant melanoma, 15 (56%) had a confirmed response. In BRAF V600E-mutant melanoma, responses were durable, with 17 patients (47%) on treatment for more than 6 months.

A single-arm phase II study of dabrafenib also showed promising clinical results. The BREAK-2 study enrolled 92 patients, in which 76 harbored BRAF V600E and 16 harbored BRAF V600K mutations.61 The objective response rate was 59% in the BRAF V600E group, with two responses seen in the BRAF V600K cohort. The median PFS rates were 27 weeks and 20 weeks, respectively. Results from BREAK-3, a phase III randomized, multicenter trial comparing dabrafenib with dacarbazine in 250 patients with previously untreated BRAF V600E-mutant melanoma showed improved PFS and objective response rates with dabrafenib.62 The median PFS rates were 5.1 and 2.7 months, respectively. Confirmed response rates were 53% and 19% for dabrafenib and dacarbazine, respectively. This study further emphasized the efficacy of RAF inhibitors for the treatment of BRAF-mutant melanoma.

Of note, the original phase I study of dabrafenib by Falchook et al60 was also significant because its results demonstrated the potential for therapeutic efficacy of targeted RAF inhibition against melanoma brain metastases. Of the 10 patients with BRAF V600E-mutant melanoma and untreated brain metastases who were given dabrafenib, brain metastases in most patients reduced in size, with complete resolution of the metastases in 4 patients. Intracranial disease reduction was accompanied by extracranial disease reduction. Although patients with melanoma metastatic to the brain typically survive less than 5 months, all 10 patients in this study were alive at 5 months and 2 patients had durable antitumor activity with survival beyond 1 year. Although dabrafenib was designed to prevent blood-brain barrier penetration due to the potential neurotoxic effects on abundant wild-type BRAF in the brain, disruption of the blood-brain barrier by melanoma metastases could be the mechanism by which dabrafenib accesses these lesions.

The successes of vemurafenib59 and dabrafenib against cerebral metastatic disease in melanoma support the inclusion of patients with BRAF-mutant melanoma and brain metastases in future trials.60,63 Results from the BREAK-MB trial, a multicenter, phase II study examining dabrafenib in patients with BRAF V600E/K-mutant melanoma and brain metastases, also highlighted the efficacy of RAF inhibition on these lesions (NCT01266967).64 Of the 139 patients with a BRAF V600E mutation, objective responses were observed in 29 of 74 patients (39%) who had no previous treatment for their brain metastases and 20 of 65 patients (31%) who had received prior local treatment. In 33 patients with a BRAF V600E mutation, 15% had an objective response to dabrafenib. Therapeutic regimens combining targeted agents with different locoregional modalities are also being explored. A phase II prospective trial of dabrafenib with stereotactic radiosurgery for the treatment of BRAF V600E-mutant melanoma brain metastases commenced in April 2013 (NCT01721603).

**BRAF Resistance**

Primary and secondary resistance mechanisms exist in which tumors can evade treatment. Primary resistance implies initial refractoriness of a tumor to treatment, which may be due to an alteration in part of the gene locus not targeted by the drug. Secondary, or acquired, resistance denotes tumor progression following an initial response to the drug.

**Primary Resistance:** Although the reasons behind primary resistance remain unclear, it has been demonstrated in preclinical studies of BRAF V600E melanoma cell lines showing a wide range of IC50 values to various BRAF inhibitors.65-68 One molecule thought to play a role in intrinsic BRAF inhibitor resistance in melanoma is PTEN.31,65 Loss of PTEN, a negative regulator of Akt, was demonstrated in a subset of BRAF-mutant melanoma resulting in Akt activation.31 When BRAF is inhibited, increased Akt signaling is present, limiting the accumulation of FOXO3a in the nucleus and leading to decreased apoptosis. When BRAF melanoma cell lines are treated with selumetinib, a small molecule inhibitor of MEK1/2, insulin-like growth factor-1 (IGF-1)–mediated Akt signaling increases, thus decreasing apoptosis. This increased Akt signaling and decreased cell death can be overcome by combination therapy using MEK and Akt or mammalian target of rapamycin (mTOR) 1/2 inhibitors.69 In addition, concomitant PTEN and retinoblastoma protein (RB) loss results in cell cycle checkpoint dysregulation and intrinsic resistance to BRAF and MEK inhibitors.65

Cyclin D and protein kinase D3 (PRKD3) may also play a role in the primary resistance to BRAF inhibitors. Inhibition of BRAF in cell lines leads to the weakening of MEK/ERK signaling, a reduction in cyclin D1 expression, and cell cycle arrest.66 However, a small subset of BRAF-mutant melanoma cell lines harbor cyclin D1 amplifications. Cell lines that possess both amplifications in cyclin D1 and BRAF mutations demonstrate intrinsic resistance to BRAF inhibition and continue to freely enter the cell cycle even when a BRAF inhibitor is administered.68 PRKD3 may also play a role in intrinsic resistance to RAF inhibitors. PRKD3 knockdown by small interfering RNA (siRNA) reduced the IC50 of the RAF inhibitors in melanoma cells lines. MAPK pathway signaling was also blocked
be observed after the RAF inhibitors were administered. Melanoma cell lines harboring the \textit{BRAF} V600E mutation, which also had PTEN loss, demonstrated that PRKD3 caused a reactivation of the PI3K/Akt cascade following RAF inhibition, further adding to resistance.\textsuperscript{70}

The tumor microenvironment may also play a role in innate tumor resistance to therapy. Straussman et al\textsuperscript{71} reported that stroma-mediated resistance to targeted agents was common. In \textit{BRAF}-mutant melanoma, stromal cell secretion of hepatocyte growth factor (HGF) resulted in the activation of MET and reactivation of the MAPK and PI3K/Akt signaling pathways, conferring immediate resistance to RAF inhibition. Analysis of the immunohistochemistry of \textit{BRAF}-mutant melanoma tumors demonstrated the stromal cell expression of HGF and revealed a relationship between HGF expression and the innate resistance to RAF inhibitor treatment. Thus, dual inhibition by RAF and HGF or MET inhibitors represents a potential therapeutic combination in \textit{BRAF}-mutant melanoma.

\textbf{Secondary Resistance:} Acquired or secondary resistance is common in patients treated with small-molecule BRAF inhibitors. Despite impressive response rates with these drugs, complete responses have been rare and usually of a short duration (approximately 7 months), and secondary resistance ultimately developed in most cases.\textsuperscript{12,72} Unlike other targeted therapies such as imatinib in chronic myeloid leukemia and gastrointestinal stromal tumors (GISTs) in which acquisition of secondary mutations in the kinase domain leads to loss of drug efficacy, no similar gatekeeper mutations were found in BRAF inhibitor-resistant cell lines or biopsies taken from patients who failed vemurafenib.\textsuperscript{73,74} Multiple mechanisms play a role in acquired resistance to BRAF inhibitors such as the constitutive signaling of receptor tyrosine kinases (IGF-1R and PDGFR-\textgreek{g}), increased expression of \textit{COT} (\textit{MAP3K8}, \textit{TPL}-2) in the MAPK cascade, \textit{NRAS} and \textit{MEK1} mutation acquisition, and resultant \textit{BRAF} alterations.\textsuperscript{68,75-79} The significance and frequency of these mechanisms of resistance in melanoma remain unknown and are under clinical investigation.\textsuperscript{77,80-84}

Poulikakos et al\textsuperscript{82} identified a new mechanism of RAF-acquired resistance. They generated five independent vemurafenib-resistant melanoma cell lines by exposing the lines to high doses of the drug for 2 months. Three of the five clones were found to have an in-frame deletion of exons 4 to 8 (61kDa), including a domain critical for RAF activation, the RAS-binding domain. This \textit{BRAF} V600E variant, p61 \textit{BRAF} V600E, showed enhanced dimerization in cells with low levels of RAS activation compared with full-length \textit{BRAF} V600E. In cells that express p61 \textit{BRAF} V600E, ERK signaling was resistant to RAF inhibition. Mutations that eliminated the dimerization of p61 \textit{BRAF} V600E restored RAF inhibitor sensitivity. In addition, \textit{BRAF} splice variants lacking the RAS-binding domain were identified in the tumors of 6 of the 19 patients with acquired RAF inhibitor resistance.\textsuperscript{82} These data suggest that effective RAF inhibition is dependent on levels of RAS–guanosine triphosphate (GTP) too low to support RAF dimerization, and alterations in BRAF structure that alter its function in the MAPK pathway affect sensitivity to targeted agents.

\textbf{MEK and ERK as Therapeutic Targets:} High constitutive activation of the MAPK pathway exists in the vast majority of melanomas. Because of this, as well as the eventual resistance of \textit{BRAF} V600E-mutant melanoma to BRAF inhibition, recent efforts have focused on other targets in the MAPK cascade. Several preclinical studies have demonstrated that the inhibition of MEK in melanoma cells led to G\textsubscript{1} phase cell cycle arrest, which was associated with cyclin D inhibition, increased p27\textit{KIP1} expression, and decreased phosphorylation of RB1 protein.\textsuperscript{43,85,86}

Early clinical testing of CI1040 and PD-0325901, two small molecule MEK inhibitors, did not show sufficient antitumor activity and proved too toxic to move beyond phase I/II trials. CI1040, an ATP noncompetitive inhibitor against MEK1/2, did not show any partial or complete responses in a multicenter phase II trial.\textsuperscript{87} Similarly, the MEK1 inhibitor PD-0325901 did not demonstrate any objective responses in a phase II study of patients with lung cancer.\textsuperscript{88} A phase I trial of PD-0325901, which included patients with melanoma, was halted early due to toxicity (NCT00147550).\textsuperscript{89}

However, promising results have targeted MEK in patients with advanced melanoma. Selumetinib is a selective non–ATP competitive inhibitor of \textit{MAP2K1} (MEK1) and \textit{MAP2K2} (MEK2).\textsuperscript{90} In both preclinical and clinical studies, selumetinib showed favorable pharmacological characteristics with limited toxicological effects. In the phase I trial of selumetinib, 1 patient with \textit{BRAF}-mutant melanoma treated with selumetinib demonstrated a complete durable response that persisted for more than 15 months.\textsuperscript{91} In a phase II study in which selumetinib was compared with temozolomide in unselected patients with melanoma, 6 of 104 patients treated with selumetinib achieved a partial response.\textsuperscript{92} Although no difference in PFS occurred, patients with \textit{BRAF}-mutant lesions had an 11% response rate, and 5 of the 6 responding patients had tumors harboring oncogenic \textit{BRAF} V600E.

Trametinib, a potent, selective inhibitor of \textit{MAP2K1}, has also demonstrated inhibition of phosphorylated ERK, resulting in cell death.\textsuperscript{93} In a phase I study of 20 patients with \textit{BRAF}-mutation melanoma, 5 achieved a partial response and a reduction in tumor burden by 50%.\textsuperscript{94} Eight patients had stable disease. Eleven of these patients had \textit{BRAF}-mutant melanoma, with 3 achieving partial responses, 5 achieving stable disease, and 3 demonstrating progressive disease. The
phase II trial also produced promising results. Of the 57 patients with 
\textit{BRAF}-mutant melanoma previously treated with immunotherapy or chemotherapy, complete responses were seen in 2 patients, partial responses in 13, and stable disease in 29. The disease control rate was 75%, with a response rate of 25%. This was similar between 
\textit{BRAF} mutation types V600E and V600K. In patients previously untreated with a BRAF inhibitor, the median PFS was 4 months, with a median duration of response of 5.7 months. However, in patients previously treated with a BRAF inhibitor, response rates were reduced when patients were treated with trametinib. The phase III open-label trial results of 322 patients with metastatic melanoma with 
\textit{BRAF} V600E or 
\textit{BRAF} V600K mutations randomized to receive trametinib or chemotherapy (dacarbazine or paclitaxel) were recently published.99 Patients who progressed on chemotherapy were allowed to cross over to receive the MEK inhibitor. The median PFS was 4.8 months in the trametinib group and 1.5 months in the chemotherapy group. Six-month OS rates were 81% for the trametinib group and 67% for the chemotherapy group despite patient crossover. Therefore, although BRAF inhibitors still yield the best clinical responses to date, trametinib demonstrated the greatest clinical efficacy of the MEK inhibitors thus far evaluated in cases of 
\textit{BRAF}-mutant melanoma.

ERK inhibitors such as AEZS-131 and SCH772984 are currently being developed and tested. Data on the clinical efficacy of these compounds are not yet published; however, the results of such studies may be of future use in treating resistant 
\textit{BRAF}-mutant melanoma.

Advances in the field of mutational screening identifying novel, actionable mutations in RAF and other pathway molecules are likely to present therapeutic targets for MEK and ERK inhibition, highlighted by the work of Dahlman et al\textsuperscript{97} in which a somatic 
\textit{BRAF} L597R mutation was identified in exon 15 in wild-type 
\textit{BRAF} V600E melanoma during whole genome sequencing. In vitro, this 
\textit{BRAF} L597R-mutant melanoma showed sensitivity to MEK inhibition. In addition, a patient with 
\textit{BRAF} L597R-mutant melanoma had radiographically proven tumor regression to treatment with TAK-733, a MEK inhibitor, suggesting that routine screening and therapy of 
\textit{BRAF} L597R mutations should be initiated. Although single-mutation interrogation studies lack depth and whole genome sequencing is costly, the authors advocate for multiplex tests to allow for broader screening capabilities by testing for common mutations concurrently such as 
\textit{BRAF} (V600E/K/R/D), \textit{NRAS} (G12/13, Q61), \textit{KRAS} (G12, Q61), and several known alterations in \textit{Akt}, \textit{MEK}, \textit{EGFR}, \textit{ERBB2} (HER2/neu), and \textit{PIK3CA}. One such assay simultaneously screens for several known melanoma mutations such as 
\textit{BRAF} (V600E/K/M/R/D), \textit{NRAS} (G12/13, Q61), \textit{KIT} (W557, V559, L576, K642, and D816), \textit{GNAQ} (Q209), and \textit{GNA11} (Q209) and provides similar advantages, thus allowing for both cost effectiveness and comprehensiveness.\textsuperscript{97} These tests are advantageous because they concurrently examine several genes and require a smaller quantity of DNA per test. Thus, moving forward, these assays will allow for a more efficient identification of commonly mutated genes and reserve the more costly, whole genome sequencing for screening less frequent mutations, which may represent future pharmacological targets in wild-type melanoma.

\textbf{Combination Therapy:} Due to the limited duration of clinical efficacy and the ultimate development of resistance to BRAF inhibitors, dual pathway inhibition and therapeutic combinations of different pharmacological classes of are currently being investigated in the treatment of 
\textit{BRAF} V600E-mutant melanoma. A phase I/II trial is underway in patients with 
\textit{BRAF} V600E-mutant melanoma examining the combination treatment of the BRAF inhibitor, vemurafenib, and the anti-CTLA-4 monoclonal antibody ipilimumab (NCT01400451).

Because MEK inhibition alone has not been found to induce high levels of cell death, dual pathway inhibition or combination therapies may be needed to increase efficacy. Reactivation of MAPK signaling is commonly found in BRAF inhibitor-resistant melanoma; however, the combination of MEK and BRAF inhibitors has been effective in overcoming resistance mediated by specific molecular aberrations such as 
\textit{COT} overexpression, \textit{MEK1} mutations, \textit{RAS} mutations, and 
\textit{BRAF} truncations.\textsuperscript{66,77,82,83} This suggests that treatment with combination therapy, in which multiple alterations in more than one pathway are overcome, may be required to effectively treat 
\textit{BRAF}-mutant melanoma.

A phase I/II trial combining the inhibitors of BRAF and MEK demonstrated promising results.\textsuperscript{98} In 
\textit{BRAF}-mutant tumors, including melanoma, combination therapy using the MEK inhibitor trametinib and the BRAF inhibitor dabrafenib in 16 evaluable patients resulted in 13 patients with a partial response, 3 with stable disease, and an objective response rate of 81%. Another trial combining the MEK inhibitor GDC-0973 with vemurafenib is underway (NCT01271803).

\textit{BRAF} V600E may be a negative regulator of the Akt pathway. The presence of the 
\textit{BRAF} V600E mutation suppresses MEK inhibitor (RG7167)- or mTOR inhibitor-induced Akt phosphorylation and downstream activation. Aberrant activation of the Akt pathway by PTEN loss overrides the negative impact of 
\textit{BRAF} V600E on phosphorylated Akt, suggesting that induction of the PI3K/Akt cascade through the loss of PTEN controls the sensitivity to targeted inhibition.\textsuperscript{99,100} Due to the dual activation of RAS/RAF/MEK/ERK and the PI3K/Akt cascades in melanoma progression and the
role of enhanced Akt signaling in BRAF resistance, clinical trials have commenced to test combinations of agents targeting both the MAPK and PI3K pathways (NCT01347866, NCT01363232, NCT01392521, NCT01357765, NCT01390818).

Combinations of MEK and PI3K inhibition or MEK inhibition combined with chemotherapy agents such as paclitaxel have shown some enhanced antitumor effects.85,101 IGF-1R mediated resistance has been effectively treated with dual MEK and PI3K inhibition, while PDGFR-α mediated resistance has been overcome by inhibiting the mTOR/PI3K/Akt pathway.84,102 Lastly, the PI3K/mTOR inhibitor BEZ235 in combination with the MEK inhibitor MEK162 is undergoing clinical evaluation for the treatment of melanoma (NCT01357765).27,84,101,105

NRAS Mutations
Oncogenic Mechanism of Action
The NRAS family of GTPases consists of the homologous proteins KRAS, HRAS, and NRAS. The N-terminus is common to all proteins; the C-terminal end, or the hypervariable region, is where RAS proteins structurally diverge.101 These proteins are small GT-Pases that alternate between the inactive form bound to guanosine diphosphate (GDP) and the active form bound to GTP.105,106 RAS is a regulator of cellular responses to extracellular stimuli, including growth factors. When growth factors bind to cell-surface receptors, intracellular binding sites are created for molecules to then recruit and activate guanine nucleotide-exchange factors (GNEFs). These activate RAS by displacing guanine nucleotides, allowing the passive binding of GTP in the cytosol to RAS. GTP-RAS can then stimulate multiple effector pathways, including PI3K, RAF, and Ral guanine nucleotide-dissociation stimulator to regulate cell proliferation, survival, and differentiation.107,108 RAS proteins are negatively regulated by GTPase activating proteins (GAPs). These proteins profoundly enhance intrinsic GTPase activity by providing stability to the high-energy transition state that occurs during the hydrolysis reaction of RAS-GTP. Glutamine at position 61 is required for GTP hydrolysis, and the substitution of any other amino acid at this position prevents hydrolysis. Similarly, replacing glycine at position 12 of RAS with any other amino acid except proline also results in constitutively activated RAS.109

RAS-GTP binds to and activates several classes of effector molecules, including class 1 PI3K, RAF kinases, Ral guanine exchange factors (RalGEFs), the RAC exchange factor TIAMI, and phospholipase C.110 The RAF/MEK/ERK (MAPK) cascade is the most thoroughly described RAS effector pathway.108 Strong evidence exists to support that the RAF/MEK/ERK pathway is critical for RAS-induced cell proliferation, migration, and survival.111 When triggered by RAS, the three RAF serine/threonine kinases ARAF, BRAF, and RAF1 serve to activate the MEK/ERK kinase cascade. ERK kinases can phosphorylate substrates located in both the nucleus and the cytosol, including transcription factors such as JUN and ETS domain-containing protein (ELK1). Activation of these regulators of transcription can lead to the expression of proteins that influence cell cycle progression such as cyclin D1, which is required for RAS-dependent malignant transformation.112-114

RAS also plays a role in the PI3K/Akt cascade. RAS-GTP binds to class I PI3K at the catalytic subunit, which results in the transfer of PI3K to the plasma membrane where it becomes activated.115,116 PI3K phosphorylates phosphatidylinositol-4,5-biphosphate (PIP2) to generate phosphatidylinositol-3,4,5-triphosphate (PIP3), which further triggers downstream kinase activation of ligands such as 3-phosphoinositide-dependent protein kinase 1 (pDK1) and Akt.117 Akt is a kinase that phosphorylates, and therefore inactivates, several proapoptotic proteins, serving to enhance survival of many cell types.118,119

Approximately 30% of all human cancers contain oncogenic RAS mutations. KRAS mutations are most prevalent in human neoplasms, including pancreatic, colorectal, endometrial, biliary tract, lung, and cervical malignancies.120 Mutations in NRAS, which codes for a small GTP-binding protein, are usually found in primary melanoma and melanoma metastases, with approximately 15% to 18% of cases containing mutations.20,121-124 The most frequent sites of somatic missense mutations are exons 1 (codon 12) and 2 (codon 61), or, less commonly, exon 13.125,126 More than 80% of HRAS and NRAS mutations involve position Q61; however, approximately 70% of NRAS mutations in melanoma occur at position G12.127 These mutations lead to impaired GTPase activity, the accumulation of RAS-GTP, and a lack of sensitivity to the normal regulation of RAS signaling by guanine nucleotide exchange factors (GEFs; Sos1/2, RASGRP1-4, and RAS-GRF1/2 proteins) and GAPs (nuclear factor 1 [NF1] and p120GAP).110,128

Both NRAS and BRAF mutations can be found in benign neoplasms such as cutaneous nevi, suggesting that other genetic alterations are required for malignant transformation of these lesions.17 The vast majority of activating mutations BRAF and NRAS are mutually exclusive, which suggests that they may function on the same pathway.18,124,129,130 PTEN loss, a common genetic alteration in melanoma, is uncommon in NRAS-mutant melanomas. This finding in melanoma cell lines suggests that PTEN and the neuroblastoma RAS viral oncogene homolog (NRAS) may function in a common molecular cascade.131 Together, these findings indicate that NRAS-mutant melanomas activate
both the RAS/RAF/MEK/ERK pathway and the PI3K/Akt pathway to induce malignant transformation.

NRAS and BRAF mutations are not equally distributed among all types of melanoma. Curtin et al. analyzed genome alterations in DNA copy number and BRAF and NRAS mutational status in four distinct groups of melanoma that differed by levels of ultraviolet light exposure, anatomical location, or both. These types of melanoma — melanoma arising from CSD skin, melanoma arising from skin without CSD, acral melanoma, and mucosal melanoma — demonstrated varying rates of NRAS and BRAF mutations. In skin lacking damage due to prolonged ultraviolet light, mutations in NRAS and BRAF occurred in 81% of melanomas. Alternatively, acral or mucosal melanomas often were wild-type for BRAF and NRAS mutations, but they demonstrated an increase in DNA copy number for cyclin-dependent kinase 4 (CDK4) and cyclin D1, downstream substrates of NRAS. The majority of melanomas found on CSD skin were also wild-type for NRAS and BRAF mutations (76%) and were significantly more likely to have gains involving the cyclin D1 gene \( (P = .001) \), losses involving chromosome 4q \( (P = .004) \), and gains involving regions of chromosome 22 \( (P = .004) \) than melanomas from non–CSD skin, suggesting that the development of BRAF and NRAS mutations may be unrelated to ultraviolet damage.21 Several reports suggest that NRAS-mutant tumors are distinct from BRAF-mutant melanoma in both pathological and clinical behavior, and several studies have shown that NRAS-mutant tumors are correlated with greater thickness when compared with BRAF V600E-mutant or wild-type tumors.\(^{125,129,130}\) Conflicting evidence exists on whether or not NRAS-mutant status correlates with survival outcomes. Multiple studies have found on multivariate analyses that the presence of an NRAS mutation, when compared with wild-type tumors, is an adverse prognostic factor for melanoma-specific survival (hazard ratio [HR], 2.96; \( P = .04 \))\(^{120}\) and OS rates (HR, 2.05; \( P = .005 \)).\(^{132}\) NRAS was also found to correlate with higher rates of mitosis.\(^{129}\) Other studies examining hundreds of primary melanoma specimens found that NRAS-mutant melanomas were more often located on the extremities.\(^{130,132}\) In addition, in comparison to wild-type tumors, one study found that melanomas containing NRAS and BRAF mutations were more likely to have advanced stage at diagnosis, according to the American Joint Committee on Cancer classification.\(^{130}\) This study found no survival difference between wild-type, NRAS-mutant, or BRAF-mutant melanoma. One possible explanation for the correlation of mutant NRAS with increased tumor thickness is that activating RAS mutations may be associated with the vertical growth phase of melanoma. Evidence to support this theory includes the phenotypical changes seen with RAS oncogene transfection into melanoma cell lines, which include an epithelioid cell morphology, increased protease production, and increased cell motility, all of which are features of the vertical growth phase of melanoma.\(^{29,133}\)

Oncogenic NRAS may play a pivotal role in melanomas containing less common BRAF (non–V600E) mutations. Davies et al. showed that when melanoma cell lines were microinjected with Y13-259, a RAS-targeting monoclonal antibody, proliferation was not inhibited in cell lines containing BRAF V600D/E mutations. However, in cell lines containing less common BRAF mutations (G464V, G463V, and L596V), of which two also harbored RAS mutations, proliferation was inhibited by Y13-259. This finding suggests that BRAF V600E-mutant melanoma does not require NRAS for BRAF signaling, whereas other BRAF mutations still require RAS interaction for activation.

**Therapeutic Considerations**

The role of oncogenic NRAS in the growth and progression of melanoma suggests the importance of developing therapies to target mutant NRAS in melanoma. Therapeutic approaches to the treatment of NRAS-mutant melanoma include (1) targeting the downstream signaling pathways activated by NRAS, (2) inhibiting NRAS expression through siRNA, and (3) inhibiting the membrane translocation of NRAS. Direct inhibition of NRAS has been challenging. RAS possesses a high affinity for GTP. That fact, combined with the high concentrations of GTP in the cytosol, has hindered the development of small molecules to prevent the buildup of RAS-GTP because of the complexity in repairing the defective GTPase activity in mutant NRAS and in disallowing its return to an inactive GDP-RAS form.\(^{134}\)

RAS activation is heavily dependent on translocation to the inner layer of the cell membrane. This localization requires farnesylation, a post-translational process mediated by enzymes called farnesyltransferases.\(^{135}\) Therefore, farnesyltransferase inhibitors (FTIs) have been an area of interest in targeting RAS. Niessner et al. examined the role of lonafarnib, one such FTI, alone and in combination with MAPK or Akt pathway inhibitors on cell proliferation, survival, and tumor growth of melanoma cell lines, two of which harbored NRAS mutations. Lonafarnib alone or in combination with Akt pathway inhibitors did not alter melanoma growth or survival. Combinations of lonafarnib and MAPK pathway inhibitors demonstrated some decrease in cell growth. In particular, the combination of lonafarnib and sorafenib demonstrated synergistic effects on the inhibition of melanoma cell growth, with enhanced apoptosis and suppressed tumor growth. Lonafarnib affected mTOR signaling,
although it had no effect on MAPK or Akt signaling. These findings suggest that lonafarnib inhibits mTOR signaling and enhances sorafenib-induced cell death in melanoma cells.

**MEK as a Therapeutic Target:** MEK and ERK both rely on RAS for activation. Thus, they present attractive downstream therapeutic targets in NRAS-mutant melanoma. Solit et al. demonstrated that some melanoma cell lines harboring NRAS mutations are sensitive to MEK inhibition. Dedicated trials in patients with NRAS-mutant melanoma have not been conducted; however, clinical studies of MEK inhibitors included patients with NRAS-mutant melanoma. Although few studies revealed no clinical efficacy or survival benefit, more recent data suggest that MEK inhibition may be of clinical use. In a phase II study, MEK162, a selective inhibitor of MEK1/2, demonstrated antitumor effects in patients with both BRAF- and NRAS-mutant melanoma. Among the 29 patients evaluable for efficacy, 13 had tumors harboring an NRAS mutation. In addition to patients with BRAF-mutant melanoma who demonstrated a response to MEK162 by Response Evaluation Criteria in Solid Tumors (RECIST) criteria, 2 patients with confirmed partial responses, 1 with an unconfirmed partial response, and 4 with stable disease were seen in the mutant NRAS group. This was the first trial in which a targeted molecular agent showed activity against NRAS-mutant melanoma.

In melanoma, multiple mechanisms by which tumors are able to acquire resistance to therapy have been described. NRAS oncogenic mutations can be acquired or NRAS can be upregulated as part of the BRAF V600E resistance to RAF inhibitors. Subsequent knockdown of acquired mutant NRAS in these cells reduced the reactivated signaling through MEK and ERK and reduced growth of BRAF inhibitor-resistant tumors. Often ERK signaling is reactivated as part of these resistance mechanisms, which has led to trials assessing the combination of MEK and BRAF inhibitors. This combination may be effective for the treatment of BRAF-mutant tumors secondarily harboring acquired NRAS mutations. However, it is unlikely that BRAF inhibitors such as vemurafenib or dabrafenib in combination with MEK inhibitors have therapeutic activity in primary NRAS-mutant tumors. This is due to the fact that in NRAS-mutant tumors in which BRAF mutations are lacking, BRAF inhibitors are unable to block RAF signaling.

Recent data suggest that other combinations of targeted agents, such as CDK4 and MEK, may be effective for the treatment of NRAS-mutant melanomas. Using an inducible mouse model of NRAS-mutant melanoma, inhibition of MEK with either selumetinib or trametinib induced apoptosis and caused tumor stasis but did not trigger cell cycle arrest. By contrast, genetic extinction of NRAS Q61K resulted in both cell cycle arrest and apoptosis, causing tumor regression. Further investigation using network modeling concluded that CDK4, a regulator of the G1/S cell-cycle checkpoint, was a key driver of the phenotypic difference between MEK inhibition and mutant NRAS extinction. In vivo, the combination of MEK inhibition using selumetinib or trametinib and CDK4 inhibition using palbociclib, a dual selective inhibitor of CDK4 and CDK6, led to both cell cycle arrest and apoptosis and resulted in clear tumor regression. This therapeutic synergy was apparent both in a xenograft model and in NRAS-mutant human melanoma cell lines, suggesting novel pathway targets for the future treatment of NRAS-mutant melanoma.

**PI3K/Akt as a Therapeutic Target:** RAS has multiple downstream effectors and is involved in both the MEK/ERK and PI3K pathways. Recent evidence suggests that the dual inhibition of the MEK/ERK and PI3K pathways may be effective for the treatment of RAS-mutant tumors due to interdependence of the two pathways. Evidence of pathway codependence includes the finding that BRAF or NRAS mutations promote the formation of benign nevi, but the activation of ERK inhibits further tumor development. Akt3 activation is then required to bypass the effect of ERK and allow for the progression to melanoma. In addition, Akt3 has been shown to promote melanoma proliferation through the phosphorylation of Ser364 and Ser428 on BRAF V600E, reducing the levels of BRAF V600E to levels that promote tumor spread. Jaiswal et al. provided further evidence to support the use of this dual pathway inhibition. Using an in vivo mouse model of NRAS-mutant melanoma, they showed that inhibiting NRAS alone or the downstream effectors of mutant NRAS leads to cell cycle arrest and cell death. This further emphasizes the concept that to treat melanoma tumors containing mutant NRAS, the sustained downstream inhibition of Akt and ERK is required.

**mTOR as a Therapeutic Target:** mTOR, a serine/threonine kinase, is activated downstream in the PI3K pathway and stimulates cell proliferation through control of cell-cycle progression regulators and may present another target in NRAS-mutant melanoma. A phase II clinical trial in patients with advanced melanoma examined sorafenib in combination with tipifarnib, an FTI, or temsirolimus, an mTOR inhibitor, and failed to show significant clinical efficacy. However, patient mutational status was not assessed in this study, thus limiting the ability to determine whether the drugs would be effective for the treatment of NRAS-mutant melanoma. Lastly, although no therapies exist that specifically target mutant NRAS, clinical trials are currently underway exploring the use of dual therapy against downstream substrates of NRAS.
**KIT Mutations**

**Oncogenic Mechanism of Action**

The type III transmembrane receptor tyrosine kinase KIT gene was first described in 1987 by Yarden et al.\(^\text{145}\) and Woodman et al.\(^\text{146}\) Structurally, KIT is characterized by five unique domains: a glycosylated extracellular ligand-binding domain containing five immunoglobulin-like repeats (encoded by exons 1–9), a hydrophobic transmembrane domain (encoded by exon 10), an intracellular segment consisting of a juxtamembrane domain (encoded by exon 11), and two intracellular tyrosine kinase domains separated by a kinase insert region (encoded by exons 12–21).\(^\text{146,147}\) Distinct domains serve unique functional roles. The juxtamembrane domain serves an autoinhibitory role; when a ligand is absent, it prevents KIT activation. Activation occurs with binding of stem cell factor, a glycosylated transmembrane protein and the ligand of KIT, to the extracellular domain, causing the KIT tyrosine kinase receptor to dimerize. This dimerization results in autophosphorylation of the intracellular tyrosine kinase domains. Activated KIT then initiates signaling through a variety of downstream pathways, including in the MAP/MEK/ERK, PI3K/Akt, and JAK/STAT pathways.\(^\text{146-148}\) Loss-of-function studies have shown that KIT activation and downstream pathway signaling may be important in multiple types of cells, including germ cells, mast cells, hematopoietic stem cells, and the interstitial cells of Cajal.\(^\text{149-151}\)

Several studies have demonstrated the significant role of KIT in the development of normal melanocytes. Mutations resulting in loss of functional KIT in mice and humans lead to loss of pigmentation of fur and skin/hair, respectively.\(^\text{152-155}\) Although now recognized as an oncogene, KIT was initially believed to serve as a melanoma tumor suppressor. Transfection of the highly metastatic melanoma cell line A375SM with KIT led to reduced tumor growth and metastases in mice injected with A375SM-KIT cells. In addition, KIT-positive melanoma tumor cells demonstrated a higher level of apoptosis when stem cell factor was given.\(^\text{156}\) Other studies demonstrated that loss of KIT expression correlated with malignant melanocytic transformation, invasion, and metastases.\(^\text{157,159}\)

Much of the current knowledge of KIT and its function as an oncogene has come through experience with other tumor models, most significantly GISTs. Activating mutations in KIT have been identified both in and outside of the juxtamembrane domain in GIST tumors.\(^\text{160,161}\) Imatinib mesylate, a tyrosine kinase inhibitor known to target KIT,\(^\text{162}\) was administered in a phase II trial in which patients with unresectable or metastatic GISTs received 400 or 600 mg of the drug daily.\(^\text{163}\) In this trial, 79 out of 147 patients (53.7%) had a partial response and 41 patients (27.9%) had stable disease. This trial revolutionized the concept of targeted therapy and advocated for the use and efficacy of targeted therapy in the treatment of malignancies harboring specific mutations.

More recently, KIT mutations have been identified in melanoma. Similar to BRAF and NRAS mutations, KIT mutations in melanoma have different frequencies depending on the distinct type of melanoma. Although BRAF and NRAS mutations most often arise from non-CSD skin,\(^\text{21}\) KIT-activating mutations and/or increased copy number are more common in acral and mucosal melanomas and in those arising from CSD skin.\(^\text{164-166}\) In addition, distinct anatomical locations may have unique molecular characteristics.\(^\text{167,168}\) For example, vulvar melanomas have been found to contain a higher rate of KIT mutations than melanomas arising from other sites, including vaginal melanomas.\(^\text{168}\)

The prevalence of KIT mutations in melanoma varies by domain. Exon 9, which encodes for the extracellular domain, is mutated in 6% of cases. Exon 11, which encodes for the juxtamembrane domain, is mutated in 46% of cases. Exon 13, which encodes for the proximal tyrosine kinase domain, is mutated in 19% of cases. Lastly, exons 17 and 18, which encode for the distal tyrosine kinase domain, are mutated in 10% and 19%, respectively.\(^\text{169}\)

**Therapeutic Considerations**

Of the 102 melanomas analyzed by Curtin et al,\(^\text{165}\) the majority of KIT mutations identified affected exon 11, encoding the juxtamembrane domain. Mutations in this domain, which is responsible for the autoinhibition of KIT when unbound to its ligand, result in enhanced ligand-independent receptor dimerization and activation.\(^\text{170}\) GIST tumors that possess exon 11 mutations of KIT are sensitive to imatinib treatment,\(^\text{171}\) suggesting that KIT-mutant melanoma may similarly respond to this targeted therapy.

Studies have examined KIT sensitivity to tyrosine kinase inhibitors in vitro. One such study by Antonescu et al.\(^\text{172}\) examined anal tumors with KIT exon 11 L576P substitutions. The authors demonstrated sensitivity of exon 11 KIT L576P-mutant tumors to dasatinib as well as to imatinib. Another study similarly showed KIT L576P-mutant cell line sensitivity to dasatinib but not to imatinib, nilotinib, or sorafenib.\(^\text{173}\)

Other studies demonstrated in vitro sensitivity of KIT-mutated acral and mucosal melanomas to KIT inhibitors suitinib\(^\text{174}\) and imatinib.\(^\text{175}\)

**Early Clinical Trials and Case Reports of KIT**

**Treatment:** Early phase II trials of imatinib in the treatment of metastatic melanoma demonstrated limited clinical responses to therapy. This was largely attributed to the fact that the presence of KIT mutations in melanoma was not known at the time these studies were initiated; thus, patient selection by molecular criteria was not possible.\(^\text{176}\) Several studies assessed for
KIT expression by immunohistochemistry in a subset of enrolled patients, with many demonstrating weakly positive KIT staining.\textsuperscript{177,178} Similar findings were documented in a phase II study of dasatinib for the treatment of patients with melanoma.\textsuperscript{179} Patients were not selected for enrollment based on documented mutational status; however, 1 patient who harbored an exon 13 KIT mutation did exhibit a response to treatment.

Although these early phase II clinical trials were a disappointment in the efforts to develop targeted treatments for melanoma, smaller case reports support the ongoing investigation of targeted molecular therapy against KIT-mutant tumors. Two unique case reports involving 2 women 79 years of age with recurrent metastatic rectal melanoma treated with imatinib were documented to have significant benefit.\textsuperscript{180,181} The first woman was found to have a 7-codon duplication in exon 11 of KIT and strong immunohistochemistry staining for KIT.\textsuperscript{180} With a dose of 400 mg daily of imatinib, she achieved measurable reduction in metastatic burden, as well as symptomatic relief of her rectal and vaginal bleeding. The second patient had an exon 11 KIT L576P missense mutation.\textsuperscript{181} She also had a documented reduction in metastatic burden and improvement in her performance status with treatment. In several other case reports, patients harboring melanoma characterized by KIT mutations demonstrated major durable responses to treatment with other KIT tyrosine kinase inhibitors such as nilotinib, dasatinib, sunitinib, and imatinib.\textsuperscript{175,182-184}

**Selected Clinical Trials of Mutant KIT:** Based on the findings in these case reports that suggest the efficacy of imatinib in KIT-mutant melanoma, three subsequent phase II trials were conducted. Unlike prior studies, documentation of somatic alterations of KIT (either mutation or amplification) was required for enrollment.

In one study led by the Memorial Sloan-Kettering Cancer Center, 25 patients with melanoma arising from acral, mucosal, or CSD skin were treated with imatinib 400 mg twice daily.\textsuperscript{169} Of the evaluable 25 patients, 6 achieved a radiographic response, and 4 experienced durable responses, each lasting for more than 1 year. Two patients achieved complete responses lasting 94 and 95 weeks, 2 durable partial responses lasting 53 and 89 weeks, and 2 transient partial responses lasting 12 and 18 weeks. Specific KIT mutations corresponded to clinical responses. Exon 11 KIT L576P and exon 13 KIT K642E mutations were stronger predictors of clinical response. All 6 patients who responded to imatinib carried an exon 11 KIT L576P or an exon 13 KIT K642E mutation. Although 2 patients who progressed harbored exon 11 KIT L576P mutations, both patients with complete responses to imatinib had this alteration as well as concomitant amplification.

Similar results were shown in a phase II trial of Chinese patients with metastatic melanoma harboring KIT alterations treated with imatinib 400 mg daily (NCT00881049).\textsuperscript{185} Of 43 evaluable patients, 10 (23.3%) had a partial response, 13 (30.2%) had stable disease, and 20 (46.5%) demonstrated progressive disease (46.5%). Similar to the findings by Carvajal et al,\textsuperscript{169} 9 of the 10 patients demonstrating partial responses harbored mutations in exon 11 or 13. These findings suggest that patients with specific KIT mutations, such as exon 11 and 13, may benefit from targeted therapy more than those without such mutations.

Second-generation dual Src/Bcr/Abl receptor tyrosine kinase inhibitors such as nilotinib and dasatinib are also being studied. Dasatinib has demonstrated preclinical activity in KIT-mutant melanoma, especially in patients harboring the exon 11 L576P mutation. Presentation of preliminary results of a phase II trial of dasatinib in patients with unresectable locally advanced or stage IV mucosal, acral, or solar melanomas demonstrated poor response in patients with wild-type KIT.\textsuperscript{186} Of the 54 patients in the trial (26 mucosal, 13 acral, 15 solar melanomas), 1 had a complete response (unknown KIT status), 2 achieved a partial response, 13 had stable disease, 29 had disease progression, and 6 were unevaluable. Forty of the 54 patients died. KIT status was assessed in 42 patients, with 39 wild-type KIT (93%) and 3 KIT-mutant tumors (7%). All patients with wild-type KIT and 2 of the 3 patients with mutant KIT died. This prompted a modification to include KIT-mutant patients, given the lack of efficacy in patients with wild-type KIT melanoma. Accrual to this amended protocol is ongoing.

**KIT Resistance:** Primary and secondary resistance to imatinib can arise. Potential mechanisms of secondary resistance include the development of secondary KIT or NRAS mutations, genomic KIT amplifications, activation of downstream pathway signaling, and decreased imatinib plasma concentrations due to inhibitor-binding proteins.\textsuperscript{187} Overcoming these mechanisms may require the selection of alternative KIT inhibitors specific for resistant mutant tumors, combination therapy of several KIT inhibitors, or a dual blockade with KIT and other pathway inhibitors.

Several lessons on secondary resistance mechanisms of KIT can be learned from the GIST model to improve efficacy in the future treatment of melanoma. In GIST, the development of secondary mutations, especially those in the tyrosine kinase domains encoded by exons 13 and 17, leads to the development of secondary resistance.\textsuperscript{188} Study results in which patients with acral, mucosal, or CSD melanoma were tested for KIT and other molecular mutations revealed that patients with KIT mutations had clinically effective (both partial and complete) responses to treatment with sunitinib.\textsuperscript{184} A patient with mutant KIT
who initially had no other detectable mutations in 
*BRAF* or *NRAS* responded to treatment with sunitinib; however, the patient progressed after 7 months and the resistant tumor was found to harbor a new *NRAS* Q61K mutation in addition to the previously present *KIT* mutation. Subsequent treatment with imatinib was not effective. Thus, the development of additional mutations may contribute to tyrosine kinase inhibitor resistance in melanoma.

Si et al\(^{189}\) reported a patient with nasal cavity mucosal melanoma harboring an exon 11 *KIT* mutation. Although the patient initially achieved a partial response to imatinib, she progressed after 1 year. Subsequent evaluation of the tumor recurrence showed no new mutations; however, the increased phosphorylation of Akt and ERK1/2 was seen in samples obtained following imatinib treatment compared with tumor samples taken before imatinib treatment. No increased expression of cyclin D or phosphorylated MEK1/2 was present, suggesting that the mTOR pathway was selectively activated. When treated with the mTOR inhibitor everolimus, the patient achieved a partial response, with an overall tumor shrinkage of 35%.

In GIST, treatment with alternative KIT inhibitors following progression on imatinib has been effective.\(^{190}\) Such a strategy in melanoma is being tested in a phase II study for *KIT*-mutant melanoma in which nilotinib is being administered after resistance or intolerance to prior KIT inhibitor therapy (NCT01028222). Nilotinib has similar potency to imatinib but differs in that it is rapidly transported into the cell via passive diffusion as opposed to the time-dependent, active transport of imatinib.\(^{191}\) When treated with the mTOR inhibitor everolimus, the patient achieved a partial response, with an overall tumor shrinkage of 35%.

In patients with UM that is wild-type for *KIT*, activation of the MAPK pathway in UM is critical to the development and progression of this malignancy.\(^{197}\) Guanine nucleotide-binding protein Q polypeptide (*GNAQ*) encodes for the alpha subunit of a heterotrimeric G protein (Gaq), which couples transmembrane domain receptors to intracellular signaling pathways such as MAPK in 45% to 50% of patients with UM.\(^{198,199}\) The alpha subunit serves as the molecular control for the G protein, which is active when bound to GTP and inactivated when GTP is hydrolyzed to GDP.\(^{200,201}\) If alterations of specific glutamine or arginine residues exist in the alpha subunit that contact the GTP molecule, then its fundamental GTPase activity is obstructed. Thus, the alpha subunit locks the G protein in a constitutively active, GTP-bound state (Fig 3).\(^{201,202}\) An activating somatic mutation in this critical glutamine in exon 5, the RAS-like domain of *GNAQ*, located at position 209 (Q209) in Ga, is mutated to either a leucine or proline in melanocytic lesions (designated as *GNAQ* Q209L/P).\(^{199}\) Van Raamsdonk et al\(^{199}\) sequenced the entire coding region of *GNAQ* in both benign and malignant melanocytic tumors. They found 46% of primary UMs contained mutations in the glutamine residue at position 209 of *GNAQ*. In mice, this mutation leads to the melanocytic proliferation and cooperates with other oncogenes to cause malignant transformation.\(^{199,203}\) Onken et al\(^{204}\) also independently identified activating mutations of *GNAQ* at position 209 in 49% of primary UM specimens, including 22% of iris melanomas and 54% of posterior UMs.

Human melanocytes, when transfected with mutant *GNAQ* Q209L, exhibited increased levels of phosphorylated ERK compared with control cells transfected with wild-type *GNAQ* or an empty vector.\(^{199}\) These transfected human melanocytes containing *GNAQ* Q209L demonstrated anchorage-independent growth. When nude mice were injected with melanocytes transfected with *GNAQ* Q209L, heavily pigmented tumors developed, which was not observed with cells transfected with wild-type *GNAQ*. In addition, knockdown of *GNAQ* in *GNAQ*-mutated UM cell lines resulted in a reduction of phosphorylated ERK levels, a reduction in cell number, and a loss of anchorage-independent growth. These findings are also demonstrated when OMM1-3, a UM cell line containing the *GNAQ* Q209L mutation, is treated with the MEK inhibitor UO126.\(^{199}\)

In patients with UM that is wild-type for *GNAQ*, more than 50% possess a gain-of-function mutation in *GNA11*, a paralog of *GNAQ*.\(^{205}\) *GNA11*, similar to *GNAQ*, encodes for a member of the G class of G-protein alpha subunits and signals through phospholipase C (PLC) and protein kinase C (PKC). The *GNA11* exon 5 mutation most commonly seen results in a Q209L substitution, which is identical to that seen in *GNAQ*. *GNAQ* and *GNA11* hypermorphic mutations result in amplified numbers of intradermal melanocytes in mouse models.\(^{203}\) Similar to findings seen with *GNAQ* transfection, Melan-A cells transfected with mutant *GNA11* lead to the activation of phosphorylated ERK.\(^{205}\) A study by Van Raamsdonk et al\(^{205}\) found that the majority of benign uveal nevi and 83%
of UMs contained either the GNAQ Q209 mutation or the GNA11 R183 mutation in a mutually exclusive pattern. These mutations cause constitutive activation of the Gαq or Gα11 subunits by blocking their intrinsic GTPase activity required to return them to the inactive state. However, these mutations alone are not adequate for complete malignant transformation to melanoma, suggesting that GNAQ/GNA11 mutations may be initiating events in UM progression.199

Conversely, other mutations such as BAP1 tend to occur later in UM progression and have been linked to the development of metastatic disease. BRCA1-associated protein 1 (BAP1) is a deubiquitinating enzyme that forms part of a tumor-suppressor heterodimeric complex.206 In sequencing for metastasis-related mutations in UM, Harbour et al207 discovered that inactivating somatic mutations of BAP1 on chromosome 3q21.1 were present in 26 of 31 (84%) metastasizing UM tumors. Of these 26 mutations, 15 caused premature protein termination and 6 altered the ubiquitin carboxy-terminal hydrolase (UCH) domains. These mutations usually occur in metastatic tumors that have also lost the other copy of chromosome 3, implicating BAP1 as a recessive cancer gene. BAP1 germline mutations, associated with a familial cancer syndrome involving UM and cutaneous melanoma as well as mesothelioma, have also been described.208,209 Thus, BAPI appears to be involved in the metastasis development of UM and may represent a future therapeutic target.

**Therapeutic Considerations**

Current efforts in the development of targeted therapy for melanoma harboring GNAQ/GNA11 mutations focus on the inhibition of downstream signaling arising from these mutations.210,211 Similar to the difficulties in targeting mutations in the RAS family of oncogenes, directly targeting mutant GNAQ or GNA11 may prove problematic.196 Mutations in GNAQ and GNA11 alter the intrinsic GTPase activity that would normally allow the proteins to return to their inactivated forms in which they are bound to GDP. Potential downstream targets include MEK, which is involved in the MAPK pathway activated by GNAQ/GNA11 mutations, PLC, which is activated by Gq, and PKC, which is activated downstream of PLC.210,211

**MEK as a Therapeutic Target:** UM cell lines containing GNAQ or GNA11 mutations are characterized by elevated levels of phosphorylated ERK. When treated with increasing concentrations of selumetinib, wild-type GNAQ cells demonstrated resistance; however, GNAQ- and GNA11-mutant cell lines exhibited a

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**Fig 3.** — Mechanism of GNAQ signaling. GNAQ polypeptide encodes the alpha subunit of heterotrimeric G proteins, which couples the cell surface, 7-transmembrane domain receptors to intracellular signaling pathways. Ligand binding triggers receptor activation that catalyzes the exchange of GTP for GDP bound to the inactive G protein alpha subunit, resulting in a conformational change and dissociation of the G protein beta-gamma subunits. A somatic mutation of glutamine in exon 5 located at position 209 (Q209) in G-alpha subunit inactivates the catalytic domain, preventing hydrolysis of GTP to GDP and thus fixing GNAQ in its constitutively active, GTP-bound, oncogenic state. GDP = guanine diphosphate, GNAQ = guanine nucleotide-binding protein Q, GTP = guanosine triphosphate.
high degree of sensitivity to the drug at IC50s in the range of 100 to 250 nmol/L. Recently, Ambrosini et al identified the MEK-dependent transcriptional output unique to GNAQ-mutant cells, with MEK inhibition resulting in the downregulation of a unique set of genes involved in cell proliferation, tumor cell invasion, and drug resistance.

Given the known MEK-ERK pathway activation in UM as a result of GNAQ and GNA11 mutations, inhibition of this pathway is of interest. A multicenter phase I study of trametinib, a reversible, selective allosteric inhibitor of MEK1/2 activation and kinase activity, was performed in patients with advanced melanoma. Sixteen of the 97 patients enrolled had UM, 6 of whom had a documented GNAQ or GNA11 mutation. Of the 16 patients with UM, most of whom were heavily pretreated, none had a documented partial or complete response, although stable disease was noted in 4 patients (25%).

A phase II randomized clinical trial of temozolomide vs hydrogen sulfate selumetinib has been initiated in patients with either wild-type or mutant GNAQ or GNA11 metastatic UM (NCT01143402). Accrual to the GNAQ/GNA11-mutant cohort of the trial was completed, with study results anticipated to be available in the near future.

**PKC as a Therapeutic Target:** Through its multiple cellular functions, such as the regulation of proliferation, apoptosis, differentiation, angiogenesis, and tumor development, PKC provides another therapeutic target through which GNAQ- and GNA11-mutant melanoma can be treated. PKC molecules are serine/threonine kinases that exist in multiple functionally distinct isoforms. The conventional PKCs (PKCα, PKCβ, and PKCγ) are activated by diacylglycerol (DAG) and phospholipids and are calcium-dependent, while the novel PKCs (PKCδ, PKCε, PKCθ, and PKCη) are activated by DAG and phospholipids and, by contrast, are calcium-independent. Lastly, the atypical PKCs (PKCζ and PKCe) do not require DAG or calcium for activation.

Recent work by Wu et al demonstrated the significance of PKC as a therapeutic target. Through the treatment of both wild-type and GNAQ-mutant UM cell lines with the PKC inhibitors sotrastaurin and enzastaurin, they showed that PKC inhibitors selectively block UM viability in a mutation-dependent manner. Sotrastaurin and enzastaurin, through the induction of G1 cell cycle arrest and apoptosis, significantly inhibited the growth of UM cells harboring GNAQ mutations, while wild-type UM growth was not appreciably altered. Sotrastaurin suppressed the expression and phosphorylation of PKCζ, PKCe, PKCδ, PKCε, and PKCθ in GNAQ-mutated UM cells. Additional findings from small hairpin RNA (shRNA)-mediated knockdown studies revealed that these PKC isoforms are functionally important for UM cells harboring GNAQ mutations. Furthermore, the inhibition of PKC in GNAQ-mutant UM resulted in the inhibition of the MAPK pathway by targeting the PKC/ERK1/2 and PKC/NF-κB pathways. Thus, PKC inhibition offers novel therapeutic potential for UM with GNAQ mutations. A phase I clinical trial examining sotrastaurin for the treatment of UM is underway (NCT01430416), and trials to explore the PKC blockade in combination with other pathway inhibitors, such as the PI3Ka inhibitor BYL719, are in development.

**PI3K/Akt as a Therapeutic Target:** PI3K activation has been shown in UM cell lines. PI3K is activated by both G-protein receptors and receptor tyrosine kinases. Activated PI3K is responsible for the conversion of PIP2 to PIP3, which then mediates the translocation Akt to the membrane where it is activated. Akt is involved in several signaling pathways of cell proliferation and survival. PTEN, which converts PIP3 to PIP2, antagonizes PI3K signaling and decreases Akt activation. A decrease in PTEN levels or loss of PTEN expression has been demonstrated in aggressive primary UM and portends a worse survival when compared with normal levels of PTEN.

Inhibition of PI3K with LY294002 in UM cell lines resulted in decreased cell proliferation. Recent work by Khalili et al showed that knockdown of GNAQ or GNA11 resulted in decreased MAPK phosphorylation in UM cell lines containing GNAQ or GNA11 mutations, respectively, but not in wild-type cells, confirming that activated GNAQ or GNA11 signals through the MEK/MAPK pathway in GNAQ- or GNA11-mutant UM, respectively. They also demonstrated that loss of GNAQ or GNA11 mutations had no significant effect on Akt phosphorylation in either GNAQ- or GNA11-mutant or wild-type cells. Inhibition of MEK, and therefore MAPK signaling, resulted in the reciprocal activation of Akt regardless of GNAQ- or GNA11-mutant status. They further demonstrated that MEK or PI3K inhibition alone achieved cell-cycle arrest and reduced growth in most UM cells but resulted in small amounts of apoptotic death. However, combination treatment with MEK and PI3K inhibition resulted in a significant amount of apoptosis, which was most appreciable in GNAQ-mutant cells, but it was also evident in the majority of GNA11-mutant cells. These preclinical data support the use of combination targeted therapy in the treatment of UM containing GNAQ/GNA11 mutations. Clinical trials assessing the safety and efficacy of dual-pathway inhibition for advanced UM are currently in development.

**mTOR as a Therapeutic Target:** mTOR, a serine/threonine kinase activated downstream in the PI3K pathway, stimulates cell proliferation by controlling cell-cycle progression regulators. There are two distinct mTOR complexes: mTORC1 (mTOR complex 1,
that completely inhibit downstream mTOR signaling, treated with rapamycin, an mTOR inhibitor, at levels to mTOR inhibitors, and mTOR inhibition alone is unlikely to be effective alone in the treatment of UM.

A recent study examining combination treatment of UM cells with MEK and mTOR inhibitors demonstrated that efficacy was linked to tumor genotype. The ATP-competitive mTOR kinase inhibitor AZD8055 and the MEK inhibitor selumetinib did not show cooperative antitumor effects in wild-type cells. In both BRAF- and GNAQ-mutant cells, combination therapy suppressed cell viability; however, apoptotic death was seen in BRAF-mutant cells. This distinction may reflect differences in how BRAF and GNAQ mutations activate MAPK signaling. BRAF directly activates MEK and ERK activity, while GNAQ activates MAPK through PKC activation, which can facilitate cell survival signals through several pathways in parallel to the MAPK pathway. When GNAQ was suppressed in GNAQ-mutant cells, higher resultant apoptosis was present when treated with combination AZD8055/selumetinib, suggesting that GNAQ activity in these cells activates MEK and mTOR-independent prosurvival signals. Further study is needed for the development of agents directly targeting GNAQ and GNA11, as well as other downstream targets, to achieve better therapeutic efficacy.

Conclusions

Although it has long been considered a clinically diverse disease, advanced melanoma has been, until recently, uniformly treated. This has been an unsuccessful therapeutic strategy both in controlling disease progression and in improving OS rates. Increasing evidence suggests that future successful melanoma treatment requires a better understanding of the inherent biological and molecular alterations associated with melanoma. As demonstrated by numerous preclinical and clinical studies, one treatment will not work for all tumors or all molecular types. The development of targeted therapy aimed at individual molecular alterations, specific to each individual melanoma, is likely to result in more successful clinical outcomes.

The MAPK and PI3K pathways have emerged as key aberrant pathways in melanoma for which specific inhibitors now exist. However, the complexity of these cascades is an ongoing challenge in terms of developing novel therapies. In addition to overcoming mechanisms of resistance, future directions for the development of effective melanoma therapies include the identification of novel oncogenic mutations in the subset of wild-type melanoma for currently known molecular drivers, which may predict sensitivity or resistance to specific pharmacological agents. Multitarget therapy using molecular inhibitors, molecular inhibitors combined with chemotherapy, or pathway inhibition combined with immunotherapy may be all that is needed for the effective treatment of this heterogeneous disease.

Current progress and increased knowledge into the biological basis of melanoma will aid in the creation and advancement of new therapeutic agents, allowing therapy to be tailored to specific patients based on the molecular characteristics of their tumors that is likely to yield insightful results for the future treatment of melanoma.

References


35. Halaban R, Zhang WG, Bacchioccio A, et al. XL8032, a selective BRAFV600E kinase inhibitor, activates the ERK pathway and enhances cell migration and proliferation of BRAFV600E melanoma cells. Pigment Cell Mela-


49. Paraiso KHT, Fedorenco IV, Cantipe LP, et al. Recovery of phospho-


October 2013, Vol. 20, No. 4 Cancer Control 279


138. Asciento PA, Berking C, Aparnaa SS. Efficacy and safety of oral MEK162 in p < 0.05 both locally advanced and unresectable or metastatic cutaneous melanoma harboring BRAFV600 or NRAS mutations. J Clin Oncol. 2012;30(suppl):8511.


218. Abdel-Rahman MH, Yang Y, Zhou X-P, et al. High frequency of submi-