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Targeting Drivers of Melanoma with Synthetic Small Molecules and Phytochemicals

Leah Ray Strickland¹, Harish Chandra Pal¹, Craig A. Elmetts^{1,2}, and Farrukh Afaq^{1,2}

¹Department of Dermatology, University of Alabama at Birmingham, Birmingham, AL, 35294, USA

²Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, 35294, USA

Abstract

Melanoma is the least common form of skin cancer, but it is responsible for the majority of skin cancer deaths. Traditional therapeutics and immunomodulatory agents have not shown much efficacy against metastatic melanoma. Agents that target the RAS/RAF/MEK/ERK (MAPK) signaling pathway—the BRAF inhibitors vemurafenib and dabrafenib, and the MEK1/2 inhibitor trametinib—have increased survival in patients with metastatic melanoma. Further, the combination of dabrafenib and trametinib has been shown to be superior to single agent therapy for the treatment of metastatic melanoma. However, resistance to these agents develops rapidly. Studies of additional agents and combinations targeting the MAPK, PI3K/AKT/mTOR (PI3K), c-kit, and other signaling pathways are currently underway. Furthermore, studies of phytochemicals have yielded promising results against proliferation, survival, invasion, and metastasis by targeting signaling pathways with established roles in melanomagenesis. The relatively low toxicities of phytochemicals make their adjuvant use an attractive treatment option. The need for improved efficacy of current melanoma treatments calls for further investigation of each of these strategies. In this review, we will discuss synthetic small molecule inhibitors, combined therapies and current progress in the development of phytochemical therapies.

Keywords

Melanoma; signaling pathways; targeted therapy; phytochemicals

*Send correspondence to: Farrukh Afaq, Ph.D., Department of Dermatology, University of Alabama at Birmingham, Volker Hall, Room 501, 1670 University Blvd., Birmingham, AL, 35294, USA, Phone: (205) 934-5190; Fax: (205) 934-5745, farrukhafaq@uabmc.edu.

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Introduction

Melanoma is a malignant tumor of melanocytes, cells responsible for producing the skin pigment melanin. Of all skin cancers, melanoma is the least common, but because of its metastatic potential, it is responsible for approximately 80% of deaths related to skin cancer.¹ The American Cancer Society estimates that the lifetime probability of Caucasian men being diagnosed with melanoma is 1/35, while for Caucasian women it is 1/54.² Furthermore, it is projected that there will be ~76,710 new cases of melanoma in the United States in 2014, and ~9,710 of those cases will result in death.² Established risk factors for the development of melanoma include fair features (light skin, hair and eye color) and ultraviolet exposure. In particular, blistering sunburns early in life have been shown to play a causal role.³ For cutaneous melanomas of low thickness (Breslow depths of up to 1.0 mm) surgery is curative for the majority patients.⁴ Rates of survival drop precipitously with increased tumor thickness due to the increased risk of metastasis.⁴ This transition from a mostly benign disease to one with a more serious prognosis occurs as melanoma progresses through the radial and vertical growth phases. The prognosis for metastatic melanoma is grim: 5-year survival ranges from 12 to 28%, depending on the location of the metastasis.⁴

Traditional cytotoxic therapy and immunomodulatory agents have failed to demonstrate significant efficacy, with fewer than 5% of patients having complete responses at 5 years.⁵ Fortunately, the last decade has been an exciting time for melanoma research, with advances in oncogene related therapies as well as immunotherapies. Immunotherapies that block inhibitory checkpoint molecules, CTLA-4 and PD-1, have been shown to improve survival for patients with metastatic melanoma and have gained FDA approval.^{6,7,8} Likewise, pivotal advances in oncogene directed therapies have led to improvements in patient survival, resulting in FDA approval of agents that target the RAS/RAF/MEK/ERK (MAPK) pathway, such as vemurafenib, dabrafenib and trametinib. Yet, in spite of these advancements, the extension of life offered by these agents is only a matter of months due to the rapid development of resistance. Also, they only target a fraction of the oncogenic signaling that leads to melanoma. Current research in this area is focused on the discovery of additional inhibitors of the MAPK pathway and inhibitors of other pathways that play key roles in melanomagenesis and resistance, such as PI3K/AKT/mTOR (PI3K) and c-kit signaling. The study of combination therapy with existing agents and the further elucidation of mechanisms of resistance are also underway. Furthermore, preclinical studies of phytochemicals, both alone and in combination with traditional cytotoxic and targeted therapies, have recently yielded promising results. The relatively low toxicities of these substances make the adjuvant use of natural agents an attractive treatment option for metastatic melanoma. In summary, the need for improved efficacy of current melanoma treatments calls for innovative strategies, such as the elucidation of combination therapies, continued discovery of novel therapeutic targets, and preclinical investigation of natural agents as adjuvant therapy. In this review, we will discuss progress in targeting MAPK, PI3K, and c-kit signaling pathways, preclinical studies of phytochemicals, and combined oncogene directed therapies (Tables 1, 2, 3; Figures 1, 2, 3).

RAS/RAF/MEK/ERK (MAPK) Signaling in Melanoma

Activation of the MAPK has been described in roughly 90% of melanomas.⁹ Activation of the MAPK pathway occurs when RAS-GTP causes RAF kinase dimerization.⁹ An important target of activated RAF kinases is MEK1/2, which catalyzes the phosphorylation of ERK1/2.^{10,11} ERKs can translocate into the nucleus and regulate numerous cellular processes, including proliferation, differentiation, survival, motility, and angiogenesis.¹²

RAS

Some of the first oncogenes described in humans were RAS proteins. Through cellular stimuli, such as receptor tyrosine kinases (RTKs), RAS transmits extracellular signals to intracellular effector pathways, which include the RAS/RAF/MEK/ERK (MAPK) and the PI3K/AKT/mTOR (PI3K) signaling pathways.¹³ RAS signaling regulates a multitude of functions, including cell cycle progression, apoptosis, and differentiation.^{14,15} The conversion between inactive RAS-GDP and active RAS-GTP is regulated by guanine nucleotide exchange factors (GEFs) and by GTPase-activating proteins (GAPs). GEFs promote the exchange of GDP for GTP leading to RAS activation. GAPs accelerate RAS-mediated GTP hydrolysis and lead to inactivation of RAS.¹⁶ There are three main RAS isoforms: HRAS, KRAS, and NRAS.¹⁶ The most common RAS gene mutation in melanoma is NRAS, which is mutated in 15–20% of all melanomas.¹⁷ In accordance with the importance of NRAS mutations in maintaining melanoma cell growth, inactivation of NRAS in melanoma cell lines by RNA interference leads to induction of apoptosis.¹⁸ The most common NRAS mutation is at codon 61; this prevents RAS GTP hydrolysis, causing the NRAS protein to be constitutively active.^{19,20} Less common mutations at codon 12 and 13 prevent the association of GAP proteins with the NRAS complex.²¹

Association with the inner face of the plasma membrane is necessary for RAS function. Inhibition of post-translational farnesylation blocks RAS activation by impeding translocation of RAS to the plasma membrane. In mouse models, farnesyltransferase inhibitors (FTIs) were shown to have powerful anti-tumor activity and caused minimal toxicity to normal tissue in various cancer cell lines.^{22,23} Despite this evidence of the efficacy of RAS inhibition by FTIs, results from clinical trials were disappointing. A phase II clinical trial examining tipifarnib (a FTI) as a single agent in advanced melanoma was curtailed due to a lack of initial response to treatment, thus halting further clinical investigations.²⁴ It is now believed that the RAS proteins can escape FTI through prenylation by a geranylgeranyl transferase that results in the transfer of an alternate isoprenoid group to RAS and allows continued activity.^{25,26} Despite unfavorable results as a monotherapy, there is still hope for the utility of FTIs when combined with other agents. It has been shown that combination treatment with cisplatin and lonafarnib (SCH66336), another FTI, amplified cisplatin-induced apoptosis in human and mouse melanoma cell lines.^{27,28} In melanoma cells, lonafarnib has also been shown to block mammalian targets of rapamycin (mTOR) signaling and enhance sorafenib-induced apoptosis.²⁹ A phase I clinical trial combining tipifarnib and sorafenib (a BRAF inhibitor) showed stable disease in patients with various cancers, including one with metastatic melanoma.³⁰ Further, an *in vitro* study showed that the combination of lonafarnib and sorafenib led to significant enhancement of

sorafenib-induced apoptosis and complete suppression of melanoma cell invasion in raft culture.²⁹ Blockade of NRAS signaling through inhibition of BRAF with vemurafenib has also been attempted, but was unsuccessful due to paradoxical hyperactivation of MEK-ERK signaling, causing activation of CRAF and induction of growth in cells with mutated RAS.^{31,32} In contrast to the results obtained with BRAF inhibitors, a recent study using NRAS mutant, patient-derived melanoma cell cultures showed that MEK inhibition reduced ERK1/2 phosphorylation and induced apoptosis.³³ Promisingly, results of a phase II clinical trial of the MEK inhibitor, MEK162, exhibited objective responses in patients with NRAS mutations, providing support for the clinical use of MEK inhibitors for NRAS mutant metastatic melanoma treatment.³⁴ There is a phase III study currently underway to compare the efficacy of MEK162 to dacarbazine in patients with NRAS mutations, along with a phase II trial of another MEK inhibitor, pimasertib, in patients with NRAS mutant melanoma (NCT01763164, NCT01693068).

RAS-driven melanomas represent a high percentage of metastatic melanomas.¹⁷ Despite the well-established role of NRAS in melanomagenesis, the development of effective therapies for patients with NRAS-driven melanoma remains elusive. Direct inhibition of RAS, thus far, has not been effective and RAS inhibition through blockade of BRAF has been shown to be ineffective.^{24,35,36} However, despite the failure of FTIs in monotherapy, these agents may support modulation of RAS signaling when used in combination with other treatment regimens. Moreover, MEK inhibition has shown promise as a therapy for NRAS mutant melanoma.^{33,34} These treatment strategies and other means of RAS inhibition are actively being pursued.

BRAF

The RAF isoforms include ARAF, BRAF, and CRAF/RAF-1.³⁷ BRAF mutations are found in approximately 60% of all melanomas, and the oncogenic contribution of BRAF in melanoma has been validated in numerous cell and animal models.^{38,39,40} The BRAF^{V600E} mutation accounts for nearly 90% of all such mutations found in melanoma.³⁸ A substitution of valine for glutamic acid at position 600 results in the BRAF^{V600E} mutation, causing the protein to remain in the active conformation permanently. Less common mutations (V600D, V600K, V600R) contribute another 5–6%, and are due to alternative point mutations at the same position.³⁸ Of note, BRAF mutations are also found in many benign nevi.⁴¹ In fact, BRAF expression in human melanocytes has been shown to cause cell cycle arrest.⁴² Based on this evidence, BRAF is believed to induce the cancer sequence and with additional mutations, namely in tumor suppressor genes, transformation to melanoma ensues.⁴¹

The development of agents targeted at BRAF mutations, specifically the BRAF^{V600E} mutation, is responsible for much-needed advancement in the treatment of metastatic melanoma. The first targeted agent to be tested in clinical trials for BRAF mutant melanoma was sorafenib.⁴³ Sorafenib is a nonspecific kinase inhibitor, and has been shown to inhibit BRAF, CRAF, and the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and various other RTK.⁴³ However, a phase II clinical trial of sorafenib monotherapy showed a lack of response in patients with metastatic melanoma.⁴⁴ Further trials evaluated the effectiveness of sorafenib in combination with cytotoxic agents.

Unfortunately, a phase III clinical trials of sorafenib with carboplatin and paclitaxel similarly failed to shown a significant survival benefit.^{45,46} It is believed that due to sorafenib's BRAF-independent cellular effects, therapeutic doses could not be achieved because of significant toxicity.⁴⁷ The development and use of second-generation BRAF inhibitors with greater selectivity has been met with great success. Vemurafenib binds selectively to the ATP-binding site of the BRAF^{V600E} mutation, resulting in reduced proliferation and downstream inhibition of ERK phosphorylation.⁴⁸ Preclinical studies showed vemurafenib-induced RAF inhibition reduced the proliferation of BRAF mutant melanoma cell lines, but did not inhibit melanoma cell lines without BRAF mutations.⁴⁹ Phase I and II clinical trials showed tumor shrinkage and vemurafenib-induced clinical responses in more than half the patients treated and showed improvement in rates of overall survival (OS) and progression-free survival (PFS) in patients with BRAF^{V600E} mutant metastatic melanoma.^{50,51} A pivotal phase III study (BRIM-3) validated vemurafenib's superiority to cytotoxic therapy in patients with the BRAF^{V600E} mutation and also in patients with the BRAF^{V600K} mutation. In patients with the BRAF^{V600E} mutation, the estimated median PFS in the vemurafenib group was 6.9 months compared to 1.6 months for the dacarbazine group. For the patients with the BRAF^{V600K} mutation, median PFS in the vemurafenib and dacarbazine group was 5.9 months and 1.7 months, respectively.⁵² These landmark results led to the FDA approval of vemurafenib for treatment of patients with BRAF^{V600E} metastatic melanoma in 2011 along with a BRAF^{V600} mutation test. Another agent targeting BRAF mutant melanoma, dabrafenib, has recently received FDA approval. Phase I and II clinical trials of dabrafenib showed PFS ranging from 5.5–6.3 months for BRAF^{V600E} melanoma and 4.5–5.6 months for BRAF^{V600K} melanoma.^{53,54} A phase III trial comparing dabrafenib to dacarbazine showed a median PFS of 5.1 months for dabrafenib and 2.7 months for dacarbazine.⁵⁵

Despite this exciting progress, 10% of patients with BRAF inhibitor-responsive melanoma show tumor progression early in the course of therapy and a majority of patients relapse in less than a year.^{50,56} The aberrations that result in resistance are numerous, but most reactivate the MAPK pathway by bypassing BRAF inhibition and restoring ERK activation. Resistance mechanisms that restore ERK activation include, but are not limited to, elevated expression of RAF kinases,^{57,58} activating mutations in NRAS or MEK1,^{59,60} stimulation of receptor tyrosine kinases^{59,61} and a splice variant of BRAF^{V600E}.⁶² Moreover, the use of BRAF inhibitors comes with many troubling side effects, including development of keratoacanthomas and invasive squamous cell carcinoma.^{52,55} These proliferations occur in BRAF wild-type cells with mutated RAS and are due to up-regulation of BRAF-CRAF leading to ERK1/2 hyperactivation.^{63,32} This paradoxical ERK1/2 hyperactivation has been shown to lead to the development of RAS-driven squamous cell carcinomas and keratoacanthomas while patients are on vemurafenib and other BRAF inhibitors and, as mentioned above, is thought to be responsible for the inefficacy of BRAF inhibitors in NRAS mutant melanoma.⁶⁴

Other BRAF inhibitors are currently in development or in clinical trials for metastatic melanoma (NCT01436656, NCT00304525).⁶⁵ RAF265 is a dual inhibitor of mutant BRAF^{V600E} and VEGFR2; a phase II clinical trial in patients with metastatic melanoma has been recently completed with pending results (NCT00304525). Preclinical studies with

BRAF inhibitors lacking paradoxical ERK1/2 hyperactivation in cell lines with wild-type BRAF are also currently underway. For example, a non-paradox inducing BRAF inhibitor, PLX7904, was shown recently to reduce ERK1/2 phosphorylation and growth of NRAS mutant, vemurafenib-resistant melanoma cell lines.⁶⁵

BRAF inhibitors have produced exciting and much-needed progress in the treatment of metastatic melanoma, but unfortunately, they have only been shown to inhibit the RAF/MEK/ERK pathway in cell lines that harbor BRAF^{V600E} or BRAF^{V600K} mutations. Of note, a clinical report on patients with the BRAF^{V600R} mutation who were treated with vemurafenib or dabrafenib has shown promise; however, the activity of BRAF inhibitors in other, more rare, BRAF mutations remains unknown.⁶⁶ It is clear that progress has been made for patients with certain BRAF mutations, but unfortunately, this only represents about half of the patients with metastatic melanoma. Responsive melanomas also develop resistance to BRAF inhibition quickly, and the mechanisms of resistance to dabrafenib and vemurafenib have been shown to be similar.⁶⁷ The numerous mechanisms of resistance to BRAF inhibitors support the need for combined therapies of BRAF inhibitors with agents that target other locations in the RAF/MEK/ERK or alternative pathways. Most recently, the combination of MEK and BRAF inhibitors has shown great promise.⁶⁸

MEK

BRAF mutated cells have been shown to possess enhanced sensitivity to MEK inhibition.⁶⁹ MEK inhibitors are believed to function by inducing apoptosis through suppression of Mcl-1, a member of the anti-apoptotic B-cell CLL/lymphoma 2 (Bcl-2) family.⁷⁰ There are two major classes of MEK inhibitors, ATP non-competitive and ATP competitive inhibitors.⁷¹ Currently, most MEK inhibitors are noncompetitive, indicating that they do not compete for the ATP-binding site and instead bind to an adjacent allosteric site, which explains their high specificity.⁷¹ Selumetinib, a selective, noncompetitive inhibitor of MEK1/2, has been shown to reduce the growth of melanoma cells by inducing G1-phase cell cycle arrest.^{72,73,74} Furthermore, cell growth inhibition was demonstrated in melanoma lines possessing the BRAF^{V600E} mutation.^{75,76} A phase I clinical trial of selumetinib resulted in disease stabilization and tumor biopsies demonstrated reduced ERK phosphorylation in patients with metastatic melanoma.⁷² In a phase II clinical trial, patients with metastatic melanoma and an unknown NRAS/BRAF status received therapy with selumetinib or temozolomide.⁷⁷ Results showed no significant difference in PFS. However, it was later found that five of the six patients who showed a partial response to selumetinib had BRAF mutant tumors.⁷⁷ Therefore, more recent studies with MEK inhibitors have selected patients with BRAF mutant melanoma. Of note, a randomized phase II trial combining selumetinib and dacarbazine showed an improved median PFS of 5.6 months compared to 3.0 months with dacarbazine monotherapy; however, no significant change was seen in OS.⁷⁸

The second generation ATP noncompetitive MEK1/2 inhibitors have shown improved effectiveness and the MEK1/2 inhibitor, trametinib (GSK1120212) has recently gained FDA approval for the treatment of metastatic melanoma. Preclinical trials with BRAF^{V600E} melanoma cell lines and xenografts showed trametinib to be a reversible allosteric inhibitor of both MEK1/2 activation and kinase activity.⁷⁹ A phase I trial of trametinib showed a

response rate of 33% in patients with BRAF mutant melanoma and 10% in patients with wild-type BRAF, confirming the importance of selecting for mutational status.⁸⁰ A phase II clinical trial of trametinib showed a median PFS of 4.0 months in BRAF mutant melanoma that had not been treated previously with a BRAF inhibitor, however no efficacy was seen in patients who had been treated previously with a BRAF inhibitor.⁸¹ The pivotal phase III trial, METRIC, enrolled patients with metastatic melanoma and BRAF^{V600E} or BRAF^{V600K} mutations that had not been previously treated with a BRAF or MEK inhibitor. Results showed significant improvements in OS and PFS, with a median PFS of 4.8 months in the trametinib group and 1.5 months in the chemotherapy group.⁸² Because of these results, the FDA approved trametinib in May 2013 for the treatment of patients with metastatic melanoma and BRAF^{V600E} or BRAF^{V600K} mutations who had not formerly received BRAF inhibitor treatment.

As discussed above, the MEK1/2 inhibitor, MEK162, has also recently shown promise for treatment of patients with NRAS-mutated melanoma in both preclinical and clinical trials.^{33,34} Of interest, it has been shown that variances in the activation state of MEK exist in KRAS versus BRAF mutant tumors.⁸³ Specifically, inhibition of the RAF/MEK/ERK pathway in BRAF mutated melanoma occurs through inhibition of active, phosphorylated MEK, whereas inhibitors that block feedback phosphorylation by wild-type BRAF may be more effective for KRAS inhibition.^{83,84} Inhibitors that target the unique activation states of MEK are in clinical trials (NCT01689519, NCT01271803).⁸³ Other MEK1/2 inhibitors (PD-0325901, TAK733, pimasertib, and RO4987655) are also currently in clinical development.^{85,86,12,87} Clinical Trials of targeted therapies in the treatment of metastatic melanoma are summarized in Table 1.

MEK and BRAF combined therapy

Numerous preclinical and clinical studies have demonstrated that BRAF and MEK co-inhibition is a successful treatment strategy for metastatic melanoma. Preclinical studies have shown that the combination of a BRAF and MEK inhibitor reduces tumor growth and delays onset of resistance when compared to monotherapy.^{88,68} In a phase II trial dabrafenib, either as a monotherapy or in combination with trametinib showed a significant improvement in PFS.⁸⁹ The median PFS was 9.4 months in the combination group and 5.8 months in the monotherapy group.⁸⁹ Moreover, the addition of trametinib was shown to reduce the appearance of cutaneous squamous cell carcinomas.^{64,89} These results led to the accelerated approval of the combination of dabrafenib and trametinib for the treatment of patients with metastatic melanomas that carry the BRAF^{V600E} or BRAF^{V600K} mutation in January 2014. Recent results of a phase III trial comparing the combination of dabrafenib and trametinib to dabrafenib monotherapy in patients with BRAF-mutant melanoma likewise showed improvements in PFS, with a median PFS of 9.3 months in the dabrafenib and trametinib group and 8.8 months in the dabrafenib monotherapy group.⁹⁰ The rate of cutaneous SCC was also lower in the dabrafenib trametinib combination group compared to the dabrafenib-only group.⁹⁰ A phase III clinical trial comparing dabrafenib and trametinib to vemurafenib monotherapy (COMBI-v) is also in progress (NCT01597908). Other BRAF and MEK inhibitor combinations have also shown promise and are in phase III trials (NCT01909453, NCT01689519).^{91,89} Unfortunately, it has been shown that resistance to

MEK inhibition may cross over from BRAF inhibitor resistance.⁶⁰ However, the combination of BRAF and MEK inhibition seems to delay this resistance,^{60,92} and a recent study has shown benefit for patients treated with a BRAF inhibitor after MEK inhibitor failure, suggesting that the mechanisms of resistance for MEK inhibition may be different.⁹³ Moreover, a dual BRAF/MEK inhibitor, RO5126766, has recently shown activity in patients with metastatic melanoma, suggesting tandem inhibition of BRAF and MEK may one day be a possibility.⁹⁴ Clinical Trials of synthetic small molecule inhibitor combinations in metastatic melanoma are summarized in Table 2.

PI3K/AKT/mTOR (PI3K) Signaling in Melanoma

The PI3K/AKT/mTOR (PI3K) pathway promotes cell survival and proliferation, and is hyperactivated in most malignancies, including melanoma.^{95,96} Stimulation of the PI3K pathway arises via GTP binding of RAS proteins and stimulation of RTK.⁹⁶ Activation of the pathway generates phosphoinositide 3-kinase regulating subunit, allowing the catalytic subunit to phosphorylate membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) converting it to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which is a key propagator of intracellular signaling. The tumor suppressor phosphatase and tensin homolog (PTEN) is a key regulator of the PI3K pathway. Lack of PTEN antagonism leads to association of phosphoinositide-dependent protein kinase 1 to the cell membrane and phosphorylation of AKT, a widely known oncogene.^{97,98} The numerous substrates of AKT include cellular regulators of insulin signaling, proliferation, and survival.^{99,100}

AKT induces protein synthesis and cell proliferation by causing abrogation of TSC1/2, which leads to activation of the mTORC1 complex.^{101,102} Both PI3K and AKT participate in activation of mTOR,^{103,104} and the full activation of AKT requires phosphorylation of another of its kinase domains via mTOR complex 2 (mTORC2).¹⁰⁵ These two distinct complexes of mTOR, mTORC1 and mTORC2, are believed to have differing functions, but their regulation overlaps in important ways. A primary role of mTORC2 is regulation of the actin cytoskeleton.¹⁰⁶ However, as stated above, activation of mTORC2 leads directly to phosphorylation of AKT, linking this complex to the activation of the mTORC1 pathway.^{107,98} Enhanced protein translation is the result of mTORC1 activation and occurs through targeting of eukaryotic translation initiation factor (eIF4E) and p70S6 kinase.¹⁰⁸

Elevated AKT phosphorylation and/or activated mTOR functioning arises in 70% of malignant melanomas.^{109,110} Elevated phospho-AKT levels are linked with reduced survival in melanoma patients¹¹¹ and promote motility, invasion, and angiogenesis.¹¹² The major mechanisms of PI3K pathway activation in melanoma are loss of PTEN and NRAS mutations, as discussed above. Loss of PTEN is seen in 10–30% of melanoma cell lines, with most exhibiting concurrent BRAF^{V600E} mutations.^{113,114} In a mouse melanoma model, the BRAF^{V600E} mutation alone led to benign melanocytic hyperplasias, and metastasis was induced with the concurrent loss of PTEN, suggesting a model for melanomagenesis.¹¹⁴

Agents that inhibit mTOR have demonstrated anti-proliferative effects against many human cancers.¹¹⁵ Most studies of PI3K signaling blockade in melanoma have used the first-generation agent rapamycin and the second-generation agents, everolimus (RAD001) and

temsirolimus (CCI-779), which allosterically inhibit the mTORC1 complex.^{116,117} These agents have long been used as immunosuppressants in patients with organ transplants and are tolerated reasonably well. However, clinical trials in melanoma have shown a lack of objective responses to mTORC1 inhibitors as single agents or in combination with BRAF inhibitors.^{118,119,120} The reason for this seems to be interruption of negative feedback loops mediated by mTORC1, causing activation of PI3K, AKT and ERK.^{121,122} Specifically, compensatory PI3K pathway activation occurs due to interruption of baseline mTORC1 mediated inhibition of insulin receptor substrate 1, a second messenger of the insulin-like growth factor 1 RTK.^{123,122}

The efficacy of mTORC1 inhibitors is limited by dysregulation of negative feedback loops and a lack of mTORC2 inhibition.^{117,124} Promisingly, a preclinical study evaluating the efficacy of dual mTORC1/2 inhibition showed blockade of compensatory AKT hyperactivation in sensitive cell lines.¹²⁵ Dual PI3K-mTOR inhibitors are also being examined as a means to block compensatory activation of PI3K signaling.¹²⁶ In melanoma, dual PI3K-mTOR inhibitors have shown impressive antiproliferative activity and durable suppression of AKT phosphorylation in both *in vitro* and *in vivo* studies.^{127,128} Furthermore, previous trials of mTOR inhibitors have not been performed in patients with mTOR mutations.¹¹⁸ There is currently a phase II clinical trial underway in patients selected for mTOR mutations (NCT01960829).

Combined therapy with PI3K inhibitors

Resistance to BRAF inhibitors and MEK inhibitors underscores the need to target alternative pathways.^{129,130} In particular, acquired resistance after BRAF therapy has been shown to occur through activation of PI3K signaling and can be overcome by MEK inhibitor therapy.^{131,61} Crosstalk between the MAPK and PI3K pathway is known to be a major cause of resistance, and inhibition of the PI3K pathway is being examined as a means of combating BRAF and MEK inhibitor resistance.^{61,129} *In vivo* models showing that activation of PI3K signaling with mutant BRAF enhances melanomagenesis more than either mutation alone supports the use of PI3K and MEK inhibitor combinations in vemurafenib-resistant, BRAF-mutant melanoma.^{114,132} Moreover, vemurafenib and selumetinib co-resistant BRAF-mutant melanoma cell lines have shown dependence on AKT induction for survival.^{130,133} In one study, the addition of an AKT inhibitor overcame acquired resistance to both vemurafenib and selumetinib, with the only exception being vemurafenib-resistant cell lines that secondarily acquired a NRAS mutation.¹³⁰ In another study, BRAF^{V600E} mutant melanoma cell lines harboring MEK or NRAS mutations also showed resistance to dabrafenib and trametinib monotherapy. However, when these cell lines were treated with a combination of a BRAF inhibitor and the PI3K inhibitor GSK2126458, they showed improved cell growth inhibition.¹²⁹ Furthermore, in a RAS-driven, Ink4a/Arf-deficient mouse model of melanoma, the combination of BEZ235, a dual PI3K-mTOR inhibitor, with the MEK inhibitor AZD6244, produced significant tumor regression and improved survival.¹³⁴ A recent preclinical study has shown superior growth inhibition and a delay in resistance in a melanoma cell line with homozygous PTEN loss when treated with the triple therapy of dabrafenib, trametinib and the AKT inhibitor

GSK2141795B.¹³⁵ Currently, a phase I study is examining the efficacy of selumetinib alone and in combination with the AKT inhibitor MK2206 (NCT01021748).

c-kit Signaling in Melanoma

The receptor tyrosine kinase c-kit is universally expressed in mature melanocytes and promotes proliferation and survival. Binding of c-kit to its ligand, stem cell factor, results in receptor dimerization, autophosphorylation, and stimulation of several signaling pathways, which include MAPK, PI3K, and janus kinase-signal transducer and activator of transcription (JAK-STAT) pathways. A recent preclinical study demonstrated that c-kit-driven PI3K activation led to MAPK pathway activation and increased melanocyte proliferation and melanoma survival.¹³⁶ In addition, common variants at the c-kit locus have been shown to affect nevus number and increase the risk of melanoma.¹³⁷ In melanomas that harbor c-kit mutations, point mutations resulting in substitutions at L576P and K642E have been shown to account for 55% of c-kit mutated melanomas.¹³⁸ Recently, it has been shown that tumors retaining c-kit overexpression number fewer than 3% in unselected melanomas.¹³⁹ In contrast to the overall scarcity of c-kit mutations, melanomas that occur in mucosal, acral, or chronically sun-damaged skin have been reported to have high rates of c-kit-activating mutations or amplifications (~28–39%).^{139,140}

Imatinib mesylate is an ATP-competitive inhibitor of several tyrosine kinases, including c-kit. Interest in imatinib as a treatment for melanoma began with two separate case reports that showed striking responses to imatinib in metastatic melanomas with c-kit mutations.^{141,142} More recently, another case report has shown similar results.¹⁴³ Preclinical evidence has also shown that, when treated with the tyrosine kinase inhibitor imatinib, melanoma cell lines with c-kit mutations exhibit reduced rates of melanoma cell proliferation and increased apoptosis, along with suppression of the MAPK, PI3K, JAK-STAT, and anti-apoptotic pathways.¹⁴⁴ As imatinib is known to exhibit activity at several targets and to have efficacy in other tumor types, initial phase II studies in metastatic melanoma did not select for specific c-kit aberrations. As a probable result of this lack of clinical selectivity, these early trials did not show statistically significant results.^{145,146} A third phase II trial also failed to demonstrate clinical efficacy; however, it showed a dramatic response in one patient with a known c-kit mutation.¹⁴⁷ Therefore, most recent trials of imatinib have selected for patients with c-kit-mutated metastatic melanoma.^{148,149} With this selected patient population, two recent phase II trials have shown clinically significant results. In the first study, 23.4% of the cases demonstrated c-kit mutations and/or amplifications, with a median PFS of 12.0 weeks and an OS of 46.3 weeks.¹⁴⁸ This study also found that certain c-kit variants achieved greater responses; specifically, either K642E or L576P substitutions were present in all responses observed.¹⁴⁸ In the second phase II trial of imatinib, all patients harbored c-kit aberrations, which resulted in a median PFS of 3.5 months and an OS of 14 months. Conversely, correlations between response and kit mutations were not seen in this study.¹⁴⁹ Another phase II trial of imatinib in patients with c-kit aberrations and mucosal or acral metastatic melanoma is in progress (NCT00470470).

Other RTK inhibitors are currently being tested in clinical trials (NCT00700882, NCT01099514). In one study, two metastatic melanoma patients with the c-kit^{L576P}

mutation demonstrated responses to another RTK inhibitor, dasatinib.¹⁵⁰ Like imatinib, further trials with dasatinib have shown a lack of efficacy in unselected melanoma patients.¹⁵¹ Preliminary results of a phase II clinical trial of another c-kit inhibitor, nilotinib, resulted in durable responses of 8.4 months and 10.0+ months, respectively, in two patients with c-kit mutations (NCT01099514).¹⁵² A randomized phase II trial (NCT01028222) comparing the efficacy of nilotinib vs. dacarbazine in patients with c-kit mutations and mucosal or acral metastatic melanoma is currently in progress.

Phytochemicals

Metastatic melanoma treatment has made great strides in recent years; however, these new signal transduction inhibitors have notable adverse side effects and the development of resistance progresses rapidly. It is apparent that new strategies and novel agents are needed to complement current therapies. Naturally occurring chemical compounds are referred to collectively as phytochemicals. Phytochemicals have gained attention as promising therapeutics due to studies demonstrating their ability to prevent the development of cutaneous malignancies.^{153,154} There are several classes of phytochemicals that have been studied, including polyphenols, flavonoids, isoflavonoids, phytoalexins, phenols and carotenoids. Here, we discuss and summarize recent research on classes of phytochemicals in the treatment of melanoma (Table 3, Figure 3).

Flavonoids are polyphenolic compounds that are ubiquitous in plants. Flavonoids have been shown to modulate various cancer signaling pathways, including proliferation, progression and metastasis.¹⁵⁵ Several preclinical studies have shown that the flavonoid fisetin is active against melanoma. Specifically, fisetin is a flavone sub-class found in the Rhus family, which includes strawberries, mangoes and other plants.¹⁵⁶ Studies have demonstrated that fisetin reduces melanoma cell proliferation, invasion and tumor growth by inhibiting β -catenin/Microphthalmia-associated transcription factor (MITF),¹⁵⁷ MAPK,¹⁵⁸ NF κ B,¹⁵⁸ and PI3K signaling¹⁵⁹. In one study, inhibition of AKT and mTOR was linked with a significant reduction in the transition from the radial to the vertical growth phase in cell and xenograft models.¹⁵⁹ It has similarly been shown that fisetin suppresses melanoma cell invasion by promoting mesenchymal to epithelial transition, which occurred through inhibition of the MAPK and NF κ B signaling pathways.¹⁵⁸ Fisetin has also been shown to cause G1 cell cycle arrest and to decrease β -catenin/MITF signaling, leading to inhibition of melanoma cell proliferation and progression.¹⁵⁷

The stilbenoid resveratrol is a polyphenol that is found in peanuts, red wine, grape skins, and mulberries.¹⁶⁰ In plants, resveratrol is synthesized in response to stress.¹⁶¹ Resveratrol has been shown to induce apoptosis in both A375 and SK-MEL-28 melanoma cell lines.¹⁶² Ceramide accumulation and AKT/mTOR pathway inhibition has been shown to be another possible mechanism of apoptosis induction.¹⁶³ Furthermore, resveratrol has been shown to reduce proliferation and induce apoptosis of melanoma cells by causing down regulation of cyclin D1/cdk4 and increased p53 expression.^{164,165} In one study, a reduction in invasion was associated with a significant reduction in activator protein-1 (AP-1)/JunD, MMP-1, Bcl-2, and iNOS protein levels.¹⁶⁶ Resveratrol treatment has also been shown to suppress invasion and expression of alpha-melanocyte-stimulation hormone (α -MSH) signaling-

related molecules, including β -catenin, c-kit, and MITF.¹⁶⁷ A syngeneic mouse model of melanoma showed that treatment with resveratrol reduced tumor volume and metastasis, which was thought to occur through reduced AKT expression.¹⁶⁸ Evidence also suggests that resveratrol reduces melanoma-induced angiogenesis. In this study, the growth inhibition of vascular endothelial cells in co-culture with melanoma cells was associated with amplified melanoma cell expression of p53, matrix protein TSP1, and inhibition of VEGF production.¹⁶⁹ Resveratrol has also shown promise as an adjuvant chemotherapeutic agent. Resveratrol treatment enhanced cisplatin cytotoxicity in a mouse melanoma model, which was believed to occur through increased Connexin 43, a ubiquitous gap junction protein.¹⁷⁰ In another study, exposure of melanoma cells to resveratrol inhibited Ref-1-activated AP-1 DNA-binding and endonuclease activities, rendering melanoma cells more sensitive to dacarbazine treatment.¹⁷¹

The polyphenolic flavonoid silymarin is found in milk thistle.¹⁷² Silymarin has been shown to inhibit melanoma cell proliferation through suppression of MEK- and RSK-mediated signaling pathways that results in decreased activation of NF κ B, AP-1, and STAT3.¹⁷³ In another study, human melanoma cell lines treated with silymarin showed decreased melanoma cell migration via β -catenin inactivation and reduced MMP-2 and MMP-9 levels.¹⁷⁴ Silymarin was also shown to amplify the pro-apoptotic actions of anti-Fas agonistic antibody CH11 in A375-S2 melanoma cells.¹⁷⁵

Catechins, which are extracted from green tea leaves, have well-established anti-carcinogenic activity.¹⁷⁶ Epigallocatechin gallate (EGCG) is a polyphenol flavonoid that is the most abundant green tea catechin.¹⁷⁶ EGCG has been shown to cause significant reduction in melanoma cell growth and increased apoptosis through alterations in the cki-cyclin-cdk network and Bcl-2 family proteins.¹⁷⁷ In one study, EGCG resulted in NF κ B inhibition, which was associated with reduced melanoma cell interleukin-1beta (IL-1 β) secretion, downregulation of the inflammasome component, nuclear localization leucine-rich-repeat protein 1 (NLRP1), and decreased caspase-1 activation.¹⁷⁸ Further, both *in vitro* and *in vivo* evidence supports the anti-invasive and anti-metastatic actions of EGCG; specifically, it has been shown to reduce cell adhesion¹⁷⁹ and decrease cell motility.¹⁸⁰ EGCG has also been shown to suppress melanoma cell invasion/migration by targeting epithelial to mesenchymal transition via inhibition of endogenous expression of COX-2 and PGE(2) receptors.¹⁸¹ In an *in vivo* study, EGCG treatment showed reduced cell spreading, cell-extracellular matrix, and cell-cell interactions, along with inhibition of MMP-9 and focal adhesion kinase (FAK) activities.¹⁸² In this same study, the combination of EGCG and dacarbazine inhibited melanoma growth and metastasis significantly when compared to monotherapy.

Curcumin is a polyphenol found in turmeric, a widely used spice. In melanoma, curcumin has been shown to activate apoptosis through p53-independent pathways.^{183,184} In one study, curcumin treatment resulted in growth inhibition of B16BL6 melanoma cells and p53 independent down regulation of phosphatase of regenerating liver-3.¹⁸³ Similarly, curcumin was shown not to induce p53, but to suppress the NF κ B cell survival pathway and the apoptotic inhibitor, X-linked inhibitor of apoptosis protein (XIAP).¹⁸⁴ Other mechanisms that have been shown to induce apoptosis in melanoma cell lines after curcumin treatment

include down regulation of anti-apoptotic signaling molecules, including Bcl-2,^{185,186} proliferating cell nuclear antigen (PCNA),¹⁸⁶ and induced myeloid leukemia cell differentiation protein (Mcl-1) protein.¹⁸⁷ Suppression of NFκB signaling has also been demonstrated in several cell lines.^{188,189,190,184} Macrophage stimulating 1/hepatocyte growth factor-like (MST1) activation is also believed to play a role.¹⁹¹ The antiproliferative effects of curcumin have been shown to occur through blockade of cyclic nucleotide phosphodiesterases.¹⁹² One preclinical study showed that the combination of tamoxifen and curcumin increased phosphatidyl serine flipping, mitochondria depolarization, and reactive oxygen species generation in A375 and G361 melanoma cell lines.¹⁹³

Lupeol is a phytosterol and triterpene that is found in white cabbages, green peppers, strawberries, olives, mangoes and grapes.¹⁹⁴ Tumor growth inhibition after lupeol treatment has also been associated with suppression of Wnt target genes (c-myc and cyclin D1, proliferation markers proliferating cell nuclear antigen and Ki-67) and the invasion marker osteopontin.¹⁹⁵ In mouse melanoma models, lupeol was shown to decrease melanoma tumor growth and promote cell cycle arrest.^{196,197} Lupeol has been shown to induce melanoma cell differentiation in B162F2 melanoma cells.^{198,199,200} This induction of differentiation has been suggested to occur via activation of the MAPK pathway.²⁰¹ Lupeol has also been shown to suppress the migration of human melanoma cells by promoting disassembly of the actin cytoskeleton.²⁰²

Honokiol is a biphenolic compound from a species of magnolia native to China. It has long been used in traditional Chinese and Japanese medicine.²⁰³ Honokiol has been shown to inhibit proliferation in melanoma.^{204,205} This has been suggested to occur through attenuation of AKT/mTOR and Notch signaling.²⁰⁵ Further, honokiol has been shown to induce apoptosis through interaction with glucose-regulated protein 78, a sensor of endoplasmic reticulum stress.²⁰⁶

Phytochemicals have demonstrated their potential utility in the treatment of metastatic melanoma, and several studies have already shown their potential as adjuvant therapies.^{170,193} Moreover, phytochemicals are remarkably nontoxic. The proposed mechanisms of these phytochemicals are yet to be fully elucidated, however, it has been proposed that due to their ubiquity in nature, through evolution, phytochemicals inherently possess diverse mechanisms of action.²⁰⁷ Furthermore, development of phytochemical analogs with more specific spectra of activity is also underway. For some of these agents, the ability to achieve physiologically relevant concentrations has also been a challenge. Therefore, further studies are needed to delineate their target molecules, create novel vehicles to improve bioavailability, and/or develop effective analogs.

Conclusions and future directions

The elucidation of melanoma cell signaling pathways and development of cell signaling inhibitors represent a momentous accomplishment in the treatment of metastatic melanoma, and have led to much-needed new treatments. The combination of dabrafenib and trametinib is now the current treatment of choice for metastatic melanoma with BRAF mutations and offers improved survival for these patients. Despite these accomplishments, however, a cure

for patients with metastatic melanoma remains a distant goal. Current research is focused on discovering even more effective combined regimens that will lead to inhibition of coexistent melanoma signaling pathways in the hope of stalling or preventing resistance. The discovery of agents, or agent combinations, with superior toxicity profiles is also a major concern. Development of therapies for patients with other mutational aberrations, such as NRAS and c-kit, is also underway. Moreover, phytochemical therapies are on the horizon and have shown promise in preclinical studies; they also show low toxicity to non-neoplastic cells. The clinical utility of these agents will be determined by efforts to characterize their mechanisms, improve bioavailability, and/or develop effective analogs.

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Abbreviations

OS	Overall survival
MAPK	mitogen-activated protein kinase
RTKs	receptor tyrosine kinases
PI3K	phosphatidylinositide 3-kinases
GEFs	guanine nucleotide exchange factors
GAPs	GTPase-activating proteins
FTIs	farnesyltransferase inhibitors
mTOR	mammalian targets of rapamycin
VEGF	vascular endothelial growth factor
PDGF	platelet-derived growth factor
PFS	progression-free survival
PIP2	phospholipid phosphatidylinositol 4,5-bisphosphate
PIP3	phosphatidylinositol 3,4,5-trisphosphate
PTEN	phosphatase and tensin homolog
eIF4E	eukaryotic translation initiation factor
JAK-STAT	janus kinase-signal transducer and activator of transcription
MITF	β -catenin/Microphthalmia-associated transcription factor
NFκB	nuclear factor kappa B
MMP	matrix metalloproteinase
AP-1	activator protein-1
α-MSH	alpha-melanocyte-stimulation hormone
EGCG	Epigallocatechin gallate

IL-1β	interleukin-1beta
NLRP1	nuclear localization leucine-rich-repeat protein 1
FAK	focal adhesion kinase
XIAP	X-linked inhibitor of apoptosis protein
Bcl-2	B-cell CLL/lymphoma 2
PCNA	proliferating cell nuclear antigen
Mcl-1	induced myeloid leukemia cell differentiation protein
MST1	Macrophage stimulating 1/hepatocyte growth factor-like

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Highlights

- Agents targeting members of the MAPK pathway have increased survival in patients with metastatic melanoma
- Combined therapy with agents that target members of the MAPK pathway has been shown to be superior to single agent therapy in clinical trials
- Studies of agents that target MAPK, PI3K, c-kit, and other signaling pathways are currently being pursued both alone and in combination
- Phytochemicals have been shown to inhibit signaling pathways with well-established roles in cell proliferation, survival, invasion and melanomagenesis

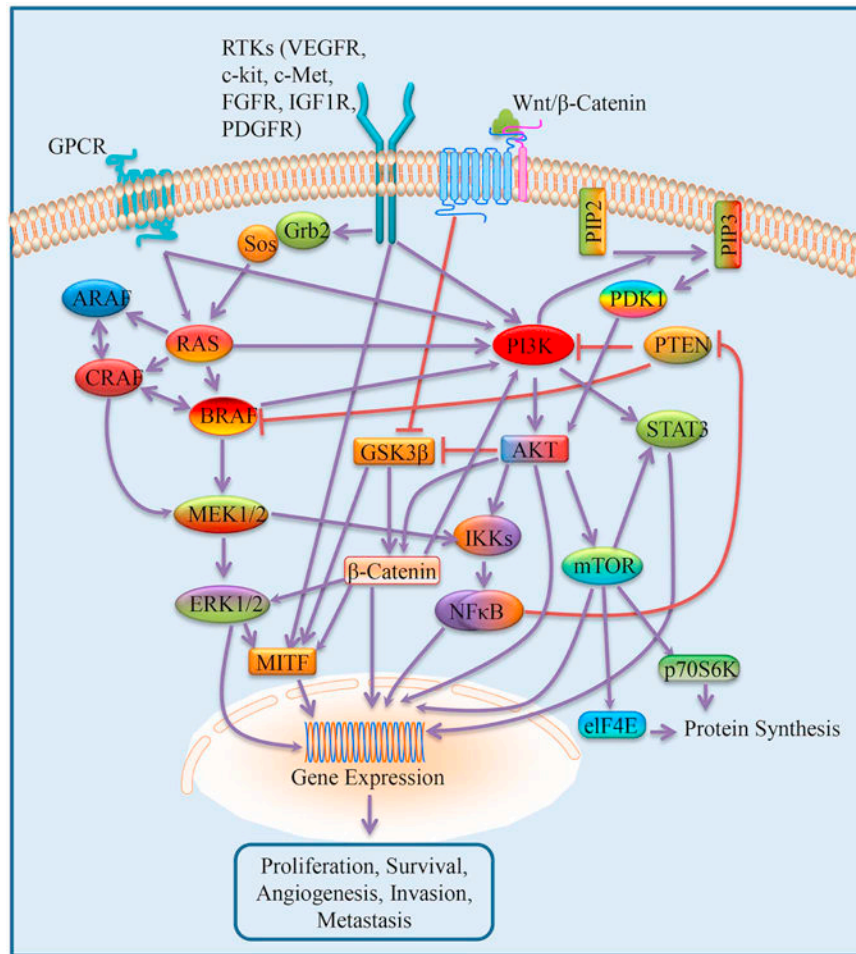


Figure 1.
Signaling pathways activated in melanoma

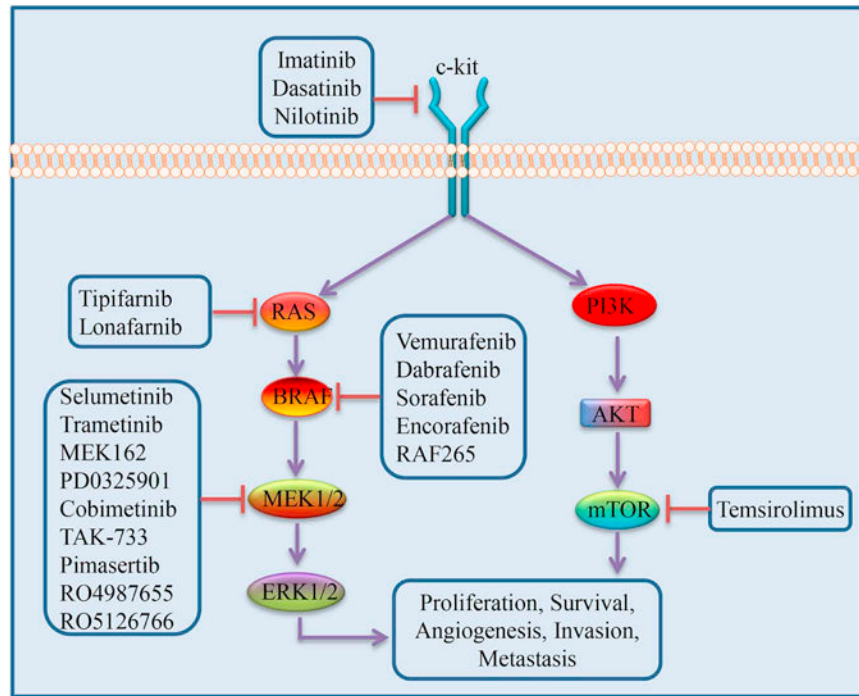


Figure 2. Signaling pathways targeted by synthetic small molecule inhibitors

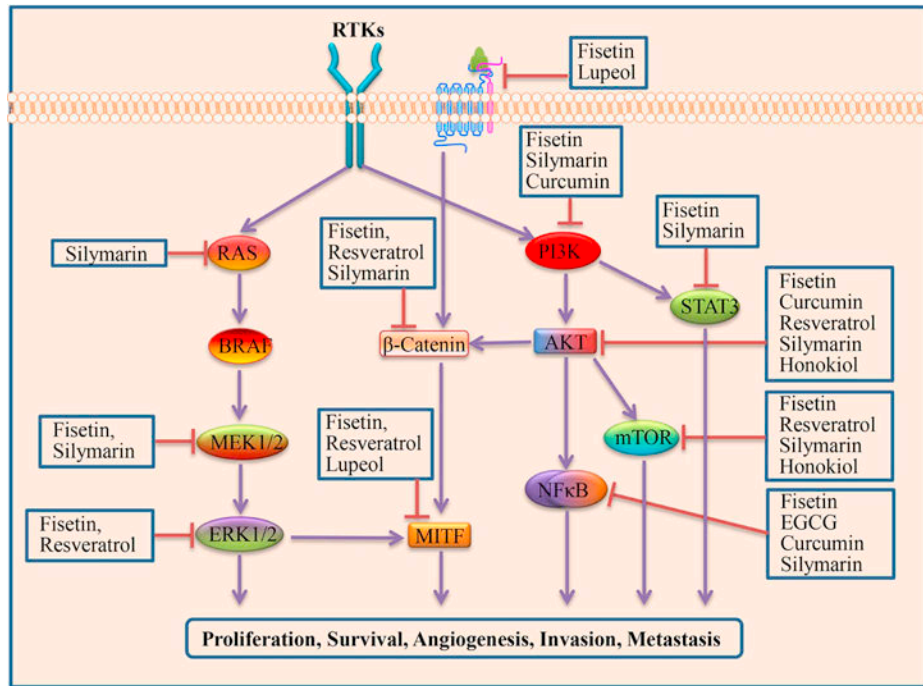


Figure 3. Signaling pathways targeted by phytochemicals

Table 1

Selected clinical trials of synthetic small molecule inhibitors in metastatic melanoma

Inhibitor (code and common name)	Target(s)	Mechanism of action	Phase of clinical trial	Treatment effect or trial status	Mutations selected in patient population	Reference or NCT trial number (if not completed)
Tipifarnib (R115777)	RAS	Inhibition of posttranslational farnesylation of RAS	Phase II	No evidence of clinical activity	None selected	24
Sorafenib (BAY 43-9006; Nexavar)	BRAF CRAF VEGF PDGF	Multikinase inhibitor	Phase III	No significant improvement in PFS, OS or RR	None selected	45
Vemurafenib (PLX4032; Zelboraf)	BRAF ^{V600E} BRAF ^{V600K}	Selective BRAF kinase inhibitor	Phase III	Significant improvement in PFS and OS	BRAF ^{V600E} BRAF ^{V600K}	52
Dabrafenib (GSK 2118436)	BRAF ^{V600E} BRAF ^{V600K}	Selective BRAF kinase inhibitor	Phase III	Significant improvement in PFS	BRAF ^{V600E} BRAF ^{V600K}	55
Encorafenib (LGX818)	BRAF ^{V600}	Selective BRAF kinase inhibitor	Phase I	In progress	BRAF ^{V600}	NCT01436656
RAF265 (CHIR-265)	BRAF ^{V600E} VEGFR2	Multikinase inhibitor	Phase II	Completed results pending	None selected	NCT00304525
Cobimetinib (GDC-0973; XL518)	MEK1/2	MEK1/2 inhibitor	Phase I	In progress	None selected	NCT01271803
Selumetinib (ARRY-142886; AZD6244)	MEK1/2	MEK1/2 inhibitor	Phase II	Significantly improved PFS when combined with dacarbazine but no significant improvement in OS	BRAF ^{V600}	78
Trametinib (GSK1120212; JTP-74057; Mekimist)	MEK1/2	MEK1/2 inhibitor	Phase III	Significantly improved PFS and OS	BRAF ^{V600E} BRAF ^{V600K}	82
MEK162 (ARRY-438162; PD0325901)	MEK1/2	MEK1/2 inhibitor	Phase II	Partial responses observed	NRAS BRAF ^{V600}	34
Pimasertib (AS703026; MSC1936369B)	MEK1/2	MEK1/2 inhibitor	Phase III	In progress	NRAS	NCT01763164
Temsirolimus (CCI-779; Torisel)	mTOR	Rapamycin analog, inhibitor of mTORC1	Phase II	In progress	NRAS	NCT01693068
Imatinib (CGP 57148; CPG57148B; STI-571; Gleevec)	c-kit	Tyrosine kinase inhibitor	Phase II	Objective responses observed	None selected	118
Dasatinib (BMS-354825; Sprycel)	c-kit	Tyrosine kinase inhibitor	Phase II	No significant improvement in PFS or RR	mTOR	NCT01960829
			Phase II		c-kit	148, 149
			Phase II		c-kit	NCT00470470

Inhibitor (code and common name)	Target(s)	Mechanism of action	Phase of clinical trial	Treatment effect or trial status	Mutations selected in patient population	Reference or NCT trial number (if not completed)
Nilotinib AMN 107; Tasigna)	c-kit	Tyrosine kinase inhibitor	Phase II	In progress	c-kit	NCT00700882
			Phase II	Preliminary results showed objectives responses	c-kit	152, NCT01099514
			Phase II	In progress	c-kit	NCT01028222

OS – Overall survival, PFS – Progression free survival, RR – Response Rate

Table 2
Selected clinical trials of synthetic small molecule inhibitor combinations in metastatic melanoma

Inhibitor combination	Target(s)	Phase of clinical trial	Treatment effect or trial status	Mutations selected in patient population	Reference or NCT trial number (if not completed)
Dabrafenib and trametinib compared to dabrafenib monotherapy	BRAF + MEK1/2	Phase II Phase III	No significant improvement in PFS In progress	BRAF ^{V600} BRAF ^{V600}	89 NCT01584648
Dabrafenib and trametinib compared to vemurafenib monotherapy	BRAF + MEK1/2	Phase III	In progress	BRAF ^{V600E} BRAF ^{V600K}	NCT01597908
Sorafenib with temsirolimus or Sorafenib with tipifarnib	BRAF + mTOR or BRAF + RAS	Phase II	No significant improvement in PFS	None selected	120
LGX818 plus MEK162 and LGX818 monotherapy to vemurafenib monotherapy	BRAF + MEK1/2	Phase III	In progress	BRAF ^{V600}	NCT01909453
Cobimetinib in combination with vemurafenib	BRAF + MEK1/2	Phase Ib	Anti-tumor activity	BRAF ^{V600}	91
Cobimetinib in combination with vemurafenib vs vemurafenib alone	BRAF + MEK1/2	Phase III	In progress	brafV ⁶⁰⁰	NCT01689519

OS – Overall survival, PFS – Progression free survival, RR – Response Rate

Table 3

A Summary of the molecular mechanisms(s)/cellular targets of phytochemicals in the treatment of melanoma

Phytochemical	Dietary Source	Category	In vitro Studies	In vivo Studies	Molecular Mechanism(s)/Cellular Targets	References
Fisetin	Strawberries, mangoes, apples, grapes, peaches, persimmons, onions, tomatoes and cucumbers	Flavonoid	A375 cells, 3-D melanoma skin constructs of A375 cells	451Lu melanoma xenografts	Inhibited cell growth and cell cycle progression Inhibited mTOR and p70S6K through direct binding and phosphorylation of AKT	159
			A375, SK-MEL-28, RPMI-7951, SK-MEL-119 and Hs294T cells, 3-D melanoma skin constructs of A375 cells	Reduced cell invasion Inhibited phosphorylation of MEK1/2 and ERK1/2 Inhibited NFκB activation And EMT	158	
EGCG	Green Tea	Catechins	451Lu cells		Inhibited cell growth, cell cycle progression Wnt/β-catenin signaling, MITF and c-myc	157
			A375 and Hs294T cells		Inhibited cell growth Induced cell cycle arrest and apoptosis by reducing Cyclin D1 and cdk2, p16INK4a, p21WAF1/CIP1 and p27KIP1 Modulated expression of Bcl-2 family proteins	177
			I205Lu and Hs294T cells		Inhibited cell growth Decreased IL-β secretion Down regulated NLRP1 Reduced caspase-1 activation Inhibited NFκB activities	178
			B16 cells		Inhibited B16 cells adhesion to laminin	179
Resveratrol	Grapes	Stilbene	B16-F10		Increased cell stiffness and inhibited cell migration	180
			A375 and Hs294T cells		Inhibited cell migration and invasion Reduced COX-2 and PGE2 receptors Inhibited NFκB activation and EMT	181
			B16-F3m cells	B16-F3m cells injected intraperitoneally in to Balb/c mice	Inhibited cell spreading on extracellular matrix and cell-cell interactions Inhibited phosphorylation of FAK Inhibited MMP-9 activity Reduced lung metastasis in mice Increased the survival of melanoma bearing mice	182
Resveratrol	Grapes	Stilbene	A375 and SK-MEL-28 cells		Inhibited cell growth Induced apoptosis Induced phosphorylation of ERK1/2 in A375, but not in SK-MEL-28 cells No effect on phosphorylation of p38 or JNK MAP kinases in either cell line	162
			B16 cells		Induced autophagy through ceramide accumulation Inhibited AKT/mTOR pathway	163

Phytochemical	Dietary Source	Category	In vitro Studies	In vivo Studies	Molecular Mechanism(s)/Cellular Targets	References
	B16 cells			Inhibited growth of an established B16/DOX melanoma and prolonged survival	Inhibited cell growth Induced cell cycle arrest and apoptosis Down regulated cyclin D1/cdk4 Increased p53 expression	164
	Autologous human melanoma cell line (weakly metastatic Line IV clone 3 and on autologous, highly metastatic Line IV clone) originally established from a primary malignant melanoma lesion				Inhibited cell growth and colony formation Induced cell cycle arrest Increased expression of p53	165
	Lu1205 cells				Reduced cell invasion Decreased AP-1/JunD, MMP-1, Bcl-2, and iNOS protein levels	166
	B16 cells				Inhibited cell growth and invasion Increased expression of α -MSH signaling-related molecules, including β -catenin, c-kit, and MITF Inhibited MMP-9 expression	167
	B16-F10 and B16-BL6 cells			B16-F10 cells injected in tail vein of C57BL/6 mice	Reduced tumor volume and metastasis; reduced AKT expression	168
	YUZA26 and M14 cells				Reduced melanoma induced angiogenesis Increased expression of p53, matrix protein TSP1, Inhibition HIF-1 α and VEGF production	169
	K1735 and B16-F10 cells			C57BL/6 mice	Enhanced cisplatin cytotoxicity Increased Connexin 43	170
	WM3211, c81-46A and c83-2c cells				Enhanced dacarbazine cytotoxicity Inhibition of Ref-1-activated AP-1 DNA-binding and endonuclease activities	171
	SK-MEL-5 and SK-MEL-28 cells				Inhibited cell growth Induced cell cycle arrest Inhibited kinase activity MEK1/2 and ribosomal S6 kinase-2 Reduced phosphorylation of ERK1/2 Reduced activation of NF κ B, AP-1, and STAT3	173
	A375, Hs294T, Mel 1241 and Mel 1011 cells	Flavonoid			Inhibited cell migration and invasion Enhanced the levels of casein kinase 1 α and GSK-3 β Inhibited β -catenin signaling Reduced MMP-2 and MMP-9 levels	174
Silymarin	Milk Thistle			A375-S2 cells	Enhanced pro-apoptotic actions of anti-Fas agonistic antibody CH11 Increased the expression of Fas-associated proteins with death domain Increased cleavage of procaspase-3 Increased digestion of the inhibitor of caspase-activated DNase	175

Phytochemical	Dietary Source	Category	<i>In vitro</i> Studies	<i>In vivo</i> Studies	Molecular Mechanism(s)/Cellular Targets	References
Curcumin	Turmeric	Polyphenol	B16 and B16-BL6 cells	Spontaneous metastatic tumor model of C57BL/6J mice	Inhibited cell invasion to the draining lymph nodes Inhibited Src and STAT3 phosphorylation through PRL-3 down-regulation	183
			MMAN, MMRU, RPEP, PMWK, SK-MEL-2, SK-MEL-5, SK-MEL-28 and MeWo cells		Induced apoptosis independent of p53 Activated caspases-3 and -8 Inhibited NFκB Induced Fas receptor aggregation in FasL-independent manner Suppressed the apoptotic inhibitor, XIAP	184
			B78H1, SK-MEL-28 and MeWo cells	C57BL/6 mice	miRNA expression signature in tumors was substantially altered by curcumin intake with mmu-miR-205-5p over 100 times higher expressed when compared to controls Induced apoptosis Down regulated Bcl-2 and PCNA	186
			B16-F10 cells		Induced apoptosis, ER stress, p23 cleavage Down regulated Mel-1 protein	187
			4046T cells		Induced apoptosis Suppressed NFκB, COX-2 and cyclin D1	189
			C32, G-361, and WM 266-4 cells		Induced apoptosis Suppressed NFκB activation independent of BRAF/MEK/ERK and AKT pathway	190
			A375 and MeWo cells		Induced cell cycle arrest and apoptosis Down regulated NFκB activation, iNOS Up regulated p53, p21(Cip1), p27(Kip1) and checkpoint kinase 2	188
			B16 and WM-115 cells		Induced ROS production and apoptosis Induced MST1 and JNK activation, Foxo3a nuclear translocation	191
			B16-F10 cells		Inhibited cell growth Induced cell cycle arrest Enhanced expression of cyclin-dependent kinase inhibitors (cyclin A, p21 and p27) Decreased expression of DNMT1 Inhibition of cyclic nucleotide phosphodiesterase 1–5 activities	192
			B16-2F2 cells		Induced differentiation	198, 199
Lupeol	White cabbages, green peppers, strawberries, olives, mangoes and grapes	Triterpene	B16-2F2 cells		Induced differentiation Increased expression of melanosome transport proteins: tyrosinase, Rab27a, and myosin-Va	200
			B16-2F2 cells		Induced differentiation Activation of p38 MAPK,	201
			B16-2F2 cells		Suppressed migration; Disassembly of the actin cytoskeleton	202
			Mel 928, Mel 1011 and Mel 1241 cells	Mel 928 and Mel 1011 xenograft	Inhibited cell growth Induced apoptosis Decreased β-catenin transcriptional activity Decrease in the expression of Wnt target genes	195

Phytochemical	Dietary Source	Category	<i>In vitro</i> Studies	<i>In vivo</i> Studies	Molecular Mechanism(s)/Cellular Targets	References
			B16-2F2	C57BL/6 mice subcutaneously injected into with B16 2F2 cells	Suppressed c-myc, cyclin D1, PCNA, Ki-67 and osteopontin expression Suppressed c-myc, cyclin D1, PCNA, Ki-67 and osteopontin expression Suppressed tumor growth Induced cell cycle arrest Decreased Ki-67 and PCNA expression	197
			WM35 and 451Lu cells	451Lu xenograft	Inhibited cell growth Induced cell cycle arrest Decreased expression of Cyclins and cdk2 Modulated Bel-2 family proteins Reduced 451Lu tumor growth, proliferation and cell cycle regulatory proteins	196
			SK-MEL2 and MeWo cells		Inhibited cell growth Increased cytosolic cytochrome c, caspase activity, and mitochondrial depolarization	204
Honokiol	Magnolia	Biphenol	B16-F10 cells		Inhibited cell growth Inhibited AKT/mTOR and Notch signaling	205
			CHL-1 and WM266-4 cells		Induced apoptosis due to ER stress from an interaction with glucose regulated protein 78	206