Published in final edited form as:
Surg Oncol Clin N Am. 2011 January ; 20(1): 165–180. doi:10.1016/j.soc.2010.09.003.

Targeted Therapy for Melanoma - A Primer

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Keywords

targeted therapy; BRAF; NRAS; c-KIT; PTEN; GNαQ

Introduction

Melanoma is the most aggressive form of skin cancer. Unfortunately, despite recent improvements for some solid tumors, the prevalence and mortality of melanoma continue to increase. For patients with distant metastases, treatment with single-agent or combination chemotherapy regimens have generally resulted in very low response rates, with no significant impact on patient survival [1]. Immunotherapies (i.e. interleukin-2, ipilimumab) have also yielded overall low response rates, although a small subset of patients have achieved durable responses and long-term survival [2–5]. These modest achievements are further limited by noting that immunotherapies may also result in significant toxicities, including treatment-related deaths. Thus, there is a critical need for new therapeutic approaches for this aggressive disease.

The treatment of many cancers is entering into a new era based on an improved understanding of the molecular pathogenesis of these diseases. While some cancers appear to be primarily driven by viral infection, the majority are caused by genetic events that alter the expression and/or function of normal genes and proteins. These events, which include gene amplifications, deletions, and mutations, disrupt the regulatory processes that normally control the growth and survival of cells. Multiple analyses have shown that while there is a spectrum of genetic abnormalities in cancer cells, most of them affect certain signaling pathways and functions. Cancer cells are subsequently often critically dependent upon these pathways for survival, a phenomenon termed ‘oncogene addiction.’ This reliance on pathways that are hyperactivated by genetic events that occur specifically in cancer cells presents a therapeutic opportunity to block those targets in order to inhibit the growth and survival of the cancer while sparing the normal cells of the body. This approach, termed

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Disclosures: M. Davies receives research support from GlaxoSmithKline, Merck, and AstraZeneca

'targeted therapy,' has been shown to be an effective and FDA-approved strategy for a number of cancers, including chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GIST), and renal cell carcinoma (RCC) [6]. Targeted therapies have also proven effective in treating specific subpopulations of patients with other cancers, such as the use of trastuzumab (Herceptin) for HER2/neu-amplified breast cancer [7]. For each of these examples, successful implementation of a targeted therapeutic approach was critically dependent upon the identification of activating genetic events, as well as the affected pathways that were present for each specific tumor type.

There is now evidence that the majority of melanomas harbor one or more mutations in critical kinase signaling pathways. Interestingly, accumulating data supports that the prevalence of these events varies greatly among the subtypes of melanoma that have been defined by clinical and pathologic characteristics [8]. Most cutaneous melanomas (CM) arise from melanocytes on sun-exposed skin. Exposure to ultraviolet radiation is thought to play a major causative role in these tumors. However, the role of ultraviolet radiation is less clear for cutaneous melanomas arising from relatively sun-protected sites. Examples of such melanomas include those arising on the palms and soles; these are termed acral lentiginous melanomas (ALM). Melanomas may also arise from melanocytes in the mucosa of the head and neck, the gastrointestinal tract, and the genitourinary tract. Melanomas arising in such sites are classified as mucosal melanomas (MuM), and clearly arise in the absence of exposure to ultraviolet radiation. Consistent with the hypothesis that these melanoma subtypes are caused by different factors, comparative genomic hybridization (CGH) analysis has demonstrated that these clinically-defined groups of tumors have markedly different patterns of DNA copy number changes, including subtype-specific gene amplifications and deletions [9]. CMs that arise in areas with chronic sun exposure, and that have histologic evidence of chronic sun damage (CSD), also exhibit markedly different chromosomal and gene copy number changes compared to CMs without chronic sun damage. Melanomas may also arise from melanocytes in the uveal tract of the eye (iris, ciliary body, and choroid), and are referred to as uveal melanomas (UvM). These tumors are also characterized by chromosomal changes that are distinct from CMs, ALMs, and MuMs [10, 11].

The identification of activating mutations in melanoma, combined with a growing appreciation of the different pattern of genetic changes in the anatomically defined melanoma subtypes, has become the focus of a concerted effort to translate these discoveries into personalized therapeutic approaches for this disease. In this article, we will review: (1) the known mutations, amplifications, and deletions in kinase signaling pathways that have been implicated in melanoma; (2) the prevalence of these genetic events in clinicopathologically defined melanoma subtypes; and (3) the results of clinical trials that utilize targeted therapy approaches to block aberrantly activated pathways resulting from such mutations. Importantly, we will also discuss the challenges that must be overcome in order to achieve improved outcomes with targeted therapies in melanoma in the future.

BRAF

The RAS-RAF-MEK-MAPK signaling pathway is a critical regulator of cellular growth and survival [Figure 1] [12, 13]. The first components of the pathway are the RAS-family GTP-ases. The RAS family members (HRAS, KRAS, NRAS) are guanine-nucleotide binding proteins that are embedded in the inner surface of the cell membrane. Normally, the RAS proteins are GDP-bound and inactive. A variety of activating signals result in the exchange of GTP for GDP, which activates RAS. RAS family members are also frequently affected by mutations in cancer that result in constitutive GTP-binding. The activated RAS members physically interact with the RAF family of serine-threonine protein kinases (ARAF, BRAF, CRAF) downstream of RAS. This interaction activates the RAF proteins, which then

translocate to the cytoplasm and phosphorylate the MEK protein kinases (MEK1, MEK2). The MEK proteins are activated by this phosphorylation, and subsequently phosphorylate the P44/42 MAPK serine-threonine kinases (ERK1, ERK2). The activated MAPKs phosphorylate a variety of transcription factors and cytosolic proteins to promote proliferation and survival.

In 2002, an experimental screen for mutations in RAF family members in cancer cell lines and tumors identified point mutations in *BRAF* in approximately half of the melanomas that were examined in the study, as well as occasional (3–18%) colon, lung, breast, and ovarian cancer specimens [14]. Since this sentinel observation, the high frequency of point mutations in *BRAF* in melanoma has been confirmed in multiple studies. A recent meta-analysis of over 200 published studies reported an overall mutation rate of 65% in melanoma cell lines and 42% in tumors [15]. The higher frequency of mutations in cell lines likely reflects a positive selection for cells with the *BRAF* mutation to propagate *in vitro*. Analysis of anatomic subtypes demonstrated that *BRAF* mutations are relatively common in CMs (42.5%), but uncommon in MuMs (5.6%) and rare in UvMs (<1%) [Table 1]. Among CMs, *BRAF* mutations are common in superficial spreading melanomas (SSM) (53%), but are less prevalent in acral lentiginous (ALM) (18%) and lentigo maligna melanomas (LMM) (9%) [15]. LMMs are associated with CSD, and often originate in the head and neck region. The low prevalence of *BRAF* mutations in these tumors is consistent with the different patterns of DNA copy number gains and losses observed in the CGH analysis of cutaneous melanomas with or without chronic sun damage. *BRAF* mutations are also detectable in up to 80% of common acquired nevi [16–18]. However, *BRAF* mutation rates are lower in several of the less-common nevi types, including congenital, Spitz, and blue nevi [18–20].

Approximately 50 different point mutations in *BRAF* have been identified in cancer [21]. A single substitution, the *BRAF V600E* mutation, comprises ~85% of the *BRAF* mutations detected in melanoma [15]. The V600E mutation increases the *in vitro* kinase activity of the BRAF protein more than 400-fold [21]. Most of the other reported somatic *BRAF* mutations, particularly other changes involving the V600 residue, also increase BRAF's catalytic activity (5-fold to 700-fold). Interestingly, a few of the *BRAF* mutations that have been detected in cancer cells (G466E, G466V, G596R, D594V) decrease the catalytic activity of the BRAF protein [21, 22]. As *BRAF V600E* is the predominant mutation identified in tumors, including melanoma, most functional studies have examined the function of the protein encoded by this change. Expression of the BRAF V600E protein results in constitutive phosphorylation and activation of MEK and MAPK [14, 21]. Inhibition of BRAF V600E with small interfering RNA (siRNA, shRNA) inhibits MAPK activation, growth, and survival of human melanoma cell lines with this mutation [23, 24]. These data support that melanomas with the *BRAF* V600E mutation depend on it for survival, and thus implicated it as a therapeutic target.

Sorafenib was the first BRAF inhibitor to be used in clinical trials in melanoma. Sorafenib is a small molecule inhibitor of multiple tyrosine kinases, including BRAF, CRAF, c-KIT, vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR) [25]. In preclinical studies, sorafenib slowed the growth of melanoma xenografts in nude mice, but it did not cause tumor regression [26]. Among 34 evaluable patients with metastatic melanoma in a phase II trial, only 1 partial response was observed [27]. Subsequently, more promising results were reported in a phase I/II clinical trial of sorafenib combined with paclitaxel and carboplatin[28]. While the trial enrolled patients with multiple tumor types, all of the clinical responses were achieved in melanoma patients; among these patients, the response rate was 42% and the median progression-free survival was ~10 months. Although these results were encouraging when compared to previous trials in melanoma with paclitaxel and carboplatin alone (response rates of <10–20%) [29, 30], a

subsequent randomized phase III trial showed that sorafenib did not improve the response rate or progression-free survival compared with the doublet of paclitaxel and carboplatin alone [31]. These results raised the possibility that mutant BRAF was not a good therapeutic target in melanoma. The observation that activating *BRAF* mutations are present in up to 80% of benign nevi - indolent lesions with almost no malignant potential - is certainly consistent with the hypothesis that this genetic alteration alone cannot fully explain the aggressive biology of melanoma [16]. Similarly, introduction of the *BRAF* V600E mutation alone was not sufficient to transform melanocytes into invasive lesions in multiple models, but required complementation by other genetic events to transform cells [32–34]. An alternative explanation for the failure of sorafenib is that mutant BRAF is actually a good therapeutic target, but that the drug did not inhibit BRAF effectively. Interestingly, sorafenib has demonstrated marked clinical efficacy in renal cell carcinoma, a tumor characterized by dependence upon signaling by VEGFR, but not by BRAF [35, 36]. Of note, in the phase II trial of paclitaxel, carboplatin, and sorafenib, there was no association noted between the presence of *BRAF* mutations and clinical responses, but there was a positive association with expression of the VEGFR2 protein and clinical response [28, 37].

The potential of the BRAF V600E protein as a therapeutic target for melanoma has now been validated by clinical trials with second-generation BRAF inhibitors. PLX4032 (also known as RG7204) is a more potent BRAF inhibitor than sorafenib, and it is selective for the V600E mutant form of the protein. PLX4032 inhibits the catalytic activity of the BRAF V600E protein at an IC50 of 13 nM, which is more than 10-fold lower than the dose that inhibits the wild-type protein [38]. In contrast to sorafenib, an inhibitor of multiple protein kinases at therapeutic drug levels, PLX4032 has minimal activity against most other protein kinases, with an IC50 >1,000 nM for many related proteins. Preclinical studies demonstrated that PLX4032 inhibited the growth of melanoma cells with a BRAF mutation at 10x-100x lower concentrations than melanoma cell lines without a mutant BRAF [39]. PLX4032 also caused the regression of BRAF-mutant melanoma xenografts in mouse models [39].

Recently, the results of a Phase I clinical trial with PLX4032 have been reported [40, 41]. In the initial phase of the trial, the drug was well tolerated but no clinical responses were observed. Importantly, however, the serum levels achieved were below those that correlated with anti-tumor activity *in vitro.* The drug was then reformulated from a crystalline compound to a microprecipitated bulk powder. Subsequently, linear dose-dependent increases in serum levels were observed, as were clinical responses. In the Phase I trial dose expansion cohort of 32 *BRAF V600E* mutant melanoma patients treated with 960 mg twice daily (the dose selected for further testing), two complete responses and 24 partial responses were observed, for an overall response rate of 81% [41]. The major toxicity was the development of cutaneous squamous cell carcinomas, mostly keratocanthomas, which developed in 31% of patients. These lesions were treated by surgical resection and did not result in any patient coming off study. No squamous cell carcinomas were observed at non-cutaneous sites. While this high overall response rate is unprecedented for a single agent in melanoma, several patients developed secondary resistance after their initial response noted by recurrence and/or progression of their tumors. The median duration of response to PLX4032 in the initial trial report was ~7 months [41].

The efficacy of targeting mutant BRAF is also supported by early results from the Phase I trial of GSK2118436, another potent, mutant-specific BRAF inhibitor [42]. While the maximum tolerated dose has yet to be reached, patients with BRAF-mutant melanoma treated with 150 – 200 mg twice daily had a 63% response rate, and 39% of patients treated with lower doses had also responded. The duration of these responses is currently unknown. Similar to the PLX4032 trial described above, cutaneous squamous cell carcinomas also represented the major toxicity observed in the GSK2118436 trial.

The reported activities of PLX4032 and GSK2118436 in melanoma patients with *BRAF V600* mutations suggests that a new standard of care will likely soon exist for these patients. However, it is also apparent that these agents should not be used in unselected melanoma patients. In the Phase I trial of PLX4032, 5 patients were included who did not have a BRAF mutation. None of these patients responded; in fact, 4 of the patients had tumor progression in the first 2 months of treatment [41]. The lack of clinical response, and possible rapid progression, in patients not harboring a *BRAF* mutation is consistent with observations in preclinical models by four different groups suggesting potential promotion of tumor growth when mutant-selective BRAF inhibitors are used to treat *BRAF* wild-type melanomas [22, 43–45]. In these experiments, inhibition of BRAF in melanoma cell lines expressing wild-type BRAF resulted in the hyperactivation of the MAPK pathway. While the results varied somewhat between the groups, the BRAF inhibitors promoted the formation of heterodimers of BRAF and CRAF that potently activate MEK in cells without a *BRAF* mutation. The cells were then dependent upon CRAF for MAPK pathway activation and survival. Interestingly, low catalytic activity BRAF mutants seem to activate MAPK similarly, and preclinical evidence suggests that melanoma cells with these mutations are sensitive to CRAF inhibition, including treatment with sorafenib [22, 46]. Thus, while sorafenib failed to demonstrate activity in unselected patients, it is possible that it may be effective for certain genetic subtypes of melanoma.

NRAS

Mutations in the members of the RAS family of GTP-ases are one of the most frequent events in cancer [47]. While mutations of *HRAS* and *KRAS* are common in many cancer types, they are very rare in melanoma. However, *NRAS* mutations have been reported in 14% of human melanoma cell lines and 15–25% of melanoma clinical specimens [15, 48–50]. The mutations affecting *NRAS* are highly conserved; mutations affecting positions 12 and 61 constitute approximately 90% of the mutations reported in melanoma [15]. The prevalence of *NRAS* mutations varies across the different anatomically-defined melanoma subgroups, although not as dramatically as is observed for *BRAF* mutations [Table 1]. *NRAS* mutations are detected in approximately 26% of CMs, 14% of MuMs, and <1% of UvMs [15]. Among CMs, 22% of superficial spreading melanomas and 28% of nodular melanomas have *NRAS* mutations, while significantly lower rates are observed in acral lentiginous (4%), spitzoid (10%), and lentigo maligna (0%) melanomas [49–51]. *NRAS* mutations are also present in common acquired nevi (6–20%) at a similar rate as has been detected in melanomas, and potentially at an even higher rate in congenital nevi [17–20]. Interestingly, although rare in melanoma and other types of melanocytic nevi, *HRAS* mutations have been reported in 12 – 29% of Spitz nevi [51, 52].

With very rare exceptions, the common activating *BRAF* and *NRAS* mutations are mutually exclusive in melanoma tumor and cell lines [48, 49, 53]. This is likely due to the fact that both of these mutations potentially activate the MAPK pathway, and the presence of both would be functionally redundant. In contrast, approximately 10% of melanomas with *BRAF* mutations that are catalytically inactive (i.e. D594V) also have activating *NRAS* mutations [22]. While the activated mutant forms of BRAF and NRAS both activate MEK and MAPK, the activation of these downstream elements is CRAF-dependent in melanomas with *NRAS* mutations, whereas it is BRAF-dependent in *BRAF*-mutant cells [54].

The development of direct RAS inhibitors, a priority in a number of cancer types, has proven to be quite challenging [55]. One approach has involved the development of farnesyl transferase inhibitors (FTIs), as RAS proteins must be farnesylated in order to translocate to the plasma membrane and activate their related signaling pathways. However, when farnesylation is inhibited, both NRAS and KRAS undergo an alternative modification,

geranylgeranylation, that allows the proteins to be recruited to the plasma membrane [56]. In addition, since over 60 cellular proteins have been shown to be farnesylated, FTIs will likely have off-target effects (and potentially dose-limiting toxicities) that compromise the ability to reach levels that effectively inhibit RAS. This lack of specificity also makes it challenging to determine the role of RAS in both the activity and toxicity of FTIs.

An alternative strategy to treat *NRAS*-mutant tumors is to inhibit pathways that are downstream of the mutant RAS protein. Experiments in multiple tumor types have demonstrated that mutant RAS activates multiple pro-survival and proliferative pathways in addition to the RAF-MEK-MAPK cascade [57]. Other RAS effectors include PI3K, RALGDS, and PKCε. Among these, PI3K has gained particular attention, as the PI3K-AKT pathway has been implicated in melanoma by other genetic events, and it is the target of aggressive drug development [Figure 1] [58, 59].

PI3K-AKT Pathway

The PI3K-AKT pathway is one of the most important signaling networks in cancer [60]. PI3K is a lipid kinase that consists of a regulatory subunit (*PIK3R*; p85) and a catalytic subunit (*PIK3C;* p110). PI3K's catalytic activity is activated by a number of different signals, including growth factor tyrosine kinase receptors and activated RAS proteins. Activation of PI3K results in the phosphorylation of the 3′-OH of phosphatidylinositols (PI) in the plasma membrane, generating PI (3,4)P_2 and PI(3,4,5)P_3. These 3′-phospholipids recruit proteins to the cell membrane that have a pleckstrin homology (PH) domain, such as AKT and PDK1. AKT, a serine-threonine kinase that normally exists in an inactive state in the cytoplasm, has been extensively studied as one of the key molecules that are regulated by PI3K. Upon recruitment to the plasma membrane, AKT is phosphorylated at two critical residues, Ser473 (by the mTORC2 complex) and Thr308 (by PDK1), which activates its serine-threonine kinase activity. The activated AKT molecule translocates to the cytoplasm where it phosphorylates a variety of substrates, including FOXO, GSK3α/β, BAD, TSC2, and MDM2 [Figure 1]. Through these and other substrates, activation of AKT regulates a number of processes that contribute to the malignant phenotype, including proliferation, survival, invasion, and angiogenesis [60].

The PI3K-AKT pathway is affected by mutations that activate it more than any other signaling pathway in cancer [61]. The PI3K-AKT pathway was initially implicated in melanoma by the identification of activating *NRAS* mutations. In addition, loss of function of PTEN, a critical negative regulator of the pathway, is a frequent event in melanoma. PTEN inhibits the activation of AKT by dephosphorylating phosphatidylinositols at the 3′ position, thereby antagonizing PI3K-mediated signaling [Figure 1] [62]. Loss of PTEN results in constitutive activation of AKT in multiple cancer types, including melanoma [63]. Loss of PTEN, by both genetic and epigenetic mechanisms, has been reported in 10–30% of melanomas [64–66]. The prevalence of PTEN loss has been defined predominantly in cutaneous melanomas, and so the relative prevalence in anatomically-defined subtypes is not known at this time. Nonetheless, loss of PTEN is frequently detected in melanoma tumors and cell lines with a concurrent *BRAF* mutation, but it appears to be mutually exclusive with *NRAS* mutations [50, 67–69]. While this pattern of mutations suggests that PTEN loss and *NRAS* mutations may have functional redundancy, quantitative analysis of AKT activation in melanoma tumors and cell lines showed that loss of PTEN correlated with much higher levels of activated AKT [70]. This finding is similar to previous studies that showed non-equivalent activation of, and functional dependence upon, different PI3K-AKT pathway effectors by PTEN loss and *PIK3CA* mutations [71]. *P IK3CA* mutations are relatively common in breast and colon cancer, but have been detected in ≤ 3% of melanomas [72, 73]. Activating mutations of *AKT*, initially identified in breast, colon, and ovarian cancers, have

also been detected as rare events in melanoma (2%) [74, 75]. Each melanoma with an *AKT* mutation also had a *BRAF* mutation. While activating mutations of *AKT* in other cancers all involved the *AKT1* isoform, some of the mutations in melanoma affected the *AKT3* gene. A role for AKT3 in melanoma is supported by previous studies that showed a frequent switch from AKT1 to AKT3 expression and dependence in metastatic melanomas [76, 77].

Inhibitors against multiple components of the PI3K-AKT pathway have been developed and are in various stages of clinical testing [Figure 1] [58]. Initial clinical trials were performed with mTOR inhibitors, in part because the safety of these agents had been previously established by the use of Rapamycin (an mTOR inhibitor) in transplant patients. Similar to the experience in several other cancers, mTOR inhibitors have shown little activity in melanoma. In a phase II clinical trial, the Rapamycin analog (rapalog) CCI-779 produced only one short-lived partial response among 33 patients with metastatic melanoma [78]. In contrast to at least some other targeted therapies in which drug levels sufficient to significantly inhibit their intended target are not attained, it does appear that mTOR inhibitors reach levels that significantly inhibit their target *in vivo* [79, 80]. However, studies in both clinical specimens and cell lines have demonstrated that rapalogs activate AKT, thus contributing to their lack of efficacy [80, 81]. The mTOR protein participates in 2 different complexes, referred to as mTORC1 and mTORC2. The mTORC1 complex, which is inhibited by rapalogs, regulates the activation of protein translational machinery by activating P70S6K. However, it also negatively regulates PI3K as part of a feedback regulatory loop of the PI3K-AKT pathway. The mTORC2 complex, which is not inhibited by the rapalogs, phosphorylates and activates AKT [82]. In preclinical models, combined inhibition of the mTORC1 and mTORC2 complexes blocked the activation of P70S6K and AKT, and more effectively inhibited growth and survival of cancer cells, than inhibition of mTORC1 alone [83]. Clinical testing has yet to be reported for this agent. Similarly, clinical trials of PI3K and AKT inhibitors are ongoing. However, preclinical experiments with RAS-mutant tumors, including melanomas, showed synergistic inhibition of tumor growth and survival when inhibitors against the RAS-RAF-MEK-MAPK and the PI3K-AKT pathways were combined [84, 85]. The high frequency of *BRAF* mutations in melanomas with PTEN loss suggests that combinatorial regimens may be necessary in other melanoma genotypes as well [86].

c-KIT

While the majority of sun-exposed cutaneous melanomas harbor an activating mutation in BRAF or NRAS in the MAPK signaling pathway, such changes are relatively rare in non-cutaneous melanomas. This disparity led to investigations that attempted to identify other genetic changes that could activate the same or other kinase signaling pathways in these tumors. In the CGH analysis of melanoma subtypes, the chromosomal region 4q12 was selectively amplified in ALMs and MuMs [9]. This region harbors a number of candidate genes, but detailed analysis demonstrated that the *c-KIT* gene was the focal target of copy number gain in this region [87]. Extra copies of *c-KIT* were identified in 8% of MuMs and 7% of ALMs. The *c-KIT* gene was also amplified in 6% of CMs with evidence of CSD, whereas no amplifications were detected in CMs without CSD. In addition, sequencing of *c-KIT* identified missense mutations in 21% of the mucosal, 11% of the acral, and 17% of the CSD cutaneous, but 0% of the non-CSD cutaneous melanomas [87]. Subsequent studies have reported similar rates of *c-KIT* mutations in mucosal and acral melanomas, but they have reported lower rates of mutations in CSD cutaneous tumors, as well as different rates of gene copy number gain across the subtypes [Table 1] [88–90].

The *c-KIT* gene encodes a membrane tyrosine kinase receptor. Mutations of *c-KIT* are the most common mutation detected in gastrointestinal stromal tumors (GISTs) [91]. These

mutations result in constitutive activation of the c-KIT tyrosine kinase, and activation of multiple pro-survival signaling pathways, including the MAPK and PI3K-AKT pathways [Figure 1]. GISTs exhibit oncogene addiction to the mutant c-KIT proteins, and c-KIT inhibitors (e.g., imatinib) have become the standard treatment for this disease [92, 93].

The mutations that affect *c-KIT* in melanoma occur in the same regions of the gene as are observed in GIST. The finding of activating mutations of *c-KIT* in melanoma was surprising, as previous studies had demonstrated that c-KIT protein expression is frequently lost in melanoma progression [94]. While c-KIT is required for normal melanocyte development, enforced expression of c-KIT in melanoma cells lines resulted in decreased growth and tumorigenicity [94]. Perhaps most importantly, in three phase II clinical trials of imatinib in melanoma patients, the clinical response rate was only 1.5% [90]. However, these clinical trials were overwhelmingly comprised of patients with cutaneous primary melanomas, and thus unlikely included patients with *c-KIT* mutations or amplifications. There are now multiple case reports of metastatic melanoma patients with *c-KIT* mutations achieving dramatic clinical responses to various c-KIT inhibitors [95–97]. Clinical trials that are restricted to metastatic melanoma patients with *c-KIT* mutations or amplifications are currently ongoing.

GNαQ

Mutations in *BRAF*, *NRAS*, and *c-KIT* are detected in <1% of uveal melanomas [Table 1]. Recently, two different groups reported point mutations in the gene encoding the stimulatory α-subunit of G-protein coupled receptors, *GNαQ* [98, 99]. The mutations were detected in approximately 45% of uveal melanoma clinical specimens and cell lines. The mutations were highly conserved, affecting the *RAS*-like domain of the protein, and specifically occur at the Q209 residue that is analogous to the Q61 residue that is frequently mutated in *NRAS* [99]. Expression of the mutant GNαQ protein in melanocytes cooperated efficiently with other genes to induce both anchorage-independent growth and tumor formation in mice. While the pathways that are activated by and critical to the function of the GNαQ protein remain to be fully elucidated, it does appear that the RAS-RAF-MEK-MAPK pathway is one of its effectors [99]. This finding suggests that inhibitors of this pathway, which were previously believed to be indicated for cutaneous melanomas only, may also have a role in uveal melanoma.

Summary

More than 10 years have passed since the FDA approved a systemic therapy for the treatment of metastatic melanoma. After years of negative clinical trials, melanoma now appears to be entering a new era in which multiple new therapeutic options will be available. The identification of clinically active targeted therapy approaches has been a gradual process, built upon an improved understanding of the appropriate use of these agents. While the recent results described here have generated great enthusiasm in the melanoma community, there clearly remain many critical hurdles to overcome.

The development of effective BRAF inhibitors, associated with unprecedented clinical response rates in appropriately selected patients, is both exciting and informative. The initial negative clinical trials with sorafenib have been followed by promising studies with PLX4032 and GSK2118436. In retrospect, the failure of sorafenib was likely due to insufficient inhibition of the MAPK pathway. Indeed, PLX4032 initially failed to demonstrate clinical activity, and only achieved clinical responses when its formulation was changed, increasing drug exposure to levels that correlated with efficacy *in vitro*. Thus, the successful development of this highly active therapy for *BRAF*-mutant melanomas was

dependent upon detailed evaluation beyond simply the measurement of clinical efficacy, but also studies to determine if the drug was being appropriately dosed. Going forward, the evaluation of other experimental therapies must include assessment of the drug levels and on-target effects in order to determine if failures are due to the selection of a poor target, or alternatively are due to pharmacodynamic failure of the therapy. Melanoma presents the rare opportunity to evaluate easily accessible tumor tissue in many patients due to the frequent spread of this disease to cutaneous, subcutaneous, and lymphatic sites, and thus may be an ideal clinical venue for the evaluation of new therapies against important targets.

While targeted therapies against both BRAF and c-KIT have demonstrated marked activity in patients with mutations in these genes, secondary resistance has rapidly developed in many patients [41, 96]. If the mutations or pathways that cause this resistance can be identified, rational targeted therapy combinations to overcome and/or prevent relapses may be identified. Alternatively, targeted therapies may need to be combined with other therapeutic modalities to improve outcomes. For example, while targeted therapies are seemingly characterized by high response rates but relatively short duration of response, immunotherapies are characterized by infrequent responses that are often durable. It is reasonable to hope that combining these and other modalities may lead to treatments with high rates of durable responses. Indeed, evidence exists that targeting activated pathways in melanoma may enhance the immunologic response to the tumors [100]. This suggests that evaluating the effects of new therapies on the interaction of tumor cells with, and their effects on, the immune response may be important to study [101].

The discovery of *BRAF*, *NRAS*, *PTEN*, and *c-KIT* alterations in melanoma has supported the development of a variety of rational therapeutic approaches. While tremendous effort is being focused on the optimization of targeted therapies against these proteins and related pathways, approximately 30% of melanoma patients have no detectable abnormality in these genes. In order to improve outcomes in these patients, it will be critical to determine if their tumors are activating similar pathways by as yet unidentified genetic alterations or if they are characterized by dependence on completely separate and heretofore underappreciated signaling cascades in this disease.

Recently, the first whole-genome sequence of a melanoma was published [102]. This study identified over 33,000 changes in the melanoma genome as compared to the germline, including almost 200 non-synonymous coding region substitutions. A second high-throughput study to identify mutations in protein tyrosine kinase family members identified somatic mutations in 19 members of this family alone [103]. These initial findings suggest that identifying critical mutational events will require the sequencing of many melanomas to identify recurrent events that are most likely to be functional, which will then need to be investigated further. However, as the identification of *c-KIT* mutations has demonstrated, such analyses will need to incorporate the recognition of the possible molecular diversity of melanomas arising from different anatomic sites. As the recent experiences with targeted therapies have demonstrated, such investment in the understanding of the molecular biology of this disease may rapidly translate into improved outcomes in patients with this highly aggressive disease.

Acknowledgments

This work was supported in part by The University of Texas MD Anderson Cancer Center Melanoma SPORE (P50 CA93459); the American Society of Clinical Oncology Career Development Award (MAD); .MD Anderson Physician-Scientist Award (MAD); Melanoma Research Alliance Young Investigator Award (MAD); Grossman Family Foundation (JEG).

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Figure 1. Kinase Signaling Pathways and Targeted Therapies for Melanoma

The diagram illustrates key proteins in the RAS-RAF-MEK-MAPK and the PI3K-AKT kinase cascades. Arrows represent activation, while bars represent inhibition. Genes that are affected by activating mutations in melanoma (BRAF, NRAS, PI3K, C-KIT, and AKT) are shaded; the degree of shading reflects the relative prevalence of these mutations in cutaneous melanomas. Genes that are affected by genetic inactivation (PTEN) are shown with white type against a black background. The feedback regulation of PI3K and AKT by mTORC1 and mTORC2, respectively, is shown by the dashed lines. Classes and examples of targeted therapies against various effectors in the pathways are shown as free text beside the pathways.

Table 1

Patterns of gene mutations and amplifications in melanoma.

Melanoma Subtype	***BRAF* Mutation**	***NRAS* Mutation**	***c-KIT* Mutation**	***c-KIT* Amplification**	***GNαQ* Mutation**
Sun-exposed Cutaneous (CM)	43%	26%	Non-CSD: < 2% CSD: 2–17%	Non-CSD: 0–7% CSD: 6%	< 1%
Acral (ALM)	18%	4%	18%	24%	< 1%
Mucosal (MuM)	6%	14%	24%	26%	< 1%
Uveal (UvM)	< 1%	< 1%	< 1%	< 1%	45%

The table shows the prevalence of the listed genetic events in melanoma clinical specimens. CSD, chronic-sun damaged