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Driver Mutations in Melanoma: Lessons Learned From Bench-to-Bedside Studies

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Abstract

The identification of somatic driver mutations in human samples has allowed for the development of a molecular classification for melanoma. Recent breakthroughs in the treatment of metastatic melanoma have arisen as a result of these significant new insights into the molecular biology of the disease, particularly the development of inhibitors of activating BRAF^{V600E} mutations. In this article the roles of several mutations known to be involved in the malignant transformation of melanocytes are reviewed including BRAF, PTEN, NRAS, ckit, and p16 as well as some of the emerging mutations in cutaneous and uveal melanoma. The bench to bedside collaborations that resulted in these discoveries are summarized, and potential therapeutic strategies to target driver mutations in specific patient subsets are discussed.

Keywords

Melanoma; Somatic mutations; Drug targeting

Introduction

The incidence of melanoma continues to rise; an estimated 76,250 new diagnoses will be made in 2012, significantly more than only 5 years ago, when the annual incidence in the United States was 59,940 [1]. This rise in incidence is due both to improved awareness leading to additional diagnoses and due to life style changes that have resulted in an increase in sun exposure over the past decades. While many melanomas arise in pre-existing cutaneous nevi, others do not, and the somatic genetic characteristics of melanomas arising in sun-exposed versus sun-protected areas are starting to emerge. Similarly, specific mutations have been identified in melanomas of mucosal or acral-lentiginous origin, which

differ from ocular or sun-induced cutaneous melanomas, and mutations in chronic sun damaged skin differ from those found in skin exposed to intermittent ultraviolet irradiation.

Melanoma has traditionally been difficult to treat, given its near universal resistance to standard chemotherapy [2]. Early stage disease can often be cured by surgery, but unresectable disease has to be treated with systemic therapy. Although a larger percentage of patients are being diagnosed with early stage disease than in previous years because of heightened patient awareness and increased surveillance, the death rate from unresectable melanoma continues to rise [1]. While a small subset responds to immune therapy, the majority still does not, and the quest for improved targeted therapies has gone hand in hand with efforts to identify new mutations and other molecular aberrations in the different clinical subtypes that can be used for drug development. Identification of novel mutations has led to progress in systemic therapy for patients with unresectable disease, and the survival for patients with advanced melanoma has finally started to improve.

Identification of “driver” somatic mutations (mutations that are necessary to promote the malignant process) in this disease have, for the most part, resulted from studies employing human samples and newer high throughput screening platforms. Careful validation of these observations using specimens from multiple institutions and mechanistic studies of identified driver mutations have been the basis of the recent progress, along with development of novel drugs to target these mutations.

The first frequently occurring somatic mutation to be identified in cutaneous melanomas was the activating mutation in BRAF [3], an observation that was followed by mechanistic studies demonstrating that mutated BRAF is critical for the malignant process. Subsequently, drug development efforts focused on targeting BRAF in this and other diseases featuring an activating BRAF mutation, and specific inhibitors of mutant BRAF have already been shown to improve survival in advanced melanoma patients whose disease harbors BRAF mutations [4•]. These advances occurred over a period of approximately 10 years, and concurrent studies identified other driver mutations present in subsets of melanomas. While much additional research is needed, the bulk of the evidence suggests that more than one critical mutation is necessary to transform a benign melanocyte into an invasive, malignant tumor. In this article, we review some of the recently discovered unique driver mutations, discuss the paradigms of population specific target identification, and present the supporting preclinical and, when available, clinical evidence to drive pharmacological targeting of these mutations.

Activating Mutations in BRAF

In 2002, Davies et al, conducted a genome-wide screen in a number of tumor types using array- based comparative genomic hybridization focusing on the mitogen-activated protein kinase (MAPK) pathway, one of the major intracellular signal transduction pathways responsible for cellular proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis, and reported that 66 % of melanoma samples harbored a single substitution (V599E) mutation in the activating segment of the kinase domain [3]. They found that melanoma cell lines with BRAF^{V599E} mutations (subsequently found to be at BRAF^{V600E})

can signal through the MAPK pathway without upstream activation by RAS, indicating that these cells lose their dependence on upstream signaling. Moreover, these early studies showed that when BRAF^{V600E} is ectopically expressed in immortalized cell lines, it causes hyperstimulation of the MAPK cascade and malignant cellular transformation. Subsequent validation studies on melanoma cell lines were conducted, which similarly demonstrated frequent activating mutations in codon 600 [5]. This led to speculation that BRAF might be an important drug target in melanoma [6].

Subsequent studies in particular melanoma subtypes further elucidated the molecular biology of histologically similar, yet biologically and therapeutically different subsets at the same time as BRAF-directed therapy was developed. BRAF mutations were not found in uveal melanoma, as demonstrated by Rimoldi et al. and Cruz et al. [7, 8]. Maldonado et al., in a study of primary invasive cutaneous melanomas, reported that BRAF mutations were more common in melanomas occurring on skin that had been exposed to intermittent sun exposure, whereas skin on the palms, soles, and under nailbeds (acral lentiginous) as well as melanomas arising in mucosal surfaces only rarely harbor BRAF mutations (15 % and 10 %, respectively) [9]. A specific study of oral mucosal melanoma demonstrated that only one out of 15 (6 %) cases had an activating BRAF mutation, and similar validation studies on acral lentiginous cutaneous melanomas demonstrated that BRAF mutations were a rare event [10, 11].

Early stage primary melanomas and metastatic lesions appear to have BRAF mutations at a similar frequency. Moreover, benign nevi harbor BRAF mutations in a high percentage, indicating that this is an early event in development of pigmented neoplasias, and insufficient in itself to result in malignant transformation [12]. However, in metastatic melanoma patients, BRAF mutations were associated with worse prognosis if not treated with BRAF inhibitors, providing clinical evidence that although BRAF mutations are an early event, BRAF remains activated, even once tumors have metastasized [13, 14].

Since the identification a decade ago of mutations in BRAF in about half of cutaneous melanomas, intense efforts have been made to develop pharmacologic agents that inhibit BRAF for treatment of advanced melanoma. The first such inhibitor, sorafenib, had little activity as a single drug; however, a phase I/II trial in which the drug was given in combination with carboplatin and paclitaxel to unselected patients with metastatic melanoma showed a high response rate and longer progression-free survival than typically seen in this patient population [15]. This led to two phase III randomized trials comparing carboplatin, paclitaxel, and sorafenib to carboplatin, paclitaxel, and placebo, both of which failed to show a benefit with the sorafenib-containing therapy [16, 17]. Additional, more specific pan-RAF inhibitors, such as RAF265, have been tried, and responses have been seen in both BRAF mutant and WT tumors, as supported by preclinical data showing that sensitivity to this drug is not associated with BRAF mutation status [18]. The most promising clinical trial results for patients whose tumors harbor BRAF mutations have been obtained with drugs specifically designed to target the mutated form of BRAF. Two such inhibitors have been studied in advanced stage clinical trials; PLX4032 and GSK2118436, now known as vemurafenib and dabrafenib, respectively. These studies only include patients whose tumors harbor BRAF mutations. PLX4032 was shown to be superior to dacarbazine

in a randomized phase III trial, which was stopped at interim analysis when a survival benefit on the PLX4032 arm was demonstrated [4•]. Based on similarly promising activity in Phase II trials, Phase III trials of GSK2118436 in comparison with dacarbazine have been completed, as reviewed [19, 20].

While BRAF inhibitors are clearly active in the subset of patients whose tumors harbor BRAF^{V600E} mutations, a number of questions remain, which are the focus of both clinical and preclinical investigation. The activity of these drugs in BRAF mutant tumors that are not BRAF^{V600E} is unknown, although anecdotal reports of activity have been published [21]. An ongoing study is assessing activity of vemurafenib in this patient population. Predictive assays to determine activity of specific BRAF inhibitors are still being perfected. For example, the real-time polymerase chain reaction (RT-PCR)-based assay was developed to predict sensitivity to vemurafenib and used in the pivotal vemurafenib trials. The assay was compared with a massive parallel pyrosequencing method that was selected as the “gold standard” and was felt to be superior to Sanger sequencing [22]. The assay was approved by the Federal Drug Administration for predicting response to vemurafenib. However, the assay was less sensitive in detecting BRAF^{V600K} or BRAF^{V600D} mutations, and it cannot differentiate between heterozygous and homozygous BRAF mutant cells. While activity in humans can be prolonged, the median progression-free survival with vemurafenib in the randomized phase III trial was only in the order of 5.5 months [4•]. A number of preclinical studies have been conducted to identify and overcome mechanisms of resistance, which have primarily been attributed to secondary molecular alterations including new mutations in NRAS, nonmutational up-regulation of PDGFR, MEK, C-Raf, and COT activation [23–26]. These preclinical results have led to newer generation clinical trials, such as studies of combinations of BRAF and MEK inhibitors, with the hope of prolonging responses to these inhibitors in this patient population, as reviewed [19]. The bench-to-bedside and bedside-to-bench paradigms that have been employed in the past decade have thus successfully started to change the therapeutic approach to metastatic melanomas harboring BRAF mutations, and additional advances are anticipated in the near future.

NRAS

NRAS, or neuroblastoma RAS viral v-ras oncogene, is a member of the RAS gene family of signaling proteins, oncogenes which have GTP/GDP binding and GTPase activity and function in the maintenance of cell growth [27]. Mutations in NRAS, reported in 15 %–20 % of human melanomas at exon 2 and exon 3 (formerly exon 1 and exon 2), were among the first described in malignant melanoma [28]. The most common mutation occurs at codon 61, exon 3, resulting in the replacement of the Q61 glutamine residue usually by lysine or arginine [29]. This substitution irreversibly activates the RAS protein, resulting in an inability to cleave GTP, which would otherwise terminate downstream growth [30]. Of note, the presence of NRAS mutations in melanoma causes a switch in MAPK signaling from BRAF to CRAF, initiating dysregulated cAMP signaling that allows CRAF to signal to MEK [31]. Mutations in BRAF and NRAS are almost always mutually exclusive [32]. In contrast to BRAF, mutations in NRAS are also exclusive of alterations in PTEN [32], implying that mutations in NRAS alone may activate signaling through both the MAPK and PI3K pathways [33].

Like BRAF, NRAS mutations have also been identified in benign nevi, appearing most associated with congenital melanocytic nevi, particularly those of medium to large size [34]. Indeed, RAS-dependent transformation of melanocytes has been demonstrated in transgenic mice, which express the oncogenic form of human NRAS in melanocytes on an INK4a-deficient background, resulting in the development of metastatic melanomas that share salient features of the human disease [35]. But at least in nevogenesis, BRAF and NRAS mutations may play distinct roles. The exact role of both BRAF and NRAS mutations in melanocyte development has not been elucidated, but it has been proposed that the NRAS mutation event may prime the environment of the nevus to be receptive to abnormal growth signals. This is in contrast to BRAF, where mutations have been shown to be polyclonal in nature [36, 37], suggesting that they may be secondary events in nevogenesis and thus may promote growth of acquired nevi [38].

Multiple recent studies seeking to analyze clinical characteristics of NRAS mutant melanomas, with the intention of improving biologic insights to ultimately lead to more sophisticated drug development and clinical trials for this patient subset, have yielded conflicting results [32, 39, 40]. A meta-analysis of studies from 1989 to 2010 reported that NRAS mutations were associated with nodular histology and location on the extremities [41]. This meta-analysis also found that NRAS mutations were more frequently observed on chronically sun damaged skin, in contrast to previous published reports [42]. Two recent, large (>240 samples) studies of melanomas with NRAS mutations indicate that these tumors appear to exhibit more aggressive behavior, being associated with shorter overall survival. These tumors have also exhibited higher rates of mitosis [43] and are thicker at presentation [43, 44]. Finally, patients with BRAF and NRAS mutations were more likely to present with advanced AJCC stages, specifically stage III disease [44], and were more likely to have CNS disease at diagnosis of stage IV melanoma [45].

Historically, targeting mutant NRAS has proven to be challenging, in part due to the dynamics of the Ras cycle; this approach would require displacement of GTP from Ras, which has strong affinity for GTP binding, in a GTP rich environment, or reconstitution of its GTPase activity [30, 46]. Multiple alternative strategies have thus been proposed [30], including (1) targeting membrane localization of Ras, required for Ras activity, through inhibitors of farnesyl transferase or galectin 1; (2) targeting NRAS mRNA with interfering RNAs; and (3) targeting signaling downstream of NRAS through inhibitors of PI3K/Akt and Mek, an approach supported by preclinical models [47]. Meanwhile, additional targets continue to emerge. For instance, activated c-Met has been demonstrated in NRAS mutant tumor cell lines, which also showed sensitivity to pharmacologic c-Met inhibition, suggesting a novel therapeutic target [48]. As with BRAF inhibition, it is expected that a winning therapeutic strategy will ultimately target multiple signaling networks, which will require investigation into known active pathways in melanoma but also the identification of novel downstream mediators [46].

c-Kit Mutations

Two decades ago, Larue et al showed that mutations in the kinase domain of c-Kit can transform melanocytes to the malignant phenotype, suggesting that c-Kit might have a role

as a therapeutic target in this disease, although expression levels of the receptor tyrosine kinase have not been shown to increase with melanoma progression [49–52]. Although expression of c-kit was found to be increased in ocular melanoma, high expression has not been shown to be critical for malignant transformation of uveal melanocytes [53, 54].

Imatinib, a small molecule inhibitor of PDGFR, c-Kit, and Bcr-Abl was studied in both clinical and preclinical models. In the latter, the drug effectively inhibited PDGFR α and β , yet it was ineffective at inhibiting growth of melanoma tumors, regardless of whether they expressed c-Kit or not [55]. In uveal melanoma models, however, where c-Kit was upregulated but not mutated, imatinib demonstrated some preclinical activity [56, 57]. Imatinib was studied in phase II trials for activity in metastatic melanoma. In a study of 18 patients by Ugurel et al, no responses were seen, despite expression of both PDGFR and c-Kit in pretreatment specimens [58]. In a phase II trial conducted in the United States, no objective responses were seen among 26 patients [59]. However, a large tissue-based study employing comparative genomic hybridization data from 102 primary melanomas of mucosal, acral, and cutaneous origin showed DNA copy number aberrations of the c-Kit gene specific to melanoma subtypes where mutations in BRAF and NRAS are infrequent. When amplification on 4q12 was noted, targeted sequencing revealed oncogenic mutations in c-kit in 39 % of mucosal, 36 % of acral, and 28 % of melanomas arising in chronically sun damaged skin, while no mutations were found in cutaneous melanomas without chronic sun damage [60]. In a clinical trial that specifically selected patients based on expression of one of the imatinib targets (c-Kit, PDGFR, or c-abl), one patient with an acral lentiginous melanoma had a dramatic response [61]. This patient had the highest c-Kit protein expression, yet did not have an activating mutation in exon 9, 11, 13, 15, or 17. In a trial conducted in Asia for patients with C-kit mutation or amplification, a response rate of 23 % was observed, with most responses seen in patients with mutations in exons 11 or 13 [61, 62]. Studies with other inhibitors of c-Kit have since been conducted and show tumor shrinkage in subsets of patients with melanomas harboring c-kit mutations, as reviewed [63]. For example, clinical response to the multi-kinase inhibitor, dasatinib, which inhibits c-Kit as well as PDGFR and src kinases, was seen in two patients with a L576P mutations in exon 11 of c-Kit [64]. A study of unselected melanoma patients treated with dasatinib showed a dramatic response in one patient with an exon 13 c-Kit mutation and disease progression in a second patient with an exon 11 mutation, while protein expression analysis showed no association between c-Kit expression and clinical response or in vitro sensitivity to dasatinib [65, 66]. In summary, c-Kit is currently viewed as a good therapeutic target in a very small subset of metastatic melanomas, primarily patients with exon 11 and 13 mutations, based on both clinical and preclinical evidence. Expression of c-Kit protein does not appear to correlate with sensitivity to c-Kit inhibitors, whereas presence of activating c-Kit gene mutations does correlate. A multicenter trial lead by the Eastern Cooperative Oncology Group (ECOG) assessing activity of dasatinib in unresectable acral, mucosal, or vulvovaginal melanomas harboring mutations in exons 9, 11, 13, 17, or 18 of the c-Kit gene (ECOG 2607) is ongoing. Studies of human tissues obtained from patients enrolled in clinical trials, coupled with preclinical studies demonstrating the importance of certain mutations in melanoma tumorigenesis, were necessary for gaining this insight [64].

PTEN Silencing

Phosphatase and tensin homolog deleted from chromosome ten (PTEN) is a tumor suppressor gene identified after its positional cloning from a region of chromosome 10q24 [67] and its recognized deletion in multiple tumor types [67, 68]. Germline mutations in PTEN result in Cowden syndrome, an autosomal dominant disorder in which patients develop hamartomas and are at increased risk for certain cancers [69]. The importance of PTEN as a tumor suppressor in melanoma pathogenesis has been supported by functional studies showing that PTEN is targeted for LOH in melanoma, and that both ectopic expression and overexpression of PTEN suppress tumor growth [68, 70]. Loss of PTEN activity has been shown to reduce apoptosis and promote cell survival [71], as well as result in PI3K pathway activation [72], implicating this event as critical in melanoma tumorigenesis. In addition to mutational inactivation or deletion of the PTEN gene, loss of PTEN activity can occur through epigenetic silencing, potentially by hypermethylation of the PTEN promoter [73], a feature that has recently been reported as having an independent unfavorable impact on patient survival [74]. PTEN function can also be suppressed because of subcellular localization of the protein, as it suppresses the PI3K pathway when it is membrane-bound [71].

Loss of PTEN function through mutation or deletion has been seen in up to 60 % of melanoma cell lines but 10 % of uncultured tumor material [71, 75, 76]. With reports suggesting that epigenetic silencing may occur in up to 30 %–40 % of metastatic tumors [71, 73], loss of PTEN function could be an important event in 40 %–50 % of sporadic melanomas [71]. Whether alterations of PTEN activity occur early or late in the development of melanoma is under investigation, and it is unlikely that PTEN inactivation alone is sufficient for melanoma genesis [77]. While NRAS mutation alone is sufficient for downstream activation of the PI3K pathway [70], somatic mutations of PTEN often occur in association with BRAF [32, 78•], a finding possibly explained by preclinical murine models in which inactivation of PTEN in the presence of mutant BRAF leads to spontaneous development of metastatic melanoma [78•]. While PTEN itself is not a directly “druggable” target, it has been suggested that agents that can induce expression of PTEN in PTEN-deficient melanoma cells or restore PTEN regulation, or drugs that target the PI3K pathway downstream of PTEN, are likely to play a role in the treatment of melanoma and are worthy of further study [79, 80]. Clinical trials of novel PI3K pathway inhibitors, alone and in combination with MAPK pathway inhibitors, are underway, and additional studies are being planned. Co-targeting of these two pathways is an area of active research, and predictive biomarkers, including but not limited to BRAF mutations and PTEN deletions will likely facilitate future patient selection.

CDKN2A (p16)

Cyclin-dependent kinase 2a (CDKN2A, also called MTS1 or p16INK4A), located on chromosome 9p21, is mutated in a variety of cancers, including a small percentage of familial melanoma [81]. Unlike the somatic mutations described in the rest of this review, p16 mutations are hereditary (germline) mutations. The gene encodes proteins p16 and p14 ARF, with germline mutations occurring in both proteins in cases of familial melanoma,

albeit much more commonly in p16 [82]. When mutated, the ability of p16 to interact with cyclin-dependent kinase 4 (CDK4) in regulatory arrest of the cell cycle is disrupted, allowing unchecked progression through the cell cycle [83]. Germline CDK4 mutations have also been described in a smaller percentage of familial melanoma kindreds. These familial syndromes are characterized by multiple members with melanoma, multiple primary melanomas within individual members, and diagnosis of additional tumors within the family, particularly pancreatic cancer [84]. Somatic mutations in both p16 and CDK4 have also been observed in sporadic melanomas [85, 86]. Therapeutic targeting of these mutations may be possible with the development of newer, selective CDK4 inhibitors [46].

Emerging Somatic Mutations

Additional, rarer genetic aberrations have been identified in melanoma specimens, and shown to be important for malignant transformation. Microphthalmia-associated transcription factor (MITF), a protein involved in development and survival of melanocytes, and MITF pathway members are activated or amplified in subsets of melanomas [87]. Garraway et al showed that MITF can transform primary melanocytes in conjunction with BRAF mutations [88]. Two recently published studies showed that germline mutations of MITF can confer a familial melanoma risk, further supporting a role for MITF in this disease [89, 90]. Rare mutations in AKT1 and AKT3 have been found in melanoma samples; the functional importance of these mutations has not yet been determined [91].

High throughput sequencing technologies are leading to identification of novel driver mutations in melanoma. Most of the studies to date have been conducted on small cohorts of melanomas; however, as the cost of sequencing declines, we are likely to identify new driver mutations in subsets of melanomas. Nikolaev et al. showed that 8 % of melanomas harbor mutations in MAP2K1 (MEK1) and MAP2K2 (MEK2), which lead to constitutive ERK activation [92]. This study was published simultaneously with a study by Stark et al. showing that inactivating mutations in MAP3K5 and MAP3K9 in a small percentage of melanomas are similarly important for maintaining the malignant phenotype [93]. A study of 14 melanoma samples and matched normal DNA subjected to exome sequencing showed that GRIN2A, the gene that encodes a part of the ionotropic glutamate receptor, was mutated in some. This finding was validated in an independent melanoma cohort, showing that approximately 25 % of melanomas harbor this mutation [94]. This effort also identified mutations in TRRAP, a co-activator of several transcription factors including Mdm-2, MYC, and E2F. Additional exome sequencing efforts are underway which are likely to identify new driver and “passenger” mutations in melanoma.

Mutations in Uveal Melanomas

Uveal melanomas are genetically different than cutaneous melanomas. By subjecting murine uveal melanoma models to genetic screening, Van Raamsdonk et al identified two q-class G-protein alpha subunits that are mutated in intradermal (but not epidermal) melanocytes, GNAQ and GNA11 [95]. These studies were complemented with analysis of a cohort of 48 paraffin embedded uveal melanoma samples sequenced for mutations in GNAQ and GNA11, which showed that the former was mutated in 46 % of uveal melanomas. No

GNA11 mutations were found in this set of tumors, yet additional studies conducted by Van Raamsdonk et al. on a larger set of 186 specimens did show mutually exclusive mutations in GNA11 or GNAQ [96]. The majority of the mutations were in codon Q209 of exon 5; 45 % of primary and 22 % of metastatic uveal melanomas harbored a Q209 mutation in GNAQ, whereas 32 % of primary and 57 % of metastatic specimens harbored Q209 mutations in GNA11. A less common mutation was seen in R183 (exon 4). Forced expression of mutated GNA11 resulted in metastases in uveal melanoma mouse models. GNAQ and GNA11 mutations might have therapeutic implications, as they result in constitutive Ras activation and result in downstream signals that may be therapeutic targets [97]. Stable transfection of GNAQ^{Q209L} into normal melanocytes resulted in increased anchorage-independent growth with efficiency similar to that of transfection with NRAS^{Q61R}, and tumorigenicity in mice was similarly demonstrated with GNAQ^{Q209L} transfection, resulting in activation of the MAPK pathway. Preclinical studies have shown that uveal melanoma cell lines harboring GNAQ or GNA11 mutations are more resistant to MAPK pathway inhibitors than WT cell lines [98]. Further clinical studies are needed to determine whether mutations in GNAQ or GNA11 predict sensitivity to MAPK pathway inhibitors.

High throughput sequencing of uveal melanomas led to identification of mutations in BAP1 (BRCA1 associated protein 1) in uveal melanomas with high metastatic potential [99•]. Discovery studies were done on two tumors and validated on 29 poor prognosis tumors and 26 good prognosis tumors. Mutations leading to loss of protein expression were found in 84 % of the poor prognosis tumors and only 4 % of the good prognosis tumors. One patient was found to have a germline mutation in BAP1, indicating that in rare cases, this might be a tumor susceptibility gene. Knock-down of BAP1 gene expression in uveal melanoma lines using small interfering RNA sequences that inhibit gene transcription resulted in the acquisition of a more aggressive phenotype, suggesting that pharmacologic targeting of the products of genes whose expression is up-regulated by BAP1 might be a useful strategy for treating aggressive uveal melanoma [99•].

Summary and Conclusions

In recent years, a challenge was issued to the melanoma community to identify the year 2010 as a target date for improving survival of patients with metastatic disease [100]. Indeed, 2011 saw the FDA approval of vemurafenib [4•], a highly specific targeted agent that does not result in the cure of advanced melanoma, but is nonetheless destined to change the natural history of the disease. This development would not have been possible without the multidisciplinary efforts that led to new insights into melanoma biology, resulting in the rational development of targeted therapies designed to benefit a specific subset of patients. As the field moves forward toward unraveling mechanisms of vemurafenib resistance for patients with BRAF mutant melanoma, additional advances are anticipated in the development of therapies for molecularly selected patients within other melanoma subsets. The discovery of additional somatic driver mutations in human melanomas, as well as strategies for inhibiting their activity and those of the emerging mutations discussed in this review, will require continued collaboration between basic and clinical scientists. Following the examples of rigorous preclinical and clinical studies that incorporated correlative studies of human melanomas, future efforts will be enhanced by increasing technological advances,

inclusive of new high throughput screening techniques that include robust statistical analysis and rigorous clinical application. In summary, we are at the dawn of a new era where targeting the complex heterogeneity of signaling networks of melanoma is possible, with the ultimate goal of improving therapeutic outcomes for specific patient subsets.

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