Oncogene-directed small molecule inhibitors for the treatment of cutaneous melanoma

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Practice points

Background

- The American Cancer Society has projected that in 2014, 76,100 new melanomas will be diagnosed and 9710 melanoma patients will die from the disease.
- Since 2011, three small molecule inhibitors have been approved by the US FDA for use in unresectable or metastatic melanoma patients: vemurafenib, dabrafenib and trametinib; many more are in the clinical testing pipeline.

Targeting the MAPK pathway

- The latest The Cancer Genome Atlas data set reveals *BRAF* mutations in 50.8% of cutaneous melanomas, with 75.2% of these harboring V600E-type mutations.
- BRAF/MEK dual-inhibitor regimens result in increased efficacy and decreased toxicity compared with their respective monotherapies; as further combination therapies are explored, they will likely be built into triple or quadruple combination therapies on top of the BRAF/MEK dual-inhibitor backbone.

Targeting the PI3K pathway

- PI3K/PTEN/AKT/mTOR signaling is one of the central pathways in melanomagenesis; additionally, BRAF/MEK and PI3K/AKT signaling likely cooperate in melanoma progression.
- 69.5% of cutaneous melanomas in the latest The Cancer Genome Atlas data set have alterations in one or more of the major players in this pathway.
- Several small molecule inhibitors of PI3K, AKT or mTOR are now accessible through clinical trials; most of these trials are testing such inhibitors in combination therapy with BRAF and MEK inhibitors given the reciprocal aberrant signaling networks that have been observed in preclinical studies of MAPK and PI3K pathway inhibition.

KIT inhibition

- Oncogenic *KIT* mutations and amplifications are uncommon in melanoma, yet are enriched in, and help define a particular genetic subset of melanomas originating on acral lentiginous surfaces, mucosal surfaces and chronically sun-damaged skin.
- It was not until patient selection based on *KIT* mutation/amplification status became possible that KIT inhibition demonstrated promising therapeutic potential.

p53 restoration

- Because melanoma is an exception to the customarily high rates of mutated *TP53* in cancer, rescuing wild-type p53 function is a point of vulnerability in the majority of cases.

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Practice points (cont.)

Cyclin-dependent kinase inhibition

- As one of most commonly altered circuits involved in melanomagenesis, interest in pharmacologically targeting the p16^{INK4A}:cyclin D-CDK4/6:RB pathway is intensifying, and inhibition of CDK4/6 has become the most studied option.

Additional oncogene-directed small molecule inhibitors

- Discovering effective approaches to targeting oncogenic transcription factors and chromatin modifiers that act as nodes of coalescence downstream of intracellular signaling pathways is a particularly exciting area of investigation.
- An enormous effort is also dedicated to effectively merging targeted therapies with immunotherapies to maximize benefit and minimize toxicity.

Conclusion & future perspective

- The future of combination therapy is bright; it will likely not be long before we see Phase III trials for therapeutic regimens that simultaneously target two, three or even four oncogenic pathways, perhaps with a strategic element of redundancy

SUMMARY Achievements in cancer genetics and molecular biology have revolutionized the treatment options available for advanced melanoma. Patients with certain molecularly defined melanomas have been the most fortunate beneficiaries of recently US FDA-approved therapies that target aberrant MAPK pathway signaling, yet response rates and duration of response remain suboptimal. Furthermore, many patients harbor melanomas for which no approved targeted therapies currently exist. Since the approval of vemurafenib, a selective BRAF V600E inhibitor, in 2011, there has been a surge of preclinical and clinical studies aimed at developing novel targeted therapies for a wide range of molecularly defined melanomas. In this review, we will examine the present status and future potential of molecularly targeted therapies directed at the most significant oncogenic signaling pathways in melanoma.

KEYWORDS

- KIT • MAPK pathway
- melanoma • oncogene-directed therapy • PI3K pathway • small molecule inhibitor • targeted therapy
- TCGA

Melanoma is the most aggressive form of skin cancer, and over the past few decades there has been an alarming rise in worldwide incidence at a rate that has outpaced all other malignancies [1]. The devastating impact of this disease can be appreciated by the fact that melanomas represent roughly 4% of skin cancers, yet account for 80% of the deaths attributable to skin cancer [2]. Based on the latest statistics from numerous cancer registries in the USA, the American Cancer Society has projected that in 2014, 76,100 new melanomas will be diagnosed, and 9710 melanoma patients will die from the disease [3]. When diagnosed early, melanoma can be effectively treated with wide local excision, but the survival statistics for patients with advanced disease have historically been dismal, with lackluster treatment options, until recently. In the last 4 years, there has been a very encouraging shift in the landscape of treatments available for advanced melanoma.

During the last decade, advances in cancer genomics have created a molecular revolution in the search for novel oncogene-directed targeted therapies. Since 2011, the US FDA has

approved three such small molecule inhibitors for use in advanced (unresectable or metastatic) melanoma patients, and as of early 2014, two of those agents are now approved for used in combination. In addition to major innovations in melanoma genomics, recent breakthroughs in the understanding of melanoma immunobiology have provided enormous benefit through new immunotherapies, the other major category of available treatment options. Like targeted therapies, three immunotherapies have been FDA-approved in the last few years; most recently, in September 2014, pembrolizumab, an anti-programmed cell death 1 receptor monoclonal antibody, was approved. In this review, we will narrow our focus to molecular therapies by highlighting major developments of the past few years, current areas of intense interest and potential avenues of future investigation and innovation.

Targeting the MAPK pathway

• BRAF inhibition

CRAF was the first identified RAF kinase, and was subsequently found to have two closely

Figure 1. Critical oncogene-targeted signaling pathways in cutaneous melanoma. Activation of KIT, a receptor tyrosine kinase, leads to dual activation of the MAPK (NRAS, BRAF, MEK, ERK) and PI3K signaling pathways. The MAPK signaling pathway results in cell proliferation by activating cyclin-CDK complexes that promote cell cycle progression and gene transcription, as well as by influencing other transcriptional regulators. Signal output through the PI3K pathway leads to antagonism of apoptosis and increased cell growth and proliferation. The varying sizes of each protein in this figure are relative to the percentage of 262 cutaneous melanomas (the latest from The Cancer Genome Atlas dataset) that harbor alterations (mutations, amplifications, homozygous deletions and mRNA up/downregulation) in each protein's respective encoding gene.

Figure 1. Critical oncogene-targeted signaling pathways in cutaneous melanoma (cont.). For example, BRAF is the largest because the *BRAF* gene has the highest burden of alterations among the 262 melanoma samples. Small-molecule, targeted therapies are listed next to target proteins. The following protein–gene associations were used when extracting data from the cancer genome atlas (those not listed share the same name): MEK–*MAP2K1/2*, ERK–*MAPK3/1*, Cyclin D1–*CCND1*, PI3K–*PI3KCA*, AKT–*AKT3*, p53–*TP53*, BIM–*BCL2L11*. Additionally, p14^{ARF} and p16^{INK4A} are alternative splice products of the same gene, *CDKN2A*.
†Drugs approved by the US FDA for treatment of advanced melanoma.

related isoforms, ARAF and BRAF [4–6]. A decade after the discovery of CRAF, the family of RAF serine-threonine kinases was implicated as an integral component of the MAPK signaling pathway; RAF proteins were recognized as direct substrates of activated RAS proteins, and contemporaneously identified as upstream activators of MAP kinase (MEK) via phosphorylation [7,8]. Once activated by RAF, MEK phosphorylates MAP kinase (ERK), which ultimately phosphorylates many substrates involved in regulating cellular proliferation, survival, division and migration (Figure 1) [7,9].
The landmark discovery by Davies *et al.*, in 2002, that a substantial subset of melanomas is driven by an oncogenic, gain-of-function mutation in *BRAF* was a major impetus for research in oncogene-directed targeted melanoma therapies [10]. Of the dozens of distinct mutations

in *BRAF* that have been identified, 70–90% of *BRAF*-mutated melanomas have the same T1799A missense mutation [10–12]. This mutation results in valine being substituted by glutamic acid at amino acid 600 (V600E), which lies in BRAF’s activation loop [7]. The latest whole exome sequencing data of 262 cutaneous melanomas from The Cancer Genome Atlas (TCGA) reveals *BRAF* mutations in 133 cases (50.8%), with 75.2% harboring V600E-type mutations (Figure 2) [13]. The next most common *BRAF* mutations result in V600K and V600R substitutions [13–16]. The prevalence of *BRAF* mutations in melanoma does not hold true for *ARAF* and *CRAF* mutations, as these are uncommon in melanoma [9]. While the association between BRAF V600E and melanoma is quite clear, the finding that about 80% of benign melanocytic nevi also possess this mutation implies that it

Figure 2. Alterations in genes associated with important melanoma signaling pathways. The darkened area within each donut graph of this chart represents the proportion of cutaneous melanomas harboring a specific type of genetic alteration (left) across different genes (top). Generated with the latest from The Cancer Genome Atlas data set of 262 cutaneous melanomas. ↑: Upregulation; ↓: Downregulation.

Table 1. Select Phase II and Phase III trials investigating MAPK pathway-directed small molecule inhibitors as monotherapy for melanoma.

Test drug	Phase	Control drug	n	RR (95% CI); %	mPFS (95% CI); months	mOS (95% CI); months	OSR (95% CI); %	Clinicaltrials.gov identifier (NCT number)
Target: BRAF								
Vemurafenib	II	–	132	53 (44–62)	6.8 (5.6–8.1)	15.9 (11.6–18.3)	77 (70–85)	NCT00949702; Sosman <i>et al.</i> [31]
	III	Dacarbazine	675	48 (42–55) vs 5 (3–9) p < 0.001	5.3 vs 1.6 HR: 0.26 (0.2–0.3) p < 0.001	–	84 (78–89) vs 64 (56–73) HR: 0.37 (0.26–0.55) p < 0.001	NCT01006980; Chapman <i>et al.</i> [29]
Dabrafenib	II	–	92	BRAF V600E: 59 (48.2–70.3) BRAF V600K: 13 (0–28.7)	BRAF V600E: 6.3 BRAF V600K: 4.5	BRAF V600E: 13.1 BRAF V600K: 12.9	–	NCT01153763; Ascierto <i>et al.</i> [33]
	III	Dacarbazine	250	50 (42.4–57.1) vs 6 (1.8–15.5)	5.1 vs 2.7 HR: 0.3 (0.18–0.51) p < 0.0001	HR: 0.61 (0.25–1.48)	–	NCT01227889; Hauschild <i>et al.</i> [35]
LGX818	II	–	R	–	–	–	–	NCT01894672
	III	Vemurafenib	R	–	–	–	–	NCT01909453
Target: Multi-RAF/ VEGFR								
RAF265	II	–	104	–	–	–	–	NCT00304525
Target: MEK								
Trametinib	II	–	97	Prior BRAFi: 0 BRAFi naive: 25 (14.1–37.8)	Prior BRAFi: 1.8 (1.8–2.0) BRAFi naive: 4 (3.6–5.6)	Prior BRAFi: 5.8 (4.1–9.0) BRAFi naive: 14.2	–	NCT01037127; Kim <i>et al.</i> [50]
	III	Dacarbazine	322	22 (17–28) vs 8 (4–15) p = 0.01	4.8 vs 1.5 HR: 0.45 (0.33–0.63) p < 0.001	–	81 vs 67 HR: 0.54 (0.32–0.92) p = 0.01	NCT01245062; Flaherty <i>et al.</i> [51]
MEK162	II	–	71	BRAF-mut: 20 (9–35) NRAS-mut: 20 (8–39)	BRAF-mut: 3.6 (2.0–3.8) NRAS-mut: 3.7 (2.5–5.4)	–	–	NCT01320085; Ascierto <i>et al.</i> [53]
Pimasertib	II	Dacarbazine	194	–	–	–	–	NCT01693068

BRAFi: BRAF inhibitor; mOS: Median overall survival; mPFS: Median progression-free survival; n: Study size; OSR: Overall survival rate; R: Recruiting; RR: Response rate.

does not act alone; *BRAF* mutations are drivers of melanoma through the initiation of melanomagenesis, rather than players in its progression [17,18]. Constitutively active, mutant BRAF no longer requires activating phosphorylation by RAS, and leads to unregulated MAPK signaling, which stimulates growth factor independent proliferation. Additionally, constitutive MAPK signaling has been implicated in melanoma adhesion, migration and angiogenesis, as well as a melanoma specific prosurvival response

by respectively inactivating and downregulating the proapoptotic proteins BAD and BIM (Figure 1) [9,19–23].

The multikinase inhibitor sorafenib was the first nonselective RAF inhibitor studied in Phase II and Phase III trials for advanced melanoma. Results from these trials were unfavorable, as sorafenib failed to benefit patients either alone or in combination with traditional cytotoxic, anti-cancer drugs [24–26]. The second-generation RAF inhibitors, vemurafenib and dabrafenib, were

designed to specifically target V600E-mutated BRAF proteins. The first of these, vemurafenib, was shown in preclinical studies to have a low-nanomolar IC_{50} , and promote cell cycle arrest and apoptosis in melanoma cells [27]. Vemurafenib's clinical success was first demonstrated in a Phase I trial extension cohort resulting in complete or partial response in over 80% of participants (26 of 32), and further validated in a Phase III trial (BRAF Inhibitor in Melanoma 3 [BRIM-3]) trial of 675 patients with advanced, unresectable, *BRAF* V600E-mutated melanoma (Table 1) [28]. The early interim results of the BRIM-3 trial, where vemurafenib reduced the risk of death or disease progression by 74% compared with dacarbazine, were enough to convince the FDA of vemurafenib's utility [29,30]. After FDA approval for late stage and unresectable melanoma in August of 2011, additional support came from BRIM-2, a Phase II trial; 47% of patients treated with vemurafenib had a partial response, while 6% had a complete response, and the median progression-free survival and median overall survival were 6.8 and 15.9 months, respectively (Table 1) [31].

Dabrafenib is the second BRAF inhibitor currently FDA-approved (May 2013) for *BRAF* V600E-mutant unresectable or metastatic melanoma. In a Phase I trial, a response rate of 69% was observed in patients with *BRAF* V600E- or V600K-mutated melanoma [32]. A consequent Phase II trial, BREAK-2, also enrolled patients with either the V600E (76 patients) or V600K mutations (16 patients). The overall response rate (59% vs 13%) and progression-free survival (6.3 vs 4.5 months) were both better in the V600E cohort (Table 1) [33]. The BREAK-3 trial, a Phase III trial comparing dabrafenib to dacarbazine corroborated the earlier findings, and produced results similar to those observed in the BRIM-3 trial (Table 1). Compared to patients treated with dacarbazine, patients treated with dabrafenib had a 70% reduction in risk of disease progression. Patients receiving dabrafenib also fared much better with regard to progression-free survival, with a median of 5.1 months compared with 2.7 months for dacarbazine treatment [34,35].

There are several other BRAF inhibitors at different stages of clinical testing, the most notable of which is LGX818. This agent appears to be even more potent and selective than both vemurafenib and dabrafenib, and has the unique feature of having a very slow

dissociation for BRAF once bound [36]. This may account for the broad range of doses at which responses have been observed and the lower rate of certain toxicities characteristic of vemurafenib and dabrafenib, even when given at the highest tolerable doses. LGX818 monotherapy is currently being compared with vemurafenib for treatment of advanced melanoma in a Phase III trial (COLUMBUS), and is additionally being studied in Phase I and Phase II trials in melanoma (Table 1) (NCT01909453, NCT01894672, NCT01436656). Several clinical trials are also investigating LGX818 in dual- and triple-therapy combination with a variety of novel small molecule inhibitors targeting other oncogenic programs, such as the PI3K pathway. The multi-RAF inhibitors XL281 (BMS908662) and ARQ736, and the multi-RAF/VEGFR inhibitor RAF265 (CHIR-265), have completed monotherapeutic Phase I and/or Phase II trials, but no Phase III trials have begun (NCT00451880, NCT01225536, NCT01352273, NCT00304525) [37,38].

The widely celebrated success of these selective BRAF inhibitors, while warranted for being a milestone in the field of targeted therapeutics for melanoma, only impacts a fraction of advanced melanoma patients, and for these patients the impact is often transient. A patient with melanoma harboring a *BRAF* V600E mutation has about a 50% chance of responding to selective BRAF inhibition, and as was demonstrated in the BRIM-3 and BREAK-3 Phase III trials, the median progression-free survival time is 5.3 and 5.1 months for vemurafenib and dabrafenib, respectively [14,29,35]. If patients' disease is not primarily, or intrinsically, resistant to BRAF inhibition, then acquired resistance will, in almost all cases, limit the durability of their response. Nevertheless, having a *BRAF* V600E mutation has gone from being a poor prognostic factor before the existence of BRAF inhibitors, to affording patients longer median survival times, when treated appropriately, compared with *BRAF*-wild-type melanoma patients [7,15].

The landscape of resistance to BRAF inhibitors is sprawling, with an enormous effort expended on exploring the intricate mechanisms and pathways melanomas exploit to overcome BRAF inhibition, or avoid it altogether. Acquired resistance results in a dampened drug response through dynamic changes that occur during treatment. Reactivation of the MAPK signaling cascade is the most common hallmark

of acquired resistance to BRAF inhibition, occurring in about 70% of cases through various mechanisms, including: activating mutations in upstream and downstream kinases (NRAS and MEK, respectively), *BRAF* amplification and alternative *BRAF* splice-products, among others [14,39–44]. Resistance to BRAF inhibitors is a complex story that will not fit within the limitations of this review article, but has been comprehensively reviewed elsewhere (see Holderfield *et al.*, 2014 and Shi *et al.*, 2014) [7,41]. Beyond the ubiquitous challenge of resistance, other challenges to BRAF inhibition are the relatively common adverse effects of developing a secondary malignancy, predominantly keratoacanthoma-type squamous cell carcinoma (SCC), and triggering volatility in nevi [45,46]. Similar to resistance mechanisms that activate MAPK signaling, BRAF inhibitors causing paradoxical activation of wild-type BRAF kinase activity in squamous cells is one of the prevailing mechanisms thought to account for SCC development [30,45,47]. As noted above, there is diversity in the properties of the selective BRAF inhibitors and the most recently developed agent, LGX818 appears to have the lowest rate of proliferative skin lesions, perhaps relating to unique effects on the MAPK pathway.

• MEK inhibition

In an effort to overcome resistance following BRAF inhibitor treatment and explore other potential targets along the constitutively activated BRAF signaling cascade, interest turned to small molecules capable of inhibiting downstream substrates of BRAF, namely MEK (*MAP2K1/2*) and ERK (*MAPK3/1*). In May of 2013, alongside dabrafenib, the FDA approved trametinib as the first selective MEK inhibitor for treatment of BRAF V600E/K-mutant advanced melanoma. Like BRAF inhibitors, trametinib was demonstrated to reduce cell growth and induce apoptosis in preclinical studies [48]. Important insights were gained from early Phase I and Phase II trials of trametinib therapy in melanoma: patients harboring BRAF mutant melanoma had better response rates compared with those with BRAF-wild-type disease (33% vs 10%), and the drug only benefits BRAF inhibitor-naïve patients, who had a 25% response rate compared with 0% in patients who had developed resistance to prior BRAF inhibitor treatment (Table 1) [49,50]. In 2012, Flaherty *et al.* published their results from the METRIC study, a Phase III trial that

demonstrated extended progression-free survival with trametinib treatment (4.8 months), when compared with dacarbazine or paclitaxel (1.5 months) (Table 1) [51]. Interestingly, in contrast to targeted BRAF inhibition, no patients receiving trametinib developed SCC during clinical trials; however, other worrisome adverse events such as serous retinopathy and ventricular dysfunction did occur [51,52]. MEK inhibitors appear to induce toxicity by inhibiting MAPK pathway signaling in skin and other tissues, as opposed to the stimulatory effects observed with BRAF inhibitors.

There are many other MEK inhibitors being tested in melanoma (Figure 1: MEK162, cobimetinib, pimasertib and refametinib). MEK162 is perhaps the most promising, as it is currently involved in several combination therapy clinical trials and also has been demonstrated to benefit patients with NRAS-mutant melanoma, a large melanoma demographic with no FDA-approved targeted therapies [34]. Generally consistent with many other reports, the latest TCGA data reveal that 30.2% of cutaneous melanomas harbor *NRAS* mutations (Figure 2) [13,26,30]. In a Phase II trial, 20% of NRAS-mutant melanomas responded to MEK162 (Table 1), which was promising considering that past efforts at targeting NRAS have not been fruitful, most likely because it lies upstream of both the MAPK and PI3K pathways (Figure 1) [53]. While no targeted treatment options exist for patients with NRAS mutant melanoma, new strategies combining MEK162 with other small molecule inhibitors of AKT and CDK4/6 are currently under clinical investigation in this population (NCT01941927, NCT01781572).

• ERK inhibition

ERK is a direct substrate of MEK-mediated phosphorylation. ERK is the ultimate mediator of the MAPK signaling cascade, relaying the extracellular mitogenic stimuli by activating many cytoplasmic substrates and also translocating into the nucleus to activate numerous transcription factors necessary for growth and survival (Figure 1) [54]. Although MEK inhibitor monotherapy failed to produce a response in patients resistant to BRAF inhibition, multiple preclinical studies indicate that the ERK inhibitors SCH772984 and VX-11e are capable of overcoming acquired resistance to BRAF and MEK inhibition that develops via MAPK pathway-dependent mechanisms [30,55–58]. The

ERK inhibitors GDC-0994 and BVD-523 are the subjects of Phase I and Phase I/II studies, respectively, evaluating targeted ERK inhibition for cancer treatment (NCT01875705, NCT01781429).

• Combination BRAF/MEK inhibition

Preclinical studies demonstrating that co-targeting BRAF and MEK kinases augmented the induction of apoptosis in melanoma cells and delayed the onset of resistance were part of the impetus for challenging BRAF-inhibitor resistance through combination BRAF/MEK inhibitor therapy [14,59,60]. These preclinical results were recapitulated in Phase I/II and Phase III trials comparing combination trametinib and dabrafenib to dabrafenib alone; in both studies, combined inhibitor therapy improved overall response rates and progression-free survival [61,62]. Based on the results of the Phase I/II trial, the FDA granted accelerated approval for the combination therapy in January of 2014, which was contingent on the results of the Phase III trial that were recently published in November of 2014 [63]. The other important, perhaps counterintuitive, revelation that came from these trials was that the adverse effect profile of combination therapy was actually better than either drug alone. The combination of vemurafenib with cobimetinib (GDC-0973, XL-518) has also shown promising results in a Phase Ib trial, and is currently being investigated in comparison to vemurafenib monotherapy in the coBRIM study, a Phase III trial that stopped recruiting in late 2013 (NCT01689519) [64]. Similarly, LGX818 and MEK162 are a third BRAF/MEK dual-inhibition therapy with encouraging preliminary results out of a Phase I/II trial and an ongoing Phase III trial (NCT01543698, NCT01909453) [65]. Of note, the dose of LGX818 used in combination with MEK162 is higher than patients can tolerate when LGX818 is given alone. This combination uniquely takes advantage of counteracting effects of a MEK inhibitor to maximize the combination dose, and will possibly translate to greater anti-MAPK pathway effects and efficacy. Unlike the other combinations, however, neither LGX818 nor MEK162 have FDA approval as monotherapy.

Despite improving the clinical outcomes of advanced melanoma, vertically targeting the MAPK signaling cascade through combination

therapy does not entirely abrogate the development of acquired resistance, and patients eventually progress. We now know, through genetic analysis of several of the patients that progressed on dabrafenib/trametinib therapy that the resistance mechanisms at play closely resemble those involved in resistance to BRAF inhibitor monotherapy [14,44]. Interestingly, this finding could help explain why patients who have failed BRAF inhibitor monotherapy have much worse outcomes with MEK inhibitor or BRAF/MEK dual-inhibitor treatment compared with BRAF inhibitor-naïve patients [14,50,64]. Beyond the MAPK signaling pathway, many other oncogene-targeted therapies are currently in the pipeline of clinical testing for patients that have failed the current FDA-approved treatments, and for the less fortunate patients that have other driver mutations without any approved therapies. Because BRAF/MEK dual-inhibitor regimens result in increased efficacy and decreased toxicity compared with their respective monotherapies, it makes sense that as further combination therapies are explored, they will likely be built into triple or quadruple combination therapies on top of the BRAF/MEK dual-inhibitor backbone.

Targeting the PI3K pathway

The PI3K/AKT/mTOR pathway positively regulates numerous downstream intracellular proteins involved in development, growth and survival, while also negatively regulating intrinsic proapoptotic proteins (Figure 1) [9]. A surprising proportion of melanomas have been found to have aberrations leading to constitutive signaling through this pathway. Over activation of AKT3 was shown to promote tumor development in 43–60% of sporadic melanomas [66], PTEN dysfunction is estimated to be involved in 40–77% of sporadic melanomas [9] and 73% of melanomas harbor an activated mTOR pathway [34,67,68]. The latest TCGA data set of 262 melanomas with mRNA expression Z-scores, copy number analysis and whole exome sequencing data reveals that 182 of the cases, or 69.5%, have alterations (mutation, amplification, homozygous deletion, mRNA up or downregulation) in one or more of the major players in this pathway (Figure 2) [13].

In addition to the many studies that have implicated PI3K/PTEN/AKT/mTOR signaling as one of the central pathways in melanomagenesis, it

has additionally been known for some time now that BRAF/MAPK and PI3K/AKT signaling likely cooperate in melanoma progression. PTEN loss/inactivation or increased AKT activity is frequently accompanied by oncogenic *BRAF* mutations [23,69,70]. The theory that coincident MAPK and PI3K pathway activation are required for melanoma development, either through separate alterations in the signaling of both pathways or activating mutations of NRAS upstream of both pathways, was validated via a genetically engineered mouse model. Dankort *et al.* generated a model of conditional melanocyte-specific expression of V600E-mutated *BRAF* and were only able to elicit the development of melanoma when *BRAF* V600E was expressed in the presence of *PTEN* gene silencing [71,72]. Additionally, this pathway has been recognized as a route of therapeutic resistance to inhibitors of the MAPK pathway. It has been demonstrated *in vitro* that BRAF and MEK inhibition have the potential to upregulate PI3K pathway signaling, thus conveying a survival advantage by means of reducing apoptosis [14,73,74]. This was clinically corroborated when Shi *et al.* reported that PI3K/AKT-upregulating genetic alterations were detected in 22% of tumors from patients who progressed on BRAF inhibitor treatment (vemurafenib or dabrafenib), often times overlapping with recovered MAPK signaling [41].

This signaling pathway carries great therapeutic potential for patients with advanced stage melanoma given the prevalence of its oncogenic alterations in both treatment-naïve and treatment-resistant patients. Increased investigative efforts in this direction have led to several small molecule inhibitors of PI3K, AKT or mTOR being accessible through clinical trials (Figure 1: BKM120, PX-866, BEZ235, GSK2141795, MK2206, temsirolimus and everolimus). It remains unclear which is the optimal point of intervention in the pathway and whether targeting specific isoforms of PI3K or AKT might yield greater therapeutic index. Furthermore, most of the clinical trials involving these agents are testing them in combination therapy with BRAF and MEK inhibitors given the reciprocal aberrant signaling networks that have been observed in preclinical studies of MAPK and PI3K pathway inhibition [23].

KIT inhibition

c-KIT (KIT) is a member of the receptor tyrosine kinase family and has been demonstrated to

play a role in melanocyte differentiation, proliferation, and survival [75,76]. Oncogenic *KIT* mutations and amplifications are uncommon in melanoma, yet are enriched in, and help define a particular genetic subset of melanomas originating on acral lentiginous surfaces, mucosal surfaces and chronically sun-damaged skin [77–79]. The abovementioned TCGA dataset of 262 cutaneous melanomas revealed that six harbored *KIT* mutations (2.3%), seven harbored *KIT* amplifications (2.7%) and three contained both (1.1%) (Figure 2) [13]. This is in contrast to a study of 102 primary melanomas that reported *KIT* mutations and/or amplifications in 36% of ALM, 39% of mucosal melanoma and 28% of melanoma on chronically sun-damaged skin [79].

Oncogenic *KIT* mutations and amplifications enhance signaling through two major downstream cascades, the MAPK and PI3K pathways (Figure 1) [30,80]. It has been shown that oncogenic KIT-driven melanomagenesis employs MAPK as a redundant survival pathway to strengthen PI3K signaling, and that cell death requires combined inhibition of both pathways [81]. Interest in therapeutically targeting KIT led to imatinib being the first KIT inhibitor tested in melanoma, with initially disappointing results [76,80]. It was not until patient selection based on *KIT* mutation/amplification status became possible that KIT inhibition demonstrated promising potential as a therapeutic target.

The aggregate response rate across three Phase II trials of imatinib for advanced melanoma patients with either *KIT* mutations or amplifications was 25% (23 of 92 patients), with KIT inhibition being notably more effective in patients with activating *KIT* mutations versus amplifications [78,80,82]. When analyzed more closely, by adjusting the focus across these trials to only those patients with mutations of *KIT* exons 11 or 13, the response rate jumps to 39.6% (21 of 53 patients), a rate similar to what has been reported for BRAF V600E melanoma treated with selective BRAF inhibitors [75]. Effects on progression-free and overall survival compared with historically standard therapies have not been explored in randomized trials to date, though such evidence may emerge for nilotinib in the near future (NCT01028222).

Clinical trials investigating KIT inhibitors as a therapeutic option for advanced melanoma are not limited to imatinib. Nilotinib is

another KIT inhibitor that was derived from imatinib and binds KIT with greater affinity [83]. The preliminary results of a Phase II study of nilotinib in patients with melanoma harboring *KIT* aberrations reported that two of nine patients (22.2%) attained durable, partial responses; these responders were two of the three patients that had *KIT* mutations in exon 11 [84]. Sunitinib is a small molecule inhibitor that targets VEGF receptors in addition to KIT; because of this additional mechanism, it has been proposed that sunitinib may be a more effective therapy than imatinib [85,86]. A completed Phase II study of sunitinib therapy for patients with *KIT*-mutated or amplified melanoma reported responses in four of ten evaluable patients, with three of four patients with *KIT* mutations in exon 11 responding [86]. Dasatinib, a KIT inhibitor similar to sorafenib in terms of its broad multikinase activity, generated a poor clinical response rate in a Phase II study of unselected melanoma patients (5.6%, or two of 36 patients), with the exception being patients with *KIT* mutations (one of two patients responded) [87]. Corroborating this finding is a report of two patients with *KIT* mutations who were not a part of this trial but who also achieved response with dasatinib treatment [83,88]. Based on these findings, a Phase II trial of dasatinib in patients preselected for *KIT*-mutated melanoma has been initiated (NCT00700882).

p53 restoration

One interesting therapeutic strategy differs from the other pharmacologic efforts described above, which all aim to suppress oncogenic drivers of melanoma. A parallel, but opposite approach involves the use of small molecule inhibitors to indirectly target, and thus functionally restore tumor suppressor proteins, such as p53 (*TP53*). It has been estimated that the vast majority of cutaneous melanomas, between 80–95%, carry wild-type *TP53* [89,90]; however, functional dysregulation of p53, if not by mutation, then via upstream or downstream signaling interactions, is believed to be a necessary influence for tumorigenesis [90–92]. This idea was supported by the demonstration that loss of p53 activity was necessary for the development of melanoma in genetically engineered mice [90,93]. MDM2 and MDM4 are two proteins known to inhibit the regulatory functions of p53 (Figure 1). *MDM2* is reportedly amplified in a small fraction of

cutaneous melanoma cases, and additionally, the functional loss of p14ARF (*CDKN2A*) stimulates the inhibitory interaction between MDM2 and p53 (Figure 1) [89,94,95]. MDM4 has been reported to be strongly overexpressed in a subset of cutaneous melanoma, with expression levels correlating to metastatic disease [96]. Because melanoma is an exception to the customarily high rates of mutated *TP53* in cancer, wild-type p53 function is a point of vulnerability in the majority of cases.

In two preclinical studies, Ji *et al.* demonstrated that the MDM2 inhibitor nutlin-3 was able to suppress melanoma viability across a large panel of cell lines, and synergize with vemurafenib to decrease melanoma growth *in vitro* and *in vivo*; the growth suppressive effect of MDM2 antagonism was additionally linked to restored p53 activity [97,98]. Antagonizing MDM4 with SAH-p53–8 has also been shown to suppress melanoma cell viability and xenograft tumor growth by inducing growth arrest and apoptosis [99]. A dual MDM2/4 small molecule inhibitor, RO-5963, has also been reported to have antitumor activity [100]. In addition to these studies, there is accumulating support that small molecule inhibitors of MDM2, MDM4 and various other p53-inhibiting interactions may work in combination regimens to enhance current targeted melanoma therapies. An MDM2 inhibitor, DS-3032b, has recently been started in a Phase I trial for patients with advanced tumors, including melanoma (NCT01877382).

Cyclin-dependent kinase inhibition

Tightly regulated cell cycle progression is central to normal cell growth, and when disrupted, can result in aberrant proliferation and tumorigenesis [101]. One of the key regulating mechanisms that drives cells past the restriction point, and into a committed cell cycle is the interaction between cyclin D and the serine/threonine kinases CDK4 and CDK6 [102]. Once activated by coupling to cyclin D, CDK4/6 phosphorylate retinoblastoma (RB) protein and set off a series of transcriptional events necessary for G1 to S Phase cell cycle progression (Figure 1) [103,104]. This mechanism is maintained through several negative regulators, the most important being the tumor suppressor protein p16^{INK4A}, a splice product of *CDKN2A* (Figure 1) [104]. It is believed that the p16^{INK4A}:cyclin D-CDK4/6:RB pathway is altered in greater than 90% of

melanomas [103,105,106]. How deregulation of the cyclin D-CDK4/6 unit interacts with, or promotes, tumorigenic programs in melanocytes remains incompletely understood, but for a recent, comprehensive review of the literature see Miller and Flaherty [103].

As one of most commonly altered circuits involved in melanomagenesis, interest in pharmacologically targeting the p16^{INK4A} cyclin D-CDK4/6:RB pathway is intensifying, and inhibition of CDK4/6 has become the most studied option. The first selective CDK4/6 small molecule inhibitor designed was palbociclib (PD-0332991), which is currently being investigated in a Phase II trial as monotherapy for an array of cancers, including melanoma (NCT01037790). This drug was shown to inhibit tumor growth in melanoma xenografts primarily through induction of cellular senescence, suggesting that employing this drug, or others like it, as monotherapy may be ineffective in the clinical setting [103,107]. There have been preclinical reports that combining prosenescence CDK4/6 inhibition with proapoptotic MAPK pathway inhibition may be a potent therapeutic cocktail for melanoma [103,108,109]. Indeed, in addition to the clinical trials studying CDK inhibitors as monotherapy, others are studying these novel drugs in combination with potent BRAF and MEK inhibitors.

Additional oncogene-directed small molecule inhibitors

While we have given a brief overview of the major oncogenic signaling pathways currently targeted by approved and/or investigational small molecule inhibitors, there are many drugs that we did not have room to cover. Exciting preclinical studies investigating the ability HSP90 inhibitors, such as XL888 and ganetespib, to delay acquired BRAF inhibitor resistance have produced positive results [14]. Moreover, XL888 is part of a Phase I trial in combination with vemurafenib (NCT01657591). There is also a lot of interest in potential combination regimens with small molecule inhibitors that target regulators of the intrinsic apoptosis pathway, such as the BCL-2 family of proteins, known as BH3-mimetics. Additional targets that may gain momentum in the near future include EGFR/HER2, GSK3 β , the notch signaling pathway, as well as a host of transcription factors, most notably MTF, and chromatin modifiers that act as nodes of coalescence downstream of

intracellular signaling pathways. An enormous effort is also dedicated to effectively merging targeted therapies with immunotherapies to maximize benefit and minimize toxicity; Wargo, Cooper and Flaherty recently reviewed this field of study [110].

Conclusion & future perspective

The FDA's approval of vemurafenib in 2011 heralded in what has become a wave of novel targeted therapies for melanoma that are aimed at important, aberrant signal transduction programs involved in melanomagenesis. Since the approval of vemurafenib, the FDA has only added dabrafenib and trametinib to its list of small molecule inhibitors approved for the treatment of advanced melanoma, but as the rate of progress in melanoma therapeutics continues to increase, it is likely that we will see many more drugs, or drug combinations, approved in the near future. The numerous small molecule inhibitor regimens currently in the pipeline of clinical testing can be categorized as either monotherapy or combination therapy, with the latter being increasingly recognized as the more promising source of enhanced melanoma therapeutics. Acquired resistance remains the most challenging limitation of targeted therapies, but the complex and heterogenic network of mechanisms underlying resistance is becoming better understood as it is examined through RNA, epigenomic and proteomic 'lenses' in addition to the classic DNA 'lens'. New insights into resistance mechanisms go beyond activated oncogenic signaling programs, with recent studies implicating altered metabolism, specifically increased oxidative phosphorylation, as another important driver of resistance. Future advances in molecular imaging may one day make *in vivo* detection of alterations in specific signaling proteins possible, facilitating optimized treatment. Research using ¹⁸F-labeled PET probes, tagged nanoparticles and theranostic nanoparticles (those with the dual roles of diagnosis and treatment) for the early detection of primary and metastatic melanoma is paving the way for improved molecular imaging modalities capable of identifying molecular signatures in treatment-resistant melanoma. We should remain optimistic about future approaches to the challenge of resistance. It will likely not be long before we begin to see Phase III trials of therapeutic regimens that simultaneously target two, three or even four oncogenic pathways, perhaps with

a strategic element of redundancy; the notion being that highly effective combination regimens will enhance killing upfront, therefore crippling the emergence of anticipated resistance mechanisms..

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