MINIREVIEW

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Beyond BRAF: where next for melanoma therapy?

I V Fedorenko[1], G T Gibney[2], V K Sondak[2] and K S M Smalley*[,1,2]

[1]The Department of Molecular Oncology, The Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA and [2]The Department of Cutaneous Oncology, The Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA

In recent years, melanoma has become a poster-child for the development of oncogene-directed targeted therapies. This approach, which has been exemplified by the development of small-molecule BRAF inhibitors and the BRAF/MEK inhibitor combination for *BRAF*-mutant melanoma, has brought new hope to patients. Despite these successes, treatment failure seems near inevitable in the majority of cases—even in individuals treated with the BRAF/MEK inhibitor doublet. In the current review, we discuss the future of combination strategies for patients with *BRAF*-mutant melanoma as well as the emerging therapeutic options for patients with *NRAS*-mutant and *BRAF*/*NRAS*-wild-type melanoma. We also outline some of the newest developments in the in-depth personalisation of therapy that should allow melanoma treatment to continue shaping the field precision cancer medicine.

BEYOND BRAF: DEVELOPING COMBINATION STRATEGIES THAT ABROGATE RESISTANCE

The era of targeted therapy in melanoma began with the identification of driver mutations in the serine threonine kinase *BRAF* (Davies *et al*, 2002). It is now known that 40–60% of all cutaneous melanoma patients harbour position 600 mutations in *BRAF*, with the majority of these (~80%) being V600E mutations (Dhomen and Marais, 2009). The next most frequent BRAF mutation is V600K, which occurs in ~20% of *BRAF*-mutant patients (Long *et al*, 2011) and is associated with advancing age—with chronic sun damage being a risk factor (El-Osta *et al*, 2011). It has also been suggested that *BRAF* V600K-mutant melanoma shows an increased propensity to metastasise to the brain and lungs (El-Osta *et al*, 2011). The *BRAF* V600R mutation is the third most prevalent position 600 *BRAF* mutation and occurs in 5–7% of patients (Lovly *et al*, 2012).

In the clinical setting, the BRAF inhibitors vemurafenib and dabrafenib lead to significant levels of tumour shrinkage (according to RECIST criteria) in the majority of patients whose melanomas harbour position 600 *BRAF* mutations (Table 1) (Chapman *et al*, 2011; Hauschild *et al*, 2012). The three most common *BRAF* mutations (V600E/K/R) all exhibit BRAF inhibitor sensitivity, with response rates in the *BRAF* V600E group being ~50% (Chapman *et al*, 2011; Klein *et al*, 2013). Regardless of these encouraging results, most patients have responses that are relatively short-lived (progression-free survival (PFS) for vemurafenib and dabrafenib was 5.3 and 5.1 months, respectively in phase III trials) with resistance eventually occurring in most cases (Chapman *et al*, 2011; Hauschild *et al*, 2012). Despite this and preclinical data suggesting that some resistant melanoma patient xenografts as well as cell lines with *BRAF* splice mutants may depend upon continuous BRAF inhibition for their fitness, there seems to be benefit to keeping patients on therapy beyond disease progression (Das Thakur *et al*, 2013; Azer *et al*, 2014; Hartsough *et al*, 2014). A recent study of 114 patients treated with either dabrafenib or vemurafenib showed an increase in OS when patients were kept on therapy despite progressing, compared with those who stopped drug (17.8 *vs* 7.0 months, $P<0.001$) (Azer *et al*, 2014). Studies are currently ongoing to determine whether resistance is better forestalled through continuous or discontinuous dosing schedules.

The efficacy of these inhibitors is not restricted to extra-cranial sites, with vemurafenib and dabrafenib found to cross the blood–brain barrier and to have efficacy against melanoma brain metastases (Long *et al*, 2012). At the same time, a subset

*Correspondence: Professor KSM Smalley; E-mail: keiran.smalley@moffitt.org

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Table 1. Clinical trials for BRAF-mutant only patients

NCT ID	Genotype	Drug	Target	Phase	Status	Results	Reference
NCT01006980	BRAF	Dacarbazine Vemurafenib	Chemotherapy BRAF	3	Active	Vemurafenib group: OS at 6 months 84% (95% CI), 48% response rate Dacarbazine group: OS at 6 months 64% (95% CI, 56–73), 5% response rate	Chapman *et al* (2011)
NCT01227889	BRAF	Dacarbazine Dabrafenib	Chemotherapy BRAF	3	Active	Dabrafenib: mPFS 5.1 months Dacarbazine: mPFS 2.7 months With hazard ratio (HR) of 0.30 (95% CI 0.18–0.51; $P<0.0001$)	Hauschild *et al* (2012)
NCT01245062	BRAF	Dacarbazine or Paclitaxel Trametinib	Chemotherapy MEK	3	Active	Trametinib: mPFS 4.8 months, OS at 6 months 81% Chemotherapy: mPFS 1.5 months, OS at 6 months 67% Hazard ratio for disease progression or death in the trametinib group, 0.45; 95% CI, 0.33 to 0.63; $P<0.001$. Hazard ratio for death, 0.54; 95% CI, 0.32–0.92; $P=0.01$	Flaherty *et al* (2012b)
NCT01072175	BRAF	Dabrafenib Trametinib	BRAF MEK	2	Active	Combination group: mPFS 9.4 months, 76% complete or partial response Dabrafenib group: mPFS 5.8 months, 54% complete or partial response Hazard ratio for progression or death, 0.39; 95% confidence interval, 0.25–0.62; $P<0.001$	Flaherty *et al* (2012a)
NCT00304525	BRAF	RAF265	Pan-RAF	2	Completed		[a]
NCT01657591	BRAF	Vemurafenib XL888	BRAF HSP90	1	Recruiting		[a]
NCT02068079	BRAF	Vemurafenib Trientine	BRAF Copper chelator	1	Recruiting		[a]
NCT01902173	BRAF	Dabrafenib GSK2141795	BRAF AKT	1/2	Recruiting		[a]
NCT01820364	BRAF	LGX818 and: MEK162 LEE011 BGJ398 BKM120 INC280	BRAF MEK CDK4/6 FGFR PI3K c-MET	2	Recruiting		[a]
NCT00936221	BRAF	Dacarbazine Selumetinib	Chemotherapy MEK	2	Active	OS did not differ significantly between groups, PFS improved with selumetinib. Selubetinib + dacarbazine group: mOS 13.9 months (80% CI 10.2–15.6), mPFS 5.6 months Dacarbazine group: mOS 10.5 months (80% CI 9.6–14.7), mPFS 3.0 months	Robert *et al* (2013)
NCT01495988	BRAF	Vemurafenib Bevacizumab	BRAF angiogenesis	2	Recruiting		[a]
NCT01826448	BRAF	Vemurafenib PLX3397	BRAF CSF1R, KIT, FLT3	1	Recruiting		[a]
NCT01841463	BRAF	Vemurafenib P1446A	BRAF CDK4	1	Recruiting		[a]
NCT01616199	BRAF	Vemurafenib PX-866	BRAF PI3K	1/2	Recruiting		[a]
NCT02097225	BRAF	AT13387 Dabrafenib Trametinib	HSP-90 BRAF MEK	1	Recruiting		[a]
NCT01519427	BRAF, failed BRAFi	Selumetinib MK2206	MEK AKT	2	Terminated	Study terminated due to slow accrual, total of 2 patients. OS 153 days, PFS 105 days, SD in 1 patient and PD in 1 patient	[a]

Abbreviations: CI = confidence interval; CR = complete response; mOS = median overall survival; mPFS = median progression-free survival; OS = overall survival; ORR = objective response rate; PD = progressive disease; PFS = progression-free survival; PR = partial response; SD = stable disease.
[a]Clinicaltrials.org.

of *BRAF*-mutant melanoma patients—~10%—have been identified who did not meet the RECIST criteria for a response to vemurafenib or dabrafenib, suggesting the existence of complex genetic profiles that may convey intrinsic (or pre-existing) resistance (Salama and Flaherty, 2013).

Resistance to BRAF inhibitors in melanoma is complex and mediated through multiple mechanisms with heterogeneous patterns of progression observed (for extensive reviews, see Solit and Rosen, 2011 and Holderfield *et al*, 2014). A recent analysis of a small cohort of patients showed the majority to progress at new metastatic sites (50%), with a slightly small percentage showing disease progression at existing sites (Menzies *et al*, 2014). In the majority of cases (>70%), acquired BRAF inhibitor resistance (here defined as the diminishment of drug response that that occurs following chronic BRAF inhibitor treatment) is characterised by the reactivation of MAPK signalling that can be mediated through multiple mechanisms including *NRAS* (Q61K) mutations, *BRAF* amplification, activating *MEK1* (C121S and P124L) mutations, *MEK2* (Q60P) mutations concurrent with *BRAF* amplification and BRAF splice-form mutants (Emery *et al*, 2009; Wagle *et al*, 2011; Villanueva *et al*, 2013; Shi *et al*, 2014). *In vitro*, MAPK signalling recovers rapidly following BRAF inhibition, in part through the relief of feedback inhibition in the pathway and an increased sensitivity to growth factors such as epidermal growth factor (EGF), neuregulin (NRG-1), hepatocyte growth factor (HGF) and fibroblasts growth factor (FGF) (Lito *et al*, 2012). In this context, reactivation of MAPK signalling following BRAF inhibition is important for therapeutic escape with increased levels of cell death and tumour regression being seen when BRAF and MEK are co-targeted (Paraiso *et al*, 2010; Lito *et al*, 2012). Clinical trials have confirmed these preclinical observations with the BRAF/MEK inhibitor combination (dabrafenib plus trametinib) showing an increased PFS compared with BRAF inhibitor alone (Table 1). A later trial—the phase III COMBI-D regimen—further showed an increase in the overall response rate to the combination compared with monotherapy and an improvement in a range of clinical end points. Another BRAF/MEK inhibitor combination—vemurafenib + cobimetinib—also appears promising, with newly released data from the BRIM-7 trial demonstrating an 87% confirmed response rate by RECIST and a median PFS of 13.7 months (Ribas *et al*, 2014).

Despite hopes that vertical MAPK pathway targeting would limit resistance, treatment failure still occurs with the resistance mechanisms observed to the BRAF/MEK inhibitor combination being analogous to those seen in patients on BRAF inhibitor monotherapy. A preliminary genetic analysis of five patients failing dabrafenib + trametinib revealed similar mechanisms of resistance to those seen in patients on BRAF inhibitor monotherapy and highlighted the role of *MEK2* Q60P mutations, *BRAF*-splice mutants and *BRAF* amplification (Wagle *et al*, 2014). It is therefore perhaps not surprising that failure on BRAF inhibitor therapy also confers resistance to MEK inhibition, with minimal clinical activity being seen to trametinib in patients failing dabrafenib and a 15% response rate (median PFS 2.8 months) observed in patients failing BRAF inhibitor following treatment with vemurafenib + cobimetinib (Ribas *et al*, 2014).

Attention is now being turned to further targeted agents that can be added to the BRAF/MEK inhibitor backbone. There is already preclinical evidence that ERK inhibitors, such as SCH772984, can overcome acquired resistance to single agent BRAF and MEK inhibition (Morris *et al*, 2013). Although clinical trials of ERK inhibitors in the monotherapy setting have been initiated, little information has been yet presented on their efficacy or toxicity. Another surprise recent finding was the dependency of the MAPK signalling pathway upon copper ions, with the copper-MEK1 interaction being required for efficient ERK phosphorylation (Brady *et al*, 2014). Chelation of copper or the knockdown of the copper transporter CRT1 had anti-proliferative activity against *BRAF*-mutant melanoma cells and could overcome vemurafenib resistance mediated through the C121S MEK1 mutations (Brady *et al*, 2014).

Many components of the MAPK pathway, including mutant BRAF, CRAF and COT proteins, are clients of the chaperone protein HSP90 (da Rocha Dias *et al*, 2005; Grbovic *et al*, 2006; Paraiso *et al*, 2012). This, along with the requirement for HSP90 in multiple BRAF inhibitor resistance mechanisms, has raised interest in co-targeting the HSP90 'clientome' along with mutant *BRAF*. This concept has already been validated in other cancers, with studies showing that HSP90 inhibitors reverse trastuzamab resistance in HER2-positive breast cancer as well as bortezomib resistance in multiple myeloma (Modi *et al*, 2011; Richardson *et al*, 2011). At least three preclinical studies have now demonstrated that HSP90 inhibitors (including XL888 and ganetespib) can overcome or abrogate the onset of BRAF inhibitor resistance (Paraiso *et al*, 2012; Wu *et al*, 2013; Acquaviva *et al*, 2014). At this time, a phase I dose escalation study of vemurafenib + XL888 in patients with metastatic *BRAF* V600-mutant melanoma is ongoing (Table 1) (NCT01657591).

Although reactivation of MAPK signalling is frequently associated with BRAF and BRAF/MEK inhibitor failure, other pathways such as the PI3K/AKT pathway have also been implicated in resistance. In melanoma cell lines, constitutive PI3K/AKT signalling is commonly observed and can result from multiple mechanisms, including the loss/mutation of the tumour suppressors PTEN or neurofibromin (NF1) or increased expression of AKT3 (Stahl *et al*, 2004; Tsao *et al*, 2004; Maertens *et al*, 2012). *In vitro* studies have suggested that BRAF and MEK inhibition may sometimes lead to rebound PI3K/AKT signalling, resulting in therapeutic escape mediated through the suppression of apoptosis (Gopal *et al*, 2010; Paraiso *et al*, 2011). These lab-based findings have also been supported by the observation that increased PI3K/AKT signalling occurs in some early (4–25 days) on-therapy specimens from patients treated with a BRAF inhibitor (Shi *et al*, 2014). One study showed that 22% of tumours from patients failing therapy exhibited increased AKT activity (Shi *et al*, 2014). In the majority of cases (81%) increased AKT signalling overlapped with recovery of signalling in the MAPK pathway, with the AKT pathway representing the primary resistance pathway in only a minority of cases (Shi *et al*, 2014). A number of potential mechanisms of PI3K/AKT activation were identified including mutations in *PI3KCA* (D350G and E544G), *PI3KCG* (V983E), *PTEN* (134M_ and fs.40), *PI3KR2* (N561D), *AKT1* (Q79K), *AKT3* (E17K) and *PHLPP1* (K596E) (Shi *et al*, 2014). Of these, loss of PTEN function, either through mutation or loss of expression, has been the most extensively investigated with somewhat conflicting results about its relevancy. Although there is preclinical evidence suggesting that loss of PTEN expression predicts for a reduced cytotoxic response to BRAF inhibition, some *BRAF* V600E/PTEN-null melanoma cell lines have also been identified with sensitivity to vemurafenib (Atefi *et al*, 2011; Paraiso *et al*, 2011). In addition, good levels of tumour regression have been observed in *BRAF* V600E/PTEN-null GEMM models of melanoma following BRAF inhibitor treatment (Marsh Durban *et al*, 2013). A similarly nuanced picture also emerged when patients receiving dabrafenib were stratified according to their PTEN status, with a trend being seen towards a lower PFS in individuals whose tumours lacked PTEN function (Nathanson *et al*, 2013).

The identification of PI3K/AKT signalling as a core pathway for melanoma development and therapeutic escape suggested the possibility of co-targeting MAPK and PI3K/AKT signalling in *BRAF*-mutant melanoma (Table 1). A number of early preclinical studies demonstrated the utility of concurrently targeting the MAPK and PI3K/AKT pathways across multiple melanoma cell lines and xenografts (Bedogni *et al*, 2006;

Smalley *et al*, 2006). In *BRAF* V600E/PTEN-null GEMM models, the combination of the BRAF inhibitor LGX818 with the PI3K inhibitor BKM-120 was associated with a more rapid and durable pattern of tumour regression compared with LGX818 alone (Marsh Durban *et al*, 2013). With the clinical availability of PI3K, AKT and mTOR inhibitors, multiple trials are now underway with combined inhibition of the MAPK and PI3K pathways (Tables 1 and 2).

Melanomas have the highest mutational loads of all cancers (Alexandrov *et al*, 2013). One central question that has arisen is whether resistance is mediated through pre-existing clones that are *BRAF* wild type or occurs through drug-induced selection pressure that drives the mutational landscape. Evidence in favour of drug-induced selection pressure comes from a recent whole-exome sequencing study of multiple progressing lesions from one patient failing dabrafenib therapy after 383 days (Shi *et al*, 2014). Of the nine distinct progressing lesions analysed, at least five co-existent mechanisms of resistance were identified, including an acquired *KRAS* mutation, a *BRAF* splice-mutant, *BRAF* amplification, a *PTEN* indel and one mechanism that remains unknown (Shi *et al*, 2014). At the same time, the mutational spectra of the progressing tumours significantly altered on BRAF inhibitor therapy, with a reduction in the frequency of C>T transition mutations being observed in the resistant tumours compared with the pre-treatment tumours (Shi *et al*, 2014).

NRAS-MUTANT AND *BRAF/NRAS*-WILD-TYPE MELANOMA

Although the majority of therapeutic advancements in the past few years have been largely focused on patients with mutations in *BRAF*, *NRAS* was actually the first oncogene identified in melanoma (Albino *et al*, 1984). NRAS is part of family of low-molecular weight GTP-binding proteins that are associated with the plasma membrane. Ras proteins control a wide array of cellular functions, including growth, survival and invasion, by relaying signals from activated RTKs at the cell surface to downstream effectors in the nucleus, including cell-cycle proteins and transcription factors (Malumbres and Barbacid, 2003; Cully and Downward, 2008). Activated Ras can trigger a number of intracellular signalling pathways such as the Raf/MEK/ERK mitogen-activated protein kinase (MAPK) pathway and the PI3K/AKT pathway (Downward, 2003). Mutated Ras can mediate cellular transformation through a network of signal-transduction pathways independent of upstream RTK activation (Malumbres and Barbacid, 2003). The role of *NRAS* in driving growth of melanoma cells was confirmed through knockdown of *NRAS* in melanoma cell lines using small-interfering RNA, which showed a marked reduction in cell growth and with decreased expression of cyclins D1 and E2 (Eskandarpour *et al*, 2009). It is currently known that *NRAS*, *KRAS* and *HRAS* mutations are present in 20%, 2% and 1% of all melanomas, respectively, with the most common *NRAS* mutation occurring at position Q61 (Milagre *et al*, 2010).

NRAS-mutant melanomas differ from *BRAF*-mutant melanomas in clinical presentation and prognostic features (Devitt *et al*, 2011; Ellerhorst *et al*, 2011). Patients who present with *NRAS*-mutant melanomas tend to be older and have a history of chronic UV exposure (Devitt *et al*, 2011). These individuals tend to have thicker primary tumours that are located on the extremities and have higher rates of mitosis (Devitt *et al*, 2011). While MAPK signalling in melanocytes is typically driven through BRAF, BRAF activity is not required for MAPK activation in *NRAS*-mutant melanomas, which alternatively rely on CRAF signalling (Dumaz *et al*, 2006). In *NRAS*-mutant melanoma, the switch to CRAF signalling is dependent on both the phosphorylation and inactivation of BRAF at S151, T401, S750 and T753 and the deregulation of protein kinase A (PKA) activity (which serves to prevent CRAF from being phosphorylated at inhibitory sites) (Dumaz *et al*, 2006; Marquette *et al*, 2011). The role of PKA in the regulation of CRAF suggests the possibility for therapeutic intervention, with studies showing selective phosphodiesterase IV inhibitors to be growth inhibitory and pro-apoptotic in *NRAS*-mutant cell lines (Marquette *et al*, 2011). Similarly, PI3K/AKT pathway regulation in *NRAS*-mutant melanoma cells proceeds differently than in those harbouring a *BRAF* mutation, and occurs directly through the Ras-mediated recruitment of PI3K, rather than the concurrent loss of PTEN or NF1 function (Tsao *et al*, 2004; Maertens *et al*, 2012).

So far, the direct targeting of *NRAS* has proven to be a challenge. Several approaches have been explored for targeting Ras directly by designing drugs that prevent the post-translational modifications required for the insertion of Ras into the plasma membrane. Farnesyl transferase inhibitors initially showed great preclinical potential, but have ultimately been disappointing in the clinical setting (Konstantinopoulos *et al*, 2007). The recent years have seen renewed interest in the development of small-molecule RAS inhibitors that bind to domains unique to the mutant protein. One approach has utilised the binding of drug to the cysteine in the G12-mutant form of *KRAS* to achieve selectivity over the wild-type protein (Ostrem *et al*, 2013). In preclinical studies, compounds directed against *KRAS*-G12 had anti-proliferative and pro-apoptotic activity against *KRAS*-mutant lung cancer cell lines in the low micromolar range. Another RAS targeting approach is to inhibit the interaction between KRAS and the prenyl binding protein PDEδ, which in turn prevents KRAS signalling by altering its localisation to endomembranes (Zimmermann *et al*, 2013). Although these new inhibitors have been developed for mutant *KRAS*, it is likely that a similar concept can be applied to *NRAS*-mutant tumours in the near future.

One other strategy being actively explored is the inhibition of the downstream mediators of Ras signalling, including MEK, ERK, PI3K and CDK4. The greatest focus of preclinical studies for *NRAS*-mutant melanoma has been upon MAPK pathway inhibition in combination with inhibitors of other pathways activated by Ras. Preclinical work using shRNA to knock down Ras targets demonstrated that good levels of tumour regression could be achieved *in vivo* following the ablation of either BRAF + CRAF or BRAF + PI3K (Jaiswal *et al*, 2009). Other studies, in which small-molecule inhibitors of the MAPK and PI3K/AKT pathway were evaluated in *NRAS*-mutant melanoma xenograft models, demonstrated the combination of MEK/PI3K inhibitors to be superior to a combination targeting MEK + mTOR (Posch *et al*, 2013). It was further found that the combination of a MEK inhibitor with a PI3K/mTOR inhibitor was synergistic and was associated with profound levels of tumour regression (Posch *et al*, 2013). Further support for this combination comes from studies of *BRAF*-mutant melanoma cell lines in which resistance was mediated through the acquisition of an *NRAS* mutation(Greger *et al*, 2012). There is also evidence from unbiased bioinformatic studies of synergy between MEK and CDK4/6 inhibitors in *NRAS* (Q61K)/*CDKN2A*^{Null} mouse melanoma models, and this combination is currently being evaluated clinically in patients with *NRAS*-mutant melanoma as well as those with *BRAF*-mutant melanoma (Table 2) (NCT01781572, NCT01777776) (Kwong *et al*, 2012).

Despite the focus upon the co-targeting of the MAPK and PI3K/AKT pathways in *NRAS*-mutant melanoma, other Ras-driven pathways may also be important for tumour initiation and maintenance—and significantly less is known about these. A recent study in which ARF-null immortalised melanocytes were transformed with active forms of PI3K, MEK or Ral-GDS revealed distinct roles for all of these signal transduction mediators in tumour initiation, with Ral-GDS being a prerequisite for anchorage-independent growth (Mishra *et al*, 2010). Other work

Table 2. Clinical trials for genotypes other than BRAF-mutant only

NCT ID	Genotype	Drug	Target	Phase	Status	Results	Reference
NCT01781572	NRAS	LEE011 MEK162	Cyclin D1/CDK4, cyclin D3/CDK6 MEK	1/2	Recruiting		[a]
NCT01763164	NRAS	MEK162	MEK	3	Recruiting		[a]
NCT02138292	BRAF WT	Trametinib Digoxin	MEK cardiac glycoside	1	Not yet recruiting		[a]
NCT01941927	BRAF WT	Trametinib GSK2141795	MEK AKT	2	Recruiting		[a]
NCT00470470	c-KIT	Imatinib	c-KIT	2	Active	The overall durable response rate was 16% (95% confidence interval (CI), 2–30%), with a median time to progression of 12 weeks (interquartile range (IQR), 6–18 weeks; 95% CI, 11–18 weeks), and a median OS of 46.3 weeks (IQR, 28 weeks-not achieved; 95% CI, 28 weeks-not achieved)	Robert *et al* (2013)
NCT00631618	c-KIT	Sunitinib	Multiple RTKs	2	Completed	Of 4 patients with KIT mutations, 1 had a CR for 15 months and 2 had PR (1 and 7 months). 1 of the 6 patients with only KIT amplification or overexpression alone had a PR. In 1 responder with rectal melanoma who later progressed, the recurring tumour had a previously undetected mutation in NRAS, which was found in addition to the persisting mutation in KIT	Minor *et al* (2012)
NCT00591734	Not specified	Everolomus Bevacizumab	mTOR angiogenesis	2	Completed	12% major response, 58% stable disease, mPFS 4.0 months, OS 8.6 months	Hainsworth *et al* (2010)
NCT00591734	Not specified	Paclitaxel Carboplatin Everolimus	mTOR	2	Completed	ORR 17%, PFS 4.04 months, OS 10.12 months	[a]
NCT00338130	Not specified	Temozolomide *vs* Selumetinib	Chemotherapy *vs* MEK	2	Active	Selumetinib group: mPFS 78 days, ORR 5.8% Temozolomide group: mPFS 80 days, ORR 9.4% Five of the six selumetinib partial responders were BRAF mutated	Kirkwood *et al* (2012)
NCT00827177	NRAS WT/ mutant	Sorafenib Tivantinib	Pan-RAS c-Met	1	Completed	CR in 1 pt, PR in 3 pts, and SD in 3 pts. 4 pts had progressive disease and 5 pts were not evaluable. ORR and disease control rate were 25% and 44%, respectively. mPFS was 5.3 mo (95% CI, 1.6–12.9 mo). Among 8 pts with NRAS mutations, mPFS was 9.2 mo (95% CI, 5.3–12.9 mo) and responses were 1 CR, 1 PR and 2 SD	Means-Powell *et al* (2012)
NCT01363232	BRAF/NRAS	BKM120 MEK162	PI3K MEK	1	Active	No results reported	[a]
NCT01337765	BRAF/NRAS	BEZ235 MEK162	PI3K MEK	1	Active	No results reported	[a]

Table 2. (Continued)

NCT ID	Genotype	Drug	Target	Phase	Status	Results	Reference
NCT01320085	BRAF/NRAS	MEK162	MEK	2	Active	No patients had a complete response. Six (20%) of 30 patients with NRAS-mutated melanoma had a partial response (three confirmed) as did 8 (20%) of 41 patients with BRAF-mutated melanoma (two confirmed)	Ascierto *et al* (2013)
NCT00866177	BRAF/NRAS	Selumetinib	MEK	2	Completed	Tumour regression was seen in 3/5 patients with BRAF-mutated, low pAKT melanomas; no responses were seen in the high pAKT cohort. The estimated mPFS was 2.2 months in the high pAKT cohort and 7.1 months in the low pAKT cohort	Catalanotti *et al* (2013)

Abbreviations: CI = confidence interval; CR = complete response; mOS = median overall survival; mPFS = median progression-free survival; OS = overall survival; ORR = objective response rate; PD = progressive disease; PFS = progression-free survival; PR = partial response; SD = stable disease.
[a]Clinicaltrials.org.

showed the RAL-GEF effector TANK-binding kinase 1 (TBK1) to be a key mediator of RAL activity in *NRAS*-mutant melanoma, with its siRNA-mediated knockdown found to prevent tumour invasion and migration (Vu and Aplin, 2014).

In the same vein, the HSP inhibitor XL888 demonstrated efficacy against a panel of *NRAS*-mutant cell lines through simultaneous suppression of a number of targets crucial for *NRAS*-mutant melanoma cell survival, including CDK4, AKT and WEE1 (Haarberg *et al*, 2013). The potential role of WEE1 in *Ras*-mutant tumours has also been confirmed in other systems, with positive interactions seen between the WEE1 inhibitor MK-1775 and PI3K/AKT/mTOR inhibitors in various models including acute leukaemia (Weisberg *et al*, 2014).

Despite strong preclinical evidence for treating *NRAS*-mutant melanoma using inhibitors of MEK, clinical trials so far have demonstrated only modest activity in patients. At first, early generation MEK inhibitors exhibited only a 10% objective response rate. However, excitement at the idea of targeting MEK resurfaced with the development of potent third-generation MEK inhibitors trametinib (GSK1120212) and MEK162 (ARRY-438162) (Table 2). In the recent phase II clinical trial of MEK162, 20% of *NRAS*-mutant patients exhibited objective responses while a further 43% showed stable disease; however, median PFS was 3.7 months (Ascierto *et al*, 2013). A phase III trial is currently ongoing comparing MEK162 with dacarbazine specifically in *NRAS*-mutant metastatic melanoma patients (Table 2) (NCT01763164). The mechanisms by which *NRAS*-mutant melanomas escape from MEK inhibition are starting to be elucidated. Treatment of *NRAS*-mutant melanoma cell lines with the MEK inhibitors AZD6244 and trametinib is associated with recovery of MAPK signalling, an effect that can be overcome through dual MEK/ERK inhibition. Another potential mechanism of escape from MEK inhibition in *NRAS*-mutant melanoma is adaptive (here defined as a change in signalling that occurs following the administration of a first drug) RTK signalling, with increased PDGFR-B signalling being observed in some cell lines following treatment with the MEK inhibitor AZD6244 (Rebecca *et al*, 2014b). In these instances, therapeutic escape was abrogated and cytotoxicity was enhanced when MEK inhibitors were combined with the PDGFR inhibitor crenolanib (Rebecca *et al*, 2014b).

NRAS-mutant melanoma cell lines are known to have constitutive activity in many RTKs and there is growing preclinical evidence that multi-RTK targeted inhibitors have efficacy against subsets of *NRAS*-mutant melanoma (Tworkoski *et al*, 2011; Fedorenko *et al*, 2014). Studies have already shown RAF-265 (which inhibits CRAF, BRAF, VEGFR and FLT-3 among other things) to have good anti-tumour activity in some *BRAF*-wild-type melanoma patient-derived xenografts and that amuvatinib (inhibits c-KIT, c-MET, Axl and RAD51) is effective in some *NRAS*-mutant melanoma cell lines (Su *et al*, 2012; Fedorenko *et al*, 2014). In the clinical setting, *NRAS*-mutant melanoma patients also showed better responses to the combination of sorafenib with carboplatin + paclitaxel than their *BRAF*-mutant counterparts (Wilson *et al*, 2014).

Strategies to target melanomas that are *BRAF/NRAS/KIT*-wild type (so-called 'triple-negative' or 'pan-negative' melanomas) have proven even more elusive. The recent whole-exome sequencing of 21 melanomas that were *BRAF/NRAS*-wild type painted a complex picture involving mutations in *NF1* as well as rare *CRAF* and *MAP2K1* mutations (Hodis *et al*, 2012). Some of the *BRAF/NRAS*-wild-type melanomas in this study showed focal amplification in potential oncogenes such as cyclin D1 and CDK4, although it was not determined whether this conveyed sensitivity to CDK4 inhibition (Hodis *et al*, 2012). In a second more recent study, the next-generation sequencing of 623 cancer-related genes across 241 melanoma samples included 69 tumours that were 'triple-negative'. In this cohort, a number of potential driver mutations were identified including ALK (3.5%), RAC1 (2.9%), STK31 (8.7%), DGK1 (4.7%), NF1 (7.6%), KDR (6.4%) and ERBB4 (11.6%) (Xia *et al*, 2014). Although both these sequencing studies identified mutations in *NF1* (which is a negative regulator of Ras signalling) as a potential driver of *BRAF/NRAS*-wild-type melanoma, its loss seems to also play a role in *BRAF*-mutant melanoma (Maertens *et al*, 2012; Whittaker *et al*, 2012; Nissan *et al*, 2014). In the *BRAF*-mutant context, loss of NF1 function leads to increased CRAF-mediated MAPK signalling and activation of the PI3K/AKT pathway, and it is implicated in both BRAF inhibitor resistance and melanoma development (Maertens *et al*, 2012; Whittaker *et al*, 2012; Nissan *et al*, 2014). It is likely that melanoma patients lacking NF1 function (regardless of other co-operating oncogenes) will require combination therapy treatment; with preclinical studies suggesting the utility of the MEK + mTOR inhibitor combination, a pan-RAF inhibitor or an ERK inhibitor (Maertens *et al*, 2012; Whittaker *et al*, 2012; Nissan *et al*, 2014).

RAC1 is a small GTPase that has been linked to cancer cell motility (Sanz-Moreno *et al*, 2008). Recurrent P29S mutations in *RAC1* were recently reported in 3.3–9.2% of cutaneous melanomas, with mutations occurring at a greater frequency in male patients (Krauthammer *et al*, 2012; Mar *et al*, 2014). Although an association was reported between *RAC1* mutations and *BRAF/NRAS*-wild-type mutational status, this was not found in a second, larger study (Krauthammer *et al*, 2012; Mar *et al*, 2014). From a functional standpoint, the presence of a *RAC1* mutation was associated with a greater risk of nodal metastasis and it was suggested that the acquisition of a *Rac1* mutation led to a greater risk of early disease dissemination (Mar *et al*, 2014).

Although triple-negative melanomas may lack *BRAF* mutations, they may still be dependent upon BRAF signalling, with two recent reports identifying the potential role of BRAF fusion proteins (Botton *et al*, 2013; Hutchinson *et al*, 2013). These fusion events, which typically involve the fusion of the BRAF kinase domain to other N-terminal binding partners including PASSP1, CDC27, TAX1BP1 and TRIM24, occur in 4–8% of cases of triple-negative melanomas (Hutchinson *et al*, 2013). Preliminary evidence suggests that these fusion proteins activate the MAPK pathway in melanoma cell lines and convey sensitivity to either sorafenib or MEK inhibition (Botton *et al*, 2013; Hutchinson *et al*, 2013). At the same time there have also been reports of melanomas with non-position 600 mutations in *BRAF* such as K601, L597R and L597Q showing sensitivity to MEK inhibition (Dahlman *et al*, 2012; Bowyer *et al*, 2014).

Inhibition of the MAPK and PI3K/AKT signalling pathways leads to adaptive RTK signalling in multiple cancer types (Chandarlapaty *et al*, 2011; Duncan *et al*, 2012; Lito *et al*, 2012). *BRAF/NRAS*-wild-type melanoma is no exception and there is evidence that inhibition of MEK signalling leads to increased endothelin-B receptor (EDNRB) expression that limits therapeutic efficacy (Asundi *et al*, 2014). In an *in vivo* model of *BRAF/NRAS*-wild-type melanoma, the co-targeting of MEK with an antibody drug conjugate targeted against EDNRB was more efficacious than either agent alone and was associated with good levels of tumour suppression (Asundi *et al*, 2014). Similarly, inhibition of AKT in combination with paclitaxel and carboplatin suppressed the long-term growth of *BRAF/NRAS*-wild-type melanoma cell lines *in vitro*, and was associated with stable disease (>10 months) in two cases of *BRAF*-wild-type melanoma (Rebecca *et al*, 2014a).

Another potential therapeutic target that is frequently either amplified or overexpressed in *BRAF*-wild-type and *BRAF/NRAS*-wild-type melanoma is p21-activated kinase (PAK)-1 (Ong *et al*, 2013). This kinase, which is downstream of both RAC1 and CDC42, stimulates the MAPK pathway by directly phosphorylating CRAF at S338 and MEK1 at S298. In *NRAS*-mutant and *BRAF/NRAS*-wild-type melanomas, inhibition of PAK1 through either siRNA knockdown or the PAK1 inhibitor PF-3758309 suppresses ERK phosphorylation and was associated with the reduction growth in a *BRAF/NRAS*-wild-type melanoma xenograft model (Ong *et al*, 2013). In this instance, the effect seemed to be more cytostatic than cytotoxic: suggesting that other drugs may need to be combined with PF-3758309 to achieve cytoreduction and durable responses.

CONCLUSION

Tremendous progress has been made in developing oncogene-directed therapies for treating *BRAF*-mutant melanoma. Resistance remains a major problem that limits the long-term responsiveness of the majority of the patients to these drugs. Current strategies to improve the durability of response are now focused on the development of personalised combination therapy strategies, the majority of which centre upon the suppression of adaptive MAPK and PI3K/AKT signalling (Table 1). Given the current success of the BRAF/MEK inhibitor doublet, future combinations will likely be based upon this backbone. At this time, the relationship between the genetic prolife of the tumour and patterns of adaptive signalling are not well understood. Better assays and biomarkers will be needed to interrogate the early treatment responses so that combinations can be rapidly personalised before resistance ensues. Strategies being explored in this realm include the analysis of circulating tumour cells, circulating tumour DNA and proteomic methods. Considerably less progress has been made in the development of precision medicine strategies for *BRAF*-wild-type melanoma. Although there are hints that MEK inhibitors may be effective in *NRAS*-mutant melanoma, responses have been sub-optimal and combinations (likely highly personalised ones) will be needed.

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