

## Minireview

# Targeting BRAF for patients with melanoma

H-T Arkenau<sup>\*,1,3</sup>, R Kefford<sup>2</sup> and GV Long<sup>2,3</sup>

<sup>1</sup>Sarah Cannon Research UK, 93 Harley Street, London W1G 6AD, UK; <sup>2</sup>Melanoma Institute Australia and Westmead Hospital, University of Sydney, New South Wales, Australia

The prognosis of patients with metastatic melanoma is poor and not influenced by systemic therapy with cytotoxic drugs. New targeted agents directed against the RAS-RAF-MEK-ERK pathway show promising activity in early clinical development and particular interest is focused on selective inhibitors of mutant BRAF, which is present in one half of the cases of metastatic melanoma. The majority of patients on early trials of these drugs develop secondary resistance and subsequent disease progression and it is, therefore, critical to understand the underlying escape mechanisms leading to resistance.

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## MELANOMA: A HETEROGENEOUS DISEASE

Metastases develop in 10–15% of patients with cutaneous melanoma and there is no evidence from phase-III trials that systemic treatment prolongs survival, nor is there an effective adjuvant therapy after resection of high risk Stage I–III disease (Thompson *et al*, 2005). Only 5% of patients with visceral metastases survive for 2 years (Balch *et al*, 2001).

Understanding the genetic heterogeneity in melanoma has become increasingly important with the development of therapies aimed at targeting specific genetic aberrations. Of particular importance in melanoma is the mitogen-activated protein kinase (MAPK) pathway, which normally regulates cell growth, proliferation and differentiation (Figure 1). Aberrant activation of the MAPK pathway is present in over 80% of primary melanomas (Platz *et al*, 2008), and mutations in proteins along the RAS-RAF-MEK-ERK pathway are thought to be mutually exclusive. Such mutations have been documented in all subtypes of melanoma (Curtin *et al*, 2005), including cutaneous (50–60% BRAF, 15% NRAS and 17% CKIT chronic sun damage), mucosal (11% BRAF, 5% NRAS and 21% CKIT) and uveal (50% GNAQ) melanomas. The BRAF and NRAS mutations have not been reported in uveal melanoma to date (Populo *et al*, 2010).

## TYPES OF BRAF MUTATIONS

Constitutively activating somatic missense mutations in BRAF were discovered to be present in a wide variety of human cancers, including papillary thyroid cancer (39–69%), cholangiocarcinoma (22%), colorectal cancer (5–12%) and borderline ovarian cancer (30%) (Catalogue of Somatic Mutations in Cancer (COSMIC)

website). The mutations are either in the activating segment in exon 15 or the glycine-rich loop (P-loop) in exon 11 of the kinase domain of the BRAF protein (Figure 2). The point mutation in DNA (1799T→A) resulting in a single amino-acid substitution at Valine 600 to Glutamic acid in the activating segment (V600E, previously known as V599E) is the most common change, and is found in 80% of the mutated tumours (Davies *et al*, 2002). This is distinct from the CC→TT or C→T changes associated with ultraviolet light exposure in non-melanoma skin cancers. BRAF V600E results in elevated kinase activity compared with BRAF wild type and stimulated phosphorylation of downstream endogenous ERK (Dhomen and Marais, 2009).

Today more than 75 somatic mutations in the BRAF gene have been identified in melanoma, and all mutations at V600 in Exon 15 constitutively activate BRAF (Figure 2). In BRAF mutant melanoma, 74–90% are V600E (Platz *et al*, 2008) and 16–29% are V600K mutations (Thomas, 2006; Long *et al*, 2010). One hypothesis for the mechanism of uncontrolled activation is the increased exposure of the activation segment for interaction when a small hydrophobic amino acid at 600 (Valine) is switched to a hydrophilic residue. Normally, the RAF kinase domain in the inactive conformation is hidden in a hydrophobic pocket (Wan *et al*, 2004). A small number of mutations have reduced kinase activity compared with wild type, for example, G465E, G465V, D593V and G595R, but cause increased ERK activation, possibly via binding and activation of CRAF (Houben *et al*, 2004; Wan *et al*, 2004).

## PROGNOSIS OF BRAF MUTATION IN METASTATIC MELANOMA

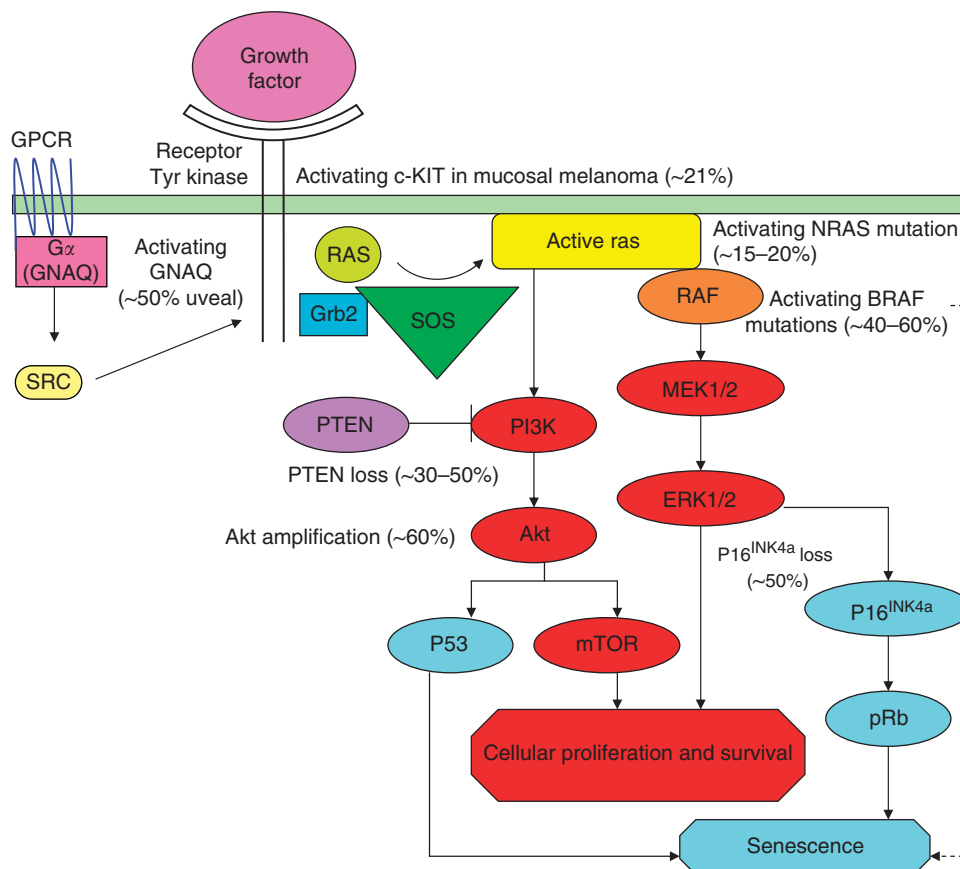
The frequency of BRAF mutation in primary melanomas ranges from 36 to 45% (Curtin *et al*, 2005; Liu *et al*, 2007; Thomas *et al*, 2007) and 42–55% in metastatic melanoma (Houben *et al*, 2004; Ugurel *et al*, 2007; Long *et al*, 2010). The presence of a BRAF mutation in early melanoma shows no association with disease-free interval or overall survival (Shinozaki *et al*, 2004). In contrast, the presence of a BRAF mutation in metastatic melanoma is

\*Correspondence: Dr H-T Arkenau;

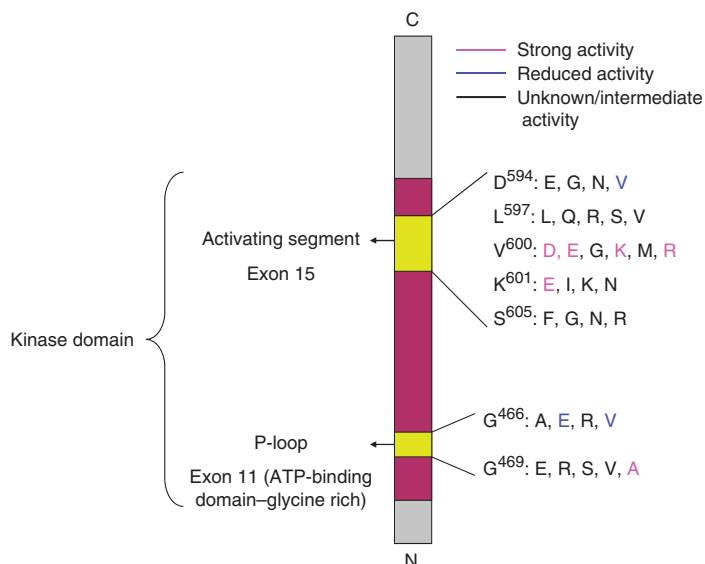
E-mail: tobias.arkenau@sarahcannonresearch.co.uk

<sup>3</sup> These authors contributed equally to this work.

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**Figure 1** The MAPK pathway activation in melanoma. Oncogenic NRAS, BRAF, GNAQ and CKIT signal through the MAPK pathway. Oncogenic NRAS also induces the phosphatidylinositol-3' kinase (PI3K) cascade. MAPK signalling can lead to proliferation in transformed cells, but also induces a potent form of growth arrest, known as senescence in normal melanocytes. The approximate proportion of melanomas with mutations are shown. GPCR = G-protein coupled receptor.



**Figure 2** Common types of BRAF mutations in melanoma (Wan *et al*, 2004).

associated with a poorer survival from time of first metastasis (Long *et al*, 2010) or time from first resected metastasis (Houben *et al*, 2004), although not consistently observed in smaller studies (Ugurel *et al*, 2007).

**RATIONALE FOR BRAF INHIBITION IN MELANOMA**

Advanced melanomas often have multiple genetic defects affecting diverse biochemical pathways. It was, therefore, surprising that

those with activating BRAF mutations displayed the hallmarks of oncogene addiction. When this single oncogenic alteration was targeted in melanoma cell lines with specific inhibitory nucleic acids or chemical RAF inhibitors, the cell lines displayed growth arrest and induction of apoptosis (Calipel *et al*, 2003). The behaviour of mutant BRAF melanomas in murine xenograft models supported these preclinical findings and confirmed mutant BRAF as an attractive target for melanoma therapy, particularly as it occurs in at least half the tumour population, and does not occur in normal cells. In addition, its serine/threonine kinase domain was amenable to the rational design of selective inhibitors.

## EARLY CLINICAL DATA OF RAF INHIBITORS IN MELANOMA PATIENTS

The first RAF kinase inhibitor entering early clinical trials was the oral diphenyl urea, sorafenib (Bay 43-9006). Sorafenib has potent RAF isoform kinase inhibitor activities (CRF (IC<sub>50</sub> 6 nM) > wild type (wt) BRAF (IC<sub>50</sub> 22 nM) > mutant BRAF<sup>V599E</sup> (IC<sub>50</sub> 38 nM)), but was also found to have a much broader inhibitory profile, including kinases of the vascular endothelial growth factor receptor 2 and 3 (VEGFR-2 (IC<sub>50</sub> 90 nM) and VEGFR-3 (IC<sub>50</sub> 20 nM)), the platelet-derived growth factor receptor- $\beta$  (IC<sub>50</sub> 57 nM), Flt-3 (IC<sub>50</sub> 58 nM) and c-Kit (IC<sub>50</sub> 68 nM).

In human xenograft models sorafenib resulted in prolonged growth stabilisation rather than tumour response, perhaps prewarning the negligible clinical activity in melanoma patients who were treated within a discontinuation phase-II trial

(Karasarides *et al*, 2004; Eisen *et al*, 2006). These results raised questions about the *in human* BRAF inhibitory activity of sorafenib and raised scepticism about the relevance of mutant BRAF as a target in melanoma. Activity of the drug in renal cell and hepatocellular carcinoma has since been attributed to promiscuous inhibitory effects on receptor tyrosine kinases, including VEGFR, PDGFR and cKIT.

Further research led to the development of second generation more selective RAF inhibitors, which are currently in clinical trials (Table 1).

XL281 (famotidine) is an orally administered inhibitor of the wt BRAF (IC<sub>50</sub> 4.5 nM), CRAF (IC<sub>50</sub> 2.5 nM) and mutant BRAF<sup>V600E</sup> (IC<sub>50</sub> 6 nM) kinases and demonstrated potent inhibitory effect in a variety of human xenograft models. The phase-I study of XL281 (NCT00451880) in an unselected patient population showed prolonged disease stabilisation in two patients with BRAF<sup>V600E</sup> mutant papillary thyroid cancer (>15 and 17 months, respectively) and some minor responses in nine patients with colorectal, ovarian and non-small-cell lung cancer. Overall XL281 was well tolerated with acceptable rates of grade 3/4 nausea, vomiting and diarrhoea (Schwartz *et al*, 2009). Currently, a three-arm dose expansion part is expected to recruit a total of 180 patients, including patients with melanoma, papillary thyroid and colorectal and non-small-cell lung cancer. Results of an ongoing melanoma phase-I/II trial (RAF265-MEL01; wt BRAF and BRAF mutant patients included) are awaited.

PLX4032 (RO 5185426) is an ATP competitive, orally administered BRAF inhibitor (wt BRAF IC<sub>50</sub> 100 nM) with high selectivity

**Table 1** Clinical development of RAF kinase inhibitors in patients with advanced melanoma

RAF inhibitor	Chemical class	Target	Additional target	Status	n	Clinical outcome	Toxicity profile grade $\geq 2$
<i>Non-selective RAF inhibitors</i>							
Sorafenib	Diphenyl urea	CRAF, BRAF <sub>wt</sub> , BRAF <sup>V599E</sup>	VEGFR-2&3, PDGFR- $\beta$ , FGFR-1, Flt-3, c-Kit	Phase-II	37	SD 19%	Diarrhea, HFS
+ Temsirolimus				Phase-I/II	69		Diarrhea, rash Hyperlipidemia
+/- Dacarbazine				Phase-II	101	Improved PFS = NS, OS = NS	Haemtox, nausea, hypertension, bleeding lipase
+/- Carboplatin/ paclitaxel				Phase-III (first-line)	270	PFS = NS	Haemtox, nausea, neuropathy, rash, HFS
+/- Carboplatin/ paclitaxel				Phase-III (second-line)	823	OS = NS	Haemtox, nausea, neuropathy, rash, HFS
RAF265	Benzazole	CRAF, BRAF <sub>wt</sub> , BRAF <sup>V600E</sup>	VEGFR-2	Phase-I/II (ST)	211	Recruiting	
<i>Selective RAF inhibitors</i>							
PLX4032	Pyrrolo, pyridine	BRAF <sup>V600E</sup> , BRAF <sub>wt</sub> , CRAF		Phase-I (ST)		RR 58% <sup>a</sup> (PFS 9 months <sup>a</sup> )	Fatigue, rash, arthralgia, SCC
				Phase-II	100	Results awaited	
				Phase-III	680	Recruiting	
GSK 2118436	Thiazole	BRAF <sup>V600E,K,D</sup> , CRAF, BRAF <sub>wt</sub>		Phase-I/II (ST)	100	RR 63% <sup>a</sup>	Nausea, Fatigue Headache Fever Rash SCC
				Phase-III		Planned	
+ GSK 1120212 (MEK-I)				Phase-I	93	Recruiting	
XL281		CRAF, BRAF <sub>wt</sub> , BRAF <sup>V600E</sup>		Phase-I (ST)	180	Results awaited	Fatigue, N/V Diarrhea

Abbreviations: HFS = hand-foot syndrome; NS = not significant; OS = overall survival; PFS = progression-free survival; RR = response rate; SCC = squamous cell carcinoma; SD = stable disease; ST = solid tumour. <sup>a</sup>At the recommended phase-II dose level in melanoma subgroup

for the mutant allele (BRAF<sup>V600E</sup> IC<sub>50</sub> 31 nM) and has shown selective tumour suppression in mutant BRAF cell lines and xenograft models. In a phase-I clinical trial enriched for patients with mutant BRAF metastatic melanoma 11/16 (68%) achieved partial response (PR) and four patients had minor responses leading to a progression-free survival (PFS) of 8–9 months (Flaherty *et al*, 2009). The dose expansion cohort enrolled a selected group of BRAF<sup>V600E</sup> melanoma patients ( $n = 32$ ) at the MTD dose level of 960 mg twice daily (Chapman *et al*, 2009). In this group, a total of 26 patients (81%) had response (two CR and 24 PR). Overall PLX4032 was well tolerated with mild nausea and vomiting, skin rash and diarrhoea, but 21% of the patients on active doses developed cutaneous neoplasms, including keratoacanthoma (KA), low-grade squamous cell carcinoma (SCC) and verrucal-like lesions. These lesions occurred within 8–12 weeks from treatment start and were resectable (Flaherty *et al*, 2010a, b). On the basis of these promising results, a phase-II trial (BRIM 2, NCT00949702) is now fully accrued and a phase-III trial (BRIM3, NCT01006980) comparing PLX4032 to standard chemotherapy with the alkylating agent dacarbazine in untreated patients with BRAF<sup>V600E</sup> mutant metastatic melanoma is underway.

GSK 2118436 is an ATP competitive, reversible inhibitor of the mutant BRAF<sup>V600E/K/D</sup> (IC<sub>50</sub> 0.5, 0.6, 1.9 nM, respectively), wt BRAF (IC<sub>50</sub> 12 nM) and CRAF (IC<sub>50</sub> 5 nM) kinases with promising preclinical efficacy data in melanoma. A phase-I trial (NCT00880321) of this oral compound enrolled >100 patients with BRAF mutations (primarily melanoma patients) (Kefford *et al*, 2010). Overall GSK 2118436 showed good tolerability with grade 1/2 nausea, fatigue, fever, headaches and skin rash as the main side effects. In total, 9% of the patients developed cutaneous neoplasms, including low-grade SCC that occurred between weeks 2 and 14. Preliminary pharmacodynamic data showed dose-dependent phospho-ERK inhibition and a correlation with clinical response. Clinical responses (63% PR) were seen at the recommended phase-II dose (150 mg twice daily), with responses in lung, liver, bone and brain metastases. Importantly, these responses were not only seen in patients with BRAF<sup>V600E</sup> but also in BRAF<sup>V600K</sup> and BRAF<sup>V600G</sup> mutations, and possibly shows the wider range of activity of selective BRAF inhibitors that are not limited only to BRAF<sup>V600E</sup>-mutant tumours. These findings were also supported by a recent case report of the BRAF inhibitor PLX4032 in a BRAF<sup>V600K</sup> mutant patient (Rubinstein *et al*, 2010).

A phase-II study of GSK 2118436 is currently underway as salvage therapy in mutant BRAF metastatic melanoma and a phase-I study has commenced of GSK 2118436 in combination with the MEK-inhibitor, GSK1120212, (NCT01072175) in BRAF mutant melanoma patients, a strategy of tandem MAPK inhibition.

Other selective B-RAF inhibitors are currently in preclinical and early clinical development and include GDC-0879, ARQ736 and AZ628, among others.

### SPECIFIC SIDE EFFECTS OF SELECTIVE RAF INHIBITORS

Selective RAF inhibitors display good tolerability with infrequent severe toxicities. Among the common grade 1–2 adverse events are skin changes (50–70%), fatigue (30–50%), diarrhoea (10–30%) and nausea (10–20%). In the GSK 2118436 phase-I trial, 29% of patients reported mild grade 1/2 headaches and a possible cytokine-related fever in 37% of the patients.

Most concerning was the development of cutaneous lesions, including KA and SCC in 15–30% of patients on the GSK 2118436 and the PLX4032 studies (Flaherty *et al*, 2010a, b; Kefford *et al*, 2010). The biology and natural history of these lesions, in comparison to their spontaneous counterparts is unknown, and no systemic spread has been reported to date. One possibility to explain the underlying mechanism could be an exacerbation

of upstream RAS mutations in pre-existing skin lesions. RAS mutations occur in approximately 15% of SCCs and, under these conditions, alterations in downstream dimerisation of BRAF by selective BRAF inhibitors could lead to paradoxical activation of the MAPK pathway in squamous cells.

Notably, the phenomenon of paradoxical activation of the MAP kinase pathway has been reported with mutant-selective BRAF inhibitors under certain conditions (Hatzivassiliou *et al*, 2010; Heidorn *et al*, 2010; Poulikakos *et al*, 2010). Although inhibition of MEK and ERK phosphorylation was achieved in BRAF<sup>V600E/K</sup> mutant melanoma, significantly increased tumour growth was seen in BRAF wild-type melanomas with upstream RAS mutations. It is proposed that in BRAF wild-type tumours BRAF signalling is usually inactivated via BRAF-induced autophosphorylation, but this self-inhibitory process is interrupted by selective BRAF inhibition. Subsequently, in a RAS-dependent manner BRAF is recruited to the plasma membrane and hyper-activates CRAF, which in turn signals downstream to MEK and ERK. This phenomenon was not observed with non-selective RAF inhibitors, probably because of the promiscuous pan-RAF inhibitory effects. Similar mechanisms are thought to underlie the induction of pre-malignant and malignant changes in keratinocytes. Some of the skin changes seen in patients on selective RAF inhibitors mimic those seen in patients with hereditary gain-of-function mutations in the MAP kinase pathway (Roberts *et al*, 2006), and the frequent induction of SCCs may be due to the same process in keratinocytes carrying RAS mutations.

These findings have important clinical implications, and highlight the need for genotyping tumours before treatment with selective RAF inhibitors and screening for secondary tumours.

### MECHANISM OF RESISTANCE

Similar to other kinase inhibitors (i.e., erlotinib and imatinib), selective RAF inhibitors can lead to drastic and impressive early tumour responses in humans, which may be of short duration in some patients. Approximately 20% of patients with mutant BRAF melanoma show no response, and most patients relapse, with a median PFS of 8–9 months.

In preclinical studies, a subset of resistant BRAF<sup>V600E</sup> mutant cell lines demonstrated increased CRAF signalling via BRAF/CRAF heterodimerisation and resulted in a shift from B-RAF to CRAF dependency. In addition post-transcriptional elevation of CRAF protein levels are thought to decrease intracellular bioavailability of the selective RAF inhibitor AZ628 and subsequent development of resistance (Montagut *et al*, 2008).

Amplification of the *CCND1* gene resulting in cyclin D1 over-expression has been reported to be present in 17% of mutant BRAF<sup>V600E</sup> melanomas with independent stimulatory effects on cell-cycle progression via CDK4 (Smalley *et al*, 2008). Furthermore, point mutations of the downstream kinase isoform MEK1 (P124L, P124S and Q56P), have resulted in changes of the allosteric drug-binding pocket within helices A and C leading to sub-optimal drug binding of the selective RAF inhibitor PLX4720 in mutant melanoma cells in cell culture and *in vivo* (Emery *et al*, 2009).

### RELEVANCE OF THE AKT PATHWAY

Preclinical studies have demonstrated a close interconnection of the RAS-RAF-MEK-ERK and the PI3K-AKT-mTOR signalling pathways, with complex inter-related feedback loops. For example, NRAS is mutated in about 15% of melanoma and can activate both the signalling pathways. Although BRAF and NRAS mutations are mutually exclusive in the majority of melanomas, dual pathway signalling is also frequently seen in melanoma through functional loss (deletion, silencing and/or mutation) of the tumour suppressor gene *PTEN* (Figure 1). Both, genetic changes are seen in

approximately 20% of melanomas (Dankort *et al*, 2009). As a major regulator of the PI3K-AKT axis PTEN loss leads to activation of the AKT/mTOR pathway and, via feedback loops, to phosphorylation of MEK and ERK (Tsao *et al*, 2004).

AKT has a central role in regulating apoptosis and over-expression (via amplification or mutation) of the isoform AKT3 correlates with tumour progression. Recent preclinical studies have shown that inhibitors of PI3K and AKT3 increased apoptosis and stimulated tumour regression (Cheung *et al*, 2008). In BRAF<sup>V600E</sup> mutant cells, AKT activation was required for melanoma initiation, demonstrating the inter-dependence of these two pathways in melanoma.

Downstream of AKT, increased signalling via mTOR regulates translation of pro-proliferative proteins. In preclinical studies, the mTOR inhibitor temsirolimus reversed these effects, however, this was not reproducible in clinical melanoma trials (Margolin *et al*, 2005). These findings could be in part explained by the dual signalling complex of mTOR, including TORC1 and TORC2. Although temsirolimus (and other rapalogs) inhibits mTOR via TORC1, the uninhibited TORC2 complex continues to stimulate AKT through phosphorylation (Feldman and Shokat, 2011). Trials of dual TORC1 and TORC2 inhibitors are currently in phase-I studies to inhibit both AKT and mTOR signalling.

## RATIONALLY DESIGNED COMBINATION DRUG THERAPY

There is increasing evidence that combination therapies targeting the RAS-RAF-MEK-ERK and the PI3K-AKT-mTOR may be more effective than single-agent therapies. For example in three-dimensional cell cultures of BRAF mutant melanoma the combination of BRAF and AKT3 directed siRNAs demonstrated significantly higher reduction of tumour growth compared with weak growth inhibition by single-agent administration (Cheung *et al*, 2008). These findings were confirmed in a melanoma xenograft model (Bedogni *et al*, 2006). There is evidence of synergism when MEK and PI3K inhibitors are combined and increased apoptotic activity was also demonstrated with a combination of the mTOR inhibitor rapamycin and sorafenib or an MEK inhibitor (Lasithiotakis *et al*, 2008). In contrast to single-agent activity, these combinations resulted in complete down-regulation of the anti-apoptotic proteins Bcl-2 and Mcl-1.

Preclinical studies have also shown a synergism between BRAF and MEK inhibitors, with significantly increased apoptosis and prolonged phospho-ERK inhibition compared with BRAF inhibition alone (Paraiso *et al*, 2010). This hypothesis is currently tested in two phase-I studies. The study, NCT01072175, combines the selective RAF inhibitor, GSK 2118436, and MEK inhibitor, GSK1120212, in patients with BRAF mutant metastatic melanoma and the study, NCT01037127, explores the efficacy of the MEK inhibitor, GSK1120212, in patients with BRAF mutant tumours who previously failed a selective BRAF inhibitor. The design of these trials rests on the observation that MEK activation persists in melanoma cell lines that develop resistance to BRAF inhibition (Montagut *et al*, 2008; Smalley *et al*, 2008).

Currently, clinical trials of selective RAF inhibitors in combination with other kinase inhibitors, such as MEK, mTOR, PI3K or AKT are underway or planned. Issues related to these combinations include overlapping or synergistic toxicities and mechanisms of resistance.

## COMBINATIONS OF RAF INHIBITORS WITH CHEMOTHERAPY

Although the combination of sorafenib and dacarbazine resulted in 24% response rates compared with 12% with dacarbazine alone,

there was no effect on the primary endpoint of PFS (McDermott *et al*, 2008). Two large phase-III trials of sorafenib in combination with carboplatin/paclitaxel in chemotherapy-naïve (Flaherty *et al*, 2010a,b) and pre-treated (Hauschild *et al*, 2009) patients with BRAF undefined metastatic melanoma did not meet the primary endpoint of improved overall survival.

Whether combinations of selective RAF inhibitors in patients with BRAF mutant melanoma will result in better outcomes remains to be investigated.

Moreover, there is compelling evidence to combine chemotherapy with other inhibitors of the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR pathways.

For example, preclinical data suggest that taxane resistance may be due to increased MEK signalling resulting in anti-apoptotic changes (Haass *et al*, 2009). On the basis of these results a phase-II trial of a taxane-based chemotherapy plus the MEK inhibitor AZD 6244 is in preparation and patients will be randomised according to their BRAF/NRAS mutational status.

The commonly used chemotherapy agents, cisplatin and temozolomide, have the potential to trigger pro-survival and anti-apoptotic effects by activating the AKT signalling pathway (Sinnberg *et al*, 2009). Interestingly, AKT inhibitors reverse these effects in preclinical studies by reducing the anti-apoptotic proteins Bcl-2 and Mcl-1. On this basis, clinical trials of combinations of PI3K/AKT/mTOR inhibitors with temozolomide or cisplatin are underway.

## OTHER COMBINATIONS OF SELECTIVE RAF INHIBITORS

Heat-shock protein 90 (HSP90) is a molecular chaperone responsible for degradation, stabilisation and activation of a variety of proteins, including HER2, CRAF, BRAF, AKT and MET. In melanoma, HSP90 stabilises CRAF and ARAF and is required for the activity of BRAF<sup>V600E</sup>. In patients with mutant BRAF melanoma a combination of a selective RAF inhibitor with an HSP90 inhibitor may, therefore, inhibit multiple pathways involved in resistance. This rationale is supported by preclinical data of the HSP90 inhibitor geldanamycin (Banerji, 2009), which promotes the degradation of CRAF, a protein known to be commonly over-expressed in resistant BRAF mutant cells.

Epigenetic changes via DNA hyper-methylation or changes in DNA remodelling have an important role in cancer development. For example, epigenetic silencing is a frequent mechanism of functional loss of the tumour suppressor gene PTEN in melanoma. Agents, such as the histone deacetylating inhibitors (HDACIs), may restore the tumour suppressor function by reversing the effects of epigenetic silencing (Kuwajima *et al*, 2007). Current single-agent HDACI trials show promising results in melanoma patients (Ryan *et al*, 2005) and combinations with selective BRAF inhibitors are planned.

## CONCLUSION

Clinical trials of new selective RAF inhibitors are currently recruiting, and two recent 'proof of concept' phase-I studies have shown activity in metastatic melanoma, a disease notoriously resistant to systemic treatment.

Despite this progress there are important questions to be addressed in current and planned clinical trials. These include the impact of selective BRAF inhibitors on overall survival and quality of life, the identification of biomarkers of early resistance and relapse, the selection and scheduling of drugs to combine with BRAF inhibitors, and the mechanism and prevention of adverse events, such as SCC.

## REFERENCES

- Balch CM, Soong SJ, Gershenwald JE, Thompson JF, Reintgen DS, Cascinelli N, Urist M, McMasters KM, Ross MI, Kirkwood JM, Atkins MB, Thompson JA, Coit DG, Byrd D, Desmond R, Zhang Y, Liu PY, Lyman GH, Morabito A (2001) Prognostic Factors Analysis of 17 600 Melanoma Patients: validation of the American Joint Committee on Cancer Melanoma Staging System. *J Clin Oncol* 19(16): 3622–3634
- Banerji U (2009) Heat shock protein 90 as a drug target: some like it hot. *Clin Cancer Res* 15(1): 9–14
- Bedogni B, Welford SM, Kwan AC, Ranger-Moore J, Saboda K, Powell MB (2006) Inhibition of phosphatidylinositol-3-kinase and mitogen-activated protein kinase 1/2 prevents melanoma development and promotes melanoma regression in the transgenic TPRas mouse model. *Mol Cancer Ther* 5(5): 1136–1144
- Calipel A, Lefevre G, Pouponnot C, Mouriaux F, Eychene A, Mascarelli F (2003) Mutation in B-Raf in human choroidal melanoma cell lines mediates cell proliferation and transformation through the MEK/ERK pathway. *J Biol Chem* 278: 42409–42418
- Catalogue of Somatic Mutations in Cancer (COSMIC) website. <http://www.sanger.ac.uk/genetics/CGP/cosmic/> : Wellcome Trust Sanger Institute
- Chapman P, Puzanov I, Sosman J, Kim K, Ribas A, McArthur G, Lee R, Grippo J, Nolop K, Flaherty K (2009) Early efficacy signal demonstrated in advanced melanoma in a phase I trial of the oncogenic BRAF-selective inhibitor PLX4032. *Eur J Cancer* 7(3): 5 (abstract 6LB)
- Cheung M, Sharma A, Madhunanpantula AV, Robertson G (2008) Akt3 and mutant V600E BRAF cooperate to promote early melanoma development. *Cancer Res* 68(9): 3429–3439
- Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Bröcker EB, LeBoit PE, Pinkel D, Bastian BC (2005) Distinct sets of genetic alterations in melanoma. *N Engl J Med* 353(20): 2135–2147
- Dankort D, Curley DP, Carlidge RA, Nelson B, Karnezis AN, Damsky WE, You MJ, DePhino RA, McMahon M, Bosenberg M (2009) BRAFV600E cooperates with Pten silencing to elicit metastatic melanoma. *Nat Genet* 41(5): 544–552
- Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA (2002) Mutations of the BRAF gene in human cancer. *Nature* 417(6892): 949–954
- Dhomen N, Marais R (2009) BRAF signaling and targeted therapies in melanoma. *Hematol Oncol Clin of North Am* 23(3): 529–545
- Eisen T, Ahmad T, Flaherty KT, Gore M, Kaye S, Marais R, Gibbens I, Hackett S, James M, Schuchter LM, Nathanson KL, Xia C, Simantov R, Schwartz B, Poulin-Costello M, O'Dwyer PJ, Ratain MJ (2006) Sorafenib in advanced melanoma: a Phase-II randomised discontinuation trial analysis. *Br J Cancer* 5(2): 120–130
- Emery CM, Vijayendran KG, Zipsper MC, Sawyer AM, Niu L, Kim JJ, Hatton C, Chopra R, Oberholzer PA, Karpova MB, MacConaill LE, Zhang J, Gray NS, Sellers WR, Dummer R, Garraway LA (2009) MEK1 mutations confer resistance to MEK and B-RAF inhibition. *Proc Natl Acad Sci USA* 106(48): 20411–20416
- Feldman ME, Shokat KM (2011) New Inhibitors of the PI3K-Akt-mTOR Pathway: Insights into mTOR Signaling from a New Generation of Tor Kinase Domain Inhibitors (TORKinibs). *Curr Top Microbiol Immunol* 347: 241–262
- Flaherty K, Puzanov I, Sosman J, Kim K, Ribas A, McArthur G, Lee RJ, Grippo JF, Nolop K, Chapman P (2009) Phase I study of PLX4032: proof of concept for V600E BRAF mutation as a therapeutic target in human cancer. *J Clin Oncol* 27: 15s (abstract 9000)
- Flaherty KT, Lee SJ, Schuchter LM, Flaherty LE, Wright JJ, Leming PD, Flaherty LE, Wright JJ, Kirkwood JM (2010a) Final results of E2603: a double blind, randomized phase-III trial comparing carboplatin/paclitaxel with or without sorafenib in metastatic melanoma. *J Clin Oncol* 28: 5s (abstract 8511)
- Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB (2010b) Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 363(9): 809–819
- Haass NK, Sproesser K, Nguyen TK, Contractor R, Medina CA, Nathanson KL, Herlyn M, Smalley KS (2009) The mitogen activated protein/extracellular signal-regulated kinase kinase inhibitor AZD6244 (ARRY-142886) induces growth arrest in melanoma cells and tumor regression when combined with docetaxel. *Clin Cancer Res* 15(12): 4147–4156
- Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, Ludlam MJ, Stokoe D, Gloor SL, Vigers G, Morales T, Aliagas I, Liu B, Sideris S, Hoeflich KP, Jaiswal BS, Seshagiri S, Koeppe H, Belvin M, Friedman LS, Malek S (2010) RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature* 464(7287): 431–435
- Hauschild A, Agarwala SS, Trefzer U, Hogg D, Robert C, Hersey P, Eggermont A, Grabbe S, Gonzalez R, Gille J, Peschel C, Schadendorf D, Garbe C, O'Day S, Daud A, White JM, Xia C, Patel K, Kirkwood JM, Keilholz U (2009) Results of a phase-III, randomized, placebo-controlled study of sorafenib in combination with carboplatin and paclitaxel as second-line treatment in patients with unresectable stage III or stage IV melanoma. *J Clin Oncol* 27(17): 2823–2830
- Heidorn SJ, Milagre C, Whittaker S, Noury A, Niculescu-Duvas I, Dhomen N, Hussain J, Reis-Filho JS, Springer CJ, Pritchard C, Marais R (2010) Kinase-Dead BRAF and Oncogenic RAS cooperate to drive tumor progression through CRAF. *Cell* 140(2): 209–221
- Houben R, Becker JC, Kappel A, Terheyden P, Bröcker EB, Goetz R, Rapp UR (2004) Constitutive activation of the Ras-Raf signaling pathway in metastatic melanoma is associated with poor prognosis. *J Carcinog* 3(1): 6
- Karasarides M, Chiloeches A, Hayward R, Niculescu-Duvaz D, Scanlon I, Friedlos F, Ogilvie L, Hedley D, Martin J, Marshall CJ, Springer CJ, Marais R (2004) B-RAF is a therapeutic target in melanoma. *Oncogene* 23: 6292–6298
- Kefford R, Arkenau H, Brown MP, Millward M, Infante JR, Long GV, Ouellet D, Curtis M, Lebowitz PF, Falchook GS (2010) Phase I/II study of GSK2118436, a selective inhibitor of oncogenic mutant BRAF kinase, in patients with metastatic melanoma and other solid tumors. *J Clin Oncol* 28: 15s (abstract 8503)
- Kuwajima A, Iwashita J, Murata J, Abe T (2007) The histone deacetylase inhibitor butyrate inhibits melanoma cell invasion of matrigel. *Anticancer Res* 27(6B): 4163–4169
- Lasithiotakis KG, Sinnberg TW, Schittek B, Flaherty KT, Kulms D, Maczey E, Garbe C, Meier FE (2008) Combined inhibition of MAPK and mTOR signaling inhibits growth, induces cell death, and abrogates invasive growth of melanoma cells. *J Invest Dermatol* 128(8): 2013–2023
- Liu W, Kelly JW, Trivett M, Murray WK, Dowling JP, Wolfe R, Mason G, Magee J, Angel C, Dobrovic A, McArthur GA (2007) Distinct clinical and pathological features are associated with the BRAFT1799A(V600E) mutation in primary melanoma. *J Invest Dermatol* 127(4): 900–905
- Long GV, Menzies AM, Nagrial AM, Haydu LL, Hamilton AL, Mann G, Hughes TM, Thompson JF, Scolyer RA, Kefford R (2010) Clinicopathologic correlates of BRAF mutation status in 207 consecutive patients with metastatic melanoma. *J Clin Oncol* 28: 15s (abstract 8548)
- Margolin K, Longmate J, Baratta T, Synold T, Christensen S, Weber J, Gajewski T, Quirt I, Doroshow JH (2005) CCI-779 in metastatic melanoma: a phase-II trial of the California Cancer Consortium. *Cancer* 104: 1045–1048
- McDermott DF, Sosman JA, Gonzalez R, Hodi FS, Linette GP, Richards J, Jakub JW, Beeram M, Tarantolo S, Agarwala S, Frenette G, Puzanov I, Cranmer L, Lewis K, Kirkwood J, White JM, Xia C, Patel K, Hersh E (2008) Double-blind randomized phase II study of the combination of sorafenib and dacarbazine in patients with advanced melanoma: a report from the 11715 study group. *J Clin Oncol* 26(13): 2178–2185
- Montagut C, Sharma SV, Shioda T, McDermott U, Ulman M, Ulkus LE, Dias-Santagata D, Stubbs H, Lee DY, Singh A, Drew L, Haber DA, Settleman J (2008) Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. *Cancer Res* 68(12): 4853–4861
- Paraiso KH, Fedorenko IV, Cantini LP, Munko AC, Hall M, Sondak VK, Messina JL, Flaherty KT, Smalley KS (2010) Recovery of phospho-ERK activity allows melanoma cells to escape from BRAF inhibitor therapy. *Br J Cancer* 102(12): 1724–1730
- Platz A, Eghazi S, Ringborg U, Hansson J (2008) Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. *Mol Oncol* 1: 395–405

- Populo H, Soares P, Rocha AS, Silva P, Lopes JM (2010) Evaluation of the mTOR pathway in ocular (uvea and conjunctiva) melanoma. *Melanoma Res* 20(2): 107–117
- Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N (2010) RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* 464(7287): 427–430
- Roberts A, Allanson J, Jadico SK, Kavamura MI, Noonan J, Opitz JM, Young T, Neri G (2006) The cardiofaciocutaneous syndrome. *J Med Genet* 43: 833–842
- Rubinstein JC, Sznol M, Pavlick AC, Ariyan S, Cheng E, Bacchiocchi A, Kluger HM, Narayan D, Halaban R (2010) *J Transl Med* 8: 67
- Ryan QC, Headlee D, Acharya M, Sparreboom A, Trepel JB, Ye J, Figg WD, Hwang K, Chung EJ, Murgo A, Melillo G, Elsayed Y, Monga M, Kalnitskiy M, Zwiebel J, Sauville EA (2005) Phase-I and pharmacokinetic study of MS-275, a histone deacetylase inhibitor, in patients with advanced and refractory solid tumors or lymphoma. *J Clin Oncol* 23(17): 3912–3922
- Sinnberg T, Lasithiotakis K, Niessner H, Schitteck B, Flaherty KT, Kulms D, Maczey E, Campos M, Gogel J, Garbe C, Meier F (2009) Inhibition of PI3K-AKT-mTOR signaling sensitizes melanoma cells to cisplatin and temozolomide. *J Invest Dermatol* 129(6): 1500–1503
- Shinozaki M, Fujimoto A, Morton DL, Hoon DSB (2004) Incidence of BRAF Oncogene Mutation and Clinical Relevance for Primary Cutaneous Melanomas. *Clin Cancer Res* 10(5): 1753–1755
- Smalley KS, Lioni M, Dalla Palma M, Xiao M, Desai B, Egyhazi S, Hansson J, Wu H, King AJ, Van Belle P, Elder DE, Flaherty KT, Herlyn M, Nathanson KL (2008) Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. *Mol Cancer Ther* 7(9): 2876–2883
- Schwartz GK, Robertson S, Shen A, Wang E, Pace L, Dials HD, Mendelson D, Shannon P, Gordon M (2009) A phase-I study of XL281, a selective oral RAF kinase inhibitor, in patients with advanced solid tumors. *J Clin Oncol* 27: 15s (abstract 3513)
- Thomas NE (2006) BRAF somatic mutations in malignant melanoma and melanocytic naevi. *Melanoma Res* 16(2): 97–103
- Thomas NE, Edmiston SN, Alexander A, Millikan RC, Groben PA, Hao H, Tolbert D, Berwick M, Busam K, Begg CB, Mattingly D, Ollila DW, Tse CK, Hummer A, Lee-Taylor J, Conway K (2007) Number of Nevi and Early-Life ambient UV exposure are associated with BRAF-mutant melanoma. *Cancer Epidemiol Biomarkers Prev* 16(5): 991–997
- Thompson JF, Scolyer RA, Kefford RF (2005) Cutaneous melanoma. *Lancet* 365(9460): 687–701
- Tsao H, Goel V, Wu H, Yang G, Haluska FG (2004) Genetic interaction between NRAS, and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. *J Invest Dermatol* 122: 337–341
- Ugurel S, Thirumaran RK, Bloethner S, Gast A, Sucker A, Mueller-Berghaus J, Rittgen W, Hemminki K, Becker JC, Kumar R, Schadendorf D (2007) B-RAF and N-RAS mutations are preserved during short time *in vitro* propagation and differentially impact prognosis. *PLoS ONE* 2(2): e236
- Wan PTC, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R, Cancer Genome Project (2004) Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 116(6): 855–867