Published in final edited form as:

Pigment Cell Melanoma Res. 2015 September ; 28(5): 607–610. doi:10.1111/pcmr.12388.

Clinical activity of the MEK inhibitor trametinib in metastatic melanoma containing *BRAF* kinase fusion

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Keywords

BRAF; fusion; translocation; trametinib; MEK; wild-type; melanoma

Dear Editor

The classification of melanoma based upon the molecular profile of the patient's tumor has changed the way metastatic melanoma is treated. For *BRAF*^{V600}-mutant melanoma, *BRAF* and MEK inhibitors in combination have become standard care. MEK inhibitors have activity in patients with *NRAS*-mutant melanoma, imatinib has activity in *KIT*-mutant melanoma, and MEK and PKC inhibitors have activity in *GNAQ* and *GNAI1*-mutant uveal melanomas. At present, approximately 30% of melanomas do not have a driver mutation targeted by an approved drug (Xia et al., 2014), and for these patients, the only available current treatments are immunotherapy and chemotherapy.

BRAF kinase fusions, occurring as a result of rearrangements that fuse the *BRAF* kinase domain to 5' partner genes, have been shown to activate the MAPK pathway in pilocytic astrocytoma, pancreatic acinar cell carcinoma, and gastric, thyroid and prostate cancers. Recently, *BRAF* fusions have been reported in 4-8% of melanomas without oncogenic point mutations in *BRAF*, *NRAS* or *KIT* (Botton et al., 2013; Hutchinson et al., 2013). *BRAF* fusions have a full and intact kinase domain comparable to the one of wild-type *BRAF*, and selective mutant-*BRAF* inhibitors (e.g. vemurafenib, dabrafenib) appear ineffective and in some cases paradoxically activate the MAPK pathway. However, sensitivity to inhibitors of wild-type *RAF* (e.g. sorafenib) and MEK inhibitors has been demonstrated *in vitro* (Botton et al., 2013; Hutchinson et al., 2013; Sievert et al., 2013). Retrospective identification of an *AGK-BRAF* fusion in a melanoma of a patient with clinical response to sorafenib has been

reported (Botton et al., 2013; Passeron et al., 2011), and a *KIAA1549-BRAF* fusion was identified in an undifferentiated sarcomatoid malignant spindle cell tumor of indeterminate histogenesis from a patient with response to a combination of sorafenib, temsirolimus and bevacizumab (Subbiah et al., 2014). Recently, a phase 1 trial has shown efficacy of AZD6244 (selumetinib) in paediatric gliomas harbouring *KIAA1549-BRAF* fusions (Banerjee et al., 2014). To date, however, there is no prospective evidence of targeted therapy for *BRAF* kinase fusions in any patient with melanoma.

Case 1

A 47-year old Caucasian female had a 1.7mm ulcerated superficial spreading primary melanoma over the right scapula resected in June 2009. Axillary sentinel lymph node biopsy (SNB) was negative. Non-sentinel node axillary recurrence was resected in October 2012, and further subcutaneous axillary recurrence was resected in February 2013. In May 2013 she developed arm paraesthesia and gait disturbance. Two small brain metastases were detected on MRI. Stereotactic radio surgery (SRS) was given, and adjuvant whole brain radiotherapy was performed as part of a clinical trial. No extracranial disease was evident on FDG-PET imaging. Targeted molecular testing was performed using the OncoCarta Panel v1.0 Kit on the Sequenom MassArray platform (<http://agenabio.com/oncocarta-panel>) and no hotspot exon 15 *BRAF*, *NRAS* or *KIT* mutations were identified.

By September 2013, the patient's disease had progressed intra-cranially, as well as in five subcutaneous sites and a mesenteric lymph node. Further SRS was delivered to several brain metastases, and ipilimumab was commenced. After four doses of ipilimumab, restaging showed progressive disease in both intra- and extra-cranial sites. A large brain metastasis was resected and an induction regimen of fotemustine was commenced, but by February 2014 the patient had developed progressive microcytic anemia and a CT scan demonstrated three new bowel metastases, several new lymph node metastases and several new subcutaneous metastases. Three brain metastases had progressed, and these were resected and sent for further molecular testing (outlined below). Nab paclitaxel was commenced, and after two cycles the patient had a mixed response with some growth of existing metastases in intra- and extra-cranial sites, on going anemia, and persistent lethargy (ECOG performance status 2).

At this time, in April 2014, a *PPFIBP2-BRAF* fusion was discovered in DNA extracted from a resected brain metastasis. This fusion joined intron 3 of *PPFIBP2* to intron 10 of *BRAF*, generating a predicted in-frame fusion transcript similar to other *BRAF* fusions reported previously (Figure 1). Trametinib 2mg once-daily was commenced as a single agent, and after three weeks the anemia had resolved and performance status improved (ECOG 1). Progress imaging at week 6 demonstrated a 90% reduction in extracranial metastases (sum of diameters of all metastases, from 181mm to 19mm), and 19% reduction in intracranial metastases (from 64mm to 52mm) (Figure 2). No new metastases were seen, and no existing metastases progressed. At this time, pembrolizumab became available via a compassionate access scheme. As the patient was thought to be at the likely nadir of response, trametinib was ceased and pembrolizumab 2mg/kg 3-weekly was commenced so as to offer the best chance of efficacy (Joseph et al., 2014).

After 3 cycles of pembrolizumab, anemia was stable and imaging showed largely stable disease, but growth in a mesenteric lymph node. By cycle 5, the patient started to decline, with increasing anemia, a decline in performance status (to ECOG 2), and a PET/CT demonstrated numerous progressing small bowel metastases. Pembrolizumab was ceased, and the patient was recommenced on trametinib. There was no clinical or radiological response with re-induction treatment, and over the next three months the patient continued to decline with increasing GI bleeding and anemia, and died in December 2014.

Case 2

A 65 year-old Asian male had a 1.65mm non-ulcerated acral lentiginous melanoma resected from the left foot in May 2011. Lymphatic mapping was complex and therefore SNB was not performed. Disease recurred in a popliteal lymph node in July 2012, and following popliteal (1/2 nodes) and ilio-inguinal (0/44) dissection, adjuvant radiotherapy was administered to the popliteal fossa.

In April 2013 the patient developed a subcutaneous metastasis in the calf, just below the radiotherapy field. He was otherwise asymptomatic. FDG-PET imaging revealed an additional small para-aortic lymph node metastasis and probable nodal recurrence deep in the popliteal fossa. Targeted molecular testing was performed similar to above, and again no hotspot *BRAF*, *NRAS* or *KIT* mutations were identified. Sanger sequencing of exon 11, 13 or 17 of *KIT* did not reveal any mutation. The patient was not eligible for clinical trials given a history of recent prostate cancer. The in-transit metastasis was excised, and the patient remained under observation.

By August 2013, the para-aortic lymph node and popliteal fossa node metastases had progressed but the patient remained well. Ipilimumab was commenced, and after four cycles imaging demonstrated progressive disease with progressive para aortic lymphadenopathy and new lung and adrenal metastases. By this time the patient had become fatigued (ECOG 1). The subcutaneous calf metastasis that was resected in April 2013 was sent for further molecular testing (outlined below), and fotemustine was commenced in January 2014.

By May 2014 disease had progressed in lung, nodal and soft tissue metastases, fatigue had increased (ECOG 2), and a *KIAA1549-BRAF* fusion was detected. The fusion joined intron 15 of *KIAA1549* and intron 8 of *BRAF*. This fusion has been previously reported in pilocytic astrocytomas and has constitutive kinase activity (Jones et al., 2008). Fotemustine was ceased and trametinib 2mg daily was commenced. Despite the patient noticing a rapid improvement in fatigue in the first week of treatment (to ECOG 1), imaging performed after two weeks on trametinib (compared to 2 weeks prior to trametinib, and as a baseline in preparation to commence pembrolizumab), demonstrated slight disease progression (sum of diameters grew from 432mm to 459mm, +6%). Of 15 measurable metastases, 9 were stable, but 6 grew (e.g. a soft tissue mass grew from 36mm to 40mm). All lung metastases were stable, no new metastases were evident, but no metastases regressed.

The patient's therapy was switched to pembrolizumab 2mg/kg 3-weekly in June 2014. After six weeks of treatment, he had declined symptomatically (to ECOG 2) and there was major radiological disease progression with several new metastases and growth of all

existing lesions (sum of diameters 459mm to 757mm, +65%), so trametinib was recommenced in July 2014. Again, the patient felt improved in the first 3 weeks of treatment but later deteriorated and died in November 2014.

Discussion

Here we report the first evidence of clinical activity of trametinib in two heavily pre-treated metastatic melanoma patients with *BRAF* fusions. Both patients had symptomatic improvement. Radiological response in both extracranial and intracranial sites was demonstrated in one patient, while the other patient appeared to have had slower disease progression on trametinib. The duration of benefit of trametinib is not known as both patients switched to pembrolizumab before disease progression.

Nevertheless, any evidence of clinical activity of trametinib in this setting is encouraging, not only for use as monotherapy, but potentially also in combination with immunotherapy (Frederick et al., 2013; Wilmott et al., 2012), or other downstream (e.g. CDK4/6 or MDM2) or alternative pathway targets (e.g. PI3K, mTOR). Pre-clinical models may inform of the best strategy to target this rare but important subtype of melanoma (Botton et al., 2013) under the framework of a formal clinical trial. Other aberrations in melanoma that activate the MAPK pathway, such as *NF1* loss, also display sensitivity to MEK inhibitors (Nissan et al., 2014), and similarly should be considered for MEK inhibitor therapy, alone and in combination with other pathway inhibitors.

Molecular testing

Tumor DNA was extracted from micro dissected formalin-fixed, paraffin embedded (FFPE) sections. Multiplex library preparation was performed using the Next flex DNA Sequencing Kit (Bioo Scientific, Austin, TX, p/n No. 5140-53) according to the manufacturer's specifications with 200ng of input tumor DNA. Hybrid capture, sequencing, and analysis were performed as previously described (Botton et al., 2013).

Acknowledgments

AMM is supported by a Cancer Institute NSW fellowship. IY, TB and BCB are supported by National Institutes of Health Grant P01 CA025874. IY is supported by the Dermatology Foundation and the Melanoma Research Alliance. GVL is supported by The Sydney Medical School Foundation, The University of Sydney. RAS is supported by an Australian National Health and Medical Research Council fellowship. Additional support from Australian National Health and Medical Research Council, Cancer Institute New South Wales and Melanoma Institute Australia is also gratefully acknowledged.

Disclosures: AMM – Honoraria and travel support from GSK and BMS

IY, TB, BCB, RAS – None

GVL – Consultant advisor to Amgen, BMS, GSK, Merck-MSD, Novartis, Provectus

Roche. Honoraria from GSK, BMS, Merck-MSD

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Figure 1.

PPFIBP2-BRAF fusion identified by targeted DNA sequencing from patient 1. Left panel: Supporting reads over the breakpoint in *BRAF* intron 10. The rainbow colored portion upstream corresponds to the portion of the read from *PPFIBP2* intron 3. Reads from pairs that span the breakpoint are highlighted brown when mapped to *BRAF* and blue when mapped to *PPFIBP2*.

Figure 2.

Imaging of patient 1 at time of commencement of trametinib (BL) and 6 weeks later showing a major response in a) intra-abdominal and b) subcutaneous sites, with c) brain metastases also demonstrating regression.