Resistance surveillance in a BRAF mutant melanoma patient on long-term BRAF-inhibitor treatment
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Treatment responses of BRAF mutant melanoma to BRAF inhibitors are often limited by the development of resistance. This case report describes the use of multiplatform molecular profiling in sequential surgical samples of a treatment-resistant tumour site subjected to ongoing treatment with dabrafenib in a patient with metastatic cutaneous BRAF mutant melanoma. Next-generation sequencing showed the presence of the V600E, fibroblast growth factor receptor 2 (FGFR2), phosphatase and tensin homologue (PTEN) and p53 gene mutations. With a continuous presence of the BRAF V600E, FGFR2 and PTEN mutations and appearances of new mutations in the PTEN gene at R137H and T321fs and p53 R273C genes during ongoing treatment, this case report indicates intratumoural clonal evolution as a resistance mechanism. Two new mutations, the G542E exon 12 mutation variant of the FGFR2 gene and the R273C mutation variant of the p53 gene, are reported for the first time in BRAF mutant melanoma. Melanoma Res 24:408–412 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins

Keywords: metastatic melanoma, molecular profiling, treatment resistance

Background
Treatment of metastatic v-Raf murine sarcoma viral oncogene homologue B1 (BRAF) mutant cutaneous melanoma has been revolutionized with the use of drugs targeting the mitogen-activated protein kinase (MAPK) pathway. Although survival is improved, resistance typically occurs within 5 to 7 months for patients treated with single-agent BRAF or mitogen-activated protein kinase kinase (MEK) inhibitors, and 9.4 months with a BRAF/MEK combination [1–3]. In this context, multiple mechanisms of adaptive resistance have been reported. Molecular profiling technologies have widely become available, allowing for the use of genome sequencing, immunohistochemical (IHC) analysis and in-situ hybridization techniques to help identify individual biomarkers within tumours for clinical application.

We report a case of a patient with metastatic cutaneous BRAF mutant melanoma treated with dabrafenib who underwent repeat debulking surgery for a resistant lesion while disease in other metastatic sites was controlled. Molecular profiling was performed on metachronous resected tumour samples, providing an insight into the molecular changes between samples.

Case presentation
In 1995, a 22-year-old man underwent completely resection of a localized cutaneous melanoma in the neck. He was diagnosed in September 2008 with a lytic lesion in the left eighth rib, a soft tissue mass invading the transverse process and pedicle of the left fourth lumbar vertebra (L4) and a nodule inferior to the right lung hilum. These lesions were intensely FDG-PET avid. The lesions in the rib and L4 were surgically removed in November 2008 and confirmed to be metastatic melanoma. Postoperative radiotherapy was administered to L4. A month later, the lung nodule was resected and treated with radiotherapy. In March 2009, new disease was detected in the right upper lobe of the lung and manubrium. In addition, residual PET avid disease was seen in the right hilum and the L4 region (SUVmax 7.0).

The resected right hilar lymph node was tested for the BRAF mutation by Sanger sequencing. This showed the presence of a V600E mutation in exon 15 of the BRAF gene. On the basis of this, he enrolled in a phase I clinical trial in July 2009 and received dabrafenib 100 mg t.d.s. The sites of disease recorded at enrolment were the lesions at L4, manubrium and lung. The patient tolerated dabrafenib without significant side effects. A PET scan in September 2009 showed resolution of the manubrial lesion and less PET avidity in the L4 lesion (SUVmax 5.5). A further PET scan in November 2009 showed further decrease in avidity of the L4 lesion (SUVmax 5.2) and no evidence of new metastases.

In late 2010, the patient complained of paraesthesia along the left L4 dermatome. Investigations indicated a shape change in the L4 lesion and no other sites of active disease. After discussion between the clinical trial and...
surgical teams, a second debulking procedure was performed in January 2011 after 18 months on dabrafenib. Dabrafenib was briefly suspended for the operation and recommenced postoperatively. In March 2012, scans indicated disease progression at the L4 site and a third surgical procedure by an anterior approach was performed. Optimal debulking was not achieved and in July 2012, further debulking was done by the posterior approach. He received postoperative radiotherapy. Subsequently, the patient had symptoms of L5 nerve root compression and another debulking procedure of the persistent residual L4 soft tissue mass was performed in April 2013. Dabrafenib was continued throughout on the basis that it continued to suppress other metastatic disease. He subsequently received the dabrafenib–trametinib combination, but this failed to stop disease progressing at L4 and he underwent a final debulking procedure in August 2013. Postoperatively, he received ipilimumab (Table 1).

**Methods**

We performed molecular profiling (Caris Life Sciences Phoenix, Arizona, USA) on four surgical specimens from the recurrently progressing L4 metastatic site – November 2008, January 2011, March 2012 and April 2013 (Table 2). Platforms used included next-generation sequencing, protein expression IHC analysis, and fluorescence and chromogenic in-situ hybridization techniques. Written informed consent was obtained from the patient for publication of this case report.

**Results**

**Immunohistochemistry**

Topoisomerase 2A staining was positive throughout all four samples. The secreted protein acidic and rich in cysteine (SPARC) protein was positive in the first and third sample, topoisomerase 1 was only positive in the second and third samples, whereas O-6-methylguanine-DNA methyltransferase (MGMT) staining was positive in the first and fourth samples. P-glycoprotein expression was stained positive in the first sample, thymidylate synthase staining was positive in the first three samples and cMET (tyrosine kinase receptor for hepatocyte growth factor and scatter factor) showed positive staining only in the last sample. Other IHC biomarkers stained negatively throughout the samples. Changes in staining intensity were observed for the phosphatase and tensin homologue (PTEN).

**In-situ hybridization**

Human epidermal growth factor receptor 2 (HER2/Neu) was undetectable by chromogenic in-situ hybridization throughout all four samples (Table 2).

**Next-generation sequencing**

Detectable mutations have been reported with the alteration frequency – being the ratio between mutation and wild-type genes. The patient’s tumour showed the BRAF V600E mutation throughout as well as mutations of the fibroblast growth factor receptor 2 (FGFR2) (G542E exon 12) and PTEN (K267fs exon 12) genes. During the treatment, a new PTEN mutations (R137H in exon 6, third sample, and T321fs in exon 8, fourth sample) occurred. New tumour suppressor p53 (TP53) gene mutations were detected in the third and fourth sample (Table 2).

**Discussion**

In patients with BRAF V600E mutant melanoma, multiple diverse mechanisms of primary and acquired resistance have been described as a result of treatment with BRAF inhibitors. These aberrations can occur at multiple levels of the MAPK pathway, as well as bypass signalling pathways (Fig. 1) [4–7].

Alterations upstream of BRAF can maintain the MAPK pathway signalling through the neuroblastoma RAS viral oncogene homologue gene (NRAS) proto-oncogene c-RAF (CRAF) signalling axis [8]. Downstream of BRAF, mutations in MEK1 can cause reactivation of the MAPK pathway in the presence of BRAF inhibition [9,10]. At the level of BRAF, multiple abnormalities have been identified, namely, BRAF amplification, gain of BRAF copy numbers [11], truncation of BRAF (p61 BRAF V600E) [12] and overactivity of CRAF and COT/Tpl2 [13]. Other resistance mechanisms include signalling through the tyrosine kinase receptors of the insulin-like growth factor 1 and platelet-derived growth factor receptor-β.

The persistence of the V600E mutation in all samples of our patient is consistent with the reported molecular

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**Table 1** Summary of locoregional and systemic treatments received by the patient

| Disease activity | Locoregional treatment | Systemic treatment | Molecular profiling undertaken | September 2008 | November 2008 | July 2009 | January 2011 | March 2012 | July 2012 | April 2013 | August 2013 |
|-----------------|------------------------|-------------------|-------------------------------|----------------|---------------|----------|-------------|------------|-----------|-----------|-----------|
| Rib, lung, L4   | Surgery to L4, rib and lung | Dabrafenib       | Yes                           | No            | No            | Dabrafenib | No          | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Rib, lung, L4   | Radiation to L4        | Dabrafenib       | Yes                           | L4, lung, manubrium | L4, lung, manubrium | Surgery to L4 | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Surgery to L4   | L4                     | Dabrafenib       | Yes                           | L4           | Surgery to L4 | Dabrafenib | L4         | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Surgery to L4   | L4                     | Dabrafenib       | Yes                           | Surgery to L4 | L4           | Dabrafenib | L4         | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Surgery to L4   | L4                     | Dabrafenib       | Yes                           | Radiation to L4 | Surgery to L4 | Dabrafenib | L4         | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Surgery to L4   | L4                     | Dabrafenib       | Yes                           | Surgery to L4 | L4           | Dabrafenib | L4         | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Surgery to L4   | L4                     | Dabrafenib       | Yes                           | Surgery to L4 | L4           | Dabrafenib | L4         | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Surgery to L4   | L4                     | Dabrafenib       | Yes                           | Surgery to L4 | L4           | Dabrafenib | L4         | L4         | Surgery to L4 | L4        | Surgery to L4 |
| Surgery to L4   | L4                     | Dabrafenib–trametinib | Yes                         | Iplimumab | Iplimumab | Iplimumab | Iplimumab | Iplimumab | Iplimumab | Iplimumab | Iplimumab |

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analyses from the pivotal vemurafenib studies, which showed that tumours that developed acquired resistance maintained their V600 mutations [14].

The phosphoinositide kinase/protein kinase B/mammalian target of the rapamycin (PI3K/AKT/mTOR) axis has been described as one of the most prominent bypass signalling pathways accounting for ~20% of resistance [15]. In this context, loss of function of the tumour suppressor gene PTEN has been described and associated with resistance and shorter progression-free survival [16]. In our patient, we detected the K267fs PTEN mutation in exon 12 throughout, but also found new mutations in exon 6 (R137H) and exon 8 (R273C), respectively. On a protein level, IHC staining was negative in all four samples, indicating loss of function of PTEN.

Recent evidence suggests that enhanced activation of FGFR is linked to Ras and MAPK activation, therefore conferring resistance to BRAF inhibitors [17]. In this context, overexpression of FGFR2 and FGFR3 through autocrine feedback loops has been identified as one of the key signalling mechanisms. Interestingly, however, there are now emerging data that FGFR2 mutations may result in receptor loss of function through several distinct mechanisms, including loss of ligand binding affinity, impaired receptor dimerization, destabilization of the extracellular domains and reduced kinase activity [18]. Whether our newly described FGFR2 exon 12 mutation falls into this category needs to be investigated further.

The inactivation of the p53 tumour suppressor pathway, which often occurs through mutations in TP53, is common in human cancers, but rare in melanoma (3–5%) [18–20]. Inactivation of p53 signalling can be a result of various mechanisms such as mutation or deletion of TP53, inactivation of ATM, amplification of MDM2, expression of viral oncoproteins or alteration in cofactors or downstream effectors which, in turn, can lead to enhanced growth and genomic instability. The appearance of the exon 8 R273C mutation in the third and fourth sample may have contributed towards further genomic instability and subsequent progression.

Table 2 Biomarker results – immunohistochemical, chromogenic in-situ hybridization and next-generation sequencing

| Biomarker | Platform | 15 November 2008 | 18 January 2011 | 13 March 2012 | 12 April 2013 |
|-----------|----------|-----------------|----------------|--------------|--------------|
| MGMT      | IHC      | Positive        | Negative       | Negative     | Positive     |
|           |          | 1 + 70%         | 1 + 10%        | 1 + 10%      | 1 + 30%      |
| SPARC     | IHC      | Positive        | Negative       | Negative     | Positive     |
|           |          | 2 + 30%         | 2 + 10%        | 2 + 30%      | 2 + 90%      |
| SPARC     | IHC      | Negative        | Negative       | Negative     | Negative     |
|           |          | 1 + 90%         | 2 + 70%        | 1 + 90%      | 1 + 90%      |
| TLE3      | IHC      | Negative        | Negative       | Negative     | Negative     |
|           |          | 1 + 30%         | 1 + 30%        | 1 + 10%      | 1 + 20%      |
| TUBB3     | IHC      | Negative        | Negative       | Negative     | Negative     |
|           |          | 2 + 15%         | 2 + 15%        | 2 + 20%      | 0 + 100%     |
| PGP       | IHC      | Positive        | Negative       | Negative     | Negative     |
|           |          | 1 + 80%         | 2 + 10%        | 2 + 30%      | 1 + 90%      |
| RRM1      | IHC      | Negative        | Negative       | Negative     | Negative     |
|           |          | 0 + 100%        | 0 + 100%       | 0 + 100%     | 0 + 100%     |
| TOPO1     | IHC      | Negative        | Positive       | Positive     | Negative     |
|           |          | 0 + 100%        | 2 + 30%        | 2 + 30%      | 0 + 100%     |
| TS        | IHC      | Positive        | Positive       | Positive     | Negative     |
|           |          | 1 + 10%         | 1 + 20%        | 1 + 20%      | 1 + 2%       |
| PTEN      | IHC      | Negative        | Negative       | Negative     | Negative     |
|           |          | 1 + 5%          | 2 + 30%        | 0 + 100%     | 0 + 100%     |
| TOP2A     | IHC      | Positive        | Positive       | Positive     | Negative     |
|           |          | 2 + 10%         | 2 + 20%        | 2 + 20%      | 2 + 20%      |
| HER2      | IHC      | Negative        | Negative       | Negative     | Negative     |
|           |          | 0 + 100%        | 0 + 100%       | 0 + 100%     | 0 + 100%     |
| HER2      | CISH     | Negative        | Negative       | Negative     | Negative     |
|           |          | 42%             | 52%            | 47%          |
| BRAF V600E exon 15 | NGS | Not performed* | 19% | 30% | 22% |
| FGFR2 G542E exon 12 | NGS | Not performed | 14% | 37% | 32% |
| FGFR2 K267fs exon 12 | NGS | Not performed | 14% | 37% | 32% |
| PTEN R137H exon 6 | NGS | Not performed | 21% |
| PTEN T321fs exon 8 | NGS | Not detected | 22% |
| TP53 R273C exon 8 | NGS | Not detected | 22% |

*BRaf testing was performed in March, 2009 separate from this molecular profiling; a V600E mutation was found.
A recent report by Romano et al. [21] supports our findings showing the coexistence of different molecular mechanisms of resistance to BRAF inhibition. In this case study, molecular profiling was performed on pretreatment tumour and two metastatic sites: one that was present at baseline and responded to vemurafenib and a second site that occurred after reintroduction of vemurafenib. The genetic alterations detectable in the two metastatic sites were tumour specific, mutually exclusive and not detectable in the pretreatment tumour [21].

Our patient is currently being treated with the CTLA-4 monoclonal antibody ipilimumab, and in case of disease progression, we are planning to rebiopsy and repeat molecular profiling to track potential new changes, which may guide us for further management. With emerging new drug therapies and combination strategies for patients with BRAF mutant melanoma, this report highlights the usefulness for serial/longitudinal biopsies to monitor disease response/progression and select patients for appropriate clinical trials.

Classification of resistance mechanisms to BRAF inhibitors. The figure shows a classification system for both de-novo and acquired resistance mechanisms to the selective BRAF inhibitors relative to the BRAF mutation. Such resistance mechanisms may lie upstream, downstream or at the same level along the mitogen-activated protein kinase pathway, or act through a bypass signalling pathway. AKT, protein kinase B; BRAF, v-raf murine sarcoma viral oncogene homologue B1; COT, P MAP3K8/mitogen-activated protein kinase kinase kinase 8; ERK, extracellular signal-regulated kinase; GCN, gene copy number; IGF-1R, insulin-like growth factor 1 receptor; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NRAS, neuroblastoma RAS viral oncogene homologue; PDGFR-β, platelet-derived growth factor receptor β; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homologue; RAS, rat sarcoma; RTK, receptor tyrosine kinase. Modified from Lemech et al. [4].
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Conflicts of interest
There are no conflicts of interest.

References
1 Floherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 2012; 367:1694–1703.
2 Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 2012; 366:707–714.
3 Hauschild A, Grob J-J, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2012; 380:358–365.
4 Lemeh C, Infante J, Arkenau HT. Combination molecularly targeted drug therapy in metastatic melanoma: progress to date. *Drugs* 2013; 73:767–777.
5 Salama AKS, Floherty KT. BRAF in melanoma: current strategies and future directions. *Clin Cancer Res* 2013; 19:4326–4334.
6 Fedorenko IV, KHT Paraiso, KSM Smalley. Acquired and intrinsic BRAF inhibitor resistance in BRAF V600E mutant melanoma. *Biochem Pharmacol* 2011; 82:201–209.
7 Pritchard AL, Hayward NK. Molecular pathways: mitogen-activated protein kinase pathway mutations and drug resistance. *Clin Cancer Res* 2013; 19:2301–2309.
8 Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, et al. Melanomas acquire resistance to B-RAF (V600E) inhibition by RTK or N-RAS upregulation. *Nature* 2010; 468:973–977.
9 Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ, et al. MEK1 mutations confer resistance to MEK and B-RAF inhibition. *Proc Natl Acad Sci USA* 2009; 106:20411–20416.
10 Trnzer K, Pavlick AC, Schuchter L, Gonzalez R, McArthur GA, Hutson TE, et al. Pharmacodynamic effects and mechanisms of resistance to vemurafenib in patients with metastatic melanoma. *J Clin Oncol* 2013; 31:1767–1774.
11 Shi H, Monicaceau G, Kong X, Lee MK, Lee H, Koya RC, et al. Melanoma whole-exome sequencing identifies V600EB-RAF amplification-mediated acquired B-RAF inhibitor resistance. *Nat Commun* 2012; 3:724.
12 Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF (V600E). *Nature* 2011; 480:387–390.
13 Montagut C, Sharma SV, Shioda T, McDermott U, Ulman M, Ullius LE, et al. Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. *Cancer Res* 2008; 68:4853–4861.
14 McArthur G, Ribas A, Chapman P, Floherty K, Kim K, Puzanov I, et al. Molecular analyses from a phase I trial of vemurafenib to study mechanism of action (MOA) and resistance in repeated biopsies from BRAF mutation-positive metastatic melanoma patients (pts). *J Clin Oncol* 2011; 29:526, (Suppl; abstr 8502).
15 Shi H, Hugo W, Kong X, Hong A, Koya RC, Monicaceau G, et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. *Cancer discov* 2014; 4:80–93.
16 Nathanson KL, Martin AM, Wubbenhorst B, Greshock J, Letrero R, D’Andrea K, et al. Tumor genetic analyses of patients with metastatic melanoma treated with the BRAF inhibitor dabrafenib (GSK2118436). *Clin Cancer Res* 2013; 19:4868–4878.
17 Yadav V, Zhang X, Liu J, Estrem S, Li S, Gong XQ, et al. Reactivation of mitogen-activated protein kinase (MAPK) pathway by FGF receptor 3 (FGFR3)/Ras mediates resistance to vemurafenib in human B-RAF V600E mutant melanoma. *J Biol Chem* 2012; 287:28087–28098.
18 Gartside MG, Chen H, Ibrahimi OA, Byron SA, Curtis AV, Wellens CL, et al. Loss-of-function fibroblast growth factor receptor-2 mutations in melanoma. *Mol Cancer Res* 2009; 7:41–54.
19 Jochemsen AG. Reactivation of p53 as therapeutic intervention for malignant melanoma. *Curr Opin Oncol* 2014; 26:114–119.
20 Carson C, Omolo B, Chu H, Zhou Y, Sambade MJ, Peters EC, et al. A prognostic signature of defective p53-dependent G1 checkpoint function in melanoma cell lines. *Pigment Cell Melanoma Res* 2012; 25:514–526.
21 Romano E, Fraderand S, Paulluson A, Weber J, Harshman K, Muehlthaler K, et al. Identification of multiple mechanisms of resistance to vemurafenib in a patient with BRAF(V600E)-mutated cutaneous melanoma successfully rechallenged after progression. *Clin Cancer Res* 2013; 19:5749–5757.

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