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.

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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 (SUV_{max} 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 (SUV_{max} 5.5). A further PET scan in November 2009 showed further decrease in avidity of the L4 lesion (SUV_{max} 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 V600* 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

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

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

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 1 + 70%	Negative 1 + 2%	Negative 1 + 10%	Positive 1 + 60%
SPARC monoclonal	IHC	Positive 2 + 30%	Negative 2 + 10%	Positive 2 + 30%	Negative 1 + 90%
SPARC polyclonal	IHC	Negative 1 + 90%	Negative 2 + 70%	Negative 1 + 70%	Negative 1 + 90%
TLE3	IHC	Negative 1 + 30%	Negative 1 + 30%	Negative 1 + 10%	Negative 1 + 20%
TUBB3	IHC	Negative 2 + 15%	Negative 2 + 15%	Negative 2 + 20%	Negative 0 + 100%
PGP	IHC	Positive 1 + 80%	Negative 0 + 100%	Negative 0 + 100%	Negative 0 + 100%
RRM1	IHC	Negative 2 + 10%	Negative 2 + 30%	Negative 2 + 30%	Negative 1 + 90%
TOPO1	IHC	Negative 0 + 100%	Positive 2 + 30%	Positive 2 + 30%	Negative 0 + 100%
TS	IHC	Positive 1 + 10%	Positive 1 + 20%	Positive 1 + 20%	Negative 1 + 2%
PTEN	IHC	Negative 1 + 5%	Negative 2 + 30%	Negative 0 + 100%	Negative 0 + 100%
TOP2A	IHC	Positive 2 + 10%	Positive 2 + 20%	Positive 2 + 20%	Positive 2 + 20%
HER2	IHC	Negative 0 + 100%	Negative 0 + 100%	Negative 0 + 100%	Negative 0 + 100%
HER2	CISH	Negative	Negative	Negative	Negative
BRAF V600E exon 15	NGS	Not performed[a]	42%	52%	47%
FGFR2 G542E exon 12	NGS	Not performed	19%	30%	22%
PTEN K267fs exon 12	NGS	Not performed	14%	37%	32%
PTEN R137H exon 6	NGS	Not performed	Not detected	21%	Not detected
PTEN T321fs exon 8	NGS	Not performed	Not detected	Not detected	22%
TP53 R273C exon 8	NGS	Not performed	Not detected	22%	21%

IHC results presented as positive/negative according to ranges, staining intensity and staining percentage.
NGS − where mutation is detected, % refers to alteration frequency, the ratio between mutant gene and wild-type gene.
NGS could not be carried out on sample 15 November 2008 as the sample was decalcified.
NGS − genes tested without alterations throughout include − ABL1, AKT1, ALK, APC, ATM, c-KIT, CDH1, cMET, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FLT3, GNA11, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KRAS, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, STK11, VHL.
BRAF, v-raf murine sarcoma viral oncogene homologue B1; CISH, chromogenic in-situ hybridization; FGFR2, fibroblast growth factor receptor 2; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemical; MGMT, 0-6-methylguanine-DNA methyltransferase; NGS, next-generation sequencing; SPARC, secreted protein acidic and rich in cysteine; PGP, P-glycoprotein; PTEN, phosphatase and tensin homologue; TOP2A, topoisomerase 2A; RRM1, ribonucleotide reductase subunit M1; TLE3, transducin-like enhancer protein 3; TOPO1, topoisomerase 1; TP53, tumour suppressor p53; TS, thymidylate synthase; TUBB3, tubulin beta-3.
[a]BRAF testing was performed in March, 2009 separate from this molecular profiling; a V600E mutation was found.

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.

Fig. 1
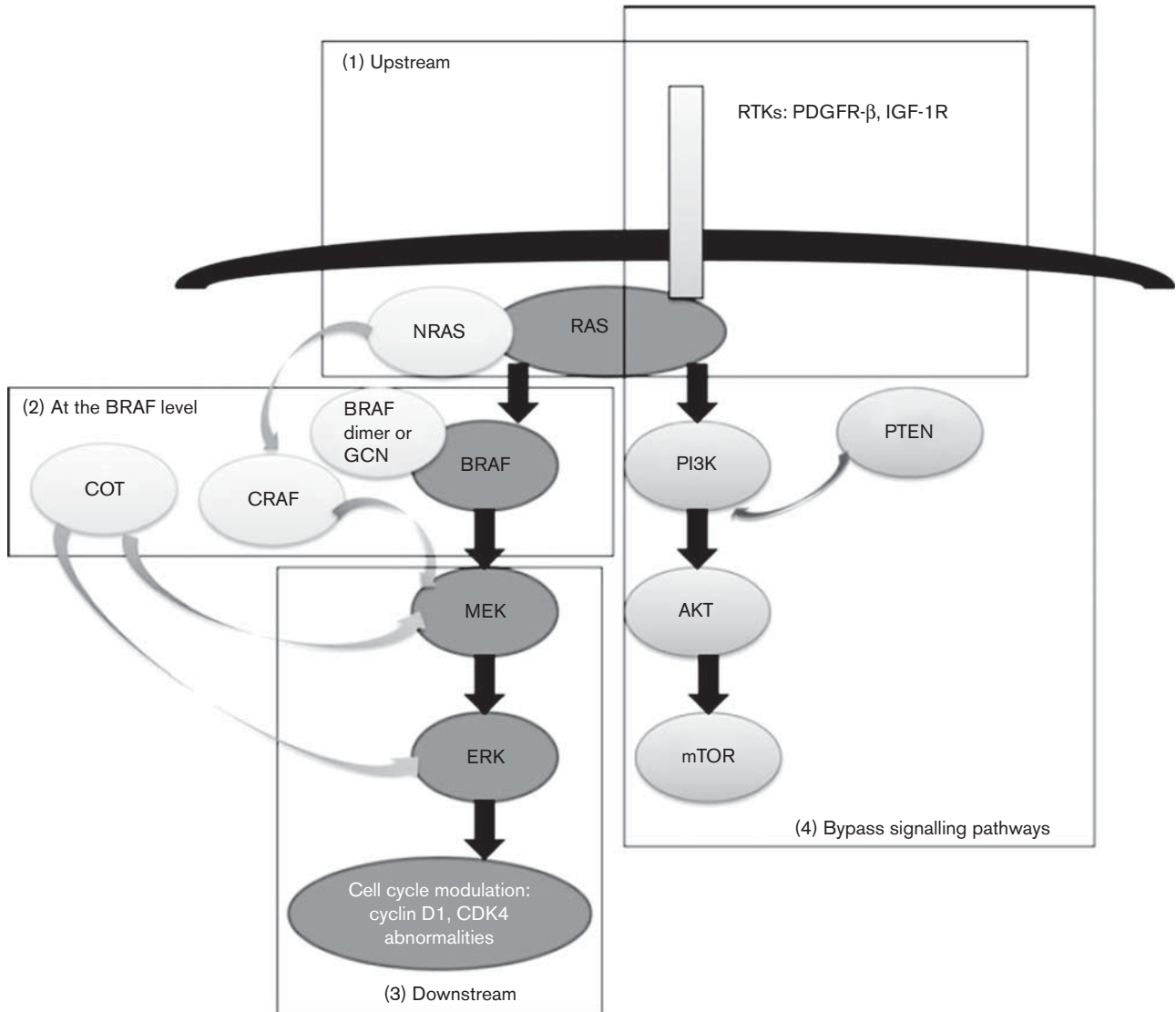
Classification of resistance mechanisms to *BRAF* inhibitors. The figure shows a classification system for both de-novo and acquired resistance mechanisms to the selective *BRAF* inhibitors relative to the *BRAF* mutation. Such resistance mechanisms may lie upstream, downstream or at the same level along 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].

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 subcutaneous metastases: 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.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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