FULL PAPER

BJC

British Journal of Cancer (2014) 111, 292–299 | doi: 10.1038/bjc.2014.287

Keywords: *BRAF*; *NRAS*; melanoma; prognosis; BRAF inhibitors; MEK inhibitors

Correlation of BRAF and NRAS mutation status with outcome, site of distant metastasis and response to chemotherapy in metastatic melanoma

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Background: The prognostic significance of BRAF and NRAS mutations in metastatic melanoma patients remains uncertain, with several studies reporting conflicting results, often biased by the inclusion of patients treated with BRAF and MEK (MAPK) inhibitors. We therefore interrogated a historical cohort of patients free of the confounding influence of MAPK inhibitor therapy.

Methods: Patients with available archival tissue first diagnosed with metastatic melanoma between 2002 and 2006 were analysed. Mutational analysis was performed using the OncoCarta Panel. Patient characteristics, treatment outcome and survival were correlated with BRAF/NRAS mutation status.

Results: In 193 patients, 92 (48%) melanomas were BRAF-mutant, 39 (20%) were NRAS-mutant and 62 (32%) were wild-type for BRAF/NRAS mutations (wt). There was no difference in response to chemotherapy based on mutation status (35–37%). The distant disease-free interval (DDFI) was significantly shorter in patients with wt melanoma (27.9 months *vs* 35.1 for BRAF and 49.1 for NRAS) although this was not significant in multivariate analysis. Survival from stage IV melanoma diagnosis was not significantly different based on mutation status. The DDFI was significantly shorter in patients with $BRAF^{V600K/R}$ versus $BRAF^{V600E}$ melanoma in univariate and multivariate analyses.

Conclusions: BRAF and NRAS mutation status does not influence survival in metastatic melanoma.

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Revised 8 April 2014; accepted 30 April 2014; published online 10 June 2014

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Activating mutations in the oncogenes *BRAF or NRAS* occur in approximately 40 and 20% of melanomas, respectively, and result in constitutive activation of the mitogen-activated kinase (MAPK) cell signalling pathway (Davies *et al*, 2002; Platz *et al*, 2008). Small molecule inhibitors of mutant BRAF and the downstream kinase MEK (MAPK inhibitors) have transformed the management of BRAF-mutant metastatic melanoma and improved overall survival (OS) compared with standard chemotherapy in patients with $BRAF^{V600}$ mutant metastatic melanoma (Chapman *et al*, 2011; Flaherty *et al*, 2012; Hauschild *et al*, 2012). Similarly, although to a lesser extent, single agent MEK inhibition has shown activity in NRAS-mutant metastatic melanoma (Falchook *et al*, 2012; Ascierto *et al*, 2013), with a phase III trial currently underway (NCT01763164).

The presence of a *BRAF* mutation in metastatic colorectal cancer is associated with a shorter OS compared with *KRAS* mutant or *RAS/RAF* wild-type disease (Van Cutsem *et al*, 2011; Yokota *et al*, 2011; Toland *et al*, 2012). Similarly *BRAF* mutations are associated with an increased risk of recurrence in papillary thyroid cancer (Elisei *et al*, 2012; Prescott *et al*, 2012; Fernandez *et al*, 2013). The prognostic significance of a *BRAF* mutation in metastatic melanoma is less clear. Recent analysis of survival in metastatic melanoma patients were performed when BRAF and MEK inhibitors were available and some patients included received these therapies (Long *et al*, 2011; Jakob *et al*, 2012), making comparisons between the *BRAF*-mutant and wild-type populations difficult. One study examining *BRAF* status only (Long *et al*, 2011) reported no difference in survival from stage IV diagnosis between patients with *BRAF*-mutant and wild-type metastatic melanoma; however, when the analysis was limited to patients with *BRAF*-mutant melanoma who did not receive a MAPK inhibitor, a significantly shorter survival in BRAF-mutant patients was observed. It is unclear if this difference in survival was due to differences in the biology of *BRAF*-mutant versus wild-type melanoma or a selection bias due to the non-random selection of *BRAF*-mutant patients for entry into the early phase clinical trials of MAPK inhibitors. Another study examining *BRAF* and *NRAS* status reported that NRAS-mutant melanoma was associated with the poorest survival (Jakob *et al*, 2012). However, an earlier study found that *NRAS*-mutant melanoma was associated with improved survival compared with *BRAF*-mutant or *BRAF/NRAS* wild-type disease (Ugurel *et al*, 2007).

This uncertainty regarding the prognostic significance of *BRAF* and *NRAS* mutations in metastatic melanoma led us to perform a retrospective analysis in a cohort of patients with advanced melanoma who were treated before the availability of MAPK inhibitors. We sought to correlate *BRAF* and *NRAS* mutation status with clinicopathologic characteristics, response to chemotherapy and survival, as well as to determine the frequency of other oncogenic mutations in metastatic melanoma.

MATERIALS AND METHODS

Patient selection and data collection. This study was undertaken at the Melanoma Institute Australia (MIA) in conjunction with Westmead Hospital and Royal Prince Alfred Hospital with human ethics review committee approval (Protocol No. X11-0023 and HREC/11/RPAH/32). All patients consented to data collection and enrolment in the melanoma research database (MRD). Patients with newly diagnosed metastatic melanoma (stage IV) managed at MIA between 2002 and 2006 with available archival paraffin-embedded melanoma tissue suitable for DNA extraction were included. To exclude the effect of survivor bias, which may occur at a quaternary referral cancer centre, patients not seen at the MIA before or within 4 weeks of developing metastatic melanoma were excluded.

Patient demographics, primary tumour characteristics (date of primary diagnosis, Breslow thickness, ulceration, mitotic rate, ulceration, N stage), clinical details at the time of diagnosis of stage IV melanoma (M stage, serum lactate dehydrogenase (LDH), organ involvement), and data regarding progress after development of stage IV disease (development of brain metastasis, treatment with systemic therapy and response to chemotherapy) were collected from the MRD and further review of the clinical record. For patients with more than one primary melanoma, the 'culprit' primary deemed responsible for subsequent metastatic disease was designated using a previously described algorithm (Murali *et al*, 2012; Mann *et al*, 2013). Chemotherapy included dacarbazine, temzolomide, fotumustine, combined carboplatin and paclitaxel or experimental combinations including these agents. Immunotherapy included vaccines and experimental agents. No patient was treated with IL-2, ipilimumab, class 1 BRAF inhibitors or MEK inhibitors. Treatment benefit was determined prospectively by the clinician, with either disease stability or a reduction in tumour burden during treatment considered as a beneficial response.

Tumour samples and molecular testing. Distant metastatic samples were preferentially sampled over lymph nodes or primaries where available. DNA was extracted from one core sample taken from one archival formalin-fixed, paraffin-embedded (FFPE) tissue block of melanoma for each patient in the study. DNA was extracted using NucleoSpin FFPE DNA Kit (Macherey Nagel, Düren, Germany) according to the manufacturer's instruction with an overnight proteinase digestion. The quality and quantity of the extracted DNA was assessed using NanoDrop ND-1000 Spectrophotometer. A minimum of 500 ng of DNA was required for successful mutational analysis. All samples were successfully amplified and analysed for 238 variant targets in a 24 multiplex polymerase chain reactions (PCR) using the OncoCarta Panel v1.0 Kit including 19 tumour-related genes such as *BRAF*, *NRAS*, *KIT* and *PIK3CA* (http://bioscience.sequenom.com/oncocarta-panel). The genotypes were called based on the matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) technology on the Sequenom MassArray platform. Specifically, the key targeted mutational hotspots in this assay were G464R/V/E, G466R, F468C, G469A/E/R/S/V, D594V/G, F595L, G596R, L597Q/R/S/V, T599I, V600E/K/R/L, K601N/E for *BRAF* and G12V/A/D/C/R/S, G13V/A/D/C/R/S, A18T, Q61L/R/P/H/E/H/K for *NRAS*.

Statistical methods. Clinical and pathologic features were tested for associations with *BRAF* or *NRAS* mutation status using simple cross-tabulations, independent samples *t*-test, Fisher's exact test, Pearson c2, and/or the Mann–Whitney *U* test. The distant disease-free interval (DDFI) was measured from the date of culprit primary melanoma diagnosis to diagnosis of distant metastatic disease. Overall survival was calculated from the date of diagnosis of stage IV melanoma to last follow-up (censored) or death from melanoma (event). Univariate survival analyses was carried out using the Kaplan–Meier method together with the log-rank (Mantel–Cox) test to calculate statistical significance. Univariate hazard ratios (HRs), 95% confidence intervals (95% CI), and corresponding *P*-values were obtained using Cox regression. A Bonferroni correction was applied to all *P*-values resulting from the univariate DDFI and survival analyses to adjust for multiple comparisons. Multivariate survival analyses were conducted with Cox proportional hazards method. The proportionality assumption was inspected visually for each categorical covariate. A two-tailed *P*-value of less than 0.05 was considered statistically significant. All analyses were prespecified and carried out with the IBM SPSS Statistic 19.0 software package.

RESULTS

Patients, tumour samples and mutation frequency. Between 2002 and 2006, 322 patients with a new diagnosis of metastatic melanoma were seen at MIA. Nine patients were excluded because they were first diagnosed with metastatic disease more than 4 weeks before their first consultation at MIA. Archival FFPE melanoma tissue sufficient for DNA analysis was available in 193 of the 313 eligible patients. Mutations were identified in tumours from 140 (73%) patients, and 10 (5%) patients had more than one mutation. *BRAF* mutations were detected in 92 patients (48%), and *NRAS* mutations in 39 patients (20%) (Table 1). No targeted mutations were identified in 53 patients (27%). Of the patients with *BRAF* mutations, 65 (71%) were V600E and 18 (20%) were V600K. Of the patients with an *NRAS* mutation, 33 (85%) were substitutions for glutamine at position 61 (Q61H/K/L/R) and 6 (15%) were substitutions for glycine at amino acids 12 (G12C/D) or 13 (G13C/S). No tumours harboured both an *NRAS* and *BRAF* mutation. Twenty-three mutations, in 19 (10%) patients, were detected in genes other than *BRAF/NRAS*; the most common were mutations in *KIT* ($n=7$, 4%) or *PIK3CA* ($n=7$, 4%) (Supplementary Table S1).

Correlations with clinical features (Supplementary Table S2) and survival analyses were not performed based on mutations other than *BRAF* or *NRAS* genes because of the small numbers and the heterogeneity of the mutation types. Subsequent analyses were based on a patient's tumour BRAF and NRAS status, and three cohorts were compared and analysed: *BRAF*-mutant ($n=92$); *NRAS*-mutant ($n=39$); and those in whom no targeted mutation was found in *BRAF* or *NRAS* (wt, $n=62$).

Patient demographics and clinicopathologic features of primary melanoma based on BRAF and NRAS mutation status. Patients with *BRAF*-mutant melanoma were significantly younger at diagnosis of the culprit primary melanoma than those with wt melanoma (Median 53 versus 59 years, $P=0.002$) (Table 2). Acral lentiginous and demoplastic melanoma subtypes appeared to be more common in the wt cohort; however, the small numbers in each subtype precluded statistical analysis (Table 2). There was no significant difference in Breslow thickness, mitotic rate, presence of ulceration and nodal status between the three cohorts at diagnosis of the culprit primary melanoma (Table 2).

Clinical characteristics and treatment received for stage IV melanoma. Patients with *BRAF*-mutant disease were significantly younger than those with wt at first diagnosis of stage IV melanoma (median age 56 versus 63 years, $P=0.03$) (Table 3). A higher proportion of patients with *NRAS*-mutant melanoma had M1c (anatomically defined) melanoma at first diagnosis of stage IV compared with patients with BRAF-mutant or wt melanoma (Table 3). Serum LDH at stage IV diagnosis was not associated with mutation status (Table 3). There was a trend to an increased incidence of liver and CNS metastasis at diagnosis of stage IV disease in patients with *NRAS*-mutant melanoma, although the risk of developing CNS metastasis at any time was similar between the three groups (40–45%) (Table 3).

The number of patients who received systemic therapy, either chemotherapy or immunotherapy, was not different between the three cohorts (Table 3). There was no difference in clinician-assessed benefit from chemotherapy, (35–37%) (Supplementary Table S3).

Distant disease-free interval and survival analysis. Although distant disease-free interval (DDFI) was significantly shorter in the wt cohort (27.9 months) compared with either the *BRAF* (35.1 months, $P=0.03$) or *NRAS*-mutant (median 49.1 months ($P=0.01$) populations (Figure 1A), this difference did not remain significant in multivariate analysis when known prognostic factors for DDFI were included (Table 4). There was no difference in OS between the three cohorts from the time of diagnosis of stage IV melanoma (Figure 1B) or in OS from culprit primary (Supplementary Figure S1).

When analysed by *BRAF* mutation genotype within the BRAF-mutant cohort, patients with $BRAF^{V600K\ or\ R}$ genotype melanoma had a significantly shorter DDFI ($n=20$, median 22 months) than those with $BRAF^{V600E}$ ($n=65$, median 45 months, $P=0.001$) (Figure 1C); this difference remained significant in multivariate analysis (Table 5). There was no difference in OS from diagnosis of stage IV disease between the $BRAF^{V600K/R}$ and the $BRAF^{V600E}$ patients (Figure 1D). There was a trend towards a shorter survival from culprit primary in patients with $BRAF^{V600K\ or\ R}$ compared with $BRAF^{V600E}$ genotype melanoma (Supplementary Figure S2). The small numbers ($n=7$) of non-V600 *BRAF* mutations precluded further analysis of this subgroup.

There was a trend towards a shorter DDFI in patients who had an exon 2 (codon 61, $n=23$) compared with those with an exon 1 (codon 12/13, $n=6$) *NRAS* mutation (Supplementary Figure S3, $P=0.091$). There was no difference in OS between the NRAS genotypes from diagnosis of stage IV disease ($P=0.66$).

Table 1. Frequency of BRAF and NRAS mutations

Mutation	Number of patients (%)	
BRAF	92 (48)	% Of BRAF
V600E	65 (34)	71
V600K	18 (9)	20
V600R	2 (1)	2
G469R	2 (1)	2
K601E	3 (2)	3
K601N	1 (0.5)	1
L597Q	1 (0.5)	1
NRAS	39 (20)	% Of NRAS
Q61H	1 (0.5)	3
Q61K	13 (7)	33
Q61L	4 (2)	10
Q61R	15 (8)	38
G12C	1 (0.5)	3
G12D	3 (2)	8
G13C	1 (0.5)	3
G13S	1 (0.5)	3

DISCUSSION

This is one of the largest studies to examine the prognostic significance of *BRAF* and *NRAS* mutation status in patients with metastatic melanoma, diagnosed and treated before the availability of BRAF and MEK inhibitors. In contrast to other large studies, our survival analyses were not confounded by the availability BRAF and MEK inhibitors (Long *et al*, 2011; Jakob *et al*, 2012). Previous large studies in primary melanomas were not powered to examine survival, as only 10% of patients with early-stage melanoma develop metastatic disease (Maldonado *et al*, 2003; Chang *et al*, 2004; Houben *et al*, 2004; Shinozaki *et al*, 2004; Akslen *et al*, 2005), although one study found *NRAS*-mutant disease was associated with a poorer survival (Devitt *et al*, 2011a). *NRAS* mutations have also been found to be associated with fast growing primary melanomas (Nagore *et al*, 2013). In the metastatic

Table 2. Clinicopathologic characteristics of the patient cohort based on mutation status

Feature	*N*	Value	WT, *N* = 62	%	NRAS, *N* = 39	%	BRAF, *N* = 92	%	Three group *P*-value
Age at first primary Dx	171	Median (Range)	59 (26–80)	—	54 (25–76)	—	53 (16–82)	—	0.006[a]
Breslow thickness (mm)	170	Median (Range)	2.5 (0.2–25)	—	2.5 (0.3–8.1)	—	2.15 (0.3–25)	—	0.426[a]
Mitotic rate (per mm^2)	139	Median (Range)	3 (0–15)	—	3 (0–33)	—	4 (0–34)	—	0.267[a]
Sex	193	Male	44	71	24	62	62	67	0.616
		Female	18	29	15	38	30	33	
Multiple primary	193	No	52	84	35	90	82	89	0.562
		Yes	10	16	4	10	10	11	
Primary site	193	Cutaneous	48	77	31	79	78	85	NA[b]
		Plantar surface of the foot	3	5	0	0	4	4	
		Toenail	2	3	0	0	0	0	
		Mucosal	3	5	2	5	0	0	
		Occult	6	10	6	15	10	11	
Melanoma subtype	130	Acral lentiginous	4	10	1	4	2	3	NA[b]
		Nodular melanoma[c]	16	40	15	58	34	53	
		Superficial spreading	6	15	3	11	5	8	
		Other[d]	14	35	7	27	23	36	
Ulceration	136	Absent	17	40	16	64	36	53	0.132
		Present	26	60	9	36	32	47	
N stage of culprit primary or occult melanoma	193	N0	50	81	29	74	62	67	NA[b]
		N1a/b	4	6	5	13	12	13	
		N2a/b/c	7	12	4	11	11	12	
		N3	1	2	1	3	7	8	

Abbreviations: Dx = diagnosis; NA = not applicable; WT = wild-type.
[a]Kruskal–Wallis independent samples test.
[b]Low expected cell frequency.
[c]Including superficial spreading with a nodular melanoma component.
[d]Lentigo maligna melanoma and desmoplastic.

melanoma population, the data regarding associations of melanoma genotype and survival are conflicting (Edlundh-Rose *et al*, 2006; Ugurel *et al*, 2007; Long *et al*, 2011; Brissy *et al*, 2012; Jakob *et al*, 2012; Ekedahl *et al*, 2013). One study showed that patients with *NRAS*-mutant tumours had an improved OS compared with those with *BRAF*-mutant or wt tumours (Ugurel *et al*, 2007), whereas another study suggested *NRAS*-mutant melanoma predicted a poorer OS from stage IV disease (Jakob *et al*, 2012). A further study found that the presence of either *NRAS* or *BRAF* mutations was associated with a poorer survival in the setting of metastatic disease (Houben *et al*, 2004). Our finding that mutation status is not prognostic in the setting of stage IV melanoma is in keeping with other studies before the availability of BRAF and MEK inhibitors (Chang *et al*, 2004; Edlundh-Rose *et al*, 2006). The data regarding the prognostic impact of mutation status in patients with stage III disease are similarly conflicting. Some studies found no prognostic impact of mutation status (Rutkowski *et al*, 2012) and others found an association between *BRAF*-mutant melanoma and poorer OS (Moreau *et al*, 2012; Mann *et al*, 2013). Although we found a significantly shorter DDFI in patients with wt disease, which has not been shown in prior studies that tested both *BRAF* and *NRAS* mutations (Jakob *et al*, 2012), it was not significant in multivariate analysis suggesting important differences in prognostic variables in the culprit primary melanoma.

There are many possible reasons for the lack of consistent results regarding the prognostic impact of *BRAF* and *NRAS* mutation status in both early and advanced melanoma, including the mutation testing method, patient selection and geographic variations in the risk of specific melanoma mutations. Different mutation testing methodologies with different sensitivities and specificities were used in various studies. In contrast to our study, very few prior studies analysed for a comprehensive range of melanoma-associated *BRAF* and *NRAS* mutations. As an example, a subset of studies did not test for exon 1 (codon 12/13) *NRAS* mutations (Edlundh-Rose *et al*, 2006; Devitt *et al*, 2011a) whereas others limited survival analysis only to *BRAF* substitutions for valine at codon 600 (Jakob *et al*, 2012). The OncoCarta assay is robust for FFPE samples and sensitive (detection limit of 10%) for the targeted hotspots within *BRAF* and *NRAS*. Although this assay does not analyse the complete genes or exons of interest, it does include all the key melanoma-associated *NRAS* mutations in exons 1 and 2 and *BRAF* mutations in exons 11 and 15 (Greaves *et al*, 2012).

Patient selection varied substantially between studies, and may be the most important factor influencing the different results between them. Methods of patient selection include selection of consecutive patients (Long *et al*, 2011; Moreau *et al*, 2012), selecting patients from a clinical database (Jakob *et al*, 2012), or, as in this study, selecting patients on the basis of available tissue (Houben *et al*, 2004; Rutkowski *et al*, 2012; Mann *et al*, 2013). This study minimised other selection biases by including all patients seen within a defined time period and limited the effect of survival bias by excluding those referred to our clinical service more than 4 weeks after the first diagnosis of metastatic melanoma. Each method of patient selection is associated with potential biases; clinically accrued data sets are likely to enhance for survivors, particularly as initially testing was performed for entry onto clinical trials or access to novel therapies, with a consequent referral bias of healthier and fitter patients who are willing to travel for experimental treatments (Long *et al*, 2011; Jakob *et al*, 2012). Studies in which patients are selected based on available archival tissue may skew the population towards patients who have had

Table 3. Stage IV clinical characteristics based on mutation status

Feature	*N*	Value	WT, *N* = 62	%	NRAS, *N* = 39	%	BRAF, *N* = 92	%	Three group *P*-value
Age	193	Median (Range)	63 (29–84)	—	57 (29–78)	—	56 (17–85)	—	0.096
DFI (months)	171	Median (Range)	28 (0–112)	—	49 (6–137)	—	35 (0–366)	—	0.156[a]
Site of stage IV diagnosis	193	Skin	36	58	31	79	62	67	0.083
		Lung	41	66	26	67	47	51	0.099
		GI	6	10	5	13	5	5	0.333
		Liver	21	34	18	46	24	26	0.079
		Bone	6	10	4	10	18	20	0.163
		Brain	8	13	8	21	16	17	0.581
		Other[b]	12	19	8	21	20	22	0.937
M stage	169	M1a	1	2	1	3	11	14	0.027
		M1b	12	22	4	11	9	12	
		M1c	42	76	31	86	58	74	
Number of metastatic sites	193	1	22	35	8	21	33	36	NA[c]
		2	23	37	12	31	28	30	
		3	7	11	10	26	23	25	
		4	9	15	7	18	6	7	
		5	1	2	2	5	2	2	
LDH	134	Not elevated	24	53	15	54	36	59	0.872
		Elevated	21	47	13	46	25	41	
ECOG PS	171	0	39	67	20	59	48	61	0.134
		1	16	28	9	26	29	37	
		2	3	5	5	15	2	2	
Brain metastases ever	193	No	37	60	23	59	51	55	0.854
		Yes	25	40	16	41	41	45	
Surgery	191	No	27	44	17	44	28	31	0.169
		Yes	34	56	22	56	63	69	
Chemotherapy	183	No	20	33	15	42	32	37	0.797
		Yes	40	67	21	58	55	63	
Immunotherapy	188	No	57	93	35	90	72	82	0.134
		Yes	4	7	4	10	16	18	

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; DFI = disease-free interval; LDH = serum lactate dehydrogenase; NA = not applicable; WT = wild-type.
[a]Kruskal–Wallis independent samples test.
[b]Spleen, pancreas, adrenal, omentum, thyroid, kidney, stomach, pleura, gallbladder.
[c]Low expected cell frequency.

surgery for stage III or IV disease, who may represent a separate prognostic group with different mutational profiles. Geographical variations may also explain the differences in the impact of mutation status on prognosis. $BRAF^{V600K}$ mutation is associated with chronic UV damage (Menzies *et al*, 2012) and varies by geography (Houben *et al*, 2004; Edlundh-Rose *et al*, 2006; Ugurel *et al*, 2007; Long *et al*, 2011; Amanuel *et al*, 2012; Jakob *et al*, 2012; Menzies *et al*, 2012).

Little is known about other genetic or epigenetic factors, which can occur concurrently with *BRAF* and *NRAS* mutations, and may vary between regions, with possible prognostic implications, for example, PTEN loss is uncommon in NRAS-mutant melanoma but occurs in BRAF-mutant melanoma and can activate the PI3K pathway (Hodis *et al*, 2012). Although NRAS mutations cause both PI3K/AKT and MAPK pathway activation (Tsao *et al*, 2000), it remains to be determined if activation of the PI3K pathway is prognostic in melanoma. This dual pathway activation is one hypothesis to explain the association between NRAS mutations and a poorer prognosis compared with BRAF mutations in the previous studies of melanoma (Jakob *et al*, 2012). However, this is not the case in colorectal cancer where BRAF mutations carry a poorer prognosis compared with KRAS mutant disease (Van Cutsem *et al*, 2011; Yokota *et al*, 2011; Toland *et al*, 2012). A meta-analysis may help to clarify the effect of mutation status on survival.

We found no differences in the clinicopathologic factors of the antecedent primary melanoma based on the mutation status, similar to prior studies (Shinozaki *et al*, 2004; Edlundh-Rose *et al*, 2006), but in contrast to one study which reported association between *BRAF* positivity and thinner primaries with lower numbers of mitoses (Devitt *et al*, 2011b).

There was no association between the patterns of organ involvement of metastatic disease both at the time of distant metastasis (stage IV) diagnosis and *BRAF/NRAS* mutation status, although we found non-lung visceral metastases (M1c disease) more common in patients with *NRAS*-mutant disease. One previous study reported an association between *BRAF* or *NRAS* mutations and the presence of CNS metastases at first occurrence of stage IV disease (Jakob *et al*, 2012). Our data show a trend towards higher rates of brain metastasis at initial stage IV diagnosis in keeping with this, but we show for the first time that the risk of developing brain metastasis at any time is comparable at 40–45% of patients irrespective of *BRAF/NRAS* mutation status.

The response to chemotherapy was not influenced by mutation status. Although *BRAF* wild-type melanomas have been reported to have a higher response rate to regional chemotherapy (Gallagher *et al*, 2008), in keeping with our data, mutation status did not influence response or survival to systemic therapy with nab-paclitaxel or dacarbazine (Hersh *et al*, 2013). Exploratory

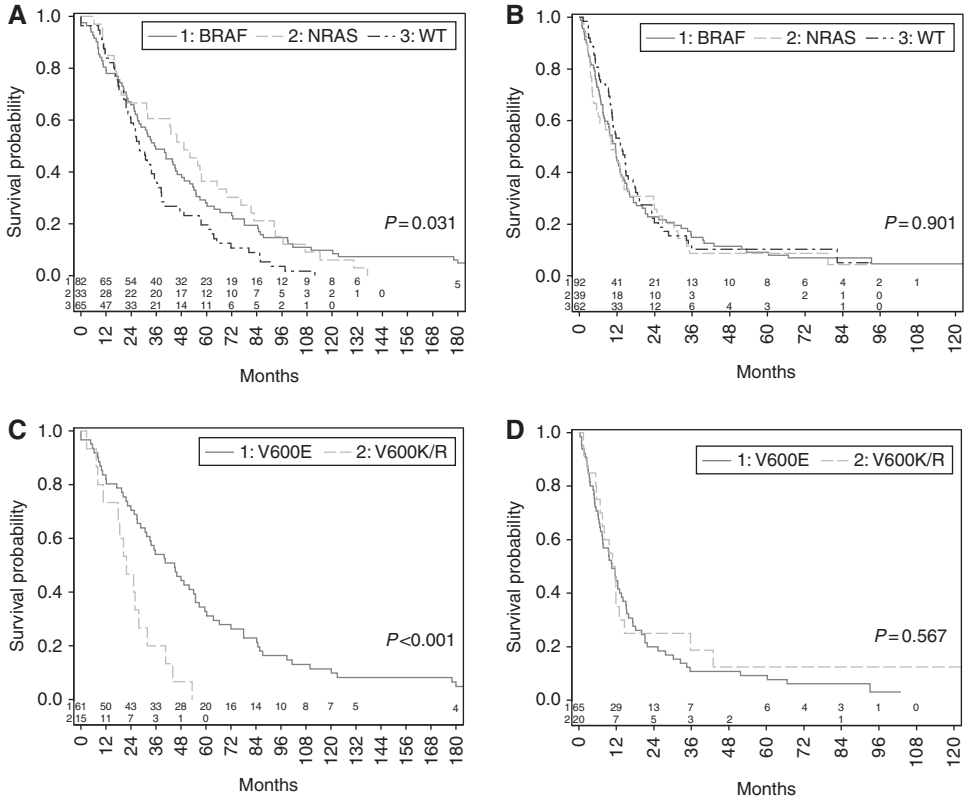

Figure 1. Impact of mutation status on DDFI and survival from stage IV melanoma. (**A**) DDFI from culprit primary melanoma based on *BRAF* and *NRAS* status. (**B**) Survival from diagnosis of stage IV melanoma based on *BRAF* and *NRAS* mutation status. (**C**) DDFI from culprit primary melanoma based on *BRAF* mutation genotype. (**D**) Survival from diagnosis of stage IV melanoma based on *BRAF* mutation genotype.

Table 4. Multivariate analysis of distant disease-free interval ($n=170$)

			95% Confidence interval		
Factor	**Value**	**Hazard ratio**	**Lower**	**Upper**	***P*-value**
Breslow thickness groups	T1	Reference			
	T2	1.225	0.750	2.000	0.417
	T3	1.443	0.918	2.270	0.112
	T4	3.013	1.811	5.012	<0.001
N stage	N0	Reference			
	N1a	1.952	1.172	3.252	0.010
	N1b	14.494	1.755	119.661	0.013
	N2a	1.566	0.853	2.875	0.148
	N2b	0.614	0.144	2.614	0.509
	N3	2.680	1.119	6.420	0.027
Mutation	WT	Reference			
	NRAS	0.679	0.429	1.073	0.097
	BRAF	0.715	0.489	1.045	0.083

Table 5. Multivariate analysis of distant disease-free interval according to genotype in BRAF-mutant patients ($n=76$)

			95% Confidence interval		
Factor	**Value**	**Hazard ratio**	**Lower**	**Upper**	***P*-value**
Breslow thickness groups	T1	Reference			
	T2	1.350	0.657	2.774	0.414
	T3	2.054	0.998	4.229	0.051
	T4	4.575	1.990	10.518	<0.001
N stage	N0	Reference			
	N1	3.887	1.773	8.522	0.001
	N2	3.434	1.493	7.897	0.004
	N3	1.238	0.387	3.958	0.719
Mutation	V600E	Reference			
	V600K/R	2.248	1.112	4.543	0.024

analysis have found an association between *BRAF* wild-type tumours and response to an investigational combination including the anti-angiogenic therapy bevacizumab (von Moos *et al*, 2011), *NRAS* mutations have also been reported to be associated with an improved response to immunotherapy as compared with patients having *BRAF/NRAS* wild-type tumours (Johnson *et al*, 2013). Analysis as part of future clinical trials should determine if mutation status is predictive in treatments beyond those involving inhibitors of the MAPK pathway.

Our finding that tumours with a $BRAF^{V600K/R}$ mutation have a significantly shorter DDFI than those with the more common $BRAF^{V600E}$ mutation confirms prior reports (Menzies *et al*, 2012; Bucheit *et al*, 2013). We found no impact of *BRAF* mutation genotype on OS from the time of first diagnosis of stage IV melanoma, as previously reported in a different cohort from our institution (Menzies *et al*, 2012), although another study found V600K mutations were associated with a poorer survival from stage IV (Bucheit *et al*, 2013). Analysis of DDFI and survival from stage IV diagnosis based on exon 1 or 2 *NRAS* mutations showed no significant difference based on genotype, in keeping with prior

studies (Bucheit *et al*, 2013). Nevertheless, this exploratory analysis is limited by the relatively small number of patients with *NRAS* mutations in this cohort.

In conclusion, our data show that *BRAF* and *NRAS* mutations are not prognostic in advanced melanoma. We confirmed the association between the $BRAF^{V600K/R}$ genotype and a shorter DDFI compared with $BRAF^{V600E}$ in an independent cohort, but there was no difference in survival from stage IV diagnosis. Given the activity of BRAF and MEK inhibitors, future studies examining the prognostic impact of *BRAF* mutation status in advanced melanoma will be difficult to interpret and future studies should examine other genetic factors which may explain the conflicting results seen across multiple studies.

ACKNOWLEDGEMENTS

This work was supported by Program Grant 633004 and project grants of the National Health and Medical Research Council of Australia (NHMRC), and an educational grant from Roche Products, Pty Limited (Australia). RA Scolyer, GV Long and SA O'Toole are recipients of Cancer Institute New South Wales, Research Fellowships and RA Scolyer is supported by an NHMRC Practitioner Fellowship. MS Carlino is supported by a Rotary Health Australia scholarship. WA Cooper is supported by Sydney Foundation for Medical Research.

CONFLICT OF INTEREST

Carlino MS received honoraria from Novartis, Roche, GlaxoSmithKline and Bristol-Myers Squibb. Menzies AM received travel support from Roche and GlaxoSmithKline, and honoraria from Roche. Hamilton AL received travel support from Roche. Cooper WA received consultancies and honararia from Pfizer and Merck. O'Toole S received honoraria from Roche, Astra Zeneca and Pfizer. Thompson JF received consultancies and honoraria from Roche and GlaxoSmithKline. Kefford RF received consultancies and honoraria from Roche, GlaxoSmithKline and Novartis. Scolyer RA received consultancies from Roche and GlaxoSmithKline, honoraria from Abbott Molecular. Long GV received consultancies from Roche, Bristol-Myers Squibb, GlaxoSmithKline, Novartis and Amgen; honoraria from Roche; travel support from GlaxoSmithKline and Roche Melanoma Institute Australia received research support from Roche. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)