Human Cancer Biology

Distinguishing Clinicopathologic Features of Patients with V600E and V600K *BRAF*-Mutant Metastatic Melanoma

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Abstract

Purpose: Certain clinicopathologic features correlate with *BRAF* mutation status in melanoma including younger age and primary subtype. This study sought to determine the *BRAF* mutation status by age-decade and whether *BRAF*-mutant genotypes correlated with clinicopathologic features and outcome in patients with metastatic melanoma.

Methods: A prospectively assembled cohort of Australian patients were followed from diagnosis of metastatic melanoma (N = 308). Clinicopathologic variables were correlated with *BRAF* mutational status, genotype, and survival.

Results: Forty-six percent of patients had a *BRAF* mutation; 73% V600E, 19% V600K, and 8% other genotypes. An inverse relationship existed between *BRAF* mutation prevalence and age-decade ($P < 0.001$). All patients <30 years and only 25% ≥70 years had *BRAF*-mutant melanoma. Amongst *BRAF*-mutant melanoma, the frequency of non-V600E genotypes (including V600K) increased with increasing age. Non-V600E genotypes comprised <20% in patients <50 years and >40% in those ≥70 years. A higher degree of cumulative sun-induced damage correlated with V600K but not V600E melanoma ($P = 0.002$). The disease-free interval from diagnosis of primary melanoma to first distant metastasis was shorter for patients with V600K compared with V600E melanoma (17.4 vs. 39.2 months, $P = 0.048$), with no difference in survival thereafter. In patients *BRAF* tested at diagnosis of metastatic melanoma, one year survival from diagnosis of metastasis was significantly longer for patients with *BRAF*-mutant melanoma treated with an inhibitor (83%), than those not treated with an inhibitor (29%, $P < 0.001$), or patients with *BRAF* wild-type melanoma (37%, $P < 0.001$).

Conclusion: Different genotypes exist within *BRAF*-mutant metastatic melanoma, representing biologically and clinically discrete subtypes, suggesting distinct etiology and behavior. *Clin Cancer Res; 18(12); 3242–9. ©2012 AACR.*

Introduction

The recent characterization of molecular subtypes of melanoma (1, 2) has led to the rapid clinical application of highly active and potent inhibitors of oncogenic mutated *BRAF* and *CKIT* proteins in patients with metastatic melanoma (3, 4). Inhibitors of the mutant *BRAF* oncogene, vemurafenib and dabrafenib, are transforming the manage-ment of patients with *BRAF*-mutant metastatic melanoma because of high patient response rates and their rapid mode of action (5–10). Furthermore, vemurafenib has showed an overall survival (OS) benefit over dacarbazine in patients with *BRAF*-mutant (V600E) metastatic melanoma (11).

BRAF is a protein critical in the mitogen-activated protein kinase (MAPK) pathway and is mutated in approximately 50% of melanoma patients (12, 13). More than 80 somatic mutations in exon 15 of the *BRAF* gene have been identified in melanoma (14). Ninety-five percent of *BRAF* mutations occur at V600 (single amino acid substitution at valine 600) and constitutively activate this oncogene (14). The most common genotype is V600E (valine to glutamic acid), with early reports suggesting this accounts for up to 95% of *BRAF*-mutant melanoma (1). Large studies recently conducted in Australia (13), Texas (15), and Florida (16) show that the V600E genotype is not as prevalent, and that V600K (valine to lysine), may comprise 20% or more of *BRAF*-mutant melanomas.

BRAF-mutant melanoma is associated with younger patient age, lack of CSD at the primary site, truncal location,

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Translational Relevance

This study shows there are clinically and biologically distinct subtypes of *BRAF*-mutant metastatic melanoma, defined by their genotype, with distinct etiology and behavior. In particular, cumulative sun-induced damage (CSD) in the primary cutaneous melanoma and older age are associated with V600K *BRAF* mutations. It establishes the prevalence of the *BRAF* mutation by age-decade, a practical clinical statistic for treatment planning and pretest counseling, and in the era of widespread optimism surrounding mitogen-activated protein kinase (MAPK) pathway therapies, improving one's ability to predict the probability of an individual patient's *BRAF* genotype may lead to more tailored discussions about possible future treatments. This is the first study to report a one year survival rate of 83% from diagnosis of metastatic melanoma in patients with *BRAF*-mutant melanoma treated with a *BRAF* inhibitor, comparing favorably both with patients with *BRAF* wild-type and *BRAF*-mutant melanoma who did not receive a *BRAF* inhibitor.

high body nevus counts, and certain pathologic characteristics (12, 13, 15–25). Just as *BRAF* wild-type and mutant melanoma have differing clinicopathologic features, we hypothesized that the mutant genotypes are also distinct. To the authors' knowledge, there have been no published reports about clinicopathologic differences between mutant *BRAF* genotypes, particularly V600E and V600K. We sought to correlate such features with *BRAF* genotype in a prospectively accrued cohort of patients with metastatic melanoma. In addition, as age is the strongest correlate of *BRAF* mutation status, we sought to explore this in detail, by determining the *BRAF* mutation prevalence and genotype proportions by age-decade. Furthermore, we explored the effect of a selective *BRAF* inhibitor on OS from diagnosis of first distant metastasis.

Patients and Methods

Patient selection and data collection

The study was undertaken at Melanoma Institute Australia (MIA, Sydney, NSW) with Human Ethics Review Committee approval. Patients with unresectable stage IIIC and IV (metastatic) melanoma, excluding ocular primary, were prospectively and consecutively accrued from June 2009 to September 2010. The first 197 patients comprised consecutively seen patients with metastatic melanoma at MIA. The remaining 111 patients were consecutive patients who underwent *BRAF* testing. Initial data on the first 197 patients have been published (13); new data were collected on these patients for this study.

Data from clinical notes and the MIA database were collected for patient demographics, details of the primary melanoma (date, type, site, melanoma histogenetic subtype, total number of primary melanomas), and details of

the metastatic disease (date of diagnosis, targeted therapy received, date of death from melanoma). For patients with multiple primary melanomas, the primary melanoma responsible for metastasis (culprit primary melanoma) was assigned using a predefined structured algorithm (26). This lesion was used for analysis.

All patients with primary melanoma tissue available had CSD scored. The degree of solar elastosis (as a marker of the degree of CSD ref. 19) was scored at the primary tumor site using a semiquantitative scoring system as previously defined (0 = no solar elastosis = absence of elastotic fibers; 1 = mild solar elastosis = scattered elastotic fibers lying as individual units, not as bushels, between collagen bundles; 2 = moderate solar elastosis = densely scattered elastotic fibers distributed predominantly as bushels rather than individual units; and 3 = severe solar elastosis = amorphous deposits of blue–gray elastotic material with lost fiber texture; refs. 20, 25, 27). Scoring was carried out by a single pathologist (R.A. Scolyer) who was blinded as to the *BRAF* mutation status of each patient.

BRAF mutation testing

Melanoma tissue from every patient was tested for the presence of a *BRAF* mutation, with preferential choice of tissue being distant metastatic tissue (most recent) first, then locoregional/in-transit metastasis, and finally primary melanoma. If no suitable melanoma tissue was available, a biopsy was conducted for the purpose of *BRAF* testing. The primary melanoma was tested only if no metastatic tissue was available. Sections from archival paraffin-embedded samples were tested at the Peter MacCallum Cancer Centre, Department of Diagnostic Molecular Pathology (Melbourne, Australia). Samples were macrodissected and subjected to high-resolution melting (HRM) analysis using primers flanking codon 600 in the *BRAF* gene. These primers identify variations in exon 15 of the *BRAF* gene between nucleotides c.1788 and c.1823 in reference sequence NM_004333.4, corresponding to codons 597 to 607. All abnormal HRM traces were subjected to bidirectional DNA sequencing using the primers described earlier (13).

More extensive sequencing was carried out on melanomas from patients considered possible candidates for the GlaxoSmithKline (GSK) phase I/II clinical trial (NCT00880321) of the selective *BRAF* inhibitor dabrafenib (6). Samples were amplified with M13-tagged primers flanking exon 15 of *BRAF* and sequenced using M13 primers. Sequence data were obtained for the whole of exon 15 comprising nucleotides c.1742 to c.1860 in reference sequence NM_004333.4, corresponding to codons 581 to 620.

BRAF and *MEK* inhibitors

Eligible patients with *BRAF*-mutant melanoma were enrolled into the GSK phase I/II trial (NCT00880321) of dabrafenib (previously GSK2118436; refs. 6, 7), the Roche phase II (NCT00949702) and III (NCT01006980) trials of vemurafenib (previously RG7204/RO5185426/

PLX4032; refs. 10, 11), and/or the GSK phase I/II trial (NCT01072175) of dabrafenib in combination with the *MEK* inhibitor trametinib (previously GSK1120212; ref. 9), and/or the GSK phase II trial (NCT01037127) of trametinib (28).

Statistical analysis

For all patients, clinical and pathologic features were tested for associations with *BRAF* mutation status and genotype using simple cross-tabulations, independent samples *t* test, Fisher exact test, Pearson χ^2, and/or the Mann–Whitney *U* test. Age at diagnosis of metastatic melanoma was tested as a continuous variable and also grouped by decade.

The primary melanoma was categorized as cutaneous, mucosal, or occult. Primary site was coded as: extremity (no distinction between proximal and distal), head and neck, trunk, mucosal, or occult. Melanoma histogenetic subtype was grouped for analysis as superficial spreading melanoma (SSM), nodular melanoma (NM), or other types (including desmoplastic, acral lentiginous, malignant blue naevus, mucosal, lentigo maligna melanoma, not classified and mixed).

The disease-free interval (DFI) was measured from date of primary melanoma diagnosis to diagnosis of metastatic melanoma. OS was calculated from diagnosis of metastatic melanoma to last follow-up (censored) or death from melanoma (event). Univariate survival analysis was carried out using the Kaplan–Meier method together with the log-rank (Mantel–Cox) test to calculate statistical significance. Univariate 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 DFI 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. For all analyses, 2-tailed *P* values less than 0.05 were considered statistically significant. All analyses were prespecified and carried out with the IBM SPSS Statistic 19.0 software package.

Results

Three hundred and eight patients were accrued, with a median age at diagnosis of metastatic melanoma of 61.5 years, ranging from 20 to 87 years (Fig. 1A). There was no significant difference in age between male and female patients.

For the patients with complete information available on their primary melanoma (N = 301), the majority had primary cutaneous melanoma (84%), and the remainder had an occult (14%) or mucosal (2%) primary melanoma (Table 1). Mutated *BRAF* was found in tumors from 143 patients (46%); 105 patients (73%) had V600E, 27 (19%) had V600K, and 11 (8%) had other genotypes [V600E and V600K ($n = 1$), V600E and L597V ($n = 1$), V600E and K601E ($n = 1$), K601E ($n = 4$), V600_K601E ($n = 1$), D594N ($n = 1$), T599dup ($n = 1$), V600R ($n = 1$)]. There was no significant difference in *BRAF* genotypes between male and female patients.

Primary melanoma and *BRAF* genotype

BRAF-mutant metastatic melanoma was significantly associated with superficial spreading and nodular primary melanoma histogenetic subtypes ($P < 0.001$) and not associated with mucosal or acral primary melanoma types ($P = 0.048$) when compared with wild-type disease.

BRAF wild-type metastatic melanoma was associated with a history of multiple primary melanomas ($P = 0.048$; Table 1). *BRAF* mutation status was not associated with the anatomic site of the primary cutaneous melanoma ($P = 0.260$, Table 1).

There was no association between the specific *BRAF*-mutant genotype and the histogenetic subtype of the primary cutaneous melanoma. However, there were significant differences between V600E and V600K genotypes and the site of primary melanoma (Table 2). The site of the primary melanoma was most commonly the trunk for both genotypes (38% vs. 41%), however, V600E mutant melanomas had a higher proportion on the extremities (33% vs. 11%, $P = 0.030$) and V600K mutant melanomas had a higher proportion involving the head and neck region (33% vs. 11%, $P = 0.013$). The proportion of occult primary melanoma was similar in the two *BRAF*-mutant genotypes.

Cumulative sun-induced damage and *BRAF* genotype

Histopathologic slides were available to score the degree of CSD at the primary tumor site for 138 of 253 (54.5%) primary cutaneous melanomas from the overall cohort (Table 3). There was no significant difference in the degree of CSD (low, scores 0 or 1 vs. high, scores 2 or 3) between *BRAF*-mutant and wild-type melanoma overall ($P = 0.21$). However, there was a significant difference between *BRAF*-mutant genotypes, with V600K mutant melanomas associated with high levels of CSD (scores 2 or 3), and V600E mutant melanomas associated with little or no CSD (scrores 0 or 1; $P = 0.002$). These results were not influenced by patient age as they persisted when the patients aged >50 years ($P = 0.029$) and ≤50 years ($P = 0.051$) were analyzed separately.

Age at diagnosis of metastatic melanoma and *BRAF* genotype

BRAF-mutant metastatic melanoma was associated with significantly younger age at diagnosis of first distant metastasis (mean, 53.9 years; SD, 15.4) compared with *BRAF* wild-type melanoma (mean, 62.7 years; SD, 12.0, $P < 0.001$). There was an inverse relationship between the *BRAF* mutation prevalence and age-decade ($P < 0.001$; Fig. 1A.). All patients <30 years and only 25% of patients ≥70 years had *BRAF*-mutant metastatic melanoma.

Age was associated with the *BRAF*-mutant genotype. The frequency of non-V600E genotypes (including V600K) increased with increasing age-decade. Less than 20% of patients under 50 years with *BRAF*-mutant melanoma were
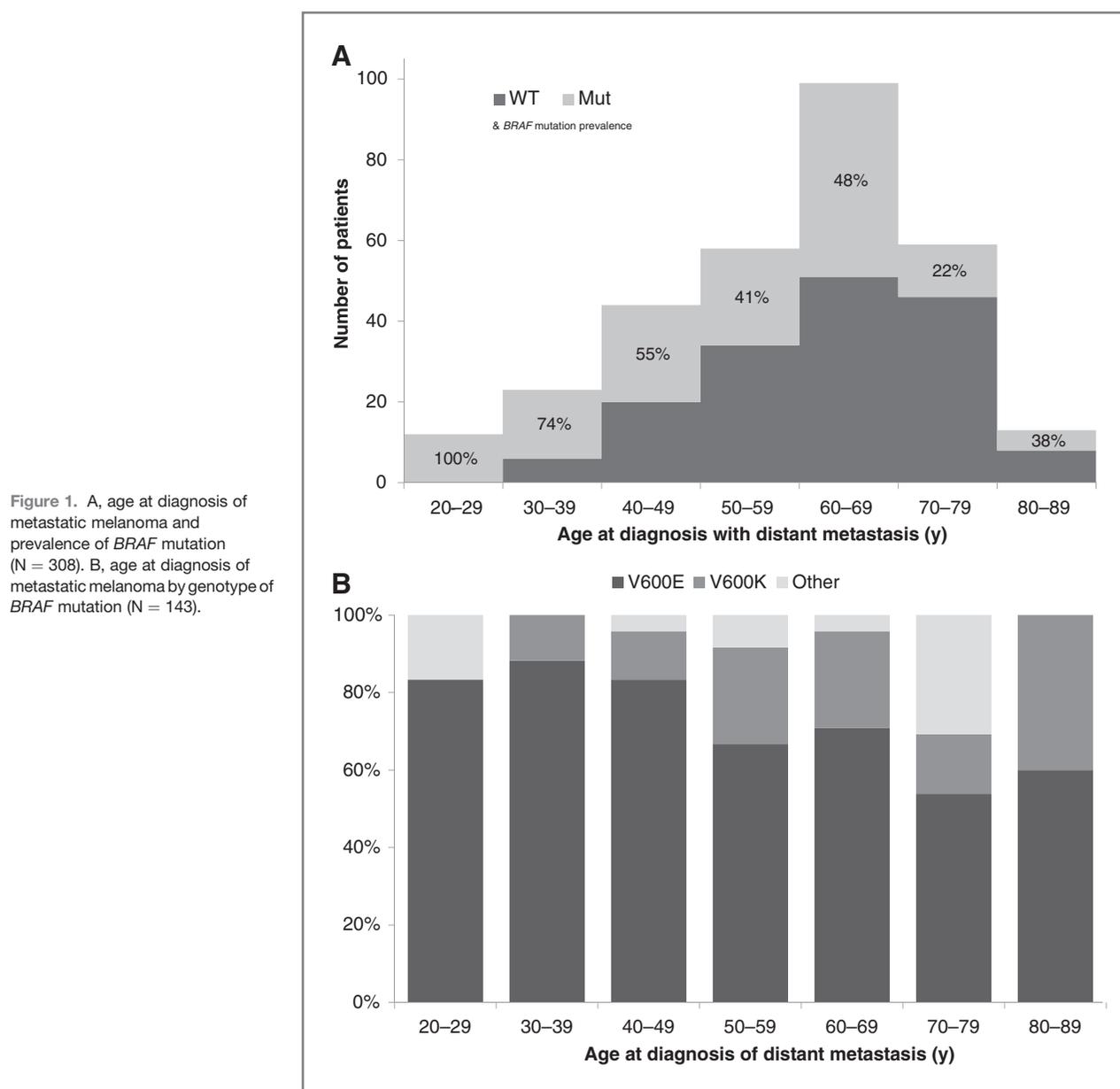
Figure 1. A, age at diagnosis of metastatic melanoma and prevalence of *BRAF* mutation (N = 308). B, age at diagnosis of metastatic melanoma by genotype of *BRAF* mutation (N = 143).

non-V600E, and >40% of patients ≥70 years were non-V600E (Fig 1B).

V600E mutant metastatic melanoma was associated with significantly younger age at diagnosis of first distant metastasis (median = 53 years) than V600K mutant melanoma (median = 61 years, *P* = 0.031) or *BRAF* wild-type melanoma (median = 64 years, *P* < 0.001). There was no significant difference between the median age of *BRAF* wild-type and V600K melanoma (*P* = 0.152). Sex did not influence the associations between age and *BRAF* status, nor age and *BRAF*-mutant genotype.

DFI and survival analysis
There was no significant difference in DFI from diagnosis of primary melanoma to first distant metastasis when

comparing patients with *BRAF* wild-type and mutant metastatic melanoma. However, within the *BRAF*-mutant melanoma cohort, the DFI was significantly shorter for patients with the V600K genotype compared with V600E genotype (17.4 vs. 39.2 months, *P* = 0.048; (HR, 1.90; 95% CI, 1.18–3.06, *P* = 0.009; Fig. 2). This difference remained significant in multivariate analysis adjusted for age, sex, and primary melanoma histogenetic subtype.

Fifty-three of 143 (37%) patients with *BRAF*-mutant metastatic melanoma received therapy with *BRAF*, *MEK*, or combination (*BRAF* and *MEK*) inhibitors during the study. Reasons for not obtaining targeted therapy included a limited number of places on the dabrafenib phase I trial, rapidly progressive disease with clinical deterioration during trial screening, poor performance status, the presence of

Table 1. *BRAF* mutation status according to characteristics of the primary melanoma (n = 301)

	Mutant *BRAF* (*n* = 139)	Wild-type *BRAF* (*n* = 162)	
Primary type	**No. of patients (%)**	**No. of patients**	**P**
Cutaneous	115 (45)	138 (55)	P = 0.183
Occult	23 (55)	19 (45)	
Mucosal	1 (17)	5 (83)	
Histologic subtype			
Superficial spreading	56 (64)	31 (36)	P< 0.001
Nodular	43 (50)	43 (50)	
Desmoplastic	2 (15)	11 (85)	
Acral lentiginous	0 (0)	5 (100)	
Other[a]	2 (18)	10 (82)	
Not classified	5 (50)	5 (50)	
Missing data	31	57	
Primary site			
Trunk	54 (50)	53 (50)	P = 0.260
Extremities	37 (41)	51 (59)	
Head and neck	24 (42)	34 (58)	
Occult	23 (55)	19 (45)	
Mucosal	1 (17)	5 (83)	
Number of primary melanomas			
Occult or single	127 (50)	125 (50)	P = 0.048
Multiple	11 (32)	23 (68)	
Missing data	1	14	

NOTE: Seven of 308 patients did not have primary melanoma history data available (2%).
[a]Other includes mucosal SSM type (*n* = 2), mucosal lentiginous (WT = 1, Mut = 1), mixed (*n* = 1), malignant blue naevus (WT = 2, Mut = 1), lentigo maligna melanoma (*n* = 4).

brain metastases, and patient preference. Median OS from date of diagnosis of metastatic melanoma was significantly longer for patients with *BRAF*-mutant melanoma treated with an inhibitor (45.2 months) than patients with *BRAF*-mutant melanoma not treated with an inhibitor (9.0

months, $P < 0.001$; HR, 0.35; 95% CI, 0.21–0.57, $P < 0.001$) and patients with *BRAF* wild-type melanoma (15.1 months, $P = 0.002$; Supplementary Fig. S1).

As the study examined a consecutive population of metastatic melanoma, not just newly diagnosed patients, it was enriched for longer term survivors who present for follow-up at regular intervals and were therefore more likely to be *BRAF* tested. When only patients with newly diagnosed metastatic melanoma during the study period were analyzed, survival differences were more pronounced, with 1 year survival rates of 83%, 29%, and 37% in patients with *BRAF*-mutant melanoma treated with an inhibitor, *BRAF*-mutant melanoma not treated with an inhibitor, and *BRAF* wild-type melanoma, respectively (Fig. 3). The median OS for patients with *BRAF*-mutant melanoma treated with an inhibitor was far greater than for those not treated with an inhibitor (>18.0 vs. 6.5 months, $P < 0.001$; HR, 0.25; 95% CI, 0.13–0.50, $P < 0.001$). The difference in OS between the patients with wild-type and *BRAF*-mutant melanoma not treated with an inhibitor was not statistically significant.

OS was not significantly different in patients with the V600E and V600K genotypes from the date of diagnosis of metastatic melanoma, including when analyzed according to the subgroups treated with or without a *BRAF* inhibitor.

Table 2. Comparing site of primary melanoma for V600E and V600K *BRAF* genotypes (n = 129)

	V600K	V600E	
Primary site	**No. of patients (%)**	**No. of patients (%)**	**P**
Extremity	3 (11)	34 (33)	0.030
Trunk	11 (41)	39 (38)	NS
Head and neck	9 (33)	11 (11)	0.013
Mucosal	0 (0)	1 (1)	NS
Occult	4 (15)	17 (17)	NS
Total	27 (100)	102 (100)	

NOTE: 129 patients had V600E or V600K genotypes and information on their site of primary melanoma ("other" mutant *BRAF* genotypes with information on primary melanoma excluded from this analysis *n* = 10).
Abbreviation: NS, not significant.

Table 3. Association of *BRAF* status with degree of CSD at the primary melanoma site (n = 138)

BRAF genotype	Low CSD No. of patients (%)	High CSD No. of patients (%)	Total	*P*
V600E	29 (74)	10 (26)	39	Reference
V600K	4 (25)	12 (75)	16	0.002
Other[a]	3 (43)	4 (57)	7	NS
Wild-type	36 (49)	40 (51)	76	0.009

NOTE: 138 patients had primary tissue available for CSD scoring.

Abbreviation: NS, not significant.

[a]Other includes V600E and V600K (*n* = 1), V600E and L597V (*n* = 1), K601E (*n* = 1), V600_K601E (*n* = 1), D594N (*n* = 1), T599dup (*n* = 1), V600R (*n* = 1).

Discussion

This study shows there are clinically distinct subtypes of *BRAF*-mutant metastatic melanoma, defined by their genotype, and can only be analyzed in a study of this size. As far as we are aware, this is the first study to (i) determine the *BRAF* mutation prevalence and genotype proportions by age-decade, (ii) show an association between *BRAF*-mutant genotype and CSD, (iii) explore the impact of *BRAF*-mutant genotype on prognosis. The results of this study are strengthened by the fact that it analyses a prospectively assembled cohort of patients with metastatic melanoma, with central review by a single pathologist to score the degree of CSD. It incorporates a comprehensive *BRAF* mutational testing method conducted at a single laboratory, coupled with a

broad range of clinicopathologic variables over a long follow up period. Despite the consecutive accrual of a large number of patients, the possibility that referral bias may have influenced the results cannot be excluded in this study from a single quaternary referral center.

This study determined the prevalence of the *BRAF* mutation by age-decade, a useful statistic for complex discussions in the clinic about possible treatment pathways, and showed that the prevalence of the *BRAF* mutation is inversely proportional to age. Younger patients with metastatic melanoma had a higher prevalence of *BRAF* mutation and a predominance of the V600E genotype. Older patients had a lower prevalence of *BRAF* mutation and a higher proportion of non-V600E genotypes, predominantly V600K. In addition, the V600K genotype was independently associated with higher degrees of CSD change at the primary site compared with that seen in V600E melanoma. These
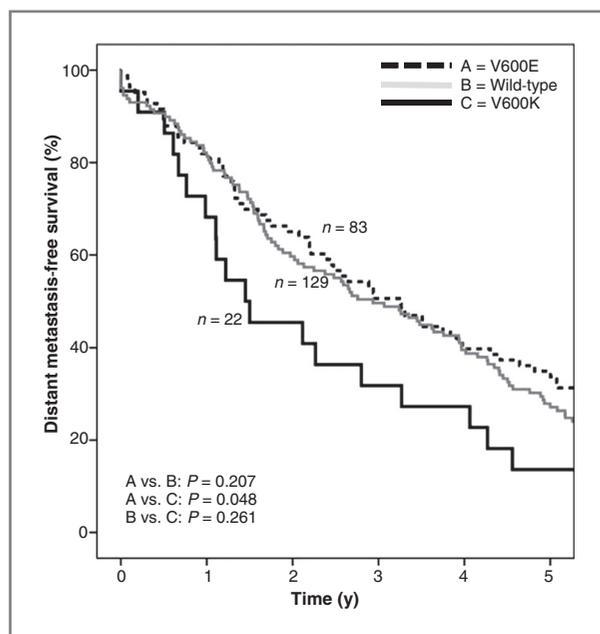

Figure 2. DFI from primary diagnosis to distant metastasis based on *BRAF* genotype (N = 234). Of the 301 patients with details on their primary, this survival analysis excludes patients with "other" *BRAF*-mutant genotypes (*n* = 10), remaining patients with occult primary melanoma (*n* = 40), and remaining patients with no recorded date of primary diagnosis (*n* = 17).
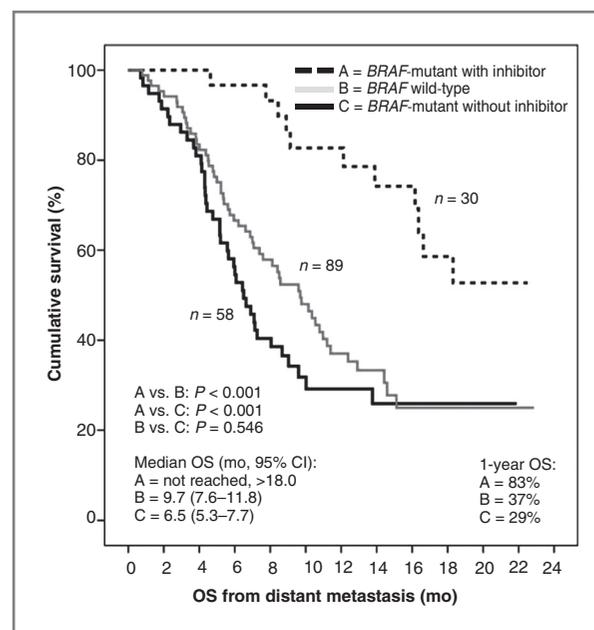

Figure 3. OS from distant metastasis for patients *BRAF* tested at diagnosis of metastatic disease (N = 177). *BRAF* inhibitor only (*n* = 20), *MEK* inhibitor only (*n* = 2), or combination (*BRAF* + *MEK*) inhibitors (*n* = 8).

findings suggest that the etiology of V600K melanoma differs from that of V600E melanoma and, unlike the latter, the cumulative effects of UV irradiation may play an important role in its pathogenesis. This is supported by the higher prevalence of V600K *BRAF* mutations in melanoma-observed patients from in geographic regions with higher ambient UV exposure (15, 16) than in patients from regions with lower UV levels (18, 29, 30).

Neither the T → A change in V600E, or the GT → AA change in V600K *BRAF* mutations, have the classic UVB signature (CC → TT or C → T) associated with pyrimidine dimer formation following exposure to UV light (31). However, evidence is emerging that UV irradiation may cause other DNA alterations, including G → T and G → A mutations (32), thus a direct role for cumulative UV exposure cannot be ruled out as a contributing causative mechanism for the development of V600K melanoma. A complex pathogenic mechanism is a more likely explanation for V600K melanoma, where the direct and indirect effects on melanocytes of cumulative sun exposure coupled with age-related genetic alterations (endogenous or exogenous) may provide a genetic foundation on which V600K is selected. Cumulative sun exposure may in addition provide a microenvironment in which emergence of V600K clones are favored. Conversely, melanoma in the young almost exclusively carries the *BRAF* mutation and is strongly associated with the V600E genotype, implying a more unifying etiology and a specific carcinogen and/or genetic predisposition.

As well as appearing etiologically distinct, the V600E and V600K genotypes show differing clinical behavior. Despite no statistical difference being detected in OS, possibly due to limitations in the sample size, patients with V600K melanoma had a significantly shorter DFI from the date of primary melanoma diagnosis. There is no clear explanation for this DFI difference however it does provide further evidence in support of our hypothesis that these genotypes represent biologically and clinically distinct subtypes of melanoma. The DFI result is subject to potential bias, for example patients with V600K melanoma are older and therefore are more likely to die from other causes before developing metastatic disease. To be more certain of any true difference in DFI, prospective assessment should be conducted on a large population of patients with early-stage melanoma, considering other prognostic features including TN stage at primary diagnosis, and accounting for extremes of age.

Despite the apparent differences in phenotype, there is evidence that drugs selectively inhibiting mutant *BRAF* are active in both V600E and other V600 genotypes (7, 11). It is critical that diagnostic tests for the *BRAF* mutation have the ability to detect the full range of V600 genotypes, as some in clinical use fail to do so. As an example, failure to detect V600K mutations may prevent 20% to 30% (13, 15, 16) of patients with *BRAF*-mutant melanoma (i.e., up to 10%–15% of the total metastatic melanoma population) from gaining access to active therapies. This proportion could increase with ageing of the melanoma-prone population.

Identifying clinicopathologic correlates of *BRAF* genotypes, particularly an age-decade–specific prevalence, does not obviate the need for *BRAF* mutation testing in patients with metastatic melanoma. It should, however, assist clinical judgement, planning, and pretest counseling due to the considerable window period between requesting *BRAF* mutation testing and receiving the *BRAF* genotype.

This is the first study to report a median OS from time of diagnosis of metastatic disease for patients with *BRAF*-mutant metastatic melanoma treated with and without a *BRAF* inhibitor. Patients with *BRAF*-mutant melanoma treated with a *BRAF* inhibitor had a clinically significant prolongation of survival when compared with patients with *BRAF*-mutant melanoma not treated with an inhibitor. Referral and selection bias may have confounded this result, as *BRAF* inhibitor medication was only available to these patients through participation in a clinical trial. Independent prognostic factors which could have precluded study entry, such as performance status and the presence of brain metastases, may have influenced this result. Nevertheless, the survival results in those patients with *BRAF*-mutant melanoma who did not receive an inhibitor reflect published survival data, suggesting that this cohort were not unduly negatively affected by selection bias (33). Furthermore, the 1-year survival of patients with *BRAF*-mutant metastatic melanoma treated with an inhibitor in this study (83%) is consistent with the result for the phase II vemurafenib study (58%; ref. 10), which calculated survival from commencement of *BRAF* inhibitor after prior systemic therapy, rather than from first distant metastasis.

The differences in the clinicopathologic phenotype amongst *BRAF*-mutant genotypes suggest that *BRAF*-mutant melanoma consists of specific disease subtypes with different etiology and behavior. Despite this, current *BRAF* inhibitors in clinical practice and in development are active against V600 *BRAF* mutant melanoma, meaning that it is essential to detect all these mutations when carrying out *BRAF* mutation testing in patients with metastatic melanoma. Furthermore, large collaborative studies are required to confirm these findings given the low proportion of non-V600E *BRAF* mutations.

Disclosure of Potential Conflicts of Interest

Authors' Contributions

Conception and design: A.M. Menzies, J.R. Howle, J.F. Thompson, R.F. Kefford, G.V. Long
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.M. Menzies, L.E. Haydu, M.S. Carlino, R.F. Kefford, R.A. Scolyer, G.V. Long
Writing, review, and/or revision of the manuscript: A.M. Menzies, L.E. Haydu, M.S. Carlino, J.F. Thompson, R.F. Kefford, R.A. Scolyer, G.V. Long
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L.E. Haydu, J.R. Howle, J.F. Thompson, R.F. Kefford, G.V. Long
Study supervision: R.A. Scolyer, G.V. Long

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