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MELANOCYTIC TUMOUR PATHOLOGY

The molecular profile of metastatic melanoma in Australia

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Summary

Targeted therapy directed at driver oncogenic mutations offers an effective treatment option for select patients with metastatic melanoma. The aim of this study was to assess the prevalence of clinically significant somatic mutations, specifically *BRAF, NRAS* and *KIT*, in a large cohort of Australian patients with metastatic melanoma. We performed a cross-sectional cohort study of consecutive patients with American Joint Committee on Cancer (AJCC) stage IIIc unresectable or stage IV melanoma managed at Melanoma Institute Australia, and affiliated sites, that underwent molecular testing between 22 June 2009 and 19 July 2013. Additionally, we examined the change in *BRAF* testing methodology and patient population over time, and how this influenced the prevalence of mutations. A total of 767 molecular tests were conducted for 733 patients. *BRAF* V600 mutation testing was performed for 713 patients (97.2%), with an overall mutation prevalence of 37.7% (269/713); 74.3% (200/269) were the V600E genotype and 22.3% (60/269) V600K. The *BRAF* mutation prevalence and proportion of *BRAF* V600E and V600K genotypes varied across the study period, as did testing methodology and the median age of the cohorts. Of 222 patients who underwent *NRAS* testing, 58 (26.1%) had a mutation identified. The overall prevalence of *KIT* mutations was 3.7% (11/296). In Australia the prevalence of *BRAF* mutations is lower than initially reported, although this remains the most common mutation identified in metastatic melanoma and an important therapeutic target. *NRAS* mutations are more prevalent than initially described; however, other mutations reported in melanoma, including *KIT*, are rare in an unselected population of patients.

Key words: BRAF; diagnosis; KIT; melanoma; molecular testing; NRAS; OncoCarta; pathology; somatic mutations; targeted therapy.

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INTRODUCTION

The identification of constitutively activating mutations in the *BRAF* gene in cutaneous melanomas[1] and the subsequent development of highly effective therapies targeting this aberration[2–4] have fundamentally changed the management of patients with unresectable American Joint Committee on Cancer (AJCC) stage III and IV melanoma. Although an increasing number of driver mutations are being identified,[5–7] mutations in *BRAF*, *NRAS* and *KIT* are of most clinical relevance due to the efficacy and availability of targeted therapies or relevant clinical trials.

BRAF mutation prevalence in cutaneous melanoma is reported as ranging from 40 to 60%, and was 41% in a meta-analysis.[8–13] However, clinical experience suggests that the prevalence in the general population of patients with metastatic melanoma is likely to be lower. Similarly, although *NRAS* mutations are reported to occur in 15–20% of patients, the prevalence in an unbiased cross-sectional population has not been well established.[10–13] *KIT* aberrations, including mutations and increased copy number, occur particularly in acral lentiginous and mucosal melanoma subtypes, and only rarely (~3%) in non-acral cutaneous melanoma.[10,14–16]

Multiplex gene testing that simultaneously tests for a range of oncogenes facilitates stratification of metastatic melanoma patients to enable access to available therapies or enrolment in clinical trials.[10,17,18] A number of different technologies are available, including mass spectrometry and next generation sequencing platforms. This strategy of comprehensive molecular profiling is becoming standard of care for many solid tumours, including at specialist melanoma centres.

The aim of this study was to assess the prevalence of clinically significant somatic mutations in a large cohort of Australian patients with metastatic melanoma in a setting where testing was performed in all patients as part of routine clinical care. Additionally, the change in *BRAF* testing methodology and patient population over time, and how this influenced the prevalence of mutations, were analysed.

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METHODS

Patient selection and data collection

Consecutive patients with AJCC stage IIIc unresectable or stage IV melanoma managed at Melanoma Institute Australia (MIA), and affiliated sites Westmead Hospital and Royal Prince Alfred Hospital (RPAH), who underwent molecular testing coordinated by the Molecular Diagnostic Oncology unit of the Department of Tissue Pathology RPAH between 22 June 2009 and 19 July 2013 were included in the cohort. Clinical data were retrospectively collected for patient demographics (median age at stage IV diagnosis and sex) and disease stage. The availability of drug treatment and the funding of molecular testing throughout the study period were recorded. The study was undertaken with institutional Human Ethics Review Committee approval.

Molecular testing techniques and tissue processing

Molecular testing was performed on sections from archival formalin fixed, paraffin embedded (FFPE) tissue with macrodissection as required. Data on the type of melanoma tissue used for molecular testing (primary, locoregional or metastatic), and the organ from which the tissue was obtained, were collected and analysed to assess for any difference in mutation prevalence.

Four methods of molecular testing were used in this study. High resolution melt (HRM) and Sanger sequencing of *BRAF* exon 15 was performed at Peter MacCallum Cancer Centre, Department of Diagnostic Molecular Pathology (Melbourne, Australia). *BRAF* V600 allele-specific hotspot polymerase chain reaction (PCR) assay was performed at Healthscope Pathology (Clayton, Australia). The Sequenom OncoCarta v1.0 mass array panel (http://bioscience.sequenom.com/oncocarta-panel) was performed at Royal Prince Alfred Hospital, Department of Clinical and Molecular Pathology (Sydney, Australia). The Sequenom OncoCarta panel v1.0 tests for 238 somatic mutations in 19 oncogenes, including *NRAS* and limited *KIT* hotspots (Sequenom, USA) (Supplementary Table 1). HRM and Sanger sequencing of *KIT* exons 11, 13, 17 was performed at Peter MacCallum Cancer Centre, Department of Diagnostic Molecular Pathology (Melbourne, Australia). All testing was performed in Australian National Association of Testing Authorities (NATA) accredited diagnostic laboratories to the ISO15189 standard. The methodologies of these molecular tests have been previously published.[14,19]

Statistical analysis

Associations between the prevalence of *BRAF* or *NRAS* mutations and variables including age, tumour site and degree of spread were tested using chi-square, Kruskal–Wallis and Mann–Whitney U tests with a threshold of significance of $p < 0.05$. IBM SPSS Statistics v21 (Chicago, USA) software was used to conduct all analyses.

RESULTS

Patients and molecular testing techniques

A total of 767 molecular tests were conducted for 733 evaluable patients between 22 June 2009 and 19 July 2013. Four patients with discrepant results in whom the true molecular profile could not be definitively determined were excluded. Two patients had conflicting results on the same tumour specimen using different methodologies (*BRAF* V600 positive on one test but wild-type on another), one patient had different *BRAF* genotypes reported on two tests (V600K on one test and V600E on a later specimen) and a final patient had conflicting results on two different specimens (*BRAF* mutant on one specimen but wild-type on another).

BRAF V600 mutation testing was performed for 713 patients (97.2%); in 420 patients (58.9%) this was performed by *BRAF* exon 15 HRM and Sanger sequencing, in 223 (31.3%) using the OncoCarta panel, in 49 (6.9%) utilising *BRAF* V600 allele-specific PCR assay and 21 (2.9%) patients underwent testing using a combination of these methods. *NRAS* mutation test was performed on 222 patients (30.3%), all using the OncoCarta panel. *KIT* mutation testing was performed on 296 patients (40.1%); 212 (71.6%) by OncoCarta panel, 74 (25%) had *KIT* exon 11, 13, 17 Sanger sequencing and 10 patients (3.4%) had testing using both platforms. Using the OncoCarta panel, 222 patients (30.3%) also underwent testing for additional somatic mutations.

Time trends in the number and type of *BRAF* mutation tests and the prevalence of *BRAF* V600E and V600K genotypes were analysed by defining eight cohorts of patients delineated by 6-month intervals from 1 July 2009 to 30 June 2013 (n = 738) (Fig. 1). The proportion of molecular tests performed by each method varied (Supplementary Table 2). In the initial two cohorts of 1 July – 31 December 2009 (n = 147) and 1 January – 30 June 2010 (n = 43) only *BRAF* V600 HRM and Sanger sequencing was used (100%). In contrast, in the last two cohorts of 1 July – 31 December 2012 and 1 January – 30 June 2013, the OncoCarta panel comprised 95.1% (116/122) and 97.7% (84/86) of tests, respectively.

The first cohort was the largest since it comprised the patients diagnosed over the preceding months to years who underwent *BRAF* testing once this became a pathway for access to potentially effective therapy as part of clinical trials. In addition, at that time the testing was subsidised under research protocols. Another peak in demand was seen in the second half of 2012, a time when *BRAF* testing was funded by a pharmaceutical company scheme. Each patient individually funded the test at other times.

Throughout the study period patients had access to clinical trials of BRAF inhibitor, with or without MEK inhibitor, therapy (Fig. 1) and from 2011 there was also access to treatment via compassionate schemes. No BRAF inhibitor was subsidised by the pharmaceutical benefit scheme (PBS) until after the study period (December 2013).

The median age at stage IV diagnosis of patients undergoing molecular testing varied across the study period (Fig. 1). In the initial cohort (1 July – 31 December 2009) the median age was 60 years, in contrast to 69 years in the most recent cohort (1 January – 30 June 2013). The male to female ratio ranged from 1.6 to 2.7.

Tumour tissue used for testing

The melanoma specimen used for molecular testing was recorded in all cases and was classified by organ/tissue type and also as primary site, locoregional disease (including local recurrence, in-transit or regional lymph node metastasis), or distant metastasis (Supplementary Table 3). Primary tissue was used in 88 cases (11.5%), locoregional metastases in 379 (49.4%) and distant metastases in 300 (39.1%). The prevalence of *BRAF*, *NRAS* and *KIT* mutations did not vary significantly between these groups, or by organ.

Prevalence of mutations

The overall prevalence of *BRAF* V600 mutations was 37.7% (269/713); 74.3% (200/269) were the V600E genotype, 22.3% (60/269) V600K, 3.0% (8/269) V600R and 0.4% (1/269) V600M. The proportion of *BRAF* V600E and V600K genotypes varied across the study period (Fig. 1 and 2). *BRAF*