Mutation status among patients with sinonasal mucosal melanoma and its impact on survival

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Background: Sinonasal mucosal melanoma (SNMM) comprises <1% of all melanomas and lacks well-characterised molecular markers. Our aim was to determine the frequencies of common mutations and examine their utility as molecular markers in a large series of primary SNMMs.

Methods: SNMM patients seen at our institution from August 1991 through July 2016 were identified. Genomic DNA was extracted from 66 formalin-fixed paraffin-embedded tumours and screened for mutations by direct sequencing. We investigated the association of mutations with clinicopathological features and survival outcomes.

Results: Overall, 41% (27 out of 66) of the SNMMs harboured mutations. BRAF and KIT mutations were identified in 8% (five patients) and 5% (three patients) of SNMMs, respectively, whereas NRAS mutations were detected in 30% (20 patients) of SNMMs. Mutation rates in these oncogenes were similar between SNMMs located in the paranasal sinuses and those in the nasal cavity (30% and 13%, respectively, \( P = 0.09 \)). In a multivariate analysis, patients with negative margins had significantly better overall survival (hazard ratio 5.43, 95% confidence interval 1.44–21.85, \( P = 0.01 \)) and disease-specific survival (hazard ratio 21.9, 95% confidence interval 3.71–180, \( P = 0.0004 \)). The mutation status of the tumours showed no association with survival outcomes.

Conclusions: In SNMM, mutation status does not affect survival outcomes, but NRAS mutations are relatively frequent and could be targeted in this disease by MEK inhibitors.

Mucosal melanoma represents approximately 1.3% of all melanomas (Gal et al, 2011). While mucosal melanoma can arise from any mucosa-lined body surface, approximately half of all mucosal melanomas occur in the head and neck, most frequently in the sinonasal cavity (Lourenco et al, 2014; Sun et al, 2014). Sinonasal mucosal melanomas (SNMMs) account for ~4% of sinonasal malignancies and <1% of all melanomas (Moreno et al, 2010; Gal et al, 2011; Lourenco et al, 2014).

Sun exposure is a well-known risk factor for cutaneous melanoma, but the risk factors for SNMM are less well defined (Spencer and Mehnert, 2016). Patients usually present later in life, with no obvious sex predilection (Spencer and Mehnert, 2016). The mitogen-activated protein kinase (MAPK) pathway has been shown to be important in the development of melanoma (Curtin et al, 2005a). In cutaneous melanoma, between 22 and 72% of cases have BRAF mutations, and 0 to 50% have NRAS mutations (Lee et al, 2011); however, molecular markers in mucosal melanoma are less well characterised. While recent studies suggest that BRAF inhibition has a promising effect in cutaneous melanoma, its role in SNMM has yet to be defined (Zebary et al, 2013a; Spagnolo et al, 2016).
SNMM is an aggressive tumour, and patients with SNMM often present with advanced disease (Ledderose and Leunig, 2015). Despite advances in treatment, survival is poor, with a 5-year survival rate of ~20–30% (Moreno et al, 2010). Single-modality therapy with surgery is rarely adequate for this disease, particularly for SNMMs, in which anatomical and quality-of-life constraints make obtaining adequate margins very difficult and sometimes impossible (Samstein et al, 2016). Therefore, adjuvant therapy is a keystone in the treatment of SNMM. As more options arise for targeted therapy, the need to characterise molecular markers in SNMM has become increasingly important.

This study quantifies molecular features and attempts to identify molecular markers in SNMM. We also investigated the correlation of molecular features with clinicopathological features and survival outcomes to determine their prognostic utility in this disease.

**MATERIALS AND METHODS**

This retrospective review was approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center (Protocol RCR04-0636). We surveyed 170 consecutive patients seen at our institution from August 1991 through July 2016 with a pathologically confirmed diagnosis of head and neck mucosal melanoma involving the sinonasal cavity. The inclusion criteria for the analysis were: (1) pathologically confirmed mucosal melanoma; (2) sinonasal origin; (3) available outcome data; (4) available tissue for molecular analysis; and (5) adequate genetic material for analysis. Patient demographic features (age, sex, smoking status and alcohol intake), disease stage, tumour characteristics, treatment modalities used, pathological data (ulceration, perineural status), and survival outcomes were collected. All staging was completed according to the American Joint Committee on Cancer Staging Manual, 7th edn (Edge et al, 2010).

The primary aim was the incidence of hotspot mutations. The secondary aim was the association between hotspot mutations and survival outcomes—overall survival (OS), disease-specific survival (DSS), disease-free survival (DFS) and distant metastasis-free survival (DMFS)—and with clinicopathological features. The index date for survival outcomes for OS and DSS was set as the date of treatment initiation. DFS was defined as the time from the date of completion of primary treatment to the earliest evidence of disease recurrence. DMFS was defined as the time from the date of completion of primary treatment to the earliest evidence of distant metastasis.

**Mutation analysis.** Tumour cells were identified in regions with >20% nuclei. Genomic DNA was extracted from formalin-fixed paraffin-embedded tumours and subjected to PCR sequencing using a next-generation sequencing platform to screen for mutations in the coding sequences of 50 key signalling genes in melanoma (see Supplementary Table 1 for the full list of covered genes, exons and codons). The results of the next-generation sequencing were confirmed by a second independent PCR and sequencing reaction. The genomic reference sequence used was genome GRCh37/hg19. The sensitivity of the assay is related in part to depth of coverage, percentage of tumour cells with the mutation, and allelic frequency of the mutation. We determined the effective lower limit of detection of this assay (that is, analytical sensitivity) for single-nucleotide variations to be in the range of 5% (one mutant allele per 19 wild-type alleles) to 10% (one mutant allele per nine wild-type alleles) by considering the depth of coverage at a given base and the ability to confirm low-level mutations using independent conventional platforms. The variants detected by our assay were determined on the basis of both analytic findings, such as allelic frequency, and the currently available information in the curated reference databases COSMIC version 64 (Catalogue Of Somatic Mutations In Cancer, Wellcome Trust Sanger Institute, Hinxton, UK) and dbSNP version 137 (National Institute of Health, Bethesda, MD, USA).

**Statistical analysis.** Basic baseline descriptive statistics were generated. Continuous data were compared according to mutation status using the Student t-test, and categorical variables were compared according to mutation status using the χ²-test. The Kaplan–Meier method was employed for all survival analyses. Survival curves were stratified according to the presence of mutations and compared using the log-rank test. Univariate and multivariate Cox proportional hazards models were used to compare survival outcomes according to mutation status and clinicopathological features. All statistical tests were two-tailed. Significance was defined by an alpha set to 0.05. All statistical testing was completed on SAS JMP Pro version 12.1.0 (SAS Institute, Cary, NC, USA).

**RESULTS**

**Clinicopathological features.** Sixty-six patients met all inclusion and exclusion criteria. Patient and tumour characteristics are

| Characteristic | N (%) |
|---------------|-------|
| Age (mean ± s.d.) | 63 ± 13 years |
| Sex | |
| Female | 33 (50%) |
| Male | 33 (50%) |
| Smoking status | |
| Current | 6 (9%) |
| Former | 22 (33%) |
| Never | 38 (58%) |
| Alcohol consumption | |
| Current | 26 (39%) |
| Former | 6 (9%) |
| Never | 34 (52%) |
| Site | |
| Nasal cavity | 53 (80%) |
| Paranasal sinuses | 13 (20%) |
| T classification | |
| 3 | 35 (53%) |
| 4a | 23 (35%) |
| 4b | 8 (12%) |
| N classification | |
| N0 | 59 (89%) |
| N+ | 7 (11%) |
| Mitosis rate | |
| < 1 | 44 (66%) |
| ≥ 1 | 22 (33%) |
| Ulceration | |
| Absent | 37 (56%) |
| Present | 29 (44%) |
| Margins | |
| Positive | 22 (33%) |
| Negative | 44 (66%) |
| Treatment | |
| Surgery | 26 (39%) |
| Surgery + radiation | 28 (42%) |
| Surgery + chemotherapy | 2 (3%) |
| Surgery + chemoradiation | 2 (3%) |
| Chemotherapy | 4 (6%) |
| Chemoradiation | 2 (3%) |
| Follow-up time (mean ± s.d.) | 40.1 ± 5.6 months |

*Unless otherwise indicated.*
Table 2. Mutations identified in sinonasal mucosal melanoma

| Patient # | Gene | Mutation | Age | Sex | Tumour epicentre | Exon | Nucleotide change | Amino acid change |
|-----------|------|----------|-----|-----|------------------|------|------------------|------------------|
| 1         | TP53 | Missense | 44  | Male | Maxillary sinus  | 5    | c.404G>A         | p.C135Y          |
| 2         | TP53 | Missense | 62  | Female | Nasal cavity | 5    | c.488A>G         | p.Y163C          |
| 3         | NRAS | Missense | 68  | Male | Nasal cavity    | 2    | c.182A>G         | p.Q61R           |
| 4         | NRAS | Missense | 64  | Male | Maxillary sinus | 1   | c.37G>C          | p.G13R           |
| 5         | NRAS | Missense | 64  | Female | Maxillary sinus | 1   | c.37G>C         | p.G13R           |
| 6         | NRAS | Missense | 67  | Male | Nasal cavity    | 1    | c.37G>T          | p.G13C           |
| 7         | NRAS | Missense | 51  | Male | Nasal cavity    | 1    | c.38G>c          | p.G12A           |
| 8         | NRAS | Missense | 82  | Male | Maxillary sinus | 1    | c.38G>c        | p.G12A           |
| 9         | NRAS | Missense | 78  | Male | Nasal cavity    | 1    | c.38G>A          | p.G13D           |
| 10        | NRAS | Missense | 65  | Female | Nasal cavity | 2    | c.181C>A        | p.Q61K           |
| 11        | NRAS | Missense | 75  | Female | Nasal cavity | 2    | c.181C>A        | p.Q61K           |
| 12        | NRAS | Missense | 62  | Female | Maxillary sinus | 2   | c.181C>A       | p.Q61K           |
| 13        | NRAS | Missense | 68  | Female | Nasal cavity | 2    | c.181C>A       | p.Q61K           |
| 14        | NRAS | Missense | 35  | Male | Nasal cavity    | 2    | c.181C>A       | p.Q61K           |
| 15        | NRAS | Missense | 36  | Female | Maxillary sinus | 2   | c.182A>G         | chr.8:38271444-38315644 |
| 16        | NRAS | Missense | 69  | Male | Nasal cavity    | 2    | c.182A>G        | p.Q61R           |
| 17        | NRAS | Missense | 62  | Female | Nasal cavity | 2    | c.182A>T        | p.Q61L           |
| 18        | NRAS | Missense | 77  | Female | Nasal cavity | 1    | c.34G>C        | p.G12R           |
| 19        | NRAS | Missense | 60  | Male | Maxillary sinus | 1   | c.35G>C        | p.G12A           |
| 20        | NRAS | Missense | 63  | Male | Nasal cavity    | 1    | c.35G>T        | p.G12V           |
| 21        | NRAS | Missense | 86  | Male | Nasal cavity    | 1    | c.37G>C        | p.G13R           |
| 22        | KIT  | Missense | 46  | Female | Nasal cavity | 2    | c.146G>A      | p.R49H           |
| 23        | BRAF | Missense | 37  | Male | Nasal cavity    | 15   | c.1799T>A     | p.V600E          |
| 24        | BRAF | Missense | 55  | Male | Nasal cavity    | 15   | c.1799T>A    | p.V600E          |
| 25        | BRAF | Missense | 64  | Male | Maxillary sinus | 15   | c.1799T>A   | p.V600E          |
| 26        | BRAF | Missense | 56  | Female | Nasal cavity | 15   | c.1799-1800GT>AA | p.D543E          |
| 27        | BRAF | Missense | 55  | Male | Nasal cavity    | 15   | c.1781A>G    | p.D594G          |

summarised in Table 1. There were 33 women and 33 men with a median age at diagnosis of 64 years (range 34–85 years). The tumour epicentre was located in the nasal cavity in 53 (80%) patients and in the paranasal sinuses in 13 patients (eight in the maxillary sinus, three in the sphenoid sinus, one in an ethmoid sinus and one in a frontal sinus). Thirty-five (53%) patients had T3 disease, 23 (35%) had T4a disease and eight (12%) had T4b disease. Nodal metastases were present in seven patients (11%). Surgery was the mainstay of treatment in 58 (88%) cases, and in 26 (39%) patients surgery was the only treatment modality. Adjuvant radiotherapy was administered in 30 (45%) patients, and four (6%) patients were treated with adjuvant chemoradiotherapy.

**Mutation analysis.** Of the 66 primary SNMMs analysed, 27 (41%) harboured at least one identified mutation, and 39 (60%) had no identified mutations. The most common mutation was NRAS mutation (n = 20, 30%, P < 0.001). Mutations in BRAF, KIT and TP53 occurred in five (8%), three (5%) and two (3%) patients, respectively (Table 2). In 24 patients (89% of the patients with at least one mutation), mutations in KIT, NRAS and BRAF were mutually exclusive.

The NRAS mutations involved codons 12 (G12A, G12R and G12V), 13 (G13R, G13C and G13D) and 61 (Q61K, Q61L, and Q61R). Eleven of the NRAS mutations were located in exon 1. The three KIT mutations were missense; of those, one was the hotspot mutation p.M541L in exon 10 with simultaneous KIT mutation (patient 26, Table 2). One tumour harboured a KIT mutation in exon 13 simultaneously with ERBB2, NOTCH1, P13K1 and TP53 mutations. No mutations were observed in exon 17 of KIT. Among the five BRAF mutations, four were in codon 600 (BRAF^V600E and BRAF^V600K), and one was in codon 594 (D594G). Both TP53 mutations were in exon 5; interestingly, one of the patients with TP53 mutation carried a germline polymorphism, but not mutation, of KIT (c.1621A>C p.M541L).

**Association of mutations with clinicopathological features.** The clinicopathological features of tumours with NRAS, KIT, TP53 or BRAF mutations and tumours lacking these mutations are compared in Table 3. Tumours with these mutations were more likely to be located in the paranasal sinuses (30%), whereas the lesions without identified mutations were more often found in the nasal cavity (13%); however, the difference in location was not
Mutations in sinonasal mucosal melanoma

In this study, we comprehensively screened primary SNMMs for over a hundred different mutations in more than 50 key genes in melanoma and found that NRAS mutations were prevalent (30%). In this retrospective, single-institution analysis, we did not find an association between mutation status and survival outcomes but did find that tumours with identified mutations had a higher mitosis rate.

Genomic aberrations are present in most melanomas (Hodis et al, 2012; Akbani et al, 2015). An increasing understanding of melanocytic biology and melanoma pathogenesis has led to the development of targeted therapies and the potential for major improvements in the care of patients with advanced melanoma. For now, large-scale genomic data in melanoma, derived mainly from cutaneous melanoma, focus on specific genes such as NRAS and its downstream mediator BRAF (Omholt et al, 2003). Targeting these pathways in patients with previously untreated melanoma with these mutations showed promising outcomes (Chapman et al, 2011). However, despite these breakthroughs, the prognosis of patients presenting with SNMM remains poor. Thus, we sought to characterise potential molecular markers in patients with these uncommon melanomas.

Published studies have reported slightly lower overall mutation rates in head and neck mucosal melanoma (10–25%) (Chraybi et al, 2013; Zebary et al, 2013b; Lyu et al, 2016; Ozturk Sari et al, 2016; Ozturk Sari et al, 2016). An initial report from a single institution of NRAS mutations in sinonasal mucosal melanoma (11%) (Thompson et al, 2003), including epithelioid, spindle, pleomorphic, rhabdoid, pagetoid, and undifferentiated (small) cells, was similar for patients with and without identified mutations. These differences were more significant when comparing tumour characteristics with respect to age at diagnosis, sex, smoking status, T classification, N classification or bone invasion. The occurrence rates of perineural and lymphovascular invasion were too low for analysis (n = 2 for both).

Association of mutations and clinicopathological features with survival outcomes. In the whole cohort, the 5-year OS rate was 39%, and the 5-year DSS rate was 54%. The 5-year OS rate was 43% in patients carrying a mutation and 37% in those without an identified mutation (log-rank P = 0.55; Figure 1A). The 5-year DSS rate was 54% for both mutation groups (log-rank P = 0.91; Figure 1B).

Recurrence occurred in 59 (89%) patients over the follow-up period; of these, 27 (40%) had distant metastases. The 5-year DFS was 24% in patients carrying a mutation and 11% for those without an identified mutation (log-rank P = 0.64; Figure 1C). A subgroup analysis of patients with NRAS mutations showed no association of NRAS mutations with DFS or DMFS (log-rank P = 0.31 and P = 0.57, respectively). In patients without identified mutations there was a trend toward a higher 5-year distant metastasis rate compared with patients carrying NRAS, KIT, TP53 or BRAF mutations (78% and 55%, respectively; log-rank P = 0.07; Figure 1D).

Univariate analysis comparing patients with and without detected mutations in their tumours showed no association of mutation status with either OS or DSS. To further assess the ability of mutation status to predict outcome in a more homogeneous population and to account for the potential impact of adjuvant treatment, we performed subgroup analyses of each of the following treatment groups: patients undergoing surgery alone (n = 30), patients undergoing postoperative radiotherapy (n = 30), and patients undergoing adjuvant chemoradiotherapy (n = 6). In all treatment groups, mutation status was not an independent predictor of OS or DSS (log-rank analysis, Supplementary Figure 1).

Patients with T3 disease had a significantly better prognosis than those with a T4a or T4b disease, with 5-year OS rates at 58%, 48% and 18%, respectively (log-rank P = 0.02, Figure 2A). Similarly, patients with negative margins had a better 5-year OS rate than patients with positive margins (54% and 27%, respectively; log-rank P = 0.009; Figure 2B). Of note, patients with tumours in the nasal cavity had a marginally better 5-year OS rate than those with tumours in the paranasal sinuses (48% and 22%, respectively; log-rank P = 0.06; Figure 2C). Multivariate Cox regression modelling of these data revealed that only margin status was a significant prognostic factor for OS (hazard ratio 5.43, 95% confidence interval 1.44–18.15, P = 0.01) and DSS (hazard ratio 21.9, 95% confidence interval 3.71–180, P = 0.0004). To control for margin status, we performed survival analyses separately in patients with positive and negative margins. This analysis revealed no difference in OS and DSS between patients with and without detected mutations in their tumours (Supplementary Figure 2).

DISCUSSION

Table 3. Association of identified mutations with clinicopathologic features

| Characteristic | Mutations not identified (n = 39) | Mutations identified (n = 27) | P-value |
|---------------|----------------------------------|-----------------------------|---------|
| Age (mean ± s.d.) | 65.2 ± 12.6 years | 61.3 ± 13.4 years | 0.23 |
| Sex | | | |
| Female | 17 (44%) | 16 (59%) | 0.20 |
| Male | 22 (56%) | 11 (41%) | |
| Smoking | | | |
| Current/former | 19 (49%) | 9 (33%) | 0.14 |
| Never | 20 (51%) | 18 (67%) | |
| Site | | | |
| Nasal cavity | 34 (87%) | 8 (30%) | 0.09 |
| Paranasal sinuses | 5 (13%) | 19 (70%) | |
| T classification | | | |
| 3 | 22 (56%) | 13 (48%) | 0.76 |
| 4a | 13 (33%) | 10 (37%) | |
| 4b | 4 (10%) | 4 (15%) | |
| N classification | | | |
| N0 | 36 (92%) | 23 (85%) | 0.36 |
| N+ | 3 (8%) | 4 (15%) | |
| Mitosis rate | | | |
| <1 | 27 (69%) | 10 (37%) | 0.01 |
| ≥1 | 12 (31%) | 17 (63%) | |
| Ulceration | | | |
| Present | 27 (69%) | 17 (63%) | 0.59 |
| Absent | 12 (31%) | 10 (37%) | |
| Cell morphology | | | |
| Pagetoid | 8 (21%) | 6 (22%) | 0.43 |
| Epithelioid | 7 (18%) | 7 (25%) | |
| Spindled | 7 (18%) | 9 (33%) | |
| Rhabdoid | 8 (21%) | 4 (15%) | |
| Undifferentiated (small cell) | 13 (33%) | 9 (33%) | |
| Bone invasion | | | |
| Absent | 33 (85%) | 20 (74%) | 0.35 |
| Present | 6 (15%) | 7 (21%) | |
| Treatment | | | |
| Surgery | 18 (46%) | 12 (44%) | 0.88 |
| Surgery and radiation | 18 (46%) | 12 (44%) | |
| Surgery and chemoradiation | 3 (8%) | 3 (12%) | |

a Unless otherwise indicated.
Recently, a combination of anti-BRAF agents have resulted in an increased response rate and longer duration of response in cutaneous melanoma patients (Larkin et al, 2017) than that in the current study (40%); however, there was a considerably similar distribution of specific mutation rates in these studies: NRAS, 14%-60%; BRAF, 0%-6%; and KIT, 3%-12% (Cohen et al, 2004; Beadling et al, 2008; Carvajal et al, 2011; Turri-Zanoni et al, 2013; Zebary et al, 2013b). The Cancer Genome Atlas and other large-scale genomic analysis efforts in melanoma have identified hotspot NRAS mutations, thought to be important drivers of oncogenesis, in 25–30% of cutaneous melanomas (Akbani et al, 2015; Krauthammer et al, 2015). Our data show a similar rate (30%) of NRAS mutations. However, in cutaneous melanoma, mutations at codon 61 (Q61R and Q61K) represent the two most common NRAS mutations. In the current study, only 40% of the patients carrying NRAS mutations had Q61R or Q61K mutations, whereas 55% of these patients had mutations in codons 12 (G12V, G12A, G12R and G12D) and 13 (G13R, G13C and G13D). These NRAS mutations at codons 12 and 13 are also prevalent in haematological malignancies (Ward et al, 2012). The different patterns of NRAS mutations in mucosal melanoma compared with cutaneous melanoma support an aetiology other than sun exposure. Another important risk factor in head and neck cancer is smoking. We found a trend towards a lower mutation rate in smokers; however, this difference did not reach significance.

We identified BRAF<sub>V600E</sub> and BRAF<sub>V600K</sub> mutations in only four out of 66 SNMMs. This frequency is similar to the incidence of BRAF mutations in mucosal melanomas from other sites such as the vulva, vagina and anorectum (Omholt et al, 2003; Curtin et al, 2005b).

Most melanoma samples that harboured a hotspot mutation in NRAS, KIT or BRAF did so in a mutually exclusive fashion. The two exceptions harboured BRAF<sup>V600</sup> mutations together with an oncogenic NRAS or KIT mutation. Two cases harboured a TP53 missense mutation in exon 5. Interestingly, one patient presented with NOTCH1, PI3KIR1, TP53 and KIT mutations, all of which have been previously shown to have a role in melanoma oncogenesis (Liu et al, 2006).

We found a mutation in KIT in only three out of 66 SNMMs. Of those cases, two had additional identified mutation (patients 2 and 26, Table 2). KIT mutations are associated with chronic sun damage in cutaneous melanoma, which is not an aetiologic risk factor in SNMMs (Curtin et al, 2005b). However, previous observations suggested that KIT is the most commonly mutated gene in mucosal melanoma, with up to 45% of vulvovaginal and anorectal melanomas carrying a mutation in KIT (Omholt et al, 2011; Schoenewolf et al, 2012). These findings suggest that KIT mutations differ between mucosal melanomas at different sites and are very rare in SNMMs.

We found a significantly higher mitosis rate in patients carrying an identified mutation. There also were trends toward a higher rate of mutations in tumours originating in the paranasal sinuses rather than the nasal cavity and worse prognosis in patients with disease originating from the sinuses compared with those with tumours originating from the nasal cavity. Our finding that mutation status, for all known mutations or for NRAS alone, did not affect survival in the setting of SNMM is in keeping with studies conducted before the availability of MEK inhibitors and immune checkpoint inhibitor antibodies (Ellerhorst et al, 2011). The high proportion...
of NRAS-mutated tumours suggests that further studies investigating the use of MEK inhibitors, which have shown promising phase II results in cutaneous melanoma with NRAS mutations, may be worthwhile in SNMMs (Ascierto et al., 2013). A phase III study comparing the MEK inhibitor binimetinib with dacarbazine in patients with NRAS-mutant cutaneous melanoma showed longer progression-free survival in patients treated with binimetinib (Dummer, 2016). However, the adverse events profile of these agents, including cardiomyopathy, hypertension, coagulopathies and rash, makes them good candidates for a combined treatment regimen rather than single-agent therapy.

In the present study, we included only patients seen at a single tertiary cancer centre. Although mutation testing was done prospectively in patients with SNMM, data were collected and analysed retrospectively, which might limit our ability to control for patient comorbidities and different treatments administered. Also, matched non-tumour tissue was not tested, so the possibility of a detected mutation being a germline mutation cannot be completely ruled out. In our cohort, 24 patients had one mutation, two patients had two mutations, and one patient had eight mutations. Because of the low number of events, we could not analyse the correlation between the number of mutations and the outcome. However, our study represents the largest single-institution cohort to date of SNMM patients undergoing characterisation of mutation status. The role of mutation status, particularly NRAS mutations in G12 and G13, as a biomarker for response to MEK inhibition in SNMM needs to be addressed in future studies.

In conclusion, NRAS, BRAF and KIT mutations do not affect survival outcomes in SNMM. As MEK inhibitors have shown promise in the treatment of cutaneous melanoma, their prognostic impact in SNMM should be further investigated, especially in the relatively frequent cases with NRAS mutations.

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The authors declare no conflict of interest.

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