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Title: **Clinico-pathological Characteristics Associated With BRAF^{K601E} and BRAF^{L597} Mutations in Melanoma**

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Summary:

BRAF mutations at codons L597 and K601 occur uncommonly in melanoma. Clinical and pathological associations of these mutations were investigated in a cohort of 1,119 patients with known *BRAF* mutation status. A *BRAF* mutation was identified in 435 patients; Mutations at L597 and the K601E mutation were seen in 3.4% and 3.2% of these, respectively. K601E melanomas tended to occur in male patients, a median age of 58 years, were generally found on the trunk (64%) and uncommonly associated with chronically sun-damaged (CSD) skin. *BRAF* L597 melanomas occurred in older patients (median-66 years), but were associated with CSD skin (extremities or head and neck location – 73.3%, p=0.001). Twenty-three percent of patients with V600E and 43% of patients with K601E mutant melanomas presented with nodal disease at diagnosis compared to just 14% of patients with BRAF wild-type tumors (p=0.001 and 0.006 respectively). Overall, these mutations represent a significant minority of *BRAF* mutations, but have distinct clinico-pathological phenotypes and clinical behaviors.

Significance:

Current understanding of the clinico-pathological associations of *BRAF* mutated melanomas is limited to the more common *BRAF* V600 mutations and very little is understood about the

BRAF L597 and K601E mutations. We present data from the largest cohort of these primary melanomas, which represent almost 7% of all BRAF mutations, and show that they represent distinct clinic-pathological phenotypes. These mutations represent a significant minority of melanomas and testing for these mutations and examining potential therapeutic interventions in a prospective manner is warranted.

Introduction:

Melanoma is the fourth most common malignancy in men and women in Australia (AIHW, 2013). Recently, significant advances have been made in the management of advanced melanoma, in large part due to an improved molecular understanding of melanoma. Discovery of oncogenic drivers such as mutations in the gene encoding for the BRAF protein in the mitogen activating protein kinase (MAPK) pathway has been a critical advancement in our understanding.

Clarification of the molecular basis of melanoma has resulted in the development of efficacious targeted therapies such as BRAF and MEK inhibitors in patients with melanoma harboring a *BRAF* mutation. BRAF inhibitors (vemurafenib, dabrafenib) and MEK inhibitors (trametinib, cobimetinib) have been shown, as single agents and in combination, to be effective in providing a rapid tumor response, prolongation of progression free survival and most importantly improving overall survival .

Reported *BRAF* mutation rates are between 40 and 60% , and greater than 90% of these are due to V600 mutations, in particular V600E and V600K. Codons L597 and K601 are adjacent to V600 in exon 15 of the *BRAF* gene and a small proportion of *BRAF* mutant melanomas harbor mutations at these codons. The K601E mutation results in an amino acid substitution at position 601 in *BRAF*, from a lysine (K) to a glutamic acid (E). The L597 mutations result in an amino acid substitution at position 597 in *BRAF*, from a leucine (L) to a serine (S),

glutamine (Q) or arginine (R). Mutations at both codons result in elevated kinase activity similar to mutations at V600 (Wan et al., 2004). Previously, there has been a reported clinical response of a L597 *BRAF* mutant metastatic melanoma to a MEK inhibitor as well as *in vitro* sensitivity of a K601 mutant melanoma to MEK inhibition .

Whilst we have a greater understanding of the common *BRAF* V600 mutations, in terms of clinical associations, prognostic and predictive data, there is little published data on the less common mutations . These uncommon mutations might have clinical relevance as there is preliminary evidence of sensitivity to targeted therapies . We aimed to describe the relevant clinico-pathological characteristics of L597 and K601E *BRAF* mutant melanomas.

Results:

At the time of analysis, the primary melanomas of 1162 patients were included in the Melbourne Melanoma Project database. 1159 (99.7%) successfully underwent molecular analysis. In-situ melanomas, cutaneous metastases and benign nevi including spitzoid tumors of unknown malignant potential were excluded from the final analysis that included a total of 1,119 invasive melanomas. 435 (38.9%) of these harbored a *BRAF* mutation. The remaining 684 patients were considered to be *BRAF* 'wild-type', with 41 patients harboring either a *NRAS* or *KIT* mutation (37 and 4 patients respectively). V600 mutations constituted the majority of *BRAF* mutations (93.3%, n=406) with the V600E mutation the most common of these V600 mutations (76.4%, n=310). The less common V600 mutations included V600K (n=86, 21.2%), V600R (n=5, 1.2%), V600D (n=4, 1.0%) and V600M (n=1, 0.2%). A minority of all patients (2.8%) had known metastases at entry.

The most common non-V600 mutations were L597 (c.1789_1790delinsTC: p. Leu597Ser) and K601E (c.1801A>G: p.Lys601Glu). 15 patients had a L597 mutation and 14 had a K601E mutation, 3.3% and 3.1% of all *BRAF* mutations respectively. 3 patients had other *BRAF* mutations not previously described (c.1798_1799insATACAG: p.Thr599_Val600insAspThr, c.1799-1802delinsAGAT: p.Val600-Lys601delinsGlulle, c1794_1796dup: p.Thr599dup).

Clinical and pathological data is summarized in Table 1. There was a significant association between mutation status and gender (p=0.002). The majority of patients with a K601E and L597 mutation were male, 71.4% and 73.3% respectively, whilst fewer V600E (49.8%) and *BRAF* wild-type patients (62.0%) were male.

There was a significant association between mutation status and age (p=0.001), which was mostly attributable to V600E patients being younger than non-V600E and *BRAF* wild-type patients. Patients with a L597 mutation were significantly older than patients harboring a V600E mutation (median 66 years, IQR 58-71 versus 50 years IQR 40-61 p=0.001), as were *BRAF* wild-type (median 63 years, IQR 51-71, p<0.001) and V600K patients (median 63 years IQR 53-72, p<0.001). Patients with a K601E mutation had a median age of 58 years (IQR 31-86), which was not significantly older than V600E patients (p=0.2). There were no patients with L597 melanomas that were under the age of 50, compared to 35.7% (n=5) of K601E melanomas and 50.6% (n=157) of V600E melanomas.

We examined various surrogates for prior sun exposure in melanoma patients. As expected, patients with *BRAF* wild-type and V600K mutant primary melanomas were more likely to have a history of solar keratoses (50.2% and 46.7% respectively) compared to patients with *BRAF* V600E mutant primaries (29.5%, p<0.001). A history of solar keratoses was also more common in L597 patients (58.3%) compared to V600E (p=0.04). Patients with a K601E mutation, however, were similar to V600E patients, with only a minority (16.7%) having a history of solar keratoses. In keeping with this, K601E and V600E mutant primaries were more likely to arise on the trunk (64.3% p=0.01 and 39.8% p=0.001 respectively) compared to *BRAF* wild-type melanomas (25.8%), which were more common at sun-exposed sites (Table 1). It is worth noting that a similar proportion of V600E primaries occurred on the trunk (39.8%) and on an extremity (41.8%). Conversely, L597 melanomas were most commonly found on the extremities (40%), though this association was not significant. The proportion of patients with a history of sunburn was similar amongst V600E, K601E, L597 and *BRAF* wild-type patients (60.5%, 66.7%, 66.7% and 65.5 % respectively, p=0.4).

The median Breslow thickness was significantly greater in *BRAF* wild-type (1.7mm, p=0.008) and V600K (1.5mm, p=0.04) mutant tumors compared to V600E (1.3mm). *BRAF* K601E mutant melanomas were thicker (2.15mm) compared to both V600E (1.3mm) and L597 melanomas (1.25mm) although this did not reach statistical significance (p=0.3). 21.4 % of

K601E melanomas were less than 1.0 mm thick as compared to 35.5% of V600E melanomas, 42.9% of L597 melanomas and 27.9% of *BRAF* wild-type melanomas.

There was a significant association between mutation status and histologic subtype ($p<0.001$). Superficial spreading melanomas (SSM) were the predominant histopathologic subtype identified across all *BRAF* mutated melanomas; 66.7% of L597 and 69.3% of V600 melanomas were SSM subtype. However, K601E melanomas had a greater proportion of NM (35.7%) and fewer SSM (57%) compared to other mutational types, though this did not reach statistical significance ($p=0.2$). *BRAF* V600K and L597S mutant melanomas were more likely to be LMM than SSM subtype when compared to V600E mutant tumors ($p=0.02$), which is in keeping with their propensity to sun exposed sites.

The presence of ulceration and an elevated mitotic count were two poor prognostic features observed at a similar frequency in melanomas across mutational subgroups. The presence of ulceration was seen in 33.3%, 28.6% and 26.2% of L597, K601E and V600E melanomas respectively, ($p=0.7$). *BRAF* wild-type melanomas had a similar ulceration rate (25.2%) to V600E melanomas in our series. There was no significant association between mitotic rate and mutation status in this cohort. Median mitotic rates are shown in table 2. L597 melanomas had $\geq$1 mitoses/mm^2 in 85.7%, compared to 76.9% of K601E, 75.8% of V600E and 75.9% of *BRAF* wild-type melanomas,.

Twenty-three percent of patients with *BRAF* V600E and 43% of patients with K601E mutant melanomas presented with nodal disease at diagnosis compared to just 14% of patients with *BRAF* wild-type tumors ($p=0.001$ and 0.006 respectively). This association remained significant after adjusting for thickness. Patients with L597S mutations were similar to wild-type patients, with only 14% presenting with nodal disease at diagnosis.

Discussion

Our current understanding of the clinical and pathological characteristics of *BRAF* mutated melanomas is largely limited to *BRAF* V600 mutations . We present here the clinico-pathologic characteristics of the uncommon *BRAF* K601E and L597 mutated primary melanomas and show that these are associated with distinct tumour characteristics. Whilst

tumours harboring L597 mutations have similarities with *BRAF* wild-type tumors (association with sun exposed sites and LMM subtype), tumors with K601E mutations are more similar to V600E mutant melanomas (more common on the trunk, not associated with markers of chronic sun exposure and significantly more likely to present with nodal disease at diagnosis).

Despite the large size of our cohort, given the low frequency of K601E and L597 mutations, the absolute number of these melanomas identified was relatively small. This might limit the statistical power with which analyses might be undertaken but is still the largest series presented to date. Another potential limitation of the data presented here is the absence of outcome data, particularly in terms of rates of progression, recurrence and overall survival. Our study is therefore not able to clarify the prognostic implications of these uncommon but important *BRAF* mutations.

Melanomas with the *BRAF* K601E mutation, compared to V600 melanomas, seemed to occur more frequently in male patients although no statistically significant difference in the median age was observed. However, 64% of these K601E tumours occurred on the trunk and only a minority were associated with chronically sun damaged skin. A similar proportion of patients with K601E and V600E melanomas reported previous sunburn (66.7% and 60.5% respectively). Therefore, the lack of association with chronic sun damage would suggest that this mutation is more likely to occur in low mutation load melanomas (Figure 1). The 'divergent pathway' hypothesis for cutaneous melanoma development proposes that patients with a lower tendency to develop naevi ('naevus resistant') are more likely to have melanomas develop on CSD skin with significant UV exposure (Whiteman et al., 2003). BRAF wild-type and L597 mutated melanomas have a clinical phenotype consistent with this group. However, patients with 'naevus prone' skin, are more likely to develop melanomas at a younger age with less prior UV damage. This phenotype is consistent with V600 and K601E mutated melanomas. Recently published data from The Cancer Genome Atlas shows that the mutation load of K601E melanomas is similar to, although with a trend to being slightly higher than, the V600E melanomas (mean 859.4, standard deviation (SD) 793.6 and mean 430.2, SD 436.0 respectively, P=0.08 with Wilcox rank sum test) (TCGA Network, 2015).

In contrast, whilst melanomas harboring the L597 mutation were more common in older patients compared to V600E, most of the L597 melanomas were found on either the extremities or head and neck (73.3%). Just over half (66.7%) of these patients admitted to

previous sunburn. These findings suggest that L597 melanomas were associated with chronically sun damaged and sun exposed skin, more so than that seen in K601E, and V600E melanomas and are therefore more likely to arise in high mutation load tumors . This is clinically relevant as high mutation load may be predictive of a response to immune checkpoint inhibition through production of neoantigens (Snyder et al., 2014). It has previously been shown that melanomas with a higher mutation load may be associated with an improved response to immune checkpoint inhibition, for example with CTLA-4 inhibition.

K601E melanomas were commonly of either the superficial spreading or nodular melanoma subtypes (92.8%) that were associated with poor prognostic histopathological features. Of this group, 71.4% had mitoses present, 28.6% were ulcerated and 35.7% had a Breslow thickness greater than 4.0 mm. In keeping with these high-risk features, K601E mutant tumors were significantly more likely to present with nodal disease at diagnosis (42.9% compared to just 14% of *BRAF* wild-type patients, p=0.003). Interestingly, V600E mutant melanomas were also significantly associated with the presence of nodal metastases at diagnosis despite having more favorable tumor characteristics compared to K601E melanomas. We have previously reported an association between *BRAF* mutant tumors and *RAC1* immunoreactivity and hypothesize that *RAC1* is important for melanoma migration and metastasis in these cases . This may explain why some melanomas metastasize early, despite being thin. The association between K601E and significantly increased tumor thickness may be related to either a more aggressive phenotype, delay in diagnosis (which may be a function of these melanomas being more common on the trunk of elderly patients), or a combination of the two.

In contrast, L597 melanomas generally had more favorable prognostic characteristics compared to K601E. Despite mitoses and ulceration being commonly seen in these melanomas (80.0% and 33.3% respectively), at a rate greater than V600E melanomas (68.9% and 23.6%) and K601E melanomas (71.4% and 28.6%), they were thinner and less likely to have nodal involvement.

Although the proportion of *BRAF* mutant melanomas that harbor either L597 or K601E mutations is small, the absolute number of patients is certainly not insignificant. The estimated total number of patients in the US diagnosed with melanoma in 2012 was approximately 76,250 . If the rate of L597 and K601E mutations were assumed to be 2.6% (as demonstrated in our study), then the absolute number of these patients would be approximately 1,976 in 2012.

The clinical impact of the prospective identification of these uncommon *BRAF* mutations is still unclear. The large clinical trials examining BRAF and MEK inhibitors usually only include V600E or V600K mutations so the therapeutic implications are uncertain for L597 and K601E. However, there is some emerging evidence that L597 melanomas might respond, in a clinically meaningful way, to MEK inhibitor therapy. Dahlman and colleagues reported a case of a patient with *BRAF* L597S mutant metastatic melanoma that responded radiologically to a MEK inhibitor . They also described *in vitro* sensitivity of L597 and K601 mutant melanomas to MEK and BRAF inhibition. More recently, it has been shown in a retrospective analysis of 4 patients with *BRAF* K601E metastatic melanoma and 1 patient with a *BRAF* L597Q melanoma, 3 of these patients achieved a partial response to trametinib, an oral MEK inhibitor (including the solitary L597Q melanoma) . Clearly, these very early findings need to be assessed prospectively in a clinical trial in order to change clinical practice with regards to these mutations. An ongoing phase II clinical trial is investigating the efficacy of trametinib in patients with *BRAF* nonV600 mutations (NCT02296112).

It is interesting to note that the overall *BRAF* mutation rate in our series was 39.0%. Compared to previous series where rates of *BRAF* mutations have been reported between 40% and 60% , the rate that we describe appears slightly lower than anticipated. One possibility is that the mutation rate in patients with advanced melanoma is higher due to the worse prognosis of *BRAF* mutant primary melanomas. This would then result in a disproportionately high rate of *BRAF* mutant melanomas in patients with advanced disease compared to those with early, primary melanomas. It is also possible that melanomas in our cohort of Australian patients would be enriched for *BRAF* wild-type as they have an association with chronic UV exposure.

In conclusion, our study provides a description of the incidence of L597 and K601E *BRAF* mutant melanomas and their clinico-pathological characteristics in a large series of primary melanomas. Both L597 and K601E mutated melanomas appear to be associated with various poor prognostic features although this would need to be examined further in future studies. Our study emphasizes the importance of these uncommon melanomas and we would encourage prospective evaluation of these melanomas with regards to their sensitivity to BRAF and MEK inhibitors.

Methods:

Consecutive patients from the Melbourne Melanoma Project (MMP) dataset that have had their primary melanoma managed at one of three tertiary referral centres (The Victorian Melanoma Service (VMS), Peter MacCallum Cancer Centre or the Austin Hospital) were tested for a *BRAF* mutation. Patients recruited from 2009 until May 2013 were included for analysis. Human Research Ethics Committee approval was granted at all three institutions involved in the MMP (Project number 07/38).

Tumor tissue from the primary melanomas was tested for *BRAF* mutations at the Peter MacCallum Cancer Centre, Department of Diagnostic Molecular Pathology (Melbourne, Australia). Variations in exon 15 of *BRAF* were identified between nucleotides c.1788 and c.1823 in reference sequence NM_004333.4, corresponding to codons 597 to 607 by high-resolution melting (HRM) analysis following macrodissection of the paraffin embedded tumour specimens as described previously . According to the Catalogue of Somatic Mutations in Cancer (COSMIC) this region contains 97% of all *BRAF* mutations. All abnormal HRM traces were subjected to DNA Sanger sequencing according to methods previously described . Tumors were therefore evaluated for *BRAF* mutations in V600 as well as K601E, L597 and other rare mutations.

Clinical data were collected prospectively, including patient age, gender, eye color, previous history of sunburn and site of melanoma. The primary site was coded as either extremity, head and neck, trunk or other/unknown. Melanoma histopathologic subtype was classified as superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM), mucosal, other or unknown. The stage at diagnosis was recorded according to the American Joint Committee on Cancer (AJCC) cancer staging version 7, 2010. Other pathological information including Breslow thickness (mm), mitotic count, ulceration and nodal status was also recorded.

Clinical and pathologic features were assessed for associations with BRAF mutation status using chi squared and kruskal-wallis tests as well as multinomial logistic regression. All analyses were performed using Stata statistical software version 12.1.

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Table 1. Clinical Characteristics of melanomas according to mutational subtype.

Total (n= 1119)	**BRAF Wild-type (n=684)**	**V600E (n=310)**	**V600K (n=86)**	**V600 other (-D, -R, -M) (n=10)**	**K601E (n=14)**	**L597 (n=15)**	**p value**
	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	
Sex							
• Female	257 (37.6)	155 (50.2)	29 (33.7)	6 (60)	4 (28.6)	4 (26.7)	0.002
• Male	426 (62.4)	154 (49.8)	57 (66.3)	4 (40)	10 (71.4)	11 (73.3)	
Age at diagnosis (years)							
• <29	17 (2.5)	23 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0001
• 30-49	135 (19.8)	134 (43.2)	13 (15.1)	2 (20.0)	5 (35.7)	0 (0.0)	
• 50-69	340 (49.8)	118 (38.1)	46 (53.5)	5 (50.0)	8 (57.1)	12 (80.0)	
• >70	191 (28.0)	35 (11.3)	27 (31.4)	3 (30.0)	1 (7.1)	3 (20.0)	
• Median(IQR)	63 (51-71)	50 (40-61)	63 (53-72)		58 (31-86)	66 (58-71)	0.0001
Eye Color							
• Blue	379 (59.0)	150 (50.5)	46 (54.8)	6 (60)	4 (33.3)	9 (75.0)	0.1
• Brown/Hazel	198 (30.8)	100 (33.7)	30 (35.7)	4 (40)	5 (41.7)	2 (16.7)	
• Green	65 (10.1)	47 (15.8)	8 (9.5)	0 (0)	3 (25.0)	1 (8.3)	
Previous sunburn							
• Yes	422 (65.5)	179 (60.5)	48 (57.8)	8 (80)	8 (66.7)	8 (66.7)	0.4
• No	222 (34.5)	117 (39.5)	35 (42.17)	2 (20)	4 (33.3)	4 (33.3)	
Solar Keratoses							
• Present	305 (50.2)	83 (29.5)	35 (46.7)	1 (11.1)	2 (16.7)	7 (58.3)	<0.0001
• Absent	303 (49.8)	198 (70.5)	40 (53.3)	8 (88.9)	10 (83.3)	5 (41.67)	
Site of primary melanoma*							
• Extremity	313 (45.9)	129 (41.8)	31 (36.5)	4 (40.0)	3 (21.4)	6 (40.0)	0.001

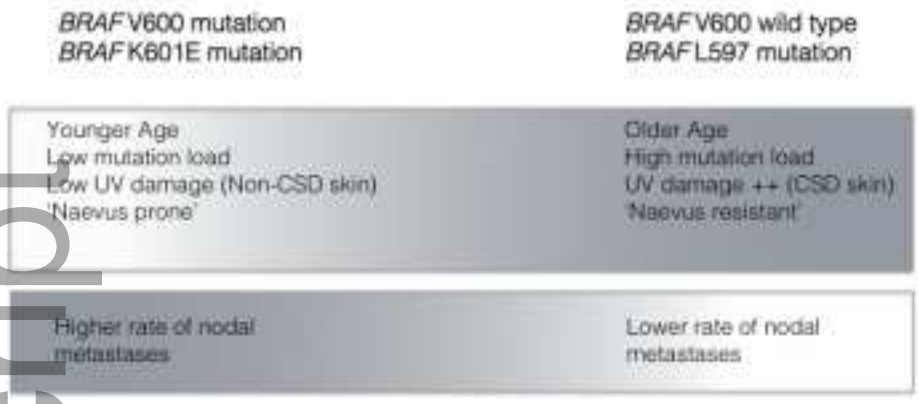
• Head and neck	171 (25.1)	51 (16.5)	26 (30.6)	3 (30.0)	1 (7.1)	5 (33.3)	
• Trunk	176 (25.8)	123 (39.8)	26 (30.6)	3 (30.0)	9 (64.3)	4 (26.7)	
• Other	22 (3.2)	6 (1.94)	2 (2.4)	0 (0)	1 (7.1)	0 (0.0)	

Table 2. Pathological characteristics according to mutational subtype.

Total (n= 1119)*	**BRAF Wild-type (n=684)**	**V600E (n=310)**	**V600K (n=86)**	**V600 other (-D, -R, -M) (n=10)**	**K601E (n=14)**	**L597 (n=15)**	**p-value**
	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	
Breslow Thickness, mm							
<1.00	171 (27.9)	99 (35.5)	22 (29.0)	3 (37.5)	3 (21.4)	6 (42.9)	0.3
1.00-1.99	159 (26.0)	85 (30.5)	23 (30.3)	2 (25.0)	4 (28.6)	2 (14.3)	
2.00-2.99	101 (16.5)	41 (14.7)	9 (11.8)	1 (12.5)	1 (7.1)	1 (7.1)	
3.00-3.99	66 (10.8)	21 (7.5)	7 (9.2)	1 (12.5)	1 (7.1)	2 (14.3)	
>4.00	116 (18.9)	33 (11.8)	15 (19.7)	1 (12.5)	5 (35.7)	3 (21.4)	
Median	1.7 mm	1.3 mm	1.45 mm	1.78 mm	2.15 mm	1.25 mm	0.05
Histopathologic subtype							
SSM	336 (50.5)	223 (69.3)	53 (60.2)	4 (40)	8 (57.1)	10 (66.7)	<0.0001
NM	134 (20.2)	68 (21.1)	23 (26.1)	4 (40)	5 (35.7)	1 (6.7)	
Lentigo Maligna	56 (8.4)	7 (2.2)	6 (6.8)	1 (10)	0 (0)	3 (20.0)	
Mucosal	6 (0.9)	1 (0.3)	0 (0.0)	0 (0)	0 (0)	0 (0.0)	
Other	107 (16.1)	15 (4.7)	6 (6.8)	1 (10)	0 (0)	1 (6.7)	
Mitoses							
Present (≥1)	461 (69.3)	222 (68.9)	63 (71.6)	7 (70)	10 (71.4)	12 (80.0)	0.9
Absent (<1/mm^2)	185 (27.8)	73 (22.7)	22 (25)	1 (10)	3 (21.4)	3 (20.0)	
Median (IQR)	2 (1-6)	2 (1-6)	2 (0-8)	3.5 (1.5-5.5)	2 (1-6)	2.5 (1-5)	0.9
Mean	4.41	4.22	4.90	3.88	4.85	4.71	
Ulceration							
Present	163 (25.2)	76 (26.2)	21 (25.6)	4 (50.0)	4 (28.6)	5 (33.3)	0.7
Absent	485 (74.9)	214 (73.8)	61 (74.4)	4 (50.0)	10 (71.4)	10 (66.7)	
Lymph node status at diagnosis							
Negative	564 (86.0)	232 (76.8)	68 (82.9)	7 (70.0)	8 (57.1)	12 (85.7)	0.003
Positive	92 (14.0)	70 (23.2)	14 (17.1)	3 (30.0)	6 (42.9)	2 (14.3)	

AJCC stage at diagnosis							
IA	129 (19.0)	78 (25.2)	17 (20.2)	2 (20.0)	3 (21.4)	3 (20.0)	0.0001
IB	183 (27.1)	96 (31.1)	23 (27.4)	3 (30.0)	3 (21.4)	5 (33.3)	
IIA	134(19.7)	29 (9.4)	9 (10.7)	0 (0.0)	0 (0.0)	2 (13.3)	
IIB	81 (11.9)	27 (8.7)	10 (11.9)	1 (10.0)	0 (0.0)	1 (6.7)	
IIC	39 (5.7)	7 (2.3)	3 (3.6)	1 (10.0)	2 (14.3)	1 (6.7)	
IIIA	37 (5.5)	31 (10.0)	8 (9.5)	0 (0.0)	4 (28.6)	1 (6.7)	
IIIB	40 (5.9)	14 (4.5)	2 (2.4)	0 (0.0)	1 (7.1)	2 (13.3)	
IIIC	19 (2.8)	20 (6.5)	5 (6.0)	3 (30.0)	1 (7.1)	0 (0.0)	
IV (M1A)	4 (0.6)	2 (0.7)	3 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	
IV (M1B)	1 (0.2)	1 (0.3)	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	
IV (M1C)	12 (1.8)	4 (1.3)	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	

Figure 1. Clinico-pathologic phenotype and association with *BRAF* mutation



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