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BRAF, *NRAS* and *C-KIT* Advanced Melanoma: Clinico-pathological Features, Targeted-Therapy Strategies and Survival

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Abstract. *Background/Aim: The mutational status of stage III and IV melanomas should be recognized in order to allow for targeted therapies. The aim of our study was the characterization of BRAF, NRAS and C-KIT melanoma patients, in order to define their optimal management. Patients and Methods: Between 1991 and 2015, 63 mutated melanoma patients were treated and monitored during their diagnostic and therapeutic management at a single institution. Results: BRAF-mutated melanoma patients were the most common, representing 70% of the study population, while NRAS- and C-KIT-mutated melanoma represented 19% and 11% respectively. BRAF-mutated melanomas were mostly located at sites of intermittent sun exposure, and were associated with higher Breslow thickness and an increased number of mitosis. NRAS mutated melanoma were mainly observed in chronic sun-damaged areas and had a negative prognostic value, with shorter time to progression and a high incidence of central nervous system involvement. C-KIT mutated melanoma were located at acral and mucosal sites. Overall survival observed in the three groups of patients revealed wide differences. Conclusion: BRAF, NRAS and C-KIT melanomas constitute distinct clinico-pathological entities. BRAF-mutated melanoma benefit from both anti-BRAF and anti-MEK targeted therapies while triple-negative melanomas could benefit from novel anti-CTLA-4 and anti-PD-L1 immunotherapeutic approaches.*

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Recent advances in the field of molecular biology and genetics, allowed for identification of several mutations that play a key role in the development and progression of malignant melanoma (MM). The main genes involved in the melanocytic cancerogenesis are: RAS/RAF/MEK/ERK *Mitogen Activated Protein Kinases* (MAPK) and PI3K-AKT. Mutations along *BRAF, NRAS, C-KIT* and *GNAQ* are the main causes of MAPK pathway disregulation in MM (1-3). The mutational status of *BRAF, NRAS* and *C-KIT* was correlated with body site and histopathological characteristics of MM (1-3). Some recent metanalyses presented the incidence of *BRAF* mutations of 36-52% of MM (2, 3), with the identification of more than 40 different MM BRAF mutations, mainly at the aminoacidic position 600 (95%), where valine is substituted with another aminoacid. The most common is the *BRAF V600E*, that occurs in 71-75% of cases, followed by the *V600K* that occurs in 7-22% of cases. Other, less frequent mutations are *V600R, V600D* and *V600M* (4, 5). NRAS mutations are the second most frequent mutations in MM. RAS proteins play a key role in signal transduction of MAPK and PI3K pathway. Regarding *C-KIT*, it is an important key-player in the pathogenesis of melanoma, especially acral and mucosal melanoma, gastro-intestinal stromal tumours (GIST) and chronic myeloid leukemia (6, 7). The frequency of *C-KIT* mutated melanoma is 1-7% (2, 8-10). *C-KIT* mutations are associated to older age at diagnosis (>60) (11, 12). The determination of the mutational status of unresectable III and IV stage melanoma, and high risk (IIc, IIIa, IIIb e IIIc) resectable melanomas should be performed in order to allow

Table I. *Clinical and familial features of mutated melanoma patients.*

Mutation type	Patients (%)	Gender		Average and median age at diagnosis	Other tumors in patient (%)	Other tumors in family (%)	Site of primary (%)
		M	F				
*BRAF**	44 (69.8%)	25	19	57-56	NS/NE 9/44 Present 14/35 (40%) Absent 21/35 (60%)	NS/NE 10/44 Present 9/34 (26.5%) Absent 25/34 (73.5%)	Unknown primary 8/44 Head and neck 11/36 (30.6%) Trunk 10/36 (27.8%) Upper limb 5/36 (13.9%) Lower limb 10/36 (27.8%, 2 acral)
V600E	32 (72.7%)	16	16	56.2-54.5	NS/NE 6/32 Present 13/26 (50%) Absent 13/26 (50%)	NS/NE 7/32 Present 8/25 (32%) Absent 17/25 (68%)	Unknown primary 8/32 Head and neck 5/24 (20.8%) Trunk 6/24 (25%) Upper limb 4/24 (16.7%) Lower limb 9/24 (37.5%, 2 acral)
V600K	10 (22.7%)	8	2	59.2-58.5	NS/NaE 3/10 Present 1/7 (14.3%) Absent 6/7 (85.7%)	NS/NE 3/10 Absent 7/7 (100%)	Head and neck 5/10 (50%) Trunk 4/10 (40%) Lower limb 1/10 (10%)
V600 not E/K	3 (6.8%)	2	1	61-65	Absent 3/3 (100%)	Present 1/3 (33.3%) Absent 2/3 66.7%)	Head and neck 1/3 (33.3%) Upper limb 2/3 (66.7%)
NRAS	12 (19%)	9	3	64.9-69	NE/NS 2/12 Present 4/10 (40%) Absent 6/10 (60%)	NS/NE 3/12 Present 2/9 (22.2%) Absent 7/9 (77.8%)	Trunk 9/12 (75%) Lower limb 2/12 (16.7%) Mucosal 1/12 (8.3%)
C-KIT	7 (11.1%)	2	5	65.9-66	Present 2/7 (28.6%) Absent 5/7 (71.4%)	Present 2/7 (28.6%) Absent 5/7 (71.4%)	Unknown primary 1/7 Mucosal 3/6 (50%) Acral 2/6 (33.3%) Head and neck 1/6 (16.7%)

NS/NE: Not Specified or not evaluable, n: number of patients. *1 patient with double mutation V600E+V600M.

the usage of target therapies. Differently from *BRAF* melanomas, for *NRAS* and *C-KIT* mutated melanomas targeted therapies are not available. The aim of our study was the clinical, epidemiological, histopathological and molecular characterization of a cohort of patients affected by MM in order to better understand the optimal management of MM patients.

Patients and Methods

Patient selection. A total of 63 patients diagnosed with MM and harbouring *BRAF, NRAS* or *C-KIT* gene mutations were monitored during their diagnostic and therapeutic management at the the University of Modena and Reggio Emilia. The diagnosis was made at the identification of a primary tumour or metastasis between 1991-2015. Patients were divided into 3 groups based on the melanoma mutational status: *BRAF, NRAS* or *C-KIT*.

Therapies Patients with *BRAF* mutated melanomas were treated with anti-*BRAF* monotherapy; Vemurafenib (Zelboraf, 960 mg twice daily) or Dabrafenib (Tafinlar, 150 mg twice daily), or the combination Dabrafenib (Tafinlar, 150 mg twice daily) + Trametinib (Mekinist, 2 mg, once daily). Patients with disease progression despite anti-*BRAF* monotherapy or combination anti-*BRAF*+anti-MEK (combination Dabrafenib (Tafinlar, 150 mg twice daily) + Trametinib (Mekinist, 2 mg, once daily), were treated with immunotherapy based on Ipilimumab (Yervoy, 3 mg/kg every 3 weeks x 4 times).

Parameters and measurements. Clinical and pathologic stage was classified according to the American Joint Committee on Cancer criteria (AJCC 7th edition). RECIST criteria were applied. Patients receiving treatments were monitored on a monthly basis with clinical visits, and total body imaging (TC or PET/TC+TC) was performed every 3-6 months.

Statistical analysis. The statistical analysis was performed using software SATA (version 13). Kaplan-Meier curves were drawn.

Results

Our study population included 63 patients with MM. Melanomas were located on the head and neck area (n=12, 22.2%), on the trunk (n=19, 35.2%), on the upper limb (n=6 of which 1 was acral, 11.1%) and on the lower limb (n=13 of which 3 were acral, 24.1%), 4 patients had mucosal melanoma (n=4, 7.4%). Nine patients (14.2%) had an unknown primary site.

The prevalence of *BRAF, NRAS* and *C-KIT* mutations, the average and median patient age at diagnosis, clinical details and histopathological features for each group of patients are reported in Tables I and II. *BRAF* mutated melanomas were located at sites of intermittent sun exposure and were associated with high Breslow thickness and an increased number of mitosis, while *NRAS* mutations occurred in

Table II. *Clinical and histopathological features of mutated melanoma patients.*

Mutation type	Patients (%)	T	Mitotic index (%)	TILs (%)	Stage at diagnosis (%)	Average and median time to metastatic progression (n)
*BRAF**	44 (69.8%)	NS/NE 2/44 Unknown primary 8/44 T1 5/34 (14.7%) T2 12/34 (35.3%) T3 7/34 (20.6%) T4 10/34 (29.4%)	NS/NE 11/44 >1/mm^2 22/33 (66.7%) ≤1/mm^2 11/33 (33.3%)	NS/NE 17/44 Present 18/27 (66.7%) Absent 9/27 (33.3%)	NS/NE 1/44 I 8/43 (18.6%) II 5/43 (11.6%) III 22/43 (51.2%) IV 8/43 (18.6%)	42 months- 23 months (33/44)
V600E	32 (72.7%)	NS/NE 1/32 Unknown primary 1/32 T1 2/23 (8.7%) T2 8/23 (34.8%) T3 5/23 (21.8%) T4 8/23 (34.8%)	NS/NE 10/32 >1/mm^2 17/22 (77.3%) ≤1/mm^2 5/22 (22.7%)	NS/NE 13/32 Present 13/19 (68.4%) Absent 6/19 (31.6%)	I 4/32 (12.5%) II 3/32 (9.4%) III 18/32 (56.2%) IV 7/32 (21.9%)	42 months- 17 months (24/32)
V600K	10 (22.7%)	NS/NE 1/10 T1 3/9 (33.3%) T2 3/9 (33.3%) T3 1/9 (11.1%) T4 2/9 (22.2%)	NS/NE 1/10 >1/mm^2 5/9 (55.6%) ≤1/mm^2 4/9 (44.4%)	NS/NE 4/10 Present 3/6 (50%) Absent 3/6 (50%)	NS/NE 1/10 I 4/9 (44.4%) II 1/9 (11.1%) III 3/9 (33.3%) IV 1/9 (11.1%)	44 months- 44 months (7/10)
V600 not E/K	3 (6.8%)	T2 1/3 (33.3%) T3 1/3 (33.3%) T4 1/3 (33.3%)	>1/mm^2 3/3 (100%)	Present 3/3 (100%)	II 1/3 (33.3%) III 2/3 (66.7%)	30 months- 31 months (3/3)
NRAS	12 (19%)	NS/NE 2/12 Tis 1/10 (10%) T2 1/10 (10%) T3 4/10 (40%) T4 4/10 (40%)	NS/NE 3/12 >1/mm^2 9/9 (100%)	NS/NE 3/12 Present 5/9 (55.6%) Absent 4/9 (44.4%)	NS/NE 2/12 II 3/10 (30%) III 7/10 (70%)	65 months- 1 4 months (10/12)
C-KIT	7 (11.1%)	Unknown primary 1/7 T1 2/6 (33.3%) T3 3/6 (50%) T4 1/6 (16.7%)	NS/NE 3/7 >1/mm^2 2/4 (50%) ≤1/mm^2 2/4 (50%)	NS/NE 3/7 Present 3/4 (75%) Absent 1/4 (25%)	I 2/7 (28.6%) II 1/7 (14.3%) III 3/7 (42.9%) IV 1/7 (14.3%)	20 months- 20 months (2/7)

NS/NE: Not Specified or Not Evaluable, n: number of patients. *1 patient with double mutation V600E+V600M.

chronic sun damaged (CSD) skin areas, *i.e.* arms and legs, and had a negative prognostic value, because of a shorter time to progression and a high incidence of central nervous system (CNS) involvement. *NRAS* and *C-KIT* melanoma patients showed an older age at diagnosis with respect to the *BRAF* group. Kaplan-Meier curves represent global survival of the cohort of patient (Figure 1). Most *BRAF* mutated patients (40/44; 91%) received anti-*BRAF* therapy as a single line of treatment or in association with anti-MEK target therapy. Average and median treatment times were 10.6 and 8.0 months (range 2-38) respectively. Table III summarizes the objective response rate, treatment time and progression free survival. Eight patients encountered disease progression during anti-BRAF single or association therapy and were treated with ipilimumab.

There were 12 *NRAS* mutated melanoma patients; five of whom were treated with chemotherapy as a first line treatment (2 patients with temozolomide, 2 patients with dacarbazine and 1 patient fotemustine), one patient was treated with ipilimumab and another patient was treated with anti-MEK agent (pimasertib) according to a clinical protocol. As second line of treatment, 3 patients were treated with ipilimumab and 1 with chemotherapy. Average and median treatment time of the 5 patients that received chemotherapy as first line of treatment were 3 months, with 100% of no response to the treatment and an average and median progression free survival of respectively 2.6 and 3 months. A patient participating in a clinical trial received pimasertib, and initially showed a partial response and progression of the disease after 6 months of treatment.

Among 7 patients with *C-KIT* mutated melanomas, 4 underwent clinical and radiologic follow-up, 3 were treated respectively with imatinib (as an off-label), ipilimumab and chemotherapy (temozolomide). The patient that received imatinib had a course of 8 months of therapy, going into progression after 6 months of therapy and showing stable disease as the better objective response. The patient that received ipilimumab shows stable disease after 9 months

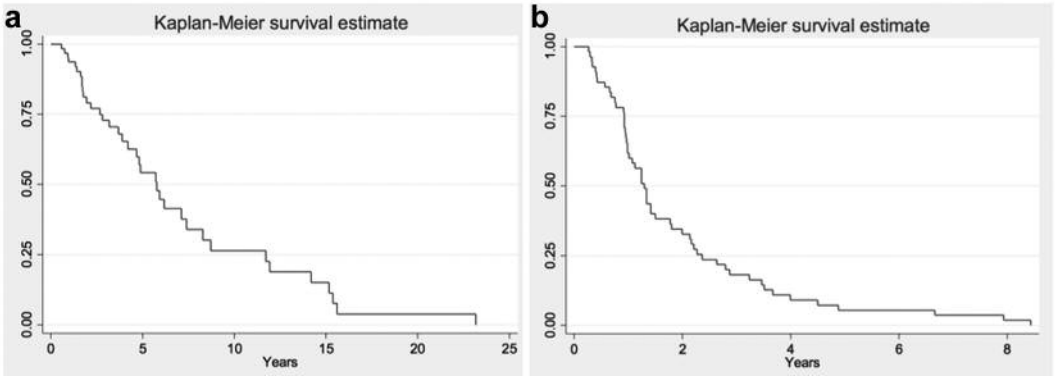

Figure 1. *Kaplan-Meier curves represent global survival of the cohort of patient (a) and metastatic subgroup (b).*

Table III. *Clinical efficacy and impact of anti-BRAF and the association of anti-BRAF and anti-MEK target therapies.*

	No. of treated patients	Treatment duration		Objective response (%)	PFS (No. of patients with PD)	
		Average	Median		Average	Median
Monotherapy with anti-BRAF	32	11 months	8 months	4 CR (12.5%) 18 PR (56.3%) 4 SD (12.5%) 6 NR (18.7%)	8.2 months (29)	6 months (29)
Dabrafenib + Trametinib	8	9.1 months	9 months	1 CR (12.5%) 6 PR (75%) 1 NR (12.5%)	7.3 months (3)	6 months (3)
Total	40	10.6 months	8 months	5 CR (12.5%) 24 PR (60%) 4 SD (10%) 7 NR (17.5%)	8.1 months (32)	6 months (32)

CR: Complete Response; PR: partial response; SD: stable disease; NR: non-response; PD: progression disease.

from the beginning of the treatment. The patient that received chemotherapy received temozolomide for 4 months, without any objective response, and with progression of the disease.

Cutaneous erythema, rash, hyper-transaminasemia and alopecia were more commonly associated with vemurafenib, whereas headache and fever were more commonly reported in association with dabrafenib.

Discussion

The clinical, epidemiological and histopathological characterization of patients affected by MM with distinct molecular signatures is crucial for the adoption of personalized therapeutic patient management. There is a correlation between different clinical characteristics, histopathological features and sensitivity to different therapeutic approaches. The genes involved in this new melanoma "molecular classification" are *BRAF, NRAS* and *C-KIT*. The most common mutations associated with MM are the *BRAF* mutations (2, 3), that are also the most frequent in this series, representing almost 70% of the population. The most common *BRAF* mutation was the V600E, identified in over 70% of the *BRAF* MM. *BRAF* mutations have been associated with superficial spreading melanoma (SSM); skin void of chronic solar sun damage (non-CSD); history of intermittent sun exposure; localization to the trunk, arms or legs; presence of multiple melanocytic nevi; young age at diagnosis (<50 years); high risk of metastasis to lymph node and brain, and aggressive behaviour (9, 13-15). These features are valid for the *V600E* mutations, while melanoma harbouring the V600K mutation usually shows different clinical, pathological and prognostic characteristics (16, 17). The age at onset of *BRAF* and *NRAS* mutated melanomas is slightly higher than what is reported in

literature: *BRAF* MM were more frequent on the trunk and legs, but a high number of V600K mutated melanomas were localized on the head and neck site (50%) (17). *NRAS* mutated melanomas were most frequently localized on the trunk, contrary to that reported by Ellerhorst *et al*., who found a higher prevalence of *NRAS* MM on the arms and legs (18). *BRAF* mutated melanomas were often characterised by a discrete mitotic index ($\geq 1/mm^2$) and an advanced stage at diagnosis (stage III or IV). *NRAS* mutated MMs were correlated to a higher Breslow thickness at diagnosis. The mitotic index was $>5/mm^2$ in 78% of the *NRAS* MMs. Comparatively, half of the *C-KIT* MM were associated with a $<1/mm^2$ mitotic index. The *C-KIT* MMs were predominately acral and mucosal distribution, confirming similar results previously reported in literature (11).

In the current study, metastatic progression was evidenced in 75% and 83% of *BRAF* and *NRAS* patients respectively. Comparatively, only 28.6% of *C-KIT* patients progressed to metastasis. BRAF and NRAS MMs exhibited a more advanced clinical stage (70% stage III and IV) with earlier progression to metastasis. The mean time to metastatic progression for *BRAF* and *NRAS* MMs was 23 and 14 months respectively. Among the *BRAF* MMs there was however, a large range; the *V600E* subgroup had a time to metastatic progression of 17 months and the *V600K* subgroup 44 months. The *C-KIT* MMs had a relatively short time to progression (20 months).

The recent development of anti-BRAF and anti-MEK targeted therapies has been a major improvement in the management of *BRAF* MM patients. Targeted therapies are still to be developed for *NRAS* and *C-KIT* MMs, but currently there are clinical trials evaluating anti-MEK therapies for *NRAS* MMs and tyrosine-kinase inhibitors, *i.e.* imatinib, for *C-KIT* MMs. In this study, the objective response rate (partial or complete) for *BRAF* MM patients, was higher for the group treated with the association therapy compared to the monotherapy anti-*BRAF (*87.5% *vs*. 68.8%). These results are similar to those reported by of the COMBI-v and COMBI-d studies (19, 20). The median treatment time and progression free survival were similar for the groups treated with single and association therapy.

The current study is not based on a large population of mutated advanced MM patients that would allow a complete statistical evaluation, our analysis has the remarkable value of being a monocentric study which permits to accurately describe the different biomolecular signatures underneath the MM pathogenesis: this is a comprehensive retrospective study that followed up a specific cohort of MM patients. Being advanced melanoma a rare disease, the collaboration between several national and international referral centres is auspicable and necessary in order to recognize the best management of advanced MM patients and the novel therapeutic approaches (21-25).

Conclusion

In conclusion, *BRAF, NRAS* and *C-KIT* melanomas constitute distinct clinicopathological entities. The advances in the molecular characterization of melanoma and the identification of the involvement of *BRAF, NRAS* and *C-KIT* genes in the pathogenesis of the disease lead to the development of new targeted therapies that significantly improved the survival of patients with metastatic disease. *BRAF* mutated melanomas seem to benefit from both anti-BRAF and anti-MEK targeted therapies while triple-negative melanomas could benefit of novel anti-CTLA-4 and anti-PD-L1 immunotherapeutic approaches. The adoption of the single anti-BRAF therapy (dabrafenib/vemurafenib) or the combination therapy (dabrafenib/vemurafenib + trametinib) is an important step in the management of *BRAF*-mutated melanoma patients. Patients affected by advanced malignant melanoma that do not harbour *BRAF* mutations are still waiting for approved and effective target therapies, and are currently treated only with the immunotherapeutic approach (ipilimumab, nivolumab, pembrolizumab) or chemotherapy.

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References

1 Sullivan RJ and Fisher DE: Understanding the biology of melanoma and therapeutic implications. Hematol Oncol Clin North Am *3*: 437-453, 2014.

2 Yaman B, Akalin T and Kandiloğlu G: Clinicopathological characteristics and mutation profiling in primary cutaneous melanoma. Am J Dermatopathol *5*: 389-397, 2015.

3 Cancer Genome Atlas Network: Genomic classification of cutaneous melanoma. Cell *7*: 1681-1696, 2015.

4 Ponti G, Tomasi A, Maiorana A, Ruini C, Maccaferri M, Cesinaro AM, Depenni R, Manni P, Gelsomino F, Giusti F, Garagnani L and Pellacani G: BRAFp.V600E, p.V600K, and p.V600R mutations in malignant melanoma: do they also differ in immunohistochemical assessment and clinical features? Appl Immunohistochem Mol Morphol *1*: 30-34, 2016.

5 Ponti G, Pellacani G, Tomasi A, Loschi P, Luppi G, Gelsomino F and Longo C: Molecular targeted approaches for advanced BRAF V600, N-RAS, c-KIT, and GNAQ melanomas. Dis Markers *2014*: 671283, 2014.

6 Beadling C, Jacobson-Dunlop E, Hodi FS, Le C, Warrick A, Patterson J, Town A, Harlow A, Cruz F 3rd, Azar S, Rubin BP, Muller S, West R, Heinrich MC and Corless CL: KIT gene mutations and copy number in melanoma subtypes. Clin Cancer Res *21*: 6821-6828, 2008.

7 Curtin JA, Busam K, Pinkel D and Bastian BC: Somatic activation of KIT in distinct subtypes of melanoma., J Clin Oncol *26*: 4340-4346, 2006.

8 Carlino MS, Haydu LE, Kakavand H, Menzies AM, Hamilton AL, Yu B, Ng CC, Cooper WA, Thompson JF, Kefford RF,

O'Toole SA, Scolyer RA and Long GV: Correlation of BRAF and NRAS mutation status with outcome, site of distant metastasis and response to chemotherapy in metastatic melanoma. Br J Cancer *2*: 292-299, 2014.

9 Pracht M, Mogha A, Lespagnol A, Fautrel A, Mouchet N, Le Gall F, Paumier V, Lefeuvre-Plesse C, Rioux-Leclerc N, Mosser J, Oger E, Adamski H, Galibert MD and Lesimple T: Prognostic and predictive values of oncogenic BRAF, NRAS, c-KIT and MITF in cutaneous and mucous melanoma. J Eur Acad Dermatol Venereol *8*: 1530-1538, 2015.

10 Handolias D, Salemi R, Murray W, Tan A, Liu W, Viros A, Dobrovic A, Kelly J and McArthur GA: Mutations in KIT occur at low frequency in melanomas arising from anatomical sites associated with chronic and intermittent sun exposure. Pigment Cell Melanoma Res *2*: 210-215, 2010.

11 Sakaizawa K, Ashida A, Uchiyama A, Ito T, Fujisawa Y, Ogata D, Matsushita S, Fujii K, Fukushima S, Shibayama Y, Hatta N, Takenouchi T, Uehara J, Okuyama R, Yamazaki N and Uhara H: Clinical characteristics associated with BRAF, NRAS and KIT mutations in Japanese melanoma patients. J Dermatol Sci *1*: 33-37, 2015.

12 Jin SA, Chun SM, Choi YD, Kweon SS, Jung ST, Shim HJ and Yun SJ: BRAF mutations and KIT aberrations and their clinicopathological correlation in 202 Korean melanomas. J Invest Dermatol *2*: 579-582, 2013.

13 Kim SY, Kim SN, and Hahn HJ.: Metaanalysis of BRAF mutations and clinicopathologic characteristics in primary melanoma;. J Am Acad Dermatol *6*: 1036-1046, 2015.

14 Hacker E, Hayward NK, Dumenil T, James MR and Whiteman DC: The association between MC1R genotype and BRAF mutation status in cutaneous melanoma: findings from an Australian population. J Invest Dermatol *1*: 241-248, 2010.

15 Menzies AM, Haydu LE and Visintin L: Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. Clin Cancer Res *12*: 3242-3249, 2012.

16 Ponti G, Manfredini M and Tomasi A: Distinctive clinical and dermoscopic features of BRAF V600K mutated melanomas. Br J Dermatol *5*: 1438-1440, 2015.

17 Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, Chiarion-Sileni V, Lebbe C, Mandalà M, Millward M, Arance A, Bondarenko I, Haanen JB, Hansson J, Utikal J, Ferraresi V, Kovalenko N, Mohr P, Probachai V, Schadendorf D, Nathan P, Robert C, Ribas A, DeMarini DJ, Irani JG, Swann S, Legos JJ, Jin F, Mookerjee B and Flaherty K: Dabrafenib and trametinib *versus* dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. Lancet *9992*: 444-451, 2015.

18 Ellerhorst JA, Greene VR, Ekmekcioglu S, Warneke CL, Johnson MM, Cooke CP, Wang LE, Prieto VG, Gershenwald JE, Wei Q and Grimm EA: Clinical correlates of NRAS and BRAF mutations in primary human melanoma Clin Cancer Res *2*: 229-235, 2011.

19 Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, Lichinitser M, Dummer R, Grange F, Mortier L, Chiarion-Sileni V, Drucis K, Krajsova I, Hauschild A, Lorigan P, Wolter P, Long GV, Flaherty K, Nathan P, Ribas A, Martin AM, Sun P, Crist W, Legos J, Rubin SD, Little SM and Schadendorf D: Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med *1*: 30-39, 2015.

20 Grob JJ, Amonkar MM, Karaszewska B, Schachter J, Dummer R, Mackiewicz A,Stroyakovskiy D, Drucis K, Grange F, Chiarion-Sileni V, Rutkowski P, Lichinitser M, Levchenko E, Wolter P, Hauschild A, Long GV, Nathan P, Ribas A, Flaherty K, Sun P, Legos JJ, McDowell DO, Mookerjee B, Schadendorf D and Robert C: Comparison of dabrafenib and trametinib combination therapy with vemurafenib monotherapy on health-related quality of life in patients with unresectable or metastatic cutaneous BRAF Val600-mutation-positive melanoma (COMBI-v): results of a phase 3, open-label, randomised trial. Lancet Oncol *13*: 1389-1398, 2015.

21 Pollio A, Tomasi A, Seidenari S, Pellacani G, Rodolfo M, Frigerio S, Maurichi A, Turchetti D, Bassoli S, Ruini C and Ponti G: Malignant and benign tumors associated with multiple primary melanomas: just the starting block for the involvement of MITF, PTEN and CDKN2A in multiple cancerogenesis? Pigment Cell Melanoma Res *5*: 755-757, 2013.

22 Manfredini M, Pellacani G, Losi L, Maccaferri M, Tomasi A and Ponti G: Desmoplastic melanoma: a challenge for the oncologist. Future Oncol *4*: 337-345, 2017.

23 Spencer C, Montalvo J, McLaughlin SR and Bryan BA: Small molecule inhibition of cytoskeletal dynamics in melanoma tumors results in altered transcriptional expression patterns of key genes involved in tumor initiation and progression. Cancer Genomics Proteomics *2*: 77-85, 2011.

24 Weidle UH, Birzele F, Kollmorgen G and Rüger R: The multiple roles of exosomes in metastasis. Cancer Genomics Proteomics *1*: 1-15, 2017.

25 Pittaka M, Kardamakis D and Spyropoulou D: Comparison of international guidelines on mucosal melanoma of the head and neck: a comprehensive review of the role of radiation therapy. In Vivo *3*: 165-170, 2016.

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