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Clinicopathologic associations of *BRAF* mutation status in patients with metastatic melanoma

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Summary. *Aim: BRAF* is frequently mutated in cutaneous melanoma. The aim of the study was to assess *BRAF* status association with clinicopathologic features and outcome in patients with metastatic melanoma. *Methods:* We identified 197 consecutive Polish patients with metastatic melanoma treated in one oncological center and tested for *BRAF* mutation. We performed a retrospective chart review of patients to identify clinicopathologic characteristics in *BRAF*-mutant and *BRAF* wild-type patients. *Results:* 122/197 patients (61.9%) had *BRAF* mutation. The age at diagnosis of primary melanoma (PM) was significantly younger for *BRAF*-mutant (median age 52, n=122, range 19–78) than for *BRAF* wild-type (median age 58, n=75, range 19-85; p<0.05) patients. The most common site of PM in *BRAF*-mutant patients was the trunk (45.9%). The most common locations of first distant metastasis were the lungs, regardless of the *BRAF* mutation status. There was no difference in the time to occurrence of metastatic disease between *BRAF*-positive and *BRAF*-negative cohorts (p=0.75). Patients without *BRAF* mutations had non-significantly better overall survival (OS) when calculated from diagnosis of metastatic disease as compared to *BRAF*-mutant patients not treated with tyrosine kinase inhibitors (median OS: 337 vs. 270 days, respectively), but OS was significantly better for *BRAF*-mutant patients treated with BRAF/MEK inhibitors (median OS not reached; p<0.05). *Conclusions:* Age at diagnosis differed between the groups. The presence of mutant *BRAF* had no impact on the interval from diagnosis of melanoma to first distant metastasis, but had some impact on the natural course of metastatic disease.

Key words: metastatic melanoma, *BRAF* mutation, clinicopathologic features, tyrosine kinase inhibitors

«Associazione clinico-patologica rispetto alla mutazione BRAF nei pazienti con melanoma metastatico»

Riassunto. *Obiettivo:* BRAF è spesso mutato nel melanoma cutaneo. Lo scopo dello studio è stato quello di valutare l'associazione tra lo status BRAF con le caratteristiche clinico-patologiche e la prognosi nei pazienti affetti da melanoma metastatico. *Metodi:* Sono stati identificati 197 pazienti consecutivi polacchi affetti da melanoma metastatico Trattati in un centro oncologico e testati per mutazione BRAF. Abbiamo eseguito una revisione retrospettiva dei pazienti per identificare le caratteristiche clinico-patologiche in pazienti BRAF-mutanti e wild-type. *Risultati:* 122/197 pazienti (61,9%) presentavano una mutazione BRAF. L'età alla diagnosi di melanoma primitivo (PM) era significativamente più giovane per i pazienti BRAF-mutanti (età media 52 anni, n=122, range 19-78) rispetto ai BRAF wild-type (età media 58 anni, n=75, range 19- 85;

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p<0.05). La sede più comune di PM nei pazienti BRAF-mutanti era il tronco (45,9%). La sede più comune di prima metastasi a distanza è stato il polmone, indipendentemente dallo stato di mutazione BRAF. Non c'era alcuna differenza nel tempo di comparsa della malattia tra coorti BRAF-positivi e BRAF-negativi (p=0.75). I pazienti senza mutazioni BRAF non avevano una migliore sopravvivenza globale (OS) rispetto ai pazienti BRAF-mutante non trattati con inibitori della tirosina chinasi (OS mediana: 337 vs 270 giorni, rispettivamente), ma l'OS era significativamente migliore per i pazienti BRAF-mutante trattati con inibitori BRAF/MEK (OS mediana non raggiunta; p<0,05). *Conclusioni:* L'età alla diagnosi differiva tra i gruppi. L'intervallo tra la diagnosi di PM e la prima metastasi a distanza è identico nei due gruppi. I pazienti con melanoma metastatico con mutazione BRAF hanno la tendenza a una minore sopravvivenza.

Parole chiave: melanoma metastatico,mutazione BRAF, caratteristiche clinico-patologiche, inibitori della tirosin-chinasi

Introduction

The incidence of cutaneous melanoma is constantly increasing. According to statistics from the Polish National Cancer Registry we can still expect an increase of morbidity due to cutaneous melanoma for both sexes during the next ten years, and the incidence among young women (25–44) will be higher than among young men (1). The number of new melanoma cases in the whole population may even double by 2025 (1). Metastatic melanoma still has a poor prognosis with median overall survival reaching 6–10 months from the time of diagnosis of metastatic disease (before the era of BRAF/MEK inhibitors).

At least 50% of all sporadic cutaneous melanomas harbor *BRAF* mutations (over 90% V600E: single nucleotide mutation resulting in substitution of glutamic acid for valine) (2). The second most common mutation is V600K, followed by V600R, V600 'E2' and V600D (2, 3). RAF serine/threonine kinases are the key signaling components in the RAS pathways and the *BRAF* V600E mutation leads to a significant increase in BRAF kinase activity (4, 5). Tumor grows as a consequence of the change in the RAS-RAF-MEK-ERK signaling pathway. Several studies have shown some clinicopathologic differences between *BRAF*-mutant and *BRAF* wild-type patients, but the data about the prognostic role of BRAF mutational status are contradictory (5–12). Mutation analysis for *BRAF* and optionally *NRAS* and *KIT* are recommended by ESMO (European Society for Medical Oncology), as well as by national guidelines in patients with metastatic melanoma (13, 14).

Vemurafenib was the first approved inhibitor of mutant BRAF kinase approved in Europe in 2012, based on the positive results of a randomized phase III trial demonstrating improvement of progression-free survival (PFS) and overall survival (OS) (15, 16). Another BRAF inhibitor named dabrafenib was approved in 2013. Additionally, MEK inhibitor trametinib has been approved among this group of patients. Vemurafenib and dabrafenib have similar efficacy and a slightly different toxicity profile (17).

The aim of the study is to correlate *BRAF* status with clinicopathologic features and outcome in the Polish group of patients with metastatic melanoma treated in one tertiary oncological center.

Materials and methods

Patients

Consecutive metastatic cutaneous melanoma patients treated in one tertiary oncological center, whose tumor samples were tested for BRAF mutation between 2012 and mid 2013 with the use of the cobas 4800 BRAF V600 Mutation Test, were included in this study.

We performed a retrospective chart review of the patients treated in our department to identify those with the following criteria: pathologically confirmed

melanoma, American Joint Committee on Cancer (AJCC) stage IV and patients tested for *BRAF* mutation. Ocular and mucosal melanoma patients were not included in the study. Patients with melanoma of unknown primary site were included in the study. Data regarding age, sex, Eastern Cooperative Oncology Group (ECOG) performance status, characteristics of primary melanoma (PM), e.g. histopathologic subtype, Clark level invasion, Breslow thickness, ulceration, site, or number of positive lymph nodes, site of the first distant metastases, treatment details, serum lactate dehydrogenase level (LDH) at diagnosis of metastatic disease were collected retrospectively from the medical charts of patients. New data on treatment and survival after diagnosis of metastatic disease, new locations and number of distant metastases, biochemical blood analysis (e.g. LDH) were collected prospectively. Not all of the necessary information were available from patients` charts, e.g. all pathological features of primary melanoma (PM) which can be partly explained by the inclusion of patients with melanoma of unknown primary site (n=32).

We also evaluated outcomes of 25 patients treated at our center with BRAF inhibitor and 2 patients treated with BRAF and MEK inhibitors. Some of them (n=11) had previously been enrolled in MO25515 and MEK116513 clinical trials with BRAF inhibitor or a combination of BRAF inhibitor and MEK inhibitor, the results of these trials being previously reported (18–24).

Finally, we divided the patients into two main groups: those with melanomas harboring *BRAF* mutation or wild-type cases. We completed our data collection in July 2013.

The study was approved by the local Bio-Ethics Committee according to Good Clinical Practice Guidelines.

BRAF mutation testing

Genomic DNA was extracted from FFPE (formalin-fixed, paraffin-embedded) tissue samples of malignant melanoma. Microtome section was stained with H&E (hematoxylin and eosin) and evaluated by the pathologist for tumor tissue content. If the sample contained less than half tumor cells, the area with the highest concentration of tumor cells was chosen and macrodissected. DNA was isolated from one unstained 5 μm section containing at least 50% of tumor nucleus. The sample was deparaffinized with xylene and absolute ethanol. After paraffin removal, the tissue was dried at 56°C and lysed with proteinase K (Roche Molecular Systems Inc., U.S.A.). DNA was purified using the cobas DNA Sample Preparation Kit (Roche Molecular Systems Inc., U.S.A.) according to the manufacturer's protocol. The concentration of extracted DNA was measured using a NanoDrop spectrophotometer. Each sample was diluted to 5 ng/μl and 125 ng of DNA template was used per reaction. The presence of *BRAF* gene V600 mutation was evaluated by the cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems Inc., U.S.A.) following the manufacturer's recommendations. The target DNA was amplified and the mutation was detected on the cobas z480 analyzer (Roche Molecular Systems Inc., U.S.A.).

Statistical analysis

Time of occurrence of metastatic disease was calculated from diagnosis (excision) of primary melanoma to first information of metastasis. Overall survival time (OS) was calculated from the date of first distant metastasis of melanoma to the patient`s date of death (n=103) or last follow-up. Statistical calculations were conducted according to *BRAF*-mutational status. Chi-square and Mann Whitney U tests were used to assess the association of clinicopathologic features with BRAF status. Survival was calculated using the Kaplan-Meier method with log-rank tests for univariate comparisons. $P<0.05$ was considered statistically significant. Statistical analysis was performed using Statistica software (version 7.0).

Results

One hundred ninety-seven consecutive patients (men/women 113/84) with metastatic melanoma tested for *BRAF* mutation were included in this analysis. Patient characteristics are summarized in Table 1. 122

Table 1. Patients' characteristics.

	Mutant *BRAF* (n=122)	Wild-Type *BRAF* (n=75)
Sex – no. (%)		
Female	54 (44.3)	30 (40.0)
Male	68 (55.7)	45 (60.0)
Age at diagnosis of primary melanoma – median – yr (range)	52 (19–78)	58 (19–85)
Site of primary melanoma – no. (%):		
Upper extremity	10 (8.2)	22 (29.3)
Lower extremity	25 (20.5)	15 (20.0)
Trunk	56 (45.9)	22 (29.3)
Head	9 (7.4)	3 (4.0)
Neck	1 (0.8)	0 (0)
Unknown primary	19 (15.6)	13 (17.4)
No data	2 (1.6)	0 (0)
Histopathologic subtype – no. (%)		
NM	36 (29.5)	16 (21.4)
SSM	9 (7.4)	5 (6.7)
ALM	0 (0)	4 (5.3)
LMM	2 (1.6)	1 (1.3)
Other	1 (0.8)	1 (1.3)
Unknown	74 (60.7)	48 (64.0)
Breslow thickness – mm – no. (%)		
0.01-1.0	4 (3.3)	3 (4.0)
1.01-2.0	14 (11.5)	5 (6.7)
2.01-4.0	19 (15.6)	11 (14.7)
> 4.0	30 (24.6)	21 (28.0)
Unknown	55 (45.0)	35 (46.6)
Clark scale/level of invasion – no. (%)-		
1	0 (0)	0 (0)
2	6 (4.9)	2 (2.7)
3	19 (15.6)	6 (8.0)
4	19 (15.6)	16 (21.3)
5	8 (6.5)	6 (8.0)
Unknown	70 (57.4)	45 (60.0)
Ulceration – no. (%)		
Yes	19 (15.6)	7 (9.3)
No	40 (32.8)	27 (36.0)
Unknown	63 (51.6)	41 (54.7)
AJCC stage at diagnosis of melanoma – no. (%)		
0	0 (0)	0 (0)
I	10 (8.2)	2 (2.7)
II	28 (22.9)	22 (29.3)
III	44 (36.1)	31 (41.3)
IV	14 (11.5)	9 (12.0)
Unknown	26 (21.3)	11 (14.7)

(continued)

Table 1. Patients' characteristics.

	Mutant *BRAF* (n=122)	Wild-Type *BRAF* (n=75)
Lymphadenectomy – no. (%):		
Yes	77 (63.1)	51 (68.0)
No	41 (33.6)	24 (32.0)
Unknown	4 (3.3)	0 (0)
No. of positive LNs at pts with LND – no. (%):	n=77	n=51
0	2 (2.6)	2 (3.9)
1	19 (24.7)	10 (19.6)
2-3	17 (22.1)	8 (15.7)
≥4	21 (27.2)	20 (39.2)
Unknown	18 (23.4)	11 (21.6)

NM, nodular melanoma; SSM, superficial spreading melanoma; ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; AJCC, American Joint Committee on Cancer; LN, lymph node; LND, lymphadenectomy

(61.9%) patients had a confirmed *BRAF* mutation. Patients with mutant *BRAF* melanoma were younger than patients with wild-type *BRAF* melanoma at diagnosis of primary melanoma (median age, 52 *vs* 58 years, respectively, $p<0.05$) and at diagnosis of distant metastases (median age, 54 *vs* 61 years, respectively, $p<0.05$). There was no difference in the sex distribution between two groups.

Primary melanoma

Histopathological features: Breslow thickness, Clark level invasion, ulceration and AJCC stage at diagnosis of primary melanoma were not significantly different between *BRAF*-mutant and *BRAF*-wild-type patients (Table 1). The most common site of PM in *BRAF*-mutant patients was the trunk (45.9%), followed by extremities (28.7%: upper extremity 8.2%, lower extremity 20.5%). In the *BRAF*-wild-type group the most common site of PM was the trunk and upper extremity in the same percentage (29.3%) followed by the lower extremity (20%). There was no statistically significant difference in truncal location between the two groups, although there was a preponderance of this PM location in *BRAF*-mutant patients.

More than 60% of patients in both groups had undergone lymphadenectomy before diagnosis of metastatic disease.

Metastatic melanoma

There was no difference in the time of occurrence of metastatic disease - as calculated from PM excision (diagnosis) - between *BRAF*-positive and *BRAF*-negative cohorts ($p=0.75$) (Fig. 1.). At least 60% of patients in both groups had 0 or 1 ECOG performance status at diagnosis of metastatic melanoma. The most common AJCC stage IV at the first diagnosis of distant metastasis was M1c in both groups (including LDH level).

The most common locations of the first distant metastasis were the lungs alone or synchronous with metastasis in other organs, both in patients with mutant *BRAF* and wild-type *BRAF*. The first distant metastases were more frequent in three or more locations in *BRAF*-mutant than *BRAF* wild-type patients ($p=0.05$). In the *BRAF* positive group 44.3% patients and 38.7% in the *BRAF* negative group had second metastases (not synchronous with the first one). The most common locations of the second distant metastasis were the brain and lung in *BRAF*-mutant and *BRAF* wild-type patients, respectively.

The type of distant metastases, development of brain metastases ever, LDH level were not significantly different between the two groups. Serum LDH level was elevated in nearly 50% in both groups of patients. All details of patients with melanoma metastasis are shown in Table 2.

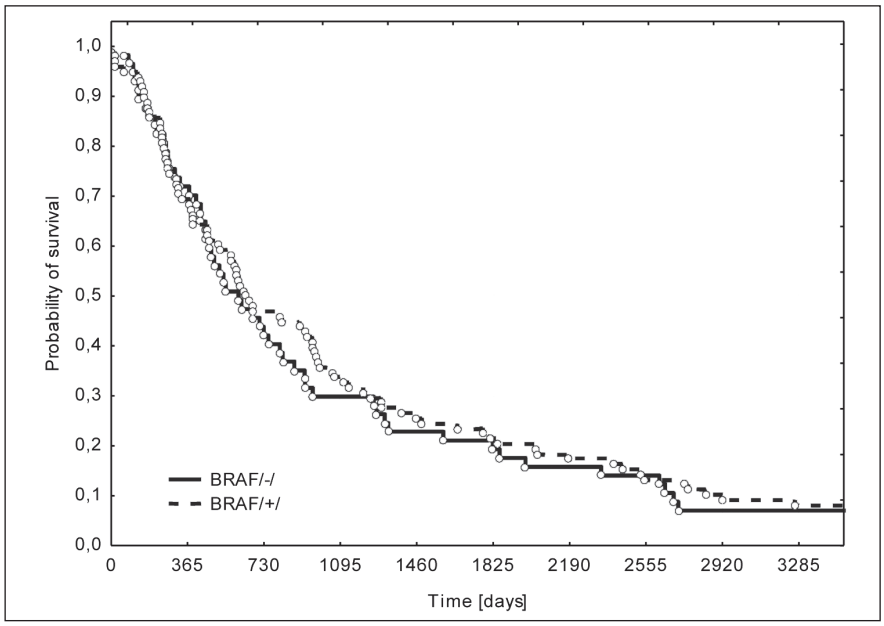

Figure 1. The interval from diagnosis of melanoma to distant metastases in *BRAF*-positive and *BRAF*- negative groups.

Twenty seven patients (22.1%) from the *BRAF*-positive group were treated with BRAF inhibitor with or without MEK inhibitor (Table 3). Eight patients treated with tyrosine kinase inhibitors (TKI) had brain metastases. Seven of them were still alive when the analysis was conducted. In two cases the diagnosis of brain metastasis led to termination of TKI therapy.

Patients without *BRAF* mutations had non-significantly better OS when calculated from diagnosis of metastatic (M1) disease as compared to *BRAF*-mutant patients not treated with TKI (median OS: 337 vs. 270 days), but OS was significantly better for *BRAF*-mutant patients treated with TKI (median OS not reached; $p<0.05$) (Fig. 2).

Discussion

Vemurafenib is the first BRAF inhibitor approved for treatment of patients with unresectable or metastatic melanoma with *BRAF* mutation, and is available in Europe in routine clinical practice. Vemurafenib was registered on the basis of positive BRIM-3 study results (15). This was a randomized, open label, multicenter study comparing vemurafenib with dacarbazine (DTIC) in patients with treatment-naïve unresectable stage IIIC or stage IV melanoma with *BRAF* mutation. Vemurafenib increased PFS and OS in this group of patients (15). Later, similar results were demonstrated for dabrafenib, leading to registration of this drug (17).

BRAF mutations occur in 41% – 70% of melanoma patients (2, 10, 25, 26). The percentage of patients with mutation in our study (61.9%) is consistent with the data available in the literature, although lower rates within the range mentioned have been reported quite often recently (2, 27).

The cobas BRAF V600 Mutation Test was used in our study. This test is more sensitive in detecting V600E mutations than Sanger sequencing and it may also detect other V600 mutations such as V600K (cobas 4800 BRAF V600 Mutation Test FDA-IVD package insert), but with lower sensitivity (28, 29). Perhaps the use of this test may have had an impact on the percentage of patients with *BRAF* mutation. However, in our previous studies the results of *BRAF* mutation detection by Sanger sequencing and by cobas test were comparable.

Investigators have tried to find a specific clinicopathologic profile of patients with mutant *BRAF*. Till now, we know only some features relating to *BRAF*-positive tumors, e.g. that younger age and primary histopathologic subtype correlate with *BRAF*-mutation status (10, 30). The results of our study confirm some

Table 2. Characteristics of metastatic melanoma patients.

	Mutant *BRAF* (n=122)	Wild-Type *BRAF* (n=75)
Age at first distant metastases – yr - median (range)	54 (20–88)	61 (20–87)
AJCC – Stage of the first distant metastasis – no. (%)		
M1a	24 (19.7)	17 (22.7)
M1b	38 (31.1)	24 (32.0)
M1c	60 (49.2)	34 (45.3)
Serum LDH[1] level at diagnosis of the first distant metastasis – no. (%)		
Normal	15 (12.3)	11 (14.7)
Elevated	10 (8.2)	7 (9.3)
Unknown	97 (79.5)	57 (76.0)
Serum LDH elevated ever – no. (%)		
Yes	60 (49.2)	37 (49.4)
No	33 (27.0)	22 (29.3)
Unknown	29 (23.8)	16 (21.3)
Location of the first distant metastasis - no.[2]:		
Skin/soft tissue	40	20
Lymph node	29	16
Lung	53	36
Liver	20	6
Peritoneal cavity/DT	20	8
Skeletal system	12	2
Brain	17	6
Other	5	5
ECOG performance status at diagnosis of MM - no. (%):		
0 or 1	75 (61.5)	45 (60.0)
2 or 3	13 (10.6)	6 (8.0)
Unknown	34 (27.9)	24 (32.0)
Number of locations of 1st distant metastasis (synchronous) – no. (%):		
1	54 (44.3)	51 (68.0)
2	50 (41.0)	22 (29.3)
≥3	18 (14.7)	2 (2.7)
Second metastasis – no. (%):		
Yes	54 (44.3)	29 (38.7)
No	68 (55.7)	46 (61.3)
Location of the second distant metastasis – no.[2]:		
Skin/soft tissue	11	5
Lymph node	8	5
Lung	10	8
Liver	9	3
Peritoneal cavity/DT	9	5
Skeletal system	8	6
Brain	21	6
Other	1	1
BRAF inhibitor ever – no. (%):		
Yes	27* (22.1)	0 (0)
No	95 (77.9)	75 (100)

BRAF, v-raf murine sarcoma viral oncogene homolog B1; AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase, DT, digestive tract; ECOG, Eastern Cooperative Oncology Group; MM, metastatic melanoma.
[1] LDH level on day of diagnosis of MM or -/+ 7 days before or after that (only in n=2); [2] including synchronous
* n=2 treated with BRAF and MEK inhibitors

Table 3. Selected clinical parameters of *BRAF*-mutant patients treated with BRAF inhibitor.

No.	M/F	Age at diagnosis of PM	Site of PM	AJCC (PM)	Location of the first distant metastasis	Brain metastasis ever	ECOG PS at diagnosis of MM	AJCC – stage of first metastasis (including LDH level)	Death
1.	M	61	T	ND	L	+	1	M1b	+
2.	F	36	T	III	Liver	-	3	M1c	+
3.	F	40	LE	ND	L	+	1	M1b	-
4.	M	75	T	III	N, L, PC/DT	-	0	M1c	+
5.	F	54	I	III	N, L	-	2	M1b	+
6.	M	36	H	III	B	+	1	M1c	-
7.	F	52	T	ND	N	-	1	M1a	-
8.	M	52	T	III	N, L	-	1	M1b	-
9.	M	69	H	ND	N	-	0	M1a	-
10.	M	57	UE	II	N, Liver	-	0	M1c	-
11.	F	41	UE	II	N, L	+	0	M1b	-
12*.	M	45	I	III	Skin/ST, N	-	0	M1a	-
13.	M	31	I	IV	Skin/ST, N, PC/DT, Skeletal system	+	2	M1c	-
14.	F	27	T	II	N	-	0	M1a	-
15.	F	62	LE	II	L	-	1	M1b	-
16.	F	60	T	II	Skin/ST, L	+	0	M1b	-
17.	F	28	H	ND	Skin/ST	+	0	M1a	-
18.	M	38	LE	III	Skin/ST, N, L, Skeletal system, B	+	2	M1c	-
19.	M	54	T	III	PC/DT	-	1	M1c	-
20.	M	60	T	I	L	-	0	M1b	-
21.	M	41	H	III	Skeletal system	-	1	M1c	-
22*.	F	50	T	III	PC/DT, Skeletal system	-	1	M1c	-
23.	M	65	T	II	Skin/ST, L	-	1	M1b	+
24.	F	72	T	I	L	-	ND	M1b	-
25.	F	26	LE	II	PC/DT	-	ND	M1c	-
26.	M	24	H	III	Liver	-	1	M1c	-
27.	M	56	UE	II	L	-	0	M1b	-

M, male; F, female; PM, primary melanoma; AJCC, American Joint Committee on Cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; MM, metastatic melanoma; LDH, lactate dehydrogenase; ND, no data; T, Trunk; UE, upper extremity; LE, lower extremity; H, head; I, ignotus; N, lymph nodes; PC, peritoneal cavity; DT, digestive tract; L, lung; B, brain; ST, soft tissue; *pts treated with BRAF and MEK inhibitors (No. 12, 22).

findings from the literature (10, 30). Patients with mutant *BRAF* melanoma were younger than patients with wild-type *BRAF* melanoma at diagnosis of PM (median age, 52 vs 58 years) and at diagnosis of distant metastases. All other features at diagnosis of PM: specifically sex distribution, Breslow thickness, histopathologic subtype, site of PM, were not significantly different between *BRAF*-mutant and *BRAF* wild-type cases. According to the literature, superficial spreading (SSM) and nodular melanomas (NM) present a much higher frequency of *BRAF* and *NRAS* mutations than other melanoma types (10, 13, 30). In our study the most common histopathologic subtype in *BRAF*-positive patients was NM, followed by SSM but without significant differences as compared with *BRAF*-negative patients. Furthermore, the most common histopathologic subtypes in *BRAF*-negative patients were also NM and SSM. These results should be interpreted with caution because access to the information about histopathologic subtype was only possible in a subgroup of patients.

The most common site of primary melanoma in *BRAF*-mutant and *BRAF* wild-type patients was the trunk and extremities, respectively. The proportion of

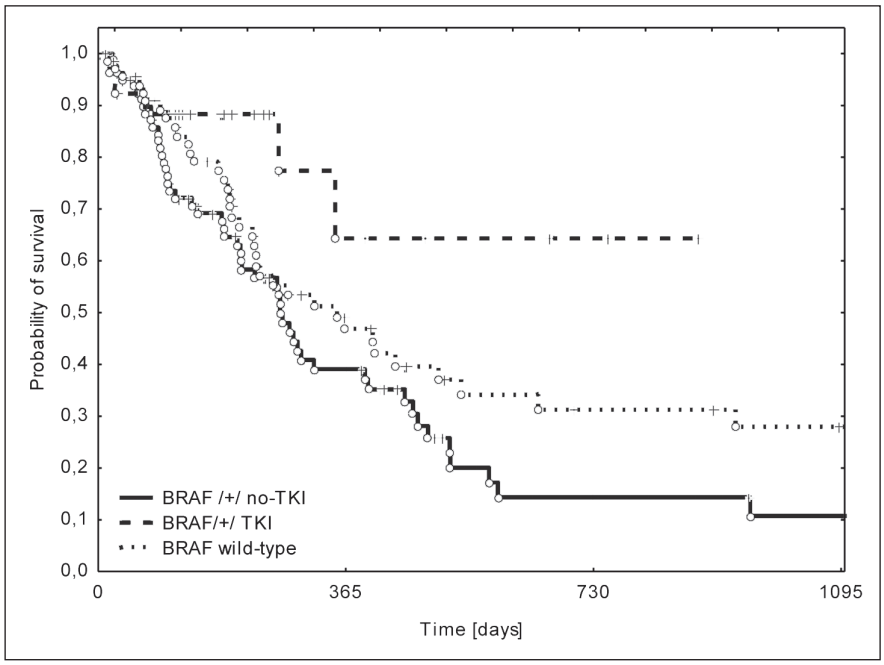

Figure 2. Overall survival from diagnosis of metastatic melanoma according to *BRAF* mutation status, and treatment with a BRAF inhibitor (tyrosine kinase inhibitor TKI – BRAF with/or without MEK inhibitors).

patients with a trunk site of PM was much higher in *BRAF*-mutant patients. These results are comparable to those available in other studies (10, 30). In the study by Long et al. the truncal location was significantly associated with the presence of mutant *BRAF* (10).

Our study also confirms that at the time of diagnosis of melanoma, *BRAF* mutation status does not affect the time of onset of metastasis. There was no difference in the time to occurrence of metastatic disease when calculated from PM excision (diagnosis) between *BRAF*-positive and *BRAF*-negative cohorts, just as generally the natural course of primary disease is independent of the *BRAF* mutational status. This may be related to the fact that BRAF is an early oncogenic event, but not sufficient per se for malignant transformation of melanocytes (31). However, once the disease is diagnosed as metastatic melanoma, *BRAF*-positive patients tend to have shorter overall survival. Mutational analysis for *BRAF* is recommended for patients with metastatic melanoma to select the proper candidates for BRAF inhibitor therapy, but also for possible patient prognostication (10, 13, 15, 16, 30).

The most common location of the first distant metastasis was the lungs in both groups. There was more frequent first distant metastasis in three and more locations in *BRAF*-mutant than *BRAF* wild-type patients. We also evaluated location of the second echelon of distant metastasis. The most common locations of the second distant metastasis were the brain and lung in *BRAF*-mutant and *BRAF* wild-type patients, respectively. The majority of investigators report only the location of the first occurrence of distant metastasis. That is why there is not enough information about the second distant metastasis in metastatic melanoma patients in the literature.

Long *et al.* in their study showed that the overall prognosis of patients with *BRAF*-mutant metastatic melanoma is not better than for those with *BRAF* wild-type metastatic melanoma. Patients with *BRAF* mutation had poorer survival unless treated with a BRAF inhibitor (10). In our study there was a non-significant difference in OS between *BRAF*-mutant and *BRAF* wild-type patients. However, there was a tendency for shorter OS in patients with *BRAF* mutation. Overall survival was significantly better for *BRAF*-mutant patients treated with TKI (median OS not reached). In our study there was a small sample size of patients (n=27) treated with BRAF inhibitor or combination (BRAF/MEK inhibitors), as in the Long *et al.* study (38 patients were treated with BRAF inhibitor ever) (10). In 2012 Menzies *et al.* published a study where 53 patients (37%) with *BRAF*-mutant metastatic mel-

anoma received therapy with BRAF, MEK or BRAF and MEK combination inhibitors (30). The outcome of these studies and our analysis shows that median OS from the date of diagnosis of metastatic melanoma is significantly longer for patients with *BRAF*-mutant melanoma treated with an inhibitor than patients with *BRAF*-mutant melanoma not treated with an inhibitor (10, 30). It is worth underlining that in our study patients with very advanced disease - with brain metastases (n=6) or with ECOG performance status 2 or more (n=4) were also treated with BRAF inhibitor.

To the best of our knowledge, we have presented the first analysis of a large series of Polish patients with metastatic melanoma tested for *BRAF* mutation, some of whom were treated with BRAF/MEK inhibitor.

Conclusions

The results of our study confirm the significant survival improvement in a group of *BRAF*-mutated melanoma, when treated with TKI, as well as a probably better natural course of the disease in the case of metastatic melanomas without *BRAF* mutation (10, 30).

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References

1. Didkowska J, Wojciechowska U, Zatonski W. Prediction of cancer incidence and mortality in Poland up to the year 2025. Melanoma of skin. Warsaw 2009; 39-44.
2. Ascierto PA, Kirkwood JM, Grob JJ, *et al.* The role of BRAF V600 mutation in melanoma. J Transl Med 2012; 10: 85.
3. Catalogue of Somatic Mutations in Cancer (COSMIC) at http://www.sanger.ac.uk/cosmic.
4. Gray-Schopfer VC, da Rocha Dias S, Marais R. The role of B-RAF in melanoma. Cancer Metastasis Rev 2005; 24: 165-83.
5. Kumar R, Angelini S, Czene K, *et al. BRAF* Mutations in Metastatic Melanoma: A possible association with clinical outcome. Clin Cancer Res 2003; 9: 3362-8.
6. Liu W, Kelly JW, Trivett M, *et al.* Distinct clinical and pathological features are associated with the BRAF (T1799A(V600E)) mutation in primary melanoma. J Invest Dermatol 2006; 127: 900-5.
7. Viros A, Fridlyand J, Bauer J, *et al.* Improving melanoma classification by integrating genetic and morphologic features. PLoS Med 2008; 5:e120.
8. Thomas NE, Edmiston SN, Alexander A, *et al.* Number of nevi and early-life ambient UV exposure are associated with BRAF-mutant melanoma. Cancer Epidemiol Biomarkers Prev 2007; 16: 991-7.
9. Edlundh-Rose E, Egyhazi S, Omholt K, *et al.* NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: A study based on mutation screening by pyrosequencing. Melanoma Res 2006; 16: 471-8.
10. Long GV, Menzies AM, Nagrial AM, *et al.* Prognostic and clinicopathologic associations of oncogenic *BRAF* in metastatic melanoma. J Clin Oncol 2011; 29: 1239-46.
11. Chang DZ, Panageas KS, Osman I, *et al.* Clinical significance of BRAF mutations in metastatic melanoma. J Transl Med 2004; 2: e46.
12. Ugurel S, Thirumaran RK, Bloethner S, *et al.* B-RAF and N-RAS mutations are preserved during short time in vitro propagation and differentially impact prognosis. PLoS One 2007; 2: e236.
13. Dummer R, Hauschild A, Guggenheim M, *et al.* Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 (suppl 7): vii86-vii91.
14. Rutkowski P, Wysocki PJ, Nowecki ZI, *et al.* Cutaneous melanoma – diagnostic and therapeutic guidelines in 2013. Onkol Prak Klin 2012; 6: 219-33.
15. Chapman PB, Hauschild A, Robert C, *et al.* Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011; 364: 2507-16.
16. Chapman PB, Hauschild A, Robert C, *et al.* Updated overall survival (OS) results for BRIM-3, a phase III randomized, open-label, multicenter trial comparing BRAF inhibitor vemurafenib (vem) with dacarbazine (DTIC) in previously untreated patients with BRAF V600E-mutated melanoma. J Clin Oncol 2012; 30 (suppl.): abstract 8502.
17. Hauschild A, Grob J, Demidov LV, *et al.* Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 2012; 380: 358-65.
18. www.rochetrials.com/studyResultGet.?studyResultNumber MO25515
19. Blank C, Del Vecchio M, Ascierto P, *et al.* An open-label, multicentre safety study of vemurafenib in patients with metastatic melanoma. ESMO Congress, Vienna, Austria, 2012. Poster 1124P.
20. Larkin J, Queirolo P, Aranceet AM, *et al.* An open-label, multicenter safety study of vemurafenib (PLX4032, RO5185426) in patients with metastatic melanoma. J Clin Oncol 2012; 30 (suppl; abstr 8517).
21. Larkin J, Del Vecchio M, Ascierto PA, *et al.* Vemurafenib in

patients with BRAF(V600) mutated metastatic melanoma: an open-label, multicentre, safety study. Lancet Oncol 2014; 15: 436-44.
22. http://www.europeanpharmaceuticalreview.com/26147/news/industry-news/trametinib mekinist-dabrafenib-tafinlar-combination-demonstrated-overall-survival-benefit compared-vemurafenib-phase-iii-braf-v600-mutant-metastatic-melanoma-study-stoppe/#.U81ZrXa6OZk
23. http://www.investegate.co.uk/glaxosmithkline-plc--gsk-/rns/mek-braf-combination-phase-iii-study-update/201407171400026316M/
24. http://www.medscape.com/viewarticle/828536
25. Dhomen N, Marais R. New insight into *BRAF* mutations in cancer. Curr Opin Genet Dev 2007; 17: 31-9.
26. Davies H, Bignell GR, Cox C, *et al.* Mutations of the BRAF gene in human cancer. Nature 2002; 417: 949-54.
27. Turajlic S, Furney SJ, Stamp G, *et al.* Whole-genome sequencing reveals complex mechanisms of intrinsic resistance to BRAF inhibition. Ann Oncol 2014; 25: 959-67.
28. http://molecular.roche.com/assays/Pages/cobas4800BRAFV600MutationTest.aspx
29. Menzies AM, Long GV. New combinations and immunotherapies for melanoma: latest evidence and clinical utility. Ther Adv Med Oncol 2013; 5: 278-85.
30. Menzies AM, Haydu LE, Visintin L, *et al.* Distinguishing clinicopathologic features of patients with V600E and V600K *BRAF*-mutant metastatic melanoma. Clin Cancer Res 2012; 18: 3242-9.
31. Kumar R, Angelini S, Snellman E, Hemminki K. BRAF mutations are common somatic events in melanocytic nevi. J Invest Dermatol 2004; 122: 342-8.

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