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ORIGINAL ARTICLE

BRAF mutation status might contribute an effect on both disease-free and overall survival in stage III cutaneous melanomas treated with intermediate dose interferon-alpha

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Abstract

Purpose The interaction between interferon treatment and BRAF mutation status among melanoma patients have yet to be evaluated. The present study aims to assess the intermediate dose interferon (IDI) in stage III melanoma patients with respect to BRAF mutation status.

Methods A total of 46 adult lymph node-positive primary skin melanoma patients (23 BRAF-mutant and 23 BRAF-wild) with available information on the mutational status of the oncogene BRAF V600E were included in the analysis. BRAF V600E mutation was detected using the real-time PCR in the formalin-fixed paraffin-embedded samples. All the patients were treated with adjuvant IDI. IFN-alpha-2b was administered 10 MU per day, subcutaneously, three times per week for 1 year.

Results The distribution of patient numbers between the clinicopathological variables and BRAF mutation status was well balanced. Most of the patients relapsed (83%); however, no significant differences were found between recurrence frequencies and recurrence sites in accordance with BRAF mutation status. BRAF-mutant melanomas were found to be significantly advantageous in disease-free survival (HR 0.464, $p=0.03$). More deaths occurred in BRAF-wild-type patients (67%) ($p=0.03$), and BRAF mutation was found to be a favorable prognostic factor for overall survival (HR 0.373, $p=0.04$).

Conclusion The presence of BRAF mutation in stage III melanoma patients treated with IDI might contribute a favorable effect on both disease-free survival and overall survival.

Keywords Melanoma · BRAF mutation · Interferon · Survival

Introduction

Stage III melanoma makes up 9% of new diagnoses, and in 2017, 5-year relative survival rate is predicted around 62% in the US [1]. In node-positive disease, the number of metastatic lymph nodes is one of the most significant independent prognostic factors apart from others, such as tumor thickness and ulceration [2–3]. Five-year survival rates in pathologic stage III melanoma patients were found to demonstrate diversities for defined subgroups, i.e., ranging from 69 to 13% [2] and from 70% for patients with T1-4N1a to 39% for those with T1-4N3 [3]. Clearly, there is a need for an effective adjuvant therapy in these patients.

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Interferon (IFN) has been extensively used for years as the most effective agent in the adjuvant therapy of melanoma [4]. High-dose interferon (HDI) has been the only adjuvant drug that was approved by FDA in high-risk melanomas after resection and it has shown statistically significant improvement in relapse-free and sometimes overall survival; however, both of these effects appear to decrease with longer follow-up, thus its clinical benefit may be limited to a subset of patients [4, 5]. Moreover, intermediate dose interferon (IDI) adjuvant therapy was not found to improve overall survival either, and results for relapse-free survival were contradictory across reports; however, IDI therapy was found to be associated with advantages such as acceptable tolerability and lower cost [5]. As do many other cancer centers [4], our institute prefers IDI to HDI for adjuvant treatment particularly in stage III melanomas because of these advantages mentioned earlier [6].

BRAF is a proto-oncogene and is one of the growth signal transduction RAF kinases that accounts for the regulation

of the mitogen-activated protein kinases (MAPK) pathway that mediates cell division, differentiation and secretion [7]. In malignancies, a number of BRAF mutations have been discovered; most of these mutations result from an amino acid substitution at position 600 in BRAF, from a valine to a glutamic acid (BRAF V600E) that eventually causes the activation of kinase pathway and cancer development [7]. It is suggested that approximately 50% of skin melanomas harbor BRAF mutations [7]. BRAF V600E is the most common BRAF mutation in melanoma and it causes augmented kinase activity that leads to uncontrolled melanocyte cell growth, increased mitotic activity and accelerated melanoma development. The similar argument regarding the association between the tumor BRAF genotype and survival in metastatic melanoma patients exists also for non-metastatic melanoma patients; some studies argue in favor of [8] and some others disprove [9] the presumption that BRAF mutation is a favorable prognostic factor in non-metastatic melanoma patients; however, there are also publications that found no association between BRAF mutation and survival in such patients [10].

BRAF V600E mutation leads to protracted activation of MAPK pathway which mediates proliferation and survival of melanoma cells and regulates molecules that mediate interactions between melanoma cells and host immunity. The activation of this pathway also down-regulates type I IFN-alpha receptor-1 (IFNAR1) level and signaling that mediates the effects of IFN-alpha [11, 12]. Therefore, BRAF inhibitor enhances the antiproliferative and immunomodulatory effects of IFN-alpha on BRAF V600E melanoma cells because inhibition of ERK activation by BRAF inhibitor upregulates IFNAR1expression [11, 12].

The interaction between IFN and BRAF mutation status among melanoma patients have yet to be uncovered. The present study aims to assess IDI in stage III melanoma patients with respect to BRAF mutation status.

Materials and methods

A total of 46 adult Turkish Caucasian lymph node-positive (stage III) primary skin melanoma patients with available information on the mutational status of the oncogene BRAF V600E were included in the analysis. The records were retrieved from the cancer registry for review of the clinical and pathological features and outcome. The disease was staged according to AJCC (8th edition) staging system. Lymph node status was determined by either sentinel lymph node (SLN) biopsy or elective lymph node dissection. Patients with pathologically positive SLN underwent a completion lymphadenectomy. Data were retrieved retrospectively from the charts of the patients who had been admitted, treated and followed up at Istanbul University Institute of Oncology, a single tertiary referral center. In case of disease relapse, patients were treated with various therapeutic options such as BRAF/MEK inhibitors, immunomodulatory

Table 1 Comparisons of the clinicopathological variables based on BRAF mutation status

Variable	BRAF (−)	BRAF (+)	*p*
Age, years			0.01
≥ 50	14	6	
< 50	9	17	
Sex			1.00
Male	16	16	
Female	7	7	
Site of lesion			0.29
Axial	9	12	
Limbs	13	9	
Histopathology			0.58
Nodular	8	8	
Others	6	9	
Clark invasion level			0.32
I–III	0	1	
IV–V	17	17	
Breslow thickness, mm			0.15
< 2	2	0	
≥ 2	16	19	
Ulceration			0.24
Positive	13	11	
Negative	4	8	
Mitotic rate, /mm^2			0.06
≥ 3	14	12	
< 3	1	6	
Lymphovascular invasion			0.47
Positive	5	5	
Negative	8	14	
Tumor-infiltrating lymphocytes			1.00
Positive	6	6	
Negative	11	11	
Regression			0.67
Positive	3	3	
Negative	9	12	
Node stage (*N*)			0.77
1	10	9	
2–3	13	14	
Relapse at follow-up			0.12
No	2	6	
Yes	21	17	
Site of relapse			0.17
Locoregional only	6	9	
Distant only	8	4	
Last status			0.03
Alive	9	16	
Dead	14	7	

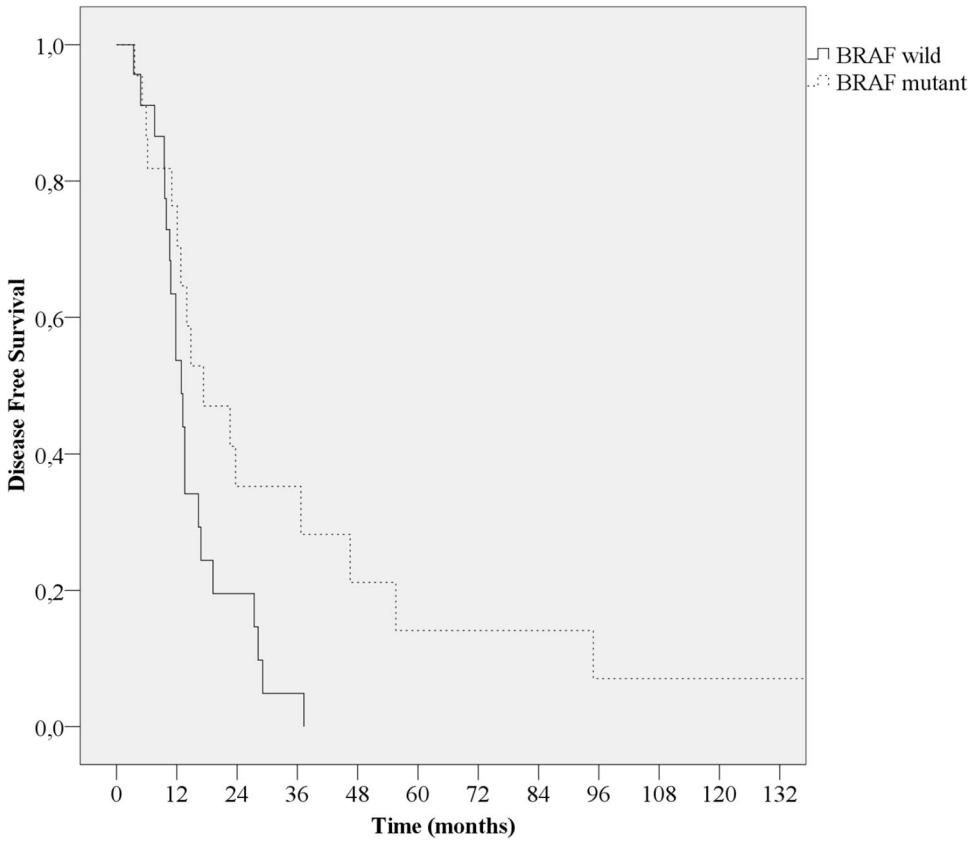

Fig. 1 Disease-free survival curves based on BRAF mutation status ($p = 0.03$)

agents (CTLA-4 or/and PD-1 inhibitors) and cytotoxic agents (temozolomide and platinum agents) depending on clinical indication when their cases emerged and whether such treatments were available.

BRAF V600E mutation was detected using the real-time PCR in the formalin-fixed paraffin-embedded samples. Genomic DNA was extracted from melanoma cell-positive paraffin-embedded samples via the commercial kit Exgene FFPE Tissue DNA Kit (GeneAll Biotechnology Co, Seoul, Korea; Catalog no: 38-150) according to the kit's instructions. Fifty-nanogram DNA was then applied for BRAF V600E mutation analysis using a commercial kit with a detection sensitivity of 1% mutation load (BRAF Mutation Analysis Kit II, Entrogen, USA; Catalog no: BRAFX-RT64) in a ABI 7500 real-time PCR machine (Applied Biosystems, USA) as described previously [13]. The allele-specific probe used to identify the V600E mutation was labeled with FAM.

All patients were treated with adjuvant IDI. IFN-alpha-2b was administered 10 MU per day, subcutaneously, three times per week for 1 year. All the patients were followed up according to standard international guidelines including National Comprehensive Cancer Network guidelines.

Comparisons between patient/disease variables and BRAF V600E mutation status were done using Chi-square tests. Kaplan–Meier analysis was used for estimation of survival and differences in survivals were assessed by the log-rank statistics. Disease-free survival (DFS) was calculated from the date of pathologic diagnosis to the date of the clinical recurrence which was defined as detected by imaging studies or by clinical examination. Overall survival (OS) was determined from the date of pathologic diagnosis to death resulting from any cause. A p value ≤ 0.05 was considered significant. Statistical analysis was carried out using SPSS 21.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

At the time of diagnosis, all patients ($n = 46$) had stage III disease [N1 ($n = 19$, 41%), N2 ($n = 19$, 41%) and N3 ($n = 8$, 18%)]. The demographic, histopathological and clinical characteristics with respect to BRAF mutation statuses are listed in Table 1. The median age was 48.5 years (ranging from 27 to 70 years) and men were predominant ($n = 32$). The majority of the patients had thick Breslow depth (≥ 2mm) (95%), high mitotic rate ($\geq 3/\text{mm}^2$) ($n = 79\%$) and ulcerated lesions (67%). The distribution of patient numbers between the clinicopathological variables and BRAF mutation status was well balanced ($p > 0.05$); however, younger patients were more significantly associated with BRAF mutation ($p = 0.01$) (Table 1).

Table 2 Univariate analyses of variables associated with disease-free survival (DFS) and overall survival (OS)

Variables	DFS			OS		
	HR	95% CI	*p*	HR	95% CI	*p*
Age						
< 50 vs ≥ 50 years	1.742	0.857–3.538	0.1	1.258	0.516–3.068	0.6
Sex						
Male vs female	0.696	0.339–1.429	0.3	0.872	0.315–2.414	0.8
Site of lesion						
Axial vs limbs	1.632	0.815–3.270	0.1	1.805	0.727–4.478	0.2
Histopathology						
Nodular vs others	1.098	0.453–2.658	0.8	1.296	0.409–4.110	0.6
Breslow depth, mm						
< 2 vs ≥ 2	0.476	0.109–2.072	0.3	0.641	0.083–4.986	0.6
Ulceration						
Positive vs negative	4.392	1.460–13.21	0.008	4.315	1.102–19.31	0.05
Mitotic rate						
< 3 vs ≥ 3/mm^2	9.542	1.255–72.55	0.02	2.210	0.48–10.179	0.3
Lymphovascular invasion						
Positive vs negative	1.913	0.751–4.871	0.1	2.648	0.827–8.482	0.1
Tumor-infiltrating lymphocytes						
Positive vs negative	0.778	0.332–1.821	0.5	0.957	0.339–2.701	0.9
Regression						
Positive vs negative	2.405	0.861–6.721	0.09	1.207	0.319–4.565	0.7
Node stage (*N*)						
1 vs 2–3	0.842	0434–1.634	0.6	1.006	0.402–2.520	0.9
Relapse at follow-up						
No vs yes	–	–	–	0.333	0.092–1.209	0.09
BRAF mutation						
Negative vs positive	0.464	0.229–0.940	0.03	0.373	0.143–0.974	0.04

Most of the patients relapsed ($n = 38$, 83%); however, no significant differences were found between recurrence frequencies and recurrence sites in accordance with BRAF mutation status (Table 1). The median disease-free survival times were 12.8 months (ranging from 3.1 to 180.0) for BRAF-mutant and 11.8 months (ranging from 3.4 to 37.3) for BRAF-wild-type patients ($p = 0.03$) (Fig. 1). Moreover, the minority of BRAF-mutant patients (14%) was disease free in the fifth year of follow-up; on the other hand all of the BRAF-wild-type patients relapsed before reaching this time. Apart from ulceration and mitotic rate, BRAF mutation status also had a significant prognostic effect on disease-free survival (HR 0.464, $p = 0.03$) (Table 2).

Twenty-one patients died (46%), of which 67% were BRAF-wild type ($p = 0.03$) (Table 1). The median time and 5-year overall survival rates for BRAF-mutant cases were 24.5 months (ranging from 5.1 to 184.5) and 60%, respectively. These numbers were lower in BRAF-wild-type patients: 18 months (ranging from 4.1 to 100) and 28.6% (Fig. 2). Furthermore, the presence of the BRAF mutation was found to be a favorable prognostic factor for overall survival (HR 0.373, $p = 0.04$) (Table 2).

Discussion

In this study, we observed that BRAF-mutant stage III melanoma patients who were treated with adjuvant IDI have more favorable disease-free and overall survivals compared to their BRAF-wild counterparts. The data and reports presented on this subject so far have been scarce [11, 14]. The effect of BRAF inhibitors on IFNAR1 expression was probed using three melanoma cell lines and four biopsies of BRAF V600E metastases in 60 melanoma patients who did not receive treatment [11]. Three melanoma cell lines were used to evaluate in vitro and in vivo antiproliferative, pro-apoptotic and immunomodulatory activities of the combination of BRAF inhibitors and IFN-alpha. The IFNAR1 level was significantly lower in BRAF V600E melanomas than in BRAF-wild-types ($p < 0.001$). IFNAR1 reduction was turned backward by BRAF inhibitor administration in three cell lines ($p \leq 0.02$) and in three of four metastases. These changes were associated with (a) an increased sensitivity in vitro of melanoma cells to the antiproliferative, pro-apoptotic and immunomodulatory activities of BRAF inhibitor and IFN-alpha combination and, (b) an increased

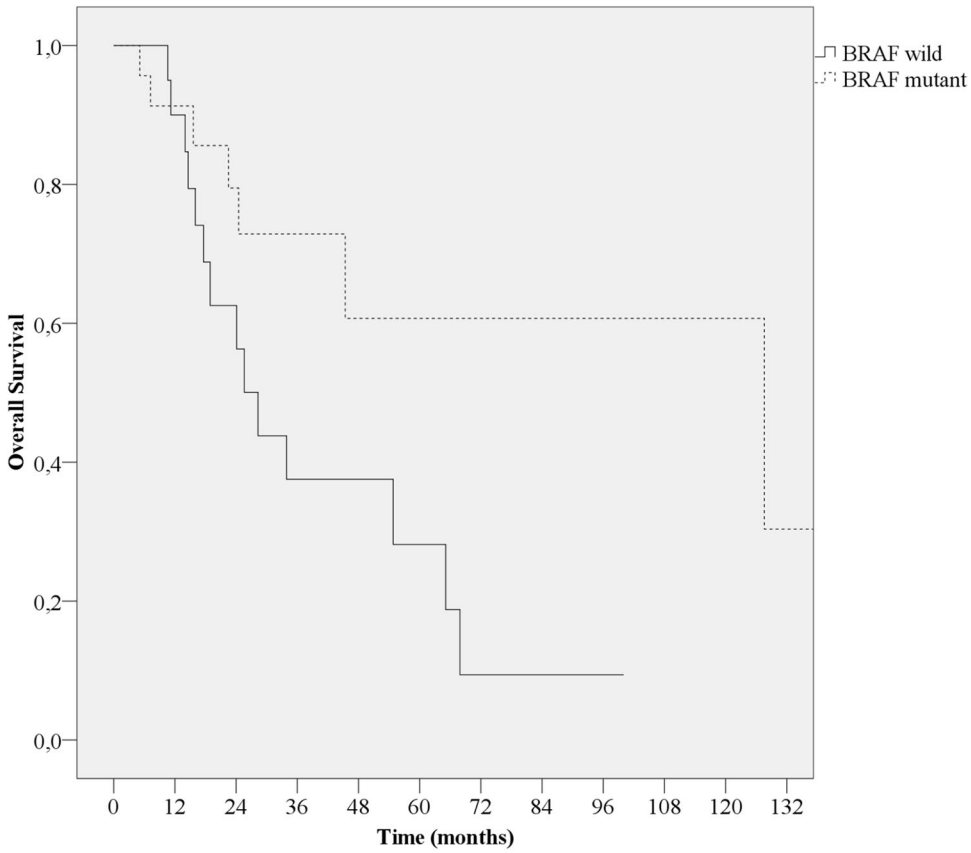

Fig. 2 Overall survival curves based on BRAF mutation status ($p = 0.04$)

survival and inhibition of tumor proliferation of melanoma cells in vivo by BRAF inhibitor and IFN-alpha combination. The authors concluded that these results provided a strong rationale for the clinical trials in BRAF V600E melanoma patients with BRAF inhibitor and IFN-alpha combination.

Experiments done by Sabbatino et al. concurred that IFNAR1degradation in BRAF-mutant melanomas may regulate immunogenicity and rescind antitumor immune responses of the host [11]. However, the effects of IFN-alpha in melanoma are recondite, composite and in some cases time dependent; IFN-alpha therapy causes expected changes in pSTAT1/3 and IFNAR expression but no congruous effects on MHC class I/II expression [12, 15]. Prior US Intergroup and EORTC adjuvant studies of HDI-alpha and pegylated IFN-alpha did not restrict or stratify enrollment by BRAF mutation status [12]. Retrospective evaluation of archived tumor samples with mature follow-up data from US Intergroup trials (E1684, E1690, and E1694) of IFN-alpha will provide some evidence as to whether BRAF mutation status and/or IFNAR1 expression modify disease-free and/or overall survival [12].

A Chinese clinical study investigated HDI-alpha in BRAF-mutant stage IIB–IIIC cutaneous melanoma patients ($n = 88$, 45% was stage III) and discovered improved disease-free survival in stage III melanomas ($p = 0.021$); this result concurs with our report [14]. They concluded that HDI therapy may contribute a beneficial effect to the BRAF-mutant high-risk melanoma patients who underwent tumor resection. The hypothesis claiming that IFNAR1 degradation in BRAF-mutant melanoma sensitizes cells to antitumor effects of IFN-alpha might be the possible explanation for the results of this study as well as our study [12].

In conclusion, there are conflicting reports regarding association between IFN treatment and BRAF mutation status in high-risk melanoma patients after resection. In this study, with a small number of patients that was a limitation to the study we showed that stage III BRAF-mutant melanoma patients treated with IDI have better disease-free and overall survivals compared to their BRAF-wild counterparts. The potential predictive role of interferon with respect to the BRAF mutation status in managing of high-risk melanoma patients needs to be investigated in larger studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Informed consent was obtained from all individual participants included in the study. The study was reviewed and approved by our local ethical committee.

Ethical approval All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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