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REVIEW

BRAF mutational status as a prognostic marker for survival in malignant melanoma: a systematic review and meta-analysis

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ABSTRACT

Background: The analysis of the BRAF mutational status has been established as a standard procedure during diagnosis of advanced malignant melanoma due to the fact that BRAF inhibitors constitute a cornerstone in the treatment of metastatic disease. However, the general impact of BRAF mutational status on survival remains unclear. Our study aimed to assess the underlying prognostic significance of BRAF mutant versus wild type (WT) malignant melanoma on overall survival (OS), disease-free survival (DFS) and progression-free survival (PFS).

Material and methods: A systematic literature search in EMBASE, Medline and Cochrane CENTRAL was performed. Studies were included if they reported survival outcomes for BRAF mutant versus WT patients as hazard ratios (HR) or in Kaplan-Meier (KM) curves. Random-effects meta-analysis models were used to pool HRs across the studies.

Results: Data from 52 studies, representing 7519 patients, were pooled for analysis of OS. The presence of a BRAF mutation was statistically significantly associated with a reduced OS (HR [95% confidence interval (CI)]: 1.23 [1.09–1.38]), however, with substantial heterogeneity between the studies (I^2: 58.0%). Meta-regression and sensitivity analyses showed that age, sex and BRAF mutation testing method did not have a significant effect on the OS HR. BRAF mutant melanoma showed comparable effect on DFS to non-BRAF mutant melanoma in stage I–III melanoma (combined HR: 1.16, 95% CI: 0.92–1.46), and on PFS in stage III–IV (HR: 0.98 (95% CI: 0.68−1.40)).

Conclusion: Although there was substantial heterogeneity between the studies, the overall results demonstrated a poorer prognosis and OS in patients harbouring BRAF mutations. Future studies should take this into account when evaluating epidemiological data and treatment effects of new interventions in patients with malignant melanoma.

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Introduction

Malignant melanoma is the most aggressive skin cancer entity accounting for approximately 5% of cancer incidence globally. Over the past few decades, its incidence and mortality have increased significantly in many countries [1,2]. In early-stage melanoma, effective excision with an appropriate surgical margin can cure the disease with excellent prognosis. However, distant metastases are considered life-threatening and are associated with an impaired 5-year survival rate ranging between 10% and 30% [1–3]. However, due to the implementation of novel therapies like immunotherapy in monotherapy using PD-1 inhibitors alone or combined with CTLA-4 and PD-1 inhibitors in concert, these figures have recently been improved.

Recent advances in molecular biology demonstrate that transformation of melanocytes to malignant melanoma is associated with the activation of proto-oncogenes and inactivation of tumour suppressor genes into their malignant derivatives [4,5]. Mutations regulating genes like p53, NRAS, and BRAF lead to activation of pathways involved in melanoma carcinogenesis and progression. In particular, mutations in BRAF, a serine/threonine protein kinase activating the MAP kinase/ERK-signalling pathway which plays an important role in cell proliferation and apoptosis [4,5] have been reported in 40–60% of melanoma patients [6–10]. Lastly, in

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Supplemental data for this article can be accessed here.

about 80–90% of BRAF mutations glutamic acid (E) replaces valine (V) at the amino acid position 600 (V600E) [4,5,11,12].

BRAF was identified as a therapeutic target and valuable predictive marker in the treatment of advanced malignant melanoma, thus, BRAF mutation analysis has become increasingly common in the diagnostic procedure. Patients harbouring the V600 mutation showed improved OS after novel therapies targeting the BRAF/MEK pathway (e.g., vemurafenib [13,14], dabrafenib [15,16], trametinib [17], cobimetinib [18], and combinations thereof) [19]. However, the impact of BRAF mutation status on overall survival (OS) remains controversial [3,20], especially in conjunction with other factors such as age, sex, European Cooperative Oncology Group (ECOG) performance status, and metastatic sites which play critical roles in the prognosis and risk of disease recurrence [21,22].

The aim of this study was to assess the impact of BRAF activating mutations versus BRAF wild type (WT) malignant melanomas on OS, progression-free survival (PFS) and disease-free survival (DFS), by integrating available evidence in a meta-analysis.

Material and methods

Search strategy and study selection

A systematic literature review was conducted following the PRISMA guidelines [23]. Medline, EMBASE, and Cochrane CENTRAL databases were searched for studies published between 1 January 2002 and 7 December 2016. The search focussed on publications from 2002 onwards to align with Davies et al. [7], the first publication to describe the BRAF mutation in cancer. Search strategy is provided in Supplement Table 1. A manual search of 2015–2016 conference abstracts from meetings of the American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO), European Association of Dermato Oncology (EADO), American Association for Cancer Research (AACR), and Society for Melanoma Research (SMR) was also performed.

Studies were included if they reported survival outcomes for BRAF mutant versus WT patients as a hazard ratio (HR) or in a Kaplan-Meier (KM) curve. The outcomes of interest were OS for all malignant melanoma patients, DFS/relapse-free survival (RFS) for stage I–III patients, and PFS for stage III–IV patients. Studies with no adult melanoma patients, no survival analysis, or no WT versus mutant BRAF comparison were excluded. Studies employing checkpoint blockade immunotherapy or reporting survival data from patients currently undergoing treatments with BRAF or MEK inhibitors were also excluded along with exploratory studies enrolling less than 15 patients.

Data extraction and quality assessment

When only KM data were available, curves were digitised and HRs were estimated as per the methodology developed by Guyot et al. [24]. Each study was critically appraised using the Quality In Prognosis Studies (QUIPS) appraisal tool for assessing risk of bias [25,26].

Statistical analysis

Random-effects meta-analysis models were used to pool HRs across all studies and by disease stage according to the seventh edition of the American Joint Committee on Cancer (AJCC) cancer staging system [27]. The individual studies were weighted through inverse variance weights following DerSimonian and Laird [28]. To assess the heterogeneity across studies, we performed a chi-square test to estimate the I^2 statistic (0–100%, 0% meaning no heterogeneity) [29].

To explain potential heterogeneity, pre-specified potential effect modifiers, i.e., age, and sex, were investigated *via* meta-regressions. Subgroup analyses were conducted per disease stage, mutation subgroups, and excluding specific groups of studies: (1) with outlier definitions of OS (i.e., based on melanoma-specific death only), (2) using non-polymerase chain reaction (PCR)-based techniques for detecting BRAF mutation, (3) with the definition of WT not restricted to BRAF WT only, and (4) with the HR estimated from KM curves.

Small study effects and publication bias were assessed by funnel plots and Egger's tests [30]. Analyses were performed using the meta [31] and metaphor [32] packages in *R* [33] statistical software.

Results

Overview of identified studies

An overview of the study selection process is presented in Figure 1. A total of 54 studies (8 interventional, 46 non-interventional studies) published from 2006 to 2016 were identified and met the inclusion criteria for meta-analysis. The characteristics of the included studies are presented in Table 1. In Roh et al. [65], 158 patients from an archived primary Korean melanoma cohort (KMC) were studied, and 234 patients from The Cancer Genome Atlas melanoma (TCGA-MEL) cohort were included for validation. Hence, results were reported separately for the two cohorts.

The sample size ranged from 17 to 770, with 25 studies including less than 100 patients and with only two studies of over 500 patients [8,77]. The median age ranged from 50 to 70 years for all melanoma patients, and from 50 to 64 years for stage IV melanoma. The percentage of males ranged from 32% to 76%, without any particular outlier. Twenty-five studies included patients with only cutaneous melanoma, and 13 studies included 26% to 92% patients with cutaneous melanoma, whilst 23 studies did not indicate melanoma type. Additionally, 24 studies did not provide information regarding treatment exposure (see Supplement Table 2).

The disease stage of included patients was diverse. Six studies [40,45,51,56,77,82] that did not specify the stage distribution were assigned to stage I–IV. WT was defined as BRAF/NRAS dual WT tumours in 19 studies and as solely

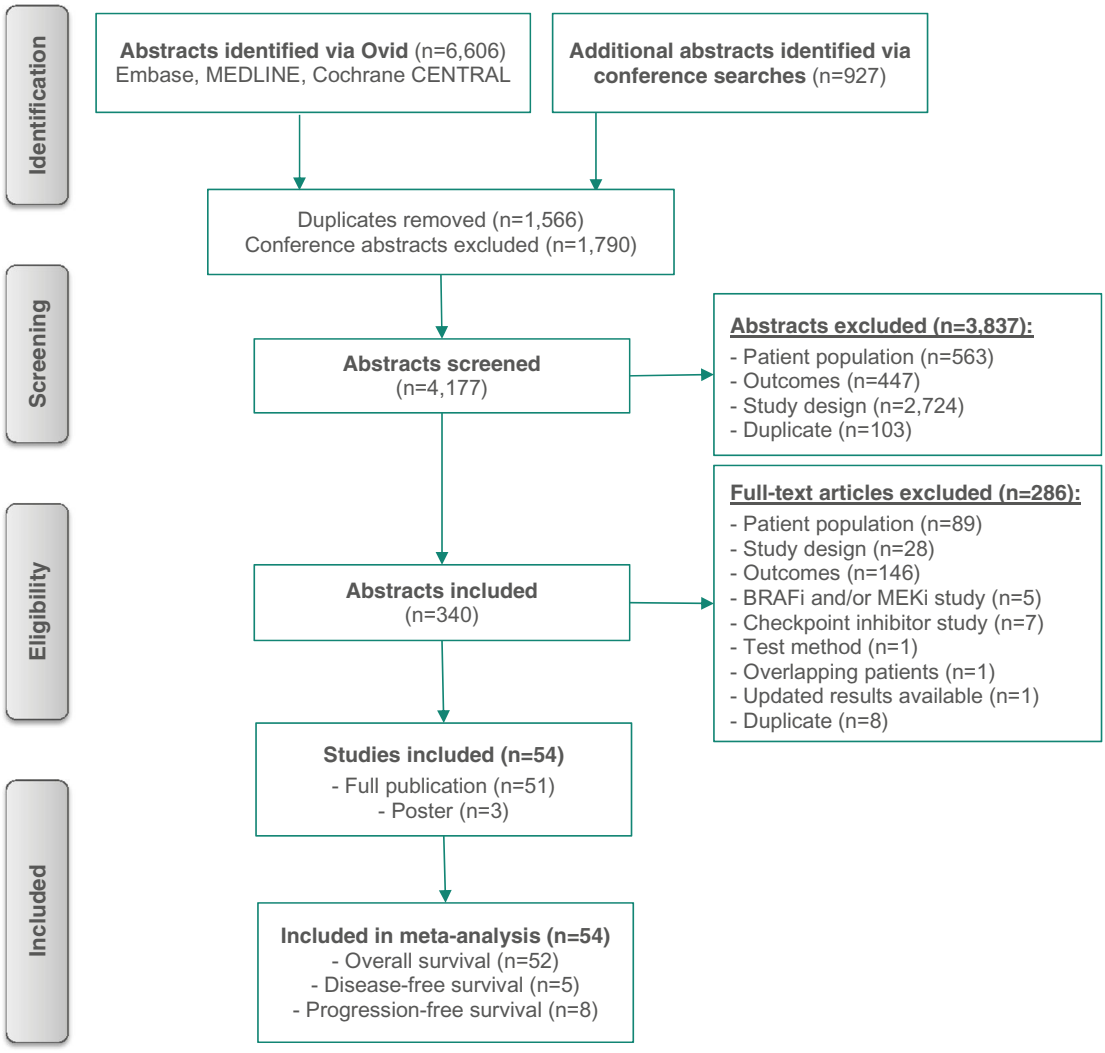

Figure 1. Flow diagram of study selection.

BRAF WT tumours in 42 studies. The BRAF mutation-positive rates in individual studies ranged from 14% to 75%.

Fifty-one studies reported the methods used for detecting BRAF mutations, with 47 studies using PCR-based genetic techniques and four studies [38,41,42,67] using immunohistochemistry (IHC) (see Supplement Figure 1).

Results of the risk of bias assessment [25,26] found that one third of the included studies were associated with moderate to high risk of bias due to inadequately reporting the study attrition and confounding (see Supplement Table 3).

Impact of BRAF mutation on OS

Figure 2 presents the results of the meta-analysis for OS. The overall pooled effect of 56 HRs from 52 studies showed that BRAF mutant melanoma was likely to be associated with shorter OS compared to non-BRAF mutant melanoma (combined HR: 1.23, 95% CI: 1.09–1.38). The heterogeneity between the studies can be considered moderate to substantial ($I^2 = 58\%$). Most of the subgroup HRs suggested a similar trend to the overall HR: stage I–II, I–III, III, I–IV, III, III–IV and IV patients, with a statistically significant difference for stage III–IV.

For stage I, I–II and I–III patients, each subgroup had one or two studies only. BRAF mutant melanoma was associated with better but not statistically significant OS compared to non-BRAF mutant melanoma at stage I and stage I–III (see Supplement Figure 2). Two studies [21,63] examined OS among 557 patients in BRAF mutated and WT stage I–II melanoma patients. The HR in Mechbach et al. [21] was estimated from a KM curve. The pooled effect showed no statistically significant impact of BRAF mutation on OS for stage I–II patients (HR: 2.03, 95% CI: 1.27–3.24).

Eleven studies included stage III–IV patients without stage-specific survival data. Apart from Ugurel et al. [78], showing a statistically significant higher risk of death due to melanoma for BRAF mutated versus WT patients, no other studies found any prognostic significance for BRAF mutation on risk of death due to any cause. The overall pooled results showed no statistically significant prognostic impact on BRAF mutations for stage III–IV melanoma (HR: 1.23, 95% CI: 1.02–1.48).

The pooled effect from the six studies on stage III melanoma also lacked statistical significance (HR: 1.52, 95% CI: 0.99–2.33). The largest HR (4.49) was reported in a small retrospective study [64] with 72 patients assessed for BRAF-

Table 1. Characteristics of Included Studies.

Author year	AJCC stage	Study design	Follow-up (years)*	Definition of WT	N included in MA	Age (years)*	Tx received/allowed**	KM	OS	DFS	PFS
Akman 2015 [34]	Stage II: 46% Stage III: 54%	Retrospective cohort study	3.1	nonBRAFm	BRAFm: 21 WT: 29	51.2	Adjuvant interferon therapy + tumour surgery	Y	Y	Y	N
Algazi 2015 [35]	Stage III: 3% Stage IV: 97%	Prospective, single arm, phase II study	NR	nonBRAFm	BRAFm: 6 WT: 32	NR	Axitinib + carboplatin/ paclitaxel	Y	N	N	Y
Amaravadi 2009 [36]	Stage IV: 100%	Four-arm clinical trial (Phase II)	NR	nonBRAFm/nonNRASm	BRAFm: 26 WT: 28	61.0					
		Temozolomide + sorafenib	N	N	N	Y					
Barbour 2014 [37]	Stage III: 100%	Registry (retrospective analysis)	2.5	nonBRAFm	BRAFm: 57 WT: 67	57.4	Surgery	Y	Y	Y	N
Bhandaru 2014 [38]	Stage I: 25% Stage II: 35% Stage III: 17% Stage IV: 24%	Retrospective analysis	5.0	low BRAFm	BRAFm: 229 WT: 98	NR	NR	N	Y	N	N
Birkeland 2013 [39]	Stage III: 4% Stage IV: 96%	Single institution, retrospective cohort study	NR	nonBRAFm/nonNRASm	BRAFm: 26 WT: 31	62.4	Dacarbazine	Y	Y	N	Y
Brown 2012 [40]	Stage I–IV: 100%	Retrospective series	5.4	nonBRAFm	BRAFm: 43 WT: 101	53.3 (mean)	NR	Y	Y	N	N
Capper 2012 [41]	Stage IV: 100%	Retrospective analysis	NR	nonBRAFm	BRAFm: 32 WT: 29	57.4	NR	Y	Y	N	N
Carlino 2014 [20]	Stage IV: 100%	Retrospective cohort study	NR	nonBRAFm/nonNRASm	BRAFm: 92 WT: 62	58.8					
		Chemotherapy + immunotherapy	Y	Y	N	N					
Cheng 2015 [42]	Stage I: 21% Stage II: 36% Stage III: 28% Stage IV: 15%	Retrospective analysis	5.0	low BRAFm	93***	60.0	NR	N	Y	N	N
Davies 2012 [43]	Stage III: 40% Stage IV: 60%	Clinical trial/ phase I	NR	nonBRAFm/nonNRASm	BRAFm: 10 WT: 8	51.0	Sorafenib + temsirolimus	Y	Y	N	Y
Devitt 2011 [44]	Stage I: 66% Stage II: 25% Stage III: 9%	Retrospective cohort study	3.9	nonBRAFm/nonNRASm	BRAFm: 112 WT: 101	55.3 (mean)	NR	N	Y	Y	N
Edlundh-Rose 2006 [45]	Stage I–IV: 100%	Clinical study (sample analysis)	NR	nonBRAFm/nonNRASm	BRAFm: 120 WT: 40	58.5 (mean)	Surgery	Y	Y	N	N
Ekedahl 2013	Stage III–IV: 100%	Cohort study	2.0	nonBRAFm/nonNRASm	BRAFm: 54 WT: 32	60.2	Surgery	Y	Y	N	N
Frauchiger 2016 [46]	Stage IV: 100%	Retrospective review	NR	nonBRAFm	BRAFm: 34 WT: 74	57.3	Radiotherapy or systemic therapy	Y	Y	N	N
Gallaher 2016 [47]	Stage IV: 100%	Retrospective review	2.5	nonBRAFm	BRAFm: 11 WT: 8	53.9	Stereotactic radiosurgery	Y	Y	N	N
Gorayski 2015 [48]	Stage III–IV: 100%	Retrospective cohort study	1.6	nonBRAFm	BRAFm: 69 WT: 72	54.7	Radiotherapy and systemic chemotherapy: 67%	Y	Y	N	N
Griewank 2014 [49]	Stage I: 16% Stage II: 30% Stage III: 30% Stage IV: 7%	Retrospective cohort study	2.9	nonBRAFm/nonNRASm	BRAFm: 125 WT: 126	58.0	NR	N	Y	N	N
Gupta 2015 [50]	Stage 0–II: 44.4% Stage III–IV: 46.6%	Retrospective cohort study	NR	nonBRAFm/nonNRASm	BRAFm: 119 WT: 79	55.7 (mean)	NR	N	Y	N	N
Hugdahl 2016 [51]	Stage I–IV: 100%	Patient series	2.6	nonBRAFm	BRAFm: 78 WT: 113	70.0	NR	Y	Y	N	N

(*continued*)

Table 1. Continued.

Author year	AJCC stage	Study design	Follow-up (years)*	Definition of WT	N included in MA	Age (years)*	Tx received/allowed**	KM	OS	DFS	PFS
Ide 2016 [52]	Stage I: 21% Stage II: 38% Stage III: 31% Stage IV: 5%	Retrospective cohort study	NR	nonBRAFm	BRAFm: 54 WT: 59	64.8 (mean)	NR	Y	Y	N	N
Jacquelot 2016 [53]	Stage III: 87% Stage IV: 13%	Retrospective analysis	NR	nonBRAFm	BRAFm: 21 WT: 17	60 (mean)	NR	N	Y	N	N
Jakob 2012 [8]	Stage IV: 100%	Retrospective cohort study	NR	nonBRAFm/nonNRASm	BRAFm: 112 WT: 94	53.8	NR	Y	Y	N	N
Joon 2015 [54]	Stage IV: 100%	Retrospective cohort study	NR	nonBRAFm	106***	NR	NR	N	Y	N	N
Joseph 2012 [55]	Stage III: 12% Stage IV: 88%	Retrospective cohort study	2.5	nonBRAFm/nonNRASm	BRAFm: 62 WT: 26	50.0	IL-2	Y	Y	N	Y
Jovanovic 2008 [56]	Stage I–IV: 100%	Registry (retrospective analysis)	NR	nonBRAFm/nonNRASm	BRAFm: 22 WT: 22	57.0	Surgery	Y	Y	N	N
Linardou 2015 [57]	Stage III–IV: 100%	Prospective phase II study	NR	nonBRAFm	BRAFm: 13 WT: 11	58.5 (mean)	Cisplatin-vinblastine-temozolomide	Y	Y	N	Y
Lyubchenko 2016 [58]	Stage I–II: 81% Stage III–IV: 13%	Retrospective cohort study	1.8	nonBRAFm	BRAFm: 50 WT: 52	54.0 (mean)	NR	Y	Y	N	N
Mar 2015 [59]	Stage I: 58% Stage II: 31% Stage III: 11%	Prospective cohort study	7.7	nonBRAFm/nonNRASm	BRAFm: 87 WT: 76	55.0	NR	N	Y	N	N
Meckbach 2014a [21]	Stage I: 81% Stage II: 19%	Cohort study	7.8	nonBRAFm	BRAFm: 169 WT: 268	57.0	Surgery	Y	Y	N	N
Meckbach 2014b [60]	Stage IV: 100%	Observational study	NR	nonBRAFm	BRAFm: 89 WT: 126	64.0	Monochemotherapy: dacarbazine or temozolomide	Y	Y	N	N
Menzies 2012 [61]	Stage III–IV: 100%	Prospective cohort study	NR	nonBRAFm	BRAFm: 90 WT: 165	58.6 (mean)	NR	Y	Y	N	N
Moreau 2012 [62]	Stage III: 100%	Retrospective cohort study	NR	nonBRAFm	BRAFm: 42 WT: 63	61.8	Surgery	Y	Y	N	N
Nagore 2014 [63]	Stage I–II: 100%	Retrospective cohort study	4.0	nonBRAFm/nonNRASm	BRAFm: 38 WT: 82	52.5	No BRAFi treatment	N	Y	Y	N
Picard 2014 [64]	Stage III: 100%	Retrospective cohort study	range: 3–13	nonBRAFm	BRAFm: 32 WT: 37	53.7 (mean)	NR	N	Y	N	N
Roh 2016 (KMC) [65]	Stage I–II: 62% Stage III–IV: 38%	Retrospective cohort study	3.6	nonBRAFm/nonNRASm	BRAFm: 26 WT: 103	59.4	NR	N	Y	N	N
Roh 2016 (TCGA) [65]	Stage 0–II: 49% Stage III–IV: 41%	Retrospective cohort study	5.3	nonBRAFm/nonNRASm	BRAFm: 107 WT: 65	56.6 (mean)	NR	N	Y	N	N
Rutkowski 2014 [66]	Stage III: 100%	Prospective study	20.8	nonBRAFm	BRAFm: 154 WT: 54	55.1	Surgery (no BRAF or MEK inhibitors)	Y	Y	Y	N
Safaee 2013 [67]	Stage I: 34% Stage II: 29% Stage III: 16% Stage IV: 21%	Cohort study	5.0	low BRAFm	BRAFm: 239 WT: 131	NR	NR	N	Y	N	N
Saiag 2015 [68]	Stage III: 100%	Prospective cohort study	4.7	nonBRAFm	BRAFm: 80 WT: 78	57.7	NR	N	Y	N	N
Schlaak 2013 [69]	Stage IV: 100%	Retrospective analysis	NR	nonBRAFm	BRAFm: 22 WT: 20	58.2 (mean)	NR (no BRAFi allowed)	Y	Y	N	N
Schoenewolf 2014 [70]	Stage IV: 100%	Retrospective cohort study	NR	nonBRAFm	BRAFm: 40 WT: 22	55 (mean)	NR	Y	Y	N	N
Sekulovic 2015 [71]	Stage IV: 100%	Single-centre retrospective registry	1.0	nonBRAFm	BRAFm: 37 WT: 41	60.0	Targeted and immunotherapy not allowed	N	Y	N	N

(*continued*)

Table 1. Continued.

Author year	AJCC stage	Study design	Follow-up (years)*	Definition of WT	N included in MA	Age (years)*	Tx received/allowed**	KM	OS	DFS	PFS
Shankar 2016 [72]	Stage IV: 100%	Retrospective cohort study	NR	nonBRAFm	BRAFm: 6 WT: 12	NR	Surgery	N	Y	N	N
Sheen 2016 [73]	Stage I: 33% Stage II: 39% Stage III: 19% Stage IV: 8%	Retrospective review	4.4	nonBRAFm	BRAFm: 17 WT: 102	NR	Treatments allowed: vemurafenib, encorafenib, or selumetinib and binimetinib	Y	Y	N	N
Shinozaki 2007 [74]	Stage IV: 100%	Pilot study	NR	nonBRAFm	BRAFm: 8 WT: 12	44.9 (mean)	Biochemotherapy	Y	Y	N	N
Si 2012 [75]	Stage I: 5% Stage II: 37% Stage III: 25% Stage IV: 27%	Retrospective cohort study	2.0	nonBRAFm	BRAFm: 322 WT: 110	51 (mean)	NR	Y	Y	N	N
Slingluff 2013 [76]	Stage III: 6% Stage IV: 94%	Phase II trial	NR	nonBRAFm	BRAFm: 6 WT: 11	63.5	temsirolimus + bevacizumab	Y	Y	N	N
Thomas 2015 [77]	Stage I–IV: 100%	Population based study	7.6	nonBRAFm/nonNRASm	BRAFm: 268 WT: 502	NR	NR	Y	Y	N	N
Ugurel 2007 [78]	Stage III: 25% Stage IV: 75%	Retrospective analysis	2.6	nonBRAFm	BRAFm: 53 WT: 44	56.0	NR	Y	Y	N	N
Ulivieri 2015 [79]	Stage IV: 100%	Cohort study	NR	nonBRAFm/nonNRASm	BRAFm: 13 WT: 7	50.9	Surgery	Y	Y	N	N
von Moos 2012 [80]	Stage IV: 100%	Single-arm, open-label Phase II trial	1.7	non-BRAF	BRAFm: 22 WT: 22	59.0	Temozolomide + bevacizumab	Y	Y	N	N
Wilson 2015 [81]	Stage III: 13% Stage IV: 87%	Prognostic evaluation of a phase III DB RCT (ECOG 2603)	NR	nonBRAFm/nonNRASm	BRAFm: 80 WT: 59	58.0	Carboplatin-paclitaxel +/− sorafenib	N	Y	N	Y
Wu 2014 [82]	Stage distribution unclear	Retrospective cohort study	30.0	nonBRAFm/nonNRASm	BRAFm: 24 WT: 72	63.7	NR	Y	Y	N	N
Xu 2016 [83]	Stage IV: 100%	Single-center retrospective study	0.3	nonBRAFm	BRAFm: 13 WT: 35	56.9	Stereotactic radiosurgery	Y	Y	N	Y

*BRAF*i: *BRAF* inhibitor; *BRAF*m: *BRAF* mutant; DB: double-blind; DFS: disease free survival; MA: meta-analysis; N: number; N: no; NR: not reported; OS: overall survival; PFS: progression free survival; Tx: treatment; WT: wild type; Y: yes.

*Median unless otherwise stated as mean or range.

**Data included in analysis were required to exclude patients receiving *BRAF*i if study allowed use in *BRAF*m patients.

***Total number of patients with *BRAF*m and WT were included in the meta-analysis.

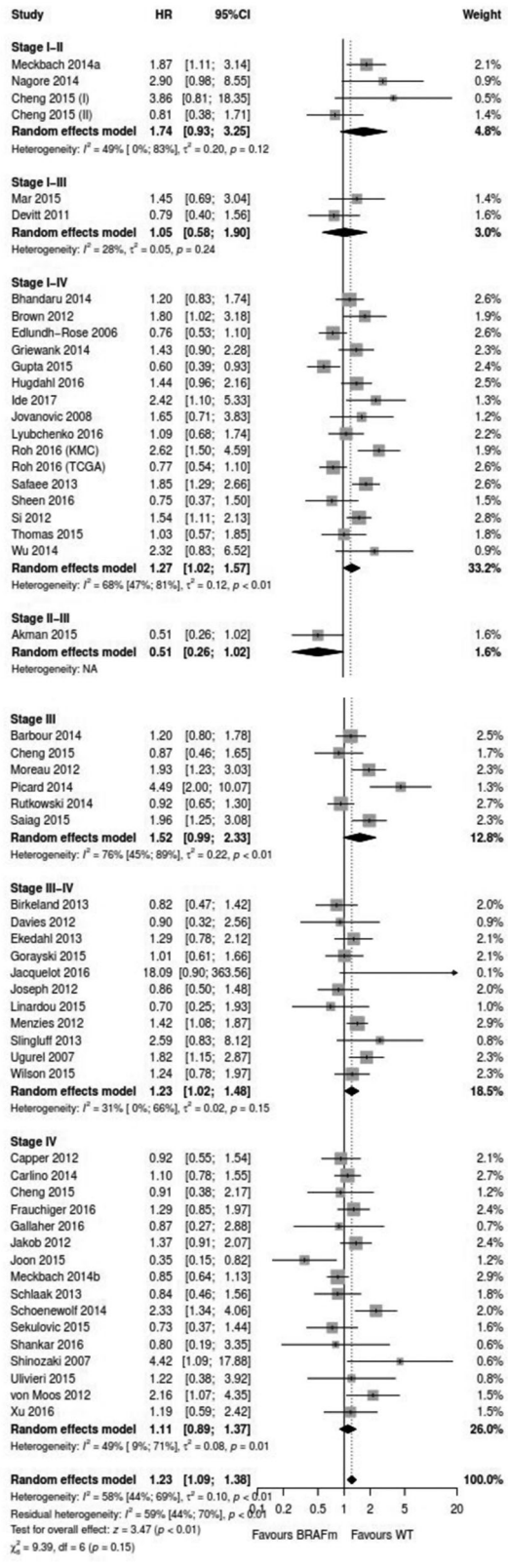

Figure 2. Meta-analysis of the prognostic impact of BRAF status for determining OS.

mutation in stage III melanoma with a positive sentinel lymph node. BRAF-mutated patients had a statistically significant higher frequency of metastases ≥2 mm in their lymph nodes. For this subgroup, Moreau et al. [62] showed a poorer prognosis for BRAF-mutated versus WT patients, among 105 patients with lymph node metastases of >2 mm that underwent surgical lymph node resection. Barbour et al. [37] included 134 resected stage IIIb-IIIc melanoma patients, treated without neoadjuvant therapy and reported a trend towards impaired survival for BRAF mutated patients, without reaching statistical significance. The other two studies reported conflicting results. Rutkowski et al. reported a high BRAF mutation rate (74%) [66] and Cheng et al. used IHC to detect the BRAF mutation and assessed melanoma-specific survival (MSS) [42].

For stage IV melanoma, 16 studies reported data on BRAF-mutated and WT and half of them assessed small patient samples (<50). However, BRAF-mutations did not have a statistically significant prognostic impact on OS (HR: 1.11, 95% CI: 0.89–1.37). HRs were reported in five studies, ranging from 0.35 to 0.91. Among those in which HRs were derived from KM curves ($n = 12$), two studies (sample size: 108–206 patients) [8,46] excluded patients treated with BRAF inhibitors in the analysis and reported slightly higher HRs (1.29 and 1.37) than the overall pooled effect. The largest HR (4.42) came from a small pilot study [74] where 20 patients received bio-chemotherapy. Two small studies with patients who underwent surgery reported similar HRs (1.19 [83] and 1.22 [79]) derived from KMs.

Sixteen studies also investigated prognostic impact without stage-specific estimated risk. The pooled effect showed a statistically significant prognostic impact for BRAF mutations on OS (HR: 1.27, 95% CI: 1.02–1.57) for stage I–IV, in line with the overall effect from all included studies. Meta-regressions with pre-specified potential effect modifiers did not reveal any statistically significant covariate effects (Table 2). Age showed a statistically significant effect for stage IV melanoma, but not for all pooled patients. This study showed a possible higher risk in men, but the difference was not statistically significant.

A sensitivity analysis excluding studies detecting BRAF mutations using non-PCR-based methods showed similar results to the base case. WT patients were reported to be dual BRAF/NRAS WT in one third of the included studies. Removing these studies, BRAF-mutation still showed statistically significant impact on the OS. Individual studies applied various definitions on the OS. Excluding the seven studies which defined OS from time of diagnosis or treatment to death due to melanoma showed similar results to the pooled studies at base case. The HR for patients with V600E mutation only versus WT was higher than in all BRAF mutant base case (HR: 1.65, 95% CI: 1.29–2.10) suggesting an even shorter survival for patients harbouring a V600E mutation, while the HRs for patients with V600E mutation and other mutants versus WT were close to the base case (HR: 1.27, 95% CI: 1.11–1.46). The subgroup analysis excluding studies with estimated HR from KM curves reported similar results to the base case.

Sensitivity analyses on stage IV melanoma studies showed a similar trend to the base case, however the uncertainty was higher and the results were not statistically significant except in the subgroup of patients with V600E mutations. The meta-regression using median age as a covariate found

Table 2. Meta-regression and sensitivity analyses to assess the impact of suspected effect modifiers on prognostic impact of BRAF status for determining OS in stage I–IV patients [all stages pooled] and stage IV patients of Included studies.

Description of analysis/effect modifier	No. of studies	I^2 (%) [95% CI]	HR [95% CI]	β [95% CI]*	p-Value
All AJCC stages pooled					
Base case	53	58.73 [45.08, 79.51]	1.23 [1.09, 1.38]	–	–
Adj. Median age [imputation – median value]	52	59.37 [45.72, 79.82]	1.23 [1.09, 1.38]	0.00 [−0.03, 0.03]	.8431
Adj. Median age [complete case]	43	61.26 [45.86, 81.81]	1.24 [1.08, 1.42]	0.00 [−0.03, 0.03]	.8484
Adj. Sex [imputation - mean value]	52	59.69 [45.84, 79.81]	1.23 [1.09, 1.39]	0.38 [−1.33, 2.09]	.6643
Limit to studies with same BRAFm testing	42	59.55 [44.37, 81.72]	1.25 [1.1, 1.42]	–	–
Limit to studies defining WT as nonBRAFm	34	60.59 [46.05, 85.52]	1.29 [1.11, 1.49]	–	–
Exclude outlier definitions of OS	46	57.66 [42.24, 80.51]	1.26 [1.11, 1.43]	–	–
Subgroup V600E mutants	14	45.60 [0.00, 80.00]	1.65 [1.29, 2.10]	–	–
Subgroup V600E + other mutants	27	55.58 [36.17, 86.07]	1.27 [1.11, 1.46]	–	–
Subgroup of unknown mutation	12	15.19 [0.00, 71.09]	0.84 [0.71, 1.01]	–	–
Exclude studies with <50 patients	4	0.00 [0.00, 98.98]	0.88 [0.69, 1.11]	–	–
Exclude HRs estimated from KM curves	6	0.01 [0.00, 92.14]	1.19 [0.96, 1.48]	–	–
Stage IV pooled					
Base case	16	49.23 [6.78, 86.31]	1.11 [0.89, 1.37]	–	–
Adj. Median age [imputation - median value]	15	22.97 [0.00, 80.08]	1.13 [0.95, 1.35]	−0.07 [−0.12, −0.02]	0.0058
Adj. Median age [complete case]	12	9.20 [0.00, 72.71]	1.21 [1.02, 1.43]	−0.07 [−0.11, −0.02]	0.0024
Adj. Sex [imputation – mean value]	16	45.73 [0.00, 82.37]	1.05 [0.84, 1.31]	2.91 [−0.32, 6.13]	0.0775
Limit to studies with same BRAFm testing	11	46.15 [6.18, 93.51]	1.09 [0.87, 1.36]	–	–
Limit to studies defining WT as nonBRAFm	13	59.25 [17.05, 89.49]	1.08 [0.81, 1.44]	–	–
Exclude outlier definitions of OS	13	49.46 [0.00, 88.63]	1.21 [0.94, 1.55]	–	–
Subgroup V600E mutants	6	47.02 [0.00, 92.63]	1.60 [1.06, 2.44]	–	–
Subgroup V600E + other mutants	5	28.69 [0.00, 90.55]	1.06 [0.86, 1.30]	–	–
Subgroup of unknown mutation	5	0.00 [0.00, 82.36]	0.67 [0.44, 1.00]	–	–
Exclude studies with <50 patients	2	80.57 [2.38, >99.74]	1.67 [0.34, 8.20]	–	–
Exclude HRs estimated from KM curves	2	65.01 [0.00, >99.52]	1.43 [0.75, 2.43]	–	–

Adj.: adjusted; BRAFm: BRAF mutated; HR: hazard ratio; KM: Kaplan-Meier; OS: overall survival; WT: wild type.

a statistically significant negative effect. When the median age of the patients increases, the HR of BRAF mutation versus WT should decrease.

Impact of BRAF mutations on DFS and PFS

Five studies [34,37,44,63,66] were included in the meta-analysis for DFS with stage I–III melanoma patients pooled based on disease stage whenever possible (see Supplement Figure 3(A)). The overall pooled effect for all included studies showed that BRAF-mutant melanoma was comparable to non-BRAF mutant melanoma regarding DFS (combined HR: 1.16, 95% CI: 0.92–1.46). There was no heterogeneity found between the studies ($I^2 = 0\%$). The pooled results also suggested comparability in patient populations at stage II–III patients (HR: 1.09, 95% CI: 0.68–1.76) and stage III (HR: 1.06 (95% CI: 0.79–1.41). Three studies [34,37,66] indicated that patients had surgery.

The results for PFS including nine HRs from eight studies are presented in Supplement Figure 3(B), of which Wilson et al. [81] had investigated prognosis of BRAF mutations in two arms, where patients were treated with carboplatin and paclitaxel in group 1 and carboplatin, paclitaxel and sorafenib in group 2. The pooled HR for PFS showed that BRAF mutant malignant melanoma was comparable to non-BRAF mutant melanoma (HR: 0.98 (95% CI: 0.68 − 1.40). The heterogeneity observed was moderate to substantial ($I^2 = 58\%$).

The funnel plot shows no evidence of asymmetry and Egger's test for assessing funnel plot asymmetry failed to detect publication bias in studies with OS, DFS and PFS (see Supplement Figure 4).

Discussion

The BRAF protein has become one of the major therapeutic targets in the treatment of both stage III and stage IV melanoma. Although an increasing number of studies investigate the prognostic impact of BRAF mutations on survival in melanoma, its prognostic role in melanoma initiation and progression remains controversial. The present meta-analysis assessed the prognostic significance of BRAF-mutant versus WT malignant melanoma for OS, DFS and PFS, both overall and at different melanoma stages.

The overall results demonstrated a poorer prognosis in patients harbouring BRAF mutations. From a pooled population of 7519 patients BRAF mutations were associated with a 23% higher relative mortality risk compared to BRAF WT melanoma. A moderate to substantial heterogeneity was identified between the studies and the degree of heterogeneity was not reduced in any of the pre-specified sensitivity analyses, demonstrating the robustness of the base case results. This was consistent with findings of a previous meta-analysis by Safaee Ardekani et al. [84] of four studies (three cohort studies [10,75,85] and one randomised controlled trial [80]). The BRAF V600E mutation was associated with a 1.7 times higher relative mortality risk compared to BRAF WT in melanoma patients.

The present study is, to our knowledge, the largest meta-analysis investigating the correlation between BRAF mutations and survival in malignant melanomas patients. Due to large differences in the clinical-pathological features among different melanoma stages, subgroup analyses were also performed by AJCC stages [27]. Only three studies [21,42,63] examined OS in BRAF-mutated and WT melanoma patients at stage I–II. Our review highlighted the dearth of evidence

concerning the role of BRAF mutational status in survival in early-stage melanoma. This may be due to the fact that only approximately 10–15% of primary melanomas develop metastatic disease [20] and therefore OS might not be the primary outcome of interest in early stage studies or even feasible to collect due to limited data on long-term follow-up in this group of patients. In addition, BRAF-mutational analysis is not established as a standard procedure for primary malignant melanoma.

Several studies have examined the prognostic importance of BRAF mutations in advanced melanoma. As known, stage III and IV melanoma include tumours of any thickness with known spread to lymph nodes, or distant sites with a wide spectrum of treatment modalities (i.e., surgery, radiation, medical oncological treatments). In order to analyse the treatment-independent prognostic impact of BRAF mutations, our study excluded patients receiving a BRAF and/or MEK-targeted treatment, as these BRAF mutant patients are expected to be biased towards favourable prognosis compared with WT patients. Similarly, patients receiving checkpoint inhibitors were excluded, as this type of immunotherapy can significantly modify prognosis [86,87]. Patients who received chemotherapy, other types of immunotherapy and surgery were all included without further selection, although all of these treatments may have effect on survival. However, many of the included studies did not report primary or later drug intervention(s), and some patients may have been treated with BRAF and/or MEK-targeted treatments as well as checkpoint inhibitors resulting in improvement of the survival in BRAF mutant cohort. Our findings from six studies covering stage III melanoma patients showed that the overall pooled effect (HR: 1.52, 95% CI: 0.99–2.33) was numerically – but not statistically significant higher than other stages. Unlike stage IV melanomas, surgery is still the first-line treatment for many stage III melanoma and the metastatic burden in the lymph nodes serves as an important prognostic factor.

For early-stage melanoma, age, sex, tumour thickness of primary melanoma, presence of ulceration, and mitotic rate were known prognostic factors for the survival of patients [88]. For advanced melanoma, the survival was also associated with factors such as site of metastases, level of LDH in serum and ECOG status. Pre-specified subgroup analyses and meta-regressions were conducted exploring some of these potential effect modifiers.

One of the meta-regression analyses showed a counter-intuitive result for stage IV patients. Previous studies reported that BRAF mutations were likely associated with younger melanoma patients, suggesting that age had a negative impact on OS [21,88]. Given that BRAF mutant patients tended to have poorer OS than WT (HR $>$ 1) in the base case for stage IV, we could expect that HR of mutant versus WT increases even further when adjusting for median age. However, in this study, for stage IV, median age has been shown to have a reverse effect. This may be explained by the fact that (1) median age of the whole population has been used instead of the median age of BRAF-mutant patients only, and (2) it is more difficult to get a high multiplicative factor on a high baseline risk (old patients) than a low baseline risk (young patients). Survival has been consistently reported to be better and disease less likely to progress in women [89]. The reasons remain unclear, though hormonal influences, sex differences in immunity or oxidative stress may play a role. This study found no statistically significant treatment effect modifier role of sex in the estimation of the prognostic impact of BRAF mutation.

BRAF and NRAS mutations are not mutually exclusive, thus, the definition of the control group (i.e., WT group) varied across the identified studies, either as solely BRAF WT or BRAF/NRAS dual WT tumours. In some studies [90], NRAS-mutated appeared to show a poorer outcome compared with those who were BRAF/NRAS dual WT.

Apart from age, sex and WT definition, there are also differences between the studies in factors such as clinic-pathological features (reported/unreported), treatments and follow-up time. Given the heterogeneity across the included studies, potential effect modifiers might be further explored in sensitivity analyses, although assumptions, such as grouping of treatments, might be required.

Nagore et al. [63] included 147 patients with localised invasive melanoma (stage I–II) and found that patients with localised BRAF-mutant melanomas experienced poorer DFS than those with BRAF WT. In contrast, Rutkowski et al. [66] included 250 clinical stage III melanoma patients treated with surgery revealing that BRAF mutational status was not a prognostic marker. Other smaller studies also reported similar findings [34,37,44]. The pooled effect found that BRAF mutational status had a neutral role on DFS.

PFS measures time to disease progression or death from any cause, which is widely used to assess whether a treatment makes a meaningful impact on patients' quality of life or as a surrogate for OS. As this meta-analysis excluded studies with patients treated with a BRAF and MEK inhibitor or a checkpoint inhibitor, only eight studies, with patients treated with chemotherapy, immunotherapy or surgery were pooled. Except for one small study [35] with 38 patients that showed significantly poorer PFS (HR: 5.5) in BRAF mutated versus WT patients, no prognostic impact on PFS was reported. The pooled effect showed that BRAF mutational status had a neutral role on PFS.

Using the QUIPS tool [25,26], about one third of the studies were deemed methodologically poor due to the lack of information on recruitment and study confounders. The study designs also varied with 34 retrospective cohort studies, 12 prospective cohort studies (seven clinical trials) and eight observational (unclear if retrospective or prospective). All study designs had the same weight in the performed analysis. In order to use all available information, this meta-analysis also included studies where survival was only reported in a KM curve. These data are probably less reliable than data directly reported in the primary studies and a calculation bias might be present. However, the respective subgroup analyses found similar results to the base case. A proportional hazard assumption was used to summarise the individual study results, as well as the overall combined effect. This means that BRAF mutation multiplies the risk of

dying compared to WT by 1.20 at any time [24]. This may be a strong assumption requiring further investigation.

To evaluate treatment effects of new interventions, future studies should take into account the fact that BRAF activating mutations are in general associated with a shorter OS compared to WT melanoma cases. BRAF mutant and BRAF wild-type melanomas have to be seen as two fundamentally different populations, and treatment recommendations may differ according to BRAF status [91] also when treatment strategies like immunotherapy etc. are considered.

Conclusion

To the best of our knowledge, we present here the largest meta-analysis investigating the correlation between BRAF mutational status and OS in patients with malignant melanoma. Although substantial heterogeneity between the used trials was registered, as expected, our meta-analysis demonstrates that the presence of a BRAF mutation is associated with a shorter OS in patients with malignant melanoma compared to WT. Moreover, BRAF mutant melanoma showed comparable effect on DFS and PFS to WT melanoma in stage I–III and III–IV, respectively. Future studies should consider this observation when evaluating epidemiological data and treatment effects of new interventions.

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- MSN has participated in ad-boards for Novartis, Pierre Fabre, BMS and Incyte.
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- LO and MRY are employed by Novartis. LO and MRY have equity in Novartis.
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