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Journal
JAMA Oncology, 1(3)

ISSN
2374-2437

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Publication Date
2015-06-01

DOI
10.1001/jamaoncol.2015.0493

Peer reviewed

Published in final edited form as:

JAMA Oncol. 2015 June ; 1(3): 359–368. doi:10.1001/jamaoncol.2015.0493.

Association Between *NRAS* and *BRAF* Mutational Status and Melanoma-Specific Survival Among Patients With Higher Risk Primary Melanoma

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Author Attributions and Financial Disclosures

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Analysis and interpretation of data: Thomas, Armstrong, Anton-Culver, Berwick, Bramson, Busam, Conway, Cust, Dwyer, Edmiston, Frank, From, Gallagher, Groben, Gruber, Kanetsky, Kricker, Luo, Marrett, Ollila, Orlow, Rosso, Zanetti

Drafting of the manuscript: All authors

Critical revision of the manuscript for important intellectual content: All authors.

Obtained funding: Thomas, Armstrong, Anton-Culver, Berwick, Conway, Gruber

Administrative, technical, and material support: Thomas, Armstrong, Anton-Culver, Begg, Berwick, Gruber, Kricker.

Study supervision: Thomas, Armstrong, Anton-Culver, Begg, Berwick, Gruber, Kricker.

Conflict of Interest Disclosures: None reported.

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Abstract

Importance—*NRAS* and *BRAF* mutations in melanoma inform current treatment paradigms but their role in survival from primary melanoma has not been established. Identification of patients at high risk of melanoma-related death based on their primary melanoma characteristics before evidence of recurrence could inform recommendations for patient follow-up and eligibility for adjuvant trials.

Objective—To determine tumor characteristics and survival from primary melanoma by somatic *NRAS* and *BRAF* status.

Design, Setting, and Participants—A population-based study with median follow-up of 7.6 years for 912 patients with first primary cutaneous melanoma analyzed for *NRAS* and *BRAF* mutations diagnosed in the year 2000 from the United States and Australia in the Genes, Environment and Melanoma Study and followed through 2007.

Main Outcomes and Measures—Tumor characteristics and melanoma-specific survival of primary melanoma by *NRAS* and *BRAF* mutational status.

Results—The melanomas were 13% *NRAS*+, 30% *BRAF*+, and 57% with neither *NRAS* nor *BRAF* mutation (*wildtype*). In a multivariable model including clinicopathologic characteristics, *NRAS*+ melanoma was associated ($P<.05$) with mitoses, lower tumor infiltrating lymphocyte (TIL) grade, and anatomic site other than scalp/neck and *BRAF*+ melanoma was associated with younger age, superficial spreading subtype, and mitoses, relative to *wildtype* melanoma. There was no significant difference in melanoma-specific survival for melanoma harboring mutations in *NRAS* (HR 1.7, 95% CI, 0.8–3.4) or *BRAF* (HR, 1.5, 95% CI, 0.8–2.9) compared to *wildtype* melanoma adjusted for age, sex, site, AJCC tumor stage, TIL grade, and study center. However, melanoma-specific survival was significantly poorer for higher risk (T2b or higher stage) tumors with *NRAS* (HR 2.9; 95% CI 1.1–7.7) or *BRAF* (HR 3.1; 95% CI 1.2–8.5) mutations but not for lower risk (T2a or lower) tumors ($P=.65$) adjusted for age, sex, site, AJCC tumor stage, TIL grade, and study center.

Conclusions and Relevance—Lower TIL grade for *NRAS*+ melanoma suggests it has a more immunosuppressed microenvironment, which may impact its response to immunotherapies. Further, the approximately three-fold increased death rate for higher risk tumors harboring *NRAS* or *BRAF* mutations compared to *wildtype* melanomas after adjusting for other prognostic factors indicates that the prognostic implication of *NRAS* and *BRAF* mutations deserves further investigation, particularly in higher AJCC stage primary melanomas.

Keywords

oncogene; epidemiology; pathology; RAS; RAF; b-raf; n-ras; neoplasm staging; tumor microenvironment; tumor-infiltrating lymphocytes

Melanomas frequently harbor mutually exclusive *BRAF* or *NRAS* mutations that arise early in tumor progression and persist throughout the course of the disease.[1,2] These mutations influence tumor development and maintenance through constitutive activation of the RAS-RAF-MEK-ERK pathway.[1,3] Their clinical relevance is underscored by improved survival of Stage IV patients with *BRAF*-mutant melanomas treated with BRAF inhibitors alone or in combination with MEK inhibition.[4–6] These targeted therapies along with new immunotherapies[7,8] are rapidly changing treatment paradigms for metastatic melanoma, and some are under investigation as adjuvant therapies.[9] Identification of patients at high risk of death from melanoma based on their primary melanoma tumor characteristics before sign of recurrence remains important to inform evidence-based follow-up of patients and adjuvant trials. Equally important is identification of patients who rarely die from melanoma as they can be spared the risks of adjuvant therapy. However, it remains unknown whether the primary melanoma *NRAS/BRAF* mutational status influences survival from melanoma during the natural course of the disease.

To date, studies of *NRAS* and *BRAF* mutations in primary melanoma have mostly been retrospective and examined all-cause rather than disease-specific survival.[10–17] Many selected cases based on referral to a particular center,[11–15,17] applied additional criteria such as selection of frozen[16] or metastatic[14] tissues for analysis, or included only nodular[18] or vertical growth phase[19] melanoma. Several studies determined *BRAF* but not *NRAS* mutations.[12,17,20] Only two studies included more than one center and examined *NRAS* and *BRAF* mutations in relationship to melanoma-specific survival. Of these, Devitt et al.[21] found that *NRAS* exon 3 and *BRAF* V600E mutations translated into worse melanoma-specific survival in a prospective cohort of 249 primary melanoma cases from two Australian tertiary melanoma referral centers. Wu et al.[22] found *BRAF* V600E mutation to be associated with an unfavorable melanoma-specific survival for 127 primary melanomas diagnosed in women enrolled in the Nurse's Health Study.

We examined tumor characteristics and melanoma-specific survival by *NRAS* and *BRAF* mutation status in 912 incident first primary cutaneous invasive melanomas from patients diagnosed in 2000 from Australia (New South Wales) or the United States (North Carolina, Michigan, and California) enrolled in the population-based Genes, Environment, and Melanoma (GEM) Study. The primary melanomas were analyzed for *NRAS* and *BRAF* mutations. Our median 7.6-year observation period concluded prior to 2011 when the US Food and Drug Administration and Australian Therapeutic Goods Administration began approving new systemic therapies that improve overall survival in metastatic melanoma patients.

METHODS

Study Population

The GEM study included single and multiple primary cutaneous melanoma patients diagnosed between 1998 and 2003 from Australia, Canada, Italy and the United States.[23–27] The institutional review board at the coordinating center, Memorial Sloan Kettering Cancer Center, and each participating institution approved the study protocol. Each study participant provided informed written consent. We sought tumor sections from 1,547 participants' first

primary invasive melanoma diagnosed in 2000 from New South Wales (Australia), California, North Carolina, and Michigan.

Histopathology slides were centrally reviewed as previously described.[28,29] Mitoses were defined as present or absent.[30] TIL grade was scored as absent, nonbrisk, or brisk using a previously defined grading system.[31] All data items were available for the T classification describing the state of the primary tumor in the AJCC TNM (tumor, regional nodes, distant metastasis) melanoma staging system; data on regional nodal and distant metastases were not available.

Melanoma treatment information was not available; however, the follow-up period at all study centers ended before recent approvals of new systemic agents that alter the natural course of disease.[4–8] Information about deaths from melanoma or other causes was obtained for participants from the National Death Index for the US study centers and the cancer registry for the Australian study center as previously described.[28] Patient follow-up for vital status was complete to the end of 2007.

NRAS and *BRAF* Mutational Analysis

Of eligible GEM participants, 912 (59% of 1,547) had formalin-fixed, paraffin-embedded melanomas successfully analyzed for *NRAS* and *BRAF* mutations. When indicated because of small tumor size or admixture of nonmalignant cells, tumor cells were selectively procured using laser capture microdissection. Tumor DNA was analyzed for *BRAF* exon 15 (including codon 600) and *NRAS* exon 2 and 3 (including codons 61, 12, 13) mutations using single-strand conformational polymorphism (SSCP) analysis and radiolabeled sequencing of SSCP-positive samples as previously described.[32,33] All mutations were confirmed by sequencing an independently amplified DNA fragment to eliminate mutational artifacts. The *NRAS*/*BRAF* status of 214 (98% of 218) cases from North Carolina previously had been reported.[33]

Statistical Methods

BRAF and *NRAS* mutations were mutually exclusive, and melanomas were grouped as: *NRAS*+ (exon 2 or 3 mutation), *BRAF*+ (exon 15 mutation), or *wildtype* (neither *NRAS* nor *BRAF* mutation) for analyses. Pearson's chi-square tests and Wilcoxon tests were used to compare cases analyzed for *NRAS* and *BRAF* mutations to those not analyzed.

To identify factors that independently distinguished *NRAS*+ or *BRAF*+ from *wildtype* melanoma, a multivariable model was developed that included all clinicopathologic features and study center. We used polytomous logistic regression for this purpose to estimate simultaneously the odds ratios (OR) and 95% confidence intervals (CI) with *NRAS*+ and *BRAF*+ compared to *wildtype* melanoma adjusted for study center. Statistical significance was assessed using Wald tests. Linear trend was tested when appropriate using the Wald statistic with those variables treated as a single ordinal variable. We also report results from a similar model examining the association of *NRAS*+ and *BRAF*+ compared to *wildtype* melanoma with AJCC tumor stage. . Statistical tests were two-sided with $P<0.05$ considered statistically significant.

Survival time was accumulated from the diagnosis date until date of death due to melanoma or the end of follow-up (censored patients). Patients were censored at the time of death from any cause other than melanoma. Of the 912 patients who entered the study with first primary melanoma, 40 developed a second primary melanoma during the ascertainment period, and the occurrence of a second primary was included as a time-dependent covariate. The *NRAS/BRAF* mutational status and pathologic characteristics of their thicker melanoma was utilized in the survival analysis, as previously published.[28,29]

Survival curves by *NRAS* and *BRAF* status were visualized using the Kaplan-Meier method and compared using a log-rank test. Hazard ratios (HR) and 95% CI by *NRAS/BRAF* status were estimated in Cox regression models adjusted for age, sex, study center, and the time-dependent covariate and then in fully adjusted models that also included anatomic site, TIL grade, and AJCC tumor stage. Scalp/neck and face/ears were included as separate covariates as scalp/neck, but not face/ear, melanoma predicts worse survival.[34–36] TIL grade was included as higher TIL grade of primary melanoma is associated with better melanoma-specific survival.[29] To account for the competing risk of death from other causes, we performed Fine and Gray's proportional subdistribution hazards regression models[37] to assess the effects of covariates on the subdistribution hazard for death as a result of melanoma. The likelihood ratio test was used to test each interaction, comparing a model with the main effects to a model with the main effects and the interaction term with an *a priori* alpha of 0.2[38]

Tests based on Schoenfeld residuals and graphical methods using Kaplan-Meier curves showed no evidence that proportional hazards assumptions were violated for mutational status. SAS (SAS Institute, Cary, NC) version 9.3 was used for all analyses except for Kaplan-Meier curves, which were implemented in STATA/IC 12.1 (StataCorp LP, College Station, TX).

RESULTS

The participants whose tumors were analyzed for *NRAS* and *BRAF* mutations (n=912) were compared to 635 participants whose tumors were unavailable (n=560), insufficient (n=43), or failed molecular analysis (n=32). There were no significant differences (all $P>.05$) based on median age, sex, site, median Breslow thickness, or melanoma death.

Of the 912 participants with *NRAS/BRAF* mutational status of their first primary invasive melanomas available, 54% were from Australia and 46% from the United States (Table 1). The participants were 54% male with a median age of 57 years. The median melanoma Breslow thickness was 0.74 mm.

NRAS and *BRAF* Mutational Frequencies and Spectra

The melanomas were 13% *NRAS*+, 30% *BRAF*+, and 57% *wildtype* (with neither *NRAS* nor *BRAF* mutation (Table 1 and eTable 1). Of *NRAS*+ melanomas, 92% harbored mutations in exon 3 and 8% in exon 2; 93% of exon 3 mutations were at codon 61. Of *BRAF*+ melanomas, 72% carried *BRAF* V600E, 21% *BRAF* V600K, and 7% other *BRAF* exon 15 mutations.

Clinicopathologic Features

We examined age, sex, and pathologic characteristics comparing *NRAS*+ and *BRAF*+ to *wildtype* melanoma for the 892 melanomas with complete data for all variables (Table 2). After adjustment for study center, *NRAS*+ melanoma was significantly associated ($P<.05$) with each of the pathologic characteristics, but not sex or age; and *BRAF*+ melanoma was associated ($P<.05$) with each of the clinicopathologic characteristics, but not sex, ulceration or TIL grade.

When all clinicopathologic characteristics were included in one model adjusted for study center, *NRAS*+ tumors were significantly associated ($P<.05$) with anatomic site other than scalp/neck (OR 0.1, 95% CI, 0.01–0.6 for scalp/neck vs. trunk/pelvis), presence of mitoses (OR 1.8, 95% CI, 1.0–3.3), and lower TIL grade (ORs 0.5, 95% CI, 0.3–0.8 for nonbrisk and 0.3, 95% CI, 0.5–0.7 for brisk, vs. absent TILs). In this model, *BRAF*+ melanoma was associated with younger age (ORs 0.7, 95% CI, 0.5–1.0 for ages 50–69 and 0.5, 95% CI, 0.3–0.8 for >70, vs. <50 years), superficial spreading subtype (ORs 0.5, 95% CI, 0.2–1.0 for nodular, 0.4, 95% CI, 0.2–0.7 for lentigo maligna, and 0.2, 95% CI, 0.1–0.5 for unclassified/other, vs. superficial spreading), and presence of mitoses (OR 1.7, 95% CI, 1.1–2.6) (Table 2).

The relationships between *NRAS*+ and *BRAF*+ tumors with AJCC tumor stage relative to *wildtype* tumors were examined, adjusted for other prognostic factors (age, sex, anatomic site, and TIL grade) and study center (Table 3). *NRAS*+ and *BRAF*+ melanomas were each more frequent among higher tumor stages (P for trend<.001 and P for trend=.04, respectively).

Melanoma-Specific Survival

There were 62 melanoma deaths in 892 patients with complete AJCC tumor stage and TIL grade information during a median follow-up time of 7.6 years. Five-year survival was 91% (95% CI, 86–96%) with *NRAS*+; 95% (95% CI, 93–98%) with *BRAF*+; and 95% (95% CI, 94–97%) with *wildtype* melanoma (log-rank test $P=.088$) (Figure 1a).

In a Cox model adjusted for age, sex, and study center, *NRAS*+ (HR 1.8, 95% CI, 0.9–3.4) and *BRAF*+ (HR 1.3, 95% CI, 0.7–2.4) relative to *wildtype* melanoma were not significantly associated with melanoma-specific survival ($P=.19$). Further adjusting for anatomic site, tumor stage, and TIL grade, the HR for *NRAS*+ melanoma was 1.7 while the HR of *BRAF*+ melanoma increased to 1.5; the results remained non-significant ($P=.27$) (Table 4). In the fully adjusted model, younger age, upper extremities relative to trunk, and lower tumor stage were significantly ($P<.05$) associated with improved melanoma-specific survival, while scalp/neck site was associated with worse melanoma-specific survival (HR 2.1; 95% CI, 0.9 to 5.1) (eTable 2). We found a significant interaction of *NRAS/BRAF* mutational status with tumor stage (P for interaction=.04) but not with age, sex, site, TIL grade, or study center in the full model.

Given the significant interaction with stage, we categorized tumors as in higher (T2b/T3a/T3b/T4a/T4b) and lower (T1a/T1b/T2a) risk AJCC stages[39] (Table 4). In our study, 25% (36/144) of patients with higher risk tumors died of melanoma compared to

3.5% (26/748) with lower risk tumors. For higher risk tumors, 5-year survival was 73% for *NRAS*+; 71% for *BRAF*+; and 82% for *wildtype* melanoma (log-rank test *P*=.28) (Figure 1b). For lower risk tumors, 5-year survival was 98% for *NRAS*+; 99% for *BRAF*+; and 98% for *wildtype* melanoma (log-rank test *P*=.61) (Figure 1c).

For higher risk tumors adjusted for age, sex, and study center, the HRs were 1.7 (95% CI, 0.8–3.9) for *NRAS*+ and 2.3 (95% CI, 1.0–5.1) for *BRAF*+ compared to *wildtype* melanoma (*P*=.13) (Table 4). Further adjusting for anatomic site, tumor stage, and TIL grade, the HRs for *NRAS*+ and *BRAF*+ melanoma strengthened to 2.9 (95% CI, 1.1–7.7) and 3.1 (95% CI, 1.2–8.5), respectively, compared to *wildtype* melanoma (*P*=.04). Addition of anatomic site in the model explained the strengthening of the estimates for *NRAS* and *BRAF* mutations in the full model. For lower risk tumors, *NRAS/BRAF* mutational subtype was not positively associated with hazard of death in either the partially or fully adjusted models. Similar patterns of higher ORs for higher compared to lower risk tumors were seen in reanalyses stratified by continent despite.

In a reanalysis including only *NRAS* codon 61 and *BRAF* V600E and *wildtype* melanomas, melanoma-specific survival differences based on mutational status remained limited to higher risk tumors (Table 4).

The associations remained similar in competing risk models (Tables 4 and eTable 2)

DISCUSSION

We present data from the largest population-based study to date analyzing tumor characteristics and melanoma specific survival by *NRAS* and *BRAF* mutational subtypes. *NRAS*+ melanoma was associated with anatomic site other than the scalp/neck, presence of mitoses, and lower TIL grade and *BRAF*+ melanoma with younger age, superficial spreading subtype, and presence of mitoses independently of other clinicopathologic characteristics. We found no significant difference for the risk of melanoma-related death from *NRAS*+ or *BRAF*+ compared to *wildtype* melanoma adjusted for other prognostic factors. However, there was an approximately three-fold increase in melanoma-related death for higher risk (T2b or higher stage) *NRAS*+ and *BRAF*+ tumors compared to *wildtype*, but not for lower risk (T2a or lower stage) tumors adjusting for other prognostic factors.

The *NRAS* and *BRAF* mutational frequencies, 13% and 30%, respectively, in our study are within previously reported ranges for primary melanoma.[13,21,40] Other studies similarly reported associations of *NRAS*+ melanoma with older age, trunk and extremity locations, nodular subtype, increased Breslow thickness, and mitoses.[13,14,21,40,41] We also confirm *BRAF*+ melanoma associations with younger age, trunk location, superficial spreading melanoma, mitoses, and vertical growth phase.[11,13,14,21,40–44] Ellerhorst et al. in a hospital-based study similarly found that *NRAS*+ and *BRAF*+ melanomas tended to present at more advanced AJCC tumor stage,[13] while Devitt et al.[21] found that *NRAS*+ tended to be higher stage.

No prior study has reported an association of mitoses with *NRAS*+ and *BRAF*+ compared to *wildtype* melanoma independently of Breslow thickness and other clinicopathologic

characteristics. This association may reflect *NRAS* and *BRAF* oncogenic activation of the mitogenic RAS-RAF-MEK-ERK pathway.[1] Mitoses are considered as a marker for tumor growth.[45] Melanoma growth rate, based on self-report, correlates positively with mitotic rate,[46] and, thus, *NRAS*+ and *BRAF*+ melanomas' associations with mitoses suggests that they may grow faster than *wildtype* melanomas. It is in agreement with a significant association between either *BRAF* or *NRAS* mutation and fast growing melanomas, calculated by using self-reported time on the skin and Breslow thickness.[47]

Similar to our results, *NRAS*+ melanoma has been identified frequently arising on the trunk[40] or on the upper[13,14] or lower extremities.[22] We further refine this knowledge with our report of an inverse association of *NRAS*+ melanoma for scalp or neck location; the majority of scalp/neck melanomas in GEM were *wildtype*. This finding and the 2-fold worse survival in GEM for scalp/neck melanoma adjusted for mutational subtype indicate that the poor prognosis of scalp/neck melanoma[34–36] is unlikely to be related to *NRAS*/*BRAF* mutational status.

To our knowledge, our study is the first to report lower TIL grade for *NRAS*+ compared to *wildtype* melanoma. Notably, TIL grade remained associated with *NRAS*+ melanoma independently of other factors (age, anatomic site, histologic subtype, and Breslow thickness) that we previously found to be associated with TIL grade in GEM.[29] Our observation is plausible as oncogenic RAS pathway activation can disrupt antitumor immunity by decreasing expression of antigen-presenting major histocompatibility complexes on the surface of tumor cells and recruiting immunosuppressive regulatory T cells and myeloid-derived suppressor cells to the tumor site.[48] Unlike Edlundh-Rose et al.,[14] we did not find *BRAF*+ relative to *wildtype* melanoma to be associated with higher lymphocyte infiltration; however, their study design and lymphocyte scoring differed from GEM.

We compare our results to other multi-site studies examining melanoma-specific survival by *NRAS/BRAF* primary melanoma status. Although not reaching statistical significance, our findings of poorer melanoma-specific survival for *NRAS*+ and *BRAF*+ (adjusted HRs of 1.7 and 1.5, respectively) compared to *wildtype* melanoma are in the same direction found by Devitt et al. for *NRAS*+ and *BRAF*+ (adjusted HRs of 2.96 and 1.7, respectively) melanoma despite different study designs and adjustments.[21] Wu et al. similarly found that *NRAS*+ and *BRAF*+ had shorter melanoma-specific survival than *wildtype* melanoma, with *BRAF*+ compared to *wildtype* reaching statistical significance. Thus, these studies and our results combined indicate a modestly worse prognosis for *NRAS*+ and *BRAF*+ tumors overall for melanoma-specific survival.

Our study suggests that melanoma-specific survival differences based on *NRAS* and *BRAF* mutational status are limited to higher risk tumors. Few deaths occurred in lower risk tumors, and we found no effect of mutational status on survival among lower risk tumors. Thus, our results provide evidence that *NRAS/BRAF* mutational status may add prognostic information for higher risk tumors. A possible explanation for the increased proportion of deaths for *NRAS*+ and *BRAF*+ melanoma limited to higher risk tumors is that higher risk tumors may have acquired another contributing genetic alteration during their progression.

Our finding, however, requires confirmation. We are not aware of another study that has analyzed survival by *NRAS* and *BRAF* status stratified by tumor stage.

Advantages of our study are its large size, use of current AJCC tumor staging, centralized pathology review by expert dermatopathologists, and comparatively long observational period ending before recent approvals of new systemic agents that alter the natural course of disease.[4–8] Any future study examining *NRAS* and *BRAF* mutations in primary melanomas in relationship to survival will be confounded by these new treatments.

Our tumor collection and mutational analysis rate of all eligible primary melanomas is similar to or higher than comparable melanoma studies.[21,22,49–51] Further, our results are representative of the entire population of melanoma participants enrolled into GEM, as we found no significant differences comparing clinicopathologic characteristics of cases with and without mutation analysis. Population-based prevalence estimates of mutations provided may be useful for budgetary and economic evaluations in present and future pharmacoeconomics studies. Some mutations may have been misclassified, but we minimized this possibility by using laser capture microdissection for all small samples and independently confirming mutations on a separately amplified DNA fragment.

A limitation is that we did not obtain sentinel lymph node (SLN) status so we could not determine whether *NRAS/BRAF* status provides information beyond SLN status for outcome prediction. We also did not obtain information regarding therapies potentially utilized, such as regional radiation, systemic interferon, or clinical trial participation, which could confound our results. Information on relapse was also not available.

In conclusion, our finding that *NRAS*+ and *BRAF*+ melanomas are associated with higher tumor stage at diagnosis indicates that *NRAS*+ and *BRAF*+ are less likely than *wildtype* melanoma to be diagnosed when lower risk and surgically curable. *NRAS*+ melanoma's association with lower TIL grade may influence its response to immunotherapies. In GEM, the approximately three-fold increased risk of death from *NRAS*+ and *BRAF*+ compared to *wildtype* melanoma limited to higher risk tumors after adjusting for other prognostic factors indicates that mutational status may be prognostic for this group. This finding could be useful in the identification of patients at high risk of death from melanoma based on their primary melanoma tumor characteristics to inform evidence-based follow-up of patients and determination of eligibility for novel systemic therapy adjuvant trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: National Cancer Institute (NCI) grants R01CA112243, R01CA112524, R01CA112243-05S1, R01CA112524-05S2, U01CA83180, CA098438, R33CA10704339, P30CA016086, P30CA014089, and P30CA008748; National Institute of Environmental Health Sciences (P30ES010126); University of Sydney Medical Foundation Program grant (Bruce Armstrong).

Role of the Sponsors: The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of data; in the preparation of the manuscript; or in the review or approval of the manuscript.

Abbreviations

AJCC	American Joint Committee on Cancer
GEM	Genes, Environment, and Melanoma Study
HR	hazard ratio
IQR	interquartile range
OR	odds ratio

GEM Study Group

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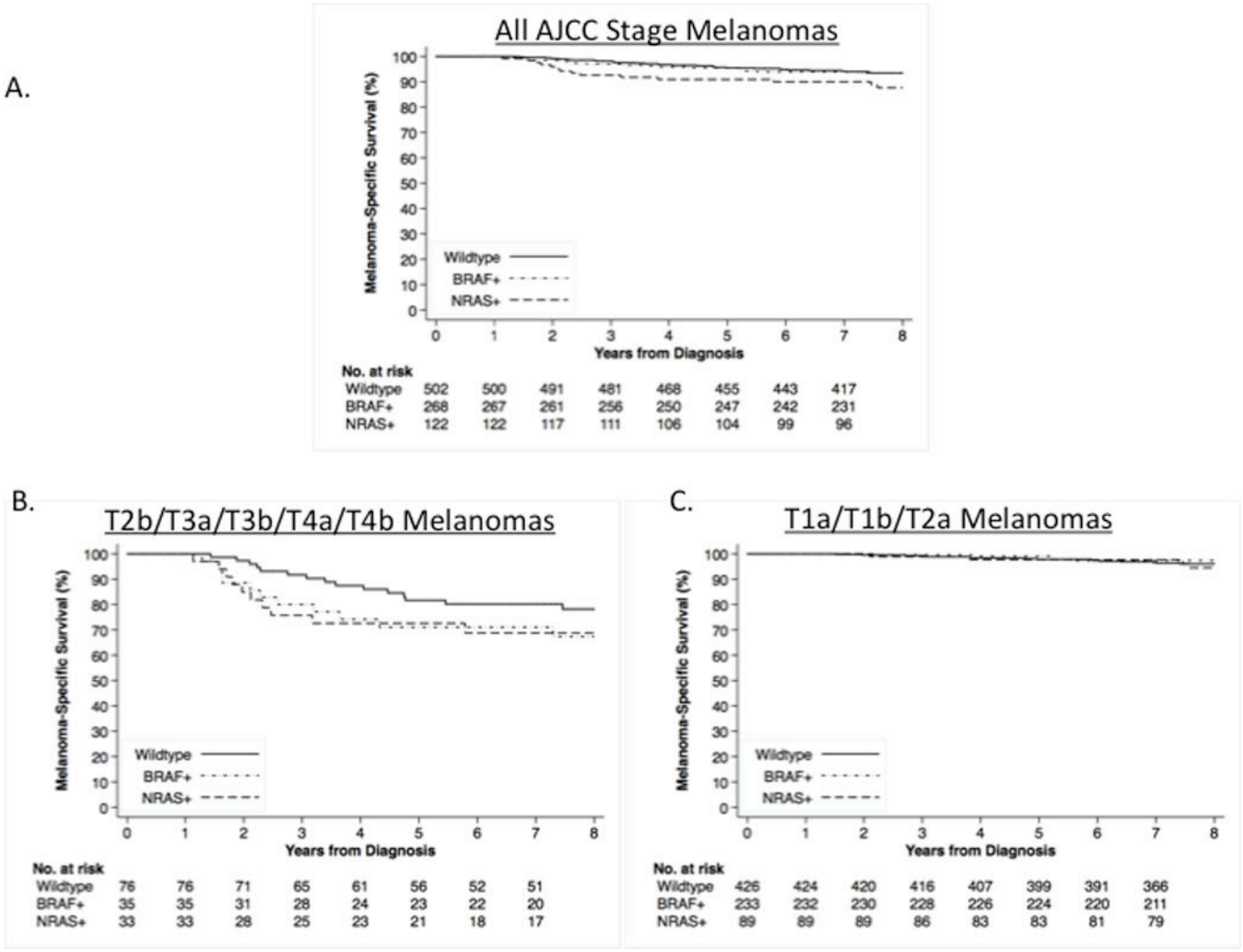

Fig. 1.

Kaplan-Meier melanoma-specific survival probabilities by primary melanoma *NRAS* and *BRAF* mutational status are shown for participants with melanomas (n=892). Patients with single primary melanoma were diagnosed in 2000. Patient follow-up for vital status was complete to the end of 2007. A. Melanoma-specific survival for all primary melanomas; B. Melanoma-specific survival for higher risk (T2b or higher AJCC stage) primary melanomas); C. Melanoma-specific survival for lower risk (T2a or lower AJCC stage) primary melanomas.

Table 1

Characteristics of 912 First Primary Invasive Cutaneous Melanoma Analyzed for *BRAF* and *NRAS* Mutations

Characteristic	**No. (%)**
Country	
Australia	488 (54)
United States	424 (46)
Sex	
Male	501 (55)
Female	411 (45)
Age at diagnosis, years	
Median (IQR)	57 (25)
Breslow thickness, mm	
Median (IQR)	0.74 (0.89)
BRAF and *NRAS* mutation	
Wildtype (NRAS−/BRAF−)	516 (57)
NRAS+	123 (13)
BRAF+	273 (30)

Abbreviations: IQR, interquartile range.

Table 2

Relationship between Tumor *NRAS*+ and *BRAF*+ Mutational Status and Cliniopathologic Features for First Primary Melanomas from 892 Patients[a]

				Adjusted for Study Center[b]				Fully Adjusted[c]			
	No. (%)			Compared with *Wildtype* Melanomas				Compared with *Wildtype* Melanomas			
	Wildtype	*NRAS*+	*BRAF*+	Adjusted *NRAS*+		Adjusted *BRAF*+		Adjusted *NRAS*+		Adjusted *BRAF*+	
Characteristic	**(n = 503)**	**(n = 122)**	**(n = 267)**	**OR (95% CI)**	***p*[d]**	**OR (95% CI)**	***p*[d]**	**OR (95% CI)**	***p*[d]**	**OR (95% CI)**	***p*[d]**
Sex											
Male	279 (55)	70 (57)	144 (54)	Reference	.83	Reference	.54	Reference	.59	Reference	.33
Female	224 (45)	52 (43)	123 (46)	1.0 (0.6–1.4)		1.1 (0.8–1.5)		1.1 (0.7–1.8)		1.2 (0.8–1.7)	
Age at diagnosis, years											
<50	149 (30)	25 (21)	125 (47)	Reference	.07[d]	Reference	<.001[d]	Reference	.11[d]	Reference	.001[d]
50–69	201 (40)	54 (44)	100 (37)	1.6 (1.0–2.7)		0.6 (0.4–0.9)		1.5 (0.9–2.6)		0.7 (0.5–1.0)	
>70	153 (30)	43 (35)	44 (16)	1.7 (1.0–3.0)		0.4 (0.2–0.5)		1.7 (0.9–3.1)		0.5 (0.3–0.8)	
Anatomic site											
Trunk/pelvis	198 (39)	54 (44)	147 (55)	Reference	.007	Reference	<.001	Reference	.011	Reference	.13
Scalp/neck	40 (8)	1 (1)	11 (4)	0.1 (0.01–0.7)		0.4 (0.2–0.7)		0.1 (0.01–0.6)		0.6 (0.3–1.3)	
Face/ears/other[e]	69 (14)	10 (8)	18 (7)	0.5 (0.3–1.1)		0.4 (0.2–0.6)		0.6 (0.3–1.3)		0.7 (0.4–1.3)	
Upper extremities	95 (19)	37 (30)	43 (16)	1.4 (0.9–2.3)		0.6 (0.4–1.0)		1.4 (0.8–2.3)		0.6 (0.4–1.0)	
Lower extremities	101 (20)	20 (16)	48 (18)	0.7 (0.4–1.3)		0.7 (0.4–1.0)		0.6 (0.3–1.2)		0.6 (0.4–0.9)	
Histologic subtype											
Superficial spreading	320 (64)	80 (66)	227 (85)	Reference	.002	Reference	<.001	Reference	.17	Reference	<.001
Nodular	38 (8)	21 (17)	17 (6)	2.2 (1.2–4.0)		0.7 (0.4–1.2)		1.1 (0.6–2.3)		0.5 (0.2–1.0)	
Lentigo maligna	90 (18)	9 (7)	15 (6)	0.4 (0.2–0.8)		0.2 (0.1–0.4)		0.5 (0.2–1.1)		0.4 (0.2–0.7)	
Unclassified/other[f]	55 (11)	12 (10)	8 (3)	0.9 (0.5–1.8)		0.2 (0.1–0.5)		0.6 (0.3–1.3)		0.2 (0.1–0.5)	
Breslow thickness, mm											
0.01–1.00	349 (69)	59 (48)	164 (61)	Reference	<.001	Reference	.01	Reference	.87	Reference	.34
1.01–2.00	86 (17)	34 (28)	74 (28)	2.3 (1.4–3.8)		1.8 (1.2–2.6)		1.3 (0.7–2.3)		1.4 (0.9–2.3)	

				Adjusted for Study Center[b]				Fully Adjusted[c]			
	No. (%)			Compared with *Wildtype* Melanomas				Compared with *Wildtype* Melanomas			
	Wildtype	*NRAS*+	*BRAF*+	Adjusted *NRAS*+		Adjusted *BRAF*+		Adjusted *NRAS*+		Adjusted *BRAF*+	
Characteristic	**(n = 503)**	**(n = 122)**	**(n = 267)**	**OR (95% CI)**	***P*[d]**	**OR (95% CI)**	***P*[d]**	**OR (95% CI)**	***P*[d]**	**OR (95% CI)**	***P*[d]**
2.01–4.00	49 (10)	19 (16)	21 (8)	2.2 (1.2–4.1)		0.9 (0.5–1.5)		1.1 (0.5–2.4)		0.9 (0.4–1.9)	
>4.00	19 (4)	10 (8)	8 (3)	3.2 (1.4–7.3)		0.9 (0.4–2.1)		1.3 (0.4–3.7)		0.9 (0.3–2.7)	
Ulceration											
Absent	467 (93)	104 (85)	248 (93)	Reference	.013	Reference	.84	Reference	.85	Reference	.54
Present	36 (7)	18 (15)	19 (7)	2.2 (1.2–4.0)		0.9 (0.5–1.7)		1.1 (0.5–2.4)		1.3 (0.6–2.6)	
Mitoses											
Absent	319 (63)	43 (35)	129 (48)	Reference	<.001	Reference	<.001	Reference	.04	Reference	.02
Present	184 (37)	79 (65)	138 (52)	3.1 (2.1–4.8)		1.8 (1.3–2.4)		1.8 (1.0–3.3)		1.7 (1.1–2.6)	
Growth phase											
Radial	196 (39)	22 (18)	68 (25)	Reference	<.001	Reference	.002	Reference	.11	Reference	.12
Vertical	307 (61)	100 (82)	199 (75)	3.2 (1.9–5.3)		1.7 (1.2–2.4)		1.7 (0.9–3.2)		1.4 (0.9–2.2)	
TIL grade											
Absent	96 (19)	38 (31)	45 (17)	Reference	<.001[d]	Reference	.39[d]	Reference	.002[d]	Reference	.74[d]
Nonbrisk	334 (66)	75 (61)	186 (70)	0.6 (0.4–0.9)		1.3 (0.8–1.9)		0.5 (0.3–0.8)		1.0 (0.7–1.6)	
Brisk	73 (15)	9 (7)	36 (13)	0.3 (0.1–0.6)		1.2 (0.7–2.2)		0.3 (0.1–0.7)		1.1 (0.6–2.0)	

Abbreviations: OR, odds ratio; TIL, tumor infiltrating lymphocyte.

[a] We used polytomous logistic regression to estimate the odds ratios and 95% confidence intervals with *NRAS*+ and *BRAF*+ melanoma simultaneously compared to wildtype. Melanomas (n = 20) with one or more data points missing for ulceration (n = 19), mitoses (n = 19), growth phase (n = 19), or TIL grade (n = 20) were excluded.

[b] Adjusted for study center.

[c] Included all variables in the table and adjusted for study center.

[d] Where noted for, linear trend was tested using the Wald statistic with the variable treated as a single ordinal variable.

[e] Other includes melanomas on the face/ears and other head/neck melanomas with unspecified sites.

[f] Other includes acral lentiginous, spindle cell, nevoid, and Spitzoid melanomas.

Table 3

Relationships between Tumor *NRAS* and *BRAF* Mutational Status and AJCC Tumor Stage for 892 First Primary Melanomas[a]

	No. (%)			Compared with *Wildtype*			
	Wildtype	*NRAS*+	*BRAF*+	Adjusted *NRAS*+[c]		Adjusted *BRAF*+[c]	
AJCC Tumor Stage[b]	(n = 503)	(n = 122)	(n = 267)	OR	P_{trend}[d]	OR	P_{trend}[d]
T1a	286 (57)	36 (30)	121 (46)	Reference	<.001	Reference	.04
T1b/T2a	143 (28)	53 (43)	111 (41)	2.7 (1.6–4.3)		1.8 (1.3–2.6)	
T2b/T3a	38 (8)	16 (13)	19 (7)	2.9 (1.4–5.8)		1.4 (0.7–2.5)	
T3b/T4a	26 (5)	11 (9)	11 (4)	3.1 (1.3–7.1)		1.3 (0.6–2.7)	
T4b	10 (2)	6 (5)	5 (2)	3.8 (1.2–12.0)		1.9 (0.6–5.9)	

Abbreviations: AJCC, American Joint Committee on Cancer; OR, odds ratio; TIL, tumor infiltrating lymphocyte.

[a] We used polytomous logistic regression to estimate the odds ratios and 95% confidence intervals with *NRAS*+ and *BRAF*+ melanoma simultaneously compared to wildtype. Melanomas (n = 20) with one or more data points missing for ulceration (n = 19), mitoses (n = 19), or TIL grade (n = 20) were excluded.

[b] T1a, Breslow thickness ≤1.0 mm and no ulceration and absent mitoses; T1b, Breslow thickness ≤1.0 mm and presence of ulceration or present mitoses; T2a, Breslow thickness 1.01–2.0 mm without ulceration; T2b, Breslow thickness 1.01–2.0 mm with ulceration; T3a, Breslow thickness 2.01–4.0 mm without ulceration; T3b, Breslow thickness 2.01–4.0 mm with ulceration, T4a, Breslow thickness >4.0 mm without ulceration; T4b, Breslow thickness >4.0 mm with ulceration.

[c] Adjusted for age (<50, 50–69, >70), sex, anatomic site (scalp/neck, face/ears/other, trunk/pelvis, upper extremities, lower extremities), TIL grade, study center.

[d] Linear trend was tested using the Wald statistic when AJCC tumor stage was treated as a single ordinal variable.

Table 4

Hazard Ratios for Melanoma-Specific Death According to Tumor *BRAF* and *NRAS* Mutational Status Among 892 Patients with Primary Melanoma

				Cox Proportional Hazards Model				Proportional Subdistribution Hazards Model for Competing-Risks			
	Censored	Melanoma Death	Death from Other Cause	Partially Adjusted[b]		Fully Adjusted[c]		Partially Adjusted[b]		Fully Adjusted[c]	
Characteristic	**No. (%)**	**No. (%)**	**No. (%)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**sHR (95% CI)**	***P***	**sHR (95% CI)**	***P***
All primary melanomas[a]											
All stage melanomas	(n=750)	(n = 62)	(n=80)								
NRAS/BRAF Status											
Wildtype (NRAS−/BRAF−)	420 (84)	31 (6)	51 (10)	Reference	.19	Reference	.27	Reference	.18	Reference	.28
NRAS+	94 (77)	14 (11)	14 (11)	1.8 (0.9–3.4)		1.7 (0.8–3.4)		1.8 (1–3.5)		1.7 (0.8–3.6)	
BRAF+	236 (88)	17 (6)	15 (6)	1.3 (0.7–2.4)		1.5 (0.8–2.9)		1.3 (0.7–2.4)		1.6 (0.8–3.2)	
Stratified by AJCC Stage											
Stage T1a/T1b/T2a	(n=667)	(n = 26)	(n=55)								
NRAS/BRAF Status											
Wildtype (NRAS−/BRAF−)	374 (88)	16 (4)	36 (8)	Reference	.84	Reference	.65	Reference	.83	Reference	.67
NRAS+	77 (87)	4 (4)	8 (9)	1.1 (0.4–3.4)		0.9 (0.3–3.0)		1.2 (0.4–3.5)		0.9 (0.3–2.9)	
BRAF+	216 (93)	6 (3)	11 (5)	0.8 (0.3–2.1)		0.6 (0.2–1.7)		0.8 (0.3–2)		0.6 (0.2–1.7)	
Stage T2b/T3a/T3b/T4a/T4b	(n=83)	(n = 36)	(n=25)								
NRAS/BRAF Status											
Wildtype (NRAS−/BRAF−)	46 (61)	15 (20)	15 (20)	Reference	.13	Reference	.04	Reference	.09	Reference	.02
NRAS+	17 (52)	10 (30)	6 (18)	1.7 (0.8–3.9)		2.9 (1.1–7.7)		1.8 (0.8–4.2)		3 (1.1–8.2)	
BRAF+	20 (57)	11 (31)	4 (11)	2.3 (1.0–5.1)		3.1 (1.2–8.5)		2.4 (1.1–5.3)		3.6 (1.3–9.5)	
Primary melanomas limited to *NRAS* codon 61 and *BRAF* V600E mutant and *wildtype* melanomas											
All stage melanomas	(n=685)	(n = 57)	(n=67)								
NRAS/BRAF Status											
Wildtype (NRAS−/BRAF−)	420 (84)	31 (6)	51 (10)	Reference	.12	Reference	.17	Reference	.11	Reference	.19
NRAS codon 61+	90 (79)	13 (11)	11 (10)	1.9 (1.0–3.6)		1.9 (0.9–4.0)		1.9 (1–3.7)		1.9 (0.9–4.2)	

				Cox Proportional Hazards Model				Proportional Subdistribution Hazards Model for Competing-Risks			
	Censored	Melanoma Death	Death from Other Cause	Partially Adjusted[b]		Fully Adjusted[c]		Partially Adjusted[b]		Fully Adjusted[c]	
Characteristic	**No. (%)**	**No. (%)**	**No. (%)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**sHR (95% CI)**	***P***	**sHR (95% CI)**	***P***
BRAF V600E+	175 (91)	13 (7)	5 (3)	1.6 (0.8–3.0)		1.7 (0.8–3.5)		1.6 (0.8–3.2)		1.8 (0.8–4)	
Stratified by AJCC Stage											
Stage T1a/T1b/T2a	(n=607)	(n = 25)	(n=45)								
NRAS/BRAF Status											
Wildtype (NRAS−/BRAF−)	374 (88)	16 (4)	36 (8)	Reference	.92	Reference	.87	Reference	.91	Reference	.89
NRAS codon 61+	74 (88)	4 (5)	6 (7)	1.3 (0.4–3.8)		1.1 (0.3–3.6)		1.3 (0.4–3.7)		1.1 (0.4–3.4)	
BRAF V600E+	159 (95)	5 (3)	3 (2)	1.0 (0.4–2.8)		0.8 (0.3–2.3)		1 (0.4–2.8)		0.8 (0.2–2.5)	
Stage T2b/T3a/T3b/T4a/T4b	(n=78)	(n = 32)	(n=22)								
NRAS/BRAF Status											
Wildtype (NRAS−/BRAF−)	46 (61)	15 (20)	15 (20)	Reference	.13	Reference	.04	Reference	.10	Reference	.03
NRAS codon 61+	16 (53)	9 (30)	5 (17)	1.7 (0.7–3.9)		3.2 (1.1–9.7)		1.8 (0.7–4.2)		3.3 (1.1–10.1)	
BRAF V600E+	16 (62)	8 (31)	2 (8)	2.4 (1.0–6.0)		3.8 (1.2–11.8)		2.6 (1–6.6)		4.3 (1.3–14.8)	

Abbreviations: AJCC, American Joint Committee on Cancer; HR, hazard ratio; sHR, subdistribution hazard ratio; TIL, tumor infiltrating lymphocyte.

[a] Of the 912 patients who entered the study with first primary melanoma, 40 developed a second melanoma during the ascertainment period and were treated as time-dependent and the *BRAF/NRAS* mutational status and pathologic characteristics of their thicker melanoma was utilized in the survival analysis. Excluded from this analysis wee 20 participants with missing AJCC tumor stage or TIL grade for their melanoma. In the cox models death from other causes were considered as censored.

[b] The Cox models /proportional subdistribution hazards models were adjusted for age (continuous), sex, and study center.

[c] The Cox models/proportional subdistribution hazards models were adjusted for age (continuous), sex, anatomic site (trunk/pelvis, scalp/neck, face/ears/other, upper extremities, lower extremities), AJCC tumor stage (T1a, T1b/T2a, T2b/T3a, T3b/T4a, T4b), TIL grade, and study center.