original report

PTEN Loss-of-Function Alterations Are Associated With Intrinsic Resistance to BRAF Inhibitors in Metastatic Melanoma

abstract

Purpose The clinical use of BRAF inhibitors in patients with melanoma is limited by intrinsic and acquired resistance. We asked whether next-generation sequencing of pretreatment tumors could identify coaltered genes that predict for intrinsic resistance to BRAF inhibitor therapy in patients with melanoma as a prelude to rational combination strategies.

Patients and Methods We analyzed 66 tumors from patients with metastatic *BRAF*-mutant melanoma collected before treatment with BRAF inhibitors. Tumors were analyzed for > 250 cancer-associated genes using a capture-based next-generation sequencing platform. Antitumor responses were correlated with clinical features and genomic profiles with the goal of identifying a molecular signature predictive of intrinsic resistance to RAF pathway inhibition.

Results Among the 66 patients analyzed, 11 received a combination of BRAF and MEK inhibitors for the treatment of melanoma. Among the 55 patients treated with BRAF inhibitor monotherapy, objective responses, as assessed by Response Evaluation Criteria in Solid Tumors (RECIST), were observed in 30 patients (55%), with five (9%) achieving a complete response. We identified a significant association between alterations in *PTEN* that would be predicted to result in loss of function and reduced progression-free survival, overall survival, and response grade, a metric that combines tumor regression and duration of treatment response. Patients with melanoma who achieved an excellent response grade were more likely to have an elevated *BRAF*-mutant allele fraction.

Conclusion These results provide a rationale for cotargeting BRAF and the PI3K/AKT pathway in patients with *BRAF*-mutant melanoma when tumors have concurrent loss-of-function mutations in *PTEN*. Future studies should explore whether gain of the mutant *BRAF* allele and/or loss of the wild-type allele is a predictive marker of BRAFi sensitivity.

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INTRODUCTION

BRAF inhibitors (BRAFis) and RAF/MEK inhibitor combinations are standard-of-care therapies for patients with BRAF V600E/K melanoma. Despite the profound clinical activity of BRAFis in this setting, the degree and durability of response are highly variable.[1-6] Prior studies have identified mechanisms of acquired resistance to BRAFis, many of which have been validated using tumor tissues collected at the time of disease progression. These resistance mechanisms can be divided into two classes. Class 1 alterations confer drug resistance by abrogating the ability of the drugs to inhibit ERK signaling and include mutations in *NRAS*, *NF1*, *MEK1/MEK2*, and *BRAF* splice variants.[7-14] Class 2 alterations result in reduced dependence on RAF signaling and include alterations that activate parallel pathways such as inactivating *PTEN* mutations[15-17] and alterations in genes that dysregulate the downstream machinery that mediates cell cycle progression (eg, *RB1*, *CDKN2A*, *CCND1*)[17-19] or apoptosis (eg, *TP53*, *MCL1*).[20] In addition, alterations that overcome BRAF-mediated feedback-induced suppression of upstream receptor tyrosine kinases likely function by both mechanisms.[8,21-27]

The molecular basis of intrinsic resistance is less well characterized. Here, we used next-generation

sequencing methods to determine whether genomic alterations present in pretreatment tumor tissue are predictive of intrinsic resistance to BRAFi therapy in patients with *BRAF*-mutant melanoma.

PATIENTS AND METHODS

Patient Clinical Characteristics

This study was conducted after institutional review board approval. Patients had stage IV or unresectable stage III BRAF V600E/K melanoma. Response was assessed using both Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST) and response grade, a composite measure of the average of the percentage of lesion shrinkage and the duration of response.[28] For response grade, patients were segregated into the following three classes: excellent ($\geq$ 50% tumor shrinkage for $\geq$ 7 months or any shrinkage for $\geq$ 12 months), poor (tumor growth, any new lesions, or $\leq$ 50% shrinkage for $<$ 4 months), or intermediate (neither excellent nor poor). Patients who died or were lost to follow-up before the first assessment or who had incomplete imaging were graded as not evaluable. Progression-free survival (PFS) was defined as the time from the start of BRAFi therapy until the date of progression on BRAFi, date of death, or the date of last follow-up; patients who were switched to immune checkpoint blockade were censored at the date of withdrawal from BRAFi therapy. Overall survival (OS) was measured as the time from the start of BRAFi therapy until the date of death; patients alive at last follow-up were censored at the date of last documented contact. The cutoff date was March 2016, with a median follow-up time for the entire cohort of 14.6 months.

Exon Capture Sequencing

DNA from tumor and blood was analyzed using a custom, exon capture DNA sequencing assay designed to capture all protein-coding exons and selected introns of $>$ 250 cancer-associated genes.[29,30] See Data Supplement for gene lists. Single nucleotide variants were detected using MuTect,[31] and indels were detected using the SomaticIndelDetector tool in GATK. All candidate mutations were reviewed manually using the Integrative Genomics Viewer.[32,33] Mean sequence coverage was calculated using the DepthOfCoverage tool in GATK and used to compute copy number.[29,30] The FACETS algorithm was used to estimate tumor purity, ploidy, and allele-specific copy number.[34] For correlations with response and outcome, we restricted the analysis to a subset of 56 genes selected based on their mutation frequency in melanoma and their ability to activate MAPK and/or PI3K signaling (Data Supplement). All clinical and genomic data are available through the Memorial Sloan Kettering cBio Portal for Cancer Genomics (http://cbioportal.org/).

Biostatistics

A Kruskal-Wallis test and one-way analysis of variance were used to assess the association between mutations and response. Fisher's exact *t* test was used to compare the percentage of mutations for each designated gene between The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering Cancer Center/Vanderbilt-Ingram Cancer Center cohorts. *BRAF*-mutant allele frequencies were *z* score normalized relative to allele frequencies of other mutations in the same sample; deviation from the null distribution was computed using a one-sided Mann-Whitney *U* test. A Cox proportional hazards model was fit to obtain hazard ratios (HRs) and *P* values for *PTEN* mutation (Wald test) from univariable and multivariable models. Survival curves (OS and PFS) were obtained with Prism (GraphPad, La Jolla, CA), and a *P* value from a log-rank test is presented.

RESULTS

Patient Characteristics and BRAFi Response

We analyzed pretreatment tumors from 66 patients with metastatic *BRAF*-mutant melanoma. Forty-nine patients received vemurafenib, five dabrafenib, and 11 a BRAFi plus MEK inhibitor (MEKi) combination. Notably, in the BRAFi therapy group, one patient received cobimetinib (MEKi) for the treatment of chronic myelomonocytic leukemia 75 weeks after the start of BRAFi therapy.[35] For this patient, the clinical response to BRAFi monotherapy was only assessable before the administration of the MEKi. The clinical characteristics of the two cohorts of patients are listed in Table 1. Approximately 50% of the patients also received immunotherapy during their treatment course (29 of 55 BRAFi patients and three of 11 BRAFi plus MEKi patients), whereas 19.7% of patients received chemotherapy. Two (3%) of 66 patients had previously received the MEKi selumetinib before treatment with a BRAFi (Table 1).

Objective responses, as assessed by RECIST criteria, to BRAFi monotherapy were observed in 30 (55%) of 55 patients, with five (9%) of 55 patients achieving a complete response and 25 (45%) of 55 patients achieving a partial response. Among the patients who achieved a complete response, three

Table 1. Patient Characteristics

Characteristic	BRAFi (n = 55)	BRAFi + MEKi (n = 11)	All Patients (N = 66)
Sex, No.			
Female	34	8	42
Male	21	3	24
Median age, years (range)	56 (23-83)	49 (21-62)	54 (21-83)
Stage at treatment,			
IIIC	1	1	2
IVA	4	2	6
IVB	2	0	2
IVC	48	8	56
Pretreatment LDH levels, No.			
Normal	30	8	38
Elevated	21	3	24
Not measured	4	0	4
Brain metastases, No.	9	1	10
BRAFi, No.			
Vemurafenib	49	0	49
Vemurafenib + cobimetinib	1*	3	4
Dabrafenib	5	0	5
Dabrafenib + trametinib	0	8	8
Previous treatment, No.			
None	23	0	23
Chemotherapy	12	1	13
MEKi	2	0	2
Immunotherapy, No.			
Prior to BRAFi	7	0	7
Ipi	2	0	2
IL-2	4	0	4
IFN	1	0	1
During or after BRAFi	22	3	25
Ipi	17	3	20
Ipi + Nivo	2	0	2
Nivo	1	0	1
Pem	2	0	2

Abbreviations: BRAFi, BRAF inhibitor; IFN, interferon; IL-2, interleukin-2; Ipi, ipilimumab; LDH, lactate dehydrogenase; MEKi, MEK inhibitor; Nivo, Nivolumab; Pem, pembrolizumab.

*Cobimetinib was given for treatment of chronic myelomonocytic leukemia, from which the patient died.[35]

remained alive, with two being disease free 4.9 and 4.8 years after initiating therapy. Stable disease was observed in 11 (20%) of 55 patients, whereas 10 (18%) of 55 patients experienced primary progressive disease. Four (7%) of 55 patients were not evaluable for response as a result of rapid clinical deterioration (Fig 1A, left panel). In addition to RECIST, patients were stratified using a response grade classification that incorporates measures of both lesion shrinkage and response duration. Seventeen (31%) of 55 patients achieved an excellent response grade, and 19 (35%) of 55 patients had a poor response, whereas for 15 (27%) of 55 patients, the response was scored as intermediate. For four (7%) of 55 patients, the response grade was not evaluable (Fig 1B, left panel). PFS and OS in the three classes of responders are shown in the Data Supplement. As highlighted in Figure 1C, RECIST 1.1 and response grade classifications were not always concordant, with eight of 66 patients classified as partial responders on the basis of RECIST criteria but as poor responders on the basis of response grade because their responses to BRAFi therapy were of short duration.

Pattern of Genes Co-Mutated With *BRAF*

The primary objective of this study was to determine whether the pattern of genes co-mutated with *BRAF* was predictive of the clinical benefit from BRAFi treatment. Samples were sequenced as outlined in Patients and Methods to a mean coverage of 622-fold, identifying on average nine single nucleotide variants or indels per tumor (range, two to 39 variants or indels; Data Supplement). We confirmed the presence of a BRAF V600E/K mutation in all tumors. In contrast to patients treated with immune checkpoint blockade,[36] no correlation was observed between the number of somatic mutations and treatment response (Data Supplement). *BRAF* amplification was observed in three tumors, all with intermediate responses. Although *BRAF* amplification has been associated with acquired resistance to BRAFis,[37] these data indicate that *BRAF* amplification does not preclude BRAFi response. An increase in *BRAF*-mutant allele fraction relative to the mean allele fraction of other variants identified in a sample could indicate allelic imbalance resulting from selective gain of the mutated *BRAF* allele or loss of the wild-type allele. Significantly elevated *BRAF*-mutant allele fractions (more than twice the median of all variants detected in the sample) were observed in a total of nine patients (four excellent responders, four intermediate responders, and one poor responder; Data Supplement) in the BRAFi cohort. Normalizing relative to the mean in each case to account for differences in tumor content or purity, we observed a significant enrichment for elevated BRAF V600–mutant allele fraction in excellent responders ($P < .001$) compared with poor responders ($P = .02$). Quantitative estimates of tumor ploidy and purity could be calculated for a subset of patients and confirmed allelic imbalance in two excellent responders (Data

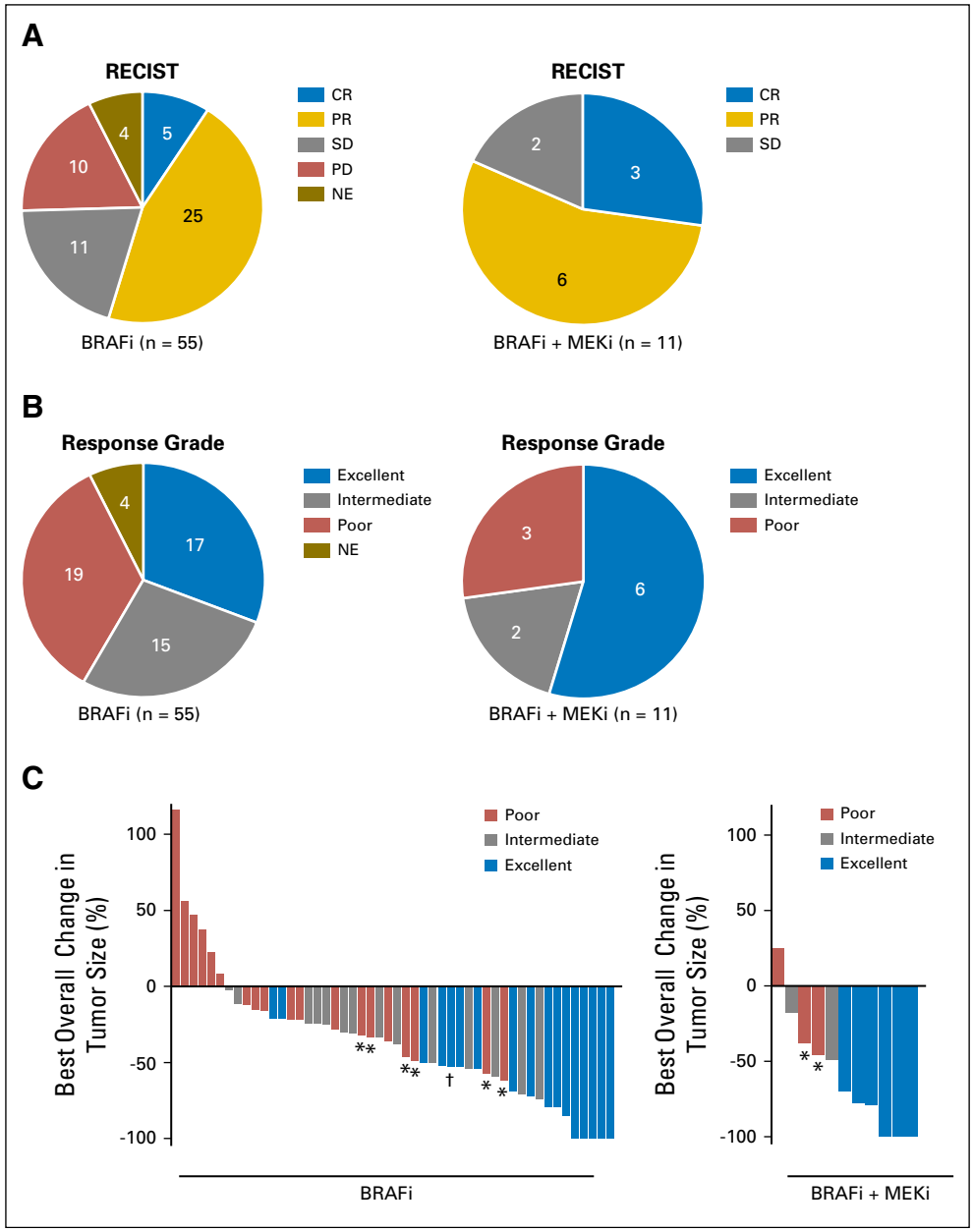

Fig 1. Response and response grade of patients with melanoma to *BRAF*-targeted therapy. (A) Classification of patients according to Response Evaluation Criteria in Solid Tumors (RECIST). (B) Patients were also stratified accordingly to response grade, which incorporates both tumor regression and response duration. (A and B) Left panel: patients treated with BRAF inhibitor (BRAFi) monotherapy; right panel: patients treated with the BRAFi plus MEK inhibitor (MEKi) combinations. (C) Waterfall plot showing best overall response. Left panel: BRAFi cohort (50 patients; in one patient, best overall response was not evaluable as a result of rapid clinical deterioration); right panel: BRAFi + MEKi (11 patients). Each bar represents an individual patient. CR, complete response; NE, not evaluable; PD, progression of disease; PR, partial response; SD, stable disease. (*) Indicates patients who had a PR according to RECIST but a poor response grade. (†) Indicates patient was treated with vemurafenib plus cobimetinib for chronic myelomonocytic leukemia.

Supplement), suggesting that selective amplification of the mutant allele or loss of the wild-type allele may be a sensitizing event in these tumors.

To confirm that the tumors in our cohort had a co-mutation pattern consistent with the genomic landscape of *BRAF*-mutated melanomas reported in other studies, we compared the 66 tumors analyzed here to the 151 BRAF V600E/K–mutated tumors from TCGA. In both cohorts, *CDKN2A* and *PTEN* were among the genes most commonly co-mutated with *BRAF* (Fig 2). A minority of tumors also harbored coalterations in a second RAS/MAPK pathway gene (*NRAS*, *NF1*, or *MAP2K1*) or in the PI3K/AKT/mTOR axis (*PI3KCA*, *AKT1/2/3*, *PTEN*, *TSC1/2*, or *MTOR*). We did observe enrichment for alterations in the *RB1* and *MDM2* genes, which may reflect the more aggressive clinical profiles of the patients in this study versus the TCGA (Fig 2A). The phosphatases *PTPRT* and *PTRTD* and the lysine methyltransferase *KMT2C* were among the genes mostly commonly coaltered with *BRAF* (Fig 2). Loss-of-function mutations have been reported in each of these tumor suppressor genes.[35,36] In melanoma, the alterations were primarily missense variants of unknown significance (Appendix Tables A1-A3). Given the large size of these genes, the high mutation rate of melanomas and the distribution

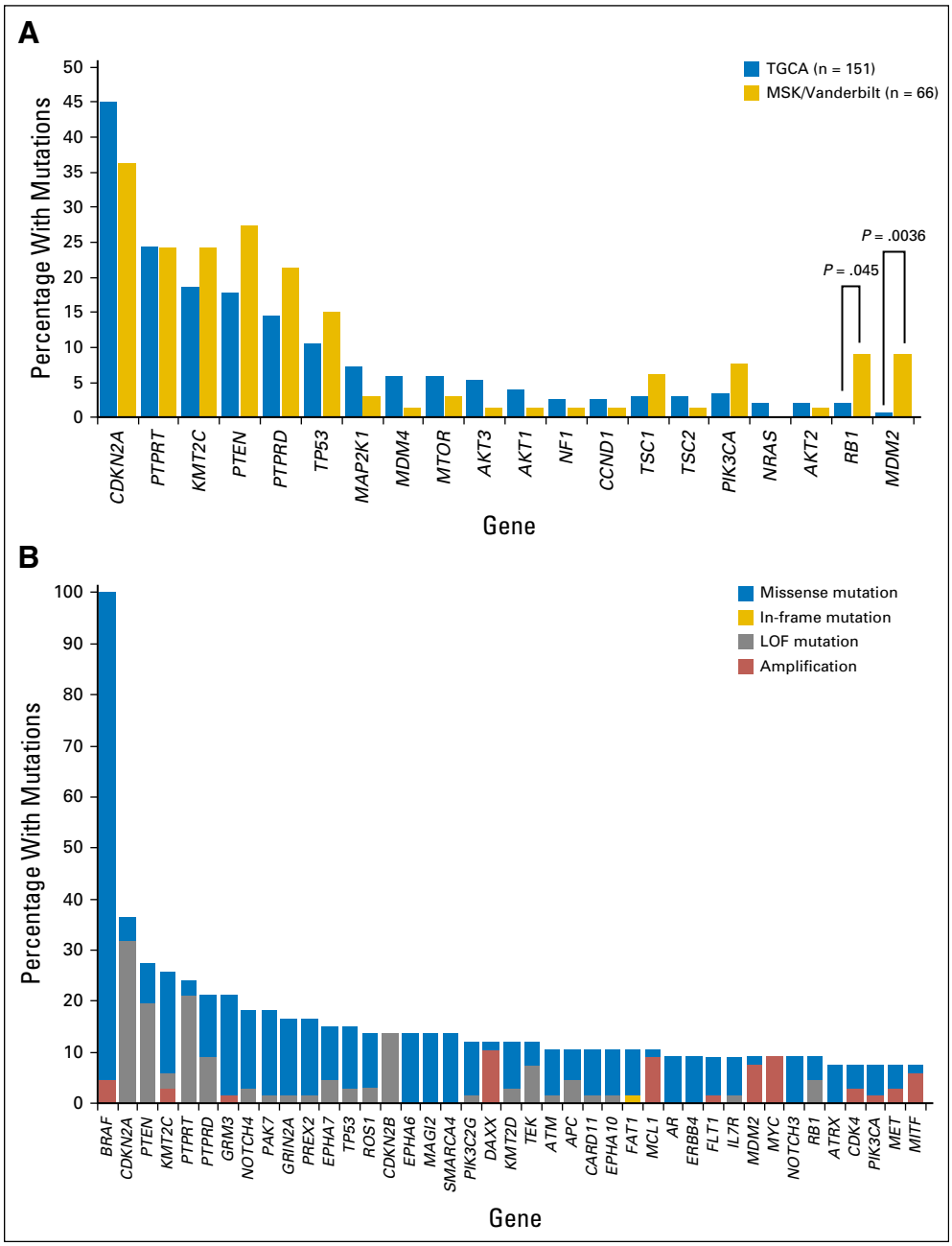

Fig 2. Landscape of most frequently mutated genes across the 66 patients with melanoma treated with BRAF inhibitors. (A) Each bar represents the total percentage of alterations found in each gene in either The Cancer Genome Atlas (TCGA) or Memorial Sloan Kettering/Vanderbilt cohort. $P < .05$ was considered significant. (B) *BRAF* mutations were found in all samples (100%), with three of 66 exhibiting coamplification of *BRAF*. Loss-of-function (LOF) mutations included deep deletions, nonsense mutations, and frameshift alterations predicted to result in early truncation and loss of expression.

of the variants throughout the genes, most of the variants observed were likely passenger events (Data Supplement).

Genomic Predictors of Sensitivity to BRAFi Therapy

The co-mutational pattern identified in individual patients is shown as an OncoPrint in Figure 3. A prior report had suggested an association between *CDKN2A* alteration and BRAFi response.[18] Twenty-one (41%) of 51 patients receiving BRAFi monotherapy had alterations in *CDKN2A* (Fig 3, left panel), of which 19 were putative loss-of-function alterations.[38] Contrary to previous studies,[7,18,39] mutation or homozygous deletion of *CDKN2A* did not correlate with response grade (Fig 4A).

Alterations in *PTEN* were significantly more common in patients with poor response grade treated with BRAFi monotherapy (11 alterations in patients with poor response, three in patients with intermediate response, and two in patients with excellent response; Fig 4B). Of the 11 *PTEN* alterations identified in the poor response grade cohort, 10 were likely inactivating, including deep deletions consistent with homozygous loss (n = 5),

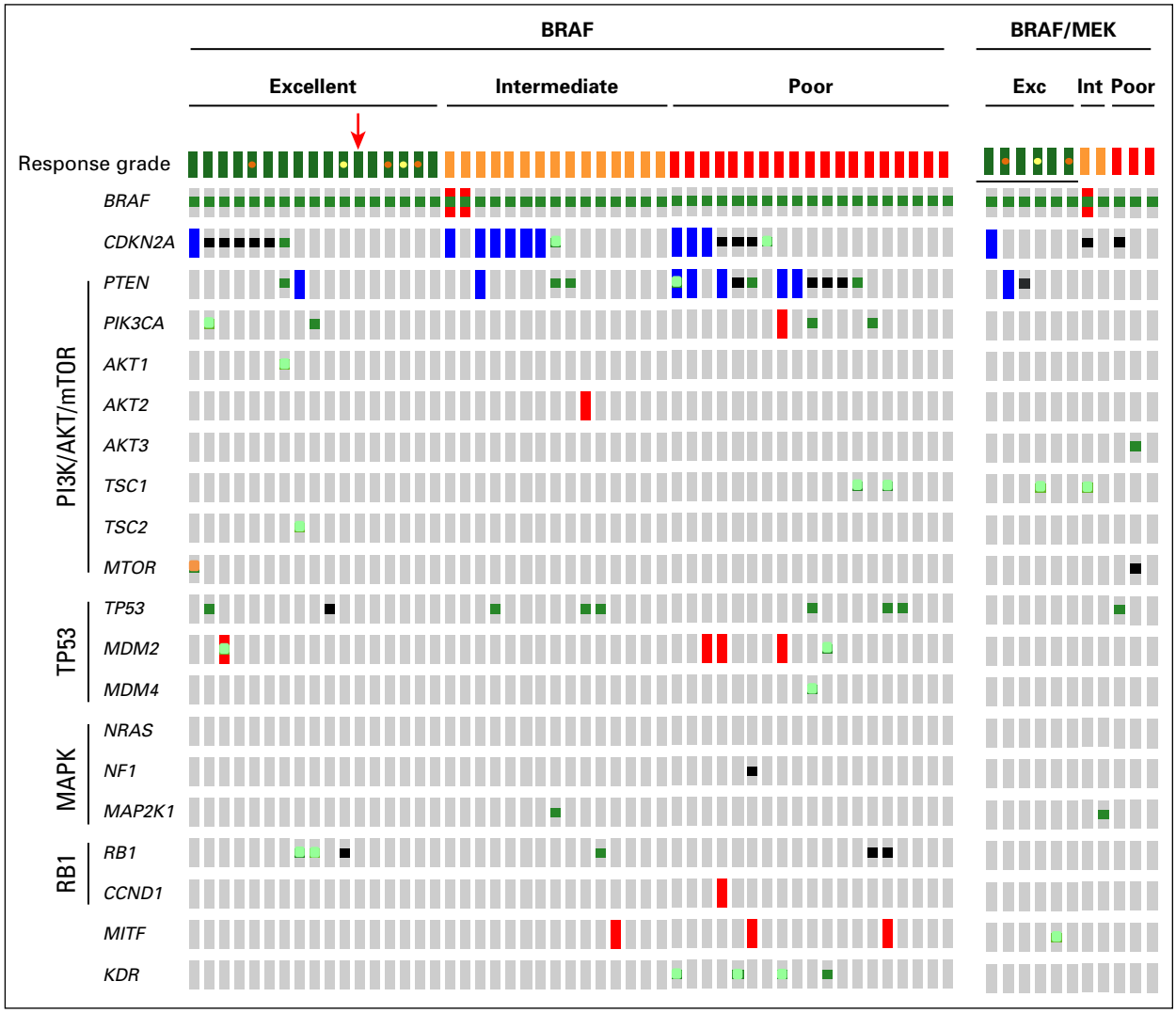

Fig 3. OncoPrint of key pathways altered in the three groups of responders. The OncoPrint highlights the pattern of comutation of 20 genes stratified by response grade into excellent (Exc), intermediate (Int), or poor cohorts. The red arrow identifies the patient tumor treated with the MEK inhibitor for chronic myelomonocytic leukemia. Orange dots indicate complete response and still alive; yellow dots indicate complete response and dead or disease progression. Dark green indicates recurrent and/or activating missense mutation;

truncating mutations (n = 4), and one missense mutation (P204S) located in the C2 domain of the protein and known to affect its stability and catalytic activity.[40] The one variant of unknown significance (P38S) was located in the phosphatase domain. Patients whose tumors had alterations in *PTEN* had shorter PFS (HR, 3.46; 95% CI, 1.79 to 6.71; $P < .001$) and reduced OS (HR, 3.10; 95% CI, 1.59 to 6.05; $P < .001$; Figs 4C and 4D). The association of *PTEN* mutation with PFS and OS remained statistically significant after controlling for stage and Eastern Cooperative Oncology Group performance status (PFS: HR, 3.30; 95% CI, 1.70 to 6.43; $P < .001$; OS: HR, 3.37; 95% CI, 1.70 to 6.70; $P < .001$). Despite the association between alterations in *PTEN* and response grade, *PTEN* alterations were not exclusive to the poor response grade cohort. Specifically, two excellent and three intermediate responders harbored either deep deletions or recurrent missense variants in PTEN (R15S, G132C, and Y177H; Fig 3).[41]

Given the association between *PTEN* alteration and BRAFi response, we assessed additional nodes in the PI3K/AKT/mTOR pathway. We detected four missense mutations in *PIK3CA*, which encodes for the catalytic subunit of PI3K, three of which are hotpot mutations (V344G, E545K, and H1047R). These mutations were not exclusive to patients with a poor response grade (Fig 3 and Data Supplement). In fact, two excellent responders harbored missense mutations in *PIK3CA*, one of which was an activating hotspot mutation (H1047R).[42,43] No hotspot mutations in *AKT* were observed in the 51 patients treated with BRAFi alone (Fig 3, left panel). A hotspot E17K AKT3 mutation was observed in one poor responder treated with the RAF/MEK inhibitor combination (Fig 3, right panel). Nonrecurrent

a cutoff of six recurrences or more reported in the Catalogue of Somatic Mutations in Cancer (COSMIC) in at least two tumor types was used to distinguish a recurrent mutation from a rare event. Light green indicates nonrecurrent missense variant of unknown significance; red indicates amplification; blue indicates deep deletion; black indicates truncating deletion; and orange indicates in-frame mutation.

missense variants of unknown significance in *TSC1*, *TSC2*, and *MTOR* were rare and present in both excellent and poor responders (Fig 3, left panel).

We observed that 25% of the patients (13 of 51 patients) harbored likely functional alterations in the *TP53* or *MDM2* genes. *TP53* was mutated in eight patients (Appendix Table A4), and *MDM2* was amplified in four patients and mutated in one patient (Appendix Table A4 and Data Supplement). When analyzed together, alterations in *TP53* and *MDM2* did not correlate with response grade or shorter PFS and OS (Fig 3, left panel, and Data Supplement).

Alterations that abrogate the ability of BRAFis to inhibit ERK activation (*NRAS*, *MAP2K1*, and *NF1* mutations) have been shown to be common mechanisms of acquired resistance to BRAFis. None of the 66 pretreatment tumors analyzed harbored an *RAS* mutation (Fig 3 and Data Supplement). One patient had an alteration in *NF1* that would be predicted to result in loss of expression (X2441_splice). Consistent with preclinical data indicating that loss of *NF1* confers resistance to BRAFis,[11,44] this patient had a poor response grade. *MAP2K1* mutations were rare; only one patient, an intermediate responder, harbored an MEK1 mutation (P124S; Fig 3, left panel). A second patient, an intermediate responder treated with a BRAFi plus MEKi combination, had a known activating mutation in MEK1 (Q56P) with an allele frequency of 0.04 (compared with 0.19 for the *BRAF* mutation), consistent with the presence of a subclonal population. Both the MEK1 P124S and Q56P mutations have previously been shown to be associated with context-dependent resistance to both MEKis and BRAFis.[12]

We observed mutations in *RB1* or amplifications in *CCND1* in 11% of patients treated with BRAFi monotherapy; however, no association with response was observed (Data Supplement). Three patients had amplification of *MITF*, and consistent with a prior report,[15] *MITF* amplifications were observed exclusively in poor or intermediate responders. However, given the rarity of this event, this association did not reach statistical significance (Data Supplement). Finally, four mutations in *KDR* (kinase insert domain receptor, VEGFR2) were identified, all in patients who exhibited a poor response grade (Fig 3, left panel, and Data Supplement). Of these mutations, only one (VEGFR2 R1032Q) is a hotspot,[45] whereas the others are variants of unknown significance. Both OS and PFS were significantly shorter in patients with *KDR* mutations (Data Supplement). Although this association was statistically significant, given the small number of mutational events and the lack of functional data regarding the significance of these events, this association should be interpreted with caution.

DISCUSSION

BRAFis and MEKis are now US Food and Drug Administration–approved treatments for *BRAF*-mutant melanoma. Prior studies of small cohorts of patients who initially responded to BRAFis but later developed acquired resistance have identified molecular alterations that underlie acquired drug resistance.[7-14,46,47] In this study, we sought to determine whether pre-existent co-occurring alterations predict for intrinsic resistance to BRAFis. As the benefit with anticancer agents has been shown to associate with both depth of response and response duration, we also correlated the genomic findings with response grade, a composite measure of tumor regression and duration of response. We found that loss-of-function alterations of *PTEN* correlate with poor response to BRAFis in *BRAF*-mutant melanoma. Furthermore, we observed that patients with *PTEN* alterations had shorter PFS and OS compared with the *PTEN* wild-type cohort.

Notably, *PTEN* and other PI3K pathway alterations were identified in a small number of excellent and intermediate BRAFi responders. This observation is consistent with prior clinical and laboratory studies that suggested that alterations in *PTEN* do not preclude an antitumor response to BRAFis.[9,17,18] The data also suggest that additional comolecular events likely cooperate with *PTEN* loss to confer BRAFi resistance in patients who derive no clinical benefit from these agents. In sum, the results support the testing of PI3K inhibitors in combination with BRAFi and MEKi alone or in combination in patients with concurrent *BRAF* and *PTEN* alterations.[48,49] Because complete responses were infrequent even in *PTEN* wild-type patients, coadministration of a PI3K inhibitor could also result in further incremental tumor regression or a longer duration of treatment response in patients who lack PI3K pathway mutations.

A notable observation from this study was a trend toward increased *BRAF*-mutant allele fraction in excellent responders. This allelic imbalance is consistent with a relative gain of the mutant *BRAF* allele. Because increased expression of wild-type *BRAF* would be predicted to confer resistance to vemurafenib, it is biologically plausible that loss of the wild-type allele leading to a decrease in the

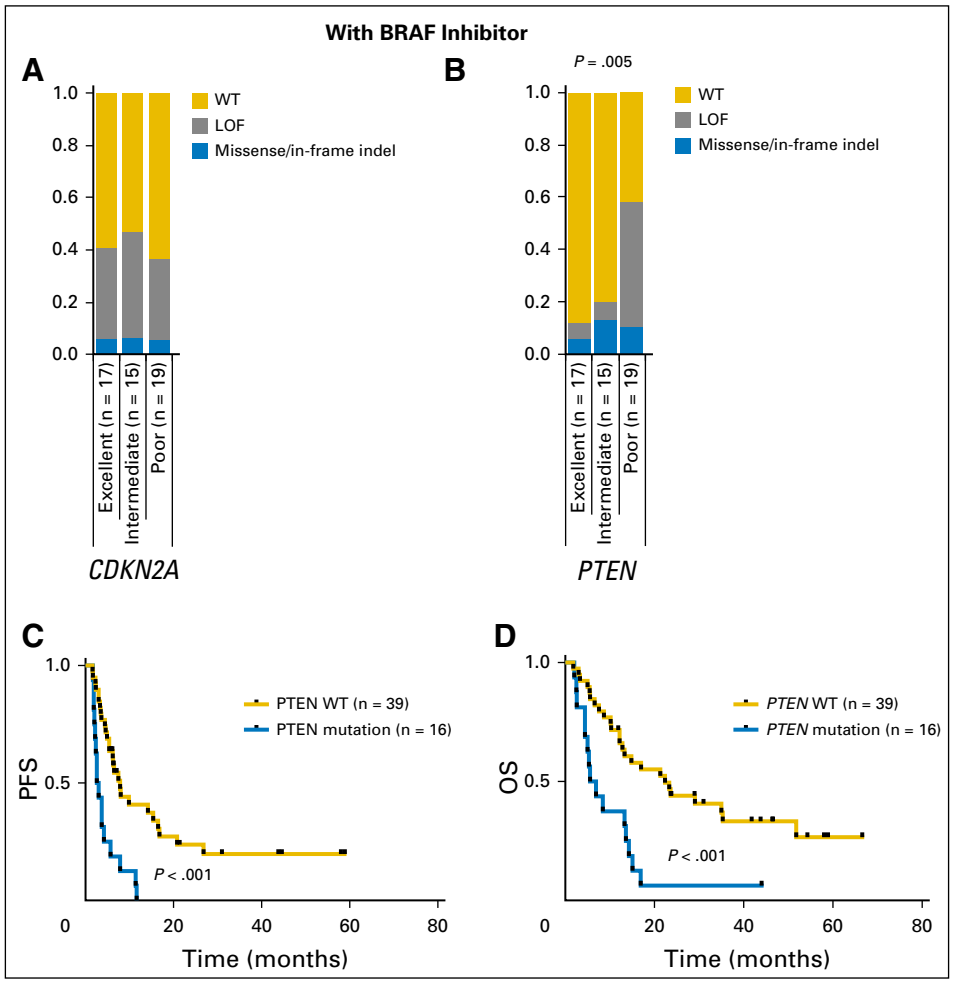

Fig 4. *CDKN2A* and *PTEN* mutation status as a function of response. Percentage of excellent, intermediate, and poor responders with wild-type (WT), loss-of-function (LOF), or missense mutations in (A) *CDKN2A* or (B) *PTEN*. Gold indicates WT; blue indicates missense mutation; gray indicates LOF mutation. (C) Progression-free survival (PFS) and (D) overall survival (OS) for patients with *PTEN*-mutant (gold line) and *PTEN* WT (blue line) melanomas.

fraction of RAF dimers that contain a wild-type RAF protein could increase BRAFi sensitivity.[50] The results support broader whole-exome or whole-genome sequencing of *BRAF*-mutant tumors to explore whether pre-existent *BRAF* allelic imbalance is a predictive marker of BRAFi sensitivity.

There were several limitations inherent to the sample set analyzed and methodology used in this study. First, the size of the cohort was too small to define associations between response and alterations in genes that are infrequently altered in melanoma. In addition, the current standard of care for *BRAF*-mutant melanoma has been shifting toward coadministration of BRAFi and MEKi, and *PTEN* alterations may have less effect in patients treated with the combination. A second limitation is that the approach used could not detect changes in the expression of genes such as *PTEN* resulting from epigenetic mechanisms. There was also variability in the duration between collection of the pretreatment sample and the initiation of BRAFi therapy, and the molecular status of some genes may have changed during this interval. In addition, only a single pretreatment tumor site was profiled, and thus, some alterations may not have been detected as a result of intratumoral or lesion-to-lesion tumor heterogeneity. Given these latter challenges to the use of pretreatment tumors, we are now exploring the analysis of tumor-derived DNA in plasma, which may better represent the spectrum of molecular alteration present in individual patients. Finally, broader sequencing methodologies such as whole-genome sequencing may identify predictive biomarkers in genes not included in the capture-based approach used in this study.

In summary, our results suggest that pre-existent mutations in *PTEN* are associated with poor BRAFi response and shorter survival in patients with *BRAF*-mutant melanoma. Additional studies using broader analysis methods and cell-free DNA may identify additional molecular signatures of intrinsic BRAFi resistance that could be used to guide first-line combinatorial strategies.

DOI: https://doi.org/10.1200/PO.16.00054
Published online on ascopubs.org/journal/po on June 23, 2017.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

***PTEN* Loss-of-Function Alterations Are Associated With Intrinsic Resistance to BRAF Inhibitors in Metastatic Melanoma**

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Research Funding: Bristol-Myers Squibb, Immunocore
Travel, Accommodations, Expenses: Bristol-Myers Squibb

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Consulting or Advisory Role: Bristol-Myers Squibb, Genoptix, Merck
Research Funding: Incyte
Patents, Royalties, Other Intellectual Property: Intellectual property and patents pending surrounding use of MHC-II and response to immune therapy

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No relationship to disclose

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No relationship to disclose

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No relationship to disclose

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No relationship to disclose

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No relationship to disclose

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Research Funding: GlaxoSmithKline, Genentech, Bristol-Myers Squibb, Pfizer
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ACKNOWLEDGMENT

We thank Efsevia Vakiani, Gopakumar Iyer, and Tara Soumerai for technical assistance; Cyrus Hedvat for directing the core facility that performed the *BRAF* and *NRAS* typing; Armando Sanchez and Sherie Mar-Chaim for management of the clinical data; Nikolaus Schultz for the uploading of the data on the cBio Cancer Genomics Portal; and Valerio Donato for critical discussion and review of the article.

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Support

Supported by the Melanoma Research Alliance, the National Institutes of Health (NIH), Cycle for Survival, and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology. Also supported in part by the Sloan Kettering Institute for Cancer Research Cancer Center Support Grant No. P30CA008748 and NIH Grant No. K23 CA204726 (to D.B.J.). J.J.H. was supported by a Young Investigator Award from the Conquer Cancer Foundation.

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APPENDIX

Table A1. *PTPRT* Mutations (n = 66)

Patient No.	Mutation	TCGA	No. of Recurrences in COSMIC	Function	Response Grade	Drug
19	R328C	Yes	5	Unknown	Intermediate	BRAFi
28	E324K	Yes	5	Unknown	Intermediate	BRAFi
7	R721C	No	4	Unknown	Excellent	BRAFi
21	P485S	No	1	Unknown	Intermediate	BRAFi
47	N466S	No	1	Unknown	Poor	BRAFi
9	G369D	No		Unknown	Excellent	BRAFi
51	E468K	No		Unknown	Poor	BRAFi
38	P1238L	No		Unknown	Poor	BRAFi
19	X969_splice	No		Inactivating	Intermediate	BRAFi
2	S80F	No		Unknown	Excellent	BRAFi
20	D659N	No		Unknown	Poor	BRAFi
6	L225F	No		Unknown	Excellent	BRAFi
22	R1349C	No		Unknown	Intermediate	BRAFi
21	R999Q	Yes		Unknown	Intermediate	BRAFi
55	P352L	No	2	Unknown	Excellent	BRAFi + MEKi
59	Y860*	No		Inactivating	Intermediate	BRAFi + MEKi
59	Y1431H	No		Unknown	Intermediate	BRAFi + MEKi
60	R359Q	No	1	Unknown	Poor	BRAFi + MEKi
63	E1432K	No		Unknown	NE	BRAFi

Abbreviations: BRAFi, BRAF inhibitor; COSMIC, Catalogue of Somatic Mutations in Cancer; MEKi, MEK inhibitor; NE, not evaluable; TCGA, The Cancer Genome Atlas.

Table A2. *PTPRD* Mutations (n = 66)

Patient No.	Mutation	TCGA	No. of Recurrences in COSMIC	Function	Response Grade	DRUG
28	R1088C	No	3	Inactivating	Intermediate	BRAFi
12	G1819R	No	1	Unknown	Excellent	BRAFi
22	R1496*	No	1	Inactivating	Intermediate	BRAFi
12	G285E	Yes	1	Unknown	Excellent	BRAFi
12	R427Q	Yes	1	Unknown	Excellent	BRAFi
47	H1477Y	No	1	Inactivating	Poor	BRAFi
7	Y1290N	No		Unknown	Excellent	BRAFi
3	P1200S	No		Unknown	Excellent	BRAFi
6	Q508P	No		Unknown	Excellent	BRAFi
28	D1521A	No		Unknown	Intermediate	BRAFi
25	D1521A	No		Unknown	Intermediate	BRAFi
41	X227_splice	No		Inactivating	Poor	BRAFi
12	G1001R	No		Unknown	Excellent	BRAFi
3	E1576K	No		Unknown	Excellent	BRAFi
20	Deletion	Yes		Inactivating	Intermediate	BRAFi
21	Deletion	Yes		Inactivating	Intermediate	BRAFi
35	Deletion	Yes		Inactivating	Poor	BRAFi
42	Deletion	Yes		Inactivating	Poor	BRAFi
64	M754I	No		Unknown	NE	BRAFi

Abbreviations: BRAFi, BRAF inhibitor; COSMIC, Catalogue of Somatic Mutations in Cancer; NE, not evaluable; TCGA, The Cancer Genome Atlas.

Table A3. *KMT2C* Mutations (n = 51)

Patient No.	Mutation	TCGA	Function	Response Grade	Drug
28	S2638F	No	Unknown	Intermediate	BRAFi
30	F4738L	No	Unknown	Intermediate	BRAFi
43	P1570L	No	Unknown	Poor	BRAFi
20	D958N	No	Unknown	Intermediate	BRAFi
21	S931L	No	Unknown	Intermediate	BRAFi
10	S144F	No	Unknown	Excellent	BRAFi
25	Q2462*	No	Inactivating	Intermediate	BRAFi
12	H2563Q	No	Unknown	Excellent	BRAFi
3	P1909S	No	Unknown	Excellent	BRAFi
51	R526C	No	Unknown	Poor	BRAFi
30	L4729V	No	Unknown	Intermediate	BRAFi
48	T107A	No	Unknown	Poor	BRAFi
42	C963W	No	Unknown	Poor	BRAFi
19	Ampl	Yes	Unknown	Intermediate	BRAFi
27	Ampl	Yes	Unknown	Intermediate	BRAFi
55	Q2348E	No	Unknown	Excellent	BRAFi + MEKi
61	E864K	No	Unknown	Poor	BRAFi + MEKi

Abbreviations: Ampl, amplification; BRAFi, BRAF inhibitor; MEKi, MEK inhibitor; TCGA, The Cancer Genome Atlas.

Table A4. *TP53/MDM2* Mutations in the BRAFi Cohort (n = 51)

Patient No.	Mutation	Frequency	Function	Response Grade	Drug
5	V216G	Hotspot	Unknown	Excellent	BRAFi
28	E258Q	Recurrent	Inactivating (does not bind DNA)	Intermediate	BRAFi
10	R342*		Truncating	Excellent	BRAFi
21	S241F	Hotspot	Disrupts binding with BARD1 and CstF1	Intermediate	BRAFi
27	P34F	Recurrent	Unknown	Intermediate	BRAFi
27	H168L	Recurrent	Unknown	Intermediate	BRAFi
42	I251L	Recurrent		Poor	BRAFi
47	R248W	Hotspot	Gain of function (abolishes TS function)	Poor	BRAFi
48	R337C	Recurrent	Unknown	Poor	BRAFi
43	MDM2 S304F		Unknown	Poor	BRAFi
4	MDM2 S196F	Recurrent	Unknown	Excellent	BRAFi
4	MDM2 S160F		Unknown	Excellent	BRAFi
4	*MDM2* ampl			Excellent	BRAFi
36	*MDM2* ampl			Poor	BRAFi
35	*MDM2* ampl			Poor	BRAFi
40	*MDM2* ampl			Poor	BRAFi

Abbreviations: ampl, amplification; BRAFi, BRAF inhibitor; TS, tumor suppressor.