Copy number changes are associated with response to treatment with carboplatin, paclitaxel and sorafenib in melanoma

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Abstract

Purpose—Copy number alterations have been shown to be involved in melanoma pathogenesis. The randomized, phase III clinical trial E2603: carboplatin, paclitaxel, +/− sorafenib (CP vs. CPS) offers a large collection of tumor samples to evaluate association of somatic mutations, genomic alterations, and clinical outcomes, prior to current FDA approved therapies.

Materials and Methods—Copy number and mutational analysis on 119 pretreatment samples was performed.

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Results—CPS therapy was associated with improved PFS compared to CP in patients with tumors with *RAF1* (cRAF) gene copy gains (HR=0.372, P=0.025) or *CCND1* gene copy gains (HR=0.45, P=0.035). CPS therapy was associated with improved OS compared to CP in patients with tumors with *KRAS* gene copy gains (HR=0.25, P=0.035). *BRAF* gene copy gain and *MET* amplification were more common in samples with V600K vs V600E mutations (P<0.001), which was validated in the TCGA data set.

Conclusion—We observed improved treatment response with CPS in melanoma patients whose tumors have *RAF1* (cRAF), *KRAS* or *CCND1* amplification, all of which can be attributed to sorafenib targeting CRAF. These genomic alterations should be incorporated in future studies for evaluation as biomarkers.

Keywords

Carboplatin; Copy Number Changes; Metastatic Melanoma; Paclitaxel; Sorafenib

INTRODUCTION

Despite recent improvements in the treatment of metastatic melanoma, it remains the deadliest form of skin cancer. In addition to tumor specific somatic mutations (e.g. in *BRAF*, *NRAS*, *KIT*), copy number alterations, both gains and losses, are thought to play integral roles in melanoma pathogenesis. Global genomic gain of chromosomes 1, 6p, 7, 17q, and 20 and loss of chromosomes 4, 6q, 9, 10, 11, 13, 16, and 18 have been observed [1-10]. Gains of chromosome 7 and loss of chromosome 10 have been associated with melanomas with *BRAF* mutations, whereas loss of chromosome 11 has been associated with melanomas with *NRAS* mutations [3,6,8,9]. Furthermore, a number of treatment options have been FDA approved in recent years, including both immunotherapies and targeted therapies. However, there is still a need to identify effective mechanisms to stratify patients to optimize treatment decision and improve clinical outcomes with many studies evaluating the use of biomarkers in the selection of patients for appropriate therapies. Despite the expansion of correlative studies, currently most cannot discriminate between the identification of predictive or prognostic biomarkers. In part, this issue is due to significant advances both in technologies since natural history studies were done, and revolutionary changes in therapies [11,12].

Prior to the development of the targeted mutant BRAF inhibitors, vemurafenib (Zelboraf, Genentch) and dabrafenib (Tafinlar, GlaxoSmithKline) [13,14], sorafenib was used in clinical trials in order to attempt to inhibit the MAPK signaling pathway and target angiogenesis. Sorafenib (Nexavar, Bayer Pharmaceuticals) is an oral multikinase inhibitor, including RAF kinases, BRAF and CRAF [15-17], approved by the U.S. Food and Drug Administration (FDA) for the treatment of renal cell carcinoma, hepatocellular carcinoma, and thyroid cancer [18-21]. E2603 was a randomized phase III clinical trial investigating carboplatin, paclitaxel, +/− sorafenib in advanced stage melanoma patients, and demonstrated no difference in clinical outcome with the addition of sorafenib to chemotherapy in unselected melanoma populations [22-24]. However, our recent observations suggest that melanoma patients whose tumors carry *NRAS* mutations may benefit from targeting CRAF. Patients with *NRAS* mutant melanoma with chemotherapy alone had poorer responses as compared to patients with *BRAF* mutant and WT melanoma, and the addition of sorafenib to chemotherapy

improved treatment responses to a level similar to those observed in patients with *BRAF* mutant and WT melanoma in E2603 [25].

In the current study, we used pretreatment tumor samples from patients enrolled on E2603 to explore whether copy number alterations were associated with somatic mutations and clinical outcome in patients with melanoma. E2603 provides a large, clinically annotated dataset, treated prior to the current FDA approved therapies, which can be used to evaluate associations with clinical outcome and discriminate between predictive and prognostic biomarkers for melanoma.

MATERIALS AND METHODS

Patients

Patients were enrolled on the double-blind phase III ECOG 2603 clinical trial and randomized to receive carboplatin/paclitaxel (CP, control arm) or carboplatin/paclitaxel plus sorafenib (CPS, experimental arm) as detailed in Flaherty et al [22]. Dosing was carboplatin at area under the curve (AUC) of 6 and paclitaxel at 225 mg/m2 every three weeks, and sorafenib at 400mg orally twice daily for days 2-19 of every 21-day cycle. Trial enrollment required confirmed diagnosis of unresectable or metastatic melanoma, excluding uveal melanoma and patients with brain metastases. Eligibility criteria also included age greater than 18, ECOG performance status (PS) of 0 or 1, measurable disease, and normal baseline laboratory studies. Patient demographics, disease characteristics, and treatment history were all documented including disease stage, primary tumor site, numbers of involved sites, age at diagnosis, ECOG PS, Breslow thickness, ulceration, and lactate dehydrogenase (LDH).

Melanoma tumor samples and Tumor genotyping

Tumor samples from patients enrolled on E2603 were genotyped as described in Wilson et al [25]. From the 179 tumor samples which were genotyped, 20 samples had inadequate DNA to undergo labeling and 40 samples failed multiple attempts at labeling, most likely due to decreased DNA integrity or inhibition of the reaction by melanin. In total, 119 tumor samples were labeled and underwent copy number analysis.

Copy number and genomic instability analysis

Tumor DNA was labeled using BioPrime® Array CGH Genomic Labeling System (Life Technologies, Grand Island, NY) according to manufacturer's instructions. Array-based comparative genomic hybridization (aCGH) and data analysis was performed as in [26] using the Agilent SurePrint G3 Human CGH 2×400K M microarrays following manufacturer's instructions. Extracted data were analyzed using BioDiscovery's Nexus 7 copy-number software (Nexus Genomics Inc.). Copy number variation was assessed using Nexus 7 and gene mapping was done to hg19, Feb 2009 build. Copy number gain was defined as log2 scale value > 0.3 and loss as log2 scale value < -0.3, with at least three contiguous probes needed to call a gain or loss. High copy gains were defined as log2 scale value > 1.14, and homozygous loss as log2 scale value < -1.1. We measured genomic instability as the number of non-diploid copy number changes in each tumor sample. *BRAF* and *MET* mutation and copy number data were downloaded from the public TCGA data repository

website of the Broad Institute (http://gdac.broadinstitute.org). We used Level 4 GISTIC copy number data in the analysis.

Statistical analysis

Along with analysis of global copy number gains and losses, copy number changes of 26 genes (Supplemental Table 1) known to be involved in melanoma pathogenesis were analyzed, and correlation between gene copy number gains and losses and outcomes were performed. Statistical analysis was performed as in Wilson et al [25]. The Fisher's exact test was used to compare gene CNV by patients' demographic and disease characteristics.. Kaplan-Meier methods were used to estimate the distribution of OS and PFS. Cox proportional hazards models were used to test prognostic and predictive value of CNV for each gene. The same set of covariates were adjusted in all multivariable Cox models, including age, gender, race, AJCC stage, ECOG PS, prior therapy, number of involved sites, and LDH. Pearson's Chi-squared tests were used to analyze TCGA *BRAF* and *MET* copy number data. Due to the exploratory nature of the study, no adjustment was made for multiple comparisons. All tests were two-sided and $p<0.05$ was considered statistically significant. All analysis was conducted using STATA 11.2 [27].

RESULTS

Overall copy number analysis

One hundred and nineteen pretreatment tumor samples from patients on E2603 had sufficient tumor tissue for CNV analysis for 26 genes via aCGH. The demographics and disease characteristics of the 119 patients were similar to those patients who did not have DNA samples available for or who failed aCGH analysis (Supplemental Table 2). Moreover, no differences were observed in clinical outcomes in patients with samples for analysis and those without (Supplemental Table 3). As no difference was seen in the overall outcome of the study, treatment arms were collapsed for analysis about prognostic markers. In these 119 tumor samples, 45% (54/119) had BRAF mutations, 24% (28/119) had NRAS mutations, and 31% (37/119) had neither, designated as WT (Figure 1A), consistent with the entire sample set from E2603 (Wilson, Zhao et al. 2014), as well as many other genetic studies of melanoma [28-30].

Genomic alterations were evaluated in all tumor samples and frequency of copy gains and losses across the entire genome were identified (Supplemental Figure 1). We observed global genomic gain of chromosomes 1, 6p, 7, 17q, and 20 and loss of chromosomes 4, 6q, 9, 10, 11, 13, 16, and 18. Genomic instability was also assessed in all tumor samples, but no correlation was identified between genomic instability (non-diploid genome) and patient characteristics, treatment arms, or clinical outcomes (data not shown). As has been previously reported [1-10], we identified regions throughout the genome which demonstrated similar copy gains and copy losses in all melanoma tumor samples (e.g. chromosomes 1 and 6). We also observed genomic regions which exhibited differences in copy gains and losses in tumor samples stratified by somatic mutation cohorts, such as chromosome 7.

We next evaluated copy gains and losses in an initial set of 26 genes known to be involved in melanoma pathogenesis (Figure 1B). *BRAF*, *CDK4*, and *MDM4* were found exclusively either as being diploid or copy gains. Conversely, the tumor suppressors, *CDKN2A* and *PTEN*, were identified mainly either as diploid or having copy loss, with rare copy gains identified. *CDK4* copy number gain was more frequent in tumor samples with *BRAF* mutation (31/54, 57.4%) compared to tumors with *NRAS* mutation (9/28, 32.1%) or WT (15/37, 40.5%) (P=0.066). Moreover, *PTEN* copy number loss was more frequent in tumor samples with *BRAF* (28/54, 51.9%) and *NRAS* (13/28, 46.4%) mutations compared to WT (10/37, 27.0%) (P=0.051). These comparisons did not reach statistical significance due to small sample size.

Association of BRAF amplification with BRAF somatic mutations and clinical outcome

We observed gains of chromosome 7, most notably in the region around *BRAF* (7q34); *BRAF* gene amplification was present in 66% of the tumor samples (Figure 1B). In tumor samples with *BRAF* mutations, 82% had *BRAF* copy gains, compared to 64% of samples with *NRAS* mutations or 46% of samples that were WT (P=0.002) (Supplemental Table 4). Using Kaplan-Meier analysis in all samples, the presence of *BRAF* amplification was significantly associated with decreased PFS (median PFS 6.3 vs. 3.9 months for *BRAF* diploid and copy gain, logrank P=0.023) and OS (median OS 11.0 vs. 8.5 months respectively, logrank P=0.046) (Figure 2A and 2B), regardless of BRAF mutation status. The associations were no longer statistically significant after adjusting for patient characteristics in Cox models (HR=1.52, 95% CI: 0.97, 2.37, P=0.067 for PFS, HR=1.16, 95% CI: 0.73, 1.86, P=0.53 for OS).

Copy number aberrations associated with response to sorafenib

Twenty-six pre-selected genes known to play a role in melanomagenesis were evaluated individually for their predictive values to explore whether sorafenib could provide survival benefit in any subgroups. In patients with *KRAS* copy gain, CPS was associated with better OS compared to CP (adjusted HR=0.25, 95% CI:0.07, 0.91, P=0.035) (Figure 3 A and B). In patients with *CCND1* or *RAF1* copy gain, CPS was associated with better PFS compared to CP (CCND1: adjusted HR=0.45, 95% CI:0.22, 0.95, P=0.035 (Figure 3 C and D), RAF1: adjusted HR=0.37, 95% CI: 0.16, 0.88, P=0.025 (Figure 3 E and F) . Figure 3 demonstrates the altered outcomes associated with copy number changes of *KRAS*, *CCND1* and *RAF1* in each treatment arm. For all three genes, patients whose tumors carried copy gains had worsened outcomes on CP treatment, but improved outcomes with CPS treatment. Results from copy number changes in remaining genes did not identify significant alterations in PFS or in outcomes in response to treatment arms (data not shown).

RAF1 was found to be amplified in 29% of tumor samples (Figure 1B) and *RAF1* amplification was associated with worse performance status (PS) compared to diploid or copy loss (p=0.002) (Supplemental Table 5). We recently demonstrated a trend towards improved response with the addition of sorafenib to carboplatin and paclitaxel in patients with *NRAS* mutant melanoma [25]. Therefore, we performed a conjoined analysis of *NRAS* somatic mutation status and *RAF1* (cRAF) copy gain. We evaluated the effect of *NRAS* somatic mutations, *RAF1* copy gains, both, or neither on PFS and OS. Our results

demonstrate that there is no significant association of these variables, either alone or in combination, on PFS or OS, independent of treatment arm (Supplemental Figure 2). In patients without a *NRAS* mutation, *RAF1* copy gain remained predictive of improved response to treatment with CPS compared to CP (N=90, HR=0.27, p=0.018). In patients with a *NRAS* mutation, *RAF1* copy gain was no longer statistically significantly predicting response to CPS treatment, however, the sample size was small (N=25, HR=0.43, 95% CI: 0.03, 6.04, p=0.532). We did not observe a correlation between *RAF1* amplification and *NRAS* mutations in our sample set, which is confirmed in TCGA melanoma data as well (www.cbioportal.org).

Associations with BRAF V600K tumor samples

During our analysis of copy number changes within the genome and specific genes of interest, we noted differences between the subsets of tumor samples with the *BRAF* V600E and V600K mutations. We observed a focal amplification of 7q34 in tumor samples with V600E mutations; however, we noted a more global amplification pattern along the q-arm in tumor samples with V600K mutations (Figure 4A). Thus, we further evaluated copy number changes in association with specific *BRAF* V600 mutations. We found that the *BRAF* gene was amplified in all *BRAF* somatic mutation cohorts, but amplification was greater in samples with V600K vs V600E mutations (p<0.001) (Supplemental Figure 3A). Given our observation, we did a global analysis of copy number changes and identified an increase in copy gains of *MET*, another gene located on chromosome 7 (7q31) in tumor samples with *BRAF* somatic mutations (59%) compared to *NRAS* somatic mutations (29%) and WT (38%) (P=0.03). Additionally, although *MET* was found to be amplified in both V600E and V600K mutation cohorts, we found a 2-fold increase in amplification in V600K vs. V600E (p<0.05) (Supplemental Figure 3B).

We then independently validated our finding using data from the cutaneous melanoma TCGA. We found that *BRAF* CN (GISTIC +2) levels were higher in *BRAF* V600K (17%) compared to V600E/R or K601E (13%), atypical mutants (0%) or wild-type (2%) samples (P=0.002) (Figure 4B). We demonstrated differential *MET* amplification in somatic mutation cohorts with copy gains observed in 76% of BRAF mutant, 27% of NRAS mutant, and 51% of WT melanoma tumor samples (P=0.003). Furthermore, *MET* copy gains were increased in V600K samples, as compared to V600E. It was observed that *MET* CN (GISTIC +2) levels are higher in *BRAF* V600K (17%) compared to V600E/R or K601E (6%), atypical mutants (0%) or wild-type (2%) samples (P=0.075) (Figure 4C).

DISCUSSION

We investigated the association between copy number alterations, point mutations and clinical outcome in melanoma patients treated on the E2603 randomized, phase III clinical trial of carboplatin, paclitaxel, +/− sorafenib. Copy number alterations were identified across the entire genome in the melanoma tumor samples. Similar copy gains in chromosome 1 and 6p and loss in chromosome 6q were observed in all tumor samples, although differential copy gains of chromosomes 7, 8, and 17 and copy loss of chromosome 10 were seen associated with specific somatic mutation sub-sets. As in other studies, which primarily

analyzed copy number in melanoma cell lines and a small number of tumor samples, we observed global genomic gain of chromosomes 1, 6p, 7, 17q, and 20 and loss of chromosomes 4, 6q, 9, 10, 11, 13, 16, and 18 [2,3,5,6,8-10]. In addition, we observed a similar rate of copy number changes of individual genes including, amplifications of *BRAF*, *CCND1*, *CDK4*, *MDM2*, and *MET* and deletions of *CDKN2A* and *PTEN*, as well as copy number changes in additional genes including *AKT1*, *MAP2K2*, *RAC1* and *RAF1* [3,8,10]. Our results did not identify significant amplifications in *NOTCH2*, as were previously identified [3,8] nor global changes associated with chromosomes 8q34 and 11q13 [4]. Gains at 8q34 and 11q13 were previously identified using fluorescence *in situ* hybridization probes specific for genes encoded at these loci which can account for the differences in observations. We also detected specific copy number changes specific to the different somatic mutation cohorts, which have been previously observed, including gain on chromosome 7 and loss on chromosome 10q as more frequent in tumor samples with *BRAF* mutations [3,6,9] and loss on chromosome 11q was more frequent in tumor samples with *NRAS* mutation [6,9]. Our study results provide information regarding genetic alterations in a large collection of melanoma tumor samples, adding to prior work evaluating genetic aberrations identified in melanoma cell lines.

We observed that *BRAF* copy number gain, regardless of mutation status, was associated with worsened clinical outcomes, with decreased PFS and OS in patients, as determined by univariate analysis, although this association was no longer statistically significant in multivariate analysis. Acquisition of *BRAF* amplification at varying levels of copy gain (between 3-75) have recently been identified to play a role in treatment resistance to BRAF and MEK inhibitors, either alone or in combination, in melanoma [1,31-33]. Additionally, copy number gains of *BRAF* have been shown to be associated with decreased response to BRAF inhibition [26]. Thus, it has been postulated that copy gain of *BRAF* is a predictive marker (i.e. intrinsic copy gain or acquired amplification is associated with response to BRAF inhibition). Our data suggest that BRAF copy gain may predict for poor clinical outcome as compared to diploid. It may be that some of the prior studies suggesting that *BRAF* copy number gain is predictive of outcome upon treatment with BRAF inhibition were uncovering a prognostic association that was not well delineated prior to the era of BRAF targeted therapy. With advances in technology and increased attention to *BRAF* amplification as potential biomarker of response, we are now able to make this observation. However, this finding should not be taken to imply that high level *BRAF* amplification, acquired upon treatment with BRAF inhibition, is not associated with resistance to therapy [1].

We found differential amplification of the *BRAF* gene, with increased copy gains associated with V600K mutations, as compared to V600E mutations. We also observed an association between the *BRAF* V600K mutation and amplification of *MET*, located proximal to *BRAF* on chromosome 7. Both associations were validated in the TCGA data set, which shows the same pattern of increased amplification of *BRAF* V600K mutations, which also correlates with *MET* amplification. It is known that *BRAF* V600K mutations occur in older patients and in patients with chronic sun damage [30], which we also observed in our recent analysis of this E2603 patient cohort [25]. Although our sample set was too small to formally evaluate an association between V600K mutations and genomic instability, prior data suggest that V600K mutations may be associated with more chromosomal abnormalities [29,34].

Amplification of *MET* may be associated with treatment resistance to BRAF inhibitors, or the combination of BRAF and MEK inhibitors, particularly since studies have demonstrated that the HGF/MET pathway has been implicated in the development of treatment resistance to BRAF inhibitors [35,36], as well as in lung cancer [37,38].

Our data suggest that *RAF1* copy number gain, independent of *NRAS* mutation status, is predictive of benefit from treatment with CPS. We found that *RAF1* was amplified in 29% of our samples; in the TCGA cohort, it is gained in 16% of cases (45/278) increasing amplification correlates with increased expression (Pearson correlation 0.67) (cbioportal.org). Increased levels of CRAF lead to upregulation of the MAPK signaling pathway, known to be critically important in melanomagenesis [39]. We postulate that the addition of sorafenib, a known CRAF inhibitor [15,16], to chemotherapy targets MAPK signaling resulting from increased levels of CRAF. Not surprisingly, the inhibition of the MAPK signaling pathway through CRAF appears to result in improved PFS. The improved response to treatment with CPS in this patient cohort is consistent with our previous finding that treatment with CPS increased treatment response in melanoma patients whose tumors had a *NRAS* mutation [25], as *NRAS* mutants have increased signaling through the MAPK pathway through CRAF. These results are reminiscent of those observed with addition of monoclonal antibodies trastuzimab, in breast cancer with HER2 overexpression [40], and panitumumab, in *KRAS* wild-type metastatic colorectal cancer [41]. In these studies, distinct improvement in PFS and OS was observed in select patient cohorts, despite otherwise poor responses to standard therapies, with the addition of these monoclonal antibodies, respectively. Furthermore, improved OS with CPS treatment was identified in patients with *KRAS* amplification, which also signals through CRAF, strengthening the importance of CRAF inhibition in select melanoma patient cohorts. In the TCGA database, *KRAS* has been shown to be altered in 3% of melanoma tumor samples. Although it is unlikely that further clinical investigation will continue with sorafenib as an agent in melanoma, these results suggest that targeting of CRAF in melanoma, as defined by *RAS*-mutant or *RAF1* amplification, may provide alternative treatment options for these select group of patients. As large scale sequencing of tumor samples becomes standard of care, this approach may become more feasible in the near future. Currently, there are ongoing clinical trials investigating pan-RAF inhibitors, as single agents or in combination with MEK inhibitors or alternative pathway inhibitors (www.clinicaltrials.gov). Correlative studies in association with these trials will be critical to identify determinants of response, and we would predict that patients with *RAS*-mutant or *RAF1* amplification would demonstrate improved response upon treatment with these agents. Interestingly, in the TCGA data, 13% of papillary thyroid cancers and 2.6% of liver cancers have *RAS* mutations. One mechanism by which sorafenib may be effacious in these cancers, may be through inhibition of MAPK signaling through CRAF.

We observed that amplification of *CCND1* predicted for improved PFS with treatment with CPS. These results differ from prior studies in melanoma cell lines showing that that Cyclin D1 amplification may contribute to resistance to mutant BRAF inhibitors in melanoma [42]. Additionally, recent analyses of patient tumor samples also have identified an association of *CCND1* copy number gain and decreased PFS upon treatment with BRAF inhibitors [26]. Hweover, prior studies have demonstrated that Cyclin D1 is downregulated by sorafenib. In

both hepatocellular carcinoma and prostate cancer cells, sorafenib demonstrated anti-proliferative effects through inhibition of the MAPK pathway, shown by decreased MEK and ERK phosphorylation [43,44]. These effects of sorafenib were associated with increased apoptosis, along with increases in caspase-3, as well as decreased levels of Cyclin D1 upon treatment with drug [43,44]. We postulate that as sorafenib is an inhibitor which targets multiple kinases [15,16]; unlike specific mutant BRAF inhibitors, one of its effects is the downregulation of CCND1, as was observed in a hepatocellular carcinoma cell lines [17]. Further studies are needed to determine the mechanism by which the addition of sorafenib to chemotherapy results in improved PFS in melanoma patients whose tumors have *CCND1* amplification.

There are several limitations to our current study, including sample size. Initial clinical trial enrollment comprised 823 patients; however, tumor samples were not available for all patients and there were further technical limitations precluding aCGH from being done on all available samples. Nevertheless, the 119 tumors which underwent aCGH were representative of the overall clinical trial patient population. Analysis of tumor samples from the E2603 clinical trial, done prior to the implementation of the current FDA approved regimens of targeted therapy and immunomodulatory immunotherapies, provides an opportunity to evaluate prognostic markers for melanoma. Moreover, despite the fact that E2603 did not stratify patients on enrollment and did not demonstrate a difference between the two treatment arms, CP vs. CPS, we were able to identify markers associated with sorafenib as predictive of response to treatment with CPS, and which importantly, serve as a guide for analysis of future trials. In the era of targeted therapy and continual development of new treatment options, it is imperative to identify biomarkers which select patients who will benefit from particular treatments. The results of this study reinforce the critical value of correlative studies, even in negative clinical trials. Lastly, multiple comparisons are not adjusted in the study due to its exploratory nature.

In conclusion, our analysis of this large cohort of tumors unselected for somatic mutations and genomic alterations provides important information on copy number changes associated with treatment outcome. BRAF gain may be a potential prognostic biomarker, which should be investigated further in future clinical trials. In addition, *RAF1* amplification predicted for improved response to CRAF inhibition, as did *CCND1* and *KRAS* amplification, supporting the hypothesis that signaling thru CRAF is important in select melanoma cohorts. We also observed *MET* amplification associated with BRAF somatic mutation, and specifically increased amplification in BRAF V600K mutation cohorts, results consistent with TCGA data. These copy number changes suggest potential biomarkers that may be important in identifying prognostic markers, markers of response to treatments, or markers of mechanisms of resistance and should be evaluated further in future studies. Finally, *RAS* mutations have been identified in papillary thyroid cancer and *RAF1* is commonly amplified in a number of cancers, including bladder cancer, that are treated with sorafenib suggesting that our results and observations may have implications in other tumor types.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Translational Relevance

We present correlative studies for E2603, the randomized ECOG phase III clinical trial. Patients with advanced stage melanoma were randomized to carboplatin and paclitaxel, with and without sorafenib (CPS vs. CP), prior to current immunotherapies and *BRAF* targeted therapies. Although in the clinical trial, no overall benefit was demonstrated for CPS over CP, in the correlative studies, we identified three markers associated with significantly improved clinical outcomes upon CPS treatment. These copy number aberrations, including *RAF1* (cRAF) itself, a target of sorafenib, have been associated with changes in MAPK signaling through cRAF. Identification of these markers expands upon mechanisms to stratify patients for benefit from targeted therapies, and has implications for development of pan-RAF inhibitors. Moreover, this study emphasizes the importance of correlative studies, even in the setting of a negative clinical trial, which can identify sub-sets of patients that respond to therapy.

## Somatic Mutations

A.

B.
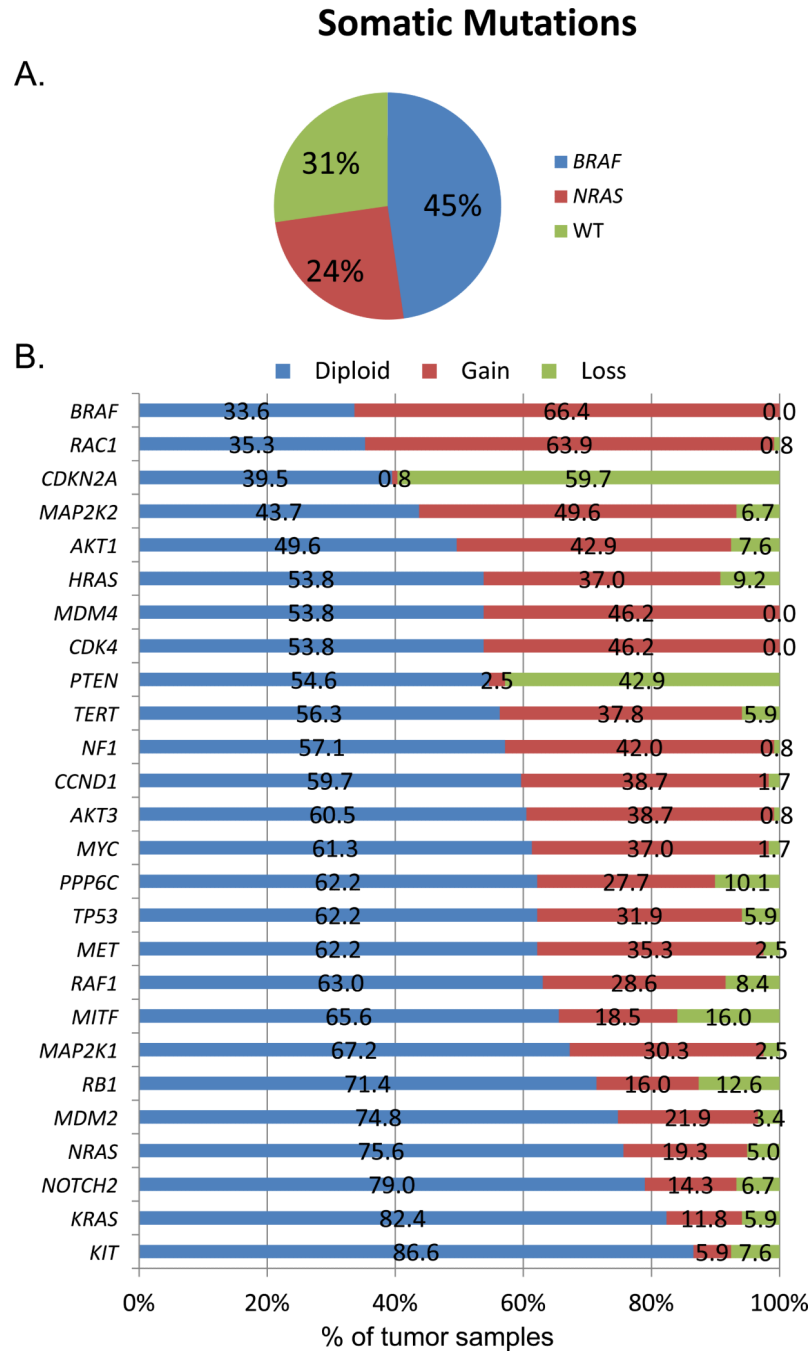
Figure 1. Somatic Mutations and Copy Number Changes in Melanoma Tumor Samples
A. Genotype of melanoma tumor samples evaluated using array Comparative Genomic Hybridization (aCGH). WT – wildtype.

B. Aggregate copy numbers of specified genes in melanoma tumor samples. The genes evaluated are listed on the left and the legend, shown on the top, depicts copy number status, demonstrating diploid, gain, or loss, in tumor samples. Numbers represent percentage of tumors with indicated copy numbers.

BRAF
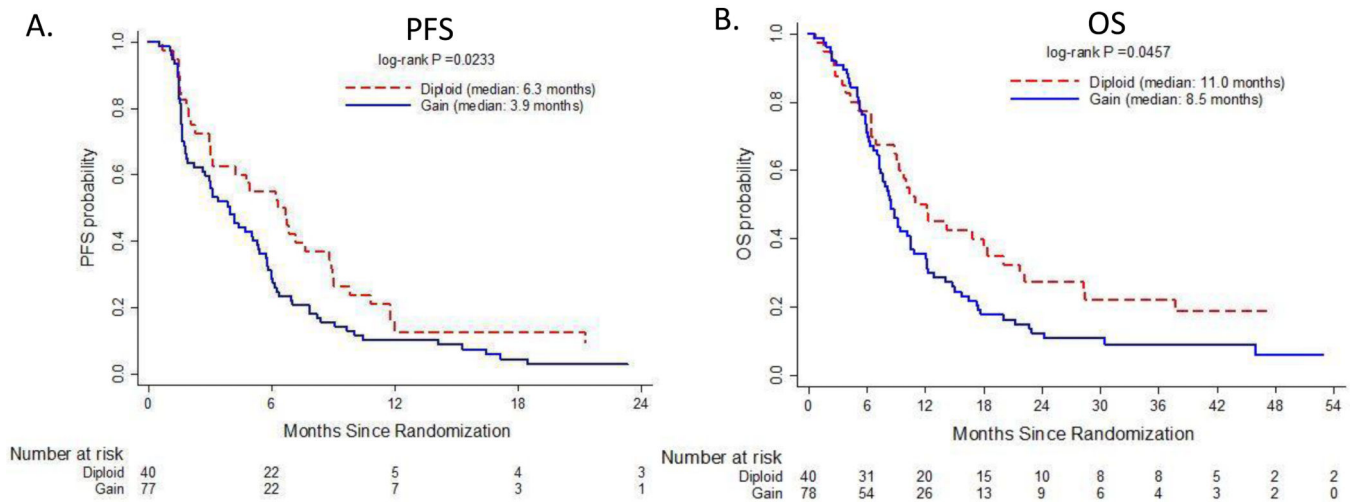
Figure 2. *BRAF* gene amplification is associated with somatic mutations and worse clinical outcomes

Kaplan-Meier curves of progression free survival (PFS) (A) and overall survival (OS) (B) based on *BRAF* gene copy number status, diploid versus copy gain. Median PFS for *BRAF* diploid and copy gain, 6.3 (95% CI: 3.0, 8.8) months and 3.9 (95% CI: 2.6, 5.3) months (P=0.023), respectively (A). Median OS for BRAF diploid and copy gain, 11.0 (95% CI: 8.8, 18.4) months and 8.5 (95% CI: 7.3, 10.4) months (P=0.046), respectively (B).
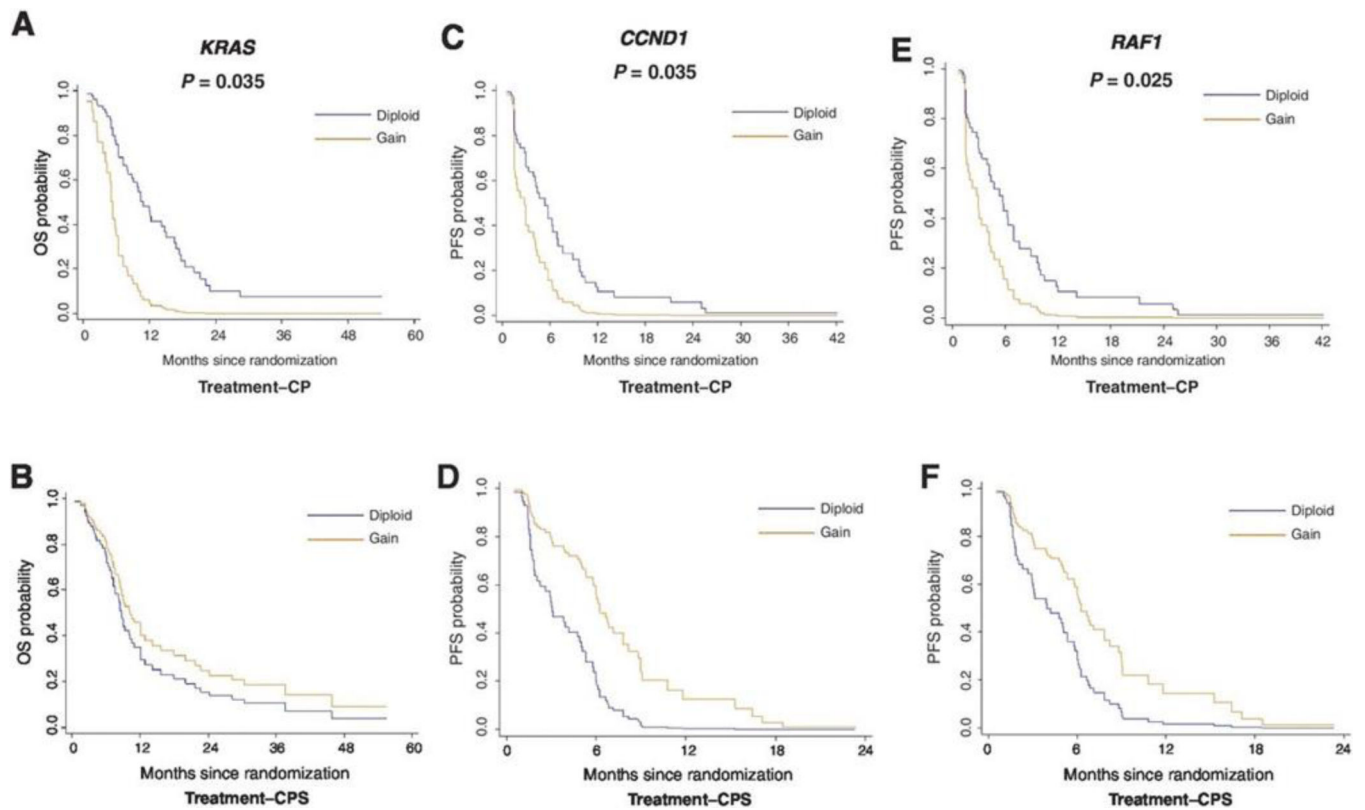
Figure 3. Association of gene copy number changes in treatment arms

Adjusted survival curves from Cox models for gene copy number changes in each treatment arm. Gene copy changes are *KRAS* (A, B), *CCND1* (C,D), and *RAF1* (cRAF) (E,F). Adjusted covariates include age, gender, race,AJCC status, ECOG performance status, prior treatment, number of involved sites and LDH. CP – carboplatin and paclitaxel; CPS – carboplatin, paclitaxel, and sorafenib.
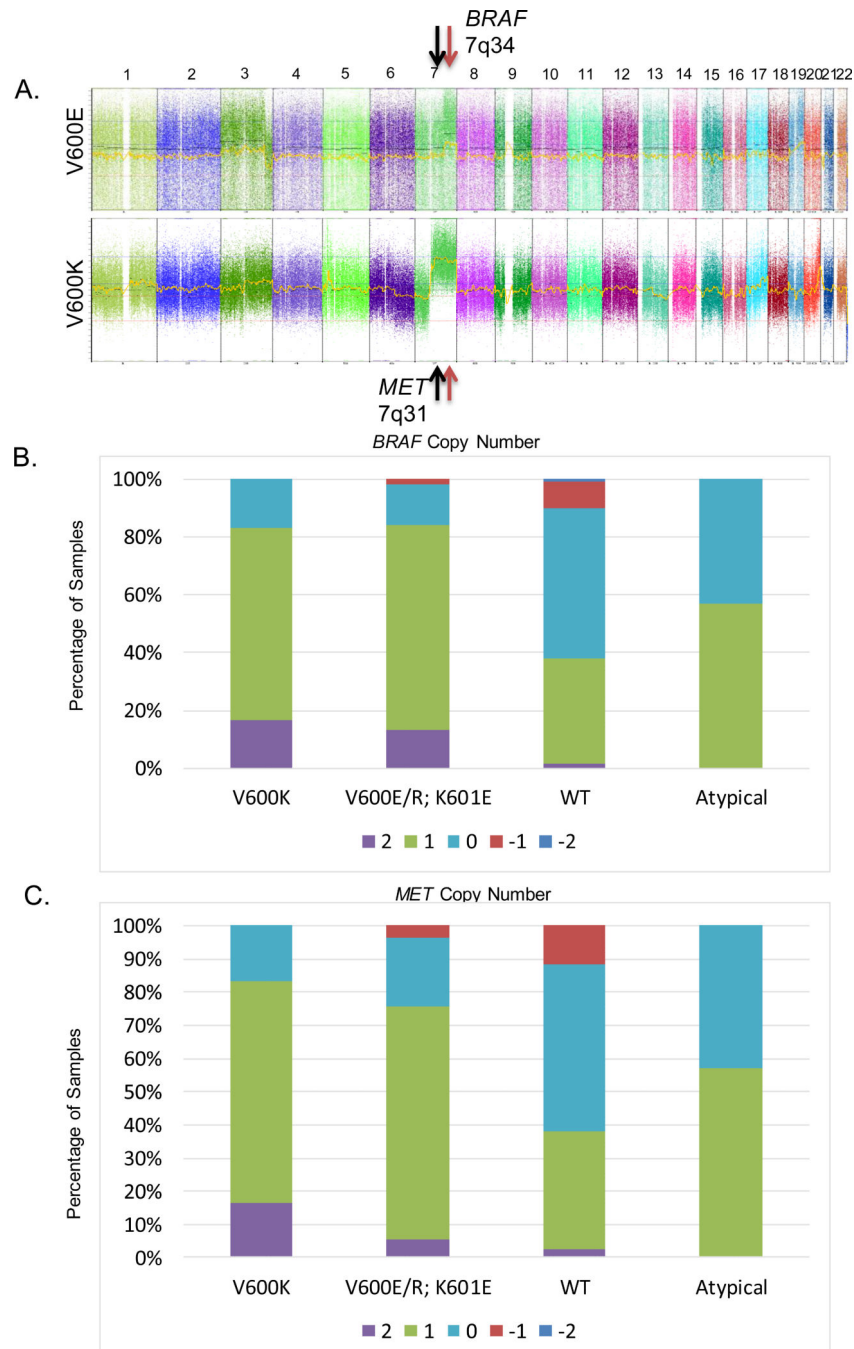
Figure 4. *BRAF* and *MET* gene amplifications are associated with *BRAF* V600K mutation cohort
A. Representative plots of copy gains and losses in tumor samples with *BRAF* V600E and *BRAF* V600K mutations. *BRAF* and *MET* genomic locations are denoted with arrows. Numbers across the top designate chromosomes.

B. *BRAF* copy number from TCGA data in designated somatic mutation cohorts. X-axis, Designated somatic mutation cohorts. Y-axis, percentage of samples. Legend designates GISTIC copy number (CN) where +2 is high level copy gain, +1 is copy gain, 0 is diploid, −1 is copy loss, and −2 is homozygous loss.

C. *MET* copy number from TCGA data in designated somatic mutation cohorts. X-axis, Designated somatic mutation cohorts. Y-axis, percentage of samples. Legend designates GISTIC copy number (CN) where +2 is high level copy gain, +1 is copy gain, 0 is diploid, −1 is copy loss, and −2 is homozygous loss.