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Clinical, molecular and immune analysis of dabrafenib and trametinib in metastatic melanoma patients that progressed on BRAF inhibitor monotherapy: a phase II clinical trial:

CombiDT in BRAF inhibitor refractory melanoma patients

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Abstract

Importance—Combined treatment with dabrafenib and trametinib (CombiDT) achieves clinical responses in only ~15% of BRAF inhibitor (BRAFi)-refractory metastatic melanoma patients, in contrast to the high activity observed in BRAFi-naïve patients. Identifying correlates of response and mechanisms of resistance in this population will facilitate clinical management and rational therapeutic development.

Objective—To determine correlates of benefit from CombiDT therapy in BRAFi-refractory metastatic melanoma patients.

Design—Single-center, single-arm prospective phase II study of CombiDT in patients with *BRAF*^{V600} metastatic melanoma resistant to BRAFi monotherapy conducted between September 2012 and October 2014.

Setting—University of Texas MD Anderson Cancer Center.

Participants—28 patients were screened and 23 enrolled. Key eligibility criteria included: *BRAF*^{V600} metastatic melanoma, prior BRAFi monotherapy, measurable disease (RECIST 1.1), and accessible tumor for biopsy.

Intervention—Patients were treated with dabrafenib (150 mg twice daily) and trametinib (2 mg daily) continuously until disease progression or intolerance. All participants underwent a mandatory baseline biopsy, and optional biopsies were performed on-treatment and at progression. Whole-exome sequencing, RT-PCR for *BRAF* splicing, RNAseq and IHC were performed on tumor samples, and blood was analyzed for levels of circulating *BRAF*^{V600}.

Main outcome measures—Primary endpoint was overall response rate (ORR). Progression-free survival (PFS) and overall survival (OS) were secondary clinical endpoints.

Results—Among evaluable patients, the confirmed ORR was 10%, disease control rate (DCR) was 45%, and median PFS was 13 weeks. Clinical benefit was associated with duration of prior BRAFi >6 months (DCR 73% vs. 11% for ≤6 months, *p*=0.02) and decrease in circulating *BRAF*^{V600} at day 8 of cycle 1 (DCR 75% vs. 18% for no decrease, *p*=0.015), but not by pre-treatment MAPK pathway mutations or activation. On-treatment biopsies demonstrated that CombiDT failed to achieve significant MAPK pathway inhibition or immune infiltration in most patients.

Conclusions and relevance—The baseline presence of MAPK pathway alterations was not associated with benefit from CombiDT in BRAFi-refractory metastatic melanoma patients. Failure to inhibit the MAPK pathway provides a likely explanation for the limited clinical benefit of

CombiDT in this setting. Circulating BRAF^{V600} is a promising early biomarker of clinical response.

Introduction

Approximately 50% of cutaneous melanomas harbor a somatic BRAF^{V600} mutation that activates the MAPK signaling pathway.^{1,2} Two mutant-selective BRAF inhibitors (BRAFi), dabrafenib and vemurafenib, that have been FDA-approved for treating BRAF^{V600} metastatic melanoma achieve RECIST-criteria responses in ~50% of patients and disease control in ~90%.^{3,4} However, the development of resistance is nearly inevitable, and the median duration of response for these agents is ~6 months.

Multiple molecular changes that cause reactivation of MAPK pathway signaling have been identified in melanomas with acquired resistance to BRAFi. These include alternate BRAF splicing or amplification,^{5–7} NRAS and MEK mutations,^{8–11} and COT overexpression.¹² Resistance can also be mediated by epigenetic upregulation of receptor tyrosine kinases (RTKs).⁸ Preclinical studies have demonstrated that combining BRAFi and MEK inhibitors (MEKi) is often able to both overcome such resistance mechanisms and forestall acquired resistance.^{8,9,12–14} In addition, there is growing evidence that the anti-tumor immune response is important to the clinical activity of BRAFi.^{4,15,16}

Clinical studies have demonstrated that combined BRAFi and MEKi (BRAFi+MEKi) therapy in BRAFi-naïve BRAF^{V600}-mutant melanoma patients achieves response rates of ~70%, disease control rates (DCR) of >90%, and median progression-free survival (PFS) of 10–12 months.^{3,17} However, in BRAF^{V600}-mutant melanoma patients who have developed resistance to BRAFi, the response rate to BRAFi+MEKi is only ~15%.¹⁸ It is unclear which BRAFi-refractory patients would likely benefit from BRAFi+MEKi. Further, it is unknown why the combination has low activity in this setting.

We conducted a prospective phase II trial of dabrafenib (BRAFi) and trametinib (MEKi) combination (CombiDT) therapy in metastatic melanoma patients who previously progressed on single-agent BRAFi. Biospecimens collected as part of this trial underwent integrated multi-platform analysis of tumor biopsies and serum specimens to elucidate molecular mechanisms of resistance and correlates of response. We report here the clinical outcomes and molecular and immunological effects of CombiDT in these patients.

Methods

Clinical Trial

We conducted an IRB-approved, open-label phase II study of CombiDT in patients with BRAF^{V600}-mutant metastatic melanoma resistant to BRAFi monotherapy. Patients were recruited from a tertiary cancer hospital. Inclusion criteria for this study included age ≥16; ECOG performance status 0–2; histologically confirmed stage IV or unresectable stage III melanoma with a BRAF^{V600} mutation; disease progression on single-agent vemurafenib or dabrafenib; measurable disease, defined by RECIST 1.1;¹⁹ at least 1 accessible tumor for

biopsy; adequate organ function; no active brain or leptomeningeal metastasis within 4 weeks; and no history of significant cardiac or ocular illness.

Written informed consent was obtained. Patients were treated with dabrafenib (150 mg twice daily) and trametinib (2 mg daily) continuously until disease progression or intolerance. Treatment response was assessed by RECIST 1.1¹⁹ every 2 cycles (8 weeks). Severity of toxicity was graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0).²⁰

Biospecimens

Mandatory baseline tumor biopsies were collected 0–4 days before the first dose. Optional tumor biopsies were performed between days 4 to 10 of cycle 1 and at time of disease progression. Blood samples were collected at baseline and on cycle 1 day 8 (C1D8), and intermittently afterwards.

Tumor biopsies were split; two-thirds were frozen in optimal cutting temperature (OCT) compound, and one-third was formalin-fixed and paraffin-embedded (FFPE). A haematoxylin and eosin (H&E)-stained slide from each block was subjected to pathology review to determine the percentage of viable tumor cells. Frozen samples with >70% viable tumor cells were subjected to DNA and RNA extraction as previously described.²¹ Peripheral blood mononuclear cells (PBMC) isolated from blood were subjected to total RNA isolation using mirVana™ kit (AM1560).

Molecular and Immune Analyses

Amount of circulating *BRAF*^{V600} was determined from PBMC-derived RNA as previously described.²² *BRAF* splice variants were detected by RT-PCR as previously described.⁵ WES and RNAseq were performed at the Broad Institute (eMethods in Supplement 1). MAPK z-scores were determined by averaging normalized RNAseq gene expression of 14 known transcriptional targets of MAPK in *BRAF*^{V600}-mutant melanoma,^{15,23} and immune z-scores using 105 genes that define the high-immune cluster of TCGA melanoma samples.¹ Targeted NGS of 200 cancer-related genes (T200) was performed as previously described.²⁴ IHC on FFPE tumor tissues was performed using antibodies against *BRAF*^{V600E}, pMAPK (ERK1/2), pS6, pPRAS40, CD8, and PD-L1. IHC scores were determined by multiplying pathologist-assessed IHC intensity with fraction of positive cells.

Statistical Analysis

The trial was designed to enroll 30 patients with target response rate of 25% and with interim efficacy and safety monitoring. Though the prespecified stopping criteria were not met, this trial was terminated early due to slowed accrual following approval of front-line CombiDT therapy. The primary efficacy end point was response rate by RECIST1.1.¹⁹ PFS and overall survival (OS) were secondary endpoints. Kaplan-Meier curves were used to estimate the distribution of PFS and OS, and distributions were compared using the log-rank test. Univariate Cox proportional hazards regression was used to assess the association between PFS and the covariates of interest (age, gender, stage, LDH, performance status, *BRAF* mutation, duration of BRAFi monotherapy, response to BRAFi monotherapy, interval

from BRAFi monotherapy, and baseline molecular features). Univariate logistic regression and/or Fisher's exact test were used to assess the association between DCR and the same covariates. Pearson correlation analysis was used to assess the correlation between IHC and MAPK/immune scores. P-values of <0.05 were considered significant. R (v3.2.2) and Prism 6 were used for data analysis.²⁵

Results

Patient characteristics and clinical outcomes

Twenty-three patients were treated between September 28, 2012, and October 23, 2014 (Figure 1). Sixty-one percent of patients had M1c disease and 39% had elevated LDH (Table 1). The majority (87%) had a *Braf*^{V600E} mutation. With prior BRAFi monotherapy, 78% of patients had a confirmed response and 57% had duration of response >6 months. Most patients had no interval between BRAFi monotherapy and CombiDT initiation except for 3 patients with inter-treatment intervals of 3.5–12 months.

Among the 20 patients evaluable for response, 6 (30%) patients achieved transient clinical responses (1 complete response [CR], 5 partial responses [PR]) (Figure 2A). However, only 2 (10%) patients had confirmed responses on consecutive CT scans; 7 patients had confirmed stabilization of disease (SD), for an overall DCR of 45% (95% CI, 23%–68%) (eTable 1 in Supplement 1). The median PFS was 13 weeks (95% CI, 8–16) (Figure 2B). With a median follow-up of 5.9 months, the median OS was 10.2 months (95% CI, 4.9–NR) (eFigure 1 in Supplement 1). The spectrum and rate of adverse events were similar to those reported previously with CombiDT¹⁷ (eTable 2 in Supplement 1) and only 3 patients required dose reduction.

Clinical features and circulating BRAF as correlates of response

PFS and DCR did not correlate significantly with LDH, stage, BRAF mutation, performance status, prior immunotherapy, or interval from prior BRAFi monotherapy (eTable 3 in Supplement 1). Patients with duration of prior BRAFi monotherapy >6 months had longer PFS (median 16 vs 8 weeks, HR 0.29, p=0.01) (Figure 2C) and higher DCR (73% vs. 11%, OR 21.3, p=0.015). Patients who had progressive disease as best response to prior BRAFi monotherapy had shorter PFS with CombiDT than patients who had achieved disease control (median 8 vs. 16 weeks, p=0.04). A decrease in the level of circulating BRAF^{V600} at C1D8 was associated with improved disease control (75% DCR for C1D8-to-baseline BRAF^{V600} log2 ratio <0 vs. 18% with ratio>0, p=0.02) (Figure 2D).

BRAFi resistance mechanisms and clinical outcomes with CombiDT

Thirteen patients had DNA extracted from baseline tumor biopsies of sufficient quality and amount for analysis by both WES and T200; 2 additional patients had T200 alone.²⁴ Thirteen patients had tumor RNA sufficient for analysis by RT-PCR for BRAF alternative splicing. The results of these analyses are shown in Figure 3 (full WES results in Supplement 2).

Alterations known to mediate BRAFi resistance by reactivating the MAPK pathway were detected at baseline in 5 of 15 evaluable patients (33%), including *NRAS*^{Q61R} (Patient 19), *NRAS*^{G13R} (Patient 20), and *MAP2K1*^{E203K} (Patient 18) mutations; *BRAF* amplification (Patients 2 and 12); and *BRAF* aberrant splicing (Patients 2 and 18^a) (Figure 3). There was no significant difference in PFS (p=0.73) or DCR (p=0.36) with CombiDT between patients with or without these alterations, nor did they correlate with baseline clinical characteristics or duration of prior BRAFi monotherapy.

Other genetic aberrations previously implicated in either melanoma pathogenesis or MAPK pathway inhibitor resistance are indicated in Figure 3. Two patients with baseline *RAC1*^{P29S} mutation had disease progression as their best response to CombiDT; both patients also received BRAFi monotherapy 6 months.

WES of the CombiDT baseline sample for Patient 9 failed to detect the presence of a BRAF mutation, although a BRAF^{V600E} mutation had been detected in the primary tumor and the patient had a CR to prior vemurafenib monotherapy. BRAF^{V600} mutation was not detectable in the pre-CombiDT sample, which had ~80% tumor content on pathology review, by T200 sequencing (621 reads), RNAseq, or BRAF^{V600E} IHC. Interestingly, high levels of circulating BRAF^{V600} were detected in the blood before and during CombiDT, suggesting that the patient likely had both BRAF^{V600E}-mutant and BRAF-wildtype clones/metastases, although no other tumor samples were available for confirmatory analysis. This patient rapidly progressed on CombiDT.

Patient 27 was evaluable for acquired resistance to CombiDT, as the patient achieved a PR and had evaluable baseline, on-treatment, and disease progression tumor biopsies. WES of the lesion progressing on CombiDT detected a *NRAS*^{Q61K} mutation that was not present in the pre-CombiDT lesion. Notably, both patients (#19, #20) with an activating *NRAS* mutation in their pre-CombiDT tumor had disease progression as best response with CombiDT.

Direct analysis of MAPK pathway activity

MAPK pathway activity was assessed directly by pMAPK (pERK1/2) IHC in FFPE tumor samples, and transcriptionally by MAPK transcriptional output (z-score) by RNAseq on available frozen tumors (n=14) (Figure 4A–C). The pMAPK IHC scores and MAPK z-scores were highly concordant ($R^2=0.8$, $P=0.003$) in tumors with sufficient tissues for both assays (n=8) (eFigure 2 in Supplement 1). Combining the results from the two methods, 15 patients were evaluable for MAPK activity in their baseline tumors. Ten of the 15 patients had high baseline MAPK activity (pMAPK IHC score > mean or MAPK z-score >0). There was no significant difference in either PFS ($P=0.71$) or DCR ($P=1.0$) between these patients and those without baseline MAPK activation.

Eight patients had both baseline and on-treatment biopsies suitable for MAPK activity analysis. Analysis of the paired tumors demonstrated that 5/8 (62.5%) patients did not achieve MAPK inhibition on CombiDT (Figure 4B–C). Patient 27 was the only patient to

^aAn in-frame insertion between exons 4 and 5 was found in patient 2. Skipping of exon 2 was found in patient 18.

achieve >90% inhibition of pMAPK, which was previously shown to correlate with clinical response with single-agent BRAFi therapy.²⁶ As noted above, this patient achieved a PR, and at disease progression had a new *NRAS*^{Q61K} mutation (Figure 3) and reactivation of MAPK signaling (Figure 4A).

Signaling by the mTOR complex was assessed by IHC for pS6. High pS6 expression, which was identified in 7/12 evaluable pre-treatment samples, did not correlate significantly with PFS (HR 1.76, p=0.38) or DCR (p=1.0) (eTable 4 in Supplement 1). Increased expression of pPRAS40, a marker of activation of the PI3K-AKT pathway, was elevated in 3/7 evaluable patients, and correlated with a non-significant trend for shorter PFS (HR 5.72, p=0.13) (eFigure 3 in Supplement 1).

Analyses of tumor immune infiltration

Tumor biopsies were analyzed by IHC to quantify CD8+ T cell infiltration and for immune-related gene expression (by RNAseq) to assess the anti-tumor immune response. The estimated fraction of CD8-positive cells by IHC correlated with z-scores for genes overexpressed by TCGA “high-immune” tumors ($R^2=0.7$, p=0.004) (eFigure 2 in Supplement 1).¹ Of 14 baseline tumors evaluated by IHC, only one (Patient 02) had >5% infiltration by CD8+ T cells. This patient, who also achieved inhibition of pMAPK, demonstrated a marked increase in CD8+ T cell infiltration with CombiDT treatment, and achieved SD (Figure 4D and eFigure 4A in Supplement 1). None of the other 8 patients with matched baseline and on-treatment samples developed increased immune infiltrate, as detected by IHC (Figure 4D) or RNAseq (eFigure 4B–4C in Supplement 1).

IHC analysis revealed a lack of PD-L1 expression (< 2% of tumor cells) in 10/13 baseline tumors analyzed (eFigure 5 in Supplement 1). Only 1/6 patients with matched baseline and on-treatment tumors demonstrated an increase in PD-L1 with CombiDT (Patient 20).

Discussion

This study represents the first prospective interrogation of the molecular, immunological, and circulating correlates of response and mechanisms of resistance to CombiDT therapy in BRAFi-refractory metastatic melanoma patients. This multi-platform analysis of biospecimens allows for in-depth characterization of the heterogeneity of response and resistance to CombiDT in these patients. Our findings underscore the heterogeneity of BRAFi-resistant metastatic melanoma and provide new insights into the lack of efficacy of CombiDT therapy in this population.

The treatment outcomes in this trial (10% confirmed response, 45% DCR) are similar to a recent larger independent study of BRAFi-refractory melanoma patients treated with CombiDT which did not require baseline biopsies.¹⁸ Improved outcomes with CombiDT in patients previously treated with BRAFi monotherapy for >6 months compared to <6 months have been observed in both studies, suggesting a potential clinical selection criteria for CombiDT.¹⁸ Notably, we did not observe any significant clinical differences between these groups at the start of CombiDT in factors known to correlate with outcomes in advanced melanoma (i.e. stage, LDH).

Decrease in the level of mutant BRAF RNA in the blood after only 1 week of treatment correlated with better DCR. This result is consistent with previous findings in the front-line BRAFi monotherapy setting.²² Further validation is needed to confirm the utility of this highly sensitive and specific blood-based assay. However, our results suggest that such assays could contribute significantly to therapeutic development in BRAFi-refractory patients, as early blood-based markers of efficacy could lead to rapid identification of effective therapies and spare patients prolonged treatment with ineffective therapies.

We hypothesized that the presence of baseline molecular events that reactivate the MAPK pathway would predict clinical benefit from CombiDT in BRAFi-refractory melanoma, as multiple studies have shown efficacy with BRAFi+MEKi in preclinical models.^{8,12,13} However, neither the presence of mechanisms of resistance (MOR) that reactivate the MAPK pathway, nor direct assessment of MAPK activity, correlated significantly with outcomes. This is likely due to the failure of CombiDT to meaningfully inhibit the pathway in this setting, demonstrated here for the first time by our analysis of on-treatment tumor biopsies. This lack of pathway inhibition contrasts with the nearly universal pathway inhibition observed in BRAFi-naïve metastatic melanoma patients treated with MAPK pathway inhibitors.²⁷ It remains unclear why CombiDT fails to achieve pathway inhibition in the majority of BRAFi-refractory patients, but our results support the critical need to obtain such biopsies to interpret results in future trials with other agents predicted to inhibit the pathway, such as ERK inhibitors.²⁸ Further, the failure to identify a putative genetic MOR in every patient is consistent with prior studies and supports the need for inclusion of other platforms (i.e. epigenetics) in investigating resistance.⁷

Our in-depth characterization also resulted in informative observations in individual patients. *NRAS* mutations are detected in ~20% of melanomas with acquired resistance to BRAFi monotherapy,⁷ and BRAFi+MEKi overcomes this resistance mechanism in preclinical models.⁸ However, we observed a new *NRAS*^{Q61K} mutation in a progressing lesion of a patient who initially responded to CombiDT, which corresponded with reactivation of MAPK signaling. Moreover, both patients in the study with pre-treatment *NRAS* mutations failed to respond to CombiDT. These results are consistent with a recent retrospective analysis of melanoma patients characterized for MORs to BRAFi,²⁹ and together support the need for alternative strategies to overcome resistance mediated by *NRAS* mutations.^{28,30} Our cohort also included two patients with baseline *RAC1*^{P29S} mutations, both of whom had disease progression as the best response with CombiDT. *RAC1*^{P29S} mutation is enriched in patients with rapid progression on BRAFi monotherapy³¹ and has been shown to mediate resistance to single-agent BRAFi and single-agent MEKi in preclinical models.³² Our observed clinical resistance to CombiDT provides further validation that *RAC1*^{P29S} is a clinically significant target and supports the need for therapies to overcome it.

Our cohort also included one patient with a tumor biopsied after progression on BRAFi monotherapy in which no *BRAF*^{V600} mutation could be detected by NGS, RNAseq, or IHC. This patient had a documented *BRAF*^{V600E} mutation in the primary melanoma, previously had a complete response to vemurafenib monotherapy lasting 7 months, and had mutant BRAF RNA detected in circulating blood. This finding again underscores the potential heterogeneity of BRAFi-refractory melanoma, including of different tumors within

individual patients, and the potential clinical utility of mutation detection with blood-based assays.

Immune cell infiltration and PD-L1 expression were low at baseline in the majority of these BRAFi-refractory patients, consistent with previous studies demonstrating loss of immune infiltration at progression on BRAFi monotherapy.³³ While previous studies demonstrated that robust immune infiltration of tumors can be achieved with CombiDT in BRAFi-naïve melanomas,³³ we observed no significant increase in 8/9 patients. Interestingly, the patient with the highest pre-treatment CD8+ tumor infiltration demonstrated an increased infiltration of T cells by IHC, increased expression of immune-related genes in the tumor by RNAseq, and achieved disease control with CombiDT. This patient was also one of the few patients that achieved MAPK pathway inhibition with CombiDT. These findings suggest that the effects of CombiDT that promote the anti-tumor immune response in BRAFi-naïve patients are largely absent in BRAFi-refractory patients, which has potential implications for the optimal sequencing of targeted and immune therapies in this disease.

Conclusion

Together, our findings reinforce the clinical and molecular heterogeneity of metastatic melanoma patients that have progressed on BRAFi monotherapy. Given the recent approvals of CombiDT and vemurafenib+cobimetinib⁴ as front-line therapies, this study likely represents one of the last opportunities to assess combination therapy after BRAFi monotherapy. Our studies identify potential early markers of clinical activity for future therapies, and improve our understanding of the likely basis for the limited activity of CombiDT in this setting. These findings also support the rationale for the interrogation of blood and tumor samples in future studies to expedite the development of more effective treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
CONSORT study flow diagram. QC=quality control.

Figure 2.

Clinical outcomes with CombiDT. **(A)** Waterfall plot of maximum tumor reduction with CombiDT treatment. CR, confirmed complete response; PD, progressive disease; PR, confirmed partial response; SD, stable disease. **(B)** Kaplan-Meier (K-M) analysis of PFS for all patients treated with CombiDT. **(C)** Kaplan-Meier PFS for patients whose prior BRAFi monotherapy treatment was ≤ 6 months (n=10, Red line) versus > 6 months (n=13, Blue line). The difference in PFS between the two groups of patients is statistically significant ($P=0.007$ by Log-rank test). **(D)** Log2 ratio of levels of circulating BRAF^{V600} mutation in blood at cycle 1 day 8 compared to baseline in patients who did (Blue) or did not (Red) achieve disease control (DC) with CombiDT treatment. Log2 ratio was <0 in 6 of 8 patients that achieved disease control, but only 2 of 11 patients that did not (PD) ($P=0.02$ by Fisher's exact test).

Figure 3.

Pre-treatment molecular features and clinical outcomes with CombiDT. Thirteen baseline tumor biopsies were subjected to WES analysis. Clinical characteristics, WES results (top to bottom: selected mutations, mutation burden and copy number variations), BRAF splicing by RT-PCR, and baseline pMAPK and CD8 immune scores determined by IHC are displayed. * pMAPK and immune scores not available for patient 22.

Figure 4.

MAPK activity and immune infiltrate analyses. **(A)** Representative IHC images of pMAPK expression in baseline, early on-treatment, and at disease progression on CombiDT.

Dynamic changes in pMAPK were observed in Patient 27 who achieved a partial response and then progressed, whereas no significant pMAPK inhibition was observed in Patient 20 (best response = disease progression). **(B)** IHC scores of pMAPK in baseline and on-treatment tumors of 6 patients with paired evaluable samples. Three of the 6 patients had no decrease in pMAPK with CombiDT. **(C)** Heatmap of log₂ gene expression (mean-centered) of 14 known BRAF^{V600}-driven transcriptional targets of MAPK in 12 BRAF^{V600} tumors analyzed by RNAseq. MAPK z-score represents the normalized and averaged expression level of the 14 genes (Red = high activity, Blue = low activity). Arrows point from baseline tumors to their matched on-treatment tumors. Patients 1 and 14, who were not evaluable for on-treatment MAPK inhibition by IHC, showed no decrease in MAPK z-score after CombiDT. **(D)** IHC-determined CD8 fraction in baseline (n=14) and on-treatment (n=8) tumors. Matched tumors from the same patient are connected by solid lines with arrowheads pointing to on-treatment tumor.

Table 1

Patient Demographics and Clinical Characteristics

Age, years	
Mean	52
Median	54
Range	27–54
Sex	
Male	16 (70%)
Female	7 (30%)
ECOG Performance status	
0	13 (57%)
1	7 (30%)
2	3 (13%)
Stage	
IIIc	3 (13%)
IVa	2 (9%)
IVb	4 (17%)
IVc	14 (61%)
BRAF mutation	
BRAF ^{V600E}	20 (87%)
BRAF ^{V600K}	1 (4%)
BRAF ^{V600R}	2 (9%)
Baseline LDH	
Normal	14 (61%)
>ULN	9 (39%)
Duration of prior BRAF inhibitor, months	
6	10 (43%)
>6	13 (57%)
Best response to prior BRAF inhibitor	
CR	5 (22%)
PR	12 (52%)
SD	2 (9%)
PD	3 (13%)
Time from treatment with prior BRAF inhibitor, months	
Median	0
Range	0–12
Prior BRAF inhibitor ^a	
Dabrafenib	5 (21%)

Vemurafenib	19 (82%)
Prior immunotherapy	10 (43%)

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