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Clinical validation study of circulating tumour DNA in patients with advanced melanoma treated with dabrafenib or dabrafenib plus trametinib

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Abstract

Background: Melanoma lacks validated blood-based biomarkers for monitoring and predicting treatment efficacy. Cell-free circulating tumour DNA (ctDNA) is a promising biomarker; however, various detection methods have been used, and, to date, there have been no large studies examining the association between serial changes in ctDNA and survival after BRAF and/or MEK

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Contributors

MMS, MS, CR, JCB, DP contributed to conception and design. MMS, BCC, CR, JG, DP contributed to development of methodology. MMS, BCC, DS, PDN, GVL, CR, AR, JJG contributed to acquisition of data, including patient recruitment. MMS, JMW, KTF, DS, PDN, CR, JJG, EG, MM, JG, JCB, DP contributed to analysis of data. JG and MM have accessed and verified the underlying data. MMS, JMW, BCC, GVL, KTF, DS, PDN, CR, AR, MAD, JJG, EG, MS, MM, JG, JCB, DP contributed to writing, review and/or revision of the manuscript. MS, GVL, DS, DP contributed to administrative, technical, or material support. EG, MS, JCB, DP contributed to study supervision.

Data sharing

Novartis is committed to sharing with qualified external researchers, access to patient-level data, and supporting clinical documents from eligible studies. Requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided is anonymised to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations. This trial data availability is according to the criteria and process described on ClinicalStudyDataRequest.com.

inhibitor therapy. We aimed to evaluate whether baseline ctDNA levels and kinetics could predict survival outcomes.

Methods: We used analytically validated droplet digital polymerase chain reaction assays to measure *BRAF* V600–mutant ctDNA in pretreatment and on-treatment plasma samples from patients aged ≥ 18 years old enrolled in two clinical trials. COMBI-d (NCT01584648) is a double-blind, randomised phase 3 study of dabrafenib plus trametinib vs dabrafenib plus placebo in previously untreated patients with *BRAF* V600 mutation-positive unresectable or metastatic melanoma. Patients had Eastern Cooperative Oncology Group performance status (ECOG PS) 0 or 1. The primary endpoint was progression-free survival. COMBI-MB (NCT02039947) is an open-label, phase 2 study evaluating dabrafenib plus trametinib in patients with *BRAF* V600 mutation-positive metastatic melanoma and brain metastases. Patients in cohort A of COMBI-MB had asymptomatic brain metastases, no previous local brain-directed therapy, and an ECOG PS 0 or 1. The primary outcome was intracranial response in cohort A. Biomarker analysis was a prespecified exploratory endpoint and performed in the intention-to-treat population in COMBI-d and COMBI-MB. We investigated the relationship between mutant copy number (baseline or week 4 or zero conversion status) and efficacy endpoints (progression-free survival, overall survival, and best overall response). We used Cox models, Kaplan-Meier plots, and log-rank tests to explore the relationship with progression-free survival and overall survival. The impact of additional prognostic variables such as lactate dehydrogenase were also investigated in addition to the mutant copy number.

Findings: In COMBI-d, pretreatment and on-treatment (week 4) plasma samples were available from 345 of 423 (82%) and 224 of 423 (53%) patients, respectively. In cohort A of COMBI-MB, pretreatment and on-treatment samples were available from up to 38 of 76 patients (50%) with intracranial and extracranial metastatic melanoma. ctDNA was detected in pretreatment samples from 320 of 345 patients (93%; COMBI-d) and 34 of 38 patients (89%; COMBI-MB). When assessed as a continuous variable, elevated baseline *BRAF* V600 mutation-positive ctDNA levels predicted worse overall survival outcomes, independent of baseline lactate dehydrogenase levels, in COMBI-d. A ctDNA cut point of 64 copies/mL of plasma stratified patients enrolled in COMBI-d with respect to survival outcomes and was validated in the COMBI-MB cohort. In COMBI-d, undetectable ctDNA at week 4 was significantly associated with extended progression-free and overall survival, particularly in patients with elevated lactate dehydrogenase levels.

Interpretation: Pretreatment and on-treatment *BRAF* V600–mutant ctDNA measurements may serve as independent, predictive biomarkers of clinical outcome with targeted therapy.

Funding: Novartis.

INTRODUCTION

Although early scan assessments are useful for monitoring antitumour responses, noninvasive methods with increased accuracy are needed to help predict clinical outcome. There are currently no validated blood-based biomarkers to monitor treatment efficacy in patients with advanced melanoma. Lactate dehydrogenase (LDH) is an established prognostic factor; however, it lacks sufficient specificity and sensitivity to routinely inform on-treatment decision-making. Cell-free circulating tumour DNA (ctDNA) has emerged as a promising biomarker in many types of cancers. Released primarily by dead or dying tumour

cells, cancer-relevant genetic abnormalities have been measured in plasma or serum using various analytical techniques, and their detection/quantification has been associated with tumour burden, minimal residual disease, treatment response and clonal evolution.[1] However, studies showing clinical utility are less common. Examples include the use of ctDNA assays to identify mutations in the epidermal growth factor receptor and PIK3CA genes to determine eligibility for targeted therapies among non-small cell lung cancer and breast cancer patients.[2,3]

In melanoma, *BRAF* V600 or *NRAS* Q61 hot spot mutations are found mutually exclusively in approximately two-thirds of metastatic tumours. Patients with *BRAF* V600-mutant tumours can be treated with highly efficacious BRAF plus MEK targeted therapies. ctDNA studies in patients with metastatic melanoma receiving either targeted therapies or immune checkpoint inhibitors demonstrated associations between baseline and on-treatment ctDNA levels and several clinical parameters and outcomes. These include changes in tumour burden and response rates, early detection of disease progression, pseudoprogression, mutations associated with resistance to targeted therapies, and survival;[4–7] suggesting the potential for ctDNA to serve as a biomarker to help guide patient management. However, nearly all studies have involved small, mostly retrospectively collected data, and have used a variety of detection methods with differing sensitivities, often lacking analytical validation details. In particular, two studies observed that ctDNA detection after a few weeks of treatment may be used to identify intrinsic resistance to immune checkpoint inhibitors.[7,8] The only comparable study of targeted therapy with BRAF and MEK inhibitors in the metastatic melanoma setting examined 25 patients enrolled in a phase 2 trial.

To our knowledge, we present the first large-scale ctDNA evaluation of patients with *BRAF* V600 mutation-positive unresectable or metastatic melanoma treated with dabrafenib plus trametinib or dabrafenib plus placebo in two clinical trials using an analytically validated droplet digital polymerase chain reaction (ddPCR) method.

METHODS

Study design and participants

Patients with a minimum of 8 × 0·5 mL aliquots or 4 × 1 mL aliquots of plasma available were included. ctDNA analysis was performed on 345 plasma samples collected at baseline and 224 plasma samples collected at week 4 (222 had corresponding baseline plasma samples) from 423 patients enrolled in the COMBI-d trial (registered at ClinicalTrials.gov, NCT01584648). COMBI-d was a double-blind, randomised, phase 3 study of oral dabrafenib 150 mg twice daily plus oral trametinib 2 mg once daily vs dabrafenib plus placebo (screened for enrolment between May 4, 2012, and Nov 30, 2012).[9] Eligible patients were aged at least 18 years and had histologically confirmed, unresectable or metastatic melanoma with *BRAF* V600E/K mutations. Patients were ineligible if they had previous systemic treatment for advanced or metastatic cancer. Patients with brain metastases that had been definitively treated and stable for at least 12 weeks were eligible. Patients continued treatment until disease progression, death, unacceptable toxicity, or withdrawal of consent. Randomisation and masking in COMBI-d are detailed in the appendix.

We included cohort A of COMBI-MB as a validation cohort using plasma samples collected at baseline and at weeks 4, 8, 16, 24, 32, and 40. COMBI-MB was an open label, nonrandomized, phase 2 trial evaluating dabrafenib plus trametinib in patients with *BRAF* V600–mutant metastatic melanoma and brain metastases (registered at ClinicalTrials.gov, NCT02039947).[10] Cohort A enrolled patients with *BRAF* V600E mutation–positive asymptomatic melanoma brain metastases who had received no previous local brain-directed therapy and had an Eastern Cooperative Oncology Group performance status of 0 or 1. Additional study design details for COMBI-MB are included in the appendix.

For both trials, whole blood was collected in EDTA tubes, gently inverted (8–10 times), and plasma was separated by immediate centrifugation (10 minutes at 1500g ± 150g followed by 10 minutes at 3000g ± 150g), and plasma supernatant was stored at −70°C or colder prior to use.

The protocols for both COMBI-d and COMBI-MB (available with the primary publications[9,10]) were approved by the independent review board at each participating institution. The studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent.

Procedures

Each sample was labeled with a unique identifier assigned by the sponsor, and laboratory staff conducting the ctDNA measurements were blinded to all patient data with the exception of the specific tumour-associated *BRAF* mutation.

Plasma samples were thawed at room temperature and centrifuged (10 minutes at 16000*g*) immediately before ctDNA extraction using the QIAamp DSP Circulating NA Kit (Qiagen; Hilden, Germany), according to the manufacturer's instructions, and eluted in 85 μL of elution buffer. Total eluate from each sample was divided into eight replicate wells, and the concentration of *BRAF* V600E or V600K ctDNA was measured using analytically validated ddPCR assays run on a QX200 Droplet Digital PCR system and analysed by QuantaSoft Analysis Pro (version 1.0.596) (Bio-Rad Laboratories; Hercules, CA, USA) as previously described. The choice of assay (V600E vs V600K) was based on genotyping information determined during screening for trial enrolment. The normal assay range was determined using plasma from 30 healthy donors (BioIVT; Westbury, NY, USA), and the limit of blank was 0·28 copies/mL or 0·019% mutant fraction (*BRAF* V600E) and 0·34 copies/mL or 0·022% mutant fraction (*BRAF* V600K) using standard methods. Samples with concentrations exceeding these cutoffs were considered positive for ctDNA. Patients with detectable ctDNA at baseline were determined to have a ctDNA zeroconversion if levels decreased below the limit of blank at any follow-up time point.

Outcomes

The primary endpoint in COMBI-d was investigator-assessed progression-free survival, defined as the time from randomisation until progression or death from any cause. In COMBI-MB, the primary endpoint was intracranial response rate in cohort A, defined as the percentage of patients with a confirmed intracranial complete or partial response assessed by investigator using modified RECIST version 1.1. Analysis of ctDNA and association with

clinical outcomes was an exploratory endpoint of both trials. Secondary endpoints are included in the appendix.

Statistical analysis

ctDNA analysis data from the two arms of COMBI-d were pooled after an initial analysis revealed no significant difference in the correlative analyses between the arms, and Cox models were adjusted for treatment arm information.

We used Cox models to investigate whether the mutant copy number (after logarithmic transformation) at baseline interacts with treatment arm in its relationship to progression-free survival; we found no interaction. To search empirically for an optimal cutoff for mutant copy number (considered as a continuous variable) at baseline, we sought to maximise a log-rank test. We used the method of Lausen and Schumacher, as implemented in the maxstat R package (R Foundation), to conduct the search and return an adjusted p-value assessing evidence that apparent difference in risk is not random. In accordance with Faraggi and Simon, we estimated cutoff performance by a two-fold cross-validation exercise, then estimated the final cutoff on the full data set. We evaluated the cutoff using Cox models, log-rank tests, and Kaplan-Meier plots. For each Kaplan-Meier plot, we calculated a p-value derived from the log-rank test for difference between the subgroups, and we fitted a Cox model to estimate the hazard ratio. We derived 95% Wald-type confidence intervals for each hazard ratio. Note that it is possible, in borderline cases, for the Wald-type confidence interval to contain 1·0 (indicating lack of evidence of difference at the 5% level) while the log-rank test yields a p-value less than 0·05, as they are not identical methods and each has a nominal 5% rate of indicating a difference when in truth no difference is present.

The linear form for logarithmic transformation of ctDNA at baseline was evaluated using plots of the cumulative Martingale residuals against the values of the covariate log ctDNA at baseline. The p value of a Kolmogorov-type supremum test based on a sample of 1000 simulated residual patterns was assessed.

In model-based analysis, missing data imputations or variable selection methods were not implemented. We first examined effects based on clinical relevance. Multivariate models were also fit to examine if other prognostic/baseline characteristics affected the relationship between mutant copies and efficacy. When performing model based analysis for mutant copy number, the impact of the following baseline characteristics and factors was examined: treatment, LDH strata, number of metastatic sites, *BRAF* status, ECOG status, gender, age, stage of disease at baseline.

We used R (version 3.4.3) and R packages maxstat (version 0.7.25), survival (version 2.41.3), and Hmisc (version 4.0.3) as well as SAS (version 9.4; SAS Institute; Cary, NC, USA) and SAS macro %fincut.

Role of the funding source

The study was designed by the authors and the funder of the study. Data were collected by the study site staff and authors and monitored by the funder. The funder was also involved in

data analysis, data interpretation, and writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

The number of pretreatment and on-treatment plasma samples from the COMBI-d trial are summarised in the appendix p 10. Baseline characteristics were similar between patients with and without available plasma samples at baseline and between patients with and without paired baseline and week 4 samples (table 1). For the COMBI-d study, median follow-up was 20 months (range, 0 to 76 months) in the combination therapy group and 16.0 months (range, 0 to 76 months) in the dabrafenib monotherapy (from randomization to last contact). Amongst the 345 patients analysed, 290 had *BRAF* V600E, 54 had *BRAF* V600K, and 1 had *BRAF* V600E and V600K. Plasma samples (median volume, 3·6 mL; [IQR, 3·0–4·2 mL]) were analysed using mutation-specific ddPCR. Median cell-free DNA/sample was 28·6 ng (range, <1 to 5990 ng). *BRAF* V600E/K mutations were detected in 320 (93%) of 345 baseline plasma samples.

Amongst the subset of patients with paired pretreatment and on-treatment plasma samples, 201 (90%) had detectable ctDNA at baseline, and 121 (60%) of 201 remained positive at week 4. Nearly all patients (111 of 121 [92%]) had a considerable decrease in mutant ctDNA copies after 4 weeks of therapy (appendix p 11). Median ctDNA concentrations at baseline and week 4 were 66·7 copies/mL (range, 0–266902 copies/mL) and 0·55 copies/mL (range, 0–11078 copies/mL), respectively. The median mutant fraction at baseline was 3·1% (range, 0%−79%) and at week 4 was 0·02% (range, 0%−47%). In 25 patients, a duplicate pretreatment sample was analysed by a different operator, on a different day, using a different lot of reagents, and there was a high correlation between log ctDNA values (R^2=0·94) (appendix p 3).

Baseline characteristics were similar between patients with detectable vs undetectable ctDNA levels at screening and week 4, although LDH level, number of metastatic sites, and median sum of baseline lesion diameters were higher in patients with detectable ctDNA levels at baseline and week 4 than in patients with undetectable ctDNA levels at the respective time points (appendix p 4).

We tested the associations between pretreatment ctDNA levels and progression-free survival and overall survival with ddPCR, using ctDNA as a continuous variable. The analysis revealed that the quantity of ctDNA at baseline was directly associated with survival; higher ctDNA levels were associated with significantly shorter progression-free and/or overall survival. Specifically, log ctDNA was associated with progression-free survival and overall survival in univariate analysis adjusted for treatment arm (progression-free survival: hazard ratio [HR], 1·08 [95% CI, 1·04–1·12]; p<0·0001; overall survival: HR, 1·13 [95% CI, 1·09–1·18]; p<0·0001) as well as in multivariable analysis for overall survival when adjusting for pretreatment LDH and treatment arm information (HR, 1·08 [95% CI, 1·03–1·13]; p=0·0020).

Baseline ctDNA subgroups (positive vs negative; 320 [93%] of 345 patients with detectable ctDNA) were not associated with progression-free survival or overall survival (appendix p 12). At the time of the analysis, 253 (79%) of 320 patients in the positive baseline ctDNA subgroup and 18 (72%) of 25 patients in the negative baseline ctDNA subgroup had a progression-free survival event (progressed or died). There were 237 (74%) and 15 (60%) deaths, respectively. We explored potential cutoff values to stratify patients by high and low risk for disease progression and survival. We identified 64 copies/mL of plasma as a robust cut point to separate patients with shorter and longer survival. Specifically, patients with pretreatment ctDNA <64 copies/mL had a median progression-free survival of 12·7 months (95% CI, 10·8–16·3 months) compared with 6·5 months (95% CI, 5·6–7·4 months) in patients with ctDNA ≥64 copies/mL (HR, 1·74 [95% CI, 1·37–2·21]; p<0·0001]. At the time of the analysis, 122 (72%) of 170 patients and 149 (85%) of 175 patients, in each group respectively, progressed or died. Similarly, patients with ctDNA <64 copies/mL had a median overall survival of 35·1 months (95% CI, 27·1–48·8 months) compared with 13·4 months (95% CI, 11·9–16·1 months) in patients with ctDNA ≥64 copies/mL (HR, 2·23 [95% CI, 1·73–2·87]; p<0·0001). At the time of the analysis, 105 (62%) and 147 (84%) patients in each group, respectively, had died (figure 1).

We compared pretreatment and week 4 on-treatment plasma ctDNA levels in 224 patients with available on-treatment ctDNA results. In the subgroup with detectable ctDNA at baseline (n=201), achieving zeroconversion (ie, undetectable ctDNA) at week 4 was significantly associated with prolonged survival. Median progression-free survival and overall survival in patients who achieved zeroconversion was prolonged compared with patients with detectable ctDNA at week 4 (figure 2). There were 57 (71%) of 80 patients who achieved zeroconversion and 102 (84%) of 121 patients with detectable ctDNA at week 4 who progressed or died; 52 (65%) and 96 (79%) patients had died, respectively.

Amongst patients with detectable ctDNA at baseline and an evaluable ctDNA result at 4 weeks, there was a strong association between best overall response and zeroconversion status (figure 3; appendix p 6). Objective response rates in patients with detectable ctDNA at week 4 vs those who had zeroconversion were 53% (63 of 118) vs 81% (65 of 80); complete responses were achieved by 14% (16 of 118) vs 23% (18 of 80) of patients, respectively. All patients with zeroconversion had disease control (complete response, partial response, or stable disease) as best overall response. A proportional odds likelihood ratio test for association between zeroconversion status and the four best overall response categories yielded a p value of 0·0002, which strongly suggests that the association cannot be explained by chance. A similar association was found between best overall response and zeroconversion status in COMBI-d regardless of treatment arm (appendix p 13).

When patients were stratified by baseline LDH level (> or ≤ upper limit of normal), achieving undetectable ctDNA at week 4 was significantly associated with progression-free survival and overall survival in patients with LDH levels above the upper limit of normal (appendix p 14). Of these patients, 58 (91%) of 64 in the positive ctDNA subgroup and 13 (76%) of 17 in the negative ctDNA subgroup progressed or died; 58 (91%) and 11 (65%) patients in each subgroup had died, respectively. Detectable vs undetectable ctDNA at week 4 was not significantly associated with survival endpoints amongst patients with LDH levels

at or below the upper limit of normal. Of these patients, 49 (77%) of 64 in the positive ctDNA subgroup and 56 (71%) of 79 patients in the negative ctDNA subgroup progressed or died; 42 (66%) and 52 (66%) patients in each subgroup had died, respectively. A test for interaction between baseline LDH and week-4 ctDNA groups in a Cox model for overall survival was significant (p=0.022), while that for progression-free survival was not significant (p=0.19).

We investigated the role of clinical markers using a multivariable Cox regression model that included baseline ctDNA status using the optimised cut point of 64 copies/mL (appendix p 7) and ctDNA status at week 4 (table 2). These models indicated that, even adjusting for treatment and clinical prognostic factors, baseline ctDNA and week 4 zeroconversion status had independent, predictive value. No evidence of interaction with treatment group was identified (data not shown).

We analysed plasma samples from 38 patients with intracranial and extracranial unresectable metastatic melanoma enrolled in cohort A of the COMBI-MB trial (all patients in COMBI-MB were recruited between Feb 28, 2014 and Aug 5, 2016 from 32 hospitals and institutions in Europe, North America, and Australia). Patient characteristics are summarised in appendix p 8. ctDNA was measured longitudinally for up to 40 weeks (n=157 samples) and was detectable in 34 of 38 patients (89%) before treatment initiation (appendix p 9). Baseline ctDNA levels were correlated with baseline sum of extracranial lesion diameters (Pearson r=0·48; p=0·0042); no correlation was observed with baseline sum of intracranial lesion diameters (Pearson r=0·16; p=0·33) (appendix p 15). ctDNA levels at baseline were not correlated with best overall response (either extracranial or intracranial) when analysed as a continuous or categorical value (appendix p 16).

Baseline ctDNA levels as a continuous variable were significantly associated with progression-free survival (HR, 1·17 [95% CI, 1·05–1·30]; p=0·0024) and overall survival (HR, 1·21 [95% CI, 1·07–1·38]; p=0·0020). Using a cutoff of 64-copies/mL, we stratified patients into groups with median progression-free survival of 5·3 months (95% CI, 3·7–7·2 months) and 11·6 months (95% CI, 5·6 months-not reached) in the ctDNA-high and -low groups, respectively (HR, 3·20 [95% CI, 1·39–7·34]; p=0·0047). All patients in the ctDNA-high group and 10 (91%) of 11 patients in the ctDNA-low group progressed or died. Similarly, median overall survival in patients with high or low ctDNA level at baseline was 9·0 months (95% CI, 8·1–13·5 months) and 34·5 months (95% CI, 21·0 months-not reached), respectively (HR, 2·94 [95% CI, 1·18–7·32]; p=0·016) (figure 4). At the time of the analysis, 23 (85%) of 27 patients in the ctDNA-high group and 6 (55%) of 11 patients in the ctDNA-low group had died. ctDNA zeroconversion (including all available longitudinal on-treatment samples) was associated with extracranial best overall response (odds ratio [OR], 5·8; p=0·041), but less so with intracranial best overall response (OR, 4·2; p=0·060) (appendix p 17).

DISCUSSION

To our knowledge, we present the first large-scale analysis of ctDNA measurements and their association with survival outcomes in patients with unresectable or metastatic

melanoma treated with targeted therapy. Strengths of this report include use of an analytically validated ddPCR method to obtain quantitative ctDNA measurements and analysis of patients from two prospective clinical trials. Taking advantage of the quantitative nature of ddPCR, we show that elevated pretreatment *BRAF* V600–mutant ctDNA levels predicted worse overall survival, independent of LDH levels. We also identified an optimised cut point for pretreatment samples that stratified patients with respect to survival outcomes in the COMBI-d cohort and validated that cut point in a second independent clinical trial cohort (COMBI-MB). Undetectable ctDNA at week 4 was significantly associated with extended progression-free survival and overall survival, especially in patients with elevated LDH levels.

In the current analysis, we detected ctDNA in 93% (COMBI-d) and 89% (COMBI-MB) of pretreatment samples, the highest liquid biopsy detection rates amongst melanoma studies. Detectable ctDNA levels were associated with LDH level, number of baseline metastatic sites, and median sum of lesion diameters, similar to other studies that found an association between ctDNA level and increased tumour burden.[4,11] The ability of week 4 ctDNA measurement to identify patients with “high LDH” who had a more favourable response to targeted therapy could be due to differences between patients with respect to the number and distribution of metastatic sites with differing ctDNA shedding characteristics (eg, visceral vs skin), or differences in metastatic tumour volume, all of which have been associated with survival outcomes.[12–14]

Numerous studies have evaluated ctDNA as a blood-based biomarker for melanoma; most have described fewer than 90 patients and have used several different platforms to detect hot spot mutations in *BRAF* and other genes. Pretreatment ctDNA detection rates have varied from 33%−77%.[15] In the largest of these studies, the overall detection rate was 76% and patients with undetectable pretreatment ctDNA had significantly longer progression-free survival and overall survival.[4] Although in this study baseline ctDNA detection was described as a categorical variable, the findings were consistent with our quantitative finding that lower pretreatment levels are associated with improved survival. Another smaller study found that decreases in on-treatment ctDNA levels were associated with improved survival outcomes in patients treated with targeted therapies.[16]

With respect to immunotherapies, ctDNA measurements have also been associated with clinical outcomes. Pretreatment detection of *BRAF* or *NRAS* ctDNA was associated with shorter progression-free survival in patients receiving immunotherapy, and two other studies showed that patients with undetectable ctDNA after weeks 5 or 6 of anti–programmed death receptor 1–based therapies had durable responses and long survival. These findings are in slight contrast to our results, which show that patients with undetectable ctDNA after 4 weeks of BRAF/MEK therapy have an improved clinical outcome but may still develop acquired resistance over time.

The current study provides evidence of the clinical validity of ddPCR-based detection of *BRAF* V600–mutant ctDNA and the association with clinical outcome and response to targeted therapy in patients with metastatic melanoma, demonstrating utility of ctDNA as a predictive biomarker. The next step in biomarker development is to determine clinical utility

from analysis of prospective studies. One possibility would be to use this test as a liquid biopsy in cases where there may be difficulties in obtaining tumour material or where there is an urgency for treatment initiation while awaiting results of a tumour biopsy. The turnaround time following blood collection is a matter of days compared with tissue-based testing, which can take weeks for sample acquisition, tissue retrieval, and genomic testing. These benefits are supplemented by the reduced risk and burden of liquid biopsies for patients. Future studies that identify blood-based biomarkers which aid selection between targeted treatment and immunotherapies would also be beneficial. Other potential applications include longitudinal monitoring of patients for disease progression while they are receiving treatment, as we and others have demonstrated previously. The resulting detection of "ctDNA relapse" might inform changes in radiographic scanning schedules outside of the central nervous system. Additionally, future studies could evaluate the added benefit of monitoring ctDNA levels with parallel scan assessments for adjusting treatment strategy, as needed. Finally, mutant ctDNA detection associated with resistance to targeted therapies, such as *NRAS* mutations in melanoma, could motivate a change in treatment plan, as indicated in non-small cell lung cancer with the detection of acquired resistance mutations and treatment with corresponding mutant-selective agents.

The current analysis has several limitations. Only one on-treatment plasma sample was available for ctDNA testing in COMBI-d. Because patients with undetectable ctDNA after 4 weeks of therapy may develop acquired resistance over time, additional longitudinal plasma collections may be needed to accurately predict clinical outcome. Also, only *BRAF* V600 was analysed. Additional variants, such as those associated with acquired resistance, may improve the accuracy of monitoring strategies. With respect to patients with melanoma brain metastases, we found that ctDNA may not be a useful monitoring tool for intracranial disease, confirming reports from other groups. Twenty of 21 samples from nine patients with isolated intracranial disease had no detectable ctDNA (data not shown). There were associations between baseline ctDNA levels and progression-free survival and overall survival as well as ctDNA zeroconversion and extracranial response, but the longitudinal ctDNA results beyond 4 weeks were hard to assess. Furthermore, because a relatively low number of patients composed the validation cohort, including 4 patients who had undetectable ctDNA levels at baseline, confirmational analyses are needed. The poor prognosis of patients with brain metastases limited the ability to analyse response and progression in the brain with sequential ctDNA measurements.

In conclusion, this large-scale study provides a foundation for investigating the clinical utility of ctDNA measurements to guide the management of patients with *BRAF* V600 mutation-positive unresectable or metastatic melanoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

MMS, JMW, and BCC reported a research grant from Novartis for the submitted work. JMW reported their spouse is an employee at Regeneron Pharmaceuticals. GVL reported an advisory role for Aduro, Amgen, Bristol Myers Squibb, Highlight Therapeutics, Mass-Array, Merck, Merck Sharp & Dohme, Novartis, OncoSec Medical, Pierre Fabre, Roche, QBiotics, SkylineDx, and Sandoz. KTF reported consulting for Novartis, Genentech, Bristol Myers Squibb, Merck, Takeda, Verastem, Boston Biomedical, Pierre Fabre, Cell Medica, and Debiopharm; advisory role for X4 Pharmaceuticals, PIC Therapeutics, Sanofi, Amgen, Asana BioSciences, Fount Therapeutics, Aeglea, Shattuck Labs, Tolero, Apricity Therapeutics, Oncoceutics, FogPharma, Array BioPharma, Neon Therapeutics, Tvardi, Monopteros Therapeutics, and Cell Signaling Technologies; board of directors membership for Loxo Oncology, Clovis Oncology, Strata Oncology, Vivid Biosciences, and Checkmate Pharmaceuticals; and stock ownership for Loxo Oncology, Clovis Oncology, Strata Oncology, Vivid Biosciences, Checkmate Pharmaceuticals, X4 Pharmaceuticals, PIC Therapeutics, Fount Therapeutics, Shattuck Labs, Apricity Therapeutics, Oncoceutics, Fog Pharma, Tvardi. DS reported consulting or advisory role for Roche/Genentech, Novartis, Bristol Myers Squibb, Merck Sharp & Dohme, Merck Serono, Amgen, Immunocore, Incyte, 4SC, Pierre Fabre, Mologen, and Sanofi/Regeneron; honoraria from Roche/Genentech, Novartis, Merck Sharp & Dohme, Bristol Myers Squibb, Merck Serono, Amgen, Immunocore, Incyte, 4SC, Pierre Fabre, Array BioPharma, InflaRx, Philogen, Sanofi/Regeneron, Sandoz/Hexal, and Neracare; patient fees to the institution from Roche/Genentech, Novartis, Bristol Myers Squibb, Merck Serono, Amgen, 4SC, Pierre Fabre, Sanofi/Regeneron, Philogen, and Merck Sharp & Dohme; travel, accommodation, and expenses from Roche/Genentech, Novartis, Bristol Myers Squibb, Merck Serono, Amgen, and Merck Sharp & Dohme; speakers bureau for Novartis, Bristol Myers Squibb, and Incyte; and research funding from Novartis and Bristol Myers Squibb. PDN reported advisory role for AstraZeneca, Bristol Myers Squibb, Immunocore, Merck, Merck Sharp & Dohme, Novartis, Pfizer, and 4SC. CR reported an advisory role for Bristol Myers Squibb, Novartis, Amgen, Roche, Merck, and Merck Sharp & Dohme, Pierre Fabre, and Sanofi and personal fees from Biothera and Ultimovacs. AR reported honoraria for consulting from Amgen, Chugai, Roche/Genentech, Novartis, Merck, Sanofi, Vedanta, and Nurix; advisory role and equity ownership for Arcus, Highlight Therapeutics, Compugen, CytomX, Five Prime, RAPT, Merus, Rgenix, PACT Pharma, Tango Therapeutics, Advaxis, Kite Pharma (Gilead), Apricity, ImaginAb, Isoplexis, Lutris Pharma, MapKure, 4C Biomed; and research grant (to his institution) from Agilent and Bristol Myers Squibb. MAD reported consulting for Novartis, GlaxoSmithKline, Genentech, Sanofi Aventis, Array Biopharma, and Bristol Myers Squibb; grant to their institution from GlaxoSmithKline, Genentech, Oncothyreon, and Sanofi Aventis. JJG reported an advisory role for Bristol Myers Squibb, Merck Sharp & Dohme, Novartis, Roche, Amgen, Pierre Fabre, Sanofi, Merck, Pfizer; speaker's role for Bristol Myers Squibb, Merck Sharp & Dohme, Novartis, and Pierre Fabre; and personal fees from Sun Pharma. EG, MS, and JCB reported employment and stock ownership for Novartis. JG and MM reported employment with Novartis. DP reported a research grant from Novartis and National Institutes of Health (NIH) for the submitted work; consulting/advisory role for Novartis and MolecularMD; and laboratory research support from Bio-Rad Laboratories.

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RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed from Jan 1, 2015, to June 1, 2020, for studies including the terms "melanoma" and "circulating DNA" and "therapy" with and without "BRAF". Most publications identified included small patient cohorts involving fewer than 90 patients and used different detection methods with varying degrees of sensitivity. The largest of these studies, which was limited to pretreatment samples, included 732 patients from four clinical trials of targeted therapies and showed that patients negative for *BRAF* V600E/K circulating tumour DNA (ctDNA) had prolonged survival. The only comparable study that evaluated *BRAF* V600-mutant ctDNA levels in pretreatment and on-treatment (after 2 weeks, every 2 months, and at disease progression) samples from patients with metastatic melanoma treated with targeted BRAF and/or MEK inhibitor therapy was a phase 2 trial involving 25 patients, which found a correlation between ctDNA level and disease progression. Additional reports that included analysis of pretreatment and on-treatment (at weeks 5 or 6) *BRAF* or *NRAS* ctDNA levels in patients treated with immune checkpoint inhibitor therapy also demonstrated an association with survival outcomes and durable responses.

Added value of this study

We report findings from an evaluation of *BRAF* V600–mutant ctDNA assessed using analytically validated droplet digital polymerase chain reaction in blood samples collected before treatment and on treatment (week 4) in patients enrolled in the phase 3 COMBI-d and phase 2 COMBI-MB (cohort A) trials. To our knowledge, this study represents the first large-scale analysis of baseline ctDNA and on-treatment modulations and association with progression-free and overall survival in patients with *BRAF* V600 mutation-positive unresectable or metastatic melanoma who were treated with dabrafenib plus trametinib or dabrafenib plus placebo. Our findings support other studies that demonstrated an association between pretreatment and early on-treatment ctDNA levels and clinical survival outcomes. Furthermore, we identified an optimised cut point of 64 copies/mL of plasma for pretreatment samples to separate patients with shorter and longer progression-free and overall survival which was validated in an independent cohort from a second clinical trial (COMBI-MB).

Implications of all the available evidence

Reliable blood-based biomarkers are needed for clinical practice to predict and monitor antitumour activity of targeted treatments in patients with metastatic or unresectable melanoma. Our results provide evidence that pretreatment and on-treatment *BRAF* V600–mutant ctDNA may serve as a blood-based prognostic and predictive biomarker to identify patients who could derive clinical benefit from targeted therapy. These findings can inform future studies to evaluate the clinical utility of assessing *BRAF* V600–mutant ctDNA levels for improved patient management.

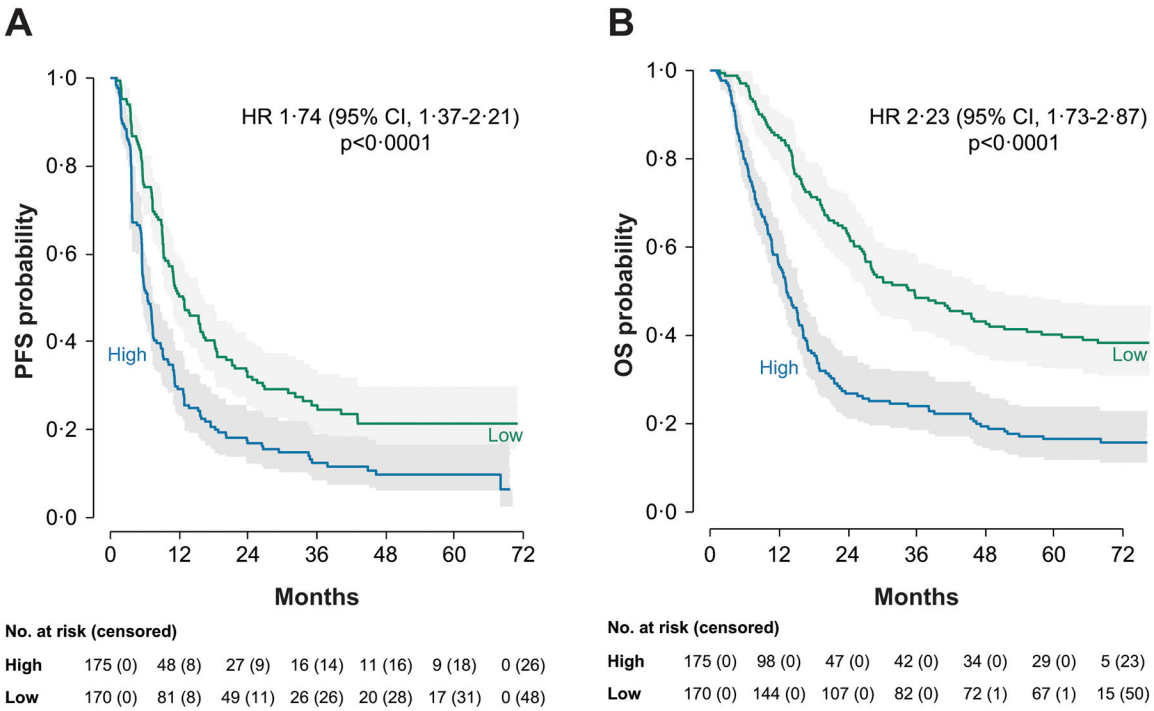
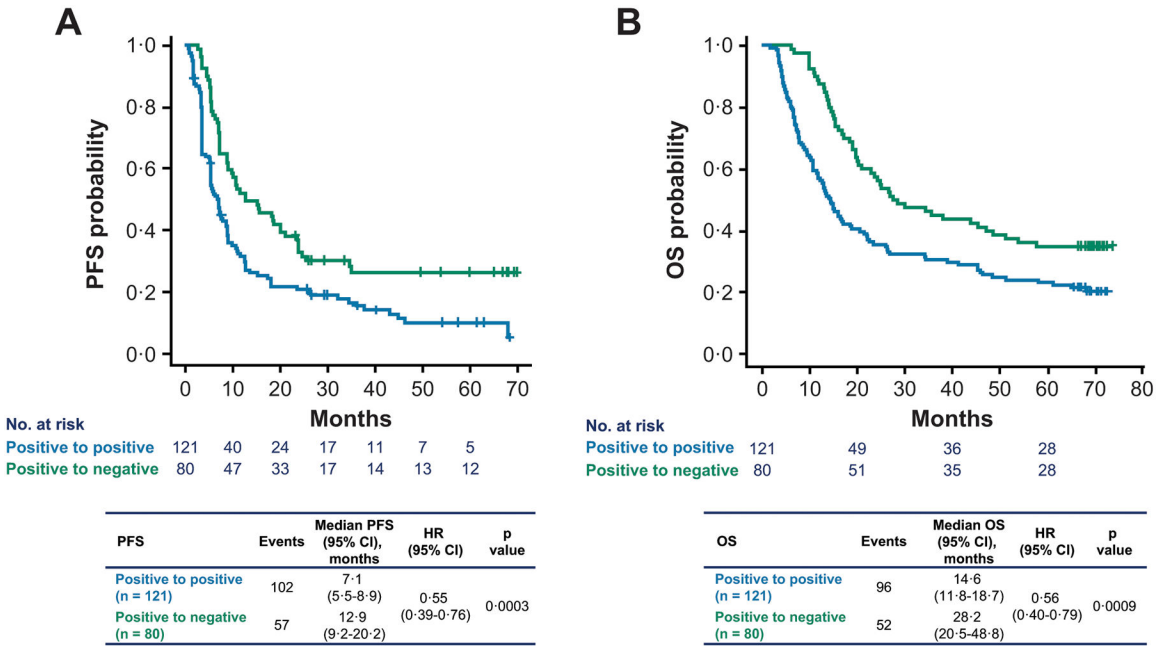


Figure 1. Progression-free survival (A) and overall survival (B) by baseline ctDNA using a cutoff of 64 copies per mL in COMBI-d.

ctDNA=circulating tumour DNA. HR=hazard ratio.



PFS	Events	Median PFS (95% CI), months	HR (95% CI)	p value
Positive to positive (n = 121)	102	7·1 (5·5-8·9)	0·55 (0·39-0·76)	0·0003
Positive to negative (n = 80)	57	12·9 (9·2-20·2)		

OS	Events	Median OS (95% CI), months	HR (95% CI)	p value
Positive to positive (n = 121)	96	14·6 (11·8-18·7)	0·56 (0·40-0·79)	0·0009
Positive to negative (n = 80)	52	28·2 (20·5-48·8)		

Figure 2. Progression-free survival (A) and overall survival (B) by ctDNA status at week 4 in COMBI-d.

ctDNA=circulating tumour DNA. HR=hazard ratio.

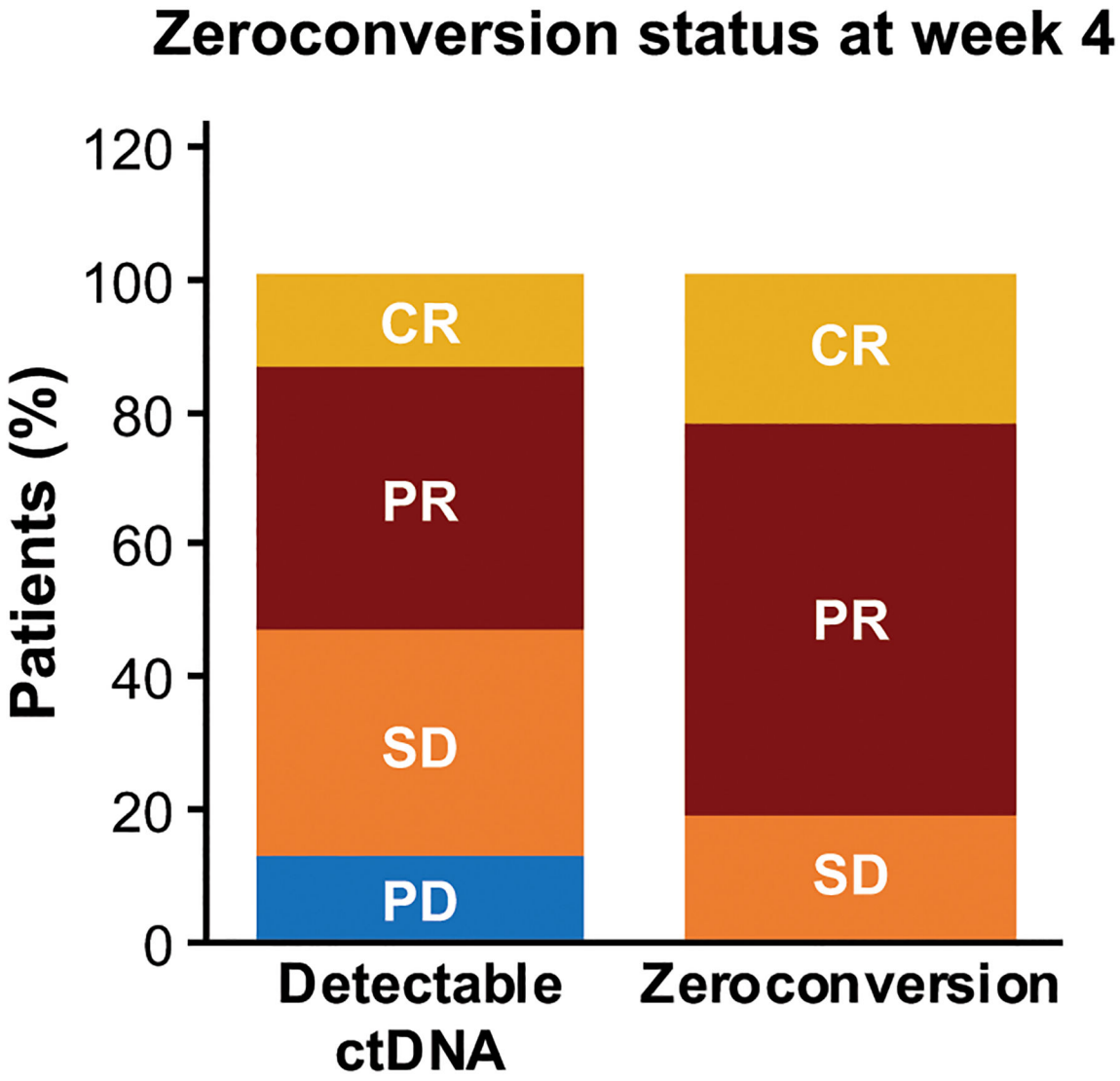


Figure 3: Best overall response by zeroconversion status at week 4 in COMBI-d.
CR=complete response. ctDNA=circulating tumour DNA. PD=progressive disease. PR=partial response. SD=stable disease.

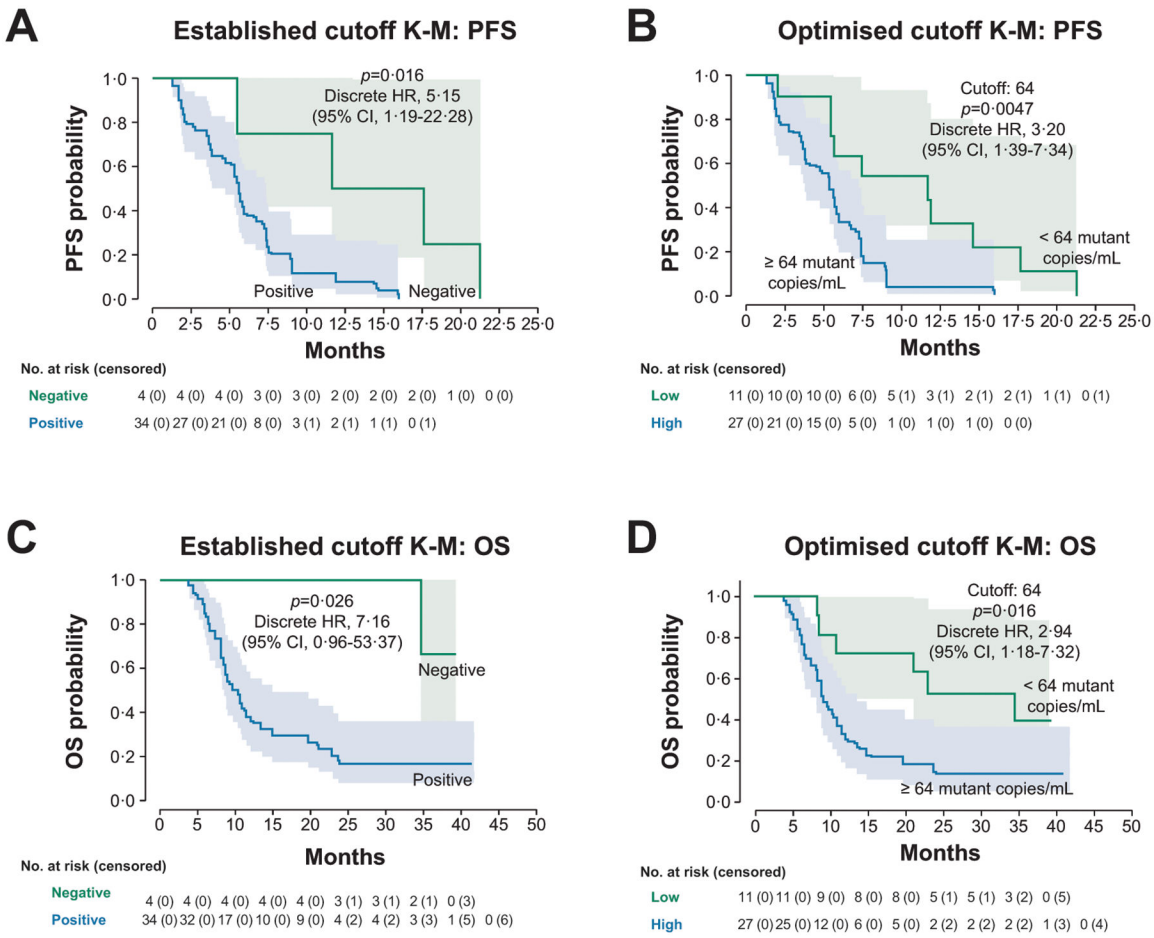


Figure 4. Progression-free survival (A, B) and overall survival (C, D) by baseline ctDNA in COMBI-MB patients.

The established cutoff used to identify ctDNA positive samples was 0·28 copies per mL or 0·019% mutant fraction for BRAFV600E and 0·34 copies per mL or 0·022% mutant fraction for BRAFV600K. The optimised cutoff was 64 copies per mL of plasma. ctDNA=circulating tumour DNA. HR=hazard ratio.

Table 1:

COMBI-d baseline characteristics

Comparison of Patients With and Without Baseline Plasma Samples			
	Present (n = 345)	**Absent (n = 78)**	**Total (N = 423)**
Median age (IQR), years	56·00 (45·00–65·00)	53·50 (45·25–65·75)	56·00 (45·00–65·00)
Female, n (%)	162 (47)	36 (46)	198 (47)
Received dabrafenib plus trametinib, n (%)	170 (49)	41 (53)	211 (50)
LDH strata			
≤ ULN	220 (64)	51 (65)	271 (64)
> ULN	125 (36)	27 (35)	152 (36)
ECOG PS, n (%)			
0	250 (72)	55 (71)	305 (72)
1	95 (28)	21 (27)	116 (27)
Not available	0	2 (3)	2 (< 1)
BRAF mutation, n (%)			
V600E	290 (84)	70 (90)	360 (85)
V600K	54 (16)	8 (10)	62 (15)
V600E and V600K	1 (< 1)	0	1 (< 1)
Number of metastatic sites, n (%) *			
< 3	184 (53)	44 (56)	228 (54)
≥ 3	161 (47)	32 (41)	193 (46)
Not available	0	2 (3)	2 (< 1)
Disease stage, n (%)			
IIIC	13 (4)	2 (3)	15 (4)
IV	332 (96)	75 (96)	407 (96)
Not available	0	1 (1)	1 (< 1)
Median sum of lesion diameters (IQR), mm	57·5 (34·00–97·00)	52·00 (31·00–111·25)	57·00 (33·00–98·75)
Comparison of Patients With and Without Paired Baseline and Week 4 Samples			
	Present (n = 224)†	**Absent (n = 199)**	**Total (N = 423)**
Median age (IQR), years	56·00 (46·00–65·00)	55·00 (45·00–64·00)	56·00 (45·00–65·00)
Female, n (%)	97 (43)	101 (51)	198 (47)
Received dabrafenib plus trametinib, n (%)	109 (49)	102 (51)	211 (50)
LDH strata at baseline			
≤ ULN	143 (64)	128 (64)	271 (64)
> ULN	81 (36)	71 (36)	152 (36)
ECOG PS, n (%)			
0	166 (74)	139 (70)	305 (72)
1	58 (26)	58 (29)	116 (27)
Not available	0	2 (1)	2 (< 1)

BRAF mutation, n (%)			
V600E	187 (83)	173 (87)	360 (85)
V600K	36 (16)	26 (13)	62 (15)
V600E and V600K	1 (< 1)	0	1 (< 1)
Number of metastatic sites, n (%) *			
< 3	124 (55)	104 (52)	228 (54)
≥ 3	100 (45)	93 (47)	193 (46)
Not available	0	2 (1)	2 (< 1)
Disease stage, n (%)			
IIIC	8 (4)	7 (4)	15 (4)
IV	216 (96)	191 (96)	407 (96)
Not available	0	1 (< 1)	1 (< 1)
Median sum of lesion diameters (IQR), mm	57·00 (35·50–93·00)	57·00 (31·00–110·00)	57·00 (33·00–98·75)

ctDNA=circulating tumour DNA. ECOG PS=Eastern Cooperative Oncology Group performance status. IQR=interquartile range. LDH=lactate dehydrogenase. RECIST=Response Evaluation Criteria in Solid Tumours. ULN=upper limit of normal.

*Number of body sites with disease based on unique RECIST target and nontarget lesions identified by the investigator, not the number of metastases.

†Includes two patients with week 4 results who lacked baseline results.

Table 2:

Multivariable Cox model for progression-free and overall survival in COMBI-d including ctDNA detection at week 4 and clinical factors at baseline

	Coef	HR (95% CI)	SE (coef)	*z*	p value
PFS					
Treatment with dabrafenib plus trametinib	−0·34	0·71 (0·52–0·97)	0·16	−2·08	0·037
ctDNA positive at week 4	0·52	1·68 (1·18–2·39)	0·18	2·94	0·0033
Baseline LDH level > ULN	0·39	1·48 (1·06–2·06)	0·17	2·31	0·021
Three or more organ sites with metastases	0·43	1·54 (1·12–2·10)	0·16	2·62	0·0089
BRAF V600K mutation	0·56	1·75 (1·14–2·69)	0·22	2·49	0·013
ECOG PS 1	0·19	1·21 (0·85–1·72)	0·18	1·03	0·30
OS					
Treatment with dabrafenib plus trametinib	−0·25	0·78 (0·51–0·99)	0·17	−1·50	0·13
ctDNA positive at week 4	0·47	1·60 (1·18–2·39)	0·18	2·61	0·009
Baseline LDH level > ULN	0·60	1·83 (1·04–2·10)	0·18	3·41	0·0007
Three or more organ sites with metastases	0·42	1·52 (1·10–2·15)	0·17	2·46	0·014
BRAF V600K mutation	0·68	1·97 (1·14–2·69)	0·22	3·13	0·0018
ECOG PS 1	0·52	1·68 (0·85–1·72)	0·18	2·83	0·0046

Analysis includes 224 patients with ctDNA plasma samples available at week 4; 96 plasma samples were negative, and 128 plasma samples were positive. Progression-free and overall survival analyses suggest that hazards may not have been proportional between the week 4 ctDNA groups; for overall survival, the baseline LDH groups may not have had proportional hazards.

coef=coefficient. ctDNA=circulating tumour DNA. ECOG PS=Eastern Cooperative Oncology Group performance status. exp (coef)=exponentiated coefficient. HR=hazard ratio. LDH=lactate dehydrogenase. OS=overall survival. PFS=progression-free survival. SE=standard error. ULN=upper limit of normal.