Changes in Circulating Tumor DNA Reflect Clinical Benefit Across Multiple Studies of Patients With Non–Small-Cell Lung Cancer Treated With Immune Checkpoint Inhibitors

Diana Merino Vega, PhD1; Katherine K. Nishimura, PhD, MPH2; Névine Zariffa, PhD3; Jeffrey C. Thompson, MD1; Antje Hoering, PhD2; Vanessa Ciento, MPH4; Adam Rosenthal, MS5; Valsamo Anagnostou, MD, PhD5; Jonathan Baden, MS5; Julia A. Beaver, MD1; Aadel A. Chaudhuri, MD, PhD8,9,10,11; Darya Chudova, PhD12; Alexander D. Fine, PharmD13; Rachel Hodge, PhD13; Darren Hodgson, PhD16; Nathan Hunkapiller, PhD17,18; Daniel M. Klass, PhD19; Julie Koblis, PhD20; Carol Peña, PhD21; Gene Pennello, PhD, MS22; Neil Peterman, PhD23; Reena Philip, PhD24; Katie J. Quinn, PhD12; David Raben, MD25; Gary L. Rosner, ScD5; Mark Sausen, PhD6; Ayse Tezcan, MPH, PhD23; Qi Xia, PhD26; Jing Yi, PhD25; Amanda G. Young, PhD13; Mark D. Stewart, PhD1; Erica L. Carpenter, MBA, PhD27; Charu Aggarwal, MD, MPH27; and Jeff Allen, PhD1

PURPOSE As immune checkpoint inhibitors (ICI) become increasingly used in frontline settings, identifying early indicators of response is needed. Recent studies suggest a role for circulating tumor DNA (ctDNA) in monitoring response to ICI, but uncertainty exists in the generalizability of these studies. Here, the role of ctDNA for monitoring response to ICI is assessed through a standardized approach by assessing clinical trial data from five independent studies.

PATIENTS AND METHODS Patient-level clinical and ctDNA data were pooled and harmonized from 200 patients across five independent clinical trials investigating the treatment of patients with non–small-cell lung cancer with programmed cell death-1 (PD-1)/programmed death ligand-1 (PD-L1)–directed monotherapy or in combination with chemotherapy. CtDNA levels were measured using different ctDNA assays across the studies. Maximum variant allele frequencies were calculated using all somatic tumor-derived variants in each unique patient sample to correlate ctDNA changes with overall survival (OS) and progression-free survival (PFS).

RESULTS We observed strong associations between reductions in ctDNA levels from on-treatment liquid biopsies with improved OS (OS; hazard ratio, 2.28; 95% CI, 1.62 to 3.20; \( P < .001 \)) and PFS (PFS; hazard ratio 1.76; 95% CI, 1.31 to 2.36; \( P < .001 \)). Changes in the maximum variant allele frequencies ctDNA values showed strong association across different outcomes.

CONCLUSION In this pooled analysis of five independent clinical trials, consistent and robust associations between reductions in ctDNA and outcomes were found across multiple end points assessed in patients with non–small-cell lung cancer treated with an ICI. Additional tumor types, stages, and drug classes should be included in future analyses to further validate this. CtDNA may serve as an important tool in clinical development and an early indicator of treatment benefit.

INTRODUCTION The recent approval of programmed cell death-1 (PD-1)/programmed death ligand-1 (PD-L1) inhibitors as frontline therapy for advanced non–small-cell lung cancer (NSCLC) has changed the treatment paradigm for this disease.1,6 However, not all patients respond to immune checkpoint inhibitors (ICI), and some may experience clinically significant, and sometimes long-lived, toxicity.7 Disease response is currently assessed with clinical and radiographic evaluation, with the first imaging assessment usually after 8 weeks on ICIs. However, clinical assessments are subjective, difficult to standardize, may lack the necessary sensitivity to identify very early stages of progressive disease, and may misinterpret tumor responses in the case of pseudoprogression.8 Hence, accurate, early, and objective predictors of response to ICI therapy are needed. Next-generation sequencing of circulating tumor DNA (ctDNA) has been recently established as a sensitive, less invasive, and accurate means to detect therapeutically actionable mutations in patients as well as to identify the emergence of resistance mutations in patients receiving targeted therapies. However, the use of this technology to monitor response to therapy is less

ASSOCIATED CONTENT Data Supplement Author affiliations and support information (if applicable) appear at the end of this article.

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**CONTEXT**

**Key Objective**
Can changes in circulating tumor DNA (ctDNA) reflect clinical benefit across multiple, independent studies of patients with non–small-cell lung cancer treated with immune checkpoint inhibitors?

**Knowledge Generated**
Analyses confirm an association between changes in ctDNA levels and clinical benefit for patients with non–small-cell lung cancer treated with varying lines of anti–programmed cell death-1 (PD-1)/programmed death ligand-1 (PD-L1) therapy. Harmonization strategies were developed to help address differences in ctDNA collection timing, ctDNA assay results, and clinical variables across different clinical studies.

**Relevance**
Our study provides supporting evidence that ctDNA may serve as an early predictor of treatment response. Given the multitude of recent studies investigating the use of ctDNA as a minimally invasive way to measure treatment outcome, these results are timely by confirming observations seen across multiple, independent studies and by outlining harmonization strategies to support future studies and meta-analyses to validate ctDNA as an end point in drug development.

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defined for ICIs. Serial ctDNA measurements may yield additional insights into a patient’s disease, providing a more timely assessment of response to treatment than traditional clinical and radiologic assessments. If shown to correlate with treatment response, monitoring ctDNA changes during treatment may improve disease management.

Several recent studies suggest a potential role for ctDNA in monitoring response to ICI therapy and have investigated how changes in ctDNA levels may be associated with outcomes. These studies have identified a correlation between on-therapy reductions in ctDNA and objective response rate, progression-free survival (PFS), and overall survival (OS). However, uncertainty exists in the generalizability of these studies, since they often used different methods of ctDNA assessment, had variable on-treatment blood collection time points, had heterogeneity in the patient populations, and implemented a variety of methods to calculate ctDNA changes over time.

To address the need for a standardized approach to assess the role of ctDNA as a potential tool for monitoring response to ICI treatment as well as to develop a robust data set evaluating the relationship between ctDNA changes during ICI treatment and clinical outcomes, Friends of Cancer Research (Friends) launched the ctDNA for Monitoring Treatment Response (ctMoniTR) pilot project. The first step of ctMoniTR pooled and harmonized data from five independent studies focused on patients with NSCLC receiving PD-(L)1–directed monotherapy or combination with chemotherapy. The results from this multi-institutional study are presented and discussed, providing further evidence of ctDNA as a noninvasive and dynamic indicator of clinical outcome to ICI.

**PATIENTS AND METHODS**

**Patients**
Anonymized patient-level clinical and ctDNA data from five independent clinical trials were collected and included 254 patients (Data Supplement). Each study reviewed patients’ informed consent approved by the local institutional review board to ensure their data were suitable for secondary use beyond their original intent. Patients with NSCLC who had been treated with varying lines of anti–PD-(L)1 therapy, either as monotherapy or in combination with standard chemotherapy, and who had a pretreatment ctDNA sample (no earlier than 14 days before the start of treatment) and at least one on-treatment ctDNA sample (no later than 70 days from the initiation of treatment) were included. As this was a pilot project, these time points were selected to allow inclusion of the largest number of samples. The five data sets were split into seven cohorts, with each cohort representing a unique study or trial arm. Initial criteria for patient inclusion/exclusion and strategies for minimizing bias in a combined data set were established before analysis (Data Supplement).

**Clinical Outcomes and Covariates**
OS and PFS were defined as the number of days between treatment initiation and death resulting from any cause, and the number of days between treatment initiation and death from any cause or progression, respectively. Tumor response was evaluated according to the RECIST, version 1.1, and confirmed by local or central review. Durable clinical benefit was defined as maintenance of PFS at 6 months from treatment initiation (PFS6). Patients who did not progress on study but were lost to follow-up within 6 months of treatment initiation (n = 11) were excluded from the PFS6 analysis. Additional clinical descriptors were collected and harmonized according to a common set of definitions (Data Supplement).

**ctDNA Data**
All studies used similar plasma collection methods (Data Supplement) that met the minimum prespecified assay standards (Data Supplement) and provided ctDNA results according to their individual protocols. Various next-generation
sequencing–based ctDNA assays (Data Supplement), including targeted panels and whole-genome sequencing, were used and, as a result, performance metrics may vary across the platforms. Variant allele frequencies (VAF), defined as the number of mutant alleles divided by the total number of mutant and wild-type alleles, were reported from four of the five studies. The fifth study assessed ctDNA changes with a whole-genome sequencing approach using copy-number alterations and local changes in ctDNA fragment length to determine a tumor fraction ratio. Variants contributing to the calculation of VAF met internal assay-specific quality standards. Germline and clonal hematopoiesis variants were removed according to each study’s original protocol (Data Supplement) or, for one study, by the independent analysis center (Data Supplement).

**Derived ctDNA Metrics**

Mean, median, and maximum VAF values were calculated using all somatic tumor-derived variants eligible for analysis in each unique patient sample, regardless of whether they were detected at baseline. For patients with nondetectable (ND) ctDNA, the VAFs were assumed to be indeterminately low and were set to a value of 0; additional data handling details are in the Data Supplement. The percent change of the mean, median, or maximum VAF value from baseline (T0) to the first on-treatment sample collected within 70 days of treatment initiation (T1) was calculated as

\[
\text{Percent Change of Mean VAF} = \left( \frac{\text{mean VAF}_{T1}}{-\text{mean VAF}_{T0}} \right)
\]

\[
\text{Percent Change of Median VAF} = \left( \frac{\text{median VAF}_{T1}}{-\text{median VAF}_{T0}} \right)
\]

\[
\text{Percent Change of Maximum VAF} = \left( \frac{\text{maximum VAF}_{T1}}{-\text{maximum VAF}_{T0}} \right)
\]

Then, three types of ctDNA metrics were calculated for analysis: (1) continuous percent change variable using the raw percent change value, with a cap in cases with percentage increase of 500% to mitigate the impact of outliers; (2) binary variable using a cutpoint of −50% change in VAF as the threshold, where this optimal cutpoint was determined using the running log-rank method, and (3) the three-level variable, which used cohort-specific thresholds to identify the 50% most extreme patients within each cohort exhibiting a strong decrease in ctDNA from baseline (decrease), the 50% most extreme patients exhibiting a strong increase in ctDNA (increase), and the remaining patients in a middle category with modest reductions or increases in ctDNA (intermediate; Data Supplement).

**Statistical Analyses**

The three-level ctDNA metric was modeled as an ordinal variable with three categories representing patients with a decrease in ctDNA from baseline, an intermediate change, or an increase. Kruskal-Wallis tests were used to compare the medians of continuous variables, and Wald chi-Square tests were used to compare proportions of categorical variables, with Fisher’s exact test used in cases where assumptions for utilization of the chi-square test were not met. Survival probabilities (OS and PFS) were estimated using the Kaplan-Meier method, using a 70-day landmark from treatment initiation to ensure that the ctDNA metric reflected a change in ctDNA that occurred before patients were assessed for survival outcome. Overall and pairwise comparisons between strata in Kaplan-Meier analyses were calculated using log-rank tests. Univariate and multivariate Cox proportional hazards models were used to assess associations with OS and PFS, with P values derived from the log-likelihood test, and covariates that were measured after treatment initiation modeled as time-dependent covariates. Univariate and multivariate logistic regression models were used to assess associations with binary clinical end points (partial response [PR] or better, and PFS). All models accounted for cohort-specific risks using cohort-stratified models, where cohort was adjusted by stratification, which allows for a different baseline risk within each cohort group. All statistical tests with P value < .05 were considered statistically significant. As this was an exploratory pilot project, P values were not adjusted for multiple tests. Analyses were done using the SAS statistical software package (SAS Institute, Cary, NC) or R (R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Analysis Data Set**

A total of 254 patients were considered for inclusion, with 200 patients included in the final data set after excluding patients who failed to meet study criteria (Fig 1; full population demographics shown in the Data Supplement). Broad heterogeneity was observed across cohorts with noticeable differences in age, sex, stage at enrollment, histology, programmed death ligand-1 (PD-L1) expression, and number of prior lines of therapy (Table 1). Among all clinical covariates, smoking history was the only one to be univariately associated with changes in ctDNA values (Data Supplement).

**ctDNA Collection Timing and ctDNA Metrics**

Descriptive analyses revealed that the timing and frequency of ctDNA samples varied between cohorts because of differences in the protocols used within each study (Fig 2). There was also variability across cohorts in the number of variants detected, the magnitude of VAF values, and the range of baseline mean, median, and maximum VAF values (Data Supplement). Considering the likelihood that differences in these data could be related to the assay used, the 3-level Max VAF Percent Change Group results are shown here, since this metric accounted for differences in distributions by using cohort-specific thresholds to categorize patients. This metric also demonstrated the most consistent results for OS, PFS, and durable clinical benefit. The results for the other ctDNA metrics are available in the Data Supplement. Within the 3-level Max VAF Percent Change
Group metric, 63 (32%) patients had a decrease, 103 (51%) had an intermediate change, and 34 (17%) an increase in ctDNA levels from baseline while on treatment.

**Changes in ctDNA Are Associated With Survival End Points**

Strong and consistent associations between reductions in ctDNA levels and improved OS were observed in unadjusted Cox models (Data Supplement) and adjusted Cox models with cohort stratification and adjustment by baseline clinical covariates (Fig 3A). For example, each increase in the category of the three-level Max VAF Percent Change Group variable (from decrease, to intermediate, to increase in Max VAF) was associated with an increased risk of death (adjusted hazard ratio, of 2.28 [95% CI, 1.62 to 3.20; \( P \), .001]), after adjusting for baseline clinical covariates. Baseline ctDNA values, including ND samples, were not found to be associated with OS (Data Supplement). OS Kaplan-Meier plots showed a strong separation in the different ctDNA categories, with statistically significant differences in the pairwise comparisons, and 1-year survival rates of 75%, 58%, and 32% for patients with a decrease, intermediate change, or increase in Max VAF, respectively (Fig 3B). Additional Kaplan-Meier and univariate associations for OS are available in the Data Supplement. Of note, in the adjusted Cox models for both OS and PFS, smoking history was associated with improved survival outcomes. This finding is consistent with previous studies that argued that cancers resulting from the accumulation of tobacco-related mutations may have increased tumor mutational burden and respond especially favorably to immunotherapies.30,31 Additionally, there was a lack of association with PD-L1 positivity, which was likely because of variation in how it is measured and defined in each clinical trial.

**Changes in ctDNA Are Associated With Improved Tumor Response**

Reductions in ctDNA were also associated with improved tumor response, defined as achieving a RECIST classification of PR or complete response. Logistic regression models with cohort stratification and adjustment by baseline clinical covariates yielded an adjusted odds ratio of 0.19 (95% CI, 0.08 to 0.45; \( P < .001 \) ) for intermediate versus decrease and 0.11 (0.03 to 0.38) for increase versus decrease, suggesting that each increase in the strata of the three-level Max VAF Percent Change Group variable was associated with a decreased likelihood in achieving PR or better, after adjusting for baseline clinical covariates (Table 2). Baseline ctDNA values were not univariately associated with achieving PR or better (Data Supplement). Additional univariate associations and results for other ctDNA metrics are included in the Data Supplement.

**Changes in ctDNA Are Associated With Durable Clinical Benefit**

Logistic regression models with cohort stratification and adjustment by baseline clinical covariates found that
| Trait                      | Description                                      | Cohort 1  | Cohort 2  | Cohort 3a | Cohort 4a | Cohort 4b | Cohort 5a | Cohort 5b | P | Overall, n/N (%) |
|----------------------------|--------------------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---|-----------------|
| Age, years                 | Age ≥ 66 years at enrollment, No. (%)            | 19/20 (95)| 9/16 (56) | 19/43 (44)| 16/55 (29)| 5/15 (33) | 12/24 (50)| 14/27 (52)| < .001 | 94/200 (47)    |
| Sex                        | Female, No. (%)                                 | 9/20 (45) | 8/16 (50) | 10/43 (23)| 22/55 (40)| 2/15 (13) | 15/24 (63)| 11/27 (41)| .013  | 77/200 (39)    |
| Race                       | White, No. (%)                                  | 13/20 (65)| 8/14 (57) | 32/43 (74)| 28/55 (51)| 11/15 (73)| 15/24 (63)| 22/27 (81)| .094  | 129/198 (65)   |
| Smoking status             | Ever smoked, No. (%)                            | 15/20 (75)| 12/14 (86)| 37/43 (86)| 47/55 (85)| 12/15 (80)| 23/24 (96)| 24/27 (89)| .577a | 170/198 (85.9) |
| ECOG                       | ECOG performance status ≥ 1, No. (%)            | 13/19 (68)| ND        | 26/43 (60)| 35/55 (64)| 12/15 (80)| 11/24 (46)| 18/27 (67)| .360  | 115/183 (63)   |
| Stage at enrollment        | Advanced stage (stage IV), No. (%)              | 18/20 (90)| 14/16 (88)| 43/43 (100)| 39/55 (71)| 13/15 (87)| 24/24 (100)| 27/27 (100)| < .001a | 178/200 (89)   |
| Histology                  | Squamous, No. (%)                               | 3/20 (15) | 7/16 (44) | 12/43 (28)| 13/55 (24)| 9/15 (60) | 7/24 (29) | 0/27 (0)  | < .001 | 51/200 (26)    |
| PD-L1 expression           | PD-L1–positive (TPS ≥ 1%, or TC/IC)              | 14/19 (74)| 11/11 (100)| 30/42 (71)| 48/53 (91)| 12/15 (80)| 23/24 (96)| 13/26 (50)| < .001a | 151/190 (79)   |
| Prior therapy              | Prior lines of systemic treatment ≥ 1, No. (%)  | 8/20 (40) | 11/15 (73)| 43/43 (100)| 55/55 (100)| 11/15 (73)| 6/24 (25) | 0/27 (0)  | < .001 | 134/199 (67)   |

NOTE. Each cohort represents a unique study (cohort number) or trial arm (cohort letter) within a study. The proportion is calculated as the percent of patients with a given trait, within each cohort. If a patient was missing data on a given trait, this was reflected in the total count for the cohort; therefore, N may be < 200. Studies were blinded for analyses.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ND, no data; PD-L1, programmed death ligand-1; TC/IC, tumor cells/immune cells; TPS, tumor proportion score.

*P value from Fisher’s exact test, otherwise χ².
decreases in ctDNA were associated with achieving durable clinical benefit, defined as PFS \( \geq 6 \) months (PFS6). This analysis yielded an adjusted odds ratio of 0.13 (95% CI, 0.05 to 0.34; \( P < .001 \)) for intermediate versus decrease, and 0.06 (95% CI, 0.02 to 0.22) for increase versus decrease, interpreted as a decreasing likelihood of achieving PFS6 with each increase in the ctDNA Max VAF metric category (Table 2). No other clinical covariates were statistically significant in the adjusted model, and the ctDNA values at baseline were also not found to be associated with PFS6. Additional univariate associations and results for other ctDNA metrics are included in the Data Supplement.

**DISCUSSION**

Among patients with NSCLC treated with ICI whose data were analyzed in aggregate, consistent and robust associations between reductions in ctDNA and clinical benefit were found across multiple end points. Although the results presented in this manuscript are consistent with recent reports, these individual studies have limited sample sizes, and were constrained in their generalizability, given that each study used a particular treatment and a specific ctDNA assay on a carefully selected group of patients.\(^{19-23,25,32}\) The heterogeneity of the data sets included required various harmonization strategies to address differences in ctDNA collection timing, ctDNA assay results, and clinical variables. These strategies successfully minimized bias and confounding factors and were equally valuable in establishing useful methodologies for combining data sets collected from disparate sources. By pooling and harmonizing the results from independent studies, the results of this study show that, even when analyzed across five different clinical trials, using multiple ICIs in differing NSCLC populations, with different sample collection time points and different ctDNA assays, the on-treatment changes in ctDNA levels correlate with outcome. These correlations hold true in analyses using ctDNA as a dichotomized, trichotomized, or continuous variable, and using all outcome measures evaluated (OS, PFS, best response, and PFS6).

In the literature, there is a lack of standardization in the methods used to quantitate ctDNA changes and evaluate their association with clinical outcomes. Previous studies have generally used different metrics, such as mean, median, or maximum VAF, mutant molecules per unit volume of blood or plasma, or absolute numbers of mutations observed at one point in time.\(^{24,32}\) Moreover, different thresholds have been used to determine significant changes in ctDNA, such as one log reduction, two-fold change or statistically distinguishable changes with non-overlapping CIs, percent change in the absolute ctDNA levels, or a ratio of on-treatment VAF to baseline VAF, with a molecular response set at > 50% decrease.\(^{19,21,23,32,33}\)
Thus, one aim of this study was to compare different ctDNA metrics to identify those that yielded the most consistent and robust associations across multiple technologies and clinical outcomes. The analyses presented in this manuscript were focused on metrics on the basis of VAF or tumor fraction values, since these were available for studies in this evaluation.

When comparing changes in the mean, median, or maximum VAF values, it was generally observed that the mean and maximum VAF ctDNA values showed similarly strong and consistent univariate associations with different outcomes, whereas median VAF had a weak and inconsistent signal (Data Supplement). One possibility is that median values minimize the impact of large, outlier VAF values that
are clinically meaningful, suggesting that large VAF values may be the most informative when assessing treatment responses. Thus, the single highest somatic VAF value, regardless of the gene and mutation that contributed to the calculation, may be a superior proxy for disease burden, as opposed to other summary measurements that give more weight to rare variants with low VAFs. However, capturing a single highest variant will be sensitive to the panel used, and a mean VAF may be more robust across tumor types and molecular subtypes, especially those without defined driver mutations.

When comparing the continuous, two-level, and three-level ctDNA metrics, the three-level, and to a lesser
### TABLE 2. Multivariate Testing for Association With PR or Better, or PFS at 6 Months

| Factor                               | PR or Better (N = 187) |            | P      | PFS at 6 Months (N = 178) |            | P      |
|--------------------------------------|------------------------|------------|--------|---------------------------|------------|--------|
|                                      | With Factor With Factor | Without Factor | OR (95% CI) | P            | With Factor With Factor | Without Factor | OR (95% CI) | P      |
| Age ≥ 66, years, No. (%)             | 28/88 (32)             | 32/99 (46) | 0.85 (0.39 to 1.89) | .696         | 43/84 (51)             | 43/94 (46) | 0.86 (0.38 to 2.04) | .735 |
| Female, No. (%)                      | 25/70 (36)             | 35/117 (30) | 2.31 (1.00 to 5.31) | .050         | 35/65 (54)             | 51/113 (45) | 1.84 (0.78 to 4.37) | .165 |
| White, No. (%)                       | 40/122 (33)            | 20/65 (31) | 0.77 (0.35 to 1.71) | .521         | 64/119 (54)            | 22/59 (37) | 2.12 (0.93 to 4.85) | .075 |
| Ever smoked, No. (%)                 | 57/160 (36)            | 3/27 (11)  | 3.23 (0.80 to 13.01) | .100         | 81/153 (53)            | 5/25 (20)  | 2.44 (0.76 to 7.86) | .134 |
| Advanced stage (stage IV), No. (%)   | 56/168 (33)            | 4/19 (21)  | 3.45 (0.85 to 14.04) | .097         | 82/160 (51)            | 4/18 (22)  | 3.52 (0.86 to 14.49) | .081 |
| Squamous, No. (%)                    | 18/46 (39)             | 42/141 (30)| 2.62 (1.06 to 6.49) | .037         | 18/42 (43)             | 68/136 (50)| 1.12 (0.45 to 2.81) | .812 |
| PD-L1 3-level, No. (%)               |                        |            |        |                           |            |        |
| 0%                                   | 15/39 (38)             | Reference  | —      |                           | 21/38 (55) | Reference | —      |
| 1%-49%                               | 14/62 (23)             | 0.45 (0.15 to 1.31) | .144 | 24/61 (39) | 0.51 (0.17 to 1.55) | .236 |
| ≥ 50%                                | 31/86 (36)             | 0.75 (0.24 to 2.30) | .614 | 41/79 (52) | 0.64 (0.20 to 2.08) | .462 |
| Prior systemic treatment ≥ 1, No. (%)| 27/61 (44)             | 27/61 (44) | 0.77 (0.19 to 3.12) | .714         | 44/121 (36)            | 42/57 (74) | 0.77 (0.20 to 2.95) | .706 |
| 3-level max VAF percent change group, No. (%) | 34/59 (58) | Reference | — | 43/56 (77) | Reference | — |
| Decrease                             | 22/95 (23)             | 0.19 (0.08 to 0.45) | < .001 | 36/90 (40) | 0.13 (0.05 to 0.34) | < .001 |
| Intermediate                         | 4/33 (12)              | 0.11 (0.03 to 0.38) | < .001 | 7/32 (21.8) | 0.06 (0.02 to 0.22) | < .001 |

NOTE. The proportion is calculated as the percent of patients with a given factor, who had the outcome (PR, or better, or PFS, at 6 months). Conversely, among the 99 patients younger than 66 years, 32 patients (or 46%) achieved PR, or better.

Abbreviations: NA, not applicable; OR, odds ratio; PD-L1, programmed death ligand-1; PFS, progression-free survival; PR, partial response; P, P-value from Wald \( \chi^2 \) test in logistic regression; VAF, variant allele frequency.
extent, the two-level ctDNA metric (Data Supplement), consistently showed strong associations with patient outcomes. The continuous ctDNA metric on the basis of the raw percent change value had inconsistent associations with patient outcomes. Modeling the continuous variable was challenging, as the natural range of a percent change calculation (potentially ranging from –100% to +infinity) made data transformations problematic to implement and produced a distribution of the values that resulted in several outliers that could strongly bias a model that assumes a linear association. Conversely, the three-level ctDNA metric grouped extreme and moderate patients (who unquestionably had a substantial change in their ctDNA levels from baseline) and appeared to classify patients into appropriate categories despite potential differences that may exist across ctDNA platforms or clinical situations. Absolute ctDNA values, such as mutant molecules per volume of plasma, were not evaluated because these data were not available for all studies but should be examined in greater depth in future studies. Assessment of overall tumor fraction from plasma data is a field with ongoing development. Incorporating analytical characteristics of specific assays, like limit of detection and precision as well as further improvements on filtering and dynamics of variant VAFs over time, could be hypothesized to further improve predictive power of response assessment. These should continue to be integrated into assessment of molecular response, building off the standardized VAF-based approaches established within the ctMoniTR Project.

Other lines of inquiry include determining how early a change in ctDNA can accurately reflect a patient’s response to treatment, especially if it can reveal tumor responses earlier than radiographic evaluation, and whether baseline ctDNA values are associated with clinical outcomes, as this has been reported previously.¹² In the current study, however, we did not observe an association between baseline ctDNA VAF and clinical outcomes, which could be related to all patients harboring advanced NSCLC or failing a prior line of systemic therapy. Still, our data suggest that ctDNA measurements may help guide treatment decisions, either independently or in conjunction with radiographic evaluation, especially in tumors that are challenging to assess.

Future work from the ctMoniTR Project will expand the scope to include additional tumor types, stages, and drug classes to further validate the association between harmonized ctDNA levels and clinical outcomes in different clinical settings. More specifically, future analyses will focus on better understanding how early changes in ctDNA could be associated with treatment outcomes, and how longitudinal ctDNA measurements can reflect ongoing changes in an actively evolving tumor. Larger data sets will also enable subgroup analyses where relevant covariates can be further investigated. Future efforts will aim to recommend common standards for ctDNA evaluation for use in pharmaceutical trials and clinical practice. Additionally, standardization of ctDNA sampling time points is recommended for future studies, and additional modeling techniques to account for left-truncated data may be considered in future analyses.²⁴ Ongoing work in the ctMoniTR Project will focus on improving measurements and comparability in ctDNA studies, facilitating acceleration in the regulatory adoption of reliable ctDNA measures of responsiveness to treatment, and investigating ctDNA as an intermediate measure of treatment success.

**AFFILIATIONS**

1Friends of Cancer Research, Washington, DC
2Cancer Research And Biostatistics (CRAB), Seattle, WA
3NMD Group LLC, Bala Cynwyd, PA
4Division of Pulmonary, Allergy and Critical Care Medicine, Thoracic Oncology Group, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
5Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD
6Translational Medicine, Bristol Myers Squibb, Princeton, NJ
7Oncology Center of Excellence, Bristol-Myers Squibb, Princeton, NJ
8Department of Radiation Oncology, Washington University School of Medicine, St Louis, MO
9Department of Genetics, Washington University School of Medicine, St Louis, MO
10Department of Computer Science and Engineering, Washington University, St Louis, MO
11Siteman Cancer Center, Washington University School of Medicine, St Louis, MO
12Guardant Health Inc, Redwood City, CA
13Research and Development, Foundation Medicine Inc, Cambridge, MA
14Oncology Development, Bristol Myers Squibb, Princeton, NJ
15Late Oncology Statistics, Oncology Biometrics, AstraZeneca, Cambridge, United Kingdom
16Translational Medicine, Oncology Research & Development, AstraZeneca, Waltham, MA
17GRAIL, Menlo Park, CA
18During the conduct of this work and development of the manuscript, N.H. was affiliated with GRAIL, Inc; however, is not affiliated with GRAIL, Inc at the time of submission.
19Assay Development, Roche Sequencing Solutions, Pleasanton, CA
20Translational Oncology, Early Oncology Statistics, Merck Research Laboratories, Kenilworth, NJ
21Companion Diagnostics, Oncology Early Development, Merck Research Laboratories, Kenilworth, NJ
22Division of Imaging, Diagnostics, and Software Reliability, Office of Science and Engineering Laboratories, Food and Drug Administration (FDA), Silver Spring, MD
23Foundation Medicine Inc, San Diego, CA
24Division of Molecular Genetics, Office of Health Technology 7 (In Vitro Diagnostics and Radiological Health), Food and Drug Administration (FDA), Silver Spring, MD
25Product Development Oncology, Genentech Inc, South San Francisco, CA
26Product Development Data Sciences, Genentech Inc, South San Francisco, CA
27Division of Hematology and Oncology, Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
Changes in ctDNA Reflect Clinical Benefit in NSCLC

CORRESPONDING AUTHOR
Mark D. Stewart, PhD, Friends of Cancer Research, 1800 M St NW, Suite 1050 South, Washington, DC 20036; e-mail: mstewart@focr.org.

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AUTHOR CONTRIBUTIONS
Conception and design: Diana Merino Vega, Katherine K. Nishimura, Névine Zariffa, Vanessa Cilento, Valsamo Anagnostou, Jonathan Baden, Julia A. Beaver, Darya Chudova, Joseph Fiore, Rachel Hodge, Darren Hodgson, Nathan Hunkapiller, Julie Kobie, Carol Peña, Gene Pennello, Reena Philip, David Raben, Mark Sausen, Ayse Tezcan, Qi Xia, Jing Yi, Mark D. Stewart, Erica L. Carpenter, Charu Aggarwal, Jeff Allen
Financial support: Amanda G. Young
Provision of study materials or patients: Jonathan Baden, Charu Aggarwal
Collection and assembly of data: Diana Merino Vega, Katherine K. Nishimura, Névine Zariffa, Jeffrey C. Thompson, Vanessa Cilento, Valsamo Anagnostou, Julia A. Beaver, Joseph Fiore, Rachel Hodge, Darren Hodgson, Daniel M. Klass, Neil Peterman, Katie J. Quinn, Ayse Tezcan, Qi Xia, Jing Yi, Mark D. Stewart, Charu Aggarwal
Data analysis and interpretation: Diana Merino Vega, Katherine K. Nishimura, Névine Zariffa, Antje Hoering, Vanessa Cilento, Adam Rosenthal, Valsamo Anagnostou, Jonathan Baden, Julia A. Beaver, Aadel A. Chaudhuri, Alexander D. Fine, Joseph Fiore, Rachel Hodge, Darren Hodgson, Carol Peña, Reena Philip, Katie J. Quinn, David Raben, Gary L. Rosner, Mark Sausen, Ayse Tezcan, Qi Xia, Jing Yi, Amanda G. Young, Mark D. Stewart, Erica L. Carpenter, Charu Aggarwal
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
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Diana Merino Vega
Employment: AstraZeneca/MedImmune, AllStripes Research Inc
Stock and Other Ownership Interests: AstraZeneca/MedImmune
Travel, Accommodations, Expenses: Aetion

Katherine K. Nishimura
Research Funding: EUSA Pharma (Inst)

Névine Zariffa
Stock and Other Ownership Interests: AstraZeneca, Inovio Pharmaceuticals, GlaxoSmithKline, Pfizer, Moderna Therapeutics, Takeda, Vaxart, VIR Biotechnology, Janssen, Merck, Sanofi, Translate Bio, ANOVA, Intelligencia
Consulting or Advisory Role: Genentech, Bristol Myers Squibb, Cytokinetics, ANOVA, Intelligencia
Other Relationship: 2S Associates

Jeffrey C. Thompson
Consulting or Advisory Role: AstraZeneca

Antje Hoering
Research Funding: eUSA, Hookipa (Inst)

Valsamo Anagnostou
Research Funding: Bristol Myers Squibb (Inst), AstraZeneca (Inst)

Jonathan Baden
Employment: Bristol Myers Squibb, Johnson & Johnson
Stock and Other Ownership Interests: Bristol Myers Squibb, Johnson & Johnson

Aadel A. Chaudhuri
Leadership: Droplet Biosciences, LiquidCell Dx
Stock and Other Ownership Interests: Genescopy, Droplet Biosciences, LiquidCell Dx

Honoraria: Roche, Dava Oncology
Consulting or Advisory Role: Genescopy, Roche, Tempus, AstraZeneca/ Daiichi Sankyo, NuProbe, Alphasights, Guidepoint Global
Research Funding: Tempus
Patents, Royalties, Other Intellectual Property: Patent filings related to cancer biomarkers, and have licensed intellectual property to Droplet Biosciences, Tempus Labs, and Biocognitive Labs
Travel, Accommodations, Expenses: Roche, Droplet Biosciences, LiquidCell Dx

Other Relationship: Roche

Darya Chudova
Employment: Guardant Health
Leadership: Guardant Health
Stock and Other Ownership Interests: Guardant Health
Patents, Royalties, Other Intellectual Property: Patents filed

Alexander D. Fine
Employment: Foundation Medicine
Stock and Other Ownership Interests: Roche

Joseph Fiore
Employment: Bristol Myers Squibb
Stock and Other Ownership Interests: Bristol Myers Squibb

Rachel Hodge
Employment: AstraZeneca
Stock and Other Ownership Interests: AstraZeneca

Darren Hodgson
Employment: AstraZeneca
Stock and Other Ownership Interests: AstraZeneca

Nathan Hunkapiller
Employment: Grailbio, Genentech (I), Pacific Biosciences (I)
Leadership: Grailbio, Pacific Biosciences (I)
Stock and Other Ownership Interests: Grailbio, Genentech (I), Pacific Biosciences (I)
Consulting or Advisory Role: Pacific Biosciences (I)
Patents, Royalties, Other Intellectual Property: Patents generated while employed at Grailbio, patents generated while at Pacific Biosciences (I)
Travel, Accommodations, Expenses: Grailbio, Pacific Biosciences (I), Genentech (I)

Daniel M. Klass
Employment: Roche Molecular Diagnostics
Stock and Other Ownership Interests: Roche
Patents, Royalties, Other Intellectual Property: Coinventor on a patent from Stanford University
Travel, Accommodations, Expenses: Roche Molecular Diagnostics

Julie Kobie
Employment: Merck
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