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

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abstract

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.

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ASSOCIATED CONTENT

Data Supplement

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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

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.

defined for ICIs.[9-13] 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.[14-18]

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).[19-23] 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).[20-22,24,25] 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.[26] Durable clinical benefit was defined as maintenance of PFS at 6 months from treatment initiation (PFS6).[27] 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.[21] 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 indeterminably 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

$$\begin{aligned}\text{Percent Change of Mean VAF} &= (\text{mean VAF}_{T1} - \text{mean VAF}_{T0})/\text{mean VAF}_{T0}\\ \text{Percent Change of Median VAF} &= (\text{median VAF}_{T1} - \text{median VAF}_{T0})/\text{median VAF}_{T0}\\ \text{Percent Change of Maximum VAF} &= (\text{maximum VAF}_{T1} - \text{maximum VAF}_{T0})/\text{maximum VAF}_{T0}\end{aligned}.$$

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[28]; 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,[29] 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 PFS6). 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

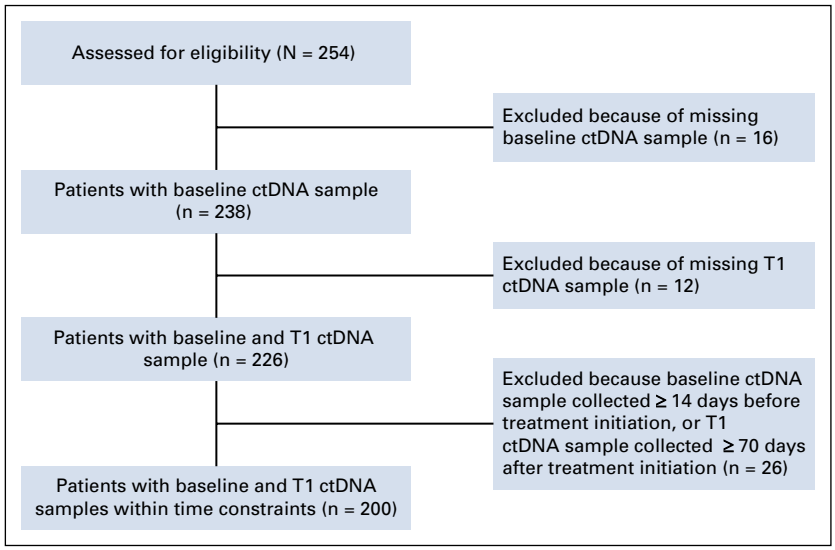

FIG 1. Flow diagram. ctDNA, circulating tumor DNA.

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.

Similar observations occurred when examining the ctDNA associations with PFS in unadjusted Cox models (Data Supplement) and adjusted Cox models with cohort stratification and adjustment by baseline clinical covariates (Fig 4A), where the adjusted hazard ratio of 1.76 (95% CI, 1.31 to 2.36; $P <$ .001) indicated that each increase in the categories 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 progression or death, after adjusting for baseline clinical covariates. Similar to the OS analysis, baseline ctDNA values, including ND samples, were not associated with PFS. The PFS Kaplan-Meier plot revealed that patients with a decrease in the maximum VAF had better PFS compared with the other two groups, but there was no apparent separation in PFS between patients in the intermediate and increase categories (Fig 4B). Additional Kaplan-Meier and univariate associations for PFS 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

TABLE 1. Patient Demographics

Trait	Description	Cohort							*P*	Overall, n/N (%)
		1	2	3a	4a	4b	5a	5b		
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)	.577[a]	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)	< .001[a]	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 PD-L1–positive), No. (%)	14/19 (74)	11/11 (100)	30/42 (71)	48/53 (91)	12/15 (80)	23/24 (96)	13/26 (50)	< .001[a]	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.

[a]*P* value from Fisher's exact test, otherwise χ^2.

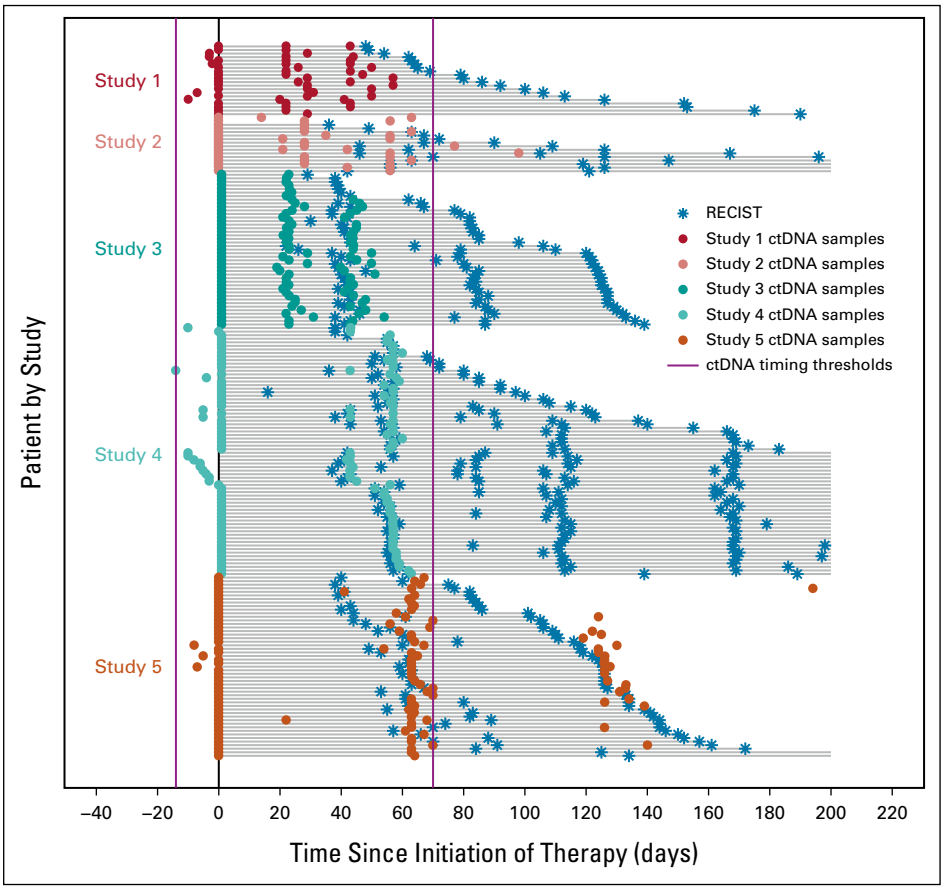

FIG 2. Timing of plasma collection and tumor response per patient by study in the analysis data set (N = 200). Unique patients are represented as horizontal lines, with markers denoting the timing of RECIST evaluations and ctDNA samples. The *x*-axis is truncated at 200 days, with some patients having longer follow-up. ctDNA, circulating tumor DNA.

decreases in ctDNA were associated with achieving durable clinical benefit, defined as PFS ≥ 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]

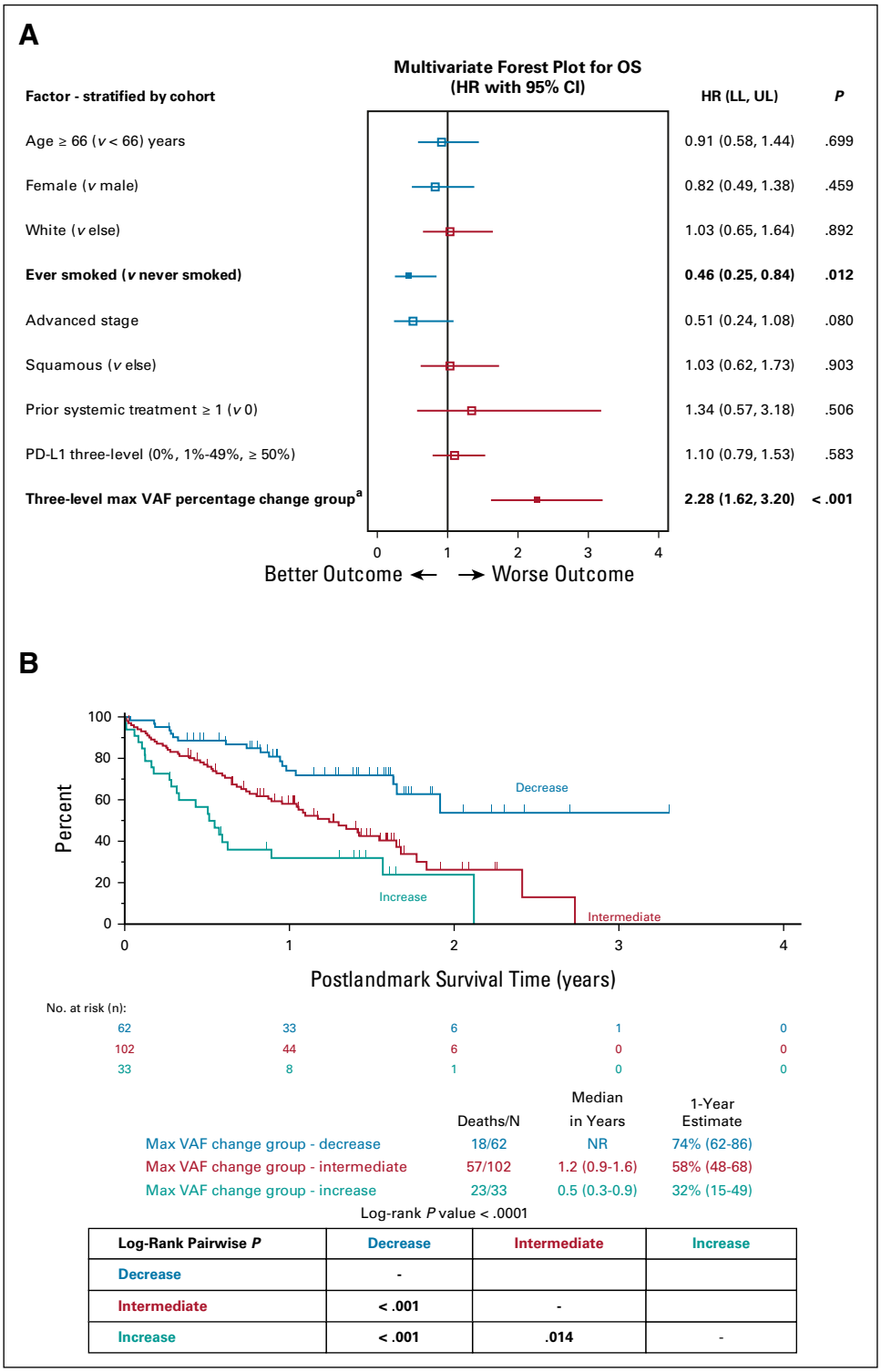

FIG 3. (A) Forest plot with Cox regression results for OS and the three-level max VAF percent change group variable, adjusted by baseline clinical covariates. Red means the HR is > 1.0 (increased risk of death) and blue means the HR is < 1.0 (decreased risk of death); unfilled box = nonsignificant *P* value, filled box = significant $P < .05$. (B) Kaplan-Meier plot for OS and the three-level max VAF percent change groups, landmarked at 70 days from treatment initiation (the sampling window for the first on-treatment ctDNA sample); patients with an event during the 70-day landmark were excluded from the analysis. [a]Denotes a time-dependent variable. ctDNA, circulating tumor DNA; HR, hazard ratio; LL, lower limit; OS, overall survival; PD-L1, programmed death ligand-1; UL, upper limit; VAF, variant allele frequency.

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

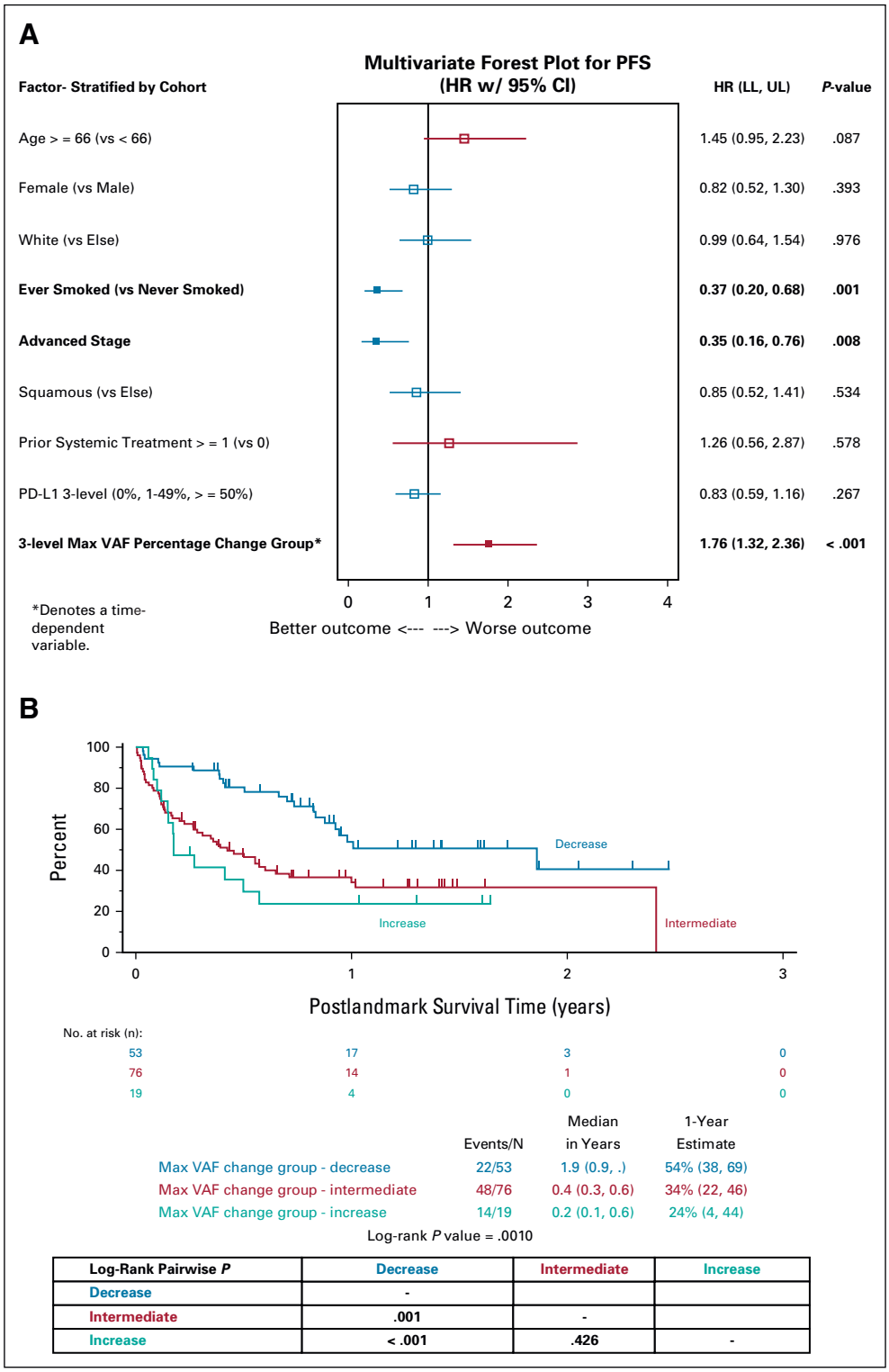

FIG 4. (A) Forest plot with Cox regression results for PFS and the three-level max VAF percent change group variable, adjusted by baseline clinical covariates. Red means the HR is > 1.0 (increased risk of death/progression) and blue means the HR is < 1.0 (decreased risk of death/progression); unfilled box = nonsignificant *P* value, filled box = significant $P < .05$. (B) Kaplan-Meier plot for PFS and the three-level max VAF percent change groups, landmarked at 70 days from treatment initiation (the sampling window for the first on-treatment ctDNA sample); patients with an event during the 70-day landmark were excluded from the analysis. [a]Denotes a time-dependent variable. ctDNA, circulating tumor DNA; HR, hazard ratio; LL, lower limit; PD-L1, programmed death ligand-1; PFS, progression-free survival; UL, upper limit; VAF, variant allele frequency.

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

	PR or Better (N = 187)				PFS at 6 Months (N = 178)			
Factor	**With Factor**	**Without Factor**	**OR (95% CI)**	***P***	**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)	0.134
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. (%)								
Decrease	34/59 (58)		Reference	—	43/56 (77)		Reference	—
Intermediate	22/95 (23)		0.19 (0.08 to 0.45)	< .001	36/90 (40)		0.13 (0.05 to 0.34)	< .001
Increase	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 χ^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.[23,32] 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.[34] 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.

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DISCLAIMER

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