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Dynamic Changes of Circulating Tumor DNA Predict Clinical Outcome in Patients With Advanced Non–Small-Cell Lung Cancer Treated With Immune Checkpoint Inhibitors

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abstract

PURPOSE Immune checkpoint inhibitors (ICIs) are increasingly being used in non–small-cell lung cancer (NSCLC), yet biomarkers predicting their benefit are lacking. We evaluated if on-treatment changes of circulating tumor DNA (ctDNA) from ICI start (t_0) to after two cycles (t_1) assessed with a commercial panel could identify patients with NSCLC who would benefit from ICI.

PATIENTS AND METHODS The molecular ctDNA response was evaluated as a predictor of radiographic tumor response and long-term survival benefit of ICI. To maximize the yield of ctDNA detection, de novo mutation calling was performed. Furthermore, the impact of clonal hematopoiesis (CH)–related variants as a source of biologic noise was investigated.

RESULTS After correction for CH-related variants, which were detected in 75 patients (44.9%), ctDNA was detected in 152 of 167 (91.0%) patients. We observed only a fair agreement of the molecular and radiographic response, which was even more impaired by the inclusion of CH-related variants. After exclusion of those, a ≥ 50% molecular response improved progression-free survival (10 *v* 2 months; hazard ratio [HR], 0.55; 95% CI, 0.39 to 0.77; $P = .0011$) and overall survival (18.4 *v* 5.9 months; HR, 0.44; 95% CI, 0.31 to 0.62; $P < .0001$) compared with patients not achieving this end point. After adjusting for clinical variables, ctDNA response and *STK11*/*KEAP1* mutations (HR, 2.08; 95% CI, 1.4 to 3.0; $P < .001$) remained independent predictors for overall survival, irrespective of programmed death ligand-1 expression. A landmark survival analysis at 2 months (n = 129) provided similar results.

CONCLUSION On-treatment changes of ctDNA in plasma reveal predictive information for long-term clinical benefit in ICI-treated patients with NSCLC. A broader NSCLC patient coverage through de novo mutation calling and the use of a variant call set excluding CH-related variants improved the classification of molecular responders, but had no significant impact on survival.

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INTRODUCTION

Circulating tumor DNA (ctDNA) has been extensively studied to identify predictive markers and has improved the delivery of targeted therapy for advanced non–small-cell lung cancer (NSCLC).[1,2] More recently, next-generation sequencing (NGS) blood tests have been used in patients treated with immune checkpoint inhibitors (ICIs),[3–7] as currently available candidate biomarkers such as tumor mutational burden and programmed death ligand-1 (PD-L1) expression are not sufficiently specific to discern responders from nonresponders.[8–11] Although some studies evaluated tumor mutational burden in cfDNA,[12,13] others focused on the on-treatment assessment of ctDNA trajectories.[3,5,14,15] Several recent studies reported that molecular ctDNA responses correlate with radiographic responses to ICI,[3–6,15] whereas others suggested a multiparameter model integrating ctDNA and circulating immune cell profiling to improve the prediction of tumor response.[15] Most studies were performed using comprehensive laboratory developed tests, the development and validation of which are complex and often not feasible for routine diagnostic laboratories with respect to time and costs. Others have used centralized testing facilities[7]; however, outsourcing adds to the costs, requires efficient sample, and results in transmission networks, which can also result in sample-to-answer delay. Moreover, most of these vendors do not correct for clonal hematopoiesis (CH), which refers to a clonal expansion of

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

The search for predictive or prognostic biomarkers for response to immune checkpoint inhibitors (ICIs) in patients with non–small-cell lung cancer (NSCLC) is of high clinical relevance. Given that circulating tumor DNA (ctDNA) reflects the total body tumor burden, we assessed if the on-treatment ctDNA trajectories (before ICI and on average 4 weeks later) are associated with radiographic response and survival.

Knowledge Generated

To this end, we used a broadly applicable, easy-to-implement commercial ctDNA test that achieves a broad NSCLC patient coverage through de novo mutation calling. Our data indicated that changes in ctDNA levels are highly predictive for long-term benefit in ICI-treated advanced NSCLC. The use of a tumor-specific variant call set by excluding variants related to clonal hematopoiesis improved the discrimination of survival by a molecular ctDNA response.

Relevance

Our approach provides guidance for clinicians on the long-term benefit of the therapy and may enable stratification of patients for clinical trials.

mutations in blood cells. Besides mutations in driver genes for hematologic malignancies, there is increasing evidence of CH-related mutations in genes usually mutated in solid tumors, including *TP53 or KRAS*.[16,17] Compared with clonal hematopoiesis of indeterminate potential, in solid malignancies, variant allele frequencies (VAFs) of CH-related variants often overlap with the range of ctDNA-derived variants.[17] Therefore, these variants represent important biologic confounders and paired analyses of cfDNA and peripheral blood mononuclear cells (PBMCs) are suggested.

Therefore, the use of commercial NGS kits may accelerate a broad application of ctDNA. To this end, analytical validation of ctDNA assays[18] and the use of validated ctDNA workflows are essential.[19–21] Within the framework of Cancer-ID,[22] we have assessed the variability of cfDNA extraction kits and commercial NGS kits that can be easily implemented and standardized for clinical applications in many laboratories.[23–25] Here, we describe the use of a commercial platform for liquid profiling in a large cohort of patients with NSCLC undergoing ICI treatment. On-treatment changes of ctDNA, the presence of specific genetic alterations, and the influence of variants related to CH on outcome were analyzed.

PATIENTS AND METHODS

Patients

Patients with advanced NSCLC (N = 177) treated with ICI were recruited from December 2015 to May 2018 at the University Medical Center Groningen (the Netherlands), of which 167 patients were evaluable (Fig 1A and Table 1). Blood was taken prospectively before ICI administration (t_0) and after two cycles (on average 4 weeks for nivolumab and 6 weeks for pembrolizumab; t_1). Tumor response was assessed by diagnostic computed tomography using RECIST, version 1.1, by an observer blinded to the biomarker outcome. Early response (defined as complete [CR] or partial [PR] tumor response at the first response evaluation) was observed in 44 of 167 patients (26.4%), and 60 of 167 patients (35.9%) achieved a durable clinical response (defined as CR or PR or persistent stable disease for more than 6 months). Patients without a durable response (no durable response [NDR]) progressed within 6 months progressive disease [PD]. For follow-up time and median survival, see the Data Supplement. All patients provided written informed consent. The ISO-certified biobank initiative (9001:2008 Healthcare) was approved by the medical ethics committee of the University Medical Center Groningen (No. 2010/109) and made available to the CANCER-ID consortium.

Molecular Profiling From Plasma

Molecular profiling was performed at t_0 and t_1 using the AVENIO ctDNA Expanded kit (Roche, Basel, Switzerland; Data Supplement) according to the manufacturer's recommendations. Performance assessment confirmed the reported sensitivity down to 0.1% (Data Supplement). Single nucleotide polymorphism (SNPs) and CH-related variants were filtered on the basis of public databases and patient-matched PBMCs (for details, see the Data Supplement).

Assessment of ctDNA Levels

ctDNA levels were assessed as VAFs (percentage of alternate sequence reads divided by the total number of reads at that locus) and the number of mutant molecules (MMs) per mL plasma. For both proxies, the average of all identified variants per patient (average mutant molecules [aMMs] and aVAF) and the value of the highest variant (hMM and hVAF) were determined. Here, only the results from the aMM are presented (for details, see the Data Supplement).

Study Design and Statistics

The primary hypothesis of this observational exploratory study was that changing levels of ctDNA may serve as early predictors of response to ICI in NSCLC. The prespecified primary end point was the agreement between a molecular

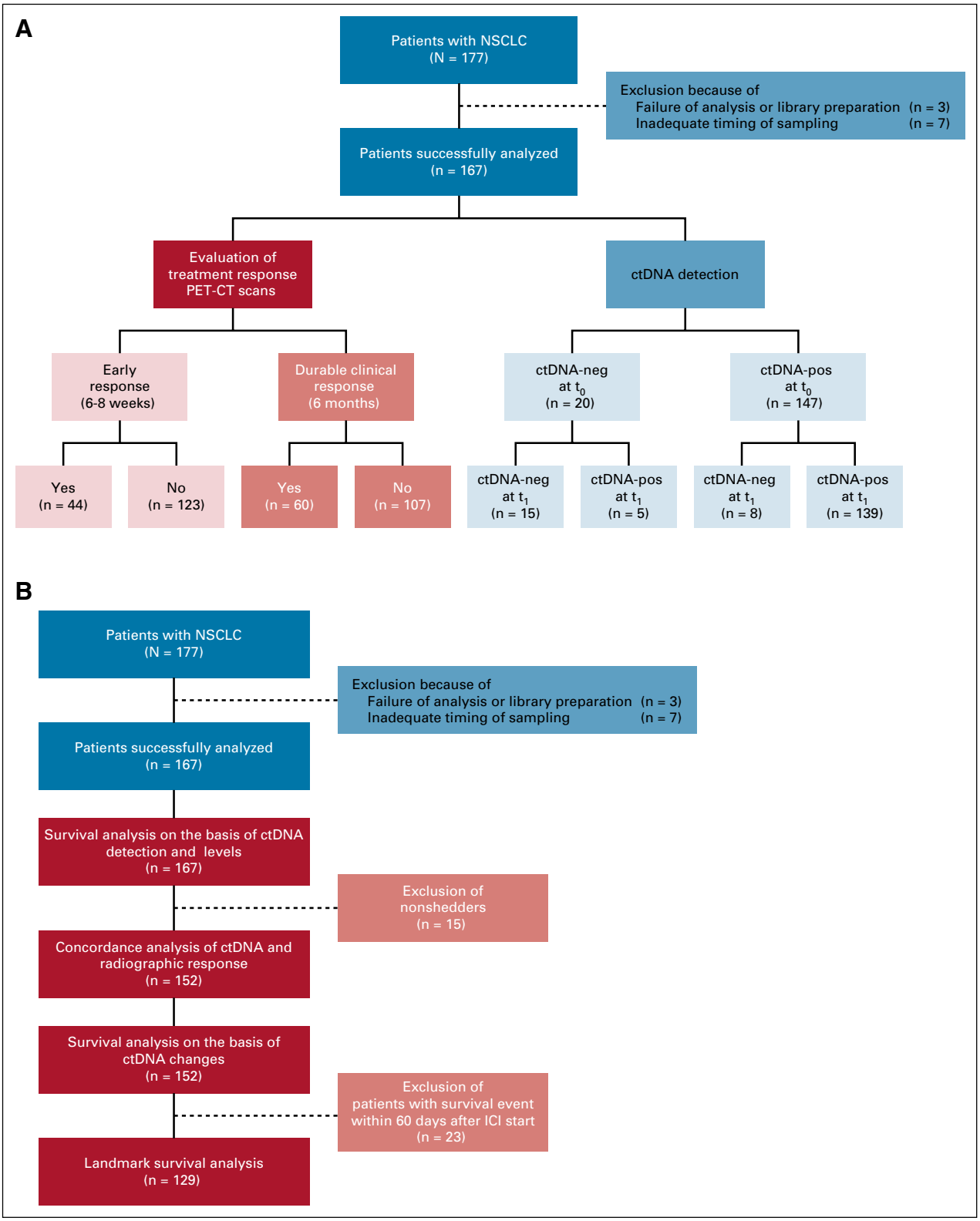


FIG 1. Flowchart and study design. (A) Flowchart of patients included in the study. In 139 of 152 (91.4%) patients, at least one mutation was identified at both time points, and in 91 of 152 patients (59.9%), additional mutations unique to a single time point (either t_0 or t_1) were observed. (B) Analysis workflow of the study. CT, computed tomography; ctDNA-neg, no tumor-specific somatic mutation detected; ctDNA-pos, detection of at least one mutation using the AVENIO ctDNA Expanded Kit; ctDNA, circulating tumor DNA; ICI, immune checkpoint inhibitor; NSCLC, non–small-cell lung cancer; PET, positron emission tomography; t_0, blood draw before initiation of ICI; t_1, blood draw at first response assessment.

TABLE 1. Baseline Patient Characteristics (n = 167)

Variable	Parameter	No. of Patients (%)
Age, years	Median (range)	66 (29-87)
Sex	Male	96 (57.5)
	Female	71 (42.5)
Performance score	0	69 (41.3)
	1	89 (53.3)
	2	8 (4.8)
	3	1 (0.6)
Smoking history	Smoker	107 (64.1)
	Former smoker	47 (28.1)
	Nonsmoker	13 (7.8)
Stage	IIIB	22 (13.2)
	IV	145 (86.8)
Metastatic sites	0	3 (1.8)
	1	32 (19.2)
	2	55 (32.9)
	3	41 (24.5)
	≥ 4	36 (21.6)
Line of therapy	First	13 (7.8)
	Second	119 (71.2)
	Third	35 (21.0)
Therapy	Nivolumab	140 (83.8)
	Pembrolizumab	15 (9.0)
	Atezolizumab	7 (4.2)
	Nivolumab plus ipilimumab	3 (1.8)
	Durvalumab	2 (1.2)
No. of cycles	Mean (SD)	13 (15)
ICI ended because of	PD	121 (72.5)
	Toxicity	25 (15.0)
	2 years duration	19 (11.4)
	Infection	2 (1.2)
Histology	Adenocarcinoma	112 (67.1)
	Squamous cell carcinoma	51 (30.5)
	Others	4 (2.4)
PD-L1 expression	< 1%	56 (33.5)
	1%-49%	31 (18.6)
	≥ 50%	23 (13.8)
	NE or unknown	57 (34.1)
Tumor response	CR	8 (4.8)
	PR	36 (21.6)
	SD	39 (23.4)
	PD	70 (41.9)
	NE	14 (8.4)
Early response	Response at first CT	44 (26.3)
Durable response	SD + PR + CR ≥ 6 months	60 (35.9)

Abbreviations: CR, complete response; CT, computed tomography; NE, not evaluable; PD, progressive disease; PD-L1, programmed death ligand-1; PR, partial response; SD, standard deviation.

ctDNA response (25% and 50% drop, see the Data Supplement) from t_0 to t_1 and the radiographic tumor response. The secondary aim was to compare overall survival (OS) and progression-free survival (PFS) in patients who presented a ctDNA response versus those who did not achieve this end point. Further prespecified outcome measures included the baseline ctDNA levels and the number and types of mutations in association with survival.

Descriptive statistics were used for patient and tumor characteristics. For comparisons of ctDNA levels, response, and concordance between ctDNA and clinical response, nonparametric and parametric tests were used. Analyses of PFS and OS (defined from ICI start until radiographic progression or death, the primary outcome was censored on the date of last follow-up) were performed using the Kaplan-Meier method and the log-rank test. Moreover, a landmark analysis at 2 months (60 days after therapy for survival analysis), which represents a critical time point for clinicians to decide whether to continue with ICI, was performed. For this analysis, 144 (OS) and 99 (PFS) patients were available (Fig 1B).

Multivariate Cox regression analysis was used to adjust for clinical factors. Data were analyzed and visualized using GraphPad PRISM, SPSS, and R (maftools and ggplot2 packages; for details, see the Data Supplement).

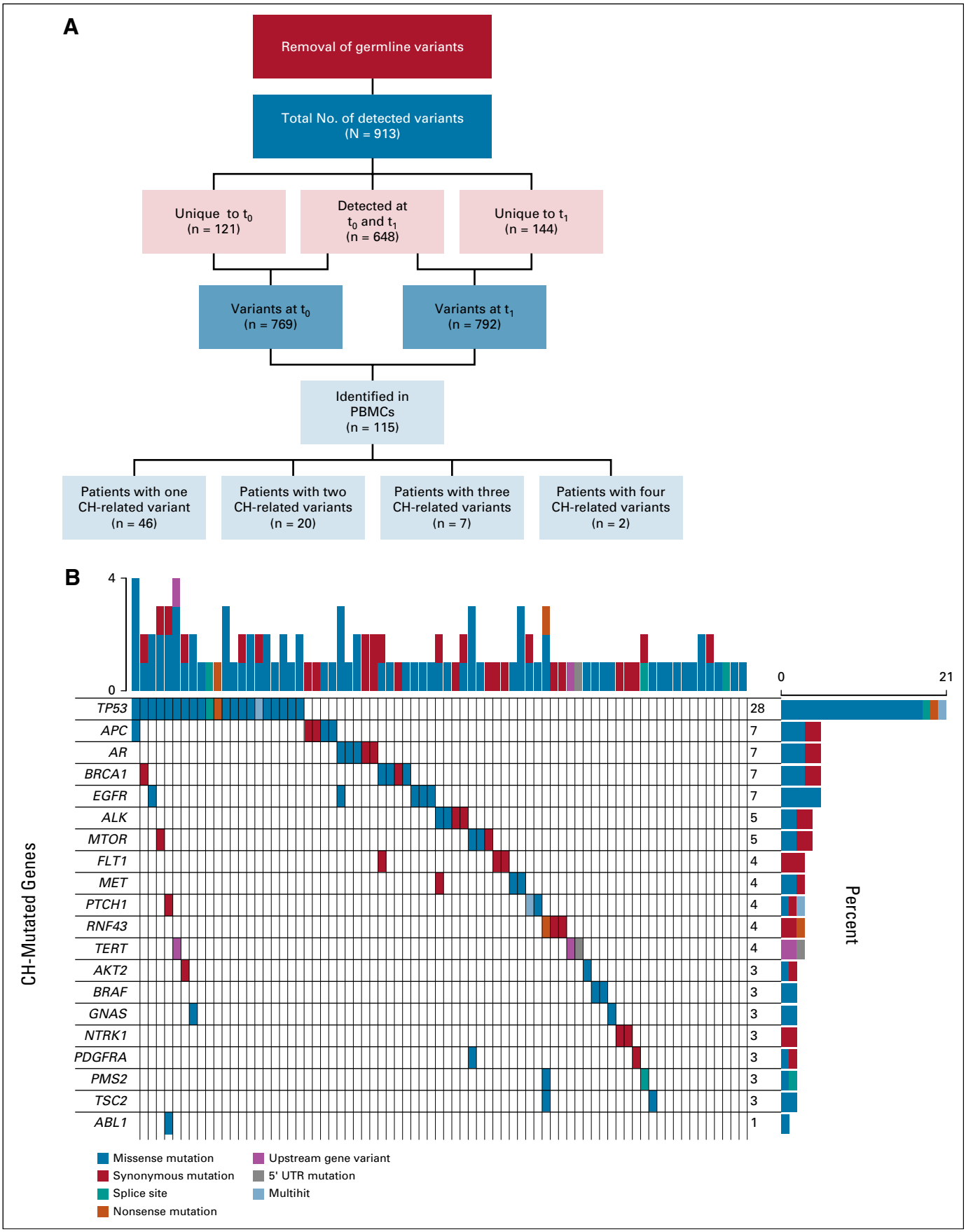
RESULTS

Clonal Hematopoiesis–Related Variants Are a Source of Biologic Noise

Recent studies have indicated that CH—a process that leads to the expansion of mutations in blood cells—affects the specificity of ctDNA detection.[19,26] Therefore, we included PBMCs in our analyses to ensure a tumor-specific variant call set. Of 913 somatic variants, 115 (12.6%) from 75 of 167 patients (44.9%) were also detected in PBMCs (Fig 2A), whereas most CH-related variants were identified in *TP53* (Fig 2B).[19,27,28] The number and presence of CH-related variants were associated with higher age (Fig 2C and Data Supplement). The median VAF of CH-related mutations (0.59%; range, 0.07-14.65) was significantly lower than that of the tumor-derived mutations (median VAF of 0.91%; range, 0.04-79.12; Fig 2D). In contrast to tumor-specific variants (average fold change from t_0 to t_1 2.6, range 0-218), CH-related variants largely remained unchanged (average fold change 0.95, range 0-3.0). Consistent with previous reports, DNA fragments carrying tumor-specific variants were slightly shorter than those carrying CH-related variants (Kolmogorov-Smirnov test, $P < 1 \times 10^{-10}$; Fig 2E).[19] The presence of CH-related variants was neither associated with prior chemotherapy nor survival (Data Supplement).

De Novo Mutation Calling Shows Good Concordance With Tissue and Leads to a Higher Yield ctDNA Detection Rate

After removing CH-related variants, an average of 5.25 tumor-specific mutations (median 4, range 1-68) were



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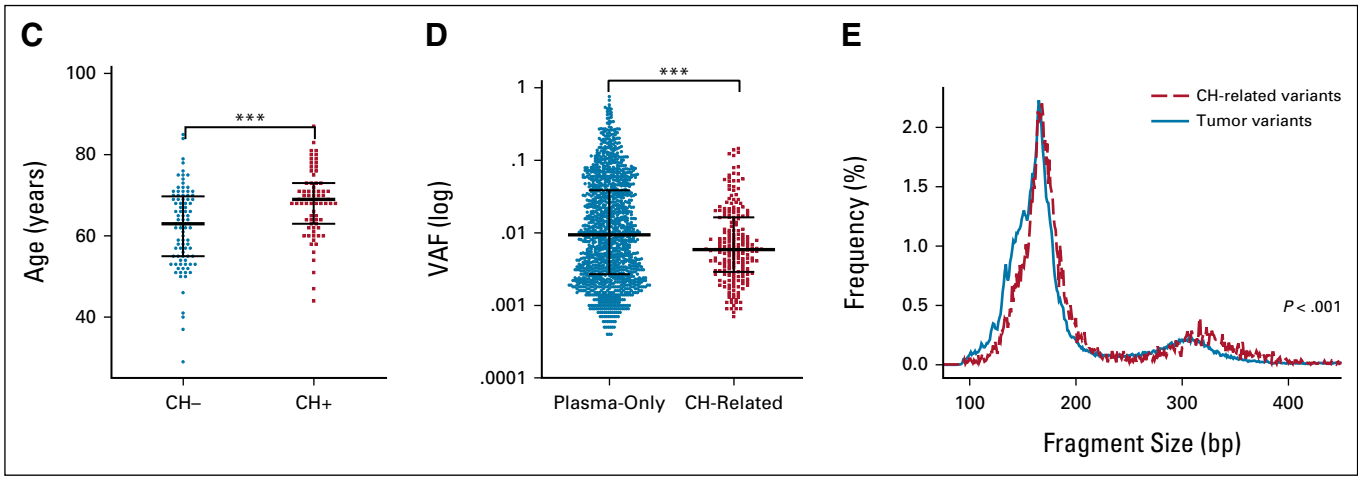

FIG 2. CH-related variants are frequently observed in patients with advanced NSCLC. (A) After removal of germline variants, a total of 769 and 792 somatic variants were identified at t_0 and t_1, respectively. Of those, 648 were identified at both time points, whereas 121 and 144 variants were either unique to t_0 or t_1, respectively, resulting in a total of 913 detected variants. Of these, 115 variants (12.6%) from 75 of 167 patients (44.9%) were also detected in PBMCs with 30 of 167 patients (17%) having more than one CH-related variant. (B) Oncoprint of the most frequent CH-mutated genes (top 20). Shown is an overview of the top 20 altered genes and its genomic alterations (legend), in particular, genes (rows) affecting individual samples (columns). Of the 144 identified TP53 mutations in 114 patients, a total of 30 (20.8%) mutations in 21 patients were associated with CH. (C) Age distribution of patients with (CH+, red) or without (CH-, blue) detected CH-related variants (median age 63 *v* 69 years, two-sided Mann-Whitney test, $P < .001$). (D) Distribution of the variant allele frequency (VAF) detected in plasma only (tumor-specific variants, blue) was significantly increased compared with variants that were additionally observed in corresponding PBMCs (CH-related, red; two-sided Mann-Whitney test, $P = .0007$). (E) Fragments carrying tumor-specific variants (blue) were significantly lower compared with CH-related variants (red) (Kolmogorov-Smirnov test, $P < .0001$). CH, clonal hematopoiesis; NSCLC, non–small-cell lung cancer, PBMCs, peripheral blood mononuclear cells; ctDNA, circulating tumor DNA; t_0, blood draw before initiation of ICI; t_1, blood draw at first response assessment; ICI, immune checkpoint inhibitor. $^{***}P \leq .001$.

detected in 152 (91.0%) patients (Data Supplement), excluding 15 patients without detectable ctDNA (nonshedders) at both time points. Interestingly, six of those nonshedders had CH-related variants and would have been mistakenly classified as ctDNA-positive.

Tumor profiling data were available from 116 patients. In 71 (61.2%), a total of 85 mutations were detected in the tissue. From these, 64 were also detected in plasma from 54 patients, resulting in an overall concordance of 75.3% (Data Supplement), which is consistent with previous reports.[28] Of 17 discordant samples, six were classified as nonshedders, and in 11 patients, at least one other mutation was detected. Not surprisingly, discordant plasma samples had significantly lower ctDNA fractions (Data Supplement). Of the 45 (38.8%) patients for whom no mutation was detected in the tumor tissue, six were classified as nonshedders. In the remaining 39 patients, at least one mutation could be identified with AVENIO, demonstrating an improved breadth of patient coverage compared with a hot spot panel.

Changes in ctDNA Levels and Tumor Response

Overall ctDNA levels at t_0 were low, with 50.9% (85 of 167) of patients having an average VAF (aVAF) < 1% (median 0.9%; range, 0-48.4) and aMM ranging from 0-57,500 molecules per mL of plasma. Responders had significantly lower ctDNA levels at t_1 compared with t_0 (Data Supplement). When assessing ctDNA response patterns from t_0 to t_1, the majority of responders (DCR and ER) presented decreasing ctDNA levels (Figs 3A and 3B), whereas nonresponders (NDR and NR) had mixed molecular responses (Figs 3C and 3D and Data Supplement). In eight patients (5.3%), ctDNA was cleared completely at t_1, whereas in five patients (3.3%), ctDNA was only detected at t_1 (Fig 1A). When using a 50% threshold for aMM changes, on average, 54.5% of patients were evaluable, whereas all others did not reach the cutoff in either direction (Fig 3E). Without excluding CH-related variants, this proportion even decreased and a total of 20 patients would have been misclassified (Data Supplement). For example, patient 1147, who presented a partial response, achieved a 51% molecular response (Fig 3F). However, on the basis of all identified plasma variants, the ctDNA level increased by more than 100%. Overall, early and durable responders were 4.5- and 3.6-fold more likely to achieve a 50% molecular ctDNA response compared with nonresponders (Fisher's exact test, $P = .0003$ and $P = .0009$; Fig 3G). When assessing the agreement between radiographic and molecular response from t_0 to t_1, only a fair agreement (Cohen's kappa coefficients 0.31 and 0.28 for 50% ctDNA response and 0.27 and 0.23 for 25% ctDNA response, respectively) was observed between early and durable radiographic and ctDNA response.

A comparison of a tumor-informed approach (n = 71 patients), that is, considering only ctDNA variants identified also in tumor tissue, and the CH-corrected de novo variant

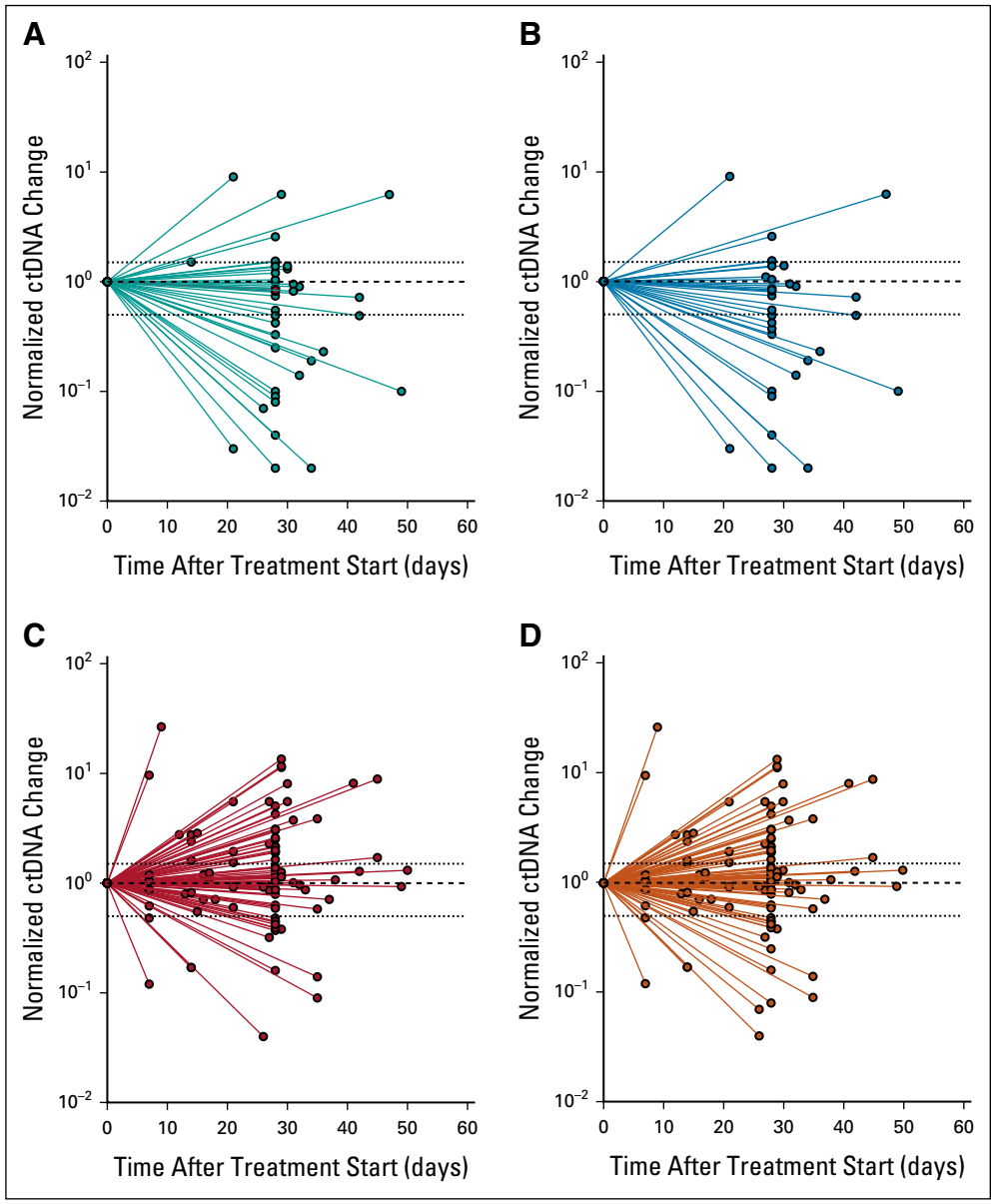

FIG 3. Correlation of dynamic ctDNA changes with response to ICI. ctDNA levels (aMMs, average mutant molecules per mL plasma) normalized to pretreatment ctDNA levels of (A) durable responders (DCR; green) and (B) early responders (ER; blue). The dashed line indicates no change, and the dotted line indicates the 50% cutoff for ctDNA drop and increase. The same as in (A) but for patients (C) without a durable (NDR; red) or (D) early response (ER; orange). (E) Plotted is the fraction of patients stratified by early or durable response that reached a ≥ 50% ctDNA drop (left) or rise (right) for all variants (including CH-related variants) and a corrected variant call set without CH-related variants. (F) Shown are exemplary cases for whom the molecular ctDNA response on the basis of all variants and those after removal of CH-derived variants revealed different results. (G) Shown are ORs of responders versus nonresponders for a 50% increase or decrease for all variants including CH-related variants and a corrected variant call set (without CH-related variants) calculated from a two-sided Fisher's exact test with 95% CI. n.s. $P > .05$; $^{*}P \leq .05$; $^{**}P \leq .01$, $^{***}P \leq .001$, and $^{****}P \leq .0001$. CH, clonal hematopoiesis; ctDNA, circulating tumor DNA; DCR, durable clinical response; NDR, no durable response; ER, early response; NR, no response; n.s., not significant; OR, odds ratio; t_0, blood draw before initiation of ICI; t_1, blood draw at first response assessment.

call set revealed no substantial differences (Data Supplement). For the 25% threshold, similar results were obtained (Data Supplement).

Plasma ctDNA Dynamics and Survival

Patients with detectable, CH-corrected ctDNA at both time points (n = 147) had a significantly worse OS compared

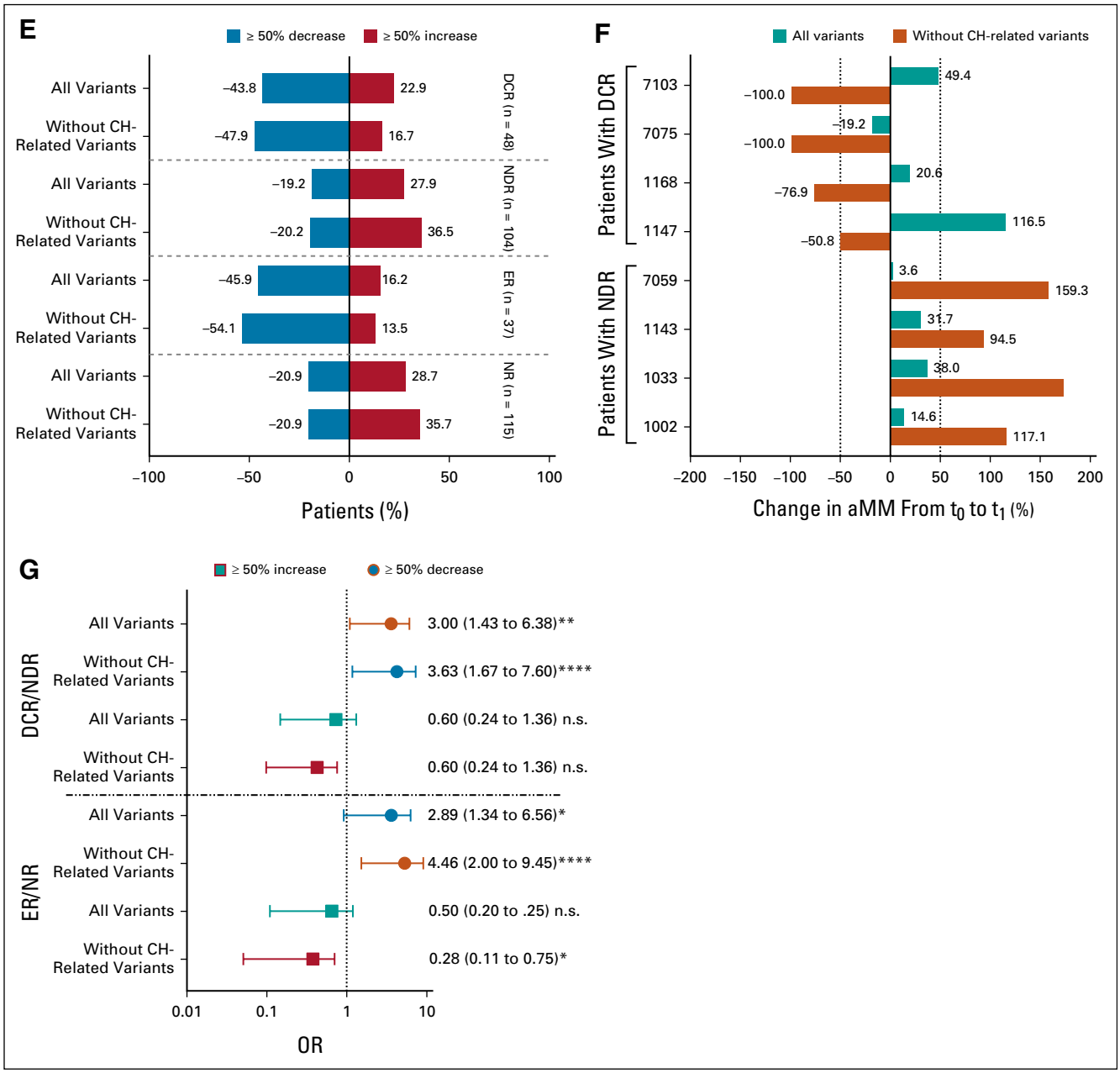

FIG 3. (Continued).

with nonshedders (no ctDNA at both time points; n = 15; median OS 7.6 *v* 20.3 months; hazard ratio [HR], 0.48; 95% CI, 0.26 to 0.48; *P* = .0147; Data Supplement). Moreover, patients with elevated ctDNA levels (above the median) had significantly worse PFS and OS for both t_0 and t_1 (for details, see the Data Supplement).

Interestingly, patients with a ≥ 50% molecular ctDNA response had a similar PFS and OS like nonshedders, whereas patients with a < 50% decrease or an increase of ctDNA at t_1 had a similarly worse outcome (Data Supplement). When stratifying patients by a ≥ 50% molecular ctDNA response, the median PFS and OS of patients without a ≥ 50% drop were 2.0 and 5.9 months, respectively, compared with 10 and 18.4 months for patients with a ≥ 50% decrease (PFS HR, 0.55; 95% CI, 0.39 to 0.77; log-rank *P* = .0011; OS HR, 0.44; 95% CI, 0.31 to 0.62; log-rank *P* < .0001; Figs 4A and 4B). Without the exclusion of the CH-related variants, survival curves were still significantly different, but the stratification was less pronounced (PFS HR 0.63 *v* 0.55 and OS HR 0.53 *v* 0.44; Figs 4A and 4B). By contrast, stratification on the basis of a tumor-informed approach (n = 55 patients) revealed the same outcome as the CH-corrected de novo approach (Data Supplement). A 2-month landmark analysis (60 days after t_0) again revealed a significant stratification for

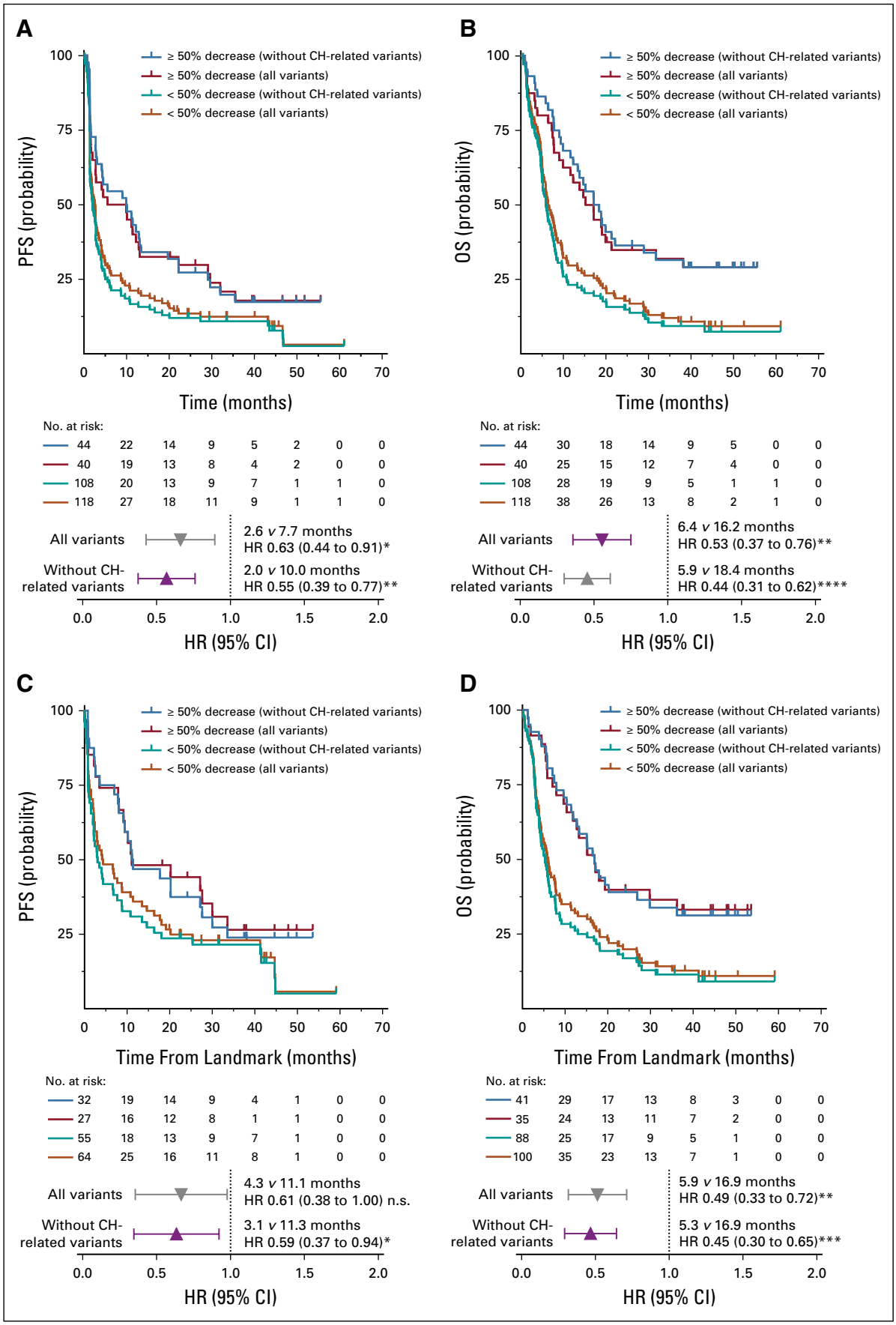

FIG 4. Dynamic ctDNA changes are associated with survival. Kaplan-Meier curves of (A) PFS and (B) overall survival of patients with detected ctDNA (n = 152 patients) stratified by a ≥ 50% decrease in the average number of mutant molecules from t_0 to t_1 calculated from all somatic variants (all variants) and CH-corrected variant call set (without CH-related variants). Lower plots indicate HRs. (C) and (D) The same as in (A) and (B), but using a 2-month landmark for OS and PFS calculation. By then, 23 patients had already died and only 77% (129 of 167) of patients were evaluable. *$P \leq .05$; **$P \leq .01$, ***$P \leq .001$, and ****$P \leq .0001$. HR, hazard ratio plus 95% CI. n.s. *P* values were calculated from log-rank tests. CH, clonal hematopoiesis; ctDNA, circulating tumor DNA; n.s., not significant; OS, overall survival; PFS, progression-free survival.

a ≥ 50% molecular ctDNA response (PFS HR, 0.59; 95% CI, 0.37 to 0.94; log-rank $P = .0319$ and OS HR, 0.45; 95% CI, 0.30 to 0.65; log-rank $P = .001$; Figs 4C and 4D). A lower cutoff of 25% for ctDNA response revealed a similar but slightly weaker stratification (Data Supplement).

Association of Genetic Alterations and Patient Outcome

Since genetic alterations may interfere with ICI outcome, we tested whether any of the recurrently identified mutated genes in our cohort are associated with survival. Except for mutations in *STK11* and/or *KEAP1* (PFS HR, 0.53; 95% CI, 0.34 to 0.82; $P = .0003$; OS HR, 0.38; 95% CI, 0.24 to 0.61; $P = .0001$), none of the other evaluated genes were associated with survival (Figs 5A and 5B). Interestingly, the addition of *NFE2L2* mutations did not improve the stratification (Data Supplement). Although patients with *STK11*- and/or *KEAP1*-mutated tumors died quickly, the 2-month landmark comparing patients with and without detected *STK11/KEAP1* mutations again showed significantly different PFS (HR 0.48; 95% CI, 0.24 to 0.97; $P = .0063$) and OS (HR, 0.42; 95% CI, 0.33 to 0.71; $P < .0001$; Figs 5C and 5D).

Multivariable Analysis of Biomarkers

To investigate the influence of clinical variables on survival, Cox regression analysis was performed. The number of metastases as a marker of disease burden, performance score, and sex were the most significant clinical variables. When including ctDNA features in the clinical model (n = 152), both a molecular response of ≥ 50% response (HR, 0.58; 95% CI, 0.39 to 0.86; $P < .007$) and the presence of *STK11/KEAP1* mutations (HR, 1.56; 95% CI, 1.04 to 2.33; $P < .03$) were independent predictors for PFS (interaction $P = .78$; Data Supplement). For OS and the landmark survival analysis (n = 129), both biomarkers provided a similar result (Data Supplement).

PD-L1 expression was available for 110 patients and represented a borderline significant baseline biomarker for PFS (HR, 0.77; 95% CI, 0.59 to 1.01; $P = .057$) and a significant biomarker for OS (HR, 0.70; 95% CI, 0.53 to 0.93; $P = .01$). However, when adding the molecular ctDNA response of ≥ 50% to the model, PD-L1 expression became insignificant for both end points (Data Supplement).

DISCUSSION

Here, we evaluated whether dynamic ctDNA features may identify patients with advanced NSCLC who benefit from ICI treatment. Although similar studies have been reported, our data include several novel aspects such as (1) the use of a broadly applicable easy-to-implement ctDNA test that achieves a broad NSCLC patient coverage through de novo mutation calling, (2) a landmark survival analysis at a time point when all ctDNA data are available and when clinicians decide to continue or discontinue treatment, and (3) the use of a tumor-specific variant call set by excluding CH-related variants.

We used the commercial AVENIO platform, which provides a user-friendly, generic end-to-end workflow. In contrast to routine diagnostic workup, in which mutations were identified in only 60% of patients, our de novo mutation calling approach identified mutations in more than 90% of the patients, which demonstrates a high sensitivity and a broad patient coverage of the AVENIO platform that is comparable with panels such as the Guardant360 assay offered by a large service provider. A comparison of tumor-informed and de novo mutation calling in plasma revealed similar performances with respect to treatment response and survival, confirming ctDNA as a reliable cancer marker without the need for tissue sequencing.

Consistent with a recent study[6], we observed lower pre- and on-treatment levels of MMs for both early and durable responders compared with nonresponders. In addition to higher ctDNA levels at t_0 and t_1, the presence of *STK11/KEAP1* mutations was independently predictive of worse survival. However, these mutations are not uniquely associated with response to ICI, but confer a poor prognosis regardless of treatment.[29] As not only a static assessment of ctDNA levels but also changes in ctDNA levels may predict response and long-term outcome,[5–7,14,15] we assessed ctDNA dynamics as a surrogate for tumor response and survival. Our data indicate that on-treatment changes of ctDNA predict survival, but did not well correlate with radiographically assessed tumor response, which is in line with previous reports.[5,15] Although initial studies proposed an association of early ctDNA dynamics and pathologic response,[3,5] we confirmed from a recent pan-cancer study including 333 patients with NSCLC that ctDNA changes provide prognostic information, but do not mirror the immediate tumor response.[7] Similarly, Nabet et al[15] reported that, on the basis of a molecular ctDNA response alone, more than 25% of patients would be incorrectly classified for early response assessment—a fraction similar to what we observed—and that an early prediction of tumor response may require an integration of additional parameters.

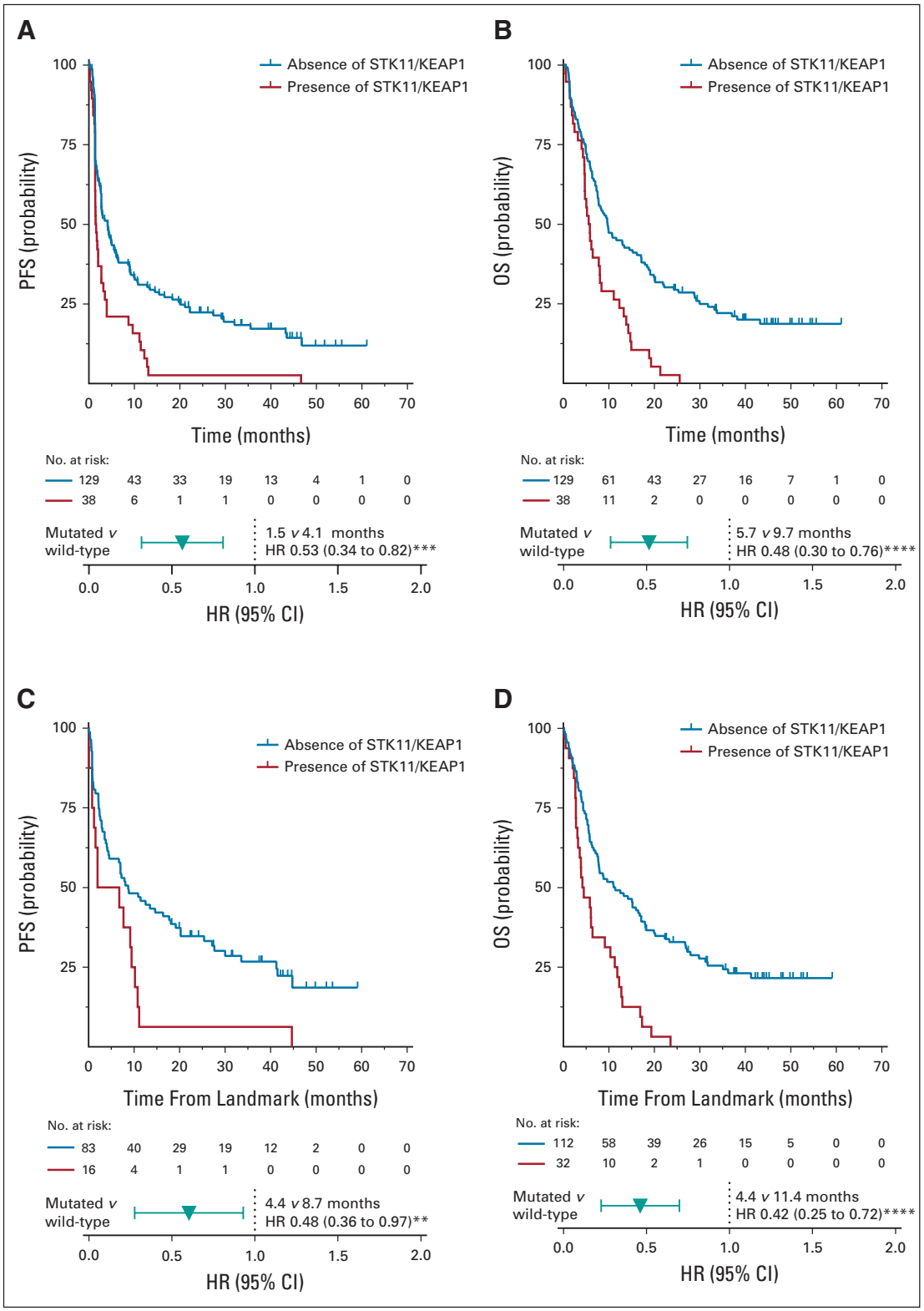

FIG 5. Association of *KEAP1*/*STK11* mutations and survival: Kaplan-Meier curves of (A) PFS and (B) OS of the entire cohort (n = 167 patients) stratified by the presence of *KEAP1* and/or *STK11* mutations at t_0 and/or t_1 and (C) and (D) the same as in (A) and (B), but using a 2-month landmark for OS and PFS calculation. HR, hazard ratio plus 95% CI. *P* values were calculated from log-rank tests. OS, overall survival; PFS, progression-free survival.

Therefore, the optimal time point for response assessment still needs to be determined. Although some patients might initially show short-term responses before progression, others may demonstrate a delayed response. Therefore, a continuous monitoring at several time points may harbor important dynamic information[30] and might better reflect the pathologic response.

Nevertheless, on-treatment changes of ctDNA had an independent impact on PFS and OS, with patients without or a complete clearance of ctDNA having the longest survival. Moreover, we confirmed the observation from the study by Nabet et al[15] that tumor PD-L1 expression does not add value to the outcome classification and is therefore dispensable in this setting.

Since the outcome of on-treatment trajectories of ctDNA can only be evaluated after the second blood sample, we performed a landmark analysis 60 days after treatment initiation, a time point when clinicians may inform their patients about the efficacy of the treatment. In particular, when the radiologic response is difficult to interpret, the ctDNA result may help guide further procedures. At this landmark, 23 of 152 (15.1%) patients had already died, which emphasizes the need for a quick turnaround time and an early assessment of the molecular response.

We confirm NSCLC as a low-ctDNA cancer[31] since half of our patients had a VAF below 1%. At these VAFs, CH-related variants comprise an important source of biologic noise.[19,21,32,33] Indeed, CH-related variants were detected in 43% of our patients. In addition to *TP53*, many other genes that are not canonically associated with CH were affected.[19] Since CH-related variants in patients with solid cancer are not related to changes in tumor burden, the incorporation of CH-related variants might disguise the actual ctDNA response and lead to misclassification of the patient's molecular response, as seen in more than 10% of our cohort. In addition, although not significant, the exclusion of CH-related variants improved the discrimination of survival by a molecular ctDNA response.

Limitations of our study include the heterogeneous ICI regimens, variable blood collection times, and predominantly second-line ICI. However, a subgroup analysis including only patients with the second blood draw after 4 weeks (±2 days) revealed the same result for OS (Data Supplement). Yet, a major benefit of our study is the use of a broadly applicable commercial ctDNA test including CH correction that might facilitate a widespread implementation of ctDNA-based clinical decision making. With respect to ICI, our approach provides guidance for clinicians on the long-term benefit of the therapy and may enable stratification of patients for clinical trials, but an early prediction of tumor response may require the integration of additional parameters.

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DISCLAIMER

Roche Diagnostics provided AVENIO ctDNA Expanded Kits in the framework of an investigator-initiated study, but had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

EQUAL CONTRIBUTION

S.W., P.v.d.L., E.H., and H.J.M.G. contributed equally to this work.

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CLINICAL TRIAL INFORMATION

Trial registration number: Dutch Trial Register NL7839.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding authors upon request. All sequencing raw data have been deposited at the European Genome-phenome Archive (EGA; http://www.ebi.ac.uk/ega/), which is hosted by the EBI, under the accession number EGAS00001004847 (data set EGAD00001007930).

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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