ORIGINAL ARTICLE

Clinical Utility of Circulating Tumor DNA in Patients With Advanced *KRAS*^{G12C}-Mutated NSCLC Treated With Sotorasib

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ABSTRACT

Introduction: For patients with *KRAS*^{G12C}-mutated NSCLC who are treated with sotorasib, there is a lack of biomarkers to guide treatment decisions. We therefore investigated the clinical utility of pretreatment and on-treatment circulating tumor DNA (ctDNA) and treatment-emergent alterations on disease progression.

Methods: Patients with *KRAS*^{G12C}-mutated NSCLC treated with sotorasib were prospectively enrolled in our biomarker study (NCT05221372). Plasma samples were collected before sotorasib treatment, at first-response evaluation and at disease progression. The TruSight Oncology 500 panel was used for ctDNA and variant allele frequency analysis. Tumor response and progression-free survival were assessed per Response Evaluation Criteria in Solid Tumors version 1.1.

Results: Pretreatment *KRAS*^{G12C} ctDNA was detected in 50 of 66 patients (76%). Patients with detectable *KRAS*^{G12C} had inferior progression-free survival (hazard ratio [HR] 2.13 [95% confidence interval [CI]: 1.06–4.30], $p = 0.031$) and overall survival (HR 2.61 [95% CI: 1.16–5.91], $p = 0.017$). At first-response evaluation ($n = 40$), 29 patients (73%) had a molecular response. Molecular nonresponders had inferior overall survival (HR 3.58 [95% CI: 1.65–7.74], $p = 0.00059$). The disease control rate was significantly higher in those with a molecular response (97% versus 64%, $p = 0.015$). *KRAS* amplifications were identified as recurrent treatment-emergent alterations.

Conclusions: Our data suggest detectable pretreatment *KRAS*^{G12C} ctDNA as a marker for poor prognosis and on-treatment ctDNA clearance as a marker for treatment response. We identified *KRAS* amplifications as a potential recurring resistance mechanism to sotorasib. Identifying patients with superior prognosis could aid in optimizing time of treatment initiation, and identifying patients at risk of early progression could allow for earlier treatment decisions.

Keywords: NSCLC; ctDNA; Sotorasib; Biomarker

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Introduction

Activating mutations in the *KRAS* gene are the most prevalent oncogenic driver alterations in Western patients with NSCLC, occurring in almost a third of nonsquamous NSCLC.[1] Of all *KRAS* mutations, $KRAS^{G12C}$ occurs most frequently with a prevalence of approximately 13% in nonsquamous NSCLC.[2] The CodeBreaK 200 trial revealed that sotorasib, an oral covalent $KRAS^{G12C}$-specific inhibitor, is superior to docetaxel in pretreated patients with advanced $KRAS^{G12C}$-mutated NSCLC for progression-free survival (PFS) (hazard ratio [HR] 0.66 [95% confidence interval [CI]: 0.51–0.86], $p = 0.0017$) and overall response rate (ORR) (28.1% [95% CI: 21.5%–35.4%] versus 13.2% [95% CI 8.6%–19.2%], $p < 0.001$), with a one-year PFS of 24.8% versus 10.1% for docetaxel.[3] On the basis of findings from the earlier phase I/II CodeBreaK 100 trial, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) granted sotorasib approval for adult patients with advanced $KRAS^{G12C}$-mutated NSCLC who have progressed after at least one prior line of systemic therapy.[4] Despite this considerable progress in the treatment of $KRAS^{G12C}$-mutated NSCLC, it remains challenging to properly select patients who will benefit from sotorasib.

Circulating tumor DNA (ctDNA) is released into the bloodstream through apoptosis and necrosis of tumor cells and is part of the total circulating cell-free DNA (cfDNA) that is present in plasma.[5,6] ctDNA has been found to have promise as a blood-based biomarker in early and advanced stage disease in different cancer types.[7–11] Major advantages of ctDNA are its noninvasive nature, the ability to track tumor evolution, and presumed better representation of tumor heterogeneity than tissue biopsies. In addition, especially in advanced disease, ctDNA could potentially allow for noninvasive real-time treatment monitoring and treatment response prediction.[5,6] An ongoing challenge of the treatment of *KRAS*-mutated NSCLC is its clinical heterogeneity and the lack of clear prognostic and predictive biomarkers to select individual patients for treatment. The 2-year analysis of the phase I/II CodeBreaK 100 trial recently revealed that patients with long-term benefit of sotorasib tended to have lower ctDNA levels at baseline.[12] In addition, serial plasma analysis of the same trial revealed that patients with undetectable ctDNA after three weeks on treatment had longer median PFS compared with those with detectable ctDNA.[13] Nevertheless, currently, there are no real-world studies that have independently investigated serial on-treatment ctDNA changes in patients with $KRAS^{G12C}$-mutated NSCLC treated with sotorasib. Furthermore, data on mechanisms of acquired resistance to $KRAS^{G12C}$-inhibitors remain limited.[14–16]

Here, we present our real-world prospective cohort of patients with metastatic $KRAS^{G12C}$-mutated NSCLC receiving sotorasib in which serial plasma samples were taken for cfDNA analysis. The TruSight Oncology 500 (TSO500) ctDNA panel, one of the most comprehensive targeted panels used for ctDNA analysis in patients receiving sotorasib to date, was used for plasma-based genomic profiling. Pretreatment and on-treatment ctDNA dynamics were correlated with clinical outcome data. In addition, we investigate treatment-emergent alterations in ctDNA at time of disease progression on sotorasib. Last, an explorative analysis was performed to investigate the potential exposure-response relationship of sotorasib.

Materials and Methods

Patients and Study Design

Patients with advanced $KRAS^{G12C}$-mutated NSCLC who were eligible for treatment with sotorasib were prospectively enrolled in our biomarker START-TKI study (NCT05221372) before treatment initiation. The START-TKI study is a prospective, observational multicenter study in which additional blood samples are collected during the standard outpatient visits of patients with oncogene-driven advanced NSCLC for which they receive treatment with tyrosine kinase inhibitors (TKIs) or small molecules. The study was approved by the medical ethical committee of the Erasmus Medical Center, Rotterdam, The Netherlands (MEC 16-643), and is conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki's ethical principles for medical research. All patients provided written informed consent before enrolment. Demographic, clinical, and laboratory information including age, sex, Eastern Cooperative Oncology Group Performance Status (ECOG PS), smoking history, prior systemic treatment for NSCLC, albumin levels, and presence of liver, bone, or central nervous system (CNS) metastasis was collected at baseline. Histology and programmed death-ligand 1 (PD-L1) tumor proportion score (TPS) from tumor tissue were retrieved from the clinical pathology reports in the electronic patient records. Sotorasib was supplied by Amgen (Thousand Oaks, CA) through the named patient program and post-approval program that was available in the Netherlands until March 31, 2023. TSO500 cfDNA analysis was supported by a research grant from AstraZeneca (2604226313) and consumables from Illumina (San Diego, CA).

Blood Sampling and cfDNA Isolation

Blood samples were drawn before start of sotorasib and at every outpatient visit after treatment start,

typically 4 weeks after start and then every 8 weeks thereafter. Here, we present the cfDNA analysis of the samples taken before treatment start, defined as T0, the samples taken at first response evaluation per computed tomography scan (±1 wk), defined as T1, and the samples taken at disease progression per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v.1.1) (±1 wk), defined as PD. In addition, the sample taken at the outpatient visit before disease progression per RECIST v.1.1, defined as PD-1, was sequenced to evaluate the potential lead-time between ctDNA increase and disease progression. Two CellSave preservative 10 mL Vacutainer tubes (Menarini Silicon Biosystems SpA, Bologna, Italy) were collected for cfDNA isolation. Further details on cfDNA isolation are available in the Supplementary methods.

cfDNA Analysis

The TSO500 ctDNA panel (Illumina, San Diego, CA), which comprises 523 genes and has 1.94 Mb genome coverage was used for mutation detection in cfDNA and blood-based tumor mutational burden (bTMB) analysis. Further information on library preparation and data analysis is available in the Supplementary methods. Pathogenicity was classified according to the ClinVar, Franklin, and OncoKB databases, and variants are presented if they were classified as likely pathogenic or pathogenic. The variant allele frequency (VAF) was calculated as the ratio of observed variant allele to the total reads. bTMB assessment was performed with the TSO500 TMB data analysis pipeline. In addition, a subset of samples with sufficient cfDNA concentrations after TSO500 testing was tested with digital droplet polymerase chain reaction (ddPCR) on the Droplet Digital PCR System of Bio-Rad (Lunteren, The Netherlands). The PD-1 samples were exclusively analyzed with ddPCR.

ctDNA detectability was defined by a cutoff of greater than or equal to two detected unique molecules for both TSO500 and ddPCR. The relative change in VAF between T0 and T1 was defined as ΔVAF. A ΔVAF of less than 0 reflects a decline in ctDNA at T1. Patients with detectable *KRAS*^{G12C} ctDNA at T0 and available T1 sample were evaluated for molecular response. A molecular response was defined as a decrease in VAF at T1 of greater than or equal to 50% as informed by previous studies investigating ctDNA in patients with lung cancer treated with immunotherapy.[8,17] In addition, complete ctDNA clearance was defined as less than two detected variants at T1 and incomplete ctDNA clearance as a decrease in VAF at T1 without complete clearance. In case of undetectable *KRAS*^{G12C} ctDNA at T0, the T1 sample was not sequenced unless the T1 time point coincided with disease progression.

Acquired resistance was defined as disease progression after a prior partial or complete response or stable disease for at least 12 weeks according to RECIST v.1.1. Acquired mutations were defined as mutations that were absent at T0 and present at disease progression. As subclonal mutations are likely to be below the detection limit of the ctDNA assay in samples in which the clonal *KRAS*^{G12C} mutation is present in low levels, we only included patients with greater than or equal to 10 *KRAS*^{G12C} unique molecules present at T0 and at PD for further acquired resistance analysis. Pathogenic variants that are strongly associated with clonal hematopoiesis of indeterminate potential (CHIP) were filtered out. Variants of unknown significance (VUS) are only presented if the gene is part of the RTK/KRAS or PIK3 pathway. Supplementary Figure 1 depicts the filtering technique used.

Exploratory Sotorasib Plasma Concentrations

A 4.0 mL lithium-heparin tube was collected at T1 for plasma sotorasib concentration analysis. All samples were taken after steady state had been reached. Sotorasib plasma concentrations were measured by validated liquid chromatography-mass spectrometry and were used to extrapolate trough concentrations (C_{trough}). Further information on the assay and the method of extrapolation is available in the Supplementary Methods. The reliability of extrapolation decreases for samples taken shortly after reaching the maximum concentration. Consequently, C_{trough} values extrapolated beyond twice the half-life of sotorasib were excluded from subsequent statistical analyses.

Clinical End Points

PFS was defined as time from the start of sotorasib treatment to disease progression per RECIST v.1.1 as assessed by the local investigator or death from any cause. Patients who had not yet progressed at data cutoff or who discontinued treatment due to reasons other than disease progression were censored at the date of the last tumor assessment before the cutoff date. Best overall response (BOR) was evaluated by the local investigator per RECIST v.1.1. Patients who were not assessable for response assessment according to RECIST v.1.1 were classified as nonresponders. Durable benefit was defined as a PFS of greater than or equal to 6 months and early progression as a PFS of less than 3 months. Overall survival (OS) was defined as the time from the start of sotorasib to death from any cause. Patients who were not known to have died at data cutoff were censored at the last date on which they were known to be alive.

Statistical Analysis

Statistical analysis was performed on IBM SPSS version 25.0 software. Categorical baseline characteristics and tumor response were compared using the Pearson chi-square test or the Fisher's exact test as appropriate. Differences in continuous data between groups were compared with the Mann-Whitney *U* test. Median follow-up time was estimated by reverse Kaplan-Meier methodology. Survival curves were estimated by Kaplan-Meier methodology and compared with the log-rank test. Univariate Cox proportional hazards regression was used to estimate the HRs. The 95% CIs for ORR and disease control rate (DCR) were estimated by Clopper-Pearson method. Correlations were evaluated using Pearson's correlation coefficient. All analyses were two sided, and *p* values less than 0.05 were considered statistically significant. Categorical data are presented as a number (percentage, %) and continuous data as the median (interquartile range [IQR]).

Results

Patient Characteristics

Between May 2021 and April 2023, 71 patients with available pretreatment plasma samples were included for serial cfDNA analysis. Data cutoff was November 14, 2023. After a median follow-up time of 15.1 months (95% CI: 9.6–20.6), 53 patients (75%) had experienced disease progression and 47 patients (66%) had died. Key clinical and demographical characteristics are summarized in Table 1. All patients had received previous platinum-based chemotherapy, immunotherapy, or both

Table 1. Baseline Characteristics of Study Population

Characteristic		Entire Cohort ($N = 71$)	Durable Benefit[a] ($n = 28$)	Early Progression[b] ($n = 23$)
Sex, *N* (%)	Male	19 (27%)	6 (21%)	6 (26%)
	Female	52 (73%)	22 (79%)	17 (74%)
Age, y	Median (IQR)	63 (56-70)	68 (58-73)	63 (53-68)
Smoking history, *N* (%)	Current	15 (21%)	5 (18%)	7 (30%)
	Former	47 (66%)	19 (68%)	13 (57%)
	Never	0	0	0
	Unknown	9 (13%)	4 (14%)	3 (13%)
Previous lines of systemic therapy for advanced disease, *N* (%)	1	44 (62%)	17 (61%)	15 (65%)
	2	25 (35%)	10 (36%)	8 (35%)
	≥3	2 (3%)	1 (4%)	0
Histology, *N* (%)	Adenocarcinoma	65 (92%)	27 (96%)	20 (87%)
	Squamous cell	2 (3%)	0	1 (4%)
	NSCLC NOS	3 (4%)	1 (4%)	2 (9%)
	LCNEC	1 (1%)	0	0
PD-L1 expression (TPS), *N* (%)	<1%	24 (34%)	14 (50%)	6 (26%)
	1%-49%	21 (30%)	8 (29%)	5 (22%)
	≥50%	20 (28%)	4 (14%)	10 (43%)
	Unknown	6 (9%)	2 (7%)	2 (9%)
Location of metastasis, *N* (%)	Liver			
	Yes	16 (23%)	4 (14%)	9 (39%)
	No	55 (76%)	24 (86%)	14 (61%)
	Bone			
	Yes	17 (24%)	5 (18%)	8 (35%)
	No	54 (76%)	23 (82%)	15 (65%)
	CNS			
	Yes	19 (27%)	6 (21%)	4 (17%)
	No	17 (24%)	9 (32%)	4 (17%)
	Unknown[c]	35 (49%)	13 (46%)	15 (65%)
ECOG PS at baseline, *N* (%)	0-1	46 (65%)	21 (75%)	11 (48%)
	≥2	25 (35%)	7 (25%)	12 (52%)

Note: Percentages may not add up to 100% due to rounding.
[a]Durable benefit was defined as a PFS of ≥6 months.
[b]Early progression was defined as a PFS of <3 months.
[c]Patients had unknown central nervous system metastases if no imaging of the brain had been performed that showed the absence of brain metastases within 6 weeks prior to treatment initiation.
IQR, interquartile range; LCNEC, large cell neuro-endocrine carcinoma; PD-L1, programmed death-ligand 1; TPS, tumor proportion score; CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

as subsequent treatment lines or as combination therapy. Six patients had also received docetaxel as a second- or third-line treatment before inclusion.

In the entire cohort, median PFS was 5.6 months (95% CI: 3.1–8.1) with a one-year PFS of 19%, and median OS was 9.1 months (95% CI: 7.0–11.2). ORR was 25% (95% CI: 16–37), and DCR was 86% (95% CI: 76–93). At first response evaluation, 18% of patients had achieved an objective response. Furthermore, 28 patients (39%) experienced durable benefit and 23 patients (32%) experienced early progression. Durable benefit was more frequent in patients with tumors with PD-L1 TPS less than 1% than with greater than or equal to 1% (odds ratio [OR] 3.3 [95% CI: 1.18–9.19], $p = 0.020$). Moreover, PD-L1 positivity was associated with numerically shorter OS (8.3 mo [95% CI: 6.8–9.9] versus 16.7 mo [95% CI: 5.7–27.7], $p = 0.088$) but was not associated with ORR ($p = 0.60$) or DCR ($p = 0.15$). Early progression was more frequent in patients with liver metastases (OR 3.77 [95% CI: 1.18–12.00], $p = 0.021$) and ECOG PS of greater than or equal to 2 (OR 2.94 [95% CI: 1.04–8.28], $p = 0.038$).

Pretreatment Detectability of ctDNA

Of the 71 included patients, 66 baseline samples were sequenced successfully. Reasons for sequencing failure can be found in Supplementary Data 1. A total of 50 patients (76%) had pretreatment-detectable *KRAS*^{G12C} ctDNA with a median VAF of 2.8% (IQR 0.8–8.7). Median bTMB of the patients with detectable *KRAS*^{G12C} ctDNA was 14.8 (IQR 10.6–20.0) mutations per megabase (mut/Mb). All patients with liver metastases had detectable *KRAS*^{G12C} ctDNA versus 68% without liver metastases ($p = 0.0073$). *KRAS*^{G12C} ctDNA detectability was not associated with any other baseline characteristics, although we did find that most patients with ECOG PS greater than or equal to two (88%) had detectable *KRAS*^{G12C} ctDNA versus 69% of patients with ECOG PS zero to one ($p = 0.092$). Patients with undetectable *KRAS*^{G12C} at baseline more frequently experienced durable benefit than patients with detectable *KRAS*^{G12C}, although not statistically significant (56% versus 34%, $p = 0.11$). *KRAS*^{G12C} detectability at T0 was not associated with subsequent objective response ($p = 0.74$) or with disease control ($p = 0.43$).

Median PFS of patients with detectable *KRAS*^{G12C} ctDNA was 4.6 months (95% CI: 3.8–5.3) versus 10.1 months (95% CI: 4.4–15.8) in patients with undetectable *KRAS*^{G12C} ctDNA (HR 2.13 [95% CI: 1.06–4.30], $p = 0.031$) (Fig. 1*A*). Median OS was also significantly shorter in patients with detectable *KRAS*^{G12C} ctDNA compared with patients with undetectable *KRAS*^{G12C} ctDNA (7.8 mo [95% CI: 4.5–11.1] versus 18.9 mo [95% CI: 8.8–29.0], HR 2.61 [95% CI: 1.16–5.91], $p = 0.017$) (Fig. 1*B*). The survival outcome data are summarized in Supplementary Data 1.

To further evaluate the potential prognostic value of pretreatment VAF and bTMB, we analyzed the patients in the highest and lowest quartiles of pretreatment *KRAS*^{G12C} VAF and bTMB. We found that patients with a pretreatment *KRAS*^{G12C} VAF in the highest quartile ($\geq$8.7%) had significantly shorter OS than those with a VAF in the lowest quartile ($\leq$0.8%) (3.8 mo [95% CI: 0.7–6.9] versus 16.7 mo [95% CI: 5.4–28.0], respectively, HR 3.66 [95% CI: 1.23–10.86], $p = 0.012$). In addition, in patients with detectable *KRAS*^{G12C} ctDNA, median *KRAS*^{G12C} VAF was significantly lower in patients with durable benefit compared with those without durable benefit (0.8% [IQR 0.5–6.9] versus 3.8% [IQR 1.9–9.9], $p = 0.048$). OS did not differ significantly between those with a bTMB of greater than or equal to 20.0 mut/Mb

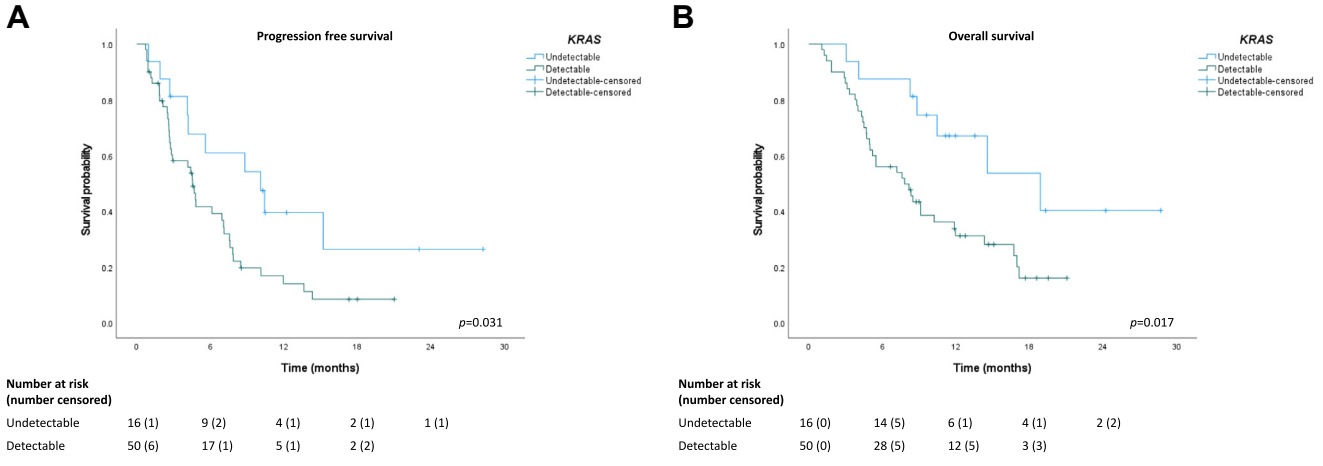

Figure 1. Survival in patients according to pretreatment *KRAS*^{G12C} ctDNA detectability. Kaplan-Meier estimates of (*A*) progression-free survival and (*B*) overall survival by *KRAS*^{G12C} detectability at baseline. *p* values are determined by log-rank test.

and those with a bTMB of less than or equal to 10.6 mut/Mb (7.6 mo [95% CI: 3.7–11.5] versus 8.5 mo [95% CI: 0.0–18.1], respectively, HR 1.70 [95% CI: 0.63–4.60], $p = 0.30$).

Co-Occurring Mutations in ctDNA at Baseline

Co-occurring mutations in ctDNA at T0 are found in Figure 2. The most frequently co-occurring mutations were *TP53* (56%), *STK11* (26%), and *KEAP1* (15%). For outcome analyses, only the samples with detectable $KRAS^{G12C}$ ($n = 50$) were analyzed. Patients with detectable *STK11* ctDNA at T0 had numerically shorter PFS (2.8 mo [95% CI: 2.4–3.1] versus 4.8 mo [95% CI: 2.8–6.8], HR 1.45 [95% CI: 0.73–2.89], $p = 0.28$) and OS (4.7 mo [95% CI: 3.9–5.5] versus 8.5 mo [95% CI: 7.1–9.9], HR 1.76 [95% CI: 0.87–3.57], $p = 0.11$) than those with undetectable *STK11* ctDNA (Supplementary Fig. 2*A* and *B*). DCR did not differ significantly between those with detectable and undetectable *STK11* ctDNA (80% versus 83%, $p = 1.00$). Patients with detectable *KEAP1* ctDNA at T0 had significantly shorter PFS (2.1 mo [95% CI: 0.6–3.7] versus 4.8 mo [95% CI: 2.1–7.5], HR 4.17 [95% CI: 1.66–10.51], $p = 0.0010$) and shorter OS (4.3 mo [95% CI 3.1–5.4] versus 9.1 mo [95% CI: 7.8–10.4], HR 2.24 [95% CI: 1.01–4.99], $p = 0.042$) compared with those with undetectable *KEAP1* ctDNA (Supplementary Fig. 2*C* and *D*). DCR was lower in patients with detectable

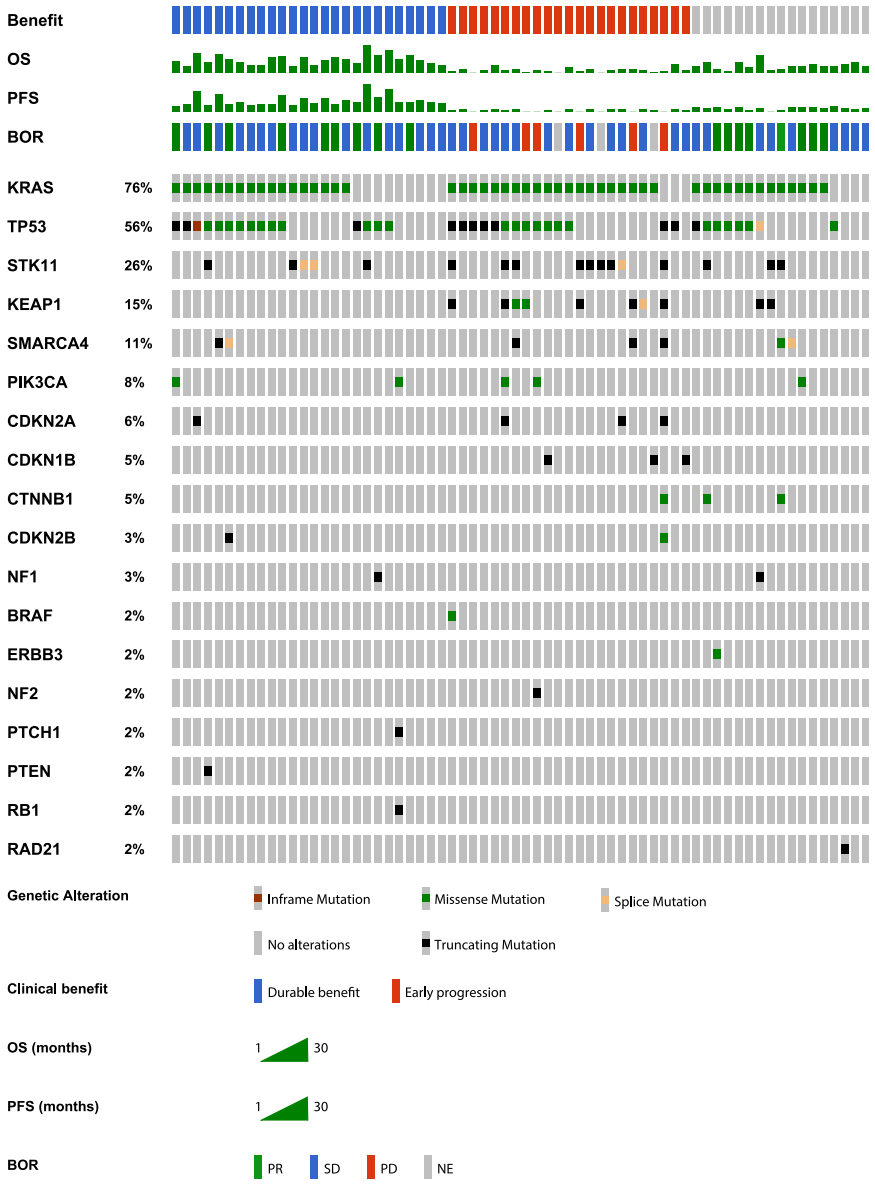

Figure 2. Heatmap of baseline alterations with clinical outcomes. Each column represents one patient. Best objective response is according to RECIST version 1.1. BOR, best overall response; NE, not evaluable; OS, overall survival; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

KEAP1 ctDNA versus in those with undetectable *KEAP1* ctDNA; however, this did not meet statistical significance (67% versus 85%, $p = 0.33$). None of the patients with detectable *KEAP1* ctDNA experienced durable benefit versus 41% of patients with undetectable *KEAP1* ctDNA ($p = 0.020$). In addition, 78% of patients with detectable *KEAP1* ctDNA experienced early progression versus 32% of patients with undetectable *KEAP1* ctDNA ($p = 0.021$). PFS, OS, and DCR did not differ between those with detectable or undetectable *TP53* ctDNA ($p = 0.79$, $p = 0.19$, and $p = 0.45$, respectively) (Supplementary Fig. 2*E* and *F*).

Molecular Response at First Response Evaluation

Of the 50 patients with detectable $KRAS^{G12C}$ ctDNA at T0, 40 subsequent T1 plasma samples were sequenced successfully. Median time between treatment start and T1 was 28 (IQR 28–30) days. A total of 29 patients (73%) had a molecular response, of which 17 patients (59%) had complete clearance of $KRAS^{G12C}$ ctDNA. Of the 11 molecular nonresponders, five patients had incomplete $KRAS^{G12C}$ clearance with a median ΔVAF of −23.1% (IQR −13.7 to −38.5) and six patients had VAF increase with median ΔVAF of 26.6% (IQR 10.4–415.6). The ΔVAF between T0 and T1 of the *TP53*, *STK11*, and *KEAP1* co-mutations mirrored that of $KRAS^{G12C}$. Nevertheless, in a few cases, there was complete clearance of $KRAS^{G12C}$, whereas the co-mutations remained detectable at low levels (Supplementary Data 1).

The clinical outcomes of the molecular responders and nonresponders are summarized in Figure 3. Molecular responders had a higher DCR than nonresponders (97% versus 64%, $p = 0.015$) but did not have a higher ORR at T1 or subsequently ($p = 1.00$ and $p = 0.23$, respectively). There was no significant difference between patients with complete or incomplete clearance for ORR at T1 ($p = 1.00$), ORR subsequently ($p = 1.00$), or DCR ($p = 0.061$). Early progression occurred more frequently in molecular nonresponders than in

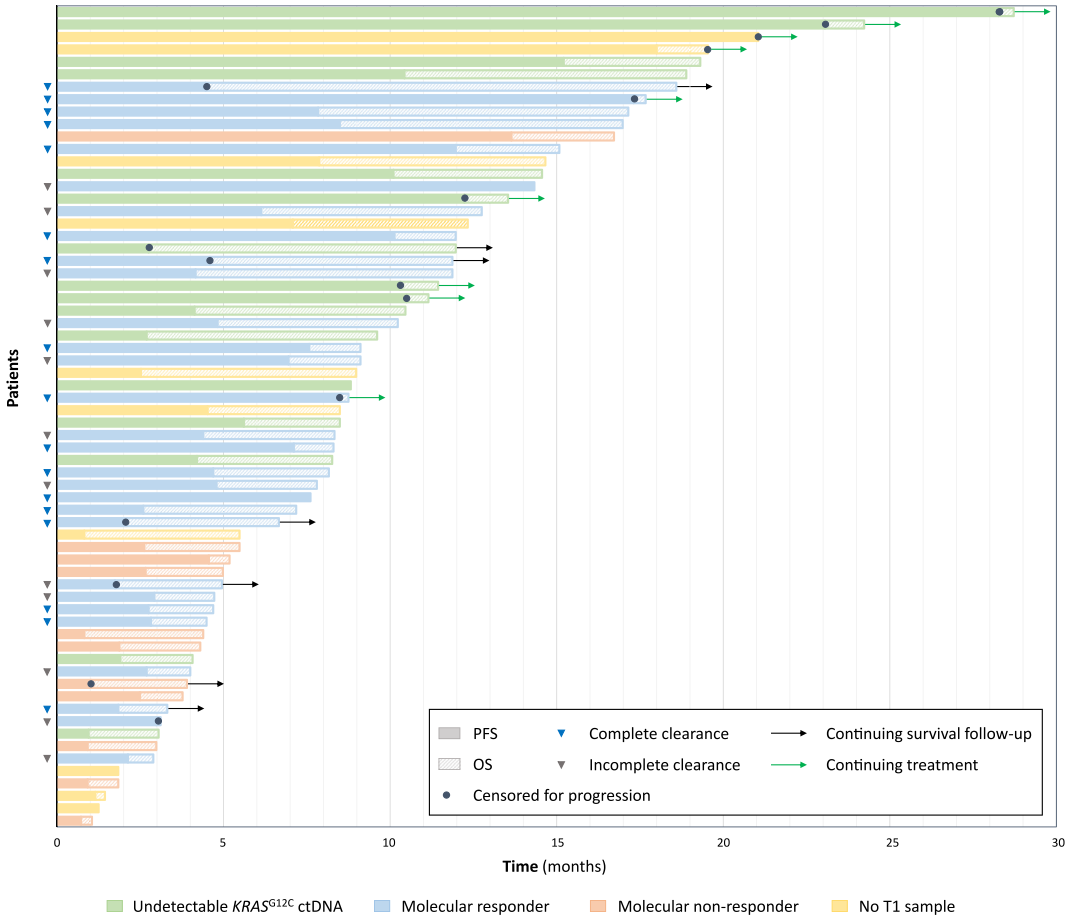

Figure 3. Progression-free and overall survival and molecular response. Swimmer's plot revealing time to progression per RECIST version 1.1 or censoring and time to death or censoring for patients with undetectable $KRAS^{G12C}$ ctDNA at T0 and for molecular responders and nonresponders. A molecular response is defined as a decrease of greater than or equal to 50% in $KRAS^{G12C}$ variant allele frequency. Each bar represents one subject. RECIST, Response Evaluation Criteria in Solid Tumors; T0, pretreatment; T1, at first response evaluation.

molecular responders (73% versus 24%, OR 8.38 [95% CI: 1.73–40.53], $p = 0.0090$), and durable benefit occurred more frequently in molecular responders than in molecular nonresponders, although this did not meet statistical significance (41% versus 9%, OR 7.06 [95% CI: 0.79–62.72], $p = 0.068$).

Median PFS of the molecular nonresponders was 2.5 months (95% CI: 0.2–4.8) versus 6.1 months (95% CI: 2.9–9.4) of the molecular responders (HR 3.03 [95% CI: 1.39–6.61], $p = 0.0035$) (Fig. 4*A*). Median OS was 4.3 months (95% CI: 3.6–5.0) in molecular nonresponders versus 9.1 months (95% CI: 6.7–11.5) in molecular responders (HR 3.58 [95% CI: 1.65–7.74], $p = 0.00059$) (Fig. 4*B*).

Progressive Disease

A total of 29 samples at time of disease progression per RECIST v.1.1 were sequenced successfully. Five samples were collected at T1 as those patients had disease progression at first evaluation. In the patients with unique T1 and PD samples ($n = 16$), increase in $KRAS^{G12C}$ ctDNA VAF between T1 and PD was found in 13 patients (81%). Interestingly, in the five patients with progression at T1, only one exhibited increase in $KRAS^{G12C}$ ctDNA VAF.

Furthermore, 17 samples taken at the outpatient visit before disease progression per RECIST v.1.1 (PD-1) were successfully sequenced by ddPCR. Median time between PD-1 sampling and disease progression was 53 (IQR 40–61) days. In 10 of 17 samples, a rise in ctDNA $KRAS^{G12C}$ VAF at PD-1 compared with T1 was observed. Two of the samples that did not have a rise in ctDNA were negative for $KRAS^{G12C}$ ctDNA at baseline.

After the filtering depicted in Supplementary Figure 1, seven PD samples were included for further exploratory acquired resistance analysis. The treatment-emergent variants are summarized in Table 2, and the unfiltered sequencing data are available in Supplementary Data 1. We found *KRAS* amplifications in three of seven samples at time of disease progression. No other clear resistance mechanisms were detected.

Correlation Between TSO500 and ddPCR

A total of 26 samples were also analyzed by ddPCR. We found a strong correlation between TS0500- and ddPCR-detected $KRAS^{G12C}$ VAFs ($r = 0.99$, $p < 0.0001$, Supplementary Fig. 3). Among these samples, in five cases where the TSO500 analysis did not detect $KRAS^{G12C}$ ctDNA, ddPCR did. The median VAF detected by ddPCR in these cases was 0.17%, ranging from 0.03% to 0.40%.

Systemic Sotorasib Exposure

A total of 28 patients had available sotorasib plasma concentrations at T1. Median sotorasib plasma concentration was 113 ng/mL (IQR 59–414 ng/mL). Median PFS was 4.2 months (95% CI: 0.1–8.2) for patients with a sotorasib concentration above 113 ng/mL and 7.6 months (95% CI: 3.5–11.6) for those with a concentration below 113 ng/mL (HR 1.68 [95% CI: 0.66–4.23], $p = 0.27$). Median OS was significantly shorter for patients with a sotorasib concentration above 113 ng/mL at T1 compared with those with a concentration below 113 ng/mL (4.4 mo [95% CI: 2.2–6.6] versus 16.7 mo [95% CI: 8.5–24.9], HR 3.35 [95% CI: 1.13–9.98], $p = 0.022$). ORR at T1 was 29% (95% CI: 8–58) in patients with a sotorasib plasma concentration below 113 ng/mL and 8% (95% CI: 1–34) in patients with a sotorasib plasma concentration above 113 ng/mL ($p = 0.33$). Patients with a sotorasib plasma concentration above 113 ng/mL

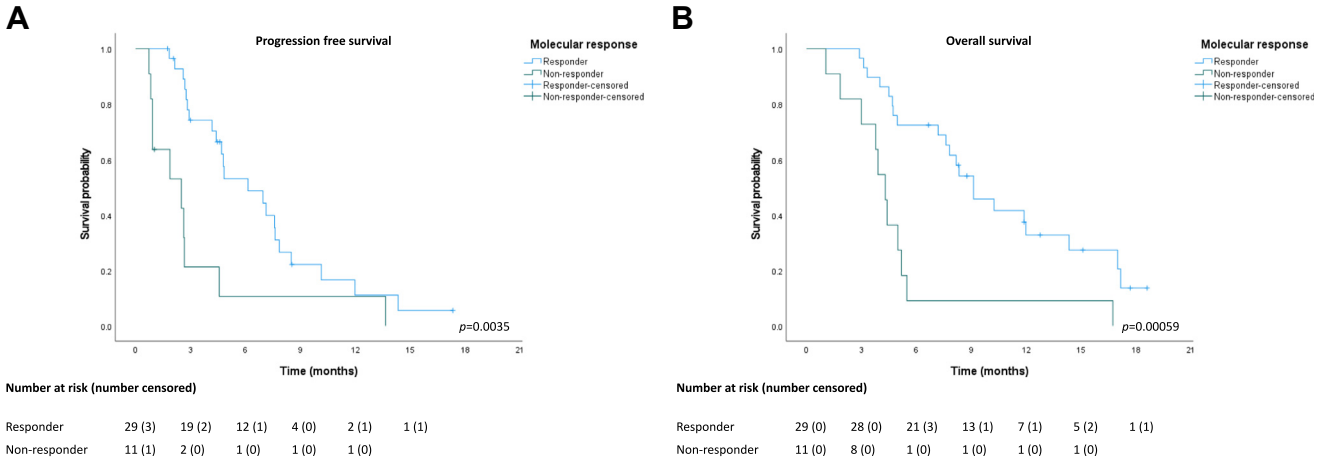

Figure 4. Survival in patients according to molecular response at first response evaluation. Kaplan-Meier estimates of (*A*) progression-free survival and (*B*) overall survival by molecular response at first response evaluation. *p* values are determined by log-rank test. T1, at first response evaluation.

Table 2. Treatment-Emergent Variants at Disease Progression

Patient	Tumor Response	PFS (mo)	*KRAS*^{G12C} VAF (Altered/Reference Molecules)		Treatment-Emergent Alterations	
			T0	PD	Alteration VAF (Altered/Reference Molecules)	Pathogenicity
1	PR	4.8	3.8 (59/1505)	40.8 (425/617)	*KRAS*^{Amp}	Pathogenic
2	PR	8.5	8.4 (78/852)	10.3 (248/2157)	*PRKDC*^{R2899Cfs*47} 0.40 (14/3493)	Likely pathogenic
					SPEN^{F356Sfs*10} 0.23 (4/1726)	Likely pathogenic
3	PR	4.6	46.7 (199/227)	80.7 (6253/1500)	*KRAS*^{Amp}	Pathogenic
					MED12^{R621Q} 0.38 (10/2620)	Likely pathogenic
					ERBB3^{V89M} 0.16 (5/3160)	VUS
4	PR	4.4	6.2 (143/2168)	2.2 (11/493)	*NTRK3*^{R306P} 0.44 (4/909)	VUS
5	SD	4.2	1.8 (36/1962)	14.1 (159/966)	*KRAS*^{Amp}	Pathogenic
6	SD	7.0	14.5 (114/688)	66.0 (3793/1950)	*ARID5B*^{F827Lfs*54} 0.09 (3/3434)	Likely pathogenic
7	PR	7.1	3.1 (41/1272)	8.4 (16/174)	*PIK3CD*^{G346V} 0.10 (5/4468)	VUS
					EGFR$^{L210=}$ 0.10 (6/5616)	VUS
					BRCA2^{E731*} 2.8 (16/174)	Likely pathogenic

Note: (Likely) pathogenic variants and variants of unknown significance when they occur in genes in the RTK/RAS or PIK3 pathway are presented. For cases with *KRAS* amplifications, baseline tissue was checked to confirm the absence of the amplification at baseline.
PFS, progression-free survival; VAF, variant allele frequency; T0, pretreatment; PD, at disease progression; PR, partial response; SD, stable disease; VUS, variant of unknown significance.

had significantly lower albumin levels at T0 compared with those with a concentration below 113 ng/mL (30 g/L versus 38 g/L, $p < 0.00027$) and were more frequently ECOG greater than or equal to two, although not significantly (57% versus 36%, $p = 0.26$).

Discussion

In the current study, we investigated the clinical utility of *KRAS*^{G12C} ctDNA in patients with advanced NSCLC who were treated with sotorasib. To the best of our knowledge, this is the first real-world study to investigate the relationship between both pretreatment and on-treatment *KRAS*^{G12C} ctDNA dynamics and clinical outcomes in this patient population. We revealed that serial cfDNA analysis with the comprehensive TSO500 panel is a feasible treatment monitoring method, and our data suggest that *KRAS*^{G12C} ctDNA could aid in predicting clinical outcomes in patients with NSCLC who are treated with sotorasib. Given the strong correlation between the TSO500 and ddPCR VAFs, ddPCR may present a more cost-effective alternative for routine clinical follow-up of *KRAS*^{G12C} ctDNA.

In most patients (76%), *KRAS*^{G12C} ctDNA was detectable before treatment initiation with sotorasib. We found that the presence of liver metastases at baseline was associated with *KRAS*^{G12C} ctDNA detectability. This is probably explained by the vascular nature of the liver as the proximity of metastatic sites to vasculature is likely to affect the extent of ctDNA shedding into the bloodstream. Plasma ctDNA concentrations have also previously been associated with tumor load and metastatic burden, suggesting it as a prognostic biomarker.[5,18] We indeed found that pretreatment undetectable *KRAS*^{G12C} ctDNA was associated with improved PFS and OS. There was no difference in tumor response between those with detectable or undetectable *KRAS*^{G12C} ctDNA, which suggests that pretreatment *KRAS*^{G12C} detectability mainly has prognostic, rather than predictive, value. Besides *KRAS*^{G12C}, we were able to detect co-occurring alterations in most cases, primarily in *TP53, STK11,* and *KEAP1*. Several previous studies have suggested that *STK11* and *KEAP1* co-mutations could influence clinical outcomes.[4,19–21] In our cohort, primarily detectable *KEAP1* inferred inferior survival. Patients with detectable *KEAP1* ctDNA were found to have lower disease control; however, this did not reach statistical significance, possibly due to the limited sample size of patients with detectable *KEAP1*. Furthermore, none of the patients with detectable *KEAP1* ctDNA experienced durable benefit, with 78% experiencing early progression, further suggesting *KEAP1* as a negative marker for clinical outcomes. Last, we also found that PD-L1 positivity could be a potential negative prognostic marker in patients with *KRAS*^{G12C}-mutated NSCLC. Although this association has been reported previously,[22] the underlying biological mechanisms contributing to the worse prognosis remain unclear. Interestingly, in our exploratory sotorasib plasma concentration analysis, we found that higher sotorasib trough concentrations at T1 were associated with worse OS. Nevertheless, it is likely that baseline disease burden or body composition could influence sotorasib pharmacokinetics and clearance. For instance, we found that the patients in our cohort with higher sotorasib concentrations had lower albumin levels and worse performance status at baseline. This aligns with the exposure-efficacy analyses of the CodeBreaK

100, in which a higher area under the curve (AUC) at steady state was found in patients with low albumin (<34 g/L) or an ECOG PS of 2.[23] Because of the potential confounding effect of baseline disease status on sotorasib clearance and exposure, these analyses are considered inconclusive.

Molecular response at first response evaluation was associated with improved PFS, OS, and DCR, which suggests that early ctDNA clearance could serve as a predictive marker for sotorasib response. Currently, there is no consensus on the definition of molecular response, which can hamper comparisons between studies. Some studies define molecular response as undetectable ctDNA, whereas others define it as a decrease in VAF.[6,8,24,25] Our data suggest that the relative change in *KRAS*^{G12C} VAF might be more informative than solely the presence or absence of *KRAS*^{G12C} ctDNA as DCR was significantly higher in molecular responders than nonresponders but did not differ between those with complete clearance and those with incomplete clearance. Last, we identified *KRAS* amplifications as a potential acquired resistance mechanism to sotorasib, consistent with the findings of two previous cohort studies.[14,15] Nevertheless, we were unable to confirm other resistance mechanisms reported in those studies. This discrepancy might be due to our analysis being limited to patients with higher levels of ctDNA at both baseline and disease progression. Our approach aimed to minimize the likelihood of misclassifying a variant as acquired when it could have been below the ctDNA assay's detection limit at baseline but became detectable during disease progression due to an increase in tumor load. We did not detect any other treatment-emergent variants that plausibly would contribute to resistance to sotorasib.

Our study has a few limitations that should be considered. First, due to our limited sample size, we were unable to confirm the independent prognostic or predictive significance of *STK11* and *KEAP1* alterations, given that numerous patients had both *STK11* and *KEAP* co-occurring alterations. Next, the C_{trough} extrapolation method has not been validated for sotorasib. Therefore, these analyses should be considered exploratory. In addition, because of limited sampling, we were not able to calculate sotorasib clearance on an individual patient basis as this requires the development and validation of a pharmacokinetic model. Consequently, we were not able to assess whether sotorasib plasma concentrations serve as an independent marker for clinical outcomes. It is likely that the difference in clinical outcomes between the high- and low-exposure groups in our cohort is mainly driven by differences in baseline disease burden. Last, none of the patients underwent tumor biopsies at disease progression. Consequently, it is possible that certain acquired alterations remained undetected due to sensitivity constraints of the ctDNA assay. In addition, we were not able to evaluate histologic transformations.

cfDNA profiling before treatment initiation and during treatment could have several clinical implications. Importantly, cfDNA could provide a rationale for determining the place of sotorasib in daily clinical practice as the CodeBreaK 200 trial was not able to fully answer this question due to several limitations in the study design as also discussed by Olivier et al.[26] For example, the study was not adequately powered to detect OS differences and permitted crossover to sotorasib. In addition, PFS estimates could be unreliable due to an imbalance in censoring rates between treatment arms. Owing to these constraints, the Food and Drug Administration's Oncologic Drug Advisory Committee recently voted that the PFS of the CodeBreaK 200 trial could not be reliably interpreted. Therefore, there is an urgent need for additional tools to aid clinicians in determining which patients would benefit from sotorasib. Pretreatment cfDNA analysis baseline could aid in identifying those patients with superior prognosis which could provide a rationale for reducing the frequency of radiological response evaluations. In addition, identifying those patients who are less likely to deteriorate quickly could aid in optimizing time of treatment initiation. This can be of special interest in patients who recently received immunotherapy, as those patients are at a higher risk of developing hepatotoxicity when treated with sotorasib shortly after receiving immunotherapy.[27,28] Next, early on-treatment cfDNA analysis could allow to identify patients who are at risk of early progression. In such cases, intensifying the frequency of radiological response evaluations could allow for timely detection of radiological disease progression. In turn, a switch of systemic therapy could be achieved before the patients' clinical condition deteriorates. Last, as we found that radiological disease progression coincided with increase in *KRAS*^{G12C} VAF in most cases and in certain cases was also preceded by an increase in *KRAS*^{G12C} VAF, it would be of clinical interest to further investigate whether there is a lead-time between ctDNA increase and radiological progression, which would allow for early treatment decisions. Owing to the limited number of samples available at the PD-1 time point in our cohort, we cannot currently draw definitive conclusions from these findings.

In conclusion, our data reveal pretreatment ctDNA detectability and lack of early ctDNA clearance as important markers of inferior outcomes in patients with NSCLC who are treated with sotorasib. These markers could aid in guiding both treatment decisions and follow-up approaches in daily clinical practice.

CRediT Authorship Contribution Statement

Sophie M. Ernst: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review and editing.

Ronald van Marion: Data curation, Formal analysis, Resources, Investigation, Software, Writing—review and editing.

Peggy N. Atmodimedjo: Data curation, Investigation, Writing—review and editing.

Evert de Jonge: Data curation, Investigation, Writing—review and editing.

Ron H.J. Mathijssen: Conceptualization, Writing—review and editing.

Marthe S. Paats: Conceptualization, Writing—review and editing.

Peter de Bruijn: Data curation, Formal analysis, Resources, Investigation, Software, Writing—review and editing.

Stijn L. Koolen: Conceptualization, Data curation, Writing—review and editing.

Jan H. von der Thüsen: Conceptualization, Writing—review and editing.

Joachim G.J.V. Aerts: Conceptualization, Writing—review and editing.

Ron H.N. van Schaik: Conceptualization, Data curation, Writing—review and editing.

Hendrikus J. Dubbink: Conceptualization, Funding acquisition, Supervision, Writing—review and editing.

Anne-Marie C. Dingemans: Conceptualization, Supervision, Writing—review and editing.

Disclosure

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2024.04.007.

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