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# Clinical validation of Guardant360 CDx as a blood-based companion diagnostic for sotorasib

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ABSTRACT

*Objectives:* Effective therapy for non–small-cell lung cancer (NSCLC) depends on morphological and genomic classification, with comprehensive screening for guideline-recommended biomarkers critical to guide treatment. Companion diagnostics, which provide robust genotyping results, represent an important component of personalized oncology. We evaluated the clinical validity of Guardant360 CDx as a companion diagnostic for sotorasib for detection of *KRAS* p.G12C, an important oncogenic NSCLC driver mutation.

*Materials and Methods: KRAS* p.G12C was tested in NSCLC patients from CodeBreaK100 (NCT03600833) in pretreatment plasma samples using Guardant360 CDx liquid biopsy and archival tissue samples using *therascreen*® KRAS RGQ polymerase chain reaction (PCR) kit tissue testing. Matched tissue and plasma samples were procured from other clinical trials or commercial vendors, and results were compared. Demographics and clinical characteristics and objective response rate (ORR) were evaluated.

*Results:* Of 126 CodeBreaK patients, 112 (88.9%) were tested for *KRAS* p.G12C mutations with Guardant360 CDx. Among 189 patients in the extended analysis cohort, the positive and negative percent agreement (95% CI) for Guardant360 CDx plasma testing relative to *therascreen*® KRAS RGQ PCR kit tissue testing were 0.71 (0.62, 0.79)

*Abbreviations:* AAAS, assay agreement analysis set; *ALK*, anaplastic lymphoma kinase; CDx, companion diagnostics; CI, confidence interval; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; CR, complete response; DCR, disease control rate; DOR, duration of response; ECOG, Eastern Cooperative Oncology Group; *EGFR*, epidermal growth factor receptor; FDA, US Food and Drug Administration; G360, Guardant360; GCLP, Good Clinical Laboratory Practice; GTP, guanosine-5′-triphosphate; IQR, interquartile range; *KRAS*, Kirsten rat sarcoma; LBx, liquid biopsy; NGS, next-generation sequencing; NPA, negative percentage agreement; NSCLC, non–small-cell lung cancer; ORR, objective response rate; PCR, polymerase chain reaction; PD, progressive disease; PD-1, programmed cell death protein-1; PD-L1, programmed cell death protein ligand-1; PPA, positive percentage agreement; PR, partial response; QC, quality control; RECIST, Response Evaluation Criteria in Solid Tumors; RGQ, Rotor-Gene-Q MDx instrument; *ROS1*, receptor tyrosine kinase; SAAS, sensitivity analysis prevalence sub-study; SD, stable disease; SMM, sum of mutant molecules; TAT, turnaround time; TTR, time to response.

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and 1.00 (0.95, 1.00), respectively; overall percent agreement (95% CI) was 0.82 (0.76, 0.87). *TP53* co-mutations were the most common regardless of *KRAS* p.G12C status (*KRAS* p.G12C–positive, 53.4%; *KRAS* p.G12C–negative, 45.5%). *STK11* was co-mutated in 26.1% of *KRAS* p.G12C–positive samples. The ORR was similar among patients selected by plasma and tissue testing.

*Conclusion:* Comprehensive genotyping for all therapeutic targets including *KRAS* p.G12C is critical for management of NSCLC. Liquid biopsy using Guardant360 CDx has clinical validity for identification of patients with *KRAS* p.G12C–mutant NSCLC and, augmented by tissue testing methodologies as outlined on the approved product label, will identify patients for treatment with sotorasib.

## 1. Introduction

In patients with non–small-cell lung cancer (NSCLC), the treatment paradigm has markedly evolved with the emergence of therapies that target specific molecular abnormalities [1–3], such as activating alterations in epidermal growth factor receptor *(EGFR*), anaplastic lymphoma kinase (*ALK*), and proto-oncogene 1, receptor tyrosine kinase (*ROS1*) [4,5]. An important target for therapy, and one of the most frequently mutated oncogenes in NSCLC [6,7], is the Kirsten rat sarcoma viral oncogene homology p.G12C mutation (*KRAS* G12C), which is found in approximately 13% of all patients with NSCLC [8,9] and associated with decreased overall survival compared to *KRAS* wild-type [10,11]. Sotorasib, a covalent small-molecule inhibitor of $KRAS^{G12C}$, was recently approved for adults with *KRAS* G12C–mutated locally advanced or metastatic NSCLC [12,13], and phase 1 and 2 studies support its tolerability and antitumor efficacy [14,15].

As effective treatment in NSCLC depends on genomic classification of individual patients [5,16], companion diagnostics (CDx) represent a category of *in vitro* diagnostic devices that provide results needed for the safe and effective use of a corresponding therapy and, as such, are a critical component of personalized medicine [17–19]. Failure to correctly identify actionable mutations can result in selection of suboptimal treatment options, delays in administration of more appropriate treatment, and shortened patient survival [20–22].

Despite this, insufficient genotyping remains common in NSCLC. Although testing rates have improved over time for commonly targeted genes such as *EGFR*, *ALK,* and *ROS1,* testing rates vary widely worldwide, and improvements in testing frequency, completeness, and rapidity are critically needed [16]. The reasons for incomplete testing are numerous and include tissue insufficiency, use of single-biomarker tests, suboptimal physician education, lengthy turnaround times (TAT) required for some test results, and incomplete and inconsistent healthcare payer coverage for such testing [16].

Comprehensive liquid biopsy (LBx) addresses some of these limitations. In certain settings, LBx may provide a more viable testing option when key elements for clinical decision-making are well-balanced, including testing sensitivity and country-specific differences in molecular testing availability, TAT, and cost. Most LBx are performed using a whole blood sample collected in a specialized blood collection tube that preserves cell-free DNA (cfDNA) at ambient temperatures for up to 7 days, allowing facile worldwide collection and shipment to testing laboratories even from sites without any in-house laboratory or sample processing facilities [23,24]. Because circulating tumor DNA (ctDNA) typically comprises only a small minority of all cfDNA present in circulation, even in advanced cancer patients, the sensitivity of an LBx test is important [23]. Previous studies have shown that LBx tests for *KRAS* mutations correlate with tissue biopsy results, with approximately 85%–95% overall concordance depending on the tumor type and timing of the test, and driven by higher specificity and lower sensitivity [25–27].

The objective of this study was to clinically validate the use of Guardant360 CDx, a US Food and Drug Administration (FDA)–approved [28] next-generation sequencing (NGS)–based LBx test, as a CDx to identify patients with NSCLC and *KRAS* p.G12C who are eligible for sotorasib therapy.

## 2. Materials and methods

The phase 2 multicenter, open-label clinical trial evaluating the efficacy of sotorasib in patients with advanced solid tumors (phase 2: NSCLC; CodeBreaK100; NCT03600883) has been previously described [15,29]. Briefly, adults ≥ 18 years of age were eligible for participation if they had Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1–measurable, pathologically documented, locally advanced or metastatic malignancy with *KRAS* p.G12C. Tumor mutation status was confirmed prior to enrollment by central tissue testing with *therascreen*® KRAS RGQ polymerase chain reaction (PCR) kit (KRAS RGQ PCR kit; Qiagen, Germantown, MD, USA). Patients had to provide archival tumor tissue samples (collected within 5 years) or undergo pretreatment tumor biopsy. Patients were included if they had progressed after anti–PD-1 or anti–PD-L1 immunotherapy and/or platinum-based combination chemotherapy. Patients were not eligible if they had active brain metastases from non-brain tumors, a myocardial infarction within 6 months of study start, or any gastrointestinal disease precluding oral medication. The study was designed to assess the efficacy, safety, and tolerability of sotorasib.

Patients enrolled in the phase 2 trial with informed consent for blood sample use for diagnostic development were included in this analysis. The primary endpoint was objective response rate (ORR) and secondary endpoints included disease control rate (DCR) and duration of response (DOR) [15].

### 2.1. Diagnostic study populations

Pretreatment plasma samples were collected from patients enrolled in the phase 2 NSCLC cohort of CodeBreaK100 (NCT03600833). Whole blood samples were drawn and plasma was isolated and stored frozen for 5 to 12 months until testing. Matched tissue and plasma samples were procured from patients in other clinical trials or from commercial vendors using similar selection criteria; the archival tumor tissue samples were required to have been collected within 5 years of the matched plasma sample. Patient cohorts were defined as outlined in Fig. 1.

### 2.2. Study objectives

The primary objective of the current analysis was to establish the clinical validity of Guardant360 CDx using data and samples from the CodeBreaK100 (NCT03600883) study. The secondary objectives were to assess the concordance between *KRAS* p.G12C mutation status determined by the *therascreen*® KRAS RGQ PCR kit and Guardant360 CDx in patients with NSCLC; to assess representativeness of the Guardant360 CDx–positive cohort compared with the full analysis group; and to assess DOR, DCR, and time to response (TTR) in patients with *KRAS* p.G12C–mutant NSCLC as detected by Guardant360 CDx relative to the full analysis group.

### 2.3. Description of testing processes

A designated central laboratory (Neogenomics, Houston, Texas) was used to determine *KRAS* p.G12C status for each tissue sample. De-identified archival formalin-fixed, paraffin-embedded tissue samples

were shipped at ambient temperature and stored until tested. Mutation status was assessed using the *therascreen*® KRAS RGQ PCR kit in accordance with the laboratory standard operating procedures, Good Clinical Laboratory Practice (GCLP) guidelines, and manufacturer protocols. De-identified plasma samples were shipped from the central laboratory to Guardant Health (Redwood City, CA, USA), where *KRAS* p.G12C mutation status was determined. De-identified whole blood samples were processed to plasma and stored at −80 °C until testing. Mutation status was assessed using Guardant360 CDx in accordance with the laboratory standard operating procedures, GCLP guidelines, and manufacturer protocols.

### 2.4. Tumor DNA shedding analyses

Patients were grouped into categories based on the presence or absence of plasma tumor DNA and whether *KRAS* p.G12C was detected. The groups were 1) Non–G12C Shedder, which included patients in whom tumor DNA variants were reported but *KRAS* p.G12C not detected; 2) G12C Shedder, in which *KRAS* p.G12C was detected; 3) Non-Shedder, in whom no tumor DNA variants were reported from a sample passing laboratory quality control (QC) checks; and 4) Fail, which included patients with samples failing QC. Tumor DNA content and cfDNA content (ng) were compared between these groups to characterize Non–G12C Shedders and G12C Shedders. Tumor DNA content within the plasma cfDNA was represented by the sum of mutant molecules (SMM), where mutant molecules were defined as each variant's allele frequency multiplied by the corresponding sequencing depth. Because allele frequency was not reported for copy number variations, they did not contribute to the SMM calculation. The Kruskal-Wallis test was used for comparisons of SMM between patient groups.

### 2.5. Statistical analyses

Based on an initial enrollment estimate of 105 patients, and 75% of patients being tissue-positive based on the Guardant360 CDx test, the study had a power of 90% to exclude a benchmark standard of care ORR of 23%. Demographics and clinical characteristics for the Guardant360 CDx–evaluable cohort and Guardant360 CDx–unknown cohort were compared using a chi-square test. Imbalance among baseline covariates was not adjusted for due to small sample sizes. The ORR for the Guardant360 CDx–positive population was considered acceptable if the lower limit of its 95% confidence interval (CI) was greater than 22%, the bridging study size–adjusted equivalent of the 23% benchmark ORR reported in the phase 2 study of sotorasib.

To assess an estimate of the efficacy of sotorasib in patients that were Guardant360 CDx–positive, regardless of their underlying tumor tissue *KRAS* p.G12C status, a supplementary population of 132 patients with NSCLC was investigated.

Sensitivity analysis was undertaken based on a "missing at random" approach [30] to account for patients without a valid Guardant360 CDx result. Guardant360 CDx results were simulated for this population, and the primary efficacy analysis repeated using these imputed results. The ORR for a hypothetical Guardant360 CDx–positive intended use population was estimated as a weighted average of two ORR, calculated as follows:

Observed ORR*P($Tissue^+/G360^+$) + Postulated ORR*P($Tissue^-|G360^+$)

where the observed ORR was from the primary analysis and represents the Guardant360 CDx–positive tissue-positive population, the two postulated ORRs for the Guardant360 CDx–positive tissue-negative population were the observed ORR and 0, and the weighting was determined based on the P($Tissue^+$) and the positive percentage agreement (PPA) and negative percentage agreement (NPA) of Guardant360 CDx relative to the *therascreen*® KRAS RGQ PCR test; P($Tissue^+$) indicates the probability of a positive tissue test, and P($Tissue^-|G360^+$) indicates the probability of a negative tissue test in patients with a positive Guardant360 CDx test result. Concordance results are presented in a 2×2 table with 95% CIs.

## 3. Results

### 3.1. Patient population and summary of KRAS p.G12C mutation status

Overall, 126 patients with NSCLC were enrolled in the phase 2 portion of the study and comprised the Full Analysis Set (Fig. 1). Among these, samples from 112 patients (88.9%) were tested for *KRAS* p.G12C mutations with Guardant360 CDx (Guardant360 Analysis Set). Testing was successful in 109 patients (86.5%; Guardant360 Evaluable cohort). Overall, 78 patients (61.9%) in the Guardant360 Analysis Set tested positive for the *KRAS* p.G12C mutation (Guardant360 Positive cohort). In addition, matched tissue and plasma samples procured from 132 patients with NSCLC for the sensitivity analysis prevalence sub-study (Assay Agreement Analysis Set; AAAS cohort) were included. Among these, samples from 128 patients were tested with both Guardant360 CDx and *therascreen*® KRAS RGQ PCR kit. Overall, 189 patients had valid Guardant360 CDx and *therascreen*® KRAS RGQ PCR kit results. Testing failures for Guardant360 CDx were due to insufficient cfDNA. Although not a device "failure," the failure to identify mutations could

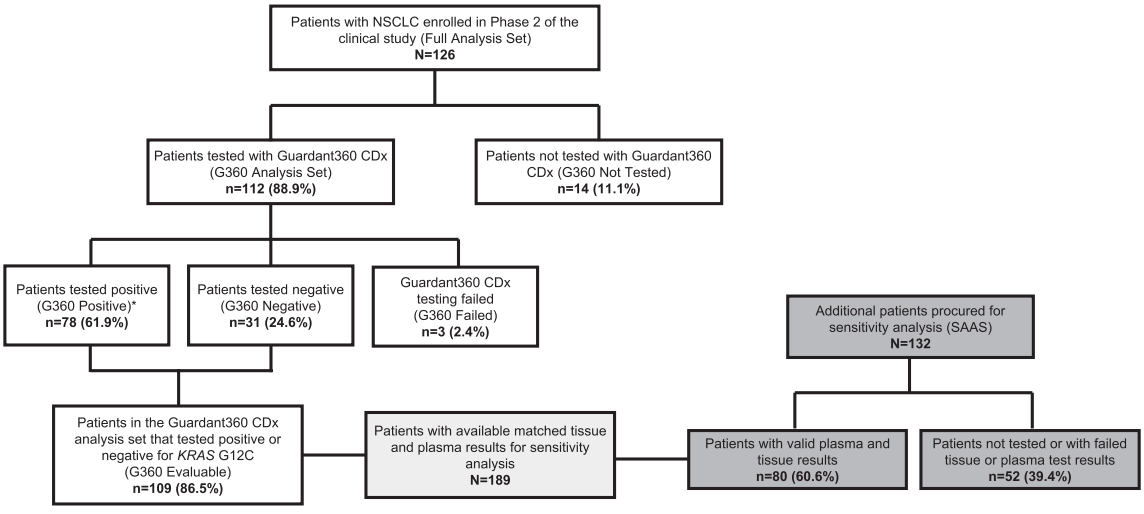


**Fig. 1.** Overview of patients in each of the diagnostic study cohorts.*Positive for *KRAS* p.G12C. CDx, companion diagnostic; G360, Guardant360; *KRAS*, Kirsten rat sarcoma; SAAS, sensitivity analysis prevalence sub-study.

only be identified after testing. Testing failures for the *therascreen*® KRAS RGQ PCR kit were mainly due to poor tissue quality or failure to identify tumor cells. Age of the tissue samples did not appear to affect testing outcomes (Supplemental Table S1).

### 3.2. Baseline demographics and disease characteristics

Baseline demographic and clinical characteristics were similar among the diagnostic cohorts (Table 1). The median age ranged between 63 years to 64 years. Eastern Cooperative Oncology Group performance status of 1 was reported for most patients in all cohorts. Most patients (94.9% in the Guardant360 Positive cohort, 100% in the Guardant360 Negative cohort) had metastatic disease, with bone being the most common site of metastasis. Although most patients had stage IV disease at initial diagnosis, the proportion of patients in the Guardant360 - Negative cohort with prior stage I (n = 4/31 [12.9%]) and II (n = 6/31 [19.4%]) disease was numerically higher compared with the other cohorts (<10% and <12%, respectively). Furthermore, tumor tissue surface area and age were not significantly different among groups with concordant or discordant test results between tissue and LBx (Supplemental Table S2).

### 3.3. Agreement between Guardant360 CDx and tissue testing

The concordance of plasma and tissue samples for the extended cohort, including matched tissue and plasma samples from patients in other clinical trials or from commercial vendors, is summarized in Table 2. For the 189 patients in the AAAS cohort, the PPA and NPA (95% CI) for Guardant360 CDx relative to *therascreen*® KRAS RGQ PCR test were 0.71 (0.62, 0.79) and 1.00 (0.95, 1.00), respectively. The overall percent agreement (95% CI) was 0.82 (0.76, 0.87).

In the phase 2 trial, Non-Shedders (no variants detected in a sample passing QC) had lower cfDNA than Non–G12C Shedders and G12C Shedders, as expected (Fig. 2A). In the phase 2 trial, higher ctDNA levels, as defined by the SMM, were detected in G12C Shedders (ie, *KRAS* p.G12C–positive samples) versus the Non–G12C Shedders (ie, *KRAS* p.G12C–negative samples) from the phase 2 trial (Fig. 2B). One Non–G12C Shedder only had one copy number variation reported, and a placeholder value of 0.5 SMM was use for illustrative purposes because the SMM calculation does not include copy number variations. Median SMM differed between Non–G12C Shedders and G12C Shedders (18 and 269, respectively; $P = 2.8\times10^{-7}$, Kruskal-Wallis test).

### 3.4. Prevalence of co-mutations

Panel-wide data were analyzed to determine the presence of other mutations in the combined NSCLC population (phase 2 clinical trial and prevalence sub-study) used in this study. A total of 231 subjects with valid Guardant360 CDx LBx results were included (*KRAS* p.G12C detected, n = 88; *KRAS* p.G12C not detected, n = 143). Differences in the baseline demographics and clinical characteristics between the phase 2 clinical trial and prevalence sub-study cohorts were present (Supplemental Table S3). Specifically, the sub-study had a high proportion of patients who were male (phase 2 trial, n = 56 [51.4%]; sub-study, n = 91 [74.6%]) and fewer patients who had received prior anti-cancer therapy (phase 2 trial, n = 109 [100%]; sub-study, n = 12 [9.8%]). The top five genes with an identified mutation in *KRAS* p.G12C–positive and *KRAS* p.G12C–negative samples are listed in Table 3. Mutations in *TP53* were most commonly found in the NSCLC cohort, irrespective of the *KRAS* p.G12C status. *STK11* was co-mutated in 26.1% of the *KRAS* p.G12C–positive patients. Mutations in *TP53* and *STK11* occurred in 47 and 22 patients, respectively. Based on the odds ratios for objective response, there was no association between outcome and co-mutation status (mutated *TP53*, 0.86 [$P = 0.71$]; mutated *STK11*, 0.71 [$P = 0.49$]). Among actionable mutations, *MET* and *EGFR* were prevalent in *KRAS* p.G12C–positive patients, whereas coalteration of *ALK* and

**Table 1**
Baseline demographics and clinical characteristics by diagnostic cohort.*

| | Full Analysis Set (n = 126) | G360 Evaluable Cohort (n = 109) | G360 Positive Cohort† (n = 78) | G360 Negative Cohort (n = 31) |
|---|---|---|---|---|
| Age, median (IQR) | 63.5 (56.0–70.0) | 63.0 (56.0–70.0) | 63.0 (56.0–72.0) | 64.0 (55.0–69.0) |
| Female, n (%) | 63 (50.0) | 53 (48.6) | 42 (53.8) | 11 (35.5) |
| Race, n (%) | | | | |
| White | 103 (81.7) | 87 (79.8) | 65 (83.3) | 22 (71.0) |
| Asian | 19 (15.1) | 19 (17.4) | 11 (14.1) | 8 (25.8) |
| Other | 4 (3.2) | 3 (2.8) | 2 (2.6) | 1 (3.2) |
| Region, n (%) | | | | |
| North America | 79 (62.7) | 67 (61.5) | 50 (64.1) | 17 (54.8) |
| Europe | 30 (23.8) | 25 (22.9) | 18 (23.1) | 7 (22.6) |
| Asia | 12 (9.5) | 12 (11.0) | 7 (9.0) | 5 (16.1) |
| Rest of the world | 5 (4.0) | 5 (4.6) | 3 (3.8) | 2 (6.5) |
| ECOG status at baseline, n (%) | | | | |
| 0 | 38 (30.2) | 33 (30.3) | 20 (25.6) | 13 (41.9) |
| 1 | 88 (69.8) | 76 (69.7) | 58 (74.4) | 18 (58.1) |
| Prior line of anti-cancer therapy | | | | |
| 1 | 54 (42.9) | 46 (42.2) | 33 (42.3) | 13 (41.9) |
| 2 | 44 (34.9) | 37 (33.9) | 28 (35.9) | 9 (29.0) |
| 3 | 28 (22.2) | 26 (23.9) | 17 (21.8) | 9 (29.0) |
| Type of prior anti-cancer therapy, n (%) | | | | |
| Chemotherapy | 115 (91.3) | 101 (92.7) | 73 (93.6) | 28 (90.3) |
| Immunotherapy | 116 (92.1) | 100 (91.7) | 72 (92.3) | 28 (90.3) |
| Platinum-based chemotherapy and anti–PD-1/PD-L1 | 102 (81.0) | 89 (81.7) | 66 (84.6) | 23 (74.2) |
| Targeted biologics | 30 (23.8) | 28 (25.7) | 17 (21.8) | 11 (35.5) |
| Targeted small molecules | 9 (7.1) | 6 (5.5) | 3 (3.8) | 3 (9.7) |
| Other | 1 (0.8) | 1 (0.9) | 1 (1.3) | 0 (0.0) |
| Disease stage at initial diagnosis, n (%) | | | | |
| I | 11 (8.7) | 10 (9.2) | 6 (7.7) | 4 (12.9) |
| II | 14 (11.1) | 12 (11.0) | 6 (7.7) | 6 (19.4) |
| III | 22 (17.5) | 21 (19.3) | 19 (24.4) | 2 (6.5) |
| IV | 78 (61.9) | 65 (59.6) | 46 (59.0) | 19 (61.3) |
| Missing | 1 (0.8) | 1 (0.9) | 1 (1.3) | 0 (0.0) |
| Smoking history, n (%) | | | | |
| Never smoked | 6 (4.8) | 6 (5.5) | 4 (5.1) | 2 (6.5) |
| Current smoker | 15 (11.9) | 12 (11.0) | 7 (9.0) | 5 (16.1) |
| Former smoker | 102 (81.0) | 88 (80.7) | 66 (84.6) | 22 (71.0) |
| Missing | 3 (2.4) | 3 (2.8) | 1 (1.3) | 2 (6.5) |
| Histopathology type, n (%) | | | | |
| Squamous | 1 (0.8) | 1 (0.9) | 1 (1.3) | 0 (0.0) |
| Nonsquamous | 125 (99.2) | 108 (99.1) | 77 (98.7) | 31 (100.0) |
| Metastatic, n (%) | | | | |
| Yes | 122 (96.8) | 105 (96.3) | 74 (94.9) | 31 (100.0) |
| No | 4 (3.2) | 4 (3.7) | 4 (5.1) | 0 (0.0) |
| Common metastases, n (%) | | | | |
| Liver | 26 (20.6) | 19 (17.4) | 17 (21.8) | 2 (6.5) |
| Brain | 26 (20.6) | 21 (19.3) | 17 (21.8) | 4 (12.9) |
| Bone | 61 (48.4) | 51 (46.8) | 41 (52.6) | 10 (32.3) |
| Best response to last prior line of therapy, n (%) | | | | |
| CR | 1 (0.8) | 1 (0.9) | 1 (1.3) | 0 (0.0) |
| PR | 12 (9.5) | 11 (10.1) | 9 (11.5) | 2 (6.5) |
| SD | 33 (26.2) | 28 (25.7) | 19 (24.4) | 9 (29.0) |
| PD | 48 (38.1) | 43 (39.4) | 33 (42.3) | 10 (32.3) |
| Unknown/ unevaluable/ missing | 32 (25.4) | 26 (23.9) | 16 (20.5) | 10 (32.3) |

CR, complete response; ECOG, Eastern Cooperative Oncology Group; G360, Guardant360; IQR, interquartile range; PD, progressive disease; PD-1/PD-L1, programmed cell death protein-1/programmed cell death protein ligand-1; PR, partial response; SD, stable disease.

* Cohorts defined in Fig. 1.

† Positive for *KRAS* p.G12C.

**Table 2**
Concordance between Guardant360 CDx test and tissue testing.

| | *therascreen*® KRAS RGQ PCR Kit Positive | *therascreen*® KRAS RGQ PCR Kit Negative | Total |
|---|---|---|---|
| Guardant360 CDx Positive, n (%) | 82 (70.7) | 0 (0.0) | 82 (43.4) |
| Guardant360 CDx Negative, n (%) | 34 (29.3) | 73 (100.0) | 107 (56.6) |
| Positive percent agreement* (95% CI) | 0.71 (0.62, 0.79) | | |
| Negative percent agreement† (95% CI) | 1.00 (0.95, 1.00) | | |
| Overall percent agreement‡ (95% CI) | 0.82 (0.76, 0.87) | | |

AAAS, assay agreement analysis set; CI, confidence interval; CDx, companion diagnostic; PCR, polymerase chain reaction; RGQ, Rotor-Gene-Q MDx instrument.

* Number of patients with both Guardant360 CDx positive and *therascreen*® KRAS RGQ PCR Kit positive, divided by the number of patients with *therascreen*® KRAS RGQ PCR Kit positive alone in the AAAS.

† Number of patients with both Guardant360 CDx negative and *therascreen*® KRAS RGQ PCR Kit negative, divided by the number of patients with *therascreen*® KRAS RGQ PCR Kit negative alone in the AAAS.

‡ Number of patients with both Guardant360 CDx positive and *therascreen*® KRAS RGQ PCR Kit positive, or both Guardant360 CDx negative and *therascreen*® KRAS RGQ PCR Kit negative, divided by the number of patients in the AAAS.

*ROS1* with *KRAS* p.G12C was not seen.

### 3.5. Efficacy outcomes

The ORR (95% CI; patients with objective response/all patients in dataset) for all patients was 37.1% (28.6%, 46.2%; n = 46/124) in the Full Analysis Set, 36.4% (25.7%, 48.1%; n = 28/77) in the Guardant360 Positive cohort, and 46.7% (28.3%, 65.7%; n = 14/30) in the Guardant360 Negative cohort (Table 4; Supplementary Fig. 1). Rates of PD, SD, and PR were similar among the cohorts (Supplementary Fig. 1), with SD being the most common outcome (Full Analysis Set, n = 54/124 [43.5%]; Guardant360 Evaluable, n = 46/107 [43.0%]; Guardant360 Positive, n = 32/77 [41.6%]; Guardant360 Negative, n = 14/30 [46.7%]). DCR (95% CI; patients with disease control/all patients in dataset) was 80.6% (72.6%, 87.2%; n = 100/124) in the Full Analysis Set and 77.9% (67.0%, 86.6%; n = 60/77) in the Guardant360 Positive cohort. Among responders, DOR was $\geq$ 3 months in 38/46 (82.6%) patients in the Full Analysis Set and 24/28 (85.7%) patients in the Guardant360 Positive cohort; DOR was $\geq$ 6 months in 28/46 (60.9%) and 15/28 (53.6%) patients in the Full Analysis Set and Guardant360 Positive cohort, respectively. Of the four cohorts, DOR $\geq$ 3 months among responders was numerically highest in the Guardant360 Positive cohort (n = 24/28 [85.7%]), whereas DOR $\geq$ 6 months was numerically highest in the Guardant360 Negative (n = 9/14 [64.3%]) cohort. The median time to objective response was similar among all cohorts.

## 4. Discussion

Accurate and timely detection of oncogenic driver mutations continues to be critically important in the management of NSCLC patients [5]. In the current study, the primary endpoint for determining clinical validity was the ORR of Guardant360 CDx–selected patients, which was similar to that of patients selected by tissue testing. Key secondary endpoints of DOR and median time to objective response were also similar between populations (Table 4). Additionally, good concordance was observed between blood and tissue testing, consistent with other studies assessing concordance of tissue- and blood-derived samples for *KRAS* mutations [25]. This observation demonstrates that patients with *KRAS* p.G12C–mutant NSCLC may be similarly and effectively treated with sotorasib irrespective of the testing modality or analyte by which these patients were identified, and builds on the results of previous studies in which patients selected for targeted therapy by LBx demonstrated similar outcomes to those selected by tissue testing. For example, patients with NSCLC and *EGFR* mutations identified by tissue or cfDNA demonstrated similar clinical benefit from osimertinib as first-line therapy and in later lines [31–33]. Concordance between tissue and LBx testing for *EGFR* driver mutations was high; however, LBx demonstrated superiority in the detection of the *EGFR* T790M acquired resistance mutation. These data demonstrate that LBx may identify biomarkers that could be missed due to issues of tissue adequacy. Similar findings have been observed in NSCLC with *MET* exon 14-skipping and *BRAF* mutations and in gastrointestinal cancers [34–36]. To assess the clinical validity of Guardant360 CDx to identify *KRAS* p.G12C–positive patients, a clinical trial dataset enriched with patients with *KRAS* p.G12C disease was used. An additional cohort from an unselected population matching the intent-to-treat population of the clinical study provided a control group and enabled a sensitivity analysis to assess the validity of Guardant360 CDx to accurately exclude *KRAS* p.G12C–negative patients. Although differences between the clinical trial and unselected patient population were present, we do not anticipate that these would affect the conclusions regarding the clinical validity of Guardant360 CDx.

The primary limitation of LBx is that not all tumors shed detectable ctDNA into circulation. In the current study, 30% of tissue-positive patients were negative by LBx; similarly, up to 33% of patients with *EGFR*-activating mutations were missed in previous studies [37]. No clinical or pathological factors were identified in this study that were significantly linked to ctDNA shedding or non-shedding tumors. As such, and similar to expert consensus guidelines for *EGFR* testing [38], it is important to reflex test all patients who are negative for all driver mutations on LBx by an approved tissue testing methodology to ensure false negatives on LBx are not overlooked [37]. However, previous studies have shown that reliance on tissue genotyping alone may miss a substantial proportion of patients [31–36]. Indeed, multiple prospective studies comparing biomarker discovery rates between LBx and tissue genotyping in NSCLC patients have shown that LBx identifies similar or higher numbers of biomarker-positive patients in all-comer populations relative to tissue testing, with the increased genotyping success rate of LBx being balanced by the lack of tumor shedding [31–33]. Without the need for scheduling and performing the biopsy and tissue processing, it would be expected that LBx would typically deliver results faster than tissue genotyping, as supported by results from a recent study in NSCLC (LBx TAT of 9 days versus 15 days for tissue testing [$P < 0.001$]) [39], allowing treatment decisions to be made more frequently with full genotyping information available [16,40]. The LBx testing landscape continues to evolve with efforts to augment assay sensitivity and specificity, as well as the increasing number of actionable mutations for targeted therapy.

A benefit of comprehensive NGS LBx testing is the ability to detect mutations that may either be linked to or be mutually exclusive from *KRAS* p.G12C. A recent study reported multiple and diverse acquired mutations that confer resistance to $KRAS^{G12C}$ inhibitors [41]. In addition to allowing a personalized approach to treatment of NSCLC, information regarding the presence of certain mutations can also be used to identify potential mechanisms of resistance and inform potential treatment combination strategies. In the case of *KRAS* p.G12C, we observed two primary co-mutated genes, *TP53* and *STK11*, which may be associated with poor survival in *KRAS*-mutated patients [42]. The current study recapitulates previous findings of consistent sotorasib response across molecular subgroups of commonly mutated genes, including co-mutated *TP53* and co-mutated *STK11*. Due to the heterogeneity inherent in samples from patients with NSCLC and the increasing number of treatment options available, a variety of combination studies are underway

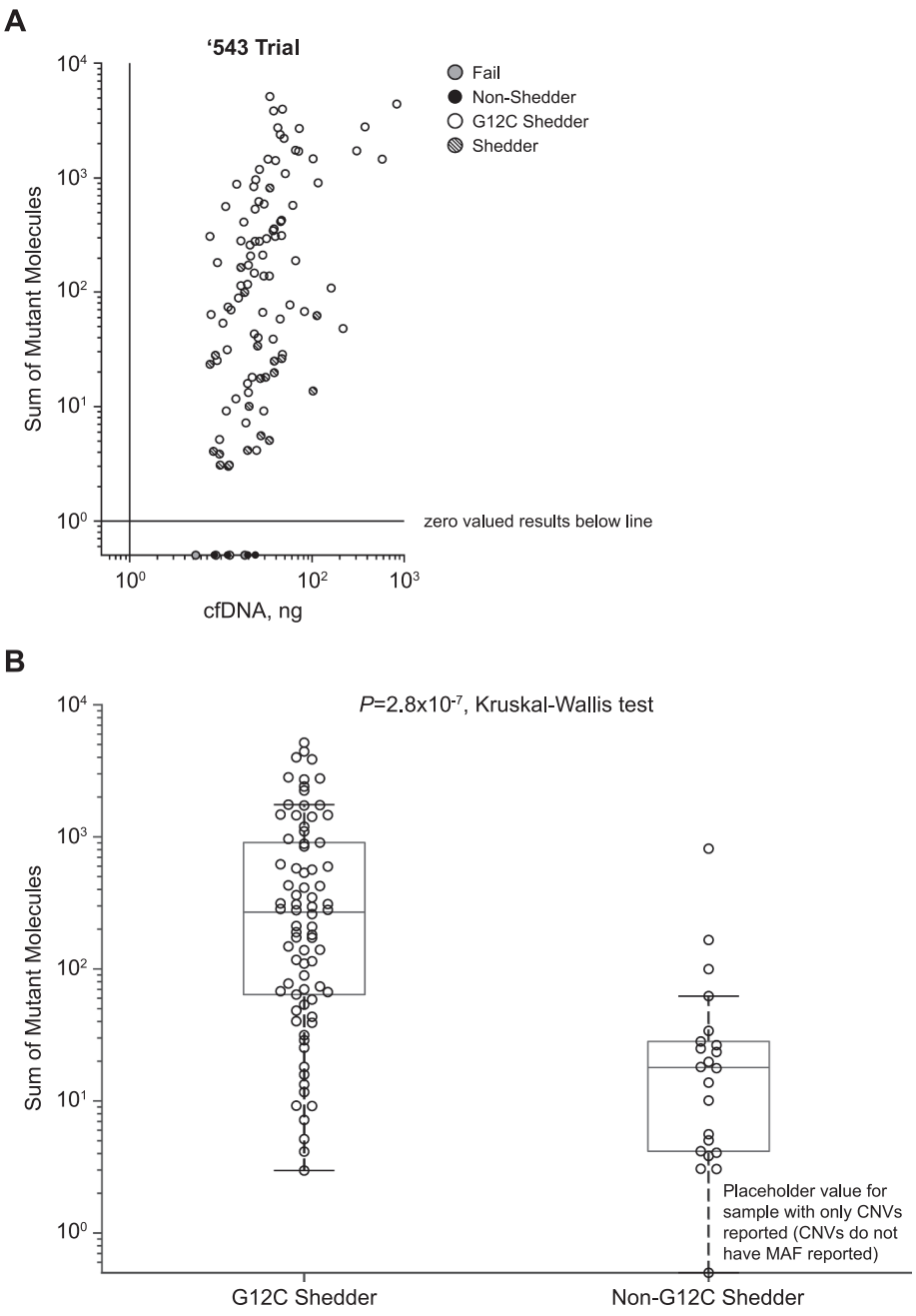


**Fig. 2.** Sum of mutant molecules (SMM). (A) SMM and cfDNA distributions in the phase 2 trial study for the Non–G12C Shedder, G12C Shedder, Non-Shedder, and Fail groups. (B) Distribution of SMM for Non–G12C Shedders and G12C Shedders. Fail, patients with samples that failed quality control checks; G12C Shedder, patients in whom *KRAS* p.G12C was detected; Non-Shedder, patients in whom no tumor DNA variants were reported, and sample passed laboratory quality control checks; Non–G12C Shedder, patients in whom tumor DNA variants were reported but *KRAS* p.G12C was not detected.

with the goal of evaluating the efficacy of *KRAS*-targeting agents like sotorasib alone and in combination with other targeted therapies. A benefit of comprehensive NGS LBx testing is the ability to interrogate a panel of cancer-associated genes, including those with actionable mutations with available targeted therapies [43], and may inform future strategies for combination therapy.

The results reported herein are consistent with trends observed in other reports in NSCLC and other cancer types [7,44,45]. The discrepancy in results obtained with Guardant360 CDx versus tissue testing in our study may be related to the difference in shedding of DNA in patients with the *KRAS* p.G12C mutation versus patients without *KRAS* p.G12C. Mechanisms of release may differ in patients harboring the *KRAS* p.G12C mutation, resulting in the higher SMM observed in this study [23]. Outcomes associated with shedding status may also merit further examination in studies with a larger dataset.

Comprehensive genotyping is critical for proper management of NSCLC patients. As $KRAS^{G12C}$ is an emerging therapeutic target, it is equally critical that genotyping for this marker is validated and incorporated into all genotyping platforms. The current study shows that properly validated LBx, with patients with negative LBx results reflex tested using approved tissue testing, has clinical validity in the identification of patients with *KRAS* p.G12C–mutant NSCLC for treatment with sotorasib.

### *CRediT authorship contribution statement*

**Joshua M. Bauml:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Bob T. Li:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Vamsidhar Velcheti:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Ramaswamy Govindan:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – review & editing.

**Table 3**
Prevalence of most common cancer-associated genes identified in *KRAS* p.G12C–positive versus *KRAS* p.G12C–negative patients by the Guardant360 CDx liquid biopsy test.

| Gene | *KRAS* p.G12C–Positive Samples, % | *KRAS* p.G12C–Negative Samples, % |
|---|---|---|
| ***TP53*** | 53.4 | **45.5** |
| *STK11* | 26.1 | 11.2 |
| ***ATM*** | 17.1 | **18.2** |
| *MET** | 13.6 | 7.7 |
| ***EGFR**** | 12.5 | **14.0** |
| ***No mutation*** | 0.0 | **16.8** |
| ***PIK3CA*** | 6.8 | **11.9** |
| *BRAF** | 8.0 | 8.4 |
| *RET** | 4.6 | 0.7 |
| *NTRK1** | 3.4 | 1.4 |
| *ALK** | 1.1 | 1.4 |
| *ROS1** | 1.1 | 3.5 |

CDx, companion diagnostic.
**Bold text** indicates the top five most common genes in patients who were *KRAS* p.G12C–negative.
* Genes with available targeted therapies.

**Table 4**
Efficacy endpoints with sotorasib according to Guardant360 CDx in all cohorts.

| Cohort,* n† | Primary Endpoint | Secondary Endpoints | | | |
|---|---|---|---|---|---|
| | ORR, % (95% CI) | DCR, % (95% CI) | DOR ≥ 3 mo,‡ n/n1 (%) | DOR ≥ 6 mo,‡ n/n1 (%) | Median Time to Objective Response,‡ mo (IQR) |
| Full Analysis Set (n = 124) | 37.1 (28.6, 46.2) | 80.6 (72.6, 87.2) | 38/46 (82.6) | 28/46 (60.9) | 1.4 (1.3, 2.7) |
| G360 Evaluable (n = 107) | 39.3 (30.0, 49.2) | 82.2 (73.7, 89.0) | 34/42 (81.0) | 24/42 (57.1) | 1.4 (1.3, 2.8) |
| G360 Positive§ (n = 77) | 36.4 (25.7, 48.1) | 77.9 (67.0, 86.6) | 24/28 (85.7) | 15/28 (53.6) | 1.4 (1.3, 2.7) |
| G360 Negative (n = 30) | 46.7 (28.3, 65.7) | 93.3 (77.9, 99.2) | 10/14 (71.4) | 9/14 (64.3) | 1.4 (1.2, 2.8) |

CI, confidence interval; CDx, companion diagnostic; DCR, disease control rate; DOR, duration of observed response; G360, Guardant360; IQR, interquartile range; ORR, objective response rate; RECIST 1.1, Response Evaluation Criteria in Solid Tumors version 1.1.
* Cohorts defined in Fig. 1.
† n is the number of patients who are in the cohort and received at least 1 dose of sotorasib, and have one or more measurable lesions at baseline as assessed by independent radiologic review using RECIST 1.1.
‡ Calculated among number of responders (n1).
§ Positive for *KRAS* p.G12C based on G360 liquid biopsy testing.

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## Conflicts of Interest

**J M Bauml** reports grants or contracts from Merck, Clovis, Carevive Systems, Novartis, Bayer, Janssen, Astra Zeneca, Takeda, and Carisma Therapeutics; consulting fees from Clovis, BMS, Astra Zeneca, Celgene, Boehringer Ingleheim, Janssen, Merck, Guardant Health, Genentech, Takeda, Regeneron, Inivata, and Novartis; participation on a data safety monitoring board or advisory board for Ayala. **B T Li** reports support for the present manuscript from Amgen Inc. (clinical trial funding, uncompensated consulting/advisory) and the National Institutes of Health (Memorial Sloan Kettering Cancer Center Support Grant P30 CA008748); support for attending meetings and/or travel from MORE Health and Jiangsu Henrui Medicine; patents, royalties, other intellectual property (institutional patents through Memorial Sloan Kettering Cancer Center) in US62/514,661 and US62/685,057; royalties or licenses (personal) in Karger Publishers (intellectual property rights as a book author) and Shanghai Jiao Tong University Press (intellectual property rights as a book author); grants or contracts (clinical trial or research funding) from Lilly, Genentech, AstraZeneca, Daiichi Sankyo, Hengrui Therapeutics, MORE Health, and Bolt Biotherapeutics; and uncompensated consulting/advisory role for Genentech, Boehringer Ingelheim, Lilly, AstraZeneca, and Daiichi Sankyo. **V. Velcheti** reports consulting fees from BMS, Merck, Novartis, Amgen, Foundation Medicine, and AstraZeneca. R. Govindan reports consulting or advisory role for GlaxoSmithKline, Genentech/Roche, Abbvie, Celgene, AstraZeneca/MedImmune, Merck Serono, Pfizer, Bristol-Myers Squibb, EMD Serono, Lilly, Ignyta, Nektar, Phillips Gilmore Oncology, Jounce Therapeutics, Roche, Janssen, Amgen Inc., and Achilles Therapeutics; honoraria from Genentech/Abbvie, Abbvie, and Geneplus. **A Curioni-Fontecedro** reports fees for advisory board participation from Amgen Inc., AstraZeneca, Bristol Meyer Squibb, Boehringer Ingelheim, F. Hoffmann-La Roche, Merck Sharp & Dohme, Novartis, and Takeda; fees for an educational event from Roche; and served as principal investigator of clinical trials for Amgen Inc. BMS, MSD, Roche, and Takeda. **C Dooms** has no disclosures to report. **T Takahashi** reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from AstraZeneca KK, Chugai Pharmaceutical Co Ltd, Eli Lilly Japan K.K., ONO Pharmaceutical Co Ltd, MSD K.K., Pfizer Japan Inc., Boehringer Ingelheim Japan Inc., Roche Diagnostics K.K., Takeda Pharmaceutical Co Ltd, and Yakult Honsha Co Ltd; and grants or contracts (to institution) from AstraZeneca KK, Chugai Pharmaceutical Co Ltd, Eli Lilly Japan K.K., ONO Pharmaceutical Co Ltd, MSD K.K., Pfizer Japan Inc., Amgen Inc, and Boehringer Ingelheim Japan. **A Duda** is an employee and stockholder of Guardant Health. **J Odegaard** is an employee and stockholder of Guardant Health; reports fees for honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events, expert testimony, and travel/meeting support from Guardant Health; holds patents with Guardant Health; and holds leadership or fiduciary roles for Guardant Health. **F Cruz-Guilloty** is an employee and stockholder of Amgen Inc., and reports travel/meeting support from Amgen Inc. **L Jin, Y Zhang** and **A Anderson** are employees and stockholders of Amgen Inc. **F Skoulidis** reports consulting fees from Amgen Inc.; lecture fees from BMS and RV Mais Promocao Eventos LTDS; fees for travel, and food and beverage from Tango Therapeutics, AstraZeneca Pharmaceuticals, and Amgen Inc.; stock or stock options in

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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*Data Sharing*

Qualified researchers may request data from Amgen clinical studies. Complete details are available at the following: http://www.amgen.com/datasharing.

## Appendix A. Supplementary data

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