ORIGINAL ARTICLE

Clinical Utility of Combined Tissue and Plasma Next-Generation Sequencing in Patients With Advanced, Treatment-Naïve NSCLC

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ABSTRACT

Introduction: Tissue and plasma-based next-generation sequencing (NGS) have complementary roles in patients with advanced NSCLC. Nevertheless, whether there is any added clinical value in combining both methods in the treatment of naïve patients remains unclear.

Methods: We retrospectively collected clinical and genomic data from 275 patients with treatment-naïve advanced NSCLC who had undergone plasma-based NGS at diagnosis in our institution. We analyzed patient data in two separate cohorts, each assessed with a different plasma-based NGS method: cohort 1 (n = 127, Guardant360), and cohort 2 (n = 148, FoundationACT/FoundationOne Liquid CDx). Ninety-five patients (75%) in cohort 1 and 108 patients (73%) in cohort 2 underwent concurrent amplicon-based tissue NGS testing locally.

Results: Forty-three patients in cohort 1 (34%) and 49 patients in cohort 2 (33%) harbored European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT) I or II targetable driver alterations. The addition of orthogonal biopsy (tissue to liquid, or liquid to tissue) offered no relevant clinical value in cases with ESCAT I or II targetable drivers already detected by one method. In contrast, adding orthogonal biopsy incremented the detection of ESCAT I or II targetable drivers not only in cases with uninformative testing (undetectable circulating tumor DNA, unavailable/inadequate tissue) but also in about 5% of the patients with seemingly informative but driver undetected molecular results. The prevalence of ESCAT I or II targetable drivers in plasma was significantly higher in patients with adenocarcinoma, 20 pack-year or less smoking history, and abdominal metastases.

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Conclusions: Our study suggests that the addition of sequential orthogonal biopsy should be considered whenever an ESCAT I or II targetable driver has not been detected by the initial method, including cases with seemingly informative molecular analysis.

Keywords: Non-small cell lung cancer; Next-generation sequencing; Liquid biopsy; Targetable driver alterations

Introduction

Molecular profiling is required for appropriate clinical management of patients with NSCLC. Globally, about 40% of patients with advanced NSCLC have tumors with clinically actionable genomic alterations, although this percentage may vary depending on age, ethnicity, smoking status, histology, and other factors.[1–3] These molecular aberrations predict sensitivity to currently approved drugs in the front-line setting, in which the use of targeted therapies is mandatory and should be prioritized.[4–6]

Tissue testing has been traditionally accepted as the standard of care for genomic analysis in routine clinical practice.[7] More recently, advances in the molecular profiling of circulating tumor DNA (ctDNA) have enabled the use of plasma-based next-generation sequencing (NGS) methods to facilitate personalized treatment guiding in patients with NSCLC. Liquid biopsy has reported clinical utility as a complement or alternative to tumor biopsy when tissue is unavailable or inadequate for molecular analysis.[8–10] In addition, its convenience due to the ease of sampling and its faster turnaround time (TAT) has prompted its use in the initial workup of patients with diagnosed or suspected lung cancer.[11,12] Some studies have indeed suggested that a "plasma-first" approach might shorten the time to treatment and increase the proportion of patients selected for matched targeted therapies.[13,14] However, the success in detecting targetable driver alterations in plasma largely relies on the amount of ctDNA shed in blood,[15–17] and the optimal way to integrate tissue and liquid biopsy in the clinic remains unclear. Moreover, whether there is any added clinical value in combining both methods in patients with advanced, treatment-naïve NSCLC has not been properly assessed.

In this study, we aimed to analyze the clinical utility of combining plasma- and tissue-based NGS in the initial molecular diagnosis of patients with advanced, treatment-naïve, NSCLC. Our primary objective was to assess the added clinical value of using both liquid and tissue biopsy for detecting targetable molecular drivers in the front-line setting.

Material and Methods

Patient Cohorts

We retrospectively identified patients with advanced, treatment-naïve NSCLC who had prospectively undergone plasma-based NGS as part of their initial molecular diagnostic workup between January 2019 and December 2022 in our institution. To verify reproducibility in clinical utility findings, we analyzed patients' clinical and molecular data in two separate cohorts, each assessed by a different plasma-based NGS method. Cohort 1 contained 127 patients whose plasma samples were analyzed with Guardant360 (Redwood City, California) from November 2020 to December 2022. Cohort 2 consisted of 148 patients whose plasma samples were analyzed with FoundationACT and FoundationOne Liquid CDx (Cambridge, Massachusetts) from January 2019 to October 2020 within the BFAST trial molecular screening (ClinicalTrials.gov identifier: NCT03178552). A total of 95 patients (75%) in cohort 1 and 108 patients (73%) in cohort 2 had available tumor tissue in quantity and quality for molecular analysis and underwent concurrent tissue-based NGS testing locally. The baseline clinical characteristics of both cohorts are summarized in Table 1. All patients provided signed informed consent for plasma NGS analysis, and retrospective data collection was done under Ethics Committee approval (#23/264).

NGS Analysis and Clinical Interpretation of Genomic Data

All patients in the study had their peripheral blood submitted for externalized plasma-based NGS analysis using hybrid-capture–based sequencing methods. In cohort 1, the 73-gene Guardant360 assay[18] (Guardant Health, Redwood City, California) was used. In cohort 2, liquid biopsy and plasma-based NGS were performed under the BFAST molecular pre-screening platform using the FoundationACT (n = 82) assay or the FoundationOne Liquid CDx assay[19] (n = 67) (Foundation Medicine, Cambridge, Massachusetts) testing 62 and 324 genes, respectively.

In patients with available tissue in sufficient quantity and quality, we performed concurrent tissue-based NGS onsite with targeted amplicon sequencing. We used the Oncomine Focus Assay[20] (n = 190) or the Oncomine Precision Assay[21] (OPA, n = 13) (Thermo Fisher Scientific, Waltham, Massachusetts), testing 52 and 50 genes, respectively. For each formalin-fixed and paraffin-

Table 1. Baseline Clinical Characteristics of Patients From Cohort 1 and Cohort 2

Characteristic	Cohort 1 (n = 127)	Cohort 2 (n = 148)
Tissue-based NGS performed, No. (%)		
Yes	95 (75)	108 (73)
No	32 (25)	40 (27)
Gender, No. (%)		
Male	73 (57)	98 (66)
Female	54 (43)	50 (34)
Median age, y (range)	66 (41-89)	66 (31-94)
Pack-year history, No. (%)		
≤20	53 (42)	43 (29)
>20	74 (58)	105 (71)
Histology, No. (%)		
Adenocarcinoma	97 (76)	102 (69)
Squamous NSCLC	16 (13)	32 (22)
Carcinoma NOS	14 (11)	14 (9)
Liver metastases, No. (%)		
Yes	25 (20)	29 (20)
No	102 (80)	119 (80)
CNS metastases, No. (%)		
Yes	30 (24)	36 (24)
No	97 (76)	112 (76)
Abdominal metastases[a], No. (%)		
Yes	50 (39)	44 (30)
No	77 (61)	104 (70)
Bone metastases, No. (%)		
Yes	49 (39)	47 (32)
No	78 (61)	101 (68)

[a]Abdominal metastases are defined as metastases in, at least, one of the following sites: liver, spleen, peritoneum, abdominal nodes, kidney, adrenal glands, or subcutaneous abdominal nodes. CNS, central nervous system; NGS, next-generation sequencing; NOS, not otherwise specified.

embedded tumor sample, the first section was stained with hematoxylin-eosin and reviewed by a pathologist to confirm the percentage of tumor cells was 30% or higher. Freshly cut 5-μm-thick formalin-fixed and paraffin-embedded sections were collected for nucleic acid extraction (DNA and RNA). Library preparation and sequencing analysis were developed on the Ion S5XL sequencer with automated library preparation using the Ion Chef System (Thermo Fisher Scientific) for the Oncomine Focus Assay test and on the Ion Torrent Genexus Purification System (Thermo Fisher Scientific) integrated with the Ion Torrent Genexus System (Thermo Fisher Scientific) for OPA test.

We annotated genomic variants detected in plasma or tissue samples in two main categories on the basis of their functional and biological relevance: (1) variants of unknown significance, those genomic alterations whose functional consequences and clinical significance have not been established; and (2) pathogenic or deleterious variants, those genomic alterations validated or predicted to affect gene function. We then subclassified these deleterious variants into four levels of actionability according to the European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT).[6] For the purposes of this study, we focused our main analysis on the detection of ESCAT I or II targetable driver alterations[22] because these were genomic alterations covered by all plasma or tissue assays used in our study and are more relevant to inform molecularly guided therapies in the clinic.

Finally, we classified plasma and tissue molecular reports into three categories: (1) driver positive: cases with informative molecular results with ESCAT I or II targetable driver alteration detected; (2) uninformative: cases with undetectable ctDNA in the case of liquid biopsy, and cases with unavailable or inadequate tissue or failed molecular analysis in the case of tissue biopsy; and (3) driver undetected: cases with seemingly informative molecular results in which ESCAT I to II targetable driver alterations were not detected.

Statistical Considerations

We used IBM SPSS Statistics software (version 23) for all statistical analyses. The TAT for tissue NGS was calculated from the date of tissue biopsy to the date of tissue molecular results, whereas the TAT for liquid biopsy was calculated from the date of blood sample collection to the date of plasma molecular results. The turn around time for plasma and tissue molecular reports was compared through two-sample t test analysis. We used chi-square and binary logistic regression to analyze the association between categorical variables. The positive percent agreement (PPA) for liquid biopsy was calculated as the percentage of patients who had ESCAT I or II alterations detected in tissue who were also positive for the same ESCAT I or II alteration in plasma. All hypothesis testing was performed at a two-sided significance level (α) of 0.05.

Results

We included a total of 275 patients in the study. One-hundred twenty-two patients in cohort 1 (96%) and 139 patients in cohort 2 (94%) had informative plasma NGS results, of which 36 (28%) and 35 patients (24%) had ESCAT I to II targetable driver alterations detected in ctDNA, respectively (Fig. 1). A total of 95 patients in cohort 1 (75%) and 108 patients in cohort 2 (73%) had available tissue in quantity and quality and obtained informative tissue NGS results, of which 31 (24%) and 36 patients (24%) harbored ESCAT I or II targetable driver alterations, respectively (Fig. 1). The median TAT was 29 calendar days for tissue biopsy as compared with 11 days for liquid biopsy in cohort 1 ($p < 0.001$), and 27

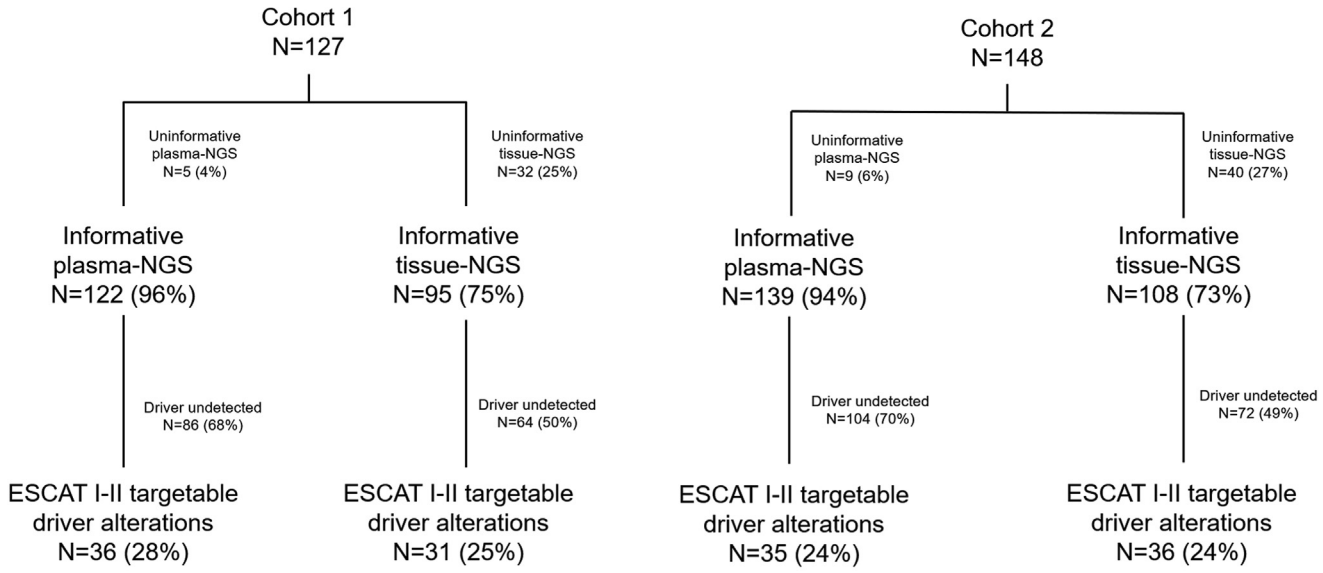

Figure 1. Study flowchart. ESCAT, European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets; NGS, next-generation sequencing.

days compared with 15 days in cohort 2 ($p < 0.001$) (Supplementary Fig. 1).

Forty-three patients in cohort 1 (34%) and 49 patients in cohort 2 (33%) harboured ESCAT I or II targetable driver alterations detected by either tissue and/or liquid biopsy (Fig. 2*A*). The distribution of ESCAT I or II targetable drivers was comparable between both cohorts (Fig. 2*B*). Using tissue NGS as the reference standard, the PPA of liquid biopsy for detecting ESCAT I to II targetable driver alterations was 77% in cohort 1 and 61% in cohort 2. Individually, none of the ESCAT I to II targetable drivers reported a poor PPA (<50%) consistently in both cohorts. In general, point mutations were the most concordantly detected genomic alterations in plasma and tissue, whereas gene fusions, and other structural variants (e.g., *METex14* alterations), tended to have a lower overall PPA (Fig. 2*C*).

To analyze the incremental value of performing orthogonal biopsy (tissue-to-liquid biopsy and liquid-to-tissue biopsy) to detect ESCAT I or II targetable driver alterations, we considered three distinct clinical scenarios: (1) driver-positive cases by liquid or tissue biopsy; (2) uninformative cases by liquid or tissue biopsy; and (3) driver undetected cases by liquid or tissue biopsy. Firstly, in plasma driver-positive cases, the addition of tissue biopsy detected co-occurring ESCAT I or II targetable drivers in 1/36 cases in cohort 1 (3%) (one ESCAT II driver alteration [*MET* amplification] missed by liquid biopsy in a patient with an *EGFR*-mutant tumor), and none in cohort 2 (Fig. 3*A*). The addition of liquid biopsy in tissue driver positive cases did not find ESCAT I/II co-occurring drivers in any of the cohorts (Fig. 3*B*).

Next, we analyzed the utility of orthogonal testing in cases with uninformative liquid or tissue NGS testing. In cases with undetectable ctDNA, tissue-based NGS detected ESCAT I or II targetable driver alterations in 3/5 patients in cohort 1 (60%) (five ESCAT I drivers [one *KRAS-G12C* and two *EGFR* mutations in one patient, one *ALK* fusion and one *BRAF-V600E* mutation]), and in 8/9 patients in cohort 2 (89%) (eight ESCAT I drivers [four *KRAS-G12C*, one *BRAF-V600E* and two *METex14* mutations, and one *ROS1* fusion]) (Fig. 3*A*). As expected, in cases with uninformative tissue molecular testing, adding liquid biopsy detected ESCAT I or II targetable drivers in 10/32 patients in cohort 1 (31%) (11 ESCAT I driver alterations [eight *EGFR*—two in the same patient—and two *KRAS-G12C* mutations, and one *ALK* fusion]), and in 8/40 patients in cohort 2 (20%) (eight ESCAT I drivers [one *EGFR* and six *KRAS G12C* mutations, and one *RET* fusion]) (Fig. 3*B*).

We then assessed the added value of orthogonal biopsy in patients that received a seemingly informative but driver undetected molecular result. In cases with driver undetected by liquid biopsy, tissue-based NGS found ESCAT I or II targetable driver alterations in 4/86 patients (5%) in cohort 1 (four ESCAT I driver alterations [one *EGFR* and two *KRAS*-G12C mutations and one *ALK* fusion]), and in 6/104 patients (6%) in cohort 2 (six ESCAT I drivers [three *EGFR* mutations, one *RET* fusion, one *MET*ex14 mutation, and one *ROS1* fusion]) (Fig. 3*A*). Similarly, in cases with driver undetected by tissue NGS analysis, the addition of liquid biopsy resulted in the detection of ESCAT I or II targetable driver alterations missed by tissue in 2/64 of the patients (3%) in cohort 1

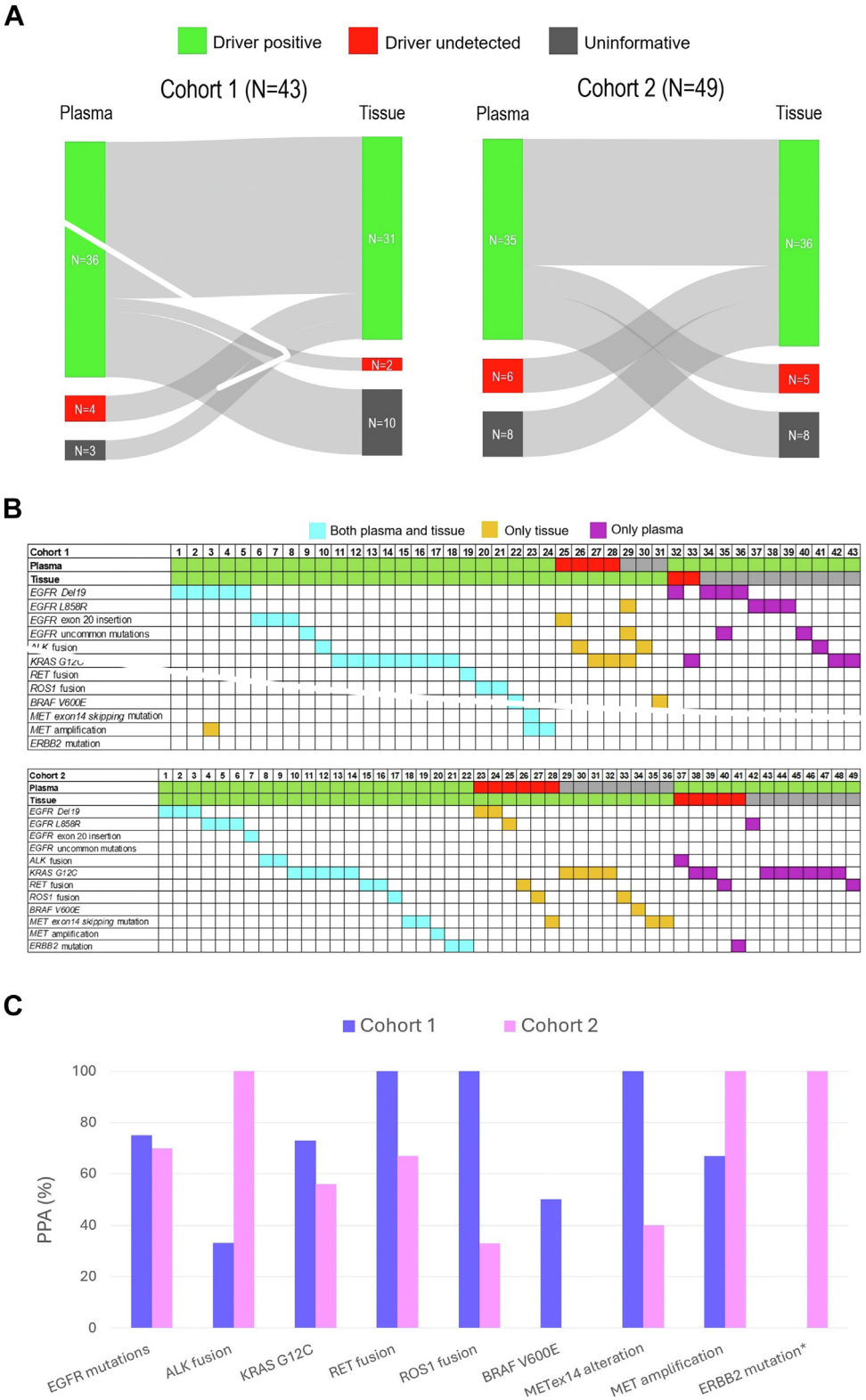

Figure 2. ESCAT I or II targetable driver alterations detected in plasma and tissue. *(A)* Patients with ESCAT I or II targetable drivers detected by plasma and tissue NGS in cohort 1 and cohort 2. Driver-positive, driver-undetected, and uninformative NGS results are shown in green, red, and grey, respectively. *(B)* Plasma and tissue NGS results for ESCAT I or II targetable driver alterations were found in cohort 1 and cohort 2. Patients are shown in columns and actionable alterations are represented in rows in blue if detected on both plasma and tissue samples, purple if only on plasma, and orange if only on tissue. *(C)* PPA for each ESCAT I or II targetable driver alteration in cohort 1 and cohort 2. *No *ERBB2* mutations were detected in cohort 1; PPA is not calculable for this alteration in this cohort. ESCAT, European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets; NGS, next-generation sequencing; PPA, positive percent agreement.

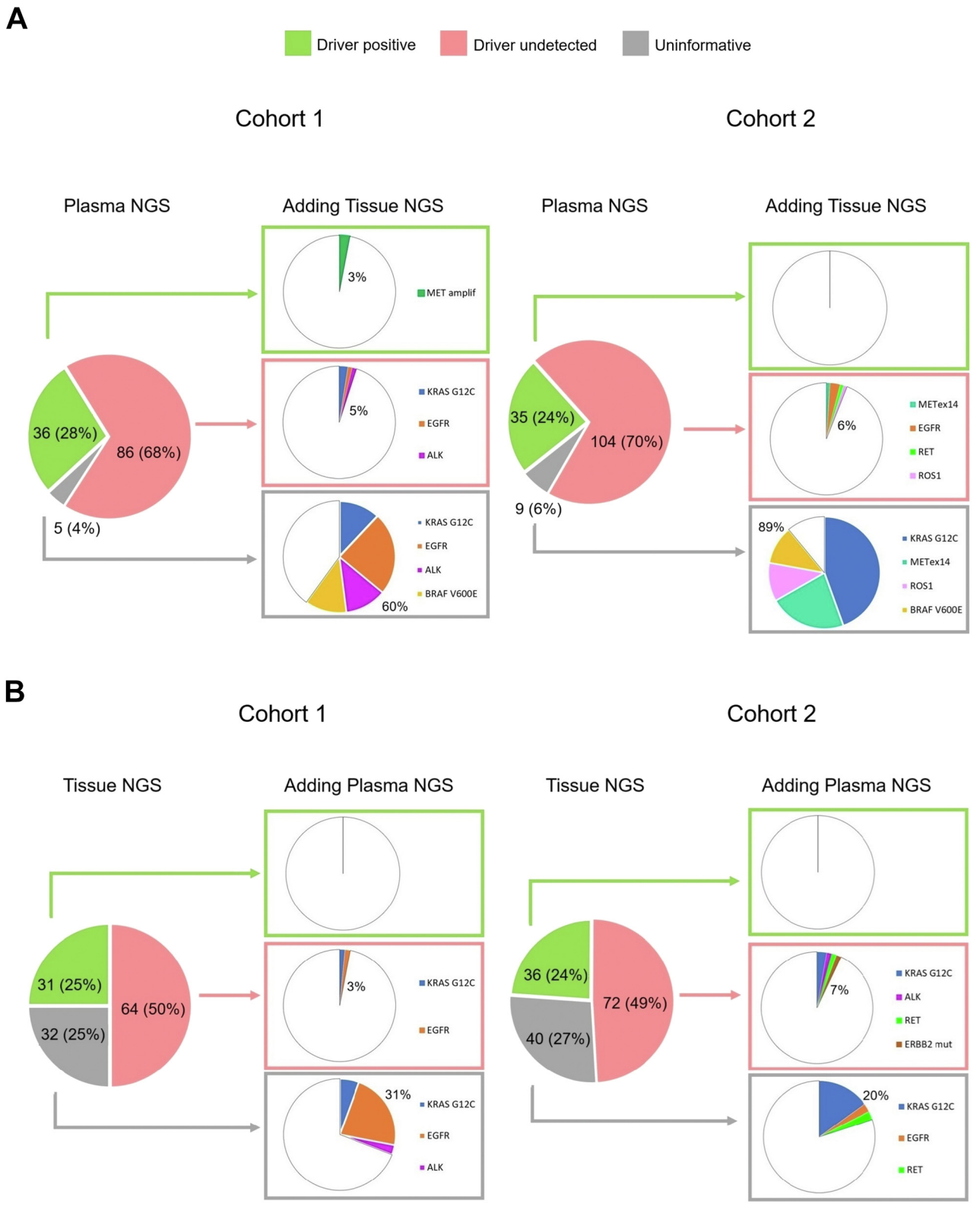

Figure 3. The utility and added value of orthogonal biopsy to detect ESCAT I to II targetable driver alterations. *(A)* The incremental value of adding tissue to liquid biopsy to detect ESCAT I to II targetable driver alterations in patients with driver-positive (green), driver-undetected (red), and uninformative (grey) plasma NGS results in cohort 1 and cohort 2. *(B)* The incremental value of adding liquid to tissue biopsy to detect ESCAT I to II targetable driver alterations in patients with driver-positive (green), driver-undetected (red), and uninformative (grey) tissue NGS results in cohort 1 and cohort 2. ESCAT, European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets; NGS, next-generation sequencing.

(two ESCAT I driver alterations [one *EGFR* and one *KRAS*-G12C mutations]), and in 5/72 of the patients (7%) in cohort 2 (four ESCAT I [one *RET* and one *ALK* fusion and two *KRAS-G12C* mutations] and one ESCAT II [one *ERBB2* mutation] drivers) (Fig. 3*B*).

A total of 26 patients in cohort 1 (20%) and 21 patients in cohort 2 (14%) received targeted therapies on the basis of tissue and/or liquid biopsy results during the whole course of their disease. Most cases that had discordant ESCAT I or II targetable driver results by tissue or liquid biopsy and received targeted therapies (n = 11 in cohort 1, and n = 9 in cohort 2) achieved durable partial responses (10/11 in cohort 1, and 6/9 in cohort 2) (Supplementary Table 1).

Finally, with the aim of trying to identify a subgroup of patients that could be ideal candidates for a "plasma-first" genomic profiling approach, we explored if any baseline clinical characteristic could predict the detection of ESCAT I or II targetable driver alterations in liquid biopsy. Consistently in both cohorts, the prevalence of ESCAT I or II targetable drivers in plasma was significantly higher in patients with lung adenocarcinoma (cohort 1: odds ratio [OR] = 7.55, $p = 0.003$; cohort 2: OR = 4.58, $p = 0.004$), 20 pack-year or less history (cohort 1: OR = 4.27, $p < 0.0001$; cohort 2: OR = 2.68, $p = 0.013$), M1b-c staging (cohort 1: OR = 3.25, $p = 0.010$; cohort 2: OR = 2.55, $p = 0.026$), and abdominal disease involvement (cohort 1: OR = 4.19, $p < 0.0001$; cohort 2: OR = 3.00, $p = 0.005$) (Table 2).

Discussion

In the present study, we have assessed the complementary roles and added value of combining liquid and tissue biopsy in detecting targetable molecular drivers for the initial clinical management of patients with advanced NSCLC. We show that orthogonal biopsy seems to offer limited or no significant clinical value in those cases with ESCAT I or II targetable drivers already detected by one method (either liquid or tissue biopsy), supporting sequential orthogonal testing for selected cases rather than concomitant testing in all patients with NSCLC. We further confirm the complementary roles of tissue and liquid biopsy for detecting driver alterations and selecting patients for targeted therapies when either source (tissue or plasma) is unavailable or uninformative. Finally, our study suggests that orthogonal sequential biopsy (tissue to liquid, or liquid to tissue) can meaningfully increment the detection of ESCAT I or II targetable driver alterations also in those cases with seemingly informative but driver undetected molecular results.

To our knowledge, this is the first study evaluating the clinical utility of reflex tissue and liquid biopsy in two separate cohorts of unselected, newly diagnosed patients with advanced-stage NSCLC who were separately assessed with two different Food and Drug Administration–approved liquid biopsy methods. We found that 34% of the patients in cohort 1 and 33% of the patients in cohort 2 harbored ESCAT I or II targetable drivers detected in tissue, plasma, or both. These proportions compare similarly to other studies assessing advanced and patients with treatment-naïve NSCLC[23,24] and, as expected, are somewhat lower to those assessing only patients with non-squamous NSCLC unselected for prior therapies.[25]

Despite the well-established complementary roles of tissue and plasma-based NGS analysis in patients with NSCLC,[26] the optimal approach for successful and efficient clinical integration of both methods in patients with advanced and treatment naïve NSCLC is still unclear. The first relevant question is whether a "plasma-first" approach can be widely adopted in routine clinical practice. The utility and clinical relevance of performing a liquid biopsy first have been shown in several studies.[14,27–29] Most remarkably, the LIBELULE study has recently reported in a randomized setting that performing liquid biopsy even before histological diagnosis in patients with radiological suspicion of advanced NSCLC can significantly reduce time to molecular testing and time to first-line treatment, particularly in those cases with ESCAT I driver alterations.[13] Our results do support this approach, confirming a significantly shorter TAT as compared with tissue biopsy and the capacity to detect ESCAT I to II targetable driver alterations in about 25% of unselected NSCLC cases. Here, we further propose that certain subgroups of patients (i.e., patients with lung adenocarcinomas with 20 pack-year or less smoking history and extrathoracic metastases) seem ideal candidates for initial liquid biopsy testing, on the basis of their higher likelihood of detecting ESCAT I to II targetable drivers. Importantly, patients with ESCAT I to II targetable drivers detected in plasma would not need to undergo concurrent tissue NGS testing[13,14,28] and could have their tissue preserved for further immune-based biomarker analysis or other sort of molecular testing if needed (Fig. 4).

However, one of the main limitations of a "plasma-first" approach is that a non-negligible fraction of advanced NSCLC cases do not shed enough ctDNA for successful plasma-based NGS testing. In line with prior reports,[30] our study does confirm the absolute need for tissue NGS in cases with uninformative (ctDNA-negative) liquid biopsy results because complementary tissue sequencing could detect ESCAT I or II targetable driver alterations in most of these cases (Fig. 4). Certain clinical characteristics typically associated with some driver positive tumors (i.e., M1a stage or central nervous

Table 2. Association Between Clinical Characteristics and the Presence of ESCAT I to II Targetable Driver Alterations in Liquid Biopsy

	Cohort 1 (n = 127 pts)			Cohort 2 (n = 148 pts)		
	Positive ESCAT I-II Drivers in Plasma (n = 36 pts)	Undetected ESCAT I-II Drivers in Plasma (n = 91 pts)	*p* Value	Positive ESCAT I-II Drivers in Plasma (n = 35 pts)	Undetected ESCAT I-II Drivers in Plasma (n = 113 pts)	*p* Value
Median age, y (range)	63 (41-84)	66 (43-88)	0.82	63 (31-86)	66 (42-94)	0.09
Gender, No. (%)			0.28			0.94
Male	18 (50)	55 (60)		23 (66)	75 (66)	
Female	18 (50)	36 (40)		12 (34)	38 (34)	
Pack-year history, No. (%)			<0.0001			0.013
≤20	24 (66)	29 (32)		16 (46)	27 (24)	
>20	12 (34)	62 (68)		19 (54)	86 (76)	
Histology, No. (%)			0.003			0.004
Adenocarcinoma	34 (94)	63 (69)		31 (85)	71 (63)	
Others	2 (6)	28 (31)		4 (15)	42 (37)	
Staging TNM, No. (%)			0.010			0.026
M1a	7 (19)	40 (44)		9 (26)	53 (47)	
M1b, M1c	29 (81)	51 (56)		26 (74)	60 (53)	
Liver metastases, No. (%)			0.96			0.012
Yes	7 (19)	18 (20)		12 (34)	17 (15)	
No	29 (81)	73 (80)		23 (66)	96 (85)	
CNS metastases, No. (%)			0.40			0.12
Yes	10 (28)	20 (21)		12 (33)	24 (21)	
No	26 (72)	71 (79)		23 (67)	89 (79)	
Abdominal metastases[a], No. (%)			<0.0001			0.005
Yes	23 (64)	27 (30)		17 (49)	27 (24)	
No	13 (36)	64 (70)		18 (51)	86 (76)	
Bone metastases, No. (%)			0.65			0.23
Yes	15 (42)	34 (37)		14 (40)	33 (29)	
No	21 (58)	57 (63)		21 (60)	80 (71)	

[a]Abdominal metastases are defined as metastases in, at least, one of the following sites: liver, spleen, peritoneum, abdominal nodes, kidney, adrenal glands, or subcutaneous abdominal nodes.
ESCAT, European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets; CNS, central nervous system; NGS, next-generation sequencing; pts, patients.

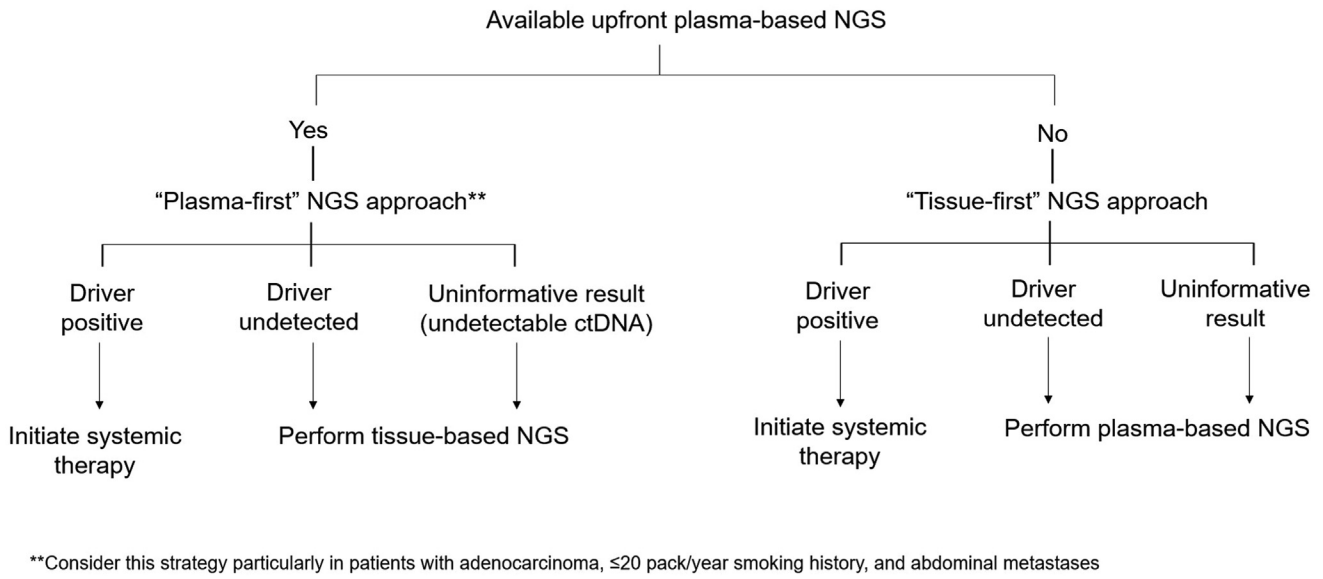

Figure 4. Proposed molecular diagnostic management algorithm in patients with advanced, treatment-naïve NSCLC. ctDNA, circulating tumor DNA; NGS, next-generation sequencing.

system only disease) are also predictive of lower ctDNA shedding, which could at least partially explain the high rates of ESCAT I or II targetable driver detection in tissue in this subgroup of uninformative liquid biopsy cases.

Remarkably, we also observed that the addition of tissue biopsy was capable of detecting ESCAT I or II targetable drivers missed by plasma in about 5% of the patients that received a seemingly informative with driver undetected liquid biopsy result. The interpretation of plasma-based NGS reports is challenged by the fact that some of the pathogenic genomic variants that are detected in plasma are a result of clonal hematopoiesis of indeterminate potential.[31,32] In cases with insufficient ctDNA shed, the detection of clonal hematopoiesis of indeterminate potential–related genomic variants can lead to the misinterpretation of a liquid biopsy result as tumor informative.[33] Collectively, we believe that these results suggest that in patients that undergo a "plasma-first" approach, only the detection of ESCAT I to II targetable driver alterations should be considered informative results to guide treatment in the lack of a tissue biopsy, underscoring the need of complementary sequential tissue NGS testing in the rest of the cases (Fig. 4). Of note, as indicated in a recent large-scale report, perhaps the quantification of ctDNA tumor fraction could help distinguish true negative from false negative plasma NGS results, thereby avoiding confirmatory tissue testing in those cases more likely to be true driver negatives in plasma.[34]

Despite "plasma-first" being a compelling approach for most patients with advanced-stage NSCLC in the front-line setting, it needs to be considered that plasma-based NGS is not globally available or widely adopted as a standard of care in the initial management of patients with advanced NSCLC yet, and most institutions in fact perform tissue-based NGS upfront. In line with what we observed in the "plasma-first" approach, there was no added significant clinical value of performing concomitant orthogonal liquid biopsy in those cases with ESCAT I to II targetable driver alterations detected in tissue, which does not therefore seem to support concomitant tissue and liquid biopsy for all patients with NSCLC (Fig. 4).

In contrast, the clinical value of using liquid biopsy when tissue NGS is uninformative for molecular testing (unavailable or inadequate) has been extensively reported in several studies.[16,35–37] This strategy is guideline-recommended[4,38–43] and widely adopted to facilitate personalized therapy in patients with advanced NSCLC in many institutions.[44,45] In this setting, plasma-based NGS can detect a broad spectrum of driver alterations that recapitulate the tissue-based molecular landscape of NSCLC.[46–49] Consistent with this paradigm, our study found that liquid biopsy can detect ESCAT I to II targetable driver alterations in approximately 25% of the cases with uninformative tissue testing, further reinforcing the established clinical value of liquid biopsy in this setting (Fig. 4).

What is still unclear is the added clinical value of performing liquid biopsy in patients that have a seemingly informative with driver undetected tissue NGS result. Some studies have shown that liquid biopsy does not increase the overall detection of driver alterations in patients with informative tissue NGS testing using large

hybrid-capture-based tissue assays,[26] whereas other studies that have used more limited amplicon-based tissue assays found that liquid biopsy could increase the detection of actionable drivers in about 5% of the cases in this particular setting.[50] In our study, in which we also used a more limited amplicon-based tissue NGS testing in real-world clinical practice conditions, we found that there was a meaningful increment in the detection rate of ESCAT I or II targetable driver alterations with the addition of liquid biopsy in those cases with apparently informative but driver undetected tissue NGS result (i.e., either successful sequencing with no genetic alterations detected, or pathogenic variants that do not rank within ESCAT I or II targetable driver alterations). Adding liquid biopsy in this scenario resulted in 3% and 7% more patients being detected with ESCAT I or II targetable drivers missed by tissue NGS in cohort 1 and cohort 2, respectively. Collectively, we believe that these results support the use of reflex liquid biopsy sequentially to tissue biopsy not only when tissue is unavailable or inadequate for molecular analysis (uninformative), but also in those cases with a seemingly informative but driver undetected tissue NGS result (Fig. 4).

This study needs to be interpreted in the context of two main limitations. First, this was a single-center retrospective study. We included consecutive front-line patients with NSCLC selected to undergo liquid biopsy as part of their routine diagnostic and clinical workup, most of which had concomitantly undergone successful tissue-based NGS testing, but the study was not designed to prospectively compare the utility and performance of tissue and liquid biopsy in this setting. And second, we used multiple panels with different sequencing methodologies, most notably hybrid-capture-based in the case of plasma NGS, and amplicon-based in the case of tissue NGS. Although we focused our clinical utility analysis by assessing only the detection of ESCAT I or II targetable drivers, which were covered by all liquid- and tissue-based NGS panels used in the study, the existing differences in the analytical performance of the different panels need to be considered when interpreting the discordant results between liquid and tissue biopsy observed in this study. Some of these differences could also at least partially explain the slight discrepancies in PPA values between both cohorts. Despite this limitation, we believe that our data represent real-world clinical practice, in which different panels for liquid and tissue biopsy might be available for routine clinical care. Moreover, our study was not intended or designed to compare the analytical performance between liquid and tissue biopsy, but rather to analyze the clinical utility and added value of combining tissue and liquid biopsy to detect ESCAT I or II targetable driver alterations in the front-line setting, regardless of the assay. In fact, the observed incremental value of combining liquid and tissue biopsy in certain clinical scenarios seems to be assay-independent, on the basis of the comparable findings observed in cohort 1 and cohort 2.

In conclusion, this work underscores the utility of combining liquid and tissue biopsy to maximize the detection of ESCAT I or II targetable driver alterations in patients with newly diagnosed advanced NSCLC. Our data seem to favor a sequential rather than a concomitant approach, adding orthogonal biopsy sequentially (tissue to liquid in cases selected for a "plasma-first" approach, or liquid to tissue in cases selected for a "tissue-first" approach) whenever an ESCAT I or II targetable driver alteration has not been detected by the initial method, which includes cases with uninformative testing, but also those with seemingly informative but driver undetected molecular results. Our study also suggests that patients with lung adenocarcinoma, lower tobacco history, and involvement of extrathoracic disease seem to be appropriate candidates for "plasma-first" NGS testing.

CRediT Authorship Contribution Statement

Helena Bote-de Cabo: Conceptualization, Data curation, Investigation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing.

Marco Siringo: Conceptualization, Data curation, Investigation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing.

Esther Conde: Methodology, Validation, Writing - review & editing.

Susana Hernández: Methodology, Validation, Writing - review & editing.

Fernando López-Ríos: Methodology, Validation, Writing - review & editing.

Alicia Castelo-Loureiro: Data curation, Writing - review & editing.

Esther García-Lorenzo: Data curation, Writing - review & editing.

Javier Baena: Writing - review & editing.

Mercedes Herrera: Writing - review & editing.

Ana Belén Enguita: Methodology.

Yolanda Ruano: Methodology.

Jon Zugazagoitia: Conceptualization, Supervision, Validation, Writing - review & editing.

Luis Paz-Ares: Supervision, Validation, Funding acquisition, Writing - review & editing.

Disclosure

Dr. Bote-de Cabo reports receiving payment or honoraria for lectures, presentations, or speakers' bureau

from Astra Zeneca, Janssen, and support for attending meetings or travel from Roche, Takeda. Dr. Siringo reports receiving travel or accommodation expenses from Novartis. Dr. Conde reports receiving grants or contracts from Roche, Thermo Fisher, and Astra Zeneca. She has received payment or honoraria for lectures, presentations, or speakers' bureau from Pfizer, Roche, Thermo Fisher, Astra Zeneca, Lilly, and Janssen. She reports receiving consulting fees from Roche, Janssen, and Astra Zeneca. She has received support for attending meetings or travels from Thermo Fisher, Janssen, and Lilly. She reports participation on the data safety monitoring or advisory boards for Roche, and Janssen. Dr. Hernández reports receiving grants or contracts from Roche, Thermo Fisher, and Astra Zeneca. She has received payment or honoraria for lectures, presentations, or speakers' bureau from Pfizer, Roche, Thermo Fisher, Astra Zeneca, and Lilly. She has received support for attending meetings or travels from Thermo Fisher. Dr. Lopez-Ríos reports receiving grants or contracts from Roche, Thermo Fisher, Astra Zeneca, and Pfizer. He has received payment or honoraria for lectures, presentations, or 8 speakers' bureau from Abbvie, Astellas, Astra Zeneca, Bayer, BMS, Daiichi Sankyo, Janssen, Lilly, MSD, Merch Pfizer, Roche, Sanofi, Takeda, Thermo Fisher. Dr. Castelo-Loureiro reports receiving payment or honoraria for lectures, presentations, or speakers' bureau from Astra Zeneca, Pfizer, and Rovi and support for attending meetings or travel from GSK and Pfizer. Dr. García-Lorenzo has nothing to disclose. Dr. Baena reports receiving honoraria from Roche, Astra Zeneca, BMS, consulting or advisory roles for Roche, and travel or accommodation expenses from MSD. Dr. Herrera reports receiving payment or honoraria for lectures, presentations, or speakers' bureau from Astra Zeneca, and support for attending meetings or travel from Roche, Takeda. Dr. Enguita has nothing to disclose. Dr. Ruano reports receiving grants or contracts from Roche, Astra Zeneca, and Pfizer for lectures, presentations, and support for attending meetings or travels. Dr. Zugazagoitia has served as a consultant for Merck, Sanofi, Astra Zeneca, BMS, Roche, Pfizer, Novartis, and Guardant Health. He reports speakers' honoraria from Pierre Fabre, Janssen, Sanofi, Takeda, BMS, Pfizer, Roche, Astra Zeneca, NanoString and Guardant Health. He reports travel honoraria from Janssen, Sanofi, Takeda, BMS, Pfizer, Roche, Astra Zeneca, and NanoString. He has received research support/funds from BMS, Astra Zeneca, and Roche. Dr. Paz-Ares reports receiving honoraria from Amgen, AstraZeneca, Bayer, Beigene, Bristol Myers Squibb, Boehringer-Ingelheim, Daiichi Sankyo, Eli Lilly, Jazz Pharmaceuticals, Merck Serono, Mirati Therapeutics, Merck Sharp & Dohme, Novartis, Pfizer, PharmaMar, Roche /Genentech, Sanofi, and Takeda; leadership role (founder and Board member) in ALTUM Sequencing and Stab therapeutics; research funding (to Institution) from AstraZeneca, Bristol Myers Squibb, Kura Oncology, PharmaMar, and Merck Sharp & Dohme; speaker fees from Bristol Myers Squibb, Eli Lilly, Merck Serono, Merck Sharp & Dohme Oncology, Pfizer and Roche/Genentech; and travel, accommodation, and expenses from AstraZeneca, Bristol Myers Squibb, Merck Sharp & Dohme, Pfizer, Roche, and Takeda.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100778.

References

1. Tan AC, Tan DSW. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. *J Clin Oncol*. 2022;40:611–625.
2. Jordan EJ, Kim HR, Arcila ME, et al. Prospective comprehensive molecular characterization of lung adenocarcinomas for efficient patient matching to approved and emerging therapies. *Cancer Discov*. 2017;7:596–609.
3. Lee VHF, Mok TSK, Goto Y, et al. Differences between the east and the west in managing advanced-stage non-small cell lung cancer. *Clin Oncol (R Coll Radiol)*. 2020;32:e1–e9.
4. Hendriks LE, Kerr KM, Menis J, et al. Non-oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34:339–357.
5. Otano I, Ucero AC, Zugazagoitia J, Paz-Ares L. At the crossroads of immunotherapy for oncogene-

addicted subsets of NSCLC. *Nat Rev Clin Oncol*. 2023;20:143–159.
6. Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020;31:1491–1505.
7. Hofman P. Matched tissue and liquid biopsies for advanced non-small cell lung cancer patients A potentially indispensable complementary approach. *Transl Oncol*. 2023;35:101735.
8. Aggarwal C, Thompson JC, Black TA, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. *JAMA Oncol*. 2019;5:173–180.
9. Malapelle U, Pisapia P, Pepe F, et al. The evolving role of liquid biopsy in lung cancer. *Lung Cancer*. 2022;172:53–64.
10. Zugazagoitia J, Ramos I, Trigo JM, et al. Clinical utility of plasma-based digital next-generation sequencing in patients with advance-stage lung adenocarcinomas with insufficient tumor samples for tissue genotyping. *Ann Oncol*. 2019;30:290–296.
11. Thompson JC, Aggarwal C, Wong J, et al. Plasma genotyping at the time of diagnostic tissue biopsy decreases time-to-treatment in patients with advanced NSCLC-results from a prospective pilot study. *JTO Clin Res Rep*. 2022;3:100301.
12. Lee Y, Clark EW, Milan MSD, et al. Turnaround time of plasma next-generation sequencing in thoracic oncology patients: a quality improvement analysis. *JCO Precis Oncol*. 2020;4. PO.20.00121.
13. Swalduz A, Curcio H, Ambasager B, et al. LIBELULE: a randomized phase III study to evaluate the clinical relevance of early liquid biopsy (LB) in patients with suspicious metastatic lung cancer. *J Clin Oncol*. 2023;16:9019.
14. García-Pardo M, Czarnecka-Kujawa K, Law JH, et al. Association of circulating tumor DNA testing before tissue diagnosis with time to treatment among patients with suspected advanced lung cancer: the ACCELERATE nonrandomized clinical trial. *JAMA Netw Open*. 2023;6:e2325332.
15. Cescon DW, Bratman SV, Chan SM, Siu LL. Circulating tumor DNA and liquid biopsy in oncology. *Nat Cancer*. 2020;1:276–290.
16. Lam VK, Zhang J, Wu CC, et al. Genotype-specific differences in circulating tumor DNA levels in advanced NSCLC. *J Thorac Oncol*. 2021;16:601–609.
17. Tomasik B, Skrzypski M, Bieńkowski M, Dziadziuszko R, Jassem J. Current and future applications of liquid biopsy in non-small-cell lung cancer-a narrative review. *Transl Lung Cancer Res*. 2023;12:594–614.
18. Food and Drug Administration. Guardant360 CDx - P200010/S008. https://www.fda.gov/medical-devices/recently-approved-devices/guardant360-cdx-p200010s008. Accessed December 29, 2023.
19. Food and Drug Administration. FoundationOne CDx (F1CDx). https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P170019S017. Accessed December 29, 2023.
20. Thermo Fisher Scientific. Oncomine focus assay. https://www.thermofisher.com/es/es/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-focus-assay.html. Accessed December 29, 2023.
21. Thermo Fisher Scientific. Oncomine Precision Assay. https://www.thermofisher.com/es/es/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-precision-assay.html. Accessed December 29, 2023.
22. Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2024;35:588–606.
23. Cui W, Milner-Watts C, O'Sullivan H, et al. Up-front cell-free DNA next generation sequencing improves target identification in UK first line advanced non-small cell lung cancer (NSCLC) patients. *Eur J Cancer*. 2022;171:44–54.
24. Mack PC, Banks KC, Espenschied CR, et al. Spectrum of driver mutations and clinical impact of circulating tumor DNA analysis in non-small cell lung cancer: analysis of over 8000 cases. *Cancer*. 2020;126:3219–3228.
25. Page RD, Drusbosky LM, Dada H, et al. Clinical outcomes for plasma-based comprehensive genomic profiling versus standard-of-care tissue testing in advanced non-small cell lung cancer. *Clin Lung Cancer*. 2022;23:72–81.
26. Schwartzberg LS, Li G, Tolba K, et al. Complementary roles for tissue- and blood-based comprehensive genomic profiling for detection of actionable driver alterations in advanced NSCLC. *JTO Clin Res Rep*. 2022;3:100386.
27. Makarem M, Leighl NB. Molecular testing for lung adenocarcinoma: is it time to adopt a "plasma-first" approach? *Cancer*. 2020;126:3176–3180.
28. Garcia-Pardo M, Czarnecka K, Law JH, et al. Plasma-first: accelerating lung cancer diagnosis and molecular profiling through liquid biopsy. *Ther Adv Med Oncol*. 2022;14:17588359221126151.
29. Cui W, Milner-Watts C, McVeigh TP, et al. A pilot of Blood-First diagnostic cell free DNA (cfDNA) next generation sequencing (NGS) in patients with suspected advanced lung cancer. *Lung Cancer*. 2022;165:34–42.
30. Lin LH, Allison DHR, Feng Y, et al. Comparison of solid tissue sequencing and liquid biopsy accuracy in identification of clinically relevant gene mutations and rearrangements in lung adenocarcinomas. *Mod Pathol*. 2021;34:2168–2174.
31. Ptashkin RN, Mandelker DL, Coombs CC, et al. Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors. *JAMA Oncol*. 2018;4:1589–1593.
32. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371:2477–2487.
33. Hu Y, Ulrich BC, Supplee J, et al. False-positive plasma genotyping due to clonal hematopoiesis. *Clin Cancer Res*. 2018;24:4437–4443.
34. Rolfo CD, Madison RW, Pasquina LW, et al. Measurement of ctDNA tumor fraction identifies informative negative

liquid biopsy results and informs value of tissue confirmation. *Clin Cancer Res*. 2024;30:2452–2460.
35. García-Pardo M, Aparicio I, Martínez Í, et al. Brief report: clinical outcomes using plasma-based molecular profiling to guide treatment decisions in patients with advanced NSCLC and limited access to broad tissue testing. *Clin Lung Cancer*. 2023;24:366–370.
36. Thompson JC, Yee SS, Troxel AB, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. *Clin Cancer Res*. 2016;22:5772–5782.
37. Schwaederlé MC, Patel SP, Husain H, et al. Utility of genomic assessment of blood-derived circulating tumor DNA (ctDNA) in patients with advanced lung adenocarcinoma. *Clin Cancer Res*. 2017;23:5101–5111.
38. Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2022;33:750–768.
39. Lockwood CM, Borsu L, Cankovic M, et al. Recommendations for cell-free DNA assay validations: a joint consensus recommendation of the association for molecular pathology and College of American Pathologists. *J Mol Diagn*. 2023;25:876–897.
40. Hanna NH, Robinson AG, Temin S, et al. Therapy for Stage IV non-small-cell lung cancer with driver alterations: ASCO and OH (CCO) joint guideline update. *J Clin Oncol*. 2021;39:1040–1091.
41. Kalemkerian GP, Narula N, Kennedy EB, et al. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. *J Clin Oncol*. 2018;36:911–919.
42. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med*. 2018;142:321–346.
43. Álvarez-Alegret R, Rojo Todo F, Garrido P, et al. Liquid biopsy in oncology: a consensus statement of the Spanish Society of Pathology and the Spanish Society of Medical Oncology. *Rev Esp Patol*. 2020;53:234–245 [in Spanish].
44. Rolfo C, Mack P, Scagliotti GV, et al. Liquid biopsy for advanced NSCLC: a consensus statement from the International Association for the Study of Lung Cancer. *J Thorac Oncol*. 2021;16:1647–1662.
45. Aggarwal C, Rolfo CD, Oxnard GR, Gray JE, Sholl LM, Gandara DR. Strategies for the successful implementation of plasma-based NSCLC genotyping in clinical practice. *Nat Rev Clin Oncol*. 2021;18:56–62.
46. Remon J, Lacroix L, Jovelet C, et al. Real-world utility of an amplicon-based next-generation sequencing liquid biopsy for broad molecular profiling in patients with advanced non-small-cell lung cancer. *JCO Precis Oncol*. 2019;3:PO.18.00211.
47. Palmero R, Taus A, Viteri S, et al. Biomarker discovery and outcomes for comprehensive cell-free circulating tumor DNA versus standard-of-care tissue testing in advanced non-small-cell lung cancer. *JCO Precis Oncol*. 2021;5:93–102.
48. Pritchett MA, Camidge DR, Patel M, et al. Prospective clinical validation of the InVisionFirst-lung circulating tumor DNA assay for molecular profiling of patients with advanced nonsquamous non-small-cell lung cancer. *JCO Precis Oncol*. 2019;3:PO.18.00299.
49. Leighl NB, Page RD, Raymond VM, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. *Clin Cancer Res*. 2019;25:4691–4700.
50. Sugimoto A, Matsumoto S, Udagawa H, et al. A large-scale prospective concordance study of plasma- and tissue-based next-generation targeted sequencing for advanced non-small cell lung cancer (LC-SCRUM-liquid). *Clin Cancer Res*. 2023;29:1506–1514.