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Clinical utility of a plasma-based comprehensive genomic profiling test in patients with non-small cell lung cancer in Korea

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ABSTRACT

Objectives: Plasma-based comprehensive circulating cell-free DNA (cfDNA) next generation sequencing (NGS) has shown utility in advanced non-small cell lung cancer (aNSCLC). The aim of this study was to determine the feasibility of cfDNA-based NGS to identify actionable gene alterations in patients with aNSCLC.

Patients and methods: This single-center non-interventional retrospective study evaluated Korean patients with biopsy-confirmed stage III/IV non-squamous aNSCLC. Tissue biopsy samples were collected at baseline, and/or at progression and analysed with Standard of Care (SOC) testing; cfDNA was analyzed by NGS in some patients concurrently.

Results: aNSCLC patients with cfDNA test results ($n = 405$) were categorized into three groups: treatment naïve ($n = 182$), progressive aNSCLC after chemotherapy and/or immunotherapy ($n = 157$), and progressive aNSCLC after tyrosine kinase inhibitors (TKIs) ($n = 66$). Clinically informative driver mutations were identified for 63.5% of patients which were classified as OncoKB Tiers 1 (44.2%), 2 (3.4%), tier 3 (18.9%), and 4 (33.5%). Concordance between cfDNA NGS and tissue SOC methods for concurrently collected tissue samples ($n = 221$) with common *EGFR* mutations or *ALK/ROS1* fusions was 96.9%. cfDNA analysis identified tumor genomic alterations in 13 patients that were unidentified with tissue testing, enabling initiation of targeted treatment.

Conclusions: In clinical practice, results of cfDNA NGS are highly concordant with those of tissue SOC testing in aNSCLC patients. Plasma analysis identified actionable alterations that were missed or not evaluated by tissue testing, enabling the initiation of targeted therapy. Results from this study add to the body of evidence in the support routine use of cfDNA NGS for patients with aNSCLC.

1. Introduction

In 2020, lung cancer was reported by the World Health Organization (WHO) to be the second most common cause of new cases of cancer, after breast cancer, and the leading cause of cancer-related deaths [1]. In Korea, lung cancer was responsible for >20% of the cancer- related deaths in 2016 [1] and is predicted to further increase [2]. These numbers highlight the need for improved treatment approaches in this disease, and the development of molecular targeted therapies is providing a source for optimism. Over the last 20 years the treatment paradigm for advanced non-small cell lung cancer (aNSCLC), the predominant subtype accounting for about 85% of all lung cancers, has changed dramatically [3]. It has transformed from a disease managed empirically with cytotoxic drugs to one where targeted therapy with small molecule tyrosine kinase inhibitors and immunotherapy has resulted in markedly improved survival benefits in selected groups of patients [4].

These advances in aNSCLC management have been driven by the identification of biomarkers to select patients who will benefit from targeted therapy. Initial trials at the turn of the century investigated epidermal growth factor receptor (EGFR) for targeted therapy [5,6]. More recently, clinical practice guidelines from professional societies

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such as the College of American Pathologists, the International Association for the Study of Lung Cancer (IASLC) and the Association for Molecular Pathology have recommended somatic genomic testing for all patients with newly diagnosed aNSCLC [7]. Today, inhibitors targeting *EGFR* mutations, *ALK* fusions, *ROS1* fusions, *BRAF* V600E mutation, *NTRK* fusions, *MET* amplification and *MET* exon 14 skipping variants, *RET* fusions, and *KRAS*-G12C have been recommended as targeted treatment of specific aNSCLC subtypes [8]. This has resulted in increased median survival for aNSCLC patients from an average of <2 years pre-2010, to over 3 and 5 years for patients receiving targeted therapy with EGFR or ALK inhibitors, respectively [3,9–11].

Immunotherapeutic approaches for the treatment of aNSCLC have also advanced in the last two decades with the introduction of immune checkpoint inhibitors, antibodies against PD-1 or PD-L1 [12] and monoclonal antibodies that target cytotoxic T-lymphocyte antigen-4 (CTLA4) [4]. Prescribing information for such agents recommends identification of EGFR mutations and ALK fusions as exclusion criteria for such treatment [12], and attempts have been made to identify other genomic alterations that may influence immune checkpoint inhibitor efficacy, such as the presence of microsatellite instability [4] With an ever-increasing number of relevant biomarkers being identified in patients with aNSCLC, the pressure on speedy and cost-effective diagnosis has been a priority to help guide treatment decisions. Traditionally, tumor tissue-based testing (biopsy) has been the standard method for biomarker assessment, but this suffers from some major drawbacks such as its invasive nature and inconvenience to the patient. It is both slow and costly, and associated with a number of failures because of the 'hit-or-miss' nature of the sampling technique and/or lack of tumor material. It is limited to a single tumor focus and may not represent the complete molecular profile of aNSCLC, which can be a heterogeneous disease. Furthermore, it is not ideal for serial testing to monitor tumor progression [13,14]. In recent years plasma-based comprehensive circulating cell-free DNA (cfDNA) next generation sequencing (NGS) has shown diagnostic potential in patients with aNSCLC and offers several advantages over tissue biopsy-based assays such as providing a global perspective based upon all tumor DNA shed into the blood, speed, it is non-invasive so preferred by patients, it is less costly, serial testing is much more practicable, and plasma samples can be obtained without the need for deep technical training.

Plasma-based comprehensive cfDNA analysis has shown high concordance with standard- of-care (SOC) tissue-based genotyping has been reported in a number of clinical studies [13,15–17]. Despite this, there is still a perception that plasma-based genotyping to identify relevant biomarkers for aNSCLC is not as efficient as tissue-based SOC genotyping [16]. Thus, the goal of the current study was to determine the feasibility of using a plasma-based NGS to identify actionable gene mutations in patients with aNSCLC as a diagnostic approach supporting targeted treatment of the individual. In addition, the medical records of all patients were reviewed to ascertain prior SOC tissue-test including tissue-based NGS findings and treatment history, and the concordance of the different techniques was evaluated for concurrent samples.

2. Patients and methods

2.1. Study design and patients

This single-center non-interventional prospective/retrospective study was undertaken at the Division of Medical Oncology, Yonsei Cancer center, Seoul from 5/12/2018 until 11/3/2020. Yonsei Cancer center is a high-volume referral center with a patient population enriched for aNSCLC cases lacking common genomic biomarkers detected by conventional testing. The study population included all patients who underwent plasma-based (cfDNA) testing with Guardant360 for biopsy confirmed stage III or IV non-squamous cell aNSCLC. Patients with early-stage NSCLC with recurrence at least 6 months after surgery or curative-intent chemoradiotherapy who completed chemotherapy at least 6 months prior to enrolment were also eligible. aNSCLC patients with any other concurrent malignancy were not included in this analysis.

2.2. Study procedures and tumor mutation detection

As part of routine testing, all eligible patients provided blood samples for plasma-based cfDNA NGS prior to initial systemic treatment for aNSCLC or after disease progression on prior therapy for aNSCLC [8]. The medical records of these patients were reviewed to identify results of tumor tissue biomarker testing using Standard of Care (SOC) techniques according to National Comprehensive Cancer Network (NCCN) guidelines [18]. These included NGS, allele-specific polymerase chain reaction (PCR) hotspot testing, fluorescence in-situ hybridization (FISH) and/or immunohistochemistry (IHC). Some of the commercial assays include PNAClamp EGFR Mutation Detection Kit (Panagene, Daejeon, Korea), PANAMutyper EGFR Kit (Panagene), ALK (rabbit monoclonal, clone D5F3, Cell Signaling Technology, Danvers, MA), ROS1 (rabbit monoclonal, clone D4D6, Cell Signaling Technology) antibodies, all the test were performed according to manufacturer's instructions Analysis of cfDNA by NGS was performed using Guardant360 (Guardant Health), a commercially available assay [13]. At the time of our study, the assay assessed single- nucleotide variants and insertions/deletions in 74 genes, including EGFR, KRAS, ERBB2, BRAF, ALK, ROS1, RET, and MET; copy-number variants in 18 genes, including MET; and gene rearrangements in 6 genes, including ALK, ROS1, RET, and NTRK1. All the patients in this study had results of cfDNA testing done at diagnosis or at progression. Any tissue biopsy collected and tested within 90 days of cfDNA was considered to be concurrent for statistical calculations.

2.3. Genomic mutation classification using OncoKB tier of evidence

Therapeutically targetable tumor mutations were classified according to the OncoKB expert- guided precision oncology knowledge base. [19] OncoKB uses a four tier classification system to define the clinical utility of somatic mutations and molecular alterations in specific tumor types to support targeted treatment decisions. The overall goal is to utilize clinical and laboratory data, treatment guidelines, oncology expert and advocacy group recommendations, and the clinical literature to communicate best-evidence-based recommendations related to biomarker-guided use of FDA-approved therapies. [19,20]

Supplementary Table 1 provides details of mutations detected and classified by OncoKB criteria.

2.4. Ethical approval

The study was approved by the Institutional Review Board of Yonsei Cancer Center (IRB No. YCC 4-2019-0886) and performed under Good Clinical Practice guidelines and conducted according to the guidelines of the Declaration of Helsinki. All patients provided a broad level written informed consent for data analysis and publication before providing blood sample for cfDNA NGS.

2.5. Statistical analyses

Descriptive statistics were used for all variables and endpoints. According to the NCCN- recommended genomic testing guidelines for NSCLC, the following genomic alterations were assessed: clinically actionable mutations in EGFR, ALK fusions, ERBB2 exon 20 insertions, KRAS mutations, RET fusions, ROS1 fusions, MET exon 14 skipping events, MET amplifications, and BRAF V600E mutations. Common EGFR mutations were defined as the conventional sensitizing mutations, exon 19 deletions and L858R but not exclusively to these mutations. The results of tissue-based and cfDNA-based NGS tests from each cohort were compared using the OncoKB tier of evidence The positive percent agreement (PPA) and negative percent agreement (NPA), and positive

predictive value (PPV) and negative predictive value (NPV), were calculated using an online diagnostic test evaluation calculator [21]. Results from the NGS tests were also compared with standard test results from routine clinical procedures, including EGFR polymerase chain reaction; ALK IHC or FISH; KRAS polymerase chain reaction; ROS1 IHC, FISH, or reverse transcriptase–polymerase chain reaction; RET FISH; MET IHC, FISH, or reverse transcriptase–polymerase chain reaction; and PD-L1 IHC.

3. Results

All patients with aNSCLC and cfDNA NGS test results ($n = 405$; 263 men, 64.9%; and 142 women, 35.1%) were categorized into three groups depending on treatment history: treatment naïve (Group A, $n = 182$), progressive aNSCLC after chemotherapy and/or immunotherapy (Group B, $n = 157$), and progressive aNSCLC after tyrosine kinase inhibitors (TKIs) (Group C, $n = 66$). The median age of the overall group was 64 years (range 25–87 years), 38.3% had never smoked 37.8% were ex-smokers, while 23.5% were current smokers (Table 1). Adenocarcinoma was the most commonly diagnosed histology (>80%) and almost three- quarters had stage IV disease.

A patient enrolment and testing flowchart is shown in Fig. 1 and includes details of the biomarker tests performed. Compared to treatment naïve patients (Group A), a lower proportion of patients in Group B (disease progression after chemotherapy and/or immunotherapy) underwent tissue biopsy. Groups A and B had similar proportions of alterations classified by OncoKB classification. Group C had a higher percentage of Tier 1 mutation at progression as these patients were known to have targetable mutations before being treated by targeted therapy.

In group A (treatment naïve) aNSCLC patients, tissue SOC identified EGFR as the most common actionable mutation (Table 1). While a few patients from group B with prior treatment of chemotherapy/ immunotherapy were also found to have driver mutations such as EGFR, ALK, or ROS1. Tissue SOC was not performed in 14.3% and 12.1% of patients in Groups A and B, respectively.

Table 1
Patient demographics and clinical features.

Parameter	**Group A** ($n = 182$)	**Group B** ($n = 157$)	**Group C** ($n = 66$)
Disease classification	Treatment naive	PD after chemotherapy and/or immunotherapy	PD after EGFR/ ALK/ ROS1 TKIs
Gender (male/female)	120/62	114/43	29/37
Age: median [range] (years)	65 [25-85]	65 [32–84]	63 [36–87]
Disease histology:			
Adenocarcinoma	143	125	62
Other	39	32	4
Disease status:			
Stage III	22	2	0
Stage IV	127	119	50
Recurrence after surgery	17	22	11
Recurrence after definitive chemoradiotherapy	16	14	5
Smoking history:			
Never	68	47	40
Former	59	72	22
Current	55	38	2
Unknown	0	0	2
Tissue SOC genotyping result:			
EGFR/ALK/*ROS1* wt	130	123	0
EGFR mutation	22	7	64
ALK mutation	3	2	1
ROS1 mutation	1	6	1
Not done	26	19	0

Abbreviations: *ALK*, anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; *ROS1*, c-ros oncogene1; SOC, standard of care; TKI, tyrosine kinase inhibitor; wt, wild-type.

A summary of the mutations found by cfDNA and/or tissue SOC is shown in Fig. 2. The most frequent mutations detected were common EGFR mutations (17.3%), followed by KRAS mutations (9.9%) including KRAS G12C (2.7%). An Oncoplot showing mutation profiles of all samples analyzed with cfDNA NGS ($N = 405$) is shown in Fig. 3. Mutations were classified depending on the OncoKB -defined tier of supporting evidence from Tier 1 (highest) to Tier 4 (lowest) as per Supplementary Table 1 [22]. Over half (52.8%) of all samples had mutations categorized as OncoKB Tiers I-IV: 25.4% were Tier 1, 2% were Tier 2, 8.6% were Tier 3 and 14.8% were Tier 4, and 13% of samples had no mutations detected. Concordance analyses between cfDNA NGS and tissue SOC methods for common EGFR mutations and ALK / ROS1 fusions is shown in Table 2. These analyses only compare concurrently collected tissue samples ($n = 221$). Overall, the concordance between the two methods was 96.9% and was consistent for the four groups of mutations analysed: concordance ranged from 94.7% for EGFR T790M mutations to 98.8% for ALK / ROS1 fusions. Overall sensitivity and specificity, comparing cfDNA to SOC tissue testing, were 77.8% and 98.3%, respectively.

In a subset of 64 patients whose tissue NGS results were available, a comparison with cfDNA is shown in Table 3 for EGFR mutations or ALK / ROS1 fusions; overall concordance was 98.4% and sensitivity and specificity were 100.0% and 98.3%, respectively. Concordance analyses between cfDNA and tissue NGS ($n = 64$) for genomic alterations are shown in Supplementary Table 2. Concordance between methods for Tiers 1, 2, 3 and 4 genomic alterations was 91.8%, 91.8%, 90.2% and 73.8%, respectively, and the overall concordance for these groups was 86.9%. High tier concordance rates were found for Tier 1 alterations, both mutations (95.1%) and Tier gene rearrangements (96.7%). The proportion of the various tier s of mutation was similar in Groups A and B while Group C had a higher proportion of Tier 1 and 2 mutations as all these patients were known to have actionable mutations (data not shown).

Compared with SOC tissue test results, cfDNA testing identified actionable mutations in 13 patients missed by tissue SOC testing. Mutation identification enabled initiation of targeted therapy which, produced a partial response or stable disease (Table 4). The clinical benefit of cfDNA NGS is illustrated by a patient who was originally diagnosed (February 2018) with Stage IIIA lung adenocarcinoma. SOC tissue testing did not detect any clinically informative mutations. The patient was treated with a combination of pemetrexed, cisplatin, and radiotherapy with curative intent. Two months later, radiographic disease progression was detected. cfDNA NGS identified an EGFR T725M exon 18 mutation with allelic frequency 0.8%. The patient was treated with erlotinib with stable disease for 7 months before subsequent disease progression.

4. Discussion

In this real-world study, we compared the performance of a cell-free NGS assay with physician-choice tissue-based SOC testing. This study of 405 Korean patients with aNSCLC is one of the largest real-world analyses of cfDNA NGS published. Compared with treatment naïve patients (Group A), a lower proportion of patients in Group B and C underwent tissue biopsy at progression This reflects routine clinical practice in Korea due to potential convenience offered by liquid biopsy to avoid a repeat biopsy at progression.

Among all patients the most frequent alterations detected in cfDNA and/or tissue SOC were common EGFR mutations (17.3%), followed by KRAS mutations (9.9%) including KRAS G12C (2.7%). BRAF mutations were identified in three patients (1.4%), two with the V600E BRAF and one with a non-V600 SNV (single nucleotide variant) mutation. The

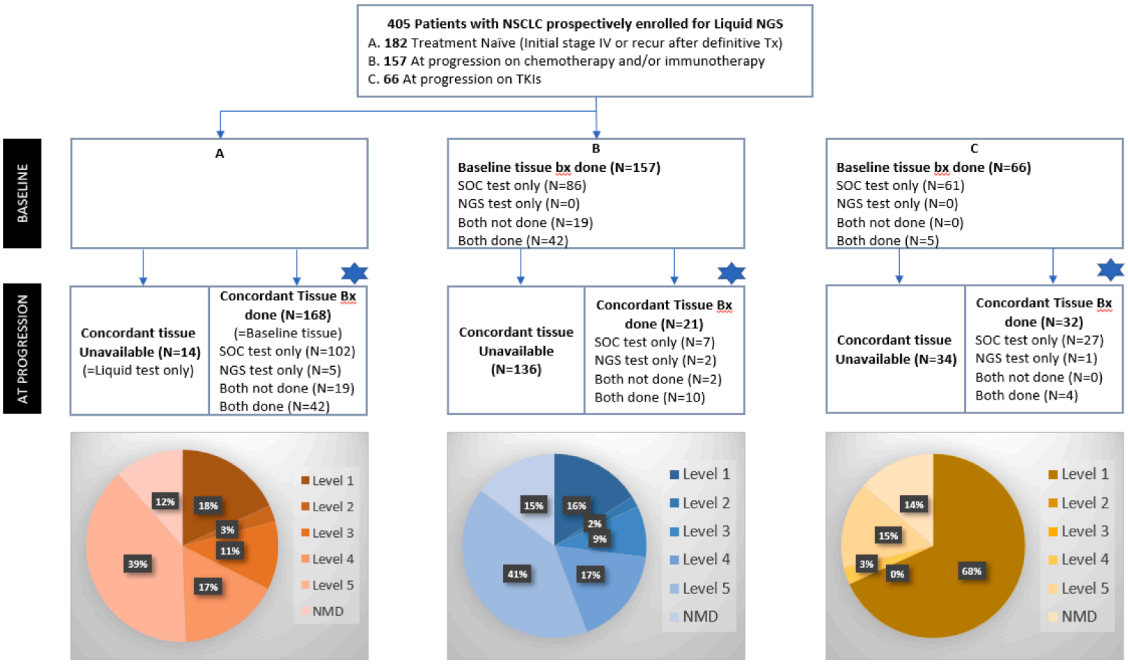

Fig. 1. Patient enrolment and testing flowchart. bx, biopsy; NGS, next generation sequencing; SOC, standard of care; TKI, tyrosine kinase inhibitor sample timepoint for concordance analysis.

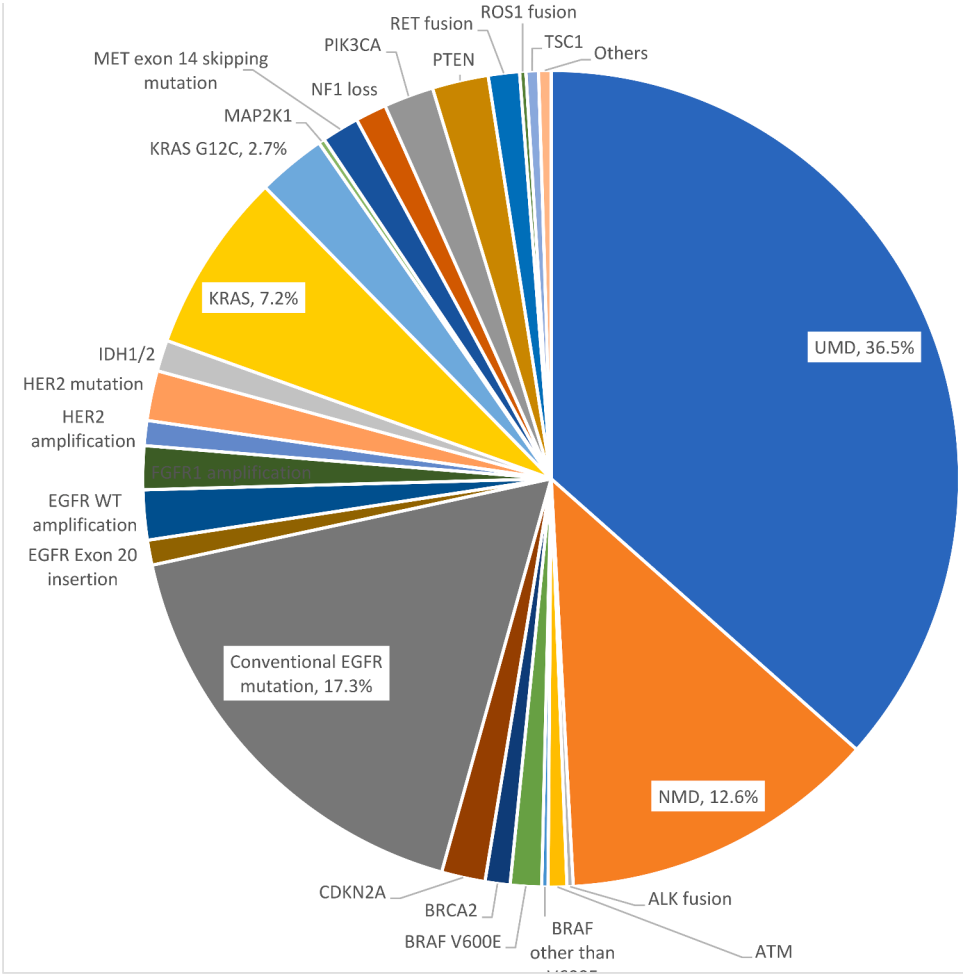

Fig. 2. Genomic alterations identified in cfDNA in 405 patients. ALK, ALK receptor tyrosine kinase; ATM, ATM serine/threonine kinase; BRCA2, BRCA2 DNA repair associated; BRAF, B-Raf proto-oncogene; CDKN2A, cyclin dependent kinase inhibitor 2A; EGFR, epidermal growth factor receptor; FGFR1, fibroblast growth factor receptor 1; HER2, human epidermal growth factor receptor 2; IDH1/2, isocitrate dehydrogenase 1/2; KRAS, KRAS proto-oncogene; MAP2K1, mitogen-activated protein kinase kinase 1; MET, MET proto-oncogene; NF1, Neurofibromatosis type 1; NMD, no mutation detected; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; RET, ret proto-oncogene; ROS1, ROS proto-oncogene 1; TSC1, TSC complex subunit 1; UMD, unknown mitogenic driver mutation.

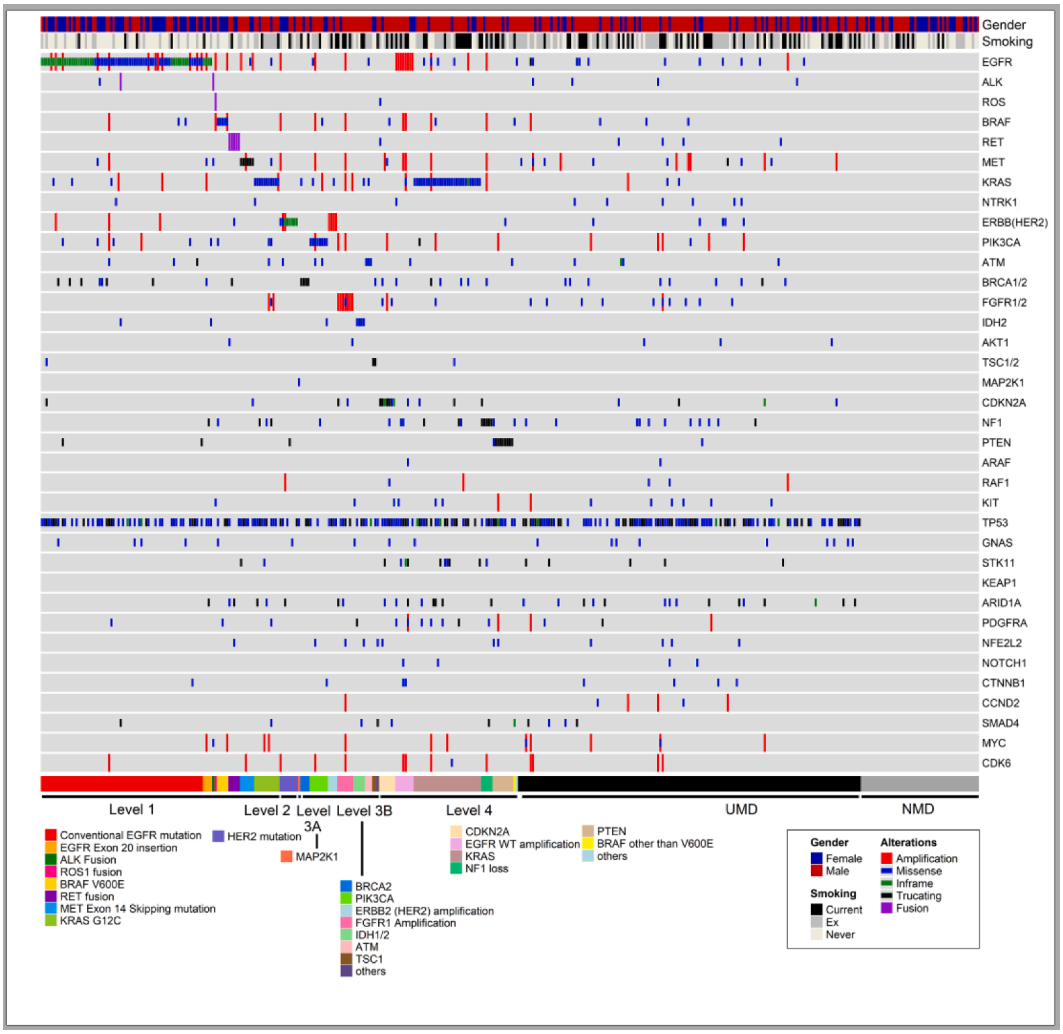

Fig. 3. Oncoplot depicting mutation profile of 405 patients in this study using cfDNA NGS. AKT1, AKT serine/threonine kinase 1; ALK, ALK receptor tyrosine kinase; ARAF, A-Raf proto-oncogene, serine/threonine kinase; ARID1A, AT-rich interaction domain 1A; ATM, ATM serine/threonine kinase; BRCA1, BRCA1 DNA repair associated; BRCA2, BRCA2 DNA repair associated; BRAF, B-Raf proto-oncogene; CDK6, cyclin dependent kinase 6; CDKN2A, cyclin dependent kinase inhibitor 2A; CTNNB1, catenin beta 1; EGFR, epidermal growth factor receptor; ERBB2, erb-b2 receptor tyrosine kinase 2; FGFR1, fibroblast growth factor receptor 1; FGFR2, fibroblast growth factor receptor 2; GNAS, GNAS complex locus; IDH2, isocitrate dehydrogenase 2; KEAP1, kelch like ECH associated protein 1; KIT, KIT proto-oncogene, receptor tyrosine kinase; KRAS, KRAS proto-oncogene; MAP2K1, mitogen-activated protein kinase kinase 1; MET, MET proto-oncogene; MYC, MYC proto-oncogene, bHLH transcription factor; NFE2L2, NFE2 like bZIP transcription factor 2; NMD, no mutation detected; NOTCH1, notch receptor 1; NTRK1, neurotrophic receptor tyrosine kinase 1; NTRK2, neurotrophic receptor tyrosine kinase 2; NTRK3, neurotrophic receptor tyrosine kinase 3; NF1, neurofibromin 1; PDGFRA, platelet derived growth factor receptor alpha; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3- kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; RAF1, Raf-1 proto-oncogene, serine/threonine kinase; RET, ret proto-oncogene; ROS1, ROS proto-oncogene 1; SMAD4, SMAD family member 4; STK11, serine/threonine kinase 11; TP53, tumor protein p53; TSC1, TSC complex subunit 1; TSC2, TSC complex subunit 2; UMD, unknown mitogenic driver mutation.

prevalence of EGFR mutations in newly diagnosed non-squamous aNSCLC patients in Asia is expected to be around 50% [23]; however, we found a prevalence of only 17.3% by cfDNA NGS. A possible explanation for the lower EGFR mutation prevalence in this cohort is that most patients were evaluated elsewhere using SOC testing for common actionable mutations (EGFR and ALK), and were only referred to this tertiary center for further evaluation when negative. The relatively high proportion of unknown mitogenic driver mutations (UMD) detected by cfDNA NGS (in over a third of patients), is a reflection of clinicians using cfDNA NGS during a later line of therapy, mostly in the absence of informative tissue SOC results.

In some patients with disease progression, repeat tissue biopsy is not an option because of the patient's condition and limited resources. In such cases, a liquid biopsy provides a less invasive approach. Here, we demonstrate that the performance of a specific cfDNA NGS assay is similar to tissue testing, which should assure clinicians when considering this approach as an alternative. The performance of the cfDNA NGS assay used in our study in aNSCLC patients with insufficient tumor samples has been demonstrated in other studies. For example, in a prospective study of patients with advanced lung adenocarcinomas, 13% of patients without tumor tissue results received matched therapies based on their cfDNA results alone [24].

In our study, concordance between cfDNA NGS and tissue SOC methods for common EGFR mutations and ALK / ROS1 fusions in concurrently collected tissue samples was high (96.9%), Similarly, high concordance of liquid and tissue biopsy has been shown in prospective randomised studies of patients with previously untreated advanced non-squamous NSCLC [16,17].

With the increasing number mutations that can be targeted with effective therapy, it becomes critical for clinicians to identify and fully evaluate actionable genomic alterations prior to initial therapy. This could be performed through serial testing of multiple genes or by

Table 2
Concordance analysis between cell-free DNA next generation sequencing (NGS) and tissue standard of care (SOC) for common *EGFR* mutations and *ALK / ROS1* fusions ($n = 221$).

	Tissue SOC+	Tissue SOC–	Tissue not assessed	Total	Concordance (%)	PPA (%)	NPA (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
		EGFR Exon 19del							
cfDNA+	13	1	1	15	97.06	76.47	99.35	92.86	97.44
cfDNA–	4	152	26	182					
cfDNA undetected	3	19	2	24					
Total	20	172	29	221					
		EGFR L858R (exon 21)							
cfDNA+	19	2	0	21	97.06	86.36	98.65	90.48	97.99
cfDNA–	3	146	27	176					
cfDNA undetected	3	19	2	24					
Total	25	167	29	221					
		EGFR T790M (exon 20)							
cfDNA+	2	7	1	10	94.71	50.00	95.78	22.22	98.76
cfDNA–	2	159	26	187					
cfDNA undetected	0	22	2	24					
Total	4	188	29	221					
		ALK / ROS1 fusion							
cfDNA+	1	1	0	2	98.82	50.00	99.40	50.00	99.40
cfDNA–	1	167	27	195					
cfDNA undetected	2	20	2	24					
Total	4	188	29	221					

ALK,ALK receptor tyrosine kinase; *EGFR*, epidermal growth factor receptor; *ROS1*, ROS proto-oncogene 1.

Table 3
Concordance analysis between cell-free DNA next generation sequencing (NGS) and tissue NGS for common *EGFR* mutations and *ALK / ROS1* fusions ($n = 64$). This is a subset of patients from Table 1 who have tissue NGS results available.

	Tissue SOC+	Tissue SOC–	Total	Concordance (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
	EGFR Exon 19del							
cfDNA+	2	0	2	100.0	100.0	100.0	100.0	100.0
cfDNA–	0	59	59					
cfDNA undetected	0	3	3					
Total	2	62	64					
	EGFR L858R (exon 21)							
cfDNA+	2	0	2	100.0	100.0	100.0	100.0	100.0
cfDNA–	0	59	59					
cfDNA undetected	0	3	3					
Total	2	62	64					
	EGFR T790M (exon 20)							
cfDNA+	1	1	2	98.36	100.0	98.33	50.00	100.0
cfDNA–	0	59	59					
cfDNA undetected	0	3	3					
Total	1	63	64					
	ALK / ROS1 fusion							
cfDNA+	0	0	0	98.36	0.0	100.0	0.0	98.36
cfDNA–	1	60	61					
cfDNA undetected	0	3	3					
Total	1	63	64					

ALK, ALK receptor tyrosine kinase; EGFR, epidermal growth factor receptor; NPA, negative percent agreement; PPA, positive percent agreement; ROS1, ROS proto-oncogene.

comprehensive genomic profiling using either tumor tissue or plasma. Even after the failure of tumor tissue testing, cfDNA NGS results identified 13 aNSCLC patients with disease progression and facilitated the utilization of matched therapy. Most mutations ($n = 8$) were in the EGFR gene, and other genomic alterations were RET or ROS1 gene fusions, and one ERBB2 mutation.

Although repeat tissue biopsy at progression is an option in some patients, tissue biopsies may not be feasible in all the patients and may not represent the complete global picture of the current mutation profile of the tumor due to heterogeneity. Liquid biopsy is a convenient alternative to invasive tissue biopsy and provides results similar to those expected with tissue testing [25].

In our case study, a patient with disease recurrence was found to have EGFR T725M by cfDNA NGS, which was not detected by other means. Treatment with matched targeted therapy (erlotinib) provided meaningful clinical benefit. The EGFR T725M mutation is very rare but has been described [26–28]. This case emphasizes the importance of whole exome sequencing for EGFR, which can be performed with NGS

Table 4
Change of treatment in patients ($n = 13$) following cell-free DNA (cfDNA) test results.

Patient	Highest-tier actionable alteration	VAF (%)	Co-mutations	Line of therapy	Treatment	Treatment context	Best response
1	*EGFR* (T725M)	0.8	No	2	Erlotinib	Standard care	Stable disease
2	*EGFR* (exon 19 del)	1.0	Yes	2	Gefitinib	Standard care	Partial response
3	*ERBB2* (G660D)	6.2	Yes	2	Neratinib + Trastuzumab	Clinical trial	Stable disease
4	*RET* (*KIF5B-RET* fusion)	1.7	Yes	2	Pralsetinib (BLU-667)	Clinical trial	Stable disease
5	*EGFR* (L858R)	0.9	Yes	3	Gefitinib	Standard care	Partial response
6	*EGFR* (exon 20 ins)	0.09	No	3	Amivantamab (JNJ-61,186,372)	Clinical trial	Stable disease
7	*EGFR* (L858R)	1.2	Yes	2	Gefitinib	Standard care	Partial response
8	*RET* (*NCOA4-RET* fusion)	5.6	Yes	2	Selpercatinib (LOXO-292)	Clinical trial	Stable disease
9	*ROS1* (*CD74-ROS1* fusion)	10.1	Yes	3	Crizotinib	Standard care	Stable disease
10	*RET* (*KIF5B-RET* fusion)	0.4	No	3	Selpercatinib (LOXO-292)	Clinical trial	NE (referred to another hospital)
11	*EGFR* (T790M)	0.2	Yes	2	Osimertinib	Standard care	NE (lost to follow-up)
12	*EGFR* (T790M)	0.3	Yes	2	Osimertinib	Standard care	Stable disease
13	*EGFR* (T790M)	0.1	Yes	2	Osimertinib	Standard care	Partial response

but not with hotspot testing. Other studies have shown the diagnostic capability of ctDNA NGS. In the KLLIP study, plasma-based NGS testing detected genomic alterations in 11 patients (4.2%) that were missed by tissue NGS [29]. In the NILE prospective, multicenter North American study of patients with advanced non-squamous NSCLC ($n = 282$), 31.6% had an actionable NCCN - defined biomarker which were detected by tissue (21.3%) and/or cfDNA (27.3%) NGS. One of the additional benefits of ctDNA NGS is a faster turnaround time compared to tissue genotyping (9 vs. 15 days) [23,30]. Using the same cfDNA NGS in Asian patients a turnaround time of 7 days has been reported in real life patients.

Limitations of this study include its retrospective nature, and it represents the experience of a single tertiary care facility. Most of the patients are referred to Yonsei Cancer Center when initial biomarker testing was not informative and therefore the landscape of mutations reported in this study may not be representative of the overall Korean NSCLC patient population. In addition, tissue genomic testing was not standardized and was performed at the physician's discretion.

Advances in targeted therapies and immunotherapies are changing the treatment paradigm for patients with NSCLC. Personalized treatment based upon a full understanding of the underlying genomic aberrations, including the complex and heterogeneous mechanisms leading to resistance, will be key to future improvements [3,31]. NGS facilitates the detection of multiple genomic alterations simultaneously. In patients with metastatic NSCLC, this will support a precision-based approach to patient management aimed at targeting treatment to the tumor characteristics of the individual. Acquisition of tumor tissue is invasive, time-consuming and adds to costs; it is ideally performed in large specialty hospitals that can successfully perform biopsy procedures and conduct sophisticated testing. Therefore, plasma- based comprehensive genotyping assays may extend the availability of NGS testing to a wider population of patients with similar accuracy and a shorter turnaround time, and should provide more diagnostic information on which oncologists can base their treatment decisions.

5. Conclusions

In this study, ctDNA NGS test reported highly concordant results with tissue SOC and tissue NGS testing in aNSCLC in Korean NSCLC patients. For some patients, this approach identified actionable alterations that were missed by tissue SOC testing. These results add to the existing body of evidence supporting the incorporation of ctDNA NGS test in standard clinical practice.

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CRediT authorship contribution statement

Beung-Chul Ahn: Conceptualization, Methodology, Writing – review & editing. **Seoyoung Lee:** Methodology. **Jiyun Lee:** Methodology, Formal analysis. **Jii Bum Lee:** Conceptualization, Formal analysis. **Min Hee Hong:** Investigation. **Sun Min Lim:** Writing – review & editing. **Suyog Jain:** Writing – original draft, Writing – review & editing. **Steve Olsen:** Writing – original draft, Writing – review & editing. **Byoung Chul Cho:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

B-C.A. has served in a consulting role for AstraZeneca, Boehringer-Ingelheim, Roche, BMS, Yuhan, Pfizer, Eli Lilly, Guardant, HK Inno-N, Janssen, Takeda, MSD, Janssen; and has received research funding and/or an honorarium from AstraZeneca, Boehringer-Ingelheim, Roche, BMS, Yuhan, Pfizer, Eli Lilly, Guardant, HK Inno-N, Janssen, Takeda, MSD, Janssen. B.C.C. has received research funding and/or an honorarium from Novartis, Bayer, AstraZeneca, MOGAM Institute, Dong-A ST, Champions Oncology, Janssen, Yuhan, Ono, Dizal Pharma, MSD, AbbVie, Medpacto, GIInnovation, Eli Lilly, Blueprint Medicines and Guardant Health; served in a consulting role for Novartis, AstraZeneca, Boehringer- Ingelheim, Roche, BMS, Ono, Yuhan, Pfizer, Eli Lilly, Janssen, Takeda, MSD, Medpacto, Blueprint Medicines; and owns shares in TheraCanVac Inc., Gencurix Inc., Bridgebio therapeutics, KANAPH Therapeutic Inc. S.J. is an employee and owns shares in Guardant Health AMEA. S.O. is an employee of Guardant Health AMEA and owns shares in AstraZeneca and Guardant Health. All other authors have no relevant conflicts of interest to disclose. The final approval of the content of this manuscript was made by the academic authors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ctarc.2023.100715.

Appendix A. Supplementary data

Supplementary data to this article can be found online at:

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