의학박사 학위논문

비소세포폐암 환자에서 액체생검을 통한 EGFR 유전자변이 검출의 유용성 확인에 대한 연구

Feasibility of liquid biopsy in detection of epidermal growth factor receptor mutation status in patients with non-small cell lung cancer

울 산 대 학 교 대 학 원

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이 논문을 의학박사 학위 논문으로 제출함

2022 년 6 월

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감사의 글

부족함이 많은 저이지만 많은 분들의 도움으로 무사히 박사 과정을 마무리할 수 있었습니다. 이 글을 통해 감사의 인사를 드리고자 합니다.

먼저 이 연구의 방향을 제시해 주시고, 아낌없는 지도를 해 주신 최창민 교수님께 감사드립니다. 교수님께서는 학문에 대한 끝없는 열정으로 참된 연구자의 자세를 보여주셨을 뿐만 아니라 전반적인 박사 과정동안 큰 도움을 주셨고, 논문이 마무리될 때까지 세심한 조언을 아끼지 않으셨습니다. 이 글을 통해 다시 한번 진심으로 감사드립니다.

그리고 바쁘신 와중에도 논문 심사를 위해서 귀중한 시간을 내주시고 논문의 내용과 연구 전반에 대하여 조언을 해 주신 이재철 교수님, 장승훈 교수님, 김형렬 교수님, 김호철 교수님께 다시 한번 감사드립니다. 훌륭하신 교수님들을 본받아 항상 최선을 다하는 자세로 학문에 임하겠습니다.

한결 같은 전폭적인 지지와 저에 대한 믿음을 보여준, 세상에서 가장 사랑하는 저의 반려자 이정우에게 언제나 저를 믿어주고 격려해주며 채찍질해 준 것에 대해 감사하며 사랑한다고 전합니다. 언제나 제가 바른 길을 가도록 인도해주시는 아버지, 생각만으로도 늘 편안한 마음의 쉼터가 되어 주시는 어머니, 언제나 든든한 지원군, 버팀목이 되어주는 사랑하는 동생 소연이, 엄마의 뱃속에서 열심히 지지해준 저의 쌍둥이 딸들, 우리 가족들이 없었다면 박사 과정을 무사히 마치기 힘들었을 것입니다. 가족들의 건강을 기원하며 감사의 인사를 드립니다.

더불어 무사히 대학원 박사 과정에 진학하고 수료까지 할 수 있도록 도와주신 서울아산병원 호흡기내과 교수님들께도 감사의 인사를 드립니다.

지면으로 미처 언급하지 못했지만 저를 아끼고 격려해 주셨던 모든 분들께도 진심으로 감사드립니다. 박사 과정을 통하여 학문의 길로 한 발짝 더 들어선만큼, 앞으로 더욱 정진하고 매진하겠습니다.

Abstract

Background: Liquid biopsy refers to assays capable of sampling, isolating and testing analytes from a biological fluid which is minimally invasive, reproducible, and cost-saving. Clinical application of liquid biopsy in patients with advanced non-small cell lung cancer (NSCLC) mainly focuses on detecting sensitizing epidermal growth factor receptor (EGFR) mutations. We aimed to evaluate the feasibility of liquid biopsy technique using circulating tumor cell (CTC) or circulating tumor DNA (ctDNA) from blood of patients with NSCLC in detection of EGFR sensitizing mutations, as well as the detectability of sensitizing EGFR mutation in plasma using next-generation sequencing (NGS) and promer-based technique.

Methods: Two independent prospective cohort study was conducted. Firstly, patients who were diagnosed as advanced NSCLC by tissue biopsy and agreed to conduct blood sampling were prospectively enrolled. Medical records were reviewed and EGFR status from tissue sample, blood CTC, and ctDNA was analyzed to compare diagnostic performance of CTC and ctDNA. The other cohort study enrolled NSCLC patients benefited from previous EGFR-tyrosin kinase inhibitor treatment followed by treatment failure. Re-biopsy of tissue or plasma sampling was conducted. EGFR mutation was detected by extracting ctDNA from plasma, using both PANA mutyper and promer-based EGFR assay. Diagnostic performance of promer-based assay compared to PANA mutyper was evaluated, as well as further detectability of C797S mutation from plasma of patient who underwent at least 3 months of osimertinib treatment. Objective response rate (ORR) with osimertinib treatment was also evaluated.

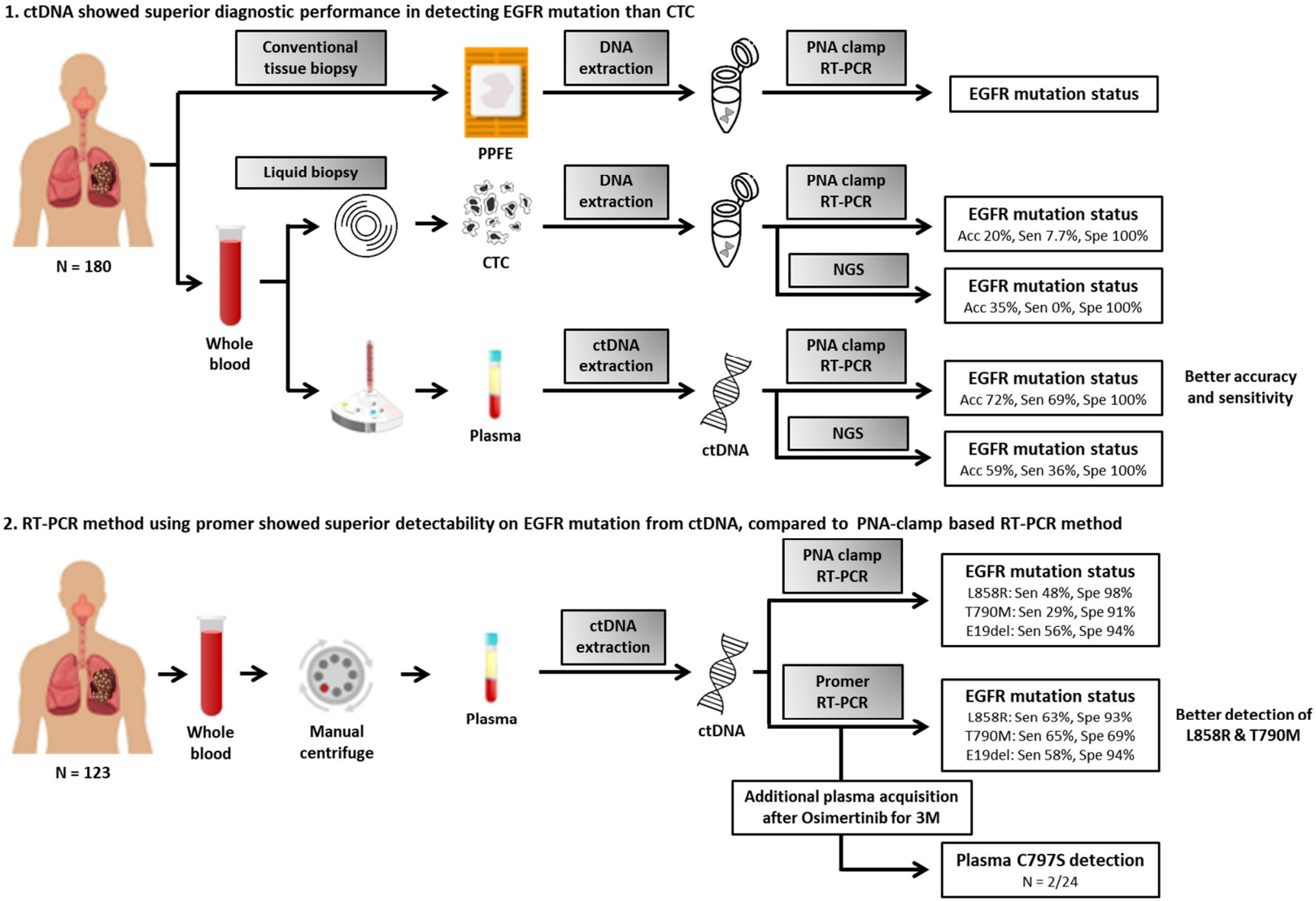
Results: In the first cohort study, 180 patients were enrolled between November 2019 and February 2022. Real-time polymerase chain reaction (RT-PCR) method using ctDNA detected EGFR mutation with superior accuracy (72.0% *vs.* 20.0%) and sensitivity (68.8% *vs.* 7.7%), compared to CTC (p-value < 0.01). NGS method using ctDNA detected EGFR mutation with 59.4% accuracy, 36% sensitivity, and 100% specificity. The other cohort study enrolled 123 NSCLC patients between January 2018 and December 2019. Median age was 63 years and 52 (42.3%) were male. 80 patients received osimertinib treatment and ORR was 66.3%. Promer-based EGFR assay showed superior sensitivity and specificity compared to PANA Mutyper in

detection of L858R and T790M mutation. In addition, promer-based EGFR assay detected C797S mutation in plasma of two out of twenty-four patients who were treated over 3-months of osimertinib treatment.

Conclusion: EGFR detection rate using plasma ctDNA was higher to blood CTC. RT-PCR method using promer showed superior detectability on EGFR mutation from ctDNA compared to PANA Mutyper, with possibility to detect further C797S mutation in patients treated with osimertinib, demonstrating clinical utility in predicting resistance to osimertinib.

Keywords: Non-small cell lung carcinoma, Liquid biopsies, T790M, C797S, osimertinib

Graphical abstract



Contents

Tables

Figures

Introduction

Recent evolutions in molecular targeted therapies, especially the use of epidermal growth factor receptor (EGFR) tyrosin kinase inhibitors (TKIs) have conferred significant clinical benefits in patient with advanced *EGFR* mutant non-small cell lung cancer (NSCLC)[1, 2]. In this context, *EGFR* mutations are the most commonly and routinely assessed molecular test for predicting whether a patient will benefit from *EGFR*-TKI therapy[3]. Clinical diagnosis of NSCLC harboring sensitizing EGFR mutations typically requires repeated solid biopsy which usually accompany discomfort and risk of procedure-related complications and may not always supply enough tumor tissues for genetic profiling[4-7]. Liquid biopsies, which are less invasive, more convenient, and easily repeatable therefore have emerged and became the alternative tests[8, 9].

Liquid biopsy refers to the analysis of biologic elements that are isolated from various body fluids[4]. Circulating tumor DNA (ctDNA) extracted from plasma is the most studied for characterization of tumor-associated genetic alterations[10, 11], since Mandel and Metais first reported its presence in the circulation[12]. ctDNA is highly fragmented, ranging from 130 to 170 base pairs[13] and rapidly disappears from the bloodstream, with a reported average half-life of 15 minutes[14]. To face the short half-life of ctDNA and the detection of low-frequency mutations against a high background level of wild-type ctDNA fragments, highly sensitive analytical techniques as well as standardized procedures are needed[15]. Commercial cell stabilizer tubes to prevent white blood cells degradation[16] and immediate cooling at 4°C and then storage in frozen conditions are strictly required[17]. QIAamp Circulating Nucleic-Acid kit (Quiagen, Antwerp, Belgium) and Maxwell RSC ccfDNA plasma kit (Promega, Leiden, the Netherlands) are commonly used cfDNA isolation kits[18].

Circulating tumor cells (CTCs), tumor cells that fall fall off from primary or metastatic lesions and enter the peripheral blood, are another biological analyte detected by liquid biopsy[19]. CTCs are distinguished from other blood cells by their positive expression of epithelial cell adhesion molecules (EpCAM) and cytokeratins (CK) and negative expression of CD45[20]. Since CTCs undergo epithelial-to-mesenchymal transition (EMT) or

mesenchymal-to epithelial transition (MET) during extravasation, they have heterogenous biomarker expression[21]. CTCs contain various cellular and subcellular components including DNA, RNA, and proteomic biomarkers that can be used for further downstream analysis[19]. However, CTCs are extremely rare in comparison to other types of blood cells (1 - 10 CTCs/10^6 blood cells in 1 mL of blood)[22] and are heterogeneous, casting a great challenge to be isolated with high sensitivity and specificity. CellSearch system, a positive enrichment product using immunomagnetic bead method, is widely used to enrich CTCs[23]. Microfluidic chips, based on immunoadsorption method is under various investigations and known to be fast and high-throughput method[24].

Once ctDNA or CTCDNA is extracted and concentrated from plasma or CTC, various assays can be applied to detect *EGFR* mutations including cobas real time polymerase chain reaction (RT-PCR) based *EGFR* mutation test (Roche diagnostics)[9, 25], PANA Mutyper with peptide nucleic acid (PNA) clamping-assisted fluorescence melting curve analysis[26], droplet digital PCR (ddPCR)[27], and next generation sequencing (NGS)[28]. Recently, *EGFR* mutation detection method using promer, which is a newly designed hydrolysis probe that consists of primer group, cleavage group, and blocking group is developed and under evaluation for clinical utility.

To evaluate the clinical efficacy of various liquid biopsy methods in patients with NSCLC, we aimed to compare the diagnostic performance of ctDNA and CTC in detection of *EGFR* sensitizing mutations, as well as to investigate clinical availability of promer-based *EGFR* mutation detecting method in patients with advanced NSCLC.

Methods

Study population and sample collection

We established two independent prospective cohort study. 180 patients who were diagnosed as advanced NSCLC by tissue biopsy and agreed to conduct blood sampling between November 2019 and February 2022 were prospectively enrolled to compare ability to detect *EGFR* mutations of CTC and ctDNA. Medical records were reviewed and *EGFR* status from tissue sample, blood CTC, and ctDNA was analyzed to compare diagnostic performance. RT-PCR and NGS was used to determine EGFR status in liquid biopsy samples.

The other cohort study was constructed to evaluate clinical efficacy of promer-based *EGFR* detecting assay using ctDNA, compared to PANA Mutyper method. Among patients diagnosed and treated for NSCLC at Asan Medical Center between January 2018 and December 2019, we enrolled 123 patients who met following inclusion criteria: (1) patients who are aged ≥ 20 years and histologically or cytologically diagnosed as inoperable stage IIIB or IV NSCLC according to the 7th edition of the TNM staging system by the international association for the study of lung cancer, and patients who understand information about the trial and voluntarily agree to participate the trial; (2) patients with *EGFR* sensitizing mutation (E19Del, L858R, L861Q, G719X) positive, who had shown clinical benefits (complete responders [CR] or partial response [PR] and stable disease ≥ 6 months) from EGFR-TKIs and had developed progressive disease. Patients who received drugs targeting T790M mutations prior to enrolment, who have coexisting malignancies, severe or unstable medical conditions were excluded.

Each study protocol was approved by the Institutional Review Board of Asan Medical Center (IRB No. 2019-1169 and 2017-0295) and written informed consent was obtained from all patients.

Tissue and liquid biopsy sample preparation

Tissue sample including initial biopsy and re-biopsy samples were collected in a form of formalin-fixed, paraffin embedded (PPFE) sections of tumor tissues. Twenty millimiters of

blood sample was obtained in ethylenediaminetetraacetic acid (EDTA) bottle from subjects at the time of screening.

For isolation of CTCs from whole blood, we used lab-on-a-disc platform which allows rapid size-based isolation of CTCs with relatively high purity from whole blood with a fluid-assisted separation technology (FAST), termed "FAST disc" (ref: Anal. Chem. 2014, 86, 11349−11356.). Before the isolation of the CTCs, the surface of the disc was passivated with a 1% BSA solution, 1mL of PBS, and the washing buffer (need 30 minutes of incubation). After the surface passivation, 3 mL of whole blood was introduced to the disc without any sample pretreatment steps. The CTCs were isolated on the filter by spinning the disc, and then the filter was washed two times with 1 mL of PBS solution. The total filtration time was less than 1 minute[29].

For isolation of plasma before extracting ctDNA, we used two different methods: lab-on-a-disc platform using microfluidic device[30] and conventional manual centrifugation method. The lab-on-a-disc platform, termed as "CD-LBx2" conduct fully automated 3-step centrifugation of 10 mL of whole blood with added 5 mL of Ficoll solution, within 15 minutes. Manual centrifugation of whole blood was conducted by performing a 2000g centrifugation of 5 mL blood sample for 10 minutes and carefully removing the 1 mL of supernatant.

DNA extraction

DNA was extracted from five 5 μm PPFE sections of tumor tissues. The sections were deparaffinized in xylene and washed in ethanol prior to DNA extraction. Genomic DNA from deparaffinized tissue sample and CTCs was extracted using the QIAamp DNA blood kit (Qiagen). ctDNA was isolated from 3 - 4 mL of plasma with QIAamp Circulating Nucleic Acid Kit (Quiagen, Hilden, Germany) according to manufacturer's instructions. The quantity and quality of the cfDNA were assessed using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and 4150 TapeStation System (Agilent, Santa Clara, CA, USA), respectively.

Detection of EGFR mutations

We generally used PANA Mutyper R *EGFR* kit with PNA clamping-assisted fluorescence melting curve analysis (Panaegene, Daejeon, Korea) of *EGFR* mutation detection and genotyping of tissue or liquid biopsy samples[31]. The PNA clamp probe tightly binds only to wild-type DNA sequences and thus suppresses their amplification during PCR. The PNA probe specifically detects target mutant DNA and each mutation is then genotyped by melting peak analysis. As the probe is conjugated with a fluorescent dye and a quencher, the mutant DNA can be visualized[26].

For NGS library preparation, Total 10ng of ctDNA was needed using the customized cancer panel which allows the detection of mutations in 51 actionable genes. Library preparation was performed using Ion Ampliseq Library Kit 2.0 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction. We used the Ion Express Barcode Adaptors Kit (Thermo Fisher Scientific, Waltham, MA, USA) for the samples multiplexing and libraries were purified using Agencourt AMPure XP (Beckman Coulter, Danvers, MA, USA) reagent. The libraries were quantified using the Qubit 3.0 fluorometer and the 4150 TapeStation System. Template preparation for the libraries was performed using the Ion Chef Instrument (Thermo Fisher Scientific) with Ion 540 Chef Kit (Thermo Fisher Scientific). Multiplexed templates were subjected to sequencing on the Ion S5 XL system (Thermo Fisher Scientific). After library preparation, data analysis was performed using Torrent Suite software (5.8.0). Sequencing coverage analysis was performed using coverage Analysis (5.8.0.1) plugins and VCF files were generated using the variantCaller (5.8.0.19) plugins. Annotation for the variants was performed using the Ion Reporter (5.10.2.0) software. We defined SNVs according to the following criteria: 1) minimum number of total coverages $\geq$ 500, 2) Phred-scaled minimum average evidence per read $\geq$ 10, 3) minimum variant allele frequency (VAF) $\geq$ 1%.

Another *EGFR* mutation detection method using promer, referred as "PRIME *EGFR*" in this study, was used to assess *EGFR* mutation status from ctDNA samples. Promer is composed of DNA-RNA-DNA structure and performs primer and probe function simultaneously, unlike primer and probe system consisting only of DNA. RNase H enzyme, which degrades the RNA-DNA structure within promer, is added and only when the promer RNA site binds precisely to

the target gene. After the RNA-DNA structure is degradaded, amplification of target mutant gene begins. Therefore, only the target gene can be selectively amplified, so that it is possible to realize high performance with low cost without additional experiments with fewer components (**Figure 1**).

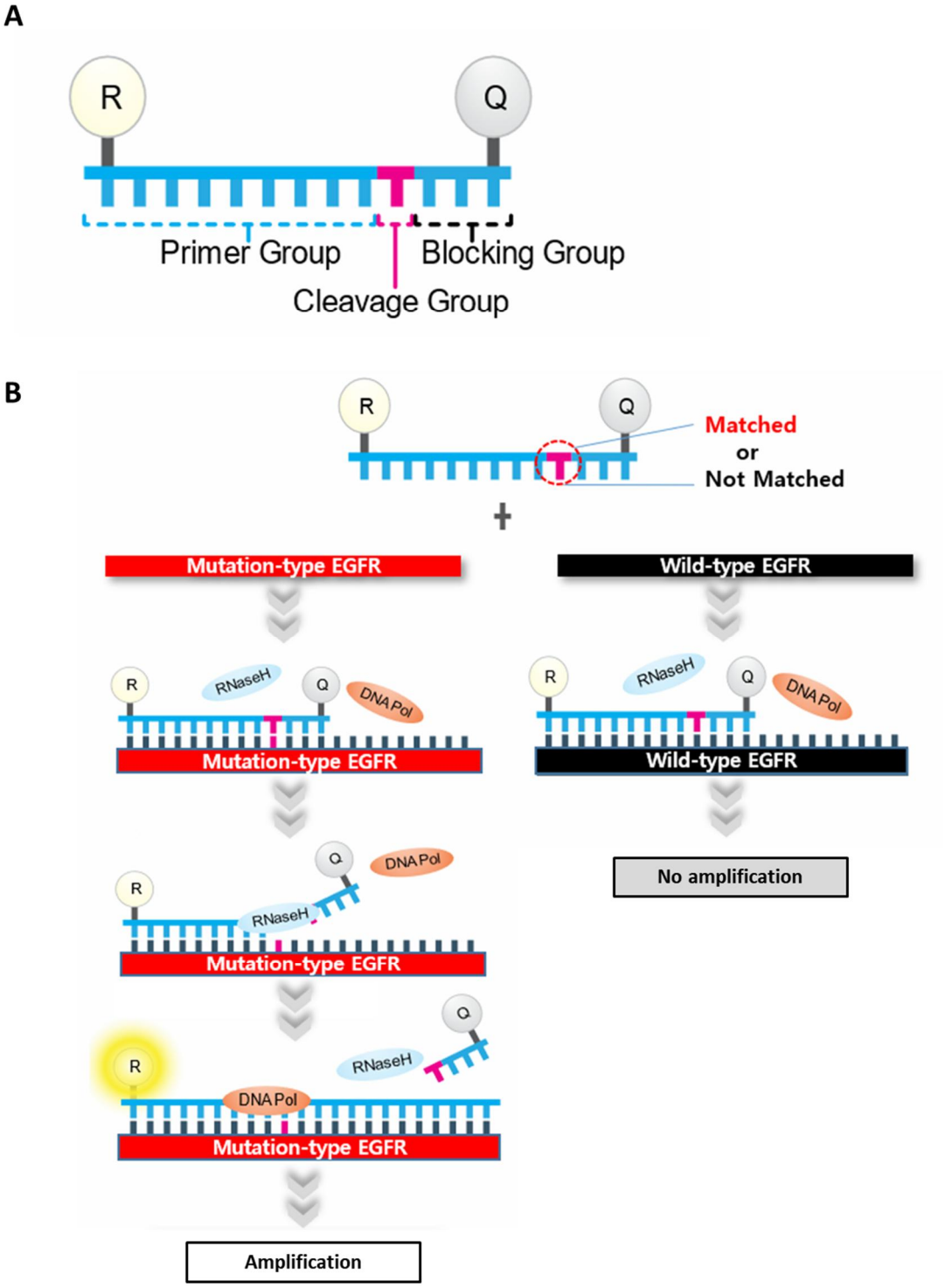


Figure 1. Scheme for promer-based EGFR mutation assay. (A) Structure of promer, (B) Mechanism of EGFR mutation assay by promer

Therapeutic methods

For patients who were candidate for the osimertinib treatment, osimertinib was administered as 80 mg once daily, and dose reduction to 40 mg once daily was permitted under physician's judgement based on individual safety and tolerability. A cycle of study treatment was defined as 28 days, day 1 of next cycle being day 29 of previous cycle, and the time window for each visit being ±7 days. Each cycle was scheduled as D29±7(cycle 2), D57±7(cycle 3), D85±7(cycle 4), D113±7(cycle 5) from cycle 1 day 1, and then every 8 weeks. Response evaluation was performed every 8 weeks (±7 days) from day 1 of first cycle. Each subject was recommended to continue the study drug until disease progression or manifestation of unacceptable toxicity.

Study variables and statistical analyses

Baseline demographic and clinical characteristics such as age, sex, performance status, EGFR mutation status, and the presence or absence of previous surgery or irradiation were extracted from each patient's medical record.

Objective response rate (ORR) was defined as the proportion of patients achieving a best clinical response to osimertinib of either CR or PR, as recorded in the patient's medical record, based on Response Evaluation Criteria in Solid Tumors ver. 1.1. Progression-free survival (PFS) was defined as the time (in months) from the first date of Osimertinib treatment until the date of objective disease progression or death, whichever comes first.

All clinical data are presented as mean ± standard deviation or median (interquartile range [IQR]) for continuous variables, and numbers (%) for categorical variables. Data categorized according to tissue *EGFR* availability were compared using the One-way ANOVA or the Kruskal-Wallis test (for continuous variables) and the χ^2 or the Fisher's exact test (for categorical variables). The diagnostic performance of each method for detecting *EGFR* mutations in blood samples was expressed in terms of the sensitivity, specificity, and accuracy, with the mutation status determined in tissue sample as the reference standard. $p< 0.05$ was considered statistically significant for all tests. All analyses were conducted using the IBM

SPSS ver. n 25.0 (IBM Corp., Armonk, NY) or the R statistical package ver. 3.5.3 (Institute for Statistics and Mathematics, Vienna, Austria; http://www.R-project.org).

Results

Clinical characteristics of study population from comparing EGFR detectability of CTC and ctDNA

In the cohort study evaluating diagnostic performance of CTC and ctDNA in detection of EGFR mutation status using RT-PCR or NGS, one hundred eighty patients diagnosed as NSCLC by tissue biopsy from November 20 to February 2022. While analyzing CTC DNA and ctDNA samples for EGFR mutation status, we came to figure out CTC DNA had significantly poor diagnostic performance in detecting EGFR mutation, with both RT-PCR and NGS test. Therefore, we decided to discontinue to isolate CTC and extracted only ctDNA from blood samples, and conducted NGS study to further evaluate diagnostic feasibility of NGS test using ctDNA samples (**Figure 2**). **Table 1** presents the clinical characteristics of the 180 study patients. The median age was 63 years, with a preponderance of women (57.2%). 117 patients (65.0%) were initially diagnosed as advanced stage NSCLC. Tissue EGFR was detected in 63.3% of patients and 37.7% of patients received EGFR-TKI. Osimertinib treatment after failure of 1st-line EGFR TKI was conducted to 28 (15.5%) subjects. 12 (6.7%) patients died during the follow-up.

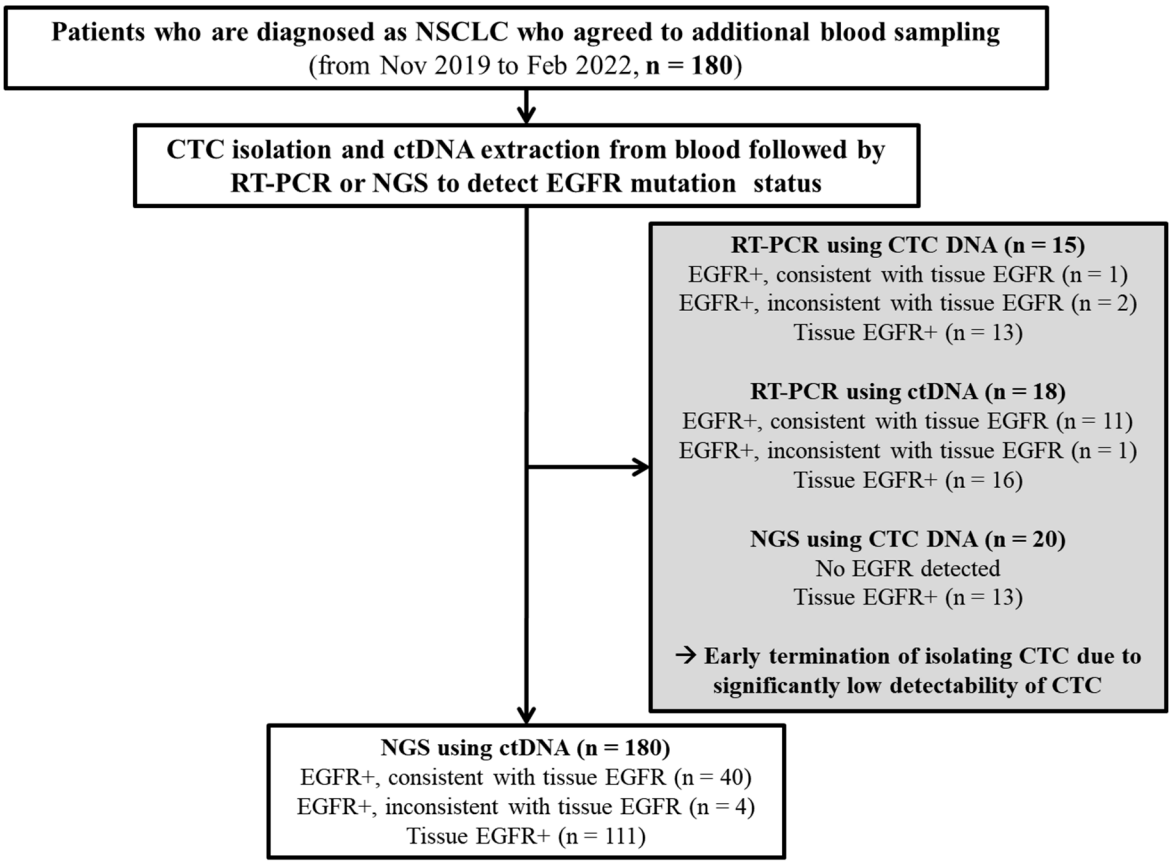


Figure 2. Study flow of evaluating diagnostic performance of CTC or ctDNA in detecting EGFR mutation using RT-PCR or NGS method.

NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; CTC, circulating tumor cell; ctDNA, circulating tumor deoxyribonucleic acid; RT-PCR, real-time polymerase chain reaction; NGS, next-generation sequencing

Table 1. Clinical characteristics of the NSCLC patients who underwent CTC-ctDNA comparison study

	Total (n = 180)
Age, yr, median (range)	63.0 (29-83)
Male gender, n (%)	77 (42.8%)
Smoking status	
Ever-smoker, n (%)	69 (38.3%)
Pack-years, median IQR	0 (0-15)
Pathology, n (%)	
Adenocarcinoma	178 (98.9%)
Squamous cell carcinoma	2 (1.1%)
Stage, n (%)	
I-II	55 (30.6%)
IIIA	12 (6.7%)
IIIB-IV	117 (65.0%)
Unknown	3 (1.7%)
Tissue mutation status, n (%)	
EGFR	114 (63.3%)
ALK	14 (7.7%)
ROS1	13 (7.2%)
Initial chemotherapy regimen	
Conventional chemotherapy, n (%)	38 (21.1%)
Afatinib, n (%)	28 (15.6%)
Erlotinib, n (%)	2 (1.1%)
Gefitinib, n (%)	37 (20.6%)
Osimertinib, n (%)	1 (0.6%)
Crizotinib, n (%)	11 (6.1%)
Immunotherapy (pembrolizumab combination), n (%)	1 (0.6%)
No chemotherapy, n (%)	62 (34.4%)
Follow-up duration months, median (IQR)	20.5 (16.8–23.9)
Patients who received osimertinib treatment, n (%)	29 (16.1%)
1st line	1
2nd line	19
3rd line	6
4th line	1
5th line	1
6th line	1
Death, n (%)	12 (6.7%)

Diagnostic performance of CTC and ctDNA for detection of EGFR mutation

We compared the diagnostic yields of CTC and ctDNA from blood for detecting *EGFR* mutations in 15 cases with matched CTC, ctDNA, and tissue samples. 2 cases tested *EGFR* negative (ALK positive instead). *EGFR* mutation test from liquid biopsy samples was conducted using two different methods; RT-PCR and NGS. Accuracy (73.3%), sensitivity (69.2%), and specificity (100.0%) for predicting tissue *EGFR* mutation using ctDNA with RT-PCR method was significantly higher compared to using CTC (20.0%, 9.1%, 50.0%, respectively). When using plasma NGS method to detect *EGFR* mutation from ctDNA and CTC, ctDNA showed better performance in detecting sensitizing mutations (accuracy 40.0% vs. 13.3%, sensitivity 30.8% vs. 0.0%) compared to CTC (**Table 2**). With this result, we decided early termination of isolating CTC due to significantly low detectability of CTC. **Table 3** presents final comparison of *EGFR* mutation detectability using RT-PCR or NGS technique with CTC or ctDNA samples. Plasma NGS using ctDNA samples showed accuracy of 59.4%, sensitivity of 36.0% (95%CI, 27.1 to 45.7), and sensitivity of 100% (95%CI, 15.8 to 100). Sensitivity to detect *EGFR* mutation was significantly higher using RT-PCR method than using NGS method with ctDNA samples.

Table 2. Result of EGFR mutation detected by RT-PCR or NGS technique using CTC or ctDNA only in patients who have matched samples

Patient		Tissue		Plasma RT-PCR		Plasma NGS	
No.	**Age/Sex**	**Pathology**	**EGFR**	**CTC**	**ctDNA**	**CTC**	**ctDNA**
1	55/F	Adenoca	Negative	Negative	Negative	Negative	Negative
2	69/F	Adenoca	E19del	Negative	E19del	Negative	Negative
3	66/F	Adenoca	E19del	Negative	E19del	Negative	Negative
4	63/M	Adenoca	L858R	Negative	L858R	Fail to seq	Negative
5	74/M	Adenoca	G719X	Negative	Negative	A859T	Negative
6	56/F	Adenoca	E20ins	Negative	E20ins, L858R	Negative	E20ins
7	39/M	Adenoca	E19del	Negative	E19del	Negative	E19del
8	64/M	Adenoca	E19del	Negative	Negative	Negative	Negative
9	66/F	Adenoca	L858R	Negative	Negative	Negative	Negative
10	57/M	Adenoca	L858R	E19del	Negative	Negative	Negative
11	67/M	Adenoca	L858R	Negative	L858R	Negative	L858R
12	57/F	Adenoca	E19del	E19del	E19del	Negative	E19del
13	65/F	Adenoca	E19del	Negative	L858R	Negative	Negative
14	41/F	Adenoca	Negative	Negative	Negative	Negative	Negative
15	65/F	Adenoca	E19del	L858R	E19del	Negative	Negative
Accuracy, n (%)		Reference: Tissue EGFR		3/15 (20.0)	11/15 (73.3)	2/15 (13.3)	6/15 (40.0)
Sensitivity (95%CI)				7.7 (0.2-36.0)	69.2 (38.6-90.9)	0.0 (0.0-24.7)	30.8 (9.1-61.4)
Specificity (95%CI)				100.0 (15.8-100.0)	100.0 (15.8-100.0)	100.0 (15.8-100.0)	100.0 (15.8-100.0)

EGFR, epidermal growth factor receptor; CTC, circulating tumor cell; ctDNA, circulating tumor deoxyribonucleic acid; EGFR mutation: E19del, (c.2235del15; p.E746_A750del); L858R, (c.2573T>G; p.Leu858Arg); E20ins, EGFR exon 20 insertion; A859T, (c.2575G>A; p.A859T)

Table 3. Comparison of EGFR mutation detectability using RT-PCR or NGS technique from CTC or ctDNA samples

	Plasma RT-PCR		Plasma NGS	
	CTC	**ctDNA**	**CTC**	**ctDNA**
Accuracy, n (%)	3/15 (20.0)	13/18 (72.2)	7/20 (35.0)	107/180 (59.4)
Sensitivity (95%CI)	7.7 (0.2-36.0)	68.8 (41.3-89.0)	0.0 (0.0-24.7)	36.0 (27.1-45.7)
Specificity (95%CI)	100.0 (15.8-100.0)	100.0 (15.8-100.0)	100.0 (15.8-100.0)	100.0 (15.8-100.0)
Positive predictive value (95%CI)	100.0	100.0	-	95.2 (83.3-98.8)
Negative predictive value (95%CI)	14.3 (12.5-16.3)	28.6 (16.2-45.3)	35.0 (35.0-35.0)	48.6 (44.9-52.2)
TP	1	11	0	40
TN	2	2	7	67
FP	0	0	0	2
FN	12	5	13	71

EGFR, epidermal growth factor receptor; RT-PCR, real-time polymerase chain reaction; NGS, next-generation sequencing; CTC, circulating tumor cell; ctDNA, circulating tumor deoxyribonucleic acid; TP, true positive; TN, true negative; FP, false positive; FN, false negative

Clinical characteristics of study population of promer-based EGFR analysis

In the cohort study evaluating clinical effectiveness of Promer-based *EGFR* analysis in detection of *EGFR* mutation status from ctDNA, one hundred twenty-four patients with acquired resistance after treatment with EGFR-TKIs sere screened for eligibility from January 2018 to December 2019. Excluding 1 patients who refused to participate, 123 patients were enrolled and proceeded to additional tissue biopsy and blood test. Tissue EGFR result was obtained in 87 patients and plasma samples were obtained in 123 patients (**Figure 3**). The median age was 63 years, with a preponderance of women (57.7%). Comparing into two groups according to the availability of tissue *EGFR* mutation result, there were no significant differences in age, ECOG PS, previous surgery or irradiation history, extrathoracic metastasis, plasma T790M positivity, follow-up durations, proportions of patients who received osimertinib, and ORR between the two groups. **Table 4** presents the clinical characteristics of the 123 study patients.

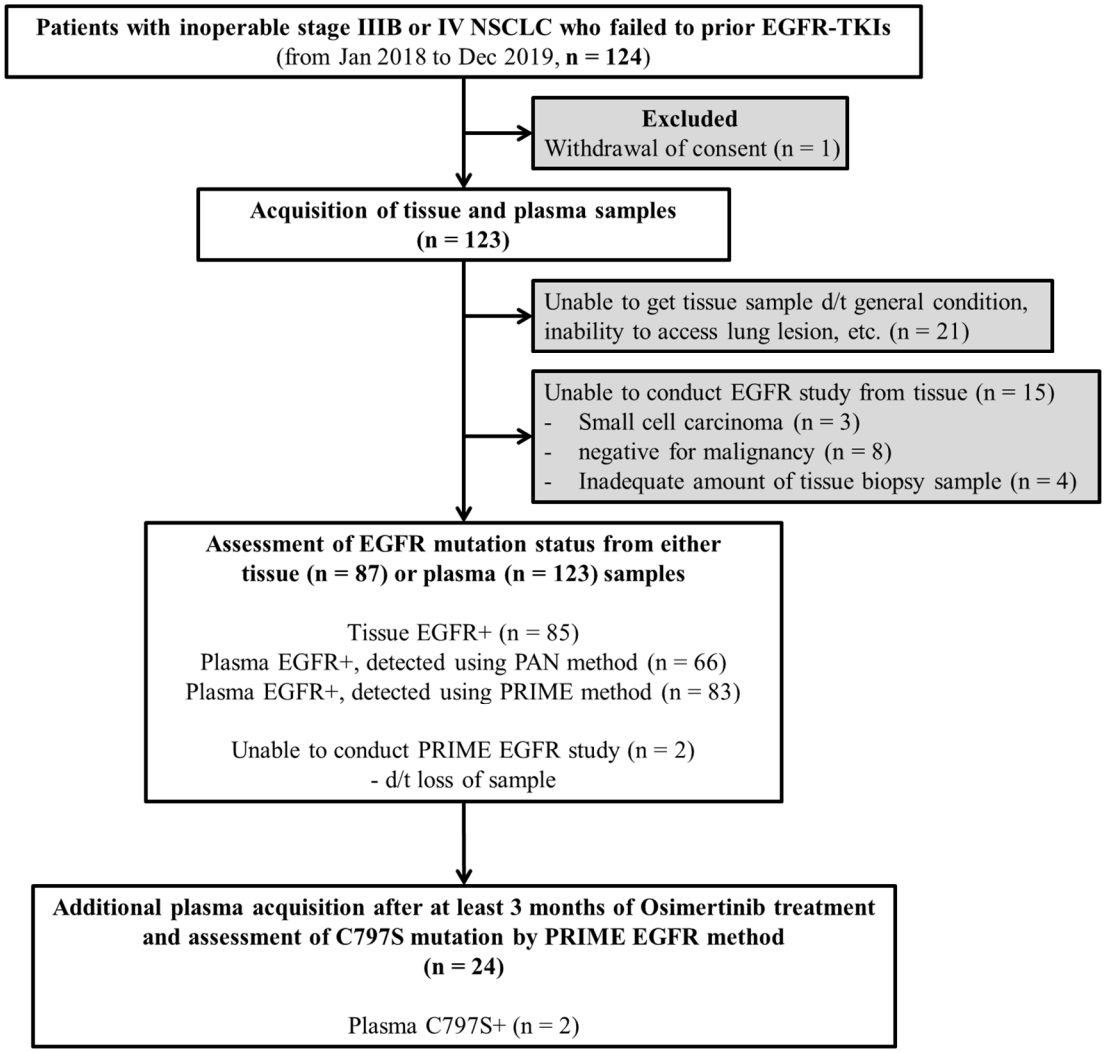


Figure 3. Study flow of study evaluating feasibility of PRIME EGFR method.

NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; PAN, PANAMutyper; PRIME, promer based EGFR detecting system; C797S; c.2389T>A; p.Cys797Ser

Table 4. Clinical characteristics of study population who underwent PRIME EGFR study

	Total (n = 123)
Age, yr, median (range)	63.1 (37.0-84.0)
Male gender, n (%)	52 (42.3%)
ECOG	
0-1	116 (94.3%)
2	7 (5.7%)
Previous surgery, n (%)	23 (18.7%)
Previous RTx, n (%)	41 (33.3%)
Extrathoracic metastasis	
Brain, n (%)	36 (29.3%)
Extrathoracic visceral metastases, n (%)	54 (43.9%)
T790M positivity, n (%)	
Tissue	48 (39.0%)
Plasma - detected by PANA Mutyper	25 (20.3%)
Plasma - detected by PRIME	58 (47.2%)
Follow-up duration months, median (IQR)	23.5 (11.8–28.3)
Patients who received osimertinib treatment, n (%)	80 (65.0%)
Objective response rate (%)	66.3

Table 5. Clinical characteristics of study population whose tissue EGFR status was unavailable in PRIME EGFR study

	Tissue EGFR unavailable (n = 36)	**Tissue EGFR measured (n = 87)**	**p-value**
Age, yr, median (range)	65.0 (48.0-81.0)	62.0 (37.0-84.0)	0.06
Male gender, n (%)	12 (33.3%)	40 (46.0%)	0.20
ECOG			1.00
0-1	34 (94.4%)	82 (94.3%)	
2	2 (5.6%)	5 (5.7%)	
Previous surgery, n (%)	7 (19.4%)	16 (18.4%)	1.00
Previous RTx, n (%)	12 (33.3%)	29 (33.3%)	1.00
Extrathoracic metastasis			
Brain, n (%)	11 (30.6%)	25 (28.7%)	0.83
Extrathoracic visceral metastases, n (%)	16 (44.4%)	38 (43.7%)	1.00
T790M positivity, n (%)			
Tissue	-	48 (55.2%)	
Plasma - detected by PANA Mutyper	8 (22.2%)	17 (19.5%)	0.81
Plasma - detected by PRIME	16 (44.4%)	42 (48.3%)	0.85
Reason for absence of EGFR mutation test in tissue sample			
Unable to conduct tissue biopsy	21 (58.3%)		
Inadequate amount of sample	4 (11.1%)		
Small cell carcinoma	3 (8.3%)		
No malignant cells	8 (22.2%)		
Follow-up duration months, median (IQR)	24.6 (11.8–27.0)	23.0 (18.8–28.3)	0.78
Patients who received osimertinib treatment, n (%)	23 (63.9%)	57 (65.5%)	1.00

Objective response rate (%)	69.5	64.9	0.62

Distribution of EGFR mutation results detected by PANA Mutyper or PRIME *EGFR* kit using tissue and plasma ctDNA samples are shown in **Table 6**. PRIME *EGFR* kit appeared to detect T790M mutation more frequently compared to PANA Mutyper. When grouping total subjects by tissue E19del, T790M, and L858R positivity, 40 patients had both E19del and T790M positivity with tissue *EGFR* test (**Figure 4A**), 47 patients had other types of EGFR mutation result (harboring only E19del or T790M or L858R positivity, both T790M and L858R positivity, or wild type) (**Figure 4B**), and 36 patients did not have available EGFR mutation results (**Figure 4C**).

Table 6. Distribution of EGFR mutations detected by PANA Mutyper or PRIME *EGFR* kit

	Tissue	**Plasma**	
		PAN	**PRIME**
Mutation			
Exon 18 G719X	-	1 (0.8)	-
Exon 18 G719X + Exon 20 T790M	1 (1.1)	-	-
Exon 18 G719X + Exon 21 L861Q	1 (1.1)	-	-
Exon 19 del	15 (17.2)	24 (19.5)	11 (8.9)
Exon 19 del + Exon 20 T790M	40 (46.0)	17 (13.8)	32 (26.1)
Exon 20 S768I	1 (1.1)	-	-
Exon 20 C797S		1 (0.8)	-
Exon 20 T790M	-	1 (0.8)	10 (8.1)
Exon 20 T790M + Exon 21 L858R	7 (8.0)	7 (5.7)	16 (13.0)
Exon 21 L858R	20 (23.0)	15 (12.2)	14 (11.3)
Wild type	2 (2.3)	57 (46.3)	38 (30.9)
Total	87	123	121
Not measured	36	0	2

Data are presented as n (%). EGFR, epidermal growth factor receptor; PAN, PANAMutyper; PRIME, promer based EGFR detecting system; EGFR mutation: T790M, (c.2369C>T; p.Thr790Met); E19del, (c.2235del15; p.E746_A750del); L858R, (c.2573 T>G; p.Leu858Arg)

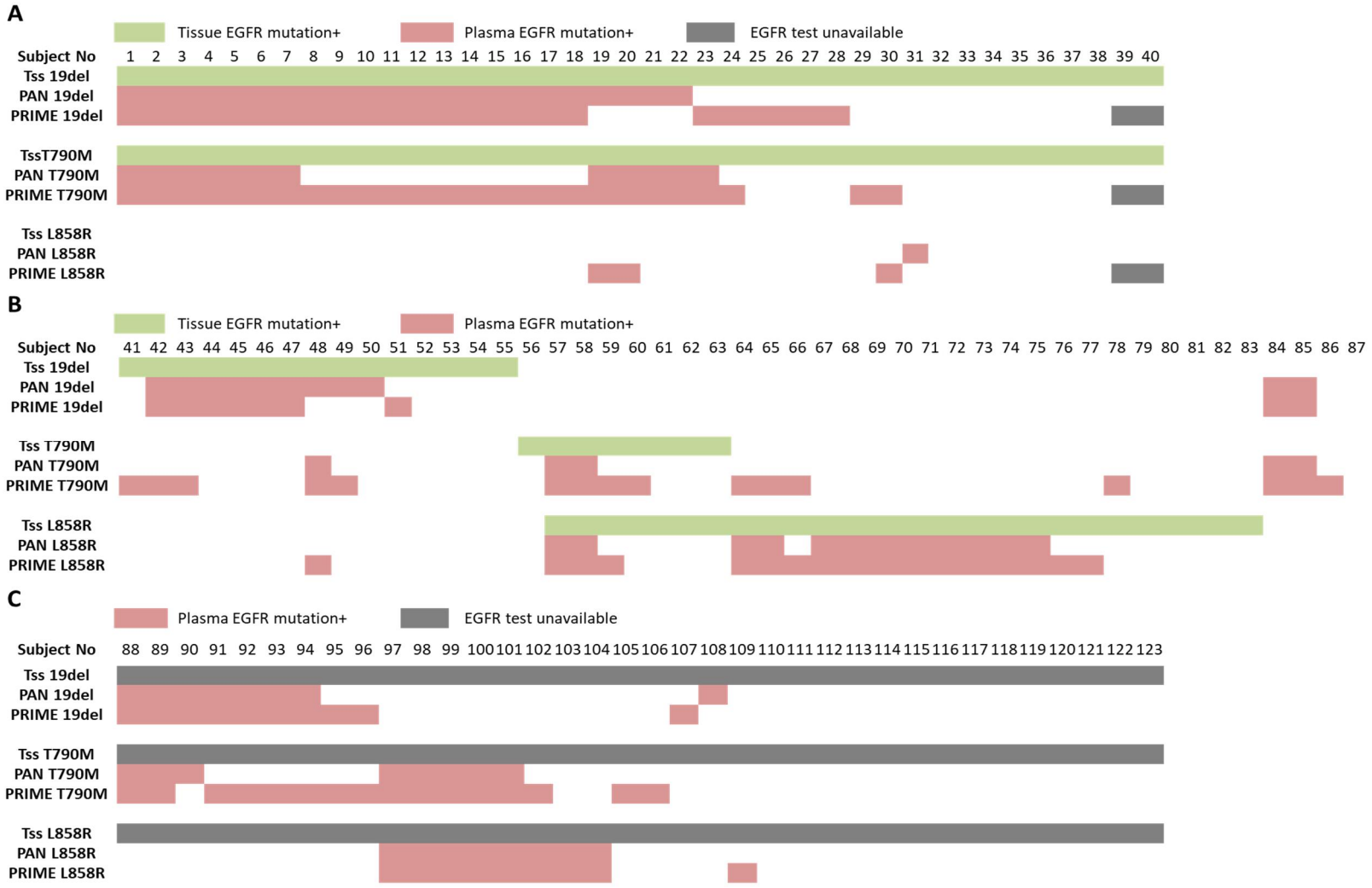

A
Tissue EGFR mutation+
Plasma EGFR mutation+
EGFR test unavailable
Subject No
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Tss 19del
PAN 19del
PRIME 19del
TssT790M
PAN T790M
PRIME T790M
Tss L858R
PAN L858R
PRIME L858R
B
Tissue EGFR mutation+
Plasma EGFR mutation+
Subject No
41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87
Tss 19del
PAN 19del
PRIME 19del
Tss T790M
PAN T790M
PRIME T790M
Tss L858R
PAN L858R
PRIME L858R
C
Plasma EGFR mutation+
EGFR test unavailable
Subject No
88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123
Tss 19del
PAN 19del
PRIME 19del
Tss T790M
PAN T790M
PRIME T790M
Tss L858R
PAN L858R
PRIME L858R

Figure 4. Diagram of *EGFR* mutation status in tissue or plasma samples

(A) Subjects who tested positive for both E19del and T790M in tissue sample, (B) Subjects who tested positive for other types of *EGFR* mutation in tissue sample, (C) Subjects with unavailable tissue *EGFR* mutation test

Concordance of PANAMutyper and PRIME EGFR in detection of each EGFR mutations

The degree of diagnostic concordance between PANA Mutyper and PRIME EGFR for detecting L858R, E19del, and T790M in plasma ctDNA is presented in **Table 7**. Concordant cases were those in which one diagnostic method detected a mutation, and the same, plus additional mutations were detected by the other. Discordance between the two diagnostic methods occurred in detection of L858R (p=0.021) and T790M (p<0.001) mutation.

Table 7. Concordance between PANA Mutyper and promer based EGFR kit in detection of each EGFR mutations

	PRIME					
	L858R	19del	T790M	Wild-type/invalid	K coefficient (95%CI)	McNemar's test P-value
PAN						
L858R	21			1	0.76 (0.67-0.82)	0.021
Wild-type/invalid	9			90		
19del		33		8	0.67 (0.56-0.76)	0.814
Wild-type/invalid		10		70		
T790M			24	1	0.41 (0.27-0.52)	<0.001
Wild-type/invalid			34	62		

EGFR, epidermal growth factor receptor; PAN, PANAMutyper; PRIME, promer EGFR detecting system; CI, confidence interval; EGFR mutation: T790M, (c.2369C>T; p.Thr790Met); E19del, (c.2235del15; p.E746_A750del); L858R, (c.2573 T>G; p.Leu858Arg)

Diagnostic performance of PRIME EGFR kit for detection of EGFR mutation in ctDNA

We compared the diagnostic yields of PANA Mutyper and PRIME EGFR for detecting *EGFR* mutations from ctDNA in 87 cases with adequate tissue samples. Sensitivity for predicting tissue L858R mutation using PRIME *EGFR* was 62.96% (95% confidence interval [CI], 42.37 to 80.60), significantly higher compared to PANA Mutyper (48.15% [95%CI, 28.67-68.05], p=0.021). Similar results were shown in detecting tissue T790M (65.22% [95%CI, 49.75 to 78.65] *vs*. 29.17% [95%CI, 16.95 to 44.06], p<0.001). Specificity, however, was lower in PRIME EGFR (69.23% [95%CI, 52.43 to 82.98]) for detecting T790M mutation than in PANA Mutyper (95.31 [95%CI, 79.13 to 98.39]). There was no significant difference in sensitivity or specificity for the diagnosis of E19del evaluated by PRIME EGFR and PANA Mutyper. Discordance between the tissue EGFR result and PANA Mutyper or PRIME EGFR did not occur in L858R detected with PRIME EGFR (p=0.180) and T790M detected with PRIME EGFR (p=0.571) (**Table 8**).

Table 8. Diagnostic performance of two methods in plasma with matched tissue for detecting *EGFR* mutation

		Mutation	Wild-type	Total	Sensitivity (95%CI)	Specificity (95%CI)	PPV(95%CI)	NPV(95%CI)	K coefficient (95%CI)
						Reference standard: Tissue			
L858R	**PAN**								P<0.001*
	Mutation	13	1	14	48.15	98.33	92.86	80.82	0.54
	Wild-type	14	59	73	(28.67-68.05)	(91.6-99.96)	(64.16-98.95)	(74.53-85.86)	(0.38-0.66)
	PRIME								P=0.180*
	Mutation	17	4	21	62.96	93.10	80.95	84.38	0.60
	Wild-type	10	54	64	(42.37-80.60)	(83.27-98.09)	(61.26-91.95)	(76.67-89.87)	(0.44-0.72)
T790M	**PAN**								P<0.001*
	Mutation	14	3	17	29.17	92.31	82.35	51.43	0.20
	Wild-type	34	36	70	(16.95-44.06)	(79.13-98.39)	(59.08-93.78)	(46.36-56.47)	(0.04-0.35)
	PRIME								P=0.571*
	Mutation	30	12	49	65.22	69.23	71.43	62.79	0.34
	Wild-type	16	27	37	(49.75-78.65)	(52.43-82.98)	(59.88-80.73)	(51.89-72.53)	(0.14-0.52)
E19del	**PAN**								P<0.001*
	Mutation	31	2	33	56.36	93.75	93.94	55.56	0.44
	Wild-type	24	30	54	(42.32-69.70)	(79.19-99.23)	(79.88-98.37)	(47.75-63.10)	(0.27-0.58)

PRIME								P<0.001*
Mutation	31	2	33	58.49	93.75	93.94	57.69	0.46
Wild-type	22	30	52	(44.13-71.86)	(79.19-99.23)	(79.90-98.37)	(49.46-65.52)	(0.30-0.60)

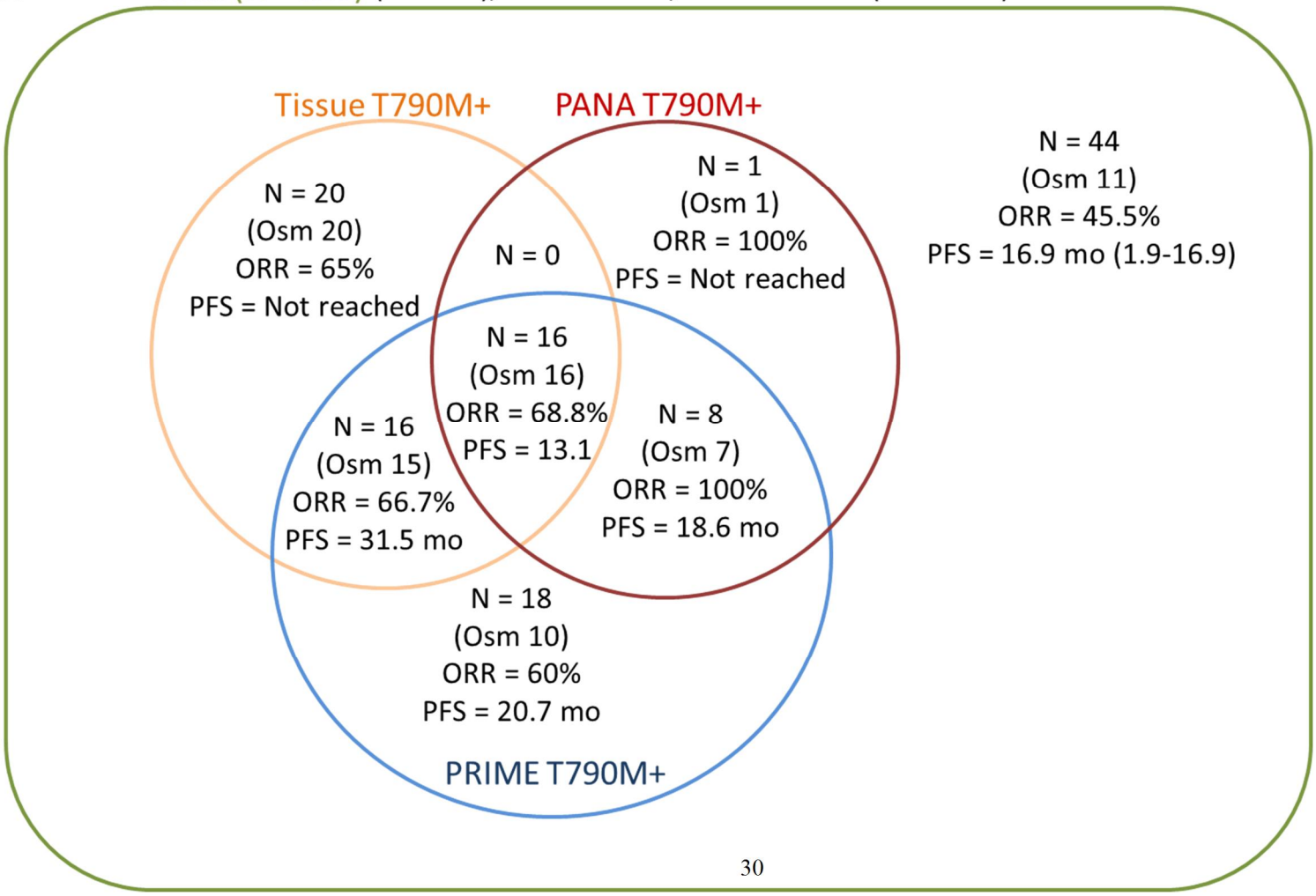
EGFR, epidermal growth factor receptor; PAN, PANAMutyper; PRIME, promer EGFR detecting system; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; EGFR mutation: T790M, (c.2369C>T; p.Thr790Met); E19del, (c.2235del15; p.E746_A750del); L858R, (c.2573 T>G; p.Leu858Arg)

*p-value of McNemar's test for concordance compared to tissue EGFR result was calculated.

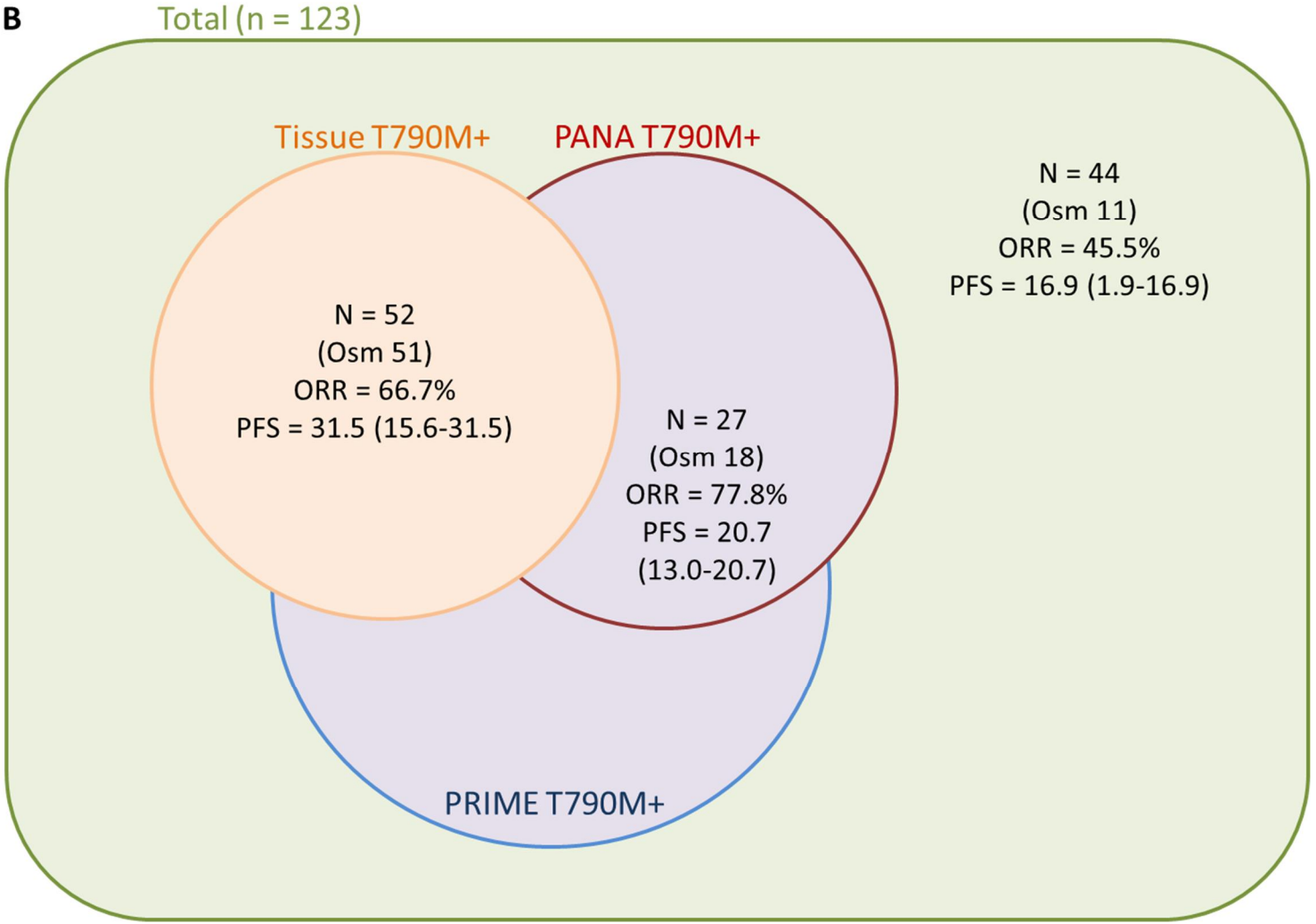
Clinical efficacy of osimertinib according to T790M positivity status in tissue or liquid biopsy

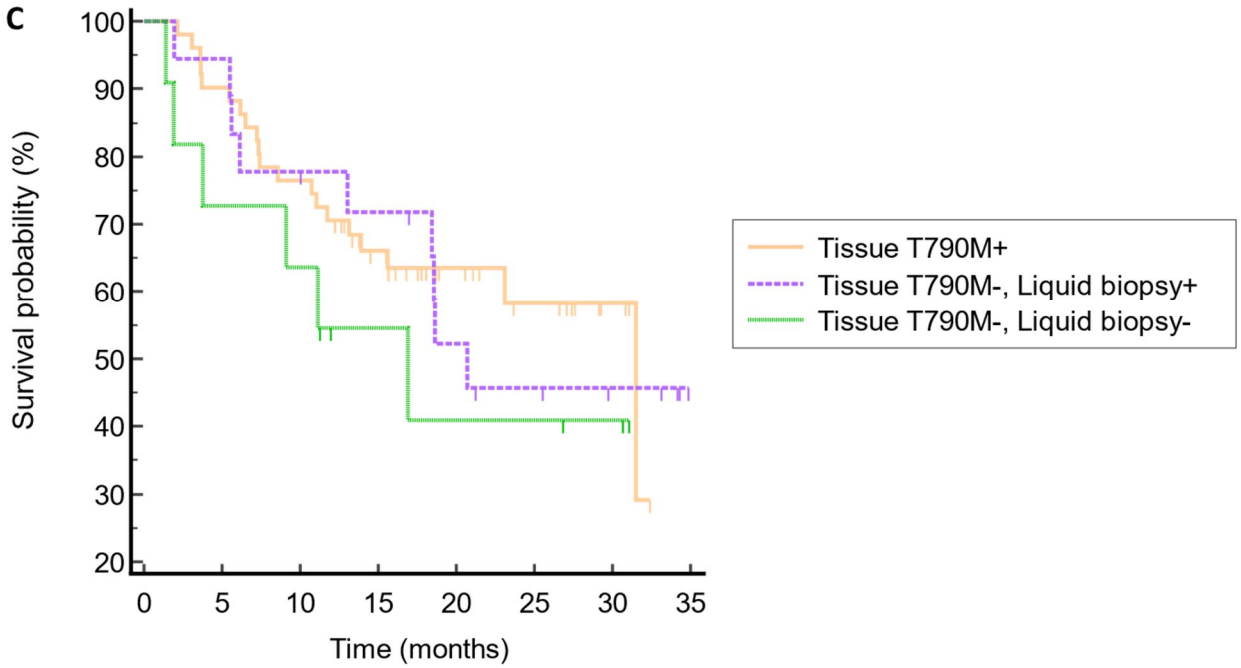
The response to osimertinib was evaluated in all 80 patients at the data analysis. In the overall popilation, ORR was 66.3%. Subjects who had T790 positivity only in tissue showed ORR of 65%, while subjects with T790M positivity only with PRIME EGFR or PANA Mutyper test was 60% and 100%, respectively. Subjects who did not shown to harbor T790M mutation detected by tissue or plasma sample had ORR of 45.5% (**Figure 5A**). PFS for each group of patients with different tissue or plasma T790M positivity was also shown in **Figure 5A**, which was not significantly different. We stratified the subjects as they harbor T790M mutation (tissue or liquid biopsy) again in **Figure 5B**, as group of subjects who harbor T790M mutation in tissue (group orange), only in liquid biopsy samples (group purple), or not in any of tissue or plasma samples (group green). ORR in group purple seemed to have highest ORR and group green had lowest ORR, there was no significant difference. The final analysis of PFS on the 80 patients was performed on data cut-off date of December 3, 2020 and the median follow-up duration was 20.6 months (95% CI, 17.2 to 24.0). The median PFS in all 80 patients was 31.5 months (95% CI, 18.4 to 31.5) (**Figure 5A**). PFS according to detection of T790M by tissue or liquid biopsy was as follows: patients who tested as tissue T790M positive, 31.5 months (95% CI, 15.6 to 31.5); patients who tested as only plasma T790M positive, 20.7 months (95% CI, 13.0 to 20.7); patients who tested not having T790M positivity either on tissue or plasma, 16.9 months (95% CI, 1.9 to 16.9). Although patients with T790M positivity not detected in tissue or plasma seemed to have numerically shorter PFS than the other group of patients, there were no statistically significant difference in PFS (p=0.80) (**Figure 5B, 5C**).

A Total (N = 123) (Osm 80), ORR = 66.3%, PFS = 31.5 mo (18.4-31.5)



B


Total (n = 123)
Tissue T790M+
PANA T790M+
PRIME T790M+
N = 52
(Osm 51)
ORR = 66.7%
PFS = 31.5 (15.6-31.5)
N = 27
(Osm 18)
ORR = 77.8%
PFS = 20.7
(13.0-20.7)
N = 44
(Osm 11)
ORR = 45.5%
PFS = 16.9 (1.9-16.9)



Number at risk

Tissue T790M+							
51	46	39	27	15	10	4	0
Tissue T790M-, Liquid biopsy+							
18	17	14	12	8	6	4	0
Tissue T790M-, Liquid biopsy-							
11	8	7	4	3	3	2	0

Figure 5. Objective response rates and progression-free survivals after osimertinib treatment according to tissue or plasma T790M status. (A) ORR and PFS of overall and each detailed group of patients, (B) ORR and PFS by tissue or liquid biopsy T790M status, (C) PFS by tissue or liquid biopsy T790M status. T790M, (c.2369C>T; p.Thr790Met)

Clinical efficacy of PRIME EGFR in detecting additional C797S mutation after treatment with osimertinib

Among 123 patients who tested for tissue or plasma *EGFR* mutation using PANA Mutyper or PRIME EGFR, 80 patients received osimertinib treatment. *EGFR* mutation analysis by PRIME *EGFR* was performed using ctDNA extracted from plasma of 24 patients who agreed to additional blood sampling after at least 3 months of osimertinib treatment. 2 out of 24 patients revealed to harbor C797S mutation, who had previously benefited from osimertinib treatment (**Figure 6**). The final analysis of PFS on the 24 patients was performed on data cut-off date of December 3, 2020 and the median follow-up duration was 20.6 months (95% CI, 17.2 to 24.0). The median PFS in all 24 patients was 9.1 months (95% CI, 5.5 to 15.6) (**Figure 7A**). PFS according to further detection of C797S by PRIME EGFR was as follows: patients who tested as C797S negative, 9.1 months (95% CI, 3.8 to 16.9); patients who tested as C797S positive, 7.3 months (95% CI, 7.3-10.7). Although patients with C797S positivity detected after osimertinib treatment seemed to have numerically shorter PFS than the other group of patients, there were no statistically significant difference in PFS (p=0.50) (**Figure 7B**).

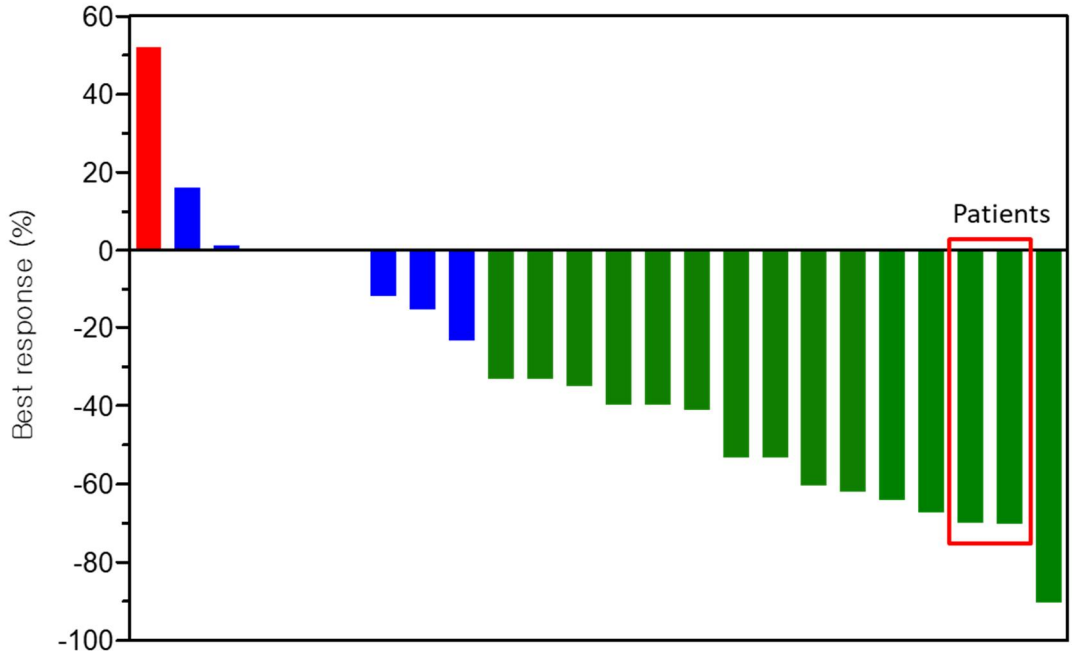


Figure 6. Tumor response to osimertinib in patients who measured C797S mutation status after at least 3-months of treatment. Red box indicates two patients who were tested positive to harbor C797S mutation by PRIME *EGFR*. PD (red bar), progression of disease; PR (green bar), partial response; SD (blue bar), stable disease; C797S, (c.2389T>A; p.Cys797Ser)

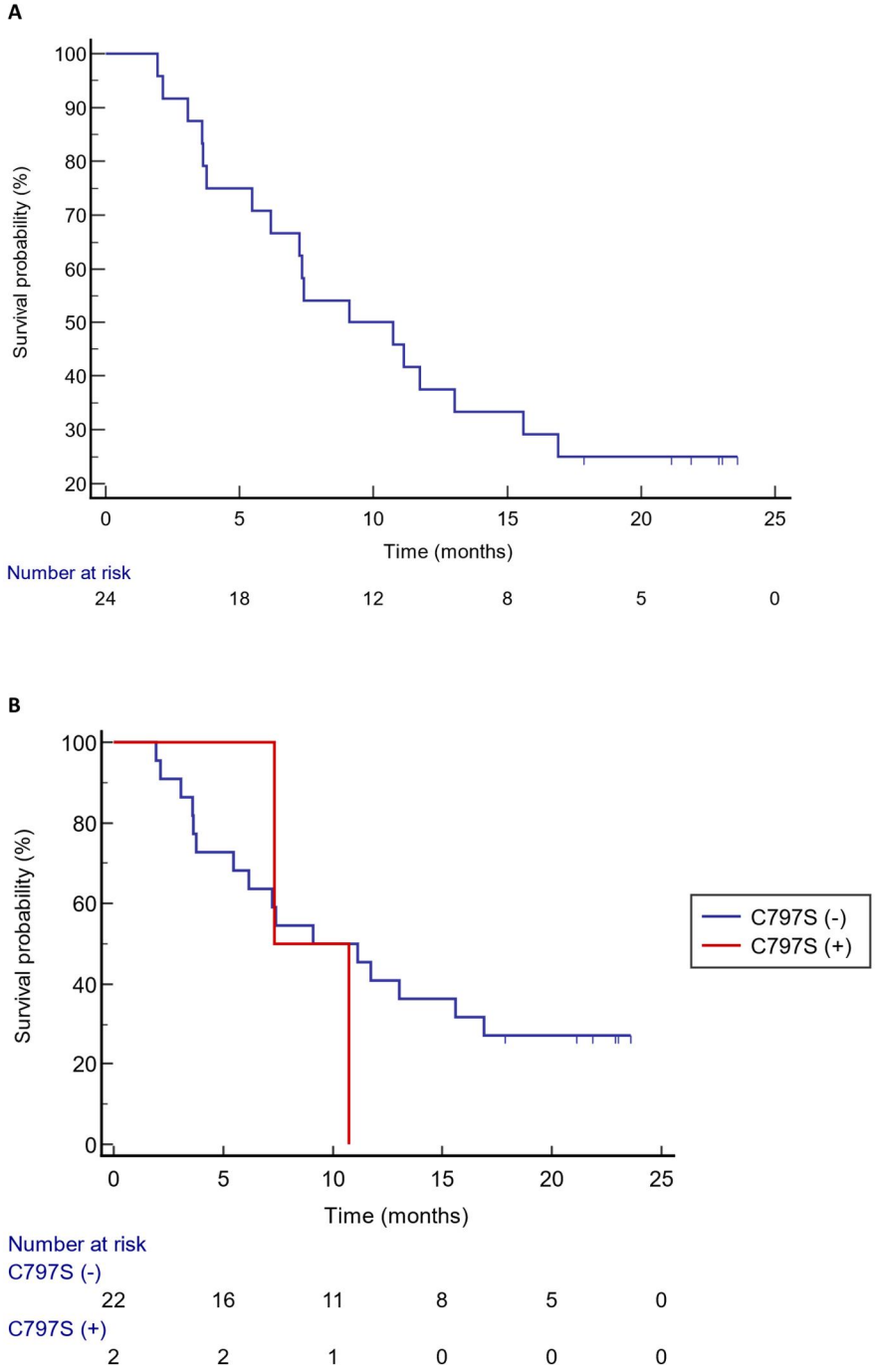


Figure 7. Progression-free survival after osimertinib treatment. Progression-free survival after treatment with osimertinib in patients who additionally tested for harboring C797S mutation after treatment of osimertinib. (A) PFS of overall twenty-four patients, (B) PFS by C797S mutation result. C797S, (c.2389T>A; p.Cys797Ser)

Discussion

Although increasing studies have profiled the clinical efficacy of liquid biopsy using blood samples from lung cancer patients, debate still exists about real diagnostic performance of liquid biopsy including CTC or ctDNA, enhancing EGFR mutation detection rate from liquid biopsy, and finding more sensitive EGFR mutation analysis using liquid biopsy samples. In this study, we demonstrate ctDNA extracted from plasma acquired by using lab-on-a disc microfluidic device with fully automated centrifugation technique show better detectability of EGFR mutations compared to CTC isolated by FAST disc. When we conducted EGFR mutation analysis from ctDNA with PRIME EGFR, a novel promer-based RT-PCR method, significant superiority detecting L858R and T790M mutation compared to PNA clamp-based RT-PCR method was noted. Furthermore, we found PRIME EGFR can capture C797S mutation, which is the most common tertiary EGFR mutation that induce resistance to osimertinib treatment.

Unlike traditional tissue biopsy, which usually cast risk of procedure related complications and lack of availability in some patients with inaccessible tumor site or poor performance status, liquid biopsy using blood is minimally invasive and repeatable, cost saving, and can reflect tumor heterogeneity[5, 6, 32]. With its promising role of providing individualized cancer therapy with molecular analysis, liquid biopsy is therefore recommended in cases with insufficient or unobtainable tumor tissue specimens[33] and Korean National Health Insurance Service (NHIS) has covered ctDNA tests for EGFR mutations in advanced since 2018[34]. However, some limitations remain regarding feasibility of liquid biopsy. Proportion of analytes including CTC or ctDNA in blood sample is generally low and half-life of CTC or ctDNA is short, casting challenges with respect to low sensitivity and high false-negative rates. Molecular diagnosis of lung cancer using CTCs isolated by various CTC isolation devices demonstrated detection rate of EGFR mutation ranging from 16.7% to 85.7% for L858R, 28.6% to 66.7% for T790M[35-39]. Previous studies also reported widely ranged sensitivity with plasma ctDNA in detection of genotype of lung cancer, with 39%-86% sensitivity for EGFR mutations and 27-75% sensitivity for T790M mutation[25, 40-42]. A *post hoc* analysis of AURA phase III

trial demonstrated detection rate of T790M as 51 to 66% (51% by cobas plasma, 58% by ddPCR, and 66% by next-generation sequencing)[43]. In the current study, we used a lab-on-a chip microfluidic based centrifugation method to isolate CTC and ctDNA from blood, which was expected to show high detection rate of EGFR mutation because of its automated, less-contaminated procedure. The sensitivity and specificity of EGFR mutation of CTC by RT-PCR was 9.1% and 50% respectively, which was much lower than ctDNA (69% sensitivity and 100% specificity), thus causing premature cessation of conducting CTC isolation. Although CTC failed to demonstrate satisfying result of detecting EGFR mutations, ctDNA extracted using microfluidic disc method revealed favorable diagnostic performance, thus leading to further confirmative study with larger cohort. NGS technique we used in this study presented sensitivity of 30.8% and specificity of 100% in detecting EGFR mutations with ctDNA, which are not far inferior to other study results and also needs further investigation.

We used PRIME EGFR, a novel promer based EGFR mutation detection method to find out its diagnostic performance in detecting L858R, T790M, and E19del mutations from plasma ctDNA, in comparison with PANA Mutyper in the current study. PRIME EGFR is constructed to detect EGFR mutations in exons 19, 20, 21, which include E19del, T790M, L858R, as well as C797S with 4 tubes, while PANA Mutyper targets exons 18, 19, 20, 21, but not C797S with 6 tubes. Total analysis time of PRIME EGFR is 1 hour 40 minutes, shorter than 2 hour 30 minutes of PANA Mutyper. We observed that PRIME EGFR have superior sensitivity in detecting L858R and T790M, which indicate more patients might benefit from EGFR-TKI treatment with higher detection rate of druggable EGFR mutations. Compared to previous studies used BEAMing, cobas, or ddPCR method to detect EGFR mutations[25, 44], detection rate of EGFR mutation by PRIME EGFR does not seem to have superiority. However, we observed significantly higher diagnostic performance of PRIME EGFR than PANA Mutyper, which is widely used in majority of lung cancer institutes of Korea. Furthermore, its promising role of capturing C797S mutation would help patients taking osimertinib to predict resistance to therapy and prognosis.

EGFR C797S mutation, is the most common tertiary EGFR mutation, which occurs in exon 20 and accounts for 10-26% of cases of resistance to second-line osimertinib treatment[45]. With

front-line treatment of osimertinib, the frequency of C797S mutation was 7%[46]. In C797S mutation, cysteine at codon 797 within the ATP-binding site is substituted for by serine, results in the loss of the covalent bone between between osimertinib and the mutant EGFR. It is also predicted to harbor cross-resistance to other third-generation TKIs, including rociletinib, olmutinib and narzatinib[47-49].

The present study have several limitations. First, study population of comparing the feasibility of CTC and ctDNA extracted by microfluidic disc was small. However, it was enough to consider CTC isolated by the FAST disc is not appropriate analyte to detect EGFR mutation and further investigation is ongoing. In addition, we only used ctDNA to evaluate feasibility of PRIME EGFR, which is known to have relatively low sensitivity compared to EV-derived liquid biopsy tests. But ctDNA is simple and cost-effective, and our study showed permissive sensitivity and specificity in detecting EGFR sensitizing mutations. Furthermore, clinical assessment predicting prognosis of patients in relation to tumor mutation burden was not available. Finally, C797S detectability of PRIME EGFR was not confirmed with tissue biopsy samples, which needs further investigation with larger population.

Conclusion

In conclusion, we showed that microfluidic chip derived ctDNA have significantly higher diagnostic performance in detecting EGFR mutation than CTC isolated by FAST chip. And promer based RT-PCR method revealed superior detectability on L858R and T790M mutation, compared to PNA-clamp and melting based RT-PCR method, and its additional capability of identifying C797S mutation was uncovered. These findings suggest that the various methods we investigated in the current study might benefit discovering druggable mutations in patients with NSCLC so that provide further individualized treatment options.

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국문 요약

배경: 액체생검법은 체액에서 분석물을 채취하고 분리하여 실험하는 것으로 최소침습적이고, 반복검사가 가능하며, 비용을 절감할 수 있다는 장점을 갖는다. 진행성 비소세포 폐암 환자에서 액체생검법은 주로 상피세포 성장인자 수용체 (EGFR) 변이를 확인하는 데 응용된다. 본 연구에서는 circulating tumor cell (CTC)와 circulating tumor DNA (ctDNA)를 이용한 액체생검법의 EGFR 변이 검출율을 확인하여 그 임상적 유용성을 평가하고, 프로머 (promer)를 이용한 유전자변이 검출 기법의 진단율을 평가하고자 하였다.

방법: 본 연구에서는 두 개의 전향적 코호트를 구축하여 연구를 진행하였다. 첫번째 코호트는 서울아산병원에서 진행성 비소세포폐암으로 초진단받은 환자를 등록하여 혈액을 채취, CTC 와 ctDNA 의 *EGFR* 변이 검출 능력을 비교하였다. 또다른 코호트는 기존에 *EGFR* 티로신 인산화효소 억제제 (TKI)에 치료 반응이 있었다가 병이 진행한 환자를 등록하여 재조직검사 또는 혈액에서 ctDNA 를 채취하여 PANA Mutyper 와 promer 를 이용한 EGFR 변이 진단키트의 유전자변이 검출능력을 비교분석 하였다. Osimertinib 을 썼을 때 객관적 반응률 (ORR) 및 osimertinib 투약 후 3 개월 이상이 지난 환자의 혈장에서 promer 기반 기법을 통해 C797S 변이가 검출되는지 함께 확인하였다.

결과: 첫번째 코호트에서 15 명의 환자의 조직과 혈액의 CTC 및 ctDNA 를 채취하여 EGFR 유전자변이 검출율을 비교하였고, ctDNA 를 이용하였을 때 EGFR 검출율이 CTC 에 비해 우세하였다. 다른 코호트 연구에서 123 명의 환자를 등록하였고, promer 기반 기법이 PANA Mutyper 기법보다 ctDNA 에서 L858R 과 T790M 변이의 진단에 우월함을 보였다. 또한 promer 기반 기법이 osimertinib 치료를 3 개월 이상 시행한 환자의 혈액에서 C797S 변이를 검출할 수 있음을 확인하였다.

결론: 본 연구에서는 비소세포폐암 환자에서 혈액의 ctDNA 가 CTC 보다 *EGFR* 변이 검출에 보다 우세함을 확인하였다. Promer 기반 기법은 PANA Mutyper 기법보다 L858R, T790M 변이 검출율이 높으며, C797S 변이 또한 함께 검출함으로써 osimertinib 의 치료효과를 예측할 수 있음을 시사하였다.

중심단어: 비소세포폐암, 액체생검법, T790M, C797S, osimertinib