Title: 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)

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Running Title (limit of 60 chracteristics including space)

Concordance of Plasma cfDNA Analysis in Prospective Cohort

Keywords

Plasma cfDNA sequencing, Next-generation sequencing (NGS), Non-small cell lung cancer (NSCLC), Concordance

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Declaration of interests

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Ono Pharmaceutical Co. Ltd., outside the submitted work. Dr. Kato reports grants and other from Abbvie, grants and other from Amgen K.K., grants, personal fees and other from AstraZeneca K.K., grants and personal fees from Bristol-Myers Squibb K.K., grants, personal fees and other from Chugai Pharmaceutical Co, Ltd., grants, personal fees and other from Eli Lilly Japan K.K., grants, personal fees and other from Merck Biophama Co., Ltd, grants, personal fees and other from MSD K.K., grants, personal fees and other from Novartis Pharma K.K., grants, personal fees and other from Ono Pharmaceutical Co., Ltd., grants, personal fees and other from Pfizer Japan Inc., grants and other from Taiho Pharmaceutical Co., Ltd., grants from Regeneron, personal fees from Boehringer Ingelheim Japan, Inc., personal fees and other from Daiichi Sankyo Co., Ltd., personal fees from Roche Diagnostics, other from Nippon Kayaku Co., Ltd., other from Takeda Pharmaceutical Co., Ltd., outside the submitted work. Dr. Sakakibara-Konishi reports grants from Eli Lilly Japan K.K., outside the submitted work. Dr. Nakagawa reports personal fees from AstraZeneca K.K., personal fees from Chugai Pharmaceutical Co, Ltd., personal fees from Eli Lilly Japan K.K., personal fees from Ono Pharmaceutical Co., Ltd., personal fees from Pfizer Japan Inc., personal fees from Taiho Pharmaceutical Co., Ltd., personal fees from Boehringer Ingelheim Japan, Inc., personal fees from MSD K.K., personal fees from Pfizer Japan Inc., outside the

submitted work. Dr. Kawaguchi reports personal fees from AstraZeneca K.K., personal fees from Bristol-Myers Squibb K.K., personal fees from Chugai Pharmaceutical Co, Ltd., personal fees from Eli Lilly Japan K.K., personal fees from Ono Pharmaceutical Co., Ltd., personal fees from Pfizer Japan Inc., personal fees from Taiho Pharmaceutical Co., Ltd., personal fees from Boehringer Ingelheim Japan, Inc., personal fees from Astellas, personal fees from Kyorin, personal fees from Kyowa Hakko, outside the submitted work. Dr. Sakai reports personal fees from AstraZeneca K.K., personal fees from Chugai Pharmaceutical Co, Ltd., outside the submitted work. Dr. Shibata reports personal fees from AstraZeneca K.K., grants and personal fees from Ono Pharmaceutical Co., Ltd., personal fees from Pfizer Japan Inc., personal fees from Bristol-Myers Squibb K.K., personal fees from Eli Lilly Japan K.K., outside the submitted work. Dr. Izumi reports grants from Amgen Inc., personal fees from AstraZeneca K.K., grants and personal fees from Ono Pharmaceutical Co. Ltd., outside the submitted work. Dr. Nosaki reports personal fees from AstraZeneca K.K., personal fees from Chugai Pharmaceutical Co, Ltd., personal fees and other from Pfizer Japan Inc., personal fees from MSD K.K., personal fees from Bristol-Myers Squibb K.K., other from Eli Lilly Japan K.K., personal fees from Nippon Kayaku Co., Ltd., personal fees from Ono Pharmaceutical Co., Ltd., personal fees from Taiho Pharmaceutical Co.,

Ltd., personal fees from Takeda Pharmaceutical Co., Ltd., other from Daiichi Sankyo Co., Ltd., outside the submitted work. Dr. Zenke reports personal fees from AstraZeneca K.K., personal fees from Ono Pharmaceutical Co., Ltd., personal fees from Bristol-Myers Squibb K.K., personal fees from Eli Lilly Japan K.K., personal fees from Chugai Pharmaceutical Co, Ltd., personal fees from Takeda Pharmaceutical Co., Ltd., personal fees from Boehringer Ingelheim Japan, Inc., outside the submitted work. Dr. Yoh reports grants and personal fees from AstraZeneca K.K., personal fees from Chugai Pharmaceutical Co, Ltd., grants from Pfizer Japan Inc., grants from MSD K.K., personal fees from Bristol-Myers Squibb K.K., grants and personal fees from Eli Lilly Japan K.K., grants and personal fees from Taiho Pharmaceutical Co., Ltd., grants from Takeda Pharmaceutical Co., Ltd., grants and personal fees from Daiichi Sankyo Co., Ltd., grants from Abbvie, personal fees from Janssen Pharmaceutical K.K., personal fees from Novartis Pharma K.K., personal fees from Kyowa Kirin Co., Ltd., personal fees from Boehringer Ingelheim Japan, Inc., outside the submitted work. Dr. Goto reports grants from Merck Biophama Co., Ltd, grants from Takeda Pharmaceutical Co., Ltd., non-financial support from Guardant Health Inc., during the conduct of the study; grants and personal fees from Amgen Astellas Biopharma K.K., grants and personal fees from Amgen K.K., grants and personal fees from Boehringer Ingelheim Japan, Inc.,

grants and personal fees from Bristol-Myers Squibb K.K., grants and personal fees from Bayer, grants and personal fees from Chugai Pharmaceutical Co, Ltd., grants and personal fees from Daiichi Sankyo Co., Ltd., grants and personal fees from Eisai Co., Ltd., grants and personal fees from Eli Lilly Japan K.K., grants from Ignyta, Inc., grants, personal fees and other from Janssen Pharmaceutical K.K., grants from KISSEI PHARMACEUTICAL CO., LTD., grants and personal fees from Kyowa Kirin Co., Ltd., grants from Loxo Oncology, Inc., grants from MEDICAL & BIOLOGICAL LABORATORIES CO., LTD., grants from Merck Biophama Co., Ltd, grants from Merus N.V., grants and personal fees from MSD K.K., grants from NEC Corporation., grants and personal fees from Novartis Pharma K.K., grants and personal fees from Ono Pharmaceutical Co., Ltd., grants and personal fees from Pfizer Japan Inc., grants from Sumitomo Dainippon Pharma Co., Ltd., grants from Spectrum Pharmaceuticals, Inc., grants from Sysmex Corporation., grants from Haihe Biopharma Co., Ltd., grants and personal fees from Taiho Pharmaceutical Co., Ltd., grants and personal fees from Takeda Pharmaceutical Co., Ltd., grants from Turning Point Therapeutics, Inc., personal fees from Amoy Diagnosties Co., Ltd., personal fees from Guardant Health Inc., personal fees from Life Technologies Japan Ltd., personal fees from Otsuka Pharmaceutical Co., Ltd., outside the submitted work. The other authors declare no

conflict of interest.

Statement of translational relevance (150 words)

The extent to which extent plasma cfDNA sequencing can diagnose rare driver oncogenes has not been fully evaluated. Our large-scale study revealed the clinical performance of plasma cfDNA sequencing, especially for the detection of a rare fraction of oncogenic drivers. Plasma cfDNA sequencing in patients with advanced NSCLC had a relatively high detectability for gene mutations, but a low detectability for gene fusions and *MET* exon 14 skipping. Plasma cfDNA sequencing cannot fully complement tissue assays in terms of detection of oncogenic alterations because the concordance was not high especially in fusions and MET exon 14 skipping. On the other hand, when oncogenic alterations were detected by plasma cfDNA sequencing, they were useful for the selection of the corresponding genotype-matched therapy. Plasma cfDNA sequencing may be an alternative assay only when a tissue assay is unavailable due to insufficient DNA and RNA.

Abstract

Purpose: We evaluated plasma cell-free DNA (cfDNA) and tissue-based sequencing concordance for comprehensive oncogenic driver detection in non-small cell lung cancer (NSCLC) using a large-scale prospective screening cohort (LC-SCRUM-Liquid).

Methods: Blood samples were prospectively collected within four weeks of corresponding tumor tissue sampling from advanced NSCLC patients to investigate plasma cfDNA sequencing concordance for alterations in eight oncogenes (*EGFR*, *KRAS*, *BRAF*, *HER2, MET, ALK*, *RET*, and *ROS1*) compared to tissue-based next-generation targeted sequencing.

Results: Paired blood and tissue samples were obtained in 1062/1112 enrolled NSCLC patients. Oncogenic alteration was detected by plasma cfDNA sequencing and tissue assay in 455 (42·8%) and 537 (50·5%) patients, respectively. The positive percent agreement (PPA) of plasma cfDNA sequencing compared with tissue DNA and RNA assays were 77% (*EGFR*, 78%; *KRAS*, 75%; *BRAF*, 85%; *HER2*, 72%) and 47% (*ALK*, 46%; *RET*, 57%; *ROS1*, 18%; *MET* 66%), respectively. Oncogenic drivers were positive for plasma cfDNA and negative for tissue due to unsuccessful genomic analysis from poor-quality tissue samples (70%), and were negative for plasma cfDNA and positive for tissue due to low sensitivity of cfDNA analysis (61%). In patients with positive

oncogenic drivers by plasma cfDNA sequencing but negative by tissue assay, response rate of genotype-matched therapy was 85% and median progression-free survival was 12·7 months.

Conclusions: Plasma cfDNA sequencing in advanced NSCLC patients showed relatively high sensitivity for detecting gene mutations but low sensitivity for gene fusions and *MET* exon 14 skipping. This may be an alternative only when tissue assay is unavailable due to insufficient DNA and RNA.

Abbreviations

cfDNA: Cell-free DNA

NSCLC: Non-small cell lung cancer

PPA: Positive percent agreement

NGS: next-generation sequencing

CLIA: Clinical Laboratory Improvement Amendments

CAP: College of American Pathologists

OCA: Oncomine Comprehensive Assay

EDC: Electronic data capture

NPA: Negative percent agreement

PPV: Positive predictive value

NPV: Negative predictive value

PPV: Positive predictive value

NPV: Negative predictive value

OPA: Overall percent agreement

TAT: Turnaround time

PFS: Progression-free survival

cfRNA: cell-free RNA

Introduction

A variety of oncogenic drivers have been identified in non-small cell lung cancer (NSCLC), and molecular targeted therapy has greatly improved the clinical outcomes of patients with oncogenic drivers [1]. Plasma cell-free DNA (cfDNA) sequencing has been developed as a less invasive method than conventional tissue genotyping for detecting various genomic alterations. Some previous retrospective studies have examined the concordance between plasma cfDNA sequencing and tissue genotyping. Previous small studies (n = 72–287) reported positive percent agreement (PPA) of plasma cfDNA sequencing compared with tissue genotyping as 58·8%–95·8% for *EGFR* mutations, 75·0% for *KRAS* G12X, 40·0%–100·0% for *ALK* fusions, and 33·3%–100·0% for *BRAF* V600E [2-6]. However, the concordance between plasma cfDNA sequencing and tissue genotyping has not been evaluated in detail because these results are based on smaller cohorts, and in particular, the number of patients with rare fractions of oncogenic drivers was extremely low. Therefore, to evaluate the detectability of oncogenic alterations in plasma cfDNA sequencing precisely, prospective comparative analyses with the corresponding tumor tissue genotyping in a large-scale sample size study are needed. We evaluated the concordance between plasma cfDNA sequencing and tissue assays for the detection of oncogenic alterations in advanced NSCLC patients

using a large-scale prospective study.

A large-scale lung cancer genomic screening project, LC-SCRUM-Asia, was started in February 2013, and tissue genotyping was performed to identify lung cancer patients with oncogenic drivers (UMIN number: 000010234 and 000036871) [7]. As of October 2021, more than 14,000 patients were already enrolled in this study.

Methods

Study design and patients

This liquid biopsy study, LC-SCRUM-Liquid, has been conducted as an additional study in LC-SCRUM-Asia since December 2017. Blood samples were prospectively collected from patients with advanced or recurrent NSCLC within four weeks of tissue biopsy. Plasma cfDNA was extracted from blood samples and analyzed using next-generation sequencing (NGS). The concordance of oncogenic drivers in plasma cfDNA sequencing was evaluated, compared to tissue genotyping, which was performed independently and blindly by plasma cfDNA sequencing. The clinical outcomes of patients who received genotype-matched therapy, were also prospectively investigated.

Patients who met the following eligibility criteria were enrolled: 1) above the age of

20; 2) with histologically/cytologically-confirmed NSCLC; 3) clinical stage III or, IV, or recurrence; 4) diseases were unsuitable for operation or thoracic radiotherapy, but suitable for chemotherapy; 5) chemonaive or one or two prior systemic treatments for lung cancer, 6) already enrolled in LC-SCRUM-Asia, and 7) with blood samples taken within four weeks after tissue sample biopsy.

LC-SCRUM-Asia and LC-SCRUM-Liquid were approved by the Institutional Review Board of the National Cancer Center (approval number 2012-257 and 2017-222, respectively) and by each institution participating in these studies. Written informed consent was obtained from all the patients. Our studies were conducted in accordance with the guidelines for medical and health research involving human subjects specified in the Declaration of Helsinki.

Plasma-based NGS assay

Blood samples, collected using a blood collection tube, Streck Cell-Free DNA BCT (Streck Corporate, NE), were submitted to Guardant Health, a Clinical Laboratory Improvement Amendments (CLIA)-certified, and College of American Pathologists (CAP)-accredited laboratory, and was subjected to plasma cfDNA sequencing, Guardant 360 panel (Guardant Health, CA), targeting 73 (until April in 2019) or 74 (afterward)

cancer-related genes.

Tissue-based NGS assay

Tissue samples were mainly collected from previously untreated patients. Tissue genotyping was performed within LC-SCRUM-Asia. Tumor tissue analysis was mainly performed using fresh frozen biopsy samples. Tissue samples were submitted to a CLIA-certified clinical laboratory (SRL Incorporation, Tokyo, Japan). DNA and RNA extracted from the tissue samples were subjected to a tissue-based NGS assay, Oncomine Comprehensive Assay (OCA) version 1 or 3 (Thermo Fisher Scientific, MA), targeting 143 (version 1) or 161 (version 3) cancer-related genes. In this assay, gene mutations were analyzed by DNA assay, and fusions and *MET* exon 14 skipping were analyzed by RNA assay.

Clinical data capturing

Clinical data of patients were collected using an electronic data capture (EDC) system of LC-SCRUM-Asia. The patients' baseline characteristics were collected when the patients were enrolled in LC-SCRUM-Asia, and follow-up clinical data, including the start dates of systemic anti-cancer drug therapy, therapeutic regimens, tumor responses,

dates of disease progression, and prognosis, were periodically collected.

Statistical analysis

Mutations in *EGFR*, *KRAS*, *BRAF*, *HER2*, *NRAS*, *HRAS*, *AKT1*, and *MAP2K1*, fusions in *ALK*, *RET*, *ROS1*, and *FGFR3*, and *MET* exon 14 skipping, were defined as targetable gene alterations. Among these targetable gene alterations, the concordance for alterations of eight oncogenic drivers (mutations of *EGFR* [insertion, deletion and missense mutation in exons 18-21]; *KRAS* [G12X, G13X, and Q61X]; *BRAF* [V600E]; and *HER2* [insertions in exon 20]: fusions of *ALK*, *RET*, and *ROS1*; and *MET* exon 14 skipping) in plasma cfDNA sequencing was assessed by estimating PPA, negative percent agreement (NPA), positive predictive value (PPV), negative predictive value (NPV) and overall percent agreement (OPA) of plasma cfDNA sequencing compared to the results of the tissue assays. These concordance analyses were performed in variants of the eight oncogenic drivers, which were covered by both the two assays.

Turnaround time (TAT) was defined as the duration from sample submission to reporting the sequencing results, and the results of plasma cfDNA sequencing and tissue assay were compared using the Wilcoxon sum rank test.

The Kaplan-Meier method was used to estimate the progression-free survival (PFS) of

patients who received genotype-matched therapy. EZR software (Saitama Medical Center, Jichi Medical University, Japan) was used for the statistical analyses.

Role of the funding source

The funder of LC-SCRUM-Liquid and LC-SCRUM-Asia had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Data availability

The data generated in this study are available upon reasonable request from the corresponding author. The request is reviewed by research group whether if it is able to approve.

Results

Patient characteristics

From December 2017 to January 2021, 1,112 patients with advanced or recurrent NSCLC were enrolled in LC-SCRUM-Liquid. Of these, 1,065 paired blood and tissue samples were available for this study analyses. Three patients who were ineligible for inclusion were excluded. Thus, 1,062 patients (95%) were analyzed in this study

317 (Supplementary Figure S1).

318 The patient characteristics are shown in Table 1. The median age was 69 years (range:
319 25–91). The majority were male (61%), smokers (69%), and had stage IV disease (80%).
320 Almost all the patients were previously untreated (93%). The histology of tumors
321 comprised 77% adenocarcinoma, 14% squamous cell carcinoma and other NSCLCs.
322 Number of metastatic sites was 0 in 14%, 1 in 33%, 2 in 22%, 3 or more in 15%. There
323 were brain metastasis in 17%, pulmonary metastasis in 31%, pleural dissemination or
324 pleural effusion in 24%, liver metastasis in 6%, adrenal metastasis in 7%, and bone
325 metastasis in 24%. Tissue samples for tissue assays were mainly obtained as fresh
326 frozen (90%) and from primary lung tumor (60%), metastatic sites (29%), or pleural
327 effusion (11%).

328

329 *Availability of genomic analysis and detection of oncogenic alterations*

330 The success rates of genomic analysis by plasma cfDNA sequencing and tissue assay
331 were 91% (964/1,062) and 97% (1,025/1,062), respectively. TAT in plasma cfDNA
332 sequencing was significantly shorter than that in the tissue assay (10 days [range: 6–27]
333 vs. 22 days [range: 12–57], $p < 0{\cdot}01$).

334 In plasma cfDNA sequencing, targetable gene alterations were detected in 473 patients

(44·5%). Of these, the number of eight oncogenic alterations were 255 *EGFR* mutations (24·0%), 129 *KRAS* mutations (12·1%), 10 *HER2* exon 20 insertions (0·9%), 7 *BRAF* V600E mutation (0·7%), 26 *ALK* fusions (2·4%), 9 *RET* fusions (0·8%), 3 *ROS1* fusion (0·3%), and 16 *MET* exon 14 skipping (1·5%) (Figure 1A). In contrast, eight oncogenic alterations were detected by tissue assay in 549 patients (51·6%). There were 281 *EGFR* mutations (26·4%), 145 *KRAS* mutations (13·6%), 11 *HER2* exon 20 insertions (1·0%), 7 *BRAF* V600E mutation (0·7%), 45 *ALK* fusions (4·2%), 14 *RET* fusions (1·3%), 16 *ROS1* fusion (1·5%), and 18 *MET* exon 14 skipping (1·7%) in tissue assay (Figure 1B). Among 147 patients with squamous cell carcinoma, targetable gene alterations were detected in 19 patients (12.9%) by plasma cfDNA sequencing, and in 16 patients (10.8%) by tissue assay (Supplementary Figure S2). One of the eight oncogenic alterations was detected by plasma cfDNA sequencing or tissue assay in 18 patients with squamous cell carcinoma; 8 *EGFR* mutations, 6 *KRAS* mutations, 1 *ALK* fusion, 3 *MET* exon 14 skipping (Supplementary Table S1).

Concordance between plasma cfDNA sequencing and tissue assay

As shown in Figure 2A, the overall PPA of plasma cfDNA sequencing was 72% (389/537). Other performance indexes of plasma cfDNA sequencing were as follows:

NPA, 87% (459/525); PPV, 85% (389/455), NPV, 75% (459/607); and OPA, 79% (848/1,062) (Supplementary Table S2).

For the DNA assay, PPA of plasma cfDNA sequencing was 78% (345/444) (Figure 2A): *EGFR*, 78% (221/281); *KRAS*, 75% (110/145); *BRAF*, 85% (6/7); *HER2*, 72% (8/11) (Figure 2B). Other performance indexes of plasma cfDNA sequencing for DNA assay were as follows, NPA, 90% (562/618); PPV, 86% (345/401), NPV, 85% (562/661); OPA, 85% (907/1062) (Supplementary Table S2).

For the RNA assay, PPA of plasma cfDNA sequencing was 47% (44/93) (Figure 2A): *MET* exon14 skipping, 66% (12/18); *ALK*, 46% (21/45); *ROS1*, 18% (3/16); *RET*, 57% (8/14) (Figure 2B). Other performance indexes of plasma cfDNA sequencing were as follows: NPA, 98% (959/969); PPV, 81% (44/54); NPV, 95% (959/1,008); and OPA, 94% (1,003/1,062) (Supplementary Table S2).

The breakdown of discordant results between plasma cfDNA sequencing and tissue assays is shown in Figure 3. Among the 1,062 patients, 389 showed concordant results between each assay. Among patients with oncogenic alterations detected by plasma cfDNA sequencing only, the results of tissue assay were unavailable due to unsuitable tissue samples in 70% (46/66) and no detection of oncogenic alterations in only 30% (20/66); among patients with oncogenic alterations detected by tissue assay only, the

results of plasma cfDNA sequencing showed no detection of oncogenic alterations in 61% (90/148).

Patient characteristics and concordance between plasma cfDNA sequencing and tissue assay

To investigate whether if there were any subpopulations in which plasma cfDNA sequencing was more sensitive, we evaluated PPA of plasma cfDNA sequencing according to patient characteristics. PPA of plasma cfDNA sequencing was similar regardless of smoking status ($p = 0.84$), stage ($p = 0.47$) or histology ($p = 1.00$), and higher in patients with 3 or more metastatic sites than in those with 2 or less metastatic sites (0, 69%; 1, 63%, 2, 71%; 3 or more, 87%) ($p < 0.01$) (Supplementary Figure S3).

Metastatic sites and concordance between plasma cfDNA sequencing and tissue assay

We also evaluated metastatic site and PPA of plasma cfDNA sequencing to identify subpopulations in which plasma cfDNA sequencing was more preferable. PPA was higher in patients who had brain metastasis (Brain +, 80%; Brain -, 68%) ($p = 0.01$), liver metastasis (Liver +, 88%; Liver -, 69%) ($p = 0.01$), adrenal metastasis (Adrenal +, 90%; Adrenal -; 69%) ($p = 0.01$), and bone metastasis (Bone +, 85%; Bone-, 63%) ($p <$

389 0.01), and was not different between patients with and without lung metastasis (p =
390 0.59), or pleural dissemination and effusion (p = 0.05) (Supplementary Figure S4).
391 There were 54 patients whose distant metastasis was present only in brain. In the 54
392 patients, PPA of plasma cfDNA sequencing was not different between mutation
393 detection and fusion/exon skipping detection (60% [12/20] vs. 62% [5/8]) (p = 1.00)
394 (Supplementary Table S3).
395
396 *Clinical outcomes of patients treated with genotype-matched therapy based on plasma*
397 *cfDNA sequencing and tissue assay*
398 To clarify whether oncogenic alterations detected by plasma cfDNA sequencing are
399 correctly diagnosed and accurately reflect the efficacy of genotype-matched therapy, we
400 analyzed the clinical outcomes of patients treated with genotype-matched therapy based
401 on plasma cfDNA sequencing and tissue assays. Clinical outcome data of 115 patients
402 treated with genotype-matched therapy were available. Among these patients, the
403 oncogenic alterations were detected only by tissue assay in 31 patients (T group), by
404 both tissue assay and plasma cfDNA sequencing in 71 patients (TP group), and only by
405 plasma cfDNA sequencing in 13 patients (P group). The median PFS of T, TP, P groups
406 were 23·0 months (95% confidence interval [CI]: 12·4 – not reached [NR]); 12·4

months (95% CI: 9·1–16·3); and 12·7 months (95% CI: 5·0–13·5), respectively (Figure 4A). Therefore, the median PFS for each group was > 12 months. The median PFS of the T and P groups was not inferior to that of the TP group. In 13 patients in the P group, in which tissue samples were unsuitable for genomic analysis due to insufficient quantity or quality of the DNA, RNA or both, the response rate of genotype-matched therapy was 85% (11/13) (Supplementary Table S4).

As for patients with *EGFR* mutations, there were 19, 63, 11 patients in the T, TP, P groups, respectively. In the treatment with EGFR-TKIs, the median PFS of the T, TP, P groups was 23·0 months (95% CI: 4·7 – NR); 10·4 months (95% CI: 7·8–15·0); and 12·7 months (95% CI: 5·0–13·5), respectively (Figure 4B). The median PFS of the T and P groups was not inferior to that of the TP group.

Discussion

To our knowledge, this is the largest prospective concordance study for plasma cfDNA sequencing, in which tissue- and plasma-based NGS assays were simultaneously performed in advanced NSCLC patients. The within four-week interval for the tissue and plasma sample collections for all patients made the accurate evaluation of the concordance possible. Moreover, this study included 74 patients with rare fractions of

425 oncogenic drivers, such as *BRAF* V600E (n = 8), *HER2* exon 20 insertions (n = 13),
426 *MET* exon 14 skipping (n = 22), and fusions of *ROS1* (n = 16) and *RET* (n = 15). For
427 concordance analysis, previous studies included only a few patients with rare fractions
428 of oncogenic drivers, such as *BRAF* V600E mutation, *ROS1* fusions, and *RET* fusions [2-6].
429 This large-scale study enabled us to evaluate the clinical performance of plasma cfDNA
430 sequencing, especially for detecting a rare fraction of oncogenic drivers, which had not
431 been previously proven precisely.
432 Previous reports have shown that the PPA of plasma cfDNA sequencing compared to
433 tissue assay was 58·8%–95·8% for *EGFR* mutations, and 40%–100% for *ALK* fusions
434 [2-6]. However, these reports were not sufficient to evaluate the PPA of plasma cfDNA
435 sequencing accurately because the studies were mostly conducted retrospectively, and
436 they excluded tissue or plasma samples that were unavailable due to insufficient DNA
437 or RNA. In this study, the PPA of plasma cfDNA sequencing was 72%–85% for
438 mutations in *EGFR*, *KRAS*, *HER2*, or *BRAF*, and 18%–57% for fusions in *ALK*, *RET*, or
439 *ROS1* compared to those of tissue assays. We reveal that the detection of oncogenic
440 alterations by plasma cfDNA sequencing was not as sensitive as previously reported but
441 was inferior to that by tissue assay. In particular, the PPA of plasma cfDNA sequencing
442 for gene fusions against tissue RNA assay was extremely low (less than 60%) compared

443 to that for mutations against tissue DNA assay in our study. In a prospective report, the
444 PPA of plasma cfDNA sequencing compared to tissue assay was 81·8%–90% for *EGFR*
445 mutations, and 62.5% for *ALK* fusions [3]. PPA of plasma cfDNA sequencing in gene
446 fusions was reported to be lower than that in gene mutations, because gene fusions
447 include various variants and the capture of fusion DNA fragments is technically difficult
448 due to the low capturing efficiency and shortness of cfDNA fragments, as indicated in a
449 previous report [8]. *ROS1* fusion is known to have many partner genes compared with
450 *ALK* and *RET* fusions; therefore, the poor detectability of *ROS1* fusion in plasma
451 cfDNA sequencing (PPA, 18%) might also be caused by the existence of various variant
452 types. In addition, bioinformatic technologies could also influence the detectability of
453 gene fusions. A previous study demonstrated that PPA of plasma cfDNA sequencing for
454 *ALK* fusions was improved by updating bioinformatic systems for fusion detection [3, 9].
455 Plasma cell-free RNA (cfRNA) analysis also showed a higher sensitivity for detecting
456 fusion genes than plasma cfDNA sequencing (cfRNA, 78%; cfDNA, 33%)[10]. Thus,
457 detection sensitivity for fusions in plasma cfDNA sequencing could be improved by
458 further advances in technology, including DNA capturing methods, bioinformatics and
459 plasma cfRNA analysis.
460 There were some discordant results between plasma cfDNA sequencing and tissue

assays. The main discordant reasons, in which oncogenic alterations were positive by plasma cfDNA sequencing and negative by tissue assay, were due to the unavailability of tissue samples because of the insufficient quality or quantity of DNA or RNA. When the quality and quantity of tissue samples are acceptable for genomic analysis and the results of tissue assays are negative, plasma cfDNA sequencing does not provide additional information because oncogenic alterations are rarely detected by plasma cfDNA sequencing. Therefore, plasma cfDNA sequencing could be useful for detecting oncogenic alterations only when tissue assay is unavailable.

The utility of biomarker-matched precision medicine based on plasma cfDNA sequencing has not been well investigated. In particular, the efficacy of genotype-matched therapy in patients whose oncogenic drivers are detected only by plasma cfDNA sequencing is not fully understood, although one previous study reported the responses to plasma genotype-matched therapy [11]. Our study also demonstrated that, in 13 patients with oncogenic alterations identified only by plasma cfDNA sequencing, the corresponding genotype-matched therapy showed robust clinical activities. Moreover, the median PFS of patients with oncogenic alterations detected only by plasma cfDNA sequencing was over 12 months. These data were comparable to the median PFS of patients treated with tissue genotype-matched therapy [12-15]. However, the

479 median PFS of patients with oncogenic alterations detected only by plasma cfDNA
480 sequencing tended to be shorter than that of patients with oncogenic alterations detected
481 only by tissue assay. This is because patients with oncogenic alterations detected by
482 plasma cfDNA sequencing often have more advanced cancers and a higher tumor
483 burden [11, 16]. Indeed, higher positivity by cfDNA sequencing was demonstrated in
484 patients with 3 or more metastatic sites, and in patients with brain, liver, adrenal or bone
485 metastasis in the present study. Our results suggest that oncogenic alterations detected
486 by plasma cfDNA sequencing are genuine for selecting the corresponding
487 genotype-matched therapy. Therefore, treatments selected using plasma cfDNA
488 sequencing could be suitable for advanced NSCLC patients, especially when tissue
489 assays are unavailable. To further validate the clinical utility of plasma cfDNA
490 sequencing, we are presently conducting prospective umbrella trials of
491 genotype-matched therapy stratified based on this liquid biopsy study (JapicCTI
492 number: JapicCTI-205154 and JapicCTI-205155).
493 This study has some limitations. First, although our study was large-scaled, patients
494 with oncogenic alterations in *HER2*, *BRAF*, *MET*, *RET*, or *ROS1* were only 74 in total.
495 Accurate evaluation of concordance in rare fractions of oncogenic alterations was
496 limited even in this large-scale analysis, and it requires larger-scale concordance studies

with over 10,000 patients. Second, the efficacy of genotype-matched therapy in each patient was evaluated by investigators in clinical practice.

In conclusion, plasma cfDNA sequencing in advanced NSCLC patients had a relatively high detectability for gene mutations but a lower detectability for gene fusions and *MET* exon 14 skipping. Our data indicated that plasma cfDNA sequencing could not fully replace tissue assays for oncogenic alterations detection. However, when positive results are obtained, plasma cfDNA sequencing has a diagnostic value equivalent to that of the tissue assay in predicting the efficacy of genotype-matched therapy for plasma oncogenic-driver-positive patients. Therefore, plasma cfDNA sequencing can be a promising alternative to tissue genotyping when the tissue is unavailable because of insufficient DNA/RNA. Further, new technologies for plasma cfDNA sequencing could improve its clinical utility for NSCLC.

Acknowledgements

We are grateful to participating patients and their families. We also thank Ms. Yuri Murata, Ms. Akiko Iizuka, and PREMIA Inc. for administrative assistance in managing clinical samples, molecular screening and clinico-genomic database in LC-SCRUM-Liquid and LC-SCRUM-Asia.

LC-SCRUM-Liquid was funded by Guardant Health, Inc., Merck Biopharma Co., Limited. and Takeda Pharmaceutical Company., Limited. This work was supported by the National Cancer Center Research and Development Fund 28-A-6 (K.G.), and 31-A-5 (Atsushi Ohtsu), AMED Grant Number JP21ck0106289 (K.G.), JP21ck0106568 (K.G.), JP17Ack0106148 (K.G.), JP21ck0106294 (K.Y.), JP21ck0106483 (K.No.), JP20ck0106411 (S.Ma.), JP20ck0106449 (Isamu Okamoto), JP20ck0106450 (Seiji.Niho.), JP20ak0101050 (Katsuya Tsuchihara), JP18Ik0201056 (A.O.), JP18kk0205004 (Hitoshi Nakagama), and JP17Ack0106147 (Seiji Yano). Tissue NGS analysis in LC-SCRUM-Asia was supported by Amgen, Astellas, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Chugai, Daiichi Sankyo, Eisai, Janssen, Kyowa Kirin, Lilly, Merck, MEDICAL & BIOLOGICAL LABORATORIES, MSD, Novartis, Ono, Pfizer, Sumitomo Dainippon, Taiho, and Takeda.

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AS and SMa have directly accessed and verified the underlying data.

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Figure captions

Figure 1. Frequency of the targetable gene alterations detected by plasma cfDNA sequencing (A) and tissue assay (B).

(A) Plasma cfDNA sequencing (N= 1062)

(B) Tissue assay (N= 1062)

606

607 **Figure 2. Positive percent agreement of plasma cfDNA sequencing compared to tissue assay.**

608 **PPA, positive percent agreement.**

609

610 **(A) PPA of plasma cfDNA sequencing compared to tissue DNA or RNA assays**

611 **(B) PPA of plasma cfDNA sequencing for eight oncogenic alterations**

612

613 **Figure 3. Discordant cases between plasma cfDNA sequencing and tissue assay**

614

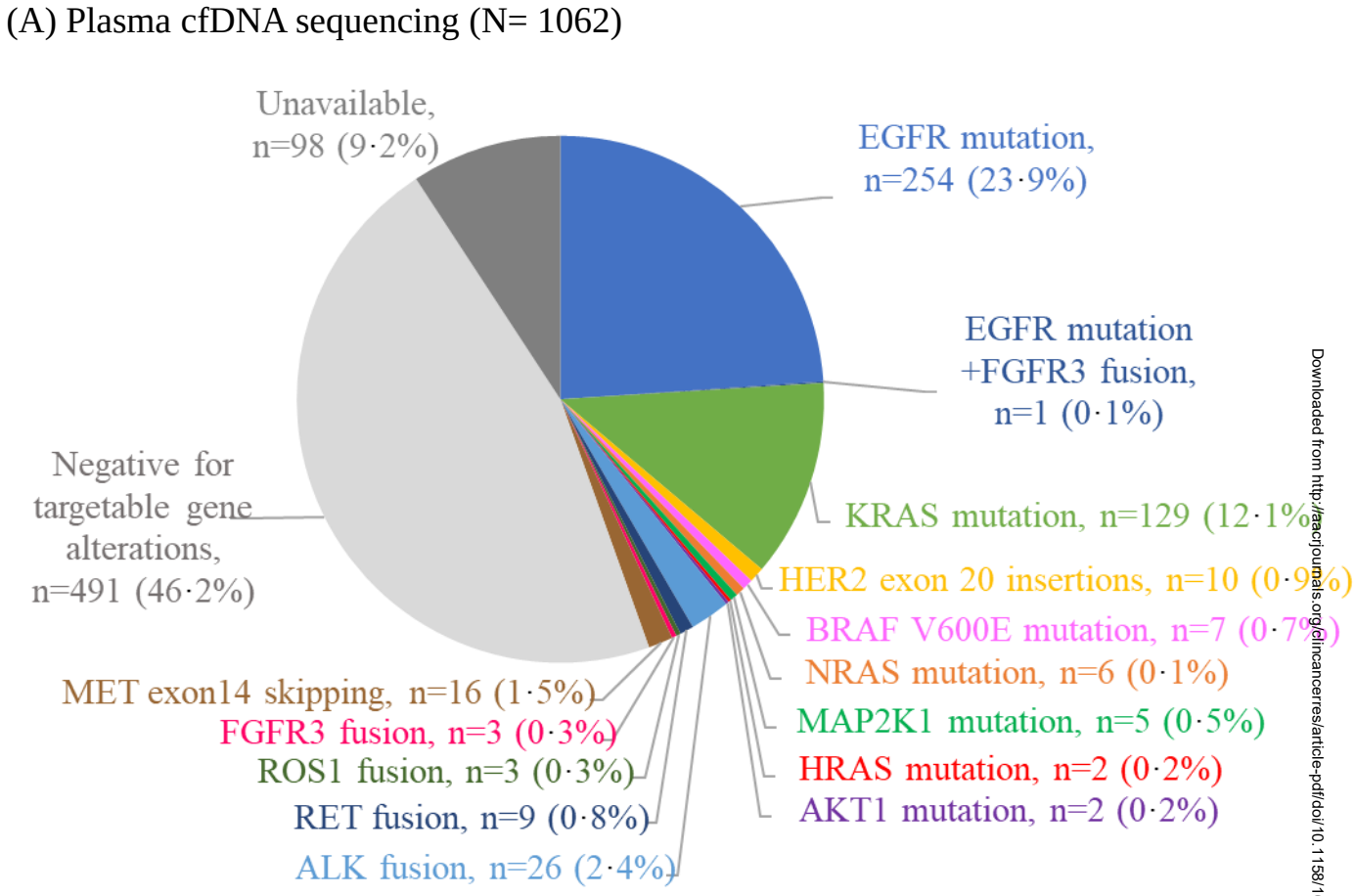
615 **Figure 4. Progression-free survival of patients treated with genotype-matched therapy (A), and**

616 **EGFR-TKI(B) according to the results of plasma cfDNA sequencing and tissue assay.**

617 **(A) Genotype-matched therapy**

618 **(B) EGFR-TKI**

Figure 1. Frequency of the targetable gene alterations detected by plasma cfDNA sequencing (A) and tissue assay (B).



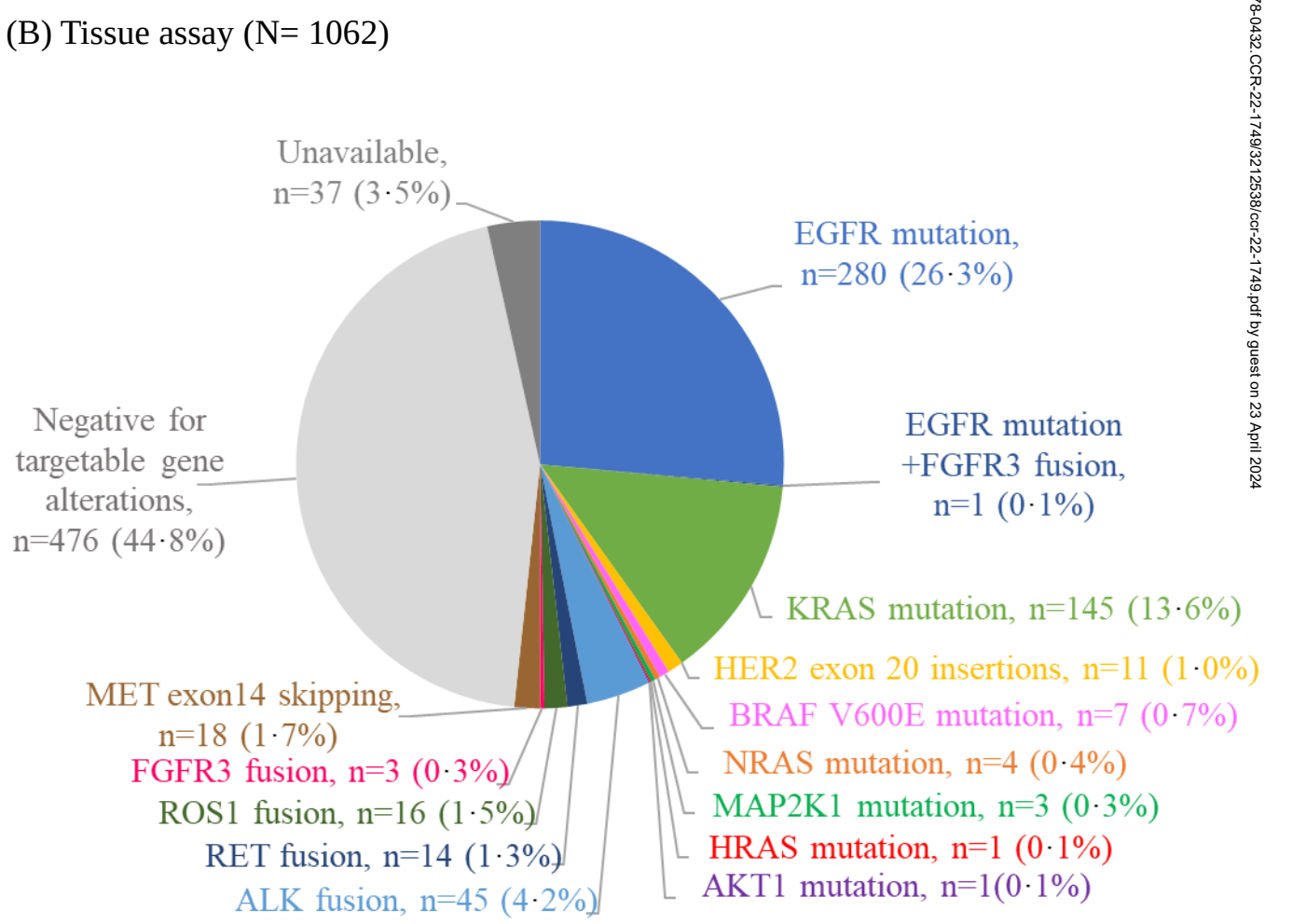
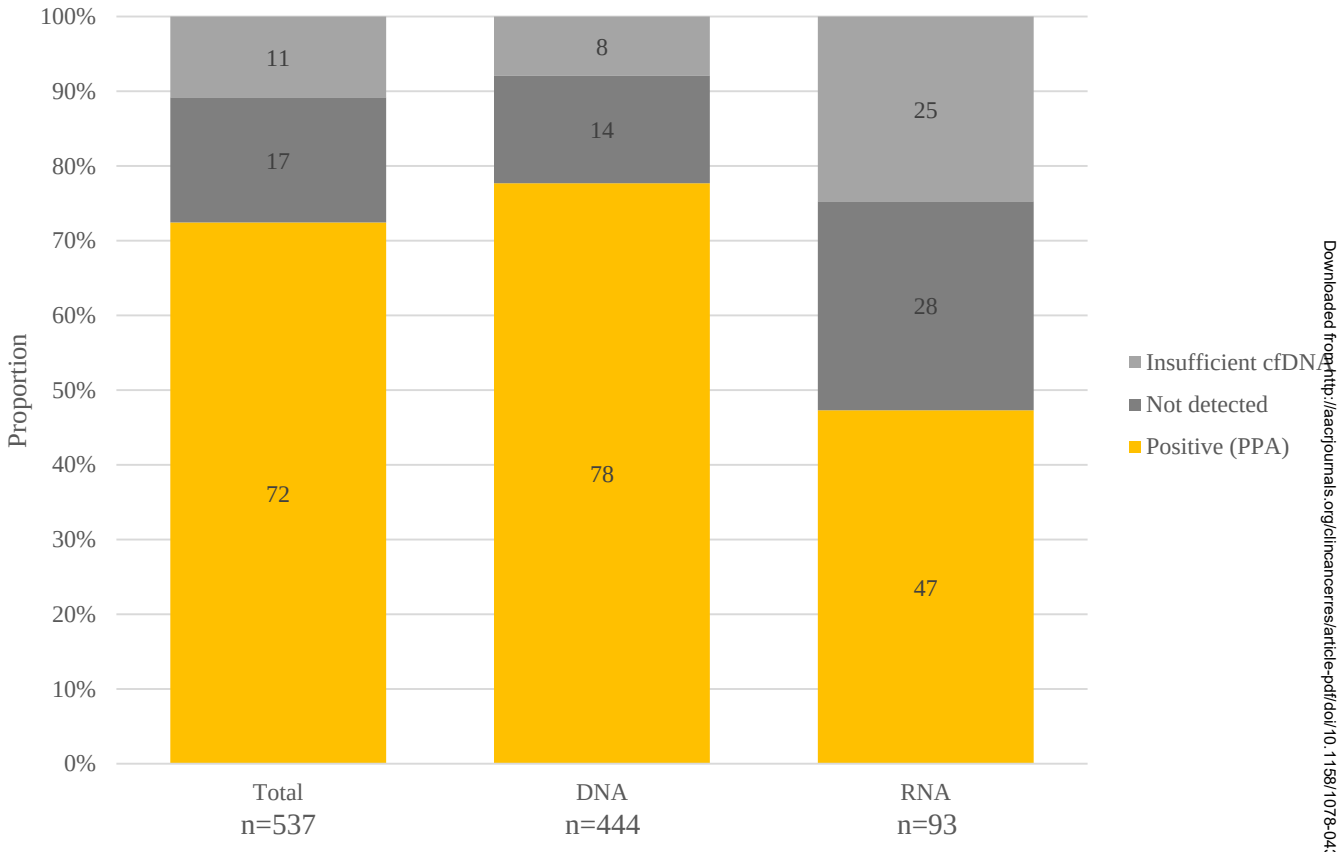


Figure 2. Positive percent agreement of plasma cfDNA sequencing compared to tissue assay. PPA, positive percent agreement.

(A) PPA of plasma cfDNA sequencing compared to tissue DNA or RNA assays.



(B) PPA of plasma cfDNA sequencing for eight oncogenic alterations

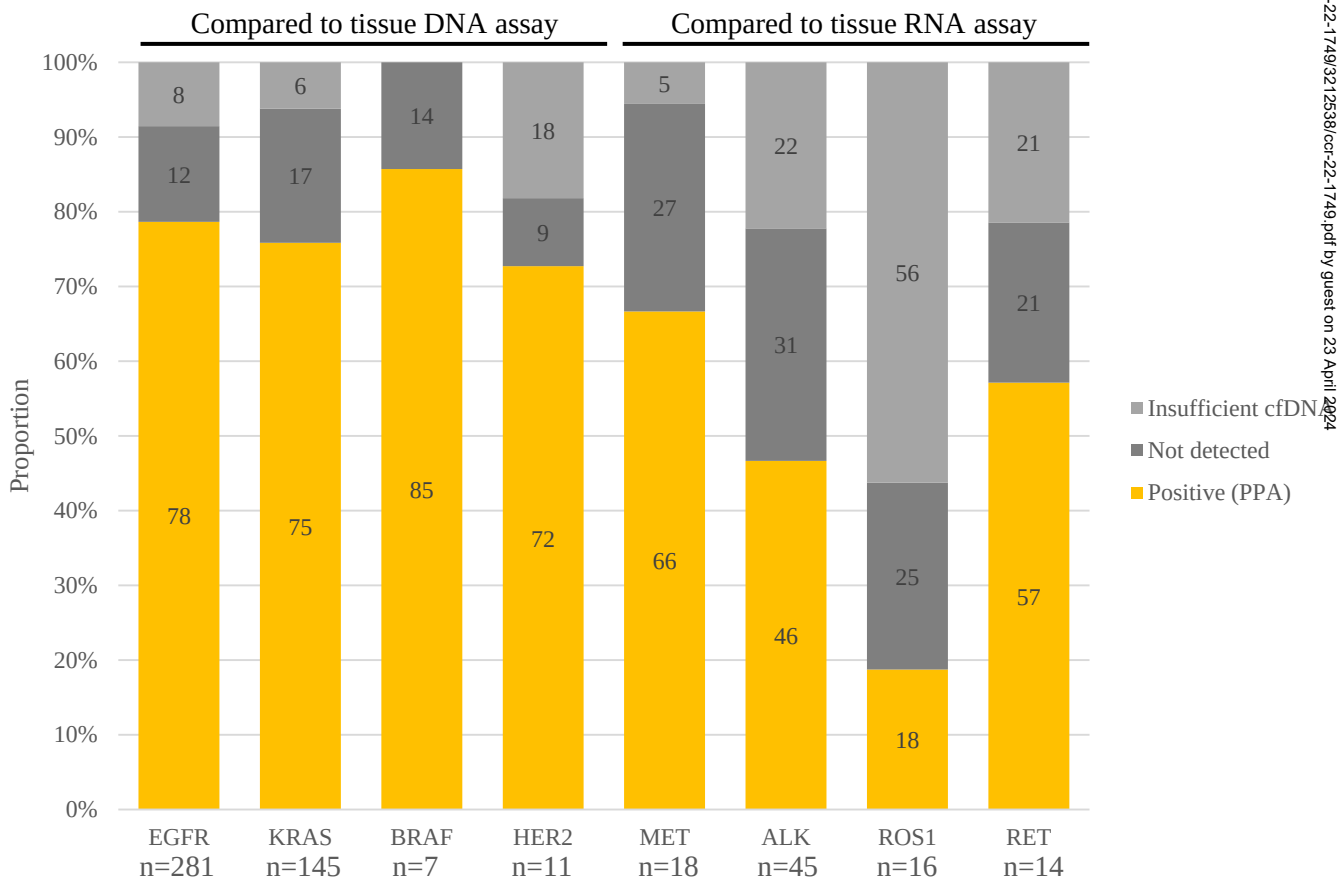


Figure 3. Discordant cases between plasma cfDNA sequencing and tissue assay.

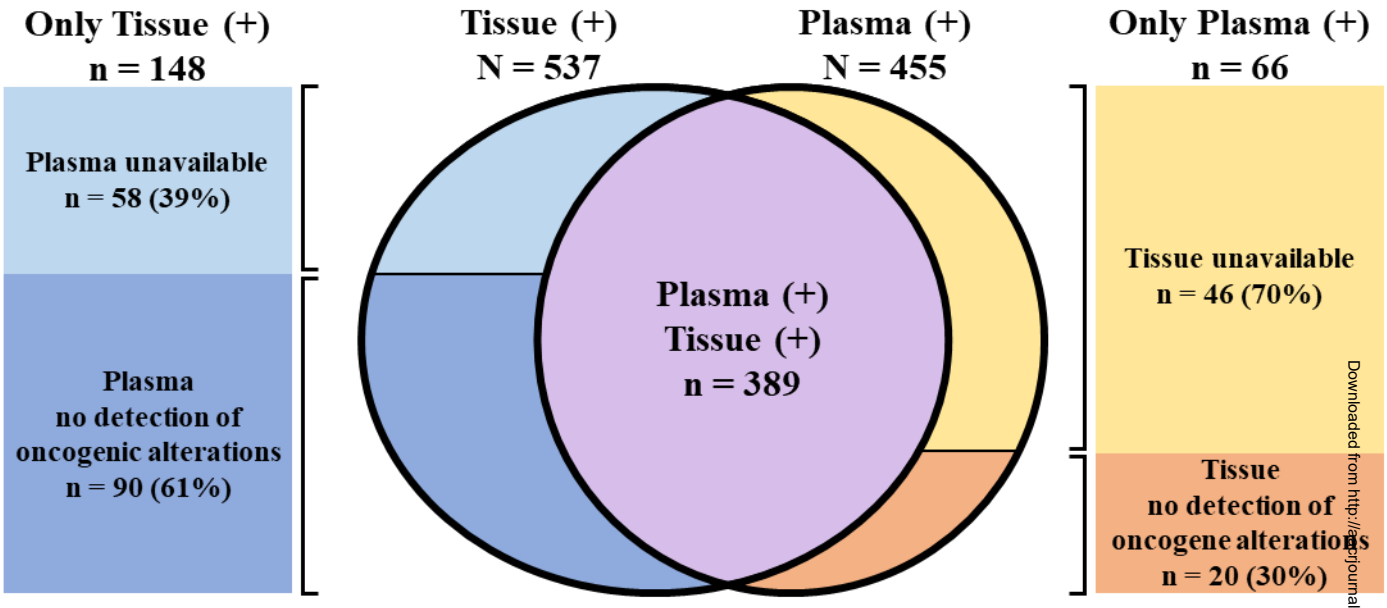
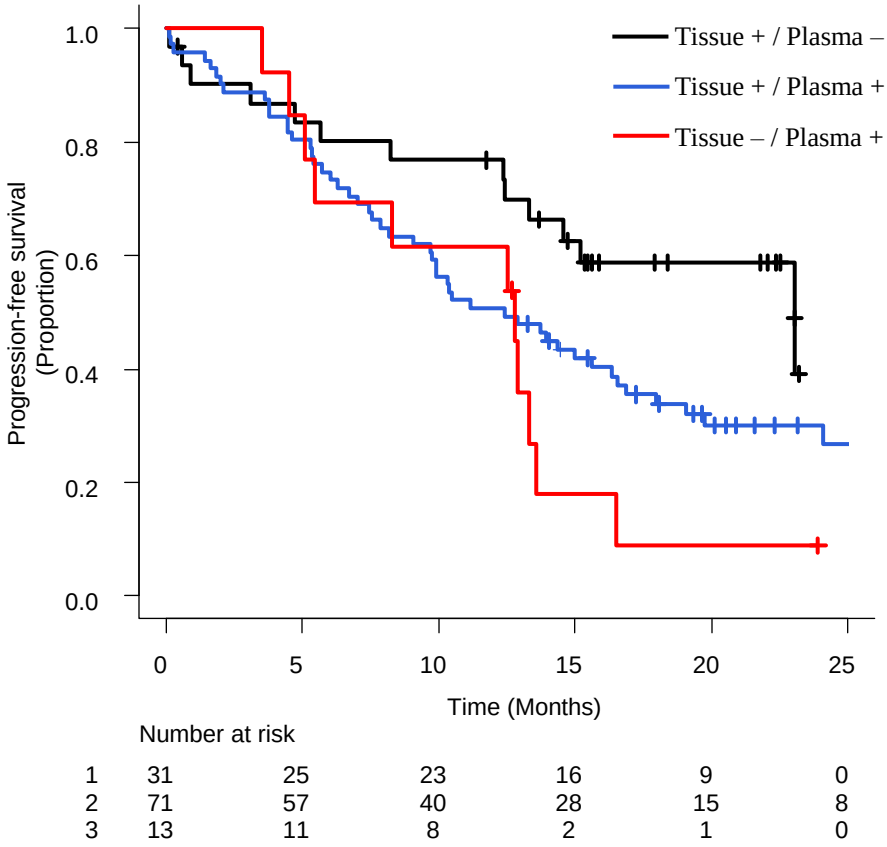


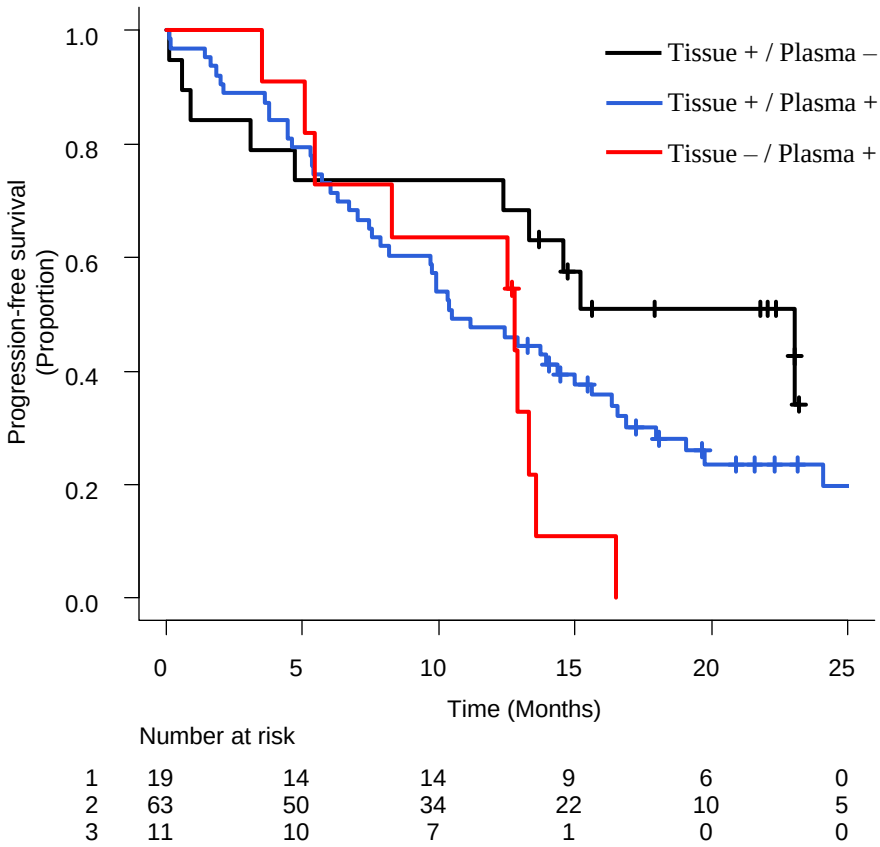
Figure 4. Progression-free survival of patients treated with genotype-matched therapy (A), and EGFR-TKI(B) according to the results of plasma cfDNA sequencing and tissue assay.

(A) Genotype-matched therapy



	n	Median PFS month (95% CI)
Tissue + / Plasma – (T group)	31	23·0 (12·4–NR)
Tissue + / Plasma + (TP group)	71	12·4 (9·1–16·3)
Tissue – / Plasma + (P group)	13	12·7 (5·0–13·5)

(B) EGFR-TKI



	n	Median PFS month (95% CI)
Tissue + / Plasma – (T group)	19	23·0 (4·7–NR)
Tissue + / Plasma + (TP group)	63	10·4 (7·8–15·0)
Tissue – / Plasma + (P group)	11	12·7 (5·0–13·5)

Table1 Patient characteristics

Characteristics	Total (N=1062)
Age, median (range), years	69 (25-91)
Sex, n (%)	
Male	644 (61)
Female	418 (39)
Smoking history, n (%)	
Never	324 (31)
Current or former	733 (69)
Unknown	5 (0·4)
ECOG-PS, n (%)	
0	419 (39)
1	643 (61)
Stage, n (%)	
III	152 (14)
IV	851 (80)
Recurrence	59 (6)
Line of therapy, n (%)	
0	992 (93)
1-2	70 (7)
Histology, n (%)	
Adenocarcinoma	818 (77)
Squamous cell carcinoma	149 (14)
Others	95 (9)
Number of metastatic sites, n (%)	
0	151 (14)
1	348 (33)
2	235 (22)
3 or more	154 (15)
Unknown	174 (16)
Site of Metastasis, n (%)	
Brain	181 (17)
Lung	324 (31)
Pleural dissemination or pleural effusion	258 (24)
Liver	66 (6)
Adrenal grand	71 (7)
Bone	258 (24)
Type of tissue biopsy, n (%)	
Fresh frozen	956 (90)
FFPE	20 (2)

Cytology specimen	86	(8)
Tissue biopsy site, n (%)		
Lung	640	(60)
Lymph node	225	(21)
Pleural effusion	113	(11)
Pleura	26	(2)
Brain	17	(2)
Skin and soft tissue	12	(1)
Bone	14	(1)
Others	15	(1)

ECOG-PS, Eastern Cooperative Oncology Group performance status

FFPE, formalin fixed paraffin embedded