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Circulating Tumor DNA Analysis for Patients with Oncogene-Addicted NSCLC With Isolated Central Nervous System Progression

Mihaela Aldea, MD, PhD,^a Lizza Hendriks, MD, PhD,^b Laura Mezquita, MD, PhD,^a Cécile Jovelet, PharmD, PhD,^c David Planchard, MD, PhD,^a Edouard Auclin, MD, PhD,^d Jordi Remon, MD,^{a,e} Karen Howarth, PhD,^f Jose Carlos Benitez, MD,^{a,g} Anas Gazzah, MD,^h Pernelle Lavaud, MD,^a Charles Naltet, MD,^a Ludovic Lacroix, PharmD, PhD,^c Frank de Kievit, BAS,^f Clive Morris, MD, MBA,^f Emma Green,^f Maud Ngo-Camus,^h Etienne Rouleau, PharmD, PhD,^c Christophe Massard, MD, PhD,^h Caroline Caramella, MD,ⁱ Luc Friboulet, PhD,^{j,k} Benjamin Besse, MD, PhD^{a,k,*}

^aDepartment of Cancer Medicine, Gustave Roussy Cancer Centre, Villejuif, France

^bDepartment of Pulmonary Diseases, GROW - School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands

*Corresponding author.

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Address for correspondence: Benjamin Besse, MD, PhD, Department of Cancer Medicine, Gustave Roussy Cancer Centre, Villejuif, France. E-mail: benjamin.besse@gustaveroussy.fr

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^cDepartment of Medical Biology and Pathology, Gustave Roussy Cancer Campus, Villejuif, France

^dDepartment of Medical and Gastrointestinal Oncology, Hôpital Européen Georges Pompidou, René Descartes University, Paris, France

^eDepartment of Medical Oncology, Centro Integral Oncología Clara Campal Barcelona, HM-Delfos, Barcelona, Spain

^fInivata Ltd., Granta Park, Cambridge, United Kingdom

^gHospital Universitari Mutua Terrassa, University of Barcelona, Terrassa, Spain

^hDrug Development Department (DITEP), Gustave Roussy, Villejuif, France

ⁱDepartment of Radiology, Gustave Roussy Cancer Centre, Villejuif, France

^jINSERM U981, Gustave Roussy Cancer Campus, Paris Saclay University, Villejuif, France

^kParis-Sud University, Orsay, France

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ABSTRACT

Introduction: In patients with oncogene-addicted NSCLC and isolated central nervous system progression (iCNS), tissue biopsy is challenging, and the clinical utility of plasma liquid biopsy (i.e., circulating tumor DNA [ctDNA]) is unknown.

Methods: Patients with advanced NSCLC with known baseline genomic alteration (GA) (*EGFR*, *ALK*, *BRAF*, *KRAS*, *HER2*, *ROS1*, *MET*, *PIK3CA*, *STK11*, *TP53*) on tissue were divided into three groups on the basis of their disease progression pattern: iCNS, extra-CNS only (noCNS), or both (cCNS). All patients with available plasma ctDNA were included and were analyzed by next-generation sequencing InVisionFirst-Lung. ctDNA was considered positive if at least one GA was detected. Cell-free tumor DNA was analyzed in cerebrospinal fluid when available.

Results: Out of 517 patients screened, 247 were included: 54 had iCNS, 99 had noCNS, and 94 had cCNS progressive disease (64, 128, and 110 ctDNA samples, respectively). CtDNA was positive in 52% iCNS versus 84% in noCNS and 92% in cCNS ($p < 0.00001$), with lower detection of driver (37% versus 77% and 73%, respectively) and resistance alterations (6% versus 45% and 44%). Patients with iCNS and positive ctDNA were more at risk of extra-CNS progression (32% versus 7%, $p = 0.026$). In 12 patients with iCNS, ctDNA was positive in six (50%) plasma and in 10 (83%) paired cerebrospinal fluid ($p = 0.193$).

Conclusions: Although tagged amplicon-based next-generation sequencing has high detection rates of GA in plasma ctDNA in patients with NSCLC with extra-CNS disease, detection rate of GAs (52%) is lower in the subset of patients with iCNS disease. Complementary tests such as cerebrospinal fluid cell-free DNA may be useful. Further evidence would be beneficial to understand the genomic landscape in patients with NSCLC and iCNS.

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Keywords: Liquid biopsy; ctDNA; NSCLC; Brain; Leptomeningeal

Introduction

Central nervous system (CNS) metastases are frequently found in oncogene-addicted NSCLC, with percentages reaching up to 50% for brain and 10% for leptomeningeal metastases.¹⁻³ Especially in patients treated with first-generation tyrosine kinase inhibitors (TKIs), isolated CNS progression (iCNS) is common, potentially because of the blood-brain and blood-tumor barriers.⁴ If the planned treatment is to change the TKI, subsequent TKIs are preferentially selected on the basis of the resistance mechanism to the current TKI.

Although genomic profiling on tissue is considered the standard procedure, analysis fails in up to 25% of the cases.⁵ One potential alternative source of tumor DNA can be identified from plasma. Circulating tumor DNA (ctDNA), also referred to as “liquid biopsy” has emerged as an ideal minimally invasive tool, especially in the case of hard-to-biopsy lesions or insufficient tissue material.^{6,7} It is well established that oncogenic drivers and resistance mutations are detected in ctDNA with high sensitivity and specificity.^{6,8}

In the case of iCNS, physiological and/or pathologic factors such as blood-tumor barrier could reduce the presence of ctDNA in the bloodstream and subsequently reduce the accuracy of liquid biopsy for genomic alteration (GA) detection. This is supported by several studies in patients with primary brain tumors or CNS-restricted metastases.^{3,9-11} However, the current evidence is limited in the absence of large series performed with adequate and highly sensitive genomic assays, in particular in patients with oncogene-addicted NSCLC. In this study, we aimed to assess the clinical utility of liquid biopsy through detection of GA in plasma ctDNA from patients with oncogene-addicted NSCLC and isolated CNS progression.

Materials and Methods

Patients and Samples

Patients with NSCLC were prospectively enrolled in a study evaluating liquid biopsy (NCT02666612) from January 2016 to November 2018 at Gustave Roussy.

Cases were selected for our study on the basis of the following inclusion criteria: (1) stage IV disease; (2) known tissue GA at baseline in *EGFR*, *ALK*, *BRAF*, *KRAS*, *HER2*, *ROS1*, *MET*, *PIK3CA*, *STK11*, or *TP53*; and (3) at least one liquid biopsy collected at baseline or at the time of disease progression and successfully analyzed by next-generation sequencing (NGS) with InVisionFirst-Lung assay.

Eligible patients were stratified in three groups: (1) iCNS: isolated CNS progression while stable or no extra-CNS disease; (2) noCNS: extra-CNS progression and no CNS involvement; and (3) cCNS: CNS involvement and extra-CNS progression (Table 1).

Radiologic assessment consisted of at least one extra-CNS and one CNS imaging method (body computed tomography [CT] scan or positron emission tomography-CT scan, and brain magnetic resonance imaging [MRI], brain CT scan, and/or spinal MRI). Leptomeningeal disease was diagnosed by cerebrospinal fluid (CSF) cytology or a combination of compatible clinical symptoms and typical MRI findings.¹² Disease progression was assessed per investigator’s criteria. For leptomeningeal involvement, a neurologic aggravation was considered as clinical progression.

Liquid biopsy was performed at baseline and/or at disease progression, within 1 month of the radiologic assessment, resulting in serial plasma ctDNA results for a limited number of patients. CSF were collected when available.

Clinical, pathologic, molecular, and imaging data were retrospectively collected.

Genomic Profiling of ctDNA

Blood (10–20 mL) was collected in ethylenediaminetetraacetic acid or Cell-Free DNA BCT STRECK tubes. Plasma was isolated using a standard operating procedure, and ctDNA analysis was centralized (Inivata, Cambridge, UK, and Research Triangle Park, Durham, NC) using InVisionFirst-Lung, a tagged amplicon-based NGS comprehensive genomic profiling assay that identifies single nucleotide variants, insertions and deletions, copy number variations, and fusions (variant 1 to 3 of *EML4-ALK* fusions and *CD74-*, *SLC34A2-*, *SDC4-*, and *EZR-ROS1*) with whole gene and gene hotspots across a 36-gene panel (Supplementary Fig. 1); methods were previously described.^{8,13,14}

Table 1. Patients’ Stratification According to Their Disease Evolution Pattern

Groups	CNS Progression	Extra-CNS Progression
iCNS	+	-
noCNS	-	+
cCNS ^a	+	+

^astable CNS disease was also allowed in cCNS group.

CSF ctDNA was performed from 2 mL of CSF. Samples were analyzed by direct sequencing (*EGFR* exon 19, 20, 21, 18) or NGS (Sentosa NSCLC Panel, Vela Diagnostics, Singapore) at Gustave Roussy.

Outcomes

The primary objective of the study was to determine the proportion of patients with positive ctDNA, defined as the identification of at least one somatic GA of known pathogenicity, irrespective of its mutant allele fraction (MAF%).

Identified GAs were categorized under the following: actionable drivers (*EGFR* exon 19, 21, 18 and exon 20 insertion, *BRAF*, *HER2* exon 20 insertion, *MET* exon 14 skipping mutations, *ALK* and *ROS1* rearrangements), resistance alterations (*EGFR* T790M, *EGFR* C797S/G, *EGFR* G729S/V, *EGFR* L747P, *ALK* mutations, *MET* D1246N, *EGFR*, *MET* and *HER2* amplifications), or other GA (e.g., *KRAS/NRAS/HRAS*, *MAPK*, *TP53*, *STK11*, *PIK3CA*). GAs were considered actionable if treatment options were available within an FDA/EMA approval or ongoing clinical trials at our institution.

For positive plasma ctDNA, comparisons among MAF % in the different groups were performed. When more than one GA was detected per sample, the highest MAF% was selected for analysis.

For the iCNS group, the occurrence of an extra-CNS progression and the subsequent development of a CNS progression were evaluated. Extra-CNS progression-free survival (PFS) and CNS PFS were calculated and compared between patients with negative and positive iCNS ctDNA.

Statistical Analysis

Statistical analyses were performed using R-Studio version 3.5.2 and IBM SPSS Statistics 20. Median (interquartile-range) values and proportions (percentage) were provided for the description of continuous and categorical variables, respectively. Mean and proportions were compared using *t* test (or ANOVA if appropriate) and chi-square test (or Fisher’s exact test, if appropriate), respectively.

The association between clinical or molecular variables and ctDNA positivity was explored with a logistic regression. First, a univariate analysis was performed. All variables reaching a *p* value less than 0.1 were included in a multivariable. Any *p* value less than 0.05 was considered statistically significant.

Extra-CNS PFS and CNS PFS were defined as the time between the date of ctDNA collection and extra-CNS and CNS disease progression, respectively. Median PFS was estimated with the Kaplan-Meier method and compared with the log-rank test. The median follow-up was calculated with the reverse Kaplan-Meier method.

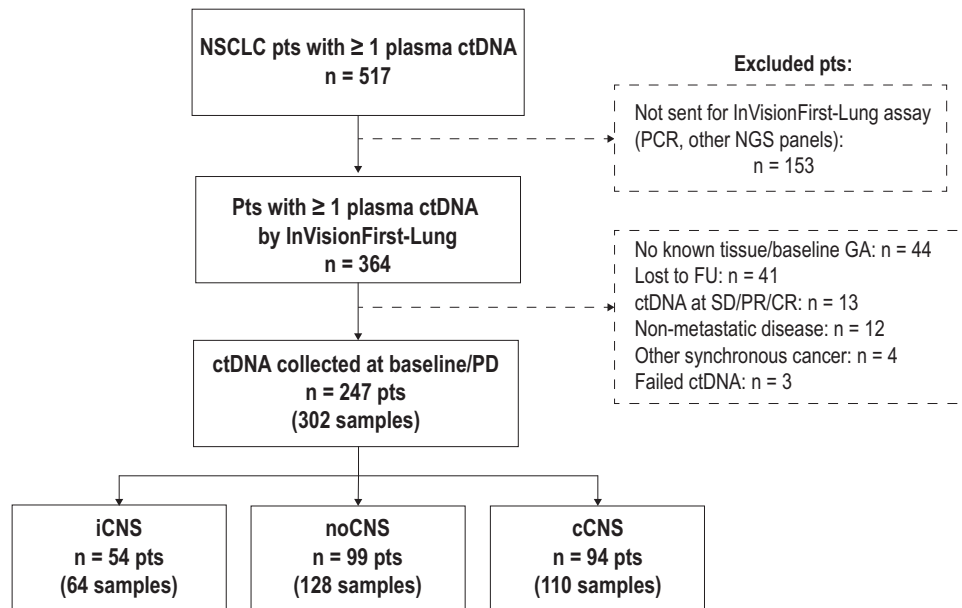


Figure 1. Flowchart of the study population. CR, complete response; ctDNA, circulating tumor DNA (liquid biopsy); GA, genomic alterations; FU, follow-up; n, number; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD, progressive disease; PR, partial response; Pts, patients; SD, stable disease.

Results

Patient Selection and Characteristics

Between January 2016 and June 2018, 517 patients with NSCLC had at least one plasma ctDNA extracted,

and 247 had an InVisionFirst-Lung analysis collected at baseline or at disease progression. A total of 302 plasma samples were available (Fig. 1 – Flowchart), 54 patients had iCNS (64 samples), 99 had noCNS (128 samples),

Table 2. Patients Characteristics

Characteristics	iCNS (n = 54 pts)	noCNS (n = 99 pts)	cCNS (n = 94 pts)	p Value
Age at first-line treatment	–	–	–	0.001
Median (y), range (min, max)	59 (23-83)	65 (23-89)	59 (26-75)	–
Sex	–	–	–	0.072
Male	14 (26%)	40 (40%)	42 (45%)	–
Female	40 (74%)	59 (60%)	52 (55%)	–
History of smoking	–	–	–	0.694
Never/light smoker (<15 PY)	41 (76%)	67 (68%)	64 (68%)	–
Smoker more or equal to 15 PY	13 (24%)	28 (28%)	28 (30%)	–
Histology	–	–	–	0.151
Adenocarcinoma	53 (98%)	89 (90%)	88 (94%)	–
Squamous carcinoma	0	3 (3%)	2 (2%)	–
Other	1 (2%)	7 (7%)	4 (4%)	–
GA at baseline tissue biopsies	–	–	–	–
EGFR mutation exon 19, 21, 18 exon 20 insertions	28 (51%) 0	38 (38%) 4 (4%)	43 (46%) 2 (2%)	–
ALK fusion	17 (31%)	8 (8%)	12 (13%)	–
BRAF V600E mutation	2 (4%)	15 (15%)	8 (9%)	–
KRAS mutation	3 (6%)	17 (17%)	11 (12%)	–
ROS1 fusion	2 (4%)	4 (4%)	4 (4%)	–
HER2 mutation	1 (2%)	3 (3%)	7 (7%)	–
MET mutation	0	2 (2%)	2 (2%)	–
PIK3CA mutation	1 (2%)	3 (3%)	1 (1%)	–
Other (TP53 only, STK11)	0	5 (5%)	4 (4%)	–
Number of metastatic sites at baseline	–	–	–	0.005
Median, range	1 (1-5)	2 (1-6)	2 (1-6)	–
CNS involvement at baseline, yes	38 (70%)	NA	66 (70%)	0.983

CNS, central nervous system; GA, genomic alterations; NA, not applicable; pts, patients; PY, pack-year; y, years.

and 94 had cCNS (110 samples). Patients' and samples' characteristics are depicted in Table 2 and Supplementary Table 1.

The iCNS group included 22 patients with CNS-restricted metastases and 32 patients with isolated CNS progression. Leptomeningeal involvement was more common in the iCNS group compared with the cCNS group (36% versus 10%, $p < 0.0001$). The patients with CNS metastases (iCNS and cCNS groups) were younger, were more likely to have ALK+ adenocarcinoma, and had in 70% of the cases the CNS lesions present at diagnosis (Table 2). Patients with systemic progression had more metastatic sites at diagnosis of advanced disease (2 versus 1) and at the moment of sample collection (3 versus 2), and more failure sites (2 versus 1) ($p < 0.001$, Supplementary Table 1).

The genomic landscape of the overall populations included 46% EGFR mutations, 15% ALK fusions, 13% KRAS, 10% BRAF, 4% HER2 alterations, 4% ROS1 fusions, and in smaller percentages, MET alterations, PI3KCA, STK11, and TP53 mutations (Table 2). Diversity of GA in the systemic progression group was higher than that in the iCNS group, with a higher prevalence of ALK rearrangements in iCNS than in noCNS/cCNS (31% versus 8% versus 13%, $p < 0.01$) (Fig. 2).

iCNS progression was observed in 72% after first-generation TKI, in 23% after second-generation TKI, and in 5% after third-generation TKI (Supplementary Table 1).

Plasma ctDNA Positivity and CNS Invasion

Plasma ctDNA was positive in 52% in iCNS versus 84% in noCNS and 92% in cCNS ($p < 0.00001$) (Fig. 3, Table 3).

In iCNS compared with noCNS/cCNS, the performance of ctDNA analysis was lower for the detection of drivers ($p < 0.00001$), resistance mutation ($p =$

0.00001), and for other GAs ($p < 0.00001$). In line with these results, the detection of EGFR T790M resistance mutation was also significantly lower in patients with progressive iCNS compared with other groups ($p = 0.0002$) (Table 3). There was a slight tendency of the occurrence of more comutations across cCNS/noCNS (75%) compared with iCNS (61%), however without reaching statistical significance ($p = 0.09$).

Serial samples were available for 18 patients in the iCNS group (Supplementary Fig. 2). In seven of 18 cases (38.8%), a negative ctDNA at the time of iCNS shifted to positive when the patient had a systemic progression.

The number of metastatic sites (>2) at sample collection was independently associated with a positive ctDNA in multivariate analysis (odds ratio = 3.4, 95% confidence interval [CI]: 1.7–7.2, $p = 0.001$) (data of univariate analysis are depicted in Supplementary Table 2). The median of MAF% was lower in iCNS group (1% versus 2.7% noCNS and 9.4% cCNS, $p < 0.0001$) (Supplementary Fig. 3).

Clinical Outcomes of Patients With Positive ctDNA in the iCNS Group

In the iCNS group, median follow-up was 36.5 months (95% CI: 31–not reached [NR]). Subsequent CNS progression occurred more frequently than extra-CNS progression (79% versus 19%, $p < 0.0001$). Positive ctDNA was more frequently associated with a subsequent extra-CNS progression compared with negative ctDNA (32% versus 7%, $p = 0.026$), although there were no differences in the clinical characteristics of the two groups. In addition, we did not observe any correlation between positive samples and the burden of CNS disease (we considered multiple brain metastases/leptomeningeal disease as high CNS tumor burden) ($p = 0.93$). The median time to the occurrence of extra-CNS progression was shorter in the positive ctDNA group: 5.5 months (95% CI: 3.22–23)

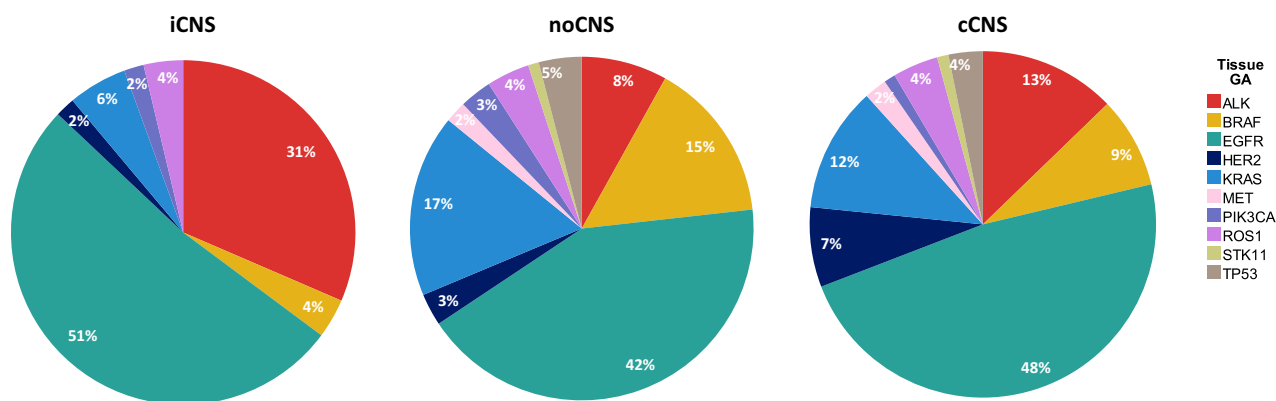


Figure 2. Distribution of GAs at tissue baseline. GA, genomic alteration.

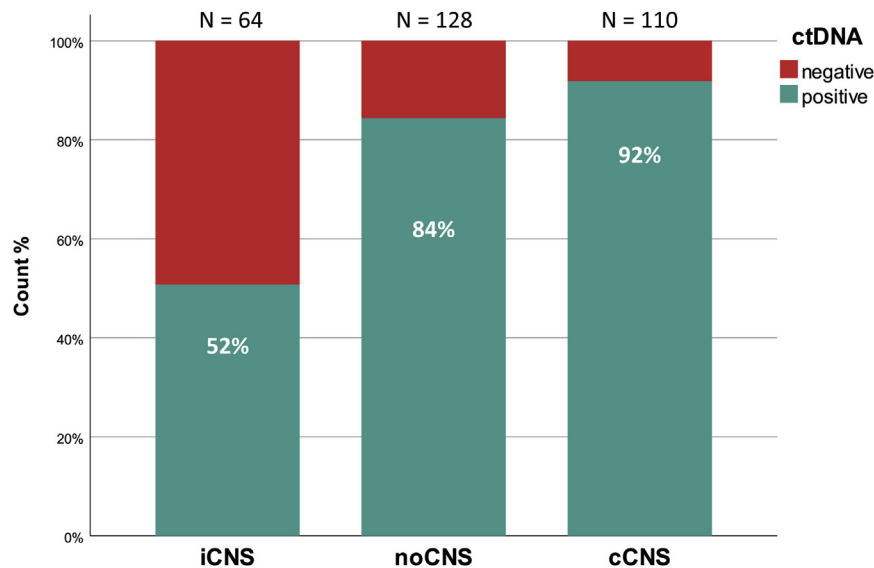


Figure 3. Positive versus negative ctDNA. Results are represented as percentage from the total number of samples from each group. Positive (green); negative (red).

versus NR in negative ctDNA (95% CI: 6.31–NR) (log-rank 0.02).

Paired Plasma - CSF ctDNA

In the iCNS group, paired plasma–CSF liquid biopsies were available for 12 patients (11 with leptomeningeal progression and one with brain progression). Cytology in CSF was positive in all cases with leptomeningeal involvement. Samples were collected from patients with known *EGFR* mutations ($n = 11$) and one patient with *ALK* rearrangement. Plasma ctDNA was positive in six patients (50%), versus 10 patients (83%) in whom tumor mutations were detected in paired CSF samples ($p = 0.193$).

Discussion

We evaluated the clinical utility of plasma ctDNA in patients with oncogene-addicted NSCLC with isolated CNS progression using a highly sensitive NGS assay. The detection rate of 52% positive ctDNA in patients with iCNS was significantly lower than in patients with noCNS

or cCNS, 84% and 92%, respectively. However, 52% positive ctDNA is relatively high compared with results previously reported by De Mattos Arruda et al., in which no GAs were detected in a limited number of patients ($n = 7$, including primary brain tumors).¹¹ One possible explanation is the cutoff of 2% MAF used in the study of De Mattos Arruda et al., compared with a higher sensitivity assay (as low as 0.02%) in our study.^{8,13,15} Our results are in line with a study in patients with primary brain tumors ($N = 419$) in which the rate of positive ctDNA was 51% with the Guardant360 assay.¹⁶ Another study using the Guardant360 assay evaluated patients with NSCLC with brain metastases and GAs were found in 75% of 12 cases, but the status of extra-CNS disease was unknown.¹⁷ To our knowledge, our study is the largest that evaluates isolated CNS progression in a cohort of patients with various known baseline GAs: *EGFR*, *ALK*, *BRAF*, *HER2*, *KRAS*, *MET*, *PIK3CA*, *STK11*, and *TP53*.

Isolated CNS progression mostly occurs in patients with oncogenic drivers when TKIs are used with

Table 3. Prevalence of GA in Plasma ctDNA Samples

Type of GA	iCNS	noCNS	cCNS	p Value
Overall (all)	52% (33/64)	84% (108/128)	92% (101/110)	<0.00001
Actionable drivers (<i>EGFR</i> , <i>ALK</i> , <i>BRAF</i> , <i>HER2</i> , <i>ROS1</i> , <i>MET</i>) ^a	37% (22/60)	77% (79/102)	73% (67/92)	<0.00001
Resistance ^b (all <i>EGFR</i> , <i>ALK</i> , <i>MET</i> at progression) ^a	71% (22/31)	93% (79/85)	82% (68/83)	0.008
	6% (3/50)	45% (18/40)	44% (23/52)	0.00001
<i>EGFR</i> T790M in <i>EGFR</i> patients at progression ^a	13% (3/23)	46% (18/39)	49% (23/47)	0.01
	7% (2/30)	48% (16/33)	50% (20/40)	0.0002
	13% (2/16)	48% (16/33)	53% (20/38)	0.019
Other	31% (20/64)	65% (83/128)	68% (75/110)	<0.00001

^apositive ctDNA only.

^b*EGFR* T790M, *EGFR* C797S/G, *EGFR* G729S/V, *EGFR* L747P, *ALK* mutations, *MET* D1246N, *EGFR*, *MET*, and *HER2* amplifications.

insufficient CNS penetration. Another cause could be the supposed CNS tropism of some oncogenic drivers such as *ALK* rearrangements. In our iCNS cohort, *ALK* rearrangement was detected in a higher frequency compared with patients with systemic progression as most patients were on crizotinib. This is consistent with other studies that report a high CNS tropism for patients with *ALK* rearrangements, reaching a risk of CNS disease of 58% at 3 years¹⁸ and 70% at progression during crizotinib treatment.^{1,19,20}

Importantly, plasma ctDNA detected a very low percentage of resistance mutations (6%) in patients with iCNS, compared with more than 40% in patients with systemic progression, for patients with alterations in *EGFR*, *ALK*, or *MET*. This is in line with the results of Zhang et al, in a prospective observational study of patients with *EGFR*-mutated NSCLC failing first-generation TKI (n = 307). *EGFR T790M* resistance mutation, assessed by plasma ddPCR, was less frequently detected in patients with progression limited to the brain (n = 32) than other sites (21.9% versus 72.3% respectively, $p < 0.001$).²¹ Interestingly, they reported that the resistance mutation was less likely to be detected in patients with disease limited to the chest (40.6%). The main hypothesis is that the tumor burden drives the quantity of ctDNA released into the blood. This is supported by the correlation between the number of tumor sites and the positivity of ctDNA in our study and others.²²⁻²⁴ This might also explain why plasma positivity of iCNS patients is related to higher number of patients with subsequent extra-CNS progression in our study. It is likely that ctDNA is released by infraradiologic lesions that subsequently become detectable. It has also been hypothesized that reduced CNS drug penetration and low exposure of first-generation TKI in CSF might cause a different tumor selection in the CNS with the possibility of retaining sensitizing mutations²⁵⁻²⁸ or with the development of different resistance mechanisms.²⁹ This might explain the different prevalence of *EGFR T790M* that has been reported within studies that compared CSF with plasma.^{9,25,30}

The reliability of GA detection by plasma ctDNA was assessed also by the comparison between paired plasma-CSF samples. There were more positive samples in CSF compared with plasma (10 versus 6), although not statistically significant, possibly because of the small sample number (n = 12). This was in line with other studies that specifically compared CSF with plasma ctDNA and found that CSF ctDNA had higher mutation detection rates and higher MAF%, including patients with NSCLC with CNS-unrestricted metastases (n = 72), *EGFR* (n = 26) and *ALK* cohorts (n = 11).^{3,9,11,30} Moreover, more unique resistance mutations, including a higher number of acquired resistance mutations, were identified in CSF

than in plasma.⁹ Indeed, in patients with low levels of ctDNA, mutations may be more easily detected in CSF as the tumor/normal DNA ratio is higher in CSF compared with plasma. This is due to low levels of non-tumor derived DNA in CSF.^{31,32}

The main limitation of our study is the absence of a comparison with the accepted standard tissue biopsy-derived genomic profile, which for the iCNS subgroup is not feasible to obtain. Other study limitations included the retrospective collection of data (however, patients were included in a prospective study at our institution), the limited number of paired CSF samples, and the fact that it was not possible to distinguish between ctDNA and circulating free DNA in the absence of an identified GA. Thus, correlations between positive/negative samples and ctDNA concentrations were not possible. Another limitation is that InVisionFirst-Lung does not identify all kind of *ROS1* and *ALK* fusions, leaving rarer partners or variants uncovered if present (e.g., *EML4-ALK V4, 5, 6*, other than *EML4* partner fusion). This might result in false-negative liquid biopsies in the *ALK/ROS1* subgroup; however, it is unlikely that this would considerably affect the results, because such variants have a small prevalence in NSCLC.³³

Alternative strategies should be explored in patients with isolated CNS progression. CSF cell-free DNA analysis seems highly promising; however, its role in characterizing patients with asymptomatic and with limited brain lesions is less clear and might also be challenged by low tumor-derived DNA levels in CSF. As in several countries, first-line osimertinib is not yet reimbursed and the presence of the *EGFR T790M* resistance mutation remains mandatory for the use of osimertinib in second-line treatment; therefore, better detection methods for *T790M* or other treatment approaches are needed. In the absence of resistance mutations after first- and second-generation TKIs, an interesting strategy would also be the investigation of plasma and CSF TKI concentrations, which could guide inpatient drug dosage escalations.

Conclusions

Although tagged amplicon-based NGS has high detection rates of GA in plasma ctDNA in patients with NSCLC with extra-CNS disease, detection rate of GA (52%) is lower in the subset of patients with iCNS disease. Complementary tests such as CSF cell-free DNA assay may be useful. Further evidence would be beneficial to understand the genomic landscape in patients with NSCLC and iCNS.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at <https://doi.org/10.1016/j.jtho.2019.11.024>.

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