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Clinical utility of plasma-based digital next-generation sequencing in oncogene-driven non-small-cell lung cancer patients with tyrosine kinase inhibitor resistance

Jon Zugazagoitia[a,b,c,d], Ana Gómez-Rueda[e], Eloisa Jantus-Lewintre[c,f], Dolores Isla[g], Carlos Camps[c,h], Inmaculada Ramos[i], Jose Manuel Trigo[i], Reyes Bernabé[j], Oscar Juan-Vidal[k], Jose Miguel Sanchez-Torres[l], Rosario García-Campelo[m], Mariano Provencio[n], Enriqueta Felip[o], Javier de Castro[p], Iris Faull[q], Richard B. Lanman[r], Santiago Ponce-Aix[a,b], Luis Paz-Ares[a,b,c,s,**], Pilar Garrido[c,e,*]

[a] Medical Oncology Department, Hospital Universitario 12 de Octubre and i+12 Research Institute, Madrid, Spain
[b] Lung Cancer Group, Clinical Research Program, Spanish National Cancer Research Center (CNIO), Madrid, Spain
[c] CIBERONC, Spain
[d] Department of Pathology, Yale School of Medicine, New Haven, CT, USA
[e] Medical Oncology Department, IRYCIS Hospital Universitario Ramón y Cajal, Universidad Alcalá, Madrid, Spain
[f] Molecular Oncology Laboratory, Fundación para la Investigación del Hospital General Universitatio de Valencia, Biotechnology Department, Universitat Politècnica de València, Spain
[g] Medical Oncology Department, Hospital Universitario Lozano Blesa, Zaragoza, Spain
[h] Medical Oncology Department, Hospital General Universitario de Valencia, Medicine Department, Universidad de Valencia, Spain
[i] Medical Oncology Department, Hospital Universitario Virgen de la Victoria, Málaga, Spain
[j] Medical Oncology Department, Hospital Universitario Vírgen del Rocío, Sevilla, Spain
[k] Medical Oncology Department. Hospital Universitari i Politècnic La Fe, Valencia, Spain
[l] Medical Oncology Department, Hospital Universitario La Princesa, Madrid, Spain
[m] Medical Oncology Department, Hospital Universitario Da Coruña, A Coruña, Spain
[n] Medical Oncology Department, Hospital Universitario Puerta de Hierro, Madrid, Spain
[o] Medical Oncology Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain
[p] Medical Oncology Department. Hospital Universitario La Paz, Madrid, Spain
[q] Medical affairs, Guardant Health, Barcelona, Spain
[r] Medical affairs, Guardant Health, Redwood City, California
[s] Complutense University, Madrid, Spain

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ABSTRACT

Objectives: Resistance to tyrosine-kinase inhibitors (TKIs) is a clinical challenge in patients with oncogene-driven non-small-cell lung cancers (NSCLC). We have analyzed the utility of next-generation sequencing (NGS) of cell-free circulating tumor DNA (ctDNA) to impact the clinical care of patients with TKI resistance.

Materials and methods: We conducted a multi-institutional prospective study including consecutive *EGFR*, *ALK*, or *ROS1*-altered NSCLC patients with TKI resistance from 12 Spanish institutions. Post-progression ctDNA NGS was performed by Guardant Health (Guardant360 assay).

Results: We included 53 patients separated in 3 cohorts: 31 *EGFR*-mutant NSCLCs with first/second-generation TKI resistance (cohort 1), 15 *EGFR* T790M + NSCLCs with osimertinib resistance (cohort 2), and 7 *ALK/ROS1*-rearranged NSCLCs with crizotinib and/or next-generation TKI resistance (cohort 3). Besides Guardant360, 22 patients from cohort 1 (71%) underwent post-progression tumor biopsies and/or alternative plasma-based genotyping. In the entire study population, 34 patients (64%) had reliable evidence of tumor-DNA shed for resistance assessment, and 24 patients (45%) had actionable alterations. Target-independent pathogenic alterations were frequently detected, particularly at osimertinib resistance. Eleven patients (20%) received subsequent molecular-guided therapies indicated by plasma NGS alone (n = 9, 17%), or plasma NGS and tissue

* Corresponding author at: Servicio de Oncología Médica, Universidad Alcalá, Hospital Universitario Ramon y Cajal, Ctra. Colmenar Viejo, km. 9,100, 28034 Madrid, Spain.
** Corresponding author at: Servicio de Oncología Médica, Hospital Universitario 12 de Octubre, Av. de Córdoba Km 5.4, 28041 Madrid, Spain.
E-mail addresses: lpazares@seom.org (L. Paz-Ares), pilargarridol@gmail.com (P. Garrido).

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sequencing (n = 2, 4%), deriving the expected clinical benefit. Of these, 9 had *EGFR* T790 M mutation and received osimertinib, 1 had *ALK* G1202R mutation and received lorlatinib, and 1 had *ROS1* G2032R mutation and received cabozantinib. Two additional cases from cohort 1 (6%) had undetectable *EGFR* T790 M by Guardant360 but were T790M + by tissue and BEAMing digital PCR respectively, and also received osimertinib. *Conclusion:* NGS of ctDNA detects actionable alterations in a large proportion of oncogene-driven NSCLC patients with TKI resistance, and can be used to guide subsequent treatments as a complement or alternative to tissue or PCR-based plasma genotyping in the real-world clinical setting.

1. Introduction

Tyrosine-kinase inhibitors (TKIs) induce relatively durable responses in most patients with *EGFR*, *ALK*, or *ROS1*-altered non-small-cell lung cancer (NSCLC) patients, but resistance invariably develops at a median of about 10–15 months of therapy [1–5].

Next-generation sequencing (NGS) of cell-free circulating tumor DNA (ctDNA) could be utilized to select appropriate molecularly-guided therapies in oncogene-driven NSCLC patients progressing on TKI therapy. The main advantage of this technology in the resistance setting includes the assessment of target-dependent as well as target-independent actionable resistance alterations in a single assay, avoiding the need of repeated tumor biopsies in some cases [6]. However, the utility of plasma-based NGS to impact the therapeutic management of TKI-resistant NSCLC patients in routine clinical practice has not been properly addressed in prospective studies thus far.

In the present study, we have performed targeted ctDNA NGS in a multi-institutional prospective cohort of *EGFR*, *ALK*, or *ROS1*-altered NSCLC patients with TKI resistance. Our primary objective was to prospectively assess the real-world clinical utility of plasma-based NGS in the TKI resistance setting.

2. Methods

Institutional Ethics Committee approval was obtained before this study was initiated. A single protocol was contemporaneously distributed across 12 Spanish academic institutions that participated in this prospective study. Between January and September 2017, consecutive patients with *EGFR*, *ALK*, or *ROS1*-altered advanced-stage NSCLC who experienced clinical or radiological progression on prior TKI therapy were eligible for plasma-based NGS, irrespective of the timepoint when TKI progression had occurred or what the most recent treatment was. All patients provided signed informed consents before plasma genotyping and were subsequently registered in the study.

We obtained blood samples from patients and ctDNA was isolated from plasma. Digital NGS of ctDNA was performed by Guardant Health, Inc. (Redwood City, CA) in all patients, using a hybrid-capture-based NGS panel detecting all four major types of genetic alterations in 73 genes (Guardant360 assay, supplementary methods). Detailed protocols for ctDNA isolation, sequencing and data analysis have been previously described [7,8] (supplementary methods).

Patients were considered to have reliable evidence of tumor DNA shed for adequate clinical interpretation of plasma findings in the resistance setting (classified as “shedders”) when their known initial driver alteration was detected in ctDNA [9].

We annotated pathogenic genomic alterations detected after TKI resistance in 3 levels of actionability according to OncoKB precision oncology knowledge base [10]: Level R1, standard of care resistance alterations that predict sensitivity to FDA or EMA-approved drugs in that indication (*EGFR* T790 M mutations); Level R2, clinically described resistance alterations with compelling clinical evidence for drug response, but neither the alteration nor the drug is standard or care in the resistance setting (e.g. *MET* amplifications); Level R3, resistance alterations with biological but not clinical evidence for drug response (e.g. *PI3KCA* mutations). In the case of *MET* amplifications, only high-level amplifications (reported as 3+, [supplementary methods]) were considered therapeutically actionable. Variants not clustering within any of these actionable subgroups were annotated as non-actionable genomic alterations [10].

We used *t*-test to compare the mean number of genomic alterations between two groups. Progression-free survival (PFS) was defined as the time interval between the date of TKI initiation and the date of radiological and/or clinical disease progression or loss of follow-up. All hypothesis testing was performed at a two-sided significance level of $\alpha = 0.05$.

3. Results

We included 53 patients that were divided in three cohorts: 31 *EGFR*-mutant NSCLCs with resistance to first/second-generation EGFR TKIs (cohort 1), 15 *EGFR* T790M + NSCLCs with osimertinib resistance (cohort 2), and 7 *ALK/ROS1*-rearranged NSCLCs with resistance to crizotinib and/or next-generation ALK/ROS1 TKIs (cohort 3). The baseline characteristics of these patients (at the time of plasma sequencing) are summarized in Table 1.

The median time interval between TKI progression and plasma collection was 18 days (range 0–481), and the median turnaround time for ctDNA results was 12 days (range 8–19). Besides Guardant360, post-progression tumor biopsies or alternative plasma-based genotyping methods (supplementary table S1) were concurrently (within 1 month) performed in 17 patients (55%) and 10 patients (32%) respectively in cohort 1 (Fig. 1a). NGS of ctDNA (Guardant360) was the only method for resistance assessment in cohorts 2 and 3.

In the entire study population, 34 patients (64%) had reliable evidence of tumor-DNA shed for resistance assessment, and actionable alterations were detected in 24 patients (45%). The mean number of metastatic sites was higher among shedders (2.3 vs. 1.6, p = 0.06), and the presence of liver metastasis was significantly more frequent among them (p = 0.009).

3.1. First/second-generation EGFR TKI-resistant cohort

Most patients (n = 28, 90%) received only one TKI before plasma NGS (Table 1), and the median PFS on prior TKI was 13.8 months (range 3.9–36.7).

Twenty patients (65%) were tumor DNA shedders. At least one pathogenic alteration in addition to the initial *EGFR* sensitizing mutation was found in 17 patients (55%), and 14 patients (45%) had actionable genomic alterations (Fig. 1a).

Using NGS of ctDNA (Guardant360), *EGFR* T790 M mutations were seen in 9 patients (29% of the entire cohort, 45% of the shedders). In 2 additional cases (6%), one shedder (*EGFR* del19 plus *EGFR* D761 N mutations detected) and the other non-shedder, the *EGFR* T790 M mutation was not detected by the Guardant360 assay but found with BEAMing digital PCR and tissue genotyping, respectively.

The mean number of non-*EGFR* pathogenic alterations detected with NGS of ctDNA was similar in T790M + and T790M- patients, but those alterations co-occurring with *EGFR* T790 M were mostly non-actionable alterations (only one patient had co-occurrence of T790 M and *BRAF* V600E mutations) (Fig. 1b).

Nine patients (29%), all of them with *EGFR* T790 M mutations, received subsequent osimertinib therapy indicated by plasma NGS alone

Table 1
Baseline clinical characteristics of the patients.

Characteristic	Cohort 1 (1st/2nd-gen EGFR TKI)	Cohort 2 (Osimertinib)	Cohort 3 (ALK/ROS1 TKI)
Total	31	15	7
Median age (range)	68 (36–85)	69 (53–86)	48 (39–74)
Gender			
Female	22 (71)	10 (67)	2 (29)
Male	9 (29)	5 (33)	5 (71)
Smoking history			
Never smoker	19 (61)	11 (73)	5 (71)
Former smoker	10 (32)	4 (27)	1 (14)
Current smoker	2 (7)	0	1 (14)
Performance status			
0	13 (42)	2 (13)	4 (57)
1	14 (45)	10 (67)	2 (29)
> 1	4 (13)	3 (20)	1 (14)
Stage			
III	1 (3)	0	0
IV M1a	10 (32)	2 (13)	2 (29)
IV M1b	0	0	1 (14)
IV M1c	20 (65)	13 (87)	4 (57)
Nº of metastatic sites			
< 3	22 (71)	10 (67)	4 (57)
≥3	9 (29)	5 (33)	3 (43)
Brain metastasis			
Yes	8 (26)*	4 (27)*	3 (43)*
No	23 (74)	11 (73)	4 (57)
Liver metastasis			
Yes	7 (23)	5 (33)	2 (29)
No	24 (77)	10 (67)	5 (71)
Bone metastasis			
Yes	13 (42)	7 (47)	2 (29)
No	18 (58)	8 (53)	5 (71)
Nº of lines of TKI			
1	28 (90)	0	5 (71)
2	2 (7)	9 (60)	2 (29)
> 2	1 (3)	6 (40)	0
TKI before plasma NGS			
Gefitinib	8 (26)		
Erlotinib	11 (35)		
Afatinib	15 (48)		
Osimertinib		15 (100)	
Crizotinib			5 (71)
Brigatinib			1 (14)
Lorlatinib			1 (14)

*A total of 3 patients (5%, 1 per cohort) had disease confined only in the central nervous system (CNS) at baseline (isolated CNS progression).

(n = 7, 23%) or plasma NGS and tissue sequencing (n = 2). The 2 patients with *EGFR* T790 M mutations detected by tissue or BEAMing also received osimertinib (Fig. 1a). The median PFS in the 11 patients receiving subsequent osimertinib was 10.4 months (range 0.7–16.8). The presence of pre-treatment non-*EGFR* pathogenic alterations in plasma did not seem to result in shorter PFS (Fig. 1c).

3.2. Osimertinib-resistant cohort

All 15 patients had T790M + disease and received osimertinib as a second or later line of treatment (Table 1). The median PFS on prior osimertinib was 9.1 months (range 1.3–32.2) (Supplementary Fig. 1).

Ten patients (67%) were tumor DNA shedders. At least one pathogenic alteration in addition to the *EGFR* sensitizing and/or T790 M mutation was detected in 9 patients (60%), and actionable alterations were seen in 6 patients (40%), including (1 patient each): *EGFR* C797S mutation (7%), *EGFR* G724S mutation (7%), *MET* amplification (13%), *MAP2K1* D67 N mutation (7%), *BRAF* V600E mutation (7%), *PI3KCA* E545 K mutation (7%), and *STRN-ALK* fusion (7%) (Fig. 2a). Only 2 cases (13%) had T790 M mutation detectable in plasma at osimertinib progression, and both had late resistance on prior osimertinib (supplementary Fig. 1). Among shedders, the mean number of non-*EGFR* pathogenic alterations was higher at osimertinib resistance (3.5) than at first/second-EGFR TKI resistance (1.7) (p = 0.06) (Fig. 2b). No patient received subsequent matched targeted therapies in this cohort.

3.3. ALK/ROS1 TKI-resistant cohort

Four patients had *ALK*+ disease and 3 had *ROS1*+ disease (Table 1). The median PFS on the last TKI was 7 months (range 2.9–29.6).

Four patients (57%) were shedders (*ALK/ROS1* fusions and/or kinase domain mutations detected in ctDNA), and actionable alterations were seen in all these 4 cases. Kinase-domain mutations were found in 3 patients (43%), in 2 of them co-existing with non-*ALK/ROS1* pathogenic alterations. The non-*ALK/ROS1* actionable alterations were: *BRAF* V600E and D594 G mutations (2 patients), *NF1* mutations (1 patient), and *PI3KCA* E545 K mutation (1 patient) (Fig. 3a).

Six patients received subsequent ALK/ROS1 TKIs (Fig. 3b), but these were molecularly-informed based on NGS of ctDNA only in 2 cases (28%), both of which had received prior crizotinib and a next-generation TKI. In the first patient, plasma NGS after brigatinib revealed multiple *ALK* kinase domain mutations (including G1202R) and was subsequently treated with lorlatinib, showing early resistance (3.8 months). In the second patient, plasma NGS after lorlatinib revealed a *ROS1* G2032R kinase domain mutation and was subsequently treated with cabozantinib, experiencing a partial response (8 months).

4. Discussion

In this multicenter prospective study, we show that NGS of ctDNA detects both target-dependent and target-independent actionable alterations in the majority of NSCLC patients with TKI resistance, and informs subsequent treatments in a relatively high proportion of these patients in routine clinical practice.

For the purposes of this study, we considered patients as tumor DNA shedders only when their known initial driver alteration was detected in plasma. Although the detection of any ctDNA alteration could potentially indicate tumor DNA release, in patients with oncogene-driven NSCLCs the detection of the ubiquitously present driver alteration is considered as the strongest indicator of the presence of tumor DNA [9], and this is perhaps a more appropriate definition for sufficient ctDNA to assess for resistance alterations at TKI progression. Thus, in this context, the detection of actionable alterations in plasma can be considered informative and reliable to correctly inform subsequent therapies in clinical practice [9]. Using this criterion, about 65% of the *EGFR*-mutant NSCLCs and 57% of the *ALK/ROS1*-rearranged NSCLCs in this study had reliable evidence for tumor DNA shed after TKI resistance. These proportions, particularly in the case of *EGFR*-mutant NSCLCs, are somewhat lower than those reported in other series or clinical trial cohorts (≈ 70–80 % of shedders in *EGFR*-mutant NSCLC) [11–15]. Of note, three patients (5%) in our study had isolated central nervous system (CNS) progression when they underwent plasma-based NGS, a clinical situation where the performance of ctDNA analysis has been shown to be suboptimal [16]. In addition, plasma-based NGS was not performed immediately after TKI progression in some of the patients included in our study (n = 9 patients [17%] underwent plasma sequencing beyond 3 months after TKI progression), which could have potentially underestimated the proportion of tumor DNA shedders. Alternatively, these results might truly reflect the sensitivity of plasma-based NGS to detect known driver alterations after TKI resistance in the real-world clinical setting.

In our study, 55% of the *EGFR*-mutant patients with resistance to first/second-generation TKIs, 60% of *EGFR*-mutant patients with resistance to osimertinib, and 57% of *ALK/ROS1*-altered cases with resistance to ALK/ROS1 TKIs, had detectable actionable alterations in ctDNA. Overall, these results can be considered comparable to those reported in other series, including cohorts from clinical trials

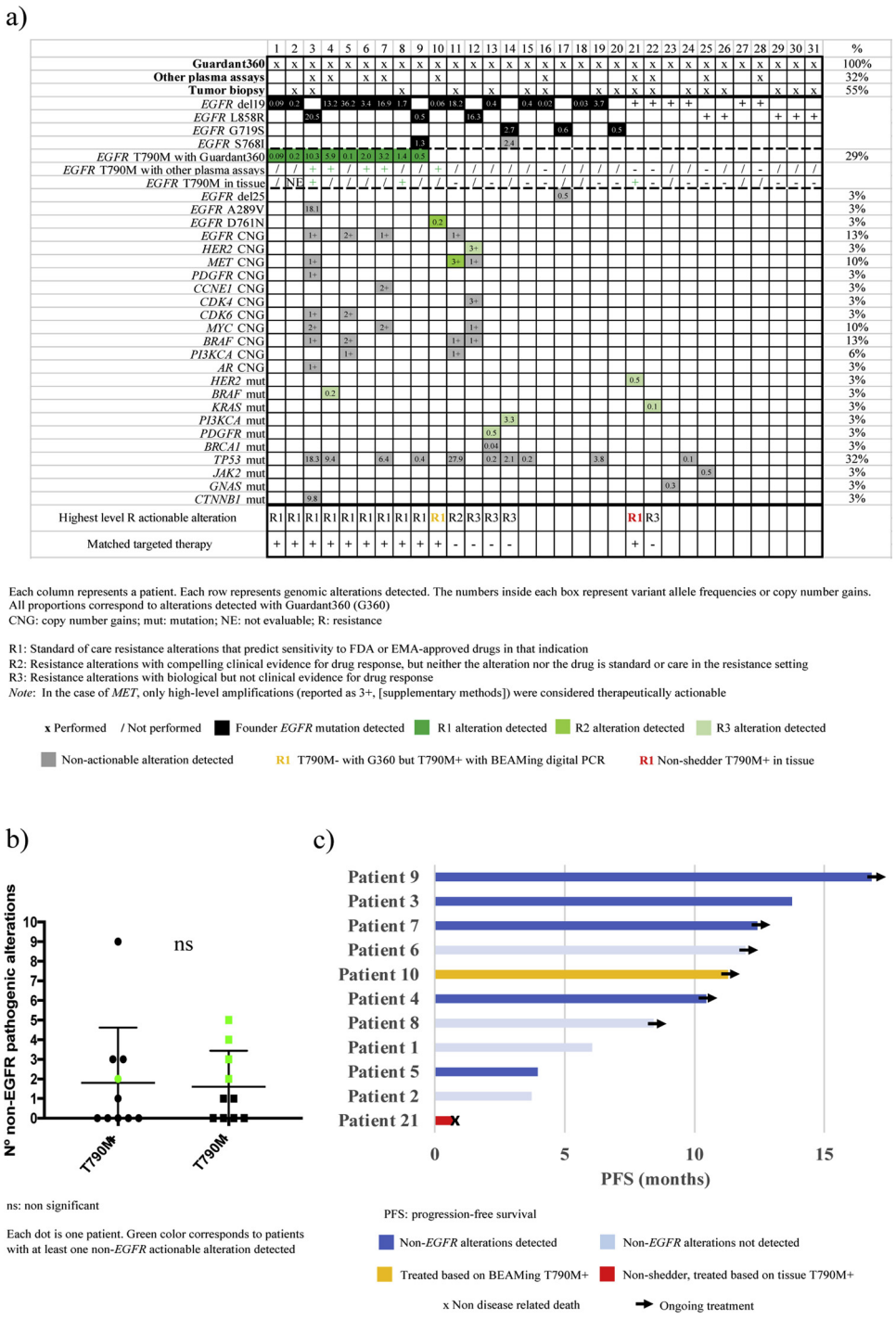


Fig. 1. Summary of ctDNA findings and clinical management of patients with first/second-generation EGFR TKI resistance (cohort 1) (a) Heatmap summarizing the findings of plasma and tissue sequencing in each patient; (b) Number of non-*EGFR* pathogenic alterations per patient in T790M + compared to T790M- subgroups; (c) Progression-free survival of patients receiving subsequent osimertinib based on plasma or tissue T790 M positivity.

[12–15,17–19]. We did detect well described R1 and R2 actionable resistance alterations, including *EGFR* T790 M and *MET* amplifications in cohort 1, *EGFR* C797S and *MET* amplifications in cohort 2, and common *ALK/ROS1* kinase domain mutations in cohort 3. The prevalence of *MET* amplification in cohort 1 and 2 seemed slightly lower that previously reported in other plasma studies [13,14,15], probably because of the somewhat lower fraction of higher shedders in an admittedly small sized study. In addition, we also detected other rare but potentially clinically relevant alterations, such as the *STRN-ALK* rearrangement in a patient with acquired resistance to osimertinib. This translocation has been recently described in osimertinib-resistant patients [18], reinforcing its importance as another potentially targetable fusion event in these patients.

Eleven patients (20%) received molecularly-guided therapies indicated by NGS of ctDNA, deriving the expected clinical benefit. This proportion is clinically meaningful, considering also that 9 of them (82%) had insufficient or unavailable tissue biopsies and thus would have otherwise probably not accessed matched therapies. A larger number of available molecularly-driven clinical trials would have likely increased the number of patients treated with targeted therapies. This

a)

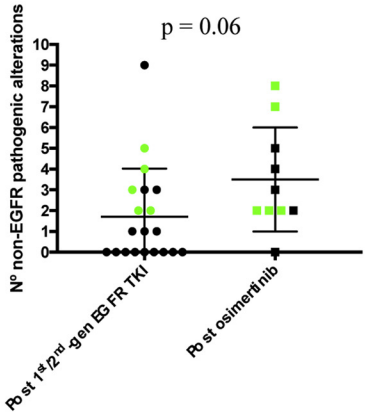
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	%
Guardant360	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	100%
EGFR del19		20.8	0.7	3.4	1.5	17.2	11.8	5.7	0.5	0.09	+	+	+	+		
EGFR L858R	69.9														+	
EGFR T790M		19.7			1.0											
EGFR C797S		5.1														7%
EGFR G724S				0.5												7%
EGFR CNG	3+	1+					2+									20%
MET CNG	3+						1+									13%
PDGFR CNG	1+															7%
KIT CNG	1+															7%
CCNE1 CNG		2+														7%
CDK6 CNG	2+						1+									13%
MYC CNG	3+	2+				2+										13%
BRAF CNG	2+						1+									13%
PI3KCA CNG						2+										7%
AR CNG						3+	1+									13%
BRAF mut					0.9											7%
MAP2K1 mut			0.1													7%
PI3KCA mut						35.9										7%
MTOR mut											0.1					7%
TP53 mut	46.0			1.9	0.1		11.3	1.0	1.3			0.3				47%
RB1 mut	38.7															7%
CTNNB1 mut			1.0			30.4										13%
SMAD4 mut						80.0										7%
ARID1A mut		1.6														7%
NFE2L2 mut						51.7										7%
ALK-STRN fusion				0.3												7%
Highest level R actionable alteration	R2	R2	R3	R3	R3	R3										
Matched targeted therapy	-	-	-	-	-	-										

Each column represents a patient. Each row represents genomic alterations detected. The numbers inside each box represent variant allele frequencies or copy number gains
CNG: copy number gains; mut: mutation; R: resistance. All proportions correspond to alterations detected with Guardant360 (G360)

R1: Standard of care resistance alterations that predict sensitivity to FDA or EMA-approved drugs in that indication
R2: Resistance alterations with compelling clinical evidence for drug response, but neither the alteration nor the drug is standard or care in the resistance setting
R3: Resistance alterations with biological but not clinical evidence for drug response
Note: In the case of *MET*, only high-level amplifications (reported as 3+, [supplementary methods]) were considered therapeutically actionable

Founder *EGFR* mutation detected | R2 alteration detected | R3 alteration detected | Non-actionable alteration detected

b)



Each dot is one patient. Green color corresponds to patients with at least one actionable alteration detected

Fig. 2. Summary of ctDNA findings and clinical management of patients with osimertinib resistance (cohort 2) (a) Heatmap summarizing the findings of plasma-based NGS in each patient; (b) Number of non-*EGFR* pathogenic alterations in patients from cohort 1 as compared to patients from cohort 2.

was particularly the case for patients progressing on osimertinib. As patients with osimertinib resistance had limited access to clinical trials during the study period (January-September 2017), we could not actually demonstrate the clinical utility of plasma-based NGS in this setting. On the other hand, in patients with *ALK/ROS1* + disease, the clinical utility of plasma-based NGS was more obvious in those progressing on at least one next-generation TKI, whose treatment was molecularly guided based on distinct sensitivities of these drugs to specific kinase-domain mutations [20].

In cohort 1, two T790M + cases were undetected by NGS of ctDNA (Guardant360). This finding underlies that tissue biopsies, and eventually alternative plasma-based genotyping methods if tissue is unavailable, should be strongly considered in cases with uninformative plasma results [9,21]. One exception might be higher shedders where the maximum variant allele fraction of the *EGFR* driver mutation in the sample is 1% or higher, as at this level the sensitivity for *EGFR* T790 M rose to 93% in the AURA3 trial [22].

A total of 8 patients (13%) (five from cohort 1, two from cohort 2, and one from cohort 3) did not have reliable evidence of tumor DNA shed for resistance assessment (initial known driver alteration was not detected in ctDNA), but had detectable pathogenic alterations in plasma. Although we cannot definitely exclude that these mutations do truly represent tumor genotype, some mutations detected in plasma might not be derived from tumor DNA but from hematopoietic cell DNA

a)

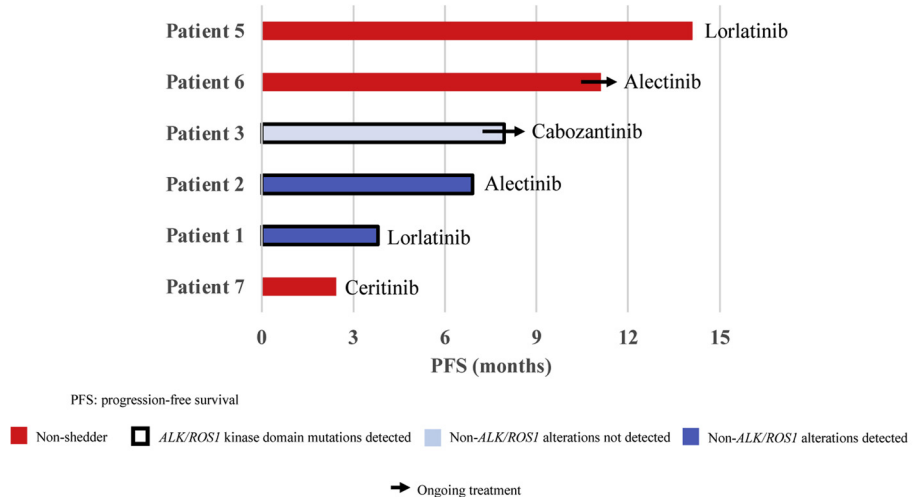
	1	2	3	4	5	6	7	%
Guardant360	x	x	x	x	x	x	x	100%
ALK fusion	2.8	+				+	+	
ROS1 fusion			0.1	2.0	+			
ALK G1202R	0.9							25%
ALK F1174V	0.1							25%
ALK F1174C	0.05							25%
ALK G1269A		3.6						25%
ROS1 G2032R			0.2					33%
CDK6 CNG	1+							14%
BRAF mut		0.3		2.4				28%
NF1 mut	0.2							14%
PI3KCA mut		5.8						14%
TP53 mut					1.0			14%
CDKN2A mut				1.0				14%
Highest level actionable R alteration	R2	R2	R2	R3				
Post-progression TKI therapy	+	+	+	-	+	+	+	
Matched targeted therapy	+	-	+	-	-	-	-	

Each column represents a patient. Each row represents genomic alterations detected. The numbers inside each box represent variant allele frequencies or copy number gains
CNG: copy number gains; mut: mutation; R: resistance; TKI: tyrosine kinase inhibitor. All proportions correspond to alterations detected with Guardant360 (G360)

R1: Standard of care resistance alterations that predict sensitivity to FDA or EMA-approved drugs in that indication
R2: Resistance alterations with compelling clinical evidence for drug response, but neither the alteration nor the drug is standard or care in the resistance setting
R3: Resistance alterations with biological but not clinical evidence for drug response

Founder *ALK/ROS1* fusion detected | R2 alteration detected | R3 alteration detected | Non-actionable alteration detected

b)



PFS: progression-free survival

Non-shedder | *ALK/ROS1* kinase domain mutations detected | Non-*ALK/ROS1* alterations not detected | Non-*ALK/ROS1* alterations detected

Ongoing treatment

Fig. 3. Summary of ctDNA findings and clinical management of patients with ALK/ROS1 TKI resistance (cohort 3) (a) Heatmap summarizing the findings of plasma-based NGS in each patient; (b) Progression-free survival in patients receiving post-progression ALK/ROS1 TKIs.

as a consequence of clonal hematopoiesis [23], underscoring the need for cautious and expert interpretation of plasma findings in this setting.

In line with other studies, non-*EGFR* pathogenic alterations were frequently detected in *EGFR*-mutant NSCLCs, particularly in those with osimertinib-resistance [24]. Although the co-occurrence of target-independent alterations seems to predict poorer responses to TKIs [24–26], their clinical interpretation when found in ctDNA is still evolving. In our study, many of the co-occurring target-independent alterations were mutations with low variant allele frequencies (potentially subclonal events), or low-level copy number gains in multiple genes simultaneously, which could be more a consequence of polysomy than of focal amplifications. For example, co-occurring amplifications in *EGFR*, *MET*, *BRAF*, and *CDK6,* which were detected in patients 3 and 5 from cohort 1, and patients 1 and 7 from cohort 2, may be interpreted as non-focal, as all these genes cluster in chromosome 7. This phenomenon has also been described in other series [18]. Remarkably, in *EGFR*-mutant NSCLCs, only clonal *MET* amplification, but not *MET* polysomy, predicts poorer responses to EGFR TKIs [27]. Our study lacks of statistical power to compare outcomes, but we observed that the presence of pretreatment non-*EGFR* pathogenic alterations did not seem to result in shorter PFS with osimertinib in our limited sized cohort. Although no formal conclusions can be drawn from this observation, it also highlights the importance of adequate clinical interpretation of plasma findings in the resistance setting. In this regard, the clinical significance of target-independent pathogenic alterations detected in ctDNA will need to be addressed in larger prospectively conducted cohorts.

This study has to be interpreted in the context of a number of limitations, some of which have already been discussed. First, the sample size was admittedly low, particularly for cohorts 2 and 3. Secondly, the fact that some patients underwent plasma sequencing not immediately after TKI resistance could have affected the sensitivity to detect ctDNA. Finally, only one patient from cohort 2 (patient 4) had pre and post-treatment plasma samples, limiting our capacity to assess for alterations

that are newly acquired at the time of TKI resistance.

In conclusion, NGS of ctDNA (Guardant360), used as a complement or alternative to tissue or other plasma-genotyping methods, can appropriately select TKI-resistant NSCLC patients for subsequent molecularly-guided therapies in the real-world clinical setting. Methods to discern between dominant and subclonal resistance alterations are needed to deliver effective combination therapies in these patients.

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Disclosures

J. Zugazagoitia has received consulting honoraria from Guardant Health. L. Paz-Ares (or relatives) has received honoraria from Lilly, MSD, BMS, Roche, Pharmamar, Merck, Astra-Zeneca, Novartis, Boehringer, Celgene, Servier, Sysmex, Amgen, Incyte, Pfizer, Sanofi, outside the submitted work. P. Garrido has received honoraria from Roche, Eli Lilly, Boehringer Ingelheim, Astra Zeneca, Celgene and GlaxoSmithKline, outside the submitted work. M Provencio has received honoraria from Lilly, MSD, BMS, Roche, Astra-Zeneca, Novartis, Boehringer, and Celgene. I. Faull and R. Lanman are Guardant Health employees. The rest of the authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.05.032.

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