

# Clinical Impact of Plasma and Tissue Next-Generation Sequencing in Advanced Non-Small Cell Lung Cancer: A Real-World Experience

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Disclosures of potential conflicts of interest may be found at the end of this article.

**Key Words.** Oncogene addiction • Actionable genetic alterations • Genetic characterization • Next-generation sequencing • Liquid biopsy

## ABSTRACT

**Background.** Targeted agents have improved the outcome of a subset of non-small cell lung cancer (NSCLC). Molecular profiling by next-generation sequencing (NGS) allows screening for multiple genetic alterations both in tissue and in plasma, but limited data are available concerning its feasibility and impact in real-world clinical practice.

**Methods.** Patients with advanced NSCLC consecutively referring to our Institution for potential eligibility to VISION trial (NCT02864992) were prospectively enrolled. They were already screened with standard method, and EGFR/ALK/ROS-1 positive cases were excluded. NGS was performed in plasma and tissue using the Guardant360 test covering 73 genes and the OncoPrint Focus Assay covering 59 genes, respectively.

**Results.** The study included 235 patients. NGS was performed in plasma in 209 (88.9%) cases; 78 of these (37.3%) were evaluated also in tissue; tissue only was analyzed in 26 cases (11.1%). Half of the tissue samples were deemed

not evaluable. Druggable alterations were detected in 13 (25%) out of 52 evaluable samples and 31 of 209 (14.8%) of plasma samples. Improved outcome was observed for patients with druggable alterations if treated with matched targeted agents: they had a longer median overall survival (not reached) compared with the ones who did not start any targeted therapy (9.1 months; 95% confidence interval, 4.6–13.6;  $p = .046$ ). The results of NGS testing potentially also affected the outcome of patients treated with immunotherapy.

**Conclusion.** Systematic real-life NGS testing showed the limit of tissue analysis in NSCLC and highlighted the potentiality of genetic characterization in plasma in increasing the number of patients who may benefit from NGS screening, both influencing the clinical decision-making process and affecting treatment outcome. *The Oncologist* 2020;25:e1996–e2005

**Implications for Practice:** Genetic characterization of cancer has become more important with time, having had positive implications for treatment specificity and efficacy. Such analyses changed the natural history of advanced non-small cell lung cancer (aNSCLC) with the introduction of drugs targeted to specific gene alterations (e.g., EGFR mutations, ALK and ROS-1 rearrangements). In the field of cancer molecular characterization, the applicability of the analysis of a wide panel of genes using a high-throughput sequencing approach, such as next-generation sequencing (NGS), is still a matter of research. This study used NGS in a real-world setting to systematically and prospectively profile patients with aNSCLC. The aim was to evaluate its feasibility and reliability, as well as consequent access to targeted agents and impact on clinical outcome whenever a druggable alteration was detected either in tumor tissue samples or through liquid biopsy.

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## INTRODUCTION

The availability of targeted therapies has profoundly changed the diagnostic and therapeutic pathways in solid tumors, especially in advanced non-small cell lung cancer (aNSCLC). In several instances, therapeutic algorithms based on genetic characterization have led to the use of on-label targeted agents in a subset of patients with oncogene-addicted tumors. Until recently most molecular information used for clinical decision has stemmed from single genetic alteration testing [1–7], whereas the potentialities of wide genetic screening in clinical practice are now emerging [8–10].

In aNSCLC, according to the European Society for Medical Oncology clinical practice guidelines, testing for *EGFR* mutations and rearrangements involving *ALK* and *ROS-1* genes are considered mandatory, and *BRAF* V600E mutations are rapidly approaching this status, as first-line *BRAF*/MEK inhibitors have been approved by some regulatory agencies. *HER2*, *MET* exon 14 mutations, and gene fusions involving *RET* and *NTRK-1* are rapidly gaining the role of potentially interesting targets [11]. The molecular testing guidelines of the American Society of Clinical Oncology also highlight the importance of *BRAF* testing in patients with advanced lung adenocarcinoma (LADC) [12, 13] as well as testing *RET*, *HER2*, *KRAS*, and *MET* as part of larger panels.

Next-generation sequencing (NGS) allows screening for multiple genetic alterations and can be performed both in tissue and in blood [14]. The real clinical impact of systematic NGS analysis in aNSCLC compared with standard methods is still under investigation.

Tissue biopsies represent the current standard for molecular testing, but availability of adequate material to perform a wide genetic characterization is challenging in non-small cell lung cancer (NSCLC). Even considering standard molecular testing, tissue biopsies are not diagnostic in 20% to 30% of patients, highlighting the need for alternative sources of tumor DNA [14, 15].

Circulating tumor DNA (ctDNA) is a subset of cell-free DNA (cfDNA) that can be found in plasma and represents genetic material both from the primary tumor and from metastases [16, 17]. Plasma ctDNA harboring specific somatic mutations is relatively specific for cancer; it might be usefully representative of tumor burden and mirror intratumor heterogeneity [17, 18]. Thus, cfDNA analysis holds considerable promise as a surrogate marker in multiple contexts: monitoring response to local or systemic therapies, definition of minimal residual disease and risk of relapse, following radical-intent treatments, and identifying of acquired drug-resistance mechanisms [19–21].

However, limited data are available concerning the real impact of NGS plasma characterization in clinical practice and the correct application of NGS results for the management of patients with NSCLC [22, 23].

The present article reports a real-world prospective experience of systematic NGS analysis in patients with aNSCLC with the aim of analyzing feasibility, reliability, access to targeted agents, and impact on clinical outcome.

## MATERIALS AND METHODS

### Patients and Molecular Analyses

From August 2017 to September 2019, patients with stage IIIB/IV NSCLC treated at Rete Oncologica Veneta and referring to our institution for potential eligibility for the VISION trial (NCT02864992) were prospectively screened, and clinical data were collected. VISION is a single-arm, open-label, phase II trial, aimed to assess activity and tolerability of tepotinib, a highly selective small molecule inhibitor of c-Met in patients with aNSCLC harboring *MET* exon 14 skipping alterations or *MET* amplification.

Patients' data recorded at baseline included patient demographics, Eastern Cooperative Oncology Group performance status (ECOG PS) at time of first-line systemic treatment start, smoking history, and weight loss of more than 5% during the 6 months before cancer diagnosis [24]. Tumor data collected included histology; *EGFR*, *ALK*, and *ROS-1* status as they determined for LADCs; programmed death ligand 1 (PD-L1) status, when available; and radiological staging. Information about treatments undergone during the course of the disease and their response were collected.

The ethics committee of our institution approved the study on February 20, 2017. The latest version of the protocol was approved by the ethics committee of our institution on the December 6, 2018.

Written informed consent was signed before any trial-related activities were carried out. Prescreening informed consent was obtained prior to NGS testing in tumor tissue and/or plasma. The prescreening informed consent approved by our ethics committee was version 3.0. The study was performed in accordance with the Declaration of Helsinki.

Tumor tissue for NGS testing was obtained from archived samples or from freshly obtained formalin-fixed paraffin-embedded (FFPE) tumor tissue. ctDNA was isolated and tested from freshly collected plasma samples.

*MET* alterations were searched in either plasma samples or tissue tumor samples, using the Guardant360 test (Guardant Health, Redwood City, CA), covering 73 genes and all somatic alterations recognized as potential targets by National Comprehensive Cancer Network, and OncoPrint Focus Assay (Thermo Fisher Scientific, Waltham, MA; testing done by MolecularMD Inc., Portland, OR) covering 59 genes, respectively.

Additional details about inclusion criteria, study procedures, and protocol are provided in the supplemental online material.

### Statistical Analysis

The primary aim of the study was to describe the feasibility of routine use of multiple genetic testing by NGS in aNSCLC and its impact on clinical management in real-world clinical practice.

After assessing feasibility, we analyzed the impact of the presence of potentially druggable alterations (not previously found with standard methods) on outcome according to the access to matched targeted drugs.

The secondary aims of the study were to explore the association of the presence of druggable alterations with outcome in the whole study population and among patients treated with immunotherapy.

Because of the observational exploratory nature of the work, a sample size was not calculated.

Druggable alterations according to NGS results were defined as *EGFR* sensitizing mutations or deletions, *ALK* rearrangements (that were not identified with standard diagnostics), *RET* fusions, *ROS-1* translocations, *MET* exon 14 skipping mutations, *MET* amplifications, *HER2* exon 20 mutations, *HER2* amplifications, and *BRAF* V600E mutations [25].

Progression-free survival (PFS) was calculated from the first day of systemic treatment to the first radiological or clinical disease progression (PD) or death from any cause. Overall survival (OS) was calculated from the first day of any systemic treatment to death from any cause. Radiological response (RR) was assessed according to RECIST version 1.1; disease control rate was defined as complete response plus partial response (PR) plus stable disease (SD).

Variables were presented by using median value for continuous variables and percentages (numbers) for categorical variables and their relationship with the presence of target alteration was assessed using the Mann-Whitney test, Kruskal-Wallis test, and the chi-squared test as appropriate.

Univariate logistic regression models and results were reported using odds ratios (ORs) with their 95% confidence intervals (CIs). Median PFS and OS were estimated by using Kaplan-Meier methods, and a log-rank test was used to compare survival between groups. Hazard ratios (HRs) and their 95% CIs were calculated with the Cox regression method. Statistical significance level was set at  $p < .05$  for all tests. All statistical analyses were performed with SPSS version 20.0 software (SPSS Inc., Chicago, IL).

## RESULTS

### Patients and Treatments

A total of 235 patients were included: 130 (55.3%) men and 105 (44.7%) women. Clinical-pathological features of the study population are summarized in Table 1. Median follow-up time was 11.7 (range, 1.6–26.6) months; 54.9% of enrolled patients was still alive at the time of this analysis.

First-line systemic therapy was started in 215 cases (91.5%): 165 (76.7%) received a platinum-based doublet, 35 (16.3%) were treated with pembrolizumab, 9 (4.2%) were eligible only for single-agent chemotherapy because of comorbidities, and 2 (0.9%) started targeted treatment after the results of the NGS testing (supplemental online Table 1).

Among the 20 patients who did not undergo any systemic treatment, 6 patients (2.6%) received first-line locoregional treatments for oligometastatic disease, and 14 (5.9%) patients experienced a fast decline of performance status, preventing the chance of any kind of active treatment.

Seventy-one of 129 (55.0%), 10 of 36 (27.8%), and 2 of 15 (13.3%) patients were treated with immune-checkpoint inhibitors (ICIs) in the second, third, and fourth line of

**Table 1.** Patients' clinical features

Variable	Patients' population (n = 235), n (%)
Gender	
Male	130 (55.3)
Female	105 (44.7)
Age at diagnosis of aNSCLC, median (range), years	68.5 (31.8–88.2)
Performance status	
0	78 (33.2)
≥1	157 (66.8)
Weight loss	40 (17.0)
Smoking status	
Never smoker	63 (26.8)
Active smoker	54 (23.0)
Former smoker	118 (50.2)
Cancer histology	
Adenocarcinoma	204 (86.8)
Squamous cell carcinoma	12 (5.1)
Not otherwise specified carcinoma	12 (5.1)
Sarcomatoid carcinoma	3 (1.3)
Large cell carcinoma	2 (0.9)
Adenosquamous carcinoma	2 (0.9)
Number of metastatic sites	
1	100 (42.6)
2	81 (34.5)
3	38 (16.2)
4	11 (4.7)
Extrathoracic metastases	118 (50.2)
Liver metastasis	26 (11.1)
Bone metastasis	64 (27.2)
PD-L1 testing	
Not done	34 (14.5)
Done	201 (85.5)
PD-L1 status cutoff 1%	
<1%	104 (51.7)
≥1%	97 (48.3)
PD-L1 status cutoff 50%	
<50%	152 (75.6)
≥50%	49 (24.4)
Total number of systemic treatments	
0	20 (8.5)
1	86 (36.6)
2	94 (40.0)
3	20 (8.5)
4	10 (4.3)
5	4 (1.7)
6	1 (0.4)

Abbreviations: aNSCLC, advanced non-small cell lung cancer; PD-L1, programmed death ligand 1.

systemic treatment, respectively. Further details on the administered therapies and patients' responses are shown in supplemental online Table 1.

### NGS Analyses

Molecular analyses were performed exclusively in tumor tissue in 26 cases (11.1%), in plasma in 131 cases (55.7%), and both in tissue and plasma in 78 cases (33.2%).

Overall, tumor tissue was analyzed in 104 cases: 18 samples (17.3%) were obtained from archived surgical tissue, whereas 86 (82.7%) were obtained from FFPE biopsy tissue. Fifty-two samples (50.0%) were defined as not evaluable by central pathology review because of insufficient tumor tissue available. Almost all not-evaluable samples were obtained from nonsurgical biopsies (49 of 52; 94.2%), whereas 17 of 52 (32.7%) of evaluable samples were surgical samples. Median time needed to receive the report of druggable MET alterations and to have the complete NGS testing report was 8 (range, 1–29) and 11 (range, 1–31) working days, respectively.

Plasma samples of 209 patients were analyzed, and all samples were evaluable. Twenty-nine samples were found negative for the presence of any genetic alterations: among these, eight patients underwent liquid biopsy before any systemic treatment. Seven patients were tested twice, at the time of PD to sequential lines of systemic treatment. The first assessment for five of these cases showed no detectable tumor-related somatic alterations; three of five turned out positive for somatic genetic alterations at subsequent plasma testing performed after PD. Median time needed to have the complete NGS testing report was 10 working days (range, 4–29).

Seventy-eight patients were tested both in tissue and in plasma: 43 tissue tumor samples were not suitable for NGS testing, and 4 showed no evidence of oncogenic alterations; 14 plasma samples showed no detectable alterations. Among the remaining 28 cases, 20 genetic alterations were detected in tissue and 14 confirmed in plasma. Four of eight druggable alterations were found both in tissue and in plasma.

Thirty-two of 209 patients (15.3%) tested in plasma were found positive for potentially druggable alterations; nine patients had also tissue evaluable, and the alterations were confirmed in four cases.

Details about genetic alterations found in tissue and plasma are summarized in supplemental online Figure 1.

### Druggable Alterations: Tissue Analyses

A druggable oncogenic alteration was found in 13 patients (24.5% of evaluable samples) in tumor. We found four *KIF5B-RET* translocations, three *BRAF* V600E mutations, one *MET* exon 14 fusion skipping alteration, one case of *MET* exon 14 fusion skipping alteration and *MET* gene amplification, one common sensitizing *HER2* exon 20 insertion (G776delinsVC), and one *HER2* amplification, later confirmed using fluorescence in situ hybridization (FISH) test. Besides these 11 cases, an *EZR-ROS-1* translocation was detected with NGS in a former smoker (16 pack-years) male patient with 3+ for ROS-1 at immunohistochemistry; confirmative FISH diagnostic for this patient turned out not evaluable. An *EGFR* sensitizing mutation on exon 21 was also found: this patient's tissue had not

been routinely tested for *EGFR* before because the patient had a light smoking habit and a squamous cell carcinoma.

### Druggable Alterations: Plasma Analyses

Plasma NGS allowed detection of druggable oncogenic driver alterations in 32 of 209 patients (15.3%).

*EGFR* sensitizing alterations were found in four cases. Two patients were diagnosed with squamous cell carcinoma, had previous smoking habits, and had not been tested on tissue for *EGFR*; one did not have enough tissue available to perform the test. These three patients were found positive by real-time polymerase chain reaction (RT-PCR) testing after additional confirmatory liquid biopsy. Another patient carried an uncommon exon 20 mutation (A767\_V769dup), which was not confirmed either in tissue, using RT-PCR, or in liquid biopsy, also by using RT-PCR.

*ALK* translocations were observed in three cases, previously negative at immunohistochemistry. In two of them, subsequent FISH testing for *ALK* rearrangement was deemed not evaluable. In the other, material was insufficient to perform FISH testing, and the patient underwent rebiopsy: the sample was evaluable and positive for *ALK* rearrangement.

*KIF5B-RET* translocation was detected in two cases: one was also analyzed and confirmed in tissue.

Among four patients positive for *BRAF* V600E mutation in plasma, one had also tissue tested, and the alteration was confirmed.

Fourteen patients carried *MET* alterations: seven patients had *MET* exon 14 fusion skipping alteration, five had *MET* gene amplification, and two had both these alterations (one also positive in tissue).

Four patients carried common sensitizing exon 20 insertion involving *HER2* gene (G776delinsVC); one of them had the same mutation in tissue.

*CD74-ROS-1* gene fusion was detected in one patient, who was diagnosed of LADC, immunohistochemically negative for ROS-1 protein. Subsequent FISH testing was deemed not evaluable.

### Clinical and Pathological Features of Patients

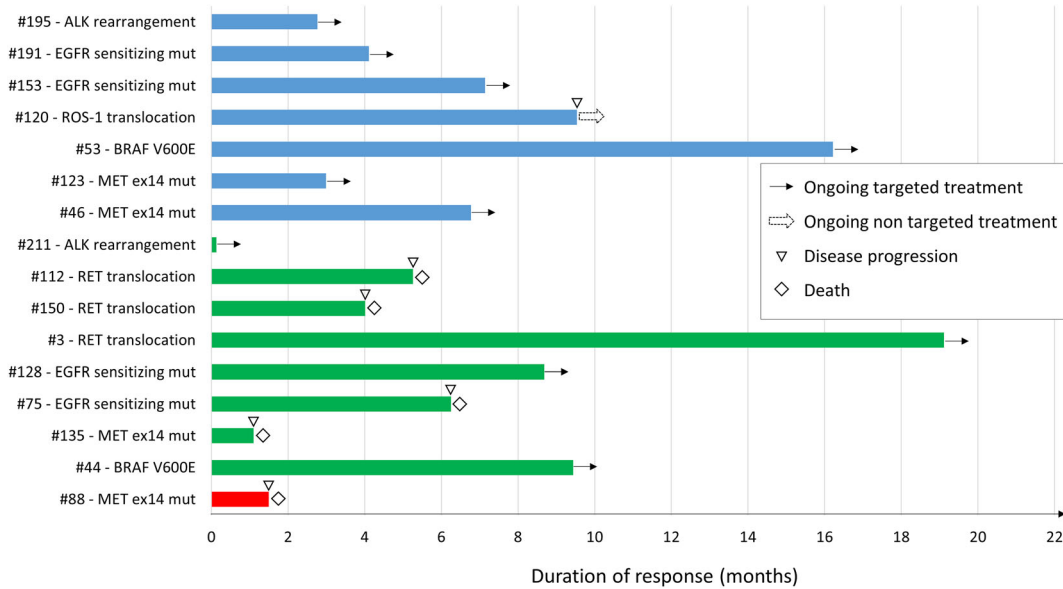
#### Carrying a Potentially Druggable Genetic Alteration

In the whole study population, being a current or a former smoker was associated with lower chance of carrying druggable molecular alterations (OR, 0.21; 95% CI, 0.10–0.42;  $p < .001$ ). No other clinical features, such as gender or age, showed such a correlation (OR, 1.22; 95% CI, 0.62–2.41;  $p = .557$  for female; OR, 1.00; 95% CI, 0.97–1.03;  $p = .979$  for older age; supplemental online Table 2).

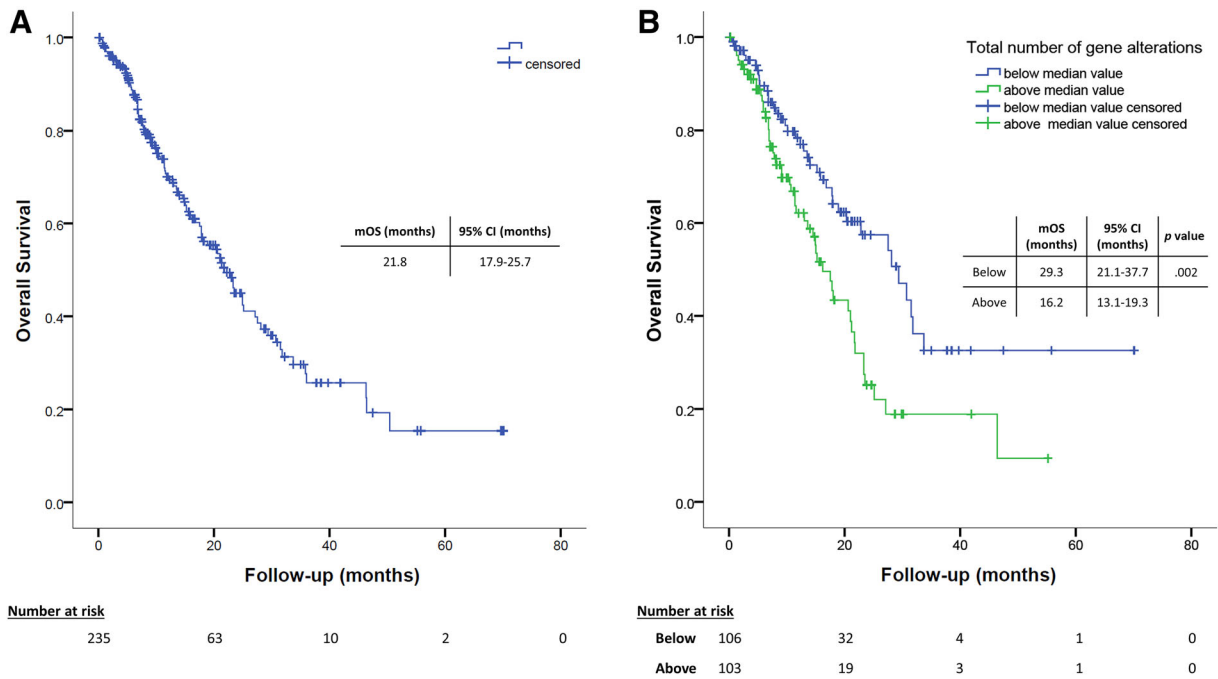
The distribution of PD-L1 tumor proportion score values showed higher values for the subgroup of patients carrying druggable alterations ( $p = .037$ , Mann-Whitney test).

### Druggable Alterations and Targeted Treatments

Five patients were positive for *RET* rearrangement; three of them received off-label cabozantinib, experiencing SD as best RR. One of them is still on treatment after 76 weeks. The remaining two patients died because of rapid progression while on ICI treatment and never received targeted therapy.



**Figure 1.** Response to targeted agents among patients found positive for potentially druggable alterations after next-generation sequencing analysis in tissue or in plasma (previously screened for EGFR/ALK/ROS-1 with standard method and found negative or not evaluable). In blue, patients who achieved partial response as best response, in green patients with stable disease as best response and in red patients who experienced disease progression as best response. Abbreviations: ex14, exon 14; mut, mutation.



**Figure 2.** Survival analyses. **(A):** Estimation of mOS of the whole study population. **(B):** Estimation of mOS according to the number of genetic alterations found in plasma, dichotomized according to the median number of alterations (median value, 2). Abbreviations: CI, confidence interval; mOS, median overall survival.

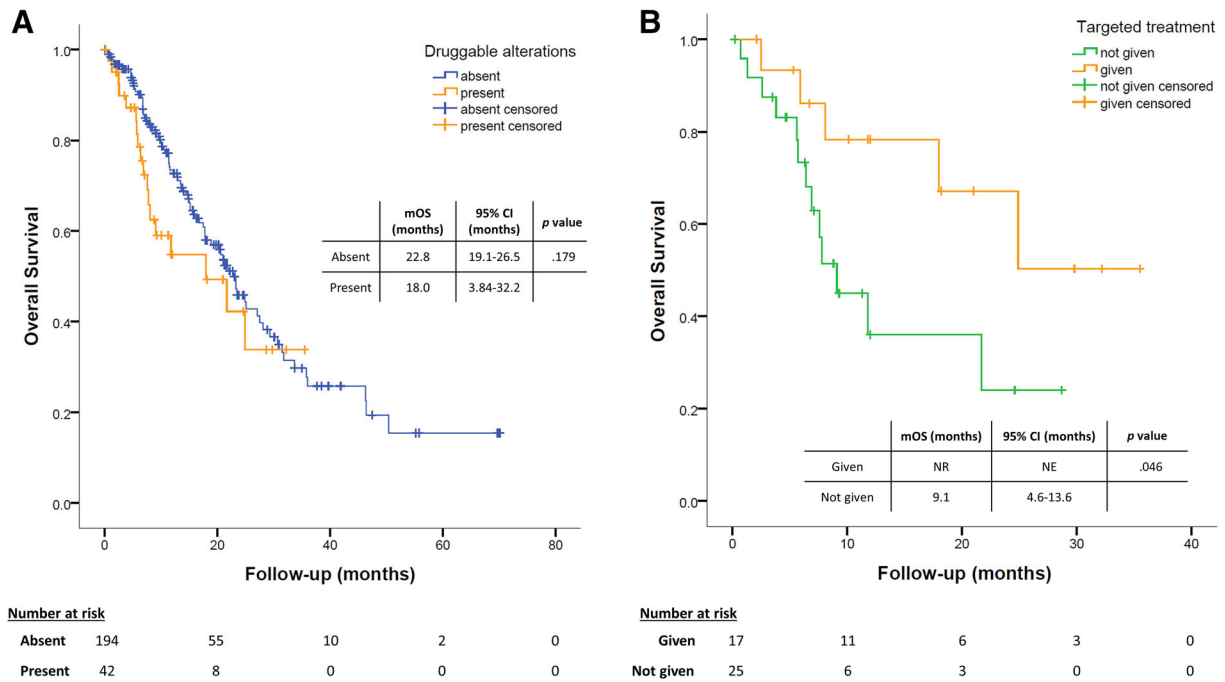
*BRAF* V600E mutation was found in six cases: two died before starting any treatment, one died after first-line chemotherapy, one is receiving second-line ICI treatment, and two started off-label dabrafenib and trametinib. These two patients experienced a good disease control, still ongoing after 37 and 64 weeks, respectively.

Druggable *MET* alterations were detected in 15 cases. Four patients received tepotinib as part of the VISION

clinical trial, and RR cannot be disclosed yet. Five patients are undergoing first-line therapy, and six either died or experienced a fast decline of performance status that prevented any other active treatment.

Somatic gene alterations involving *HER2* gene were found in five patients: three died after completion of first-line chemotherapy, and two are receiving second-line standard systemic treatment.





**Figure 3.** Survival analyses. **(A):** Estimation of overall survival according to the presence of a potentially druggable genetic alteration found by next-generation sequencing analyses. **(B):** Kaplan-Meier comparison of patients who were found positive for a potentially druggable alteration and received targeted agents accordingly versus patients who did not. Abbreviations: CI, confidence interval; mOS, median overall survival; NE, not evaluable; NR, not reached.

Two patients tested positive for *ROS-1* gene rearrangement; one is still receiving maintenance pemetrexed, whereas the other had clinical benefit from crizotinib for 38 weeks.

Among the five patients with *EGFR* alterations, four began EGFR tyrosine kinase inhibition: two achieved SD and two PR as best RR. The one with the uncommon exon 20 insertion is still receiving first-line chemotherapy.

Three patients were positive for *ALK* rearrangement; two are currently receiving alectinib after 15 and 5 weeks, respectively, whereas one is still on treatment with first-line chemotherapy.

Details about response to targeted treatments and duration of clinical benefit in patients with a potentially druggable alteration are summarized in Figure 1.

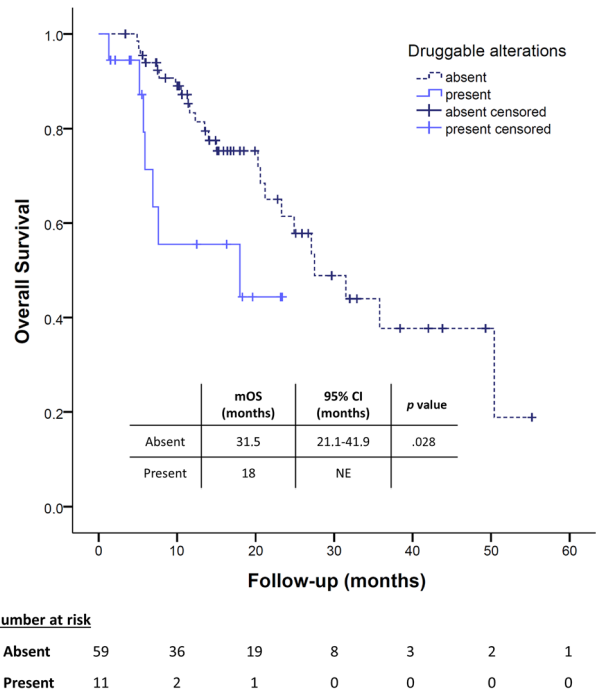
### Druggable Alterations and Immune-Checkpoint Inhibitors

Four of five patients carrying *RET* rearrangement received prior treatment with ICIs, achieving one PR and four PD as best RR.

Among patients carrying *BRAF* V600E mutation, one has just started second-line treatment with pembrolizumab, whereas another patient was treated with nivolumab prior to targeted therapy, obtaining SD for over 9 months.

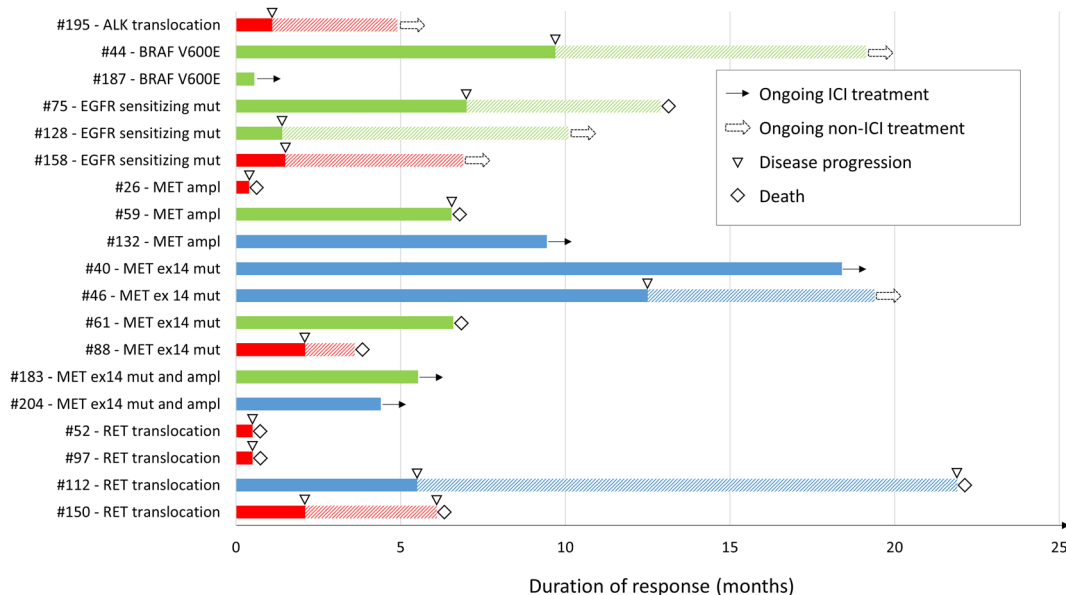
ICIs were part of prior oncologic treatment for 10 patients with *MET* alterations, and best responses recorded were four PR, four SD, and two PD.

No patients with either somatic gene alterations involving *HER2* gene or *ROS-1* gene rearrangement ever received ICIs. Conversely, immunotherapy was administered in one of three patients with *ALK* translocation, having PD as best



**Figure 4.** Overall survival analyses among patients who received immunotherapy during the course of the disease according to the presence of a potentially druggable alteration. Abbreviations: CI, confidence interval; mOS, median overall survival; NE, not evaluable.

response, and in three of five patients carrying *EGFR* sensitizing alterations, with their best response being one stability and two PD.



**Figure 5.** Response to ICI agents among patients found positive for potentially druggable alterations after next-generation sequencing analysis in tissue or in plasma (previously screened for EGFR/ALK/ROS-1 with standard method and found negative or not evaluable). In blue, patients who achieved partial response as best response, in green patients with stable disease as best response and in red patients who experienced disease progression as best response.

Abbreviations: ampl, amplification; ex14, exon 14; ICI, immune-checkpoint inhibitor; mut, mutation.

### Survival Analyses

Median OS of the study population was 21.8 (95% CI, 17.9–25.7) months (Fig. 2).

Impact on OS of gender, age at diagnosis, smoking status, ECOG PS, weight loss before cancer diagnosis, histology, presence of druggable target alterations, and number and subtype of metastatic sites were evaluated in univariate analysis (supplemental online Table 3). ECOG PS at diagnosis of stage IV disease (HR, 1.93; 95% CI, 1.28–2.93;  $p = .002$ ), weight loss (HR, 2.43; 95% CI, 1.54–3.93;  $p < .001$ ), number of metastatic sites (HR, 1.41; 95% CI, 1.14–1.74;  $p = .002$ ), and having bone (HR, 2.26; 95% CI, 1.49–3.42;  $p < .001$ ) or liver (HR, 1.79; 95% CI, 1.01–3.17;  $p = .047$ ) or extrathoracic metastasis (HR, 1.53; 95% CI, 1.03–2.28;  $p = .038$ ) had a significant impact on OS. ECOG PS, weight loss, and bone metastasis were confirmed to independently affect OS in multivariate analysis (HR, 1.56; 95% CI, 1.04–2.36;  $p = .033$ ; HR, 2.46; 95% CI, 1.53–3.96;  $p < .001$  and HR, 2.22; 95% CI, 1.30–3.78;  $p = .0013$ , respectively).

The same analyses were performed in the 209 patients with plasma samples tested, confirming the results of the whole population (supplemental online Table 4).

The presence of potentially druggable alterations had no significant impact on outcome: median OS was 22.8 (95% CI, 19.1–26.5) months in the absence of druggable alterations versus 18 (95% CI, 3.84–32.2) months among positive patients ( $p = .179$ ; Fig. 3A). Of interest, patients carrying druggable alterations who started a matched targeted agent had longer median OS (not reached), compared with the ones who did not (9.1 months; 95% CI, 4.6–13.6;  $p = .046$ , log-rank test; Fig. 3B). Although the difference was not statistically significant, patients with druggable alterations who started a matched targeted agent showed a

numerically longer median OS (not reached) in comparison with the rest of the study population (21.7 months; 95% CI, 17.4–25.9;  $p = .173$ , log-rank test).

In the subgroup of patients who received immunotherapy and who had their plasma tested before ICI ( $n = 69$ ), those who carried a druggable molecular alteration had a median PFS comparable to the others (6.5 months; 95% CI, 0.3–12.8 vs. 5.8 months; 95% CI, 3.1–8.5;  $p = .316$ ) but showed a trend for a shorter median OS: 8.1 (95% CI, 9.8–9.4) versus 22.8 months (95% CI, 17.6–27.9;  $p = .133$ , log-rank test; HR, 2.01; 95% CI, 0.78–5.68;  $p = .143$ , Cox regression test; Fig. 4), although it was not statistically significant. On the other side, different patterns of response have been shown according to the kind of potentially druggable alteration found, as described above (Fig. 5).

### DISCUSSION

Molecular characterization of aNSCLC has led to considerable clinical benefit for patients carrying molecular alterations for which targeted agents are available [11–13, 26]. Indeed, as an example, patients with *ROS-1* rearrangement treated with crizotinib reached a median OS of 51 months [27], a result considered unreachable before the introduction of targeted agents.

Routine molecular testing in lung cancer is performed in tissue, and analysis includes molecular alterations with matched drugs available in clinical practice. In parallel, less invasive diagnostic procedures to obtain tumor samples are increasingly used in the context of a disease for which surgical approach is rare [28, 29], whereas extensive molecular testing for potentially druggable alterations [30, 31] might also allow the development of more personalized treatments

also for patients with wild type *EGFR/ALK/ROS-1*. This promising approach can be further fostered by the use of NGS analysis in plasma, which can overcome the limitation of tissue availability, also allowing for dynamic evaluation during treatment [21, 32].

Outside of limited reports, evidence that this approach can have clinical impact on real-world patients is still debatable [20, 33, 34]. In 2014 a pivotal work showed the benefit of multiple molecular testing for LADC in clinical practice and the impact of targeted agents in this setting [35]. However, it must be stressed that among the molecular alterations tested, Kris and colleagues included *EGFR* and *ALK* sensitizing alterations, which are now routinely tested in current clinical practice, and other driver mutations, such as *AKT1*, *MEK1*, or *NRAS*, that are currently orphans of a targeted treatment [35]. On the other hand, more recent studies including different solid tumors showed that increasing genetic information and offering off-label drugs might not be translated into clinical benefit for patients with cancer [36, 37].

Our study is based on real-world data collection aiming to describe the impact of genetic characterization through NGS performed in plasma and in tissue in clinical practice. Real-world evidence, although characterized by several methodological limitations, can provide useful information about reliability and transferability of molecular diagnostic in clinical practice, thus helping in planning new organizational models to better apply NGS screening in clinical practice [38].

In our study, we first tested the feasibility of the analysis in clinical practice, showing that tissue genetic characterization was feasible in only 50% of samples. Moreover, 32.7% of these evaluable cases came from surgical samples, underlining the difficulties of obtaining material suitable for NGS analysis from biopsies in real-life patients. On the contrary, liquid biopsy was always evaluable; however, 29 samples were found negative for the presence of any genetic alterations. This potential limit can be related to tumor burden and tumor shedding [39, 40]. This consideration is supported by our finding that genetic alterations were found by repeating liquid biopsy at the moment of progression in three of five negative cases. These observations confirm that plasma NGS is feasible in clinical practice, opening the opportunity to apply this technique for disease monitoring evaluation of tumor genetic heterogeneity and early detection of acquired treatment resistance mechanisms [32, 41, 42]. The feasibility of NGS analysis in plasma in NSCLC was also recently evaluated in other series including a comparable number of patients [22, 43, 44].

In our series, we also highlighted another potential limitation of performing genetic screening only in plasma. Tumor heterogeneity and disease burden, in addition to potential technical issues, might account for potential discrepancy between tissue and plasma results. The number of patients having both tissue and plasma available was not sufficient to draw any conclusions on the topic. Overall, whenever feasible, both tissue and plasma NGS should still be performed.

After assessing feasibility and reliability, our data allowed us to estimate the clinical impact of multigene screening in a real-world setting. First of all, we observed that even in a population already tested for the presence of *EGFR*, *ALK*, and

*ROS-1* sensitizing alterations with standard techniques, NGS was able to find about 18% of patients carrying potentially druggable alterations. The identification of a potentially druggable alteration and the availability of a matched agent can have a significant impact on prognosis (Fig. 3). Indeed, in the presence of druggable alterations, only patients treated with targeted agents had improved outcome.

These observations are potentially relevant for clinical practice, even though the analysis is not able to fully exclude intrinsic biological differences related to different potentially druggable alterations. Even selecting genetic alterations with matched targeted drugs potentially available, we could not establish the potential influence of intrinsic prognostic value of each alteration.

Unfortunately, in our experience, a matched targeted treatment was available only for a limited subset of the study population: 39% of the patients carrying a potentially druggable alteration and 6.8% of the overall study population (including also the not evaluable samples, which were 50% of the tissue samples). This low rate is due to many factors, mirroring real-world clinical potential limitations to this approach. First of all, patients have been screened but started systemic treatment according to clinical practice, and several patients carrying potentially druggable alterations are still on standard treatment and might be subsequently eligible. Some patients also experienced a rapid decline of clinical conditions preventing any further treatments. Limited access to matched targeted drugs is also due to limitations for the off-label use of targeted agents and limited availability of clinical trials for rare molecular alterations. Notably, however, three patients who were classified with standard methods as having wild-type tumors turned to be positive for *EGFR/ALK/ROS-1* alterations and received the matched approved drug after NGS testing.

In addition to the potential for matched targeted agents, plasma NGS may allow the identification of patients at different prognosis or might influence sensitivity to other treatments, thus potentially impacting on clinical decision-making process. In our experience, a higher number of genetic alterations has been associated with worse prognosis (Fig. 2B). Moreover, the presence of a potentially druggable alteration has been associated with higher level of PD-L1 expression and with a trend for shorter survival in patients treated with ICIs, even though heterogeneity of response and duration was found (Fig. 5). These observations are in line with prior reports and highlight the potentialities of liquid biopsy in clinical practice in evaluating indication and timing for immunotherapy [45, 46].

Our data also highlight the importance of formally established molecular tumor boards in cancer institutes and networks of community hospitals to define criteria for patient selection, material to be tested and tests to be used, interpretation of results, access to matched therapies, and clinical outcomes [47, 48]. This need stems from the complexity of analyzing and interpreting the results of multigene NGS analysis in clinical practice, the issues related to access to targeted drugs, and the importance of integrating molecular information in diagnostic-therapeutic pathways of patients with aNSCLC.



## CONCLUSION

We describe a real-world experience with NGS analysis systematically and prospectively performed in patients with aNSCLC, providing insight into its feasibility and its potential clinical implications, including the effective use of matched targeted agents, but also its potential limitations in clinical practice of the near future.

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providing the molecular analyses as part of the screening for the VISION trial.

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## DISCLOSURES

The authors indicated no financial relationships.

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