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Next-Generation Sequencing in Public Healthcare NSCLC Management: Molecular Profiles, Actionable Alterations, and Survival Benefits

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ABSTRACT

Next-generation sequencing (NGS) has emerged as a molecular technique capable of generating an extensive genomic characterization of non-small cell lung cancer (NSCLC). Because a wide range of biomarker-based therapeutic options now exist, molecular laboratories have become essential in guiding clinical decision-making. In this report, we present the performance of an NGS assay accredited under UNE-EN ISO 15189:2022 in a series of 350 individuals. The most common alterations involved TP53 (51.0%), KRAS (26.6%), and EGFR (12.9%). We also identified patterns of coexistence and mutual exclusivity among variants, together with distinct mutational distributions linked to sex and tobacco exposure. Actionable variants appeared at a significantly higher rate in women (80.5%, $p < 0.001$) and in patients who never smoked (87.7%, $p < 0.001$). After NGS became the standard molecular platform, 36.4% of patients accessed at least one targeted therapeutic line. For the subgroup of 200 stage IV cases, use of targeted agents in first-line therapy correlated with extended progression-free survival (PFS) (13.4 months (95% CI, 10.2-16.6) ($p = 0.001$)). Likewise, overall survival (OS) improved notably among individuals who received any targeted agent (26.2 months (95% CI, 11.8-40.5) ($p < 0.001$)). Overall, these findings indicate that broader application of NGS within the public system has advanced the incorporation of precision oncology.

Keywords: Non-small cell lung cancer, Molecular diagnosis, Translational research, Next-generation sequencing, Quality management system

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Introduction

Insights into the genomic drivers sustaining malignant growth have helped establish precision oncology as a central treatment strategy in non-small cell lung cancer (NSCLC) [1]. This shift has placed molecular characterization at the forefront of patient evaluation, particularly since predictive alterations and effective matched therapies have been identified.

The expansion of precision-based therapies has intensified the need for rapid, translational molecular research capable of updating clinically relevant genetic findings [2, 3]. Nonetheless, the adequacy of tumor material continues to be a major constraint. Limited cellularity in cytology specimens or biopsies, along with potential degradation of nucleic acids caused by fixation and paraffin embedding, may negatively affect diagnostic yield [4].

Given the demands of modern NSCLC management and the inherent challenges of sample quality, high-capacity analytical platforms have become essential. NGS has rapidly taken a central role because it can evaluate multiple clinically important alterations across numerous genes in a single workflow.

Transitioning from isolated single-gene testing to NGS involves a detailed and controlled multi-step process, from nucleic acid extraction to final reporting. Current recommendations highlight the need for rigorous quality assurance programs in laboratories performing such testing [5]. Similar to single-gene methods, UNE-EN ISO

15189 accreditation for NGS requires extensive technical validation, consistent tracking of materials, and continuous internal and external quality monitoring. Accreditation ensures a functioning quality management system, technical expertise, and dependable output [6, 7].

NGS has clarified the genetic architecture of NSCLC, allowing assessment of intratumoral diversity and identification of variant combinations that either co-occur or exclude each other. Distinct molecular signatures have also been tied to demographic and clinical variables such as sex, smoking history, histologic subtype, and ethnicity [8, 9].

Because numerous targetable genomic alterations define the therapeutic landscape of NSCLC, current clinical guidelines strongly advocate using multigene panels for routine diagnostic evaluation [10-13]. In addition, NGS enables research centers to match patients with investigational targeted therapies in ongoing clinical trials [11].

In the present study, we describe how NGS was integrated into a public reference hospital under UNE-EN ISO 15189:2022 accreditation. We examined how NGS contributed to comprehensive genomic characterization in NSCLC cases, evaluated its role in identifying candidates for targeted therapies, and analyzed its impact on treatment-related patient outcomes.

Materials and Methods

Patients and Samples

A total of 350 individuals diagnosed with NSCLC between 2015 and May 2020 at Hospital Universitario y Politécnico La Fe (Valencia, Spain) were included. **Table 1** summarizes all epidemiological and clinicopathological features. Samples were considered acceptable for molecular testing when they contained at least 150 total cells and a minimum tumor fraction of 10%. The study received approval from the Drug Research Ethics Committee (CEIm) of IIS La Fe, and written informed consent was obtained from every participant. All procedures followed the principles of the Declaration of Human Rights and the Helsinki Convention.

Table 1. Epidemiological and clinical-pathological characteristics of the recruited patients.

Variable	Value
Age, mean ± SD	63.2 ± 0.6
Sex, n (%)	
Male	217 (62.0)
Female	133 (38.)
Smoking history, n (%)	
Never	65 (18.6)
Former smoker	127 (36.3)
Current smoker	158 (45.1)
Smoking load (former and current smokers), median (IQR)	36 (23-50)
Years since quitting smoking (former smokers), median (IQR)	12 (5-20)
Histology, n (%)	
Adenocarcinoma	288 (82.3)
Large-cell carcinoma	9 (2.6)
Squamous	14 (4.0)
Sarcomatoid carcinoma	11 (3.1)
Adenosquamous carcinoma	3 (0.9)
Large-cell neuroendocrine carcinoma	8 (2.3)
NOS	17 (4.9)
Stage, n (%)	
IA	38 (10.9)
IB	24 (6.9)
IIA	2 (0.6)
IIB	17 (4.9)
IIIA	28 (8.0)
IIIB	19 (5.4)
IIIC	9 (2.6)

IV	200 (57.1)
Unknown	13 (3.7)

SD: standard deviation. IQR: interquartile range.

At the beginning of 2021, following the implementation of NGS assays accredited under UNE-EN ISO 15189:2022 [14], the hospital's Lung Cancer Committee elected to make NGS the default platform for routine NSCLC genomic characterization. During this timeframe, 128 specimens underwent NGS evaluation. The median time from sample arrival to finalized report was 10 days, with a span of 5-25 days.

Nucleic acid isolation

Genomic DNA was obtained from five FFPE slices, each 5 µm thick, using the Deparaffinization Solution together with the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany). RNA was isolated from five 10 µm FFPE curls using the RecoverAll™ Total Nucleic Acid Isolation Kit (ThermoFisher Scientific, Waltham, MA, USA), following vendor guidelines. DNA and RNA concentrations were determined with a Qubit 3.0 fluorometer using the appropriate HS kits (ThermoFisher Scientific, Waltham, MA, USA). All NGS procedures were performed within the Molecular Biology Unit of the Clinical Analysis Department, certified under ISO 15189 (ENAC Nº1302/LE2445).

Next-generation sequencing studies

Of the total samples, 104 were processed using the Oncomine Solid Tumor panel (OST; ThermoFisher Scientific, Waltham, MA, USA), while the remaining 246 were assessed with the Oncomine Focus Assay (OFA; ThermoFisher Scientific, Waltham, MA, USA). The OST panel targets point variants and small indels across 22 genes and detects fusion transcripts for 4 genes. The OFA panel covers point mutations and small indels in 35 genes, copy number alterations in 19 genes, and fusion events involving 23 drivers. Library construction and emulsion PCR were performed either manually or through the Ion Chef™ system (ThermoFisher Scientific, Waltham, MA, USA). Sequencing was run on either the Ion PGM™ platform or the Ion GeneStudio™ S5 (ThermoFisher Scientific, Waltham, MA, USA). Data processing and alignment to the hg19 reference genome were carried out using Torrent Server, and variants were identified and annotated with Ion Reporter. Synonymous and intronic changes were removed from downstream interpretation. Variants required ≥500 total reads and ≥20 variant reads to be retained. All selected calls were visually inspected in Integrative Genomics Viewer (IGV; Broad Institute, Cambridge, MA, USA).

Statistical analyses

Quantitative variables are described using either mean ± SD or median with IQR, while categorical variables appear as counts and percentages. Associations between categorical variables were tested with chi-square or Fisher's exact tests. For PFS and OS analyses, individuals lacking complete clinical records or without progression and/or still alive at data lock were censored. Time-dependent outcomes were estimated using the Kaplan-Meier method, with comparisons made through log-rank testing. Cox regression was applied to evaluate predictors of survival. Statistical computations were conducted using GraphPad Prism 7.0.2 (San Diego, CA, USA) and SPSS v.21 (IBM, Armonk, NY, USA). A p-value < 0.05 indicated significance. Drug-related annotations and trial-related evidence for variants were reviewed as of May 2022.

Results and Discussion

Molecular alterations identified by NGS

Within the cohort, 54.3% showed a single genomic alteration, 22.3% carried two or more (range: 2-4), and 23.4% exhibited no detectable variants **(Figure 1)**. TP53 mutations were most common (51.0%), followed by changes in KRAS (26.6%), EGFR (12.9%), BRAF (6.9%), and PIK3CA (5.4%). Fusions involving ALK (4.0%), MET (3.6%), and ROS1 (1.4%) constituted the predominant rearrangements. The most frequent copy number gains involved EGFR (2.4%) and MYC (2.0%).

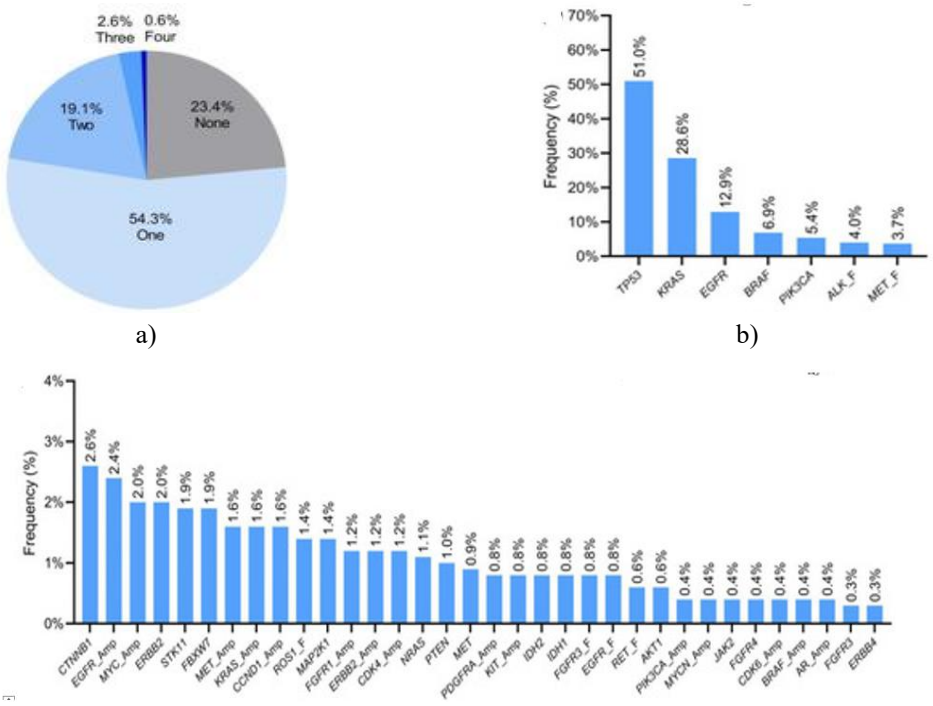

Figure 1. Molecular findings detected by NGS. (a) Proportion of individuals classified by the number of alterations identified. (b, C) Percentage distribution of alterations across all surveyed genes, separated into those occurring >3% (a) and <3% (b). Amp: amplification. F: fusion.

Clinical-pathological correlates of molecular findings

Sex and smoking categories substantially influenced mutation patterns. EGFR ($p < 0.001$), ERBB2 ($p = 0.013$), and ALK rearrangements ($p = 0.049$) appeared more often in women, whereas TP53 alterations were more typical among men ($p = 0.045$). KRAS variants were strongly linked to current or former smokers ($p < 0.001$). In contrast, EGFR ($p < 0.001$), ERBB2 ($p = 0.029$), ALK ($p < 0.001$), ROS1 ($p = 0.030$), RET ($p = 0.012$), and METEx14 events ($p < 0.001$) predominated in never smokers **(Figure 2)**.

Among former smokers, the pack-year burden was lower in patients with EGFR alterations (median 15) compared with those without them (median 35; $U = 302.5$, $p = 0.03$). Patients with METEx14 were significantly older at diagnosis (mean 75.1 ± 12.1) than individuals negative for this event (mean 62.6 ± 11.4), $t(244) = -3.2$, $p = 0.002$. No meaningful associations emerged with tumor histology.

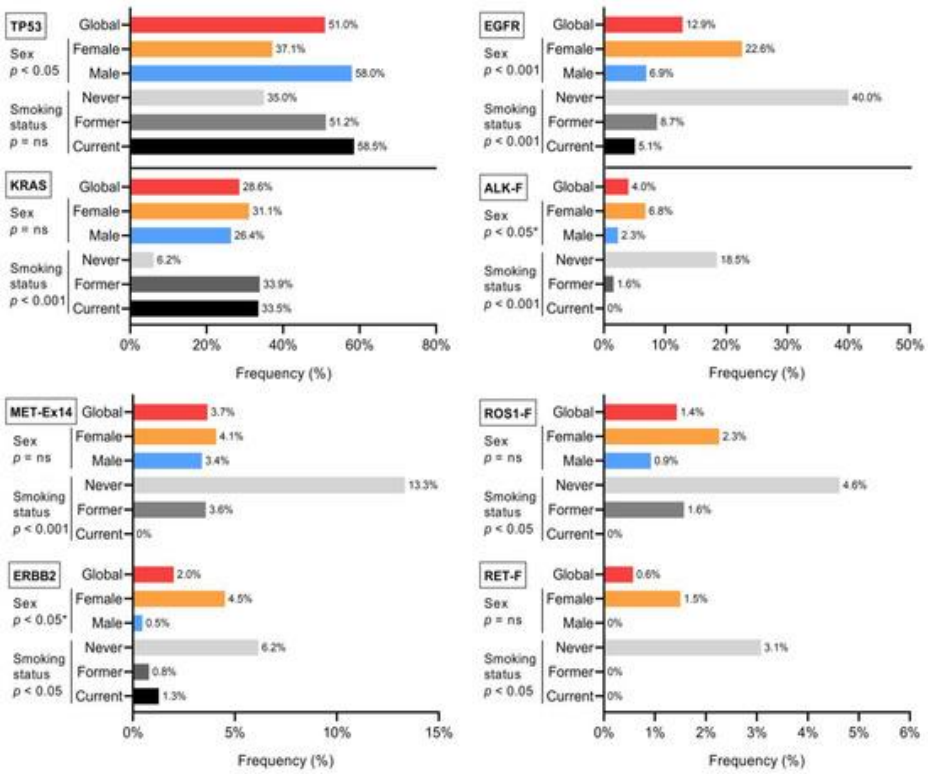

Figure 2. Distribution of selected genomic alterations by sex and smoking category. Frequencies are shown for the full cohort and each subgroup. ns: not significant. * Fisher's exact test.

Co-occurring or mutually exclusive genetic alterations

Comprehensive NGS characterization of NSCLC cases revealed patterns in how individual genomic lesions either tend to appear together or avoid one another. A pronounced mutual exclusivity was seen between KRAS variants and EGFR mutations ($p < 0.001$), and similar separation existed between KRAS alterations and both TP53 aberrations ($p = 0.01$) and ALK rearrangements ($p = 0.01$). Individuals carrying EGFR mutations also showed a higher likelihood of EGFR gene amplification ($p < 0.01$). MET alterations demonstrated strong alignment with METEx14 skipping ($p < 0.001$). In addition, KRAS gene amplifications frequently coincided with CCND1 amplification events ($p = 0.01$) **(Figure 3)**.

Several other genomic pairings appeared suggestive but did not surpass statistical thresholds, including KRAS mutation + KRAS amplification, NRAS mutation + MYC amplification, CCND1 + MET amplification, and a tendency for TP53 mutations to diverge from ALK fusions.

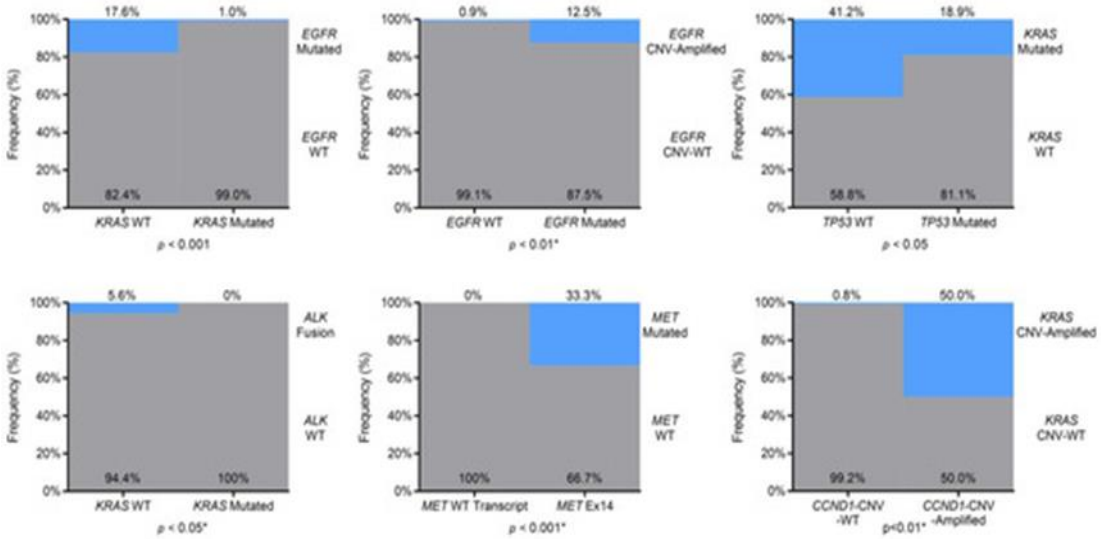

Figure 3. Mosaic visualization displaying how often two given alterations appear together or separately. The gene with the higher alteration frequency is placed along the X-axis. The resulting four combinations (presence/absence of each alteration) are shown. Fisher's exact test was used for each comparison.

Clinically relevant genetic variants

Across the cohort, 65% of patients harbored molecular findings with therapeutic implications. Among these, 54.4% corresponded to biomarkers linked to approved targeted drugs, while 45.6% represented alterations that would qualify individuals for phase I-II clinical trials. Women displayed a significantly higher prevalence of actionable markers compared with men ($p < 0.001$), and they more frequently carried variants connected to already-available targeted agents ($p < 0.001$) **(Figure 4)**.

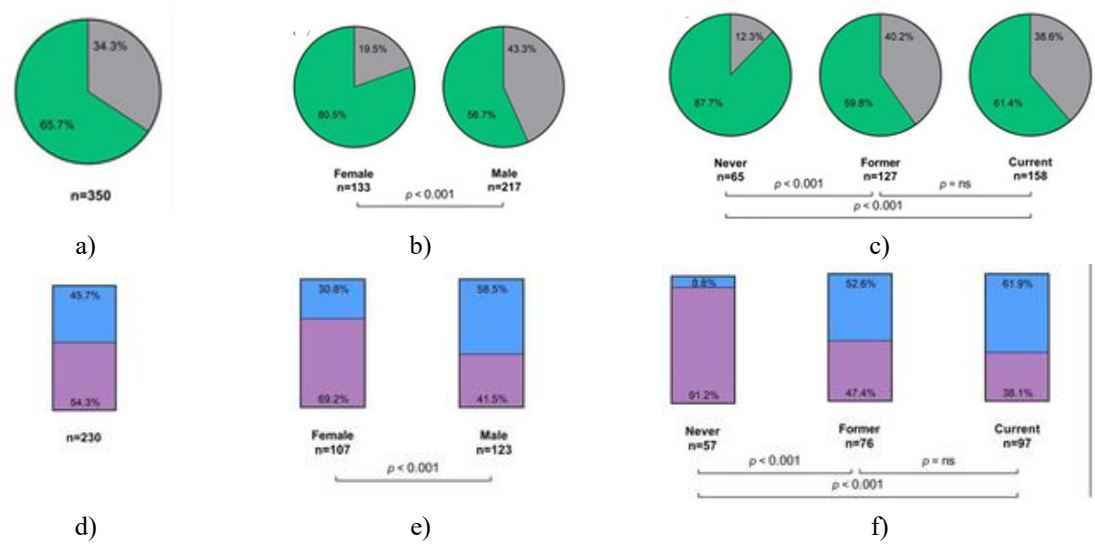

Figure 4. Distribution of targetable genomic findings from NGS. Proportions are shown for the full cohort (a), by sex (b), and by smoking category (c). Evidence tiers (d-f): purple = approved therapies available; blue = trial-eligible variants. ns = not statistically significant.

Smoking history also affected the frequency of therapeutically meaningful variants ($p < 0.001$). Among never-smokers, 87% exhibited molecular alterations, and 91% of these had links to currently approved targeted therapies. Former and active smokers did not differ meaningfully in either the proportion of actionable alterations or the strength of associated clinical evidence. Examining sex and smoking jointly revealed that women consistently had more targeted options than men, regardless of smoking behavior. Within each sex group, former and current smokers again showed no significant differences **(Figure 4)**.

Since NGS became the routine profiling method, 128 samples were prospectively examined. Of these, 83 individuals had newly diagnosed stage IV NSCLC, and 55 initiated systemic therapy. Based on genomic findings, 20 patients (36.4%) ultimately received at least one targeted regimen **(Table 2)**. Among the other 35 patients, 8 (22.9%) began targeted therapies later, after progression (6 started KRAS p.(Gly12Cys) inhibitors, and 2 received new EGFR exon 20-directed treatments).

Table 2. Targeted therapies are administered to patients with stage IV NSCLC based on NGS results.

Molecular Alteration	n	Drug
EGFR: p.(Leu858Arg)	4	Osimertinib
EGFR: p.(Glu746_Ala750del)	3	Osimertinib
EGFR: p.(Leu858Arg) + EGFR Amplification	1	Osimertinib
EGFR: p.(Gly719Ala) + p.(Ser768Ile)	1	Osimertinib
EGFR: p.(Leu861Gln)	1	Osimertinib
EGFR: p.(Glu709_Thr710delinsAsp)	1	Osimertinib
EGFR: p.(Ala767_Val769dup)	1	Amivantamab

EML4(13)-ALK(20)	1	Alectinib
KIF5B(17)-ALK(20)	1	Brigatinib
ALK Fusion (Unknown Partner)	1	Alectinib
MET(13)—MET(15)	1	Capmatinib
KRAS: p.(Gly12Cys)	1	Sotorasib
BRAF: p.(Val600Glu)	1	Dabrafenib + trametinib
SLC34A2(13)-ROS1(32)	1	Crizotinib
KIF5B(15)-RET(12) + IDH1: p.(Arg132His) + MYC Amplification	1	Selpercatinib

First-line treatment analyses

Table 3 summarizes the principal demographic and pathological characteristics of the 200 patients with de novo stage IV NSCLC. Among them, 146 underwent first-line treatment: 69 (47.3%) received chemotherapy, 33 (22.6%) were given targeted therapy, and 44 (30.1%) began an immunotherapy-based regimen. Progression-free survival (PFS) differed among the three treatment categories (p = 0.01). Targeted therapy achieved the longest PFS—13.4 months (95% CI, 10.2-16.6)—compared with chemotherapy—5.2 months (95% CI, 4.2-6.2) (p = 0.001). The chemotherapy group had a markedly elevated progression risk: HR 2.3 (95% CI, 1.4-3.8). Immunotherapy recipients also showed extended PFS—7.8 months (95% CI, 3.5-12.1) (p = 0.011)—yet their progression risk remained higher than that of the chemotherapy group (HR 1.8 (95% CI, 1.1-3.0)). Although targeted therapy numerically outperformed immunotherapy in PFS, this difference was not statistically significant. Responses to immunotherapy varied widely: 31.6% progressed within 5 months, while 20.5% maintained durable responses exceeding 60 months **(Figure 5)**.

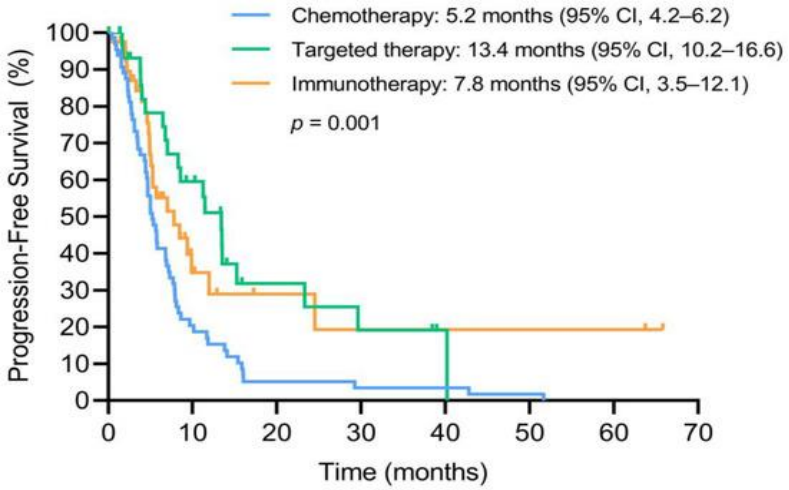

Figure 5. First-line PFS curves for stage IV NSCLC patients, separated by therapeutic strategy.

Table 3. Clinical, epidemiologic, and pathological characteristics of stage IV NSCLC cases.

Variable	Value
Age, mean ± SD	63.1 ± 12.1
Sex, n (%)	
Male	78 (39.0)
Female	122 (61.0)
Smoking history, n (%)	
Never	46 (23.0)
Former smoker	63 (31.5)
Current smoker	91 (45.5)
Sex and smoking history, n (%)	
Never-smoker female	31 (15.5)

Former smoker female	16 (8.0)
Current smoker female	31 (15.5)
Never-smoker male	15 (7.5)
Former smoker male	47 (23.5)
Current smoker male	60 (30.0)
Histology, n (%)	
Adenocarcinoma	162 (81.0)
Large-cell carcinoma	5 (2.5)
Squamous	7 (3.5)
Sarcomatoid carcinoma	5 (2.5)
Adenosquamous carcinoma	2 (1.0)
Large-cell neuroendocrine carcinoma	5 (2.5)
NOS	14 (7.0)
Systemic treatment, n (%)	
No	37 (19.0)
Yes	158 (81.0)

SD = standard deviation.

Overall survival

To assess how the different therapeutic approaches influenced overall survival (OS) in stage IV NSCLC (n = 200), patients were classified according to the regimens they received: those treated exclusively with chemotherapy (41, 28.1%), those exposed to at least one targeted agent (40, 27.4%), individuals who began an immunotherapy-based protocol (55, 37.7%), and a smaller subset treated with a combination of targeted therapy and immunotherapy (10, 6.9%). Survival outcomes differed markedly across these categories ($p < 0.001$). The chemotherapy-only group demonstrated the shortest OS, reaching 8.8 months (95% CI, 4.5-13.1) **(Figure 6)**. Relative to chemotherapy, the risk of death was substantially lower for patients treated with targeted therapies (HR 0.3, 95% CI 0.2-0.6), immunotherapy (HR 0.2, 95% CI 0.1-0.4), or the combined approach (HR 0.2, 95% CI 0.1-0.6).

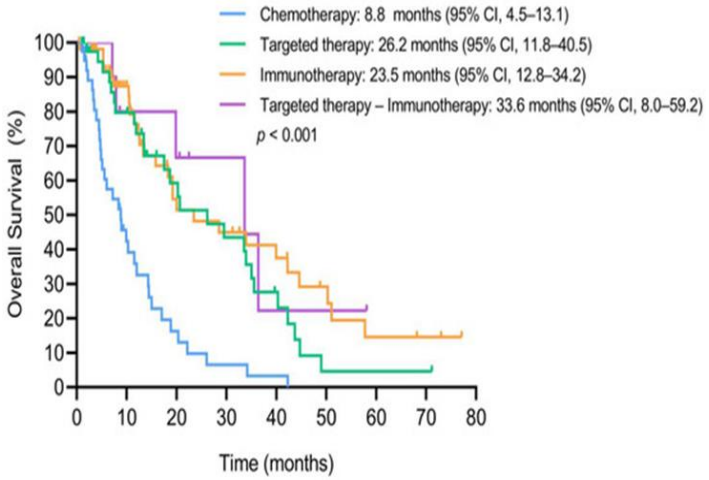

Figure 6. Overall survival curves for stage IV NSCLC patients grouped by therapeutic strategy.

A wide array of targeted drugs is now available for molecularly defined subgroups of NSCLC, creating an ongoing need for laboratories to employ modern, high-resolution profiling tools. In this context, NGS has rapidly become the preferred diagnostic platform for advanced NSCLC, as it enables simultaneous analysis of numerous genomic alterations while requiring minimal tissue. Nevertheless, integrating NGS into the routine workflow of public health systems remains a challenge, a concern highlighted by both SEAP and SEOM [15]. Here, we present how NGS—accredited under UNE-EN ISO 15189:2022—helps clarify the molecular architecture of NSCLC and informs clinical decisions in our public hospital.

The demographic and clinicopathological traits of the recruited individuals indicate that our cohort mirrors the typical presentation of NSCLC in community settings [16]. The prevalence of key genomic alterations in our series aligns with earlier reports [17-19]. Clear molecular distinctions emerged when stratifying by sex and smoking exposure. Sex has long been linked to differences in lung cancer susceptibility, with female patients frequently described as having unique disease features [20-22]. Clinical outcomes have also been shown to diverge by sex, with suggestions that sex may serve as a prognostic factor [23, 24]. Consistent with previous findings, EGFR mutations and ALK rearrangements were more frequent in women [25, 26], while TP53 mutations appeared more often in men [27]. Our observations regarding ERBB2 alterations also match studies indicating a male association [28, 29].

Smoking fundamentally shapes the biological trajectory of lung cancer, to the extent that NSCLC in never smokers is often regarded as a biologically distinct entity [30, 31]. Our data reinforce this concept: tumors from never-smokers were enriched for EGFR and ERBB2 mutations and for ALK, ROS1, RET, and METEx14 fusion events [32-36]. Former smokers, however, represent a heterogeneous group reflecting varied smoking histories. In our cohort, former smokers harboring EGFR mutations showed a markedly lower cumulative tobacco exposure, suggesting that these cases resemble “never-smoker-like” NSCLC [32].

Routine use of NGS provided a broader view of genomic diversity and uncovered patterns of coexistence and mutual avoidance between alterations. Such insights are especially relevant in the setting of targeted therapy, where selective pressure can favor the growth of resistant subclones [37, 38]. EGFR-activating mutations and EGFR amplification tended to appear together, a relationship that has been described previously, with amplification frequently arising in the mutated allele. The elevated proportion of mutant EGFR may characterize a subgroup with distinct responses to EGFR-TKIs [39, 40]. In accordance with published work, KRAS mutations showed strong exclusion patterns with major driver events such as EGFR mutations and ALK fusions [41, 42]. At the same time, KRAS mutations were positively associated with KRAS amplifications, implying a cooperative role in tumor progression [43]. Our findings also showed concurrent KRAS mutations and KRAS amplifications, further pointing to potential synergistic activity [44]. MET exon 14 skipping was linked to MET amplification, a pairing that may influence responses to MET-targeted agents [45, 46].

Sex- and smoking-associated genomic differences contributed to the variable relevance of targeted therapies across subgroups. Actionable alterations were most commonly observed in women and in never smokers [47], and these groups showed the highest proportion of variants linked to approved treatments. In particular, among never-smoker women, up to 91% had genomic findings suitable for targeted therapy, emphasizing the importance of ensuring access to NGS in this subgroup [18, 48]. In contrast, current and former smokers exhibited comparable genomic landscapes in our cohort. Consistent with prior literature, tobacco-associated NSCLC displayed a significantly lower prevalence of actionable biomarkers [49].

In patients with advanced NSCLC, first-line PFS varied significantly depending on the treatment modality. Those managed with chemotherapy demonstrated the poorest PFS, whereas individuals receiving targeted agents had the longest progression-free intervals. The immunotherapy group showed highly variable trajectories: nearly one-third progressed within the first 5 months, yet roughly 20% achieved long-lasting disease control. This wide separation in outcomes underscores the ongoing absence of reliable biomarkers to pinpoint which patients will derive consistent benefit from immunotherapy [50, 51].

The overall survival analysis further highlighted the influence of targeted and immune-based approaches in NSCLC. Patients who were administered at least one line of either treatment modality exhibited substantially extended survival when compared to those treated solely with chemotherapy. These findings reinforce the rationale for conducting NGS testing, enabling the identification of individuals who might qualify for existing targeted therapies or for clinical trial enrollment [52-54].

Consistent with previously published real-world evidence in larger NSCLC cohorts, we likewise observed significantly improved outcomes among patients exposed to targeted therapies or immunotherapy at any point during treatment [55, 56].

Aligned with current guidelines, NGS profiling of advanced NSCLC was incorporated into our routine diagnostic pipeline [10-13]. Certification of this method under UNE-EN ISO 15189:2022 ensures compliance with required analytical standards, supporting dependable results that can guide therapy choices within practical clinical timelines. This transition has been pivotal for implementing biomarker-driven treatments, as 36.4% of patients initiating first-line therapy ultimately received targeted agents based on their NGS findings.

Conclusion

This study documents the integration of a UNE-EN ISO 15189:2022-certified NGS workflow into routine NSCLC molecular diagnostics at a public reference center. Our data highlight clear molecular distinctions tied to clinical and pathological variables and enhance understanding of tumor heterogeneity. Crucially, targeted therapies were linked to improved outcomes, demonstrating how NGS expands access to precision oncology for a broader population of patients.

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Conflict of Interest: None

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Ethics Statement: None

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