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ORIGINAL RESEARCH

Molecular profiling and feasibility using a comprehensive hybrid capture panel on a consecutive series of non-small-cell lung cancer patients from a single centre

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Background: Targeted next-generation sequencing (NGS) is recommended to screen actionable genomic alterations (GAs) in patients with non-small-cell lung cancer (NSCLC). We determined the feasibility to detect actionable GAs using TruSight™ Oncology 500 (TSO500) in 200 consecutive patients with NSCLC.

Materials and methods: DNA and RNA were sequenced on an Illumina® NextSeq 550 instrument and processed using the TSO500 Docker pipeline. Clinical actionability was defined within the molecular tumour board following European Society for Medical Oncology (ESMO) guidelines for oncogene-addicted NSCLC. Overall survival (OS) was estimated as per the presence of druggable GAs and treatment with targeted therapy.

Results: Most patients were males (69.5%) and former or current smokers (86.5%). Median age was 64 years. The most common histological type and tumour stage were lung adenocarcinoma (81%) and stage IV (64%), respectively. Sequencing was feasible in most patients (93.5%) and actionable GAs were found in 26.5% of patients. A high concordance was observed between single-gene testing and TSO500 NGS panel. Patients harbouring druggable GAs and receiving targeted therapy achieved longer OS compared to patients without druggable GAs. Conversely, patients with druggable GAs not receiving targeted therapy had a trend toward shorter OS compared with driver-negative patients.

Conclusions: Hybrid capture sequencing using TSO500 panel is feasible to analyse clinical samples from patients with NSCLC and is an efficient tool for screening actionable GAs.

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INTRODUCTION

Lung cancer is the leading cause of cancer-related death worldwide.[1] A significant proportion of patients diagnosed with non-small-cell lung cancer (NSCLC), mostly lung adenocarcinomas, harbour actionable genomic alterations (GAs).[2] Patients with oncogenic driver alterations receiving appropriate targeted therapy have better survival than their counterparts not receiving targeted therapy, or those patients not harbouring any actionable driver alteration.[3,4] International guidelines recommend molecular testing before initiating systemic treatment in patients with NSCLC.[5-7]

The incorporation of molecular testing in the routine clinical practice in NSCLC is challenging due to the scarcity of tumour tissue to carry out all required tests and the growing number of GAs to be tested.[8] Also, there is a clinical need to reduce the turnaround time from sample collection to final molecular test report. Sequential molecular testing is associated with higher cost and longer turnaround time, so parallel testing is recommended.[9,10] Next-generation sequencing (NGS) allows simultaneous testing of multiple GAs in a short time frame, saving tissue sample and time, while being more affordable. For this reason, NGS has become the preferred technology to screen actionable molecular alterations in NSCLC.[11]

Several NGS technologies are currently available, mainly the PCR capture-based sequencing of predefined areas in oncogenes where actionable alterations are usually found ('hotspots') or hybrid capture-based NGS assay which analyses the entire coding sequence of oncogenes and tumour suppressor genes and achieves higher sensitivity to detect small insertions and deletions (indels), but also gene fusions and copy number alterations (CNAs) in formalin-fixed paraffin-embedded (FFPE) specimens.[12,13]

TruSight™ Oncology 500 (TSO500) is a hybrid capture-based NGS assay that covers the full coding DNA regions of 523 genes and the RNA transcripts of 55 genes and can detect base substitutions and small indels, CNAs, splice variants and gene fusions. In addition, it can accurately measure microsatellite instability (MSI) and tumour mutational burden (TMB).

To our knowledge, the TSO500 NGS panel has been used in ring trials for measuring TMB and to detect *NTRK* fusions,[14-16] but the feasibility of using this panel as a tool for routine molecular testing of NSCLC has not been reported yet. Here, we aim to assess the rate of success and frequency of actionable GAs detected with the TSO500 panel in a cohort of 200 patients with NSCLC from a single institution. In this work, we also evaluate the prognostic impact of detecting actionable alterations with access to matched targeted therapy.

MATERIALS AND METHODS

Study population

We conducted a single-centre observational study including patients diagnosed with NSCLC sequenced using the Illumina® TSO500 NGS panel (Illumina®, San Diego, CA) between July 2020 and March 2022. Between July 2020 and July 2021, only patients with non-squamous histology and without *EGFR* activating mutations, or *ALK* and *ROS1* rearrangements detected by conventional techniques [PCR for *EGFR* mutations, and immunohistochemistry (IHC) or FISH for *ALK* and *ROS1* rearrangements] were sequenced. From August 2021, NGS was carried out on patients with non-squamous histology or patients with squamous histology under 50 years of age or never smoked. Demographic, clinical, pathological and molecular data were collected and the end of follow-up was 31 August 2022. The project was approved by the Ethical Committee of the Hospital Universitari de Bellvitge (L'Hospitalet, Barcelona, Spain) and conducted in accordance with the Declaration of Helsinki.

Nucleic acid extractions, quality assessment and library preparation

DNA and RNA were extracted from FFPE samples and DNA and RNA libraries were prepared using the hybrid capture-based TSO500 Library Preparation Kit (Illumina®). Individual and pooled libraries were stored at −20°C. More details are provided in Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2023.102197.

NGS and bioinformatics analysis

The pooled amplicon libraries from eight DNA and eight RNA libraries were loaded on a NextSeq 500/550 High Output Kit v2.5 (300 cycles) and paired-end reads (2 × 101 bp) were sequenced on a NextSeq 550 Dx instrument (Illumina®). Raw data were analysed using the Illumina® TSO500 Local App version 2.2.0.2 in a Docker container. *Homo sapiens* GRCh37/hg19 was used as reference genome. Bioinformatics analysis outputs include MSI, TMB, small variants including single nucleotide variants (SNVs) and small indels, gene amplifications, gene fusions and splice variants. Samples with 10 or more mutations per megabase were classified as high TMB samples.

To enrich and annotate the analysis results, additional resources such as ClinVar[17] or gnomAD release 2.1[18] were used to annotate the variants. Then, to reduce the large number of small variants, variants located farther from 20 bp in the intron or the untranslated regions, as well as polymorphisms and synonymous variants, were excluded. These analyses have been carried out using the language

and statistical environment R v4.0.4 (R Foundation for Statistical Computing, Vienna, Austria).

SNVs with an allelic frequency <3%, CNAs with <5 copies and gene fusions and splice variants with a percentage of supporting reads <3% were also excluded. Variants were named following the HGVS nomenclature version 20.05[19] and ranked following the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets (ESCAT).[20] The gnomAD non-Finnish European, non-cancer subpopulation (Genome Aggregation Database, v2.1.1, https://gnomad.broadinstitute.org/) was used as a control population. Variants with a minor allele frequency >0.1% or >1% in at least one ethnic population are classified as likely benign or benign, respectively.

As the clinical interpretation is based on the biological classification, only pathogenic and likely pathogenic variants were clinically reported according to the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP)/College of American Pathologists (CAP) guidelines of classification system.[21,22] Actionability was discussed in our multidisciplinary molecular tumour board.

Genes of interest for this study were based on actionability, predisposition to hereditary cancer, identification of novel targets in the context of early drug development and genes predicting response to immune checkpoint inhibitor (ICI). Actionability for small variants was considered following the latest ESMO Clinical Practice Guidelines for advanced NSCLC.[23] Additionally, CNA gains (CNA ≥5 copies) were analysed in *ERBB2* and *MET* genes, but only amplifications (CNA ≥10 copies) were considered actionable. To detect potential putative alterations in cancer susceptibility genes, guidelines from Mandelker et al.[24] were used, and all pathogenic or likely pathogenic indels ≥20% allele frequency or SNVs ≥30% allele frequency in the described genes were taken into account (*BAP1, BRCA1, BRCA2, BRIP1, FLCN, FH, MLH1, MSH2, MSH6, MUTYH, PALB2, PMS2, POLE, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TSC2, VHL*). Small variants in *PIK3CA, HRAS, FGFR1-FGFR3 and NRG1*, as well as fusions in *FGFR1-FGFR3* were considered for the early drug development category, while small variants in *TP53, STK11, SMARCA4, ARID1A, ARID1B, ARID2, KEAP1* and *PBRM1* genes were assessed for their correlation with ICI response.

Statistical analysis

Exploratory data analysis of clinicopathological and molecular features was carried out using R. To evaluate the association between characteristics across groups, the 'compareGroups' package[25] was used. For categorical variables, a χ^2 test or a Fisher's exact test was carried out depending on sample distribution and continuous variables were tested using the Kruskal–Wallis test. Survival curves of overall survival (OS) for patients with advanced disease with available covariates were estimated using the Kaplan–Meier and Cox proportional hazards models were adjusted for sex, stage, age, histology, smoking status and TMB status. OS was calculated from the date of advanced disease diagnosis until death or last follow-up.

RESULTS

Patients and tumour description

Here, we present the results of the first 200 consecutive patients diagnosed with NSCLC that were sequenced with the TSO500 NGS panel. Clinical and pathological features are summarised in Table 1. The median age at diagnosis was 64 years (range 35-88 years). Most patients were male (69.5%, $n = 139$) and current or former smokers (46.0%, $n = 92$ and 40.5%, $n = 81$, respectively). The most common histological subtype was lung adenocarcinoma (81.0%, $n = 162$), followed by lung squamous cell carcinoma (6.5%, $n = 13$). Most patients were diagnosed with stage IV (63.5%, $n = 127$) or III (23.5%, $n = 47$).

The origin of samples utilised for sequencing was diverse: 102 biopsy (51.3%), 44 cytology (22.1%) and 53 surgical specimens (26.6%). Most specimens were obtained through computed tomography-guided lung or bronchoscopy biopsies of the primary lung lesion (49.0%, $n = 98$), or by

Table 1. Baseline characteristics

	All patients $n = 200$ (%)
Age, median (range), years	64 (35-88)
Gender	
Male	139 (69.5)
Female	61 (30.5)
Smoking status	
Never smoker	27 (13.5)
Former smoker	81 (40.5)
Current smoker	92 (46.0)
ECOG PS	
0	30 (15.0)
1	135 (67.5)
≥2	35 (17.5)
Histology	
Adenocarcinoma	162 (81.0)
Squamous cell carcinoma	13 (6.5)
NOS	10 (5.0)
Other	15 (7.5)
Stage at diagnosis	
I	11 (5.5)
II	15 (7.5)
III	47 (23.5)
IV	127 (63.5)
PD-L1 status	
<1%	78 (39.0)
1%-49%	52 (26.0)
≥50%	50 (25.0)
Missing	20 (10.0)
Type of sample	
Biopsy	102 (51.3)
Surgical sample	53 (26.6)
Cytology	44 (22.1)
Anatomical site of biopsy	
Lung	98 (49.0)
Pleura/pleural effusion	16 (8.0)
Adenopathy	44 (22.0)
Central nervous system	12 (6.0)
Bone	11 (5.5)
Other	19 (9.5)

ECOG PS, Eastern Cooperative Oncology Group performance status; NOS, not otherwise specified; PD-L1, programmed death-ligand 1.

endobronchial ultrasound-guided transbronchial needle aspiration of a mediastinal lymphadenopathy (22.0%, $n = 44$). All samples were assessed by an expert lung pathologist and 178 specimens (89.0%) showed at least 20% of tumour cells.

Library quality failure rate

The quality metrics of the DNA and RNA libraries were not reached (NR) only in 2 (1.0%) and 11 (5.5%) patients, respectively. The failure rate of RNA libraries was significantly higher in surgical specimens, accounting for 7 out of 53 samples (13.2%), compared with 3 out of 102 biopsies (2.9%) and 1 out of 44 cytological samples (2.3%; $P = 0.017$). The mean time from sample collection to molecular analysis was significantly longer for RNA libraries failed (20.1 versus 7.1 months; $P = 0.021$). Low tumour content in the tumour sample or the anatomical site of the biopsy was not associated with failure of RNA libraries. To evaluate the contribution of these factors, we conducted a logistic regression which showed that surgical specimens and longer time from collection were associated with a higher risk of RNA failure in the univariate analysis (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.102197). However, in the multivariate analysis, none of the variables remained statistically significant.

Detection of actionable genomic alterations with the TSO500 NGS panel

Overall, we identified actionable GAs in 53 patients (26.5%) using the TSO500 NGS panel taking into consideration nine oncogenic drivers defined by the ESMO guidelines consisting of *EGFR, ALK, ROS1, BRAF, KRAS, MET, RET, NTRK* and *ERBB2* (Figure 1A). The most recurrent oncogenic alteration was *KRAS* mutation found in 55 (27.5%) tumour samples, and the G12C variant was observed in 19 (9.5%) patients (Supplementary Figure S1A, available at https://doi.org/10.1016/j.esmoop.2023.102197). *EGFR* activating mutations were found in nine (4.5%) tumour samples, consisting of five (2.5%) patients harbouring common sensitising *EGFR* mutations (exon 19 deletion or L858R), one patient (0.5%) had an *EGFR-S768I* mutation, one patient (0.5%) with exon 20 insertion and two patients (1.0%) with multiple mutations. Additionally, two patients (1.0%) harboured *EGFR* truncating mutations that were not considered actionable. *BRAF* mutations were found in 16 (8%) tumour samples and the actionable V600E variant was seen in 5 (2.5%) tumours. Actionable gene fusions were detected in 11 (5.5%) patients, consisting of *ALK* in 3 (1.5%), *RET* in 5 (2.5%), *ROS1* in 2 (1.0%) and *NTRK1* rearrangement in 1 (0.5%) patient. All these alterations were mutually exclusive. *MET* exon 14 skipping alterations were detected in two (1.0%) patients. Also, other potentially actionable *MET* alterations were found: *MET* amplification in two patients (1.0%) and one patient (0.5%) harboured a *CD47-MET* fusion. *ERBB2* actionable alterations were detected in seven (3.5%) patients: five (2.5%) exon 20 insertions, one (0.5%) exon 19 mutation and one (0.5%) exon 17 mutation. Furthermore, *ERBB2* amplification was detected in two patients (1.0%) and a non-actionable mutation in exon 24 was also found in another patient (0.5%).

On the contrary, two actionable alterations were detected in two patients with an allele frequency below 3%. These alterations were further confirmed by an orthogonal method and consisted of an *EML4-ALK* fusion (allele frequency 2.64%) which was validated by IHC, and a *MET* exon 14 skipping mutation (allele frequency 1.23%) confirmed by NGS in liquid biopsy. None of the samples had MSI and the median TMB was 10.2 mutations/Mb (range 0.0-119.7 mutations/Mb). An oncoplot showing the frequency of all pathogenic or likely pathogenic GAs observed in these genes is shown in Figure 1B, regardless of being actionable or not.

Detection of additional clinically relevant genomic alterations with the TSO500 NGS panel

Pathogenic or likely pathogenic variants in seven genes associated with cancer predisposition were identified in 10 tumours (5.0%): *MUTYH* ($n = 4$, 2.0%), *BRCA2* ($n = 2$, 1.0%), *PALB2* ($n = 1$, 0.5%), *BAP1* ($n = 1$, 0.5%), *SDHA* ($n = 1$, 0.5%) and 1 patient (0.5%) with alterations in both *BRIP1* and *FLCN*. More details about the variant allele frequency and the specific variants are provided in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.102197. *BRCA2* mutations were confirmed at the germline level by Sanger sequencing. Both patients had familiar history of cancer and were referred to the Genetic Counselling Unit, where the variant was studied in multiple relatives who were carriers that will be followed up in the upcoming years. All *MUTYH* mutations were found in heterozygosis and were not deemed candidates for subsequent germline testing. Two patients died before being referred to the Genetic Counselling Unit (one harbouring an *SDHA* mutation and another with a *BAP1* mutation). *PALB2* mutation was not validated by Sanger sequencing at the germline level and *BRIP1* mutation is still being studied.

The utilisation of TSO500 panel was helpful to identify additional oncogenic alterations involving genes that have not been yet established as actionable targets in NSCLC, such as *HRAS*, *PIK3CA and FGFR1* (Supplementary Figure S1B, available at https://doi.org/10.1016/j.esmoop.2023.102197). Specifically, an *FGFR1* fusion and an *HRAS* mutation were detected in this cohort, but also *PIK3CA* mutations in nine patients (4.5%), of which five (2.5%) involved the catalytic domain. Those genes could be classified according to the ESCAT as ESCAT IIIA, since clinical benefit has been demonstrated in patients with these specific alterations albeit in other tumour types. Despite the limited evidence of clinical benefit of drugs targeting these GAs, their identification is relevant since there are current clinical trials or expanded access programmes ongoing that could potentially benefit those patients.

An additional advantage of using a large panel is the ability to account for concurrent mutations in tumour

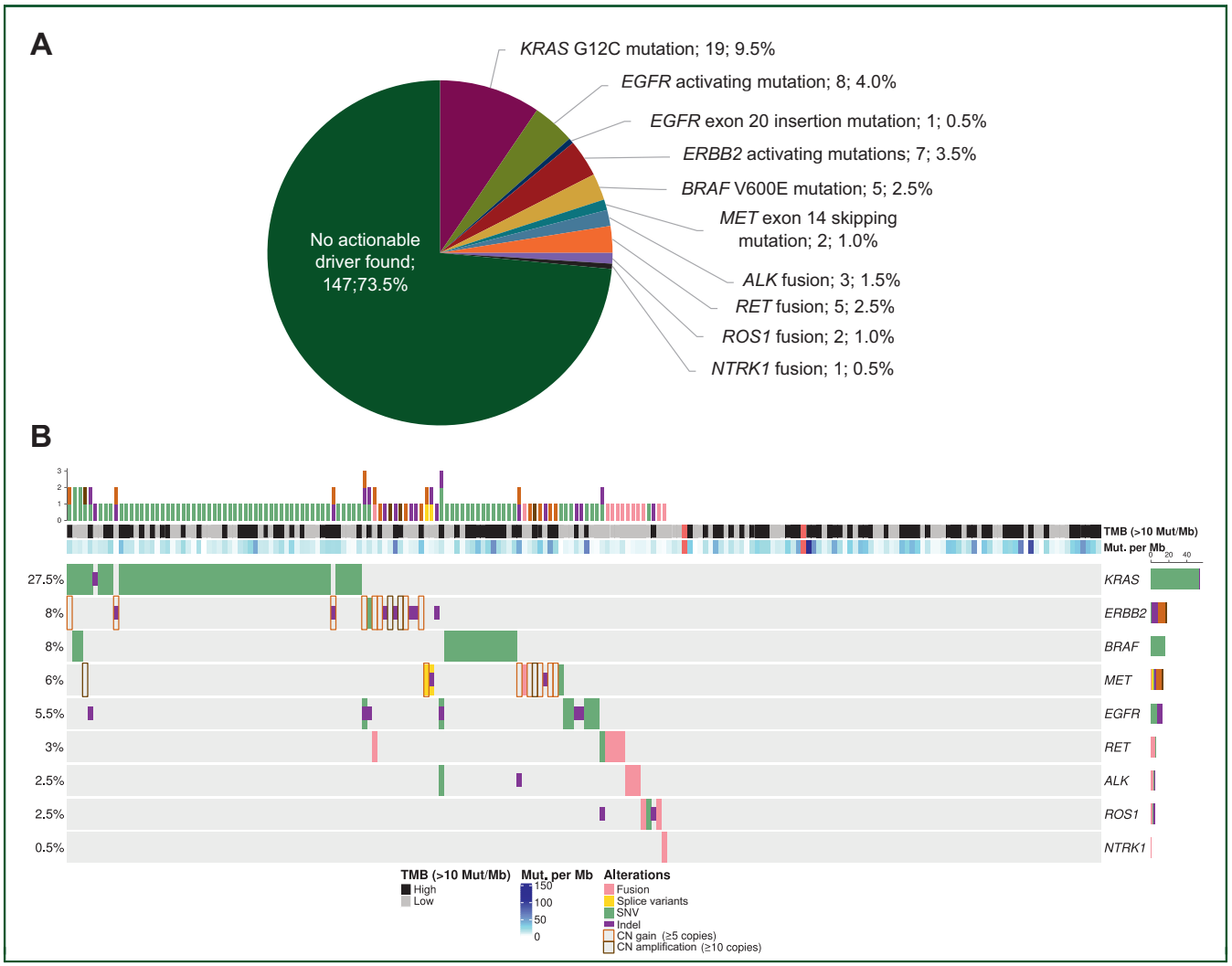

Figure 1. Actionable alterations found in non-small-cell lung cancer (NSCLC) specimens using the TruSight™ Oncology 500 (TSO500) panel in this cohort of 200 patients. (A) Distribution of actionable driver alterations in clinically relevant genes according to the European Society for Medical Oncology (ESMO) guidelines. (B) Oncoplot showing all pathogenic or likely pathogenic genomic alterations in clinically relevant genes, regardless of being actionable or not. Missing tumour mutational burden (TMB) values are depicted in red.
CN, copy number; indel, insertions and deletions; SNV, single nucleotide variants.

suppressor genes, as for example the presence of *TP53, STK11* or *KEAP1* mutations which is gaining clinical relevance in the context of *KRAS*-mutant tumours. In this regard, we analysed which mutations are more likely to co-occur focusing on a predefined list of actionable drivers and tumour suppressor genes (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.102197). Only concurrent mutations of *STK11* and *KRAS* remained statistically significant, after adjusting for multiple comparisons.

Comparison of TSO500 panel results with alternate single-gene testing

Single-gene testing was conducted before NGS in 131 patients (65.5%). Specifically, an RT—PCR for assessing *EGFR* mutation was carried out in 130 patients (65.0%), while IHC or FISH for *ALK* and *ROS1* rearrangement in 126 (63.0%) and 103 (51.5%) patients, respectively. Additional information about the single-gene testing is provided in Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2023.102197.

We obtained discordant results between single-gene testing and NGS in four cases. Firstly, a patient with an *EGFR L861Q* mutation detected by RT—PCR, but not confirmed by NGS due to a DNA library failure. This patient received frontline osimertinib and achieved a partial response lasting for only 6 months. Secondly, a patient harboured an *RET* rearrangement diagnosed by FISH, which was not confirmed by NGS, despite testing the same specimen and the good quality of DNA and RNA libraries. This patient achieved a confirmed partial response to pralsetinib lasting for 12 months. Thirdly, a patient with a negative FISH for *RET* rearrangement, but with a *KIF5B-RET* fusion detected by NGS. This patient had an ongoing partial response to pralsetinib lasting for >18 months. Lastly, in a patient with a previously negative *ROS1* expression by IHC and an *ROS1-EZR* fusion detected by NGS. This patient had

Table 2. Access to targeted therapies in 63 patients harbouring an actionable oncogenic driver alteration detected by TSO500 panel or by other testing methods

	Access to targeted therapy	Reasons for not receiving targeted therapy			
		Clinical deterioration	No access in the first line	Clinical trial/expanded access not available	Stage I-III
n = 63 (%)	28 (44.4)	12 (19.1)	12 (19.1)	4 (6.3)	7 (11.1)
EGFR mutations *n* = 10 (%)	10 (100.0)	—	—	—	—
NTRK1 fusion *n* = 1 (%)	1 (100.0)	—	—	—	—
FGFR1 fusion *n* = 1 (%)	1 (100.0)	—	—	—	—
RET fusion *n* = 6 (%)	5 (83.3)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)
ALK fusion *n* = 4 (%)	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)
ROS1 fusion *n* = 2 (%)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)
ERBB2 alterations *n* = 9 (%)	3 (33.4)	2 (22.2)	2 (22.2)	2 (22.2)	0 (0.0)
BRAF-V600E *n* = 5 (%)	1 (20.0)	2 (40.0)	0 (0.0)	1 (20.0)	1 (20.0)
MET alterations *n* = 6 (%)	1 (16.7)	2 (33.3)	0 (0.0)	2 (33.3)	1 (16.7)
KRAS-G12C *n* = 19 (%)	3 (15.8)	6 (31.6)	8 (42.1)	0 (0.0)	2 (10.5)

Reasons for not receiving personalised treatment are described according to the type of driver alteration.
TSO500, TruSight™ Oncology 500.

an ongoing partial response to entrectinib lasting for >25 months. In all these cases, NGS and single-gene testing were conducted in the same specimen.

Access to targeted therapy in patients with NSCLC

We analysed the impact on survival of having access to targeted therapies in this cohort, including not only 53 harbouring actionable oncogenic alterations detected by the TSO500 panel, but also two patients with low allele frequency alterations that were validated by orthogonal methods (*ALK* fusion and *MET* exon 14 skipping) and six additional patients who carried driver alterations that might be potentially actionable (*MET* fusions and amplifications, *ERBB2* amplifications and *FGFR1* fusions). We also considered two additional cases detected only by single-gene testing (*EGFR* L861Q and *RET* rearrangement). Of those 63 patients, only 28 (44.4%) were eligible to receive matched targeted therapy. The access to targeted therapy is highly influenced not only by the specific GA and tumour stage but also by regulatory constraints. More details about the percentage of patients receiving targeted therapy according to the oncogenic driver are shown in Table 2.

In this cohort, patients with *EGFR* mutations and those with *RET* or *NTRK1* rearrangements had a higher access rate to targeted therapy, compared to those with *KRAS*-G12C mutation, *BRAF*-V600E mutation, *MET* and *ERBB2* alterations. Thirty-five patients (55.6%) did not receive targeted therapy, despite harbouring a druggable GA. The main reasons for not receiving matched targeted therapy were clinical deterioration (n = 12, 19.1%), lack of access to specific therapy in the first line (n = 12, 19.1%), no therapies approved and lack of clinical trials or expanded access programmes (n = 4, 6.3%) or no evidence of metastatic disease (n = 7, 11.1%). Patients with *ALK* and *ROS1* rearrangements had a lower access rate to targeted therapy than expected because half received therapy with curative intent and were free of recurrence during the study follow-up.

Survival analysis according to the presence of actionable oncogenic alterations and targeted therapy in advanced NSCLC

We defined three groups of patients based on the presence of actionable alterations and whether they received targeted therapy. The patients' characteristics are shown in Table 3. Patients harbouring actionable alterations were more likely to be women, never smokers and had lower TMB. The number of treatment lines was higher among patients who received targeted therapy, while the proportion of patients who received chemotherapy and immunotherapy was similar between these three groups. The survival analysis was restricted to 183 patients with advanced NSCLC with sufficient clinicopathological information; among this group, median follow-up was 14.5 months (interquartile range 7.5-32.6 months). The median OS for all patients was 20.7 months [95% confidence interval (CI) 15-58.6 months]. Patients harbouring a druggable GA receiving a targeted therapy had significant longer median OS (not reached, NR) compared to patients without an actionable driver [27.7 months, 95% CI 15.02 months-NR; hazard ratio (HR) = 0.32; 95% CI 0.14-0.75; Figure 2]. Patients with an oncogenic driver not receiving a targeted therapy had a trend toward shorter median OS (10 months, 95% CI 9.18 months-NR) compared to patients without an actionable driver (27.7 months, 95% CI 15.02 months-NR; HR = 1.71, 95% CI 0.93-3.19).

DISCUSSION

In this study, we proved the feasibility of using the large hybrid capture-based NGS panel TSO500 to characterise the GAs in a cohort of 200 patients with NSCLC. Our study demonstrates that this NGS panel, even after conducting single-gene testing for *EGFR* and *ALK*, was able to detect genomic druggable alterations in 26.5% of the patients, with major impact in treatment decisions. This result is consistent with previous works in advanced NSCLC. Drilon et al. sequenced 31 samples from patients diagnosed with NSCLC without any alteration by single-gene testing and using a hybrid capture NGS panel was able to detect a

Table 3. Association between clinicopathological variables with distinct subgroup of advanced patients based on the presence of genomic driver alteration (gDA) and access to targeted therapies

	n	No gDA found	gDA found (untreated with targeted therapy)	gDA found (treated with targeted therapy)	*P* value
		$n = 127$	$n = 28$	$n = 28$	
Age, median (range), years	183	63.90 (35.28-82.82)	66.37 (38.64-87.92)	59.57 (42.26-85.58)	0.240
Sex, *n* (%)	183				0.012
Male		94 (74.02)	15 (53.57)	14 (50.00)	
Female		33 (25.98)	13 (46.43)	14 (50.00)	
Stage, *n* (%)	183				0.463
I		10 (7.87)	0 (0.00)	1 (3.57)	
II		5 (3.94)	1 (3.57)	2 (7.14)	
III		29 (22.83)	4 (14.29)	4 (14.29)	
IV		83 (65.35)	23 (82.14)	21 (75.00)	
Histology, *n* (%)	183				0.120
Adenocarcinoma		99 (77.95)	26 (92.86)	23 (82.14)	
Squamous		12 (9.45)	0 (0.00)	0 (0.00)	
Other		16 (12.60)	2 (7.14)	5 (17.86)	
ECOG PS, *n* (%)	183				0.076
0		21 (16.54)	0 (0.00)	7 (25.00)	
1		80 (62.99)	22 (78.57)	18 (64.29)	
≥2		26 (20.47)	6 (21.43)	3 (10.71)	
Smoking status, *n* (%)	183				<0.001
Never smoker		6 (4.72)	8 (28.57)	9 (32.14)	
Former smoker		54 (42.52)	10 (35.71)	10 (35.71)	
Current smoker		67 (52.76)	10 (35.71)	9 (32.14)	
Smoking (pack-years), median (range)	151	40.00 (2.00-100.00)	40.00 (10.00-76.00)	27.00 (5.00-75.00)	0.028
PD-L1 category, *n* (%)	169				0.610
<1%		50 (43.10)	12 (46.15)	13 (48.15)	
1%-49%		32 (27.59)	8 (30.77)	10 (37.04)	
≥50%		34 (29.31)	6 (23.08)	4 (14.81)	
Sample type, *n* (%)	182				0.748
Biopsy		68 (53.54)	15 (53.57)	11 (40.74)	
Surgical piece		33 (25.98)	6 (21.43)	9 (33.33)	
Cytology		26 (20.47)	7 (25.00)	7 (25.93)	
Tumour purity, *n* (%)	183				0.275
<20%		10 (7.87)	3 (10.71)	5 (17.86)	
≥20%		117 (92.13)	25 (89.29)	23 (82.14)	
TMB status, *n* (%)	181				0.002
Low		51 (40.48)	17 (60.71)	20 (74.07)	
High		75 (59.52)	11 (39.29)	7 (25.93)	
Treatment lines received, *n* (%)	183				0.002
0		29 (22.83)	6 (21.43)	0 (0.00)	
1		55 (43.31)	15 (53.57)	8 (28.57)	
2		21 (16.54)	5 (17.86)	14 (50.00)	
≥3		22 (17.32)	2 (7.14)	6 (21.43)	
Received chemotherapy, *n* (%)	183				0.778
No		45 (35.43)	8 (28.57)	10 (35.71)	
Yes		82 (64.57)	20 (71.43)	18 (64.29)	
Received ICI, *n* (%)	183				0.072
No		50 (39.37)	15 (53.57)	17 (60.71)	
Yes		77 (60.63)	13 (46.43)	11 (39.29)	

ECOG, Eastern Cooperative Oncology Group; ICI, immune checkpoint inhibitors; PD-L1, programmed death-ligand 1; TMB, tumour mutational burden.

druggable alteration in 26% of patients.[13] Suh et al. analysed 6832 NSCLC samples using hybrid capture NGS panel and found a druggable alteration in 33.6% of the samples.[26] Noteworthy, when this study was published, neither *KRAS*-G12C mutation nor *NTRK* rearrangement was considered actionable alterations. In a more recent study, Perdrizet et al. evaluated 134 patients (107 not harbouring *EGFR* mutations or *ALK* rearrangements by single-gene testing) using the Oncomine Comprehensive Assay NGS panel and found a potentially actionable alteration in 31% of patients. This cohort was more enriched for females (59.7%) and never smokers (53.0%) than our series.[27]

In our study, the failure rate of the TSO500 panel was very low and was mostly related to the unsuccessful preparation of the RNA libraries. Failure of the RNA libraries was more likely in surgical specimens and in older samples, as previously reported.[28] RNA library failure may reduce the ability to detect gene rearrangements.[28,29] A previous work validating the TSO500 panel using 170 FFPE tumour samples from distinct tumour types and specimens, including a small subset of NSCLC, reported a 7% failure rate in the RNA library preparation, which was slightly higher than in our study. The analytical sensitivity and specificity were high for detecting SNVs, indels, CNVs, MSI and gene

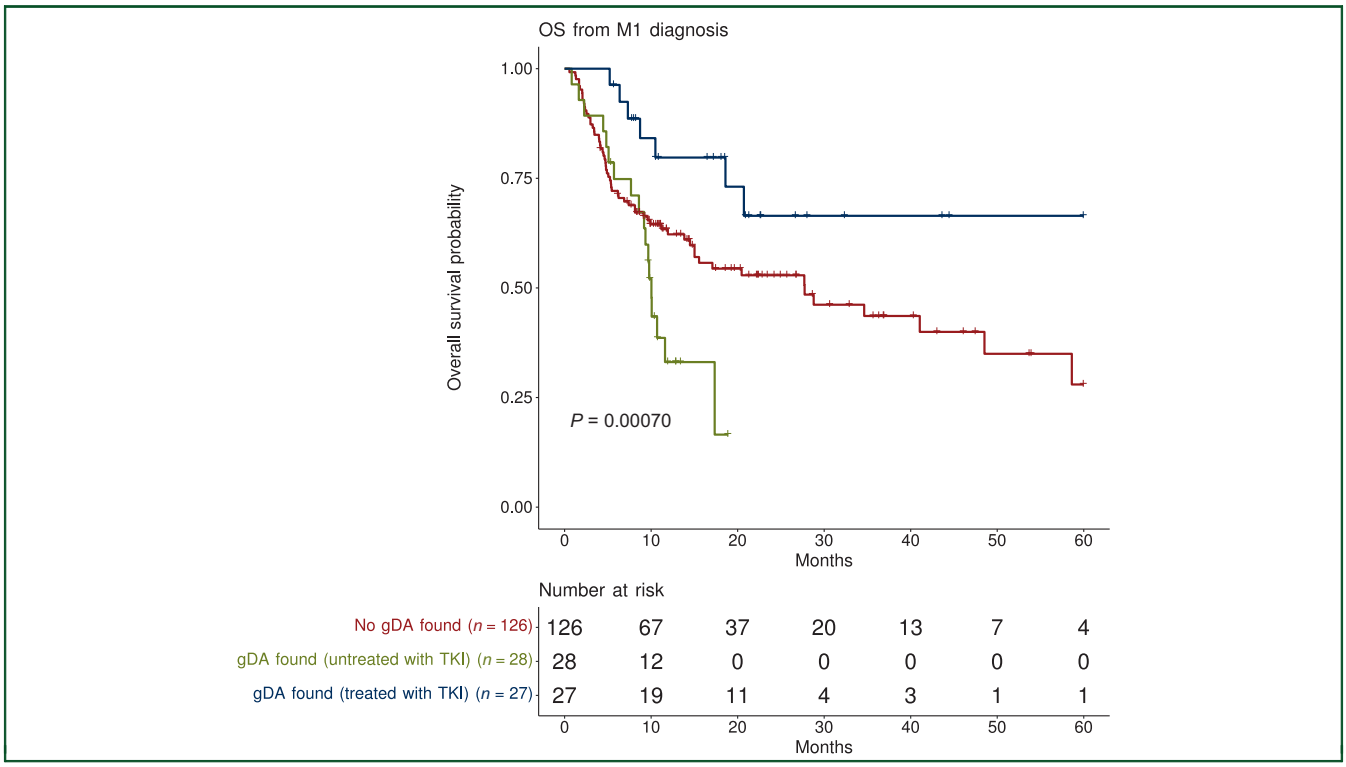

Figure 2. Kaplan–Meier plot of overall survival in patients with advanced non-small-cell lung cancer (NSCLC) according to the presence or absence of an actionable alteration and its treatment with a targeted therapy. *P* value from a Cox proportional hazards model adjusted for sex, stage, age, histology, smoking status and tumour mutational burden (TMB) status.
gDA, genomic driver alteration; TKI, tyrosine kinase inhibitor.

rearrangements.[30] A recent study assessed the feasibility of the TSO500 panel using Diff-Quick cytology smears from 27 patients with NSCLC, but sequencing failed in 5 cases (1 due to library preparation failure and 4 due to low median coverage).[31]

In our study, we obtained discordant results between NGS and single-gene testing only in four patients, despite using the same specimen for the analysis. An *EGFR* mutation was not detected due to failure in the DNA library and one *RET* fusion found by FISH was not validated by NGS. These discordances occur in the real clinical practice and should always be discussed in the multidisciplinary molecular tumour board.

A significant number of patients with actionable GAs were able to receive matched targeted therapy in this series. As previously reported, patients receiving tailored targeted therapies had improved survival outcomes.[3,4] The major reasons for not getting access to targeted therapy were diverse, but some were avoidable in principle such as the lack of access to personalised therapies especially in the frontline setting due to hurdles in the drug approval process.

When using large NGS panels to sequence somatic tumour DNA, it is likely to detect incidental findings as pathogenic variants in cancer susceptibility genes. In our study, we found pathogenic variants in those genes at an allele frequency $\geq$20% for indels or $\geq$30% for SNVs in 12 patients (6%). Those cases were discussed in our multidisciplinary molecular tumour board and we followed the ESMO recommendations[24] to manage those cases and decide which patients should be referred to the Genetic Counselling Unit.[32]

Our study has several limitations. Firstly, the single-gene testing overlap may introduce a bias selection in our cohort. During the first period of this study, only patients without *EGFR* mutations and *ALK* or *ROS1* rearrangements were sequenced; this partly explains the lower rate of these alterations observed in our study. Secondly, our study was carried out at a single institution with a limited sample size but included consecutive cases. The limited sample size did not allow us to assess the prognostic impact of concurrent mutations such as *TP53* mutations in *EGFR*- or *ALK*-positive patients receiving targeted therapy or *STK11-KEAP1* co-mutations in *KRAS*-mutant patients receiving immunotherapy. Moreover, the study population was heterogeneous and received a wide range of different treatments. This variability could yield biases in the survival analysis. Finally, the limited access to specific targeted therapies which are not reimbursed in our country could restrain the expected survival benefit obtained by NGS.

In conclusion, the utilisation of the TSO500 hybrid capture NGS panel is feasible and highly informative in clinical practice, with reasonable turnaround time and low failure rate in this cohort of patients with NSCLC. Additionally, detection of oncogenic driver alterations and access to targeted therapies have a major impact on patients' life expectancy. Ensuring access to NGS testing and to targeted therapy is currently essential to treat patients with NSCLC.

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DISCLOSURE

EN has participated in advisory board or lectures from Roche, Bristol Myers Squibb, Merck Sharp Dohme, Merck-Serono, Sanofi, Pfizer, Lilly, Amgen, Daiichi-Sankyo, Boehringer-Ingelheim, AstraZeneca, Pierre Fabre, Qiagen, Takeda, Sanofi, Janssen, Regeneron and Bayer. All other authors have declared no conflicts of interest.

DATA SHARING

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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