# Actionable non-small cell lung cancer mutation identification by comprehensive genomic profiling for clinical trial enrollment: the European Program for the ROutine testing of Patients with Advanced lung cancer (EPROPA)

Francesco Passiglia, Angela Listì, Paolo Bironzo, Alessandra Merlini, Federica Benso, Francesca Napoli, Francesca Alice Barbu, Vanessa Zambelli, Fabrizio Tabbò, Maria Lucia Reale, Claudio Sini, Elisa Roca, Paola Adriana Taveggia, Francesca Simionato, Lucio Buffoni, Laura Mazilu, Vito Barbieri, Daniele Pignataro, Antonio Araujo, Luis Paz-Ares, Enriqueta Felip, Nevena Secen, Alina Comanescu, Kleida Mati Ramizi, Anna Cecilia Bettini, Vieri Scotti, Helena Linardou, Katja Mohorcic, Giulia Meoni, Diana Giannarelli, Marco Volante, Umberto Malapelle, Stefania Vallone, Giorgio Scagliotti, Luisella Righi, Silvia Novello

PII: S1556-0864(24)02529-2

DOI: https://doi.org/10.1016/j.jtho.2024.12.010

Reference: JTHO 3223

To appear in: *Journal of Thoracic Oncology*

Received Date: 3 June 2024

Revised Date: 15 November 2024

Accepted Date: 7 December 2024

Please cite this article as: Passiglia F, Listì A, Bironzo P, Merlini A, Benso F, Napoli F, Barbu FA, Zambelli V, Tabbò F, Reale ML, Sini C, Roca E, Taveggia PA, Simionato F, Buffoni L, Mazilu L, Barbieri V, Pignataro D, Araujo A, Paz-Ares L, Felip E, Secen N, Comanescu A, Ramizi KM, Bettini AC, Scotti V, Linardou H, Mohorcic K, Meoni G, Giannarelli D, Volante M, Malapelle U, Vallone S, Scagliotti G, Righi L, Novello S, Actionable non-small cell lung cancer mutation identification by comprehensive genomic profiling for clinical trial enrollment: the European Program for the ROutine testing of Patients with Advanced lung cancer (EPROPA), *Journal of Thoracic Oncology* (2025), doi: https://doi.org/10.1016/j.jtho.2024.12.010.

# Actionable non-small cell lung cancer mutation identification by comprehensive genomic profiling for clinical trial enrollment: the European Program for the ROutine testing of Patients with Advanced lung cancer (EPROPA)

Francesco Passiglia[1]*, Angela Listì[1]*, Paolo Bironzo[1], Alessandra Merlini[1], Federica Benso[1], Francesca Napoli[1], Francesca Alice Barbu[1], Vanessa Zambelli[1], Fabrizio Tabbò[2], Maria Lucia Reale[3], Claudio Sini[4], Elisa Roca[5], Paola Adriana Taveggia[6], Francesca Simionato[7], Lucio Buffoni[8], Laura Mazilu[9], Vito Barbieri[10], Daniele Pignataro[11], Antonio Araujo[12], Luis Paz-Ares[13], Enriqueta Felip[14], Nevena Secen[15], Alina Comanescu[16], Kleida Mati Ramizi[17], Anna Cecilia Bettini[18], Vieri Scotti[19], Helena Linardou[20], Katja Mohorcic[21], Giulia Meoni[22], Diana Giannarelli[23], Marco Volante[1], Umberto Malapelle[24], Stefania Vallone[25], Giorgio Scagliotti[1], Luisella Righi[1]^ and Silvia Novello[1]^

1. Department of Oncology, University of Turin, S. Luigi Gonzaga Hospital, Orbassano (TO), Italy
2. Medical Oncology, ASLCN2 Alba e Bra, Michele e Pietro Ferrero Hospital, Verduno (CN), Italy
3. Medical Oncology, Vito Fazzi Hospital, Lecce, Italy
4. Medical Oncology and CPDO, Giovanni Paolo II Hospital, Olbia, Italy
5. Thoracic Oncology - Lung Unit P. Pederzoli Hospital, Peschiera d/G (VR), Italy
6. Medical Oncology, P.O.U. Villa Scassi Hospital, Genova, Italy
7. Medical Oncology, AULSS 8 - San Bortolo Hospital, Vicenza, Italy
8. Medical Oncology, Humanitas Gradenigo Hospital, Torino, Italy
9. Medical Oncology, Ovidius Clinical Hospital, Ovidius University of Constanta, Romania.
10. Medical Oncology, Renato Dulbecco University Hospital, Catanzaro, Italy
11. Medical Oncology, Cardinal Massaia Hospital, Asti, Italy
12. Dept of Medical Oncology, Centro Hospitalar Universitário de Santo António, Portugal
13. Medical Oncology Dept, University Hospital 12 De Octubre, Madrid, Spain
14. Medical Oncology Dept, Vall d'Hebron University Hospital, Barcelona, Spain
15. Pulmonology- oncology Dept., AcibademBelmedic, Belgrade, Serbia
16. Community Health Association, Romania
17. Medical Oncology, Salus Hospital, Tirana, Albania
18. Medical Oncology, ASST Papa Giovanni XXIII, Bergamo, Italy
19. Dept of Radiotherapy, AOU Careggi, Firenze, Italy
20. 4th Oncology Dept & Comprehensive Clinical Trials Center, Metropolitan Hospital, Athens, Greece
21. Medical Oncology Dept., University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia
22. SOS Medical Oncology, San Giovanni di Dio Hospital, Firenze, Italy
23. Fondazione Policlinico Universitario A. Gemelli, IRCCS - Facility of Epidemiology and Biostatistics, Rome, Italy.
24. Department of Public Health, University Federico II of Naples, Naples, Italy.
25. WALCE, Orbassano (TO), Italy

**#Correspondence to**: Silvia Novello, Department of Oncology, University of Turin, St. Luigi Hospital, Orbassano (TO), Italy; email: silvia.novello@unito.it.

**Conflict of interest Statement:**

F.P received consultants' and advisory fee from AstraZeneca, BMS, Novartis, Pfizer, Roche, MSD, Amgen, Johnson and Johnson, Sanofi, Beigene, Gilead, Regeneron, Pharmamar, Thermo Fisher Scientific. A.L. has nothing to declare. P.B. reports personal fees (as advisor) from Seagen, Regeneron, Pierre Fabre, Janssen and for Research Grant (Institution) from Pfizer and Roche F.T. reports personal fees (as speaker bureau) from AstraZeneca, Novartis, Roche, Takeda L.B. reports personal fees (as advisor) from AstraZeneca, BMS, MSD, Novartis L.M. reports personal fees (as speaker bureau, advisor, manuscript writer or travel expenses) from Accord, Angelini, Astellas, AstraZeneca, Bayer, BMS, Eli Lilly, Ipsen, Johnson & Johnson Romania, Merck, MSD, Novartis, Pfizer, Roche, Sandoz, Sanofi D.P. reports personal fees (as speaker bureau) from AstraZeneca, BMS, Novartis, Roche, Takeda A.A. reports personal fees (as speaker bureau, advisor, manuscript writer or travel expenses) from Astellas, AstraZeneca, BMS, Eli Lilly Oncology, Janssen, MSD, Novartis, Pfizer, Roche. L.P.A. reports personal fees (honoraria or travel expenses) from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Incyte, Ipsen, Lilly, Merck Serono, Mirati, MSD, Novartis, PharmaMar, Pfizer, Roche/Genentech, Sysmex. Other relationships (immediate family member) with Amgen, Ipsen, Merck, Novartis, Pfizer, Roche, Sanofi, and Servier. Cofounder of Altum Sequencing and an external board member for Genomica. E.F. reports personal fees (as speaker bureau, advisor or travel expenses) from Abbvie, Amgen, AstraZeneca, Bayer, BeiGene, BMS, Boeringher Ingelheim, Daichii Sankyo, Eli Lilly, F. Hoffmann-La Roche, Genentech, Gilead, GlaxoSmithKline, Janssen, Medical Trends, Medscape, Merck Serono, Merck Sharp & Dohme, Novartis, Peervoice, Peptomyc, Pfizer, Regeneron, Roche, Sanofi, Takeda, Touch Oncology, Turning Point A.C. reports personal fees (as advisor) from Novartis A.C.B. reports personal fees (as advisor or support for attending meetings) from BMS, Sanofi, Takeda V.S. reports personal fees (as speaker bureau or advisor) from AstraZeneca, BMS, MSD, Roche, Novatis, Takeda. Research grant from AstraZeneca H.L. reports personal fees (as advisor) from Amgen, AstraZeneca, BMS, GSK, MSD, Novartis, Pfizer, Roche K.M. reports personal fees (as advisor or support for attending meetings) from Amgen, BMS, Boehringer Ingelheim, Elli Lilly, Janssen, MSD, Pfizer, Roche, Takeda. G.S. reports personal fees (as consultant/advisor) from AstraZeneca, Bayer, BeiGene, Eli Lilly, J&J, MSD, Pfizer, Roche, Takeda U.M. reports personal fees (as speaker bureau or advisor) from Amgen Inc., AstraZeneca, Boehringer Ingelheim, Diatech, Eli Lilly & Company, GlaxoSmithKline, Hedera, Janssen Biotech, Merck Sharpe & Dohme Corporation, Novartis, Qiagen, Roche Health Solutions Inc., Thermo Fisher Scientific. Leadership Role: International Society of Liquid Biopsy and The Journal of Liquid Biopsy - Elsevier Spouse Dependent: Roche. L.R. received consultants' fee from AstraZeneca, Novartis, Roche, Amgen, BeiGene, Novartis, EliLilly. S.N reports personal fees (as speaker bureau or advisor) from Eli Lilly, MSD, Roche, Takeda, Pfizer, Astra Zeneca, Amgen, Thermo Fisher, Novartis, Sanofi, Janssen, unrelated to the current work. The other authors have nothing to declare.

**Abstract**

**Background:** To reduce the gap about the relevant heterogeneity of molecular testing and cancer care across Europe, Women Against Lung Cancer in Europe (WALCE) promoted the European Program for ROutine testing of Patients with Advanced lung cancer (EPROPA) and provided a free-of-charge molecular profiling platform for non-small cell lung cancer sample characterization with the aim of increasing the detection of targetable drivers and improving patients' access to clinical trials.

**Methods:** From January 2021 to December 2023, 20 centres located at 5 different European countries (Greece, Slovenia, Romania, Albania and Italy) joined EPROPA, with 555 advanced NSCLC patients registered into the program. Anonymized patients' clinical-pathological data were shared through the EPROPA web platform and tissue samples were collected to the Molecular Pathology Unit of the Reference Center (University of Turin) for molecular analyses. A comprehensive genomic profiling by targeted next-generation sequencing approach has been performed and molecular reports have been discussed within the molecular tumour board (MTB) in order to assess patients' eligibility for clinical trials.

**Results:** The average turnaround time was 8 days, with only 30 out of 555 (6%) tissue samples not suitable for molecular analysis. Among the 525 analyzed samples, a total of 570 molecular alterations have been identified, including 264 pathogenic targetable oncogenic alterations and 113 cases with co-occurring mutations. A total of 18 molecular alterations with potential germline and hereditary cancer syndrome implications have been reported. The identification of a clinical trial was considered for 205 patients. After MTB discussion, 30 patients were enrolled and treated in clinical studies available in Europe. Survival outcome were significantly improved in patients with targetable molecular alterations receiving a matched targeted therapy.

**Conclusion:** This data confirmed the feasibility and usefulness of the program in the real-world practice scenario, supporting the implementation of NGS-based molecular characterization of NSCLC samples, in order to reduce the unequal access to tests, drugs and clinical trials in Europe.

**Keywords:** Precision medicine, lung cancer, biomarker testing, targeted therapy, clinical trials, advocacy.

## Background

The stepwise implementation of precision medicine in cancer care has radically changed the therapeutic management of different tumor types, including non-small cell lung cancer (NSCLC) generating a paradigm shift, characterized by the mutational model for patients' personalized treatment. A biological classification of the disease based on the tumor molecular profiling has definitively entered in the routine clinical practice, and in lung adenocarcinomas at least nine (EGFR, KRAS, ALK, ROS1, RET, ERBB2, BRAF, NTRK, MET) different molecular biomarkers are identifiable, requiring different therapeutic approaches in terms of targeted therapies, as well as sequencing strategies to be carefully personalized at individual patient level (1). Different studies have clearly demonstrated that advanced NSCLC patients harbouring a targetable molecular alteration and receiving a matched targeted treatment, live longer and better (2). Furthermore, recent epidemiological reports clearly showed that the implementation of precision medicine in clinical practice has significantly contributed to the reduction of lung cancer related mortality (3), with a percentage of patients alive at 5 years from diagnosis around 23% now, as compared to 15% few years ago (4). Based on this data, the lung cancer international guidelines currently recommend tumour molecular profiling for all newly diagnosed advanced NSCLC patients with non-squamous subtype, in order to personalize therapeutic strategies and ultimately optimize patients' outcomes (4, 5). Considering the increasing number of targetable molecular biomarkers approved for NSCLC treatment, the use of next-generation sequencing (NGS) is currently recommended as the most suitable approach for complete tumour genomic profiling, enabling the simultaneous identification of all relevant predictive biomarkers, covering either full length or hot spot regions in target genes, detecting either known or unknown genomic alterations within the gene panel reference range, ensuring higher diagnostic accuracy, faster turnaround time (TAT) for low sample volumes, and reducing costs, compared to the old standard single-gene technology approach (5-7). International cancer scientific societies have recently endorsed NGS-based molecular profiling as a key component of NSCLC patients' standard management and care (5, 6), definitively ensuring adequate characterization of some elusive targets, like EGFR exon 20 insertion variants and NTRK rearrangements. However, biomarker testing recommendations vary across Europe, while molecular tumor boards (MTB) national guidelines are lacking in most countries (8). In addition to that, logistic, cultural, technological and socioeconomic barriers limited patients' access to NGS-based molecular profiling and matched targeted treatments worldwide (9, 10). In addition, compared to Unites States (US), European patients with metastatic NSCLC are often penalized due to delay in drug and diagnostic test approval, registration and reimbursement processes, with a significant negative impact on their life expectancy and quality.

To fill the gap affecting the relevant heterogeneity of molecular testing and cancer care across Europe, the Women Against Lung Cancer in Europe (WALCE) Advocacy Group promoted the European Program for ROutine testing of Patients with Advanced lung cancer (EPROPA) and provides a free-of-charge molecular

screening platform for NSCLC samples Comprehensive Genomic Profiling (CPG) with the aim of increasing the detection of targetable drivers and optimizing patients' access to biomarker-driven clinical trials. Here we report an interim report summarizing molecular testing results, clinical trials enrollment and therapeutic management of advanced NSCLC patients during the first two years of EPROPA activity.

## Methods

### *Patients' selection criteria*

Patients aging ≥ 18 years old, with histologically or cytologically confirmed diagnosis of NSCLC**,** stage IV (according to the 8th edition of the International Association for the Study of Lung Cancer (IASLC) TNM Staging System) and availability of adequate tumor samples for molecular analysis, were included. All the patients provided a written informed consent (IC) according to ICH/GCP and national/local regulations, before enrollment. The EPROPA protocol was initially approved (n. 132/2020) by the Ethical Committee (EC) of the reference center, St. Luigi Hospital (Orbassano, Italy), and subsequently received the EC approvals by all other participating centers across different European countries.

### *Patients' registration and samples collection*

Following signing the IC, patients have been registered to the EPROPA portal (https://www.epropa.eu/portal/) by the reference oncologist and a sequential identification number ("seqID") has been assigned. Substantial clinical and pathological data have been electronically collected through the online portal. In parallel, the relevant Formalin-Fixed and Paraffine-Embedded (FFPE) tissue samples were collected to the central laboratories of the reference center by WALCE shipment logistics. Samples included at least one tissue block (or sections) from the primary tumor, recurrent tumor or metastasis, obtained at the time of disease diagnosis (primary surgery or biopsy) or progression (re-biopsy). Each collected tissue has been centrally evaluated by a reference Pathologist for molecular analysis adequacy.

### *Comprehensive Genomic Profiling*

A tumor comprehensive genomic profiling (CGP) sequencing for molecular screening has been performed at the central laboratory of the reference center. A targeted next-generation sequencing (tNGS) analysis has been performed by using the Ion Torrent platform (ThermoFisher Scientific, MA, USA) with the broad gene panel OCAv3 (Oncomine Comprehensive Assay v.3; ThermoFisher Scientific, Waltham, MA, USA), a solid tumor DNA and RNA kit assay allowing to cover 161 cancer-associated genes in hot spot region and full length, including CNV analysis and fusion detection. Briefly, tumor DNA and RNA were extracted by automated purification kits (Maxwell). The amplicon libraries were prepared with Ion AmpliSeq Library Kit (ThermoFisher). After PCR amplification and barcodes ligation, the amplicon libraries were equalized and

pooled in equal molar ratio. Emulsion PCR and template preparation were performed using the Ion Chef and sequenced. Data analysis have been conducted automatically by Ion Reporter Software. Post-sequencing bioinformatics analysis matched the complementary strands of each barcoded DNA fragment to remove false-positive results. The variant allele frequency (VAF) was computed by dividing the number of mutated DNA molecules by the total count of DNA fragments (mutated and wild-type) at that allele. Variants were called if the VAF was ≥5%. Biomarker assessment was performed according to separately defined standards of quality control, in accordance with applicable national and international quality standards.

The results of the analysis were reported using an automated genomic analysis software, a tool developed specifically for further examination of NGS data (Oncomine Reporter, ThermoFisher) and uploaded to the EPROPA platform. The level of certification of the biomarker test used were provided with the results, in order to help the treating physician at local center to evaluate whether the results can match for the enrolment in relevant therapeutic biomarker-driven trial or drive routine clinical practice.

***Enrollment in downstream biomarker-driven clinical trials***

Upon request of the medical oncologist who was taking care of the patient at the local center, molecular reports have been discussed within the molecular tumour board (MTB) of the reference center in order to assess patients' eligibility for targeted therapies available, either in clinical trials or in real-world practice. When downstream therapeutic biomarker-driven trials were available, the treating physicians were informed about patient potential eligibility for clinical trial across European sites. For those patients enrolled, Women Against Lung Cancer Europe (WALCE) provided logistic and financial support to both patients and caregivers during the entire treatment journey.

***Statistical analysis***

The number and percentage of participants as well as their clinical, pathological, molecular characteristics, and administered therapies/clinical trials have been summarized either by descriptive statistics or categorical tables and descriptive analysis has been performed. The Overall Response Rate (ORR) was compared among subgroups using the chi-square test. Survival analysis was performed using the Kaplan-Meier method and differences among curves were assessed using the log-rank test. IBM SPSS Statistics Software for Windows, v.28.0 (Armonk, NY: IBM Corp) was used for analysis.

## Results

***Patients' characteristics***

From January 2021 to December 2023, 20 centres sited at 5 different European countries (Greece, Slovenia, Romania, Albania and Italy) joined EPROPA, with overall 555 advanced NSCLC patients registered to the website platform (https://www.epropa.eu/portal/). Among these, 161 (29%) were over 75 years/old, 172 (31%) aged between 65 and 75 years/old, 178 (32%) aged between 50 and 65 years/old, and 44 (8%) < 50 years/old. About half of the patients (250/555, 45%) were female, 144/555 (26%) never smokers, 272/555 (49%) former smokers, and 139/555 (25%) current smokers. The majority of analyzed samples were adenocarcinoma (433/78%), 78/14% squamous cell carcinoma, and 44/8% other histological subtypes. All the patients have a metastatic disease (stage IVA-B) at the time of inclusion, according to the 8$^{th}$ TNM Staging System. Previous molecular profiling was not performed for 270/555 (49%) patients with newly diagnosed metastatic disease, while 285/555 (51%) of them had previously received a molecular testing and were negative for standard oncogenic drivers and/or progressing to a targeted therapy. Patients' characteristics were detailed in **Table 1**. Clinical outcomes (treatment response and date of progression/death) were available for 250 out of 525 patients included in EPROPA who received antitumor systemic treatments. In this population the ORR was 34.4% (95% CI: 28.5-40.3), mPFS was 8.3 months (95% CI: 6.4-10.3), and mOS was 14.1 months (95% CI: 11.1-17.0).

***Molecular analysis***

Among the 555 patients included in the EPROPA project, a total of 30 out of 555 tissue samples were not suitable for molecular analysis for scarce or deteriorated tumor cell amount with an overall failure rate of 6%. The mean turnaround time (from sample delivering at the reference molecular laboratory to the molecular report upload on platform) was of 8 days (**Supplementary Figure 1**).

Among the 525 analyzed samples, a total of 570 molecular alterations have been identified (**Figure 1**), including 264 potentially targetable pathogenic alterations with different levels of actionability according to the ESCAT score (11) (**Figure 2**), matching with targeted therapies available either in daily practice or in clinical trials. Higher absolute numbers and rates of targetable pathogenic alterations were found in female (142/250: 57%) versus male (122/305: 40%) patients as well as in never/former (209/416: 50%) vs current smokers (55/139: 40%).

Focusing on EGFR mutations, 25 patients received first-line Osimertinib and were tested at the time of disease progression. A molecular mechanism of resistance has been reported in 6 (25%) of cases, with MET amplification reported as the most common, as detailed in **Figure 3**.

Overall, 113 cases of co-occurring mutations were reported, including TP53 plus EGFR/KRAS mutations, STK11 plus KRAS/EGFR mutations, and EGFR mutations plus MET amplification, among the most common associations. Other frequently co-occurring mutated genes were NFE2L2, IDH1-2, PIK3CA and RB1. Among the most relevant oncogene EGFR, KRAS, BRAF and MET were the most frequently associated with other

mutations, while HER2 along with ALK, ROS1 and RET, were less frequently co-mutated. No molecular alterations have been reported in 41/525 (8%) of patients.

Among the 525 patients undergoing NGS molecular testing within EPROPA a total of 18 molecular alterations with potential germline and hereditary cancer syndrome implications have been identified (**Figure 4**), with genetic counselling recommended by the molecular tumor board (MTB), as detailed below.

***Molecular Tumor Board and Targeted Treatments***

The MTB located at St. Luigi Hospital (composed by thoracic oncologists, lung pathologists, molecular biologists, advocacy personnel and data manager) discussed the results of molecular profiling, providing treatment recommendations, upon request of treating medical oncologists, at local centers **(Figure 5).** Among the 264 patients harboring a targetable molecular alteration, 194 (73%) received a matched targeted therapy, while the possibility of enrollment into a clinical trial was requested for 205 patients. After MTB discussion, a clinical trial was not available, in Europe, for 122 patients, while 53 patients were not clinically eligible because of worsening of patient's performance status (46%), availability of alternative standard treatment at local site (36%), logistic issue (13%) and/or patients' refusal (5%). Thus finally, 30 patients received a biomarker-driven experimental targeted treatment in the context of clinical trials across different European countries (**Figure 5**).

Among the 250 patients included in the survival analysis, 76 harbored a targetable molecular alterations and received a matched targeted therapy, 36 harbored a targetable alterations without receiving a matched targeted therapy, 100 harbored an untargetable molecular alterations and 38 were wild-type, respectively. All clinical outcomes were significantly improved in patients with targetable molecular alterations receiving a matched targeted therapy as compared to the other groups: ORR (48.7% vs 30.6% vs 24% vs 30.8%; p:0.007), mPFS (16.3 vs 6.9 vs 6.7 vs 5.8 months; p<0.001), and mOS (22.3 vs 12.6 vs 11.8 vs 10.8; p: 0.015) (Figure 6).

## Discussion

This paper provides an interim report of the first two years of EPROPA activity, recruiting more than 500 patients with advanced NSCLC, from 20 centres, across 5 different European countries (Greece, Slovenia, Romania, Albania and Italy). Overall, the preliminary data emerging from this report confirmed the feasibility and the utility of EPROPA in the real-world practice scenario, supporting the implementation of NGS-based comprehensive genomic profiling for patients with advanced NSCLC in Europe. The limited failure rate and the low TAT (8 days), confirmed the reliability and the robustness of the EPROPA samples management workflow, supporting the timely actionability of treatment recommendations in the real-world clinical context.

EPROPA was promoted by WALCE to reduce the current gap about the relevant heterogeneity of molecular testing and targeted treatments across Europe (8), with the aim of increasing the detection of targetable drivers and improving lung cancer patients' access to biomarker-driven clinical trials. Over the years, EPROPA has offered great support to clinicians, patients and caregivers. The oncologists consider it as a valuable program, because they have been able to offer patients new diagnostic and treatment opportunities not available in their geographical areas, while patients recognized EPROPA as a great opportunity for their life. Even if this program had not any research purposes, however the rate of molecular alterations identified in the overall tested population largely reflect that reported in clinical trials as well as in real-world studies (12, 13), providing a reliable picture of the lung cancer biomarkers distribution across the included countries. In detail, 570 significant (in terms of pathogenicity and allele frequency) molecular alterations across 59 genes were identified in 92% of patients, thus confirming the tendency of a high molecular burden in NSCLC. This data is in line with The Cancer Genome Atlas (TCGA), revealing that lung cancer ranks among the most genomically-complex tumors within the 12 investigated cancer types (14, 15).

On the contrary, in only 41/525 (8%) patients, no molecular alterations were found: this is an acceptable rate considering the technical amplicon-based approach (16). Unfortunately, information about programmed-death ligand 1 (PD-L1) expression was missing in almost all the patients.

It's interesting to note that even if a molecular alteration was identified in 92% of the patients, only 50% of them were considered potentially targetable, based on the current availability of tailored drugs either in clinical practice or in clinical trials, while the biological significance as well as the clinical relevance of all others mutations remain largely unknown, thus questioning the current impact of comprehensive genomic profiling in clinical practice. Conversely the evidence of an increased survival for patients with targetable alterations receiving matched targeted therapies reinforces once again the paradigm of precision medicine in lung cancer, showing that timely NGS testing is associated with the quality of patient care.

Of note, 113/525 (22%) of cases had concurrent mutations, in particular TP53 with EGFR/KRAS mutations and STK11 with KRAS/EGFR mutations were the most frequently identified. Among the most relevant mutated oncogenes, HER2, ALK, ROS1 and RET were less frequently associated with other molecular alterations, thus suggesting a potentially differential tumor clonal evolution among the actionable gene groups (17). Despite this high rate of co-occurring molecular alterations emerging from our analysis, we were not able to assess their potential impact on patients' clinical outcomes, since treatment responses and survival data were not available from the website platform at the time of the present report.

Another relevant finding to be pointed out concerns the identification of molecular alterations with potential germline and hereditary cancer syndrome implications. In a small fraction (3,4%) of our patients who underwent NGS analysis for somatic mutation identification with anticancer treatment purposes, gene alterations with known potential implications in hereditary cancer prevention were found, highlighting the

actual and controversial issue of the optimal management of these patients in the real-world scenario (18). All these cases were discussed within the MTB, and according to the updated American College of Medical Genetics and Genomics as well as the European Society of Medical Oncology Guidelines (19, 20), a genetic counselling has been suggested to the patients and/or their families. Beyond providing a deep molecular characterization of NSCLC samples, EPROPA also supported patients' enrollment in clinical trials, providing logistic and financial support to both patients and caregivers during the different stages of the therapeutic process. In this regards the high discrepancy between clinical trials requests from treating physicians at local centers and definitive patients' enrollment was mostly related to the lack of biomarker-driven clinical studies for a relevant fraction of oncogenic drivers in Europe compared to the US cancer centers. In this patients-centered context, advocacy groups may play a crucial role promoting joined initiatives aimed at raising awareness among public decision makers in order to overcome political, economic, cultural, and logistic barriers and ultimately generating ethical and sustainable cancer care models worldwide. From the European patients' perspective of care, WALCE is convinced that only the establishment of National and International Research Networks, like EPROPA, may allow to adequately address the urgent challenges of precision medicine, overcoming the disparities as well as the multifaced barriers which limit the access to clinical trials within individual countries, improving survival outcomes of patients with NSCLC and decreasing health inequalities across Europe.

**References**


1. Tan AC, Tan DSW.J Targeted Therapies for Lung Cancer Patients With Oncogenic Driver Molecular Alterations. J Clin Oncol. 2022 Feb 20;40(6):611-625.
2. Aggarwal C, et al. Association of comprehensive molecular genotyping and overall survival in patients with advanced non-squamous non-small cell lung cancer. *Journal of Clinical Oncology* 40, no. 16_suppl (June 01, 2022) 9022-9022
3. Howlader N, et al. The Effect of Advances in Lung-Cancer Treatment on Population Mortality. N Engl J Med. 2020 Aug 13;383(7):640-649.
4. https://ascopost.com/news/november-2019/2019-state-of-lung-cancer-report-released/, accessed on March, 2nd, 2021).
5. Mosele F, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2020 Nov;31(11):1491-1505.
6. Hendriks LE, et al. Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol. 2023 Apr;34(4):339-357.
7. Pisapia P, et al. Next generation diagnostic algorithm in non-small cell lung cancer predictive molecular pathology: The KWAY Italian multicenter cost evaluation study. Crit Rev Oncol Hematol. 2022 Jan;169:103525.
8. de Jager VD et al. Developments in predictive biomarker testing and targeted therapy in advanced stage non-small cell lung cancer and their application across European countries. *The Lancet Regional Health - Europe* 2024;38: 100838
9. Robert NJ, et al. Biomarker testing and tissue journey among patients with metastatic non-small cell lung cancer receiving first-line therapy in The US Oncology Network. *Lung Cancer* 2022;166:197–204
10. Bayle A, et al. ESMO study on the availability and accessibility of biomolecular technologies in oncology in Europe. Ann Oncol. 2023 Oct;34(10):934-945.
11. Mosele M.F. et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024. S0923-7534(24)00111-X
12. Harada G, et al. Rare molecular subtypes of lung cancer. Nat Rev Clin Oncol. 2023 Apr;20(4):229-249.
13. Otano I, et al. At the crossroads of immunotherapy for oncogene-addicted subsets of NSCLC. Nat Rev Clin Oncol. 2023 Mar;20(3):143-159.
14. Kandoth C et al. Mutational landscape and significance across 12 major cancer types. Nature 2013 Oct 17;502(7471):333-339.
15. Lawrence MS et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013 Jul 11;499(7457):214-218.

16. Samorodnitsky E, et al. Evaluation of Hybridization Capture Versus Amplicon-Based Methods for Whole-Exome Sequencing. Hum Mutat 2015 Sep;36(9):903-14.
17. Passaro A, et al. Genomic Characterization of Concurrent Alterations in Non-Small Cell Lung Cancer (NSCLC) Harboring Actionable Mutations. Cancers 2021 Apr 30;13(9):2172.
18. Farinea G, et al. The Role of Germline Mutations in Thoracic Malignancies: Between Myth and Reality. J Thorac Oncol. 2023 Sep;18(9):1146-1164.
19. Kalia, S. S. *et al.* Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med* **19**, 249–255 (2017).
20. Kuzbari Z, et al. Germline-focused analysis of tumor-detected variants in 49,264 cancer patients: ESMO Precision Medicine Working Group recommendations. Ann Oncol. 2023 Mar;34(3):215-227.

## Acknowledgements

To the Women Against Lung Cancer Europe (WALCE) Advocacy Group, Francesca Arizio, Cristina Destro, Ewelina Smzytke (LuCE), Angela Dicorato (SC Oncology – ASU GI, Trieste, Italy), Ravzan Curca (Spitalul Juedetean de Urgenta, Alba Iulia, Romania) and Daniela Elvira Sirbu (Centrul de Oncologie Oncohelp, Timisoara, Romania)

## Funding

This work was supported by an unrestricted educational grant by AstraZeneca, Amgen, BeiGene, Blueprint Medicines, Boeringher Ingelheim, Gilead, Incyte, Eli Lilly, Merck, MSD, Novartis, Pfizer, Roche, Sanofi, Thermo Fisher Scientific with no role in study design, data collection, data analysis, data interpretation as well as report writing.

**CRediT Author Statement:**

SN: Conceptualization, methodology, and supervision. FP, AL, LR, SV: data curation and elaboration; writing -original draft preparation; DG: statistical analysis. All other authors: reviewing editing and approval of the final mauscript.

**Figures' and Table Legend:**

**Table 1.** Patients' Characteristics

**Figure 1.** Molecular landscape of advanced NSCLC patients from EPROPA.

**Figure 2.** Frequency of potentially targetable molecular alterations from EPROPA

**Figure 3.** Mechanisms of acquired resistance to Osimertinib in EGFR-mutated NSCLC patients

**Figure 4.** Molecular alterations with potential germline and hereditary cancer syndrome implications

**Figure 5.** EPROPA workflow overview for advanced NSCLC patients

**Figure 6.** Survival outcomes of advanced NSCLC patients from EPROPA

**Supplementary Figure 1:** Men turnaround time of NGS molecular analysis from EPROPA

**Table 1.** Patients' Characteristics

| **Patients' Characteristics** | **Number (%)** |
|---|---|
| **Age** | |
| ≥75 years/old | 161 (29) |
| 65 - 75 years/old | 172 (31) |
| 50 – 65 years/old | 178 (32) |
| < 50 years/old | 44 (8) |
| **Gender** | |
| Male | 305 (55) |
| Female | 250 (45) |
| **Smoking Status** | |
| Current | 139 (25) |
| Former | 272 (49) |
| Never | 144 (26) |
| **Histological Subtypes** | |
| Adenocarcinoma | 433 (78) |
| Squamous Cell Carcinoma | 78 (14) |
| Other | 44 (8) |
| **Stage (8$^{th}$ TNM version)** | |
| IVA | 195 (35) |
| IVB | 360 (65) |
| **Tumor sample** | |
| Histological | 499 (90) |
| Cytological | 56 (10) |
| **Previous Genotyping/Treatment** | |
| No | 270 (49) |
| Yes | 285 (51) |

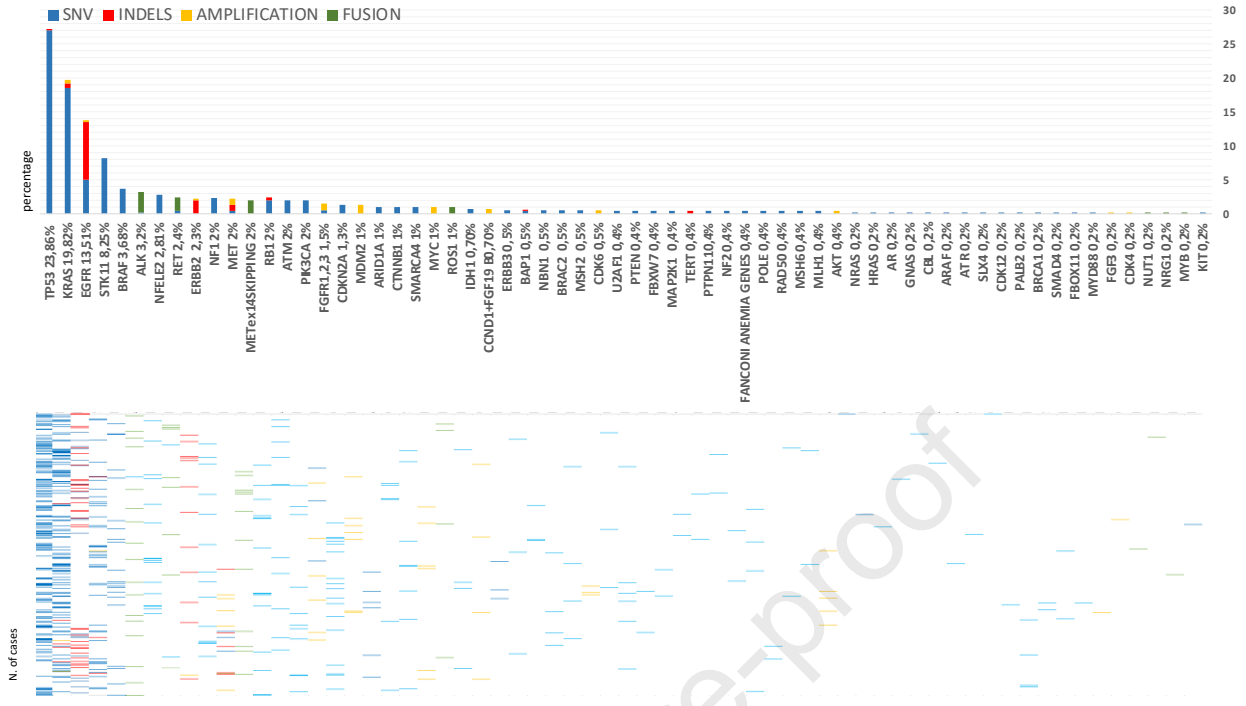



**Figure 1. Molecular landscape of advanced NSCLC patients.** Pathogenic/likely pathogenic molecular alterations (SNVs, MNVs, ins/del in blue; CNVs in orange; fusions in green) reported with frequencies among 525 tested cases.

| Gene | Prevalence |
|---|---|
| KRAS G12C ESCAT IA | 6% |
| EGFR ex18 ESCAT IA/IB*p.G719 | 1,52% |
| EGFR ex19 ESCAT IA | 7,60% |
| EGFR ex20ins ESCAT IA | 2,30% |
| EGFR ex21 ESCAT IA/IB*p.L861Q | 4% |
| ERBB2 ex20ins ESCAT IIB | 2,30% |
| MET ex14skipping ESCAT IB | 2,86% |
| MET amplifications ESCAT IIB | 0,76% |
| ROS1 fusions ESCAT IB | 1,14% |
| ALK fusions ESCAT IA | 3% |
| RET fusions ESCAT IA | 1,40% |
| FGFR1,2,3 mut. ESCAT IC | 1,14% |
| BRAF V600E ESCAT IB | 0,40% |
| NRG1 fusions ESCAT IIB | 0,20% |
| BRCA1 ESCAT IIIA | 0,20% |
| BRCA2 ESCAT IIIA | 0,60% |
| HRD ESCAT IIIA | 2,30% |

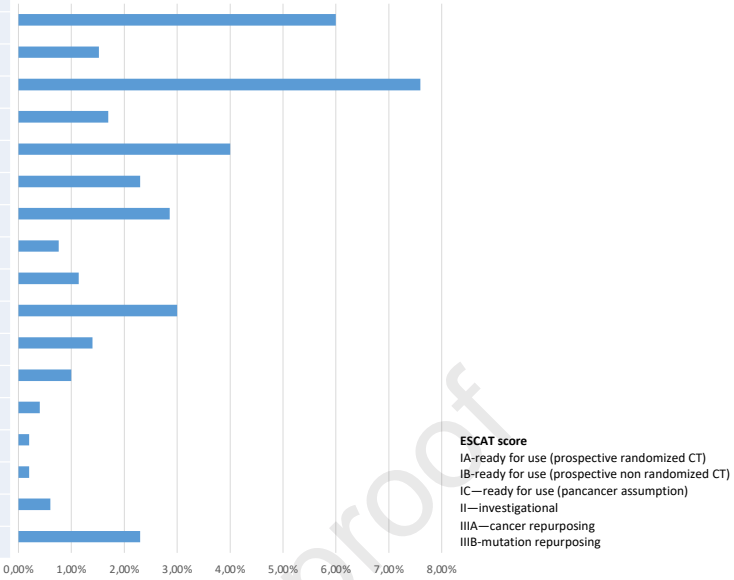



**Figure 2. Potentially targetable molecular alterations from EPROPA.** The percentages and the ESCAT score of clinically actionable molecular alterations from overall 525 tested patients have been reported.

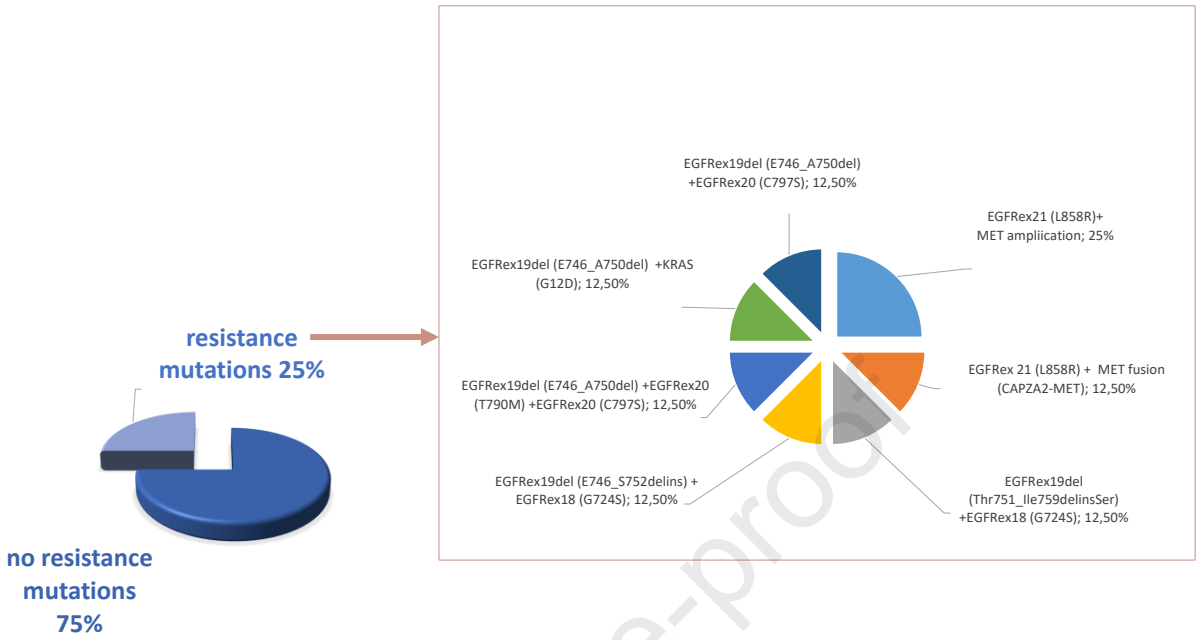



**Figure 3. Mechanisms of acquired molecular resistance to Osimertinib in EGFR-mutant patients.** The percentages of molecular alterations from overall 25 Osimertinib treated patients have been reported.

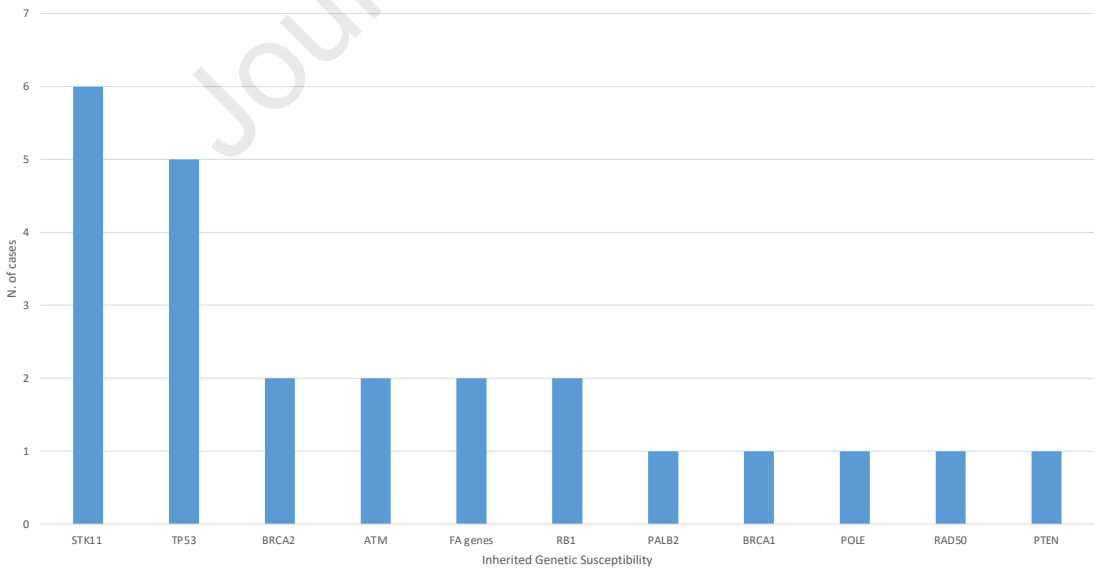



**Figure 4. Molecular alterations in genes potentially involved in cancer hereditary susceptibility**

Number of cases per gene from overall 525 tested patients have been reported.

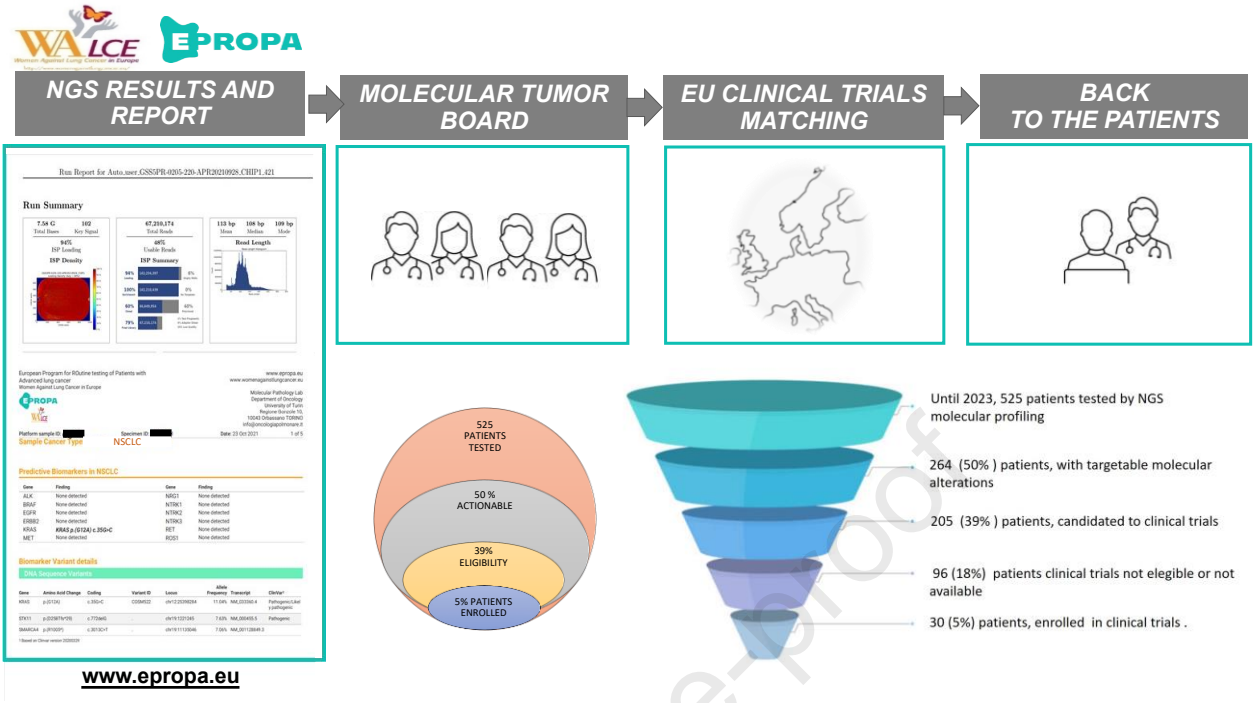



**Figure 5. EPROPA workflow overview for advanced NSCLC patients.** Numbers and percentages of patients at different stages of EPROPA workflow have been reported

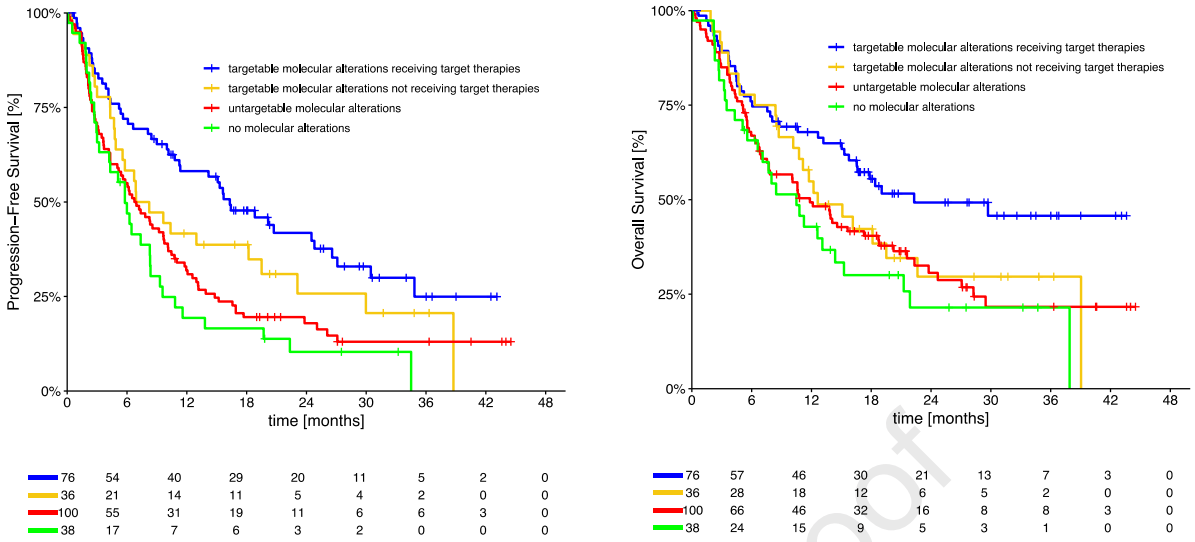
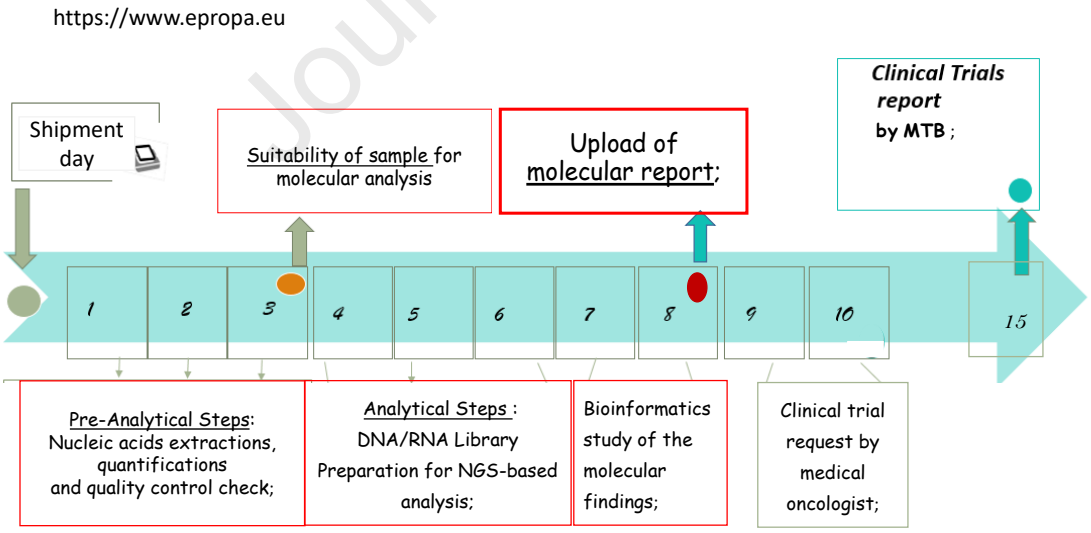



**Figure 6. Survival outcomes of advanced NSCLC patients from EPROPA.** mPFS and mOS of the 250 patients who received systemic antitumor treatments according to their genomic profile.




**Supplementary Figure 1:** Mean turnaround time of NGS molecular analysis and MTB report from EPROPA

**CRediT Author Statement:**

SN: Conceptualization, methodology, and supervision. FP, AL, LR, SV: data curation and elaboration; writing -original draft preparation; DG: statistical analysis. All other authors: reviewing editing and approval of the final mauscript.