HER2 in advanced NSCLC: an Observational, prospective, multi-centric Study exploring HER2 mutations incidence and therapeutic management in Italy. The HEROS study - GOIRC-01-2022

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AMENDMENTS

NUMBER	VERSION	DESCRIPTION	APPROVAL

INVESTIGATOR AGREEMENT

Study titol: *"**HER2 in advanced NSCLC: an Observational, prospective, multi-centric Study exploring HER2 mutations incidence and therapeutic management in Italy. The HEROS study**".*

Coordinating Investigator

Prof. Marcello Tiseo

Signature _____

Date _____

I have read the Protocol and I agree to conduct the study as detailed herein and in compliance with ICH Guidelines for Good Clinical Practice and applicable regulatory requirements.

Principal Investigator

Dr./Prof. _____

Signature _____

Date _____

TABLE OF CONTENT

1. PROTOCOL SYNOPSIS

Study Title	HER2 in advanced NSCLC: an Observational, prospective, multi-centric Study exploring HER2 mutations incidence and therapeutic management in Italy. The HEROS study.
Sponsor **Version**	Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC) 1.0 of 02.05.2024
Rationale	Considering the clinical and molecular heterogeneity of HER2-aberrant NSCLC, currently lacking real-world evidence about prevalence and specific treatment algorithms, the HEROS study aims to investigate the current diagnostic and therapeutic approach towards HER2-mutated NSCLC in the Italian clinical practice, with the final aim of enriching the current knowledge, as well as to provide exploratory data regarding outcome with different treatments, according to the specific type of HER2 mutation.
Primary objective	To describe the prevalence of HER2 mutations in advanced NSCLC in Italy.
Secondary objective	To describe the therapeutic management in clinical practice of HER2 mutated advanced NSCLC patients.
Exploratory objectives	• To describe potential correlations among baseline demographics, clinicopathological variables and HER2 mutations in advanced NSCLC. • To explore the clinical outcome according to the type of anti-HER2 therapies applied in HER2 mutated advanced NSCLC patients. • To investigate potential correlations between PD-L1 expression and HER2 mutations. • To analyse the association between co-mutations and clinical outcome in HER2 mutated advanced NSCLC patients treated with anti-HER2 agents. The subsequent exploratory objectives are only for a Prospective Biomarker Analysis that will be performed in a subset of centers (25 centers): • To analyse the association between FcγR polymorphisms (evaluated in blood and/or tissue) and clinical outcome in HER2 mutated advanced NSCLC patients treated with anti-HER2 agents. • To analyse the IHC expression of HSP90 on NSCLC tissue samples to test its potential impact as a prognostic/predictive factor during treatment with anti-HER2 agents. • To analyse the serum level of HSP90 and circulating level of HER2 mutation in cell-free DNA as possible prognostic/predictive factors during treatment with anti-HER2 agents.
Study design	The HEROS study is an Italian observational multicenter prospective study aimed to investigate the current diagnostic and therapeutical approach towards HER2 mutated NSCLC in clinical practice. The enrolment will start in

	September 2024 until September 2025. A 12-months follow-up window will be performed. The HEROS study will be conducted in partnership with ATLAS project: all patients prospectively enrolled in ATLAS project will be used to fulfil primary and secondary objectives. Information will be obtained by querying ATLAS database (59 centers) in aggregated manner. ATLAS project is a multi-centric retrospective/prospective Italian study aimed to describe prevalence of different oncogene alterations in all new diagnosed advanced NSCLC patients (5,000 patients/year). In particular, prevalence of HER2-mutated patients will be calculated on all patients enrolled in ATLAS project. Approximately 100 HER2 mutated patients enrolled in ATLAS study will be studied for secondary and explorative objectives. Moreover, a subset of centers (25 centers) will enrol patients also for Prospective Biomarker Analysis in order to perform exploratory analysis on blood and tissue samples according to the availability of tissue specimens from the primary tumor or metastatic sites. All centers that will participate for Prospective Biomarker Analysis necessarily will be included into ATLAS project.
Number of Patients	The study will include three cohorts of patients: 1. Cohort 1: Prevalence study population (5,000 patients from ATLAS project in 59 Italian centers) 2. Cohort 2: HER2 mutations study population (100 patients from Cohort 1) 3. Cohort 3: Prospective Biomarker Analysis population (50 patients from Cohort 2 in 25 Italian centers)
Inclusion Criteria	<u>Cohort 1 (Prevalence study population)</u> 1. Male or female, aged at least 18 years. 2. Pathologically confirmed diagnosis of NSCLC from September 2024 to September 2025. 3. Locally advanced (IIIC), not amenable to multimodal approach (chemo-radiotherapy), or metastatic (IV) NSCLC according to TNM VIII edition. 4. Enrolled in ATLAS project. <u>Cohort 2 (HER2 mutations study population)</u> 5. Included in Cohort 1. 6. Presence of HER2 mutation. 7. Enrolled in ATLAS project. <u>Cohort 3 (Prospective Biomarker Analysis population)</u> 8. Included in Cohort 2. 9. Availability of tissue sample from the first 50 patients enrolled in cohort 2. 10. Written informed consent (HEROS project) must be obtained before any study-related procedure.
Study duration	The duration of the study is expected to be a maximum of 24 months. The study recruitment period is expected to be approximately 12 months.

Primary Endpoint	Percentage of advanced NSCLC with HER2 mutations in Italy clinical practice.
Secondary Endpoints	Percentage of type of therapies in advanced HER2 mutated NSCLC patients.
Exploratory Endpoints	• Percentage of clinicopathological variables (i.e. central nervous system metastases, ECOG PS, smoking status, etc) in advanced HER2 mutated NSCLC patients. • Real world objective response rate (RW-ORR), real world disease control rate (RW-DCR), real world progression-free survival (RW-PFS), time to next treatment (TTNT), time to treatment discontinuation (TTD) and overall survival (OS), according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients; • Percentage of PD-L1 expression among advanced HER2 mutated NSCLC patients; • RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies according to the most represented co-mutations (e.g. *TP53*); • RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the FcγR polymorphisms (evaluated in blood and/or tissue); • RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the IHC tissue expression of HSP90; • RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the serum level of HSP90 and circulating level of HER2 mutation in cell-free DNA.
Statistical methods	The sample size was estimated according to the available literature and to the patients' population expected to fulfil the pre-specified inclusion criteria at the participating centers during the enrolment time. Considering the incidence of HER2 mutations in advanced NSCLC, the number of centers, trial duration, as well as the current diagnostic management of lung cancer, we plan to enroll from ATLAS project (approximately 5,000 advanced NSCLC patients per year) information regarding 100 HER2-mutated patients, of which 50 patients (2-3 patients/center) will be enrolled also for Prospective Biomarker Analysis. Descriptive statistics will be performed to analyze the characteristics and outcomes of patients. In summary: Prevalence study population: 5,000 patients HER2 mutations study population: 100 patients Prospective Biomarker Analysis population: 50 patients.

2. BACKGROUND AND RATIONALE

2.1 Background

In recent years, HER2 alterations emerged as candidate actionable targets in advanced non-small-cell lung cancer (NSCLC). Although a clear identification and definition of HER2 alterations in lung cancer are still missing, three main subgroups were recognized: mutation, amplification, and protein overexpression. The assessment of HER2 alterations in NSCLC is performed by immunohistochemistry (IHC) for overexpression, fluorescent in situ hybridization (FISH), and next generation sequencing (NGS) for genetic alterations [1].

The incidence of HER2 mutations in NSCLC is 2-4%, mainly insertions in exon 20-21 causing constitutive activation of the tyrosine kinase domain. The most common is duplication or insertion of the amino acids YVMA at codon 75 in exon 20, the A775_G776 ins YVMA, accounting for around 80%-90% of all HER2 mutations [2]. HER2 overexpression is detected in 2-38% of cases, while amplification is observed in 2-5% [3]. Differently from breast and gastric cancer, the association between amplification and overexpression is controversial with no significant correlation between increased gene copy number and protein expression at IHC [4, 5]. As a potential explanation, the lack of standardized testing techniques and cut-offs for defining HER2 positivity represents a crucial issue [6]. In terms of clinical characteristics, patients harbouring HER2 alterations are more frequently women, young, never smokers, and mainly adenocarcinomas [7, 8].

To date, phase III prospective randomized studies are missing. Available data exploring anti-HER2 approaches in NSCLC derive mainly from retrospective studies and early phase prospective trials. HER2-mutated NSCLC harbour a worse prognosis with chemotherapy compared with wild-type tumors [9].

Regarding tyrosine kinase inhibitors (TKI), afatinib showed modest activity in this setting (response rate, RR 13-19%, with heterogeneous outcomes according to the type of HER2 insertion) [10, 11]. Pyrotinib, an irreversible pan-HER receptor TKI, resulted in a RR of 53.3% with a median progression-free survival (PFS) of 6.4 months [12]. Another irreversible pan-HER TKI, poziotinib, demonstrated in the phase II ZENITH20 trial, an ORR of 35.1% and a mPFS of 5.5 months in pre-treated patients affected by NSCLC harbouring HER2 exon 20 insertions. Use of poziotinib was limited by relevant toxicities (grade 3 diarrhea and rash in 26% and 30%, respectively), requiring a dose reduction in 78% of patients [13].

Recently, the RAIN-701 trial suggested a promising activity (RR 22% and disease control rate, DCR 67%) of tarloxotinib, a hypoxia-activated prodrug of a pan-HER TKI, in HER2 exon 20 insertion mutations [14]. No significant clinical benefit was observed using lapatinib [15], dacomitinib [16], and neratinib [17]. Finally, TKIs were not active in HER2-amplified/-overexpressed NSCLC patients [18].

Trastuzumab in combination with chemotherapy (vinorelbine, docetaxel, or paclitaxel) was active in pre-treated HER2-mutant lung cancer patients [2, 19, 20]. Ado-trastuzumab emtansine (T-DM1), an antibody-drug conjugate targeting HER2, induced a RR of 44% in HER2-mutant lung cancer [21]. Moreover, T-DM1 demonstrated efficacy in patients with HER2 overexpression (IHC 3+), especially when concomitant HER2 mutations are present [22]. Based on the results of a promising phase 1 trial, trastuzumab deruxtecan, an antibody-drug conjugate composed of an anti-HER2 antibody and a topoisomerase I inhibitor payload, was recently evaluated in phase 2 DESTINY-Lung01 trial demonstrating a very promising clinical activity (RR 61.9%, DCR 90.5%, mPFS 14 months) in patients with HER2-mutated NSCLC [23].

Several monoclonal antibodies (MoAB), including rituximab in follicular lymphoma, cetuximab in colon cancer, and trastuzumab in breast cancer, demonstrated to induce antibody-dependent cell-mediated cytotoxicity (ADCC), based on the interaction of the Fc domain of the antibody with the Fc-γ receptors (FcγR) on the surface of the immune cells, such as natural killer (NK) and macrophages. Specific polymorphisms of FcγRIIA, homozygous for histidine expression at position 131, and of FcγRIIIA, homozygous for valine at position 158, are associated with an increased ADCC and with better clinical outcomes with trastuzumab in both metastatic and adjuvant HER2-positive breast cancer, when compared with other FcγR genotypes [24, 25].
Another promising marker in this setting is represented by the evaluation of HSP90 expression levels in HER2 positive NSCLC since its role in regulation and stabilization of HER2 receptor is recognized in preclinical and clinical models [26–29]. In this regard, available data showed the co-targeting of HSP90 and HER2 with HSP90 inhibitors (such as tanespimycin 17-AAG or ganetespib) and trastuzumab, in patients affected by trastuzumab-refractory HER2-amplified breast cancer, seemed to restore sensitivity to anti-HER2 MoAB, improving clinical outcomes [30, 31].
Another field of interest is represented by the occurrence of co-mutations together with HER2 alterations, which have been shown to impact patients' prognosis and response to targeted treatments. In this regard, co-occurring TP53 mutation was found in 52-66% of HER2-mutated NSCLC [32, 33], and was associated with a worse prognosis [32]. In addition, TP53, as well as PI3K/AKT/mTOR co-mutations, were significantly correlated with worse clinical outcomes in HER2-mutated NSCLC patients treated with afatinib [34].
In conclusion, HER2 alterations, mainly mutations, recently emerged as a promising actionable target in lung cancer, deserving deepened investigations regarding diagnostic and therapeutical management, as well as the underlying mechanisms of activity/resistance of anti-HER2 agents.

2.2 Study rationale

Considering the clinical and molecular heterogeneity of HER2-aberrant NSCLC, currently lacking specific treatment algorithms, the HEROS study aims to investigate the current diagnostic and therapeutic approach towards HER2-mutated NSCLC in the Italian clinical practice, with the final aim of enriching the current knowledge, as well as to provide exploratory data regarding outcome with different treatments (MoAb, TKI, chemotherapy), according to the specific type of HER2 mutations.

3 STUDY OBJECTIVES

3.1 Primary objective

To describe the prevalence of HER2 mutations in advanced NSCLC.

3.2 Secondary objectives

To describe the therapeutic management in clinical practice of HER2 mutated advanced NSCLC patients.

3.3 Exploratory objectives

- To describe potential correlations among clinicopathological variables and HER2 mutations in advanced NSCLC.

- To explore the clinical outcome according to the type of anti-HER2 therapies applied in HER2 mutated advanced NSCLC patients.
- To investigate potential correlations between PD-L1 expression and HER2 mutations.
- To analyse the association between co-mutations and clinical outcome in HER2 mutated advanced NSCLC patients treated with anti-HER2 agents.

The subsequent exploratory objectives are only for the prospective biological analysis that will be performed in a subset of centers (25 centers):

- To analyse the association between FcγR polymorphisms (evaluated in blood and/or tissue) and clinical outcome in HER2 mutated advanced NSCLC patients treated with anti-HER2 agents.
- To analyse the IHC expression of HSP90 on NSCLC tissue samples to test its potential impact as a prognostic/predictive factor during treatment with anti-HER2 agents.
- To analyse the serum level of HSP90 and circulating level of HER2 mutation in cell-free DNA as possible prognostic/predictive factors during treatment with anti-HER2 agents.

4 STUDY DESIGN

The HEROS study is an Italian observational multicenter prospective study aimed to investigate the current diagnostic and therapeutical approach towards HER2-mutated NSCLC in clinical practice.
The enrolment will last a year, approximately from September 2024 until September 2025. A minimum of 12-months follow-up window will be performed following the prospective phase.
The HEROS study will be conducted in partnership with ATLAS project: all patients prospectively enrolled in ALTAS project will be used to fulfil primary and secondary objectives. Information will be obtained by querying ATLAS database (59 centers) in aggregated manner.
A subset of centers (25 centers) will enrol patients also for the Prospective Biomarker Analysis, that will be performed on blood and tissue samples according to the availability of tissue specimens from the primary tumor or metastatic site.

The study will include three cohorts of patients:

- Cohort 1: Prevalence study population (5,000 patients)
- Cohort 2: HER2 mutations study population (100 patients)
- Cohort 3: Prospective Biomarker Analysis population (50 patients).

5 STUDY POPULATION

5.1 Inclusion criteria

Cohort 1 (Prevalence study population)

Patients enrolled in ATLAS project that met all the following criteria will be included into the study for the primary objective analysis:

1. Male or female, aged at least 18 years
2. Pathologically confirmed diagnosis of NSCLC during Heros enrolment period, approximately from September 2024 to September 2025
3. Locally advanced (IIIC), not amenable to multimodal approach (chemo-radiotherapy), or metastatic (IV) NSCLC according to TNM VIII edition

4. Enrolled in ATLAS project

Cohort 2 (HER2 mutations study population)

5. Included in Cohort 1
6. Presence of HER2 mutation
7. Enrolled in ATLAS project

Cohort 3 (Prospective Biomarker Analysis population)

8. Included in Cohort 2
9. Availability of tissue sample from the first 50 patients enrolled in cohort 2
10. Written informed consent (HEROS project) must be obtained before any study-related procedure

6 VARIABLES AND EPIDEMIOLOGICAL MEASUREMENTS

6.1 Exposures

Data on previous/ongoing anticancer treatment will be collected. Patients will receive anticancer treatments as per clinical practice, consisting of chemotherapy, TKIs, immunotherapy or anti-HER2 agents (i.e., trastuzumab, T-DM1, trastuzumab deruxtecan). Available clinical data about medical history, demographic data, tumor molecular typing, treatment compliance and treatment activity for patients in cohorts 1, 2 and 3 will be collected from the ATLAS eCRF. Biological exploratory data of cohort 3 patients will be collected in a dedicated database, filled out with the results of the biomarker analyses.

6.2 Medical history and demographic data

Medical history includes:
Clinically significant diseases (active or resolved)
Cancer history, including prior cancer therapies and procedures
Prior surgeries
Reproductive status
Smoking history
ECOG PS
Presence/absence of central nervous system metastases

Demographic data includes age, sex and self-reported ethnicity.
The initial cancer diagnosis and the current cancer stage at the time of screening, along with tumor histology and all sites of disease, should be recorded per The American Joint Committee on Cancer (AJCC), 8th edition (http://www.cancerstaging.com). Any previously identified mutations and the dates of identification, must be recorded.

6.3 Tumor molecular typing

Data regarding molecular typing of the tumor obtained before enrolment as per clinical practice will be collected and will include EGFR, ALK, ROS1 and PD-L1 status. When available, all additional

molecular alterations will be recorded (i.e., KRAS, RET, BRAF, MET, NTRK). If NGS analysis is available, all the molecular alterations will be reported.

Regarding HER2 mutation history, any previously identified mutation, the dates and methods of identification, must be recorded at screening.

7 DESCRIPTION OF STUDY ASSESSMENTS

Cohort 1 (Prevalence study population)

Patients must be enrolled in ATLAS project.
Data collected for ATLAS project compliant with HEROS project will be collected from ATLAS database.

Cohort 2 (HER2 mutations study population)
Patients must be enrolled in ATLAS project.
Data collected for ATLAS project compliant with HEROS project will be collected from ATLAS database.

Cohort 3 (Prospective biological analysis)
Written informed consent for participation in the HEROS study must be obtained before collecting biological samples. Informed Consent Forms for enrolled patients and for patients who are not subsequently included will be maintained at the study site.
Flow-chart of scheduled study assessments is provided in ***Section 6.6***. All assessments must be performed and documented for each patient.

7.1 Data collection

During the study, clinical data of patients will be collected (See ***Section 5***). Data will be stored for 15 years at the Medical Oncology Unit of University Hospital of Parma, in a dedicated password-protected storage folder on a PC in charge of the aforementioned operating unit, under the responsibility of Prof. Marcello Tiseo.

7.2 Samples collection (only for Prospective Biomarker Analysis)

Patients who will start anticancer treatment will undergo a basal blood sample collection, concurrently with the planned routinary blood tests according to clinical practice, that should be performed no more than 7 days before starting anticancer treatment, whatever it is. During the routine blood test, an extra 20 ml of blood will be drawn for study purposes.
Tissue samples already collected as per clinical practice will be reviewed.

7.3 Storage, Re Use and Destruction of Biological Samples (only for Prospective Biomarker Analysis)

Tissue samples will be labelled at the recruiting center, then delivered to the Department of Diagnostics and Public Health, University of Verona, P. le L.A. Scuro 10, 37134; Verona (Italy) where they will be stored and processed. The tissue samples will be stored for 5 years so that the planned evaluations can be repeated or completed. Liquid biopsies will be labelled at the recruiting center, then delivered to the Laboratory of Medical Oncology Unit of University Hospital of Parma, where they will be stored and processed.

The data collected during the study will be stored at the Laboratory of Medical Oncology Unit of University Hospital of Parma, under the responsibility of Prof. Marcello Tiseo. The processes adopted for the coding and storage of samples for molecular analysis are important to maintain subject confidentiality. Each sample will be pseudonymized so that it will be impossible to identify the patient but related information will remain available and will be used for future studies. In this regard, future studies will always concern the same pathology and/or drug of interest of this research, and they will be submitted to the ethics committee for approval.
Samples will be destroyed or returned to the competent Institution, when feasible, after 5 years to the end of the study.at the end of the study. The results of any further analyses will be reported either in the Clinical Study Report itself or as an addendum, or separately in a scientific report or publication.

7.4 Radiological Assessments

All eligible patients who will receive anticancer treatment as per clinical practice will undergo a tumor assessment as per clinical practice. Radiological assessments during treatment will be performed according to clinical practice.
Measurable and non-measurable disease must be documented at screening prior to start of treatment. Screening assessment should include:

- CT scan (with contrast) of the chest, abdomen and pelvis; MRI of the chest, abdomen, and pelvis with a non-contrast CT scan of the chest may be used in patients for whom CT scans with contrast are contraindicated (i.e., patients with contrast allergy or impaired renal clearance);
- Brain CT (with contrast) or MRI scan should be performed at baseline to evaluate CNS metastases in all patients.

7.5 Molecular Analyses (only for Prospective Biomarker Analysis)

Laboratory of Medical Oncology Unit of University Hospital of Parma will coordinate the collection and processing of blood samples.
Blood samples will be collected from all patients enrolled in the Prospective Biomarker Analysis and will be sent to the central laboratory. Each center will process the blood sample in order to obtain an aliquot of blood for FcγR analysis, serum sample for quantification of circulating HsP90 levels, and plasma samples for cell-free DNA (cfDNA) extraction for HER2 analysis. Genomic DNA (gDNA) and cfDNA will be extracted with proper kit from blood and plasma samples, respectively. Where blood sample is not available, gDNA will be extracted from normal tissue micro-dissected from tissue biopsy.
The analysis of FcγR will be performed by a quantitative PCR (qPCR) and specific probes for FcγRIIA and FcγRIIIA polymorphisms (rs1801274 and rs396991, Applied Biosystems) following the manufacturer's instructions.
Hsp90 levels will be quantified with ELISA kit (Thermofisher) following the manufacturer's instructions.
The analysis of HER2 on cfDNA will be performed by a digital droplet PCR (ddPCR) and specific ddPCR Mutation Assay (BioRad®, Hercules, CA, USA) following the manufacturer's instructions.

Laboratory of Medical Oncology Unit of University Hospital of Verona will coordinate the collection and processing of tissue samples.
Tissue from biopsy obtained at the time of diagnosis must be submitted in order to perform IHC of HSP90 and when feasible, NGS analysis to study co-mutations.

Immunostaining for HSP90 (clone EPR16621-67, dilution 1:1500, Abcam) will be performed in biopsy and surgical NSCLC specimens. All samples will be processed using a sensitive 'Bond Polymer Refine' detection system in an automated Bond immunohistochemistry instrument (Leica Biosystems). Labelling for the marker will be recorded as the percentage of positive cells and intensity of the staining.

Genomic profiling will be performed using the CORE NGS panel developed by the International Cancer Genome Consortium that encompasses mutations, copy number alterations, and structural variants in a panel of 174 clinically actionable genes, genomic signatures, regulatory elements, immunological features, sample integrity/contamination, tumor mutational burden, microsatellite instability, and relevant fusion genes.

7.6 Schedule of activities for Prospective Biomarker Analysis

Study procedures	**Enrollment phase (12 months)**	**Follow-up (min 12 months)**
Signed Informed Consent Form(s)[a]	X	
Review of eligibility criteria	X	
Medical history, including cancer and demographic information[b]	X	
Data on treatment compliance and administration	X	X
Blood samples for gDNA, serum, and cfDNA analysis[c]	X	
Collection of tumor tissue[d]	X	
IHC analysis	X	
NGS analysis	X	
Tumor assessment[e]	X	
Survival status[f]		X

[a]Written informed consent is required for performing any study-specific tests or procedures. Patient may allow for the collection and testing of archival or fresh tumor tissue by signing the main consent form.
[b]Cancer history includes stage, date of diagnosis and prior antitumor treatment. Demographic information includes sex, age and self-reported race/ethnicity.
[c]Patients who will receive anticancer treatment will undergo a basal blood sample collection, to be performed no more than 7 days before starting treatment.
[d] Tissue samples already collected as per clinical practice will be reviewed.
[e]All eligible patients who will receive anticancer treatment will undergo a tumor assessment as per clinical practice. Radiological assessments during treatment will be performed according to clinical practice.
[f]Survival status information will be collected every 3 months via telephone calls, patient medical records, and/or clinic visits. All patients will be followed for survival unless the patient withdrawn consent; this request must be documented in the source documents and signed by the investigator.

8 CRITERIA OF EVALUATION

A summary of objectives and corresponding endpoints is provided below.

Primary Objective	**Primary Endpoint**	**Study cohort**
To describe the prevalence of HER2 mutations in advanced NSCLC.	Percentage of advanced NSCLC with HER2 mutations in Italy clinical practice	Cohort 1
Secondary Objectives	**Secondary Endpoints**	
To describe the therapeutic management in	Percentage of type of therapies in advanced	Cohort 2

clinical practice of HER2 mutated advanced NSCLC patients.	HER2 mutated NSCLC patients.	
Exploratory Objectives	**Exploratory Endpoints**	
To describe potential correlations among clinicopathological variables and HER2 mutations in advanced NSCLC.	Percentage of clinicopathological variables (i.e. central nervous system metastases, ECOG PS, smoking status, etc) in advanced HER2 mutated NSCLC patients.	Cohort 2
To explore the clinical outcome according to the type of anti-HER2 therapies applied in HER2 mutated advanced NSCLC patients.	RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients.	Cohort 2
To investigate potential correlations between PD-L1 expression and HER2 mutations.	Percentage of PD-L1 expression among advanced HER2 mutated NSCLC patients.	Cohort 2
To analyse the association between co-mutations and clinical outcome in HER2 mutated advanced NSCLC patients treated with anti-HER2 agents.	RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies according to co-mutations.	Cohort 2
To analyse the association between FcγR polymorphisms (evaluated in blood and/or tissue) and clinical outcome in HER2 mutated advanced NSCLC patients treated with anti-HER2 agents.	RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the FcγR polymorphisms (evaluated in blood and/or tissue).	Cohort 3
To analyse the IHC expression of HSP90 on NSCLC tissue samples to test its potential impact as a prognostic/predictive factor during treatment with anti-HER2 agents.	RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the IHC tissue expression of HSP90.	Cohort 3
To analyse the serum level of HSP90 and circulating level of HER2 mutation in cell-free DNA as possible prognostic/predictive factors during treatment with anti-HER2 agents.	RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the serum level of HSP90 and circulating level of HER2 mutation in cell-free DNA.	Cohort 3

8.1 Primary endpoints

The primary endpoint is the percentage of advanced NSCLC with HER2 mutations in Italy clinical practice. The prevalence will be calculated by dividing the number of advanced NSCLC with HER2 mutations enrolled in the ATLAS study by the number of advanced NSCLC patients who underwent NGS at the Centers participating to ATLAS project.

8.2 Secondary endpoint

The secondary endpoint is the percentage of type of therapies performed in advanced HER2 mutated NSCLC patients as per clinical practice.

8.3 Exploratory endpoints

- Percentage of clinicopathological variables (i.e. central nervous system metastases, ECOG PS, smoking status, etc) in advanced HER2 mutated NSCLC patients.
- Real world objective response rate (RW-ORR), real world disease control rate (RW-DCR), real world progression-free survival (RW-PFS), time to next treatment (TTNT),

time to treatment discontinuation (TTD) and overall survival (OS), according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients;
- Percentage of PD-L1 expression among advanced HER2 mutated NSCLC patients;
- RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies according to co-mutations;

The subsequent exploratory endpoints are only for Prospective Biological Analysis:

- RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the FcγR polymorphisms (evaluated in blood and/or tissue);
- RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the IHC tissue expression of HSP90;
- RW-ORR, RW-DCR, RW-PFS, TTNT, TTD and OS according to the type of anti-HER2 therapies applied in advanced HER2 mutated NSCLC patients and the serum level of HSP90 and circulating level of HER2 mutation in cell-free DNA.

8.3.1 Objective response

Objective Response (OR) is defined as a complete response (CR) or a partial response (PR) at the radiological evaluation, based on the Investigator's assessment, and will be measured according to standard RECIST criteria v1.1.

8.3.2 Real World Objective Response Rate

Real World Objective Response rate (RW-ORR) is defined as the proportion of patients in which an objective response will be observed.

8.3.3 Disease Control Rate

Real World Disease Control Rate (DCR) is defined as the proportion of patients who achieved a complete response (CR) or a partial response (PR) or a stability of disease (SD) at the radiological evaluation, based on the Investigator's assessment.

8.3.4 Definition of disease progression

Disease progression will be defined as any unequivocal progression of disease according to RECIST criteria.

8.3.5 Real World Progression-free survival

Real World Progression-free survival (RW-PFS) is calculated as the interval from the date of treatment start to either the date of disease progression or the date of death, whichever occurs first and whatever the cause. The date of first documented radiographic progression as assessed by RECIST1.1, if available, will be used as the date of event. Patients alive with no evidence of disease progression at the time of their last visit are censored at the time of the last examination.

8.3.6 Time to next treatment

Time to next treatment (TTNT) is calculated as the interval from the date of treatment start to the date of commencement of the next line of therapy, if applicable.

8.3.7 Time to treatment discontinuation

Time to treatment discontinuation (TTD) is calculated as the interval from the date of treatment start to the date of treatment end, whatever the cause. Patients alive with treatment ongoing at the time of their last visit are censored at the time of the last examination.

8.3.8 Overall survival

Overall survival (OS) is defined as the time from the date of treatment start to death, whatever the cause. The follow-up of patients still alive will be censored at the moment of last visit/contact.

9 STUDY DISCONTINUATION

The Investigator has the right to discontinue a patient from study or withdraw a patient from the study at any time. In addition, patients have the right to voluntarily discontinue study or withdraw from the study at any time for any reason. Discontinued patients will be not replaced. Reasons for withdrawal from the study may include but are not limited to the following:

- Patient withdrawal of consent at any time
- Investigator determines it is in the best of interest of the patient

Every effort should be made to obtain information on patients who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate CRF. However, patients will not be followed for any reason after consent has been withdrawn.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical design and sample size

The sample size was estimated according to the patients' population expected to fulfil the pre-specified inclusion criteria at the participating centers during the enrolment time. Considering the incidence of HER2 mutations in advanced NSCLC, the number of centers, trial duration, as well as the current diagnostic management of lung cancer, we plan to collect, from ATLAS project (approximately 5,000 advanced NSCLC patients per year), information regarding 100 patients and to enroll in HEROS study 50 patients (2-3 patients/center) for biological exploratory analysis.
Descriptive statistics will be performed to analyze the prevalence of HER2 mutations and the prevalence of characteristics of patients in the overall registered population. Correlations with treatment outcomes will be analyzed.
Assuming from literature that prevalence of HER2 mutation is 2% we plan to consider as denominator of this rate about 5,000 NSCLC patients. This sample size will allow to estimate this prevalence with a 95% confidence interval based on the exact binomial calculation ranging from 1.6% to 2.4%.

10.2 Statistical analysis plan

10.2.1 Analysis populations'

Registered population*:* registered population will include all patients who were enrolled into the ATLAS study.
Study population: patients fulfilling selection criteria

10.2.2 Statistical methods

Primary endpoint: the prevalence will be calculated by dividing the number of advanced NSCLC with HER2 mutations enrolled in the study by the number of advanced NSCLC patients who underwent NGS at the selected Centers in the study period participating to ATLAS project reported with the exact 95% confidence interval.

Secondary endpoint: distribution of type of therapies performed in advanced NSCLC patients HER2 mutated as per clinical practice calculated as absolute counts and percentages on the study population

Exploratory endpoints:

Clinical-pathological characteristics of HER2 mutated NSCLC patients
They will be summarized using absolute counts and percentages if referred to categorical items or by calculating mean and standard deviations as well as median, interquartile range, minimum and maximum for quantitative variables.

Real World Objective response rate
The RW-ORR will be reported including the exact 95% CI. Patients that for any reason will not be evaluated for the objective response will be considered as non-responder for this analysis and included in the denominator.

Real World Disease control rate
The RW-DCR will be reported including the exact 95% CI. Patients that for any reason will not be evaluated for the objective response will be considered as non-responder for this analysis and included in the denominator.

Real world progression-free survival, time to next treatment, time to treatment discontinuation and overall survival
The distributions of RW-PFS, TTNT, TTD and OS probabilities and the estimates of the corresponding median times will be obtained by the Kaplan Meier technique. The 95% CI for the median times will be calculated using the Brookmeyer and Crowley method. Estimates of the PFS and OS rates at a fixed time point (6-months and 1-year) will be obtained using the Kaplan Meier technique and 95% CI will be calculated by the Greenwood's formula for standard deviation. Patients who will not experience the events will be censored.

Percentage of PD-L1 expression among advanced NSCLC patients HER2 mutated
PD-L1 expression will be summarized as a quantitative item and categorized according to the usual cut-points (0, 1%, 50%)

Allelic frequency (AF) of HER2 mutation in the blood
Allelic frequency of HER2 mutation in cfDNA will be calculated with fractional abundance method by QuantaSoft (BioRad).

FcγR polymorphisms
Differences in characteristics among the genotypes will be assessed using Fisher exact test for categorical data. Genotypes will be analyzed separately and as combined allelic groups.

HSP90 IHC expression
Chi-square test or Fisher's exact test will be performed to analyze the relationship between HSP90 status and patients' characteristics.

10.2.3 Data recording and display
Frequency tables will be tabulated for all categorical variables by the levels of the variables as they appear on the ATLAS database eCRF (with percentages). Categories with a text field specification will be tabulated as categories and then supplemented by a listing with the following information for the patients fulfilling the condition for the specification (patient id, institution, value of the item and text field contents).
Other continuous variables (for example age, dose) are presented using the median and range (minimum, maximum), mean and standard deviation.
Continuous data may also be presented in categories (for example, age may also be grouped in decades).
Continuous variables for which a coding system exists (such as for laboratory data) will be recoded into categories.
Laboratory data derived from exploratory analyses will not be recorded in the ATLAS eCRF, but collected in a dedicated separate database under the supervision of the Coordinating Investigator.

10.2.4 End of study
The final analysis will occur at the earliest when the last patient with HER2 mutation has been followed up in the study for at least 12 months. Prior to the final analysis, the database will be fully clean and frozen.

11 DATA MONITORING BOARD

A Board formed by the Coordinating and Co-Coordinating Investigator, with the Scientific Coordinators and Clinical Trial Office will monitor the recruitment and the data quality at least twice a year. Arising problems will be discussed with the Coordinating and Co-Coordinating Investigator, with the Scientific Coordinators who will respond appropriately. Relevant information will be included in the study status

reports. No results will be presented at investigators meetings before the trial is closed to recruitment and data are mature for the analysis of the primary endpoint, unless recommended otherwise by the Board.

12 INVESTIGATOR AUTHORIZATION PROCEDURE

Investigators will be authorized to register patients in this study only when all regulatory requirements are being fulfilled and a site initiation visit is performed.

13 PATIENT REGISTRATION PROCEDURE

This is a multicenter observational study. All patients must be registered in the ATLAS eCRF at the time of study enrolment. Patient registration will only be accepted from authorized investigators, who will receive a personal user ID to access the registration system.
Registration will be centralized and performed online. At the end of the procedure, a patient sequential identification number will be allocated to the patients. The sequential identification number attributed to the patient at the end of the registration procedure identifies the patient.
All patients in the Cohort 3 must be registered in the HEROS CRF at the time of study enrolment. They will maintain the same ATLAS ID.

14 FORMS AND PROCEDURES FOR COLLECTING DATA

The primary study database for data analysis to assess the primary and secondary objectives of the study is the ATLAS case report form. All data concerning the collection and analysis of biological samples will be recorded in the HEROS paper CRFs.
The HEROS CRFs pertaing the results of blood and tissue analysis will be completed, dated and signed by the Investigator of the centralized laboratory.
Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, X-rays, patient files, and records kept at pharmacies, laboratories and medico-technical departments involved in a clinical study.

15 DATA QUALITY ASSURANCE

Computerized and manual consistency checks will be performed on newly entered forms; queries will be issued in case of inconsistencies. Consistent forms will be validated by the Clinical Trial Office to be entered on the master database. Inconsistent forms will be kept "pending" until resolution of the inconsistencies.
Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, patients' medical records and eCRFs. The investigator will permit national and local health

authorities, Sponsor monitors, representatives and collaborators and the IRBs/ECs to inspect facilities and records relevant to this study.

16 ETHICAL CONSIDERATIONS

16.1 Patient protection

The responsible investigator will ensure that this study is conducted in agreement with the Declaration of Helsinki. The protocol has been written, and the study will be conducted according to the International Conference on Harmonisation (ICH) Harmonized Tripartite Guideline for Good Clinical Practice (GCP).
This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the IRB/EC and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC. Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the IRB/EC in accordance with local regulatory requirements.

16.2 Subject identification

The name of the patient will not be asked for nor recorded in the study Data Base. Patient enrolled in Heros study will keep the identification number assigned for ATLAS study. This number will identify the patient and must be included on all case report forms. A subject identification list will be stored at the selected Centers.

16.3 Informed consent

All patients will be informed of the aims and procedures of the study. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for study purposes by authorized individuals other than their treating physician. Being an observational study, there are no possible hazards to which the patients will be exposed, except for incidental genetic findings which could potentially reveal a hereditary predisposition to other malignancies, with potential risk for the patient's relatives. Only in this last case, the patient will be informed and sent to genetic counselling, if the patient has given the consent to be informed on incidental genetic findings. Otherwise, considering the non-interventional nature of the study, results will not be returned.
The informed consent form is part of the documents to be submitted to the ethic committee for approval. For deceased or untraceable patients for whom it is not possible to obtain the informed consent form, every effort would be made to reach the patient and obtain his consent, otherwise the General Data Protection Regulation will be consulted.
It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all alive patients. This must be done in accordance with the national and local regulatory requirements.
For European Union member states, the informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative". All the personal data will be collected, stored and used according to the General Data Protection Regulation n. 679/2016, the Legislative Decree n. 196/2003 (Code on personal data protection) and

Decision of Guarantee for privacy protection n. 146/2019.

17 ADMINISTRATIVE RESPONSABILITIES

17.1 Overall study management

The Coordinating and Co-Coordinating Investigator, with the Scientific Coordinators (in cooperation with the Clinical Trial Office) will be responsible for writing the protocol, reviewing all case report forms and documenting his/her review on evaluation forms, discussing the contents of the reports with the Clinical Trial Office and for publishing the study results. The Coordinating and Co-Coordinating Investigator with the Scientific Coordinators will also generally be responsible for answering all clinical questions concerning eligibility, treatment, and the evaluation of the patients.

17.2 Clinical Trial Office

The Clinical Trial Office will be responsible for reviewing the protocol, collecting case report forms, controlling the quality of the reported data, and generating reports and analyses in cooperation with the Coordinating and Co-Coordinating Investigator and Scientific Coordinators. All methodological questions should be addressed to the Clinical Trial Office.
Clinical Trial Office will supervise CRO's activities.

17.3 CRO

The CRO will be responsible of ethical submissions and site activation and site close out activities.

18 SPONSORSHIP

This study is sponsored by GOIRC (Gruppo Oncologico Italiano di Ricerca Clinica). The protocol will be supported by partial economic support from Astra-Zeneca.

19 INSURANCE

A study insurance is not necessary considering that the patients enrolled receive standard treatment according to clinical practice.

20 PUBLICATION POLICY

The final publication of the study results will be written by the GOIRC on the basis of the final analysis performed at the Clinical Trial Office. After revision by the Clinical Trial Office and other co-authors the manuscript will be sent to a major scientific journal. Authors of the manuscript will include at least the Study Staff, the members of the writing committee, the investigators who have included more than 10% of the eligible patients in the trial (by order of inclusion).
The manuscript will include an appropriate acknowledgment section, mentioning all investigators who have contributed to the trial, the data center staff involved in the study, as well as supporting bodies.
All publications including data from the present trial will be submitted for review to the Clinical Trial Office and to all co-authors prior to submission. The Study Staff must approve all publications, abstracts and presentations of data pertaining to patients included in this study.

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