I.R.C.C.S. Ospedale San Raffaele

CLINICAL STUDY PROTOCOL

Study Code: The HER Project

Title: The HER Project: Homologous Recombination Deficiency in EGFR-Mutated NSCLC, a retrospective and prospective translational single-center study

Principal Investigator: Francesca Rita Ogliari, MD

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Study Identifier	The HER Project
RSO number	N/A
Other Protocol Codes	N/A
Version	1.0
Date	30/SEP/2025

Sponsor	IRCCS Ospedale San Raffaele Via Olgettina, 60 20132 – Milano, Italy
Authorized Sponsor Representative	Prof. Michele Reni Head of Medical Oncology Unit IRCCS Ospedale San Raffaele
Authorized Co-Sponsor Representative	N/A
Funding Source(s)	Medical Oncology internal Funding (Eredità Buffa)
Principal Investigator	Francesca Rita Ogliari, MD Medical Oncologist IRCCS Ospedale San Raffaele

DISCLAIMER

VERSION HISTORY

Protocol version n.	Reason of changes	Date issued
1.0	First version submitted to the Ethics Committee (EC)	

PROTOCOL SIGNATURE PAGE

Study Title: The HER Project: Homologous Recombination Deficiency in EGFR-Mutated NSCLC, a retrospective and prospective translational single-center study
Study Code: The HER Project
Protocol Version and Date: Version 1.0 30/SEP/2025

The undersigned has read and understood all the aspects of the protocol detailed within this document and agrees to supervise and conduct the study in accordance with the protocol, the Declaration of Helsinki, Guideline for Good Clinical Practice ICH E6 (R2), and all applicable regulatory requirements.

Prof. Michele Reni		IRCCS Ospedale San Raffaele	
Authorized Sponsor Representative Name	**Signature**	**Affiliation**	**Date**

Dott.ssa Francesca Rita Ogliari		IRCCS Ospedale San Raffaele	
Principal Investigator Name	**Signature**	**Affiliation**	**Date**

TABLE OF CONTENTS

1. KEY STUDY CONTACTS

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Principal Investigator	Francesca Rita Ogliari, MD Medical Oncologist IRCCS Ospedale San Raffaele ogliari.francesca@hsr.it
Study Clinical Unit	Medical Oncology Unit
Participant Clinical Units	Pathology Unit, Lung cancer research lab (Experimental Oncology Division)
Funding source(s)	Medical Oncology internal Funding (Eredità Buffa)
Clinical Trial Center	Email: ctc.firstcontact@hsr.it; ctc.trialstartup@hsr.it; ctc.datamanagement@hsr.it; ctc.quality@hsr.it

2. SYNOPSIS

Study Identifier	The HER Project	
Study Title	The HER Project: Homologous Recombination Deficiency in EGFR-Mutated NSCLC, a retrospective and prospective translational single-center study	
Protocol Version and Date	Ver 1.0 – 30/SEP/2025	
Sponsor	IRCCS Ospedale San Raffaele Via Olgettina, 60 20132 – Milano, Italy	
Co-Sponsor	N/A	
Funding Source(s)	Medical Oncology internal Funding (Eredità Buffa)	
Principal Investigator	Francesca Rita Ogliari, MD Medical Oncologist IRCCS Ospedale San Raffaele	
Study Description	The study aims to evaluate the prevalence of homologous recombination deficiency (HRD) in metastatic EGFR-mutated NSCLC and to assess its correlation with clinical and molecular features. Based on the hypothesis that HRD identifies a distinct EGFRm subgroup with prognostic value and a potential sensitivity to PARP inhibitor–based strategies, translational analysis will be performed with multiple pre-clinical models, ranging from human cancer cells to murine models.	
Study Design	Observational, retrospective and prospective single-center study	
Primary Objective To evaluate prevalence of HRD in our internal cohort of metastatic EGFRm NSCLC	**Primary Endpoint** Proportion of EGFRm NSCLC with an HRD positive score evaluated by AmoyDx HRD Panel or similar (GSS ≥ 50).	**Time point(s)** At T0 on baseline tumour samples
Secondary Objectives To assess correlations between HRD score (pos/neg) and: - Clinical variables (PFS, OS, metastatic sites) - Molecular events (type of EGFR mutations, VAF, drug resistance mechanisms i.e. gene amplification)	**Secondary Endpoints** Overall survival (OS), Progression-free survival (PFS), tumour genomic mutations.	**Time point(s)** At T0 on baseline tumour samples
Exploratory Objectives - To assess the efficacy of osimertinib and olaparib combination in TKI-naïve EGFRm NSCLC cell lines (HCC-4006, HCC-827, and HCC-2935, ranging from low to high HRD score); - To test the drug combination in patient-derived models of EGFRm tumours (PBMCs, organoids, and PDXs);	Tumor growth, cellular viability, drug toxicity.	N/A

- To rule out toxicity the drug combination and assess treatment response, thanks to our immune competent GEMM model of EGFRm16 and TP53 wt/null.		
Study Population	Adult patients (≥18 years) with metastatic EGFR-mutated non-small cell lung cancer (NSCLC) eligible for treatment with EGFR-TKI according to standard clinical practice, with available tumor tissue suitable for HRD testing.	
Inclusion Criteria	The population of interest will include patients affected by EGFR-mutated NSCLC. Individuals must meet all the following inclusion criteria in order to be eligible to participate in the study: 1. Participant is willing and able to give informed consent for participation in the study. However, concerning the retrospective cohort, the large sample sizes needed and considering that the disease involved in this study affects elderly subjects with co-morbidities and presents a significant mortality rate based on the stage of the disease, deceased and untraceable patients will also be included. 2. Age ≥18 years old 3. Have a metastatic histologically confirmed NSCLC 4. Presence of EGFR common mutations (i.e. ex19del and L858R) 5. Patients treated in first-line setting with 4th generation EGFR-TKI inhibitors (i.e. osimertinib, lazertinib) or beyond 6. Have archival tumor tissue suitable for HRD testing with AmoyDx Focus panel or similar 7. Survival follow-up available (for retrospective cohort)	
Exclusion Criteria	All individuals meeting any of the following exclusion criteria at baseline will be excluded from the study participation: 1. Insufficient baseline tumour tissue (unsuitable for HRD scoring) 2. Presence of EGFR uncommon mutations (i.e. ex20ins) 3. Known presence of co-occurring uncommon EGFRm, ALK, ROS1, or oncogenic drivers.	
STUDY OBJECT		
DRUG		
Sample Size	N= 100 Total sample size (N=50 retrospective cohort; N=50 prospective cohort)	
Statistical Design	According to the literature, to obtain an adequate number of patients to allow subgroup analyses and explore clinical and molecular correlations, we calculated a sample size of 100 patients (50 retrospective; 50 prospective) to conduct an exploratory proof-of-concept study, in which we expect to observe around 25 HRD-positive cases (the precision of the estimate corresponds to a 95% confidence interval with a half-width of approximately 8.5 percentage points, which is considered clinically relevant). Based on the historical caseload at IRCCS Ospedale San Raffaele (≈15 eligible patients/year), recruiting the planned sample over 4 years is feasible. For the retrospective cohort, we estimate that ≈30% of patients have insufficient residual archival tumor tissue, therefore the planned 50 patients will be included considering a longer interval (1/1/2020 - 30/09/2025). For the Primary and Secondary Objectives, continuous variables will be summarized using mean, median, range, and standard deviation. Categorical variables will be described using frequency and percentage. To test association between categorical variables, chi-squared test will be applied. To compare survivals between groups (i.e. HRD positive vs negative, cut-offs to be explored) log-rank test will be applied. Time-to-event outcomes will be represented using Kaplan-Meier curves.	

	For the Exploratory Objectives, parametric and non-parametric analyses will be used to compare differences in cell lines and phenotypes between groups. For the in vivo studies, differences in tumour size and phenotype will be evaluated at different timepoints and compared with t-test. For all statistical tests, $p < 0.05$ will be considered statistically significant.
Duration of the Study	Duration of enrolment: 4 years, starting from ethical approval Duration of total study period: 7 years, including 4 years of enrolment and 3 years of follow-up (01/01/2026-31/12/2032). Based on historical caseload at IRCCS Ospedale San Raffaele, we estimate an average of ≃ 15 EGFRm metastatic NSCLC patients eligible per year. Retrospective data collection will include patients respecting the eligibility criteria from 1/1/2020 to 30/09/2025.

3. ABBREVIATIONS AND DEFINITIONS

3.1. Abbreviations

AE	Adverse event
CRF	Case Report Form
CRO	Contract Research Organization
CTC	Clinical Trial Center
DPIA	Data Protection Impact Assessment
EC	Ethics Committee
EGFR	Epidermal Growth Factor Receptor
GCP	Good Clinical Practice
GSS	Genomic Scarring Score
HRD	Homologous Recombination Deficiency
ICF	Informed Consent Form
ICH	International Conference on Harmonization
NSCLC	Non-small cell lung cancer
OS	Overall Survival
OSR	Ospedale San Raffaele
PARP inhibitors	Poly (ADP-ribose) polymerase inhibitors
PDX	Patient-derived xenograft
PFS	Progression-Free Survival
PI	Principal Investigator
SOP	Standard Operating Procedure
TKI	Tyrosine Kinase Inhibitor

3.2. Definitions

AmoyDx® HRD Focus Panel	A homologous recombination deficiency (HRD) test designed to simultaneously detect genetic aberrations of BRCA1 and BRCA2 genes and HRD status [0].

GSS	A machine learning-based model which assesses genomic instability by analyzing different types of copy number events across the genome [0]. It can be calculated as the sum of the events of loss of heterozygosity (LOH), large-scale state transition (LST) and telomere allele imbalance (TAI) [20].
HRD	A condition linked to an impaired DNA repair mechanism that contributes to genomic instability.
PDX	A type of cancer model in which tumour tissues or cells from patients are implanted into immunodeficient or humanized mice.

4. BACKGROUND AND RATIONALE

Introduction

Lung cancer is the most common diagnosed cancer, with almost 2.5 million new cases per year, and the leading cause of cancer-related death (18.7%), according to GLOBOCAN (2022) [1,2]. Non-small cell lung cancer (NSCLC) is the most prevalent subtype, accounting for 85% of lung cancers [2]. Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor that regulates survival, proliferation and differentiation in mammalian cells [3]. EGFR mutations occur in around 30% of patients affected by NSCLC, representing the second most frequent targetable oncogenic driver in NSCLC [4,5].

The treatment landscape for metastatic EGFR-mutated (EGFRm) NSCLC has significantly evolved since the introduction of 1st generation tyrosine kinase inhibitors (TKIs) in 2010. While osimertinib (a 3rd generation TKI) is currently the standard first-line treatment worldwide in the advanced setting, emerging combination strategies, such as osimertinib plus chemotherapy (FLAURA2 trial) [6] or lazertinib plus amivantamab (MARIPOSA trial) [7], improved progression-free survival (PFS) and overall survival (OS) compared to TKI alone. However, these drug combinations often cause increased treatment-related toxicities, impacting both patients' quality of life and healthcare costs [8]. For this reason, it becomes crucial to identify predictive biomarkers to guide patient selection for combination therapies. For example, post-hoc analyses of FLAURA2 were conducted on poor-risk populations, such as EGFRm patients with high tumour burden or TP53 co-mutations [9,10]. Despite better outcomes of chemotherapy plus osimertinib across all patient subgroups, only 1 out of 4 patients had baseline tissue samples available for wider genome sequencing. Therefore, understanding the role of co-alterations in EGFRm tumours remains to be explored, particularly those associated with shorter OS and/or more aggressive clinical presentations.

Targeting HRD pathway with PARP-inhibitors in Non-small cell lung cancer

Homologous recombination deficiency (HRD) is a condition linked to an impaired DNA repair mechanism that contributes to genomic instability in several cancers, including NSCLC. PARP-inhibitors (PARPi), drugs targeting a key protein involved in DNA repair, are already available in clinical practice for the treatment of other HRD-positive subgroups of solid tumours (e.g. breast, ovarian, prostate cancer) [11]. In NSCLC, however, their clinical benefit remains unclear. Previous studies evaluating PARPi in NSCLC, including combinations with chemotherapy or immunotherapy, have shown modest or non-significant improvements in PFS and OS [12, 13, 14, 15, 16]. However, a significant limitation of these studies is the lack of HRD status assessment, which may have diluted the expected benefits in HRD-positive patients.

Interestingly, emerging evidence suggests that a higher Genomic Scarring Score (GSS), a surrogate for HRD, may predict PARPi sensitivity better than the traditional assessment of the mutational status of homologous recombination repair (HRR) genes [17]. In this study, the administration of PARPi significantly delayed tumour growth in patient-derived xenograft (PDXs) with high GSS, half of which carried pathogenic/likely pathogenic somatic HRR mutations [17]. Importantly, favourable responses to the PARPi-olaparib were observed regardless of HRR gene mutation status, underscoring the potential of GSS as a robust biomarker to more effectively select patients for PARPi-based therapies [17].

HRD in EGFR mutated NSCLC

In a Chinese cohort of HRD-positive NSCLC, EGFR-mutated tumours are associated with poor TKI response [18], which might be explained by gene amplifications like MET, a common resistance mechanism that can be promoted by HRD [19]. Studies also showed the co-occurrence of biallelic TP53 mutations and high HRD score in poor-risk EGFRm NSCLC, suggesting a link between this tumour genotype and TKI-resistance [20]. In preclinical models of NSCLC, osimertinib-resistant EGFRm cell lines exhibited sensitivity to olaparib , leading to increased DNA damage and cell death via a pro-oxidant state. Moreover, combining osimertinib with a PARPi *in vivo* significantly improved tumour regression compared to osimertinib alone [21]. Altogether, these findings lead to hypothesize that the HRD positive score/GSS may define a patient subgroup that could benefit from PARPi-based combination therapy.

Clinical trials evaluating EGFR-TKIs with PARPi failed to achieve their primary endpoint of PFS, possibly due to a lack of patient selection based on HRD score or other molecular criteria [22]. However, subsequent analyses of the GOAL trial found a stronger synergy between gefitinib (a 1st generation TKI) and olaparib in tumours with higher

BRCA1 expression [23], supporting a predictive role for targeting DNA damage repair pathways. In operable NSCLCs, the percentage of HRD positive tumours is ~20-30% [24], suggesting that the acquisition of HRD status could be an early event. Importantly, a similar incidence was observed in a Chinese cohort of any-stage EGFRm or ALK-rearranged NSCLC patients in which the cases with high HRD score are ~33% of the total [18]. The sensitivity of EGFRm tumours to platinum-based doublets [25], which is a known characteristic of HRD-positive tumours, and a larger compendium evidence of chromosomal instability further support exploring the role of HRD in EGFR-mutated lung cancer, paving the way for new targeted therapeutic approaches [26].

The HER Project

With the HER project, we will investigate the role of HRD and genomic instability (GSS) in EGFRm NSCLC by i) defining the fraction of metastatic EGFRm tumours with HRD positive score, ii) correlating HRD score with patient outcomes, and iii) assessing the synergic efficacy of EGFR-TKIs plus PARPi based on the HRD score in different preclinical settings, ranging from cell lines to a murine model of spontaneous EGFRm lung cancer. We expect that this drug combination will be effective in a specific subset of EGFR tumors. However, even if we won't observe any relevant findings by targeting this pathway, our work will increase our knowledge about DNA damage repair and drug resistance in EGFRm tumours.

5. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints	Time point(s)
Primary Objective To evaluate prevalence of HRD in our internal cohort of metastatic EGFRm NSCLC	**Primary Endpoint** Proportion of EGFRm NSCLC with an HRD positive score evaluated by AmoyDx HRD Panel or similar (GSS ≥ 50).	At T0 on baseline tumour samples
Secondary Objectives To assess correlations between HRD score (pos/neg) and: - Clinical variables (PFS, OS, metastatic sites) - Molecular events (type of EGFR mutations, VAF, drug resistance mechanisms i.e. gene amplification)	**Secondary Endpoints** Overall survival (OS), Progression-free survival (PFS), tumour genomic mutations.	At T0 on baseline tumour samples
Exploratory Objectives - To assess the efficacy of osimertinib and olaparib combination in TKI-naïve EGFRm NSCLC cell lines (HCC-4006, HCC-827, and HCC-2935, ranging from low to high HRD score); - To test the drug combination in patient-derived models of EGFRm tumours (PBMCs, organoids, and PDXs);	Tumor growth, cellular viability, drug toxicity.	N/A

- To rule out toxicity the drug combination and assess treatment response, thanks to our immune competent GEMM model of EGFRm16 and TP53 wt/null.		

6. STUDY DESIGN

The study aims to investigate the prevalence of HRD profiles in advanced EGFR-mutated NSCLC patients. The design is retrospective/prospetive observational study, and 100 patients will be enrolled (50 in the retrospective cohort and 50 in the prospective cohort). We plan 4 years of enrollment based on the number of patients with those characteristics accessing at OSR Medical Oncology Unit. Moreover, to calculate clinical parameters such as PFS and OS we plan to follow-up patients for 3 additional years, based on literature survival analysis.

6.1. Study duration

Duration of enrolment: 4 years, starting from ethical approval.

Duration of total study period: 7 years, including 4 years of enrolment and 3 years of follow-up (01/01/2026-31/12/2032). Based on historical caseload at IRCCS Ospedale San Raffaele, we estimate an average of ≃ 15 EGFRm metastatic NSCLC patients eligible per year.

Retrospective data collection will include patients respecting the eligibility criteria from 1/1/2020 to 30/09/2025.

7. STUDY POPULATION

7.1. Study Participants

The study population will include adult patients (≥18 years) with metastatic EGFR-mutated non-small cell lung cancer (NSCLC). Patients may be included either retrospectively, if already diagnosed and treated with available archival tumor tissue, or prospectively, if newly diagnosed and with tumor samples collected prior to treatment initiation. All participants must have tumor tissue suitable for HRD testing.

7.2. Inclusion Criteria

Individuals must meet all the following inclusion criteria in order to be eligible to participate in the study:

1. Participant is willing and able to give informed consent for participation in the study. However, since a part of the study is retrospective and, considering the large sample sizes required and considering that the disease involved in this study affects elderly subjects with co-morbidities and presents a significant mortality rate based on the stage of the disease, deceased and untraceable patients will also be included.
2. Age ≥18 years old
3. Have a metastatic histologically confirmed NSCLC
4. Presence of EGFR common mutations (i.e. ex19del and L858R)
5. Patients treated in first-line setting with 4th generation EGFR-TKI inhibitors (i.e. osimertinib, lazertinib) or beyond
6. Have archival tumor tissue suitable for HRD testing with AmoyDx Focus panel or similar
7. Survival follow-up available (for retrospective cohort)

7.1. 7. Survival follow-up available (for retrospective cohort)Exclusion Criteria

All individuals meeting any of the following exclusion criteria at baseline will be excluded from the study participation:

1. Insufficient baseline tumour tissue (unsuitable for HRD scoring)
2. Presence of EGFR uncommon mutations (i.e. ex20ins)
3. Known presence of co-occurring uncommon EGFRm, ALK, ROS1, or oncogenic drivers.

7.2. Screening Failures

Screen failures are defined as participants who consent to participate in the clinical study but does not meet the criteria for participation in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants. Minimal information includes demography, screening failure details, eligibility criteria. Based on the study features, we do not expect a high rate of screening failure.

8. STUDY OBJECT

The object of the study is to evaluate the prevalence of HRD in metastatic EGFRm NSCLC, investigating any correlation with clinical and/or molecular characteristics. As exploratory analysis, the project will assess the combination of Osimertinib and Olaparib in different pre-clinical models, ranging from tumour cell lines to GEMM animal models.

8.1. Study object description

N/A

9. STUDY PROCEDURES

Patients will follow normal clinical practice for advanced EGFR-mutated NSCLC. For the purpose of the study, only data on disease progression/death/survival will be collected, in addition to molecular analysis about HRD on tumor tissue. The flow-chart below is just representative of a normal path of a patient receiving EGFR-TKI in first-line setting, but can be slightly modified according to clinical practice, without any substantial issue.

Legenda: R=for research purposes, C=per clinical practice, *radiological assessments per clinical practice are accepted every 12-16 weeks

	Enrollment	**Screening**	**Baseline**	**Study Visit**	**End of Study Visit**	**Follow-Up Visit**
Day **Procedures**		-28 days (+/-7)	0	As per clinical practice (at least once a month)	At disease progression upon first-line treatment	As per clinical practice (at least every three months)
Informed consent	R					
Demographics	C					
Medical history	C		C			
Eligibility assessment		R				
Physical examination (including height and weight)	C		C	C	C	C
Vital signs	C		C	C	C	C
Performance status	C		C	C	C	C
Laboratory test	C		C	C		
Availability of tumor tissue for HRD testing		R				
EGFR-TKI administration			C	C		
Radiologic/Imaging assessment (CT-scan and brain imaging, CT or MRI)	C			C*		
Adverse event				C		

9.1. Informed Consent

Consent forms will be EC-approved, and the participant will be asked to read and review the document. The investigator (according to applicable regulatory requirements) or a person designated by the investigator, and under the investigator's responsibility, will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant MUST sign the informed consent document prior to any procedures being done specifically for the study/any data is collected. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedure and any data is collected. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.
However, deceased and untraceable patients will also be included. These patients will be pseudo-anonymized according to Institutional procedures. The inclusion of those patients will avoid bias resulting from the exclusion of such subjects (patients selected for outcomes) that could compromise the scientific quality of the study and the purpose of the research. That is, provided the data controller adopts appropriate measures to protect the rights, freedoms and legitimate interests of the individual's data.

9.2. Subject Recruitment and Screening

In this single-center study will be enrolled 100 outpatients affected by advanced EGFR-mutated NSCLC, accessing to Medical Oncology Unit of IRCCS Ospedale San Raffaele. In the retrospective cohort will be enrolled 50 patients, whereas in the prospective cohort we will enroll 50 patients in about 4 years, considering an accrual rate of 15 patients/years. The patients enrolled in the prospective cohort will follow the normal clinical practice, and no additional clinical requirements are needed to be involved in the study. The single parameter that will be investigated in the "screening" phase will be the availability of sufficient tumor tissue to perform HRD analysis.

9.3. Subject Identification

Starting from the signature of the informed consent by the patient or the patient's legal tutor, the subject is considered enrolled in the clinical study. For the retrospective cohort of the study, deceased and untraceable patients will be included as well.

A subject identification code will be assigned consecutively (e.g.: HER_YYY; where HER is the abbreviation of the Protocol Identifier, and YYY is the progressive subject number in increasing order starting from 001).

A patient identification list will be kept.

9.4. Baseline Assessments

Baseline assessment is considered as the visit during which the patient receives EGFR-TKI therapy for the first time (Day 0). This time point will serve as the reference for all subsequent evaluations and will be considered the starting point for survival analyses (Progression-Free Survival and Overall Survival). At baseline, medical history, physical examination (including performance status and vital signs) and routine laboratory data (as per clinical practice) will be recorded.

9.5. Study Visit and Follow Up

For the prospective cohort, study visits will follow routine clinical practice for patients receiving first-line EGFR-TKI.

- Treatment visits: patients will be assessed approximately every month (in accordance with standard clinical practice), including physical examination and vital signs, performance status, adverse event reporting, and laboratory tests as per clinical practice.
- Radiological evaluation: imaging (CT-scan of thorax and abdomen plus brain imaging as per clinical practice) will be performed every 12 to 16 weeks and as per local use.
- Follow-up after treatment discontinuation: patients who discontinue first-line treatment will be followed every 3 months, until death or study end.

The retrospective cohort will be followed from the date of diagnosis/first EGFR-TKI treatment until last available follow-up or death.
All patients (retrospective and prospective) will be followed for survival outcomes up to 3 years after the enrollment of the last patient, consistent with the study design.

9.6. Definition of End of Study

According to the study design, the end of the study is defined as the date on which the last patient completes the last visit (approximately 3 years after the last patient enrollment).

9.7. Premature termination or suspension of a study

As this is an observational, non-interventional study, premature termination or temporary suspension is not expected on the basis of patient safety concerns related to study treatment. However, the study may be suspended or terminated early by the Sponsor or Principal Investigator if deemed necessary.
Circumstances that may warrant early termination or suspension include, but are not limited to:

- inadequate patient recruitment or follow-up;
- data quality concerns, such as incomplete or non-evaluable data;
- administrative or financial reasons;
- withdrawal of approval by the Ethics Committee or other regulatory authorities.

In such cases, the Sponsor and the Principal Investigator will ensure that appropriate measures are taken to protect participants' interests. Patients already enrolled will continue to receive standard medical care in accordance with institutional clinical practice.
Written notification documenting the reasons for study suspension or termination will be provided by the Sponsor/PI to the Ethics Committee and, if applicable, to participants.

10. DISCONTINUATION AND WITHDRAWAL

Participants are free to withdraw from participation to the study at any time upon request.

The reason for participant discontinuation or withdrawal from the study will be recorded on the clinical records and in the electronic Case Report Form (eCRF). Subjects who sign the informed consent form and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced.

11. SAMPLE HANDLING

Subject included in the study are patients affected by metastatic EGFRm NSCLC, and diagnostic tissue samples are taken from each participant to the study protocol as part of the standard of care pathway. Archival tumour samples to be used in the study will be those already stored in the Pathology Unit, and tested for HRD status with AmoyDx at the Molecular biology laboratory (in the Pathology Unit). HRD testing costs will be covered by research funding as stated above. No further analysis are planned for this study, and any further and new research will be approved by the EC. The residual material will be stored in the Pathology Unit for 10 years.

12. PATIENT SAFETY

No additional risks are expected for participants, as this is an observational study and all procedures are part of standard clinical practice.

13. DATA MANAGEMENT

13.1. Definition of source data and source documents

Clinical data will be retrieved from patient medical records stored in the electronic medical record system of IRCCS Ospedale San Raffaele (e.g.: "Galileo", "Xero"). These records serve as the primary source documents and include hospital charts, laboratory results, diagnostic imaging reports, and other relevant clinical information.

Source Data: All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies). Any data recorded directly on the CRFs (i.e., no prior written or electronic record of data), is considered to be source data.
Source Documents: Original documents, data, and records (e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subjects' diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, or records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study).
All parameters asked for in the case report form (CRF) should be documented in the source documents.

A record of patient screen failures will be maintained for patients who do not qualify for enrollment, including the reason for the screen failure.

13.2. Documentation of data in Case Report Forms (CRFs)

Data relevant to the study will be extracted from the source documents and entered into the eCRF CGP (Cohort Genomics Platform). The eCRF can be accessed only to the PI and the involved staff, prohibiting access to unauthorized third parties. CGP is an electronic data capture system specifically developed around HIPAA-Security guidelines.

The creation of eCRFs is done by a team from the Center for Omics Sciences OSR (COSR) in collaboration with Medical Oncology data managers to define the variables and structure. The platform (instance at The OSR clinic servers) is accessed via credentials (with institutional hsr email), account configuration is done by the team itself upon request.

The electronic systems used for data handling and storage are validated and comply with established requirements for completeness, accuracy, reliability, and consistent performance.

A record of patient screen failures will be maintained, including the reason for screen failure, and will also be stored securely.

All parameters required for the CRF will be documented in the source documents.

13.3. Data Recording and Record Keeping

The investigator shall arrange for the retention of Essential Documents for the Conduct of a Clinical study (e.g., patient files, other source data, and the Trial Master File/Investigator Site File) after the completion or discontinuation of the study according to institutional procedures and applicable laws.

13.4. Data Protection

The Investigator (or the Center that will receive the data) undertakes to:
- use the data only for the purposes of the foreseen analyses and within the limits established by the study and approved by the competent EC;
- store data in a secure network system;
- prohibit unauthorised third parties from accessing data, even partially;
- guarantees to limit access to and processing of data only to its employees and collaborators who, upon appointment as an authorized person:
1. need to process the data in order to carry out their work in relation to the study;
2. have undertaken to maintain the confidentiality of the Data and of any information deriving from it or that is communicated to them.

If, within the context of the Study, the Investigator (or the Center that will receive the data) needs to make use of its own suppliers, the latter undertakes to appoint these subjects as Data Processors /Responsabili dei Trattamenti with a specific agreement or other legal act suitable for this, before the start of any data processing by them, according to Regulation (EU) n. 2016/679, art. 28.
The Investigator (or the Center that will receive the data) also undertakes to adopt suitable measures to facilitate the exercise of the rights of the subject provided for by Regulation (EU) n. 2016/679, art. 15 – 22, including the rights of access, rectification, cancellation, limitation, opposition and portability, within 30 days of receiving the relative request.

In case the Investigator need to communicate the data outside OSR (the Data Controller /Titolare) in pseudonymized form, the Investigator (or the Center that will receive the data) will refrain from carrying out any activity aimed at identifying the identity of the subjects to whom such data refer. In the event, however, that the data could not be communicated in pseudonymized form by the Data Controller, the Investigator (or the Center that will receive the data) undertakes to adopt all the security and organizational measures aimed at protecting the confidentiality of the Data Subject).
Within 30 days following the end of the study, the Investigator (or the Center that will receive the data) undertakes to cancel the data communicated by the Data Controller or to make them irreversibly anonymous and to promptly communicate it in writing.
A study specific DPIA (Data Protection Impact Assessment), which includes all these aspects and revised by OSR DPO, has been conducted according to applicable data protection law.

14. STATISTICS

14.1. Description of Statistical Methods

The statistical analysis will address the primary, secondary, and exploratory objectives of the study. For descriptive statistics, continuous variables will be summarized using mean, median, range, and standard deviation, while categorical variables will be described using frequency and percentage. Comparisons between groups (e.g., HRD positive vs HRD negative) and time-to-event analyses will be performed with appropriate statistical tests, as detailed in Section 14.4. Statistical significance will be set at a two-sided p-value < 0.05. Analyses will be performed through statistical software such as Microsoft Excel, R, and SPSS.

14.2. Sample Size Determination

According to the literature, the expected prevalence of HRD positivity in NSCLC ranges from 20–30% in early-stage tumours [24]. A similar incidence was observed in a Chinese cohort of any-stage EGFRm or ALK-rearranged NSCLC patients in which the cases with high HRD score are ~33% of the total [18].

Therefore, to obtain an adequate number of patients to allow subgroup analyses and explore clinical and molecular correlations, we calculated a sample size of 100 patients (50 retrospective; 50 prospective) to conduct an exploratory proof-of-concept study, in which we expect to observe around 25 HRD-positive cases (the precision of the estimate corresponds to a 95% confidence interval with a half-width of approximately 8.5 percentage points, which is considered clinically relevant). Based on the historical caseload at IRCCS Ospedale San Raffaele (≈15 eligible patients/year), recruiting the planned sample over 4 years is feasible. For the retrospective cohort, we estimate that ≈30% of patients have insufficient residual archival tumor tissue, therefore the planned 50 patients will be included considering a longer interval (1/1/2020 - 30/09/2025).

14.3. Analysis Populations

The analysis population will include all patients meeting the eligibility criteria, with available tumour tissue suitable for HRD testing. The retrospective cohort will comprise patients already diagnosed and treated with archival tumour tissue available; the prospective cohort will include newly diagnosed patients with tumour samples collected prior to treatment initiation. Patients with insufficient baseline tumour tissue will be excluded from the analyses.

14.4 Statistical Design

For the Primary and Secondary Objectives, continuous variables will be summarized using mean, median, range, and standard deviation. Categorical variables will be described using frequency and percentage. To test association between categorical variables, chi-squared test will be applied. To compare survivals between groups (i.e. HRD positive vs negative, cut-offs to be explored) log-rank test will be applied. Time-to-event outcomes will be represented using Kaplan-Meier curves.

For the Exploratory Objectives, parametric and non-parametric analyses will be used to compare differences in cell lines and phenotypes between groups. For the in vivo studies, differences in tumour size and phenotype will be evaluated at different timepoints and compared with t-test. For all statistical tests, $p < 0.05$ will be considered statistically significant.

14.5 Potential biases and mitigation strategies

The study is a pilot investigation with a primarily descriptive intent and will include all advanced lung cancer patients with an EGFR mutation treated with TKI inhibitors as standard of care. Retrospective cohort will include patients in the interval indicated (1/1/2020 - 30/09/2025) and those who have received at least one dose of treatment and for whom survival can be estimated (i.e., at least a date of death available). Only patients for whom insufficient archival tumor tissue is available will be excluded, which should not introduce a selection bias. To limit as much as possible bias related to sample size, a prospective cohort has been planned, in order to broaden the study while maintaining its exploratory nature.

15. ETHICAL AND REGULATORY CONSIDERATIONS

This clinical study will be conducted in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments established by the World Medical Assemblies, and the ICH guidelines for Good Clinical Practice.
This clinical study will be conducted in compliance with all international laws and regulations; national laws and regulations of the country in which the clinical study is performed; as well as any other applicable guidelines.

15.1. Responsibilities of the Investigator(s)

The Investigator(s) undertake(s) the responsibility to perform the study in accordance with this Protocol, Good Clinical Practice, and the applicable regulatory requirements. The Investigator is required to ensure compliance with the investigational product schedule, visits schedule, and procedures required by the protocol. The Investigator agrees to provide all information requested in the Case Report Form (CRF) in an accurate and legible manner. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to study subjects without prior EC approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted. The investigator must have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the study to conduct the study properly and safely.

15.2. Ethics Committee (EC) Approvals

This clinical study protocol as well as the Informed Consent are to be submitted to the appropriate Ethics Committee, and it is mandatory to obtain the written and dated approval, signed by the chairman with Ethics Committee(s) composition.
The clinical study the documents reviewed, the list of voting members and their qualifications, and the date of the review should be clearly stated on the written Ethics Committee approval.

15.3. Other Ethical Considerations

N/A

16. GENDER MEDICINE IN RESEARCH PROTOCOL

N/A

17. QUALITY ASSURANCE AND QUALITY CONTROL

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations, and standard operating procedures. Investigators involved in the study will permit study-related audits, EC review, and regulatory inspections by providing direct access to all study records.
Study audits can be conducted by or on behalf of CTC, according to OSR quality standards. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the auditors will verify that the clinical study is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.
The Investigator should notify the CTC promptly of any inspection scheduled by any regulatory authorities and will promptly forward copies of any inspection reports received.

17.1. Deviation from study protocol

A deviation from the protocol is an unintended departure from the procedures or processes described in the protocol and approved by the EC.
The Investigator or designee must document and explain in the patient's source documentation any deviation from the approved protocol.
Every deviation from the study protocol must be specified and documented separately for each patient. The investigator should discuss the type and extent of deviation as well as the possible consequences for further participation of the patient in the study.

17.2. Surveillance for observational studies with additional procedure

- NA

18. FINANCE AND INSURANCE

18.1. Funding

The study is funded by Medical Oncology internal funds (Eredità Buffa).

18.2. Patient Insurance

N/A

19. END OF CLINICAL STUDY

In accordance with applicable regulation, ICH GCP and SOPs, the PI shall notify the end of the clinical study within 15 days from the end of the clinical study and the reasons for such action.

19.1. Summary of the results of the clinical study

Irrespective of the outcome of a clinical study, within one year from the end of a clinical study, the PI shall submit a summary of the results of the clinical study.

20. INTELLECTUAL PROPERTY

IRCCS San Raffaele Hospital and the Principal Investigator is the sole owner of data, without prejudice to the provisions of the regulations in force regarding the publication of the data.

21. PUBLICATION POLICY

The Principal Investigator will have full access to all study data and will have full and total responsibility for:
1. the preparation of the manuscript(s) and the data collected in this study;
2. the choice of the journal or conferences to which the manuscript(s) will be submitted;
3. the final decision on the number, order, and names of contributing authors.

All manuscripts will include an appropriate acknowledgments section, mentioning all researchers who contributed to the trial. Prior to submission, all publications (articles, abstracts, presentations) that include data related to the patients of this study will be reviewed by all co-authors. The above-mentioned rules are applicable to publications involving any patient registered in the trial.

22. REFERENCES

[0] https://www.amoydiagnostics.com/products/amoydx-hrd-focus-panel [1] Bray, Freddie et al. “Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.” *CA: a cancer journal for clinicians* vol. 74,3 (2024): 229-263. doi:10.3322/caac.21834
[2] Hendriks, Lizza E L et al. “Non-small-cell lung cancer.” *Nature reviews. Disease primers* vol. 10,1 71. 26 Sep. 2024, doi:10.1038/s41572-024-00551-9
[3] Oda, Kanae et al. “A comprehensive pathway map of epidermal growth factor receptor signaling.” *Molecular systems biology* vol. 1 (2005): 2005.0010. doi:10.1038/msb4100014
[4] Hendriks, L E et al. “Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up.” *Annals of oncology : official journal of the European Society for Medical Oncology* vol. 34,4 (2023): 339-357. doi:10.1016/j.annonc.2022.12.009
[5] Skoulidis, Ferdinandos, and John V Heymach. “Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy.” *Nature reviews. Cancer* vol. 19,9 (2019): 495-509. doi:10.1038/s41568-019-0179-8
[6] Planchard, D. et al. Osimertinib with or without Chemotherapy in EGFR -Mutated Advanced NSCLC. New England Journal of Medicine 389, 1935–1948 (2023).
[7] Cho, B. C. et al. Amivantamab plus Lazertinib in Previously Untreated EGFR -Mutated Advanced NSCLC. New England Journal of Medicine 391, 1486–1498 (2024)
[8] Planchard, D. et al. 512MO FLAURA2: Safety and CNS outcomes of first-line (1L) osimertinib (osi) ± chemotherapy (CTx) in EGFRm advanced NSCLC. Annals of Oncology 34, S1668 (2023).
[9] Yang, J. C. et al. MA12.03 FLAURA2: Resistance, and Impact of Baseline TP53 Alterations in Patients Treated With 1L Osimertinib ± Platinum-Pemetrexed. Journal of Thoracic Oncology 19, S101–S102 (2024).
[10] Valdiviezo, N. et al. MA12.04 FLAURA2: Impact of Tumor Burden on Outcomes of 1L Osimertinib ± Chemotherapy in Patients with EGFR-mutated Advanced NSCLC. Journal of Thoracic Oncology 19, S102 (2024).

[11] Mekonnen, N., Yang, H. & Shin, Y. K. Homologous Recombination Deficiency in Ovarian, Breast, Colorectal, Pancreatic, Non-Small Cell Lung and Prostate Cancers, and the Mechanisms of Resistance to PARP Inhibitors. Front Oncol 12, (2022).
[12] Fennell, Dean A et al. "Olaparib maintenance versus placebo monotherapy in patients with advanced non-small cell lung cancer (PIN): A multicentre, randomised, controlled, phase 2 trial." *EClinicalMedicine* vol. 52 101595. 11 Aug. 2022, doi:10.1016/j.eclinm.2022.101595
[13] Hochmair, Maximilian et al. "Pembrolizumab With or Without Maintenance Olaparib for Metastatic Squamous NSCLC That Responded to First-Line Pembrolizumab Plus Chemotherapy." *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* vol. 20,2 (2025): 203-218. doi:10.1016/j.jtho.2024.10.012
[14] Ramalingam, Suresh S et al. "JASPER: Phase 2 trial of first-line niraparib plus pembrolizumab in patients with advanced non-small cell lung cancer." *Cancer* vol. 128,1 (2022): 65-74. doi:10.1002/cncr.33885
[15] Ahn, Myung-Ju et al. "Durvalumab in Combination With Olaparib Versus Durvalumab Alone as Maintenance Therapy in Metastatic NSCLC: The Phase 2 ORION Study." *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* vol. 18,11 (2023): 1594-1606. doi:10.1016/j.jtho.2023.06.013
[16] Clarke, Jeffrey M et al. "Veliparib and nivolumab in combination with platinum doublet chemotherapy in patients with metastatic or advanced non-small cell lung cancer: A phase 1 dose escalation study." *Lung cancer (Amsterdam, Netherlands)* vol. 161 (2021): 180-188. doi:10.1016/j.lungcan.2021.09.004
[17] Tsilingiri, Katerina et al. "Genomic scarring score predicts the response to PARP inhibitors in non-small cell lung cancer." *NPJ precision oncology* vol. 8,1 291. 26 Dec. 2024, doi:10.1038/s41698-024-00777-6
[18] Wang, Y. et al. Clinical and molecular significance of homologous recombination deficiency positive non-small cell lung cancer in Chinese population: An integrated genomic and transcriptional analysis. Chin J Cancer Res 36, 282–297 (2024)
[19] Fischer, A. et al. Mutational Landscape and Expression of PD-L1 in Patients with Non-Small Cell Lung Cancer Harboring Genomic Alterations of the MET gene. Target Oncol 17, 683–694 (2022).
[20] Feng, J. et al. Combination of genomic instability score and TP53 status for prognosis prediction in lung adenocarcinoma. NPJ Precis Oncol 7, 110 (2023)
[21] Marcar, L. et al. Acquired Resistance of EGFR-Mutated Lung Cancer to Tyrosine Kinase Inhibitor Treatment Promotes PARP Inhibitor Sensitivity. Cell Rep 27, 3422-3432.e4 (2019).
[22] Garcia-Campelo, R. et al. Combination of gefitinib and olaparib versus gefitinib alone in EGFR mutant non-small-cell lung cancer (NSCLC): A multicenter, randomized phase II study (GOAL). Lung Cancer 150, 62–69 (2020)
[23] Karachaliou, N. et al. BRCA1 Expression and Outcome in Patients With EGFR-Mutant NSCLC Treated With Gefitinib Alone or in Combination With Olaparib. JTO Clin Res Rep 2, 100113 (2021).
[24] Klinakis, A. et al. 1277P HRD status of patients with early stage non-small cell lung cancer. Annals of Oncology 34, S737–S738 (2023).
[25] Wang, Z. et al. Correlation between EGFR mutation status and response to first-line platinum-based chemotherapy in patients with advanced non-small cell lung cancer. Onco Targets Ther 1185 (2014) doi:10.2147/OTT.S63665.
[26] Drews, R. M. et al. A pan-cancer compendium of chromosomal instability. Nature 606, 976–983 (2022).

23. APPENDIX A: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Rationale	Study status	Details of Changes

24. APPENDIX B: LIST OF CLINICAL SERVICES / LABORATORIES

Unit/Service	**Referent** (name, email and telephone)	**Specific activity** (study activity, diagnostic procedures, lab tests, other)
Oncologia Medica	Francesca Rita Ogliari ogliari.francesca@hsr.it +39 02-26437627	Patient enrolment and study coordination

Lung Cancer Research Lab (Experimental Oncology Division)	Giorgia Foggetti foggetti.giorgia@hsr.it +39 02-26438011	Protocol-associated pre-clinical research
Anatomia Patologica	Maria Giulia Cangi cangi.mariagiulia@hsr.it +39 02-26437076	Histological and molecular analysis

25. APPENDIX C: LIST OF ENROLLING SITES

Name and Address	IRCCS Ospedale San Raffaele Via Olgettina, 60 20132 – Milano, Italy
Site Role	Coordinating site
Principal Investigator of Site	Francesca Rita Ogliari, MD Medical Oncologist IRCCS Ospedale San Raffaele
Number of subjects planned	N=100