Non-Interventional Study (NIS) Protocol

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A diagnostic Study of European and Japanese advanced NSCLC patients to evaluate suitable sample types for EGFR testing: The ASSESS Study

Sponsor:

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NON-INTERVENTIONAL STUDY PROTOCOL SYNOPSIS

A diagnostic Study of European and Japanese advanced NSCLC patients to evaluate suitable sample types for EGFR testing: The ASSESS Study

Medicinal Products (type, dose, mode of administration) and concomitant medication

The assignment of a subject to a particular therapeutic strategy is not decided in advance by a protocol but falls within current practice and marketing authorisation.

Study Rationale

This study will be conducted in Japan and Europe and will assess the concordance of *EGFR* mutation status derived from tumour samples and blood based circulating free DNA. The data generated will inform the use of less-invasive sample types in diagnostic practice. The study also aims to assess the current status of *EGFR* mutation testing across Japan and Europe and gaps in currently available data including *EGFR* mutation frequency in particular populations and demographic subgroups, *EGFR* mutation frequency in histological subtypes of NSCLC, *EGFR* mutation test process and methodology, utility of multiple sample types in the assessment of *EGFR* mutation status, and impact of *EGFR* mutation status on therapy choice. The data may be used to drive improvements to the *EGFR* mutation testing process, ensuring that patients have access to testing and are treated appropriately on the basis of the molecular features of their disease.

Study Objectives

(a) Primary objective

To determine the level of concordance between *EGFR* mutation status obtained via tissue/cytology and blood (plasma) based testing.

(b) Secondary objectives

1. To determine the *EGFR* mutation frequency (including mutation subtypes) in patients with advanced NSCLC (aNSCLC) of adenocarcinoma and non-adenocarcinoma histologies.

2. To describe first line therapy choice following *EGFR* mutation testing.

3. To describe second line therapy choice following discontinuation of first line treatment for patients confirmed as *EGFR* mutation positive via tissue/cytology.

4. To summarise *EGFR* mutation testing practices in terms of methods, sample types, success rate, mutation detection rate, testing turnaround time and reasons for not testing.

5. To determine the correlation between *EGFR* mutation status from tumour and demographic data and disease status.

6. To determine the correlation between *EGFR* mutation status derived from plasma (blood) and demographic data and disease status.

Study Design

This is a non-interventional diagnostic, international, multicenter and non-comparative study of *EGFR* mutation status in aNSCLC patients (locally advanced and/or metastatic disease) with adenocarcinoma and non-adenocarcinoma histologies.

Target subject population

- Patients with locally advanced (stage IIIA/B) or metastatic NSCLC who are newly diagnosed, have not received any local or systemic chemotherapy. Locally advanced patients should not be eligible for curative treatment (including surgery and chemoradiotherapy). The tumour samples of these patients are planned to undergo EGFR mutation testing, or have had an EGFR mutation test within 2 months of screening, or

- Patients who have newly diagnosed locally advanced (stage IIIA/B) NSCLC who are not eligible for curative treatment (including surgery and chemoradiotherapy) or metastatic NSCLC and have not received systemic chemotherapy. Previous or planned palliative radiotherapy is allowed. The tumour samples of these patients are planned to undergo EGFR mutation testing, or have had an EGFR mutation test within 4 months of screening, or

- Patients who have had surgical resection with or without adjuvant chemotherapy and have experienced recurrent disease. In this case if a new/contemporary biopsy is available, it should be used for *EGFR* mutation testing. If the only sample available is a historical diagnostic/resected sample, then this may be used for *EGFR* mutation testing (no time-limit from screening)

Study variable(s):

Primary variable of the study will be *EGFR* mutation status and subtype from tumour sample and *EGFR* mutation status and subtype from blood (plasma) sample.

Statistical methods

There will be no formal statistical hypothesis tests made in this study. Basic summary statistics will be used to describe the data to meet the objectives.

For the primary objective, the concordance rate, sensitivity, specificity, positive and negative predictive values and their exact 2-sided 95% confidence interval for comparing mutation status between tumour and plasma samples will be calculated for evaluable population for Europe and Japan.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this NIS Protocol.

Abbreviation or special term	Explanation
AE	Adverse event
ADR	Adverse Drug Reaction
aNSCLC	Advanced non-small-cell lung carcinoma
Assessment	An observation made on a variable involving a subjective judgement (assessment)
AZ	AstraZeneca
cfDNA	Circulating free DNA
CI	Confidence interval
CRF	Case Report Form
DNA	Deoxyribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
eCRF	Electronic Case Report Form
EGFR	Epidermal Growth Factor Receptor
EGFR-TKI	EGFR tyrosine kinase inhibitors
FFPE	Formaldehyde Fixed-Paraffin Embedded (tissue)
FSI	First subject in
GCP	Good Clinical Practice
H&E	Haematoxylin and eosin
HR	Hazard ratio
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
International Co-ordinating investigator	If a study is conducted in several countries, the International Co-ordinating Investigator is the Investigator co-ordinating the investigators and/or activities internationally
IRB	Institutional Review Board
LSLV	Last subject last visit
MC	Marketing Company
NIS	Non-Interventional Study
NSCLC	Non-small-cell lung carcinoma

Abbreviation or special term	Explanation
OR	Odds ratio
ORR	Objective response rate
PFS	Progression free survival
PI	Principal Investigator responsible for the conduct of a NIS at a site
QoL	Quality of life
SAE	Serious Adverse Event
SEER	Surveillance, Epidemiology and End Results
Tumour sample	Biopsy, cytology or other sample type containing tumour cells
Variable	A characteristic of a property of a subject that may vary e.g., from time to time or between subjects
WBDC	Web based data capture

1. STUDY OBJECTIVES

1.1 Primary objective

To determine the level of concordance between *EGFR* mutation status obtained via tissue/cytology and blood (plasma) based testing.

The study will establish whether blood (plasma) is a suitable sample type for reliably determining the *EGFR* mutation status of patients with NSCLC compared with cytology/tumour tissue, used in routine diagnostic & clinical practice in many countries.

1.2 Secondary objectives

Secondary objectives of the study are:

1. To determine the *EGFR* mutation frequency (including mutation subtypes) in patients with advanced NSCLC (aNSCLC) of adenocarcinoma and non-adenocarcinoma histologies.

2. To describe first line therapy choice following *EGFR* mutation testing.

3. To describe second line therapy choice following discontinuation of first line treatment for patients confirmed as *EGFR* mutation positive via tissue/cytology.

4. To summarise *EGFR* mutation testing practices in terms of methods, sample types, success rate, mutation detection rate, testing turnaround time and reasons for not testing.

5. To determine the correlation between *EGFR* mutation status from tumour and demographic data and disease status.

6. To determine the correlation between *EGFR* mutation status derived from plasma (blood) and demographic data and disease status.

1.3 Exploratory objective

Optional collection of tumour material in order to investigate exploratory biomarkers which may help to define molecular features of NSCLC. Prevalence, co-occurrence, and correlation with demographic data will be investigated.

2. STUDY PLAN AND PROCEDURES

This Non-Interventional Study Protocol has been subject to an internal review according to AstraZeneca standard procedures.

2.1 Overall study design

This is a non-interventional diagnostic, international, multicenter and non-comparative study of *EGFR* mutation status in aNSCLC patients (locally advanced and/or metastatic disease) with adenocarcinoma and non-adenocarcinoma histologies.

Approximately 1300 (1000 in Europe and 300 in Japan) aNSCLC patients who fulfil the inclusion/exclusion criteria will be recruited by the investigational sites throughout Europe and Japan. Participating countries from Europe will be: France, Germany, Italy, Spain, Netherlands, UK and Sweden.

Patients will be recruited over approximately 18 months.

It will be mandatory for every enrolled patient to provide a diagnostic tumour sample (i.e. biopsy, cytology or other sample type containing tumour cells) and a diagnostic blood (plasma) sample upon inclusion to be tested for *EGFR* mutations.

Once each patient has signed and dated the informed consent on enrolment, the patient demographics will be recorded and a blood (plasma) sample will be collected.

The tumour sample which was used to diagnose NSCLC will undergo *EGFR* mutation testing according to local practices. The plasma sample will be transported to a designated testing laboratory for *EGFR* mutation testing.

Optional collection of material from the diagnostic tumour sample will be requested from all patients to assess the exploratory objective. Samples will be used to investigate biomarkers which may help define molecular features of NSCLC (e.g. KRAS, EML4-ALK) as an exploratory objective. Biomarker prevalence, co-occurrence and correlation with demographic data will be investigated.

The first line therapy choice will be recorded for all patients. The second line therapy choice will be recorded following discontinuation of first line treatment, only for patients confirmed as *EGFR* mutation positive via tissue/cytology, where applicable.

Assignment of a subject to a particular therapeutic strategy is not decided in advance by this protocol. Medicinal products will be used according to current practice and marketing authorisation.

A Non-Interventional Study is a study in which no additional diagnostic or monitoring procedures shall be applied to the patients.

Table 1: Study plan

Visit description	**Screening/enrolment Day -28 to 0**	**Post EGFR Testing Data** **When data becomes available**
Main study informed consent (including tumour and plasma sample *EGFR* analysis)	X	
Inclusion / Exclusion criteria	X	
Demography / Smoking History	X	
Diagnostic tumour* sample for *EGFR* mutation analysis	X	
Optional tumour* collection for exploratory biomarker analysis	X	
Diagnostic plasma sample for *EGFR* mutation analysis in participating countries	X	
WHO performance status	X	
Disease status (date of first diagnosis, histological type, TNM (AJCC) disease stage, number of organs with metastasis, location of metastasis)	X	
Diagnostic tumour sample* for *EGFR* mutation test results (status, mutation sub-type)		X
Diagnostic tumour sample* for *EGFR* testing practice		X
Diagnostic plasma *EGFR* mutation test result (status, mutation sub-type) in participating countries		X
Diagnostic plasma *EGFR* testing practice in participating countries		X
1st line treatment information		X
2nd line treatment information**		X

* "Tumour sample" refers to a biopsy, cytology or other sample type containing tumour cells
** Only for patients confirmed as EGFR mutation positive via tissue/cytology

2.2 Biomarker samples in the study

Biomarker samples that will be collected in the study are described in the table below.

Table 2: Biomarker samples in the study

Sample	Optional or mandatory	Testing	Time point
Tumour sample for *EGFR* mutation analysis	Mandatory	Local – according to established practice	Screening/Enrolment
Blood (Plasma) sample for *EGFR* mutation analysis	Mandatory	Central – Nominated laboratories	Screening/Enrolment
Tumour sample for exploratory biomarker analysis	Optional	Central – Nominated CRO	Screening/Enrolment

2.2.1 *EGFR* mutation assessment of Tumour Samples

As a mandatory requirement for enrollment to the study, patients will be asked to provide consent for analysis of their tumour material for analysis of *EGFR* mutation status in a local laboratory according to established local practices. Tissue or cytology samples used to establish the diagnosis of NSCLC, including samples from the primary or metastatic sites, will be accepted.

If an EGFR mutation test has previously been performed and the patient meets the definition of the subject population, then a repeat of the *EGFR* mutation test is not required. In this case the *EGFR* mutation result and all other relevant testing data from the original test will be recorded.

2.2.2 *EGFR* mutation assessment of cfDNA from Blood (plasma) Samples

All screened patients will be asked to provide a mandatory blood (plasma) sample. These samples will be used for the extraction of cfDNA and determination of *EGFR* mutation status a nominated central laboratory.

All patients will be required to provide the following:

- 1 x 10 mL blood, from which the plasma will be isolated.

Samples will be collected, labelled, stored and shipped as detailed in the laboratory manual.

Residual material may be used for optional exploratory biomarker research if the patient consents to this research.

2.2.3 Tumour sample for exploratory biomarker analysis (Optional)

All screened patients will be asked to provide consent (optional) for the collection and exploratory biomarker analysis of residual tumour material. These samples will be collected

and stored by AstraZeneca for future investigation of biomarkers which may help define molecular features of NSCLC.

The investigator will be asked to provide one of the following for each consenting patient, depending on which format is more convenient or available:

- Formalin-fixed, paraffin-embedded tumour tissue blocks
- Cytology blocks
- Cytology Smears
- 10 to 20 unstained sections re-cut from formalin-fixed paraffin-embedded tumour tissue block or cytology blocks presented on slides. Each section is to be 5 μm thick.

Unused tumour samples will be stored for a period of maximum of 25 years (or as per local regulations) unless requested to be repatriated. For further details see the laboratory manual.

3. SELECTION OF SUBJECT POPULATION

- Patients with locally advanced (stage IIIA/B) or metastatic NSCLC who are newly diagnosed, have not received any local or systemic chemotherapy. Locally advanced patients should not be eligible for curative treatment (including surgery and chemoradiotherapy). The tumour samples of these patients are planned to undergo EGFR mutation testing, or have had an EGFR mutation test within 2 months of screening, or

- Patients who have newly diagnosed locally advanced (stage IIIA/B) NSCLC who are not eligible for curative treatment (including surgery and chemoradiotherapy) or metastatic NSCLC and have not received systemic chemotherapy. Previous or planned palliative radiotherapy is allowed. The tumour samples of these patients are planned to undergo EGFR mutation testing, or have had an EGFR mutation test within 4 months of screening, or

- Patients who have had surgical resection with or without adjuvant chemotherapy and have experienced recurrent disease. In this case if a new/contemporary biopsy is available, it should be used for *EGFR* mutation testing. If the only sample available is a historical diagnostic/resected sample, then this may be used for *EGFR* mutation testing (no time- limit from screening).

3.1 Investigators

Investigators must be oncologists experienced in the treatment of advanced NSCLC patients and based in the participating European countries (i.e. France, Germany, Italy, Spain, Netherlands, UK or Sweden) or Japan.

It is estimated that approximately 15 to 20 patients will be enrolled per investigator/centre in Europe, i.e. aproximately 60 centres.

In Japan, it is estimated that approximately 60 patients will be enrolled per investigator/centre, i.e. approximately 6 centres.

3.2 Inclusion criteria

The subject population that will be observed in the NIS, must fulfil all of the following criteria:

1. Provision of written informed consent

2. Patients aged 18 years and older from European countries and patients aged 20 years and older for patients from Japan

3. Histological or cytological confirmed locally advanced NSCLC (stage IIIA/B) not suitable for curative treatment or metastatic (stage IV) NSCLC.

4. Newly diagnosed patients with locally advanced and/or metastatic NSCLC who are systemic treatment Naïve (i.e. no chemotherapy or EGFR-TKI) or patients with recurrent disease who have previously received adjuvant chemotherapy (not including EGFR-TKI)

5. Provision of diagnostic cancer tissue or cytology sample upon inclusion (surgical specimen, biopsy sample, or cytology sample is acceptable)

6. Provision of a routine blood (plasma) sample

The prescription of any medicinal product is clearly separated from the decision to include the subject in the NIS.

3.3 Exclusion criteria

1. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study site)

2. Previous enrolment in the present study

3. As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g. unstable or uncompensated respiratory, cardiac, hepatic or renal disease)

4. Evidence of any other significant clinical disorder or laboratory finding that made it undesirable for the patient to participate in the study

5. Pregnancy or breast-feeding

4. DISCONTINUATION OF SUBJECTS

4.1 Criteria for Discontinuation

Subjects may be discontinued from the NIS at any time. Specific reasons for discontinuing a subject from this NIS are:

1. Voluntary discontinuation by the subject who is at any time free to discontinue his/her participation in the NIS, without prejudice to further treatment

2. Incorrect enrolment (inclusion/exclusion criteria are not fulfilled)

Discontinuation of the patient treatment is not a criterion for discontinuation from the

study.

4.2 Procedures for discontinuation

Patients who discontinue should be asked about the reason(s) for their discontinuation. No specific procedures after discontinuation are required per study but continuation of patient assessment and treatment according to clinical practice.

No further data will be collected on patients following discontinuation from the study.

Discontinued subjects will not be replaced.

5. THERAPEUTIC STRATEGY

5.1 Therapeutic strategy of a Non-Interventional Study

The assignment of a subject to a particular therapeutic strategy is not decided in advance by a protocol but falls within current practice and marketing authorisation.

6. STUDY CONDUCT

6.1 Subject enrolment

Every Investigator will:

1. Obtain signed informed consent from the potential subject or their guardian/legal representative before any study specific procedures are performed.

2. Assign potential subject a unique enrolment number

3. Determine subject eligibility.

7. MEASUREMENTS OF STUDY VARIABLES AND DEFINITIONS OF OUTCOME VARIABLES

7.1 Primary variables

EGFR mutation status and mutation subtype from tumour sample and *EGFR* mutation status and mutation subtype from blood (plasma) sample.

7.2 Secondary variables

The following data will be collected in order to fulfil the secondary objectives of the study:

Table 3: Secondary variables

Patient demographics	• Gender
	• Age
	• Race
	• Ethnicity
	• WHO Performance status
	• Smoking status:
	  o Intensity (cigarettes per day)
	  o Duration (years)
	  o Time since cessation (years)
	• Disease status:
	  o Date of first diagnosis
	  o Histological type
	  o TNM (AJCC) disease stage (Edition 7)
	  o Number of organs with metastasis
	  o Location of metastasis/metastases

1st-line treatment decision after testing	• Name of treatment • Treatment start date • Reason for treatment choice (including Clinical Trial enrolment)
2nd-line treatment decision (for patients confirmed as *EGFR* mutation-positive via tissue/cytology)	• Name of treatment • Treatment start date • Reason for treatment choice (including Clinical Trial enrolment)

EGFR mutation testing endpoints	• Test result
	  o Overall mutation result (positive negative, unknown)*
	  o Mutation subtype
	• Testing methodology
	• Sample type and availability
	• Testing turnaround time
	• Reason(s) for testing not being performed

Footnote:
Smoking is a multi-dimensional phenomenon with many characteristics, such as intensity (in cigarettes per day), duration (in years) and time since cessation (in years). In this study, every patient should report the all information list above to determine the smoking status.

- Deletion, substitution, and/or insertion mutations of tyrosine kinase domain of the *EGFR* gene will be identified and recorded in the CRF. Methods used for measuring *EGFR* mutation status should also be recorded.

**EGFR* mutation status will be recorded for each patient as:

- Positive: *EGFR* mutation detected (one or more individual mutations)
- Negative: *EGFR* mutation not detected
- Unknown: *EGFR* mutation status not determined as *EGFR* mutation positive or *EGFR* mutation negative

It is accepted that mutation assays (methods and individual mutation assays) may differ between labs and institutions. Thus a patient can be positive or negative depending on the method used. As a minimum, laboratories should assess the common *EGFR* mutations: Exon 19 deletions, and Exon 21 L858R point mutations.

Mutation subtypes will also be recorded:

- Exon 18:
  - G719C/S/A
  - other
- Exon 19:
  - deletions
  - other
- Exon 20:
  - T790M
  - insertions
  - other
- Exon 21:
  - S768I
  - L858R
  - L861Q
  - Other

The following outcome variables will be calculated in order to fulfil the secondary objectives of the study:

EGFR Testing Overall Endpoints

- Test failure rate, calculated as, tests not performed successfully providing an "unknown" or "fail" test result/ number of tests requested
- Mutation detection rate, calculated as performed successfully providing a "positive" mutation result / total number of successful tests providing a mutation result
- Testing success rate, calculated as, tests performed successfully providing a mutation result (positive/negative) / total number of tests requested

8. BIOLOGICAL SAMPLING PROCEDURES

Laboratories for tumour analysis will be selected according to local practice including academic, hospital and commercial laboratories.

In Europe, central/regional expert labs for blood (plasma derived cfDNA) based testing will be identified, selected and agreed in association with local AZ representatives, the AZ diagnostic study team (global) and Study Steering Committee.

In Japan, both tumour and blood based testing will occur at a commercial laboratory.

Details of the acquisition and processing of tumour material, including suggested preparation

methods should follow the routine practices of the hospital where the patient is enrolled and/or the laboratory at which the sample will be tested. Central laboratories (outside the hospital where the patient is enrolled) deemed appropriate by the study site may be used as an alternative where appropriate according to local practices or investigator preference.

8.1 Specification of biological samples

8.1.1 Pathology review

Histopathological review of tumour material should take place to ensure that samples are adequate for use. Where appropriate tissue or cytology samples should be reviewed by suitably qualified pathologist and classified according to the most recent WHO classification. Reasons for the exclusion of the material for use in subsequent analyses may include:

1. Inappropriate sample (i.e., provision of materials inconsistent with NSCLC)
2. Insufficient tumour sample
3. Inadequate cellular morphology (e.g., due to poor or inappropriate fixation)

8.1.2 Somatic (non-inheritable) *EGFR* mutation testing

Where suitable tumour/cytology material is available, tumour DNA extraction and somatic mutational analysis should be carried out.

8.1.3 Reporting of *EGFR* mutation testing results

The *EGFR* mutation test result obtained from the patient's tumour will be sent to the investigator as per local practices and will be recorded in the study eCRF. The result will not be made available to any insurance company, the patient's employer, or the patient's family without the patient's specific consent.

9. HANDLING, STORAGE AND DESTRUCTION OF BIOLOGICALSAMPLES

Procedures for the handling and storage of samples will follow local lab requirements and practices. Sample processing should follow study site or central lab standard procedure in the context of applicable local regulation.

Study investigators will be encouraged to retain the residual DNA derived from tumour samples for possible future verification of results.

9.2 Withdrawal of informed consent for donated biological samples

Collection of mandatory tumour and blood (plasma) samples is an integral part of the study. If a subject withdraws consent to the use of any mandatory samples, the subject is withdrawn from further study participation and the samples will not be analysed.

If a subject withdraws consent to the use of blood (plasma) samples for molecular analysis at the nominated expert laboratory, residual blood (plasma) samples (and any derivatives thereof) if remaining after analysis, will be destroyed.

If a subject withdraws consent to the use of optional tumour samples for exploratory biomarker analysis, the subject can continue in the study. If a subject withdraws consent to the use of optional tumour samples during the study and the sample is held at a central facility (nominated by AstraZeneca), then the tumour blocks will be repatriated, slides and any residual material or derivatives of the sample will be destroyed.

If tumour (mandatory or optional) or blood (plasma) samples have already been analysed before withdrawal of consent, AstraZeneca is not obliged to destroy the results of this research.

10. SAFETY REPORTING

Due to the non-interventional character of this study, no pro-active safety data collection should take place. Only spontaneously mentioned safety events should be reported as required by the post-marketing pharmacovigilance regulations.

11. ETHICAL CONDUCT OF THE NON-INTERVENTIONAL STUDY

The Non-Interventional Study will be performed in accordance with ethical principles that are consistent with the Declaration of Helsinki, ICH GCPs and the applicable legislation on Non-Interventional Studies.

The Investigator will perform the NIS in accordance with the regulations and guidelines governing medical practice and ethics in the country of the NIS and in accordance with currently acceptable techniques and know-how.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of outcome variables in relation to objectives and hypotheses

No formal statistical hypothesis tests will be made in this study.

The relationship between the objectives and outcome variables is described in the table below.

Table 4: Study objectives and outcome variables

Objectives	Outcome variables
Primary objectives	
To determine the level of concordance between *EGFR* mutation status obtained via tissue/ cytology and blood (plasma) based testing.	Concordance rate, sensitivity, specificity, positive and negative predictive values between sample types.
Secondary objectives	
- To determine the *EGFR* mutation frequency (including mutation subtypes) in patients with advanced NSCLC (aNSCLC) of adenocarcinoma and non-adenocarcinoma histologies.	*EGFR* mutation frequency overall and by adenocarcinoma and non-adenocarcinoma histologies and by country/region.
- To describe first line therapy choice following *EGFR* mutation testing.	% first line treatment choices by mutation status and by country/region and mutation subtype.
- To describe second line therapy choice following discontinuation of first line treatment for patients confirmed as *EGFR* mutation positive via tissue/cytology.	% patients on 2nd line treatment, treatment sequence, % second line treatment choices by country/region and mutation subtype for *EGFR* mutation positive for tumour.
- To summarise *EGFR* mutation testing practices in terms of methods, sample types, success rate, mutation detection rate, testing turnaround time and reasons	*EGFR* mutation test method, sample type, success rate, mutation detection rate, testing turnaround time and reasons for testing not being performed summarised overall and by

for not testing.	country/region.
- To determine the correlation between *EGFR* mutation status from tumour and demographic data and disease status.	Correlation of mutation status from tumour with demographic characteristics and disease status data.
- To determine the correlation between EGFR mutation status derived from plasma (blood) and demographic data and disease status	Correlation of mutation status from plasma (blood) with demographic characteristics and disease status data.

13. DESCRIPTION OF ANALYSIS SETS

13.1 Enrolled Population

The Enrolled Population consists of all patients who sign an informed consent.

13.2 Evaluable Population

The Evaluable Population consists of all enrolled patients with all non-missing or unknown:

- Tumour sample *EGFR* mutation test status.
- Tumour sample *EGFR* mutation test sub-type.
- Plasma *EGFR* mutation test status.
- Plasma *EGFR* mutation test sub-type.

Unless otherwise specified all summaries will be based on the Evaluable Population.

14. METHOD OF STATISTICAL ANALYSIS

Descriptive summary of patient demographics and disease characteristics will be presented for all enrolled population and the evaluable population.

14.1 Primary Objective

The concordance rate, sensitivity, specificity, positive and negative predictive values and their exact 2-sided 95% confidence interval for comparing mutation status between tumour and plasma samples will be calculated for evaluable population for Europe and Japan.

14.2 Secondary Objectives

EGFR Mutation Frequency

The *EGFR* mutation frequency from the tumour sample in the evaluable population will be summarised overall and by country/region by adenocarcinoma and non-adenocarcinoma histologies.

First line treatment choice

1st line treatment choice following *EGFR* mutation testing will be summarised descriptively for the overall evaluable population by *EGFR* mutation status, *EGFR* mutation subtype and

by country/region.

Second line treatment choice

The percentage of patients on 2nd line treatment, including treatment choice following discontinuation of first line treatment for patients confirmed as *EGFR* mutation positive via tissue/cytology will be summarised descriptively for the evaluable EGFR mutation positive population by *EGFR* mutation status, *EGFR* mutation subtype and by country/region. Treatment sequence i.e. 1st line treatment choice and 2nd line treatment choice will be summarised descriptively.

Summaries of EGFR mutation testing practices

The testing methodology, sample type and availability, testing turnaround time, testing success rate and mutation detection rate will be summarised using appropriate descriptive statistics for evaluable population. The reasons for testing not being performed will be listed.

Correlation between EGFR mutation status from tumour sample and demographic characteristics and disease status

The patient characteristics of the enrolled population will be compared to the characteristics of the evaluable population in order using summary statistics to understand any relationship between all aNSCLC patients and those whose samples undergo *EGFR* mutation testing**.**

The correlation between *EGFR* mutation status and both demographic characteristics and disease status will be analysed using a multivariate logistic regression model, constructed for the evaluable population to model *EGFR* tumour mutation status at baseline (*EGFR* mutation positive versus *EGFR* mutation negative from tumour sample) and key demographic characteristics such as histology (adenocarcinoma versus non-adenocarcinoma), smoking status (never- versus eversmoker) gender (female versus male), age (≤65 versus 65+) and WHO performance score (PS; 0-1, 2) and key disease status characteristics (time since diagnosis, disease stage at progression and number of organs with metastasis).

Correlation between *EGFR* mutation status derived from plasma sample and demographic characteristics and disease status

The *EGFR* mutation status from plasma sample and demographic and disease characteristics will be summarised for the evaluable population with available samples. This summary will be compared with summaries of the *EGFR* mutation status from tumour samples within the same demographic and disease status characteristics to understand any patterns of differences in the mutation detection rate in the plasma sample within key patient sub-groups.

The correlation between *EGFR* mutation status from plasma sample and both demographic characteristics and disease status will be analysed using a multivariate logistic regression model, constructed for the evaluable population to model *EGFR* tumour mutation status at baseline (*EGFR* mutation positive versus *EGFR* mutation negative from tumour sample) and key demographic characteristics such as histology (adenocarcinoma versus non-adenocarcinoma), smoking status (never- versus eversmoker) gender (female versus male), age (≤65 versus 65+) and WHO performance score (PS; 0-1, 2) and key disease status characteristics (time since diagnosis, disease stage at progression and number of organs with metastasis).

15. DETERMINATION OF SAMPLE SIZE

The number of patients required to be tested for *EGFR* mutation status in this study in Europe and Japan is a total of 1000 patients from Europe and 300 patients from Japan. This is to determine the sensitivity between tumour and blood based testing methodologies for each region; for 100 *EGFR* mutation positive patients, assuming the sensitivity is 50%, the 95% CI will be 40-60%. Considering the prevalence of *EGFR* mutations in Europe, 1000 patients will need to be tested (*EGFR* mutation frequency of 10%) to obtain 100 mutation positive patients. As an independent replication, the sensitivity will also be assessed in Japanese patients; 300 patients will need to be tested (*EGFR* mutation frequency of 30%) to obtain 100 mutation positive patients.