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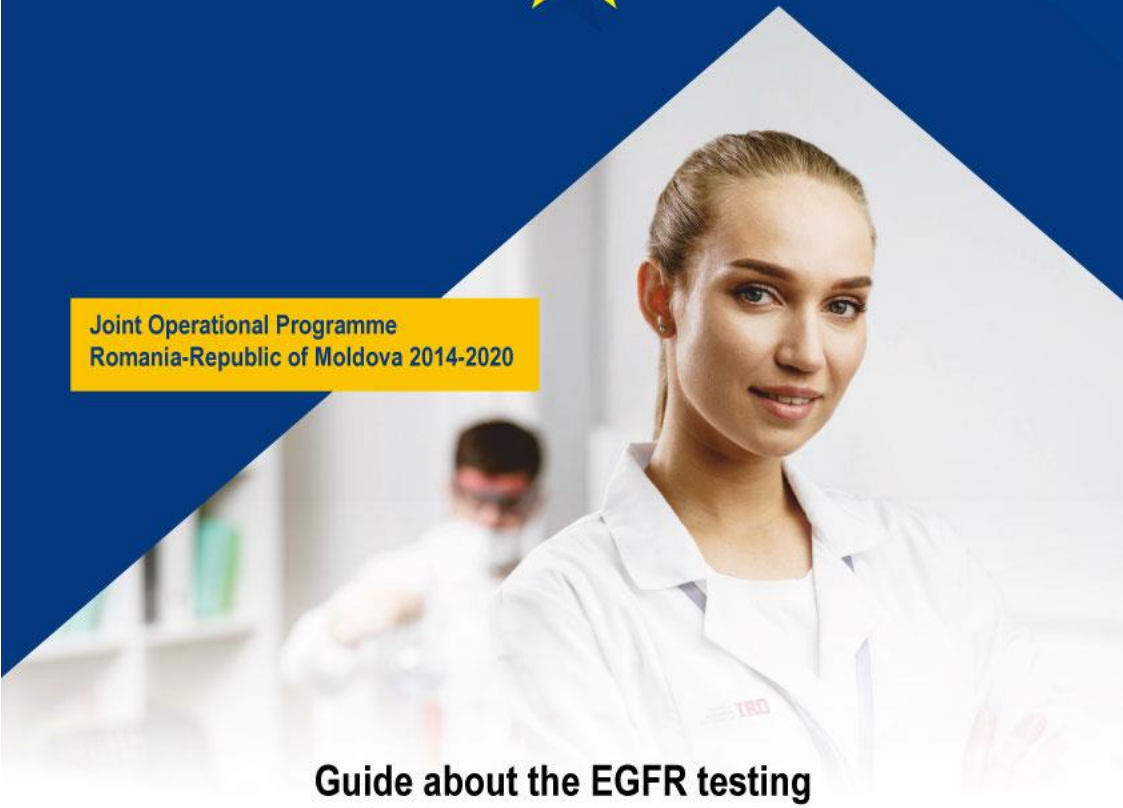

Guide about the EGFR testing

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Institutul Regional de Oncologie Iasi IRO
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Guide about the EGFR testing

Content

1. EGFR MUTATIONS

Epidermal growth factor receptor (EGFR) activating mutations are found in exons 18 to 21 of the EGFR gene, which is part of the gene coding for the tyrosine kinase domain of the EGFR protein.

Exon 19 deletions and an L858R point mutation in exon 21 account for around 90% of all EGFR mutations in advanced non-small cell lung cancer (NSCLC).

Personalised treatment for advanced NSCLC

EGFR tyrosine kinase inhibitors (TKIs) have high affinity for mutant EGFR and block aberrant EGFR signalling pathways to inhibit proliferation, tumour growth, cell survival and angiogenesis. Treatment of EGFR mutation-positive advanced NSCLC tumours with EGFR-TKIs results in tumour shrinkage in the majority of patients.

Evidence to support treatment of advanced NSCLC according to EGFR mutation status

Several randomised trials have demonstrated the importance of epidermal growth factor receptor (EGFR) mutation status in determining the most appropriate first-line treatment for patients with advanced non-small cell lung cancer (NSCLC). Clinical trials have shown that patients with EGFR mutation-positive tumours have significantly improved progression-free survival (PFS) and objective response rate (ORR) when treated with first-line EGFR tyrosine kinase inhibitors (TKIs) compared with standard platinum-based doublet chemotherapy regimens.

The IRESSA Pan-Asian Study (IPASS) showed that in clinically selected patients from Asia with advanced NSCLC, EGFR mutation-positive and EGFR mutation-negative tumours responded differently to first-line gefitinib or platinum-based doublet chemotherapy (carboplatin/paclitaxel). Patients with EGFR mutation-positive tumours had significantly improved PFS, ORR, quality of life (QoL) and lung cancer symptoms when treated with gefitinib compared with carboplatin/paclitaxel. Patients with EGFR mutation-negative tumours had significantly better PFS, ORR, QoL and lung cancer symptoms when treated with carboplatin/paclitaxel compared with gefitinib.

2. SAMPLE SOURCES

Suitable samples for EGFR mutation testing

In order to perform a successful epidermal growth factor receptor (*EGFR*) mutation test, a sufficient quantity of tumour cells is required. This ensures that an adequate amount of tumour DNA is extracted for analysis. The three main sample types are discussed in the following sections: **tumour biopsy samples, cytology samples** and **ctDNA (plasma) samples.**

Tumour biopsy samples are the most suitable and commonly available samples for *EGFR* mutation testing and are therefore the preferred sample type. Cytology samples are frequently used to diagnose advanced non-small cell lung cancer (NSCLC) and, in the absence of a biopsy, should be analysed, taking into consideration that the sample quantity and tumour cell content may be low.

Once obtained, the sample should be sent for *EGFR* mutation testing as soon as possible to avoid delays in treatment decisions.

Other sample types, such as circulating tumour DNA (ctDNA) extracted from blood (serum or plasma), can be analysed. Studies have shown that when *EGFR* mutations are identified in ctDNA it is highly predictive of an *EGFR* mutation-positive tumour. The IFUM study demonstrated that ctDNA *EGFR* testing is reasonably sensitivity (65.7% of *EGFR* mutation tumour samples had a matched *EGFR* mutation-positive ctDNA sample) and highly specific (99.8% of *EGFR* mutation-negative tumour samples had a matched *EGFR* mutation-negative ctDNA sample).

EGFR testing using ctDNA should only be used when biopsy and cytology samples are unavailable, as there is a high *EGFR* mutation test false negative rate, i.e. not all patients harbouring an *EGFR* mutation-positive tumour will be determined to be *EGFR* mutation-positive from the ctDNA (plasma) samples.

EGFR mutation test methods for tissue and cytology samples

The choice of epidermal growth factor receptor (*EGFR*) mutation test method is often influenced by a laboratory's expertise and available equipment. Labs can use commercially available kits, develop their own tests (lab-developed tests) or send the samples to an external specialist testing laboratory. It is important to consider using highly sensitive

testing methods for cytology samples because of the limited number of tumour cells present in such samples.

Kits: These are ready to use, generate accurate results, if used according to the kit's instructions, and will be quality-controlled. They tend to have been well-tested on clinical samples, and many are regulated as in vitro diagnostics (IVDs).

Lab-developed tests (LDTs): These may be less expensive, but they take time to develop and validate, requiring known samples for development and validation. Quality control may be limited.

Tests fall into two main categories:

Screening methods detect all potential activating and novel mutations by analysing the entire mutation-containing region in *EGFR* exons 18 to 21. They tend to be less sensitive but are able to detect a greater number of mutations (analytical sensitivity).

Targeted methods focus on detecting only well-characterised mutations. These tend to be more sensitive (lower limit of detection) but will detect only the mutations the test is designed to detect.

Both approaches have their relative merits, and it is essential to optimise the testing process to ensure that a reliable result is obtained for every patient.

***EGFR* mutation testing and turnaround time**

Some mutation detection methods take longer to perform than others, with a range from two to three hours to two to three days. Batching together samples may reduce cost and effort but can significantly increase the time taken to generate decision-making results. Different methods also have different numbers of process steps, which can increase both the turnaround time for the testing process and the potential for contamination.

Test fails

If a reliable result is not obtained because of an insufficient sample or a low-quality sample, another sample may be requested, depending on other factors.

3. SAMPLE PROCESSING

Tumour DNA extraction and macrodissection procedures

The quality and the quantity of DNA extracted from the tumour sample are the keys to a successful epidermal growth factor receptor

(*EGFR*) mutation test. Extraction methods need to be highly reliable and yield as much DNA as possible.

A poor DNA extraction method can lead to increased assay failure and increase the risk of false negative results, as well as limiting the amount of analysis that can be performed.

Prior to DNA extraction, **macrodissection** can be performed to enrich for tumour. This can improve the ability to detect potentially low levels of tumour DNA in subsequent testing. Macrodissection is highly recommended if the percentage of tumour in the sample is <50%. The procedure is easy to perform in any lab and does not require specialised equipment.

DNA extraction methods

Many DNA extraction methods are available, including the kit-based methods listed below as exemple.

Method	Manufacturer	Additional information
QIAamp DNA FFPE Tissue Kit	QIAGEN www.qiagen.com	Can be automated on the QIAGEN QIAcube
QIAamp DNA Mini Kit	QIAGEN www.qiagen.com	Can be automated on the QIAGEN QIAcube
Arcturus PicoPure DNA Extraction Kit	Applied Biosystems www.appliedbiosystems.com	
Cobas® DNA Sample Preparation Kit	Roche Molecular Systems www.molecular.roche.com	Integral to the cobas® EGFR Mutation Test

DNA quantification

Measuring how much DNA is present in the sample (DNA quantification) is optional, but may be useful for prioritising exons or mutations to be screened if only small amounts of DNA are extracted. It can also help with determining the cause of test failure.

Spectrophotometry is a rapid and simple method of quantifying the amount of extracted DNA and is used by many labs. However, as this

method includes DNA fragments not suitable for downstream testing, it may over-estimate the amount of DNA available. Quantitative PCR (Q-PCR) is a more accurate method, as it quantifies only the DNA that is large enough to be amplified by PCR.

4. EGFR mutation testing guidelines

In patients with advanced non-small cell lung cancer (NSCLC), accurate and accessible epidermal growth factor receptor (EGFR) mutation testing is important to guide treatment decisions, as EGFR tyrosine kinase inhibitors (TKIs) have demonstrated superior efficacy to platinum-based doublet chemotherapy in patients with EGFR mutation-positive advanced NSCLC. In general, it is recommended that all patients with NSCLC and adenocarcinoma histology be tested for EGFR mutations. EGFR mutation testing is not recommended for patients with a confident diagnosis of squamous cell carcinoma lacking any adenocarcinoma component.

In the EU, the European Society for Medical Oncology Clinical Practice Guidelines for metastatic NSCLC recommend that EGFR mutation testing is systematically performed with a validated mutation detection platform in a laboratory participating in an external quality assurance scheme. Choice of methodology will vary but should provide the test sensitivity required for the tumour content of the sample, and provide an adequate coverage of all clinically relevant mutations.

In the US, guidelines from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology outline many recommendations and expert consensus opinions on EGFR testing:

- Testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease.
- Primary tumours or metastatic lesions are equally suitable for testing.
- Laboratories may use any validated EGFR testing method with sufficient performance characteristics.
- Immunohistochemistry for total EGFR is not recommended for selection of EGFR-TKI therapy.
- EGFR copy number analysis (i.e. fluorescence or chromogenic in situ hybridisation) is not recommended for selection of EGFR-TKI therapy.

5. ESTABLISHING ADEQUATE CONTENT FOR TESTING

Building quality into the *EGFR* mutation testing process

To ensure that the epidermal growth factor receptor (*EGFR*) mutation testing process is reliable and consistent, it is essential to build quality assurance into every step of the procedure.

This includes:

- Pathology review to ensure that a sample of sufficient quality and quantity is available for testing.
- Independent quality control of data and reports.
- Validation of new assays.
- Use of good laboratory practice, including control of PCR product contamination.
- An optimal fixative (10% neutral-buffered formalin) and a fixation time as short as possible, yet sufficient to permit diagnosis.
- Use of positive and negative controls.

In the US, information regarding best practice in sample handling and laboratory processes can be obtained through the Clinical Laboratory Standards Institute (CLSI):

- *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline* CLSI document MM13-A. Wayne, PA: CLSI; 2006. (The US Food and Drug Administration has evaluated and recognised this approved-level consensus guideline for use in satisfying a regulatory requirement).
- *Design of Molecular Proficiency Testing/External Quality Assessment; Approved Guideline-Second Edition*. CLSI document MM14-A2. Wayne, PA: CLSI; 2013.
- *Establishing Molecular Testing in Clinical Laboratory Environments; Guideline*. CLSI document MM19-A. Wayne, PA: CLSI; 2011.
- *Quality Management for Molecular Genetic Testing; Approved Guideline*. CLSI document MM20-A. Wayne, PA: CLSI; 2012.

Some laboratories have established networks whose members independently assess each other and share best practices. This helps ensure that diagnostic tests are being performed robustly and that data are

reliable. Any laboratory offering *EGFR* mutation testing should consider joining or establishing such a network. Examples of networks running quality assurance schemes are the European Molecular Genetics Quality Network (EMQN, www.emqn.org), the United Kingdom National External Quality Assessment Service (UKNEQAS, www.ukneqas.org.uk), the European Society of Pathology EQA (ESP, http://lung.eqascheme.org) and the College of American Pathologists (CAP, www.cap.org).

The EMQN promotes quality in molecular genetic testing by establishing, harmonising and disseminating best practices. Assessment schemes run by the EMQN have shown improvements in testing quality through reduced processing (e.g. wrong sample identifier assigned) and genotyping errors, and better report accuracy and clarity.

Various guidelines and consensus papers have been developed that highlight best practices in *EGFR* mutation testing in advanced non-small cell lung cancer (NSCLC), for example:

- The guideline **'Metastatic non-small-cell lung cancer: consensus on pathology and molecular tests, first-line, second-line, and third-line therapy'** was published in the *Annals of Oncology* following the first ESMO Consensus Conference in Lung Cancer in 2010.
- The **'Consensus for *EGFR* mutation testing in non-small cell lung cancer'** was developed following the International Association for the Study of Lung Cancer/European Thoracic Oncology Platform multidisciplinary workshop. It recommends high-quality *EGFR* mutation testing throughout Europe and highlights the importance of co-operation and communication flow among the various disciplines involved.
- The **'Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors'** from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology.

6. EGFR MUTATION DETECTION: AN OVERVIEW

The epidermal growth factor receptor (EGFR) mutation testing process involves a number of different people from different disciplines. Crucial to this testing process is the provision of suitable samples:

Laboratories should follow good laboratory practice, including control of PCR product contamination.

The biopsy site should be selected by the clinician who will perform the biopsy and, in general, the biopsy should be taken from the most easily accessible tumour or metastasis site.

Several well-established biopsy techniques can be used to obtain high-quality tissue samples: needle core biopsy, transbronchial biopsy, endobronchial biopsy, computed tomography-guided needle biopsy, mediastinoscopy, video-assisted, thoracic surgery and thoracotomy.

It is recommended that specimens be formalin-fixed, paraffin-embedded or fresh, frozen, or alcohol-fixed.

The optimal fixative is 10% neutral-buffered formalin and the fixation time should be 6–12 hours for small samples and 8–18 hours for larger surgical specimens.

The cancer cell content and DNA quantity and quality of specimens should be determined, and the minimum proportion and number of cancer cells needed for mutation detection during validation established.

In the EU and China, when considering gefitinib treatment for locally advanced or metastatic non-small cell lung cancer (NSCLC), circulating tumour DNA (ctDNA) obtained from a blood (plasma) sample may be used if a patient's tumour sample is not evaluable.

The EGFR mutation testing process varies among countries and even among hospitals, but EGFR mutation testing typically includes the patient, the treating physician, pathologists and molecular biologists. In some situations, other professionals may also be involved (e.g. bronchoscopists or thoracic surgeons).

The key to accurate and rapid EGFR mutation testing is having a well-defined and coordinated process.

- If lung cancer is suspected, a sample of the tumour is required for initial diagnosis.

- The sample will be preserved and fixed in the pathology lab and then analysed by a pathologist to provide a diagnosis. The pathologist may also mark the area of tumour to assist in downstream testing.
- If a diagnosis of advanced NSCLC is made, the treating physician may request an *EGFR* mutation test. In some centres, this may be done automatically for all patients with advanced NSCLC (also known as reflex testing).
- The sample will be transferred to a pathologist's reference lab or molecular biology lab for *EGFR* mutation testing.
- In the EU and China, when considering gefitinib treatment for locally advanced or metastatic NSCLC, ctDNA obtained from a blood (plasma) sample may be used if a patient's tumour sample is not evaluable.
- The *EGFR* mutation test results will be reported back to the treating physician.

***EGFR* mutation testing kits**

Epidermal growth factor receptor (*EGFR*) mutation testing kits are ready to use and will generate consistent and reliable results, if used according to the kits' instructions. They tend to have been well-tested on clinical samples during the assay validation process.

Please note that turnaround times listed below do not include pre-test steps such as DNA extraction, which are a pre-requisite for all of these tests (with exception of the cobas® EGFR mutation detection kit), or post-test activities such as quality control or report writing.

7. TURNAROUND TIME

EGFR mutation test turnaround time

The turnaround time for epidermal growth factor receptor (*EGFR*) mutation testing is generally defined as the time period between requesting an *EGFR* mutation test and reporting the results back to the treating physician.

It is vital that the turnaround time is as short as possible to ensure that a treatment decision can be made quickly. Ideally this should be no more than seven working days, to ensure that all possible treatment options are available to a patient as soon as possible.

There are many steps involved in determining a patient's *EGFR* mutation status. Small delays at each step can lead to a significant delay in reporting the *EGFR* mutation test results to the physician.

Turnaround time can mean different things

It is important to understand the steps involved in *EGFR* mutation testing in order to identify bottlenecks in the process.

- Treatment decision turnaround time: measured from patient presentation to treatment decision by the treating physician.
- Test turnaround time: measured from test order to test result reported by pathologist to the treating physician.
- *EGFR* mutation analysis method turnaround time: measured from DNA extraction of the sample to result coming off the instrument (does not include report).

Tips for improving turnaround time

- If *EGFR* mutation testing is not routine for all patients with advanced non-small cell lung cancer (NSCLC), then a request to perform the testing should be submitted as soon as possible after diagnosis is confirmed.
- Ensure that the transport of sample between stages is rapid.
- Ensure that all parties involved are aware of their role in streamlining the process.
- Perform the *EGFR* mutation testing within two to four days of receiving the sample rather than waiting and batching samples, where possible.
- Use an *EGFR* mutation detection method that does not take excessive time
- Report data electronically (email, web-based systems) rather than by external post.

8. REPORTING FORMAT

EGFR genotyping report model
MAIN PAGE EN

EGFR MUTATION ANALYSIS in FFPE TISSUE			
PATIENT	**Last Name**: --	**Name**: --	
	Date of birth: ---		**Sex**: M
Reffering doctor: --- Date of request: ---			
Sample registration number: --- Lab. date of registration: ---			
PATHOLOGY DATA			
Tissue : --			
Date of collection : --			
Specimen ID : --			
Sample type : --			
Diagnosis : --			
HE selection : --			
Procentage of Tumoral Area : --			
Procentage of Tumoral Cells : --			
REZULT			
Mutation detected: L858R. Percentage of tumoral area: 25-30%; percentage of tumoral cells: 20%.			
CLINICL SIGNIFICANCE			
The L858R mutation detected on exons 21 confers increased sensitivity to first generation EGFR TKIs.			

Team:

Molecular Diagnostic Department Responsiv: Date:

VERSO EN

DNA EXRACTION

DNA Extraction Kit: *Cobas DNA Sample Preparation Kit, lot: Y01172/31-08-2018*

DNA Evaluation: NanoDrop2000:
DNA Quantity: - - ng/ µl
ADN Quality: -- (A260/A280)

RT- PCR

Procedure:

- RT-PCR: ***Cobas EGFR Mutation Test, lot: Y08114/30-11-2018***
- Instrument: ***Cobas 480 z analyser***
- Following mutations could be detected :
    - Exon 18: G719X(G719A,G719C, and G719S)
    - Exon 19: deletions and complex mutations
    - Exon 20: S768I, T790M, and insertions
    - Exon 21: L858R and L861Q

- Detection limits :

Exon 19 deletions	1.4 -13.4%
S768I	2.4%
L858R	4.0-5.3%
T790M	2.0%
G719X	2.5-5.6%
Exon 20 insertions	1.3%

- Procedure according to **Cobas EGFR Mutation test v2, Doc.Rev.1.0**
- Secvența de referință **NM_005228.3**
- Control negativ: conform
- Control pozitiv: conform

NGS report model

REPORT EN

GENETIC ANALYSIS (NGS) FOR LUNG CANCER

SAMPLE DATA		**PATIENT DATA**
REFERENCE		LAST NAME
SAMPLE TYPE		NAME
ENTRY DATE		BIRTH DATE
DNA EXTRACTION DATE		SEX

INFORMED CONSENT: ASSUMED

STUDY: *NGS SEQUENCING for the following genes:* AKT1, ALK, AXL, BRAF, CALCA, CCND1, CTNNB1, DDR2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, GNAS, HRAS, IDH1, IDH2, KRAS, KRT20, KRT7, MAP2K1, MET, NRAS, NRG1, NTRK1, NTRK2, NTRK3, PIK3CA, PPARG, PTH, RAF1, RET, ROS1, SLC5A5, THADA, TTF1

CLINICAL INDICATION:

BIOLOGICAL BASIS FOR THE STUDY: FusionPlex Comprehensive Thyroid and Lung Kit from ArcherDX, is a targeted NGS panel to detect gene fusions, SNV/indels, splicing and gene expression. This panel is expertly designed to cover relevant exons in 34 genes when used in combination with the VariantPlex CTL Panel. Libraries are created by using the FusionPlex assay in conjunction with the Archer MBC Adapters. This panel complements the Archer VariantPlex® CTL Panel for comprehensive mutation profiling of fusions, CNVs and variants. Studies are performed from RNA extracted from several types of starting sample. Using Archer's proprietary Anchored Multiplex PCR

(AMP™)-based enrichment, fusions of all genes in this kit can be identified in a single sequencing assay, even without prior knowledge of fusion partners or breakpoints. The Archer® FusionPlex® Lung Kit is focused to detect EGFR vIII and MET exon 14 skipping events along with prominent ALK, BRAF, FGFR, NRG1, NTRK, RET, and ROS1 fusions and select point mutations in 14 key gene targets associated with lung cancer.

METHODOLOGY:

1. RNA extraction and purification from FFPE and RT samples
2. Multiplex PCR amplification of regions of interest
3. Sequencing the amplified fragments by NGS
4. Bioinformatic analysis of the obtained DNA fragments by comparison with the reference sequences

TECHNICAL LIMITATIONS:

RESULTS: Results obtained by NextGen sequencing of the genes: AKT1, ALK, AXL, BRAF, CALCA, CCND1, CTNNB1, DDR2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, GNAS, HRAS, IDH1, IDH2, KRAS, KRT20, KRT7, MAP2K1, MET, NRAS, NRG1, NTRK1, NTRK2, NTRK3, PIK3CA, PPARG, PTH, RAF1, RET, ROS1, SLC5A5, THADA, TTF1 from the sample linked to the reference _____ sunt summarized in the following table:

PACIENT (REFERENCE)	GENE	EXON	PATHOGENIC VARIANT (HGVS* SEQUENCE)	PATHOGENIC VARIANT (HGVS PROTEIN)	DATABASE ID	CLINICAL SIGNIFICANCE

* Variants are noted in accordance with the *Human Genome Variation Society (HGVS)* and numbered in relation to the reference sequences.

The variants identified as probably not pathogenic in the international databases with frequencies less than <1% are listed in **Annex I.**

The variants identified as polymorphism in the international databases, with frequencies >1% are listed in **Annex II.**

BIOLOGICAL INTERPRETATION:

CLINICAL INTERPRETATION: The results are consistent with the patient's clinical diagnosis.

NOTE

1. 1. The data of this study are confidential and must be handled with strict privacy criteria.
2. The biological and clinical interpretation of this report has been performed in accordance to the current scientific literature available and international standards.
3. Data storage: the raw data will be destroyed _____ months/years after the delivery of the report. The processed data will be stored for _____ months/years.

ANNEX I: Rare variants, with frequencies <1% described as probably not pathogenic in
The international databases

GENE	EXON	POSITION	NUCLEOTIDE CHANGE	PROTEIN	CLINICAL SIGNIFICANCE	DATABASE ID	REFERENCE

ANNEX II: SNP variants (Single Nucleotide Polymorphism), with frequencies >1%
described as polymorphisms in the international databases

GENE	CROMOSOME	POSITION	REFERENCE GENOTYPE	SAMPLE GENOTYPE	VARIANT ID

9. PATHOLOGIST'S ROLES

Importance of pathology review

Pathology review is required to confirm the diagnosis of advanced non-small cell lung cancer (NSCLC) and to ensure that there is adequate tumour tissue present in the sample for successful epidermal growth factor receptor (*EGFR*) mutation testing.

Before the pathologist reviews the sample to establish a diagnosis, the tumour biopsy is fixed in formalin and embedded in a block of paraffin for storage. The block is cut into sections using a microtome and then stained to allow the morphology of the samples to be assessed under a microscope.

The pathologist will provide a report confirming the underlying histology:

- Small cell lung cancer, NSCLC or other
- Squamous or adenocarcinoma

Once the histological diagnosis and disease stage are established, any additional tests, such as *EGFR* mutation testing, can be performed on the remaining tissue.

Additional steps required for *EGFR* mutation testing

A number of unstained sections are required for *EGFR* mutation testing. These can be cut at the same time as the histology samples to reduce the turnaround time for the *EGFR* mutation testing process.

The pathologist should record information on tissue adequacy. There should ideally be >200 tumour cells present, but successful *EGFR* mutation testing can be performed on fewer tumour cells, provided a sensitive method is used. Information on the percentage of tumour in the sample, and the amount of necrosis and/or sample quality, is also useful for the *EGFR* mutation testing lab for troubleshooting difficult samples. This information should be recorded and passed on with the samples to the testing lab.

For samples with a low percentage of tumour cells, the pathologist should either mark on the slide the area containing the tumour or provide a haematoxylin and eosin (H&E) stained section marked with the area of tumour to be used as a template to identify the tumour-containing regions. This will assist the testing lab to select the tumour area and maximise the chance of a successful test. Histological sections

may be returned to the pathologist on completion of *EGFR* mutation testing.

Tips for the pathologist responsible for sending the biopsy for mutation testing

- Check sample requirements with the molecular pathology lab (e.g. sample type, quantity, number of blocks, sections and any other requirements)
- Check whether the molecular pathology lab requires the H&E (hematoxylin and eosin) stained sections in order to perform macro- or microdissection to enrich for tumour cells
- Mark the area of tumour on the slide if required for macro- or microdissection

10. PERSPECTIVES AND ADDITIONAL RECOMMENDATIONS

Reporting an *EGFR* mutation test result

The detailed structure of clinical laboratory reports deviate by region and institution. Reports should fundamentally contain a unique patient identifier, information on sample adequacy for testing from the pathologist, histopathology results (non-small cell lung cancer [NSCLC]: adenocarcinoma, squamous, large cell, not otherwise specified) from the pathologist and epidermal growth factor receptor (*EGFR*) mutation status (mutation detected, mutation not detected, unknown) and mutation subtype. Reports may also contain associated clinical data and treatment options.

Reports should be prepared by a trained scientist and authorised by a registered scientist to ensure that there are no errors in reporting. They should be returned to the requesting physician or oncologist and, if required, the pathologist. It is important to report the *EGFR* mutation results as soon possible (ideally less than seven working days after receiving the sample) for the benefit of the patient, whose treatment may depend on the *EGFR* mutation results.

EGFR mutation data should be reported using the Human Genome Variation Society Recommendations so that the *EGFR* mutation test results are understood and can be interpreted to allow a treatment decision to be made.

Using *EGFR* mutation status to inform treatment decisions

Approximately 90% of *EGFR* mutations are either exon 19 deletions or L858R point mutations in exon 21, for which there are extensive randomised clinical data to support sensitivity of tumours harbouring these mutations to EGFR-TKI treatment. For example, post-hoc analyses from the IPASS study showed that progression-free survival (PFS) was significantly longer for gefitinib versus carboplatin/paclitaxel in both the exon 19 deletion and the exon 21 L858R mutation subgroups, although a direct comparison between exon 19 deletion and L858R in the gefitinib group was not done. Data from two randomised trials conducted in Japan, which compared gefitinib with platinum-based doublet chemotherapy as first-line treatment for *EGFR* mutation-positive advanced NSCLC, showed that the PFS achieved with gefitinib did not differ significantly between patients with exon 19 deletions and those with L858R point mutations. Additionally, in the **I**RESSA **F**ollow-**U**p **M**easure (IFUM) study, ORR for the exon 19 deletion and L858R subpopulations was 72.5% (95% CI 61.0, 81.6) and 63.6% (95% CI 46.6, 77.8), respectively. In a meta-analysis of randomised controlled trials, EGFR-TKIs were shown to prolong PFS compared with chemotherapy in all patients with *EGFR* mutation-positive advanced NSCLC (63% overall reduction in the risk of disease progression or death (HR 0.37, 95% CI 0.32, 0.42, $p<0.001$). Across all EGFR-TKIs, greater PFS benefit over chemotherapy was observed in those patients with *EGFR* exon 19 deletion mutations than with exon 21 L858R substitution mutation (50% greater benefit, interaction $p<0.001$), suggesting the relative benefits of EGFR-TKIs compared with chemotherapy were greatest in patients with exon 19 deletions.

There are a number of less common mutations (~8% of all *EGFR* mutations) that occur within the *EGFR* gene. These include mutations G719X (G719C, G719S, G719A), L861Q, S768I and so-called double mutations: L858R and exon 19 deletions, L858R and T790M, exon 19 deletions and T790M, etc. There are limited data supporting the use of EGFR-TKIs in patients with these mutations.

In addition, there are a number of rare mutations that have been observed within the *EGFR* gene. These include lone T790M point mutations, exon 20 insertions and other rare mutations, making up ≤2% of all *EGFR* mutations. Some screening methodologies such as

sequencing may identify novel *EGFR* mutations for which there will be no clinical or pre-clinical data to guide use of EGFR-TKIs. Based on the available information from randomised clinical trials and the extremely low number of patients in which they have been observed, there are currently no data supporting the use of EGFR-TKIs in patients whose tumours harbour these mutations.

11.CONCLUSION

Recent advances in molecular pathology and targeted therapies have opened a new era of personalized medicine for lung cancer treatment. Driver genetic alterations such as epidermal growth factor receptor (*EGFR*) mutations, as well as Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and anaplastic lymphoma kinase (*ALK*) rearrangements, have been identified and are currently used as predictive biomarkers for targeted therapies. Activating somatic mutations in the *EGFR* gene are known to be major driver mutations in that they exhibit a high incidence in lung cancers and have played an important role in the development of targeted molecular therapies for lung cancer.

Rapid and accurate *EGFR* mutation testing is essential for patient selection and establishing targeted therapies with EGFR TKIs. Thus, a standard set of guideline recommendations for *EGFR* mutation testing is necessary.

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Institutul Regional de Oncologie Iasi IRO

www.ro-md.net

A project implemented by
Regional Institute of Oncology Iași
Adress: Str. G-ral Henri Mathias Berthelot nr. 2-4
Phone: +40374278810
Fax: +40374278802

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"The European Union is made up of 28 Member States who have decided to gradually link together their knowhow, resources and destinies. Together, during a period of enlarge- ment of 50 years, they have built a zone of stability, democra- cy and sustainable development whilst maintaining cultural diversity, tolerance and individual freedoms. The European Union is committed to sharing its achievements and its values with countries and peoples beyond its borders".