Horizon Scanning report No. 4

Diagnostic tests for the identification of EGFR mutations in Non-Small Cell Lung Cancer (NSCLC) patients to be treated with tyrosine kinase inhibitors

April 2010

Methods

Agenas is a public body. Its mission is to promote innovations and developments within the Italian national healthcare service and develop a Horizon Scanning (HS) function in the field of healthcare technologies. A full description of the methods used for the production of the present HS report can be found at www.agenas.it

This document should be cited as follow:
Perrini MR, Migliore A, Corio M, Ferroni E, Jefferson T, Cerbo M. Diagnostic tests for the identification of EGFR mutations in NSCLC patients to be treated with tyrosine kinase inhibitors. Agenas, National Agency for Regional Healthcare. Rome, April 2010.

For further information contained in this report please contact:
Agenas – Agenzia nazionale per i servizi sanitari regionali
Sezione Iss – Innovazione, sperimentazione e sviluppo
Via Puglie, 23 - 00187 Roma
e-mail: hta@agenas.it

Limitations

This report is based on information available when the searches were made and does not contain data on subsequent developments or improvements of the evaluated technology. The observations made on effectiveness, safety or cost-effectiveness of the technology evaluated in the report are to be considered temporary.

Authors

This HS report was prepared by:

Maria Rosaria Perrini

Antonio Migliore

Mirella Corio

Eliana Ferroni

Tom Jefferson

Marina Cerbo

Declaration of Conflict of Interest

The authors declare that they will not receive either benefits or harms from the publication of this report. None of the authors have or have held shares, consultancies or personal relationships with any of the producers of the devices assessed in this document.

Acknowledgements

Prof. Luigi Portalone (Head of the Second Pneumological Oncology Department, S. Camillo-Forlanini Hospital, Rome), Dr. Alvaro Leone (Pathological Anatomy Department, S. Camillo-Forlanini Hospital, Rome), Dr. Marco Ratti (Health Economics and Outcome Research Manager, Boehringer Ingelheim Italia S.p.A., Milan).

Name of the technology/procedure: **Diagnostic tests for the identification of EGFR mutations in NSCLC patients to be treated with tyrosine kinase inhibitors**

Target population

The technology aims to identify mutations in the EGFR (epidermal growth factor receptor) gene in patients with Non-Small Cell Lung Cancer (NSCLC), to select those suitable for treatment with Tyrosine-Kinase inhibitors (TKIs).

Description of the procedure and technology

In oncology, genetic testing procedures are performed to detect gene mutations in a sample of DNA extracted from the patient's tumour [www.eurogentest.org]. Diagnostic kits based on real-time PCR (polymerase chain reaction) technology are among the methods used for this purpose. Generally, a specimen of the patient's tumour is obtained by biopsy and then processed to extract the DNA, according to specific protocols depending on the type of the diagnostic kit used. The genomic material is treated in a real-time PCR procedure and the mutations are analysed by reading the curves (i.e. comparing the mutant DNA with the normal one).
This report is focused on diagnostic kits, based on real-time PCR technology, able to detect EGFR gene mutations in NSCLC patients to select those eligible for treatment with TKIs.

Clinical importance and burden of disease

Lung cancer is the primary cause of death in industrialised countries. Recently in Italy, the epidemiology of this pathology significantly changed, principally due to smoking habits changes which present different gender-based characteristics. After a historic increasing trend, incidence and mortality rates are now decreasing among males. This situation appears different for the female population, where incidence and mortality rates are still rising [AIRT Working group]. Data on lung cancer epidemiology can be obtained from the Cancer Register which, to date, only covers 32% of the Italian population. Data on all kinds of cancer are collected by any single accredited register and then recorded into the AIRTUM database, which represents the national archive hosted by the Italian National Health Institute (ISS) [www.tumori.net].
According to the 2010 estimates made by the Cancer Epidemiology department of the CNESPS (National Center for Epidemiology, Surveillance and Health Promotion), in the Italian population in

the age group 0-84 years, there will be 23,969 new cases of “lung cancer” (ICD-9-CM 162) in males and 7,082 in females. The prevalence estimate is 78,515 cases (62,463 males and 16,052 females). Mortality rates are based on data collected in 2008, when 20,599 males and 5,612 females died from lung cancer.
From a clinical point of view, a distinction is usually made between small cell lung cancer (microcytoma) and non-small cell lung cancer (which includes squamous cell carcinoma, adenocarcinoma and large cell carcinoma). Table 1 indicates the frequency percentages by histological type by gender for 2008 [Italian Journal of Health Technology Assessment, 2008].

Table 1: Lung cancer in Italy by histological type and gender. Year 2008

	Males	Females
Adenocarcinoma NSCLC	33.9 %	46.1 %
Squamous NSCLC	28.5 %	16.0 %
Large cell NSCLC	2.6 %	2.9 %
SCLC (microcytomas)	8.8 %	11.0 %
Not specified	26.2 %	24.0 %
	100.0 %	100.0 %

NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer.

Source: AIRTUM data

The importance of this histological distinction is linked to the different treatment as unlike microcytomas, NSCLCs show low sensitivity to chemotherapy [Non-Small Cell Lung Cancer Collaborative Group, 2000]. For the latter new therapies with biological drugs have been implementing. These substances work in a highly selective way on neoplastic cell regulatory mechanisms. In particular, therapies against EGFR (epidermal growth factor receptor involved in cell proliferation) have already been used [www.cancer.gov]. In NSCLC the expression modes of the EGFR gene are correlated to unfavourable prognosis, a greater capacity for metastatisation and reduced survival [Hirsch FR, 2003]. In NSCLC the principal studies with anti-EGFR drugs prevalently used TKIs and demonstrated net improvements in symptoms and quality of life both as first-line treatment and in patients previously treated with other therapies [Sanford M, 2009]. Further, sub-groups of patients who were particularly sensitive to treatment with these inhibitors were identified: women, Asian, non smokers, and with adenocarcinoma histo-type [Yang CH, 2006; Jiang H, 2009].
Patients with mutated variants of the EGFR gene show greater sensitivity to TKIs. EGFR gene mutations associated with a response to TKIs have been found in some 10-20% of NSCLC patients. The most common mutations of the kinase dominion of the EGFR gene are the Exon 19 deletions and the mutation L858R in Exon 21, which constitute approximately 90% of all EGFR gene mutations [Yu J, 2009]. The remaining 10% are rare missense mutations found mainly in Exon 18 but also in Exons 20 and 21 [Shigematsu H, 2005].
Data currently available indicate the high value of identifying EGFR gene mutations in patients with NSCLC, to select those sensitive to the treatment with TKIs. This technology may support the development of a “personalised medicine”, which represents one of the greatest challenges for the

decades to come. These specific diagnostic technologies would enable the definition of the molecular profile of the pathology (in this case NSCLC) and would provide useful information for prognostic and prediction purposes. In this approach, patient sub-groups, sharing biological or genetic characteristics, may be treated with therapies suitable for their specific condition, as currently happens in patients with breast cancer where HER2 mutation was detected. [Jørgensen JT, 2008; Gianni L, 2010].

Products, manufacturers, distributors and approval

The device identified is the TheraScreen® EGFR29 kit, produced by DxS Ltd and consisting of a series of reagents which enable the analysis of a number of bioptic specimens (20 or 80 depending on the kit format). According to the manufacturers' instructions, in a procedure lasting less than 3 hours, the device can identify 29 EGFR gene mutations relative to Exons 18, 19, 20 and 21. These mutations seem to be correlated to the efficacy of or resistance to treatment with TKI [Sanford M, 2009].

The kit obtained CE marking as an in vitro diagnostic in April 2007 for use on the MX3000 Real-Time PCR system (produced by Stratagene Inc.) but is also compatible with all the other real-time PCR systems capable of running the DxS Ltd protocol. However the manufacturer does not guarantee the same performance when used on real-time PCR systems other than that indicated. The FDA approval application procedure is expected in 2011.

As indicated by the manufacturer (DxS Ltd) *"Its intended use is as an adjunct to other prognostic factors currently used to select suitable patients for treatment with tyrosine kinase inhibitor therapies"*. In particular, given the evidence, it is important to take into consideration if the patients belong to one of the following sub-groups: Asian origin, non smokers, women and those with adenocarcinoma histo-type [Yang CH, 2006; Jiang H, 2009].

Following the acquisition of DxS Ltd by Qiagen S.p.A. the kit will be distributed directly in Italy by Qiagen Italia from July 2010; in the past it could be purchased exclusively from the manufacturers DxS Ltd (based in the UK, with no Italian distributor).

Manufacturers	Distributors	CE Mark	RDM	FDA
DxS Ltd.	Qiagen S.p.A.	☑	□*	□

* Not applicable as the Health Ministry RDM (Repertory of Medical Devices) does not collect in vitro diagnostics.

Setting

This genetic test can be performed by trained personnel at any Pathological Anatomy laboratory equipped with real-time PCR.

□ Home	☑ Hospital	□ Out Patients
□ Accident and Emergency	☑ Other: Pathological Anatomy laboratory	

Roll out in Italy

Distribution of the TheraScreen® EGFR29 kit in Italy is expected to start in July 2010. To date the device is used only in 3 centres (two hospitals and one laboratory) where it has been purchased directly from the manufacturer. The usage volumes, that is the number of tests purchased or executed, are not known.

□ Pre-marketing	□ On the market for 1-6 months	□ On the market for 7-12 months
☑ On the market for more than 12 months	□ Not identified	

Comparators

EGFR gene mutations are commonly detected by direct DNA sequencing which is considered the methodology of reference (gold standard). However, direct DNA sequencing requires high quality bioptic specimen, with significant costs also in terms of time and cannot be used for non surgical samples (i.e. serum, plasma or cytological preparations). These limitations make its use rather unfeasible in the clinical practice [Fassina A, 2009].
Other molecular diagnostics methods, when used to characterise the specimen, do not detect the specific mutations, though they enable observations attributable thereto. For example, immunohistochemistry (IHC) does not detect the mutations but detects the expression of EGFR protein; FISH (fluorescent in situ hybridization analysis) does not detect the mutations but rather the number of copies of the EGFR gene DNA; DHPLC (denaturing high-performance liquid chromatography) enables detection of the mutations, of the micro-deletions and micro-insertions, yet does not enable their characterisation [Eberhard DA, 2008].

Effectiveness and safety

We searched the EuroScan database (27 January 2010) to identify *Horizon Scanning* reports and *Rapid Health Technology Assessments*, published in English, on the TheraScreen diagnostics kit for identification of EGFR gene mutation in NSCLC patients for treatment with TKI. The search only found reports on the effects deriving from the administration of a specific drug after detection of a certain mutation.

The literature search was undertaken in three database Embase (15 February 2010), Medline (25 January 2010) and the Cochrane Library (5 February 2010), to identify studies about the technology, published from 2007 to date in English and Italian. The search did not find studies which assessed the use of the specific TheraScreen EGFR29® Diagnostic kit. We identified only one study [Mok TS, 2009] in which this test was used to address Asian patients with advanced pulmonary adenocarcinoma, non or ex smokers (subjects who had quit smoking for at least 15 years and who smoked less than 10 packets per year) to first-line treatment with TKIs.
As this is an emerging technology, an analysis of "grey literature" was also undertaken (registers, presentations, posters, etc.). Only one poster was identified relative to a study, conducted in 2009, regarding a comparison between direct DNA sequencing and the TheraScreen EGFR29® kit, on 96 tumours (in NSCLC patients) [Angulo B, 2009]. The study compared only 88 tumours because insufficient DNA collected from 5 tumours impeded execution of both procedures, while a further 3 tumours were not analysed due to poor DNA quality. In the 88 tumours, both procedures confirmed the same results, i.e. they found 10 mutated tumours and 78 wild type tumours. According to the authors, the TheraScreen EGFR29® kit allowed to detect also 1% of mutant DNA in the specimen, while direct sequencing required at least 10%. This conclusions were, however, reached comparing the results of the 10 mutated specimens. Given the nature of the sources and the low number of specimens observed, the conclusions may be considered of limited general application.
There are no significant concerns on the safety of the technology: TheraScreen EGFR29® is a non directly invasive diagnostic test.

Potential benefits to patients

This technology would enable definition of the molecular profile of the pathology (NSCLC) and thus would provide information useful to both the prognostic and the therapeutic profiles, enabling appropriate selection of the patients most responsive to the treatment with TKIs.

☐ Mortality reduction or increased survival	☐ Reduction of the morbidity	☐ Improved quality of life (patient/users)
☐ Improved patient monitoring	☑ Other: Improved appropriateness of treatment	☐ Not identified

Cost of the technology / procedure

At the moment the technology is available at an indicative price of € 160.00 (price before VAT declared by the manufacturer) referable to the analysis of a single specimen. The price of the kit in the two formats (20 and 80 tests) is not available at this time.
The test requires the availability of ordinary equipment for real-time PCR. This equipment can be

used for many other analyses (molecular biology) but is not usually available as normal equipment of Pathological Anatomy laboratories.
In laboratories already using real-time PCR, the technology does not require dedicated and specifically trained personnel (no additional training costs are foreseen).

☐ Increased costs compared to alternative treatments	☐ Increased costs due to increased demand	☐ Increased costs due to the required investments
☑ New costs	☐ Other:	

Potential structural and organisational impact

Structural impact

The technology has no significant structural impact when used in a Pathological Anatomy laboratory fitted with equipment for real-time PCR.

☐ Increase in requirement of instruments	☐ Always be used	☑ Can be used only under specific circumstances
☐ Decrease in requirement of instruments	☐ Other:	☐ Not identified

Organisational impact

The technology impacts the workflow at Pathological Anatomy laboratory where it tends to substitute mutation analyses by direct sequencing (relative to EGFR analysis in NSCLC samples). Further, personnel with specific training in the molecular biology fields is required.

☐ Increase in the number of procedures	☑ Re-organisation required	☐ Training required for users
☐ Reduction in the number of procedures	☐ Other:	☐ Not identified

Conclusions

Diagnostic tests for genetic mutations based on real-time PCR technology are an emerging technology in oncology. By contrast with direct DNA sequencing methods, these may also be executed on biological specimens with a low number of cells (e.g. needle aspiration biopsy), and present a potentially significant impact in terms of integration in clinical-hospital work flows (due to low complexity and reduced times). However these tests enable only the detection of specific mutations, already identified and described. In certain cases, this limit may have little relevance. In the specific case of NSCLC, the principal EGFR gene mutations have been identified and affect Exons 19 and 21, which together account for some 90% of the mutations discovered and Exons 18 and 20 [Yu J, 2009].

As patients with these mutations showed a greater sensitivity to TKIs drugs [Sanford M, 2009], their identification before choosing the treatment could only generate positive effects in terms of appropriateness of treatment.

The technology enables the identification of 29 EGFR gene mutations relative to Exons 18, 19, 20 and 21 in specimens taken from NSCLC patients and may have a positive impact on clinical oncology practice. However this potential may be considered expressed only performing comparative studies to evaluate the diagnostic efficacy of the new technology with respect to the technologies employed today. At now the sole source of evidence is represented by a poster presentation which cannot be considered sufficient [Angulo B, 2009].

Particular attention should be paid to the analysis of the context of use of the new technology. The structural and organisational impact on Pathological Anatomy laboratory already using real-time PCR would be minimal while it could be fairly significant otherwise, as the molecular biology skills and the equipment required are not present in all Pathological Anatomy laboratories.

Future prospects

- **Population:** increasingly frequent use of the test can be hypothesised to detect EGFR gene mutations in oncology patients for the identification of candidate patients for concomitant treatment with other drugs.
- **Intervention:** during 2010 there will be an update of the diagnostic kit which should provide better compatibility with other real-time PCR systems (further to that indicated by the manufacturer).
- **Comparators:** diagnostic procedures which are intended to detect EGFR gene mutations in blood samples are at the study phase.
- **Outcome:** clinical validation of this technology to identify EGFR gene mutations will allow greater appropriateness with consequent improvement in the definition of therapy for the single patient.

Evidence searches

Searches of the databases were executed using the following key words to indicate:

- ***the technology of interest:*** TheraScreen® EGFR29, EGFR29 Mutation Kit, mutation detection, DxS mutation test, “amplification refractory mutation system” OR ARMS, “Scorpion real-time PCR”;
- ***the pathology of reference:*** NSCLC, Non-Small Cell Lung Cancer, squamous cell lung carcinoma, large cell lung carcinoma, Bronchioloalveolar carcinoma, “non small cell lung carcinoma”, “lung cancer”, “lung adenocarcinoma”;
- ***the typology of the genetic mutations which the TheraScreen EGFR29 kit is used to detect:*** EGFR mutations (EGFR/HER2), epidermal growth factor receptor mutations, EGFR aberrations OR epidermal growth factor receptor aberrations, EGFR amplification OR epidermal growth factor receptor amplification, EGFR expression OR epidermal growth factor receptor expression, EGFR status OR epidermal growth factor receptor status, EGFR copy number changes OR epidermal growth factor receptor copy number changes.

Bibliography

AIRTUM. I tumori in Italia, rapporto 2009. Associazione Italiana dei Registri Tumori.

AIRT Working group. I tumori in Italia – rapporto 2006. Incidenza, mortalità e stime. Epidemiologia & Prevenzione 2006; 30 (Suppl. 1): 64-65.

Angulo B, Conde E, Martinez R, et al. Comparison of Direct Sequencing and a Commercial Real-Time PCR Kit for Detection of Mutations in EGFR Gene. Poster presented at the 13th World Conference on Lung Cancer, San Francisco, 2009.

Eberhard DA, Giaccone G, Johnson BE. Biomarkers of Response to Epidermal Growth Factor Receptor Inhibitors in Non–Small-Cell Lung Cancer Working Group: standardization for use in the clinical trial setting. J Clin Oncol 2008; 26 (6): 983-993.

Fassina A, Gazziero A, Zardo D, et al. Detection of EGFR and KRAS mutations on transthoracic needle aspiration of lung nodules by high resolution melting analysis. J Clin Pathol 2009 62: 1096-1102.

Gianni L, Eiermann W, Semiglazov V et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet. 2010 Jan 30;375(9712):377-384.

Giornale Italiano di Health Technology Assessment 2008; 1(1): 15-20.

Hirsch FR, Varella-Garcia M, Bunn PA, et al. Epidermal Growth Factor Receptor in Non–Small-Cell Lung Carcinomas: Correlation Between Gene Copy Number and Protein Expression and Impact on Prognosis Journal of Clinical Oncology, Vol 21, Issue 20 (October), 2003: 3798-3807.

Jiang H. Overview of gefitinib in non-small cell lung cancer: an Asian perspective Jpn J Clin Oncol. 2009 Mar;39(3):137-50.

Johnson DH. Targeted therapy in non-small cell lung cancer: myth or reality. Lung Cancer. 2003 Aug;41 Suppl 1:S3-8.

Jørgensen JT. From blockbuster medicine to personalized medicine. Personalized medicine. 2008, Vol. 5, No. 1, Pages 55-63.

Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009 Sep 3;361(10):947-57.

Non-small Cell Lung Cancer Collaborative Group. Chemotherapy for non-small cell lung cancer. Cochrane Database Syst Rev 2000;(2):CD002139.

Sanford M, Scott LJ. Gefitinib: a review of its use in the treatment of locally advanced/metastatic non-small cell lung cancer. Drugs. 2009 Nov 12;69(16):2303-28.

Shigematsu H, Lin L, Takahashi T, et al. Clinical and Biological Features Associated With Epidermal Growth Factor Receptor Gene Mutations in Lung Cancers. J Natl Cancer Inst 2005;97:339 – 46.

Yang CH, Shih JY, Chen KC, et al. Survival outcome and predictors of gefitinib antitumor activity in East Asian chemonaive patients with advanced non small cell lung cancer. Cancer. 2006 Oct 15;107(8):1873-82.

Yu J, Kane S, Wu J, et al. Mutation–specific antibodies for the detection of EGFR mutations in non-small-cell lung cancer. Clinical Cancer Research. 2009 May 1;15(9):3023-8.

www.cancer.gov National Cancer Institute - Dictionary of Cancer Terms (last access 11 February 2010).

www.eurogentest.org Definitions of Genetic Testing (Sequeiros J, Guimarães B) EuroGentest Network of Excellence Project.

http://www.eurogentest.org/patient/public_health/info/public/unit3/DefinitionsGeneticTesting-3rdDraf18Jan07.xhtml. (last access 2 February 2010).

Glossary

AIRTUM: Italian Association of Cancer Registers (www.registri-tumori.it/cms/)

HER2-positive breast cancer: particularly aggressive form of breast cancer distinguished by rapid growth and high probability of relapse.

Kinase Domain: intracellular portion of the protein of enzymatic type with activity connected with the protein kinase.

EGFR: *Epidermal Growth Factor Receptor*, epidermal growth factor receptor involved with cellular proliferation; also denominated HER1 or ErbB1.

FDA: *Food and Drug Administration*.

FISH: *Fluorescent In Situ Hybridization analysis*, analysis of fluorescent hybridisation *in situ*; a cytogenetic technique which can be used to detect and locate the presence or absence of specific DNA sequences in chromosomes.

IHC: Immunohistochemistry, method which permits the highlighting of determined substances in a tissue sample by use of antigen-antibody reactions and enzymatic or fluorescent methods.

Tyrosine-Kinase inhibitors: drugs whose action blocks the activity of Tyrosine-Kinase receptors. These molecules are able to inhibit the binding of ATP (adenosine triphosphate) in the cytoplasmatic region of the EGFR thus blocking all the consequent reactions.

ISS: Italian National Health Institute.

Istat: Italian National Institute of Statistics.

Histo-type: type of cells present in a determined tissue.

Micro-deletion: a type of cytogenetic anomaly, or chromosome mutation, consisting of the absence of a tract of a chromosome, with consequent loss of genetic material.

Micro-insertion: a type of cytogenetic anomaly, or chromosome mutation, consisting of the insertion of a tract of a chromosome, with consequent loss of genetic material.

Missense mutation: also called “nonsense” mutations, these determine the formation of a stop tail within a sequence.

NSCLC: Non-Small Cell Lung Cancer; one of the 4 principal histological types of lung cancer.

RDM: Medical device Repertory (http://www.salute.gov.it/dispositivi/paginainternasf.jsp?id=499&menu=repertorio).

Real-time PCR: also known as quantitative PCR or quantitative real time PCR (qrt-PCR), a

method of amplification (polymerase chain or PCR) and simultaneous quantification of DNA.

SCLC: Small Cell Lung Cancer; one of the 4 principal histological types of lung cancer.

First-line treatment: recommended medical therapy for initial treatment of a disease, indication or symptom.

Wild-type tumour: Tumour with “non mutated” phenotype or genotype; in general wild-type indicates the most common phenotype (or genotype) in nature.