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Implementation of a companion diagnostic in the clinical laboratory: The *BRAF* example in melanoma

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ABSTRACT

A companion diagnostic test provides information that is essential for the safe and effective use of a corresponding therapeutic product as indicated in the drug instructions. The implementation of a companion diagnostic follows the rules of a molecular test for somatic mutations in a routine clinical laboratory environment and needs guidance on practical aspects, including the choice of the proper analytical method and the procedures for internal and external quality controls. Selection of the appropriate assay for detection of genetic alterations depends on several factors: the type of mutation under study, the sample to be assayed and its preparation procedure. In addition, the results of a molecular assay require a complex interpretation process of the analytical data as the patient's genotype, the translation of the identified variant into a predicted phenotype and knowledge on restrictions of the method used. In relation to these aspects herein we report an opinion paper of the Working Group Personalized Laboratory Medicine jointly constituted by the European Federation of Laboratory Medicine (EFLM) and by the European Society of Pharmacogenomics and Theranostics (ESPT) using, as an example, the BRAF genotype analysis in tumor tissue samples for identification of melanoma patients that can benefit treatment with BRAF inhibitors. The manuscript is focused on the following aspects: i) medical rationale, ii) methodologies of analysis, iii) laboratory performance evaluation and iv) the laboratory specific report for the clinicians. The critical evaluation of these aspects would be useful for the implementation of a companion diagnostic in the clinical laboratory.

1. Introduction

Over the past decade, nucleic acid testing for infectious diseases, human genetics and molecular oncology has grown rapidly. Although molecular testing was recently offered exclusively by specialized reference laboratories possessing appropriate resources and technical expertise, new instruments for sequencing and IVD-labeled assays allow routine clinical laboratories to offer molecular analysis without large investments in research and development. As these technologies will further develop in the future, it can be expected that molecular diagnostic techniques will become easier and more applicable in a routine clinical laboratory.

Abbreviations: IVD, in vitro diagnostics; BRAF, V-RAF murine sarcoma viral oncogene homolog B1; SSCP, single-stranded conformation polymorphism analysis; dHPLC, denaturing high performance liquid chromatography; HRMA, high resolution melting analysis; LDTs, Laboratory Developed Tests; AS real-time PCR, allele-specific real-time polymerase chain reaction; FFPE, formalin-fixed paraffin-embedded; NGS, next-generation sequencing; MALDI TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NRAS, neuroblastoma RAS viral oncogene homolog; KIT, V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; EQA, external quality assessment.

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However, the implementation of molecular diagnostic tests in a routine clinical laboratory needs guidance on several practical aspects during set-up including the choice of the proper analytical method and the procedures for internal and external quality controls. The results of molecular assay require a complex interpretation process of the analytical data based on several factors as the patient's genotype, the translation of the identified variant into a predicted phenotype and knowledge on limitations of the assay method used in addition to specific skills on the disease area (infectious diseases, molecular genetics and molecular oncology).

The approach to new molecular assays in a routine clinical laboratory environment requires expert laboratory specialists able to advise clinicians to select the appropriate biological specimen, to request a suitable test, to evaluate the performance of the pre-analytical and analytical phases and to generate clinically useful and patient-specific reports including the availability of consulting.

Several publications and reviews on molecular genetic and genomic tests produced by international organizations, such as the Agency for Health Quality Research (AHRQ) (www.ahrq.gov), the Cochrane Collaboration (www.cochrane.org/reviews), the Clinical Laboratory Standards Institute (www.clsi.org) and the Organisation for Economic Co-operation and Development (www.oecd.org/science/biotech/) or developed from activities of European projects such as EuroGentest (www.eurogentest.org/) are available. In particular the recent CLSI MM19 document *Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline* [1] details all these aspects.

Whereas we suggest referring to the above-mentioned documents in the case of planning the incorporation of molecular diagnostics in a clinical laboratory, the implementation itself poses a number of challenges to which no readily available answer can be found in these documents including the choice of analytical method and the procedure for internal and external quality controls. Here we report the experience of the Clinical Biochemistry Unit of the University of Florence in performing molecular testing for *BRAF* somatic mutations in melanoma patients. This test belongs to the area of the so called "companion diagnostics" whereby molecular tests that identify specific mutations are used to provide a specific therapy for the condition of an individual. An up-to-date list of companion diagnostic devices linked to a specific drug and approved by Food and Drug Administration (FDA) can be consulted at the following link:http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm.

The approach used at the University of Florence has been discussed within the joint Working Group Personalized Laboratory Medicine (WG-PLM) nominated by the European Federation of Laboratory Medicine (EFLM, http://www.efcclm.eu/) and by the European Society of Pharmacogenomics and Theranostics (ESPT, http://www.esptnet.eu/) to produce an opinion paper focused on the following aspects: i) medical rationale, ii) methodologies of analysis, iii) laboratory performance evaluation and iv) the laboratory's specific report for the clinicians.

2. BRAF: medical rationale

BRAF mutations and BRAF-inhibitors are an emblematic example of companion diagnostics with improved clinical response and survival in metastatic melanoma (MM) patients.

Belonging to RAF family, the *BRAF* gene encodes for a serine–threonine protein kinase [2] and its mutations account for approximately 50% of all the genetic alterations in primary cutaneous melanoma [3–5]. About 90% of all the clinically relevant mutations affect exon 15 and they arise as a single-point mutation at position 1799 (thymine to adenine) that converts valine to glutamic acid at 600 position of the amino acid sequence [6–8].

Among the remaining *BRAF* mutations, the most common one involves the variations of two adjacent nucleotides and it is identified as c.1798_1799delinsAA (p.Val600Lys).

Other mutations affecting the same region, such as c.1798_1799delinsAG p.Val600Arg, c.1801A>G p.Lys601Glu and c.1799_1800delinsAA p.Val600Glu are extremely rare (COSMIC, *Catalogue of Somatic Mutations in Cancer Database*, http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/).

The identification of such a frequent mutation in kinase has provided a hub for the development of a new inhibitor molecule that targets toward the mutated *BRAF* gene product and thus affecting only cancer cells by suppression of essential tumor-growth pathways.

Vemurafenib (RG7204/PLX4032), identified by Plexxikon Inc. (Berkeley, CA, USA) is a first-in-class selective inhibitor of BRAF. The effectiveness of Vemurafenib in comparison to the traditional treatment with dacarbazine has been established by the results of several clinical trials, in particular by the BRIM-3 (BRAF inhibitor in melanoma-3) trial performed on 675 MM patients affected by a BRAF-mutated tumor [9].

On August 2011, the drug Zelboraf (Vemurafenib) was approved by the Food and Drug Administration as "*a drug to treat patients with late-stage (metastatic) or unresectable (cannot be removed by surgery) melanoma*". In the label, it is also clearly specified that the drug is "*for the treatment of patients with melanoma whose tumors express a gene mutation called BRAF V600E*".

On 15 December 2011 European Medicines Agency and the Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion, recommending the granting of a marketing authorization for the medicinal product Zelboraf for the treatment of adult patients with BRAF V600 mutation-positive unresectable or metastatic melanoma.

However, the recurrence of side effects and relapse using BRAF inhibitors is an important aspect to be considered [10] and the mechanisms surrounding the resistance should be better defined and investigated especially in the context of the targeted therapy [11].

3. *BRAF*: methods for mutation analysis

Selection of the appropriate assay for the detection of genetic alterations depends on several factors: firstly the type of mutation under study, the kind of sample to be assayed and then the sample preparation procedure.

In somatic mutations molecular analysis procedures, the contribution of the pathologist is fundamental to evaluate the fraction of neoplastic cells into the sample as this can influence the choice of the analytical method. As a matter of fact, the most important aspect to be considered is the large excess of wild type DNA, when dealing with the detection of rare mutated DNA molecules in tumor samples. Therefore, techniques with a defined specificity and sensitivity are required to detect "mutant genomes" in a background of wild-type DNA.

Each method has its own characteristics in regard to sensitivity, specificity, coverage (i.e. spectrum of identifiable variants), cost and turnaround time. An overview of the main methods used to date in a routine clinical laboratory to assess mutation status of cancer samples is briefly reported in the following paragraphs (see also Table 1).

3.1. Methods of pre-screening analysis

Pre-screening analysis is a heterogeneous group of methods based on different intrinsic features of the target sequence. The most currently used techniques are the single-stranded conformation polymorphism analysis (SSCP) [12], the denaturing high performance liquid chromatography (dHPLC) [13] and the high resolution melting analysis (HRMA) [14].

Since these techniques are Laboratory Developed Tests (LDTs), they should be used only by expert personnel and after a validation procedure that has demonstrated reliability of the analytical performances. An extensive optimization may be required. Methodological aspects that should be taken into account are listed in Table 1.