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Introduction

The Italian Association of Medical Oncology and the Italian Society of Anatomic Pathology and Diagnostic Cytopathology organized an external quality assessment (EQA) scheme for anaplastic lymphoma kinase (ALK) rearrangement by florescence in situ hybridization (FISH) analysis in non–small-cell lung cancer (NSCLC).

Methods

Sections from tissue microarrays, each including 10 NSCLC samples with known ALK status, were first validated in five referral laboratories and then provided to 37 participating centers. The laboratories were requested to perform the FISH test, using their usual protocols, and to complete the analysis within 3 weeks. By using a predefined scoring system, two points were assigned in case of correct genotype and zero points to false-negative or false-positive results. The threshold value to pass the EQA scheme was set at 18 points. Two rounds were planned.

Results

Thirty-four centers submitted the results within the established deadline. Several errors in the evaluation of genotype (*n* = 18) were reported, with both false-positive (*n* = 7) and false-negative (*n* = 11) results. Test failure occurred in seven cases. Two samples were found to be critical by two referral laboratories and seven participating centers. Twenty-six (70%) laboratories passed the first round and six the second round. Overall, 32 (86%) laboratories passed the ALK EQA scheme.

Conclusions

The results of this first EQA scheme for ALK testing in NSCLC cancer patients indicate that ALK analysis is performed with adequate quality in most Italian laboratories and highlight the importance of EQA in revealing methodological problems that need to be addressed to further increase the reproducibility of molecular tests.

Key Words

- Non–small-cell lung cancer;
- ALK rearrangement;
- FISH;
- Quality assessment

Genetic aberrations that drive human malignancies, known as driver mutations, can be used as therapeutic targets for specific drugs. In patients with non–small-cell lung cancer (NSCLC) and epidermal growth factor receptor (EGFR)-sensitizing mutations, EGFR tyrosine kinase inhibitors such as gefitinib or erlotinib induced dramatic tumor responses and improved survival.[1, 2 and 3]

More recently, similarly remarkable outcomes have been reported in lung cancer patients with ALK gene rearrangement who underwent a crizotinib-based treatment (PF-02341066 Pfizer).[4]

Chromosomic rearrangements involving the anaplastic lymphoma kinase (ALK) gene were first identified as oncogenic events in anaplastic large-cell lymphomas in 1994.[5] More recently, an inversion event on the short arm of chromosome 2, resulting in the fusion of ALK gene with the EML4 gene locus, was identified as the most common ALK aberration in NSCLC patients. The chimeric protein (EML4-ALK) resulting from this rearrangement confers a strong proliferative stimulus to the neoplastic cells.[6,7] More than 10 EML4-ALK fusion variants with transforming activity in vitro have so far been identified in lung cancer patients.[8,9] Other fusion patterns including tyrosine receptor kinase (TRK)-fused gene and kinesin family member 5B (KIF5B)[10] have been reported, but clinical data for patients harboring these variants are very limited.

In unselected patients with NSCLC, the prevalence of ALK rearrangement ranges from 1% to 7%,[6] depending on the population studied and the ALK detection methods used. However, a prevalence of more than 30% has been observed in patients with EGFR and KRAS wild-type, adenocarcinoma histology and absent or light smoking history.[11] This is consistent with the notion that ALK rearrangement defines a unique molecular subset of NSCLC, with distinct clinical and pathologic characteristics. Indeed, patients who most likely harbor EML4-ALK translocation tend to be younger, never/light smokers with lung adenocarcinoma.

Phase 1 and 2 clinical studies have shown that crizotinib is active in patients with advanced, ALK-positive NSCLC.[12, 13 and 14] These data led to the accelerated approval of crizotinib by the U.S. Food and Drug Administration (FDA) in August 2011.[15] The use of the drug has been restricted to patients with advanced, ALK-positive NSCLC evaluated by an FDA-approved test, the Vysis ALK Break Apart florescence in situ hybridization (FISH) Probe Kit, that has become the gold standard for detecting ALK rearrangement in NSCLC.[16] In October 2012, the European Medicine Agency granted conditional marketing authorization in the European Union for crizotinib for the treatment of patients with previously treated ALK-positive, advanced NSCLC. Following this conditional approval, ALK testing has become mandatory to select patients to be treated.

In Italy, recommendations for ALK rearrangement analysis were elaborated in 2012 by a steering committee of members of the Italian Association of Medical Oncology (AIOM) and the Italian Society of Pathology and Cytopathology (SIAPEC-IAP).[17] Following the publication of the guidelines, the AIOM-SIAPEC societies started an educational program, presenting and discussing the recommendations at national meetings. Moreover, an external quality assessment (EQA) scheme for ALK testing was organized to evaluate the effects of recommendations on molecular diagnostics in Italy and establish interlaboratory consistency, with the aim of improving the qualitative standard for this analysis and allowing an appropriate NSCLC patient selection for treatment with crizotinib. This article describes the development and the results of the first Italian EQA scheme for ALK testing, which was completed in March 2013.

MATERIALS AND METHODS

Organization of the Scheme

AIOM and SIAPEC identified a board of Italian pathologists and oncologists with particular experience in lung cancer who were assigned to organize the EQA scheme and that are coauthors of this document. Within the team, five surgical pathology departments (Department of Pathology, University-Foundation, Chieti, Italy; Division of Pathology and Laboratory Medicine, European Institute of Oncology, Milan, Italy; Division of Anatomic Pathology, San Luigi Hospital

and University of Turin, Orbassano, Italy; Diagnostic and Laboratory Medicine, National Cancer Institute "Fondazione Pascale," Naples, Italy; Pathology Department, Policlinico of Modena, Italy) were identified as referral centers of the EQA program for ALK testing. They were in charge of selecting and validating the samples for the EQA. AIOM and SIAPEC decided to conduct the EQA scheme for ALK rearrangement by FISH analysis, as this is currently the method of choice for ALK assessment following the results obtained in clinical trials which led to the approval of crizotinib for treatment of NSCLC patients. However, a specific kit was not recommended, since the European Medicine Agency does not demand for a particular one. The scheme included two rounds: the laboratories which failed the first round had the chance to register for a second round.

Selection and Validation of Samples

In the five referral centers, a series of 30 resected formalin-fixed paraffin-embedded (FFPE) NSCLC specimens were selected from a large cohort of more than 1000 consecutive patients affected by NSCLC underwent to radical resection of the primary tumor. Twenty out of the 30 selected samples harbored ALK rearrangement by FISH. Samples with ALK rearrangement were also subjected to immunohistochemical analysis for ALK protein expression using the D5F3 monoclonal antibody (Cell Signaling Technology, Beverly, MA) that confirmed an alteration of ALK in all cases. Ten FFPE specimens (six negative and four positive for ALK rearrangement) were further selected on the bases of the thickness of the tissues available, the content of ALK-positive tumor cells, and the pattern of ALK alteration (split signal, single orange signal). After careful histological revision of the selected samples, areas with the highest percentage of tumor cells positive by FISH analysis and reacting with ALK D5F3 monoclonal antibody were selected for tissue microarrays (TMAs) construction (Fig. 1). The construction of a series of TMAs was performed at the Center of Predictive Molecular Medicine, University of Chieti. Selected neoplastic areas were withdrawn with a 2-mm puncher (mta1 Beecher Instruments, Sun Prairie, WI) and assembled in a 10-core TMA (one core per sample) (Fig. 2). Then, from each TMA paraffin block, one 5-µm-thick blank histological section was sent to the five referral surgical pathology laboratories for ALK testing by FISH. All the centers used the Vysis ALK Break Apart Rearrangement Probe (Abbott Diagnostics, Abbott Park, IL), but the prehybridization protocol was not the same in all referral laboratories.

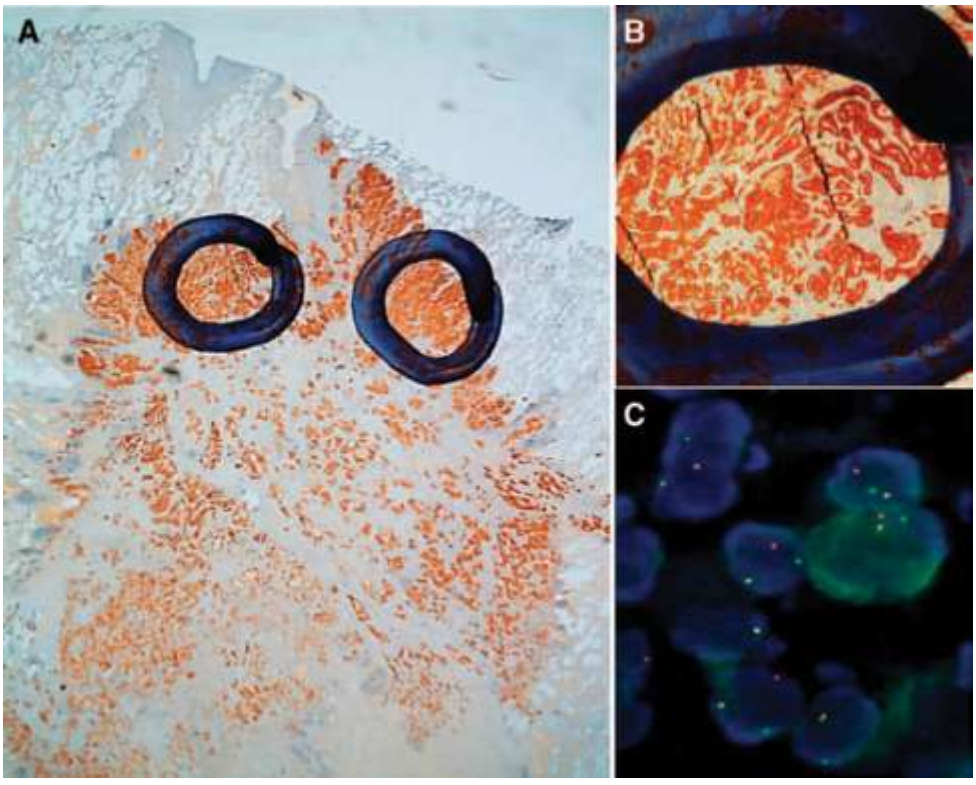

FIGURE 1.

Example of a tumor sample (*A*) in which areas with the highest percentage of tumor cells immunoreactive with the anti-ALK D5F3 monoclonal antibody (magnified in *B*) and positive by FISH analysis (*C*) were selected for TMA construction. ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization; TMA, tissue microarray.

Figure options

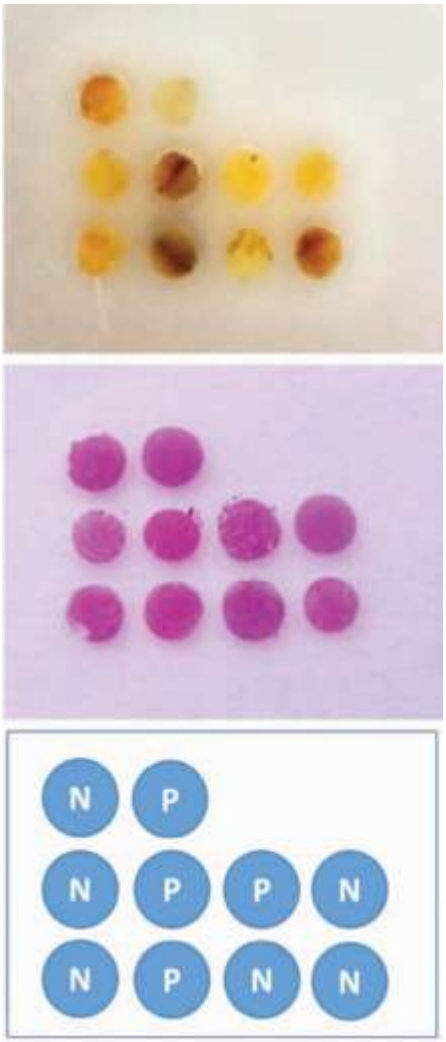

FIGURE 2.

Example of a tissue microarray used in the ALK EQA scheme: *A*, paraffin block; *B*, section stained with hematoxylin and eosin showing the good quality of 2 mm cores; *C*, scheme of the distribution of ALK-positive (P) and ALK-negative (N) cases in this particular microarray. ALK, anaplastic lymphoma kinase; EQA, external quality assessment.

Registration of the Participants and Shipment of the Samples

Italian laboratories that performed FISH analysis for diagnostic evaluation of ALK rearrangement in NSCLC patients were invited to join the EQA scheme. Participating laboratories, registered at the http://www.alkquality.it Web site, were requested to perform FISH analysis using their usual method. A 5-µm-thick TMA slide was sent to each of the registered laboratories. Before sending the sections, the first, twentieth, and last sections obtained from TMA blocks were subjected to FISH analysis at the Referral Center of Chieti to confirm the results previously obtained. The

characteristics of the four positive sample on the last section are summarized in Table 1. All positive samples showed a high percentage of cells with ALK rearrangement, two samples showed a high percentage of single red signals, and two samples a high percentage of split signals. Random codes, different for each center, were automatically assigned to the samples by an application of the Web site to avoid exchange of information among the participants. The laboratories were given 3 weeks to complete the analysis and submit the results through the ALK quality Web site. The centers were asked to define cases as positive, negative, or not assessable according to the criteria reported in the Italian Recommendation for ALK gene rearrangement.[17] Information on the number and percentage of positive nuclei was not requested. The centers were also demanded to provide information about the type of probe and prehybridization type used. Samples for the first and the second round were sent on December 12, 2012, and on February 28, 2013, respectively.

TABLE 1.

FISH Analysis of the Four Samples Carrying ALK Alterations

ALK-Positive Cores	Cells with ALK Alterations	Predominant Alteration
Core 4	60%	Split signal (90%)
Core 5	65%	Single red signal (95%)
Core 6	80%	Single red signal (95%)
Core 8	90%	Split signal (90%)

FISH, fluorescence in situ hybridization; ALK, anaplastic lymphoma kinase.

Evaluation of the Results

The AIOM/SIAPEC board of assessors evaluated the results using a scoring system, in agreement with the European guidelines for EQA in molecular pathology.[18] The scoring system assigned two points in case of accurate evaluation of genotype and zero points to false-negative or false-positive results. The threshold value to pass the EQA scheme was set at 18 points. In case of an analytical error (test failure with no result on the sample), one point was assigned for the first error, and zero from the second onward.

Statistical Analysis

The variables measured in the study were investigated for association by using the Fisher's exact test or chi-square test as appropriate. A *p* value less than 0.05 was considered as significant. All statistical analyses were performed using the SPSS pack (version 15; SPSS, Chicago, IL).

RESULTS

Validation of the Samples for the EQA Scheme in the Referral Centers

A high degree of concordance was obtained by FISH analysis on sections from TMAs in the five referral centers (Table 2). However, in two centers two cases (case n. 2 and case n. 9) were judged

as not assessable due to lack of probe signal. These samples were considered as “critical or borderline cases.” Nevertheless, it was decided to include them in the EQA scheme, with the aim of pointing out possible latent weaknesses in test performance and interpretation of the results. Obviously, where an approach as this is followed, more laboratories might fail. Therefore, as suggested in the recent guidelines on the requirements of EQA programs in molecular pathology, we decided not to consider as failure the inability to evaluate the two critical cases. Test failure in these cases has been reported as not assessable in critical sample.

TABLE 2.

Results Obtained by the Five Referral Centers in the Validation Study

Center	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
1	N	N	N	P	P	P	N	P	N	N
2	N	N	N	P	P	P	N	P	N	N
3	N	N	N	P	P	P	N	P	N	N
4	N	NA	N	P	P	P	N	P	NA	N
5	N	NA	N	P	P	P	N	P	NA	N

N, negative; P, positive; NA, not assessable.

Table options

First Round

Thirty-seven laboratories registered to the Italian ALK EQA scheme. Thirty-four participating centers submitted the results within the established deadline. Overall, 26 (70%) laboratories passed the first round, having reached a score of 18 points or greater (Fig. 3).

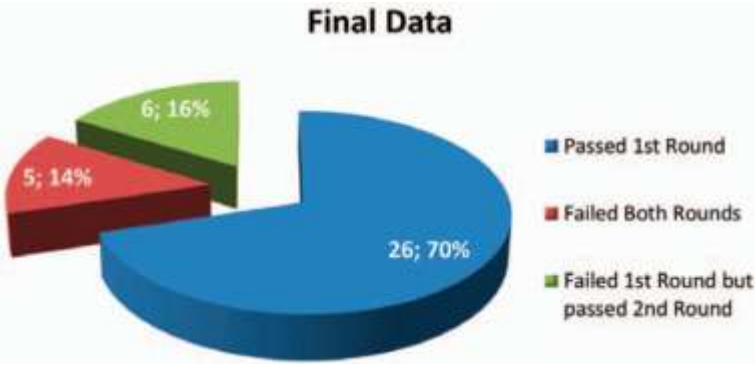

FIGURE 3.

Results of the first Italian external quality assessment for ALK testing. ALK, anaplastic lymphoma kinase.

Several errors in the evaluation of genotype (*n* = 18) were found, with both false-positive (*n* = 7) and false-negative (*n* = 11) results. Moreover, test failure in suitable sample occurred in seven cases (reported as not assessable in suitable sample) (see Table 3). The errors (false positive/false positive) reported were randomly distributed in the different cores of the TMA. However, six of the 34 centers (18%) failed on sample N.5 (Table 4). Three (50%) of the laboratories that failed with sample N.5 made errors on other cores. This relatively difficult sample, re-evaluated at the Referral Center of Chieti on the last TMA section (see Materials and Methods), showed 65% of rearranged nuclei (95% with single red signals) (Table 1).

TABLE 3.

Results of the First Round of the Italian EQA Scheme for ALK Rearrangement Testing in NSCLC Samples

Center	Outcome	Type of Error
1	Passed	1FP
2	Not passed	3FP + 1NASS
3	Passed	
4	Passed	
5	Not passed	2FP
6	Passed	
7	Not passed	1FN + 2NASS
8	Not passed	Data not submitted
9	Not passed	Data not submitted
10	Passed	
11	Not passed	1FN + 1NASS
12	Passed	
13	Passed	1NASS
14	Passed	
15	Passed	
16	Passed	
17	Passed	

Center	Outcome	Type of Error
18	Passed	
19	Passed	
20	Passed	
21	Passed	
22	Passed	
23	Passed	
24	Passed	1NACS
25	Passed	2NACS
26	Passed	1FN + 1NACS
27	Passed	1FN + 2NACS
28	Passed	1FN + 2NACS
29	Not passed	2NASS
30	Not passed	2FN
31	Not passed	2FN + 2NACS
32	Not passed	1FP + 1FN
33	Passed	1FN
34	Passed	
35	Passed	1NACS
36	Passed	
37	Not passed	Data not submitted

EQA, external quality assessment; ALK, anaplastic lymphoma kinase; NSCLC, non–small-cell lung cancer; FP, false positive; FN, false negative; NACS, not assessed in critical sample; NASS, not assessed in suitable sample.

TABLE 4.

Number of Errors (FP/FN) per TMA Core

TMA Core Number	Errors (N)
Core 1 (wild type)	FP (1)
Core 2 (wild type)	FP (1)
Core 3 (wild type)	FP (1)
Core 4 (ALK rearrangement)	FN (2)
Core 5 (ALK rearrangement)	FN (6[a])
Core 6 (ALK rearrangement)	FN (1)
Core 7 (wild type)	FP (1)
Core 8 (ALK rearrangement)	FN (2)
Core 9 (wild type)	FP (2)
Core 10 (wild type)	FP (1)

FP, false positive; FN, false negative; TMA, tissue microarray; ALK, anaplastic lymphoma kinase.

a

Three of the six centers made errors in other cores (core 5 + core 3; core 5 + core 4; core 5 + core 1).

Second Round

The 11 centers that did not pass the first round were given information about the number and type of errors made, but they were not informed on the results of the specific samples previously analyzed. Subsequently, according to the guidelines of the scheme, the 11 centers were invited to participate in a second round. TMAs with the same samples, but differently arranged and provided of code number different from the one given in the first round, were sent to the laboratories.

Five centers did not pass the second round: three laboratories did not submit the results within the established deadline (same centers that did not submit data in the first round), and two laboratories once again scored below 18. Of these two latter laboratories, one reported errors in the evaluation of genotype (both false-positive and false-negative results), and the other one did not get probe signals on the samples (complete test failure) (Table 5).

TABLE 5.

Results of the Second Round of the Italian EQA Scheme for ALK Rearrangement Testing in NSCLC Samples

Center	Outcome	Type of Error
1	Not passed	Data not submitted
2	Not passed	Data not submitted
3	Passed	
4	Not passed	Complete test failure
5	Passed	
6	Passed	
7	Not passed	Data not submitted
8	Passed	
9	Passed	
10	Passed	
11	Not passed	1 FN and 3 FP

EQA, external quality assessment; ALK, anaplastic lymphoma kinase; NSCLC, non–small-cell lung cancer; FP, false positive; FN, false negative.

Overall, 32 out of 37 Italian laboratories (86%) passed the ALK EQA scheme, having reached a score of 18 points or higher in the first or in the second round of the EQA (Fig. 3). The list of the centers that passed the EQA has been published on the AIOM and SIAPEC Web sites (http://www.aiom.it; http://www.siapec.it).

Several commercial kits are available to evaluate ALK rearrangements by FISH. In Table 6, the performance of the different commercial tests used by the laboratories participating in the first round of the EQA scheme are reported. For each test, the number and percentage of the correct genotypes and the different type of errors are indicated. Of the 34 centers that submitted data, 25 (74%) utilized the FDA-approved LSI Vysis ALK Break Apart FISH Probe Kit (Abbott Diagnostics), 4 the ALK FISH DNA Probe, Dako (Via Real Carpinteria, CA), 3 the ZytoLight SPEC ALK/EML4 TriCheck Probe, ZytoVision (Bremerhaven, Germany), and 2 the ON ALK (2p23) Break, Kreatech (Amsterdam, The Netherlands). Due to the low number of laboratories that used commercial kits different from the ALK breakApart test, the power of a statistical comparison among the various kits utilized is very limited. No significant differences were observed.

TABLE 6.

Performance of the Different FISH Tests Used in the Italian EQA Scheme

Report		Commercial Kit			Total	*p*
	Vysis[a]	Dako[b]	Zytovision[c]	Kreatech[d]		
Correct genotype	221 (88.4%)	36 (90%)	27 (90%)	20 (100%)	304	NS (0.45)
False positive	6 (2.4%)	0 (0%)	1 (3.3%)	0 (0%)	7	NS (0.67)
False negative	8 (3.2%)	3 (7.5%)	0 (0%)	0 (0%)	11	NS (0.26)
NASS	6 (2.4%)	1 (2.5%)	0 (0%)	0 (0%)	7	NS (0.74)
NACS	9 (3.6%)	0 (0%)	2 (6.6%)	0 (0%)	11	NS (0.36)
Total	250	40	30	20	340[e]	

FISH, fluorescence in situ hybridization; EQA, external quality assessment; NASS, not assessable in suitable sample; NACS, not assessable in critical sample; NS, not significant.

a

LSI Vysis ALK Break Apart FISH Probe Kit, (Abbott Diagnostics, Abbott Park, IL).

b

ALK FISH DNA Probe, Dako (Via Real Carpinteria, CA).

c

ZytoLight SPEC ALK/EML4 TriCheck Probe, ZytoVision (Bremerhaven, Germany).

d

ON ALK (2p23) Break, Kreatech (Amsterdam, The Netherlands).

e

Ten cases were sent on a tissue microarray to each center; three centers did not submit data.

Regarding the two cases considered as critical, seven of the 32 centers (22%) that passed the EQA scheme defined these samples as not assessable. An accurate analysis of the data obtained during the validation of the samples by the five referral laboratories indicated that the inability to evaluate the critical cases was related to differences in the prehybridization step.

DISCUSSION

Molecular testing of biological specimens to guide therapeutic protocols is of increasing relevance. In the last decade, several drugs have been approved for the treatment of specific subgroups of patients having tumors harboring distinct molecular alterations. In the European Union, the ALK inhibitor crizotinib has recently received conditional marketing authorization for treatment of patients with previously treated ALK-positive advanced NSCLC. After this approval, the accurate detection of ALK rearrangement has become mandatory, as the result of such testing is key to manage therapy, with both false-negative and false-positive results being harmful for patients.

The Italian health system is structured on a regional basis, and there is no limit in the number of laboratories that can perform molecular analyses, with few exceptions in restricted geographical areas. When crizotinib was approved, several centers were offering ALK testing in different areas of the country. Since in Italy there is no national or regional health authority that releases guidelines or organizes EQA programs for molecular pathology, AIOM and SIAPEC decided to launch a program to improve ALK testing in the country. The program comprised the publication of guidelines, the development of training courses, and the organization of an EQA scheme to ensure that ALK testing is performed with high quality in every Italian center that provides this service.

Different methods are available for the analysis of ALK rearrangement, and each of them has advantages and disadvantages. The European regulatory agency has not linked the authorization of crizotinib to a specific type of analysis, whereas the FDA restricted the use of the drug to detection by FISH. The Italian EQA scheme was based on FISH testing, as this is widely considered the elective method for ALK assessment. This method can be performed on FFPE samples and was used in the clinical trials which led to the approval of crizotinib for treatment of NSCLC patients. However, a specific kit was not recommended, since the EMA does not demand for a particular one.

The main purpose of the EQA scheme for the detection of ALK rearrangements in NSCLC patients promoted by AIOM and SIAPEC was to assess the rate of analytical errors. Laboratories were neither required to indicate the percentage of neoplastic cells nor the percentage of tumor cells with rearrangement. Therefore, samples for the analysis were selected on the basis of a high percentage of tumor cells and a diffusely rearranged ALK genotype.

Different types of samples can be used for EQA. For the specific purpose of this investigation, we decided to generate TMAs with 2 mm cores containing a total of 10 samples which has been suggested as the adequate number of cases for a proficiency testing.[19] Since the great majority (approximately 70%) of lung cancer patients present in an advanced stage of disease, in most cases no resection specimens are available and only tissues collected at the initial diagnostic work-up can be used for histopathological diagnosis of lung cancer and subsequent molecular analyses. Hence, the use of small tissue specimens in an EQA scheme can better mimic the routine clinical activity. However, we believe that the use of TMA cores smaller than 2 mm is not practical, since core sections are more prone to detach from the slide. Another characteristic of the samples used in this EQA which guarantees the similarity with the daily clinical practice is the different source of the specimens, as the ten samples came from five different surgical pathology departments.

Overall, the results of this EQA suggest that ALK testing is performed with adequate quality in the majority of Italian centers, in that 32 (86%) out of 37 laboratories passed the EQA. However, this conclusion may be limited by the type of samples used which had a very high percentage of tumor cells. Nevertheless, we noticed a high number of analytical errors. In the first round, 12

laboratories reported at least one false-positive or false-negative result. Both these results are detrimental for NSCLC patients, causing the application of inadequate treatment protocols.

False-positive results might be caused by prehybridization issues. If pretreatment of the tissue is inadequate, the hybridization reaction could occur in an improper way, resulting in a weak or an absent probe signal. This could particularly affect green signals, as for this probe the signal–noise ratio is usually lower than that obtained with the orange one because of the high background fluorescence typically emitted by lung tissue. In these conditions, nuclei with an orange signal without a corresponding green signal could be considered as rearranged.

The rate of false-negative results was higher when compared with false-positive (11 versus 7). False-negative results might be caused by an incorrect probe signal evaluation. Cells are considered negative (non-rearranged) when orange and green signals are fused or adjacent and positive when signals are two or more signal diameter apart.[17] The misinterpretation of the distance between probe signals could be the main cause of false-negative results.

The decision to admit the two critical cases emerged during the sample validation step highlighting weaknesses in test performance. The analysis of data obtained by the five referral centers suggests that the prehybridization protocol is a critical step for the success of the reaction in borderline cases. As is well known, the specifications for fixation and handling of tissue specimens could be slightly different among laboratories or even among tissues processed in the same laboratory. These variables can influence the analytical step, especially if tissues are not processed under optimal conditions with an appropriate digestion pretreatment. To overcome this difficulty, several commercial kits have been developed for the pretreatment of specific type of tissue, but in particular cases it could be necessary to make adjustments even to standardized protocols. When dealing with very small amount of tissue, however, it may not be possible to carry out several attempts. For this reason, every molecular laboratory should be able to perform an effective FISH assay for each sample to be analyzed, without wasting the precious tissue material. In future EQA schemes, the use of TMA sections containing different specimens, including critical samples, may allow to test the capability of each center to evaluate differently processed tissues and provide useful information to overcome technical problems related to tissue characteristics and handling. In this respect, we are planning to organize a further ALK EQA scheme (scheduled to start in 2015) in which we will accurately select tissue samples for TMA construction and collect detailed information on the technical protocols used by the laboratories.

The list of the centers that passed the EQA is published on the Web sites of AIOM and SIAPEC (http://www.aiom.it; http://www.siapec.it). The publication of this list offers to both patients and clinicians the opportunity to choose among a wide number of certified laboratories, which are located in different regions of the country and that are able to provide ALK FISH testing with an adequate quality.

CONCLUSION

In conclusion, the results of this first Italian EQA scheme for ALK testing in NSCLC cancer patients indicate that the evaluation of ALK rearrangement is performed with adequate quality in most of the Italian diagnostic centers. In addition, our data highlight the importance of EQA in revealing methodological problems that must be addressed to fur ther increase the reproducibility and accuracy of molecular tests. As for the other EQA programs previously activated for KRAS, EGFR, and BRAF testing in Italy,[20],[21] the success of the ALK EQA scheme might be in part attributable to the publication of specific recommendations and the development of local educational

programs.[17] The activity of AIOM and SIAPEC in this field might represent a model for other national and international associations.

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