

Second ESMO consensus conference on lung cancer: pathology and molecular biomarkers for non-small-cell lung cancer

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To complement the existing treatment guidelines for all tumour types, ESMO organises consensus conferences to focus on specific issues in each type of tumour. The Second ESMO Consensus Conference on Lung Cancer was held on 11–12 May 2013 in Lugano. A total of 35 experts met to address several questions on management of patients with non-small-cell lung cancer (NSCLC) in each of four areas: pathology and molecular biomarkers, early stage disease, locally advanced disease and advanced (metastatic) disease. For each question, recommendations were made including reference to the grade of recommendation and level of evidence. This consensus paper focuses on recommendations for pathology and molecular biomarkers in relation to the diagnosis of lung cancer, primarily non-small-cell carcinomas.

Key words: ESMO, consensus, non-small-cell lung cancer, pathology, molecular testing, recommendations

Lung cancer is the leading cause of cancer-related death and most currently used chemotherapeutic agents lack adequate specificity and efficacy. The past decade has seen the emergence of histology (squamous cell versus non-squamous) as an important determinant of therapy in non-small-cell carcinomas (NSCLC). In addition, a significant proportion of patients have tumours with therapeutically targetable molecular characteristics (mutations, fusion genes etc.) [1], and currently, the vast majority of these actionable molecular abnormalities occur in pulmonary adenocarcinomas. Laboratory testing to identify those likely to respond to such targeted therapy, based upon a predictive biomarker, represents a paradigm shift in lung cancer diagnosis and has now become a standard of care [2–6]. Potential targets more often found in squamous cell carcinomas are emerging. Pathological diagnosis of lung cancer is now a multi-step process, beginning with morphological diagnosis to determine histological type, refined by immunohistochemistry

(IHC) where required (see Recommendation 2), followed by appropriate molecular characterisation of the tumour.

This increasingly complex diagnostic algorithm poses many challenges for those managing patients with lung cancer [1–7]. Important among these is the availability of sufficient tumour tissue to perform all required tests. The majority of NSCLC are diagnosed at an advanced stage, so that large tumour samples (e.g. from surgical resection) are available in only a minority of cases. In patients with advanced disease, interventions required to obtain tumour tissue are often difficult due to tumour location and patient comorbidity, thus, most diagnostic lung cancer samples are very small with minimal tumour content. It is now imperative that, whenever possible and without compromising patient safety, any diagnostic biopsy/tissue sampling procedure carried out should aim to maximise the amount of tissue acquired [3]. The role of a multidisciplinary team, including thoracic surgeons, radiologists, pathologists, oncologists and pulmonologists, is essential in determining the best approach in individual patients. Tissue sampling may be achieved from the primary tumour via bronchoscopy, video-assisted thoracoscopic surgery, thoracotomy or via image-guided, percutaneous, trans-thoracic, transbronchial or transoesophageal techniques [3, 7]. Some of these approaches, as well as many other interventions

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accessing extra-thoracic sites, may be selected, where appropriate, to sample metastatic disease. There is no current evidence to suggest that there is any significant difference in the clinical relevance of biomarker tests carried out on the primary lung tumour versus any resultant metastatic disease. The choice of site sampled will usually be determined by ease of access, patient safety and likely tissue yield.

Tissue samples will range from the whole or large parts of the tumour to smaller tissue biopsy and a variety of cytology type samples. The yield from any procedure will vary according to the nature of the technique (open surgical, closed, image guided etc.), the equipment used (gauge or type of sampling needle etc.) and the skill and tenacity of the operator. Tissue biopsy and cytology type specimens are suitable for diagnosis and molecular testing but must be handled appropriately in order to facilitate the necessary molecular diagnostic procedures.

This guideline follows from that published in 2011 [8]. For the following recommendations, levels of evidence and grades of recommendation have been applied using the system shown in Table 1. Statements without grading were considered to be justified standard clinical practice by the experts and the ESMO faculty.

Recommendation 1: Guidance on tissue handling

- Specimen processing
 - Standard fixation using 10% neutral buffered formalin (4% formaldehyde) is recommended [V, A]
 - Fixation time should be no less than 6 h, and no greater than 48 h [IV, A]
 - Sections for biomarker testing should ideally be cut immediately before analysis [IV, A]
- Cytology samples (cellblocks, stained direct smears or liquid-based preparations) can be used reliably to detect EGFR mutations and ALK rearrangements [III, A]. At this time, a cell block is the most widely applicable cell source
- The same pathologist should, if possible, review all available tumour material from the same patient including biopsies and cytology specimens to select the most suitable for biomarker analyses [IV, A]
- A pathologist should be involved in sample preparation for DNA extraction [V, A]
- Enrichment of samples by micro- or macrodissection to maximise tumour cell content before DNA extraction is recommended [III, A]

Pre-analytical factors are critical for reliable biomarker testing on any diagnostic specimen, yet standardisation of tissue processing remains a challenge. The time to fixation (cold ischaemia time) should be minimised, ideally only a few minutes and certainly less than an hour, to avoid degradation of proteins and nucleic acids (NAs) [9, 10]. Fixation inhibits decay or autolysis of cells and preserves tissue morphology. Specimens should be fixed in 10% neutral buffered formalin (4% formaldehyde solution), which is widely available, and which preserves protein, RNA and DNA for subsequent biomarker analysis. In general, fixation time should be between 6 and 48 h [3, 11]. Longer or shorter fixation time may adversely affect the quality of IHC, *in situ* hybridisation (ISH) and mutation testing; under-fixation also results in poor tissue morphology [3, 12]. If rapid

Table 1. Levels of evidence and grades of recommendation [adapted from the Infectious Diseases Society of America-United States Public Health Service Grading System [98] (by permission of the Infectious Diseases Society of America)]

Levels of evidence	
I	Evidence from at least one large randomized controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomized trials without heterogeneity
II	Small randomized trials or large randomized trials with a suspicion of bias (lower methodological quality) or meta-analyses of such trials or of trials with demonstrated heterogeneity
III	Prospective cohort studies
IV	Retrospective cohort studies or case-control studies
V	Studies without control group, case reports, experts opinions
Grades of recommendation	
A	Strong evidence for efficacy with a substantial clinical benefit, strongly recommended
B	Strong or moderate evidence for efficacy but with a limited clinical benefit, generally recommended
C	Insufficient evidence for efficacy or benefit does not outweigh the risk or the disadvantages (adverse events, costs, optional
D	Moderate evidence against efficacy or for adverse outcome, generally not recommended
E	Strong evidence against efficacy or for adverse outcome, never recommended

processing routines with short fixation times are employed, these hazards should be borne in mind and results interpreted with due caution. Acidic fixatives (e.g. Bouin) are not recommended since they lead to rapid NA degradation [13], and accelerated fixation with heated formalin is discouraged as it degrades morphology and molecular studies [14]. Samples from bone metastases which have been decalcified by acidic agents known to degrade DNA should be avoided for molecular analysis if at all possible. Ethylenediaminetetraacetic acid is an effective decalcifying agent, has no adverse effect on DNA quality and should be used in samples of suspected lung cancer where decalcification is required and molecular studies are anticipated.

Ageing of formalin-fixed, paraffin-embedded tissue sections causes degradation of epitopes and DNA. Sections should be freshly cut, and biomarker analysis carried out within 4–6 weeks to avoid non-informative or false-negative results. Integrity of stored tissue sections can be prolonged by paraffin coating, or by sealing using special tapes [3, 13].

Up to 40% of all NSCLC diagnoses are made by cytology alone. Biomarker analyses are applicable to cytological specimens, including cellblocks, smears, cytospins or liquid-based cytology [15]. Cellblocks are preferred for marker analysis by many institutions since the same protocols as for histology can be used. Ethanol-based fixation of cytological smears also permits mutation analysis, fluorescence *in situ* hybridisation

(FISH) and IHC, although pathologists must be aware that laboratory protocols may well differ from those for formalin-fixed tissue [3, 15–17]. The same pre-analytical factors (time to fixation, fixation time, and material ageing) apply to cytology specimens.

A pathologist, preferably with expertise in lung cancer, should, if at all possible, review all available material on an individual patient, in order to select that which is optimal for analysis. This will include marking sections for macro/microdissection as required (see below). Where cytology and biopsy samples are reported independently, close communication between responsible pathologists is encouraged to facilitate best sample selection. Pathologists are also responsible for the training and supervision of technical staff preparing and processing the specimens before molecular analysis.

For DNA or RNA-based biomarker testing, it is usually important to enrich the tumour cell content of the tissue used for NA extraction. Sections of tissue with high tumour content may be used directly. In poorer samples, where feasible, suitable areas identified by pathologists may be scraped (manual macrodissection) from tissue slides. Laser capture microdissection can facilitate high purification of tumour for NA extraction, even from very poor samples, but this technology is not widely available, and requires pathologist skills, and the additional labour and cost involved must be weighed against the potential to avoid repeat biopsy sampling.

Recommendation 2: What is an acceptable rate for NSCLC not otherwise specified (NOS) diagnosis in the small biopsy/cytology diagnostic setting and how can this be achieved?

- A diagnosis of NSCLC-NOS should be given in <10% of cases [IV, A]
 - This figure is achieved with the judicious use of immunohistochemistry in morphologically indeterminate cases. A recommended approach should include TTF-1 to predict adenocarcinoma. For predicting squamous cell carcinoma, p63 or p40 and CK5/6 testing are useful [IV, A]
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A specific histologic diagnosis (i.e. squamous versus adenocarcinoma) is of importance in therapeutic decision-making. Drugs such as pemetrexed and bevacizumab are only licensed for use in patients with non-squamous NSCLC, on grounds of efficacy and toxicity, respectively [18, 19]. The recommended triage of tumours selected for molecular testing is partly histologically based.

Diagnostic criteria in the WHO classification of lung carcinomas [20] require examination of the entire tumour. However, most lung cancers are not surgically resected and diagnosis, classification and molecular biomarker testing are carried out on small diagnostic biopsy or cytology type samples (see above). The lack of applicable diagnostic criteria, and a tendency for pathologists to diagnose on inadequate evidence, probably accounts for the reported inaccuracy of morphological classification of NSCLC subtypes on small samples [21–28]. NSCLC lacking definitive morphological evidence of squamous or glandular differentiation in a small tumour sample should be classified as NSCLC-NOS [21, 28]. These cases should NOT be classified as large-cell carcinoma [29, 30]. Reported NSCLC-NOS rates are highly variable, reflecting variation in pathologist

approach and the case mix of published studies but rates of 25%–30% in small biopsy samples and up to 40% in cytology type samples probably reflect a ‘real world’ situation for consecutive, unselected cases.

Predictive IHC can reduce the NSCLC-NOS rate to <10%, typically 5%–6%, but never to zero. Most NSCLC-NOS samples are the result of non-representative sampling of differentiated lung cancers, mostly adenocarcinomas [22]. Markers such as p63, p40 and cytokeratin CK 5/6 are associated with squamous cell carcinomas, while TTF1, Napsin A and CK7, as well as mucin stains, are associated with adenocarcinomas [31–36]. These markers are, however, not specific for either diagnosis. TTF1 is expressed in only 75%–80% of lung adenocarcinomas. These markers only have predictive value when expressed at certain levels in the diagnostic sample; levels which have been determined by predictive power in small samples when compared with final resection diagnosis [31–33].

When an NSCLC subtype is predicted by this approach, the recommended diagnosis is ‘NSCLC, probably (or favour) squamous cell carcinoma’ or ‘probably adenocarcinoma’. The predictive accuracy is ~85%. Thus, in small biopsy or cytology samples, a more limited range of primary carcinoma diagnoses should be given (Table 2) [29, 30]. Cases with a ‘favoured’ diagnosis should be treated as those with definitive subtyping.

Recommendation 3: Routine EGFR somatic mutation testing has been recommended

- All non-squamous tumours in patients with advanced/recurrent disease should be tested for EGFR mutation [I, A]
 - Selected squamous tumours (from patients with minimal or remote smoking history) should strongly be considered for testing [IV, B]
 - A wide coverage of mutations in exons 18–21 is strongly encouraged, including those associated with resistance to some therapies. At a minimum, when resources or material are limited, the most common activating mutations (Exon19del, L858R) should be determined [I, A]
 - Any methodology employed should be validated by an external quality assurance programme [V, A]
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Activating mutations in the epidermal growth factor receptor gene (*EGFR*) tyrosine kinase domain are found in 10%–16% of cases of adenocarcinoma in European patients [37–39]. These mutations are only rarely reported in bona fide squamous cell carcinomas. The cause of *EGFR* mutation is unknown, but is not related to tobacco carcinogenesis. These mutations are therefore more common in, though by no means exclusive to, never smokers and distant ex-smokers, and females of younger age [40, 41]. These clinical parameters should not, therefore, be used to select patients for *EGFR* mutation testing. However, recent anecdotal evidence indicates that patients with any type of primary lung carcinoma, including small-cell carcinoma, who have a minimal or remote smoking history, should be strongly considered for testing.

Phase III trials involving Asian, European and North American patients with metastatic disease whose tumours have activating *EGFR* mutations have demonstrated high response rates (~70%) and significantly longer progression-free survival

Table 2. Recommended nomenclature for small sample diagnosis of lung cancer

Small cell carcinoma
Squamous cell carcinoma
NSCLC, probably squamous cell carcinoma
Adenocarcinoma
NSCLC, probably adenocarcinoma
NSCLC-NOS ^a
Carcinoid tumour ^b
Others ^c

^aThis diagnosis is still acceptable if the immunohistochemistry (IHC) cannot be carried out, or is not predictive, the latter sometimes referred to as 'null IHC'.

^bThe distinction between typical and atypical carcinoid cannot be made reliably on small samples.

^cRarely, for example, there may be sufficient tissue and morphological features to reliably diagnose rare tumours such as adenoid cystic or mucoepidermoid carcinoma.

(PFS) (though not overall survival) in patients treated with EGFR tyrosine kinase inhibitors (EGFR TKIs, gefitinib, erlotinib, afatinib) as initial treatment when compared with those receiving chemotherapy [42–46]. The use of EGFR TKIs is now well established in clinical practice, requiring routine testing of appropriate cases for EGFR mutations.

Most clinically significant *EGFR* mutations are either deletions in exon 19 or the L858R substitution mutation (80%–90% of mutations) [47, 48], but rarer mutations in exons 18 and 21 may also predict response to EGFR TKIs. Some mutation testing strategies may be allele-specific for a pre-determined range, or detect only certain types of mutation (e.g. fragment length analysis for exon 19 deletions), while other techniques can detect any mutation in *EGFR* exons 18–21. Methodology used will often depend on that available in individual centres. Users should be aware of any limitation in mutation coverage offered by the technology used, as well as the sensitivity for detecting mutations against a background of wild-type genome (see concluding remarks). This caution also applies to the use of *EGFR* mutation-specific antibodies in IHC to detect *EGFR* mutations. Given the range of clinically important mutations not reliably detected by currently available antibodies, this approach as a primary method of detection is not recommended.

Some mutations, especially those in exon 20, most commonly exon 20 insertions and the T790M substitution, are activating but confer resistance to first-generation EGFR TKIs [48]. These mutations are uncommon, but may exist as minor clones in EGFR TKI-naïve tumours, detectable only by very high-sensitivity, targeted methods. A T790M mutation is found in ~50% of tumours relapsing during EGFR TKI therapy [49]. Testing strategy around EGFR TKI resistance is still in development, but it is likely that specific recommendations on T790M mutation testing will evolve, since agents targeting this mutation, as well

as the more common sensitising mutations, have reported activity in this setting.

Recommendation 4: Is there sufficient evidence to support routine testing for ALK rearrangement?

- All non-squamous tumours in patients with advanced/recurrent disease should be tested for ALK rearrangement [II, A]
 - Selected squamous tumours (from patients with minimal or remote smoking history) should strongly be considered for testing [III, B]
 - Definitive assessment of ALK rearrangement is determined by FISH [I, A]
 - IHC methods may be employed for screening and may become validated for therapy [IV, B]
 - Methodologies employed should be validated by an external quality assurance programme [V, A]
-

ALK gene rearrangement occurs in ~3%–5% of adenocarcinomas and predicts response to ALK-targeted inhibitors. *ALK*-rearranged tumours are associated with distinct clinicopathological features, including onset at a young age, never or light smoking history, and adenocarcinoma, particularly with signet ring or acinar histology [50–55]. As with *EGFR* mutations, however, these clinicopathological features are insufficiently sensitive to screen for testing an individual patient as a significant proportion of *ALK*-rearranged tumours may go undetected. *ALK* gene rearrangements are very infrequent in squamous cell carcinoma [50, 51, 55], have been reported in adenosquamous carcinoma [54] and are largely mutually exclusive with *EGFR* or *KRAS* mutations [56].

The ALK tyrosine kinase inhibitor crizotinib is effective in patients whose tumours show an *ALK* gene rearrangement. In Europe, crizotinib is currently indicated for second-line therapy in NSCLC patients who have received one prior platinum-based regimen. There is no current indication in early disease or as first-line therapy in advanced disease. *ALK* testing will therefore not necessarily be required at initial tumour diagnosis but could be carried out in anticipation of future need.

In the majority of the cases, *ALK* fuses with *EML4* through a small intra-chromosomal inversion event and FISH analysis detecting chromosome 2 inversions and other *ALK* translocations is the current standard for diagnosis of *ALK* rearrangement. The US Food and Drug Administration mandates the *ALK* Break Apart FISH Probe Kit companion diagnostic for crizotinib in lung cancer [57], but the European Medicines Agency is less specific, requiring only 'a validated ALK test' [58]. FISH testing is relatively expensive, and can be difficult to interpret, as the most common alteration, the intra-chromosomal inversion, leads to subtle alterations with a consequent risk of both false-negative and false-positive results [59]. IHC detection of elevated ALK protein in *ALK*-rearranged tumour cells offers a practical and cost-effective approach to pre-screening cases for confirmation by FISH analysis, based on the high probability of rearrangement when IHC testing is positive, and a very low probability of *ALK* rearrangement when IHC testing is negative [16, 60–64]. Protein levels are relatively low and detection requires a high-affinity antibody and a sensitive IHC detection system. The potential for IHC as a screening tool, or even as a primary biomarker, is still evolving. IHC pre-screening is widely

accepted, and practiced in many centres. Recommendations for utilising these methods have been published [65, 66].

Recommendation 5: Do we require guidelines for testing other somatic gene mutations or other targetable genetic alterations (KRAS, BRAF, HER2, ROS1 fusion, RET fusion etc.)

- Routine testing for these biomarkers is not currently recommended [III–IV, C]
 - Patients with demographic variables (e.g. minimal or remote smoking history) whose tumours have tested negative for EGFR mutation and ALK rearrangement might be considered for testing for ROS1 rearrangements, RET rearrangements, BRAF mutation and other abnormalities for which specific therapeutics are currently available, even if evidence of activity is from case series (see text) [III, A]
 - Emerging data from clinical trials of agents targeting tumours with other abnormalities may lead to recommendations for additional testing
-

The revolution in gene and other biomarker analysis has led to the identification of a number of biomarkers for which potentially active agents are already approved for other indications (e.g. crizotinib for ALK gene rearrangements). These biomarkers include ROS1 and RET gene rearrangements, HER2 amplification and mutation, BRAF mutations and others [4, 67, 68]. In addition, though no active agent has been clinically proven, KRAS mutation analysis has become common, given the widespread availability of validated tests. MET and PDL1 expression are other potential future biomarkers. As none of the respective targeted agents has regulatory approval, routine testing is not advised.

However, prospective case series have demonstrated patient benefit (in terms of response and prolonged PFS) for treatment with crizotinib, and vemurafenib and dabrafenib, in ROS1-rearranged and BRAF-mutated tumours, respectively. The panel members therefore felt that testing those at substantial risk of some abnormalities, if that risk can be identified (e.g. never, scant or remote smokers lacking EGFR or ALK abnormalities), is reasonable if a sequential testing strategy is employed [68–70]. The panel members recognise that there is increasing evidence that a history of significant tobacco use does not exclude the possibility of activating and actionable mutations/rearrangements, particularly for BRAF mutation. This population can be further enriched by excluding from further testing patients bearing a KRAS mutation, should this test be available [71]. While mutations are not entirely mutually exclusive, the incidence of disease characterised by ‘double mutations’ is <1% [72], making this a logical and cost-effective approach. At this time, there is only anecdotal evidence for benefit of various agents in patients with RET mutations, HER2 mutations and amplification and other biomarkers. It is likely that this recommendation will evolve as prospective studies validating patient benefit in these subsets are reported. Furthermore, the increasing availability of ‘multiplex’ testing may render this point moot. We emphasise that this is not a core recommendation since approved treatments are not available. Individual laboratories will have to judge whether this approach is the best use of

resources, including tissue, especially in the context of supporting clinical trials.

Recommendation 6: Is there a role for testing markers predictive of response to cytotoxic chemotherapy (ERCC1, RRM1, TS, etc.)?

- Routine testing for these biomarkers is not recommended. No biomarker has been validated to predict benefit or resistance to currently available cytotoxic agents [II, D]
-

Numerous factors have been identified as potential predictive markers for specific chemotherapies in NSCLC, including ERCC1, BRCA1, RRM1 and thymidylate synthase (TS). Low ERCC1 expression by IHC [73] and mRNA [74–77] have been associated with sensitivity to cisplatin-based chemotherapy, but IHC results have not been reproducible [78, 79] and ERCC1 mRNA assessment requires further optimisation in prospective trials before this marker can be considered for routine use. Low BRCA1 mRNA expression also appears to confer sensitivity to the platinum agents, among other drugs, but confers resistance to the tubulin-targeting taxanes and vinca alkaloids [76, 77, 80–83]. BRCA1 may also be assessed by IHC [82–84], and both it and mRNA testing [76, 77, 80] appear reproducible, each lending themselves to evaluation in prospective randomised controlled trials in NSCLC. Data from translational studies suggest that IHC and mRNA expression of RRM1 are both prognostic and associated with benefit from gemcitabine therapy, but a prospective tailored treatment study has failed to bear this out [79, 80]. Low TS has been associated with improved clinical outcomes in patients with non-squamous NSCLC treated with pemetrexed. In a prospective phase II study, high nuclear TS expression was correlated with poorer outcome, with a shorter overall survival, after treatment with second-line, single-agent pemetrexed [85]. While encouraging, further prospective phase III studies are required to validate the reproducibility of the IHC and scoring systems used.

A number of other markers are currently under evaluation for predicting benefit or resistance to chemotherapy. There is sufficient evidence to encourage research and suggest success, but at present no biomarker has been adequately evaluated for use in the clinic to select patients for any chemotherapeutic agent.

Recommendation 7: What is the role of emerging technologies in contemporary biomarker testing?

- Multiplex platforms, including so-called ‘next-generation’ technologies, for specific mutation panels are emerging as a cost- and resource-effective approach to simultaneous analysis of multiple potential targets. These are appropriate methods to test for mutations, provided that adequate external quality controls are in place [III, A]
 - Global genomic analysis using next-generation sequencing technology is a valuable research tool but has not yet been validated for clinical use [IV, C]
-

Next-generation sequencing (NGS) is a powerful technology that allows sequencing of large numbers of DNA templates in a single test with high sensitivity and at relatively low cost [86]. Vast amounts of data have been generated using NGS for genome-wide analyses of limited series of NSCLC samples, identifying potentially targetable genetic alterations that may be used to develop companion diagnostic tests for new drugs [87–91].

Multiplex testing platforms (SNaPshot, MassARRAY MALDI-TOF mass spectrometry etc.) for detection of specific alterations [92] or targeted resequencing by NGS allows parallel testing for panels of validated genetic alterations which are therapeutic biomarkers. Published data on NSCLC patients in the usual clinical setting of small biopsy/cytology samples remain relatively few.

Another potentially important application of NGS is ultra-deep sequencing, an extremely high-sensitive approach for detecting mutations correlating with those in the primary tumour, in body fluids, circulating tumour cells (CTCs), plasma or sera [93–95]. Sampling at different time points using this method may help to identify mutations evolving after different lines of treatment.

NGS holds great promise for the future, and although the technology is not yet being routinely used to guide treatment in NSCLC, this will soon change. Problems associated with the uptake of NGS include the lack of central regulation and standardisation for the platforms used, interpretation and validation of findings, reimbursement, DNA requirements and the implications of identifying rare mutations.

Recommendation 8: Should laboratories participate in quality assurance programmes?

- Yes. Laboratories should have accreditation to conduct the test and should participate in external quality assurance to maintain accreditation [V, A]
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Biomarker tests that are required for clinical decision-making should be carried out in laboratories that are compliant with country-specific standards for clinical diagnostic testing. These standards normally require participation and success in an external quality assurance (EQA) programme [96, 97]. A number of different agencies oversee EQA programmes for molecular pathology in Europe (UKNEQAS, ESP, EMQN). Laboratories should also conduct frequent internal quality assurance assessments.

Recommendation 9: How should data from pathological, molecular and other biomarker testing be reported?

- Reports of pathological, molecular and other biomarker testing should include both results and interpretation that are readily understandable by oncologists and by non-specialist pathologists [V, A]
 - Where appropriate, the analytic methodology, nature and quality of the sample should be described
 - Integration of pathology, molecular and other biomarker data for a case, usually in the form of a single report, is recommended [V, A]
-

Reports should be as clear, concise and unambiguous as possible. Recommendations for reporting NSCLC subtypes in small diagnostic samples were mentioned in Recommendation 2 and Table 2. Molecular data should always be reported in conjunction with the pathology of the tested sample and interpreted in that context [1–6], since the relevance of the molecular findings is dependent upon the tumour type and the quality of the test sample. Test methodology determines test sensitivity and specificity and this information should be available, with appropriate explanation, to allow the reader to assess test outcome significance. Sufficient detail should be present in the report to adequately describe test findings, allow their interpretation, and thereby guide therapy. The relevance of the findings to potential therapy choices should be indicated.

conclusion

Molecular pathology and biomarker testing is an important development in the diagnosis and therapeutic stratification of lung cancer. The complexity of molecular abnormalities and their assessment, combined with the challenge of delivering as much robust data as possible on very small diagnostic samples, poses many questions for all involved in this process.

There is no single correct answer as to who should order the test; practice will depend on local organisation and healthcare systems, and decisions about how this is done require discussion between those involved. Reflex testing, ordered by the pathologist on making an appropriate diagnosis expedites testing, leading to faster turnaround time but may result in unnecessary tests being carried out. Bespoke testing, at the behest of the oncologist, should ensure that only tests which are required are carried out, but such testing will occur later, after the initial diagnosis is made.

Until a clinical role has been established for biomarkers in squamous cell carcinoma, routine biomarker testing in lung cancer cases in Europe is largely confined to adenocarcinomas, or in cases where this diagnosis cannot be excluded (non-squamous tumours). The mutual exclusivity, for all practical purposes, of clinically relevant mutations in adenocarcinoma underpins the sequential testing approach where the most common mutation (in Europe, KRAS mutation) would be sought, and if absent, the next most common (EGFR mutation) and so on. While economical in a service using individual mutation tests, this approach is very time-consuming, potentially wasteful of materials and not recommended. Parallel testing of multiple mutations on the same tumour (DNA) sample is becoming the standard, especially where multiplex mutation testing platforms are used. ALK testing may be practiced in parallel, perhaps using an IHC pre-screen, or may follow a negative mutation screen, although the benefits, in terms of time and tissue preservation, of parallel testing have already been discussed. In Recommendation 5, we considered the potential use of sequential testing for rare *ROS1* and *RET* fusions in cases lacking other, more frequent, mutations. In addition, accumulating evidence of change in molecular status post-treatment, and the emergence of molecular resistance may indicate a requirement for repeat biopsy at the time of tumour progression,

if any change in biomarker status indicates a new targeted therapy validated by appropriate data. This is an evolving field.

These recommendations provide a framework for sound molecular pathology testing practice based upon current published evidence and expert opinion. This is a rapidly changing field, due to developments in molecular testing technology and in terms of emerging new targeted therapies which have predictive biomarkers. It is likely that these recommendations will require further updates to reflect changing practice.

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conflict of interest

Prof. Stahel has reported Consultancy/honoraria: Abbott, Amgen, Astellas, Boehringer Ingelheim, Daiichi-Sankyo, GlaxoSmithKline, Genentech, Eli Lilly, Roche. Dr. Felip has reported Consultancy/honoraria: Lilly, GlaxoSmithKline, Pfizer, Roche, Boehringer Ingelheim. Dr. Peters has reported Consultancy/honoraria: Roche, Eli Lilly, AstraZeneca, Pfizer, Boehringer Ingelheim, Bristol-Myers Squibb, Merck Serono, Daiichi Sankyo, Tesaro. Prof. Kerr has reported Advisory Board and/or Speakers' bureau: Abbott Diagnostics, Roche, AstraZeneca, Eli Lilly, Pfizer, Boehringer Ingelheim, Novartis, Merck Serono. Dr. Besse has reported Research grants: Pfizer, Roche, Boehringer Ingelheim, AstraZeneca. Prof. Vansteenkiste has reported that he is the Eli-Lilly Chair in Respiratory Oncology at the Leuven University (research funding) and is the AstraZeneca Chair in Personalised Lung Cancer Care at the Leuven University (research funding). Dr. Eberhardt has reported Advisory board: GSK, Amgen, Novartis, Merck, Teva, Roche, AstraZeneca, Lilly, Boehringer, Pfizer, BMS; Speakers' bureau: Roche, AZ, Lilly, Boehringer, Pfizer, GSK, Amgen, Novartis, Hexal, Merck; Research grants: Eli Lilly. Dr. Edelman has reported Advisory board and/or research funding: Genentech, Boehringer Ingelheim, Lilly, Endocyte. Prof. Baas has reported Research grants: Pfizer. Dr. Reck has reported Advisory board: Hoffmann-La Roche, Lilly, Pfizer, AstraZeneca, Bristol-Myers Squibb, Daiichi-Sankyo; Speakers' honoraria: Hoffmann-La Roche, Lilly, Pfizer, Bristol-Myers Squibb, AstraZeneca, Daiichi-Sankyo. Dr. Paz-Ares has reported Scientific advisor / Speakers' bureau: Lilly, Roche, Pfizer, Merck, Boehringer Ingeheim, Desi pharma, Celgene. Dr. Meldgaard has reported Speakers' bureau: Roche. Dr. Nicolson has reported Speakers' bureau: Roche, Lilly, Boehringer Ingelheim, Pfizer, Otsuka; Research grants: Roche, Lilly, Boehringer Ingelheim, Pfizer, Novartis. Prof. Senan has reported Research grants and honoraria: Varian Medical Systems; member of phase III trial management group conducted by Lilly Oncology. Dr. Faivre-Finn has reported Research grants AstraZeneca, Eli Lilly. Dr. Rocco has reported Speakers' bureau/grants: Covidien. Mr. Lim has reported Research support: ScreenCell and PointHope; previously Speakers' bureau for Roche and Imedex and Advisory board for Strategen, Abbott Molecular and GSK;

patent pending with Clearbridge BioMedics; stock in Pfizer. Dr. Westeel has reported Consultancy/honoraria: Lilly, Roche, Boehringer Ingelheim and AstraZeneca (for lectures); Advisory role: Lilly, Roche and AstraZeneca; currently conducting research sponsored by Merck Serono. Prof. Mok has reported: Speaker's bureau and Honoraria from: AstraZeneca, Roche, Eli Lilly, Merck Serono, Eisai, BMS, BeiGene, AVEO, Pfizer, Taiho, Boehringer Ingelheim, GSK Biologicals, Clovis Oncology; Research funding from AstraZeneca. Prof. Bubendorf has reported Speakers' honoraria: Pfizer, Roche, and Abbott Molecular Inc.; research supported by and stock held in Roche. Dr. Novello, Dr. Marchetti, Prof. Syrigos, Prof. Smit, Dr. Adjei, Prof. Van Schil, Prof. Douillard, Prof. Weder, Prof. De Ruyscher, Dr. Le Pechoux, Prof. De Leyn, Dr. Veronesi and Dr. Doooms have declared no potential conflicts of interest. Dr. Crinò and Prof. O'Byrne have not reported any potential conflicts of interest.

references

- Kerr KM, Loo PS, Nicolson MC. Pathology and personalized medicine in lung cancer. *Lung Cancer Manage* 2013; 2: 35–46.
- Kerr KM. Personalized medicine for lung cancer: new challenges for pathology. *Histopathology* 2012; 60: 531–546.
- Thunnissen E, Kerr KM, Herth FJ et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. *Lung Cancer* 2012; 76: 1–18.
- Cardarella S, Johnson BE. The impact of genomic changes on treatment of lung cancer. *Am J Respir Crit Care Med* 2013; 188: 770–775.
- Cagle PT, Allen TC, Dacic S et al. Revolution in lung cancer: new challenges for the surgical pathologist. *Arch Pathol Lab Med* 2011; 135: 110–116.
- Pirker R, Herth FJF, Kerr KM et al. Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. *J Thorac Oncol* 2010; 5: 1706–1713.
- Lim E, Baldwin D, Beckles M et al. Guidelines on the radical management of patients with lung cancer. *Thorax* 2010; 65(suppl III): iii1–iii27.
- Felip E, Gridelli C, Baas P et al. Metastatic non-small-cell lung cancer: consensus on pathology and molecular tests, first-line, second-line, and third-line therapy: 1st ESMO Consensus Conference in Lung Cancer; Lugano 2010. *Ann Oncol* 2011; 22: 1507–1519.
- Neumeister VM, Anagnostou V, Siddiqui S et al. Quantitative assessment of effect of preanalytic cold ischemic time on protein expression in breast cancer tissues. *J Natl Cancer Inst* 2012; 104: 1815–1824.
- Portier BP, Wang Z, Downs-Kelly E et al. Delay to formalin fixation 'cold ischemia time': effect on ERBB2 detection by in-situ hybridization and immunohistochemistry. *Mod Pathol* 2013; 26: 1–9.
- Engel KB, Moore HM. Effects of preanalytical variables on the detection of proteins by immunohistochemistry in formalin-fixed, paraffin-embedded tissue. *Arch Pathol Lab Med* 2011; 135: 537–543.
- Arber DA. Effect of prolonged formalin fixation on the immunohistochemical reactivity of breast markers. *Appl Immunohistochem Mol Morphol* 2002; 10: 183–186.
- Babic A, Loftin IR, Stanislaw S et al. The impact of pre-analytical processing on staining quality for H&E, dual hapten, dual color in situ hybridization and fluorescent in situ hybridization assays. *Methods* 2010; 52: 287–300.
- Chafin D, Theiss A, Roberts E et al. Rapid two-temperature formalin fixation. *PLoS One* 2013; 8: e54138.
- Lindeman NI, Cagle PT, Beasley MB et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol* 2013; 8: 823–859.

16. Savic S, Bode B, Diebold J et al. Detection of ALK-positive non-small-cell lung cancers on cytological specimens: high accuracy of immunocytochemistry with the 5A4 Clone. *J Thorac Oncol* 2013; 8: 1004–1011.
17. Dejmek A, Zendejrokh N, Tomaszewska M, Edsjö A. Preparation of DNA from cytological material: effects of fixation, staining, and mounting medium on DNA yield and quality. *Cancer Cytopathol* 2013; 121: 344–353.
18. Scagliotti GV, Parikh P, von Pawel J et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naïve patients with advanced-stage non-small cell lung cancer. *J Clin Oncol* 2008; 26: 3543–3551.
19. Johnson DH, Fehrenbacher L, Novotny WF et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small cell lung cancer. *J Clin Oncol* 2004; 22: 2184–2191.
20. Travis WD, Brambilla E, Muller-Hermelink HK et al. (eds). *World Health Organisation Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon: IARC Press 2004.
21. Thomas JS, Lamb D, Ashcroft T et al. How reliable is the diagnosis of lung cancer using small biopsy specimens? Report of a UKCCCR Lung Cancer Working Party. *Thorax* 1993; 48: 1135–1139.
22. Edwards SL, Roberts C, McKean ME et al. Preoperative histological classification of primary lung cancer: accuracy of diagnosis and use of the non-small cell category. *J Clin Pathol* 2000; 53: 537–540.
23. Schreiber G, McCrory DC. Performance characteristics of different modalities for diagnosis of suspected lung cancer: summary of published evidence. *Chest* 2003; 123: 115S–128S.
24. Rivera MP, Detterbeck F, Mehta AC, American College of Chest Physicians. *Diagnosis of lung cancer: the guidelines*. *Chest* 2003; 123: 129S–136S.
25. Burnett RA, Howatson SR, Lang S et al. Observer variability in histopathological reporting of non-small cell carcinoma on bronchial biopsy specimens. *J Clin Pathol* 1996; 49: 130–133.
26. Cataluña JJ, Perpiñá M, Greses JV et al. Cell type accuracy of bronchial biopsy specimens in primary lung cancer. *Chest* 1996; 109: 1199–1203.
27. Matsuda M, Horai T, Nakamura S et al. Bronchial brushing and bronchial biopsy: comparison of diagnostic accuracy and cell typing reliability in lung cancer. *Thorax* 1986; 41: 475–478.
28. Chuang MT, Marchevsky A, Teirstein AS et al. Diagnosis of lung cancer by fiberoptic bronchoscopy: problems in the histological classification of non-small cell carcinomas. *Thorax* 1984; 39: 175–178.
29. Travis WD, Brambilla E, Noguchi M et al. International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol* 2011; 6: 244–285.
30. Travis WD, Brambilla E, Noguchi M et al. Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society Classification. *Arch Pathol Lab Med* 2013; 137: 668–684.
31. Loo PS, Thomas SC, Nicolson MC et al. Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens. *J Thorac Oncol* 2010; 5: 442–447.
32. Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6. *Am J Surg Pathol* 2011; 35: 15–25.
33. Terry J, Leung S, Laskin J et al. Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell carcinomas in small tumor samples. *Am J Surg Pathol* 2010; 34: 1805–1811.
34. Righi L, Graziano P, Fornari A et al. Immunohistochemical subtyping of non-small cell lung cancer not otherwise specified in fine-needle aspiration cytology: a retrospective study of 103 cases with surgical correlation. *Cancer* 2011; 117: 3416–3423.
35. Bishop JA, Teruya-Feldstein J, Westra WH et al. p40 (Δ Np63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol* 2012; 25: 405–415.
36. Wallace WA, Rassi DM. Accuracy of cell typing in non-small cell lung cancer by EBUS/EUS-FNA cytological samples. *Eur Respir J* 2011; 38: 911–917.
37. Sartori G, Cavazza A, Sgambato A et al. EGFR and K-ras mutations along the spectrum of pulmonary epithelial tumors of the lung and elaboration of a combined clinicopathologic and molecular scoring system to predict clinical responsiveness to EGFR inhibitors. *Am J Clin Pathol* 2009; 131: 478–489.
38. Marchetti A, Martella C, Felicioni L et al. EGFR mutations in non-small cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005; 23: 857–865.
39. Rosell R, Moran T, Queralt C et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* 2009; 361: 958–967.
40. Tsao AS, Tang XM, Sabloff B et al. Clinicopathologic characteristics of the EGFR gene mutation in non-small cell lung cancer. *J Thorac Oncol* 2006; 1: 231–239.
41. Pham DK, Kris MG, Riely GJ et al. Use of cigarette-smoking history to estimate the likelihood of mutations in epidermal growth factor receptor gene exons 19 and 21 in lung adenocarcinomas. *J Clin Oncol* 2006; 24: 1700–1704.
42. Rosell R, Carcereny E, Gervais R et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; 13: 239–246.
43. Zhou C, Wu YL, Chen G et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label randomized phase 3 study. *Lancet Oncol* 2011; 12: 735–742.
44. Mok TS, Wu YL, Thongprasert S et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361: 947–957.
45. Mitsudomi T, Morita S, Yatabe Y et al. Gefitinib versus cisplatin plus docetaxel in patients with non small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomized phase 3 trial. *Lancet Oncol* 2010; 11: 121–128.
46. Sequist LV, Yang JC, Yamamoto N et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* 2013; 31: 3327–3334.
47. Li AR, Chitale D, Riely GJ et al. EGFR mutations in lung adenocarcinomas: clinical testing experience and relationship to EGFR gene copy number and immunohistochemical expression. *J Mol Diagn* 2008; 10: 242–248.
48. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007; 7: 169–181.
49. Sequist LV, Waltman BA, Dias-Santagata D et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; 3: 75ra26.
50. Soda M, Choi YL, Enomoto M et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007; 448: 561–566.
51. Shaw AT, Yeap BY, Mino-Kenudson M et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* 2009; 27: 4247–4253.
52. Inamura K, Takeuchi K, Togashi Y et al. EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. *J Thorac Oncol* 2008; 3: 13–17.
53. Takahashi T, Sonobe M, Kobayashi M et al. Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. *Ann Surg Oncol* 2010; 17(3): 889–897.
54. Chaft JE, Rekhman N, Ladanyi M, Riely GJ. ALK-rearranged lung cancer: adenocarcinoma lung cancer masquerading as pure squamous carcinoma. *J Thorac Oncol* 2012; 7: 768–769.
55. Wong DW, Leung EL, So KK et al. University of Hong Kong Lung Cancer Study Group. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. *Cancer* 2009; 115: 1723–1733.
56. Zhang X, Zhang S, Yang X et al. Fusion of EML4 and ALK is associated with development of lung adenocarcinomas lacking EGFR and KRAS mutations and is correlated with ALK expression. *Mol Cancer* 2010; 9: 188.
57. http://www.accessdata.fda.gov/cdrh_docs/pdf11/p110012a.pdf (26 November 2013, date last accessed).
58. http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion_-_initial_authorisation/human/002489/WC500130138.pdf. (26 November 2013, date last accessed).

59. Camidge DR, Kono SA, Flacco A et al. Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. *Clin Cancer Res* 2010; 16: 5581–5590.
60. Wallander ML, Geiersbach KB, Tripp SR, Layfield LJ. Comparison of reverse transcription polymerase chain reaction, immunohistochemistry and fluorescence in situ hybridization methodologies for detection of echinoderm microtubule-associated protein-like 4/anaplastic lymphoma kinase fusion positive non-small cell lung carcinoma: implications for optimal clinical testing. *Arch Pathol Lab Med* 2012; 136: 796–803.
61. Mino-Kenudson M, Chirieac LR, Law K et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. *Clin Cancer Res* 2010; 16: 1561–1571.
62. Park HS, Lee JK, Kim DW et al. Immunohistochemical screening for anaplastic lymphoma kinase (ALK) rearrangement in advanced non-small cell lung cancer patients. *Lung Cancer* 2012; 77: 288–292.
63. Yi ES, Boland JM, Maleszewski JJ et al. Correlation of IHC and FISH for ALK gene rearrangement in non-small cell lung carcinoma: IHC score algorithm for FISH. *J Thorac Oncol* 2011; 6: 459–465.
64. Paik JH, Choe G, Kim H et al. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J Thorac Oncol* 2011; 6: 466–472.
65. Marchetti A, Ardizzone A, Papotti M et al. Recommendations for the analysis of ALK gene rearrangements in non-small-cell lung cancer. A consensus of the Italian Association of Medical Oncology and the Italian Society of Pathology and Cytopathology. *J Thorac Oncol* 2013; 8: 352–358.
66. Thunnissen E, Bubendorf L, Dietel M et al. EML4-ALK testing in non-small cell carcinomas of the lung: a review with recommendations. *Virchows Arch* 2012; 461: 245–257.
67. Wang R, Hu H, Pan Y et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J Clin Oncol* 2012; 30: 4352–4359.
68. Bergethon K, Shaw AT, Ou SH et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* 2012; 30: 863–870.
69. Davies KD, Le AT, Theodoro MF et al. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. *Clin Cancer Res* 2012; 18: 4570–4579.
70. Planchard D, Mazieres J, Riely GJ. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation-positive non-small cell lung cancer (NSCLC) patients. *J Clin Oncol* 2013; 31(Suppl): (abstr 8009).
71. Horn L, Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. *J Clin Oncol* 2009; 27: 4232–4235.
72. Barlesi F, Blon H, Beau-Faller M et al. Biomarkers (BM) France: results of routine EGFR, HER2, KRAS, BRAF, PI3KCA mutations detection and EML4-ALK gene fusion assessment on the first 10,000 non-small cell lung cancer (NSCLC) patients (pts). *J Clin Oncol* 2013; 31(Suppl): (abstr 8000).
73. Olausson KA, Dunant A, Fouret P et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med* 2006; 355: 983–991.
74. Cobo M, Isla D, Massuti B et al. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. *J Clin Oncol* 2007; 25: 2747–2754.
75. Ceppi P, Volante M, Novello S et al. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. *Ann Oncol* 2006; 17: 1818–1825.
76. Papadaki C, Sfakianaki M, Ioannidis G et al. ERCC1 and BRAC1 mRNA expression levels in the primary tumor could predict the effectiveness of the second-line cisplatin-based chemotherapy in pretreated patients with metastatic non-small cell lung cancer. *J Thorac Oncol* 2012; 7: 663–671.
77. Wang L, Wei J, Qian X et al. ERCC1 and BRCA1 mRNA expression levels in metastatic malignant effusions is associated with chemosensitivity to cisplatin and/or docetaxel. *BMC Cancer* 2008; 8: 97.
78. Friboulet L, Olausson KA, Pignon JP et al. ERCC1 isoform expression and DNA repair in non-small-cell lung cancer. *N Engl J Med* 2013; 368: 1101–1110.
79. Bepler G, Williams C, Schell MJ et al. Randomized international phase III trial of ERCC1 and RRM1 expression-based chemotherapy versus gemcitabine/carboplatin in advanced non-small-cell lung cancer. *J Clin Oncol* 2013; 31: 2404–2412.
80. Boukovinas I, Papadaki C, Mendez P et al. Tumor BRCA1, RRM1 and RRM2 mRNA expression levels and clinical response to first-line gemcitabine plus docetaxel in non-small-cell lung cancer patients. *PLoS One* 2008; 3: e3695.
81. Quinn JE, Kennedy RD, Mullan PB et al. BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. *Cancer Res* 2003; 63: 6221–6228.
82. Busacca S, Sheaff M, Arthur K et al. BRCA1 is an essential mediator of vinorelbine-induced apoptosis in mesothelioma. *J Pathol* 2012; 227: 200–208.
83. Paul I, Savage KI, Blayney JK et al. PARP inhibition induces BAX/BAK-independent synthetic lethality of BRCA1-deficient non-small cell lung cancer. *J Pathol* 2011; 224: 564–574.
84. Carser JE, Quinn JE, Michie CO et al. BRCA1 is both a prognostic and predictive biomarker of response to chemotherapy in sporadic epithelial ovarian cancer. *Gynecol Oncol* 2011; 123: 492–498.
85. Nicolson MC, Fennell DA, Ferry D et al. Thymidylate synthase expression and outcome of patients receiving pemetrexed for advanced nonsquamous non-small-cell lung cancer in a prospective blinded assessment phase II clinical trial. *J Thorac Oncol* 2013; 8: 930–939.
86. Ross JS, Cronin M. Whole cancer genome sequencing by next-generation methods. *Am J Clin Pathol* 2011; 136: 527–539.
87. Ding L, Getz G, Wheeler DA et al. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* 2008; 455: 1069–1075.
88. Voelkerding KV, Dames SA, Durtschi JD. Next-generation sequencing: from basic research to diagnostics. *Clin Chem* 2009; 55: 641–658.
89. Imielinski M, Berger AH, Hammerman PS et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* 2012; 150: 1107–1120.
90. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012; 489: 519–525.
91. Govindan R, Ding L, Griffith M et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell* 2012; 150: 1121–1134.
92. Su Z, Dias-Santagata D, Duke M et al. A platform for rapid detection of multiple oncogenic mutations with relevance to targeted therapy in non-small-cell lung cancer. *J Mol Diagn* 2011; 13: 74–84.
93. Buttitta F, Felicioni L, Del Grammastro M et al. Effective assessment of EGFR mutation status in bronchoalveolar lavage and pleural fluids by next-generation sequencing. *Clin Cancer Res* 2013; 19: 691–698.
94. Su KY, Chen HY, Li KC et al. Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small-cell lung cancer. *J Clin Oncol* 2012; 30: 433–440.
95. Punnoose EA, Atwal S, Liu W et al. Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: association with clinical endpoints in a phase II clinical trial of pertuzumab and erlotinib. *Clin Cancer Res* 2012; 18: 2391–2401.
96. van Krieken JH, Normanno N, Blackhall F et al. Guideline on the requirements of external quality assessment programs in molecular pathology. *Virchows Arch* 2013; 462: 27–37.
97. van Krieken JH, Siebers AG, Normanno N. Quality Assurance for Molecular Pathology Group. European Consensus Conference for external quality assessment in molecular pathology. *Ann Oncol* 2013; 24: 1958–1963.
98. Dykewicz CA. Summary of the guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2001; 33: 139–144.

appendix

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