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Review of the use of pretest probability for molecular testing in non-small cell lung cancer and overview of new mutations that may affect clinical practice

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Abstract: This article considers the use of pretest probability in non-small cell lung cancer (NSCLC) and how its use in *EGFR* testing has helped establish clinical guidelines on selecting patients for *EGFR* testing. With an ever-increasing number of molecular abnormalities being identified and often limited tissue available for testing, the use of pretest probability will need to be increasingly considered in the future for selecting investigations and treatments in patients. In addition we review new mutations that have the potential to affect clinical practice.

Keywords: *EGFR* mutation, molecular testing, non-small cell lung cancer, pretest probability

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Introduction

Pretest probability is used in medicine on a daily basis. Clinical guidelines detailing the selection of patients for a particular test are often established based on the pretest probability of having a particular disorder. The importance of pretest probability is increasingly being recognized, particularly with the recent discovery of an increasing number of molecular alterations in patients with non-small cell lung cancer (NSCLC). This is particularly relevant because many of these recently identified gene anomalies [c-ros Oncogene 1, Receptor Tyrosine Kinase Gene (*ROS1*), v-Raf Murine Sarcoma Viral Oncogene Homolog B1 (*BRAF*), Human Epidermal Growth Factor Receptor 2 Gene (*HER2*), MNNG-HOS Transforming Gene (*MET*), Rearranged During Transfection Gene (*RET*)] have been detected in small subsets (1–7%) of patients, and with limited tissue available it is often necessary to use pretest probability to identify patients who are candidates for potential new treatments.[1–5] In this review we discuss the use of pretest probability and how its use in *EGFR* testing has helped establish clinical guidelines on selecting patients for *EGFR* testing. We also discuss new mutations in NSCLC where ongoing clinical trials are establishing targeted therapies. As newer treatments come on-stream for specific molecular abnormalities, the principles of pretest probability testing used in *EGFR* testing may be modified for this use. Recent advances in immunotherapy also need to be now considered when assessing treatment options.

In the last decade, the introduction of molecularly targeted therapies in a wide range of cancers has led to increasing demands on health care resources. Molecular profiling of tumours has aided in the identification of molecular subsets of cancer with distinct biological and clinical characteristics that guide treatment with targeted therapies. This is preferable to traditional chemotherapy, targeting cell cycle, which non-selectively interferes with rapidly dividing cells. The decision to perform molecular testing on a patient is influenced by a number of factors.

A literature search was conducted using PubMed, the proceedings of the American Society of Clinical Oncology annual meeting, the World Conferences on Lung Cancer and www.ClinicalTrials.gov to identify relevant clinical trials.

Pretest probability definition

Pretest probability is the chance of having a disorder before a diagnostic test result is known.[6–10] It

can help decide whether molecular testing should be performed, help select the appropriate test and aid in the interpretation of results. Pretest probability can be calculated from the formula: P(D+) = D+/(D+ + D–), where P(D+) is the probability of the target disorder, D+ equals the number of patients with the target disorder, and D– equals the number of patients without the target disorder.[7] The pretest probability depends not only on the prevalence of a disease, but also on individual factors such as pathologic subtype, environmental and lifestyle factors, ethnicity. The threshold for testing in a clinical setting also depends on several factors, including geographical variation in prevalence, and available funding for testing.[10,11] It is important to recall that the pretest probability of disease is an evolving estimate and can change depending on the results of other investigations. For example, if a patient is being investigated for metastatic NSCLC, the pretest probability of having an epidermal growth factor receptor (*EGFR*) mutation would be 5–15% in an unselected population.[12] However, if following pathological confirmation a diagnosis of metastatic pulmonary adenocarcinoma is made and the patient is Asian, the pretest probability of having an *EGFR* mutation will increase to 36–60%.[13,14] The decision to consider molecular testing depends not only on the pretest probability, but also on the sensitivity and specificity of a test.[15]

Threshold approach

The threshold approach, originally described by Pauker and Kassirer in 1980, uses the physician's estimate of the probability that a patient has a particular disease to determine whether to withhold treatment, perform further testing, or treat without subjecting to further testing.[16] The upper threshold describes a probability of disease greater than the treatment threshold, where no further investigation is required. The lower threshold describes the probability of disease as lower than the treatment threshold. Between the two thresholds lies the testing zone, where testing is performed and treatment is dependent on the test outcome. The testing zone is a function of the test properties, risk attributes of the test relative to the disease, and the risk–benefit profile of available treatment options.[16–18] Although the underlying principle continues to be useful today and helps aid with the interpretation of diagnostic tests, initiating targeted treatment usually requires confirmation of the target molecular aberration. This ensures better identification of potential responders to treatment, is often used for reimbursement of testing and treatment, and avoids the potential for harm of ineffective or inappropriate therapy. For example, advanced NSCLC patients with a high pretest probability of having a tumoral *EGFR* mutation (such as never-smoking Asians with pulmonary adenocarcinoma), but that in fact have *EGFR* wild-type tumours, have inferior progression-free survival and quality of life if treated with initial EGFR-tyrosine kinase inhibitor (EGFR-TKI) instead of platinum-based chemotherapy.[14]

The posttest probability determines the probability of having the disease after the test result is known.[8] The posttest odds of disease can be calculated using Bayes theorem, by multiplying the likelihood ratio (LR) by the pretest probability of having the disease, where the LR is 'the likelihood that a particular test result would be found in a patient with the target disorder, relative to the likelihood of that same test result occurring in a patient without the target disorder'.[19,20] In addition, consideration of the potential harm of missed diagnoses should be considered, as well as the risks and cost that additional biopsies and molecular testing entail. In molecular oncology, clinicians rarely consider that the test result may not reflect the disease status, with little available information on false-positive rates of testing, and more known about the potential for false-negative results.

Prediction models can be useful as a guide to pretest probability; however, including all potential variables that may affect outcome is a limitation of this approach.[21] Rather than single-genotype testing, multiplex-genotype testing will facilitate assessment of multiple simultaneous genes. However, this will also lead to unique challenges as not all mutations are found in the same patient subgroups. For example, not all are seen in non-smokers. Therefore, testing guidelines are being revised to reflect the increasing knowledge that we are gaining regarding molecular subtypes, and incorporate the use of immunotherapy.

Setting parameters

Medical guidelines advising on pretest probability parameters must also consider the potential harm that a missed diagnosis carries. For example, in one study determining testing threshold for computed tomographic angiography (CTA) and D-Dimer in the evaluation of thoracic aortic

dissection (TAD), it was found that CTA should be considered for patients with diagnostic probability >0.3–2.1%, and D-Dimer testing is recommended for pretest probability of 0.01–0.6%.[22] The testing threshold for TAD is low, reflecting the large mortality associated with a potential missed diagnosis. In molecular testing in NSCLC, threshold levels are not quantified to the same extent, perhaps reflecting lack of available prediction models. In the molecular testing guidelines of lung cancer, treatment thresholds are not quantified.[23] Prior to proceeding with *EGFR* mutation testing, physicians consider several variables, including histology, sex, smoking history and ethnicity. These factors when taken together can affect the pretest probability of having an *EGFR* mutation. However, no pretest probability model currently exists which comprises all possible variables that may impact *EGFR* mutation prevalence. However, prevalence parameters are provided and these help guide the recommendations. For example, *EGFR* mutation prevalence is approximately 5% in squamous cell histology, and the guidelines do not recommend testing for *EGFR* mutation unless certain clinical and histological features are present.[23] In the acute medical setting, where a missed diagnosis can carry high acute mortality, guidelines often suggest testing at a much lower frequency. Defining an exact threshold for pretest probability in molecular oncology may not be feasible, not only due to multiple variables that can affect outcome, but also due to the financial implications of testing large cancer subpopulations.

Multiplex-genotype testing will add additional considerations beyond *EGFR* testing and guidelines are currently being revised to reflect these changes.

Real-life examples

While the examples presented here focus on *EGFR* mutations, there is an increasing number of actionable mutations being identified and therefore patient selection will become even more important.

Physicians assessing a patient for molecular testing have guidelines available that have been established by international organizations.[23,24] However, with the accelerated pace of scientific data, guidelines often lag behind, leaving physicians to decide on testing without the support of established consensus on best practice. In addition, guidelines do not account for individual diversity among patients.[25] Difficulty often arises in establishing acceptable pretest probability parameters. For example, in metastatic adenocarcinoma, the pretest probability of a patient having an *EGFR* mutation is approximately 25%. Therefore, *EGFR* genotyping of all pulmonary adenocarcinoma cases is recommended.[25] Whereas, in squamous cell carcinoma, the pretest probability is lower, at 5% [pretest probability (squamous cell carcinoma) = squamous cell carcinoma *EGFR* mutation positive (5%)/squamous cell carcinoma *EGFR* mutation positive (5%) + squamous cell carcinoma *EGFR* mutation negative (95%)]. Testing for *EGFR* mutations in adequate squamous cell carcinoma samples is not routinely recommended due to the low pretest probability, but it could be argued that some patients who may benefit from EGFR-TKIs are being excluded from potentially beneficial treatment, particularly those with characteristics that may increase the pretest probability, such as non-smoking status.

Pretest probability must consider multiple variables in a clinical scenario when making a decision to determine the lower threshold for testing guidelines. For example, consider the pretest probability of having an *EGFR* mutation in three new patients presenting to a thoracic oncology clinic: Patient A, a 40-year-old never-smoking Asian female with a diagnosis of stage IV adenocarcinoma; Patient B, a 68-year-old Caucasian male with a 50 pack per year smoking history diagnosed with metastatic NSCLC, squamous cell histology; and Patient C, a 40-year-old never-smoking Asian female with a diagnosis of stage IV lung squamous cell carcinoma (large tissue sample). The additive effect of each variable in Patient A's case – histological subtype, Asian, female, never-smoker – increases the pretest probability of having an *EGFR* mutation. Guidelines clearly state that *EGFR* mutation testing should be performed to select patients for EGFR-TKI in those with stage IV adenocarcinomas. *EGFR* mutation testing is performed by direct sequencing or real-time quantitative PCR-based approach. For Patient B, when we consider the additional variables along with squamous histology – male, Caucasian, heavy smoking history – the probability of having an *EGFR* mutation is further reduced. The lower threshold for testing is not felt to be breached in Patient B, and patients would therefore not be tested and treated with an EGFR-TKI.

In the case of Patient C, when we assess the individual variables that comprise the pretest probability of this patient: P(D+) = D+/(D+ + D–), female (58/58 + 42) + age (38/38 + 62) + never-smoker (58/58+42) + squamous (5/5 + 95) = 40% – the pretest probability in this clinical scenario would be 40%.[23] In this situation, most clinicians would consider testing for an *EGFR* mutation.

Discussion

Although the threshold for testing cannot always be defined on an individual basis, recommended guidelines often highlight the need to account for additional clinical parameters when making a decision on molecular testing, particularly if this will increase the probability of a positive test. One example of this is when a limited tissue sample exists and where adenocarcinoma cannot be excluded; guidelines suggest: '*EGFR* and *ALK* testing may be performed in cases showing squamous or small cell histology but clinical criteria (eg, young age, lack of smoking history) may be useful in selecting a subset of these samples for testing.'[23] Therefore, the physician must consider all variables that may impact on the pretest probability prior to ordering a molecular test.

The published guidelines on the use of molecular testing for *EGFR* and *ALK* in lung cancer aid the oncologist in the decision-making process around molecular testing.[23] However, adequate tissue samples, qualified laboratories and economic resources differ across regions, and so international guidelines must often be adapted for use in the local clinic.[23] It is recognized that clinical characteristics in patients with adenocarcinoma should not be used to exclude patients for testing, as this could exclude a large number of patients who could potentially benefit from EGFR-TKI therapy. However, other testing strategies are driven by local institutional policy and are often acknowledged in the consensus guidelines. One example of this would involve *EGFR* testing of tumours in patients with stage I, II or III NSCLC. Identification of an *EGFR* mutation in this subgroup will not change therapeutic management, but may have value at a later stage in the event of relapsed disease (common in NSCLC). Therefore testing in these cases is left to the discretion of the individual institution. In addition, although double mutations in *ALK, EGFR* and *KRAS* have been described, these are rare and more commonly are mutually exclusive.[26–29] Therefore, identification of molecular abnormalities where established therapeutic interventions are available should take precedence and further mutation testing following a positive result has limited therapeutic benefit from our current understanding of molecular abnormalities in NSCLC. Although the guidelines advise on patient selection for testing, cases should also be considered on an individual basis, particularly where there is a suspicion of a high pretest probability of having a mutation and where guidelines are not always clear.

Overview of other mutations affecting clinical practice

Several other biomarkers have been investigated and may affect clinical practice. Therefore the concepts of pretest probability should be considered. Some of these biomarkers include the following.

ALK

The *EML4–ALK* fusion gene results when echinoderm microtubule-associated protein-like 4 (*EML4*) gene is fused to the anaplastic lymphoma kinase (*ALK*) gene.[30] *ALK* translocations have been described in 2–7% of NSCLC patients and is more commonly seen in adenocarcinoma histology and never- or light smokers.[31–33] Two phase III studies in the first- and second-line setting of crizotinib *versus* chemotherapy in *ALK*-positive patients have demonstrated an improvement in progression-free survival (PFS) and response rate (RR) in patients treated with crizotinib.[32,34] No difference in overall survival (OS) was seen, which was likely a result of patients being allowed to cross over to crizotinib following progression.

Resistance to crizotinib eventually develops, with secondary *ALK* mutations being one of the main mechanisms of resistance. Second-generation ALK inhibitors (e.g. ceritinib, alectinib, brigatinib) have demonstrated promising results in patients treated following progression on crizotinib in *ALK*-positive patients, with median PFS greater than 5.7 months.[35–37,38] These second-generation ALK inhibitors have greater specificity than crizotinib and do not inhibit MET and ROS1. In addition, they are able to cross the blood–brain barrier.

Brigatinib, an oral tyrosine kinase inhibitor, has demonstrated promising results in a phase I/II

trial. Patients with prior crizotinib exposure demonstrated RR 72% (95% CI, 60–82%) and median PFS 13.2 months (95% CI, 9.2–NR).[37] Trials are ongoing comparing these second-generation ALK inhibitors in the first-line setting with crizotinib in *ALK*-positive patients. Significant PFS has been demonstrated in a phase III Japanese trial comparing alectinib with crizotinib in the first-line setting.[39] However, we will have to wait for the completion of trial data to assess survival outcomes in order to know the optimal timing of treatments in the first- and second-line setting of *ALK*-positive patients and those who develop resistance to treatment.

KRAS mutation

The RAS oncogene family, including KRAS, NRAS and HRAS, are membrane-bound intracellular GTPase which act as central mediators activating multiple downstream pathways including RAF (MAP kinase pathway), PI3K (AKT/MTOR pathway), ERK, RLIP and RALGDS, which regulate cell proliferation, apoptosis, angiogenesis and cellular metabolism.[40] *KRAS* mutations are the most common mutation in lung cancer and are more frequently seen in adenocarcinoma (25%) *versus* squamous histology (5%), non-Asian ethnic origin and in current or ex-smokers.[41–43] Studies to date have not conclusively identified KRAS as a prognostic or predictive marker, with the exception of negative predictive value of *KRAS* mutations and response to EGFR-TKI.[44–45] The majority of *KRAS* mutations in NSCLC are single amino acid substitutions in codon 12 and to a lesser extent in codon 13 and 61.[46]

Therapeutic targets to date inhibiting KRAS have largely been discouraging, likely as a result of its functional complexity. *KRAS* mutations can be associated with other genetic alterations and these different genetic profile combinations may require alternative therapeutics.[42]

Phase II trials of the MEK inhibitor selumetinib plus docetaxel demonstrated promising survival results in *KRAS*-mutant NSCLC.[47] However, in the phase III, double-blind, randomized SELECT-1 trial, selumitinib plus docetaxel did not significantly improve PFS, OS or objective response rate (ORR) *versus* placebo plus docetaxel.[48]

While *KRAS* remains an elusive target for therapy, it could potentially affect the testing algorithm as many mutations are mutually exclusive with these mutations.

MET amplification or mutation

MET is a tyrosine kinase receptor for the ligand HGF.[49] Activation of the receptor results in downstream signalling pathways [mitogen-activated protein kinase (MAPK), PI3K (phosphoinositide 3-kinase protein kinase B)/AKT, signal transducer and activator of transcription proteins (STAT) and nuclear factor-κB].[50] MET abnormalities can result from gene amplification, exon 14 splice mutations of the MET receptor gene or decreased degradation.[51] *MET* amplification occurs in approximately 2–4% of lung cancer cases, and in addition might contribute to progression after treatment with EGFR inhibitors in 5–20% of patients (www.mycancergenome.org/content/disease/lung-cancer/met/59). Exon 14 *MET* splice mutations occur in 3% of patients with lung cancer.[52,53] MET inhibitors include monoclonal antibodies targeting HGF or the MET receptor (e.g. onartuzumab, ficlatuzumab) or MET TKIs (foretinib, crizotinib, tivantinib, and cabozantinib).[41,51] A phase II trial comparing onartuzumab plus erlotinib *versus* erlotinib alone in pretreated patients with advanced NSCLC demonstrated an improvement in PFS and OS in the MET-positive population.[54] However, a phase III trial was stopped early due to futility, as the addition of onartuzumab to erlotinib did not improve PFS, ORR or OS, even in patients with high expression of MET.[55]

Crizotinib, which inhibits cMET, ALK and ROS-1, demonstrated promising anti-tumour activity in patients with cMET amplified NSCLC in a small (n = 13) pilot study.[56] In the expansion cohort of the PROFILE 1001 study, 10 of 15 patients with *MET* exon 14-altered NSCLC demonstrated anti-tumour activity.[57]

MET exon 14 skipping mutations occur in 3% of patients with NSCLC. Early studies have demonstrated promising results in patients with *MET* exon 14 skipping mutations treated with crizotinib and cabozantinib.[58,59]

cMET dysregulation is seen in 15–20% of NSCLC patients with acquired EGFR-TKI resistance. Capmatinib (INC280) is a highly selective cMET inhibitor.[60] A phase Ib/II trial investigated the safety and efficacy of capamatinib plus gefitinib in *EGFR* mutant, cMET positive

NSCLC who progressed on gefitinib, erlotinib, or afatinib. In the phase II expansion phase, capamatinib plus gefitinib was well-tolerated and demonstrated encouraging clinical activity, particularly in patients with high cMET GCN (gene copy number) [partial responses in 12/65 evaluable patients (ORR 18%) and 40/65 (62%) patients had stable disease (SD); 10/53 patients with IHC 3+ or IHC 2+ and GCN ⩾5 had PRs (ORR 19%) and 7/23 patients with GCN ⩾6 had PRs (ORR 30%)].

ROS-1 rearrangements

The *ROS-1* oncogene encodes an orphan receptor tyrosine kinase with structural similarity to the ALK.[61] It occurs in approximately 2% of NSCLC patients.[1] Characteristics of *ROS-1* patients are similar to those with *ALK*-rearrangements – young, never-smokers and adenocarcinomas.[1] *ROS-1* rearrangements have been reported to be mutually exclusive with other oncogenic driver alterations (*EGFR, KRAS, ALK, HER2, RET*).[62,63] Nine fusion protein variants have been described in NSCLC; fluorescence *in-situ* hybridization (FISH) assay is currently considered the gold standard for *ROS-1* fusion detection.[1,43,61] A phase I expansion phase cohort study – 50 patients with advanced NSCLC who were pretreated with chemotherapy – demonstrated an ORR of 72% (95% CI, 58–84) and median PFS of 19.2 months (95% CI, 14.4 not reached).[61] A retrospective European study of 31 patients with *ROS-1* rearrangement treated with crizotinib demonstrated a median PFS of 9.1 months.[64] Other *ROS-1* inhibitors are being investigated.

Conclusion

Pretest probability by definition is a concise number. However, in clinical practice it often cannot be determined precisely due to individual variation.[9] The concept of using pretest estimates of disease probability is widely used in practice, including in molecular oncology. This allows a broad identification of the most suitable patients to test, but pressing questions remain in the current economic climate. These include questions prior to ordering molecular testing, such as whether a positive test result will impact a treatment decision. And if a test is not performed (due to a low estimated pretest probability), could a missed positive result have an adverse effect on the individual patient's outcome?

Traditionally, diagnostic testing has been performed when it will change medical management or provide further information regarding a patient's prognosis.[18] However, in many cancer centres, multiplex genotyping or sequencing assays that test a large number of genes simultaneously are offered to oncology patients. Turnaround time for results with these assays may be several weeks, and many mutations identified have no proven therapeutic options. Although the identification of such genomic abnormalities may add scientific merit to future research, molecular testing where potential therapeutic interventions are available should take precedence. In those jurisdictions where testing resources are limited, pretest probabilities of positive results may be important to factor into policy decisions about molecular testing. An increasing number of molecular abnormalities have been identified in NSCLC patients, and also there has been an increasing interest in using circulating DNA for the diagnosis and monitoring of disease. Currently no guidelines have been established to guide the clinician on which patients to test for these new anomalies. In situations where limited tissue is available, it is likely that results from clinical trials will provide information on pretest probability and allow the establishment of clinical guidelines. This will enable the clinician to advise patients of the potential benefits, risks and limitations of testing.

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References

1. 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.

2. Paik PK, Arcila ME, Fara M, *et al.* Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J Clin Oncol* 2011; 29: 2046–2051.

3. Heinmöller P, Gross C, Beyser K, *et al.* HER2 status in non-small cell lung cancer: results from patient screening for enrollment to a phase II

study of herceptin. *Clin Cancer Res* 2003; 9: 5238–5243.

4. Ou SH, Kwak EL, Siwak-Tapp C, *et al.* Activity of crizotinib (PF02341066), a dual mesenchymal–epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a nonsmall cell lung cancer patient with de novo MET amplification. *J Thorac Oncol* 2011; 6: 942–946.
5. Ju YS, Lee WC, Shin JY, *et al.* A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. *Genome Res* 2012; 22: 436–445.
6. Attia JR, Nair BR, Sibbritt DW, *et al.* Generating pre-test probabilities: a neglected area in clinical decision making. *Med J Aust* 2004; 180: 449–454.
7. Centre for Evidence Based Medicine. *Pretest probability*, www.cebm.net/pre-test-probability (accessed 7 January 2017).
8. Akobeng AK. Understanding diagnostic tests 2: likelihood ratios, pre- and post-test probabilities and their use in clinical practice. *Acta Paediatr* 2007; 96: 487–491.
9. Reed MH. Pretest probability: should we care? *J Am Coll Radiol* 2013; 10: 486–487.
10. Richardson WS. Five uneasy pieces about pre-test probability. *J Gen Intern Med* 2002; 17: 882–883.
11. Kroenke K. Diagnostic testing and the illusory reassurance of normal results: comment on 'reassurance after diagnostic testing with a low pretest probability of serious disease'. *JAMA Intern Med* 2003; 173: 416–417.
12. Perez-Moreno P, Brambilla E, Thomas R, *et al.* Squamous cell carcinoma of the lung: molecular subtypes and therapeutic opportunities. *Clin Cancer Res* 2012; 18: 2443–2451.
13. Tsao MS, Sakurada A, Cutz JC, *et al.* Erlotinib in lung cancer: molecular and clinical predictors of outcome. *N Engl J Med* 2005; 353: 133–144.
14. Mok TS, Wu YL, Thongprasert S, *et al.* Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361: 947–957.
15. Agoritsas T, Courvoisier DS, Combescure C, *et al.* Does prevalence matter to physicians in estimating post-test probability of disease? A randomized trial. *J Gen Intern Med* 2011; 26: 373–378.
16. Pauker SG and Kassirer JP. The threshold approach to clinical decision making. *N Engl J Med* 1980; 302: 1109–1117.
17. Cahan A, Gilon D, Manor O, *et al.* Probabilistic reasoning and clinical decision-making: do doctors overestimate diagnostic probabilities? *QJM* 2003; 96: 763–769.
18. Gilbert R, Logan S, Moyer VA, *et al.* Assessing diagnostic and screening tests: part 1 – concepts. *West J Med* 2001; 174: 405–409.
19. Gallagher EJ. Clinical utility of likelihood ratios. *Ann Emerg Med* 1998; 31: 391–397.
20. Phelps MA and Levitt MA. Pretest probability estimates: a pitfall to the clinical utility of evidence-based medicine? *Acad Emerg Med* 2004; 11: 692–694.
21. Girard N, Sima CS, Jackman DM, *et al.* Nomogram to predict the presence of EGFR activating mutation in lung adenocarcinoma. *Eur Resp J* 2012; 39: 366–372.
22. Taylor RA and Iyer NS. A decision analysis to determine a testing threshold for computed tomographic angiography and D-dimer in the evaluation of aortic dissection. *Am J Emerg Med* 2013; 31: 1047–1055.
23. 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.
24. Ellis PM, Blais N, Soulieres D, *et al.* A systematic review and Canadian consensus recommendations on the use of biomarkers in the treatment of non-small cell lung cancer. *J Thorac Oncol* 2011; 6: 1379–1391.
25. Taron M, Ichinose Y, Rosell R, *et al.* Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor are associated with improved survival in gefitinib-treated chemorefractory lung adenocarcinomas. *Clin Cancer Res* 2005; 11: 5878–5885.
26. Zhou JX, Yang H, Deng Q, *et al.* Oncogenic driver mutations in patients with non-small-cell lung cancer at various clinical stages. *Ann Oncol* 2013; 24: 1319–1325.
27. Schmid K, Oehl N, Wrba F, *et al.* EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. *Clin Cancer Res* 2009; 15: 4554–4560.
28. Zhu CQ, da Cunha Santos G, Ding K, *et al.* Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group

Study BR.21. *J Clin Oncol* 2008; 26: 4268–4275.

29. Shaozhang Z, Xiaomei L, Aiping Z, *et al.* Detection of EML4–ALK fusion genes in non-small cell lung cancer patients with clinical features associated with EGFR mutations. *Genes Chromosomes Cancer* 2012; 51: 925–932.

30. Katayama R, Lovly CM and Shaw AT. Therapeutic targeting of anaplastic lymphoma kinase in lung cancer: a paradigm for precision cancer medicine. *Clin Cancer Res* 2015; 21: 2227–2235.

31. Kwak EL, Bang YJ, Camidge DR, *et al.* Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010; 363: 1693–1703.

32. Shaw AT, Kim DW, Nakagawa K, *et al.* Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* 2013; 368: 2385–2394.

33. 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.

34. Solomon BJ, Mok T, Kim DW, *et al.* First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* 2014; 371: 2167–2177.

35. Kim DW, Mehra R, Tan DS, *et al.* Activity and safety of ceritinib in patients with ALK-rearranged non-small-cell lung cancer (ASCEND-1): updated results from the multicentre, open-label, phase 1 trial. *Lancet Oncol* 2016; 17: 452–463.

36. Ou SH, Ahn JS, De Petris L, *et al.* Alectinib in crizotinib-refractory ALK-rearranged non-small-cell lung cancer: a phase II global study. *J Clin Oncol* 2016; 34: 661–668.

37. Langer CJ, Gettinger SN, Bazhenova L, *et al.* Activity and safety of brigatinib (BRG) in patients (pts) with ALK+ non-small cell lung cancer (NSCLC): phase (ph) 1/2 trial results. *J Clin Oncol* 2016; 34(Suppl. 15): abstract 9057.

38. Mok T, Spigel D, Felip E, *et al.* ASCEND-2: a single-arm, open-label, multicenter phase II study of ceritinib in adult patients (pts) with ALK-rearranged (ALK+) non-small cell lung cancer (NSCLC) previously treated with chemotherapy and crizotinib (CRZ). *J Clin Oncol* 2015; 33(Suppl.): abstract 8059.

39. Nokihara H, Hida T, Kondo M, *et al.* Alectinib (ALC) versus crizotinib (CRZ) in ALK-inhibitor naive *ALK*-positive non-small cell lung cancer (*ALK*+ NSCLC): primary results from the J-ALEX study. *J Clin Oncol* 2016; 34(Suppl.): abstract 9008.

40. Bhattacharya S, Socinski MA and Burns TF. KRAS mutant lung cancer: progress thus far on an elusive therapeutic target. *Clin Transl Med* 2015; 4: 35.

41. Korpanty GJ, Graham DM, Vincent MD, *et al.* Biomarkers that currently affect clinical practice in lung cancer: EGFR, ALK, MET, ROS-1, and KRAS. *Front Oncol* 2014; 11: 204.

42. Hirsch FR, Suda K, Wiens J, *et al.* New and emerging targeted treatments in advanced non-small-cell lung cancer. *Lancet* 2016; 388: 1012–1024.

43. Hirsch FR, Scagliotti GV, Mulshine JL, *et al.* Lung cancer: current therapies and new targeted treatments. *Lancet* 2017; 389: 299–311.

44. Linardou H, Dahabreh IJ, Kanaloupiti D, *et al.* Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. *Lancet Oncol* 2008; 9: 962–972.

45. Mao C, Qiu LX, Liao RY, *et al.* KRAS mutations and resistance to EGFR-TKIs treatment in patients with non-small cell lung cancer: a meta-analysis of 22 studies. *Lung Cancer* 2010; 69: 272–278.

46. Prior IA, Lewis PD and Mattos C. A comprehensive survey of Ras mutations in cancer. *Cancer Res* 2012; 72: 2457–2467.

47. Jänne PA, Shaw AT, Pereira JR, *et al.* Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol* 2013; 14: 38–47.

48. Jänne PA, van den Heuvel M, Barlesi F, *et al.* Selumetinib in combination with docetaxel as second-line treatment for patients with KRAS-mutant advanced NSCLC: results from the phase III SELECT-1 trial. *Ann Oncol* 2016; 27(Suppl. 6): abstract LBA47_PR.

49. Ma PC, Maulik G, Christensen J, *et al.* c-Met: structure, functions and potential for therapeutic inhibition. *Cancer Metastasis Rev* 2003; 22: 309–325.

50. Garajová I, Giovannetti E, Biasco G, *et al.* c-Met as a target for personalized therapy. *Transl Oncogenomics* 2015; 7(Suppl. 1): 13–31.

51. Sadiq AA and Salgia R. MET as a possible target for non-small-cell lung cancer. *J Clin Oncol* 2013; 31: 1089–1096.

52. Onozato R, Kosaka T, Kuwano H, *et al.* Activation of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers. *J Thorac Oncol* 2009; 4: 5–11.

53. Awad MM, Oxnard GR, Jackman DM, *et al.* MET exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and c-Met overexpression. *J Clin Oncol* 2016; 34: 721–730.

54. Spigel DR, Ervin TJ, Ramlau R, *et al.* Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. *ASCO Meet Abstr* 2011; 29(Suppl. 15): abstract 7505.

55. Spigel DR, Edelman MJ, O'Byrne K, *et al.* Onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIb or IV NSCLC: results from the pivotal phase III randomized, multicenter, placebo-controlled METLung (OAM4971g) global trial. *ASCO Meet Abstr* 2014; 32(Suppl. 15): abstract 8000.

56. Camidge DR, Ou S-HI, Shapiro G, *et al.* Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC). *ASCO Meet Abstr* 2014; 32(Suppl. 15): abstract 8001.

57. Drilon AE, Ross Camidge D, Ignatius Ou SH, *et al.* Efficacy and safety of crizotinib in patients (pts) with advanced *MET* exon 14-altered non-small cell lung cancer (NSCLC). *J Clin Oncol* 2016; 34(Suppl.): abstract 108.

58. Paik PK, Drilon AE, Yu HA, *et al.* Response to crizotinib and cabozantinib in stage IV lung adenocarcinoma patients with mutations that cause MET exon 14 skipping. *J Clin Oncol* 2015; 33: abstract 8021.

59. Frampton GM, Ali SM, Rosenzweig M, *et al.* Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Discov* 2015; 5: 850–859.

60. Wu YL, Kim DW, Felip E, *et al.* Phase (Ph) II safety and efficacy results of a single-arm Ph Ib/II study of capmatinib (INC280) + gefitinib in patients (pts) with EGFR-mutated (mut), cMET+ non-small cell lung cancer (NSCLC). *ASCO Meet Abstr* 2016; 34(Suppl.): abstract 9020.

61. Shaw AT, Ou SH, Bang YJ, *et al.* Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* 2014; 371: 1963–1971.

62. Cha YJ, Lee JS, Kim HR, *et al.* Screening of ROS1 rearrangements in lung adenocarcinoma by immunohistochemistry and comparison with ALK rearrangements. *PLoS One* 2014; 9: e103333.

63. Yoshida A, Kohno T, Tsuta K, *et al.* ROS1-rearranged lung cancer: a clinicopathologic and molecular study of 15 surgical cases. *Am J SurgPathol* 2013; 37: 554–562.

64. Mazières J, Zalcman G, Crinò L, *et al.* Crizotinib therapy for advanced lung adenocarcinoma and a ROS1 rearrangement: results from the EUROS1 cohort. *J Clin Oncol* 2015; 33: 992–999.