The evolving genomic classification of lung cancer

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Abstract

EGFR gene mutations and ALK gene fusions are well-characterized molecular targets in NSCLC. Activating alterations in a variety of potential oncogenic driver genes have also been identified in NSCLC, including ROS1, RET, MET, HER2, and BRAF. Together with EGFR and ALK, these mutations account for ~20% of NSCLCs. The identification of these oncogenic drivers has led to the design of rationally targeted therapies that have produced superior clinical outcomes in tumours harbouring these mutations. Many patients, however, have de novo or acquired resistance to these therapies. In addition, most NSCLCs are genetically complex tumours harbouring multiple potential activating events. For these patients, disease subsets are likely to be defined by combination strategies involving a number of targeted agents. These targets include FGFR1, PTEN, MET, MEK, PD-1/PD-L1, and NaPi2b. In light of the myriad new biomarkers and targeted agents, multiplex testing strategies will be invaluable in identifying the appropriate patients for each therapy and enabling targeted agents to be channelled to the patients most likely to gain benefit. The challenge now is how best to interpret the results of these genomic tests, in the context of other clinical data, to optimize treatment choices in NSCLC.

Keywords: ALK; biomarkers; EGFR; genomic classification; molecular targets; monoclonal antibodies; multiplex testing; lung cancer; NSCLC; tyrosine-kinase inhibitors

Introduction

Historically, lung cancers have been sub-divided by histology into small-cell and non-small-cell lung cancers (NSCLCs) [1], with NSCLC further classified into squamous cell carcinoma (SCC), large-cell carcinoma, and adenocarcinoma. More than half of all lung cancers are adenocarcinomas [2]. While treatment advances have been made with the use of platinum-based chemotherapy [3], lung cancer remains the most frequent cause of cancer-related mortality worldwide [4] and has a 5-year overall survival (OS) rate of just 16% for all stages [5].

Crucial to enhancing outcomes for patients with lung cancer is the ability to build a detailed profile of the disease, to guide treatment decisions and to enable the development of more effective therapeutic strategies. The last decade has seen a shift to a more molecular-based classification, in which information about genetic alterations and protein expression level is considered alongside histology in order to better understand the pathogenesis of the disease [6,7].

In NSCLC, multiple genetic alterations have already been identified as therapeutic targets, including mutations of the epidermal growth factor receptor (EGFR) gene and rearrangements of the anaplastic lymphoma kinase (ALK) gene. Drugs designed specifically as inhibitors of these molecular targets have significantly extended the survival times for patients with NSCLC whose tumours harbour these mutations [8–14].

As novel molecular targets are discovered, and ultimately new therapies developed, we may edge ever closer to a personalized treatment approach in NSCLC and further extend survival for patients. Within this article, we review recently identified molecular targets in NSCLC, new genetic techniques for classifying the disease, and the implications of these findings for clinical practice and future clinical trial design.
patients with rationally targeted therapies (ie drugs directed against activated oncogenes, such as EGFR or ALK gene-fusion products) as opposed to the modest benefits achieved in unselected patients [15]. However, de novo or acquired resistance often develops, driving the search for novel targets and treatment mechanisms. In addition, EGFR and ALK alterations account for only a small minority of NSCLC cases, and both alterations occur predominantly in adenocarcinomas from non-smokers [11,16]. At present, the community does not have an answer for patients who have or will get lung cancer as a result of exposure to tobacco carcinogens.

Modern treatment strategies focus on the pathological classification of NSCLC, which includes assessment of protein expression by immunohistochemistry (IHC) to assess cell differentiation markers such as TTF1 and p63 (the splice variant p40), as well as the detection of molecular predictive markers, including validated driver mutations in genes involved in cell growth and survival. A variety of novel driver mutations or molecular targets have recently been identified in NSCLC (Figure 1 and Table 1). Here, we review some of these key targets (and interventions), including known oncogenic drivers (EGFR, ALK, ROS1, and RET), non-driver targets [MET, fibroblast growth factor receptor 1 (FGFR1), PTEN, and phosphatidylinositol 3-kinase (PI3K)], immunotherapies [programmed death ligand 1 (PD-L1/PD-1)], and antibody–drug conjugates (ADCs; NaPi2b). Mutations of a number of other important molecular targets identified in NSCLC, such as HER2, BRAF, and MEK1 (Table 1), have been described in detail elsewhere [2,15,17] and will not therefore form the focus of this review. In addition, the RAS oncogenes (KRAS and NRAS) have been excluded from this review, as we are not aware of any molecules in clinical development that directly inhibit RAS.

Oncogenic drivers

For the purposes of this review, a target was considered an oncogenic driver if it is genetically activated in NSCLC and if there is an approved inhibitor (clinically validated target) or convincing proof-of-concept data (high response rates in a targeted population or a positive randomized phase II trial).

EGFR

Mutations of the EGFR gene are a well-established example of an oncogenic driver in NSCLC. EGFR activating mutations are present in ~10% of NSCLCs in Caucasians and ~40% in Asian patients, and are primarily seen in adenocarcinomas [18]. In prospective phase III trials, patients with previously untreated EGFR mutation-positive NSCLC achieved significantly longer progression-free survival (PFS) with the reversible EGFR tyrosine-kinase inhibitors (TKIs) erlotinib and gefitinib than with platinum-doublet chemotherapy [8,12,14]. Erlotinib has been approved by the FDA for the first-line treatment of patients with EGFR activating mutation-positive NSCLC detected by the approved cobas® EGFR Mutation Test. Several other platforms (mostly sequencing assays) are

Figure 1. Evolving genomic classification of NSCLC. Li T et al: J Clin Oncol 2013; 31: 1039–1049. Reprinted with permission. © 2013 by American Society of Clinical Oncology. All rights reserved [17].
used to study EGFR mutations in DNA extracted from tumour tissue specimens. Gefitinib is also approved as monotherapy for EGFR mutation-positive NSCLC following failure of platinum- and docetaxel-based chemotherapy.

The second-generation irreversible EGFR TKI afatinib recently gained FDA approval as first-line therapy for EGFR mutation-positive NSCLC in conjunction with Qiagen’s therascreen PCR diagnostic test. Another second-generation irreversible EGFR TKI, dacomitinib, demonstrated preclinical efficacy in NSCLC tumours harbouring the T790M gatekeeper mutation [19,20], which is present in ~50% of NSCLCs that have acquired resistance to erlotinib or gefitinib [21,22]. In a randomized phase II study, dacomitinib demonstrated significantly improved PFS versus erlotinib in patients with advanced NSCLC [23]. A phase III study of dacomitinib versus erlotinib as second-/third-line therapy for advanced NSCLC is currently underway (NCT01360554) [24].

**ALK**

Rearrangements of the **ALK** gene are another recent example of oncogenic drivers in NSCLC. **ALK** is a transmembrane tyrosine-kinase receptor expressed in the small intestine, testes, and brain, but not normally in the lung. In NSCLC, **ALK** signalling is activated by the creation of oncogenic fusions of the **ALK** gene with an upstream partner, **EML4** [25], although other fusion partners exist [26]. **EML4**–**ALK** rearrangements occur in 2–7% of NSCLC patients [11,27], usually in young never-smokers with adenocarcinoma [28–31]. **ALK**-rearranged tumours are resistant to the EGFR TKIs gefitinib and erlotinib [28].

The first-in-class **ALK** inhibitor crizotinib was approved by the FDA for the treatment of **ALK**-positive advanced NSCLC, with the concurrent approval of a companion fluorescence in situ hybridization (FISH) diagnostic test, based on impressive results in phase I/II trials. In the subsequent phase III trial, **ALK**-rearranged tumours demonstrated superior PFS and response rates to chemotherapy alone in patients with locally advanced or metastatic **ALK**-positive NSCLC [13]. A first-line phase III trial of crizotinib in newly diagnosed **ALK**-positive NSCLC is currently recruiting patients (NCT01154140). Results of a recent study confirm that **ALK** rearrangements in lung adenocarcinoma can also be effectively detected using IHC for **ALK** expression in malignant cells [32].

**ROS1**

**ROS1** is a tyrosine-kinase receptor of the insulin receptor family. **ROS1** gene rearrangements are known oncogenic drivers in NSCLC, and several fusion partners have been identified, including **CD74**, **SLC34A2/NaPi2b**, and FIG [33,34]. **ROS1** fusions are present in ~2% of NSCLC cases and are often seen in young never-smokers with adenocarcinoma, a population similar to those with **ALK**-rearranged NSCLC [33]. **ROS1** rearrangements rarely present simultaneously with **EGFR**, **ALK** or **Kras** alterations [35].

Crizotinib has shown inhibitory growth effects on **ROS1**-positive cell lines, and a near-complete response was reported in a patient with advanced **ROS1**-positive NSCLC treated with crizotinib in a phase I clinical trial [33]. In an expansion cohort of the trial, 14 patients received crizotinib for **ROS1**-rearranged NSCLC (as tested by FISH) and nine (64%) had a confirmed response [36]. A further case of a complete metabolic response to crizotinib was reported in a patient with advanced **ROS1**-positive NSCLC [37]. A **ROS1** monoclonal antibody (D4D6) has recently been developed and validated for use in IHC assays [34].

**RET**

The tyrosine-kinase receptor **RET** is involved in cell proliferation, migration, and differentiation. A novel fusion oncogene between the **RET** gene and **KIF5B** was recently described in a young never-smoker with adenocarcinoma and no family history of lung cancer [38,39]. Fusions between the **RET** gene and **CCDC6** have since been identified [40]. **RET** fusions are known to occur in ~2% of lung adenocarcinomas [38], are usually independent of other oncogenic drivers [35], and can be targeted with TKIs such as sunitinib, sorafenib, vandetanib, and cabozantinib [15,41]. Preliminary data have been published for the first three patients with **RET** fusion-positive NSCLC enrolled in a phase II trial of cabozantinib; confirmed partial responses occurred in two patients (one with a novel **TRIM33**–**RET** fusion), with prolonged disease stabilization (31 weeks) in the third patient [42].
Other targets

MET

Binding of the hepatocyte growth factor (HGF) to the transmembrane tyrosine-kinase receptor MET activates multiple signalling pathways involved in cell proliferation, survival, motility, and invasion [43]. Dysregulation of the MET/HGF pathway can occur via several mechanisms and is observed in many human malignancies, including NSCLC [43]. Mutations in MET are rare, but high MET gene copy number has been detected in 1–11% of NSCLC cases and is often associated with high MET protein expression and poor prognosis [44–46]. MET amplifications have also been linked with secondary resistance to EGFR TKIs in patients with EGFR mutation-positive NSCLC [47,48]; MET amplifications can be found in up to 20% of these patients [45].

A number of therapeutic agents targeting the MET/HGF pathway are in clinical development, including small molecule MET inhibitors (eg cabozantinib), specific MET TKIs (eg crizotinib), antagonistic antibodies against MET (eg onartuzumab), and neutralizing antibodies against HGF (eg rilotumumab). Although tivantinib was initially believed to be a MET inhibitor, several recent reports suggest that, at least preclinically, tivantinib does not appear to inhibit MET signalling [49,50].

Onartuzumab is a humanized monovalent (one-armed) monoclonal antibody that binds to the extra-cellular domain of MET to prevent HGF binding and activation [51,52]. In a randomized phase II trial, onartuzumab plus erlotinib improved PFS and OS versus placebo plus erlotinib in patients with tumours predefined as MET-positive by IHC (≥ 50% of tumour cells expressing moderate-to-strong staining intensity) [53]. Clinical outcomes were worse in MET-negative patients treated with onartuzumab plus erlotinib, exemplifying the need for parallel diagnostic testing in drug development [54]. A randomized phase III study is investigating the combination of onartuzumab and erlotinib in patients with MET-positive advanced or metastatic NSCLC (NCT01456325). The MET IHC assay is being developed as a companion diagnostic for onartuzumab within this study, based on the 50% cut-off used in the phase II trial [54].

FGFR1

FGFR1 is a membrane-bound tyrosine-kinase receptor involved in the regulation of cell proliferation and angiogenesis [15]. FGFR1 amplification occurs more frequently in SCC (21%) than in adenocarcinoma (3%) [55]. Activation of FGFR2 and FGFR3 has also been reported in NSCLC cell lines treated with EGFR inhibitors [56]. Recently, high FGFR1 gene copy number was reported to be an independent favourable prognostic factor in NSCLC [57].

Several small molecule FGFR TKIs are currently under clinical investigation, including AZD4547, a selective inhibitor of FGFR1/2/3 [58,59], and S49076, an ATP-competitive TKI of MET, AXL, and FGFR1/2/3, which demonstrated marked antitumour activity in MET- and FGFR-dependent tumour xenografts [56]. In addition, the novel FGFR inhibitor ponatinib suppressed the growth of NSCLC cells overexpressing FGFR1, and significantly inhibited the growth of primary lung cancer cultures in vitro, suggesting that ponatinib may also be effective in patients whose tumours overexpress FGFR1 [60].

PTEN

Many cancers are associated with deletions or mutations of the PTEN tumour suppressor gene, which plays a significant role in cell cycle progression, apoptosis, growth, proliferation, and migration via negative control of the PI3K/Akt pathway [61]. PTEN mutations [62] and loss of PTEN protein expression are relatively common in SCC of the lung [63]. However, in many cases, the functional consequences of PTEN mutations remain to be elucidated. PTEN loss has also been linked with acquired resistance to EGFR TKIs in EGFR mutation-positive NSCLC [18]. In a meta-analysis of mutation incidence in NSCLC, PTEN mutations were influenced by ethnicity, with a higher frequency amongst Asian patients with SCC (9.8%) versus adenocarcinoma (1.6%), and in western patients with adenocarcinoma (6.0%) versus SCC (0%) [64]. The TKI vandetanib has shown efficacy against EGFR mutation-positive lung cancer cell lines showing loss of PTEN, suggesting that it may also be effective in patients with EGFR mutation-positive NSCLC whose tumours lack PTEN expression [65].

PI3K

PI3Ks are lipid kinases involved in the regulation of cell growth, proliferation, and survival. Mutations in the PIK3CA gene that encodes the catalytic subunit of PI3Kα have been identified in several cancers [66]. Furthermore, aberrant signalling through the PI3K/Akt/mTOR pathway has been observed in a number of human cancers, including NSCLC [67]. PIK3CA mutations occur in less than 5% of NSCLCs [15] and often co-exist with other oncogenic mutations, particularly EGFR, KRAS or ALK [68]. PTEN loss is thought to be a marker for PI3K dependency in some tumours [69].

Several PI3K inhibitors are in clinical development (eg GDC-0941, BKM120, BEZ235, XL-147, XL-765, perifosine), but the response rate to single agents has been low [2,70]. Ongoing phase II trials in lung cancer are examining combinations of PI3K inhibitors and chemotherapy (NCT01297491) or targeted agents (NCT01493843, NCT01487265). In human NSCLC lines, the novel PI3K inhibitor imidazopyridine demonstrated anti-proliferative effects, including the induction of apoptosis, in a dose-dependent manner [71].
Immunotherapies

PD-L1/PD-1

Interaction of PD-L1 with the PD-1 and B7.1 receptor on activated T cells plays a key role in tumour evasion of the host immune system [72–75]. Whether PD-L1 is overexpressed in solid tumours and associated with increased tumour aggressiveness remains unclear. However, high levels of PD-L1 were recently reported in patients with sarcomatoid lung cancer, a rare, high-grade, poorly differentiated form of NSCLC [76]. Furthermore, in a 5-year follow-up study in patients with NSCLC, PD-L1 was a significant independent poor prognostic factor, with PD-L1-positive patients having a shorter 5-year OS than PD-L1-negative patients [77].

Nivolumab (BMS-936558), an anti-PD-1 monoclonal antibody, has shown anti-tumour activity in a phase I clinical trial in patients with advanced solid tumours, including NSCLC [78,79]. Of 129 patients with NSCLC treated with nivolumab, 22 (17.1%) achieved an objective response (13 with non-squamous cell histology and nine with squamous cell histology) and 13 patients (10.1%) had disease stabilization for at least 24 weeks [78]. The 24-week PFS rate was 33% [79]. Patients are currently being enrolled in a phase II study of nivolumab in SCC (NCT01721759) and a phase III study in non-SCC (NCT01673867).

MPDL3280A, an engineered anti-PD-L1 monoclonal antibody, has also demonstrated anti-tumour activity in a phase I NSCLC clinical trial. MPDL3280A treatment was associated with a 22% objective response rate in 41 heavily pretreated NSCLC patients, with 12% of patients having disease stabilization for at least 24 weeks [80]. A correlation between PD-L1 status and efficacy was reported: 4/5 patients (80%) with PD-L1-positive tumours achieved an objective response and 0/4 had disease progression, while 4/28 patients (14%) with PD-L1-negative tumours had an objective response and 0/4 had disease progression, while 4/28 patients (14%) with PD-L1-negative tumours achieved an objective response (13 with non-squamous cell histology and nine with squamous cell histology) and 13 patients (10.1%) had disease stabilization for at least 24 weeks [78]. The 24-week PFS rate was 33% [79]. Patients are currently being enrolled in a phase II study of nivolumab in SCC (NCT01721759) and a phase III study in non-SCC (NCT01673867).

The impact of molecular testing on lung cancer management

The identification and characterization of molecular targets are having a growing impact on the management of patients with lung cancer. Several clinical practice guidelines, including those published by the National Comprehensive Cancer Network (NCCN), the American Society of Clinical Oncology (ASCO), and the International Association for the Study of Lung Cancer (IASLC)/College of American Pathologists (CAP)/Association of Molecular Pathology (AMP), now recommend that all patients with NSCLC containing an adenocarcinoma component undergo biomarker testing for EGFRT mutations and ALK rearrangements [6,84,85]. The French National Cancer Institute, in collaboration with the French Ministry of Health, recently introduced a programme of free molecular diagnostic testing for all patients with solid tumours, which includes testing for EGFTR mutations and EML4–ALK rearrangements in lung cancer [86]. Not only has this mandate granted equal access to molecular tests and appropriate targeted therapies for all patients across France, but it has also reduced the unnecessary treatment of unselected patients.

Across the USA, the Lung Cancer Mutation Consortium (LCMC), a collaborative approach between 16 academic centres, is actively promoting molecular mutation testing in lung cancer in order to match patients to optimal treatment strategies [87]. The LCMC is conducting an observational study with the aim of genotyping ten driver mutations in tumour specimens from 1000 patients with advanced lung adenocarcinoma (NCT01014286); results of EGFTR testing are passed to treating physicians, while patients with other driver mutations are offered enrolment into LCMC-linked clinical trials of various targeted agents.
Preliminary reports from the first LCMC study confirm the presence of several key target genes, including \textit{KRAS} mutations (24%), \textit{EGFR} mutations (20%), and \textit{ALK} rearrangements (8%) [88–90].

**New techniques in the genomic classification of lung cancer**

The landmark studies that led to the approval of the first targeted agents in NSCLC used gene-based molecular tests that were focused on single biomarkers. However, with the advent of many more potential molecular targets, and the challenges associated with obtaining tissue from patients with late-stage NSCLC, there is a growing need to develop and utilize molecular technologies that can determine the expression or mutation status of several genes simultaneously, so-called multiplex testing, in order to obtain the maximum diagnostic information from the limited tumour tissue available (Figure 2).

**Predictive and prognostic gene signatures**

A number of research groups have developed predictive and prognostic gene signatures in surgically resected lung cancer [91–93]. However, the use of these signatures in clinical practice is often hampered by issues such as reproducibility, cost, and limited availability, as well as lack of validation [92]. Using samples from the JBR.10 clinical trial, Zhu et al developed a 15-gene expression signature that demonstrated the potential to select patients with stage IB/II NSCLC most likely to benefit from adjuvant chemotherapy with cisplatin/vinorelbine [91]. Kratz et al developed a prognostic gene signature that was able to identify patients with early-stage, non-squamous NSCLC at high risk for mortality after surgical resection [92]. The 14-gene mRNA expression assay was based on quantitative PCR using formalin-fixed, paraffin-embedded (FFPE) tissue samples and improved prognostic accuracy beyond NCCN criteria for stage I high-risk tumours ($p < 0.0001$). Blinded and independent validation of the assay was confirmed in a cohort of 433 patients from the USA and in a larger sample of 1006 patients from China [92].

More recently, Tang et al developed an 18-gene prognostic signature in resectable NSCLC, which was then integrated with genome-wide functional data and genetic aberration data to derive a 12-gene predictive signature for survival benefits with adjuvant chemotherapy [93]. The prognostic signature predicted the prognosis of patients with adenocarcinoma in all validation datasets across four microarray platforms, including Illumina® (Illumina Inc, San Diego, CA, USA), Affymetrix® (Affymetrix, Santa Clara, CA, USA), and Agilent® (Agilent Technologies Inc, Santa Clara, CA, USA). The prognostic signature was successfully validated in two independent datasets in 266 patients. Prospective clinical trials are needed to further understand disease.

![Figure 2. Lung diagnostic testing today and in the future.](image-url)
validate the use of prognostic and predictive signatures in lung cancer [93,94].

**Multiplex PCR (mutation detection and gene expression)**

Multiplex PCR involves the simultaneous amplification of two or more cDNA/DNA targets in a single reaction vessel with uniquely labelled probes for each target [95]. A number of multiplexed PCR-based assays are available, including SNaPshot® (Applied Biosystems, Foster City, CA, USA), which detects hotspot mutation sites in key cancer genes using fluorescently-labelled primer extension products [96], and Sequenom MassARRAY® (Sequenom Inc, San Diego, CA, USA), which analyses primer extension products using mass spectrometry [97]. A high-throughput microfluidics method (Fluidigm, South San Francisco, CA, USA) has been developed for mutation detection [mutation multi-analyte panel (MUT-MAP)] based on quantitative PCR, which includes ~120 hotspot mutations and works effectively with less than 100 ng of FFPE tissue [98].

Similar assays and formats are widely used in the cancer research community and are starting to be applied in clinical trials. For example, the LCMM is predominantly utilizing the SNaPshot® and Sequenom MassARRAY® platforms, together with the FDA-approved FISH test for ALK gene rearrangement in their ongoing genotyping trial. Multiplex PCR has the advantage of needing only a small sample of tumour compared with conventional tests, but it is restricted to codons previously determined as mutation hotspots, and is unable to detect chromosomal rearrangements or determine gene copy number [70].

**Next-generation sequencing (NGS)**

High-throughput NGS technology has been commercially available since 2004 and offers the ability to analyse DNA, mRNA, transcription factor regions, and DNA methylation patterns throughout the entire genome [99]. Several NGS platforms are available, including Illumina® HiSeq 2500 (Illumina Inc, San Diego, CA, USA), SOLID™ System (Applied Biosystems, Foster City, CA, USA), and Ion Torrent™ (Applied Biosystems, Foster City, CA, USA) [17,99]. NGS has been applied to clinical settings in almost all tumour types and is being used as a research tool, as well as to screen patients for clinical trial enrolment. NGS can detect chromosomal rearrangements and gene copy number alterations at a very high resolution [70]. Indeed, identification of the KIF5B–RET fusion was made possible through the application of NGS [38].

NGS has huge potential over traditional sequencing techniques; however, currently each platform requires a specific investment in computational analysis and bioinformatic support to produce and interpret the data, and the assays are expensive and often cumbersome [99]. Over the next few years, the cost and complexity of NGS-based testing will continue to decrease rapidly and testing is likely to become even more widespread. In the USA, NGS-based clinical assays are already being offered as laboratory-developed tests (LDTs) in several Clinical Laboratory Improvement Amendments (CLIA)-approved laboratories (eg Foundation Medicine and laboratories in academic institutions). However, there are currently no NGS-based FDA-approved companion diagnostic tests. Given the high-resolution data that NGS can provide, traditional prospective clinical validation can be challenging, particularly for rare genomic alterations. Overcoming this challenge, and/or refining the definition of prospective clinical validation, will require the cooperation of clinical scientists, regulatory authorities, and payers. Collaboration between institutions and patient referral centres are necessary to identify these rare patients, as are innovative clinical trial designs, as described later. NGS-based companion diagnostics will also require the flexibility to be updated as additional clinical information emerges. As the cost of NGS-based tests continues to drop to the point where whole genome sequencing becomes routine clinical practice, the test update required may simply be a software update that changes the clinical report to include, for example, detection of a newly validated rare EGFR mutation or ROS1 fusion partner.

Clearly, other important platforms for identifying molecular targets, such as FISH (as exemplified with crizotinib and ALK rearrangements) and IHC (as exemplified with onartuzumab and MET expression), should be considered alongside these newer techniques. The key for clinicians and pathologists, therefore, will be to determine the optimal method for molecularly classifying lung cancers moving forward.

**Challenges of widespread genetic testing**

The merits of molecular testing in lung cancer are clear; however, there are a number of challenges to overcome in the widespread use of these tests. Firstly, the community will need to come to some consensus as to what an actionable test result might be. A valid test result can mean very different things depending on the technology, bioinformatics pipeline, and what the investigator or treating physician perceives to be clinically relevant, and there are obvious potential dangers in this. Additionally, the quantity, quality, and type of tumour tissue available for testing vary extensively between different centres and countries. One of the greatest challenges is obtaining adequate tumour samples for all genomic tests, while avoiding contamination with normal and necrotic cells, in a minimally invasive manner [70]. Substrates derived
from peripheral tissues, such as circulating tumour cells and circulating tumour DNA, are less invasive alternatives to surgical or biopsy specimens and have yielded comparable results in molecular tests [100], although further research in this field is required [70]. Intratumour heterogeneity can result in a mixed response to a molecularly targeted agent in different tumour sites, and throughout the course of successive treatment lines, due to alterations in the genetic make-up of the tumour as the disease progresses or in response to therapy [17]. Such heterogeneity may represent a major treatment challenge if the therapy choice is based on genomic analysis of a single tumour biopsy sample at a specific time point [101]. Thus, serial biopsy or cytology sampling during the course of the disease may provide a more accurate genomic analysis of the tumour (Figure 3).

Another challenge with genomic testing will be for clinicians to decide which of the genomic data is of relevance to an individual patient’s treatment choice [102]. Importantly, in cases where a patient has more than one activating alteration, the physician will need to decide which lesion to treat first. An additional consideration will be the time it takes to perform the tests – should physicians start treating a patient with the current standard of care for unselected patients while testing is taking place, and then switch the patient once a positive result is identified? What decisions should be made in the event that a patient has a mutation in a gene for which there is no currently approved therapy in NSCLC, but where a targeted treatment is approved in other indications? At a minimum, a multidisciplinary team approach will be required to accurately interpret the results of the tests, and central to this will be engaging patients to help them realize the importance of molecular testing in the first instance [17]. Several institutions have implemented multidisciplinary molecular tumour boards to discuss the management of patients whose lung tumours harbour rare genetic abnormalities with no validated targeted therapy available.

The costs of widespread genetic testing will also come into question, in terms of the cost–benefit ratio of the newer platforms versus the more conventional tests, and the challenges faced by diagnostic laboratories in keeping up with the costs of buying new equipment and validating new assays as the sequencing platforms continually evolve [103]. Finally, as the current regulatory environment does not allow for the rapid adoption of new technology, we may face unavoidable delays in the implementation of genomic testing and, ultimately, the optimization of treatment for patients with lung cancer.

Failure, genomic testing of NSCLCs must be included alongside histological testing, such that candidate patients can be identified and treatment choices optimized. For example, non-mucinous bronchoalveolar carcinoma (BAC) is now classified as lepidic predominant adenocarcinoma (100% TTF1, ~45% EGFR mutation-positive, 5% BRAF mutation-positive), while mucinous BAC is classified as mucinous invasive carcinoma (15% TTF1, ~80–100% KRAS mutation-positive, 0% EGFR mutation-positive) [7,104]. The potential impact of targeting different molecular targets or histologies within the context of historical ‘all-comer’ studies is illustrated in Figure 4; not only do the specific targeted subsets need to be considered in terms of their particular prognostic behaviour (EGFR mutation-positive patients do much better on both chemotherapy and EGFR TKIs than EGFR wild-type patients), but the impact of removing these patients from the remaining pool should also be considered if spurious interpretations of trial data are to be avoided.

Furthermore, it is likely that most patients with NSCLC will test positive for at least two potential molecular targets when IHC and genetic tests are considered together. Thus, there is a real need to understand how these molecular targets fit into current and future treatment algorithms, especially for patients with multiple biomarkers.

Earlier clinical trials were not designed adequately for the testing of multiple molecular targets, but rather restricted enrolment to patients with a known single mutation. Evaluating biomarker combinations or overlap (ie between mutations, gene expression, and IHC) could inform rational drug combinations or sequencing trials in the future. Ongoing programmes, such as the LCMC study and the MD Anderson BATTLE trials, are already utilizing novel designs to evaluate multiple targeted therapies in NSCLC [70,106]. Careful ethical consideration must also be given to the design of control arms in clinical trials of biomarker-selected patients. From the patients’ and treating physicians’ perspective, strong arguments can be made to permit crossover in biomarker enabled trials, such that patients whose tumours have the relevant biomarker can gain benefit from the targeted agent at some point; however, from regulatory and payer perspectives, similarly strong arguments are made to prevent crossover, to demonstrate differences in OS. In addition, recently obtained tumour samples should be used, rather than archival tissue from surgical resection, as the tumour profile can change considerably over time, and trial enrolment should be based on the current disease profile, rather than that at the initial diagnosis.

Implications for clinical trial design
Lung cancer is a competitive landscape with many new drugs in development and several failed phase III clinical trials. To reduce the risk of further clinical trial

Conclusions
A deeper understanding of the molecular classification of lung cancer may ultimately lead to personalized treatment strategies, which will improve care for
Genomic classification of lung cancer

Figure 3. The future of oncology testing in NSCLC. BC, breast cancer; CTCs, circulating tumour cells; qRT, real-time reverse transcription; WGS, whole genome sequencing.
those patients most likely to benefit, and spare the cost and morbidity associated with failed treatment interventions. Multiplex PCR assays, high-throughput technologies such as NGS, and hopefully some form of multiplex protein-based platform will play an important role in lung carcinoma management and rational therapy selection, but there are many challenges ahead. Careful design of clinical trials will help to evaluate molecularly targeted agents in the context of those populations most likely to benefit, but clinicians will be faced with difficult decisions, such as how to include an ethically fair control arm, what treatment to choose when a new patient subset is no longer part of the first-line population, and what the preferential order of treatment should be where multiple molecular targets are present. Only through a better understanding of the disease can treatment choices be enhanced and the outlook for patients with lung cancer improved.

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Author contribution statement

DSS and IIW conceived and wrote this manuscript.

References

1. Travis WB, Brambilla A, Müller-Hermelinck HK, et al. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. IARC Press: Lyon, 2004.
2. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. Lancet Oncol 2011; 12: 175–180.
3. Schiller J, Harrington D, Belani C, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346: 92–98.
4. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011; 61: 69–90.
5. American Cancer Society. Cancer Facts & Figures 2012. Atlanta: American Cancer Society; 2012. Available from: http://www.cancer.org/acs/groups/content/@epidemiology/surveillance/documents/document/acspc-033423.pdf.
6. 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.
7. 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.
8. 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, randomised, phase 3 study. Lancet Oncol 2011; 12: 735–742.
9. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129–2139.
10. Pazé JG, Janne PA, Lee J, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004; 304: 1497–1500.
11. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010; 363: 1695–1703.
12. 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.
13. Shaw TA, Kim D-W, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013; 368: 2385–2394.
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. Oxnard GR, Binder A, Janne PA. New targetable oncogenes in non-small-cell lung cancer. J Clin Oncol 2013; 31: 1097–1104.
16. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005; 97: 339–346.
17. Li T, Kung H-J, Mack PC, et al. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. J Clin Oncol 2013; 31: 1039–1049.
18. Sos ML, Koker M, Weir BA, et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. Cancer Res 2009; 69: 3256–3261.
19. Engelman JA, Zejnullahu K, Gale CM, et al. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. Cancer Res 2007; 67: 11924–11932.
20. Gonzales AJ, Hook KE, Allthaus IW, et al. Antitumor activity and pharmacokinetic properties of PF-00299804, a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor. Mol Cancer Ther 2008; 7: 1880–1889.
21. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005; 352: 786–792.
22. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005; 2: e73.

23. Ramalingam SS, Blackhall F, Krazkowski M, et al. Randomized phase II study of dacarbazine (PF-00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2012; 30: 3337–3344.

24. Brzezniak C, Carter CA, Giaccone G, Dacombitin, a new therapy for the treatment of non-small cell lung cancer. Expert Opin Pharmacother 2013; 14: 247–253.

25. Soda M, Choi YL, Enomoto M, et al. Identification of the fusing EML4–ALK gene in non-small-cell lung cancer. Nature 2007; 448: 561–566.

26. Cardarella S, Johnson BE. The impact of genomic changes on treatment of lung cancer. Am J Respir Crit Care Med 2013; 188: 770–775.

27. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4–ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008; 14: 4275–4283.

28. 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.

29. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol 2011; 12: 1004–1012.

30. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res 2009; 15: 5216–5223.

31. Sasaki T, Rodig SJ, Chirieac LR, et al. The biology and treatment of EML4–ALK non-small cell lung cancer. Eur J Cancer 2010; 46: 1773–1780.

32. To KF, Tong JH, Yeung KS, et al. Detection of ALK rearrangement by immunohistochemistry in lung adenocarcinoma and the identification of a novel EML4–ALK variant. J Thorac Oncol 2013; 8: 883–891.

33. Bergethon K, Shaw AT, Ou SH, et al. ROSI rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012; 30: 863–870.

34. Rimkunas VM, Crosby KE, Li D, et al. Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: identification of a FIG–ROS1 fusion. Clin Cancer Res 2012; 18: 4449–4457.

35. Gainor JF, Shaw AT. Novel targets in non-small cell lung cancer: ROSI and RET fusions. Oncologist 2013; 18: 865–875.

36. Shaw AT, Camidge DR, Engelman JA. Clinical activity of crizotinib in advanced non-small cell lung cancer (NSCLC) harboring ROSI gene rearrangement. J Clin Oncol 2012; 30 (Suppl): abstract 7508.

37. Bos M, Gardizi M, Schildhaus HU, et al. Complete metabolic response in a patient with repeatedly relapsed non-small cell lung cancer harboring ROSI gene rearrangement after treatment with crizotinib. Lung Cancer 2013; 81: 142–143.

38. 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.

39. Kohno T, Ichikawa H, Totoki Y, et al. KIF5B–RET fusions in lung adenocarcinoma. Nature Med 2012; 18: 375–377.

40. Takeuchi K, Soda M, Togashi Y, et al. RET, ROSI and ALK fusions in lung cancer. Nature Med 2012; 18: 378–381.

41. Dacic S. Molecular genetic testing for lung adenocarcinomas: a practical approach to clinically relevant mutations and translocations. J Clin Pathol 2013; 66: 870–874.

42. Drilon A, Wang L, Hasanovic A, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov 2013; 3: 630–635.

43. Birchmeier C, Birchmeier W, Gherardi E, et al. Met, metastasis, motility and more. Nature Rev Mol Cell Biol 2003; 4: 915–925.

44. Capuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol 2009; 27: 1667–1674.

45. Toschi L, Capuzzo F. Clinical implications of MET gene copy number in lung cancer. Future Oncol 2010; 6: 239–247.

46. Dziadziuszko R, Wynes MW, Singh S, et al. Correlation between MET gene copy number by silver in situ hybridization and protein expression by immunohistochemistry in non-small cell lung cancer. J Thorac Oncol 2012; 7: 340–347.

47. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007; 104: 20932–20937.

48. Engelmann JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007; 316: 1039–1043.

49. Katayama R, Aoyama T, Yamori T, et al. Cystotoxic activity of tivantinib (ARQ 197) is not due solely to c-MET inhibition. Cancer Res 2013; 73: 3087–3096.

50. Basilico C, Pennacchietti S, Vigna E, et al. Tivantinib (ARQ197) displays cystotoxic activity that is independent of its ability to bind MET. Clin Cancer Res 2013; 19: 2381–2392.

51. Martens T, Schmidt NO, Eckerich C, et al. A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo. Clin Cancer Res 2006; 12: 6144–6152.

52. Jin H, Yang R, Zheng Z, et al. MetMAb, the one-armed 5D5 anti-c-Met antibody, inhibits orthotopic pancreatic tumor growth and improves survival. Cancer Res 2008; 68: 4360–4368.

53. Spigel DR, Ervin TJ, Ramala R, et al. Randomized phase II trial of onartuzumab (MetMab) in combination with erlotinib in patients with advanced non-small-cell lung cancer. J Clin Oncol 2013; 31: 4105–4114.

54. Spigel DR, Edelman MJ, Mok T, et al. Treatment rationale study design for the MetLung trial: a randomized, double-blind phase III study of onartuzumab (MetMab) in combination with erlotinib versus erlotinib alone in patients who have received standard chemotherapy for stage IIIb or IV Met-positive non-small-cell lung cancer. Clin Lung Cancer 2012; 13: 500–504.

55. Dutt A, Ramos AH, Hammerman PS, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 2011; 6: e20351.

56. Burbridge MF, Bossard CJ, Saurier C, et al. S49076 is a novel bioavailable, potent, and selective inhibitor of the fibroblast growth factor receptor 1 (FGFR1) copy number in lung cancer patients. Oncotarget 2014; 5: 13650–13665.

57. Zhang J, Zhang L, Su X, et al. Translating the therapeutic potential of AZD4547 in FGFR1-amplified non-small cell lung cancer through the use of patient-derived tumor xenograft models. Clin Cancer Res 2012; 18: 6658–6667.
60. Ren M, Hong M, Liu G, et al. Novel FGFR inhibitor ponatinib suppresses the growth of non-small cell lung cancer cells overexpressing FGFR1. *Oncol Rep* 2013; 29: 2181–2190.

61. Abdalkareem IH, Blair M. Phosphatase and tensin homologue deleted on chromosome 10. *Niger Med J* 2013; 54: 79–86.

62. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012; 489: 519–525.

63. Maris CJ, Zheng S, Alldape K, et al. PTEN expression in non-small cell lung cancer: evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. *Hum Pathol* 2005; 36: 766–768.

64. Dearden S, Stevens J, Wu YL, et al. Mutation incidence and coincidence in non-small cell lung cancer: meta-analyses by ethnicity and histology (mutMap). *Ann Oncol* 2013; 24: 2371–2376.

65. Takeda H, Takigawa N, Ohashi K, et al. Vandanetib is effective in EGFR-mutant lung cancer cells with PTEN deficiency. *Exp Cell Res* 2013; 319: 417–423.

66. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004; 304: 554.

67. Trigka EA, Levidou G, Saetta AA, et al. A detailed immunohistochemical analysis of the PI3K/AKT/mTOR pathway in lung cancer: correlation with PIK3CA, AKT1, K-RAS or PTEN mutational status and clinicopathological features. *Oncol Rep* 2013; 30: 623–636.

68. Chaft JE, Arcila ME, Paik PK, et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma: rationale for comprehensive mutation profiling. *Mol Cancer Ther* 2012; 11: 485–491.

69. Pfeifer M, Grau M, Lenze D, et al. PTEN loss defines a PI3K/AKT pathway-dependent germinal center subtype of diffuse large B-cell lymphoma. *Proc Natl Acad Sci U S A* 2013; 110: 12420–12425.

70. Thomas A, Rajan A, Lopez-Chavez A, et al. From targets to targeted therapies and molecular profiling in non-small cell lung carcinoma. *Ann Oncol* 2013; 24: 577–585.

71. Lee H, Kim SJ, Jung KH, et al. A novel imidazopyridine PI3K inhibitor with anticancer activity in non-small cell lung cancer cells. *Oncol Rep* 2013; 30: 863–869.

72. Hall RD, Gray JE, Chiappori AA. Beyond the standard of care: a review of novel immunotherapy trials for the treatment of lung cancer. *Cancer Control* 2013; 20: 22–31.

73. Brahmer JR, Tykodi SS, Chow LQM, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; 366: 2455–2465.

74. Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation programmed death-1 ligand-1 expression in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). *J Clin Oncol* 2013; 31 (Suppl): abstract 8008 and updated data presented at the meeting.

75. Chen YB, Mu CY, Huang JA, et al. A novel imidazopyridine PI3K inhibitor with anticancer activity in non-small cell lung cancer cells. *Oncol Rep* 2013; 29 (Suppl): abstract 8030.

76. Brahmer JR, Horn L, Antonia SJ, et al. Survival and long-term follow-up of the phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with previously treated advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2013; 31 (Suppl): abstract 8030.

77. Gottinger S, Horn L, Antonio SJ, et al. Clinical activity and safety of anti-programmed death-1 (PD-1) (BMS-936558/MDX-1106/ONO-4538) in patients with advanced non-small cell lung cancer (NSCLC). *Ann Oncol* 2012; 23 (Suppl 9): abstract 1234TP.

78. Spigel DR, Gettinger SN, Hom L, et al. Clinical activity, safety and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). *J Clin Oncol* 2013; 31 (Suppl): abstract 8008 and updated data presented at the meeting.

79. Gryshkova K, Goncharuk I, Gurtovy V, et al. The study of phosphate transporter NAPII2B expression in different histological types of epithelial ovarian cancer. *Exp Oncol* 2009; 31: 37–42.

80. Kiyamova R, Shyam M, Lyzogubov VV, et al. Immunohistochemical analysis of NaPi2b protein (MX35 antigen) expression and subcellular localization in human normal and cancer tissues. *Exp Oncol* 2011; 33: 157–161.

81. Gordon MS, Gerber DE, Infante JR, et al. A phase I study of the safety and pharmacokinetics of DNB1060A, an anti-NaPi2b antibody–drug-conjugate (ADC), in patients (pts) with non-small cell lung cancer (NSCLC) and platinum-resistant ovarian cancer (OC). *J Clin Oncol* 2013; 31 (Suppl): abstract 2507.

82. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Version 3; 2012 [Accessed 22 August 2013]. Available from: http://www.nccn.com.

83. Keedy VL, Temin S, Somerfield MR, et al. American Society of Clinical Oncology Provisional clinical opinion: epidermal growth factor receptor (EGFR) mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. *J Clin Oncol* 2011; 29: 2121–2127.

84. Andre F, Nowak F, Arnedos M, et al. Biomarker discovery, development, and implementation in France: a report from the French National Cancer Institute and cooperative groups. *Clin Cancer Res* 2012; 18: 1555–1560.

85. Lung Cancer Mutation Consortium (LCMC). [Accessed 22 August 2013]. Available from: http://www.golcncm.com/index.html.

86. Kris MG, Johnson BE, Kwiatkowski DJ, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI’s Lung Cancer Mutation Consortium (LCMC). *J Clin Oncol* 2011; 29 (Suppl): abstract CRA7506.

87. Larsson CJ, Källberg K, Cristalli A, et al. Identification of driver mutations in tumors from 1,000 patients with lung adenocarcinoma: the NCI’s Lung Cancer Mutation Consortium (LCMC). *J Clin Oncol* 2011; 31 (Suppl): abstract 8019.

88. Liu Y, Wang J, Lin J, et al. A phase II study of nivolumab (BMS-936558; ONO-4538) as first-line therapy for patients with advanced non-small cell lung cancer: results from the phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with previously treated advanced non-small cell lung cancer (NSCLC). *Cancer Immunol Immunother* 2013; 62: 2651–2661.

89. Li H, Zhang L, Wang L, et al. A novel imidazopyridine PI3K inhibitor with anticancer activity in non-small cell lung cancer cells. *Oncol Rep* 2013; 30: 863–869.

90. Hall RD, Gray JE, Chiaipori AA. Beyond the standard of care: a review of novel immunotherapy trials for the treatment of lung cancer. *Cancer Control* 2013; 20: 22–31.

91. Brahmer JR, Tykodi SS, Chow LQM, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; 366: 2455–2465.

92. Chen DS, Irving BA, Hodi FS, Molecular pathways: next-generation immunotherapy – inhibiting programmed death-ligand 1 and programmed death-1. *Clin Cancer Res* 2012; 18: 6580–6587.

93. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 2013; 39: 1–10.

94. Velchetti V, Rimm DL, Schulpa KA. Sarcomatoid lung carcinomas show high levels of programmed death-ligand-1 (PD-L1). *J Thorac Oncol* 2013; 8: 803–805.

95. Chen YB, Mu CY, Huang JA, et al. Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: a 5-year-follow-up study. *Tumori* 2012; 98: 751–755.

96. Brahmer JR, Horn L, Antonia SJ, et al. Survival and long-term follow-up of the phase I trial of nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with previously treated advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2013; 31 (Suppl): abstract 8030.

97. Gettinger S, Horn L, Antonio SJ, et al. Clinical activity and safety of anti-programmed death-1 (PD-1) (BMS-936558/MDX-1106/ONO-4538) in patients with advanced non-small cell lung cancer (NSCLC). *Ann Oncol* 2012; 23 (Suppl 9): abstract 1234TP.
97. 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.
98. Patel R, Tsan A, Tam R, et al. Mutation scanning using MUT-MAP, a high-throughput, microfluidic chip-based, multi-analyte panel. *PLoS One* 2012; 7: e51153.
99. Daniels M, Goh F, Wright GM, et al. Whole genome sequencing for lung cancer. *J Thorac Dis* 2012; 4: 155–163.
100. Smouse JH, Cibas ES, Janne PA, et al. EGFR mutations are detected comparably in cytologic and surgical pathology specimens of non-small cell lung cancer. *Cancer* 2009; 117: 67–72.
101. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 2012; 366: 883–892.
102. Wistuba II, Gelovani JG, Jacoby JJ, et al. Methodological and practical challenges for personalized cancer therapies. *Nature Rev Clin Oncol* 2011; 8: 135–141.
103. Levy MA, Lovly CM, Pao W. Translating genomic information into clinical medicine: lung cancer as a paradigm. *Genome Res* 2012; 22: 2101–2108.
104. Travis WD, Brambilla E, Riely GJ. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. *J Clin Oncol* 2013; 31: 992–1001.
105. Yauch RL, Januario T, Eberhard DA, et al. Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. *Clin Cancer Res* 2005; 11(24 Pt 1): 8686–8698.
106. Kim ES, Herbst RS, Wistuba II, et al. The BATTLE trial: personalizing therapy for lung cancer. *Cancer Discov* 2011; 1: 44–53.

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