

Understanding lung cancer molecular subtypes

Practice points

- Histological examination of a tissue biopsy is required to differentiate between different lung cancer histiotypes.
- In non-small-cell lung cancer, judicious use of a limited immunohistochemistry panel is recommended to differentiate between squamous cell and adenocarcinoma, and maximize tumor material for molecular analyses.
- Clinical selection criteria should not be used to select patients for tumor genotyping.
- All patients with advanced nonsquamous non-small-cell lung cancer should undergo tumor genotyping for the presence of EGFR-activating mutations and if found, receive first-line treatment with an EGFR tyrosine kinase inhibitor.
- Rebiopsy of a progressive site is recommended for patients with acquired EGFR kinaseinhibitor resistance, to exclude small cell (or high-grade neuroendocrine) carcinoma change and identify additional molecular aberrations for trial therapy.
- All advanced nonsquamous (and selected squamous cell carcinomas) should undergo ALK testing, and if positive receive crizotinib within its licensed indication.

In-depth analysis of the cancer genome has revolutionized our approach to treating solid organ malignancies. Therapeutic strategies are now driven by tumor genotyping and the identification of drug targets. Non-small-cell lung cancer is a prime example of a tumor in which specific disease subtypes are now defined by molecular genotype, rendering a homogenous approach to lung cancer management inappropriate. Herein, we review molecular characterization of non-small-cell lung cancer to date, its clinical and therapeutic impact and data that underpin routine use of molecular targeted therapy for specific tumor genotypes. We discuss molecular mechanisms of acquired drug resistance and potential therapeutic strategies to overcome this, as well as identifying genotype-directed approaches to treating newly identified somatic aberrations in development.

Keywords: ALK • EGFR • lung cancer • mutation • NSCLC

Developments in molecular diagnostics and genomic sequencing have transformed the treatment of advanced lung cancer over the last 10 years. Morphological subclassification of lung cancer on histological examination into small cell (SCLC) and non-small-cell lung cancer (NSCLC) historically identified two subtypes of lung cancer with very different clinical characteristics. NSCLC can then be further subdivided based on pathological criteria into the broad categories of squamous cell carcinoma, adenocarcinoma and other histological subtypes such as large cell carcinoma [1]. This histological subclassification now has therapeutic implications; most notably the preferential sensitivity of adenocarcinoma to pemetrexed [2] and for certain adenocarcinoma molecular subtypes to EGFR kinase inhibitors [3] and ALK kinase inhibitors [4]. It is now established that NSCLC

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molecular status is as critical as histological phenotype and potentially dictates the clinical course of disease and response to therapeutic agents. Method and site of diagnostic biopsies should therefore maximize tumor yield for downstream molecular analyses. Moreover, conservative management of these small volume diagnostic biopsies/aspirates by judicious use of immunohistochemical stains to maximize tissue for molecular testing is therefore crucial [5].

Systems biology approaches including genomic sequencing of tumor samples has characterized variation in molecular subtypes of adenocarcinoma and squamous cell carcinoma to date. These subtypes are typically defined by the presence of a specific somatic aberration (e.g., mutation) in an oncogene that drives tumor proliferation [6]. Thus, panel molecular testing of 516 stage IV adenocarcinoma patients by the Lung Cancer Mutation Consortium demonstrated a variety of aberrations. In total, 54% of cases had detectable driver aberrations (mutations); 22% in KRAS, 17% in EGFR, 7% in ALK, 2% in BRAF, 2% with MET amplification and less than 2% mutations in PIK3CA, HER2, MEK1, NRAS and AKT1. Aberrations were almost always (97%) mutually exclusive. Contingent on the genotyping panel, just under half of stage IV adenocarcinoma cases do not harbor a current potentially 'actionable' aberration, although other mutations, for example, in TP53, NF1, STK11, KEAP1 and other currently directly unactionable genes have been characterized [7].

A lower proportion of squamous cell carcinomas have currently targetable molecular aberrations. Approximately 40% of cases have been shown to carry potentially targetable somatic mutations or amplifications. *FGFR1* amplification occurs in approximately 20%, and mutations are observed in *PTEN* (10%), *AKT1* (6%), *DDR2* (4%) and *PIK3CA* (4%) [8]. Dense characterization of the squamous cell carcinoma genome has also noted frequent *TP53* mutations (81%), *BRAF* mutations, *EGFR* amplification and mutations in genes involved in the oxidative stress response [9]. In total, 3% of tumors have loss of MHC class 1 genes, suggesting a potential role for immunotherapy in this molecular subtype [10].

These molecular aberrations can initiate a malignant phenotype, drive proliferation and sustain tumor development through a variety of downstream effects. The key downstream signaling pathways for the commonest molecular aberrations found in adenocarcinoma are illustrated in Figure 1.

Molecular subtypes of lung adenocarcinoma EGFR mutations

In total, 5–10% of stage IV NSCLC in Western populations are EGFR mutated [11]. Analysis of over

15,000 cases of metastatic adenocarcinoma in France suggests a mean prevalence of 10.5% [12]. Approximately 80% are in cases of adenocarcinoma, 70% are in females and the majority are in light or never smokers [11].

Somatic *EGFR* mutations have been identified between exons 18–21. The commonest mutation, seen in 45–60% of cases, is a deletion or deletion–insertion in exon 19, while the L858R mutation in exon 21 accounts for 37–45% of cases [13–15]. Both mutations engender increased EGFR signaling and importantly, sensitivity to EGFR kinase inhibitors [12]. Such mutations are commoner in adenocarcinomas [15], never smokers and East Asian patients [16] for reasons unidentified.

The routine genotyping of NSCLC to identify EGFR mutations for therapeutic benefit was directly due to the IPASS trial of gefinitib versus carboplatinpaclitaxel chemotherapy in treatment-naive advancedstage patients clinically selected to harbor an EGFR mutation (East Asian patients, adenocarcinoma, never/ ex-light smokers) [17]. Here, despite clinical criteria, only 60% were shown to harbor an EGFR mutation, demonstrating the need to genotype rather than use clinical selection criteria. Planned subgroup analyses identified a significant progression-free survival (PFS) benefit (the primary end point) for gefitinib in EGFR mutant patients (HR: 0.48; p < 0.001), whereas EGFR wild-type patients were harmed with gefitinib, gaining greater benefit with chemotherapy (HR: 2.85; p < 0.001). Response rates (43%) and quality of life were superior for gefitinib in EGFR mutant patients compared with chemotherapy [3]. This superiority for EGFR kinase inhibitors over chemotherapy in treatment-naive EGFR mutant NSCLC, has been subsequently confirmed by six randomized trials including those using erlotinib and afatinib (Table 1) [18-22]. Current data suggest that patients with EGFR exon 19 deletions derive greatest benefit from EGFR kinase therapy [12,23].

Uncommon *EGFR* mutations include exon 20 insertions (~4%), exon 18 substitutions (~3%) and others, for example, exon 18 deletions or insertions, and exon 20 substitutions. Current data suggest that while some are sensitive to EGFR therapy (e.g., exon 18 point mutations, most commonly G719X) many are relatively insensitive (e.g., exon 20 insertions) and chemotherapy may be recommended [37]. However, recent data have suggested that the specific exon 20 insertion may modulate kinase drug sensitivity [38,39].

Patients with drug-sensitizing *EGFR* mutations usually develop therapy resistance at a median of 9–13 months [40]. Molecular analysis of repeat biopsies on acquired EGFR kinase resistance after a period of initial sensitivity has identified new molecular changes



Figure 1. Overview of selected downstream signaling mechanisms in oncogene-driven non-small-cell lung cancer. EGFR signals via the MAPK pathway, PI3K and the JAK–STAT pathway. *RAS* mutations can drive growth by enhancing MAPK pathway activity. Constitutively active ALK kinase, due to the expression of an ALK fusion protein, signals via RAS and the PI3K pathway to enhance survival and proliferation.

in approximately 70% of cases [41]. The acquisition of an EGFR second mutation is the commonest resistance mechanism observed, principally through over-representation of the exon 20 T790M mutation, detectable in 50% of such acquired resistance tumors, compared with a prevalence of approximately 1% in treatmentnaive cases [11,39]. T790M is associated with marked insensitivity to gefitinib and erlotinib. Other resistance mechanisms include recruitment of additional kinases. MET amplification is present in 4-25% of cases, and other observed changes include upregulated signaling through HER2 and IGF1 receptor. Approximately 6% of tumors seem to be SCLC or high-grade neuroendocrine carcinomas retaining the same somatic EGFR mutation on rebiopsy, which after platinum/etoposide chemotherapy may return to adenocarcinoma [42]. Other cases without a current defined resistance mechanism show signs of epithelial–mesenchymal transition but accurate prevalence estimates are unknown [40,43].

Given the insensitivity of T790M to gefitinib/ erlotinib, a number of second generation EGFR kinase inhibitors have been developed with preferential activity for T790M as well as typical sensitizing mutations in *in vitro/vivo* models, including afatanib (BIBW2992, Boehringer Ingelheim, Germany) and dacomitinib (PF-00299804, Pfizer, NY, USA). Both drugs have pan-HER (human EGFR tyrosine kinase family) activity, and both have been taken forward into Phase III trials, with afatinib demonstrating a PFS but no overall survival (OS) benefit for relapsed NSCLC with acquired resistance (LUX-Lung 1 [28]), and no subsequent license in this setting. Dacomitinib was evaluated in molecularly unselected NSCLCs against best supportive care (NCICC CTG BR.26) or erlo-

Table 1. Summary of relevant EGF receptor tyrosine kinase inhibitor clinical trials.							
Trial population	Trial	Molecule	Outcome	Ref.			
Treatment-naive adenocarcinoma	IPASS	Gefitinib	PFS benefit for gefitinib in EGFR mutant patients (HR: 0.48; p < 0.001) PFS benefit for carboplatin-paclitaxel chemotherapy for EGFR wild-type patients (HR: 2.85; p < 0.001)	[3]			
Treatment-naive metastatic EGFR mutant NSCLC, European	EURTAC	Erlotinib	PFS benefit for erlotinib vs cisplatin- docetaxel or cisplatin-gemcitabine (HR: 0.37; p < 0.0001)	[18]			
Treatment-naive EGFR mutant NSCLC, Asian	OPTIMAL	Erlotinib	PFS benefit for erlotinib vs carboplatin- gemcitabine (HR: 0.16; $p < 0.0001$)	[19]			
Treatment-naive EGFR mutant NSCLC	LUX-LUNG 3	Afatanib	PFS benefit for afatanib vs cisplatin- pemetrexed (HR: 0.47; p < 0.001)	[20]			
Treatment-naive EGFR mutant NSCLC, Asian	LUX-LUNG 6	Afatanib	PFS benefit for afatanib vs cisplatin- gemcitabine (HR: 0.28; p < 0.0001)	[21]			
Unselected NSCLC, one or more prior treatment regimens	BR.21	Erlotinib	PFS benefit of 0.4 months for erlotinib over placebo (HR: 0.61; $p < 0001$), OS benefit of 2 months (HR: 0.70; $p < 0.001$)	[24]			
Unselected NSCLC, one or more prior treatment regimens	INTEREST	Gefitinib	Gefitinib not inferior to docetaxel for overall survival (HR: 1.02)	[25]			
Unselected NSCLC, one or more prior treatment regimens	ISEL	Gefitinib	No OS benefit of gefitinib over placebo in all patients (HR: 0.89; $p = 0.087$) or in adenocarcinoma (HR: 0.84; $p = 0.089$)	[26]			
Unselected NSCLC, two or more prior treatment regimens	IDEAL2	Gefitinib	Significant symptom improvement in 43% patients (95% CI: 33–53), 70% patients adenocarcinoma	[27]			
Unselected adenocarcinoma, one or more prior treatment regimens with EGFR TKIs	LUX-Lung 1	Afatanib	PFS benefit of 2.2 months for afatanib vs placebo (HR: 0.38; p < 0.0001), no OS benefit	[28]			
EGFR mutant NSCLC with acquired resistance to prior TKI therapy		Afatanib and cetuximab	Disease control in all patients enrolled in Phase I study, 94% disease control in expanded cohort with 40% OR	[29]			
Treatment-naive NSCLC	INTACT 2	Gefitinib	No OS or PFS benefit of carboplatin-paclitaxel-gefitinib followed by gefitinib maintenance vs carboplatin-paclitaxel-placebo with placebo maintenance	[30]			
Treatment-naive NSCLC with nonprogressive disease following platinum-doublet chemotherapy	SATURN	Erlotinib	PFS benefit for erlotinib maintenance vs placebo (HR: 0.71; p < 0.0001)	[31]			
EGFR mutant NSCLC with prior TKI therapy	AURA(in progress)	AZD9291	Good tolerability and partial responses seen in early dose–escalation cohorts	[32]			
	CO-1686 Phase I(in progress)	CO-1686	Prolonged PFS for patients with sustained plasma concentrations >200 ng/ml for over 16 h	[33]			
Unselected NSCLC, one or more	ARCHER 1009	Dacomitinib	No PFS benefit vs erlotinib	[34]			
prior treatment regimens	NCICC CTG BR.26	Dacomitinib	No PFS benefit vs placebo	[35]			
Stage III unselected NSCLC treated with radical chemo-radiation	SWOG S0023	Gefitinib	No OS benefit with consolidation gefitinib vs placebo (p = 0.013)	[36]			
HR: Hazard ratio; NSCLC: Non-small-cell lung cancer; OR: Overall response; OS: Overall survival; PFS: Progression-free survival; TKI: Tyrosine kinase inhibitor.							

tinib [34] (ARCHER 1009), again with primary end points not met (Table 1) [35]. Based on preclinical data demonstrating synergy with cetuximab (an EGFR monoclonal antibody) and afatinib combination, not observed with erlotinib [44], significant activity of the combination has been demonstrated in an expanded Phase I cohort of acquired resistant NSCLC, including those with known T790M [29].

A number of 'third' generation EGFR kinase inhibitors are currently in development; specifically AZD2921 (AstraZeneca, London, UK) and CO-1686 (Clovis Oncology, CO, USA). Both drugs selectively target sensitizing and T790M mutant *EGFR* with minimal effect on wild-type *EGFR*. Responses in Phase I trials have been observed in patients with proven T790M (Table 1) [32,33]. Given these various EGFR kinase-acquired resistance mechanisms, rebiopsy on progression should be considered to best direct ubsequent therapy.

While EGFR kinase inhibition in untreated advanced disease patients with gefitinib, erlotinib or afatinib is standard of care, the clinical utility of *EGFR* mutation in management of radically treatable NSCLC is unknown with conflicting prognostic data for operable patients [45], and trials of adjuvant EGFR kinase inhibitors reporting in 2014–2015. For stage III NSCLC treated with radical chemoradiotherapy in a mainly *EGFR* wild-type population (unselected NSCLC), consolidation gefitinib was associated with a significantly inferior OS for reasons unknown [36].

MET amplification

MET amplification has been measured at varying prevalence in EGFR tyrosine kinase inhibitor (TKI)resistant tumors using different molecular assays complicated by frequent chromosome 7 polysomy in EGFR mutant NSCLC [43]. In total, a 4–7% prevalence is reported in resistant tumors, and is observed more commonly with a concurrent resistance mechanism, for example, T790M mutation or small cell transformation than as a solitary secondary aberration [46]. MET activates PI3K/AKT signaling via ERBB3 to circumvent EGFR and drive cell growth. Preclinical data and early-phase trials combining MET and EGFR inhibitors have shown some efficacy in EGFR kinase-resistant mutant NSCLC [47].

Up to 54% of molecularly unselected NSCLC overexpresses MET, mostly in adenocarcinoma [48]. Two major therapeutic strategies to MET inhibition have been pursued; antibodies and kinase inhibitors. Onartuzumab (MetMAb) is a monovalent humanized antibody against MET that has also shown promising synergism with EGFR kinase inhibitors in early-phase clinical trials, increasing PFS (HR: 0.53; p = 0.04) and

overall survival (HR: 0.37; p = 0.002) compared with erlotinib monotherapy in patients with MET-positive tumors. In contrast, this combination was potentially harmful in MET-negative tumors [48]. A Phase III study of this combination in MET-positive NSCLC is currently recruiting.

The MET kinase inhibitor, tivantinib (ARQ197) has been reported in a Phase III trial of erlotinib versus erlotinib–tivantinib combination in unselected relapsed NSCLC (Table 2) [49]. Here, while combination therapy did not improve OS (primary end point) in a MET-positive subset, a significant OS advantage for the combination was observed (HR: 0.7; p = 0.03). A number of other potent MET kinase inhibitors are in development, most notably crizotinib which has marked MET kinase inhibitory activity with case reports demonstrating activity in *MET*-amplified NSCLC [50].

KRAS mutations

KRAS substitution mutations are detectable in 15–25% of advanced lung adenocarcinomas, mostly in smokers, and as with other tumor types are principally observed in exons 2 and 3 [52]. The commonest *KRAS* mutation is G12C (~40% prevalence), followed by G12V (~20%) and G12D (15–20%) [53].

There is conflicting data from large multicentre trials on the prognostic significance of somatic KRAS mutation and no definitive prospective evidence that it is predictive of response to chemotherapy [54,55]. Metaanalysis suggests that KRAS mutation may be a negative prognostic factor in adenocarcinoma [52], but this may be a reflection of the mutual exclusivity of KRAS and EGFR mutations and the differential response to EGFR kinase therapy between EGFR mutant and wild-type populations [24]. Randomized trial data of outcomes between EGFR kinase inhibitors and taxanes [56] have shown poor survival in KRAS mutants regardless of therapy, and this has been observed in other trials datasets. This correlation is likely due to activation of RASmediated signaling downstream of the EGF receptor, which negates reliance on EGFR-mediated growth signals [57]. Limited retrospective exploratory analyses of clinical outcomes of NSCLC patients and cell line data have suggested differential activity of differing KRAS genotypes and systemic therapy [57,58].

A variety of strategies to therapeutically inhibit KRAS signaling have previously failed [59]. Most recently, focus has shifted to inhibiting downstream effectors of KRAS. In a mouse coclinical trial, selumetinib (AZD6244), a MEK1/2 kinase inhibitor has shown activity in combination with DNA-damaging chemotherapeutics in animal models. Significant tumor cell death was seen with docetaxel and selumetinib, in particular when

Table 2. Clinical trials in <i>MET</i> -amplified and <i>KRAS</i> mutant non-small-cell lung cancer.								
Trial population	Trial	Molecule	Outcome	Ref.				
MET amplification								
Unselected NSCLC, one or more prior treatment regimens	MARQUEE	Tivantinib and erlotinib	OS advantage (HR: 0.7; p = 0.03) with combination in MET-positive tumors	[49]				
KRAS mutation								
KRAS mutant NSCLC, one prior treatment regimen	NCT00890825	Docetaxel and selumetinib (AZD6244)	PFS benefit (HR: 0.58; $p = 0.014$) and OS advantage (HR: 0.8; $p = 0.21$) with combination	[51]				
HR: Hazard ratio; NSCLC: Non-small-cell lung cancer; OS: Overall survival; PFS: Progression free survival.								

given concurrently or with docetaxel preceding selumetinib [51]. In a KRAS mutant transgenic mouse coclinical trial, combination selumetanib/docetaxel was associated with synergistic tumor response compared with docetaxel monotherapy [60], translating in the human Phase II placebo-controlled trial of selumetanib/ docetaxel to a significant PFS advantage and a nonsignificantly improved OS (PFS – HR: 0.58, p = 0.014; OS - HR: 0.8, p = 0.21). Impressively, the overall response rate for selumetanib/docetaxel was 37% compared with 0% for docetaxel monotherapy (Table 2) [61]. The corresponding Phase III trial (SELECT) is now recruiting. Other approaches to targeting KRAS mutant NSCLC are ongoing, including KRAS G12C irreversible allosteric inhibitors which preferentially bind the mutant protein [62].

ALK rearrangements

3–7% of adenocarcinomas harbor *ALK* fusions, due to chromosome 2p inversion (usually with *EML4*, although *TFG* and *KIF5B* are two other fusion partners [63]) causing aberrant expression and activation of the oncogenic ALK kinase fusion protein [64]. At least 13 different *ALK* fusions are documented. The most common fusion variants are E13;A20 (*EML4* exon 13 fused to *ALK* exon 20) and E6a/b; A20. The fusion results in constitutive activation of the preserved ALK kinase domain and the varying transcripts are oncogenic *in vitro* [65].

ALK fusions are mostly observed in younger never/ex-light smokers and are associated with more advanced disease. Whilst *ALK* fusions are generally mutually exclusive to other somatic aberrations [66], other somatic mutations, for example *EGFR* and *BRAF*, have been observed concurrently. *ALK* fusions can be identified by a FISH assay which identifies separation of the *ALK* 5' and 3' ends (break-apart) [65]. Alternatively, ALK overexpression by immunohistochemistry correlates well with ALK fusions [67] and is validated and suited for use in routine screening, given the labor intensiveness of FISH.

Crizotinib, a potent MET, ALK and ROS1 kinase inhibitor showed remarkable activity in patients with ALK fusion from early-phase trials (Table 3). In the PROFILE 1005 single-arm Phase II trial, in pretreated ALK fusion-positive NSCLC patients, the response rate (RR) was 60.8% with median PFS 9.7 months, and in a small cohort of treatment-naive patients (n = 24) median PFS was 18.3 months [68]. The subsequent confirmatory Phase III study, PROFILE 1007, comparing crizotinib with chemotherapy (pemetrexed or docetaxel monotherapy) in ALK FISH-positive NSCLC following one prior line of platinum-based treatment, confirmed superiority for crizotinib (median PFS 7.7 vs 3.0 months, HR: 0.49; p < 0.001; RR 66 vs 29 and 7% [pemetrexed and docetaxel, respectively]) [4]. This efficacy underpinned marked improvements in quality of life [69]. Therefore, patients with advanced NSCLC should undergo ALK testing and if available, receive crizotinib.

As with mutation-driven NSCLC subsets, resistance to crizotinib can be mediated through a second ALK acquired mutation or upregulation of alternative signaling pathways. Defined molecular resistance mechanisms have been identified in approximately 70% of published cases of crizotinib acquired resistance [72]. These are classified as ALK-dominant and ALK nondominant [73]. ALK dominant mechanisms include; up to one-third with a second ALK mutation, most commonly L1196M (comparable to EGFR T790M as it also markedly reduces ALK-kinase inhibitor affinity [74]) ALK amplification, and ALK deletion. ALK nondominant mechanisms include upregulation of EGFR activity (either wild-type or through acquisition of activating EGFR mutations), KIT amplification, KIT overexpression and KRAS mutations [74].

More potent 'second-generation' ALK kinase inhibitors are in development for crizotinib-refractory and crizotinib-naive ALK-positive advanced NSCLC (Table 3). These include ceritinib (LKD378; Novartis, Basel, Switzerland), alectinib (AF802, CH5424802, RO5424802; Roche, Basel, Switzerland) and AP26113 (Ariad Pharmaceuticals, MA, USA). Both ceritinib and alectinib have shown activity in crizotinib-naive, and crizotinib-resistant patients in early-phase trials [70,71], and also those with brain and meningeal disease, a common site of treatment failure with crizotinib [75,76]. *In vitro* data suggest that the specific mechanism mediating crizotinib resistance may dictate sensitivity of tumors to second generation inhibitors [74] emphasizing further the influence of molecular subtype on treatment of NSCLC.

RET fusions

As well as *ALK* fusions, *RET* oncogene fusions (usually with *KIF5B*) have been identified in 1–2% of adenocarcinomas. *RET-KIF5B* fusions are secondary to an inversion event between the long and short arms of chromosome 10, and are transforming [77]. *CCDC6–RET* and *NCOA4-RET* fusions have also been identified in resected adenocarcinoma specimens. These fusion genes are also observed in papillary thyroid cancers and thyroid cancer secondary to radiation exposure.

While series are limited, *RET* fusions tend to occur in patients younger than *EGFR* mutant NSCLC, with more poorly differentiated tumors [78]. Vandetanib is a clinically available RET kinase inhibitor that has shown preclinical activity here [79], and clinical trials of vandetinib, sunitinib, cabozantinib and lenvatinib in *RET* fusion NSCLC are currently recruiting.

ROS1 fusions

Up to 1.7% of all NSCLC harbor *ROS1* fusions [80], increasing to approximately 7% in tumors negative for *EGFR/KRAS* mutations and *ALK* fusions [81]. Such fusions are transforming [79]. ROS is a receptor tyrosine kinase with sequence homology to ALK kinase [82], with poorly understood biology [81]. A number of dif-

ferent fusion partners have been identified including *SLC34A2*, *SDC4*, *LRIG3* and *CD74*.

Limited clinical cohorts evaluated thus far have shown that *ROS1* fusion positive patients are more likely to be never-smokers with more advanced disease, with conflicting reports on median age and sex distribution. As with *ALK* rearrangements, *ROS1* fusions are detectable using a break-apart FISH assay [81].

Crizotinib is a known inhibitor of ROS1 kinase and in a Phase I trial of crizotinib in unselected patients, patients with a *ROS1* rearrangement (n = 25) showed an RR of 57% [83] similar to that seen in *ALK* rearrangement patients [84]. *In vitro* data suggest that acquired resistance to crizotinib in *ROS1* positive patients may be mediated by EGFR pathway activation, suggesting the potential rationale to trial dual ROS1 and EGFR inhibitor therapy [85].

BRAF mutations

In total, 1–3% of adenocarcinomas harbor somatic *BRAF* mutations. Unlike other cancer types, only 50% of patients have an activating exon 15 V600E mutation. 40–50% have exon 11 or 15 mutations that are either non-V600E activating mutations, such as G469A, or mutations that confer low kinase activity, for example, G466V [86]. The majority of *BRAF* mutations are observed in smokers, but it has been suggested that *BRAF* V600E mutations are commoner in females and nonsmokers [87]. V600E is associated with a poorer prognosis than wild-type tumors [88].

BRAF inhibitors vemurafenib [89] and dabrafenib have both been reported to show efficacy in individual patients with *BRAF* V600E positive advanced adenocarcinoma, with interim Phase II efficacy data for dabrafenib [90] demonstrating an RR of 54%. Further clinical trials are in progress with both agents. V600E

Table 3. Summary of clinical trials in ALK fusion-positive non-small-cell lung cancer.							
Trial population	Trial	Molecule	Outcome	Ref.			
ALK rearrangements							
ALK fusion-positive NSCLC, one or more prior treatment regimens	PROFILE 1005	Crizotinib	RR: 60.8%, overall median PFS: 9.7 months; treatment-naive subgroup, median PFS: 18.3 months	[63]			
ALK fusion-positive NSCLC, one prior line of platinum-based treatment	PROFILE 1007	Crizotinib	PFS benefit over single-agent chemotherapy (median PFS: 7.7 vs 3.0 months; HR: 0.49; p < 0.001)	[4]			
<i>ALK</i> fusion-positive NSCLC, one or more prior treatment regimens, ALK inhibitor naive	AF001-JP	Alectinib (CH5424802, RO5424802)	93.5% (95% CI: 82.1–98.6) RR with 300 mg dose	[70]			
ALK fusion-positive NSCLC (including prior ALK inhibitor treatment)		Ceritinib	ORR: 58% (95% CI: 48–67), RR: 56% (95% CI: 45–67) if previously received crizotinib, median PFS: 7.0 months if ≥400 mg ceritinib daily	[71]			
HR: Hazard ratio; NSCLC: Non-small-cell lung cano	er; ORR: Overall respo	onse rate; OS: Overa	all survival; PFS: Progression free survival; RR: Response rate.				

melanomas develop acquired resistance through MEK signaling and dabrafenib–trametinib combination therapy significantly increased PFS as compared with dabrafenib alone (HR: 0.39; p < 0.001) [91]. Trials of the BRAF-MEK combination inhibitors dabrafenib–trametinib in NSCLC are recruiting. Lung cancer cell lines carrying non-V600E activating mutations are vemurafenib resistant but sensitive to MEK inhibition [92]. Kinase-dead BRAF is known to use CRAF to sustain MEK signaling and can be inhibited *in vitro* with sofarenib or dasatanib [93,94]. Phase II studies of dasatinib in this setting are currently underway but have shown significant toxicity [95].

Molecular subtypes of lung squamous cell carcinoma

Histological identification of squamous cell lung cancer facilitates treatment decisions, since both bevacizumab and pemetrexed are contraindicated in squamous cell carcinomas [2,96]. Comprehensive genomic sequencing of squamous cell carcinomas by The Cancer Genome Atlas and others has identified potential molecular therapeutic targets including *FGFR1* amplification [97], *DDR2* mutations [98], *EGFR* amplification and *BRAF* mutations [9].

FGFR1 amplification

FGFR1 amplification is identified in 20–25% of squamous cell lung cancers and enhances signaling through the MAPK pathway, promoting cell proliferation [10]. Preclinical data suggest that *FGFR1* amplification confers sensitivity to *FGFR1* inhibitors and tumor shrinkage, and trials of AZD4547 are ongoing for *FGFR* amplified NSCLC [97]. To date no gender, age or pathological features have been shown to correlate with the presence of *FGFR1* amplification, although it is more common in current smokers than former smokers, and is rare in never smokers [99]. There are conflicting reports of the effect of *FGFR1* amplication on prognosis [10,100].

DDR2 mutations

DDR2 is a tyrosine kinase receptor with structural similarities to ABL kinase and IGF1 receptor [101]. *In vitro DDR2* mutant expression promotes interleukinindependent growth and colony formation [98]. Up to approximately 5% of squamous cell lung cancers harbor a *DDR2* mutation and *in vitro* data suggest sensitivity to dasatanib, nilotinib and imatinib [10,98]. Phase II trials of dasatanib in *DDR2* mutant NSCLC are ongoing.

PIK3CA amplification/PTEN loss/AKT mutations

Mutation in components of the PI3K/AKT signaling pathway have been identified in nearly half of all lung squamous cell carcinomas analyzed by The Cancer Genome Atlas [9]. These mutations were mutually exclusive of *EGFR* alterations. The commonest aberrations in the pathway are *PIK3CA* mutations (16%), homozygous loss or mutation of the tumor suppressor *PTEN* (15%) and *AKT* mutation, overexpression or amplification (16%).

Both *PIK3CA* amplification and exon 9 and 20 mutations are observed in up to 6.5% of squamous cell lung cancer, with the commonest mutation E454K. Gains in chromosome 3q (the site of *PIK3CA*), occur in just over 40% of squamous cell carcinomas [8].

PTEN mutations are found in up to 15% of squamous cell lung cancer [9]. PTEN is a tumor suppressor that moderates the activity of PI3K. Deletion of part of PTEN can also lead to homozygous or heterozygous loss. This is one mechanism that has been shown *in vitro* to contribute to secondary resistance to EGFR TKIs in EGFR mutant NSCLC [102]. Drugs that target the PI3K/AKT pathway, namely PI3K inhibitors and dual PI3K/mTOR inhibitors, have shown efficacy in earlystage clinical trials in patients with other solid tumors harboring a PI3KCA mutation or PTEN loss and trials in squamous cell lung cancer patients are ongoing [103,104].

AKT3 and *AKT1* mutations have been identified in NSCLC. The frequency of *AKT1* mutations is estimated to be approximately 1% and most commonly is due to the E17K substitution mutation that alters the membrane binding of AKT1 [105]. *AKT3* expression is altered in 16% of squamous cell lung cancers [9].

Allosteric pan-AKT inhibitors such as MK2206 have been developed and show promising results in early clinical trials [105]. These therapeutic agents may increase signaling through other parts of PI3K downstream pathway, however, and so are also in development with other molecularly targeted agents [106].

Conclusion & future perspective

Significant progress has been made in elucidating the molecular subtypes of NSCLC over the last 10 years. This has translated to considerable benefits for subsets of patients such as those with *ALK* rearrangements and *EGFR* mutations. The rapid development and approval of crizotinib illustrates that designing a therapeutic agent against a specific molecular target remains a viable strategy despite concerns over tumor heterogeneity and clonal resistance [107].

Clear elucidation of molecular mechanisms of resistance to targeted agents such as has been achieved with EGFR kinase therapy [108] helps inform future drug development strategies and underlines the critical role of rebiopsy and tumor tissue re-evaluation. The limitations of available material in clinical practice and the risk of sampling bias from one site of disease in a patient with advanced lung cancer is driving exploration of other methods of reliably sampling the tumor genome. Analysis of circulating tumor DNA shed into the blood is a very active area of current research [109,110]. Tracking oncogenic mutations derived from tumor subclones on treatment may become a very sensitive measure of tumor response and of assessing treatment resistance [111,112]. Current data already suggest that circulating tumor DNA changes may be able to detect progression of disease months before it is evident on imaging and that the rate of decline of circulating tumor DNA is predictive of treatment response and outcome [109,113]. Detection and monitoring of tumor subclones and developing resistance to EGFR kinase inhibitors in EGFR-mutant lung cancer is detectable in the circulating tumor DNA [114,115].

Elucidating the molecular subtype of advanced NSCLC at initiation of systemic therapy and on progression is key to ensuring the most effective therapies are used appropriately. Over the next 10 years, circulating tumor DNA may enhance our ability to ascertain this information in real time to aid decision making and

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Papers of special note have been highlighted as: • of interest; •• of considerable interest.

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influence patient care. In addition, a vast array of potentially tractable targets in lung squamous cell carcinoma are now becoming apparent and it is likely that these targets and associated therapeutics will become more established in the very near future. Finally, the early success of immune checkpoint inhibitors in lung cancer thus far, suggests that over the next 10 years immunotherapy will play an increasing role, potentially in conjunction with molecular targeted personalized therapy [116].

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