REVIEW ARTICLE

Targeted therapy in lung cancer: IPASS and beyond, keeping abreast of the explosion of targeted therapies for lung cancer

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ABSTRACT Advances in the treatment of non-small cell lung cancer (NSCLC) over the last decade have predominantly involved the development of therapies directed at molecular targets such as mutations in the epidermal growth factor receptor (EGFR) or rearrangements in the anaplastic lymphoma kinase (ALK) gene. Other targets have been discovered at low frequency, with multiple agents approved or in development for treatment of these rare molecular subtypes. The tumour microenvironment has also provided opportunities for therapies targeting angiogenesis and the host immune response. This review will provide an overview of current targeted therapies in NSCLC and promising treatment approaches on the horizon.

KEY WORDS Non-small-cell lung carcinoma (NSCLC); molecular targeted therapy; immunotherapy; epidermal growth factor receptor (EGFR); anaplastic lymphoma kinase (ALK)

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Introduction

Delivering a high chance of benefit and avoiding futile treatment is crucial in the management of advanced lung cancer where quality of life is constantly at risk from disease progression or treatment toxicity. This ideal is now achievable with the realisation of targeted therapy in non-small cell lung cancer (NSCLC). Targeted therapy refers to pharmaceutical agents that affect a known molecular target in the cancer cell or tumour microenvironment. In some cases, the presence of the target is determined prior to treatment by interrogating tumour samples with a variety of histological and molecular techniques. In other cases, the presence of the target is assumed to be present in the majority of patients on the basis of prior analyses on large numbers of samples. Detectable targets that indicate a high chance of treatment benefit with a given therapy are termed predictive biomarkers. This is in contrast to prognostic biomarkers, which merely indicate an influence on prognosis rather than treatment response. Testing for mutations in the epidermal growth factor receptor (*EGFR*) gene and rearrangements of the anaplastic lymphoma kinase (*ALK*) gene in adenocarcinoma of the lung are now in routine clinical use as predictive genomic biomarkers in the management of advanced lung cancer. The group of patients with lung adenocarcinomas that harbour either of these genomic alterations (15-50% depending on the population studied) are already benefiting from targeted therapy with oral kinase inhibitors such as erlotinib and crizotinib. Other potential predictive genomic biomarkers in known oncogenes such as *BRAF, ROS1, MET* and *PIK3CA* have been identified in a systematic fashion and efforts are underway to target them with novel drug compounds.

It is clear now that lung cancer represents a constellation of diseases with distinct molecular profiles and sensitivity to treatment. This re-imagining of the classification of lung cancer has been paralleled by the discovery that squamous cell carcinoma and adenocarcinoma of the lung have very different molecular architectures, and distinguishing the two on histological grounds remains a crucial first step to guide subsequent molecular analyses. Determining the molecular subtypes of lung cancer in the clinic requires an ongoing effort to develop reliable molecular diagnostics, as has occurred with testing for *EGFR* mutation and *ALK* rearrangement. Lung cancer therapy is also likely to benefit from the nascent field of cancer immunotherapy, with preliminary evidence that targeting the host immune response to lung cancer will be a successful and versatile treatment modality in the future. This review

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Table 1. Phase III trials of EGFR TKIs in exclusively *EGFR*-mutant advanced NSCLC.

Trial	Patients	Targeted agent	Comparator arm	Primary endpoint
Western Japan Thoracic Oncology Group 3405 (12)	172	Gefitinib	Cisplatin + Docetaxel	Median PFS 9.2 versus 6.3 months (HR 0.49, 95% CI: 0.34-0.71, P<0.0001)
North East Japan Study Group 002 (13)	230	Gefitinib	Carboplatin + Paclitaxel	Median PFS 10.8 versus 5.4 months (HR 0.3, 95% CI: 0.22-0.41, P<0.001)
OPTIMAL (14)	165	Erlotinib	Carboplatin + Gemcitabine	Median PFS 13.1 versus 4.6 months (HR 0.16, 95% CI: 0.1-0.26, P<0.0001)
EURTAC (15)	174	Erlotinib	Cisplatin + Docetaxel or Gemcitabine	Median PFS 9.7 versus 5.2 months (HR 0.37, 95% CI: 0.25-0.54, P<0.0001)
LUX-Lung 3 (16)	345	Afatinib	Cisplatin + Pemetrexed	Median PFS 11.1 versus 6.9 months (HR 0.58, 95% CI: 0.43-0.78, P=0.001)
LUX-Lung 6 (17)	364	Afatinib	Cisplatin + Gemcitabine	Median PFS 11 versus 5.6 months (HR 0.28, P<0.0001)

PFS, Pogression free survival; HR, Hazard ratio; CI, Confidence interval.

will summarise the current state of targeted therapy for lung cancer with a focus on NSCLC, and discuss promising agents in development.

Targeting oncogenic mutations and chromosomal aberrations in NSCLC

EGFR-mutant NSCLC

Mutations in the *EGFR* gene found in adenocarcinoma of the lung was the first biomarker predictive of benefit from a targeted therapy in NSCLC, and was exemplary of the impressive efficacy that could be expected from this paradigm. Small molecule inhibitors of EGFR were originally developed and tested in unselected lung cancer populations, where some patients were noted to have dramatic responses (1,2). Subsequent studies revealed that tumours with mutations in the intracellular tyrosine kinase domain that mediates downstream signalling of the *EGFR* gene product had substantial clinical responses to oral tyrosine kinase inhibitors (TKIs) such as gefitinib or erlotinib (3-5).

Before *EGFR* mutation was known to be a predictive biomarker, certain patient populations were seen to benefit more from EGFR TKIs, namely those with lung adenocarcinomas, Asian ethnicity, females and never-smokers. It is now known that the enhanced efficacy in these populations is explained by the greater likelihood that their tumours harbour *EGFR* mutations (5-8) and that such mutations are almost exclusively found in adenocarcinoma of the lung (7-9). There is however no clinical characteristic that can be used in lieu of *EGFR* mutation testing.

The efficacy of EGFR TKIs in advanced *EGFR*-mutant lung cancer has now been established in eight randomised phase III clinical trials. The first of these was the pivotal IPASS study which evaluated the efficacy of gefitinib versus first line chemotherapy with carboplatin and paclitaxel in an Asian population of light or never smokers with advanced lung cancer (10). As part of this study which involved over 1,200 patients, 437 patients had tumour samples assayed for *EGFR* mutations. In the overall population, the study showed a non-inferior progression free survival for gefitinib compared to chemotherapy. It was also found that *EGFR* mutation was a very strong predictor of improved progression free survival with gefitinib, and that gefitinib was inferior to chemotherapy in patients without *EGFR* mutations. These results were confirmed in the phase III First-SIGNAL study which also compared gefitinib to chemotherapy in never-smokers with advanced lung cancer (11).

In addition to IPASS and First-SIGNAL, there have been six randomised controlled phase III trials comparing the EGFR TKIs gefitinib, erlotinib or afatinib to chemotherapy in patients with exclusively *EGFR*-mutant lung cancer, both in Asian and Caucasian populations. These studies which are summarised in Table 1 (12-17), uniformly show superior response rates, progression free survival and quality of life with EGFR TKIs compared to cytotoxic chemotherapy. Despite mature follow up data (18-20), no trial of a first line EGFR TKI has shown an overall survival benefit, most likely explained by the large numbers of patients in the chemotherapy arms of these trials that crossed over to EGFR TKI treatment after progression. Although there has been no direct comparison, the second generation EGFR TKI afatinib appears to have more toxicity compared to gefitinib and erlotinib, with higher rates of severe diarrhoea and skin rash (16).

It is now recommended that all patients with advanced adenocarcinoma of the lung be tested for *EGFR* mutations (21), which is typically carried out using DNA sequencing of archival formalin fixed tumour tissue obtained at biopsy. The frequency of *EGFR* mutation in current or former smokers is approximately

10%, and in never smokers can be up to 40-50% (8,22). Due to the superior response rates and quality of life seen with erlotinib or gefitinib compared to chemotherapy, it is also recommended that all patients with *EGFR*-mutant NSCLC receive these treatments as first line therapy (23-25).

EGFR TKIs continue to have a role in NSCLC without *EGFR* mutations, where they may inhibit the overexpressed non-mutant protein, so-called wild-type *EGFR*. Erlotinib was found to improve overall survival in advanced NSCLC compared to placebo following progression on second or third line chemotherapy in the NCIC Clinical Trials Group BR.21 phase III study (26). This study was conducted before the link between *EGFR* mutation and EGFR TKI response was known, but subsequent subgroup analysis showed that the benefit was maintained in patients with wild-type *EGFR* and non-adenocarcinoma histology. A similar phase III study comparing gefitinib to placebo in a heavily pre-treated population failed to meet statistical significance, but there was a trend towards improved survival (27) with gefitinib.

Only one phase III study has compared EGFR TKIs to chemotherapy as second line therapy in a population that is specifically *EGFR* wild-type (28). Although this study suggested that docetaxel was a superior treatment in this group, final publication of results is awaited. A variety of studies have been conducted in unselected populations, showing that EGFR TKIs are non-inferior to second line chemotherapy (29), have a role as maintenance therapy after first line chemotherapy (30), and have similar efficacy to second line chemotherapy in patients that have failed to respond to first line treatment (31). There are no data to suggest the use of EGFR TKIs as first line therapy in *EGFR* wild-type disease, and this strategy appeared to be detrimental in IPASS (10) and also in the phase III TORCH study of erlotinib followed by chemotherapy versus chemotherapy followed by erlotinib (32).

Second generation EGFR TKIs are irreversible inhibitors of mutant *EGFR*, and also inhibit other receptors in the epidermal growth factor family. Afatinib, an ErbB receptor family blocker, is one such drug that has progressed furthest in development. In a phase IIb/III study of afatinib versus best supportive care in an unselected population of patients who had progressed on two chemotherapy regimens as well as either erlotinib or gefitinib, there was a modest prolongation of progression free survival by 2 months, but no overall survival benefit (33). Afatinib has also been tested in two phase III randomised trials as first line therapy in patients with *EGFR*-mutant NSCLC (Table 1) where it showed superior progression free survival compared to chemotherapy (16,17). It has been approved by the United States Food and Drug Administration (FDA) for this indication. Another second generation EGFR TKI dacomitinib has shown superior progression free survival compared to erlotinib when given after failure of prior chemotherapy in a phase II study of 188 patients (34), and is currently under investigation in two phase III studies compared to erlotinib (ARCHER) or placebo (BR26).

An alternative approach to targeting EGFR in NSCLC has been the use of monoclonal antibodies engineered to have strong affinity for the EGFR protein, such as cetuximab (35). Two randomised phase III trials have been conducted comparing chemotherapy to chemotherapy plus cetuximab in advanced NSCLC. The FLEX study of 1,125 patients with advanced NSCLC showed a modest improvement in overall survival of around 1 month with the addition of cetuximab to chemotherapy (36). A similar study failed to show benefit in the primary endpoint of progression free survival (37). Data about the role of EGFR protein expression in predicting benefit have been conflicting, although a retrospective subgroup analysis showed high EGFR expression was predictive of longer survival with cetuximab in the FLEX study (38,39). The lack of clear benefit and uncertainty over an appropriate biomarker has limited the use of cetuximab.

Acquired treatment resistance to EGFR TKIs

There is now little doubt about the effectiveness of EGFR TKIs in *EGFR*-mutant NSCLC. However, despite high initial response rates, drug resistance and clinical failure is inevitable with the use of these agents over the course of a patient's treatment, so-called acquired resistance. In contrast to cytotoxic chemotherapy, the well defined mechanism of action of EGFR TKIs means that treatment resistance is a potentially tractable problem. Serial biopsies of tumours before and after treatment with EGFR TKIs have provided insight into the mechanisms of treatment failure (40-43), and have now been performed in sufficient numbers of patients to give an overview of the most common resistance mechanisms. In approximately 60% of cases, treatment failure is mediated by the presence of the secondary *EGFR* mutation T790M that is resistant to inhibition by current EGFR TKIs (40,43). This is presumed to develop from a resistant population of cells already present in low numbers before treatment with EGFR TKIs (44). In another 5-15% of cases, activation of alternative pathways within the cell that free it from dependence on *EGFR* signalling occurs, most commonly involving amplification of the *MET* gene (40-42,45) and mutations in *PIK3CA* (41). Mutations in *BRAF* have also been seen, and confirmed to confer resistance in cell line models (46), as has amplification of *HER2* (47). Activation of the *AXL* kinase appears to be another mechanism of acquired resistance (48). Unexpectedly, transformation to small cell histology has been observed in approximately 5% of cases (41,42) and several of these patients responded to conventional chemotherapy regimens used for small cell lung cancer (41). It is of note that several mechanisms of resistance may co-exist

in the same tumour (41-43), such as T790M mutation and *MET* amplification.

The great value in understanding the mechanism of acquired resistance is that it provides a pathway to developing improved therapeutic strategies. Given that T790M mutations are the most common mechanism of acquired resistance, developing EGFR TKIs that inhibit T790M mutant *EGFR* is a logical next step. There is *in vitro* evidence that second generation EGFR TKIs such as afatinib may have better efficacy against T790M mutations (49), although response rates in trials with populations expected to have significant numbers of T790M mutations have been poor (33). A phase II study of afatinib combined with cetuximab has however shown promising results, controlling disease in all 22 patients enrolled with 36% showing partial responses (50). Toxicity has been a problem with this combination however. Finally, third generation mutation-selective EGFR TKIs such as CO-1868 have been developed that specifically inhibit the T790M mutant EGFR protein. CO-1868 is currently being tested in a phase I trial in patients with advanced *EGFR*-mutant NSCLC that have progressed on other EGFR TKIs, where it has shown preliminary evidence of efficacy in resistant disease and a favourable toxicity profile (51). AP26113 is another third generation EGFR TKI with T790M activity that is in phase I/II testing (52).

Targeted therapies already exist or are in development for other molecular pathways that may mediate acquired resistance, such as those involving *HER2, BRAF, PIK3CA* and *MET*. Combining such therapies with EGFR TKIs may provide an avenue for preventing or delaying acquired resistance. This has been applied *in vitro* where EGFR TKI resistance was reversed by co-administration of a MET inhibitor (53,54). Challenges remain in designing trials of tailored drug combinations in this setting and managing the potential toxicities that arise.

ALK-positive NSCLC

ALK was first detected as a fusion oncogene in lung adenocarcinoma in 2007 (55,56), although it had previously been identified as a fusion oncogene arising from a translocation between chromosome 2p and 5q in a subset of anaplastic large cell lymphomas (57). In the context of NSCLC the most frequent *ALK* gene rearrangement arises due to a short inversion in chromosome 2p where the *ALK* gene is fused with the echinoderm microtubule-associated protein-like 4 gene (*EML4*). The aberrant fusion protein EML4-ALK promotes cell growth, and is sufficient to transform cells into a malignant phenotype *in vitro* (55). *ALK*-positive cells seem to rely almost exclusively on the fusion protein to drive cell growth and survival, a concept termed 'oncogene addiction' that also applies to *EGFR*-mutant NSCLC (58). In this context, inhibition of oncogene function in EML4-ALK addicted tumours should result in growth arrest and cell death, and this was observed in animal models using small molecule kinase inhibitors targeting *ALK* (59,60).

Although developed originally as a small molecule inhibitor of the oncogene *c-MET*, crizotinib was also found to inhibit the *ALK* kinase (61), and was already in phase I trials when *ALK* was discovered to play a role in lung cancer. A reliable diagnostic method was also developed to detect *ALK* fusions in archival lung tissue using fluorescence in situ hybridisation (FISH) with break-apart probes. This enabled patients with advanced *ALK*-positive lung cancer to be enrolled rapidly into a phase I trial of crizotinib, where an impressive response rate of 60% was demonstrated (62,63). Most of these patients had received prior chemotherapy. A subsequent report with more mature data compared the overall survival of patients who received crizotinib in the phase I study to *ALK*-positive patients that were not enrolled and also *ALK* negative patients. Although not a randomised comparison, use of crizotinib was associated with improved survival compared to historical cohorts (64). It was also noted that the presence of an *ALK* fusion was not prognostic for survival in the absence of crizotinib.

Of the 1,500 patients screened for *ALK* fusions in the phase I study, only 5% were positive (62). In a similar fashion to *EGFR* mutations, some clinicopathologic characteristics predict a higher likelihood of *ALK* positivity, including young age, lack of smoking history and adenocarcinoma with solid, acinar or signet-ring histologic patterns. In an unselected population with NSCLC the frequency of *ALK* positivity is approximately 4% (62,65-68). *ALK* fusions are only very rarely found in lung cancers that have mutations in other oncogenes such as *EGFR* or *KRAS* (67).

Crizotinib has since been compared to standard second line chemotherapy in a multi-centre phase III randomised controlled trial in 342 patients with advanced *ALK*-positive lung cancer that had progressed after first line chemotherapy (69). Almost all of the patients in the standard arm received pemetrexed or docetaxel. The study was clearly positive for the primary endpoint with a median progression free survival of 7.7 months in the crizotinib arm and 3.0 months in the chemotherapy arm, shown in Figure 1 (HR 0.49, 95% CI: 0.37-0.64, P<0.0001) (69). Crizotinib also improved baseline symptoms and delayed subsequent worsening to a greater degree than chemotherapy in quality of life analyses. There was no overall survival benefit seen, most likely because at least 64% of patients in the chemotherapy arm subsequently received crizotinib. A phase III trial of crizotinib as first line treatment for *ALK*-positive lung cancer has recently completed accrual. Crizotinib has received regulatory approval in Europe and the United States. It is recommended by international guidelines that testing for the presence of an *ALK* fusion be considered for all patients with adenocarcinoma of the lung (23,70).

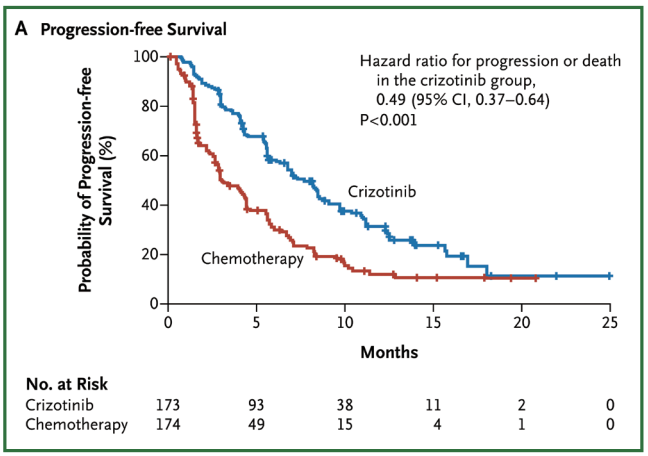

Figure 1. Progression free survival for second line crizotinib versus chemotherapy in *ALK*-positive NSCLC. From "Shaw AT, Kim DW, Nakagawa K, *et al*. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013;368:2385-94. Copyright © 2013 Massachusetts Medical Society". Reprinted with permission.

Crizotinib and *ALK* positive lung cancer is a unique example of the promise of targeted therapy. It has taken only 4 years from the original discovery of the *EML4-ALK* fusion in lung cancer to the FDA approval of crizotinib and its widespread clinical use for this indication.

Acquired resistance to crizotinib

With time, resistance to *ALK* inhibition with crizotinib is inevitable. The median progression free survival in the largest study of crizotinib was 7.7 months (69). In a similar fashion to EGFR TKIs, biopsy of progressing lesions in patients treated with crizotinib has provided insight into resistance mechanisms (71-74). Mutations in the *ALK* gene appear to mediate resistance in around one third of patients, although there is a much wider spectrum of mutations than that seen in *EGFR*-mutant lung cancer where T790M dominates as discussed previously. Activation of alternate signalling pathways involving *EGFR* and *c-KIT* (an oncogene targeted by imatinib) may also play a role in mediating resistance (71). *In vitro* studies suggest that targeting the alternative pathway with existing agents such as gefitinib in the case of *EGFR* or imatinib for *c-KIT* may reverse resistance to crizotinib (71). The mechanism of crizotinib resistance in *ALK* positive tumours currently remains unknown in around one third of cases (75). Of concern, multiple different resistance mechanisms may occur simultaneously in the same patient (71).

Next generation ALK inhibitors with different properties to crizotinib have been developed to have greater potency and potentially target resistance mutations. One agent CH5424802, has been tested in phase I and phase II trials in crizotinib naïve *ALK*-positive NSCLC, and is notable for the 93% overall response rate seen (76). Another agent LDK378 has shown efficacy in a phase I trial which included both crizotinib resistant and naïve *ALK*-positive NSCLC (77), with a response rate of 70%. LDK378 also appeared effective in the presence of resistant *ALK* mutations.

KRAS-mutant NSCLC

KRAS mutations occur in around 30% of NSCLC (73), making them the most common driver mutation seen in an unselected population. Adenocarcinomas make up the majority of NSCLC with *KRAS* mutations (78), and there is a positive association with smoking history (79). *KRAS* mutations may predict a lack of benefit from EGFR TKIs in patient with wild-type *EGFR*, but data have been conflicting (80-82). Despite much research, it has not proved possible to directly target *KRAS*, although recent progress has been made (83). Alternative strategies have involved targeting the down stream signalling pathway of *KRAS* (84), a role fulfilled by the *MEK* inhibitor selumetinib (85). In a randomised phase II trial of second line therapy in *KRAS*-mutant advanced NSCLC, selumetinib plus docetaxel was superior to docetaxel in response rate and progression free survival (86). Other approaches to targeting *KRAS*-mutant NSCLC in early phase trials include PIK3CA/mTOR/AKT pathway inhibitors in combination with *MEK* inhibitors to effectively block downstream *KRAS* signalling (87).

Other oncogenes in NSCLC

With the advent of next generation sequencing technology, driver oncogenes beyond *EGFR*, *ALK* and *KRAS* have been characterised in NSCLC, often at frequencies of less than 5% (88). As targeted therapies already exist for several of these altered genes and are in use in other cancer types, there is currently a focus on identifying lung cancer patients with these alterations and matching them to appropriate therapies within early phase trials (89). There are clear differences between squamous cell and adenocarcinoma histologies in terms of driver oncogenes (9,90), so these will be discussed separately. The pattern and frequency of alterations are summarised in Figure 2.

Adenocarcinomas

ROS1 translocation

Fusion genes involving the receptor tyrosine kinase *ROS1* have been found in 1-2% of NSCLC typically in never or light smokers with adenocarcinoma (91,92). This fusion is notable as it appears sensitive to inhibition with crizotinib (91,93), and defines a molecular subclass of lung cancers with clinical similarity to *ALK*-positive cancers.

MET amplification

MET is the gene for the hepatocyte growth factor receptor (HGFR). Activation of *MET* signalling is sufficient to transform cells to a malignant phenotype, and has effects on the cell cycle and survival. NSCLC cells commonly overexpress *MET*, and *MET* amplification is a defined pathway of resistance to EGFR TKIs (40-42,45). The monoclonal antibody onartuzumab (MetMAb) blocks binding of HGF to the *MET* receptor. It was combined with erlotinib in a randomised phase II trial in advanced NSCLC after failure of prior therapy. In patients with *MET* over-expression, combination therapy significantly prolonged overall survival from 4.6 to 12.6 months (HR 0.37, 95% CI: 0.2-0.71, P=0.002) compared to erlotinib alone. Tivantinib, a small molecule MET inhibitor was tested in a phase III trial in combination with erlotinib, but the study was closed early for futility (Press Release, ArQule Inc. and Daiichi Sankyo Co.).

BRAF mutations

BRAF is a well characterised driver mutation in metastatic melanoma, where it is treated with oral *BRAF* inhibitors such as vemurafenib or dabrafenib. A phase II trial of dabrafenib in *BRAF* mutant NSCLC is ongoing, with 7 out of the first 17 patients on trial demonstrating a partial response (94). The frequency of BRAF mutation in NSCLC is 1-5% (88,95,96), and appears to be at least equally as common in current or former smokers as non-smokers. The classic sensitising V600E mutation was only found in 50% of the *BRAF* mutant lung cancers, which may limit the use of currently available *BRAF* inhibitors (95).

HER2 amplification and mutations

HER2 amplification or mutation is known to exist in some lung cancers with a frequency of around 3% (97). Attempts at treating HER2 amplified NSCLC with the monoclonal anti-HER2 antibody trastuzumab were unsuccessful (98). *HER2* mutation in exon 20 is a more promising molecular subgroup, and there exist several small molecule inhibitors of the *HER2* tyrosine kinase such as afatinib or dacomitinib (99). There have been early reports of some responses to these drugs in patients with *HER2* mutations (100), and trials are ongoing.

RET translocations

Fusions involving the receptor tyrosine kinase *RET* gene have recently been indentified in lung adenocarcinomas, and *in vitro* studies have confirmed the oncogenic potential of at least some of the identified fusions (101). The prevalence of *RET* rearrangements is estimated at between 1-2%, being higher in never or light smokers (92,101). The *RET* kinase inhibitor vandetanib (102) is a well established treatment for medullary thyroid carcinoma and may be a treatment option for *RET* positive adenocarcinoma of the lung.

PIK3CA mutation

PIK3CA is a known oncogene central to the phosphatidylinositide 3-kinase (PI3K) pathway that is deregulated in multiple cancer types (103). *PIK3CA* has been found altered in 1-2% of lung adenocarcinomas, and may co-exist with other mutant oncogenes (104-106). There is considerable effort to target this gene in other cancer types, and early phase trials are underway with *PIK3CA* targeted therapy for lung cancer both as monotherapy and in combination with other targeted agents and chemotherapy.

Squamous cell carcinomas

Recent progress has identified three potential therapeutic targets in squamous cell carcinoma of the lung. The fibroblast growth factor receptor 1 (FGFR1) is one such target, which is amplified in 21-22% of squamous cell carcinomas in recent studies (107,108). These studies also showed that *FGFR1* amplified cells underwent apoptosis when treated with a small molecule FGFR1 inhibitor, and *FGFR1* amplified tumours in mice shrank with inhibitor therapy, suggesting that FGFR1 is an important driver in some squamous cell carcinomas. Multiple small molecule inhibitors of FGFR1 are in development and entering early phase trials, with promising preliminary activity (109).

Mutations in the receptor tyrosine kinase *DDR2* gene have been seen in 2% of squamous cell carcinomas of the lung (9,110). TKIs widely used in treating chronic myeloid leukaemia such as dasatinib also have activity against *DDR2*. Dasatinib has produced partial responses in some squamous NSCLC patients in phase I trials (111,112). In one of the patients with a response, sequencing of a tumour biopsy revealed a *DDR2* mutation (110). Phase II trials of dasatinib specifically in squamous cell carcinoma of the lung are underway.

Alterations in genes playing a role in the PI3K pathway are present in 30-50% of squamous cell carcinomas, mostly comprising *PIK3CA* amplification and mutation, and deletion of the tumour suppressor gene *PTEN* (9,106). This pathway is important to maintaining cell survival and promoting growth (103), but the relationship between alterations in this pathway and response to inhibitors is complex. Phase I trials of *PIK3CA* inhibitors are underway in squamous NSCLC.

Targeting the tumour microenvironment

Angiogenesis in lung cancer

Angiogenesis has emerged as a broadly available target in multiple cancer types, as any sizeable tumour requires the ability to form a new blood supply to survive (113,114). The most well studied pathway mediating angiogenesis involves the vascular endothelial growth factor (VEGF) family of ligands and associated receptors which have intracellular tyrosine kinase

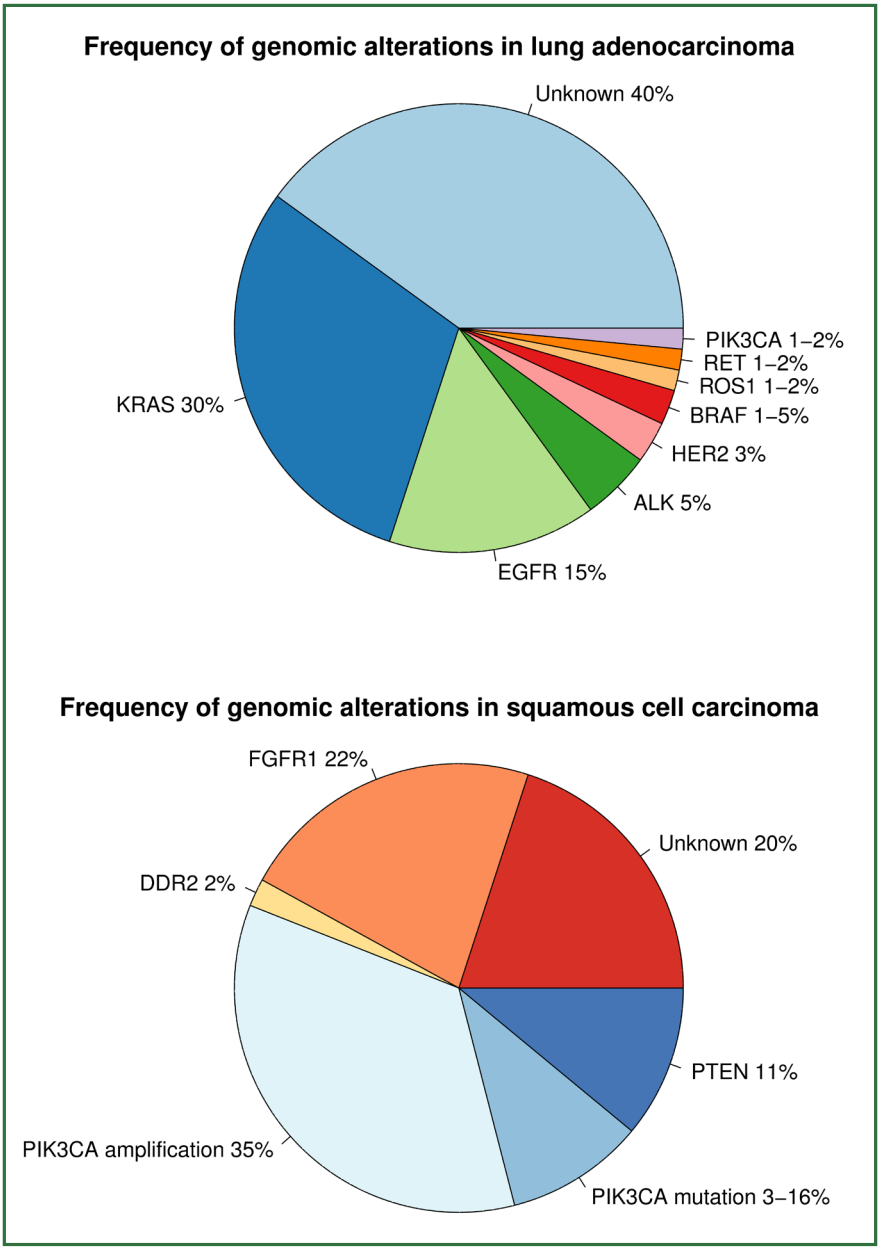

Figure 2. Relative frequency of genomic alterations in adenocarcinoma and squamous cell carcinoma. Data adapted from multiple references (see text) and are estimates only.

domains that mediate downstream signalling (115). Targeting VEGF receptor tyrosine kinase signalling using small molecule inhibitors has generally proven unsuccessful, despite multiple agents having been tested in phase III trials (116-122). The VEGF and FGF receptor inhibitor nintedanib combined with chemotherapy has shown a marginal benefit of less than one month in progression free survival over chemotherapy alone, as second line treatment of advanced NSCLC in two phase III trials (123,124).

Bevacizumab is the most widely used anti-angiogenic agent in routine practice. It is a recombinant humanised monoclonal antibody that binds to VEGF, specifically the VEGF-A isoform, and prevents activation of the VEGF receptor (125). The Eastern Cooperative Oncology Group E4599 trial was performed in 878 patients with advanced NSCLC, and compared bevacizumab plus chemotherapy with carboplatin and paclitaxel to chemotherapy alone (126). Bevacizumab was continued as maintenance therapy until progression after 6 cycles of chemotherapy. Median overall survival was superior with bevacizumab at 12.3 versus 10.3 months (HR 0.79, 95% CI: 0.67-0.92; P=0.003). Progression free survival and response rate were also superior with bevacizumab in a second phase III

trial AVAiL, although overall survival was no different (127). Toxicities of bevacizumab include arterial thromboembolism, hypertension, augmented chemotherapy-related haematological toxicity and bleeding (126). Due to the higher risk of significant haemoptysis, bevacizumab should not be used for squamous cell histology. Bevacizumab has not had widespread uptake as standard first line therapy outside of the United States due to concerns about toxicity, cost and the lack of a biomarker predictive of benefit.

Immunotherapy

Recent advances in tumour immunology have revealed that the immune system plays an important role in controlling malignant growth, and shapes the characteristics of the tumour that eventually manifests clinically (128). Harnessing the immune system as a therapeutic modality has already shown success in advanced melanoma (129) and prostate cancer (130). Although traditionally not considered to be an immunogenic tumour type, there is evidence that markers of a host immune response to lung cancer have a significant prognostic impact in both the adjuvant setting and advanced disease (131-134). Enhancing the immune response may therefore represent a rational therapeutic target. Immunotherapy in lung cancer consists primarily of two approaches: vaccines derived from lung cancer cell lines or tumour associated antigens, and immuno-stimulatory checkpoint antibodies.

Vaccines

Several vaccines have shown promising results in phase II trials, and are currently being evaluated in randomised phase III trials. The largest trials will be discussed here.

Belagenpumatucel-L is an irradiated whole cell product consisting of multiple lung cancer cell lines reflecting adenocarcinoma, large cell carcinoma and squamous cell carcinoma histologies together with an immuno-adjuvant (135). A small single arm phase II trial conducted in a mixed population of early stage and advanced lung cancer demonstrated radiological responses in 15% of patients with measurable disease and a positive correlation between prolonged overall survival and higher vaccine dose (135). Belagenpumatucel-L is being further evaluated in a phase III trial recruiting patients with stage III-IV disease that is stable or responding after first line therapy.

Other vaccines consist of antigens expressed exclusively or predominantly in lung cancer cells. Melanoma-associated antigen-A3 (MAGE-A3) is expressed in 35% of NSCLC (136), and has been prepared as a mono-antigenic vaccine. This was tested in a randomised placebo-controlled phase II trial following resection of stage I-II NSCLC showing cellular expression of MAGE-A3 (137). Following surgery, the disease free survival and overall survival were no different between vaccine and placebo groups, but there were numerically fewer recurrences in the vaccine group after a median of 44 months post surgery (35% versus 43% in placebo group). 2,270 patients have been recruited to a phase III trial of the MAGE-A3 vaccine, with results awaited.

MUC-1 is an epithelial cell protein that is differentially glycosylated in malignant cells (138) and overexpressed in NSCLC (139,140). The BLP25 vaccine contains the MUC-1 peptide and an immuno-adjuvant encased in a liposomal delivery system (141). In a phase III randomised trial comparing BLP25 to placebo after concurrent or sequential chemoradiotherapy for stage III NSCLC, patients who had received concurrent treatment showed a median overall survival of 30.8 months compared to 20.6 months with placebo (HR 0.78, 95% CI: 0.64-0.95; P=0.016) (142). BLP25 also prolonged survival in a phase II study in advanced NSCLC compared to best supportive care but this was not statistically significant (141). TG4010 is an alternative approach to MUC-1 vaccination, incorporating an attenuated but replication competent vaccinia virus that encodes for the MUC-1 protein and interleukin-2 (143). In a randomised phase II study, cisplatin and gemcitabine plus TG4010 was compared to cisplatin and gemcitabine alone in 148 patients with advanced NSCLC (144). Progression free survival at 6 months was 43% with the vaccine versus 35% without, but this difference was not statistically significant. Further studies with BLP25 and TG4010 are awaited.

Immune checkpoint blockade

Immune checkpoints refer to the molecular mechanisms that control T-cell responses to foreign antigens. Part of the immune checkpoint system encompasses stimulatory or suppressive co-receptors that modulate the interaction of the T-cell receptor (TCR) with human leukocyte antigen (HLA) expressed on the target cell. Two such receptors have emerged as important therapeutic targets in cancer. The cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptor is expressed on T-cells following activation by antigen, and serves to dampen the T-cell response to promote self-tolerance and prevent autoimmune activation. Programmed cell death protein 1 (PD1) is also expressed on T-cells and similarly provides a mechanism for down-regulating the T-cell response if the ligand (programmed cell death 1 ligand 1 or PD-L1, also known as B7) is encountered. Preventing T-cell suppression at the tumour-immune interface by disrupting immunosuppressive signals forms a promising therapeutic strategy for advanced lung cancer that may also extend to adjuvant treatment.

The toxicities of the various immune checkpoint antibodies are similar and relate to autoimmune phenomena such as colitis, skin rash, pneumonitis and endocrinopathies. As these do not overlap with chemotherapy toxicity, combining these treatments

Table 2. Upcoming trials of anti-PD-1 therapy in advanced NSCLC.

Population	Treatment arms	Phase
Squamous cell carcinomas of the lung	Nivolumab versus Docetaxel	Phase III
Non-squamous carcinoma of the lung	Nivolumab versus Docetaxel	Phase III
All NSCLC, no previous therapy	Nivolumab monotherapy; Nivolumab + cisplatin/pemetrexed; Nivolumab + carboplatin/paclitaxel; Nivolumab + cisplatin/gemcitabine	Phase I
	Standard first line chemotherapy followed by nivolumab and bevacizumab maintenance	Phase I
	Ipilimumab + nivolumab	Phase I
EGFR-mutant NSCLC	Nivolumab + erlotinib	Phase I
All NSCLC	Lambrolizumab monotherapy; Lambrolizumab + standard chemotherapy; Lambrolizumab in NSCLC overexpressing PD-L1	Phase I

with chemotherapy is a feasible approach. Ipilimumab is a humanised IgG1 anti-CTLA-4 receptor antibody, and is already an established therapy for advanced melanoma (129). A randomised placebo controlled trial was conducted comparing ipilimumab plus carboplatin and paclitaxel chemotherapy to placebo plus chemotherapy in 204 patients with advanced NSCLC (145). Ipilimumab was given in two schedules in the treatment arms: concurrent treatment starting from the first cycle of chemotherapy and phased treatment starting after two cycles of chemotherapy. In light of experience with melanoma that ipilimumab may cause an initial worsening in the radiological appearance of lesions used to assess progression free survival, modified immune-related radiological response criteria were used (146). The study was positive for the primary endpoint of immune-related progression free survival, which was 5.7 months in the phased treatment group compared to 4.6 months in the control group (HR 0.72, P=0.05). Efficacy was most pronounced in patients with squamous cell histology. A similar randomised phase II trial was carried out in 130 patients with extensive stage small cell lung cancer, and showed a trend towards improvement in immune-related progression free survival for the phased regimen in combination with chemotherapy compared to chemotherapy alone (6.4 versus 5.3 months; HR 0.64; 95% CI: 0.4-1.02; P=0.03) (147). Further trials for squamous cell lung cancer and small cell lung cancer are planned.

Multiple tumour types express the PD-L1 ligand on their cell surface, highlighting the role of the PD-1 receptor in suppressing anti-tumour T-cell responses (148). Monoclonal antibodies to both PD-1 and PD-L1 have been tested in several phase I trials that enrolled considerable numbers of patients with NSCLC (148,149). In one such trial the anti-PD-1 antibody nivolumab (formerly known as BMS-936558/MDX-1106) produced an unprecedented response rate of 18% amongst 129 NSCLC patients that were heavily pre-treated, with half of these patients having received three or more previous lines of therapy (148). In addition, the anti-PD-L1 antibody BMS-936559 produced response rates of 10% in a phase I trial that included 49 patients with NSCLC (149). The benefit was evident for both squamous cell carcinomas and adenocarcinomas. From these two trials there is early evidence that expression of the PD-L1 ligand in the tumour microenvironment, which can be evaluated with immunohistochemistry, may predict benefit from anti-PD-1/PD-L1 therapies. In addition to nivolumab, lambrolizumab is another anti-PD-1 antibody that has shown efficacy in melanoma and is being evaluated in lung cancer. Upcoming trials involving nivolumab and lambrolizumab are shown in Table 2.

Conclusions

The last ten years have seen a revolution in the way that lung cancer is conceptualised and treated, born out by advances in genomics, cell biology and drug development technologies. The same advances that facilitated this revolution will continue to provide a roadmap for ongoing improvements by identifying new targets and defining the mechanisms of treatment failure and resistance. The transition of crizotinib from an investigational compound to an approved therapy in a mere 4 years also provides hope that there will be a rapid expansion in therapeutic options available to patients in the near feature. Similarly, immunotherapy represents an entirely new class of agents with a promising efficacy and toxicity profile. With the arrival of targeted therapy come multiple challenges however. The development of targeted therapies is often at odds with the traditional clinical trial structure required by regulatory authorities, where phase III trials illustrating an overall survival benefit are considered the gold standard. In addition, targeted therapies carry high costs to the patient or funding agency, and the long term economic viability of the current drug development cycle is uncertain. Finally, it is still the case that the majority of patients with advanced lung cancer have no targeted therapy available to them at the current time, either due to a lack of known targets in their tumour or poor access to novel

agents. Addressing both these issues will remain a priority if the successes of the past decade are to be maintained.

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