UPDATE ON THE TREATMENT OF METASTATIC NON-SMALL CELL LUNG CANCER (NSCLC) IN NEW ERA OF PERSONALISED MEDICINE

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UPDATE ON THE TREATMENT OF METASTATIC NON-SMALL CELL LUNG CANCER (NSCLC) IN NEW ERA OF PERSONALISED MEDICINE

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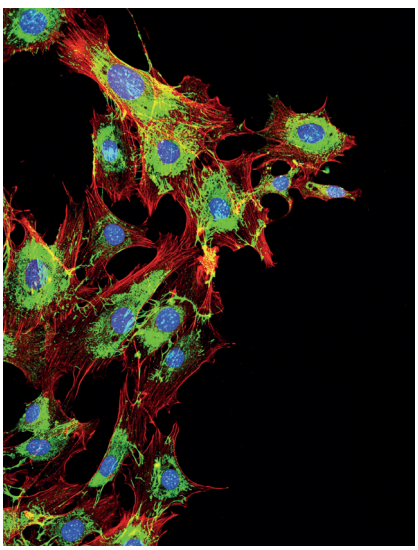

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Lung Cancer remains a major cause of death in of both women and men in our society. Lung cancer treatment paradigms have changed enormously as we've started to understand the genetic complexity and the multiple driver mutations influencing the disease. Therapeutics directed towards, or to inhibit signaling pathways has resulted in increased life spans for our patients. Over the last two decades, we have gone from simple chemotherapy used to treat all, to a personalized medicine approach for the majority. For non-small cell lung cancer patients without driver mutations, the world of immune oncology has arrived. These improved long-term outcomes mean that now our lung cancer patients can live with their cancers, but without progression.

The aim of this book is to catalogue the current state of knowledge for the many facets of advanced lung cancer. It describes current treatment approaches for driver mutations, rare mutations, and rare thoracic malignancies such as neuroendocrine tumors. Most importantly, this book addresses the topics of palliative treatment and care which allow our patients to enjoy longer survival with the highest quality of life. We hope you enjoy this e-book. The future is brighter for lung cancer patients and as lung cancer specialists; we finally feel sense optimism about treatment options for our patients.

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EDITORIAL
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Editorial: Update on the Treatment of Metastatic Non-small Cell Lung Cancer (NSCLC) in New Era of Personalised Medicine

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Keywords: NSCLC, advanced, targeted therapy, immunotherapy, editorial

Editorial on the Research Topic

Update on the Treatment of Metastatic Non-small Cell Lung Cancer (NSCLC) in New Era of Personalised Medicine

We are honored and privileged to present 11 review articles in the context of "An Update on the Treatment of Non Squamous Non-small Cell Lung Carcinoma (NSCLC) in the era of Personalized Medicine." Gone are the days when all lung cancers are treated the same. Treatment is now personalized for multiple biomarkers leading to major advances in survival and quality of life.

It has been over a decade since the IPASS trial was published showing us that patients with an EGFR mutation fare better with a first-generation EFGR TKI targeted to inhibit that mutation versus chemotherapy. In this update, results of clinical trials of the second-generation EGFR inhibitor, afatinib are explored (Morin-Ben Abdallah and Hirsh). The benefit in treatment naïve, refractory, and squamous histology is reviewed reflecting a benefit of irreversibly inhibiting all ErbB family members. As we identify resistance mechanisms to both first- and second-generation EGFR TKI's, the use and benefit of third-generation inhibitors has become standard of care (Barnes et al.). The treatment paradigm of ALK rearrangement is rapidly changing. This incredible journey is leading to prolonged survival in these patients. With next generation sequencing, rare oncogenic drivers are found and successful drug development has occurred (Daoud and Chu). Histology is itself a biomarker and with multiple mutations now being found, the practicing oncologist is challenged. A series of algorithms will be presented for non-squamous histology (Melosky). Squamous histology deserves its own attention and potential molecular targets and novel agents are explored (Soldera and Leighl).

You cannot mention advances in lung cancer without discussing the amazing story of immune-oncology. Activating one's own immune system has led to impressive survival in thoracic malignancies. Future investigations combining PD-1/L1 with chemotherapy, targeted therapy, or other immune-oncology agents aim to improve the number of patients to benefit are ongoing (Iafolla and Juergens).

Targeting angiogenesis is recognized as an effective treatment strategy in a multitude of malignancies including lung cancer. Adding angiogenesis inhibitors to EGFR inhibitors has promising results. Preclinical evidence suggesting an immunosuppressive effect of pro-angiogenic factors leads also to the rationale of adding these agents to immune checkpoint inhibitors (Tabchi and Blais).

Addressing and maintaining quality of life has always been on the forefront goal of lung oncologists. Because of this and impact on lifespan, brain metastases have been an important issue to

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address. Both targeted therapy and immunotherapy have changed the natural history and our treatment in this important metastatic site (Wong). Thoracic oncologists have had to become experts in systemic therapy of pain. The field of interventional pain management needs to be highlighted as it leads to comprehensive patient care (Morin-Ben Abdallah and Hirsh).

Finally, carcinoid and atypical carcinoid tumors of lung origin are reviewed as new treatment options exist and education in these rare tumors is lacking (Melosky).

As far as we have come in the past few years, we still have to strive for improvement in patients with advanced disease. Molecular testing for should be standardized. Patients with non-squamous histology and never smokers should have molecular testing to include at least EGFR, ALK, ROS 1, and BRAF. In addition, genes that could and should be tested include RET, HER2, NTRK, and C MET Exon 14 Skip. Driver mutations continue to be discovered and therapeutics directed toward them continue to be developed. We should not stop trying to inhibit KRAS, the most prevalent mutation in adenocarcinoma. Patients with advanced disease squamous histology should also be tested with molecular panels. CMET Exon 14 Skip is just one example of a driver in squamous histology that when identified may be treated appropriately. PD-L1 testing should be done reflexively on all patients. Although immuno-oncology has changed the treatment landscape, many still do not benefit or respond. Combination therapy may be the answer but toxicity must be low. Our goal for our patients it to prolong their survival duration while maintaining a high quality of their life.

We welcome you to read this issue. The treatment has become complex but the winner is our patients who are now living longer and sustaining a higher quality of life as their treatment is now focused on their tumor factors. The world of lung cancer has changed dramatically with personalized medicine.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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MINI REVIEW
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Current Treatment Algorithms for Patients with Metastatic Non-Small Cell, Non-Squamous Lung Cancer

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The treatment paradigm for metastatic non-small cell, non-squamous lung cancer is continuously evolving due to new treatment options and our increasing knowledge of molecular signal pathways. As a result of treatments becoming more efficacious and more personalized, survival for selected groups of non-small cell lung cancer (NSCLC) patients is increasing. In this paper, three algorithms will be presented for treating patients with metastatic non-squamous, NSCLC. These include treatment algorithms for NSCLC patients whose tumors have *EGFR* mutations, *ALK* rearrangements, or wild-type/wild-type tumors. As the world of immunotherapy continues to evolve quickly, a future algorithm will also be presented.

Keywords: metastatic non-squamous non-small cell lung cancer, systemic therapy, chemotherapy, targeted therapy, epidermal growth factor receptor, anaplastic lymphoma kinase, algorithm

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INTRODUCTION

The previous standard of care in metastatic non-small cell lung cancer (NSCLC) was to treat patients with a platinum doublet for four to six cycles and to offer second-line therapy upon progression (1).

The emergence of molecular tests allows us to tailor treatment strategies based on the presence of driver mutations. Patients who have genetic alterations to epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) now benefit from targeted therapies in the first line and beyond. In patients with no known driver mutations, the efficacy of immunotherapy with checkpoint inhibitors has revolutionized treatment. This area is evolving rapidly.

As new treatment options emerge, algorithms must balance the need to give the best drugs first with ensuring that there are still beneficial options available for later. The treatment algorithms discussed in this paper are based on Canadian recommendations. Although other health authorities may have different therapeutics available, many basic principles apply.

This paper discusses treatments for patients with non-squamous histology only.

Tumor mutation testing allows us to divide patients into three groups: patients with *EGFR*-positive tumor mutations (10–30%) (2); patients with *ALK* rearrangements (4–7%) (2); and patients with tumors who either do not have *EGFR* or *ALK* mutations, or their mutation status is unknown. As mutation testing expands to include new targets including human epidermal growth factor receptor 2 (*HER2*), *BRAF*, *RET* and *MET* and effective treatments are found, the treatment algorithms will increase in complexity (3).

Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; HR, hazard ratio; HER 2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer; OS, overall survival; ORR, overall response rate; RR, response rate; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

EGFR MUTATION POSITIVE

First-line Therapies: Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors (TKIs) that inhibit the EGFR are now standard of care for first-line treatment in patients with metastatic, non-squamous NSCLC whose tumors harbor an *EGFR* mutation (**Figure 1A**). Randomized trials have shown that patients experience superior overall response rates (ORR) and progression-free survival (PFS) when treated with EGFR TKIs versus chemotherapy for first-line therapy [erlotinib: EURTAC (4), OPTIMAL (5); gefitinib: NEJGSG_ 002 (6), WJTOG 3405 (7), IPASS (8, 9); afatinib: LUX LUNG 3 (10, 11), LUX LUNG 6 (11, 12)].

Erlotinib and gefitinib are first generation TKIs, while afatinib is a second generation TKI. Second generation TKIs block more ligands of the HER family and are non-competitive inhibitors at the kinase site so confer a longer period to resistance (13). Patient performance status, comorbidities, and age come into play in the decision making. EGFR mutation subtype is also important to consider. Unlike chemotherapy, TKIs are continued past progression as long as there is a clinical benefit to the patient.

LUX LUNG 7, a recently reported randomized phase IIb trial, compared afatinib to gefitinib in patients with advanced NSCLC and common *EGFR* mutations (14). The coprimary endpoint of PFS hazard ratio (HR) was met for superiority of afatinib, HR = 0.73 ($p = 0.0165$). This benefit was independent of mutation subtype. Response rate (RR), a secondary endpoint, was 70% versus 56% (HR = 1.873, $p = 0.0083$) favoring afatinib. Toxicities were as expected, with a preponderance of diarrhea and rash for afatinib and transaminitis for gefitinib. The overall survival (OS) was 3 months longer for afatinib (27.9 versus 24.5 months) but did not meet statistical significance [HR: 0.86 (95% CI: 0.66–1.12), $p = 0.2580$] (15). ARCHER 1050, a 452 patient phase III randomized trial of first-line treatment of *EGFR*-positive NSCLC comparing gefitinib with dacomitinib, will shed light on the question of which EGFR TKI is superior (16).

The inhibition of both EGFR and angiogenesis pathways deserves comment. The results of a randomized phase II trial from Japan illustrated a benefit for the combination erlotinib–bevacizumab over erlotinib for common EGFR mutations (17). Median PFS for the combination was 16.0 versus 9.0 months for erlotinib monotherapy, with no statistical difference in RRs or OS (18). In June 2016, the European Commission approved the combined use of erlotinib and bevacizumab for the first-line treatment of EGFR positive NSCLC patients. A larger phase III trial of this EGFR TKI–bevacizumab combination is needed to confirm and quantify the benefit (18).

Retesting for EGFR Mutations on Progression

An acquired mutation in *EGFR* exon 20, *T790M*, which leads to drug resistance, may be found in up to 60% of patients progressing on TKIs (19). Repeat testing for mutations is now recommended. Testing plasma cell free (cf) DNA has been suggested as an alternative to repeat biopsy. Different testing platforms are

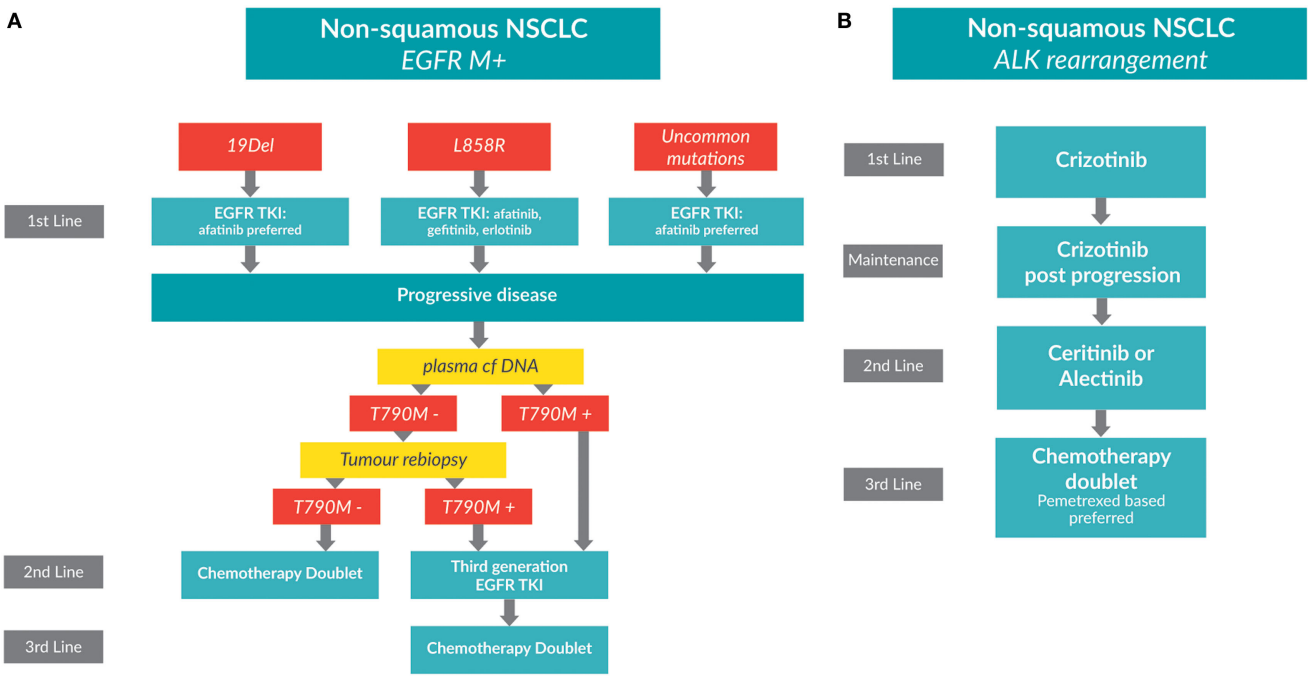

FIGURE 1 | Treatment algorithms for non-small cell lung cancer (NSCLC) patients whose tumors have driver mutations. (A) A treatment algorithm for patients with *EGFR*-positive metastatic, non-squamous NSCLC [adapted from Melosky, Popat, and Gandara (submitted)]. **(B)** A treatment algorithm for patients with *ALK*-positive metastatic, non-squamous NSCLC.

being developed and validated, and concordance between cfDNA and tumor tissue is improving (20–23). Patients who initially test negative for the presence of a *T790M* mutation by cfDNA testing should undergo a tumor rebiopsy. Biopsy is still considered to be the gold standard for *T790M* molecular testing.

Second-line Therapy

For patients with a *T790M* positive disease, third generation EGFR TKIs have demonstrated RRs of over 60% and prolonged PFS, resulting in the approval of osimertinib (AZD9291) in several countries. Pooled results from AURA phase I and II trials was recently presented, which evaluated osimertinib in patients with *T790M*-positive disease who progressed on previous EGFR TKIs (24). Patients from the pooled cohort ($n = 411$) had a RR of 66%, and a PFS of 11 months (24, 25).

For patients without a *T790M*, second-line therapy is a chemotherapy doublet. Patients who are *T790M* mutation negative who progress on chemotherapy have few other options and may consider a clinical trial.

ALK MUTATION POSITIVE NCSLC

Rearrangements in the *ALK* gene are found in adenocarcinomas and more commonly in light or non-smokers. *ALK* rearrangements occur in approximately 4–7% of lung cancers (2). A treatment algorithm for patients with *ALK*-positive metastatic, non-squamous NSCLC is shown in **Figure 1B**.

First-line Therapy with Crizotinib

For patients whose tumors are positive for an *ALK* rearrangement, crizotinib is superior to standard chemotherapy. The phase III PROFILE 1014 trial randomized 343 treatment-naïve patients with advanced *ALK* rearrangement positive NSCLC to receive either crizotinib or intravenous chemotherapy (26). The primary endpoint of PFS was significantly longer in patients treated with crizotinib at 10.9 months as compared to those treated with chemotherapy at 7.0 months [HR: 0.45 (95% CI: 0.35–0.60); $p < 0.001$]. Overall RRs were 74% for crizotinib and 45% for chemotherapy ($p < 0.001$). Median OS was not reached in either group due to cross-over [HR: 0.82 (95% CI: 0.54–1.26); $p = 0.36$] (26). Crizotinib was associated with a greater reduction in symptoms and better quality of life. As with other TKIs, crizotinib can be continued past progression if there is continuing clinical benefit to the patient.

Second-line Therapy with Ceritinib, Alectinib, or Brigatinib

New agents are proving valuable as second-line treatments for NSCLC patients with *ALK*-positive tumors. As the brain is a frequent site of metastasis for patients with *ALK*-positive tumors, the intracranial activity of these agents is important to consider.

Ceritinib

Ceritinib is a second-generation ALK inhibitor that has demonstrated impressive RRs and has improved survival in patients who have progressed on crizotinib. The results of the ASCEND 1, 2, and 3 trials demonstrated the efficacy of ceritinib in treating both systemic disease and brain metastasis.

The ASCEND 1 phase I trial evaluated the efficacy and safety of ceritinib in 246 patients with advanced *ALK*-positive NSCLC (27). The ORR for all patients was 61.8%; 56.4% in pretreated patients and 72.3% in inhibitor-naïve patients. The PFS was 6.9 months for all trial participants (28). Of the 28 subjects with measurable brain metastases at baseline, 35% ($n = 10$) had a partial response (29). As a result of this trial, the FDA approved ceritinib for patients with advanced *ALK*-positive NSCLC following treatment with crizotinib in April 2014.

The ASCEND 2 single-arm phase II trial evaluated ceritinib efficacy in patients with advanced *ALK*-positive NSCLC who had progressed on both standard chemotherapy and crizotinib. With an ORR of 38.6% and a PFS of 5.7 months, ASCEND 2 confirmed the efficacy of ceritinib (30).

The ASCEND 3 single-arm phase II trial evaluated ceritinib efficacy in treatment-naïve patients with advanced *ALK*-positive NSCLC (31). In this trial, PFS was 11.1 months, with a RR of 36.3%. ASCEND 3 demonstrated that ceritinib has intercranial activity; a blinded independent central review demonstrated a 58.8% intracranial response in 50 (40.3%) of subjects with brain metastases (31).

Alectinib

Alectinib, another second-generation ALK inhibitor, also demonstrated impressive RRs and has improved survival in patients who have progressed on crizotinib. A phase I/II trial first evaluated the efficacy of alectinib in patients with crizotinib-refractory *ALK*-positive NSCLC; the dose determined in the phase I component was 600 mg orally twice a day (32). Two large phase II trials conducted in North America and internationally evaluated the efficacy and safety of alectinib in patients with *ALK*-positive NSCLC who had progressed on crizotinib. In the international study, an ORR of 50.8% was observed, the CNS ORR was 58.8% with 20.6% complete responses (33). In the North America trial, similar results were seen with an ORR of 52.2%. The CNS ORR in patients with measurable CNS metastases was 75%, with 25% complete responses (34). In both studies, Grade ≥ 3 adverse events were rare (33, 34).

Japanese researchers have studied alectinib in the first-line setting. The AF-001JP phase I study conducted in ALK treatment-naïve patients showed impressive efficacy. Because Japan has regulations on the use of sodium lauryl sulfate, the dose was set at just 300 mg twice daily (35).

Primary results of the phase III J-ALEX trial were presented at the 2016 American Society of Clinical Oncology meeting (36). In this trial, patients were randomized to receive either alectinib (300 mg twice a day) or crizotinib (250 mg twice a day) in the first-line setting. Alectinib demonstrated significant prolonged PFS [median PFS not reached (95% CI: 20.3 months–not estimated)] compared to crizotinib [PFS: 10.2 months (95% CI: 8.2–12.0)] (36). Although J-ALEX trial used a different dose than the global and North American trials, it led to FDA granting alectinib breakthrough therapy designation for first-line treatment (37).

Investigational Agents: Brigatinib and Lorlatinib

Brigatinib and the third-generation ALK inhibitor lorlatinib are being investigated for their efficacy and safety in *ALK*-positive NSCLC patients who have progressed after crizotinib and/or ceritinib (38, 39). Results of a phase II trial testing two doses of brigatinib demonstrated that patients who received the higher dose achieved a PFS of 12.9 months (40). As a result, the FDA gave brigatinib break through designation. Lorlatinib demonstrated efficacy in a phase I study in heavily pretreated patients; the ORR of 46% and a PFS of 11.4 months were impressive as most patients had received two or more lines of previous therapy (41). Both agents are active in CNS disease. We look forward to adding these agents to the algorithm.

ROS-1

The rare *ROS-1* rearrangement is now recognized as a standard biomaker in many countries, and several ALK inhibitors including crizotinib show activity in these patients. In May 2016, crizotinib was approved in the United States for patients with *ROS-1* rearranged NSCLC (42).

MUTATION STATUS NEGATIVE ("WILD-TYPE/WILD-TYPE") NSCLC

First-line Therapy: Platinum Doublet

Patients with advanced NSCLC whose tumors do not have *EGFR* mutations or *ALK* rearrangements, or who have unknown mutation status, receive the standard of care: a platinum doublet (pemetrexed-based preferred) for four to six cycles (see **Figure 2A**). The Scagliotti trial demonstrated that NSCLC patients with adenocarcinoma experience greater benefit when treated with cisplatin/pemetrexed than with cisplatin/gemcitabine in the first line [OS: 12.6 versus 10.9 months; HR: 0.84 (95% CI: 0.71–0.99); $p = 0.033$] (1, 43).

Maintenance Therapy

Maintenance therapy is administered after completion of first-line therapy but before disease progression. The PARAMOUNT trial demonstrated that pemetrexed maintenance after first-line chemotherapy significantly reduced disease progression over placebo for patients with non-squamous tumor histology (44). Studies have shown that pemetrexed improves both PFS and OS when administered as maintenance therapy (45). Although erlotinib was also an accepted option for switch maintenance based on the SATURN trial (46), the IUNO trial (HR: 1.02; 95% CI: 0.85–1.22; $p = 0.82$) did not support these results. As a consequence, erlotinib is no longer considered as a maintenance option for people with negative or unknown tumor mutation status (47).

Second-line Therapy: Immune Checkpoint Inhibitors

The most important change in the NSCLC treatment paradigm has been the introduction and success of PD-L1 immune checkpoint inhibitors. The programmed cell death receptor (PD-1) is an inhibitory receptor on T lymphocytes that binds PD-L1 and PD-L2 ligands. When ligands PD-L1 and PD-L2 bind, the immune response is suppressed. PD-L1 overexpression by tumor cells allows them to escape T cell detection. Monoclonal antibodies targeting PD-1 or PD-L1 can lead to reactivation of the T lymphocyte and stimulate the natural immune response against tumor cells. Patients tested for PD-L1 overexpression can

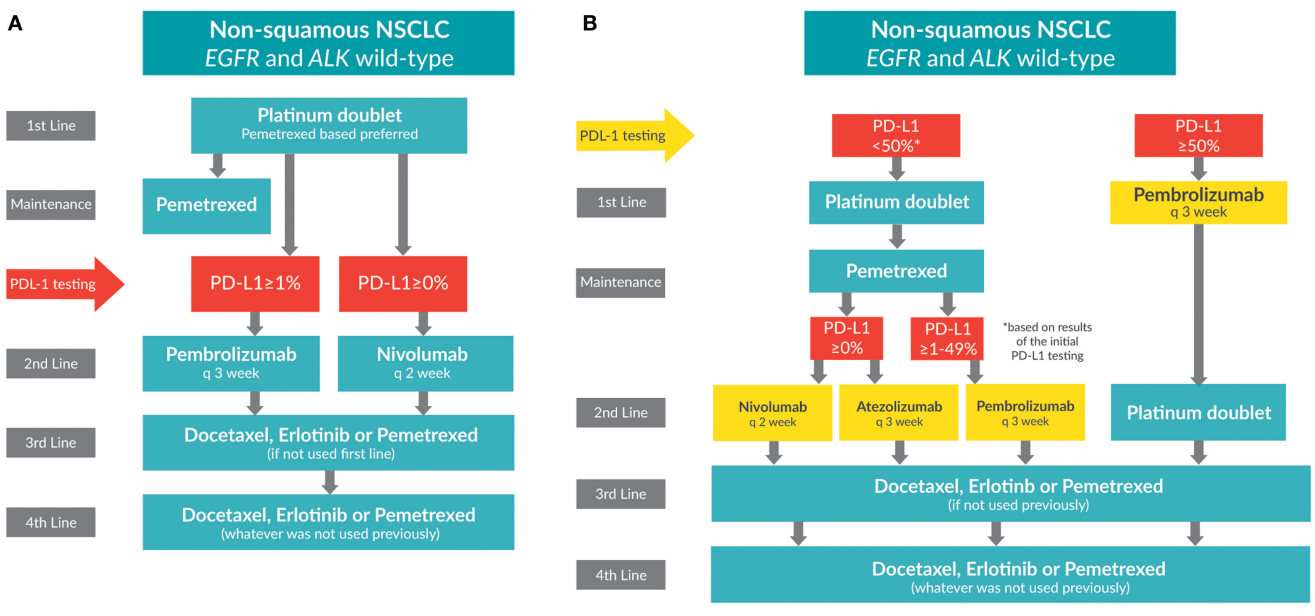

FIGURE 2 | Treatment algorithms for non-small cell lung cancer patients whose tumors do not have *EGFR* or *ALK* mutations (wild-type). **(A)** Current treatment algorithm. **(B)** Future treatment algorithm.

be categorized into PD-L1 expressers (≥1% expression) and non-expressers (<1% expression).

Trials evaluating three immunotherapy agents targeting the PD-1 pathway in NSCLC patients have demonstrated durable clinical activity and manageable toxicity (48–51).

Pembrolizumab

The KEYNOTE 010 trial compared pembrolizumab, a PD-1 monoclonal antibody, to docetaxel in the second-line NSCLC setting. The trial was positive for OS, favoring pembrolizumab at 10.4 months as compared to docetaxel at 8.5 months (HR: 0.71; $p = 0.0008$) (52). This trial included only patients whose tumors tested positive (> 1%) for the biomarker PD-L1. Pembrolizumab is administered intravenously every 3 weeks.

Nivolumab

Nivolumab, a monoclonal antibody against PD-1, was the first checkpoint inhibitor to show efficacy in a randomized phase III trial. The CHECKMATE 057 randomized phase III trial compared the efficacy of nivolumab with docetaxel as second-line treatment for patients with non-squamous NSCLC. Results showed OS benefits favoring nivolumab, at 12.2 months compared to 9.4 months for docetaxel (HR: 0.73; $p = 0.0015$) (53). Although survival was independent of whether the PD-L1 biomarker was present, there was a positive relationship between the degree of positivity of the biomarker and the level of benefit of the drug. Nivolumab is administered intravenously (3 mg/kg) every 2 weeks.

The decision about which antibody to use in the second line will depend on many factors. Determining the level of PD-L1 expression is complex. Biomarker testing and results, scheduling of drug administration (every 2 or every 3 weeks), cost, and availability all play a role.

Third-Line Therapies and Beyond

Now that checkpoint inhibitors are used in the second line, the previously second-line therapies become third-line options for patients whose tumors are mutation negative or mutation unknown. Options include docetaxel (54), erlotinib (55), and pemetrexed (56). Pemetrexed can only be prescribed if it was not used in first line or maintenance therapy. The REVEL trial showed a benefit of adding the angiogenesis inhibitor ramucirumab to docetaxel, with PFS of 10.5 months for the combination versus 9.1 months for docetaxel alone (HR: 0.86; $p = 0.23$) (57).

It follows from above that fourth line therapies may include whatever agents were not administered in previous lines. A significant limitation of therapy selection is that no trials have tested these different agents in later lines of therapy. Patients with satisfactory performance status can be considered for clinical trials.

Future Algorithm for Speculation Only

With many investigational agents in development, it is enticing to speculate what future treatment algorithms for patients with non-squamous NSCLC, mutation negative, or unknown mutation status (see **Figure 2B**).

High PD-L1 Expressers: Checkpoint Inhibitors in First-line Treatment

Recently, immune checkpoint inhibitors were tested in the first-line setting. The KEYNOTE O24 trial randomized patients whose tumors expressed >50% PD-L1 to pembrolizumab or a platinum doublet. The primary endpoint of PFS was met with 10.3 months favoring pembrolizumab, as compared to 6.0 months for chemotherapy (HR: 0.50; $p < 0.001$) (58). KEYNOTE 024 results will quickly be accepted due to the checkpoint inhibitor's unique mechanism of action and low toxicity profile; we anticipate using pembrolizumab in the first line soon.

This contrasts with the results of the CHECKMATE 026 first-line trial of nivolumab, which randomized patients whose tumors expressed >5% PD-L1 to either nivolumab or a platinum doublet. The primary endpoint of PFS was not met, with 5.9 months favoring chemotherapy as compared to 4.2 months for nivolumab (HR: 1.15; $p = 0.2511$) (59).

For the high PD-L1 expressers, we speculate that second-line treatment will be a platinum doublet.

Low PD-L1 Expressers

In the future, patients who are low PD-L1 expressers will likely be treated with a platinum doublet in the first line. After progression, patients will be subdivided further based on the results of their initial PD-L1 test. In addition to pembrolizumab or nivolumab, many other agents are also in development.

Atezolizumab

Atezolizumab is a PD-L1 monoclonal antibody. The results of the OAK second-line trial comparing atezolizumab with docetaxel in patients with positive or negative PD-L1 expression were recently presented (60). The endpoint of OS was met, with results favoring atezolizumab at 13.8 months as compared to 9.6 months for docetaxel (HR: 0.73; $p = 0.0003$) (60). Atezolizumab is administered intravenously at a dose of 1200 mg/kg, every 3 weeks.

While speculating on the future of targeted therapy, will PD-1/PD-L1 checkpoint inhibitors will be prescribed for patients whose tumors are driven by *EGFR* mutations or *ALK* rearrangements? None of the immune therapy agents tested in the CHECKMATE 057 (59), KEYNOTE 010 (52), or OAK (60) trials showed efficacy in these patients. One reason for this may be because tumors with driver mutations have a low mutational load and low PD-L1 expression.

CONCLUSION

Treatment algorithms for NSCLC have changed dramatically over the last few years. Researchers continue to elucidate many molecular pathways involved in thoracic malignancy. Our understanding of tumor mutations and their contribution to therapeutic efficacy is expanding. The treatment selection is complex, with many new target therapies being developed.

For patients with *EGFR*-driven tumors, treatment with osimertinib, a third-generation inhibitor, can lead to improvements in survival in patients whose tumors have acquired

a *T790M* mutation. For patients with *ALK*-driven tumors who have progressed on crizotinib, new treatment options to improve survival include second-generation inhibitors ceritinib and alectinib. For patients without driver mutations or have an unknown tumor mutation status, chemotherapy remains the standard first-line treatment. The efficacy of checkpoint inhibitors has revolutionized treatment in the second-line setting; they now occupy the second-line setting and, on completion of KEYNOTE 024, we hope to see them in the first-line setting as well.

Targeted therapies are shifting the treatment paradigms and increasing survival for patients with NSCLC, a group that used to have a very poor prognosis. The ultimate winner is the patient.

AUTHOR CONTRIBUTIONS

BM wrote this article and created the algorithms. One algorithm is adapted from another paper (submitted).

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frontiers in Oncology

MINI REVIEW
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Update on the Treatment of Metastatic Squamous Non-Small Cell Lung Cancer in New Era of Personalized Medicine

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Despite advances in molecular characterization and lung cancer treatment in recent years, treatment options for patients diagnosed with squamous cell carcinoma of the lung (SCC) remain limited as actionable mutations are rarely detected in this subtype. This article reviews potential molecular targets and associated novel agents for the treatment of advanced SCC in the era of personalized medicine. Elements of various pathways including *epidermal growth factor receptor*, *PI3KCA*, *fibroblast growth factor receptor*, *retinoblastoma*, *cyclin-dependent kinases*, *discoidin domain receptor tyrosine kinase 2*, and *mesenchymal-to-epithelial transition* may play pivotal roles in the development of SCC and are under investigation for drug development.

Keywords: targeted therapy, personalized medicine, lung cancer, squamous cell carcinoma, molecular sequence data

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INTRODUCTION

In 2016, lung cancer remains the most commonly diagnosed malignancy and accounts for the most cancer-related deaths worldwide, representing a significant global health burden (1). The majority of these neoplasms are pathologically categorized as non-small cell lung cancer (NSCLC), which is further divided into three main pathological subtypes: adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma. SCC represents an estimated 20% of NSCLC in developed countries and is mainly attributed to tobacco consumption (2). In the past decade, breakthroughs in molecular characterization of cancers have revolutionized the classification and therapeutic arsenal for lung malignancies. With the discovery of oncogenic driver mutations in epidermal growth factor receptor (EGFR) and rearrangements in *anaplastic lymphoma kinase* (*ALK*) and *ROS1*, there has been a paradigm shift from a "one size fits all" approach to lung cancer treatment to more precise and rational targeted therapy (3, 4). Targeted agents such as EGFR and ALK tyrosine kinase inhibitors (TKI) are now routinely used in clinical practice and have contributed to improving the previously dismal prognosis of this malignancy (5–12). Unfortunately, the impact of these developments to date is largely limited to lung adenocarcinoma as these actionable mutations are rarely detected in other subtypes such as pure SCC (13). This article reviews potential molecular targets and associated novel treatments for advanced lung SCC in the new era of personalized medicine (**Figure 1**; **Table 1**).

In recent years, comprehensive molecular profiling of SCC has revealed that these cancers harbor numerous genomic and epigenomic alterations with a reported mean of 360 exonic mutations, 165 rearrangements, and 323 segments of copy-number alteration per tumor (14). Relative to

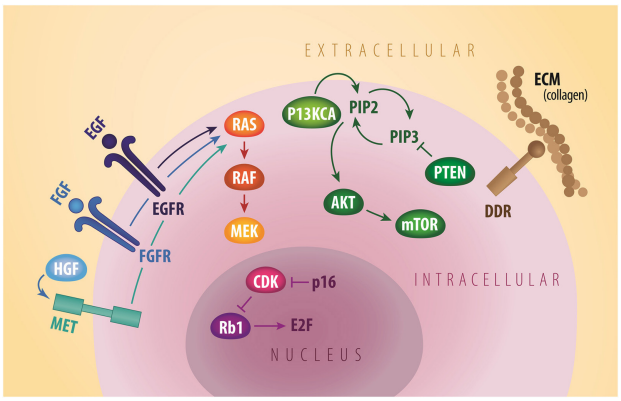

FIGURE 1 | General signaling schema of cell membrane (EGFR, FGFR, MET, and DDR2), cytoplastic (PI3KCA, AKT, mTOR, and PTEN), and nuclear (Rb1 and CDK) molecular targets in squamous NSCLC. CDK, cyclin dependent kinases; DDR2, discoidin domain receptor tyrosine kinase 2; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; HGF, hepatocyte growth factor; mTOR, mammalian target of rapamycin; MET, mesenchymal-to-epithelial transition; PTEN, phosphatase and tensin homolog. Credit to Matthew Villagonzalo, graphic artist, University Health Network.

other tumor types, only malignant melanomas contain a higher burden of genetic abnormalities (24). This is not surprising since both of these cancers are associated with significant exposure to carcinogens. In fact, SCC is known to be strongly associated with chronic tobacco exposure (25). With such a complex genetic landscape and associated high immunogenicity, this tumor type has been an interesting target for immunotherapy and chemotherapy, but the development of targeted agents has thus far represented a significant challenge (26). To address this lack of targeted therapies, the Cancer Genome Atlas Project compared SCC samples to normal pulmonary tissue in order to identify potential actionable mutations (14). Eleven recurrent genomic abnormalities were reported, including *tumor protein 53*, *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*), *phosphatase and tensin homolog* (*PTEN*), *PIK3CA, Kelch-like ECH-associated protein 1*, *mixed-lineage leukemia protein 2*, *human leukocyte antigens A, nuclear factor erythroid-derived 2-like 2*, *NOTCH1*, and *retinoblastoma (Rb1)* (**Figure 1**; **Table 1**). Aberrations in these genes are thought to promote oncologic transformation and progression through their effect on cell survival and proliferation, cell cycle progression, metastatic spread, genetic instability, and response to oxidative stress. Other series have demonstrated similar recurring mutations, while also demonstrating significant abnormalities in *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*), *PI3KCA*, *mesenchymal-to-epithelial transition (MET)*, *human epidermal growth factor receptor 2*, *fibroblast growth factor receptor (FGFR)*, *platelet-derived growth factor receptors* (*PDGFR*), *BRAF*, and *discoidin domain receptor tyrosine kinase 2 (DDR2)* (15–23, 27) (**Figure 1**; **Table 1**). These findings have fueled the development of multiple targeted agents directed against these pathways (**Table 2**).

TABLE 1 | Estimated incidence of targetable molecular aberrations in squamous non-small cell lung cancer (NSCLC).

Gene and aberration	Incidence (%)	Reference
EGFR		
Mutation	0–4.9	Lindeman et al. (13)
	1.1	TCGA (14)
	4	Spoerke et al. (15)
Amplification	7	TCGA (14)
ALK		
Rearrangement	0	Lindeman et al. (13)
FGFR		
Mutation	0.8[b]	CLCGP/NGM (16)
	8[c]	TCGA (14)
Amplification	9.7–22	Weiss et al. (17)
	16[e]	Heist et al. (18)
PI3KCA		
Amplification	37	Spoerke et al. (15)
	33	Yamamoto et al. (19)
Mutation	9	Spoerke et al. (15)
	16	TCGA (14)
	3.6	Yamamoto et al. (19)
	6.5	Kawano et al. (20)
PTEN		
Loss	21	Spoerke et al. (15)
Mutation	8	TCGA (14)
	10.2	Jin et al. (21)
Rb1		
Mutation	7	TCGA (14)
CDK		
Amplification[d]	Significantly amplified	TCGA (14)
CDKN2A		
Mutation	15	TCGA (14)
Loss[a]	72	TCGA (14)
DDR2		
Mutation	1.1	CLCGP/NGM (16)
	3.8	Hammerman et al. (22)
MET		
amplification	6.2–10.3	Go et al. (23)

[a]*Via epigenetic silencing by methylation, inactivating mutation, exon 1β skipping and homozygous deletion.*
[b]*All FGFR3 mutations.*
[c]*FGFR1, 2, 3, and 4 mutations.*
[d]*Significant amplification of CDK6 and CCND1.*
[e]*FGFR1 amplification.*

EPIDERMAL GROWTH FACTOR RECEPTOR

EGFR TKIs improve outcomes for patients with lung cancer harboring activating *EGFR* mutations. While these mutations are commonly found in adenocarcinoma, women, Asians and light or never smokers (3, 5–10), they are rarely found in pure SCC with series reporting a rate in the range of 0–5% (13). Despite this, EGFR TKI have shown significant benefit compared to placebo in patients with advanced lung cancer (all genotypes) having progressed on first or second-line chemotherapy, including SCC (28–30). More recently, Soria et al. reported further advantage of afatinib over erlotinib in the treatment of advanced unselected SCC (including mixed NSCLC) in terms of both PFS (median 2.6 versus 1.9 months; HR 0.81, 95% CI 0.69–0.96, $p = 0.0103$)

TABLE 2 | Clinical trials of targeted therapies in squamous NSCLC.

Agents	Trial	Phase	Outcome (95% CI)	Reference
EGFR				
Erlotinib versus placebo	BR21	III	OS HR 0.70 (0.58–0.85)	Shepherd et al. (28)
Gefitinib versus D	INTEREST	III	OS HR 1.020 (0.905–1.150)	Kim et al. (29)
Afatinib versus erlotinib	LUX-Lung 8	III	PFS HR 0.81 (0.69–0.96) OS HR 0.81 (0.69–0.95)	Soria et al. (30)
C + T ± cetuximab	BMS 099	III	PFS HR 0.902 (0.761–1.069) OS HR 0.890 (0.754–1.051)	Lynch et al. (31)
Cis + V ± cetuximab	FLEX	III	OS HR 0.871 (0.762–0.996)	Pirker et al. (32)
Chemo ± cetuximab	Pujol et al.	Individual patient data meta-analysis	PFS HR 0.90 (0.82–1.00) OS HR 0.88 (0.79–0.97)	Pujol et al. (33)
Cis + G ± necitumumab	SQUIRE	III	OS HR 0.84 (0.74–0.96)	Thatcher et al. (34)
P ± matuzumab (1 versus 3 week)	Schiller et al.	Randomized II	ORR 5 versus 11% ($p = 0.332$)[a] OS 1 week HR 0.67 (0.3–0.21) OS 3 week HR 1.66 (0.9–0.86)	Schiller et al. (35)
C + T ± panitumumab	Crawford et al.	Randomized II	TTP HR 0.9 (0.66–1.21)	Crawford et al. (36)
FGFR				
D ± nintedanib	LUME-lung 1	III	PFS HR 0.79 (0.68–0.92) OS HR 0.94 (0.83–1.05)	Reck et al. (37)
Dovitinib	Lim et al.	Single arm II	ORR 11.5% (0.8–23.8)	Lim et al. (38)
AZD4547	Paik et al.	Ib	0 CR, 1 PR, 4 SD, 9 PD[b]	Paik et al. (39)
BGJ398	Nogova et al.	I	15.4% PR, 34.6% SD 23.1% PR, 26.9% unknown	Nogova et al. (40)
PI3KCA				
Everolimus	Soria et al.	Single arm II	ORR 4.7%	Soria et al. (41)
Everolimus + D	Ramalingam et al.	Single arm II	ORR 8%	Ramalingam et al. (42)
Erlotinib ± everolimus	Besse et al.	Randomized II	PFS 0.769 (0.506–1.167)	Besse et al. (43)
Buparlisib	BASALT-1	Single arm II	12 week PFS 23.3% (9.9–42.3)	Vansteenkiste et al. (44)
D ± PX-866	Levy et al.	Randomized II	med PFS 2 versus 2.9 mo ($p = 0.65$) med OS 7.9 versus 9.4 mo ($p = 0.9$)	Levy et al. (45)
Rb1/CDK				
Palbociclib	Gopalan et al.	Single arm II	ORR 0%, SD 50% (8/16) Med PFS 12.5 week	Gopalan et al. (46)
Abemaciclib	Patnaik et al.	I	ORR 3%, DCR 49%	Patnaik et al. (47)
DDR2				
Dasatinib	Johnson et al.	Single arm II	DCR 43%, ORR 3% Med PFS 1.36 mo Med OS 11.4 mo	Johnson et al. (48)
Dasatinib + erlotinib	Haura et al.	I/II	DCR 62%, ORR 7% Med PFS 2.7 mo Med OS 5.6 mo	Haura et al. (49)
MET				
PL + TAX ± onartuzumab	Hirsch et al.	Randomized II	PFS HR 0.95 (0.63–1.43) OS HR 0.90 (0.55–1.47)	Hirsch et al. (50)
Erlotinib ± tivantinib	Sequist et al.	Randomized II	PFS HR 0.81 (0.57–1.16) OS HR 0.87 (0.59–1.27)	Sequist et al. (51)
Erlotinib ± onartuzumab	METLung	III	PFS HR 0.99 (0.81–1.20) OS HR 1.27 (0.98–1.65)	Spigel et al. (52)
Erlotinib ± onartuzumab	Spigel et al.	Randomized II	PFS HR 1.09 (0.73–1.62) OS HR 0.80 (0.50–1.28)	Spigel et al. (53)

C, carboplatin; Cis, cisplatin; CR, complete response; D, docetaxel; DCR, disease control rate; G, gemcitabine; HR, hazard ratio; Med, median; ORR, objective response rate; OS, overall survival; P, pemetrexed; PD, progressive disease; PFS, progression-free survival; PL, platinum; PR, partial response; SD, stable disease; T, taxane; TAX, paclitaxel; TTP, time to progression; V, vinorelbine.

[a]*ORR in pem versus all matuzumab containing arms.*

[b]*Represents number of patients with measured response as detailed.*

and OS (median OS 7.9 versus 6.8 months; HR 0.81, 95% CI 0.69–0.95, $p = 0.0077$) (30). Of note, patients were previously treated with first-line platinum doublet and had no prior EGFR TKI directed therapies.

Monoclonal antibodies directed against EGFR have also been investigated in this setting. For example, several trials explored the use of cetuximab in combination with chemotherapy in treatment naïve patients, including two phase III trials with conflicting

results (31, 32). A meta-analysis reported a HR of 0.878 (95% CI, 0.795–0.969; $p = 0.01$) for overall survival favoring the use of cetuximab in all lung cancer subtypes (33). Necitumumab, a second-generation recombinant human IgG1 monoclonal antibody, has also shown minor improvements in PFS and OS when added to gemcitabine/cisplatin first-line in advanced SCC versus gemcitabine/cisplatin alone (HR OS 0.84, 95% CI 0.74–0.96; $p = 0.01$) (34). No predictive markers of benefit were identified, although *EGFR* copy number may be promising (54). Conversely, other agents such as matuzumab and panitumumab have failed to show a benefit (35, 36). Despite the low frequency of actionable mutations, SCC shows high rates of *EGFR* amplification and protein expression that could explain these results (55–57). To date, different trials have reported inconsistent results using these findings as predictive biomarkers for response to EGFR directed therapies and their significance remains controversial (58).

FIBROBLAST GROWTH FACTOR RECEPTOR

Genomic abnormalities in the *FGFR* pathway have also been frequently reported in various malignancies including SCC of the lung (59). Most of these aberrations are *FGFR* amplifications with reported rates ranging from approximately 10–25%, while mutations are present in approximately 0–8% of cases (14, 16–18). It is hypothesized that this family of transmembrane receptors participates in many cellular processes including cell survival, differentiation, migration, angiogenesis, tissue homeostasis and repair, and inflammation (60–62). Clinically, *FGFR* amplifications are associated with smoking history and worse prognosis in SCC (63). In recent years, multiple FGFR-directed molecules, including both selective and non-selective FGFR inhibitors, have been developed but remain investigational to date. In the phase III LUME-lung 1 trial, nintedanib, an oral multiple TKI targeting FGFR1–3, vascular endothelial growth factor receptor 1–3, PDGFR α and β, RET, FLT3, and Src family kinases, was investigated in combination with docetaxel after failure of first-line therapy versus placebo (37). Despite marginal improvement in PFS in the overall study population, OS benefit was limited to adenocarcinomas. Dovitinib, a multikinase inhibitor of FGFR1–3, VEGFR1–3, PDGFR β, c-KIT, and FLT3, investigated in a phase II trial of SCC lung cancers showed modest antitumor activity and acceptable toxicity profile with most common significant side effects including gastrointestinal toxicity (nausea, diarrhea, and anorexia), skin rash, and fatigue (38). Selective FGFR inhibitors, such as FGFR1–3 and VEGFR2 inhibitor AZD4547 and pan-FGFR inhibitor BGJ398, remain largely investigational, as early phase trials have reported mixed results in terms of efficacy (39, 40) (NCT00979134, NCT02154490, NCT02160041, NCT01004224). Other agents such as lucitanib (64) (NCT01283945, NCT02109016), ponatinib (NCT01935336), Bay1163877 (NCT02592785, NCT01976741), ARQ087 (NCT01752920), and JNJ-42756493 (NCT02699606) are also in development. Most trials enrolled molecularly enriched populations according to *FGFR* amplification. To date, there is however no standardized method or cut-off for amplification status with significant heterogeneity across trials.

PI3KCA

Alterations in the *PI3KCA* pathway have also been implicated in the development and progression of advanced lung cancer (14). Its activation, triggering downstream AKT and mammalian target of rapamycin signaling, has been linked to gene amplification and mutations, which are both found predominantly in SCC in the range of 35 and 3–15%, respectively (14, 15, 19–21). This pathway is also upregulated through inactivating mutations and loss of its negative regulator *PTEN* and rarely *via AKT* mutations (14, 21, 65). In response to various growth factors, PI3KCA-AKT-mTOR participates in many cellular functions including cell growth, proliferation, differentiation, motility, and survival (66). In preclinical models, cells harboring *PI3KCA* alterations present aggressive phenotype and express markers of epithelial-to-mesenchymal transition (67). Clinically, these aberrations are also linked to EGFR inhibitor resistance (68). Previously, multiple trials have investigated the use of everolimus, an mTORC1 inhibitor, with disappointing results (41–43). Currently, various newer agents targeting this pathway are in development including isoform-specific and pan-isoform PI3KCA inhibitors, AKT inhibitors, and dual PI3KCA-mTOR inhibitors. Buparlisib, an oral inhibitor of class I PI3K (α, β, γ, and d), showed disappointing response rates in a phase II trial meeting futility criteria despite enrichment for PI3KCA pathway activation positive tumors (44). In phase I trials of advanced solid tumors including NSCLC, pilaralisib, an oral pan-class I PI3K inhibitor, has shown acceptable toxicity profile both as a single agent and in combination with EGFR inhibitors with preliminary efficacy limited to monotherapy use (69, 70). PX-866, an irreversible pan-isoform inhibitor of PI3K, failed to show benefit in terms of PFS and OS in a randomized phase II trial in combination with docetaxel compared to placebo (45). Trials investigating other selective PI3K inhibitors such as taselisib (NCT02785913, NCT02389842, NCT02154490, NCT02465060) and pictilisib (NCT01493843, NCT02389842) are currently ongoing both as single agents and in combination with chemotherapy.

Rb1 AND CYCLIN-DEPENDENT KINASES (CDK)

The *Rb1* pathway is also commonly disrupted in various cancers. In association with D-type CDK, CDK4 and CDK6 promote cell cycle progression from the G1 to S phase *via* phosphorylation of the tumor suppressor *Rb1*. P16, a tumor suppressor protein encoded by *CDKN2A*, also influences this pathway through its negative regulation of CDK4 and CDK6, which ultimately causes inhibition of Rb phosphorylation. Once phosphorylated, Rb is rendered inactive, driving cells into synthesis thus contributing to oncogenesis. Deregulation of this pathway occurs as a result of various mechanisms in SCC including *CDKN2A* inactivation *via* promoter methylation, deletions, and mutations, *Rb* mutations and deletions, and *CDK* amplifications (14, 71–74). Furthermore, preclinical data suggest activity of CDK inhibitors in lung cancer xenograft models, and therefore, CDK4/6 inhibitors are currently under investigation for the treatment of advanced lung cancers (74). In a phase II trial, Gopalan et al.

found no responses to palbociclib, a highly specific CDK4/6 inhibitor, in patients with advanced lung cancers and negative p16 expression by immunohistochemistry (46). Interestingly, approximately half of evaluable patients had stable disease (SD) suggesting treatment may induce replicative senescence. Abemaciclib, another CDK4/6 inhibitor, showed acceptable toxicity profile and preliminary efficacy in a phase I trial of multiple tumor types, including NSCLC (47). Further trials investigating these agents are currently underway (NCT02411591, NCT02450539, NCT02152631, NCT02079636, NCT02022982, NCT02389842, NCT02897375, NCT02785939).

DISCOIDIN DOMAIN RECEPTOR TYROSINE KINASE 2

Discoidin domain receptor tyrosine kinase 2 is a widely expressed receptor tyrosine kinase (RTK) in normal cells that is activated through its interaction with various types of extracellular matrix protein collagen. Once activated by ligand binding and phosphorylation, DDR2 has been shown to promote various cellular functions such as migration, differentiation, proliferation, and survival (75). This RTK has been proposed as a potential treatment target in various cancers. Sequencing data has in fact shown mutations in the kinase domain of *DDR2* in approximately 1–4% of SCC (16, 22). Furthermore, *in vitro* studies have also demonstrated that cells harboring these mutations are sensitive to silencing of DDR2 by RNA interference. Multikinase inhibitors have been found to have *DDR2* directed activity in cell lines (76). Dasatinib, a multikinase inhibitor that targets *BCR-ABL*, Src family, c-KIT, PDGFR-β, and ephrin receptor approved for the treatment of chronic myelogenous leukemia (CML), has been investigated for the treatment of NSCLC. Pitini et al. reported a case of a patient with *DDR2* mutated SCC who presented a nearly complete response following treatment with dasatinib for a concurrent CML (77). In a phase II trial, this agent demonstrated moderate clinical activity in patients with unselected treatment naive advanced NSCLC (48). Its use was however limited by significant toxicity, in particular pleural effusion. Notably, one patient responded markedly to treatment with four others showing prolonged SD, suggesting potential benefit in a subset of patients. Unfortunately, investigators failed to identify a predictive biomarker in this subpopulation of responders. Another phase II trial of dasatinib in combination with erlotinib in heavily pretreated NSCLC showed modest efficacy with two patients having PR, one with an *EGFR* mutated adenocarcinoma and one with SCC (49). It is however challenging to estimate the antitumor activity of dasatinib in this setting as responses are more likely related to erlotinib.

MESENCHYMAL-TO-EPITHELIAL TRANSITION

The proto-oncogene *MET* is disrupted in various cancers including NSCLC (78). It encodes a RTK that, once activated by its ligand hepatocyte growth factor, promotes downstream signaling *via* multiple pathways such as PI3KCA, AKT, signal transducer and activator of transcription 3, and mitogen-activated protein kinase (79). Various activating alterations in *MET* have been reported in NSCLC. For example, *MET* amplification has been reported in approximately 6–10% of SCC, while mutations, particularly in exon 14, are more common in adenocarcinomas (23). Once upregulated, MET signaling contributes to cell survival, invasion, migration, and proliferation (79). Clinically, *MET* amplification has been linked to *EGFR* TKI resistance and poor prognosis (80). Cells harboring alterations in this pathway were found to be responsive to MET inhibitors that are commonly used in other tumor types such as crizotinib and cabozantinib (81, 82). Several clinical trials have investigated various TKI with MET directed activity for the treatment of advanced NSCLC with disappointing results in the SCC subpopulation so far (50–53). For example, onartuzumab, a monoclonal antibody directed against MET, failed to show significant antitumor activity in a phase II trial in combination with platinum-doublet chemotherapy (50). Moreover, a phase III trial of onartuzumab in combination with erlotinib was terminated early due to futility in terms of its primary outcome (OS) despite selection of patients with positive MET expression by immunohistochemistry (52). Tivantinib, a small-molecule MET inhibitor, showed modest antitumor activity in combination with erlotinib in unselected NSCLC (51). In subgroup analysis, benefit was however mostly noted in *KRAS* mutated patients and the subsequent phase III trial enrolled only non-squamous histology (83). Finally, identifying responding subpopulations represents a significant challenge in the development of these agents. In fact, selection of patients across trials has been inconsistent, with no clear definition of *MET* enriched populations. Overexpression has been defined using various methods including protein overexpression by immunohistochemistry, gene copy-number gain, and amplification by fluorescent *in situ* hybridization. Despite these challenges, multiple MET-directed molecules are currently under investigation for advanced NSCLC, including SCC (NCT02499614, NCT02034981, NCT00585195, NCT02925104, NCT02414139, NCT02929290, NCT02296879, etc).

IMMUNE THERAPY

In recent years, immunotherapy agents have elicited great interest for the treatment of several tumor types. Various immune checkpoint inhibitors including antibodies directed against cytotoxic T-lymphocyte associated protein 4, programmed cell death protein 1 (PD-1), and programmed death ligand-1 (PD-L1) are under investigation or approved for clinical practice, revolutionizing the approach to lung cancer treatment. Patients diagnosed with SCC in particular have benefited from these advancements, as alternative treatments are sparse, and they have higher mutation burden, which may be associated with benefit. Agents such as nivolumab, pembrolizumab, and atezolizumab have demonstrated improvement in survival outcomes in the second-line setting including in SCC (84–87). Furthermore, Pembrolizumab showed improvement both PFS and OS for patients with strongly PDL-1-expressing tumors treated in the first-line setting. This was however not the case for first-line nivolumab, another PD-1 inhibitor that used less restrictive PDL-1 selection, which had similar PFS and OS but not superior outcomes (88)

(NCT02041533). Much like targeted agents, the selection of patients seems to be an important factor when choosing the best course of therapy. Unfortunately, a predictive biomarker to guide this decision is lacking with PD-L1 expression status, a promising biomarker for the selection of the subgroup likely to benefit from PD-1 and PD-L1 inhibiting drugs, having shown mixed results so far. For example, in the Checkmate 017 study of nivolumab in advanced pretreated SCC patients, PDL-1 expression was not predictive of benefit and even those without PDL-1 expression derived survival gain (84). Conversely, PD-L1 expression was predictive in the Checkmate 057 trial of nivolumab in a similar setting in non-squamous NSCLC (85). Finally, smoking status, a simple clinical characteristic, could also represent a possible predictive marker of response.

CONCLUSION

SCC represents complex tumors with alterations in various interacting pathways (14). Despite the current wealth of available molecular data and a vast array of clinical trial results, multiple challenges remain in the development of targeted therapies for this cancer. One recurring obstacle is the definition of subgroups that derive optimal benefit from investigational agents. With the current understanding of NSCLC now refined according to molecular profiles, individual subpopulations represent rare tumor types limiting their accrual into traditionally designed clinical trials. The revolutionized classification of lung cancer therefore requires an equally novel approach to clinical trial design. In fact, a growing number of "master protocols" with innovative schemes such as "basket" and "umbrella" biomarker-driven trials have been completed or are currently underway (89, 90) (NCT01042379). The LUNG-MAP trial, one such biomarker-based master protocol, is currently ongoing in multiple centers (90). Enrolled patients with advanced SCC are assigned to treatment arms according to detected targetable mutations identified through a comprehensive genomic profiling platform. Targeted agents such as taselisib, palbociclib, talazoparib, ABBV-399, rilotumumab, and AZD4547 have been included in this study. Furthermore, patients without actionable mutations are included in immune therapy sub-studies investigating various immune checkpoint inhibitors such as nivolumab, ipilimumab, durvalumab, and tremelimumab. Considering the dismal prognosis of patients diagnosed with advanced SCC, a greater focus on drug development and clinical trials remains of upmost importance to improve outcomes in this disease.

AUTHOR CONTRIBUTIONS

SS researched data for review topic, drafted manuscript, and edited manuscript revisions. NL researched data for review topic and edited manuscript.

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Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Treatment of Metastatic Non-Small Cell Lung Cancer, with a Focus on Afatinib

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Somatic epidermal growth factor receptor (EGFR) mutations are present in around 50% of Asian patients and in 10–15% of Caucasian patients with metastatic non-small cell lung cancer (NSCLC) of adenocarcinoma histology. The first-generation EGFR-tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib have demonstrated improved progression-free survival (PFS) and response rates but not overall survival (OS) benefit in randomized phase III trials when compared with platinum-doublet chemotherapy. All patients treated with EGFR-TKIs will eventually develop acquired resistance to these agents. Afatinib, an irreversible ErbB family blocker, has shown in two randomly controlled trials in patients with EGFR-activating mutations, a significant improvement in PFS and health-related quality of life when compared to platinum-based chemotherapy. Afatinib improved OS in patients with Del19 mutations. In patients having progressed on first-generation EGFR-TKIs, afatinib did lead to a clinical benefit. A randomly controlled trial showed that PFS was significantly superior with afatinib vs. erlotinib in patients with squamous NSCLC in the second-line setting. A phase IIb trial comparing afatinib and gefitinib in first-line EGFR positive NSCLC showed significantly improved PFS with afatinib but OS was not significantly improved.

Keywords: non-small cell lung cancer, epidermal growth factor receptor, tyrosine kinase inhibitor, afatinib, gefitinib, erlotinib

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INTRODUCTION

The advent of targeted therapy has had a dramatic effect on the treatment of cancer. Few treatment landscapes have shifted more in recent years than in metastatic non-small cell lung cancer (NSCLC). The identification of several oncogenic driver mutations has led to the development of targeted agents (1). The principal targets identified include rearrangements in the anaplastic lymphoma kinase gene and mutations of the epidermal growth factor receptor (EGFR) (1–4).

Epidermal growth factor receptor is a receptor that is part of the ErbB family (5, 6). This family of receptors includes four members; human epidermal growth factor 1 (HER1; EGFR, ErbB1), HER2 (Neu, ErbB2), HER3 (ErbB3), and HER4 (ErbB4) (5, 6). The physiological role of these receptor tyrosine kinases is to regulate cellular proliferation (5). Somatic EGFR mutations are present in

around 50% of patients in Asia and in 10–15% of Caucasian patients with metastatic NSCLC with adenocarcinoma histology (7). Most of these mutations are caused by deletions on the exon 19 or L858R point mutations on exon 21 (8). EGFR-activating mutations lead to aberrant constitutive signaling by EGFR and its associated cell signaling pathways. As a consequence, proliferation often becomes completely dependent on EGFR activation in a phenomenon known as oncogene addiction. Because of this, inhibition of EGFR interrupts proliferation and induces apoptosis (9).

Epidermal growth factor receptor inhibition with oral tyrosine kinase inhibitors (TKIs) has shown proven clinical benefit in patients with NSCLC harboring activating EGFR mutations. The first-generation EGFR-TKIs gefitinib and erlotinib have demonstrated improved progression-free survival (PFS) and response rates but not overall survival (OS) in randomized phase III trials when compared with platinum-doublet chemotherapy (10–16).

FIRST-GENERATION EGFR TKIs: GEFITINIB AND ERLOTINIB

The first-generation EGFR-TKIs, gefitinib and erlotinib, bind reversibly to the kinase domain of the receptor. This leads to the inhibition of both mutant and, to a lesser extent, wild-type EGFR (17). In the early phase III trials of gefitinib conducted in Asia, IPASS, and First SIGNAL (**Table 1**) (10, 13), patients were not initially selected for their EGFR mutation status. Several subgroup analyses of these trials in addition to smaller subsequent trials, however, showed that the presence of EGFR-activating mutations was a strong predictor of clinical benefit with gefitinib when compared with platinum-doublet chemotherapy (10, 13, 18, 19). As a result, subsequent phase III trials of EGFR-TKIs included exclusively patients with activating EGFR mutations (11, 12, 14, 16). Two additional phase III trials, NEJ002 and WJTOG3405, also showed significant PFS advantages of first-line gefitinib when compared to chemotherapy, this time in a Japanese EGFR-mutant population (**Table 1**) (11, 12).

The benefit of EGFR-TKIs was also demonstrated in a European population with advanced NSCLC and EGFR-activating mutations. The phase III EURTAC trial compared erlotinib with platinum-based chemotherapy. Erlotinib was associated with a significant benefit in PFS and was better tolerated than chemotherapy (**Table 1**) (14). The OPTIMAL trial also showed similar results with erlotinib in a Chinese population (16).

Gefitinib and erlotinib have also shown efficacy in second and third line treatment of NSCLC (2). Erlotinib may be an option in both EGFR mutated and wild-type patients. This is based on the results of NCIC BR21 placebo-controlled phase III trial in which patients were not selected for EGFR status. The trial demonstrated a PFS advantage with docetaxel (27). When compared with docetaxel, however, erlotinib did not appear to benefit patients with wild-type EGFR tumors in two phase III trials. In the TAILOR trial, PFS was significantly longer in wild-type EGFR NSCLC patients treated with second line docetaxel (28). In the DELTA trial, no PFS or OS improvement was shown in an EGFR-unselected population treated in the second or third line (29).

Unfortunately, NSCLC with EGFR-activating mutations treated with first-generation EGFR-TKIs inevitably develop resistances (30). Several resistance mechanisms have been described. The development of a T790M missense mutation in exon 20 is the most common of these and has been described in 50–60% of patients (31–33). This mutation causes steric hindrance, which obstructs binding of EGFR-TKIs to their target receptor (34). Other reported resistance mechanisms include alterations to the MET receptor (35–37) and amplification of HER2 (35–37) and HER3 (38).

AFATINIB

Afatinib irreversibly inhibits the tyrosine kinase activity of EGFR, HER2, and ErbB4 by forming covalent bonds to the receptors (39). Although ErbB3 lacks intrinsic kinase activity, it does form active heterodimers by interacting with ErbB family receptors and with HER2 in particular (40). Afatinib suppresses the activity of all four ErbB family members (39). Its irreversible inhibition is also more potent and prolonged than the reversible first-generation EGFR-TKIs (17, 39, 41).

FIRST-LINE AFATINIB IN PATIENTS WITH NSCLC AND ACTIVATING EGFR MUTATIONS: LUX-LUNG 3 (LL3) AND LUX-LUNG 6 (LL6)

The largest randomized phase III trials in treatment-naive advanced NSCLC with EGFR-activating mutations were the LL3 and LL6 trials. The LL3 trial was a global trial, which recruited 345 patients while the LL6 trial recruited 364 patients in Asia (15, 21, 25). Patients were randomized (2:1) to afatinib (40 mg/day) or up to six cycles of platinum-doublet chemotherapy. LL3 used cisplatin and pemetrexed as a control group while LL6 used cisplatin and gemcitabine (42). The primary endpoint of these trials was PFS by prespecified independent central review. The trials also included comprehensive patient-reported outcomes (PROs) related to functional health status/quality of life (QoL) and lung cancer-related symptoms (**Table 2**) (15, 25, 43).

Both trials demonstrated a significant median PFS benefit with first-line afatinib [11.1 vs. 6.9 months; hazard ratio (HR) 0.58 $p = 0.001$ in LL3 and 11.0 vs. 5.6 months; HR 0.28; $p = 0.0001$ in LL6; **Table 1**] (15, 25). A preplanned analysis indicated that the PFS advantage was greater in patients with common EGFR mutations (Del19 and/or L858R). However, afatinib also showed activity in some patients with select uncommon EGFR-activating mutations. A pooled analysis of LL3, LL6, and the phase II LUX-Lung 2 (44) trials showed a median PFS of 10.7 months in 38 patients with uncommon mutations of EGFR (45). The pooled analysis also demonstrated particularly poor outcomes with afatinib in patients with exon 20 insertions (median PFS 2.7 months, $n = 23$).

Afatinib also showed clinical benefit in patients with brain metastases (46). A subgroup analysis of 35 patients in LL3 demonstrated a trend toward improved median PFS when compared

TABLE 1 | Randomized phase III trials comparing EGFR TKIs to standard platinum-based chemotherapy for first-line treatment of advanced *EGFR* mutation-positive NSCLC [adapted from Ref. (20)].

TKI	Reference	Study	Geography	Comparator	No. of pts[a]	RR (%)	Median PFS[b] (months)	Difference in PFS, HR (95% CI); *p*-value	Median OS (months)	Difference in OS, HR (95% CI); *p*-value	Difference in OS—Del19 mutation, HR (95% CI); *p*-value
Gefitinib	(13, 21, 22)	IPASS	East Asia	Carboplatin + paclitaxel	261	71 vs. 47	9.5 vs. 6.3[d]	0.48 (0.36–0.64); $p < 0.001$	21.6 vs. 21.9	1.00 (0.76–1.33); p = n.s.	0.79 (0.54–1.15)[c] p = n.s.
	(10)[c]	First-SIGNAL[c]	South Korea	Cisplatin + gemcitabine	42	85 vs. 38	8.0 vs. 6.3[d]	0.54 (0.27–1.1); p = n.s	27.2 vs. 25.6[b]	1.04 (0.50–2.18)[e] p = n.s.	n/a p = n.s.
	(12)	WJTOG 3405[f]	Japan	Cisplatin + docetaxel	177	62 vs. 32	9.2 vs. 6.3[d]	0.49 (0.34–0.71); $p < 0.0001$	34.8 vs. 37.3[b]	1.25 (0.88–1.78)[e] p = n.s.	n/a p = n.s.
	(11, 23)	NEJGSG 002[c]	Japan	Carboplatin + paclitaxel	230	74 vs. 31	10.8 vs. 5.4[g]	0.30 (0.22–0.41); $p < 0.001$	27.7 vs. 26.6	0.89 (0.63–1.24); p = n.s.	0.83 (0.52–1.34)[e] p = n.s.
Erlotinib	(16, 24)	OPTIMAL	China	Carboplatin + gemcitabine	154	83 vs. 36	13.1 vs. 4.6[d]	0.16 (0.10–0.26); $p < 0.0001$	22.7 vs. 28.9[b]	1.04 (0.69–1.58); p = n.s.	n/a p = n.s.
	(14)	EURTAC	France, Italy, Spain	Cisplatin or carboplatin[h] + docetaxel or gemcitabine	173	58 vs. 15	9.7 vs. 5.2[g]	0.37 (0.25–0.54); $p < 0.0001$	19.3 vs. 19.5[b]	1.04 (0.65–1.68); p = n.s.	0.94 (0.57–1.54)[e] p = n.s.
Afatinib	(21, 25)	LL3	Global	Cisplatin + pemetrexed	345	56 vs. 23	13.6 vs. 6.9[g]	0.47 (0.34–0.65); $p = 0.001$	31.6 vs. 28.2[b]	0.78 (0.58–1.06); p = n.s.	0.54 (0.36–0.79); $p = 0.0015$
	(21, 26)	LL6	China, South Korea	Cisplatin + gemcitabine	364	67 vs. 23	11.0 vs. 5.6[g]	0.28 (0.20–0.39); $p < 0.0001$	23.6 vs. 23.5[b]	0.83 (0.62–1.09); p = n.s.	0.64 (0.44–0.94); $p = 0.023$

CI, confidence interval; EGFR, epidermal growth factor receptor; EURTAC, European tarceva vs. chemotherapy; First-SIGNAL, First-line single-agent iressa vs. gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung; HR, hazard ratio; IPASS, Iressa Pan-Asia study; n/a, not available; n.s., not significant; NEJGSG, North East Japan Gefitinib Study Group; LL3, LUX-Lung 3; LL6, LUX-Lung 6; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; RR, response rate; TKI, tyrosine kinase inhibitor; WJTOG, West Japan Thoracic Oncology Group.

[a]*Number of patients enrolled with EGFR mutations.*

[b]*In patients with common activating mutations (Del19 and/or L858R).*

[c]*Patients with EGFR mutations were a subgroup of all enrollees.*

[d]*Based on investigator assessment.*

[e]*No p-value reported.*

[f]*Including patients with either postoperative recurrent or stage IIIb/IV NSCLC.*

[g]*Based on independent central review.*

[h]*Carboplatin plus docetaxel or gemcitabine was allowed for patients for whom cisplatin was contraindicated.*

TABLE 2 | Patient-reported outcome assessments in first-line *EGFR* mutation-positive clinical trials vs. platinum-doublets [adapted from Ref. (20)].

Trial	Treatments	QoL assessments	Methodology	Outcomes
IPASS (13)	Gefitinib vs. carboplatin + paclitaxel	FACT-L and FACT-TOI	Randomization, week 1, every 3 weeks until day 127, once every 6 weeks from day 128 until disease progression, and when the study drug was discontinued	Significantly more patients in the gefitinib group than in the carboplatin + paclitaxel group had a clinically relevant improvement in QoL and by scores on the FACT-TOI. Rates of reduction in symptoms were similar
EURTAC (14)	Erlotinib vs. cisplatin + docetaxel or gemcitabine	Completion of the lung cancer symptom scale	Baseline, every 3 weeks, end of treatment visit, and every 3 months during follow-up	Insufficient data collected for any analysis to be done—due to low compliance
LL3 (25, 43)	Afatinib vs. cisplatin + pemetrexed	EORTC QLQ-C30, EORTC QLQ-LC13	Baseline, every 3 weeks until disease progression	Afatinib improved lung cancer-related symptoms and QoL and delay of deterioration of symptoms compared with chemotherapy
LL6 (26)	Afatinib vs. gemcitabine + cisplatin	EORTC QLQ-C30, EORTC QLQ-LC13	Baseline, every 3 weeks until disease progression	Afatinib improved lung cancer-related symptoms of cough, dyspnea, and pain and global health status/QoL compared with chemotherapy

EGFR, epidermal growth factor receptor; EURTAC, European tarceva vs. chemotherapy; EORTC, QLQ European Organization for Research and Treatment of Cancer Quality of Life Questionnaire; FACT-L, Functional Assessment of Cancer Therapy—Lung; FACT-TOI, Functional Assessment of Cancer Therapy—Trial Outcome Index; IPASS, Iressa Pan-Asia study; LL3, LUX-Lung 3; LL6, LUX-Lung 6; QLQ-LC13, Quality of Life Questionnaire—Lung Cancer Module; QoL, quality of life.

to chemotherapy [11.1 vs. 5.4 months (HR 0.52 $p = 0.13$)]. For 10 patients with intracranial progression, median time to progression was 11.6 months with afatinib and 5.5 months with chemotherapy (46).

The median OS results of both trials did not show significant statistical differences between afatinib and chemotherapy. The LL3 trial had a median follow-up of 41 months. Median OS was 28.2 months in the afatinib arm and 28.2 months in the chemotherapy arm (HR 0.88, $p = 0.39$). In LL6, the median OS was 23.1 months for afatinib and 23.5 months for chemotherapy (HR 0.93, $p = 0.61$). However, in a preplanned analysis including only patients harboring Del19 mutations in both trials, a significant median OS advantage was shown in favor of afatinib (33.3 vs. 21.1 months; HR 0.54, $p = 0.0015$ in LL3 and 31.4 vs. 18.4 months; HR 0.64, $p = 0.0229$; **Table 1**) (21).

Both the LL3 and the LL6 trials integrated comprehensive PRO evaluation, including both the EORTC QLQLC12 and QLQ-C30 questionnaires, to determine the effect of afatinib on QoL (47). This differed from the past trials such as IPASS (which used Functional Assessment of Cancer Therapy indices) and EURTAC (analysis of PROs was not possible due to insufficient data). This showed that prespecified lung cancer-related symptoms, including cough, dyspnea, and pain were improved with afatinib. In addition, time to deterioration was longer with afatinib when compared to the chemotherapy arms. LL3 demonstrated statistically significant delayed time to deterioration and improved mean scores over time for cough and dyspnea (25, 43). Pain was not statistically different. Similar results were seen in LL6 with the addition that both time to deterioration and mean score over time were improved for pain. Overall, afatinib was associated with statistically significant improvements from baseline in global health status/QoL in both trials (26).

In comparison to platinum-based chemotherapy, afatinib was relatively well tolerated in both LL3 and LL6. Common grade 3 or higher treatment-related adverse events (AEs) of afatinib (LL3/LL6) included diarrhea (14/5%), rash and acne (16/15%), stomatitis and mucositis (9/5%), and paronychia (11/0%). There were more treatment discontinuations due to AEs in the chemotherapy arm than in the afatinib arm in both trials (12 vs. 8% in LL3 and 40 vs. 6% in LL6). No patient discontinued treatment due to diarrhea as a lone AE.

The relatively low rate of treatment discontinuations of afatinib in both trials may be due to effective symptom control and/or protocol defined dose reductions (25, 26). The trials recommended dose reductions in 10 mg decrements to a minimum dose of 20 mg for grade 3 AEs or grade 2 AEs lasting a prolonged length of time (25, 26). These reductions were shown to decrease excessive plasma concentrations of afatinib and, therefore, reduced toxicity without compromising efficacy. In fact, dose reduction was not associated with an inferior PFS (25).

AFATINIB IN PATIENTS WITH RELAPSED/REFRACTORY NSCLC: LUX-LUNG 1 (LL1) AND LUX-LUNG 5 (LL5)

The phase IIb/III trial LL1 compared afatinib at a dose of 50 mg/day to placebo in 585 patients with stage IIIb/IV NSCLC. It included patients who had failed up to two lines of chemotherapy and had been exposed to at least 12 weeks of a first generation EGFR-TKI (gefitinib and/or erlotinib) (48, 49). Although a positive EGFR mutation status was not required, EGFR status was known for 141 patients and, of these, 68% were EGFR positive. Patients were randomly assigned to afatinib or placebo. Afatinib did not lead to a benefit in the primary endpoint of median OS. The median OS was 10.8 months for afatinib and 12.0 months for the placebo arm (HR 1.08, $p = 0.74$). Despite the absence of benefit in OS, an improvement in median PFS was seen with afatinib (3.3 vs. 1.1 months; HR 0.38, $p < 0.0001$) (49). The prolongation of PFS was also associated with an overall improvement in lung cancer-related symptoms and EORTC global health status (48).

Another phase III trial, LL5, included 202 EGFR mutation-positive patients with progressive disease on a prior EGFR-TKI (gefitinib, erlotinib, or afatinib) (46). Patients were randomly assigned to a combination of afatinib and paclitaxel or to investigator's choice of chemotherapy without an EGFR-TKI.

The trial achieved its primary endpoint of PFS. The median PFS was 5.6 months with afatinib and paclitaxel and 2.8 months with chemotherapy alone (HR 0.60, $p = 0.003$). The secondary endpoint of objective response rate (ORR) was also significantly improved (32.1 vs. 13.2%, $p < 0.005$), but median OS was not significantly different (12.2 vs. 12.2 months, HR 1.00, $p = 0.994$). The results of LL5 demonstrated prospective evidence of the benefit of maintaining EGFR blockade beyond disease progression in oncogene-addicted lung cancer.

COMPARING REVERSIBLE AND IRREVERSIBLE ERBB FAMILY BLOCKADE: LUX-LUNG 7 (LL7)

Lux-Lung 7 was an open-label trial comparing first-line afatinib (40 mg/day) to gefitinib (250 mg/day) in 319 EGFR mutation-positive advanced NSCLC patients. This was an exploratory phase IIb trial. In the primary analysis, afatinib significantly improved the co-primary endpoints of PFS and time-to-treatment failure (TTF) when compared to gefitinib. At a median follow-up of 27.3 months, the median PFS was 11.0 months with afatinib and 10.9 months with gefitinib (HR 0.73, $p = 0.017$). TTF was 13.7 months with afatinib and 11.5 months with gefitinib (HR 0.73, $p = 0.007$). The key secondary endpoint of ORR was also significantly improved ($p = 0.008$). The treatment discontinuation rate was 6% in both arms (50). The OS data were recently updated with a median follow-up of 42.6 months. The median OS was 27.9 vs. 24.5 months with a non-significant trend in favor of afatinib (HR 0.86, $p = 0.2580$). Analysis by EGFR mutation subtype showed a median OS of 30.7 months for afatinib compared to 26.4 months for gefitinib (HR 0.83, $p = 0.2841$) in patients with exon 19 deletion. In patients with a L858R mutation, there was a median OS of 25.0 months for afatinib compared to 21.2 months for gefitinib (HR 0.91, $p = 0.6585$) (51). LL7 again demonstrated that dose reductions of afatinib reduced drug-related AEs without compromising efficacy. Overall, irreversible ErbB family blockade with afatinib provided improved clinical benefit over the reversible EGFR-TKI gefitinib for patients with EGFR mutation-positive NSCLC (50).

AFATINIB IN SECOND-LINE TREATMENT FOR NSCLC OF SQUAMOUS CELL (SCC) HISTOLOGY: LUX-LUNG 8 (LL8)

Approximately 30% of NSCLC are of squamous histology (52). Platinum-doublet chemotherapy remains recommended first-line treatment for the majority of these patients. The phase III LL8 trial compared second-line afatinib (40 mg/day) and erlotinib (150 mg/day) in 795 patients with stage IIIb/IV SCC of the lung that were EGFR-TKI-naïve and had failed treatment after four or more cycles of platinum-based chemotherapy. The primary endpoint of PFS by independent radiological review was significantly improved with afatinib. The median PFS was 2.6 months with afatinib compared to 1.9 months with erlotinib (HR 0.81, $p = 0.010$). In addition, the secondary endpoint of OS was also significantly improved with afatinib (7.9 vs. 6.8 months; HR 0.81, $p = 0.008$). Furthermore, results for disease-control rate (50.5 vs. 39.5%, $p = 0.002$), ORR (5.5 vs. 2.8%, $p = 0.055$), and global health status/QoL (35.7 vs. 28.3%, $p = 0.041$) were all also in favor of afatinib (53). Overall, the benefit of EGFR-TKIs in squamous cell NSCLC has been limited. Immune-checkpoint inhibitors are now the preferred second-line option or even first-line option for patients with positive PD-L1 expression (54).

CONCLUSION

The development of ErbB-family blockers has significantly improved patient outcomes for patients with metastatic NSCLC. This is particularly true in patients with EGFR-activating driver mutations where three EGFR-TKIs, gefitinib, erlotinib, and afatinib were shown to have significant survival advantage over first-line platinum-based chemotherapy. Afatinib, an irreversible ErbB family blocker, was designed to decrease resistance to reversible EGFR-TKIs and, therefore, prolong response in the first-line setting. Afatinib remains the only EGFR-TKI to have demonstrated a significant OS advantage in comparison to chemotherapy in patients with EGFR Del19 mutations. Furthermore, head-to-head data of LL7 trial demonstrated an improvement in PFS and PROs with afatinib regardless of mutation type. The results of afatinib in brain metastases have also been promising. There continues to be significant developments in the field of EGFR mutation-positive NSCLC, a third-generation of EGFR-TKIs is already seeking to improve outcomes, especially with osimertinib in patients resistant to EGFR-TKIs due to T790M mutations.

AUTHOR CONTRIBUTIONS

SM and VH contributed to the conception and design of the work, the drafting and revising of its content, and gave final approval of the version to be published. They agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

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Third-Generation Tyrosine Kinase Inhibitors Targeting Epidermal Growth Factor Receptor Mutations in Non-Small Cell Lung Cancer

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Sensitizing mutations in the epidermal growth factor receptor (*EGFR*) predict response to *EGFR* tyrosine kinase inhibitors (TKIs) and both first- and second-generation TKIs are available as first-line treatment options in patients with advanced *EGFR*-mutant non-small cell lung cancer. Eventual resistance develops with multiple mechanisms identifiable both upon repeat biopsy and in plasma circulating tumor DNA. The *T790M* gatekeeper mutation is responsible for almost 60% of cases. A number of third-generation TKIs are in clinical development, and osimertinib has been approved by the US Food and Drug Administration for the treatment of patients with *EGFR T790M* mutant lung cancer after failure of initial *EGFR* kinase therapy. Resistance mechanisms are being identified to these novel agents, and the treatment landscape of *EGFR*-mutant lung cancer continues to evolve. The sequence of *EGFR* TKIs may change in the future and combination therapies targeting resistance appear highly promising.

Keywords: lung cancer, lung cancer treatment, epidermal growth factor receptor, tyrosine kinase inhibitors, *T790M*

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INTRODUCTION

Non-small cell lung cancer (NSCLC) accounts for over 80% of lung cancer cases and is a leading cause of morbidity and mortality internationally (1, 2). When treated with platinum-based chemotherapy, the median survival in patients with metastatic disease is 8 months (3). Mutations in the epidermal growth factor receptor gene (*EGFR*) are found in 10–15% of lung cancers in Caucasians, and 30–40% of East Asian patients (4, 5). These patients most commonly have adenocarcinoma, are lifetime non- or light smokers and are more frequently female. The most commonly described mutations are deletions in exon 19 (del19) and the exon 21 L858R point mutation (from leucine to arginine). The discovery of *EGFR* mutations as a predictor of response to tyrosine kinase inhibitors (TKIs) heralded a paradigm shift in the treatment of NSCLC (6–8).

In the advanced setting, options for first-line treatment of *EGFR*-positive lung cancer include first-generation TKIs (erlotinib, gefitinib) and afatinib, a second-generation kinase inhibitor. These agents have an impressive body of evidence confirming better response, improved progression-free survival (PFS), and quality of life compared to chemotherapy (9–16). The pooled analysis of the LUX Lung 3 and 6 studies also suggested an overall survival advantage of afatinib relative to chemotherapy in the first-line setting for the subgroup of patients with exon 19 deletions (17). Recently, afatinib has been shown to have improved PFS compared to gefitinib, however, at the expense of greater

toxicity (18). Resistance to both first- and second-generation TKIs is common and develops at a median time of 9–16 months (9, 11, 14, 19). This review summarizes known mechanisms of TKI resistance, clinical approaches to resistance with a focus on third-generation *EGFR* TKIs, their preclinical and clinical evidence for use, and future directions to improve the outcomes of patients with *EGFR* mutation-positive lung cancer.

RESISTANCE TO FIRST- AND SECOND-GENERATION INHIBITORS

By performing biopsies in patients with progression on first-generation TKIs, Yu et al. elucidated the common mechanisms of resistance to first-generation TKIs (20). In approximately 60% of cases, a *T790M* point mutation in exon 20 was identified. Other mechanisms include downstream signaling pathway mutations in *BRAF* or *PIK3CA*, or parallel signaling pathway activation via changes in *MET, HER2, FGFR,* and *AXL*. A small group (3%) had histologic transformation: epithelial to mesenchymal transition or high-grade neuroendocrine transformation. A total of 18% did not have an identifiable cause. Multiple mechanisms of resistance were shown in 10% cases and have been postulated to be up to 15% of cases in other series (20–22).

More recently, the rate of *T790M* mutation have been reported to be much higher when analyzing circulating tumor DNA (ctDNA), highlighting the limitations of a single biopsy in the context of tumor heterogeneity (23). Tissue biopsies are associated with risks, delays, and an increased economic burden (24). Liquid biopsies are an attractive alternative to this and can accurately detect *T790M* mutations in ctDNA with a high positive predictive value. In the study by Oxnard et al., of 58 patients with a *T790M* negative tissue biopsy, one-third had *T790M* detected in plasma with similar response rates (RRs) to patients with the mutation identified in tumor biopsy samples (25). Recently, two studies have reported the detection of *T790M* several weeks to months prior to radiological progression, which emphasizes the potential use of serial plasma monitoring in this population (26, 27). However, plasma genotyping may still result in false negatives and it is unlikely that repeat tumor biopsies in clinic can be completely eliminated for all patients. But an approach whereby initial blood-based screening is used, followed by biopsy in only those without the mutation identified, may decrease the morbidity and delays involved in serial genomic testing.

MANAGING RESISTANCE TO INITIAL TKI THERAPY

Platinum-based chemotherapy has been considered the standard treatment upon progression for patients on initial EGFR kinase therapy; however, few patients are well enough or agree to have cytotoxic chemotherapy (28). Intercalation or combination with chemotherapy has been minimally successful with added toxicity and no consistent survival benefit (29). The IMPRESS study showed that continuing TKI therapy with chemotherapy did not provide a PFS benefit and was associated with increased toxicity (30).

For oligo progressive disease, administering local therapy and continuing the original kinase inhibitor is a common approach (31). In a small single-arm phase II study (ASPIRATION), patients with minimally symptomatic or asymptomatic progression were randomized to continue erlotinib past progression or to stop, and those continuing remained on treatment for a median of an additional 3.7 months after the initial PFS of 11 months (32).

Despite *in vitro T790M* inhibition, the second-generation TKIs have not demonstrated significant single-agent activity in *T790M* mutation positive disease. Dual inhibition of *EGFR* signaling has generated interest, with a phase II study of afatinib and cetuximab in TKI-resistant patients, demonstrating a RR of 29% in *T790M*-positive and -negative subgroups. Thus, *EGFR* pathway signaling remains an important driver of disease, with trials ongoing (33).

The most significant development in treating resistance has been through third-generation kinase inhibitors that target *T790M*.

THIRD-GENERATION TKIs

Not only do these agents have activity in *T790M* mutant lung cancer but many have the advantage of limited wild-type inhibition, therefore, overcoming toxicities associated with first- and second-generation TKIs. WZ4002, a covalent pyrimidine *EGFR* TKI, was one of the first compounds to show *in vitro* and *in vivo EGFR T790M* inhibition with relative WT sparing (34). Several agents have now been tested in clinical trials, with osimertinib recently approved by the US Food and Drug Administration (FDA) and other regulatory agencies in patients with *EGFR T790M* mutant NSCLC post failure of first-/second-generation TKIs.

Osimertinib (AZD9291, Previously Merelitinib)

Osimertinib is an oral, irreversible TKI that forms a covalent bond with the cysteine residue in position 797 of *EGFR* within the ATP-binding site. Osimertinib and its active metabolites also interact with a number of other kinases harboring the cysteine residue. Osimertinib is a potent inhibitor of *T790M* with little wild-type activity and shows tumor regression in murine models (35).

AURA (a phase I dose escalation study) (36) was performed in patients with *EGFR* mutation-positive advanced NSCLC with acquired resistance to TKI. No dose-limiting toxicity (DLT) was observed; the most common adverse events were diarrhea, rash, anorexia, and nausea (see **Table 1**). The overall RR was 51% [95% confidence interval (CI), 45–58]; higher in the *T790M* mutation-positive group than the *T790M* mutation-negative group (61 versus 21%). The median PFS was 9.6 months (95% CI, 8.3 to not reached) in *EGFR T790M*-positive patients.

Updated results of the 80 mg (RP2D) cohorts from three AURA studies were presented (37), and confirm a high RR (66% in phase I study and 71% in phase II studies). Encouraging duration of response (12.5 months in pooled phase II studies) and PFS (11.0 months in same pooled analysis) was seen (37).

AURA2 is a single-arm, open-label phase II study of osimertinib 80 mg daily in *T790M* mutation positive NSCLC after

TABLE 1 | Toxicities of third-generation tyrosine kinase inhibitors.

Agent	Osimertinib (36–39)			Rociletinib (41, 43)	Olmutinib (46–48)	Nazartinib (50, 51)	Avitinib (55)
Study	Phase I	Phase II	Phase III	Phase I/II	Phase I/II	Phase I/II	Phase I
Number of patients	253	210	419	92	93	111	25
Response rate (RR) overall	51 (45–58)	70 (64–77)	71 (65–76)	38 (NR)	54 (NR)	47 (39–55)	NR
RR T790M Positive	61 (52–70)	As above	As above	45 (31–60)	NR	NR	NR
Overall grade 3–4 toxicity	32	34	23	NR	NR	NR	4
Rash	40 (1)	40 (1)	34 (1)	<1 (0)	39 (5)	39 (14)	20 (4)
Dry skin	20 (0)	30 (0)	23 (0)	NR	NR	28 (0)	NR
Pruritus	NR	NR	NR	NR	39 (1)	32 (0)	16 (0)
Diarrhea	47 (2)	33 (<1)	41 (1)	22 (0)	55 (0)	40 (6)	44 (0)
Loss of appetite	21 (1)	NR	18 (1)	20 (1)	NR	17 (0)	NR
Nausea	22 (<0.5)	NR	16 (1)	35 (2)	38 (0)	13 (0)	16 (0)
Fatigue	17 (1)	NR	16 (1)	24 (4)	NR	21 (NR)	NR
Dyspnea	11 (2)	<1 (<1)	4 (<1)	1.5	NR (1)	NR	12 (0)
Hyperglycemia	2 (0)	NR	NR	47 (22)	NR (0)	NR	0 (0)
QTc prolongation	NR	NR	NR	12 (5)	NR (0)	NR	8 (0)
Anemia	NR	NR	NR	NR	NR	NR (6)	NR
Stomatitis	NR	NR	NR	NR	NR	23 (NR)	NR
Muscle spasms	NR	NR	NR	11 (1)	NR	NR	NR
Dose reduction	7%	3%	NR	51%	NR	NR	NR
Discontinuation of drug	6%	5%	7%	11%	4%	NR	0

RRs reported in % (95% confidence interval).
Toxicity reported in overall rate % (grade 3–4%).
NR, not reported.
Dyspnea, dyspnea/ILD/pneumonitis reported together.

first-line TKI. A total of 140 (70%; 95% CI 64–77) of 199 patients (with measurable disease) achieved an objective response. There was a disease control rate (dCR) of 92%. Toxicity was manageable with low rates of grade 3 or higher toxicity (see **Table 1**) (38).

AURA3 was a phase III randomized trial that assessed the efficacy and safety of osimertinib (80 mg daily) versus platinum-doublet chemotherapy after initial TKI failure in 419 patients with *T790M* mutation-positive advanced NSCLC (39). The trial demonstrated superior PFS 10.1 versus 4.4 months (HR 0.30; 95% CI 0.23–0.41; $p < 0.001$) and higher RR with osimertinib, 71%, versus chemotherapy, 31% (39). Grade 3 or 4 adverse events occurred in 23% of patients on osimertinib, compared to 47% with chemotherapy (**Table 1**). Quality of life results are pending.

In November 2015, osimertinib received accelerated approval by the FDA, representing rapid progress through drug development—the first AURA patient was enrolled in March 2013 and FDA accelerated approval was granted in November 2015.

Rociletinib (CO1686)

Rociletinib is an irreversible orally delivered third-generation TKI that targets *L858R*, del19, and *T790M* mutations of *EGFR* with little WT activity. Rociletinib also modifies the C797 site through covalent binding. Tumor xenograft and transgenic models documented tumor regression in preclinical studies (40). In the phase I/dose expansion study, TIGERX, 130 patients with progression following *EGFR* TKI were enrolled but the maximum tolerated dose was not reached (41). The RR was 59% in *T790M*-positive patients; however, pooled data from this TIGER-X study and the phase II TIGER-2 was initially reported to be 30.2% (42) and later updated and published as 45% (43).

The most frequent grade 3/4 AEs, which occurred in more than 10%, included hyperglycemia and QTc prolongation (**Table 1**). The hyperglycemia is thought to be mediated by a metabolite of rociletinib that inhibits insulin-like growth factor-1 receptor and to a lesser extent, insulin receptor kinases. Clovis has suspended development of rociletinib and terminated enrollment in clinical trials in 2016, soon after the FDA rejected the request for accelerated approval (44).

Olmutinib (HM61713; Formerly BI148269)

Olmutinib is an oral selective inhibitor for *EGFR* including *T790M* mutant kinases and acts by binding to a cysteine residue close to the kinase domain. Potent inhibition of representative cell lines and *in vivo* activity have been reported (45). A phase I trial of 173 patients with *EGFR*-mutant lung cancer that had failed previous TKI therapy demonstrated a favorable safety profile and promising antitumor activity (46, 47). The MTD was established as 800 mg once daily. Treatment-related adverse events occurred in 87.3% of 165 patients, mainly diarrhea, rash, skin exfoliation, nausea, pruritus, decreased appetite, and dry skin. Grade 3 or greater toxicity was 2% in the initial study report (2/93), although not in the updated results (**Table 1**) (46–48).

In 34 patients with centrally confirmed *T790M* tumor mutations who received olmutinib at a dose greater than 650 mg daily, the RR was 59% (10 confirmed, 10 unconfirmed partial responses) and 13 achieved disease stabilization (dCR 97%). The phase II study has been suspended early with three cases of severe skin toxicity, including two reports of toxic epidermal necrolysis (one fatal), and one case of non-fatal Stevens–Johnson syndrome. The future of the drug's development is uncertain.

Nazartinib (EGF816)

EGF816 is an oral, irreversible *EGFR* TKI that also forms a covalent bond with C797. Low IC_{50} values and *in vivo* activity against

L858R–T790M and del19–*T790M* have been reported (49). Data from the phase I/II study of EGF816 in advanced *T790M*-positive lung cancer are now available (50, 51). Dose escalation began at 75 mg daily up to 350 mg daily for capsules and 100–225 mg daily for tablets. Diarrhea, stomatitis, rash, and pruritus were the most common toxicities (see **Table 1**), and the confirmed RR was 44% (56/127) and dCR was 91% (NCT02108964). Combination studies with immunotherapy are now recruiting (NCT02323126; NCT02335944; NCT02900664).

ASP8273

ASP8273 is an *EGFR* TKI that selectively and irreversibly inhibits mutant *EGFR* kinases including T790M by the formation of a covalent bond with C797. Both *in vitro* and *in vivo* studies confirm activity in *T790M* mutant lung cancer with relative WT sparing (52). In a phase I study, doses were escalated to 500 mg but the RP2D has been deemed 300 mg, although the details of DLT and maximum tolerated dose levels are not published (53). Of 60 patients treated with ASP8273 at the 300 mg dose, there was no DLT. All patients were *EGFR* positive with 90% having a *T790M* mutation. PR was demonstrated in 16 of 45 evaluable patients; dCR was 62% ($n = 28/45$). For the 40 *T790M* mutation-positive subjects with evaluable data, 38% (15/40) had PR and dCR was 65% (26/40) (NCT02113813). The Phase III SOLAR study is underway comparing initial ASP8273 with a first-generation TKI in patients with *EGFR*-mutant lung cancer (NCT02588261) (53).

PF06747775

PF0677775 is another oral inhibitor of *EGFR T790M* with 26-fold increased selectivity of mutant versus wild-type *EGFR*. It is currently under evaluation in a phase I/II study in patients with advanced *EGFR* mutation-positive lung cancer (del19 or L858R, *T790M* positive and negative) (NCT02349633) and early results have demonstrated activity and tolerability (54).

Avitinib (AC0010)

Avitinib is a new, irreversible, *EGFR* mutation selective TKI being evaluated in a phase I/II clinical trial (NCT02274337). In the reported dose escalation study (55), 25 patients were treated. The most common AEs were diarrhea, rash, and pruritus. Although diarrhea and rash increased in frequency in a dose-dependent manner, the majority of them were grade 1 (**Table 1**). There was no drug discontinuation in all treated patients. Outcomes for two patients with T790M-positive lung cancer showed partial responses. The clinical characteristics and efficacy outcomes of the remaining patients are not reported (55).

SPECIAL POPULATIONS

Uncommon Mutations

The "uncommon" *EGFR* mutations represent a heterogeneous group and can account for up to 10–18% of *EGFR* mutations (56, 57). The most frequent include exon 20 insertions (exon-20ins), and point mutations G719X, L861Q, and S768I. The latter three mutations may have a superior response to afatinib (58). The majority of (exon20ins) are thought resistant to *EGFR* TKIs with the exception of A763_Y764insFQEA. In a preclinical study, osimertinib demonstrated potency with a wide therapeutic window in the exon20ins studied (Y764_V765insHH, A767V-769dupASV, and D770_N771insNPG) (59). More recently, it has been revealed that *EGFR* amplification may occur in a subset of exon20ins. The dual *EGFR* blockade with osimertinib and cetuximab has demonstrated significant growth inhibition in *in vivo* models (60). EGF816 has shown both *in vitro* and *in vivo* efficacy in a number of exon20ins and in a patient-derived xenograft model, 100 mg/kg dosing resulted in tumor regression of 81% (49). AP32788 has also been shown to inhibit exon20ins in BA/F3 cell lines (61). The activity of third-generation TKIs in preclinical models has led to clinical trials for exon20 insertions including the phase I/II study of AP32788 (NCT02716116).

Brain Metastases

Approximately 30–50% of patients with NSCLC develop central nervous system (CNS) disease (62, 63). The association between *EGFR* mutation-positive NSCLC and the incidence of brain metastases is controversial with some studies suggesting an increased risk of CNS disease at diagnosis (64, 65). CNS disease can reduce survival and both brain metastases and loco regional therapies can impact neurological function and quality of life. First-generation *EGFR* TKIs have shown intracranial activity with erlotinib exhibiting higher CSF concentrations than gefitinib (66). Afatinib in patients pretreated with chemotherapy and a first-generation TKI has demonstrated a CNS dCR of 66% (67). A recent preclinical study has shown superior blood–brain barrier penetration of osimertinib compared to gefitinib, afatinib, and rociletinib (68). Sustained tumor regression in a murine brain metastases model has also been reported with osimertinib, doses of 80 mg in humans were predicted to target human brain metastases using an adaptive pharmacokinetic/pharmacodynamics model (69). AZD3759 is an innovative *EGFR* TKI developed to penetrate the blood–brain barrier but does not have *T790M* activity. BLOOM (NCT02228369) is a study testing the safety and efficacy of osimertinib 160 mg/day and AZD3759 in NSCLC patients with leptomeningeal disease; early data have reported disease control in three-quarters of patients and responses in 7 of 20 patients (70).

Osimertinib CNS activity was also confirmed in AURA and AURA2 (71). In the recently reported phase III study of osimertinib versus platinum-pemetrexed doublet, a significant improvement in PFS in patients with brain metastases was evident in the osimertinib group (8.5 versus 4.2 months; hazard ratio 0.32; 95% CI, 0.21–0.49) (39).

RESISTANCE TO THIRD-GENERATION TKIs AND COMBINATION TREATMENT

C797S and Other *EGFR*-Dependent Mechanisms

The point mutation *C797S* in exon 20 represents the most common resistance mechanism identified in third-generation *EGFR* TKIs. Most third-generation TKIs use the site of the cysteine amino acid located at position 797 for covalent binding, and

the serine amino acid substitution reduces the capacity for TKI binding. Analysis of both plasma and tissue has confirmed this as a mechanism of resistance to osimertinib and olmutinib (72, 73). *C797S* as a cause of rociletinib resistance has been found to be much lower, but emergence of other uncommon *EGFR* mutations including *L798I*, *L692V,* and *E709K* have been implicated (23). Interestingly in preclinical models, if the activating mutation (del19 or *L858R*) is retained in the presence of *C797S* but without *T790M*, the tumor remains sensitive to gefitinib or afatinib. If *T790M* is present, *in vitro* analysis has demonstrated partial cetuximab sensitivity (74). Cetuximab with EAI045, a novel *EGFR* resistance mutation selective allosteric inhibitor was also effective in a mouse model of the triple-mutant *EGFR L858R/T790M/C797S* (75). Necitumumab is also being trialed in combination with osimertinib (**Table 2**) (NCT02496663, NCT02789345). Notably, brigatinib with or without the combination of an anti-EGFR antibody has demonstrated activity in preclinical models for the "triple-positive" tumors (76). First- and third-generation TKIs may also be combined effectively, but this is only likely if the *C797S* and *T790M* mutations occur *in trans* (77). Other acquired *EGFR* mutations have been reported by Chabon et al. including L798I, L762V, and E709K (23). *EGFR* amplification and copy number alterations are also important resistance mechanisms.

RAS/RAF/MEK

Cell line studies have identified the RAS pathway as important in emerging osimertinib resistance, including mutations in NRAS as well as copy number gains. The *BRAF V600E* mutation is also a known acquired resistance mutation (78). The addition of selumetinib (MEK inhibitor) delayed and prevented resistance in preclinical models and tumor regression has been documented in an osimertinib-resistant transgenic mouse model (79). The TATTON phase 1b study is a three-arm trial of TKI naive and pretreated patients that includes combinations of osimertinib with selumetinib (AZD6094), savolitinib, a MET inhibitor, and durvalumab (NCT02143466).

MET Amplification

MET amplification as a cause for *EGFR TKI* acquired resistance has been described in case reports and crizotinib led to a response in one of these (80–82). In a further study of rociletinib resistance, *MET* amplification accounted for 26% of patients. Patient-derived xenograft models in this study were again successfully targeted using crizotinib (83). Notable in the xenografts and in the case report by Planchard et al., selective pressure permitted the emergence of *MET* amplifications without detectable T790M suggesting that *MET* may induce resistance to third-generation TKIs. Chabon et al. also described the preexistence of co-occurring *MET* amplification with T790M, which correlated with inferior responses to rociletinib (23).

Immunotherapy Combinations

Although immune checkpoint inhibitors have made huge advances in shifting the treatment paradigm in NSCLC, their role in *EGFR*-mutant disease is unclear. The third arm of TATTON investigated the combination of osimertinib with durvalumab and has reported early data (84). Patients were treated with osimertinib 80 mg daily with varying dosing and scheduling of durvalumab. In *EGFR* TKI naïve patients, the RR was 70% and in pretreated *T790M*-positive patients and *T790M*-negative patients RR was 67 and 21%, respectively. The combined rate of interstitial lung disease was 38% and as such this arm is currently on hold. Similarly, the CAURAL (NCT02454933) study investigating the durvalumab combination versus single-agent osimertinib has been halted due to toxicity concerns.

TABLE 2 | Ongoing combination studies targeting resistance mechanisms.

Target	Trial name	Clinical trial identifier	Status
Epidermal growth factor receptor (EGFR)	*EGFR* inhibitor AZD9291 and necitumumab in treating patients with *EGFR*-positive stage IV or recurrent non-small cell lung cancer (NSCLC) who have progressed on a previous *EGFR* tyrosine kinase inhibitor (TKI)	NCT02496663	Recruiting
EGFR Vascular endothelial growth factor (VEGF)	A study of ramucirumab (LY3009806) or necitumumab (LY3012211) plus osimertinib in participants with lung cancer	NCT02789345	Recruiting
VEGF	Osimertinib and bevacizumab as treatment for *EGFR*-mutant lung cancers	NCT02803203	Recruiting
JAK1	An open-label phase 1/2 study of INCB039110 in combination with osimertinib in subjects with NSCLC	NCT02917993	Recruiting
BCL-2	Osimertinib and navitoclax in treating patients with *EGFR*-positive previously treated advanced or metastatic NSCLC	NCT02520778	Recruiting
ABL1/SRC	Dasatinib and osimertinib (AZD9291) in advanced NSCLC with *EGFR* mutations	NCT02954523	Recruiting
TORC1/2	TORC1/2 inhibitor INK128 and *EGFR* inhibitor AZD9291 in treating patients with advanced *EGFR* mutation-positive NSCLC after progression on a previous *EGFR* TKI	NCT02503722	Recruiting
MET	Study of safety and efficacy of EGF816 in combination with INC280 in NSCLC patients with *EGFR* mutation	NCT02335944	Recruiting
PD-1 MET	Study of efficacy and safety of nivolumab in combination with EGF816 and of nivolumab in combination with INC280 in patients with previously treated NSCLC	NCT02323126	Recruiting

Other Resistance Mechanisms

Other potentially targetable resistance mechanisms include *HER2* amplification, *FGFR1* amplification, and the *PIK3CA E545K* mutation. Epithelial to mesenchymal transition (EMT) and small cell transformation are also well recognized. Preclinical models have successfully targeted EMT with Akt inhibitors (40). Navitoclax, a BCL-2 inhibitor when combined with WZ4002, was shown to induce greater apoptosis than with the *EGFR* TKI alone (85). A phase 1b study is accruing (NCT02520778). Vascular endothelial growth factor (VEGF) and *EGFR* pathways are intimately related. The upregulation of VEGF receptors may be responsible for *EGFR* resistance and combination studies are ongoing (86) (**Table 2**). Early trials have already confirmed the benefit of dual inhibition with bevacizumab and erlotinib (87, 88). One small retrospective study has suggested the possibility of rechallenging with *EGFR* TKIs in addition to bevacizumab to gain further disease control (89).

FUTURE DIRECTIONS

It is not just the complexity of resistance mechanisms that poses challenges to physicians treating the *EGFR* mutation-positive population. It is also unclear as to whether third-generation TKIs should be used in the first-line setting or should remain the option for *T790M* resistance in the second line. The results of the phase III FLAURA study which compares osimertinib to either gefitinib or erlotinib are awaited. The ADAURA study will also investigate the potential role of adjuvant osimertinib in stage IB–IIIA resected NSCLC with and without chemotherapy. The roadmap of resistance continues to grow and it is very likely that ctDNA analysis will at least complement if not replace repeat tumor biopsies in building the knowledge of resistance mechanisms. Studies have already demonstrated the emergence of *T790M* prior to radiographic changes but whether this should mean a switch in TKI is uncertain at present.

AUTHOR CONTRIBUTIONS

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MINI REVIEW
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Targeting Novel but Less Common Driver Mutations and Chromosomal Translocations in Advanced Non-Small Cell Lung Cancer

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Discovery of the epidermal growth factor receptor gene mutation and the anaplastic lymphoma kinase chromosomal translocation in non-small cell lung cancer has prompted efforts around the world to identify many less common targetable oncogenic drivers. Such concerted efforts have been variably successful in both non-squamous and squamous cell carcinomas of the lung. Some of the targeted therapies for these oncogenic drivers have received regulatory approval for clinical use, while others have modest clinical benefit. In this mini-review, several of these targets will be reviewed.

Keywords: novel, non-small cell lung cancer, advanced, mutations, chromosomal rearrangement

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INTRODUCTION

Epidermal growth factor receptor (EGFR) activating mutations in exons 18–21 and their exceptional responses to its kinase inhibitors (1, 2) marked the beginning of precision medicine in non-small cell lung cancer (NSCLC). Randomized trials showed treatment naïve, recurrent, or metastatic NSCLC patients harboring these mutations, particularly for exons 19 or 21 (3–10), had improved median progression-free survival (mPFS), tolerability, and quality-of-life from EGFR inhibitors over platinum-based chemotherapy. These studies triggered ongoing research to identify novel targets in both non-squamous (11, 12) and squamous NSCLC (13, 14) (**Table 1**). Crizotinib for ROS1 and dabrafenib/trametinib for BRAF mutation have received and submitted for regulatory approval, respectively. Selected targets, excluding EGFR and ALK, which will be discussed in separate reviews, will be discussed.

BRAF MUTATIONS

BRAF is a serine/threonine intracellular kinase and is activated by RAS, subsequently activates mitogen-activated protein kinase (MAPK). Activating BRAF mutations occur in 2–5% of NSCLC (15, 16). It is rare to find concurrent driver mutations, like K-RAS or EGFR (17). Activating BRAF mutations in NSCLC can be categorized into V600 and non-V600, in contrast to the predominance of V600 mutation in melanoma (15, 16). Although non-V600 BRAF mutations are more prevalent in heavy smokers, V600 mutants are found in never or light smokers (17). There are conflicting reports regarding the prognostic difference between the two subtypes (17).

BRAF inhibitors, such as vemurafenib, have shown promising preliminary benefit in V600 BRAF mutant, advanced NSCLC patients with a response rate (RR) of 42% and mPFS of 7.3 months (18). Planchard et al. (19) reported an RR of 33% and mPFS of 5.5 months with dabrafenib. Dual inhibition of BRAF and MEK with dabrafenib and trametinib yielded an RR of 63.2% and mPFS of 9.7 months in 57 evaluable patients (20). Dual inhibition prevents mechanisms leading to MAPK

pathway reactivation (21, 22), resulting in more effective growth inhibition. However, resistant mechanisms to dual inhibition may arise as a result of RAS or ERK activation or mutation (23, 24), epigenetic EGFR alteration (25), or overexpression of MCL-1 (26). Non-V600 BRAF mutants may not be as responsive to BRAF inhibition based on BRAF-mutated melanoma data (15, 17, 20). There is no specific targeted therapy developed in this subpopulation.

DDR2 MUTATIONS

DDR2 is a receptor kinase that binds to collagen at the discoidin domain leading to its activation and subsequently to cell migration, proliferation, and survival (27, 28). Activating DDR2 mutations were identified in 4% of squamous NSCLC, with the majority in the kinase domain. Tumor growth inhibition by dasatinib was observed preclinically (29). One partial response (PR), in a patient with S768R DDR2 mutation and wild-type EGFR, of almost 1 year was reported in the Phase II trial of dasatinib and erlotinib in advanced NSCLC (30). Another PR was reported in the Phase II trial of dasatinib in previously treated, advanced NSCLC (31).

A Phase II trial of dasatinib in patients with either inactivating BRAF mutations or DDR2 mutations was conducted. It was terminated prematurely due to intolerable dyspnea, fatigue, and nausea. Patients were on therapy for 9–42 days, with no observed response (32). Trials of dasatinib and MGCD516 in DDR2 mutant solid tumors, including squamous NSCLC, are ongoing. Success in the development of DDR2 inhibitors should modulate the toxicity hindering adequate drug exposure and efficacy by careful dose and schedule selection.

TABLE 1 | Targets, mechanism(s) of target dysregulation, associated histology, and examples of current drugs in development and corresponding phase of clinical development in non-small cell lung cancer.

Target	Mechanism of target dysregulation	Histology associated	Example of targeted therapy	Phase of clinical development
BRAF	V600	Adenocarcinoma	Dabrafenib/trametinib	Awaiting approval[a]
	Non-600		Vemurafenib ± cobimetinib	I/II
			LGX 818	I/II
DDR2	Mutation	Squamous	Dasatinib	II
			Nilotinib	II
			MGDC516	I/II
FGF1	Amplification	Squamous	Ponatinib	II
			AZD4547	I/II
			BGJ 398	I/II
			INCB054828	I/II
			JNJ-42756493	I/II
			TAS120	I
			ARQ087	I
			Debio 1347	I
			E7090	I
			LY287445	I
HER-2	Exon 20 mutation	Adenocarcinoma	Afatinib	II/III and approval[b]
	HER-2 amplification		Dacomitinib	II
			Trastuzumab ± pertuzumab	II
			T-DM1	II/III
K-RAS	Point mutation	Adenocarcinoma	MEK inhibition:	III
			Selumetinib	I/II
			trametinib	I/II
			CDK4/6 inhibitor:	
			Palbociclib	II/III
			Abemaciclib	I/II
			Ribociclib	I/II
MET	Amplification	Non-squamous and squamous	Crizotinib	II-approval
	Exon 14		Cabozantinib	II
			Foretinib	II
			Tepotinib	II
			Capmatinib	I/II
			Merestinib	I/II
			Volitinib	I/II
			Lorlatinib	I/II/III
			RXDX106	I
			PLB001	I
			HS10241	I

(*Continued*)

TABLE 1 | Continued

Target	Mechanism of target dysregulation	Histology associated	Example of targeted therapy	Phase of clinical development
NTRK	Translocation Point mutation	Adenocarcinoma	Entrectinib LOXO-101 AZD7451 DS 6051b MGCD516 PLX 7486 TPX00005	II I/II I I I I I
P3K/AKT/mTOR	PI3K mutation AKT mutation	Squamous cell carcinoma	PI3K inhibitor: Pan inhibitor: Buparlisib Copanlisib GSK2126458 MNL1117 XL147 CUDC-927 (HADC) PKB inhibitor: AZD8186 Alpelisib (BLY719) BGT 226 GDC0084 PI3K/mTOR inhibitor: BEZ 235 DS 7423 LY3023414 PF 04691502 VX-5584 XL 765 AKT inhibitor: Ipatasertib (GDC 0068) AZD 5363 GSK 2141795 LY2780301 Afuresertib ARQ 092 ARQ 751 BAY 1125976 ONC201 mTOR inhibitor: Temsirolimus Everolimus Vistusertib (AZD 2014) AZD 8055 BI 860585 CC-223 GDC 0349 ME-344 P70/S6K inhibitor: LY2584701 MSC 2363318A	II II II II I I I II I I I/II I I I I I I II I/II I/II I/II I I I I I II II I/II I I I I I I I I I I I I I
RET	Translocation	Adenocarcinoma	Cabozantinib Lenvatinib Ponatinib Vandetanib BLU667	II II II II I
ROS1	Translocation	Adenocarcinoma	Crizotinib Cabozantinib Ceritinib Entrectinib Lolatinib DS 6015b TPX0005	Approval II II I/II II I I

[a]*Dabrafenib and trametinib combination has received approval from EMEA in February 2017 and has been submitted to the FDA for approval.*

[b]*Afatinib has regulatory approval for EGFR mutation positive, treatment naïve, advanced NSCLC, and previously treated squamous cell carcinoma by the FDA and EMEA.*

FGFR PATHWAY ABERRATIONS

The FGF pathway consists of four receptors, FGFR1-4, and 18 ligands. Activation of the pathway leads to downstream activation of the RAS/RAF/MAPK, PI3K/AKT/mTOR, STAT, and PLCγ, which cause cell growth, proliferation, differentiation, migration, and survival (33). Pathway dysregulation can result from overexpression of either FGFs or their receptors, alternative splicing receptor isoforms, impaired downregulation, and degradation of activated FGF signal, FGFR gene amplification, point mutations, or chromosomal translocations (34). FGFR1 amplification is found in 10–25% of squamous NSCLC, commonly in smokers (35, 36). Whether FGFR1 amplification is prognostic remains controversial (36, 37).

An RR of 11.1% and disease-control rate (DCR) of 50% were reported in 36 FGFR1-amplified squamous NSCLC patients treated with BGJ398 (38). In the dose expansion cohort of the Phase 1 erdafitinib (JNJ-42756493) study, no response was observed (39). The criterion for FGFR1 amplification was not specified in either trial (38, 39). Two studies of AZD4547 reported 0/4 and 1/14 PR in evaluable FGFR1-amplified NSCLC, respectively. The responder had high FGFR1 amplification, defined as FGFR1:CEP8 $\geq$ 2.8 (40, 41).

It is premature to declare that FGFR1 amplification is not a driver mutation. Clinically significant toxicity from FGFR-targeted agents may occur at doses below which adequate growth inhibition of amplified FGFR1 tumors can be achieved. It is still unknown if FGFR1 amplification translates to overexpression or activation of the receptor. The definition of FGFR1 amplification needs to be refined, as in MET amplification and crizotinib efficacy (42).

Chandrani et al. reported that 5.5% of adenocarcinoma NSCLC harbor FGFR3 mutations at S249C, which was previously described in squamous NSCLC, and G691R which are sensitive to FGFR kinase inhibition in preclinical models. The clinical relevance will be established by future clinical trials (43).

HER-2 MUTATIONS AND AMPLIFICATION

HER-2 is a member of the EGFR family. The most common HER-2 mutation is exon 20 in-frame deletion or insertion between codons 776–779 (44, 45), which occur in 1.7–9% of all adenocarcinoma NSCLC (44–47). The length of insertion or deletion is heterogeneous (48). They are most commonly found in females and non-smokers. HER-2 exon 20 mutation or amplification leads to HER-2 phosphorylation, RAS/RAF/MAPK and PI3K/AKT/mTOR activation, and subsequent cell growth, proliferation, survival, and metastasis.

Six patients with HER-2 3+ or amplification had an RR of 83% and mPFS of 8.5 months as compared to an RR of 41% and mPFS of 7.0 months in those without after cisplatin/gemcitabine/trastuzumab treatment (49). A retrospective series of metastatic, HER-2 exon 20 mutant NSCLC reported DCRs of 93 and 100% after trastuzumab ($N = 15$) and afatinib ($N = 3$), respectively (46). A Phase II study of dacomitinib in 30 NSCLC with HER-2 aberrations reported an RR of 12% in those with exon 20 mutation and no response in those with amplification (50). The Phase II study of afatinib in 7 exon 20 mutant NSCLC had a DCR of 71% with 1 unconfirmed PR (uPR) (51). The ETOP NICHE trial of afatinib in HER-2 exon 20 mutant NSCLC reported a disappointing DCR at 12-week of 54% and mPFS of 13 weeks (52).

Phase II trials of ado-trastuzumab emtansine in HER-2 exon 20 or point mutations and HER-2 2+/3+ overexpressed NSCLC reported an RR of 6/18 and 10/49 with mPFS of 4 and 2.7 months, respectively (53, 54). The preliminary result of the ongoing MyPathway trial of trastuzumab and pertuzumab, targeting HER-2 dimerization, reported an ORR of 13 and 19% in 16 HER-2-amplified and 12-mutated NSCLC patients, respectively (55).

The benefit of HER-2-targeted therapeutics is modest. It is plausible that HER-2 exon 20 mutation and amplification represent two distinct molecular and therapeutic entities. There may be biological and therapeutic differences to HER-2-targeted agents based on the length of HER-2 exon 20 insertion or deletion (56). The full clinical and molecular data from these trials may help to elucidate the best treatment strategies to these subpopulations of HER-2 gene aberrant NSCLC.

K-RAS MUTATIONS

K-RAS is a member of the guanosine triphosphate gene superfamily. Upon activation by upstream receptors or point mutations at codons 12, 13, 14, or 60/61, K-RAS activates RAF/MAPK and PI3K/AKT/mTOR. These pathways regulate cell proliferation, growth, motility, and apoptosis (57).

K-RAS is mutated in 20–30% of NSCLC, predominantly in adenocarcinoma, non-Asians, and smokers. The incidence of K-RAS mutations may correlate with the amount of cigarettes smoked (11, 57). The majority of K-RAS mutations in NSCLC are at codon 12 (58). In a meta-analysis (59), K-RAS mutation was associated with poorer prognosis (HR = 1.45, 95% CI: 1.29–1.62), particularly in adenocarcinoma and early-stage NSCLC. It remains controversial if K-RAS mutation is predictive of platinum-based palliative chemotherapy efficacy (57), but it is associated with resistance to EGFR inhibitors. It is unclear if K-RAS mutation predicts efficacy to EGFR antibody (60–62), and if K-RAS transversion and transition mutations have different biology and thus therapeutic strategy and outcome (63, 64).

Targeting K-RAS mutation remains elusive. RAS attaches to the cell membrane for activation of downstream pathways *via* isoprenylation by farnesyltransferase. Alternatively, this is achieved by adding geranyl group by geranylgeranyltransferase I, particularly for K-RAS and H-RAS. Farnesyltransferase inhibitors failed possibly due to this geranylgeranyltransferase pathway (57, 65).

Current therapeutic approaches to K-RAS mutations in NSCLC focus on either the RAF/MAPK pathway or novel K-RAS biology. The MAPK pathway converges at MEK, which in turn activates ERK1/2. Targeting MEK will be expected to be effective in inhibiting the MAPK pathway, regardless of the upstream stimulatory signal. Despite encouraging Phase II results, the Phase III trial of docetaxel/selumetinib, an allosteric MEK1/2 inhibitor, combination over docetaxel alone in platinum-pretreated,

advanced K-RAS mutant NSCLC (66), failed to confirm any survival improvement (67).

RAS activation drives G1/S cell cycle transition *via* cyclin-dependent kinase 2 and 4 (CDK2/4), induces cyclin D1, and downregulates the cdk inhibitor, p27KIP. Cycle D1 activates CDK4/6, which in turn phosphorylates retinoblastoma protein, leading to G1/S transition (68). K-ras mutant NSCLC animal models were particularly sensitive to CDK4/6 inhibition (69, 70). Synergistic antitumor activity was observed with trametinib and CDK4/6 inhibitor because MEK or ERK activation leads to cyclin D1 expression (71). A number of CDK4/6 inhibitors as single agent or in combination with MEK inhibitors are being studied in this population (72).

MET MUTATION AND AMPLIFICATION

MET is a receptor kinase and is activated by its ligand, hepatocyte growth factor, which plays a role in cell growth and development. It subsequently activates downstream RAS/RAF/MAPK, PI3K/AKT/mTOR, WNT/β-catenin, and STAT, promoting mitogenesis, motility, invasion, and morphogenesis (73, 74).

MET point mutation is detected in 3–4% of NSCLC. The most common is exon 14 splicing mutation (METex14) in 2–3% of NSCLC, who are older than 70 with non-squamous histology (sarcomatoid > adenosquamous and adenocarcinoma) and smokers. METex14 can have concurrent MET amplification, defined as MET/CEP7 ratio > 5.0 (75, 76). METex14 corresponds to the juxtamembrane domain, which is involved in its degradation by ubiquitin ligase, Cbl, leading to increase in MET activity (74, 77). METex14 alteration is highly variable, making it different to diagnose and predict therapeutic benefit (78). There has been encouraging preliminary antitumor activity of MET inhibitors in METex14 NSCLC (74), like an RR of 44 and 28% uPR after crizotinib (79).

It is challenging to define MET amplification. A recent study suggested the MET/CEP7 ratio > 5 as a sensitive and specific diagnostic test for MET amplification with low oncogenic driver overlap and highly predictive of crizotinib efficacy. These patients were mainly female and ex-smoker. High MET gene copy number was identified in 33% of adenocarcinoma NSCLC, however, none responded to MET inhibitor (80). The Phase II study of crizotinib in advanced NSCLC harboring MET amplification reported RR in low (>1.8–<2.2), intermediate (>2.2–<5) and high (>5) MET/CEP7 ratios of 0, 20, and 50%, respectively (42). It is important to determine MET amplification in non-responding EGFR mutants to EGFR inhibitors, as 2% of them have concurrent MET amplification (81).

Clinical development of MET inhibitors in MET aberration positive and in combination with EGFR inhibitors in EGFR mutant NSCLC is ongoing. This latter strategy may delay the emergence of MET amplification and thus prolong clinical benefit to EGFR inhibitors. Caution should be exercised in patient selection. Onartuzumab, MET antibody, or ARQ-197, MET kinase inhibitor, combined with erlotinib failed to improve survival in either unselected or non-squamous NSCLC with or without wild-type EGFR (82–84). Exploratory analysis found EGFR mutants had a trend toward poorer survival with onartuzumab/erlotinib (82).

NTRK MUTATION AND CHROMOSOMAL TRANSLOCATION

The NTRK family kinases, NTRK1–3, are activated by ligands from neurotrophin growth factor family. They are involved in neuronal development (85, 86). They subsequently activate downstream PI3K/AKT/mTOR, RAS/RAF/MAPK, PLC-γ, and protein kinase C, leading to cell proliferation, survival, and growth (86, 87). In addition, NTRK overexpression is prognostic (85, 88, 89). NTRK activation can result from translocation of the NTRK kinase to a transcription factor. NTRK1, NTRK2, and NTRK3 translocations account for 3.5, 0.2–1, and 1%, respectively, of adenocarcinoma NSCLC (87). NTRK1 and NTRK2 mutations were identified primarily in large cell carcinoma (85, 87).

Due to the structural similarity in the kinase domain of NTRK, ROS1, and ALK, several pan-inhibitors, such as entrectinib, LOXO101, and TPX-0005, are in clinical investigation. Initial Phase 1 studies reported encouraging preliminary antitumor activity and tolerability (87, 90). Identifying the primary and secondary resistant mechanisms, based on the understanding from ALK and ROS1, will help to improve the efficacy of current inhibitors and identify novel therapeutics, not limited to NTRK inhibitors targeting gatekeeper or solvent front mutation.

PIK3CA/AKT/PHOSPHATASE AND TENSION HOMOLOG (pTEN)/mTOR PATHWAY GENE ABERRATIONS

The PI3K/AKT/mTOR pathway is often activated in human cancers, leading to tumor proliferation, growth, and survival (91–93). There are three classes of PI3K. PIK3CA are heterodimers of a single p85 regulatory subunit, and one of the four isoforms of p110 catalytic subunits (α, β, γ, and δ). Different p110 subunit is preferentially expressed in different normal and malignant tissues. PIK3CA can be activated by upstream growth factor receptors, followed by AKT/mTORC1/p70S6K, which exerts a negative feedback on activated PIK3CA. In addition, tumor suppressor pTEN is a key negative regulator to PI3K/AKT/mTOR activation at PIK3CA (91, 94).

Several PI3K pathway activation mechanisms have been documented in NSCLC. Activating mutations in the exon 9 helical and exon 20 kinase domains are uncommon (92, 93, 95, 96). Amplification or polysomy is the predominant mechanism (92, 93). PIK3CA genetic alterations are thought to be more pivotal in squamous NSCLC pathogenesis. A study screening NSCLC, SCLC, extrapulmonary small cell cancer cell lines, and resected NSCLC identified PIK3CA gain in 33.1 and 6.2% of squamous and adenocarcinoma, respectively (92). Squamous NSCLC with PI3K family gene aberrations had inferior median overall survival (mOS) (8.5 versus 19.1 months, $p < 0.0001$), higher

incidence of brain metastases, especially those with truncated pTEN loss (27 versus 11%, $p < 0.0001$), higher overall disease burden and genomic heterogeneity between the metastatic and primary tumors (37).

AKT consists of three isoforms, AKT1–3. Activating mutation, E17K in exon 4 kinase domain, accounts for 1–7% of all NSCLC (97, 98) with the majority being squamous NSCLC (99). Loss of pTEN expression occurred in up to 75% of NSCLC either by allelic loss (10–20%) (100, 101) or gene methylation (100, 102). It is postulated that pTEN loss leads to PIK3CAβ and downstream pathway activations.

Therapeutics targeting this pathway are currently in progress. Preliminary single agent antitumor activity has been disappointing. Toxicity, including hyperglycemia and GI toxicity, at least in part, limits the delivery of the optimal dose or schedule and thus antitumor activity. Inhibition of specific PIK3CA or AKT isoform leads to compensatory activation of other isoforms, limiting the antitumor activity. Due to extensive negative feedback loops, inhibition of a component leads to rebound activation of the pathway upstream (103). Ongoing studies to fully understand how to best target these genetic alterations, particularly in squamous NSCLC, with single agents, such as the LUNG MAP trial, or in combination with other complementary pathways, such as EGFR, HER-2, BRAF, may help optimize their efficacy.

RET CHROMOSOMAL TRANSLOCATION

RET is a kinase receptor for the giant cell-derived neurotrophic factor ligand. Binding of ligand leads to activation of RAS/RAF/MAPK, PI3K/AKT/mTOR, and PLC-γ, which regulate cell proliferation, migration, and differentiation. RET is important for renal organogenesis and enteric nervous system development (104).

RET was first determined to be oncogenic through the identification of interchromosomal translocation or intrachromosomal inversion in papillary thyroid cancer (105). Subsequently, RET chromosomal rearrangement was identified in NSCLC. The most common 5′ partner of the fusion oncogene is kinesin family member 5B, which is translocated to the kinase domain, leading to activation (106–110).

RET translocation is reported in 1–2% of NSCLC samples and are usually younger than 60, non-smoker, equally distributed in males and females and in mixed or solid adenocarcinoma. Over 30% have signet ring features (106–110).

Preliminary antitumor activity in Phase II trials with cabozantinib (111) and vandetanib (112, 113) demonstrated an RR of 18–47% and mPFS of 4.5–8 months. A global RET inhibitors registry reported an RR of 26% and mPFS of 2.3 months (114). The modest benefit from these multitargeted RET inhibitors may be related to subtherapeutic RET inhibition due to toxicity arising from inhibition of other targets. The heterogeneity of RET fusion partners and concurrent driver mutations may also impact the sensitivity to RET inhibitors. Highly selective RET inhibitors and better understanding of the biological differences in the fusion partners and concurrent mutations may help to improve the outcome of this NSCLC subtype.

ROS-1 CHROMOSOMAL TRANSLOCATION

ROS is a kinase receptor in the insulin receptor superfamily. Rearrangement occurs in 1–2% of non-squamous NSCLC (115, 116). ROS-1 chromosomal rearrangement leads to STAT3, PI3K/AKT/mTOR, and RAS/RAF/MAPK activation, followed by cell growth, proliferation, and survival (117). ROS-1 translocation NSCLC patient is described to be young, female, non-smoker, and with advanced stage adenocarcinoma (115, 117–120). The 5′ partners and the breakpoints of the ROS1 gene are variable (115, 116), which may impact on the biology and benefit to therapy.

The RR of 72%, mPFS of 19.2 months, and 1-year OS rate at 85% in 50 ROS-1 translocation NSCLC patients treated with crizotinib led to recent regulatory approval (121). Based on 77% homology in ALK and ROS-1, especially the kinase domain (121), ALK inhibitors are potentially efficacious. The Phase II study of ceritinib had an RR of 84% and mPFS of 19.3 months (122). In addition, pemetrexed-based chemotherapy may be effective, as ROS1 NSCLC have low thymidylate synthase mRNA levels (123). Further clinical validation is needed.

Overall, ROS1-rearranged NSCLC may have better prognosis with mOS of 36 months after standard chemotherapy and exceeding 5 years with chemotherapy and crizotinib. The incidence of brain metastases may be lower (123). Ongoing development of novel ROS1 inhibitors or combination to improve the benefit and to overcome resistance is important. It is conceivable that the resistant mechanisms to ROS1 inhibition parallel to those to ALK (124), such as secondary kinase domain mutations (125–127), which are sensitive to cabozantinib and lorlatinib, KIT mutation (128), RAS or EGFR pathway activation (129, 130).

CONCLUSION

Multiple driver mutations have been identified in non-squamous and squamous NSCLC. There is regulatory approval of EGFR-, ALK-, ROS-1-, and BRAF-targeted agents. Benefits from therapies to other targets are preliminary.

To bring targeted therapeutics into the clinic, emphasis should be made on careful selection of true drivers. The criteria remain to be defined (131). Early clinical development efforts to identify and validate the most predictive biomarkers are key. With increasing number of driver mutations and therapies, and limited diagnostic tissues in advanced NSCLC, it is important to optimize diagnostic tissue accruement, minimize unnecessary pathological tests, and implement multiplex mutation analysis. The latter approach and basket trials, such as the LUNG MAP trial, exploring multiple targets simultaneously, can reduce the number and risk of biopsy, increase enrollment, and improve clinical trial efficiency.

Continual basic, translational, and clinical investigations are crucial to understand the targets, their resistance mechanisms, and corresponding therapies. For treatment tumor or plasma biopsies are necessary.

AUTHOR CONTRIBUTIONS

AD is an internal medicine rotating through medical oncology rotation. This manuscript served as one of her research projects. She was responsible to review the literature on a number of the therapeutic targets reviewed in the manuscript and provided her part of the corresponding manuscript. QC is the corresponding author who reviewed AD's part of the manuscript, in addition to the review of other therapeutic targets in this manuscript.

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REVIEW
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Update on Programmed Death-1 and Programmed Death-Ligand 1 Inhibition in the Treatment of Advanced or Metastatic Non-Small Cell Lung Cancer

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Purpose: Non-small-cell lung cancer (NSCLC) has a large worldwide prevalence with a high mortality rate. Chemotherapy has offered modest improvements in survival over the past two decades. Immune checkpoint modulation with programmed death-1 (PD-1) or programmed death-ligand 1 (PD-L1) inhibition has shown the promise of changing the future landscape of cancer therapy. This update reviews recent advances in the treatment of NSCLC with immune checkpoint modulation.

Methods: Publications and proceedings were identified from searching PubMed and proceedings from the annual meetings of the American Society of Clinical Oncology, European Society for Medical Oncology, and European Lung Cancer Conference.

Results: Atezolizumab, nivolumab, and pembrolizumab increase overall survival in second-line treatment of Stage III/IV squamous and non-squamous NSCLC when compared to docetaxel. Pembrolizumab increases progression-free survival in the first-line treatment of Stage IV NSCLC with 50% PD-L1 expression when compared to platinum-based chemotherapy. Combination therapy with chemotherapy and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors has shown promise in early trials.

Conclusion: Immune checkpoint modulation produces durable responses and overall survival benefits with less toxicity compared to conventional chemotherapy. Future investigations are combining PD-1/L1 inhibition with chemotherapy, targeted therapy, or other immuno-oncology agents in an effort to further improve efficacy.

Keywords: non-small-cell lung cancer, immuno-oncology, programmed death-1, programmed death-ligand 1, CTLA-4

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INTRODUCTION

More than 100 years of research in the field of cancer immunotherapy has produced several modalities capable of producing clinical response (1). Most notably, immune checkpoint modulation has shown the promise of changing the future landscape of cancer therapy through its durable clinical responses (2–4) and safety profiles of some agents that are either milder and/or more manageable compared to traditional anti-neoplastic therapies (5). Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), the first immune checkpoint receptor to be clinically targeted, is exclusively expressed on the surface of $CD4^+$ and $CD8^+$ T cells in lymphatic tissue and is involved in T-cell regulation,

proliferation, and tolerance (6). The repertoire of immune modulation was expanded with the advent of programmed death-1 (PD-1) immune checkpoint inhibitor antibodies, which restores T-cell effector function and augments the host anti-tumor response by blocking the binding of either programmed death-ligand 1 (PD-L1) and/or PD-L2 to PD-1 receptors (7).

Following the clinical success of treating melanoma with immune checkpoint modulation (8), trialists have expanded the application of checkpoint inhibitors to multiple tumor types, including lung cancer (9). Globally, 1.8 million new diagnoses of lung cancer occurred in 2012; with a mortality rate of nearly 90%, lung cancer is the first and second cause of cancer mortality in men and women, respectively (10). Eighty-five percent of lung cancers are non-small-cell lung cancer (NSCLC), further divided into non-squamous (70%) and squamous (30%) histologic subtypes (11). Metastatic disease is present in 50% of new NSCLC diagnoses (12, 13), which harbors an untreated median overall survival (mOS) of 4.0 months (14) and a metastatic 5-year survival rate ranging from 2 to 9% (15). Although mortality has improved with the use of targeted drugs for driver mutations (16–20), few patients harbor these mutations and resistance to targeted treatment frequently occurs (21). Currently, NSCLC has numerous checkpoint inhibitors being evaluated for clinical efficacy (22). The possible treatment of NSCLC is being further enriched through the addition of other immune modulation targets and combination therapy. At present, the Food and Drug Administration has approved three immuno-oncology agents for the treatment of NSCLC: atezolizumab, nivolumab, and pembrolizumab in the relapsed, refractory setting as well as pembrolizumab for the first-line treatment of metastatic NSCLC with a tumor proportion score (TPS) ≥50%. This update will offer guidance into the current application and pending developments for treatment NSCLC with immune modulating pharmacotherapy.

METHODS

Current studies investigating the use of immune checkpoint modulation in NSCLC were reviewed by searching PubMed (January 1, 2015 to December 30, 2016) using the following search terms: non-small-cell lung cancer and immune checkpoint modulation (or aliases). Any proceedings from the American Society of Clinical Oncology (ASCO) (2015–2016), European Society for Medical Oncology (ESMO) (2015–2016), and European Lung Cancer Conference (ELCC) (2015–2016) annual meetings involving both NSCLC and immune checkpoint modulation were reviewed. **Table 1** summarizes the search results and each trial's pertinent characteristics.

RESULTS

Third Line

CheckMate 063 is a Phase 2, open-label, global, multicenter, single-arm trial investigating the use of nivolumab, a fully human immunoglobulin G4 (IgG4) monoclonal antibody that selectively inhibits the PD-1 receptor, dosed 3 mg/kg every 2 weeks ($n = 117$) in patients with either Stage IIIb/IV squamous NSCLC who have received prior platinum-doublet and one additional systemic treatment. Treatment with nivolumab continued until progressive disease (PD) or an unacceptable treatment-related adverse event (TRAE), although treatment beyond PD was permitted as per protocol. The primary endpoint was overall response rate (ORR) by independent radiology review (per RECIST v1.1). The ORR was 14.5% (95% CI 9–22). mOS was 8.2 months (95% CI 6.1–10.9), with 12-month OS and 18-month OS rates of 39% (95% CI 30–48) and 27% (95% CI 19–35), respectively. TRAE of any Grade occurred in 75% of patients, Grade 3–4 TRAEs occurred in 17%, TRAE lead to nivolumab discontinuation in 12%, and death occurred in two patients secondary to nivolumab, although these patients had multiple comorbidities in the setting of PD (23, 24). These results are similar to those obtained from two smaller Japanese trials (25). To put this in historical perspective, a retrospective analysis looking at third-line treatment (58% received cytotoxic chemotherapy, 42% EGFR received tyrosine kinase inhibitors) in patients who had not received any immunotherapy found a 6.5-month mOS, 3.4-month median progression-free survival (mPFS), and 8% ORR (26).

Second Line

CheckMate 017 is a Phase 3, global, multicenter, open-label, 1:1 randomized trial comparing nivolumab 3 mg/kg every 2 weeks ($n = 135$) or docetaxel 75 mg/m^2 every 3 weeks ($n = 137$) in patients with Stage IIIb/IV squamous NSCLC histology that recurred or progressed following prior platinum-doublet therapy. Treatment continued until PD or unacceptable toxicity, and treatment beyond PD was allowed. The primary endpoint was OS. Nivolumab had an mOS of 9.2 months (95% CI 7.33–12.62) versus docetaxel with 6.0 months (95% CI 5.29–7.39). The risk of death was 41% lower with nivolumab versus docetaxel [hazard ratio (HR) 0.59, 95% CI 0.44–0.79; $p < 0.001$]. The ORR with nivolumab was 20 versus 9% with docetaxel. PD-L1 expression stratified to 1, 5, and 10% was not predictive of benefit. Nivolumab had less TRAEs compared to docetaxel: TRAEs of any grade were reported in 59 versus 87% of patients, respectively, and Grades 3–4 TRAEs were reported in 8 versus 56% of patients, respectively. Study discontinuation due to a TRAE was reported for 5% of nivolumab versus 10% of docetaxel patients. No treatment-related deaths occurred with nivolumab and two deaths occurred in patients receiving docetaxel that were determined to be treatment-related (27). This efficacy is similar to the single-arm CheckMate 063 results (23). OS was updated at the ASCO 2016 Annual Meeting: the 1- and 2-year OS for nivolumab was 42 and 23%, respectively, in comparison to 24 and 8% for docetaxel (28).

CheckMate 057 is a Phase 3, global, multicenter, open-label, 1:1 randomized trial comparing nivolumab 3 mg/kg every 2 weeks ($n = 292$) to docetaxel 75 mg/m^2 every 3 weeks ($n = 290$) in patients with Stage III/IV non-squamous NSCLC that recurred or progressed on platinum-doublet chemotherapy. Treatment continued until PD or discontinuation due to toxicity; treatment beyond PD was permitted per protocol. This study met its primary endpoint of OS, with nivolumab mOS 12.2 months versus docetaxel 9.4 months, yielding a 27% reduction in risk of death (HR 0.73; $p = 0.002$) and improved ORR (19 versus 12%; $p = 0.02$). Using pre-defined PD-L1 expression levels of ≥1, ≥5,

TABLE 1 | Summary of advanced or metastatic non-small-cell lung cancer immuno-oncology trials.

Type of treatment		Trial name	Trial phase	Stage	Histology	Programmed death-ligand 1 (PD-L1) tumor expression level	Study arm (*n*)	Comparative arm (*n*)	Primary endpoint (study versus comparator)[a]	Secondary endpoint(s) (study versus comparator, when appropriate)[a,b]	TRAEs (study versus comparator)[a]	Death from study drug (patients)[a]	Reference
Monotherapy anti-PD-1/L1	Third line	CheckMate 063	2	IIIb/IV	Squamous	≥1, ≥5, ≥10, and ≥5% for analysis	Nivolumab 3 mg/kg every 2 weeks (*n* = 117)	N/A	ORR: 14.5% (95% CI 8.7–22.2%)	mOS: 8.2 months (95% CI 6.1–10.9) median progression-free survival (mPFS): 1.9 months (95% CI 1.8–3.2)	Grade 3–4: 17%	2	(23)
	Second line	CheckMate 017	3	IIIb/IV	Squamous	≥1, ≥5, and ≥10%	Nivolumab 3 mg/kg every 2 weeks (*n* = 135)	Docetaxel 75 mg/m^2 every 3 weeks (*n* = 137)	mOS: 9.2 versus 6.0 months [hazard ratio (HR) 0.59; 95% CI 0.44–0.79; $p < 0.001$]	mPFS: 3.5 versus 2.8 months (HR 0.62; 95% CI 0.47–0.81; $p < 0.001$) ORR: 20 versus 9% ($p = 0.008$)	Grade 3–4: 8 versus 56%	0	(26)
		CheckMate 057	3	IIIb/IV	Non-squamous	≥1, ≥5, and ≥10%	Nivolumab 3 mg/kg every 2 weeks (*n* = 292)	Docetaxel 75 mg/m2 every 3 weeks (*n* = 290)	mOS: 12.2 versus 9.4 months (HR 0.73; 95% CI 0.59–0.89; $p = 0.002$)	mPFS: 2.3 versus 4.2 months (HR 0.92; 95% CI 0.77–1.11; $p = 0.39$) ORR: 19 versus 12% ($p = 0.02$)	Grade 3–4: 10 versus 54%	0	(27)
		Keynote 010	2 and 3	IIIb/IV	Squamous and non-squamous	≥1 and ≥50%	Pembrolizumab 2 mg/kg every 3 weeks (*n* = 345)	Docetaxel 75 mg/m2 every 3 weeks (*n* = 343)	mOS (PD-L1 expression ≥1%; pembro 2 mg/kg): 10.4 versus 8.5 months (HR 0.71; 95% CI 0.58–0.88; $p = 0.0008$)	ORR (PD-L1 expression ≥1%; pembro 2 mg/kg): 18 versus 9% ($p = 0.0005$)	Grade 3–5: 13% (pembro 2 mg/kg) and 16% (pembro 10 mg/kg) versus 35%	3 (pembro 2 mg/kg) and 3 (pembro 10 mg/kg)	(29)
							Pembrolizumab 10 mg/kg every 3 weeks (*n* = 346)		mOS (PD-L1 expression ≥1%; pembro 10 mg/kg): 12.7 versus 8.2 months (HR 0.61; 0.49–0.75; $p < 0.0001$)	ORR (PD-L1 expression ≥1%; pembro 10 mg/kg): 18 versus 9% ($p = 0.0002$)			
									mPFS (PD-L1 expression ≥1%; pembro 2 mg/kg): 3.9 versus 4.0 months (HR 0.88; 95% CI 0.74–1.05; $p = 0.07$)	ORR (PD-L1 expression ≥50%; pembro 2 mg/kg): 30 versus 8% ($p < 0.0001$)			

(*Continued*)

TABLE 1 | **Continued**

Type of treatment	Trial name	Trial phase	Stage	Histology	Programmed death-ligand 1 (PD-L1) tumor expression level	Study arm (*n*)	Comparative arm (*n*)	Primary endpoint (study versus comparator)[a]	Secondary endpoint(s) (study versus comparator, when appropriate)[a,b]	TRAEs (study versus comparator)[a]	Death from study drug (patients)[a]	Reference
								mPFS (PD-L1 expression ≥1%; pembro 10 mg/kg): 4.0 versus 4.0 months (HR 0.79; 95% CI 0.66–0.94; $p = 0.004$) mOS (PD-L1 expression ≥50%; pembro 2 mg/kg): 14.9 versus 8.2 months (HR 0.54; 95% CI 0.38–0.77; $p = 0.0002$) mOS (PD-L1 expression ≥50%; pembro 10 mg/kg): 17.3 versus 8.2 months (HR 0.50; 95% CI 0.36–0.70; $p < 0.0001$) mPFS (PD-L1 expression ≥50%; pembro 2 mg/kg): 5.0 versus 4.1 months (HR 0.59; 95% CI 0.44–0.78; $p = 0.0001$) mPFS (PD-L1 expression ≥50%; pembro 10 mg/kg): 5.2 versus 4.1 months (HR 0.59; 95% CI 0.45–0.78; $p < 0.0001$)	ORR (PD-L1 expression ≥ 50%; pembro 10 mg/kg): 29% vs 8% ($p < 0.0001$)			
	OAK	3	IIIb/IV	Squamous and non-squamous	TC/IC 0 = <1% TC/IC; TC/IC 1 = ≥1% TC/IC; TC/IC 2 = ≥5% TC/IC; TC/IC 3 = ≥50% TC/≥10% IC	Atezolizumab 1,200 mg (*n* = 425) every 3 weeks	Docetaxel 75 mg/m^2 every 3 weeks (*n* = 425)	mOS: 13.8 versus 9.6 months (HR 0.73; 95% CI 0.62–0.87; $p = 0.0003$)	mPFS: 4.0 versus 2.8 months (HR 0.95; 95% CI 0.82–1.10; $p = 0.493$)	Grade 3–4: 15 versus 43%	0	(31)

(*Continued*)

TABLE 1 | **Continued**

Type of treatment	Trial name	Trial phase	Stage	Histology	Programmed death-ligand 1 (PD-L1) tumor expression level	Study arm (*n*)	Comparative arm (*n*)	Primary endpoint (study versus comparator)[a]	Secondary endpoint(s) (study versus comparator, when appropriate)[a,b]	TRAEs (study versus comparator)[a]	Death from study drug (patients)[a]	Reference
								mOS (PD-L1 expression ≥1%): 15.7 versus 10.3 months (HR 0.74; 95% CI 0.58–0.93; $p = 0.0102$)				
	First line	Keynote 024	3	IV	Squamous and non-squamous	≥50%	Pembrolizumab 200 mg every 3 weeks ($n = 154$)	Investigator's choice of five different platinum-based chemotherapy regimens ($n = 150$)	mPFS (PD-L1 expression ≥50%): 10.3 versus 6.0 months (HR 0.50; 95% CI 0.37–0.68; $p < 0.001$)	mOS (yet to be reached). Six-month survival (PD-L1 expression ≥5%): 80.2% versus 72.4% (HR 0.60; 95% CI 0.41–0.89; $p = 0.005$) ORR (PD-L1 expression ≥5%): 44.8 versus 42% (p value not reported)	Grade 3–5: 26.6 versus 53.3%	0	(32)
		CheckMate 026	3	IV or recurrent	Squamous and non-squamous	≥1 and ≥5%	Nivolumab 3 mg/kg every 2 weeks ($n = 271$)	Investigator's choice of platinum-based doublet chemotherapy ($n = 270$)	mPFS (PD-L1 expression ≥5%): 4.2 versus 5.9 months (HR 1.15, 95% CI 0.91–1.45; $p = 0.25$)	mPFS: not reported mOS: 14.4 versus 13.2 months (HR = 1.02; 95% CI: 0.80–1.30) (p value not stated) ORR: not reported	Grade 3–4: 18 versus 51%	Not stated	(33)
Combination chemotherapy and anti-PD-1 therapy		CheckMate 012	1	IIIb/IV	Squamous and non-squamous	<1 and ≥1%	Squamous: nivolumab 10 mg/kg every 3 weeks plus gemcitabine-cisplatin ($n = 12$) Non-squamous: nivolumab 10 mg/kg every 3 weeks plus pemetrexed-cisplatin ($n = 15$)	N/A	Tolerability: 21% discontinuation from TRAE; TRAE (grade 3–4): 45%	ORR (nivo 10 mg/kg plus gem-cis): 33% ORR (nivo 10 mg/kg plus pem-cis): 47%	Grade 3–4: 45%	0	(35)

(*Continued*)

TABLE 1 | **Continued**

Type of treatment	Trial name	Trial phase	Stage	Histology	Programmed death-ligand 1 (PD-L1) tumor expression level	Study arm (*n*)	Comparative arm (*n*)	Primary endpoint (study versus comparator)[a]	Secondary endpoint(s) (study versus comparator, when appropriate)[a,b]	TRAEs (study versus comparator)[a]	Death from study drug (patients)[a]	Reference
						All histologies: nivolumab 5 or 10 mg/kg every 3 weeks plus paclitaxel-carboplatin (*n* = 14 and *n* = 15, respectively)			ORR (nivo 10 mg/kg plus pacli-carbo): 47% ORR (nivo 5 mg/kg plus pacli-carbo): 43% progression-free survival (PFS) (24 weeks; nivo 10 mg/kg plus gem-cis): 51% PFS (24 weeks; nivo 10 mg/kg plus pem-cis): 71% PFS (24 weeks; nivo 10 mg/kg plus pacli-carbo): 38% PFS (24 weeks; nivo 5 mg/kg plus pacli-carbo): 51%			
	Keynote 021	1 and 2	IIIb/IV	Non-squamous	<1 and ≥1%	Pembrolizumab 200 mg every 3 weeks plus pemetrexed-carboplatin (*n* = 60)	Pemetrexed-carboplatin (*n* = 63)	ORR: 55 versus 29% (95% CI 9–42%; *p* = 0.0016)	mPFS: 13.0 versus 8.9 months (HR 0.53; 95% CI 0.31–0.91; *p* = 0.010) OS (12 months): 75 versus 72% ORR (<1versus ≥1% in pembro arm): 57 versus 54% ORR (PD-L1 1–49% in pembro arm): 26% ORR (PD-L1 ≥50% in pembro arm): 80%	Grade 3–5: 39 versus 26%	1	(36)

(*Continued*)

TABLE 1 | **Continued**

Type of treatment	Trial name	Trial phase	Stage	Histology	Programmed death-ligand 1 (PD-L1) tumor expression level	Study arm (*n*)	Comparative arm (*n*)	Primary endpoint (study versus comparator)[a]	Secondary endpoint(s) (study versus comparator, when appropriate)[a,b]	TRAEs (study versus comparator)[a]	Death from study drug (patients)[a]	Reference
Combination anti-PD-1/L1 therapy and anti-CTLA-4 therapy	Keynote 021	1 and 2	IIIb/IV	Non-squamous	<1 and ≥1%	Maximum dose: Pembrolizumab 10 mg/kg every 3 weeks plus ipilimumab 1 or 3 mg/kg every 3 weeks (only four cycles) Final dose selected: pembrolizumab 2 mg/kg and ipilimumab 1 mg/kg	N/A	Dose-limiting toxicities: none TRAE (all grades): 10 patients (66.7%)	None defined	All grade: 10 patients (66.7%)	0	(37)
	CheckMate 012	1	IIIb/IV	Squamous and non-squamous	<1 and ≥1%	Nivolumab 1 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks (data not reported in publication)	N/A	TRAE (grade 3–4; ipi every 6 weeks): 33%	ORR (ipi every 6 weeks): 38% (95% CI 23–55)	TRAE (grade 3–4; ipi every 6 weeks): 33%	0	(39)
						Nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 12 weeks (*n* = 38)		TRAE (grade 3–4; ipi every 12 weeks): 37%	ORR (ipi every 12 weeks): 47% (95% CI 31–64)	TRAE (grade 3–4; ipi every 12 weeks): 37%		
						Nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks (*n* = 39)			PFS (24 weeks; ipi every 6 weeks): 65% (95% CI 42–81)			
									PFS (24 weeks; ipi every 12 weeks): 80% (95% CI 55–92)			

(*Continued*)

TABLE 1 | Continued

Type of treatment	Trial name	Trial phase	Stage	Histology	Programmed death-ligand 1 (PD-L1) tumor expression level	Study arm (*n*)	Comparative arm (*n*)	Primary endpoint (study versus comparator)[a]	Secondary endpoint(s) (study versus comparator, when appropriate)[a,b]	TRAEs (study versus comparator)[a]	Death from study drug (patients)[a]	Reference
	D4190C00006	1b	III/IV	Squamous and non-squamous	Unknown, 0, <25, and ≥25%	Maximum dose: durvalumab 20 mg/kg with tremelimumab 3 mg/kg Final dose selected: durvalumab 10 mg/kg and tremelimumab 1 mg/kg, both every 4 weeks (*n* = 102)	N/A	Serious adverse event ("serious" not formally defined): 37%	ORR (durvalumab 10–20 mg/kg every 2 weeks or 4 weeks plus tremelimumab 1 mg/kg): 23% (95% CI 9–44)	Serious adverse event ("serious" not formally defined): 37%	3	(40)

mOS, median overall survival; mPFS, median progression-free survival; n, number of patients; ORR, overall response rate; TRAE, treatment-related adverse event.

[a]*Results are for all studied patients, unless otherwise stated.*

[b]*Only selected secondary endpoints reported in* ***Table 1***

and ≥10% from archival tumors, nivolumab showed improved efficacy across all endpoints. PD-L1 expression predicted the benefit of nivolumab, even at the lowest expression level of 1%. mOS approximately doubled with nivolumab versus docetaxel across PD-L1 expression levels; conversely, survival was equivocal with negative PD-L1 expression. Grades 3–4 TRAEs occurred in 10 and 54% of nivolumab and docetaxel patients, respectively. There were no nivolumab-related deaths, whereas docetaxel led to one death. Subsequent systemic therapy was given to 42.1 and 49.7% of nivolumab and docetaxel patients, respectively (29). Investigators at the ASCO 2016 Annual Meeting presented an update to the 1- and 2-year OS for nivolumab noting 51 and 29%, respectively, in comparison to 39 and 16% for docetaxel (28).

Keynote 010 is Phase 2/3, global, multicenter, open-label, randomized, controlled study to evaluate the safety and efficacy of pembrolizumab, a humanized monoclonal IgG4 antibody that selectively inhibits the PD-1 receptor. Patients with NSCLC and tumor cell PD-L1 expression ≥1% who progressed after platinum-doublet chemotherapy were randomized 1:1:1 to receive pembrolizumab 2 mg/kg ($n = 345$), pembrolizumab 10 mg/kg ($n = 346$), or docetaxel 75 mg/m^2 ($n = 343$) every 3 weeks; no crossover was allowed. Treatment was continued for 24 months, or until PD or discontinuation due to toxicity; treatment beyond PD was allowed. The primary endpoints were OS and progression-free survival (PFS) (by independent radiology review as per RECIST v1.1) in both all patients and those with PD-L1 expression ≥50% of tumor cells (TCs) from either archival or new biopsies. Compared to docetaxel, risk of death was decreased with both pembrolizumab 2 mg/kg (HR 0.71, 95% CI 0.58–0.88; $p = 0.0008$) and pembrolizumab 10 mg/kg (HR 0.61, 0.49–0.75; $p < 0.0001$). Patients with PD-L1 expression ≥50% had better mOS with pembrolizumab 2 mg/kg (14.9 versus 8.2 months; HR 0.54, 95% CI 0.38–0.77; $p = 0.0002$) and pembrolizumab 10 mg/kg (17.3 versus 8.2 months; HR 0.50, 95% CI 0.36–0.70; $p < 0.0001$) versus docetaxel. Grades 3–5 TRAEs were less common with pembrolizumab versus docetaxel: 13, 16, and 35% for those treated with pembrolizumab 2 mg/kg, pembrolizumab 10 mg/kg, and docetaxel, respectively (30). Investigators at the ASCO 2016 Annual Meeting presented an update on the patients with 1–49% PD-L1 expression: OS was longer for both pembrolizumab 2 mg/kg (9.4 months) versus docetaxel (8.6 months) (HR 0.79, 95% CI 0.61–1.04) and pembrolizumab 10 mg/kg (10.8 months) versus docetaxel (8.6 months) (HR 0.71, 0.53–0.94) (31).

The OAK study is a Phase 3, global, multicenter, open-label, randomized, controlled study to evaluate the efficacy and safety of atezolizumab, a humanized IgG1-kappa monoclonal antibody that binds PD-L1 and inhibits PD-L1/PD-1 and PD-L1/B7.1 interactions (32). Patients with Stage IIIb/IV or recurrent non-squamous NSCLC following failure of platinum-based treatment were randomized 1:1 to receive either fixed dose atezolizumab 1,200 mg ($n = 425$) or docetaxel 75 mg/m^2 ($n = 425$) every 3 weeks; no crossover was allowed. Treatment continued until disease progression or unacceptable toxicity occurred; treatment beyond PD was allowed. The study had co-primary endpoints of OS in the full study population, in addition to OS in patients with PD-L1 expression ≥1% of TCs or tumor-infiltrating immune cells (IC) by the Ventana SP142 assay. mOS was significantly longer

for atezolizumab versus docetaxel (13.8 versus 9.6 months, stratified HR 0.73, 95% CI 0.62–0.87; $p = 0.0003$), and had 12- and 18-month OS of 55 versus 41% and 40 versus 27%, respectively. OS was significant regardless of presence of PD-L1 expression: 55% of patients had PD-L1 expression ≥1% and had OS of 15.7 months versus docetaxel 10.3 months (stratified HR 0.74, 95% CI 0.58–0.93; $p = 0.0102$); 45% of patients had no TC or IC with PD-L1 expression with a respective OS of 12.6 months versus docetaxel 8.9 months (HR 0.75, 95% CI 0.59–0.96; $p = 0.0215$); and 16% of patients had high TC (≥50%) or IC (≥10%) PD-L1 expression and had OS of 20.5 months versus docetaxel 8.9 months (HR 0.41, 95% CI 0.27–0.64; $p < 0.0001$). Further, OS was also significant regardless of NSCLC histology: non-squamous NSCLC had OS of 15.6 months versus docetaxel 11.2 months (HR 0.73, 95% CI 0.60–0.89; $p = 0.0015$); squamous NSCLC had OS of 8.9 months versus docetaxel 7.7 months (HR 0.73, 95% CI 0.54–0.98; $p = 0.0383$). Five percent of the atezolizumab group went on to receive subsequent immunotherapy versus 17% in the docetaxel group. Atezolizumab was well tolerated: only 15% of patients treated with atezolizumab had Grades 3–4 adverse events compared to 43% of the patients treated with docetaxel (33).

First Line

Keynote 024 is Phase 3, global, multicenter, open-label, 1:1 randomized trial comparing fixed dose pembrolizumab 200 mg every 3 weeks ($n = 154$) to the investigator's choice of five different platinum-based chemotherapy regimens ($n = 150$) in patients with both squamous and non-squamous Stage IV NSCLC who have not received prior systemic therapy for their metastatic disease and have PD-L1 expression on ≥50% of TCs. Treatment with pembrolizumab and platinum-based chemotherapy continued for a total of 35 cycles (~2 years) and 4–6 cycles, respectively, or until the patient had radiologic disease progression or unacceptable toxicity. Pemetrexed maintenance was allowed for patients with non-squamous histology. Crossover from chemotherapy to pembrolizumab was allowed if PD occurred. The primary end point was PFS (by independent radiology review as per RECIST v1.1). mPFS was longer for pembrolizumab versus chemotherapy (10.3 versus 6.0 months) and disease progression or death was significantly better for pembrolizumab (HR 0.50, 95% CI 0.37–0.68; $p < 0.001$). mOS has yet to be reached; however, the 6-month OS for pembrolizumab versus chemotherapy was 80.2 and 72.4%, respectively (HR 0.60, 95% CI 0.41–0.89; $p = 0.005$). Pembrolizumab had fewer TRAEs of any grade compared to chemotherapy (73.4 versus 90.0%), less grade 3–5 TRAEs (26.6 versus 53.3%), and although had higher rates of immune-TRAEs (29.2 versus 4.7%) most were grade 1–2 severities and did not lead to any deaths. The second interim analysis by the data and safety monitoring committee determined that the benefit of pembrolizumab was large enough to warrant stopping the trial and offer the chemotherapy group pembrolizumab (34).

CheckMate 026 is a Phase 3, global, multicenter, open-label, 1:1 randomized controlled trial comparing nivolumab 3 mg/kg every 2 weeks ($n = 271$) to the investigator's choice of platinum-based doublet chemotherapy ($n = 270$) in patients with Stage IV or recurrent squamous and non-squamous NSCLC who have not received previous systemic therapy for their disease and have PD-L1 expression on ≥1% of TCs. Nivolumab continued until disease progression or unacceptable toxicity. Treatment beyond progression was allowed. Platinum-doublet chemotherapy was given for up to six cycles and pemetrexed maintenance was allowed for non-squamous patients. Crossover from chemotherapy to nivolumab was allowed if PD occurred. The primary end point was PFS (by independent radiology review as per RECIST v1.1) in patients with PD-L1 expression ≥5%. Nivolumab did not improve mPFS compared to platinum-doublet among patients with PD-L1 expression ≥5%: 4.2 and 5.9 months, respectively (HR 1.15, 95% CI 0.91–1.45; $p = 0.25$). Surprisingly, in contrast to the Keynote 024 results, the PFS was not superior in the ≥50% PD-L1 cohort (PFS HR 1.07) in the subgroup analysis. The OS for the nivolumab and chemotherapy groups were similar with an mOS of 14.4 and 13.2 months, respectively (HR = 1.02, 95% CI 0.80–1.30). Nivolumab had less TRAEs of any grade and grade 3–4 (71 and 18%), respectively, when compared to platinum-doublet (92 and 51%) (35). Further knowledge on the use of first-line nivolumab will be forthcoming from the ongoing CheckMate 227 trial where the 1% PD-L1 positive arm includes patients randomized to combined immunotherapy with nivolumab and ipilimumab versus nivolumab alone versus chemotherapy in the first-line setting.

Combinations of Immunotherapy and Chemotherapy

CheckMate 012 is a multi-cohort phase I clinical trial evaluating nivolumab as a single agent or in combination with chemotherapy, targeted therapy, or ipilimumab (a recombinant human IgG1 immunoglobulin that inhibits the CTLA-4 receptor). The data have been published from the platinum-doublet combinations (36). Three chemotherapy backbones were evaluated in 56 patients: pemetrexed/cisplatin, gemcitabine/cisplatin, and paclitaxel/carboplatin. All three backbones were paired with nivolumab 10 mg/kg. An additional cohort of paclitaxel/carboplatin was accrued that was combined with nivolumab 5 mg/kg. Maintenance chemotherapy was not allowed but patients continued on maintenance of nivolumab until progression. The primary objective, ORR, was 47, 33, and 47% for the pemetrexed, gemcitabine and paclitaxel platinum combinations with the 10 mg/kg nivolumab dose, respectively; ORR for paclitaxel/carboplatin combination with 5 mg/kg nivolumab was 43%. Responses were seen irrespective of presence or absence of PD-L1 expression on the tumor. Two-year OS rates were 33, 25, and 27%, for the pemetrexed, gemcitabine and paclitaxel platinum combinations with the 10 mg/kg nivolumab dose, respectively. The 2-year OS rate for the paclitaxel/carboplatin combination with 5 mg/kg nivolumab was 62%. These objective response and 2-year survival rates for the nivolumab combinations were numerically increased over what we would have expected historically from platinum doublet. No dose-limiting toxicities occurred during the first two cycles of treatment. Forty-five percent of patients reported Grade 3–4 TRAEs; 21% of patients discontinued all study therapy as a result of TRAEs.

Keynote 021 is a multi-cohort Phase 1/2 randomized trial investigating the safety, tolerability, and efficacy of pembrolizumab in combination with platinum doublets, targeted therapy, and ipilimumab. The data were recently published from the randomized phase 2 cohort G which compared pemetrexed and carboplatin followed by pemetrexed maintenance with or without a maximum of 2 years of pembrolizumab in 123 patients. Patients have Stage IIIb/IV non-squamous NSCLC and were stratified according to their PD-L1 TPS <1 versus ≥1%. Crossover from the chemotherapy group to the pembrolizumab group was permitted in the event of PD. The primary endpoint was ORR (by independent radiology review as per RECIST v1.1). Pembrolizumab combined with chemotherapy has a superior ORR versus chemotherapy alone (55 versus 29%, 95% CI 9–42%; $p = 0.0016$). Subgroup analysis of PD-L1 stratification <1% versus ≥1% showed similar ORR for the pembrolizumab group (57 versus 54%, respectively) while the chemotherapy alone group showed a difference in ORR (13 versus 38%, respectively). Further stratification of PD-L1 to 1–49% and ≥50% had an ORR of 26 and 80%, respectively, for the pembrolizumab with chemotherapy group, versus 39 and 35%, respectively, for the chemotherapy alone group. Pembrolizumab with chemotherapy was able to achieve a superior mPFS versus chemotherapy alone (13.0 versus 8.9 months, HR 0.53, 95% CI 0.31–0.91; $p = 0.0102$). mOS has not yet been met, and the 12-month OS has been 75% for those with pembrolizumab and chemotherapy versus 72% for chemotherapy alone. Grade 3–5 TRAEs were similar between groups (39% in the pembrolizumab plus chemotherapy group versus 26% in the chemotherapy alone group), with similar treatment discontinuation rates (10% for the pembrolizumab arm compared to 13% for the chemotherapy only arm) and treatment-related deaths (one death in the pembrolizumab group secondary to sepsis, and two deaths in the chemotherapy alone group due to sepsis and pancytopenia) (37).

Immunotherapy Doublets

Based on the success of dual immunotherapy combinations in melanoma, PD-1 inhibitors have been combined with the CTLA-4 inhibitor, ipilimumab. Cohort C from the Keynote 021 trial described above was presented at the ASCO Annual Meeting in 2015 (38). This cohort was a dose finding and safety study. The initial doses of pembrolizumab were 10 mg/kg and doses of 1 or 3 mg/kg of ipilimumab were planned. There were no safety signals at the 10 mg/kg dose of pembrolizumab with either dose of ipilimumab in the six patients treated, but, based on the emerging results from the CheckMate 012 trial, the final dose selected for further dose expansion was pembrolizumab 2 mg/kg and ipilimumab 1 mg/kg. Results were presented for the 15 patients treated at all dose levels. The ORR was 39% and the disease control rate was 83%. Immune TRAEs were identified in 33% of patients, half of whom had Grade 3 events (adrenal insufficiency and skin eruptions). This combination is being explored further in a randomized two cohort of Keynote 021 (cohort H).

The CheckMate 012 trial had several arms assessing the optimal dosing of nivolumab and ipilimumab in chemotherapy-naïve patients with NSCLC. Initial dose combinations with higher doses of ipilimumab (3 mg/kg) given every 3 weeks, similar to those used in melanoma, were not tolerable and had very high rates of TRAEs (49%) and treatment-related deaths in the NSCLC population (39). This prompted reassessment of dose and schedule. The results of nivolumab 3 mg/kg every 2 weeks in combination with ipilimumab 1 mg/kg every 6 or 12 weeks in 77 previously untreated metastatic NSCLC patients have been recently published (40). The ORR was 38 and 47% for the Q6 and Q12 week ipilimumab cohorts, respectively. In patients with PD-L1 expression of 1% or greater, the ORR was 57% for both cohorts. OS at 1 year in the ipilimumab Q6 week cohort was 69%. The follow-up data were too immature at the time of publication to report the OS in the Q12 week cohort. Grade 3–4 TRAEs occurred in 33 and 37% of patients in the Q6 and Q12 week cohorts, respectively. The majority of these TRAEs were auto-immune phenomena. No treatment-related deaths occurred. The results of the CheckMate 012 trial are the basis for the CheckMate 227 trial where PD-L1 positive (1%) patients are randomized to nivolumab 3 mg/kg every 2 weeks with ipilimumab 1 mg/kg every 6 weeks versus nivolumab 3 mg/kg every 2 weeks versus platinum doublet. The PD-L1 negative patients are randomized to nivolumab with ipilimumab at the dose and schedule above versus nivolumab with platinum-doublet chemotherapy versus standard of care chemotherapy.

The phase Ib experience with the combination of durvalumab, an IgG1 antagonist antibody that binds PD-L1 and inhibits its function, with tremelimumab, a fully human IgG2 isotype that inhibits the CTLA-4 receptor, in NSCLC has recently been published (41). In this dose-finding study, 102 patients were enrolled; 94% of the patients had prior systemic therapy. The final tolerable dose was established as durvalumab 10 mg/kg and tremelimumab 1 mg/kg both given every 4 weeks. Serious TRAEs occurred in 37 (36%) of 102 patients. Three treatment-related deaths occurred from suspected or confirmed autoimmunity (myasthenia gravis, pericardial effusion, and neuromuscular disorder). In the final cohort of 26 patients treated at the tremelimumab dose of 1 mg/kg, patients with both PD-L1 high (25%) as well as PD-L1 negative (0%) tumors had ORR 22 and 40%, respectively. Further work with this combination is being done in chemotherapy refractory (ARCTIC study) (42) and chemotherapy-naïve (MYSTIC study) (43) NSCLC patients.

PD-L1 Expression

Currently there are at least six monoclonal antibodies to assay PD-L1. The 28-8 antibody has been developed in conjunction with nivolumab. The 22C3 antibody has been developed with pembrolizumab. The 78-10 antibody has been developed with avelumab. Each of these three antibodies was developed initially on the DAKO autostainer platform. The SP142 antibody has been developed with atezolizumab. The SP263 antibody has been developed with durvalumab. Both of these antibodies were validated initially using the Ventana platform. Additional work has been published with the E1L3N antibody, which is commercially available and has been used in multiple laboratory-developed tests at numerous academic centers with both the DAKO and Ventana platforms. Work is now underway to cross compare the

antibodies. Several studies have now been published including phase 1 of the BluePrint PD-L1 Assay Comparison Project (44). The results of this work consistently note that the 28-8, 22C3, and SP263 antibodies are comparable when staining tumor cells. The SP142 antibody has more variability when compared to the other three antibodies. In general, all four antibodies have greater variability when assaying ICs. Work is also underway to assess the reliability of some of the antibodies on alternative staining platforms. Recently, the Ventana platform has been shown to also be reliable for 22C3 analysis (45). While the FDA has approved many of these antibodies as either companion or complementary diagnostics, due to the high cost of these tests, globally, laboratory-developed assays for PD-L1 are likely to predominate. At this point, the authors recommend that a well-validated assay be used to determine the presence or absence of PD-L1 staining. The key to this is the requirement for rigorous validation methodology if a laboratory-developed assay is going to be used. This sentiment has been demonstrated in the recently presented Multi-center French Harmonization Project (46). The 22C3, 28-8, SP263, and E1L3N antibodies were generally comparable. This study did show significant variability in the detection of PD-L1-positive tumor cells when laboratory-developed tests were used. The key is a thorough initial and ongoing validation process for laboratory-developed tests.

With that background, understanding the molecular determinants of response to immunotherapies is a difficult clinical challenge. Presently, PD-L1 expression levels have shown a variable ability to predict response to checkpoint inhibition. CheckMate 017 did not show any clear predictive benefit of PD-L1 analysis at the reported 1, 5, and 10% cut points for squamous histology patients. CheckMate 057 did show significant improvement in ORR, PFS, and OS with nivolumab for non-squamous patients expressing any level of PD-L1, but there is clear escalating benefit with increasing PD-L1 expression in the published 1, 5, and 10% cut points (47). The OAK study also showed that atezolizumab, when compared to docetaxel, produced OS benefit regardless of PD-L1 expression on either TC or IC, but again, increased magnitude of benefit is seen when patients with increasing PD-L1 expression are identified (33). The Keynote 010 trial only included PD-L1 positive patients, and although it does not offer information about the PD-L1-negative patients, there was increasing benefit when the patients with low expression (1–49%) are contrasted with the patients with high expression (≥50%) (31). Consistently in patients with non-squamous tumors who have progressed on platinum doublet, there is increased chance of benefit with increased PD-L1 expression. The struggle for clinicians is that the benefit in the PD-L1 low and/or negative groups is not zero, nor is it clinically insignificant, making use of PD-L1 as a biomarker in the refractory setting a challenge.

There has been documented success when stratifying patients for PD-L1 using the 22C3 antibody at the 50% cut point. This cut point was clinically validated during the Keynote 010 phase I trial that showed both pembrolizumab at either 2 or 10 mg/kg dose significantly improved OS in patients with ≥50% PD-L1 expression, which was numerically greater than the benefits seen in the low expressing cohort (48). Keynote 024 also demonstrated dramatic benefits of pembrolizumab in comparison to platinum-doublet chemotherapy in previously untreated patients with PD-L1 expression ≥50%. This comes in contrast to what has been seen with the 28-8 antibody. The results of the CheckMate 026 trial are perplexing. If the 22C3 and 28-8 antibodies select patients similarly, as is suggested by several recent publications including the initial publication of the BluePrint PD-L1 Assay Comparison Project, one would expect the patients treated with nivolumab who had ≥50% PD-L1 expression to do better with immunotherapy than chemotherapy, but this was not demonstrated (44, 49). The CheckMate 026 study was not designed nor powered to look at this subgroup. Confirmatory information about the benefits of PD-L1 inhibition in the chemotherapy-naïve first-line setting is needed. The Keynote 042 trial is ongoing (50). This trial is similar to the CheckMate 026 trial where patients with ≥1% PD-L1 staining are eligible to enroll. Patients are stratified by PD-L1 expression using the 50% cut point and randomized to pembrolizumab versus standard platinum-doublet chemotherapy. The high expressing group can then be used to confirm the Keynote 024 data. As mentioned earlier, the ongoing Checkmate 227 trial has a nivolumab monotherapy arm for patients with ≥1% PD-L1 expression. Again, this trial was not designed to look specifically at the 50% PD-L1-positive group but may yield a signal as to whether there is benefit of nivolumab alone in the first-line setting.

Other Potential Biomarkers

A multivariate exploratory analysis of baseline serum cytokines levels in 222 nivolumab-treated patients in either Checkmate 017 or 063 trials was presented at the April 2016 ELCC (51). The effect of the cytokine set on OS was quantified by generating an SQ-cytoscore defined as "high" or "low" based on the median cut-off. Those with a high SQ-cytoscore ($n = 102$) achieved an mOS of 15.6 months, approximately three times longer than 5.3 months of those with a low SQ-cytoscore ($n = 120$) (HR 0.48, 95% CI 0.36–0.64; $p < 0.0001$), respectively. While clinical factors are not suitable to determine sensitivity to PD-1 inhibition and PD-L1 expression does not predict response in squamous-NSCLC, the SQ-cytoscore may serve as a predictive marker for anti-PD-1 therapy. Prospective validation of these preliminary findings is required.

Due to tumor heterogeneity and the fluctuant infiltration of ICs into the tumor microenvironment (52), future biomarker investigations may look at other checkpoint molecules (53), TIIC (54), blood-based immune analyses (55), inflammatory gene signatures (56), and mutational load (57). In two independent cohorts, whole-exome sequencing of patients with NSCLC treated with pembrolizumab found an improved ORR, PFS, and durability in patients with higher tumor non-synonymous mutational load. Mutation burden was also associated with DNA repair pathway mutations, larger neoantigen burden, and molecular smoking signature; each factor was also correlated with clinical efficacy (57). Mutational burden is largely a consequence of chronic exposure to mutagens, and hence non-smoking NSCLC patients tend to harbor fewer mutations (58). Future prospective studies will need to explore the role of smoking exposure to durability of response.

Future Work

Future trials will continue to explore the potential of combination therapy of PD-1 inhibition with chemotherapy, targeted therapy, or other immuno-oncology agents. Chemotherapy has been shown to increase PD-L1 expression on TCs (59, 60), in addition to possibly reducing the quantity and activity of suppressive ICs, inducing immunogenic cell death, activating and maturing dendritic cells, enhancing tumor antigen presentation, and increasing effector T-cell function (61). Beyond cytotoxic chemotherapy combinations, the PD-1 inhibitors are being combined with targeted therapies such as the EGFR and ALK inhibitors. As listed above, there are numerous clinical trials currently investigating the potential of combination immunotherapy in NSCLC, of which the majority are investigating the combination of PD-1/PD-L1 inhibition with CTLA-4 inhibition, but other novel checkpoint inhibitors are also entering phase I development. Studies are also investigating the role of PD-1 inhibition in the adjuvant setting in the ANVIL (nivolumab), PEARLS (pembrolizumab), IMpower010 (atezolizumab), and BR31 (durvalumab) trials as well as in locally advanced disease (PACIFIC). Beyond investigating immunotherapy in earlier stages of NSCLC, further work needs to be done to understand the mechanisms of resistance to this class of drugs. Loss-of-function mutations in the interferon-receptor-associated Janus kinase 1 (JAK1) and/or Janus kinase 2 (JAK2) genes in melanoma and mismatch repair-deficient colon cancer have been implicated in acquired resistance, or possibly even primary resistance, to PD-1 inhibition (62, 63). JAK1/2 is required for signaling through the interferon-γ receptor, a process required for TC PD-L1 expression (9). Hence inactivating JAK1/2 mutations lead to loss of PD-L1 expression and lack of response to anti-PD-1/L1 therapy. While this mechanism has yet to be studied in NSCLC, it warrants further exploration as a possible biomarker of resistance. This paper also noted changes in the folding and localization of major histocompatibility complex (MHC) 1 to the cell surface in patients who have developed resistance to PD-1/L1 checkpoint inhibition through mutations in the beta-2-microglobulin gene. The presence of MHC on the surface of tumor cells is required for T-cell cytotoxicity and lack of presence of MHC on the surface will mitigate the effect of PD-1/L1 checkpoint inhibition.

CONCLUSION

Based on the data we have to date, in patients who have not received prior therapy for metastatic NSCLC, pembrolizumab could be offered as an alternative to platinum-doublet chemotherapy for those patients who express PD-L1 on ≥50% of their TCs based on the Keynote 024 trial. Confirmatory data will be forthcoming to support this single positive trial. In the refractory setting, nivolumab, pembrolizumab, and atezolizumab have all shown benefit in phase 3 clinical trials. Nivolumab is the only agent tested in a phase 3 trial with both known and unknown PD-L1 expression and demonstrated an OS benefit. Atezolizumab has shown a significant survival benefit in both the PD-L1 positive and negative patients (patients in this trial were required to have adequate tissue to document PD-L1 status). Pembrolizumab should only be given to patients who have known PD-L1 expression of at least 1%. We look forward to further phase 3 randomized data of the immunotherapy combination strategies but for now this strategy should be reserved for clinical trials.

Anti-PD1 and PD-L1 in NSCLC treatment have durable response rates of approximately 20% that produce remarkable long-term survival. Toxicity is more favorable than chemotherapy, however, unique to immune checkpoint blockade are immune TREAs, of which the Grade 3–4 occurring in 5% of patients leads to treatment discontinuation. PD-L1 expression levels show a variable response to checkpoint inhibition, and at present they are not essential to guide therapy in the patients who have failed platinum doublet. In contrast, PD-L1 expression appears to be critical in assessing the potential benefit of PD-1 inhibition in the chemotherapy-naïve patient population. Determining predictive biomarkers to response is still undergoing further investigation. The rapidly evolving future of immunotherapy will continue with future studies investigating the potential of PD-1 inhibition in combination with chemotherapy, targeted therapy, or other immuno-oncology agents. We have entered a new era of lung cancer treatment.

AUTHOR CONTRIBUTIONS

RJ performed the search for new publications and proceedings relevant to this study; reviewed the paper prior to submission. MI drafted the paper.

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The Emerging Role of Targeted Therapy and Immunotherapy in the Management of Brain Metastases in Non-Small Cell Lung Cancer

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Lung cancer is the worldwide leading cause of cancer-related mortality in men and second leading in women. Brain metastases (BM) account for 10% of non-small cell lung cancer (NSCLC) patients at initial presentation, with another 25–40% developing BM during the course of their disease. In the last decade, the field of precision oncology has led to the discovery of a multitude of heterogenous molecular abnormalities within NSCLC as well as the development of tyrosine kinase inhibitors that target them. In this review, the focus will be on targeted therapy and immunotherapy that show efficacy in BM rather than conventional treatment for multiple BM (such as surgical resection, WBRT, or stereotactic radiosurgery).

Keywords: brain metastases, non-small cell lung cancer, targeted therapies, immunotherapy, intracranial responses

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INTRODUCTION

Lung cancer is the worldwide leading cause of cancer-related mortality in men and second leading in women (1). Brain metastases (BM) account for 10% of non-small cell lung cancer (NSCLC) patients at initial presentation (2), with another 25–40% developing BM during the course of their disease (3). The general metastatic NSCLC population survival is approximately 12 months, with a median progression-free survival (PFS) range from 3 to 6 months (4). BM are associated with poor prognosis, and the median survival ranges from 2.4 to 4.8 months for patients with BM who receive whole-brain radiation therapy (WBRT) (5). While the standard of care for BM remains radiotherapy, determining the optimal treatment between high-dose-focused radiations via stereotactic radiosurgery (SRS) alone versus WBRT remains controversial. A retrospective multi-institutional retrospective study showed a survival advantage in patients with fewer than four BM less than 4 cm in size ($n = 189$ for NSCLC) who were treated with SRS compared to those treated with WBRT [adjusted hazard ratio (HR) for NSCLC, 0.58; 95% confidence interval (CI), 0.38–0.87; $P = 0.01$] (6).

The treatment of BM is important in maintaining a good quality of life and limiting cognitive impairment and neurological dysfunction. In the last decade, the field of precision oncology has led to the discovery of a multitude of heterogenous molecular abnormalities within NSCLC as well as the development of tyrosine kinase inhibitors (TKIs) that target them (7).

Patients with untreated BM have been excluded from most clinical trials of systemic therapy for two reasons: (1) historically poor prognosis and (2) presumed poor blood–brain barrier (BBB)

penetration by experimental drugs. Thus, the efficacy of these drugs in controlling NSCLC-related BM remains controversial.

In this review, the focus will be on targeted therapy and immunotherapy that show efficacy in BM rather than conventional treatment for multiple BM (such as surgical resection, WBRT, or SRS), or some combination of the three.

ANAPLASTIC LYMPHOMA KINASE (*ALK*)-REARRANGED NSCLC

The control and prevention of BM have emerged as an important therapeutic issue as systemic therapies with TKIs continue to improve the duration of disease control for patients with oncogene-driven NSCLCs (8). BM have been reported in about 24% of *ALK*-rearranged NSCLC patients at diagnosis, making intracranial activity an important feature of all *ALK*-targeted therapies (9).

Crizotinib

Crizotinib was the first *ALK* and ROS1 (c-ros oncogene 1) inhibitor, approved for treatment of *ALK*-rearranged NSCLC. Crizotinib has shown evidence of potential clinical benefit in patients with a baseline of BM. PROFILE 1014, a phase 3 prospective study in *ALK*-positive NSCLC, demonstrated higher intracranial disease control rate (IDCR) with first-line crizotinib compared to chemotherapy in patients with treated BM. Although intracranial time to progression was improved, it was not significant (ITT population: HR, 0.60; 95% CI, 0.34–1.05; $P = 0.069$; treated BM present: HR, 0.45; 95% CI, 0.19–1.07; $P = 0.063$; BM absent: HR, 0.69; 95% CI, 0.33–1.45; $P = 0.323$) (10). In patients with BM, the PFS was greatly improved with crizotinib versus chemotherapy (BM present: HR, 0.40; $P < 0.001$; median, 9.0 versus 4.0 months, respectively) and in the intent-to-treat population (HR, 0.45; $P < 0.001$; median, 10.9 versus 7.0 months, respectively). IDCR in patients with BM was significantly higher with crizotinib compared with chemotherapy (56 versus 25% at 24 weeks, respectively) (10) (**Table 1**).

Furthermore, a retrospective pooled analysis of single-arm phase 1 and 2 studies of crizotinib in advanced *ALK*-positive NSCLC, PROFILE 1007 (13) and 1005 (12), demonstrated a median overall survival (OS) of 29.6 months for 120 patients who were allowed to continue crizotinib beyond progressive disease (PD) because they continued to derive clinical benefit from it. Only 18% of previously untreated BM patients achieved an overall intracranial response rate (ICRR), with an IDCR of 56% (95% CI, 46–66%) at 12 weeks (11) (**Table 1**).

Inevitably, the brain is the most common site of PD after resistance to crizotinib because of inadequate central nervous system (CNS) penetration of the drug or the biological change in the tumor (28). Hence, progression of preexisting or development of new intracranial lesions in up to 70% of patients while receiving therapy was a common manifestation of acquired resistance to crizotinib (29). Most systemic cytotoxic chemotherapies and some TKIs seem to cross the intact BBB inefficiently (30).

Limited intracranial response of crizotinib might be related to lower concentrations of the drug in cerebrospinal fluid compared with the plasma concentration (0.616 versus 237 ng/mL, respectively, 5 h after administration of a 250 mg dose) (31).

Ceritinib

Ceritinib is another *ALK* inhibitor approved for *ALK*-rearranged NSCLCs that have progressed on crizotinib.

In vitro studies have found that ceritinib has a 20-fold greater potency to inhibit *ALK* than crizotinib and a 12-fold greater potency than alectinib (32). Ceritinib was found to cross the intact BBB in rats with a brain-to-blood exposure ratio of approximately 15%, although no human data exists (33).

In the phase 1 ASCEND-1 study, ceritinib demonstrated activity in *ALK*-rearranged locally advanced or metastatic cancer NSCLC patients, including both *ALK*-naïve and *ALK*-pretreated patients who had progressed following multiple lines of chemotherapy. Thirty one percent of the *ALK* inhibitor-naïve patients and 60% of *ALK* inhibitor-pretreated patients had BM, respectively. There were 94 patients with retrospectively confirmed BM and at least one post-baseline imaging. IDCR was 79% (15 of 19) in *ALK* inhibitor-naïve patients and 65% (49 of 75) in *ALK* inhibitor-pretreated patients (14). Overall ICRR was 34.5% (34) (**Table 1**).

In the ASCEND-2 phase 2 study, ceritinib showed a durable response in *ALK*-rearranged NSCLC patients who progressed on chemotherapy and crizotinib, including patients with BM. Moreover, 20 of the 100 patients with baseline BM had active target lesions at baseline. The investigator-assessed overall ICRR was 45% (95% CI, 23.1–68.5%), while the IDCR was 80% ($n = 20$, 95% CI, 56.3–94.3) (15) (**Table 1**).

In the ASCEND-3 phase 2 study of ceritinib in *ALK*-inhibitor-naïve NSCLC, 40.3% (50/124 patients) presented with BM at baseline. Fifty-four percent (27/50 patients) had received prior radiotherapy to BM. Updated data from ESMO 2016 showed an ICRR of 61.5% (8/13) in patients with measurable BM at baseline and an IDCR of 76.9% (10/13) (16) (**Table 1**).

Alectinib

Alectinib is another powerful *ALK* inhibitor that has shown activity in crizotinib-resistant patients. A phase 2 study in *ALK*-positive NSCLC patients observed an objective response rate of 48% (35).

A hurdle in treating BM is achieving a higher rate of drug concentration in the brain because of the BBB. In animal models, alectinib has a high brain-to-plasma ratio (0.63–0.94) and activity in intracranial tumor implantation models (36). Alectinib penetrates into the CNS (2.69 nmol/L) where it is able to exceed the *in vitro* concentration for *ALK* inhibition (1.9 nmol/L) (35, 37). Alectinib human studies show a 50% CNS distribution, but of a 12-fold lesser potency than ceritinib (33).

Unlike crizotinib and ceritinib, studies also suggest that alectinib is not a substrate of P-glycoprotein (P-gp), a key drug efflux pump typically expressed in the BBB (36), thus allowing for a higher rate of drug penetration through the BBB.

Pooled data analysis of NP28761 and NP28673, two single-arm phase 2 trials, evaluated the CNS effect of alectinib in pretreated *ALK*-rearranged NSCLC patients (19). NP28761 was limited

TABLE 1 | Intracranial effect of tyrosine kinase inhibitors ALK inhibitors and epidermal growth factor receptor (EGFR) inhibitors in trials in non-small cell lung cancer (NSCLC).

Trial	Treatment	IDCR	ICRR
ALK inhibitors			
PROFILE 1014 (10)	Crizotinib	56% at 24 weeks	Not described
	PEM + CBDCA or CDDP	25% at 24 weeks	Not described
Pooled analysis of Ref. (11) PROFILE 1005 (12) PROFILE 1007 (13)	Crizotinib	56% at 12 weeks (previously untreated)	18% (previously untreated)
ASCEND-1 (14)	Ceritinib	65% (pretreated) 79% (naïve)	34.5%
ASCEND-2 (15)	Ceritinib	80%	45%
ASCEND-3 (16)	Ceritinib	76.9%	61.5%
NCT01449461 (17)	Brigatinib	83% (measurable) 85% (non-measurable)	50% 31%
NP28673 (18)	Alectinib	85.3% 84.5% (pretreated)	58.8% (measurable) 46.4% (non-measurable)
NP28673 and NP28761 (19)	Alectinib	90.0% (measurable BM) 85.3% (measurable and/or non-measurable BM)	64.0% (measurable BM) 42.6% (measurable and/or non-measurable BM) 35.8% (prior RT) 58.5% (non-prior RT)
J-ALEX (20)	Alectinib	92.9%	85.4%
ALTA (21)	Brigatinib	88% (90 mg) 83% (180 mg)	36% (90 mg) 67% (180 mg)
NCT01970865 (22)	Lorlatinib	Not described	44% (targetable and non-targetable) 60% (targetable)
EGFR inhibitors			
Pooled analysis of published data Fan et al. (23)	Erlotinib or gefitinib	75.7%	51.8%
Retrospective analysis Grommes et al. (24)	Pulsatile high-dose weekly erlotinib	Not described	67%
LUX-Lung 3 and LUX-Lung 6 (25)	Afatinib	Not assessed	Not assessed
BLOOM (26)	Osimertinib	Not described	76% (33% LM improvement and 43% LM SD)
BLOOM (27)	AZD3759	Not described	52.4% (measurable)

IDCR, intracranial disease control rate; ICRR, intracranial response rate; PEM, pemetrexed; CBDCA, carboplatin; CDDP, cisplatin; BM, brain metastases; RT, radiotherapy; LM, leptomeningeal metastases; SD, stable disease.

to North America only (NCT01871805), while NP28673 was a global study (NCT01801111). One hundred thirty-six patients had baseline measurable BM (60% of the overall study populations). For patients with baseline measurable BM, the ICRR was 64.0% (95% CI, 49.2–77.1%) with 11 (22%) complete responses (CR) in the brain, the IDCR was 90.0% (95% CI, 78.2–96.7%), and the duration of response (DOR) was 10.8 months (95% CI, 7.6–14.1 months) (19) (**Table 1**). For patients with measurable and/or non-measurable baseline BM, the IDCR was 42.6% (95% CI, 34.2–51.4%), the IDCR was 85.3% (95% CI, 78.2–90.8%), and the median DOR was 11.1 months (95% CI, 10.3 months to not evaluable) (19). For patients with prior radiotherapy ($n = 95$), the ICRR was 35.8% (95% CI, 26.2–46.3%) and 58.5% (95% CI, 42.1–73.7%) for patients without prior radiotherapy ($n = 41$) (**Table 1**).

Updated intracranial response data on the 61/138 patients with baseline BM the global phase 2 NP28673 study was presented at ESMO 2016. In the measurable BM group ($n = 34$), the ICRR was 58.8% (95% CI, 40.7–75.4), while IDCR was 85.3% (95% CI, 68.9–95.1) and the median DOR was 11.1 months (**Table 1**). In the measurable and non-measurable group ($n = 84$), the ICRR was 46.4% (95% CI, 35.5–57.7), while IDCR was 84.5% (95% CI, 78–91.5) and median DOR was 11.2 months (18) (**Table 1**).

More recently, J-ALEX, a phase 3 study comparing alectinib and crizotinib in treatment naive patients in Japan, showed an ORR of 85.4% in the alectinib group versus 70.2% in the crizotinib group (20). In patients with BM, the HR for alectinib versus crizotinib was 0.08 (95% CI, 0.01–0.61). The J-ALEX trial enrolled 14 patients with asymptomatic BM in the alectinib arm. Only one of the patients with BM treated with alectinib had progressed by the time of data cut-off (IDCR of 92.9%) (20) (**Table 1**). Thus, reducing CNS progression in patients with *ALK*-positive NSCLC with alectinib could be achievable if alectinib is used in the first-line setting.

The Global ALEX trial is currently ongoing, also comparing alectinib versus crizotinib in first-line *ALK*-positive NSCLC but on a global scale. If the J-ALEX results are confirmed with this trial, alectinib could likely replace crizotinib as the standard first-line therapy for *ALK*-positive NSCLC in the future, especially those with BM.

Brigatinib, Lorlatinib, and Others

Brigatinib is an *ALK* inhibitor with preclinical activity against rearranged *ALK* and clinically identified crizotinib-resistant mutants. NCT01449461, a phase 1/2 single-arm, open-label, multicenter study in patients with advanced malignancies is ongoing. In a *post hoc* independent radiological review of patients with baseline BM, 6/12 patients with lesions ≥10 mm had a brain response (≥30% decrease in sum of longest diameters of target lesions) and 8/26 patients with only non-measurable lesions had disappearance of all lesions. ICRR for brigatinib with measureable BM was 50% and the IDCR was 83% (17). In non-measurable BM, the ICRR was 31%, IDCR was 85%, median intracranial PFS was 97 weeks, and median duration of intracranial response 82 weeks (**Table 1**). In ALTA, a phase 2 trial of brigatinib, ORR in arm A (90 mg qd) was 46% while ORR in arm B (90 mg qd for 7 days followed by 180 mg qd) was 54%. Seventy-one percent (arm A) and 67% (arm B) had BM (21) (**Table 1**).

Since CNS progression is a common site of relapse in NSCLC *ALK*/*ROS1* mutation patients, lorlatinib was developed as a selective brain-penetrant *ALK*/*ROS1* TKI active against most known resistance mutations. The phase 1 portion of the ongoing phase 1/2 study NCT01970865 enrolled patients with *ALK*+ or *ROS1*+ NSCLC with or without BM and were treatment naïve or had disease progression after ≥1 TKIs. Preliminary results revealed an objective ICRR of 44% in targetable and non-targetable lesions and 60% in targetable lesion, respectively (22) (**Table 1**).

Additional second-generation *ALK* inhibitors shown to have efficacy in the brain include ASP3026, X396, and entrectinib (38).

EPIDERMAL GROWTH FACTOR RECEPTOR (*EGFR*) TKIs

First-Generation TKIs

Approximately 33% of patients with NSCLC harboring tumors with *EGFR*-TKI-sensitizing mutations develop BM during treatment (39). Evidence suggests that *EGFR* TKIs have some limited BBB penetration (40, 41). In a pooled analysis including 464 patients from 16 trials to study the efficacy of *EGFR* TKIs in NSCLC patients with activating *EGFR* mutations with BM showed that *EGFR* TKIs produce significant beneficial effects, with a pooled objective ICRR of 51.8%, IDCR of 75.7%, median PFS of 7.4 months, and OS of 11.9 months (23) (**Table 1**).

Although erlotinib is effective for *EGFR* mutant NSCLC, CNS penetration is limited at standard daily dosing. Concentrations in cerebrospinal fluid exceeding the half maximal inhibitory concentration for *EGFR* mutant lung cancer cells in patients with BM and leptomeningeal metastases (LM) that developed despite standard daily erlotinib or other *EGFR* TKIs were achieved with weekly intermittent "pulsatile" administration of high-dose (1,500 mg) erlotinib (24). ICRR was 67% (**Table 1**). Median time to CNS progression was 2.7 months (range, 0.8–14.5 months), and median OS was 12 months (range, 2.5 months–not reached) (24).

Second-Generation TKI

In both LUX-Lung 3 and LUX-Lung 6 studies, there was a non-significant trend toward improved PFS with afatinib versus chemotherapy in patients with asymptomatic BM (LUX-Lung 3:11.1 versus 5.4 months, HR = 0.54, $P = 0.1378$; LUX-Lung 6:8.2 versus 4.7 months, HR = 0.47, $P = 0.1060$) (25). In combined analysis, PFS was significantly improved with afatinib versus with chemotherapy in patients with BM (8.2 versus 5.4 months; HR = 0.50; $P = 0.0297$) (25).

Afatinib significantly improved the ORR versus chemotherapy in patients with BM. For LUX-Lung 3, ORR for afatinib was 70.0% (95% CI, 45.7–88.1) versus chemotherapy 20.0% (95% CI, 4.3–48.1) in patients with BM. The LUX-Lung 3 DCR for afatinib was 95.0% (95% CI, 75.1–99.9) versus chemotherapy 80.0% (95% CI, 51.9–95.7) in patients with BM. In LUX-Lung 6, ORR for afatinib was 75.0% (95% CI, 55.1–89.3) versus chemotherapy 27.8% (95% CI, 9.7–53.5) in patients with BM. The LUX-Lung 6 DCR for afatinib was 89.3% (95% CI, 71.8–97.7) versus chemotherapy 72.2% (95% CI, 46.5–90.3). There was no significant difference in OS in patients with BM who were treated with afatinib or chemotherapy.

These findings perhaps demonstrate a clinical benefit of afatinib in *EGFR* mutation–positive patients with NSCLC and asymptomatic BM. However, the role of afatinib in active BM remains to be clarified since this was an exclusion criterion in this study. ICRRs were not assessed in this study (**Table 1**). Therefore, no direct conclusions can be made regarding afatinib's ability to cross the BBB in concentrations sufficient to elicit CNS responses.

Despite limited evidence of *EGFR* TKIs providing benefit in a few patients with *EGFR* mutation-positive NSCLC with BM, a clinical need for novel *EGFR* TKIs with improved efficacy against BM still exists.

Osimertinib in Leptomeningeal Disease

Leptomeningeal metastases are seen in 3–5% of NSCLC (42) and in 9% of *EGFR* mutation-positive patients (43). Osimertinib is an irreversible *EGFR* TKI that targets activating mutations (*EGFR*m) and resistance mutations (T790M). Osimertinib induced sustained tumor regression in an *EGFR*m PC9 mouse BM model. PET imaging showed higher levels of osimetinib levels in NHP and mouse models, in contrast to rociletinib and gefitinib (39).

In previous trials, osimertinib demonstrated robust systemic activity in patients with *EGFR*m NSCLC and BM and has shown CNS penetration with sustained tumor regression in BM (44). In the phase 1 BLOOM study, two third-generation *EGFR* TKIs—osimertinib and AZD3759—were studied in patients with *EGFR* mutation-positive advanced NSCLC (26). Neurological function improved from baseline in 24% (5/21) patients. Radiological improvements in LM were seen in 33% (7/21) patients, and 43% (9/21) had stable disease (SD) (**Table 1**). Clearance of tumor

cells from the CSF occurred in two patients at two consecutive visits. Time on treatment suggests durable clinical benefit, with 15 patients remaining on treatment, 7 of whom have been on treatment for >9 months.

AZD3759 in BM

AZD3759 is a reversible inhibitor of *EGFR*-activating mutations that was designed to achieve high exposure in the plasma and CNS. AZD3759 has high passive permeability (29.5×10^{-6} cm/s) and is not a substrate of the efflux transporters Pgp or BCRP at the BBB. *In vivo*, AZD3759 reached distribution equilibrium in rats, mice, and monkeys ($K_{puu,brain}$ and $K_{puu,CSF} > 0.5$), suggesting BBB penetration (45). AZD3759 induced significant tumor regression and dramatically improved animal survival in the BM model (45).

The AZD3759 cohort of the BLOOM trial evaluated the safety, tolerability, and early efficacy of AZD3759 in 29 patients with advanced *EGFR* mutation-positive NSCLC and metastases, including LM (27). Patients with non-LM BM were required to have at least one measurable intracranial or extracranial lesion. Patients with LM had a diagnosis confirmed by positive CSF cytology. All patients received at least one prior line of *EGFR* TKI therapy and chemotherapy. In addition, 34% of patients underwent prior whole-brain radiotherapy.

AZD3759 demonstrated encouraging intracranial antitumor activity. Among 21 patients with measurable BM, 11 patients demonstrated tumor shrinkage in the target brain lesion at AZD3759 doses of ≥50 mg BID (**Table 1**). In this group, there were three confirmed partial responses (PR) and three unconfirmed PRs. Among 22 patients with measurable extracranial lesions, 8 experienced tumor shrinkage, with 1 unconfirmed partial response. At the time of data cutoff, 5 of 29 patients remained on treatment with AZD3759. The longest duration of treatment was 48 weeks.

Beyond *EGFR* and *ALK*

Apart from *EGFR* mutation and *ALK* translocations other distinct molecular subtypes of NSCLC depend on oncogenic molecular aberrations (driver mutations) for their malignant phenotype. Limited but promising data exist for the treatment of BM on novel molecular targets such as *ROS1*, *BRAF*, *KRAS*, *HER2*, *c-MET*, *RET*, *PIK3CA*, *FGFR1*, and *DDR2* (46).

IMMUNOTHERAPY

Nivolumab

Nivolumab, a human IgG4 anti-PD-1 monoclonal antibody is active in the second-line treatment of metastatic NSCLC after progression on a platinum-based chemotherapy. Experience in routine clinical practice may differ from that seen in a controlled clinical trial. In a randomized phase 3 trial (CheckMate 017), the effect of nivolumab was studied in patients with advanced squamous NSCLC and central CNS metastases in a real-world, expanded access program (EAP) in Italy (47). Three hundred seventy-one patients participated in the EAP at 96 centers in Italy. Thirty-seven of 371 (10%) patients had asymptomatic and controlled CNS metastases. The DCR was 49% among patients with CNS metastases, with CR in 1 patient, PR in 6 patients, SD in 11 patients, and PD in 19 patients (**Table 2**), while the ORR in patients with CNS metastases was 7/37 (19%) (**Table 2**). OS rate at 12 months was 35% for patients with CNS metastases and 39% for all patients. The median OS was 5.8 months (95% CI, 1.8–9.8) for patients with CNS metastases and 7.9 months (95% CI, 6.2–9.6) for all patients. The PFS rate at 12 months was 31% for patients with CNS metastases and 27% for all patients. The median PFS was 4.9 months (95% CI, 2.7–7.1) for patients with CNS metastases and 4.2 months (95% CI, 3.4–5.0) for all patients.

In the Goldman et al. abstract 9,038 analysis presented ASCO 2016, pooled data from nivolumab studies [CheckMate 063 (50), CheckMate 017 (47), and CheckMate 057 (51)] were assessed to determine efficacy and safety of nivolumab in patients with previously treated, asymptomatic CNS metastases at baseline and patients with untreated, asymptomatic CNS metastases at baseline. The best response to most recent prior therapy demonstrated in the nivolumab with CNS metastases arm was CR/PR of 13/46 (28%), SD of 15/46 (33%), and PD of 18/46 (39%), compared to the docetaxel with CNS metastases arm with CR/PR 8/42 (19%), SD 13/42 (31%), and PD 18/42 (43%) (48). Among patients with pretreated CNS metastases, median OS was longer in the nivolumab group (8.4 months; 95% CI, 4.99–11.6) compared to the docetaxel group (6.2 months; 95% CI, 4.4–9.23). The frequency of and time to new CNS lesions were similar across treatment groups. Furthermore, 8/46 (17%) patients developed new CNS lesions in the nivolumab with CNS metastases arm with a median (range) of 3 (1.9–10.4) months, while 9/42 (21%) patients developed new CNS lesions in the docetaxel with CNS metastases arm with a median (range) of 2 (0.5–8.0).

Moreover, in CheckMate 012 Arm M, 2 of 12 patients (16.7%) with untreated CNS metastases achieved intracranial responses, including one intracranial CR lasting >10.5 months (48) (**Table 2**). These results support further investigation of nivolumab monotherapy in patients with NSCLC and asymptomatic CNS metastases.

Pembrolizumab

Pembrolizumab, a fully human anti-PD-1 monoclonal antibody is approved in first- and second-line treatment of metastatic NSCLC. NCT02085070 is a phase 2 study of pembrolizumab in patients with metastatic melanoma and NSCLC with untreated or progressive BM. The effect of drugs on untreated BM remains unclear because most clinical trials exclude these patients. Early data demonstrated that there was an ICRR of 6/18 (33%) in the NSCLC on pembrolizumab 10 mg/kg arm, similar to the systemic response rate (49) (**Table 2**). The median OS was 7.7 months to date.

TABLE 2 | Effect of immunotherapy on BM in non-small cell lung cancer trials.

Trial	Treatment	IDCR	ICRR (%)
CheckMate 017 (47)	Nivolumab	49%	19
CheckMate 012 (48)	Nivolumab	Not described	16.7
NCT12085070 (49)	Pembrolizumab	Not described	33

BM, brain metastases; IDCR, intracranial disease control rate; ICRR, intracranial response rate.

The available data for the use of anti-PD-1 agents in the treatment of BM do not yet include data on PD-L1 status. These data when available could suggest higher response rates based on the level of PD-L1 positivity.

CONCLUSION

Current standard of care for BM that require immediate local intervention (based on symptoms, location, size, or other concerning features) is craniotomy with resection or radiation therapy. There is still a role in integrating locally ablative therapy (LAT) in combination with targeted therapy and immunotherapy in patients with oligometastatic BM that are limited or have low metastatic tumor burden (52).

Prior to the advent of second-generation therapies for BM developing while on crizotinib, the only alternatives were ablation of oligometastatic brain lesion with LAT and continuing crizotinib (28). Using WBRT with concurrent erlotinib (53) was also a viable option rather than changing to traditional chemotherapy.

However, recent data showing dramatic and prolonged responses in BM patients treated with *EGFR* and *ALK* TKIs have suggested that delaying LAT and WBRT may be a valid treatment option for patients with asymptomatic BM from NSCLC, especially for those with *EGFR*-activating mutations or harboring *ALK* rearrangement.

The challenge will be to determine the optimal sequence of agents and modalities (WBRT and SRS). Perhaps serial genotyping, the degree of BM symptoms, and the toxicity profiles will serve to individualize treatments and determine the role of these targeted therapies in the therapeutic armamentarium of BM.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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Antiangiogenesis for Advanced Non-Small-Cell Lung Cancer in the Era of Immunotherapy and Personalized Medicine

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Over the past decade, patients with advanced non-small-cell lung cancer (NSCLC) have witnessed substantial advances in regards to therapeutic alternatives. Among newly developed agents, angiogenesis inhibitors were extensively tested in different settings and have produced some favorable outcomes despite several shortcomings. Bevacizumab is the most examined agent in this context and has demonstrated significant survival benefits when combined with standard chemotherapy in eligible patients. Preliminary results on the addition of bevacizumab to erlotinib in patients with EGFR-mutated NSCLC seem promising. Other antiangiogenic agents were also tested, but ramucirumab and nintedanib are the only agents with a positive impact on survival. More recently, immune checkpoint inhibitors (ICIs) have had considerable success due to their prolonged durations of response, yet response rates are still deemed suboptimal, and various combination therapies are being tested in an effort to improve efficacy. Preclinical evidence suggests an immunosuppressive effect of pro-angiogenic factors, which sets up a plausible rationale for combining ICIs and antiangiogenic agents. Herein, we review the landmark data supporting the success of angiogenesis inhibitors, and we discuss the potential for combination with immunotherapy and targeted agents.

Keywords: antiangiogenesis, combination therapy, immunotherapy, non-small-cell lung cancer, angiogenesis

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INTRODUCTION

A decade has now passed since bevacizumab, the first promising antiangiogenic agent, was approved for the treatment of non-small-cell lung cancer (NSCLC), and the lessons learned revealed that clinical applications of antiangiogenesis are somewhat more challenging than initially believed (1). As a fully humanized monoclonal antibody (mAb) that binds vascular endothelial growth factor-A (VEGF-A) and prevents interaction with VEGFR-1 and VEGFR-2 (the primary receptors involved in endothelial cell proliferation and migration), bevacizumab was thought of as a "silver bullet" capable of targeting multiple types of cancer since tumor proliferation and spread depend on neo-vasculature (2–4). However, despite survival gains attributed to this agent, clinical trial results did not fully meet with the expectations and management of patients with advanced NSCLC still requires significant improvements in order to clearly affect outcomes in this first ranking cancer in terms of cancer-related mortality (5). Nevertheless, angiogenesis remained an area of active research, and numerous agents have been tested. These agents bind VEGFR-2 directly (e.g., ramucirumab), act as VEGF

inhibitors (e.g., aflibercept), or block intracellular downstream signal transduction by the inhibition of the tyrosine kinases of VEGF receptors (e.g., sorafenib and nintedanib) (6–8).

In the era of immunotherapy and refined precision medicine, the value of antiangiogenic agents and their cost-efficiency could be put into question in the face of more successful biologic agents such as immune checkpoint inhibitors (ICIs) that demonstrated significant clinical activity both in the first- and second-line setting with much promise attributed to the durable responses they achieve in responding patients (9). On the other hand, combining immunotherapy and angiogenesis inhibitors could prove to be a successful undertaking, which might improve the efficacy of both agents. Herein, we will provide a review of noteworthy data relating to successful antiangiogenic agents in NSCLC, be it in combination with chemotherapy or with newer agents.

TARGETING VEGF

Bevacizumab

Combination with Cytotoxic Therapy

The initial randomized phase II study of this anti-VEGF-A mAb evaluated two different doses of bevacizumab (7.5 and 15 mg/kg) in addition to paclitaxel/carboplatin vs. chemotherapy alone, and the results demonstrated significant improvements in terms of response rate (RR) (31.5 vs. 18.8%) and median time to progression (7.4 vs. 4.2 months, $p = 0.023$) in favor of the arm with the highest dose of bevacizumab compared with the control arm (10). A noteworthy outcome of this trial was the identification of clinical features that were associated with high rates of life-threatening hemoptysis. Therefore, centrally located tumors with proximity to major blood vessels, cavitation, and squamous cell histology became exclusion criteria in most of the subsequent studies. However, ensuing data from the phase 4 SAiL study and the ARIES Observational Cohort study called into question whether cavitation and centrally located tumors did affect the rate of severe hemoptysis (11). Consequently, expert opinion suggests that squamous histology and the presence of hemoptysis are the most important contraindications to bevacizumab (12).

Following the success of the phase II study, a large phase III trial with a similar design conducted by the Eastern Cooperative Oncology Group (ECOG)—ECOG 4599—confirmed the benefits of bevacizumab (at a dose of 15 mg/kg), in the same setting, in terms of overall survival (OS) (12.3 vs. 10.3 months, $p = 0.003$), RR (35 vs. 15%, $p < 0.001$), and progression free survival (PFS) (6.2 vs. 4.5 months, $p < 0.001$) (13). In Europe, the AVAiL phase III trial also attempted to confirm the benefit of bevacizumab but in combination with the cisplatin/gemcitabine doublet and at two different dose levels (7.5 and 15 mg/kg) (14). Although the improvements in PFS were statistically significant for both dose levels of bevacizumab (6.5 vs. 6.1 months, $p = 0.03$ for the higher dose and 6.7 vs. 6.1, $p = 0.003$ for the lower dose), the study design did not allow for a direct comparison between both dose levels. Additionally, a subsequent survival analysis failed to demonstrate any OS benefit (15). Considering the modest absolute value of PFS improvements and the absence of any OS benefit, some experts favor the addition of bevacizumab to a paclitaxel/carboplatin regimen and support a theory that paclitaxel might be more susceptible to positive modulation by bevacizumab (16–18). The results of the BEYOND study are in line with this reasoning. This more recent phase III study, evaluating the addition of bevacizumab (15 mg/kg) to a carboplatin/paclitaxel backbone chemotherapy regimen in a Chinese cohort, demonstrated significant improvements in PFS [9.2 vs. 6.5 months; hazard ratio (HR), 0.40; 95% CI, 0.29–0.54; $p < 0.001$] and OS (24.3 vs. 17.7 months; HR, 0.68; 95% CI, 0.50–0.93; $p = 0.0154$) (19). Of particular note, the very favorable outcomes in terms of PFS and OS in the control arm seem to reflect a better selection of patients along with improvements in supportive care measures. Additionally, subsequent lines of therapy have most definitely impacted survival results in both arms as the EGFR mutation rates were 27 and 26% and the subsequent use of EGFR-TKI was 36 and 38% for the experimental and standard arms, respectively (**Table 1**) (19).

To date, the available data were compiled in two different meta-analyses of platinum doublets combined with bevacizumab and both concluded to significant PFS and RR benefit from the addition of bevacizumab to standard cytotoxic therapy (20, 21). However, only one of these studies demonstrated a 10% relative reduction in the risk of death with the addition of bevacizumab to chemotherapy (HR: 0.90, 95% CI, 0.81–0.99) (21).

Bevacizumab was also tested in the adjuvant setting at a dose of 15 mg/kg in combination with cisplatin and vinorelbine, docetaxel, gemcitabine, or pemetrexed (for non-squamous histology) per physician's choice. The results of the E1505 phase III study were released after an interim analysis showed futility 41 months of follow-up. Additionally, patients receiving bevacizumab-containing therapy had significantly higher rates of grade 3–5 toxicities, mostly in the form of hypertension (8 vs. 30%), neutropenia (33 vs. 38%), and overall worst grade (67 vs. 84%) (22).

Maintenance Therapy and Dosing

Besides the issue of optimal backbone chemotherapy, other pivotal questions involve the duration of therapy with bevacizumab and the optimal dose of this agent.

In the landmark ECOG 4599 study, bevacizumab was continued until progression or limiting toxicities, and a retrospective analysis demonstrated superior PFS and OS in patients where bevacizumab was continued (PFS: 4.4 vs. 2.8; HR: 0.64, and OS: 12.8 vs. 11.4; HR: 0.75) (13, 23). Since then, three important studies have addressed this issue. The POINTBREAK trial did not demonstrate any OS advantage when pemetrexed/carboplatin/bevacizumab (15 mg/kg) followed by bevacizumab/pemetrexed maintenance was compared with paclitaxel/carboplatin/bevacizumab followed by bevacizumab maintenance, but PFS favored the pemetrexed containing regimen (6.0 vs. 5.6 months; $p = 0.012$). The AVAPERL study comparing cisplatin/pemetrexed/bevacizumab followed by either pemetrexed or pemetrexed/bevacizumab maintenance, in non-progressing patients, demonstrated a substantial PFS advantage in favor of the doublet maintenance (7.4 vs. 3.7 months; HR, 0.57; 95% CI, 0.44–0.75; $p < 0.0001$), but OS did not reach statistical significance. The PRONOUNCE study did not demonstrate a survival difference

TABLE 1 | Results of landmark trials evaluating antiangiogenic agents in metastatic non-small-cell lung cancer.

Study/phase	Chemotherapy	Number of patients (*n*)	ORR (%)	Median PFS (months)	HR (95% CI); *p*	Median OS (months)	HR (95% CI); *p*
ECOG 4599/ phase III	Pac/Carbo Pac/Carbo/Bev	444 434	15 35	4.5 6.2	HR = 0.66 (0.57–0.77); $p < 0.001$	10.3 12.3	HR = 0.79 (0.67–0.92); $p = 0.003$
AVAiL/phase III	Cis/Gem Cis/Gem/Bev –7.5 mg/kg –15 mg/kg	345 –345 –351	21.6 –37.8 –34.6	6.1 –6.7 –6.4	–HR = 0.75 (0.64–0.87); $p = 0.0003$ –HR = 0.85 (0.73–1.00); $p = 0.0456$	13.1 –13.6 –13.4	–HR = 0.93 (0.78–1.11); $p = 0.420$ –HR = 1.03 (0.86–0.54); $p < 0.01$
BEYOND/phase III	Pac/Carbo Pac/Carbo/Bev	138 138	26 54	6.5 9.2	HR = 0.40 (0.29–0.54); $p < 0.01$	17.7 24.3	HR = 0.68 (0.50–0.93); $p = 0.0154$
AVAPERL/phase III	Cis/Pem/Bev Pem/Bev maintenance Bev maintenance	376 128 125	– – –	– 7.4 3.7	– HR = 0.57 (0.44–0.75); $p < 0.0001$	– 17.1 13.2	– HR = 0.87 (0.63–1.21); $p = 0.29$
POINTBREAK/ phase III	Carbo/Pem/Bev → Bev/Pem maintenance Carbo/Pac/Bev → Bev maintenance	472 467	34 33	6 5.6	HR = 0.83 (0.71–0.96); $p = 0.012$	13.4 12.6	HR = 1.0 (0.86–1.16); $p = 0.949$
PRONOUNCE/ phase III	Carbo/Pem → Pem Carbo/Pac/Bev → Bev	182 179	23.6 27.4	4.44 5.49	HR = 1.06 (0.84–1.35); $p = 0.610$	10.5 11.7	HR = 1.07 (0.83–1.36); $p = 0.615$
JO25567/phase II	Erlotinib Erlotinib/Bev	75 77	64 69	9.7 16.0	HR = 0.54 (0.36–0.79); $p = 0.0015$	– –	–
BELIEF/phase II	Erlotinib/Bev All patients T790M-mutated EGFR	109 60	– 70.3	13.6 15.4	–	– –	–
REVEL[a]/phase III	Docetaxel Docetaxel/ramucirumab	625 628	14 23	3.0 4.5	HR = 0.76 (0.68–0.86); $p < 0.0001$	9.1 10.5	HR = 0.86 (0.75–0.98); $p = 0.023$
LUME-lung 1[a]/ phase III	Docetaxel Docetaxel/nintedanib	659 655	1.5 4.9	1.5 3.6	HR = 0.63 (0.48–0.83); $p = 0.0008$	9.1 10.1	HR = 0.94 (0.83–1.05); $p = 0.2720$
LUME-lung 2[a]/ phase III	Pem Pem/nintedanib	360 353	8.3 9.1	3.6 4.4	HR = 0.83 (0.70–0.99); $p = 0.0435$	12.7 12.2	HR = 1.03 (0.85–1.21); $p = 0.8940$

HR, hazard ratio; PFS, progression free survival; OS, overall survival; Pac, paclitaxel; Carbo, carboplatin; Bev, bevacizumab; Cis, cisplatin; Gem, gemcitabine; Pem, pemetrexed; ECOG, Eastern Cooperative Oncology Group.

[a]*Trials in the second-line setting.*

when pemetrexed/carboplatin followed by pemetrexed maintenance was compared with paclitaxel/carboplatin/bevacizumab (15 mg/kg) followed by bevacizumab maintenance (**Table 1**) (24–26). When all these trials are taken together, it remains unclear whether the demonstrated benefit of maintenance pemetrexed is improved by bevacizumab. An ongoing phase III study with three different maintenance therapies (ECOG 5508; pemetrexed vs. bevacizumab vs. pemetrexed/bevacizumab) will provide further data in that regard.

Different doses of bevacizumab were tested in different settings, and in NSCLC both the higher (15 mg/kg every 3 weeks) and lower (7.5 mg/kg every 3 weeks) doses were tested, but direct comparison of both dose levels for efficacy was not performed in the larger landmark trials. However, the ABIGAIL trial, designed as a correlative biomarker finding study of bevacizumab combined to a platinum doublet, randomized patients to receive 7.5 or 15 mg/kg. Although survival was not the primary endpoint of this study and with the caveat of an insufficient patient cohort ($n = 303$) to adequately compare the clinical effect of dose, no difference in PFS and OS was observed between both dose levels of bevacizumab (27). Considering these data and results from the aforementioned meta-analyses suggesting similar clinical benefit from bevacizumab at both dose levels, the optimal dose of bevacizumab is still debatable (20, 21).

Combination with Tyrosine Kinase Inhibitors

The addition of bevacizumab to erlotinib was initially attempted in patients with refractory NSCLC who were unselected for activating EGFR mutations, but no improvements in survival were obtained with the combination therapy (28). More recently, a Japanese phase II study evaluated the same combination of erlotinib/bevacizumab in patients with treatment-naïve EGFR-mutated (exon 19 and 21 alterations) NSCLC (29). The results demonstrated a substantial improvement in PFS (16.0 vs. 9.7 months, HR 0.54, $p = 0.0015$), but the study was not powered to compare OS (29).

These encouraging results have been suggested to be due to an increased uptake of erlotinib in tumor cells that is potentiated by bevacizumab in addition to the actual blockade of angiogenic signaling (30).

The preliminary results of another open label single arm phase II trial from Europe, the BELIEF study, yielded provocative results

and met its 1-year PFS endpoint for the entire cohort [55.6% (95% CI: 44.7–66.6%); median: 13.6 months], including patients with T790M-mutated NSCLC [1-year PFS: 60.2% (95% CI: 45.6–74.8%); median: 15.4 months] (31). Based on these results, erlotinib/bevacizumab received approval as first-line treatment of patients with EGFR-mutated NSCLC in June 2016 in Europe. Another ongoing study, the ACCRU (NCT01532089) trial, has completed accrual in the US, and its results will help confirm the available data.

TARGETING VEGF-R

Ramucirumab

This fully human mAb targeting VEGFR-2 first demonstrated its efficacy in gastric and colorectal cancers (32–34). The development in NSCLC was somewhat more challenging. After the initial open label phase II data demonstrated favorable responses, another phase II study randomized patients to cisplatin/pemetrexed followed by pemetrexed maintenance vs. cisplatin/pemetrexed/ramucirumab followed by ramucirumab-pemetrexed maintenance (35, 36). Unfortunately, the latter trial did not meet its primary endpoint (PFS: 7.2 vs. 5.6 for the ramucirumab arm; $p = 0.132$) (36). Further development of ramucirumab in the first-line setting was subsequently halted.

The activity of ramucirumab in NSCLC was nonetheless demonstrated in the phase III REVEL trial, where a docetaxel/ramucirumab combination was compared to docetaxel alone in the second-line setting (**Table 1**) (37). Of note, patients who previously received bevacizumab and those who had squamous histology were not excluded. Modest but statistically significant improvements in OS [10.5 vs. 9.1 months; HR: 0.86 (0.75–0.98); $p = 0.023$] and PFS [4.5 vs. 3.0 months; HR: 0.76 (0.68–0.86); $p < 0.0001$] led to FDA approval in combination with docetaxel regardless of histological subtype. However, the use of ramucirumab is not widely adopted since some experts believe that the OS improvement, although statistically significant, might not be clinically meaningful in accordance with the ASCO definition for expensive drugs, particularly if these improvements come at the expense of added toxicities (38).

TYROSINE KINASE INHIBITORS OF ANGIOGENESIS

The appeal of antiangiogenic TKIs stemmed from their success in renal cell carcinoma as well as from their ease of administration, which led to further development in different other cancer types. Unfortunately, different TKIs failed to produce consistent success in the treatment of advanced NSCLC.

Combining sorafenib with a platinum doublet in the first-line setting did not demonstrate any survival benefit (39). Two studies evaluating sunitinib combined with erlotinib in the second-line setting in patients with wild-type EGFR, or with pemetrexed, also failed to demonstrate efficacy of the combination therapies (40, 41). Combining pazopanib with a platinum doublet resulted in excessive toxicities (42).

Among newer multi-kinase inhibitors, the phase II/III study evaluating cediranib in addition to frontline carboplatin/paclitaxel was halted for futility on the basis of excessive toxicities, and the phase III MONET trial testing motesanib, also in combination with frontline carboplatin/paclitaxel, did not result in significant OS improvements (43, 44). Another TKI, vandetanib, was assessed in four phase III trials, two of which (ZEAL and ZODIAC trials) evaluated the agent in combination with docetaxel or pemetrexed maintenance, whereas the other two studies tested vandetanib as a single agent in second or subsequent lines of therapy, but neither of these studies had a positive impact on survival, and the combination therapies mostly resulted in increased toxicities (45–48).

Nintedanib

The triple angiokinase inhibitor nintedanib is the only TKI agent that has shown significant results when tested in the phase III LUME-Lung1 study (**Table 1**). This agent was tested in the second-line setting of patients with advanced NSCLC (both squamous and non-squamous histologies were included) in combination with docetaxel, and the study met its primary PFS endpoint in comparison with docetaxel monotherapy (3.4 vs. 2.7 months; HR, 0.79; 95% CI, 0.68–0.92; $p = 0.0019$) but failed to demonstrate differences in survival for the global population (49). When patients were evaluated in a prespecified subgroup analysis, the combination therapy showed improvements in OS for patients with an adenocarcinoma histology who progressed within 9 months of first-line therapy (10.9 vs. 7.9 months; HR, 0.75; 95% CI, 0.60–0.92; $p = 0.0073$) and for all patients with adenocarcinoma (12.6 vs. 10.3 months; HR, 0.83; 95% CI, 0.70–0.99; $p = 0.0359$). A confirmatory phase III trial, the LUME Columbus study (NCT02231164), with the same design, but excluding patients with squamous histology, was terminated for slow accrual. The LUME-Lung 2 study, examining a pemetrexed/nintedanib combination in the second-line setting, also demonstrated a modest but significant PFS improvement in comparison with pemetrexed monotherapy (PFS: 4.4 vs. 3.6 months; HR, 0.83, 95% CI, 0.70–0.99; $p = 0.0435$) but failed to demonstrate a survival benefit (**Table 1**) (50). As such, this agent has received approval in Europe for the second-line treatment of NSCLC in combination with docetaxel, but FDA approval has not been granted.

COMBINATIONS WITH IMMUNOTHERAPY

The demonstration of durable responses in patients with advanced NSCLC, through the use of ICIs, has led to considerable enthusiasm within the scientific community. The first anti-programmed death-1 (PD-1) agents, nivolumab and pembrolizumab, gained accelerated approval for the treatment of metastatic NSCLC in the second-line setting after demonstrating significant clinical activity in this context (51–53). Additionally, agents targeting programmed death-ligand 1 (PD-L1)—such as atezolizumab, durvalumab, and avelumab—are also in advanced stages of development, and some have gained approval in several other indications (54–56). Most recently, pembrolizumab was also found to be superior to standard platinum-based chemotherapy

and gained approval for the first-line treatment of metastatic NSCLC with positive PD-L1 expression—defined as tumor proportion score of 50% or more (57). Despite their efficacy, reported overall RRs are less than optimal (20–25% in the second-line and 45% in the frontline setting for selected patients), which gives rise to different strategies aimed at improving responses to ICIs. Therefore, investigators are attempting combinations of ICIs with chemotherapy, radiation therapy, cancer vaccines, oncolytic viruses, and targeted therapies in order to overcome resistance mechanisms (58). Some evidence suggests that angiogenesis might be associated with immunosuppression within the tumor microenvironment thereby potentiating immune-escape of tumor cells (59).

The complex relationship between VEGF and tumor-related immune regulation involves several key pathways that lead to an immunosuppressive microenvironment. VEGF is effectively capable of inducing inhibitory immune cells such as T-regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs) (60, 61). Additionally, exposure to VEGF at pathologic levels might inhibit the differentiation and/or emigration T-cell progenitors from the thymus resulting in a state of systemic cancer-related immunosuppression (62). Moreover, it seems that lymphocyte influx across the vascular endothelium toward the tumor is affected by VEGF, which leads to a defect in intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 clustering at the endothelial cell surface through nitric oxide production and subsequently leads to defective lymphocyte adhesion and migration toward the tumor environment (63).

On the other hand, preclinical models have shown that the use of antiangiogenic agents such a sunitinib or cabozantinib lead to an increase in CD4+ and CD8+ T-cells infiltration and reduce PD-1 expression within these cells while the influx of MDSCs and Tregs toward tumor tissue seems to be decreased (64–66).

In light of these findings, multiple trials are currently investigating combinations of immunotherapy and antiangiogenic drugs in different types of cancer. The most encouraging results in this context come from the experience with melanoma, where immunotherapy achieved its first successes. Promising phase I data indicated that a combination of ipilimumab and bevacizumab is both safe and effective with a median OS of 25.1 months and a disease control rate of 67.4%, thereby supporting the preclinical rationale of VEGF impact on immune regulation (67).

In NSCLC, preliminary data from a phase I study evaluating a nivolumab/bevacizumab combination vs. nivolumab monotherapy as maintenance after initial platinum-based chemotherapy suggested a favorable adverse-events profile for both arms (68). This study is certainly not powered to provide information in regards to optimal regimens, but the results indicated median PFS values with combination therapy that compared favorably to those obtained with the comparator arm (PFS: 37.1 weeks for nivolumab/bevacizumab, whereas nivolumab monotherapy yielded 16 and 21.4 weeks of PFS in patients with squamous and non-squamous histology, respectively) (68).

Another phase Ia/dose-limiting toxicity evaluation explored the addition of ramucirumab to pembrolizumab in patients with advanced NSCLC, gastric-esophageal cancers, and urothelial carcinoma (69). Preliminary data also indicate the safety of this combination as no dose-limiting toxicities were identified in patients with NSCLC (only one patient with urothelial carcinoma experienced severe toxicities requiring treatment discontinuation).

These encouraging safety data will certainly need to be cemented with efficacy data from larger trials exploring ICIs/antiangiogenesis combinations before any definitive conclusions can be drawn. Several challenges involving optimal dosing and treatment schedules remain to be resolved before such combinations can be considered for clinical practice especially since several combinations involving immunotherapy (with chemotherapy, radiation therapy, vaccines, etc.) are being tested and could have better efficacy when tested in larger trials.

CONCLUSION

Identifying the VEGF pathway as a key regulator in angiogenesis and in subsequent tumor growth and metastasis has led to the development of several agents targeting the pathway's different components. Bevacizumab appears to be the most successful antiangiogenic, but ramucirumab and nintedanib have also demonstrated clinical efficacy in the second-line setting. Although some experts believe that the benefits of these agents have plateaued, the promising results of an erlotinib/bevacizumab combination in EGFR-mutated lung cancer have proven otherwise. The intricate relationship between immunosuppression and angiogenesis indicates that a synergistic relationship could result from a combination of ICIs and angiogenesis inhibitors with relatively favorable toxicity profiles and has sparked a renewed interest in the study of antiangiogenic drugs. However, our comprehension of cancer-related immune modulation barely scratches at the surface of a vast compendium of knowledge. Many challenges need to be addressed before optimal combination therapies are defined.

AUTHOR CONTRIBUTIONS

ST and NB contributed equally to the production of this manuscript.

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MINI REVIEW
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Low Grade Neuroendocrine Tumors of the Lung

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The lung is the second most common site of neuroendocrine tumors (NETs). Typical and atypical carcinoids are low-grade NETs of the lung. They present a favorable prognosis comported to the more common high-grade NETs. The low- and high-grade NETs require different treatment strategies; effective management of these tumors is essential to prolong survival and to manage the symptoms in patients with secretory or functional tumors. These rare tumors have received little attention and education is needed for treating physicians. This mini-review will concentrate mainly on advanced low-grade lung NETs. The article describes the classification of lung NETs and the diagnostic work-up. Different treatment methods including somatostatin analogs, peptide receptor radioligand therapy, and biologic systemic therapy are discussed. Promising results from recent trials are presented and discussed in the context of the lung primary site.

Keywords: neuroendocrine tumors, lung, everolimus, octeotride, Carcinoid, Atypical carcinoid

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INTRODUCTION

Neuroendocrine tumors (NETs) are derived from neuroendocrine cells. As these cells exist in many organs embryologically, NETs can initiate in many parts of the body including the gastrointestinal (GI) tract, lung, thymus, and ovary. The lung is the second most common site for NETs after the GI tract, and account for 25% of all NETs (1, 2) and 1–2% of all lung cancers (1, 3, 4).

Neuroendocrine tumors are considered very rare tumors and accurate incidence and prevalence data is difficult to obtain. In 2010, (latest year available) only 315 Canadians were diagnosed with endocrine tumors of all types; the numbers of NETs and even lung NETs are lower still (5). The reported incidence of NETs is increasing, likely due to greater awareness of the disease and better diagnostic capabilities (3). As patients with NETs have a prolonged survival, prevalence rates are high.

Lung NETs are a very heterogeneous group of tumors. They possess varied pathological and clinical features and require different treatment strategies. A spectrum of cell histologies from low grade carcinoid to high-grade small cell malignancies can be observed. Although it is important for the treating physician to understand the disease spectrum of lung NETs, this review will primarily focus on the classification and treatment of low-grade, well-differentiated lung NETs.

Neuroendocrine tumors may secrete biologically active amines or peptides and are often referred to as "functional" or "secretory." As a result of this secretory activity, patients experience a spectrum of symptoms. Treatment is essential for symptom management and quality of life improvement and may prolong survival. However, as there are only small numbers of patients with lung NETs, evidence

Abbreviations: AC, atypical carcinoid; DOPA, dihydroxyphenylalanine; 5-HIAA, 5-hydroxyindoleacetic acid; HPF, high power field; HR, hazard ratio; IM, intramuscularly; NETs, neuroendocrine tumors; LAR, long-acting release; NR, non-response; OS, overall survival; PET, positron emission tomography; PFS, progression-free survival; PPRT, peptide receptor radioligand therapy; SC, subcutaneous; SSAs, somatostatin analogs; TC, typical carcinoid; TTP, time to progression; WHO, World Health Organization.

for optimal treatment strategies is lacking. The heterogeneous nature of NETs, their rarity and the lack of randomized trials in this disease area, underscores the importance of education in disease management.

CLASSIFYING LUNG NETs

The WHO classification of Lung NETs was updated in 2015 and organizes the types of lung NETs on a spectrum, shown in **Table 1** (6). A significant change made in the 2015 reclassification was grouping all four NET types into one category. Until this time, large cell and small NETs were separate from the typical carcinoid (TC) and atypical carcinoid (AC) tumors.

The WHO classification distinguishes between the low grade (TC and AC) and high grade (large cell neuroendocrine and small cell) tumors. TC tumors are quite bland in their histology, have less than 2 mitoses per 2 mm^2 and lack any evidence of necrosis. AC tumors can have the same "carcinoid morphology," but the mitotic rate is increased from 2 to 10 mitoses per 2 mm^2 and/or may be punctuated with necrotic features. Images of both TC (G1) and AC (G2) NETs are shown in **Figures 1A,B**, respectively.

Because this review focuses on low-grade NETs, pathology and clinical presentation of high-grade NETs is described only briefly here. As their name implies, large-cell neuroendocrine carcinomas have a large cell size, and a low nuclear to cytoplasmic ratio and frequent nucleoli. The mitotic rate is greater than >10 mitoses per 2 mm^2 and necrosis is frequently present.

Low-grade NETs include TC and AC tumors. NET G1 or TC tumors, account for 1% of thoracic malignancies with only 10% chance of distant spread (7). NET G2 or AC, account for 0.1% of thoracic malignancies with a 20% chance of distant spread (7). NET G3 large cell NETs have a 4.8% incidence and 50% chance of distant spread, and G3 small-cell NETs have the highest incidence at 13.9%, with the highest chance of distant spread at 70% (7).

The most important point of differentiation for the treating physician is the dichotomous distinction between low grade (carcinoid and AC) and high grade (large cell neuroendocrine and small cell carcinoma) NETs. Prognosis and management differ widely between these two groups. This article will focus on the low- and intermediate-grade NETs. It is important to note that some patients do not fall easily into a discrete category, despite this classification system. Although Ki-67 expression is not validated for use in the lung, it can be used to differentiate the high-grade large cell NETs from the G1/G2 NETs, with crush biopsies or when cells are necrotic (6). Ki-67 is not recommended by the WHO to distinguish the TC from AC tumors (6).

TABLE 1 | WHO Classification of neuroendocrine tumors (NETs).

NET type	WHO grading (6)	Histology	Mitosis per 2 mm^2	Presence of necrosis
Low grade (well-differentiated)	G1	Typical carcinoid	<2 (6)	No necrosis
Intermediate grade (well-differentiated)	G2	Atypical Carcinoid	2–10 (6)	Necrosis
High grade (poorly differentiated)	G3	Large cell	>10 (6)	Extensive necrosis
		Small cell		High necrosis

Low-grade lung NETs are subdivided into central or peripheral depending on their site of origin within the bronchial tree. Patients with central lesions may present with symptoms such as hemotypsis (bleeding), wheezing, or airway obstruction. Patients with peripheral disease rarely experience symptoms related to tumor location.

The staging of lung NETs is non-specific and follows the TNM staging of non-NET lung cancers, which follow the current WHO classification (8). This may not be the best staging for this subset of lung malignancies as many lung carcinoid and AC are <3 cm in size (9).

PROCEDURES FOR WORK UP FOR ADVANCED LOW GRADE LUNG NET

According to SEER, 12.9% of NET patients present with metastasized tumors at diagnosis (10). Although NETs are slow growing

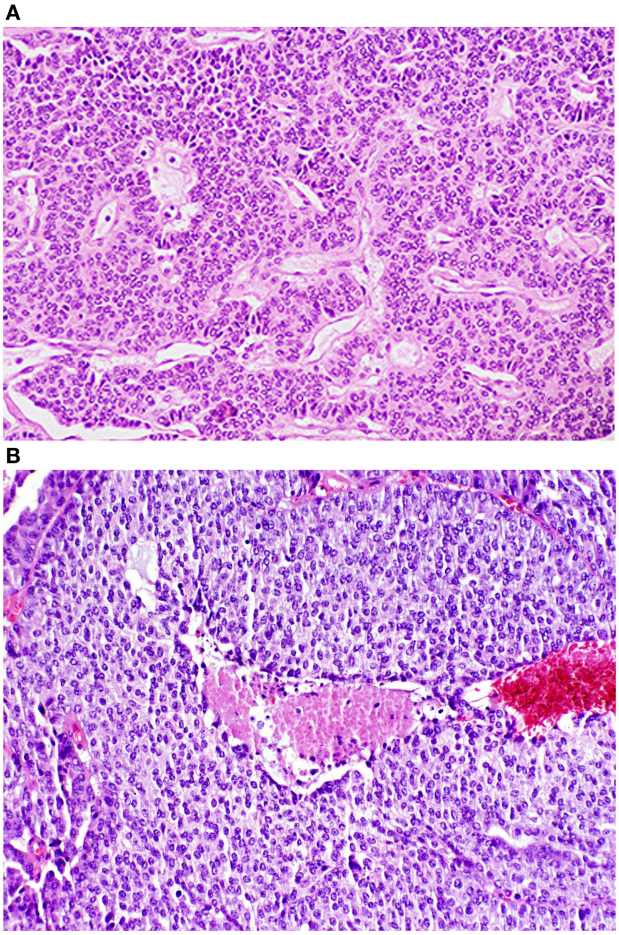

FIGURE 1 | Photomicrographs of typical and atypical pulmonary carcinoid tumors. **(A)** Low power photomicrograph of a typical pulmonary tumor. **(B)** Low power photomicrograph of an atypical pulmonary carcinoid tumor with central necrosis. Reproduced with permission from: Tazelaar HD. Pathology of lung malignancies. In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed on November 2, 2016.) Copyright © 2016 UpToDate, Inc. For more information visit www.uptodate.com.

tumors, advanced disease leads to poor survival, and in patients with well-differentiated NETs with distant metastasis, 73% will die within 5 years (1). Liver, bones, and mediastinal lymph nodes are the most common sites of metastasis (11).

Once a diagnosis of advanced low-grade lung NET (carcinoid or AC) is made, a workup to establish disease burden, determine whether the tumor is functional (secretory) or not, and document baseline cardiac status should be initiated. Baseline tests include renal function, calcium and plasma Chromogranin A (12).

For diagnosis, a CT scan of both chest and abdomen should be performed (13). A high resolution CT can be done if contrast is contraindicated. Functional imaging with 111-Indium labeled octreotide is commonly used to establish disease burden and can also indicate whether treatment with peptide receptor radioligand therapy (PPRT) is an option (described in more detail in the Section "Peptide Receptor Radioligand Therapy") (14, 15). Newer imaging technologies are more accurate, permit tumor staging and better treatment decision making, and can help localize disease. These include ^{18}F-dihydroxyphenylalanine (DOPA) positron emission tomography (PET) or preferably, ^{68}Ga-DOTATATE PET scan, which also targets somatostatin receptor expression (16, 17).

Functional or secretory NETs may secrete biologically active amines or peptides. Patients may experience a spectrum of symptoms that may include diarrhea, flushing, abdominal pain, hypotension, and vasospasm. Depending on the source, an estimated 10–30% of advanced TC and AC NETs are functional (3, 18).

In patients with functional symptoms, a 24-h urine test for 5-hydroxyindoleacetic acid (5-HIAA) should be performed at baseline (12). High levels of urine 5-HIAA may correlate with the risk of carcinoid heart disease and an attempt to lower it by treating with somatostatin analogs (SSAs) should be made. The 24-h 5-HIAA test should be repeated on disease progression or when a change is therapy is being considered. Because carcinoid complications may occur with time, a baseline echocardiogram should also be performed (19).

TREATMENT MODALITIES FOR ADVANCED LOW GRADE NET

For advanced carcinoid and AC patients, treatment is essential for symptom management and quality of life improvement in patients with functional tumors. Treatment may prolong survival in patients with both non-functional and functional tumors. As there are only small numbers of patients with lung NETs, evidence for optimal treatment strategies is lacking. Most NET clinical trials conducted to date have focused on GI NETs, particularly in those of pancreatic (pNET) and midgut origins. Although trial results may be extrapolated, lung NETs deserve individual attention. The heterogeneous nature of NETs, their rarity and the lack of randomized trials in this disease area, underscores the need for trials in this area and the importance of education in disease management.

Surgery

When TC and AC lung NETs are diagnosed at an early stage, surgical intervention is often curative. TC tumors have excellent 5- and 10-year survival rates of over 90%. This is in contrast to AC tumors where 5-year survival is approximately 70% and 10-year survival is only 50% (20–22).

Regarding adjuvant therapy, the use of chemotherapy with or without radiation has not been well studied and treatment guidelines differ (3, 23, 24). The NCCN guidelines recognize that the role of chemotherapy in the adjuvant setting for typical NET of lung origin is not known (24). However, for stage II or III atypical NET, chemotherapy with or without radiation is recommended (23). The European ENET guideline agrees with this for TC but states that for AC, adjuvant therapy may be considered if nodal disease is found (3).

Surgical treatment may also be considered in patients with advanced or metastatic disease for curative intent or symptom control, depending on the individual patient and site of disease (3).

Systemic Chemotherapy for Advanced G1 (TC) and G2 (AC) Lung NETs

Patients with low-grade TC and AC lung NETs may respond to chemotherapy, but data are historical and concrete recommendations are not supported. Multiple cytotoxic drug combinations have shown degrees of activity in lung NETS, although there is a lack of consensus regarding standard therapy.

SSAs for Advanced Low Grade NET

Patient with functional tumors need appropriate treatment to control the functional symptoms of diarrhea, flushing, abdominal pain, hypotension, and vasospasm. In addition to symptom control, randomized trials have demonstrated the benefits in slowing disease progression (25, 26). These will be described in more detail below.

Somatostatin receptors are often overexpressed on the surface of low-grade lung NETs (27). SSAs bind to the somatostatin receptor, blocking the release of peptides and amines, and thus help to control symptoms. The two SSAs currently available in clinical practice for advanced low-grade NETs are octreotide and lanreotide. Pasareoide is a third SAA, not yet in clinical use but currently being tested in a lung NETs clinical trial (28).

Octreotide is available as both intermediate acting subcutaneous (SC) and long-acting release (LAR) formulations. A 30-mg IM dose of octeotride-LAR can be repeated every 4 weeks, and increased by 10 mg increments up to an octeotride-LAR dose of 60 mg. At this dose, most receptors are saturated and increasing it beyond has little benefit (29). Lanreotide is administered as a deep SC injection at a dose of 120 mg every 4 weeks (30). Both SSAs are well tolerated, although they may also lead to increased rates of biliary stones so abdominal imaging by ultrasound is recommended every 6 months.

A carcinoid crisis is very rare and can occur when massive amines are released by NET tumors, leading to hypotension and flushing. This can occur in NET patients as a secondary effect to an operative procedure or general anesthesia (31). Most surgeons or interventional radiologists require patients to be pre-medicated with a SSA prior to a procedure to avoid such complications.

In addition to their established role in symptom control, there are now randomized trials demonstrating that SSAs have an antiproliferative effect. The PROMID trial is a randomized

phase III trial in 86 patients with midgut NETs, 40% of which are functional (secretory) tumors (25). Patients were randomized to receive either octreotide LAR 30 mg or placebo (25). Time to progression (TTP), the primary endpoint, was significantly increased with octreotide, at 14.3 months compared to 6 months with placebo (HR = 0.34, $p = 0.000072$). The CLARINET trial is a randomized phase III trial in 204 somatostatin receptor-positive patients with non-functioning (non-secretory) well or moderately differentiated-NETs of the pancreas, midgut or hindgut. Patients were randomized to either lanreotide 120 mg SC or placebo (26). Median progression-free survival (PFS), the primary endpoint, was significantly increased in patients who received the lanreotide, at an estimated 24 months as compared to 18 months for placebo (HR = 0.47, $p < 0.001$). A comparison of the PFS in the placebo arms of PROMID and CLARINET (6 and 18 months, respectively) suggests key differences in patient populations, making cross trial comparison impossible. However, both trials illustrated that SSA treatment in patients with NETs incurs an anti-proliferative effect that improves survival in both non-functional and functional pancreatic and other GI NETs. Neither the PROMID nor CLAIRNET trials included any lung NET patients. Results from the LUNA-randomized trial, which was specifically designed for lung and thymic NETS, were recently presented (28). LUNA-randomized patients to pasireotide, everolimus, or a combination of both agents, and all three arms had a promising progression-free rate at 9 months. LUNA confirms that SSA is a viable treatment option for patients with functional lung NETs as they are effective in controlling symptoms and provide antiproliferative benefits. In some jurisdictions, they are approved only for patients who are symptomatic.

Peptide Receptor Radioligand Therapy

Peptide receptor radioligand therapy specifically delivers a radiolabeled agent to a target, such as somatostatin receptors which often overexpressed on the surface of metastatic lung NETs (27). PRRT using yttrium Y-90 labeled octreotide was first used to treat this disease in the early 1990 and has been delivered and used in many centers for decades, despite the lack of phase III trials confirming benefit.

This has now changed with the results of the phase III NETTER-1 trial (32). This trial enrolled carcinoid patients whose disease was progressing on a standard dose of octreotide 30 mg LAR. Two hundred and thirty patients with grade 1–2 metastatic midgut NETs were randomized to receive either PRRT ^{177}Lu-Dotatate, 7.4 GBq every 8 weeks (×4 administrations), or octreotide LAR 60 mg every 4 weeks. The primary endpoint of PFS was not reached for ^{177}Lu-Dotatate and was 8.4 months in the control group (HR 0.21, $p < 0.0001$). The objective radiographic response rate was 18% with ^{177}Lu-Dotatate and 3% with control ($p = 0.0008$). Overall survival analysis, although preliminary, was positive as well (13 deaths in ^{177}Lu-Dotatate group and 22 in control group; $p = 0.019$). The safety profile of PRRT was favorable. Although this trial was conducted primarily in patients with midgut NET, the results may apply to lung NETs that are receptor-positive by nuclear imaging. A retrospective study which included 89 lung NET's treated with PRRT revealed a response by RECIST in 28% supporting this treatment as an option for pulmonary NETs (33).

Systemic Therapy: m-TOR Inhibition

As lung NETs have shown increased activation of the mammalian targets of the rapamycin (m-TOR) signaling pathway (34), everolimus, an m-TOR inhibitor, is another potential therapy for lung NET patients. The phase III RADIANT-2 trial evaluated everolimus plus octreotide-LAR compared to octreotide-LAR alone in advanced NETs with carcinoid syndrome (35). Although the trial included patients with lung NETs, it did not stratify by site. Patients treated with dual agents everolimus and octeotride-LAR, experienced a non-significant improvement in PFS of 16.4 months as compared to 11.3 months with octeotride-LAR alone ($p = 0.026$). The predetermined PFS significance rate was 0.0246, so with a p value of 0.026, RADIANT-2 missed its mark. In an exploratory subgroup analysis for lung NETs only ($n = 44$), there was a trend toward improved PFS (13.6 months) for dual treatment as compared to 5.6 months for octeotride alone ($p = 0.228$). As RADIANT-2 included only small numbers of patients and was not stratified per site, the trial had to be repeated to test the effect of everolimus without octeotride in a population of patients with non-functional tumors.

The RADIANT-4 trial randomized patients with non-functional NETs of the lung and GI tract to either everolimus or placebo (36). The median PFS was significantly prolonged in the everolimus arm compared to placebo arm (11 months versus 3.9 months, $p < 0.00001$) (see **Figure 2**). These improvements were independent of site of disease origin: lung, GI, or unknown.

The phase III RADIANT-2 trial (comparing everolimus and octeotride with octeotride alone) included functional tumors in both lung and GI, and demonstrated that the combination of everolimus and octreotide was not only safe but complementary. However, as the RADIANT-4 trial (comparing everolimus with placebo) excluded functional tumors, the Health Canada label limits everolimus to be used without octeotride for the treatment of non-functional lung NETS only.

Treatment of NETs Side Effects

Carcinoid heart disease may occur up to 50% of patients with functional tumors (37) and is secondary to serotonin acting on serotonin receptors on the right heart. An echocardiogram can show thickening of the tricuspid valve and surgical management may be needed. The medical management may include diuretics and SSAs to reduce levels of serotonin (38).

Importance of Multidisciplinary Management

As patients with both TC and AC tumors have prolonged survival and treatment spans across many areas such as surgery, nuclear medicine, medical and radiation oncology, a multidisciplinary approach and or team may be in the patients' best interest.

FOLLOW-UP

Patients diagnosed with low-grade lung NETs need to be frequently followed up after surgical resection. For patients with TC NETs, conventional imaging can be carried out at 3 and at 6 months, then on an annual basis. For AC, closer monitoring

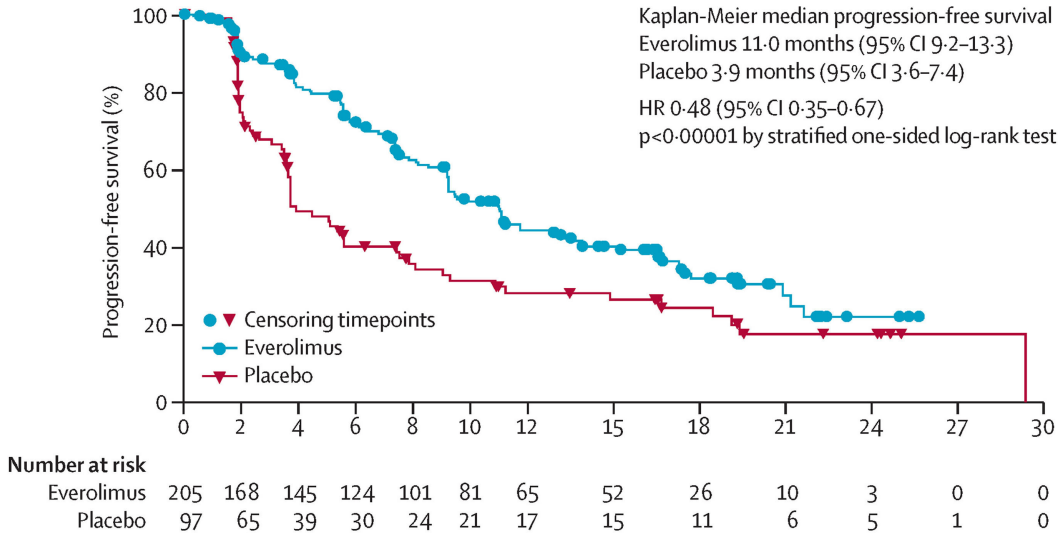

FIGURE 2 | Progression-free survival curve from Radiant 4 trial. PFS curve from the RADIANT 4 trial. Reprinted from Yao et al. (36), Copyright 2016, with permission from Elsevier.

is recommended, first at 3 and 6 months, then continuing at 6-month intervals (3). Clear instructions for the type and interval of follow-up for patients with advanced well-differentiated NETs do not exist (1, 24, 39). Follow-up and imaging needs to be individualized as it is based on the individual baseline status, new symptoms, prior treatment and if change in therapy is contemplated. Chromogranin A measurements can be used to monitor disease progression; however, the frequency and duration of measurement is not articulated. More detailed guidelines are needed to direct follow-up.

CONCLUSION

Lung NETs are a unique tumor entity. As the second most common type of NETs, they deserve attention. This heterogenous group of tumors requires a multimodality team approach for optimal treatment. A pathological review is critical to differentiate between low-grade TC and AC NETs and high-grade tumors, and radiologicial imaging is necessary to visualize the tumor and determine metastatic spread. Treatment with somatostatin receptor analogs octreotide and lanreotide can improve carcinoid symptomology and prolong PFS. Tumors that are receptor avid by octreotide may be treated with PRRT with the goal of improving PFS. Finally, m-TOR inhibitors have demonstrated efficacy toward NETs regardless of functional status. The rarity of the disease limits our knowledge, and there is a need for more trials involving lung NET patients. Until more lung-specific data are available, we will have to extrapolate data from the GI NET studies. We look forward to the global understanding of lung NET's expanding, and the disease finally receiving the attention it deserves.

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BM reviewed the literature and wrote this article. She is responsible for the content.

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REVIEW
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Interventional Analgesic Management of Lung Cancer Pain

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Lung cancer is one of the four most prevalent cancers worldwide. Comprehensive patient care includes not only adherence to clinical guidelines to control and when possible cure the disease but also appropriate symptom control. Pain is one of the most prevalent symptoms in patients diagnosed with lung cancer; it can arise from local invasion of chest structures or metastatic disease invading bones, nerves, or other anatomical structures potentially painful. Pain can also be a consequence of therapeutic approaches like surgery, chemotherapy, or radiotherapy. Conventional medical management of cancer pain includes prescription of opioids and coadjuvants at doses sufficient to control the symptoms without causing severe drug effects. When an adequate pharmacological medical management fails to provide satisfactory analgesia or when it causes limiting side effects, interventional cancer pain techniques may be considered. Interventional pain management is devoted to the use of invasive techniques such as joint injections, nerve blocks and/or neurolysis, neuromodulation, and cement augmentation techniques to provide diagnosis and treatment of pain syndromes resistant to conventional medical management. Advantages of interventional approaches include better analgesic outcomes without experiencing drug-related side effects and potential for opioid reduction thus avoiding central side effects. This review will describe various pain syndromes frequently described in lung cancer patients and those interventional techniques potentially indicated for those cases.

Keywords: lung neoplasms, cancer pain, nerve block, spinal anesthesia, cementoplasty, neurostimulation

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INTRODUCTION

Prevalence of Pain in Patients Diagnosed with Lung Cancer

Cancer and pain are clinical entities closely associated. Recent reviews suggest a prevalence of pain in cancer patients of 51% regardless of the type and stage. This prevalence increases with the type of tumor (head and neck, lung, and breast cancers are the ones with higher prevalence) and with the staging (advanced, metastatic, or terminal) reaching a 66% of cases (1).

Recent therapeutic advances have allowed increased survival rates potentially turning lung cancer into a chronic condition (2). Since pain is present in up to 39% of cases after curative intent, an increased survival could potentially impact this number of patients left with persistent symptoms despite being successfully treated.

Importance of Appropriate Symptom Control in Lung Cancer Patients

Undertreated cancer pain associates both physical and psychological consequences, causing suffering and reduced quality of life. Patients with unrelieved pain associate physical symptoms like insomnia, anorexia, profound fatigue, reduced cognition, and overall reduced vital capacity. Cancer patients presenting with unalleviated pain withdraw from social and familial interactions leading to isolation and psychological distress. Lastly, persistent pain can cause existential and spiritual suffering, which can limit the patient's coping skills (3).

Basic Pharmacological Management to Relieve Cancer Pain

The World Health Organization (WHO) responded to an essential necessity to assess and treat cancer pain by designing in 1986 the WHO Cancer Pain Relief guidelines [updated 10 years later (4)]. Adoption to the three step ladder approach leads to satisfactory cancer pain control in the majority of cases. However, in a significant proportion of patients, appropriate conventional medical management following the WHO guidelines do not warrant satisfactory analgesic control or may provoke limiting drug-related side effects (5). For those cases, interventional cancer pain management may represent a valid option.

Definition of Interventional Pain Medicine

Interventional pain management is a subspecialty of medicine devoted to the use of invasive techniques such as joint injections, nerve blocks and/or neurolysis, neuromodulation, and cement augmentation techniques to provide diagnosis and treatment of pain syndromes unresponsive to conventional medical management.

The basis of interventional pain practice lays on a profound knowledge of the anatomy and particularly the sensory innervation of different anatomical structures. When assessing a cancer pain case, aside from physiopathological considerations, the interventionalist may reflect about what is the anatomical structure that is hurting and which is the nerve supplying sensation to that structure.

As a principle, injections are avoided to be performed in the close vicinity of tumors for several reasons: (1) an increased risk of bleeding caused by abnormal tumor vascular neogenesis; (2) a risk of seeding cancer cells along the needle track, and (3) there is a risk of missing the target if the tumor has distorted the local anatomy. Routinely, nerve blocks are performed at levels where nerves are not damaged but are found proximal to the site of where the pain is coming from.

Several interventional cancer pain procedures have demonstrated effectiveness in relieving drug-resistant cancer pain symptoms (6), yet the evidence is scant. This may explain why interventional procedures have not yet been adopted in clinical guidelines for the management of cancer pain and thus remain optional to teams with trained clinicians on board.

Scope of the Paper

This article aims to review the most common pain syndromes described in patients diagnosed with lung cancer. Treating physicians must be aware that conventional medical management is sufficient to achieve satisfactory pain management in most cases. Readers are encouraged to be familiar with comprehensive medical reviews on basic pharmacological analgesic approaches beyond the principles of the WHO (7, 8). The second part of this review lists the available interventional pain techniques indicated in cases of poor response to conventional medical management. A brief explanation of each technique with its peculiarities and scientific evidences, when available, is presented.

CLINICAL PAIN SYNDROMES IN PATIENTS WITH LUNG CANCER

Pain in the Chest

Chest Wall Pain

Chest wall pain is a severe and disabling symptom. Over a half of lung cancer patients suffer from chest pain at diagnosis (9). Pain is usually ipsilateral to the tumor site and is described as dull, aching, persistent, and poorly localized. Chest pain can be particularly severe and better identified if secondary to a rib metastasis or when the primary tumor involves the chest wall or pleura. The majority of patients suffering from non-small cell lung cancer with chest wall invasion suffer from chest pain (10).

Costopleural Syndrome

It refers to the severe refractory chest pain, often observed in patients diagnosed with pleural mesothelioma. It is caused by tumor invasion of the pleural cavity and thoracic wall, and it is seen often in the early stages of the disease. This chest pain can be pleuritic in nature and also described as a dull and poorly localized pain arising and involving part of the hemi-thorax. It normally develops during the course of the disease thus worsening with disease progression and often becoming challenging to relieve with conventional drug management. Generally, the pain will present with mixed nociceptive and neuropathic pain features as the autonomic, intercostal, and occasionally brachial plexus nervous structures are involved (11).

Rib Bone Metastases

The primary symptom resulting from bone inflammation is pain, which may have a pleuritic component when the parietal pleura is involved. Since lung cancer metastases to bone are predominately lytic, periosteal inflammation and breach is the most common mechanism of pain from bone metastases (12). Additionally, metastases to the ribs often come associated with intercostal nerve damage and thus neuropathic pain. The pain usually is localized in a particular area and is often reported at night or on weight bearing and with deep breathing. Pain is characteristically described as dull in character, constant in presentation, and progressively increasing in severity. At rest, the pain severity may be better controlled, thus patients may describe breakthrough pain related to postures and volitional or involuntary chest movements (13).

Pancoast Tumor

Pancoast tumor is defined as a malignant tumor arising from the lung apex, also referred to as superior sulcus tumor. The tumor usually affects adjacent structures such as ribs, blood vessels, and

nerves (typically the lower nerve roots of the brachial plexus). As a result, patients may present with severe pain, often of neuropathic characteristics radiating toward the ipsilateral upper extremity and accompanied with sympathetic symptoms (like the Horner syndrome) caused by invasion of the cervicothoracic sympathetic ganglion. These manifestations often appear months prior to the diagnosis of the underlining disease (14).

Malignant Brachial Plexopathy

Tumor infiltration of the brachial plexus is commonly seen among patients with lung cancer. It usually affects the lower elements of the nervous plexus but at times it may evolve into a panplexopathy. Presenting symptoms are typically pain at the shoulder and upper extremity associating with weakness, muscle atrophy, and sensory deficits. As the tumor expands and invades adjacent structures, the likelihood of reaching the epidural space becomes substantial (15).

Post-Thoracotomy Pain Syndrome

Between 25 and 60% of patients undergoing thoracic surgery develop persistent postoperative pain following the procedure (16). Post-thoracotomy pain syndrome (PTPS) is defined as pain along the surgical bed lasting more than 2 months post-thoracic resection surgery (17). It may occur after thoracotomy for malignant or non-malignant lesions, it is usually restricted to one or more dermatomes. It is characterized by moderate to severe pain and typically described as numbness, tingling, burning, shooting, and sometimes itchy painful sensations. Sensory loss and allodynia are usually present as well. The exact mechanism for the pathogenesis of PTPS remains unclear and is probably a combination of neuropathic and myofascial pain (MFP) (18). Genetics, age, gender, preoperative stress, and perioperative pain have been identified as predisposition factors for PTPS. The type and extent of surgery are also factors for the development of chronic pain particularly when there is trauma to the intercostal nerves.

Postherpetic Neuralgia

Cancer patients increasingly suffer from acute herpetic neuralgia (19). The Varicella Zoster virus, which remains dormant at the dorsal root ganglion after primary infection, is reactivated under certain circumstances like aging and immunosuppression, causing a skin rash usually restricted to a dermatomal distribution. Upon resolution of the skin lesions, patients develop the commonly known as postherpetic neuralgia. This pain, neuropathic in nature (20), is found most frequently affecting thoracic dermatomes. Pain management for both acute and chronic forms is challenging and relies mostly on pharmacology-based approaches. In severe cases, when conservative treatment fails to provide satisfactory relief of postherpetic neuralgic pain, interventional approaches could be attempted (21).

Bone Pain

Metastatic disease involving the musculoskeletal system is a common problem in oncology patients, occurring in up to 85% of patients diagnosed with breast, prostate, or lung cancer at the time of death (22). Bone metastases indicate a poor prognosis, with patients experiencing a median survival of 3 years or less; however, 5–40% of patients are alive at 5 years, dependent on tumor histology and disease burden (23). Metastatic bone disease leads to complications, such as pain, that can affect the patients' quality of life. Bony metastases are frequent causes of pain among lung cancer patients as a result of pathological fractures, invasion of nearby pleural or visceral organs, involvement of neighbor nerve structures, spinal instability, and/or spinal cord compression. All of these complications are manifested as difficulty in ambulation or immobility and neurologic deficits (24). Pain symptoms arising from bone metastases present with mixed somatic and neuropathic features and are typically confined to a particular anatomical region. Pain often appears during the night and is exacerbated by weight bearing, posture change, or movement, thus with a strong dynamic component. Pain starts over weeks or months with progressive worsening becoming more severe and continuous at rest or with exacerbation triggered by dynamic changes (7). When baseline pain is well controlled but the patient experiences sudden and short lasting crisis of severe pain (also known as breakthrough pain), the case becomes more challenging since the crisis can be unpredictable and the available pharmacological options may be unsatisfactory.

Although thought to be the optimal management, surgery often cannot be offered because of underlying medical conditions, poor functional status, poor bone quality, or presence of multiple bone metastases (25). Currently, the gold standard symptomatic treatment of focal bone pain caused by metastatic disease is external beam radiation therapy. After radiation therapy most patients present with partial or complete pain relief; however, this relief is not achieved immediately but experienced after a considerable amount of time. In over 50% of patients, the pain relief is found temporary, and in 20–30% of cases the pain is not relieved (26, 27). Patients who underwent radiation therapy presenting with localized recurrent pain in the irradiated region are not usually candidates to receive more radiation because the potential toxic impact on non-cancer tissues.

Unfortunately, standard chemotherapy is often ineffective to treat metastatic-related pain. Bisphosphonates and denosumab are agents with proven benefits in decreasing severity of bone-related incidents in patients with metastatic bone disease. They may alleviate cancer-induced bone pain, yet there is insufficient evidence to recommend these therapies solely for pain relief purposes (28).

Myofascial Pain

In approximately 10% of cancer patients, pain is unrelated directly to the disease or treatment and is most often originated in muscles and connective tissues (29). MFP is started to be recognized as one of the most important causes of pain in cancer patients during treatment, at terminal stages, or after curative therapy (30). MFP is a syndrome characterized by regional chronic pain associating multiple myofascial trigger points and fascial constrictions. It can appear in any body part and characteristically features focal point tenderness, reproduction of pain and hardening of the muscle upon trigger point palpation, pseudo-weakness of the involved muscle, referred pain, and restricted range of motion (31). The treatment for MFP includes physical therapies like myofascial

trigger point needling and injections, myofascial release, and stretching exercises (32).

Pain Related to Diagnostic Procedures and Cancer Treatment

Certain diagnostic test and treatments can cause or aggravate pain because they require the patient to maintain an immobile posture like imaging test or radiotherapy (RT). Others cause pain due to their invasiveness such as transthoracic needle biopsy or thoracocentesis (33). These acute pain episodes are described as transient exacerbations of pain typically well managed with conventional analgesic medications. Chemotherapy and RT are treatments frequently associated with deleterious and persistent painful syndromes that are not easily managed.

Chemotherapy-induced painful neuropathy is one of the most common and better studied pain syndromes consequences of cancer therapy. Most of chemotherapy-induced pains are self-limited and can be managed pharmacologically and/or with dose adjustments of the chemotherapeutic regimen. Probably, the better described chronic pain syndrome consequence of cancer treatment is chemotherapy-induced peripheral neuropathic pain (34).

Cancer patients can potentially suffer from RT-related pain both immediately after the treatment and as a late complication. During the acute phase, RT causes pain due to skin or mucosa inflammation or due to the procedure itself. Patients subject to RT for bone metastasis commonly suffer from pain flare-ups in radiated areas (35) and are treated usually with breakthrough analgesics and steroids. At later stages, RT-related pain can be caused by a variety of mechanisms including soft-tissue fibrosis and sclerosis and muscle weakness, such as thoracic pain, shoulder pain, and cervical dystonia (36). Thoracic cancer patients receiving greater dosages may require opioids to treat brachial plexopathy or chest wall pain following radiation (37, 38).

INTERVENTIONAL PROCEDURES

General Principles of Interventional Pain Procedures in Cancer Patients

An interventional pain procedure is typically indicated when (a) the patient has not achieved satisfactory analgesic control despite optimal conventional medical management as suggested by the WHO guidelines or (b) when adequate pain control comes associated with intolerable side effects (39). Additional indications may include (c) favoring analgesic control with opioid sparing techniques or (d) analgesia in patients that are poor candidates to opioid analgesia.

The key to a successful partnership between treating oncologists and interventional pain physicians is communication to sharing the cases, reviewing indications and contraindications, appraising the available scientific evidences, and updating the team about the patient's status and goals of treatment, in summary creating a clinical pathway for these patients.

Overall, interventional pain procedures should be offered to patients before they are too frail to undergo the procedure, thus they should not be considered an option in isolation but rather a part of an analgesic strategy. Patients should be able to consent and they should, along with their caregivers and the treating team, understand the procedure, the expected benefits and side effects, and potential complications (40).

A bidirectional communication between teams allows for earlier identification of candidates, thus preventing drug escalation and challenging cases. Additionally, the team must be updated on those fluctuations in the patient's status that may potentially change the indication (risk of bleeding, infection, respiratory insufficiency). Following a successful procedure, the treating team must be vigilant for potential changes in analgesic requirements; ideally, opioids must be decreased to prevent central toxicity.

Peripheral Nerve Injections

When cancer pain is experienced in the vicinity of an identified peripheral nerve, a temporary interruption of the pain transmission can be an effective method to control neuropathic pain. The term "nerve block" describes any procedure that utilizes a needle to deliver a local anesthetic or an ablative agent (phenol, alcohol, glycerin, etc.) for analgesic purposes. A block can have both diagnostic and therapeutic values. In order to identify the anatomical area and/or the afferent pathway involved in originating/conveying the pain sensation, a diagnostic nerve block may be effective. A prognostic block allows the decision to indicate a more complex and permanent procedure usually with neurolytic purposes. Diagnostic and prognostic blocks consist in injecting a small volume of a local anesthetic agent onto a nerve. The duration of the effect is usually short, depending on the potency of the local anesthetic agent injected. Patients are considered responders when most of their pain is significantly relieved during the following hours after the procedure. Neurolysis implies the focal destruction of nervous tissue as by the use of chemicals or thermal methods to disrupt nerve transmission. The classical targets for nerve blocks or neurolysis are sympathetic nerves or nerves with predominant sensory component. It is very important to always preserve motor and sphincter functions and when not possible, balance potential benefits against side effects before performing a neurolysis (40).

Among lung cancer pain patients, the most frequently targeted nerve structures are obviously located inside the thorax. As a general principle, the interruption of nociception must be attempted at a proximal site to the pain generator (41). Patients with thoracic chest wall pain may benefit from procedures targeting (from distal to proximal) the intercostal nerve, the posterior root of the thoracic radicular nerve, and the paravertebral space.

Intercostal Nerve Blocks and Neurolysis

It consists in injecting the neural structure located underneath each rib. This is a simple procedure that can be performed at the patient's bed site not requiring advanced imaging guidance systems. Because the main complication is the pleural puncture and subsequent pneumothorax, it is suggested direct needle placement with ultrasonography. The injection of an intercostal nerve provides loss of sensation distal to the point of injection following the trajectory of the nerve toward the anterior chest wall. The largest series reporting intercostal nerve procedures for chest pain management include 25 patients with metastatic rib lesion undergoing intercostal blocks. In this study, 80% of the

patients noted optimal local pain control and 56% experienced reduction in analgesic use after the procedure (42).

When a temporary intercostal nerve block provides adequate analgesia but limited to a short period of time, it may be reasonable to repeat the block adding a coadjuvant (43) or opting for a more permanent relief by damaging the nerve with a chemical neurolysis with phenol (44), a thermal neurolysis with heat using radio-frequency (RF) (45, 46) or freezing the nerve with cryoneurolysis (47).

Thoracic Nerve Root and Paravertebral Procedures

This consists of injecting the thoracic nerve roots at their exit from the spinal canal. These nerve roots can be injected individually (selective thoracic nerve block/neurolysis) or several at the same time by placing a needle at the thoracic paravertebral space. The selective nerve root block technique has been suggested as a proximal alternative site of injection in cases of post-thoracotomy pain (48). Authors described the use of pulsed RF, which delivers electricity to the dorsal root ganglion without causing nerve tissue damage. Results favored this technique over treatment of intercostal nerves and over conventional pharmacological management.

The injection of neurolytic agents into the thoracic paravertebral space presents advantages since one single injection may reach several thoracic nerve roots, thus involving a larger anatomical area. Neurolytic injection of the thoracic paravertebral space has been also described in cases of lung cancer with chest wall pain. In a small case series, injection of phenol in the vicinity of thoracic nerve roots provided satisfactory yet short lasting chest pain relief (49).

Brachial Plexus Procedures

Pain to the upper limb caused by lung cancer has been reported in cases of Pancoast tumors. The involvement of the sympathetic chain and the brachial plexus may cause neuropathic symptoms radiated toward the arm and the hand. Anesthetic techniques targeting the brachial plexus may include intermittent or continuous injection of local anesthetics (50) and neurolysis with phenol (51).

Spinal Injections

Drugs injected into the spinal canal act through direct interaction with spine receptors thus achieving more potent analgesic effects with minimal doses. Additionally, the effect may be restricted to few dermatomes, hence sparing the possible side effects to a targeted anatomical area. The two modalities of intraspinal procedures available to manage drug-resistant pain secondary to lung cancer are continuous spinal drug delivery or spinal neurolytic procedures.

Continuous Drug Delivery

The basics of neuraxial analgesia consist of a catheter inserted into the spinal canal and a pump to administering medication in a continuous fashion. Opioids alone or combined with local anesthetics and other substances, such as clonidine or ziconotide, can be administered *via* epidural or intrathecal route to achieve neuraxial analgesia. Neuraxial analgesia allows the use of lower dosages of opioids, hence minimizing systemic side effects. As an example of the potency of intraspinal opioids: 300 mg PO morphine/day = 100 mg IV morphine/day = 10 mg epidural morphine/day = 1 mg intrathecal morphine/day (52).

Patient selection for spinal drug delivery includes choice of the anatomical space to deliver the drug (epidural vs. intrathecal) and choice of the administration mode (external infusion with syringe driver/pump vs. implanted reservoir with automated pump). The selection of the system is determined by factors like survival expectancy, body habit, patient admitted or ambulatory, financial resources, and/or expertise of the treating team (53).

For those patients with reduced life expectancy (<3 months), the neuraxial method of choice remains the epidural route. The main advantages of epidural opioid delivery are reduced risk of pharmacological complications, theoretical dermatomal analgesia achieved when combined with local anesthetics, decreased risk of post-dural puncture headache, and potentially, more familiarity within other specialties. On the other side, continuous epidural analgesia requires infusion of larger volumes of medication and a higher risk of catheter-related complications since it is not normally anchored or internally implanted (54).

Intrathecal drug delivery has been extensively described in the literature for the management of drug-resistant cancer pain syndromes. Available guidelines can be found to identifying the best candidates for this analgesic therapy (55). Advantages of intrathecal systems include better pain control with lower dosages, lower risk of catheter-related complications, and totally implanted systems thus, reduced rate of infections (56). Direct comparison of intrathecal drug delivery vs. conventional medical management favors the experimental arm in quality of analgesia, profile of side effects, and survival rates (57).

Intraspinal Neurolysis

Pain relief in terminal cancer cases achieved by means of injection of a neurolytic agent has been extensively reported (58). The key for a successful neurolytic procedure is balancing the expected analgesia and the potential nerve deficits associated.

These neurolytic procedures seem to be restricted to the latest option in the interventional cancer pain armamentarium (59) because they carry inevitable nerve deficits and because intraspinal drug delivery systems have become more available.

For lung cancer pain patients, the options include epidural (60) or intrathecal (58) injections of neurolytic agents such as alcohol or phenol. Because these neurolytic approaches are usually left as a last resort in the management of severe and drug-resistant cancer pain in terminally ill patients, the available evidences are only restricted to case series. From those evidences, it can be inferred that intraspinal neurolysis is a complex analgesic technique providing satisfactory analgesia but carrying a high potential for neurological deficits that must be weighted before performing the technique.

Electrical Neuromodulation Techniques

Electrical neuromodulation is a technique by which an electrode that is placed next to a nervous structure stimulates selective small nerve fibers, which in turn inhibit nociception through complex physiological mechanism. Neurostimulation can be achieved *via* placement of electrodes under the skin (subcutaneous/field

stimulation), close to peripheral nerves or to spinal nerve roots (peripheral nerve/DRG stimulation), inside the epidural space close to the ascending dorsal columns [spinal cord stimulation (SCS)], or inside the brain (deep brain stimulation). The efficacy, safety, and cost-effectiveness of neurostimulation techniques in the management of chronic pain of non-cancer origin have been sufficiently demonstrated in the last decade (61).

Conversely, because of its cost, the indication for neurostimulation in cancer pain patients is usually restricted to those cases when cancer has been successfully cured but patients are left with painful permanent consequences. There are no randomized trials addressing the benefits of SCS for cancer-related pain (62). Indications for SCS included chest wall pain (63) or chemotherapy-induced neuropathy (64), for example.

Neurosurgical Procedures

Historically, destructive procedures for cancer pain were the main line of treatment therapy in the previous two centuries; however, the availability of opioids, coadjuvants, and newer anesthetic techniques has essentially replaced such procedures (65). The indication of these techniques is restricted to anecdotal reports nowadays.

Percutaneous Cervical Cordotomy

This procedure consists of creating a lesion to the lateral spinothalamic tract. The purpose is to disrupt the pain transmission carried from the contralateral side, as the spinothalamic tract carries pain, temperature, and some tactile information. The lesion is usually done percutaneously through the C1–C2 level (66). This procedure has been shown to be most effective in patients with confined unilateral nociceptive pain, such as in the case of mesothelioma (67) or other malignant invasions of the chest wall.

The complications involved are substantial with 3% mortality, up to 11% motor weakness, and others such as respiratory, postcordotomy hypotension, bladder dysfunction, sexual dysfunction, and dysethesia (68).

Intracerebroventricular Opioid Delivery

Intracerebroventricular opioids are useful for intractable pain when other simpler techniques have failed. It consists of delivering opioids *via* a ventricular catheter attached to a subcutaneous storage (69).

Cingulotomy

This procedure refers to lesioning of the anterior cingulate cortex, which is a component of the limbic system that affects a wide array of functions involving behavior, emotions, and others. It is indicated in cancer pain patients with significant emotional distress. A case report from 2014 described bilateral anterior cingulotomy effectively relieved both pain and dyspnea for a patient with malignant mesothelioma (70).

Procedures for Localized Painful Bone Metastases

Cement Augmentation Techniques

The diagnosis and management of clinically relevant bone fractures are based on a clinical examination indicating pain localized to the level of the fracture along with confirmatory imaging studies (71). Cementoplasty refers to a technique where cement is delivered percutaneously to the spinal bones or other weight-bearing bones for stability purposes. It broadly includes procedures like vertebroplasty, kyphoplasty, sacroplasty, and osteoplasty (72).

Vertebroplasty and Kyphoplasty

Untreated vertebral compression fractures can result in a spinal cord compression with irreversible neurological symptoms and paraplegia (73, 74). Pain severity or the medications used to control pain can cause considerable functional impairment, significantly limiting patients' mobility and ability to carry out day-to-day activities (75).

Vertebral augmentation techniques—vertebroplasty or kyphoplasty—are often done at an outpatient setting, at which image-guided injection of bone cement (methyl methacrylate) is injected into a collapsed vertebral body. This approach may be valuable for patients when pain is unresponsive to conservative treatments and no other options like RT are available, and for patients whose pain causes poor functional status thus limiting their life expectancy. Compared to non-surgical management, kyphoplasty was found to be an effective and safe treatment that rapidly reduced pain and improved function. A recently published systematic review including 111 clinical reports with 4,235 patients evaluated vertebral augmentation (vertebroplasty or kyphoplasty) for cancer-related vertebral compression fractures. Researchers found these two procedures to significantly and rapidly reduced pain intensity as well as significantly decrease the need for opioid pain medication, and functional disabilities related to back and neck pain (76). Beyond the contraindications mentioned above for invasive procedures, additional contraindications for these procedures include epidural disease, a fracture with new neurological impairment attributed to it, and fractured vertebra with a burst element penetrating the spinal canal (77).

Osteoplasty

It is the percutaneous injection of bone cement into painful bone metastases at extraspinal regions. Two retrospective studies, comprising a total of 76 patients, evaluated osteoplasty under CT or fluoroscopy found this technique effective and valuable as a method for reduction of pain and improvement of patients' quality of life (78, 79). In particular, for patients with lung cancer metastatic to the bones, a large retrospective series demonstrated vertebroplasty and cementoplasty to be effective and safe as a means to decrease pain and enhance mobility in patients with vertebral and extra spinal metastases (80).

RF Ablation and Cryoablation of Painful Bone Metastases

Several new ablation treatment strategies have been reported to be effective over the last two decades. These treatments consist of image-guided (CT, fluoroscopy) destruction of soft tissues or bone tumors (either primary or metastatic). Among these techniques, RF ablation is the most studied and frequently used modality, but cryoablation, laser ablation, and microwave ablation have all been

also reported. The pain treated with these techniques should be limited to one or two sites, and patients with numerous painful tumors should be treated systemically. Ablative therapy tends to be most effective in soft-tissue tumors and bone tumors with dominant osteolytic component.

RF Ablation

High-frequency, alternating current is passed to an adjacent tissue *via* a needle electrode and results in heating of the tissue, denaturation of proteins, and cell death. It is usually performed with local anesthesia or under moderate sedation. Careful consideration of the regional anatomy should be carefully assessed and considered. As anatomy is frequently disturbed in these patients, the ablation zone should not be extended to less than 1 cm of critical structures such as the bowel, urinary bladder, or spine (81). Two multicenter clinical trials conducted on a large set of patients with a wide range of solid malignancies (the majority were lung, colon, and renal metastases) confirmed that RF ablation as a means to decrease pain due to bone metastatic disease is safe and well tolerated by patients (82, 83).

Percutaneous Cryoablation

This method uses room temperature-pressurized argon and helium gasses for tissue freezing and warming, respectively. The cryoprobes are placed into the tumor using CT/fluoroscopy for tumors within bones or deep in the pelvis. The passage of gas through the probe results in rapid cooling that reaches −100°C within a few seconds, forming a low-attenuation ice ball that is readily visible with the CT imaging (or MRI). Tissue destruction is complete at −20 to −40°C, approximately 3- to 5-mm deep to the visible edge of the ice ball. A synchronous use of multiple cryoprobes can be done to allow for a complete coverage of the tumor and its immediate surroundings. The method is less studied than RFA, but case series and reports have established its efficacy. Its main advantage over RF is its ability to be readily visualized intra-procedurally with intermittent non-enhanced CT or MR imaging (84).

INTEGRATING INTERVENTIONAL PAIN TREATMENT INTO AN ONCOLOGY PRACTICE

Multidisciplinary symptom management results in positive outcomes described in terms of significant relief of cancer pain and other cancer-related symptoms like fatigue, depression, anxiety, and drowsiness. It also impacts positively on patients' disability and eventually on opioid reduction (85).

Traditionally, interventional treatments have been regarded as a last resort to relieving cancer pain in those patients where conventional drug therapies have failed. The term "fourth step of the WHO ladder" was coined with views of placing interventional cancer pain within the well-known WHO three steps clinical algorithm (86). Major efforts are being conducted to prove that interventional pain management indicated at early stages of the disease or before the pain becomes unmanageable with drugs may be a better option. Potential benefits of early blocks include better health status and enhanced performance to face the disease and its treatment and avoiding or delaying opioid escalation to manage pain. Rather than a fourth step of the ladder, interventional cancer pain approaches should be regarded as a handrail accompanying all the three steps of the WHO ladder.

Interaction between different clinical specialties may be challenging if their mutual approaches are poorly understood or perceived ineffective and/or dangerous. In the case of interventional cancer pain management, this is more challenging since the outcomes can seldom be presented in terms of evidence-based medicine.

Oncologist must identify those patients whose pain is inadequately controlled and ask themselves if an interventional approach may be indicated. With progressive learning, the indications and contraindications become clearer, and the cases are referred in a timelier and more appropriate fashion. Interventional pain clinicians must identify, in turn, the potential implications of their techniques on the patient's status like, for example, the risk of bleeding when anticoagulated or receiving chemotherapy, the anatomical alterations a tumor may cause when attempting to target a specific nerve structure, or the changes in analgesic therapy necessary to apply after a successful nerve block or neurolysis. A fluid and bidirectional communication is key to integrate successful analgesic strategies into the oncology care.

SUMMARY

Interventional cancer pain approaches can provide valuable help to treating oncologist in cases of lung cancer with pain that is not satisfactorily relieved with conventional medical management. The indications and contraindications, the goals of treatment, the limitation of the technique, and the post-procedure care are necessary elements to be discussed between clinicians involved and the patient and their caregivers. Because the available scientific evidences are sparse, at present, interventional cancer pain remains an optional alternative rather than a natural indication. Only those teams integrating a specialist in interventional cancer pain may offer these options to selected cases presenting with challenging cancer pain syndromes.

AUTHOR CONTRIBUTIONS

JP has collected the contribution from the other co-signing authors, edited and formatted the text, and prepared the final manuscript for submission. UH and ME contributed to the preparation of the manuscript and collected data from the medical literature to prepare the review.

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