Non-small cell lung cancer targetable mutations: present and future

Peter Vu, Sandip Pravin Patel

Division of Hematology and Oncology, Department of Medicine, UC San Diego Moores Cancer Center, La Jolla, CA, USA

Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Peter Vu, MD. Division of Hematology and Oncology, Department of Medicine, UC San Diego Moores Cancer Center, 3855 Health Sciences Drive, MC #0829, La Jolla, CA 92093-0658, USA. Email: p0vu@ucsd.edu.

Abstract: Rapid advances in the utility of molecular testing to identify common and uncommon driver mutations as well as the expeditious rate of drug development have intensified the need to obtain multiplex next generation sequencing from tissue and/or blood at the time of diagnosis in order to determine the most appropriate treatment for patients with advanced lung cancers. In this review, we discuss the currently available investigational targeted therapies for the treatment of sensitizing epidermal growth factor receptor (*EGFR*) mutations, anaplastic lymphoma kinase (*ALK*) fusions, ROS proto-oncogene 1 (*ROS1*) fusions, B-Raf proto-oncogene (*BRAF*) V600E mutations, and neurotrophin receptor tyrosine kinase (*NTRK*) fusions. In addition, we have highlighted promising investigational therapies aimed at high level mesenchymal-to-epithelial transition factor (*MET*) amplifications or exon 14 skipping mutations, *EGFR* or human epidermal growth factor receptor 2 (*HER2*) exon 20 insertion mutations, rearranged during transfection (*RET*) fusions, and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) G12C mutations that lend support towards routine testing for these driver mutations as well.

Keywords: Lung cancer; targeted therapy; precision medicine; acquired resistance; next generation sequencing (NGS)

Introduction

Over the past 10 years, non-small cell lung cancer (NSCLC) has become the archetype for precision cancer medicine based on the identification of an increasing number of oncogenic driver mutations and improved survival associated with matched targeted therapy (1,2). In 2013, the molecular testing guidelines published by the College of American Pathologists/International Association for the Study of Lung Cancer/Association of Molecular Pathology recommended testing only for epidermal growth factor receptor (*EGFR*; also known as *ERBB1*) mutations (17%) using polymerase chain reaction and anaplastic lymphoma kinase (*ALK*) fusions (7%) using fluorescence *in situ* hybridization (3). Since then, the ability to identify several hundred gene alterations in one tissue sample

using commercially available DNA-based next generation sequencing (NGS) coupled with the rapid development of novel and effective tyrosine kinase inhibitors (TKI) has led to the identification of many additional actionable driver mutations, such as ROS proto-oncogene 1 (*ROS1*) fusions (2%), B-Raf proto-oncogene (*BRAF*) mutations (2%), neurotrophin receptor tyrosine kinase (*NTRK*) fusions (1%), *EGFR* and human epidermal growth factor receptor (*HER2*; also known as *ERBB2*) exon 20 insertion mutations (3%), mesenchymal-to-epithelial transition factor (*MET*) amplifications or exon 14 skipping mutations (2%), rearranged during transfection (*RET*) proto-oncogene rearrangements (1%), and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations (25%). In all, over half of advanced lung adenocarcinomas may harbor a targetable driver mutation using either currently approved

and/or investigational targeted therapies, with additional predictive genomic and proteomic biomarkers expected in the near future (4-6). A growing body of evidence suggests that the most effective and economical way to test for these alterations is to utilize a comprehensive NGS panel rather than other approaches that would require larger tissue samples and may need repeat biopsies (7).

EGFR

Sensitizing *EGFR* mutations occur in exon 19 (e.g., variable deletions of at least three amino acid residues) or exon 21 (e.g., L858R point mutation) and confer sensitivity to available EGFR TKIs such as gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib. In addition, approximately 60% of patients treated with first- or second-generation EGFR TKIs develop a secondary T790M gatekeeper mutation in exon 20 that leads to acquired resistance to EGFR inhibitors (8,9). Osimertinib is a third-generation covalent EGFR TKI that is highly selective for *EGFR* mutations, including the T790M point mutation, with excellent central nervous system (CNS) penetration and is relatively wild type EGFR sparing (10). As a result, osimertinib quickly became the standard second-line treatment in *EGFR* mutated lung cancer based on results from the AURA studies that demonstrated superior progression-free survival (PFS) and fewer adverse events (AE) compared to chemotherapy in patients found to have a T790M resistance mutation (10). In the phase III FLAURA trial, osimertinib was compared with gefitinib or erlotinib in the front-line setting for patients with sensitizing *EGFR* mutations and demonstrated superior PFS of 18.9 *vs.* 10.2 months (hazard ratio, 0.46; P<0.001). Overall survival (OS) data for FLAURA has not been formally presented yet, but was positive based on a recent pharmaceutical press release (11). Post-progression analyses demonstrated superior PFS2 (defined as time from randomization to progression on second-line therapy) for patients who received front-line osimertinib compared to a sequential strategy with gefitinib or erlotinib front-line followed by osimertinib in the second-line (PFS2; not reached *vs.* 20.0 months, respectively) (12). Together, these studies solidified the use of osimertinib as standard of care for patients who harbor sensitizing *EGFR* or T790M gatekeeper mutations. Of note, patients with uncommon *EGFR* mutations (e.g., exon 20 insertions, exon 18 p.E709X and p.G719X, exon 21 p.L861Q) were excluded from these landmark clinical trials and therefore the optimal treatment

strategy using chemotherapy only, chemoimmunotherapy, or targeted therapy for these patients is still under investigation (13-15).

Acquired resistance

Mechanisms of acquired resistance to osimertinib are extremely diverse and may even occur simultaneously, making the search for therapeutic strategies after progression on osimertinib challenging. The most commonly reported mechanisms involve altered drug binding caused by a secondary C797S (7%) or G724S point mutations or increased reliance on alternative signaling pathways mediated by a high level *MET* amplification (15–20%), *HER2* amplification (2%), *BRAF* and *KRAS* mutations (3%), *PIK3CA* mutations (7%), or histologic transformation to small cell carcinoma (16-20). However, the majority of resistance mechanisms are currently unknown (67%) (20) and therefore there has been renewed interest in combining EGFR TKIs with chemotherapy or a vascular endothelial growth factor (VEGF) inhibitors to potentially delay the onset of resistance. Two recent phase III trials have demonstrated improvements in PFS when a first-generation EGFR TKI was given with chemotherapy or a VEGF inhibitor compared to EGFR TKI monotherapy (21,22), but unfortunately these studies excluded patients with brain metastases and were designed prior to the adoption of osimertinib as first-line therapy and therefore are not considered practice changing at this time. There is an ongoing phase I/II trial of osimertinib with or without bevacizumab (NCT02803203) as well as a randomized, phase III trial of osimertinib with or without platinum/pemetrexed chemotherapy (FLAURA2; NCT04035486).

Due to the heterogeneity of osimertinib resistance mechanisms, there is currently no post-progression standard of care treatment option aside from chemotherapy and these patients should be encouraged to participate in clinical trials. Repeat tissue and/or blood-based NGS is recommended at the time of progression to identify potentially actionable resistance mutations or pathways as mentioned above or transformation to small cell carcinoma. For example, the ORCHARD clinical trial (NCT03944772) utilizes a modular design with multiple cohorts based on molecular biomarkers identified after progression on osimertinib (23) and will begin recruitment soon. There is also emerging data to suggest that patients may respond to the addition of a second-generation irreversible EGFR inhibitors such as afatinib when a specific secondary point

mutation is found (e.g., C797S in trans, L718V/Q, G724S), but this is admittedly uncommon and only effective in the absence of a T790M mutation (17,18,24-26).

Other promising treatments for *EGFR* resistance target alternative signaling pathways. For example, in a phase I dose-escalation study of an EGFR-cMET bispecific antibody (JNJ-372), responses were observed in several EGFR TKI resistant populations including those with exon 18 G719A mutations, C797S mutations, *MET* amplifications, and exon 20 insertions with low rates of grade 3 or higher AEs. JNJ-372 may be effective because it has the ability to inhibit both *EGFR* and *MET* signaling as well as initiate receptor degradation and antibody dependent cellular cytotoxicity (27). In addition, the TATTON and SAVANNAH trials are evaluating the addition of a selective MET inhibitor, savolitinib, to osimertinib in patients who progressed on prior EGFR TKIs. Interim results from the cohort of patients with T790M-negative progression after first- or second-generation EGFR inhibitors demonstrated an objective response rate (ORR) of 52% and an ORR of 25% among patients who had progressed on a third-generation EGFR inhibitor (28). There are also several case reports of acquired *RET* fusions that are highly responsive to selective *RET* inhibitors currently in development (see section below on *RET*) (29). Also, an early trial of a *HER3* targeted antibody drug conjugate linked to a topoisomerase inhibitor (U3-1402) demonstrated both tolerable safety and antitumor activity in an *EGFR*-resistant cohort of 23 patients (16). Another approach to address mutations that alter drug binding include the ongoing development of allosteric *EGFR* inhibitors that do not depend on covalent interactions within the ATP-site of the kinase (30,31).

Case example

A woman in her 60's who never smoked was found to have a 5.7-cm left upper lobe mass with mediastinal lymphadenopathy and a malignant pleural effusion. A core biopsy of the left upper lobe mass was performed that was consistent with lung adenocarcinoma and *EGFR* mutation testing by polymerase chain reaction revealed an exon 19 deletion. She was started on erlotinib 150 mg daily and developed a partial response (PR) to therapy. She then underwent stereotactic body radiotherapy as consolidation for a residual 2.1-cm left upper lobe mass. She continued on erlotinib for a total of 22 months until there was clear evidence of radiographic progression in mediastinal lymph nodes, but without evidence of new lesions outside

of the thorax. Blood-based NGS was performed at the time of progression and revealed an *EGFR* T790M mutation (allele fraction 4.5%). She was then started on osimertinib. Eventually she developed enlarging subcarinal lymphadenopathy and right hilar adenopathy after 16 months of therapy, consistent with disease progression. A repeat blood-based NGS test was performed that showed a new *BRAF* V600E mutation (allele fraction 0.4%), loss of the T790M clone, and persistence of the exon 19 deletion (allele fraction 2.2%). She was initially offered enrollment on a clinical trial for patients who have progressed after treatment with osimertinib but was ineligible due to chronic kidney disease. Instead, we opted to continue osimertinib given persistence of the original exon 19 driver mutation and add combined BRAF/MEK inhibition with dabrafenib and trametinib. On triplet therapy, her disease has remained stable for over 9 months with no new lesions and treatment is ongoing.

This case illustrates several key points: (I) *BRAF* is a potential bypass signaling pathway for osimertinib resistant *EGFR*-mutated tumor clones (32); (II) *BRAF* V600E is an actionable driver mutation in lung cancer that can be effectively targeted with dabrafenib and trametinib; (III) Triplet therapy with osimertinib, dabrafenib, and trametinib for concomitant *BRAF* and *EGFR* sensitizing mutations is a tolerable combination (33,34); (IV) serial NGS obtained at the time of disease progression can be informative in patients with oncogene driven lung cancer. Of note, repeat tissue biopsies should be performed at the time of progression in *EGFR* mutated lung cancer to evaluate for small cell transformation, however, in this case we identified a bypass pathway (*BRAF* V600E) on liquid biopsy that revealed the mechanism of resistance and therefore cancelled the plan for a tissue biopsy.

ALK

Frontline

Constitutive activation of *ALK* in lung adenocarcinomas is caused by a chromosomal rearrangement that generates a fusion protein, most commonly between echinoderm microtubule-associated protein-like 4 (EML4) and *ALK*, that leads to cellular proliferation and invasion through interactions with downstream JAK/STAT, PI3K/AKT, and MEK/ERK signaling pathways (35). At the present time, there are five highly active ALK TKIs that are commercially available (i.e., crizotinib, ceritinib, alectinib,

brigatinib, and lorlatinib) with several others in clinical development. Crizotinib was the first ALK inhibitor to demonstrate superiority over chemotherapy in untreated patients in terms of PFS and ORR, however, intracranial relapse often developed within 12 months of therapy (36). Ceritinib is a second-generation ALK TKI that is distinguished from crizotinib by its activity against acquired crizotinib resistance mutations (e.g., Leu1196Met, Gly1269Ala, Ile1171Thr, and Ser1206Tyr) and improved CNS activity. Gastrointestinal (GI) toxicity was a common AE in ceritinib clinical trials, however, this is ameliorated by a lower 450 mg daily dose when taken with food (37). Since the publication of the ALEX trial in 2017, alectinib became the standard front-line therapy for the treatment of *ALK* driven lung cancer due to its potency, more favorable AE profile, efficacy against crizotinib resistance mutations, and excellent CNS penetration (38,39). Brigatinib, a second-generation ALK inhibitor, became another off-label option for patients with untreated *ALK* rearranged NSCLC in 2018 based on results from the ALTA-1L trial that compared brigatinib to crizotinib (40). Unfortunately, there is no comparative data between second-generation ALK TKIs (e.g., alectinib, brigatinib, ceritinib) and the choice of therapy is dictated by drug availability, AE profile, as well as systemic and CNS activity. In addition, results from two additional clinical trials looking at the efficacy of ensartinib compared to crizotinib and lorlatinib compared to crizotinib in ALK inhibitor-naïve patients are expected in the next few years. At this time, lorlatinib is not approved for in the United States in the frontline setting.

Refractory

Regardless of initial therapy, all patients eventually develop treatment resistance and clinical progression. Resistance mutations within the *ALK* domain are more common after treatment with second-generation ALK inhibitors (>50%) versus crizotinib (20–30%) (35,41) and compound *ALK* mutations (more than one on the same allele) can occur after sequential treatment with first-, second-, and third-generation ALK inhibitors in around 12.5% of patients (41,42). The most common resistance mutation following treatment with second-generation ALK TKIs is the G1202R solvent front mutation, which has been estimated to occur in approximately 20% of patients following treatment with ceritinib, 30% following alectinib, and 40% following brigatinib (41). Importantly, many *EML4-ALK* variants have been described and lorlatinib appears

to be the most potent ALK inhibitor with sensitivity for most acquired resistance mutations (43). Among patients with a G1202R mutation, lorlatinib has demonstrated an ORR of 57% and a median PFS of 8.2 months (43). In addition, lorlatinib has excellent intracranial activity with an ORR of up to 87% (44). Lastly, in order to clarify the optimal sequence of therapy and to determine the most efficacious treatment for the most common *ALK* resistance mutations, the National Cancer Institute devised the ALK Master Protocol (NCT03737994) which will assign patients with G1202, C1156Y, I1171, L1196, V1180, F1174, or compound mutations to treatments with either lorlatinib, ceritinib, alectinib, brigatinib, ensartinib, crizotinib, or chemotherapy.

ROS1

ROS1 rearrangements are associated with a younger patient population, rare or never-smoking history, as well as a lower incidence of brain metastases compared to *ALK* fusion positive NSCLC (45,46). *ROS1* and *ALK* share a significant amount of amino acid sequence homology within the ATP-binding site and therefore many ALK TKIs are active in *ROS1* rearranged lung cancer, with the exception of alectinib. Crizotinib has been the most well-studied ALK/ROS1 inhibitor and has demonstrated an ORR of 72% with a median PFS of 19.3 months and duration of response (DOR) of 24.7 months among patients previously treated with one or more prior lines of chemotherapy (47,48). The 4-year OS rate was 51% in this cohort and is Food and Drug Administration (FDA) approved for *ROS1*-positive NSCLC (47). Other TKIs that have been studied primarily in the treatment-naïve setting include ceritinib and the NTRK/ROS1 inhibitor, entrectinib. In a single arm trial with ceritinib, ORR was 62% with a median PFS was 19.3 months, which is similar to results seen with crizotinib (49). However, based on data in *ALK*-positive patients and responses seen in 5 of 8 patients in the ceritinib/ROS1 trial, ceritinib may have improved CNS activity compared to crizotinib (49). Recently, entrectinib was approved by the FDA for both *ROS1* and *NTRK* positive patients based on an analysis of three single-arm trials (ALKA, STARTRK-1, STARTRK-2). A total of 51 *ROS1* positive patients with NSCLC were included and the ORR was 78% with a DOR lasting at least 12 months in 55% of patients (50). Entrectinib is CNS penetrant with preliminary results showing an intracranial ORR of 55% and a median PFS of 13.6 months (45,51).

Most oncology guidelines recommend starting treatment

with crizotinib (although this may change after entrectinib was FDA-approved), but inevitably resistance will develop. G2032R (analogous to the solvent front *ALK* G1202R resistance mutation) has been identified as the most common resistance mutation within the *ROS1* domain, occurring in around 41% of cases (52,53). Data in the post-crizotinib setting is relatively sparse, however, the first-in-human study of lorlatinib enrolled both *ALK* and *ROS1*-positive patients. In a *ROS1* expansion cohort that included 34 of 47 patients previously treated with crizotinib, objective responses were seen in 26.5% of patients with a median PFS of 8.5 months (54). In addition, all 4 patients with the G2032R resistance mutation maintained stable disease on lorlatinib for up to 9.6 months and 3 patients with a different *ROS1* mutation achieved a PR to therapy (53). These data suggest that lorlatinib has some activity in patients who have progressed after crizotinib or other ROS1 TKIs. Another promising agent is the next-generation ROS1/TRK/ALK inhibitor repotrectinib, which was designed specifically to overcome the solvent front G2032R resistance mutation. Although the study cohort was small (n=11), when repotrectinib was administered to ROS1 TKI naïve patients in the TRIDENT-1 study, the ORR was 82% (9/11 patients) and CNS response was 100% (3/3 patients). Likewise, in patients who received one or more prior ROS1 targeted therapies, confirmed ORR was 39% (7/18 patients) with a clinical benefit rate of 78% (14/18 patients). CNS responses were seen in 75% (3/4 patients). Of note, higher doses of repotrectinib may be more efficacious in patients who have received prior ROS1 targeted therapy. Dizziness and dysgeusia were the most common AEs, which is considered a class effect from TRK inhibition. The phase 2 portion of the study will begin enrollment later this year.

BRAF

BRAF alterations that lead to constitutive downstream activation of the MAPK pathway can occur as a *de novo* driver mutation in lung cancer or as a mechanism of acquired resistance to targeted therapies in oncogene driven lung cancers (34). *BRAF* fusions, though rare (estimated to account for 4% of *BRAF* alterations in NSCLC), tend to be acquired as an alternative downstream signaling pathway in patients who progressed on EGFR TKIs (32). About half of all *BRAF* mutations in lung cancer occur as a result of a V600E amino acid substitution in exon 15, which is more common among non-smokers but can also occur in patients with a smoking history (55), and can be effectively

targeted with BRAF-MEK inhibitors. The most well studied treatment combination has been dabrafenib plus trametinib; when given as front-line therapy, the ORR was 64% with a median PFS of 10.9 months and median OS of 24.6 months (56). When dabrafenib and trametinib are given as subsequent therapy, outcomes were similar with investigator assessed response rates of 67%, median PFS of 10.2 months, and median OS of 18.2 months (57,58). The number of patients with CNS metastases were limited in these trials, but based on data from metastatic melanoma, dabrafenib and trametinib does exhibit intracranial activity (59). Since there is no randomized data comparing dabrafenib plus trametinib with chemotherapy or chemoimmunotherapy in the front-line setting, most oncology guidelines suggest that either a targeted therapy or chemotherapy approach are reasonable initial treatment options.

The role of immunotherapy in *BRAF*-mutated lung cancer is controversial, as historically patients with oncogene driven lung cancer have lower response rates and poorer survival outcomes to immunotherapy compared to patients who lack driver mutations (60). However, a retrospective subgroup analysis of *BRAF*-mutated patients in the international, multicenter IMMUNOTARGET registry suggests that PD-1/L1 inhibitors may be effective in *BRAF*-mutated patients with a history of smoking or in non-V600E mutations (60). In addition, class II (non-V600 kinase activating dimers) and III (non-V600 kinase inactivating heterodimers) mutations occurred exclusively in patients with a history of smoking and were associated with the development of brain metastases (61). These data suggest that *BRAF*-mutant NSCLC is a heterogeneous disease and some subtypes may benefit from chemoimmunotherapy (62).

An area of active research is in developing therapies for non-V600E alterations as well as for patients with V600E mutations who have developed resistance to dabrafenib and trametinib. Traditionally, these patients have received chemotherapy in the second-line setting but several new agents are currently in early clinical trials, such as third-generation RAF inhibitors, pan-RAF inhibitors (e.g., LY3009120, TAK-580, PLX8394, LXH254), and selective ERK inhibitors (e.g., ulixertinib) that are expected to have activity against BRAF splice variants, amplifications, and secondary mutations that lead to downstream, BRAF-independent ERK signaling (32,63,64).

NTRK

NTRK genes normally encode TRK proteins involved

in neurotrophin binding that can activate downstream MAPK, PI3K, protein kinase C, and other SHC-independent signaling pathways normally involved in neuronal development and survival (65). Several *NTRK* alterations including mutations, splice variants, and TRK overexpression have been reported, but by far the most common oncogenic *NTRK* alteration is a gene fusion involving a transcription factor (e.g., EML4, ETV6) and *NTRK1*, *NTRK2*, or *NTRK3* genes. These fusions occur in <1% of NSCLC, but can also occur as a rare driver mutation in other common cancers such as head and neck, breast, GI, melanoma, primary brain tumors, or as a pathognomonic alteration in rare cancers like secretory breast cancer, mammary analogue secretory carcinoma, infantile fibrosarcoma, etc. In general, *NTRK* fusions are diagnosed using comprehensive DNA-based NGS panels, however, *NTRK2* and *3* fusions in particular may be more difficult to detect by DNA-based NGS due to the presence of large noncoding regions within these genes; thus, diagnostic sensitivity is improved with the addition of RNA-based sequencing (65,66).

Larotrectinib is the most well studied selective TRK inhibitor and is FDA-approved for any solid tumor with an *NTRK* gene fusion without a known acquired resistance mutation. In a pooled analysis of three phase I/II tissue agnostic trials that included pediatric and adult patients with *NTRK* fusions, 75% of patients had a confirmed response and a median PFS of 25.8 months in the adult cohort (67,68). Although only 7 patients with primary lung cancer were included in the analysis, 3 of 7 achieved a complete response, 2 of 7 achieved a PR, and 2 of 7 had stable disease (68). Overall, brain metastases appear to be less common in patients with *NTRK* fusions and only occurred in about 5% of patients treated on clinical trials with larotrectinib (69). Among these patients, 67% (4 of 6 patients) had NSCLC and larotrectinib demonstrated good clinical activity with an intracranial response rate of 60% (69). Likewise, entrectinib is an alternative first-generation TRK inhibitor with activity against ALK and ROS1 positive tumors that has demonstrated an ORR of 57% and a median OS of 20.9 months in a pooled analysis of 54 patients with *NTRK* fusions (70). Based on these results, the FDA recently approved the use of entrectinib for patients with *NTRK* positive tumors (50).

A variety of acquired resistance mutations within the TRK kinase domain have been discovered in patients previously treated with larotrectinib and entrectinib. The most frequent amino acid substitutions that result in resistance tend to occur at the solvent front region of the kinase domain at G595R in *NTRK1* fusions and G623R in *NTRK3* fusions; these (and other mutations at positions F589L, G667S, G696A) directly interfere with drug binding and are key targets for next-generation TRK inhibitors such as repotrectinib and selitrectinib currently in clinical trials (67,71-73). As a whole, TRK inhibitors are well tolerated but unique toxicities appear to be related to on-target effects on the nervous system including dizziness, paresthesia, and cognitive disturbances in addition to anemia and hepatitis (65,67).

EGFR and *HER2* exon 20 insertions

EGFR and *HER2* are both members of the HER family of tyrosine kinase receptors and, in both cases, structurally analogous exon 20 insertion mutations can result in constitutive kinase activation and downstream signal transduction. In addition, HER2 can heterodimerize with other HER receptors to induce EGFR transphosphorylation. Together, these mutations account for approximately 4% of lung adenocarcinomas and have slightly different biology than traditional *EGFR* and *HER2* mutations and therefore do not respond to currently available targeted therapies (74,75). This is primarily due to the small size of the drug binding pocket induced by exon 20 insertions that sterically hinders binding of currently available EGFR inhibitors (75). In NSCLC, *HER2* activating mutations are almost exclusively due to a duplication or insertion of 4 amino acids (YVMA) at codon 775 in exon 20 (74,76-78). On the other hand, classical sensitizing *EGFR* mutations account for approximately 85% to 90% of cases but *EGFR* exon 20 insertion mutations constitute only up to 10% of all *EGFR* mutations and are associated with *de novo* resistance to first-generation EGFR inhibitors with a poorer prognosis compared to those with sensitizing *EGFR* mutations (79). Likewise, targeting HER2 overexpression with trastuzumab or with TKIs like neratinib, lapatinib, and afatinib have been ineffective in lung cancer trials with response rates of <10% (80,81). At that time, HER2 overexpression was determined at the protein level using immunohistochemistry (IHC) and, unlike in breast cancer, was not a good predictor of response to HER2 directed therapy (78). As DNA-sequencing has become more widespread, it has allowed for the identification of new biomarkers such as exon 20 insertion mutations in *EGFR* or *HER2* that are predictive of benefit from ado-trastuzumab emtansine (78) as well as next-generation pan-HER/EGFR superfamily TKIs.

Some promising new targeted therapies include

poziotinib, TAK-788, pyrotinib, and JNJ-372 along with other agents with preclinical activity such as tarloxotinib, luminespib, TAS6417, and Compound 1A (82). Among these new agents, the pan-HER/EGFR TKIs poziotinib, TAK-788, and pyrotinib are the furthest along in clinical development. Early results from a phase II trial with poziotinib dosed at 16 mg daily demonstrated an ORR of 55% (24 of 44 patients) for *EGFR* exon 20 and 50% (6 of 12 patients) for *HER2* exon 20 insertions. However, dose reductions were needed in 63% of patients due to grade ≥3 skin rash, diarrhea, or paronychia and one report of grade 5 pneumonitis (83). Of note, *in vitro* murine models have demonstrated that acquired resistance to poziotinib may result from secondary point mutations in C805S of *HER2* or C797S of *EGFR* that can be overcome by heat shock protein 90 inhibitors (e.g., luminespib), which associates with *EGFR* exon 20 kinases and leads to their degradation (82,84).

TAK-788 has also been studied in 26 patients with *EGFR* exon 20 insertions and has demonstrated a 54% ORR and 89% DCR at doses between 80–160 mg daily with a tolerable safety profile that most commonly included diarrhea, rash, stomatitis, nausea, and fatigue (85). Responses occurred in multiple exon 20 insertion variants and an extension cohort is currently enrolling additional patients. Notably, the dose limiting toxicity was pneumonitis and observed in 2 of 34 patients (86). Pyrotinib also appears very promising based on preclinical activity in patient-derived xenograft models and a preliminary analysis of 15 treated. Using the recommended phase II dose of 400 mg/day, an ORR of 53% was seen with a median PFS of 6.4 months and no grade 3 or 4 AEs had been reported yet (87). The bispecific antibody directed against *EGFR* and *MET*, JNJ-372, also had a cohort for patients with *EGFR* or *HER2* exon 20 insertions and responses were seen in 8 of 27 patients, including one patient who had progressed after treatment with poziotinib; in addition, 14 of 27 patients achieved durable stable disease on therapy (27).

MET

Primary MET driven NSCLC

A variety of alterations in *MET* can lead to oncogenic downstream MAPK signaling, such as high-level *MET* amplifications or a splice site mutation that leads to exon 14 skipping and subsequent loss of the CBL E3 ubiquitin ligase binding site on the MET protein. The diminished

ability to degrade the MET protein then renders the cell more dependent on downstream MET signaling pathways (88,89). *MET* exon 14 skipping mutations are estimated to occur in roughly 3–4% of NSCLC (89,90) and can be detected using DNA-based NGS in tissue or blood, although blood-based sequencing may only detect *MET* alterations in about half of samples that tested positive in tissue (91). Even with tissue-based DNA testing, we are likely underestimating the number of patients who harbor *MET* exon 14 skipping mutations due to the large non-coding regions within the *MET* gene that is often not adequately sequenced; to overcome this, the use of RNA sequencing for patients who initially test negative on DNA-based tissue NGS may allow for the detection of a *MET* exon 14 skipping mutation in an additional 2–3% of cases (92).

Once a high-level *MET* amplification or exon 14 skipping mutation has been identified, currently available targeted therapies include crizotinib or cabozantinib. These two multikinase inhibitors have been FDA-approved for use in other settings and have demonstrated some degree of activity as MET inhibitors based on small case series or early clinical trials. Crizotinib is the most well studied MET TKI and, as part of the ongoing PROFILE 1001 study, has been administered to 69 patients with an ORR of 32%, stable disease in 45% of patients, and a median PFS of 7.3 months (91). Of note, crizotinib is classified as a type Ia MET inhibitor that binds MET through interactions with the hinge (Y1230) and solvent front glycine residue (G1163) as opposed to cabozantinib, which is a type II MET inhibitor that binds MET at the ATP adenine binding site and a hydrophobic back pocket region. Clinically, crizotinib may be a more specific MET inhibitor than cabozantinib with fewer off target AEs and cabozantinib is more likely to have variable potency against MET (93). Also, patients who develop solvent front resistance mutations on crizotinib theoretically may respond to cabozantinib since it does not rely on interactions with the G1163 residue (93).

Next-generation, selective type Ib MET inhibitors are currently in clinical trials and have demonstrated very positive results. For instance, capmatinib has demonstrated an ORR of 67.9% with a DCR of 96.4% in 28 treatment-naïve patients with *MET* exon 14 skipping mutations; among previously treated patients, capmatinib was less effective but still demonstrated an ORR of 40.6% and a DCR of 78.3% (94). Intracranial responses were also seen in about half of patients who had CNS metastases (94). Likewise, tepotinib is another selective MET inhibitor with ORR ranging from 45–50%, depending on whether

the *MET* alteration was identified in blood or tissue, and a median DOR lasting 14.3 months (95). As a class effect, patients taking capmatinib or tepotinib tend to experience grade 1–2 peripheral edema and GI toxicities such as nausea, vomiting, diarrhea.

Acquired MET amplification

MET exon 14 skipping mutations also tend to coexist with high-level *MET* amplification or copy number gains and are associated with higher MET protein expression and can be found in up to 20% of patients who develop resistance to third-generation EGFR inhibitors such as osimertinib (20,96). Notably, savolitinib is another selective type Ib MET inhibitor that is being explored in combination with osimertinib in the phase I TATTON trial and as a treatment arm in the phase II ORCHARD trial for patients who have both an *EGFR* mutation and *MET* amplification. Preliminary efficacy data showed an ORR of 52% with a median DOR of 7.1 months; in the cohort previously treated with a third-generation EGFR inhibitor, the combination resulted in an ORR of 25% (12 of 48 patients) and SD in 44% (21 of 48 patients), including in some patients with CNS metastases (97).

Primary resistance to MET inhibitors may be due to a lack of MET protein expression by IHC or mass spectrometry, despite the presence of a *MET* alteration identified on DNA-based sequencing (98). It is hypothesized that tumors lacking MET protein expression, potentially due to posttranslational modification events, are likely not oncogene addicted and therefore fail to respond to MET TKIs (98). Although not well characterized yet, acquired resistance mechanisms to MET inhibitors have most frequently reported to be due to increased reliance on bypass pathways, such as acquired *RAS* alterations or *EGFR/HER2* amplifications (98,99). Secondary mutations in the kinase domain that confer resistance to crizotinib can also occur, often at D1228 and Y1230 (98,100-102).

RET

RET alterations occur in about 2% of lung cancer and can be identified by DNA-based NGS in tissue or blood, however, RNA sequencing has the ability to identify uncommon *RET* fusion partners and may significantly increase the detection rate (92). RET is activated by two major mechanisms in cancer: *RET* fusions occur most often in papillary thyroid cancers and NSCLC (with the most common fusion partner being KIF5B in 70% of cases) (103,104), whereas *RET*-activating point mutations occur primarily in medullary thyroid cancer. FDA-approved targeted therapies that have been repurposed for *RET*-driven cancers with modest activity include multikinase inhibitors such as cabozantinib, vandetanib, and sunitinib (ORR ranging from 18–47%) as well as case reports of responses to nintedanib and lenvatinib (103,105,106). However, these multikinase inhibitors are not selective for RET and therefore patients also develop off-target dose-limiting toxicities related to VEGF inhibition such as hypertension, bleeding, and thrombosis.

Recently, several selective RET inhibitors have shown promising efficacy with tolerable toxicity in early phase trials. In a phase I dose escalation study using a selective RET inhibitor, LOXO-292, a total of 25 of 37 (68%) patients achieved an objective response to therapy. In addition, 4 of 4 patients with CNS metastases achieved an intracranial PR. There were four grade 3 AEs, due to diarrhea, increased liver enzymes, thrombocytopenia, and tumor lysis syndrome (107). BLU-667 is another promising RET TKI that is 90-fold more selective for RET than VEGFR2 and has demonstrated activity against some cabozantinib resistance mutations, such as the V804 gatekeeper residue (104,108). Interim results from the NSCLC expansion cohort (n=48) of the ARROW study suggests that BLU-667 is very effective with an ORR of 58%, a DCR of 96%, and intracranial responses were seen in 7 of 9 patients. The median DOR has not been reached, but some are ongoing at 2 years of follow-up. It is generally well tolerated with the most common grade 1–2 AEs being neutropenia, constipation, liver enzyme elevations, fatigue, and hypertension. Serious AEs have occurred and led to treatment discontinuation in 7% of patients due to pneumonitis, hypoxemia, mucositis, myelosuppression, gait disturbance, and anemia. BLU-667 has been granted breakthrough therapy designation status in the United States for patients who have progressive disease after platinum-based chemotherapy, which may lead to more expedited approval.

KRAS

KRAS is the most commonly mutated oncogenic driver in NSCLC, however, there are no guideline recommended targeted therapies for patients with *KRAS* mutations at this time. AMG 510 is a first in class KRAS G12C small molecular inhibitor that irreversibly binds the cysteine

Table 1 Coming attractions—what to test for and what the target may be

Gene	Available treatments	Emerging treatments	Recommended testing	Potential testing
EGFR	Gefitinib	JNJ-372	✓	
	Erlotinib	Osimertinib + Savolitinib		
	Afatinib			
	Dacomitinib			
	Osimertinib			
ALK	Crizotinib	Ensartinib	✓	
	Ceritinib	Repotrectinib		
	Alectinib	Selitrectinib		
	Brigatinib			
	Lorlatinib			
ROS1	Crizotinib	Repotrectinib ✓		
	Ceritinib			
	Lorlatinib			
	Entrectinib			
BRAF	Chemotherapy + anti-PD-1/L1	Pan-RAF inhibitors	✓	
	Dabrafenib + trametinib (V600E only)	Ulixertinib		
NTRK	Larotrectinib	Repotrectinib ✓		
	Entrectinib	Selitrectinib		
MET high level amplification or exon 14 insertion	Crizotinib	Capmatinib		✓
	Cabozantinib	Tepotinib		
		Savolitinib		
		JNJ-372		
EGFR/HER2 exon 20 insertions	Chemotherapy	Poziotinib		✓
	Afatinib	TAK-788		
	Ado-trastuzumab emtansine	Pyrotinib		
RET	Cabozantinib	BLU-667		✓
	Vandetanib	LOXO-292		
KRAS G12C	Chemotherapy + anti-PD-1/L1	AMG 510		✓
		MRTX-849		

moiety of *KRAS* G12C in an inactive state. Results from the first-in-human study conducted in patients with solid tumors were very encouraging, with mostly grade 1–2 AEs and no dose limiting toxicities. Among the subset of patients with

lung cancer, 5 of 10 heavily pretreated patients achieved a PR and remained on therapy at data cut-off. The other 4 patients had stable disease as best overall response. Mirati has also begun enrolling patients in a phase I trial of another KRAS G12C inhibitor, MRTX849, although no results have been published yet. Importantly, these KRAS TKIs only have activity for the subset of patients with G12C mutations, which accounts for approximately 44% of *KRAS* mutations in patients with advanced lung cancer (109).

Conclusions

Rapid advances in the utility of molecular testing to identify driver mutations and the expeditious rate of drug development have intensified the need for multiplex NGS testing from tissue and/or blood in order to determine the most appropriate treatment options for patients with advanced lung cancer. Presently, most guidelines recommend testing for at least sensitizing *EGFR* mutations, *ALK* fusions, *ROS1* fusions, *BRAF* V600E, and *NTRK* fusions given the availability of FDA-approved matched targeted therapies for these alterations. In addition, we have highlighted promising investigational therapies aimed at high level *MET* amplifications or exon 14 skipping mutations, *EGFR* or *HER2* exon 20 insertion mutations, *RET* fusions, and *KRAS* G12C mutations that lend support towards testing for these driver mutations as well (*Table 1*). In addition, multiplex NGS testing may help identify patients with oncogene driven lung cancers who are potentially at greater risk for a severe toxicity (e.g., pneumonitis or hepatitis) and lack of benefit when given anti-PD-1/L1 checkpoint inhibitors followed by targeted therapy.

Acknowledgments

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editors Alfredo Addeo and Giuseppe Banna for the series "Non-Small Cell Lung Cancer (NSCLC)" published in *Precision Cancer Medicine*. The article has undergone external peer review.

Conflicts of Interest: Both authors have completed the

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 2014;311:1998-2006.

2. Barlesi F, Mazieres J, Merlio JP, et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). Lancet 2016;387:1415-26.

3. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013;8:823-59.

4. Tsao AS, Scagliotti GV, Bunn PA Jr, et al. Scientific Advances in Lung Cancer 2015. J Thorac Oncol 2016;11:613-38.

5. Rotow J, Bivona TG. Understanding and targeting resistance mechanisms in NSCLC. Nat Rev Cancer 2017;17:637-58.

6. Lindeman NI, Cagle PT, Aisner DL, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol 2018;13:323-58.

7. Pennell NA, Mutebi A, Zhou ZY, et al. Economic Impact of Next-Generation Sequencing Versus Single-Gene Testing to Detect Genomic Alterations in Metastatic Non–Small-Cell Lung Cancer Using a Decision Analytic Model. JCO Precis Oncol 2019. doi: 10.1200/PO.18.00356.

8. Oxnard GR, Arcila ME, Sima CS, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. Clin Cancer Res 2011;17:1616-22.

9. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 2013;19:2240-7.

10. Mok TS, Wu YL, Ahn MJ, et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. N Engl J Med 2017;376:629-40.

11. Tagrisso significantly improves overall survival in the Phase III FLAURA trial for 1st-line EGFR-mutated non-small cell lung cancer [Internet]. 2019 [cited 2019 Aug 19]. Available online: https://www.astrazeneca.com/media-centre/press-releases/2019/tagrisso-significantly-improves-overall-survival-in-the-phase-iii-flaura-trial-for-1st-line-egfr-mutated-non-small-cell-lung-cancer-09082019.html

12. Planchard D, Boyer MJ, Lee JS, et al. Postprogression Outcomes for Osimertinib versus Standard-of-Care EGFR-TKI in Patients with Previously Untreated EGFR-mutated Advanced Non-Small Cell Lung Cancer. Clin Cancer Res 2019;25:2058-63.

13. Yamada T, Hirai S, Katayama Y, et al. Retrospective efficacy analysis of immune checkpoint inhibitors in patients with EGFR-mutated non-small cell lung cancer. Cancer Med [Internet]. 2019 Feb 21. Available online: http://dx.doi.org/10.1002/cam4.2037

14. Zhang T, Wan B, Zhao Y, et al. Treatment of uncommon EGFR mutations in non-small cell lung cancer: new evidence and treatment. Transl Lung Cancer Res 2019;8:302-16.

15. Brindel A, Althakfi W, Barritault M, et al. Uncommon

EGFR mutations in lung adenocarcinomas: clinical features and response to tyrosine kinase inhibitors. OncologyPRO. 2018 Oct 19 [cited 2019 Aug 12]. Available online: https://oncologypro.esmo.org/Meeting-Resources/ESMO-2018-Congress/Uncommon-EGFR-mutations-in-lung-adenocarcinomas-clinical-features-and-response-to-tyrosine-kinase-inhibitors

16. Janne PA, Yu HA, Johnson ML, et al. Safety and preliminary antitumor activity of U3-1402: A HER3-targeted antibody drug conjugate in EGFR TKI-resistant, EGFRm NSCLC. J Clin Oncol 2019;37:abstr 9010.

17. Brown BP, Zhang YK, Westover D, et al. On-target resistance to the mutant-selective EGFR inhibitor osimertinib can develop in an allele specific manner dependent on the original EGFR activating mutation. Clin Cancer Res 2019. doi: 10.1158/1078-0432.CCR-18-3829.

18. Fassunke J, Müller F, Keul M, et al. Overcoming EGFRG724S-mediated osimertinib resistance through unique binding characteristics of second-generation EGFR inhibitors. Nat Commun 2018;9:4655.

19. Oxnard GR, Hu Y, Mileham KF, et al. Assessment of Resistance Mechanisms and Clinical Implications in Patients With EGFR T790M-Positive Lung Cancer and Acquired Resistance to Osimertinib. JAMA Oncol 2018;4:1527-34.

20. Ramalingam SS, Cheng Y, Zhou C, et al. LBA50 Mechanisms of acquired resistance to first-line osimertinib: Preliminary data from the phase III FLAURA study. Ann Oncol 2018;29:mdy424.063.

21. Noronha V, Joshi A, Patil VM, et al. Phase III randomized trial comparing gefitinib to gefitinib with pemetrexed-carboplatin chemotherapy in patients with advanced untreated EGFR mutant non-small cell lung cancer (gef vs gef+C). J Clin Oncol 2019;37:abstr 9001.

22. Nakagawa K, Garon EB, Seto T, et al. RELAY: A multinational, double-blind, randomized Phase 3 study of erlotinib (ERL) in combination with ramucirumab (RAM) or placebo (PL) in previously untreated patients with epidermal growth factor receptor mutation-positive (EGFRm) metastatic non-small cell lung cancer (NSCLC). J Clin Oncol 2019;37:abstr 9000.

23. New data on mechanisms of acquired resistance after 1st-line Tagrisso in NSCLC support initiation of ORCHARD trial to explore post-progression treatment options [Internet]. 2018 [cited 2019 May 15]. Available online: https://www.astrazeneca.com/media-centre/press-releases/2018/new-data-on-mechanisms-of-acquired-resistance-after-1st-line-tagrisso-in-nsclc-support-initiation-of-orchard-trial-to-explore-post-progression-treatment-options-19102018.html

24. Nishino M, Suda K, Kobayashi Y, et al. Effects of secondary EGFR mutations on resistance against upfront osimertinib in cells with EGFR-activating mutations in vitro. Lung Cancer 2018;126:149-55.

25. Yang Z, Yang N, Ou Q, et al. Investigating Novel Resistance Mechanisms to Third-Generation EGFR Tyrosine Kinase Inhibitor Osimertinib in Non-Small Cell Lung Cancer Patients. Clin Cancer Res 2018;24:3097-107.

26. Liu Y, Li Y, Ou Q, et al. Acquired EGFR L718V mutation mediates resistance to osimertinib in non-small cell lung cancer but retains sensitivity to afatinib. Lung Cancer 2018;118:1-5.

27. Haura EB, Cho BC, Lee JS, et al. JNJ-61186372 (JNJ-372), an EGFR-cMet bispecific antibody, in EGFR-driven advanced non-small cell lung cancer (NSCLC). J Clin Oncol 2019;37:abstr 9009.

28. Sequist LV, Lee JS, Han JY, et al. TATTON Phase Ib expansion cohort: Osimertinib plus savolitinib for patients (pts) with EGFR-mutant, MET-amplified NSCLC after progression on prior third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI). Cancer Res 2019;79:abstr CT033.

29. Piotrowska Z, Isozaki H, Lennerz JK, et al. Landscape of Acquired Resistance to Osimertinib in EGFR-Mutant NSCLC and Clinical Validation of Combined EGFR and RET Inhibition with Osimertinib and BLU-667 for Acquired RET Fusion. Cancer Discov 2018;8:1529-39.

30. Jia Y, Yun CH, Park E, et al. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature 2016;534:129-32.

31. To C, Jang J, Chen T, et al. Single and Dual Targeting of Mutant EGFR with an Allosteric Inhibitor. Cancer Discov 2019;9:926-43.

32. Vojnic M, Kubota D, Kurzatkowski C, et al. Acquired BRAF Rearrangements Induce Secondary Resistance to EGFR therapy in EGFR-Mutated Lung Cancers. J Thorac Oncol 2019;14:802-15.

33. Ho CC, Liao WY, Lin CA, et al. Acquired BRAF V600E Mutation as Resistant Mechanism after Treatment with Osimertinib. J Thorac Oncol 2017;12:567-72.

34. Solassol J, Vendrell JA, Senal R, et al. Challenging BRAF/EGFR co-inhibition in NSCLC using sequential liquid biopsies. Lung Cancer 2019;133:45-7.

35. Lin JJ, Riely GJ, Shaw AT. Targeting ALK: Precision Medicine Takes on Drug Resistance. Cancer Discov 2017;7:137-55.

36. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 2014;371:2167-77.

37. Cho BC, Kim DW, Bearz A, et al. ASCEND-8: A Randomized Phase 1 Study of Ceritinib, 450 mg or 600 mg, Taken with a Low-Fat Meal versus 750 mg in Fasted State in Patients with Anaplastic Lymphoma Kinase (ALK)-Rearranged Metastatic Non-Small Cell Lung Cancer (NSCLC). J Thorac Oncol 2017;12:1357-67.

38. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2017;377:829-38.

39. Gadgeel S, Peters S, Mok T, et al. Alectinib versus crizotinib in treatment-naive anaplastic lymphoma kinase-positive (ALK+) non-small-cell lung cancer: CNS efficacy results from the ALEX study. Ann Oncol 2018;29:2214-22.

40. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib versus Crizotinib in ALK-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2018;379:2027-39.

41. Gainor JF, Dardaei L, Yoda S, et al. Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in ALK-Rearranged Lung Cancer. Cancer Discov 2016;6:1118-33.

42. Yoda S, Lin JJ, Lawrence MS, et al. Sequential ALK Inhibitors Can Select for Lorlatinib-Resistant Compound ALK Mutations in ALK-Positive Lung Cancer. Cancer Discov 2018;8:714-29.

43. Shaw AT, Solomon BJ, Besse B, et al. ALK Resistance Mutations and Efficacy of Lorlatinib in Advanced Anaplastic Lymphoma Kinase-Positive Non-Small-Cell Lung Cancer. J Clin Oncol 2019;37:1370-9.

44. Solomon BJ, Besse B, Bauer TM, et al. Lorlatinib in patients with ALK-positive non-small-cell lung cancer: results from a global phase 2 study. Lancet Oncol 2018;19:1654-67.

45. Ou S-HI, Zhu VW. CNS metastasis in ROS1+ NSCLC: An urgent call to action, to understand, and to overcome. Lung Cancer 2019;130:201-7.

46. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-70.

47. Shaw AT, Riely GJ, Bang YJ, et al. Crizotinib in ROS1-rearranged advanced non-small-cell lung cancer (NSCLC): updated results, including overall survival, from PROFILE 1001. Ann Oncol 2019;30:1121-6.

48. Shaw AT, Ou SH, Bang YJ, et al. Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med 2014;371:1963-71.

49. Lim SM, Kim HR, Lee JS, et al. Open-Label, Multicenter, Phase II Study of Ceritinib in Patients With Non-Small-Cell Lung Cancer Harboring ROS1 Rearrangement. J Clin Oncol 2017;35:2613-8.

50. Center for Drug Evaluation, Research. FDA approves entrectinib for NTRK solid tumors and ROS-1 NSCLC [Internet]. U.S. Food and Drug Administration. 2019 [cited 2019 Aug 19]. Available online: http://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-entrectinib-ntrk-solid-tumors-and-ros-1-nsclc

51. Drilon A, Siena S, Ou SI, et al. Safety and Antitumor Activity of the Multitargeted Pan-TRK, ROS1, and ALK Inhibitor Entrectinib: Combined Results from Two Phase I Trials (ALKA-372-001 and STARTRK-1). Cancer Discov 2017;7:400-9.

52. Gainor JF, Tseng D, Yoda S, et al. Patterns of Metastatic Spread and Mechanisms of Resistance to Crizotinib in ROS1-Positive Non-Small-Cell Lung Cancer. JCO Precis Oncol 2017. doi: 10.1200/PO.17.00063.

53. Solomon BJ, Martini J, Ou SI, et al. Efficacy of lorlatinib in patients (pts) with ROS1-positive advanced non-small cell lung cancer (NSCLC) and ROS1 kinase domain mutations. Ann Oncol 2018;29:viii493-547.

54. Ou S, Shaw A, Riely G, et al. OA02.03 Clinical Activity of Lorlatinib in Patients with ROS1+ Advanced Non-Small Cell Lung Cancer: Phase 2 Study Cohort EXP-6. J Thorac Oncol 2018;13:S322-3.

55. Marchetti A, Felicioni L, Malatesta S, et al. Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. J Clin Oncol 2011;29:3574-9.

56. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. Lancet Oncol 2017;18:1307-16.

57. Planchard D, Besse B, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. Lancet Oncol 2016;17:984-93.

58. Planchard D, Besse B, Kim TM, et al. Updated survival of patients (pts) with previously treated BRAF V600E–mutant advanced non-small cell lung cancer (NSCLC) who received dabrafenib (D) or D + trametinib (T) in the phase II BRF113928 study. J Clin Oncol 2017;35:abstr 9075.

59. Davies MA, Saiag P, Robert C, et al. Dabrafenib plus trametinib in patients with BRAFV600-mutant

melanoma brain metastases (COMBI-MB): a multicentre, multicohort, open-label, phase 2 trial. Lancet Oncol 2017;18:863-73.

60. Mazières J, Drilon A, Lusque A, et al. Immune checkpoint inhibitors for patients with advanced lung cancer and oncogenic driver alterations: results from the IMMUNOTARGET registry. Ann Oncol 2019;30:1321-8.

61. Dagogo-Jack I, Martinez P, Yeap BY, et al. Impact of BRAF Mutation Class on Disease Characteristics and Clinical Outcomes in BRAF-mutant Lung Cancer. Clin Cancer Res 2019;25:158-65.

62. Dudnik E, Peled N, Nechushtan H, et al. BRAF Mutant Lung Cancer: Programmed Death Ligand 1 Expression, Tumor Mutational Burden, Microsatellite Instability Status, and Response to Immune Check-Point Inhibitors. J Thorac Oncol 2018;13:1128-37.

63. Leonetti A, Facchinetti F, Rossi G, et al. BRAF in non-small cell lung cancer (NSCLC): Pickaxing another brick in the wall. Cancer Treat Rev 2018;66:82-94.

64. Sullivan RJ, Infante JR, Janku F, et al. First-in-Class ERK1/2 Inhibitor Ulixertinib (BVD-523) in Patients with MAPK Mutant Advanced Solid Tumors: Results of a Phase I Dose-Escalation and Expansion Study. Cancer Discov 2018;8:184-95.

65. Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nat Rev Clin Oncol 2018;15:731-47.

66. Benayed R, Offin MD, Mullaney KA, et al. Comprehensive detection of targetable fusions in lung adenocarcinomas by complementary targeted DNAseq and RNAseq assays. J Clin Oncol 2018;36:abstr 12076.

67. Drilon A, Laetsch TW, Kummar S, et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. N Engl J Med 2018;378:731-9.

68. Hong DS, Kummar S, Farago AF, et al. Larotrectinib efficacy and safety in adult TRK fusion cancer patients. J Clin Oncol 2019;37:abstr 3122.

69. Drilon AE, DuBois SG, Farago AF, et al. Activity of larotrectinib in TRK fusion cancer patients with brain metastases or primary central nervous system tumors. J Clin Oncol 2019;37:abstr 2006.

70. Demetri GD, Paz-Ares L, Farago AF, et al. Efficacy and safety of entrectinib in patients with NTRK fusion-positive (NTRK-fp) Tumors: Pooled analysis of STARTRK-2, STARTRK-1 and ALKA-372-001. Ann Oncol 2018;29:abstr LBA17.

71. Drilon A, Nagasubramanian R, Blake JF, et al. A Next-Generation TRK Kinase Inhibitor Overcomes Acquired Resistance to Prior TRK Kinase Inhibition in Patients with TRK Fusion-Positive Solid Tumors. Cancer Discov 2017;7:963-72.

72. Drilon A, Ou SI, Cho BC, et al. Repotrectinib (TPX-0005) Is a Next-Generation ROS1/TRK/ALK Inhibitor That Potently Inhibits ROS1/TRK/ALK Solvent- Front Mutations. Cancer Discov 2018;8:1227-36.

73. AACR 2019: Phase I Trial Evaluates LOXO-195 in Patients With NTRK-Positive Solid Tumors - The ASCO Post [Internet]. [cited 2019 Jul 17]. Available online: https://www.ascopost.com/News/59906

74. Pillai RN, Behera M, Berry LD, Rossi MR, Kris MG, Johnson BE, et al. HER2 mutations in lung adenocarcinomas: A report from the Lung Cancer Mutation Consortium. Cancer 2017;123:4099-105.

75. Robichaux JP, Elamin YY, Tan Z, et al. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. Nat Med 2018;24:638-46.

76. Arcila ME, Chaft JE, Nafa K, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Cancer Res 2012;18:4910-8.

77. Kosaka T, Tanizaki J, Paranal RM, et al. Response Heterogeneity of EGFR and HER2 Exon 20 Insertions to Covalent EGFR and HER2 Inhibitors. Cancer Res 2017;77:2712-21.

78. Li BT, Shen R, Buonocore D, et al. Ado-Trastuzumab Emtansine for Patients With HER2-Mutant Lung Cancers: Results From a Phase II Basket Trial. J Clin Oncol 2018;36:2532-7.

79. Yasuda H, Park E, Yun CH, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. Sci Transl Med 2013;5:216ra177.

80. Clamon G, Herndon J, Kern J, et al. Lack of trastuzumab activity in nonsmall cell lung carcinoma with overexpression of erb-B2: 39810: a phase II trial of Cancer and Leukemia Group B. Cancer 2005;103:1670-5.

81. Mazières J, Barlesi F, Filleron T, et al. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: results from the European EUHER2 cohort. Ann Oncol 2016;27:281-6.

82. Vyse S, Huang PH. Targeting EGFR exon 20 insertion mutations in non-small cell lung cancer. Signal Transduct Target Ther 2019;4:5.

83. Heymach J, Negrao M, Robichaux J, et al. OA02.06 A Phase II Trial of Poziotinib in EGFR and HER2 exon 20

Mutant Non-Small Cell Lung Cancer (NSCLC). J Thorac Oncol 2018;13:S323-4.

84. Koga T, Kobayashi Y, Tomizawa K, et al. Activity of a novel HER2 inhibitor, poziotinib, for HER2 exon 20 mutations in lung cancer and mechanism of acquired resistance: An in vitro study. Lung Cancer 2018;126:72-9.

85. Janne PA, Neal JW, Camidge DR, et al. Antitumor activity of TAK-788 in NSCLC with EGFR exon 20 insertions. J Clin Oncol 2019;37:abstr 9007.

86. Doebele RC, Riely GJ, Spira AI, et al. First report of safety, PK, and preliminary antitumor activity of the oral EGFR/HER2 exon 20 inhibitor TAK-788 (AP32788) in non–small cell lung cancer (NSCLC). J Clin Oncol 2018;36:abstr 9015.

87. Wang Y, Jiang T, Qin Z, et al. HER2 exon 20 insertions in non-small-cell lung cancer are sensitive to the irreversible pan-HER receptor tyrosine kinase inhibitor pyrotinib. Ann Oncol 2019;30:447-55.

88. Drilon A. MET Exon 14 Alterations in Lung Cancer: Exon Skipping Extends Half-Life. Clin Cancer Res 2016;22:2832-4.

89. Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov 2015;5:842-9.

90. Awad MM, Oxnard GR, Jackman DM, et al. MET Exon 14 Mutations in Non-Small-Cell Lung Cancer Are Associated With Advanced Age and Stage-Dependent MET Genomic Amplification and c-Met Overexpression. J Clin Oncol 2016;34:721-30.

91. Drilon A, Clark J, Weiss J, et al. OA12.02 Updated Antitumor Activity of Crizotinib in Patients with MET Exon 14-Altered Advanced Non-Small Cell Lung Cancer. J Thorac Oncol 2018;13:S348.

92. Benayed R, Offin M, Mullaney K, et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no driver alteration detected by DNA sequencing and low tumor mutation burden. Clin Cancer Res 2019;25:4712-22.

93. Reungwetwattana T, Liang Y, Zhu V, et al. The race to target MET exon 14 skipping alterations in non-small cell lung cancer: The Why, the How, the Who, the Unknown, and the Inevitable. Lung Cancer 2017;103:27-37.

94. Wolf J, Seto T, Han JY, et al. Capmatinib (INC280) in METΔex14-mutated advanced non-small cell lung cancer (NSCLC): Efficacy data from the phase II GEOMETRY mono-1 study. J Clin Oncol 2019;37:abstr 9004.

95. Paik PK, Veillon R, Cortot AB, et al. Phase II study of tepotinib in NSCLC patients with METex14 mutations. | 2019 ASCO Annual Meeting Abstracts. J Clin Oncol 2019;37:abstr 9005.

96. Tong JH, Yeung SF, Chan AWH, et al. MET Amplification and Exon 14 Splice Site Mutation Define Unique Molecular Subgroups of Non-Small Cell Lung Carcinoma with Poor Prognosis. Clin Cancer Res 2016;22:3048-56.

97. Yu H, Ahn MJ, Kim SW, et al. CT032 - TATTON Phase Ib expansion cohort: Osimertinib plus savolitinib for patients (pts) with EGFR-mutant, MET-amplified NSCLC after progression on prior first/second-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) [abstract]. Proceedings of the 110th Annual Meeting of the American Association for Cancer Research [Internet]. 2019. Available online: https://www.abstractsonline.com/pp8/#!/6812/presentation/9826

98. Guo R, Offin M, Brannon AR, et al. MET inhibitor resistance in patients with MET exon 14-altered lung cancers. J Clin Oncol 2019;37:abstr 9006.

99. Ding G, Wang J, Ding P, et al. Case report: HER2 amplification as a resistance mechanism to crizotinib in NSCLC with MET exon 14 skipping. Cancer Biol Ther 2019;20:837-42.

100. Ou SI, Young L, Schrock AB, et al. Emergence of Preexisting MET Y1230C Mutation as a Resistance Mechanism to Crizotinib in NSCLC with MET Exon 14 Skipping. J Thorac Oncol 2017;12:137-40.

101. Heist RS, Sequist LV, Borger D, et al. Acquired Resistance to Crizotinib in NSCLC with MET Exon 14 Skipping. J Thorac Oncol 2016;11:1242-5.

102. Bahcall M, Sim T, Paweletz CP, et al. Acquired METD1228V Mutation and Resistance to MET Inhibition in Lung Cancer. Cancer Discov 2016;6:1334-41.

103. Gautschi O, Milia J, Filleron T, et al. Targeting RET in Patients With RET-Rearranged Lung Cancers: Results From the Global, Multicenter RET Registry. J Clin Oncol 2017;35:1403-10.

104. Gainor JF, Lee DH, Curigliano G, et al. Clinical activity and tolerability of BLU-667, a highly potent and selective RET inhibitor, in patients (pts) with advanced RET-fusion+ non-small cell lung cancer (NSCLC). J Clin Oncol 2019;37:abstr 9008.

105. Lee SH, Lee JK, Ahn MJ, et al. Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: a phase II clinical trial. Ann Oncol 2017;28:292-7.

106. Yoh K, Seto T, Satouchi M, et al. Vandetanib in patients with previously treated RET-rearranged advanced non-

small-cell lung cancer (LURET): an open-label, multicentre phase 2 trial. Lancet Respir Med 2017;5:42-50.

107. Oxnard G, Subbiah V, Park K, et al. OA12.07 Clinical Activity of LOXO-292, a Highly Selective RET Inhibitor, in Patients with RET Fusion+ Non-Small Cell Lung Cancer. J Thorac Oncol 2018;13:S349-50.

108. Subbiah V, Gainor JF, Rahal R, et al. Precision Targeted Therapy with BLU-667 for RET-Driven Cancers. Cancer Discov 2018;8:836-49.

109. Arbour KC, Jordan E, Kim HR, et al. Effects of Co-occurring Genomic Alterations on Outcomes in Patients with KRAS-Mutant Non-Small Cell Lung Cancer. Clin Cancer Res 2018;24:334-40.