



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

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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.

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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)

Received: 21 August 2019; Accepted: 13 November 2019; Published: 15 March 2020.

doi: 10.21037/pcm.2019.11.03

View this article at: <http://dx.doi.org/10.21037/pcm.2019.11.03>

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

None.

Footnote

Conflicts of Interest: Dr. SP Patel receives scientific advisory income from: AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Illumina, Nektar, Novartis, Tempus. Dr. Patel's university receives research funding from: Bristol-Myers Squibb, Eli Lilly, Fate, Incyte, AstraZeneca/MedImmune, Merck, Pfizer, Roche/Genentech, Xcovery. Fate Therapeutics, Genocoea, Iovance. Dr. P Vu has no conflicts of interest to declare.

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.

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doi: 10.21037/pcm.2019.11.03

Cite this article as: Vu P, Patel SP. Non-small cell lung cancer targetable mutations: present and future. *Precis Cancer Med* 2020;3:5.