REVIEW

Beyond epidermal growth factor receptor: *MET* amplification as a general resistance driver to targeted therapy in oncogene-driven non-small-cell lung cancer

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Available online XXX

The rapidly changing treatment paradigm for patients with metastatic oncogene-driven lung cancer continues to evolve, and consequently our understanding of the landscape of resistance must also advance. *MET* amplification is an established and frequent driver of resistance in *EGFR*-mutant non-small-cell lung cancer (NSCLC). Recently, the combination of MET proto-oncogene (MET) and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) has shown promise in overcoming this molecularly defined resistance in clinical trials, and this combination strategy is being pursued in ongoing trials. Emerging data also demonstrate *MET* amplification as a resistance driver to TKI-treated *ALK*-, *RET*-, and *ROS-1*-fusion NSCLC, consistently at the range of 15%, while the resistance profiling data are maturing for other molecular targets. In this review, we discuss *MET* amplification as a driver of acquired resistance in well-defined molecular subsets of NSCLC, explore the biology behind this mechanism of resistance, and summarize the recently published clinical data, including the proposed combination strategies in the clinic achieving success in overcoming acquired *MET* amplification-dependent resistance.

Key words: EGFR, MET, amplification, resistance, NSCLC, targeted therapy

INTRODUCTION

Genomic profiling and the implementation of prospective tumor molecular analysis have revolutionized the way that lung cancer is diagnosed and treated in the clinic. As of 2021, various clinical guidelines [National Comprehensive Cancer Network (NCCN)/College of American Pathologists (CAP)/International Association for the Study of Lung Cancer (IASLC)/Association for Molecular Pathology (AMP)/American Society of Clinical Oncology (ASCO)/European Society of Medical Oncology (ESMO)] recommend genetic profiling of non-small-cell lung cancer (NSCLC) for > 10 genetic alterations, including *EGFR, BRAF, KRAS, ERBB2* (*HER2*) mutations; *ALK, ROS1, NTRK, RET* fusions; and *MET*ex14 skipping and amplification to capture actionable molecular targets.[1-7] Seven of these oncogene alterations now have Food and Drug Administration (FDA)-approved targeted therapies. With targeted therapies, patients with the oncogene-driven NSCLC have remarkable response rates and documented improved progression-free survival (PFS); however, resistance to these targeted therapies inevitably develops despite initial responses.

Understanding and addressing the development of targeted therapy resistance that occurs with tyrosine kinase inhibitors (TKIs) represent a key challenge of the precision medicine era. *MET* amplification is a well-established mechanism of acquired resistance to epidermal growth factor receptor (EGFR) inhibitors,[8,9] and now increasing evidence, reported recently, suggests *MET* amplification is a consistent mechanism of acquired resistance in a number of other oncogene-driven molecular subsets of NSCLC post-tyrosine kinase inhibition. Combination strategies may overcome the genomic heterogeneity of drug resistance; however, the risk of overlapping toxicities and resulting dose adjustments can often hamper effective drug combinations.[10] In this review, we discuss *MET* amplification as a driver of acquired resistance in NSCLC, review the clinical evidence to date in well-defined molecular subsets of NSCLC, discuss the possible mechanisms driving *MET* amplification in this setting, and summarize the proposed TKI combination strategies to overcome *MET* amplification-dependent resistance.

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MET structure and function

MET was first identified in a chemically transformed osteosarcoma-derived cell line.[11] The proto-oncogene was later discovered to encode the receptor tyrosine kinase (RTK) MET, an RTK activated by an endogenous ligand, scatter factor, or hepatocyte growth factor (HGF).[12-15] In general, RTKs such as MET contain an N-terminal extracellular binding domain, a single transmembrane α helix, and a cytosolic C-terminal domain with tyrosine kinase activity.[16] MET is a disulfide-linked heterodimeric RTK consisting of an extracellular α chain, a β chain that encompasses the remainder of extracellular domain, the juxtamembrane, and the kinase domains (Figure 1). The intracellular component contains a juxtamembrane region responsible for signal downregulation and receptor degradation, a catalytic region with the enzyme activity, and a C-terminal region acting as a docking site for adaptor proteins, which leads to downstream signaling via phosphoinositide 3-kinase (PI3K), signal transducer and activator of transcription proteins (STAT), and mitogen-activated protein kinase (MAPK).[17-19] HGF, typically produced and secreted by mesenchymal cells, is the only natural ligand of MET (Figure 1); binding of HGF to MET leads to receptor dimerization and phosphorylation of tyrosine residues in the kinase domain and autophosphorylation of the carboxy-terminal bidentate substrate-binding sites.[20-22] The resulting phosphotyrosines function as docking sites for other proteins involved in RTK-mediated signal transduction,[20-22] and bind to activate distinct downstream signaling pathways,[12,14,21] including PI3K/AKT (protein kinase B), MAPK, STAT, and nuclear factor-kB (Figure 1).[23-25] MET signaling can become dysregulated through several mechanisms, including overexpression of MET protein or MET gene alterations, such as mutations, amplifications, or rearrangements.[26,27] In this review, we focus on amplification of the MET gene in the setting of other oncogene-driven lung cancer.

Detection of MET amplification

MET amplification can occur as a focal amplification or as a result of chromosome 7 polysomy. Polysomy occurs when there are multiple copies of chromosome 7 in tumor cells, secondary to factors such as chromosomal duplication, whereas true amplification occurs in the setting of focal or regional gene duplication, via processes such as breakage—fusion—bridge mechanisms.[28] Clinically, focal high-copy amplification of MET represents an oncogenic driver event for cancer, while polysomy is typically not.[29] Traditionally, MET amplification has been detected using a FISH method, with the challenges of the technical complexity and interpretation of the test.[30] Using FISH, the mean MET per cell and chromosome 7 centromere ratio (MET/CEP7) ratio, the ratio of MET relative to chromosome 7 centromere, is used to distinguish between polysomy and true amplification. In polysomy, each copy of MET is associated with a corresponding centromere, preserving the MET/CEP7 ratio as copy number increases, while in true MET amplification, copy number increases without an increase in CEP7, and the
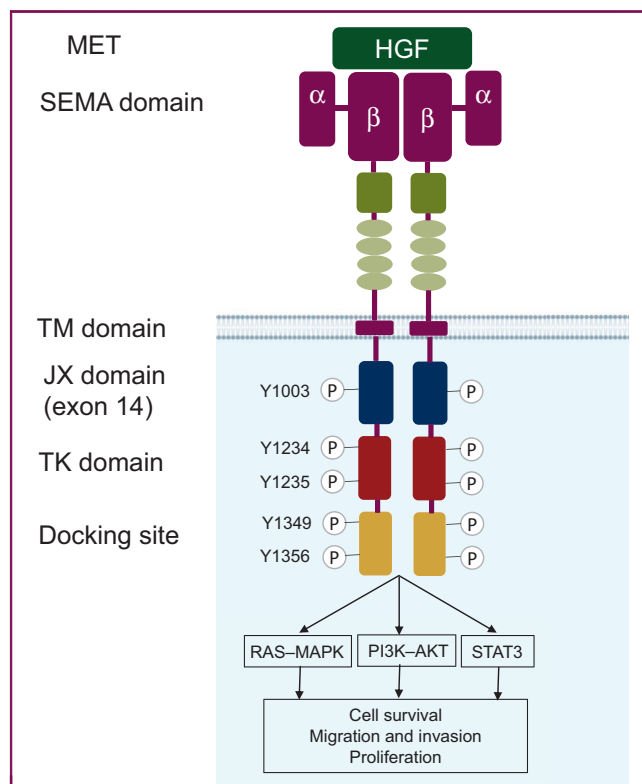

Figure 1. MET and its downstream signaling pathways.
MET is a disulfide-linked heterodimeric RTK consisting of an extracellular α chain, a β chain that encompasses the remainder of extracellular domain, the juxtamembrane, and the kinase domains. HGF is an MET ligand. The intracellular component contains a juxtamembrane region, a catalytic region with the enzyme activity, and a C-terminal region that acts as a docking site for adaptor proteins. MET activates RAS—MAPK, PI3K—AKT, and other oncogenic signaling pathways. AKT, protein kinase B; HGF, hepatocyte growth factor; MAPK, mitogen-activated protein kinase; MET, MET proto-oncogene; NSCLC, non-small-cell lung cancer; PI3K, phosphoinositide 3-kinase; RAS, rat sarcoma virus; RTK, receptor tyrosine kinase.

MET/CEP7 ratio increases.[31] In general, a cut-off of MET/CEP $\geq$2.0 is now used as the FISH criteria for MET amplification; however, historically various cut-offs have been used in studies for MET amplification and MET copy number gain: for instance, a MET/CEP7 threshold of 5 was set as minimum for high MET (FISH $\geq$5 MET signals/cell) in a study by Cappuzzo et al.,[32] MET/CEP7 ratio of $\geq$2 was used by Tanaka et al.,[33] and MET gene copy number (GCN) $\geq$ 10 was ultimately used in the GEOMETRY study.[34]

Broad, hybrid-capture next-generation sequencing (NGS) assays are able to detect amplification events and are now increasingly used in clinical practice for MET amplification detection. In contrast to FISH for single-gene testing, NGS may provide additional information on other, potentially clinically relevant, concurrent genomic alterations.[35] However, some NGS-based assays do not control for CEP7, and therefore may detect increase in copy number as in polysomy rather than true MET amplification. As with FISH, copy number gains detected via NGS are reported as continuous variables, and cut-offs can vary significantly between assays.

An ongoing difficulty with MET copy number studies has been to define a threshold for any given methodology for which MET-directed therapy will likely be active. In theory, increases in MET copy number are postulated to cause

excessive amounts of MET protein, and subsequent auto-aggregation, ligand-independent MET signaling, and subsequent oncogenic addiction to the MET pathway.[27] The challenge is that changes in *MET* copy number represent a continuous variable, and this variable has been assessed in different ways, defined as either the ratio of MET relative to another region of chromosome 7, such as CEP7, or the absolute number of MET copies.[27] A persistent challenge with any defined cut-off criteria, however, is that a more flexible criteria could include more patients but then dilute the clinical benefit, and thus conversely, a more stringent criteria while identifying fewer patients, may include patients who could potentially derive the greatest clinical benefit. The risk is always the potential of excluding patients who may still derive some benefit.

Very high level of *MET* amplification (MET/CEP7 $\geq$5 by FISH) or GCN $\geq$ 10 by NGS often has an absence of other oncogenic drivers, and is therefore viewed as a *de novo* primary oncogene driver in NSCLC.[36-38] In the setting of acquired resistance, the definition of MET amplification can be different; ongoing and future studies will inform the selection criteria for this important biomarker.

MET amplification as a resistance mechanism

MET-dependent resistance is triggered by the activation of common downstream pathways of oncogene receptors, directly by the homodimer formation or indirectly by trans-activating other tyrosine kinase receptors. In the setting of *EGFR*-mutant NSCLC, *MET* amplification leads to resistance by persistent activation of signaling pathways downstream of EGFR, such as those mediated by MAPK, STAT, and PI3K/AKT, independent of EGFR activation and signaling.[39] Signaling occurs through two adaptors: human epidermal growth factor receptor 3 (HER3 or ERBB3), when MET is triggered by genomic amplification, or Grb2-associated binder 1 (GAB1), when MET is activated by HGF.[39] Higher levels of HGF expression have also been detected in tumor samples from patients resistant to the EGFR TKI gefitinib or erlotinib than in treatment-naïve tumor specimens.[39] Resistance has been found even in the absence of *MET* amplification via HGF-induced activation of the AKT pathway.[40,41] Thus, EGFR signaling becomes redundant and, as preclinical and recent clinical studies suggest, targeting both receptors by adding an anti-MET agent to EGFR TKIs is required to obtain an effective antitumor activity.[42] In other oncogene-driven lung cancers, similar downstream signaling pathways were activated, and *MET* amplification functions similarly in a redundant manner to render resistance to TKI treatment to the original driver oncogene.

MET amplification in EGFR-mutant lung cancer

Genomic alterations in the *EGFR* gene account for up to 50% of NSCLC in Asian patients and 10% in Western patients.[43] While the resistance mechanism spectrum evolves with the introduction of third-generation EGFR TKIs, amplification of the *MET* gene as an acquired resistance mechanism to EGFR TKI treatment has been reported in all

generations of TKIs.[8,9,44-46] *MET* amplification as an acquired mechanism resistance occurs in approximately 10%-15% of patients with NSCLC who have received erlotinib, gefitinib, or afatinib.[26,39,45] Osimertinib, a third-generation EGFR TKI, is now used in the first-line setting for patients with advanced *EGFR*-mutant NSCLC.[47-49] Despite this more potent inhibition to EGFR signaling, *MET* amplification remains a major resistance mechanism, occurring still in about 15% of patients with treatment failure of first-line osimertinib,[50,51] and 10%-22% of patients following second-line osimertinib.[8,52,53] In the AURA3 study, *MET* amplification was the most common (19%) resistance mechanism,[52] where it co-occurred with *EGFR* C797S in 7% of cases, and was also likely to be associated with *CDK6* and *BRAF* amplifications.[54] Because of the relative proximity of *CDK6*, *MET*, and *BRAF* on chromosome 7q (7q21.2, 7q31.2, and 7q34, respectively), a single genomic event could be hypothesized to be responsible for gene amplifications.[54] In the FLAURA study, *MET* amplification was also the most common acquired resistance mechanism (15%).[55] MET amplification can also occur as a mechanism of resistance to third-generation EGFR TKIs, with or without loss of the T790M mutation (Table 1).[54,56,57] It is important to note that using diverse patient materials, such as tissue or plasma for analysis of MET amplification, may result in different levels of MET amplification being detected, and so a caveat should be that different levels of MET amplification detected using different samples and techniques in various studies may simply reflect these differences.

Strategies to overcome resistance — MET TKI + EGFR TKI.
Dual inhibition of EGFR and MET is a rationale treatment strategy for overcoming acquired EGFR TKI resistance due to *MET* amplification.[26] Several preclinical studies have demonstrated that concomitant use of MET inhibitors with osimertinib overcame resistance in osimertinib-resistant *EGFR*-mutant NSCLC cell lines with *MET* amplification.[54,58] More recently, preclinical models using the potent and selective MET TKI tepotinib with EGFR TKI (erlotinib, gefitinib, afatinib, or rocentinib) overcame resistance to EGFR TKIs in *EGFR*-mutant NSCLC with *MET* amplification and high MET expression in *in vivo* and *in vitro* models,[59] and led to tumor shrinkage, and even complete regression of established tumors.[59]

The INSIGHT study confirms these data clinically[60]: Wu and colleagues investigated the combination of tepotinib and gefitinib in patients with resistant *EGFR*-mutant NSCLC and with MET overexpression [immunohistochemistry (IHC) 2+ or IHC3+] or *MET* amplification (or both). Nearly 67% of patients with *MET* amplification, treated with tepotinib plus gefitinib, achieved an objective response in both phase Ib and phase II of the study. The combination was also found to be safe and tolerable.[60] Median PFS was 16.6 months with tepotinib plus gefitinib versus 4.2 months with chemotherapy (hazard ratio 0.13 90% confidence interval 0.04-0.43) and median overall survival was 37.3 months with tepotinib plus gefitinib versus 13.1 months with chemotherapy (hazard ratio 0.08, 90% confidence interval 0.01-0.51).[60] This study

Table 1. Summary of key clinical studies identifying *MET* amplification as a mechanism of resistance in oncogene-driven NSCLC

Molecular subset of NSCLC	Number of lung cancer samples + Type	Prior targeted therapy	Incidence of MET amplification	Method of MET amplification testing	Reference
EGFR	Following second-line osimertinib: range 10%-22%				
	83 Plasma		19% (14/83)	NGS	52
	32 Tumor tissue		22% (7/32)	FISH	8
	42 Tumor tissue		14% (6/42)	FISH and/or NGS	54
	41 Tumor tissue		10% (4/41)	NGS and FISH	53
EGFR	Following first-line osimertinib: range 7%-15%				
	91 Plasma		15% (14/91)	NGS	55
	27 Tumor tissue		7.4% (2/27)	NGS	51
ALK	Post-treatment tissue (*n* = 101) or Plasma (*n* = 106)	Crizotinib, or next-generation ALK inhibitors (e.g. lorlatinib)	11 (13%)	FISH and/or NGS	77
RET	23	Selpercatinib or pralsetinib	15%	FISH or NGS	82
ROS1	17	Lorlatinib	6%	NGS and FISH	85
KRAS	10 Tumor tissue and/or plasma	Adagrasib	20%	NGS	95

NGS, next-generation sequencing; NSCLC, non-small-cell lung cancer.

highlights the need for tailored therapy, as the combination of tepotinib and gefitinib was less effective in patients with low MET expression (IHC2+ or less) but greatly beneficial for high *MET* amplification.

In the TATTON study, a number of novel combinations were investigated in previously treated *EGFR*-mutant NSCLC, including the combination of MET TKI savolitinib with osimertinib.[61] In this trial, patients with *EGFR*-mutant NSCLC had received prior EGFR TKI and had evidence of *MET* amplification, defined using FISH as ≥5 copies of MET averaged over 50 cells scored. The objective response rate was 44% (22%-69%) and 30% of patients progressing on a third-generation EGFR TKI showed an objective response. An expansion cohort of this trial included patients with *EGFR*-mutant T790M-negative NSCLC who had not previously received a third-generation EGFR TKI, and objective partial responses of 64% were observed in 23 patients.[62]

There are additional early phase trial data to support the promising combination of MET TKI with EGFR TKI in patients with *MET* amplification-dependent resistance: in a phase Ib/II single-arm trial, capmatinib plus gefitinib in patients with *EGFR*-mutant NSCLC who progressed on an EGFR TKI showed activity in patients with MET-dysregulated NSCLC, particularly in *MET*-amplified tumors [47% of patients with *MET*-amplified tumors (GCN ≥6) and 32% patients with MET overexpression (IHC3+) achieved an objective response].[63] In another phase Ib study investigating MET TKI savolitinib plus gefitinib, up to 52% of patients with *EGFR*-mutant NSCLC with *MET*-amplified tumors (defined as MET to CEP7 ratio ≥2 or GCN ≥5), having relapsed on previous EGFR TKI treatment, achieved an objective response.[64] Some studies have investigated MET-targeted antibodies onartuzumab and emibetuzumab in combination with erlotinib; however, these trials relied on MET overexpression as a stratification marker, and as such did not meet their primary endpoints.[65,66] These data support

the importance of selecting the appropriate drug—drug combination for the appropriate patient (here high-level *MET* amplification).

There are a number of ongoing studies aiming to bring the EGFR plus MET TKI combination to this *EGFR*-mutant *MET*-amplified NSCLC patient population, including INSIGHT 2 (NCT03940703; tepotinib and osimertinib) and SAVANNAH (NCT03778229; savolitinib and osimertinib).[67]

MET amplification in ALK-fusion-positive lung cancer

ALK-fusion-positive NSCLC is another established molecular subtype of lung cancer occurring in 3%-5% of lung adenocarcinomas.[68] Similar to *EGFR*-mutant NSCLC, the clinical benefit of targeting anaplastic lymphoma kinase (ALK) using TKIs is limited by the emergence of drug resistance. Historically, the standard first-line therapy for advanced *ALK*-fusion NSCLC has been the multikinase *ALK/ROS1/MET* TKI crizotinib,[49] which has recently been surpassed by more potent and selective second- and third- generation ALK TKIs, such as ceritinib, alectinib, brigatinib, and lorlatinib.[69-72] Despite initial sensitivity to ALK TKIs, *ALK*-fusion-positive tumors invariably develop resistance, and a number of diverse mechanisms of resistance to ALK TKIs have now been discovered.[73] In ~50% of cases, resistance to second-generation ALK TKIs is due to ALK-independent resistance mechanisms, most often due to activation of bypass signaling pathways, including activation of *MET, EGFR, SRC,* and *IGF-1R*.[74,75] The availability of potent MET TKIs makes MET a particularly attractive target.[74-76]

MET amplification has emerged as a prominent ALK-independent mediator of resistance, and recently *MET* amplification was detected in 15% of tumor biopsies from patients relapsing on selective ALK inhibitors, including 12% and 22% of biopsies from patients progressing on second-generation inhibitors and lorlatinib, respectively.[77] Several

case reports have also confirmed this, and suggest that the *ALK/ROS1/MET* TKI crizotinib may be able to overcome *MET*-dependent resistance.[78,79]

In a comprehensive analysis of *MET* alterations in *ALK*-fusion-positive NSCLC,[77] FISH and/or NGS were performed on 207 post-treatment tissue ($n = 101$) or plasma ($n = 106$) specimens from patients with *ALK*-fusion-positive lung cancer to detect MET genetic alterations. The analysis also evaluated ALK inhibitor sensitivity in cell lines with *MET* alterations and assessed antitumor activity of the *ALK/MET* dual blockade in *ALK*-fusion cell lines and two subsequent patients with *MET*-dependent resistance. Eleven (13%) biopsies harbored *MET* amplification, including four with low-level *MET* amplification (MET/CEP7 2.4—3.9) and six with high-level *MET* amplification based on FISH (MET/CEP7 5.2 to >25) or NGS (16—19 MET copies). One sample had focal MET amplification by NGS, and FISH was too variable to estimate copy number. Of note, no coalterations in other genes potentially associated with resistance or bypass signaling were identified in the 11 biopsies, and *MET* amplification was mutually exclusive with ALK-resistance mutations, with the exception of one case.[77] Patients treated with a second-generation ALK inhibitor in the first-line setting were more likely to develop *MET* amplification than those who had received next-generation ALK inhibitors after crizotinib ($P = 0.019$).[77] This study demonstrated in preclinical models that treatment with MET-specific TKIs capmatinib or savolitinib, none of which have anti-ALK activity, partially suppressed proliferation.[77] However, combination therapy using lorlatinib with capmatinib/savolitinib, or crizotinib potently suppressed cell proliferation and only dual inhibition of ALK and MET by crizotinib or by utilizing the combination of lorlatinib plus a MET TKI effectively suppressed both ALK and downstream signaling pathways.[77] Based on these preclinical findings, two TKI-resistant ALK-fusion-positive lung cancer patients with acquired MET alterations were treated with a combination of lorlatinib and crizotinib and achieved rapid responses to ALK/MET combination therapy, validating that MET was the resistance driver and the combination's therapeutic potential.[77] Although this study confirmed the rationale for exploring ALK/MET combinations in the clinic, in the setting of MET-dependent resistance to ALK TKIs, the optimal combination needs to be evaluated in prospective clinical trials, especially taking into consideration newer generation MET inhibitors' potency and activities for brain metastasis.

This study demonstrated a comparable prevalence of *MET* amplification in patients relapsing on ALK TKIs to that seen in *EGFR*-mutant NSCLC, and interestingly, presented similar findings of increased frequency of *MET* amplification in tumors resistant to the third-generation, broad-spectrum ALK TKI lorlatinib.[77]

MET amplification in RET-fusion lung cancer

The *RET* proto-oncogene encodes an RTK which is activated by gene fusion in 1%-2% of NSCLC, and has been recently defined as a druggable molecular subset of NSCLC.

Selpercatinib[80] and pralsetinib[81] are highly selective RET kinase inhibitors that have recently been FDA approved for advanced *RET*-fusion NSCLC based on impressive efficacy data from the LIBRETTO-001 and ARROW studies, respectively. Molecular mechanisms of acquired resistance to RET inhibitors are not yet fully understood; however, recent data confirm *MET* amplification as a recurrent mechanism of resistance to targeted therapy in NSCLC patients treated with selpercatinib. Lin et al.[82] recently performed a multi-institutional analysis of repeat tumor or plasma biopsies from a cohort of patients with *RET*-fusion NSCLC following treatment with selpercatinib and pralsetinib, to systematically characterize acquired resistance mechanisms to these inhibitors (patients received pralsetinib or selpercatinib in clinical trials).

A strikingly similar prevalence was noted, comparable to *EGFR*-/*ALK*-resistance cases, as three resistant cases (15%) harbored acquired *MET* amplification without concurrent *RET*-resistance mutations[82]; acquired *RET* mutations were identified in two cases (10%) and *KRAS* amplification in one case. A recently published work confirmed these data, and provided further preclinical evidence that that *MET* amplification is sufficient to cause selpercatinib resistance in *in vitro* models.[80] Rosen et al.[80] identified that patients treated with selpercatinib have *MET* amplification associated with resistance to selpercatinib, and proposed a dual targeting strategy to overcome *MET*-dependent resistance to *RET*-directed therapy, piloting a combination strategy using selpercatinib and crizotinib to rescue the phenotype.

Rosen and investigators[80] demonstrated that this combination strategy with selpercatinib and crizotinib overcame *MET*-dependent resistance to selective RET inhibition in patients with *RET*-fusion lung cancer (one case of clinical activity and tolerability, with response lasting 10 months), and that this strategy was tolerable and feasible. The numbers in this report are admittedly small ($n = 4$) and further prospective work is needed, as our collective clinical experience with *RET*-fusion NSCLC is increased. Interestingly, while the level of *MET* gene amplification was shown to clearly increase during selpercatinib monotherapy, in three of four cases, some degree of MET gain was already present before exposure to selpercatinib: this is reminiscent of *EGFR*-mutant NSCLC, in which rare clones with high-level *MET* amplification may be detected at baseline, before EGFR inhibitor therapy.[32,66]

ROS1-fusion-positive lung cancer

Genetic rearrangements of the *ROS1* gene account for 1%-2% of NSCLC. ROS1 can be targeted by TKIs such as crizotinib, entrectinib, and lorlatinib, which results in dramatic responses[6,83,84]; however, *ROS1*-independent resistance mechanisms remain poorly characterized. Recent evidence suggests a role for *MET* amplification.[85]

Lin et al. recently analyzed repeat tumor biopsies derived from advanced ROS1-fusion-positive NSCLC patients progressing on lorlatinib using NGS ($n = 17$) or whole-exome sequencing ($n = 1$) to detect potential drivers of

resistance.[85] While on-target *ROS1* kinase domain mutations featured prominently as a mechanism of resistance, 38.9% harbored a *ROS1*-resistance mutation. NGS analyses also identified MET copy number gain in a lorlatinib-resistant case, validated by FISH as high-level focal *MET* amplification without a concomitant ROS1-resistance mutation. There are no published data investigating combination strategies in *MET*-amplified *ROS1*-positive lung cancer. Thus there is a clear need to further elucidate *ROS1*-independent resistance mechanisms and develop strategies to tackle resistance.

NTRK-fusion-positive lung cancer

Similar to *ALK*- and *ROS1*-fusion-positive lung cancers, neurotrophic tropomyosin-related kinase (*NTRK*)-fusion-positive cancers can develop on- and off-target resistance to TKI therapy. Recently, published data suggest that resistance to TRK inhibition is mediated by genomic alterations that converge to activate the mitogen-activated protein kinase (MAPK) pathway, and *MET* amplification has been identified as a mediator of resistance,[86] together with *BRAF V600E* mutation or hotspot mutations involving *KRAS*. To date, there are a paucity of published reports regarding *NTRK*-fusion-positive lung cancer resistance after TKI therapy; a recent combination of a TRK and MET inhibitor achieved a confirmed response to therapy in a patient with a *NTRK*-fusion-positive cholangiocarcinoma with *MET* amplification-dependent resistance to a first-generation TRK inhibitor, accompanied by the disappearance of detectable *NTRK* fusion and *MET* amplification in circulating free DNA.[86]

BRAF-mutant-positive lung cancer

BRAF gene is mutated in up to 5% of lung adenocarcinomas[87] and represents another actionable target in lung cancer. Scant evidence has been available concerning the mechanisms of resistance to BRAF/MEK inhibitors in BRAF V600E NSCLC. Recently, circulating tumor DNA genomics have demonstrated that mutations in effectors of the MAPK and PI3K pathways may play a role in resistance.[88] Oncogenic mutations in KRAS, which encode immediate upstream regulators of the RAF kinases have also been suggested.[89] *MET* amplification has not been identified as a driver of resistance; however, complete molecular analyses were available for only seven patients.[90]

KRAS G12C-mutant NSCLC

The *KRAS* G12C mutation occurs in ～13%-34% of NSCLC[91,92] and is emerging as the newest actionable target in lung cancer.[93] While previously KRAS has proven to be an elusive target, Hong and colleagues[7] recently demonstrated that a potent and selective small-molecule KRAS G12C inhibitor, sotorasib (AMG 510), can induce impressive, durable responses in *KRAS G12C*-mutant NSCLCs. The phase II CodeBreak 100 trial has recently further validated these impressive data: a response rate of 37.1%, a disease control rate of 80.6%, and a median PFS of 6.8 months.[94] Therefore

understanding the resistance spectrum will become critically important.

Serial biopsy data and serial circulating tumor DNA data have yet to be reported in the CodeBreak study; however, recently published data from Awad et al.[95] have confirmed *MET* amplification as a mechanism for resistance in patients with *KRAS* G12C-mutant NSCLC previously treated with the KRAS G12C inhibitor adagrasib. Preclinical models have highlighted the importance of RTK and SHP2 activation in acquired resistance to KRAS inhibitors,[96] and there are published data which show that *MET*ex14 and *KRAS* G12 mutations can co-occur, at a rate of co-occurrence significantly higher than in other major driver-defined lung cancer subsets.[97] In the study by Awad et al.,[95] 10 patients with KRAS G12C-mutant NSCLC had samples available for assessment on progression of therapy; furthermore, acquired *MET* amplification was the only potential genomic mechanism of adagrasib resistance, which was identified in two of these patients (20%). This is again a frequency rate similar to other oncogene-driven NSCLCs, and suggests that *MET* amplification may also be a driver of resistance to selective small-molecule KRAS G12C inhibitors.

Conclusions

MET amplification is a well-defined mechanism of resistance in *EGFR*-mutant NSCLCs, and in this review, we highlight the increasing number of reports which have underscored that *MET* amplification plays a role in acquired resistance in a number of other oncogene-driven NSCLCs, including *ALK*-, *RET*-, *ROS*-fusion-positive and more recently, *KRAS* G12C-mutant lung cancers. The presence and strikingly similar prevalence of *MET* amplification as a driver of resistance in ～15% of the TKI-treated population across various oncogene-driven NSCLCs is remarkable (Figure 2). In the more recently defined rare molecular subsets of NSCLCs, such as *TRK* fusion, *BRAF* mutant, the relative frequency of off-target resistance, such as *MET* amplification, has yet to be elucidated. Recent data have proved that in *KRAS* G12C-mutant NSCLCs following adagrasib therapy, *MET* amplification is a consistent mechanism of resistance.[95] We eagerly await the serial biopsy data and serial circulating tumor DNA data from the seminal sotorasib *KRAS* G12C study,[7] which will likely confirm adagrasib findings.

As our collective understanding of the molecular mechanisms underpinning TKI resistance in oncogene-driven NSCLCs continues to mature, the development of rationale combination strategies will be crucial in overcoming acquired resistance. For combination therapy, the EGFR story provides the highest level of evidence and proof of concept, where combining potent and selective MET inhibitor with EGFR inhibitor in patients with *EGFR*-mutant *MET*-amplified resistant tumors has produced clinically meaningful responses and tolerable safety profiles.[61,98] *MET* amplification-dependent TKI resistance therefore demands attention from the field because of the need to prospectively identify and treat these patients with efficacious and safe combinations.
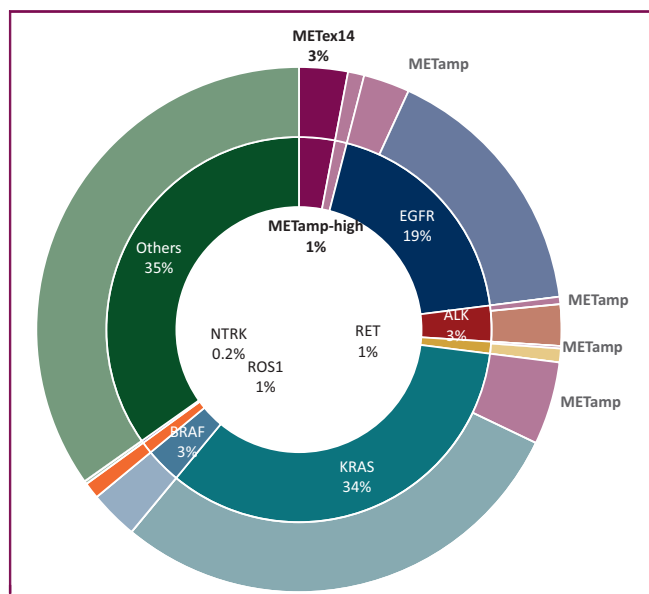
Figure 2. Frequency of MET dependency in lung cancer.
The inner ring represents known primary oncogenic driver alterations in metastatic lung cancers, such as *EGFR, ALK, RET, KRAS,* and *BRAF*. The outer ring illustrates known resistance mechanisms in these oncogenic-driven NSCLC subsets: frequency of *MET* amplification (red) is ∼15% in *EGFR, KRAS,* and *ALK*- and *RET*-fusion-positive NSCLC. Taken together, these data highlight that ∼7%-10% of NSCLC tumors are *MET* dependent, including *de novo* METex14 and high MET amplification. EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer.

FUNDING

DISCLOSURE

XL is supported by Paul Calabresi Award at MDACC (K12/ NIH), Rexanna's Foundation, Khalifa Scholarship, and Conquer Cancer Foundation; received research funds from Eli Lilly, Boehringer Ingelheim, and Spectrum Pharmaceuticals (all outside of the submitted work); received consultant and advisory fee from Eli Lilly, AstraZeneca, and EMD Serono (all outside of the submitted work). DH reports receiving consulting fees and research/grant funding from AbbVie, Adaptimmune, Adlai Nortye, Amgen, Astra-Zeneca, Bayer, Bristol Myers Squibb, Daiichi-Sankyo, Eisai, Eli Lilly, EMD Serono, Erasca, Fate Therapeutics, Genentech, Genmab, GlaxoSmithKline, Ignyta, Infinity, Kite, Kyowa, LOXO, Merck, MedImmune, Millennium, Mirati, miRNA, Molecular Templates, Mologen, Navier, NCI-CTEP, Novartis, Numab, Pfizer, Seattle Genetics, Takeda, Turning Point Therapeutics, Verstatem, VM Oncology; travel, accommodations, and expenses covered by AACR, Amgen, ASCO, Astra Zeneca, Bayer, Celgene, Eli Lilly, Genentech, Genmab, GlaxoSmithKline, Janssen, LOXO, miRNA, Pfizer, Philips, SITC, Takeda; consulting, speaker, or advisory role with Alpha Insights, Acuta, Amgen, Axiom, Adaptimmune, Baxter, Bayer, Boxer Capital, COG, Ecor1, Genentech, GLG, Group H, Guidepoint, HCW Precision, Infinity, Janssen, Merrimack, Medscape, Numab, Pfizer, Prime Oncology, Seattle Genetics, ST Cube, Takeda, Tavistock, Trieza Therapeutics, and WebMD;

other ownership interests in Molecular Match (Advisor), OncoResponse (Founder), and Presagia Inc (Advisor). JH reports advisory/consulting fees from Bristol-Myers Squibb, GlaxoSmithKline, Kairos Venture Investments, BrightPath Therapeutics, Hengrui Therapeutics, Eli Lilly, EMD Serono, and Foundation One Medicine, Spectrum, AstraZeneca; and research funding from NIH/NCI, American Cancer Society, Checkmate Pharmaceuticals, AstraZeneca, and Spectrum; and royalties and patents from Spectrum. JZ is supported by MD Anderson Physician Scientist Award (NIH R01), AACR Johnson and Johnson Innovative Cancer Research Award, Khalifa Scholarship, and Conquer Cancer Foundation; receives research funding from Merck and Johnson and Johnson; personal fees from AstraZeneca, Bristol-Myers Squibb, Geneplus-Beijing Institute, and InnoVent outside the submitted work. NC and LH have no disclosures.

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