MET Exon 14 Skipping Alterations in Non-small Cell Lung Carcinoma—Current Understanding and Therapeutic Advances

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Abnormal activation of mesenchymal epithelial transition (MET) receptor tyrosine kinase is associated with oncogenesis. Various underlying mechanisms, including alteration, amplification, gene rearrangement, and exon skipping in the transcript account for abnormal MET signaling. One of the critical alterations in MET leading to non-small cell lung carcinoma (NSCLC) is MET exon 14 (METex14) skipping, a driver mutation, which accounts for approximately 3–4% of lung adenocarcinoma. METex14 skipping results in the formation of a functionally active and stable truncated receptor lacking the juxtamembrane regulatory domain responsible for MET ubiquitination. Several MET kinase inhibitors have been developed targeting MET receptors, and many are in clinical trials. The US Food and Drug Administration has recently approved capmatinib (Tabrecta®; Novartis, Basel, Switzerland) for the treatment of NSCLC with METex14 skipping alteration. We review the current understanding of the implications of aberrant MET activation in NSCLC harboring METex14 skipping alteration, available diagnostic options, potential therapies in the pipeline, and the future clinical landscape for such alterations.

Advanced non-small cell lung carcinoma (NSCLC) treatment paradigms have evolved during the past decade. Identification of tumor-specific molecular alteration in cancer driver genes has led to the development of targeted therapies. Most of the tumors harboring such alterations are sensitive to tyrosine kinase inhibitor (TKI) drugs, making such oncogenic drivers promising targets for the development of antitumor therapeutics.

MET is a proto-oncogene that can act as an oncogenic driver after certain genomic alterations. It is expressed in many epithelial as well as mesenchymal cells, including hepatocytes, hematopoietic cells, and neuronal cells, and is essential for important biological processes, such as embryonic development and organogenesis. However, mutations and its aberrant activation can promote tumor development and cancer progression by dysregulating downstream signaling pathways. Initially, abnormal MET signaling was believed to be the mechanism of resistance acquired by NSCLC tumor cells against certain therapeutics. Further reports demonstrated the role of MET alterations in sustained MET pathway dysregulation, leading to oncogenesis. Clinically, NSCLCs with MET alterations are associated with poor prognosis, and these alterations have been recognized as an important therapeutic target in various cancers, including NSCLC.

In this review, we discuss the current understanding of the implications of aberrant MET activation in NSCLC harboring MET exon 14 (METex14) skipping alteration, available diagnostic options, potential therapies in the pipeline, and the future clinical landscape.

**Structure and function of the MET receptor**

MET was first identified in a chemically treated human-osteosarcoma-derived cell line as a transforming gene from a fusion of TPR-MET. The MET gene is located on chromosome 7q31 in the human genome, which spans about 125 kb DNA and contains 21 exons and 20 introns. MET is encoded as a precursor, which is modified into a mature protein by proteolytic cleavage between its α and β subunits. A mature MET protein is composed of a small α subunit (50 kDa) and a larger β (145 kDa) subunit linked together by a disulfide bridge. The α subunit and a portion of the β subunit together form the extracellular region of the heterodimer protein, while the remainder of the β subunit comprise the transmembrane and intracellular regions (Figure 1A).
MET signaling and its dysregulation in NSCLC

HGF binding to MET causes dimerization of the receptor leading to the autophosphorylation of intracellular residues Y1234 and Y1235 in the kinase domain followed by phosphorylation of two additional tyrosine residues, Y1349 and Y1356, in the C-terminal outside of the kinase domain (Figure 1B). Phosphorylation of the C-terminal residues leads to the formation of the C-terminal multifunctional docking site. The juxtamembrane (JM) domain, a tyrosine kinase (TK) catalytic domain, and a C-terminal multifunctional docking site lead to the transmembrane helix of MET through the immunoglobulin-plexins-transcription factor domain. The intracellular portion of the receptor includes a juxtamembrane (JM) domain, a tyrosine kinase (TK) catalytic domain, and a C-terminal multifunctional docking site. Binding of its ligand, hepatocyte growth factor (HGF), which is also known as scatter factor, is essential for the activation of the kinase activity. HGF is the only MET receptor ligand known so far and binds to the receptor with high affinity. The extracellular component of MET contains three domains. N-terminal Sema (Sema-pherin) is the largest domain comprising 500 residues, which encompasses the α and a part of β subunits. The domain is essential for the ligand binding, dimerization, and activation of MET. The Sema domain is followed by the plexins-plexin-integrins (PSI) domain, containing four disulphide bonds, which are essential for the proper orientation of the receptor for ligand binding. The PSI domain is connected to the transmembrane helix of MET through the immunoglobulin-plexin-transcription factor domain. The intracellular portion of the receptor includes a juxtamembrane (JM) domain, a tyrosine kinase (TK) catalytic domain, and a C-terminal multifunctional docking site. Binding of its ligand, hepatocyte growth factor (HGF), which is also known as scatter factor, is essential for the activation of the kinase activity. HGF is the only MET receptor ligand known so far and binds to the receptor with high affinity.

MET signaling and its dysregulation in NSCLC

HGF binding to MET causes dimerization of the receptor leading to the autophosphorylation of intracellular residues Y1234 and Y1235 in the kinase domain followed by phosphorylation of two additional tyrosine residues, Y1349 and Y1356, in the C-terminal outside of the kinase domain (Figure 1B). Phosphorylation of the C-terminal residues leads to the formation of the docking site, which is necessary for the engagement of signaling partners. Subsequently, adapter and effector proteins, such as GRB2 (growth factor receptor bound protein 2), GAB1 (GRB2 associated binding protein 1) and SHC (Src homology 2 domain-containing), bind to the docking site triggering downstream signaling. MET signaling plays a crucial role in executing various cellular functions. To maintain functional balance and cellular integrity, MET activity is regulated through various mechanisms. The active MET receptor can phosphorylate at residue Y1003 in the JM domain, a site for the recruitment of E3-ligase Casitas B-lineage lymphoma (CBL), and subsequently undergo ubiquitin-mediated lysosomal degradation, leading to the downregulation of MET (Figure 2A). Additionally, it has been shown that phosphorylation of S985 at JM domain acts as a counterbalance to receptor activation, by negatively regulating its activity, even in the presence of HGF. Furthermore, proteolytic cleavage of MET by ADAMs (a disintegrin and metalloproteinase) and gamma-secretase may also contribute to the downregulation of MET receptor activity.

Alterations in MET can result in the dysregulation of MET signaling, which is present in various solid tumors including NSCLC and is associated with tumor progression and metastasis. Gene amplification, rearrangement, and skipping alterations, which lead to the overexpression and impaired degradation of MET, are the major underlying factors of aberrant MET activation. Alteration or deletion of crucial residues in regulatory domain interfere with mechanisms that help to maintain MET receptor turnover leading to its accumulation and hyperactivation.

METex14 skipping alteration in NSCLC

Skipping of METex14 in NSCLC was first reported in 2005. Substitutions or deletions at 3’ splice site in intron 13 or the 5’ end splice site of
intron 14 results in METex14 skipping. This somatic alteration, at or around the splice junction of METex14, leads to the loss of exon 14 in the transcript and synthesis of the MET protein with an in-frame deletion of 47 amino acids in the JM domain (including residue Y1003) ablating the CBL-mediating ubiquitination and degradation of the receptor (Figure 2B). Consequently, METex14 skipping results in increased levels of MET protein, which can drive activation of downstream signaling pathways that promote tumor development.

Splicing occurs through two sequential steps involving various parts of the intron. The splice donor site and the splice acceptor site are present at the 5′ and 3′ ends, respectively. The splice acceptor site is flanked upstream by the branch point and poly-pyrimidine tract sites (Figure 3A). First, the branch point nucleotide performs a nucleophilic attack on the first nucleotide of the intron at the splice donor site. This forms an intermediate loop or lariat. Subsequently, the 3′ end of the released exon performs a similar nucleophilic attack on the last nucleotide of the SA thereby fusing the exons and releasing the intron lariat. Most METex14 skipping alterations involve the branch point, poly-pyrimidine tract or splice acceptor site in intron 13 or the splice donor site in intron 14. As shown in Figure 3B, these alterations interfere with the splicing mechanism leading to exon 14 skipping.

Interestingly, METex14 skipping alterations are primary oncogenic drivers in NSCLC, as these alterations are most likely to be mutually exclusive to other known oncogenic drivers, such as KRAS, EGFR, ALK, ROS1 or RET. Approximately 3–4% of NSCLCs harbor the METex14 alteration (Table 1). They are associated with some histologic subtypes of NSCLC but are not related to tumor stage. Among the histological subtypes, METex14 skipping alteration is commonly found in sarcomatoid carcinoma (4.9–31%), adenosquamous carcinoma (4–8%), adenocarcinoma (3–4%), and squamous cell carcinoma (2%). Also, among adenocarcinomas, the predominant subtypes are acinar (35–52.9%) or solid subtypes (35.3–53%). Clinically, METex14 skipping abnormality is found mostly in patients of advanced age.

Detection of METex14 skipping alteration

Immunohistochemical analysis is a routine practice for the detection of MET overexpression. However, this technique on its own cannot specifically confirm METex14 skipping or an underlying alteration. Therefore, DNA- and RNA-based molecular assays are preferred methods for the detection of METex14 alteration. DNA-based sequencing assay can detect MET alterations such as insertions, deletions, point mutations, or duplications in splice sites, which may cause exon 14 skipping. Identification of such mutational hotspots leading to METex14 skipping alteration are used to predict the possible skipping event. However, METex14 skipping is associated with more than 120 reported sequence variants in splice sites, which makes it challenging to detect these mutations using only DNA-based assays. Therefore, analysis of RNA transcripts allows for the verification of fusion between exons 13 and 15. In ideal cases, both DNA- and RNA-based assays are used to complement each other for reliable detection of METex14 alterations (Table 1).

Reverse transcription polymerase chain reaction (RT-PCR), quantitative real time RT-PCR, and Sanger sequencing are the routine approaches used for the analysis of mutations and METex14 alteration. mRNAs can be reverse transcribed using RT-PCR and corresponding complementary DNA is sequenced using Sanger techniques to verify exon 14 skipping from the sample. However, efficiency of the method relies on the quality of RNA, which is often derived from formalin-fixed paraffin-embedded or frozen tissue. Sanger sequencing of METex14 and its splice sites is still in routine practice for small scale analysis covering a portion of genomic region, which is performed using the PCR amplicon from genomic DNA covering exon 13 and exon 15, or cDNA from MET transcript. However, the European Society for Medical Oncology (ESMO) guidelines has proposed next-generation sequencing (NGS) and RNA sequencing, if possible, to detect METex14 alteration in its updated guidelines on September 2020.

Recently, NGS has become a common diagnostic method to identify METex14 alterations. This high throughput method allows the large-scale analysis of multiple samples in a short time with comprehensive genomic coverage. The two most popular NGS sequencing panels used for targeted sequence profiling are hybridization capture and amplicon-based sequencing panels. The hybridization capture panel allows more comprehensive profiling for all alteration types, whereas the amplicon-
MET Exon 14 Skipping Alterations in NSCLC—Current Understanding and Therapeutic Advances

Table 1: Prevalence of METex14 skipping alteration and diagnostic method for detection used in various studies

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of patients</th>
<th>Histology</th>
<th>Diagnostic method</th>
<th>Median age, years (range)</th>
<th>METex14 alterations</th>
<th>Reference</th>
</tr>
</thead>
</table>
| USA      | 230                | ADC       | WES               | 68.5 (42–86)             | 4.3% (10/230)       | CGARN (2014)
| Korea    | 70                 | ADC       | RT-PCR followed by gDNA sequencing | 65 | 2.9% (2/70) | Park et al. (2015)
| Hong-Kong| 154                | ADC       | PCR-sanger sequencing | 64.3 (28–90) | 3.9% (6/154) | Yeung et al. (2015)
| USA      | 36                 | PSC       | WES, RT-PCR followed by sanger sequencing | 69.3 (38–87) | 22.2% (8/36) | Liu et al. (2015)
| USA      | 54                 | NSCLC     | RNA sequencing followed by gDNA sequencing | 75 (43–84) | 18.3% (10/54) | Heist et al. (2016)
| China    | 1,296              | NSCLC     | NGS, RT-PCR sanger sequencing | 59 (41–77) | 0.9% (12/1,296) | Liu et al. (2016)
| USA      | 933                | NSCLC     | NGS, confirmation by qRT-PCR | 72.5 (59–84) | 3.0% (28/933) | Awad et al. (2016)
| Hong Kong| 687                | NSCLC     | PCR followed by sanger sequencing (using gDNA) | 74 | 2.6% (18/687) | Tong et al. (2016)
| China    | 1,770              | NSCLC     | qRT-PCR             | 66 (47–77) | 1.3% (23/1,770) | Zheng et al. (2016)
| USA      | 11,205             | Lung cancers | Hybrid capture-based NGS | 73 (43–95) | 2.7% (298/11,205) | Schrock et al. (2016)
| USA      | 860                | ADC       | Hybrid capture-based NGS | – | 3.0% (26/860) | Jordan et al. (2017)
| Korea    | 795                | NSCLC     | qRT-PCR followed by sequencing | 73 (55–81) | 2.1% (17/795) | Lee et al. (2017)
| Korea    | 102                | ADC, PSC  | qRT-PCR             | 73 (59–82) | 8.8% (9/102) | Kwon et al. (2017)
| France   | 231                | ADC, PSC  | MassARRAY iPLEX genotyping technology, sanger sequencing | 61 (41–79) | 5.3% (8/150) | Saffroy et al. (2017)
| Taiwan   | 850                | Lung cancers | RT-PCR followed by sequencing | 77 (36–95) | 3.3% (28/850) | Gow et al. (2017)
| China    | 77                 | PSC       | RT-PCR, NGS        | 62 (37–80) | 20.8% (16/77) | Li et al. (2018)
| China    | 461                | NSCLC     | RT-PCR, sanger Sequencing | 60 (31–87) | 2.0% (9/461) | Qiu et al. (2018)
| USA      | 3,632              | NSCLC     | Hybrid capture-based NGS | – | 3% (113/3,632) | Suzawa et al. (2018)
| Korea    | 414                | NSCLC     | qRT-PCR and/or Sanger sequencing (followed by hybrid capture-based NGS in some of the patients) | 69 (54–80) | 3.14% (13/414) | Kim et al. (2019)
| China    | 46                 | PSC       | NGS sequencing      | 46 (45–80) | 8.7% (4/46) | Yu et al. (2019)
| Netherlands | 1,497            | Non-SC NSCLC | Amplicon-based NGS | 76.5 (53–90) | 2.1% (32/1,497) | Pruis et al. (2020)
| France   | 2,369              | NSCLC     | Sanger sequencing, NGS | 75 (46–97) | 2.6% (62/2,369) | Champagne et al. (2020)

ADC = adenocarcinoma; ALK = anaplastic lymphoma kinase; ASC = adeno-squamous cell carcinoma; CGARN = Cancer Genome Atlas Research Network; EGFR = epidermal growth factor receptor; gDNA = genomic DNA; gDNA = next-generation sequencing; NSCLC = non-small cell lung carcinoma; PSC = pulmonary sarcomatoid carcinoma; qRT-PCR = quantitative real time RT-PCR; RT-PCR = reverse transcription polymerase chain reaction; SC = squamous cell; SCC = squamous cell carcinoma; TCGA = the cancer genome atlas; WES = whole exome sequencing; WT = wild type.

Therapeutic intervention of NSCLC with METex14 skipping alteration

NSCLC characterized with METex14 skipping alterations is targetable. Although many METex14 skipping tumors were found to express programmed death-ligand-1 (PD-L1), the overall response rate to PD-1/PD-L1-directed immune checkpoint inhibitors has been found to be low, and median progression-free survival (mPFS) was found to be short in patients with NSCLC. It should be noted that the mutation burden is generally low in such tumors. There are three therapeutic approaches to target tumors harboring METex14 skipping alteration:
anti-MET and anti-HGF antibodies targeting the extracellular domain of the receptor;

- MET TKIs targeting the intracellular ATP binding pocket of target kinase to inhibit the autophosphorylation of the receptor; and

- antibody–drug conjugates.

Clinical trials and case-reports have suggested varying degrees of responsiveness to experimental and FDA-approved small molecule TKIs against METex14 skipping NSCLC (Table 3). A multicenter retrospective analysis determined that the treatment with a MET TKI was associated with a significant prolongation in survival with a hazard ratio of 0.11 compared to patients who did not receive any MET inhibitor.106 MET TKIs are commonly divided into two types based on their targeting mechanism. Type I MET TKIs—such as crizotinib, capmatinib, tepotinib, and savolitinib—bind to MET in its catalytically active conformation where the aspartic acid-phenylalanine-glycine (DFG) motif projects into the ATP-binding site (DFG-in).87,107,108 Type II MET TKIs—such as cabozantinib, merestinib, and glesatinib—bind to MET in its inactive DFG-out conformation.109,110 Type II MET TKIs are further subdivided into type la (crizotinib and ib (capmatinib, tepotinib, and savolitinib) based on the interaction of TKI with G1163, a solvent residue. Various MET TKIs currently being used in clinical trials are listed in Table 4.

### Multi-kinase MET inhibitors and their response against METex14 skipping NSCLC

This group of inhibitors targets multiple TKs and has been used to target MET kinase. Crizotinib, cabozantinib, merestinib, glesatinib, and TPX-0022 are the major target agents in this group.

### Crizotinib

Crizotinib (PF-02341066; Xalkori®, Pfizer, New York, NY, USA) was originally developed as a MET inhibitor, which showed activity against ALK and ROS1 rearrangement, and was approved as an ALK and ROS inhibitor in NSCLC.111,112 Crizotinib was reported to have potent antitumor activity in NSCLC harboring MET amplification and exon 14 skipping alteration.127,128,129 The phase I study PROFILE 1001 (ClinicalTrials.gov identifier: NCT00585195) was expanded to evaluate the efficacy and safety of crizotinib in 69 patients with NSCLC with METex14 alteration. Among 65 response-evaluable patients, 5% had a confirmed complete response, 28% had a confirmed partial response, 45% had stable disease, and mPFS was 7.4 months.12 In 2018, crizotinib received FDA breakthrough therapy designation for the treatment of patients with NSCLC with METex14 alterations based on a promising response rate of up to 44% in an earlier-phase study.107 A retrospective study of 22 patients treated with crizotinib reported a similar mPFS of 7.4 months. However, a phase II study (METROS; ClinicalTrials.gov identifier: NCT0249961) reported an objective response rate (ORR) of 27% with an mPFS of 4.4 months in patients with NSCLC (n=26) with MET dysregulation with crizotinib.114 Similarly, the AcSé phase II study (by French National Cancer Institute; ClinicalTrials.gov identifier: NCT02034981) reported insufficient ORR (10.7%), even after two cycles of crizotinib in 28 patients with NSCLC with METex14 alteration.115 Furthermore, neoadjuvant treatment with crizotinib in a locally advanced unresectable METex14 mutated lung adenocarcinoma converted the unresectable tumor into a resectable one.116

### Cabozantinib

Cabozantinib (XL-184, BMS-907351; Cabometyx®, Exelixis, Alameda, CA, USA) is a multi-kinase inhibitor targeting multiple TKs including MET. Patients with NSCLC with METex14 skipping alteration have shown the partial response to therapy after treatment with cabozantib.109,117 In a phase II study of solid tumors (ClinicalTrials.gov identifier: NCT00940225) ORR was 10% and mPFS was 4 months.18 Importantly, some case studies of patients with NSCLC with METex14 alteration treated with cabozantinib showed intracranial response.118,119 Phase II studies in patients with NSCLC with MET deregulation are ongoing (ClinicalTrials.gov Identifier: NCT03911193 [CABinMET study], and ClinicalTrials.gov Identifier: NCT01639508).120

### Merestinib

Merestinib (LY2801653) is another multi-kinase ATP-competitive inhibitor of MET.121 After the demonstration of an acceptable safety profile and potential antitumor activity in a phase I trial,122 a phase II clinical study (ClinicalTrials.gov identifier: NCT02920996) is ongoing for the treatment of advanced NSCLC harboring METex14 alterations. A preclinical study demonstrated the antitumor response of merestinib in combination with embetuzumab in

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**Table 2: Next-generation sequencing assays used in the detection of METex14 skipping in clinical studies**

<table>
<thead>
<tr>
<th>NGS assay</th>
<th>Target</th>
<th>Sample quantity</th>
<th>Number of genes covered</th>
<th>Detection of mutations</th>
<th>Turn-over time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor based</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmpliSeq for Illumina Focus Panel</td>
<td>DNA or RNA</td>
<td>1–10 ng DNA or RNA</td>
<td>52</td>
<td>5% frequency</td>
<td>Not available</td>
</tr>
<tr>
<td>Archer® FUSIONPlex™ Lung</td>
<td>DNA</td>
<td>FFPE tissue, 2–250 ng DNA</td>
<td>14</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>FoundationOne® CDx</td>
<td>DNA</td>
<td>50–1,000 ng DNA</td>
<td>324</td>
<td>As low as 2–5% allele frequency</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>Oncomine focus</td>
<td>DNA or RNA</td>
<td>FFPE tissue; 7 mm thick and &gt;5 mm sq</td>
<td>52</td>
<td>100% if mutation is &gt;5% allele frequency</td>
<td>3 days</td>
</tr>
<tr>
<td>TruSight Oncology 500A</td>
<td>DNA or RNA</td>
<td>40 ng DNA or RNA</td>
<td>523</td>
<td>96%</td>
<td>4–5 days</td>
</tr>
<tr>
<td>Liquid based</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archer® LiquidPlex™</td>
<td>Cell free DNA</td>
<td>5–10 ng DNA</td>
<td>28</td>
<td>if present in &gt;1% sample</td>
<td>Not available</td>
</tr>
<tr>
<td>FoundationOne® Liquid</td>
<td>Circulating tumour DNA</td>
<td>2 × 8.5 mL blood samples</td>
<td>70</td>
<td>if present in &gt;0.5% sample</td>
<td>&lt;2 weeks</td>
</tr>
<tr>
<td>Guardiian360®</td>
<td>Circulating free DNA</td>
<td>10 mL blood sample for S–50 ng DNA</td>
<td>73</td>
<td>if present in &gt;0.1%</td>
<td>7 days</td>
</tr>
<tr>
<td>PlasmaSELECT™ 64, PGDx</td>
<td>Circulating tumor DNA</td>
<td>2 × 10 mL blood samples</td>
<td>64</td>
<td>Not available</td>
<td>Not available</td>
</tr>
</tbody>
</table>

**FFPE = formalin-fixed paraffin-embedded; NGS = next-generation sequencing.**
**Table 3: Agents that target METex14 in development**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Company</th>
<th>Type of agent</th>
<th>Targets</th>
<th>Stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multi-kinase inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crizotinib (Xalkori®, PF-02341066)</td>
<td>Pfizer</td>
<td>Ia / ATP competitive TKI</td>
<td>MET, ALK, RON, ROS1</td>
<td>Phase II</td>
</tr>
<tr>
<td>Cabozantinib (Cabometyx®, XL184)</td>
<td>Exelixis</td>
<td>II / ATP competitive TKI</td>
<td>MET, VEGFR-1/2, RET, KIT, TIE2, FLT1/3/4, AXL</td>
<td>Phase III</td>
</tr>
<tr>
<td>Merestinib (LY2801653)</td>
<td>Eli Lilly</td>
<td>II / ATP competitive TKI</td>
<td>MET, AXL, RON, MERTK, ROS1, NTRK1/2/3, TEK, DDR1/2, FLT3</td>
<td>Phase II</td>
</tr>
<tr>
<td>Glesatinib (MGC265)</td>
<td>Mirati Therapeutics</td>
<td>II / ATP competitive TKI</td>
<td>MET, AXL</td>
<td>Phase II</td>
</tr>
<tr>
<td>TPX-0022</td>
<td>Turning Point Therapeutics</td>
<td>II / ATP competitive TKI</td>
<td>MET, CSF1R, SRC</td>
<td>Phase I</td>
</tr>
<tr>
<td><strong>Selective MET TKI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capmatinib (Tabrecta™, INC280)</td>
<td>Novartis</td>
<td>Ib / ATP competitive TKI</td>
<td>MET</td>
<td>Phase II</td>
</tr>
<tr>
<td>Tepotinib (Teometko®, EMD1214063, MSC2156119 J)</td>
<td>Merck</td>
<td>Ib / ATP competitive TKI</td>
<td>MET</td>
<td>Phase II</td>
</tr>
<tr>
<td>Savolitinib (AZD6094, HMPL-504, volitinib)</td>
<td>AstraZeneca</td>
<td>Ib / ATP competitive TKI</td>
<td>MET</td>
<td>Phase II</td>
</tr>
<tr>
<td>Bozitinib (APL-101/PLB-1001, CBT-101)</td>
<td>Apollomics</td>
<td>Ib / ATP competitive TKI</td>
<td>MET</td>
<td>Phase I</td>
</tr>
<tr>
<td>Glumetinib (SCC244)</td>
<td>Shanghai Haihe Pharmaceutical</td>
<td>II / ATP competitive TKI</td>
<td>MET</td>
<td>Phase I/II</td>
</tr>
<tr>
<td><strong>Antibodies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emibetuzumab (LY2875358)</td>
<td>Eli Lilly</td>
<td>IgG4 MoAb</td>
<td>MET</td>
<td>Phase III</td>
</tr>
<tr>
<td>Sym015</td>
<td>Symphogen</td>
<td>IgG1 MoAb mixture</td>
<td>MET</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>REGN5093</td>
<td>Regeneron Pharmaceuticals</td>
<td>MET bispecific Ab</td>
<td>MET</td>
<td>Phase I/II</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telisotuzumab vedotin (ABBV-399)</td>
<td>AbbVie</td>
<td>Antibody-drug conjugate</td>
<td>MET</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Ab = antibody; ALK = anaplastic lymphoma kinase; ATP = adenosine triphosphate; CSF1R = colony stimulating factor 1 receptor; DDR1/2 = discoidin domain receptor tyrosine kinase 1/2; FLT3 = fms-like tyrosine kinase 3; Ig = immunoglobulin; MERTK = MER receptor tyrosine kinase; MoAb = monoclonal antibody; NTRK = neurotrophic-tropomyosin receptor kinase; RON = receptor originated from Nantes; ROS1 = c-ros oncogene 1; TIE2 = tyrosine-protein kinase receptor; TKI = tyrosine kinase inhibitor; VEGFR = vascular endothelial growth factor receptor.

MET inhibitors have produced a promising response in patients with METex14 skipping alteration. Capmatinib, tepotinib, savolitinib, and APL-101 are among the promising agents in this group.

**Glesatinib**

Glesatinib (MGC265) is also a multi-kinase inhibitor of MET, AXL, VEGFR1/2/3, RON, and TIE2, which demonstrated antitumor activity in preclinical and clinical studies with METex14 alteration. It showed antitumor activity of glesatinib in patients who had METex14 skipping alteration and acquired resistance against crizotinib.

**TPX-0022**

TPX-0022 is a novel multi-kinase inhibitor of MET, CSF1R, and SRC, which demonstrated antitumor activity in preclinical xenograft models. A recent phase I clinical study reported that TPX-0022 was well tolerated, and responses were observed in patients with advanced solid tumors harboring genetic MET alterations.

**Selective kinase MET inhibitor on METex14 skipping NSCLC**

This group of inhibitors specifically target the MET receptor by binding to the ACP binding pocket of the MET kinase. Application of such selective MET inhibitors has produced a promising response in patients with METex14 skipping alteration. Capmatinib, tepotinib, savolitinib, and APL-101 are among the promising agents in this group.

**Capmatinib**

Capmatinib (INC280; Trabecta™, Novartis, Basel, Switzerland) is a highly-selective, ATP-competitive MET inhibitor and the first and only MET inhibitor approved by the FDA to target metastatic NSCLC with METex14 skipping alteration as determined by an FDA-approved test. The approval is based on the results from the pivotal GEOMETRY phase II study (ClinicalTrials.gov Identifier: NCT02414139). The primary efficacy outcome based on ORR was 68% and 41% among 28 treatment-naive and 69 previously treated patients, respectively, based on the blinded independent review committee assessment. The median DOR was 12.6 months (n=19) for treatment-naive patients and 9.7 months (n=28) for pre-treated patients. Importantly, capmatinib also exhibited antitumor activity in patients with brain metastases in previously treated NSCLC harboring METex14 alterations.

**Tepotinib**

Tepotinib (END 1214063; Tepmetko®, Merck KGaA, Darmstadt, Germany) is an ATP-competitive and highly-selective oral MET inhibitor, which showed MET inhibitory activity in vitro and in vivo models. It suppressed MET activation by both ligand-dependent and independent mechanisms.
In March 2020, regulatory authority in Japan approved tepotinib for the treatment of NSCLC with METex14 skipping alteration. The approval was based on data from 99 patients with NSCLC with METex14 skipping alteration who had been followed up for 9 months in the ongoing single-arm phase II VISION study (ClinicalTrials.gov Identifier: NCT02864992). The primary endpoint ORR of the study, as assessed by an independent review committee, was 46% with median DOR of 11.1 months for patients identified by combined biopsy (liquid/tissue biopsy). The response rate was 48% for patients in the liquid biopsy group (n=66), and was 50% for those in the tissue biopsy group (n=60). The FDA granted tepotinib a breakthrough therapy designation for the treatment of NSCLC harboring METex14 skipping alterations in September 2019. Recently, a case of antitumor activity of tepotinib in a patient with NSCLC with brain metastasis harboring a MET gene rearrangement was reported.

Table 4: Clinical studies of various MET TKIs in NSCLC with METex14 skipping alteration and observation (final/interim reports)

<table>
<thead>
<tr>
<th>Clinicaltrials.gov Identifier/phase/location</th>
<th>Recruited patients</th>
<th>Population and prior treatment</th>
<th>Drug/dose</th>
<th>Responses evaluated patients</th>
<th>Observations (final/interim reports)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00585195 (PROFILE-1001)/phase I/US</td>
<td>NSCLC with METex14 skipping</td>
<td>69 (62% pre-treated)</td>
<td>crizotinib, 250 mg BID</td>
<td>65 ORR: 32% (95% CI: 21–45); DOR: 9.1 months (95% CI: 6.4–12.7); mPFS: 7.3 months (95% CI: 5.4–9.1)</td>
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</tr>
<tr>
<td>NCT02499614 (METROS)/phase II/Italy</td>
<td>NSCLC with METex14 skipping or MET amplification</td>
<td>26 (100% pre-treated)</td>
<td>crizotinib, 250 mg BID</td>
<td>26 ORR: 27% (95% CI: 11–47); mPFS: 4.4 months (95% CI: 3.0–5.8); OS: 5.4 months (95% CI: 4.2–6.6)</td>
<td>ORR: 27% (95% CI: 11–47); mPFS: 4.4 months (95% CI: 3.0–5.8); OS: 5.4 months (95% CI: 4.2–6.6)</td>
</tr>
<tr>
<td>NCT02034981 (AcSé)/phase II/France</td>
<td>NSCLC with METex14 skipping or MET amplification</td>
<td>28 (96% pre-treated)</td>
<td>crizotinib, 250 mg BID</td>
<td>25 ORR: 10.7% (95% CI: 3.8–22.3); mPFS: 4.0 months (95% CI: 2.0–6.6)</td>
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</tr>
<tr>
<td>NCT02644935 (National Lung Matrix)-Arm D3/phase II/UK</td>
<td>NSCLC including METex14 skipping</td>
<td>12 (100% pre-treated)</td>
<td>Multi-drug (including crizotinib, 250 mg BID)</td>
<td>8 ORR: 65% (95% CI: 39–86); DCR: 68% (95% CI: 39–86)</td>
<td>ORR: 65% (95% CI: 39–86); DCR: 68% (95% CI: 39–86)</td>
</tr>
<tr>
<td>NCT01234479/phase I/global NSCLC with MET dysregulated</td>
<td>55 (100% pre-treated)</td>
<td>capmatinib, 400 or 600 mg BID</td>
<td>55 ORR: 20% (95% CI: 10.4–33); Tumour responses in all 4 patients with METex14</td>
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<td></td>
</tr>
<tr>
<td>NCT02414139 (GEOMETRY mono 1)/phase II/global</td>
<td>NSCLC with cMET mutation or gene copy number or cMET dysregulation</td>
<td>97 (28 prior treatment-naive and 69 pre-treated)</td>
<td>capmatinib, 400 mg BID</td>
<td>28 treatment-naive and 69 pre-treated Treatment-naive: ORR: 68% (95% CI: 48–84); median DOR: 12.6 months (95% CI: 5.5–25.3) Pretreated patients: ORR: 41% (95% CI: 29–53); mPFS: 9.7 months (95% CI: 5.5–13.0)</td>
<td>ORR: 68% (95% CI: 48–84); median DOR: 12.6 months (95% CI: 5.5–25.3) Pretreated patients: ORR: 41% (95% CI: 29–53); mPFS: 9.7 months (95% CI: 5.5–13.0)</td>
</tr>
<tr>
<td>NCT02864992 (VISION)/phase II/global</td>
<td>NSCLC with METex14 skipping or MET amplification</td>
<td>99 (43 prior treatment-naive and 56 pre-treated)</td>
<td>tepotinib, 500 mg QD</td>
<td>99 combined biopsy; 66 liquid biopsy; 60 tissue biopsy Combined biopsy (n=99); ORR: 46% (95% CI: 36–57); DOR: 11.1 months (95% CI: 7.2–NE); mPFS: 8.5 months (95% CI: 6.7–11.0) Liquid biopsy (n=66): ORR: 48% (95% CI: 36–61); DOR: 9.9 months (95% CI: 7.2–NE); mPFS: 8.5 months (95% CI: 5.1–11.0) Tissue biopsy (n=60): ORR: 50% (95% CI: 37–63); median DOR: 15.7 months (95% CI: 9.7–NE); mPFS: 11.0 months (95% CI: 5.7–17.1)</td>
<td>ORR: 46% (95% CI: 36–57); DOR: 11.1 months (95% CI: 7.2–NE); mPFS: 8.5 months (95% CI: 6.7–11.0) Liquid biopsy (n=66): ORR: 48% (95% CI: 36–61); DOR: 9.9 months (95% CI: 7.2–NE); mPFS: 8.5 months (95% CI: 5.1–11.0) Tissue biopsy (n=60): ORR: 50% (95% CI: 37–63); median DOR: 15.7 months (95% CI: 9.7–NE); mPFS: 11.0 months (95% CI: 5.7–17.1)</td>
</tr>
<tr>
<td>NCT02897479/phase II/China</td>
<td>NSCLC (PSC and other NSCLC with METex14 skipping)</td>
<td>70 (prior MET treatment-naive)</td>
<td>savolitinib, 600 mg QD or 400 mg QD</td>
<td>61 ORR: 47.5% (95% CI: 34.6–60.7); DCR: 93.4% (95% CI: 84.1–98.2); mPFS: 6.8 months (95% CI: 4.2–13.8)</td>
<td>ORR: 47.5% (95% CI: 34.6–60.7); DCR: 93.4% (95% CI: 84.1–98.2); mPFS: 6.8 months (95% CI: 4.2–13.8)</td>
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</table>

BID = twice daily; CI = confidence interval; DCR = durable clinical benefit; DOR = disease control rate; DCR = duration of response; mPFS = median progression-free survival; NE = not evaluable; NSCLC = non-small cell lung cancer; ORR = objective response rate; OS = overall survival; PSC = pulmonary sarcomatoid carcinoma; QD = once daily; TKI = tyrosine kinase inhibitor.
MET Exon 14 Skipping Alterations in NSCLC—Current Understanding and Therapeutic Advances

Savolitinib
Savolitinib (volitinib, AZD6094, AstraZeneca, Cambridge, UK) is also a highly selective MET inhibitor.12,13 Interim data from a phase II study (ClinicalTrials.gov Identifier: NCT02897479) reported encouraging antitumor activity and an acceptable safety profile of savolitinib in patients with METex14 skipping NSCLC, including pulmonary sarcomatoid and other histologies. The ORR from preliminary data (n=61) was 47.5% and mPFS was 6.8 months.139

APL-101
APL-101 (bozitinib, CBT-101, PLB-1001), another highly selective MET TKI, has demonstrated robust anticancer activity in various human xenograft tumor models with MET dysregulation and bears the potential to cross the blood–brain barrier in glioblastoma.149,150 Currently, SPARTA, a phase I/II study (ClinicalTrials.gov Identifier: NCT03175224) is evaluating antitumor activity of APL-101 in patients with NSCLC with METex14 skipping and solid tumors with MET aberrations.

Glimetinib
Glimetinib (SSC244) is a highly-selective, ATP-competitive MET inhibitor. The antitumor activity of this agent was demonstrated as equivalent to capmatinib in a preclinical study.151 Currently, phase I/II studies (ClinicalTrials.gov Identifier: NCT03466268) in patients with NSCLC with MET alterations, and another study (ClinicalTrials.gov Identifier: NCT03457532) in patients with solid tumors harboring MET alterations are ongoing for the evaluation of the safety and antitumor activity of glimetinib. A global phase I/II study (ClinicalTrials.gov Identifier: NCT04270591) for patients with NSCLC with MET alterations is also ongoing.

The effect of MET antibodies on METex14 skipping NSCLC
Emibetuzumab
Emibetuzumab (LY2875358) is a humanized bivalent anti-MET antibody that has high neutralization and internalization activities. It showed potent antitumor activity to inhibit HGF-dependent and HGF-independent tumor growth in mouse xenograft models and in MET-positive (including NSCLC) patients.152,153 A preclinical study revealed more antitumor activity of emibetuzumab combined with merestinib on gastric cancer with METex14 mutation.152

Onartuzumab
Onartuzumab is another antibody drug that has shown antitumor activity in preliminary studies. However, it failed to improve the clinical outcomes of MET-positive patients compared with placebo in phase III studies.154

SYM015
SYM015 is a combination of two humanized antibodies directed at the elimination of the MET receptors.155 In a phase I/II study (ClinicalTrials.gov Identifier: NCT02648724), the safety and efficacy of Sym015 in patients with advanced NSCLC with MET amplification and exon 14 deletion were observed. Of 20 patients with NSCLC, the ORR was 25% and the disease control rate was 80%, with median PFS of 5.5 months.157

REGN5093
REGN5093 is a MET bivalent antibody that blocks HGF binding and causes rapid internalization and degradation of MET.158 A phase I/II study (ClinicalTrials.gov Identifier: NCT04077099) demonstrated the safety and tolerability of REGN5093 in patients with NSCLC with MET alterations.159 This study is ongoing and is open for enrollment of patients.

The impact of antibody–drug conjugates on METex14 alteration
Telisotuzumab vedotin
Telisotuzumab vedotin (ABBV-399) is a conjugate of a MET-targeted antibody and monomethyl auristatin E. This antibody–drug conjugate has demonstrated antitumor activity in patients NSCLC with MET dysregulation in a preliminary analysis from a phase I study.159,161

Resistance of METex14 skipping alterations to MET TKIs
Reports suggest that patients with NSCLC with METex14 skipping alterations are sensitive to MET TKI treatment.162,163,164 However, emergence of primary or acquired resistance may challenge the efficacy of MET TKI-based monotherapy.11,12,165 Clinically, the analysis of pre- and post-MET TKI treatment data from 20 patients showed 35% on-target and 45% off-target resistance acquired after the treatment.166 Even though MET is exclusively a driver gene in many cancers, in some cases, METex14 skipping alterations may exist with alterations in other driver genes, such as amplifications of MDM2 (25–35%), CDK4 (3–21%), and EGFR (6–29%), leading to MET TKI resistance. Furthermore, mutations or amplification of KRAS (3–7%) and PIK3CA (3–10%), and loss of PTEN expression (23%) may exist with METex14 skipping alterations contributing to resistance.

The pre-existence of MET Y1230C on-target mutation in addition to METex14 skipping alteration has accounted for the primary resistance to crizotinib.174,175 MET-D1228N-acquired mutation was found to be responsible for the resistance to crizotinib in a patient with METex14 skipping alteration, who did not have any additional mutation in MET or other driver genes before the treatment started.176 A comprehensive analysis of secondary mutations using a Ba/F3 model resistance to eight TKIs reported that D1228 and Y1230 are common sites for resistance mutations for type I TKIs, whereas L1195 and F1200 are the mutations leading to resistance to type II TKIs. D1228A/Y accounts for resistance to both type I and II MET TKIs.177

In addition, tumor cells may activate other signaling pathways to counterbalance the MET TKI suppressed signaling. In such cases, alterations leading to overexpression of key proteins drive the activation of alternative receptors, which leads to the sustained activation of major signaling pathways (bypass signaling) and contributes to the therapeutic resistance, regardless of effective MET inhibition by MET TKI drugs. On the other hand, MET dysregulation, mostly due to METex14 skipping alteration, has also been observed in NSCLC tumors because of off-target acquired resistance to EGFR TKIs.178

Identification of the resistance mechanism to MET TKIs is crucial for the effective treatment of NSCLC. For example, tumors developing acquired resistance against glesatinib (type I) through the amplification of the mutated METex14 allele showed partial response after switching to crizotinib (type Ia).160 Merestinib (type II) was reported to function better against D1228N-mediated acquired resistance, which developed after the application of capmatinib (type IIb).161 Similarly, glesatinib (type II) revealed better antitumor activity in Y1230H/S mutation, which developed after crizotinib (type Ia) treatment.162 Preclinical
MET abnormalities due to METex14 skipping alteration can drive cancer by upregulating receptor activity. It has become a promising target for kinase inhibitor-based targeted therapy in NSCLC, and several recent clinical trials have demonstrated the strong therapeutic effect of such inhibitors. However, it is not yet available to many potential patients who could benefit from such therapy. Firstly, current guidelines do not necessitate the analysis of METex14 skipping alterations for a standard treatment plan, missing the identification of such mutations in many patients and consequently precluding a possible target group from receiving MET inhibitors. Secondly, many early-stage patients may not be subjected to molecular profiling due to the complexity in accessing the tissue biopsy sample. In such cases, application of liquid biopsy and ctDNA genotyping can ease sample availability, which can widen the scope of diagnosis, thereby enabling more patients to get a proper diagnosis and therapy. Additionally, in a detailed understanding of primary and acquired resistance mechanisms can aid in decision making for the appropriate therapeutic intervention. More clinical trials focusing on the combination of MET inhibitors with inhibitors of other signaling pathways can help to identify an appropriate drug combination in MET inhibitor-resistant cancers. ▶

17. Inokuchi M, Otsuki S, Fujimori Y, et al. MET abnormalities due to METex14 skipping alteration can drive cancer by upregulating receptor activity. It has become a promising target for kinase inhibitor-based targeted therapy in NSCLC, and several recent clinical trials have demonstrated the strong therapeutic effect of such inhibitors. However, it is not yet available to many potential patients who could benefit from such therapy. Firstly, current guidelines do not necessitate the analysis of METex14 skipping alterations for a standard treatment plan, missing the identification of such mutations in many patients and consequently precluding a possible target group from receiving MET inhibitors. Secondly, many early-stage patients may not be subjected to molecular profiling due to the complexity in accessing the tissue biopsy sample. In such cases, application of liquid biopsy and ctDNA genotyping can ease sample availability, which can widen the scope of diagnosis, thereby enabling more patients to get a proper diagnosis and therapy. Additionally, in a detailed understanding of primary and acquired resistance mechanisms can aid in decision making for the appropriate therapeutic intervention. More clinical trials focusing on the combination of MET inhibitors with inhibitors of other signaling pathways can help to identify an appropriate drug combination in MET inhibitor-resistant cancers. ▶
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