Oncogenic mechanism-based pharmaceutical validation of therapeutics targeting MET receptor tyrosine kinase

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Abstract: Aberrant expression and/or activation of the MET receptor tyrosine kinase is characterized by genomic recombination, gene amplification, activating mutation, alternative exon-splicing, increased transcription, and their different combinations. These dysregulations serve as oncogenic determinants contributing to cancerous initiation, progression, malignancy, and stemness. Moreover, integration of the MET pathway into the cellular signaling network as an addiction mechanism for survival has made this receptor an attractive pharmaceutical target for oncological intervention. For the last 20 years, MET-targeting small-molecule kinase inhibitors (SMKIs), conventional therapeutic monoclonal antibodies (TMABs), and antibody-based biotherapeutics such as bispecific antibodies, antibody–drug conjugates (ADC), and dual-targeting ADCs have been under intensive investigation. Outcomes from preclinical studies and clinical trials are mixed with certain successes but also various setbacks. Due to the complex nature of MET dysregulation with multiple facets and underlying mechanisms, mechanism-based validation of MET-targeting therapeutics is crucial for the selection and validation of lead candidates for clinical trials. In this review, we discuss the importance of various types of mechanism-based pharmaceutical models in evaluation of different types of MET-targeting therapeutics. The advantages and disadvantages of these mechanism-based strategies for SMKIs, conventional TMABs, and antibody-based biotherapeutics are analyzed. The demand for establishing new strategies suitable for validating novel biotherapeutics is also discussed. The information summarized should provide a pharmaceutical guideline for selection and validation of MET-targeting therapeutics for clinical application in the future.

Keywords: antibody–drug conjugates, bispecific antibody, dual-targeting ADC, MET receptor tyrosine kinase, pharmaceutical validation, small-molecule kinase inhibitor, therapeutic monoclonal antibody, tumorigenic mechanism

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Introduction

MET, a name abbreviated from the carcinogen N-Methyl N nitroso guanidine from previous studies that eventually led to the discovery of truncated MET fused with sequences from the translocate promoter region (TPR-MET),1 belongs to a unique subfamily of receptor tyrosine kinases (RTKs) with distinct structural features and biological activities (Figure 1a).2 The MET gene is located in chromosome 7 (7q31.2) with 21 exons encoding a 180 kDa protein.3 The MET extracellular sequence contains several important domains, including a semaphorin (SEMA) domain followed by a plexin-semaphorin-integrin (PSI) domain, and four immunoglobulin-plexin-transcription (IPT) motifs (Figure 1b).1-3 The SEMA domain harbors a ligand-binding pocket responsible for interacting with hepatocyte growth factor (HGF) (Figure 1c) and is critical for receptor dimerization and subsequent phosphorylation.1-3 The PSI domain acts as a wedge between the SEMA domain and IPT motifs and facilitates the formation of a MET homodimer with interface formed by the SEMA domain from both the
α-chain and β-chain.1–3 The MET intracellular sequence consists of a juxtamembrane (JM) domain, a tyrosine kinase (TK) domain, and a C-terminal multifunctional docking site.1–4 The JM domain contains several important amino acid residues including Y1003, which interacts with casitas B-lineage lymphoma (Cbl) and leads to ubiquitin-dependent MET degradation.5 This process is a mechanism of a negative feedback loop, which controls the MET activation status.1,3,5 The TK domain, upon phosphorylation of Y1234 and Y1235, undergoes a conformational change resulting in increased TK activity,3,4 which leads to phosphorylation of two tyrosine residues, Y1339 and Y1356, in the docking site (Figure 1b).3,4 The docking site is responsible for recruiting adaptor molecules and transduction of different signals to activate multiple downstream signaling pathways (Figure 2).3,4

Cancerous MET expression and activation are featured by genetic recombination, gene amplification,
point mutation, alternative exon-splicing, increased transcription, increased protein accumulation, and their different combinations (Figure 3). The outcomes from these changes imply a complex picture of MET dysregulation, which provides the opportunity to target MET for cancer therapy. Currently, therapeutics such as small-molecule kinase inhibitors (SMKIs) (Table 1),\textsuperscript{15–27} conventional therapeutic monoclonal antibodies (cTMABs),\textsuperscript{28–33} and antibody-based biotherapeutics targeting MET (Table 2) have been validated in preclinical studies and many of them have advanced into clinical trials.\textsuperscript{34–46} Significantly, four SMKIs, crizotinib, cabozantinib, tepotinib, and capmatinib, have been approved for clinical application (Table 1) (www.FDA.gov). Nevertheless, MET-targeting cTMABs, although some of them under clinical trials for almost 10 years, have made little progress. Up to now, none of the cTMABs or antibody-based biotherapeutics have been approved by the FDA. In addition, recent progress in MET-targeted therapy has led to the preclinical development of MET-specific chimeric
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antigen receptor (CAR) T cells and natural killer cells for the treatment of cancers overexpressing MET. Moreover, dual-functioning CAR T cells targeting both MET and programmed death-1 (PD-1) has also been described as a strategy for therapy of solid tumors.

The communication presented here focuses on pathogenic mechanism-based validation of MET-targeting therapeutics for clinical trials. Based on complex mechanisms of MET dysregulation in different types of cancer, our objective is to summarize the latest development of strategies in pharmaceutical validation of MET-targeting therapeutics. Due to the page limitations, MET-targeting CAR T-cell therapy will not be discussed in this communication. As the first step in pharmaceutical development, mechanism-based validation serves as a key principle in selecting lead candidates for potential clinical trials. Considering the biological role of MET in tumorigenesis and its complex nature of dysregulation with various underlying mechanisms, the importance of a validation strategy used in the pharmaceutical development process should not be underestimated.

### MET dysregulation and underlying mechanism

Aberrant MET expression and activation during tumorigenic progression have multiple facets with different underlying mechanisms. At present, the identified forms of MET dysregulation include DNA recombination/rearrangement, gene amplification, point mutation, alternative exon skipping, somatic insertion or deletion, increased transcription, impaired protein degradation, and abnormal protein accumulation.

Several features of these abnormalities are worth mentioning. First, the form of MET dysregulation is different in different types of cancer. Second, the majority of MET abnormalities directly lead to...
Table 1. Pharmaceutical features of MET-targeting small-molecule kinase inhibitors approved or currently in clinical trials.

| MET-targeting agents | Institution produced | Target specificity | Type and mode of action* | Clinical stages | Patient population selection | Overall response rate in clinical trials | Selected biomarker | Reference |
|----------------------|----------------------|--------------------|--------------------------|-----------------|-------------------------------|------------------------------------------|---------------------|-----------|
| Cabozantinib (XL-184) | Exelixis, USA         | MET, RET, FLT3, KIT, TIE2, AXL, TRKB, VEGFR1-3 | Type II, ATP-noncompetitive | FDA approved, Phase II & III | Advanced solid tumors, NSCLC | All tumor: ~21% | none | Kurzrock et al.\(^{15}\), Baltschukat et al.\(^{51}\), Hellerstedt et al.\(^{92}\) [NCT03586973, NCT00704730] |
| Capmatinib (INC280)   | Incyte, USA/Novartis, Switzerland | MET, MET\(^{112350}\), AXL, YSK4, ABL1, CDK11 | Type I, ATP-competitive | FDA approved, Phase II | Advanced solid tumors, NSCLC, HCC | All tumors with MET amplification: 29%; NSCLC with MET mutation: ~40–70%; HCC with MET amplification: 10%; MET amplification, MET exon-14 mutation | Vansteenkiste et al.\(^{14}\), Baltschukat et al.\(^{51}\), Wolf et al.\(^{53}\), Schuler et al.\(^{64}\) [NCT02750215] |
| Crizotinib [PF-02341066] | Pfizer, USA           | MET, RON, ALK, AXL, TIE2, ROS1 | Type I, ATP-competitive | FDA approved, Phase I, II | Advanced solid tumors, NSCLC, papillary RCC | All tumors: ~6%; MET mutations: 36%; NSCLCs with MET exon-14 skipping: 32%; MET amplification: 31%; MET mutation exon 16–19, expression and amplification | Vansteenkiste et al.\(^{16}\), Baltschukat et al.\(^{51}\), Wolf et al.\(^{53}\), Schuler et al.\(^{54}\) (NCT02864992) |
| Tepotinib [EMD1214063] | EMD Serono, Germany   | MET | Type I, ATP-competitive | FDA approved, Phase II | Advanced solid tumors, NSCLC | All tumors: 67%; NSCLCs with MET exon-14 skipping: 45%; MET exon-14 skipping | MET amplification | Hughes et al.\(^{17}\), Van Cutsem et al.\(^{46}\) (NCT02016534) |
| AMG-337               | Amgen, USA            | MET, H1094I, Y1230H, M1250T, p-MET | Type I, ATP-competitive | Phase II | Advanced solid tumors, gastric cancer | All tumors: ~10%; ~30% in MET amplification; gastric: 18% in MET amplification | MET amplification | Friese-Hamim et al.\(^{18}\), Paik et al.\(^{19}\), Falchook et al.\(^{19}\) (NCT02864992) |
| Bozitinib [CBT-101]   | Beijing Pearl Biotech, China | MET | Type I, ATP-competitive | Phase I | Advanced brain glioma | Gioma: ~11%; MET fusion and exon 14 skipping | MET amplification | Shih et al.\(^{20}\), Hu et al.\(^{21}\) (NCT02896231) |
| Foretinib [XL880]     | Exelixis/GSK, UK      | MET, RON, VEGFR2, AKT, KIT, TIE2 | Type I, ATP-competitive | Phase II | Advanced solid tumors, Papillary RCC, gastric cancer | All tumors: 7.5%; Renal: 13.5%; Gastric: 0%; MET amplification, mutation exons 16–19 | MET amplification | Qi et al.\(^{21}\), Shah et al.\(^{22}\) (NCT00725764) |
| Glesatinib [MGCD265]  | Mirati Therapeutics, USA | MET, AXL | Type II, ATP-noncompetitive | Phase II | Advanced solid tumors | Clinical activity in NSCLC with MET exon-14 skipping | MET and p-MET expression | Engstrom et al.\(^{22}\), Reungwetwattana et al.\(^{23}\) (NCT02954991) |
| Golvatinib [E7050]    | Eisai, Tokyo, Japan   | MET, RON, TIE2, VEGFR1-3 | Type I, ATP-noncompetitive | Phase I | Advanced solid tumors | Currently unknown | MET and p-MET expression | Wang et al.\(^{23}\), Bouatour et al.\(^{24}\) (NCT01355301) |
| Merestinib [LY2801653] | Eli Lilly and Co, USA | MET, RON, AXL, TEK, ROS1, Kit | Type II, ATP-noncompetitive | Phase II | Advanced solid tumors | Active as a single agent | Unknown | He et al.\(^{26}\) (NCT03125239) |

(Continued)
| MET-targeting agents | Institution produced | Target specificity | Clinical stages | Type and mode of action | Reference |
|----------------------|----------------------|-------------------|----------------|------------------------|-----------|
| SAR125844             | Sanofi, France/Germany | Met, MET mutants  | Phase I, II    | Type I, ATP-competitive | Egile et al. 25, Iizuka et al. 26 |
| Savolitinib/Volitinib | AstraZeneca, UK        | MET               | Phase I, II    | Type I, ATP-competitive | Schuller et al. 26, Angevin et al. 65 |

**Selected biomarker**
- MET expression and amplification
- Met, MET mutants
- MET activation but not overexpression
- MET expression, mutation exons 16–19

**Clinical stages**
- Advanced solid tumors, NSCLC:
  - Overall response rate in clinical trials: 17–19%
- Renal with MET mutation:
  - MET expression, mutation exons 16–19
  - Met, MET mutants

**Type I MET SMKIs** target the ATP-binding pocket of the active form of the kinase domain in MET, ABL1, ABL proto-oncogene 1, non-receptor tyrosine kinase; ALK, anaplastic lymphoma kinase; ATP, adenosine triphosphate; CDK47-MET, GPRC5C-MET, and others.

**Type II MET SMKIs** interact with the ATP-binding pocket in the inactive form of the kinase domain in MET, ABL1, ABL proto-oncogene 1, non-receptor tyrosine kinase; ALK, anaplastic lymphoma kinase; ATP, adenosine triphosphate; CDK47-MET, GPRC5C-MET, and others.

**MET fusion** occurs when the MET extracellular sequence at a particular region is fused with different partner sequences under DNA recombination/rearrangement, resulting in various forms of MET fusion proteins such as CLIP2-MET, ST7-MET, CDK47-MET, GPRC5C-MET, and others.1,6,55,72,73,86,87

**TPR-MET** was the first fusion protein identified under the action of certain carcinogens.1 Mechanistically, MET fusion occurs through either intrachromosomal or interchromosomal rearrangements.1,6,47–50,72,86–89 The frequency of MET fusions in cancer such as those from lung, gastric, hepatic, kidney, and pancreatic tissues is relatively low, ranging from 0.1 to 2%.86–89 The only exception is glioma, in which MET fusion has been found in ~12% cases.72,89

**Gene amplification**, occurring as polysomy and focal events,86 exists with variable frequency in different types of cancer such as stomach and lung cancer.74–76

**Tumors** with identified frequencies of MET gene amplification include non-small cell lung cancers (NSCLCs, <1–5%), gastric cancers (<1–10%), colorectal cancer (CRC, 2–4%), and papillary renal cell carcinomas (3–135).86 The outcome is often characterized by accumulation of a large amount of activated MET proteins.74–76

**Somatic alterations** in the MET gene serve as another pathological feature observed in several types of cancers, particularly in hereditary and sporadic papillary renal cell carcinomas.87–79 They are often manifested by insertion, deletion, and missense mutations with different frequencies in different domains of MET.87–79,90–92 which profoundly affect the structural and functional integrity of the SEMA, JM, and TK domains (Figures 2 and 3).10,51,78–80,90–92

**The observed frequencies** are ~15% in papillary renal cell carcinomas, ~7% in hepatocellular carcinomas (HCCs), and up to 14% in patients with head and neck cancers.86
| Names of TMABs | Production institution | Antibody subtype and target | Linker properties | Binding properties | MET internalization and degradation | Drug conjugated | Biological activity in cell model | Therapeutic efficacy in mouse model | Toxicological profiles | Evaluation stages | Reference |
|----------------|------------------------|-----------------------------|-------------------|-------------------|-----------------------------------|----------------|-----------------------------|------------------------------------|--------------------------|----------------|----------|
| MET-HER1       | Roche Diagnostics GmbH, Germany | Humanized IgG1, MET & EGFR | None              | Specific; MET, 24 nM; EGFR, 5.8 nM | Unknown                          | None           | Inhibiting MET signaling decreasing cell growth, blocking scattering, migration, | Growth inhibition (1-45%) without tumor shrinking activity | Unknown                  | Preclinical | Castoldi et al.66 |
| Amivantamab (JNU-61183721) | Janssen Res & Dev, USA | Humanized IgG1, MET & EGFR | None              | Specific; MET, 40 pm; EGFR, 40 PM | Highly effective | None           | Preventing HGF binding, inhibiting MET signaling, inducing cell death & lysis, ADCC involved | Growth inhibition (80-100%) with tumor shrinking activity | Well tolerated, no obvious toxic activities | Phase II, ongoing | Yun et al.34, Park et al.69 (NCT04538664) |
| LY3164530      | Eli Lilly & Company, USA | Humanized IgG1, MET & EGFR | None              | Specific to both MET and EGFR; Affinity: N/A | Highly effective | None           | Preventing HGF binding, attenuating MET signaling, inhibiting cell growth | Inhibition of tumor growth, detail unknown | Well tolerated, no obvious toxic activities | Phase I (discontinued) | Patnaik et al.35, Liu et al.70 (NCT02221882) |
| ME22S          | Ajou University, Korea | Humanized IgG1, MET & EGFR | None              | Specific to MET & EGFR; Affinity: N/A | Moderately effective | None           | Inhibiting MET signaling, decreasing growth, migration & invasion, causing cell apoptosis | Moderate inhibition of tumor growth | Unknown                  | Preclinical | Lee et al.71 |
| MM-131         | Merrimack Pharmaceuticals, USA | Humanized IgG1, MET & EpCAM | None              | Specific; MET, 0.2 nM; EpCAM, 10 nM | Moderately effective | None           | Preventing HGF binding, inhibiting MET signaling, blocking cell growth and migration | Growth inhibition (80-93%) with tumor shrinking activity | Unknown                  | Preclinical | Casaletto et al.36 |
| BsVeMET        | Ajou University, Korea | Humanized IgG1, MET & VEGFR-2 | None              | Specific; MET, ~4 nM, VEGFR-2, 18 nM | Unknown | None           | Inhibiting MET signaling, reducing cell growth, viability, impairing tubular formation | Growth inhibition (1-75%) without tumor shrinking activity | Unknown                  | Preclinical | Choi et al.37 |
| MET-PD-1Bs/ MET/PD-1 DVD-IgM/MET/ PD-1 IgG-ScFv | Fudan University, China | Humanized IgG1, MET & PD-1 | None              | Specific to both MET & PD-1; Affinity: N/A | Highly effective | None           | Activating T cell, stimulating cytokine production, inhibiting cell growth, migration | Growth inhibition (1-50%) without tumor shrinking activity | Unknown                  | Preclinical | Hou et al.38, Sun et al.39 |

(Continued)
### Table 2. (Continued)

| Names of TMAbs | Production institution | Antibody subtype and target | Linker properties | Binding properties | MET internalization and degradation | Drug conjugated | Biological activity in cell model | Therapeutic efficacy in mouse model | Toxicological profiles | Evaluation stages | Reference |
|----------------|------------------------|----------------------------|-------------------|-------------------|-------------------------------------|----------------|----------------------------------|------------------------------------|----------------------|-----------------|-----------|
| Telisotuzumab vedotin (ABBV-399) | AbbVie Oncology USA | Humanized IgG1, MET | Dipeptide, cleavable | Specific; MET, ~0.5 nM | Highly effective | MMAE | Inhibiting cell growth and inducing cell apoptosis | Growth inhibition (70–100%) with tumor shrinking activity | In primate: bone marrow, liver & digestive | Phase II | Wang et al.40 (NCT03574753) |
| TR1801-ADC | Tanabe Research Laboratories USA, USA | Humanized IgG2, MET | Dipeptide, cleavable | Specific to MET, ~0.3 nM | Moderately to highly effective | PBD | Inhibiting cell growth and inducing cell apoptosis | Growth inhibition (80–100%) with tumor shrinking activity | Acceptable in rat; human in progress | Phase I, ongoing | Gymnopoulos et al.42 (NCT03859752) |
| SHR-A1403 (with SHR152852) | Hengrui Medicine Co Ltd, China | Humanized IgG2, MET | MC based, non-cleavable | Specific; MET, ~1.8 nM | Moderately to highly effective | Auristatin analog (SHR152852) | Inhibiting cell growth and inducing cell apoptosis | Growth inhibition (70–100%) with tumor shrinking activity | Acceptable in primates; Human in progress | Clinical, Phase I | Yang et al.41 (NCT03856541) |
| HucMET27-DGN549 and HucMET27-DM4 | ImmunoGen, Inc., USA | Humanized IgG1, MET | Site specific for DGN549 & dipeptide for DM4 | Specific; Affinity: sub-nM | Highly effective | DGN549 & DM4 | Inhibiting cell growth and inducing cell apoptosis | Strong growth inhibition with MET overexpression or amplification | Unknown | Preclinical | Yang et al.41 |
| cIRC201-dPBD | Sungkyunkwan University, Seoul, Korea | Humanized IgG1, MET | Site specific for PBD/glucuronic linker | Specific; Affinity: 1.5 nM | Highly effective | PBD | Inhibiting cell growth and inducing cell apoptosis | Growth inhibition (80–100%) with tumor shrinking activity | Unknown | Preclinical | Gymnopoulos et al.42 |
| B10v5x225-H/M-vc-MMAE | Technische Universität Darmstadt, Germany | Humanized IgG1, MET & EGFR | Dipeptide, cleavable | Specific to MET & EGFR | N/A | MMAE | Inhibiting cell growth and inducing cell apoptosis | Unknown | Unknown | Preclinical | Lai et al.43 |
| PCMdt-MMAE | PCM Targetech LLC TX, USA | Humanized IgG1, MET & RON | Dipeptide, cleavable | Specific to MET & RON | Highly effective | MMAE | Inhibiting cell growth and inducing cell apoptosis | Growth inhibition (70–100%) with tumor shrinking activity | Unknown | Preclinical | Min et al.44 |

*ADC, antibody–drug conjugates; ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; DGN549, a DNA-alkylating payload indolinobenzodiazepine; DM4, maytansinoid derivative 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; MC, Maleimidocaproyl; MET, a name abbreviated from the carcinogen N-Methyl N-nitroso guanidine used in studies leading to the discovery of the fusion protein consisting of a N-terminal truncated MET linked to the translocate promoter region; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; nM, nanomolar; PBD, pyrrolobenzodiazepines; PD-1, programmed death-1; PDX, patient-derived tumor xenografts; VC, chemical linker containing Val-Cit structures; SHR15852, a synthetic auristatin analog; VEGFR-2, vascular endothelial growth factor receptor 2.
splicing, particularly exon-14 skipping, is currently a hot topic due to its clinical significance associated with oncogenesis.\textsuperscript{9,80–82} Exon-14 encodes the JM domain of MET,\textsuperscript{3} which regulates the MET metabolic degradation through the Cbl-directed ubiquitin pathway.\textsuperscript{9,80–82} Alternative exon-14 skipping is caused by insertion/deletion in the acceptor or donor regions or by missense mutations in certain tyrosine residues including Y1003 (Figure 4). This results in the inability of

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**Figure 4.** Activating mutations in the different functional domains of MET. (a) Various mutations in the tyrosine kinase domain of MET. Point mutations in more than 16 amino acid residues in the kinase domain have been documented in different types of primary cancer samples. These mutations often result in a conformational change that facilitates the kinase domain to convert into an active mode with increased kinase activity. (b) Missense mutations in the exon 14 ubiquitination site. The JM domain is encoded by MET exon-4. The tyrosine residue Tyr\textsuperscript{1003} in the JM domain is responsible for the interaction with the ubiquitin E3 ligase, which promotes MET degradation, a negative feedback mechanism for controlling levels of MET activation. The mutation results in the inability of Tyr\textsuperscript{1003} to interact with ubiquitin E3 ligase, leading to an increase in stability of MET. (c) Alterations in the exon-14 splice site often results in exon-14 skipping, leading to formation of a MET slicing variant known as MET exon-14 skipping. The consequence is that this MET variant is resistant to ubiquitin-mediated protein degradation with increased stability and kinase activity. (d) Various mutations are documented in the SEMA domain of MET. Since the SEMA domain contains the MET-binding pocket; it is speculated that these mutations will affect the ability of HGF binding to MET with reduced affinity. However, pathological implication of these mutations in association with clinical oncological events currently are largely unknown.
the JM domain to interact with Cbl E3 ligase, and ultimately leads to the accumulation of a large amount of MET protein with increased stability and elevated kinase activity. The frequency of MET exon-14 skipping occurs 3–4% of patients with NSCLCs. The alteration is further enriched in patients with sarcomatoid carcinomas (9–22%), an aggressive subtype of NSCLC. The mechanism that causes cancerous MET overexpression is complex. Transcriptional upregulation appears to be the major cause. For instance, hypoxia-initiating factor (HIF)-1α is one of the triggering factors responsible for increased MET transcription. Activation of signaling proteins such as reticular activating system (RAS) also upregulates MET expression through the transcriptional event. Moreover, both gene amplification and exon-14 skipping are involved in abnormal accumulation of large amounts of MET protein. The documented MET overexpression in primary tumor samples determined by immunohistochemical staining include prostate cancer (~55%), gastric cancer (~65%), HCC (~50%), CRC (~55%), triple-negative breast cancer (TNBC, ~15%), and NSCLC (~50%). Thus, various mechanisms are involved in cancerous MET overexpression.

**MET therapeutics with different mechanisms of action**

**Small-molecule kinase inhibitors**

SMKIs discussed here are structurally designed and chemically synthesized small molecules that are specific to a unique kinase domain of MET and other proteins with similar kinase structure. The use of SMKIs has several pharmaceutical advantages and has been clinically proven to be effective. The principle of using SMKIs for cancer therapy is based on cellular oncogenic signaling addiction/dependence. Currently, chemical design and large-scale synthesis of SMKIs are not a technical challenge due to the use of computer-aided structural analysis and synthetic chemistry platforms. The use of these advanced technologies, in general, ensure to generate MET-specific SMKIs with variable targeting specificity. Besides four SMKIs specific to MET, including crizotinib, caboazantinib, tepotinib, and capmatinib, that have already been approved by the FDA (Table 1), additional SMKIs such as AMG-337, bozitinib (APL-101), glesatinib (MGCD265), Golvatinib (E7050), merestinib (LY2801653), savolitinib, Sar125844, and others appear to be promising in clinical trials (Table 1). Mechanistically, SMKIs are the choice for inhibiting both cell-surface and intracellular MET protein that displays both an inactive and active status in the TK domain. An inhibitory effect is achieved by SMKIs binding to the critical region in the TK domain, either competing with adenosine triphosphate (ATP) for binding to the ATP-binding pockets in the TK domain or by preventing the conversion of the TK domain from an inactive conformation into an active mode. Moreover, the therapeutic activity of SMKIs is independent of HGF-mediated MET activation regardless of the presence or absence of HGF in the tumor microenvironment or via a cancer cell autocrine-producing fashion. The major disadvantage of SMKIs is that their anticancer action is heavily dependent on the strength of MET signaling integrated into the cellular signaling network and the addictive levels acquired by cancer cells for growth and survival. In the preclinical studies, mechanism-based validation appears to be able to objectively determine the effectiveness of individual MET-targeting SMKIs. Nevertheless, in clinical trials and practice, the status of MET signaling addiction by cancer is difficult to assess. Although immunohistochemical (IHC) staining, fluorescence in situ hybridization (FISH), and next-generation sequencing (NGS) have been used as biomarkers for patient selection, these methods are unable to determine the addictive status of cancer cells to MET signaling.

**Therapeutic monoclonal antibodies**

Therapeutic monoclonal antibodies (mAbs) described here are defined as natural or recombinant mAbs specific to MET (cTMABs) or to both MET and other signaling proteins (bispecific mAbs) without drug, cytotoxin, or radioisotope conjugation. Both cTMABs and bispecific antibodies have been evaluated as MET-targeting biotherapeutics. Representative cTMABs are ARGX-111, emibetuzumab, onartuzumab, SAIT301, telisotuzumab, and Sym015, which have been in different phases of clinical trials. Anti-HGF TMBAs ficlatuzumab and rilotumumab are also under clinical trials. However, none of the therapeutic mAbs specific to MET or HGF have currently been approved by the FDA. The objective of using cTMABs is to suppress HGF-dependent and -independent MET activation, resulting in inhibition of cell proliferation,
induction of cellular apoptosis, and regulation of host immune activity.\textsuperscript{28–33,93–95} In this sense, the induction of these activities is a biological criterion for the selection of MET-targeting cTMABs for clinical application. However, the mechanisms of action by these cTMABs rely on the levels of cellular addiction to MET signaling. Preclinical studies have demonstrated that anti-MET cTMABs have therapeutic activities against different types of cancer. Nevertheless, the observed efficacies vary significantly among individual TMAbs tested.\textsuperscript{28–33,93–95} Moreover, outcomes from clinical studies at different phases are disappointing.\textsuperscript{93–100} Currently, conventional anti-MET TMAbs, although under clinical trials for almost 10 years, have not been approved for clinical application, mainly due to the lack of therapeutic efficacy but not pharmacokinetic or toxicological issues.\textsuperscript{93–100}

Five MET-based bispecific antibodies targeting partner proteins, including EGFR, VEGFR-2, epithelial cell adhesion molecule (EpCAM), and programmed cell death (PD)-1, have been preclinically evaluated (Table 2).\textsuperscript{34–39} The rationale to select these partner targets is either to achieve a coordinated inhibition of two signaling pathways or to regulate the immune response by targeting immunomodulatory molecules to enhance anticancer activity.\textsuperscript{34–39} Inhibition of two signaling pathways has clinical relevance for treatment of tumors that develop resistance to chemotherapeutics or kinase inhibitors. Similarly, restoration of T-cell activity by targeting PD-1 is an approach in the format of a bispecific antibody.\textsuperscript{38,39,101,102} Currently, only two bispecific antibodies, amivantamab and LY3164530 (both targeting MET and EGFR), have entered into clinical trials (Table 2).\textsuperscript{34,35,80,95} Amivantamab is effective in NSCLC patients with EGFR exon-20 insertion mutation, which has led the FDA to grant it the Breakthrough Therapy Designation status (www.FDA.gov). Interestingly, the role of amivantamab in targeting MET is not mentioned in this group of NSCLC patients. LY3164530 has been terminated in clinical trials due to toxicity.\textsuperscript{35}

**Single and dual-targeting antibody–drug conjugates**

Antibody–drug conjugates (ADCs) are a class of targeted biotherapeutics consisting of a target-specific mAb, a versatile chemical linker, and a highly potent cytotoxic payload.\textsuperscript{103,104} The combination of antibody-based antigen specificity with payload cytotoxic potency results in an increased therapeutic index, favorable pharmacokinetic profile, and acceptable toxicological activity.\textsuperscript{40–46} Up to now, the FDA has approved nine ADCs, including gemtuzumab ozogamicin, brentuximab vedotin, trastuzumab deruxtecan, sacituzumab govetecan and others, for oncological application (www.FDA.gov). These ADCs target HER2, CD22, CD30, Trop-2, and others for treatment of various types of cancer. Currently, all MET-targeting ADCs are still under clinical trials without any approval by the FDA. The major mechanisms of action by ADCs are mediated by antibody-directed delivery of a cytotoxic payload for cancer cell killing. Other activities exerted by antibodies, such as antibody-dependent cell-mediated cytoxicity, are also involved in cancer cell killing.\textsuperscript{40–46} Currently, five single targeting ADCs specific to MET, namely ABBV-399 (telo-sotuzumab vedotin), SHR-A1403, TR1801-ADC, HucMet27-based ADC, and ciRCR201-dPBD have been preclinically validated (Table 2).\textsuperscript{40–44} The obtained results indicate that these MET-targeting ADCs are highly effective against cancer cellular models and patient-derived xenografts (PDXs) that harbor different forms of MET dysregulation. These forms of dysregulation include overexpression, amplification, exon-14 skipping, and activation mutation regardless of the level of MET signaling status involving cancer cell addiction.\textsuperscript{40–44} Two MET-based dual-targeting ADCs, including B10v5x225-H/M-vc-MMAE (targeting both MET and EGFR) and PCMdt-MMAE (targeting both MET and RON) have been preclinically studied (Table 2).\textsuperscript{45,46} B10v5x225-H/M-vc-MMAE is a dual-targeting ADC specific to both MET and EGFR.\textsuperscript{45} Preclinical studies indicate that B10v5x225-H/M-vc-MMAE coordinately binds to both MET and EGFR, blocks ligand-induced MET and EGFR activation, and induces both receptors to internalize. These activities in vitro result in inhibition of MET/EGFR-mediated tumorigenic signals and cytotoxicity of various types of cancer cells.\textsuperscript{43} PCMdt-MMAE is a MET and RON dual-targeting ADC developed by PCM TargeTech in Texas.\textsuperscript{46} RON belongs to the MET family, important in epithelial tumorigenesis, and is a validated drug target.\textsuperscript{105} Results from both in vitro and in vivo studies have demonstrated that PCMdt-MMAE is highly effective against the growth of xenograft tumors mediated by various types of cancers that express different levels of MET, RON, or both receptors with a favorable pharmacokinetic profile.\textsuperscript{46} Currently, PCMdt-MMAE is ready for
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government-regulatory approval and transition into clinical development.

Mechanism-based evaluation of MET-targeted therapeutics

Tremendous efforts have been made during the last 20 years to optimize mechanism-based validation strategies for MET-targeting SMKIs and cTMABs.15–33 Pharmaceutical innovation resulting in novel biotherapeutics also pushes for the development of new strategies to meet validation demands. The principle of a mechanism-based validation strategy depends on the type of MET-targeting therapies being tested. Practically, the therapeutic efficacy of SMKIs, cTMABs, and bispecific antibodies highly rely on the addictive status of cellular models to MET signaling for growth and survival.15–39 In contrast, the activity of ADC-based biotherapeutics is associated with levels of MET expression and sensitivity of cancer cells to cytotoxic payloads attached to the mAb.40–46 Thus, logical selection of a proper mechanism-based drug validation strategy is the first step required for drug evaluation.

Increased MET expression as a validation mechanism

Quantitative MET analysis has made this model highly attractive for initial drug screening. For instance, 49 gastric cancer cell lines with integrated genomic profiling have been analyzed to establish a pattern of MET expression as a reference.106 Moreover, MET amplification, HGF production, and expression of other oncogenic kinases such as RAS, EGFR, HER2, and PI-3 kinase have been matched in many individual cell lines.106 The use of this 49-cell-based model is highly valuable for validating various types of MET-targeting therapeutics, particularly ADCs, which depends on the level of MET expression and their subsequent internalization for delivering cytotoxic payloads. As indicated in a previous study, the ADC-mediated responsiveness in vitro is proportionally correlated with levels of cancerous MET expression.40 A similar correlation trend has also been observed in animal studies, in which the effectiveness of MET-targeting ADCs is positively correlated with xenograft tumors expressing different levels of MET expression.40–44 Moreover, the use of advanced drug-linker technologies and the selection of highly potent payloads have dramatically lowered the threshold of MET expression required for an ADC to exert significant cytotoxicity.42,44 These observations have potential implication in clinical trials for selecting patient populations showing variable levels of MET expression.

Levels of MET expression as a validation marker has limitations. Increased MET expression is only a phenotypic appearance, which reflects only alterations by a particular genetic or cellular pathway. However, these aberrations, alone or in combination, contribute to increased MET expression.6–14,74–76,83–85 Importantly, levels of MET expression, including overexpression, are not equivalent to a MET-dependent or addictive status by cancer cells.86,87 Nevertheless, overexpression indeed results in MET phosphorylation with activation of downstream signaling pathways, which leads to increased cellular activities such as malignant phenotypes.72,55,73–85 However, the detection of MET signaling activation by no means implies that cancer cells are addicted to MET for growth and survival.86,87 Clinical studies show that increased MET expression is not directly associated with the efficacy of MET-targeted therapy using either SMKIs or cTMABs.86,87 The lack of signaling addiction or low levels of MET signaling addiction is the major reason for the inefficacy of MET-targeted therapeutics regardless the level of MET expression. Thus, MET overexpression is not a reliable biomarker and performs poorly for predicting clinical benefits for MET-targeting SMKIs and conventional TMABs.15–33,86,87

MET amplification as a validation mechanism

Validation of therapeutics for MET-amplified tumors is an essential pharmaceutical step. Amplification is a distinctive feature of MET dysregulation and often shows increased signaling activation with advanced oncogenesis.74–76 Currently, more than 20 cancer cell lines harboring variable degrees of amplification (Table 3) have been used to evaluate the effectiveness of MET-targeting therapeutics.15–33 This evaluation has helped identify those, such as AMG-337, that are highly effective against tumor models caused by MET-amplified cancer cells.19 The cellular MET amplification model is also suitable for analysis of MET-targeting cTMABs, bispecific antibodies, ADCs, and dual-targeting ADCs. This is mainly due to MET overexpression by MET-amplified cancer cells. In this sense, the pharmaceutical principle of applying the MET-amplified validation strategy is highly similar to
Table 3. Biochemical features of different types of cancer cell lines with MET dysregulation and their responsiveness to MET-targeting therapeutics.

| Cancer cell lines | Tissue origination | Cancer type | MET expression | MET activation | Exon-14 skipping | Sensitivity to SMKI | Sensitivity to TMABs | Cytotoxicity by ADCs | MET signaling addiction | SMKI effect in vivo | TMAB effect in vivo | ADC activity in vivo | Reference |
|-------------------|-------------------|-------------|----------------|----------------|----------------|-------------------|----------------------|---------------------|------------------------|----------------|----------------|----------------|----------|
| Hs746T            | Stomach           | Carcinoma   | 350,000 mol/cell (++++) | Strong | A: 6.6; B: 3.7; C: 14–28 | Yes, exon-14 skipping | High, >90% inhibition | Low, ~40% inhibition | High, 0.11 nM/87% death | Highly addictive | >90% tumor volume reduction | | Qian et al.21, He et al.25, Gavine et al.26, Bentell et al.20, Yun et al.24, Patnaik et al.29, Gymnopoulos et al.42, Mok et al.93, Attimos et al.36 |
| NCI-H820          | Lung              | Adeno-carcinoma | 320,000 mol/cell (++++) | Strong | A: 1.7; B: 1.5 | No | Low, <50% inhibition | Low, <40% inhibition | High, 0.2 nM/87% death | Lightly addictive | >90% tumor volume reduction | Unknown | Unknown | Qian et al.21, Yun et al.24, Patnaik et al.29, Gymnopoulos et al.42 |
| MKN-45            | Stomach           | Carcinoma   | 295,000 mol/cell (++++) | Strong | A: 4.5; B: 3.7; C: 13–25 | No | High, >90% inhibition | Moderate, ~50% inhibition | High, ~0.02 nM/98% killing | Highly addictive | >90% tumor volume reduction | Significant inhibition | Unknown | Qian et al.21, Lee et al.31, Yun et al.24, Patnaik et al.29, Hou et al.38, Wang et al.40, Yang et al.41, Min et al.43, Mok et al.93, Lee et al.95 |
| SNU-5             | Stomach           | Carcinoma   | 291,000 mol/cell (++++) | Strong | A: 2.8; B: 3.1; C: 8–24 | No | High, >90% inhibition | High, ~90% inhibition | High, ~0.02 nM/97% killing | Highly addictive | >90% tumor volume reduction | Significant inhibition | Unknown | Qian et al.21, Lee et al.31, Yun et al.24, Patnaik et al.29, Hou et al.38, Gymnopoulos et al.42, Min et al.43, Mok et al.93, Lee et al.95 |
| OE-33             | Esophagus         | Adeno-carcinoma | 258,000 mol/cell (++++) | Strong | A: 3.1; B: 2.6; C: 18 | No | Moderate, ~40% inhibition | Moderate, ~70% inhibition | High, ~1 nM/>90% killing | Moderately addictive | Unknown | Unknown | Unknown | Qian et al.21, Yun et al.24, Patnaik et al.29, Mok et al.93 |
| EBC-1             | Lung              | Squamous carcinoma | 233,000 mol/cell (++++) | Strong | A: 5.2; B: 3.1; C: 24 | No | High, ~85% inhibition | Low, ~30% inhibition | High, ~0.06 nM/96% killing | Moderately additive | Moderate-significant inhibition | Unknown | Unknown | Qian et al.21, Gavine et al.27, Lee et al.31, Yun et al.24, Patnaik et al.29, Gymnopoulos et al.42, Min et al.43, Mok et al.93 |
| NCI-H1993         | Lung              | Adeno-carcinoma | 232,000 mol/cell (++++) | Strong | A: 2.8; B: 3.8; C: 34 | No | Low, <50% inhibition | Low, ~30% inhibition | High, 16.3 ng/ml | Moderately additive | Unknown | Unknown | High, 10 mg/kg, >95% inhibition | Qian et al.21, Gavine et al.27, Yun et al.24, Patnaik et al.29, Lai et al.94, Mok et al.93 |

(Continued)
| Tumor Type      | Tissue Origination | MET Expression | MET Activation | MET Amplification |
|-----------------|--------------------|----------------|----------------|-------------------|
| Gastric         | Adenocarcinoma     | Strong         | Moderate       | High, ~0% tumor volume reduction |
| Lung            | Adenocarcinoma     | Strong         | Moderate       | Moderate additive |
| HT-29           | Colorectal         | Moderate       | Unknown        | Unknown           |
| SNU-620 Stomach | Adenocarcinoma     | Strong         | Low, ~30% inhibition | Insensitive        |
| NCI-H641       | Adenocarcinoma     | Strong         | No Low, ~20% inhibition | Insensitive        |
| SNU-638        | Stomach            | Strong         | No Low, ~20% inhibition | Insensitive        |
| NCI-H1573      | Lung               | Strong         | Moderate       | Moderate additive |
| Kato II        | Stomach            | Strong         | Moderate       | Moderate additive |
| Okajima         | Stomach            | Strong         | Moderate       | Moderate additive |
| HCC-827        | Lung               | Strong         | Moderate       | Moderate additive |
| YCC-34         | Stomach            | Strong         | Moderate       | Moderate additive |
| YCC-31         | Stomach            | Strong         | Moderate       | Moderate additive |
| NCI-H1373      | Lung               | Strong         | Moderate       | Moderate additive |
| SNU-668        | Stomach            | Strong         | Moderate       | Moderate additive |
| NCI-H1375      | Lung               | Strong         | Moderate       | Moderate additive |
| SNU-620 Stomach| Adenocarcinoma     | Strong         | Moderate       | Moderate additive |
| NCI-H641       | Adenocarcinoma     | Strong         | Moderate       | Moderate additive |
| SNU-638        | Stomach            | Strong         | No Low, ~20% inhibition | Insensitive        |
| NCI-H1573      | Lung               | Strong         | Moderate       | Moderate additive |
| Kato II        | Stomach            | Strong         | Moderate       | Moderate additive |
| Okajima         | Stomach            | Strong         | Moderate       | Moderate additive |
| HCC-827        | Lung               | Strong         | Moderate       | Moderate additive |
| YCC-34         | Stomach            | Strong         | Moderate       | Moderate additive |
| YCC-31         | Stomach            | Strong         | Moderate       | Moderate additive |
| NCI-H1373      | Lung               | Strong         | Moderate       | Moderate additive |
| SNU-668        | Stomach            | Strong         | Moderate       | Moderate additive |
| NCI-H1375      | Lung               | Strong         | Moderate       | Moderate additive |

Reference: Qian et al., Yun et al., Gymnopoulos et al., Min et al., Lee et al.
Table 3. (Continued)

| Cancer cell lines | Tissue origin | Cancer type | MET expression | MET activation | MET amplification | Exon-14 skipping | Sensitivity to SMKIs | Sensitivity to TMABs | Cytotoxicity by ADCs | MET signaling addiction | SMKI effect in vivo | TMAB effect in vivo | ADC activity in vivo | Reference |
|-------------------|---------------|-------------|----------------|---------------|------------------|-----------------|---------------------|---------------------|-----------------------|----------------------|------------------|------------------|---------------------|-----------|
| Detroit 562       | Pharyngeal    | Carcinoma   | 59,000 mol/cell | Unknown       | Unknown          | No              | Insensitive         | Unknown             | Moderate, 11.3 nM/68% killing | Unknown              | Unknown          | Unknown            | Min et al.44   |
| NCI-H2342         | Lung          | Adeno-carcinoma | Unknown/ -++- | Unknown       | B: 1.4           | No              | Insensitive         | Unknown             | Unknown              | Unknown              | Unknown          | Unknown            | Qian et al.21, Yun et al.36, Patnaik et al.25 |
| HCCLM-3           | Liver         | Carcinoma   | ~80,000 mol/cell| Unknown       | Unknown          | No              | Moderate, ~60% inhibition | Unknown             | High, 3.2 mg/ml >80% killing | Unknown              | >90% inhibition | Unknown            | Lai et al.43, Mok et al.53 |
| NUGC-4            | Stomach       | Adeno-carcinoma | 43,000 mol/cell | Low           | A: 1.3; B: 1.3   | No              | Insensitive         | Unknown             | Unknown              | Unknown              | Unknown          | Unknown            | Qian et al.21, Yun et al.36, Patnaik et al.25 |
| A-549             | Lung          | Adeno-carcinoma | 43,000 mol/cell | Moderate       | A: 1             | No              | Insensitive         | Moderate, 1.6 nM/82% killing | Moderately addictive | Unknown              | Moderate inhibition | Yun et al.34, Gymnopoulos et al.62, Lai et al.53, Poulsen et al.22 |
| Kato III          | Stomach       | Adeno-carcinoma | 38,700 mol/cell | Negative       | A: 0.9; C: 2     | No              | Insensitive         | Insensitive         | Not observed         | Unknown              | Unknown          | Unknown            | Yun et al.36, Lee et al.55 |
| SW-1417           | Colorectal    | Adeno-carcinoma | 38,000 mol/cell | Unknown       | Unknown          | No              | Insensitive         | Unknown             | High, ~3.5 nM, 93% killing | Unknown              | Unknown          | Unknown            | Min et al.44   |
| HCT-116           | Colorectal    | Adeno-carcinoma | 37,000 mol/cell | Negative       | Unknown          | No              | Insensitive         | Unknown             | High, ~0.2 nM/ 99% killing | Unknown              | Unknown          | Unknown            | Gavine et al.27, Min et al.44 |
| SNU-16            | Stomach       | Carcinoma   | 37,000 mol/cell | Unknown       | C: 3             | No              | Insensitive         | Insensitive         | High, 4.7 nM/90% killing | Not observed         | Unknown          | Unknown            | Min et al.44   |
| FaDu              | Pharynx       | Squamous carcinoma | 34,000 mol/cell | Unknown       | Unknown          | No              | Insensitive         | Unknown             | High, ~0.33 nM/98% killing | Not additive         | Unknown          | Unknown            | Min et al.44   |
| MDA-MB231         | Breast        | Adeno-carcinoma | 30,000 mol/cell | Negative       | Unknown          | No              | Unknown             | Insensitive         | Low, ~40% inhibition | Lightly addictive | Unknown          | Unknown            | Ivan et al.95 |
| SW-48             | Colorectal    | Adeno-carcinoma | 26,000 mol/cell | Negative       | Unknown          | No              | Unknown             | Not additive         | Unknown              | Unknown              | Unknown          | Unknown            | Gymnopoulos et al.62 |
| U-87MG            | Brain         | Glioblastoma | 22,000 mol/cell | Negative       | A: 1             | No              | Low, ~30% inhibition | Low, ~30% inhibition | High, 1.9 nM/ ~80% inhibition | Not additive         | >95% tumor volume reduction | 90% growth inhibition | Yun et al.36, Bendell et al.20, Lee et al.31, Wang et al.30, Yun et al.34, Gymnopoulos et al.62 |
| IM-95m            | Stomach       | Adeno-carcinoma | 22,000 mol/cell | Low           | A: 1.1; B: 0.6; C: 3 | No              | Moderate, ~50% inhibition | Low, ~40% inhibition | Moderate, 1.7 nM/53% killing | Moderately addictive | Unknown          | Unknown            | Qian et al.21, Gymnopoulos et al.62 |
| Cancer cell line | Tissue origin | MET expression | Exon-14 skipping | Sensitivity to SMKIs | Cytotoxicity by ADCs | TMAB effect | ADC activity in vitro | Reference |
|-----------------|--------------|----------------|------------------|---------------------|---------------------|-------------|----------------------|-----------|
| KF-4            | PANC-1       | 15,000 mol/cell | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Gymnopoulos et al. |
| MCF-7           | Breast       | 8000 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Hou et al.     |
| SW-480          | Colorectal   | 6500 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Leta et al.    |
| NCI-H1550       | LUNG         | 1900 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Lai et al.     |
| N87             | STOMACH      | 7000 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Hou et al.     |
| CHL886          | BREAST       | 8000 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Min et al.     |
| PC-3            | PROSTATE     | 65,000 mol/cell| Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Mok et al.     |
| PRM-Lung        | LUNG         | 8000 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Lai et al.     |
| HMEC            | BREAST       | 16,000 mol/cell| Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Mok et al.     |
| NHDF            | SKIN         | 1600 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Lai et al.     |
| HNBE            | LUNG         | 6500 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Hou et al.     |
| HVEC            | SKIN         | 1600 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Lai et al.     |
| PRM-Breast      | BREAST       | 1600 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Mok et al.     |
| PRM-Skin        | SKIN         | 1600 mol/cell  | Neg          | Negative            | Moderate, Lightly   | Moderate    | Neutral              | Lai et al.     |

*More than 40 cancer cell lines originating from different tissues/ organs are summarized here. Five normal human epithelial cell types are included for comparison. Variable levels of MET expression in various types of cancer cell lines are determined by Western blotting and artificially categorized as: (+++), overexpression with more than 100,000 MET molecules per cell; (++), high expression with MET molecules from 99,000 to 10,000 per cell; (+), moderate expression with MET molecules from 49,000 to 10,000 per cell; (+), low expression with MET molecules from 9000 to 1000 per cell; and (-), not expressed. The MET activation status is determined by detecting phosphorylated MET in Western blot analysis. Amplification of the MET gene is analyzed by FISH (fluorescence in situ hybridization) and PCR. The MET gene copy number is determined by FISH analysis. Sensitivity to SMKIs is determined by detecting MET phosphorylation inhibition as follows: high sensitivity (80–100%); moderate sensitivity (50–79%); low sensitivity (20–49%), and insensitive (<20%). The ADC activity in vitro is determined by detecting internalized ADC amounts as follows: high (80–100%), moderately high (50–79%), low (20–49%), and insensitive (<20%). The TMAB effect is determined by detecting cell killing as follows: highly effective: >80%; moderately effective: 50 to 79%; lowly effective: 10 to 49%; and insensitive: <10%. Cellular addiction to MET signaling for growth and survival is determined by individual SMKIs or TMABs and indicated by levels of growth inhibition as: highly addictive, >80–100%; moderately addictive: 50–79%; lightly addictive: 20–49%; and unknown, no information is available. ADC, antibody–drug conjugate; CEP7, chromosome enumerating probe against chromosome 7; FISH, fluorescence in situ hybridization; GCN, gene copy number; PCR, polymerase chain reaction; TMAB, therapeutic monoclonal antibody; SMKI, small-molecule kinase inhibitor.
that showing MET overexpression as described above. Regardless, results from using both MET-amplified cell lines and PDXs in testing the efficacy of MET-targeting TMAB Sym015, and ADCs TR1801, ABBV-399, and SHR-A1403 have proven that this model is highly reliable.33,42–46

The limitation of the MET-amplified validation model is the extremely low frequency of MET amplification in clinical samples.74–76,86,87 In this sense, the use of MET amplification as the biomarker for patient selection is a challenge. It requires to have an advanced laboratory with sophisticated technologies for performing FISH, NGS, and other methods, resulting in an increase in clinical cost and expenditures. In addition, cancer cells with MET amplification are not always responsive to SMKIs or conventional TMABs. As described above, certain proteins with oncogenic mutations in the MET signaling pathway with disruptive cascades can support cancer cell growth and survival independent of the presence of MET-targeting SMKIs or cTMABs.21,107

**MET exon-14 skipping as a validation mechanism**

The use of MET exon-14 skipping as a validation approach has gained special attention due to exciting results from MET-targeted clinical trials of NSCLCs.22,108,109 Oncogenic evidence has shown that MET exon-14 skipping acts as a vital oncogenic driver,108,109 but its frequency is low with minor occurrence in lung (~4%), stomach (~7), and colorectal (~5%) cancers.108,109 These observations suggest that cancer patients with MET exon-14 skipping is a particular population suitable for MET-targeted therapy.

Currently, the cellular models that truly reflect the oncogenic effect of MET exon-14 skipping are still lacking. Only two cell lines, Hs746T and NCI-H596, have MET exon-14 skipping (Table 3). However, Hs746T cells are accompanied with MET overexpression and gene amplification.108,109 In contrast, levels of MET expressed by H596 cells are relatively low (Table 3). Thus, precaution must be taken in interpretation of results from using these two cell lines. Establishment of a mouse model expressing mouse MET exon-15 deletion (equivalent to human MET exon-14 skipping) through a molecular approach has been reported resulting in the formation of mouse lung adenoma, but not adenocarcinoma.110 The use of this animal model has shown that crizotinib is able to stabilize tumor progression but the efficiency is relatively low.110 Two PDX models with confirmed MET exon-14 skipping, namely LU2503 and LU5381, are available from Crown Bioscience (www.crownbioscience.com). They have been tested for their responsiveness to MET-targeting SMKIs, such as glesatinib,22 and to cTMABs including Sym015.35 Their pharmaceutical values are confirmed from results showing the responsiveness of both models to the action of MET-targeting SMKIs and conventional TMABs.22,33

**PDXs with defined MET dysregulation as a validation strategy**

The use of PDXs with different MET dysregulations has been a favored choice for the last several years.22,29,32,33,41,42,51 The underlying reasons are obvious, owing to pathogenic features of PDXs highly resembling those from primary tumors. Currently, MET-based PDX models derived from lung, gastric, CRC, and head & neck cancers with MET overexpression, amplification and exon-14 skipping have been established.22,29,32,33,41,42,51 SMKIs, cTMABs, and ADCs have all been tested...
in PDX models with acceptable therapeutic responsiveness. For instance, glesatinib at a therapeutic dose of 60 mg/kg is highly effective against PDX LU2503 and LU5381 models with MET exon-14 skipping. Similarly, TR1801-ADC, a second-generation MET-targeting ADC at a single-dose injection of 0.125 to 1 mg/kg, has been validated in PDX models derived from stomach, CRC, and head & neck cancer samples with demonstrated therapeutic activity. Thus, PDX models are an exciting addition to the list of currently used validation strategies and should have pharmaceutical advantages in conjunction with traditional models for objectively evaluating MET-targeting therapeutics.

**Additional MET alterations as a validation mechanism**

Development of novel MET-targeting therapeutics, such as bispecific antibodies and dual-targeting ADCs, demands a proper strategy for validation. A MET-based bispecific antibody has a co-targeting antigen-binding arm that regulates the partner signaling pathway or T-cell activity, respectively. Validation of these agents requires selection of proper cellular models to determine anticancer activities of both antigen-binding arms. Several models including PDX-derived ex vivo 3D spheroids have been developed to evaluate the efficacy of MET-targeting therapeutics such as TR1801-ADC. However, comprehensive analyses at mechanistic levels of these models in terms of the strength of signaling integration, levels of addictive status, biological responsiveness, and activity coordination have not been studied in detail. For instance, efficacies of three MET-based bispecific antibodies targeting PD-1, as evaluated in several cellular models, are not impressive in terms of tumor growth inhibition and levels of T-cell activation. Thus, the complexity in mechanism of action and tumorigenic feature included in the models must be considered to objectively evaluate the efficacy of these novel MET-targeting therapeutics.

**Pharmaceutical criteria for mechanism-based drug validation**

Utilizing a mechanism-based validation strategy has significantly contributed to the progress and success in the development of MET-targeting therapeutics. Approval of four SMKIs by the FDA is an example. Nevertheless, strategies used to validate the efficacy of MET-targeting cTMABs appear to have some issues. Results from preclinical studies seem to be promising; however, outcomes from clinical trials, which have been conducted for almost 10 years, are disappointing. This raises serious concerns about the reliability of these strategies for validating MET-targeting cTMABs. Thus, it is time to evaluate current approaches in order to identify deficiencies that cause unobjective conclusions, and to avoid mistakes of moving these unjustified MET-targeting TMABs into clinical trials. The following is a summary of criteria to be considered when a mechanism-based validation strategy needs to be applied.

It is vital to select a mechanism-based validation strategy that suits the purpose of a particular therapeutic to be tested. MET dysregulation occurs predominantly in certain types of tumors such as those from stomach, lung, kidney, and liver. The majority of validation programs have predetermined objectives favoring particular types of cancer. Dependent on the nature of drug candidates, some studies screen drug efficacy by employing a large number of cancer cell lines in order to find defined MET-targeting activity. For instance, AMG-337, a type I, ATP-competitive, and highly MET-selective SMKI, has been profiled against a diverse panel of 260 cancer cell lines. Only two cell lines, SNU-5 and Hs746T with MET amplification, have shown sensitivity to AM-337. Studies then focused on cellular models with MET amplification for further validation. In contrast, other studies have utilized an approach of focusing on a unique MET abnormality. An example is glesatinib, a unique type II MET SMKI, which is evaluated in lung cancer models harboring MET exon-14 skipping and mutation-associated resistance to type I MET SMKIs. Such a focused strategy increases the potential for selecting a lead candidate moving into clinical trials. Thus, selection of a mechanism-based validation strategy must be considered in a balanced way.

Understanding the mechanism of MET dysregulation helps in selecting a proper validation strategy. The mechanism of action exhibited by individual MET-targeting therapeutics is fundamentally different. For instance, type I and II SMKIs act at different regions in the TK domain of MET with different structure conformations (active versus inactive). As described above, the TK domain of MET can be activated under...
various conditions and manifested through single or multiple events. In this sense, cellular models featured by HGF-dependent and independent MET activation have to be carefully selected before different types of MET-targeting SMKIs are applied. Similarly, different MET-targeting TMABs that bind to different regions in the MET extracellular sequences result in different biochemical impacts, such as preventing HGF binding, inducing MET internalization/ degradation, attenuating MET signaling, or enhancing immune regulatory activity. All these activities must be considered when a validation strategy is selected.

The status of cellular MET signaling integration/addiction in individual cellular models is a factor determining the success of a validation strategy. The therapeutic efficacy of MET-targeting SMKIs, cTMABs, and bispecific antibodies is highly dependent on the level of addictiveness of the cancer cell to MET or partner protein signaling for growth and survival. In preclinical studies, many MET-targeting SMKIs and TMABs display only moderate inhibitory effects on cellular models showing limited levels of addiction. Clearly, these “positive results” are not sufficient to be reflected in clinical trials. In contrast, only those showing the strongest anticancer activity with complete growth inhibition in cellular models with full MET signaling addiction have the chance to achieve an objective response in cancer patients. Thus, studies validating SMKIs, cTMABs, and bispecific antibodies should select cellular models that exhibit full MET-signaling addictive status.

Consideration of acquired drug resistance is another strategy for validation of MET-targeting therapeutics. Aberrant MET expression and signaling have been established as a compensation mechanism during the treatment of cancer with SMKIs targeting EGFR and other signaling proteins. The compensated MET pathway significantly contributes to the acquired drug resistance in various types of cancer undergoing chemo and targeted therapy. In this sense, targeted inhibition of MET signaling using SMKIs or antibody-based biotherapeutics has clinical relevance. The use of MET-targeting SMKIs for treatment of tumors resistant to EGFR inhibitors is currently a recommended clinical practice. Demonstration of the effectiveness of antibody-based biotherapeutics to these drug-resistant tumors is also an objective in the validation procedures, and is highly anticipated in many MET-targeting clinical trials. Clinically, different types of cancer with variable levels of drug-resistant phenotypes have different drug sensitivity and/or treatment profiles. In this sense, the use of drug resistance as a biological criterion to validate the effect of MET-targeting therapeutics should be highly recommended.

Last but not least is the strategy of using MET-targeting therapeutics to target cancer stem cells to achieve a therapeutic objective. Aberrant MET expression and activation contribute to cancer stemness in certain types of cancer. For instance, increased MET expression in cancer stem cells from CRC and glioblastoma contributes to malignant phenotypes and behaviors, which has therapeutic value. Thus, the use of MET-targeting ADCs that have mechanisms of action independent of signaling addiction is an attractive approach to eradicate cancer stem cells as a therapeutic objective. ADCs targeting other RTKs, such as RON and leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), are examples for eradicating cancer stem cells. Thus, the same strategy should be applied to determine the effectiveness of MET-targeting ADCs to kill cancer stem cells. The outcome will help us not only dissect the pathogenic role of MET in oncogenesis, but also broaden our understanding about the underlying mechanism of MET-targeting therapeutics in clinical application.

Conclusion
Pathogenic mechanism-based evaluation of different types of MET-targeting therapeutics is critical to select and validate lead candidates for clinical trials and approval for patient application. Technological innovation resulting in novel therapeutics also requires appropriate new models to meet the pharmaceutical demand. During the last 20 years, the achievement in dissecting oncological MET dysregulation and its underlying mechanism has significantly improved the quality of mechanism-based validation by using well-defined models with characterized biochemical and biological features. These models not only try to mimic the clinical complexity of MET-driven tumorigenesis, but also serves as a pharmaceutical tool for drug screening and evaluation. At present, novel MET-targeting biotherapeutics, such as bispecific antibodies, ADCs, and dual-targeting ADCs, have emerged as new players in MET-targeted cancer therapy.
of action by these biotherapeutics are different from previously established SMKIs and cTMABs. Thus, development and optimization of novel mechanism-based drug validation strategies is an urgent need, which will greatly facilitate the clinical approval of MET-targeting therapeutics for oncological application.

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References
1. Cooper CS, Park M, Blair DG, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature 1984; 311: 29–33.
2. Malik R, Mambetsariev I, Fricke J, et al. MET receptor in oncology: from biomarker to therapeutic target. Adv Cancer Res 2020; 147: 259–301.
3. Park M, Dean M, Kaul K, et al. Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors. Proc Natl Acad Sci U S A 1987; 84: 6379–6383.
4. Onzetto C, Bardelli A, Zhen Z, et al. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. Cell 1994; 77: 261–271.
5. Peschard P, Fournier TM, Lamorte L, et al. Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. Mol Cell Biol 2001; 8: 995–1004.
6. Dean M, Park M and Vande Woude GF. Characterization of the rearranged trp-met oncogene breakpoint. Mol Cell Biol 1987; 7: 921–924.
7. Umeki K, Shiota G and Kawasaki H. Clinical significance of c-met oncogene alterations in human colorectal cancer. Oncology 1999; 56: 314–321.
8. Schmidt L, Duh FM, Chen F, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 1997; 16: 68–73.
9. Seo JS, Ju YS, Lee WC, et al. The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res 2012; 22: 2109–2119.
10. Bradley CA, Dunne PD, Bingham V, et al. Transcriptional upregulation of c-MET is associated with invasion and tumor budding in colorectal cancer. Oncotarget 2016; 7: 78932–78945.
11. Giordano S, Di Renzo MF, Narsimhan RP, et al. Biosynthesis of the protein encoded by the c-met proto-oncogene. Oncogene 1989; 4: 1383–1388.
12. Pilotto S, Carbognin L, Karachaliou N, et al. Tracking MET de-addiction in lung cancer: a road towards the oncogenic target. Cancer Treat Rev 2017; 60: 1–11.
13. Bria E, Pilotto S, Simbolo M, et al. Comprehensive molecular portrait using next generation sequencing of resected intestinal-type gastric cancer patients dichotomized according to prognosis. Sci Rep 2016; 6: 22982.
14. Christensen JG, Burrows J and Salgia R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Lett 2005; 225: 1–26.
15. Kurzrock R, Sherman SI, Ball DW, et al. Activity of XL184 (cabozantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer. J Clin Oncol 2011; 29: 2660–2666.
16. Vansteenkiste JF, Van De Kerkhove C, Wauters E, et al. Capmatinib for the treatment of non-small cell lung cancer. Expert Rev Anticancer Ther 2019; 19: 659–671.
17. Lennerz JK, Kwak EL, Ackerman A, et al. MET amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib. *J Clin Oncol* 2011; 29: 4803–4810.

18. Friese-Hamim M, Bladt F, Locatelli G, et al. The selective c-Met inhibitor tepotinib can overcome epidermal growth factor receptor inhibitor resistance mediated by aberrant c-Met activation in NSCLC models. *Am J Cancer Res* 2017; 7: 962–972.

19. Hughes PE, Rex K, Caenepeel S, et al. In vitro and in vivo activity of AMG 337, a potent and selective MET kinase inhibitor, in MET-dependent cancer models. *Mol Cancer Ther* 2016; 15: 1568–1579.

20. Shih J, Zhong B, Shi H, et al. Bozitinib, a highly selective inhibitor of cMet, demonstrates robust activity in gastric, lung, hepatic and pancreatic in vivo models. *Cancer Res* 2017; 77(Suppl. 13): abstract 2096.

21. Qian F, Engst S, Yamaguchi K, et al. Inhibition of tumor cell growth, invasion, and metastasis by EXEL-2880 (XL880, GSK1363089), a novel inhibitor of HGF and VEGF receptor tyrosine kinases. *Cancer Res* 2009; 69: 8009–8016.

22. Engstrom LD, Aranda R, Lee M, et al. Glesatinib exhibits antitumor activity in lung cancer models and patients harboring MET exon 14 mutations and overcomes mutation-mediated resistance to type I MET inhibitors in nonclinical models. *Clin Cancer Res* 2012; 18: 1663–1671.

23. Wang W, Li Q, Takeuchi S, et al. Met kinase inhibitor E7050 reverses three different mechanisms of hepatocyte growth factor-induced tyrosine kinase inhibitor resistance in EGFR mutant lung cancer. *Cancer Res* 2012; 18: 1663–1671.

24. He AR, Cohen RB, Denlinger CS, et al. First-in-human phase I study of merestinib, an oral multikinase inhibitor, in patients with advanced cancer. *Oncologist* 2019; 24: e930–e942.

25. Egile C, Kenigsberg M, Delaisi C, et al. The selective intravenous inhibitor of the MET tyrosine kinase SAR125844 inhibits tumor growth in MET-amplified cancer. *Mol Cancer Ther* 2015; 14: 384–394.

26. Schuller AG, Barry ER, Jones RD, et al. The MET inhibitor AZD6094 (savolitinib, HMPL-504) induces regression in papillary renal cell carcinoma patient-derived xenograft models. *Clin Cancer Res* 2015; 21: 2811–2819.

27. Gavine PR, Ren Y, Han L, et al. Volitinib, a potent and highly selective c-Met inhibitor, effectively blocks c-Met signaling and growth in c-MET amplified gastric cancer patient-derived tumor xenograft models. *Mol Oncol* 2015; 9: 323–333.

28. Hultberg A, Morello V, Huyghe L, et al. Depleting MET-expressing tumor cells by ADCC provides a therapeutic advantage over inhibiting HGF/MET signaling. *Cancer Res* 2015; 75: 3373–3383.

29. Liu L, Zeng W, Wortinger MA, et al. LY2875358, a neutralizing and internalizing anti-MET bivalent antibody, inhibits HGF-dependent and HGF-independent MET activation and tumor growth. *Clin Cancer Res* 2014; 20: 6059–6070.

30. Bendell JC, Hochster H, Hart LL, et al. A phase II randomized trial (GO27827) of first-line FOLFOX plus bevacizumab with or without the MET inhibitor onartuzumab in patients with metastatic colorectal cancer. *Oncologist* 2017; 22: 264–271.

31. Lee BS, Kang S, Kim KA, et al. Met degradation by SAIT301, a Met monoclonal antibody, reduces the invasion and migration of nasopharyngeal cancer cells via inhibition of EGR-1 expression. *Cell Death Dis* 2014; 5: e1159.

32. Wang J, Goetsch L, Tucker L, et al. Anti-c-Met monoclonal antibody ABT-700 breaks oncogene addiction in tumors with MET amplification. *BMC Cancer* 2016; 16: 105.

33. Poulsen TT, Grandal MM, Skartved NJØ, et al. Sym015: a highly efficacious antibody mixture against MET-amplified tumors. *Clin Cancer Res* 2017; 23: 5923–5935.

34. Yun J, Lee SH, Kim SY, et al. Antitumor activity of amivantamab (JNJ-61186372), an EGFR-MET bispecific antibody, in diverse models of EGFR exon 20 insertion-driven NSCLC. *Cancer Chemother Pharmacol* 2018; 82: 407–418.

35. Patnaik A, Gordon M, Tsai F, et al. A phase I study of LY3164530, a bispecific antibody targeting MET and EGFR, in patients with advanced or metastatic cancer. *Clin Cancer Res* 2015; 21: 3373–3383.
antitumor activity. *Mol Cancer Ther* 2013; 12: 2748–2759.

38. Hou W, Yuan Q, Yuan X, *et al.* A novel tetravalent bispecific antibody targeting programmed death 1 and tyrosine-protein kinase Met for treatment of gastric cancer. *Invest New Drugs* 2019; 37: 867–889.

39. Sun ZJ, Wu Y, Hou WH, *et al.* A novel bispecific c-MET/PD-1 antibody with therapeutic potential in solid cancer. *Oncotarget* 2017; 8: 29067–29079.

40. Wang J, Anderson MG, Oleksijew A, *et al.* ABBV-399, a c-Met antibody-drug conjugate that targets both MET-amplified and c-Met-overexpressing tumors, irrespective of MET pathway dependence. *Clin Cancer Res* 2017; 23: 992–1000.

41. Yang CY, Wang L, Sun X, *et al.* SHR-A1403, a novel c-Met antibody-drug conjugate, exerts encouraging anti-tumor activity in c-Met-overexpressing models. *Acta Pharmacol Sin* 2019; 40: 971–979.

42. Gymnopoulos M, Betancourt O, Blot V, *et al.* TR1801-ADC: a highly potent cMet antibody-drug conjugate with high activity in patient-derived xenograft models of solid tumors. *Mol Oncol* 2020; 14: 54–68.

43. Lai KC, Muvaffak A, Li M, *et al.* In vitro and in vivo activity of a novel c-Met-targeting antibody-drug conjugate using a DNA-alkylating, indolinobenzodiazepine payload. *Cancer Res* 2017; 77(Suppl. 13): abstract 45.

44. Min B, Jin J, Kim H, *et al.* cIRCR201-dPBD, a novel pyrrolobenzodiazepine dimer-containing site-specific antibody-drug conjugate targeting c-Met overexpression tumors. *ACS Omega* 2020; 5: 25798–25809.

45. Sellmann C, Doerner A, Knuehl C, *et al.* Balancing selectivity and efficacy of bispecific Epidermal Growth Factor Receptor (EGFR) × c-MET antibodies and antibody-drug conjugates. *J Biol Chem* 2016; 291: 25106–25119.

46. Yao H-P, Tong X-M, Hudson R, *et al.* MET and RON receptor tyrosine kinase in colorectal adenocarcinoma: molecule features as drug targets and antibody-drug conjugates for therapy. *J Exp Clin Cancer Res* 2020; 39: 198.

47. Tchou J, Zhao Y, Levine BL, *et al.* Safety and efficacy of intratumoral injections of Chimeric Antigen Receptor (CAR) T cells in metastatic breast cancer. *Cancer Immunol Res* 2017; 5: 1152–1161.

48. Thayaparan T, Petrovic RM, Achkova DY, *et al.* CAR T-cell immunotherapy of MET-expressing malignant mesothelioma. *Oncoimmunology* 2017; 6: e1363137.

49. Liu B, Liu ZZ, Zhou ML, *et al.* Development of c-MET-specific chimeric antigen receptor-engineered natural killer cells with cytotoxic effects on human liver cancer HepG2 cells. *Mol Med Rep* 2019; 20: 2823–2831.

50. Yuan X, Sun Z, Yuan Q, *et al.* Dual-function chimeric antigen receptor T cells targeting c-Met and PD-1 exhibit potent anti-tumor efficacy in solid tumors. *Invest New Drugs* 2021; 39: 34–51.

51. Baltschukat S, Engstler BS, Huang A, *et al.* Capmatinib (INC280) is active against models of non-small cell lung cancer and other cancer types with defined mechanisms of MET activation. *Clin Cancer Res* 2019; 25: 3164–3175.

52. Hellenstedt BA, Vogelzang NJ, Kluger HM, *et al.* Results of a phase II placebo-controlled randomized discontinuation trial of cabozantinib in patients with non-small-cell lung carcinoma. *Clin Lung Cancer* 2019; 20: 74–81.

53. Wolf J, Seto T, Han J-Y, *et al.*; GEOMETRY mono-1 Investigators. Capmatinib in MET exon 14-mutated or MET-amplified non-small-cell lung cancer. *N Engl J Med* 2020; 383: 944–957.

54. Schuler M, Berardi R, Lim W-T, *et al.* Molecular correlates of response to capmatinib in advanced non-small-cell lung cancer: clinical and biomarker results from a phase I trial. *Ann Oncol* 2020; 31: 789–797.

55. Liu J, Li X and Peng J. A novel CAV1-MET fusion in SCLC transformation responds to crizotinib and osimertinib treatment. *J Thorac Oncol* 2019; 14: e126–e128.

56. Drilon A, Clark JW, Weiss J, *et al.* Antitumor activity of crizotinib in lung cancers harboring a MET exon 14 alteration. *Nat Med* 2020; 26: 47–51.

57. Landi L, Bruno R, Fontanini G, *et al.* Crizotinib in ROS1 and MET deregulated NSCLC-response. *Clin Cancer Res* 2020; 26: 1775.

58. Paik PK, Felip E, Veillon R, *et al.* Tepotinib in non-small-cell lung cancer with MET exon 14 skipping mutations. *N Engl J Med* 2020; 383: 931–943.

59. Falchook GS, Kurzrock R, Amin HM, *et al.* First-in-man phase I trial of the selective MET inhibitor tepotinib in patients with advanced solid tumors. *Clin Cancer Res* 2020; 26: 1237–1246.

60. Van Cutsem E, Karaszewska B, Kang YK, *et al.* A multicenter phase II study of AMG
337 in patients with MET-amplified gastric/gastroesophageal junction/esophageal adenocarcinoma and other MET-amplified solid tumors. Clin Cancer Res 2019; 25: 2414–2423.

61. Hu H, Mu Q, Bao Z, et al. Mutational landscape of secondary glioblastoma guides MET-targeted trial in brain tumor. Cell 2018; 175: 1665–1678.e18.

62. Shah MA, Wainberg ZA, Catenacci DV, et al. Phase II study evaluating 2 dosing schedules of oral foretinib (GSK1363089), cMET/VEGFR2 inhibitor, in patients with metastatic gastric cancer. PLoS One 2013; 8: e54014.

63. Reungwetwattana T, Liang Y, Zhu V, et al. The race to target MET exon 14 skipping alterations in non-small cell lung cancer: the why, the how, the who, the unknown, and the inevitable. Lung Cancer 2017; 103: 27–37.

64. Bouattour M, Raymond E, Qin S, et al. Recent developments of c-Met as a therapeutic target in hepatocellular carcinoma. Hepatology 2018; 67: 1132–1149.

65. Angevin E, Spitaleri G, Rodon J, et al. A first-in-human phase I study of SAR125844, a selective MET tyrosine kinase inhibitor, in patients with advanced solid tumours with MET amplification. Eur J Cancer 2017; 87: 131–139.

66. Choueiri TK, Heng DYC, Lee JL, et al. Efficacy of savolitinib vs sunitinib in patients with MET-driven papillary renal cell carcinoma: the SAVOIR phase 3 randomized clinical trial. JAMA Oncol 2020; 6: 1247–1255.

67. Sequist LV, Han JY, Ahn MJ, et al. Osimertinib plus savolitinib in patients with EGFR mutation-positive, MET-amplified, non-small-cell lung cancer after progression on EGFR tyrosine kinase inhibitors: interim results from a multicentre, open-label, phase 1b study. Lancet Oncol 2020; 21: 373–386.

68. Castoldi R, Ecker V, Wielhe L, et al. A novel bispecific EGFR/Met antibody blocks tumor-promoting phenotypic effects induced by resistance to EGFR inhibition and has potent antitumor activity. Oncogene 2013; 32: 5593–5601.

69. Park K, John T, Kim SW, et al.; on behalf of the CHRYSLIS Investigators. Amivantamab (JNJ-61186372), an anti-EGFR-MET bispecific antibody, in patients with EGFR exon 20 insertion (exon20ins)-mutated non-small cell lung cancer (NSCLC). J Clin Oncol 2020; 38(Suppl. 15): 9512.

70. Liu L, Zeng W, Chedid M, et al. A novel MET-EGFR bispecific antibody LY3164530 shows advantage over combining MET and EGFR antibodies in tumor inhibition and overcome resistance. Cancer Res 2016; 76(Suppl. 14): abstract 873.

71. Lee BS, Kim HJ, Hwang JW, et al. The dual inhibition of Met and EGFR by ME22S, a novel Met/EGFR bispecific monoclonal antibody, suppresses the proliferation and invasion of laryngeal cancer. Ann Surg Oncol 2016; 23: 2046–2053.

72. International Cancer Genome Consortium PedBrain Tumor Project. Recurrent MET fusion genes represent a drug target in pediatric glioblastoma. Nat Med 2016; 22: 1314–1320.

73. Yeh I, Botton T, Talevich E, et al. Activating MET kinase rearrangements in melanoma and Spitz tumours. Nat Commun 2015; 6: 7174.

74. Noonan SA, Berry L, Lu X, et al. Identifying the appropriate FISH criteria for defining MET copy number-driven lung adenocarcinoma through oncogene overlap analysis. J Thorac Oncol 2016; 11: 1293–1304.

75. Smolen GA, Sordella R, Muir B, et al. Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. Proc Natl Acad Sci U S A 2006; 103: 2316–2321.

76. Lutterbach B, Zeng Q, Davis LJ, et al. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. Cancer Res 2007; 67: 2081–2088.

77. Albiger L, Guegan J, Le Formal A, et al. MET is a potential target across all papillary renal cell carcinomas: result from a large molecular study of pRCC with CGH array and matching gene expression array. Clin Cancer Res 2014; 20: 3411–3421.

78. Park WS, Dong SM, Kim SY, et al. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. Cancer Res 1999; 59: 307–310.

79. Aebersold DM, Landt O, Berthou S, et al. Prevalence and clinical impact of Met Y1253D-activating point mutation in radiotherapy-treated squamous cell cancer of the oropharynx. Oncogene 2003; 22: 8519–8523.

80. Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. Cancer Discov 2015; 5: 850–859.

81. Schrock AB, Frampton GM, Suh J, et al. Characterization of 298 patients with lung cancer
harboring MET exon 14 skipping alterations. *J Thorac Oncol* 2016; 11: 1493–1502.

82. Ma PC, Kijima T, Maulik G, et al. c-MET mutational analysis in small cell lung cancer: novel juxtamembrane domain mutations regulating cytoskeletal functions. *Cancer Res* 2003; 63: 6272–6281.

83. Kitajima Y, Ide T, Ohtsuka T, et al. Induction of hepatocyte growth factor activator gene expression under hypoxia activates the hepatocyte growth factor/c-Met system via hypoxia inducible factor-1 in pancreatic cancer. *CancerSci* 2008; 99: 1341–1347.

84. De Bacco F, Luraghi P, Medico E, et al. Induction of MET by ionizing radiation and its role in radio-resistance and invasive growth of cancer. *J Natl Cancer Inst* 2011; 103: 645–661.

85. Ivan M, Bond JA, Prat M, et al. Activated ras and ret oncogenes induce over-expression of c-met (hepatocyte growth factor receptor) in human thyroid epithelial cells. *Oncogene* 1997; 14: 2417–2423.

86. Guo R, Luo J, Chang J, et al. MET-dependent solid tumours - molecular diagnosis and targeted therapy. *Nat Rev Clin Oncol* 2020; 17: 569–587.

87. Koch JP, Aebersold DM, Zimmer Y, et al. MET targeting: time for a rematch. *Oncogene* 2020; 39: 2845–2862.

88. Pten M, Bertrand M, de Langen AJ, et al. Structural alterations of MET trigger response to MET kinase inhibition in lung adenocarcinoma patients. *Clin Cancer Res* 2018; 24: 1337–1343.

89. Bao ZS, Shen HM, Yang MY, et al. RNA-seq of 272 gliomas revealed a novel, recurrent PTPRZ1-MET fusion transcript in secondary glioblastomas. *Genome Res* 2014; 24: 1765–1773.

90. Liu S, Meric-Bernstam F, Parinianikuli N, et al. Functional consequence of the MET-T1010I polymorphism in breast cancer. *Oncotarget* 2015; 6: 2604–2614.

91. de Melo Gagliato D, Jardim DL, Falshock G, et al. Analysis of MET genetic aberrations in patients with breast cancer at MD Anderson phase I unit. *Clin Breast Cancer* 2014; 14: 468–474.

92. Mok TS, Geater SL, Su WC, et al. A randomized phase 2 study comparing the combination of ficlatuzumab and gefitinib with gefitinib alone in Asian patients with advanced stage pulmonary adenocarcinoma. *J Thorac Oncol* 2016; 11: 1736–1744.

93. Affronti ML, Jackman JG, McSherry F, et al. Phase II study to evaluate the efficacy and safety of rilotumumab and bevacizumab in subjects with recurrent malignant glioma. *Oncologist* 2018; 23: 889–898.

94. Lee D, Sung E-S, Ahn J-H, et al. Development of antibody-based c-Met inhibitors for targeted cancer therapy. *Immunotargets Ther* 2015; 4: 35–44.

95. Aftimos PG, Barthelemy P, Rolfo CD, et al. A phase I, first-in-human study of argx-111, a monoclonal antibody targeting c-met in patients with solid tumors. *J Clin Oncol* 2015; 33(Suppl. 15): 2580.

96. Scagliotti G, Moro-Sibilot D, Kollmeier J, et al. A randomized-controlled phase 2 study of the MET antibody emibetuzumab in combination with erlotinib as first-line treatment for EGFR mutation-positive NSCLC patients. *J Thorac Oncol* 2020; 15: 80.

97. Spigel DR, Edelman MJ, O'Byrne K, et al. Results from the phase III randomized trial of onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIIB or IV non-small-cell lung cancer: METLung. *J Clin Oncol* 2017; 35: 412–420.

98. Lee J, Kim ST, Park S, et al. Phase I trial of anti-MET monoclonal antibody in MET-overexpressed refractory cancer. *Clin Colorectal Cancer* 2018; 17: 140–146.

99. Strickler JH, LoRusso P, Salgia R, et al. Phase I dose-escalation and -expansion study of telisotuzumab (ABT-700), an anti-c-Met antibody, in patients with advanced solid tumors. *Mol Cancer Ther* 2020; 19: 1210–1217.

100. Drilon A, Cappuzzo F, Oo SI, et al. Targeting MET in lung cancer: will expectations finally be met? *J Thorac Oncol* 2017; 12: 15–26.

101. Peng S, Wang R, Zhang X, et al. EGFR-TKI resistance promotes immune escape in lung cancer via increased PD-L1 expression. *Mol Cancer* 2019; 18: 165.

102. Abdollahpour-Alitappeh M, Lotfinia M, Gharibi T, et al. Antibody-drug conjugates (ADCs) for cancer therapy: strategies, challenges, and successes. *J Cell Physiol* 2019; 234: 5628–5642.

103. Yaghoubi S, Karimi MH, Lotfinia M, et al. Potential drugs used in the antibody-drug conjugate (ADC) architecture for cancer therapy. *J Cell Physiol* 2020; 235: 31–64.

104. Yao HP, Zhou YQ, Zhang R, et al. MSP-RON signalling in cancer: pathogenesis and therapeutic potential. *Nat Rev Cancer* 2013; 13: 466–481.
106. Kim HJ, Kang SK, Kwon WS, et al. Forty-nine gastric cancer cell lines with integrative genomic profiling for development of c-MET inhibitor. *Int J Cancer* 2018; 143: 151–159.

107. Thein KZ, Biter AB and Hong DS. Therapeutics targeting mutant KRAS. *Annu Rev Med*. Epub ahead of print 2 November 2020. DOI: 10.1146/annurev-med-080819-033145.

108. Cortot AB, Kherrouche Z, Descarpentries C, et al. Exon 14 deleted MET receptor as a new biomarker and target in cancers. *J Natl Cancer Inst* 2017; 109: djw262.

109. Salgia R, Sattler M, Scheele J, et al. The promise of selective MET inhibitors in non-small cell lung cancer with MET exon 14 skipping. *Cancer Treat Rev* 2020; 87: 102022.

110. Lu X, Peled N, Greer J, et al. MET exon 14 mutation encodes an actionable therapeutic target in lung adenocarcinoma. *Cancer Res* 2017; 77: 4498–4505.

111. Ugolini A, Kenigsberg M, Rak A, et al. Discovery and pharmacokinetic and pharmacological properties of the potent and selective MET kinase inhibitor 1-{6-[6-(4-fluorophenyl)-[1,2,4] triazolo [4,3-b] pyridazin-3-ylsulfanyl]benzothiazol-2-yl]-3-(2-morpholin-4-ylethyl)urea (SAR125844). *J Med Chem* 2016; 59: 7066–7074.

112. Joosten SPJ, Spaargaren M, Clevers H, et al. Hepatocyte growth factor/MET and CD44 in colorectal cancer: partners in tumorigenesis and therapy resistance. *Biochim Biophys Acta Rev Cancer* 2020; 1874: 188437.

113. Nozaki Y, Tamori S, Inada M, et al. Correlation between c-Met and ALDH1 contributes to the survival and tumor-sphere formation of ALDH1 positive breast cancer stem cells and predicts poor clinical outcome in breast cancer. *Genes Cancer* 2017; 8: 628–639.

114. Boccaccio C and Comoglio PM. The MET oncogene in glioblastoma stem cells: implications as a diagnostic marker and a therapeutic target. *Cancer Res* 2013; 73: 3193–3199.

115. Sugano T, Seike M, Noro R, et al. Inhibition of ABCB1 overcomes cancer stem cell-like properties and acquired resistance to MET inhibitors in non-small cell lung cancer. *Mol Cancer Ther* 2015; 14: 2433–2440.

116. Suthe SR, Yao HP, Weng TH, et al. RON receptor tyrosine kinase as a therapeutic target for eradication of triple-negative breast cancer: efficacy of anti-RON ADC Zt/g4-MMAE. *Mol Cancer Ther* 2018; 17: 2654–2664.

117. Gong X, Azhdarinia A, Ghosh SC, et al. LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence. *Mol Cancer Ther* 2016; 15: 1580–1590.