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Review

MET alterations detection platforms and clinical implications in solid tumors: a comprehensive review of literature

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Pei Yuan, Xuemin Xue, Tian Qiu and Jianming Ying

***Abstract*:** *MET* alterations, including *MET* exon 14 skipping variants, *MET* amplification, *MET* overexpression, and *MET* fusion, play pivotal roles in primary tumorigenesis and acquired resistance to targeted therapies, especially *EGFR* tyrosine kinase inhibitors. They represent important diagnostic, prognostic, and predictive biomarkers in many solid tumor types. However, the detection of *MET* alterations is challenging due to the complexity of *MET* alterations and the diversity of platform technologies. Therefore, techniques with high sensitivity, specificity, and reliable molecular detection accuracy are needed to overcome such hindrances and aid in biomarker-guided therapies. The current review emphasizes the role of *MET* alterations as oncogenic drivers in a variety of cancers and their involvement in the development of resistance to targeted therapies. Moreover, our review provides an overview of and recommendations on the selection of various cross-platform technologies for the detection of *MET* exon 14 skipping variants, *MET* amplification, *MET* overexpression, and *MET* fusion. Furthermore, challenges and hurdles underlying these common detection platforms are discussed.

Keywords: detection platform, exon 14 skipping, *MET* alterations, *MET* amplification, *MET* fusion, *MET* overexpression, targeted therapies

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Introduction

MET (mesenchymal–epithelial transition factor) is a widely expressed tyrosine kinase receptor that binds with the natural ligand hepatocyte growth factor (HGF) and plays a vital role in embryogenesis, cell growth, cell differentiation, and angiogenesis.[1] *MET* activation negatively affects tyrosine kinase inhibitors (TKIs) effectiveness due to the intertwining between the *MET* and receptor tyrosine kinase (RTK) [epidermal growth factor receptor (*EGFR*)] signaling pathways.[2] Dysregulation of the HGF-*MET* axis potentially arises by a variety of mechanisms, including mutational activation, such as exon 14 splice site alteration, exon 14 ubiquitination site mutation, kinase domain mutation, extracellular domain mutation, and amplification of the *MET* proto-oncogene or gene copy number (GCN) gain due to polysomy or focal amplification[3] or by overexpression that occurs either due to alteration in transcription factors [erythroblast Transformation specific (Ets) and specificity protein 1 (Sp1)] or by transcriptional upregulation due to hypoxia-inducible factor activation and downregulation of repressor microRNAs (miR-1, miR-34, and miR-449a).[4] These dysregulations lead to malignant transformation (tumor growth, invasion, metastasis, and angiogenesis) through alterations in downstream cellular signaling pathways (*Ras*, PI3K/Akt, STAT3, and NF-κB).[5] A schematic illustration of the normal *MET* signaling pathway and various mechanisms underlying aberrant *MET* signaling pathways is represented in Figure 1.

In view of this pivotal role of *MET* in tissue remodeling and morphogenesis, scholars in several studies have reported that *MET* alterations, particularly *MET* exon 14 skipping variants, *MET* amplification, *MET* overexpression, and *MET*

Correspondence to:
Jianming Ying
Department of Pathology, State Key Laboratory of Molecular Oncology, National Cancer Center/ National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No.17, Panjiayuan Nanli, Chaoyang District, Beijing 100021, China
jmying@cicams.ac.cn

Pei Yuan
Xuemin Xue
Tian Qiu
Department of Pathology, State Key Laboratory of Molecular Oncology, National Cancer Center/ National Clinical Research Center for Cancer/ Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

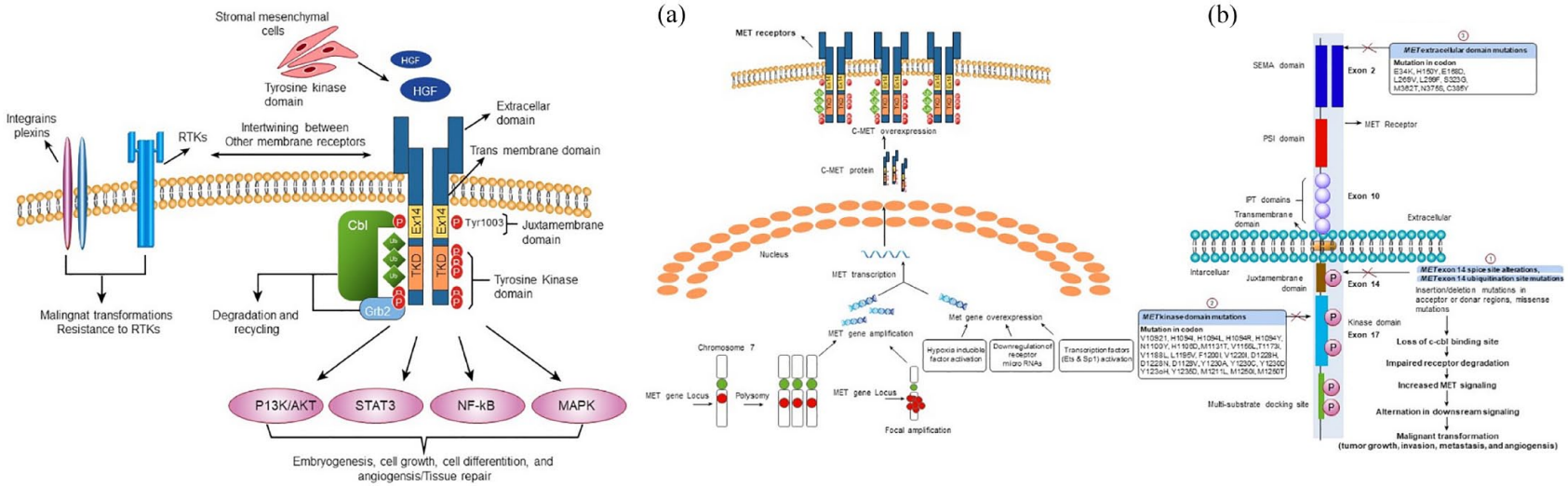

Figure 1. (1) Normal *MET* signaling pathway (left): The binding of hepatocyte growth factor (HGF) leads to receptor dimerization that activates the receptor and subsequently activates downstream signaling pathways including R*as*, PI3K/Akt, STAT3, and NF-κB. This signaling basically instigates embryogenesis, cell growth, cell differentiation, angiogenesis, and tissue repair. Furthermore, downregulation of MET receptor is initiated by CBL and ubiquitin-mediated degradation, extracellular shedding as well as proteolytic cleavage. Intertwining between MET and other membrane receptors which includes plexins, integrins, *EGFR*, and other RTKs promotes malignant transformations and drug resistance. (2) Dysregulation of MET signaling pathway (right). Major mechanisms include (a) MET amplification (polysomy and focal amplification) and overexpression. (b) MET exon 14 skipping variants. (1) *MET* exon 14 splice site variants result in exon 14 exclusion, thereby lacking ubiquitin-binding site in the juxtamembrane domain and ultimately impairing MET degradation and increasing *MET* signaling. Furthermore, Missense mutations in the juxtamembrane domain prevent spliceosome binding and modify the Y1003 ubiquitylation site in the *MET* protein. (2) Mutations in the kinase domain lead to increased activation of the *MET* kinase and can be associated with conformational changes that favor the inactive conformation state. (3) The implications of extracellular mutations that include HGF-binding site (SEMA domain) are currently unclear. All these induce alterations in downstream signaling and ultimately induce mutagenic transformations.

CBL, Casitas B lineage lymphoma proto-oncogene; Ets, erythroblast transformation specific; HGF, hepatocyte growth factor; IPT, immunoglobulin-like plexins and transcription factor; NF-κB, nuclear factor kappa-light-chain-Zkinase; PSI domain, plexin–semaphorin–integrin; SEMA, semaphorin; Sp1, specificity protein 1; STAT3, signal transducer and activator of transcription 3.

fusion, play a key role in pathogenesis and alteration in sensitivity to targeted therapies and contribute to the development of acquired tumor cell resistance to treatment with EGFR-targeting TKIs in different cancer types.[6] A few anti-*MET*-TKIs have been developed for *MET*-directed targeted therapies, such as tepotinib and capmatinib, which have been approved in the United States and Japan, respectively, and savolitinib, which was approved by the National Medical Products Administration of China in June 2021,[7–9] as well as glumetinib, which has been approved in China in March 2023. In view of the importance of *MET* gene alterations in cancer pathogenesis and therapy, the detection of *MET* abnormities has become increasingly important in clinical practice to guide patient selection for targeted therapy, and testing for *MET* exon 14 skipping variants and amplifications has already been recommended by the NCCN guidelines in treatment-naive and TKI-resistant non-small-cell lung cancer (NSCLC) patients, respectively.[10] This review will summarize the present situation and future of *MET* alteration detection technology, especially exon 14 skipping, amplification testing, and overexpression testing, to provide a landscape of *MET* alteration-associated detection and targeted therapy updates.

MET variation

MET exon 14 skipping variants in cancers

A diverse range of variations involving the kinase domain, exon 14, intronic splice site, and SEMA domain can occur within *MET* (Figure 1). Furthermore, a splicing variant in *MET* leading to loss of *MET* exon 14 emerged as a biomarker and offers a potential therapeutic target in several cancer types.[11] Therefore, robust approaches for the detection of such skipping events in *MET* exon 14 are critical in the clinical management of cancers, specifically NSCLCs, and other cancers harboring *MET* exon 14 skipping variants.[3] The prevalence of *MET* exon 14-skipping variants is widely reported in lung cancer, with a frequency of 0.9–4%.[12] Among all cancers, the prevalence of *MET* exon 14 skipping variants is widely reported in NSCLCs, and the most widely used platform for the detection of this variant includes next-generation sequencing (NGS) followed by reverse transcription polymerase chain reaction (RT-PCR). A summarization of prevalence and prognosis is presented in Table 1.

Table 1. Summarization on prevalence and prognosis of *MET* exon14 skipping variants.

Study group	Type of cancer	Disease stage	Race	Sample type	Method	Study population (*N*)	*MET* Exon 14 skipping variants detection rate (%)	Main findings determining the prognostic value
Li *et al.*[13]	NSCLC	Stage I–IV	Asian (Chinese)	Tissue	DNA- and RNA-based NGS	77	20.8 (PSCs)	–
Yu *et al.*[14]		–	–	Tissue	NGS	46	9	–
Vuong *et al.*[15]		–	Asian and Western	–	NGS, RT-qPCR, Sanger Sequencing	18,464	3.0 (Western patients) 2.0 (Asian patients)	Exon 14 skipping mutations were significantly associated with shorter OS (HR 1.82 (1.04–3.19), $p = 0.04$
Awad *et al.*[16]		Stage I–IV	White, Non-Hispanic	Tissue	NGS	933	3.0	–
Lung *et al.*[17]		Stage I–IV	–	Tissue	RT-qPCR	196	1.02	–
Okuda *et al.*[18]		Stage I–IV	–	Tissue	RT-PCR	178	1.7	–
Pruis *et al.*[19]		Stage IIIA–IV	–	Tissue	DNA-based NGS	1497	2.0	–
Heist *et al.*[20]		Stage I–IV	–	Tissue	NGS	54	19	–

(Continued)

Table 1. (Continued)

Study group	Type of cancer	Disease stage	Race	Sample type	Method	Study population (*N*)	*MET* Exon 14 skipping variants detection rate (%)	Main findings determining the prognostic value
Xu *et al.*[21]		Stage I–IV/ unknown	Asian (Chinese)	Tissue	RNA-based NGS	951	1.7	–
Dubbink *et al.*[22]		–	–	Tissue	DNA-based NGS	676	2.7	–
Frampton *et al.*[6]		–	–	Tissue	NGS	38,028	0.6	–
Lee *et al.*[23]		–	–	Tissue	RT-PCR	51	9.8	–
Hu *et al.*[24]	Glioblastoma	Grade IV	–	–	RNA-based NGS	78	14	MET ex14 showed significantly poorer OS compared to those without MET ex14 ($p=3.52\times10^{-3}$)
Lee *et al.*[23]	Gastric cancer	–	–	Tissue	RT-PCR	42	7.1	–
	Colon cancer	–	–	Tissue	RT-PCR	43	9.3	–
	Rectal cancer	–	–	Tissue	RT-PCR	23	0	–
	Hepatocellular carcinoma	–	–	Tissue	RT-PCR	15	0	–
	Sarcoma	–	–	Tissue	RT-PCR	9	0	–
	Pancreatic cancer	–	–	Tissue	RT-PCR	5	0	–
	Cholangiocarcinoma	–	–	Tissue	RT-PCR	6	0	–
	Melanoma	–	–	Tissue	RT-PCR	5	0	–
	Esophageal squamous carcinoma	–	–	Tissue	RT-PCR	1	0	–
	Renal cell carcinoma	–	–	Tissue	RT-PCR	1	0	–

'–', Not reported; AJCC, American Joint Committee on Cancer; HR, hazard ratio; NGS, next-generation sequencing; NSCLC, non-small-cell lung cancer; OS, overall survival; PSCs, pulmonary sarcomastoid carcinomas; RT-PCR, reverse transcription polymerase chain reaction; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

Detection of MET exon 14 skipping variants

Genome-wide sequencing revealed heterogenic forms of *MET* exon 14 variants at the DNA level, thereby making it challenging to detect either by amplification refractory mutation system (ARMS)-PCR or by DNA NGS panels; instead, RNA-based testing can further improve testing accuracy.[16] Furthermore, limited reports are available on the comparison of these detection platforms.

DNA-based NGS. A detailed methodology of NGS is represented in Figure 2(c). Generally, two types of NGS-based assays, namely, amplicon-based and hybrid capture-based NGS platforms differing in DNA enrichment methods, are used in clinical settings.[25] The major limitation associated with routinely used commercially available amplicon-based NGS panels for detection of *MET* exon 14 skipping variants includes the frequent emergence of new regions to be covered that lead to allelic dropouts and sequencing errors.[26] Earlier, Davies *et al.* compared amplicon-mediated DNA-based NGS *versus* RNA-based NGS in NSCLC tumor samples for *MET* exon 14 skipping variants and reported that among 286 samples tested by both assays, RNA-based testing detected 10 positive samples, 6 of which were not detected by the DNA-based assay. Further examination revealed that genomic deletion involving primer binding sequences was the likely cause of false negatives reported and led to the further conclusion that amplicon DNA-based NGS misses the detection of a substantial fraction of *MET* exon 14 alterations as they are located outside the amplified regions.[27] However, amplicon-based NGS has the advantage of improved capture of targets and sequencing of difficult regions with shorter turnaround time when compared to hybrid-based NGS.[3] The pitfalls associated with amplicon-based NGS can be addressed with the adoption of hybrid-based NGS, where it not only allows the identification of hotspot mutations but also interrogates entire coding sequences of oncogenes, tumor suppressor genes, and introns of selected genes that are involved in gene fusions and further allows the assessment of copy number alterations.[28] Furthermore, if the designed algorithm and probe/bait sufficiently cover the region of interest, DNA-based assays using hybrid capture-mediated target enrichment are less likely to produce false-negative results. This is exemplified by the studies from Frampton *et al.* and Awad *et al.*, where a wide variety of *MET* exon 14 skipping variants, including large deletions, were successfully detected by employing hybrid-based NGS assays.[6,16] Furthermore, a hybrid-based approach enables corrections for some of the sequencing bias and allele dropout issues associated with amplicon-based NGS assay.[29] Although the depth of coverage of genes of interest in both platforms was high, hybrid-based NGS, with its noteworthy advantage, outperforms amplicon-based NGS.[3]

RNA-based NGS or RT-PCR. *MET* exon 14 variants can also be detected at the RNA level. Li *et al.* conducted a study that involved the comparison of DNA- and RNA-based NGS for the detection of *MET* exon 14 skipping variants in pulmonary sarcomatoid carcinomas (PSCs) and reported a concordance of 96.1% between these platforms and concluded that RNA-based sequencing was the most accurate because some somatic variants not covering *MET* exon 14 splice sites might also induce skipping.[13] Furthermore, DNA sequencing cannot confirm the absence of the exon, as modifications such as splicing occur post-translationally.[27] At this juncture, RNA-based NGS platforms have the potential to complement DNA-based NGS platforms where RNA sequencing permits the direct recognition of the loss of exon 14 transcription.[27] In a study, Jurkiewicz *et al.* compared the performance of DNA *versus* RNA-based NGS assays for the detection of *MET*-ex14 skipping variants in 644 lung adenocarcinoma samples and concluded that DNA-based NGS panels can potentially miss *MET*-ex14 skipping when the primers do not target both the 3′ and 5′ splice sites of introns 13 and 14, respectively.[32] Furthermore, due to the diverse nature of *MET* exon 14 splice site alterations, interpreting variants that truly result in exon 14 skipping is challenging, and this becomes more strenuous when these alterations are located in deeper introns; since RNA-based platform involves sequencing mRNA that is devoid of introns, this kind of challenge can be avoided.[33]

RNA-based RT-PCR can also be used for *MET*-ex14 skipping detection and shows good concordance with RNA-based NGS.[12] Although NGS is rapid in comparison to traditional Sanger sequencing, it is still too expensive to be affordable

by small laboratories or an individual.[34] By contrast, RT-PCR is easy to perform, more widespread, and has a shorter turnaround time. RT-qPCR is usually designed in the *MET* exon 13 and 15 region primers for the detection of specific amplification products. This method has a high accuracy in detecting MET 14 variants but it can miss some special and rare forms of MET variations. Meanwhile, RNA-based analysis is highly reliant on the quality of RNA, which can be suboptimal in some clinical samples.[27] RNA-based testing is not part of the routine workflow in many molecular detection laboratories, as acquiring sufficient RNA material remains a large obstacle in contrast to DNA acquisition. Therefore, when RNA quality is at risk, an alternative is DNA-based NGS, where DNA is less difficult to obtain and less vulnerable to degradation.

Targeted therapies for MET exon 14 skipping variants

The constant discovery of actionable activating variants has led to targeted therapies with new-generation TKIs and improved overall survival and time to progress.[26] Among all reviewed literature, NSCLC lung cancer accounts for almost all. Earlier, a phase I PROFILE 1001 study reported the efficacy of crizotinib [median progression-free survival (mPFS) 7.3 months and overall response rate (ORR) of 32%] in advanced-stage NSCLC patients harboring *MET* exon 14 alterations, and these results led to the inclusion of crizotinib in the NCCN guidelines. With this breakthrough, several highly selective *MET* inhibitors, such as tepotinib, capmatinib, savolitinib, and glumetinib, have been tested in patients with *MET* exon 14-altered NSCLC and found to be more potent than crizotinib.[35]

Tepotinib was developed for the treatment of solid tumors and demonstrated promising clinical efficacy and safety profiles in patients with advanced NSCLC with a confirmed MET exon 14 skipping variant in the multinational phase II VISION study.[36] In March 2020, it was approved for use in Japan for this indication and was subsequently approved by the FDA on 3 February 2021. Capmatinib was developed for the treatment of lung cancer, inhibiting cancer cell growth driven by the mutant MET variant, including exon 14 skipping. In May 2020, capmatinib received its first global approval for the treatment of adults with metastatic NSCLC with MET exon 14-skipping variants as detected by an FDA-approved test.[8] Savolitinib was developed for the treatment of NSCLC, gastric cancer, colorectal cancer, and papillary and clear cell renal cell carcinoma. Based on the results of a pivotal phase II trial, savolitinib yielded promising activity and had an acceptable safety profile in patients with NSCLC/pulmonary sarcomatoid carcinoma and was recently approved in June 2021 in China for the treatment of metastatic NSCLC with MET exon 14-skipping alterations in patients who have progressed after or who are unable to tolerate platinum-based chemotherapy, conditional on the results of a phase III trial.[37] Glumetinib was developed for the treatment of lung cancer and showed durable antitumor activity with manageable toxicity in patients with locally advanced or metastatic MET ex14-positive NSCLC in the phase II GLORY study.[38] In March 2023, it was approved in China for this indication.

Furthermore, reports from several clinical studies cannot be compared directly due to different populations and inclusion criteria in the clinical efficacy of several *MET*-TKI inhibitors, including tepotinib[39] [independent review committee (IRC): ORR 57.3% (treatment naive), 45.0% (pretreated),[40] capmatinib (ORR: 44% (pretreated) and ORR: 68.3% (treatment naive)[35], mPFS 12.5 m (treatment naive), 5.5 m (pretreated); mOS 25.5 m (treatment naive), not reported (pretreated))],[41] and savolitinib [phase IIIb study, IRC: ORR 58.6% (treatment naive), mPFS 13.8 m (treatment naive)[42]; phase II study(36% PSC and 21% CNS metastases): TRES set: ORR 49.2%, FAS set: mPFS 6.9 m, mOS 12.5 m; in PSC, mPFS 5.5 m, mOS 10.6 m; other NSCLC (non-PSC), mPFS 7.0 m, mOS 17.3m[43]] and glumetinib[38] (BIRC: overall ORR 66%, mPFS 8.5 m, mOS 17.3 m; in treatment-naïve patients: ORR 71%, mPFS 11.7 m) in patients harboring *MET* exon 14 skipping variants in NSCLC. A summarization of *MET* exon 14 skipping variant targeted therapy outcomes in advanced NSCLC is represented in Table 2.

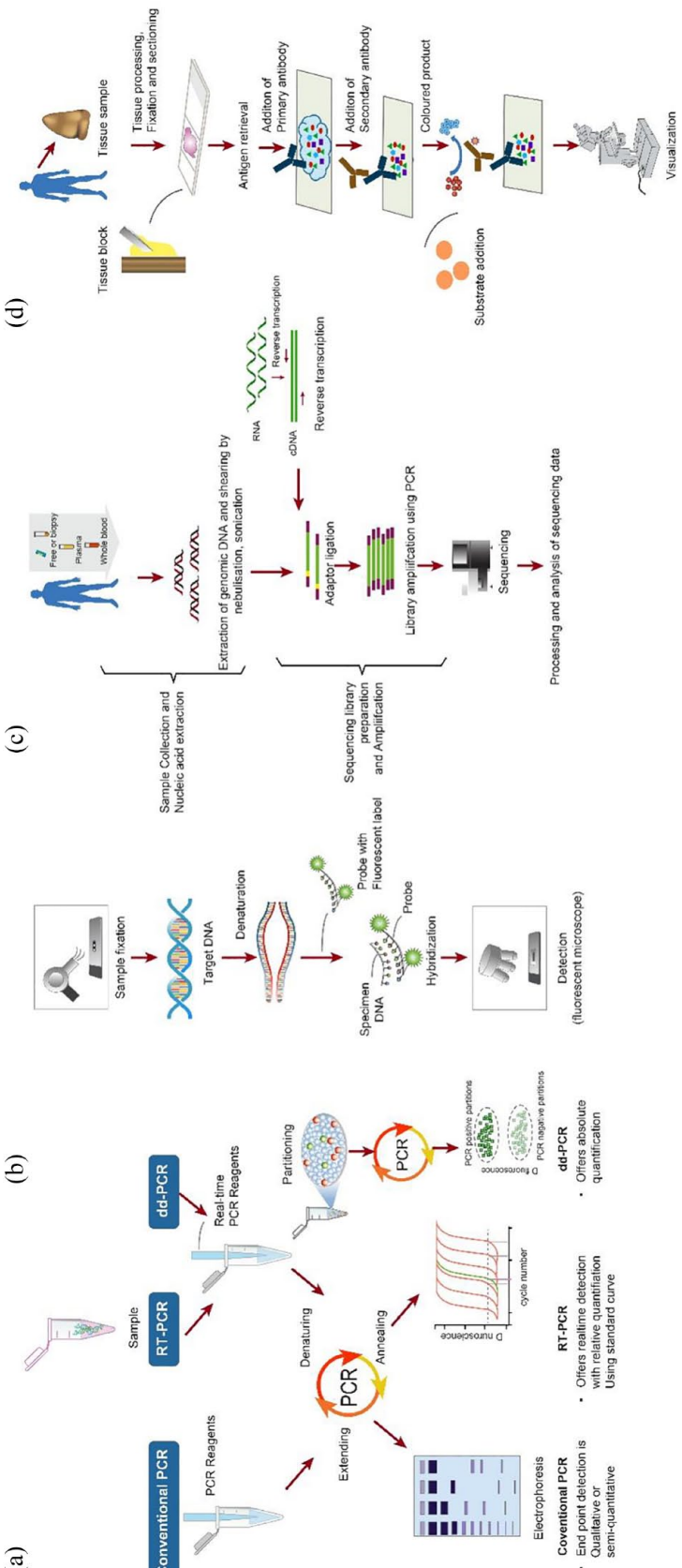

Figure 2. Schematic illustrations of workflow of various *MET* aberration detection techniques: (a) polymerase chain reaction, quantitative reverse transcription polymerase chain reaction (RT-qPCR) is based on conventional PCR which utilizes a fluorescent readout to measure the amount of PCR product after each round of amplification[30]; (b) fluorescence in situ hybridization (FISH) assay involves three steps, that is, sample fixation and denaturing the sample that involves conversion of double-stranded DNA into single-stranded DNA and subsequent hybridization where denatured single-stranded DNA was tagged with fluorescent-labeled single-stranded DNA probes and followed by visualization of the hybridized probe-target DNA complexes under fluorescent microscope.[31] (c) Next-generation sequencing (NGS) is a high-throughput technology that utilizes parallel sequencing of multiple fragments to determine the sequence. It is a complicated process with multiple steps and high requirements of quality control, different NGS platforms adopt their own specific protocol in the sequencing methods. An overview of NGS workflow (both DNA based and RNA based) was represented. (d) Immunohistochemistry: It involves the utilization of anti-*MET* antibodies [monoclonal antibodies (SP44, c*MET*, and *MET4*) or polyclonal antibodies (*MET* AF276)].
NGS, next-generation sequencing; RT-qPCR, reverse transcription polymerase chain reaction.

Table 2. *MET* exon 14 skipping variants targeted therapy outcomes in advanced NSCLC.

Study group	Type of cancer	Drug	Stage	Study phase	No. of patients	Assay used	ORR	Median PFS in months (95% CI)
Drilon *et al.*[44]	NSCLC	Crizotinib (9% PSCs)	Advanced	I	69	NGS, RT-PCR	Overall: 32% (21/65) ORR by alteration type: Indels: 25% (5/20). Point mutations: 36% (12/33). Splice acceptor site mutations: 31% (5/16). Splice donor site mutations: 32% (12/37).	7.3 (5.4–9.1).
Moro-Sibilot *et al.*[45]		Crizotinib (PSCs% unknown)	Advanced	II	28	NGS and Sanger sequencing	ORR: 10.7%	3.6 (1.6–7.1)
Wolf *et al.*[35]		Capmatinib (INC280) (none-PSCs)	Advanced	II	Pretreated: 100 treatment naive: 60	RT-PCR, NGS	Pretreated: 44%, treatment naive: 68.3%	Pretreated: 5.5 (4.2–8.1) Treatment naive: 12.5 (8.3–18.0)
Mazieres *et al.*[39]		Tepotinib (PSCs% unknown)	Advanced	II	Overall: 313 Pretreated: 149 Treatment naive: 164	RNA-based NGS	Overall: 51.4% Pretreated: 45%, Treatment naive: 57.3%	Overall: 11.2 (9.5–13.8) Pretreated: 11.0 (8.2–13.7), Treatment naive: 12.6 (9.7–17.7)
Lu *et al.*[46]	PSCs and other NSCLCs	Savolitinib (36% PSCs)	Advanced	II	PSCs: 25, non-PSCs: 45	Sanger DNA sequencing or DNA-based NGS	TRES (tumor response evaluable set): ORR: 53.2% PSCs: 50%, other NSCLCs Non-PSCs: 55%	Overall: 6.9 (4.6–8.3) PSCs: 5.5 (2.6–6.9), Other NSCLCs (non-PSCs): 7.0 (5.5–13.7)
Yu *et al.*[38]	NSCLC	Glumetinib (6% PSCs)	Advanced	II	Overall: 84 Pretreated: 38 Treatment-naive: 46	RNA-based RT-PCR	Overall: 66% Pretreated: 60% Treatment-naive: 71%	Overall: 8.5 (7.6–9.7) Pretreated: 7.6 (4.1–9.6), Treatment-naive: 11.7 (7.6–21.9)
Lu *et al.*[42]		Savolitinib (8% PSCs)	Advanced	IIIB	87 (Treatment naive)	NA	ORR: 58.6%	13.8 (9.7, NR)

mPFS, median progression free survival; NGS, next-generation sequencing; NR, not reached; NSCLC, non-small-cell lung cancer; ORR, overall response rate; PFS, progression-free survival; PSCs, pulmonary sarcomatoid carcinomas; RT-PCR, reverse transcription polymerase chain reaction.

MET amplification

MET amplification in cancers

MET GCN gain occurs either by polysomy or focal amplification (Figure 1).[3,47] Tumors harboring de novo *MET* amplifications (high level) are primarily dependent on the *MET* signaling pathway for growth and are found across a wide variety of solid tumors,[3] while tumors harboring acquired *MET* amplifications rely on other oncogene alterations (such as *EGFR* mutation) and develop a secondary dependence on the *MET* signaling pathway as a mechanism of resistance to targeted *EGFR* therapies.[48] Reports from several studies have indicated that *MET* gene amplification drives treatment resistance, particularly therapies targeting *EGFR*.[49–53]

MET amplification occurs in a broad type of cancer as protooncogenes or resistance mechanisms. A summarization of the prevalence and prognosis of *MET* amplification in various cancers is presented in Table 3.

Regarding polysomy, there is controversial evidence regarding whether *MET* polysomy is the driver of gene alteration for tumorigenesis. Lai *et al.* found that although up to 26% of TKI-naive *EGFR* mutant-positive NSCLC harbor high *MET* GCN by FISH, this did not significantly affect response to TKIs, except in patients identified as *MET*-amplified but not polysomy.[74] In the phase II Acse study, advanced NSCLC patients with *MET* polysomy and low amplification (*MET*/CET7 ratio between 1.8 and 2.2) did not respond well to crizotinib, with a mPFS of 3.2 months and a mOS of 7.7 months.[45] However, in the phase 1b/2 TATTON study, osimertinib and savolitinib showed encouraging antitumor activity in both *MET* amplification and polysomy patients recruited based on FISH positivity, with an ORR of 30% in part B1 (after prior 3G TKIs).[12] The subgroup analysis results showed that the response of focal amplification was better than that of polysomy (ORR 31% versus 28%) but more research is needed for verification. Therefore, *MET* amplification may be considered as a biomarker for *MET*-directed therapies, while polysomy still needs to be studied more.

Detection of MET amplification

MET amplification (copy number gain) can be detected by several techniques, such as FISH,

Table 3. Summarization on prevalence and prognosis of *MET* amplification in various cancers.

Study group	Type of cancer	Disease stage	Race	Sample type	Method	*MET* amplification type	Treatment	Study population (N)	*MET* amplification detection rate *N* (%)	Prognostic value
Ahn *et al.*[10]	NSCLC	Stage IV	Asian and non-Asian	Tissue	FISH	Acquired	*EGFR*-TKI + *MET*-TKI	108	32 (29.6)	FISH10+ has better survival than FISH5+
Tong *et al.*[54]	NSCLC	Stage I–IV	-	Tissue	FISH	-	-	687	29 (4.2)	-
Go *et al.* [55]		Stage I–IV	Asian (Korean)	Tissue	FISH	De novo	*EGFR*-TKI naive	180	7 (3.9)	High *MET* GCN leads to shorter survival
Okamoto *et al.*, [56]		Stage IIIB/IV	-	Tissue	FISH	-	-	229	9 (3.9)	-
Schildhaus *et al.*[49]		-	-	Tissue	FISH	De novo	Untreated	693	20 (3)	-
Suryavanshi *et al.*[57]		Stage IV		Tissue	FISH	-	-	76	5 (6.57)	
Guo *et al.*[58]		-	Asian and Non-Asian	Tissue	RT-PCR, FISH, and SISH	-	-	5516	4–18	High GCN was significantly associated with shorter OS (HR 1.90 [1.35–2.68], $p = 0.001$)
Onozato *et al.*[59]		Stage I–IV	-	Tissue	RT-PCR	-	-	148	2 (1.4)	-

(Continued)

Table 3. (Continued)

Study group	Type of cancer	Disease stage	Race	Sample type	Method	*MET* amplification type	Treatment	Study population (N)	*MET* amplification detection rate *N* (%)	Prognostic value
Bean *et al.*[60]		–	–	Tissue	RT-PCR	Acquired	*EGFR*-TKI	43	9 (21%)	*MET* amplification occurs independently of $EGFR^{T790M}$ mutation and clinically relevant to gefitinib and erlotinib resistance
						De novo	Untreated	62	2 (3%)	
Xia *et al.*[61]		Stage I–IV	Han Chinese	Tissue	RT-PCR	–	–	110	6 (5.5)	–
Xu *et al.*[62]		–	–	Tissue	fluorescent RT-PCR	De novo	–	368	33 (8.97%)	*MET* gene amplification in a primary tumor helps in guiding *MET*-targeted therapies
Chen *et al.*[63]		–	–	Tissue	RT-PCR	–	–	257	29 (11.28)	–
				Blood		–	–	318	31 (9.75)	
Beau-Faller *et al.*[64]		Stage I–IV	Non-Asian	Tissue	RT-PCR	–	–	106	22 (21)	–
Okuda *et al.*[18]		Stage I–IV	–	Tissue	RT-PCR	–	–	213	12 (5.6)	–
Tsuta *et al.* [65]		Stage I–IV	–	Tissue	BISH	–	–	844	92 (10.9)	–
Zhang *et al.*[66]	Colorectal adeno-carcinoma	AJCC Stage I–IV	–	Tissue	FISH	De novo	–	294	13 (4.4)	–
Jardim *et al.*[67]	Metastasis CRC	Advanced (metastasis)	Asian, Black, Hispanic, White	Tissue/ needle aspiration biopsies	FISH	–	–	208	4 (1.9)	–
Raghav *et al.*[68]		–	–	Tissue	FISH (*MET*/*CEP7* ratio > 2)	De novo	Untreated	208	4 (1.9)	–
		–	–	ctDNA	NGS (GCN > 4 copies)	Acquired	*EGFR*-TKI therapy	53	12 (22.6%)	–
		–	–	Tissue	NGS (GCN > 4 copies)	–	–	279	6 (2.2%)	–

(Continued)

Table 3. (Continued)

Study group	Type of cancer	Disease stage	Race	Sample type	Method	*MET* amplification type	Treatment	Study population (N)	*MET* amplification detection rate *N* (%)	Prognostic value
Yu *et al.*[69]	GC	–	Asian and Western	–	FISH, and SISH	–	–	2210	8–82	*MET* amplification was significantly associated with shorter OS (HR 2.112 [1.622–2.748])
Graziano *et al.*[70]		–	–	Tissue	FISH	De novo	–	230	1–5	–
de Melo Gagliato *et al.*[71]	Breast cancer	Stage IV	Asian, American, Indian, Black, Hispanic, White	Tissue	FISH	–	–	107	63 (4.7)	–
Jardim *et al.*[67]		Advanced (metastasis)	Asian, Black, Hispanic, White	Tissue/ needle aspiration biopsies	FISH	–	–	64	3 (4.6)	–
Jardim *et al.*[67]	Ovarian cancer	Advanced (metastasis)	Asian, Black, Hispanic, White	Tissue/ needle aspiration biopsies	FISH	–	–	110	4 (3.6%)	–
	Gastroesophageal	Advanced (metastasis)	Asian, Black, Hispanic, White	Tissue/ needle aspiration biopsies		–	–	77	5 (6.4)	–
	Melanoma	Advanced (metastasis)	Asian, Black, Hispanic, White	Tissue/ needle aspiration biopsies		–	–	61	2 (3.2)	–
	Renal cancer	Advanced (metastasis)	Asian, Black, Hispanic, White	Tissue/ needle aspiration biopsies		–	–	28	4 (14.28)	–
Pal *et al.*[72]	PRCC	Advanced	–	Tissue	FISH	De novo	–	169	13% (Type 1 PRCC) and 3% (type 2 PRCC)	–
Kato *et al.*[73]	Esophageal carcinoma	Stage I–III	–	Tissue	FISH	De novo	–	196	1 (2)	–

'–', Not reported; AJCC, American joint committee on cancer; cfDNA, circulating cell-free DNA; GC, gastric carcinoma; HR, hazard ratio; mCRC, metastatic colorectal cancer; NSCLC, non-small-cell lung cancer; PRCC, papillary renal cell carcinoma; RT-PCR, reverse transcription polymerase chain reaction; RT-qPCR, reverse transcription polymerase chain reaction; FISH, fluorescence in situ hybridization.

NGS, and ddPCR.[3] As the magnitude of *MET* amplification is a continuous variable, determining the cutoff for *MET* positivity is more challenging.[3] At present, no consensus exists on the most appropriate diagnostic cutoff point for *MET* amplification.[75] Different detection platforms for *MET* amplification testing exist and have ineligible disparities in terms of sensitivity and specificity.

Fluorescence in situ hybridization (FISH). FISH is a cytogenetic technique used for obtaining spatial genomic and quantification of nucleic acids in the cellular environment and has emerged as the gold standard technique for the detection of chromosomal abnormalities.[76]

In the FISH assay, *MET* copy number increases can be defined either by Cappuzzo criteria or by University of Colorado Cancer Center (UCCC) criteria, which calculate the ratio of *MET* to chromosome enumerating probe against chromosome 7 (*CEP7*). The Cappuzzo *et al.* criteria define *MET* amplification as a mean of five or more copies of *MET* per cell (*MET* gene copy number (GCN) $\geq$5).[77] Furthermore, other alternate definitions suggest a *MET* GCN of $\geq$6[78] and a *MET* GCN of $\geq$15.[79] However, the determination of GCN cannot differentiate between *MET* focal amplification and polysomy, and this limitation is overcome by the UCCC approach involving the calculation of the *MET-to-CEP7* ratio that adjusts the number of chromosomes present, thereby differentiating selective *MET* focal amplifications and chromosomal duplication. In general, a *MET* to *CEP7* ratio $\geq$2.0 defines *MET* focal amplification.[73] However, several other studies categorized the degree of *MET* focal amplification into low ($\geq$1.8–$\leq$2.2), intermediate (>2.2–<5), and high ($\geq$5).[75]

The FISH technique is advantageous in the detection of *MET* amplification in light of its comparable performance characteristics and potential for cost-effectiveness and limited complexity in testing when compared with NGS. Furthermore, FISH offers direct visualization of the tested samples, which was not possible by NGS, and another added benefit of this technique is that it represents a suitable technology for the detection of intratumoral heterogeneity within tissue samples. Although FISH is the current gold standard for *MET* amplification testing, the prevalence of *MET* amplification detected by FISH is variable in the literature, which is likely attributable to a lack of standardization of technique and/or patient selection criteria and different cutoffs for defining *MET* positivity.[63] In addition, the main limitation of FISH is that it can only be applied to tissue samples, while tissue feasibility is low in advanced NSCLC patients, especially patients who progressed on previous TKIs.

Next-generation sequencing. Similar to the FISH assay, there is no consensus on a single definition of *MET* amplification, and the cutoff for *MET* amplification varies across different NGS platforms.[80] Hybrid capture-based NGS is known to be more accurate in assessing copy number variation in *MET* and other genes, as it interrogates broader regions of the genome and removes sequence replicates. By contrast, amplicon-based NGS covers a limited genomic territory, and sequence replicates cannot be removed, affecting sequence coverage depth.[3] NGS can be used to analyze both tissue and plasma ctDNA or other bodily fluids, which will facilitate biomarker testing extensively. However, certain technical limitations, including sample selection, low tumor cell fraction, and low DNA quality of tumor samples can increase background noise, hindering the accurate analysis of copy number gain/loss.[80] As reported in the TATTON study, where osimertinib (3rd *EGFR*-TKI) and savolitinib (*MET*-TKI) combined therapy in NSCLC patients with *MET*-amplified *EGFR*-TKI resistance demonstrated encouraging antitumor activity, FISH positivity was defined centrally as either focal amplification (*MET:CEP7* ratio $\geq$2) or polysomy (gene copy number $\geq$5 if *MET:CEP7* <2), while tissue NGS from Foundation Medicine showed higher positive-percent agreement (PPA, also known as sensitivity) for FISH focal amplification (88%) but lower PPA for FISH polysomy (4%). Similarly, a comparison of ctDNA NGS with FISH yielded negative-percent agreement (NPA) of 90% and a modest PPA of only 25%.[45] Various factors affect the sensitivity of ctDNA detection by NGS, such as sequencing depth, threshold of bioinformatic analysis, and techniques to enrich tumor-derived signals and reduce background noise. The high specificity and low sensitivity of the currently available NGS assay indicate that copy number variation (CNV) detection by NGS needs to be optimized, and retesting with FISH should be considered to avoid missing *MET* amplification; while NGS may serve as an alternative selection for MET polysomy with higher sensitivity and specificity and guide the clinical treatment.[81]

Although plasma ctDNA testing of *MET* amplification is challenging, it is a direction for future

development to fulfill the clinical needs given that plasma specimens are dominant in late-phase patients. In addition, plasma harvested from peripheral blood is less invasive and can reflect the disease progression dynamically while avoiding tumor heterogenicity in single surgery or biopsy samples.[82]

Polymerase chain reaction. *MET* amplifications can be detected by employing a PCR technique specifically (RT-PCR). Similar to FISH and NGS, the cutoff for defining *MET* amplification positivity was not standardized.[62] A comparison of the basic principle and procedure involved in different PCR techniques is represented in Figure 2(a). The major limitation associated with RT-PCR is that the success relies on the RNA quality in the specimen tested. Inadequate fixation or prolonged ischemia of tissue leads to false-negative results. These limitations were outweighed by the introduction of droplet digital PCR (ddPCR), which has high sensitivity and accuracy levels for absolute representation of a given nucleic acid sequence. Droplet digital PCR enables the absolute quantification of nucleic acids present in the sample by partitioning the sample into independent PCR subreactions where each partition contains a few or no target sequences and is subjected to PCR wherein the fraction of amplification positive partitions is used to quantify the concentration of the target sequence with a statistically defined accuracy using Poisson's statistics.[83]

The performance of ddPCR for *MET* amplification testing is not well characterized compared to FISH and NGS.[84] However, the consistency between ddPCR and FISH is reported in some small sample studies for assay analytical validation of developed ddPCR methodology; the sensitivity and specificity of tissue ddPCR and FISH are both 100%, while the sensitivity and specificity of tissue and blood ddPCR are 66.67% and 98.86%, respectively.[85] Given that insufficient tissue can be retrieved after resistance to *EGFR*-TKIs, further studies to confirm the testing capability of blood ddPCR as an alternative detection tool for *MET* amplification is needed in the future.

Targeted therapies for MET amplification

The prevalence of acquired *MET* amplification is enriched gradually after lines of *EGFR*-TKI treatment. In lung cancer, *MET* amplification occurred in 1–4% of treatment-naive patients, while the prevalence increased to 5–22% after first- and second-generation TKI treatment and 5–50% after third-generation TKI osimertinib treatment.[86] The amplification of *MET* occurred independently of the *EGFR* T790M mutation and was clinically relevant to gefitinib and erlotinib resistance.[60]

There are several *MET*-TKIs in development for *MET* amplification-positive NSCLC patients, such as crizotinib, tepotinib, capmatinib, and savolitinib, and criteria for patient inclusion are mainly based on FISH and immunohistochemistry (IHC). The cutoff for *MET* amplification positivity varies among studies[83] Furthermore, it is evident from the literature that patients harboring higher *MET* GCN achieve better clinical outcomes from the targeted therapy.

For de novo *MET* amplification NSCLC, in a phase I PROFILE 1001 study in which NSCLC patients were stratified based on the degree of *MET* amplification and the activity of crizotinib examined in relation to the level of *MET* amplification, there was a high amplification group (*MET-to-CEP7* ratio ≥ 4) with reported ORR of 38.1% and median PFS of 6.7 months compared with a low amplification group (*MET-to-CEP7* ratio ≥ 1.8 to ≤ 2.2) with ORR of 33.3% and median PFS of 1.8 months and an intermediate amplification group (*MET-to-CEP7* ratio > 2.2 to < 4) with ORR of 14.3% and median PFS of 1.9 months.[87] Earlier, the results of the GEO*MET*RY mono-1 study demonstrated the efficacy and safety of capmatinib in patients with high-level (GCN) ≥ 10 compared with low-(GCN < 4) or mid-level (GCN 4–5 or 6–9) *MET*-amplified advanced NSCLC. Patients with GCN ≥ 10 treatment-naive and/or receiving 1 or 2 lines of therapy exhibited better ORRs of 40% and 29%, respectively.[35] It seems that high amplification status is associated with better response to *MET*-TKIs compared to low amplification status given the current evidence. Recently, the results from the VIKTORY umbrella trial showed that savolitinib monotherapy exhibited an ORR of 50% (10/20) in a subset of gastric cancer patients harboring *MET* amplifications, and further genomic analysis revealed that patients with *MET* GCN > 10 (by tissue NGS) had high response rates to savolitinib [ORR 70% (7/10)] and concluded that the subset of patients with *MET* amplifications achieved the largest absolute decrease in tumor burden.[83] Furthermore, despite there existing evidence of *MET* inhibition by foretinib, Shah *et al.* reported disappointing results for foretinib, which might be due to disparities in the selection of the study population, study design, or drug itself.[88] Furthermore, a summarization of

Table 4. *MET* amplification targeted therapy outcomes.

Study group	Cancer type	Drug	Study phase	Study population	Assay used	Amplification criteria	*MET* subgroup	Subgroup (*N*)	ORR	Median PFS (months)
Camidge *et al.*[89]	NSCLCs	Crizotinib	Phase I	38	FISH	*MET-to-CEP7* ratio ⩾ 1.8	*MET-to-CEP7* ratio ⩾ 1.8–⩽2.2	3	33% (1/3)	1.8
							MET-to-CEP7 ratio > 2.2 to < 4.0	14	14% (2/14)	1.9
							MET-to-CEP7 ratio ⩾ 4.0	20	38% (8/21)	6.7
Moro-Sibilot *et al.*[45]		Crizotinib	Phase II Acsé study	25	FISH	*MET* GCN ⩾ 6 and IHC 2+/3+	All patients	–	16% (4/25)	3.2
Schuler *et al.*[90]		Capmatinib	Phase I	44	FISH	*MET-to-CEP7* ratio ⩾ 2.0 or *MET* GCN ⩾ 5, H-score ⩾ 150, IHC 2+/3+	*MET* GCN ⩾ 5	8	63% (5/8)	–
Wu *et al.*[91]		Capmatinib + gefitinib	Phase II	100	FISH and IHC	*MET* GCN ⩾ 4	All patients	–	29% (29/100)	
							MET GCN ⩾ 6	36	47% (17/36)	
Yu *et al.*[92]		Savolitinib + osimertinib	Phase II ORCHARD study	20	NGS	-	*MET* amplification	17	41% (7/17)	–
Hartmaier *et al.*[93]		Savolitinib + osimertinib	Phase Ib TATTON study	42	FISH	*MET* GCN ⩾ 5 or *MET-to-CEP7* ratio ⩾ 2.0	Part D all patients	–	62%	9.0
Ahn *et al.*[10]		Savolitinib + osimertinib	Phase II SAVANNAH study	196	FISH and IHC	*MET* GCN ⩾ 5 and/or *MET-to-CEP7* ratio ⩾ 2.0, IHC ⩾ 50% tumor cell 3+	FISH10+ and/or IHC90+	108	49%	7.1
Liam *et al.*[94]		Tepotinib + gefitinib	Phase II INSIGHT study	31	FISH IHC	IHC ⩾ 50% tumor cell 2 + 3+ *MET* GCN ⩾ 5 or *MET-to-CEP7* ratio ⩾ 2.0	All patients	–	45.2%	4.9
							IHC 3+	19	68.4%	8.3
							MET amplification	12	66.7%	16.6
Kim *et al.* [95]		Tepotinib + osimertinib	Phase II INSIGHT 2 study	128	FISH LBx NGS	*MET* GCN ⩾ 5 and/or *MET-to-CEP7*⩾2 LBx NGS GCN ⩾ 2.3	TBx FISH LBx NGS	98(TBx FISH)	50.0%	5.6

(Continued)

Table 4. (Continued)

Study group	Cancer type	Drug	Study phase	Study population	Assay used	Amplification criteria	*MET* subgroup	Subgroup (*N*)	ORR	Median PFS (months)
Yu *et al.* [96]		Glumetinib + osimertinib	Phase Ib/II	30	FISH	*GCN* ⩾ *5* and/or *MET-to-CEP7* ⩾ *2*	*MET* amplification	11(Post 3G EGFR-TKI)	60% (36.4% in post 3G EGFR-TKI)	6.9
Qin *et al.*[97]	Hepatocellular carcinoma	Capmatinib	Phase II	30	FISH	*MET* H-score ⩾ 50 or *MET-to-CEP7* ratio ⩾ 2.0 or *MET* GCN ⩾ 5	All patients	–	10% (3/30)	–
							MET IHC score 3 + or 2 + in ⩾ 50% of tumor cells and *MET* GCN ⩾ 5	10	30% (3/10)	–
Lee *et al.*[83]	Gastroesophageal cancers	Savolitinib	Phase II	715 (25 *MET* amplified)	NGS	–	*MET* GCN > 10	20	70% (7/10)	–
Shah *et al.*[88]		Foretinib	Phase II	74	FISH	*MET-to-CEP7* ratio ⩾ 2.0	All patients	–	0% (0/71)	–
MET-amplified							3	0% (0/3)	–	
Bang *et al.*[98]	Solid tumors	Capmatinib	Phase I	38	FISH	–	*MET* GCN < 4	22	0% (0/22)	–
							MET GCN ⩾ 4 to < 6	6	0% (0/6)	
							MET GCN ⩾ 6	3	0% (0/3)	
Angevin *et al.*[99]		SAR125844	Phase I	72	FISH	*MET-to-CEP7* ratio ⩾ 2.0 and *MET* GCN > 4	All patients	–	17% (5/29)	–

'–', not reported; CEP 7, chromosome 7; FISH, fluorescence in situ hybridization; GCN, gene copy number; IHC, immunohistochemistry; NSCLC, non-small-cell lung cancer; PRCC, papillary renal cell carcinoma; NGS, next-generation sequencing; PFS, progression-free survival; ORR, overall response rate.

MET amplification targeted therapy outcomes by *MET* copy number status across different cancer types is represented in Table 4.

As shown in Table 4, the commonly used platform for *MET* amplification testing and patient screening is FISH, combined with the IHC platform for some studies. The criteria for MET amplification varied across studies, and a unified cutoff has not yet been established. Both the *MET-to-CEP7* ratio and *MET* GCN number were used separately or combined. It seems that a better response to MET-TKIs or combined therapy occurs in patients with higher *MET* amplification status.

MET overexpression

MET overexpression in cancers

MET overexpression can be caused by gene amplification, gene mutation, transcriptional enhancement [activation of specificity protein 1 (Sp1), erythroblast transformation specific (Ets)], or by post-transcriptional mechanisms that lead to malignant transformations (Figure 1).[4]

The clinicopathological impacts of *MET* overexpression in various cancers have been investigated in several studies. The prevalence of *MET* overexpression was reported to be 39.8%,[100] 33.7%,[101] and 58.8%[102] in cases of gallbladder carcinoma, triple-negative breast cancer, and NSCLC, respectively. However, the potential correlation of *MET* overexpression with patient outcome is inconsistent across tumors. Some of the studies indicated that *MET* overexpression was significantly related to shorter OS or PFS in bladder cancer[103] and glioblastoma multiforme,[104] while some indicated no correlation to prognosis in NSCLC[102] or lung adenocarcinoma[105] Thus, a detailed understanding of the relationship between *MET* overexpression and prognosis is still needed.

Detection of MET overexpression

MET overexpression can be analyzed using immunohistochemistry (IHC), which provides a semiquantitative information on *MET* expression.[3] The prevalence rate of *MET* overexpression is approximately 13.7−63.7% in all NSCLCs. Among them, the prevalence rate of *MET* overexpression is approximately 30.4−37% in advanced NSCLC after *EGFR*-TKI treatment. Different scoring systems were used to quantify the level of *MET* expression by IHC.[106] In clinical trial settings, the level of expression is quantified as a clinical score (on a scale of 0–3+). The H-score (range from 0–300) is another scoring system that involves multiplying the percent of cells with staining scores of 1+, 2+, and 3+ by their staining intensity score.[107] In general, an H-score $\geqslant$ 200 denotes overexpression, and the specific cutoff range varies among studies.[108] Recently, a Chinese expert consensus on MET immunohistochemistry detection and interpretation standards for NSCLC has been proposed, aiming to improve the quality of detecion and interpretation to further guide the clinical treatment and studies.[109]

Some *MET* IHC antibodies for *MET* overexpression testing are shown in Table 5, including SP44, D1C1, and 3077. At present, many domestic and foreign antibodies for detecting *MET* amplification have obtained domestic medical device product status (recorded in the National Medical Products Administration), involving multiple clone numbers. The staining performance of different antibodies varies, and the interpretation criteria of current clinical research combine the expression intensity and percentage of relevant antibodies in tumor cells at the same time.

Targeted therapies for MET overexpression

MET can be overexpressed in certain cancers harboring primary and/or secondary *MET* exon 14 alterations or *MET* amplifications,[3] and many studies have indicated that *MET* overexpression and gene amplification are prognostic survival factors for many cancers, including gastric carcinomas.[113] At this juncture, the results from several clinical trials involving monotherapy with anti-*MET* antibodies (onartuzumab, emibetuzumab), anti-HGF antibodies (ficlatuzumab, rilotumumab), TKIs (crizotinib, tivantinib, cabozantinib), and other therapeutic agents suggest that the overall activity of these monotherapies in *MET*-overexpressing cancers is low, indicating that *MET* overexpression is not consistently predictive of benefit from *MET*-directed therapies.[114]

By contrast, combination with *EGFR*-directed therapies is effective. Wu *et al.* conducted an INSIGHT trial aiming to evaluate the efficacy and safety of tepotinib plus gefitinib in NSCLC patients harboring *MET* overexpression or *MET* amplification and acquired resistance to previous *EGFR* inhibitors and concluded that patients with *MET* IHC3+ or *MET* amplifications showed a better

Table 5. *MET* overexpression targeted therapy outcomes.

Study	Treatment	Study phase	Study population	Assay used	Antibody	Interpretation standards	Cutoff	*MET* subgroup	Subgroup (N)	ORR	Median PFS (months)
NCT03539536[110]	Teliso-v	2	Non-squamous (*EGFR*-WT): 58 Squamous: 28	IHC	SP44	Clinical scoring	Non-squamous: ⩾25% tumor cell 3+; Squamous: ⩾75% tumor cell 1+	Non-squamous (total)	52	36.5%	–
								Non-squamous (25–50% tumor cell 3+)	29	24.1%	–
								Non-squamous (⩾50% tumor cell 3+)	23	52.2%	–
								Squamous	27	11.1%	–
TATTON[93]	Savolitinib + osimertinib	1b	180	FISH, NGS or IHC	SP44	Clinical scoring	FISH: GCN ⩾ 5 or *MET*/*CEP7* ⩾ 2 NGS: GCN ⩾ 5 IHC: ⩾50% tumor cell 3+	Overall (B2/B3/D)	111	62–67%	9.0–11.1
								IHC ⩾ 50% tumor cell 3+	B1: 13 B2: 4	B1: 46%	–
SAVANNAH Ahn et al. [10]	Savolitinib + osimertinib	2	196	FISH or IHC	SP44	Clinical scoring	FISH: GCN ⩾ 5 or *MET*/*CEP7* ⩾ 2 IHC: ⩾50% tumor cell 3+	Total	193	32%	5.3
								FISH10+ and/or IHC90+	108	49%	7.1
NCT01610336[91]	Capmatinib + gefitinib	1b/2	100	FISH or IHC	3077	Clinical scoring	FISH: GCN ⩾ 4 IHC: ⩾50% tumor cell 2+/3+	Total	100	29%	–
								IHC ⩾ 50% tumor cell 2+	16	19%	–
								IHC ⩾ 50% tumor cell 3+	78	32%	5.45
NCT04077463[111] (CHRYSALIS-2)	Amivantamab + lazertinib	1/1b	108	IHC	SP44	Clinical scoring	IHC: ⩾25% tumor cell 3+	MET+: ⩾25% tumor cell 3+ MET-: others	28 (MET+)	61%	12.2
NCT02099058[112]	Teliso-V + osimertinib	1/1b	25	IHC	SP44	Clinical scoring	IHC: ⩾25% tumor cell 3+	Total	18	58%	
								High ⩾ 50% tumor cell 3+	10	50%	
								Intermediate 25–49% tumor cell 3+	8	63%	

'–', not reported; CEP 7, Chromosome 7; FISH, fluorescence in situ hybridization; GCN, gene copy number; IHC, immuohistochemistry; NGS, next-generation sequencing; WT, wild type; PFS, progression-free survival; ORR, overall response rate.

response, where PFS and OS were longer with tepotinib plus gefitinib (PFS 8.3 months, OS 29.1 months) than with chemotherapy (PFS 4.4 months, OS 17.9 months) in patients with IHC3+ (⩾50% tumor cells with strong intensity) *MET* overexpression, while the IHC2+ (⩾50% tumor cells with moderate intensity) subgroup showed a poor response to combination therapy with a *MET* inhibitor and *EGFR* inhibitor.[115] Overall, IHC is the only detection method for *MET* expression in clinical trials. The cutoff for *MET* expression varies across studies, and 50% of tumor cells 2+/3+ are usually used as criteria. Among the studies in Table 5, subgroup analysis suggested that a higher percentage of tumor cell 3+ or the same percentage with a higher staining score generally improved clinical outcomes. Therefore, given the limited clinical studies and a small number of patients, higher *MET* overexpression can reflect better outcomes for *MET*-TKIs plus *EGFR*-TKIs treatment in *EGFR*-resistant advanced NSCLC patients harboring *MET* overexpression. More studies and specific diagnostic criteria for *MET* overexpression are required to identify patients who can benefit more from combination therapy with *MET*-TKIs and *EGFR*-TKIs in this setting.

Furthermore, *MET* overexpression is not a reliable indicator of *MET* exon 14 alterations or *MET* amplifications, and reports from several studies have indicated the same where *MET* overexpression determined by IHC does not correlate with *MET* amplification.[116] This difference in correlation might be due to the inclusion of samples (featuring a low level of amplification) that do not result in considerable protein expression or protein expression modulation by posttranscriptional and posttranslational factors.[3] Therefore, patients harboring activating alterations in *MET* can be investigated for the presence of *MET* overexpression, but unfortunately, *MET* overexpression is not a reliable indicator of *MET* amplifications/*MET* exon 14 skipping variants.[3] Furthermore, a growing number of clinical trials are in the pipeline to explore the relationship between *MET* expression and *MET* amplification; however, unifying guidelines for standard scoring systems for IHC are required to obtain consensus among different trials.

MET fusion

MET fusion in cancers

The *MET* fusion was first found in chemically transformed osteosarcoma cell lines, which was the *TPR-MET* fusion.[3] Thereafter, *MET* fusions were identified in a variety of tumors over the years, such as gastric cancers, lung adenocarcinoma, thyroid carcinoma, hepatocellular carcinoma, and glioma.[3] Beyond *TPR-MET*, multiple other fusions have been identified, including *PTPRZ1-MET*, *CLIP2-MET CAPZA2-MET*, *ST7-MET*, *TRIM24-MET*, *KIF5B-MET*, *RBPMS-MET*, and *EML4-MET*, most of which have been reported in case reports.[117–122] The exact frequency of *MET* fusion in these cancers is poorly defined; of them, glioma had the highest proportion at 15%.[123] As a result, *MET* fusions and their therapeutic implications have been largely ignored. *MET* fusions have rarely been described in NSCLC, with an overall frequency of approximately 0.29%, and half of the fusion types are intragenic fusions.[122,124] A large real-world multicenter study for the Chinese population detected putative *MET* fusions with a prevalence of 0.15% in 79,803 solid tumors, while the majority of them were lung cancer patients (75.4%).[125] It is worth considering that some patients with *MET* fusion can benefit from MET-TKI therapy.

Detection of MET fusion

Numerous assays can detect MET fusions, including FISH, RT-PCR, and NGS. However, complex/novel rearrangements may result in inadequate FISH for detecting many MET fusions.[3] Therefore, NGS has become the increasingly preferred assay in the clinic.

NGS with traditional amplicon-based enrichment is less accurate in identifying gene fusions with unknown partners, while a technique termed anchored multiplex PCR (AMP) possibly addresses this limitation.[126] In AMP, a 'half-functional' NGS adapter is ligated to cDNA fragments that are derived from input RNA, and then the amplification between gene-specific primers and a primer to the adapter leads to target enrichment. As a result, the gene fusions of interest, even if they involve a novel fusion partner, should be detected.[126] Currently, targeted RNA-based NGS (tRNA-seq) is increasingly being applied in molecular detection for gene fusion in solid tumors, which is efficient for the simultaneous detection of actionable gene fusions, splice variants, single nucleotide variants (SNVs), and indels.[127] RNA-seq is not only a well-validated tool for detecting gene fusions in fresh-frozen tumors but also in formalin-fixed, paraffin-embedded (FFPE) tumor

samples. It showed a sensitivity of 83.3% in clinical FFPE specimens, with a negative prediction value of 94.3%, and was regarded as a complement DNA-based NGS assay.[128]

In NSCLC, sequentially combining DNA NGS and RNA NGS was shown to be one of the most efficient strategies for fusion detection; it was feasible on small tissue samples and could drastically reduce the complexity and cost of molecular workup.[129] Song *et al.*[130] developed a convenient single-tube, dual-template assay, and an integrated bioinformatics pipeline for relevant variant calling, in which RNA was used for fusion detection, whereas DNA was used for SNVs and insertion and deletions (indels). This method was considered to benefit not only most patients carrying target fusion but also those with rare variations.[130] Wei *et al.*[131] designed a lung-cancer-specific targeted all-in-one transcriptome-based assay based on single primed enrichment technology which covered gene loci that are related to selecting optimal targeted therapy in advanced NSCLC and could simultaneously identify mutations, gene fusions, and exon skipping events. This assay was shown to identify all the expected mutations at the transcriptome level and to reach an accuracy of close to 100%.[131]

Targeted therapies for MET fusion

Precisely targeted therapy has been incredibly underexplored in *MET* fusion-positive cancers. Nevertheless, in recent years, there have been many clinical case reports presenting the potential for MET-TKI therapy. Among these, crizotinib (monotherapy or combination therapy) has been described as having surprising clinical responses in patients with a variety of *MET* fusion-positive glioblastoma and lung adenocarcinomas, including the gene fusion types *CUX1-MET, HLA-DRB1-MET*, *CAV1-MET, ARL1-MET, PRKAR1A-MET*, bringing substantial tumor shrinkage and associated relief of symptoms.[132–137] Blanc-Durand *et al.*[138] reported a patient with NSCLC with brain metastasis harboring an *HLA-DRB1-MET* gene fusion who successively received crizotinib and cabozantinib and the selective inhibitor tepotinib and experienced rapid responses associated with a tremendous improvement in physical function during each treatment cycle. The potential role of capmatinib was also reported in a patient with chemotherapy-refractory metastatic cholangiocarcinoma harboring a *CAPZA-2-MET* fusion.[139] Kang *et al.*[140] attempted to explain the potential resistance mechanisms of *MET* inhibitors in patients with de novo *MET* fusions and found that secondary mutations D1228H/N or D1246N are worth further exploration. Multiple clinical trials are ongoing to evaluate the efficacy of MET-TKIs in tumor patients with *MET* fusions (NCT03993873, NCT02978261, NCT01639508, and CTR20181664[141]).

Conclusion

The pivotal role of *MET* aberrations as a predictive biomarker of drug response has been reported in several clinical trials. Furthermore, several *MET* inhibitors demonstrated clinically meaningful efficacy in different cancers harboring *MET* alterations. Therefore, *MET* exon 14 skipping variant testing has gained prominence and has already been recommended in guidelines where capmatinib, tepotinib, and savolitinib have been approved for the treatment of NSCLCs. Furthermore, other small-molecule inhibitors, including cabozantinib and crizotinib, are in the pipeline. The literature suggests that assays such as NGS (DNA based and RNA based) could be a potential testing method in terms of sensitivity and operational procedures for the detection of *MET* alterations, specifically *MET* exon 14 skipping variants, in both tissue samples and plasma ctDNA, but may have limitations for CNV testing. In addition, the FISH assay remains a robust technique for *MET* amplification detection. However, it can be used only for single-gene tests and tissue samples, while NGS represents the future trend of testing choice in multialteration (*MET* exon 14 skipping variant, amplification, and fusion) multigene analysis and in situations of limitation to plasma samples. NGS seems to be a promising testing option. ddPCR is being developed for *MET* amplification testing, especially in blood. *MET* amplification and *MET* overexpression are continuous variables, so clinically meaningful cutoff points need to be standardized, particularly the cutoff for *MET* overexpression. *MET* overexpression is an emerging biomarker for *MET*-TKI treatment since an increasing amount of clinical data have been released to guide the treatment. Furthermore, prospective studies involving a wide range of cancer types and larger sample sizes are required in this direction for definite conclusions and to extend the spectrum of *MET*-targeted therapy.

Declarations

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Not applicable.

Consent for publication
Not applicable.

Author contributions
Pei Yuan: Conceptualization; Data curation; Formal analysis; Writing – original draft.

Xuemin Xue: Data curation; Methodology; Writing – review & editing.

Tian Qiu: Data curation; Methodology; Writing – review & editing.

Jianming Ying: Conceptualization; Data curation; Formal analysis; Funding acquisition; Supervision; Writing – review & editing.

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Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
Main data are shown in this article and additional data about this study could be obtained from the corresponding author on reasonable request.

ORCID iD
Pei Yuan https://orcid.org/0000-0002-4663-7731

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