Targeting MET Amplification as a New Oncogenic Driver

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Abstract: Certain genetically defined cancers are dependent on a single overactive oncogene for their proliferation and survival, a phenomenon known as “oncogene addiction”. A new generation of drugs that selectively target such “driver oncogenes” manifests a clinical efficacy greater than that of conventional chemotherapy in appropriate genetically defined patients. MET is a proto-oncogene that encodes a receptor tyrosine kinase, and aberrant activation of MET signaling occurs in a subset of advanced cancers as result of various genetic alterations including gene amplification, polysomy, and gene mutation. Our preclinical studies have shown that inhibition of MET signaling either with the small-molecule MET inhibitor crizotinib or by RNA interference targeted to MET mRNA resulted in marked antitumor effects in cancer cell lines with MET amplification both in vitro and in vivo. Furthermore, patients with non-small cell lung cancer or gastric cancer positive for MET amplification have shown a pronounced clinical response to crizotinib. Accumulating
preclinical and clinical evidence thus suggests that MET amplification is an “oncogenic driver” and therefore a valid target for treatment. However, the prevalence of MET amplification has not been fully determined, possibly in part because of the difficulty in evaluating gene amplification. In this review, we provide a rationale for targeting this genetic alteration in cancer therapy.

**Keywords:** MET; gene amplification; non-small cell lung cancer; gastric cancer; fluorescence in situ hybridization (FISH); polymerase chain reaction (PCR); crizotinib

1. Introduction

Certain genetically defined cancers are dependent on a single overactive oncogene for their proliferation and survival, a phenomenon known as “oncogene addiction” that is exemplified by the BCR-ABL fusion gene in chronic myeloid leukemia as well as by mutant forms of the epidermal growth factor receptor (EGFR) gene and by the EML4-ALK fusion gene in non-small cell lung cancer (NSCLC). A new generation of drugs that selectively target such “driver oncogenes” and which include tyrosine kinase inhibitors (TKIs) has shown a therapeutic efficacy greater than that of conventional chemotherapy in individuals with these specific molecular alterations [1,2]. The identification of additional kinase oncogenes would thus be expected to facilitate the development of new molecularly targeted therapies.

The proto-oncogene MET encodes the receptor tyrosine kinase c-MET (or MET). The binding of its ligand—the hepatocyte growth factor (HGF)—to MET results in tyrosine phosphorylation of the receptor and activation of downstream signaling pathways mediated by phosphoinositide 3-kinase (PI3K) and AKT, by signal transducer and activator of transcription 3 (STAT3), or by RAS and mitogen-activated protein kinase (MAPK). Whereas normal activation of MET is essential for wound healing and embryonic development [3,4], excessive activation of MET signaling in a subset of advanced cancers [5–9] results in the up-regulation of cell proliferation, motility, migration, and invasion [3,10]. Although such aberrant MET signaling potentially arises from genetic alteration or dysregulation of MET [11], the target potential of MET alterations including polysomy, gene amplification, and gene mutation has not been well established.

2. Preclinical Findings

To investigate the biological impact of MET amplification or mutation, we have examined the effects of a MET-TKI and of a small interfering RNA (siRNA) specific for MET mRNA on cell survival and signal transduction in NSCLC cells with or without such genetic alterations of MET [12]. Several types of MET mutation, including those that affect the kinase domain or other domains of the encoded protein, have been identified in tumors. The small-molecule drug crizotinib (PF-02341066) inhibits the tyrosine kinase activity of MET as well as that of oncogenic fusion variants of anaplastic lymphoma kinase (ALK) [13,14]. We found that inhibition of MET signaling with crizotinib or MET siRNA induced apoptosis that was accompanied by attenuation of the phosphorylation (activation) of AKT and the MAPK extracellular signal-regulated kinase (ERK) in NSCLC cells with MET amplification but not in
those positive for a non-kinase domain mutation (N375S or deletion of exon 14) of MET [12]. These results suggest that MET signaling is essential for the survival of NSCLC cells with MET amplification but not for that of those without this genetic alteration, including those with a non-kinase domain mutation of MET, although MET-TKIs have been shown to be active against MET with mutations in the kinase domain [15]. Crizotinib also showed a marked antitumor effect on lung cancer xenografts positive for MET amplification, whereas it had little effect on those negative for MET amplification, including those with a MET mutation, consistent with our results obtained in vitro. Together, these findings suggest that gene amplification, but not gene mutation, renders MET active as a driver oncogene.

In gastric cancer, in which gain-of-function mutations of MET are exceedingly rare [16–18], activation of MET has been attributed to gene amplification [19–21]. A highly selective MET-TKI, PHA-665752, was shown to have potential antitumor efficacy in gastric cancer cells with MET amplification [22]. We therefore also examined the potential antitumor action of crizotinib or MET siRNA in gastric cancer cells positive or negative for MET amplification [23]. Consistent with our results obtained with NSCLC cells [12], we found that inhibition of MET signaling by either of these agents resulted in induction of apoptosis associated with inhibition of AKT and ERK phosphorylation in gastric cancer cells with MET amplification but not in those without it, suggesting that MET signaling is essential for the survival of MET amplification-positive cells. Crizotinib also manifested a marked antitumor effect on gastric cancer xenografts positive for MET amplification, whereas it had little effect on those negative for this genetic change. Crizotinib thus showed a pronounced antitumor action both in vitro and in vivo specifically in gastric cancer cells positive for MET amplification.

In summary, our preclinical studies have shown that gene amplification, but not gene mutation, confers “oncogenic driver” potential on MET. Tumor cells positive for MET amplification are thus dependent on (“addicted to”) sustained MET activity for their growth and survival, with the result that inhibition of MET signaling either with a small-molecule MET inhibitor or by RNA interference targeted to MET mRNA has marked antitumor effects both in vitro and in vivo. These findings provide a rationale for targeting MET amplification with MET-TKIs in the clinical setting.

3. Prevalence of MET Amplification in Cancer Patients

Given the potential of MET-targeted therapy for cancer with MET amplification, it is important to determine the prevalence of this gene alteration in patients with advanced cancer. Unfortunately, however, different studies have used different methods and criteria to detect MET amplification (Tables 1 and 2). Studies based on fluorescence in situ hybridization (FISH) analysis have identified MET amplification in up to ~5% of patients with NSCLC [24–27] or gastric cancer [20,28,29], whereas an increase in MET copy number was found in up to ~20% of NSCLC [30–35] and gastric cancer [36–40] patients by Southern blot analysis or with a polymerase chain reaction (PCR)-based assay. To understand the reason for this discrepancy, it is important to recognize the difference between the two genetic mechanisms—gene amplification and polysomy—that can give rise to an increase in gene copy number in malignant tumors. Gene amplification is defined as a copy number increase for a specific gene (or group of genes) on a given chromosome arm without a change in copy number for genes located in other regions of the chromosome [41]. On the other hand, polysomy gives rise to a copy number gain for a given gene as a result of the presence of extra copies of the entire chromosome. Of note, polysomy for
chromosome 7 (the chromosome on which MET is located) was indeed observed ~30% of NSCLC [27] and gastric [29] tumors with an increased MET copy number. Furthermore, such tumors might not be MET driven, given that breast tumors with an increased copy number for the human epidermal growth factor receptor 2 (HER2) gene as a result of polysomy 17 behave as HER2-negative tumors [42]. Southern blot analysis and PCR-based assays identify a gain in gene copy number regardless of the underlying cause and are thus unable to discriminate gene amplification from polysomy (Figure 1A). This methodological limitation is sometimes overlooked in determination of the prevalence of MET amplification in cancer.

Table 1. Prevalence of MET amplification and increased MET gene copy number (GCN) in NSCLC.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Technique</th>
<th>Classification</th>
<th>Positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camidge et al. (2010) [43]</td>
<td>66</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.0</td>
<td>0</td>
</tr>
<tr>
<td>Onozato et al. (2009) [33]</td>
<td>148</td>
<td>PCR based</td>
<td>GCN &gt; 2</td>
<td>1.4</td>
</tr>
<tr>
<td>Kubo et al. (2009) [34]</td>
<td>100</td>
<td>PCR based</td>
<td>GCN &gt; 5</td>
<td>2.0</td>
</tr>
<tr>
<td>Bean et al. (2007) [30]</td>
<td>16</td>
<td>PCR based</td>
<td>GCN &gt; 5</td>
<td>3.0</td>
</tr>
<tr>
<td>Go et al. (2010) [27]</td>
<td>180</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Okamoto et al. (2014) [44]</td>
<td>229</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Cappuzzo et al. (2009) [45]</td>
<td>447</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Onitsuka et al. (2010) [32]</td>
<td>183</td>
<td>PCR based</td>
<td>GCN &gt; 1.31</td>
<td>4.4</td>
</tr>
<tr>
<td>Okuda et al. (2008) [31]</td>
<td>213</td>
<td>PCR based</td>
<td>GCN &gt; 3</td>
<td>5.6</td>
</tr>
<tr>
<td>Beau-Faller et al. (2008) [35]</td>
<td>106</td>
<td>PCR based</td>
<td>GCN &gt; mean + 2SD of 30 normal lung DNA samples</td>
<td>20.8</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; GCN, gene copy number; CEP7, centromeric portion of chromosome 7.

Table 2. Prevalence of MET amplification and increased MET gene copy number (GCN) in gastric cancer.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Technique</th>
<th>Classification</th>
<th>Positivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janjigian et al. (2011) [29]</td>
<td>38</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.0</td>
<td>0</td>
</tr>
<tr>
<td>Kawakami et al. (2013) [46]</td>
<td>266</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Lennerz et al. (2011) [28]</td>
<td>267 (junctional and gastric)</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Hara et al. (1998) [20]</td>
<td>154</td>
<td>FISH</td>
<td>NA</td>
<td>3.9</td>
</tr>
<tr>
<td>Liu et al. (2014) [47]</td>
<td>196</td>
<td>FISH</td>
<td>MET/CEP7 ratio &gt; 2.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Graziano et al. (2011) [40]</td>
<td>216</td>
<td>PCR based</td>
<td>GCN ≥ 5</td>
<td>9.7</td>
</tr>
<tr>
<td>Tsugawa et al. (1998) [21]</td>
<td>70</td>
<td>Slot blot analysis</td>
<td>Ratio &gt; 2 (relative to normal mucosa)</td>
<td>10.0</td>
</tr>
<tr>
<td>Nakajima et al. (1999) [19]</td>
<td>128</td>
<td>Southern blot analysis</td>
<td>Ratio &gt; 2 (relative to normal mucosa)</td>
<td>10.2</td>
</tr>
<tr>
<td>Lee et al. (2011) [39]</td>
<td>472</td>
<td>PCR based</td>
<td>GCN ≥ 4</td>
<td>21.2</td>
</tr>
<tr>
<td>Shi et al. (2012) [48]</td>
<td>128</td>
<td>PCR based</td>
<td>GCN ≥ 4</td>
<td>30.5</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; GCN, gene copy number; CEP7, centromeric portion of chromosome 7; NA, not available.
On the other hand, FISH analysis is a semiquantitative method that can be performed with two probes for determination of the number of signals both for a target gene and for the centromeric portion of the corresponding chromosome. Given that the number of centromeric signals directly indicates the copy number of the chromosome, FISH analysis reveals the copy number increase for the target gene from the ratio of the copy number of the gene to that of the chromosome (Figure 1). Comparative genomic hybridization (CGH) is another molecular cytogenetic approach to the identification of gene amplification. CGH analyzes copy number variation for whole chromosomes or subchromosomal regions relative to ploidy level in the DNA of a test sample in comparison with a reference sample [49]. Although CGH has proved to be an efficient and reproducible technique, it remains relatively expensive to perform and requires a well-equipped laboratory and a high level of operator expertise.

**Figure 1.** (A) Schematic comparison of gene amplification and polysomy. The ratio of the copy number for the target gene to that for the centromeric portion of the chromosome distinguishes an increased copy number of the target gene attributable to gene amplification from that resulting from extra copies of the chromosome (polysomy). (B) FISH analysis of a gastric cancer cell line (HSC58) positive for MET amplification. The image shows a single cancer cell, with green and red signals corresponding to CEP7 (CEN7p) and the MET locus, respectively.
FISH is thus currently the gold standard for detection of gene amplification. According to the recent ASCO/CAP guidelines for HER2 testing, gene amplification is defined as positive with a target gene/centromere ratio of >2.2, negative with a ratio of <1.8, and equivocal with a ratio between 1.8 and 2.2 [50]. Importantly, polysomy, which is mechanistically distinct from gene amplification, is mostly associated with a ratio in the equivocal range [51].

With the strict definition of MET amplification as a MET/CEP7 (centromeric region of chromosome 7) ratio of >2.2 as determined by FISH analysis, we identified nine out of 229 patients with advanced NSCLC (3.9%) as being positive for MET amplification [44]. We also found that four out of 266 gastric cancer patients (1.5%) were positive for MET amplification as determined with a combination of PCR-based screening and FISH confirmation [46]. These results suggest that MET amplification identifies a small but clinically important subgroup of cancer patients who are likely to respond to MET-TKIs.

4. Clinical Response to Crizotinib in MET Amplification—Positive Cancer Patients

To date, at least 17 MET-TKIs with kinase selectivity profiles ranging from highly selective to multitargeted have been or are currently being subjected to clinical evaluation [52]. Although several agents including cabozantinib [53] and foretinib [54] have made good progress, they are multitargeted MET-TKIs, and so little is known of the relation between their efficacy and MET amplification. In NSCLC, MET amplification is one of the mechanisms responsible for the development of resistance to EGFR-TKIs, with dual inhibition of EGFR and MET having been shown to induce apoptosis in such resistant cells [55]. Combination treatment with an EGFR-TKI and tivantinib, a selective MET-TKI with microtubule-disrupting activity similar to that of vincristine [56], has been evaluated in clinical trials, but the efficacy of this approach remains unclear. Among the MET-TKIs examined, however, crizotinib has consistently shown efficacy in patients with cancer positive for MET amplification.

Preliminary reports of the clinical response of patients with MET amplification-positive cancer to crizotinib have come from an enriched molecular cohort of individuals with advanced cancer in a phase I trial of this drug (A8081001, ClinicalTrials.gov identifier NCT00585195). This cohort includes patients with various tumor types harboring specific genetic alterations of MET or ALK, including MET amplification defined as a MET/CEP7 ratio of >2.2 (but not polysomy 7, kinase domain-activating mutations of MET, or other chromosomal translocations leading to altered transcriptional regulation of MET) as well as ALK chromosomal translocation or gene amplification. A patient with stage IV lung adenocarcinoma that was negative for ALK rearrangement but positive for high-level MET amplification (MET/CEP7 ratio of >5.0) started treatment with crizotinib at a dose of 250 mg twice a day [57]. The patient achieved a maximum reduction in aggregate tumor measurement of 54.8% after 4 months of such therapy and thereafter continued the study treatment showing a partial response. A patient with MET amplification-positive glioblastoma was also treated with crizotinib at 250 mg twice a day [58]. After 2 months of treatment, the first scheduled cranial magnetic resonance imaging (MRI) scan revealed a 40% reduction in tumor size, and after 4 months a restaging cranial MRI examination confirmed this effect to be stable. Administration of crizotinib was continued for a total of 6 months, until the patient manifested disease progression.

Another study revealed a pronounced clinical response to crizotinib in two of four patients with gastric cancer positive for MET amplification (MET/CEP7 ratio of >2.2) [28]. After 1 week of crizotinib
treatment, one patient experienced a rapid symptomatic response with an increase in appetite, reduction in pain, and improvement in performance status. A computed tomography (CT) scan at the end of treatment cycle 2 (8 weeks) revealed a partial tumor response, which was confirmed at 12 weeks. Another patient also showed rapid clinical improvement, with reduced pain and improved performance status, after 1 week of crizotinib treatment. Time to progression for these two patients on crizotinib treatment was ~112 and 105 days, respectively.

Crizotinib was approved by the U.S. Food and Drug Administration for the treatment of ALK rearrangement-positive NSCLC in 2011, and a recent report has addressed the clinical efficacy of this agent in a clinical practice setting [59]. A male patient with stage IV squamous cell lung cancer was found to be positive for MET amplification (MET/CEP7 ratio of >2.2) and negative for ALK rearrangement by FISH analysis. He was treated with crizotinib monotherapy at the normal dose of 250 mg twice daily. An almost complete response of tumors in the left lung and a major response of the primary tumor to therapy were demonstrated by chest CT and positron emission tomography (PET)-CT after 8 weeks of therapy.

Preliminary results of the NCT00585195 phase I study for patients with MET amplification-positive NSCLC were reported at the 2014 Annual Meeting of the American Society of Clinical Oncology (ASCO) [60]. Patients were categorized into three classes according to MET amplification status as determined by FISH analysis: low (MET/CEP7 ratio of ≥1.8 to ≤2.2), intermediate (MET/CEP7 ratio of >2.2 to ≤5.0), and high (MET/CEP7 ratio of ≥5.0). Thirteen patients with a low (n = 1), intermediate (n = 6), or high (n = 6) MET/CEP7 ratio received crizotinib. Of the 12 evaluable patients, four (33%) showed a partial response and were found to have an intermediate (n = 1) or high (n = 3) MET/CEP7 ratio. These findings are thus suggestive of an association between the MET/CEP7 ratio and the clinical benefit of crizotinib in patients with MET amplification-positive cancer.

The accumulating clinical evidence thus suggests that MET amplification as strictly defined by a MET/CEP7 ratio of >2.2 has the potential to act as an oncogenic driver and thereby to render at least a subset of affected tumors responsive to MET-TKIs such as crizotinib. Not all MET amplification-positive cancer patients respond to MET-TKI treatment, however, and most such patients who do respond, even those who show an initial marked response, eventually develop resistance to MET-TKIs. Preexisting and acquired resistance to MET-TKIs is thus an important clinical problem that is shared with other targeted therapies. Several mechanisms of resistance to MET-TKIs have been identified in preclinical models, including additional mutations in the activation loop of MET [61], ligand-dependent activation of EGFR signaling [61,62], SND1-BRAF fusion [63], and amplification and overexpression of wild-type KRAS [64]. Further characterization of such mechanisms will be important to provide a basis for the development of effective therapies for patients with MET-TKI resistance.

5. Conclusions

MET amplification has been identified as a potential oncogenic driver for several neoplasms, and targeted therapy with MET-TKIs for such tumors is thus a reasonable and effective treatment. Clinical trials of such drugs are strongly warranted for patients with advanced malignancies positive for MET amplification as strictly defined by a MET/CEP7 ratio of >2.2 determined by FISH.
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Conflicts of Interest

The authors declare no conflict of interest.

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