IN THE SPOTLIGHT

MET Receptor Juxtamembrane Exon 14 Alternative Spliced Variant: Novel Cancer Genomic Predictive Biomarker

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Summary: Clinical studies on MET-targeting cancer therapeutics have yielded mixed results in recent years, and MET-relevant predictive biomarkers remain elusive. New studies now reveal *MET*ex14 alternative splicing aberrations to represent potential predictive cancer genomic biomarker, hence renewing optimism and directions in the quest for optimized MET-targeting personalized cancer therapy. *Cancer Discov; 5(8); 802–5. ©2015 AACR.*

See related article by Paik et al., p. 842 (1).

See related article by Frampton et al., p. 850 (2).

In this issue of *Cancer Discovery*, Paik and colleagues (1) report that mutations of RNA splice acceptor and donor sites involving exon 14 of *MET* could lead to exon skipping, resulting in an in-frame deletion of the juxtamembrane domain, which normally is a negative regulator of the kinase catalytic activities. More importantly, the authors provide evidence of tumor response to MET-targeted therapies using crizotinib or cabozantinib. Another article in this issue, by Frampton and colleagues (2), identifies recurrent and diverse genomic alterations in multiple tumor types leading to *MET* exon 14 (*MET*ex14) alternative splicing aberrations. A small case series of patients harboring *MET*ex14 aberrancy is highlighted with tumor response toward crizotinib and capmatinib (INC280). Since the first report (3) of the durable complete response under onartuzumab (MetMab) treatment in a patient with chemotherapy-refractory gastric cancer metastatic to the liver, much further clinical effort has been devoted in MET-targeted therapeutics, but with only mixed results upon the completion of several advanced clinical trial studies. Aberrant MET/HGF regulation is seen in a wide variety of human cancers with a dysregulated proliferative and invasive signaling program, epithelial-to-mesenchymal transition, cell motility/migration, scattering, angiogenesis, invasion, and metastasis. MET/HGF signaling has also been implicated in the activation of invasion and metastasis, one of the "hallmarks of cancer" (4). To put into perspective the two articles by Paik and colleagues and Frampton and colleagues in this issue, recent course of clinical trial development of MET-targeting agents is briefly reviewed below (1, 2, 5).

Built upon the success of a positive phase II clinical study revealing that the anti-MET one-arm monoclonal antibody onartuzumab was efficacious in patients with advanced non–small cell lung cancer (NSCLC) selected for high MET expression, the phase III METLung trial was soon introduced as a biomarker-selected study to investigate onartuzumab/erlotinib versus erlotinib/placebo in previously treated stage IIIB to IV NSCLC with centrally confirmed MET-positive expression. The phase II results strongly suggested that MET-IHC status may predict clinical benefit from the onartuzumab/erlotinib combination; hence, the METLung trial was designed to include patients with MET-IHC 2+/3+ in ≥50% tumor cells. However, on March 3, 2014, Roche announced termination of the phase III METLung study for reason of a lack of clinically meaningful efficacy.

Tivantinib (ARQ197) is a non–ATP-competitive small molecule targeting MET. A global randomized phase II trial, ARQ197-209, initially compared erlotinib/tivantinib with erlotinib/placebo in unselected advanced NSCLC, and found progression-free survival (PFS) to be prolonged as the primary endpoint in the ET group. Biomarker analysis demonstrated that among nonsquamous tumors, 75% were MET-positive by IHC (2+/3+), compared with only 12% among the squamous subtype. Exploratory analysis demonstrated a significant delay in the time to development of new metastases among patients treated with erlotinib/tivantinib (HR, 0.49; $P < 0.01$), most notably in the nonsquamous population. A global randomized phase III trial, MARQUEE, soon followed for patients with nonsquamous NSCLC histology, enriching for MET-high expression, with overall survival (OS) as the primary endpoint (6). Unfortunately, the MARQUEE trial was again discontinued early after a planned interim analysis revealed the study's futility. Nonetheless, final analysis showed both PFS and overall response rates were improved. Additionally, the tivantinib treatment group did show significant OS improvement in the subgroup with MET-high expression, essentially recapitulating the phase II MetMab study results.

Crizotinib was approved in 2011 for *ALK* translocation–positive NSCLC based on its ALK activities, despite its initial development as a MET inhibitor. Since then, genomic *MET* amplification associated with various tumor types has been correlated with crizotinib treatment response (7). A recent report from The Cancer Genome Atlas Research Network on lung adenocarcinoma confirmed a *MET* amplification

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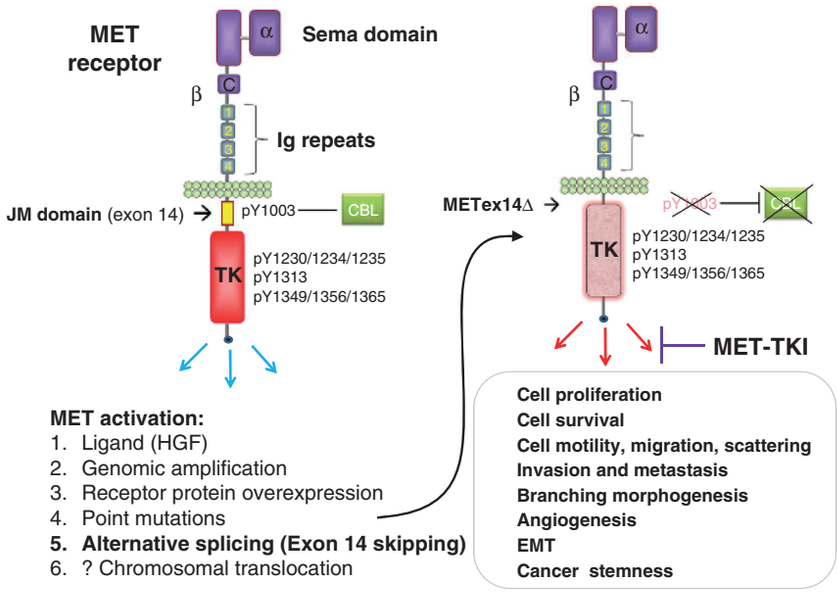

Figure 1. METex14 activates oncogenic signaling and is a potential MET-targeting therapy cancer genomic predictive biomarker. The wild-type MET receptor is known to be activated by a variety of mechanisms, including ligand HGF binding (to the ligand-binding and receptor dimerization Sema domain), genomic amplification, receptor protein overexpression, point mutations, alternative splicing, and possibly chromosomal translocation. *MET*ex14 genomic variants can occur via diverse genomic aberrations involving the splice sites, resulting in in-frame skipping of the juxtamembrane domain encoding exon 14. *MET*ex14 not only can represent an important oncogenic variant, but also can serve as genomic predictive biomarker for MET-targeting therapy. C, cystein-rich region; HGF, hepatocyte growth factor; Ig, immunoglobulin-like; JM, juxtamembrane; TK, tyroskine kinase domain; TKI, tyrosine kinase inhibitor; EMT, epithelial-to-mesenchymal transition.

frequency of 2.2% with evidence of oncogene-driver alteration (8). The first results of crizotinib treatment in *MET*-amplified NSCLC from the original phase I study of the dual MET/ALK inhibitor were presented at the American Society of Clinical Oncology (ASCO) 2014 Annual Meeting (9), with 14 patients accrued to the NSCLC cohort, predominantly with adenocarcinoma and mostly with positive smoking status. Objective partial response rates were observed: 0%, 17%, and 67% in the low-*MET* (*MET:CEP7* ratio, ≥1.8–≤2.2), intermediate-*MET* (ratio, >2.2–<5.0), and high-*MET* (ratio, ≥5.0) groups, respectively, suggesting an improved efficacy as the *MET* amplification ratio increased.

In addition to *MET* amplification, The Cancer Genome Atlas lung adenocarcinoma study report also identified 10 tumor samples harboring *MET*ex14 skipping within the RNA, in the presence of somatic in *cis* DNA exon 14 splice-site mutation, splice-site deletion, or a Y1003* mutation (8). The frequency of *MET*ex14 skipping in lung adenocarcinoma was determined to be 4.3%. Genomic alterations involving exon 14 skipping alternative splicing of *MET* were first reported in 2003 and 2005 (10, 11). Exon 14 encoding the juxtamembrane domain of MET was also found to harbor the missense mutations R988C and T1010I in lung cancer, which were shown to be activating. *MET*ex14 splicing variants, two in small cell lung cancer involving a 2 base-pair insertion in a splice acceptor site 5′ of exon 14 and one in an NSCLC tumor involving an in-frame skipping of exon 14, were identified (10, 11). In 2006, Kong-Beltran and colleagues (12) identified another series of somatic intronic mutations in lung cancer cell lines and patient samples immediately flanking exon 14, and Y1003 residue that serves as the juxtamembrane domain–binding site for Casitas B-Lineage Lymphoma (CBL) E3-ubiquitin protein-ligase to regulate MET receptor turnover. Recently, novel chromosomal fusions involving the MET kinase have been identified in various cancers. In particular, at least two fusion variants (i.e., *KIF5B–MET* in lung adenocarcinoma and *TFG–MET* in thyroid papillary carcinoma) do have the predicted chimeric protein with the classic fusion activation paradigm, joining the dimerization motifs to an intact kinase domain (13). These findings strongly suggest an oncogenic role of the MET fusion products.

The two new articles by Paik and colleagues and Frampton and colleagues (1, 2) further enrich our understanding of MET as a molecular target in precision cancer therapy (Fig. 1). In the largest tumor genomic profiling cohort performed for *MET* alteration, Frampton and colleagues reported 221 positive cases (0.6%) found to express *MET*ex14 mutations out of 38,028 profiled tumors. Most interestingly, *MET*ex14 alterations were comprised of a surprisingly diverse 126 distinct sequence variants, found most commonly but not exclusively in lung adenocarcinoma (3%), and were also seen in other lung tumor types (2.3%), brain glioma (0.4%), and tumors of unknown primary origin (0.4%). Both reports highlighted *MET*ex14 conferring sensitivity toward MET-targeting inhibitors with clinical response by either tumor measurement or metabolic PET response, further raising the specter of *MET*ex14 collectively as "actionable" genomic alterations and cancer predictive biomarkers. Because the juxtamembrane domain is a key negative regulatory region for the intracellular kinase

domain in the human kinome, its disruption through exon skipping in *MET* likely can transition the closed kinase conformation to a more open, and thus active, conformation, akin to the effects of oncogenic *FLT3–ITD* (internal tandem repeat) in acute myelogenous leukemia. *MET*ex14 variants also stabilize the altered MET receptor through decreased CBL-mediated MET ubiquitination.

These new findings also underscore the challenges in genomic tumor profiling: (i) therapeutically relevant alterations could reside within the intronic regions of splice sites, (ii) diverse mutational alterations culminating in exon 14 skipping as a common end product, and (iii) potential utility in clinical RNA-sequencing profiling. Cancer sequencing only the exons and simple hotspot mutational sequencing panels are inadequate. Of note, the finding that *MET*ex14-skipping alterations had coexisting *MET* amplification is of interest and warrants further clarification. Likewise, the highly coincident genomic events of gene copy-number amplification of *MDM2* and *CDK4* with *MET*ex14 alterations deserve further study. To this end, in the era of genomics-guided personalized cancer therapy, it is now becoming clear that it is not only important to arrive at a genomic biomarker for patient selection, a genuine predictive biomarker should also be analyzed in the context of as much genomic landscape background as possible in order to appreciate and categorize potential therapy response genomic modifiers. An example can be illustrated by the H596 adenosquamous cell line, which expresses not only the *MET*ex14-skipping variant but also *PIK3CA* mutation. The H596 cells were found to be insensitive to MET inhibitor alone but had synergistic sensitivity to combined MET/PI3K inhibitor treatment in preclinical models. Moreover, Liu and colleagues reported, at the ASCO 2015 Annual Meeting (14), that *MET*ex14 variants occurred frequently in pulmonary sarcomatoid carcinoma at a resoundingly high 22%, with one such patient with *MET*ex14 and concurrent *MET* amplification displaying substantial response to crizotinib (Balazs Halmos; personal communication).

Now the time is right to formally test in prospective clinical studies matching *MET*ex14 genomic variants with MET therapeutic agents. Clearly, other concurrent variations in *MET*/*HGF* as well as other genomic backgrounds would be important and should be deciphered simultaneously in tumor profiling, in order to enable thorough and unbiased treatment response analysis. Would *MET*ex14 tumors respond to onartuzumab as well? Also, whether there would be response variations among different MET agents does not have easy answers at present. We await further clinical–translational studies to yield the answers. Yet, a number of challenges and questions still remain on the road to optimizing MET cancer therapy. Is *MET* a legitimate molecular target for cancer therapy? Most believe it is, although the precise predictive biomarkers for response remain somewhat elusive. We now have more evidence to support *MET* amplification and *MET*ex14 alterations as potential genomic predictive determinants; but how about MET protein (over)expression and other *MET* missense mutations? Are they out of the question already? I would argue not. In the MARQUEE study, tivantinib did improve OS in the subgroup of tumors with high MET expression, suggesting a potential efficacy in a biomarker-selected population. This particular biomarker challenge perhaps is not too dissimilar to what we are witnessing in a Programmed Death-Ligand 1 expression assay under immune checkpoint therapy.

Even if all agree on *MET* being a legitimate target, how should we measure it as target and how should we optimally measure the treatment outcome? It is also important to point out that there is high heterogeneity of *MET* genomic alterations in cancer, including the large repertoire of mutations identified within the *HGF* and *MET* genes, as compiled in the cBioPortal for Cancer Genomics database, many of which have not been fully functionally tested. Besides, the spatial–temporal heterogeneity of the biomarker within the tumor itself, as well as the stromal microenvironmental influence, is not trivial. I would argue that obtaining newly biopsied pretreatment (by MET agents) tumor tissues, preferably at the site of tumor progression or metastatic site, should be a priority (if not a prerequisite) for future MET-targeting trials, so that we will not be left with more questions than answers in analyzing the clinical study outcomes. Novel technologic platforms for genomic interrogation, such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTC) as liquid biopsies, could be quite useful in this context to overcome the sampling errors of tissue biopsy. Novel quantitative biomarker expression assay technologies could also shed important new light in MET biomarker research. As the MET–HGF pathway is known to activate tumor invasion and metastasis, one of the "hallmarks of cancer," should we ask whether RECIST and OS represent the proper "measuring ruler" for treatment outcome and efficacy of MET-targeting therapies? To this end, deeper insight into the impact of MET inhibition on time-to-new metastasis in future trials could be quite illuminating. Is there any justifiable way to study and measure the clinical benefits of MET/HGF targeting if it affects new metastasis formation and progression but less so on primary tumor growth? Our current clinical trial outcome measurement, based on the conventional definition of "disease progression," could not readily discern these differences. How to combine MET-targeting therapy most effectively with other therapies, including cytotoxic, targeted, and immune therapies, to achieve the optimal clinical outcomes would thus be the most worthwhile of investigations. At this time, the quest for clinical MET therapy predictive biomarkers evidently has a new beginning. We are returning to the drawing board for a new road map in optimized MET-targeting therapy clinical study design.

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Disclaimer

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