Editorial Commentary

Devil in the detail: MET overexpression fails as surrogate marker for *MET* exon 14 splice site mutations in NSCLC

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The hepatocyte growth factor (HGF)/mesenchymal-epithelial transition tyrosine kinase (MET) axis has risen to prominence recently both in terms of its role in driving human cancers (1) as well as emerging as a rational therapeutic target in lung cancer leading to increased overall survival in patients receiving targeted therapy (2). However, therapeutic success appears to occur only in select patient subsets as there have also been prominent failures of large phase III studies in different trial populations (3). Given mixed clinical outcomes, rapid and reliable identification of those patients most likely to benefit from targeted inhibition of the HGF/MET axis is paramount.

A barrier to effective patient stratification lies in the complex signaling and regulatory systems that underpin the HGF/MET axis. Consequently, there is considerable heterogeneity in oncogenic perturbations that lead to aberrant MET signaling including transcriptional deregulation, gene translocation or amplification, activating point mutations, overexpression, constitutive kinase activation, autocrine or paracrine activation and crosstalk with other receptors and downstream signaling players (4). Such heterogeneity not only underscores the importance of reliable and accurate detection of clinically actionable alterations but also highlights the need for firm understanding of the aberrant biology at play to ensure development of effective diagnostic and therapeutic tools.

MET structure contains an extracellular ligand-binding domain, a single-pass transmembrane domain, and an intracellular segment. The cytoplasmic domain includes a juxtamembrane (JM) domain involved in MET post-translational regulation and a catalytic kinase domain responsible for tyrosine kinase activity. HGF binding results in receptor dimerization and phosphorylation of two tyrosine residues (Tyr1234 and Tyr1235) in the kinase catalytic domain (5). Subsequent phosphorylation of docking site residues leads to the recruitment of a network of intracellular adaptor and effector proteins leading to MET-mediated activation of several intracellular signaling pathways driving survival, proliferation, inhibition of apoptosis, migration, invasion and metastasis (6).

Diverse regulatory mechanisms for MET signaling exist. Degradation/recycling of the MET receptor occurs via the recruitment of casitas B lineage lymphoma proto-oncogene (CBL), a ubiquitin-protein ligase. The ubiquitination of phosphorylated MET can occur through the direct interaction of CBL with Tyr1003 in the JM domain or indirectly by its binding to Tyr1356 via the Growth factor receptor-bound protein 2 (Grb2) adaptor protein (7). Other regulatory mechanisms of MET signaling include tyrosine-specific phosphatase activity and regulated enzymatic proteolysis of the MET receptor resulting in the formation of a soluble extracellular fragment and an intracellular fragment that undergoes rapid degradation by the proteasome (8).

Alterations in the JM domain such as splice site mutations that prevent normal exon 14 (and therefore JM domain) expression—a phenomenon known as '*MET* exon 14 skipping (*MET*ex14)' or point mutations at the Tyr1003 residue preventing CBL binding (which otherwise leads to ubiquitination and downstream degradation)—were

shown to play a driving role in small cell lung cancer in 2003 and non-small cell lung cancers in 2005 (9,10). The true heterogeneity of splice site alterations was not fully appreciated until later, however, when large scale genomic profiling became increasingly common and revealed diverse changes in *MET* exon 14 and flanking introns leading to impaired JM synthesis or alternative splicing (11). Decreased degradation of the MET receptor has been hypothesized to result in MET overexpression (thus increasing oncogenic signaling) in some tumors that can be detected with immunohistochemistry (IHC) (3). So while there exists a diverse array of genomic alterations that drive oncogenesis via putative MET overexpression, the question of whether the converse may be true arises: Does MET overexpression act as a surrogate marker for underlying mutations driving oncogenesis?

*MET*ex14 alterations occur in 3–4% of lung adenocarcinomas, tend to occur in older adults with a higher percentage of former/active smokers, tend to be mutually exclusive from other drivers and have been associated with poorer overall survival (3,11-13). Further, *MET*ex14 alterations are likely to be highly predictive of response to MET inhibition(3) as dramatic and durable partial responses have been observed with inhibitors of MET such as a crizotinib, cabozantinib and capmatinib (2,3,12,14). Clinical trials are ongoing including the GEOMETRY mono-1 phase II trial (NCT02414139) for which updated efficacy data recently led to Food and Drug Administration (FDA) accelerated approval for capmatinib.

Both DNA- and RNA-based approaches can be used to detect *MET*ex14 alterations. Successful DNA-based detection of exon skipping relies on uncovering a genomic variant that alters or ablates the splice site. A potential shortcoming is that if primer binding is impaired due to such an alteration, sequencing results will fail to positively detect this error. RNA-based approaches overcome this potential false negative scenario as the direct result of impaired splicing can be observed as a "fusion" of exon 13 and 15, and this result is constant regardless of the underlying genomic event (15). Of note, RNA is inherently more unstable compared to DNA and high-quality nucleic acid from clinical samples can be comparatively more difficult to obtain. Given that DNA-based approaches risk more frequent false negatives, and that RNA-based approaches are more costly and less feasibly integrated into standard clinical sample processing, MET overexpression detected via IHC as a surrogate marker for *MET* exon 14 alterations is a highly attractive prospect for efficient patient stratification.

To this end, Baldacci and colleagues sought to assess the prevalence of *MET*ex14 mutations in a prospective cohort of treatment naïve NSCLC with high MET overexpression in their 2020 *Journal of Thoracic Oncology* publication (16). They argue that expression analysis could represent a useful prescreening tool as current DNA-based assay approaches risk not fully capturing the heterogeneous landscape of over 120 mutations associated with *MET*ex14 (12). The authors included treatment-naïve patients from the French multicentric prospective cohort IFCT-PREDICT.amm (17) found to have either high MET expression (defined as ≥50% cells showing high intensity staining) or low-to-absent MET expression and performed fragment length analysis (FLA) and next-generation sequencing (NGS) on available tumor material using a validated approach (18). Among patients found to have high MET expression, FLA did not find any mutations in 86 patients (0%) while NGS revealed two *MET*ex14 mutations in 91 patients (2.2%). Importantly, an activating *PIK3CA* mutation (E545K) and a deleterious p*TP53* mutation were found, respectively, in these patients.

Among patients from an independent cohort with low-to-absent MET expression (0 or 1+ MET immunoscore), NGS combined with FLA revealed two *MET*ex14 mutations out of 131 patients (1.5%) suggesting a similar rate of *MET*ex14 mutations independent of MET expression level. From sequencing data from the 91 patients with high MET expression, a gene copy number analysis revealed a *MET* gene copy number increase in seven patients (7.7%). Additionally, other driver oncogenic mutations were found in the 91 patient cohort with high MET expression including *KRAS* (28.6%), *EGFR* (7.7%), *PIK3CA* (4.4%), *NRAS* (2.2%), *GNAS* (1.1%) and *IDH1* (1.1%). IHC analysis revealed mutations in non-V600E *BRAF* (4.4%) and *ALK* (2.3%). NGS analysis revealed deleterious alterations in *p53* (52.7%) and *PTEN* (1.1%).

The authors conclude that there is enrichment of *MET* amplification in NSCLC with MET overexpression, a rate of oncogenic driver alterations (KRAS, PIK3CA and EGFR for example) similar to that observed in other unselected NSCLC cohorts, and that overall their data suggests MET overexpression cannot reliably be used as a surrogate marker for *MET*ex14 mutations. The authors cite low prevalence of *MET*ex14 mutations, DNA-based testing versus RNA-based, and utilization of NGS versus fluorescence *in situ*

hybridization (FISH) as limitations.

This study by Baldacci and colleagues valiantly seeks to answer a question lingering since clinical sensitivity to MET-inhibition in *MET*ex14 altered lung cancers was first demonstrated in 2015 (12): Can IHC serve as a faster, cheaper and reliable companion diagnostic for identification of patients harboring *MET*ex14-altered lung cancers? Unfortunately, their data suggests that IHC fails to serve in this capacity, and that the devil remains in the details at the genomic level—necessitating continued use of current DNA- and RNA-based sequencing approaches for patient stratification with all of the associated cost and sample processing burden.

Their patient cohort was a good representation of clinico-pathological features known to be associated with underlying *MET*ex14 alterations such as advanced age, active or past smoking histories and advanced stage, non-squamous NSCLC (3,11). There was only one patient with a pulmonary sarcomatoid tumor—the histology felt to harbor the highest frequency of *MET*ex14 mutations (19). Although inclusion of more patients with such tumors may have increased the otherwise low frequency of *MET*ex14 mutations detected, such inclusion would have skewed the overall patient cohort to a less common clinical phenotype.

While an RNA-based detection method represents the gold standard for uncovering *MET* exon 14 skipping, the authors employed a DNA-based (dual NGS and FLA) approach which is more clinically-relevant (RNA analysis is not routinely performed on patient samples) and was previously validated to have high sensitivity (12). In so doing, their results are more representative of real world clinical sample processing but do risk having missed detection of some *MET*ex14 alterations. This non-RNA based approach—combined with relatively small clinical cohort (108 patients)—could explain the low detected frequency of *MET*ex14 alterations.

Another main conclusion of the study by Baldacci and colleagues is that a co-occurrence of other driver mutations was frequent in *MET*ex14 altered patients which has implications for analyzing treatment responses to various targeted agents in clinical trials. This association is in contrast to several other reports with larger cohorts (11-13). It is important to note that in one of these reports, a positive correlation with *MET*ex14 mutations and high MET expression was observed (13). Such disparities may be due to differences in patient cohorts and/or technical/analytical approaches used. Still, these discrepancies underscore the heterogeneity of the relationship between *MET*ex14 mutations and potential co-occurring driver mutations. Thus, this association in Baldacci and colleagues' data should be interpreted with caution particularly in light of a relatively small patient cohort size.

It is unclear why MET overexpression is not observed in the setting of *MET*ex14 alterations which are hypothesized to result in impaired intracellular CBL binding and ubiquitination resulting in decreased degradation of MET. The answer likely lies buried in the details of the highly complex signaling and regulatory network that the MET/HGF axis embodies. Mitiushkina *et al.* developed a simple TaqMan polymerase chain reaction (PCR) assay specifically aimed at precise quantitation of mutant versus wild-type allele expression (20). Their assay identified 35 *MET*ex14 mutations out of 1,415 *EGFR*-negative lung adenocarcinoma samples (2.5%) which were confirmed by Sanger sequencing. Comparative expression of normal and mutant alleles revealed preferential expression of the mutant allele in 97.5% of their *MET*ex14 altered lung adenocarcinoma samples. To investigate whether this was due to increased expression of the mutant allele, or decreased expression of the wild-type allele, or both, expression of each allele was compared to a standard reference gene (*SDHA*) in both *MET*ex14-positive and *MET*ex14-negative samples as a control. Expression of the wild-type allele was found to be significantly decreased in the *MET*ex14-positive samples compared to controls. The level of expression of the mutant allele in *MET*ex14-positive samples was similar to that of the wild-type allele in *MET*ex14-negative samples. Thus, their data suggests that the loss of wild-type allele expression is responsible for the observed difference between expression levels of mutant and wild-type alleles in *MET*ex14-positive cases. While the mechanism for suppression of the wild-type *MET* allele requires further study, this observation is in keeping with Baldacci *et al.*'s demonstration that MET expression levels fail to correlate to *MET*ex14-positive status. Further study is required but the efforts by Baldacci *et al.* provide important insight that IHC is not ready to be utilized as a patient screening tool to identify underlying *MET*ex14 splice site mutations.

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