Commentary

MET exon 14 deletion (*METex14*): finally, a frequent-enough actionable oncogenic driver mutation in non-small cell lung cancer to lead MET inhibitors out of "40 years of wilderness" and into a clear path of regulatory approval

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The hepatocyte growth factor (HGF) ligand and its receptor MET (mesenchymal-epithelial transition) tyrosine kinase receptor axis has long been demonstrated to be important in oncogenesis and metastasis in multiple tumor types. Mechanisms of dysregulation of the HGF-MET axis includes over-expression of the HGF ligand, activating point mutations in *MET*, *MET* gene amplification, MET protein over-expression and potentially *MET* rearrangements. Many structural different MET tyrosine kinase inhibitors (TKIs) have been developed to target the HGF-MET axis pathway but so far the results have been disappointing (1).

In non-small cell lung cancer (NSCLC), *MET* amplification has been shown to be an actionable driver mutation as high level of de novo *MET* amplification (MET/CEP7 >5) was effectively inhibited by crizotinib, an ALK/ROS1/MET TKI (2,3). However, the incidence of true *MET* amplification and not the broad category of *MET* polysomy (copy numbers gain without respect to other gene copy number gained simultaneously) is rather rare accounting for about 1% of all NSCLC (3). Secondary acquired *MET* amplification constitutes about 5% of resistance mechanism to first- or second-generation epidermal growth factor receptor (EGFR) TKI in NSCLC patients harboring activating *EGFR* mutations (4,5). Thus the incidence of *de novo* or secondary *MET* amplification is low as compare to *EGFR T790M* which accounts for approximately 60% of the resistance mechanism. Additionally, the combination of MET TKI and EGFR TKI trials in NSCLC patients have been disappointing so far. For examples, the combination of crizotinib (MET TKI) and erlotinib (EGFR TKI) was not able to reach the approved dose of each approved agent due to toxicities of the combination (6). Additionally, the increased interstitial lung disease (ILD) observed with the combination of tivantinib and erlotinib as compared to erlotinib alone led to an early termination of a randomized phase 3 trial (ATTENTION) in Asia (7). Furthermore, the failure of the addition of tivantinib to erlotinib to improved overall survival as compared to erlotinib alone in a molecularly unselected nonsquamous NSCLC patients from a randomized phase 3 trial (MARQUEE) involving more than 1,000 patients is the latest blow to MET TKIs in gaining regulatory approval to enter clinical care (8). Given the relative low frequency of *MET* amplification as a resistance mechanism, clinical trials investigating combination of EGFR and MET TKIs in *EGFR*-positive NSCLC patients will take time to mature with no guarantee of success (e.g., ClinicalTrials.gov number: NCT01610336). Point mutations have been described throughout the *MET* gene but none of the mutations are directly activating I NSCLC to date (with the exception of Y1003N which will be discussed later) (1). More recently *KIF5B-MET*

fusion have been described in NSCLC by next generation sequencing (9). While the *KIF5B-MET* rearrangement is likely to be an activating genetic alteration in NSCLC similarly to *ALK* and *ROS1* rearrangement in NSCLC, the frequency is likely very low and to date there is no report in the literature of any NSCLC patients harboring *MET* rearrangement responding to MET inhibitors.

Another alteration in MET that is potentially actionable in NSCLC is *MET* exon 14 deletion (*METex14*) mutations resulting in defective messenger RNA (mRNA) splicing due to mutations/deletions at the splice donor or acceptor sites around or involving MET exon 14. Initially reported in both small cell lung in 2003 and then in NSCLC in 2005 (10,11), the significance of these splice site mutations/deletions were demonstrated in 2006 by Kong-Beltran and colleagues where multiple point mutations and deletions in the splice donor and acceptor sites resulted in the exon 14 of *MET* gene being spliced out of the eventual mature *MET* mRNA (12). MET exon 14 contains the Cbl ubiquitin ligases site on tyrosine residue 1003 (Y1003) where ubiquitin is attached to the tyrosine residue and led to the lysosomal degradation of the MET protein (13). Hence, missense mutation of Y1003 residue or "skipping" of the protein region that is encoded by MET exon 14 results in MET protein leads to in a relative over-expression of MET protein and enhanced MET activation and subsequent oncogenesis. The findings of *METex14* by Ma and colleagues and Kong-Beltran and colleagues were subsequently confirmed by whole genome sequencing (14,15) and estimated to be around 3–4% of adenocarcinoma from The Cancer Genome Atlas (TCGA) project (15). Interestingly, TCGA discovered that some of *MET* splice site mutations resulted in incomplete splicing so a low level of the normal size MET protein is expressed. Whether "incomplete" MET splicing is as oncogenic remain to be determined tis existence provides evidence that in order to develop companion diagnostic tests for future clinical use, and quantitative RNA approach will more accurately reflect the biological situation of the tumor environment with corresponding MET protein expression as quantified by immunohistochemistry (IHC) is also.

While there have been ample pre-clinical evidence pointing to the significance of *METex14*, the clinical evidence was lacking until Paik and colleagues demonstrated these mutations are actionable and inhibition by MET TKIs can result in clinical benefit in NSCLC patients harboring these *MET* exon 14 alterations (16). In the 2015 *Cancer Discovery* paper, Paik and colleagues first confirmed the existence of *METex14* is about 4% of adenocarcinoma of the lung similar to what was observed the TCGA, a substantial portion of a potential driver mutation according to the thoracic oncology community. Of the 8 (out of 178 adenocarcinoma samples) adenocarcinoma of lung patients with *MET* exon 14 mutation, 7 harbored splice site mutations while 1 with Y1003 mutation. Of note of the 6 samples that MET protein expression could be tested, all 6 had 3+ IHC score (H-sore of 300) indicating high MET protein expression. Importantly, in 6 out of the 8 samples *MET* was not amplified while one had intermediate level of amplification (MET/CEP7 =3.8) and one had high level of amplification (MET/CEP7 =6.0) indicating *MET* exon 14 mutations and de novo wildtype *MET* amplification is likely to be mutually exclusive. Frampton and colleagues, in an accompanying paper in the same issue of *Cancer Discovery* as Paik and colleagues, did identify that *MET* amplification (likely of the allele with the *MET* exon 14 mutations and not the wildtype *MET* gene) was associated with *METex14* (17). Importantly Frampton and colleagues survey > 38,000 clinical tumor samples submitted to Foundation Medicine Inc. and subject to hybrid-capture next generation sequencing and found that lung cancer by far is the tumor type that harbors MET exon 14 mutations. Approximately 3% of adenocarcinoma of the lung and 2.3% of non-adenocarcinoma of lung cancer harbored MET exon 14 mutations that will likely result in *METex14*. At the same time, Halmos and colleagues reported that *METex14s* occurred up to 22% of pulmonary sarcomatoid carcinoma (18) although this high frequency of *METex14* needed to be independently verified from different tumor banks.

Of the 8 patients with *MET* exon 14 mutations described by Paik and colleagues (16), 4 received anti-MET therapy and 3 out of the 4 patients had a response (*Table 1*). Contemporaneously, other investigators have also published case reports alone or embedded in larger surveys of *METex14* in solid tumors (*Table 1*). First, all histologies of NSCLC were found to harbor *METex14* (adenocarcinoma, squamous cell, large cell, and sarcomatoid). Second, both never-smokers and ever-smokers harbored *METex14*. Third, in all cases with the exception of one where IHC were performed MET protein expression is high (3+) thus providing evidence that MET protein is not degraded at the normal rate as expected. Fourth, three different MET TKIs have been shown single agent activity against *METex14* with durable partial response. In summary, given the confluence of the relative high incidence (3–4%) of *METex14* among major histologies of lung cancer reported by large clinical database from commercial diagnostic company, single

Table 1 List of NSCLC patients with *METex14* responded to MET tyrosine kinase inhibitors

Number	Age/gender	Smoking status	Histology	MET exon 14 alterations	MET IHC	MET amplification	Best (duration of) response	Reference
1	80/F	NS	Adeno	Splice donor site mutation	3+	Yes	CR (>7 months) (PERIST) to cabozantinib	Paik *et al*., Cancer Dis 2015 (16)
2	78/M	ES	Adeno	Splice donor site deletion	3+	NR	PR to crizotinib (lung); PD to crizotinib (liver)	Paik *et al*., Cancer Dis 2015 (16)
3	65/M	ES	Adeno	Splice donor site mutation	NR	NR	PR (>7 months) to crizotinib	Paik *et al*., Cancer Dis 2015 (16)
4	90/F	NS	Adeno	Splice donor site mutation	NR	NR	PR (>5 months) to crizotinib	Paik *et al*., Cancer Dis 2015 (16)
5	86/M	NS	Adeno	Splice donor site deletion	2+	NR	PR (5 weeks) to crizotinib	Jenkins *et al*., Clin Lung Cancer 2015 (19)
6	71/M	ES	Adeno	Splice donor site mutation "D1028H"	NR	No	PR (>6 months) to crizotinib	Waqar *et al*., J Thorac Oncol 2015 (20)
7	76/F	ES	Adeno	Splice donor site mutation "D1010H"	NR	No	PR (>8 months) to crizotinib	Mendenhall *et al*., J Thorac Oncol 2015 (21)
8	82/F	ES	Large cell	Splice donor site mutation	3+	Yes*	PR (>5 months) to capmatinib	Frampton *et al*., Cancer Dis 2015 (17)
9	66/F	ES	SqCC	Splice donor site mutation	3+	NR	PR (>13 months) to capmatinib	Frampton *et al*., Cancer Dis 2015 (17)
10	74/F	ES	Sarcomatoid	Splice site mutation	NR	NR**	PR (>2 months) to crizotinib	Liu *et al*., J Clin Oncol 2015 (18)
11	61/M	NS	Sarcomatoid	Splice donor site mutation/H1094Y	NR	NR	PR (> 5 months) to crizotinib	Lee *et al.,* J Thorac Oncol 2015 (22)

NSCLC, non-small cell lung cancer; *METex14*, MET exon 14 deletion; IHC, immunohistochemistry; M, male; F, Female; ES, ever-smoker; NS, never-smoker; Adeno, adenocarcinoma; SqCC, squamous cell carcinoma; NR, not reported; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. *, MET/CEP7 =2.3 (low amplification); **, polysomy (9 copies) of the *MET* exon 14 mutation allele.

institutions, and the TCGA together with case reports/series of the significant preliminary single agent activity of MET TKIs against *METex14*, suddenly the "holy grail" of eventually getting a MET TKI approved for clinical use in NSCLC is suddenly thrust upon us. Clinical trials involving MET inhibitor are now investigating their activities against MET inhibitors. For example, the on-going phase 1/2 crizotinib trial has already produced ground-breaking results in *ALK*-rearranged and *ROS1*-rearranged NSCLC is enrolling NSCLC *METex14* patients (ClinicalTrials.gov number: NCT00585195) (23,24). Besides completing clinical trials with MET TKIs in NSCLC *METex14* patients as soon as possible, several concurrent projects in *MET* exon14 deletions needed to be completed also. First, the clinicopahtologic characteristics of these NSCLC *METex14* patients remained limited and elusive (*Table 1*). Hence survey of large databases to fully characterize these MET exon1 4 NSCLC patients is urgently needed to help guide future screening and identification of these patients. Second, the development of a companion diagnostic(s) to accompany the regulatory approval of MET TKIs is urgently needed. Given the TCGA identified "incomplete skipping", any RNA based detected method is probably preferable although the mutations in the DNA level underlying the *METex14* is diverse as demonstrated by Frampton and colleagues with implication of basic science research for years to come. Finally, although not detected by Frampton and colleagues, Lee and Colleagues in Korea (25) have detected comparable

incidence of *METex14* in gastrointestinal (GI) malignances indicating the clinical benefit if MET TKIs can potentially be expanded to GI malignancies. Thus Paik and colleagues' *Cancer Discovery* paper and reports by others has suddenly provided the blueprint and started a race to finally get MET TKIs approved for clinical use after many years of searching for a frequent enough and actionable target. It is anticipated by the end of 2016, the significance of *METex14* in NSCLC will be widely appreciated.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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