Distribution of cMET by IHC, FISH, and next generation sequencing in cancer – a large cohort analysis

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Abstract

Introduction: cMET overexpression and/or activation have been implicated in signaling pathways that promote cell proliferation, invasion, and survival. It has been hypothesized as an oncogenic driver in various malignancies and is currently being investigated as a potential therapeutic target. The aim of this study is to provide insight into the distribution of cMET expression by immunohistochemistry (IHC), amplification by FISH, and mutation by next generation sequencing (NGS) across a variety of tumor types. Also, we evaluate the correlation of cMET across technology platforms in a CLIA-credited oncology reference laboratory.

Methods: In a cohort of 17292 patient samples, cMET protein expression was assessed by IHC including Caris CMET and 9P4, 17292 samples, FISH on Caris FISH 2582 samples and NGS (Humana Targeted Panel – Cancer Panel 631) samples.

Results: Our analysis has shown the highest cMET expression rates in the following tumor types: pancreatic cancer (46%, 209/360), colorectal cancer (39%, 464/1196), small intestinal malignancies (37%, 37/101), and cholangiocarcinoma (35%, 15/43). Some of the lowest expression rates of cMET by IHC included non-epithelial ovarian cancer (11%, 4/37), glioblastoma (2%, 7/351), and neuroblastoma (2%, 4/197). Analysis of cMET expression by FISH identified the highest levels amplification in peritoneal/perivascular sarcoma (7%, 21/309), melanomas (6%, 6/98), and non-small cell lung cancer (8%, 761/9311). In 6531 samples tested by NGS platform, 153 mutations were identified – all were variants of unknown significance (defined here as rare or variants with those unique theranostic significance). Twenty-five of the 153 were detected in non-small cell lung cancer samples. The most common protein changes were as follows: T1010A (n = 84, 4.6%), T1010N (n = 9, 1.6%), T1010H (n = 3, 0.6%), and G391E (n = 2, 0.1%). Concordance between all three technologies was poor, as demonstrated by Cohen’s kappa statistics.

Conclusion: Our data suggest that immunohistochemical cMET overexpression and/or activation is prevalent in various malignancies. Ongoing clinical trials targeting cMET suggest that efforts should be made to accurately interrogate tumors for cMET expression. As shown by our concordance results, full cMET analysis is enhanced utilizing multiple technologies.

Background

Hepatocyte growth factor receptor (cMET) is a receptor tyrosine kinase that is overexpressed or mutated in a variety of cancers. cMET activation by its ligand, hepatocyte growth factor, is known as scatter factor, HGF/FGF in various effects, including embryogenesis, morphogenesis, and wound healing. Activated cMET in cancer, though, leads to angiogenesis, proliferation, invasion and metastasis, making this biomarker an attractive target. Various agents targeting cMET are in development, including onartuzumab in non-small cell lung cancer and trinitumab in gastric cancer.

Other cancer types, though, demand further exploration of this biomarker. This study evaluates a large cohort spanning various cancer types in an effort to identify novel linchpins that might drive the most benefit from targeting cMET.

Methods

Data was analyzed from 17292 cancer patients who received tumor profiling at Caris Life Sciences from 2009 to 2013. IHC, FISH, CISH, Sanger SEQ, MGMT promoter methylation and next generation sequencing were performed on formalin-fixed, paraffin-embedded tumor samples in a CLIA-credited lab. Protein expression of cMET by IHC (NCL-cMET and SP44) was determined by measuring the intensity of the stain (0+, 1+, 2+, 3+) and the percent staining (0 – 100%). An intensity equal to or greater than 2+ and a percentage equal to or greater than 50% was utilized as the threshold for positivity. All IHC results were read by a board-certified pathologist. If FIFRE was sufficient, cMET amplification was then measured by either CISH or FISH, with a gene copy number (CN) > 5 used to determine positivity. Results for the FISH were determined by a molecular cytogeneticist, while CISH results were interpreted by a board-certified pathologist. MET sequencing was performed using next-generation sequencing, with results validated by board-certified molecular geneticist.

Concordance between cMET IHC, FISH/CISH, and NGS Concordance was poor based on kappa coefficients, with calculated values of 0.2 between IHC and FISH/CISH and 0.007 between FISH/CISH and NGS. Also of 2938 cases where all three tests were performed, 904 cases (31%) had only one of three possible tests. Possible causes for discordancy include tumor heterogeneity, non-specificity of the IHC antibody post-transcriptional protein regulation, and utilized cutoffs. Multiple technologies (IHC, FISH/CISH, NGS) should be considered, then, when designing cMET biomarker studies, at least until an accepted standard becomes routine for cMET testing.

Conclusions

• To the best of our knowledge, this is the first study evaluating 17,292 specimens for cMET utilizing multiple technologies to interrogate cMET DNA, RNA, and protein across various cancer types.

• Besides NGCL and gastric adenocarcinoma which are already being actively studied with cMET-targeted therapy (e.g. CMS10/8183, CMS11/8104), other cancers identified here should be considered for cMET-targeted clinical trials. This include a myriad of epithelial cancers of the gastrointestinal and pancreatobiliary tract as well as non-epithelial cancer, which include, but are not limited to sarcoma and melanoma.

• The higher percentages of positive IHC and amplified FISH/CGH results argue for incorporation of these methodologies for screening in cMET-targeted clinical trials. Mutations in the MET gene were rare but merit further study as to their theranostic significance.

• MET aberration in NGCL merits further investigation, as MET positive NGCL patients seemed to derive benefit from dual inhibition of MET and EGFR (Spigel 2013). Close examination of the methodologies, like IHC and NGS, should be investigated further in NGCL, as cMET in these tumors can be overexpressed or mutated.

• The lack of concordance argues for incorporating various methodologies to interrogate a malignancy for cMET. This comprehensive interrogation allows patients to be recruited in various clinical trials containing cMET-targeted therapy.

References

