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 identified 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 insights 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 tested in a CLIA-certified oncology reference laboratory.

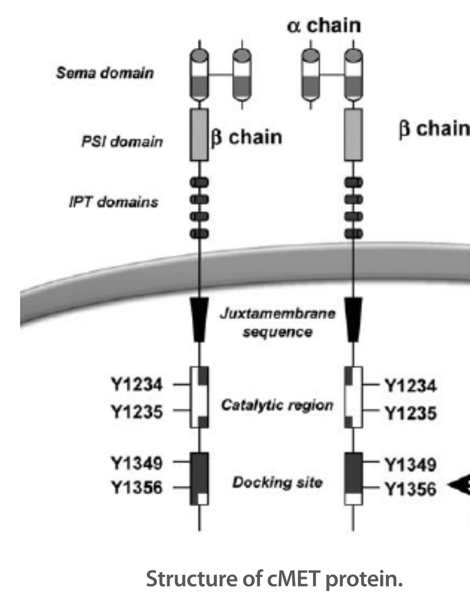
Methods: In a cohort of 17292 patient samples, cMET protein expression was assayed by IHC (NCL-cMET and SP44, 17292 samples), FISH or CISH (9328 samples) and NGS (Illumina Truseq Amplicon – Cancer Panel, 6531 samples).

Results: Our analysis has shown the highest cMET expression rates in the following tumor types: pancreatic cancer (46%, 269/587), colorectal cancer (39%, 464/1196), small intestinal malignancies (37%, 37/101), and cholangiocarcinoma (35%, 58/165). Some of the lowest expression rates of cMET by IHC included non-epithelial ovarian cancer (1.6%, 4/257), glioblastoma (2%, 7/345), and GIST (2%, 1/49). Analysis of cMET by FISH identified the highest levels amplification in peritoneal/retroperitoneal sarcoma (7%, 2/31), melanoma (6%, 65/983), and non-small cell lung cancer (6%, 78/1301). In 6531 samples tested by NGS platform, 153 mutations were identified – all were variants of unknown significance (defined here as rare mutations or those with unknown theranostic significance). Twenty-five of the 153 were detected in non-small cell lung cancer specimens. The most common protein changes were as follows: T1010I (n = 84), E168D (n = 39), S203T (n = 9), D1028H (n = 3), D1028N (n = 3), and G391E (n = 2). Concordance between all three technologies was poor, as demonstrated by Cohen's kappa statistics.

Conclusion: Our data suggest that 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 testing. 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 (also known as scatter factor, HGF/SF) results in various effects, including embryogenesis, morphogenesis, and wound healing. Activation of 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 rilotumumab in gastric cancer.



Structure of cMET protein.

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 lineages that might derive 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 NextGen SEQ (Illumina TruSeq) were performed on formalin-fixed, paraffin-embedded tumor samples in a CLIA-certified 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 FFPE tumor was sufficient, cMET amplification was then measured by either CISH or FISH, with a gene copy number (GCN) > 5 being used to determine positivity. Results for 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.

Results

cMET Expression by Immunohistochemistry (IHC)

Immunohistochemistry was read on 17292 specimens. 2431 (14.1%) specimens were positive, while 14861 (85.9%) were negative across all tumor types. Rates of cMET by IHC positivity were calculated across various malignancies (see Figure 1). Malignancies with the highest rates of cMET positivity included tumors of the gastrointestinal and pancreatobiliary tract such as pancreatic cancer (45.8%), colorectal cancer (38.8%), small intestinal malignancies (36.6%), and cholangiocarcinoma (35.2%). By comparison, epithelial tumors being actively investigated in phase III clinical trials had lower expression rates by IHC in our cohort, such as non-small cell lung cancer (26.7%), gastric adenocarcinomas (22.3%), and hepatocellular cancers (16.9%). Malignancies with no cMET IHC expression included multiple myeloma, malignant solitary fibrous tumors, and central nervous system cancers such as low grade gliomas and pituitary cancers.

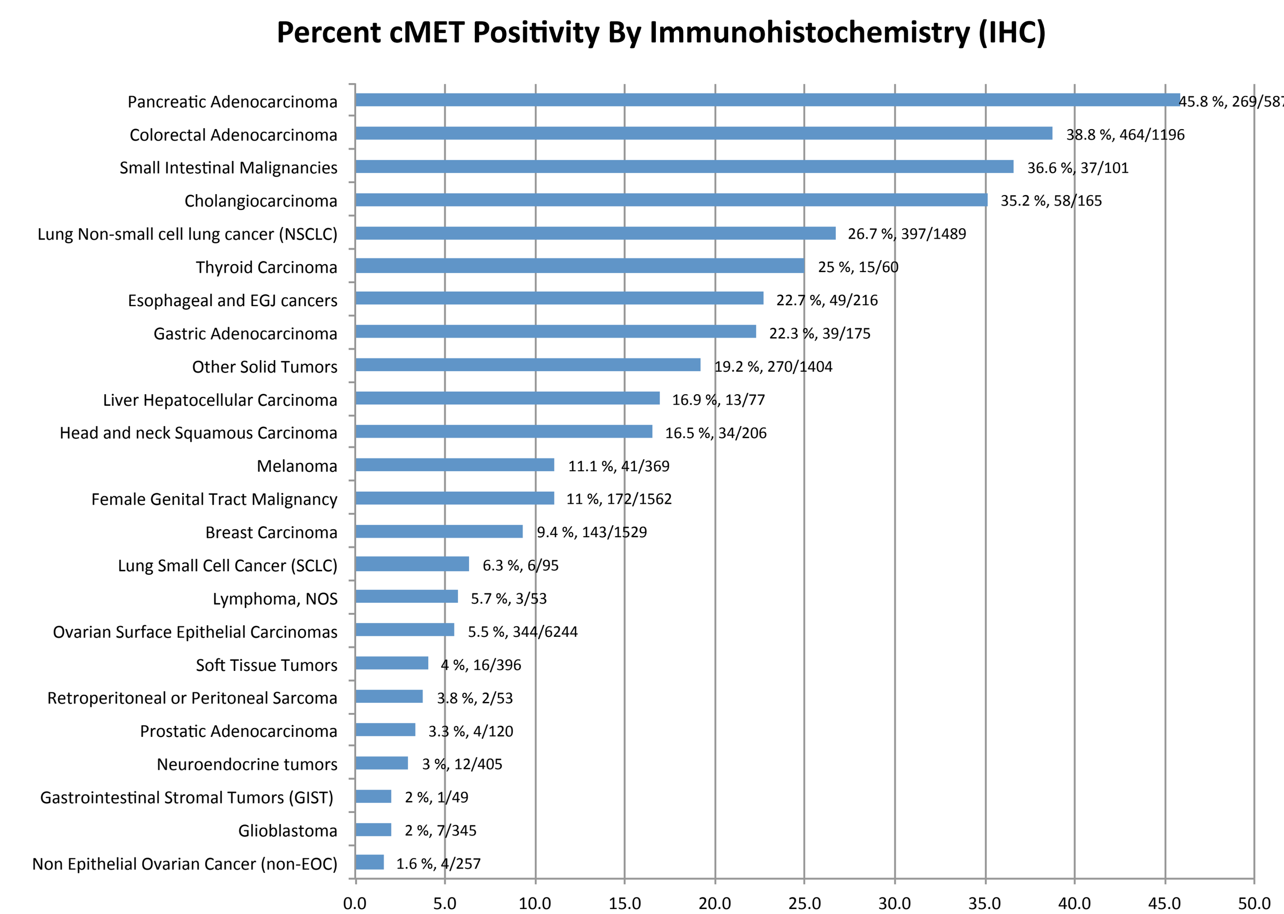


Figure 1 – Distribution of IHC results. Percent positivity, based on a threshold of at least 2+ staining in 50% of cells, sorted from highest to lowest percentage.

MET by Fluorescent in situ hybridization/Chromogenic in situ hybridization (FISH/CISH)

MET amplification rates were calculated on 9328 samples (see Figure 2). Analysis revealed the highest levels of amplification in peritoneal/retroperitoneal sarcoma (6.5%) and melanoma (6.4%), both of which contain higher rates of amplification in our cohort than more intensively studied tumors like non-small cell lung cancer, making them potential candidates in cMET-related clinical trials. Other tumors worth considering for such trials include thyroid cancer (4.7%) and non-epithelial cancers, including other soft tissue tumors (4.3%). The non-small cell lung cancer amplification rate (6%) is worth mentioning, as FISH amplification is associated with lack of clinical benefit to agents like erlotinib and gefitinib in tumors with EGFR activating mutations.

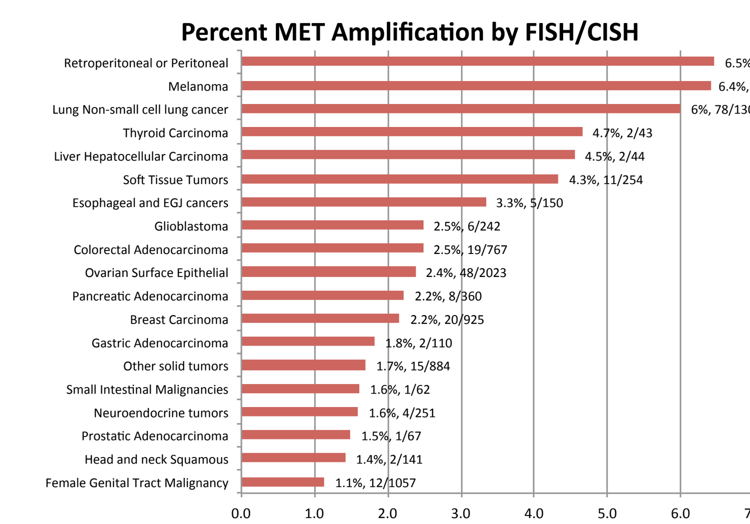


Figure 2 – Distribution of FISH/CISH results. Amplification rates were sorted from highest to lowest. Malignancies with no observed in situ hybridization (ISH) amplification (not shown) included the following: extrahepatic bile duct cancers (including cholangiocarcinomas), gastrointestinal stromal tumors, low grade gliomas, small cell lung cancer, cancers of the male genital tract, non-epithelial ovarian cancer, and uveal melanoma.

MET Overexpression and Amplification

A B

Image A and B - Example of MET Overexpression and Amplification. Image A shows cMET by IHC positivity (2+, 50%) in a urothelial bladder cancer. Image B demonstrates MET amplification by CISH in the same specimen.

MET by Next generation sequencing (NGS)

Next generation sequencing identified 153 mutations in 6531 specimens. All mutations were interpreted as variants of unknown significance (VUS), meaning the mutation was considered rare or had an unknown theranostic significance. The diagram (Figure 3) below shows the mutation rate distribution. Small cell lung cancer had the highest mutation rate (6.4%). High mutations were also found in thyroid carcinoma (6.4%), pancreatic adenocarcinoma (4.3%), and non-epithelial tumors including melanoma (4.3%).

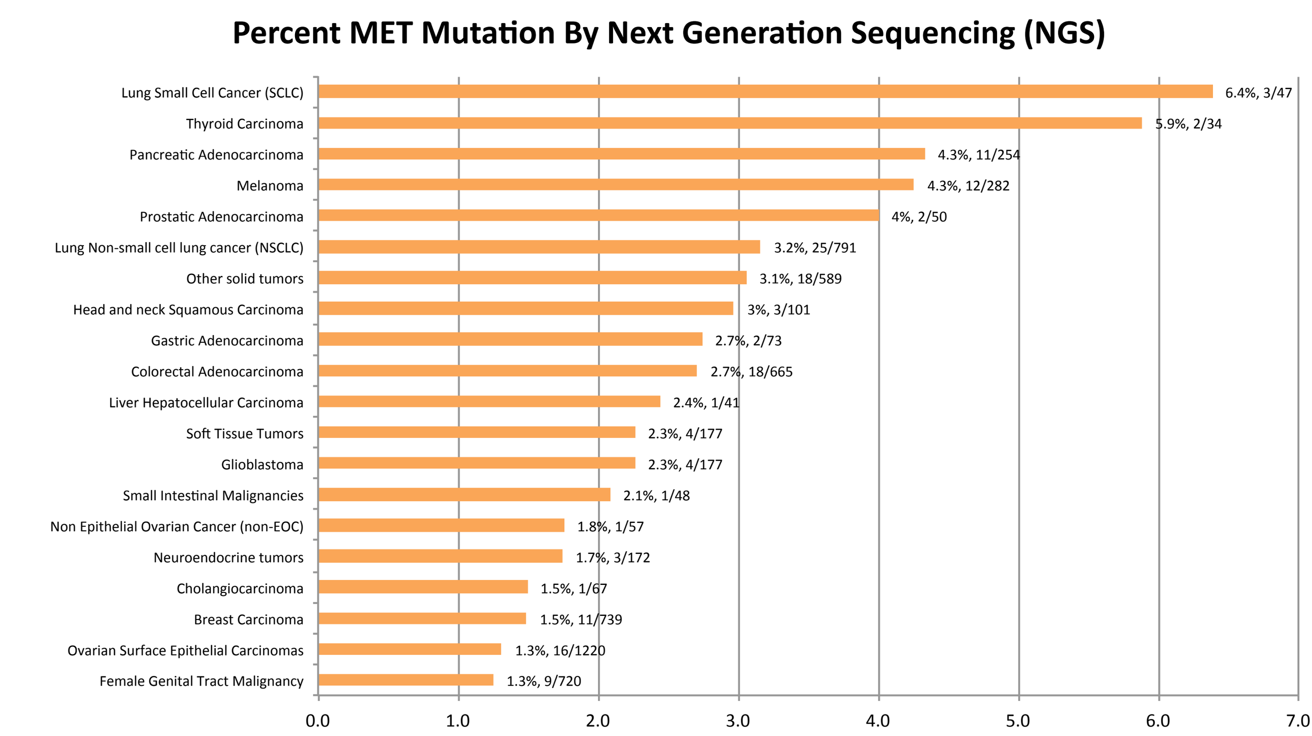


Figure 3 – Distribution of NGS results. Mutation rates were sorted from highest to lowest based on tumor type. Malignancies with no mutations detected by next generation sequencing included the following: esophageal and GEJ cancers, gastrointestinal stromal tumors, cancers of the male genital tract, thymic cancers, and uveal melanoma.

Number of cMET Mutations Across Lineages

Protein Change	Number (N)
T1010I	84
E168D	39
S203T	9
D1028H	3
D1028N	3
G391E	2
D174N	1
E1017V	1
G1113E	1
G1260V	1
G391A	1
H1112Y	1
H1256P	1
K1121N	1
K1250T	1
K380N	1
L1115S	1
N194S	1
V378I	1

A total of 153 mutations were detected in 151 patients – two patients had two alterations. The mutation most frequently detected was T1010I (n=84), which is considered an inherited missense mutation located in the juxtamembrane domain (JMD), which does not influence cMET phosphorylation. This mutation is sometimes associated with colorectal cancer risk, although penetrance is low. The next two mutations detected were E168D (39 patients) and S203T (nine patients), both missense mutations in the SEMA domain on the extracellular surface. More clinical trials will be necessary to elucidate the clinical usefulness of all mutations.

Figure 5 – Protein changes detected by NGS. The column on the left shows the exact protein change identified irrespective of tumor lineage. The column on the right refers to the number of times identified. The protein changes highlighted in **red** are those corresponding to the tyrosine kinase domain (as defined by COSMIC), a site targeted by specific agents.

Concordance between cMET IHC, FISH/CISH, and NGS

Concordance was poor based on kappa coefficients, with calculated values of 0.012 (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 positive tests. Possible causes for discrepancy include tumor heterogeneity, non-specificity of the IHC antibody, post-transcriptional protein regulation, and utilized cut-offs. 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.

Concordance – IHC, FISH/CISH, and NGS

	IHC +	IHC -
ISH+/NGS+	2	0
ISH-/NGS+	26	43
ISH+/NGS-	34	28
ISH-/NGS-	773	2032

Figure 6 – Concordance data between IHC, ISH, and NGS. The figure on the left shows IHC positivity (as defined under "Methods") compared to cases that were ISH amplified (defined as positive) or mutated (defined as positive).

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 NSCLC and gastric adenocarcinoma which are being actively studied with cMET-targeted therapy (e.g. NCT01662869, NCT1519804), other cancers identified here should be considered for cMET-targeted clinical trials. These include a myriad of epithelial cancers of the gastrointestinal and pancreatobiliary tract as well as non-epithelial cancers which include but are not limited to sarcoma and melanoma.
- The higher percentages of positive IHC and amplified FISH/CISH 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 NSCLC merits further investigation, as MET-positive NSCLC patients seemed to derive benefit from dual inhibition of MET and EGFR (Spigel 2013). Other methodologies, like IHC and NGS, should be investigated further in NSCLC, 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

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