Analysis of *MET*-amplified solid tumors using chromogenic in situ hybridization (CISH)

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Abstract #396

Introduction - *MET* amplification has 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. To date, *MET* exon 14 skipping by sequencing and *MET* amplification by FISH have been found to have potential clinical utility in predicting those patients who may derive benefit from *MET*-targeted therapy. However, little research has been conducted on alternative technologies to FISH such as CISH, which does not require a dark room and can be interpreted by a board-certified pathologist. The purpose of this study is to report our experience with *MET* amplification across solid tumors using CISH.

Methods - A retrospective analysis was done on 26,619 specimens analyzed for *MET* amplification by CISH at a CLIA-certified lab (Caris Life Sciences). The validated CISH assay, previously validated against a FISH assay, utilized a gene copy number > 5 to assess amplification. Concordance and correlative studies were done in *MET*-amplified, non-small cell lung cancer (NSCLC) specimens analyzed using a cMET IHC (SP44, 2+ or 3+ staining intensity in 50% or more tumor cell membrane) analyzing protein expression. Correlative studies involving co-existing aberrations, including PD-L1 (SP142, any intensity in at least 50% of tumor cells), in this *MET*-amplified, NSCLC cohort were also performed.

Results - *MET* amplification utilizing CISH was 0.7% (188/26,619) overall. *MET*-amplified solid tumors included carcinomas such as NSCLC (3.1%, 87/2767), gastric adenocarcinoma (3.8%, 11/293), esophageal and esophagogastric junction adenocarcinoma (3.3%, 11/338), and endometrial carcinoma (0.4%, 9/2020) along with non-carcinomas, including glioblastoma multiforme (1.0%, 5/510), uterine sarcoma (1.3%, 5/400), melanoma (0.4%, 2/538), and rare tumors such as placental-site trophoblastic tumor (100%, 1/1) and prostatic neuroendocrine tumor (100%, 1/1). A sub-analysis of *MET*-amplified, NSCLC specimens demonstrated co-occurring protein overexpression in 92.6% (75/81) of cases. These same *MET*-amplified, NSCLC specimens were found to have *EGFR* pathogenic/presumed pathogenic mutations (19.7%, 15/76), *ALK* rearrangements (2.5%, 2/80), and PD-L1 overexpression (27.5%, 14/51). *ROS1* rearrangements were not detected in this NSCLC cohort (0%, 0/76).

Conclusion - Our data suggest *MET* amplification detection utilizing CISH is a viable option for identifying *MET*-driven cancers. The presence of *MET* across various solid tumors contrasts with biomarkers like HER2, which are exclusive to carcinomas. A sub-analysis of our NSCLC population shows *MET*-amplified tumors contains a similar molecular distribution to the general NSCLC population. Future studies should incorporate *MET* CISH in clinical trials utilizing *MET*-targeted agents to determine its potential as a predictive test for evaluating who may derive the most benefit.

Methods

A retrospective analysis was done on 26,619 consecutive specimens analyzed for *MET* amplification using CISH at a CLIA-certified lab (Caris Life Sciences). The validated CISH assay, previously validated against a FISH assay, utilized a gene copy number (GCN) > 5 to assess amplification. Threshold was based on the published literature. Concordance and correlative studies were done in *MET*-amplified, non-small cell lung cancer (NSCLC) specimens analyzed using a cMET IHC (SP44, 2+ or 3+ staining intensity in 50% or more tumor cell membrane) analyzing protein expression. Correlative studies involving co-existing aberrations, including PD-L1 (SP142, any intensity in at least 50% of tumor cells), in this *MET*-amplified, NSCLC cohort were also performed.

Results

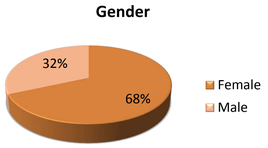

Figure 1 – Demographics (age, gender) of cohort. In total, 26,619 consecutive specimen were profiled from various solid malignancies using CISH. The age of patient cohort ranged from 1 to 89. The youngest patient with *MET*-amplification detected was 26-years-old – none were of pediatric age.

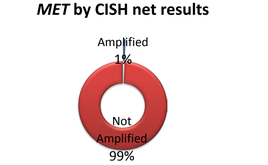

Figure 2 – Overall results of *MET* amplification by CISH. The overall rate of *MET* amplification was 0.7% across solid tumors. Details on primary tumor sites and histologies showing amplification are shown in Figure 4 and Table 1.

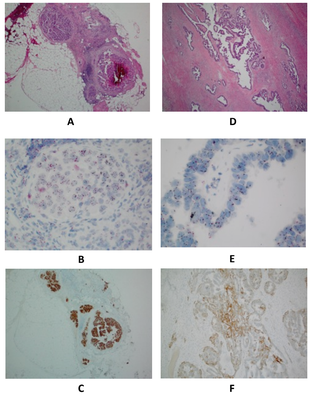

Figure 3 – Examples of H&E, *MET* amplification by CISH, and co-occurring cMET protein overexpression by IHC in NSCLC and epithelial ovarian carcinoma (EOC). Pictures (A) – (C) and (D) – (E) correspond to a metastatic adenocarcinoma of the lung and a metastatic papillary serous cystadenocarcinoma of the ovary, respectively. Photos (A) and (D) were H&Es (4x), photos (B) and (E) were CISH slides (40x and 60x), and photos (C) and (F) were the corresponding IHC (4x and 10x).

Results (cont.)

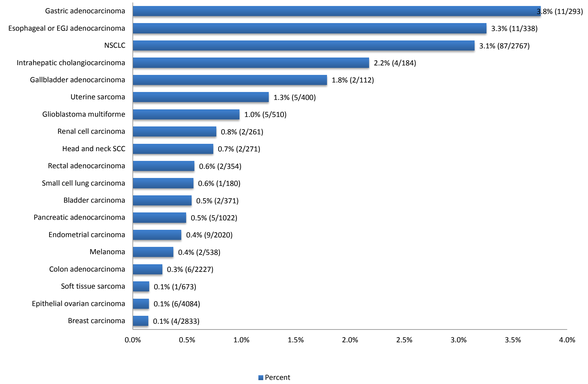

Figure 4 – *MET* Chromogenic in situ hybridization (CISH) distribution across various solid tumors profiled in over 100 specimens. The highest rates of *MET* amplification, in descending order, were in adenocarcinomas of the esophagus and stomach, NSCLC, intrahepatic bile duct and gallbladder adenocarcinoma. Unlike biomarkers like *ERBB2*/HER2, *MET* is not exclusive to epithelial malignancies as shown by amplification in glioblastoma multiforme, melanoma, and soft tissue sarcoma. When delving into histologic subtypes, *MET* amplification was found to be exclusive to urothelial bladder carcinoma in bladder cancer and invasive ductal breast carcinomas in breast cancer – no amplification was detected in non-urothelial bladder carcinomas or lobular breast carcinomas. The soft tissue sarcoma detected was a synovial sarcoma.

Other malignancies displaying *MET* amplification	
Biliary tract carcinoma, NOS	Placental trophoblastic tumor
Bone cancer – osteosarcoma	Prostatic NET
Breast phyllodes tumor/chondrosarcoma	Uveal melanoma
Head and neck cancer – adenoid cystic carcinoma	Vulvar squamous cell carcinoma

Table 1 – Rare malignancies where *MET* amplification was detected. Beyond the aforementioned tumor types shown in Figure 2, *MET* amplification by CISH was found in various rare tumors, shown here. Only one case with amplification was found in each of these tumor types. The same pattern is demonstrated here, where *MET* amplification was not limited to cancers of epithelial origin. Please note that the biliary tract carcinoma shown was one that could not be classified as intrahepatic or extrahepatic.

Results (cont.)

Biomarker	Platform	Percent
ALK	FISH	2.5% (2/80)
cMET	IHC	92.6% (75/81)
EGFR	NGS	19.7% (15/76)
PD-L1	IHC	27.5% (14/51)
ROS1	FISH	0.0% (0/76)

Table 2 – Molecular profiling results of select biomarkers in *MET*-amplified specimens in NSCLC. A subset of NSCLC containing *MET* amplification was evaluated for other genetic aberrations. As demonstrated in FISH studies, *MET* amplification is not mutually exclusive and can occur in combination with other established molecular aberrations in NSCLC. The overall percentages in biomarkers like *ALK* and *EGFR* are comparable to what has been reported in the medical literature for NSCLC in the general population.

Conclusions

- *MET* CISH may be a viable alternative to FISH in detecting patients with *MET*-driven cancer. As this technology requires only light microscopy and a pathologist, CISH is easy to incorporate in a laboratory setting.
- *MET* amplification is found in various solid tumors, whether epithelial or mesenchymal in origin.
- In NSCLC, *MET* amplification may co-occur with other molecular aberrations, necessitating further studies to look into their significance.
- *MET* amplification, along with *MET* exon 14 skipping analysis, may identify patients who derive benefit from *MET*-targeted agents currently in clinical trials. A need exists to identify patients and specific tumors who derive the most benefit.
- Further studies are warranted using alternative assays like CISH and comparing results with treatment outcomes.

References

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