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Original Contribution

Evaluation of cMET aberration by immunohistochemistry and fluorescence in situ hybridization (FISH) in triple negative breast cancers

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ABSTRACT

Purpose: To evaluate the incidence of cMET proto-oncogene aberration in a cohort of triple negative breast cancers using immunohistochemistry and fluorescence in situ hybridization (FISH) methods and correlated with patient outcome.

Patients and methods: One hundred and six female patients with diagnosis of triple negative invasive breast carcinoma at The University of Texas-M D Anderson Cancer Center from 1983 to 2009 were included in the study. Expression of cMET was assessed by IHC using rabbit monoclonal anti-total cMET antibody (SP44 from Ventana). Staining intensity was scored on a scale of 0, 1+, 2+ and 3+. cMET overexpression was defined as at least moderate membranous/cytoplasmic staining in ≥50% of tumor cells (score ≥ 2+). FISH analysis was performed using *MET* (7q31) specific probe (BAC clone RP11-95i20, Abbott Molecular Inc.) and the centromere probe (*CEP7/D7Z1*, Abbott Molecular Inc.) as internal control. *cMET* amplification was defined as gene copy numbers ≥4 per cell or *cMET/CEP7* ratio ≥ 2. cMET status was tested for correlation using Fisher's exact test with other clinicopathological parameters. The Kaplan-Meier product limit method was used to estimate the survival outcomes. Cox proportional hazards models were fit to determine the association of cMET status by IHC, or by FISH, or by copy number with survival outcomes after adjustment for other patient and disease characteristics.

Results: Medium follow up is 69.4 months (range 9–317 months). cMET was successfully evaluated by both IHC and FISH methods in ninety-six patients. There were 13 patients whose tumors overexpressed cMET was by IHC. Two patients had *cMET* amplification by FISH using definitive of *cMET/CEP7* ratio of ≥2 and four patients had *cMET* copy number > 4. Only one patient showed *cMET/CEP7* ratio of 2.53 and one was positive for cMET overexpression by IHC. No significant association between cMET overexpression by IHC and by FISH using cut-off of with either *cMET/CEP7* ratio of ≥2 or *cMET* copy number of > 4 ($P = 1.0$). There was no significant correlation between the cMET overexpression and other clinicopathological characteristics, such as patient demographics, tumor grade, stage, or chemotherapy treatment history. cMET overexpression and gene amplification did not correlate with the prognosis of TNBC regarding OS or DFS.

Conclusion: MET amplification is a rare incidence in TNBCs. cMET overexpression is infrequent in TNBCs and may not be driven by gene amplification. Neither have significant prognostic value nor do they correlate with other clinicopathological characteristics in this TNBC cohort.

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1. Introduction

Triple negative breast cancer (TNBC) is a heterogeneous group of diseases accounting for up to15~20% of breast cancers [1]. They are defined by the negative expression of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor2 (HER-2). In addition to aggressive clinical behavior and overall worse prognosis, TNBC lacks therapeutic targets unlike other subtype breast cancers [1]. Distinct functional and clinical subsets TNBC have been identified, although these have not yet been applied in clinical management [2]. Conventional cytotoxic chemotherapy remains the mainstream therapeutic regimen. There is urgent clinical need to explore novel molecules as both tumor predictive markers and therapeutic targets for this aggressive disease in clinical practice.

The proto-oncogene *cMET* on chromosome 7q21–31 (mesenchymal-epithelial transition factor gene) encodes a receptor tyrosine kinase which acts as the receptor for the hepatocyte growth factor (HGF). After binding of HGF, the receptor becomes phosphorylated and activates the downstream signaling such as the Rac1/Cdc42 pathway, the PI3k/Akt pathway, signal transducer and activator of transcription 3 (STAT3) and the Erk/mitogen-activated protein kinase cascade, etc [3]. Through these pathways cMET is involved in the regulation of cellular proliferation, motility, migration, invasion and tumorigenesis [3].

Dysregulation of cMET has been reported in tumors from various organ systems. The increased cMET activity has been indicated as a negative prognostic factor associated with poor prognosis and worse clinical outcome [4-9]. cMET overexpression was reported to be observed in 12–52% in TNBC [10-15]. Some studies found that cMET was a negative prognostic biomarker and predicted poor PFS and OS in breast cancer [14,16,17]. However, others showed cMet status did not correlate with prognosis in breast cancer [18]. The role of cMET in breast cancer still remains controversial. This study evaluated the cMET aberration using immunohistochemistry and FISH analysis in a cohort of TNBCs with attention to the relationship of cMET expression and prognosis of the patients.

2. Materials and methods

2.1. Patients

This study included 106 primary untreated tumors from patients with a pathological diagnosis of triple negative (ER/PR/HER2 negative defined by immunohistochemistry) invasive ductal carcinoma of breast undergoing segmental mastectomy or mastectomy at the University of Texas M D Anderson Cancer Center from 1983 to 2009. In our study, triple negative breast cancer was defined as ER (< 1% tumor cells staining), PR (< 1% tumor cells staining) and Her-2/neu expression (IHC score 0/1+ and/or FISH Her2/CEP17 ratio ≤ 2) all negative [19,20]. A number of variables were recorded from the UT-MD Anderson Breast Medical Oncology Database, including demographics, date of diagnosis, primary tumor type, histology, stage of disease (T,N,M), primary systemic treatment type, surgery type, response to treatment, stage of disease, date of recurrence, site of recurrence, and date of death/last follow up. Overall survival time was defined as the time (in months) between diagnosis and the date of death/date of last follow-up.

2.1.1. Immunohistochemistry

Following heat-induced antigen retrieval, immunohistochemistry targeting cMET (Rabbit monoclonal antibody [SP44], Ventana Medical Systems) was performed using the automated Benchmark XT platform (Ventana Medical Systems) in accordance to the manufacturer's recommendations on the 4-μm tissue sections from paraffin embedded formalin fixed archival blocks. Both cytoplasmic and membranous staining is considered to be positive. The breast pathologist (HZ) evaluated the immunohistochemical staining and was blinded to any clinicopathological data during the evaluation.

cMET staining is scored as the following: 0 if no staining; 1+ if weak staining in any amount of tumor cells and moderate staining in < 50% of tumor cells; 2+ if ≥ 50% of tumor cells showed incomplete membranous and/or cytoplasmic staining with at least moderate intensity but < 50% cells with strong intensity; and 3+ if ≥ 50% of tumor cells with circumferential membranous and/or cytoplasmic staining with strong intensity. The cMET is considered to be positive for overexpression if the staining is scored as 2+ or 3+ (at least ≥ 50% of tumor cells showed moderate or higher membranous staining).

2.1.2. Fluorescent In Situ Hybridization (FISH)

The *MET* probe (BAC clone RP11-95i20, CHORI; http://bacpac.chori.org) was labeled with Spectrum Orange dUTP (Abbott Molecular Inc., Des Plaines, IL) and was hybridized to full tissue sections along with the centromere probe (*CEP7/D7Z1*, Spectrum Green, Abbott Molecular Inc., Des Plaines, IL) as internal control using standard protocols. The staining was analyzed using a Zeiss fluorescent photomicroscope. A minimum of 60 cells were analyzed. *MET* amplification was defined as *MET/CEP7* ratio ≥ 2.0 or a *MET* gene copy number > 4.

2.2. Statistical analysis

Patients were categorized according to cMET expression by IHC as cMET positive or negative. Correlation between cMET status by IHC, FISH, or copy number was tested using Fisher's exact test. Patient characteristics including age, race, tumor size, lymph nodes, histology, grade, lymphovascular invasion, surgery, adjuvant radiation, hormonal therapy, chemotherapy and adjuvant chemotherapy drugs were tabulated and compared between groups using Fisher's exact test. Overall survival (OS) was measured from the date of diagnosis to the date of death or lost to follow-up. Relapse-free survival (RFS) was measured from the date of diagnosis to the date of first documented local or distant recurrence or last follow-up. Patients who died before experiencing the relevant events were censored at their dates of last follow-up date. The Kaplan-Meier product limit method was used to estimate the survival outcomes of all patients by time to chemotherapy groups; groups were compared using the log-rank statistic.

Cox proportional hazards models were fit to determine the association of cMET status by IHC, or by FISH, or by copy number with survival outcomes after adjustment for other patient and disease characteristics. Variables that showed statistical significance in the univariate log-rank test were included in the multivariable models besides the cMET measurements. Those variables included age (> 50, ≤50), tumor size (T1, T2, T3–4), nodal status (N0, N1, N2–3), and adjuvant chemotherapy (anthracycline-based, taxane-based, anthracycline/taxane-based, other). Results were expressed in hazard ratios (HRs) and 95% CIs. P values < 0.05 were considered statistically significant; all tests were two-sided. Statistical analyses were carried out using SAS 9.2 (SAS Institute, Cary, NC) and S-Plus 7.0 (Insightful Corporation, Seattle, WA).

3. Results

3.1. Patients and clinical characteristics

All 106 eligible cases of TNBC were included in the study and were followed for recurrence and survival. The median follow-up time among women was 69.4 months (range, 9–317 months). At the time of the analytical study, 23 women (21.7%) had died and 20 (18.9%) had experienced a recurrence. Patient and clinical characteristics of cMET overexpression status are displayed in Table 2.

Table 1
Correlation between cMET overexpression by IHC and amplification by FISH.

FISH results	cMET/CEP7 Radio ≥2	cMET/CEP7 Radio < 2	< 4 copy/ Nucleus	≥4 copy/ Nucleus
IHC+	0	13	12	1
IHC-	2	81	81	2

3.2. cMET aberration and its correlation with other clinicopathological characteristics

The H&E slides of all cases were reviewed by the breast pathologist (HZ) for diagnosis confirmation. The cMET overexpression and amplification were studied using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) on this cohort. Among the one hundred and six patients, cMET was successfully evaluated by both IHC and FISH methods in only ninety six patients (Table 1). There were thirteen patients whose cMET was overexpressed by IHC (moderate and strong cytoplasmic and membranous staining, Fig. 1), eighty six who had no cMET overexpression by IHC, and seven patients with un-determined cMET status due to technical issues s. With the definition of amplification of *MET/CEP7* ratio of ≥2, only two patients who had *MET* amplification by FISH (Fig. 2), ninety one patients with no *MET* amplification by FISH, and thirteen patients with unsuccessful FISH assessment for MET status. The two patients with *MET* amplification by FISH did not exhibit cMET overexpression by IHC study. There were four patients who had *MET* copy number that is > 4, with the *MET* average copy number per nucleus ranging from 4.23 to 4.83. Among these four patients, only one showed *MET/CEP7* ratio of 2.53 and one was positive for cMET overexpression by IHC (Fig. 3). Similarly, no significant association between cMET overexpression by IHC and *MET* amplification by FISH using cut-off of *MET* copy number of ≥4 ($P = 1.0$) was detected. In summary, among this cohort, two patients met criteria for MET FISH amplification using MET/CEP7 ratio of ≥2, four based on MET copy number of > 4/nucleus, and only one by both criteria, accounting for a total of five cases with MET amplification. Among these five patients, only one patient demonstrated over-expression defined by IHC.

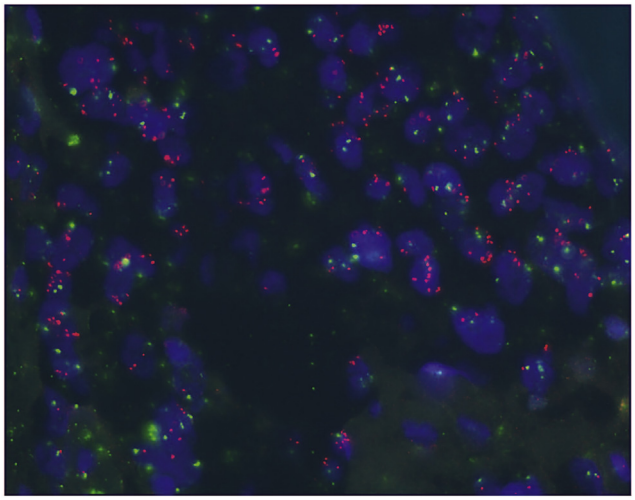

Fig. 2. *MET* amplified by FISH. *MET* amplified: *MET/CEP7* ratio of 2.0; *MET* signal of 4.4/nucleus.

Due to the low incidence of *MET* amplification assessed by FISH, the patient characteristics were only stratified by IHC status and were summarized in Table 2. No significant associations between cMET overexpression by IHC and any other clinicopathological characteristics were noted.

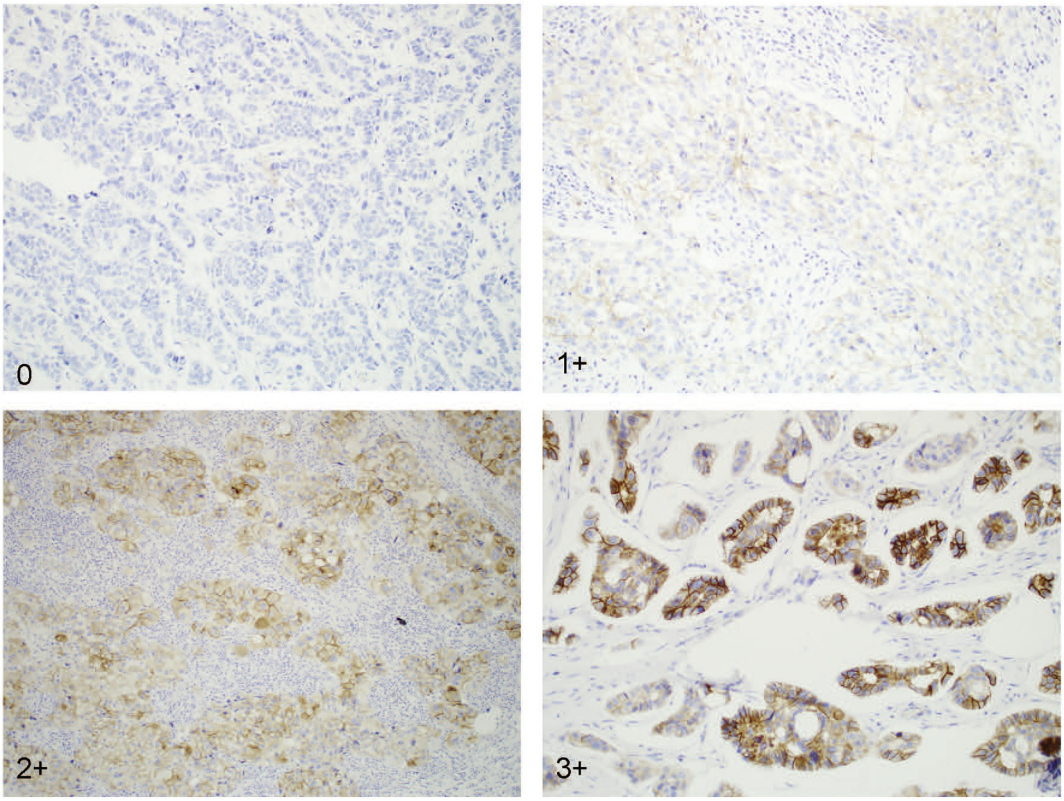

Fig. 1. cMET IHC staining. 0 if no staining; 1+ if weak staining; 2+ if ≥ 50% of tumor cells showed incomplete membranous staining with moderate or higher intensity but < 50% had complete membranous staining with strong intensity; and 3+ if ≥ 50% of tumor cells with circumferential membranous staining with moderate to strong intensity.

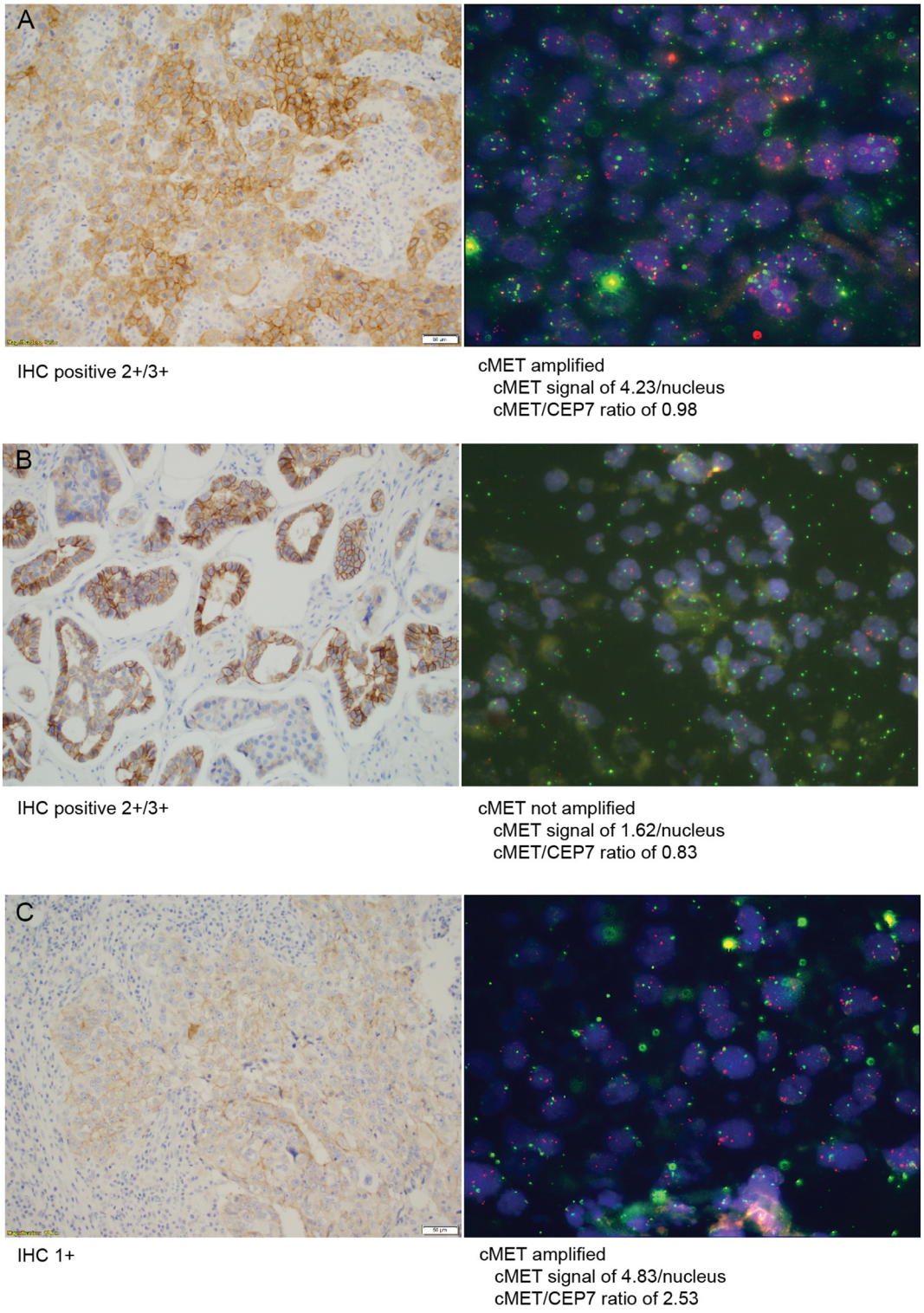

Fig. 3. (A) cMET overexpressed and amplified (B) cMET overexpressed and not amplified (C) cMET not overexpressed and amplified.

3.3. Survival estimates

Univariate analysis of survival across all clinical and pathological data was analyzed (Table 3). For the overall cohort, 5-year OS and RFS was 85% and 87%, respectively. Among all clinical factors, the 5 year survival estimate was significantly lower in the T3 and T4 groups (0), which had a significant difference in tumor size when compared to the T1 group (5 year survival estimate 0.92, 95% CI 0.79–0.9; $P < 0.001$) and T2 group (5-year survival estimate 0.79, 95% CI 0.6–0.89; $P < 0.001$). The 5-year OS estimate was 86% and 79% in patients who were IHC negative and positive ($P = 0.54$), respectively. Similarly, no significant difference across IHC groups was observed for RFS ($P = 0.44$). Similar observation was noted using cMET copy number > 4/nucleus by FISH analysis as a cutoff for these two end points.

To assess if cMET was an independent poor prognostic factor, a multivariable Cox proportional hazards model for OS and DFS was generated, adjusted for cMET status via IHC and by FISH, together with

Table 2
Patient and clinical characteristics by cMET overexpression status.

	All patients (N = 106) N(%)	cMET by IHC negative (N = 86) N(%)	cMET by IHC positive (N = 13) N(%)	p*
Age, years				
Age ≤50	47 (44.3%)	39 (88.6%)	5 (11.4%)	
Age > 50	59 (55.7%)	47 (85.5%)	8 (14.5%)	0.77
Tumor size				
T1	52 (52%)	40 (85.1%)	7 (14.9%)	
T2	44 (44%)	38 (88.4%)	5 (11.6%)	
T3–4	4 (4%)	2 (66.7%)	1 (33.3%)	0.44
Lymph nodes				
N0	65 (63.7%)	54 (91.5%)	5 (8.5%)	
N1	28 (27.5%)	19 (70.4)	8 (29.6%)	
N2	5 (4.9%)	5 (100%)	0 (0%)	
N3	4 (3.9%)	4 (100%)	0 (0%)	0.06
Histology				
Ductal	90 (84.9%)	72 (84.7%)	13 (15.3%)	
Other	16 (15.1%)	14 (100%)	0 (0%)	0.20
Nuclear grade				
I or II	6 (5.9%)	5 (83.3%)	1 (16.7%)	
III	95 (94.1%)	76 (86.4%)	12 (13.6%)	1.0
Lymphovascular Invasion				
Negative	85 (82.5%)	68 (87.2%)	10 (12.8%)	
Positive	18 (17.5%)	15 (83.3%)	3 (16.7%)	0.71
Surgery				
Breast conservation surgery	59 (55.7%)	51 (89.5%)	6 (10.5%)	
Mastectomy	47 (44.3%)	35 (83.3%)	7 (16.7%)	0.37
Adjuvant radiation				
No	34 (32.1%)	26 (83.9%)	5 (16.1%)	
Yes	72 (67.9%)	60 (88.2%)	8 (11.8%)	0.54
Adjuvant hormonal therapy				
No	99 (93.4%)	79 (85.9%)	13 (14.1%)	
Yes	7 (6.6%)	7 (100%)	0 (0%)	0.59
Adjuvant chemotherapy				
No	10 (9.4%)	9 (90%)	1 (10%)	
Yes	96 (90.6%)	77 (86.5%)	12 (13.5%)	1.0
Adjuvant chemotherapy drug				
AN	45 (44.1%)	38 (90.5%)	4 (9.5%)	
TX	5 (4.9%)	4 (80%)	1 (20%)	
AN + TX	46 (45.1%)	35 (83.3%)	7 (16.7%)	
Other	6 (5.9%)	5 (83.3%)	1 (16.7%)	0.55

AN = Anthracycline-based; TX = Taxane-based.
* Fisher's exact p-value.

other clinical and pathological factors, such as copy number, age, lymph node status, tumor size, and chemotherapy regimens. Compared with women with no cMET overexpression defined by IHC, those who had cMET overexpression by IHC (HR = 1.19; 95% confidence interval [CI], 0.25–5.63; P = 0.82) did not show a significant difference in the risk of death. Similarly, there was no significant difference in the risk of recurrence (HR = 1.34; 95% CI, 0.31–5.81; P = 0.69) (Table 4a). With respect to OS, older patients (P = 0.01), patients who had T3–4 tumor (P = 0.0002) compared to T1, patients with N2–3 nodal status (P = 0.004) compared to N0, and patients who received taxane-based chemotherapy (P = 0.001), or other chemotherapy (P = 0.022) compared to who received anthracycline-based chemotherapy had increased risk of death. With respect to RFS, patients with T3–4 tumor (P = 0.0001) compared to T1 had increased risks of recurrence. Similarly, there was no impact of *MET* amplification measured by FISH using a cut off of *MET/CEP7* ratio of ≥2 (Table 4b) or by copy numbers (Table 4c) on either OS (P = 0.17) or RFS (P = 0.1). In terms of *MET* copy numbers by FISH, using a cutoff of 4 did not have a significant impact on OS or RFS after adjustment for age, tumor size, nodal status, and adjuvant chemotherapy drugs (Table 4c). The Kaplan–Meier curves of OS and DFS according to cMET IHC groups were illustrated in Fig. 4.

4. Discussion

This is a study to assess the status of cMET on the primary tumors from a small cohort of predominantly early stage TNBC patients (96% with T1 & T2 tumors) using immunohistochemistry and FISH study. cMET is overexpressed (IHC) in 13% of this cohort of TNBC and is amplified in only rare cases (FISH). Our data showed a lack of relationship between cMET overexpression via IHC data and *MET* amplification by FISH study suggesting that cMET overexpression is not primarily driven by gene amplification. Neither overexpression nor the gene amplification of cMET correlates with the prognosis of TNBC regarding OS or DFS. There is no significant correlation between the cMET overexpression and other clinicopathological characteristics, such as patient characteristics, tumor grade, tumor stage or chemotherapy treatment history.

As a receptor tyrosine kinase, cMET aberration has been reported in cancers such as lung, kidney, stomach, head & neck, colon and cervical cancer, etc. [4-7,21-25]. Constitutive activation of cMET after phosphorylation could be achieved through either gene mutation and/or gene amplification [26]. The cMET overexpression which was shown to be often associated with gene amplification was indicated to be correlated with aggressiveness of disease and associated with worse clinical outcome, especially in EGRF-TKI resistant cancers. However, the predictive and prognostic significance of cMET in breast cancers has been controversial. This phenomenon is best exemplified by the meta-analysis including 32 retrospective studies correlating cMET levels with either OS or RFS/DFS [27]. The cMET overexpression was reported to be observed in a wide range, from 3.8% using membranous staining as positivity by IHC method in a cohort of 78 ER and HER2 positive invasive breast cancers patients [18] to 70.4% using RPPA method in a cohort of 257 patients with invasive breast cancers diagnosis regardless of hormone receptor or HER2 status [28]. Although majority of the reports indicated that cMET overexpression was connected with poor survival of breast cancer, it was linked to good prognosis in rare studies.

The previous studies showed that > 50% of TNBCs had high cMET expression using IHC method and the high cMET expression predicted recurrence and death due to disease [15,29]. This finding was supported by another study showing that increased cMET copy number was reported in 8.44% of all breast cancers and it was more frequent in TNBCs using molecular inversion probe method [30] comparing to other subtypes of breast cancers. Patients with high copy number of cMET tend to have more aggressive prognostic features, such as larger tumor size, higher nuclear grade and negative hormone receptors. With a median follow up of 7.5 years, this study found marginal worse prognostic effects of high *MET* copy number on recurrence free survival (RFS); however, it was not indicated to be an independent predictor of RFS. Interestingly, in their report, the patients with Hormone Receptor (HR)-positive and high *MET* copy number breast cancer had a significant lower 5-year RFS compared with patients with HR-positive and normal/low *MET* copy number breast cancer. The same group reported that the high level of cMET and p-cMET correlated with poor prognosis in all breast cancer subtypes using reverse phase protein array (RPPA) [28]. This is contradicted by the low incidence of high MET expression (~3%) reported in a small cohort of ER positive and HER2 positive only cases from a study accessing MET status using IHC [15] which did not yield any statistically significant result regarding overall survival or disease-free survival.

In contrast to most previous studies, our results did not indicate that cMET overexpression is a valuable prognostic biomarker in TNBCs. The reasons for the different observation are multifaceted. Lack of strict sample inclusion criteria and variability of detection methods may have confounded the studies [27]. This study includes triple negative breast cancers and most of which were lymph node negative (63.7%) and early stage (stages I to II) diseases (96%). Differences in results may also

Table 3
Survival estimates by patients' characteristics.

	Patients	N Events	5-year overall survival estimate (95%CI)	P	N Events	5-year relapse-free survival estimate (95%CI)	p
All patients	106	23	0.85 (0.75,0.91)		20	0.87 (0.79,0.92)	
Age, years							
Age ≤ 50	47	9	0.93 (0.8,0.98)		9	0.91 (0.77,0.96)	0.40
Age > 50	59	14	0.77 (0.63,0.87)	0.04	11	0.84 (0.71,0.91)	
Tumor size							
T1	52	6	0.92 (0.79,0.97)		5	0.92 (0.79,0.97)	
T2	44	13	0.79 (0.6,0.89)		10	0.85 (0.7,0.93)	
T3–4	4	3	0	< 0.001	3	0	< 0.001
Lymph nodes							
N0	65	9	0.87 (0.75,0.94)		9	0.88 (0.76,0.94)	
N1	28	5	0.92 (0.73,0.98)		5	0.89 (0.7,0.96)	
N2–3	9	8	0.44 (0.14,0.72)	0.003	4	0.67 (0.28,0.88)	0.16
Histology							
Ductal	90	19	0.86 (0.76,0.92)		15	0.88 (0.79,0.94)	
Other	16	4	0.78 (0.46,0.92)	0.9	5	0.79 (0.47,0.93)	0.25
Nuclear grade							
I or II	6	3	0.8 (0.2,0.97)		2	1	
III	95	19	0.84 (0.74,0.91)	0.52	16	0.85 (0.76,0.91)	0.56
Lymphovascular invasion							
Negative	85	14	0.88 (0.78,0.94)		14	0.89 (0.79,0.94)	
Positive	18	8	0.66 (0.35,0.84)	0.06	5	0.77 (0.5,0.91)	0.13
Surgery							
Breast conservation	59	9	0.88 (0.78,0.94)		9	0.9 (0.78,0.95)	0.38
Mastectomy	47	14	0.81 (0.65,0.9)	0.12	11	0.84 (0.68,0.92)	
Adjuvant radiation							
No	34	7	0.87 (0.68,0.95)		7	0.87 (0.68,0.95)	0.87
Yes	72	16	0.84 (0.72,0.91)	0.79	13	0.87 (0.77,0.93)	
Adjuvant hormonal therapy							
No	99	22	0.85 (0.76,0.91)		20	0.86 (0.77,0.92)	
Yes	7	1	0.8 (0.2,0.97)	0.45	0	1	0.16
Adjuvant chemotherapy							
No	4	0	1		1	1	0.56
Yes	102	23	0.84 (0.74,0.9)	0.08	19	0.86 (0.78,0.92)	
Adjuvant chemotherapy drug							
AN	45	9	0.91 (0.77,0.96)		9	0.84 (0.7,0.92)	
TX	5	3	0.53 (0.07,0.86)		2	0.8 (0.2,0.97)	
AN + TX	46	9	0.84 (0.66,0.93)		7	0.91 (0.77,0.96)	
7 Other	6	2	0.67 (0.19,0.9)	0.06	1	0.83 (0.27,0.97)	0.70
cMET by IHC							
Negative	86	18	0.86 (0.76,0.92)		16	0.88 (0.78,0.93)	
Positive	13	3	0.79 (0.37,0.94)	0.54	3	0.83 (0.48,0.96)	0.16
cMET by cMET/CEP7 ratio							
Negative	91	18	0.87 (0.77,0.93)		17	0.88 (0.79,0.94)	
Positive	2	1	0.5 (0.01,0.91)	0.12	1	0.5 (0.01,0.91)	0.44
cMET by copy Number							
≤ 4	90	19	0.86 (0.75,0.92)		18	0.87 (0.77,0.93)	
> 4	3	0	1	0.47	0	1	0.47

CI = confidence interval.

Table 4a
Multivariable Cox proportional hazards model for cMET by IHC.

	Overall survival			Relapse-free survival		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
cMET by IHC: positive v. negative	1.19	0.25 to 5.63	0.82	1.34	0.31 to 5.81	0.69
Age, years > 50v. ≤ 50	4.18	1.4 to 12.47	0.01	2.09	0.72 to 6.08	0.17
Pathologic tumor size: T2 v.T1	1.9	0.52 to 6.96	0.33	2.37	0.68 to 8.25	0.18
Pathologic tumor size: T3–4 v.T1	31.6	5.14 to 194.37	0.0002	31.42	5.48 to 180.29	0.001
Nodal status: N1v.N0	1.39	0.33 to 5.81	0.65	1.24	0.33 to 4.66	0.75
Nodal status: N2–3 v.N0	5.72	1.76 to 18.57	0.004	2.37	0.66 to 8.53	0.19
Adjuvant chemo: Taxane v. Anthracycline	18.47	3.12 to 109.31	0.001	3.67	0.63 to 21.28	0.15
Adjuvant chemo: Anthracycline/Taxane v. Anthracycline	2.81	0.85 to 9.28	0.09	0.9	0.28 to 2.86	0.86
Adjuvant chemo: other v. Anthracycline	9.3	1.39 to 62.24	0.022	2.53	0.26 to 24.84	0.43

CI = confidence interval.

be attributable in part to different scoring methods and cut-off values. Ho-Yen et al. used a semiquantitative method combining numerical scores for intensity and area of reactivity determines cMET over-expression. cMET scores were between 0 and 14, while the cut-off value applied in survival analysis is 7 [31].Whereas in our research according to the intensity and number of stained cells cMET expression was scored as 0, 1+, 2+, 3+. cMET positivity was defined as 2+ and 3+. These inherit differences in different studies inevitably led to systemic bias, manifested by the observation of wide range of cMET positivity defined by IHC (3% to 63%) in the previous studies [10,11,14,15,32,33].

Table 4b
Multivariable Cox proportional hazards model for cMET by FISH cMET/CEP7 ratio of ≥ 2.

	Overall survival			Relapse-free survival		
	Hazard ratio	95% CI	P	Hazard Ratio	95% CI	P
cMET by FISH: positive v. negative	5.05	0.5 to 50.65	0.17	6.97	0.69 to 70.82	0.1
Age, years > 50v. ≤50	4.05	1.33 to12.35	0.014	2.02	0.67 to 6.04	0.21
Pathologic tumor size: T2 v.T1	1.86	0.49 to 7.08	0.36	2.22	0.63 to 7.82	0.22
Pathologic tumor size: T3–4 v.T1	37.21	5.41 to 255.72	0.0002	31.45	5 to 197.73	0.0002
Nodal status: N1v.N0	1.75	0.44 to 6.89	0.42	1.75	0.5 to 6.15	0.38
Nodal status: N2–3 v.N0	6.1	1.69 to 22.04	0.006	3.05	0.75 to 12.4	0.12
Adjuvant chemo: Taxane v. Anthracycline	21.4	3.14 to 145.84	0.002	4.88	0.76 to 31.45	0.1
Adjuvant chemo: Anthracycline/Taxane v. Anthracycline	2.81	0.84 to 9.41	0.09	0.79	0.24 to 2.64	0.71
Adjuvant chemo: other v. Anthracycline	9.80	1.4 to 68.8	0.022	2.97	0.3 to 29.62	0.35

CI = confidence interval.

Table 4c
Multivariable Cox proportional hazards model for cMET by FISH copy number/nucleus (> 4 v ≤4).

	Overall survival			Relapse-free survival		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Copy number: > 4 v. ≤4	0.00	0 to infinity	0.99	0.00	0 to infinity	0.99
Age, years > 50v. ≤50	4.08	1.35 to 12.35	0.013	2.13	0.72 to 6.29	0.17
Pathologic tumor size: T2 v.T1	1.52	0.42 to 5.56	0.53	1.96	0.56 to 6.84	0.29
Pathologic tumor size: T3–4 v.T1	28.57	4.53 to 180.27	0.0004	24.55	4.16 to 144.93	0.0004
Nodal status: N1v.N0	1.53	0.41 to 5.7	0.52	1.54	0.46 to 5.19	0.49
Nodal status: N2–3 v.N0	5.58	1.62 to 19.24	0.007	2.91	0.75 to 11.36	0.12
Adjuvant chemo: Taxane v. Anthracycline	21.24	3.28 to 137.54	0.001	4.88	0.79 to 30.36	0.09
Adjuvant chemo: Anthracycline/Taxane v. Anthracycline	3.18	0.98 to 10.32	0.05	0.96	0.31 to 2.99	0.94
Adjuvant chemo: other v. Anthracycline	8.38	1.25 to 56.13	0.028	3.04	0.31 to 30.12	0.34

CI = confidence interval.

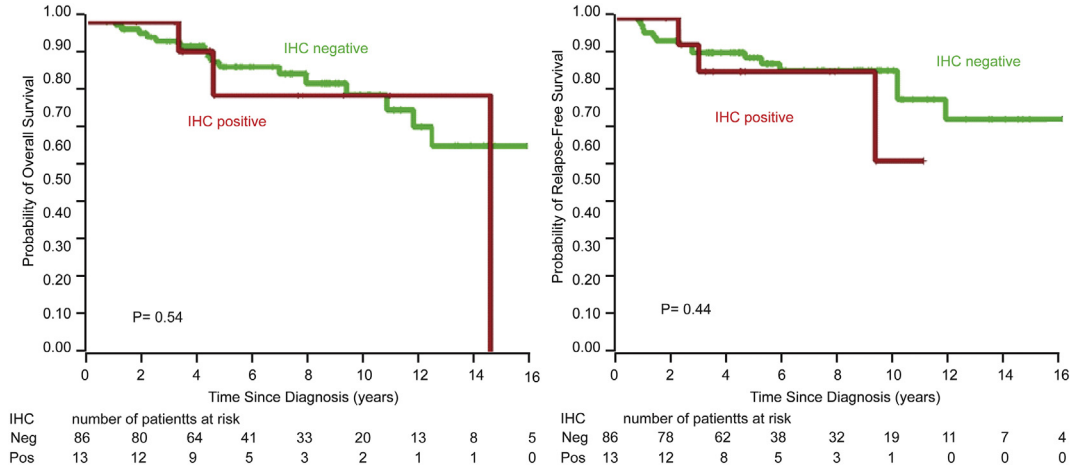

Fig. 4. Overall Survival by IHC and Relapse-free Survival by IHC.

In addition, phosphorylated cMET (p-cMET) binds Grb2 (growth factor receptor–bound protein 2) and Gab1 (GRB2-associated-binding protein 1) and then trigger downstream signaling molecules such as PI3K/AKT and MAPK pathways [34,35]. Through these cellular signaling pathways HGF/cMET plays an important role in cellular proliferation, survival, migration, and invasion [36,37]. One study revealed that total cMET and p-cMET levels were significant prognostic factors for both RFS and OS. They found that high levels of cMET and p-cMET were seen in all breast cancer subtypes and correlated with poor prognosis [28]. It can be inferred that p-cMET is most likely a potential prognostic biomarker in TNBC. However our study only detected cMET by IHC but not p-cMET. This may underestimate the prognostic value of cMET. Assessing p-cMET is needed to clarify the functional role of cMET in breast cancers.

In concordance with our results, amplification of the *MET* gene has been reported to be a rare incidence in invasive breast cancers [38]. Polysomy 7 might partially contribute to the already rare occasion of *MET* amplification by FISH, which makes the true gene amplification of *MET* an even rarer situation. It is reasonable to speculate that cMET overexpression might not be, at least partially, the direct result from increased gene copy number of *MET* gene, rather a result of increased protein productivity or protein stability at transcription and/or post-translation levels. In lieu of these observations, most recent studies have focused on the relationship of protein overexpression of cMET and survival. Most studies found cMET protein overexpression, when assessed by IHC, to be a negative prognostic factor in invasive breast cancer [13-15]. This cMET aberration seems to be more clinically significant in TNBC patients because this would provide novel potential

therapeutic application to target the disrupted cMET signaling pathways [15,39].

Overall, our results in a limited small cohort indicated that MET aberration is not a significant prognostic factor in early stage TNBCs. The standardized evaluation methods and cut off value would be indicated in larger cohort of patient population to clarify the role of cMET in breast cancers. Evaluation of the post translational modification of cMET, including p-cMET, and its function in breast tumorigenesis will further unmask its potential clinical utility.

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