

Discordant HER2 expression and response to neoadjuvant chemoradiotherapy in esophagogastric adenocarcinoma

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Background: Targeting human epidermal growth factor receptor 2 (HER2) with trastuzumab in metastatic esophagogastric adenocarcinoma (EGA) improves survival. The impact of HER2 inhibition in combination with chemoradiotherapy (CRT) in early stage EGA is under investigation. This study analyzed the pattern of HER2 overexpression in matched-pair tumor samples of patients who underwent neoadjuvant CRT followed by surgery.

Methods: All patients with EGA who underwent standard neoadjuvant CRT followed by esophagectomy at the University of Florida were included. Demographics, risk factors, tumor features, and outcome data were analyzed. Descriptive statistics, Chi-square exact test, uni- and multivariate analyses, and Kaplan Meier method were used. HER2 expression determined by immunohistochemical (IHC) was scored as negative (0, 1+), indeterminate (2+) or positive (3+).

Results: Among 49 sequential patients (41 M/8 F) with matched-pair tumor samples, 9/49 patients (18%) had pathologic complete response (pCR), 10/49 had near pCR or not enough tumor (NET) to examine in the post-treatment samples. Patients with initial HER2 negativity demonstrated conversion to HER2 positivity after neoadjuvant CRT (7/30 cases; 23%). Baseline HER2 overexpression was more common in lower stage/node negative patients (67% in stages I, IIA *vs.* 33% in stages IIB, III) and did not correlate with treatment response or survival.

Conclusions: Although limited by a relatively small sample size, our study failed to demonstrate that baseline HER2 protein over-expression in EGA predicts response to standard CRT. However, our data suggested that HER2 was up regulated by CRT resulting in unreliable concordance between pre-treatment (pre-tx) and post-treatment (post-tx) samples. Pre-therapy HER2 expression may not reliably reflect the HER2 status of persistent or recurrent disease.

Keywords: Chemoradiation; human epidermal growth factor receptor 2 (HER2); esophagogastric adenocarcinoma (EGA)

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Introduction

Human epidermal growth factor receptor 2 (HER2) is a member of the HER receptor family, which consists of four transmembrane glycoproteins (HER1-4). HER2 has no natural ligand and requires heterodimerization with other HER receptors to initiate the downstream signaling pathway. HER2 activation results in cell proliferation and survival via the RAS-MAPK pathway (1-3). HER2 is overexpressed in 10-44% of esophagogastric adenocarcinoma (EGA) (4-7). The prognostic significance of HER2 expression in EGA is unclear with conflicting results (8-11). Therapeutic inhibition of the HER2 receptor has improved outcomes when combined with chemotherapy in metastatic gastroesophageal junction (GEJ) and gastric adenocarcinoma overexpressing HER2 (12). Importantly, determination of HER2 overexpression for metastatic disease routinely utilizes molecular testing of either the initial endoscopic biopsy or the post-treatment (post-tx) resection specimen.

Treatment of localized EGA was historically surgery alone. Currently, the standard treatment for locoregional resectable esophageal and/or GEJ adenocarcinoma is trimodality therapy with neoadjuvant chemoradiotherapy (CRT) followed by surgery since combination therapy demonstrated improvement in survival compared to surgery alone (13,14). Inhibition of HER2 in localized EGA in the neoadjuvant setting is still under investigation. The Brown University Oncology Group conducted a pilot study—trastuzumab added to CRT in patients with HER2 overexpressed locally advanced adenocarcinoma of the esophagus. This study showed encouraging results with a 3-year survival of 47% (4). A phase III trial by RTOG evaluating the addition of trastuzumab to trimodality treatment of HER2 overexpressing esophageal adenocarcinoma is ongoing (Clinicaltrials.gov NCT01196390).

The objectives of our study were to determine the changes in HER2 expression pattern in matched-pair pre-treatment (pre-tx) and post-tx tissues, to determine the impact of CRT on HER2 expression and correlate pre-tx HER2 expression with response to CRT and survival. Additionally, we sought to provide a better understanding of HER2 expression in pre- and post-tx tissues and identify the most appropriate specimen (pre- vs. post-tx) for HER2 expression analysis in patients being considered for additional therapy when they develop recurrence.

Methods

We conducted a retrospective analysis of all patients who underwent neoadjuvant CRT followed by esophagectomy at the University of Florida from 2001 to 2011 in whom matched-pair tissue was available and intact. Forty-nine patients with distal esophageal or GEJ adenocarcinoma were identified through their enrollment in the UF GI Oncology Research Database and/or the presence of tumor samples in the UF Surgical Pathology database. The project was approved by the UF Health Institutional Review Board (IRB). Clinical and pathological data were obtained from the medical records.

HER2 immunohistochemical (IHC) staining

All available hematoxylin and eosin (H&E) stained slides of the pre-tx primary tumor biopsies and post-tx surgical specimens were reviewed with representative tumor blocks for each case selected. Formalin-fixed paraffin-embedded (FFPE) matched-pair tumor samples from the 49 patients were stained with HER2 monoclonal antibody (mouse monoclonal antibody; Invitrogen, Camarillo, CA, USA). The IHC results were interpreted using the standard validated scoring system for gastric cancer (*Figure 1*) consistent with previously published reports (12,15,16). IHC results were independently interpreted by three study pathologists (AA, LVD, and TZ), who were blinded to clinical data and pre-/post-tx tissue samples. In situ hybridization was not performed as part of this study.

Statistical analysis

Descriptive statistics were used for summarizing demographic, clinical and pathological characteristics, as well as molecular expressions. Chi-square exact test was used to test the association of demographic and clinicopathological features, pre- and post-CRT HER2 expression with clinical characteristics. Kruskals' Gamma coefficient was used to measure the association between pre- and post-tx HER2 expression. Odds ratio (OR) was used to measure the above association with recurrence and outcomes. Overall survival curves were compared by logrank test, and the corresponding cumulative survival rates were estimated using Kaplan-Meier method. All statistical analysis was performed using SAS 9.2 (SAS Institute Inc, NC, USA).

Results

A total of 49 patients were identified and included in this

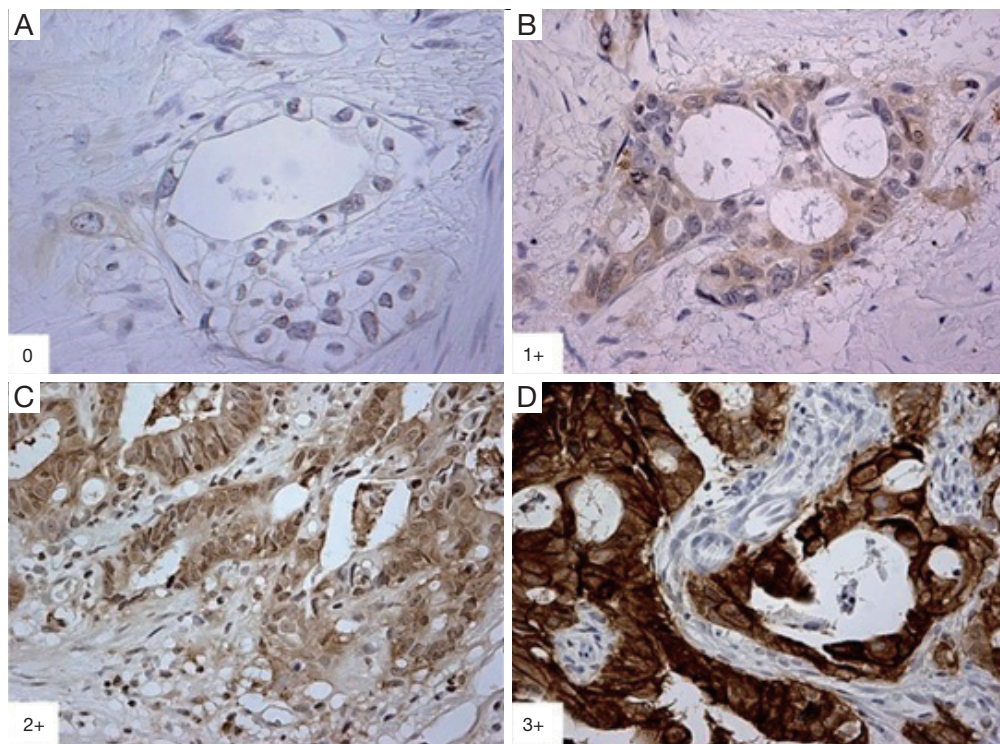


Figure 1 Immunohistochemistry of HER2 staining. (A) 0: negative (400 \times); (B) 1+: weak (400 \times); (C) 2+: moderate (100 \times); (D) 3+: strong positivity (100 \times). HER2, human epidermal growth factor receptor 2.

analysis. Mean age was 65.2 years (range, 39-87 years) and BMI was 29.4 (range, 16.9-52.0). The majority were men (84%), who were current or former smokers (80%), and had Barrett's esophagus (78%). Tumor stage and other relevant demographics are summarized in *Table 1*. Treatment uniformly consisted of platinum-based chemotherapy (cisplatin and 5-fluorouracil) concurrent with standard doses, fractionation and volumes of external beam radiotherapy (approximately 50.4 Gy). Seventeen patients (35%) recurred with a median time to recurrence of 1.0 year and median survival time of 1.2 years (*Table 1*). Overall median survival for the entire cohort was 2.3 years with a median duration of follow up of 2.0 years.

Patients with node negative disease had numerically more frequent baseline HER2 positivity compared to patients with node positive disease (20% *vs.* 6.9%; $P=0.40$) and were more likely to attain a pathologic complete response (pCR) after CRT (30% *vs.* 10.3%; $P=0.15$), although neither difference was statistically significant. Pre-tx HER2 expression was negative (0, 1+) in 63%, indeterminate (2+) in 25%, and positive (3+) in 12% of cases. Post-tx specimens revealed 18% (9/49) with pCR and another 20%

(10/49) with a near pCR or not enough tumor (NET) tissue present to perform ancillary studies. Thirty out of 49 (61%) patients did not attain pCR or near pCR/NET. Of these patients, HER2 expression was found to be negative in 20% (10/49), indeterminate in 16% (8/49), and positive in 25% (12/49) of cases. The relationship of pre- and post-tx HER2 expression is shown in *Table 2*. After CRT, patient samples with initial pre-tx HER2 negativity maintained a negative expression in 26% (8/30), but became indeterminate in 20% (6/30) and demonstrated a positive expression in 23% (7/30) of patients. Patients with pre-tx HER2 positive expression attained pCR/NET in 67% (4/6) and maintained a positive expression profile in the remaining patients (2/6); none of them became negative or demonstrated indeterminate expression after CRT. Patients with indeterminate pre-tx HER2 expression demonstrated 41% pCR/NET, with cases of persistent disease demonstrating HER2 negative (17%), indeterminate (17%) or positive expression (25%) after CRT. There was only a small to moderate association between changes in pre- and post-tx HER2 expression ($G=0.403$; 95% CI, $-0.147-0.953$; $P=0.185$). HER2 expression patterns in matched pre- and post-tx samples are

Table 1 Summary of clinicopathological characteristics of patient with esophageal and GEJ adenocarcinoma who underwent neoadjuvant standard chemoradiation followed by esophagectomy

Characteristics	N=49 [%]
Mean ± SD	
Age	65±10.9
BMI	29±6.5
Gender	
Male	41 [84]
Female	8 [16]
cStage*	
I	1 [2]
IIA	19 [39]
IIB	8 [16]
III	21 [43]
Depth of invasion (cT)	
T1	3 [6]
T2	7 [14]
T3	33 [67]
T4	1 [2]
Tx	5 [10]
Nodal involvement	
Positive	29 [59]
Negative	20 [41]
Tumor differentiation	
Well	1 [2]
Moderate	12 [28]
Poor	30 [70]
Barrett's esophagus	
Yes	31 [78]
No	9 [22]
Smoking	
Current	11 [23]
Former	28 [57]
Never	10 [20]
Recurrence	
Yes	17 [35]
No	26 [53]
Unknown	6 [12]
Patient status	
Alive with no disease	10 [20.4]
Alive disease unknown	10 [20.4]
Died	29 [59.2]

*, TNM staging—per AJCC 6th Edition. GEJ, gastroesophageal junction.

illustrated in *Figures 2,3*.

Neither pre- nor post-tx HER2 expression correlated significantly with treatment outcomes (*Table 3*). Univariate analysis did not show significant correlation of age, tumor stage and grade, smoking, presence of Barrett's esophagus, or HER2 expression with survival. However, patients with node positive disease showed a trend toward worse survival (HR =2.03; 95% CI, 0.90-4.59; P=0.08).

Discussion

Our study demonstrated that HER2 expression patterns differ from baseline specimens in several post CRT samples. Importantly, 23% of pre-tx HER2 negative (IHC 0, 1+) expressing tumors were re-classified as HER2 positive (IHC3+) after CRT. None of the pre-tx HER2 positive samples (IHC3+) became negative after CRT and 67% of these patients attained pCR or not enough tumor to examine. Based on these findings, our study suggests upregulation of this receptor expression after concurrent CRT in EGA, which is not statistically significant likely due to the relatively small sample size. Discordance in HER2 expression has been noted in the literature. Pre-clinical and clinical studies have shown correlation of HER2 overexpression with chemoradioresistance in some solid tumors (17-19). In breast cancer, studies examining HER2 expression changes after neoadjuvant chemotherapy have demonstrated conflicting results, however, more commonly showed a gain of expression (20,21). A pre-clinical study reported by Cao *et al.* demonstrated HER2 upregulation in radioresistant breast cancer cell lines via NF-κB and inhibition of HER2 re-sensitizes the resistant cell lines to radiation (19).

In the MAGIC trial, Okines and colleagues investigated the effect of HER2 expression on prognosis and benefited from perioperative chemotherapy with epirubicin, cisplatin and fluorouracil (ECF) in patients with early EGA (22). HER2 positivity (IHC3+, or IHC2+/BDISH positive) was 10.9% among all patients. HER2 expression was evaluated by IHC and brightfield dual in situ hybridization (BDISH) in tissue microarrays. Paired samples were available for 179 of 503 patients (35.6%), and 87/179 received perioperative chemotherapy. Changes in HER2 status were noted in 11 cases among which up-regulation of HER2 expression (9 cases) was more common than loss of expression (2 cases). Three patients of the 11 discordant cases received perioperative chemotherapy. The authors interpreted these changes

Table 2 Relationship between HER2 expressions in matched-pair samples

Pre CRT [n]	Post CRT [n]			
	POS [12]	IND [8]	NEG [10]	pCR or NET [19]
POS [6]	2	0	0	4
IND [12]	3	2	2	5
NEG [31]	7	6	8	10

POS, positive, HER2 IHC 3+; IND, indeterminate, HER2 IHC 2+; NEG, negative, HER2 IHC 0, 1+ by IHC. HER2, human epidermal growth factor receptor 2; CRT, chemoradiotherapy; pCR, pathologic complete response; NET, not enough tumor to examine.

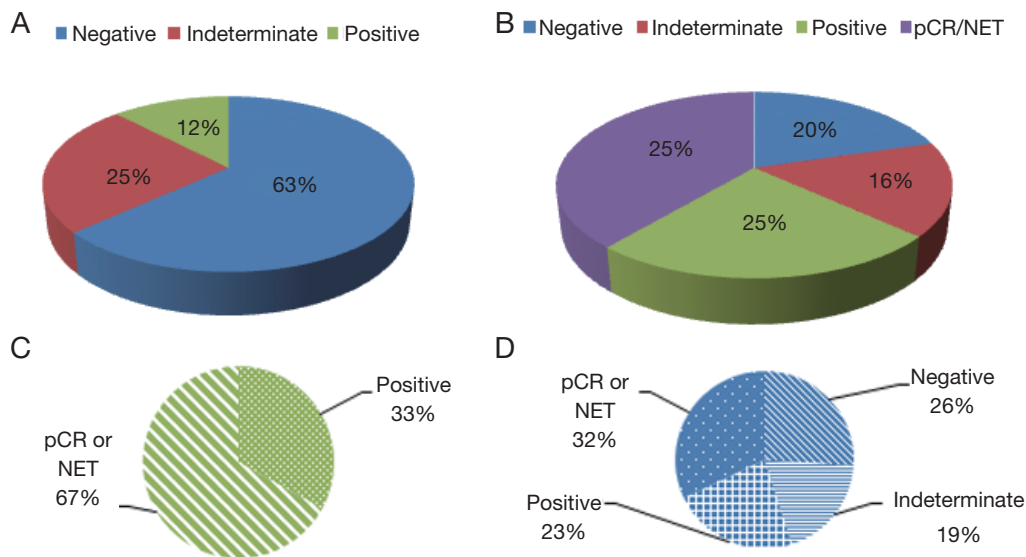


Figure 2 HER2 expressions in pre- and post-treatment specimens. (A) HER2 expression in pre-tx specimens; (B) HER2 expression in post-tx specimens; (C) post-tx HER2 expression pattern in patients with pre-tx positive HER2 expression; (D) post-tx HER2 expression pattern in patients with pre-tx negative HER2 expression. Negative, HER2 expression 0 or 1+; indeterminate, HER 2 expression 2+; positive, HER2 expression 3+ by IHC. HER2, human epidermal growth factor receptor 2; pCR, pathologic complete response; NET, not enough tumor to examine; pre-tx, pre-treatment; post-tx, post treatment.

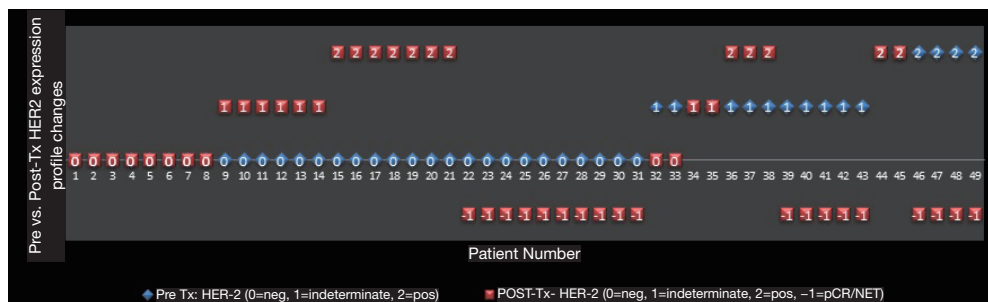


Figure 3 Changes in HER2 expression pattern in matched pre and post CRT samples. Pre-tx (blue), initial diagnostic biopsy; post-tx (red), surgical resection specimen; Neg, HER2 expression 0 or 1+; Indeterminate, HER2 expression 2+; Positive, HER2 expression 3+ by IHC. HER2, human epidermal growth factor receptor 2; CRT, chemoradiotherapy; pCR, pathologic complete response; NET, not enough tumor to examine.

Table 3 Analysis of pre- and post- treatment HER2 expression on overall survival

HER2 expression	HR	95% CI for HR	P
Pre CRT			
Negative	1.000		
Intermediate	0.584	0.234-1.460	0.2503
Positive	0.747	0.222-2.518	0.6385
Post CRT			
Negative	1.000		
pCR/NET	0.706	0.286-1.744	0.451
Intermediate	1.175	0.416-3.316	0.7607
Positive	0.367	0.112-1.203	0.0979
Association between pre- and post-tx HER2 expression	G=0.403	-0.147-0.953	0.1857

HER2, human epidermal growth factor receptor 2; CRT, chemoradiotherapy; pCR, pathologic complete response; NET, not enough tumor to examine.

in HER2 status due to heterogeneous HER2 expression rather than a true change in the biology of the disease. HER2 status was not prognostic for survival nor predicted enhanced benefit from chemotherapy (22).

Our results did not show significant association of pre-tx HER2 expression with treatment response (i.e., pCR or near pCR) or post-tx HER2 expression. However, patients with pre-tx HER2 negativity were less likely to have a pCR or near pCR compared to those patients with initial HER2 overexpression (OR =0.24; 95% CI, 0.04-1.53; P=0.16). Although this difference is not statistically different, likely due to small sample size, the trend of patients with initial positive HER2 expression who attained pCR was clearly higher than those with initial negative HER2 expression (67% *vs.* 32%). Moreover, patients with clinically node negative disease were more likely to have HER2 overexpression and attain pCR compared to those with clinical node positive disease (30% *vs.* 10%).

Consistent with previous studies, our analysis showed a non-significant trend towards worse survival in node positive patients. In addition, no significant association was found between pre- or post-tx HER2 overexpression and survival. Although some studies suggest that HER2 overexpression in EGA is associated with poor prognosis, more contemporary studies have failed to show HER2 status as an independent prognostic factor (23-25). The conflicting results are possibly due to the difference in

HER2 staining methodology and interpretation used prior to the validation of the HER2 IHC scoring system for gastric cancer described by Hoffman *et al.* in 2008 (15). Our results support the findings that HER2 overexpression in EGA is not independently associated with a poor prognosis.

In this study, HER2 overexpression was found in 12% of pre-tx samples, which is lower than that is described in the literature, however, FISH analysis was not performed on IHC2+ sample which may underestimate the determination of HER2 amplification by 10-15%. The relatively low rate of HER2 expression in pre-tx tissue from our current study is unlikely related to the smaller specimen size given the use of established criteria for scoring HER2 overexpression in biopsy samples, confirmation of adequate tumor tissue for analysis prior to IHC and consistent pathologist review of cases. Our previously reported analysis of patients with early stage EGA with Barrett's esophagus taken directly to surgery without CRT demonstrated higher HER2 overexpression (38%) (11). In support of this, Yoon *et al.* reported that HER2 positivity was significantly associated with lower tumor grade and stage and the presence of adjacent Barrett's esophagus (OR =1.8; 95% CI, 1.1-2.8; P=0.014) (26). The higher rate of HER2 overexpression in Barrett's esophagus suggests that HER2 may play an important role in the progression of dysplasia to carcinoma sequence (27-30).

Limitations of our study include relatively small sample size, lack of ISH analysis on IHC2+ cases and a short follow up. The sample size contributed to non-statistically significant results although there were trends showing possible correlation between HER2 overexpression with node negative disease and pCR. A definition of positive HER2 expression in this study included only patients with HER3+ by IHC which could underestimate the clinical impact of HER2 overexpression on clinical outcomes (31-34), but is unlikely to alter the conclusions of this study. In addition, the analysis of post-tx HER2 expression was limited to patients who had residual disease after CRT and patients with not enough tissue after CRT (near pCR) could not be examined for HER2 expression.

To date, studies that examined the expression of HER2 in matched-paired tumor samples of an individual patient (initial diagnostic primary tumor biopsy specimens and surgical tumor resections after CRT) in EGA are limited. Our study demonstrated changes in HER2 expression pattern following CRT. This has clinical implications as therapeutic selection of anti-HER2 treatments is reserved

for patients with demonstrated HER2 overexpression. Our study suggested 23% of HER2 negative patient demonstrated HER2 overexpression after CRT. Therefore, HER2 expression performed only on a pre-tx specimen could misidentify patients who became HER2 overexpressed after CRT when considering for systemic anti-HER2 therapies if they recur with metastatic disease. Therefore, HER2 testing on both pre- and post-tx specimens or re-biopsy of recurrence should be considered to avoid over or under treatment depending upon which tumor sample is chosen for molecular testing. Larger studies are needed to verify the changes in HER2 expression after neoadjuvant CRT in EGA, including the impact of whether radiotherapy or cytotoxic therapy or the combination, is accountable for the HER expression profile changes. If therapeutic HER2 inhibition during neoadjuvant therapy proves to be of clinical value, inhibition of this HER2 up-regulation may serve as an important therapeutic target for a subset of patients with HER2 negative initial assessment. Correlative analyses from the current ongoing prospective clinical trial testing the role of trastuzumab in the neoadjuvant CRT of EGA may offer further insights.

Conclusions

Changes in HER2 expressions in diagnostic biopsies compared to post-tx tumor resections are notable in this study and have the potential to impact therapeutic selection and clinical decision making in advanced disease patient management. Recurrent or residual tumor may require re-biopsy to confirm HER2 expression after neoadjuvant CRT. Consistent with the literature, our study demonstrated that baseline HER2 tumor expression does not have an impact on response to treatment nor is prognostic for survival; however, we acknowledge the limitations of a relatively small sample size. While ongoing studies are awaited to determine the potential therapeutic implications of anti-HER2 targeting in the neoadjuvant setting, the attention to the choice of sample selection for HER2 analysis for clinical decision making should be thoughtfully approached for patients who develop recurrence and are being considered for anti-HER2 therapy.

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Footnote

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

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