

## Elevated STK1p After the Second-Cycle-Chemotherapy Associated with Failure Poor Survival in I-II Stage of NscLcs- Three Cases Reports

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## 1. Abstract

**1.1. Aims:** Thymidine kinase 1 (TK1) is a precision protein molecular target for assessment of tumor proliferating rate. This investigation of 3 cases are whether the elevated STK1p after the 2-cycle **-chemotherapy** associated with failure poor survival of clinical stage I-II in non-small-cell lung carcinomas (NSCLCs).

**1.2. Main Methods:** The 3 post-operative NSCLCs with clinical stage I-II were confirmed by pathology and followed by individual chemotherapy. The serum thymidine kinase 1 protein (STK1p) was measured using the ECL dot blot assay.

**1.3. Results:** Although the 3 cases are of early clinical stage I-II, an increasing STK1p following by the second cycle of chemotherapy was observed. The elevated STK1p appeared 6-17 months early as compared the tumor metastasis by CT, and the Response Evaluation Criteria in Solid Tumors was evaluated as stable disease. The 3 patients died due to the failure poor survival.

**1.4. Significance:** The post-radical resection following by 2 cycle-chemotherapy is important critical-point time to measure STK1p if the value is increasing or decline. The elevated STK1p values can predict an earlier risk of tumor metastasis process than imaging. It can supply a strong advance advice to adapt a new individual treatment for a better prognosis in the NSCLCs in routine clinical setting.

## 2. Introduction

Lung cancer is the top cause of mortality of both men and women in the world globally, the non-small-cell lung cancer (NSCLC) as the most prevalent form [1]. According to the latest annual report of cancer registration in China, in 2015, the number of lung cancer patients was about 733.3/100,000, and the death toll was 610.2/100,000. In addition, lung cancer patients are basically diagnosed late, with an average of 5 years survival rate of  $\approx 15\%$  [2]. Currently, the clinical diagnosis of lung cancer mainly relies on chest X-ray, low dose computed tomography (CT) scans for diagnostic lung cancer. The lung cancer screening with LDCT showed a successful performance and can be benefit to reduce the high false positive results [3].

A series of serum-biomarker are available for lung diseases. The carcinoembryonic antigen (CEA) and cytokeratin 19 fragment (CYFRA21) are for routine biomarkers of lung cancer. The ROC curve analyses showed combination CEA + CA125, or combination CEA + CY211, are effective for suitable markers for lung cancer screening [4]. However, such combination still not use for the detection of early lung cancer due to low sensitivity and specificity [5] being to the CEA or AFP, such tumor-related biomarker did not directly correlation with tumor proliferating [6, 7, 8]. Therefore, the real promising candidates of biomarkers for early lung cancer screening remain a critical step in the challenging field.

Thymidine kinase 1 (TK1) was discovered in 1950s. The TK1 is one of salvage enzymes that converts deoxythymidine (dTdR) to deoxythymidine monophosphate (dTMP) and its expression in proliferating cells is closely related to cell cycle, low in G<sub>1</sub> phase, increases in S/G<sub>2</sub> phase and decreases in late G<sub>2</sub>/M phase was discovered in 1950s.. Therefore, TK1 was as a precision protein molecular target for assessment of tumor proliferating rate [9]. The concentration of serum thymidine kinase (STK1p) in malignant tumor cells is proportional to cell proliferation rate [10-11]. In normal human serum, the STK1p is almost undetectable [12-14]. Based on an enhanced chemiluminescent dot blot assay (Sino-Swed Tongkang Bio-Tech, Inc., Shenzhen, China , [www.sstkbio-tech.com](http://www.sstkbio-tech.com)), the STK1p could be an useful tool in *precise medicine* for prognosis of pre- and malignant cancer, both in oncology clinics and in health screening [9]. The STK1p increased significantly with T-values and with clinical stage and invasiveness. A meta-analysis [15] study on STK1p in early/middle stage of lung carcinoma patients (n=2,107) showed that the expressions of STK1p increased significantly in the following manner: healthy control group (n=1,694) < benign group (n=272) < carcinoma group (p<0.00001), indicating that the half-life of STK1p can monitor the response to the surgery in lung cancer patients, and STK1p could be also used for early detection and distinguish between benign and malignant tumours [15]. Previous reported that STK1p can be used as a reliable factor for prognosis, survival and recurrence in breast cancers [16, 17]. The multivariate COX analysis showed that the STK1p was an independent prognostic factor [16]. A prospective study of local advanced breast cancer patients with neo-adjuvant treatment showed that high STK1p values were positive correlated to clinical stage, tumor size, recurrence and survival [17]. The STK1p can also be used as a prognostic factor of chemotherapy-treated non-Hodgkin's lymphoma patients [18]. The TK1-activity assay (DiviTum, Biovica, Sweden) indicated that the elevated serum TK activity was an independent prognostic factor for survivals of lung cancer. It was found that an increase in TK-activity after the first and second cycle of chemotherapy was significantly associated with treatment failure and poor overall survival [19]. An recently investigation in 127 NSCLCs following a nonrandomized individual adapted treatment demonstrated that low STK1p value correlated significantly to favorable survival in early-middle-stage (I–IIIA) NSCLCs and the COX multi-variate analysis showed that STK1p was an independent prognostic biomarker [5]. Although a series of literature reviewed that the low score STK1p correlated significantly to favorable survival in early-/middle-stage (I–IIIA) of NSCLCs. In this 3 cases report that we further investigated whether the elevated STK1p after the 2-cycle -chemotherapy associated with failure poor survival of clinical stage I-II in non-small-cell lung carcinomas (NSCLCs).

### 3. Three Cases with Clinical Stage I-II and Died of Long-Term Ineffective Chemotherapy

Based on the ROC analysis, an optimal cut-off STK1p value was [clinicsofsurgery.com](http://www.clinicsofsurgery.com)

set to 0.6 pM after 2-cycle-chemotherapy of NSCLCs and divided into two groups: low STK1p ( $\leq 0.6$  p.m.) and elevated STK1p ( $\geq 0.6$  p.m.) [5]. The STK1p assay was performed on a commercial kit of enhanced chemiluminescence (ECL) dot blot assay to measure the concentration of STK1p as described by Sino-Swed Tong Kang Bio-Tech, Inc., Shenzhen, China (<http://www.sstkbio-tech.com>). The serum samples were taken on the day one-month post-operative or before chemo-treatment and after 2-cycle-chemo-treatment pateints. The serum samples stored at -20°C until analysis. The STK1p values were at least twice a quality duplicate detection.

**3.1. Case 1.** A woman with 53 years. The patient had no family history of hereditary tumor diseases and personal history of other tumor diseases.

1. Discovery of lung cancer. She had multiple small nodules in right middle lung irregularly by CT examination and then radical resection (RR) of upper right lung cancer in 2015-05-04. A histopathological examination was evaluated as NSCLC (mucinous adenocarcinoma, clinical stage IA, tumor size 2.8\*3.8. and grade G2). The STK1p was 0.27 pM one month after RR.
2. Chemotherapy. The chemotherapy was carried out using Buck Jane (0.7 mg)+Carboplatin (0.45 mg). However, the STK1 increased to 0.82 pM after 2-cycle-post-chemothrapy, and RECIST (Response Evaluation Criteria in Solid Tumors) was evaluated as Stable disease (SD). The CT examination showed that the multiple small asymptomatic malignant lung nodules expanded to in both lungs in 2016/01/26.
3. Chemotherapy continually. An 8 cycles of Dopafi (100mg) in 2016/02/05-2016/ /08/04. In the meantime, the CT examination showed that the multiple small asymptomatic malignant lung nodules in both lungs and slightly larger in 2016-07-06 and December 26-12-2016. The Dr. considered the tumor in metastasis.
4. She dead 20 months after the continually ineffective treatment. The total time of follow up was 37 months (Figure 1A).

**3.2. Case 2.** Man, 78 years. The patient had no family history of hereditary tumor diseases and personal history of other tumor diseases.

1. Discovery of lung cancer. He had a right upper adenocarcinoma lung cancer under thoracoscopy by CT examination in 2016-05-29, and RR of upper right lung cancer, was performed. Pathology conformed as NSCLC, clinical stage IIA, tumor size was of 5.5\*2 cm and grade G3. The STK1p was 0.1 pM one month after RR.
2. Maintenance therapy. His dr. used the maintenance therapy with praline plus carboplatin according to the patient individual's physical condition. The STK1p was aggressively increased to 5.29 pM and RECIST was assessed as SD

two months after the 2-cycle chemotherapy showed 2-cycle-post-chemotherapy.

3. The maintenance therapy continued from 2016-07-29 to 2018-09-22. The CT examination in 07-12-2017 found that the tumor was metastasizing to hydrothorax (partially encapsulated) and on both sides of the chest and fibrous lesions in the left upper lung, and also observed the liver cysts and left lateral adrenal branch hyperplasia.

4. The patient dead 14 months after the ineffective chemotherapy. The total time of follow up was 31 months (Figure.1B).

**3.3. Case 3.** Man, 78 years. The patient had no family history of hereditary tumor diseases and personal history of other tumor diseases.

1. Neo-chemotherapy. CT examination found that he had in left upper lobe lung cancer, clinical stage IB, tumor size was of 3.5\*3.0\*1.5 cm. the pathology diagnosed as NSCLC and grade G3. The first, a neo-chemotherapy with Lipitin 270 mg+nedaplatin 120mg, 6 cycles was used in 2013-05-21 to 2013-10-22. It found that no nodules in left upper lobe.

2. Radical resection (RR). Unfortunately, the patient had a lower right lung cancer were seen by CT in 2013-12-30. The RR of lower right lung cancer was done in 2014-08-01; The

post-operative STK1p value was litter high (0.88 pM) one months after RR as compared the cut off STK1p value (0.6 pM);

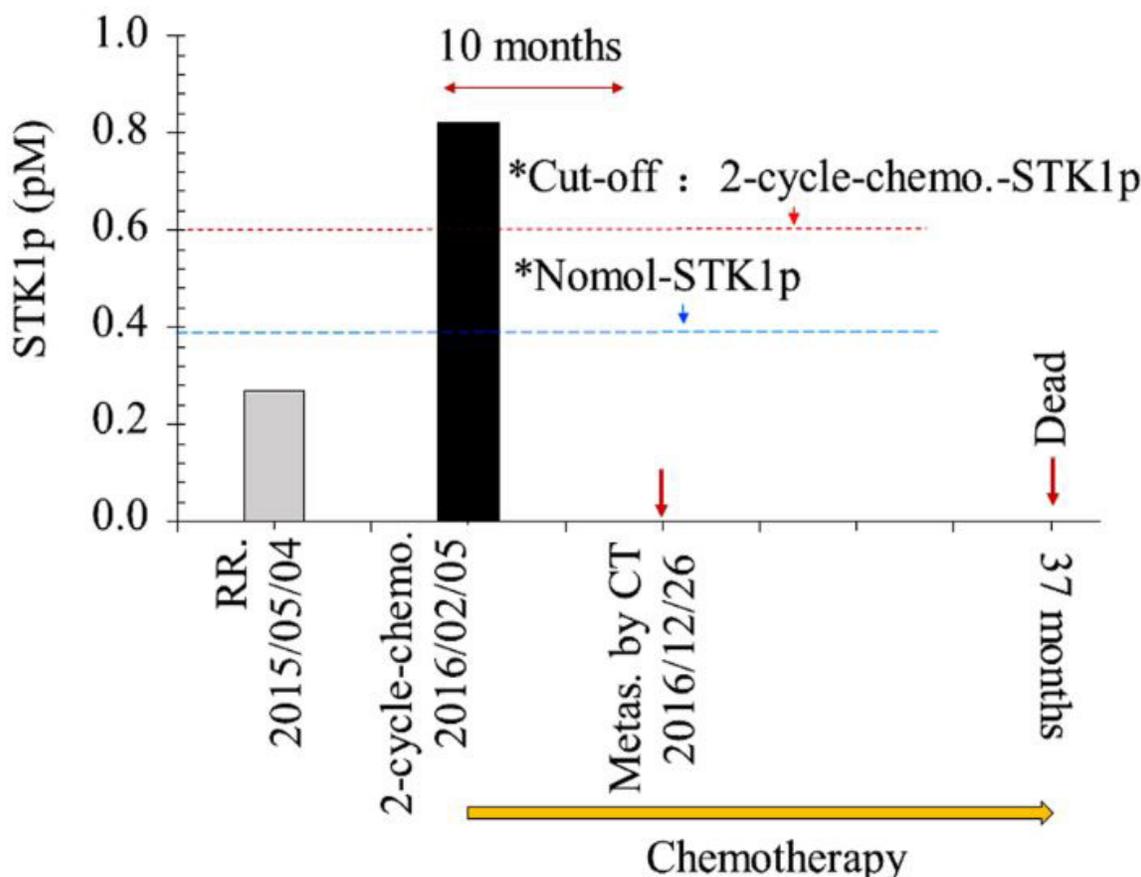
3. Chemotherapy. Of 3 cycles with Dopafi 120 mg + carbo-platin 0.4 mg was performed in 2014-11-26 to 2015-01-27. The STK1p was of 0.93 pM, no decline, and the RECIST was evaluated as SD after the 2 cycle- chemotherapy. The PET-CT examination in 2015-02-26 showed that the third and fourth posterior ribs were destroyed and local pleura was thickened and accompanied with increasing glucose metabolism. It considered tumor in metastasizing;

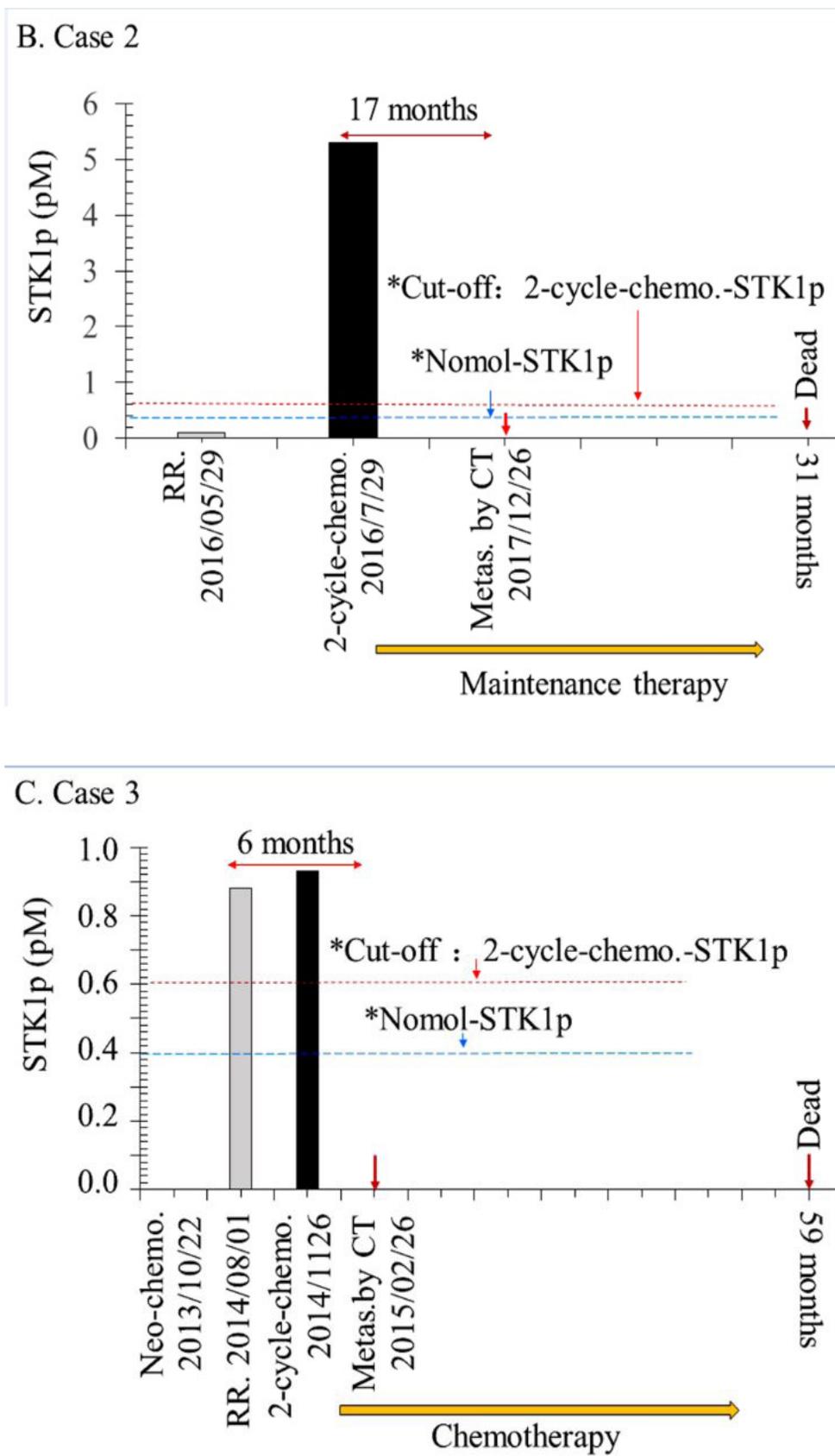
4. Continued chemotherapy. Chemotherapies arranged: 1) two cycles of gemcitabine 1.6 d1,8, in 2015-03-03 to 2015-05-03. 2) two cycles of gemcitabine 1.4d1,8+ nedaplatin 100mg in 2016-03-26 ton2016-05-03. The CT examination observed tumor metastasis in 2016-03-10, including the multiple asymptomatic malignant lung nodules, multiple right ribs and thoracic vertebrae.

5. Continued chemotherapy. Two cycles with vinom 40mg d1, 30mg d5 + vinom 100mg was done in 2016-06-01 to2016-07-23;

6. The patient dead 15 months after the long-term ineffective treatment. The total time of follow up was 59 months (Figure 1C).

**A. Case1**





**Figure 1.** The elevated STK1p after the second cycle of chemotherapy associated with poor survival in 3 NSCLCs. \*2-cycle-chemo-STK1p: The optimal cut-off STK1p value was set to 0.6 pM post-2-cycle chemotherapy of NSCLCs [5]; \*Normol-STK1p: The Normol-STK1p values were excluded all tumor diseases, moderate/severe proliferative diseases, virus-infectious, severe inflammation diseases, also including the obese, un-normal values of blood, urea or fecal, it may containing some proliferating/chronic diseases in relation with the tumor diseases. The mean and median STK1p values of 0.36-0.38 and 0.37-38 pM, respectively [9, 20].

#### 4. Discussion

A recently investigation explored that the beginning 2 cycles of chemotherapy treatment was an effectiveness time point. The cut off of STK1p (0.6 pM) is useful for a monitoring factor to assess in prognosis of NSCLCs [5].

In this case's study, an elevated STK1p was detected after the 2-cycle-chemotherapy (STK1p=0.82 pM) as compared the cut off of STK1p value (0.6 pM) in case 1 (Figure 1A). The STK1p presented early 10 months as compared the tumor metastasis by CT. In case 2, the STK1p was intensively increased to 5.29 pM after the 2-cycle-chemotherapy, it was 17 months early as compared CT (Figure 1B). In the case 3, the post-operative STK1p value was already litter high (0.88 pM) and did not decline after the 2-cycle-chemotherapy (STK1p=0.93 pM) (Figure 1C), the elevated STK1p appeared 6 months early as compared the tumor metastasis by CT. All of 3 cases were assessed as SD score after the 2-cycle-chemotherapy, it implied that RR+ Individual chemotherapy did not effectiveness and the continually chemotherapy did not give the patient any benefit.

Previous studies published on the prognosis of breast cancer patients proved that STK1p level increased 3 months [16] -18 months [17] early than the discovery of the visible cancer by imaging, it supports our the study. As well as we know that cancer is a chronic disease due to abnormal growth. Every cancer arises from a precancer, although not all precancers lead to cancer. Over time, the number of precancer cells must be abnormally proliferate, and achieve excess numbers [21]. If all preconcert were detected early, the risk to further develop into malignancy would be reduced extensively. For ex. a case report showed that a 72 years old woman was diagnosed as preconcert (ground-glass opacity) in lung while the STK1p value was high of 3.9 pM, however, 3 lung tumor-related markers were negative (CEA (-) Cyfra19 (-) NSE (-)). The STK1p continued to rise to above 10-13 pM, while CT and 3 markers were still normal. The patient underwent right lobectomy and systemic lymphadenectomy under thoracoscopy 4 months after discovery of lung cancer by CT. Pathology investigation confirmed the presence of lung adenocarcinoma (moderate differentiation). The STK1p value declined to a low level (1.3 pM) one month after surgery, no metastasis or recurrence by CT assessment so far [20].

We suggest that it is very important is the elevated STK1p values predict an earlier risk of tumor metastasis than imaging. It can be give a strong advance advice how to improve the individual adapted treatment for a better prognosis in the patient's further.

More recently, the immunotherapy revolution, specifically, the development of immune checkpoint inhibitors (ICIs), such as PD-L1 expression can improve treatment at advantage lung cancer and lead to clinical benefit. A meta-analysis revealed that immunotherapy plus chemotherapy is an effective option as a first-line

treatment for lung cancer (n= 4,887). The NSCLC patients administered combined therapy exhibited higher PFS and OS than patients treated with chemotherapy alone (P<0.001). Moreover, as the expression of PD-L1 increased, the PFS and OS benefits were more significantly [22]. However, it is challenging to obtain serial biopsies during a course of therapy to monitor the PD-L1 expression in intrathoracic tumors. An isolation of CTCs from peripheral blood can provide a minimally invasive method to monitor PD-L1 expression on tumor cells over time [23], the protocol was complicated and time-consuming. We suggest to use STK1p assay for monitoring during the time of treatment shall be simple, and low cost.

Precision molecular medicine with many biomarker tests either available or under development for several lung diseases. So far, few biomarkers are in early widespread clinical use. It is mandatory to show the performance of these biomarkers for the monitoring and prognosis in early lung tumors, such, precancers (ground-glass opacity preaches), malignant tumor (asymptomatic malignant lung nodules, invisible small tumor or early localized tumors) [21], which are potential to use monitoring and prognosis of personalized targeted therapies. TK1 is one of a precise molecule target for monitoring and prognostic factor, specifically, for early detection of growth rate of an invisible small tumors. STK1p assay is also noninvasive and low cost [9]. We proposal that STK1p combined with imaging or another biomarkers, might be a more reliable assessment for developing of premalignancy or diseases associated with the risk process of lung cancer. There would be no or very limited lung cancer development in the future if the premalignancy or early tumours were treated in time.

#### 5. Conclusion

STK1p value has benefit to early discovery premalignancy or investable tumors and give Dr's advice to adapt a reasonable treatment in time. The post-radical resection following by 2 cycle-chemotherapy is important critical-point time to measure STK1p if the value is increasing or decline. The elevated STK1p values predict an earlier risk of tumor metastasis than imaging can be give a strong advance advice how to adapt a new individual treatment for a better prognosis in the NSCLCs and also another cancer patient's further.

#### 6. Ethical Approval

This study is in accordance with the ethical standards in the 1964 Declaration of Helsinki and its later amendments. The Ethical approval supervised by the national ethical guidelines of China and approved by the Peking 301 Chinese PLA General Hospital's Ethics Committee (Ethic No. S2015-036-01).

#### 7. Financial & Competing Interests Disclosure

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