Thymidine kinase 1 in serum for prognosis, an investigation on routine individual adapted treatment of non-small cell lung carcinoma.

Zhongcheng Li
Affiliated Hospital of Suzhou University
Guoqing Zhang
Peking 301 Chinese PLA General Hospital
Jin Li (li728@163.com)
Shenzhen Precision Medicine Institute https://orcid.org/0000-0002-9641-9492
Hongbo Ma
Shenzhen Precision Medicine Institute
Zhenxin Wang
Affiliated Hospital of suzhou University
Shunchang Jiao
Affiliated Hospital of Suzhou University
Yi Hu
Peking 301 Chinese PLA General Hospital
Shengjie Sun
Affiliated Hospital of Suzhou University
Liangliang Wu
Affiliated Hospital of Suzhou University
Ji Zhou
Shenzhen Precision Medicine Institute
Ellen He
Shenzhen Precision Medicine Institute
Sven Skog
Shenzhen Precision Medicine Institute

Research article

Keywords: lung cancer, non-small cell lung cancer (NSCLC), thymidine kinase 1 (TK1), serum thymidine kinase 1 protein (STK1p), radical resection (RR), individual-chemotherapy

Posted Date: September 4th, 2019

DOI: https://doi.org/10.21203/rs.2.10552/v1

Thymidine kinase 1 in serum for prognosis, an investigation on routine individual adapted treatment of non-small cell lung carcinoma.

Zhongcheng Li[1], Guoqing Zhang[2], Jin Li[3], Hongbo Ma[3], Zhenxin Wang[4], Shunchang Jiao[4], Yi Hu, Shengjie Sun[4], Liangliang Wu[4] , Ji Zhou [3] , Ellen He[3], Sven Skog[3]

[1] Department of Thoracic Surgery, affiliated Hospital of Suzhou University, Shizi Street 188, Gusu District, Suzhou 215000, China
[2] Department of Oncology, Peking 301 Chinese PLA General Hospital, Fuxing Road 28, Haidian District, Beijing 100853, China
[3] Department of Medicine, Shenzhen Precision Medicine Institute, Building A2/19F, Kexing Science Park, C. 1st Avenue, Gaoxin, Shenzhen 518057, China
[4]Department of Oncology, affiliated Hospital of Suzhou University, Shizi Street 188, Gusu District, Suzhou 215000, China

Zhong-Cheng Li (MD, prof.), E-mail address: "13862055565@163.com, Tel: +86 13862055565.

Guo-Ging Zhang (PhD, prof.), E-mail address: zh13571113@tom.com, Tel: +86 13810996462.

Jin Li (PhD., MD., prof.), E-mail address: li728@163.com, Tel: +86 13509676365.

Hong-Bo Ma (MD., Researcher), E-mail address: jessica.ma@sstkbiotech.com, jessica.ma@sstkbiotech.com, Tel: +86 18565766376.

Zhen-Xin Wang (MD., prof.), zhenxw316@163.com, Tel: +86 13806132255.

Shu-Chang Jiao (MD.), E-mail address: Jiaosc@vip.sina.com, Tel: +86 1381380677.

Yi Hu (MD.), E-mail address: huyi0401@aliyun.com, Tel: +86 13911031186.

Sheng-Jie Sun (MD.), E-mail address: sunsj301@sina.com, Tel: +86 13810228740.

Liang-Liang Wu (MD.), E-mail address: wuliang080228@163.com, Tel: +86 15901183094.

Ji Zhou (MS., prof.), E-mail address: kevin.zhou@sstkbiotech.com, Tel: +86 18675555533.

Ellen He (PhD., prof.), E-mail address: ellen.he@sstkbiotech.com, Tel: +46 738980658.

Sven Skog (PhD., prof.), E-mail address: svenisak@icloud.com, Tel: +46 736407152.

Corresponding author

*Jin Li, Department of Medicine, Shenzhen Precision Medicine Institute, Building A2/19F, Kexing Science Park, C. 1st Avenue, Gaoxin, Shenzhen 518057, China.
e-mail address: li728@163.com, Tel: +86 13509676365.

Zhongcheng Li[1] and Guoqing Zhang[2] contributed equally as co-first authors.

Abstract

Background: Thymidine kinase 1 (TK1) is a key enzyme involved in DNA synthesis. The aim is to assess the prognostic significance of serum TK1 protein concentration (STK1p) and its role in monitoring of individual customized therapy in non-small cell lung carcinoma (NSCLC) patients in routine clinical setting.

Methods: A prospective study of 129 NSCLC patients was confirmed by imager, /pathology and treated by radical resection (RR) combined with individual-chemotherapy or individual-chemotherapy alone, in 2010 to 2017. The STK1p was measured using an ECL dot blot assay in sera of patients.

Results: Comparisons between 2-cycle-post-individual-chemotherapy and pre-individual-chemotherapy showed that STK1p was significantly associated with treatment effect ($p<0.001$), and the STK1p low-group correlated significantly to the early/middle clinical stage, as well as the treatment with RR+ individual-chemotherapy ($p < 0.05$). A significantly poor overall survival (OS) was found in elevated-risk STK1p vs. low-risk STK1p values ($p=0.016$), in advantage clinical stage vs. early/middle clinical stage ($p=0.004$), in SCC patients compare vs. AC patients ($p=0.003$) and in

chemotherapy alone vs. RR combined with individual-chemotherapy (p=0.001). The multivariate analysis showed that STK1p (hazard risk=2.295, p=0.010), and RR combined with individual-chemotherapy (hazard risk=3.04, p=0.0001), were independently survival factors.

Conclusions: STK1p correlates significantly to survival and is an independent multivariate prognostic factor in NSCLC patients. STK1p as a low-cost assay, is a useful tool to combine with imager for a rational approach to increase the efficacy in early detection of tumor in lung cancer screening and assessment of individual adjusted therapy in NSCLC patients.

Trial registration: No trial registration since this is not a case-control-study, but based on routine clinical work.

Keywords lung cancer, non-small cell lung cancer (NSCLC), thymidine kinase 1 (TK1), serum thymidine kinase 1 protein (STK1p), radical resection (RR), individual-chemotherapy

Background

Lung cancer is the top cause of mortality of both men and women in the world. Often, the symptoms of lung cancer do not appear until the cancer has been advanced, thus making the early diagnosis difficult. The 5-year survival rate for all people with lung cancer is 19% [1]. According to the 2015 report of cancer registration in China, the average 5 years survival rate was only 15% [2]. Although the false-positive low-dose computed tomography (LDCT) results in overloading of existing lung cancer clinics and multidisciplinary teams, a two large randomized controlled trials showed that early detection with LDCT and early-stage treatment can reduce mortality [3]. The USA Cancer statistics in 2019 reported that the decline in cancer mortality over the past 2 decades was primarily the result of steady reductions in smoking and advances in early detection and treatment, which were reflected in the rapid declines for the 4 major cancers (lung, breast, prostate, and colorectum) [1]. It proposals that complementation of LDCT examination with blood-based biomarkers is a rational approach to increase the efficacy for detection and treatment of early lung cancer in screening programs [4, 5, 6].

A series of serum/plasma biomarkers for routine setting are available for lung diseases, such as carcinoembryonic antigen (CEA) and cytokeratin 19 fragment (CYFRA21) [7]. Combination of CEA + CA125, or CEA + CY211 were effective for identifying suitable markers for lung cancer in screening [8]. The minimally invasive method of PD-L1 expression on circulating tumor cells (CTCs) can be performed instead of biopsy [9].

Thymidine kinase 1 (TK1) converts deoxythymidine (dTdR) to deoxythymidine monophosphate (dTMP) and its expression is closely related to cell cycle since 1950s [10]. TK1 is a precision protein molecular target for assessment of tumor proliferating rate [11, 12, 13, 14, 15, 16]. The level of serum TK activity [17, 18, 19, 20, 21] and serum TK1 protein (STK1p) concentration [18, 19, 20, 21] in malignant tumor patients is proportional to tumor proliferation, almost undetectable in normal human serum has been reviewed. We summered previous studies that STK1p could be a reliable tool in precise medicine for risk assessment of pre-malignancy and small invisible-malignancy in health screening, and for monitoring therapeutic effect in 14 type of tumors and prognostic factors of breast, non-Hodgkin's lymphoma and colorectal cancers in routing clinical oncology [22].

A meta-analysis of lung diseases showed that the expression of STK1p increased significantly in the following manner: healthy control group (n=1,694) < benign group (n=268) < carcinoma group (n=2,107) ($p<0.00001$) [23]. Similar results were obtained when measured the serum TK-activity and presented an independent prognostic factor [24]. The serum thymidine kinase 1 was an early detection marker in lung cancer using ELISA assay [21]. The ROC curve analyses showed that the STK1p combined with

CEA, CYFRA21-1 and NSE was significantly higher than that of each biomarker alone ($p < 0.0001$) in lung cancer [25].

Most standard treatment protocols for lung cancer include cytotoxic agents, which are potential modulators of STK1 [24]. In this prospective study, we investigated if STK1p could be used to assist individual customized chemotherapy treatment efficacy in NSCLC patients (n=129) with early/middle and advantage stage.

2. Methods

2.1. Patients

A prospective cooperative study of total 129 patients with non-small-cell-lung-carcinoma (NSCLC) was performed at the Department of Oncology, Affiliated Hospital of Suzhou University, JiangSu, China (stage I-IIB, n=25; stage IIIA, n=9; stage IIIB-IV, n=51; 8 cases unclear stages; from March 2011 to December 2017) and at the Department of Oncology of Chinese PLA General Hospital, China (stage IIIB-IV, n=36; from February 2014 to Dec. 2017; validated as wild type EGFR). All medical information's are summarized in table 1, including age, sex, clinical stage, pathological type (adenocarcinoma (AC), squamous cell carcinoma (SCC)) and type of treatments. In incomplete registration, 33 patients with smoking history and 55 patients without smoking history.

This is not a case-control-study, but based on routine clinical work, where serum samples were collected from time to time, pre-chemotherapy and after 2 cycle-chemotherapy (table 1 and table 2), depending on patient's medical situation, for more details, see section "Treatment" below. According to previous publications it was found that some of patients with advanced stage (IIIB-IV) had lower STK1 levels compared to patients of early/ middle stage. Therefore, the STK1 values pre-chemotherapy were not used for statistical assessment of prognosis of overall survival (OS) and COX analysis.

Serum samples from 400 healthy individuals (mean age 60.6±7.8, range 51-87 years) who had no evidence of contagious or cancerous disease were also collected between January 2016 and December 2017 and analyzed for STK1.

This study is in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. All procedures in the study involving participants of two hospitals (Affiliated Hospital of Suzhou University, JiangSu, China and Peking 301 General Hospital, China) were 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).

2.2. STK1p in relation to cycles of chemotherapy

The STK1p value 3-month-post-treatment was a best time to evaluate the prognosis of recurrence risk in breast cancer patients, which has been recommended [26]. In a previous study on NSCLC patients it was found that the TK-activity in serum (STK-activity) after 1-2 cycles of chemotherapy correlated to survival [24]. In order to find a suitable cycle chemotherapy when STK1p expression was significantly correlated to survival rate. We performed an initially testing in advantage NSCLC patients before and 2, 4, 6 and 8 cycles after chemotherapy in the oncology department of the First Affiliated Hospital of Suzhou University. We used CYFRA21-1 as a control.
The results are summarized in table 2. We found that the STK1p values correlated significantly to survival after 2-cycle individual-chemotherapy ($p< 0.05$), but not after 4, 6 or 8 individual-chemotherapy cycles. Therefore, we used the STK1p values after 2 chemotherapy-cycles in our clinical study presented here.

2.3. Treatment

According to the NCCN Guidelines for patientsTM of Non-Smal Cell Lung Cancer version 2.2010, NCCN. Org. (www.nccn.org), in this study, particular attention was paid to the agreement between each individual proposal. Each patient had an individual-chemotherapy program depending on the individual physiological and clinical symptoms, including the TNM clinical staging and the pathological type of the tumor. Briefly as follows: 1. All patients at early clinical stage (clinical I-II)/middle (IIIA) and 20 patients at advantage clinical stage (IIIB- IV) received radical resection (RR) at different time before chemotherapy; 2. The 66 patients with advantage clinical stage (IIIB-IV) received chemotherapy only (table 1); 3. According to the instruction of the Non-Smal Cell Lung Cancer version 2.2010, the patient was individual-chemotherapy with different cytotoxic agent and was given intravenous injection for 2 to 6, or more cycles. For example, docetaxel + cisplatin, or gemcitabine + cisplatin, or pemetrexed disodium + cisplatin or pemetrexed disodium + nedaplatin) or pemetrexed disodium + carboplatin or gemcitabine + nedaplatin or doxorubicin + carboplatin or vinorelbine + nedaplatin, etc.; 4. The treatment effect was evaluated by STK1p detected during the period of pre-chemotherapy and after 2 cycles chemotherapy. The chemotherapy effect was also evaluated according to WHO's RECIST (Response Evaluation Criteria in Solid Tumors) using advanced imaging, such as CT and abdominal ultrasound, MRI. ECT (electro-chemotherapy) was used for brain metastases.

2.4. Follow up

The primary endpoint was progression-free survival (PFS) and the secondary endpoint was overall survival (OS), during the follow-up period of 10-58 months. The criteria included was STK1p levels, age, sex, clinical stage, pathological type (AC and SCC), and RECIST. The criteria excluded was patient's ID, phone number, home

address, data when visiting/left the hospital, routine blood and urine test results. The patients or their families were contacted by phone every month to check if they remained alive. The survival assessment was performed only in 101 patients, due to limited access to 28 patients once they left the hospitals.

2.5. STK1p assay

STK1p concentrations were determined using a commercial kit (cat. no. 24/48T based on an enhanced chemiluminescent dot blot assay as described by the manufacturer Sino-Swed Tongkang Bio-Tech, Inc., Shenzhen, China, www.sstkbiotech.com). The collection of serum was performed between 7.30 am-10.00 am after the patients had fasted for 12-14 h. The samples were analyzed within 3 hrs. of whole blood centrifugation (400 x g, 22-25˚C, 8-10 min). If not analyzed immediately, the samples were stored at -20˚C for a maximum of 4 weeks. Although the serum samples were analyzed within 4 weeks, the serum may be stored for ≥1 year at -20˚C and still maintain TK1 in good condition. Samples comprising 3 µl serum were directly applied to nitrocellulose membranes in duplicate. Serum samples were then probed with chicken anti-human TK1 IgY polyclonal antibody (dilution 1:500) raised against a peptide (residue 195-225 of human TK1, amino acid sequence: GQPAG PDNKE NCPVP GKPGE AVAAR KLFAPQ; Multiple Peptide Systems, San Diego, CA, USA). The TK1 calibrators were dotted onto membranes at different concentrations (2.2, 6.6 and 20 pM) as an extrapolated standard. The intensity of spots on the membrane was determined using a CIS-l Imaging System (Sino-Swed Tongkang Bio-Tech Inc., Shenzhen, China). The STK1p value of each spot was calculated and expressed as pM, based on the TK1 calibrators.

2.6. Statistical analysis

SPSS version 19 was utilized for statistical analysis (IBM Corp., Armonk, NY, USA). The Kaplan–Meier method was used for overall survival analysis. Cox proportional hazards regression was used for univariate/multivariate analysis. Receiver operating characteristic (ROC) analysis was performed using the ROC program. The STK1p among the different patient groups before and after 2-cycle chemotherapy used paired–samples T-test, and one-way Anova for comparison patient group and control group, respectively. Chi-square test was used for the comparison of STK1p in related with the different clinical characteristics. The ROC curve was calculated using the ROC program. $P < 0.05$ was considered to indicate a statistically significant value.

3. **Results**

3.1. STK1p levels before and after 2 cycle-chemotherapy

The patients were divided into four groups: total number of patients of all clinical stages (n=129), follow-up patients of stage I-IIIB (n=34), follow-up patients of advance stage (IIIB+IV, n=71) and follow-up patients of all stages (n=101) (Table 3).

The pre-treatment mean STK1p value of malignant patient's is normally above 2.0 pM and the STK1p of healthy people is less than 0.5 pM (Chen et al., 2011, 2018 Chen). In this study, however, the treatment situation was complex. Before start of the type of individual-chemotherapy we focused on in this study, the all patients with clinical early/middle stage and 30.3% patients with clinical advantage stage had already received surgery and in some cases also traditional Chinese medicine treatment, reducing the mean STK1p value to about 1.0 pM and continue to significantly decrease during the individual-chemotherapy to about 0.7 pM (table 3), but still significantly higher than the normal control of 0.38 pM (table 3).

The STK1p-individual values decreased by about 30%, except for the stage I-IIIA patient group because STK1p was already decline due to radical surgery. Individual examination shows that in about 40% of the patients the STK1p value decreased by 50% or more at the end of the chemotherapy (table 4), indicating an efficient treatment, including patients with advanced stage. It should be noted that the mean STK1p value is higher in the advance stage (IIIB-IV, 1.15pM) group compared to the early stage (I-IIIA, 0.79 pM) group, although not significant different (p=0.157) (Table 5).

3.2. The number of STK1p low- and high-risk patients in relation to clinical characteristics 2-cycle-post individual-chemotherapy

To be able to identify low and high-risk groups of patients a suitable threshold level is needed to set up. This can be done by performing a Receiver Operation Characteristic (ROC) statistical analysis. The ROC test of STK1p was performed between 2 cycle-post individual-chemotherapy's patients (n = 101) and normal healthy persons (n=400), excluding a series of tumor diseases and infection diseases. The ROC analysis showed a suitable STK1p's threshold value of 0.6 pM. Based on this threshold value we divided the tumor patients into two group: STK1≤ 0.6 pM and STK1＞0.6 pM as low and high risk of prognostic assessment, respectively.

By chi-square test, the STK1p low risk and elevated risk groups 2-cycle-post-individual-chemotherapy compared with parameters of clinical characteristics are showed in table 5. The STK1p expression correlated significantly to the stages (I-IIIA vs. advanced IIIB+IV), the treatment with RR+ individual-chemotherapy vs. individual-chemotherapy alone ($p < 0.05$), but did not significantly correlated to pathology type, age, sex and RECIST, and the treatment regimen of advanced stage (IIIB+IV) only ($p > 0.05$). The STK1p low risk and elevated risk groups did not significantly correlated to smoking history ($p = 0.207$, data not show)

3.4. The overall survival (OS) rate

The total mortality was 39.6%. The patient at early/middle and at advantage stage was 11.5% and 49.3%, respectively.

The overall survival (OS) rate in relation to stage between low risk and high risk of STK1p group, I-IIIA and IIIB-IV, pathological type between AC and SCC and individual therapy program with RR+ individual-chemotherapy compared with individual-chemotherapy alone are shown in figure 1. There was a significantly higher OS in patients of low risk compared to patients of high risk (p=0.015) (Fig1 A), of early/middle stages compare to advanced stage (p=0.011) (Fig. 1 B), of AC patient compare to SCC patients (p=0.028) (Fig. C) and of patients receiving RR+ individual-chemotherapy compared with individual-chemotherapy alone (Fig. 1 D) (p=0.001), but no correlations with age (p = 0.132), sex (p = 0.097) or RECIST (p = 0.432) (data not shown). We also analyzed 24 cases with early/middle stage alone (Fig. 1 E). The OS rate was significantly (p=0.002) higher in patients with low risk STK1p values compare to high risk STK1p, indicate that STK1p expression correlates with tumor proliferation at the early/middle stage. The results of cases with advanced stage (IIIB+IV) only showed a significantly higher OS in patients with AC patient compare to SCC patients (Fig. G, p=0.02), but not between low and high risk groups (Fig.1 F, p = 0.114) or between RR+individual-chemotherapy and individual-chemotherapy alone (Fig.1 H, p=0.13).

3.5. Prognosis (COX regression analysis)

The univariate analysis showed statistically significant of OS prognosis association parameters STK1p expression (p = 0.018), stage (p = 0.008), pathology type (p = 0.004), treatment ((RR+ individual-chemotherapy vs. individual-chemotherapy alone, p = 0.002) and sex (p =0.099), but not with age (p= 0.596) and RECIST (p = 0.729) (Table 6).

The multivariate analysis showed that the treatment (RR+ individual-chemotherapy vs. individual-chemotherapy alone, p = 0.001) and the STK1p expression (p = 0.01) are independent prognostic factors for OS, while stage and pathology type and sex were not (p> 0.05, table 6).

3.6. Cases analysis of early stage dead patients

Although the patients of early stages (I-IIIA) showed a good prognosis with high OS rate (table 1, figure 2B and G), three patients died within 14-20 months after end of the treatment. The STK1p values after 2-cycle individual-chemotherapy did not returned to normal values (<0.6 pM), and were characterized as RECIST SD after 2

cycles. By the CT examination, the main reason to the death might be development of metastasis. Here we reported case analysis of the three patients.

Case 1. A 65 years old woman with a mucinous adenocarcinoma, clinical stage IIA, grade G2, tumor size 4.0*2.3. She was found to have right middle lung irregularity after operation. In 2016-01-26 she was found to have multiple small nodules in both lungs by CT observation, and was considered for metastasis. She immediately started chemotherapy with pemetrexed disodium+ carboplatin for 4 cycles from 2016-01-27 to 2016-05-04. The CT re-examination in 2016-04-01 showed multiple small asymptomatic malignant lung nodules in both lungs, similar to the observation in 2016-01-26. Therefore, she continued to treatment with docetaxe from 2016-05-04 to 2016-08-04. She was re-examined by CT in 2016-07-06 and in 2016-12-26, showing that the multiple small asymptomatic malignant lung nodules were still in both lungs and slightly larger than found in 2016-07-06, and considered for metastasis. She dead 20 months after the ineffective treatment results. The STK1p pre-chemotherapy value was 0.27 pM and 2-cycle-post-chemothyrapy increased to 0.82 pM. The RECIST was SD.

Case 2. Man, 78 years. He was found to have a right upper lung cancer and did radical resection of right upper lung cancer under thoracoscopy under general anesthesia in 2014-05-29, and conformed adenocarcinoma (clinical stage IIA, grade G3 and tumor size of 5.5 cm). He used the maintenance therapy with pemetrexed disodium + carboplatin for 4 cycle in 2015-06-09 to 2015-09-22. Abnormal changes were found in the right lung, bilateral pleural effusion (partially encapsulated), and fibrous foci in left upper lung, by CT detection in 2015-07-12. It also found liver cysts and left lateral adrenal branch hyperplasia. He dead 14 months after start of the chemotherapy. The STK1p value pre-chemotherapy was 0.1 pM and 2-cycle-post-chemothyrapy increased to 5.29 pM. The RECIST was SD.

Case 3. Man, 78 years. He was found as in left upper lobe lung cancer and conformed adenocarcinoma, clinical stage IB, grade G3, tumor size was of 3.5*3.0*1.5. The first, he did neo-chemotherapy with doxorubicin + nedaplatin for 6 cycles in 2013-05-21 to 2013-10-22. It found the right interstitial inflammation improved slightly and no nodules in left upper lobe were seen by CT in 2013-12-30. He did radical resection of in 2014 -04-17 and then continued chemotherapy. The chemotherapy planed 1) Doxorubicin + carboplatin for 3 cycles in 2014-11-26 to 2015-01-27; 2) Gemcitabine for 2 cycles in 2015-03-03 to 2015-04-03; 3) Gemcitabine + nedaplatin for 2 cycles in 2016-03-26 to 2016-05-03; 4) Vinorelbine + nedaplatin for 2 cycles in 2016-06-01 to 2016-07-23.

The PET-CT detection in 2015-02-26 after surgery and chemotherapy showed that the third and fourth posterior ribs were destroyed and local pleura was thickened as well as accompanied by increased glucose metabolism. He also considered to have metastasis. He was reexamination with CT in 2016-03-10 and suggested to have multiple asymptomatic malignant lung nodules, multiple right ribs and thoracic vertebrae metastasis. He dead 15 months after the ineffective chemotherapy treatment. The STK1p value pre-chemotherapy was 0.88 pM and after 2 cycle-post-chemothyrapy, 0.93 pM. The RECIST was SD.

4. Discussion

In clinical practice, early/middle and advanced lung cancer shows extensive difference in prognosis because of the lack of typical symptoms and effective diagnostic and prognostic methods. Most patients (≈70%) are diagnosed as advanced stage by imaging. In this study of random selected NSCLC patients, 70% of the patients were of advanced stage. However, it should be noted that these patients were faced with a number of uncertainties, including different medical background, different clinical stages and pathological grades, different treatment options (surgery + chemotherapy or chemotherapy alone), and treatment side effects. And sometimes there are false-positive results (imagers or biomarkers). In addition, it is hard to follow the recommendation of therapy from case-control trials, but try to perform the most efficient treatment based on the individual patient's medical background.

In the present study, we chose to use STK1p values after 2 cycle-individual-chemotherapy, since it correlated closely to the endpoint of the treatment, supported by a similar study of STK activity in NSCLC patients [25]. The mean STK1p values decreased by about 30%, except for the stage I-IIIA patient group. In addition, individual examination shows that in about 40% of the patients the STK1p value decreased by 50% or more after the 2-cycle-chemotherapy (table 4), indicating rather efficient individual treatment, including patients with advanced stage. Therefore, we investigated

The end of the 2-cycle individual-chemotherapy is a useful time-point when using STK1p for monitor treatment with these agents in NSCLC patients. However, the STK1p values in about 60% of the patients at the end of the 2-cycle individual-chemotherapy were still significantly higher (~0.70 pM) compare to normal health persons (0.38 pM), it means that some residual tumors are growing and might result that STK1p did not return to normal levels as described in 3.6, cases analysis of early stage dead patients. The normal STK1p value of 0.3-0.5 pM was confirmed in a 160,086-participants-health screening [27].

In this study, we divided the STK1p 2-cycle-individual-chemotherapy patients into a low risk (n=58) and an elevated risk (n=43) groups and compared them with

parameters of clinical characteristics. The only significantly difference was found that the STK1p values decreased significantly in the early/middle stage patients, but not in the patients with advance stages. The STK1p values also decreased significantly after surgery + individual-chemotherapy in the early/middle stage patients compared to individual-chemotherapy only, but not in the advanced stage patients, indicating that the STK1p low-risk value was significant correlated to the efficient combined treatment in the early/middle stage patient, but not in the advanced stage patients.

When considered elevated STK1p value, advantage stage, pathologic type of SCC and individual-chemotherapy alone correlated to poor OS in the Kaplan Meyer curves (Fig.1A, B, C and D), while low STK1p value in patients with early/middle stage (Fig. E p= 0.001) correlated to a significantly better survival rate compared to patients with high STK1p values. The multivariate Cox regression analysis showed that treatment (RR+ individual-chemotherapy vs. individual-chemotherapy alone (p =0.001) and STK1p (≦0.6 vs. ＞0.6 pM, p=0.01) were independent prognostic factors, while stage, pathology type and sex were not (p> 0.05). The present study is consistent with a previous retrospective TK-activity [24] and immunohistochemical studies on patients with lung cancer [28], the high TK1 expression was independent prognostic factor of poor survival.

Generally speaking, early/middle stage patients showed a better survival. However, there was three patients who died within 14-20 months after end of the chemotherapy. The STK1p values of the post-individual-chemotherapy did not returned to normal values (<0.6 pM), and were characterized as RECIST SD. The CT examination showed that these patients were already in a metastatic stage, and it was may be too late to start chemotherapy.

Although trying to implement a better individual customized therapy program, the Kaplan Meyer curves showed that the OS rate of the advantage stage (IIIB+IV) patients displayed slightly improvement, but not significant different between patients with low STK1p values compared to patients with high STK1p values (Fig 1 H, p=0.13). The mortality was still high (49.3%). In a previous study, it was reported that STK1p was low in 63.5 % of lung cancer patients with advantage stage with increased tumor size [29]. The high degree of necrosis is normally found in patients with stage III/IV, the decline STK1p was not corresponding to tumor size of patients at advantage stage [30]. A study on STK-activity (TKa) and circulating tumor M2 pyruvate kinase (TuM2-PK) concentration in renal cell carcinoma (RCC) demonstrated high degree necrosis in RCCs with stage III/IV. The presence of extensive tumour necrosis (>50%) was statistically correlated to high TuM2-PK concentration and low STKa [31]. A likely explanation is that advantage stage patients already had low STK1p when started the chemotherapy due to extensive necrosis with reduced growth rate [31]. Thus, it should be noted that STK1p is may not a suitable prognostic marker when there is a risk of

extensive necrosis in advantage stage of lung patients. Early detection and early treatment would lead to rapid declines in mortality [21, 22].

In this study, it was obvious that surgery in combination with individual-chemotherapy was a more efficient than individual-chemotherapy alone. Furthermore, in the COX analysis it was found that the surgery + individual-chemotherapy was an independent prognostic factor for survival. More recently, due to the immunotherapy revolution, specifically the development of immune checkpoint inhibitors (ICIs), such as PD-L1 expression, can improve the treatment of advantage lung cancer patients [32]. A meta-analysis revealed that immunotherapy in combination with chemotherapy is an effective option as a first-line treatment for lung cancer (n = 4,887) [33]. The NSCLC patients administered immune- and chemotherapy exhibited better 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 [34]. The CTCs from peripheral blood can provide a minimally invasive method to monitor PD-L1 expression on tumor cells over time [9], but the protocol is complicated and time-consuming. As an alternative to use biopsy or CTCs to measure PD-Li expression, we suggested to use STK1p assay, a non-invasive, simple, and low cost assay, for monitoring the effect of the treatment.

Of NSCLC, two most common subtypes are adenocarcinoma (AC, ~70%) and squamous cell lung cancer (SCC, ~30%) [35]. In this study, the OS was significant better in AC patients compared to SCC patients indicating that the SCC type of patients contain high-risk individuals. This is in coincide with a previous study reported that STK1 level was significantly higher in SCC as compared to AC patients [36]. In addition, in a recent study, extensive plasma lipidomics profiling of patients with SCC was performed and a panel of 2 lipid biomarkers that were able to detect SCC during the early stages were identified. The results of that study revealed the potential of using these 2 lipid markers in combination with LDCT-based screening methods in order to identify SCC patients with high-risk [37]. It is possible that STK1p in combination with such 2 lipid markers could improve the assessment of high-risk in early SCC patients.

Cancer is a chronic individual-disease with abnormal growth. Every cancer arises from a pre-cancer, although not all pre-cancers lead to cancer [22, 38]. If all pre-cancer were detected early, the risk for further development into malignancy would be reduced extensively. A series of papers proved that STK1p level increases earlier than the discovery of the visible cancer by imaging [22]. For example, a case report showed that a woman (aged 72 years) was diagnosed by imaging as having ground-glass opacity (pre-cancer) of lung. STK1p value was high of 3.9 pM while CEA, Cyfra19 and NSE were normal. The STK1p continued to rise to above 10-13 pM, while CT scanner and the three markers were still normal 4 months after discovery of right lung cancer. A radical operation was performed and confirmed AC (moderate differentiation) by

pathology. The STK1p value declined to 1.3 pM 1 month after surgery, no occurrence of metastasis or recurrence was detected by CT scanner 5 months after follow-up [27].

Precision molecular medicine with many biomarkers are either available or under development for several lung diseases. However, few biomarkers are in early widespread clinical use so far. It is mandatory to show the performance of these biomarkers for the monitoring and prognosis in early lung tumors, such, pre-cancers (ground-glass opacity preaches) or malignant tumor (asymptomatic malignant lung nodules, invisible small tumor or early localized tumors), which are potential to use for monitoring and prognosis of personalized targeted therapies. TK1 is one of a precise molecule target for monitoring and prognosis, specifically, for early detection of growth of invisible small tumors. STK1p assay is also non-invasive and low cost [22]. We proposal that STK1p combined with imaging and/or another biomarkers, for example CTCs and TK1 immunohistochemical staining, might be a more reliable assessment for developing of pre-malignancy or diseases associated with the risk process of individual lung cancer. There would be no or very limited lung cancer development in the future if the pre-malignancy or early tumors are treated in time.

Conclusions: STK1p, a low-cost assay, correlates significantly to survival and is an independent multivariate prognostic factor of NSCLC patients. The combination of the STK1p with suitable imager is a rational approach to increase the efficacy for early detection of tumor in lung cancer screening. An individual customized therapy, such as RR combined with chemotherapy, would reduce the mortality in routine clinical setting of NSCLC.

List of abbreviations

Thymidine kinase 1 (TK1)
serum TK1 protein concentration (STK1p)
non-small cell lung carcinoma (NSCLC)
radical resection (RR)
false-positive low-dose computed tomography (LDCT)
carcinoembryonic antigen (CEA)
cytokeratin 19 fragment (CYFRA21)
neoron-specific enolase (NSE)
circulating tumor cells (CTCs)
deoxythymidine (dTdR)
deoxythymidine monophosphate (dTMP)
Receiver Operating Characteristic (ROC)
adenocarcinoma (AC)

squamous cell carcinoma (SCC)
Classification of Malignant Tumor (TNM)
World Health Organization (WHO)
Response Evaluation Criteria in Solid Tumors (RECIST)
progression-free survival (PFS)
overall survival (OS)
Computerized Tomographic (CT)
Thymidine kinase activity (TKa)
tumor M2 pyruvate kinase (TuM2-PK)
renal cell carcinoma (RCC)
immune checkpoint inhibitors (ICIs)
Cox proportional hazards model (COX)

Declarations

Author contribution. ZCL, GQZ and ZXW designed the cooperative study, collected the data from their own hospitals and write manuscript, respectively. SCJ, YH and SGS were responsible for patient treatment and analyzed the results. LLW measured the blood samples and collected the data. JZ organized the research project, analyzed and checked all of results. JL, HBM, EH and SS rechecked all results from the two hospitals and rewrite the last version of the manuscript. All authors have read and approved the manuscript.

Compliance with ethical standards

Ethical approval. This study is in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. All procedures in the study involving participants of two hospitals (Affiliated Hospital of Suzhou University, JiangSu, China and Peking 301 General Hospital, China) were 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).

Informed consent. The informed consent was obtained from all individual participants by a signed written in the two hospitals, respectively.

Competing interests. Ji Zhou is the owner of Sino-Swed Tongkang Bio-Tech Inc., Shenzhen, China that produce the STK1p kit, the other authors declare no conflicts of interest.

Funding The expenses of this project was supported by the Major Special Funds of National Science and Technology in China: "Innovate New Drugs for the Prevention of Major Diseases and Establish Demonstrative Technology Platform for International Standardized Clinical Evaluation（2018ZX09201-013）and Science and Technology Projects of JiangSu city (SS201631).

Consent for publication: All of persons provided a signed written informed consent.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgements:
We acknowledge support from the Affiliated Hospital of Suzhou University, JiangSu, China, the Peking 301 General Hospital, China and the Medicine Department, Shenzhen Precision Medicine Institute, Shenzhen, China.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics 2019. CA Cancer J Clin. 2019;69: 7-34. https://doi.org/10.3322/caac.21551
2. 2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;6:115-132. https://doi.org/10.3322/caac.21338
3. Benzaquen J, Boutros J, Marquette C, Delingette H, Hofman P. Lung Cancer Screening, Towards a Multidimensional Approach: Why and How? Cancers (Basel). 2019;11:1-12. https://doi.org/10.3390/cancers11020212
4. Jelonek K, Widłak P. Metabolome-based biomarkers: their potential role in the early detection of lung cancer. Contemp Oncol (Pozn). 2018;22:135-140. https:// doi.org/10.5114/wo.2018.78942
5. Wu AC, Kiley JP, Noel PJ, Amur S, Burchard EG, Clancy JP, Galanter J, Inada M, Jones TK, Kropski JA, Loyd JE, Nogee LM, Raby BA, Rogers AJ, Schwartz DA, Sin DD, Spira A, Weiss ST, Young LR, Himes BE. (2018) Current Status and Future Opportunities in Lung Precision Medicine Research with a Focus on Biomarkers. An American Thoracic Society/National Heart, Lung, and Blood Institute Research Statement, Am. J Respir. Crit. Care Med. 198:e115-e136. https://doi.org/10.1164/rccm.201810-1895ST
6. Dama E, Melocchi V, Colangelo T, Cuttano R, Bianchi F (2019) Molecular Profile of Lung Cancer: New Strategies for the Early Detection and Prognostic Stratification. J Clin Med 8:1-14. https://doi.org/10.3390/jcm8010108

7. Crosbie PA, Shah R, Summers Y, Dive C, Blackhall F (2013) Prognostic and predictive biomarkers in early stage NSCLC: CTCs and serum/plasma markers, Transl. Lung Cancer Res 2: 2382–2397. https://doi.org/10.3978/j.issn.2218-6751.2013.09.02
8. Yang Q, Zhang P, Wu R. Lu K, Zhou H. Identifying the Best Marker Combination in CEA, CA125, CY211, NSE, and SCC for Lung Cancer Screening by Combining ROC Curve and Logistic Regression Analyses: Is It Feasible? Dis Markers. 2018;1:1-12. https://doi.org/10.1155/2018/2082840
9. Wang Y, Kim TH, Fouladdel S, Zhang Z, Soni P, Qin A, et al. PD-L1 Expression in Circulating Tumor Cells Increases during Radio(chemo)therap y and Indicates Poor Prognosis in Non-small Cell Lung Cancer. Sci Rep. 2019;9:1-9, https://doi.org/ 10.1038/s41598-018-36096-7
10. Cleaver. Thymidine metabolism and cell kinetics, A. Neuberger, E.L. Tatum (Ed.) in Frontiers of Biology. North-Holland Publishing, Amsterdam; 1967, p. 43-125.
11. Sherley JL, Kelly TJ. Regulation of human thymidine kinase during the cell cycle, Journal of Biological Chemistry. 1988;263: 8350-8358. PMID: 3372530
12. Kauffman, MG, Kelly TJ. Cell cycle regulation of thymidine kinase: residues near the carboxyl terminus are essential for the specific degradation of the enzyme at mitosis, Mol Cell Biol. 1991;11:2538-2546. PMID: 170809
13. He Q, Skog S, Wang N, Eriksson S, Tribukait B. Characterization of a peptide antibody against a C-terminal part of human and mouse cytosolic thymidine kinase, which is a marker for cell proliferation. Eur J Cell Biol. 1996;70:117-124. PMID:8793383
14. Ke PY, Chang ZF. Mitotic Degradation of Human Thymidine Kinase 1 Is Dependent on the Anaphase-Promoting Complex/Cyclosome-Cdh1-Mediated Pathway. Mol Cell Biol. 2014;24: 514–526. https://doi.org/10.1128/MCB.24.2.514-526.2004
15. Gasparri F, Wang N, Skog S, Galvani A, Eriksson S. Thymidine kinase 1 expression defines an activated G1 state of the cell cycle as revealed with site-specific antibodies and ArrayScan assays. Eur J Cell Biol. 2009;88:779-785. https://doi.org/10.1016/j.ejcb.2009.06.005
16. Weagel EG, Burrup W, Kovtun R, Velazquez EJ, Felsted AM, Townsend MH, et al. Correction to : Membrane expression of thymidine kinase 1 and potential clinical relevance in lung, breast, and colorectal malignancies. Cancer Cell Int. 2019;19:29. https://doi.org/10.1186/s12935-019-0749-6
17. Gronowitz JS, Hagberg H, Källander CF, Simonsson B. The use of serum deoxythymidine kinase as a prognostic marker, and in the monitoring of patients with non-Hodgkin's lymphoma. Br J Cancer. 1983;47: 487-495. PMID:6849793

18. Topolcan O, Lubos Holubec L. The role of thymidine kinase in cancer diseases. Expert Opin Med Diagn. 2008;2:129–140. https://doi.org/10.1517/17530059.2.2.129.
19. Zhou J, He E, Skog S. The proliferation marker thymidine kinase 1 in clinical use. Mol Clin Oncol. 2013;1:18-28. https://doi.org/10.3892/mco.2012.19
20. Alegre MM, Weyant MJ, Bennett DT, Yu JA, Ramsden MK, Elnaggar A, et al. Serum detection of thymidine kinase 1 as a means of early detection of lung cancer. Anticancer Res. 2014;34:2145-51. PMID: 24778016
21. Jagarlamudi KK, Shaw M. Thymidine kinase 1 as a tumor biomarker: technical advances offer new potential to an old biomarker. Biomark Med. 2018;12: 1035-1048. https://doi.org/10.2217/bmm-2018-0157
22. Skog S, He E, Haghdoost S. Prevention and Early Detection of Human Tumour, S. Skog (Ed.), Lambert Academic Publishing, Schaltungsdienst Lange O.H.G., Berlin; 2017, p.183-257. www.get-morebook.com
23. Lou X, Zhou J, Ma HB, Xu S, He E, Skog S, Wang HD. The Half-Life of Serum Thymidine Kinase 1 Concentration is an Important Tool for Monitoring Surgical Response in Patients with Lung Cancer: A Meta-Analysis. Genet Test Mol Biomarkers. 2017;21: 471-478. https://doi.org/10.1089/gtmb.2017.0003
24. Nisman B, Nechushtan H, Biran H, Gantz-Sorotsky H, Peled N, Gronowitz S, et al. Serum thymidine kinase 1 activity in the prognosis and monitoring of chemotherapy in lung cancer patients: a brief report. Thorac Oncol. 2014;9:1568-1572. https://doi.org/10.1097/JTO.0000000000000276
25. Jiang ZF, Wang M, Xu JL. Thymidine kinase 1 combined with CEA, CYFRA21-1 and NSE improved its diagnostic value for lung cancer. Life Sci. 2018;194:1-6. https://doi.org/10.1016/j.lfs.2017.12.020.
26 He Q, Fornander T, Johansson H, Johansson U, Hu GZ, Rutqvist LE, et al. Thymidine kinase 1 in serum predicts increased risk of distant or loco-regional recurrence following surgery in patients with early breast cancer. Anticancer Res. 2006; 26: 4753-4759. PMID:17214336
27. Chen ZH, Wang Y, Chen G, Cao X, Jiang XR, Ma HB, et al. Chapter 21. Serum-Biomarker Thymidine Kinase 1 for Early Discovery of Tumour Process of 160,086 Participants Using a Sensitive Immune-ECL-Dot-Blot Detection System. S.Y. Yurish (Ed.): In “Advances in Sensors: Reviews, Vol. 6”， International Frequency Sensor Association Publishing, Barcelona, Spain; 2018, p.529-540. http://www.sensorsportal.com
28. Xu Y, Liu B, Shi QL, Huang PL, Zhou XJ, Ma HH, et al. Thymidine kinase 1 is a better prognostic marker than Ki-67 for pT1 adenocarcinoma of the lung. Int J Clin Exp Med. 2014; 7:2120-2128. www.ijcem.com /ISSN:1940-5901/IJCEM1402040

29. Chen Y, Ying M, Chen Y, Hu M, Lin Y, Chen D, et al. Serum thymidine kinase 1 correlates to clinical stages and clinical reactions and monitors the outcome of therapy of 1,247 cancer patients in routine clinical settings. Int J Clin Oncol. 2010;15:359-368. https://doi.org/10.1007/s10147-010-0067-4

30. Nisman B, Yutkin V, Nechushtan H, Gofrit ON, Peretz T, Gronowitz S, et al. Circulating tumor M2 pyruvate kinase and thymidine kinase 1 are potential predictors for disease recurrence in renal cell carcinoma after nephrectomy. Urology. 2010;76:513.e16. https://doi.org/10.1016/j.urology.2010.04.034

31. Hemmerlein B, Kugler A, Ozisik R, Ringert RH, Radzun HJ, Thelen P. Vascular endothelial growth factor expression, angiogenesis, and necrosis in renal cell carcinomas. Virchows Arch. 2001;439: 645-652. PMID: 117643

32. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015; 21: 2018-2028. https://doi.org/10.1056/NEJMoa1501824

33. Shen K, Cui J, Wei Y, Chen X, Liu G, Gao X, Li W, et al. Effectiveness and safety of PD-1/PD-L1 or CTLA4 inhibitors combined with chemotherapy as a first-line treatment for lung cancer: A meta-analysis. J Thorac Dis. 2018;10:6636-6652. https://doi.org/ 10.21037/jtd.2018.11.72

34. Hui R, Garon EB, Goldman JW, Leighl NB, Hellmann MD, Patnaik A, et al. Pembrolizumab as first-line therapy for patients with PD-L1-positive advanced non-small cell lung cancer: a phase 1 trial. Ann Oncol. 2017;28:874-881. https:// doi: 10.1093/annonc/mdx008.

35. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017;67:7-30. doi: 10.3322/caac.21387.

36. Yu Z, Chen H, Zhu Y, Ai J, Li Y, Gu W, et al. Global lipidomics reveals two plasma lipids as novel biomarkers for the detection of squamous cell lung cancer: A pilot study. 2018;Oncol Lett. 16: 761-768. https://doi.org/10.3892/ol.2018.8740

37. Xu XH, Zhang YM, Shu XH, Shan LH, Wang ZW, Zhou YL, et al. Serum thymidine kinase 1 reflects the progression of pre-malignant and malignant tumors during therapy. Mol Med Rep. 2008;1: 705-711. https://doi.org/10.3892/mmr_00000017

38. Berman JJ, Moore G. Precancer:The Beginning and the End of Cancer. Jones and Bartlett Publisher, Masssachusetts, USA; 2010, p 69-78, p120

Legend

Figure 1. The Kaplan Meyer curves show overall survival (OS) in 101 patients in relation to STK1 (A), stage (B), , pathological type (C), and treatment (RR+individual-chemo.vs.individual-chemo. Alone, D), overall survival (OS) in 24 patients of early/middle stage in relation to STK1 (E); 71 patients of advantage stage (IIIB-IV) in relation to STK1 (F), pathological type (G) and treatment (H). Significant log rank values between the survival curves are shown in figure. The solid dots in the survival curves show the times of censored observations.

Table 1. Clinical information.

Type	Patients (n=129)	Follow up, patients (n=101)
STK1, pre-individual-chemo.	129	101
STK1,2-cycel-post-individual-chemo.	129	101
Mean age	61.80±9.37 (29-76 yes.)	61.70± 8.17(29-76 ys.)
Sex		
Men	96	68
Female	33	33
Histological type		
AC	86	69
SCC	38	28
Alveolar cell carcinoma	1	1
AC/SCC	1?	1?
missing	3	1
Clinical stage		
Early/middle: I-IIIA	34	23
I	IA(5),IB(9)	IA(5),IB(4)
II	IIA(8),IIB(3)	IIA(5),IIB(3)
IIIA	IIIA(9)	IIIA(6)
Advanced: IIIB-IV	87	71
IIIB	IIIB(9)	IIIB(9)
IV	IV(78)	IV(62)
unclear	8	6
Treatment		
Had RR pre-individual-chemo.	63	47
Stage I	IA(5),IB(10)	IA(5),IB(4)
Stage II	IIA(8),IIB(3)	IIA(6),IIB(3)
Stage IIIA	IIIA(9)	IIIA(6)
StageIIIB+ IV	IIIB+ IV (20)	IIIB+ IV (18)
Unclear stage	8	5
Non-RR pre-individual-chemo.	66	54
IIIB	IIIB(9)	IIIB(9)
IV	IV(57)	IV(45)

Table 2. STK1p and CYFRA21-1 in relation to cycles of individual-chemotherapy.

STK1p	cycles	RECIST	pre (STK1, pM）	post （STK1, pM）	P
		Total	1.07(0.49-1.89)	0.40(0.20-1.48)	0.027
	2	OR	0.96(0.38-1.87)	0.40(0.18-1.10)	0.014
		NR	1.21(0.65-1.91)	0.68(0.21-2.37)	0.913
		Total	0.40（0.18-1.54）	0.41（0.22-1.30）	0.166
	4	OR	0.45（0.22-1.56）	0.39（0.23-1.05）	0.017
		NR	0.29（0.16-1.54）	1.26（0.14-2.15）	0.445
		Total	0.49（0.25-1.26）	0.61（0.15-2.12）	0.407
	6	CR+PR	0.44（0.14-1.06）	0.45（0.10-2.04）	0.216
		SD+PD	1.01（0.29-2.37）	0.82（0.27-2.51）	0.918
		Total	0.90（0.15-2.42）	0.65（0.23-2.02）	0.935
	8	OR	1.12（0.10-2.13）	0.50（0.19-0.85）	0.241
		NR	15.25（8.25-16.70）	15.73（5.61-72.87）	0.256
CYFRA21-1	cycles	RECIST	pre（ng/ml）	post（ng/ml）	P
		Total	3.08(1.93-5.00)	2.78(1.89-3.84)	0.017
	2	~~OR~~	~~2.78(1.83-5.37)~~	~~2.41(1.78-3.58)~~	~~0.013~~
		NR	3.80(2.88-4.23)	3.39(2.65-4.23)	0.728
		Total	2.60（1.80-3.56）	2.40（1.58-3.97）	0.651
	4	OR	2.75（0.91-3.54）	2.22（1.65-3.12）	0.169
		NR	2.07（1.62-4.32）	3.87（1.52-6.33）	0.015
		Total	2.17（1.54-3.22）	2.82（1.74-4.68）	0.016
	6	OR	2.15（1.66-3.09）	2.41（1.57-3.82）	0.674
		NR	2.21（1.43-6.32）	3.63（2.10-9.58）	0.004
		Total	2.30（1.35-4.73）	2.44（1.46-6.85）	0.286
	8	OR	2.02（1.19-3.61）	1.50（1.44-2.85）	0.314
		NR	2.51（1.73-6.88）	3.62（1.57-9.22）	0.037

OR: objective response; NR: no response

Table 3. STK1p pre- and 2 cycle-post-individual-chemotherapy. A, total patients of stage I-IIIA + IIIB-IV(A); B-D, followed-up of patients: of stage I-IIIA + IIIB-IV (B), of patients of stage I-IIIA (C) , and of advantage stage IIIB-IV (D). E. Controls.

Group	Samples (n)	STK1 (pM)	p-value	t-value
A. stage I-IIIA+IIIB-IV	129			
1. Pre-chemo.		1.07± 1.54		
2. Post-chemo.		0.72±0.95	0.025	2.274
B. stage I-IIIA+IIIB-IV follow-up	101			
1. Pre- chemo.		1.08± 1.58		
2. Post- chemo.		0.75±1.03	0.022	2.33
C. stage I-IIIA	34			
1. Pre-chemo.		0.698± 0.767		
2. Post-chemo.		0.566±0.774	0.366	0.917
D. stage IIIB-IV	71			
1. Pre-chemo.		1.148± 1.70		
2. Post-chemo.		0.752±1.03	0.016	2.4698
E. Controls	400	0.38±0.31	< 0.000 (E VS. A, B, C or D)	

Table 4. Number of patients with decreasing or increasing STK1p values after 2-cycle-individual-chemotherapy.

Stage I-IIIA	
STK1p decrease by 50% or more	13/34, 38%
STK1p increase	17/34, 50%
Stage IIIB-IV	
STK1p decrease by 50% or more	28/71, 39%
STK1p increase	25/71, 35%

Table 5. The number of STK1 low and high-risk patients in relation to clinical characteristics 2-cycle-post-individual-chemotherapy (Chi-square test).

Group	STK1 ≦ 0.6 pM (n=58)	STK1 ＞ 0.6 pM (n=43)	p
Stage n=95			
Early middle (I-IIIA) n=24	19	5	
Advanced (IIIB+IV) n=71	36	35	0.015
Pathology type n=98			
CA n=70	40	30	
SCC n=28	17	9	0.149
Age n=101			
≦ 60 ys. n=34	20	14	
＞ 60 ys. n=67	36	31	0.626
Sex n=101			
M n=68	40	38	
F n=33	17	6	0.054
RECIST n=101			
PR n=32	18	14	
PD+SD n=69	40	29	0.165
Treatment all stages n=101			
RR+individual-chemo. n=47	31	16	0.0104
individual-chemo. n=54	23	31	
Only stage IIIB+IV n=73			
RR+individual-chemo. n=23	14	9	
individual-chemo. n=50	27	23	0.529
RR+chemo n=42			
Stage I-IIIA n=24	18	6	
Stage IIIB+IV n=18	8	10	0.044

R.: Radical resection; RECIST (Response Evaluation Criteria in Solid Tumors)

Table 6. Uni- and multivariate analysis.

Univariate	*p*-value	Hazard risk	95% CI
RR+individual-chemo.vs. individual-chemo. alone	0.002	2.763	1.431-5.342
STK1p (≦0.6 vs.＞0.6 pM)	0.018	2.108	1.137-3.909
Stage (I-IIIA vs. IIIB-IV	0.008	4.044	1.437-11.38
Pathology-type (AC vs. SCC)	0.004	2.538	1.355-4.294
Sex (Male vs. Female)	0.099	1.976	1.042-3.744
Multivariate	*p*-value	Hazard risk	95% CI
RR+individual-chemo.vs. individual-chemo. alone	0.001	3.04	1.543-5.987
STK1p (≦0.6 vs.＞0.6 pM)	0.010	2.295	1.222-4.308
Stage (I-IIIA vs. IIIB-IV	0.385		
Pathology-type (AC vs. SCC)	0.276		
Sex (Male vs. Female)	0.293		

RR: radical resection; chemo. : chemotherapy

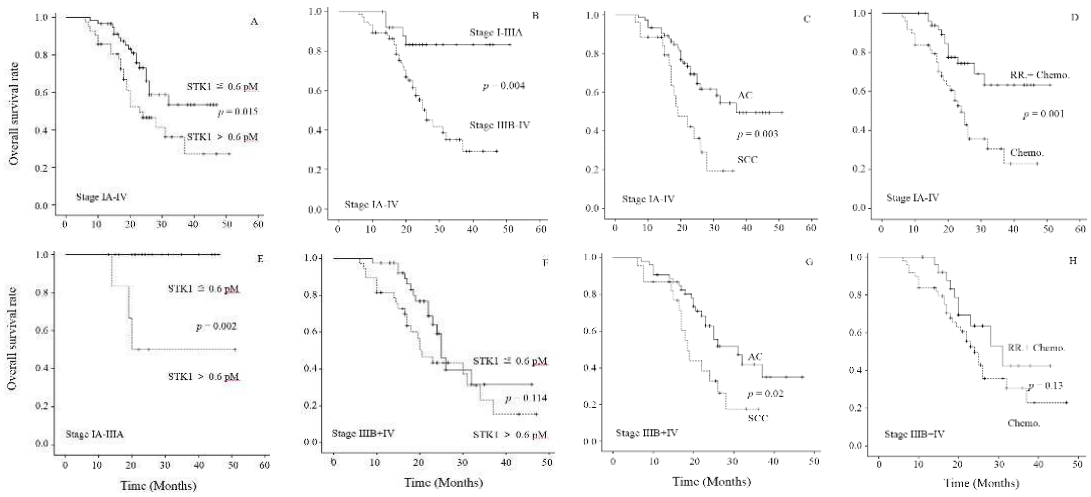
A
Overall survival rate
STK1 ≤ 0.6 pM
p = 0.015
STK1 > 0.6 pM
Stage IA-IV
B
Stage I-IIIA
p = 0.004
Stage IIIB-IV
Stage IA-IV
C
AC
p = 0.003
SCC
Stage IA-IV
D
RR.+ Chemo.
p = 0.001
Chemo.
Stage IA-IV
E
Overall survival rate
STK1 ≤ 0.6 pM
p = 0.002
STK1 > 0.6 pM
Stage IA-IIIA
Time (Months)
F
STK1 ≤ 0.6 pM
p = 0.114
Stage IIIB+IV
STK1 > 0.6 pM
Time (Months)
G
AC
p = 0.02
SCC
Stage IIIB+IV
Time (Months)
H
RR.- Chemo.
p = 0.13
Chemo.
Stage IIIB+IV
Time (Months)

Figure 1.

Figures

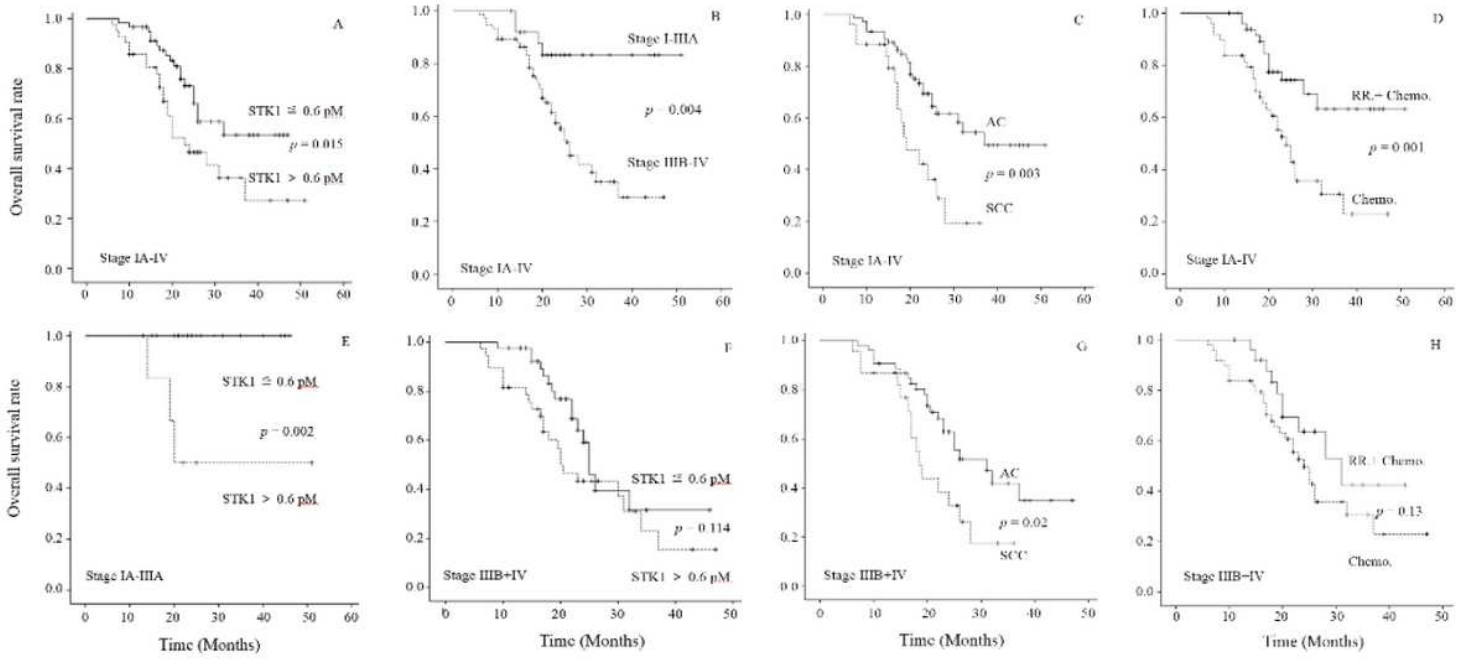

Figure 1

The Kaplan Meyer curves show overall survival (OS) in 101 patients in relation to STK1 (A), stage (B), , pathological type (C), and treatment (RR+individualchemo. vs.individual-chemo. Alone, D), overall survival (OS) in 24 patients of early/middle stage in relation to STK1 (E); 71 patients of advantage stage (IIIB-IV) in relation to STK1 (F), pathological type (G) and treatment (H). Significant log rank values between the survival curves are shown in figure. The solid dots in the survival curves show the times of censored observations.