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**Title: Monitoring of CA19-9 and SPan-1 can facilitate the earlier confirmation of progressing pancreatic cancer during chemotherapy.**

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**Short title:**

Early markers of progressing pancreatic cancer.

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## **ABSTRACT:**

Background: Measurement of objective response to chemotherapy using imaging modalities is sometimes difficult in pancreatic cancer (PC). We aimed to verify whether monitoring of serum tumor markers (TMs), namely carcinoembryonic antigen, CA19-9, DUPAN-2, SPan-1, can facilitate earlier confirmation of treatment failure. Methods: Monitoring of serum TMs and computed tomography were performed every 4 weeks until progression of disease in 90 patients with PC undergoing gemcitabine therapy. In Group A (January 2006- October 2007), we analyzed the fluctuation rates of TMs with high pretreatment positive rates, and defined the criteria of progressive disease under TM monitoring (TM-PD). In Group B (November 2007- October 2008), we calculated the time to progression (TTP) under this TM-PD criteria, which was compared with the TTP under the RECIST criteria. Results: CA19-9 and SPan-1 had the highest pretreatment positive rates: 83% and 90%, respectively. In Group A (CA19-9, n = 38; SPan-1, n = 36), TM-PD criteria were defined as follows: fluctuation rates were  $\geq 25\%$  for a month or  $\geq 10\%$  for 2 consecutive months in CA19-9, and  $\geq 10\%$  for a month in SPan-1. In Group B (CA19-9, n = 18; SPan-1, n = 17), under these criteria, one-month earlier confirmation of treatment failure was feasible in 61% by CA19-9 and 59% by SPan-1. Furthermore, the combination could facilitate this determination in 72%

(35/49), significantly better than CA19-9 alone ( $P = 0.004$ ). Conclusion: Monitoring of serum CA19-9

and SPan-1 is helpful for earlier confirmation of treatment failure during gemcitabine therapy in PC.

**Keywords:** Pancreas; Tumor marker; Gemcitabine; Diagnosis

**Abbreviations:** CEA, carcinoembryonic antigen; CT, computed tomography; PD, progressive disease;

RECIST, Response Evaluation Criteria in Solid Tumor; TM, tumor marker; TTP, time to tumor

progression; TM-PD, progressive disease under tumor marker monitoring; TM-TTP, time to progression

of tumor marker; ULN, upper limit of normal;

## **INTRODUCTION**

Pancreatic cancer is the fourth-leading cause of cancer-related mortality in the United States and the fifth

in Japan. Symptoms of pancreatic cancer include anorexia, weight loss, weakness, fatigue, abdominal

pain, and nausea. The nonspecific and mild nature of these initial symptoms often results in delayed

diagnosis; consequently, 80% or more of patients initially present with locally advanced or metastatic

disease.<sup>1</sup> Therefore, systematic chemotherapy using gemcitabine or other drugs<sup>2</sup> is considered the

treatment of choice for patients with this morbidity. Despite the low objective response rate, gemcitabine

improves survival and provides a clinical benefit. However, the median survival time is 5.7 months and the one-year survival rate 18%.<sup>3</sup> Recently, a Phase III study ‘CONKO-003’ provided at first time evidence for the benefit of second-line chemotherapy as compared to best supportive care alone for patients with pancreatic cancer.<sup>4</sup> To facilitate early second-line chemotherapy induction, earlier detection of treatment failure of the first-line chemotherapy is mandatory. Furthermore, earlier discontinuation may limit adverse effects, thereby improving quality of life, and reduce unnecessary costs.

Currently, objective measurement of response to chemotherapy using the Response Evaluation Criteria in Solid Tumors (RECIST)<sup>5</sup> is formally available. However, these criteria are sometimes difficult to apply to pancreatic cancer, which comprises inflammatory cells and fibrotic tissue as well as malignant cells.<sup>6</sup>

Under a computed tomography (CT) scan, it is difficult to discriminate the tumor component from others.<sup>7</sup> In addition, the evaluation of progressive disease for non-measurable lesions such as ascites, pleural effusion, and pericardiac effusion, is subjective and equivocal.

Tumor markers (TMs) have often been identified as surrogate markers. For example, a decrease in CA19-9 during chemotherapy has been reported to be useful for predicting the outcome of patients with pancreatic cancer in some retrospective studies.<sup>8-15</sup> We expected that use of such a TM may enable us to

detect early treatment failure of pancreatic cancer during chemotherapy, which has not been established.

The aim of this study is to verify whether monitoring of serum TMs, such as carcinoembryonic antigen

(CEA), CA19-9, DUPAN-2, and SPan-1, can facilitate earlier detection of treatment failure during

chemotherapy for pancreatic cancer than evaluation with the RECIST criteria.

## **PATIENTS AND METHODS**

### **Patients**

The patients analyzed in this study were enrolled in the randomized controlled trial UMIN ID 974,

entitled “A 4-week versus a 3-week schedule of gemcitabine monotherapy for advanced pancreatic

cancer: a randomized phase II study to evaluate toxicity and dose intensity”.<sup>16</sup> Therefore, all patients had

unresectable, histologically or cytologically proven, locally advanced or metastatic pancreatic

adenocarcinoma. Other eligibility criteria included no prior therapy, Karnofsky Performance Status  $\geq 50\%$ ,

age between 20 and 80 years, life expectancy of more than 2 months, and adequate organ function defined

as white blood cell count  $\geq 3000/\text{mm}^3$ , neutrophils  $\geq 1500/\text{mm}^3$ , platelets  $\geq 100000/\text{mm}^3$ , hemoglobin  $\geq 9.0$

g/dl, total bilirubin  $\leq 2.0$  mg/dl (or  $\leq 3.0$  mg/dl if biliary drainage was present), AST and ALT  $\leq 2$  times the

upper limit of normal (ULN) (or  $\leq 5$  times the ULN if liver metastasis was present), and creatinine  $\leq$  the



ULN. The number of patients enrolled in this original study was 90.

All patients understood the nature of the study, and written informed consent was obtained from all of them. The local ethics committee approved this treatment protocol. In addition, all patients agreed with the analysis of their clinical data including this study.

### **Chemotherapy and Assessment of Efficacy and Toxicity**

Patients were randomly assigned to either the 4-week or 3-week schedule of gemcitabine monotherapy.

For the 4-week schedule, gemcitabine was administered intravenously at 1000 mg/m<sup>2</sup> as a 30-min

infusion on days 1, 8 and 15 of a 28-day cycle, whereas in the 3-week schedules, gemcitabine was

administered at the same dose on days 1 and 8 of a 21-day cycle. Cycles were repeated every 4 or 3

weeks, respectively. Toxicity was graded according to the National Cancer Institute common toxicity

criteria, version 3.0. If grade 3-4 hematological toxicity or grade 2-4 non-hematological toxicity was

noted, administration was postponed for a week. The gemcitabine dose was then reduced by 200 mg/m<sup>2</sup>

from the previous dose, with a minimum dose of 400 mg/m<sup>2</sup>. Once a dose reduction was required,

reescalation of dose was not allowed. A delay in the cycle of up to 2 weeks during one course was

allowed when grade 2-4 hematological toxicity was recorded on day 1 of each cycle. When recovery from

treatment-related toxicity required more than 2 weeks, the gemcitabine treatment was stopped.

A CT scan was performed at baseline and every 4 weeks during chemotherapy until objective findings of disease progression were noted. Tumor response (i.e. maximum response during treatment) was assessed according to the RECIST criteria every 4 weeks. In addition, clinically severe symptoms related with progressing cancer, such as aggravated general condition, uncontrollable pain and GI obstruction, were also regarded as progressive disease (PD), namely, clinical PD. Treatment decisions were based on these radiographic and clinical grounds, and not on serum TM concentrations. Treatment was continued for at least 2 months and until progression of disease, occurrence of unacceptable toxicity, or patient refusal.

Patients who had unacceptable toxicity or refused therapy were excluded from this study.

#### **Tumor response by RECIST criteria**

Tumor response was categorized as complete response, partial response, stable disease, or PD by RECIST criteria.<sup>5</sup> All patients with evidence of a complete response or partial response or stable disease on at least one occasion were considered to have unconfirmed response. Confirmed responses were those documented with a follow-up CT scan obtained 4 weeks or longer after the scan that documented the initial response. Time to tumor progression (TTP) was defined as the time from initial therapy to the first

objective documentation of tumor progression or clinically severe symptoms such as those described above.

### **Serum tumor markers measurement**

Serum CEA, CA19-9, DUPAN-2, and SPan-1 were measured at baseline (on day 1 of first cycle of gemcitabine monotherapy) and every 4 weeks thereafter, on the same day of CT scan assessment. The CEA and CA19-9 were measured using an electrochemiluminescence immunoassay (ECLIA), DUPAN-2 using an enzyme immunoassay (EIA), and SPan-1 using an immunoradiometric assay (IRMA) at all institutions. In addition, the ULN of CEA was 5 ng/ml, that of CA19-9 was 37 U/ml, that of SPan-1 was 30 U/ml, and that of DUPAN-1 was 150 U/ml. Baseline and follow-up measurements for any given patient were performed at the same laboratory and by the same method.

### **Evaluation**

Positive rates for each TM before initial chemotherapy were calculated in patients who were enrolled in this original study. TMs having values higher than the upper limit of normal were considered as indicators which should be investigated. After selecting some TMs with higher pretreatment positive rates than the others, we made the following analysis.

In the first series of the cases (January 2006- October 2007; Group A), we analyzed the fluctuation of TM ratios in relation to tumor responses. Specifically, the changes in each TM were calculated as the TM fluctuation ratio according to the following formula:  $(A / B) \times 100 - 100$ , where  $A$  is the TM value of that day and  $B$  is the TM value of the one-month-before in each case. We calculated the median TM fluctuation ratio in the course of chemotherapy every 4 weeks until the recording of PD by tumor responses. We expected that an increase of the TM fluctuation ratio could become a sensitive indicator to predict the disease progression, and defined threshold value of this ratio on the basis of median TM fluctuation ratios at 1 and 2 months before PD under RECIST criteria by tumor responses. According to these methods, we defined this increase as PD under TM monitoring (TM-PD). Using this criterion, we calculated the time to progression of TM (TM-TTP), which was defined the time from initial therapy to documentation of TM-PD. Additionally, comparison of mean values and correlations were examined between conventional TTP by RECIST criteria and TM-TTP.

In the subsequent series (November 2007- October 2008; Group B), we calculated TM-TTP by the TM-PD criterion defined in Group A, which was compared with the conventional TTP to investigate the possibility of the early prediction of gemcitabine failure. Furthermore, TM-TTP using two or more types

of TM was compared with the conventional TTP.

In addition, median TM fluctuation ratios of selected tumor markers one month after initial chemotherapy by tumor responses were measured to support that their ratios reflected tumor progression. Overall survival was defined as the time from initial therapy to the date of either death or the last follow-up assessment.

### **Statistical analysis**

Continuous data are presented as medians and ranges. Continuous variables were compared with Mann-Whitney *U* test. Frequency distribution was compared with Fisher's exact test or the  $\chi^2$  test. Pearson's correlation coefficient was used to analyze the relationship between TTP and TM-TTP. Paired *t* test was used to compare between TTP and TM-TTP. Detection of treatment failure using the relationship between TTP and TM-TTP was evaluated by sign test. Survival analysis was assessed by the Kaplan-Meier method, and the survivals of Group A and Group B were compared using the log-rank test. All statistical analyses were performed with JMP 8.0.1 software (SAS Institute). A P-value <0.05 was considered statistically significant.

## **RESULTS;**

### **Positive rate for each TM**

The positive rate for each TM before induction of chemotherapy was 50% (45/90) for CEA, 83% (75/90) for CA19-9, 73% (59/81) for DUPAN-2, and 90% (73/81) for SPan-1, respectively. Nine of 90 patients were excluded from the SPan-1 and DUPAN-2 analyses as these markers had not been measured in these patients. From our results, since CA19-9 and SPan-1 had high positivity rates, we selected these two TMs for the subsequent analysis.

Seventy-three of 90 patients (81.1%) received gemcitabine until progression of disease and 17 patients (18.9%) discontinued gemcitabine chemotherapy due to unacceptable toxicity or refusal of treatment.

### **TM fluctuation ratio one month after initial chemotherapy by tumor responses in CA19-9 and**

#### **SPan-1**

The median TM fluctuation ratio of CA19-9 one month after initial chemotherapy induction was 27% (interquartile range [IQR]: 0% to 112%) in PD patients, -12% (IQR: -41% to 25%) in stable disease and -68% (IQR: -73% to -22%) in partial response. Similarly, the median TM fluctuation ratio of SPan-1 one month after initial chemotherapy induction was 11% (IQR: -10% to 57%) in PD patients, -24% (IQR: -43% to 6%) in stable disease and -48% (IQR: -72% to -10%) in partial response.

### **Evaluation of disease progression from the CA19-9 fluctuation ratio**

The positive rate for CA19-9 before induction of chemotherapy among the patients who received gemcitabine until progression of disease was 83.6% (60/73). However, 56 of 60 patients (93.3%) were eligible for the analysis of CA19-9, because inadequate data accumulation regarding CA19-9 was noted in 4 patients (Table 1-A). In Group A (n = 38), the changes of the CA19-9 fluctuation ratio were demonstrated by tumor responses in Figure 1. From these results, the following fluctuation ratios were defined as TM-PD of CA19-9: the value was greater than 25% for a month or greater than 10% for 2 consecutive months. Under this TM-PD criterion of CA19-9, mean TM-TTP was shorter than mean TTP (3.2 months vs. 4.6 months, respectively;  $P < 0.0001$ ) and there was statistically significant correlation between TM-TTP and TTP in Group A ( $r = 0.798$ ,  $P < 0.001$ ; Figure 3-A). Consequently, earlier confirmation of treatment failure was feasible in 61% (n = 11) of Group B (n = 18) by this criterion. Moreover, TM-PD of CA19-9 could facilitate significantly early confirmation of PD compared with PD under RECIST criteria in Group B (n = 16, 89%;  $P = 0.001$ ; Table 2-A). In this study, median difference between TM-TTP and TTP was -1.0 (95%CI: -4.4- 1.0) months.

### **Evaluation of disease progression from the SPan-1 fluctuation ratio**

Positive rates for SPan-1 before initial chemotherapy were 88.1% (59/67) in the patients who received gemcitabine until progression of disease. Fifty-three of 59 patients (89.3%) were eligible for the analysis of SPan-1, because the data accumulation of six patients regarding SPan-1 was inadequate (Table 1-B).

The changes of the SPan-1 fluctuation ratio in Group A (n = 36) are compared to tumor responses in Figure 2. From these results, TM-PD of SPan-1 was defined when its increase was greater than 10% for a month. Under this TM-PD criterion of SPan-1, mean TM-TTP was shorter than mean TTP (3.0 months vs. 5.1 months, respectively;  $P = 0.0007$ ) and there was a statistically significant correlation between TM-TTP and TTP in Group A ( $r = 0.465$ ,  $P = 0.006$ ; Figure 3-B). Similarly, earlier confirmation of treatment failure by this criterion was feasible in 59% (n = 10) of Group B (n = 17). Furthermore, TM-PD of SPan-1 can facilitate the early confirmation of PD compared with PD under RECIST criteria in Group B (n = 13, 77%;  $P = 0.049$ ; Table 2-B). In addition, median difference between TM-TTP and TTP was -1.0 (95%CI: -3.5- 2.1) months.

#### **Evaluation of disease progression from the changes of a combination of CA19-9 and SPan-1**

Forty-nine of 73 patients (67%) who had both positive CA19-9 and SPan-1 before treatment were eligible for the analysis of combination of CA19-9 and SPan-1. We could make an earlier confirmation of



treatment failure in 72% of these patients using TM-PD criteria of both CA19-9 and SPan-1 (Table 3).

Monitoring the combination of CA19-9 and SPan-1 could significantly facilitate earlier confirmation of

PD compared with CA19-9 alone (P = 0.004).

## **Discussion;**

This report is based on prospectively collected data from a cohort studied in a randomized controlled

trial.<sup>16</sup> Our data show that monitoring of serum CA19-9 and SPan-1 facilitates the earlier confirmation of

the treatment failure by approximately one month in patients with pancreatic cancer during gemcitabine

monotherapy. Furthermore, the combination of these two TMs ensures an increase in sensitivity. Certainly,

one-month earlier confirmation may be short. However, their median TTP and median survival time were

only 3.9 months and 8.2 months, respectively. Therefore, measuring the TTP-TM has a considerable

clinical impact regarding the change of chemotherapy.

CA19-9 is a tumor-associated antigen (first described by Koprowski et al.<sup>17</sup>) defined by a monoclonal

antibody (1116 NS 19-9). Using a cutoff point of 37 U/ml as the ULN, the overall sensitivity of the assay

in detecting pancreatic cancer was previously found to be approximately 80% with a specificity of 90%.<sup>18</sup>

Furthermore, CA19-9 can change in association with tumor shrinkage or disease progression<sup>19</sup>, and

therefore changes in serum CA19-9 concentration during treatment often serve as a parameter for efficacy in the setting of a clinical trial.<sup>20</sup> Regarding the usefulness of CA19-9 in patients receiving chemotherapy for advanced pancreatic cancer, a decrease in CA19-9 concentration has been proposed as a surrogate marker for survival in several retrospective studies<sup>9, 10, 11, 13, 15, 20, 21</sup>, and pretreatment CA19-9 values have been an independent predictor for survival in some other studies.<sup>12, 21, 22</sup> However, in former studies, different definitions of CA19-9 response were used (between 20% and 50% decrease from pretreatment CA19-9). In this study, we made a definition of TM-PD for earlier confirmation of PD. As mentioned in the Patients and Methods section, we defined the criteria of TM-PD from the analysis of TM fluctuation ratio in Group A, which was applicable to the analysis of early confirmation of treatment failure in Group B. As a result, we found that the TM fluctuation ratio could serve an earlier detection marker of disease progression than RECIST criteria.

Formerly, Rocha, Lima et al found a strong correlation between CA19-9 progression and TTP with CA19-9 progression preceding radiographic progression in most of their patients.<sup>23, 24</sup> However, in their studies, CA19-9 was measured every 3 weeks and imaging studies were performed every 6 weeks. In our study, both the imaging study and the measurement of TMs were performed every 4 weeks. This is the

strength of our study because our data was feasible for detailed analysis. Therefore, our study supports a more precise correlation between TM-TTP and TTP.

Ko et al reported that a rising/nondeclining CA19-9 appeared to be a clear indicator of early progressive disease and to correlate with very poor clinical outcomes.<sup>10</sup> In our study, there were marked differences in the fluctuation of TMs between PD patients and disease-controlled, i.e. stable disease and partial response, patients during chemotherapy. The TM fluctuation ratio of CA19-9 one month after initial chemotherapy induction positively increased in most PD patients (74%; 17 of 23 patients). On the other hand, significantly fewer disease-controlled patients (34%; 11/33) had a positive fluctuation ratio (P = 0.003). Furthermore, the median TM fluctuation ratio of CA19-9 one month after initial chemotherapy induction was 27% (interquartile range [IQR]: 0% to 112%) in PD patients, -12% (IQR: -41% to 25%) in stable disease and -68% (IQR: -73% to -22%) in partial response, as mentioned in the Result section.

Consequently, we can strongly support the fact that the positive fluctuation ratio of CA19-9 is a clear indicator of early disease progression.

However, CA19-9 is a sialylated Lewis<sup>a</sup> (Le)<sup>a</sup> blood group antigen and individuals with Lewis-negative phenotype (lacking the Lewis antigen glycosyltransferase), who comprise approximately 5% of the

population, are unable to synthesize CA19-9.<sup>25</sup> Kawa et al reported that DUPAN-2 was the precursor of CA19-9. Furthermore, they described that SPan-1 had an advantage over CA19-9 in the diagnosis of patients with Lewis-negative phenotype. In addition, the two markers had almost the same sensitivity for this malignancy.<sup>26</sup> In our study, although DUPAN-2 was not used as a marker for assessment of early confirmation of disease progression, SPan-1 was found to be as useful a marker as CA19-9 for the evaluation of disease control. In fact, SPan-1 could detect treatment failure earlier than RECIST criteria in 59% of the cases (Table 2-B). Moreover, in the case of the patients whose CA19-9 and SPan-1 values were both more than the baseline (81%: 66/81 in our study), we could make earlier confirmation of treatment failure by using TM-PD criteria of both CA19-9 and SPan-1 (72%, 35/49; Table 3). Accordingly, it is suggested that the monitoring of TMs during chemotherapy using our method can facilitate the change of treatment at an earlier point in the disease course.

There are some limitations to our study. The number of patients was small and the two regimens using gemcitabine were included. However, we could demonstrate that there was statistically significant correlation between TM-TTP and TTP regarding CA19-9 and SPan-1 ( $r = 0.798$ ,  $P < 0.001$ ;  $r = 0.465$ ,  $P = 0.006$ ; respectively). As for the regimen, although cases with both 4-week and 3-week

gemcitabine-monotherapy schedules were included, the regimen was randomly allocated. Furthermore, in this trial, the 3-week regimen demonstrated the same efficacy and less toxicity compared with the 4-week regimen.<sup>16</sup> Future larger studies are required to establish the role of CA19-9 and SPan-1 monitoring as a biomarker to confirm disease progression earlier in patients with pancreatic cancer treated with gemcitabine.

In conclusion, monitoring of serum CA19-9 and/or SPan-1 is helpful for earlier confirmation of treatment failure in the treatment of patients with pancreatic cancer during gemcitabine monotherapy. The only chance to improve the prognosis of patients with pancreas cancer is changing the chemotherapeutic regimens when the first such regimen fails. Despite the existence of few promising second-line therapies, it is suggested that our findings will assist physicians in deciding on changes of regimen earlier in the progression of disease, when imaging findings are still equivocal.

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**Table 1-A.** Patient Characteristics: CA19-9

	<b>Group A</b>	<b>Group B</b>	<b>P value</b>
No. of patients	38	18	
Median age, years (range)	67 (42-75)	63 (55-74)	0.50
Gender			0.18
Male, n	28	10	
Female, n	10	8	
Baseline KPS			0.97
100 %, n	16	7	
90 %, n	18	9	
80 %, n	4	2	
Stage (UICC criteria)			0.95
III, n	4	2	
IV, n	34	16	
Median basal CA19-9 value, U/ml (range)	859.3 (40.8-4237600)	3164 (52.5-381460)	0.38
Tumor response			0.77
PR, n	5	4	
SD, n	18	6	
PD, n	15	8	
Reason for decision of progression of disease			0.83
RECIST, n	33	16	
Clinical PD, n	5	2	
Introduction of second-line chemotherapy			0.59
PR, %	100	100	
SD, %	83	100	
PD, %	67	75	
PR+SD+PD, %	79	89	
Median survival time, days (range)	239 (39-1026)	269 (97-821)	0.70

Abbreviations: KPS = Karnofsky performance status scale; PR = partial response; SD = stable disease; PD = progressive disease; RECIST = Response Evaluation Criteria in Solid Tumors.

**Table 1-B. Patient Characteristics: SPan-1**

	<b>Group A</b>	<b>Group B</b>	<b>P value</b>
No. of patients	36	17	
Median age, years (range)	66 (42-75)	63 (55-74)	0.71
Gender			0.38
Male, n	27	10	
Female, n	9	7	
Baseline KPS			0.70
100 %, n	15	6	
90 %, n	17	10	
80 %, n	4	1	
Stage (UICC criteria)			0.96
III, n	5	3	
IV, n	31	14	
Median basal SPan-1 value, U/ml (range)	314.4 (30.8-190000)	547.9 (81.7-8460.3)	0.37
Tumor response			0.92
PR, n	6	3	
SD, n	17	7	
PD, n	13	7	
Reason for decision of progression of disease			0.52
RECIST, n	30	16	
Clinical PD, n	6	1	
Introduction of second-line chemotherapy			0.96
PR, %	100	100	
SD, %	88	100	
PD, %	69	71	
PR+SD+PD, %	83	88	
Median survival time, days (range)	246 (39-1026)	260 (136-560)	0.47

Abbreviations: KPS = Karnofsky performance status scale; PR = partial response; SD = stable disease; PD = progressive disease; RECIST = Response Evaluation Criteria in Solid Tumors.

**Table 2-A. Comparison of time to progression of tumor marker (TM-TTP) under TM-PD criterion of CA19-9 with time to tumor progression (TTP) under RECIST criteria in Group B**

	<b>Total</b>	<b>PD</b>	<b>SD</b>	<b>PR</b>
	N = 18	N = 8	N = 6	N = 4
	No. (%)	No. (%)	No. (%)	No. (%)
TM-TTP ≤ TTP	16 (89%)	7 (87%)	5 (83%)	4 (100%)
TM-TTP > TTP	2 (11%)	1 (13%)	1 (17%)	0
Median difference between TM-TTP and TTP (95%CI); months	-1.0 (-4.4- 1.0)	-0.4 (-1.3- 1.5)	-1.0 (-3.3- 1.0)	-2.8 (-5.7--1.0)
Comparison between median TM-TTP and median TTP	† P = 0.738			

\* P value was calculated by sign test.: P = 0.001

† P value was calculated by median test.

**Table 2-B. Comparison of time to progression of tumor marker (TM-TTP) under TM-PD criterion of SPan-1 with time to tumor progression (TTP) under RECIST criteria in Group B**

	<b>Total</b>	<b>PD</b>	<b>SD</b>	<b>PR</b>
	N = 17	N = 7	N = 7	N = 3
	No. (%)	No. (%)	No. (%)	No. (%)
TM-TTP ≤ TTP	13 (77%)	5 (71%)	5 (71%)	3 (100%)
TM-TTP > TTP	4 (23%)	2 (29%)	2 (29%)	0
Median difference between TM-TTP and TTP (95%CI); months	-1.0 (-3.5- 2.1)	0 (-1.3- 3.3)	-1.5 (-3.3- 1.0)	-3.0 (-4.0- -2.0)
Comparison between median TM-TTP and median TTP	† P = 0.727			

\* P value was calculated by sign test.: P = 0.049

† P value was calculated by median test.

**Table 2-B. Comparison of time to progression of tumor marker (TM-TTP) under TM-PD criterion of SPan-1 with time to tumor progression (TTP) under RECIST criteria in Group B**

	<b>Total</b>	<b>PD</b>	<b>SD</b>	<b>PR</b>
	N = 17	N = 7	N = 7	N = 3
	No. (%)	No. (%)	No. (%)	No. (%)
TM-TTP ≤ TTP	13 (77%)	5 (71%)	5 (71%)	3 (100%)
TM-TTP > TTP	4 (23%)	2 (29%)	2 (29%)	0
Median difference between TM-TTP and TTP (95%CI); months	-1.0 (-3.5- 2.1)	0 (-1.3- 3.3)	-1.5 (-3.3- 1.0)	-3.0 (-4.0- -2.0)
Comparison between median TM-TTP and median TTP	† P = 0.727			

\* P value was calculated by sign test.: P = 0.049

† P value was calculated by median test.

**Table 3. Comparison of time to progression of tumor marker (TM-TTP) using a combination of CA19-9 and SPan-1 with TM-TTP using CA19-9**

	<b>Total</b> N = 49		<b>PD</b> N = 19		<b>SD</b> N = 22		<b>PR</b> N = 8	
TM-TTP < TTP	30 (61%)	→ 35 (72%)	6 (31%)	→ 10 (53%)	17 (77%)	→ 18 (82%)	7 (88%)	→ 7 (88%)
TM-TTP = TTP	12 (25%)	→ 9 (18%)	10 (34%)	→ 7 (37%)	2 (9%)	→ 2 (9%)	0	→ 0
TM-TTP > TTP	7 (14%)	→ 5 (10%)	3 (16%)	→ 2 (10%)	3 (14%)	→ 2 (9%)	1 (12%)	→ 1 (12%)
Median difference between TM-TTP and TTP (95%CI); months	-1.0 (-3.9- 1.1)	-1.0 (-5.3- 0.5)	0 (-1.0- 1.0)	0 (-1.0- 0.5)	-2.4 (-4.5- 1.0)	-3.0 (-6.7- 0.5)	-1.3 (-4.1- 1.3)	-2.1 (-5.1- 1.3)
‡Comparison between median TM-TTP and median TTP	P = 0.069	P = 0.105	P = 0.324	P = 0.104	P = 0.366	P = 0.016	P = 1	P = 0.133

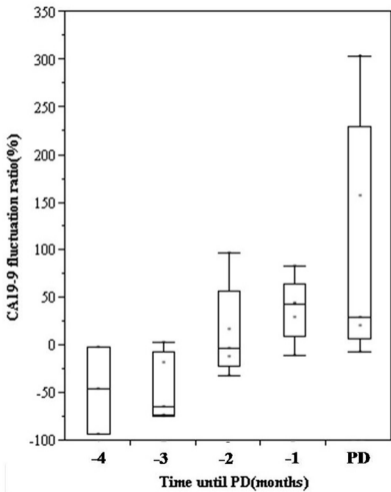
CA19-9 → CA19-9 + SPan-1; n, (%)

P values were calculated by sign test.: \* P = 0.152, † P = 0.004

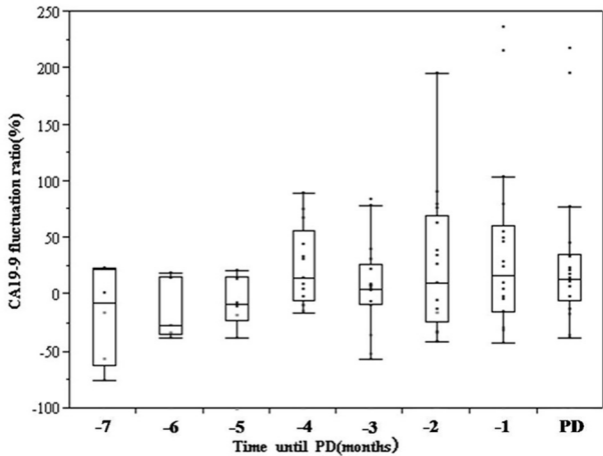
‡ P values were calculated by median test.



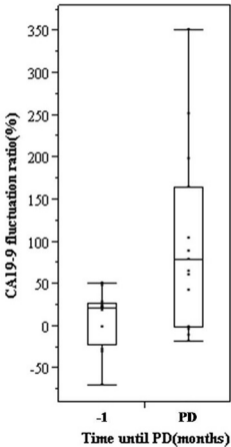
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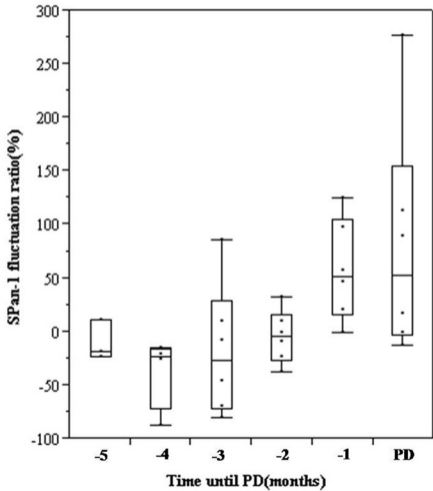
B



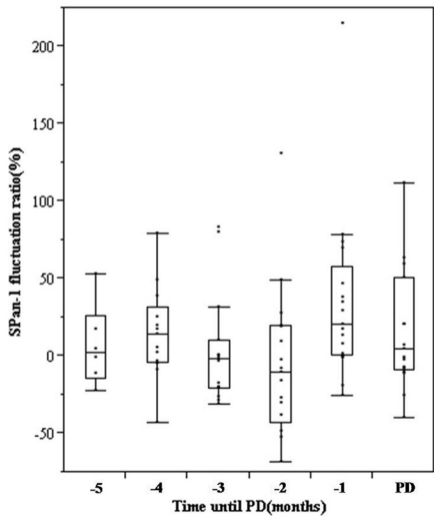
C



A



B



**C**