Original Article

Dynamic monitoring of serum soluble programmed cell death ligand 1 as a response predictor to chemotherapy in metastatic or recurrent gastrointestinal cancer

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Background: Biomarkers in serum may have important clinical implications for personalized medicine, including therapeutic guidance, and monitoring of recurrence. The role of programmed cell death ligand 1 (PD-L1) expression as a tumor biomarker remains controversial. In this study, we aimed at determining the changes of soluble PD-L1 (sPD-L1) during first-line chemotherapy and assessing the association with treatment response and progression-free survival (PFS) of patients with advanced gastrointestinal cancer.

Methods: Blood samples from 115 gastrointestinal cancer patients who have not received any previous systemic chemotherapy for recurrent or metastatic disease were collected at the time of diagnosis and each response evaluation. Serum of sPD-L1 expression was tested by enzyme-linked immunosorbent assay (ELISA). The associations between the baseline level of serum sPD-L1 and clinical-pathological characteristics and prognosis were analyzed. we further dynamically monitored the level change of serum sPD-L1 during treatment and analyzed its relationship with clinical-pathological characteristics, chemotherapy response and prognosis.

Results: Among 115 metastatic gastrointestinal patients, the median serum sPD-L1 level was 0.777 (range, <0.156–6.680) ng/mL. In most cases, changes in sPD-L1 level correlated with treatment response. Patients with values of serum sPD-L1 decreasing after chemotherapy had better tumor response and median PFS compared with patients with values increasing after chemotherapy (ORR, 88.3% *vs.* 54.0% P=0.000005 and PFS, not reached *vs.* 27 months, P=0.00026). D-values of sPD-L1 in patients with progressive disease (PD) were observed increasing from 0.406 to 1.097 ng/mL between pre- and post-chemotherapy, while in those with better tumor response D-values decreased from 1.153 to 0.791 ng/mL after chemotherapy compared with baseline. In the logistic regression analysis, the change of sPD-L1 levels in serum after chemotherapy were found to be a prognostic factor for treatment response and PFS in the multivariate analysis.

Conclusions: These results showed for the first time that sPD-L1 in serum samples of patients with advanced gastrointestinal cancer were changed after chemotherapy and increased serum sPD-L1 levels were poor prognostic factors for both tumor response and PFS of patients. Dynamic monitoring of serum sPDL1 after treatment may be served as a potential predictor to treatment response in gastrointestinal cancer

patients.

Keywords: Gastrointestinal cancer; serum programmed cell death ligand 1 (serum PD-L1); chemotherapy; tumor marker

Submitted Dec 09, 2019. Accepted for publication Feb 24, 2020.
doi: 10.21037/tcr.2020.03.23
View this article at: http://dx.doi.org/10.21037/tcr.2020.03.23

Introduction

Gastric cancer (GC) and colorectal cancer (CRC) are very common malignant tumors and leading causes of cancer-related death worldwide (1,2). Despite the development of multimodality therapies such as surgery, radiation therapy, chemotherapy and introduction of molecular targeted drugs in the past decades, the mortality rates from metastatic gastrointestinal cancer remained dismal (3). The poor prognosis highlights the urgent need for novel therapeutic approaches. Recently, breakthroughs in immune checkpoint blockade have offered new therapeutic options for many malignancies (4-7). Immunotherapy targeting the checkpoint programmed cell death protein 1 (PD-1) or programmed cell death ligand 1 (PD-L1) has been shown to be effective in the management of refractory metastatic GC (mGC) and microsatellite instability high (MSI-H) metastatic CRC (mCRC) (8,9).

PD-1 is a negative co-stimulatory receptor expressed mainly on activated T cells, which downregulates excessive immune responses by binding to its ligands, PD-L1 and PD-L2 (10,11). PD-L1 is constitutively expressed in various tissues and in different tumor types including gastrointestinal cancer (12). PD-L1 expression in tumor tissue was related to anti-PD-1/PD-L1 response (12-14). Based on the results of KEYNOTE 059 study (15), Food and Drug Administration (FDA) granted accelerated approval to Pembrolizumab (Merck & Co., Inc., USA) for patients with unresected advanced/metastatic gastric or gastroesophageal junction adenocarcinoma whose tumor express PD-L1 as determined by an FDA-approved test.

Molecular analyses are typically performed on tissues at initial diagnosis (16). However, in some cases, metastatic tumors have different molecular alterations from primary tumors (17,18). PD-L1 expression level in tumor tissue is also affected by the timing of biopsy, composition of tumor tissues, cancer treatment or host immune response (19-21). Hence, a dynamic reassessment of molecular alteration might help to optimize treatment. However, serial biopsies are not practical in practical clinical activity. Soluble PD-L1 (sPDL1) is thought to be a circulating biologically active protein which is released from PD-L1-positive tumor cells or immune cells, and binds to PD-1 receptor which contributes to systemic immunosuppression (22,23). The sPDL1 expression status has been reported to be an independent prognostic factor in various malignant tumors (24-29). In these circumstances, dynamic assessment of sPDL1 expression of metastatic gastrointestinal cancer is considered as a potential strategy with more precise clinical application.

However, the prognostic value of baseline serum sPD-L1 level in gastrointestinal cancer patients remained debate (28,30). The relationship between dynamic change of serum sPD-L1 level and treatment response to chemotherapy has not been investigated. Thus, a prospective cohort study was conducted to investigate the prognostic or predictable value of serum sPD-L1 baseline level and its dynamic change for metastatic gastrointestinal cancer patients.

Methods

Patient

Patients with histologically diagnosed gastrointestinal adenocarcinoma and radiologic confirmation of metastatic or recurrent lesions in Sun Yat-sen university cancer center were enrolled.

The inclusion criteria were as follows: (I) confirmed gastrointestinal adenocarcinoma pathologically; (II) has not received any previous systemic chemotherapy for recurrent or metastatic disease; (III) at least one computed tomography (CT) or magnetic resonance imaging (MRI) response evaluation; (IV) measurable disease based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; (V) Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) 0-1; (VI) complete follow-up medical records; (VII) available informed consent for the access to medical information and blood sample.

Baseline clinical and laboratory assessments, including age, gender, tumor stage (7th AJCC TNM stage), tumor size, tumor primary site, metastatic site, metastatic organ numbers, surgical history, cigarette smoking, drinking, HER2 status (for GC) and RAS status (for CRC) were collected from hospital database.

Treatment

Patients with HER2-negative mGC received first-line dual chemotherapeutic regimens including fluoropyrimidine (S-1, capecitabine or 5-fluorouracil) and platinum (cisplatin or oxaliplatin). For patients with HER2-positive, dual chemotherapy combined with Trastuzumab was carried out.

Patients with mCRC received first-line chemotherapeutic regimens with fluoropyrimidine (capecitabine or 5-fluorouracil) combined with oxaliplatin or irinotecan. For RAS wild type patients, chemotherapy combined with Bevacizumab or Cetuximab was recommended. For RAS mutant type patients, chemotherapy combined with Bevacizumab was recommended.

Patients continued chemotherapy until disease progression or intolerable toxicity. Response evaluation was performed every 6 or 8 weeks by contrast-enhanced CT or MRI.

Blood sample collection

Blood samples, before initiating first-line chemotherapy and within 48 h before response evaluation, was drawn into Serum Separation Tubes with polymer gel/silica activator. According to standard operating procedure, serum was prepared within 1 hour of sample collection after centrifugation (1,000 ×g) for 20 min and immediately stored at –80 ℃. In our study, the blood routine, biochemical test and blood samples in peripheral vein blood were detected or collected at the same time before treatment, CT or MRI response evaluation was taken simultaneously.

Serum sPD-L1 measurement

Serum sPD-L1 levels were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (USCN, Wuhan, China, catalogue: SEA788Hu). ELISA was conducted as follows: (I) according to the manufacturer's instructions, all chemical agent, standard dilutions, and specimen were prepared; (II) added 100 μL of the standard and sample to each well; (III) covered the plates with a plate sealer and carefully placed at 37 ℃ hatched for 120 min; (IV) after this step, added 100 μL of detection reagent A (1:100) to each well, and re-sealed the plates and hatched at 37 ℃ for another 120 min; (V) each well was washed four times by wash buffer after aspirated; (VI) added 100 μL of detection reagent B (1:100) to each well, and re-sealed the plates were and incubated for 30 min at 37 ℃; (VII) washed each well five times after aspirated, added 90 μL of substrate solution, and the plates were newly sealed and incubated in a dark room for 20 min at 37 ℃; (VIII) added 50 μL of stop solution to each well, and measured absorbance at 450 nm immediately in Bio-Tek EPOCH2 Microplate Reader (Bio-Rad Laboratories, USA).

We measured sPD-L1 protein levels by using standard curves. Four parameters logistic regression (4PL) calibration models were selected to design the standard curve of sPD-L1 ELISA. The detectable dose range of sPD-L1 was 0.057 to 20 ng/mL and minimum quantitative range was 0.156 ng/mL.

Statistical analysis

Responses to treatment were divided into complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), and non-evaluable (NE), according to the RECIST criteria version 1.1.

Progression-free survival (PFS) was defined as the time from initiation of first-line treatment to disease progression or death without evidence of progression. The prognostic factors of PFS were analyzed by univariate analysis. Continuous variables were summarized using $\bar{x} \pm s$ and median (range). The χ^2-test was used to explore the associations between sPD-L1 expression/sPD-L1 change and clinical characteristics. Differences in the distribution of more than two variables were evaluated by the Kruskal-Wallis test Means between two unrelated groups on sPD-L1/sPD-L1 change was examined by independent samples *t*-test.

The cut-off point for sPD-L1 was determined by the software named Xtiles. SPSS 20.0 statistical package (SPSS Inc., Chicago, IL, USA) was used for all the statistical analyses and graphics. $P<0.05$ was considered statistically significant. Graphs were carrying through GraphPad Prism 5.01 (GraphPad Software Inc. La Jolla, CA, USA).

Study oversight

All procedures conducted in this study were conformity with

the ethical standards of the institutional research committee and with the Helsinki Declaration and its amendments in 1960s. This research protocol was approved by the ethics committee of Sun Yat-sen University Cancer Center.

Result

Patients' clinical-pathologic characteristics

Totally 115 patients diagnosed with metastatic gastrointestinal cancer in Sun Yat-sen University Cancer Center (53 GC patients and 62 CRC patients) with detail medical records were enrolled between January 2011 to December 2017.

Table 1 summarized their clinical-pathologic characteristics. The median cycle of first-line chemotherapy was 5.5 cycles (range, 1–18). For patients with mGC, the median age was 48.6 (range, 21 to 74) years, 22 of 53 patients had proximal GC, 22 had distal GC, 9 were gastric corpus cancer. Twenty-five patients had multiple sites metastasis (≥2). For CRC, the median age was 49.6 (range, 26 to 71) years, 43 of 62 patients had left-sided CRC. Thirty-six patients had multiple sites metastasis (≥2).

Among 115 patients, 89 patients had one serum sample, 23 patients had two serial serum samples, two patients had serial three samples, and one patient had serial four samples within 48 h before response evaluation.

As of the data cutoff date of December 2017, the median duration follow-up was 16.1 (range, 2 to 81) months.

Correlation of baseline sPD-L1 expression with clinical characteristics

The mean serum sPD-L1 level of the whole cohort was 0.944 (median: 0.77; range, <0.156–6.68) ng/mL (*Figure 1*). In 33 patients (28.7%), serum PD-L1 levels was lower than limit of the ELISA detection (0.156 ng/mL). There was no difference in sPD-L1 values between in patients with mGC (median 0.802 ng/mL; range, <0.156–6.680 ng/mL) and in patients with mCRC (median sPD-L1 level was 0.772 ng/mL; range, <0.156–4.750 ng/mL). By Xtiles software, 0.944 ng/mL was chosen as cut-off value which divided all the patients into sPD-L1 level high subgroup and sPD-L1 level low subgroup.

Table 1 summarized the associations between baseline serum sPD-L1 level and the clinical-pathological features. In whole study population, there was no significant difference of baseline sPD-L1 level between GC and CRC patients. No significant difference was found in the baseline characteristics of patients with high as opposed to low serum sPD-L1 levels. However, liver metastasis was more frequently observed in patients with high levels of sPD-L1 compared with those with low levels of sPD-L1 (mean in liver metastasis group and in no liver metastasis group were 0.750 and 1.048 ng/mL respectively, P=0.036). Patients with mCRC in left-sided diseases had lower baseline sPD-L1 level compared with those with right-sided (mean: 0.714 *vs.* 1.147 ng/mL, P=0.024). The patients with distant lymph node metastasis had lower baseline sPD-L1 level (P=0.04) and those with liver metastasis had higher baseline sPD-L1 levels (P=0.035).

Correlation of dynamic change sPD-L1 expression with clinical characteristics

According to the dynamic change of Serum sPD-L1 level, the patients were divided into two groups: undetected/decrease group and elevated group. The relationship between sPD-L1 level change and clinical characteristics were evaluated (*Table 2*). In whole study population, the patients with family history (P=0.027) or no lymph node metastasis (P=0.003) were less likely to have sPD-L1 level dynamic elevation. The sPD-L1 level elevation was more common in older CRC patients (P=0.016) and lymph node metastasis (P=0.009).

Correlation of sPD-L1 expression with response to chemotherapy

As shown in *Table 1*, the baseline sPD-L1 expression significantly correlated with treatment response (P=0.00046) in whole study population. Patients with serum sPD-L1 >0.944 ng/mL had better tumor response than patients with sPD-L1 <0.944 ng/mL. There was higher baseline expression sPD-L1 in patients with PR/SD, compared with patients with PD (1.153 *vs.* 0.406 ng/mL, P<0.0001). The same phenomenon was observed in GC and CRC subgroups (P=0.043 and P=0.003, respectively).

In this study, we collected 145 pairs data of serum sPD-L1 pre- and post-chemotherapy or between each course of treatment, and 140 cases' response evaluation (five patients with no evaluation). According to the counterpart chemotherapy treatment responses, the pairs of serum samples were separated into Baseline1-PR/SD group and Baseline2-PD group. Thirty-three pairs of sPD-L1 elevated out of 62 pairs GC patients and 30 pairs

Table 1 Association between expression levels of sPD-L1 and clinical characteristics of patients with gastrointestinal cancer

Patients characteristics	All (N=115)			mGC (N=53)			mCRC (N=62)		
	sPD-L1 ≤ cutoff, n (%) (0.9442 ng/mL)	sPD-L1 > cutoff, n (%)	P value	sPD-L1 ≤ cutoff, n (%)	sPD-L1 > cutoff, n (%)	P value	sPD-L1 ≤ cutoff, n (%)	sPD-L1 > cutoff, n (%)	P value
Gender			0.756			0.340			0.647
Male	35 (60.3)	23 (39.7)		14 (53.8)	12 (46.2)		21 (65.6)	11 (35.4)	
Female	36 (63.2)	21 (36.8)		18 (66.7)	9 (33.3)		18 (60.0)	12 (40.0)	
Age (mean ± SD)	50.0±1.4	46.0±1.6	0.188	50.9±2.3	45.0±2.9	0.465	50.6±1.8	47.9±1.7	0.263
Tumor type			0.781			–			–
CRC	39 (62.9)	23 (37.1)		–	–		–	–	
GC	32 (60.4)	21 (39.6)		–	–		–	–	
Tumor site						0.124			0.024
GC									
Proximal	–	–		13 (59.1)	9 (40.9)		–	–	
Distal	–	–		16 (72.7)	6 (27.3)		–	–	
CRC									
Left-sided	–	–		–	–		31 (72.1)	12 (27.9)	
Right-sided	–	–		–	–		8 (42.1)	11 (57.9)	
Differentiation			0.278			0.581			0.207
Well/ intermediate differentiated	22 (55.0)	18 (45.0)		3 (50.0)	3 (50.0)		19 (55.9)	15 (44.1)	
Poorly differentiated	49 (65.3)	26 (34.7)		29 (61.7)	18 (38.3)		20 (71.4)	8 (28.6)	
Clinical stage (AJCC, 7th)			0.216			0.692			0.225
I and II	5	5		2	1		3	4	
III and IV	63 (61.8)	39 (38.2)		29 (59.2)	20 (40.8)		33 (63.5)	19 (36.5)	
Metastasis									
Lymph node (no/yes)	9/59 (13.2/86.8)	12/32 (27.3/72.7)	0.063	2/29 (6.5/93.5)	2/19 (9.5/90.5)	0.683	7/30 (18.9/81.1)	10/13 (43.5/56.5)	0.04
Liver (no/yes)	55/16 (77.5/22.5)	26/18 (59.1/40.9)	0.036	29/3 (90.6/9.4)	17/4 (80.9/19.1)	0.309	26/13 (66.7/33.3)	9/14 (39.1/60.9)	0.035
Multi-site transfer (no/yes)	31/37 (45.6/54.4)	20/24 (45.5/54.5)	0.989	15/15 (50/50)	11/10 (52.4/47.6)	0.867	15/22 (40.5/59.5)	9/14 (39.1/60.9)	0.914
Surgery history			0.871			0.997			0.726
None	26	14		12	8		14	6	
Radical operation	24	16		10	7		13	9	

Table 1 (*continued*)

Table 1 (*continued*)

Patients characteristics	All (N=115)			mGC (N=53)			mCRC (N=62)		
	sPD-L1 ≤ cutoff, n (%) (0.9442 ng/mL)	sPD-L1 > cutoff, n (%)	P value	sPD-L1 ≤ cutoff, n (%)	sPD-L1 > cutoff, n (%)	P value	sPD-L1 ≤ cutoff, n (%)	sPD-L1 > cutoff, n (%)	P value
Palliative operation	21	14		9	6		12	8	
Family tumor history (no/yes)	45/26 (63.4/36.6)	24/20 (54.4/45.5)	0.347	22/10 (68.8/31.2)	12/9 (57.1/42.9)	0.389	23/16 (59/41)	12/11 (52.2/47.8)	0.602
Smoking			0.927			0.448			0.469
Current	13	7		7	3		6	4	
Former	7	5		4	1		3	4	
Never	51	32		21	17		30	15	
Drinking			0.896			0.824			0.741
Current	8	4		3	1		5	3	
Former	4	2		3	2		1	0	
Never	59	38		26	18		33	20	
Treatment response (CR + PR + SD/PD)	41/28	38/4	0.000460	18/13	17/3	0.043	23/15	21/1	0.003
MMR			–			–			–
dMMR	3	2		3	1		1	0	
pMMR	22	13		12	6		10	7	
HER2			–			–			–
Negative	–	–		16	11		–	–	
Positive	–	–		2	0		–	–	
Unknown	–	–		13	10		–	–	
RAS			–			–			–
Wild	–	–		–	–		19	16	
Mutated	–	–		–	–		6	4	

sPD-L1, soluble programmed cell death ligand 1; mGC, metastatic gastric cancer; mCRC, metastatic colorectal cancer; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; MMR, mismatch repair; dMMR, deficient MMR; pMMR, proficient MMR.

increased in 83 pairs of patients with CRC (*Figure 2*). The mean sPD-L1 levels in PR/SD group decreased from pre-treatment baseline 1.153±1.115 ng/mL to post-treatment 0.791±0.574 ng/mL (P=0.004), while the mean sPD-L1 levels in PD group increased from pre-treatment baseline 0.406±0.466 ng/mL to post-treatment 1.097±0.984 ng/mL (P=0.00026) (*Figure 3A*). In GC subgroup, the mean sPD-L1 levels decreased from 1.185±1.269 to 0.850±0.616 ng/mL in PR/SD group and increased from 0.439±0.541 to 0.891±0.814 ng/mL in PD group (*Figure 3B*). In CRC subgroup, the mean sPD-L1 levels decreased from 1.132±1.010 to 0.720±0.532 ng/mL in PR/SD group and increased from 0.372±0.390 to 1.303±1.113 ng/mL in PD group, P=0.0023 (*Figure 3C*). Compared with baseline level, sPD-L1dynamically decreased in the PR/SD group and dynamically increased in the PD group.

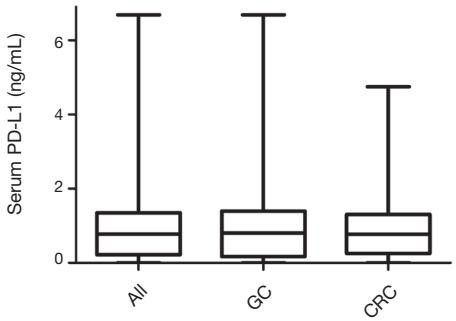

Figure 1 The boxplot of sPD-L1 level in whole patients. sPD-L1, soluble programmed cell death ligand 1; GC, gastric cancer; CRC, colorectal cancer.

Correlation between the changes of sPD-L1 during chemotherapy and treatment response was assessed in the 145 pairs data (*Figure 4*). Sixty-six pairs of sPD-L1 value were elevated during chemotherapy. Corresponding evaluation of curative effect, PD was found in 29/66 cases, and PR or SD in 34/66 cases. While among 79 pairs with declining sPD-L1 during anticancer treatment, PD was observed in 9/79 cases, and PR or SD in 68/79 cases (P=0.000005). In the follow-up courses, serum sPD-L1 values of patients with PR or SD were constantly decreasing compared with increasing in patients with PD.

Correlation of serum sPD-L1 level with prognosis

Median PFS for the whole study population was 11.1 (range, 1–72) months, with a median follow-up of 16.1 (range, 2–81) months. In cox proportional hazards model, variables showing tendencies for positive association with PFS in univariate analysis were selected. As shown in *Table 3*, the patients with advanced tumor stage, tumor diameter >5 cm or with multiple metastases (≥2) had shorter PFS.

PFS was significantly longer in sPD-L1 level decreasing/ stable group than those in sPD-L1 level increasing group (not reached *vs.* 27 months; HR, 3.032; 95% CI, 1.323–6.948; P=0.00026) (*Figure 5*). In subgroup analysis, PFS of sPD-L1 decreasing/ stable group *vs.* sPD-L1 level increasing group in GC was not reached *vs.* 27 months; HR, 0.649; 95% CI: 0.186–2.272; P=0.029. PFS of sPD-L1 decreasing/stable group *vs.* sPD-L1 level increasing group in CRC was 41 *vs.* 28 months; HR, 2.834; 95% CI, 0.910–8.833; P=0.01.

Patients with low baseline sPD-L1 level showed shorter PFS than those with high baseline sPD-L1 level (36 months *vs.* not reached; HR, 0.600; 95% CI, 0.227–1.584, P=0.016). In subgroup analysis, PFS of GC patients with low baseline sPD-L1 level *vs.* high baseline sPD-L1 level was 36 months *vs.* not reached; HR, 0.649; 95% CI, 0.186–2.272; P=0.092. PFS of CRC patients with low baseline sPD-L1 level *vs.* high baseline sPD-L1 level was 36 months *vs.* not reached; HR, 0.451; 95% CI, 0.091–2.230; P=0.069. We need to enlarge subgroup samples in later work to confirm this tendency.

Discussion

Biomarker-driven selection of immunotherapy responders and non-responders would minimize unnecessary exposure of patients to potentially immune-related toxicities. As anti-PD-1 pathway immunotherapies are effective in only a minority of gastric-intestinal cancer patients, there is a great need for reliable and easily available biomarkers of patient response. PD-L1 expression appeared to correlate with response to treatment from exploratory analyses of early reported trials, whether the level of expression of PD-L1 predicts response rate, PFS and OS in the context of anti-PD-1/PD-L1 therapy were explored in multiple malignant tumors with inconsistent results. sPD-L1 being released from immune cells or tumor cells can bind receptors in a similar manner as their membrane-bound counterparts and as a result may play a more widespread role in PD-1/PD-L1 axis. This study measured the baseline level of circulating sPD-L1 and dynamical change of sPD-L1serum level during treatment. No study has been reported to identify circulating sPD-L1 expression in patients with CRC until now. It is the first report to evaluate the relation between the baseline expression and dynamic change of sPD-L1 expression in metastatic gastrointestinal cancer and their prognostic or predictable value.

The mean value of sPD-L1 level was 0.937±0.945 ng/mL in GC group. Our data on GC was slightly higher than those reported in previous studies, the median sPD-L1 level was 0.704 ng/mL in Japanese GC population and 0.8928 ng/mL in northern Chinese GC population, respectively (28,30). Different study population, treatment stage of specimen collection and various test methods may contribute to the differences. Patients with liver metastasis had higher sPD-L1 level in whole study population and patients with mCRC. In the subgroup analysis, patients with mCRC whose family tumor history were more likely to have higher sPD-L1level and patients with right-sided CRC tended to have higher sPD-L1 level. It is well-known

Table 2 Association between sPD-L1 change and clinical characteristics of patients with gastrointestinal cancer

Patients characteristics	All (N=115)				GC (N=53)			CRC (N=62)		
	Number (%) (n=115)	Undetectable/ decrease in sPDL1	Elevation in sPDL1	P value	Undetectable/ decrease in sPDL1	Elevation in sPDL1	P value	Undetectable/ decrease in sPDL1	Elevation in sPDL1	P value
Gender (male/ female)	58/57 (50.4/49.6)	31/32 (51.2/48.8)	27/25 (54/46)	0.772	13/13 (50/50)	14/13 (51.9/48.1)	0.893	18/20 (47.4/52.6)	14/11 (56/44)	0.503
Age (young/old)	59/56 (51.3/48.7)	36/27 (57.1/42.9)	23/29 (44.2/55.8)	0.168	12/14 (46.2/53.8)	15/12 (55.6/44.4)	0.494	24/14 (63.2/36.8)	8/17 (32/68)	0.016
Tumor type				0.254						
CRC	62 (53.9)	37 (59.7)	25 (40.3)							
GC	53 (46.1)	26 (49.0)	27 (51.0)							
Tumor site				–			0.160			0.135
Proximal	–	–	–		10	12		–	–	
Distal	–	–	–		9	13		–	–	
Left-sided	–	–	–		–	–		23	20	
Right-sided	–	–	–		–	–		14	5	
Differentiation				0.225			0.413			0.282
Well/ intermediate differentiated	40	25 (62.5)	15 (37.5)		2 (33.3)	4 (66.7)		23 (67.6)	11 (32.4)	
Poorly differentiated	75	38 (50.7)	37 (49.3)		24 (51.0)	23 (49.0)		15 (51.7)	14 (48.3)	
Metastasis										
Lymph nodes (yes/no)	91/21 (81.2/18.8)	45/18 (71.4/28.6)	46/3 (93.9/6.1)	0.003	23/3 (88.5/11.5)	25/1 (96.2/3.8)	0.298	23/15 (60.5/39.5)	21/2 (91.3/8.7)	0.009
Liver (yes/no)	34/81	22/41	12/40	0.166	4/22	3/24	0.646	18/19	9/16 (36/64)	0.324
Multi-site transfer (yes/ no)	61/51 (54.5/45.5)	32/31 (50.8/49.2)	29/20 (59.2/40.8)	0.376	13/12 (52/48)	12/14 (46.2/53.8)	0.676	19/19 (50/50)	17/6 (73.9/26.1)	0.066
Clinical stage (AJCC, 7th)				0.306			0.513			0.529
I and II	10 (8.7)	7 (11.1)	3 (5.8)		2 (7.7)	1 (3.7)		32 (84.2)	21 (84.0)	
III and IV	105 (91.3)	56 (88.9)	49 (94.2)		24 (92.3)	26 (96.3)		6 (15.8)	4 (16.0)	
Surgery history				0.902			0.566			0.791
None	40	23 (36.5)	17 (32.7)		11 (42.3)	9 (33.3)		12 (31.6)	8 (32.0)	
Radical operation	40	21 (33.3)	19 (36.5)		7 (26.9)	11 (40.7)		15 (39.5)	8 (32.0)	
Palliative operation	35	19 (30.2)	16 (30.8)		8 (30.8)	7 (25.9)		11 (28.9)	9 (36.0)	

Table 2 (*continued*)

Table 2 (*continued*)

Patients characteristics	All (N=115)				GC (N=53)			CRC (N=62)		
	Number (%) (n=115)	Undetectable/ decrease in sPDL1	Elevation in sPDL1	P value	Undetectable/ decrease in sPDL1	Elevation in sPDL1	P value	Undetectable/ decrease in sPDL1	Elevation in sPDL1	P value
Family tumor history (yes/no)	46/69 (40/60)	31/32 (51.2/48.8)	15/37 (28.8/71.2)	0.027	13/13 (50/50)	6/21 (22.2/77.8)	0.035	19/19 (50/50)	9/16 (36/64)	0.274
Smoking				0.965			0.709			0.598
Current	20	11 (17.7)	9 (17.3)		4 (15.4)	6 (22.2)		7 (18.4)	3 (12.0)	
Former	12	7 (11.1)	5 (9.6)		2 (7.7)	3 (11.1)		5 (13.2)	2 (8.0)	
Never	83	38 (73.1)	45 (71.4)		20 (76.9)	18 (66.7)		26 (68.4)	20 (80.0)	
Drinking				0.906			0.913			0.598
Current	12	6 (9.5)	6 (11.5)		2 (7.7)	2 (7.4)		4 (10.5)	4 (16.0)	
Former	6	3 (4.8)	3 (5.8)		2 (7.7)	3 (11.1)		1 (2.6)	0	
Never	97	54 (85.7)	43 (82.7)		22 (84.6)	22 (81.5)		33 (86.8)	21 (84.0)	

sPD-L1, soluble programmed cell death ligand 1; GC, gastric cancer; CRC, colorectal cancer.

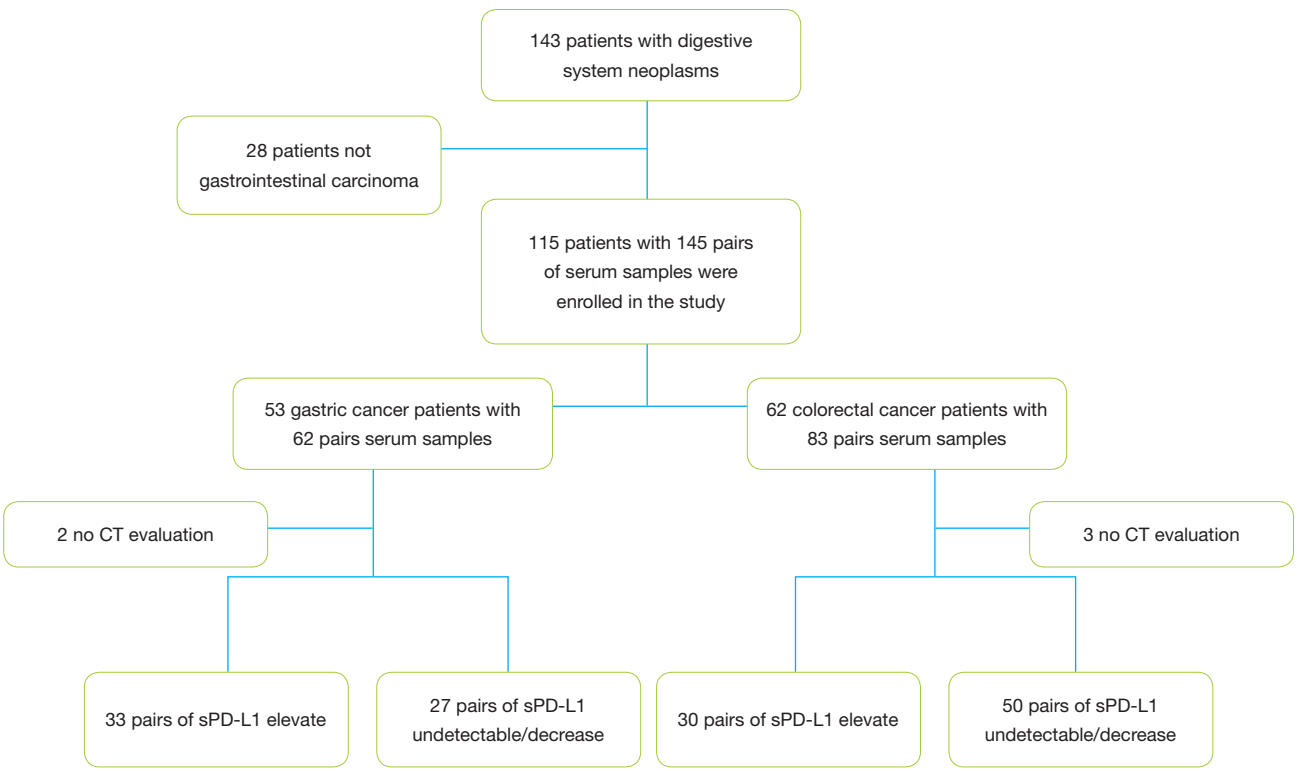

Figure 2 The flowchart of patient selections. sPD-L1, soluble programmed cell death ligand 1; CT, computed tomography.

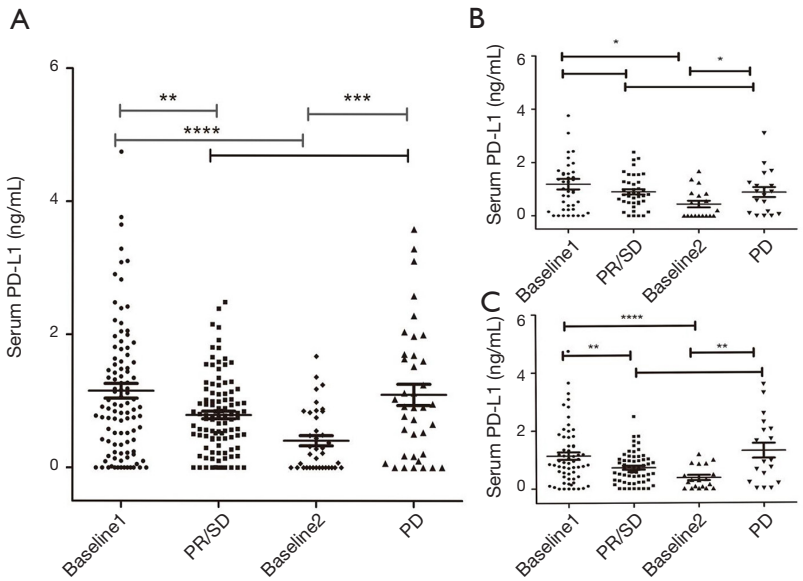

Figure 3 Levels of overall pre-treatment and post-treatment sPD-L1 expression in different chemotherapy outcome groups. The 145 pairs of cases were divided into two groups according to the treatment response: Baselin1-PR/SD group and Baseline2-PD group. sPD-L1 levels of four lines in two groups were significantly different from each other. (A) showed results in all our study population, (B) shows results in GC and (C) was in CRC. P<0.05 are considered statistically significant. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. sPD-L1, soluble programmed cell death ligand 1; PD, progressive disease; GC, gastric cancer; PR, partial response; SD, stable disease; CRC, colorectal cancer.

that right-sided colon with mucosal immune cells had stronger immunogenicity than left-side colon (8,31,32). There is more common MSI-H tumor in the right-sided colon. The proximal colon cancer shows worse OS than the distal CRC (33). The older CRC patients tended to have sPD-L1 level elevated in our study. Previous study reported that sPD-L1 levels increased in an age-dependent manner in health donors, which suggested that serum sPD-L1 was related to the immune state of human being (20). sPDL1 was thought to be released from PD-L1-positive tumor cells or immune cells and can functionally binds to PD-1 receptor then contributed to systemic immunosuppression (23,29). In clinical research, sPD-L1 could found functionally block the regulatory effect of membrane-bound PD-1 on T cell activation in Rheumatoid Arthritis (34). Serum sPD-L1 levels significantly increased in dying septic patients compared with the survivors (P<0.05) (26). In melanoma, both tumor PD-L1 and macrophage PD-L1 expression level after effective treatment were higher than intra-tumoral $CD8^+$ lymphocytes and $CD68^+$ macrophages in biopsies (35).

In our study, higher baseline sPD-L1 levels were more likely to response to chemotherapy and had better PFS. However, the clinical significance of sPD-L1 remains controversial in different tumor types. Several studies showed that high level of sPD-L1 was a negative prognosis factor, indicating lower overall response rate, shorter PFS and shorter OS (24,29,36-38). In NSCLC patients, high sPD-L1 level was significantly related to abdominal organ metastasis (P=0.004) (39). On the contrary, sPD-1/sPD-L1 levels did not appear an unfavorable outcome in advanced pancreatic cancer (40) and the author thought sPD-L1 levels may reflect inflammatory tumor type in PC patients. Zheng found that in advanced GC patients high sPD-L1 correlated with better OS (30). There were three main anticancer immunity phenotypes: the immune-desert phenotype, the immune-excluded phenotype and the inflamed phenotype. Inflamed tumors were infiltrated by a number of subtypes of immune cells. Tumor cells in inflamed tumors can express inhibitory factors, down-regulating MHC class I molecule and other pathways can de-sensitize them to anticancer immunity (31) Clinical responses to anti-PD-L1/PD-1 therapy occur most often in patients with inflamed tumors (31). Finkelmeier found that

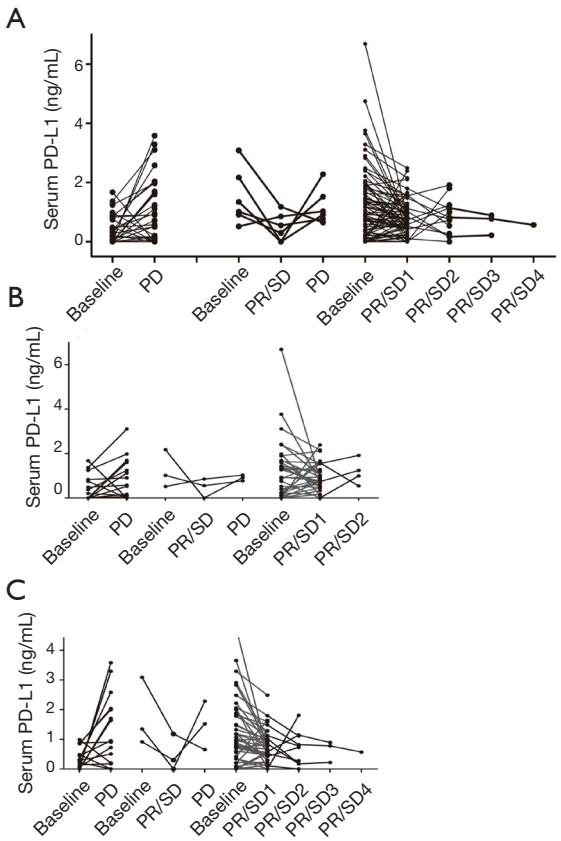

Figure 4 Dynamic changes of individual sPD-L1 levels with corresponding chemotherapy treatment evaluation. This picture illustrated changes in the levels of PD-L1 expression according to different treatment outcome. The same patient at different points of time were recorded. sPD-L1 decreased/stable in serum after effective treatment and sPD-L1 elevated during PD. (A) showed the results in all our study population, (B) showed the results in GC and (C) was in CRC. sPD-L1, soluble programmed cell death ligand 1; PD, progressive disease; PR, partial response; SD, stable disease; CRC, colorectal cancer.

in HCC sPD-L1 levels showed correlation with C-reactive protein, a typical inflammatory maker (24). Okuma inferred that sPD-L1 levels might be determined by both tumor burden and extra-tumoral inflammatory statue (29). We hypothesis that high sPD-L1 may be released by infiltrating immune cells and tumor cells, which indicate an immune-inflammatory state with better treatment outcome.

We can infer that dynamic monitor of sPD-L1 level may be used as a response predictor of chemotherapy in metastatic or recurrent gastrointestinal cancer. It is firstly reported. In our study, the patients with dynamic increase of sPD-L1 level had markedly poorer response to chemotherapy than the patients with stable or decreasing change of sPD-L1. People who got PR/SD showed a decrease/stable trend of sPD-L1 level, however, a significant increase of sPD-L1 level was found once disease progression. sPD-L1 in blood circulation can be released by antigen-presenting cell like activated mature dendritic cells or PD-L1 positive cell lines like tumor cells and bind to PD-1 receptor. We hypothesized that sPD-L1 level in blood circulation was a dynamic balance system. In pre-treatment patients, sPD-L1 is mostly released by infiltrating immune cells reflecting an immunoinflammatory state and after chemotherapy, tumor cells could be active by gene mutation and more sPD-L1 was released to escape immune surveillance which results in T cell exhaustion and dysfunction timely to avoid excessive immune damage and hold self-balance. Once disease progressed, tumor burden increased with more sPD-L1 released by tumor cells, it becomes a vicious cycle.

Table 3 Univariate analysis of PFS by Cox proportional hazards model in sPD-L1 elevated and stable/decreased groups in gastrointestinal cancer

Variables	All		Stomach	Intestinal
	P value (log-rank)	HR (95% CI)	P value (log-rank)	P value (log-rank)
Gender (male/female)	0.408	1.215 (0.754–1.956)	0.182	0.952
Age (<50/≥50)	0.177	1.368 (0.853–2.194)	0.467	0.241
Family tumor history (yes/no)	0.118	0.69 (0.426–1.118)	0.032	0.797
Smoking (yes/no/yet)	0.485	0.998 (0.824–1.21)	0.65	0.597
Drinking (yes/no/yet)	0.858	1.055 (0.836–1.333)	0.677	0.045
Tumor type (GC/CRC)	0.751	0.955 (0.592–1.54)		
Tumor site	0.852		0.649	0.533
Proximal		0.794 (0.396–1.589)		
Distal		0.884 (0.541–1.444)		
Left-sided		1.233 (0.756–2.01)		
Right-sided		0.994 (0.668–1.48)		
Clinical stage (AJCC, 7th)	0.035	0.482 (0.179–1.299)	0.025	0.67
I and II				
III and IV				
Bulky disease (yes/no)	0.047	1.376 (0.983–1.928)	0.153	0.31
Metastasis				
Lymph nodes (yes/no)	0.464	1.283 (0.645–2.552)	0.951	0.254
Liver (yes/no)	0.084	0.601 (0.330–1.093)		
Multi-site transfer (yes/no)	0.016	1.773 (1.082–2.905)	0.056	0.101
Differentiation (well/intermediate vs. poor)	0.572	1.251 (0.794–1.969)	0.808	0.102
Surgery history(none/radical/palliative)	0.067	0.724 (0.522–1.003)	0.004	0.794
sPDL1 (high/low)	0.016	0.600 (0.227–1.585)	0.092	0.097
sPD-L1 (up/down)	0.00026	3.032 (1.323–6.948)	0.029	0.01

sPD-L1, soluble programmed cell death ligand 1; GC, gastric cancer; CRC, colorectal cancer; PFS, progression-free survival.

Our study comes with some limitations. It was a single center study. Health controls are needed to be involved to estimate sPD-L1 levels between different populations. Individuals with different digestive cancer were involved. The follow-up during was not long enough.

Conclusions

Despite these limitations, to our knowledge, this study is the first to explore the association between soluble PD-1 level dynamic change and treatment response to gastrointestinal cancer patients. This is the first prospective study to explore sPD-L1 level in CRC and dynamic monitor sPD-L1 level change and its clinical significance in gastrointestinal cancer. We maybe provide an effective and easy way to predict the chemotherapeutic response to chemotherapy of gastrointestinal cancer patients.

Acknowledgments

Funding: This work was supported by National Natural Science Foundation of China (Grant number. 81602066 and 81772587); the Fundamental Research Funds for the Central Universities (Grant number. 16ykpy25); the

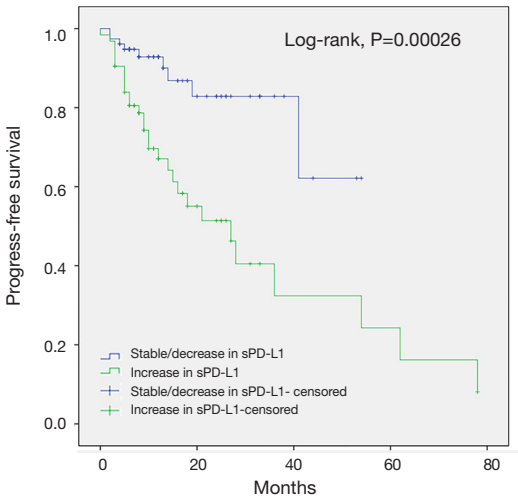

Figure 5 Kaplan-Meier survival curves of PFS according to sPD-L1 change (elevated *vs*. stable/decreased, sPD-L1 change >0 ng/mL was defined elevated here) in gastrointestinal cancer patients. sPD-L1 stable/decreased patients had a significant longer PFS (P=0.00026) than sPD-L1 elevated patients. P value was analyzed by log-rank test. PFS, progression-free survival; sPD-L1, soluble programmed cell death ligand 1.

third outstanding young talents training plan and Medical Scientist program of Sun Yat-sen University cancer center.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2020.03.23). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study is approved by the Ethics Committee of Sun Yat-Sen University Cancer Center (No. GZR2016-017). Informed consent was obtained from all individual participants included in the study.

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Cite this article as: Sun J, Qiu MZ, Mei T, Gao Y, Chang B, Zhang Y, Wang FH, Li S. Dynamic monitoring of serum soluble programmed cell death ligand 1 as a response predictor to chemotherapy in metastatic or recurrent gastrointestinal cancer. Transl Cancer Res 2020;9(4):2434-2448. doi: 10.21037/tcr.2020.03.23

