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Original Article

Assessment of TMB, PD-L1, and lymphocyte to monocyte ratio as predictive potential in a phase Ib study of sintilimab in patients with advanced solid tumors

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Abstract: Background: Sintilimab is a humanized monoclonal antibody against the programmed cell death 1 (PD-L1). We aimed to assess the safety and activity of sintilimab monotherapy or in combination with chemotherapy in advanced solid tumors. Methods: This phase Ib study included six cohorts. Cohort A-C were sintilimab monotherapy settings, and enrolled pretreated patients (2/3 L cohorts). Cohort D-F were treatment-naïve patients (1 L cohorts), and received sintilimab plus different chemotherapies. The primary endpoints were safety and objective response rate (ORR). Exploratory endpoints were potential biomarkers for the prognosis after treatment, such as tumor mutation burden scores (TMB), PD-L1 and lymphocyte-to-monocyte ratio (LMR). Results: The ORR was 14.6% in the 2/3 L cohorts (n=146), and 73.2% in the 1 L cohorts (n=61). The incidence of grade 3-4 adverse events occurred in 55 patients (37.7%) in 2/3 L cohorts, and in 38 (62.3%) in 1 L cohorts. 157 patients had available TMB scores, and in 2/3 L cohorts, patients in the high TMB groups (TMB≥10) showed a longer progression-free survival (PFS) and overall survival (OS) than those in the low TMB groups (TMB<10). No significant differences in PFS and OS were observed across different PD-L1 groups in both 1 L and 2/3 L cohorts. A high LMR was significantly associated with an improved PFS in 1 L cohorts (P=0.022). Conclusion: Sintilimab alone or combined with chemotherapy had a tolerable safety profile in solid tumors. The combination therapy showed a favorable activity with advanced non-small cell lung cancer and gastric or esophagogastric junction adenocarcinoma. LMR might be a prognostic factor for the combination regimen in these patients. Trial registration: ClinicalTrials.gov, number NCT02937116. Registered 18 October 2016.

Keywords: Sintilimab, solid tumors, programmed cell death 1, Phase I, tumor mutation burden, lymphocyte-to-monocyte ratio

Introduction

Immune checkpoint inhibitor (ICI) therapy that targets the programmed cell death 1 (PD-1) and the programmed cell death ligand-1 (PD-L1) is a new approach for tumor immunotherapy. The therapy can reverse cancer-mediated immune evasion [1]. Binding of the PD-1 receptor to its ligands negatively regulates the antitumor immune response by inhibiting T-cell proliferation, cytokine production, and cytotoxic functions, and facilitates to immune escape of tumors. The PD-1 antibodies can specifically bind to PD-1 and inhibite the apoptosis of antigen-specific T cells, thereby reducing regulatory T cell apoptosis by blocking PD-L1 activation.

Several antibodies that block PD-1/PD-L1 have been approved for multiple tumor indications, such as nivolumab and pembrolizumab [2-4]. Biomarkers are effective tools for patient selection in the response to treatments. Lots of evidence suggested the use of tumor mutation burden (TMB) and PD-L1 expression as potential biomarkers for the response to ICI therapy [5-7]. However, different results exist between

different anti-PD-1 antibodies [8-10], thus the association of these biomarkers with the response to ICIs needs to be explored.

Lymphocyte-to-monocyte ratio (LMR), the inflammation-based grading index, has been reported to be associated with prognosis in a broad spectrum of tumors, but only a few studies investigated this relationship in patients treated with ICIs [11, 12].

Sintilimab is a highly selective, humanized, monoclonal antibody that blocks the binding of PD-1 and its ligands. A phase Ia study showed an encouraging clinical benefit of sintilimab in advanced solid tumors with the recommended dose of 200 mg, every 3 weeks [13]. In addition, sintilimab has been certified as a new therapeutic option for patients with relapsed or refractory classical Hodgkin lymphoma in China [14]. In this phase Ib study, we aimed to assess the safety and activity profile of sintilimab monotherapy or in combination with chemotherapy in advanced solid tumors, and to explore the association of PD-L1 and TMB with the response to sintilimab, as well as the predictive role of LMR in the prognosis after the treatment. In this clinical trials, IHC data of PD-L1 and TMB scores were successfully obtained in 62 and 157 patients, respectively. Moreover, the mutation gene-enriched pathway that associated with survival was also investigated.

Materials and methods

Study design and participants

The multicenter, open-label, phase Ib study was performed between February 2017 and October 2018 to evaluate the safety and efficacy of sintilimab monotherapy or in combination with chemotherapy in six independent cohorts of solid tumors. Cohort A-C were all sintilimab monotherapy settings, and enrolled patients with advanced melanoma; or malignancies of digestive system after failure or intolerant to first line standard therapy; or advanced non-small cell lung cancer (NSCLC) after failure or intolerant to first line standard therapy, respectively.

Cohort D-F received the combination settings of sintilimab plus chemotherapies (cisplatin + pemetrexed in Cohort D, gemcitabine + cisplatin in Cohort E, and oxaliplatin + capecitabine in Cohort F), and the enrolled patients were with non-squamous NSCLC (nsqNSCLC), or squamous NSCLC (sqNSCLC), or gastric or esophagogastric junction (G/GEJ) adenocarcinoma, respectively. All patients in the three cohorts were treatment-naive locally advanced, recurrent or metastatic.

The major patient inclusion criteria were aged between 18-75, with Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-1, at least one measurable lesion for tumor assessment, and with adequate organ function. The inclusion and exclusion criteria details are available on the website of clinical trials.gov (number NCT02937116).

The protocol has been approved by the independent ethics committee of each participating center before study initiation. This study was conducted in accordance with Declaration of Helsinki and Good Clinical Practice guidelines, as defined by the International Conference on Harmonisation. All patients signed informed consents before enrolment.

Treatment procedures

Participants of cohort A, B and C received sintilimab 200 mg intravenously every 3 weeks (IV, Q3W) and continued the treatment until disease progression or intolerable toxicity or withdrawal of informed consent or up to 2 years.

Participants of cohort D, E and F received sintilimab 200 mg (IV) in combination with different chemotherapies: cohort D, cisplatin 75 mg/m^2 (IV, D1) and pemetrexed 500 mg/m^2 (IV, D1), Q3W for up to 4 cycles; cohort E, cisplatin 75 mg/m^2 (IV, D1) plus gemcitabine 1250 mg/m^2 (IV, D1 and D8) of a 3-week-cycle for up to 6 cycles; cohort F: oxaliplatin 130 mg/m^2 (IV, D1) plus capecitabine 1000 mg/m^2 (orally, D1-14), Q3W for up to 6 cycles. Patients in the three cohorts without progression received maintenance treatments (cohort D: sintilimab 200 mg plus pemetrexed as prior doses; cohort E and F: sintilimab 200 mg, IV, Q3W) until disease progression or intolerable toxicity or withdrawal of informed consent or up to 2 years.

Endpoints and assessments

The primary endpoint was safety profile, and the secondary endpoints were efficacy, includ-

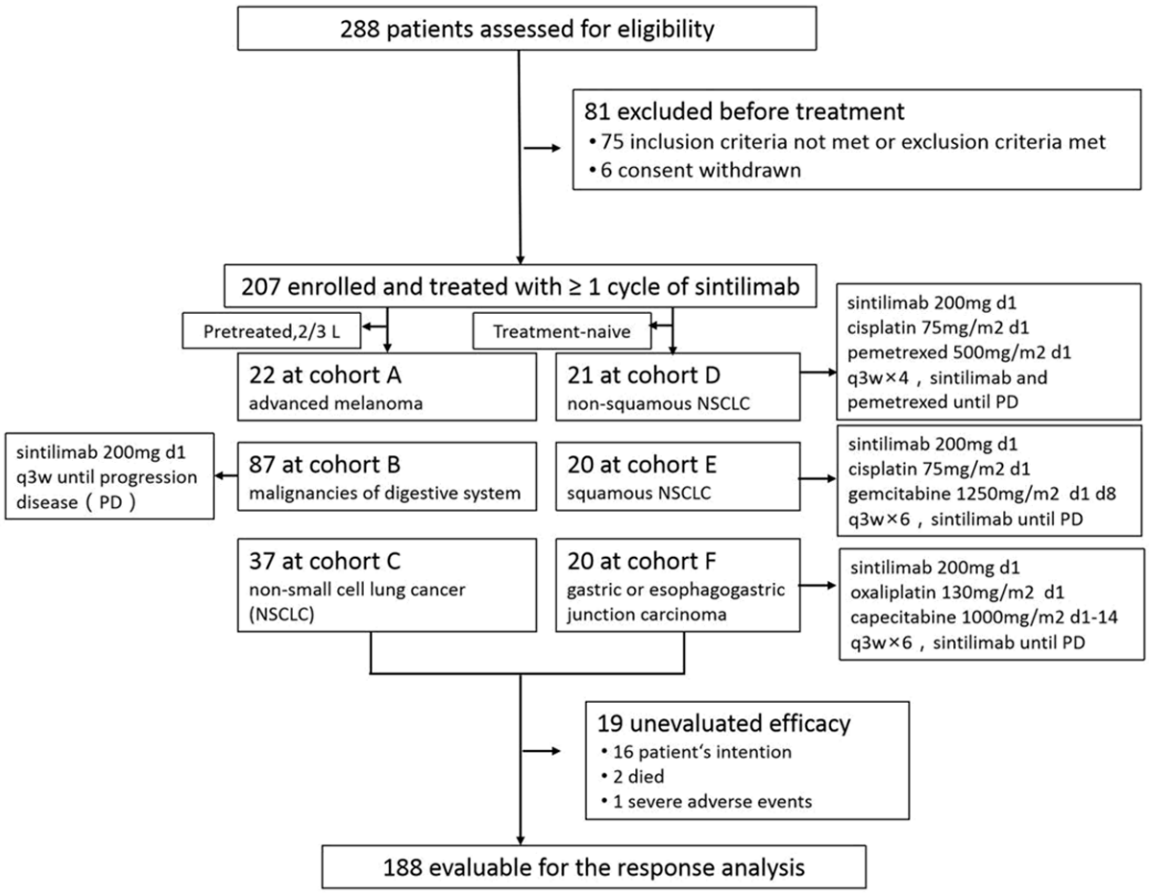

Figure 1. Flowchart of the study.

ing overall response rate (ORR), disease control rate (DCR), progression free survival (PFS) and overall survival (OS). Exploratory endpoints were potential biomarkers for the response to treatment and the prognosis after treatment.

Adverse events (AEs) were monitored throughout the study until 90 days after the last dose of the treatment, and were assessed according to the category, frequency, causality and severity graded by (CTCAE) v 4.03. The causal relation between AEs and the therapeutic schedule of the study was evaluated by investigators. Response was assessed by computed tomography or magnetic resonance imaging every 9 weeks until disease progression, new treatment initiation, withdrawal of informed consent or death. Overall responses were confirmed by two continuous complete responses (CR) or partial responses (PR) at intervals of 4 weeks. Progressive disease (PD) was confirmed by two continuous PD at intervals of 4 weeks. Efficacy was assessed by investigators according to RECIST v1.1.

PD-L1 immunohistochemistry (IHC)

PD-L1 expression of the archived tumor tissue was assessed using the 22C3 pharmDx assay (Agilent Technologies) at a central laboratory as described previously [15].

TMB analysis

Formalin-Fixed and Paraffin-Embedded (FFPE) tumor and matched blood cell samples were collected for target sequencing. The sequencing was performed on the Illumina HiSeq X-Ten platform (Illumina, San Diego, USA). TMB was calculated in mutations per one million coding bases (Mb), including somatic single-nucleotide variants (SNVs) and indel mutations in the whole exome.

Prognostics significance of LMR

The results of pre-treatment laboratory peripheral blood examinations containing the counts of lymphocytes and monocytes were recorded

Table 1. Demographics and disease characteristics

Characteristics	2/3 L N=146	1 L N=61
Age, median (range)	56 (24-72)	64 (38-72)
Sex, n (%)		
Males	103 (70.5)	53 (86.8)
Females	43 (29.5)	8 (13.2)
ECOG, n (%)		
0	54 (37.0)	20 (32.8)
1	92 (63.0)	41 (67.2)
Primary tumor, n (%)		
Malignant melanoma	22 (15.2)	0
Non-small cell lung cancer	37 (25.3)	41 (67.2)
Gastric carcinoma	1 (0.6)	20 (32.8)
Other digestive system neoplasm	86 (58.9)	0
Surgery, n (%)		
Yes	116 (79.4)	34 (55.7)
No	30 (20.6)	27 (44.3)
Radiotherapy, n (%)		
Yes	37 (25.3)	1 (1.6)
No	109 (74.7)	60 (98.4)
Chemotherapy, n (%)		
No	19 (13)	57 (93.4)
First line	127 (87)	4 (6.6)
Second line	0	0

Note: 2/3 L, cohort A, B and C (treated more than second line) with sintilimab monotherapy; 1 L, cohort D, E and F (treated naive) with sintilimab and chemotherapy.

to calculate the LMR. The LMR was defined as the absolute lymphocyte count divided by the absolute monocyte count (LMR = lymphocyte/monocyte). Patients were then categorized as high- and low-LMR groups using the median values as cut-off.

Pathway mutation analysis

For differential pathway analysis, three steps were taken as follows. Firstly, SNV mutations were mapped to gene level to obtain mutated genes. Secondly, the combined mutated genes from all samples were used to perform pathway enrichment analysis (based on GO BP terms) to select significantly enriched pathways ($P<0.05$). Thirdly, for each patient, one of these enriched pathways was considered being mutated if any gene of this pathway had mutation sites, and then the fisher's exact test method was used to identify significantly different pathways ($P<0.05$) between two patient groups (split by mPFS or mOS) in pathway mutation level.

Statistical analysis

All of the patients who received at least one treatment schedule were included in the efficacy and safety analysis. ORR was defined as the proportion of patients achieved CR or PR and its 95% CI was estimated by binomial distribution. DCR was defined as the proportion of patients achieved CR, PR or stable disease (SD) and its 95% CI was estimated by binomial distribution. Median PFS and OS were estimated by Kaplan-Meier method. Survival analysis with PFS or OS was conducted using the R survival package.

Results

Patient characteristics

Between June 1, 2017 and October 1, 2018, 288 patients were assessed and 207 eligible patients were enrolled form 11 sites in China (**Figure 1**). At data cut-off (March 1, 2020), 207 patients had received at least one dose of sintilimab and 187 patients were evaluable for the response analysis (**Table 1**). Cohort A-C enrolled 22, 87 and 37 patients, respectively; cohort D-F enrolled 21, 20 and 20 patients, respectively. Majority patients in cohort A-C received prior treatments (≥2 L), and patients in cohort D-F were previously untreated (1 L).

Safety profile

Among the patients for safety analysis (≥2 L patients, n=146; 1 L patient, n=61), treatment-related AEs (TRAEs) took place in 139 (95.2%) of patients received 2 or 3 lines of treatments (cohort A-C, **Table 2**), with the most common events of anemia (63 [43.2%]), thyroid dysfunction (53 [36.3%]) and increased aspartate aminotransferase (AST) (40 [27.4%]). Grade 3-5 TRAEs occurred in 55 (37.7%) patients and the anemia was the most frequent, 12 (8.2%). Most patients of 1 L cohorts 59 (96.7%) had at least one treatment-emergent AE; the most frequent events were anemia (41 [67.2%]), decreased neutrophil counts (36 [59.0%]), and decreased white blood cell (WBC) count (35 [57.4%]).

Table 2. Treatment-related adverse events

	2/3 L, N=146			1 L, N=61	
	Any grade	Grade 3-5		Any grade	Grade 3-5
Any events, n (%)	139 (95.2)	55 (37.7)	Any events, n (%)	59 (96.7)	38 (62.3)
Anaemia	63 (43.2)	12 (8.2)	Anaemia	41 (67.2)	9 (14.8)
Abnormal thyroid function	53 (36.3)	1 (0.7)	Decreased Neutrophil counts	36 (59)	12 (19.7)
Increased aspartate aminotransferase	40 (27.4)	4 (2.7)	Decreased white cell count	35 (57.4)	8 (13.1)
Increased alanine aminotransferase	34 (23.3)	2 (1.4)	Thrombocytopenia	35 (57.4)	13 (21.3)
Proteinuria	32 (21.9)	0 (0)	Decreased appetite	21 (34.4)	1 (1.6)
Hypoalbuminemia	32 (21.9)	0 (0)	Abnormal thyroid function	21 (34.4)	0 (0)
Fatigue	31 (21.2)	1 (0.7)	Nausea	20 (32.8)	2 (3.3)
Pyrexia	27 (18.5)	0 (0)	Increased alanine aminotransferase	16 (26.2)	0 (0)
Decreased white cell count	24 (16.4)	1 (0.7)	Vomitting	16 (26.2)	1 (1.6)
Increased Bilirubin	24 (16.4)	3 (2.1)	Pyrexia	15 (24.6)	0 (0)
Constipation	20 (13.7)	0 (0)	Fatigue	15 (24.6)	1 (1.6)
Increased γ glutamyltransferase	19 (13)	5 (3.4)	Rash	15 (24.6)	2 (3.3)
Backache	17 (11.6)	0 (0)	Constipation	14 (23)	0 (0)
Hyponatremia	17 (11.6)	3 (2.1)	Increased aspartate aminotransferase	14 (23)	0 (0)
Sinus tachycardia	17 (11.6)	0 (0)	Increased γ glutamyltransferase	11 (18)	5 (8.2)
Hypokalemia	16 (11)	2 (1.4)	Diarrhea	10 (16.4)	2 (3.3)
Lipase increased	16 (11)	7 (4.8)	Lung infection	9 (14.8)	5 (8.2)
Cough	15 (10.3)	0 (0)	Insomnia	9 (14.8)	0 (0)
Decreased Neutrophil counts	15 (10.3)	1 (0.7)	Hypertension	8 (13.1)	5 (8.2)
Lung infection	14 (9.6)	9 (6.2)	Proteinuria	7 (11.5)	0 (0)
Loss of appetite	13 (8.9)	2 (1.4)	Hypoalbuminemia	7 (11.5)	0 (0)
Thrombocytopenia	12 (8.2)	2 (1.4)	hypokalemia	7 (11.5)	2 (3.3)
Increased Blood alkaline phosphatase	11 (7.5)	1 (0.7)	The upper respiratory tract	7 (11.5)	2 (3.3)
Weight gain	10 (6.8)	1 (0.7)	Abnormality of liver function	5 (8.2)	1 (1.6)
Hyperuricaemia	10 (6.8)	1 (0.7)	Hemoptysis	5 (8.2)	1 (1.6)
Hyperuricemia	9 (6.2)	1 (0.7)	Weight loss	5 (8.2)	1 (1.6)
White blood cell count high	7 (4.8)	1 (0.7)	Hyperglycemia	5 (8.2)	1 (1.6)
Hypophosphatemia	7 (4.8)	2 (1.4)	Increased lactate dehydrogenase	5 (8.2)	1 (1.6)
Hypertension	7 (4.8)	1 (0.7)	Hyponatremia	4 (6.6)	2 (3.3)
The abdominal pain	7 (4.8)	1 (0.7)	Malnutrition	4 (6.6)	1 (1.6)
Pain	7 (4.8)	3 (2.1)	Acute kidney injury	3 (4.9)	1 (1.6)
Abnormal liver function	6 (4.1)	1 (0.7)	Increased triglyceride	3 (4.9)	1 (1.6)
Amylase increased	4 (2.7)	1 (0.7)	Decreased granulocyte count	2 (3.3)	2 (3.3)
Interstitial lung disease	4 (2.7)	3 (2.1)	Heart failure	2 (3.3)	2 (3.3)
Haemorrhage of digestive tract	4 (2.7)	4 (2.7)	Decreased Blood chloride	2 (3.3)	1 (1.6)
Increased the neutrophil count	3 (2.1)	1 (0.7)	Functional gastrointestinal disorder	1 (1.6)	1 (1.6)
The upper respiratory tract infection	2 (1.4)	1 (0.7)	Bone marrow failure	1(1.6)	1(1.6)
Gastrointestinal bleeding	2 (1.4)	1 (0.7)*	Uronephrosis	1(1.6)	1(1.6)
Fibrinogen decreased	2 (1.4)	1 (0.7)	Dysphagia	1(1.6)	1(1.6)
Bowel obstruction	1 (0.7)	1 (0.7)	Autoimmune colitis	1(1.6)	1(1.6)
The gallbladder pain	1 (0.7)	1 (0.7)			
Cachexia	1 (0.7)	1 (0.7)			
Embolism pulmonary	1 (0.7)	1 (0.7)			
Hyperkalemia	1 (0.7)	1 (0.7)			
Fracture of neck of femur	1 (0.7)	1 (0.7)			
Jaundice	1 (0.7)	1 (0.7)			
Paralysis facial	1 (0.7)	1 (0.7)			
The trachea hemorrhage	1 (0.7)	1 (0.7)			

Esophageal stenosis	1 (0.7)	1 (0.7)
Visual damage	1 (0.7)	1 (0.7)
Supraventricular tachycardia	1 (0.7)	1 (0.7)
Death	1 (0.7)	1 (0.7)*
Heart failure	1 (0.7)	1 (0.7)
Pleural effusion	1 (0.7)	1 (0.7)
Drug-induced liver injury	1 (0.7)	1 (0.7)
Uterine leiomyoma	1 (0.7)	1 (0.7)

Note: Data are n (%). Grade 1-2 adverse events reported in at least 10% of patients and all grade 3-5 events are shown; Cohort A, B and C (treated more than second line); 1 L, cohort D, E and F (treated naive); The events are listed in descending order of frequency in 2/3 L. *, Grade 5 AEs.

Table 3. Sintilimab-related adverse events with incidence of ≥10%

n (%)	Any grade	Grade 3, 4 or 5
All events	152 (73.4)	29 (14.0)
Increased aspartate aminotransferase	30 (14.5)	2 (0.9)
Fatigue	26 (12.6)	1 (0.5)
Increased alanine aminotransferase	23 (11.1)	1 (0.5)
Decreased white cell count	21 (10.1)	1 (0.5)
Hypothyroidism	17 (8.2)	1 (0.5)
Increased lipase	16 (7.7)	6 (2.9)
Increased hemobilirubin	16 (7.7)	1 (0.5)
Rash	16 (7.7)	1 (0.5)
Decreased neutrophil count	15 (7.2)	1 (0.5)
Increased γ glutamyltransferase	13 (6.3)	1 (0.5)
Anaemia	12 (5.8)	1 (0.5)
Decreased platelet count	11 (5.3)	2 (0.9)
Lung infection	10 (4.8)	5 (2.4)
Loss of appetite	8 (3.9)	1 (0.5)
Interstitial lung disease	6 (2.9)	2 (0.9)
abnormal liver function	4 (1.9)	1 (0.5)
Increased amylase	4 (1.9)	1 (0.5)
Malnutrition	2 (0.9)	1 (0.5)
Pain	2 (0.9)	1 (0.5)
Autoimmune colitis	1 (0.5)	1 (0.5)
Drug-induced liver injury	1 (0.5)	1 (0.5)
Functional gastrointestinal disorder	1 (0.5)	1 (0.5)
hypokalemia	1 (0.5)	1 (0.5)
Increased white cell count	1 (0.5)	1 (0.5)

Grade 3-4 AEs occurred in 38 (62.3%) patients, and the most common events were thrombocytopenia, 13 (21.3%) and decreased neutrophil counts, 12 (19.7%). According to the investigator's assessment, sintilimab related AEs were reported in 152 (73.4%) of 207 patients (S 1), the most common being increased AST (30 [14.5%]), fatigue (26 [12.6%]) and increased alanine aminotransferase (ALT) (23 [11.1%]). Grade 3-4 sintilimab-related AEs occurred in 29 (14.0%) patients (**Table 3**). There were no sintilimab-related AEs leading to death.

Efficacy

At the data cut-off on April 17, 2019, 2 patients achieved CR, 17 patients achieved PR, obtaining an ORR of 14.6% in cohort A-C (2/3 L cohorts). In addition, 38 patients had SD resulting in a DCR of 43.8% (**Table 4**). With a median follow-up of 13.5 months, the median PFS of patients receiving 2/3 L treatment was 2.1 months (95% CI 1.3-2.9) (**Figure 2A**), and the median OS was 12.7 months (95% CI 11.6-13.9) (**Figure 2B**).

In cohort D-F (1 L cohorts), 41 patients achieved PR, 12 patients had SD, with an ORR of 73.2% and a DCR of 94.6%. With a median follow-up of 11.3 months, the median PFS for the 1 L cohorts was 6.3 m (95% CI 5.2-7.5) (**Figure 2A**), and the median OS was 11.3 m (95% CI 10.1-12.4) (**Figure 2B**).

The largest change in tumor size compared to baseline of the six cohorts was recorded among evaluable patients (**Figure 3**).

Biomarkers and inflammatory marker analysis

PD-L1: In total, IHC data of PD-L1 was successful obtained in 62 patients including 40 receiving 2/3 L treatments and 22 receiving 1 L treatment. In both of the patients in 1 L cohorts and

Table 4. Best overall response

N (%)	A (n=21)	B (n=77)	C (n=34)	D (n=19)	E (n=17)	F (n=20)	2/3 L (n=132)	1 L (n=56)
Complete remission	1 (4.8)	1 (1.3)	0	0	0	0	2 (1.5)	0
Partial remission	0	12 (15.6)	5 (14.7)	13 (68.4)	11 (64.7)	17 (85.0)	17 (12.8)	41 (73.2)
Stable disease	6 (28.6)	19 (24.7)	13 (38.2)	3 (15.8)	6 (35.3)	3 (15.0)	38 (28.7)	12 (21.4)
Progressive disease	14 (66.7)	45 (58.4)	16 (47)	3 (15.8)	0	0	75 (56.8)	3 (5.4)
Objective response rate	1 (4.8)	13 (16.9)	5 (14.7)	13 (68.4)	11 (64.7)	17 (85.0)	19 (14.4)	41 (73.2)
Disease control rate	7 (33.3)	32 (41.6)	18 (52.9)	16 (84.2)	17 (100)	20 (100)	57 (43.2)	53 (94.6)

Note: 2/3 L, cohort A, B and C (treated more than second line); 1 L, cohort D, E and F (treated naive).

those in the 2/3 L cohorts, no significant difference was observed in PFS and OS between PD-L1 positive (TPS≥1) and negative (TPS<1) patient groups (**Figure 4**).

Tumor mutation burden (TMB) scores: In the six cohorts, there were 157 patients available with TMB scores who were categorized into TMB-high (TMB≥10) and TMB-low (TMB<10) groups. Among patients receiving 2/3 L treatment, those in the high TMB groups showed longer PFS (**Figure 2C**) and OS (**Figure 2D**) than those in the low TMB groups. With respect to ORR, the patients achieving CR and PR had a larger proportion of patients with TMB-high value than those having SD and developed PD. However, no significant association of the TMB value with treatment efficacies (PFS, OS and ORR) was observed in the 1 L cohorts (**Figure 5**).

Differential pathway mutation: To further explore the association between mutation gene-enriched-pathway and the survival time, patients were divided into two groups using the median of PFS or OS. For 2/3 L cohorts, 289 mutated genes were significantly enriched (q value <0.01) in 319 GO biological processes (BP) terms, of which 21 terms exhibited significant differences between long and short PFS groups (**Figure 6A**), and 15 terms were significantly different between long and short OS groups (**Figure 6B**). Among the 21 BP categories of the PFS groups, the most significantly different pathway was regulation of leukocyte activation. Among the 27 mutated genes of the regulation of leukocyte activation pathway, the most frequent mutated gene is JAK3 that involves in the JAK-STAT signaling pathway to regulate lymphoid homeostasis and responsiveness. Regulation of vesicle-mediated transport was the most significant pathway between the two OS groups.

For 1 L cohorts, the most significantly different pathways were development-related pathways with higher mutation rates in the short PFS groups (**Figure 7**).

Lymphocyte to monocyte ratio: The cut-off value of LMR was 2.83 (median LMR). The 1-year OS rates for patients with low- and high-LMR were 43.83% and 72.89%, respectively. The median OS for patients with low and high LMR were 10.27 and 18.97 months, respectively. Kaplan-Meier analysis and log-rank test demonstrated that low LMR level significantly predicted a poorer OS, compared with those with high LMR level (HR 2.070, 95% CI 1.44-3.01, P<0.001, **Figure 8A**). The data indicated that elevated LMR was associated with a longer OS in the patients in the 1 L cohorts (P<0.001, **Figure 8B**), but this relationship was not observed in the 2 L cohorts (P=0.069, **Figure 8C**).

The 1-year PFS rates for patients with low- and high-LMR were 19.05% and 22.91%, respectively, and the median PFS were 4.83 and 6.33 months, respectively. There were no significant differences in PFS between patients with low- and high-LMR (HR 1.304, 95% CI 0.97-1.77, P=0.077, **Figure 8D**). When stratified by treatment regimen alterations, the patients with elevated LMR showed an improved PFS versus those with low LMR in the 1 L cohorts (P=0.022, **Figure 8E**), but no statistical significance was shown in 2 L cohorts (P=0.431, **Figure 8F**).

Discussion

In this phase Ib study, sintilimab monotherapy or in combination with chemotherapy showed a well tolerable safety profile. Most AEs were manageable and mild in severity. Meanwhile, sintilimab preliminarily showed a favorable activity in patients with solid tumors, especially in combination with chemotherapy.

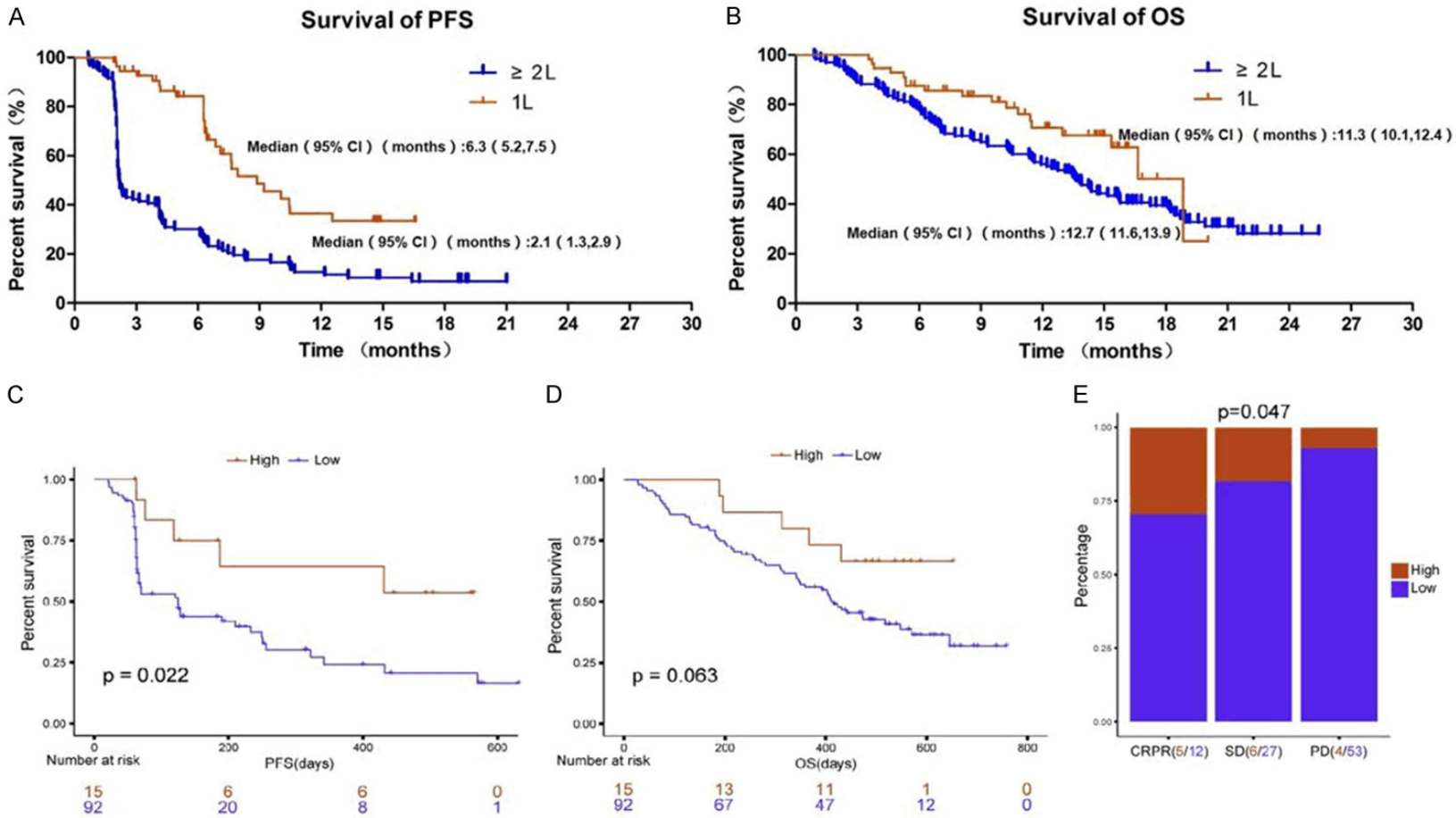

Figure 2. Outcomes of the patients (A) Progression-free survival in the cohort A, B and C (≥2 L), the cohort D, E and F (1 L). (B) Overall survival in the cohort A, B and C (≥2 L), the cohort D, E and F (1 L). (C-E) TMB analysis of the cohort A, B and C. Between TMB high (≥10) and low (<10) groups, the association analysis of PFS (C), OS (D) and ORR (E) were conducted. The ORR significance was tested using Fisher's exact test.

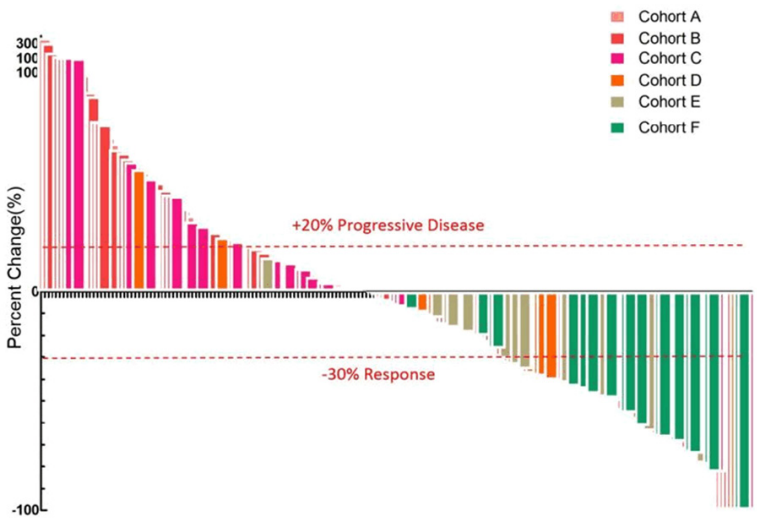

Figure 3. Shrinkage of tumor size.

The most common TRAEs in 2 L or 3 L cohorts in this study were anemia, which was also reported in other anti-PD-1/PD-L1 agents [16]. Thyroid dysfuction (as hypothyroidism, thyrotoxicosis, painless thyroiditis, or even "thyroid storm") are more frequently occurred on anti-PD-1-antibodies [17]. In our study, thyroid dysfunction was another common TRAE in the 2 L or 3 L cohorts, similar to the results in melanoma patients and advanced or metastatic NSCLC patients treated with pembrolizumab or nivolumab [18-20]. Liver injury could be induced by ICI therapies [21]. In our study, the abnormality of liver function was the TRAE with a high frequency, and was also the grade 3-4 TRAE in patients receiving sintilimab monotherapies as 2 L or 3 L settings, but this event was manageable with liver protection treatment (or without additional therapy). Overall, the sintilimab safety profile of our study was consistent to the published safety profiles of anti-PD-1 monoclonal antibodies [17, 22]. Most AEs of which were grade 1-2 and did not require additional treatment.

Pembrolizumab in combination with chemotherapies resulted in a relatively high frequency of grade 3 or worse AEs (57%-71%) in 1 L patients with nsqNSCLC, sqNSCLC and G/GEJ [23-25]. Comparable to these findings, incidence of grade 3-4 AEs with sintilimab in combination with chemotherapies in 1 L cohorts was 62.3%. Decreased WBC count and anemia were the most frequent AE or grade 3-4 AE with the combination strategy in our study, which was consistent with previous data of pembrolizumab or nivolumab combination in relevant patient cohorts [26-28].

The safety profile of sintilimab plus chemotherapy (including cisplatin and pemetrexed, gemcitabine and cisplatin, oxaliplatin and capecitabine) was first studied. No treatment-related deaths were observed during the study period, indicating that sintilimab plus chemotherapy showed a manageable safety profile for the first-line treatment of nsq/sqN-SCLC and advanced G/GEJ cancer.

Sintilimab showed a favorable anti-tumor activity in patients with solid tumors in combination with chemotherapy in this phase 1 study. In the 1 L cohorts of nsqNSCLC, sqNSCLC and G/GEJ, this combination setting showed an encouraging ORR of 73.2%, which was comparable to that with pembrolizumab or nivolumab plus chemotherapy in the aforementioned patient cohorts (33%-75%) [26-28]. Median PFS and OS of 1 L patient cohorts were 6.3 months and 11.3 months, respectively. Based on the data, the efficacy and safety of sintilimab combined with chemotherapy in first line are being examined in several phase 3 trials, including advanced or metastatic nsqNSCLC (NCT03607539), sqNSCLC (NCT03629925), esophageal squamous cell carcinoma (NCT03748134) and gastric cancer (NCT03745170). These phase 3 studies will provide a detailed examination of the therapeutic potential of sintilimab in a broad spectrum of tumor types. The ORR of patients with 2-3 L cohorts was 14.6%, and the median OS was 12.7 months, both were comparable to previous data in the phase 1 studies with other PD-1 antibodies [29, 30].

In the pre-reported clinical trials, PD-1 inhibitors such as pembrolizumab plus chemotherapy demonstrated substantially improved OS and PFS in metastatic NSCLC, regardless of PD-L1 expression [23, 24, 27]. Besides, in the KEYNOTE-062 study, pembrolizumab plus capecitabine or 5-fluorouracil plus cisplatin was not superior to chemotherapy alone in terms of OS in both PD-L1 combined positive

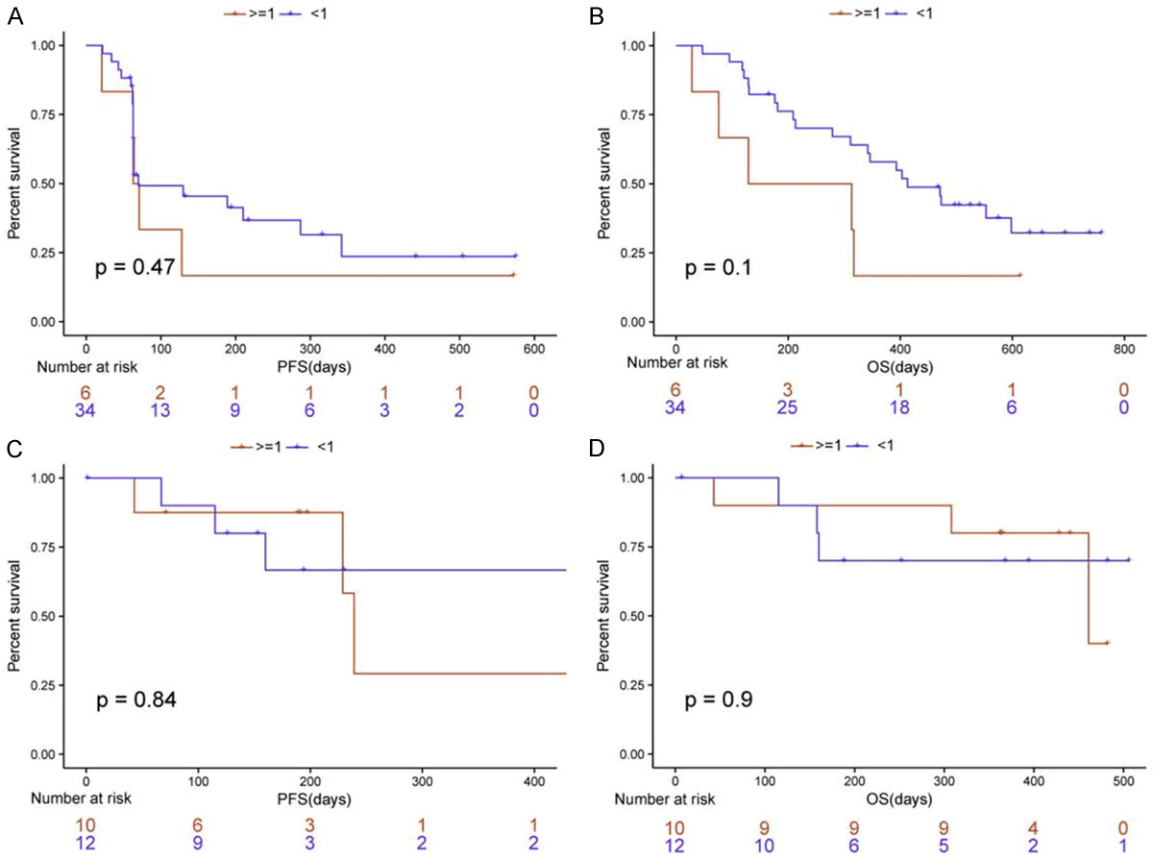

Figure 4. PD-L1 analysis of the cohort A, B and C and cohort D, E and F. Between PD-L1 positive (≥1) and negative (<1) groups, the association analysis of PFS and OS were conducted in the ABC cohort (A and B) and DEF cohort (C and D).

score (CPS) ≥1 and CPS≥10 populations, although the response rate in pembrolizumab combined with chemotherapy was much higher (48.6% versus 37.2% in CPS≥1 and 52.5% versus 37.8% in CPS≥10) [31], indicating that the level of PD-L1 expression might not predict the treatment efficacy for pembrolizumab plus chemotherapy in G/GEJ cancer patients. Consistent with these findings of the PD-1 inhibitor in combination with chemotherapy, in our study, among the PD-L1-evaluable patients treated with sintilimab and chemotherapy (n=22), no significance was observed in PFS and OS between PD-L1 positive (TPS≥1) and negative (TPS<1) groups. Anyhow, the sample size was limited, and the data was immature to make a conclusion. The relationship between PD-L1 expression and the treatment efficacy will be further studied in several phase 3 trials (NCT03607539, NCT03629925, NCT03748-134, NCT03745170).

Results in patients with anti-PD-1 monotherapies were inconsistent among different agents. The association of a high PD-L1 expression with the improved response and survival benefit with pembrolizumab was indicated in patients with NSCLC and gastric cancer [32, 33]. Nevertheless, the clinical benefit of nivolumab in these patients was regardless of PD-L1 expression [9, 34]. Regarding patients with advanced melanoma, the benefit of nivolumab appeared not affected by PD-L1 status [35]; and PD-L1 seemed a weak or uncertain predictor for the response benefit of pembrolizumab [36, 37]. In our study, among patients with previously treated melanoma, NSCLC and malignancies of digestive system, no significant differences were observed in survival outcomes (PFS and OS) across different PD-L1 status groups. These results collectively suggest that the use of PD-L1 as a biomarker for the efficacy of PD-1 inhibitor monotherapy warrants further validations.

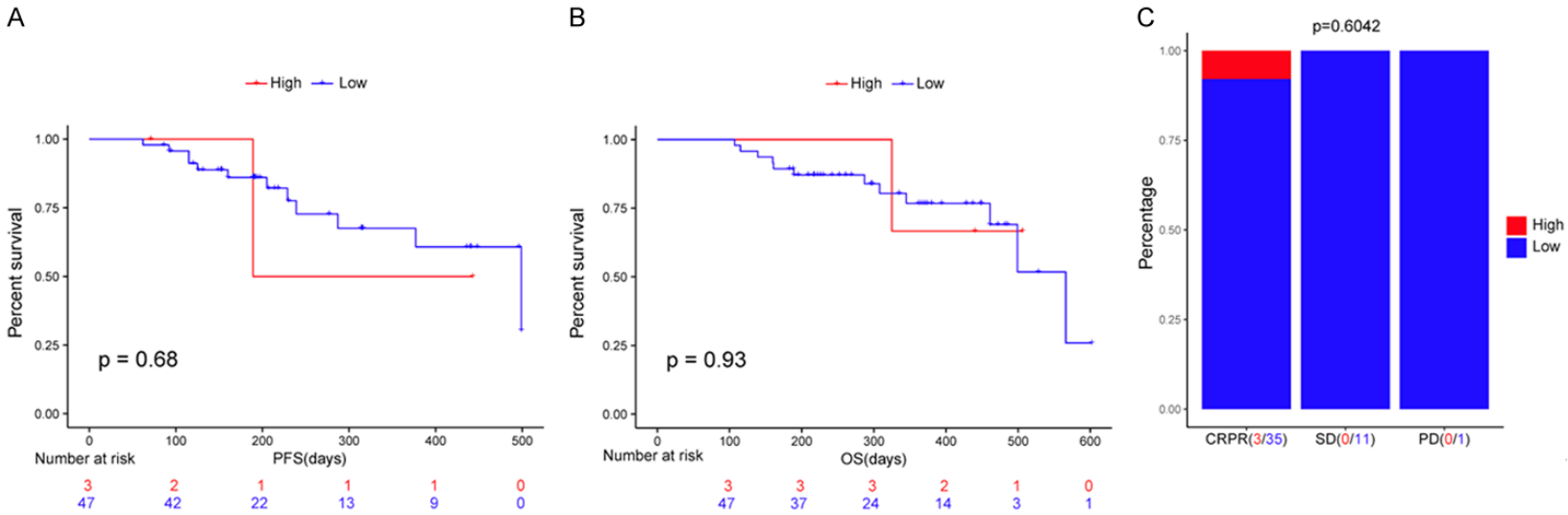

Figure 5. TMB analysis of the cohort D, E and F. Between TMB high (≥10) and low (<10) groups, the association analysis of PFS (A), OS (B) and ORR (C) were conducted. The ORR significance was tested using Fisher's exact test.

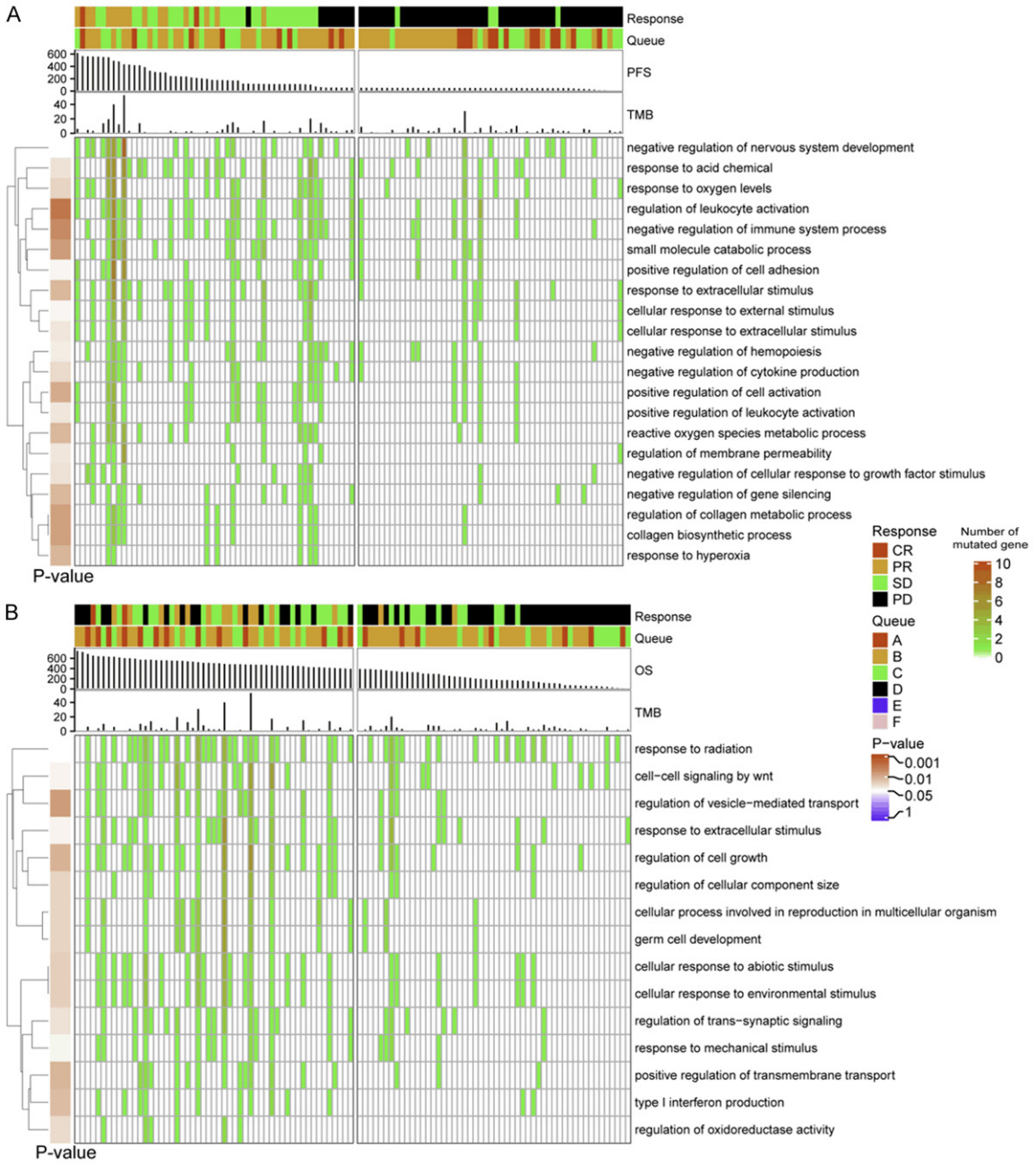

Figure 6. Pathway mutation analysis of the cohort A, B and C. Differential mutated pathways between two groups split by mPFS (A) or mOS (B) were shown. The leftmost *P*-value represents the significance of differential pathways (Fisher's exact test).

The phase III CheckMate-227 trial has indicated "mutation burden is an effective biomarker" in NSCLC [38]. In this trial, the high TMB groups showed longer PFS than the low TMB groups among patients with NSCLC treated with sintilimab monotherapy, which was consistent with findings in previous researches [39, 40]. With respect to ORR, the CR and PR group had a larger proportion of TMB-high patients than SD and PD groups. In addition, among these 2/3 L patient cohorts, enrichment analysis indicated that regulation of leukocyte activation was the most significant pathway for mutation genes between different PFS groups, suggesting mutation genes in this pathway might be predictors for the PFS outcome. However, no sig-

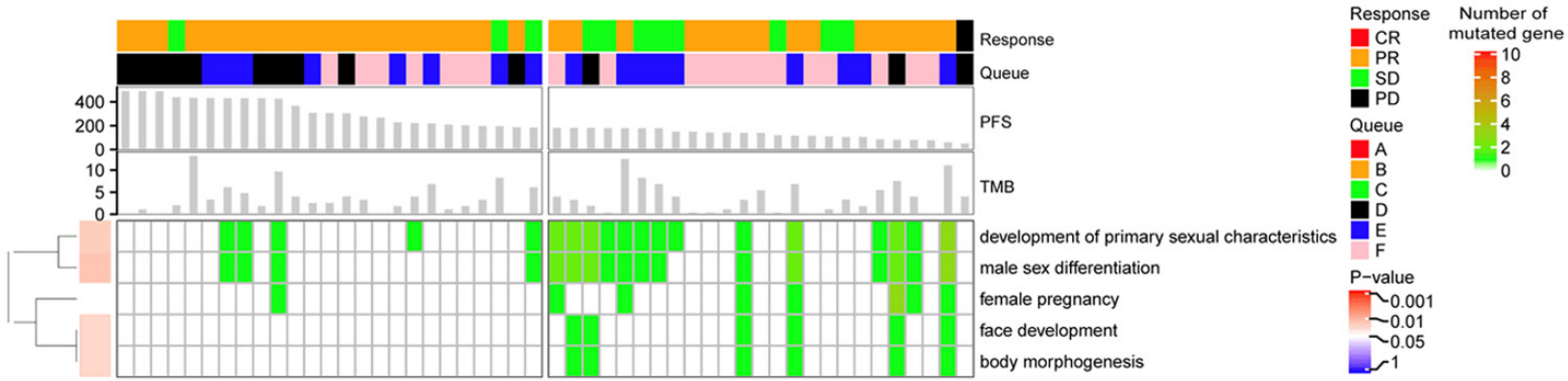

Figure 7. Pathway mutation analysis of the cohort D, E and F. Differential mutated pathways between two groups split by mPFS were shown. The leftmost *P*-value represents the significance of differential pathways (Fisher's exact test).

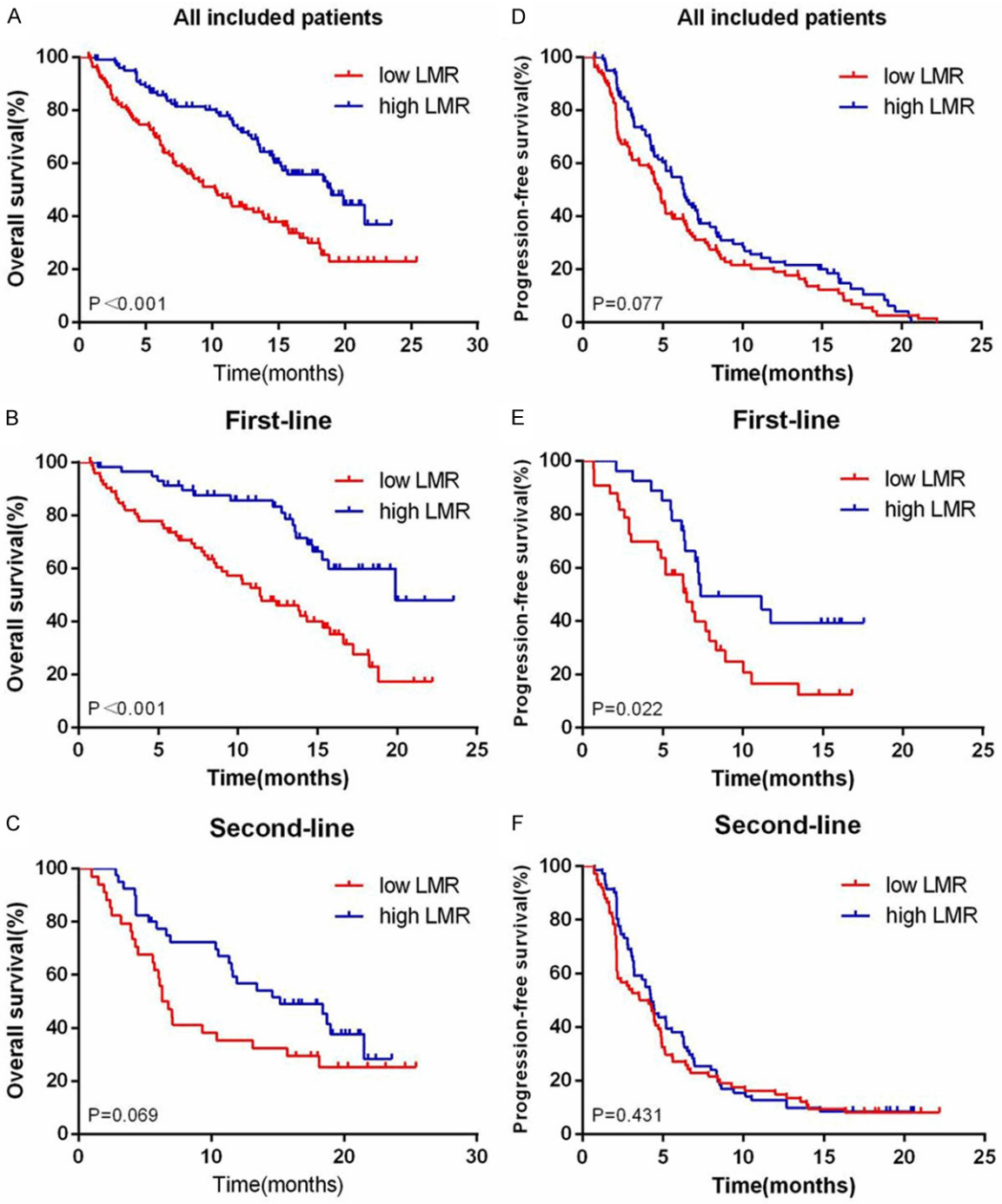

Figure 8. LMR results (A) Overall survival for all participants with low and high LMR. (B) Overall survival in the group of first line participants with low and high LMR. (C) Overall survival in the group of second line participants with low and high LMR. (D) Progression-free survival for all participants with low and high LMR. (E) Progression-free survival in the group of first line participants with low and high LMR. (F) Progression-free survival in the group of second line participants with low and high LMR.

nificant association with PFS, OS and ORR were observed in the patients treated with sintilimab plus chemotherapy, suggesting that high TMB maybe a good predictor for the prognosis to immunotherapy but not to immunotherapy combined with chemotherapy in patients with solid tumors. In a previous study, there was no significant association between the tissue TMB

(tTMB) with PFS, OS and ORR from immunotherapy with chemotherapy [41].

ICIs can improve antitumor activity of immune cells by activating cytotoxic T lymphocytes and interrupting the immune suppression in the tumor microenvironment [11, 42]. Monocytes are important drivers in the tumor progression [43]. Thus, the high lymphocyte or low monocyte counts could reflect a better efficacy of ICIs. A few studies suggested the positive prognostic role of a high LMR on the anti-PD-1 monotherapies in patients with advanced NSCLC and melanoma [11, 12, 42]. Consistent with these findings, in our study, the high LMR was associated with a longer OS in the overall population. Notably, in the 1 L patient cohorts with combination therapy, this association was significant, however, was not found in the 2/3 L patient cohorts with sintilimab monotherapy. This might be attributed to the small sample size in the 2 L cohort. The prognostic role of LMR in patients with sintilimab alone or in combination with chemotherapy warrants further investigations.

This is a phase 1 study with a relatively small patient samples, and the preliminary results need further confirmations in the phase 3 studies. In addition, in this study, PD-L1 expression was unavailable in several patients, which might influence the association between PD-L1 expression with the efficacy result.

Sintilimab monotherapy or in combination with chemotherapy exhibited acceptable safety in solid tumors. Especially, sintilimab plus chemotherapy as first-line treatment showed favorable activity in locally advanced, recurrent or metastatic NSCLC and G/GEJ adenocarcinoma patients. High TMB might indicate a better prognosis of sintilimab monotherapy. High LMR could be a good prognostic factor with sintilimab-chemotherapy combination treatment in solid tumors.

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Disclosure of conflict of interest

None.

Abbreviations

AEs, adverse events; CR, complete responses; DCR, disease control rate; ECOG, Eastern Cooperative Oncology Group; G/GEJ, gastric or esophagogastric junction adenocarcinoma; ICI, immune checkpoint inhibitor; IV, intravenously; LMR, lymphocyte-to-monocyte ratio; NSCLC, non-small cell lung cancer; nsqNSCLC, non-squamous NSCLC; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression free survival; PR, partial responses; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand-1; sqNSCLC, squamous NSCLC; SNVs, single-nucleotide variants; SD, stable disease; TMB, tumor mutation burden; TRAEs, treatment-related AEs.

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