Clinical Cancer Research

Safety, Activity, and Biomarkers of SHR-1210, an Anti-PD-1 Antibody, for Patients with Advanced Esophageal Carcinoma

Jing Huang[1], Binghe Xu[1], Hongnan Mo[1], Weilong Zhang[2], Xuelian Chen[1], Dawei Wu[1], Dong Qu[3], Xingyuan Wang[1], Bo Lan[1], Beibei Yang[2], Pei Wang[2], Hongtu Zhang[4], Qing Yang[5], and Yuchen Jiao[2]

Abstract

Purpose: The current management of advanced esophageal squamous cell carcinoma (ESCC) remains unsatisfactory. We investigated the safety, efficacy, and biomarkers of SHR-1210, an anti-PD-1 antibody, in patients with recurrent or metastatic ESCC.

Experimental Design: This study was part of a phase I trial in China. Patients with advanced ESCC who were refractory or intolerant to previous chemotherapy were enrolled. Eligible patients received intravenous SHR-1210 at a dose of 60 mg, with escalation to 200 and 400 mg (4-week interval after first dose followed by a 2-week schedule) until disease progression or intolerable toxicity. The associations between candidate biomarkers (PD-L1 and somatic mutation load) and the efficacy of SHR-1210 were also explored.

Results: Between May 11, 2016, and December 9, 2016, a total of 30 patients from one site in China were enrolled. Ten patients (33.3%) had an independently assessed objective response. Median progression-free survival was 3.6 months (95% CI, 0–7.2). Three (10.0%) treatment-related grade 3 adverse events were reported: two (6.7%) pneumonitis and one (3.3%) increased cardiac troponin I. No grade 4 or grade 5 treatment-related adverse events were reported. The exome sequencing and analysis showed that the mutational burden and the potential mutation-associated neoantigen count were associated with better responses. An objective response was more common in patients with PD-L1–positive tumors as defined by $\geq$5% staining (7 of 15 patients) than in those with PD-L1–negative tumors (1 of 9 patients).

Conclusions: In this population of ESCC patients, SHR-1210 had a manageable safety profile and promising antitumor activity.
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Introduction

Esophageal cancer is one of the most fatal malignancies globally, with a major increase in incidence over the past several decades (1). Despite improvements in the management and treatment of these patients, overall outcome remains poor. Meanwhile, esophageal squamous cell carcinoma (ESCC) is among the most common subtype and has a poor outcome with a 5-year survival rate of only about 15% to 25% (2–3).

Over the past decade, metastatic ESCC has been managed primarily with chemotherapy (such as fluorouracil, cisplatin, and taxanes; refs. 4–6). However, the long-term survival of these regimens remains poor. The poor prognosis of ESCC highlights the urgent need for improved therapies, especially novel therapeutic approaches.

Recently, breakthroughs in immune checkpoint blockade have offered new therapeutic options for many malignancies. Immunotherapy targeting the checkpoint programmed cell death protein 1 (PD1) or programmed death ligand 1 (PD-L1) has been shown to be effective in the management of melanoma, non–small cell lung cancer, and renal cell carcinoma (7–9). Nevertheless, only two clinical studies have reported the safety and efficacy of anti-PD1 antibody in ESCC patients (10–11). One study was a single-arm phase II study, which evaluated nivolumab in 65 patients with treatment-refractory ESCC. Eleven patients (17%) had a centrally assessed objective response, whereas 27 patients (42%) achieved disease control. The median progression-free survival (PFS) and overall survival (OS) were 1.5 and 10.8 months, respectively. The most common adverse events were diarrhea, decreased appetite, constipation, rash, and fatigue. Analysis to determine associations between biomarkers and the safety or activity of nivolumab was not included in that report. The other report was the result from the esophageal carcinoma cohort of the KEYNOTE-028 trial, which evaluated the safety and efficacy of pembrolizumab in

[1]Department of Medical Oncology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [2]Laboratory of Cell and Molecular Biology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [3]Department of Diagnostic Radiology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [4]Department of Pathology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [5]Jiangsu Hengrui Medicine Co., Ltd. Shanghai, China.

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J. Huang, B. Xu, and Y. Jiao contributed equally to the article.

Corresponding Authors: Jing Huang, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 17 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China. Phone: 8610-8778-8113; Fax: 8610-6773-4170; E-mail: huangjingwg@163.com; Binghe Xu, xubinghe@medmail.com.cn; and Yuchen Jiao, jiaoyuchen@cicams.ac.cn

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Translational Relevance

SHR-1210 is a selective, humanized, high-affinity IgG4-kappa mAb against PD-1. This is the first study reporting the somatic nonsynonymous mutation load and mutation-associated neoantigen count as well as the safety and antitumor activity of SHR-1210 in patients with metastatic ESCC. As part of a phase I trial in China, our findings showed that the most common treatment-related AEs were reactive capillary hemangiomas in 23 patients (76.7%). Three (10.0%) treatment-related grade 3 adverse events were reported: two (6.7%) pneumonitis and one (3.3%) increased cardiac troponin I. Treatment with SHR-1210 resulted in an ORR of 33.3%, a DCR of 56.7%, and a median PFS of 3.6 months by central review. The exploratory genomic sequencing analyses showed that the mutational burden and the mutation-associated neoantigen count were associated with better responses. In this population of ESCC patients, SHR-1210 had a manageable safety profile and promising antitumor activity.

patients with PD-L1–positive advanced solid tumors and the paper was presented at the 2016 ASCO GI meeting. Seventeen PD-L1–positive ESCC patients were enrolled in the study, whereas 5 patients had partial responses (11).

SHR-1210 is a selective, humanized, high-affinity IgG4-kappa mAb against PD-1. The cohort of patients with advanced ESCC was drawn from a larger phase I population from a phase I study (NCT02742935) conducted in China. The current report assessed the safety and activity of SHR-1210 in patients with metastatic ESCC. PD-L1 expression, mutation load, and potential mutation-associated neoantigen (MANA) counting were also assessed in this study.

Materials and Methods

Study design and participants

This is an open-label, phase I study to evaluate the safety, activity, and pharmacokinetics of SHR-1210 in patients with ESCC, gastric cancer, triple-negative breast cancer or other solid tumors. In this report, we describe the results of the advance ESCC cohort. The protocol and all amendments were approved by the institutional review board and independent ethics committee of National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences. The study was conducted in accordance with the Declaration of Helsinki and the international standards of good clinical practice. The protocol is available in the appendix. All authors had access to the study data and reviewed and approved the final article.

Eligible patients were males and females ages between 18 and 75 years old; had histologically confirmed metastatic ESCC; had experienced disease progression or recurrence after at least one systemic treatment for advanced or metastatic disease; had at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1); had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and had adequate hematologic, hepatic, and renal function. Patients with brain metastasis were enrolled only when the lesions had been stable for at least 3 months. Exclusion criteria included a history of or active autoimmune disease, a concomitant second cancer, history of organ transplantation or PD-1/PD-L1 therapy, active hepatitis B or C viral infection, or ongoing systemic immunosuppressive therapy. Previous cancer treatment, radiotherapy, or radiosurgery must have been completed at least 4 weeks before enrollment. All patients provided written informed consent before study entry.

Procedures

This study consisted of an initial dose-escalation and subsequent expansion phase. During dose-escalation, patients were treated with SHR-1210 (Jiangsu Hengrui Medicine Co, China) at a dose of 60 mg, with escalation to 200 and 400 mg. We chose these dose levels mainly based on the PK and toxicity properties of SHR-1210 given in previous phase I study conducted in Australia in 2015. In addition, the administration every 2 weeks of 200 mg of other anti-PD-1 antibodies, such as pembrolizumab and nivolumab, has been approved worldwide. A dose of 60, 200, and 400 mg was chosen for the subsequent expansion phase. All treatments were to continue until intolerable toxicity, confirmed disease progression, death, or withdrawal of consent. Tumor imaging by CT or MRI was done at baseline and every 8 weeks during the first 6 months, and every 12 weeks thereafter. The tumor response was assessed by independent radiology review according to RECIST, version 1.1. Only patients who received at least one dose of SHR-1210 were included. Treatment beyond initial disease progression was allowed in clinically stable patients (exhibiting symptom control despite radiographic progression, stable performance status or good tolerance of SHR-1210) at the discretion of the investigator.

All adverse events (AE) were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Laboratory safety evaluations (i.e., hematology, chemistry, and urinalysis) were conducted within 14 days of the initial treatment and up to 24 hours before each dose. SHR-1210 administration could be interrupted or delayed for protocol-defined reasons, but dose modification was not permitted. AEs were handled according to established safety guidelines. Patients could receive the treatment again after the AEs recovered to those of the initial state or grade 1.

We measured PD-L1 expression in pretreatment, archival tumor samples with an investigational version of the Human PD-L1 Immunohistochemistry Kit using the 6E8 antibody (Shuwen Biotech Co., Ltd., Zhejiang Province, China). For each sample, the membrane expression of PD-L1 in tumor cells or tumor-infiltrating mononuclear cells (TIMC) was determined by two independent pathologists blinded to the clinical data. PD-L1 cell scores were generally based on a single section. All the neoplastic cells were scorable. PD-L1 positivity was defined as $\geq$5% of tumor cell membrane staining. The extent of PD-L1 expression on TIMCs was scored as absent (0), mild (1), moderate (2), and severe (3). For cases in which the tissue sample had not been optimally collected or prepared or in which PD-L1 expression could not be assessed, the PD-L1 status was categorized as unevaluable.

We extracted genomic DNA from tumor tissues and matched white blood cell samples. We then performed exome sequencing and analyzed potential MANAs as described previously (12). Additional details are provided in Supplementary Materials.

Outcomes

The primary endpoints were the safety and tolerability of SHR-1210. The secondary endpoints were the objective response rate

(ORR), PFS, and the associations between candidate biomarkers (PD-L1 and exome sequences) and the efficacy of SHR-1210. ORR was defined as the percentage of complete or partial responses at any time during the study, as assessed by an independent radiology review committee. PFS was defined as the time from the date of the first dose of study medication to the date of first documented disease progression or the date of death from any cause. The pharmacokinetic profile of SHR-1210 was also a secondary endpoint, but was not assessed in this study.

Statistical analysis

Patients underwent follow-up for analysis until May 2, 2017. All patients who received at least one dose of SHR-1210 were included in the efficacy analyses (full analysis set population). The overall response was assessed by independent radiology review. All patients who received at least one dose of SHR-1210 were included in the safety analysis. The differences in rate were compared using the Fisher exact test. Clopper–Pearson exact confidence intervals (CI) were generated for the response rate and associated 95% CIs. PFS and OS were estimated via the Kaplan–Meier method. The SPSS Statistics version 22 software was used for all analyses. This study is registered with ClinicalTrials.gov, number NCT02742935.

Results

Between May 11, 2016 and December 9, 2016, a total of 30 patients were enrolled. The baseline clinical characteristics of the patients are presented in Table 1. The median age was 63 years (range, 48–75). Twenty-seven patients (90.0%) had ECOG performance status of 0, while 3 patients (10.0%) had ECOG performance-status score of 1. A large majority of the patients had been extensively pretreated, with 21 of 30 (70.0%) having received two or more previous chemotherapy, 19/30 (63.3%) had radiation and 14/30 (46.7%) had esophagectomy. The most common previous chemotherapy was platinum plus paclitaxel, which was administered in 26 patients (86.7%).

The data cut-off date was May 2, 2017. The median treatment duration was 18.1 weeks (range, 2.0 to 38.1), and 11 patients remained on study treatment. A total of 19 (63.3%) patients discontinued SHR-1210. The most common reason for treatment discontinuation was disease progression (17/19), with two exceptions of AEs (one case of upper gastrointestinal bleeding and one case of fatigue) unrelated to the study treatment.

All 30 patients were included in the safety analysis. Treatment-related AEs occurred in 25 patients (83.3%), with no clear differences according to dosage. The most common treatment-related AEs were reactive capillary hemangiomas (RCH) in 23 patients (76.7%), pruritus in 6 patients (20.0%), hypothyroidism in 4 patients (13.3%), and fever in 4 patients (13.3%; Table 2). Most events were grade 1 or 2. Three (10.0%) treatment-related grade 3 events were reported: two (6.7%) pneumonitis and one (3.3%) increased cardiac troponin I. No grade 4 or grade 5 drug-related AEs were reported. No patients discontinued SHR-1210 because of a treatment-related AE. Two patients interrupted SHR-1210 treatment: one patient due to grade 2 bilirubin elevation, and the other patient due to grade 3 cardiac troponin I elevation. Both patients resumed SHR-1210 after resolution of the AEs. One patient had grade 3 acute exacerbation of chronic obstructive pulmonary disease related to infection, but improved after receiving prednisone and antibiotics.

Immune-related AEs occurred in 25 of 30 patients (83.3%), including RCH, hypothyroidism or hyperthyroidism, and diarrhea. These AEs were predominantly grade 1 or 2 and were managed with medical therapy. RCH often manifested as red papules or macules with clear boundaries. It usually disseminated over the body, and mainly presented on the trunks, upper extremities, head and neck. Some of the lesions could be localized, or gathered. No segmental lesions were found. The most frequent

Table 1. Baseline characteristics

	60 mg ($n = 3$)	200 mg ($n = 25$)	400 mg ($n = 2$)	Total ($N = 30$)
Age (years)				
Median	63	61	65	63
Range	54–65	48–75	65–65	48–75
Sex				
Men	2 (66.7%)	24 (96.0%)	2 (100%)	28 (93.3%)
Women	1 (33.3%)	1 (4.0%)	0	2 (6.7%)
ECOG performance status				
0	2 (66.7%)	24 (96.0%)	1 (50%)	27 (90.0%)
1	1 (33.3%)	1 (4.0%)	1 (50%)	3 (10.0%)
Histologic grade				
Well or moderately differentiated	2 (66.7%)	13 (52.0%)	0	15 (50.0%)
Poorly differentiated	1 (33.3%)	11 (44.0%)	2 (100%)	14 (46.7%)
Unknown	0	1 (4.0%)	0	1 (3.3%)
Previous systemic therapies				
1	1 (33.3%)	8 (32.0%)	0	9 (30.0%)
2	1 (33.3%)	11 (44.0%)	2 (100%)	14 (46.7%)
≥3	1 (33.3%)	6 (24.0%)	0	7 (23.3%)
Previous radiotherapy				
Never	0	10 (40.0%)	1 (50%)	11 (36.7%)
Confirmed	3 (100%)	15 (60.0%)	1 (50%)	19 (63.3%)
Previous surgery				
Never	1 (33.3%)	15 (60.0%)	0	16 (53.3%)
Confirmed	2 (66.7%)	10 (40.0%)	2 (100%)	14 (46.7%)
Disease stage				
III	1 (33.3%)	5 (20.0%)	1 (50%)	7 (23.3%)
IV	2 (66.7%)	20 (80.0%)	1 (50%)	23 (76.7%)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; values are reported as number (%) unless otherwise indicated.

Table 2. Drug-related adverse events

	Patients, *n* (%)		
Adverse event	**Grade 1 or 2**	**Grade 3**	**Grade 4 or 5**
Reactive capillary hemangiomas	23 (76.7%)	0	0
Pruritus	6 (20.0%)	0	0
Fever	4 (13.3%)	0	0
Hypothyroidism	4 (13.3%)	0	0
Increased bilirubin	4 (13.3%)	0	0
Elevated transaminase	4 (13.3%)	0	0
Diarrhea	3 (10.0%)	0	0
Rash	3 (10.0%)	0	0
Nausea	2 (6.7%)	0	0
Fatigue	2 (6.7%)	0	0
Anemia	1 (3.3%)	0	0
Hyperthyroidism	1 (3.3%)	0	0
Dizziness	1 (3.3%)	0	0
Increased creatinine	1 (3.3%)	0	0
Pneumonitis	0	2 (6.7%)	0
Increased cardiac troponin I	0	1 (3.3%)	0
Palmar-plantar erythrodysesthesia syndrome	1 (3.3%)	0	0
Hyperglycemia	1 (3.3%)	0	0
Eosinophilia	1 (3.3%)	0	0
Insomnia	1 (3.3%)	0	0

NOTE: Causality assessment was provided by the investigators.

complications were bleeding, without ulcerations, pain or pruritus. The spontaneous regression of RCHs was observed both during and after treatment, but complete regression only occurred after discontinuation of SHR-1210. All patients with hypothyroidism were successfully treated with replacement therapy. Four patients developed fever after drug infusion, which resolved spontaneously in 3 patients and rapidly reversed with antipyretics in the other case.

All 30 patients were included in the response analysis. The ORR as assessed by independent radiology review was 33.3% (10/30; 95% CI, 17.9%–54.3%), with a disease control rate of 56.7% (17/30; 95% CI, 35.7%–73.6%; Fig. 1). One complete response was observed. Of the 9 patients with a partial response, one patient had received three previous systemic or adjuvant chemotherapy, three had received two previous therapies, and five had received one previous therapy. Overall, responses occurred at a median of 56 days from the start of treatment. Fifteen of 30 patients had a decrease from baseline in the size of their target lesions. Five of the ten responders had ongoing responses. Six of the ten responders continued on treatment with SHR-1210 at the data cutoff, with one exception based on patient's preference.

At the time of the data cutoff, 21 patients (70.0%) had disease progression. The median PFS as assessed by independent review was 3.6 months (95% CI, 0–7.2). The Kaplan–Meier analysis yielded an estimated rate of 6-month PFS rate of 31.5% (95% CI, 14.2–48.7). Among the 21 patients who had progressive disease, progression occurred in preexisting target lesions (17 patients), new metastatic sites (8 patients), or both (7 patients). One patient with progressive disease was clinically stable and continued on SHR-1210 treatment. The other patients with progressive disease all discontinued study treatment and started chemotherapy. Five of the 10 patients with confirmed responses developed disease progression later: four patients in new metastatic sites (one in the liver, one in the lung, one in the lymph node and one in the bone) and one patient in preexisting target lesions.

Of the 24 patients who were assessed for PD-L1 expression, 15 (62.5%) had PD-L1–positive tumors (Table 3). Objective response was more common in patients with PD-L1–positive tumors [7 of 15 patients (46.7%) than in those with PD-L1–negative tumors 1 of 9 (11.1%)], without statistical significance (all $P > 0.05$). The disease control rate was 33.3% (3 of 9 patients) in PD-L1–negative tumors and 66.7% (10 of 15 patients) in PD-L1–positive tumors. Five patients had a TIMCs score of 3 and two (40%) of these had a partial response, compared with six (31.6%) of 19 tumors with a score of 2 or less. Notably, four tumors were PD-L1 negative in tumor cells and had a TIMCs score of 1 or less. Of these 4 patients, 3 patients had progressive disease and one had stable disease after SHR-1210 treatment.

We performed exome sequencing on the DNAs from tumors and white blood cells of 23 cases whose samples were available and passed quality control. The mutation count per tumor ranged from 15 to 219, with a median of 60 mutations per tumor. The therapeutic efficacy was associated with the somatic nonsynonymous mutation load. Eight out of the 11 samples with more than 60 missense mutations (median of all samples) showed clinical benefit and only 2 out of the 12 samples with 60 or less missense mutations had clinical benefit ($P = 0.0123$, Fisher's exact test, two-sided). The nonsynonymous missense mutational load in the group with clinical benefit (CB) was much higher than that in that without clinical benefit (NCB; $P = 0.0479$, unpaired t test, two-sided, Fig. 2A; Supplementary Fig. S1A). By analyzing the immunogenic potential in the context of the patient's MHC haplotype, we identified a mean of 17.3 potential MANAs in the CB group. The NCB group harbored 8.6 MANAs on average, which was significantly lower than the CB group ($P = 0.0427$, unpaired t test, two-sided, Fig. 2B). The percentage of MANAs among missense mutations was similar in the two groups (16.74% vs. 16.66%, $P = 0.9787$, unpaired t test, two-sided, Supplementary Fig. S2).

We further found a peptide signature with shared, consensus tetrapeptide sequences that were only in CB group. There was also a pool of tetrapeptide sequences that were completely in the NCB group. The count of neoepitopes was significantly different between the two groups ($P = 0.0099$, unpaired t test, two-sided, Fig. 2C–E). All of five cases with >4 neoepitopes showed

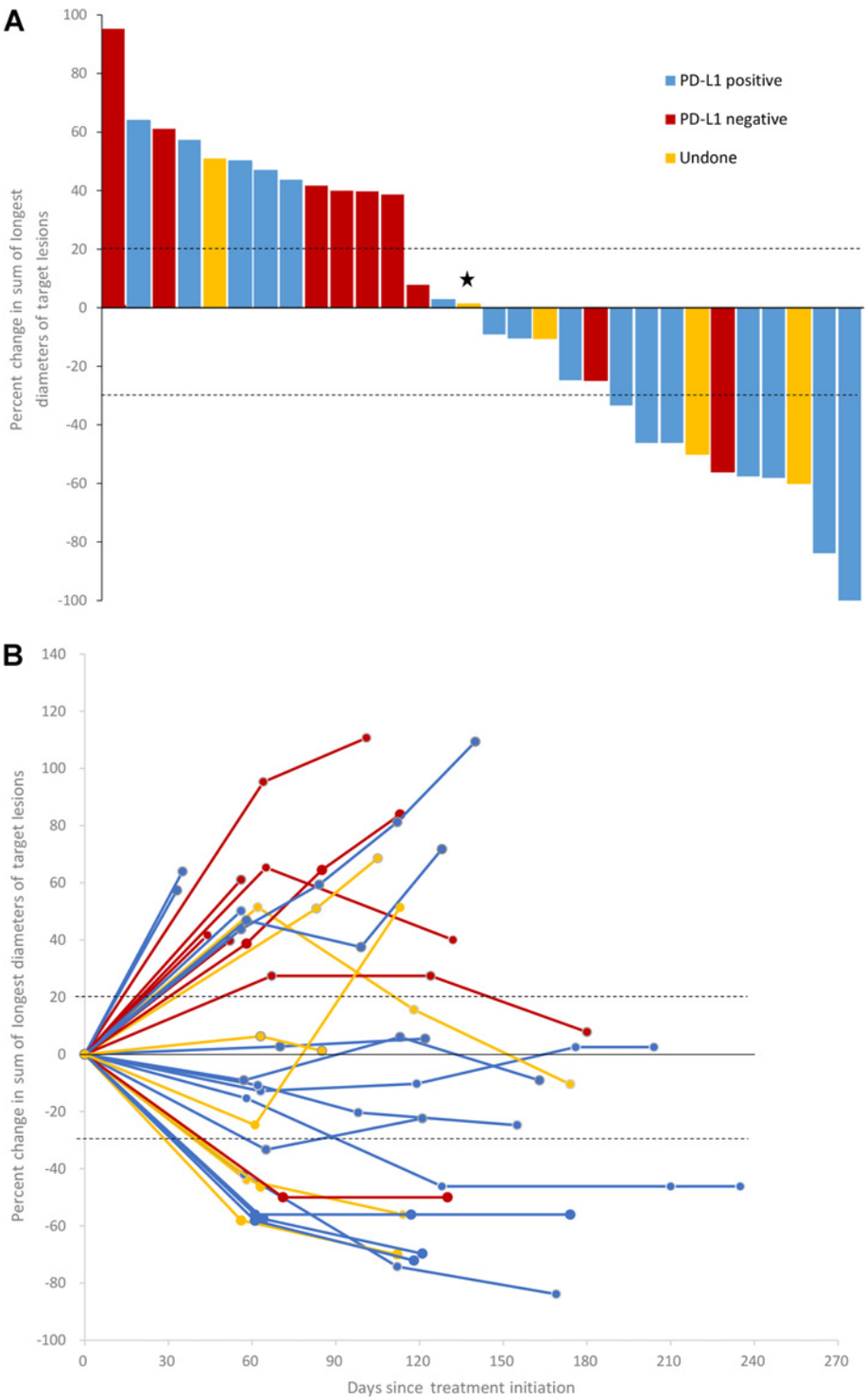

Figure 1
Efficacy of SHR-1210. Response was assessed in accordance with the Response Evaluation Criteria in Solid Tumors version 1.1 by central imaging in all 30 patients. **A,** The best change from baseline in the sum of the longest target lesion diameters per patient. The star indicates one patient who was stable regarding preexisting target lesions but had a new metastatic site. **B,** Longitudinal change from baseline in the sum of the longest target lesion diameters.

clinical benefit and only 5 out of the 18 tumors with four or less neoepitopes showed clinical benefit ($P = 0.0075$, Fisher exact test, two-sided).

We combined the genetic parameters with the previously described PD-L1 expression and tumor burden data to obtain an index (see the Supplementary Methods section) to predict the therapeutic effect. The difference ($P = 0.0024$, unpaired t test, two-sided) in the index between the CB and NCB groups was more significant than that calculated on the basis of any single factor of the index (Supplementary Fig. S3). The index also showed a predictive value to the PFS with a P value of 0.0001, which was more significant than any single factor (Fig. 3A–F).

Table 3. Responses by PD-L1 expression

	Objective responses		Disease control	
PD-L1 expression in tumor cells				
<1%	1/8 (12.5%)	$P = 0.189$	3/8 (37.5%)	$P = 0.390$
>1%	7/16 (43.7%)		10/16 (62.5%)	
<5%	1/9 (11.1%)	$P = 0.178$	3/9 (33.3%)	$P = 0.206$
>5%	7/15 (46.7%)		10/15 (66.7%)	
<50%	6/19 (31.6%)	$P = 1.000$	10/19 (52.6%)	$P = 1.000$
>50%	2/5 (40%)		3/5 (60%)	
Tumor-infiltrating mononuclear cells score				
0	1/1 (100%)	$P = 0.468$	1/1 (100%)	$P = 0.314$
1	1/5 (20%)		1/5 (20%)	
2	4/13 (30.8%)		8/13 (61.5%)	
3	2/5 (40%)		3/5 (60%)	

NOTE: Data are number of overall responses/number of patients (%). No complete response was achieved.

The mutational landscape is consistent with former reports with *TP53* as the most frequently mutated gene. There seems to be no significant association between recurrent mutated genes and therapeutic effect (Supplementary Fig. S4).

Discussion

This is the first study reporting the somatic nonsynonymous mutation load and mutation-associated neoantigen count as well as the safety and antitumor activity of anti-PD-1 antibody (SHR-1210) in patients with metastatic ESCC. Our findings indicate that SHR-1210 has promising antitumor activity and a manageable toxicity profile in extensively pretreated patients with recurrent or metastatic ESCC. Treatment with SHR-1210 resulted in an ORR of 33.3%, a DCR of 56.7%, and a median PFS of 3.6 months by independent radiology review.

Although systemic chemotherapy provides modest therapeutic gains for advanced ESCC patients, patients progress after

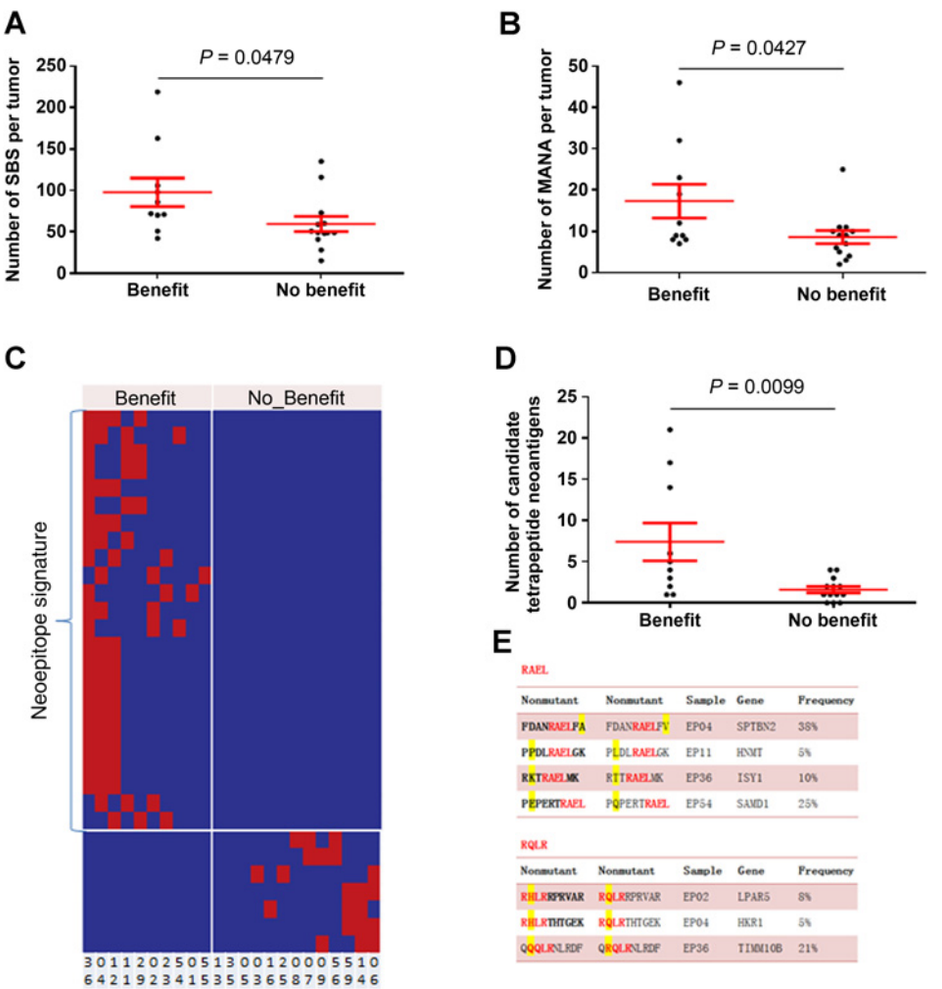

Figure 2.
Genetic features of tumors according to the clinical benefit from SHR-1210. **A,** The nonsynonymous mutation load in CB and NCB groups. **B,** The potential mutation-associated neoantigen (MANA) count in CB and NCB groups. **C,** Heatmap of tetrapeptide neoantigens that were present in patients in the CB and NCB groups. Each column represents a patient; each row represents a neoepitope. **D,** Scatter plot of the tetrapeptide neoantigen counts in the CB and NCB groups. **E,** Two examples of candidate tetrapeptide neoantigen substrings that were present in patients with a clinical benefit but absent in patients with no benefit. Data are presented as mean ± SEM in scatter plot of this article (unpaired *t* test, two-sided).

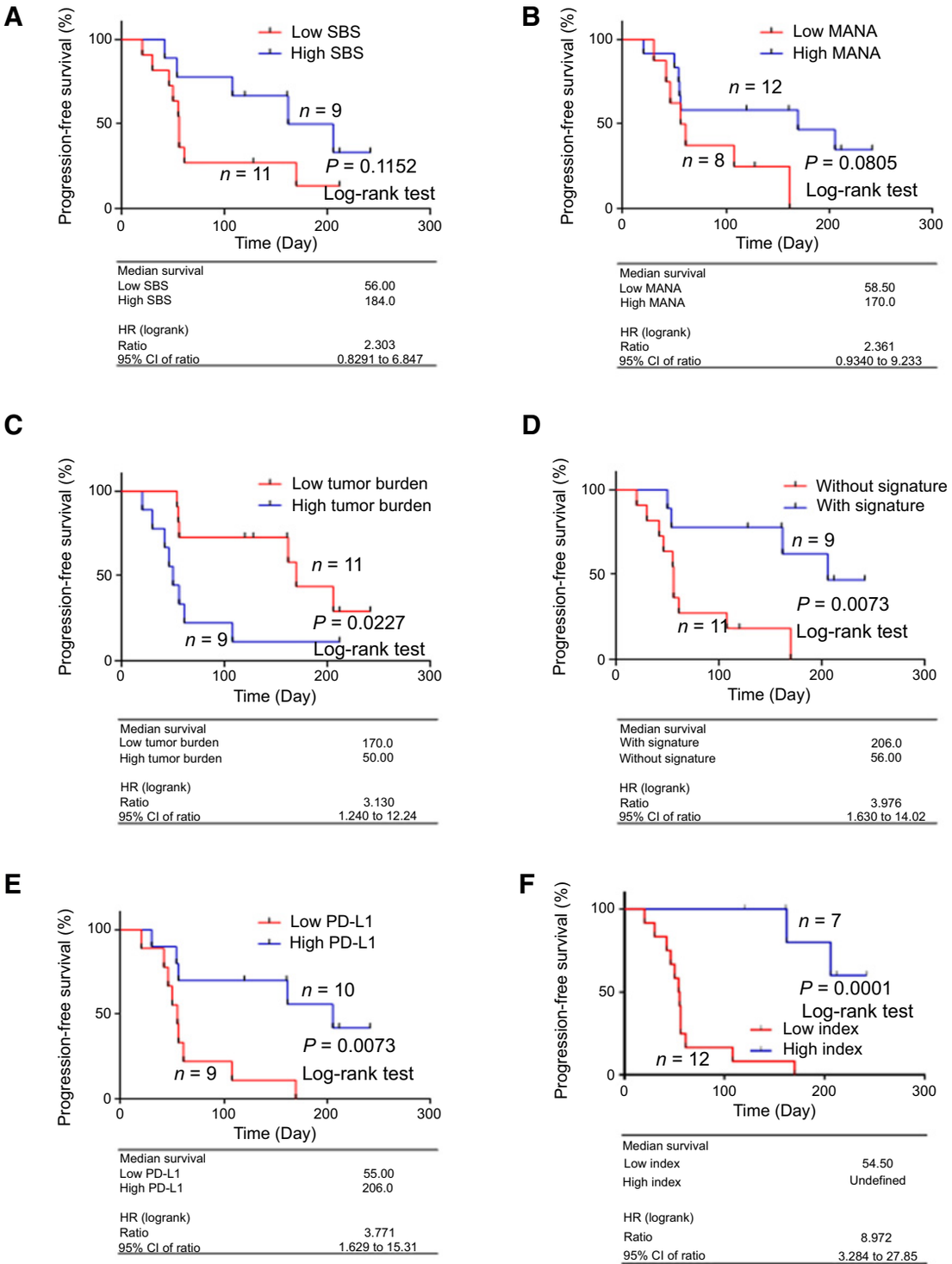

Figure 3.

The Kaplan–Meier curves for PFS of patients with therapy. **A–F,** The Kaplan–Meier curves for PFS of patients with therapy in different mutational load (number of SBSs per exome), MANAs (mutation associated neoantigens), tumor burden (sum of the longest diameters, SLD), tetrapeptide neoepitope signature, PD-L1 expression, and index (see Materials and Methods for index calculation) status. The log-rank test was used to compare Kaplan–Meier curves.

chemotherapy and have few treatment options as second or third-line treatment. Over the past decade, only one phase III trial has been performed in patients with recurrent or metastatic esophageal carcinoma. That randomized, placebo-controlled study demonstrated limited antitumor activity of gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), with a 2.7% ORR, 24% DCR, and median PFS of 44 days in unselected patients (13). Even in EGFR overexpression or amplification patients with pretreated metastatic ESCC, another EGFR-TKI, icotinib, resulted an ORR of 16.7%, a DCR of 46.3%, and a median PFS of only 52 days (6). As to immune checkpoint inhibitors, only two studies of anti-PD-1 antibody (nivolumab and pembrolizumab) in ESCC have been published. The results of our study corroborate the data from these two studies. The ORR and DCR of SHR-1210 reported here seems to be better than that of nivolumab (17% and 42%, respectively), and is similar to that of pembrolizumab (10–11). Despite the fact that more than half of our patients had received at least two previous chemotherapy regimens, 10 objective responses occurred, including one complete response. This is an encouraging improvement over previously reported efficacy of targeted therapy. However, it should be interpreted with caution because of the different patient composition and the small sample size in this study.

A majority of the toxicity profile in our study was similar to that previously reported for other anti-PD-1 antibodies (7–10, 14–15). Most of the treatment-related adverse events were grade 1 or 2, and resolved with appropriate supportive treatment. Only three in 30 patients had grade 3 treatment-related adverse events, and no grade 4 or 5 treatment-related adverse event occurred. These data indicate that SHR-1210 is well-tolerated patients with ESCC. Notably, reactive capillary hemangioma, a reactive hyperproliferative vascular response, was observed in more than two thirds of the patients. Detailed description and interpretation of this unique rash will be reported in another article.

High PD-L1 expression is generally believed to be correlative with better responses in anti-PD-1/PD-L1 therapy (15–17). Nonetheless, data observed in different trials was somewhat inconsistent (7, 18). Our data suggest a possible association between antitumor activity and higher PD-L1 expression on tumor cells, although the number of patients is too small to make definite conclusions. In spite of the mechanism of SHR-1210, one of the PD-L1–negative patients in this study had a partial response, suggesting that SHR-1210 has antitumor activity in this subgroup. Therefore, it is premature to assert the validity of PD-L1 as a predictive biomarker in ESCC.

In this study, we profiled the genetic analysis of esophageal carcinoma treated with SHR-1210. The mutation load is consistent with former reports on esophageal cancers (19–20), and is within the range of most common cancer types. We found that the mutational burden is significantly associated with the therapeutic effect, and missense mutation count of 60 could distinguish the two groups with or without clinical benefit. This is different from the former studies on melanoma or colon cancer with MSI, where <100 somatic mutations would be considered as low mutational burden indicating minimal or no clinical benefit (21–22).

Besides the mutation load analysis, the MANA count shows a better association and the neoepitope signature had a much stronger association than the former two markers. A high count (>4) of neoepitopes could be the only single marker to predict the therapeutic effect, with all the five patients show clinical benefit. These results indicate that effective neoepitopes could be the critical ones that stimulate immune response targeting esophageal tumors in the anti-PD1 therapy. Increased mutational load would increase the possibility of having more MANAs and more effective neoepitopes, but it cannot guarantee the level of the latter. This could explain some "outliners" with high-level mutation burden but poor response. For example, EP07 and EP59 had relatively high mutational load but low MANA count. These cases showed poor response to the therapy. Although highly mutated samples had more chance to harbor increased MANAs and neoepitopes, the neoepitope count was more closely associated with clinical benefit and might be a better predictor of therapeutic efficacy.

The combination of genetic markers with other factors like IHC analysis showed better predictive value than any single factor. However, the sample size in this study is not large. Further studies with large patient population would be necessary to validate the advantage of combinational analysis in prediction of anti-checkpoint therapies.

Overall, our results suggest promising efficacy and a manageable safety profile of SHR-1210 in pretreated patients with metastatic ESCC. In addition, our exploratory gene analyses revealed that a tumor whose mutational burden falls in the range of most common tumor types could benefit from SHR-1210 therapy and that mutational load/neoantigen/neoepitope analysis could become potential predictive biomarkers for patients with this tumor type. To further explore the efficacy of SHR-1210 in metastatic ESCC, a randomized phase III study (NCT03099382) of SHR-1210 versus docetaxel or irinotecan as second-line treatment has been launched in China.

Disclosure of Potential Conflicts of Interest

Q. Yang is a salaried employee of Jiangsu Hengrui Medicine Co. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J. Huang, B. Xu, Y. Jiao
Development of methodology: J. Huang, B. Xu, W. Zhang, P. Wang, Y. Jiao
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Huang, B. Xu, H. Mo, X. Chen, D. Wu, D. Qu, X. Wang, B. Lan, P. Wang, H. Zhang, Y. Jiao
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Huang, B. Xu, H. Mo, W. Zhang, X. Chen, X. Wang, B. Lan, Y. Jiao
Writing, review, and/or revision of the manuscript: J. Huang, B. Xu, H. Mo, W. Zhang, X. Wang, B. Lan, H. Zhang, Y. Jiao
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Huang, B. Xu, H. Mo, D. Wu, B. Lan, B. Yang
Study supervision: J. Huang, B. Xu, Q. Yang, Y. Jiao

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