Clinical and biomarker analyses of sintilimab versus chemotherapy as second-line therapy for advanced or metastatic esophageal squamous cell carcinoma: a randomized, open-label phase 2 trial (ORIENT-2)

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Abstract

This randomized, open-label, multi-center phase 2 study (ClinicalTrials.gov, number NCT03116152) assessed sintilimab, a PD-1 inhibitor, versus chemo in patients with advanced esophageal squamous cell carcinoma (ESCC) refractory to first-line (1L) chemotherapy. The primary endpoint was overall survival (OS), while exploratory endpoint was the association of biomarkers with treatment efficacy. The median OS in the sintilimab group was significantly prolonged compared with that of the chemotherapy group, (objective response rates 12.6% and 6.3 %, respectively). Incidence of treatment-related adverse events of grade 3–5 was lower with sintilimab than with chemotherapy (20.2 vs. 39.1 %). Patients with high TCR clonality and low mTBI showed the longest median OS (15.0 mo), while patients with low NLR at 6 wk post-treatment had a significantly prolonged median OS compared with those with high NLR. High expression of T-follicular helper cells or activated B-cell signature was significantly associated with longer progression-free survival in the sintilimab group.

Introduction

Esophageal squamous cell carcinoma (ESCC) accounts for approximately 90 % of esophageal cancer (EC), which is the seventh most frequent malignancy globally. The majority of ESCC cases (~ 80 %) have been shown to occur in central and southeastern Asia, with China alone accounting for 53 % of global cases. More than 50 % of patients with ESCC are diagnosed at an advanced stage and have a poor prognosis with a 5-y overall survival (OS) rate ≤ 15 %. Representing a promising avenue to combat such malignancies, immunotherapies have revolutionized antitumor strategies, and programmed cell death protein 1 (PD-1) inhibitors have shown promising clinical benefits in a broad spectrum of cancer types. Pembrolizumab has been approved by the U.S. Food and Drug Administration (FDA) for patients with recurrent, locally advanced, or metastatic ESCC who have received one or more prior lines of systemic treatment, as well as for patients with PD-L1 positive tumors (combined positive score [CPS] ≥ 10). However, other PD-1 inhibitors, such as nivolumab and camrelizumab have not shown a clear association of treatment efficacy with the expression of PD-L1. Therefore, the predictive role of PD-L1 and alternative biomarkers in ESCC should be further investigated.

To this end, RNA sequencing (RNA-seq) is a promising technology for the assessment of the molecular and cellular features of the tumor microenvironment, which could predict the response to immunotherapy. As a convenient liquid biopsy with minimal risk, circulating tumor DNA (ctDNA) is also widely used in biomarker discovery. In addition, the neutrophil-to-lymphocyte ratio (NLR) is an inflammatory response biomarker for the prognosis of ESCC in patients treated with chemoradiotherapy and immune checkpoint inhibitors.

One such agent, sintilimab, is a humanized, monoclonal antibody against PD-1 that has received approval by the National Medical Products Administration (NMPA) for the monotherapy of relapsed or
refractory classical Hodgkin lymphoma and for the treatment of unresectable, locally advanced or metastatic nonsquamous NSCLC in combination with pemetrexed and platinum. ORIENT-2 was a randomized, open-label, phase 2 study that evaluated sintilimab versus chemotherapy in Chinese patients with advanced or metastatic ESCC refractory to first-line chemotherapy and explored potential biomarkers for treatment efficacy.

**Results**

**Patient characteristics**

Between May 16, 2017 and August 30, 2018, 253 patients were screened and 190 eligible patients were randomly assigned to receive either sintilimab or chemo (n = 95 per group, Fig. 1). Accordingly, 94 patients in the sintilimab group and 87 patients in the chemo group received at least one dose of the assigned treatment (Fig. 1). In addition, 15 patients were treated with paclitaxel and 72 with irinotecan. Both the demographics and disease characteristics at baseline were generally balanced between the two groups (Table 1).
Table 1
Baseline demographic and disease characteristics of patients in intention-to-treat (ITT) population

|                              | Sintilimab (N = 95) | Chemo (N = 95) |
|------------------------------|---------------------|----------------|
| **Age, Mean (S.D), years**   | 58.8 (7.23)         | 59.4 (7.06)    |
| **Sex**                      |                     |                |
| Male                         | 88 (92.6%)          | 84 (88.4%)     |
| Female                       | 7 (7.4%)            | 11 (11.6%)     |
| **ECOG PS**                  |                     |                |
| 0                            | 23 (24.2%)          | 23 (24.2%)     |
| 1                            | 72 (75.8%)          | 72 (75.8%)     |
| **Disease stage**            |                     |                |
| IIIA                         | 2 (2.1%)            | 3 (3.2%)       |
| IIIB                         | 1 (1.1%)            | 0              |
| IIIC                         | 4 (4.2%)            | 3 (3.2%)       |
| IV                           | 86 (90.5%)          | 89 (93.7%)     |
| **Pathological diagnosis**   |                     |                |
| Squamous cell carcinoma      | 95 (100%)           | 95 (100%)      |
| **Previous treatment**       |                     |                |
| Chemotherapy                 | 95 (100%)           | 95 (100%)      |
| Failed to adjuvant chemo within 6 months | 11 (11.6%)   | 10 (10.5%)     |
| Failed to first-line chemo   | 84 (88.4%)          | 85 (89.5%)     |
| Radiotherapy                 | 50 (52.6%)          | 58 (61.1%)     |
| Surgery                      | 58 (61.1%)          | 47 (49.5%)     |

ECOG PS: Eastern Cooperative Oncology Group performance status.

**Efficacy**

As of the cutoff date (August 2, 2019), the median duration of follow-up was 7.2 mo (range 3.5–12.4) for the sintilimab group and 6.2 mo (3.3–10.2) for the chemo group. Overall, 69 (72.6 %) patients in the sintilimab group and 81 (85.3 %) patients in the chemo group had death events. Moreover, the median overall survival (OS) was significantly prolonged in the sintilimab group compared with that in the chemo
group (stratified hazard ratio (HR) 0.70 [95 % CI 0.50–0.97], P = 0.032, 7.2 mo [95 % CI 5.8–9.7] vs. 6.2 mo [95 % CI 5.4–7.9]), and a delayed separation of the survival curves was observed (Fig. 2A). The P-values of the weighted log-rank test were less than 0.01 from the Fleming-Harrington (0, 0.2), (0, 0.5) and (0, 1), indicating the delayed effect of the significant OS benefit with sintilimab over chemo. It was thus assumed that sintilimab did not increase the risk of death if used at an earlier time according to the Fleming-Harrington (1, 0) (P = 0.24214) (Table S3). The estimated 12-mo OS rate of patients with sintilimab versus those with chemo was demonstrated to be 37.4 % vs. 21.4 % (Fig. 2A). Moreover, the restricted mean survival time (RMST) at 9, 12, 15, and 18 mo in the sintilimab and chemo groups was 6.3 vs. 6.0 mo, 7.5 vs. 6.9 mo, 8.6 vs. 7.4 mo, 9.2 vs. 7.6 mo. The difference in RMST between the two groups gradually increased with the extension in treatment time.

78 (82.1 %) patients in the sintilimab group and 73 (76.8 %) in the chemo group had progressive disease or death events. However, the difference in median progression-free survival (PFS) per RECIST v1.1 was not significant (stratified HR = 1.00, 95 % CI 0.72–1.39, P = 0.979). The median PFS was 1.6 mo (95 % CI 1.5–2.8) in the sintilimab group and 2.9 mo (95 % CI 2.6–3.6) in the chemo group (Fig. 2B). The 12-mo PFS rate for patients treated with sintilimab versus chemo was 10.4 % vs. 1.7 % (Fig. 2B), with the median PFS in the sintilimab group per iRECIST being 3.8 mo (95 % CI 2.9–6.5).

After initial PD per RECIST v1.1, 37 patients in the sintilimab group continued to receive sintilimab (continuous subgroup), whereas treatment was discontinued for 28 patients (noncontinuous subgroup). A post-hoc analysis of OS in the above two subgroups indicated that the median OS of patients who continuously received sintilimab was dramatically improved over that of those that discontinued treatment (HR = 0.45, P = 0.008, 12.6 vs. 6.2 mo, Fig. 2C).

Based on RECIST v1.1, 12 out of 95 (12.6 %, 95 % CI 6.7–21.0) patients in the sintilimab group achieved a double overall response rate (ORR) compared with the chemo group (6/95, 6.3 %, 95 % CI 2.4–13.2; odds ratio 2.15, 95 % CI 0.77–5.98). In particular, among the responders, the median duration of response (DOR) of patients in the sintilimab group was shown to be higher than that in the chemo group (8.3 mo [95 % CI 2.9–20.9] versus 6.2 mo [95 % CI 4.6–8.4]). However, both treatment groups showed a similar disease control rate (DCR) (44.2 % vs. 43.2 %, Table 2).
Table 2
Antitumor activity of patients in intention-to-treat (ITT) population

| Best overall response per RECIST v1.1 | Sintilimab (N = 95) | Chemo (N = 95) | Odds ratio (95% CI) |
|---------------------------------------|---------------------|----------------|---------------------|
| **CR**                               | 0                   | 0              | –                   |
| **PR**                               | 12 (12.6%)          | 6 (6.3%)       | –                   |
| **SD**                               | 30 (31.6%)          | 35 (36.8%)     | –                   |
| **PD**                               | 41 (43.2%)          | 26 (27.4%)     | –                   |
| **ORR (CR + PR), % [95% CI]**         | 12 (12.6%) [6.7%, 21.0%] | 6 (6.3%) [2.4%, 13.2%] | 2.150 (0.770, 5.998) |
| **DCR (CR + PR + SD), % [95% CI]**    | 42 (44.2%) [34.0%, 54.8%] | 41 (43.2%) [33.0%, 53.7%] | 1.045 (0.586, 1.863) |
| **Median TTR, month (95% CI)**        | 1.5 (1.3, 2.8)      | 1.4 (1.3, 2.8) | –                   |
| **Median DOR, month (95% CI)**        | 8.3 (2.9, 20.9)     | 6.2 (4.6, 8.4) | –                   |
| Best overall response per iRECIST     |                     |                |                     |
| **iCR**                              | 0                   | –              | –                   |
| **iPR**                              | 14 (14.7%)          | –              | –                   |
| **iSD**                              | 30 (31.6%)          | –              | –                   |
| **iUPD**                             | 22 (23.2%)          | –              | –                   |
| **iCPD**                             | 15 (15.8%)          | –              | –                   |
| **iORR (iCR + iPR), % [95% CI]**      | 14 (14.7%) [8.3%, 23.5%] | –              | –                   |
| **iDCR (iCR + iPR + iSD), % [95% CI]**| 44 (46.3%) [36.0%, 56.8%] | –              | –                   |
| **Median iDOR, month (95% CI)**       | NA (5.6, NA)        | –              | –                   |

RECIST, Response Evaluation Criteria in Solid Tumors; iRECIST, modified RECIST v1.1 for immune-based therapeutics; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; DOR, duration of response; TTR, time to response; iCR, iPR, iSD, iORR and iDCR denote CR, PR, SD, ORR and DCR per iRECIST, respectively; iUPD, unconfirmed PD per iRECIST; iCPD, confirmed PD per iRECIST. CI, confidence interval; NA, not available.
In the subgroup analysis for OS, the HRs favored sintilimab over chemo for the following five subgroups (sintilimab vs. chemo): ages ≤ 65 y (HR 0.642, \( P = 0.017 \)), male (HR 0.692, \( P = 0.037 \)), previous treatment with paclitaxel (HR 0.616, \( P = 0.009 \)), ECOG PS score of 1 (HR 0.649, \( P = 0.024 \)), and current smokers (HR 0.261, \( P = 0.021 \)) (Fig. 3). However, no significant differences were noted in either OS or PFS between the two treatment groups across PD-L1 expression subgroups (\( P > 0.05 \), Fig. 3 and Fig. S1).

**Health-related quality of life**

Based on EQ 5D-5L and EORTC QLQ-C30, patients in the sintilimab group presented with better health-related quality of life at the early stage but exhibited no differences at the late stage from patients in the chemo group (Table. S4).

**Safety**

The median duration of treatment with sintilimab, paclitaxel, and irinotecan was 11.9 (range 3–112), 11.9 (range 3–42) and 7.0 (3–30) wk, respectively; the median cycle was 4 (range 1–36), 4 (range 1–14), and 3 (1–14), respectively. The median relative dose intensity was 100.0 % (range 66.7–107.7), 96.5 % (range 80.7–104.5), and 98.2 % (range 55.6–105.6), respectively.

Treatment emergent adverse events (TEAEs) of any grade occurred in 88 (93.6 %) patients with sintilimab, and 81 (93.1 %) patients with chemo. Treatment-related TEAEs (TRAEs) were reported in 54.3 % of patients in the sintilimab group, which was approximately half the number of TRAEs reported in the chemo group (90.8 %) (Table 3). The most common TRAEs were hypothyroidism (12.8 %), pulmonary inflammation (10.6 %), anemia (8.5 %), and decreased white blood cell (WBC) counts (8.5 %) in the sintilimab group, whereas in the chemo group, common TRAEs included decreased WBC counts (48.3 %), anemia (37.9 %), decreased neutrophil counts (34.5 %), and diarrhea (33.3 %) (Table 3). The incidence of serious TRAEs was similar between the sintilimab (18.1 %) and chemo (20.7 %) groups. The most common serious TRAEs were pulmonary inflammation (7.4 %) and abnormal hepatic function (4.3 %) in the sintilimab group and bone marrow failure (6.9 %) and diarrhea (2.3 %) in the chemo group (Table 3). However, the incidence of TRAEs of grade 3 or worse was lower in the sintilimab group (20.2 %) than in the chemo group (39.1 %). The most common grade 3 or worse TRAEs were pulmonary inflammation (5.3 %) and elevated lipase levels (4.3 %) in sintilimab group, whereas the most frequent grade 3 or worse TRAEs in the chemo group were decreased neutrophil counts (18.4 %) and decreased WBC counts (16.1 %) (Table 3). The number of patients discontinuing treatment because of TRAEs was 13 (13.8 %) and 7 (8.0 %) in the sintilimab and chemo groups, respectively. In addition, 3 (3.2 %) deaths that occurred in the sintilimab group were attributed to treatment-related adverse events; these included upper gastrointestinal bleeding, pneumonitis, and lung infection. Finally, 1 (1.1 %) death in the chemo group was attributed to a treatment-related adverse event; this was a lung infection.
Table 3
Summary of adverse events in two groups

|                      | Sintilimab (N= 94) | Chemo (N= 87) |
|----------------------|--------------------|---------------|
|                      | Any Grade 1–2 Grade ≥ 3 Any Grade 1–2 Grade ≥ 3 |                      |
| All events           | 88 (93.6) 36 (38.3) 52 (55.3) | 81 (93.1) 40 (46.0) 41 (47.1) |
| TRAEs                | 51 (54.3) 32 (34.0) 19 (20.2) | 79 (90.8) 45 (51.7) 34 (39.1) |
| Hypothyroidism       | 12 (12.8) 0 (0.0) 1 (1.1) | 1 (1.1) 1 (1.1) 0 (0.0) |
| Pulmonary inflammation| 10 (10.6) 5 (5.3) 5 (5.3) | 1 (1.1) 1 (1.1) 0 (0.0) |
| Anemia               | 8 (8.5) 8 (8.5) 0 (0.0) | 33 (37.9) 28 (32.2) 5 (5.7) |
| WBC count decreased  | 8 (8.5) 7 (7.4) 1 (1.1) | 42 (48.3) 28 (32.2) 14 (16.1) |
| ALT increased        | 7 (7.4) 7 (7.4) 0 (0.0) | 6 (6.9) 6 (6.9) 0 (0.0) |
| AST increased        | 7 (7.4) 7 (7.4) 0 (0.0) | 5 (5.7) 5 (5.7) 0 (0.0) |
| Amylase increased    | 6 (6.4) 4 (4.3) 2 (2.1) | 0 (0.0) 0 (0.0) 0 (0.0) |
| Cough                | 6 (6.4) 4 (4.3) 2 (2.1) | 1 (1.1) 1 (1.1) 0 (0.0) |
| Abnormal liver function| 6 (6.4) 5 (5.3) 1 (1.1) | 4 (4.6) 3 (3.4) 1 (1.1) |
| Neutrophils count decreased | 5 (5.3) 3 (3.2) 2 (2.1) | 30 (34.5) 14 (16.1) 16 (18.4) |
| Platelet count decreased | 5 (5.3) 3 (3.2) 2 (2.1) | 10 (11.5) 9 (10.3) 1 (1.1) |
| Fatigue              | 5 (5.3) 5 (5.3) 0 (0.0) | 19 (21.8) 19 (21.8) 0 (0.0) |
| Lipase elevated      | 4 (4.3) 0 (0.0) 4 (4.3) | 0 (0.0) 0 (0.0) 0 (0.0) |
| Diarrhea             | 4 (4.3) 4 (4.3) 0 (0.0) | 29 (33.3) 24 (27.6) 5 (5.7) |
| Lymphocyte count decreased | 3 (3.2) 1 (1.1) 2 (2.1) | 5 (5.7) 4 (4.6) 1 (1.1) |

Data are presented in %. Grade 1–2 TRAE listed with an incidence of ≥ 5% of patients in either treatment group, and grade 3–5 TRAEs with an incidence of ≥ 2% in either group. IrAEs were occurred in ≥ 2% of patients in either treatment group. TRAE, treatment-related adverse event; irAE, immune-related adverse event; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
| Side Effect                              | Sintilimab (N= 94) | Chemo (N= 87) |
|-----------------------------------------|--------------------|--------------|
| Hypochloremia                           | 3 (3.2)            | 1 (1.1)      |
| Nausea                                  | 3 (3.2)            | 28 (32.2)    |
| Proteinurina                            | 3 (3.2)            | 5 (5.7)      |
| Vomiting                                | 2 (2.1)            | 18 (20.7)    |
| Upper gastrointestinal hemorrhage       | 2 (2.1)            | 2 (2.3)      |
| Lung infection                          | 2 (2.1)            | 2 (2.3)      |
| Decreased appetite                      | 1 (1.1)            | 17 (19.5)    |
| Abdominal pain                          | 1 (1.1)            | 6 (6.9)      |
| Hypokalemia                             | 1 (1.1)            | 5 (5.7)      |
| Alopecia                                | 0                  | 13 (14.9)    |
| Bone marrow failure                     | 0                  | 10 (11.5)    |
| Hypaesthesia                            | 0                  | 6 (6.9)      |
| Immune-related AE                       | 29 (30.9)          | 21 (22.3)    |
| Pulmonary inflammation                  | 9 (9.6)            | 5 (5.3)      |
| Hypothyroidism                          | 8 (8.5)            | 8 (8.5)      |
| Rash                                    | 4 (4.3)            | 3 (3.2)      |
| Abnormal liver function                 | 3 (3.2)            | 3 (3.2)      |
| Lung infection                          | 2 (2.1)            | 1 (1.1)      |
| Psoriasis                               | 2 (2.1)            | 2 (2.1)      |
| Diarrhea                                | 2 (2.1)            | 2 (2.1)      |
| Anaemia                                 | 2 (2.1)            | 2 (2.1)      |

Data are presented in %. Grade 1−2 TRAE listed with an incidence of ≥ 5% of patients in either treatment group, and grade 3−5 TRAEs with an incidence of ≥ 2% in either group. IrAEs were occurred in ≥ 2% of patients in either treatment group. TRAE, treatment-related adverse event; irAE, immune-related adverse event; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Additionally, 29 (30.9 \%) patients in the sintilimab group experienced immune-related TEAEs (irAE), with pulmonary inflammation (9.6 \%) and hypothyroidism (8.5 \%) occurring most frequently. The majority of these irAEs were demonstrated to be of grade 1–2 severity (22.3 \%, Table 3), with only 8 (8.5 \%) patients reporting irAE of grade 3 or worse.

**Immune cell signature and signaling pathway analysis**

Among 158 patients (n = 84 in the sintilimab group and n = 74 in the chemo group) whose tumor tissue samples were available and sequenced, 118 patients (n = 64 and n = 54, respectively) had qualifiable RNA-seq data for downstream analysis. Out of the 28 immune cell populations analyzed,\(^1\) high infiltration of T-follicular helper cells (HR = 0.46, \(P = 0.007\)) and activated B-cells (HR = 0.54, \(P = 0.030\)) was significantly associated with longer PFS in the sintilimab group but not in the chemo group (Table S1, Fig. 5). However, no significant association was observed between any of the tested immune cell signatures and OS in either groups (Table S1).

Further, analysis of 45 signaling pathways revealed that 4 pathways were significantly associated with improved OS in the sintilimab group, among which the high expression of the Wnt signaling pathway showed the strongest correlation (HR = 0.46, \(P = 0.011\)) (Table S2). Moreover, patients treated with sintilimab and under high oxidative stress had longer PFS (HR = 0.50, \(P = 0.016\)) (Table S2).

**Association between neutrophil-to-lymphocyte ratio and efficacy of sintilimab**

In the sintilimab group, 81 patients had evaluable NLR levels at baseline, with 40 being classified into the low NLR (< 3) group, whereas 41 were assigned into the high NLR (\(\geq 3\)) group. Patients with a low NLR at baseline showed a significant improvement on OS (HR 0.54, \(P = 0.019\); median 14.0 vs. 6.2 mo) and PFS (HR 0.47, \(P = 0.002\); median 2.9 vs. 1.5 mo) compared with those with a high NLR at baseline (Fig. 4A and 4B). Notably, at 6 wk post-sintilimab administration, the survival benefit was also significant in patients with low versus high NLR, with a median OS of 16.6 vs. 6.2 mo (HR 0.19, \(P < 0.001\); Fig. 4C) and a median PFS of 4.3 vs. 2.3 mo (HR 0.47, \(P = 0.006\); Fig. 4D). In addition, the DCR was significantly higher in the low NLR group than in the high NLR group at baseline (65.0 \% vs. 39.0 \%, \(P = 0.019\); Fig. 4E).

**Blood T-cell receptor and ctDNA analysis**

To further explore indicators of clinical benefit in the periphery, blood T-cell receptor (TCR, n = 95) and ctDNA (n = 83) sequencing was performed for patients in the sintilimab group. Specifically, patients with low molecular tumor burden index (mTBI) showed significantly longer PFS (HR = 0.58, \(P = 0.030\)) compared with those with high mTBI (Fig. S2D). Similar trends were also observed for OS (Fig. S2C). However, TCR clonality alone could not efficiently predict the clinical outcome of patients treated with sintilimab (Fig. S2A and S2B), whereas the prediction power in the benefit of OS was shown to be enhanced when TCR was combined with mTBI. As shown in Fig. 6A, despite the limited sample size, patients with both high TCR clonality and low mTBI had a longer OS than patients with high TCR clonality and high mTBI (HR = 0.32, \(P = 0.008\)), and a longer OS than those with low TCR clonality and
high mTBI or even than those with low TCR clonality and low mTBI. This potential predictive value of the combination of TCR clonality with mTBI as a composite biomarker was further supported by PFS data (Fig. 6B).

**Discussion**

This phase 2 study revealed that sintilimab monotherapy resulted in an evidently prolonged survival outcome and had a favorable safety profile versus chemo in patients with advanced ESCC refractory to previous chemotherapy.

Both nivolumab and pembrolizumab showed encouraging results in patients with advanced ESCC refractory or intolerant to chemo (median OS > 6 mo). The KEYNOTE-181 study demonstrated a favorable survival benefit for pembrolizumab over chemo in second line (2L) patients with ESCC (HR = 0.75, P = 0.0035, median OS 8.2 vs. 7.1 mo), although the primary endpoint was not significantly reached. In the ATTRACTION-3 study, nivolumab remarkably improved the median OS in 2L patients with ESCC when compared with chemo (HR = 0.77, P = 0.019, median OS 10.9 vs. 8.4 mo). In the ESCORT study, camrelizumab also significantly improved the median OS in 2L patients with ESCC when compared with chemo (HR = 0.71, P = 0.001, median OS 8.3 vs. 6.2 mo). Although the median OS in the aforementioned studies varied based on different populations and baseline characteristics, our study showed a significant survival benefit of sintilimab over chemo, either for the overall population or several subgroups based on age, ECOG PS, and smoking status, consistent with these studies. Both the OS and PFS KM survival curves were shown to cross at an early stage and diverge beyond 5 mo, in favor of sintilimab. This was similar to the results obtained in studies on nivolumab and pembrolizumab in ESCC and other indications, suggesting a delayed response and more durable benefit of immunotherapies with these indications. In addition, the survival advantage of sintilimab was demonstrated via a prolonged follow-up duration. The superior RMST of sintilimab over chemo was increased from 0.3 mo at 9 mo to 1.6 mo at 18 mo, further suggesting a delayed survival benefit of sintilimab.

Patients in the sintilimab group had an ORR of 12.6 %, comparable to the contemporary phase 2 studies of PD-1 inhibitor monotherapies in ESCC (14–17 %). Moreover, the ORR of patients with sintilimab was twice higher than those with chemo, similar to the findings in the Chinese population of the KEYNOTE-181 study and the patients in the ESCORT study. Notably, for patients who were initially evaluated as PD per RECIST v1.1 in the sintilimab group, those continuing to receive sintilimab had a remarkable increase on median OS, compared with those that discontinued treatment. This finding suggested that continuous treatment with sintilimab could have a long-term survival benefit and should be further explored for patients after initial PD by RECIST v1.1.

Sintilimab showed a favorable safety profile over chemo, with a substantially reduced incidence of TRAEs (54.3 vs. 90.8 %) and TRAEs of grade 3 or worse (20.2 vs. 39.1 %). Most toxic effects were comparable to the historical data on treatment with sintilimab in relapsed or refractory classical Hodgkin
lymphoma and to that of treatments with other PD-1 inhibitors in EC or solid tumors. In particular, three treatment-related deaths, which were due to upper gastrointestinal bleeding, pneumonitis, and lung infection, occurred in the sintilimab group. Pneumonitis and lung infection were previously reported in pembrolizumab- or nivolumab-treated patients with ESCC or EC. While upper gastrointestinal bleeding is regarded as a common cause of death in ESCC, the estimate of cause of death from researchers might be biased as this was an open-label study.

In this trial, comprehensive biomarker studies were performed, including for tumor PD-L1 expression, tumor transcriptome analysis, NLR and peripheral blood TCR, and ctDNA analysis. Similar to the ESCORT study, none of the TPS or CPS could predict any benefit from the treatment with sintilimab, implying that alternative biomarkers are needed.

Analyzing tumor transcriptomes revealed two immune cell signatures, including infiltration of T-follicular helper cells and activated B-cells, which were significantly related to PFS in immunotherapy but not in the chemotherapy arm. On the basis of the well-established function of T-cells in cancer immunotherapy, an emerging role of B-cells and tertiary lymphoid structures in tumors has been recently reported and associated them with patient survival. Furthermore, T-follicular helper cells have been shown to play important roles in the generation of tertiary lymphoid structures in the tumor microenvironment. Thus, our results underscoring a potential relevance of tertiary lymphoid structures in the mode of action of cancer immunotherapy warrant further investigation.

Baseline peripheral TCR clonality alone did not predict the efficacy of sintilimab in this study, potentially because the PD-1 inhibitor-mediated antitumor responses relied less on T-cell quantity rather than tumor antigen specificity, which could not be reflected by TCR sequencing. Conflicting results from previous studies using TCR clonality or diversity to predict the efficacy of immunotherapy might have been due to the dilution effect of nontumor specific TCR. The mTBI is a reflection of the percentage of ctDNA detected in blood cell free DNA, and might be considered an indicator of tumor burden. Compared with TCR clonality, the mTBI index was significantly associated with clinical efficacy in the treatment with sintilimab. When mTBI was combined with TCR, the prediction was further enhanced: patients with high TCR clonality and low mTBI exhibited the longest survival. The higher TCR clonality and lower mTBI in these patients implied an existing and expanded tumor-killing immune response, which might be suppressed by the PD-1 axis, and is thus important for the response of patients to treatment with sintilimab.

As is known, NLR can reflect differences in the immune status during cancer development and progression. Our results revealed that a low NLR (< 3) prior to or at 6 wk after treatment with sintilimab was significantly correlated with a longer OS and PFS. So, NLR together with TCR and mTBI supported the validation of peripheral blood biomarkers in future clinical trials for improved patient selection.

This study had several limitations. First, the patient sample size in the phase 2 study was relatively small. Second, the open-label design of the study might have influenced the assessment of the incidence of
adverse effects. Nevertheless, this design was deemed acceptable because both dose regimens and toxicities in the two groups were disparate. Third, due to limited tumor samples available for the assessment of PD-L1 expression, it was hard to evaluate its correlation with the survival benefit of sintilimab.

In conclusion, our data favored the use of sintilimab over chemo in Chinese patients with advanced ESCC refractory to previous chemotherapies, as it suggested a prolonged survival benefit and a favorable safety profile. Both NLR (< 3) at 6 wk post-treatment and the combination of high TCR clonality with low mTBI might be potent biomarkers for the prediction of improved OS and PFS in patients with ESCC treated with sintilimab. A phase 3 study (NCT03748134), which could provide potent evidence for the potential use of sintilimab in patients with ESCC, is currently ongoing.

**Methods**

**Study design and patients**

This was a multicenter, randomized, parallel, open-label phase 2 trial conducted at 35 sites in China (NCT03116152).

Patients with histopathologically or cytologically confirmed locally advanced or metastatic ESCC aged between 18 and 75 y were enrolled in the study. Additional major eligibility criteria were at least one measurable lesion per Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1), Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1, eligible to provide a fresh or archived tumor sample for detection of PD-L1 status, radiological or clinical evidence of disease progression during or after first-line chemotherapy, and treated with at least one dose of first-line therapy (surgery, chemo, or radiotherapy).

Patients who received prior PD-1 or PD-L1 antibodies, or those who received any radiotherapy, immunosuppressive drugs (except for ≤ 10 mg/d prednisone or other glucocorticoids) or the study drug within 4 wk prior to the first drug dose, were excluded. Detailed inclusion criteria are provided in the Supplementary material.

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the ethics committee at each site. All patients provided written informed consent prior to enrollment.

**Procedures**

Patients were randomly (1:1) assigned to receive either sintilimab or the investigator’s choice of chemo (paclitaxel or irinotecan), using an interactive web response system with a block size of a mixture of 2, 4, and 6, and with the stratification factor of the ECOG PS score (0 vs. 1). Neither investigators nor patients were blinded to treatment allocation.
After randomization, patients were treated with 200 mg intravenous sintilimab, once every 3 wk, or with the investigator’s choice of chemo (175 mg/m² paclitaxel once every 3 wk, or 180 mg/m² irinotecan once every 2 wk; both given intravenously), until disease progression, death, unacceptable toxicity, or withdrawal of informed consent.

Tumor assessment was performed by investigators according to RECIST v1.1 at baseline, every 6 wk from the initiation of cycle 1 for 24 wk, and every 9 wk thereafter until either initiation of a new antitumor therapy, disease progression, withdrawal of informed consent, or death. In the sintilimab group, patients who were initially assessed as progressive disease (PD) per RECIST v1.1 could continue to receive sintilimab at the investigator’s discretion if they had a stable status, and were then reevaluated per modified RECIST v1.1 for immune-based therapeutics (iRECIST). Adverse events (AEs) and serious adverse events (SAEs) were monitored throughout the treatment period and for 90 d after the end of treatment, based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCICTCAE V. 4.03).

Measurements of PD-L1 and NLR

For patients with available tumor samples, the expression of PD-L1 in tumor cells was assessed via immunohistochemistry (Dako PD-L1 IHC 22C3 pharmDx) using the Autostainer Link 48 (clone 22C3, Dako, Carpinteria, CA, USA). PD-L1 protein staining was determined using the tumor proportion score (TPS) or the CPS. Details are provided in Supplementary material.

The NLR inflammatory biomarker, which was calculated as the total neutrophil count/total lymphocyte count, was obtained from hematology panels at baseline and 6 wk post-sintilimab treatment. High and low NLR groups were classified using the cutoff of NLR = 3.37

Biomarker analysis using next generation sequencing and bioinformatics methods

The gene sets defining 28 immune cell populations were extracted from the reference 18. The gene sets of 45 signaling pathways were downloaded from the tumor signaling 360 panel of NanoString (Seattle, Washington, US). Gene set signature scores were calculated using the GSVA algorithm38 based on the gene expression levels of TPM. High and low gene expression groups or signature scores were split according to the median value of the whole cohort. Blood TCR clonality and molecular tumor burden index (mTBI)36 were calculated in each sample. High and low TCR clonality or mTBI of baseline samples were split according to the median value of the whole cohort. Details of all data processing are shown in Supplementary material.

Outcomes

The primary endpoint was OS, defined as the time from randomization until death due to any reason. Surviving patients were censored using the data at the last follow-up. Secondary endpoints were progression-free survival (PFS), which was the time from randomization to initial disease progression or
death; objective response rate (ORR), defined as the percentage of patients achieving complete response (CR) or partial response (PR); time to response (TTR); disease control rate (DCR), which was the percentage of patients with the best overall response of CR, PR, or stable disease (SD); and duration of response (DOR). As safety endpoint was considered the incidence of all AEs across the study.

Exploratory endpoints were the association between efficacy (OS and PFS) and predefined subgroup factors (PD-L1 expression levels, age, sex, ECOG PS, previous paclitaxel treatment, and smoking history); the association between efficacy (ORR, OS, PFS) and NLR, as well as the correlations of other biomarkers with prognosis.

Health-related quality of life (HRQoL) was assessed by the European Organisation for Research and Treatment of Cancer Quality-of-Life Questionnaire-Core 30 (EORTC QLQ-C30), and the five-level version of European Quality of Life-5 Dimensions (EQ-5D-5L) visual analogue scale (VAS), at the first dose, as well as at every radiological evaluation time and at the time of the first safety follow-up.

**Statistical analysis**

The required death event number was 142, with an expected hazard ratio (HR) for OS of 0.7 ($\alpha = 0.2$, two-sided) under a statistical power of 80%. Assuming a 21% dropout rate, 180 patients were required, with 90 patients in each group.

All efficacy endpoints were assessed in the intention-to-treat (ITT) population that included all randomly assigned patients. The safety profile was assessed in the safety set (SS), which included patients receiving at least one dose of the study drug.

The Kaplan-Meier (KM) method was utilized to evaluate the time to event endpoints (median OS, PFS, and DOR), and the differences in the survival curves between the two groups were analyzed using the stratified log-rank test. A stratified Cox proportional hazards regression model was used to estimate the HR and corresponding 95% confidence interval (CI) of OS and PFS between treatment groups, using the ECOG PS score (0 vs. 1) as a stratification factor. The RMST was evaluated to compare the differences in OS between treatment groups at 9, 12, 15, and 18 mo. The binomial distribution method was used to evaluate the 95% CI of ORR and DCR, and differences were compared using the Fisher’s exact test. Chi-squared or Fisher’s exact tests were used to compare differences in tumor response (ORR and DCR) between different NLR groups.

To evaluate any factors correlated with the crossing of the survival curves in OS, different treatments were assessed using a weighted log-rank test from the FH G($^{\rho - \gamma}$), which accounts for nonproportional hazards ($\rho, \gamma = 0.1; 0, 0.5; 0, 0.2; 1, 0$). Considering the potential pseudoprogression for PD-1 inhibitors, a post-hoc analysis was performed to compare the difference in OS between patients with or without continuous treatment of sintilimab after initial PD per RECIST v1.1 in the sintilimab group.

Statistical analyses for the clinical part were performed using the SAS software (version 9.4) (Cary, North Carolina, US). The significance level for all endpoints was $\alpha = 0.05$ (two-sided) and the significance
threshold was $P < 0.05$. For biomarker identification using bioinformatics, survival analysis was performed using the survival package\textsuperscript{39} in R, by which both the $P$ value and hazard ratio between two groups were calculated using the coxph function. Survival curves were plotted using the survminer package.\textsuperscript{40}

**Declarations**

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**Author contributions**

J.M.X, Y.L and Q.X.F contributed the study design and manuscript drafting. Y.Q.S, L.Y, T.J.C, K.S. G, M.T, X.W.W, C.X.C, N.X, J.X.X, Q.L.G, Y.P.L, T.Z, W.L, Y.P.Z, G.H.D, D.M, J.D.Z, C.M.B, Y.C.H, W.J.L, L.W, X.C, Y.Y, J.Y.W, Y.X.B, and S.J.J contributed patient enrollment and data collection. Y.Q.W contributed statistical analysis. H.Z, Y.W and Z.M contributed data interpretation and medical review. B.P, J.Y.S and C.M. contributed biomarker analysis and involved in manuscript drafting.

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**Competing interests**

H.Z, Y.W, Z.M, Y.Q.W, B.P, J.Y.S and C.M. are the staff of Innovent Biologics, Inc. Other authors declare no completing interests.

**Data availability**

Data supporting the results of this study are available within the article. Supplementary material could be obtained on reasonable request from the corresponding author.

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### Tables

**Table 1.** Baseline demographic and disease characteristics of patients in intention-to-treat (ITT) population
|                                   | Sintilimab (N=95) | Chemo (N=95) |
|-----------------------------------|-------------------|--------------|
| Age, Mean (S.D), years            | 58.8 (7.23)       | 59.4 (7.06)  |
| Sex                               |                   |              |
| Male                              | 88 (92.6%)        | 84 (88.4%)   |
| Female                            | 7 (7.4%)          | 11 (11.6%)   |
| ECOG PS                           |                   |              |
| 0                                 | 23 (24.2%)        | 23 (24.2%)   |
| 1                                 | 72 (75.8%)        | 72 (75.8%)   |
| Disease stage                     |                   |              |
| IIIA                              | 2 (2.1%)          | 3 (3.2%)     |
| IIIB                              | 1 (1.1%)          | 0            |
| IIIC                              | 4 (4.2%)          | 3 (3.2%)     |
| IV                                | 86 (90.5%)        | 89 (93.7%)   |
| Pathological diagnosis            |                   |              |
| Squamous cell carcinoma           | 95 (100%)         | 95 (100%)    |
| Previous treatment                |                   |              |
| Chemotherapy                      | 95 (100%)         | 95 (100%)    |
| Failed to adjuvant chemo within 6 months | 11 (11.6%) | 10 (10.5%)   |
| Failed to first-line chemo        | 84 (88.4%)        | 85 (89.5%)   |
| Radiotherapy                      | 50 (52.6%)        | 58 (61.1%)   |
| Surgery                           | 58 (61.1%)        | 47 (49.5%)   |

ECOG PS: Eastern Cooperative Oncology Group performance status.

Table 2. Antitumor activity of patients in intention-to-treat (ITT) population
| Best overall response per RECIST v1.1 | Sintilimab (N=95) | Chemo (N=95) | Odds ratio (95% CI) |
|--------------------------------------|------------------|-------------|---------------------|
| CR                                  | 0                | 0           | --                  |
| PR                                  | 12 (12.6%)       | 6 (6.3%)    | --                  |
| SD                                  | 30 (31.6%)       | 35 (36.8%)  | --                  |
| PD                                  | 41 (43.2%)       | 26 (27.4%)  | --                  |
| ORR (CR+PR), % [95% CI]             | 12 (12.6%) [6.7%, 21.0%] | 6 (6.3%) [3.2%, 13.2%] | 2.150 (0.770, 5.998) |
| DCR (CR+PR+SD), % [95% CI]          | 42 (44.2%) [34.0%, 54.8%] | 41 (43.2%) [33.0%, 53.7%] | 1.045 (0.586, 1.863) |
| Median TTR, month (95% CI)          | 1.5 (1.3, 2.8)   | 1.4 (1.3, 2.8) | --                  |
| Median DOR, month (95% CI)          | 8.3 (2.9, 20.9)  | 6.2 (4.6, 8.4) | --                  |
| Best overall response per iRECIST   |                  |             |                     |
| iCR                                 | 0                | --          | --                  |
| iPR                                 | 14 (14.7%)       | --          | --                  |
| iSD                                 | 30 (31.6%)       | --          | --                  |
| iUPD                                | 22 (23.2%)       | --          | --                  |
| iCPD                                | 15 (15.8%)       | --          | --                  |
| iORR (iCR+iPR), % [95% CI]          | 14 (14.7%) [8.3%, 23.5%] | --          | --                  |
| iDCR (iCR+iPR+iSD), % [95% CI]      | 44 (46.3%) [36.0%, 56.8%] | --          | --                  |
| Median iDOR, month (95% CI)         | NA (5.6, NA)     | --          | --                  |

RECIST, Response Evaluation Criteria in Solid Tumors; iRECIST, modified RECIST v1.1 for immune-based therapeutics; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; DOR, duration of response; TTR, time to response; iCR, iPR, iSD, iORR and iDCR demote CR, PR, SD, ORR and DCR per iRECIST, respectively; iUPD, unconfirmed PD per iRECIST; iCPD, confirmed PD per iRECIST. CI, confidence interval; NA, not available.

**Table 3.** Summary of adverse events in two groups
| Event                        | Sintilimab (N=94) | Chemo (N=87) |
|------------------------------|-------------------|-------------|
|                              | Any               | Grade 1-2   | Grade ≥3 | Any             | Grade 1-2 | Grade ≥3 |
| All events                   | 88 (93.6)         | 36 (38.3)   | 52 (55.3) | 81 (93.1)         | 40 (46.0)  | 41 (47.1) |
| TRAEs                        | 51 (54.3)         | 32 (34.0)   | 19 (20.2) | 79 (90.8)         | 45 (51.7)  | 34 (39.1) |
| Hypothyroidism               | 12 (12.8)         | 12 (12.8)   | 0 (0)    | 1 (1.1)           | 1 (1.1)    | 0 (0)    |
| Pulmonary inflammation       | 10 (10.6)         | 5 (5.3)     | 5 (5.3)  | 1 (1.1)           | 1 (1.1)    | 0 (0)    |
| Anemia                       | 8 (8.5)           | 8 (8.5)     | 0 (0)    | 33 (37.9)         | 28 (32.2)  | 5 (5.7)  |
| WBC count decreased          | 8 (8.5)           | 7 (7.4)     | 1 (1.1)  | 42 (48.3)         | 28 (32.2)  | 14 (16.1) |
| ALT increased                | 7 (7.4)           | 7 (7.4)     | 0 (0)    | 6 (6.9)           | 6 (6.9)    | 0 (0)    |
| AST increased                | 7 (7.4)           | 7 (7.4)     | 0 (0)    | 5 (5.7)           | 5 (5.7)    | 0 (0)    |
| Amylase increased            | 6 (6.4)           | 4 (4.3)     | 2 (2.1)  | 0 (0)             | 0 (0)      | 0 (0)    |
| Cough                        | 6 (6.4)           | 4 (4.3)     | 2 (2.1)  | 1 (1.1)           | 1 (1.1)    | 0 (0)    |
| Abnormal liver function      | 6 (6.4)           | 5 (5.3)     | 1 (1.1)  | 4 (4.6)           | 3 (3.4)    | 1 (1.1)  |
| Neutrophils count decreased  | 5 (5.3)           | 3 (3.2)     | 2 (2.1)  | 30 (34.5)         | 14 (16.1)  | 16 (18.4) |
| Platelet count decreased     | 5 (5.3)           | 3 (3.2)     | 2 (2.1)  | 10 (11.5)         | 9 (10.3)   | 1 (1.1)  |
| Fatigue                      | 5 (5.3)           | 5 (5.3)     | 0 (0)    | 19 (21.8)         | 19 (21.8)  | 0 (0)    |
| Lipase elevated              | 4 (4.3)           | 0 (0)       | 4 (4.3)  | 0 (0)             | 0 (0)      | 0 (0)    |
| Diarrhea                     | 4 (4.3)           | 4 (4.3)     | 0 (0)    | 29 (33.3)         | 24 (27.6)  | 5 (5.7)  |
| Lymphocyte count decreased   | 3 (3.2)           | 1 (1.1)     | 2 (2.1)  | 5 (5.7)           | 4 (4.6)    | 1 (1.1)  |
| Hypochloremia                | 3 (3.2)           | 1 (1.1)     | 2 (2.1)  | 0 (0)             | 0 (0)      | 0 (0)    |
| Nausea                       | 3 (3.2)           | 3 (3.2)     | 0 (0)    | 28 (32.2)         | 26 (29.9)  | 2 (2.3)  |
| Proteinuria                  | 3 (3.2)           | 3 (3.2)     | 0 (0)    | 5 (5.7)           | 4 (4.6)    | 1 (1.1)  |
| Vomiting                     | 2 (2.1)           | 2 (2.1)     | 0 (0)    | 18 (20.7)         | 14 (16.1)  | 4 (4.6)  |
| Upper gastrointestinal       | 2 (2.1)           | 0 (0)       | 2 (2.1)  | 0 (0)             | 0 (0)      | 0 (0)    |
| hemorrhage                   |                   |             |          |                  |            |          |
| Lung infection               | 2 (2.1)           | 0 (0)       | 2 (2.1)  | 2 (2.3)           | 1 (1.1)    | 1 (1.1)  |
| Decreased appetite           | 1 (1.1)           | 1 (1.1)     | 0 (0)    | 17 (17)           | 15 (15)    | 2 (2.3)  |
| Condition                     | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 | Treatment 5 |
|------------------------------|-------------|-------------|-------------|-------------|-------------|
| Abdominal pain               | 1 (1.1)     | 1 (1.1)     | 0           | 6 (6.9)     | 6 (6.9)     | 0           |
| Hypokalemia                  | 1 (1.1)     | 1 (1.1)     | 0           | 5 (5.7)     | 4 (4.6)     | 1 (1.1)     |
| Alopecia                     | 0           | 0           | 0           | 13          | 13          | 0           |
| Bone marrow failure          | 0           | 0           | 0           | 10          | 4 (4.6)     | 6 (6.9)     |
| Hypaesthesia                 | 0           | 0           | 0           | 6 (6.9)     | 4 (4.6)     | 2 (2.3)     |
| Immune-related AE            | 29 (30.9)   | 21 (22.3)   | 8 (8.5)     | 0           | 0           | 0           |
| Pulmonary inflammation       | 9 (9.6)     | 5 (5.3)     | 4 (4.3)     | 0           | 0           | 0           |
| Hypothyroidism               | 8 (8.5)     | 8 (8.5)     | 0           | 0           | 0           | 0           |
| Rash                         | 4 (4.3)     | 3 (3.2)     | 1 (1.1)     | 0           | 0           | 0           |
| Abnormal liver function      | 3 (3.2)     | 3 (3.2)     | 0           | 0           | 0           | 0           |
| Lung infection               | 2 (2.1)     | 1 (1.1)     | 1 (1.1)     | 0           | 0           | 0           |
| Psoriasis                    | 2 (2.1)     | 2 (2.1)     | 0           | 0           | 0           | 0           |
| Diarrhea                     | 2 (2.1)     | 2 (2.1)     | 0           | 0           | 0           | 0           |
| Anaemia                      | 2 (2.1)     | 2 (2.1)     | 0           | 0           | 0           | 0           |

Data are presented in %. Grade 1–2 TRAE listed with an incidence of ≥5% of patients in either treatment group, and grade 3–5 TRAEs with an incidence of ≥2% in either group. IrAEs were occurred in ≥2% of patients in either treatment group. TRAE, treatment-related adverse event; irAE, immune-related adverse event; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Figures**
Figure 1

Flowchart of enrollment and allocation of the ORIENT-2 study. †, unqualified pathological type; *, patients refused to receive treatment, but received follow-up. Treatment discontinuation occurred due to prespecified conditions such as serious protocol deviation, using prohibited drug in the study, loss of follow-up, life-threatening adverse events and other unacceptable toxicities.
Figure 2

Kaplan-Meier estimates of survival in the intention-to-treat population (N=190), evaluated by the investigator per RECIST v1.1. A. Overall survival (OS); B. Progression free survival; C. OS comparison between patients received sintilimab or not after initial PD per RECIST v1.1.
Table of subgroups with HR and 95% CI for overall survival:

| Subgroup                              | Sintilimab | Paclitaxel/IRinotecan | HR (95% CI) |
|---------------------------------------|------------|-----------------------|-------------|
| Age (year): <=65                      | 55/78 (70.5%) | 68/78 (87.2%)         | 0.642 (0.444, 0.922) |
| Age (year): >65                       | 14/17 (82.4%)  | 13/17 (76.5%)         | 0.983 (0.453, 2.150) |
| Gender: Male                          | 63/88 (71.6%)  | 71/84 (84.5%)         | 0.692 (0.488, 0.977) |
| Gender: Female                        | 6/7 (85.7%)   | 10/11 (90.9%)         | 0.745 (0.247, 2.093) |
| Previous paclitaxel treatment: Yes    | 57/80 (71.3%)  | 69/77 (88.3%)         | 0.616 (0.428, 0.883) |
| Previous paclitaxel treatment: No     | 12/15 (80.0%)  | 13/18 (72.2%)         | 1.567 (0.681, 3.641) |
| PD-L1 TPS: <=1%                        | 34/44 (77.3%)  | 38/43 (88.4%)         | 0.793 (0.490, 1.277) |
| PD-L1 TPS: >1%                         | 20/30 (66.7%)  | 17/24 (70.8%)         | 0.876 (0.453, 1.708) |
| PD-L1 TPS: <=10%                       | 45/60 (75.0%)  | 46/53 (86.8%)         | 0.763 (0.498, 1.167) |
| PD-L1 TPS: >10%                        | 9/14 (64.3%)   | 9/14 (64.3%)          | 1.068 (0.413, 2.760) |
| PD-L1 CPS: <1%                         | 9/11 (81.8%)   | 13/17 (76.5%)         | 1.303 (0.513, 3.273) |
| PD-L1 CPS: >=1%                        | 45/63 (71.4%)  | 42/50 (84.0%)         | 0.729 (0.473, 1.124) |
| PD-L1 CPS: <=10%                       | 25/34 (73.5%)  | 39/47 (83.0%)         | 0.95 (0.560, 1.579) |
| PD-L1 CPS: >10%                        | 29/40 (72.5%)  | 16/20 (80.0%)         | 0.738 (0.398, 1.410) |
| ECOG PS: 0                             | 20/23 (87.0%)  | 18/23 (78.3%)         | 0.9 (0.456, 1.764) |
| ECOG PS: 1                             | 49/72 (68.1%)  | 63/72 (87.5%)         | 0.649 (0.444, 0.943) |
| Liver metastases: With                 | 17/27 (63.0%)  | 30/33 (90.9%)         | 0.563 (0.296, 1.031) |
| Liver metastases: Without              | 52/68 (76.6%)  | 51/62 (82.3%)         | 0.773 (0.519, 1.151) |
| Smoking history: Never                 | 23/30 (76.7%)  | 30/34 (88.2%)         | 0.847 (0.477, 1.479) |
| Smoking history: Former                | 39/55 (70.9%)  | 38/47 (80.9%)         | 0.751 (0.475, 1.186) |
| Smoking history: Current               | 7/10 (70.0%)   | 13/14 (92.9%)         | 0.261 (0.073, 0.754) |

**Figure 3**

Subgroups of overall survival of the treatment by age, sex, ECOG PS, previous treatment with paclitaxel, liver metastases, PD-L1 expression level and smoking.
Figure 4

Efficacy comparison of sintilimab in high and low neutrophil-to-lymphocyte ratio (NLR) groups. A, overall survival (OS) with NLR at baseline; B, progression-free survival (PFS) with NLR at baseline; C, OS with NLR at 6 weeks post-sintilimab treatment; D, PFS with NLR at 6 weeks post-sintilimab treatment.
Figure 5

TME immune cell signatures and association with PFS. Heat map of immune score of 28 immune cell populations is shown and forest plots (right panel) display the correlation of each immune subtype with PFS. Node position reflects HR (< 1 for favorable outcome with high score in respective treatment). Only significant correlations are displayed and node size reflects the p-value (the larger the node size, the more significant).
Figure 6

Association between survival and the combination of baseline blood TCR and ctDNA. (A) Overall survival analysis and (B) progression free survival analysis are shown. The high or low level groups of TCR clonality or mTBI are split by respective median value.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.pdf
- SupplementaryFigures.pdf