Journal for ImmunoTherapy of Cancer

Phase II trial of efficacy, safety and biomarker analysis of sintilimab plus anlotinib for patients with recurrent or advanced endometrial cancer

Wei Wei ,[1,2] Xiaohua Ban,[2,3] Fan Yang,[1,2] Jibin Li,[2,4] Xiaqin Cheng,[1,2] Rong Zhang,[2,3] Xin Huang,[1,2] Yongwen Huang,[1,2] Qiaqia Li,[1,2] Ya Qiu,[1,2] Min Zheng,[1,2] Xiaofeng Zhu,[2,5] Jundong Li[1,2]

To cite: Wei W, Ban X, Yang F, *et al*. Phase II trial of efficacy, safety and biomarker analysis of sintilimab plus anlotinib for patients with recurrent or advanced endometrial cancer. *Journal for ImmunoTherapy of Cancer* 2022;**10**:e004338. doi:10.1136/jitc-2021-004338

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2021-004338).

WW, XB, FY, JL and XC contributed equally.
MZ, XZ and JL contributed equally.

Accepted 09 May 2022

For numbered affiliations see end of article.

Correspondence to
Professor Jundong Li;
lijd@sysucc.org.cn

ABSTRACT

Background Although co-inhibition of the angiogenesis and programmed death 1 (PD-1) pathways is proposed as an effective anticancer strategy, studies in Chinese patients with endometrial cancer are sufficient. Anlotinib is an oral multi-targeted tyrosine kinase inhibitor affecting tumor angiogenesis and proliferation; sintilimab is an anti-PD-1 monoclonal antibody.

Methods This was a phase II trial using Simon's two-stage design. This study enrolled patients with endometrial cancer who had progressed after platinum-based chemotherapy. Sintilimab 200 mg was administered intravenously on day 1 every 3 weeks, and anlotinib 12 mg was administered on days 1–14 in a 21-day cycle. The primary endpoint was the objective response rate (ORR) using the immune-related Response Evaluation Criteria in Solid Tumors criteria. Immunohistochemistry and whole-exome sequencing were used as correlative investigations.

Results Between November 2019 and September 2020, 23 eligible patients were enrolled. The ORR and disease control rates were 73.9% (95% CI, 51.6 to 89.8) and 91.3% (95% CI, 72.0 to 98.9), respectively, with 4 complete and 12 partial responses. With a median follow-up of 15.4 months (95% CI, 12.6 to 18.3), the median progression-free survival was not reached, and the probability of PFS >12 months was 57.1% (95% CI, 33.6 to 75.0). Exploratory analysis revealed that mutations in the homologous repair pathway showed a trend for higher ORR (100% vs 0%, p=0.07). Treatment-related grade 3/4 adverse events were observed in 50.0% of the patients.

Conclusions Sintilimab plus anlotinib demonstrated robust therapeutic benefits with tolerable toxicity in endometrial cancer.

Trial registration number NCT04157491.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Pembrolizumab plus lenvatinib has demonstrated advantages over chemotherapy in patients with endometrial cancer who received at least one line of systemic therapy.

WHAT THIS STUDY ADDS

⇒ This is the first reported study in which the combination of angiogenesis inhibitor (anlotinib) and anti-programmed death 1 antibody (sintilimab) showed robust theraputic effect in Chinese patients with endometrial cancer.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ This study provided evidence for a further randomized trial investigating immunotherapy in endometrial cancer in China.

BACKGROUND

Unlike most other gynecologic malignancies with decreasing incidence, the increase in incidence and mortality of endometrial cancer (EC) in developed countries has also been noted in China.[1 2] Patients who progressed following first-line chemotherapy have a poor prognosis, with a median overall survival (OS) of approximately 1 year.[3] However, with second-line options, including chemotherapy, hormone therapy, and targeted agents, producing only modest activity, there is no standard treatment for patients who progress after first-line treatment.[4–7] Accumulated evidence indicates that EC is a rational target for immune therapy. Recently, several programmed death 1 (PD-1) antibodies have demonstrated promising activity in patients with EC with microsatellite instability-high/mismatch repair deficiency (MSI-H/dMMR), with an overall response rate (ORR) ranging from 26.7% to 57%[8–10]; however, the target population accounts for a minority of 30% of patients with EC.[11] In 2019, the Food and Drug Administration granted accelerated approval to the combination of lenvatinib plus pembrolizumab for the treatment of patients with advanced EC without MSI-H/dMMR and whose disease progresses following prior systemic therapy based on the

results of a single-arm trial—KEYNOTE-146/Study 111.[12] The following KEYNOTE-775/Study 309 was a phase III, active-controlled study conducted to confirm this accelerated approval, and the combination therapy had better progression-free survival (PFS) (median PFS, 7.2 vs 3.8 months; HR=0.56; p<0.001) and longer OS (median OS, 18.3 vs 11.4 months; HR=0.62; p<0.001) compared with chemotherapy.[13] Furthermore, to verify if pembrolizumab plus lenvatinib is superior to chemotherapy in all patients receiving first-line treatment, the ENGOT-en9/LEAP-001 trial is ongoing.[14] Although the combination of immunotherapy plus antiangiogenic therapy is a promising strategy, regardless of biomarkers, dual agents frequently cause considerable toxicity. Toxicity profiles vary among different PD-1 antibodies and tyrosine kinase inhibitors (TKIs), particularly in the context of immune combination therapies, for which clinical data are scarce. For instance, a meta-analysis reported that pembrolizumab is likely to have lower rates of adverse skin events (AEs) (5% vs 13%) and diarrhea (4% vs 13%) than nivolumab.[15] Escudier *et al* reported that patients with metastatic renal cell carcinoma favored pazopanib over sunitinib on account of the toxicity profile.[16] Thus, clinical trials utilizing novel chemo-free combination therapy are urgently needed to maximize antitumor efficacy while minimizing toxicity.

Sintilimab is a fully human anti-PD1 IgG4-k monoclonal antibody that has been approved for the treatment of classical Hodgkin's lymphoma in China.[17] Anlotinib is an oral multi-targeted anti-angiogenic TKI that inhibits the vascular endothelial growth factor receptors (VEGFRs) 1–3, fibroblast growth factor receptors 1–4, epidermal growth factor receptor, platelet-derived growth factor receptor, and c-Met.[18] Anlotinib is approved for the treatment of non-small cell lung cancer (NSCLC), small cell lung cancer, soft tissue sarcoma, and medullary thyroid carcinoma in China[19–22] The rationale for combining sintilimab and anlotinib is that anlotinib has the ability to inhibit tumor angiogenesis and modulate the tumor immune microenvironment, thereby enhancing the effect of PD-1 antibodies.[23 24] Sintilimab is currently in phase I, II, and III development in a variety of solid tumors in combination with antiangiogenic agents (including anlotinib).[25–27]

Moreover, confirmed evidence indicates genetic racial disparities in EC, emphasizing the importance of validating treatments in diverse genetic contexts.[28 29] We described the efficacy and safety of sintilimab plus anlotinib in biomarker unselected patients with EC who failed platinum-based chemotherapy, and performed an exploratory analysis, in this phase II, open-label, single-arm study.

MATERIALS AND METHODS

Study design and patients

This study was an open-label, single-arm, phase II trial conducted at a single site (online supplemental 3, figure S1). Eligible patients had recurrent or advanced EC and had progression on or after at least one line (no upper limit) of standard platinum-based chemotherapy. Additional eligibility criteria included Eastern Cooperative Oncology Group Performance Status of 0–2, sufficient organ function, and measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST). The critical exclusion criteria consisted of the previous administration of immune checkpoint inhibitors, preexisting autoimmune diseases, and ongoing steroid treatment with prednisone >10 mg/day orally or equivalent. A complete list of inclusion and exclusion criteria is provided in online supplemental 1.

Treatment and procedures

Patients (N=23) received sintilimab 200 mg intravenously on day 1 every 3 weeks and 12 mg anlotinib orally on days 1–14 every 3 weeks. Toxicity was managed with interruptions of study drugs or dose reduction of anlotinib. Specifically, three doses of anlotinib were used (12 mg, 10 mg, and 8 mg); a dose increase was allowed at 8 mg anlotinib. Therapy continued until disease progression, intolerable toxicity, withdrawal of consent, or investigator decision. Tumor response was assessed by CT or MRI before the third and fifth cycles, then every three cycles before the 11th cycle, and finally every four cycles after the 11th cycle. AEs were documented and evaluated throughout the study. All AEs were graded according to the Common Terminology Criteria for Adverse Events classification (V.4.03).

Genomic sequencing

DNA from formalin-fixed paraffin-embedded (FFPE) and whole blood control was fragmented using an M220 Focused-ultrasonicator (Covaris), followed by whole genome library preparation using KAPA Hyper Prep Kit (Kapa Biosystems). Exome capture was performed using the XGen Exome Research Panel and targeted panel (Geneseeq one; Nanjing Geneseeq). Enriched libraries were sequenced using the Illumina HiSeq 4000 platform to reach the mean coverage depths, which for whole-exome sequencing (WES) were 80× (normal control) and 250× (tumor samples), and for 425 panel, the mean coverage depths were 150× for normal control and 1200× for tumor tissues (sequencing profile is in online supplemental 2). Tumor mutation burden (TMB), as well as neoantigen and intratumor heterogeneity (ITH) were calculated using the WES data. TMB ≥10/MB and ITH >0.45 were used as cut-off values for TMB-high and ITH-high, respectively (online supplemental 3, figure S2).

Microsatellite status and programmed death ligand-1 determination

Microsatellite status (MS) was determined using WES (n=20) and immunohistochemistry (n=2). A site was considered unstable if it presented significantly altered distribution of the length species. A sample was considered MSI-H if more than 40% of the evaluated site

displayed instability; otherwise, it was defined as microsatellite instability stable (MSS). Immunohistochemistry of MLH1, MSH2, MSH6, and PMS2 was performed to define dMMR or mismatch repair proficient (pMMR). According to the manufacturer's protocol, the combined proportion score of programmed death ligand-1 (PD-L1) was calculated using a 22C3 pharmDx assay (Agilent Technologies, Santa Clara, California, USA). The specimen was considered to be PD-L1 positive if the Combined Positive Score (CPS) was ≥1 and PD-L1 high was defined if CPS was ≥8 (online supplemental 1, figure S2).

Outcomes

The primary endpoint was ORR, according to the immune-related RECIST (irRECIST). The secondary end points included disease control rate (DCR), duration of response (DOR) (defined as the time from immune-related complete response (CR) or immune-related partial response (PR), whichever was recorded first, to the date of disease progression), PFS, time to response (TTR) (defined as the time from initiation of treatment to the date of immune-related CR or immune-related PR, whichever was recorded first), clinical benefit rate (CBR) (defined as proportion of patients with immune-related CR, immune-related PR or immune-related stable disease (SD) lasting >6 months), and safety.

Statistical analyses

The previously reported ORR of pembrolizumab in PD-L1 positive EC was 13%.[9] We expected an ORR of 39% for the combination treatment refereeing to a prior similar study.[12] The planned sample size was 23 patients based on Simon's optimal two-stage design (one-sided α value of 5% and power of 80%). If ≥2 responses were observed in the 7-patient first stage, an additional 15 patients would be enrolled. Overall, if patients had more than six objective responses, the primary endpoint would be met (online supplemental 3, figure S1). There was no dose-finding stage for this study considering previously reported similar studies incorporating anlotinib.[19–22]

Full analysis set (defined as all included patients) and safety analysis set (defined as patients who had at least one cycle of treatment) were used for efficacy analysis and safety analyses, respectively. We analyzed the ORR and the exact 95% CIs using the Clopper-Pearson method, as were DCR and CBR. The medians of PFS and DOR were analyzed using the Kaplan-Meier curve, and 95% CIs were calculated with a generalized Brookmeyer and Crowley method. We calculated the probabilities of patients achieving a PFS >6 months or >12 months using the Kaplan-Meier product-limit method, and reverse Kaplan-Meier calculated the Greenwood Formula follow-up time. Cox regression models were used to test the association between clinicopathologic and genetic characteristics and PFS, and a p value <0.05 was considered significant. Statistical analyses were performed using SAS, V.9.3. For genomic analysis, p value <0.1 was considered statistically significant. The data generated in this study are not publicly available due to the ongoing expanded trial but are available on reasonable request from the corresponding author.

RESULTS

Patients

Between November 1, 2019, and September 16, 2020, 23 patients were enrolled in this study; 1 patient died of clinical progressive disease (PD) before assessing the first image. The median age was 56 years (range, 37–70). Of all the 23 patients, 11 (47.8%) received >1 line of systematic chemotherapy, and 4 (17.4%) had a history of anti-angiogenetic (including oral multi-target TKI) treatment. Twenty-one out of the 23 (91.3%) patients had endometrioid adenocarcinoma, and 8/23 (34.8%) presented lung metastasis. MSI-H/dMMR and MSS/pMMR accounted for 9/23 (39.1%) and 14/23 (60.9%) patients, respectively. PD-L1 expression was determined in 22 patients, of which 16/22 (72.7%) had CPS >1 and 4/22 (18.2%) had CPS >10 (table 1). The median follow-up time was 15.4 months (95% CI, 12.6 to 18.3). At cut-off (September 30, 2021), 10 patients were receiving treatment. Thirteen (56.5%) patients had discontinued the treatment owing to disease progression (n=9), AEs (n=3) and patient refusal. At data cut-off, five patients died during follow-up.

Efficacy

In the first seven patients enrolled, confirmed responses were noted in six (85.7%) patients. The ORR threshold for the first stage of Simon's two-stage was reached, and the trial continued to full accrual. At the cut-off, CR, PR, SD, and PD were observed in 4 (17.4%), 13 (56.5%), 4 (17.4%), and 2 (8.7%) patients (figure 1A), respectively, yielding an ORR (all confirmed) of 73.9% (95% CI, 51.6% to 89.8%) per irRECIST by investigator review. ORR_{24week} was 65.2% (95% CI, 42.7% to 83.6%). The DCR and CBR were 91.3% (95% CI, 72.0% to 98.9%) and 69.6% (95% CI, 47.1% to 86.8%), respectively. Table 2 summarizes the treatment outcomes for selected patient subgroups.

The therapeutic efficacy demonstrated in this study was rapid and durable (figure 1B,C). The median TTR was 2.8 months (95% CI, 1.5 to 5.2) (figure 1B). Although PFS (figure 2A) and DOR were not achieved with a median follow-up of 15.4 months, the probabilities of PFS >6 months and >12 months were 76.7% (95% CI, 52.7% to 89.6%) and 57.1% (95% CI, 33.6% to 75.0%), respectively (table 2). Moreover, 45.5% (10/22) of the patients with available image assessment achieved a maximum reduction of more than 50% (figure 1A). Additionally, two patients experiencing PD demonstrated tumor shrinkage, resulting in a tumor size reduction in 20 (90.1%) of 22 patients.

Notably, three of the four patients with CR were primary platinum-resistant (defined as progression during or within 6 months of first-line platinum-based adjuvant chemotherapy), who were generally regarded as extremely aggressive. In addition, 8/17 (47.1%) participants with

Table 1 Baseline characteristics (N=23)

Characteristic	No. (%)*
Age, median (range), years	56.0 (37.0–70.0)
ECOG performance status	
0	21 (91.3)
1	2 (8.7)
Tumor histologic type	
Endometrioid adenocarcinoma	21 (91.3)
FIGO grade 1	1 (4.3)
FIGO grade 2	9 (39.1)
FIGO grade 3	11 (47.8)
Other adenocarcinomas	2 (8.7)
FIGO stage	
Stage I	7 (30.4)
Stage II	1 (4.3)
Stage III	10 (43.5)
Stage IV	5 (21.7)
No. of previous lines of therapy for recurrent/metastatic disease	
1	12 (52.2)
2	8 (34.8)
3	1 (4.3)
5	2 (8.7)
Lung metastasis	8 (34.8)
Previous anti-angiogenetic treatment	4 (17.4)
Previous radiotherapy	9 (39.1)
CPS score of PD-L1 expression	
≥10	4 (17.4)
1–9	12 (52.2)
<1	6 (26.1)
Not evaluable†	1 (4.3)
Microsatellite status	
MSI-H/dMMR	9 (39.1)
MSS/pMMR	14 (60.9)
Tumor mutation burden, median (range)‡	23.0 (1.1–65.5)

*Values are presented as n (%) unless stated otherwise.
†Tissue sample missing for testing.
‡Tumor mutation burden was calculated using whole-exome sequencing.
CPS, Combined Positive Score; ECOG, Eastern Cooperative Oncology Group; FIGO, International Federation of Gynecology and Obstetrics; MSI-H/dMMR, microsatellite instability high/mismatch-repair deficient; MSS/pMMR, microsatellite instability stalble/mismatch-repair proficient; PD-L1, programmed death ligand-1.

objective responses received more than one line prior systematic chemotherapy. Post hoc analyses showed that the ORR in patients with MSI-H/dMMR was higher than their counterparts (100% vs 57.1%, p=0.048). Moreover, MSI-H/dMMR (MSS/pMMR vs MSI-H/dMMR; HR=2.96; 95% CI, 1.04 to 8.40; p=0.04) (figure 2B) was related to prolonged PFS. Additionally, clinical benefits were consistent across International Federation of Gynecology and Obstetrics stages, treatment lines, radiotherapy history, and anti-angiogenetic agent history (online supplemental 3, table S1).

Safety

The safety analysis included all 23 patients enrolled in the study. All patients experienced any-grade AEs, with the most common being palmar-plantar erythrodysesthesia (PPE)/rash and hypothyroidism (including elevated thyroid-stimulating hormone (TSH) level) (both 69.6%), followed by increased lipase (52.2%), pain/arthralgia, asthenia, and elevated hepatic enzymes (all 43.5%) (table 3). Although grade 3/4 treatment-related AEs occurred at a 50% rate, the majority were easily managed without hospitalization. The rate of severe AEs was 17.4 %, and no treatment-related death occurred. Notably, grade 3 hypertension, the most common adverse effect of TKI-induced angiogenesis, was observed in only one patient in this study. Anlotinib dose reduction occurred in 15/23 (65.2%) patients; 8/23 (37.8%) patients experienced multiple dose reductions. PPE (6/15) was the most common reason for dose reduction of anlotinib. 2/23 (8.7%) and 1/23 (4.3%) patients discontinued both study drugs and anlotinib, respectively. The median cycle of treatment was 15 (range: 2–28).

Results of next-generation sequencing

The whole-genome and targeted gene sequencing were performed on 21 patients who provided sufficient tumor tissue; 1 patient was omitted from the analysis due to poor quality control. Figure 3A depicts the targeted genomic landscapes of responders (ORR) and non-responders (non-ORR) at the single-gene level. Not a single gene was related to ORR. Mutations in the homologous repair (HR) pathway showed a trend for higher ORR (100% vs 0%, p=0.07) (online supplemental 3, table S2). Additionally, more tumors with alterations in the HR (80% vs 30%, p=0.03) or MMR (80% vs 10%, p=0.01) pathway demonstrated tumor reduction >50% (online supplemental 3, table S2). Other pathways involved in DNA repair, such as the base excision repair and non-homologous terminal connection pathways, were not associated with ORR. Additionally, increased neoantigen (figure 3D) but not ITH levels were associated with responses in our trial.

Regarding PFS, tyrosine-protein kinase Lyn (LYN) gene mutation was associated with a decreased PFS (6.5 vs NA, p=0.007) (figure 3B). PFS was significantly longer in patients with both TMB-high and HRmut than with TMB-low or HRwt (NE vs 10.1 months, p=0.03) (figure 3C). Among the aforementioned genetic characteristics, Cox regression identified no single factor with a significant p value (online supplemental 3, table S1).

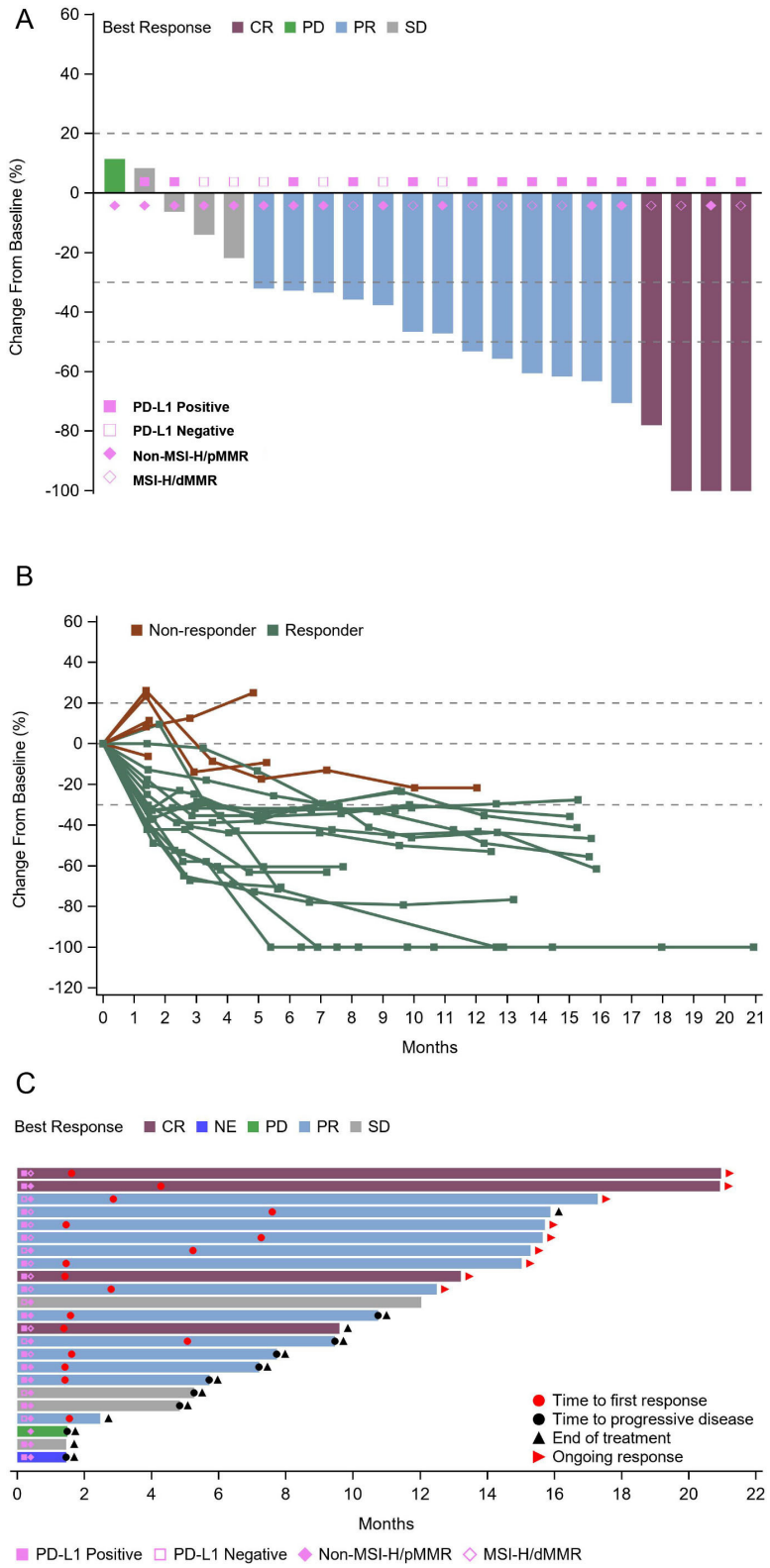

Figure 1 Response among patients with recurrent or advanced endometrial carcinoma per irRECIST. (A) Waterfall plot of the best percentage of change from baseline in the sum of the diameters of target lesions according to the irRECIST. Only patients with available tumor assessments at data cut-off are shown (n=22). Dashed line indicates threshold for partial response (30%) and disease progression (20%). (B) Longitudinal change from baseline in tumor size per irRECIST is shown in spider plots, where green lines define objective response and red lines define non-responders. Only patients with available tumor assessments at data cut-off are shown (n=22). (C) Swimmer plot of the objective responses and durations. All patients in the per-protocol population (n=23) are shown. CR, complete response; dMMR, mismatch repair deficiency; irRECIST, immune-related Response Evaluation Criteria in Solid Tumors; MSI-H, microsatellite instability-high; PD, progressive disease; PD-L1, programmed death ligand-1; pMMR, mismatch repair proficient; PR, partial response; SD, stable disease.

Table 2 Best objective response assessed per irRECIST by investigator review

Response	MSI-H/dMMR n=9	MSS/pMMR n=14	PD-L1* negative n=6	PD-L1 positive n=16	Total n=23
ORR, no. (%) (95% CI)	9 (100.0) (66.4 to 100.0)	8 (57.1) (28.9 to 82.3)	4 (66.7) (22.3 to 95.7)	13 (81.3) (54.4 to 96.0)	17 (73.9) (51.6 to 89.8)
Complete response	2 (22.2)	1 (7.1)	0	3 (18.8)	4 (17.4)
Partial response	7 (77.7)	7 (50.0)	4 (66.7)	10 (62.5)	13 (56.5)
Stable disease	0	4 (28.6)	2 (33.3)	2 (12.5)	4 (17.4)
Progressive disease	0	2 (14.3)	0	1 (6.3)	2 (8.7)
ORR_{24week}†, no. (%) (95% CI)	7 (77.8) (40.0 to 97.2)	8 (57.1) (28.9 to 82.3)	4 (66.7) (22.3 to 95.7)	11 (68.8) (41.3 to 88.9)	15 (65.2) (42.7 to 83.6)
$Complete\ response_{24week}$	1 (11.1)	2 (14.3)	0	3 (18.8)	3 (13.0)
$Partial\ response_{24week}$	6 (66.7)	6 (42.9)	4 (66.7)	8 (50.0)	12 (52.2)
$Stable\ disease_{24week}$	2 (22.2)	4 (28.6)	2 (33.3)	4 (25.0)	6 (26.1)
$Progressive\ disease_{24week}$	0	2 (14.3)	0	1 (6.3)	2 (8.7)
DCR, no. (%) (95% CI)	9 (100.00) (66.4 to 100.0)	12 (85.7) (57.2 to 98.2)	6 (100.00) (54.1 to 100)	15 (93.8) (69.8 to 99.8)	21 (91.3) (72.0 to 98.9)
Clinical benefit rate‡, no. (%) (95% CI)	9 (100) (66.4 to 100.0)	7 (50.0) (23.0 to 77.0)	4 (66.7) (22.3 to 95.7)	12 (75.0) (47.6 to 92.7)	16 (69.6) (47.1 to 86.8)
TTR, median, 95% CI, months	1.6 (1.4 to 7.3)	4.3 (1.5 to NE)	5.1 (1.5 to NE)	1.6 (1.4 to 4.0)	2.8 (1.5 to 5.2)
Probability of patients with PFS					
≥6 months, % 95% CI	100 (100 to 100)	59.6 (28.2 to 80.9)	80.0 (20.4 to 96.9)	80.4 (50.6 to 93.2)	76.7 (52.7 to 89.6)
≥12 months, % 95% CI	88.9 (43.3 to 98.4)	34.1 (10.6 to 59.6)	60.0 (12.6 to 88.2)	59.5 (30.9 to 79.5)	57.1 (33.6 to 75.0)

*The PD-L1 expression was measured by Combined Positive Score, and one patient was not available for PD-L1 assessment.
†ORR_{24week} is defined as best objective observed within 24 weeks after the treatment initiation.
‡Clinical benefit rate is defined as proportion of patients with immune-related complete response, immune-related partial response, or immune-related stable disease lasting >6 months.
DCR, disease control rate; irRECIST, immune-related Response Evaluation Criteriain Solid Tumors; MSI-H/dMMR, microsatellite instability high/ mismatch-repair deficient; MSS/pMMR, microsatellite instability stalble/mismatch-repair proficient; NE, not estimable; ORR, objective response rate; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TTR, time to response.

DISCUSSION/CONCLUSION

To the best of our knowledge, this is the first study to report on the combination of an anti-PD-1 antibody and a VEGFR inhibitor in patients with advanced/recurrent EC in China. Sintilimab plus anlotinib, demonstrated robust and durable antitumor activity with a manageable safety profile as second-line or later therapy for advanced or recurrent EC. Our trial met its primary endpoint, and the exploratory analysis suggested that the HR pathway was a novel predictive marker for immunotherapy in patients with EC.

Although PD-1 monotherapy achieved ORRs ranging from 42.3% to 57.1% in patients with EC with MSI-H/dMMR,[8–10] the response was not favorable in most patients with EC without MSI-H/dMMR (ORR less than 20%).[30 31] PD-1-/PD-L1-based combination therapy aims to promote its therapeutic effect in patients with EC. According to a previous phase II study, the ORR of niraparib plus dostarlimab was 14% in patients with recurrent EC.[32]

The ORR and median PFS of durvalumab and tremelimumab in EC and endometrial carcinosarcoma were 11.1% and 8.1 months, respectively.[33] In this study, sintilimab plus anlotinib demonstrated a promising therapeutic effect with an ORR of 73.9% (95% CI, 51.6% to 89.8%) and a probability of 57.1% of PFS >12 months, regardless of MS. Our results were in line with previous reports on lenvatinib plus pembrolizumab in patients with EC who experienced progression after first-line chemotherapy. In KEYNOTE-146/Study 111, lenvatinib and pembrolizumab achieved an ORR_{24week} of 38.0% (95% CI, 28.8% to 47.8%) and a median PFS of 7.4 months (95% CI, 5.3 to 8.7 months) in the overall population.[12] KEYNOTE-775/Study 309 further confirmed that lenvatinib plus pembrolizumab had longer PFS (7.2 vs 3.8 months; HR=0.56; 95% CI, 0.47 to 0.66) and OS (18.3 vs 11.4 months; HR=0.62; 95% CI, 0.51 to 0.75) compared with chemotherapy in the overall population.[13] We observed that MSI-H/dMMR was 39.1% in our cohort compared with 10.5% and 15.7% in KEYNOTE-146/Study 111 and KEYNOTE-775/Study 309, respectively, which partly explained the increased ORR of our study. Considering the small sample size, the prevalence and molecular features of Chinese patients with EC with MSI-H/dMMR require further study. Notably, the ORR was 57.1% in patients with MSS/pMMR in our cohort, indicating that anlotinib greatly sensitizes MSS/pMMR EC to anti-PD-1 antibody. Together with the results of pembrolizumab and lenvatinib, our study confirmed the therapeutic effect of anti-PD-1 antibodies combined with anti-angiogenetic agents in patients with recurrent/advanced EC. Additionally,sintilimab plus anlotinib has been shown to have a promising therapeutic effect in NSCLC, hepatocellular cancer, and biliary tract cancer, with an ORR ranging from 40% to 72.7%.[25 26 34] These findings indicate that sintilimab plus anlotinib is compatible with a high level of antitumor activity in a variety of solid tumors.

The safety profile of our study is consistent with that reported for other anti-PD-1/PD-L1 antibodies and VEGF pathway inhibitors. Overall, the rate of ≥grade 3 AEs in our study (50%) was similar to that in another trial (54.5%) of sintilimab and anlotinib in advanced NSCLC

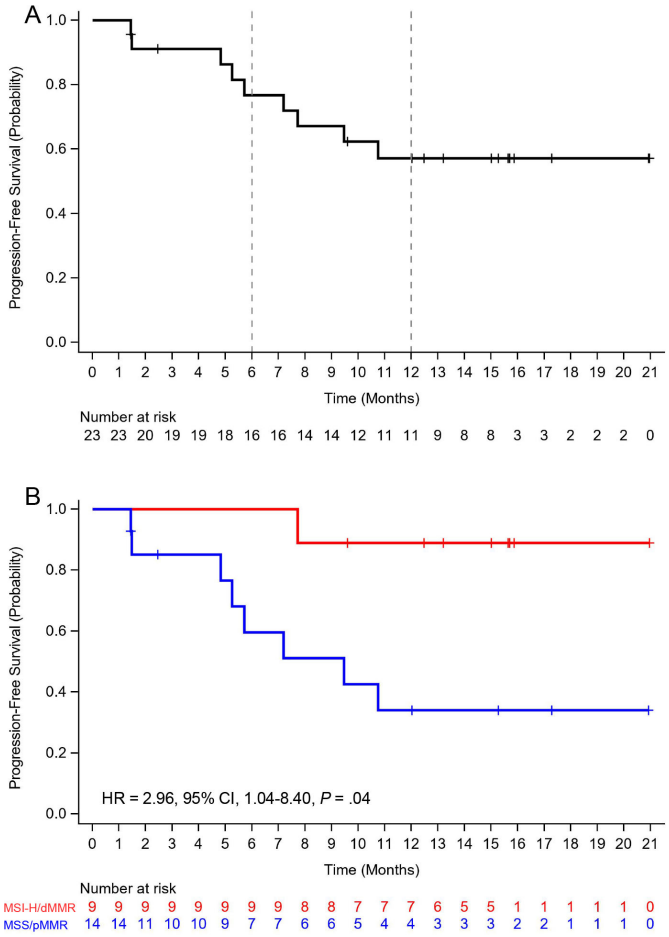

Figure 2 Kaplan-Meier plots of progression-free survival. (A) Kaplan-Meier plot of progression-free survival assessed by irRECIST in the general population. (B) Post hoc analysis by MS and PD-L1 expression. irRECIST, immune-related Response Evaluation Criteria in Solid Tumors; MS, microsatellite status; PD-L1, programmed death ligand-1.

(NCT03628521).[25] Notably, in this study, the incidence rate of ≥grade 3 hypertension was 4.3%, which is comparable with the rates of other studies including anlotinib and sintilimab in cervical cancer (4.8%) and NSCLC (9.1%).[25 35] In a previous phase III study in NSCLC, grade 3 or higher hypertension with anlotinib monotherapy was 13.6%.[19] Hypertension is the most frequently reported AE of lenvatinib. The incidence rates of ≥grade 3/4 hypertension were 32.4% and 37.9% in KEYNOTE-146/Study 111 and KEYNOTE-775/Study 309, respectively.[12 13] This is especially beneficial for patients with EC, who are prone to obesity and hypertension. Although PD-1-related AEs were common, the major AE observed was grade 1/2 hypothyroidism (including elevated TSH). In all, only one patient discontinued anlotinib after 20 cycles of treatment.

By performing exploratory analysis in our cohort, patients with MSI-H/dMMR presented higher ORR (100% vs 57.1%, p=0.048), and higher percentage of tumors shrank >50% (80% vs 10%, p=0.01). However, in KEYNOTE-146/Study 111, patients with MSI-H/dMMR demonstrated had higher ORR (63.6% vs 37.2%), but the result was not significant. Interestingly, in KEYNOTE-146/Study 111, the incidence rate of serous EC was 32.4%, which was significantly higher (8.7%) than that reported in our study.[12] Analysis of The Cancer Genome Atlas showed that the incidence rates of MSI-H in endometrioid

Table 3 Treatment-related adverse events (AEs) of any grade reported >10% and grade 3/4 AEs in all patients

	Preferred term or basket	Any grade n=23 (%)	Grade 3/4 n=23 (%)
General	Asthenia	10 (43.5)	1 (4.3)
	Weight loss	5 (21.7)	0
Gastrointestinal system	Diarrhea	9 (39.1)	0
	Uric acid increased	9 (39.1)	0
	Elevated hepatic enzymes*	10 (43.5)	0
	Abdominal pain	6 (26.1)	0
	Decreased appetite	6 (26.1)	0
	Bile acids increased	4 (17.4)	0
Musculoskeletal system	Pain and arthralgia	10 (43.5)	0
Hematologic system	Leukopenia	3 (13.0)	0
	Neutropenia	4 (17.4)	1 (4.3)
Endocrine/ metabolism	Hypothyroidism†	16 (69.6)	0
	Lipase increased	12 (52.2)	0
	Hyperthyroidism	7 (30.4)	0
	Hyperglycemia	5 (21.7)	0
Renal system	Proteinuria	6 (26.1)	1 (4.3)
	Creatinine increased	5 (21.7)	1 (4.3)
Cardiovascular system	Hypertension	9 (39.1)	1 (4.3)
	ST-T changes	5 (21.7)	0
	Myocarditis‡	1 (4.3)	1 (4.3)
	Prolonged ECG QT	1 (4.3)	1 (4.3)
Dermatologic system	PPE/rash	16 (69.6)	6 (26.1)
	Ulcer§	8 (34.8)	0
Others	Dysphonia	10 (43.5)	0
	Serum albumin decreased	9 (39.1)	0
	Hemorrhage¶	6 (26.1)	0
	Infections	6 (26.1)	0
	Abscess	2 (8.7)	1 (4.3)
	Peritonitis‡	1 (4.3)	1 (4.3)

*Elevated hepatic enzymes included aspartate aminotransferase increased, alanine transaminase increased, and γ-glutamyl transpeptidase increased.
†Hypothyroidism basket included hypothyroidism and increased blood thyroid-stimulating hormone.
‡Clinically confirmed immunotherapy-related adverse events.
§Including oral and virginal ulcers.
¶Hemorrhage basket included gingival bleeding, vaginal hemorrhage, and hemoptysis.
PPE, palmar-plantar erythrodysesthesia syndrome.

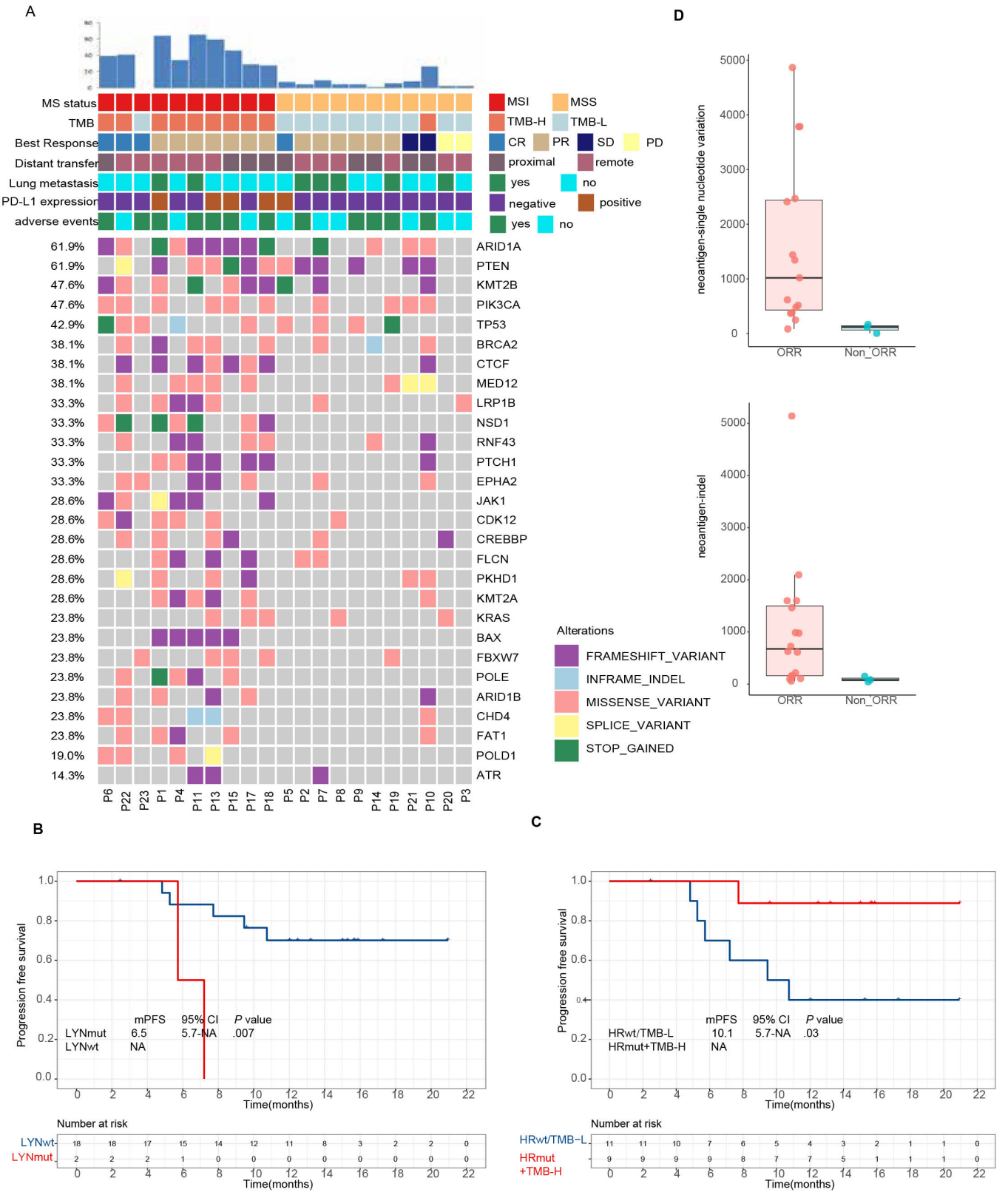

Figure 3 Exploratory analysis by next-generation sequencing and immunohistochemistry of tumor tissues. (A) Genetic profiling of 20 patients. Main clinicopathologic characteristics were also listed above the heatmap. (B) Survival analysis by tyrosine-protein kinase Lyn (LYN) status. LYNmut, LYN mutated; LYNwt, LYN wild type (C) The survival analysis by combined TMB and HR pathway mutation assessment. TMB-H +HRmut, patients with TMB-high and HR pathway mutations; TMB-L/HRwt, patients with TMB-low or HR pathway wide type. (D) Box plots of neoantigen SNV (left) and neoantigen Del (right) between ORR and non-ORR patients. CR, complete response; Del, deletion; HR, homologous repair; MSS, microsatellite instability stable; MSI, microsatellite instability; ORR, objective response rate; PD, progressive disease; PD-L1, programmed death ligand-1; PR, partial response; SNF, single nucleotide mutation; SD, stable disease; TMB, tumor mutation burden.

and serous ECs were 40% and 2%, respectively.[11] More importantly, a retrospective study, comprizing 2806 serous EC, revealed that the prognosis of individuals treated with immunotherapy was not related to MS.[36] Presumably, the predictive value of MSI-H/dMMR in KEYNOTE-146 study might be shadowed by the relatively high proportion of serous EC. Therefore, further efforts are required to clarify the different predictive role of MSI-H/dMMR in various pathological subtypes.

For the first time, the HR pathway mutation was reported to positively contribute to antitumor activity in EC in the clinical trial setting. Theoretically, the accumulated DNA damage derived from HR deficiency could increase tumor neoantigen and immunogenicity, enhance antigen presentation and promote tumor-infiltrating lymphocytes.[37] In light of accumulating evidence demonstrating additive or even synergistic effects of HR deficiency and PD1/PD-L1 inhibition in preclinical immunotherapy studies, treatment of EC with a poly-ADP-ribose polymerase inhibitor in combination with an immune checkpoint inhibitor is warranted. We are currently conducting a trial (NCT04885413) in which niraparib and sintilimab are used to investigate further the role of the HR pathway in patients with recurrent EC.

LYN mutations were observed with lower responses in our study. Similarly, Jiang reported that LYN expression could predict anti-PD-1 and anti-CTLA-4 immunotherapy responses in glioma.[38] The LYN is an essential and complex regulator of immunoreceptor signaling and mainly plays a negative role in immune activation.[39] Increased LYN expression in cancer cells was reported to promote tumor proliferation and an immune-suppressive microenvironment, particularly those activated by hormones or growth factors.[40 41] The role of LYN and the interplay between antitumor immunity in EC should be explored in further studies.

The study is not without limitations. The small sample size and single-arm design prevent the generalizability of our findings. Additionally, the use of archival FFPE tumor tissue for genomic analysis, rather than fresh samples before the initiation of dual treatment, impairs the accuracy. Further studies are required to determine the antitumor effect and safety of sintilimab plus anlotinib in comparison to chemotherapy, particularly in a biomarker-driven setting.

Sintilimab plus anlotinib demonstrated robust antitumor activity, regardless of MS with advantageous toxicity. This treatment regimen represents a promising therapy in patients with EC who progressed after first-line chemotherapy. The efficacy and safety of this regimen should be examined in a definitive randomized study.

Author affiliations
[1]Department of Gynecologic Oncology, Sun Yat-sen University Cancer Center, Guangzhou, China
[2]State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, China
[3]Department of Radiology, Sun Yat-sen University Cancer Center, Guangzhou, China
[4]Department of Clinical Research, Sun Yat-sen University Cancer Center, Guangzhou, China
[5]Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, Sun Yat-sen University Cancer Center, Guangzhou, China

Acknowledgements The authors thank the patients and their families for participating in this study. All authors had full access to the data, approved the final version of the manuscript, and accepted responsibility for submission for publication. We thank Chia Tai-Tianqing Pharmaceutical Holdings and Innovent Biologics (Suzhou) for providing anlotinib and sintilimab free of charge, respectively. The Chia Tai-Tianqing Pharmaceutical Holdings and Innovent Biologics (Suzhou) had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Contributors Data collection: XC, YQ, and QL. Study conception and design: MZ, XZ, and JL. Patients management: YH, FY, and WW. Image assessment: XB and RZ. Analysis and interpretation of results: JL, WW, and XH. Manuscript preparation: WW and JL. JL is responsible for the overall content as the guarantor.

Funding This study was supported by the Chinese National Natural Science Foundation project (grant number: 8197102869) and the Chinese Society of Clinical Oncology (grant number: Y-XD202002-0191).

Competing interests JL reported speaker fees from AstraZeneca and Chia Tai-Tianqing Pharmaceutical Holdings outside the submitted work. XZ reported receiving funding from the Chinese National Natural Science Foundation project outside the submitted work. WW reported speaker fees from AstraZeneca and Innovent Biologics (Suzhou).

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval Patient written informed consent was obtained before treatment initiation. The trial protocol was compliant with the Good Clinical Practice guidelines (as defined by the International Council on Harmonisation) and the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Sun Yat-sen University Cancer Center.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Clinical data are confidential due to the ongoing further clinical trial. Exploratory data are available upon reasonable request from the corresponding author.

ORCID iD
Wei Wei http://orcid.org/0000-0001-5929-7409

REFERENCES

1 Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209–49.
2 Chen W, Zheng R, Baade PD, *et al*. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016;66:115–32.
3 Morice P, Leary A, Creutzberg C, *et al*. Endometrial cancer. *Lancet* 2016;387:1094–108.
4 Muggia FM, Blessing JA, Sorosky J, *et al*. Phase II trial of the pegylated liposomal doxorubicin in previously treated metastatic endometrial cancer: a gynecologic Oncology Group study. *J Clin Oncol* 2002;20:2360–4.
5 Mileshkin L, Edmondson R, O'Connell RL, *et al*. Phase 2 study of anastrozole in recurrent estrogen (ER)/progesterone (PR) positive

endometrial cancer: The PARAGON trial - ANZGOG 0903. *Gynecol Oncol* 2019;154:29–37.
6 Aghajanian C, Sill MW, Darcy KM, *et al*. Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: a gynecologic Oncology Group study. *J Clin Oncol* 2011;29:2259–65.
7 Ray-Coquard I, Favier L, Weber B, *et al*. Everolimus as second- or third-line treatment of advanced endometrial cancer: ENDORAD, a phase II trial of GINECO. *Br J Cancer* 2013;108:1771–7.
8 Konstantinopoulos PA, Luo W, Liu JF, *et al*. Phase II study of avelumab in patients with mismatch repair deficient and mismatch repair proficient recurrent/persistent endometrial cancer. *J Clin Oncol* 2019;37:2786–94.
9 Ott PA, Bang Y-J, Berton-Rigaud D, *et al*. Safety and antitumor activity of pembrolizumab in advanced programmed death ligand 1-positive endometrial cancer: results from the KEYNOTE-028 study. *J Clin Oncol* 2017;35:2535–41.
10 Oaknin A, Tinker AV, Gilbert L, *et al*. Clinical activity and safety of the anti-programmed death 1 monoclonal antibody dostarlimab for patients with recurrent or advanced mismatch repair-deficient endometrial cancer: a nonrandomized phase 1 clinical trial. *JAMA Oncol* 2020;6:1766–72.
11 Kandoth C, Schultz N, Cherniack AD. The cancer genome atlas research network. integrated genomic characterization of endometrial carcinoma. *Nature* 2013;497:67–73.
12 Makker V, Taylor MH, Aghajanian C, *et al*. Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer. *J Clin Oncol* 2020;38:2981–92.
13 Makker V, Colombo N, Casado Herráez A, *et al*. Lenvatinib plus pembrolizumab for advanced endometrial cancer. *N Engl J Med* 2022;386:437–48.
14 Marth C, Vulsteke C, Rubio Pérez MJ. ENGOT-en9/LEAP-001: a phase III study of first-line pembrolizumab plus lenvatinib versus chemotherapy in advanced or recurrent endometrial cancer. *N Engl J Med* 2019;380:2317–26.
15 Wang P-F, Chen Y, Song S-Y, *et al*. Immune-related adverse events associated with anti-PD-1/PD-L1 treatment for malignancies: a meta-analysis. *Front Pharmacol* 2017;8:730.
16 Escudier B, Porta C, Bono P, *et al*. Randomized, controlled, double-blind, cross-over trial assessing treatment preference for pazopanib versus sunitinib in patients with metastatic renal cell carcinoma: PISCES study. *J Clin Oncol* 2014;32:1412–8.
17 Shi Y, Su H, Song Y, *et al*. Safety and activity of sintilimab in patients with relapsed or refractory classical Hodgkin lymphoma (ORIENT-1): a multicentre, single-arm, phase 2 trial. *Lancet Haematol* 2019;6:e12–19.
18 Shen G, Zheng F, Ren D, *et al*. Anlotinib: a novel multi-targeting tyrosine kinase inhibitor in clinical development. *J Hematol Oncol* 2018;11:1–11.
19 Han B, Li K, Wang Q, *et al*. Effect of Anlotinib as a third-line or further treatment on overall survival of patients with advanced non-small cell lung cancer: the alter 0303 phase 3 randomized clinical trial. *JAMA Oncol* 2018;4:1569–75.
20 Wu D, Nie J, Hu W, *et al*. A phase II study of anlotinib in 45 patients with relapsed small cell lung cancer. *Int J Cancer* 2020;147:3453–60.
21 Chi Y, Fang Z, Hong X, *et al*. Safety and efficacy of anlotinib, a multikinase angiogenesis inhibitor, in patients with refractory metastatic soft-tissue sarcoma. *Clin Cancer Res* 2018;24:5233–8.
22 Sun Y, Du F, Gao M, *et al*. Anlotinib for the treatment of patients with locally advanced or metastatic medullary thyroid cancer. *Thyroid* 2018;28:1455–61.
23 Shan Y, Zhong C, Ni Q, *et al*. Anlotinib enhanced penpulimab efficacy through remodeling of tumor vascular architecture and immune microenvironment in hPD-L1/hPD-1 humanized mouse model. *JCO* 2021;39:2581.
24 Yang Y, Li L, Jiang Z, *et al*. Anlotinib optimizes anti-tumor innate immunity to potentiate the therapeutic effect of PD-1 blockade in lung cancer. *Cancer Immunol Immunother* 2020;69:2523–32.
25 Chu T, Zhong R, Zhong H, *et al*. Phase 1B study of sintilimab plus anlotinib as first-line therapy in patients with advanced NSCLC. *J Thorac Oncol* 2021;16:643–52.
26 Chen X, Li W, Wu X, *et al*. 170P Sintilimab plus anlotinib as first-line therapy in patients (pts) with advanced hepatocellular carcinoma (aHCC). *Annals of Oncology* 2020;31:S1305.
27 Ren Z, Xu J, Bai Y, *et al*. Sintilimab plus a bevacizumab biosimilar (IBI305) versus sorafenib in unresectable hepatocellular carcinoma (ORIENT-32): a randomised, open-label, phase 2-3 study. *Lancet Oncol* 2021;22:977–90.
28 Althubiti MA. Mutation frequencies in endometrial cancer patients of different ethnicities and tumor grades: an analytical study. *Saudi J Med Med Sci* 2019;7:16–21.
29 John EM, Miron A, Gong G, *et al*. Prevalence of pathogenic BRCA1 mutation carriers in 5 us racial/ethnic groups. *JAMA* 2007;298:2869–76.
30 Oaknin A, Ellard SL, Leath III C, *et al*. Preliminary safety, efficacy, and PK/PD characterization from GARNET, a phase I clinical trial of the anti–PD-1 monoclonal antibody, TSR-042, in patients with recurrent or advanced MSI-H endometrial cancer. *Ann Oncol* 2018;29:viii334.
31 Hasegawa K, Tamura K, Katsumata N, *et al*. Efficacy and safety of nivolumab (Nivo) in patients (PTS) with advanced or recurrent uterine cervical or corpus cancers. *JCO* 2018;36:5594.
32 Madariaga A, Garg S, Tchrakian N, *et al*. Phase II trial assessing niraparib with or without dostarlimab (anti-PD-1) in recurrent endometrial carcinoma. *JCO* 2021;39:5574.
33 Rubinstein MM, Caird I, Zhou Q, *et al*. A phase II trial of durvalumab with or without tremelimumab in patients with persistent or recurrent endometrial carcinoma and endometrial carcinosarcoma. *JCO* 2019;37:5582.
34 Zong H, Zhong Q, Zhao R, *et al*. Phase II study of anlotinib plus sintlimab as second-line treatment for patients with advanced biliary tract cancers. *JCO* 2021;39:307–72.
35 Xu Q, Wang J, Sun Y, *et al*. Efficacy and safety of sintilimab plus anlotinib for PD-L1-positive recurrent or metastatic cervical cancer: a multicenter, single-arm, prospective phase II trial. *J Clin Oncol* 2022:2091.
36 Jones NL, Wu S, Xiu J, *et al*. Immune-response markers and actual response to immune-oncology therapy in uterine serous carcinoma. *JCO* 2021;39:5590.
37 Peyraud F, Italiano A. Combined PARP inhibition and immune checkpoint therapy in solid tumors. *Cancers* 2020;12:1502.
38 Jiang C, Zhang H, Wu W, *et al*. Immune characteristics of LYN in tumor microenvironment of gliomas. *Front Cell Dev Biol* 2021;9:760929.
39 Brian BF, Freedman TS. The Src-family kinase Lyn in immunoreceptor signaling. *Endocrinology* 2021;162:1–13.
40 Tornillo G, Knowlson C, Kendrick H, *et al*. Dual mechanisms of LYN kinase dysregulation drive aggressive behavior in breast cancer cells. *Cell Rep* 2018;25:3674–92.
41 Zardan A, Nip KM, Thaper D, *et al*. Lyn tyrosine kinase regulates androgen receptor expression and activity in castrate-resistant prostate cancer. *Oncogenesis* 2014;3:e115.