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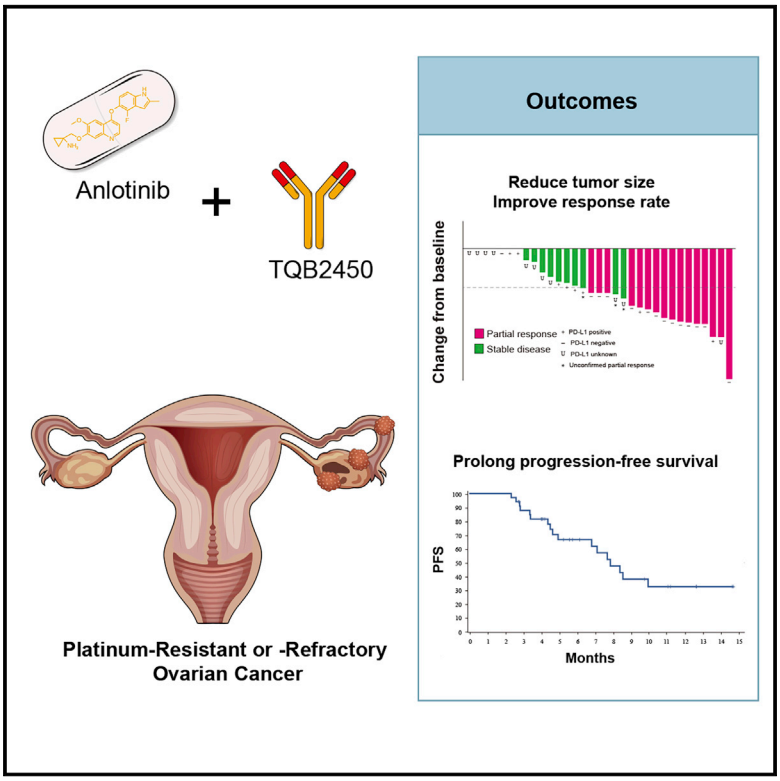
Anlotinib combined with TQB2450 in patients with platinum-resistant or -refractory ovarian cancer: A multi-center, single-arm, phase 1b trial

Graphical abstract



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In brief

Lan et al. demonstrate the efficacy, safety, and survival of anlotinib plus a PD-L1 inhibitor TQB2450 in platinum-resistant or -refractory ovarian cancer. They analyze the association between PD-L1 expression and clinical efficacy. They provide a combination regimen of anti-angiogenic agent and immune checkpoint inhibitor in the platinum-resistant and -refractory setting.

Highlights

- Anlotinib plus TQB2450 improves the response rate in platinum-resistant ovarian cancer
- Anlotinib plus TQB2450 shows durable response in platinum-resistant ovarian cancer
- A phase 3 randomized controlled trial to further validate our findings is ongoing

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Anlotinib combined with TQB2450 in patients with platinum-resistant or -refractory ovarian cancer: A multi-center, single-arm, phase 1b trial

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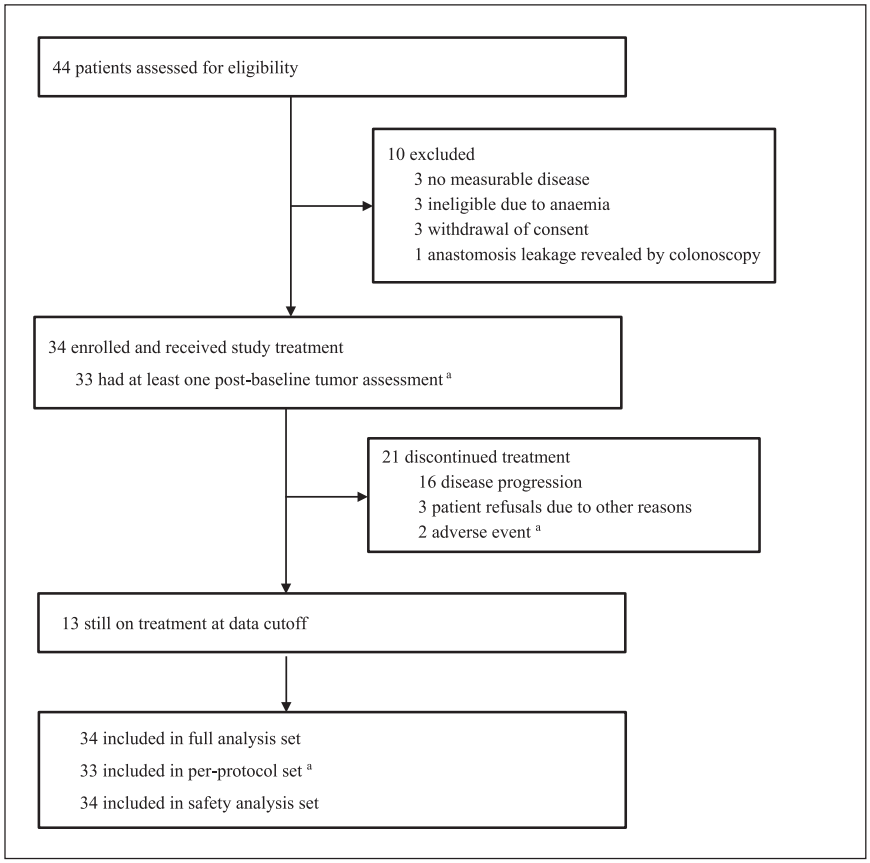
SUMMARY

This is a phase Ib study of anlotinib plus a programmed death-ligand 1 (PD-L1) inhibitor TQB2450 for platinum-resistant or -refractory ovarian cancer. Thirty-four patients are enrolled and receive treatment. The objective response rate (ORR) is 47.1%, and the disease control rate is 97.1%. The median duration of response (DOR) has not been reached, and 61.3% of patients have a DOR of at least 8 months. The median progression-free survival (PFS) is 7.8 months, and the median overall survival (OS) has not been reached. The PD-L1-positive group has an ORR of 25.0%, whereas the PD-L1-negative group has an ORR of 92.9%. Treatment-related grade 3 or 4 adverse events (AEs) occur in 70.6% of patients, with the most common being hypertension (29.4%) and palmar-plantar erythrodysesthesia syndrome (29.4%). Anlotinib plus TQB2450 show promising antitumor activity and manageable toxicities in patients with platinum-resistant or -refractory ovarian cancer. A phase 3 randomized controlled trial to further validate our findings is ongoing.

INTRODUCTION

Ovarian cancer is the eighth leading cause of cancer-related death in women worldwide and is responsible for 207,252 deaths in 2020.[1] Approximately 70% of patients with ovarian cancer are diagnosed at later stages.[2] Despite response to first-line platinum-based chemotherapy, most patients relapse within the initial 2 years after diagnosis.[3] Almost all patients with recurrent disease eventually develop resistance to platinum. The sequential use of single-agent non-platinum is considered standard treatment for platinum-resistant relapse.[4] However, the objective response rate (ORR) to single-agent therapy was low (10%–20%), and the progression-free intervals were short (3–4 months).[4–6] Thus, there is an unmet need for innovative therapy with improved response rates and duration of response (DOR) in this setting.

Immune checkpoint inhibitor (ICI) therapy has revolutionized the treatment of several cancers.[7–9] Despite the reported immunogenicity of ovarian cancer,[10] response rates to programmed cell death-1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors seem low in patients with ovarian cancer.[11–13] Single-agent PD-1 inhibitor nivolumab and pembrolizumab in heavily pretreated patients with advanced ovarian cancer achieved response rates of 15% and 8%, respectively,[11,12] and the PD-L1 inhibitor avelumab reported a response rate of 9.6% in refractory ovarian cancer.[13] Combined PD-1/PD-L1 inhibitor therapy is considered to enhance the efficacy of monotherapy, with one strategy of combining PD-1/PD-L1 inhibitors with anti-angiogenic therapy.[14,15] Thus far, two trials involving the PD-1/PD-L1 inhibitors combined with bevacizumab have shown promise in patients with recurrent ovarian cancer.[16,17] However, there is poor understanding of the efficacy of the combination of PD-1/PD-L1 inhibitors with tyrosine kinase inhibitors (TKIs) of vascular endothelial growth factor receptor (VEGFR) in this setting. Anlotinib is an oral, multi-targeted TKI with activity against VEGFR1/2/3, FGFR1/2/3, PDGFR-α, c-Kit, and Ret and is effective against various types of cancer.[18,19] In an ongoing phase 2 study of anlotinib monotherapy in patients with



[a] One patient discontinued treatment due to adverse event prior to the first post-baseline tumor assessment, thus was excluded from per-protocol set.

Figure 1. Trial profile

platinum-resistant or -refractory ovarian cancer, the preliminary results showed that the ORR was 14.3% and the disease control rate (DCR) was 71.4%.[20] TQB2450, a humanized immunoglobulin G1 (IgG1) monoclonal antibody against PD-L1, has demonstrated antitumor activity in mouse models of melanoma and colon cancer.[21] Backed by preclinical data, clinical trials of TQB2450 with or without anlotinib in treating patients with advanced refractory biliary tract cancer, triple-negative breast cancer, non-small cell lung cancer, and gynecologic cancer are ongoing.[22–24] Here, we present the efficacy and safety of anlotinib plus TQB2450 in patients with platinum-resistant or -refractory ovarian cancer.

RESULTS

Between February 27, 2020, and February 26, 2021, we screened 44 patients, of whom 34 eligible patients were enrolled and received the study treatment (full analysis set [FAS] and safety analysis set). One patient (2.9%) who discontinued the treatment before the first post-baseline tumor assessment due to adverse events (AEs) was excluded from the per-protocol set (PPS) (Figure 1). As of the data cutoff (May 31, 2021), the median follow up was 8.6 (range: 2.3–15.3) months. Thirteen (38.2%) of the 34 patients were still on treatment. Twenty-one (61.8%) patients discontinued treatment due to disease progression (n = 16, 47.1%), AEs (n = 2, 5.9%), and patient refusal (n = 3, 8.8%). Baseline characteristics of the population are summarized in Table 1.

Antitumor activity

In the first nine patients enrolled, confirmed responses were noted in five patients. The ORR threshold for the first stage of Simon's two-stage design was reached, and the trial continued to full accrual. In the FAS, 16 of 34 patients achieved partial response (PR), thus the ORR was 47.1% (95% confidence interval [CI], 29.8–64.9) (Table 2). An additional three patients had unconfirmed PR. Similar responses were recorded in PPS (Table 2). Seventeen (50.0%) of 34 patients achieved stable disease (SD), hence the DCR was 97.1% (95% CI, 84.7–99.9; Table 2). As shown in Figure 2A, 26 (78.8%) of the 33 patients who had an evaluable tumor assessment had some degree of tumor shrinkage.

Among the 16 patients with a confirmed objective response, the median time to achieve response was 2.7 (range: 1.3–7.0) months, and the median DOR was not reached (95% CI, 5.3 to not reached; Figure 3A). A DOR of at least 8 months was observed in 61.3% (95% CI, 25.1–84.0). Figure 2B presents a swimmer plot of the time to receive treatment across all patients.

As of the data cutoff, 17 (50.0%) of the 34 patients had disease progression. The median progression-free survival (PFS) was 7.8 months (95% CI, 4.9 to not reached; Figure 3B). The proportion of patients estimated to be progression free at 6 months was 66.7% (95% CI, 46.7–80.7) and at 9 months was 38.1% (95% CI, 19.1–57.0). Nine (26.5%) patients had died. The median overall survival (OS) was not reached (95% CI, 11.3 to not reached; Figure 3C).

Safety

Treatment-related AEs of any grade occurred in all 34 patients (100%) (Table 3): hypertension (n = 31, 91.2%), palmar-plantar erythrodysesthesia syndrome (n = 25, 73.5%), hypertriglyceridemia (n = 20, 58.8%), and hypothyroidism (n = 17, 50.0%; Table 3). Grade 3–4 treatment-related AEs occurred in 24 (70.6%) patients; the most common were hypertension (n = 10, 29.4%), palmar-plantar erythrodysesthesia syndrome (n = 10, 29.4%), hypertriglyceridemia (n = 5, 14.7%), fatigue (n = 3, 8.8%), and weight loss (n = 3, 8.8%; Table 3). No treatment-related deaths

Table 1. Baseline patient characteristics

Characteristic, n (%)	All patients (n = 34)
Age, years	
Median (range)	55 (26–71)
FIGO stage at diagnosis	
IA	1 (2.9)
IC	2 (5.9)
IIB	2 (5.9)
IIIA1	4 (11.8)
IIIB	5 (14.7)
IIIC	16 (47.1)
IVB	4 (11.8)
Histologic subtype	
High-grade serous carcinoma	27 (79.4)
Low-grade serous carcinoma	2 (5.9)
Endometrioid	1 (2.9)
Clear cell	4 (11.8)
ECOG performance status	
0	3 (8.8)
1	31 (91.2)
Number of prior lines of systemic therapies	
1–2	12 (35.3)
3–6	22 (64.7)
Median (range)	3 (1–6)
Target lesion size, mm	
Median (range)	48 (17–182)
Treatment-free interval (TFI)	
Progression during the last therapy/<1 month	21 (61.8)
≥1 and <2 months	9 (26.5)
≥2 and <6 months	4 (11.8)
Platinum-free interval (PFI)	
<3 months	11 (32.4)
≥3 and <6 months	9 (26.5)
≥6 months[a]	14 (41.2)
Prior bevacizumab	
Yes	9 (26.5)
No	25 (73.5)
BRCA1/2 mutation status	
gBRCA1/2 mutation	4 (11.7)
BRCA1/2 wild type	16 (47.1)
BRCA1/2 unknown	14 (41.2)
PD-L1 expression status	
Positive	8 (23.5)
Negative	14 (41.2)
Unknown	12 (35.3)
Microsatellite status[b]	
High microsatellite instability	0 (0.0)
Microsatellite stable	16 (47.1)
Unknown	18 (52.9)

FIGO, The International Federation of Gynecology and Obstetrics; ECOG, Eastern Cooperative Oncology Group.
[a]These 14 patients received at least one line of single-agent therapy with a non-platinum compound after being considered platinum-resistant (defined as disease progressing within 6 months after the last platinum therapy), which made the PFI ≥6 months. Of these 14 patients, 13 (92.9%) had TFI ≤1 month and one had TFI of 4.2 months.
[b]Assessed by fluorescent multiplex polymerase chain reaction (PCR) and capillary gel electrophoresis (CGE).

occurred. Serious treatment-related AEs were observed in 5 (14.7%) of 34 patients, including one (2.9%) each with hypertension, fatigue, pneumonitis, and arthritis and one (2.9%) with increased aspartate aminotransferase and alanine aminotransferase (Table S1).

All 34 patients received at least one complete cycle of anlotinib. Anlotinib dose interruption due to AEs occurred in 22 patients (64.7%). Sixteen (47.1%) patients required anlotinib dose reduction, of whom 12 (81.3%) required only one dose reduction and three (18.8%) required two dose reductions. The most common AE leading to anlotinib dose reduction was palmar-plantar erythrodysesthesia syndrome (10/16, 62.5%). AEs leading to anlotinib dose reduction are shown in Table S2. TQB2450 dose interruption occurred in 8 (23.5%) of the 34 patients. Eight (23.5%) patients required corticosteroids: two with pneumonitis, two with arthritis, one each with a rash, myositis, and hyperthyroidism, and one with increased aspartate aminotransferase and alanine aminotransferase. In the patients who received corticosteroids, one patient had a

Table 2. Antitumor activity assessed by RECIST 1.1

Antitumor activity	The full analysis set (n = 34)	The per-protocol set (n = 33)
ORR	16 (47.1)	16 (48.5)
95% CI	29.8–64.9	30.8–66.5
DCR	33 (97.1)	33 (100)
95% CI	84.7–99.9	89.4–100.0
Best overall response		
CR	0 (0.0)	0 (0.0)
PR	16 (47.1)	16 (48.5)
SD	17 (50.0)	17 (51.5)
Progressive disease	0 (0.0)	0 (0.0)
No assessment[a]	1 (2.9)	–

Data are presented as n (%) unless otherwise specified. Responses were assessed in accordance with the RECIST 1.1. Only confirmed responses were included. RECIST, Response Evaluation Criteria in Solid Tumors; ORR, objective response rate; DCR, disease control rate; CR, complete response; PR, partial response; SD, stable disease.
[a]One patient discontinued study treatment before the first scheduled post-baseline scan.

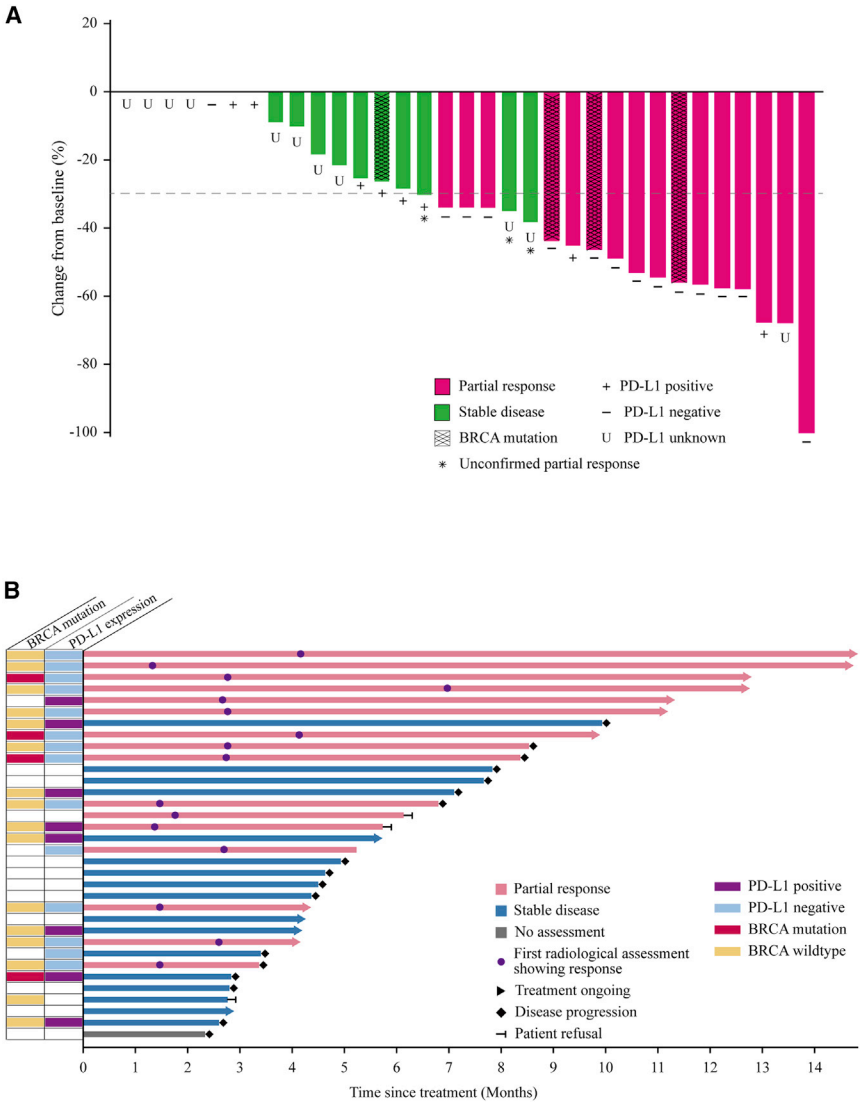


Figure 2. Tumor response assessment with waterfall plot and treatment duration with swimmer plot

(A) Waterfall plot for best percentage change in target lesion size. The patients who had at least one post-baseline tumor assessment were included (n = 33). The dashed line at −30% change represents the RECIST 1.1 cutoff to define partial response or complete response.

(B) Swimmer plot. The length of each bar represents the duration treatment for each patient.

TQB2450 dose interruption, and one patient permanently discontinued TQB2450. Two (5.9%) patients withdrew from the study due to toxicities: one with fatigue and one with grade 4 increased aspartate aminotransferase and alanine aminotransferase.

PD-L1 expression

PD-L1 expression was detected in 22 patients. Eight patients were positive for PD-L1, and 14 were negative for PD-L1 (Table S3). ORR was 25% (95% CI, 3.1%–65.1%) in patients with PD-L1-positive tumors, whereas it was 92.9% (95% CI, 66.1%–99.8%) in patients with PD-L1-negative tumors (p = 0.002; Table S3). Median PFS was 8.5 months (95% CI, 2.6 to not reached) in PD-L1-positive patients and was not reached (95% CI, 6.8 to not reached) in PD-L1-negative patients (Figure 4). The difference in PFS was not statistically significant between PD-L1-positive or -negative patients (p = 0.28; Figure 4).

DISCUSSION

In this study, anlotinib plus TQB2450 demonstrated promising antitumor activity with favorable response rate, durable response, and tolerable toxicity profile in patients with platinum-resistant or -refractory ovarian cancer. To our knowledge, this is the largest cohort of patients in this setting treated with a multi-targeted TKI plus a PD-L1 inhibitor.

Despite the immunogenic profile in ovarian cancer revealed by preclinical data, PD-1/PD-L1 inhibitor monotherapy showed only low to moderate activity in patients with recurrent ovarian cancer.[11–13] There is growing interest in combination studies of PD-1/PD-L1 and VEGF inhibitors to improve the treatment of ovarian cancer. A phase 2 trial combining the PD-1 inhibitor nivolumab and bevacizumab reported increased benefit in patients with platinum-sensitive ovarian cancer with a response rate of 40%.[16] However, this treatment combination seemed to have less activity in the platinum-resistant setting (response rate 16.7%). Limited data have reported the clinical effect of PD-1/PD-L1 inhibitor combined with VEGFR TKI in advanced ovarian cancer. Remarkably, the efficacy observed in our study is encouraging, with 16 (47.1%) confirmed responses and three unconfirmed responses and 78.8% of the patients showing some degree of tumor shrinkage. Our data suggest that anlotinib plus TQB2450 has enhanced antitumor activity in platinum-resistant ovarian cancer, given the efficacy previously reported in anlotinib monotherapy and PD-1/PD-L1 inhibitors monotherapy in this setting.[11–13,20] Yang et al. identified that PD-L1 directly interacted with VEGFR2 and that it promoted the angiogenesis and metastasis of ovarian cancer by participating in the c-JUN/VEGFR2 signaling axis.[25] Furthermore, they demonstrated the PD-L1 inhibitor durvalumab combined with the VEGFR TKI apatinib enhanced the effect of anti-angiogenesis and the inhibition of cell migration and invasion. These findings may be one of the plausible explanations for the encouraging results observed in our study, where a multi-targeted TKI anlotinib

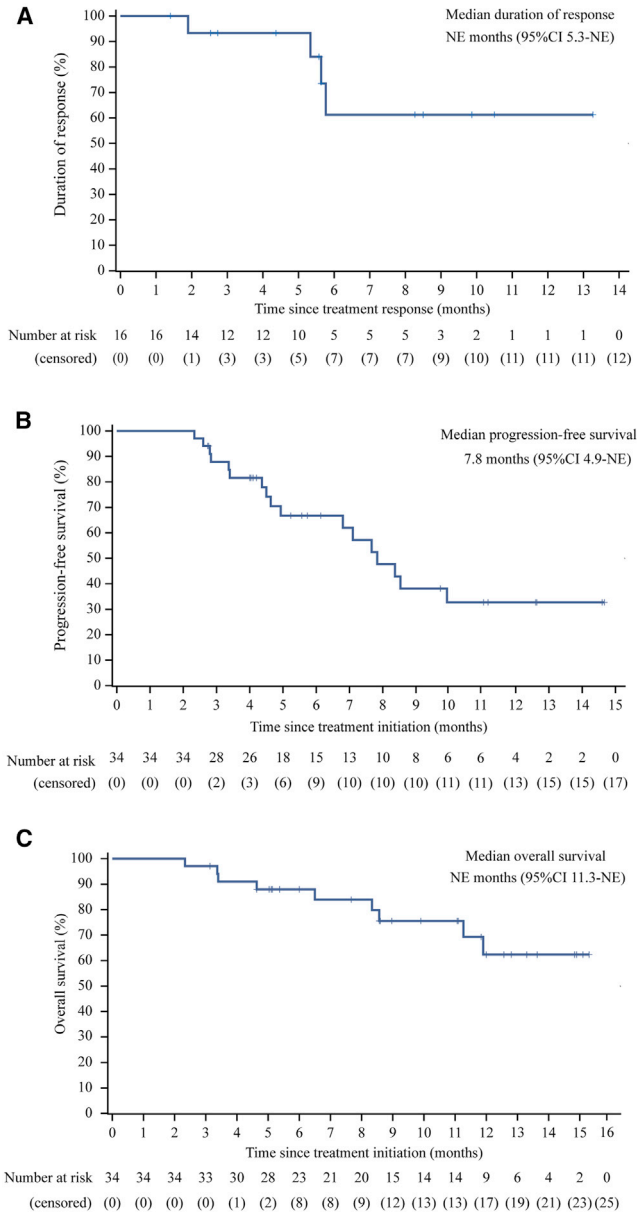


Figure 3. Kaplan-Meier curves of duration of response, progression-free survival, and overall survival

Kaplan-Meier curves of (A) duration of response, (B) progression-free survival, and (C) overall survival. NE, not estimable.

was used as a partner combined with the PD-L1 inhibitor TQB2450.

In addition, 41.2% of the patients in our study had multiple lines of single-agent therapy with a non-platinum compound after being considered resistant to the last platinum therapy, which made the platinum-free interval (PFI) ≥6 months. There is a concern that the prolonged PFI may, to some extent, better our efficacy. However, there is no evidence that the predictive and prognostic roles of PFI apply for more than two relapses, and PFI may not be a valuable criteria for molecular targeted

Table 3. Treatment-related adverse events in the total treated patients (n = 34)

Any treatment-related adverse events, n (%)	Grade 1	Grade 2	Grade 3	Grade 4
Hypertension	2 (5.9)	19 (55.9)	10 (29.4)	0
Palmar-plantar erythrodysesthesia syndrome	2 (5.9)	13 (38.2)	10 (29.4)	0
Hypothyroidism	12 (35.3)	5 (14.7)	0	0
Hypertriglyceridemia	11 (32.4)	4 (11.8)	3 (8.8)	2 (5.9)
Proteinuria	9 (26.5)	5 (14.7)	2 (5.9)	0
Stomach ache	13 (38.2)	1 (2.9)	0	0
Increased aspartate aminotransferase	12 (35.3)	1 (2.9)	0	1 (2.9)
Increased alanine aminotransferase	12 (35.3)	1 (2.9)	0	1 (2.9)
Diarrhea	9 (26.5)	3 (8.8)	1 (2.9)	0
Fatigue	8 (23.5)	2 (5.9)	3 (8.8)	0
Hoarseness	8 (23.5)	2 (5.9)	0	0
Stomatitis	6 (17.6)	4 (11.8)	1 (2.9)	0
Headache	8 (23.5)	1 (2.9)	0	0
Hypoalbuminemia	6 (17.6)	2 (5.9)	0	0
Sore throat	7 (20.6)	1 (2.9)	0	0
Weight loss	4 (11.8)	3 (8.8)	3 (8.8)	0
Myalgia	6 (17.6)	0	0	0
Thrombocytopenia	5 (14.7)	0	1 (2.9)	0
Anorexia	5 (14.7)	0	0	0
Neutropenia	3 (8.8)	2 (5.9)	1 (2.9)	0
Rash	4 (11.8)	1 (2.9)	0	0
Increased amylase	4 (11.8)	0	1 (2.9)	0
Arthritis	2 (5.9)	2 (5.9)	1 (2.9)	0
Dry mouth	4 (11.8)	0	0	0
Pruritus	3 (8.8)	1 (2.9)	0	0
Hyperthyroidism	2 (5.9)	2 (5.9)	0	0
Cough	3 (8.8)	0	0	0
Abdominal pain	3 (8.8)	0	0	0
Nausea	3 (8.8)	0	0	0
Gingival pain	3 (8.8)	0	0	0
Gamma-glutamyltransferase increased	2 (5.9)	1 (2.9)	2 (5.9)	0
Increased bilirubin	2 (5.9)	0	2 (5.9)	0
Insomnia	2 (5.9)	0	0	0
Vomiting	1 (2.9)	1 (2.9)	0	0
Increased lipase	1 (2.9)	1 (2.9)	0	0
Pneumonitis	0	2 (5.9)	0	0
Tinnitus	0	2 (5.9)	0	0
Anaemia	1 (2.9)	0	0	0
Myositis	0	1 (2.9)	0	0

No grade 5 treatment-related adverse events were reported. See also Tables S1 and S2.

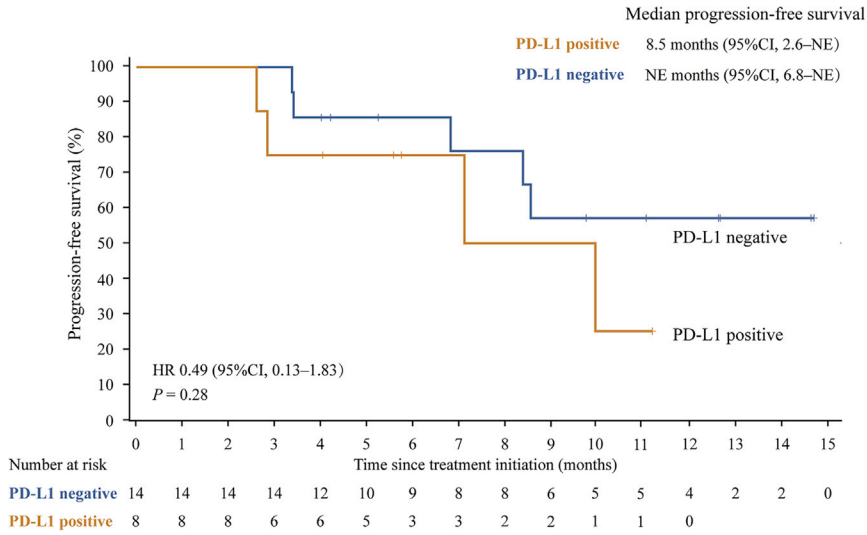


Figure 4. Kaplan-Meier curves of progression-free survival of PD-L1-positive and -negative tumors. NE, not estimable; HR, hazard ratio.
See also Table S3.

therapy.[4,26] In our study, 88.3% of patients had a treatment-free interval (TFI) <2 months, which represented a patient population with poor prognosis.[4]

Of note, the findings of our study and other trials of combination PD-1/PD-L1 inhibitor therapy demonstrated a durable clinical benefit in patients with platinum-resistant ovarian cancer. In a phase 1b study of 20 patients with platinum-resistant ovarian cancer who were treated with atezolizumab and bevacizumab, despite having a modest ORR of 15%, all three patients with PR had durable responses (11.3–18.9 months) and are still ongoing at the data cutoff, with four of eight patients with SD for >8 months.[17] In a phase 2 study combining nivolumab and bevacizumab in patients with recurrent ovarian cancer, only 3 of 18 patients (16.7%) with platinum-resistant disease achieved PR. However, two of them had a prolonged response of 16–18 months, with 16.7% of patients with SD lasting at least 24 weeks.[16] Similarly, in our study, the median PFS was 7.8 months, and the median DOR was not reached, with 61.3% of the patients showing a DOR of at least 8 months.

The safety profile of anlotinib plus TQB2450 was consistent with that reported in previous studies of other selective VEGFR TKIs combined with PD-1/PD-L1 inhibitors,[27–29] with no unexpected AEs. Although the frequency of the overall grade 3–4 AEs was as high as 70.6% in our study, the prevalence of specific grade 3–4 AEs was not so high, with hypertension and palmar-plantar erythrodysesthesia syndrome being the highest ranked with 29.4% each. These toxicities were associated with anlotinib and were manageable. Despite the grade 3–4 hypertriglyceridemia and grade 3 increased amylase observed in 14.7% and 2.9% of the patients in our study, respectively, all these patients did not have any symptoms. In the eight patients who required corticosteroids, most of them did not need TQB2450 dose interruption. In addition, the discontinuation rate due to treatment-related AEs was only 5.9% in our study. Our findings suggested that anlotinib plus TQB2450 had a manageable safety profile.

PD-L1 is the most widely investigated biomarker for predicting response to PD-1/PD-L1 inhibitor therapy; however, the predictive value of PD-L1 in ovarian cancer remains highly controversial.[11,12,16] In our study, patients with PD-L1-negative tumors had higher response rates than those with PD-L1-positive tumors (92.9% versus 25.0%). Several factors may explain the inconsistency between the efficacy and PD-L1 expression in our study, such as the spatial and temporal heterogeneity in PD-L1 expression, the variability between primary and metastatic tumor sites, and the influence by prior chemotherapy.[30–32] It should be noted that only 22 (64.7%) patients in our study had archival tumor samples for PD-L1 staining, and the small number of patients precluded definitive conclusions. Efforts are ongoing to identify more reliable biomarkers for PD-1/PD-L1 inhibitor therapy.

Anlotinib plus TQB2450 has promising antitumor activity and manageable toxicities in patients with platinum-resistant or -refractory ovarian cancer. Accordingly, a phase 3 multicenter randomized trial (ClinicalTrials.gov: NCT05145218) to investigate anlotinib plus TQB2450 as treatment for patients with platinum-resistant or -refractory ovarian cancer is ongoing.

Limitations of study

This study has limitations. This is a single-arm study lacking a comparator treatment arm; thus, selection bias could not be ruled out. Further, the small sample size reduced the certainty of effectiveness observed.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2022.100689.

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AUTHOR CONTRIBUTIONS

Conception and design, C.-Y.L. and X.H.; provision of study materials or patients, C.-Y.L., F.Y., Y.X., R.L., Y.H., J.W., C.L., X.-H.B., H.-H.J., J.M., W.-H.Z., L.Z., M.Z., and X.H.; collection and assembly of data, C.-Y.L., J.Z., F.Y., Y.X., R.L., J.W., C.L., H.-H.J., J.M., W.-H.Z., L.Z., M.Z., and X.H.; data analysis and interpretation, C.-Y.L., F.Y., Y.X., R.L., Y.H., J.W., C.L., J.M., W.-H.Z., Y.-F.W., M.Z., and X.H.; manuscript writing, C.-Y.L.; final approval of manuscript, all authors.

DECLARATION OF INTERESTS

Y.-F.W. is an employee of Chia Tai Tianqing Pharmaceutical Group Co., Ltd.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Formalin-fixed paraffin-embedded (FFPE) archival tumor specimens	This manuscript	N/A
Chemicals, peptides, and recombinant proteins		
TQB2450	Chia Tai Tianqing Pharmaceutical Group Co., Ltd.	See Table S4
Anlotinib	Chia Tai Tianqing Pharmaceutical Group Co., Ltd.	See Table S5
Critical commercial assays		
PD-L1 IHC 22C3	Dako, an Agilent Technologies, Inc. company, Santa Clara, CA, USA	Cat# 11174544
Deposited data		
The data of patients	This manuscript	https://www.researchdata.org.cn/ (ID: RDDA2021002123)
Software and algorithms		
SAS software version 9.4	SAS Institute, Cary, NC, USA	www.sas.com

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xin Huang (huangxin@sysucc.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The data of patients in this study have been recorded at Research Data Deposit: http://www.researchdata.org.cn with number RDDA2021002123. The data are available from Research Data Deposit but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Research Data Deposit public platform.
- This paper does not report the original code.
- Any additional information required to reanalyze the data reported in this work paper is available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines after approval by the China National Medical Products Administration (ID: [NMPA] 2017L04914) and local institutional review board at each participating site.

Human subjects

Chinese women with histologically confirmed epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer who had platinum-resistant or platinum-refractory disease, were enrolled in the study. Demographic information was provided in Table 1. All patients provided written informed consent prior to enrollment.

Patient eligibility

The key inclusion criteria were: (1) aged 18–70 years with histologically confirmed epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer who had platinum-resistant or platinum-refractory disease. Platinum-resistant was defined as disease progressing within 6 months after the last platinum therapy. Platinum-refractory was defined as disease progressing during the initial platinum-based therapy or within 28 days after the last dose of platinum. We also enrolled patients whose disease progressed within 6 months after the last platinum therapy and who received subsequent non-platinum treatment. (2) with radiological progression during the last treatment before enrolling in this study; (3) with measurable disease according to the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1); (4) an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; (5) adequate organ function. Major exclusion criteria were: (1) poorly controlled hypertension; (2) previous treatment with VEGFR-TKIs, anti-PD-1, anti-PD-L1, or anti-cytotoxic T-lymphocyte-associated antigen-4 antibodies; (3) active or documented history of autoimmune disease; (4) active brain metastases; (5) active hepatitis B or hepatitis C viral infection. All patients provided written informed consent prior to enrollment.

Subject allocation

The current phase Ib clinical trial was a single-arm study, with no control group, and all the patients were enrolled in one group.

METHOD DETAILS

Study design

This study (ACTION study) is part of an open-label, multi-cohort, multicenter phase Ib trial (clinicalTrials.gov identifier: NCT04236362) evaluating the efficacy and safety of anlotinib plus TQB2450 in patients with advanced gynecologic cancer at five academic medical centers in China. Here we report the results of ovarian cancer cohort. The primary endpoint was ORR, defined as the proportion of patients with complete response (CR) or PR according to RECIST 1.1 assessed by investigators. Objective responses had to be confirmed with a repeat scan at least 4 weeks later. Secondary endpoints were PFS, OS, DOR, DCR, safety, and tolerability.

Sample size estimation

A Simon optimal two-stage design is used to estimate sample size. The ORR of single-agent anlotinib in platinum-resistant ovarian cancer was approximately 15%. The combination of TQB2450 and anlotinib is expected to result in an improved ORR of 35%. The null hypothesis that the true ORR was 15% would be tested against a one-sided alternative of 35%. In the first stage, nine patients were accrued. If no response or one response was observed, the study was terminated and declared negative. Otherwise, 25 additional patients would be accrued to the second stage. The study was considered positive if > 8 responders were observed among the 34 patients. This design yielded a type I error rate of 5% and power of 80% when the true response rate was 35%.

Drug administration

Patients received anlotinib 12 mg orally once daily on days 1–14 followed by a 7-day rest and TQB2450 1,200 mg intravenously on day 1 of a 21-day cycle. This dose was determined based on the phase Ib study of anlotinib plus TQB2450 in advanced solid tumors.[24] Treatment continued until disease progression, unacceptable toxicity, or withdrawal of consent. Dose interruptions and dose reductions of anlotinib were permitted for toxicities that were not relieved by supportive care. A maximum of two dose reductions were allowed in anlotinib therapy. The first dose reduction was to 10 mg once daily, and the additional reduction was to 8 mg once daily. Dose reductions in TQB2450 were not allowed.

Assessments

Tumor responses were assessed by investigators according to RECIST 1.1 using computed tomography (CT) or magnetic resonance imaging (MRI) at baseline, every 6 weeks for the initial 54 weeks and every 9 weeks thereafter. The primary endpoint was ORR, defined as the proportion of patients with CR or PR according to RECIST 1.1 assessed by investigators. Objective responses had to be confirmed with a repeat scan at least 4 weeks later. Secondary endpoints were PFS, OS, DOR, DCR, safety, and tolerability. PFS was defined as the time from treatment initiation to the date of first documented disease progression or death from any cause, whichever occurred first. OS was defined as the time from treatment initiation to the date of death from any cause. DOR was defined as the time from the first evidence of response to disease progression in patients who achieved PR or better. DCR was defined as the proportion of patients who achieved confirmed CR or PR or SD. AEs were monitored throughout the treatment period and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE; version 5.0). The exploratory endpoint reported here included associations between antitumor activity and PD-L1 expression.

PD-L1 expression analysis

PD-L1 expression in formalin-fixed paraffin-embedded (FFPE) archival tumor specimens was assessed using the PD-L1 immunohistochemistry (IHC) 22C3 assay (Dako Inc., Agilent Technologies, Santa Clara, CA, USA) and measured using a combined positive

score (CPS), defined as the number of PD-L1-stained cells divided by the total number of viable tumor cells, multiplied by 100. PD-L1-positivity was defined as a CPS of $\geq$1. The expression of PD-L1 and its correlation with treatment efficacy were provided in Figure 4 and Table S3.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical tests were performed using the SAS software version 9.4 (SAS Institute, Cary, NC, USA). The FAS and safety analysis set were defined as all enrolled patients who received at least one dose of study treatment. The PPS consisted all FAS patients who had at least one post-baseline tumor assessment and no major protocol deviations and completed at least one treatment cycle. The primary endpoint was analyzed in both FAS and PPS. Secondary endpoints were analyzed in the FAS. A safety analysis was performed in the safety analysis set. We summarized the proportion of patients who achieved an objective response and calculated 95% CI using the Clopper–Pearson method. DOR, PFS, and OS were analyzed using the Kaplan-Meier method. Median DOR, PFS, and OS were estimated, and 95% CIs were calculated based on Greenwood's formula and log-log transformation. The AEs by the proportion of the total number of patients treated are summarized. In post hoc analyses, the association between ORR and PD-L1 status were assessed using the Chi-square test or Fisher's exact test. PFS according to PD-L1 expression status was estimated using Kaplan-Meier method and compared by log-rank tests.

ADDITIONAL RESOURCES

This study has been registered on https://clinicaltrials.gov/, ID: NCT04236362.