Clinical outcomes and safety of apatinib monotherapy in the treatment of patients with advanced epithelial ovarian carcinoma who progressed after standard regimens and the analysis of the *VEGFR2* polymorphism

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Abstract. The aims of the present study were to investigate the clinical outcomes and safety of apatinib monotherapy in the treatment of patients with advanced epithelial ovarian carcinoma (EOC) who have progressed after standard regimens, and to analyze the vascular endothelial growth factor receptor 2 (*VEGFR2*) rs2071559 polymorphism. A total of 118 patients with advanced EOC who received apatinib treatment were included in the study. Tumor response was evaluated using progression-free survival (PFS) and overall survival (OS) time, and safety data were documented. Additionally, peripheral blood and peripheral blood mononuclear cell (PBMC) specimens from the patients with EOC were collected to perform the genotyping of genetic polymorphism and assess the mRNA expression of *VEGFR2*, respectively. The objective response rate across the 118 patients with advanced EOC was 38.98%, the disease control rate was 63.56%, the median PFS time was 4.65 months and the median OS time was 15.10 months. Regarding the polymorphism analysis, the prevalence of rs2071559 in *VEGFR2* among the 118 patients with advanced EOC was recorded as the TT genotype in 72 cases (61.02%), TC genotype in 41 cases (34.75%) and CC genotype in 5 cases (4.23%), and the minor allele frequency of rs2071559 was 0.22. The distribution of the three genotypes was in accordance with the Hardy-Weinberg equilibrium (P=0.781). TC and CC genotypes were merged in the subsequent analysis. The prognosis analyses suggested that the median PFS time of patients with the TC/CC genotype and the TT genotype was 3.10 and 5.40 months, respectively (P=0.015). Moreover, the median OS time of the two genotypes was 12.60 and 17.50 months, respectively (P=0.009). However, no association was noted between genotype status of the polymorphism and adverse reactions. Additionally, the mRNA expression analysis indicated that the mRNA expression levels of *VEGFR2* in PBMC specimens were significantly different between TT and TC/CC genotypes (P<0.001). The present study suggested that the clinical outcomes of patients with advanced EOC, who progressed after standard regimens and received apatinib treatment, might be influenced by the *VEGFR2* rs2071559 polymorphism.

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Introduction

Ovarian carcinoma is a common gynecological malignancy and one of the leading causes of cancer-associated death in women worldwide. In 2018 alone, it accounted for ~295,400 new cases and ~184,800 deaths globally (1). Annually, there are ~52,100 new cases and ~22,500 associated deaths in China (2). The majority patients with ovarian carcinoma are diagnosed with epithelial ovarian carcinoma (EOC), which accounts for ~90% of ovarian carcinoma cases (3). Most of the patients are diagnosed with advanced disease due to the non-specific symptoms (4). Breakthroughs in treatment over the past two decades have improved the prognosis of EOC slightly, but the 5-year overall survival rate is still <30% for patients with advanced-stage EOC (5). According to European Society for Medical Oncology and National Comprehensive Cancer Network (NCCN) guidelines for ovarian

carcinoma (6,7), the standard initial chemotherapeutic treatment for patients with stage II-IV ovarian carcinoma is based on carboplatin plus paclitaxel regimens. Those who relapse ≥6 months or <6 months after initial chemotherapy are termed as platinum-sensitive patients and platinum-resistant patients, respectively (8). Almost all patients with recurrent disease eventually develop platinum resistance (9). The available second-line treatment regimens are liposomal doxorubicin, and weekly paclitaxel, topotecan, gemcitabine and etoposide, respectively, as single agents. These second-line treatment regimens have demonstrated an objective response rate (ORR) ranging from 10-30% and a median progression-free survival (PFS) time of 3-4 months (10,11). No appropriate regimens were available for subsequent lines of treatment (12).

It has been demonstrated that angiogenesis plays an important role in tumor growth, recurrence and metastasis of EOC (13). Anti-angiogenic therapy consists of anti-vascular endothelial growth factor (VEGF) antibodies and small molecule anti-angiogenesis tyrosine kinase inhibitors (TKIs) (14). Bevacizumab, a monoclonal antibody, has been approved for the therapy of ovarian cancer (15). Although a previous study showed that the combination of bevacizumab with chemotherapy in ovarian cancer improves the ORR, the administration of bevacizumab is ineffective and the prognosis is clinically unsatisfactory (11). Pazopanib was the first anti-angiogenic TKI, with positive clinical significance in a phase II clinical trial, to be recommended by NCCN guidelines of ovarian cancer (16). Subsequently, sorafenib demonstrated promising clinical outcomes as a maintenance therapy for patients with platinum-resistant ovarian cancer (17). These studies suggest the potential therapeutic significance of anti-angiogenic TKIs in the treatment of EOC.

Apatinib was the first anti-angiogenic TKI with demonstrable efficacy and safety in patients with advanced gastric cancer (18). Additionally, a previous study supported the effectiveness of apatinib in EOC (19). Generally, the clinical application of anti-angiogenic drugs results in a relatively low ORR. In advanced EOC, the ORR of monotherapy with pazopanib, sorafenib and apatinib were recorded as 18 (16), 3.4 (20) and 41.4% (21), respectively. Therefore, these individual differences between TKIs show that the clinical application of apatinib has a promising effect in patients with EOC. Unfortunately, no biomarker for patients with EOC who received apatinib treatment is available to determine prognosis and monitor disease progression clinically.

An important therapeutic target of apatinib is VEGF receptor 2 *(VEGFR2)*, which is located on chromosome 4q12 and contains 30 exons (22). To date, there are few studies investigating the polymorphism of *VEGFR2* in the Chinese population. rs2071559 is located in the upstream region of *VEGFR2* (23). A previous study indicated that rs2071559 is significantly associated with a pathological complete response in patients with advanced breast cancer treated with capecitabine-based neoadjuvant therapy (24). Furthermore, studies involving European and American populations have suggested that the *VEGFR2* polymorphism is associated with improved prognosis in patients with advanced renal cell carcinoma who are treated with sorafenib. However, the underlying mechanisms for this effect has not been completely interpreted or understood (25).

Therefore, the present study aimed to explore the clinical outcomes and safety of apatinib monotherapy in the treatment of patients with advanced EOC who progressed after standard regimens, and to analyze the *VEGFR2* rs2071559 polymorphism.

Materials and methods

Study design and therapeutic schedule. The present study was designed as a retrospective analysis considering patients with ovarian carcinoma receiving apatinib treatment. Therefore, patients with advanced ovarian carcinoma that progressed after standard regimens between January 2015 and December 2018 in the Department of Gynecological Oncology of Beijing Obstetrics and Gynecology Hospital (Beijing, China) were enrolled in this study. The eligibility criteria included: i) Histologically or cytologically confirmed diagnosis of epithelial ovarian carcinoma confirmed by a pathological expert; ii) pathological stage III or IV according to the International Federation of Gynecology and Obstetrics (FIGO) staging system (26); iii) female sex and age ≥18 years; iv) Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; v) patients treated with apatinib after at least two failed standard lines of treatment, including patients that had undergone secondary cytoreductive surgery after relapse; and vi) at least one measurable target lesion according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1) (27). The exclusion criteria included: i) Presence of concomitant tumors or serious diseases; ii) hemoptysis >50 ml per day; iii) presence of uncontrolled hypertension or serious presence of protein in the urine; and iv) a diagnosis of squamous cell skin cancer or *in situ* cancer of the cervix uteri. A flow chart of the study is illustrated in Fig. 1. The primary endpoint of the study was PFS time, the secondary endpoint was ORR, overall survival (OS) time and the analysis of *VEGFR2* gene polymorphism.

Apatinib was administered at the initial dosage of 500 or 750 mg per day, orally with warm water, 30 min after meals, and for 28 days as one cycle, until disease progression or intolerable adverse reactions. The precise dose of apatinib was determined according to the baseline physical conditions of the patients, namely body surface area, ECOG score and age. The dose of apatinib was adjusted according to the hematological (neutropenia) or non-hematological toxicity (hypertension) during the treatment. The treatment was discontinued when a potentially life-threatening adverse reaction occurred. The present study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital (Beijing, China). Written informed consent was provided by enrolled patients or by their relatives.

The clinical outcomes were assessed according to RECIST 1.1 criteria (27). The change of target lesions was assessed with computed tomography (CT) scans after the completion of the first cycle and then after every two cycles, or more frequently if the clinical symptoms of the patients deteriorated. The occurrence of adverse reactions during treatment was evaluated using the Common Terminology Criteria for Adverse Events version 4.03, in order to register hematological and non-hematological events that may be drug-related (28). Adverse events classified as grade ≥2 with an incidence ≥10% were recorded and analyzed.
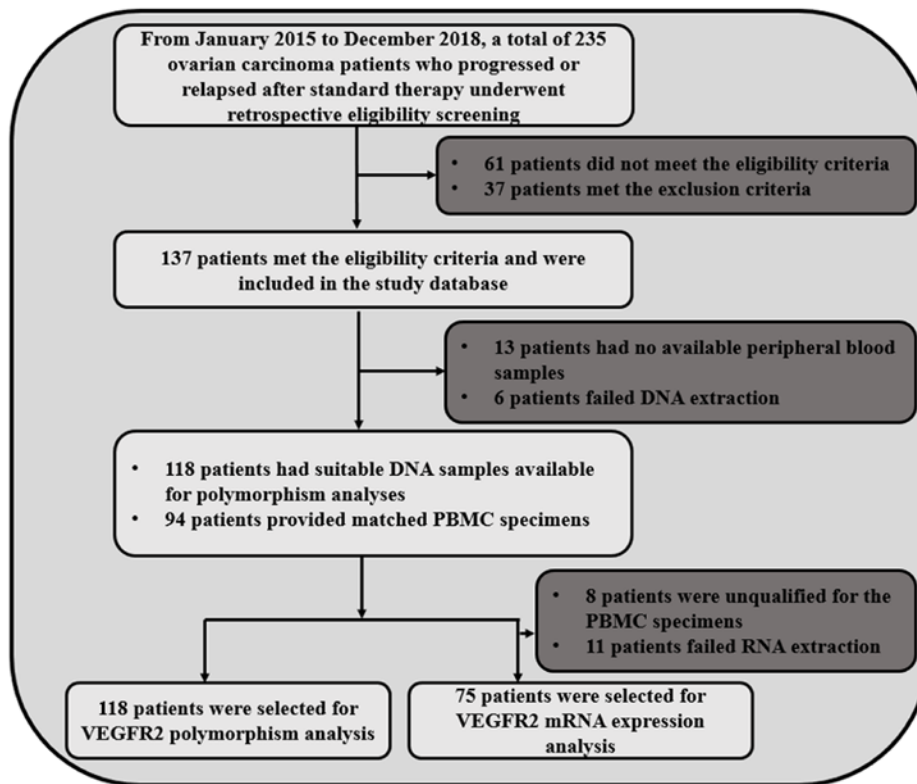
Figure 1. Flow chart of the retrospective study of apatinib monotherapy in the treatment of patients with advanced epithelial ovarian carcinoma who progressed after standard regimens. PBMC, peripheral blood mononuclear cells; VEGFR2, vascular endothelial growth factor receptor 2.

Collection of peripheral blood specimens and genotyping of VEGFR2 polymorphism. Genomic DNA was extracted from whole venous blood collected prior to treatment with apatinib during hospitalization using phenol chloroform methods, according to the standard clinical procedure. Single nucleotide polymorphisms of *VEGFR2*, with the minor allele frequency >10% in the Chinese population, were identified using the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/snp/), and included rs2071559, rs2305948 and rs11941492. As shown in Table I, of the three polymorphisms analyzed in the 118 patients with EOC, only rs2071559 was significantly associated with PFS time. Therefore, the subsequent analysis of this study was focused on the rs2071559 polymorphism. The rs2071559 polymorphism of *VEGFR2* was genotyped through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Sangon Biotech Co., Ltd.). Initially, the PCR product including this polymorphism was amplified, using SYBRGreen (Sangon Biotech Co., Ltd.). The forward primer was 5'-TCACTAGGGCTCTTCGTTGG-3' and the reverse primer was 5'-GAAGCGGATACTCAGCCAAG-3'. PCR primers were synthesized by Sangon Biotech Co., Ltd. PCR reaction conditions were as follows: 94°C for 5 min; 36 cycles at 94°C for 45 sec, 63.5°C for 45 sec and 72°C for 30 sec; 72°C for 10 min. In total, 2 μl PCR products (size, 271 bp) were digested using the restriction enzyme *Bsm*I (Thermo Fisher Scientific Inc.). Agarose gel electrophoresis (1%) was performed on the enzyme digestion products. The genotypes of the polymorphism were determined by the size of PCR bands as follows: TT genotype (one 271-bp band); CC genotype (one 108-bp band and one 163-bp band); and TC genotype (one 271-bp band, one 108-bp band and one 163-bp band).

Collection of peripheral blood mononuclear cell (PBMC) specimens and analysis of VEGFR2 mRNA expression. Initially, the PBMC specimens were collected from 94 randomly matched samples in the 118 patients with EOC. However, 8 PBMC specimens were not available and RNA extraction failed in 11 PBMC specimens. Eventually, a total of 75 PBMC specimens were available for further analysis and preserved in liquid nitrogen. Total RNA samples were extracted using the TRIzol® reagent (Bao Biological Engineering Co. Ltd.) according to the manufacturer's instructions and stored at -80°C for mRNA expression analysis. In total, 500 ng RNA extracted from each PBMC specimen was used as a template for reverse transcription-PCR to prepare the first strand of cDNA using the PrimeScript RT reagent kit (Bao Biological Engineering Co., Ltd.) according to the manufacturer's instructions. Relative quantitative analysis of *VEGFR2* mRNA expression was performed on the Roche LightCycler® 480 using a SYBR Premix EX Taq system (Bao Biological Engineering Co., Ltd.). The forward primer of *VEGFR2* was 5'-ATGCAGAGCAAGGTGCTGC-3' and the reverse primer was 5'-TTAAACAGGAGGAGAGCTCAGTG-3'. The amplification reaction (20 μl) contained 10 μl SYBR Premix EX Taq, 0.2 μl each primer (20 μM), 7.6 μl double distilled water (ddH2O) and 2 μl cDNA. PCR reaction conditions were as follows: 95°C for 5 min; 40 cycles at 95°C for 10 sec; 62°C for 10 sec; and 72°C for 10 sec. The mRNA expression of *VEGFR2* was detected using a comparative Cq method ($2^{-\Delta\Delta Cq}$) (29). The mRNA expression of *GAPDH* was used as an endogenous

Table I. Details of the polymorphisms included in the present study and the preliminary analysis between genotypes status and PFS.

Polymorphism	Primers (5'-3')	Location	MAF	Median PFS time, months	P-value
rs2071559	F: TCACTAGGGCTCTTCGTTGG R: GAAGCGGATACTCAGCCAAG	Upstream region	0.22	5.4 vs. 3.1 (TT vs. TC/CC)	0.015
rs2305948	F: TTCCAAGACCATAGCTTACCA R: AATGTTTACCAAAGCCCAGA	Coding region	0.15	4.7 vs. 4.3 (CC vs. CT/TT)	0.315
rs11941492	F: TTGAGTTCCAATCTCAGCTTCA R: CTGGCCTTGAGAAAATCACC	Intron region	0.28	4.5 vs. 4.8 (CC vs. CT/TT)	0.553

MAF, minor allele frequency; PFS, progression-free survival; F, forward; R, reverse.

control. The forward primer of *GAPDH* was 5'-GCACCGTCA AGGCTGAGAAC-3' and the reverse primer was 5'-TGGTGA AGACGCCAGTGGA-3'.

Statistical analysis. All variables in this study were statistically analyzed using the statistical analysis software SPSS version 25.0 (IBM Corp.). Hardy-Weinberg equilibrium was measured for the rs2071559 genotypes using the χ^2 test. The significance of observed differences in proportions was assessed using the χ^2 test, and Fisher's exact test was performed when the dataset was small. The analysis between continuous variables and genotype status was performed using the Mann-Whitney U non-parametric test (between two groups). The primary endpoint was PFS. The Kaplan-Meier curves were drawn using Stata 14.0 (StataCorp LP) to compare the differences in PFS and OS times according to each genotype status. Survival differences were compared using a log-rank test. PFS was defined as the period from the initial treatment with apatinib up until disease progression or patient death, whichever occurred first. OS was defined as the period from the time of treatment with apatinib to patient death from any cause. For those without disease progression or death by the end of the study follow-up, the survival end points were censored at the date of last follow-up. For the multivariable analysis, a Cox proportional hazards model was constructed for PFS time, and the backward selection procedure was used to adjust for potential confounding covariates. P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics of the 118 patients with EOC and genotypes of VEGFR2 rs2071559 polymorphism. The baseline characteristics of the 118 patients with EOC are shown in Table II. The median age of the patients was 57 years (range, 41-78 years) and the ECOG 0 score was reported in 55 patients. According to FIGO stage criteria, stage III was noted in 92 patients. The most common histology of EOC was serous, diagnosed in 87 patients. Regarding the first-line platinum-based chemotherapy response, 34 patients were identified with platinum-refractory disease, 49 patients with platinum-resistant disease and 35 patients were platinum-sensitive. In terms of the tumor differentiation, the majority of patients (58 in total) were found to present with

poorly differentiated tumors. Two lines of previous treatment were reported in 21 patients and ≥3 lines of previous treatment were confirmed in 97 patients. Apatinib was administered at an initial dosage of 500 and 750 mg in 86 and 32 patients, respectively. However, a dosage reduction had to be implemented in a total of 48 patients due to hematological or non-hematological toxicities.

Of the *VEGFR2* polymorphisms analyzed, only rs2071559 was of clinical significance (Table I). The germline mutation frequency of rs2071559 among the 118 patients with EOC were as follows: TT genotype, 72 cases (61.02%); TC genotype, 41 cases (34.75%); CC genotype, 5 cases (4.23%), and the minor allele frequency of rs2071559 was 0.22. The distribution of the three genotypes was in accordance with the Hardy-Weinberg equilibrium (P=0.781). TC and CC genotypes were merged in the subsequent analysis. As shown in Table II, patients with TT and TC/CC genotypes were well balanced, presenting similar baseline characteristics. No significant differences were observed between the two groups.

Influence of VEGFR2 rs2071559 polymorphism on the clinical outcomes of the 118 patients with EOC. All 118 patients with EOC included in this study were subjected to efficacy evaluation during the treatment with apatinib, where the best overall response of each patient was recorded. A complete response was not observed in any of the patients, whereas a partial response was identified in 46 patients, stable disease in 29 patients and progression disease in 43 patients. According to RECIST 1.1, the ORR was 38.98% and disease control rate (DCR) was 63.56%. The waterfall plot representing the best percentage change in the dimensions of the target lesion is shown in Fig. 2.

The last follow-up time of this study was June 2019. The median follow-up time of all patients from the time of enrollment to the last follow-up was 14.50 months (range, 1-36 months). The median PFS time of the 118 patients with EOC included in this study was 4.65 months [95% confidence interval (CI), 3.45-5.80]. Additionally, the median PFS time according to different baseline characteristic was further analyzed (Fig. 3). Univariate analysis according to baseline characteristics suggested that ECOG score (P=0.015), FIGO stage (P=0.008) and tumor differentiation (P=0.019) were significantly associated with PFS time. In addition, the results indicated that the median PFS time of patients with an ECOG score of 0 was significantly longer compared with that of

Table II. Baseline characteristics of the 118 patients with EOC according to TT (n=72) or TC/CC (n=46) vascular endothelial growth factor receptor 2 rs2071559 polymorphism status.

| Characteristics | Value | 4397T>C genotypes | | P-value |
		TT	TC/CC	
Median age (range), years	57 (41-81)	57 (41-79)	58 (44-81)	0.632
ECOG score, n (%)				0.832
0	55 (46.61)	33 (45.83)	22 (47.83)	
1-2	63 (53.39)	39 (54.17)	24 (52.17)	
FIGO stage, n (%)				0.951
III	92 (77.97)	56 (77.78)	36 (78.26)	
IV	26 (22.03)	16 (22.22)	10 (21.74)	
Histology of EOC, n (%)				0.971
Serous	87 (73.73)	53 (73.61)	34 (73.91)	
Mixed	31 (26.27)	19 (26.39)	12 (26.09)	
First-line platinum response, n (%)				0.793
Platinum-refractory	34 (28.81)	20 (27.78)	14 (30.43)	
Platinum-resistant, <6 months	49 (41.53)	29 (40.28)	20 (43.48)	
Platinum-sensitive, ≥6 months	35 (29.66)	23 (31.94)	12 (26.09)	
Tumor differentiation, n (%)				0.949
Well differentiated	19 (16.10)	11 (15.28)	8 (17.39)	
Intermediately differentiated	41 (34.75)	25 (34.72)	16 (34.78)	
Poorly differentiated	58 (49.15)	36 (50.00)	22 (47.83)	
Lines of previous treatment regimens, n (%)				0.927
2	21 (17.80)	13 (18.06)	8 (17.39)	
≥3	97 (82.20)	59 (81.94)	38 (82.61)	
Initial dosage of apatinib in mg, n (%)				0.517
500	86 (72.88)	54 (75.00)	32 (69.57)	
750	32 (27.12)	18 (25.00)	14 (30.43)	
Dose of reduction, n (%)				0.154
Yes	48 (40.68)	33 (45.83)	15 (32.61)	
No	70 (59.32)	39 (54.17)	31 (67.39)	

ECOG, Eastern Cooperative Oncology Group; EOC, epithelial ovarian carcinoma; FIGO, International Federation of Gynecology and Obstetrics.

patients with a score of 1-2 (5.55 vs. 3.90 months), the median PFS time of patients with FIGO stage III was significantly higher compared with that of patients with stage IV (5.40 vs. 3.25 months) and the median PFS time of patients with well and intermediate differentiation was significantly superior compared with that of patients with poor differentiation (5.25 and 4.70 vs. 3.95 months).

The analysis of the effect of the *VEGFR2* rs2071559 polymorphism in terms of the PFS, showed that the median PFS time between patients with TT and TC/CC genotypes was significantly different, being 5.40 and 3.10 months, respectively (χ^2=6.50) (Fig. 4). Furthermore, a Cox regression model was constructed. Results of the multivariate analysis are shown in Table III. After adjustment for other confounding factors, a significant difference was observed for the influence of TC/CC genotype of rs2071559 on PFS time. Results indicated that the rs2071559 polymorphism was an independent factor for PFS time [hazard ratio (HR), 1.69; P=0.021]. Additionally, ECOG

high score (HR, 1.41; P=0.018), FIGO IV stage (HR, 1.81; P=0.011) and tumor poor differentiation (HR, 1.28; P=0.046) were also independent factors for PFS.

The evaluation of OS in the present study showed that the median OS time of the 118 patients with EOC was 15.10 months (95% CI, 13.10-17.25). Regarding the effect of the *VEGFR2* rs2071559 polymorphism on OS, the results showed that the median OS time between patients with TT and TC/CC genotypes was significantly different, with an OS of time 12.60 and 17.50 months, respectively (χ^2=4.50) (Fig. 5).

Association between VEGFR2 rs2071559 polymorphism and the safety of patients with EOC. Adverse reactions classified as grade ≥2 with an incidence ≥10% were analyzed. The most common treatment-related adverse reactions were hand-foot syndrome, hypertension, nausea/vomiting, proteinuria, fatigue and diarrhea, with an incidence of 40.68, 25.42, 18.64, 13.56, 11.86 and 10.17%, respectively (Table IV). The
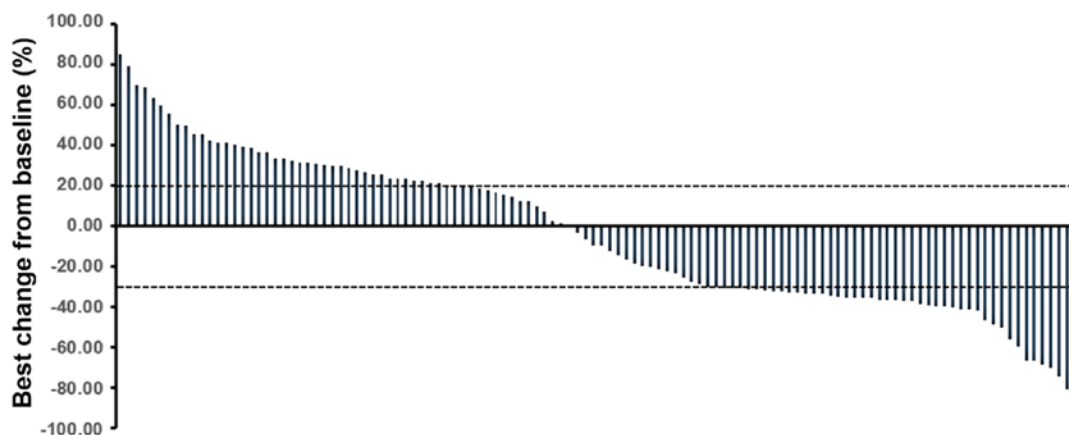
Figure 2. Waterfall plot of best change (reductions in sum of lesion diameters) from baseline (before the start of apatinib treatment) in the 118 patients with epithelial ovarian carcinoma receiving apatinib monotherapy.

Characteristics	N		mPFS (Months)	95% CI		P-value
				Lower	Upper	
Total	118		4.65	3.45	5.80	
Age (years)						0.531
>57	54		4.55	3.30	5.65	
≤57	64		4.73	3.51	5.83	
ECOG						0.015
0	55		5.55	4.85	6.20	
1-2	63		3.90	3.10	4.95	
FIGO stage						0.008
III	92		5.40	4.65	6.15	
IV	26		3.25	2.65	3.75	
Histology of EOC						0.611
Serous	87		4.78	3.68	5.70	
Mixed	31		4.45	3.55	5.30	
First line platinum response						0.323
Platinum refractory	34		4.41	3.52	5.40	
Platinum resistant (<6 months)	49		4.70	3.25	6.10	
Platinum sensitive (≥6 months)	35		4.75	3.50	5.89	
Tumor differentiation						0.019
Well differentiated	19		5.25	4.10	6.05	
Intermediate differentiated	41		4.70	3.50	5.75	
Poorly differentiated	58		3.95	3.20	4.75	
Lines of previous treatment regimens						0.655
2	21		4.80	3.55	6.05	
≥3	97		4.50	3.50	5.85	
Initial dosage of apatinib						0.532
500 mg	86		4.65	3.30	5.75	
750 mg	32		4.65	3.35	5.90	

Figure 3. Forest plot of mPFS of the 118 patients with EOC score data according to different baseline characteristics subgroups. CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EOC, epithelial ovarian carcinoma; FIGO, International Federation of Gynecology and Obstetrics; mPFS, median progression-free survival; N, number of patients.

evaluation of the effect of the rs2071559 polymorphism on the incidence of adverse events grade ≥2 showed no significant difference between the patients with the TT and TC/CC genotypes.

Association between rs2071559 polymorphism and the mRNA expression of VEGFR2. Across the 75 specimens included, the prevalence of the *VEGFR2* rs2071559 polymorphism

was as follows: TT genotype, 46 patients (61.33%); TC genotype, 26 patients (34.67%); and CC genotype, 3 cases (4.00%). The prevalence was comparable with the genotype frequency observed among the 118 patients with EOC. The distribution of the three genotypes was in accordance with the Hardy-Weinberg equilibrium (P=0.776). Similarly, CC and TC genotypes were merged in the subsequent analysis, and patients with the TC/CC genotype showed a significantly

Table III. Multivariate Cox regression analysis of progression-free survival according to baseline characteristics and vascular endothelial growth factor receptor 2 rs2071559 polymorphism.

Characteristics	HR (95% CI)	df	P-value
ECOG score		1	0.018
0	1.00[a]		
1-2	1.41 (1.21-1.68)		
FIGO stage			
III	1.00[a]		
IV	1.81 (1.38-2.36)	1	0.011
Tumor differentiation		1	0.046
Well and intermediately differentiated	1.00[a]		
Poorly differentiated	1.28 (1.02-1.51)		
rs2071559 genotype		1	0.021
TT genotype	1.00[a]		
TC/CC genotype	1.69 (1.21-1.95)		

[a]Reference. ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; CI, confidence interval; df, degrees of freedom; FIGO, International Federation of Gynecology and Obstetrics.
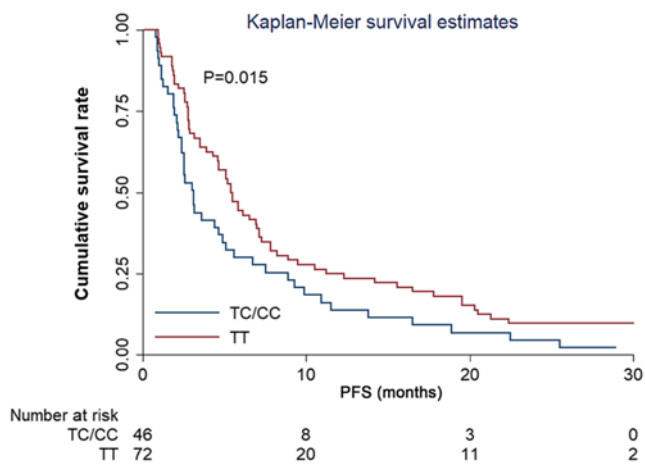

Figure 4. PFS of the 118 patients with epithelial ovarian carcinoma according to the vascular endothelial growth factor receptor 2 rs2071559 genotype status. PFS, progression-free survival.
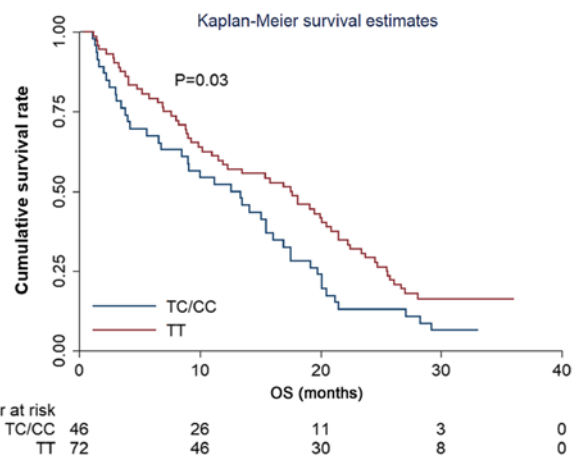

Figure 5. OS of the 118 patients with EOC according to the vascular endothelial growth factor receptor 2 rs2071559 genotype status. OS, overall survival.

higher relative expression of *VEGFR2* mRNA in PBMC specimens compared with those with the TT genotype (4.06±0.331 vs. 2.90±0.480; Fig. 6).

Discussion

The present retrospective study provides evidence regarding the clinical outcomes and safety of apatinib in the treatment for patients with EOC who progressed after standard regimens. Polymorphism analysis indicated that patients with the TC/CC genotype of the rs2071559 polymorphism were associated with worse PFS and OS times. Furthermore, the results suggested that the mRNA expression levels of *VEGFR2* were significantly different according to rs2071559 genotype status. Therefore, the clinical outcomes of patients with EOC receiving apatinib therapy may be influenced by the *VEGFR2*

rs2071559 polymorphism through the mediation of *VEGFR2* mRNA expression.

As an anti-angiogenic TKI, apatinib has been demonstrated to be effective in gastric cancer, colorectal cancer, lung cancer, soft tissue sarcoma and gynecological tumors (30-33). In the present study, the clinical outcomes of 118 patients with EOC receiving apatinib treatment were evaluated. The ORR was 38.98%, the DCR was 63.56% and the median PFS time was 4.65 months, all values that were lower than those reported in the phase II clinical trial of apatinib in recurrent EOC by Miao *et al* (21), where ORR was 41.4%, DCR was 68.9% and median PFS time was 5.10 months. It was hypothesized that the reason for these discrepancies maybe attributed to the retrospective design of the present study, with insufficient patient management compared with clinical trials, which was also observed in the other retrospective study, and the clinical outcomes in this retrospective study were inferior to that in

Table IV. Analysis between vascular endothelial growth factor receptor 2 rs2071559 genotype status and adverse reactions classified as grade ≥2.

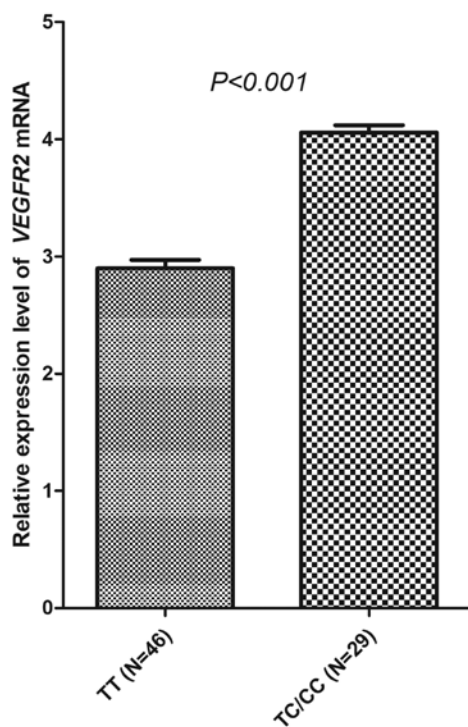| | | rs2071559 genotype status, n (%) | | |
Adverse reactions	Total, n (%)	TT (n=72)	TC/CC (n=46)	P-value
Hand-foot syndrome	48 (40.68)	30 (41.67)	18 (39.13)	0.784
Hypertension	30 (25.42)	19 (26.39)	11 (23.91)	0.763
Nausea/vomiting	22 (18.64)	14 (19.44)	8 (17.39)	0.780
Proteinuria	16 (13.56)	10 (13.89)	6 (13.04)	0.896
Fatigue	14 (11.86)	9 (12.50)	5 (10.87)	0.789
Diarrhea	12 (10.17)	8 (11.11)	4 (8.70)	0.672

Figure 6. Relative mRNA expression levels of *VEGFR2* in 75 peripheral blood mononuclear cells specimens according to *VEGFR2* rs2071559 genotype status. *VEGFR2*, vascular endothelial growth factor receptor 2.

clinical trials (34). Furthermore, patients with an ECOG score of 2 were included in the present study, in contrast to the phase II clinical trial, where these patients were excluded. However, the clinical significance of ECOG score has been confirmed in previous studies, suggesting that the higher the score, the worse the prognosis (35,36). In the present study, the results of Cox's regression analysis indicated that patients with a score of 1-2 were associated with a worse prognosis. Notably, the present median OS time was 15.10 months, which was slightly longer compared with that reported in the phase II clinical trial with a median OS time of 14.50 months (21). It is possible that the reason for this difference may be the licensing of the PARP inhibitor olaparib in 2018. Patients with EOC in China had the opportunity to be treated with olaparib in the subsequent lines of treatment, which was effective and offered an extended survival benefit for the patients (37).

Although apatinib was licensed in China ~5 years ago, only a few studies have investigated potential predictive biomarkers for the treatment with apatinib. Results from a previous study suggested that the occurrence of proteinuria or hand-foot syndrome during treatment with apatinib could predict superior clinical outcomes (38,39). However, polymorphism studies that could indicate a potential benefit for the use of apatinib are still limited Regarding EOC in particular, one study investigated the effect of polymorphisms in the susceptibility of EOC (40). Previous studies have also indicated that germline polymorphisms may play a role in the development of ovarian cancer (41,42). To the best of our knowledge, the present study was the first to research the effect of the presence of a polymorphism in Chinese patients with EOC receiving apatinib treatment. The result of polymorphism analysis was partly consistent with that of a previous study by Scartozzi *et al* (43). In the aforementioned study, a total of 148 patients with advanced hepatocellular cancer treated with sorafenib were enrolled and analyzed. Although, the prevalence of the rs2071559 polymorphism was greater compared with that in the present study (0.49 vs. 0.22), the clinical outcomes showed that the prognosis of patients with the TC/CC genotype were worse, which was consistent with the findings of the present study. Additionally, the results of a recent study by Sullivan *et al* (44) were also in line with those of the present study. In the aforementioned study, patients with advanced non-small cell lung cancer (NSCLC) treated with platinum-based chemotherapy were enrolled, and the polymorphisms of *VEGF-A*, *VEGFR1* and *VEGFR2* were investigated. The results of multivariate analysis suggested that *VEGF-A* rs2010963 and *VEGFR2* rs2071559 were significantly associated with prognosis. Specifically, patients with the TT genotype of rs2071559 were associated with an improved prognosis, which was consistent with the results of the present study, where the TT genotype was associated with improved PFS and OS times. A recent phase III clinical trial that investigated the effect of adjuvant sunitinib in patients with high-risk renal cell carcinoma (45) suggested that the median disease-free survival time was longer in patients with the TT genotype, which is consistent with the effects observed for the TT genotype in the present study. In conclusion, the *VEGFR2* rs2071559 polymorphism might be of clinical significance as a biomarker for the prediction of patients with EOC who

might show an improved response towards apatinib treatment.

With regard to the safety analysis, results from a previous study indicated that hypertension is usually the most common adverse reaction in patients with cancer receiving apatinib treatment (46). However, the present study reported that hand-foot syndrome was the most common adverse reaction, with an incidence >40%, which was consistent with the results observed in patients with EOC who received sorafenib therapy (47). It hypothesized that this effect might be attributed to a difference in sex. A previous study suggested that the incidence of hand-foot syndrome and other overall toxicity in women was higher than that in men (48). Additionally, the other adverse reactions classified as grade ≥2 with an incidence ≥10% included hypertension, nausea/vomiting, proteinuria, fatigue and diarrhea, which were similar to the adverse reactions observed in another previous study for apatinib treatment (49). In the present study, the association analysis between genotype status and adverse reactions failed to show any significant difference, which suggested that the polymorphism was not associated with apatinib disposition for specific adverse reactions.

Notably, mRNA expression analysis indicated that the *VEGFR2* mRNA expression levels were significantly different according to rs2071559 polymorphism status. VEGFR2 is the receptor with the strongest binding affinity to VEGF-A and has an important role in signal transmission. In addition, the expression levels of *VEGFR2* show a crucial role in the process of angiogenesis (50). Moreover, previous studies also suggested that higher expression levels of *VEGFR2* in tumor cells were associated with a higher likelihood for tumor cells to relapse and metastasize in gastric cancer and epithelial cancer (51,52). In addition, higher expression levels of *VEGFR2* have been associated with worse PFS and OS times in patients with NSCLC (53) and cervical cancer (54), which is consistent with the preliminary results of the present study.

There are a number of limitations to the present study. Firstly, the sample size was small. Secondly, the study was designed as a retrospective analysis, so some bias (no control group and randomization) might be unavoidable. Thirdly, the present study did not perform cell- and animal-based experiments. Lastly, the results of the present study need to be validated in clinical trials. However, the prognostic value of the *VEGFR2* rs2071559 polymorphism was fully evaluated, and the determination of *VEGFR2* mRNA expression levels might indicate the polymorphism and prognosis of patients with EOC. Therefore, the present study was of clinical significance for the evaluation of prognosis in patients with EOC who received apatinib treatment.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZY, YYG, WD and YG conceived and designed the study. ZY, XDH, QZ and MW collected the characteristics of the patients and performed the experiments. ZY and HLK analyzed the data. YYG, MW and YG contributed with reagents and analysis tools. ZY, YYG, WD and YG wrote the paper. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work was appropriately investigated and resolved.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University (Beijing, China) (approval no. 2017-KY-01105). Written informed consent was obtained from all patients or their family members. Only in circumstances where the patients had passed away or the patient was unaware of their diagnosis of EOC, was written informed consent obtained from their relatives.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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