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BRCA1/2 mutations associated with progression-free survival in ovarian cancer patients in the AGO-OVAR 16 study☆,☆☆

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HIGHLIGHTS

- To identify genetic markers associated with prognosis in ovarian cancer.
- Longer progression-free survival was observed in patients with *BRCA1/2* mutations.
- Genetic counseling plays an important role and should be offered to all patients.

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ABSTRACT

Objective. AGO-OVAR 16 demonstrated that pazopanib maintenance therapy significantly increased progression-free survival (PFS) in patients with ovarian cancer whose disease had not progressed after first-line therapy. In a sub-study, we evaluated the effect of clinically important germline *BRCA1* and *BRCA2* mutations on PFS.

Methods. Of 940 AGO-OVAR 16 participants, 664 had *BRCA1/2* exon sequencing data (pazopanib, $n = 335$; placebo, $n = 329$). A Cox model was used to test the association between genetic variants and PFS.

Results. Ninety-seven of 664 patients (15%) carried clinically important *BRCA1/2* mutations (*BRCA1/2* carriers: pazopanib 14%, placebo 16%). Median PFS was longer in *BRCA1/2* mutation carriers than in *BRCA1/2* non-carriers in the placebo arm (30.3 vs 14.1 months, hazard ratio, 0.48; 95% confidence interval [CI]: 0.29–0.78; $P = 0.0031$);

☆ Previous publication: *BRCA1/2* association results were presented in part at IGCSM 2014 (Harter P, et al. Abstract 0971). GWAS results from a combined dataset that included AGO-OVAR 16 were presented at ASCO 2014 (Scambia G, et al. Abstract 5574). Data from East Asian ancestry patients from AGO-OVAR 16 are included in a manuscript (in preparation) summarizing the integrated analysis result with a separate East Asian study.

☆☆ Clinical Trial Registration: NCT00866697 https://www.clinicaltrials.gov/ct2/show/NCT00866697.

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Germline *BRCA* mutation
Pazopanib

a similar non-significant trend was noted with pazopanib (30.2 vs 17.7 months, hazard ratio, 0.64; 95% CI: 0.40–1.03; $P = 0.069$). Among *BRCA1/2* non-carriers, PFS was longer for pazopanib-treated patients than placebo-treated patients (17.7 vs 14.1 months, hazard ratio, 0.77; 95% CI: 0.62–0.97; $P = 0.024$). Among *BRCA1/2* carriers, there was no significant PFS difference between treatments, although numbers were small (pazopanib, 46; placebo, 51), resulting in a wide CI (hazard ratio, 1.36; 95% CI: 0.66–2.82).

Conclusions. Patients with clinically important *BRCA1/2* mutations had better prognosis. *BRCA1/2* mutation status might be added as strata in future trials in primary ovarian cancer.

1. Introduction

Ovarian cancer is the fifth most common cause of cancer deaths in women [1]. The standard treatment is debulking surgery and taxane-platinum chemotherapy [2]. However, most patients will relapse after initial response to these treatments and subsequently die from their disease [3]. Therefore, new treatment agents as maintenance therapy to delay disease progression are being developed [4–6]. Pazopanib is an oral tyrosine kinase inhibitor of VEGF receptors-1/−2/−3, platelet-derived growth factor receptors-α/−β, and c-KIT [7]. In a phase III study (AGO-OVAR 16) to evaluate the efficacy and safety of pazopanib versus placebo in women with non-bulky, International Federation of Gynecology and Obstetrics (FIGO) stages II–IV epithelial ovarian, fallopian tube, or primary peritoneal cancer whose disease had not progressed after first- line chemotherapy, pazopanib maintenance therapy significantly increased progression-free survival (PFS) compared with placebo [8].

Substantial heterogeneity exists among patients with ovarian cancer in prognosis and in response to both chemotherapies and targeted therapies [9–11]. Biomarkers that are prognostic or predictive of clinical benefit would facilitate evidence-based selection of particular agents or dosages for optimal treatment of individual patients. Specific *BRCA1* and *BRCA2* (*BRCA1/2*) mutations are a well-established risk factor and may represent a prognostic factor indicating better outcome [12,13]. The latter may depend on a higher sensitivity to platinum-based chemotherapy, leading to improved disease-free intervals and overall survival [9,14].

Our previous exploratory pharmacogenetic studies in pazopanib-treated patients with advanced renal cell carcinoma suggested that germline genetic variants may be associated with efficacy or safety endpoints [15,16]. This pharmacogenetic sub-study in AGO-OVAR 16 tested the effect of clinically important germline *BRCA1/2* mutations on PFS as well as genetic associations with pazopanib efficacy and safety in a genome-wide association study (GWAS).

2. Patients and methods

2.1. Patients

The pharmacogenetic analysis used data from participants in clinical trial AGO-OVAR 16 (NCT00866697). Patient characteristics have been described previously [8]. Briefly, AGO-OVAR 16 is an international, randomized, double-blind, placebo-controlled, pivotal phase III trial to evaluate the efficacy and safety of pazopanib maintenance therapy in patients without disease progression after first-line chemotherapy for advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer [8]. Of 940 participants, 664 had *BRCA1/2* exon sequencing data (pazopanib, $n = 335$; placebo, $n = 329$), and 334 pazopanib-arm patients had GWAS data. The clinical study was conducted in accordance with the Declaration of Helsinki; protocols and informed consent forms were reviewed and approved by Institutional Review Boards and Independent Ethics Committees according to local guidelines. Patients who were included in this pharmacogenetic analysis provided additional consent and a blood sample for genetic research.

2.2. Genetic markers and genotyping

Venous blood was collected into an EDTA Vacutainer for each patient who consented for genetic research. Germline DNA was extracted from peripheral blood using the QiAmp DNA Blood Kit (Qiagen, Valencia, CA) at Covance (Indianapolis, IN; Geneva, Switzerland; or Shanghai, China). Germline mutations in *BRCA1* and *BRCA2* were genotyped using targeted exome sequencing by Beijing Genomics Institute for patients in China (library was prepared by exon capture using NimbleGen SeqCap EZ Choice and sequencing was performed using an Illumina HiSeq2500), and by Ambry Genetics (Aliso Viejo, CA) for non-China patients (library was generated by PCR amplification using the Fluidigm Access Array and sequencing was conducted using an Illumina MiSeq). *BRCA1* and *BRCA2* sequence reads were aligned to the human reference, build GRCh37, using BWA v0.7.4, and variants were called using GATK v2.39. Mutations in *BRCA1* and *BRCA2* were annotated as clinically important if they were described as such in the Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/), accessed December 17, 2013.

Genome-wide single nucleotide polymorphism (SNP) genotyping was performed using the HumanOmniExpressExome beadchip (Illumina, San Diego, CA) by Expression Analysis (Durham, NC) and ShanghaiBio Corporation (Shanghai, China), which successfully genotyped ~700,000 SNPs. Genotype imputation [17] using a reference panel of haplotypes from the 1000 Genomes Project [18] was conducted to generate a set of ~6.5 million common genetic variants with minor allele frequency (MAF) of ≥5% for GWAS.

2.3. Statistical analysis

This pharmacogenetic analysis tested germline genetic variants for association with efficacy and safety endpoints. The efficacy endpoint evaluated was PFS, which was evaluated using Kaplan–Meier estimates of the survival function, and was tested for association using a Cox proportional hazards model. Blood pressure is both a potential pharmacodynamic biomarker [19] and a safety endpoint; mean arterial pressure (mean over weeks 1–4 as change from baseline) was transformed to normality and tested using a linear model. All other safety endpoints were derived using the maximum on-treatment value for each patient. Continuous endpoints (serum alanine transaminase, serum total bilirubin, neutropenia as measured by neutrophil count, and thrombocytopenia as measured by platelet count) were transformed to normality and tested using a linear regression. Ordinal endpoints (hand-foot syndrome, diarrhea, and fatigue, graded according to Common Terminology Criteria for Adverse Events v4) were tested using ordinal regression.

The analyses of *BRCA1* and *BRCA2* sequencing data were focused on the association of clinically important mutations with PFS in both pazopanib- and placebo-treated patients. Limited pre-planned analyses considered *BRCA1/2* as part of a panel of ~80 candidate variants, with commensurate multiple testing correction. However, given the strong association observed between *BRCA1/2* mutations and survival outcomes in ovarian cancer patients receiving platinum-based therapy [9,14], as well as a specific question from a regulatory agency about the potential effect of *BRCA1/2* mutation on pazopanib efficacy, we conducted the more extensive post-hoc analyses reported here.

GWAS evaluated both PFS and safety endpoints in pazopanib-treated patients. Statistical analyses were conducted using data from patients of all race/ancestral groups, as well as for the subgroup of European ancestry. All GWAS analyses were adjusted for age and for ancestry principal components to correct for confounding by population structure [20]. GWAS analyses tested all variants with MAF of ≥5% and imputation quality of ≥0.3, assuming an additive genetic model, and the conventional $P \le 5 \times 10^{-8}$ threshold for genome-wide significance was used after genomic control [21] was applied.

3. Results

3.1. Association of clinically important mutations in BRCA1/2 with PFS in AGO-OVAR 16

The *BRCA1/2* pharmacogenetic analysis population consists of 71% of overall enrolled patients in AGO-OVAR 16 (664/940), with 71% (335/472) in the pazopanib arm and 70% (329/468) in the placebo arm (Supplementary Table S1). There was no statistically significant difference in PFS between patients included in the *BRCA1/2* analysis versus those who were not ($P = 0.31$, Supplementary Fig. S1).

Of 664 patients with *BRCA1/2* genotyping data, 97 (15%) carried a clinically important germline mutation in either the *BRCA1* gene (*BRCA1*+; $n = 68$, 10%) or the *BRCA2* gene (*BRCA2*+; $n = 29$, 4%; Supplementary Table S2). No patients were both *BRCA1*+ and *BRCA2*+; hereafter, patients who were either *BRCA1*+ or *BRCA2*+ are termed "*BRCA1/2* carriers". No clinically important mutations were detected for the remaining patients in the *BRCA1* or *BRCA2* exons sequenced ($n = 567$, 85%; hereafter "*BRCA1/2* non-carriers"). Table 1 lists specific baseline clinical characteristics by *BRCA1/2* status. As expected, *BRCA1/2* carriers were on average younger than *BRCA1/2* non-carriers (median age, 52 vs 57 years; $P = 0.0047$).

Overall, there was no imbalance between pazopanib and placebo treatment arms in the proportion of carriers of clinically important mutations in *BRCA1*, *BRCA2*, or both (Table 2). In univariate analyses, longer PFS was observed in *BRCA1/2* carriers than in *BRCA1/2* non-carriers within the placebo arm (median PFS: 30.3 vs 14.1 months, hazard ratio [HR], 0.48; 95% confidence interval [CI]: 0.29–0.78, $P = 0.0031$; Fig. 1A). A similar but non-significant trend was observed in the pazopanib arm (median PFS for *BRCA1/2* carriers vs non-carriers: 30.2 vs 17.7 months, HR, 0.64; 95% CI: 0.40–1.03; $P = 0.069$; Fig. 1A). The number of *BRCA2*+ patients (placebo, $n = 15$; pazopanib, $n = 14$) was too small to make meaningful comparisons between *BRCA2*+ and *BRCA1*+ patients (Table 2). These results were essentially unchanged in multivariate sensitivity analyses that adjusted for first-line treatment outcome and recruitment region (Table 3).

The efficacy of pazopanib versus placebo in *BRCA1/2* carrier and *BRCA1/2* non-carrier subgroups was estimated using a Cox proportional hazards model, stratified by recruitment region and first-line treatment outcome. In the *BRCA1/2* non-carrier subgroup, median PFS was 17.7 months (95% CI: 13.2–20.9) for pazopanib and 14.1 months (95% CI: 11.7–17.7) for placebo (HR, 0.77; 95% CI: 0.62–0.97; $P = 0.024$; Fig. 1A). In the *BRCA1/2* carrier subgroup, the HR point estimate was 1.36; however, the 95% CI was wide and overlapped the HR CI for the *BRCA1/2* non-carrier subgroup (Fig. 1A). Similar results were obtained using the Pike estimator for the HR (Fig. 2), as used in the primary clinical analyses [8]. Overall, the PFS benefit for pazopanib versus placebo was not significantly different between *BRCA1/2* non-carrier and carrier subgroups (no statistically significant interaction on a log-HR scale between treatment and *BRCA1/2* status, $P = 0.38$).

3.2. Association of clinically important mutations in BRCA1/2 with PFS in ancestry subgroups

The effect of clinically important *BRCA1/2* mutations on PFS was also evaluated in separate ancestry groups. The European ancestry subgroup consisted of 505 patients with self-declared White/Caucasian/European heritage, and the East or South East (E/SE) Asian ancestry subgroup consisted of 151 with self-declared East Asian, Japanese, or South East Asian heritage. Eight patients with *BRCA1/2* sequencing data were not included in the ancestry subgroup analysis (two African American/African, one of whom was a *BRCA1/2* carrier; two American Indian or native Alaskan; one Central/South Asian; three Arabic/North African). The frequency of *BRCA1/2* carriers was not significantly different ($P = 0.19$) between patients of E/SE Asian ancestry (17/151, 11%) and patients of European ancestry (79/505, 16%). No imbalance in the frequency of *BRCA1/2* carriers was seen between the two treatment arms in patients of European ancestry (pazopanib, 16%; placebo, 15%). However, there was a higher proportion of *BRCA1/2* carriers in the placebo arm (16%) than the pazopanib arm (6%) in the E/SE Asian subgroup, reflecting a chance imbalance due to the small number of patients randomized. Preliminary results from the combined analysis of pazopanib efficacy and

Table 1
Selected baseline characteristics by *BRCA1/2* mutation status.

	BRCA1/2 non-carrier			*BRCA1/2* carrier		
	Pazopanib ($n = 289$)	Placebo ($n = 278$)	Overall ($n = 567$)	Pazopanib ($n = 46$)	Placebo ($n = 51$)	Overall ($n = 97$)
Age, median years (range)	56 (25–80)	58 (23–84)	57 (23–84)	54 (37–75)	52 (26–77)	52 (26–77)
ECOG performance status, n (%)						
0	218 (75)	205 (74)	423 (75)	30 (65)	41 (80)	71 (73)
1	70 (24)	71 (26)	141 (25)	16 (35)	9 (18)	25 (26)
2	1 (<1)	2 (1)	3 (1)	0	1 (2)	1 (1)
Disease stage, n (%)						
II	22 (8)	24 (9)	46 (8)	5 (11)	3 (6)	8 (8)
III	217 (75)	201 (72)	418 (74)	30 (65)	41 (80)	71 (73)
IV	50 (17)	53 (19)	103 (18)	11 (24)	7 (14)	18 (19)
Tumor histology, n (%)						
Clear cell	11 (4)	9 (3)	20 (4)	1 (2)	1 (2)	2 (2)
Mucinous	12 (4)	10 (4)	22 (4)	2 (4)	2 (4)	4 (4)
Serous	204 (71)	208 (75)	412 (73)	37 (80)	39 (76)	76 (78)
Endometrioid	19 (7)	12 (4)	31 (5)	2 (4)	2 (4)	4 (4)
Undifferentiated carcinoma	11 (4)	7 (3)	18 (3)	1 (2)	1 (2)	2 (2)
Undifferentiated adenocarcinoma	18 (6)	17 (6)	35 (6)	1 (2)	4 (8)	5 (5)
Other	10 (3)	10 (4)	20 (4)	2 (4)	1 (2)	3 (3)
Unknown	4 (1)	5 (2)	9 (2)	0	1 (2)	1 (1)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2
BRCA1/2 mutation status by treatment and ancestry.

	Overall population		European population		East/South East Asian population	
	Pazopanib ($n = 335$)	Placebo ($n = 329$)	Pazopanib ($n = 252$)	Placebo ($n = 253$)	Pazopanib ($n = 78$)	Placebo ($n = 73$)
BRCA1/2−, *n*	289	278	211	215	73	61
BRCA1+, *n*	32	36	30	27	2	9
BRCA2+, *n*	14	15	11	11	3	3
BRCA1/2+, *n* (%)	46 (14)	51 (16)	41 (16)	38 (15)	5 (6)	12 (16)
Nominal *P* for imbalance[a]	0.58		0.71		0.071	

[a] The "nominal *P*" is simply an indication of how unlikely the imbalance is to occur, not a test of the associated null hypothesis, because no true association between genotype and randomization status, under infinite repeated sampling, is true by definition of randomization.

safety in East Asian patients from the AGO-OVAR 16 study and a pazopanib maintenance study for ovarian cancer in East Asians (NCT01227928) have been presented [22]; final analyses including pharmacogenetic data will be reported separately. In European patients, the efficacy of pazopanib versus placebo in *BRCA1/2* carrier and non-carrier subgroups was similar to the overall population. A longer PFS was observed in the pazopanib group (median PFS: 16.6 months; 95% CI: 12.3–21.4) compared with the placebo group (median PFS: 11.9 months; 95% CI: 10.2–15.8) in *BRCA1/2* non-carriers (HR, 0.68; 95% CI: 0.53–0.88; Fig. 1B). In *BRCA1/2* carriers of European ancestry, median PFS was 30.2 months (95% CI: 22.3–not reached) for pazopanib and 30.3 months (95% CI: not reached) for placebo; this analysis is limited by small sample size such that the 95% CI for HR was wide and overlapped 1.

3.3. Genome-wide association study of pazopanib efficacy and safety

GWAS was conducted using data from pazopanib-treated patients with genome-wide genotyping data ($n = 334$); 6,533,949 common genetic variants (MAF ≥ 5%) were analyzed. At the

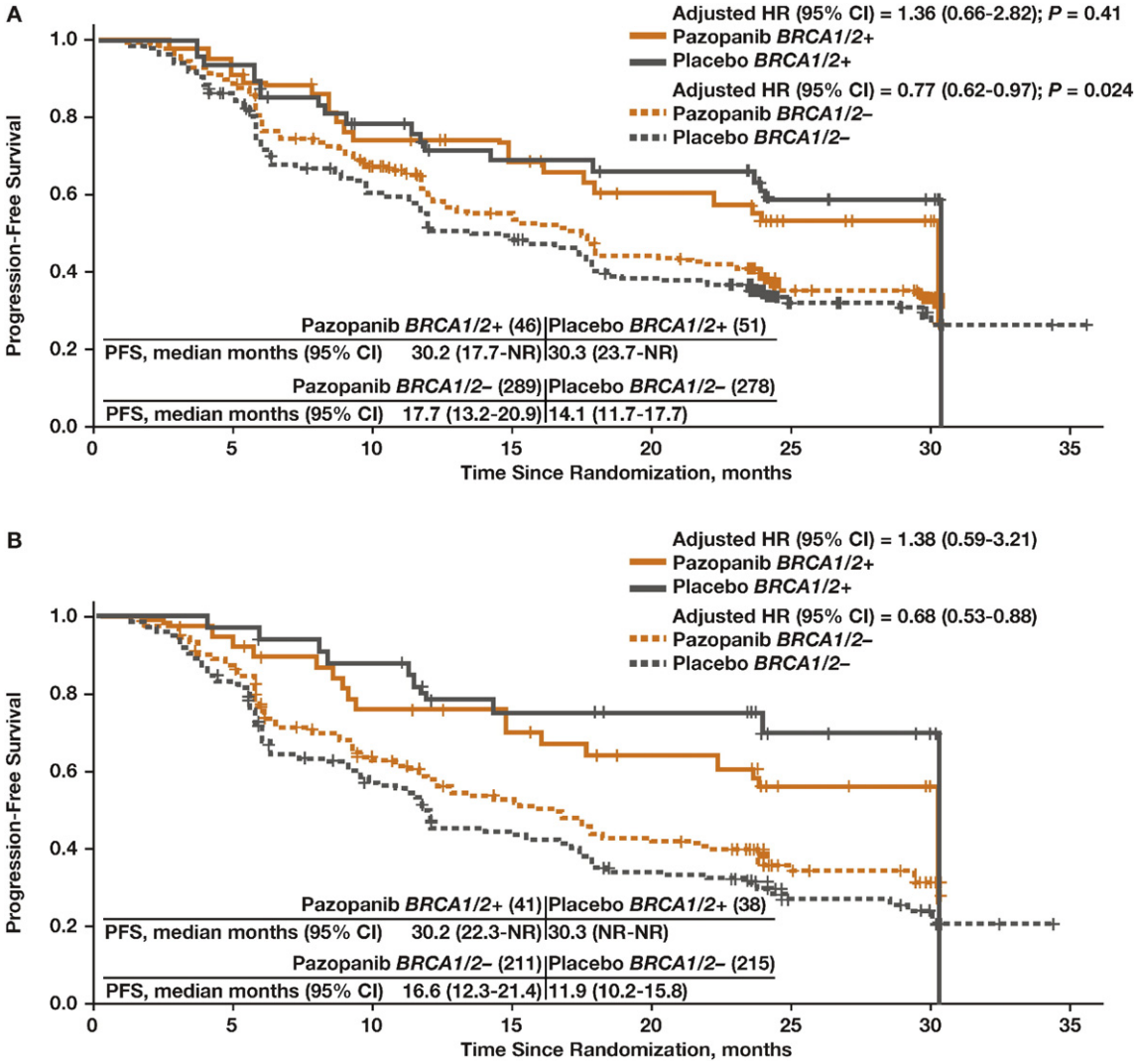

Fig. 1. (A) Progression-free survival (PFS) by treatment groups and *BRCA1/2* mutation status in the overall pharmacogenetic populations. (B) PFS by treatment groups and *BRCA1/2* mutation status in patients of European ancestry. Hazard ratios (HRs) were estimated using a Cox model stratified by recruitment region and first-line treatment outcome. Abbreviations: CI, confidence interval; NR, not reached.

Table 3
Multivariate sensitivity analysis for progression-free survival (PFS) association with first-line treatment outcome, recruitment region, and *BRCA1/2* status.

Factor	Level	PFS HR (95% CI)	*P* value[a]
Pharmacogenetic analysis population, placebo arm (n = 329)			
First-line outcome[b]	NED/NED and normal	Reference	
	RD/NED and normal	1.76 (1.25–2.47)	
	RD/RD or abnormal	2.78 (1.88–4.13)	1.88×10^{-6}
Region	Europe	Reference	
	Asia	0.62 (0.42–0.91)	
	US/Australia	1.05 (0.70–1.57)	0.027
BRCA1/2 status[c]	*BRCA1/2* −	Reference	
	BRCA1/2 +	0.45 (0.28–0.74)	0.00045
Pharmacogenetic analysis population, pazopanib arm (n = 335)			
First-line outcome[b]	NED/NED and normal	Reference	
	RD/NED and normal	1.48 (1.05–2.08)	
	RD/RD or abnormal	2.10 (1.44–3.05)	5.27×10^{-4}
Region	Europe	Reference	
	Asia	0.93 (0.66–1.33)	
	US/Australia	0.96 (0.59–1.55)	0.92
BRCA1/2 status[c]	*BRCA1/2* −	Reference	
	BRCA1/2 +	0.67 (0.41–1.08)	0.084
Pharmacogenetic analysis population, stratified by treatment arm (n = 664)			
First-line outcome[b]	NED/NED and normal	Reference	
	RD/NED and normal	1.60 (1.25–2.03)	
	RD/RD or abnormal	2.351 (1.80–3.10)	2.96×10^{-9}
Region	Europe	Reference	
	Asia	0.78 (0.60–1.01)	
	US/Australia	1.03 (0.76–1.40)	0.12
BRCA1/2 status	*BRCA1/2* −	Reference	
	BRCA1/2 +	0.55 (0.39–0.77)	0.00021

Abbreviations: CI, confidence interval; HR, hazard ratio; NED, no evidence of disease; RD, residual disease.
[a] There is one *P* value for each factor, not for each level of each factor.
[b] NED/NED and normal = NED after surgery or stages II–IIIA if unknown, NED after chemotherapy and normal CA125 at screening; RD/NED and normal = RD after surgery or stages IIIB–IV if unknown, NED after chemotherapy and normal CA125 at screening; RD/RD or abnormal = RD after chemotherapy or abnormal CA125 at screening.
[c] Main results for *BRCA1/2* status (reported in text) were from univariate analyses.

genome-wide significance level ($P \leq 5 \times 10^{-8}$), common genetic variants in the *UGT1A1* region were associated with on-treatment maximum serum total bilirubin ($P = 3.2 \times 10^{-22}$; Supplementary Fig. S2). No other common variants reached genome-wide significance for PFS or for any of the eight safety endpoints evaluated. Similar results were obtained when GWAS was conducted in the subset of European ancestry patients.

4. Discussion

To our knowledge, this is the first phase III trial in ovarian cancer with a genetic sub-study that directly evaluated the effect of *BRCA* germline mutations on the efficacy of a targeted therapy during the course of the clinical trial. Consistent with the results from all patients in the AGO-OVAR 16 clinical trial [8], PFS with pazopanib was significantly longer versus placebo in *BRCA1/2* non-carriers (85% pharmacogenetic patients). In the *BRCA1/2* carriers, the HR point estimate was above 1, but the 95% CI was wide due to the small sample size and small number of PFS events in this subgroup. Our data showed that carriers of clinically important *BRCA1/2* mutations had significantly longer PFS than non-carriers in the placebo arm; a similar, albeit non-significant, trend was seen in the pazopanib arm. The longer PFS in *BRCA1/2* carriers observed in this study is consistent with other studies in which *BRCA1/2* mutations were associated with better prognosis and survival in ovarian cancer patients receiving platinum-based chemotherapy [9,14]. All patients in the AGO-OVAR 16 study received platinum-based therapy before randomization. The longer PFS observed in patients carrying clinically important mutations in either *BRCA1* or *BRCA2* could therefore be due to improved treatment response to platinum-based therapy before randomization.

In patients in AGO-OVAR 16 for whom genetic data were available, we observed clinically important mutations in *BRCA1/2* at a frequency of 15%, which is at the high end of the overall range historically reported [23] but generally consistent with recent findings using more sensitive mutation detection methods [9,24]. This may also be a result of the patient enrollment criteria for this trial. Study patients were younger than non-study patients with ovarian cancer [25], and only patients with at least a partial remission after chemotherapy were included. Of note, we found that 20% of *BRCA1/2* carriers had non-serous histology, although a central pathologic review was not performed in this large international trial. This observation suggests that genetic counseling and testing should be an option for all patients with ovarian cancer, irrespective of primary histologic subtype.

Consistent with previous findings in renal cell carcinoma [15,26], this study showed that variants in the *UGT1A1* region were significantly associated with on-treatment maximum serum total bilirubin levels in pazopanib-treated patients with ovarian cancer. These data suggest that some instances of isolated bilirubin elevation in pazopanib-treated patients may be benign manifestations of Gilbert's syndrome. Bilirubin fractionation or *UGT1A1* genotyping would enable further characterization of the potential risk of liver toxicity.

The prospective collection of germline DNA samples enabled this exploratory pharmacogenetic analysis to be conducted during the course

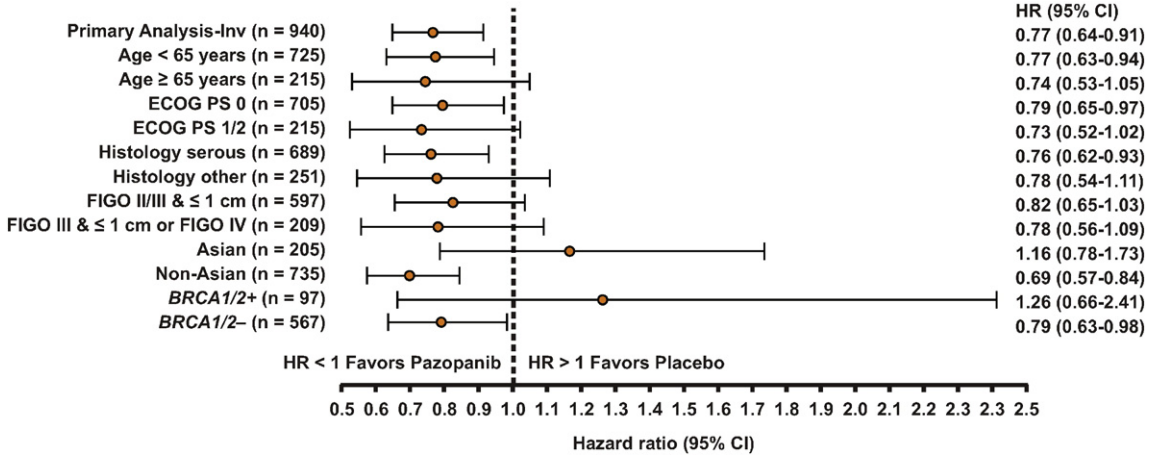

Fig. 2. Primary and exploratory subgroup analysis of progression-free survival (PFS) according to prognostic factors and *BRCA1/2* mutation status. For consistency with the primary clinical analyses [8], in this figure (only) hazard ratios (HRs) were calculated using the Pike estimator, stratified by region and first-line treatment outcome. Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; FIGO, International Federation of Gynecology and Obstetrics; Inv, investigator.

of the clinical study. The strengths of the present study include the combination of hypothesis-driven (e.g., *BRCA1/2*) and hypothesis-free (GWAS) analyses, and having detailed data on patient characteristics and outcomes. The overall sample size provided good power to detect any common genetic variants with large effects on pazopanib efficacy or safety endpoints. However, as consent to participate in genetic research was optional for patients in this clinical study, the pharmacogenetic sub-study is limited by incomplete data (pharmacogenetic patients represent 71% of intent-to-treat patients); in addition, because the AGO-OVAR 16 study is ongoing, overall survival data are not sufficiently mature to investigate the effect of *BRCA1/2* mutations on overall survival. Analyses of the *BRCA1/2* carrier subgroup were limited by small patient numbers and a small number of PFS events, resulting in wide CIs around the effect of pazopanib versus placebo, and potential for confounding by chance imbalances with respect to other prognostic factors. For this study, *BRCA1/2* sequencing was conducted in research laboratories using next-generation sequencing technologies that are not approved for diagnostic purposes. The methods used for library preparation, variant calling, and annotation of the called variants meant that we were unable to detect any large heterozygous deletion mutations that may have been present. However, previous reports have estimated that approximately <1% of patients with ovarian cancer carry large deletion mutations [9,27], and hence our association results are unlikely to have been materially different if we had used diagnostic-quality sequencing. However, for clinical trials that will stratify patients by *BRCA1/2* genotype at baseline, diagnostic-quality genotyping technology should be used.

Our results have potentially important implications for clinical management in patients with ovarian cancer, and also for the design of future clinical trials. Since the discovery of *BRCA1* and *BRCA2* two decades ago, major progress has been made in the understanding of their functions, the clinical consequences of malfunctioning BRCA proteins, and more recently, their effects on patient survival [9,10,12,28]. Our data from a large clinical trial have contributed to the body of evidence demonstrating that *BRCA1/2* mutation carriers had significantly better prognosis than non-carriers after receiving standard platinum-based chemotherapies. If patients are prospectively tested for *BRCA1/2* status, their treatment might be tailored to reflect this, with non-carriers targeted for more aggressive treatments. A current standard of care in advanced ovarian cancer is systemic treatment with carboplatin/paclitaxel with or without bevacizumab. Given the relatively good prognosis of *BRCA1/2* carriers, perhaps a less aggressive or more tailored systemic treatment could be identified for these patients without compromising prognosis; however, prospective clinical studies would be needed to definitively measure the effect of such strategies. Furthermore, different therapies could be (and indeed are being) developed for *BRCA1/2* mutation carriers versus non-carriers. Functional characterization of *BRCA1/2* led to the development of poly(ADP-ribose) polymerase (PARP) inhibitors [23] where inhibition of the PARP DNA repair pathway in *BRCA* carriers creates a synthetic lethal phenotype. Deleterious *BRCA* mutations were the major determinants of a clinically meaningful response to olaparib [11]. Our data do not demonstrate a significant difference in efficacy of pazopanib (vs placebo) for PFS according to *BRCA1/2* status. However, given the substantially worse prognosis for *BRCA1/2* non-carriers, it is possible that the overall benefit–risk ratio for pazopanib maintenance therapy might be different in *BRCA1/2* non-carriers than in *BRCA1/2* carriers, even if the true HR effect for PFS is the same in both subgroups. However, the number of *BRCA1/2* carriers might be too small for any meaningful subgroup analysis, at least if the drug under investigation does not specifically inhibit *BRCA*-associated targets. The large prognostic effect of *BRCA1/2* status also means that chance imbalances between treatment arms may confound trial outcomes, especially in trials or trial subgroups with small sample sizes. It is therefore important to consider stratifying analyses or blocking randomization by *BRCA1/2* genotyping at baseline in future clinical trials, as suggested by the Fourth Ovarian Cancer Consensus Conference [29].

Conflict of interest

- Philipp Harter reports honoraria from Roche, AstraZeneca; consulting or advisory role for Roche, Astra Zeneca, MSD, Takeda.
- Toby Johnson is an employee of GlaxoSmithKline and owns company stock.
- Dominique Berton-Rigaud has nothing to disclose.
- Sang-Yoon Park has nothing to disclose.
- Michael Friedlander reports advisory role for Astra Zeneca, Roche and Clovis.
- Josep M. del Campo reports speaker's bureau from Roche, Boehringer Ingelheim, Pharmamar, MSD.
- Muneaki Shimada has nothing to disclose.
- Frédéric Forget has nothing to disclose.
- Mansoor R. Mirza has nothing to disclose.
- Nicoletta Colombo reports honoraria from Roche, Astra Zeneca, AMGEN, Pharmamar, Clovis, MSD; consulting or advisory role for Roche, Astra Zeneca, AMGEN, Pharmamar, Clovis, MSD.
- Claudio Zamagni has nothing to disclose.
- John K. Chan has nothing to disclose.
- Martin Imhof has nothing to disclose.
- Thomas J. Herzog reports honoraria from Roche, Gradalis, and Vermillion; consulting or advisory role for Morphotek, Roche, Astra Zeneca.
- Dearbhaile O'Donnell reports travel, accommodations, or expenses from Pfizer.
- Florian Heitz has nothing to disclose.
- Karen King is an employee of GlaxoSmithKline and owns company stock.
- Sandy Stinnett is an employee of GlaxoSmithKline and owns company stock.
- Catherine Barrett is an employee of GlaxoSmithKline and owns company stock.
- Minesh Jobanputra was an employee of GlaxoSmithKline during conduct of the study and owns company stock.
- Chun-Fang Xu is an employee of GlaxoSmithKline and owns company stock.
- Andreas du Bois reports personal fees from Astra Zeneca, personal fees from MSD, personal fees from Roche, personal fees from AMGEN, personal fees from Eisai, personal fees from Mundipharm, personal fees from Pharmamar, outside the submitted work.

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Appendix A. Supplementary data

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