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Berzosertib plus gemcitabine versus gemcitabine alone in platinum-resistant high-grade serous ovarian cancer: a multicentre, open-label, randomised, phase 2 trial

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PAK contributed to study concept and study oversight, and PAK, S-CC, AEWH, ECK, ADD’A, GIS, and UAM contributed to the study design. ADD’A and GIS contributed to the literature search. AEWH, RTP, STS, LAD, ABO, ISW, MTM, and UAM contributed to patient recruitment, and BB contributed to patient care. PAK, RTP, STS, MAC, LMB, SF, RJS, JC, RSQ, BB, GIS, and UAM contributed to data collection; S-CC, RTP, ABO, ISW, SF, AF, DC, RSQ, GIS, and UAM contributed to the data analysis; and PAK, S-CC, AEWH, RTP, STS, LAD, ECK, ISW, SF, MTM, RJS, AF, DC, GIS, and UAM contributed to the data interpretation. PAK, S-CC, RTP, EKL, ECK, SF, and GIS contributed to writing the manuscript; S-CC contributed to creating the figures; MAC, LMB and RJS contributed to reviewing the manuscript, UAM contributed to final editing; and MAC and UAM approved the final version of the manuscript.

See Online for appendix

Data sharing

Upon email request, we will provide fully de-identified analysis datasets for adverse events listings (multiple records per subject), and for baseline characteristics, including treatment and stratification assignments, and the primary (progression-free survival) and secondary (overall survival, objective response rate, clinical benefit rate, duration of response) outcome endpoints (one record per subject).

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Summary

Background—High-grade serous ovarian cancers show increased replication stress, rendering cells vulnerable to ATR inhibition because of near universal loss of the G1/S checkpoint (through deleterious *TP53* mutations), premature S phase entry (due to *CCNE1* amplification, *RBI* loss, or *CDKN2A* mRNA downregulation), alterations of homologous recombination repair genes, and expression of oncogenic drivers (through *MYC* amplification and other mechanisms). We hypothesised that the combination of the selective ATR inhibitor, berzosertib, and gemcitabine could show acceptable toxicity and superior efficacy to gemcitabine alone in high-grade serous ovarian cancer.

Methods—In this multicentre, open-label, randomised, phase 2 study, 11 different centres in the US Experimental Therapeutics Clinical Trials Network enrolled women (aged ≥18 years) with recurrent, platinum-resistant high-grade serous ovarian cancer (determined histologically) and Eastern Cooperative Oncology Group performance status of 0 or 1, who had unlimited previous lines of cytotoxic therapy in the platinum-sensitive setting but no more than one line of cytotoxic therapy in the platinum-resistant setting. Eligible patients were randomly assigned (1:1) to receive intravenous gemcitabine (1000 mg/m²) on day 1 and day 8, or gemcitabine plus intravenous berzosertib (210 mg/m²) on day 2 and day 9 of a 21-day cycle until disease progression or intolerable toxicity. Randomisation was done centrally using the Theradex Interactive Web Response System, stratified by platinum-free interval, and with a permuted block size of six. Following central randomisation, patients and investigators were not masked to treatment assignment. The primary endpoint was investigator-assessed progression-free survival, and analyses included all patients who received at least one dose of the study drugs. The study is registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02595892), NCT02595892, and is active but closed to enrolment.

Findings—Between Feb 14, 2017, and Sept 7, 2018, 88 patients were assessed for eligibility, of whom 70 were randomly assigned to treatment with gemcitabine alone (36 patients) or gemcitabine plus berzosertib (34 patients). At the data cutoff date (Feb 21, 2020), the median follow-up was 53.2 weeks (25.6–81.8) in the gemcitabine plus berzosertib group and 43.0 weeks (IQR 23.2–69.1) in the gemcitabine alone group. Median progression-free survival was 22.9 weeks (17.9–72.0) for gemcitabine plus berzosertib and 14.7 weeks (90% CI 9.7–36.7) for gemcitabine alone (hazard ratio 0.57, 90% CI 0.33–0.98; one-sided log-rank test $p=0.044$). The most common treatment-related grade 3 or 4 adverse events were decreased neutrophil count (14 [39%] of 36 patients in the gemcitabine alone group vs 16 [47%] of 34 patients in the gemcitabine plus berzosertib group) and decreased platelet count (two [6%] vs eight [24%]). Serious adverse events were observed in ten (28%) patients in the gemcitabine alone group and nine (26%) patients in the gemcitabine plus berzosertib group. There was one treatment-related death in the gemcitabine alone group due to sepsis and one treatment-related death in the gemcitabine plus berzosertib group due to pneumonitis.

Interpretation—To our knowledge, this is the first randomised study of an ATR inhibitor in any tumour type. This study shows a benefit of adding berzosertib to gemcitabine in platinum-resistant high-grade serous ovarian cancer. This combination warrants further investigation in this setting.

Funding—US National Cancer Institute.

Introduction

Replication stress is defined as the slowing or stalling of replication fork progression during DNA synthesis.¹ Sources of replication stress include loss of G1/S checkpoint (eg, due to deleterious *TP53* mutations), premature entry into the S phase (eg, due to *RBI* loss or *CCNE1* amplification), oncogenic drive (eg, due to *KRAS* activating mutations or *MYC* amplification), and DNA repair deficiencies (eg, deficiencies in homologous recombination repair and nucleotide excision repair).^{2–4} All these mechanisms of increased replication stress are prevalent in high-grade serous ovarian cancer, the most common histological subtype of epithelial ovarian cancer.^{5–7} Specifically, large-scale genomic studies have shown that high-grade serous ovarian cancers have near universal loss of the G1/S checkpoint (via deleterious *TP53* mutations); premature S phase entry due to *CCNE1* amplification in about 20% of tumours, *RBI* loss in about 11% of tumours, or *CDKN2A* mRNA downregulation in about 32% of tumours; oncogenic driver activation via amplification of the *MYC* oncogene in up to 40% of tumours or *NFI* loss in about 10% of tumours; and DNA repair deficiencies, mainly due to homologous recombination repair alterations in about 50% of tumours and nucleotide excision repair alterations in 4–8% of tumours.^{5–7}

The cellular response to replication stress involves activation of the ATR kinase that mediates the activation of DNA repair pathways and stabilisation of replication forks.^{1,8} ATR is a member of the PI3K-related kinase family of proteins and is the master kinase that regulates cellular responses to stalled and collapsed replication forks, mainly by activating DNA repair proteins and suppressing dormant origin firing. Activation of ATR triggers the intra-S phase and the G2 checkpoints through phosphorylation of CHK1, which in turn phosphorylates WEE1 and CDC25 phosphatases to inhibit cell cycle progression through the coordinated suppression of cyclin-dependent kinase activity.^{1–3,9} ATR also facilitates DNA repair through phosphorylation of several proteins involved in homologous recombination repair and interstrand crosslink repair, including BRCA2, RAD51, FANCD2, and FANCE.^{1,8}

Given the prevalence of DNA replication stress in high-grade serous ovarian cancer, inhibition of ATR might be an effective strategy against these tumours. Furthermore, treatment with gemcitabine, a drug used routinely in platinum-resistant ovarian cancer, induces additional replication stress and dependence on ATR through two mechanisms: inhibition of DNA repair by incorporating gemcitabine nucleotides into the DNA, and inhibition of ribonucleotide reductase leading to depletion of the deoxyribonucleotide pool required for replication and repair.^{10–13} Preclinical models have shown a synergistic antitumour effect with gemcitabine and the ATR inhibitor berzosertib, with maximal effect when berzosertib is administered 24 h after starting gemcitabine.¹⁴ This schedule dependence is attributed to the fact that 24 h after gemcitabine administration coincides with the highest accumulation of cells in S phase, with a concomitant increase in ATR activity (as measured by phosphorylated CHK1) and thus maximal susceptibility to ATR inhibition.¹⁴ Therefore, in this study, we aimed to evaluate whether berzosertib would enhance the activity of gemcitabine and show acceptable toxicity and superior efficacy compared with

gemcitabine alone in patients with recurrent, platinum-resistant high-grade serous ovarian, primary peritoneal, or fallopian tube cancers.

Methods

Study design and participants

In this multicentre, open-label, investigator-initiated, randomised, phase 2 study, we enrolled patients at 11 different cancer centres in the USA through the Experimental Therapeutics Clinical Trials Network (appendix p 10).

Eligible patients were women aged 18 years or older with life expectancy of more than 6 months, who had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (with Karnofsky Performance Status score ≥ 70), and histologically determined high-grade serous carcinoma of the ovary, fallopian tube, or primary peritoneum (collectively referred to as ovarian cancer) that was platinum resistant, defined as progression within 6 months after the last platinum regimen. Patients were required to have measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, and adequate haematological function (white blood cell count ≥ 3000 cells per μL , absolute neutrophil count ≥ 1500 cells per μL , and platelets $\geq 100\,000$ per μL), renal function (serum creatinine concentration \leq upper limit of normal [ULN] or creatinine clearance ≥ 60 mL/min), and hepatic function (total bilirubin concentration \leq ULN and alanine and aspartate aminotransferase concentration $\leq 2.5 \times$ ULN). Patients could have received unlimited lines of cytotoxic therapy when their cancer was platinum sensitive, but no more than one line of cytotoxic therapy was allowed in the platinum-resistant setting. Previous hormonal therapies, immunotherapy, and antiangiogenic therapies were allowed and did not count as lines if given as monotherapy. Previous receipt of PARP inhibitors was allowed, and in an approved amendment to the protocol (June 14, 2018), it was clarified that previous PARP inhibitor use did not count as a separate line of treatment if it was given as maintenance after platinum therapy. Previous carboplatin plus gemcitabine therapy was allowed if there was no disease progression within 12 months after completion of that regimen. In addition, availability of archival formalin-fixed, paraffin embedded tumour specimens was required for participation, for doing exploratory correlative studies.

Patients were ineligible if they had primary platinum-refractory disease, known brain metastases, ongoing grade 2 or worse adverse events related to previous treatment, if they were taking any inhibitors or inducers of cytochrome P450 3A4 enzyme, or if they had any major comorbidities (including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness or difficulty in social situations that would limit compliance with the study requirements). Previous treatments targeting the ATR/CHK1 pathway and previous administration of gemcitabine as monotherapy were not permitted. A full list of inclusion and exclusion criteria can be found in the protocol (appendix).

Written informed consent was obtained from all patients or their guardians before enrolment in the study. The clinical trial was approved by the National Cancer Institute (NCI) Central Institutional Review Board and the US Food and Drug Administration. All procedures were

done in accordance with the Declaration of Helsinki. The full protocol is available in the appendix.

Randomisation and masking

Eligible patients were randomly assigned (1:1) to receive either gemcitabine alone or gemcitabine plus berzosertib. Randomisation was stratified by platinum-free interval (3 months *vs* >3 months but <6 months [as in the AURELIA study¹⁵]). Patient randomisation was done centrally and independently through the Theradex Interactive Web Response System. The study statistician (S-CC) generated the allocation sequence using stratified permuted blocks with a block size of six. Patients and investigators were not masked to treatment assignment.

Procedures

Eligible patients received intravenous gemcitabine (1000 mg/m² during 30 min) on day 1 and day 8 of each 21-day cycle, either alone or in combination with intravenous berzosertib (210 mg/m² during 1 h) on day 2 and day 9 of each 21-day cycle. The gemcitabine and berzosertib dose and the timing of administration was the recommended phase 2 dose that was established in a previous dose-escalation study.¹⁶ Berzosertib was administered with corticosteroid and antihistamine premedications to protect against acute hypersensitivity. Haematopoietic growth factor support was allowed.

Treatment continued until disease progression, unacceptable toxicity, patient refusal to participate in the study, intercurrent illness that prevented further administration of treatment, or general or specific changes in the participant's condition that rendered the treatment unacceptable in the opinion of the treating investigator. Toxicity-related dose delays of up to 4 weeks were allowed. Depending on the grade of the adverse event, gemcitabine could be reduced to 750 mg/m² or 500 mg/m², and berzosertib could be reduced to 165 mg/m² or 120 mg/m². Generally, for grade 3 gastrointestinal-related adverse events and other grade 4 non-haematological adverse events, treatment with both drugs was delayed until adverse events resolved to grade 2 or less, and the dose of both gemcitabine and berzosertib was reduced by one dose level each. For other grade 3 non-haematological adverse events, treatment was delayed until they resolved to grade 2 or less; gemcitabine treatment was resumed at a reduced dose level but berzosertib was resumed at the previous dose. If, after the dose reduction of gemcitabine, grade 3 non-haematological adverse events recurred, or if there was persistent grade 2 or lower non-haematological adverse event that resulted in a need for a dose delay of more than 2 weeks, then berzosertib was also reduced by one dose level. For grade 3 haematological adverse events, treatment was delayed until complete blood count recovery. For grade 4 neutropenia or grade 3 thrombocytopenia, treatment was delayed until count recovery and gemcitabine was reduced by one dose level. For grade 4 neutropenia persisting for more than 7 days, grade 4 thrombocytopenia, or febrile neutropenia, treatment was delayed until they were resolved to grade 2 or less, and gemcitabine treatment was resumed at a reduced dose level. If these adverse events recurred after the reduction in gemcitabine, then berzosertib was also reduced by one dose level. Crossover from the gemcitabine group to the gemcitabine plus berzosertib group was allowed for patients who developed disease progression according to RECIST 1.1 criteria.

Tumour reassessment was through CT or MRI of the chest, abdomen, and pelvis every 9 weeks for the first year and every 12 weeks after the first year. Assessments could be done up to 7 days before or after the designated timepoint, and were done by the investigator using RECIST version 1.1. Safety and adverse events were assessed on day 1 and day 8 of each cycle and were graded according to the NCI Common Terminology Criteria for Adverse Events version 4.0. Laboratory assessments (complete blood count, blood urea nitrogen, creatinine, sodium, potassium, calcium, carbon dioxide, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, total protein, and albumin) were done on day 1 and day 8 of each cycle, and ovarian cancer-related tumour marker (CA-125) was tested on day 1 of each cycle. After stopping study treatment, patients were followed up every 3 months for 1 year and every 6 months thereafter, for 3 years after removal from study treatment or until death, whichever occurred first.

Outcomes

The primary endpoint was progression-free survival, defined as the time from randomisation until the date of documented, investigator-assessed RECIST-based progressive disease or death (regardless of cause). Secondary endpoints were overall survival (defined as the time from randomisation to death, regardless of cause); objective response rate (defined as the percentage of participants with a complete or partial response as per RECIST version 1.1) in the whole population and in crossover patients; clinical benefit rate (defined as the percentage of patients showing complete response, partial response, or stable disease for 4 months or more); duration of response (measured from the time that criteria were met for complete or partial response [whichever came first] until the first date that progressive disease was recorded); proportion of patients with a reduction in CA-125 by more than 50%; progression-free survival at 6 months; and the safety profile of each group.

Statistical analysis

The study was designed to have 80% power to detect an improvement of median progression-free survival from 15 weeks with gemcitabine alone to 27.3 weeks with gemcitabine plus berzosertib using the stratified log-rank test (with the platinum-free interval as the stratification factor) at a one-sided alpha level of 0.1. Randomly assigning 64 patients (32 patients in each group) was assumed to achieve the required 50 progression-free survival events, allowing for a 5% dropout. Anticipating that 10% of participants would not be evaluable, the maximum total number of patients that needed to be enrolled was 70.

Progression-free survival, overall survival, and safety analyses were done in all patients who received at least one dose of the study drugs. Analysis of all other secondary efficacy endpoints were also done in all patients who received at least one dose of the study drug, including those with measurable disease who were not assessable for objective response because of early discontinuation of protocol therapy.

Patient characteristics, objective response rate, clinical benefit rate, proportion of patients with a reduction in CA-125, and adverse events were summarised by counts (percentages) and confidence intervals when appropriate. Treatment differences in clinical benefit rate were compared by Fisher's exact test (two-sided). Progression-free survival and overall

survival for each treatment group were summarised using the Kaplan-Meier method. The log-rank test was used to compare progression-free survival and overall survival between the two groups with a one-sided significance of 0.1. The Cox proportional hazards models were fit to estimate the treatment hazard ratio (HR) and the corresponding two-sided 90% CI, as well as the upper one-sided 90% CI. We used the test developed by Grambsch and Therneau¹⁷ based on Schoenfeld residuals, to examine the proportional hazards assumption; the proportional hazards assumption was not rejected. We also examined the treatment effects on progression-free survival in the two platinum-free interval strata using Kaplan-Meier curves and the Cox model (with a term for interaction between treatment and platinum-free interval) to assess whether it was suitable to fit the data with a Cox model stratified by platinum-free interval. Patients who withdrew treatment for reasons unrelated to progression were included and censored at their off-protocol treatment date in the progression-free survival analysis.

Exploratory post-hoc subgroup analysis of progression-free survival was graphically displayed with a forest plot to examine heterogeneity in treatment effects across subgroups.

For patients who crossed over, objective response rate was included as a secondary endpoint. Progression-free survival for crossover patients was a post-hoc analysis, and was measured from the time of progressive disease when receiving gemcitabine alone until they progressed on gemcitabine plus berzosertib.

Patients who started a non-protocol treatment before documented progressive disease were censored at the time that they started non-protocol therapy. By definition, crossover patients had events in the progression-free survival analysis and were included per their randomly assigned groups in the overall survival analysis. An interim futility analysis was planned at 25 progression-free survival events to stop enrolment in the case of futility, but it was not done because all patients had been enrolled before that point. Descriptive statistics were used to summarise objective response information after crossover.

All statistical analyses were done using R (version 3.6.2). This study is registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02595892), NCT02595892.

Role of the funding source

The drug was provided by Merck KGaA/EMD Serono through the NCI. The NCI had a role in the study design, data collection, data analysis, data interpretation, and writing of the report. Two authors (LAD and ECK) are from the NCI. Merck KGaA/EMD Serono had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study, were involved in writing the report, and approved the final version for submission. PAK and UAM had final responsibility for the decision to submit for publication.

Results

Between Feb 14, 2017, and Sept 7, 2018, 88 patients were assessed for eligibility, of whom 70 were randomly assigned to treatment with either gemcitabine alone (36 patients) or

gemcitabine plus berzosertib (34 patients; figure 1). Three patients, at some timepoints, had imaging scans without oral or intravenous and oral contrast. All 70 patients received treatment and were included in the efficacy and safety analyses. Baseline patient characteristics were balanced between the two groups (table 1). At the data cutoff date of Feb 21, 2020, all patients had discontinued treatment (figure 1). The median follow-up was 43.0 weeks (IQR 23.2–69.1) in the gemcitabine alone group and 53.2 weeks (25.6–81.8) in the gemcitabine plus berzosertib group.

17 (50%) of 34 patients receiving gemcitabine plus berzosertib and 25 (69%) of 36 patients receiving gemcitabine alone had progression-free survival events. Median progression-free survival was 22.9 weeks (90% CI 17.9–72.0) in the gemcitabine plus berzosertib group versus 14.7 weeks (9.7–36.7) in the gemcitabine alone group (HR 0.57, 0.33–0.98; one-sided log-rank test $p=0.044$; upper one-sided 90% CI 0.87; figure 2A). In patients with a platinum-free interval of 3 months or less, median progression-free survival was 27.7 weeks (90% CI 15.7–not reached [NR]) in those receiving gemcitabine plus berzosertib (seven progression-free survival events) versus 9.0 weeks (8.7–NR) in those receiving gemcitabine alone (11 progression-free survival events; HR 0.29, 0.12–0.71; one-sided log-rank test $p=0.0087$; figure 2B). In patients with a platinum-free interval greater than 3 months, median progression-free survival was 18.6 weeks (9.4–NR) for those receiving gemcitabine plus berzosertib (ten progression-free survival events) versus 15.3 weeks (14.7–NR) for those receiving gemcitabine alone (14 progression-free survival events; HR 1.04, 0.51–2.12; one-sided log-rank test $p=0.46$; figure 2C). Exploratory post-hoc analyses of progression-free survival are shown in figure 3.

At the data cutoff date, 24 (71%) of 34 patients in the gemcitabine plus berzosertib group and 29 (81%) of 36 patients in the gemcitabine alone group had died. Median overall survival was 59.4 weeks (90% CI 33.7–84.4) in the gemcitabine plus berzosertib group versus 43.0 weeks (34.4–67.9) in the gemcitabine alone group (HR 0.84, 0.53–1.32; one-sided log-rank test $p=0.26$; appendix p 1). In patients with a platinum-free interval of 3 months or less, median overall survival was 84.4 weeks (59.4–NR) in the gemcitabine plus berzosertib group (eight patients died) versus 40.4 weeks (27.6–92.4) in the gemcitabine alone group (11 patients died; HR 0.42, 0.19–0.94; one-sided log-rank test $p=0.034$; appendix p 1). In patients with a platinum-free interval of more than 3 months, median overall survival was 39.0 weeks (31.3–80.6) in the gemcitabine plus berzosertib group (16 patients died) versus 59.9 weeks (34.6–98.1) in the gemcitabine alone group (18 patients died; HR 1.29, 0.73–2.31; one-sided log-rank test $p=0.23$; appendix p 1).

Secondary endpoints of objective response, duration of response, 6-month progression-free survival, and clinical benefit rate were measured for all 70 patients who received at least one dose of the study drug (table 2). 18 (26%) of 70 patients had a decrease in CA-125 of more than 50%, including nine (25%) of 36 patients in the gemcitabine alone group and nine (27%) of 34 patients in the gemcitabine plus berzosertib group.

Ten (67%) of 15 patients who crossed over from the gemcitabine alone group to the gemcitabine plus berzosertib group had a disease evaluation scan after crossover. Among these ten patients, one (10%) had stable disease and then withdrew because of an adverse

event, one (10%) had stable disease and then had clinical progression, and the other eight (80%) had progressive disease. In a post-hoc analysis, median progression-free survival for these patients was 19 weeks (90% CI 8.7–NR) after crossover (ie, after progressive disease when receiving gemcitabine alone; appendix p 2).

The most common treatment-related grade 3 or 4 adverse events were decreased neutrophil count (14 [39%] of 36 patients in the gemcitabine alone group *vs* 16 [47%] of 34 patients in the gemcitabine plus berzosertib group) and decreased platelet count (two [6%] *vs* eight [24%]; table 3; appendix pp 3–7). No clinically significant bleeding was observed in the combination group, but one case was observed in the gemcitabine alone group.

There was one treatment-related death in the gemcitabine alone group due to sepsis and one treatment-related death in the gemcitabine plus berzosertib group due to pneumonitis. No other deaths due to adverse events (while on protocol treatment) were observed. Pneumonitis has a known association with gemcitabine. One patient developed pneumonitis in the gemcitabine alone group (grade 2), one developed grade 2 pneumonitis after crossing over to the gemcitabine plus berzosertib group (this patient had five cycles of gemcitabine alone, crossed over to gemcitabine plus berzosertib, and was diagnosed with pneumonitis after she received the first dose of berzosertib), and three patients developed pneumonitis in the gemcitabine plus berzosertib group (two grade 2 who discontinued protocol therapy because of pneumonitis and one for whom it was the cause of death).

Four (11%) of 36 patients in the gemcitabine alone group and six (18%) of 34 patients in the gemcitabine plus berzosertib group discontinued treatment. In the gemcitabine alone group, one patient each discontinued because of neutropenia, capillary leak syndrome, oedema, and acute renal failure. In the gemcitabine plus berzosertib group, two patients discontinued because of fatigue, one because of abdominal pain, one because of small bowel obstruction, and as stated, two patients in the combination group discontinued because of pneumonitis.

Serious adverse events were observed in ten (28%) of 36 participants in the gemcitabine alone group (predominantly fever or febrile neutropenia; two [6%] patients), and in nine (26%) of 34 participants in the gemcitabine plus berzosertib group (predominantly thrombocytopenia; four [12%] patients). All serious adverse events were deemed to be drug-related. 13 (36%) patients in the gemcitabine alone group and 13 (38%) patients in the gemcitabine plus berzosertib group had a dose reduction (appendix pp 8–9).

Discussion

The results of this multicentre, open-label, randomised, phase 2 trial show that the addition of the ATR inhibitor, berzosertib, to gemcitabine in platinum-resistant, high-grade serous ovarian cancer increased progression-free survival compared with gemcitabine alone. To our knowledge, this is the first randomised study of ATR inhibitor therapy in any tumour type, and the first suggesting a benefit of adding an ATR inhibitor to standard chemotherapy in ovarian cancer or any tumour type. Building on the mechanistic rationale behind using an ATR inhibitor in high-grade serous ovarian cancer (a tumour with high replication stress and thus high dependency on ATR), the results of its combination with gemcitabine (which

further enhances replication stress and dependency on ATR), might be applicable to additional tumour types with high replication stress (eg, uterine serous cancers or small cell lung cancers).^{18,19} Furthermore, ATR inhibitor combinations with other drugs that enhance replication stress, such as topoisomerase-I inhibitors, PARP inhibitors, and PI3K inhibitors, should be considered.^{20,21} Of note, in this study, berzosertib was administered 24 h after gemcitabine, based on preclinical studies.¹⁴ These data highlight the importance of preclinical studies in determining the optimal sequence and schedule of administration of ATR inhibitors in combination with chemotherapy.

The management of patients with platinum-resistant ovarian cancer is a substantial unmet medical need. Nonplatinum cytotoxic chemotherapy (with or without the antiangiogenic drug bevacizumab) remains the standard of care for these patients, who typically have a poor prognosis and no targeted treatment options except PARP inhibitors if their tumours are *BRCA*-mutation positive.²² Weekly gemcitabine, administered in cycles of either 2 weeks on and 1 week off, or 3 weeks on and 1 week off, has shown similar activity to pegylated liposomal doxorubicin in two randomised phase 3 studies and is a standard regimen for platinum-resistant ovarian cancer.^{23,24} The median progression-free survival of 14.7 weeks observed with gemcitabine alone in this study is consistent with that previously reported for gemcitabine (about 3 months) and other standard cytotoxic drugs, such as pegylated liposomal doxorubicin (also about 3 months), as monotherapies in platinum-resistant ovarian cancer.^{23–25} The median progression-free survival of 22.9 weeks and the HR of 0.57 for the gemcitabine plus berzosertib group are promising, considering that this study allowed unlimited lines of previous therapies and up to one line in the platinum-resistant setting. This approach is in contrast to the AURELIA study,¹⁵ in which no patient could have received more than two previous lines of therapy and 107 (60%) of 179 patients in the chemotherapy plus bevacizumab group had received only one previous line of therapy (ie, first-line chemotherapy only), compared with only three (9%) of the 34 patients in the gemcitabine plus berzosertib group in this study.

A higher objective response rate was observed in the gemcitabine alone group than in the gemcitabine plus berzosertib group, whereas progression-free survival was better in the gemcitabine plus berzosertib group. These results are not uncommon in platinum-resistant ovarian cancer. In the CLIO study reported in 2019,²⁶ in which olaparib was compared with standard chemotherapy in platinum-resistant ovarian cancer, the objective response rate was higher in patients assigned to olaparib than in patients who received chemotherapy (18% vs 6%), but no significant difference was observed in progression-free survival (median 2.9 months for olaparib vs 3.4 months for chemotherapy; HR 1.18). Furthermore, in a randomised phase 3 study of topotecan versus pegylated liposomal doxorubicin in recurrent ovarian cancer,²⁷ the objective response rate in the platinum-resistant cohort was 12.3% for pegylated liposomal doxorubicin and 6.5% for topotecan, whereas the median progression-free survival was 9.1 weeks for pegylated liposomal doxorubicin compared with 13.6 weeks for topotecan.

The progression-free survival benefit of adding berzosertib to gemcitabine was notable in patients with a platinum-free interval of 3 months or less but not for those with a platinum-free interval greater than 3 months. These findings might reflect the fact that tumours of

patients with a platinum-free interval of three months or less are enriched for biomarkers of replicative stress (such as *CCNE1* amplification), which are likely to be predisposed to respond to ATR inhibition; this hypothesis is being investigated in ongoing correlative work. Furthermore, post-hoc analysis of progression-free survival in other subgroups indicated that there was a benefit of adding berzosertib to gemcitabine in more heavily pretreated patients, including patients with at least two previous lines of therapy and patients with one previous line of therapy in the platinum-resistant setting, although the benefit was not seen in patients who had received at least three lines of therapy. Given that ATR-mediated stabilisation of the replication fork is one of the known mechanisms of resistance to platinum and other DNA-damaging chemotherapy drugs in high-grade serous ovarian cancer,^{28,29} more heavily pretreated tumours might be more likely to have developed resistance through that mechanism and therefore be more susceptible to ATR inhibition. Tumours that have been exposed to antiangiogenic therapy might be even more dependent on resistance through ATR-mediated stabilisation of the replication fork because anti-angiogenic drugs downregulate homologous recombination repair,^{30,31} thereby negating the restoration of homologous recombination repair as a mechanism of resistance in these tumours. This factor might explain the effect of the addition of berzosertib in patients who had previously received antiangiogenic therapy. All these mechanistic hypotheses are being evaluated in ongoing correlative work, which includes targeted next-generation sequencing to identify genomic determinants of response and assessment of biomarkers of replication stress and ATR activation in these tumours.

The addition of berzosertib to gemcitabine was well tolerated with a similar percentage of patients having treatment-related grade 3 or worse adverse events and dose reductions in the two groups. In terms of myelo-suppression, thrombocytopenia was more common in the gemcitabine plus berzosertib group than in the gemcitabine alone group, but no clinically significant bleeding was observed; anaemia and neutropenia were similar in the two groups. Berzosertib was administered with corticosteroid and antihistamine premedications to protect against acute hypersensitivity, resulting in only two patients having infusion-related reactions in the experimental group, which were easily manageable. Pneumonitis, a known adverse effect of gemcitabine, was observed in one patient in the gemcitabine alone group, in one patient after crossing over to the gemcitabine plus berzosertib group, and in three patients in the combination group, including one death. It is impossible to conclude whether berzosertib increases the incidence or severity of gemcitabine-induced pneumonitis on the basis of these data; of note, pneumonitis has not been reported in other studies of berzosertib alone or in combination. Nonetheless, the development of pneumonitis should be carefully monitored in future studies of the gemcitabine plus berzosertib combination. No other safety signals or unexpected adverse events were observed in this study.

One of the main limitations of this study is the small sample size; this trial was a hypothesis-generating study powered to detect an improvement in median progression-free survival at a one-sided significance level of 0.1. The study was open label, although it is reassuring that the standard gemcitabine alone group showed a median progression-free survival that was consistent with that previously reported for platinum-resistant ovarian cancer. These limitations notwithstanding, our findings warrant further investigation of gemcitabine plus berzosertib in a phase 3 trial in platinum-resistant ovarian cancer and support stratification based on platinum-free interval, number of previous lines of therapies, and previous

antiangiogenic therapy. Finally, our results support future assessment of ATR inhibitor therapy in combination with gemcitabine in additional tumour types, especially in tumours that are predicted to have high replication stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

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Research in context

Evidence before this study

We searched PubMed from database inception to Dec 28, 2019, to identify publications that included the search terms “ATR”, “randomised”, “randomized”, “cancer”, and “ovarian cancer”. We also searched PubMed for publications describing clinical evaluation of berzosertib using the terms “berzosertib”, “M6620”, or “VX-970” and assessment of other ATR inhibitors using the terms “ATR inhibitor”, “M4344”, “VX-803”, “AZD6738”, “ceralasertib”, and “BAY1895344”. We did not use any language restrictions. These searches did not identify any previous randomised trials that have investigated the use of ATR inhibitors, alone or in combination, either in ovarian cancer or in any other tumour type. Given the prevalence of DNA replication stress in high-grade serous ovarian cancer, inhibition of ATR might be an effective strategy against these tumours. Furthermore, treatment with gemcitabine, a drug used routinely in platinum-resistant ovarian cancer, induces additional replication stress (through inhibition of DNA repair and inhibition of ribonucleotide reductase, leading to depletion of the deoxyribonucleotide pool), thereby leading to higher dependency on ATR inhibition. Preclinical models have shown a synergistic antitumour effect with gemcitabine and the ATR inhibitor berzosertib, prompting our evaluation of whether berzosertib enhances the activity of gemcitabine.

Added value of the study

The results of this phase 2 trial showed that the addition of the ATR inhibitor, berzosertib, to gemcitabine in platinum-resistant high-grade serous ovarian cancer increased progression-free survival compared with gemcitabine alone. To our knowledge, this is the first randomised study of ATR inhibitor therapy in any tumour type and the first to show the benefit of adding an ATR inhibitor to standard chemotherapy in ovarian cancer or in any other tumour type.

Implications of all the available evidence

The results of this study support further investigation of gemcitabine plus berzosertib in a phase 3 trial in platinum-resistant ovarian cancer. Furthermore, when considered together with the mechanistic rationale behind using an ATR inhibitor in high-grade serous ovarian cancer (a tumour with high replication stress and thus high dependency on ATR) in combination with gemcitabine (a drug that further enhances replication stress and dependency on ATR), these results might be applicable to other tumour types with high replication stress (eg, uterine serous cancers or small cell lung cancers), as well as ATR inhibitor combinations with other drugs that enhance replication stress, such as topoisomerase-I inhibitors, PARP inhibitors, and PI3K inhibitors.

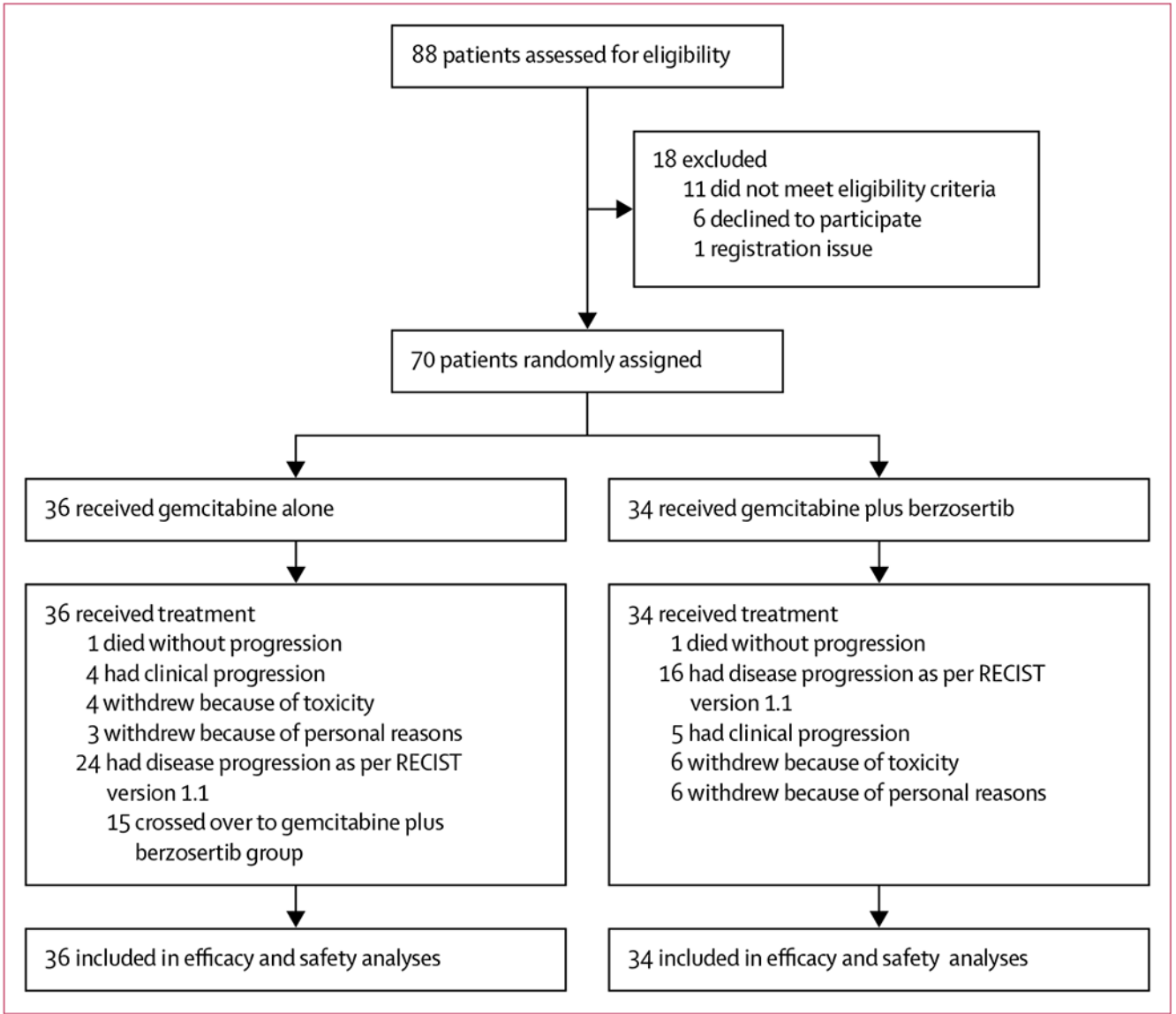


Figure 1: Trial profile
RECIST=Response Evaluation Criteria in Solid Tumors.

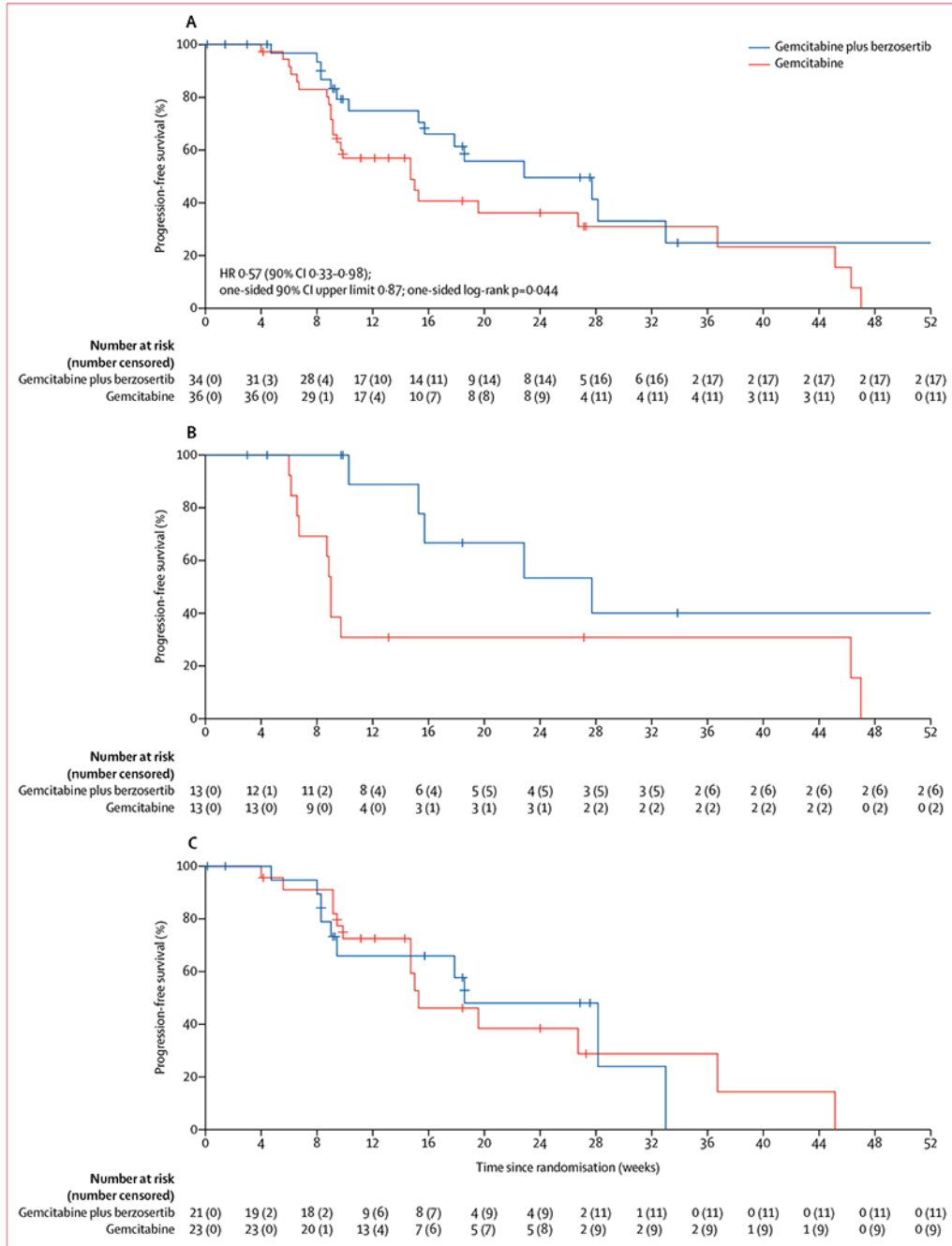


Figure 2: Progression-free survival in all patients who initiated protocol therapy (A), patients with a platinum-free interval of 3 months or less (B), and patients with a platinum-free interval of more than 3 months to less than 6 months (C)
 HR=hazard ratio.

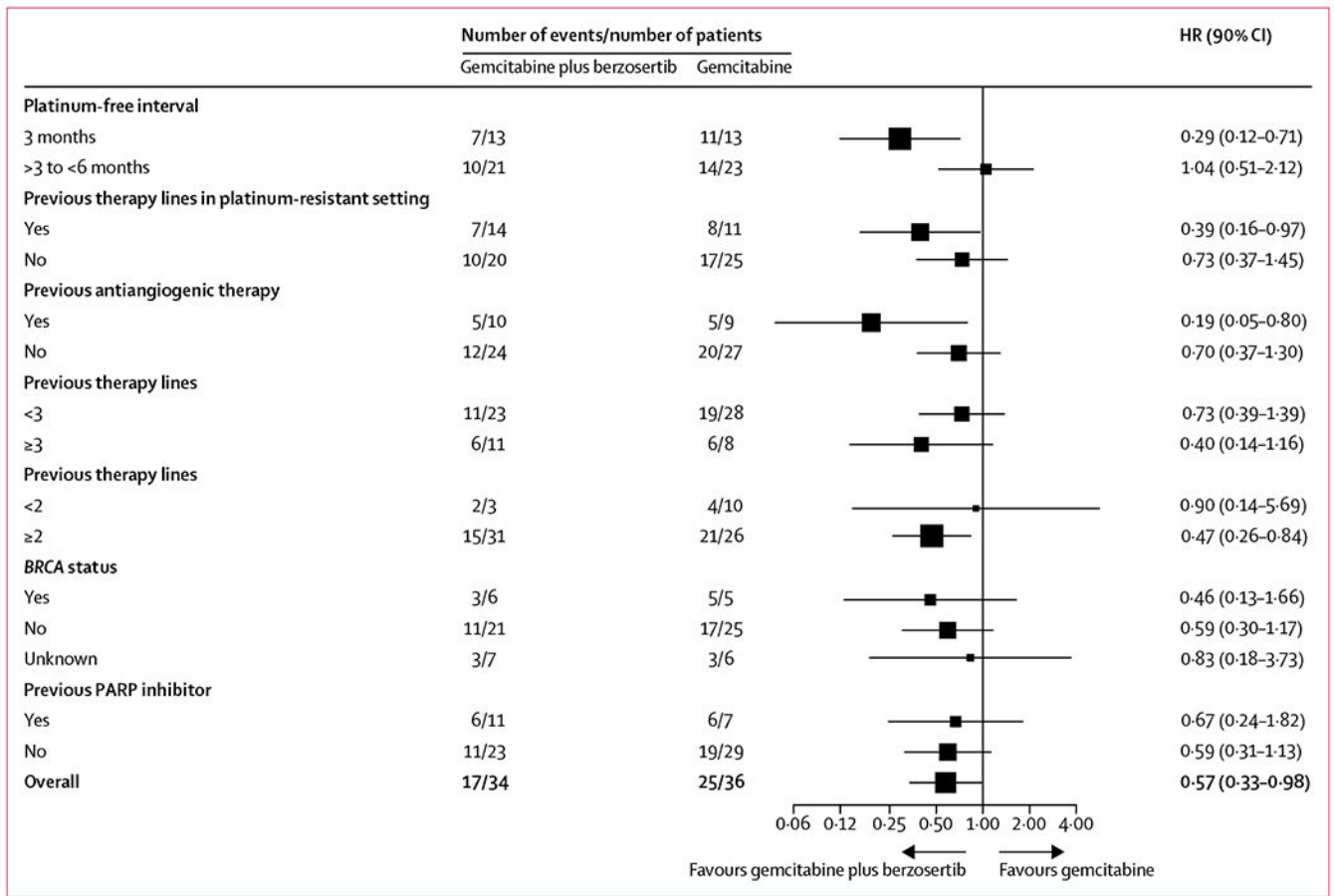


Figure 3: Post-hoc subgroup analysis of progression-free survival
 HR=hazard ratio.

Table 1:

Baseline characteristics

	Gemcitabine alone (n=36)	Gemcitabine plus berzosertib (n=34)
Race		
White	34 (94%)	30 (88%)
Asian	0	2 (6%)
Unknown	2 (6%)	2 (6%)
Ethnicity		
Not Hispanic or Latino	34 (94%)	30 (88%)
Hispanic or Latino	1 (3%)	2 (6%)
Unknown	1 (3%)	2 (6%)
ECOG performance status		
0	22 (61%)	20 (59%)
1	14 (39%)	14 (41%)
Stage at diagnosis		
II	0 (0%)	2 (6%)
III	16 (44%)	18 (53%)
IV	14 (39%)	9 (26%)
Unknown	6 (17%)	5 (15%)
Platinum-free interval*		
3 months	13 (36%)	13 (38%)
>3 to <6 months	23 (64%)	21 (62%)
Previous therapy lines		
1	10 (28%)	3 (9%)
2	18 (50%)	20 (59%)
3	5 (14%)	6 (18%)
4	2 (6%)	4 (12%)
5	0	1 (3%)
8	1 (3%)	0
Previous therapy lines in platinum-resistant setting		
1	11 (31%)	14 (41%)
0	25 (69%)	20 (59%)
Previous PARP inhibitor		
Yes	7 (19%)	11 (32%)
No	29 (81%)	23 (68%)

	Gemcitabine alone (n=36)	Gemcitabine plus berzosertib (n=34)
Previous antiangiogenic therapy		
Yes	9 (25%)	10 (29%)
No	27 (75%)	24 (71%)
BRCA mutated		
Yes	5 (14%)	6 (18%)
No	25 (69%)	21 (62%)
Unknown	6 (17%)	7 (21%)

* Stratification factor. ECOG=Eastern Cooperative Oncology Group.

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Table 2:

Secondary endpoints

	Objective response rate		Clinical benefit rate		6-month progression-free survival	
	Gemcitabine alone (n=36)	Gemcitabine plus berzosertib (n=34)	Gemcitabine alone (n=36)	Gemcitabine plus berzosertib (n=34)	Gemcitabine alone (n=36)	Gemcitabine plus berzosertib (n=34)
All patients	4 (11%)	1 (3%)	9/36 (25%, 14–40)	12/34 (35%, 22–51)	36% (24–55)	50% (35–71)
Platinum-free interval 3 months	1/13 (8%) [†]	1/13 (8%) [‡]	3/13 (23%, 7–49)	7/13 (54%, 29–78)	31% (16–61)	53% (31–91)
Platinum-free interval 3–6 months	3/23 (13%) [§]	0/21	6/23 (26%, 12–45)	5/21 (24%, 10–44)	39% (23–65)	48% (30–77)

Data are n (%), n/N (%), n/N (%), 90% CI), or % (90% CI).

* Two-sided Fisher's exact test.

[†] Duration of response 10 weeks.

[‡] Duration of response 7 weeks.

[§] Duration of response 6 weeks, 18 weeks, and 27 weeks.

Clinical benefit rate was defined as the proportion of patients showing complete response, partial response, or stable disease for 4 months or more.

Table 3:

Treatment-related adverse events

	Gemcitabine (n=36)				Gemcitabine plus berzosertib (n=34)			
	Grades 1–2	Grade 3	Grade 4	Grade 4	Grades 1–2	Grade 3	Grade 4	Grade 4
Blood and lymphatic system disorders								
Anaemia	21 (58%)	4 (11%)	0	0	18 (53%)	5 (15%)	0	0
Febrile neutropenia	1 (3%)	1 (3%)	0	0	0	1 (3%)	0	0
Haemolytic uraemic syndrome	0	1 (3%)	0	0	0	0	0	0
Thrombocytosis	0	0	0	0	1 (3%)	1 (3%)	0	0
Lymphocyte count decreased	5 (14%)	1 (3%)	0	0	0	1 (3%)	0	0
Neutrophil count decreased	7 (19%)	10 (28%)	4 (11%)	4 (11%)	8 (24%)	12 (35%)	4 (12%)	4 (12%)
Platelet count decreased	4 (11%)	0	2 (6%)	0	9 (26%)	2 (6%)	6 (18%)	6 (18%)
White blood cell decreased	4 (11%)	4 (11%)	0	0	5 (15%)	3 (9%)	0	0
Cardiac disorders								
Myocardial infarction	0	1 (3%)	0	0	0	0	0	0
Endocrine disorders								
Addisonian crisis	0	0	0	0	0	1 (3%)	0	0
Gastrointestinal disorders								
Abdominal pain	1 (3%)	0	0	0	0	1 (3%)	0	0
Constipation	4 (11%)	0	0	0	6 (18%)	0	0	0
Diarrhoea	6 (17%)	0	0	0	8 (24%)	1 (3%)	0	0
Gastric haemorrhage	0	1 (3%)	0	0	0	0	0	0
Mucositis oral	1 (3%)	0	0	0	6 (18%)	0	0	0
Nausea	17 (47%)	1 (3%)	0	0	19 (56%)	1 (3%)	0	0
Vomiting	7 (19%)	0	0	0	12 (35%)	1 (3%)	0	0
General disorders and administration site conditions								
Chills	2 (6%)	0	0	0	6 (18%)	0	0	0

	Gemcitabine (n=36)			Gemcitabine plus bezosertib (n=34)		
	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4
Oedema limbs	6 (17%)	1 (3%)	0	1 (3%)	0	0
Fatigue	18 (50%)	3 (8%)	0	19 (56%)	3 (9%)	0
Fever	3 (8%)	0	0	8 (24%)	0	0
Flu-like symptoms	4 (11%)	0	0	4 (12%)	0	0
Injury, poisoning, and procedural complications						
Fall	0	1 (3%)	0	0	0	0
Investigations						
Alanine aminotransferase increased	7 (19%)	0	0	10 (29%)	0	0
Aspartate aminotransferase increased	8 (22%)	0	0	12 (35%)	0	0
Cardiac troponin T increased	0	1 (3%)	0	0	0	0
Metabolism and nutrition disorders						
Anorexia	5 (14%)	0	0	7 (21%)	0	0
Musculoskeletal and connective tissue disorders						
Generalised muscle weakness	1 (3%)	0	0	2 (6%)	1 (3%)	0
Myalgia	3 (8%)	0	0	5 (15%)	0	0
Nervous system disorders						
Headache	3 (8%)	0	0	7 (21%)	1 (3%)	0
Respiratory, thoracic, and mediastinal disorders						
Dyspnoea	2 (6%)	1 (3%)	0	5 (15%)	0	0
Skin and subcutaneous tissue disorders						
Maculopapular rash	5 (14%)	0	0	4 (12%)	0	0
Vascular disorders						

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	Gemcitabine (n=36)		Gemcitabine plus berzosertib (n=34)		
	Grade 1-2	Grade 3	Grade 4	Grade 3	Grade 4
Capillary leak syndrome	0	1 (3%)	0	0	0

Grade 1 and 2 adverse events occurring in at least 10% of patients and all grade 3 and 4 adverse events are shown. There was one treatment-related death in the gemcitabine alone group due to sepsis and one treatment-related death in the gemcitabine plus berzosertib group due to pneumonitis. There were no other deaths due to adverse events.