Gynecologic Oncology xxx (2020) xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

A multicenter open-label randomized phase II trial of paclitaxel plus EP-100, a novel LHRH receptor-targeted, membrane-disrupting peptide, versus paclitaxel alone for refractory or recurrent ovarian cancer

Anca Chelariu-Raicu [a], Alpa Nick [a,1], Renata Urban [b], Mary Gordinier [c], Carola Leuschner [d], Linda Bavisotto [d], Graziela Zibetti Dal Molin [a,2], John K. Whisnant [d], Robert L. Coleman [a,*,3]

[a] Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
[b] Department of Gynecologic Oncology, University of Washington Medical Center and Seattle Cancer Care Alliance, Seattle, WA, USA
[c] Department of Gynecologic Oncology, Norton Cancer Institute, Louisville, KY, USA
[d] Esperance Pharmaceuticals, Inc., Houston, TX, USA

HIGHLIGHTS

- EP-100 with weekly paclitaxel was shown to be safe and well tolerated.
- Patients in the combination arm received extended treatment beyond 6 cycles.
- Liver metastasis demonstrated greater benefit from combination as compared to paclitaxel in a posthoc analysis.
- There was no difference in response rate with the addition of EP-100 to paclitaxel in the overall population.

ARTICLE INFO

Article history:
Received 8 August 2020
Accepted 12 November 2020
Available online xxxx

Keywords:
EP-100
Paclitaxel
Recurrent ovarian cancer
LHRH receptor

ABSTRACT

Objective. This randomized open-label phase II study evaluated the safety and clinical activity of EP-100 plus weekly paclitaxel in patients with recurrent ovarian cancer expressing positive LHRH receptor.

Methods. In a limited "run-in" dose escalation phase for EP-100, six patients were treated with ascending dose levels (13 mg/m^2, 20 mg/m^2, 30 mg/m^2). In the randomized phase, patients received weekly paclitaxel (80 mg/m^2 intravenously) plus twice weekly EP-100 (30 mg/m^2 intravenously; combination arm) or weekly paclitaxel alone (80 mg/m^2 intravenously; paclitaxel arm). The primary study endpoint was overall response rate (ORR).

Results. Forty-four patients were then randomized to either the experimental combination arm ($n = 23$) or the standard of care paclitaxel monotherapy arm ($n = 21$). The ORR was 35% (95%CI 16%–57%) for the combination arm and 33% (95% CI 15%–57%) for the paclitaxel arm. An interesting observation from an unplanned analysis was that a subset of patients with target liver lesions showed a greater overall response rate to the combination (69%) compared to paclitaxel alone (16%). The frequency of treatment-related grade 3–4 adverse events was similar between treatment arms: 48% vs 43% for the combination and paclitaxel arms, respectively.

Conclusions. ORR in the EP-100 combination arm was similar to that in the group treated with paclitaxel alone; however, a subset of patients with liver metastases appeared to benefit from the combination. The addition of EP-100 did not appear to augment the adverse event profile of paclitaxel and was well tolerated.

1. Introduction

Treatment of recurrent ovarian cancer remains a major clinical challenge. Paclitaxel is an effective agent for ovarian cancer. In addition to its use in combination with carboplatin after initial diagnosis, paclitaxel is commonly employed as single-agent therapy for patients with tumor progression or recurrence [1]. Single-agent paclitaxel administered weekly at a dose of 80 mg/m^2 intravenously has had an objective response rate (ORR) of 20% to 25% when administered in patients

* Corresponding author at: 10101 Woodloch Forest Drive, The Woodlands, TX 77380, USA.
E-mail address: robert.coleman@usoncology.com (R.L. Coleman).
[1] Currently at Department of Gynecologic Oncology, Tennessee Oncology, Nashville, Tennessee, USA.
[2] Currently A Beneficência Portuguesa de São Paulo, São Paulo, Brazil.
[3] Currently at US Oncology Research, The Woodlands, Texas.

https://doi.org/10.1016/j.ygyno.2020.11.013
0090-8258/

experiencing disease progression following treatment with regimens containing paclitaxel at the conventional dose of 175 mg/m^2 administered intravenously in an every 3 week treatment schedule [2].

Luteinizing hormone-releasing hormone (LHRH) receptors are expressed by many types of solid tumors [3–12]. LHRH receptors are not expressed, however, in non-cancerous tissue cells, apart from those in endocrine cells which express LHRH receptors located within the pituitary and reproductive organs. In gynecologic tumors, 80% of human ovarian and endometrial tumors express receptors for LHRH [13,14]. Thus, LHRH receptors represent a potential target for personalized medicine directed against LHRH receptor expressing tumors [15], while sparing non-cancerous tissues.

EP-100 is a 28-amino acid synthetic peptide being developed as a novel targeted, membrane-disrupting anticancer agent. Its first 18 amino acids comprise the cationic lytic peptide, which is followed by the 10-amino acid sequence of the natural LHRH hormone, synthesized without a linker using solid phase chemistry. The lytic EP-100 peptide thereby selectively targets cancer cells expressing the LHRH receptor, conferring EP-100 with both targeted cytotoxicity for LHRH receptor-positive tumors and the capacity for rapid membrane disruptive effects leading to cellular necrosis [16–18].

Monotherapy and combination studies in multidrug-resistant LHRH receptor-expressing ovarian, breast, uterine sarcoma and prostate cell lines suggested that addition of EP-100 could overcome the resistance and restore chemosensitivity to standard of care agents (paclitaxel, doxorubicin) [16,17]. More specifically, experiments utilizing the above-mentioned cancer cell lines showed showed reversion of drug resistance and potentiation of toxicity in LHRH-receptor-positive cell lines.

This open-label randomized phase II study was designed to first establish the recommended phase 2 dose (RP2D) of EP-100 when administered in combination with a standard regimen of weekly paclitaxel while evaluating the safety and tolerability of this regimen. The second aim of the study was to confirm and compare the safety and antitumor activity of this experimental combination against the concurrent control arm of standard paclitaxel monotherapy in patients with LHRH receptor-expressing advanced recurrent or refractory epithelial ovarian cancers.

2. Methods

2.1. Study design and participants

This study was conducted at 15 sites in the United States with a run-in phase and a randomized phase. The study was approved by Institutional Review Boards in every center and all patients provided written informed consent before enrollment. The trial is registered with ClinicalTrials.gov, number NCT01485848 and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice.

Eligible participants were aged 18 years or older and had histologically confirmed epithelial ovarian carcinomas. This study enrolled patients with advanced disease who had received at least two prior regimens of chemotherapy, whose disease had progressed during or had recurred, and who had no other proven therapy options. The majority of patients (81.8%) had relapsed or progressed <6 months after their last therapy. Prior regimens of chemotherapy included different durgs such as paclitaxel in combination with platinum, gemcitabine, liposomal doxorubicin, topotecan. The use of paclitaxel in combination with platinum prior to progression was allowed to be weekly as well as q3w. Patients who had received paclitaxel as single agents were excluded. Eligible patients were required to have confirmation of LHRH receptor-positive tumor tissue prior to enrollment and randomization, as reviewed by central pathologic review of tumor specimens obtained from archival tumor blocks or slides. Standardized immunohistochemical testing using a commercially available LHRH receptor-directed monoclonal antibody, with analysis of LHRH receptor staining intensity graded by a single pathologist and rated from 1+ to 3+ on the basis of semiquantitative visual assessments of the number of positive tumor cells per high-power field.

Other key eligibility criteria included measurable disease, based on Response Evaluation Criteria in Solid Tumors (RECIST 1.1) [19] or the Gynecologic Cancer Intergroup (GCIG) criteria for nonmeasurable but evaluable disease [20], respectively, within 28 days of initiating the study treatment. Patients with nonmeasurable but evaluable disease as defined above were required to have had a CA-125 tumor marker value at least twice the upper limit of normal within 14 days of initiating the study treatment, as well as a Karnofsky performance status ≥70% and adequate bone marrow, hepatic, and renal function.

2.1.1. Run-in dose-escalation phase

The objective of the dose-escalation run-in phase was to establish a RP2D of EP-100 when administered in combination with standard dosing of weekly paclitaxel, based on safety and tolerability, with monitoring for any dose-limiting toxicities (DLTs) during the first treatment cycle. Sequentially enrolled patients were assigned to one of three escalating EP-100 dose levels, starting with the lowest dose level and continuing through planned escalations of approximately 50% for each subsequent dose level (13 mg/m^2, 20 mg/m^2, 30 mg/m^2) of intravenous EP-100 infusion on weeks 1, 2, and 3 of each 4-week cycle (days 1, 4, 8, 11, 15, and 18), along with weekly 1-h 80 mg/m^2 intravenous paclitaxel infusions (days 1, 8, 15, 22). On days when both drugs were administered, the EP-100 infusion was administered first followed by the paclitaxel, to allow monitoring for any infusion reactions potentially related to EP-100. No intra-patient dose escalation was permitted during the run-in phase; each patient remained at their assigned dose level for the duration of their study participation. EP-100 has a short half-life and demonstrated few adverse effects in Phase I. Therefore, due to high tolerability, the dose was rapidly escalated to 30 mg/m^2 and 4 patients were treated with this dose to confirm safety. Dose-limiting toxicities were assessed during cycle 1, graded according the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

2.1.2. Randomized phase

The run-in dose escalation phase confirmed a RP2D of EP-100 to be 30 mg/m^2 intravenously twice weekly. An earlier first-in-human Phase I study of EP-100 in patients with advanced solid tumors had established that this dose and schedule of EP-100 as single-agent was not myelosuppressive nor was it associated with significant adverse events (AEs) [15]. Prophylaxis with granulocyte colony stimulating factor (G-CSF) was not used routinely but could be added at the investigator's discretion. Following the safe completion of the run-in phase, newly enrolled patients were randomized 1:1 to receive either standard weekly paclitaxel (80 mg/m^2) on days 1, 8, 15, and 22 of each 28-day cycle or the same regimen of weekly paclitaxel plus twice weekly doses of 30 mg/m^2 EP-100. The randomization was stratified by time to progression (TTP) or recurrence at <6 months or ≥6 months after patient's most recent regimen prior to enrollment. Randomization for patients at all study sites was accomplished via a central Interactive Voice Response System (IVRS).

Patients randomized to the weekly paclitaxel monotherapy treatment were permitted to crossover to treatment with the combination of EP-100 plus weekly paclitaxel ("crossover arm") upon confirmed development of progressive disease according to RECIST 1.1 or GCIG criteria, after receiving at least two cycles of the treatment. The imaging scan and/or CA-125 tumor marker levels which established progressive disease were taken as the new baseline for assessing potential antitumor activity of following initiation of treatment in the crossover combination arm. Patients assigned to the run-in or randomization phase dose who completed six cycles of randomized treatment without evidence of progressive disease and deemed by investigator to deriving clinical benefit were eligible for extended, continuing on the originally

assigned treatment arm until development of progressive disease or meeting another discontinuation criterion (unacceptable adverse event, serious noncompliance with the study protocol, investigator decision or withdrawal of patient consent).

2.2. Endpoints and assessments

The primary study endpoint was ORR, as defined by RECIST 1.1. Secondary endpoints included disease control rate (DCR), proportion of evaluable patients attaining complete or partial response (PR), as well as those with stable disease (SD) of at least 3 months duration, duration of response (DOR), time-to-progression (TTP) and progression-free survival (PFS). CA-125 responses were assessed according to GCIG criteria for each patient, including those with RECIST-measureable as well as non-measurable but evaluable disease. Disease assessments for all patients were conducted on fixed schedules (monthly for CA-125 levels and following every two treatment cycles by imaging of target lesions). Evidence of pharmacologic activity of EP-100 as measured by endocrinologic parameters FSH and LH were recorded at baseline and followed each cycle.

2.3. Statistical analysis

The study was not powered to test any comparison of the randomized treatment arms; all analyses were descriptive. We estimated the response rate in 20 patients receiving single agent paclitaxel to be 20% (95% CI 7.7–42.3%) and in 20 patients receiving combination paclitaxel and EP100 to be 50% (95% CI 31.1–70.1%). Enrollment of a total of 40 patients randomized equally between the two treatment arms was deemed sufficient to assess the preliminary clinical activity of the combination regimen compared to standard of care weekly paclitaxel as single agent.

3. Results

Over a 26 month period, 50 patients were enrolled: 6 in the run-in dose escalation phase and 44 in the randomized phase of the study (Fig. 1). The six patients enrolled in the run-in phase received pre-planned escalating doses of EP-100: one patient at 13 mg/m^2, one patient at 20 mg/m^2, and four patients at 30 mg/m^2. During the randomized phase of the study, an additional 44 patients were enrolled and randomized 1:1 to receive either combination therapy with EP-100 plus paclitaxel (experimental arm, $n = 23$) or standard weekly paclitaxel (active control arm, $n = 21$). Ten of the 21 patients (48%) randomized to treatment in the paclitaxel monotherapy treatment arm developed confirmed disease progression on study following at least 2 treatment cycles and qualified for crossover to receive combination treatment arm (crossover arm). Fig. 1 shows schematically the assignment of patients to the combination, paclitaxel monotherapy, and crossover arms.

Baseline demographic and clinical characteristics for all study patients are summarized in Table 1, and further reviewed in the Randomized Phase discussion below.

3.1. Run-in dose-escalation phase

No dose-limiting toxicities were reported, and no maximum tolerated dose of EP-100 in combination with weekly paclitaxel was established. The RP2D of EP-100 was established at 30 mg/m^2, in combination with standard weekly paclitaxel, after four patients were safely treated at this dose level. Individual RECIST v1.1-assessed responses for 4 patient with measurable disease is shown in Supplementary Fig. S2A. ORR by RECIST v1.1 was 33% (1 PR, 1 CR) and the disease control rate was 100%. One patient remained in remission after 18 months from treatment start, with no residual disease on imaging and normalization of the CA-125. The median TTP was 4.5 months (range 2–18 months). Individual responses determined by GCIG criteria for all 6 run-in patients is shown in Supplementary Fig. S2B: one patient achieved CR, two patients achieved PR, and the other three patients had a $< 50\%$ reduction of the tumor marker; no patients showed progression.

3.2. Randomized phase

Patients in both treatment arms received a median of four cycles of treatment with a range of 2 to16 cycles in the combination arm, and 2

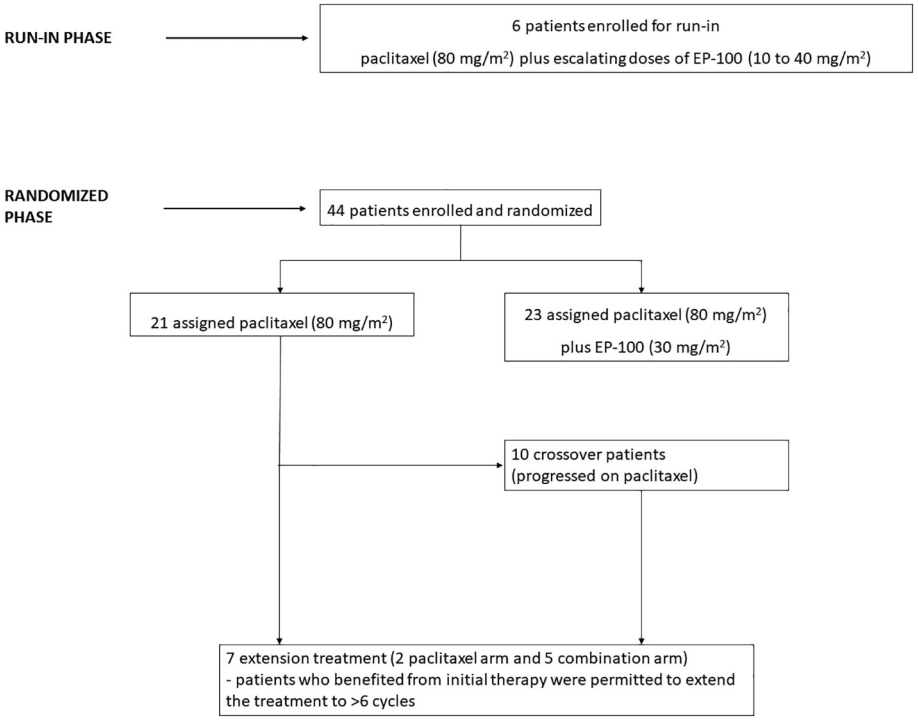

Fig. 1. Flowchart showing original randomization assignment for all patients and subsequent movements into extension treatment or crossover arm.

Table 1
Baseline demographic and clinical characteristics in the randomized population.

Characteristic	No. (%)	
	Paclitaxel, $n = 21$	EP-100 and paclitaxel, $n = 23$
Median age (range) (years)	68 (43–91)	60 (25–75)
Age group		
<65 years	8 (38)	17 (74)
65–74 years	11 (52)	5 (22)
>74 years	2 (10)	1 (4)
Race/ethnicity		
White	19 (90)	19 (83)
Black	0 (0)	1 (4)
Asian	0 (0)	1 (4)
Other	2 (10)	2 (9)
Karnofsky performance status		
70–80	11 (52)	4 (17)
90–100	10 (48)	19 (83)
Histologic subtype		
High-grade serous	17 (81)	16 (70)
Low-grade serous	5 (24)	0 (0)
Adenomatous	3 (14)	1 (4)
Clear cell	0 (0)	2 (9)
Endometrioid	0 (0)	2 (9)
Mucinous	0 (0)	1 (4)
Others	1 (5)	1 (4)
Baseline CA-125		
≤2 ULN	1 (5)	7 (30)
>2 ULN	20 (95)	16 (70)
Liver target lesions	6 (29)	10 (43)
Prior regimens		
1	1 (4)	3 (13)
2	4 (19)	5 (22)
3	2 (10)	4 (17)
>3	14 (67)	11 (48)
Time from previous therapy to relapse or progression		
<6 months	19 (90)	17 (74)
>6 months	2 (10)	6 (26)

ULN: upper limit of normal range for laboratory.

to 8 cycles in the paclitaxel arm. Roughly comparable numbers of patients completed 6 cycles of treatment in both treatment arms: 6 of 23 patients (26%) in the combination arm, and 6 of 21 patients (29%) in the paclitaxel monotherapy arm. A higher proportion of patients randomized to the combination arm, however, entered extended treatment following the completion of 6 treatment cycles: 5/23 (22%) patients in the combination arm, vs 2/21 (9%) patients in the paclitaxel monotherapy arm, supporting the tolerability of extended combination treatment. The most frequent reason for treatment discontinuation was progressive disease (65% in the combination arm and 57% in the paclitaxel arm). At the time of the primary data cutoff, the median duration of follow-up was 17.8 months in the combination arm and 16.3 months in the paclitaxel arm.

3.3. ORR and duration of response

Intention to treat analysis of the primary endpoint of ORR included all 44 patients in the randomized treatment phase, all of whom initiated treatment at the RP2D. Results are summarized in Table 2. ORR was similar between treatment arms: 35% (95% CI 16–57%) in the combination arm compared to 33% (95% CI 15–57%) in the paclitaxel arm. DCR was similar between arms: 74% (95% CI 52–90%) for the combination arm and 71% (95% CI 48–89%) for the paclitaxel arm. A waterfall plot showing the maximum percent change from baseline (max CFB) in the sum of the diameters of target lesions according to RECIST v1.1 criteria for each patient with measurable disease and having a post-baseline tumor measurement is presented in Fig. 2A.

Table 2
Response rates in the randomized population (intention-to-treat analysis).

	No. (%)	
	Paclitaxel, $n = 21$	EP-100 and paclitaxel, $n = 23$
Best objective RECIST response		
Complete response	1 (5)	2 (9)
Partial response	6 (29)	6 (26)
Overall response	7 (33)	8 (35)
Non-response		
Stable disease	8 (38)	9 (39)
Progressive disease	5 (24)	6 (26)
Not evaluable	1 (5)	0 (0)
CA-125 response rate (GCIG criteria)		
Complete response	0 (0)	0 (0)
Partial response	13 (62)	9 (39)
Stable disase	6 (29)	6 (26)
Progressive disease	1 (5)	3 (13)
Not evaluable	1 (5)	5 (22)

RECIST: Response Evaluation Criteria in Solid Tumors version 1.1; GCIG: Gynecologic Cancer Intergroup in evaluable patients.

There were no complete responders in either treatment arm per GCIG criteria. ORR per GCIG criteria, was higher in the paclitaxel monotherapy arm than in the combination arm:13/21 (62%) vs 9/23 (39%), respectively. Quantitative responses in CA-125 levels and maximum percent change from baseline for each patient are shown in the Fig. 2B.

Median DOR was doubled in the combination arm compared to the paclitaxel monotherapy arm: 8.8 vs 4.2 months. Median TTP was comparable between treatment groups, 4 months (95% CI 1.8 to 5.7 months) for the combination arm and 4.7 months (95% CI 3.3 to 8.0 months) for the paclitaxel arm.

3.4. Target liver lesion responses

Pre-clinical biodistribution studies showed that EP-100 localizes in the liver, with label persisting there for at least 72 h [Esperance confidential data]. This observation prompted an additional post-study analysis to assess and compare between treatment arms the ORR in the subset of all patients with liver metastases and measurable disease. Ten patients in the combination arm and six patients in paclitaxel monotherapy arm had 13 vs 6 target lesions in the liver respectively, as evaluated by imaging of liver at baseline. A ≥ 30% reduction in the sum of the diameters of the target liver lesions occurred in 69% of the liver target lesions in the combination arm vs. only 16% of those receiving treatment with paclitaxel monotherapy (2 CR and 7 PR in the combination arm vs 1 CR in the paclitaxel arm for target liver lesion responses). The degree of response in each liver lesion is shown in Fig. 3A and B.

3.5. Progression-free survival

The primary analysis of progression-free survival was done after 16/21 (76%) progression events in the paclitaxel arm and 20/23 (87%) progression events in the combination arm. Median PFS durations were similar between the two arms (combination arm: 4 months, 95% CI 1.8–5.7 months; paclitaxel arm: 4.6 months, 95% CI 3.85.5 months; Supplementary Fig. S2).

Post hoc analysis of the data, adjusted for age, showed that the median progression-free survival duration for patients younger than 65 years was 3.9 months (95% CI 1.8–6.4 months) in the combination arm and 2.3 months (95% CI 1.4–4.9 months) in the paclitaxel arm.

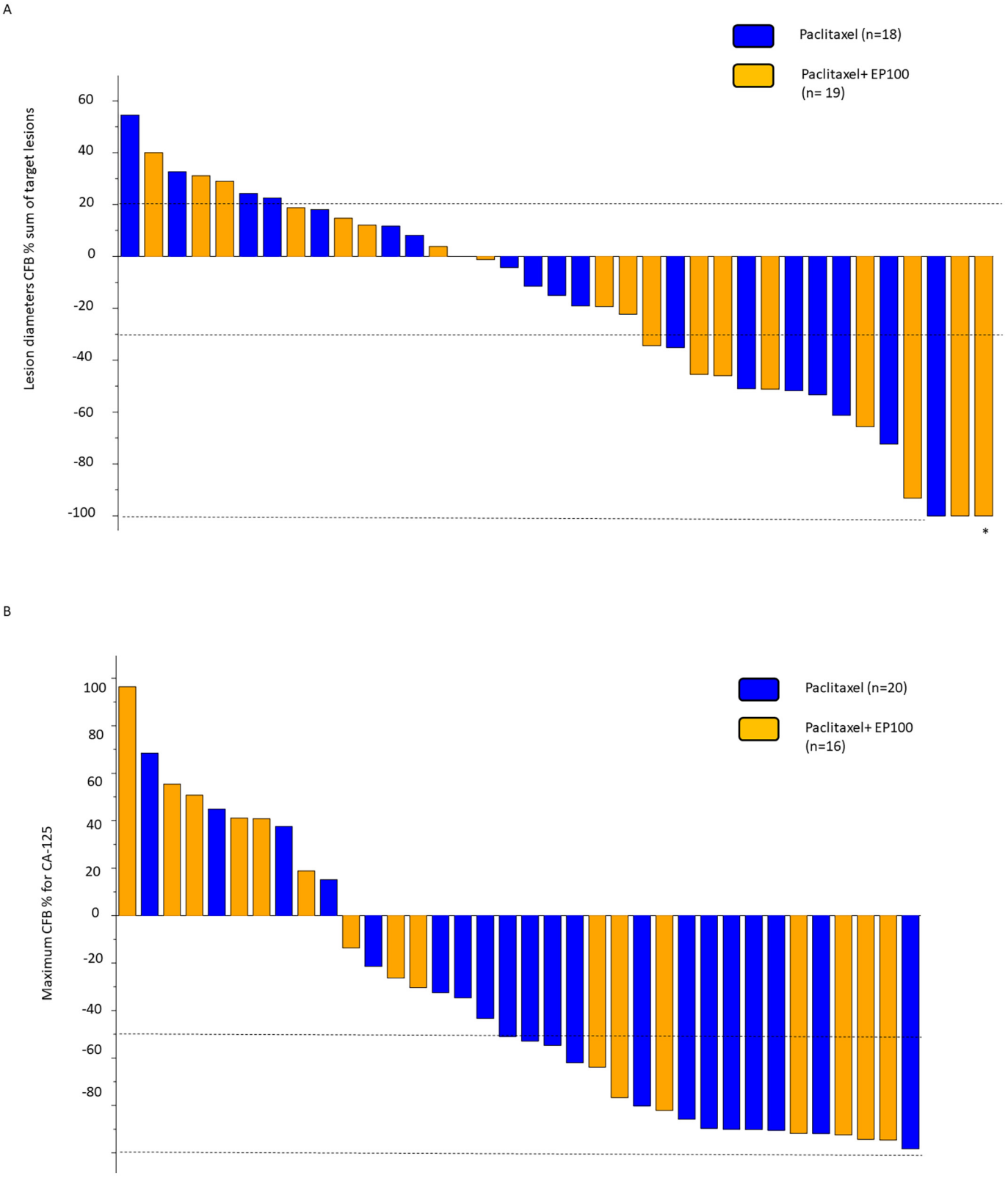

Fig. 2. Waterfall plots according to treatment arm, showing overall response rate on study for evaluable individual patients. A: Individual best overall responses in evaluable patients with RECIST-measurable disease and at least one post-treatment disease assessment, showing maximum percentage change from baseline (CFB) for sum of the diameters of target lesions. * One patient in the combination arm showed complete disappearance of a splenic target lesion, but best overall response was assessed as PR due to persistence of non-target lesions. B: Individual best overall responses in all patients, per GCIG criteria, indicating maximum percent change from baseline (CFB) in CA-125 levels.

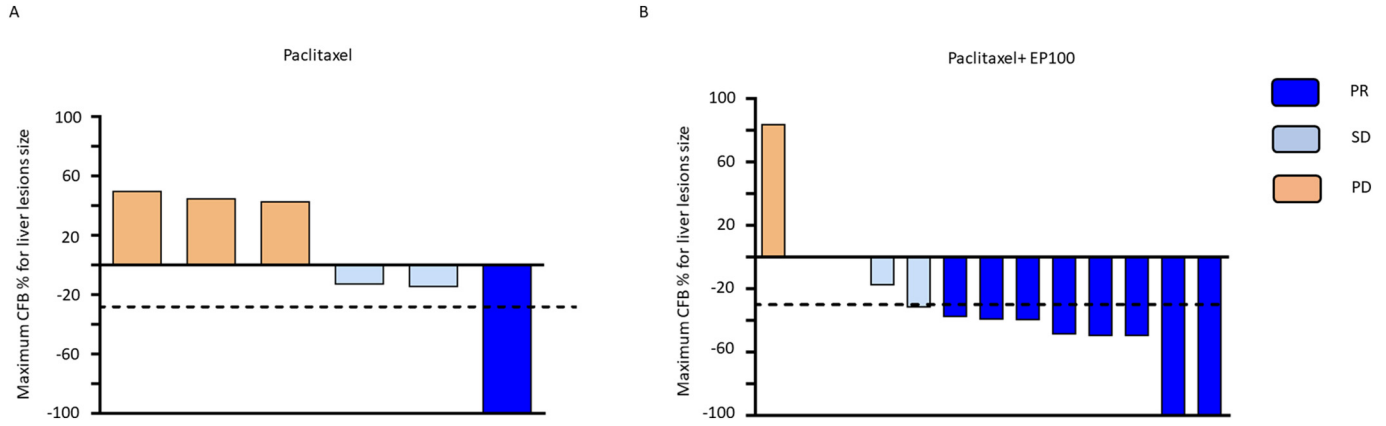

Fig. 3. Maximum CFB for liver lesions (in all patients with measurable disease, according to Response Evaluation Criteria in Solid Tumors version 1.1). PR: partial response; SD: stable disease; PD: progressive disease. A: Maximum CFB for the sum of target lesion sum of diameters in the paclitaxel arm. B: Maximum CFB for the sum of target lesion sum of diameters in the combination arm.

3.6. Safety profile

Table 3 shows the most commonly occurring AEs in both treatment arms. The incidence of high-grade (grade 3 or 4) AEs was similar between the two arms: 11 patients (48%) in the combination arm and 9 patients (43%) in the paclitaxel arm.

The addition of twice weekly EP-100 infusions to a weekly paclitaxel regimen did not significantly alter the AE profile of weekly paclitaxel, except for an increase in the incidence of infusion-related reactions, which occurred in 12 patients in the combination arm (52%) compared with 5 patients in the paclitaxel arm (24%). The most common AEs were abdominal pain, anemia, peripheral edema, vomiting, rash, flushing, back pain, pruritus, upper abdominal pain, and parasthesia (Table 3). Clinically, although treatment-emergent, many of the gastrointestinal and abdominal complaints in both treatment arms, including the occurrence of small bowel obstructions, could be related to the natural history of the underlying advanced ovarian cancer and/or to worsening of the effects of pre-existing treatment regimens with continuing taxane treatment being common to both treatment arms. There were no AE-related deaths in the study and no safety issues identified in the laboratory, vital sign, or electrocardiogram assessments.

3.7. Crossover patients

Ten patients with documented disease progression while receiving paclitaxel alone crossed over to receive combination therapy. Clinical characteristics of the crossover group including age, histology, prior chemotherapy, time from last exposure to paclitaxel and cumulative dose of paclitaxel are summarized in Supplementary Table S2. Following crossover, these patients received a median of 2 (range: 1–7) cycles of treatment. In 5 of the 10 (50%) of these patients, time on study was extended while maintaining stable disease for 3–7 months. Interestingly, two patients from the responders presented low-serous histology and one of them attained a partial response after roll-over. Of the five patients with stable disease after crossover, 3 of the 5 patients attained a TTP on crossover treatment that was three-fold more prolonged relative to their own respective previous TTP following on-study treatment in the paclitaxel monotherapy arm. Overall, five crossover patients (50%) had a TTP benefit of 3 months or more, suggesting a clinical benefit of a median TTP improvement of 3 months in these heavily pretreated patients who had been actively progressing on paclitaxel alone.

3.8. Pharmacodynamics

Pharmacologic effects of EP-100 exposure were assessed in part by monitoring each patient's circulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels. Patients showed a range of baseline values that were somewhat related to age and hormonal status, as predicted. There was a range of change from baseline values, but neither positive nor negative shifts correlated with objective disease responses in the combination arm (data not shown). No clinically significant unintended endocrine effects referable to pituitary or reproductive organs were observed in this study; all patients were naturally or surgically post-menopausal at study baseline, however.

4. Discussion

In this multicenter, phase II clinical trial, we demonstrate that EP-100, at a dose of 30 mg/m^2, in combination with paclitaxel, at the a dose of 80 mg/m^2, is safe and well tolerated in patients with refractory and recurrent measurable epithelial ovarian cancer. Given the similar ORR between the two study arms, it appears that the addition of EP-100 at this dose and schedule failed to show an improvement in a population of patients that were selected using a commercial antibody test for LHRH receptors.

One surprising finding was of more frequent objective responses in target lesions located in the liver in patients in the combination arm (69%) compared with patients in the paclitaxel arm (16%). Ovarian cancer patients who have target liver lesions are considered to have further advanced disease (stage IV) with a typically worse prognosis [21] than do those who do not have liver lesions. Most importantly, accumulation of EP-100 in the liver did not cause any liver toxicities in patients even after twice weekly infusions over 18 months. Preclinical biodistribution studies showed that EP-100 accumulated primarily in the liver and is detectable for up to 72 h [Esperance confidential data], which may explain the greater treatment response we observed in the combination arm [22,23]. Another potential explanation could be that paclitaxel is metabolized in the liver by cytochromes P450 2C8 and 3A4 that could generate potentially interaction between these pathways, paclitaxel, and EP-100. However, pre-clinical metabolic studies in human hepatocytes showed that EP-100 does not induce metabolosim activity in CYP1A2 and CYP3A4 [Esperance confidential data].

Analysis of crossover arm patients permitted a longitudinal within-subject analysis of patients originally randomized into the paclitaxel monotherapy arm who crossed over to receive combination therapy

Table 3
Treatment-emergent adverse events of any grade in patients in either arm of the randomized phase.

Primary organ system class	No. (%)			
	Paclitaxel, $n = 21$		EP-100 and paclitaxel, $n = 23$	
	All grades	Grade ≥ 3	All grades	Grade ≥ 3
Blood and lymphatic system disorders	0 (0)	0 (0)	1 (4)	1 (4)
Anemia	20 (95)	2 (10)	23 (100)	3 (13)
Leucocytes decreased	18 (86)	3 (14)	18 (78)	2 (9)
Neutrophil count decreased	13 (62)	5 (24)	13 (57)	2 (9)
Platelet count decreased	4 (19)	0 (0)	6 (26)	2 (9)
Febrile neutropenia	0 (0)	0 (0)	1 (4)	1 (4)
Metabolism and nutrition disorders	1 (5)	1 (5)	1 (4)	1 (4)
Dehydration	1 (5)	1 (5)	1 (4)	1 (4)
Decreased appetite	1 (5)	1 (5)	0 (0)	0 (0)
Hypocalcemia	15 (71)	0 (0)	16 (70)	0 (0)
Hyperkalemia	0 (0)	0 (0)	2 (9)	0 (0)
Hypokalemia	10 (48)	3 (14)	8 (35)	0 (0)
Hypernatremia	2 (10)	0 (0)	2 (9)	0 (0)
Hyperglycemia	15 (71)	0 (0)	19 (83)	0 (0)
Infections and infestations	1 (5)	0 (0)	0 (0)	0 (0)
Cellulitis	1 (5)	0 (0)	0 (0)	0 (0)
Vascular disorders	0 (0)	0 (0)	1 (4)	0 (0)
Jugular vein thrombosis	0 (0)	0 (0)	1 (4)	0 (0)
Respiratory, thoracic, and mediastinal disorders	1 (5)	1 (5)	1 (4)	0 (0)
Dyspnea	1 (5)	1 (5)	1 (4)	0 (0)
Gastrointestinal disorders	3 (14)	3 (14)	5 (22)	5 (22)
Small intestinal obstruction	2 (10)	2 (10)	3 (13)	3 (13)
Abdominal pain	0 (0)	0 (0)	1 (4)	1 (4)
Ascites	0 (0)	0 (0)	1 (4)	1 (4)
Intestinal obstruction	0 (0)	0 (0)	1 (4)	1 (4)
Nausea	0 (0)	0 (0)	1 (4)	1 (4)
Vomiting	2 (10)	2 (10)	0 (0)	0 (0)
Liver function	3 (14)	3 (14)	5 (22)	5 (22)
Alanine aminotransferase increased	2 (10)	2 (10)	3 (13)	3 (13)
Aspartate aminotransferase increased	0 (0)	0 (0)	1 (4)	1 (4)
Blood blilirubin increased	0 (0)	0 (0)	1 (4)	1 (4)
Hypoalbuminemia	10 (48)	0 (0)	10 (43)	1 (4)
Skin and subcutaneous tissue disorders	2 (10)	1 (5)	0 (0)	0 (0)
Blister	1 (5)	0 (0)	0 (0)	0 (0)
Skin ulcer	1 (5)	1 (5)	0 (0)	0 (0)
Renal and urinary disorders	1 (5)	1 (5)	0 (0)	0 (0)
Oliguria	1 (5)	1 (5)	0 (0)	0 (0)
Increased creatinine	1 (5)	0 (0)	4 (17)	0 (0)

at the time of confirmed disease progression (signifiying development of taxane resistance). Crossover patients showed a 3 times greater time to progression (TTP), following addition of EP-100 in combination, compared to their own previous TTP on study in the paclitaxel monotherapy arm. This finding is supportive in the clinical setting of this study of the hypothesis based on preclinical findings that EP-100 when combined with to standard weekly paclitaxel may reverse prior taxane resistance and re-sensitize to the effects of paclitaxel.

The results of our study showing a clinical benefit of EP-100 in combination with chemotherapy is consistent with findings from trials of a drug similar to EP-100. Zoptarelin doxorubicin acetate is a cytotoxic hybrid molecule that consists of doxorubicin linked to an LHRH analog [15]. A clinical study that included 44 patients with advanced or recurrent endometrial cancer expressing LHRH receptors demonstrated a complete response in two (5%) and a partial response in 8 (18%) patients. The median TTP was 7 months and the median OS duration was 15 months [24]. Given these promising findings a multinational, multicenter phase III study (NCT01767155) was initiated, in which AEZS-108 was compared with doxorubicin monotherapy. However, data on OS, PFS and ORR presented at ASCO 2018 failed to show disease improvement in patients who were treated with AEZS-108 when compared with doxorubicin monotherapy.

Our study is the first randomized trial of EP-100 to assess the antitumor effect of EP-100 combined with weekly paclitaxel at 80 mg/m^2 versus paclitaxel alone in patients with relapsed or recurrent LHRH receptor-positive ovarian cancer. Despite showing insufficient clinical activity among all comers, the combination of EP-100 with paclitaxel revealed more frequent objective responses in liver lesions and 3 times greater TTP in patients that recently progressed on paclitaxel alone. Together, our findings suggest that EP-100 in combination with paclitaxel shows promise for the treatment of liver metastases, and may be fruitful avenue for investigation in future studies.

Despite randomization, several potentially confounding imbalances in patient characteristics between treatment groups were identified including median age (proportion of patients greater than or less than 65 yrs), median number of prior treatment regimens, proportion of patients with higher or lower Karnofsky Performance Status, and an unequal distribution of more favorable or less favorable histologic subtypes between arms. Patients in the combination arm tended toward younger age and better performance than did those in the paclitaxel arm; the combination arm was disadvantaged, however, with a higher proportion of less responsive non-serous histologic subtypes (endometrioid, clear cell, mucinous). An imbalance in the representation of these less common histologic types between treatment arms could negatively impact the efficacy assessment of response rate and TTP analyses in the arm with the higher number of these less favorable subtypes (in this case, the combination arm). Five patients in the paclitaxel monotherapy arm had low-grade serous subtypes, which are associated with improved prognosis compared with other subtypes, whereas no patients in the combination arm had this subtype; this factor may have influenced evaluations of ORR or TTP to an extent unknown. Interestingly, two from three patients with low-serous histology who crossed over showed indeed evidence of a better clinical activity of paclitaxel in combination with EP-100, which can be a result of a higher response to other endocrine therapies of low-grade histology.

An additional confounding factor recognized post-study was that patient selection using a functional ligand assay for LHRH, rather than the commercial antibody assay as was employed for patient selection, would have more accurately determined the patients whose tumors were LHRH receptor positive. As a targeted therapy, the effectiveness of EP-100 is dependent upon tumor expression of functional LHRH receptors, thus the inclusion of patients with absence of receptors (false positives results using the commercial antibody assay) would predictably lead to a diminution of objective response rate, as seen in this enrolled patient population. A post study analysis of the same tumor sections used to screen for patient selection at enrollment were reviewed and retested using a newly developed LHRH ligand functional receptor assay. Only patients that tested positive for LHRH receptors using the functional assay showed clinical benefit to EP-100 + paclitaxel treatment with more than 3 month of TTP, while patients that tested negative had responses of less than 3 months or progressed in combination treatment group (Supplementaty Table S1). Therefore, the new functional LHRH ligand assay will be applied to select patients in future studies. Alternative dosing and schedule given the short half life of EP-100 (7.1 ± 3.8 to 15.9 ± 3.6 min) could offer better opportunity for increasing the drug's efficacy. More specifically, more frequent exposure and higher dose levels may optimize this agent's mechanism of action.

Among prognostic variables, BRCA status would have been an interesting clinical factor to have collected. Although none of the drugs included in the present trial have DNA damage related mechanism of action, BRCA status proved to be prognostic for other targeted therapies

such as angiogenic agents. A previous study demonstrated that the median PFS was longer for BRCA1/2 mutation carriers versus wild-type patients (30.3 vs 14.1 months; HR 0.48; 95% CI 0.29–0.78) [25]. Recently, other preclinical studies noted that EP-100 enhanced DNA damage accumulation, suppressed the PI3K/AKT pathway and inhibited BRCA1 in ovarian cancer cells.

Therefore, new potential mechanisms underlying an ability to the enhance the cytotoxic efficacy of PARP inhibitors would be rationale for developing future combination trials with EP-100 and PARP inhibitors [26].

5. Conclusions

In summary, EP-100 in combination with paclitaxel is well-tolerated but showed limited activity in patients who were heavily pretreated. Interestingly, liver metastases responded significantly better in the combination arm, and further investigation in this patient population is indicated. EP-100 may also increase TTP by sensitizing tumors previously resistant to paclitaxel. Using cytotoxic analogs of LHRH is a promising strategy for targeted therapy in patients with advanced disease such as liver lesions and LHRH receptor-positive cancer.

Author contribution

(I) Conception and design: All authors
(II) Administrative support: This trial was investigator initiated but sponsored by Esperance Pharmaceuticals.
(III) Provision of study materials or patients: AN, RU, MG, RLC
(IV) Collection and assembly of data: Sanofi's Clinical Sciences & Operations Department was engaged as a clinical research organization to perform trial functions including the selection, registration and management of clinical sites, the monitoring and collection of clinical and laboratory data, and the completion and reporting of the study. Sanofi's draft Complete Study Report (CSR) was transferred to Esperance for the final analyses and completion.
(V) Data analysis and interpretation: AC-R, AN, RLC
(VI) Manuscript writing: AC-R, AN
(VII) Final approval of manuscript: All authors

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2020.11.013.

Declaration of Competing Interest

Anca Chelariu-Raicu: None.
Alpa Nick: None.
Renata Urban: Royalties, editorial duties for UpToDate, Inc.
Mary Gordinier: None.
Carola Leuschner: Employment and consultant for Esperance Pharmaceuticals, Inc.
Linda Bavisotto: Consultant Esperance Pharmaceuticals, Inc.
Graziela Zibetti Dal Molina: None.
John K. Whisnant: Consultant Esperance Pharmaceuticals, Inc.
Robert L. Coleman: Research funding: Merck, AstraZeneca/Medimmune, Genentech/Roche, Novartis, Clovis Oncology, Abbvie, Janssen Pharmaceuticals; Consultant: Genmab, Tesaro, Agenus, OncoMed, Novocure, Oncoquest, Merck, AstraZeneca/Medimmune, Genentech/Roche, Novartis, Clovis Oncology, Abbvie, Janssen Pharmaceuticals, Aravive, OncoSec. Employment: US Oncology Research.

Acknowledgements

The authors thank Scientific Publications, Research Medical Library, at The University of Texas MD Anderson Cancer Center for editing the manuscript.

References

[1] D.K. Armstrong, Relapsed ovarian cancer: challenges and management strategies for a chronic disease, Oncologist. 7 (Suppl. 5) (2002) 20–28.
[2] N.C. Kampan, M.T. Madondo, O.M. McNally, M. Quinn, M. Plebanski, Paclitaxel and its evolving role in the management of ovarian cancer, Biomed. Res. Int. 2015 (2015) 413076.
[3] G. Falchook, R.L. Coleman, A. Roszak, K. Behbakht, U. Matulonis, I. Ray-Coquard, et al., Alisertib in combination with weekly paclitaxel in patients with advanced breast cancer or recurrent ovarian cancer: a randomized clinical trial, JAMA Oncol. 5 (1) (2019), e183773, .
[4] C. Fost, F. Duwe, M. Hellriegel, S. Schweyer, G. Emons, C. Grundker, Targeted chemotherapy for triple-negative breast cancers via LHRH receptor, Oncol. Rep. 25 (5) (2011) 1481–1487.
[5] S.V. Liu, A.V. Schally, D. Hawes, S. Xiong, L. Fazli, M. Gleave, et al., Expression of receptors for luteinizing hormone-releasing hormone (LH-RH) in prostate cancers following therapy with LH-RH agonists, Clin. Cancer Res. 16 (18) (2010) 4675–4680.
[6] K. Szepeshazi, A.V. Schally, G. Keller, N.L. Block, D. Benten, G. Halmos, et al., Receptor-targeted therapy of human experimental urinary bladder cancers with cytotoxic LH-RH analog AN-152 [AEZS- 108], Oncotarget. 3 (7) (2012) 686–699.
[7] G. Keller, A.V. Schally, T. Gaiser, A. Nagy, B. Baker, G. Halmos, et al., Receptors for luteinizing hormone releasing hormone expressed on human renal cell carcinomas can be used for targeted chemotherapy with cytotoxic luteinizing hormone releasing hormone analogues, Clin. Cancer Res. 11 (15) (2005) 5549–5557.
[8] B. Szende, G. Srkalovic, J. Timar, J.J. Mulchahey, J.D. Neill, K. Lapis, et al., Localization of receptors for luteinizing hormone-releasing hormone in pancreatic and mammary cancer cells, Proc. Natl. Acad. Sci. U. S. A. 88 (10) (1991) 4153–4156.
[9] F. Hohla, T. Winder, R. Greil, F.G. Rick, N.L. Block, A.V. Schally, Targeted therapy in advanced metastatic colorectal cancer: current concepts and perspectives, World J. Gastroenterol. 20 (20) (2014) 6102–6112.
[10] D. Hao, L. Sun, X. Hu, X. Hao, (99m)Tc-LHRH in tumor receptor imaging, Oncol. Lett. 14 (1) (2017) 569–578.
[11] G. Keller, A.V. Schally, T. Gaiser, A. Nagy, B. Baker, G. Westphal, et al., Human malignant melanomas express receptors for luteinizing hormone releasing hormone allowing targeted therapy with cytotoxic luteinizing hormone releasing hormone analogue, Cancer Res. 65 (13) (2005) 5857–5863.
[12] G. Keller, A.V. Schally, T. Gaiser, A. Nagy, B. Baker, G. Halmos, et al., Receptors for luteinizing hormone releasing hormone (LHRH) expressed in human non-Hodgkin's lymphomas can be targeted for therapy with the cytotoxic LHRH analogue AN-207, Eur. J. Cancer 41 (14) (2005) 2196–2202.
[13] S. Westphalen, G. Kotulla, F. Kaiser, W. Krauss, G. Werning, H.P. Elsasser, et al., Receptor mediated antiproliferative effects of the cytotoxic LHRH agonist AN-152 in human ovarian and endometrial cancer cell lines, Int. J. Oncol. 17 (5) (2000) 1063–1069.
[14] G. Srkalovic, A.V. Schally, J.L. Wittliff, T.G. Day Jr., E.L. Jenison, Presence and characteristics of receptors for [D-Trp6]luteinizing hormone releasing hormone and epidermal growth factor in human ovarian cancer, Int. J. Oncol. 12 (3) (1998) 489–498.
[15] J.B. Engel, A.V. Schally, S. Buchholz, S. Seitz, G. Emons, O. Ortmann, Targeted chemotherapy of endometrial, ovarian and breast cancers with cytotoxic analogs of luteinizing hormone-releasing hormone (LHRH), Arch. Gynecol. Obstet. 286 (2) (2012) 437–442.
[16] C. Leuschner, C. Giardina, H. Alila, Ep-100 Synergizes with Paclitaxel in Ovarian, Breast and Prostate Cancer Cell Lines AACR Conference, Chicago, 2012 (Abstract 3715).
[17] C. Leuschner, Synergistic activity of Ep-100 and chemotherapies in cancer cell lines, Cancer Res. 73 (8 Supplement) (2013) 978.
[18] K.K. Curtis, J. Sarantopoulos, D.W. Northfelt, G.J. Weiss, K.M. Barnhart, J.K. Whisnant, et al., Novel LHRH-receptor-targeted cytolytic peptide, EP-100: first-in-human phase I study in patients with advanced LHRH-receptor-expressing solid tumors, Cancer Chemother. Pharmacol. 73 (5) (2014) 931–941.
[19] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), Eur. J. Cancer 45 (2) (2009) 228–247.
[20] G.C. Stuart, H. Kitchener, M. Bacon, A. duBois, M. Friedlander, J. Ledermann, et al., 2010 Gynecologic Cancer InterGroup (GCIG) consensus statement on clinical trials in ovarian cancer: report from the Fourth Ovarian Cancer Consensus Conference, Int. J. Gynecol. Cancer 21 (4) (2011) 750–755.
[21] R.L. Coleman, B.J. Monk, A.K. Sood, T.J. Herzog, Latest research and treatment of advanced-stage epithelial ovarian cancer, Nat. Rev. Clin. Oncol. 10 (4) (2013) 211–224.
[22] S.S. Dharap, Y. Wang, P. Chandna, J.J. Khandare, B. Qiu, S. Gunaseelan, et al., Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide, Proc. Natl. Acad. Sci. U. S. A. 102 (36) (2005) 12962–12967.

[23] C. Leuschner, A. Coulter, J. Keener, H. Alila, Targeted oncolytic peptide for treatment of ovarian cancers, Int. J. Cancer Res. Molecular Mechanisms 3 (1) (2017)https://sciforschenonline.org/journals/cancer-research/IJCRMM-3-132.php.

[24] G. Emons, G. Gorchev, P. Harter, P. Wimberger, A. Stahle, L. Hanker, et al., Efficacy and safety of AEZS-108 (LHRH agonist linked to doxorubicin) in women with advanced or recurrent endometrial cancer expressing LHRH receptors: a multicenter phase 2 trial (AGO-GYN5), Int. J. Gynecol. Cancer 24 (2) (2014) 260–265.

[25] P. Harter, T. Johnson, D. Berton-Rigaud, S.Y. Park, M. Friedlander, J.M. Del Campo, et al., BRCA1/2 mutations associated with progression-free survival in ovarian cancer patients in the AGO-OVAR 16 study, Gynecol. Oncol. 140 (3) (2016) 443–449.

[26] S. Ma, S. Pradeep, A. Villar-Prados, Y. Wen, E. Bayraktar, L.S. Mangala, et al., GnRH-R-targeted lytic peptide sensitizes BRCA wild-type ovarian cancer to PARP inhibition, Mol. Cancer Ther. 18 (5) (2019) 969–979.