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Dose escalation and pharmacokinetic study of AEZS-108 (AN-152), an LHRH agonist linked to doxorubicin, in women with LHRH receptor-positive tumors

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ABSTRACT

Objectives. Receptors for luteinizing hormone-releasing hormone (LHRH) can be utilized for targeted chemotherapy of cytotoxic LHRH analogs. The compound AEZS-108 (previously AN-152) consists of [D-Lys^6] LHRH linked to doxorubicin. The objectives of this first study in humans with AESZ-108 were to determine the maximum tolerated dose and to characterize the dose-limiting toxicity, pharmacokinetics, preliminary efficacy, and hormonal effects.

Methods. The study included 17 women with histologically confirmed epithelial cancer of the ovary, endometrium, or breast that was metastatic or unresectable and for which standard curative or palliative measures could not be used or were no longer effective or tolerated. In each patient, immunohistochemistry of primary tumor or metastatic lesion confirmed that the tumors expressed LHRH receptors.

Results. One patient each received intravenous doses of 10, 20, 40, or 80 mg/m^2 of AEZS-108, six received 160 mg/m^2 and seven 267 mg/m^2 at 3 week intervals. Dose-limiting leukopenia and neutropenia were observed at the highest dose. A total of 6 patients, 3 patients each in both upper dose groups, showed responses to AEZS-108. The half-life of AESZ-108 was estimated to be about 2 h.

Conclusions. The maximum tolerated dose of AESZ-108 in the absence of supportive medication is 267 mg/m^2 and this dose is recommended as starting dose for therapeutic Phase II studies.

Introduction

Targeted drugs are increasingly used for treatment of various malignancies [1]. Receptors for luteinizing hormone-releasing hormone (LHRH) are expressed by about 50% of breast and 80% of ovarian and endometrial cancers [2]. Our group has previously reported on cytotoxic peptides consisting of analogs of hypothalamic peptides conjugated to doxorubicin (DOX) or its derivatives [2–7]. In AEZS-108, formerly known as AN-152, DOX is covalently linked to the LHRH agonist D-Lys^6-LHRH. AEZS-108 was shown to bind to LHRH receptors (LHRH-R) on human breast, ovarian and endometrial cancer cells, and biopsy specimens [8–11]. AEZS-108 was less toxic than DOX and more effective in inhibiting the growth of LHRH-R positive experimental cancers in mice [12,14]. This is likely due to receptor-mediated internalization of this conjugate and reduced induction of multi-drug resistance (MDR-1) P-glycoprotein [13,15].

In vitro studies demonstrated the facilitated uptake of AEZS-108 into LHRH-R positive cell lines; in LHRH-R negative lines, AEZS-108 was not or significantly less active than DOX [14]. *In vivo*, AEZS-108 was highly effective in nude mice bearing various human ovarian, endometrial, breast, and prostate cancer lines. At equimolar doses, AEZS-108 was more active but less toxic than DOX. AEZS-108 had no influence on neuropharmacological variables or on the motor coordination when given intravenously (IV). In dogs, AEZS-108 had no effect on cardiovascular, electrocardiographic, and respiratory variables. Pharmacokinetic investigations in rats and dogs showed a

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short elimination half-life ($t_{1/2}$<1 h) of AEZS-108 and dose linearity when based on maximum plasma concentration (C_{max}) and area under the curve (AUC). The AUCs of AEZS-108 were higher (3 to 9 times in rats, 7 to 12 times in dogs) than those of DOX after both single and multiple doses.

AEZS-108 was sensitive to hydrolytic and carboxylesterase-catalyzed deconjugation into DOX and probably D-Lys^6-LHRH-glutarate [14]. Hydrolysis in mouse serum ($t_{1/2}$≅20 min) was significantly faster than in human serum ($t_{1/2}$≅100–120 min). In acute toxicity studies in mice and rats, the signs of toxicity after AEZS-108 and DOX were similar; as were no-observed effect level (NOEL) and LD_{50} of AEZS-108 and DOX when compared on a molar basis (molecular weights: 1893 g/mol for AEZS-108 and 544 g/mol for DOX).

Based on preclinical studies, AEZS-108 is expected to provide targeted therapy for LHRH receptor-positive human cancers such as ovarian and endometrial cancers, hormone-refractory prostatic tumors, and mammary neoplasms. The present study was the first in which AESZ-108 was administered in women with ovarian, endometrial or breast cancers. The primary objective was to determine the maximum tolerated dose (MTD) in female patients without supportive medication including growth factors. The secondary objectives comprised the characterization of dose-limiting toxicity, pharmacokinetics, preliminary efficacy, and hormonal effects.

Patients and methods

This was a sequential group dose escalation study on the safety of AEZS-108, which also included pharmacokinetic investigations. Study protocol, patient information and consent forms were reviewed and approved by German and Bulgarian Ethics Committees and Regulatory Authorities. Informed written consent was obtained from each patient before enrolment. The trial was carried out in accordance with applicable local drug laws, the principles of the Declaration of Helsinki, and the ICH Guideline for Good Clinical Practice.

Eligible patients had to comply with the following criteria: female; aged 18–70; histologically confirmed epithelial cancer of the ovary, endometrium, or breast; and metastatic or unresectable disease for which standard palliative measures did not exist or were no longer effective or tolerated; positive LHRH receptor status was determined by immunohistochemistry of primary tumor or metastatic specimens. The most important exclusion criteria comprised: history of unstable or newly diagnosed angina pectoris, or myocardial infarction within the last 6 months; serious arrhythmia or congestive heart failure; left ventricular ejection fraction (LVEF) <60%; prior radiotherapy of >35 Gy to pericardial area and >50% of bone marrow; and prior use of anthracyclines or anthracenediones corresponding to >70% of the recommended lifetime cumulative dose for DOX or equivalent doses of anthracenediones.

Starting at 10 mg/m^2 in the first patient, doses were doubled between subsequent patients until first observation of a possibly drug-related toxicity of grade ≥2; then dose escalation followed a modified Fibonacci scheme until the maximum tolerated dose (MTD) was defined. When CTCAE Grade 2 leukopenia was observed at 160 mg/m^2, the cohort size was expanded to 3 patients. In the absence of a dose-limiting toxicity (DLT) at this dose, the dose was increased to 267 mg/m^2 (+67%). DLT was defined as a possibly drug-related adverse event (AE) of CTCAE grade ≥3 (non-hematological) or 4 (hematological) despite symptomatic/prophylactic treatment. Safety monitoring included weekly AE and laboratory controls and LVEF prior to each cycle, and tumor responses were evaluated per RECIST.

AESZ-108 was provided by Æterna Zentaris, Frankfurt, Germany. It was dissolved in water for injections, diluted in 250 ml physiological saline and infused intravenously over 2 h. Retreatment was scheduled at 3-week intervals, allowing for a 2 week delay in case of persisting AEs.

For a preliminary pharmacokinetic evaluation, blood samples were drawn before the first infusion, 1 and 2 h after the start of infusion, 15, 30, and 45 min and 1, 1.5, 2, 3, 4, and 6 h after the end of infusion. Analyses for AEZS-108 and metabolite (DOX) were performed by Prolytic GmbH, Frankfurt, using a validated HPLC method (unpublished) with a lower limit of quantification (LLOQ) of 5 ng/ml for DOX and 10 ng/ml for AESZ-108.

Results

Disposition, demographics, and exposure of patients

Seventeen women received at least one dose of AESZ-108. One patient each was treated at 10, 20, 40, and 80 mg/m^2. Because of the absence of clinically relevant, possibly drug-related adverse events in this dose range, the results are presented in one group. Six patients were treated at 160 mg/m^2 and 7 at 267 mg/m^2. The cohort at 160 mg/m^2 was expanded to include 6 patients, after the MTD was reached at the highest dose.

All 4 patients of the low dose group went off-study with progressive disease after 2 treatment cycles. In the two higher dose groups, 3 patients each received the anticipated maximum of 6 treatment cycles; no patient discontinued treatment because of poor tolerability.

Except for a Hispanic woman all other patients were Caucasian. The patients were somewhat heterogeneous in their baseline and background characteristics (Table 1). All patients had undergone surgery and in most cases also chemotherapy, including doxorubicin (160–300 mg/m^2) in 6 patients (3 at MTD) and mitoxantrone (1 dose) in 1 patient at MTD. Their cancers were in advanced stage, having metastasized already at screening (Table 2). Positive LHRH receptor status was verified from primary tumor tissue (13 cases), metastases (2 cases), or from a local relapse (one case of breast cancer); in one case the origin of the specimen was not specified. Most tumors had 50% or more cells staining positive for LHRH receptors (range: 20–90%).

Efficacy

Although efficacy was not a primary endpoint, tumor response was assessed whenever possible. In the low dose group, none of the patients showed a response, however, 7 patients in the two higher dose groups achieved a stabilization or remission (Table 2).

Table 1
Demographics and main baseline data.

Dose		10–80 mg/m^2	160 mg/m^2	267 mg/m^2
Patients		N=4	N=6	N=7
Age (years)	Mean±SD	55±11	59±5	48±11
Weight (kg)	Mean±SD	88±40	83±19	63±11
BMI	Mean±SD	31.4±13.2	30.7±6.8	23.7±4.3
Performance status (ECOG/WHO)	Grade 0	4 (100%)	0 (0%)	5 (71%)
	Grade 1	0 (0%)	6 (100%)	1 (14%)
	Grade 2	0 (0%)	0 (0%)	1 (14%)
Time since first diagnosis (months)	Mean±SD	46±24	42±18	83±81
Pre-treatment	Surgery	4 (100%)	6 (100%)	6 (86%)
	Radiotherapy	1 (25%)	2 (33%)	1 (14%)
	Hormone therapy	1 (25%)	0 (0%)	1 (14%)
	Chemotherapy	3 (75%)	6 (100%)	7 (100%)
	Immunotherapy	0 (0%)	0 (0%)	1 (14%)
	Other	0 (0%)	0 (0%)	1 (14%)

Table 2
Dosage, cancer type, LHRH receptor expression, and outcome.

ID	AEZS-108 dose/cycles (mg/m^2)	Cancer type	Target lesion	% LHRH-R positive	Response
1/1	10/×2	Fallopian tube	Liver metastasis	50	PD
1/2	20/×2	Ovarian	Liver metastasis	80	PD
2/3	40/×2	Endometrial	Liver metastasis	70	PD
2/4	80/×2	Uterus/ ovarian	Peritoneal metastasis	80	PD
1/3	160/×3	Breast	Left thoracic region	30	PD
1/4	160/×5	Ovarian	Former ovarian location	30	PD
1/5	160/×2	Ovarian	Spleen metastasis	60	PD
2/10	160/×6	Ovarian	Liver metastasis	20	SD
2/12	160/×6	Ovarian/ endometrial	Lymph node	90	CR
4/5	160/×6	Ovarian	Peritoneal cancer	80	SD
1/7	267/×1	Ovarian	Paraaortal metastasis	20	PD
2/8	267/×3	Breast	Liver metastasis	60	PD
2/11	267/×6	Ovarian	Spleen metastasis	50	SD
3/1	267/×5[a]	Ovarian	Spleen metastasis	80	SD
4/2	267/×6	Ovarian	Liver metastasis	60	PR[b]
4/3	267/×6	Ovarian	−(CA-125)	80	CR[c]
4/4	267/×4	Ovarian	Metastasis not specified	80	PD

% LHRH-R positive: receptor status expressed as percentage of tumor cells in the specimen that stained positive for LHRH receptor.
Overall response categorized by the investigators as CR: complete response. PR: partial response. SD: stable disease. PD: progressive disease.
[a] Patient 3/1 received 267 mg/m^2 during the first cycle, but 160 mg/m2 at all 4 following cycles. She was, however, analysed in the higher dose group in all analyses.
[b] PR of liver metastasis and normalization of CA-125 marker level.
[c] Normalization of CA-125 marker level.

Safety and tolerability

Treatment-emergent adverse events (AEs) were reported by 15 of 17 patients (88%). The 2 patients receiving 20 mg/m^2 or 80 mg/m^2 reported no AE. The patients receiving 10 mg/m^2 and 40 mg/m^2 reported one and three AEs, respectively, however none was ascribed to AEZS-108. In contrast, 5 patients from the 160 mg/m^2 group and all 7 patients from the 267 mg/m^2 group reported AEs that the investigators attributed to the study medication. The most important AEs were leukopenia and neutropenia, which were particularly frequent in the highest dose group (Table 3). Besides these, nausea, vomiting, and alopecia were attributed to study medication in >4 patients. All other AEs were attributed to study medication in one or two cases. There were no consistent effects on vital signs.

Serial monitoring of the left ventricular ejection fraction (LVEF) did not reveal signs of cardiotoxicity. Atrial fibrillation was observed in one patient, at the end of Cycle 5 at 160 mg/m^2, but the patient showed no change in LVEF and had no complaints indicating impaired cardiac function and, thus, continued treatment without cardiac events. Ventricular extrasystoles were recorded in another patient, at the end of Cycle 2 at 267 mg/m^2. At the end of Cycle 6, the patient showed signs of myocardial ischemia during an exercise tolerance test, although vital signs at rest were normal and also the performance status of Grade 0 was not suspicious at that time. In the absence of an earlier stress test, it is unclear if the exercise-induced ischemia represented an adverse change from pre-study baseline.

For each patient an ECG was recorded between Day 15 and the end of a cycle. Compared to baseline, the mean QTcB increased by 24 ms in the 160 mg/m^2 group and by 3 ms in the 267 mg/m^2 group. One patient of the 160 mg/m^2 dose group was found with QTcB of 515 ms (at a heart rate of 110) at the end of Cycle 1, while QTcB values in later cycles were similar to baseline. No other patient had a QTcB values >465 ms.

In all, laboratory data characterize hematological toxicity as dose-limiting for AEZS-108 in this study. At the dose of 267 mg/m^2, leukopenia and neutropenia reached Grade 4 in Cycle 1 of 1 and 3 of the 7 patients, respectively (Table 4).

In one patient, an extravasal application of AEZS-108 occurred in Cycle 1 at 160 mg/m^2, but resolved without sequels after appropriate local treatment.

The investigators judged the tolerability as satisfactory for all patients in the 267 mg/m^2 group (one evaluation missing); in contrast, they judged the tolerability as good or very good for 3 of the 6 patients receiving 160 mg/m^2, as well as for 3 of the 4 patients in the lowest dose group.

Table 3
AEs classified by investigator as "likely" to be drug-related (preferred AE terms grouped by system organ class).

Dose group	160 mg/m^2		267 mg/m^2	
CTCAE grade	1/2	3/4	1/2	3/4
Blood and lymphatic system				
Leukopenia	3 (50%)	0	2 (29%)	4 (57%)
Neutropenia	0	1 (17%)	1 (14%)	4 (57%)
Thrombocytopenia	0	0	2 (29%)	0
Anaemia	0	0	1 (14%)	0
Gastrointestinal				
Nausea	5 (83%)	0	1 (14%)	0
Vomiting	2 (33%)	0	2 (29%)	0
Stomatitis	0	0	2 (29%)	0
Dysphagia	0	0	2 (29%)	0
Metabolism and nutrition				
Anorexia	1 (17%)	0	2 (29%)	0
Investigations				
Weight decreased	1 (17%)	0	0	0
General disorder and administrational site				
Asthenia	0	0	1 (14%)	0
Fatigue	1 (17%)	1 (17%)+	0	0
Mucosal inflammation	0	0	1 (14%)	0
Skin and subcutaneous tissue				
Alopecia	0	0	4 (57%)	0
Pruritus/pruritus allergic	0	0	2 (29%)	0
Erythema	0	0	1 (14%)	0
Nail disorder	0	0	1 (14%)	0
Palmar plantar erythrodysesthesia	0	0	1 (14%)	0
Dermatitis allergic	1 (17%)	0	0	0
Eye				
Conjunctivitis	0	0	1 (14%)	0

Pituitary function

No relevant effect of AEZS-108 on cortisol levels was observed in the ACTH stimulation test. Similarly, there was no effect of AEZS-108 on baseline serum levels of TSH, T3, and T4 and on the increase in TSH 30 min after stimulation with 200 μg TRH.

Mean LH and FSH levels decreased after administration of AESZ-108. Initial values for both hormones were in the postmenopausal range (>20 IU/L), but LH concentrations dropped into the pre-

Table 4
Incidence of nadirs in leukocytes and neutrophils.

Dose of AEZS-108	Cycle 1					Any following cycle				
	Observed cases	CTCAE grade				Observed cases	CTCAE grade			
		G1	G2	G3	G4		G1	G2	G3	G4
Leukocyte nadirs counts										
10–80 mg/m^2	4	0	0	0	0	4	0	0	0	0
160 mg/m^2	6	2	3	0	0	6	2	4	0	0
267 mg/m^2	7	1	3	2	1	5	1	2	1	1
Absolute neutrophil nadirs counts										
10–80 mg/m^2	4	0	0	0	0	4	0	0	0	0
160 mg/m^2	6	0	2	1	0	6	1	1	1	0
267 mg/m^2	7	1	2	1	3	5	0	2	2	1

Table 5
Mean levels of LH and FSH by treatment course.

Dose of AEZS-108	160 mg/m^2			267 mg/m^2		
	N	LH [IU/L]	FSH [IU/L]	N	LH [IU/L]	FSH [IU/L]
Screening	5	33	53	6	41	93
Cycle 1	5	12	20	5	15	45

Number of observations after later cycles was 1–3, thus precluding meaningful statistics.

menopausal range after AEZS-108 doses of 160 and 267 mg/m^2 (Table 5). A smaller decrease in LH and FSH was observed after doses of 40 and 80 mg/m^2 (data not shown); no suppression was seen in the patient dosed at 20 mg/m^2 AEZS-108. Although supported by fewer observations only, the effects on LH and FSH appeared to be similar after subsequent cycles.

Pharmacokinetics

The measured plasma concentrations of DOX and AEZS-108 showed a high variability, especially for the latter. Following 160 and 267 mg/m^2 AEZS-108, maximum plasma concentrations (C_{max}) ranged from 728 to 6661 ng/ml. The high variability influenced all other pharmacokinetic parameters. Therefore, only a weak dose dependency was found in C_{max} and AUC (Table 6). The calculated $t_{1/2}$ and clearance of AEZS-108 were approximately 2 h and 1 l/min m^2, respectively.

At the dose levels of 160 and 267 mg/m^2, average C_{max} values of DOX ranged from 600 to 700 ng/ml. As expected, average C_{max} and AUC of DOX were closely correlated to the AESZ-108 levels. The $t_{1/2}$ of DOX was slightly above that calculated for AESZ-108, and the respective clearance was slightly lower. There was no evidence for accumulation of AESZ-108 or DOX after repeated cycles.

Discussion

AEZS-108 (AN-152) is a cytotoxic peptide hybrid in which the LHRH analog peptide targets the drug preferentially to cells and tissues bearing LHRH receptors. Because of this targeting mechanism, patients with LHRH receptor-positive tumors were selected even in this Phase I study. Still, as preclinical studies had shown dose-related efficacy of AEZS-108, the study aimed to determine the MTD and used an accelerated dose escalation scheme to minimize the number of patients receiving potentially non-effective doses. While LHRH receptors have been demonstrated on tumor types occurring both in males and females, this study was conducted in female patients only, as gynecological tumors were considered as a priority for subsequent Phase II studies. In all, this study design allowed to span a 27-fold dose range (10–267 mg/m^2) and define the MTD with only 6 dose levels and 17 patients, of whom 13 were treated at the two highest dose levels of 160 and 267 mg/m^2.

Hematological toxicity, specifically leukopenia and neutropenia (no febrile neutropenia), were the most severe drug-related adverse events and led to the definition of the MTD of AEZS-108 at 267 mg/m^2, where 3 and 4 out of 7 patients experienced leukopenia and neutropenia, respectively, of Grade 3/4 in Cycle 1. In addition to the effects on white blood cells, significant, but not dose-limiting changes in red blood cell parameters and thrombocytes were observed at this dose. Expansion of the 160 mg/m^2 dose group, to finally include 6 patients, confirmed the lack of DLTs at this dose.

It was anticipated that the recommended dose should be associated with DLT in no more than 1 of 6 patients. However, as no cases of febrile neutropenia were observed, and leukopenia was rapidly reversible even after repeated treatment cycles, 267 mg/m^2 was concluded to be a suitable dose of AEZS-108 in a 3-weekly regimen. Retreatment at 160 mg/m^2 should be considered for cases of delayed hematological recovery. In addition, hematopoietic growth factors could be used as supportive or rescue treatment in patients with more severe or more delayed recovery from hematoxicity.

AEZS-108 was initially infused without any prophylactic medication. As anticipated, antiemetic premedication was implemented after the observation of nausea and vomiting that was considered as drug-related. Anti-allergic premedication was implemented after the observation of an allergic skin reaction during the infusion of AEZS-108. According to the rules of ASCO guideline for antiemetics in oncology [17], AEZS-108 would be considered as an agent with *low emetic risk*, for which prophylactic treatment with 8 mg dexamethasone is recommended.

At dosages below 160 mg/m^2, no relevant changes in laboratory variables were observed. At the higher dosage, hematological toxicity was the most frequent and severe change in laboratory data, which also led to the definition of the MTD at 267 mg/m^2 as discussed above. Non-hematological reactions to AEZS-108, mainly nausea and vomiting, were limited to a severity of Grade 1 or 2, with the exception of a single case of Grade 3 fatigue. In all, with the exception of cardiotoxicity, which was not observed in this study, the spectrum of AEs after AEZS-108 resembled the profile of reactions known to be associated with DOX.

Various types of skin reactions were reported. Alopecia is a typical adverse reaction to DOX [16]. Only mild alopecia, however, was reported by 1 and 4 patients in the 160 and 267 mg/m^2 dose groups, respectively. Palmar plantar erythrodysesthesia (PPE) is another known side effect of DOX; it was reported by one patient each in the 160 and 267 mg/m^2 dose groups. Similarly, pruritus and allergic skin reactions are also known side effects of DOX. Dexamethasone, which is being recommended as an antiemetic drug for future studies, can serve as an anti-allergic premedication.

Because of the known cardiotoxic potential of DOX, the cytotoxic component of AEZS-108, cardiac safety was addressed specifically by

Table 6
Pharmacokinetic parameters.

Dose		C_{max} [ng/ml]	t_{max} [h]	$AUC_{0-\infty}$ [ng h/ml]	$t_{1/2}$[a] [h]	CL [l/min m^2]
10–80 mg/m^2	AEZS-108	(106–4905)	(2–2.25)	(213–4400)	(0.60–5.67)	(0.26–1.01)
	Doxorubicin	(19–1111)	(1–2.25)	(40–1095)	(0.17–5.34)	(0.37–2.56)
160 mg/m^2	AEZS-108 (N = 6;14)[b]	2193 (728–6661)	2 (1–2.25)	3237 (997–8742)	2.11 (0.74–4.58)	0.82 (0.37–2.67)
	Doxorubicin (N = 6:14)[b]	601 (279–1580)	2 (1–2.25)	1283 (571–2494)	3.65 (1.47–6.02)	0.64 (0.31–1.43)
267 mg/m^2 [c]	AEZS-108 (N = 6;9)[b]	2262 (796–4351)	2 (1–2)	3955 (2222–8824)	1.89 (0.82–3.17)	1.00 (0.50–2.00)
	Doxorubicin (N = 5:10)[b]	644 (177–1181)	2 (1–2)	1542 (1011–2074)	2.87 (0.78–4.71)	0.76 (0.50–1.35)

Presented are geometric means and the ranges (in brackets), except for t_{max} (median and ranges in brackets): the low number of the observations in the lower dose group precluded calculation of statistics. Data from all cycles with valid PK data were considered.

[a] Blood sampling ended 6 h after the end of infusion, thus, the half-life should have been underestimated, in particular for doxorubicin.

[b] N: number of patients evaluable for PK; number of cycles with evaluable data.

[c] PK parameters for Patient 3/1 were not included in the calculation of medians, as the samples of this patient had been thawed during shipment to the analytical laboratory and measured concentrations were not reliable.

repeated evaluation of LVEF and ECG. While ECGs suggested a small average increase in QTc, LEVF was inconspicuous up to the highest dose. Although cardiotoxicity was not seen for AESZ-108 up to 267 mg/m^2, data from larger studies are needed to substantiate this observation.

The pituitary physiologically expresses LHRH receptors and, therefore, is a potential target for cytotoxic effects of AEZS-108. However, in aged, postmenopausal patients or in younger patients who had their ovaries removed during the primary surgical treatment of their cancer, a reduction in LH or FSH would not be expected to have any adverse physiological consequences. In fact, AESZ-108 produced a reduction in the plasma levels of these hormones which still remained in the normal range for pre-menopausal women. Moreover, neither the measurements of baseline hormone levels nor the tests for release of the corresponding pituitary hormones revealed an effect of AEZS-108 on non-gonadotropic pituitary functions.

A therapeutic effect, including remissions and prolonged courses of stable disease, were observed in 3 patients each in the dose groups of 160 and 267 mg/m^2. Because of the low number of patients and the variability in patient and disease characteristics, no dose–response could be expected from these data. Due to the specific targeting mechanism of AESZ-108, even dosages lower than 160 mg/m^2 could be effective. However, as only single patients were treated, the trial allowed no conclusion on the efficacy at lower doses.

While all patients with remission had tumors with high percentage of cells expressing LHRH receptors (Pt 2/12: 90%, Pt 4/2: 60%, and Pt 4/3: 80%), there were also 2 patients who remained on treatment for 5 to 6 treatment courses at 160 mg/m^2 (Pt 1/4 and 2/10) although their tumors had only 20–30% cells with LHRH receptors. Because of the chance of a clinical benefit, patients with tumors containing lower percentage of LHRH receptor-positive cells, should be included also in further studies to assess the correlation between LHRH receptor expression and efficacy of AEZS-108 on a larger database.

The plasma concentrations of AEZS-108 showed a high variability. In some cases sampling errors, i.e. sampling after the end of the infusion, might explain a low C_{max}. In other cases, a delay during pre-analytical processing may have resulted in hydrolysis of AEZS-108 and higher concentrations of DOX. The high variability influenced all calculated pharmacokinetic parameters. Hence, a clear dose dependency could not be shown for C_{max} or for AUC of AEZS-108 and DOX, and the pharmacokinetic results can serve only for orientation on the exposure to parent drug and metabolite indicating the need for further investigations.

In conclusion, our study indicated that in female patients the recommended dose of AEZS-108 in a 3-weekly dosage regimen is 267 mg/m^2, with 8 mg dexamethasone as antiemetic premedication. Phase II studies in platinum-resistant ovarian and advanced endometrial cancer are ongoing.

Conflict of interest statement

The following authors declare a financial relationship with Æterna Zentaris (AEZS): Herbert Sindermann — employee and stock ownership of AEZS; and Jürgen Engel — Chairman and Managing Director and stock ownership of AEZS.
The other authors declare that there are no conflicts of interest.

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