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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%)