Poster
LB-407

Down-modulation of messenger ribonucleic acid (mRNA) by EZN-2968, an hypoxia-inducible factor-1α (HIF-1α) mRNA antagonist, administered in adult patients with advanced solid tumors

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Background

HIF-1 is a transcription factor that is a critical mediator of key genes important in cancer biology, including angiogenesis, neovascularization, cell proliferation, tumor metabolism, drug resistance, tumor invasion, autophagy, and cell survival.[1-4]

HIF-1 mediates adaptive responses to changes in tissue oxygenation.[1-2] HIF-1α levels increase in tumor cells in response to hypoxia and are regulated at both the level of translation and degradation.[3] In particular, in well-oxygenated cells, HIF-1α is degraded in an oxygen-regulated manner by the ubiquitin-proteasome system. In addition to intratumoral hypoxia, multiple other mechanisms may result in increased levels of HIF-1α in cancer cells.[1-2] Examples of such mechanisms include mutations (loss of function) in genes such as von Hippel Lindau (VHL), p53, and phosphatase and tensin homolog (PTEN); alterations in signaling via phosphatidylinositol 3 kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) and MEK-ERK; alterations (gain of function) in genes such as SRC and ARF; and BCL2 overexpression.[2]

HIF-1α is rarely expressed in normal tissues and is expressed in many primary malignant tumor types.[5] Hypoxic cells, which have high levels of HIF-1α, are resistant to both chemotherapy and radiotherapy. Increased HIF-1α levels are associated with poor prognosis in several neoplasms.[4] Down-regulation of HIF-1α may have broad therapeutic application.

The HIF-1α mRNA antagonist, designated EZN-2968, is a locked nucleic acid (LNA) antisense oligonucleotide that specifically down-modulates HIF-1α mRNA and consequently HIF-1α protein.[6] The oligonucleotide EZN-2968 is a 16-mer composed of 16 monomeric units, of which 6 DNA nucleotides are replaced with LNA nucleotides. The sequence of EZN-2968 is 5′-TGGCaagcatccTGTa-3′, where upper case indicates LNA residues and lower case indicates DNA residues.

A highly potent, selective, and durable antagonism of HIF-1α expression was observed under both normoxic and hypoxic conditions when human cancer cells were transfected with EZN-2968.[6] *In vivo* administration of EZN-2968 to normal mice led to specific, dose-dependent, and highly potent down-modulation of endogenous HIF-1α and vascular endothelial growth factor (VEGF) in the liver.

Clinical Study

Study Design

- 3 + 3 dose-escalation design
- Dose expansion to 6 patients to determine the maximum tolerated dose (MTD)
- MTD dose expansion up to 10 patients
- 3 centers

Objectives

- Determine the MTD
- Determine the recommended Phase 2 dose
- Evaluate the safety and tolerability
- Determine the pharmacokinetic (PK) profile
- Determine the pharmacodynamic profile
- Detect preliminary evidence of antitumor activity

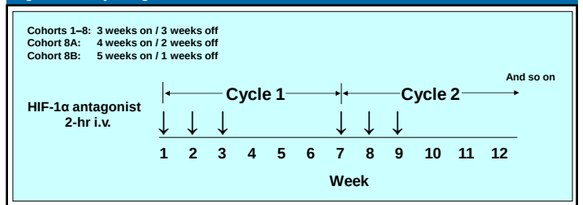
Key Eligibility Criteria

- Advanced and/or metastatic solid tumor or lymphoma; refractory to standard therapy
- Eastern Cooperative Oncology Group (ECOG) performance status = 0 to 2
- Serum creatinine and total bilirubin ≤1.5 × upper limit of normal (ULN)
- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 ×ULN

Methods

- Plasma concentrations of EZN-2968 determined by ELISA hybridization assay; lower limit of quantitation = 3.999 ng/mL
- PK parameters estimated using nonlinear mixed-effect modeling (Monolix v31s, http://wfn.software.monolix.org)

Figure 1. Study Design



Cycles repeat every 6 weeks. Dose escalation was based on toxicities observed during the first cycle. PK was evaluated in the first cycle. Objective tumor response assessed every 6 to 8 weeks using the Response Evaluation Criteria in Solid Tumors (RECIST).

Results

Patient and Treatment Information

At the time of the data cutoff, 49 patients had been treated. The primary reasons for discontinuation of EZN-2968 were progressive disease (PD) (34 patients), withdrawn consent (6 patients), adverse event (AE) (4 patients), investigator decision (4 patients), and death (1 patient).

The median age of the treated patients was 59 y (range: 28-87 y) (Table 1). Of the 49 patients, 28 (57%) were women and 21 (43%) were men; 86% of patients were white. ECOG performance status was 0 for 20 patients (41%), 1 for 28 patients (57%), and 2 for 1 patient (2%). All 49 patients had received prior chemotherapy. The median number of prior cytotoxic chemotherapies was 4 (range = 1–9).

Tumor types included colorectal cancer (12 patients); ovarian cancer (9 patients); liposarcoma/ soft-tissue sarcoma (5 patients); non-small cell lung cancer (4 patients); adenoid cystic carcinoma, hepatocellular carcinoma (HCC), and renal cell cancer (RCC) (3 patients each); pancreatic cancer (2 patients); and cholangiocarcinoma, esophageal cancer, head & neck cancer, laryngeal cancer, melanoma, penile cancer, peritoneal carcinomatosis, and unknown primary adenocarcinoma (1 patient each).

The 49 patients received between 1 and 10 treatment cycles (mean = 2).

Table 1. Demographics and ECOG Performance Status

Cohort	1	2	3	4	5	6	7	8	8A	8B	All Patients
Dose (mg/kg)	1	1.5	2.3	3.5	5.3	8	12	18	18 = MTD	18	n (%)
Patients treated	4	4	3	8	5	7	4	3	7	4	49
Age, years											
Median	55	70	52	62	50	61	63	60	57	62	59
Range	44-72	37-87	50-55	28-74	43-63	48-73	52-70	58-69	42-67	50-77	28-87
Sex, n											
Female	2	1	3	3	3	4	2	3	6	1	28 (57)
Male	2	3	0	5	2	3	2	0	1	3	21 (43)
ECOG performance status, n											
0	2	1	2	2	4	1	2	1	4	1	20 (41)
1	2	3	0	6	1	6	2	2	3	3	28 (57)
2	0	0	1	0	0	0	0	0	0	0	1 (2)

Safety and Tolerability

To date, 4 patients have had dose-limiting toxicity (DLT).

- Two patients had DLT after receiving EZN-2968 administered weekly for 3 weeks per 6-week cycle.
  - One patient in the 3.5-mg/kg group had intracranial bleeding at a site of a metastasis; this patient had a history of breast cancer with no history of signs or symptoms of brain metastases. This finding, not considered drug related, necessitated cohort expansion to 6 patients at this dose level. The intracranial bleeding resulted in death 17 days after the patient's last dose of EZN-2968.
  - One patient in the 8-mg/kg group had Grade 3 fatigue.
- Two patients had DLT after receiving the intensified EZN-2968 dosage of 18 mg/kg every week for 5 weeks per 6-week cycle.
  - One patient had Grade 3 fatigue.
  - One patient had a reversible Grade 3 increase in AST and ALT.

Overall, 48 patients (98%) had at least one treatment-emergent AE. The most commonly reported AEs (>20% of patients), regardless of relationship to study drug, were fatigue (49%), nausea (29%), anemia (27%), anorexia (22%), and constipation (20%). The intensity of most AEs was Grade 1 or 2.

Twenty-four patients (49%) had Grade 3 or 4 AEs, the most common (>1 patient) of which were fatigue (6 patients); abdominal pain and increased AST (3 patients each); and increased ALT, deep-vein thrombosis, hypokalemia, and hyponatremia (2 patients each). Two patients (11%) had Grade 4 AEs (not drug related): hypokalemia and hypocalcemia (1 patient), and respiratory failure and coma (1 patient).

Drug-related AEs reported for >10% of patients were fatigue (29%) and headache (14%) (Table 2). Seven patients (14%) had Grade 3 drug-related AEs: increased AST and fatigue (3 patients each), increased ALT (2 patients), and hyponatremia (1 patient). No patient had a drug-related Grade 4 or 5 AE.

No significant changes in blood pressure or urine protein were reported.

Table 2. Treatment-Emergent Drug-Related Adverse Events Reported for >1 Patient

Cohort	1	2	3	4	5	6	7	8	8A	8B	All Patients
Dose (mg/kg)	1	1.5	2.3	3.5	5.3	8	12	18	18 = MTD	18	n (%)
Patients treated	4	4	3	8	5	7	4	3	7	4	49
Patients with ≥1 drug-related AE	3	2	3	4	3	4	2	2	6	3	32 (65)
Patients with:											
Fatigue	1	0	1	1	3	3	0	1	3	1	14 (29)
Headache	1	1	1	1	0	0	0	1	2	0	7 (14)
Anorexia	0	0	2	0	0	0	1	0	2	0	5 (10)
Nausea	1	0	2	0	1	0	0	0	1	0	5 (10)
Arthralgia	2	0	0	0	0	0	0	0	1	0	3 (6)
AST increased	0	0	0	0	0	0	0	1	1	1	3 (6)
Chills	2	0	0	0	0	0	0	0	0	1	3 (6)
Diarrhea	0	0	0	0	0	0	0	0	2	1	3 (6)
Myalgia	2	0	0	0	0	0	0	0	1	0	3 (6)
Pyrexia	2	0	1	0	0	0	0	0	0	0	3 (6)
ALT increased	0	0	0	0	0	0	0	0	1	1	2 (4)
Anemia	0	0	0	0	0	0	0	0	1	1	2 (4)
Early satiety	0	0	1	0	0	0	0	0	1	0	2 (4)
Flushing	1	0	0	0	0	0	0	1	0	0	2 (4)
Mucosal inflammation	0	0	0	1	0	1	0	0	0	0	2 (4)
Peripheral edema	0	0	0	0	0	0	1	0	1	0	2 (4)
Vomiting	0	0	2	0	0	0	0	0	0	0	2 (4)

Pharmacokinetics

The PK of plasma EZN-2968 are nonlinear at the higher doses (Figure 2). The drug concentration and exposure increase more rapidly than the drug dosage.

The PK of EZN-2968 are best described by a one-compartment nonlinear model with saturable elimination, with maximum velocity (V_{max}) = 756 mg/h/70 kg ± 13% relative standard error (rse) and K_m = 59.3 mg/L ± 19% rse (Figure 3). The V_{max} is dependent on the patient's weight. At the MTD of 18 mg/kg, the volume of distribution (V_d) was 6 L, and clearance was 5.74 L/h.

Sources of nonlinearity have not been determined, but may include for example saturable clearance from the plasma.

Figure 2. Mean EZN-2968 Plasma Concentration Versus Time After a 2-hour Infusion of EZN-2968 (Dose 1)

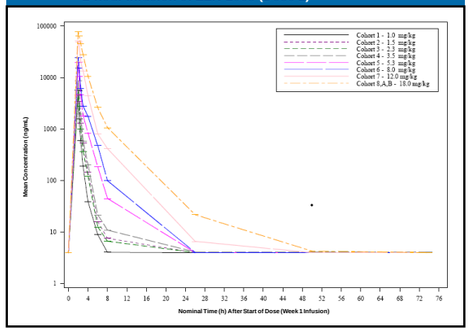
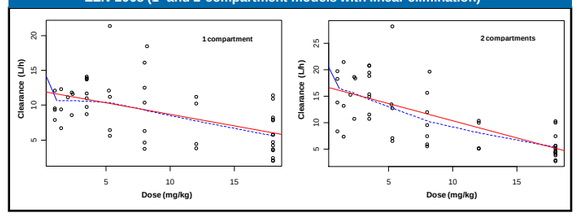


Figure 3. Relationship between empirical Bayesian estimates of CL and dose of EZN-2968 (1- and 2-compartment models with linear elimination)



Pharmacodynamics

Concentrations of the following HIF-1–regulated gene products were determined: VEGF, erythropoietin, ferritin, and ceruloplasmin. Blood samples were collected at Weeks 1 (pre-dose), 3 (pre-dose), and 5 (Study Day 29) for the first treatment cycle; at Week 1 (pre-dose) for subsequent treatment cycles; and at the end-of-study visit. No consistent changes in these gene products were observed.

HIF-1α expression was evaluated pre-treatment and on-treatment in tumor (n=6) and in skin (n=41). Tumor and skin biopsies were obtained after the third weekly administration of EZN-2968. Tumor biopsy HIF-1α mRNA decreased in 4 patients, increased in 1 patient, and did not change in 1 patient (Table 3). Skin biopsy HIF-1α mRNA decreased in 63% (26/41) of patients, remained the same in 10% (4/41) of patients, and increased in 27% (11/41) of patients (Figure 4).

Figure 4. Skin Biopsies: Fold Change in HIF-1α mRNA Concentrations Before and After EZN-2968

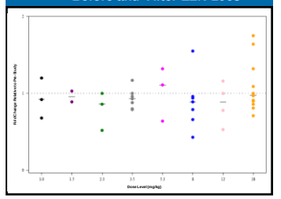


Table 3. Tumor Biopsies: Fold Change in HIF-1α mRNA Concentrations Before and After EZN-2968

Patient #	Cohort	Dose (mg/kg)	Time Between Last Dose and Biopsy	Tumor Type	Fold Change in HIF-1α mRNA	HIF-1α mRNA	
						Before	After
1	3	2.3	1 day	Colon	1.65	1.7	2.8
2	4	3.5	1 day	Liposarcoma	0.58	14.5	8.4
3	8A	18	1 day	Colon	0.54	3.5	1.9
4	8A	18	<1 day	Unknown primary adenocarcinoma	0.82	7.1	5.8
5	8B	18	3 days	Penile	0.41	2.7	1.1
6	8B	18	3 days	Esophageal	0.49	6.5	3.8

Antitumor Activity

Sixteen patients achieved stable disease (SD) (mean duration = 112.5 days; range = 36+ to 408+ days). Of the 16 patients, 7 patients had prolonged SD (duration of SD >90 days): 2 patients had RCC (120 days, 408+ days), 2 patients had soft-tissue sarcoma (117 days, 120+ days), 1 patient had HCC (211 days), 1 patient had ovarian cancer (119 days), and 1 patient had adenoid cystic carcinoma (120 days). Most patients were heavily pretreated.

Table 4. Best Overall Response

Cohort	1	2	3	4	5	6	7	8	8A	8B	All Patients
Dose (mg/kg)	1	1.5	2.3	3.5	5.3	8	12	18	18 = MTD	18	n (%)
Patients treated	4	4	3	8	5	7	4	3	7	4	49
Stable disease	1	2	0	3	2	1	2	2	2	1	16 (33)
Progressive disease	2	1	2	3	3	5	2	1	3	3	25 (51)
Not evaluated	1	1	1	2	0	1	0	0	2	0	8 (16)

The patient with RCC who had prolonged SD for more than 1 year received 1 mg/kg of EZN-2968. This 44-year-old man had a wild-type VHL genotype (methylation not assessed) and had progressed after receiving prior sunitinib and an mTOR inhibitor. For this patient, duration of treatment with EZN-2968 (60 weeks) exceeded the duration of treatment with prior therapies. Compared to the pre-study measurements, the sum of the tumor sizes had increased 27% after Cycle 1 and had decreased 14% after Cycle 6 (Figure 5). Compared to the measurements after Cycle 1, the tumor had decreased by 32% after Cycle 6. SD lasted 408+ days.

Figure 5. Renal Cell Cancer Treated with EZN-2968 (1 mg/kg)

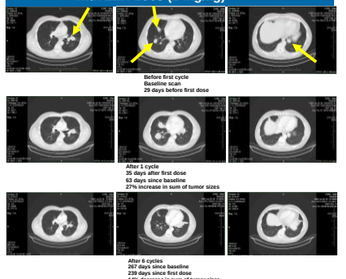
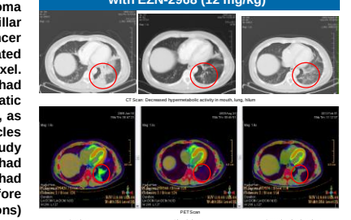


Figure 6. Hepatocellular Carcinoma Treated with EZN-2968 (1.5 mg/kg)

The patient with HCC who had prolonged SD for more than 6 months received 1.5 mg/kg of EZN-2968. This 62-year-old man had progressed after receiving prior sorafenib, 5-fluorouracil + leucovorin + bevacizumab, erlotinib, and capecitabine. Compared to the pre-study measurements, the sum of the tumor sizes had decreased 29% after Cycle 2 (Figure 6). SD lasted 211 days.

The patient with head & neck cancer received 12 mg/kg of EZN-2968. This 52-year-old man had a history of squamous cell carcinoma (3 years earlier). He underwent tonsillar irradiation while receiving cisplatin. The cancer recurred 2.5 years later, and he was treated with cetuximab + albumin-bound paclitaxel. Following EZN-2968 treatment, the patient had qualitative improvement of his metastatic disease to the posterior wall of his left lung, as measured on CT scan, and completed 2 cycles of treatment. Compared to the pre-study measurements, the sum of the tumor sizes had decreased 16% after Cycle 1 and had decreased by 33% after Cycle 2, before discontinuing the study due to PD (new lesions) (Figure 7). SD lasted 78 days.

Figure 7. Head & Neck Cancer Treated with EZN-2968 (12 mg/kg)



Conclusions

The MTD of EZN-2968, a novel HIF-1α mRNA antagonist, is 18 mg/kg given weekly for 4 of 6 weeks. EZN-2968 was well tolerated in previously treated patients with advanced tumors who received up to 10 cycles of EZN-2968. PK data support this schedule of administration of EZN-2968. The best response was SD, and multiple patients had tumor shrinkage. Evidence for down-modulation of the HIF-1α target is supported by observations in tumor and skin biopsies. Additional evaluation of EZN-2968 in clinical trials is warranted.

Reference

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*Author is a full-time employee of Enzon Pharmaceuticals, Inc., and owns company's stock options and/or units.

EZN-2968 is being developed by Enzon Pharmaceuticals, Inc. under a license with Santaris-Pharma A/S.

AACR; Orlando, FL; April 5, 2011