

EZN-2968, a novel hypoxia-inducible factor-1 α (HIF-1 α) messenger ribonucleic acid (mRNA) antagonist: results of a Phase 1, pharmacokinetic (PK), dose-escalation study in patients with advanced malignancies

Poster 2564

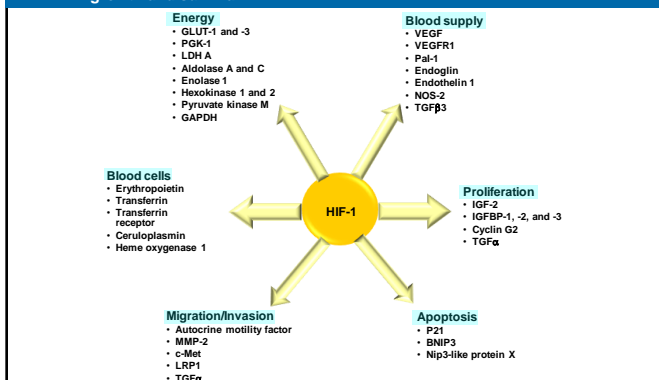
A. Patnaik,¹ E. G. Chiorean,² A. Tolcher,¹ K. Papadopoulos,¹ M. Beeram,¹ D. Kee,¹ M. Waddell,² E. Gilles,^{3*} A. Buchbinder,^{3*}

¹START (South Texas Accelerated Research Therapeutics), San Antonio, TX; ²Indiana University Cancer Center, Indianapolis, IN; ³Enzon Pharmaceuticals, Inc., Bridgewater NJ

Background

HIF-1 is a significant transcription factor that regulates expression of many key genes important in cancer biology (Figure 1), notably those switching cell metabolism to anaerobic glycolysis and inducing neovascularization in response to hypoxia.^{1,2}

Figure 1. HIF-1 regulates expression of a variety of genes that enhance tumor growth and survival.¹



HIF-1 is a critical mediator of adaptive responses to changes in tissue oxygenation.^{1,2} In well-oxygenated cells, HIF-1 α is continuously degraded in an oxygen-regulated manner by the ubiquitin-proteasome system. In tumor cells, HIF-1 α levels increase in response to hypoxia and are regulated at both the level of translation and degradation.³ In addition to intratumoral hypoxia, multiple other mechanisms may result in increased levels of HIF-1 α in cancer cells.¹ Examples of such mechanisms include 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; mutations (loss of function) in genes such as von Hippel Lindau (VHL), p53, and phosphatase and tensin homolog (PTEN); and BCL2 overexpression.¹

HIF-1 α is rarely expressed in normal tissues and is expressed in many primary malignant tumor types.⁴ Hypoxic cells, which have high levels of HIF-1 α , are resistant to both chemotherapy and radiotherapy. Increased HIF-1 α levels are associated with poor prognosis and increased mortality in several neoplasms such as melanoma and bladder, brain, breast, cervical, endometrial, and head and neck cancers.⁵ Down-regulation of HIF-1 α may have broad therapeutic application.

EZN-2968 is a locked nucleic acid (LNA) mRNA antagonist that specifically inhibits the expression of HIF-1 α mRNA and leads to its destruction.⁶ EZN-2968 is a 16-mer oligonucleotide, of which 6 deoxyribonucleic acid (DNA) nucleotides are replaced with LNA nucleotides.

A highly potent, selective, and durable antagonism of HIF-1 α 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-1 α and vascular endothelial growth factor (VEGF) in the liver.

Clinical Study

Study Design

- Schedule of administration
 - Original: Five daily 2-hour intravenous infusions per 4-week cycle
 - Amendment 3: Once-weekly administration, with two doses the first week
- 3 + 3 design

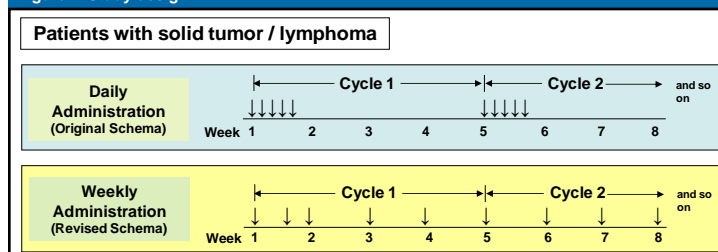
Objectives

- Determine maximum tolerated dose
- Determine recommended Phase 2 dose
- Evaluate safety and tolerability
- Determine PK profile
- Determine pharmacodynamic profile: relevant laboratory parameters, functional imaging, biopsies (skin, liver, tumor)
- Detect preliminary evidence of anti-tumor 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
- Prothrombin time (PT), partial thromboplastin time (PTT), International Normalized Ratio (INR), serum creatinine, and total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN)
- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 2.5 \times$ ULN

Figure 2. Study design.



Methods

- Plasma concentrations of EZN-2968 determined by ELISA hybridization assay
- Dose proportionality determined with linear regression between dose and area under the drug concentration-time curve (AUC)
 - Initially estimated using noncompartmental model
 - In-depth analysis then performed using two-compartment model with population-based approach and Bayesian method including patient-specific biologic variables
- PK parameters
 - PK analyzed using NONMEM Version V1.2

Results

Patient and Treatment Information

At the time of the data cutoff, 26 patients had been enrolled and treated. Three patients were still receiving study drug. For the other 23 patients, the reasons for discontinuation of EZN-2968 were progressive disease (PD) (20 patients), adverse events (AEs) (2 patients, cardio-respiratory / cardiac arrest considered unlikely related to study drug), and withdrawal of consent (1 patient).

The median age of the treated patients was 60 y (range: 42-79 y) (Table 1). Of the 26 patients, 15 (58%) were men and 11 (42%) were women; 96% of patients were white. ECOG performance status was 0 for 11 patients (42%), 1 for 13 patients (50%), and 2 for 2 patients (8%). All 26 patients had received prior chemotherapy. The median number of prior treatments was 4 (range = 1 – 10).

Tumor types included colorectal cancer (CRC) (10 patients); renal cell cancer (RCC) (5 patients); soft tissue sarcoma (STS) and ovarian cancer (3 patients each); and breast cancer, gastrointestinal stromal tumor, melanoma, pancreatic cancer, and prostate cancer (1 patient each) (Table 1).

The 23 patients who completed the study received between 1 and 7 treatment cycles (mean = 2).

Table 1. Demographics and Baseline Characteristics

	Dose (mg/kg/day)								All Patients n (%)	
	0.5	0.8	1.2	1.8	2.7	4.1	4.1W*	6.2		6.2W*
Patients enrolled and treated	3	3	3	4	3	3	4	1	2	26 (100%)
Age, years										
Median	68	57	64	60	49	63	58	66	55	60
Range	60-79	51-67	54-68	53-68	47-52	53-77	47-64	0	42-68	42-79
Sex, n										
Male	3	2	2	2	1	1	2	1	1	15 (58%)
Female	0	1	1	2	2	2	2	0	1	11 (42%)
Diagnosis, n										
Colorectal cancer (CRC)	1	0	1	2	2	1	1	1	1	10 (38%)
Renal cell cancer (RCC)	0	1	2	0	0	0	1	0	0	5 (19%)
Ovarian cancer	0	1	0	0	0	0	1	0	1	3 (12%)
Soft tissue sarcoma (STS)	1	0	0	0	0	1	0	0	0	3 (12%)
Breast cancer	0	0	0	0	1	0	0	0	0	1 (4%)
Gastrointestinal stromal tumor (GIST)	0	0	0	1	0	0	0	0	0	1 (4%)
Melanoma	1	0	0	0	0	0	0	0	0	1 (4%)
Pancreatic cancer	0	0	0	1	0	0	0	0	0	1 (4%)
Prostate cancer	0	1	0	0	0	0	0	0	0	1 (4%)
Performance status (ECOG), n										
0	1	0	2	1	2	0	3	0	2	11 (42%)
1	2	3	1	3	1	1	1	1	0	13 (50%)
2	0	0	0	0	0	2	0	0	0	2 (8%)

*This cohort received weekly administration; dose is reported as mg/kg/week.

Safety and Tolerability

No dose-limiting toxicities (DLTs) have been observed to date.

Overall, 24 patients (92%) had at least one treatment-emergent AE (Table 2). The most commonly reported AEs (>20% of patients) were vomiting (31%) and anemia, diarrhea, fatigue, and nausea (23% each). The intensity of most AEs was Grade 1 or 2.

Eleven patients (42%) had AEs (all Grade 1 or 2) considered likely related to study drug. Drug-related diarrhea was reported in three patients; drug-related fatigue, pyrexia, and vomiting were reported in two patients each; all other drug-related AEs were reported in one patient each.

Eight patients (31%) had ≥ 1 Grade 3 AE. Grade 3 diarrhea and anemia were reported in 2 patients each; all other Grade 3 AEs were reported in one patient each. No patient had a Grade 4 AE. Two patients (8%) had one Grade 5 AE each: cardio-respiratory arrest (1 patient) and cardiac arrest (1 patient). None of the Grade 3 or 5 AEs were considered likely related to study drug.

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

Table 2. Treatment-Emergent Adverse Events Reported in >10% of Patients

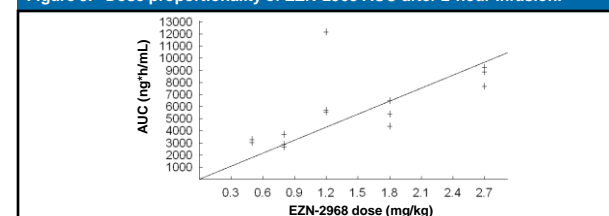
	Dose (mg/kg/day)								All Patients n (%)	
	0.5	0.8	1.2	1.8	2.7	4.1	4.1W*	6.2		6.2W*
Patients enrolled and treated	3	3	3	4	3	3	4	1	2	26 (100)
Patients with ≥ 1 AE	2	3	3	4	3	3	3	1	2	24 (92)
Patients with:										
Vomiting	1	1	0	3	2	1	0	0	0	8 (31)
Anemia	0	1	0	2	1	2	0	0	0	6 (23)
Diarrhea	1	0	0	1	1	2	0	0	1	6 (23)
Fatigue	1	1	1	1	0	1	0	1	0	6 (23)
Nausea	0	0	1	4	1	0	0	0	0	6 (23)
Constipation	1	1	0	1	1	0	1	0	0	5 (19)
Dyspnea	1	0	0	1	0	0	2	1	0	5 (19)
Tumor pain	1	1	1	1	0	1	0	0	0	5 (19)
Anorexia	0	0	0	1	1	1	0	0	0	4 (15)
Headache	0	0	0	0	3	0	1	0	0	4 (15)
Pyrexia	0	0	0	2	0	0	2	0	0	4 (15)
Abdominal pain	0	0	0	1	1	0	0	1	0	3 (12)
Back pain	0	0	0	1	1	1	0	0	0	3 (12)
Cough	1	0	0	0	0	1	0	0	0	3 (12)
Dry skin	1	0	0	0	0	1	1	0	0	3 (12)

*This cohort received weekly administration; dose is reported as mg/kg/week.

Pharmacokinetics

After a 2-hour infusion of EZN-2968, the dose is highly correlated with AUC (n=14, r=0.63, p=0.014), with a pattern consistent with dose-proportional PK within the studied range (Figure 3).

Figure 3. Dose proportionality of EZN-2968 AUC after 2-hour infusion.



The two-compartment model is shown in Figure 4A, and the goodness of fit of the model is shown in Figure 4B. The residual variability of the modeled data is illustrated by the distance separating data points from the zero value or perfect fit. One aberrant data point is observed on the top left.

Figure 4. (A) Two-compartment model with population-based approach and Bayesian method including patient-specific biologic variables. (B) Goodness of fit.

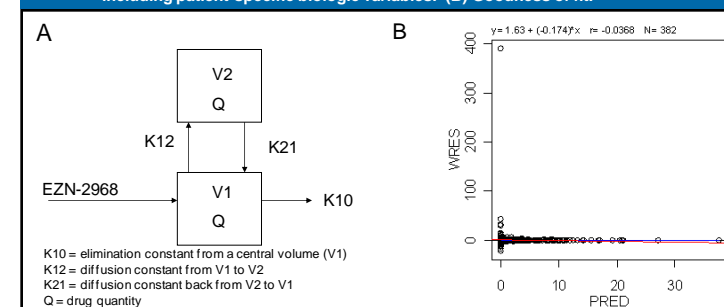


Table 3. Population PK Estimates and Bootstrap Verifications (N=400)

	Estimate	% Relative Standard Error	Median*	90% Confidence Interval
V1 (h)	4.5	8.5	4.39	3.39>>5.79
CL (L/h)	8.26	7.9	8.2	6.91>>9.91
Q (mg)	0.366	12.3	0.327	0.065>>0.68
V2 (h)	23.2	22.1	20.9	0.3>>73

*Bootstrap.

The data derived from the different model parameters described in Figure 4 are consistent with two distribution phases (Tables 3 and 4).

- First, a rapid diffusion (0.4 hours) (Table 4) and elimination of EZN-2968 from plasma (K10 [elimination half-life] = 0.46 hours; data not shown), which together determine clearance from the plasma and an apparent half-life of ~20 minutes (data not shown).
- Second, a much slower diffusion into a deeper compartment, as evidenced by a 45.9-hour half-life (Table 4)

A possible clinical interpretation would be that EZN-2968 is rapidly cleared from the plasma into peripheral tissues and elimination. In tissues, a longer diffusion further occurs to a deeper sector, while plasma levels are no longer detectable due to rapid clearance from the plasma.

Table 4. Two-Component Diffusion PK Parameters of EZN-2968

	Estimate	% Relative Standard Error	Median	2.5th>>97.5th Percentiles
T1/2 (h)				
α	0.4	8.5	0.35	0.19>>1.02
β	45.9	7.9	45.7	45.3>>47
% of Total AUC				
α Phase	82.9	12.3	85.3	64>>96.8
β Phase	17.1	22.1	14.7	3.2>>36

Statistics of Bayesian estimates. AUC = area under the drug concentration-time curve; T1/2 = half-life.

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) and 3 (pre-dose) 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.

Antitumor Activity

Thus far, the best overall response (per RECIST) was stable disease (SD) for 4 patients and PD for 17 patients (Table 5). Of the 4 patients who achieved SD, 2 patients had STS (duration of SD = 197 and 82+ days), 1 patient had RCC (166 days), and 1 patient had ovarian cancer (99 days).

Table 5. Best Overall Response

	Dose (mg/kg/day)								All Patients n (%)	
	0.5	0.8	1.2	1.8	2.7	4.1	4.1W*	6.2		6.2W*
Patients enrolled and treated	3	3	3	4	3	3	4	1	2	26 (100)
Stable disease	1	0	0	0	0	1	2 ^a	0	0	4 (15)
Progressive disease	1	3	3	3	3	2	1	1	0	17 (65)
Not evaluable	1 ^b	0	0	1 ^b	0	0	0	0	0	3 (12)
Pending data	0	0	0	0	0	0	0	0	2 ^a	2 (8)

*This cohort received weekly administration; dose is reported as mg/kg/week.

^a Two patients still receiving treatment: 1 patient receiving 4.1 mg/kg/week, and 1 patient receiving 6.2 mg/kg/week.

^b Patient was on study for 4 weeks.

Two patients with STS had SD (per RECIST).

- One patient with angiosarcoma had prolonged SD for 197 days after having received 0.5 mg/kg/day of EZN-2968. This 79-year-old man had progressed after receiving prior paclitaxel. For this patient, duration of treatment with EZN-2968 (28 weeks, 7 cycles) exceeded the duration of treatment with prior paclitaxel (<22 weeks). Near complete regression of the angiosarcoma after treatment with EZN-2968 is shown in Figure 5.
- The other patient is a 47-year-old man with leiomyosarcoma who has had SD for 82+ days. He has received 4+ cycles and continues to receive 4.1 mg/kg/week of EZN-2968. Prior chemotherapy included doxorubicin and DTIC, gemcitabine and docetaxel, trabectedin, and deforolimus.

Figure 5. Angiosarcoma treated with EZN-2968 (0.5 mg/kg/day).



The patient with RCC who had prolonged SD for 166 days received 4.1 mg/kg/day of EZN-2968. This 77-year-old man had progressed after receiving prior anti-angiogenic treatment with sunitinib and temsirolimus. For this patient, duration of treatment with EZN-2968 (24 weeks, 6 cycles) exceeded the duration of treatment with prior anti-angiogenic treatment (<13 weeks).

The patient with ovarian cancer had SD for 99 days after having received 4.1 mg/kg/week of EZN-2968 for 4 cycles. This 60-year-old woman had progressed after receiving prior carboplatin/paclitaxel, imatinib/docetaxel, doxorubicin HCl liposome injection, topotecan, satraplatin, pazopanib, carboplatin/decitabine, and gemcitabine.

Conclusions

EZN-2968, a novel HIF-1 α mRNA antagonist, was well tolerated in previously treated patients with advanced malignancies. No DLTs have been observed to date. PK data do not show accumulation of EZN-2968 in plasma and support weekly dosing. EZN-2968 has a two-component diffusion. Durable stable disease was observed in two patients with STS (angiosarcoma, leiomyosarcoma), in one patient with renal cancer, and in one patient with ovarian cancer. Dose escalation is ongoing.

References

- Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*. 2003;3:721-32.
- Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. *Physiology*. 2009;24:97-106.
- Carroll VA, Ashcroft M. Targeting the molecular basis for tumour hypoxia. *Expert Rev Mol Med*. 2005;7:1-16.
- Zhong H, De Marzo AM, Laughner E, et al. Overexpression of hypoxia-inducible factor 1 α in common human cancers and their metastases. *Cancer Res*. 1999;59:5830-5.
- Hirota K, Semenza GL. Regulation of angiogenesis by hypoxia-inducible factor-1. *Crit Rev Oncol Hematol*. 2006;59:15-26.
- Greenberger LM, Horak ID, Filipula D, et al. A RNA antagonist of hypoxia-inducible factor-1 α , EZN-2968, inhibits tumor cell growth. *Mol Cancer Ther*. 2008;7:3598-608.

*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.