Poster 398

Phase I, pharmacokinetic (PK), dose-escalation study of EZN-2968, a novel hypoxia-inducible factor-1α (HIF-1α) RNA antagonist, administered weekly in patients (pts) with solid tumors

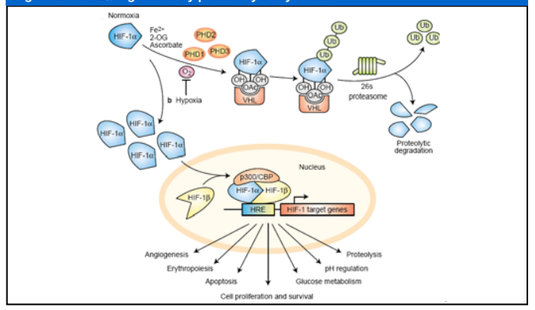
N. Lewis,[1] R.B. Cohen,[1] Y. Nishida,[1] H.I. Hurwitz,[2] C. Arrowwood,[2] H.E. Uronis,[2] E. Gilles,[3*] C. Longley,[4*] A. Buchbinder,[3*] J. Figueroa[5]

[1]Fox Chase Cancer Center, Philadelphia, PA, USA; [2]Duke University Medical Center, Durham, NC, USA; [3,4]Enzon Pharmaceuticals, Inc., Bridgewater[3] and Piscataway,[4] NJ, USA; [5]Joe Arrington Cancer Research & Treatment Center, Lubbock, TX, USA

Background

HIF-1 is a crucial transcription factor that regulates key genes important in cancer biology. HIF-1 controls processes that include tumor metabolism, pH, neovascularization, drug resistance, tumor invasion, autophagy, and cell survival (Figure 1).[1,2]

Figure 1. HIF-1α regulation by proline hydroxylation.[2]



HIF-1 mediates adaptive responses to changes in tissue oxygenation.[1] HIF-1α levels increase in tumor cells in response to hypoxia and are regulated at both the level of translation and degradation (Figure 1).[2] In particular, in well-oxygenated cells, HIF-1α is continuously 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] 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.[1]

HIF-1α is rarely expressed in normal tissues and is expressed in many primary malignant tumor types.[3] 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.

EZN-2968 is a locked nucleic acid (LNA) RNA antagonist that specifically inhibits the expression of HIF-1α messenger RNA and leads to its destruction.[5] 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.

When human cancer cells were transfected with EZN-2968, a highly potent, selective, and durable antagonism of HIF-1α expression was observed under both normoxic and hypoxic conditions.[5] In vivo administration of EZN-2968 to normal mice led to specific, dose-dependent, and highly potent down-regulation of endogenous HIF-1α and vascular endothelial growth factor (VEGF) in the liver.

Clinical Study

Study Design

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

Objectives

- Determine the MTD
- Determine the recommended Phase 2 dose
- Evaluate the safety and tolerability
- Determine the PK profile
- Determine the pharmacodynamic (PD) profile: relevant laboratory parameters, functional imaging, biopsies (skin, liver, tumor)
- Detect preliminary evidence of anti-tumor activity

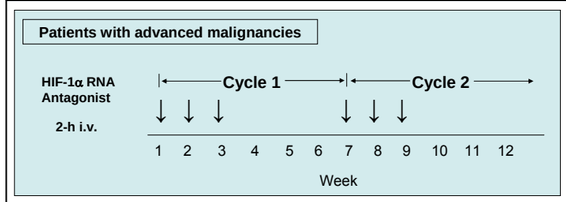
Key Eligibility Criteria

- 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 ≤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
- PK parameters estimated using noncompartmental model & analyzed using WinNonlin PK software (Version 5.1)

Figure 2. 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, 18 pts had been enrolled and treated. Three pts were still receiving study drug. For the other 15 pts, the reasons for discontinuation of EZN-2968 were progressive disease (PD) (10 pts), investigator decision (2 pts), death (1 pt), withdrawn consent (1 pt), and hip fracture (1 pt).

The median age of the treated pts was 59 y (range: 37-87 y) (Table 1). Of the 18 pts, 9 (50%) were female and 9 (50%) were male; 94% of pts were white. ECOG performance status was 0 for 7 pts (39%), 1 for 10 pts (56%), and 2 for 1 pt (6%). All 18 pts had received prior chemotherapy. The median number of prior cytotoxic chemotherapies was 3 (range = 1 - 7).

Tumor types included colorectal cancer (CRC) (6 pts); liposarcoma (3 pts); hepatocellular carcinoma and laryngeal cancer (2 pts each); and adenoid cystic carcinoma, bronchoalveolar cancer, non-small cell lung cancer (NSCLC), renal cell cancer (RCC), and pancreatic cancer (1 pt each) (Table 1).

The 15 pts who completed the study received between 1 and 10 treatment cycles (mean = 2).

Table 1. Demographics and Baseline Characteristics for Treated Patients

	Dose Cohort				
	Cohort 1 1 mg/kg	Cohort 2 1.5 mg/kg	Cohort 3 2.3 mg/kg	Cohort 4 3.5 mg/kg	All Patients
Pts enrolled and treated	4	4	3	7	18
Median age in years (range)	55 (44-72)	70 (37-87)	52 (50-55)	65 (46-74)	59 (37-87)
Sex, n					
Female	2	1	3	3	9
Male	2	3	—	4	9
Diagnosis, n					
Colorectal cancer (CRC)	2	2	2	—	6
Liposarcoma	1	—	—	2	3
Hepatocellular carcinoma (HCC)	—	1	—	1	2
Laryngeal cancer	—	1	—	1	2
Adenoid cystic carcinoma	—	—	—	1	1
Bronchoalveolar cancer	—	—	—	1	1
Non-small cell lung cancer (NSCLC)	—	—	—	1	1
Renal cell cancer (RCC)	1	—	—	—	1
Pancreatic cancer	—	—	1	—	1
Performance status (ECOG), n					
0	2	1	2	2	7
1	2	3	—	5	10
2	—	—	1	—	1

Safety and Tolerability

Dose-limiting toxicity (DLT), intracranial bleeding at a site of a metastasis, was found in one pt in the fourth cohort (3.5 mg/kg) who had a history of breast cancer with no history of signs or symptoms of brain metastases. This finding necessitated cohort expansion to 6 pts at this dose level. No other DLTs have been observed. The intracranial bleeding resulted in death 17 days after the pt's last dose of EZN-2968.

Overall, 17 pts (94%) had at least one treatment-emergent adverse event (AE) (Table 2). The most commonly reported AEs (>30% of pts) were nausea (39% of pts) and abdominal pain, fatigue, and vomiting (33% of pts each). The intensity of most AEs was Grade 1 or 2. Four pts (22%) had Grade 3 AEs: abdominal pain (1 pt); cataracts (1 pt); ascites, biliary sepsis, fatigue, hypercalcemia, and hypotension (1 pt); and hip fracture, hypertension, and osteoporosis (1 pt). Two pts (11%) had Grade 4 AEs: coma and hypocalcemia (1 pt), and respiratory failure (1 pt). None of these Grade 3 or 4 AEs were considered drug related.

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

Table 2. Treatment-Emergent Adverse Events Reported in >20% of Patients

	Dose Cohort				
	Cohort 1 1 mg/kg	Cohort 2 1.5 mg/kg	Cohort 3 2.3 mg/kg	Cohort 4 3.5 mg/kg	All Patients n (%)
Pts enrolled and treated	4	4	3	7	18
Pts with ≥1 AE	4	4	3	6	17 (94)
Pts with:					
Nausea	3	—	2	2	7 (39)
Abdominal pain	1	1	2	2	6 (33)
Fatigue	2	1	3	—	6 (33)
Vomiting	2	—	2	2	6 (33)
Pyrexia	3	—	2	—	5 (28)
Anorexia	2	—	2	—	4 (22)
Cough	2	2	—	—	4 (22)
Headache	1	1	1	1	4 (22)

Pharmacokinetics

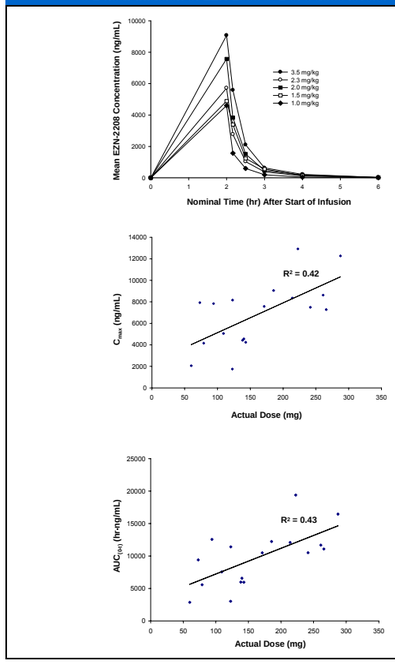
At least 3 pts from each of the 4 cohorts (total = 18 pts) provided data for PK analysis (Table 3, Figure 3). After a 2-hour infusion of EZN-2968, the half-life is short (mean = 0.8 hours) (Table 3). The dose is highly correlated with maximum plasma concentration (C_{max}) and area under the drug concentration-time curve (AUC) (R^2 = 0.42 and 0.43, respectively), with a pattern consistent with dose-proportional PK within the studied range. The human plasma PK is similar to what was observed preclinically, where prolonged tissue retention also was observed.

Table 3. PK Parameters After 2-Hour Infusion of EZN-2968

Dose (mg/kg)	N	C_{max} (ng/mL)	$AUC_{(0-t)}$ (h-ng/mL)	Terminal $t_{½}$ (h)
1	4	4598 ± 2439	5954 ± 2680	0.73 ± 0.47
1.5	3	4883 ± 3049	7719 ± 4769	1.02 ± 0.44
2	1	7573	10492	0.85
2.3	3	5716 ± 2120	7992 ± 2975	0.86 ± 0.07
3.5	7	9429 ± 2268	13349 ± 3288	0.74 ± 0.17

Mean ± standard deviation.
$AUC_{(0-t)}$, t is time of last measurable concentration.
One pt received 2 mg/kg instead of 1.5 mg/kg. This pt is included in the 1.5-mg/kg cohort for Tables 1, 2, and 4.

Figure 3. Mean EZN-2968 plasma concentration, C_{max}, and $AUC_{(0-t)}$ after a 2-hour infusion of EZN-2968 at 1 of 5 dose levels.



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.

Antitumor Activity

The best overall response was stable disease (SD) for 7 pts and PD for 7 pts (Table 4). Two pts had not completed Cycle 1 at the data cutoff; response information was not available for the other 2 pts. Of the 7 pts who achieved SD, 2 pts had hepatocellular cancer (duration of SD = 211 days, 36 days), 1 pt had RCC (408 days), 1 pt had adenocystic carcinoma (120 days), 1 pt had laryngeal cancer (78 days), 1 pt had liposarcoma (120+ days), and 1 pt had NSCLC (37+ days). Most pts were heavily pretreated.

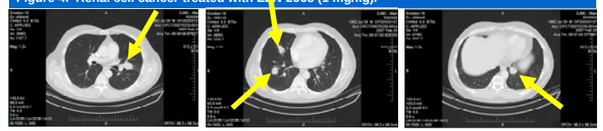
Table 4. Best Overall Response

	Dose Cohort				All Patients n (%)
	Cohort 1 1 mg/kg	Cohort 2 1.5 mg/kg	Cohort 3 2.3 mg/kg	Cohort 4 3.5 mg/kg	
Pts enrolled and treated	4	4	3	7	18
Stable disease	1	2	—	4	7 (39)
Progressive disease	2	1	3	1	7 (39)
Not evaluable	1	—	—	1	2 (11)
Pending data	—	1	—	1	2 (11)

Antitumor Activity (continued)

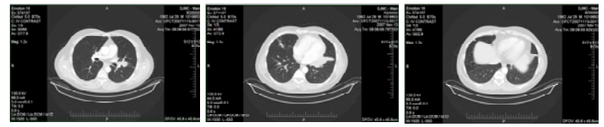
The pt with RCC who had prolonged SD for more than 1 year received 1 mg/kg of EZN-2968. This 44-year-old man had a wild-type VHL genotype and had progressed after receiving prior sunitinib and an mTOR inhibitor. For this pt, duration of treatment with EZN-2968 (60 weeks) exceeded the duration of treatment 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 4). Compared to the measurements after Cycle 1, the tumor had decreased by 32% after Cycle 6.

Figure 4. Renal cell cancer treated with EZN-2968 (1 mg/kg).



February 20, 2007
Before first cycle
Baseline scan
29 days before first dose

April 23, 2007
After 1 cycle
35 days after first dose
63 days since baseline
27% increase in sum of tumor sizes

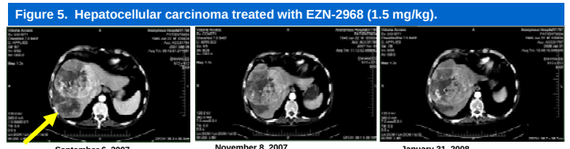


November 13, 2007
After 6 cycles
267 days since baseline
239 days since first dose
14% decrease in sum of tumor sizes

Percentages are maximum changes in tumor relative to baseline measurement.

The pt 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 21% after Cycle 1 and had increased less than 1% after Cycle 3 (Figure 5).

Figure 5. Hepatocellular carcinoma treated with EZN-2968 (1.5 mg/kg).



September 6, 2007
Baseline
29 days before first dose

November 8, 2007
After 1 cycle
64 days since baseline
36 days since first dose
21% decrease* in sum of tumor sizes

January 31, 2008
After 3 cycles
148 days since baseline
120 days since first dose
<1% increase* in sum of tumor sizes

*Tumor cut at site of maximal diameter for indicated lesion. Percentages are maximum changes in tumor relative to baseline measurement.

Conclusions

EZN-2968, a novel HIF-1α RNA antagonist, was well tolerated in previously treated pts with advanced malignancies. DLT, intracranial bleeding at the site of a metastasis, was reported in one pt in Cohort 4 (3.5 mg/kg). PK data support weekly administration of EZN-2968. Prolonged stable disease with clear evidence of tumor shrinkage was observed in one pt with RCC and one pt with HCC. Dose escalation is ongoing.

Reference

1. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*. 2003;3:721-732.
2. Carroll VA, Ashcroft M. Targeting the molecular basis for tumour hypoxia. *Expert Rev Mol Med*. 2005;7:1-16.
3. Zhong H, De Marzo AM, Laughner E, et al. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. *Cancer Res*. 1999;59:5830-5835.
4. Hirota K, Semenza GL. Regulation of angiogenesis by hypoxia-inducible factor-1. *Crit Rev Oncol Hematol*. 2006;59:15-26.
5. Greenberger LM, Horak ID, Filpula D, et al. A RNA antagonist of hypoxia-inducible factor-1α, EZN-2968, inhibits tumor cell growth. *Mol Cancer Ther*. 2008;7:in press.

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

EORTC-NCI-AACR; Geneva, Switzerland; 23 October 2008