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

**Background**

HIF-1α is a crucial transcription factor that regulates key gene products important in cancer biology. HIF-1α controls processes that include tumor metabolism, pH, neovascularization, and angiogenesis and is regulated at both the mRNA level as well as protein level. HIF-1α stabilization occurs when hypoxic cells are continuously deprived of oxygen-regulated regulatory substances. Several hypoxic mechanisms may result in increased levels of HIF-1α in cancer cells. For example, changes in microvascularity, hypoxia, and alternative mechanisms may result in increased levels of HIF-1α in cancer cells. 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. Examples of such mechanisms include mutations in genes such as von Hippel-Lindau (VHL), p53, and phosphatase and tensin homing (PTEN); alterations in signaling via phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways; and loss of function of tumor suppressor genes such as ARF and p53.

HIF-1α is highly expressed in normal tissues and is elevated in early primary malignant tumor types. Hypoxic cells, which have high levels of HIF-1α, are resistant to both chemotherapy and radiotherapy. Elevated HIF-1α levels are associated with poor patient survival. Down-regulation of HIF-1α may have broad therapeutic applications.

**EZN-2968** is a locked nucleic acid (LNA)-RNA antagonist that specifically inhibits the expression of HIF-1α by repressing its endogenous mRNA levels and leads to the destruction of HIF-1α protein. The efficacy and safety of EZN-2968 in 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′-GGAACACTAATTGAACG-3′, where upper case indicates LNA residues and lower case indicates DNA residues. The polymerase chain reaction (PCR) and DNA sequencing of a 66-bp fragment from the 5′-end of EZN-2968 revealed 100% sequence identity to the predicted sequence.

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**Clinical Study**

**Study Design**

- **3 x 3 design**: Dose escalation to 6 pts to determine the maximum tolerated dose (MTD).
- **MTD dose expansion**: Up to 18 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**
- **Determine laboratory parameters, including coagulation factors**
- **Detect any potential evidence of anti-tumor activity**

**Key Eligibility Criteria**

- **Advanced solid malignant solid tumor or lymphoma; refractory to standard therapy**
- **Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2**
- **Prothrombin time (PT), prothrombin time international normalized ratio (INR), serum creatinine, total bilirubin ≤ 1.5 upper limit of normal**

**Methods**

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

**Pharmacodynamics**

Concentrations of the following HIF-Liganded gene products were determined: VEGF, erythropoietin, fibroblast growth factor-2, and transforming growth factor-β (TGF-β). The entire array of gene products was assessed at Weeks 1 (pre-dose), 3, and 6, and each cycle (pre-dose and post-dose). Treatment with EZN-2968 resulted in a series of dose-level dependent and highly potent down-regulation of endogenous HIF-1α and vascular endothelial growth factor (VEGF) in the brain.

**Safety and Tolerability**

In-cutaneous bleeding (CTLB), an intraocular bleeding at a site of metastasis, was found in one pt in the fourth cohort (2 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 8 pts at this dose level. No other SAEs have been observed. The intraocular bleeding resulted in death 17 days after the pt’s last dose of EZN-2968.

**Results**

**Patient and Treatment Information**

At the time of the data cutoff, 14 pts had been enrolled and treated. Three pts were still receiving study drug. For the other 11 pts, reasons for discontinuation were determined: 4 pts discontinued due to progressive disease (PD) (18%) (investigator decision (2 pt), death (1 pt), withdrawn consent (1 pt), and hip fracture (1 pt)). The median age of the treated pts was 59 (range: 37-67) (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 (63 days since baseline), 1 for 1 pt (35 days after first dose) (3 years), and 2 for 1 pt (160+ days). 8 pts had received prior primary chemotherapy. The pt with HCC who had prolonged SD for more than 6 months received 1.5 mg/kg of EZN-2968.

**Safety Data**

No SAEs have been observed. The intracranial bleeding resulted in death 17 days after the pt’s last dose of EZN-2968.

**PK Parameters**

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

**Safety and Tolerability**

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**Research**

**Antitumor Activity**

**Conclusions**

EZN-2968, a novel HIF-1α RNA antagonist, was well tolerated in previously treated pts with advanced malignancies. DLT, intracranial bleeding in the site of a metastasis, was reported in one pt (in cohort 6: 2 mg/kg). For pts support weekly administration of EZN-2968. Posterior vitreous detachment was noted in one pt with HCC. Dose escalation is ongoing.

**References**

7. The author is affiliated with EonPharmaceuticals, Inc., and company’s stock options under All.

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