Poster
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Results of a Phase 1, open-label, dose-escalation study evaluating the safety and tolerability of EZN-3042, a survivin messenger ribonucleic acid (mRNA) antagonist, administered with or without docetaxel in adult patients with advanced solid tumors or lymphoma

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Background

Survivin, the smallest member of the inhibitors of apoptosis (IAP) gene family, functions as an essential regulator of mitosis and apoptosis as well as a promoter of tumor-associated angiogenesis.[1,2]

Survivin is rarely expressed in most normal adult tissues,[1] but it is preferentially and highly expressed in many types of cancer.[3-5] Overexpression of survivin in many cancers is correlated with an increased rate of disease recurrence and reduced survival.[6,7] Increased survivin expression also is associated with increased risk of metastasis, depth of invasion, and locoregional occurrence.[7] Down-regulation of survivin may have broad therapeutic application.

The survivin mRNA antagonist, designated EZN-3042, is a potent locked nucleic acid (LNA) antisense oligonucleotide that specifically down-modulates survivin mRNA and protein.[8-10] The oligonucleotide EZN-3042 is composed of 16 monomeric units, a 16-mer, of which 7 DNA nucleotides are replaced with LNA nucleotides. The sequence of EZN-3042 is 5′CTCAatccatggCAGc-3′. Capital letters denote LNA monomers, and lowercase letters denote DNA monomers.

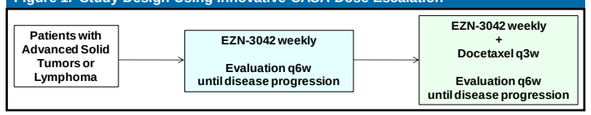
When transfected into tumor cells, the survivin antagonist was a highly potent inhibitor of survivin expression in vitro (IC_{50} <5 nM).[8,10] Preclinically, EZN-3042 enhanced apoptotic and antitumor effects of taxanes.[9,10] When EZN-3042 was tested in combination with subtherapeutic doses of paclitaxel in xenograft models of human lung cancer, EZN-3042 significantly enhanced apoptotic and antitumor effects of taxanes.[9,10]

Clinical Study

Study Design

- Combination after single agent (CASA) modified Fibonacci dose-escalation (Figure 1)
  - Allows one to follow safety and efficacy for the same patient as he/she progresses from single-agent to combination treatment
  - Independent dose escalation (3 + 3) for single agent and for combination; dose escalation based on toxicities observed during Cycle 1 of single agent or combination, respectively

Figure 1. Study Design Using Innovative CASA Dose Escalation



- Single-agent EZN-3042
  - 2-hour intravenous (i.v.) infusion
  - 2 doses during Week 1, then weekly doses; removed loading dose starting at 6.5-mg/kg cohort
  - Continue until progressive disease (PD) or unacceptable toxicity
- EZN-3042 in combination with docetaxel (Taxotere®), 75 mg/m², at progression
- Objective tumor response assessed every 6 weeks using the Response Evaluation Criteria in Solid Tumors (RECIST)

Objectives

- Determine the maximum tolerated dose (MTD)
- Determine the recommended Phase 2 dose
  - EZN-3042 administered as a single agent
  - EZN-3042 administered in combination with docetaxel
- Evaluate safety and tolerability of each regimen
- Determine the pharmacokinetic (PK) profile
- Evaluate the pharmacodynamic profile of EZN-3042
- Detect preliminary evidence of antitumor activity

Methods

- Plasma concentration of EZN-3042 determined by immunoassay using hybridization of capture and detection oligonucleotides; lower limit of quantitation = 0.25 ng/mL
- PK parameters estimated using a Bayesian approach (Monolix Version 31s; http://wfn.software.monolix.org)

Results

Patient and Treatment Information

Single-Agent EZN-3042

At the time of the data cutoff, 24 patients had received single-agent EZN-3042 (Table 1). The median age of these patients was 61 y (range: 44-75 y) (Table 1). Of the 24 patients, 13 (54%) were men and 11 (46%) were women; 96% of patients were white. Thirteen patients (54%) had an ECOG performance status of 1.

All treated patients had received prior chemotherapy. Two patients had failed prior treatment with YM155, an investigational small-molecule suppressor of survivin. The median number of prior regimens was 3 (range = 1 to 10).

Tumor types included prostate cancer (8 patients); colorectal cancer (CRC) (5 patients); breast cancer (3 patients); non-small cell lung cancer (NSCLC) (2 patients); and pancreatic cancer, lymphoma, mesothelioma, leiomyosarcoma, thymic carcinoma, and unknown primary cancer (1 patient each). The 24 patients received between 1 and 5 cycles (mean = 2).

Table 1. Single-agent EZN-3042: Demographics and ECOG Performance Status

	Cohort 1 2.5 mg/kg	Cohort 2 5 mg/kg	Cohort 2-I 6.5 mg/kg	Cohort 3 8 mg/kg	All Patients
Patients treated	3	9	6	6	24
Age, years					
Median	69	60	53	65	61
Range	67-70	49-75	44-75	46-70	44-75
Sex, n					
Male	3	3	2	5	13 (54)
Female	0	6	4	1	11 (46)
ECOG performance status, n					
0	2	2	2	2	8 (33)
1	1	6	2	4	13 (54)
2	0	1	2	0	3 (13)

EZN-3042 + Docetaxel

At the time of the data cutoff, 11 patients had received the combination regimen (Table 2), including 1 patient who did not receive single-agent EZN-3042 but was treated directly with EZN-3042 (6.5 mg/kg) + docetaxel. The median age of the 11 patients was 67 y (range: 44-75 y) (Table 2). Of the 11 patients, 7 (64%) were men and 4 (36%) were women; 91% of patients were white. Six patients (55%) had an ECOG performance status of 1.

One patient had failed prior treatment with YM155. Tumor types included prostate cancer (6 patients); breast cancer (2 patients); and NSCLC, leiomyosarcoma, and unknown primary cancer (1 patient each). The 10 patients who completed the study received between 1 and 9 cycles (mean = 3). At the time of data cutoff, one patient was still receiving EZN-3042 + docetaxel.

Table 2. EZN-3042 + Docetaxel: Demographics and ECOG Performance Status

	Cohort 1C 2.5 mg/kg EZN-3042 + Docetaxel	Cohort 2C 5 mg/kg EZN-3042 + Docetaxel	Cohort 2C-I 6.5 mg/kg EZN-3042 + Docetaxel	All Patients
Patients treated	7	3	1	11
Age, years				
Median	69	54	71	67
Range	57-75	44-55	—	44-75
Sex, n				
Male	6	0	1	7 (64)
Female	1	3	0	4 (36)
ECOG performance status, n				
0	3	0	0	3 (27)
1	4	1	1	6 (55)
2	0	2	0	2 (18)

Safety and Tolerability

Single-Agent EZN-3042

Dose-limiting toxicity (DLT), was observed in 3 patients who had received EZN-3042 at a dose of 8 mg/kg: rapidly reversible Grade 3 increased aspartate or alanine aminotransferase (AST or ALT).

- One patient had CRC with metastasis to both lobes of the liver and entered the study with Grade 2 increased AST at baseline. After receiving the first dose of EZN-3042, the AST worsened in intensity to Grade 3. By 1 week after the last dose of EZN-3042, the intensity of the AST value had decreased to Grade 1. This patient had transient Grade 1 hyperbilirubinemia from Days 4 to 8.
- One patient with prostate cancer developed Grade 3 increased AST and Grade 2 increased ALT after the first dose of EZN-3042. By 6 weeks after the last dose of EZN-3042, the intensity of the AST and ALT values had decreased to Grade 1. Bilirubin remained within normal limits for this patient.
- One patient with prostate cancer developed Grade 3 increased AST and Grade 2 increased ALT after the second dose of EZN-3042. By 4 weeks after the last dose of EZN-3042, the intensity of the AST and ALT values had decreased to Grade 1. Bilirubin remained within normal limits for this patient.

All 24 patients who received single-agent EZN-3042 had at least one treatment-emergent adverse event (AE). The most commonly reported AEs (>20% of patients), regardless of relationship to study drug, were fatigue (46%); tumor pain and increased AST (42% each); increased ALT (38%); anorexia (29%); and diarrhea, nausea, and rash (21% each).

Safety and Tolerability (continued)

Single-Agent EZN-3042 (continued)

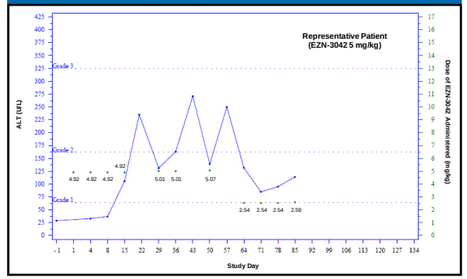
Most AEs were Grade 1 or 2 in intensity. Fourteen patients (58%) had Grade 3 AEs, the most common (>1 patient) of which were neutropenia (21%), leukopenia and increased AST (13%), and increased ALT (8%). There were no Grade 4 AEs. One patient (5 mg/kg EZN-3042) died due to PD.

The most frequently reported drug-related AEs (in >20% of patients) were increased AST (42%), increased ALT (38%), and fatigue (33%) (Table 3). Four patients, all of whom received EZN-3042 at a dose of 8 mg/kg, had drug-related Grade 3 AEs: 1 patient had increased AST and ALT, 2 patients had increased AST (both DLTs), and 1 patient had increased ALT (DLT). In all cases, the hepatoxicity was rapidly reversible. An example of the rapidly reversible hepatoxicity and the ability to continue EZN-3042 at a reduced dose in a patient with NSCLC treated at a dose of 5 mg/kg is provided in Figure 2.

Table 3. Single-Agent EZN-3042: Drug-Related Adverse Events Reported for >1 Patient

	Cohort 1 2.5 mg/kg	Cohort 2 5 mg/kg	Cohort 2-I 6.5 mg/kg	Cohort 3 8 mg/kg	All Patients n (%)
Patients treated	3	9	6	6	24
Patients with ≥1 drug-related AE	3	6	5	6	20 (83)
AST increased	1	3	2	4	10 (42)
ALT increased	1	2	2	4	9 (38)
Fatigue	2	3	1	2	8 (33)
Diarrhea	0	2	0	2	4 (17)
Anorexia	0	1	1	1	3 (13)
Nausea	0	3	0	0	3 (13)
Anemia	1	1	0	0	2 (8)
Pruritus	0	0	2	0	2 (8)
Rash	0	0	2	0	2 (8)

Figure 2. Rapid Reversibility of Hepatotoxicity and Ability to Continue EZN-3042 at Reduced Dose



EZN-3042 + Docetaxel

During the first week of treatment, a DLT of Grade 3 neutropenic fever was observed in 1 patient who received EZN-3042 (2.5 mg/kg) + docetaxel; the event resolved within 7 days of onset.

Ten of the 11 patients (91%) had at least one treatment-emergent AE. The most commonly reported AEs (>2 patients), regardless of relationship to study treatment, were fatigue (64%); neutropenia (55%); alopecia (45%); anorexia, tumor pain, and vomiting (36% each); and constipation, dyspnea, leukopenia, nausea, and peripheral sensory neuropathy (27% each).

Most AEs were Grade 1 or 2 in intensity. Eight patients (73%) who received combination treatment had Grade 3 AEs, the most common (>1 patient) of which were neutropenia (55%) and leukopenia (27%). There were no Grade 4 AEs. One patient (5 mg/kg EZN-3042 + docetaxel) died due to PD.

For EZN-3042 + docetaxel, the most frequently reported AEs (in ≥2 patients) considered likely related to the combination treatment were fatigue (64%), neutropenia (55%), alopecia (45%), anorexia (36%), and leukopenia and nausea (27% each) (Table 4). Six patients (55%) had drug-related Grade 3 AEs: leukopenia and neutropenia (3 patients), neutropenia (2 patients), and neutropenia and increased transaminases (ALT/AST) 1 patient).

Table 4. EZN-3042 + Docetaxel: Drug-Related Adverse Events Reported for >1 Patient

	Cohort 1C 2.5 mg/kg EZN-3042 + Docetaxel	Cohort 2C 5 mg/kg EZN-3042 + Docetaxel	Cohort 2C-I 6.5 mg/kg EZN-3042 + Docetaxel	All Patients n (%)
Patients treated	7	3	1	11
Patients with ≥1 drug-related AE				
Fatigue	5	2	0	7 (64)
Neutropenia	5	0	1	6 (55)
Alopecia	4	1	0	5 (45)
Anorexia	4	0	0	4 (36)
Leukopenia	3	0	0	3 (27)
Nausea	3	0	0	3 (27)
ALT increased	1	0	1	2 (18)
Mucosal inflammation	1	1	0	2 (18)
Vomiting	2	0	0	2 (18)

Pharmacodynamics

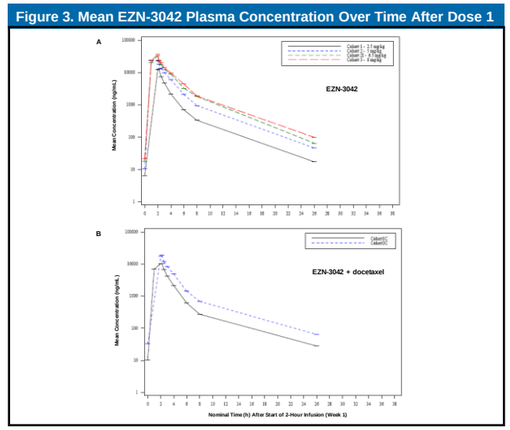
Hair follicles and tumor biopsies were collected at the prestudy visit and after the Week 3 infusion of single-agent EZN-3042. Six patients provided informative hair samples before and after EZN-3042 administration. Three patients provided tumor biopsies for survivin protein expression. No consistent down-modulation of survivin mRNA or protein was observed.

Pharmacokinetics

All 24 patients who received single-agent EZN-3042 provided data for PK analysis. EZN-3042 was infused over 2 hours. Both the drug concentration-time curve (AUC) and the maximum drug concentration (C_{max}) appear to be increasing in an approximately dose-proportional manner (Figure 3A). There appears to be little or no accumulation of drug during the fourth week through Day 28. The median elimination half-life of EZN-3042 was approximately 1.85 hours (range = 1.30–5.00 hours), the mean volume of distribution of the central compartment was 12.95 L (range = 6.38–18.99 L), and the mean clearance was 5.1 L/h (1.6–6.9 L/h).

Seven patients who received combination treatment with EZN-3042 and docetaxel (Cohorts 1C and 2C) also provided data for PK analysis (Figure 3B).These data were provided at one time point. The maximum plasma concentration of EZN-3042 at the 2.5- and 5.0-mg/kg dose levels are approximately the same in the single agent and combination cohorts (Figure 3).

Figure 3. Mean EZN-3042 Plasma Concentration Over Time After Dose 1



Antitumor Activity

The best response for single-agent EZN-3042 was stable disease in 5 patients (Table 5), including one with thymic carcinoma (5 mg/kg, 16 weeks). The best response for EZN-3042 (2.5 mg/kg) + docetaxel combination was a confirmed partial response (PR) in 1 patient with prostate cancer (27 weeks).

Table 5. Best Overall Response

Cohort	Single-Agent EZN-3042					EZN-3042 + Docetaxel			
	1	2	2-I	3	All Patients n (%)	1C	2C	2C-I	All Patients n (%)
EZN-3042 Dose (mg/kg)	2.5	5	6.5	8		2.5	5	6.5	
Patients treated	3	9	6	6	24	7	3	1	11
Partial response	0	0	0	0	0	1	0	0	1
Stable disease	1	2	1	1	5	3	0	0	3
Progressive disease	2	6	3	2	13	2	2	0	4
Not evaluated	0	1	0	3	4	1	1	0	2
No data yet	0	0	2	0	2	0	0	1	1

Conclusions

EZN-3042 was generally well tolerated in previously treated patients with advanced malignancies. The MTD for single-agent EZN-3042 is 6.5 mg/kg. Enrollment in the combination arm is ongoing. A qualitative assessment of the single-agent EZN-3042 PK data indicated that the AUC and C_{max} appear to increase in a dose-proportional manner. The CASA dose-escalation design is an innovative approach that allows one to follow the safety and efficacy of single-agent and combination therapy in the same patient. EZN-3042 also is being evaluated in an ongoing window-of-opportunity study in pediatric patients with relapsed acute lymphoblastic leukemia.

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

EZN-3042 is being developed by Enzon Pharmaceuticals, Inc. under a license with Santaris-Pharma A/S.

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