Poster C216

# Phase 1, first-in-human, dose-escalation study of EZN-2208, a novel anticancer agent, in patients with advanced malignancies

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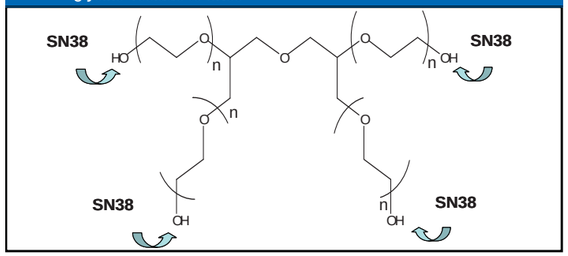
## Background

SN38 (10-hydroxy-7-ethyl-camptothecin), the active moiety of CPT-11 (Camptosar®, irinotecan), is a potent topoisomerase I inhibitor. Thus far, SN38 itself has not been used as an anticancer drug in humans due to its poor solubility in any pharmaceutically acceptable formulation.

EZN-2208 (PEG-SN38) is a water-soluble polyethylene glycol (PEG) drug conjugate of SN38 with approximately 3.5 to 4.0 SN38 molecules attached to the multi-arm PEG backbone (Figure 1).[1]

**Figure 1. EZN-2208 (PEG-SN38): Optimally loaded 4-arm PEG linked via a glycine residue to SN38[1]**




In preclinical in vitro and in vivo models of multiple solid tumors, lymphomas, large tumors, resistant tumors (including an in vivo model of CPT-11 resistance), and sensitive tumors, EZN-2208 had a significantly enhanced therapeutic effect compared with CPT-11.[2,3]

EZN-2208 enables increased solubility, parenteral delivery of SN38, longer circulating half-life, higher exposure of the active drug (SN38) in tumors, and greater preservation of the closed lactone ring (active form) in SN38 compared with SN38 derived from CPT-11.[1,2]

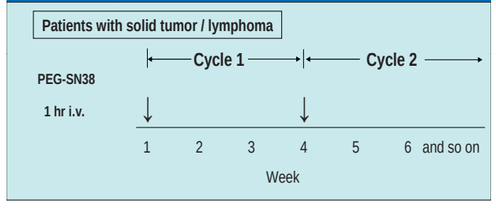
In animal models, EZN-2208 accumulates in tumors, where it releases SN38. The antitumor activity is attributed to higher exposure of tumors to SN38 via the preferential accumulation of EZN-2208 in the tumor (enhanced permeability and retention [EPR] effect) compared with CPT-11.[2] EZN-2208 also down-modulates mRNA of hypoxia-inducible factor-1α (HIF-1α) target genes, and has been shown to have anti-angiogenic and anti-apoptotic effects.[3]

## Clinical Study

### Study Design

- 3 + 3 design
- Dose expansion to 6 patients to determine the maximum tolerated dose (MTD)
- MTD dose expansion up to 10 patients
- 3 centers
- Cycles repeat every 3 weeks (Figure 2).

**Figure 2. Study schema**




- All patients were screened for UGT1A1 genotype.
- Patients with UGT1A1 *28/*28 genotype were treated in separate cohorts, at 2 dose levels below current cohort.
- Dose escalation was based on drug-related toxicities observed during Cycle 1.
- Pharmacokinetics (PK) were evaluated in Cycle 1.
- Objective tumor response assessed every 6 to 8 weeks using the Response Evaluation Criteria in Solid Tumors (RECIST).

### Objectives

- Determine the MTD
- Determine the recommended Phase 2 dose
- Evaluate the safety and tolerability
- Determine the PK profile
- Detect preliminary evidence of tumor response activity

### Methods

- Plasma concentrations of EZN-2208, SN38, and glucuronidated SN38 (SN38G) determined by HPLC using fluorescence detection
- SN38 concentration-time data analyzed via a population approach using NONMEM
- PK parameters estimated using a two-compartment open model with transit compartment to best describe a first-order sustained release of SN38 from EZN-2208

## Results

### Patient and Treatment Information

Forty-one patients were enrolled. Two patients, one in the 2.5 mg/m$^2$ cohort and one in the 5 mg/m$^2$ cohort, died due to progressive disease (PD) before receiving EZN-2208. Of the 39 patients who received EZN-2208, the reasons for discontinuation of study drug were PD (33 patients, 85%), withdrawn consent (4 patients, 10%), adverse event (drug-related dyspnea) (1 patient, 3%), and investigator's decision (1 patient, 3%).

The median age of the treated patients was 58 y (range: 24-78 y) (Table 1). Of the 39 treated patients, 23 (59%) were women and 16 (41%) were men; 69% of patients were white, and 72% had an ECOG performance status of 1. The most common UGT1A1 genotypes were *1/*28 (46%) and *1/*1 (44%); 2 patients (5%) had a homozygous *28 genotype.

All 39 patients had received prior chemotherapy. Seventeen patients (44%) had received prior irinotecan. The median number of prior cytotoxic chemotherapies was 3 (range = 1-10).

**Table 1. Demographics and Baseline Characteristics for Treated Patients**

| Cohort | 1 | 2 | 3 | 3H | 4 | 5 | 5A | 6A | All Patients |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/m$^2$) | 1.25 | 2.5 | 5 | 5 (*28/*28) | 10 | 16.5 | 16.5 + G-CSF | 25 + G-CSF | |
| Number of patients treated | 3 | 3 | 3 | 2 | 10 | 6 | 6 | 6 | 39 |
| Median age in years (range) | 53 (52-70) | 60 (51-75) | 59 (33-78) | 52 (35-69) | 58 (24-75) | 62 (56-69) | 56 (37-76) | 60 (44-71) | 58 (24-78) |
| Sex, n | | | | | | | | | |
| Female | 2 | 3 | 3 | 2 | 5 | 2 | 3 | 3 | 23 (59%) |
| Male | 1 | 0 | 0 | 0 | 5 | 4 | 3 | 3 | 16 (41%) |
| Cancer type, n | | | | | | | | | |
| Colorectal | 2 | 1 | 3 | 0 | 2 | 3 | 2 | 1 | 14 (36%) |
| Breast | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 3 (8%) |
| Head & neck | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 (8%) |
| Pancreatic | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 (8%) |
| Ovarian | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 (5%) |
| Thyroid | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 (5%) |
| Uterine | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 (5%) |
| Anal | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (3%) |
| Cholangiocarcinoma | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 (3%) |
| Esophageal | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 (3%) |
| Fallopian | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 (3%) |
| Gallbladder | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 (3%) |
| Gastric | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (3%) |
| Hepatocellular | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 (3%) |
| Lung | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 (3%) |
| Unknown primary | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 (3%) |
| Vulvar | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 (3%) |
| Performance status (ECOG), n | | | | | | | | | |
| 0 | 0 | 0 | 0 | 0 | 4 | 3 | 2 | 1 | 10 (26%) |
| 1 | 3 | 3 | 3 | 2 | 6 | 3 | 4 | 4 | 28 (72%) |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 (3%) |
| UGT1A1 genotype, n | | | | | | | | | |
| *1/*28 | 0 | 1 | 2 | 0 | 5 | 1 | 4 | 5 | 18 (46%) |
| *1/*1 | 3 | 2 | 1 | 0 | 4 | 4 | 2 | 1 | 17 (44%) |
| *28/*28 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 (5%) |
| *1/*37 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 (3%) |
| *28/*36 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 (3%) |

Tumor types included colorectal cancer (CRC) (14 patients); breast, head & neck, and pancreatic cancers (3 patients each); ovarian, thyroid, and uterine cancers (2 patients each); adenocarcinoma of unknown primary site (1 patient); cholangiocarcinoma (1 patient); and anal, esophageal, fallopian, gallbladder, gastric, hepatocellular, lung, and vulvar cancers (1 patient each) (Table 1).

Of the 39 treated patients, 27 patients received EZN-2208 without granulocyte colony-stimulating factor (G-CSF) per the original protocol (Table 1). After the dose-limiting toxicity (DLT) was found to be neutropenic fever, 12 additional patients received EZN-2208 with granulocyte colony-stimulating factor (G-CSF) per a protocol amendment (Tables 1 and 2). The 39 patients who completed the study received between 1 and 12 treatment cycles (mean = 3).

Note: The dose of EZN-2208 is stated as the dose of SN38 and not the dose of the conjugated compound.

**Table 2. Dose Escalation With and Without G-CSF**

Without G-CSF in first cycle

| Starting Dose (mg SN38/m$^2$) | Dose Level (mg SN38/m$^2$) | | | |
|---|---|---|---|---|
| | 2 | 3 | 4 | 5 |
| 1.25 | 2.5 | 5 | 10 | 16.5 |

2 DLTs Neutropenic fever

With G-CSF in first cycle

| Starting Dose (mg SN38/m$^2$) | Dose Level (mg SN38/m$^2$) |
|---|---|
| 5A | 6A |
| 16.5 | 25 |

2 DLTs Neutropenic fever

### Safety and Tolerability

The DLT was Grade 3 to 4 febrile neutropenia, which was reported in 2 of 6 patients in Cohort 5 (16.5 mg/m$^2$ without G-CSF) and in 2 of 6 patients in Cohort 6A (25 mg/m$^2$ with G-CSF). The UGT1A1 genotype was *1/*1 for both patients with DLT in Cohort 5 and *1/*28 for both patients with DLT in Cohort 6A. The MTD for EZN-2208 was determined to be 10 mg/m$^2$ without G-CSF and 16 mg/m$^2$ with G-CSF.

Overall, all 39 treated patients had at least one adverse event (AE) at any time during the study. The most commonly reported AEs (>20%), regardless of relationship to study drug, were fatigue (64%), diarrhea and nausea (38% each), alopecia (33%), anemia and anorexia (26% each), and neutropenia and vomiting (23% each). The most commonly reported AEs (>20%) considered likely related to study drug were fatigue (41%); alopecia, diarrhea, and nausea (33% each); neutropenia (23%); and vomiting (21%) (Table 3).

The intensity of most AEs was Grade 1 or 2. One case of Grade 3 diarrhea was reported in one patient who received 16.5 mg/m$^2$ of EZN-2208 with G-CSF; this event was considered likely related to study drug. One AE, Grade 5 multiorgan failure, resulted in the death of one patient in Cohort 3H (5 mg/m$^2$) who was homozygous for UGT1A1*28; the event was considered unlikely related to study drug.

**Table 3. Drug-Related Adverse Events Reported in ≥10% of Patients**

| AE / Cohort | 1 | 2 | 3 | 3H | 4 | 5 | 5A | 6A | All Patients |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/m$^2$) | 1.25 | 2.5 | 5 | 5 (*28/*28) | 10 | 16.5 | 16.5 + G-CSF | 25 + G-CSF | |
| Number of patients treated | 3 | 3 | 3 | 2 | 10 | 6 | 6 | 6 | 39 |
| Patients with ≥1 drug-related AE[a] | 0 | 2 | 0 | 1 | 8 | 6 | 5 | 6 | 28 (72%) |
| Fatigue | 0 | 2 | 0 | 1 | 2 | 5 | 3 | 3 | 16 (41%) |
| Alopecia | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 4 | 13 (33%) |
| Diarrhea | 0 | 0 | 0 | 0 | 5 | 3 | 3 | 2 | 13 (33%) |
| Nausea | 0 | 0 | 0 | 0 | 4 | 5 | 2 | 2 | 13 (33%) |
| Neutropenia | 0 | 1 | 0 | 0 | 1 | 4 | 1 | 2 | 9 (23%) |
| Vomiting | 0 | 0 | 0 | 0 | 1 | 3 | 2 | 2 | 8 (21%) |
| Anemia | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 1 | 6 (15%) |
| Leukopenia | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 5 (13%) |
| Thrombocytopenia | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 5 (13%) |
| Anorexia | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 4 (10%) |
| Febrile neutropenia | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 4 (10%) |
| Lymphopenia | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 4 (10%) |

[a] Treatment-emergent AEs considered likely related to study drug.

### Antitumor Activity

The best overall response was stable disease (SD) for 15 patients (38%) and PD for 19 patients (49%) (Table 4). The response was not evaluable for the other 5 patients (13%).

**Table 4. Best Overall Response**

| Cohort | 1 | 2 | 3 | 3H | 4 | 5 | 5A | 6A | All Patients |
|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/m$^2$) | 1.25 | 2.5 | 5 | 5 (*28/*28) | 10 | 16.5 | 16.5 + G-CSF | 25 + G-CSF | |
| Number of patients treated | 3 | 3 | 3 | 2 | 10 | 6 | 6 | 6 | 39 |
| Stable disease, n | 1 | 1 | 1 | 0 | 4 | 1 | 4 | 3 | 15 (38) |
| Progressive disease, n | 2 | 1 | 1 | 1 | 6 | 4 | 2 | 2 | 19 (49) |
| Not evaluable, n | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 (13) |

Of the 15 patients who achieved SD, 7 patients had CRC; 2 patients had uterine cancer; 1 patient had cholangiocarcinoma; and 1 patient each had breast, fallopian, head & neck, ovarian, and pancreatic cancers. Eight patients had prolonged SD (>90 days), sometimes associated with tumor shrinkage (Table 5). The 4 patients with CRC who had prolonged SD had received prior irinotecan.

**Table 5. Patients with Stable Disease (>90 Days)**

| Cancer Type | Cohort | Dose (mg/m$^2$) | Prior Camptosar® Treatment | Number Prior Regimens | Duration of Stable Disease (Days) | Tumor Shrinkage |
|---|---|---|---|---|---|---|
| Colorectal | 3 | 5 | Yes | 4 | 111 | No |
| | 4 | 10 | Yes | 2 | 145 | No |
| | 5A | 16.5 + G-CSF | Yes (*K-RAS* mutation) | 2 | 205 | No |
| | 6A | 25 + G-CSF | Yes | 5 | 126 | No |
| Breast | 2 | 2.5 | No | 10 | 134 | Yes |
| Cholangiocarcinoma | 5A | 16.5 + G-CSF | No | 1 | 121 | Yes |
| Head & neck | 6A | 25 + G-CSF | No | 1 | 259 | Yes |
| Uterine | 4 | 10 | No | 3 | 130 | No |

### Pharmacokinetics

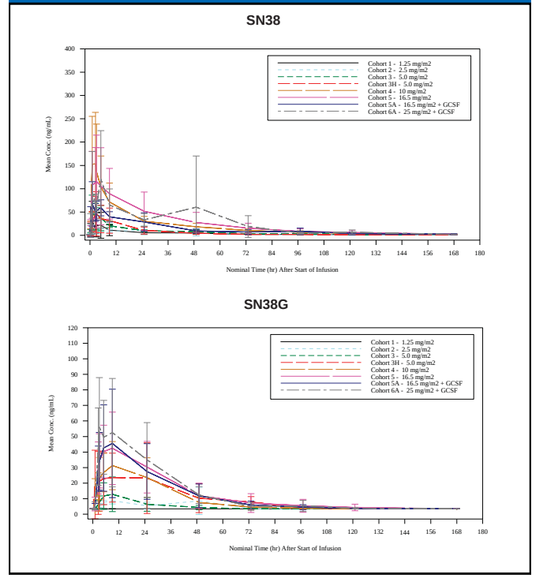
All 39 treated patients provided data for PK analysis. Mean PK parameters for SN38 are summarized by dose level in Table 6. The SN38 clearance (estimated by Bayesian approach) was 6.1 L/hour. Mean SN38 and SN38G concentrations in plasma are plotted in Figure 3.

**Table 6. PK Parameters[a] for SN38 After 1-Hour Infusion of EZN-2208**

| Dose[b] (mg/m$^2$) | N | $C_{max}$ (ng/mL) | $AUC_{0-\infty}$ (h-µg/L) | $t_{1/2}$ (h) | CL (L/h) | Vd (L) |
|---|---|---|---|---|---|---|
| 1.25 | 3 | 32.22 ± 40.88 | 1683.3 ± 1723.2 | 58.45 ± 13.27 | 3.64 ± 4.03 | 351.62 ± 424.14 |
| 2.5 | 3 | 14.58 ± 7.73 | 1286.3 ± 884.44 | 60.28 ± 24.06 | 6.04 ± 6.18 | 386.26 ± 215.50 |
| 5 | 3 | 56.63 ± 36.15 | 1924.7 ± 697.27 | 42.31 ± 16.26 | 5.37 ± 2.87 | 371.61 ± 333.61 |
| 5[c] | 2 | 48.50 ± 43.98 | 1146.5 ± 984.78 | 21.41 ± 2.12 | 11.25 ± 9.23 | 361.54 ± 319.53 |
| 10 | 10 | 144.25 ± 115.26 | 4879.8 ± 2984.6 | 29.89 ± 3.58 | 6.11 ± 5.17 | 269.88 ± 233.73 |
| 16.5 | 12 | 105.49 ± 74.03 | 3904.0 ± 2604.9 | 30.66 ± 9.93 | 11.88 ± 8.31 | 462.74 ± 219.81 |
| 25 | 6 | 181.62 ± 121.79 | 10356 ± 123285 | 37.12 ± 10.54 | 11.05 ± 9.17 | 489.11 ± 337.68 |

[a] Mean ± standard deviation.
[b] SN38 equivalents.
[c] Homozygous (*28/*28).

**Figure 3. Mean (±SE) SN38 and SN38G concentrations (ng/mL) in plasma after a 1-hour infusion of EZN-2208**




## Conclusions

EZN-2208, a novel agent, was well tolerated in previously treated patients with advanced malignancies. No cumulative toxicities were reported. The DLT for EZN-2208 administered as a 1-hour i.v. infusion every 3 weeks, with or without G-CSF, was febrile neutropenia, in distinction to the DLT of irinotecan. The MTD and recommended Phase 2 dose of EZN-2208 administered once every 3 weeks is 10 mg/m$^2$ for EZN-2208 administered without G-CSF and 16.5 mg/m$^2$ for EZN-2208 administered with G-CSF. Stable disease, sometimes prolonged and associated with tumor shrinkage, was observed as best response. For some patients, the duration of EZN-2208 was longer than the duration of their prior therapy.

Also, see Poster C221 for a dose-escalation study of EZN-2208 administered weekly x 3 weeks per 4-week cycle.[4]

References

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

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