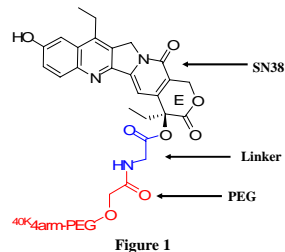


**Abstract # LB-39**

**Introduction**

K-ras encodes a small GTP-binding protein that acts as a self-inactivating signal transducer by cycling from GTP- to GTP-bound states in response to stimulation of a cell surface receptor, including EGFR (1). K-ras can harbor oncogenic mutations that yield a constitutively active protein. Recent data demonstrate that EGFR targeted monoclonal antibody, C225, lacks efficacy in patients with mutated K-ras tumors. Also, several recent clinical trials have demonstrated that the combination of C225 and CPT-11 is not active in patients with mutated K-ras colorectal cancer progressing after CPT-11 therapy. EZN-2208 is a PEGylated conjugate of SN38, which is the active moiety of CPT-11. Preclinical data suggest that EZN-2208 may be a promising anticancer agent in a wide variety of clinical settings, including tumors that are refractory to CPT-11 treatment (2). The excellent preclinical activity of EZN-2208 prompted us to investigate the efficacy of EZN-2208 in tumors that have K-Ras mutations and poor response to CPT-11 and/or C225. Beyond this, as some topoisomerase I inhibitors can inhibit HIF-1 $\alpha$  (3), we explored if EZN-2208 might preferentially inhibit HIF-1 $\alpha$  compared with CPT-11.

**Test compound (EZN-2208)**



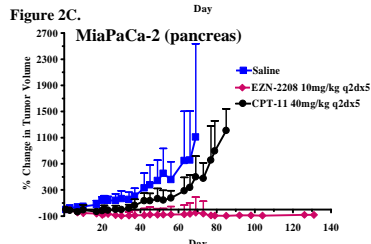
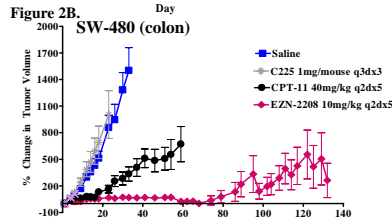
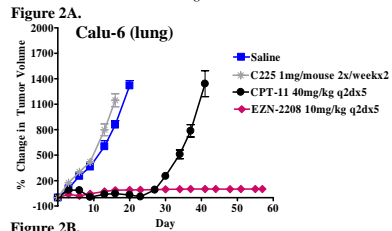
EZN-2208 (Figure 1) is a novel water soluble prodrug of SN38, generated by conjugating SN38 to multi-arm PEG (40k 4-arm-PEG) via a glycine linker. EZN-2208 is readily soluble in saline (180 mg/ml) (4).

**Objectives**

- 1) To evaluate EZN-2208 efficacy in K-ras mutated tumors that demonstrate poor response to CPT-11 and/or C225.
- 2) To explore if EZN-2208 preferentially inhibits HIF-1 $\alpha$  compared to CPT-11, as a possible mechanism for activity.

**Efficacy of EZN-2208 in K-ras mutant models**

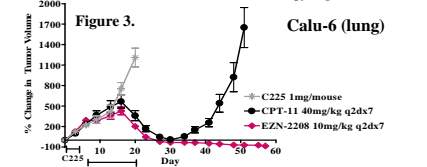
Therapeutic efficacy of EZN-2208, CPT-11 and C225 were compared in nude mice subcutaneous xenograft models of K-ras mutant lung (Calu-6), colorectal (SW-480), and pancreatic (MiaPaCa-2) cancers (Figure 2A to 2C). Treatment with EZN-2208, C225 and CPT-11, at their respective MTDs, were started when tumors on the flank reached an average volume of 100 mm<sup>3</sup>.



Treatment with EZN-2208 resulted in 100, 30, and 40% (no evidence of tumor by gross observation) cures of animals in Calu-6, SW-480, and MiaPaCa-2 xenograft models, respectively. No cures were observed with CPT-11 treatment. Initially, CPT-11 resulted in tumor growth inhibition, but tumor growth rapidly resumed and all mice had to be terminated due to excessive tumor mass. Treatment with C225 was ineffective in these models.

**Efficacy of EZN-2208 in C225 refractory, K-ras mutant models**

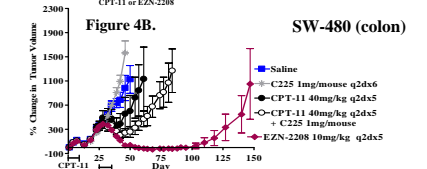
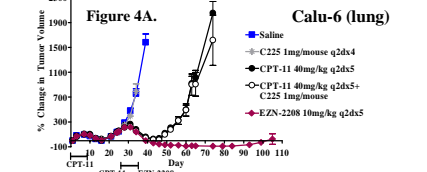
Mice bearing subcutaneous Calu-6 xenografts (100 mm<sup>3</sup>) were first treated with C225 (1 mg/mouse, qd, ip). When tumors reached ~600 mm<sup>3</sup>, these tumors were classified as C225-refractory and subsequently were treated iv. with either EZN-2208 or CPT-11 or were continued on C225 therapy (Figure 3).



Initially, both CPT-11 and EZN-2208 were effective in C225-refractory tumors. However, CPT-11 treated mice eventually relapsed and tumors growth resumed, while EZN-2208 treated mice continued to respond and resulted in 63% cures and 100% regressions.

**Efficacy of EZN-2208 in CPT-11 refractory, K-ras mutant models**

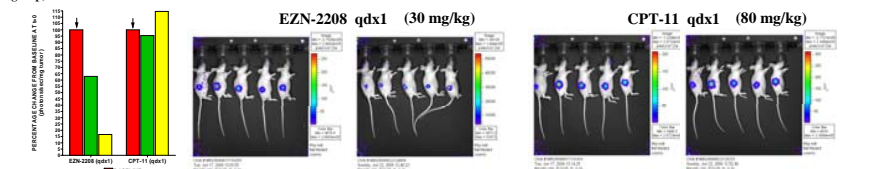
Mice bearing subcutaneous Calu-6 or SW-480 xenografts (100 mm<sup>3</sup>) were initially treated with CPT-11 (40mg/kg, q2dx4, iv.). When tumor mass reached >3x initial values, the tumors were classified as CPT-11 refractory. These mice were treated with EZN-2208, CPT-11, C225 or with a combination of CPT-11 and C225 at the doses indicated (Figure 4A and B).



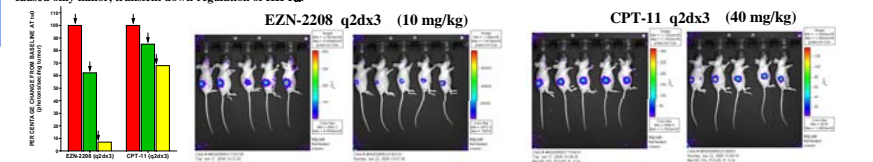
In both models, treatment with EZN-2208 was significantly better than CPT-11 alone or in a combination of CPT-11 and C225. EZN-2208 treatment resulted in either 80% tumor regressions (Calu-6) or 33% cures (SW-480). CPT-11 and C225 treatment was not significantly better than CPT-11 alone.

**Inhibition of HRE-dependent luciferase expression**

The inhibition by EZN-2208 or CPT-11 of HIF-1-dependent luciferase expression and tumor growth was evaluated in a U251-HRE (HIF-1 $\alpha$  reporter line) where a luciferase reporter gene is under the control of a hypoxia response element (3). When U251-HRE tumors (sc.) in the right axillary flank of nude mice were ~100 mm<sup>3</sup>, iv. treatment with saline, EZN-2208 or CPT-11, at their respective MTDs, was initiated as single (qdx1) (Figure 5A) or multiple (q2x3) doses (Figure 5B). Luciferase expression in U251-HRE tumors was measured using bioluminescence (Xenogen IVIS 100 Imaging Station, Xenogen Corp.). Firefly D-luciferin (150 mg/kg, ip.) was injected at the 0, 48 and 120 hours following the initiation of drug treatment. The saline-treated mice had progressive increases in luminescence, whereas both EZN-2208 and CPT-11-treated mice had diminished luminescence. Because the tumor mass was reduced by chemotherapy treatment (data not shown), the luminescence values (total flux/photon/second) were normalized for tumor mass. The percent change at each time point, relative to the zero-time baseline for the respective treatment group, was calculated.



A single MTD of EZN-2208 (30 mg/kg) induced potent, sustained down-regulation of HIF1 $\alpha$  (37% at 48h and 83% at 120h). A single MTD of CPT-11 (80 mg/kg) caused only minor, transient down-regulation of HIF1 $\alpha$ .



Multiple doses of EZN-2208 (10 mg/kg) induced potent, sustained down-regulation of HIF1 $\alpha$  (63% at 48 h and 93% at 120h). Multiple doses of CPT-11 (40 mg/kg) caused minor down-regulation of HIF1 $\alpha$  (15% at 48h and 32% at 120h).

**Conclusions**

- 1) EZN-2208 (PEG-SN38), a novel water soluble pegylated SN38 conjugate has excellent therapeutic efficacy in K-ras mutant cancer xenograft models.
- 2) Treatment with EZN-2208 is significantly better than either CPT-11 in C225 refractory K-ras mutant models.
- 3) Treatment with EZN-2208 is significantly better than CPT-11 alone, C225 alone, or in a combination of CPT-11 and C225 in CPT-11 refractory K-ras mutant models.
- 4) EZN-2208 has sustained profound inhibition of HIF-1 $\alpha$  compared with CPT-11; this data suggested that a novel method of action may account for superior efficacy of EZN-2208 in preclinical models compared to CPT-11.
- 5) EZN-2208 may be an effective therapeutic to treat K-ras mutant colorectal cancer in the clinic.

**References**

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