A novel polyethyleneglycol-SN38 conjugate, EZN-2208, downregulates HIF-1α and results in significant tumor growth inhibition in K-ras mutant cancer xenograft models

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Abstract # LB-39

Introduction

K-ras encodes a small GTP-binding protein that acts as a self-inactivating signal transducer by cycling from GDP- 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-1α (3), we explored if EZN-2208 might preferentially inhibit HIF-1α compared with CPT-11.

Test compound (EZN-2208)

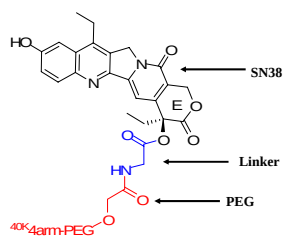


Figure 1

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 inhibit HIF-1α 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 mm³.

Figure 2A.

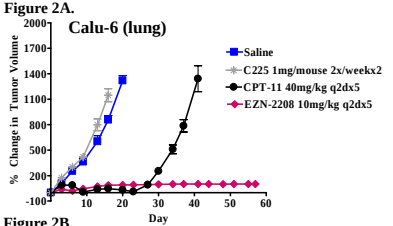


Figure 2B.

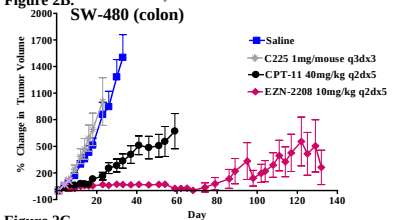
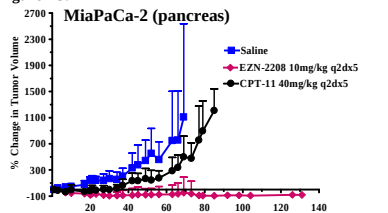


Figure 2C.

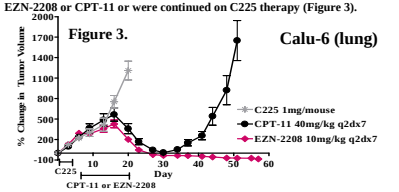


Treatment with EZN-2208 resulted in 100, 30, and 40% (no evidence of tumor by gross observation) cures of animals in Calu-6, SW-480, and MiaPaCa-2 xenograft models, respectively. No cures were observed with CPT-11 treatment. Initially, CPT-11 resulted in tumor growth inhibition, but tumor growth rapidly 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 mm³) were first treated with C225 (1 mg/mouse, q3d, ip.). When tumors reached ~600 mm³, these tumors were classified as C225-refractory and susequently were treated iv. with either EZN-2208 or CPT-11 or were continued on C225 therapy (Figure 3).

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 mm³) 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).

Figure 4A.

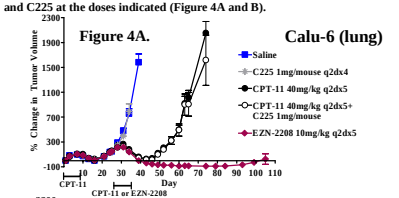
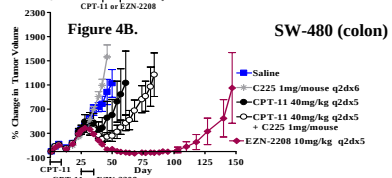


Figure 4B.

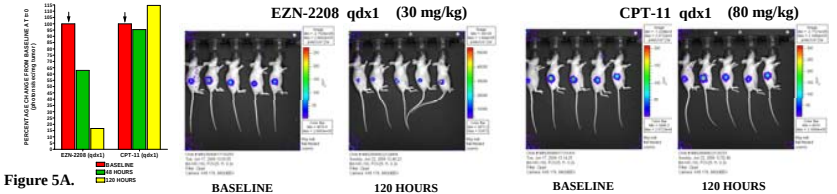


In both models, treatment with EZN-2208 was significantly better than CPT-11 alone or in a combination of CPT-11 and C225. EZN-2208 treatment resulted in either 80% tumor regressions (Calu-6) or 33% cures (SW-480). CPT-11 and C225 treatment was not significantly better than CPT-11 alone.

Inhibition of HRE-dependent luciferase expression

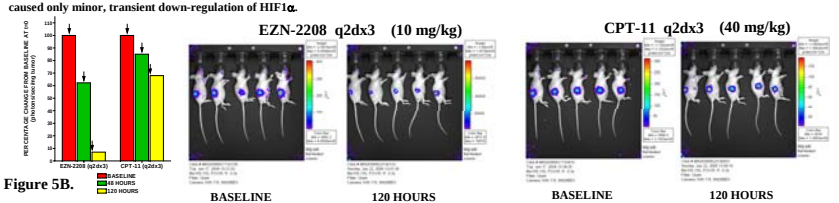
The inhibition by EZN-2208 or CPT-11 of HIF-1–dependent luciferase expression and tumor growth was evaluated in a U251-HRE (HIF-1α reporter line) where a luciferase reporter gene is under the control of a hypoxia response element (3). When U251-HRE tumors (sc.) in the right axillary flank of nude mice were ~ 100 mm³, 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 the 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.

Figure 5A.



A single MTD of EZN-2208 (30 mg/kg) induced potent, sustained down-regulation of HIF1α (37% at 48h and 83% at 120h) . A single MTD of CPT-11 (80 mg/kg) caused only minor, transient down-regulation of HIF1α.

Figure 5B.



Multiple doses of EZN-2208 (10 mg/kg) induced potent, sustained down-regulation of HIF1α (63% at 48 h and 93% at 120h) . Multiple doses of CPT-11 (40 mg/kg) caused minor down-regulation of HIF1α (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-1α 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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