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 & 2C). Treatment with EZN-2208, CPT-11 and C225, at their respective MTDs, were started when tumors on the flank reached an average volume of 100 mm³.

Figure 2A. SW-480 (colon) and Calu-6 (lung) xenografts

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 >80% tumor regressions (Calu-6) or 33% circumscribed (SW-480). CPT-11 and C225 treatment was not significantly better than CPT-11 alone.

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

Mice bearing subcutaneous Calu-6 xenografts (100 mm³) were initially treated with C225 (1 mg/mouse, qdx5). When tumors reached ~600 mm³, 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).

Figure 3. Calu-6 (lung)

Initially, both CPT-11 and EZN-2208 were effective in C225-refractory tumours, however, CPT-11 treated mice eventually relapsed and tumor growth resumed, while EZN-2208 treated mice continued to respond and resulted in 67% new and 86% regressions.

Figure 5B. EZN-2208 10mg/kg q2dx5 vs CPT-11 40mg/kg q2dx5

Multiple doses of EZN-2208 (10 mg/kg) induced potent, sustained down-regulation of HIF-1α (40k 4-arm-PEG) via a glycine linker. EZN-2208 is readily soluble in saline (180 mg/ml) (4).

Inhibition of HRE-dependent luciferase expression

The inhibition by EZN-2208 of CPT-11 of HIF-1α-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 (HRE). When U251-HRE tumors (sc.) in the right axillary flank of nude mice were ~100 mm³, is treatment with saline, EZN-2208 or CPT-11, at their respective MTDs, was initiated as single qdx5 (Figure 5A) or multiple qdx3 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 luminoscense. Because the tumor mass was reduced by chemotherapy treatment (data not shown), the luminescence values (total flux/photons/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. EZN-2208 qdx1 (30 mg/kg) vs CPT-11 qdx1 (80 mg/kg)

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 or 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 xenografts.
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
