Targeting the β-catenin oncogene in multiple myeloma disease using locked nucleic acid antisense technology

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Overview

β-catenin is a transcriptional regulator that, when activated, has been shown to lead to cancer cell proliferation. It has been exceedingly difficult to identify small molecule inhibitors that target only protein-RNA interactions involving β-catenin; this provides an ideal opportunity for the use of antisense-based therapy.

Previously we described a locked nucleic acid (LNA) oligomercatide (LNA-ON) antagonist of β-catenin, EZN-3892, with potent in vitro and in vivo anti-tumor properties in numerous solid tumors with β-catenin activation. Here, we describe the activity of EZN-3892 in models of multiple myeloma (MM). MM is characterized by uncontrolled proliferation of plasma cells whose growth is driven by various cytokines and several Wnt ligands which activate β-catenin (3). β-catenin is activated in MM since it is stabilized and translocated to the nucleus in many MM cell lines and patient samples (2). Several groups have demonstrated modulation of β-catenin in the tumor and stroma cells of the xenograft tumors.

EZN-3892 inhibits cell growth in only a few MM cell lines

Figure 1: Some MM cell lines are sensitive to EZN-3892 when used without transfection (gyromantically). Previous studies of solid tumor cell lines have shown that not all cell lines take up LNA-ON (5). MM cell lines were treated by simply adding EZN-3892 to cell cultures at doses up to 10 μM, for 5 days. This method does not use transfection, and therefore more accurately reflect in vivo conditions. Analysis by MTS assay revealed 7/28 cell lines show some growth inhibition in the presence of EZN-3892. OPMA-1 and XO36 cells were more sensitive to EZN-3892 than others (IC50 ~ 8-10 μM). Sensitivity to EZN-3892 may correlate with the ability of particular cell lines to allow EZN-3892 to enter the cell and hybridize with the β-catenin target compared to other anti-sense technology.

This study assessed the activity of EZN-3892 in 8 MM cell lines that show the ability to take up LNA-ON gyromantically (without the aid of transfection) resulting in a down modulation of β-catenin protein. Interestingly, EZN-3892 modestly inhibits cell growth in vitro in only 2/8 cell lines. In tumors derived from one of the most responsive cell lines, EZN-3892 delivered at 50 mg/kg, resulted in robust tumor inhibition. The efficacy in vivo appears mediated by a dual inhibition of β-catenin in the tumor and stroma cells of the xenograft tumors.

EZN-3892 down-modulates β-catenin mRNA and protein in most MM cell lines

Figure 2: EZN-3892 down-modulates β-catenin mRNA and protein levels in many MM cell lines. Given that only 2/28 cell lines showed anti-proliferative effects after exposure to EZN-3892, the prediction is that EZN-3892 would not down-modulate β-catenin mRNA in most cell lines. In fact, this was not true. (A) Treatment of MM cell lines with EZN-3892 for 72 h in gyromonic conditions shows that EZN-3892 down-modulated β-catenin mRNA in all MM cell lines. (B) Analysis of β-catenin protein levels with and without EZN-3892 show MM cell lines have varying levels of β-catenin protein and 7/28 cell lines show a reduction following treatment with EZN-3892.

EZN-3892 inhibits MM tumor growth via β-catenin modulation in tumor and stroma

Figure 4: Down modulation of β-catenin mRNA is evident in liver, tumor, and tumor stroma taken from mice dosed with EZN-3892. Mice bearing OPMA-2 tumor xenografts were administered multiple doses of either saline, control LNA or EZN-3892 at 50 and 50 mg/kg (as in Figure 3A) and tumor and liver tissue harvested 24 h after the 6th dose. RT-qPCR was performed on total RNA extracted from the tissues. As expected, EZN-3892 results in a robust decrease in β-catenin mRNA in the mouse liver. The inhibition of β-catenin mRNA in the human OPMA-2 tumor xenografts was modest at 100 μg/kg and difficult to detect at 50 mg/kg. Analysis of the mouse β-catenin mRNA levels within the tumor stroma, reveals a striking ~50% decrease in β-catenin mRNA.

REFERENCES

1. EZN-3892 down-modulates β-catenin mRNA and protein in most MM cell lines. However, only 2/28 cell lines show anti-proliferative effects in the presence of EZN-3892. This data suggests only a sub-set of MM responds to β-catenin inhibition.
2. Tumors derived from cells that show moderate growth inhibition in the presence of EZN-3892 in vivo, are sensitive in vitro. Efficacy in vivo appears more robust than the proliferation assay predicts.
3. Conversely, MM cell lines not sensitive to EZN-3892 in a proliferation assay in vitro are also not sensitive to EZN-3892 in vivo. This suggests the 2:10 MTT proliferation assay as a predictor of EZN-3892 efficacy.
4. Anti-tumor effects of EZN-3892 are associated with a small modulation of β-catenin in the tumor combined with a more marked inhibition of stromal β-catenin.

In summary, EZN-3892 is a potent and specific inhibitor of β-catenin and able to modulate levels in MM cell lines. The lack of anti-proliferative effect across cell lines suggests not all MM cell lines are driven by β-catenin. Further study should reveal a signature of MM tumors sensitive to β-catenin inhibition.

CONCLUSIONS

1. EZN-3892 down-modulates β-catenin mRNA and protein in most MM cell lines. However, only 2/28 cell lines show anti-proliferative effects in the presence of EZN-3892. This data suggests only a sub-set of MM responds to β-catenin inhibition.
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In vitro anti-proliferative response predicts anti-tumor efficacy in vivo

Figure 5: H&E evaluation of OPMA-2 tumors reveals striking tumor cell death in OPMA-2 tumors treated with EZN-3892. Tumors were harvested into 10x buffered formalin and stained with hematoxylin and eosin. (A) Photomicrographs below depict OPMA-2 tumors treated with saline show a homogenous tumor matrix of red, highly basophilic cells. (B) Conversely, those treated with 50 and 100 mg/kg EZN-3892 show a more disorganized histology and many neculated, dead cells. (C) Tumors taken from mice dosed with Control Oligo appeared more disorganized but cells appeared healthy.

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REFERENCES


EZN-3892 results in OPM-2 xenograft cell death in vivo

Figure 6: MM cell lines that are not sensitive to EZN-3892 in 2-D proliferation assays show no anti-tumor effect in vivo. Since β-catenin did not appear to drive the growth of AMO-1 cells in vitro (yet EZN-3892 down-modulated β-catenin from this cell line was used to address specificity of EZN-3892 in vivo. Mice bearing AMO-1 tumor xenografts were treated with saline, control oligo or EZN-3892 at 50 or 100 mg/kg, Q3 days. No growth inhibition is found supporting the concept that the MTT proliferation assay is predictive of the MM cell lines dependence on β-catenin for proliferation.