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

Melissa Dumble, Peifang Zhu, Jessica Kearney, Yixian Zhang, Lee M. Greenberger

Enzon Pharmaceuticals, Inc., Piscataway, NJ , 08854

Melissa.Dumble@enzon.com

Abstract # A74

Overview

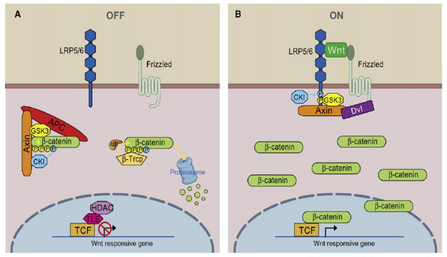
β-catenin is a transcriptional regulator that, when activated, has been shown to lead to cancer cell proliferation. It has been exceedingly difficult to identify small molecule inhibitors that target only protein-protein/DNA interactions involving β-catenin; this provides an ideal opportunity for the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

Previously, we described a locked nucleic acid (LNA) oligonucleotide (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 (1). 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 siRNA elimination of β-catenin leads to decreased MM cell viability and growth (3).

This study assessed the activity of EZN-3892 in 8 MM cell lines that show the ability to take up LNA-ON gymnotically (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, IV results in robust tumor growth inhibition. The efficacy *in vivo* appears mediated by a dual inhibition of β-catenin in the tumor and stroma cells of the xenograft tumor.

Targeting the Wnt pathway and β-catenin

The Wnt pathway as a therapeutic target for cancer treatment

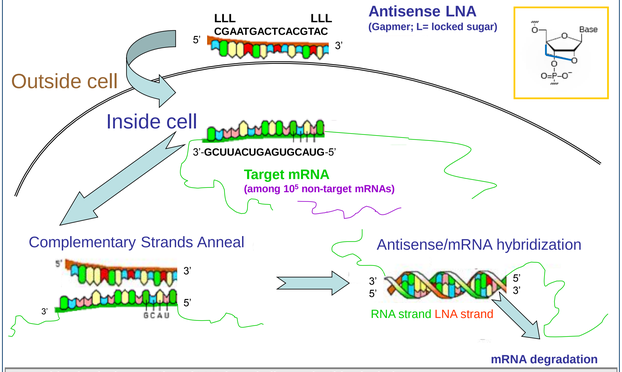


Taken from McDonald *et al*, 4.

β-catenin:

- **Transcription factor controlling cell survival, growth, cell cycle, cancer stem cells, metastasis**
- **Activated in multiple cancers via many mechanisms (e.g. APC, β-catenin, Axin mutations). Activation in MM via increased Wnt activity**

LNA-based RNA antagonism

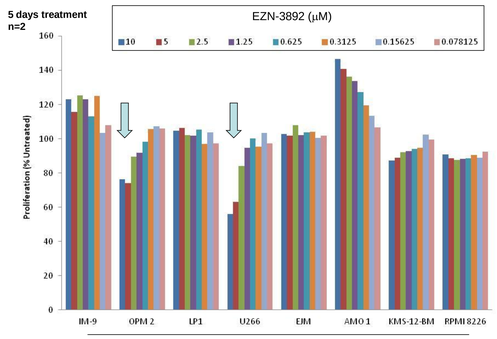


Favorable physical properties of LNA-based oligonucleotides include:

- **Highest affinity for mRNA target compared to other anti-sense technology**
- **Single stranded molecule and relatively short sequence (12-16mer) needed for effective targeting**
- **Highly stable in vitro and in vivo**
- **Prepared in saline and given IV; unlike siRNAs, no delivery vehicle required**

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

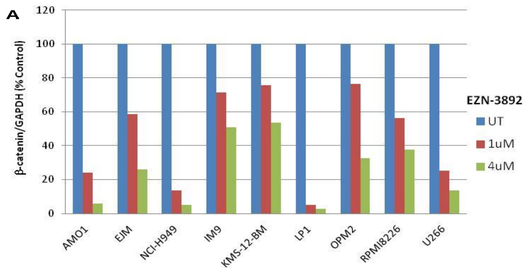
Figure 1: Some MM cell lines are sensitive to EZN-3892 when used without transfection (gymnotically) . Previous studies of solid tumor cell lines have shown that not all cell lines take up LNA-ON (5). MM cell lines were treated by simply adding EZN-3892 to cell cultures at doses up to 10 μM, for 5 days. This method does not use transfection, and therefore more accurately reflect *in vivo* conditions. Analysis by MTS assay reveals 2/8 cell lines show some growth inhibition in the presence of EZN-3892. OPM-2 and U266 cells were more sensitive to EZN-3892 than others (EC_{50} ~ 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 mRNA.



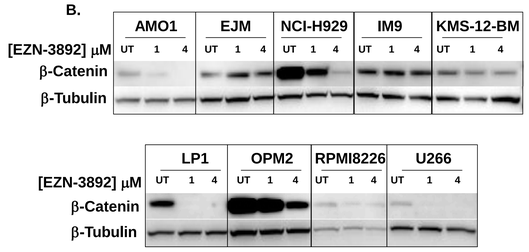
EZN-3892 down-modulates β-catenin mRNA and protein in most MM cell lines

Figure 2: EZN-3892 down-modulates β-catenin mRNA and protein levels in many MM cell lines.
Given that only 2/8 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 gymnotic conditions shows that EZN-3892 down-modulated β-catenin mRNA in all MM cell lines. (B) Analysis of β-catenin protien levels with and without EZN-3892 show MM cell lines have varying levels of β-catenin protein and 7/9 cell lines show a reduction following treatment with EZN-3892.

mRNA:



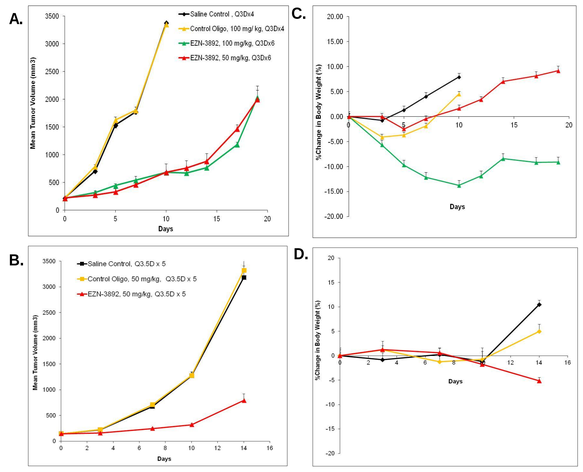
Protein:



β-catenin is a driver for tumor growth in only few MM cell lines

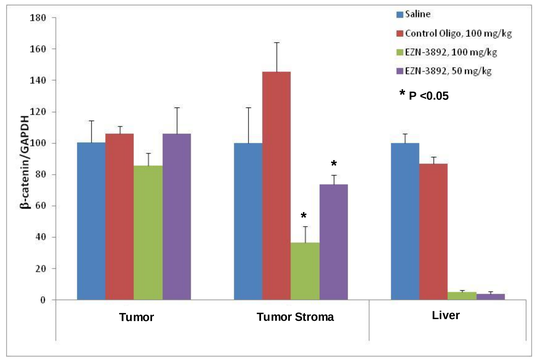
EZN-3892 has potent anti-tumor effects *in vivo*

Figure 3: EZN-3892 robustly inhibits the growth of OPM-2 MM tumor xenografts *in vivo*. To test the sensitivity of MM cell lines to EZN-3892, mice bearing OPM-2 tumor xenografts were dosed IV with saline, control LNA or EZN-3892 at 100 and 50 mg/kg. Significant tumor growth inhibition, 85 and 78%, was recorded for this OPM-2 tumor model in independent experiments (A and B, respectively). (A) Interestingly, the maximum efficacious dose of EZN-3892 is 50 mg/kg and increasing the dose further does not increase the efficacy. (C) EZN-3892 was well tolerated in mice at 50 mg/kg, with the higher dose of 100 mg/kg resulting in 13.7 % body weight loss. The mice appeared healthy and alert throughout the studies.



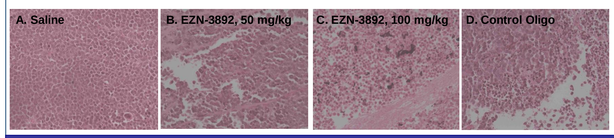
EZN-3892 inhibits MM tumor growth via β-catenin modulation in tumor and stroma

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 OPM-2 tumor xenografts were administered multiple doses of either saline, control LNA or EZN-3892 at 100 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 OPM-2 tumor xenografts was modest at 100 mg/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.



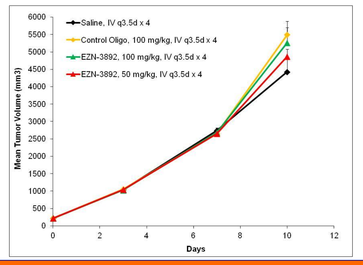
EZN-3892 results in OPM-2 tumor xenograft cell death *in vivo*

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



In vitro anti-proliferative response predicts anti-tumor efficacy *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) tumors from this cell lines were 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 100 or 50 mg/kg, Q3.5Dx4. No tumor 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.



CONCLUSIONS

1. **EZN-3892 down-modulates β-catenin mRNA and protein in most MM cell lines. However, only 2/8 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 dervied from cells that show moderate growth inhibition in the presence of EZN-3892 *in vitro,* are sensitive *in vivo*. Efficacy *in vivo* appears more robust that 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 supports the 2-D MTT proliferation assay as a predictor of EZN-3892 efficacy.**
5. **Anti-tumor effects of EZN-3892 are associated with a small modulation of β-catenin within the tumor combined with a more marked inhibition of stromal β-catenin.**

In sum, 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.

REFERENCES

1. Qiang *et al*, Oncogene, 2003, 22:1536-1545
2. Sukhedo *et al*, PNAS, 2007, 104: 7516-7521
3. Ashihara *et al*, Clinical Cancer Research, 2009, 15: 2731-2738
4. McDonald *et al*, Developmental Cell, 2009, 17:9-26
5. Zhang *et al*, Gene Therapy, 2011, 18:326-333

23rd EORTC-NCI-AACR, San Francisco, CA, 2011.