Androgen receptor locked nucleic acid antisense oligos potently knocks down target gene expression both in vitro and in vivo

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**INTRODUCTION**

Prostate cancer is the most common cancer and the second leading cause of cancer death in North American males. Growth of prostate cancer epithelial cells is androgen-dependent during the initial stage, mediated by androgen receptor (AR), a major regulatory transcription factor for genes regulating proliferation and survival of prostate cancer cells. Therefore, androgen deprivation is a mainstay therapy for prostate cancer. However, prostate cancer that is resistant to existing therapies will emerge within 1-3 years. Importantly, AR remains present and plays a key role in the progression of prostate cancer. Androgen receptor locked nucleic acid antisense oligos potently knocks down target gene expression both in vitro and in vivo.

**METHODS**

The target mRNA knockdown, growth inhibition, reduction of prostate specific antigen (PSA, an AR downstream target and biomarker of prostate cancer), and apoptosis induction effects of EZN-4176 and EZN-4187 were evaluated by qRT-PCR, western blot analysis, ELISA, MTS assay, and caspase-3/7 activity assay, respectively. In two AR positive prostate cancer cell lines (LNCaP and 22Rv1/prostate) after transfection. A scrambled LNA antisense oligo (EZN-3046) and the AR negative cell line (15PC-3 prostate cancer) served as controls. Additionally, the effect of EZN-4176 and its mismatched control oligonucleotide on DHT-induced growth of LNCaP was evaluated in charcoal-stripped serum (CSS) containing medium. In vivo, target knockdown efficacy of EZN-4176 in 22Rv1 tumors was evaluated after intravenous administration. Tumor inhibition was examined with an androgen-dependent CWR22 xenograft model.

**ANDROGEN ACTION**

- In the absence of estradiol (E), androgen receptor (AR) monomers are associated with heat shock protein complex (HSP) and other proteins.
- Upon binding to T or DHT, AR becomes phosphorylated (P). Eventually, HSP complex is dissociated and AR is able to enter the nucleus to regulated specific target genes for growth and survival.
- AR can also be activated in the absence of androgen through ligand-independent pathways (not depicted in the picture), which is an important mechanism leading to resistance to androgen ablation therapy.
- Targeting AR represents an important therapeutic approach.

**REFERENCES**

3. The design and discovery of EZN-4176 and EZN-4187 has been done in collaboration with Santenara Pharma A/S. EZN-4176 and EZN-4187 are being developed by Enzon under a license with Santenara Pharma A/S

**CONCLUSIONS**

- Selective and specific downmodulation of AR mRNA and protein in cells correlate with in growth inhibition.
- Potent inhibition of AR and PSA mRNA in 22Rv1 xenograft model.
- Significant tumor growth inhibition in CWR22 xenograft model.