Abstract # 5647

INTRODUCTION

Unlike siRNAs, single-stranded locked nucleic acid-based antisense oligonucleotides (LNA-ONs) have shown the ability to down regulate mRNAs in vitro and in vivo without any delivery systems such as transfection reagents or liposomes. Hence, LNA-ONs may have significant advantages as a therapeutic compared to siRNA. Investigation of LNA-ONs that target HIF-1α, β-catenin, and HER3 in multiple cell lines and in cells prepared directly from patient tumors, 2) the correlation of target down-regulation and growth inhibition in vitro with tumor growth inhibition in vivo, 3) the correlation of intratumoral LNA-ON concentration with target down-regulation in vivo in xenograft models, and 4) genes that are differentially expressed in LNA-ONs resistant cells as compared to permissive cells.

METHODS

LNA-ONs were either added to tissue culture media (i.e. no transfection) in vitro or prepared in saline and given IV in vivo. Endpoints were measured by qRT-PCR, MTT, Western Blot analysis, and tumor size. Gene Expression Profiling was performed by Asuragen, Inc. Concentration of LNA-ONs in tissues was measured by LC/MS/MS. Primary tumors samples obtained and treated by Precision Therapeutics Inc (PTI) and down-regulation analysis carried out by Enzon Pharmaceuticals.

WHY USE LNA TECHNOLOGY?

Locked Nucleic Acid

LNA Gampers

• 2nd generation LNA-based antisense technology results in:
  • Improved Tm (4 to 6°C higher than LNA equivalent)
  • Excellent plasma stability (T1/2 = 15 hrs)
  • Long tissue retention time (days T1/2 = weeks)
  • Short sequence (14 to 16 mer)
  • IV administration without delivery vehicle

LNA-ONs USED IN THE STUDIES

<table>
<thead>
<tr>
<th>Complements</th>
<th>Target</th>
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<tbody>
<tr>
<td>EZN-3589</td>
<td>HER2</td>
</tr>
<tr>
<td>EZN-3389</td>
<td>β-catenin</td>
</tr>
<tr>
<td>EZN-3489</td>
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<tr>
<td>EZN-2419</td>
<td>Androgen Receptor</td>
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<td>EZN-2598</td>
<td>HIF-1α</td>
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<tr>
<td>EZN-3656 scrambled control</td>
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</tbody>
</table>

Genomic analysis of resistant cell lines

Fig. 8. Characterization of type 2 resistant cell lines. Parental and resistant cells were tested for their responses to LNA-ON.

CONCLUSIONS

- LNA-based oligonucleotides (LNA-ONs) result in down-regulation of target in vitro and in vivo in many cancer cell lines including primary tumor cells without the need for transfection.
- In vivo activity is assessed by potency in growth inhibition and target down-regulation may predict in vivo anti-tumor response.
- LNA-ONs administered IV achieve tumor concentration required to effectively down regulate target in vitro (Zhang et al., 2011 Gene Therapy; 18:526).
- Cells resistant to LNA-ONs have been generated.
- Understanding the mechanism of resistance to LNA-ON may assist in the selection of tumors and patients who may benefit the most from LNA-ON therapy.