Heteroduplex oligonucleotide (HDO)
a nucleic acid pharmaceutical platform technology

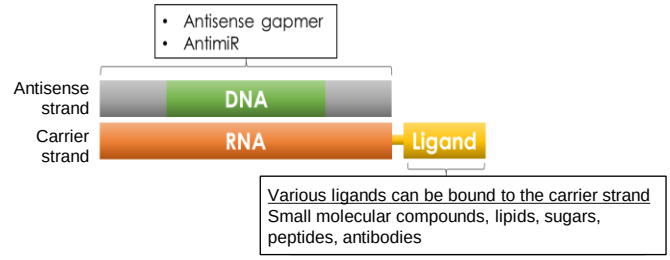
Rena Therapeutics, Inc. , Tokyo, Japan| https://www.renatherapeutics.com/ | info@renatherapeutics.com

Overview

Heteroduplex oligonucleotide (HDO) is the third platform technology for mRNA Therapeutics following short interfering RNA (siRNA) and single-stranded antisense oligonucleotide (ASO), which serves as a therapeutic agent for the modulation of specific genes at the post-transcriptional level. Rena Therapeutics Inc. (Rena) is a university-originated venture company established in 2015 to commercialize HDO technology. Rena has licensed HDO technology to Ionis Pharmaceuticals, Inc. and Takeda Pharmaceutical Co., Ltd., respectively and is focused on joint research with pharmaceutical companies on drug discovery using HDO technology.

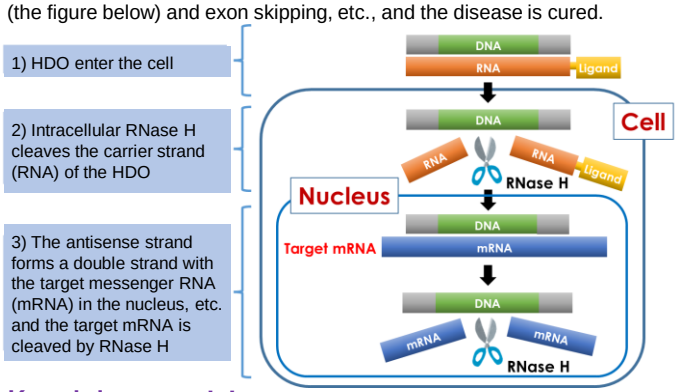
Structure

HDO is an artificial functioning nucleic acids composed of an antisense strand (DNA) that binds to a transcript of a target gene and a carrier strand (RNA) that is complementary to the antisense strand. Since a ligand is bound to the carrier strand, the activity of the antisense strand, which is an active body of the therapeutic agent, is not affected, allowing the introduction of various ligands and cell-specific delivery. Due to being a heteroduplex of DNA/RNA, its features include high nuclear translocation and low toxicity compared to ASO.



Mechanism of action

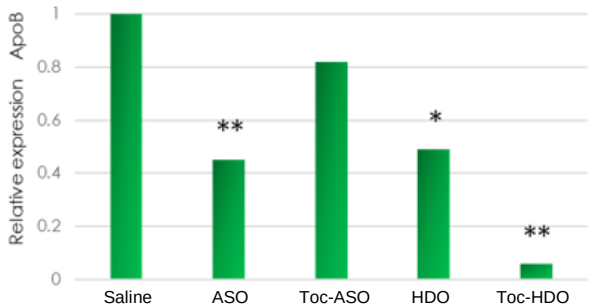
Once inside the cell, the HDO carrier strand (RNA) is cleaved by a ribonuclease (primarily RNase H). Subsequently, the antisense strand (DNA) forms a double strand with the target mRNA in the nucleus and cytoplasm, and the amount of target protein is adjusted through cleavage (the figure below) and exon skipping, etc., and the disease is cured.



Knockdown activity

Ligand-bound HDO has much better knockdown activity than ASO. When the knockdown activity was compared in an *in vivo* study, Toc-HDO knocked down 95% of the target mRNA, showing a much stronger knockdown activity than ASO and Toc-ASO.

Dose: 0.75 mg/kg of ASO, Toc-ASO, HDO, Toc-HDO; Administration: single bolus i.v.; Target: APOB mRNA (mouse liver); Observation period: 3 days after dosing

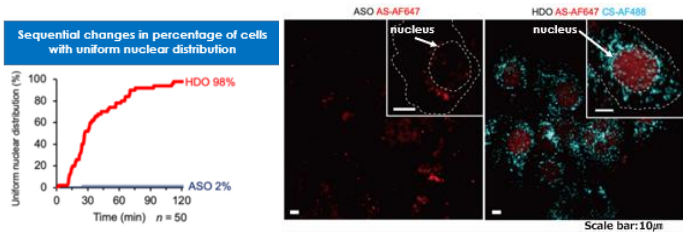


Nature Communications 2015 Aug 10;6:7969, Nishina K et al.

Nuclear translocation

HDO translocate better into the nucleus compared to ASO.

The antisense strand (AS) of HDO was labeled with AF647 and the complementary strand (CS) with AF488. Under 50nM transfection conditions of HDO or ASO (n=50) with cell line: Huh-7, target: intron ApoB, the left graph shows a distribution of 98% for HDO but 2% for ASO in 120 minutes after transfection. In the right picture, a strong and distinct nuclear signal was observed for AS in almost all HDO-transfected cells. In addition, a dotted signal of CS accumulated mainly in the cytosol was detected. On the other hand, at the same dose of ASO presented just a diffused or partly dotted weak distribution throughout the cells.



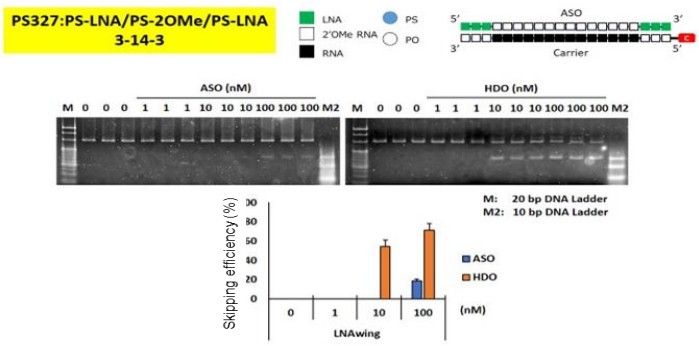
Molecular Therapy: Nucleic Acids Vol. 23 March 2021, 1360-1370

Efficient exon skipping

HDO is associated with higher skipping efficiency than ASO.

An increase in the skipping rate by at least 10-fold was seen for mouse pre-mRNAIL-1 RAcP exon 9 when comparing HDO to ASO.

*Skipping efficiency (%) = (amount of mRNA skipped)/(amount of mRNA skipped + amount of mRNA not skipped) x 100



Reduced hepatotoxicity

HDO is excellent at reducing hepatotoxicity. In an *in vivo* study, alanine aminotransferase (ALT) hepatotoxicity was seen after 2 doses in the ASO treated group, but not in the HDO treated group.

Subject: c57BL/6J mouse (n=5); Dose: Twice i.v. administration with 1000 nmol/kg/week of ASO, HDO, RL-002HDO and Saline; Target: YB-1; Data observed for 7 days after dosing