Heteroduplex oligonucleotide (HDO) a nucleic acid pharmaceutical platform technology

RenaTherapeutics has an exclusive license to HDO technology.

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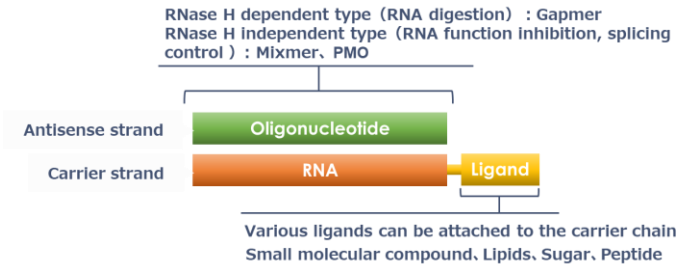
Overview

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 (gapmer, mixmer, PMO etc.) that binds to a transcript of a target gene and a carrier strand (RNA) that is complementary to the antisense strand. Since a ligand (receptor ligands, antibodies, lipids, etc.) is bound to the carrier strand, various ligands can be introduced without affecting the activity of the antisense strand enabling cell-specific delivery. HDO has high nuclear localization and low toxicity compared to ASO.



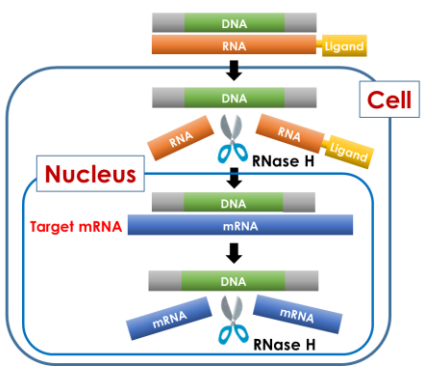
Mechanism of action

The mechanism of action of RNaseH-dependent antisense effects using DNA gapmers is as follows.

1) HDO enter the cell

2) Intracellular RNase H cleaves the carrier strand (RNA) of the HDO

3) The antisense strand forms a double strand with the target messenger RNA (mRNA) in the nucleus, etc. and the target mRNA is cleaved by RNase H

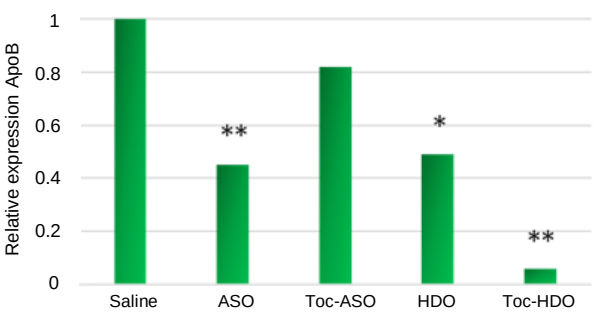


※HDO is also capable of RNaseH-independent antisense effects (eg exon skipping).

Knockdown activity

Ligand-conjugated HDO has much better knockdown activity than ASO.

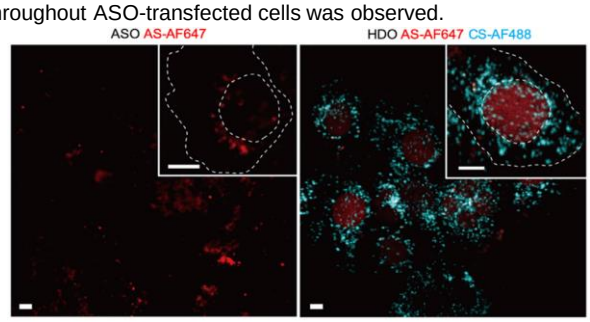
Dose : 0.75mg/kg of ASO, Toc-ASO, HDO, Toc-HDO; Administration : single bolus i.v.; Target : ApoB mRNA (mouse liver); Observation period : 3 days after dosing.



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

Nuclear translocation

HDO has better nuclear translocation than ASO. The antisense strand (AS) was labeled with AF647 and the carrier strand (CS) with AF488. Cell line: Huh-7, target: intronic ApoB, HDO or ASO under 50 nM transfection (n = 50). Right picture: A strong and distinct nuclear signal of AS was observed in HDO-transfected cells. Left picture: a weak distribution that was diffuse or partially interspersed throughout ASO-transfected cells was observed.

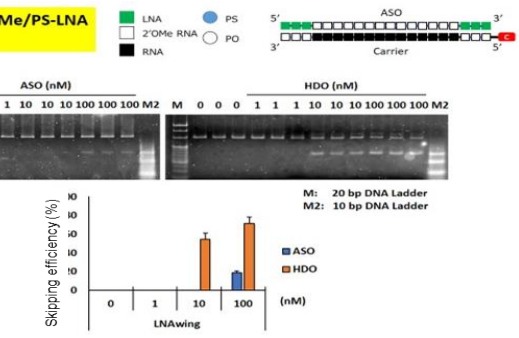


Mol Ther Nucleic Acids 2020 23; 1360-1370 **Scale bar:10μm**

Efficient exon skipping

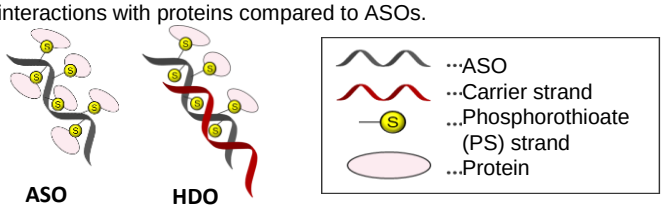
HDO is associated with higher skipping efficiency than ASO.
An increase in the skipping rate by at least 10-fold was seen for mouse pre-mRNAIL-1 RAcP exon 9 when comparing HDO to ASO.
*Skipping efficiency (%) = (amount of mRNA skipped)/(amount of mRNA skipped + amount of mRNA not skipped) x 100

PS327:PS-LNA/PS-2OMe/PS-LNA 3-14-3



Toxicity reduction

HDO is expected to reduce toxicity caused by non-specific protein binding. This is partly because 50% of the PS modifications are located within the double-helical Groove, resulting in reduced interactions with proteins compared to ASOs.



In fact, it has been reported that compared to ASO, HDO has a weak binding force of about 1/60 to albumin and about 1/500 to IgG (see table below).

<Dissociation constants between HDO or ASO and plasma proteins (kd, μM)>

	Albumin	Transferrin	IgG	Fibrinogen	A2M *1	HRG *2
ASO	10.4	7.3	0.9	0.3	0.044	0.009
HDO	762.9	450	>500	75	>3	>1

Gaus et al, Nucleic Acids Res 2019