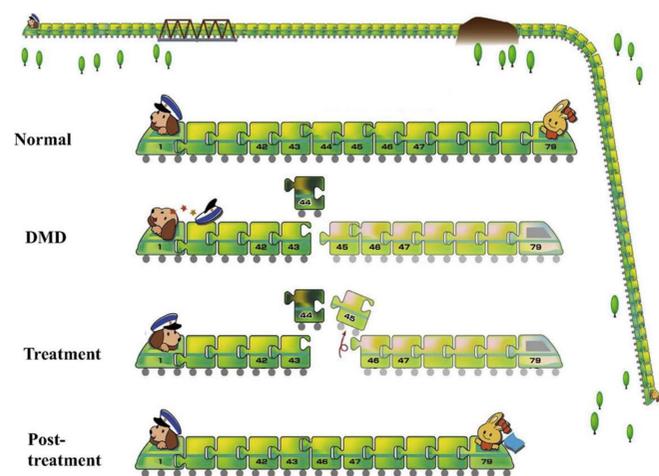


Background

- Duchenne muscular dystrophy (DMD) is a common inherited neuromuscular disorder characterized by progressive muscular degeneration.
- Antisense oligonucleotide (ASO)-mediated exon skipping is the most promising way to express internally deleted dystrophin in DMD by correcting the reading frame of dystrophin mRNA.
- DS-5141b is an ASO consisting of 2'-O,4'-C-ethylene-bridged nucleic acids (ENA[®]) and 2'-O-methyl RNA (2'-OMe) that induces dystrophin mRNA exon 45 skipping for the treatment of DMD. ENA is a registered trademark of Daiichi-Sankyo Co., Ltd.

Cartoon Explaining Exon Skipping Therapy



Brain & Development (2016) 38, 4-9

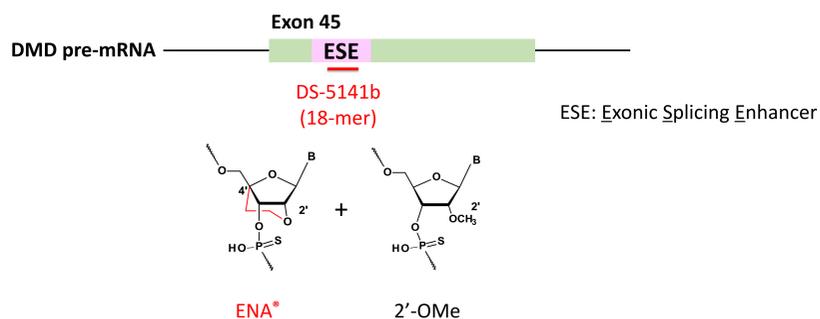
ENA[®] has Stronger Affinity for mRNA Than 2'-OMe and Morpholino

Table 1. Properties of 2'-OMe, Morpholino (PMO) and ENA[®]

	2'-OMe	PMO	ENA [®]
Structure			
Charge	Negative	Neutral	Negative
Protein binding	High	Low	High
Permeability	Low	Very low	Low
Elimination from tissues	Slow	Fast	Slow
Affinity towards mRNA (ΔT_m/modification)	+0.2 - 1.4°C*	+0.8°C*	+3.5 - 5.2°C**
Nuclease resistance	High	Very high	Very high

*Nucleic Acids Res. (1997) 25, 4429-4443.
 **Bioorg. Med. Chem. (2003) 11, 2211-2226.
 ENA is a registered trademark of Daiichi-Sankyo Co., Ltd.

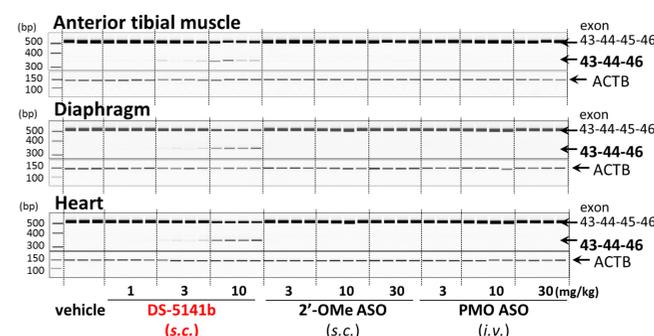
DS-5141b is an ASO Consisting of ENA[®] and 2'-OMe



Potent Exon 45 Skipping Activity of DS-5141b Compared With 2'-OMe ASO and PMO ASO in DMD Model Mice

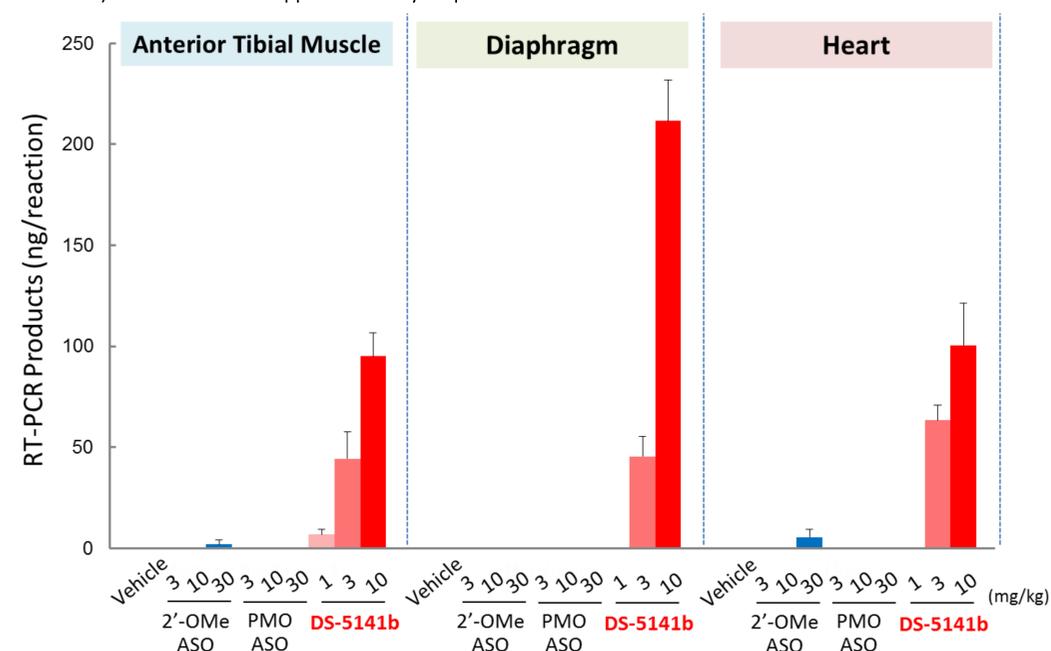
Figure 1. Exon 45 skipping profiles of DS-5141b, 2'-OMe ASO and PMO ASO in *mdx* mice after weekly administration for 4 weeks

a) Detection of exon 45-skipped mouse dystrophin mRNA by RT-PCR



- 2'-OMe ASO and PMO have the same nucleotide sequence as DS-5141b
- ACTB : Actin beta

b) Level of exon 45-skipped mouse dystrophin mRNA



- DS-5141b showed clear exon 45 skipping in all tissues examined, including the heart, at doses over 3 mg/kg.

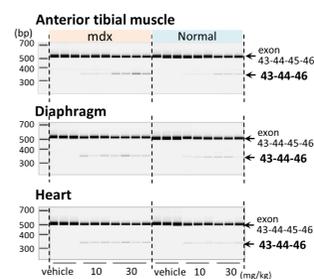
- PMO ASO did not induce any exon 45 skipping at doses up to 30 mg/kg; and 2'-OMe ASO had very slight exon skipping activity at a dose of 30 mg/kg.

(n=4, Mean + S.E.)

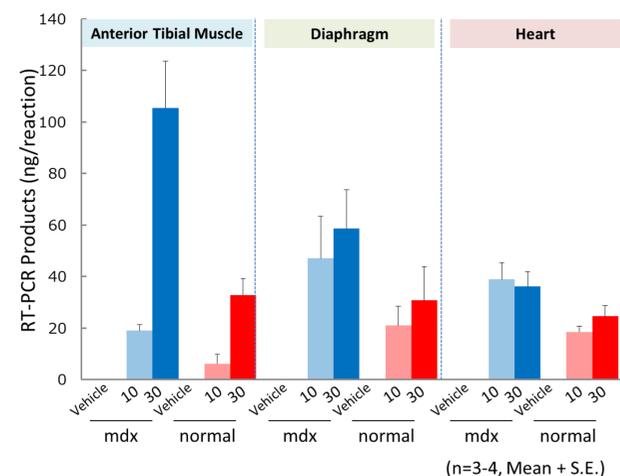
Induction of Exon 45 Skipping by DS-5141b in Normal Mice

Figure 2. Exon 45 skipping profiles of DS-5141b in *mdx* and normal mice after a single s.c. administration

a) Detection of exon 45-skipped mouse dystrophin mRNA by RT-PCR



b) Levels of exon 45-skipped mouse dystrophin mRNA



- At a dose of 30 mg/kg, DS-5141b showed clear exon 45 skipping in all tissues examined, including the heart and even in normal mice, after only a single administration.

Distribution of DS-5141b to Heart, Diaphragm and Skeletal Muscles in Normal Mice

Figure 3. Autoradiograms after single s.c. administration of [¹⁴C]DS-5141b (10 mg/kg)

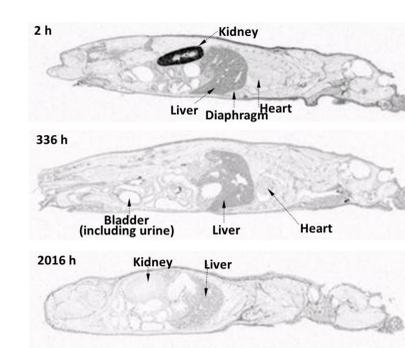
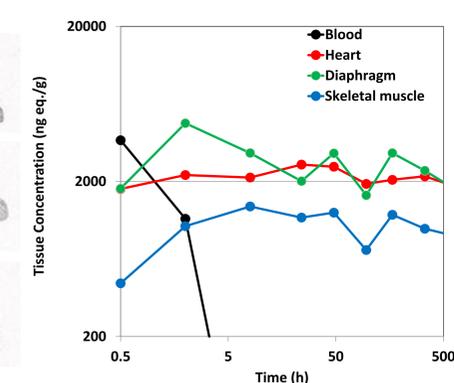


Figure 4. Time course of radioactivity concentrations in tissues



- [¹⁴C]DS-5141b was distributed throughout the tissues of mice, with a long retention time in the heart, diaphragm and skeletal muscle.

Summary and Conclusion

- DS-5141b showed clear exon 45 skipping in all muscles examined (anterior tibial muscle, diaphragm, and heart) in *mdx* mice compared with 2'-OMe ASO and PMO ASO. Notably, exon skipping was detected in heart muscle at a low dose, suggesting cardiac improvement.
- In normal mice, DS-5141b induced exon skipping in all muscle examined, including the heart, which suggests potential benefits to uninjured tissues.
- Autoradiography study in mice revealed that DS-5141b is distributed not only in skeletal muscles, but also in the heart and diaphragm.
- DS-5141b is currently undergoing a Ph1/2 clinical trial (NCT02667483) in Japan. (Will be introduced in pre-record video and live panel presentation on Restoring Dystrophin at the Virtual Annual Conference on July 23rd)