Supplementary material

Short Locked Nucleic Acid antisense oligonucleotides potently reduce apolipoprotein B mRNA and serum cholesterol in mice and non-human primates.

Ellen Marie Straarup, Niels Fisker, Maj Hedtjärn, Marie W. Lindholm, Christoph Rosenbohm, Vibeke Aarup, Henrik Frydenlund Hansen, Henrik Ørum, Jens B. Rode Hansen and Troels Koch*

Santaris Pharma A/S, Kogle Allé 6, DK-2970 Hørsholm, Denmark

Supplementary figures and experimental section

Eight Bcl-2 targeting LNA gapmers were designed and synthesized (Table 1). The length of the oligonucleotides ranged from 12- to 22-nucleotides, and seven of these gapmers were designed as truncated versions of the 22-mer sequence (Table 1). Down regulation of the Bcl-2 expression was conducted under gymnotic delivery and recorded at two different time points (day 3 & 5) and at three concentrations (2.5, 5, & 10 μM).

Oligo	Length	Target	Sequence
4743	12	Bcl-2	5'- $^{m}\mathbf{C}_{s}\ ^{m}\mathbf{C}_{s}\ c_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ c_{s}\ \mathbf{G}_{s}\ ^{m}\mathbf{C}$ -3'
4744	12	Bcl-2	5'- $\mathbf{T}_{s}\ ^{m}\mathbf{C}_{s}\ c_{s}\ c_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ ^{m}\mathbf{C}_{s}\ \mathbf{G}$ -3'
4745	12	Bcl-2	5'- $^{m}\mathbf{C}_{s}\ ^{m}\mathbf{C}_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ c_{s}\ g_{s}\ ^{m}\mathbf{C}_{s}\ ^{m}\mathbf{C}$ -3'
4746	14	Bcl-2	5'- $\mathbf{T}_{s}\ ^{m}\mathbf{C}_{s}\ c_{s}\ c_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ c_{s}\ \mathbf{G}_{s}\ ^{m}\mathbf{C}_{s}\ c$ -3'
2993	16	Bcl-2	5'- $^{m}\mathbf{C}_{s}\ \mathbf{T}_{s}\ c_{s}\ c_{s}\ c_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ c_{s}\ g_{s}\ ^{m}\mathbf{C}_{s}\ ^{m}\mathbf{C}_{s}\ a$ -3'
2989	18	Bcl-2	5'- $\mathbf{T}_{s}\ ^{m}\mathbf{C}_{s}\ t_{s}\ c_{s}\ c_{s}\ c_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ c_{s}\ g_{s}\ c_{s}\ ^{m}\mathbf{C}_{s}\ \mathbf{A}_{s}\ t$ -3'
4747	20	Bcl-2	5'- $\mathbf{T}_{s}\ \mathbf{T}_{s}\ c_{s}\ t_{s}\ c_{s}\ c_{s}\ c_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ c_{s}\ g_{s}\ c_{s}\ c_{s}\ \mathbf{A}_{s}\ \mathbf{T}_{s}\ c$ -3'
4748	22	Bcl-2	5'- $\mathbf{G}_{s}\ \mathbf{T}_{s}\ t_{s}\ c_{s}\ t_{s}\ c_{s}\ c_{s}\ c_{s}\ a_{s}\ g_{s}\ c_{s}\ g_{s}\ t_{s}\ g_{s}\ c_{s}\ g_{s}\ c_{s}\ c_{s}\ a_{s}\ \mathbf{T}_{s}\ ^{m}\mathbf{C}_{s}\ c$ -3'
3088	16	Scrambled control	5'- $^{m}\mathbf{C}_{s}\ \mathbf{G}_{s}\ \mathbf{T}_{s}\ c_{s}\ a_{s}\ g_{s}\ t_{s}\ a_{s}\ t_{s}\ g_{s}\ c_{s}\ g_{s}\ \mathbf{A}_{s}^{o}\ \mathbf{A}_{s}\ \mathbf{T}_{s}\ c$ -3'

Table1: Design and sequence of LNA ologonucleotides. Bold capital letters denote LNA nucleotides and normal case letters denote DNA nucleotides. A = nucleotide monomer with an adenin-9-yl base, C = nucleotide monomer with a cytosin-1-yl base, G = nucleotide monomer with a guanin-9-yl base, T = nucleotide monomer with a thymin-1-yl base. ^{m}C = nucleotide monomer with a 5-methylcytosin-1-yl base. Lower case "s" denote thioated phosphate group.

Methods

Cells: 518A2 human melanoma cells were grown in DMEM (Invitrogen, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, and 100 U/ml penicillin G sodium and 100 μg/ml streptomycin sulfate.

Gymnotic Delivery of Oligonucleotides: 518A2 cells were seeded at a low plating density of 75,000 cells per well in 6-well plates in complete media the day before the experiment began. The day after plating, oligonucleotides were added at the stated concentrations and mixed by gentle rocking

of the plate. LNA-oligonucleotides were used at a final concentration of 2.5, 5 and 10 μM. The total incubation time before cell lysis and RNA isolation were 3 and 5 days at 37°C.

Quantitative RT-PCR: Total RNA from 518A2 cells was extracted using the Qiagen RNeasy kit (Qiagen, The Netherlands) according to the manufacturer's instructions. The reverse transcription reaction was carried out with random decamers, 0.5 μg total RNA, and the M-MLV RT enzyme from (Applied Biosystems, Carlsbad, CA) according to protocol. First strand cDNA was subsequently diluted 10 times in nuclease-free water before addition to the Real-Time PCR reaction mixture. mRNA quantification of Bcl-2 and GAPDH genes were done using standard TaqMan assays (Applied Biosystems). A two-fold total RNA dilution series from untreated 518A2 cells served as standard to ensure a linear range (Ct versus relative copy number) of the amplification. The Applied Biosystems 7500 Real-Time PCR instrument was used for amplification.

Results

As illustrated in figure 1 the longer oligonucleotides (>14-mer) are less potent than the 12- and 14-mers. Taken together, the 4 shortmers (12-&14-mers) seemed to exert their activity faster than the longer LNA oligonucleotides.

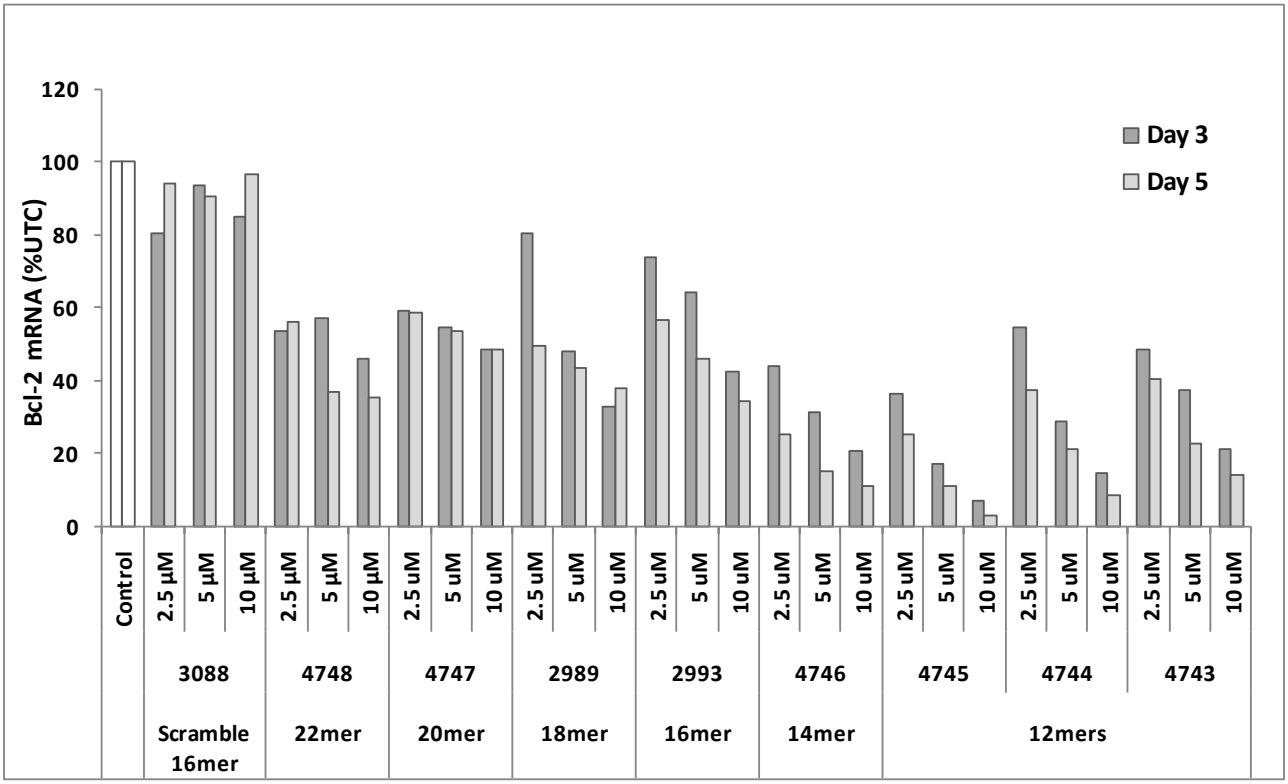

Figure 1: Bcl-2 mRNA reductions in 518A2 melanoma cells after gymnotic treatment for 3 and 5 days at 2.5, 5 and 10 μM.