TECHNOA

SPLICE TO DISCOVER SPLICE TO UNDERSTAND

Two million Antisense OligoNucleotides (AONs)
for **Splice** and **Transcript Modulations**

Transcript **switching**

Transcript **knock-down**

www.technoa.science

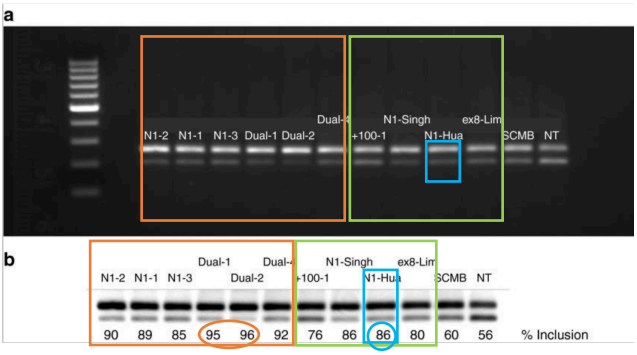
TechNOA AONs **perform better** than all the AONs designed by others.

TechNOA-designed AONs

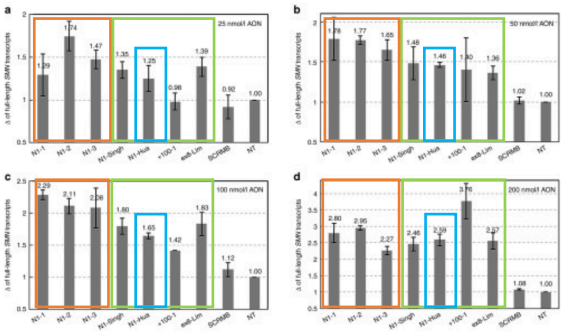
Other published AONs

IONIS Spinraza (SMA)

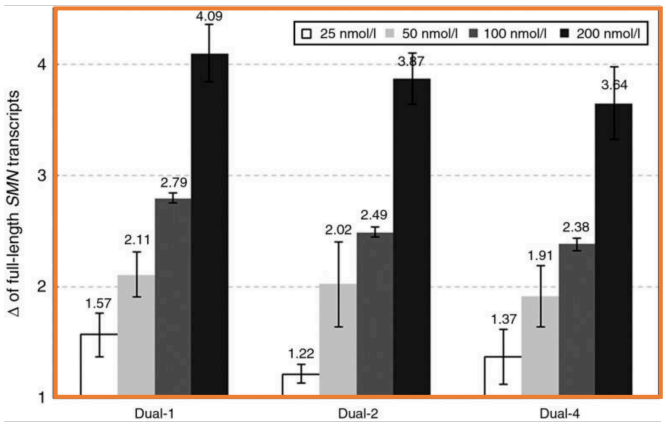
ProSensa/GSK (DMD)



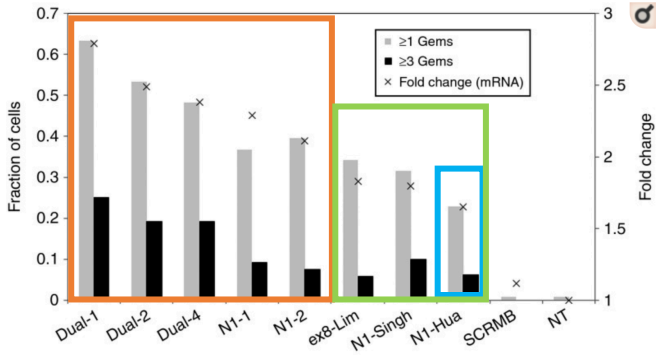
Gel electrophoresis images of *SMN* amplicons. (a) RT-PCR products were amplified using forward primer at exon 6 and reverse primer at exon 8. Bands: upper: amplicons with exon 7; lower: amplicons without exon 7. SCRM: scrambled AON. NC: negative control where cells were transfected with Lipofectamine 2000 only. Lane labels prefixed with "Dual" refer to dual-targeting AONs (see text below).



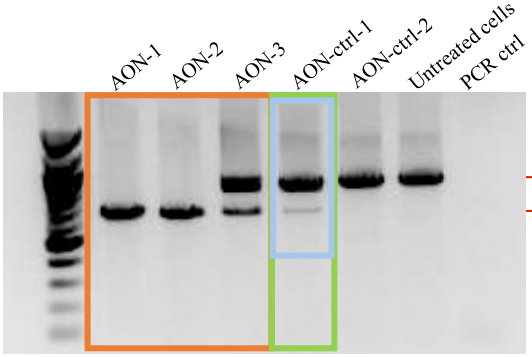
Fold increase of full-length SMN transcripts measured at various antisense oligonucleotide (AON) transfection concentrations. (a) 25 nmol/l. (b) 50 nmol/l. (c) 100 nmol/l. (d) 200 nmol/l. The fold increase of exon 7 inclusion induced by each AON was normalized with the internal control (GAPDH) and the data was further normalized to the level of the corresponding transcript in the normal control (Lipofectamine2000 only; labelled as NT).



Fold increase of full-length SMN transcripts measured at various dual-targeting antisense oligonucleotide (AON) transfection concentrations.



Fraction of cells with GEMs. Fraction of cells observed to have at least one or three nuclear GEMs upon treatment with 100 nmol/l of a specific antisense oligonucleotide (AON) were counted and plotted. A total of 120 cells were used for GEMs counting.



Products of RT-PCR amplifying dystrophin cDNA encompassing exons 43-54

Pao PW et al., Mol Therapy, 2014

Wee KB et al., Neuromusc Disorders, 2012

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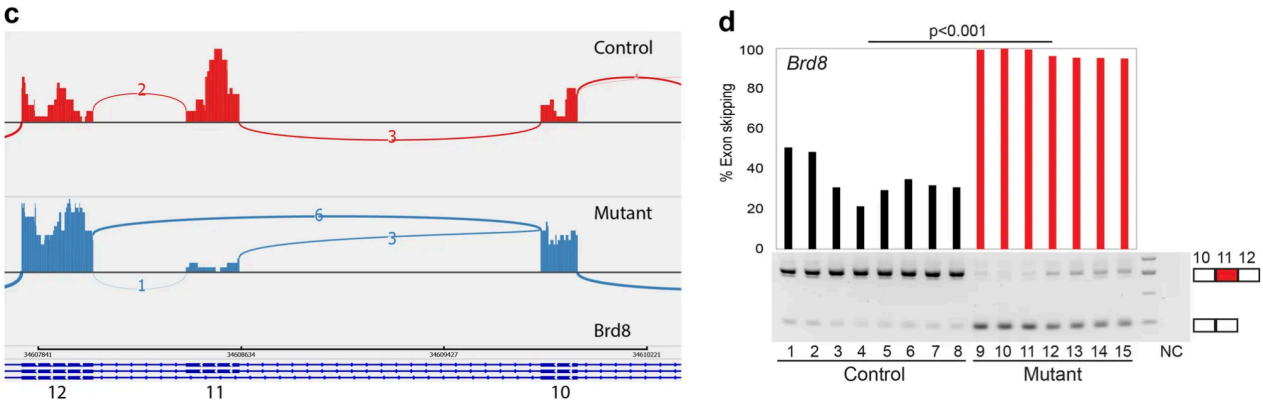
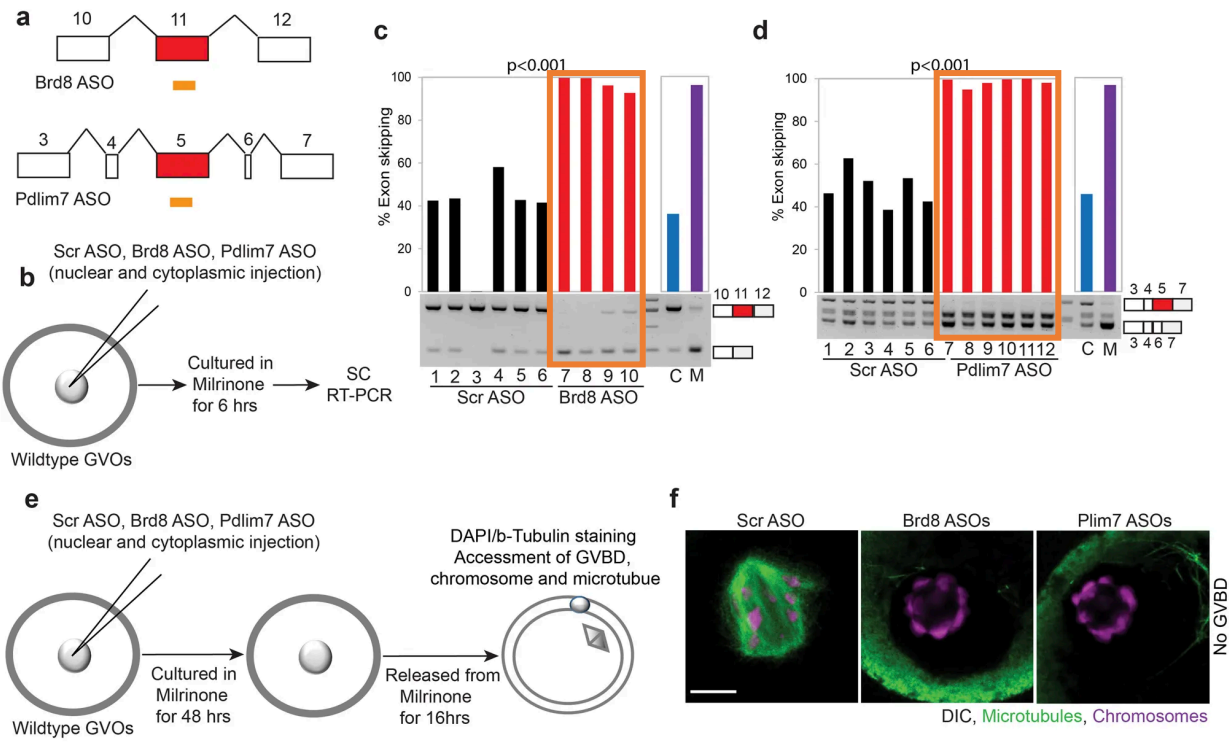
TechNOA AONs are **efficient** (10/10) to mimic a naturally-occurring splicing event.



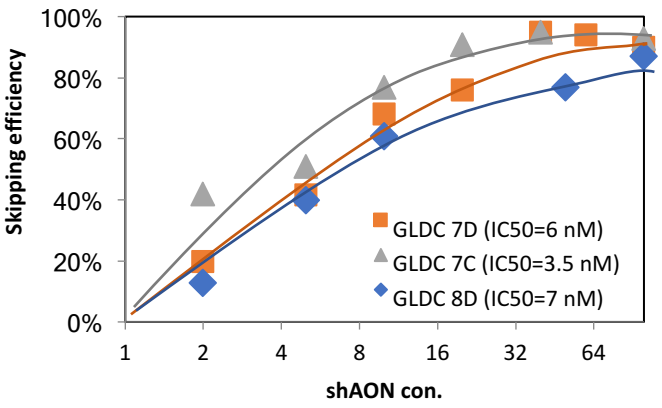
C A bar graph shows percentages of exon skipping in unannotated splicing events. All splicing events in grey, downregulated (control-specific) splicing events in blue, upregulated (mutant-specific) splicing events in red. **c** A representative sashimi plot shows an exon skipping event in *Brd8* transcript in control and mutant oocyte. The numbers of junction reads between two connecting exon are shown in the sashimi plot. **d** Validation of the exon skipping on *Brd8* transcript in control and mutant oocyte by semiquantitative PCR. Percentages of exon skipping calculated for eight control and seven mutant oocytes are shown on the top. Mann–Whitney test (Wilcoxon rank sum test) was used to calculate *p*-value



a Schematic representation of ASOs designed to induce exon skipping of *Brd8* and *Pdlim7* in wild-type oocytes. **b** Schematic illustration of an experiment to validate efficacies of ASOs in inducing exon skipping of *Brd8* and *Pdlim7* in wild-type oocytes. **c**, **d** Validation of efficacies of Brd8 (**c**) and Pdlim7 (**d**) ASOs by semiquantitative PCR. Percentage of exon skipping in individual control and mutant oocytes is shown on the top. C: control oocyte, M: mutant oocyte. Mann–Whitney test (Wilcoxon rank sum test) was used to calculate p-value. **e** Schematic illustration of an experiment to validate function of ASOs inducing exon skipping for *Brd8* and *Pdlim7* and GVBD defect in wild-type oocytes. **f** Representative confocal images of wild-type GV oocytes injected with *Brd8* or *Pdlim7* ASOs show no GVBD while oocyte injected with scramble ASO shows normal GVBD. Chromosome in magenta. Microtubule in green. Scale bar, 20 µm.

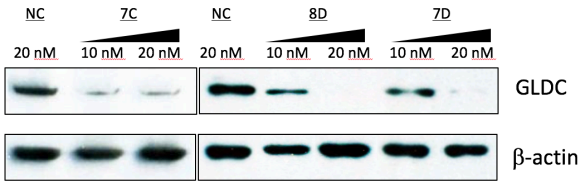
Do DV et al., Cell Discovery, 2018

TechNOA AONs can be use as a **dose-dependent** compound

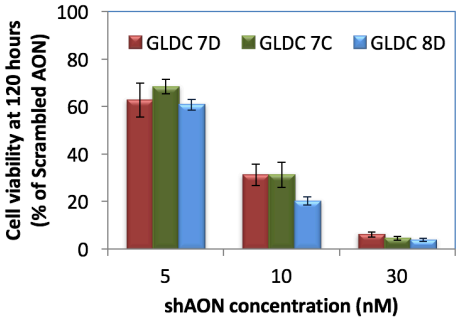


(**A**) Dose-response curves of shAON-induced exon-skipping efficiency (measured by densitometry analysis) in A549 cells. Cells were harvested 24 hr post-transfection. 100 µg/mL cycloheximide was added 5 hr after shAON transfection to inhibit the skipped transcripts from undergoing NMD. Data are presented as means ± SEM. (**C**) Western blotting of GLDC protein with β-actin as loading control showing the dose effect of shAON. Protein extraction was performed 72 hr after transfection.

c)



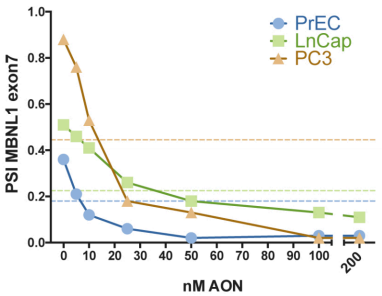
b)



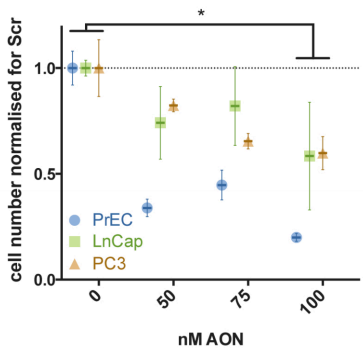
(**B**) Effect of various concentrations of shAONs on A549 cell viability at 120 hr after transfection.

Lin et al., Mol Ther Nucleic Acids, 2017

E



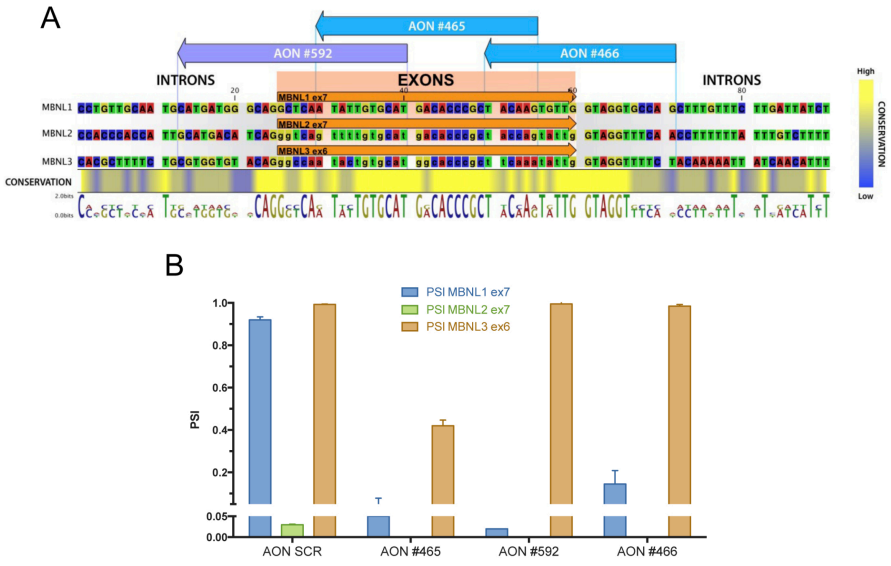
F



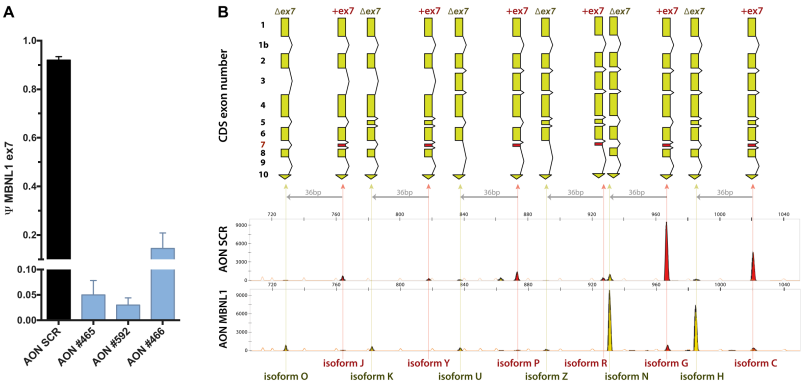
(**E**) PSI of MBNL1 ex7 in different cell lines upon transfection of AON MBNL1 at different concentrations. Cells were collected at 48 h. (**F**) AON SCR–normalized PC3 cell viability at different AON MBNL1 concentrations. Cells were counted at 48 h.

Tabaglio T et al., LSA, 2018

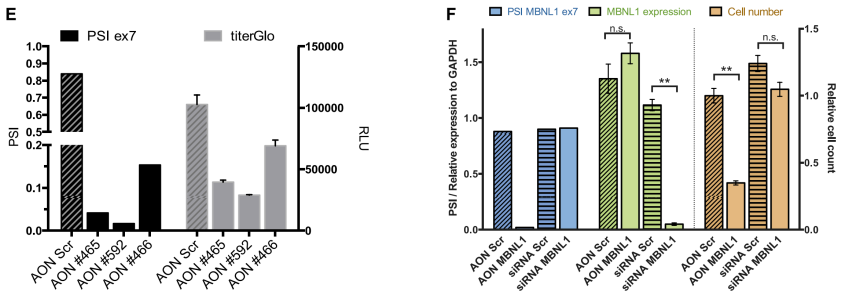
TechNOA AONs are **specific** and they enable you to **avoid overexpression.**



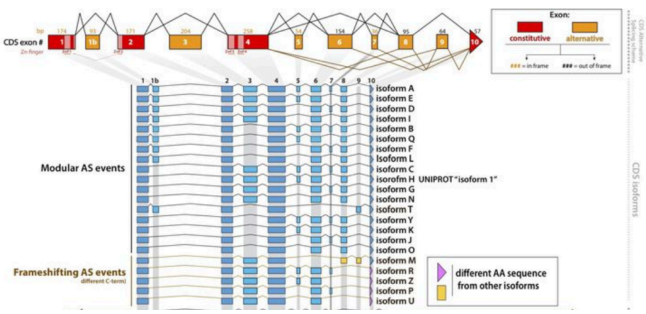
(**A**) Genomic sequence of the highly conserved 36-bp exons of *MBNL1*, *MBNL2*, and *MBNL3* (in orange) with the surrounding intronic region. Above is shown the pairing position of the tree AONs directed against *MBNL1* ex7. Below, the conservation index of the nucleotide sequence. (**B**) PSI of *MBNL1* ex7 (blue), *MBNL2* ex7 (green), and *MBNL3* exon 6 upon 100 nM AON transfection at 48 h in PC3 cells.



(**A**) MBNL1 ex7 PSI change upon AON transfections (100 nM), 48 h post-transfection. Data are representative of two independent experiments. (**B**) FLA-PCR visualization of MBNL1cDNA isoforms upon AON #592 transfection in PC3 cells (100 nM, at 48 h post-transfection). On the X axis is represented the nucleotide size and on the Y axis the fluorescence intensity of the amplicon. Data are representative of multiple independent experiments.



(**E**) MBNL1 ex7 PSI change and relative cell viability in PC3 cells. 100 nM of AON after 48 h. Cell viability quantified with TiterGlo luminescence assay. (**F**) Ex7 PSI (blue bars), overall MBNL1 relative mRNA expression (green bars) and relative cell count of PC3 cells transfected with AON SCR, AON MBNL1 (100 nM), siRNA Scrambled, and siRNA MBNL1 (25 nM).



Tabaglio T et al., LSA, 2018

List of **publications** that use TechNOA-designed AONs

TechNOA NOA-Switch
AON used to switch between splicing isoforms

TechNOA NOA-NMD
AON used to induce degradation of the transcript through NMD

SRSF3 maintains transcriptome integrity in oocytes by regulation of alternative splicing and transposable elements
Do et al. 2018. **Cell Discov**
In this work from Surani's lab, the AONs designed with our algorithm have been used in wild type oocytes. A change in the splicing landscape was detected upon SRSF3 genetic knockout. Our antisenses were successfully microinjected in wild type oocytes to mimic some of the observed splicing events in 3 different genes.

PTBP1-Mediated Alternative Splicing Regulates the Inflammatory Secretome and the Pro-tumorigenic Effects of Senescent Cells
Georgilis et al. 2018. **Cancer Cell**
Gil's lab has shown that PTBP1, an RNA binding protein, affects the alternative splicing of multiple genes that influence the inflammatory secretome of cancer cells. 15 AONs were rationally designed and tested to verify the individual contribution of the splicing switch to the phenotype.

MBNL1 alternative splicing isoforms play opposing roles in cancer
Tabaglio et al. 2018. **Life Sci Alliance**
Tabaglio and colleagues used a specific AON to prove that two different splicing isoform of MBNL1 protein can affect its dimerization properties. The AON allowed to switch between multiple isoforms, showing that they can have opposing effects in cancer cells.

Induced-Decay of Glycine Decarboxylase Transcripts as an Anticancer Therapeutic Strategy for Non-Small-Cell Lung Carcinoma
Lin et al. 2017. **Mol Ther Nucleic Acids**
In this piece of research, Lin and colleagues used our AONs to modulate the splicing of multiple exons in the GLDC gene. In this way they efficiently reduced the abundancy of the gene both in different cancer cell lines and in vivo mouse models.

RNAi Reveals Phase-Specific Global Regulators of Human Somatic Cell Reprogramming
Toh et al. 2016. **Cell Rep**
Loh's lab used our antisense oligonucleotides to modulate, both ways, the inclusion of two mutually exclusive exons of ZNF207, a gene involved in reprogramming. The use of the AONs confirmed the hypothesis on the phenotype caused by different splicing isoform of the gene.

Antisense oligonucleotide-mediated MDM4 exon 6 skipping impairs tumor growth
Dewaele et al. 2016. **J Clin Invest**
In this experimental work form Marine and Guccione lab, the AONs were used to induce a physiologically observed splice change in the MDM4 gene. The exon skipping induced by the antisense oligonucleotide was observed in multiple low-passage melanoma lines, breast cancer line, neuroblastoma and ovarian cancer, DLBCL. In vivo injection of the antisense oligonucleotide induced the splicing switch in PDX mouse models of melanoma and DLBCL.

MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis
Koh et al. 2015. **Nature**
Koh and colleagues exploited AON-mediated intron retention in multiple genes to prove that some splicing events are at the basis of lymphomagenesis.

Dual masking of specific negative splicing regulatory elements resulted in maximal exon 7 inclusion of SMN2 gene
Pao et al. 2014. **Mol Ther**
Antisense oligonucleotides can be used for the cure of some genetic diseases. Here, AONs designed with our algorithm have been used to rescue the expression of the SMN2 gene, proving that a more efficient antisense sequence can alleviate the spinal muscular atrophy symptoms.

A Prospective Study in the Rational Design of Efficient Antisense Oligonucleotides for Exon Skipping in the DMD Gene
Pramono et al. 2012. **Hum Gene Ther**
In this work, we showed how the AONs can be designed to skip specific exons of the dystrophin gene in order to restore the phenotype caused by somatic mutations.

T.P.18 A recipe for the rational design of efficient antisense oligonucleotides for DMD gene exon skipping
Wee et al. 2012. **Neuromusc Disorders**
With this analysis, Dave Wee and colleagues analyzed in depth the DMD gene and it's splicing, designing AONs that can outperform largely the ones designed by other labs.

Computational papers on AON rational design

Discovery of Influenza A Virus Sequence Pairs and Their Combinations for Simultaneous Heterosubtypic Targeting that Hedge against Antiviral Resistance
Wee et al. 2016. **PLoS Comput Biol**
Thousands of oligos were designed to identify combinations of targets that can best hedge against drug resistance in influenza virus. This large scale study analyzes more than 100,000 influenza sequences from across more than 100 different influenza subtypes.

Dynamics of Co-Transcriptional Pre-mRNA Folding Influences the Induction of Dystrophin Exon Skipping by Antisense Oligonucleotides
Wee et al. 2008. **PLoS One**
The complexity of targeting co-transcriptional pre-mRNA structure is outlined in this study. With the improved oligo designs, previously unskippable exons in the DMD gene could now be skipped and with high skipping efficiency.