# Spherical nucleic acid (SNA) construct improves functional delivery of anti-microRNA oligonucleotide in preclinical studies *in vitro* and *in vivo*

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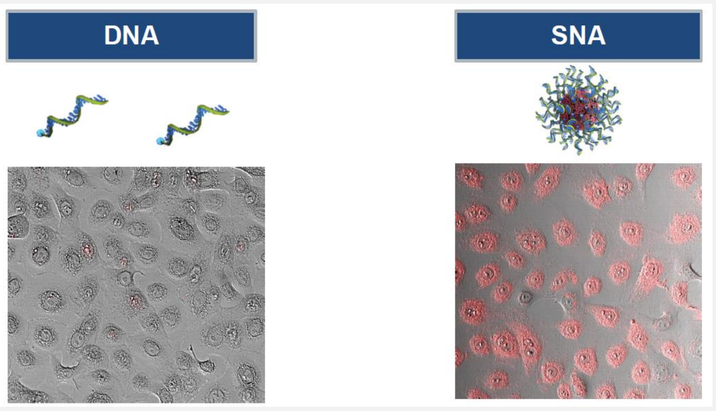
## Abstract

MicroRNAs are non-coding RNAs that play central roles in multiple cellular processes by binding to complementary target sequence-bearing mRNAs, resulting in repression of translation and eventual degradation of the targeted mRNAs. Anti-microRNA oligonucleotides (anti-miRs) are chemically-modified single-stranded oligonucleotides designed to inhibit the function of microRNA and represent a powerful therapeutic modality for multiple human diseases involving aberrant microRNA activities. Therefore, enhancing the delivery of anti-miRs is of specific interest for the development of microRNA-based therapeutics.

Spherical Nucleic Acids (SNA) are nanoscale particles consisting of densely packed oligonucleotides that are radially arranged in three-dimensions around a liposomal core. These constructs can enter cells by engaging scavenger receptors and lipid rafts and result in improved cellular uptake of the oligonucleotides.

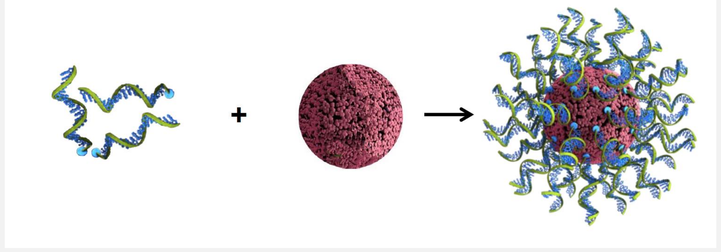
Here, we describe the preclinical characterization of an SNA construct designed to inhibit a ubiquitously expressed microRNA family called Let-7 [1, 2]. Anti-Let-7 SNA construct treatment without auxiliary transfection agents inhibited Let-7 activity and de-repressed the expression of multiple Let-7 target mRNAs in multiple cell lines *in vitro*. Moreover, anti-Let-7 SNA construct dose-dependently de-repressed multiple Let-7 target genes *in vivo* in the liver following a single intravenous (IV) or subcutaneous (SC) injection in mice. Importantly, SNA construct significantly improved functional delivery of the anti-Let-7 oligonucleotide *in vivo* compared to either cholesterol conjugated or unconjugated oligonucleotide with concomitant increases in both exposure (approximately 3- and 7-fold higher respectively IV; >6- and >2-fold higher respectively SC) and liver target gene de-repression (>10- and >30-fold higher potency respectively IV; >3- and >10-fold higher potency respectively SC).

## Spherical Nucleic Acids (SNA) Platform




Increased cellular uptake of oligonucleotides in spherical nucleic acid (SNA) format (right panel) compared with oligonucleotides (DNA) that are not in the SNA format (left panel). Equimolar oligonucleotide concentration of fluorescent dye-labeled oligonucleotide and SNA was used for uptake studies.

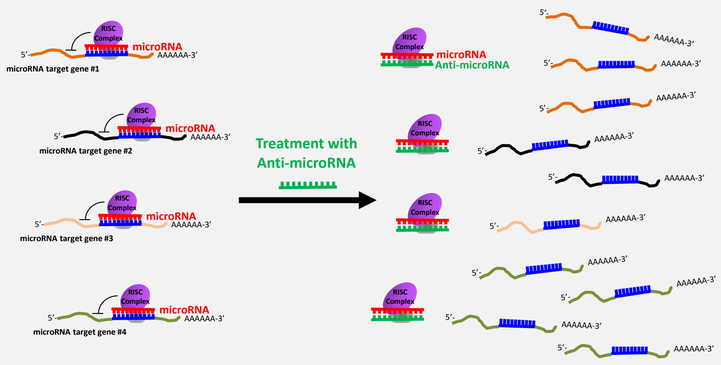
## Using SNA technology to enhance functional delivery of anti-miR oligonucleotide



Anti-miR Oligonucleotide + Scaffold from benign lipid nanoparticles → Generation of SNA with anti-miR Oligonucleotides

Synthesis of SNA is a simple and scalable process. Oligonucleotides conjugated to a lipophilic moiety are mixed with lipid nanoparticle scaffold to generate anti-miR SNA.

## Anti-miR treatment results in de-repression of multiple microRNA target genes

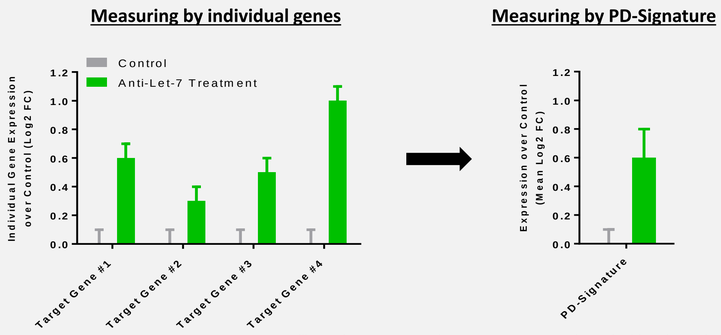



*For illustration purpose, only 4 target genes are shown in this schematic*

MicroRNA can bind to complementary target sequences located in the 3'UTR of target mRNAs, resulting in repression of translation and eventual degradation of the targeted mRNAs.

Anti-miRs inhibit the function of microRNA, resulting in de-repression of multiple target genes.

## Measurement of Let-7 activity using a Let-7 PD signature



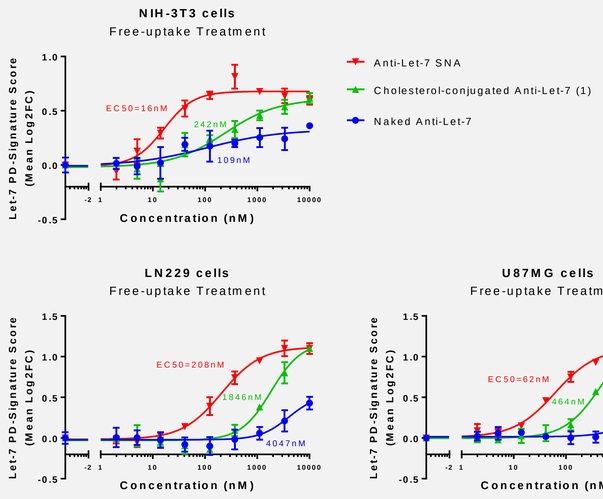

*For illustration purpose, only 4 target genes are shown in this schematic*

Inhibition of microRNA function can be assessed by individually measuring de-repression of known microRNA target genes. However, changes in individual microRNA target genes can often be small, variable and cell-type dependent.

We have previously developed a mouse Let-7 multigene PD-signature that can assess Let-7 target engagement with a dynamic window that works across different mouse cell lines and tissue-types following anti-Let-7 treatment. This mouse Let-7 PD-signature consists of 18 unique Let-7 target genes normalized by 6 reference house keeping genes. We have also developed a human Let-7 PD-signature which consists of 11 unique Let-7 target genes normalized by 5 reference house keeping genes.

Our Let-7 multigene PD-signatures provide comprehensive and unbiased assessment of Let-7 inhibition that can be used to discover, rank order and guide the development of novel microRNA-based therapeutics. The development and utilization of similar multigene PD-signatures have previously been described [3, 4].

## SNA construct improves activity of anti-Let-7 oligonucleotide in multiple cell lines *in vitro*

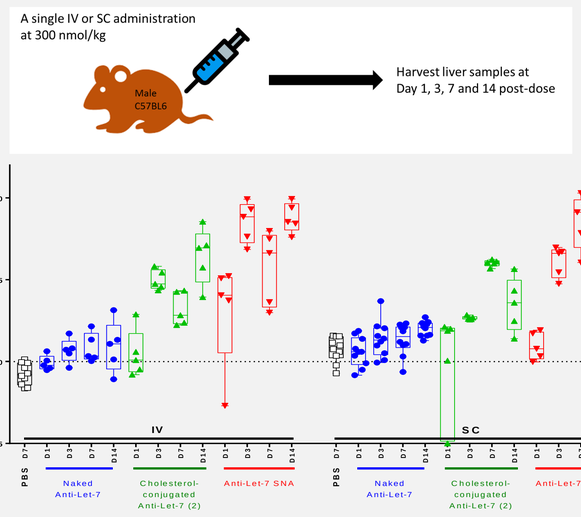



Mouse NIH-3T3 fibroblast, human LN229 or U87MG glioblastoma cells were treated with naked, cholesterol-conjugated (linker 1), or SNA construct of anti-Let-7 oligonucleotide (all without auxiliary transfection agents) at 0-to-10 μM for 24h *in vitro*. Following treatment, total RNA was extracted using RNAeasy® 96-well spin-plates per manufacturer's protocol (Qiagen) and expression of Let-7 target genes was assessed by qPCR using the respective Let-7 PD-signature.

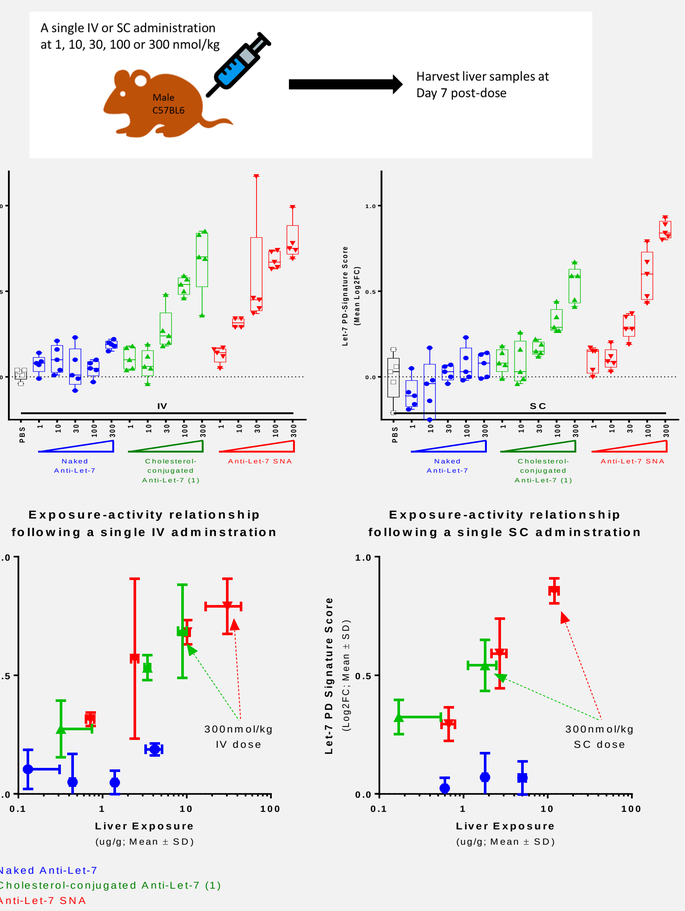
SNA construct improved the *in vitro* activity of anti-Let-7 oligonucleotides compared to the naked oligonucleotides and cholesterol-conjugate, and de-repressed the expression of multiple Let-7 target mRNAs with EC50 values of 14 nM, 208 nM and 62 nM in NIH-3T3, LN229 and U87MG cells, respectively.

## SNA construct improves activity of anti-Let-7 oligonucleotide in preclinical mouse studies *in vivo*

### Time course after a single IV or SC administration




### Dose-response after a single IV or SC administration




Male C57BL6 mice were treated with a single tail vein intravenous (IV) or subcutaneous (SC) dose of naked, cholesterol-conjugated (linker 1 or 2), or SNA construct of anti-Let-7 oligonucleotide. For time course study, mice were dosed at 300 nmol/kg and liver samples were harvested at Day 1, 3, 7 or 14 post dose. In the dose-response study, mice were dosed at 1, 10, 30, 100 or 300 nmol/kg and liver samples were harvest at Day 7 post dose. Total RNA was extracted using RNAeasy® 96-well spin-plates (Qiagen) from liver samples after homogenization in Qiazol®, and expression of Let-7 target genes was assessed by qPCR using the respective Let-7 PD-signature. To determine exposure of the anti-Let-7 oligonucleotide in liver, tissue homogenates were extracted using protein digestion/liquid-liquid extraction prior to analysis using high-performance liquid chromatography with fluorescence detection (HPLC-FL)

SNA construct improved the *in vivo* activity of anti-Let-7 oligonucleotides compared to the naked and cholesterol-conjugated oligonucleotides, and de-repressed multiple Let-7 target genes in the liver following a single IV or SC injection in mice. A concomitant increase in liver exposure was also observed, indicating that improvement in activity was driven by improvement in delivery.

## Conclusion

Taken together, our preclinical studies demonstrate that the SNA construct improves delivery of anti-miRs and target gene expression modulation *in vitro* and *in vivo*. Our data exemplify the potential utility of SNA technology to augment the functional delivery of oligonucleotide-based therapeutics such as anti-miRs.

## References

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2. Roush et al, The let-7 family of microRNAs. [Trends Cell Biol. 2008 Oct; 18(10): 505-16]
3. Pavlicek *et al*, A miR-221 multigene pharmacodynamics signature for assessing miR-221 inhibition. [Poster Presentation at AACR 2014, San Diego CA, USA]
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