Gene-silencing oligonucleotides: Innovative design of oligonucleotides with enhanced efficacy and RNAi-like mechanism of action

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Berlin, 15 - 16 November 2011
Nucleic Acids Therapeutics: Over Three Decades of research
# Gene Silencing Approaches

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Design of Gene-Silencing Oligo (GSO)

- Synthetic DNA or RNA
- Phosphorothioate modification provides stability
- Systemic delivery without lipid formulation

19 and 21-mer show optimal gene-silencing activity

TLR-mediated immune response is mitigated due to 5' end inaccessibility
Chemistry of GSO

GSO Structure

Synthetic DNA or RNA PO or PS backbone

Linker
GSO has Optimal Activity with 19-mer Arms

MyD88 RNA (R.Q.)

Length of Oligo

J774 cell culture, 48 hr incubation
• Harvest RNA 48hr post transfection
• Perform RNA-Linker-Mediated Rapid Amplification of cDNA ends (RLM-RACE)
• Sequence amplified cleavage products

5’ RLM-RACE Assay

5’ RLM-RACE Analysis: Comparison of siRNA and GSO cleavage products
siRNA and RNA GSO have similar cleavage products

MyD88 Target sequence

5’-GCAGGCTGCTA GAGCTGCTGGCCTT…..-3’

Expected Cleavage Site

Target sequence

5’-GCAGGCTGCTA GAGCTGCTGGCCTT…..-3’

siRNA cleavage product

5’-AGTAGAAA GAGCTGCTGGCCTT…..-3’

RNA GSO cleavage product

5’-AGTAGAAA GAGCTGCTGGCCTT…..-3’

2’-O-Me GSO

No Cleavage Product

AGTAGAAA - Linker
DNA GSOs induce cleavage of target mRNA at multiple sites

MyD88 Target sequence

5'-GCAGGCTGCTA GAGCTGCTGGCCTT…..-3'

Expected Cleavage Site

Target sequence

19-mer siRNA

19-mer DNA GSO

Control DNA with 3'-3'-linkage

5’-GCAGGCTGCTA GAGCTGCTGGCCTT…..-3’

5’- AGTAGAAA GAGCTGCTGGCCTT…..-3’

5’- AGTAGAAA GGAGTACTGGCCTT…..-3’

5’- GTAGAAA AGAGCTGCTGGCCTT…..-3’

5’- GGAAGAAAGAAGGAGTAAA GGAAGAAAGAAGGAGTAAA GGAAGAAAGAAGGAGTAAA -3’

AGTAGAAA - Linker

GGA - Unmatched/ambiguous sequence

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Insights into Structure-Activity Relationship Studies of GSOs

- Progress to Date:
  - Comparative Activity of GSO and Antisense
  - Affinity of GSO and Antisense to the target RNA
  - Requirement of 19-mer arms of GSOs
  - Effect of mis-matches in each arm of GSO
  - Do the 19-mer arms need to be the same
  - Effect of presence of 5’-phosphate on the activity of GSO
  - Effect of linker on the activity of GSO
GSO has greater gene silencing activity than Antisense Oligonucleotide (ASO)

GSO

Antisense oligonucleotide (ASO)

% Inhibition of MyD88 RNA

J774 cell culture, 48 hr incubation
GSO and ASO have similar affinity to target mRNA

T_m, °C

Length of Oligo

15-mer 17-mer 19-mer 21-mer 23-mer 25-mer

T_m with target RNA

GSO

ASO
GSOs require Two 19mer Arms for Optimal Activity

19-19 mer

19-25 mer

19-15 mer

% Inhibition of Protein

0

-25

-50

-75

-100

19-19 mer  19-25 mer  19-15 mer

J774 cell culture, 3 \( \mu \)g/ml, 48 hr incubation
Requirement of 19-mer arms of GSOs in vivo

19-19 mer

19-15 mer

19-25 mer

% Inhibition of induced IL-12

0
-25
-50
-75
-100

19-19 mer

19-15 mer

19-25 mer

GSO, s.c. 24 hr

TLR Agonist, s.c. 2 hr

Serum IL-12 Analysis
Mismatches in GSO sequence result in loss of activity

X = mismatch nucleotide

J774 cells: 10 µg/ml, 48 hr incubation
Effect of Presence of 5’-phosphate on the Activity of GSO

One phosphate

Two phosphates

% Inhibition of MyD88 RNA

One phosphate

Two phosphate

J774 cell culture, 48 hr incubation
Do the 19-mer Arms Need to Be the Same

DNA/DNA GSO

RNA/DNA GSO

2’-OMe RNA/2’-OMe RNA GSO

% Inhibition of MyD88 RNA

0
-25
-50
-75
-100

DNA/DNA GSO
RNA/DNA GSO
2’-OMe RNA/2’-OMe RNA GSO

J774 cell culture, 48 hr incubation
Length Dependent In Vivo Activity of GSO

Length of MyD88 GSO

% Inhibition of IL-12 induction

0 17-mer 19-mer 21-mer 23-mer 25-mer

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GSO has greater activity than ASO in Vivo

% Inhibition of IL-12 induction

Dose, mg/kg

0 5 10 15

0 0 40 80 120

GSO, s.c. 24 hr TLR Agonist, s.c. 2 hr Serum IL-12 Analysis
Comparative Duration of Activity of GSO and ASO in vivo

% Inhibition of IL-12 induction

Post Administration, Days

GSO, s.c. 24 hr  TLR Agonist, s.c. 2 hr  Serum IL-12 Analysis
GSOs Mitigate Immune Responses

- Examples of Sequences Evaluated

<table>
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<tr>
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<th>Sequence</th>
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<tr>
<td>Bcl2 antisense</td>
<td>5’-TCTCCCA\text{G}C\text{G}TG\text{G}GCCAT-3’</td>
</tr>
<tr>
<td>GSO</td>
<td>3’-TACCG\text{G}C\text{G}TCG\text{A}CCCTCTX-TCTCCCA\text{G}C\text{G}GCCAT-3’</td>
</tr>
<tr>
<td>H-ras antisense</td>
<td>5’-TC\text{G}TC\text{C}AT\text{C}GCTCCTC\text{A}GG-3’</td>
</tr>
<tr>
<td>C-raf antisense</td>
<td>5’-TCC\text{G}C\text{C}CT\text{G}TG\text{A}CAT\text{G}CAT-3’</td>
</tr>
<tr>
<td>PKC-a antisense</td>
<td>5’-GTTCT\text{C}GCT\text{G}GT\text{G}AGTTTC-3’</td>
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GSOs Mitigate Immune Responses

**Serum Cytokine Analysis**

10 mg/kg GSO or Antisense, s.c.

2 hr

**Cytokine/Chemokine, pg/ml**

- **bcl2**
  - PBS
  - Antisense
  - GSO

- **H-ras**
  - PBS
  - Antisense
  - GSO

- **C-raf**
  - PBS
  - Antisense
  - GSO

- **PKC-α**
  - PBS
  - Antisense
  - GSO

**Cytokines and Chemokines**:
- IL-1α
- IL-1β
- IL-2
- IL-4
- IL-5
- IL-6
- IL-12
- IP-10
- KC
- MCP-1
- MIG
- MIP-1α
- TNF-α
### GSOs Targeted to ApoB

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>4</th>
<th>8</th>
<th>11</th>
<th>15</th>
<th>18</th>
<th>22</th>
<th>29</th>
<th>36</th>
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<th>- GSO</th>
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- Collect blood for plasma cholesterol assessment
- Sacrifice mice for liver mRNA and protein levels, and blood for plasma cholesterol
- Collect blood for plasma cholesterol recovery assessment

LDL-c

![Diagram of cholesterol metabolism and progression](image_url)
GSOs Targeted to ApoB

Suppression of ApoB mRNA

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<th>GSO, mg/kg</th>
<th>% Change in liver mRNA expression compared to PBS control</th>
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<tr>
<td>7.5</td>
<td>53%</td>
</tr>
<tr>
<td>15</td>
<td>70%</td>
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* P < 0.05 vs PBS

Suppression of ApoB Protein

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<th>% Reduction of ApoB 100 protein</th>
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<td>PBS</td>
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<tr>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>15.0</td>
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* * P < 0.05 vs PBS
GSO Targeted to ApoB

Reduction of Cholesterol

PBS
7.5 mg/kg
15 mg/kg

* P < 0.05 vs PBS
GSO is Specific for ApoB mRNA

At day 22

% Change in mRNA expression compared to PBS control

15 mg/kg dose group
Anti-miR-21 GSO specifically silences target micro RNA

HCT-116 cells
Design of Gene-Silencing Oligo (GSO)

- Synthetic DNA or RNA
- Phosphorothioate modification provides stability
- Systemic delivery without lipid formulation
- TLR-mediated immune response is mitigated due to 5' end inaccessibility
- 19 and 21-mer show optimal gene-silencing activity

Similar cleavage product as siRNA
## Gene Silencing Technologies

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Acknowledgements

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