# Generic Oligonucleotides: Challenges and Opportunities

Likan Liang, Ph.D.

*Division of Product Quality Assessment X*
*Office of Product Quality Assessment II, OPQ, CDER*

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Everyone deserves confidence in their *next* dose of medicine.

**Pharmaceutical quality** assures the availability, safety, and efficacy of *every* dose.

# Disclaimer

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*The author declares no competing financial interest.*

# Oligonucleotide (ON) Drug Products

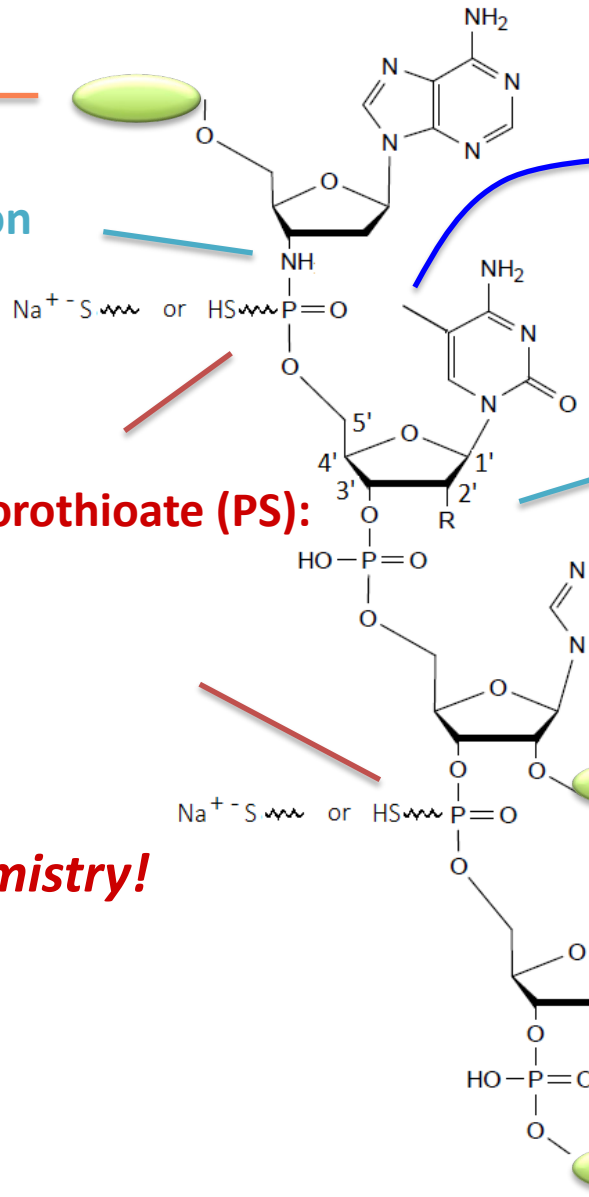
- Short strings of synthetic or semisynthetic nucleic acids with therapeutic effects
  - Potential therapies for broad range of diseases (including fatal, rare, “undruggable”)
- Nucleic acid structures
  - Unmodified (e.g., Defibrotide)
  - Modified (most CDER-approved ONs)

# Some Modifications of ON

5’-end conjugation
(e.g., PEG, fatty acid)

3’ modification

Base modification (e.g., methyl-cytosine)




Nucleotide linkage modification:
(e.g., non-stereospecific phosphorothioate (PS):
*R* and *S* ratio @ each PS.
n x PS: PS diastereomers = $2^n$

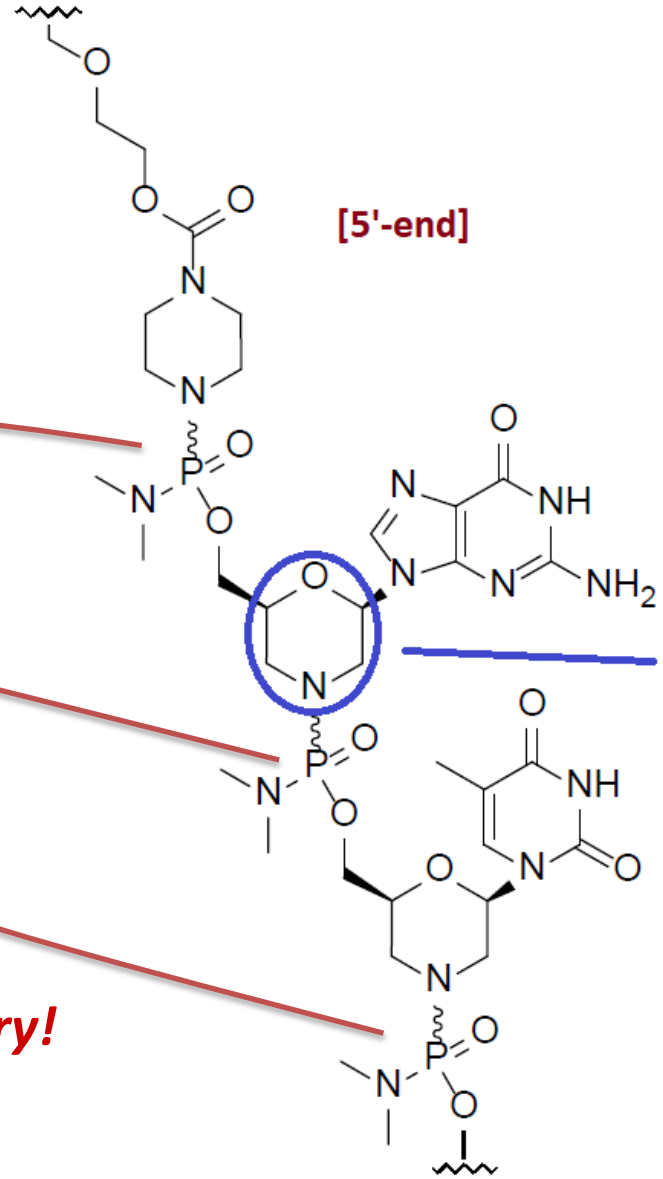
2’ modification
(R = F, O-methyl,
O-methoxyethyl, etc.)

2’ conjugation (e.g., N-acetylgalactosamine (GalNAc))

***Complexity of ON Stereochemistry!***

3’-end conjugation
(e.g., GalNAc)

# Some Modifications of ON (cont.)




**Nucleotide linkage modification:**
**(e.g., non-stereospecific phosphorodiamidate (PDA):**
***R* and *S* ratio @ each PDA**
**n x PDA: PDA diastereomers = $2^n$**

Morpholino replacement of ribose

***Complexity of ON Stereochemistry!***

# CDER-Approved Oligonucleotide Drug Products

| Type of ON | CDER-Approved ONs [a] | Length of ONs | Modifications [c] | Possible Nucleotide Linkage Diastereomers [d] |
|---|---|---|---|---|
| Antisense Oligonucleotides (ASO) | Fomivirsen (Vitravene)[b] | 21 mer | **20*PS** | > 1 million PS diast. |
| | Mipomersen (Kynamro)[b] | 20 mer | **19*PS**, 10*2'-MOE, 9*Me-C | > Half million PS diast. |
| | Eteplirsen (Exondys 51) | 30 mer | **30*PDA**, 30*morpholino | > 1 billion PDA diast. |
| | Nusinersen (Spinraza) | 18 mer | **17*PS**, 4*Me-C | > 130,000 PS diast. |
| | Inotersen (Tegsedi)[b] | 20 mer | **19*PS**, 10*2'-MOE, 5*Me-C | > Half million PS diast. |
| | Golodirsen (Vyondys 53) | 25 mer | **25*PDA**, 25*morpholino | > 33 million PDA diast. |
| | Viltolarsen (Viltepso) | 21 mer | **20*PDA**, 21*morpholino | > 1 million PDA diast. |
| | Casimersen (Amondys 45) | 22 mer | **22*PDA**, 22*morpholino | > 4 million PDA diast. |
| | Tofersen (Qalsody) | 20 mer | **15*PSH**, 10*2'-MOE, 5*Me-C | > 32,000 PSH diast. |
| | Eplontersen (Wainua) | 20 mer | **13*PS**, 10*2'-MOE, 5*Me-C, GalNAc | > 8,000 PS diast. |
| | Olezarsen (Tryngolza) | 20 mer | **19*PS**, 10*2'-MOE, 5*Me-C, GalNAc | > Half million PS diast. |
| siRNA (sense \|\| antisense) | Patisiran (Onpattro) | 21 mer \|\| 21 mer | 9*2'-M\|\|2*2'-M | N/A |
| | Givosiran (Givlaari) | 21 mer \|\| 23 mer | **2*PS**, 16*2'-M, 5*2'-F, GalNAc \|\| **4*PS**, 12*2'-M, 11*2'-F | 4 PS diast. \|\| 16 PS diast. |
| | Lumasiran (Oxlumo) | 21 mer \|\| 23 mer | **2*PS**, 17*2'-M, 4*2'-F, GalNAc \|\| **4*PS**, 17*2'-M, 6*2'-F | 4 PS diast. \|\| 16 PS diast. |
| | Inclisiran (Leqvio) | 21 mer \|\| 23 mer | **2*PS**, 18*2'-M, 2*2'-F, GalNAc \|\| **4*PS**, 13*2'-M, 10*2'-F | 4 PS diast. \|\| 16 PS diast. |
| | Vutrisiran (Amvuttra) | 21 mer \|\| 23 mer | **2*PS**,17*2'-M,4*2'-F,GalNAc \|\| **4*PS**, 18*2'-M, 5*2'-F | 4 PS diast. \|\| 16 PS diast. |
| | Nedosiran (Rivfloza) | 36 mer \|\| 22 mer | **1*PS**,23*2'-M,9*2'-F,4*GalNAc \|\| **5*PS**, 12*2'-M, 10*2'-F | 2 PS diast. \|\| 32 PS diast. |
| | Fitusiran (Qfitlia) | 21 mer \|\| 23 mer | **2*PS**,9*2'-M,12*2'-F,GalNAc \|\| **4*PS**, 14*2'-M, 9*2'-F | 4 PS diast. \|\| 16 PS diast. |
| Aptamers, Other Inhibitors | Pegaptanib (Macugen)[b] | 28 mer | PEG, 12*2'-M, 13*2'-F | N/A |
| | Avacincaptad pegol (Izervay) | 39 mer | PEG, 14*2'-M, 21*2'-F | N/A |
| | Imetelstat (Rytelo) | 13 mer | **13*PS**, 13*3'-amino, 1*fatty acid | > 8,000 PS diast. |
| Polydisperse Oligonucleotide | Defibrotide (Defitelio) | Mixture ( ~4 mer to ~100 mer) | (Predominantly single-stranded polydeoxyribonucleotide, derived from porcine intestinal tissue) | N/A |

[a] As of 4/30/2025 [b] Discontinued. [c] Based on publicly available information; 2'-F = 2'-fluoro; 2'-M = 2'-O-methyl; 2'-MOE = 2'-O-methoxyethyl; Me-C = methyl cytosine; PDA = phosphorodiamidate; PS = phosphorothioate; PSH = phosphorothioate, labeled as free acid form. [d] Derived from publicly available information; diast. = diastereomers

# Generic Oligonucleotides: RLD-Comparative Evaluations

- Active ingredient sameness
  - Sequence, chemical structures (e.g., correctness of all modifications/stereochemistries), composition (e.g., diastereomeric composition), potency, and activity where necessary, etc.
- Physicochemical characteristics
  - Higher order structures/aggregation in drug product
- Safety/quality considerations
  - Impurity profiles and levels
  - Risks in immunogenicity, inflammation, other potential risks associated with ON (e.g. off-target effects), etc.

# Generic ON Challenges: Assessing Diastereomeric Composition Sameness/Manufacturing Consistency

- Large number of diastereomers in most CDER-approved ONs
- Limitation of current analytical tools
  - Limited diastereomer resolution power + co-eluting impurities interference
    - Current siRNAs – possibility in quantifying individual diastereomers in composition
    - Current ASOs – individual diastereomers not distinguishable in distribution curves
  - Sensitivity towards composition changes
    - Use of multiple suitability test standards with intentional small perturbations in the composition (e.g. altering R/S ratios at various stereocenters)
- Product Specific Guidance (PSG) recommendations:
  - Use of multiple orthogonal methods when necessary
  - Evaluate R/S ratios at individual relevant stereocenters during synthesis
- Complexity in sameness assessment criteria

# Generic ON Challenges: Impurities

- Complexity of possible ON-related impurities
- Limitation of current analytical tools in resolving ON impurities → co-eluting impurities (particularly isobaric and isomeric impurities)
  - Use orthogonal and/or complementary methods.
  - Coupled with high resolution mass spectrometry (HR-MS) or other advanced technologies
- Potential differences in generic impurity profile/levels *vs* RLD
  - In depth RLD-comparative studies
- Comprehensive risk assessment and RLD comparison to support quality proposals (e.g., grouping, thresholds, limits, etc.)

# Generic ONs: Progress and Opportunities

- Research are being conducted to address some challenges such as diastereomer characterization methods and comparative impurity profiling methods to facilitate generic ON development and assessment
- Opportunities for innovations
  - Develop innovative analytical methodologies to address limitations in ON analysis
  - Explore computational approaches to aid sameness evaluation
  - Investigate novel strategies to address complex sameness, quality, and safety requirements

# Acknowledgement


- Various oligonucleotide ANDA/pre-ANDA review teams
- Various oligonucleotide PSG development and review teams
- PSG development oligonucleotide SME triage team
- OPQ/OPQR oligonucleotide research teams

# Questions?