Oligonucleotides: Current Thinking and Analytical Challenges Identified in the Nusinersen PSG Development

SBIA 2022: Advancing Generic Drug Development: Translating Science to Approval

Day 1, Session 1B: Oligonucleotide Active Pharmaceutical Ingredient (API) Sameness and Impurity Assessment Considerations

Deyi Zhang, PhD

Division of Therapeutic Performance I, Office of Research and Standards,
Office of Generic Drugs

CDER | U.S. FDA

September 20, 2022

Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA's views or policies

Learning Objectives

- Describe oligonucleotide-based therapeutics and the regulatory challenges
- Understand Agency's thinking in the development of product-specific guidance (PSG) on nusinersen
- Describe analytical challenges in oligonucleotide characterization

Oligonucleotide-based therapeutics

Oligonucleotide-Based Therapeutics

- Oligonucleotides: Nucleic acid polymer chains that can act in a sequence specific manner to control gene expression
- Therapeutic oligonucleotides exert their effect through suppression of, or interference with mRNA translation, immune stimulation, protein binding, or through induction of exon skipping
- Therapeutic oligonucleotides can target a broad range of mRNAs (encode all cellular proteins) including those protein targets considered “undruggable” by small molecule or protein therapeutics

Oligonucleotide-Based Therapeutics

- Oligonucleotide-based therapeutics include:
  - Antisense oligonucleotides (ASOs)
  - Small interfering RNAs (siRNAs)
  - Small hairpin RNAs (shRNAs)
  - Anti-micro RNAs (anti-miRNAs)
  - Aptamers
  - Others (messenger RNAs, etc.)
- Synthetic oligonucleotides: Are regulated as drugs by CDER, FDA
- Vector-based or promotor-driven oligonucleotides: Are regulated as biologics by CBER, FDA

FDA Approved Synthetic Oligonucleotide Drugs

Proprietary name	Active ingredient	Category	Length of Oligonucleotide
VITRAVENE	Fomivirsen sodium	Phosphorothioate ASO	21
MACUGEN	Pegaptanib sodium	Phosphate oligonucleotide aptamer	28
KYNAMRO	Mipomersen sodium	Phosphorothioate ASO	20
EXONDYS 51	Eteplirsen	Phosphorodiamidate morpholino ASO	30
SPINRAZA	Nusinersen sodium	Phosphorothioate ASO	18
ONPATTRO	Patisiran sodium	Double-stranded siRNA	19+2 (antisense)
TEGSEDI	Inotersen sodium	Phosphorothioate ASO	20
GIVLAARI	Givosiran sodium	Double-stranded siRNA	21+2 (antisense)
VYONDYS 53	Golodirsen	Phosphorodiamidate morpholino ASO	25
VILTEPSO	Viltolarsen	Phosphorodiamidate morpholino ASO	21
OXLUMO	Lumasiran	Double-stranded siRNA	21+2 (antisense)
AMONDYS 45	Casimersen	Phosphorodiamidate morpholino ASO	22
LEQVIO	Inclisiran	Double-stranded siRNA	21+2 (antisense)
AMVUTTRA	Vutrisiran	Double-stranded siRNA	21+2 (antisense)

Antisense Oligonucleotide Drugs

- Antisense oligonucleotides (ASO) are small pieces of synthetic oligonucleotides, generally 12-30 nucleotides in length that can bind to specific molecules of RNA by Watson-Crick base pairing rules
- We will focus mainly on ASO, but the discussions are generally applicable to siRNAs

Regulatory Challenges on Oligonucleotides

- No ICH* or FDA guidelines that specifically address the quality aspect/expectations for oligonucleotide drugs
- No consensus on impurity reporting, identification and qualification thresholds
- Impurity characterization:
  - Most impurities exist as mixtures of closely related molecules
  - Many impurities coelute with the active ingredient
  - Lack of analytical methods to adequately resolve impurities
- Additional challenges for generic oligonucleotide drug development

*ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Current thinking in developing product-specific guidance on nusinersen

Product-Specific Guidances

- Started in 2007, PSGs provide the Agency's current thinking and expectations on how to develop generic drug products that are therapeutically equivalent to a specific reference listed drug
  - PSGs are posted on a quarterly basis and as of June 2022, there are 2003 posted PSGs.[1]
  - In GDUFA II (FY 2018-2022), FDA committed to posting a PSG for complex products as soon as scientific recommendations are available.[2]
  - For GDUFA III (FY 2023-2027), FDA has agreed to posting a PSG for complex products approved on or after October 1, 2022, 50% two years after NDA approval and 75% three years after NDA approval.[3]
- Developing PSGs for oligonucleotides will be critical for generic oligonucleotide drug development

1. FDA Website: Product-Specific Guidances for Generic Drug Development (https://www.accessdata.fda.gov/scripts/cder/psg/)
2. GDUFA II commitment letter (https://www.fda.gov/media/101052/download)
3. GDUFA III commitment letter (https://www.fda.gov/media/153631/download)

Nusinersen for PSG development

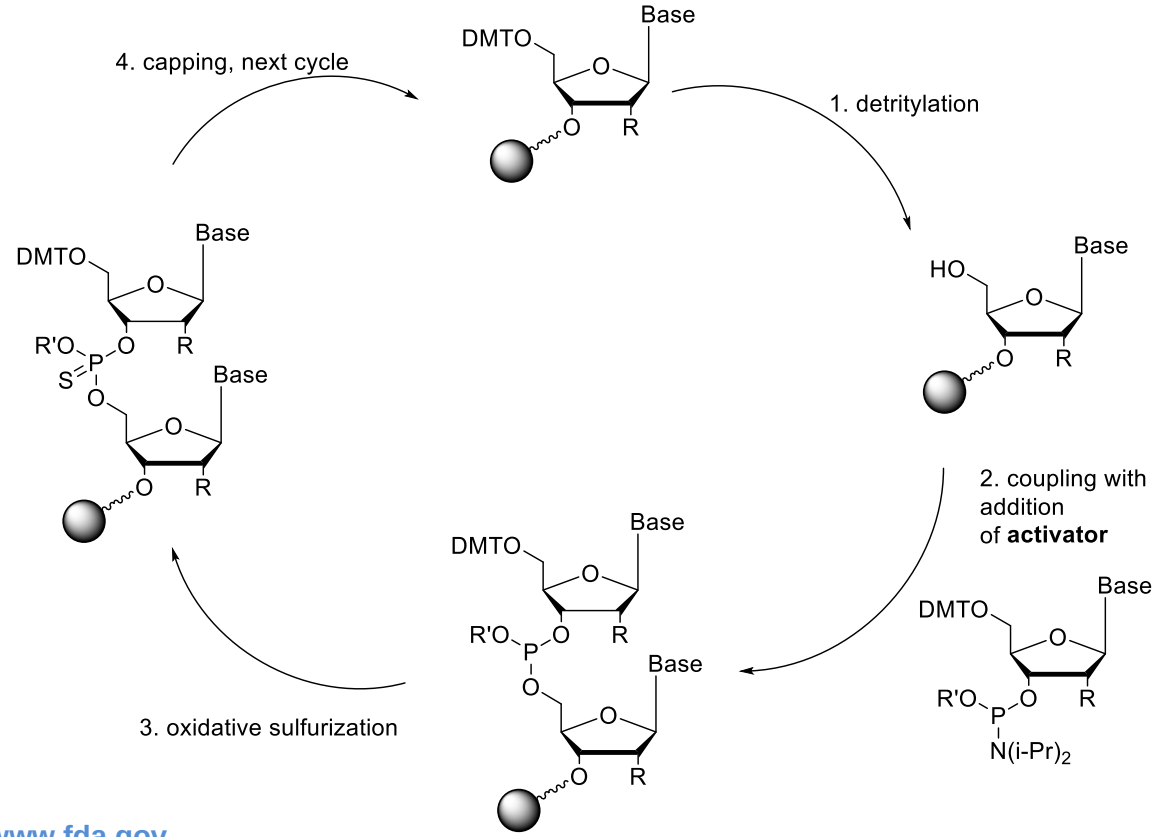
- One of the first oligonucleotides drugs approved that is still on the market
- Approved in 2016 for the treatment of spinal muscular atrophy
- Belongs to phosphorothioate oligonucleotide family
- Serves as an example for other approved ASOs
- SPINRAZA (nusinersen) is very expensive (~130K$/5 mL)

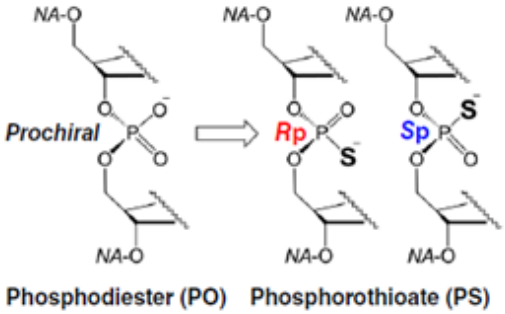
* https://www.accessdata.fda.gov/scripts/cder/psg/

Key Considerations in PSG Development

- API sameness recommendations
- Considerations on impurity profile assessment
  - — product-related impurities
  - — immunogenicity risk assessment

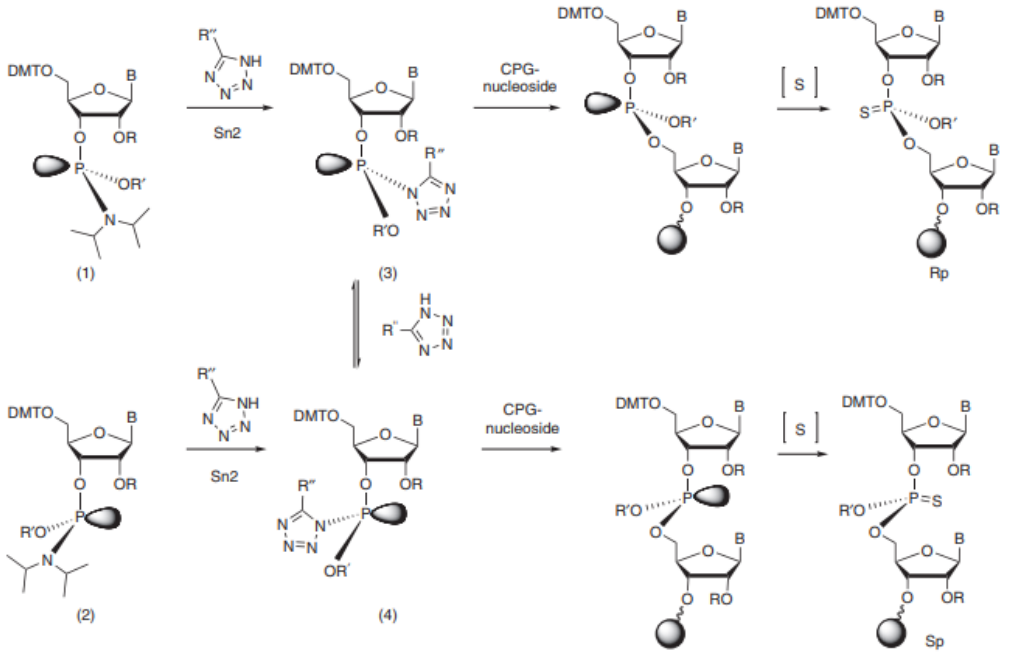
Solid Phase Synthesis of PS Oligonucleotides





PS: phosphorothioate

Solid Phase Synthesis of PS Oligonucleotides



- The product diastereomeric ratio is independent of the starting material configuration
- Activators affect the product diastereomeric ratio

Adapted from Jahns H et al *Nat Comm*, 2015, 6, 6317

Considerations for API Sameness

1. Equivalence in primary sequence, chemical structure and diastereomeric composition
   - Phosphorothioate (PS) stereochemistry affects pharmacologic properties of ASO*
   - Reaction conditions including activator selection affect PS stereochemical outcomes during ASO synthesis**
   - Employ a broad range of orthogonal analytical methods with sufficient sensitivity, discriminating and resolving power

*Iwamoto N, et al *Nat Biotechnol*. 2017, 35(9), 845-851
**Ravikumar V et al *Org. Proc. Res. Dev*. 2002, 6(6), 798-806

Characterization of Nusinersen

- Possible analytical methods/tools to explore:
  - Mass spectrometry (MS), including tandem MS (MS/MS)
  - Nuclear magnetic resonance (NMR) spectroscopy (^{1}H, ^{13}C and ^{31}P)
  - Liquid chromatography (LC)
  - Duplex melting temperature to a complementary strand

Considerations for API Sameness

2. Equivalence in physicochemical properties
   - Including aggregation state or higher order structure of the API in the product
   - Methods could include:
     - Circular dichroism (CD) spectroscopy
     - Differential scanning calorimetry (DSC)
     - Size exclusion chromatography (SEC)
     - Sedimentation velocity analytical ultracentrifugation (SV-AUC)

Impurities: Comparative Evaluation

To ensure impurities in the proposed generic nusinersen product will not alter the safety (including the immunogenicity) and efficacy compared to the reference listed drug (RLD) product

- Very complicated. Contact Office of Generic Drugs (OGD) (e.g., Pre-ANDA meeting) for questions related to generic nusinersen development including questions on immunogenicity and inflammation risk assessment, and comparability of impurities in the proposed generic product

Impurities: Things to Consider

- Impurity characterization:
  - Use of a range of suitable orthogonal methods for analyzing impurities, including those co-eluting with the API
  - If impurity levels can be controlled at or below those in the RLD
  - Criteria and justification for grouping impurities
- Immunogenicity risk assessment
  - Local inflammation and/or thrombocytopenia
  - Immunomodulatory effect

Analytical challenges in oligonucleotide characterization

Analytical Challenges

- ASO API typically is a mixture of huge number of diastereomers
  - Nusinersen: 2^{17} = 131,072 diastereomers
  - Full characterization of API, including diastereomeric composition, is challenging
- Huge number of impurities with diverse structures
  - Deletion/addition impurities: n-1, n-2, n+1, n+2, etc. Each group contains many different impurities
  - P=O impurities; base residue changes; Abasic sites; sugar moiety changes
  - Especially challenging:
    - Co-eluting impurities (mono-P=O, modified full length, n-1, n+1)
    - Isobaric impurities (deamination products: C to U; ^{Me}C to T)
    - Quantification of overlapping impurities

Analytical Challenges

- Constant trade-off between chromatographic resolution and mass spectrometry sensitivity
  - Liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) are important analytical tools
  - Ion pair reversed phase chromatography (IP-RP) as LC method of choice for oligonucleotides, quite often causes MS signal suppression due to ion pair regents used in the system
  - Hydrophilic interaction liquid chromatography (HILIC)-MS is an attractive alternative method, however, chromatographic resolution needs further improvement

Challenge Question #1

Among the different categories of synthetic oligonucleotide drugs approved by FDA, which category of is NOT one of them:

A. Antisense oligonucleotides (ASOs)

B. Aptamers

C. Small hairpin RNAs (shRNAs)

D. Small interfering RNAs (siRNAs)

Challenge Question #2

Which is NOT one of the analytical challenges we talked about for Oligonucleotide drug characterization?

A. ASO drug substance contains huge number of diastereomers

B. Oligonucleotides are big molecules, and are not soluble in aqueous media

C. Many impurities are co-eluting with each other in liquid chromatography

D. Ion pair reversed phase chromatography (IP-RP) often causes MS signal suppression

Summary

- Oligonucleotides are a class of new therapeutics that offer promising treatment solutions to a broad range of diseases. They also present unique scientific and regulatory challenges
- Product-specific guidance on nusinersen was developed to facilitate the generic development of this drug product
- Analytical challenges were identified including diastereomeric composition analysis in API characterization and product-related impurity analysis and quantification. Further research is needed in this area

Acknowledgement

Office of Generic Drugs

Rob Lionberger
Lei Zhang
Darby Kozak
Yan Wang
Utpal Mondal

Office of Pharmaceutical Quality

Andre Raw
Bing Cai
Pahala Simamora
Cameron Smith
Likan Liang
Mohan Sapru
Kui Yang
Yili Li
Daniela Verthelyi

FDA Oligonucleotide Working Group (WG) Members


FDA
U.S. FOOD & DRUG
ADMINISTRATION