Emerging Generic Oligonucleotides - Challenges and Progress

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Learning Objectives

- Discuss the complexity of generic oligonucleotides and RLD-equivalence considerations
- Review key challenges in demonstrating sameness and potential approaches
- Address comparability of oligonucleotide impurity profiles and impurity controls
- Describe Agency's efforts and progress in addressing challenges in generic oligonucleotide sameness and impurity controls

Oligonucleotide (ON) Therapeutics

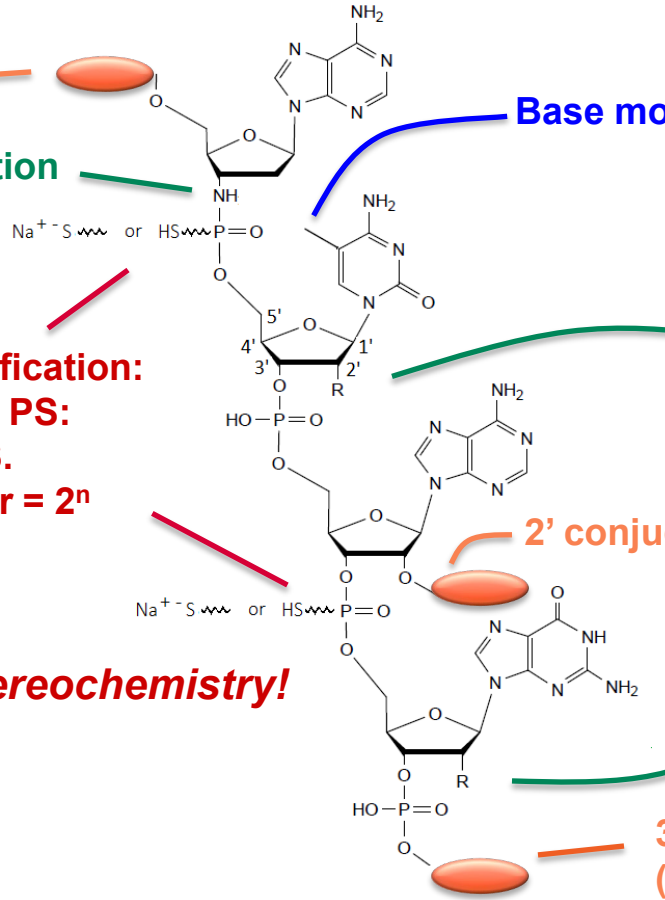
- Short strings of nucleic acids with therapeutic effects
  - Potential therapy for broad range of diseases (including fatal, rare, “undruggable”)
- Fully synthetic or semisynthetic
  - Unmodified (e.g., Defibrotide)
  - Modified (most of CDER-approved ONs)

Some Modifications of ON

5’-end conjugation
(e.g., PEG)

3’ modification

Base modification (e.g., pyrimidines)



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

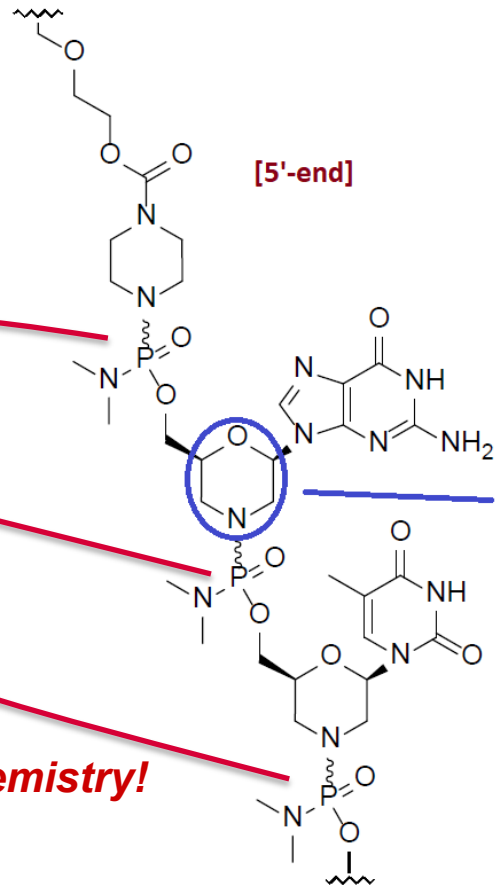
2’ modification
(R = F, O-methyl,
O-methoxyethyl, etc.)

2’ conjugation (e.g., GalNAc)

Complexity of ON Stereochemistry!

3’-end conjugation
(e.g., GalNAc)

Some Examples of ON Modifications – cont.



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

Complexity of ON Stereochemistry!

CDER-Approved ON Drug Products

Type of ON	CDER-Approved ONs (as of 6/30/2024)	Some Noted Complexity (based on RLD labeling)
Antisense Oligonucleotides (ASO)	Fomivirsen (Vitravene, withdrawn by sponsor) Mipomersen (Kynamro) Eteplirsen (Exondys 51) Nusinersen (Spinraza) Inotersen (Tegsedi) Golodirsen (Vyondys 53) Viltolarsen (Viltepso) Casimersen (Amondys 45) Tofersen (Qalsody) Eplontersen (Wainua)	> 1 million PS diastereomers (diast.) > Half million PS diast. > 1 billion PDA diast. > 130,000 PS diast., Ca/Mg > Half million PS diast. > 33 million PDA diast. > 1 million PDA diast. > 4 million PDA diast. > 32,000 PSH diast., Ca/Mg > 8,000 PS diast.
siRNA (sense|| antisense)	Patisiran (Onpattro) Givosiran (Givlaari) Lumasiran (Oxlumo) Inclisiran (Leqvio) Vutrisiran (Amvuttra) Nedosiran (Rivfloza)	ON in lipid complex 4 || 16 PS diast. 4 || 16 PS diast. 4 || 16 PS diast., other considerations 4 || 16 PS diast. 2 || 32 PS diast.
Aptamer	Pegaptanib (Macugen) Avacincaptad pegol (Izervay)	Conjugated Aptamer Conjugated Aptamer
Polydisperse Oligonucleotide	Defibrotide (Defitelio)	Polydisperse mixture, predominately single strand
Other - Inhibitors	Imetelstat (Rytelo)	> 8,000 PS diast.

TE Requirements – Applied to Generic Oligonucleotides

- Therapeutic equivalents (TE), 21CFR314.3
  - Pharmaceutical equivalents (PE)
    - Identical dosage forms, route(s) of administration
    - Identical amounts of identical active drug ingredient, …
    - Meet the identical compendial or other applicable standard of identity, strength, quality, and purity, including potency and, …
  - Bioequivalence (BE)
  - Same clinical effect and safety profile for the conditions of use specified in the labeling

Generic Oligonucleotides

- Active ingredient sameness
  - Sequence, chemical structure, composition (diastereomers, etc.), higher order structures, potency/activity, etc.
- Physicochemical characteristics
- Safety profile and quality
  - Impurities
    - Off-target effect, immunogenicity, inflammatory, general toxicity, etc.

Stereochemistry in Generic ONs

ON binding to target: largely sequence-specific

Why diastereomeric composition sameness then?

- Clinical evidence not available to confidently negate stereochemistry impact
- Some evidences - stereochemistry/conformation matters!
  - Mipomersen vs stereochemically pure isomer (Iwamoto *et al**)
  - Locked nucleic acids
- All approved ON RLDs so far counted all diastereomers, where applicable, as active ingredient

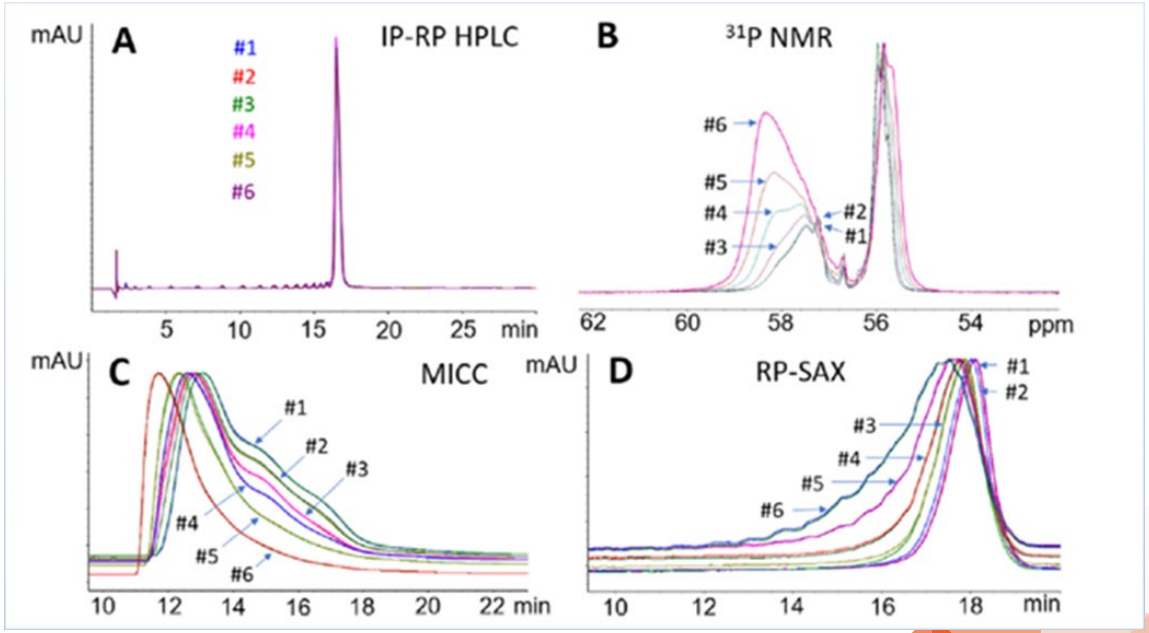
* Iwamoto, N. et al, *Nature Biotechnology* 35 (9) (2017) 845

Challenges for Generic ONs - Diastereomers

High diastereomer numbers vs resolving power / sensitivity / ability of a single method

#	DCI/ETT
1	19:0
2	18:1
3	14:5
4	9:10
5	4:15
6	0:19

> half million diastereomers in each sample



Diastereomeric Composition Sameness Considerations

- Check product specific guidance (PSG)
- Use multiple orthogonal methods
  - e.g.: Multiple distribution profiles (orthogonal separation) + spectroscopic methods + other characterizations
  - Sensitivity, specificity, resolving power
- If diastereomer # is low, develop methods to resolve each (from other diastereomers and impurities)

Diastereomeric Composition Sameness Considerations – cont.

- Demonstrate method sensitivity
  - Multiple suitability test standards (changing R/S ratios at diverse set of small number of chiral centers)
  - Include them in all diastereomer methods validations
- Diastereomer distribution profile comparison
  - Overall and individual peaks/regions
- Justify your generic ON approaches and criteria – ensure comparable efficacy & safety as RLD

Diastereomeric Composition Consistency

- Know more about your own product's baseline!

  (for manufacturing consistency, future changes)

  Measure R/S ratio of each newly created chiral center after its elongation during synthesis

<u>This may be applied to both new drugs and generics of ON w/ complex diastereomers</u>!

Challenges for Generic ONs - Impurities

- Diverse, very high number of impurities
- Relatively large molecular sizes
- Limited resolving power of a single method
- Challenges compounded by diastereomer situations in many cases

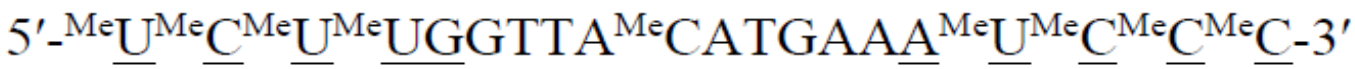
Current Regulatory Landscape for Generic ON Impurities Controls

- ON excluded in ICH guidelines
- FDA has not published guidance about ON impurity thresholds
- FDA has not recommended ON impurities classification for safety evaluation and quality control purposes for generic ONs.

ON Impurities Characterization

Rule of thumb: RLD-comparison

- Use multiple orthogonal analytical methods
- Compare with RLD – determine nature & location of structural changes in impurities



  - If any ambiguity, perform comprehensive risk assessment that includes all possible impurities

Generic ON Impurities Control

Rule of thumb: RLD-comparison

- For higher impurities levels, new impurities, proposed thresholds, specification grouping:
  - Comprehensive risk assessment (off-target effect, immunogenicity, inflammatory, general toxicity, any other risks associated with ONs) or qualification information
  - Include all possible/relevant impurities in risk assessment for thresholds/grouping based on process understanding
  - Justification with adequate supporting information

RLD-Comparative Evaluations

Active ingredient sameness, characterization, impurity profiling

- Drug substance: 3 batches each - test API, API from RLD
  - Sample processing should not change diastereomeric composition
  - Subject test API to the same sample processing as RLD (?)
- Drug product: 3 batches each

RLD-Comparative Evaluations – cont.

Sameness, characterization, impurity profiling

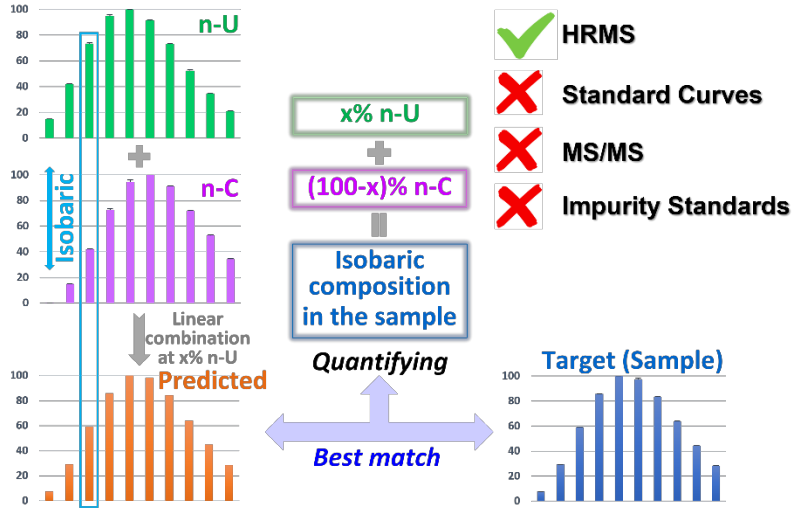
- At release and end of proposed shelf-life
  - Three exhibit batches
  - For sameness/impurity profiling initial filing ONLY: May use a development batch as supporting data for end of shelf-life consideration, if equivalent to exhibit batch in ALL CMC aspects. Additional data may be requested.
- Intrinsic property characterization - formulation impact and elimination - Tm

Some of CDER's Efforts to Address Generic ON Challenges

- OGD/OPQ collaboration in PSG development
- Pre-ANDA product development meetings
- Controlled correspondences
- OPQ's internal research
- OGD's external research grants
- Outreach programs

Selected Highlights of OPQ's Research for Generic ONs

- Impurity profiling – LC-HRMS
  - Quantifying coeluting (LC-inseparable) isobaric (MS-inseparable) impurities



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Decoding Complexity in Synthetic Oligonucleotides: Unraveling Coeluting Isobaric Impurity Ions by High Resolution Mass Spectrometry

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Challenge Question #1

ON impurities have much higher Mw than impurities of small molecule drugs. The molar amounts of ON impurities are lower. Therefore, ON impurities should generally be given higher w/w% limits than impurities of small molecule drugs.

A. True

B. False

Challenge Question #2

Which of the following statements is true?

A. Oligonucleotide binding is sequence-specific. Stereochemistry of the ON modifications need not to be considered in sameness evaluation.

B. I can use three development batches of generic ON product for sameness evaluation and characterization for ANDA submission, even if they are not true representation of the exhibit batches.

C. For complex sameness or comparative impurity profile evaluation, I can use multiple orthogonal analytical methods that give quite different and detailed distribution curves/profiles on the same sample.

D. Currently I can justify my quality control of impurities in generic ON based on literature information about ON impurities classification without comparing them to impurities of the RLD.

Summary

- RLD comparison is the rule of thumb for generic ONs.
- When unambiguous conclusion for generic ON cannot be obtained by a single method, use multiple orthogonal methods.
- Comprehensive risk assessment may help justify proposed solutions to generic ON challenges.
- PSG, pre-ANDA, controlled correspondence, and other public outreach programs are some of the ways one can obtain assistance from the Agency.