

# CMC Challenges in FDA-Approved Synthetic Oligonucleotide Drugs

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# Disclaimer



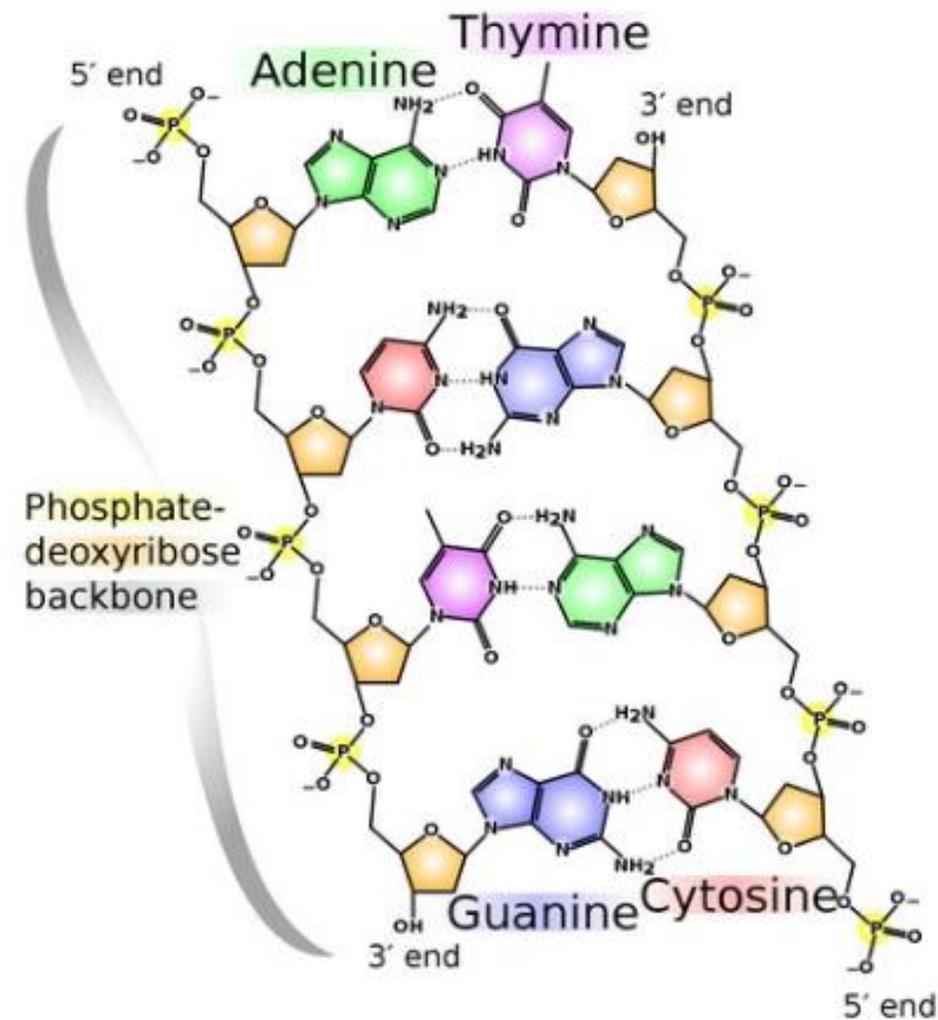
This presentation reflects the views of the author and should not be considered to represent the FDA's views or policies

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

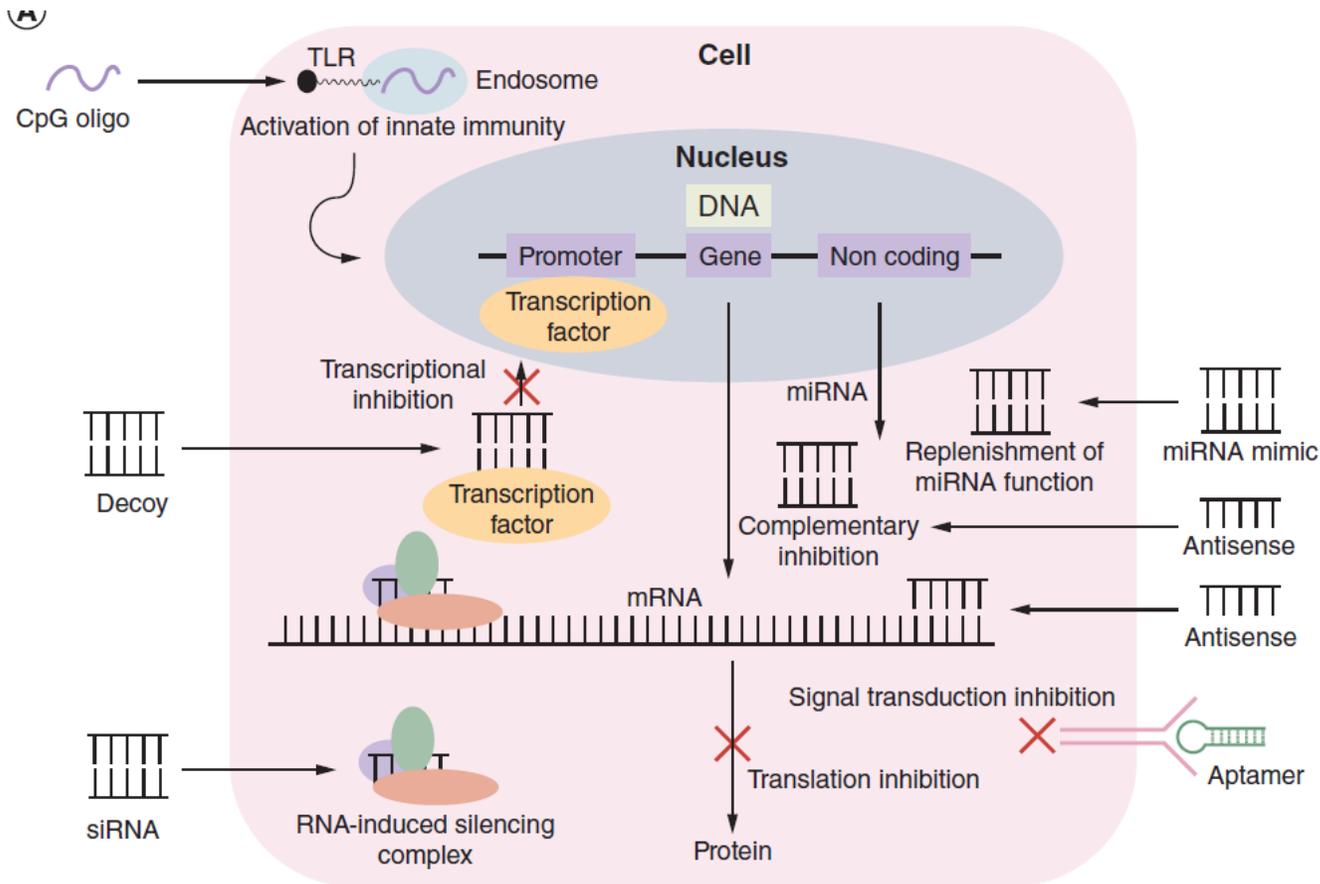
**Pharmaceutical quality**  
assures the  
availability,  
safety,  
and efficacy  
of *every* dose.

# What are Synthetic Oligonucleotides?

- Modified nucleic acids designed to bond to a complimentary DNA/RNA sequence and sometimes a protein
- Effects typically mediated by Watson-Crick base-pairing
- Manufacturing process involves synthetic organic chemistry and is not cell-based
- In CDER/FDA, synthetic oligos are evaluated under an NDA.



# Modes of Action for Synthetic Oligos

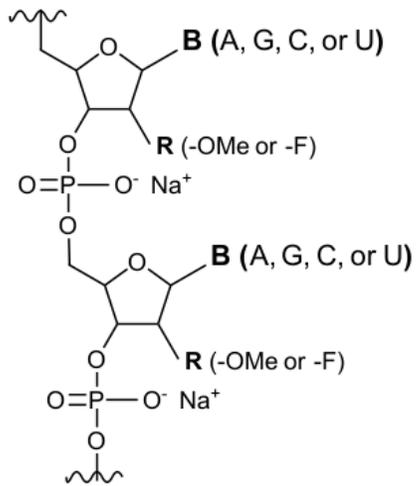
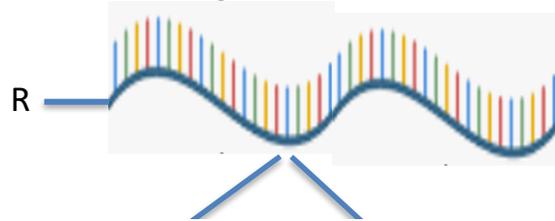


- For the biological effects of synthetic oligonucleotides, several different pathways have been investigated.
  - Overall goal is to affect downstream protein activity
- Pathways that have had commercial success are mRNA degradation, translational regulation and RNA-induced silencing

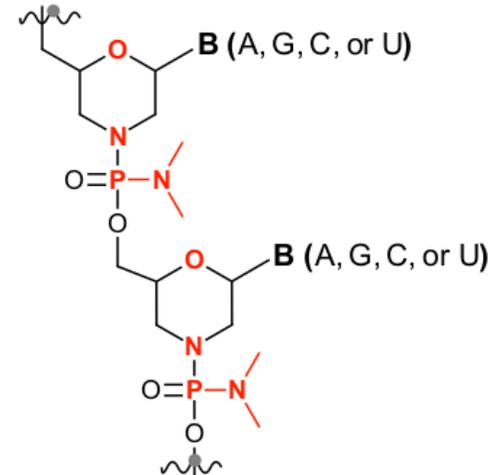
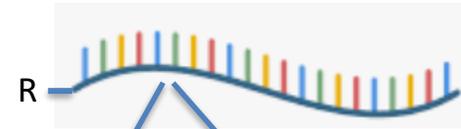
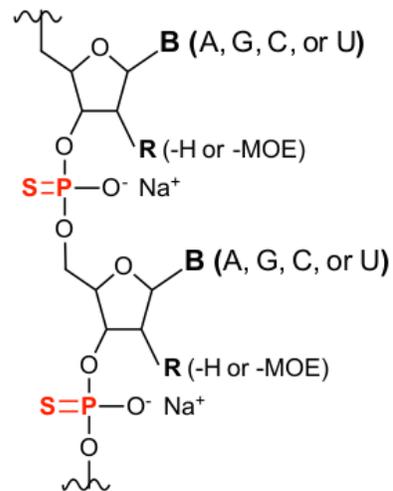
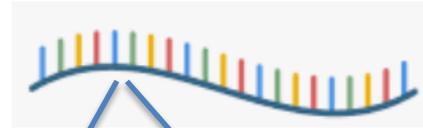
# Synthetic Oligo Drugs: 3 Classes



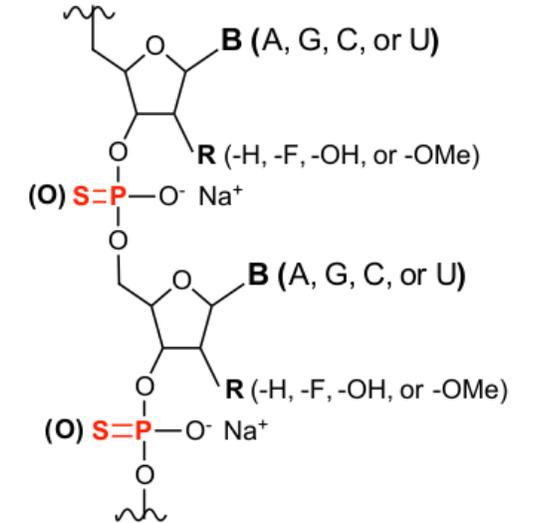
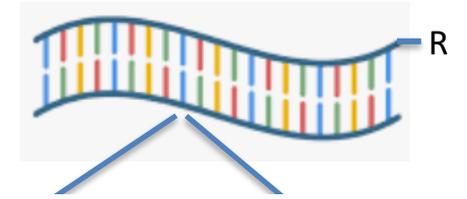
## Aptamer



## Antisense



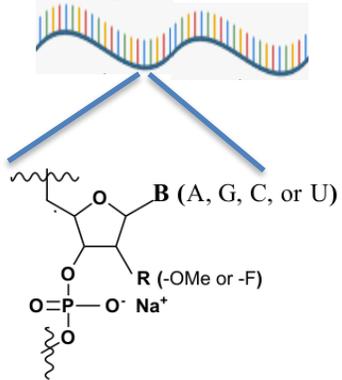
## siRNA



# FDA-Approved Synthetic Oligo Drugs



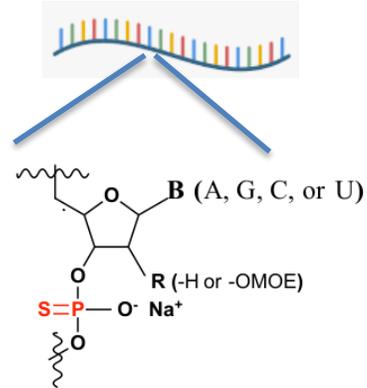
## Aptamer



**Macugen** (pegaptanib:  
*PEG-linked 28-mer PO oligo*)

**Izervay** (avacincaptad  
Pegol: *PEG-linked 39-mer  
PO oligo*)

## Antisense



**Vitravene** (fomiversen:  
*21-mer PS oligo*)

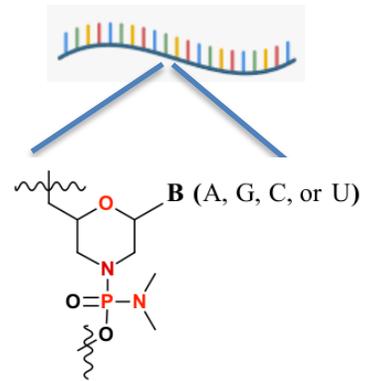
**Kynamro** (mipomersen:  
*20-mer PS oligo*)

**Spinraza** (nusinersen:  
*18-mer PS oligo*)

**Tegsedi** (inotersen:  
*20-mer PS oligo*)

**Qalsody** (Tofersen:  
*20-mer mixed PS/PO oligo*)

**Wainua** (eplontersen: *GalNAc-  
linked 20-mer mixed PS/PO oligo*)



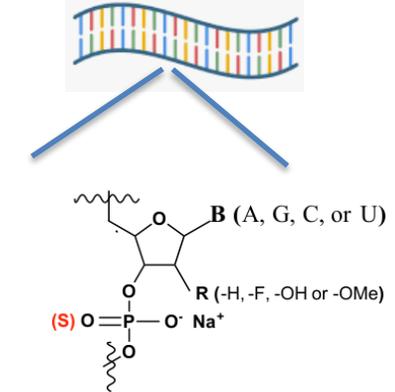
**Exondys 51** (eteplirsen:  
*30-mer PN-morpholino oligo*)

**Vyondys 53** (golodirsen:  
*25-mer PN-morpholino oligo*)

**Viltepso** (viltolarsen:  
*30-mer PN-morpholino oligo*)

**Amondys 45** (casimersen: *25-  
mer PN-morpholino oligo*)

## siRNA



**Onpatro** (patisiran:  
*21-mer/21-mer PO oligo*)

**Givlaari** (givosiran: *GalNAc-linked  
21-mer/23-mer PO/PS oligo*)

**Oxlumo** (lumasiran: *GalNAc-linked  
21-mer/23-mer PO/PS oligo*)

**Leqvio** (inclisiran: *GalNAc-linked 21-  
mer/23-mer PO/PS oligo*)

**Amvuttra** (vutrisiran: *GalNAc-  
linked 21-mer/23-mer PO/PS oligo*)

**Rivfloza** (nedosiran: *GalNAc-linked  
36-mer/22-mer PO/PS oligo*)

# CMC Issues: Specification

- For establishing the **identity, purity** and **potency/strength** of the drug substance (API) or the drug product.
- Although ICH Q6A and ICH Q3A specifically exclude oligos, the recommendations of **these** guidelines applies with some flexibility.

## Compendial Methods: Drug Substance

- Appearance
- Bacterial Endotoxins
- Bioburden
  - Total Aerobic Microbial Count
  - Total Combined Yeast and Mold

## Standard Methods: Drug Substance

- Identity by Mass
- Residual Solvents
- Elemental Impurities
- Water Content

## Oligo-Specific Methods

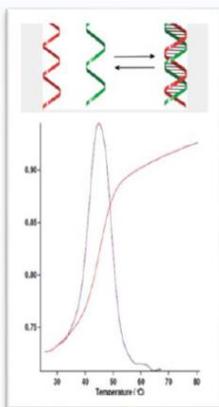
- Identity by Sequence
- Identity, Counter-ion
- Assay / Label Claim
- Purity, Product-related Impurities

## Compendial Methods: Drug Product

- Appearance
- Degree of Coloration
- Clarity and Degree of Opalescence
- pH
- Osmolality
- Particulate Matter
- Bacterial Endotoxins
- Sterility/Container Closure Integrity
- Extractable Volume
- Uniformity of Dosage Units

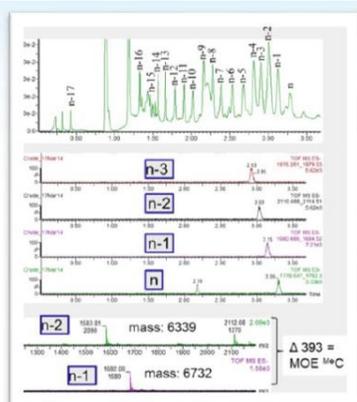
# CMC Issues: *Specification - Identity*

- An Identity test should be able to discriminate among closely related structures.
- For synthetic oligos due to their large size and polymeric composition, we recommend an additional third identity test, Identity by Sequence, to confirm the primary structure.



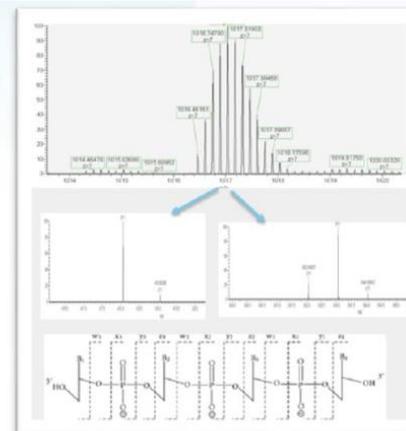
### Melting Temperature

- Good for ASO ID
- Simple instrumentation
- Requires complementary strand with confirmed ID



### Failure Sequence Analysis

- Test the crude, not final DS
- Need RHMS
- Manual data processing



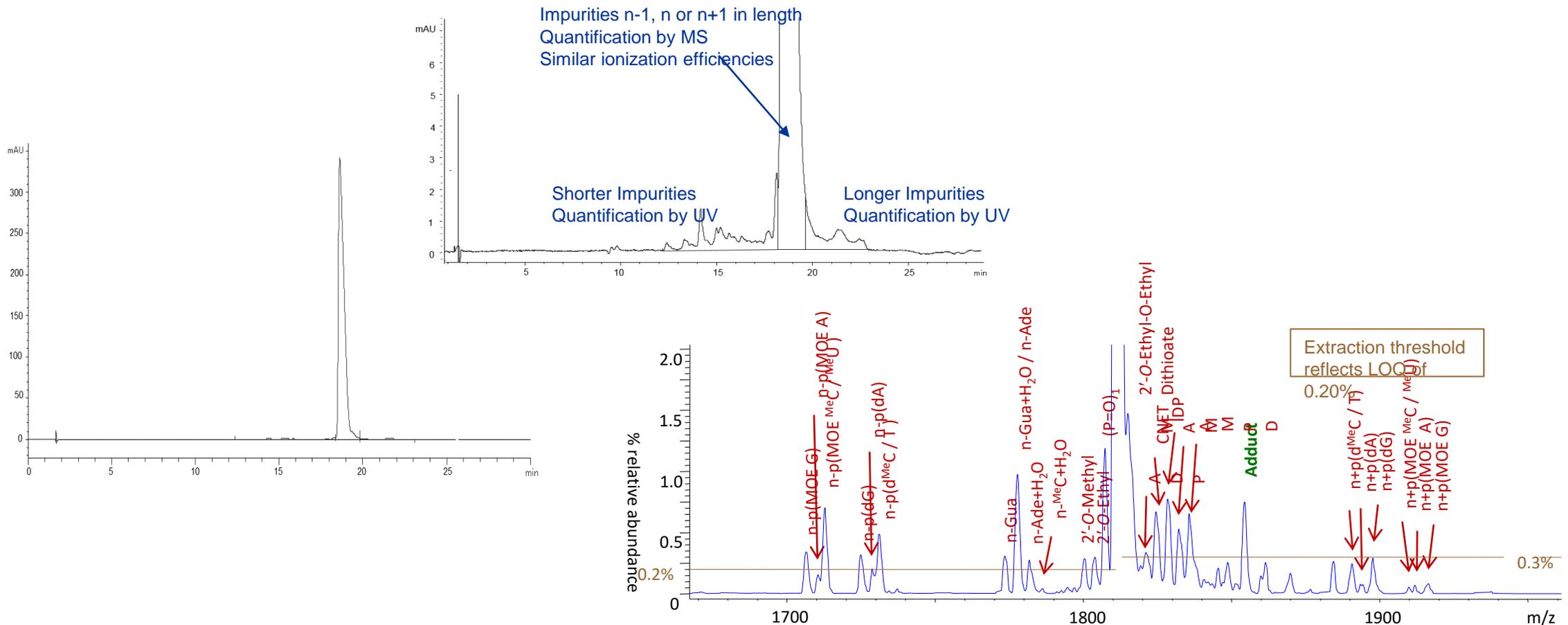
### Sequencing by MS/MS

- Need RHMS
- Complicated data processing
- Recent software development
- Good for reference characterization
- Lack of consensus on acceptance criteria if used as ID release test

# CMC Issues: *Specification - Purity*

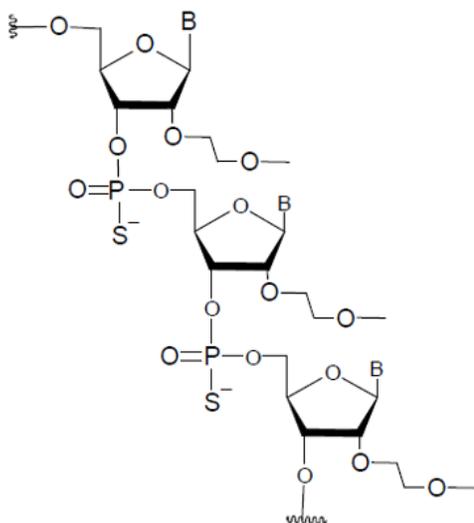


- A purity test should be able to assess the levels of process-related and product-related impurities in the drug substance or drug product.
- For synthetic oligos, the product peak in an LC is broad since the product is both a mixture of diastereomers and high molecular weights



# CMC Issues: *Impurity Profile*

- No ICH or FDA regulatory guidelines on impurity identification and qualification thresholds.
- For the impurity profile, oligo-related impurities can be grouped either based on their structural class (e.g., n-1, n+1, etc.) or on their relative retention time (RRT) ranges in the HPLC chromatogram.
- For double strand oligos, two purity tests (denaturing and non-denaturing conditions) should be used to define the impurity profile.



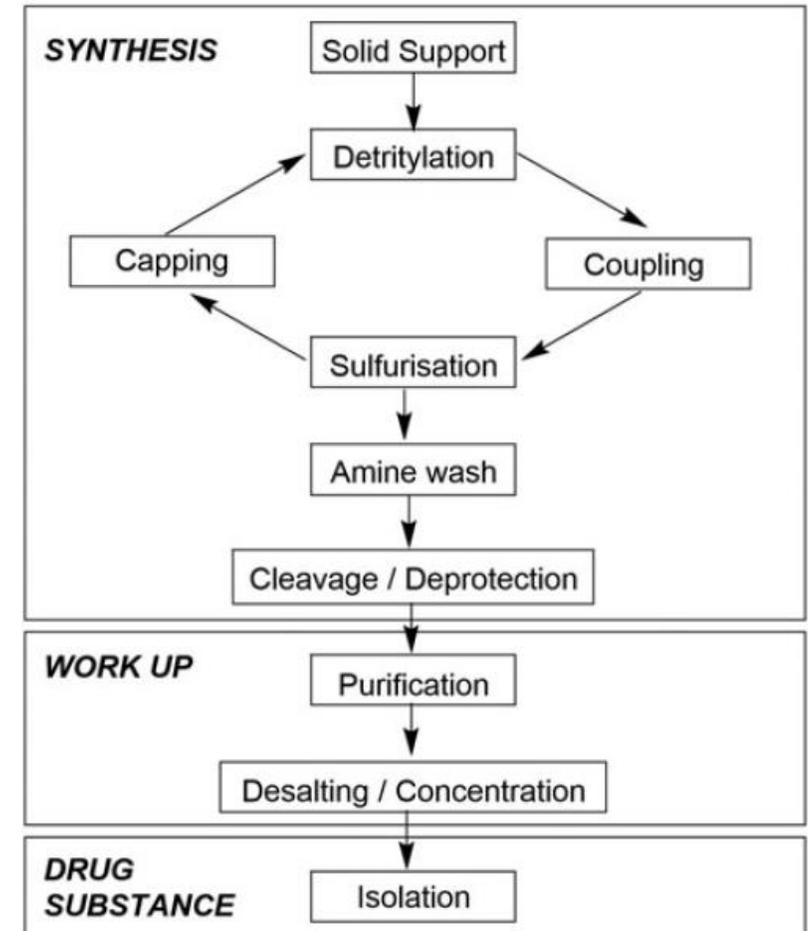
Impurity	Examples
Internucleotide	<ul style="list-style-type: none"> <li>• PO/PS (Oxidation)</li> <li>• C-Phosphonate (incomplete sulfuration)</li> </ul>
Sugar residue	<ul style="list-style-type: none"> <li>• Abasic</li> <li>• O-alkyl, O-alkoxyalkyl (e.g. 2'-O-butyl)</li> <li>• Positional isomers (2',5' ribose)</li> </ul>
Base Residue	<ul style="list-style-type: none"> <li>• Deamination</li> <li>• Acetyl Capping Impurities (N<sup>2</sup>-acetyl-guanine)</li> <li>• CNET/Acrylonitrile addition to thymine from base deprotection</li> </ul>
Deletion	<ul style="list-style-type: none"> <li>• n-1</li> </ul>
Incorporation; Single Nucleotide Repeat	<ul style="list-style-type: none"> <li>• n+1</li> </ul>
High MW Impurities	<ul style="list-style-type: none"> <li>• Pyrimidine dimers/CPD crosslinking</li> </ul>

# CMC Issues: *Specification - Assay*

- An assay (potency/strength) test should be able to determine the drug substance content in a sample of the drug substance or drug product.
- For synthetic oligos, an assay test should be a weight-based determination of the drug substance content that provides assurance that all components of the sample have been accounted for.
  - Strength/potency tests do require a reference standard.
- Cell-based assays: CDER does not require potency bioassays for most synthetic oligos since the primary structure can be confirmed by sensitive analytical methods.
  - For ASOs and siRNA drugs, the biochemical binding activity is dependent on the primary structure of the oligo.

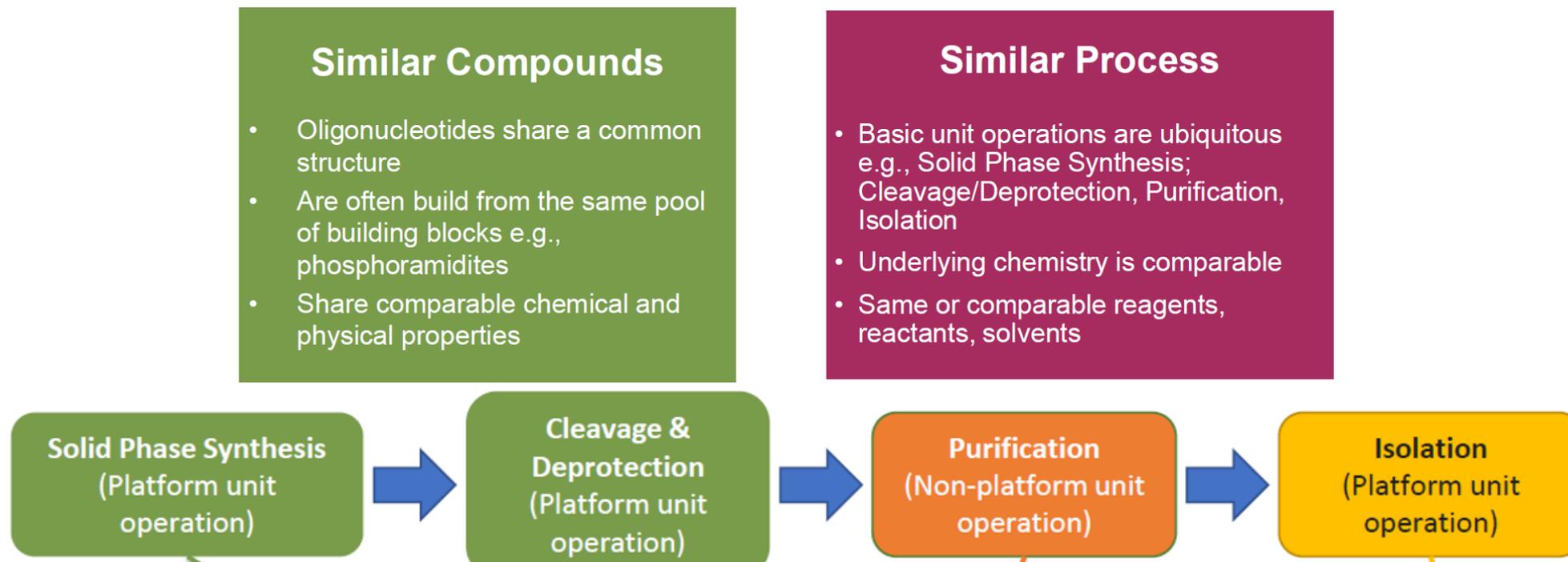
# CMC Issues: *Manufacturing*

- **Synthesis** - Phosphoramidite chemistry
  - Used commercially for over 35 years
  - Used for solid-phase and solution-phase reactions
  - Can synthesize oligos up to 200 base pairs in length
- **Starting materials**
  - Common (i.e., 2'-OMe, 2'-MOE, 2'-F) nucleoside phosphoramidites are generally recognized as regulatory starting materials
  - Conjugated GalNAc phosphoramidites need to comply with ICH Q11 guidance
- **Bioequivalence / API sameness**
  - Diastereomeric composition - sulfurization (or oxidation) of the phosphite intermediate is not stereoselective
  - Ensure that appropriate reagents and reaction conditions are adequately controlled.
  - How to analytically compare the diastereomeric composition of the test API with the RLD/API?



# CMC Issues: *Platforms*

- **Platform Manufacturing** (*from ICH Q11 glossary*): The approach of developing a production strategy for a new drug starting from manufacturing processes similar to those used by the same applicant to manufacture other drugs of the same type.



- *Platform Strategies for Synthetic Oligonucleotide Drug Substances* (Org. Process Res. Dev 2023, 27, 2211-2222).

# Definition of *Platform Technology*



FDA acknowledges that the term **platform technology** has been used by both industry and FDA to describe technologies in ways that differ from the *designated platform technology* discussed in section 506K of FD&C Act.

**Platform technology** (*from section 506K of FD&C Act*) means a well-understood and reproducible technology, which may include a nucleic acid sequence, molecular structure, mechanism of action, delivery method, vector, or a combination of any such technologies that the Secretary determines to be appropriate, that the sponsor demonstrates -

- A. is incorporated in or utilized by a drug or biological product and is essential to the structure or function of such drug or biological product;
- B. can be adapted for, incorporated into, or utilized by, more than one drug or biological product sharing common structural elements; and
- C. facilitates the manufacture or development of more than one drug or biological product through a standardized production or manufacturing process or processes.

# Designated Platform Technology Criteria



A **platform technology** would be considered eligible for designation as a “*designated platform technology*” if, per 506K(b):

- (1) the platform technology is incorporated in, or utilized by, a drug approved under section 505 of this Act, or a biological product licensed under section 351 of the Public Health Service Act;
- (2) preliminary evidence submitted by the sponsor of the approved or licensed drug described in paragraph (1), or a sponsor that has been granted a right of reference to data submitted in the application for such drug, demonstrates that the platform technology has the potential to be incorporated in, or utilized by, more than one drug without an adverse effect on quality, manufacturing, or safety; and
- (3) data or information submitted by the applicable person under paragraph (2) indicates that incorporation or utilization of the platform technology has a reasonable likelihood to bring significant efficiencies to the drug development or manufacturing process and to the review process.

Additional Information on FDA’s implementation of the *designated platform technology* program will be forthcoming in a Guidance for Industry

(Link to the 2024 CDER guidance agenda: <https://www.fda.gov/media/134778/download>)

# FDA Guidances on Synthetic Oligos

- Presently, the FDA has not released a Guidance for Industry for the commercial development of a synthetic oligonucleotide (ASO, PMO or siRNA).
- However, FDA has recently published four guidances on developing individualized (n of 1) oligonucleotide drugs

**Nonclinical Testing of Individualized Antisense Oligonucleotide Drug Products for Severely Debilitating or Life-Threatening Diseases**  
 Guidance for Sponsor-Investigators

- IND enabling studies
- Need for POC
- Safety studies to get to clinical studies
- Dose and dose escalation

**IND Submissions for Individualized Antisense Oligonucleotide Drug Products for Severely Debilitating or Life-Threatening Diseases: Clinical Recommendations**  
 Guidance for Sponsor-Investigators

- IRB and consent issues
- Diagnosis and genetics
- Dose selection and escalation
- Safety monitoring
- Assessment of response

**IND Submissions for Individualized Antisense Oligonucleotide Drug Products: Administrative and Procedural Recommendations**  
 Guidance for Sponsor-Investigators

- Efficiently interacting with FDA to guide entry into humans
- pre-IND packages and meetings
- Communications with FDA
- IND packages
- How to submit

**IND Submissions for Individualized Antisense Oligonucleotide Drug Products for Severely Debilitating or Life-Threatening Diseases: Chemistry, Manufacturing, and Controls Recommendations**  
 Guidance for Sponsor-Investigators

- Recommendations for CMC information in IND for N of 1 individualized ASO
- Unconjugated, manufactured with conventional methods, simple formulations

# Summary

- Several regulatory issues are specific for synthetic oligonucleotides
  - Control of Drug Substance – Specification
    - Identity: confirmation of the primary sequence
    - Purity: impurity profile
    - Potency: weight-based assay
  - Manufacturing
    - Starting materials
    - API sameness
    - Platform knowledge
  
- While several CMC Guidances specifically exclude oligonucleotides, the recommendations of these guidelines applies with some flexibility
  - Regulatory meetings with the FDA are encouraged for CMC-related issues for synthetic oligonucleotides



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