CMC Regulatory Considerations for Oligonucleotide Drug Products: FDA Perspective

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OVERVIEW

- ✓ Oligonucleotide-Based Therapeutics: Promises and Challenges

- ✓ Synthetic Oligonucleotides: Structural Aspects
  - Antisense Oligonucleotides
  - Double-Stranded Small Interfering RNAs (siRNAs)
  - Chemical Modifications of Oligonucleotides
  - Oligonucleotide Structure-Related Safety Considerations

- ✓ Synthetic Oligonucleotides: Major Regulatory Aspects
  - Regulatory Challenges
  - General CMC Considerations
  - Oligonucleotide-Specific CMC Considerations

Therapeutic Oligonucleotides

- Exert effects through suppression of, or interference with mRNA translation, immune stimulation, protein binding, or through induction of exon skipping

- Can target a broad range of mRNAs (encode all cellular proteins), including protein targets that are considered “undruggable” by small molecule or protein therapeutics

- An evolving class of therapeutic agents that present unique scientific and regulatory challenges

- Synthetic therapeutic oligonucleotides (*in theory no potential for incorporation into the chromatin*): Regulated by CDER, FDA

- Vector-based or promoter-driven oligonucleotides: Regulated by CBER, FDA

Synthetic Antisense Oligonucleotides: Structural Aspects

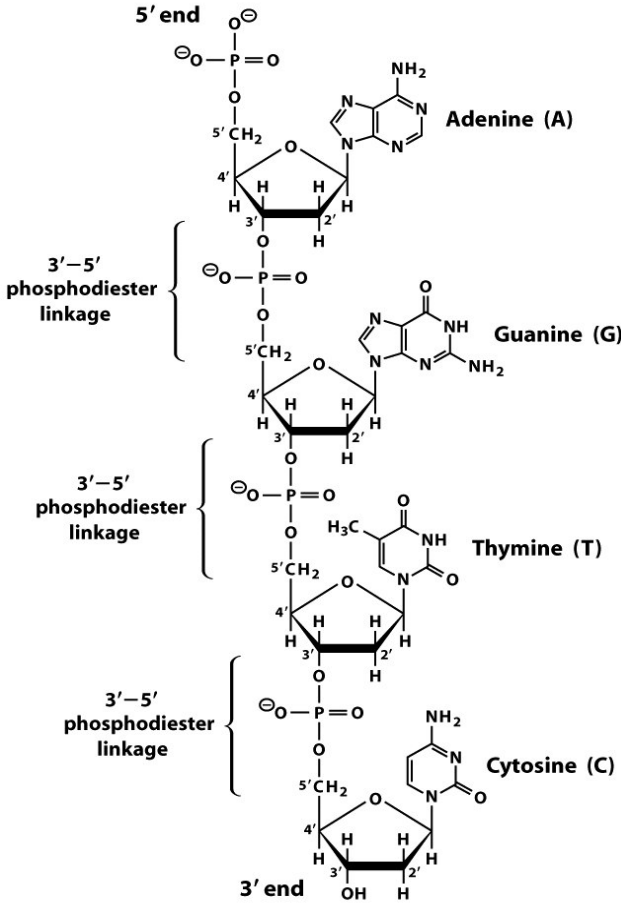
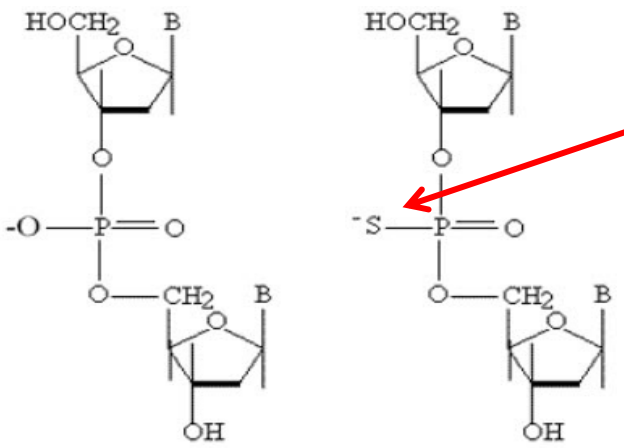


Figure 19-11 Principles of Biochemistry, 4/e

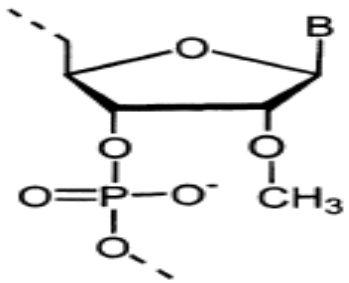
- Usually consist of 15-20 unmodified or chemically modified nucleotides (complementary to target mRNA sequence)
- Unmodified oligonucleotides are rapidly degraded by nucleases
- Chemically modified ribonucleotides are used to protect against nuclease degradation, improve target affinity and delivery to the intended target/tissue/region

Antisense Technology Challenges: Nuclease Degradation, Stabilization, Targeted Delivery, Off-target Effects, and Toxicity

Commonly Used Antisense Oligonucleotide Modifications



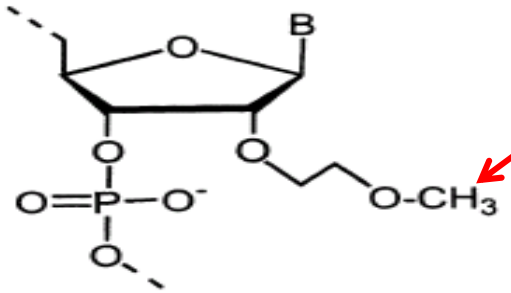
- Phosphate backbone: One of the oxygen atoms in the phosphate moiety replaced by sulfur (oligonucleotide phosphorothioate)
- Desirable effects: Nuclease resistance
- Undesirable effects at higher doses: Probability of off-target effects (binding to heparin-binding proteins)



Ribonucleotide modifications (2' position of the ribose):

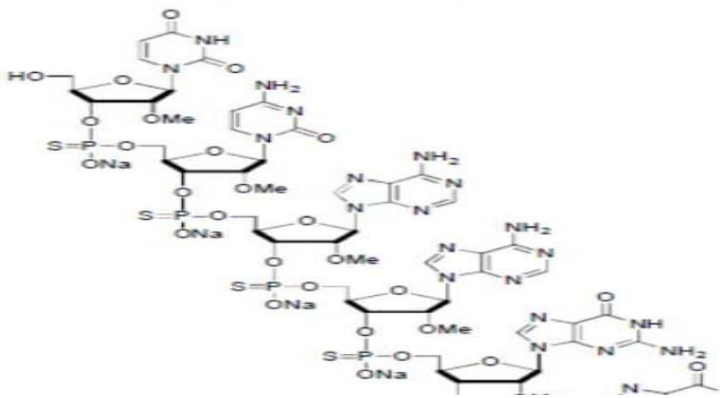
2'-O-methyl

2'O-methoxy-ethyl



Morpholino Modification: The ribose is replaced by a morpholino moiety and phosphoroamidate

Use of Both Phosphate Backbone, and 2' Ribose Modifications



Sodium 2'-O-methyl-phosphorothioate oligoribonucleotide (partial structure)

Lower Toxicity?; Higher Target Affinity

Oligonucleotide Conjugates (for improved pharmacokinetic properties)

- The covalent attachment of various ligands designed to improve bio-distribution and cellular uptake or targeting of specific tissues

- Attached ligands: peptides, proteins, carbohydrates, aptamers and small molecules, including cholesterol, tocopherol or folic acid

Example: N-Acetylgalactosamine (GalNAc) conjugates: reduced toxicity, improved potency/PK properties, lower off-target activity

Overlapping CMC and Toxicology Review Considerations

Direct relationship between oligonucleotide structure/modifications and toxicity and safety liabilities

- Example: Phosphorothioate (PS) backbone modification used to protect against rapid degradation by nucleases

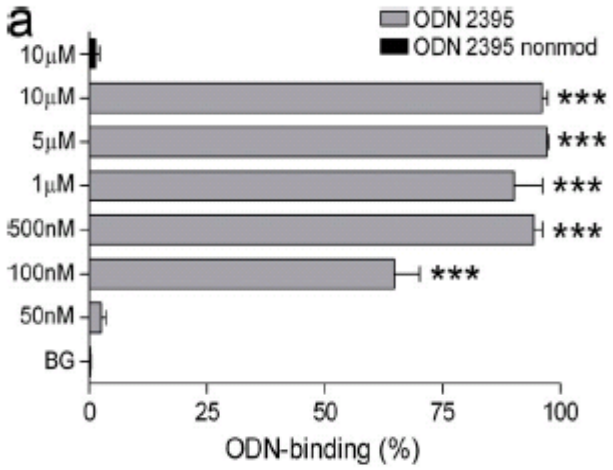
- Phosphorothioate Antisense Oligonucleotides:
  - Sequence-independent, but length-dependent binding to various cellular proteins (heparin-binding molecules)
  - Phosphorothioate modification-linked thrombocytopenia

Phosphorothioate (PS) Modification-Linked Platelet Activation

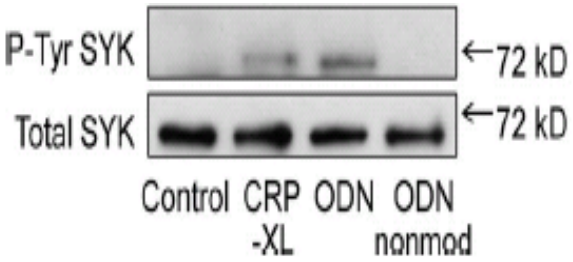
PS modification-linked thrombocytopenia mechanism not well understood

* Based on recent studies PS-modified (not unmodified) oligonucleotides bind to platelet-specific collagen receptor glycoprotein VI (GPVI)

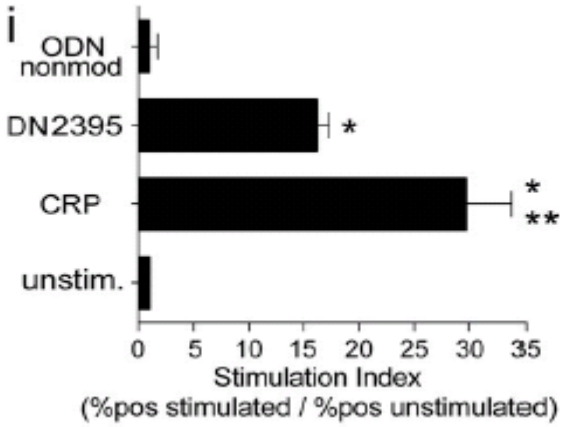
Selective Binding



Tyrosine Activation



ROS Production



*Flierl, et al. Journal of Experimental Medicine 212.2 (2015): 129-137.

Regulatory Challenges

- No ICH or FDA regulatory guidelines that specifically address the quality expectations/standards for oligonucleotide products

- Oligonucleotide Diversity: Single stranded antisense, splice modulators, aptamers and immunological modulators, and double stranded siRNAs (that function by RNAi mechanism). Unique mechanisms of action with diverse toxicology profiles/concerns

- No consensus about impurity identification and qualification thresholds

- Impurity characterizing challenges:
  - Most exist as mixtures of closely related components
  - Some impurities are largely intact parent oligonucleotide cross-linked to another molecule of the parent oligonucleotide
  - Precision of analytical methods to adequately resolve impurities

Review Considerations for Synthetic Oligonucleotides: Current Practices

- CFRs concerning CMC information apply:
  INDs: 21 CFR, part 312.23 (a) (7); NDAs: 21 CFR, part 314.50 (d) (1)
  - *to ensure the proper identity, strength or potency, quality, and purity*

- Despite their large size, synthetic oligonucleotide drugs are considered more similar to small molecule drugs than biologics in that they are manufactured by solid-phase chemical synthesis

FDA's quality-related guidances for submission of INDs, NDAs or supplements are applicable--- *graded nature of CMC information needed*

- ICH guidances covering drug substance and drug product stability, analytical method validation, specifications, GMP risk management, pharmaceutical development/quality system; and development and manufacture of drug substance are applicable

Review Considerations for Synthetic Oligonucleotides: Current Practices

- Confusion about whether USP Salt Policy applies to salt oligonucleotide drugs, partly because oligonucleotides (approx. molecular mass: 7000 to 8000) are not perceived as small molecules

- Based on antisense oligonucleotide structure, design and mechanism of action, the salt counterion does not play a critical role in mediating mode of action

- USP Salt Policy and FDA guidance *Naming the Drug Products Containing Salt Drug Substances (20125)* is applicable to synthetic oligonucleotide drugs

- ICH Q3C(R6) and ICH Q3D, the guidelines that cover residual solvents, and elemental impurities, respectively, are applicable to oligonucleotide products

Synthetic Oligonucleotide-Specific Review Considerations

Identity: Determination of oligonucleotide sequence

API Designation: Based on current availability of : a) refined analytical tools for structural characterization and resolution of different oligonucleotide species, and b) precisely controlled method for solid-phase oligonucleotide synthesis, designating the full-length intended oligonucleotide as the API and considering all the other oligonucleotide species as the process impurities is generally recommended

Calculation of "Assay": Current recommendation is not to include the process impurities such (P=0)1 as a part of API for calculation of 'assay' values for the drug substance

Aptamers with 3-D conformations: May require bioactivity assays in addition to the usual panel of quality tests to assure quality

Synthetic Oligonucleotide-Specific Review Considerations

Double-stranded oligonucleotides:

- Two orthogonal measurements assessing purity at the individual single strand level and purity of the duplex to address completeness of annealing
- Assessing completeness of annealing by measuring excess single strand as specified impurity

ICH Q3a and Q6a: Though specifically exclude oligonucleotides, the spirit of these guidelines applies with some flexibility:

- Flexibility in the limits for reporting, identification and qualification thresholds of process impurities based on toxicology qualification, product risk assessment, manufacturing/impurity resolution capability, and batch analysis/stability data

- Polymorphism characterization not relevant

Regulating Oligonucleotide Products: Agency Experience

1. Fomivirsen (Vitravene); 1998: Antisense phosphorothioate oligonucleotide for treating cytomegalovirus retinitis in AIDS patients (intraocular injection; no longer marketed)

2. (Macugen®) ; 2004: Anti-vascular endothelial growth aptamer (pegaptanib sodium) that specifically binds to the 165 isoform of VEGF. For age-related neovascular macular degeneration

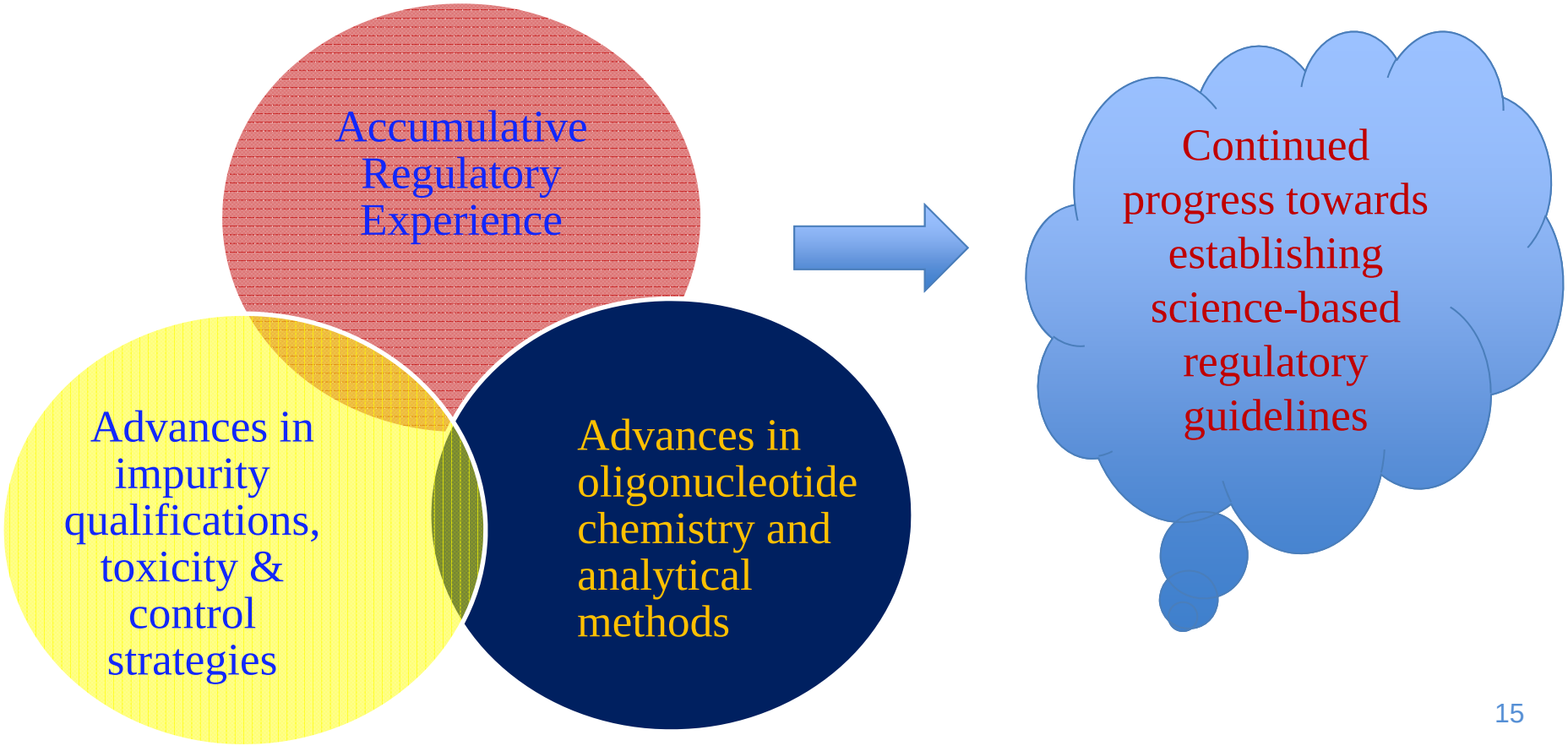
3. Mipomersen (Kynamro); 2013: Antisense phosphorothioate oligonucleotide for homozygous familial hypercholesterolemia

4. Eteplirsen (Exondys 51); 2016: phosphorodiamidate morpholino oligonucleotide indicated for patients with mutation of the dystrophin gene amenable to exon 51 skipping (Duchenne muscular dystrophy)

Review Considerations for Synthetic Oligonucleotides: An Evolving Process

5. Nusinersen (Spinraza); 2016: Splice modulating phosphorothioate oligonucleotide for the treatment of spinal muscular atrophy (SMA)

No approved RNAi (siRNA) product




Thank You!