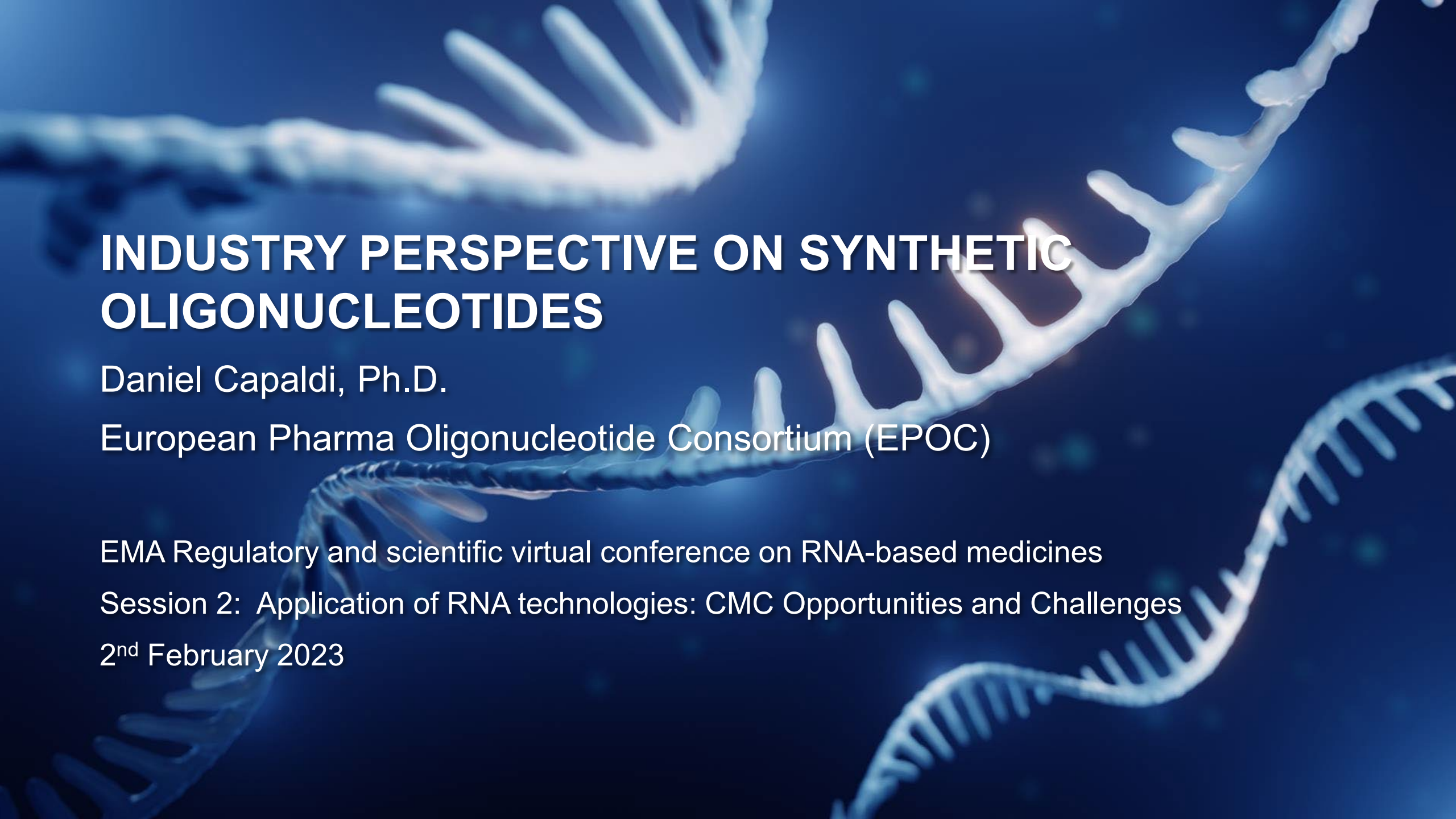


INDUSTRY PERSPECTIVE ON SYNTHETIC OLIGONUCLEOTIDES

Daniel Capaldi, Ph.D.

European Pharma Oligonucleotide Consortium (EPOC)

EMA Regulatory and scientific virtual conference on RNA-based medicines

Session 2: Application of RNA technologies: CMC Opportunities and Challenges

2nd February 2023

Agenda

- The European Pharma Oligonucleotide Consortium
- Published papers
- Drug substance starting materials
- Drug substance impurities
- Terminal sterilization
- Future papers

Classified as public by the European Medicines Agency

[European Pharma Oligonucleotide Consortium | Groups | LinkedIn]

Mission Statement

Through innovative collaboration, identify areas for harmonization of oligonucleotide* CMC development and propose solutions to influence the external regulatory environment; expediting patient access to revolutionary medicines

* Currently defined as antisense, siRNA, aptamers and sgRNA; includes small molecule conjugates. Currently excludes mRNA and antibody/protein conjugates

Publications

EPOC

1. Technical Considerations for Use of Oligonucleotide Solution API | Nucleic Acid Therapeutics (liebertpub.com)
2. Perspectives on the Designation of Oligonucleotide Starting Materials | Nucleic Acid Therapeutics (liebertpub.com)
3. Strategies for Identity Testing of Therapeutic Oligonucleotide Drug Substances and Drug Products | Nucleic Acid Therapeutics (liebertpub.com)
4. Determination of Purge Factors for Use in Oligonucleotide Control Strategies | Organic Process Research & Development (acs.org)
5. Solution Oligonucleotide APIs: Regulatory Considerations | SpringerLink
6. Terminal sterilization; in the press
7. Nitrosamine Risk Assessments in Oligonucleotides | Organic Process Research & Development (acs.org)

Non-EPOC

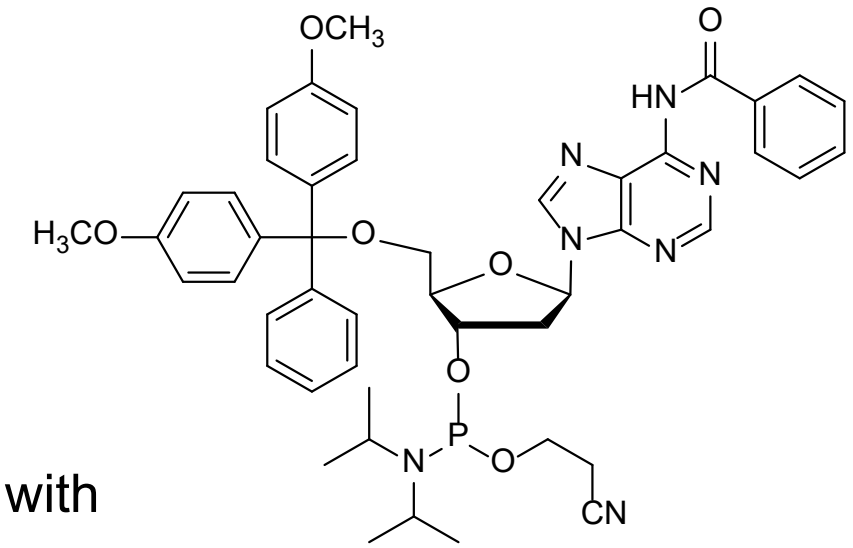
1. Quality Aspects of Oligonucleotide Drug Development: Specifications for Active Pharmaceutical Ingredients | SpringerLink
2. Impurities in Oligonucleotide Drug Substances and Drug Products | Nucleic Acid Therapeutics (liebertpub.com)

Classified as public by the European Medicines Agency

Drug Substance Starting Materials

[Perspectives on the Designation of Oligonucleotide Starting Materials | Nucleic Acid Therapeutics (liebertpub.com)]

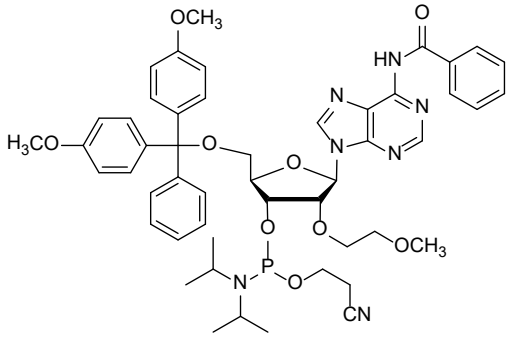
- Aim: To create harmonized starting material justification packages (to avoid inconsistencies among different sponsor companies and different regulatory agencies)
- Focus is phosphoramidite starting materials for solid phase synthesis
- Try to apply as much of Q11 as possible
  - Starting materials have defined and stable structures with characteristic chemical and physical properties
  - Emphasize requirement to provide complete proof of structure and characterization, e.g., ^{1}H, ^{13}C, ^{31}P NMR (and 2D NMR), HPLC-MS



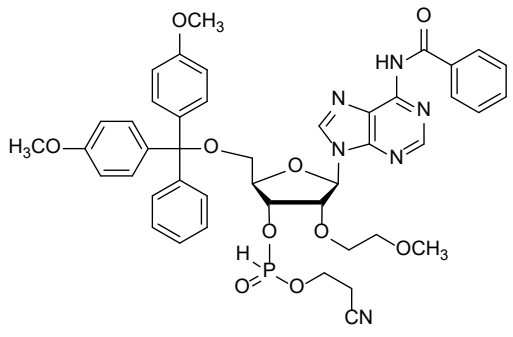
Classified as public by the European Medicines Agency

Drug Substance Starting Materials

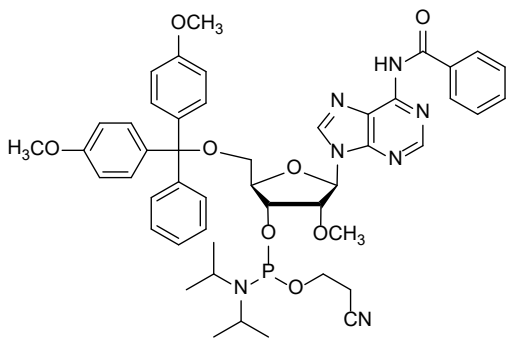
- Impurity characterization and fates of impurities
- Critical and non-critical impurities, e.g.,



Starting material



Non-critical impurity
(unreactive)



Critical impurity
(reactive)

- Major impurities are unreactive. Reasonable to assume unreactive impurities flow though column and are non-critical
  - In most cases, non-reactivity should be obvious - should be no requirement to conduct formal fate and purging studies
- Reactive impurities lead to product related impurities
  - Propinquity arguments very weak for solid phase synthesis
  - Corresponding product related impurities typically possess similar physical properties to parent oligonucleotide – limited opportunities to reduce by purification (and sometimes difficult to detect and quantify in drug substance)
  - Potential for multiplicative effects

Classified as public by the European Medicines Agency

Drug Substance Starting Materials

Specification

- Should focus on testing critical quality attributes (CQA)
- CQA
  - Identity – determines drug substance identity
  - Reactive (nucleoside) phosphoramidite impurities
    - Likely need to be controlled to low levels (due to multiplicity issues)
    - Normally determined by validated HPLC, UHPLC or LC-MS techniques (^{31}P NMR typically doesn't add much).
- Non CQA (although may be controlled for economic reasons)
  - Non-reactive nucleotide (nucleoside) impurities – subject to extensive purging during solid phase synthesis
  - Solvents - subject to extensive purging during solid phase synthesis
  - Water – solutions typically dried before use, starting materials used in excess, coupling failure typically results in yield reduction only
  - Stereochemistry at P-atom* – racemization during activation, and therefore starting material P-stereochemistry does not impact drug substance stereochemistry
  - Purity (assay) - starting materials used in excess, coupling failure typically results in yield reduction only

* Excepting P-stereopure starting materials used to make P-streopure drug substance

Classified as public by the European Medicines Agency

Drug Substance and Drug Product Impurities

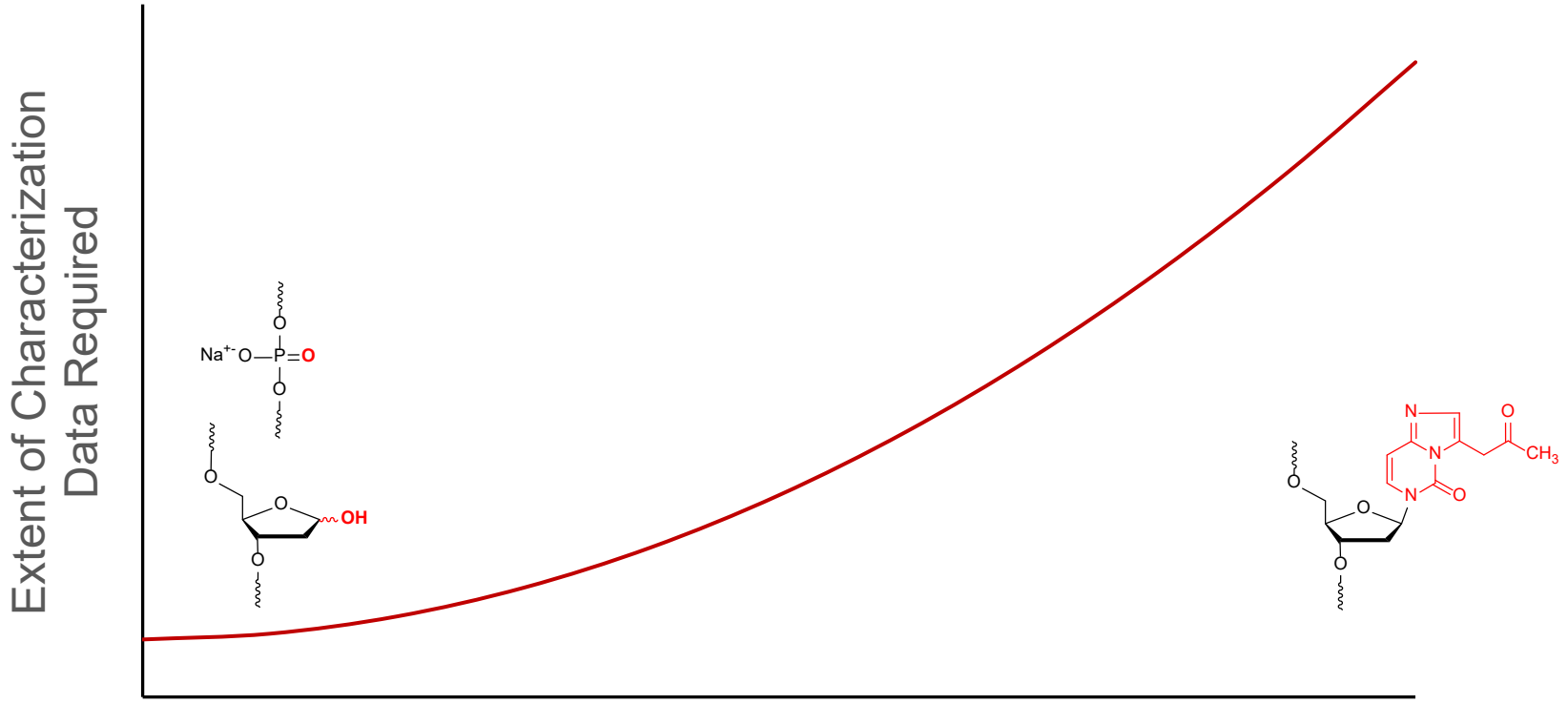
Impurities in Oligonucleotide Drug Substances and Drug Products | Nucleic Acid Therapeutics (liebertpub.com)

- Focus is product-related impurities
  - Oligonucleotide impurities derived from impurities in starting materials and reagents
  - Oligonucleotide impurities formed due to side- or incomplete reactions of the manufacturing process
  - Oligonucleotide impurities arising by degradation
- Chemistry considerations - impurity characterization and reporting (grouping, reporting threshold, specification limits)
- Safety considerations - classes of oligonucleotide impurities, qualification requirements (identification and qualification thresholds)

Classified as public by the European Medicines Agency

Impurity Characterization

- Propose a spectrum of empirical data requirements



Impurities previously characterized independently by several groups for several oligonucleotides

Impurities characterized for the first time

Classified as public by the European Medicines Agency

Impurity Reporting

- Specifications should include tests of specified impurities (frequently observed impurities that are diagnostic of the success or otherwise of a particular step or control strategy in the manufacturing process)
- Example
  - Single-stranded oligonucleotides typically contain low levels of n-1 impurity
  - Levels of n-1 largely dependent on successful completion of the detritylation step of solid-phase oligonucleotide synthesis
  - Therefore, monitoring n-1 in the drug substance may confirm that the detritylation step performed as expected
- Complete separation of impurities from likely not be practical for most (perhaps all?) oligonucleotides - will be necessary to report impurities as groups, or classes
  - Several means of assembling impurities into groups
  - Structural class: e.g., n-1, n+1, abasic oligonucleotides, CNET etc.
    - Benefit is that all members of a particular group are subject to the same mechanism of formation
    - Consistent with the idea that reported results reflect the success of a particular synthetic step or control strategy

Classified as public by the European Medicines Agency

Impurity Reporting: Reporting Threshold

- Reporting Threshold: Level above which there is an expectation to report (and sum) impurities
- For most small molecule drugs, reporting threshold = 0.05%
  - Important consideration is the LOQ of the analytical method
  - Impurities of small molecule drugs are typically single components that are readily separated chromatographically as sharp, well-defined peaks detected by UV absorbance
  - LOQ values on the order of 0.02% or less are readily achieved; therefore, 0.05% reporting threshold is technically feasible
- Product-related impurities of oligonucleotides often difficult to separate chromatographically (in some instances can only be detected using mass spectrometry)
  - When chromatographic separation is practical, composite nature of impurities contributes to chromatographic peak broadening
  - Peak broadening reduces signal-to-noise and impacts LOQ
  - Mass spectrometry detection typically has higher LOQ than UV detection
  - However, oligonucleotides typically contain a greater number of impurities than most small molecule drugs - if reporting threshold is too high, risk severely under-reporting total impurities

Reasonable compromise reporting threshold = 0.20%

Classified as public by the European Medicines Agency

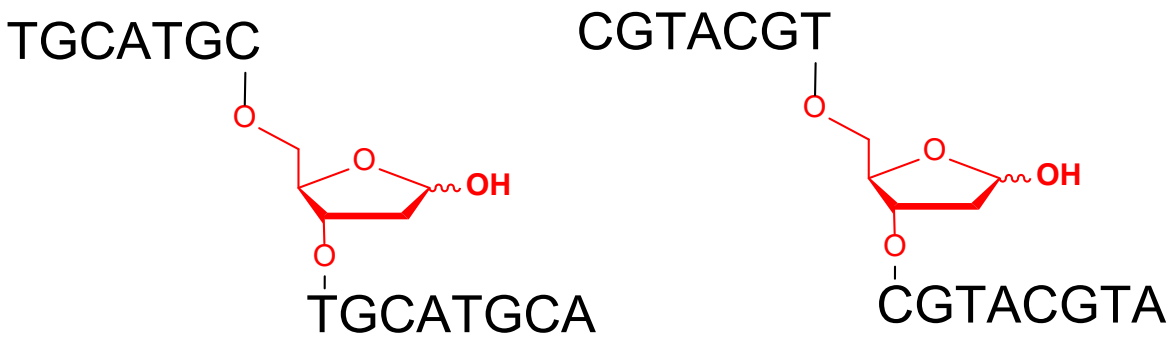
Safety Considerations

Impurity Class	Examples	Safety Assessment?
Class I Impurities that are also major metabolites (structure and sequence are the same as parent)	• Impurities that lack nucleotides from the 3´ or 5´-end of the parent oligonucleotide • Impurities formed by incomplete conjugation of (parent) conjugated oligonucleotides • Parent single-stranded impurity of double-stranded oligonucleotides	No; impurities that are also major metabolites are generally considered qualified
Class II Impurities containing only structural elements found in naturally occurring nucleic acids	• Phosphate diester impurity of phosphorothioate diester oligonucleotides	No; endogenous presence of the structural element rules out inherent safety concerns associated with the impurity
Class III Impurities that are sequence variants of the parent (impurity contains only structural elements found in the parent)	• n-1 • n+1	No; safety concerns limited to potential off-target antisense effect or generation of inflammatory motif: therefore, appropriate to consider safety of each individual impurity, which are too low to generate a pharmacologic effect
Class IV Impurities that contain structural elements not found in the parent or in naturally occurring nucleic acids	• e.g., CNET, abasic impurities • Unidentified impurities	Yes; potentially altered reactivity and metabolism, lead to potentially toxic metabolites

Classified as public by the European Medicines Agency

Safety Considerations

- Likely that the safety of the impurity is independent of the sequence context
  - Example: Potentially reactive abasic impurity



Sequence surrounding modified linkage, sugar or base unlikely to impact the physico-chemical properties of the lesion significantly

- Concept aligned with database structural alert approaches e.g., ToxAlerts, DEREK, NOXNET
- Animal studies should not need to be repeated to qualify the same impurity classes in other oligonucleotides of the same or similar chemical structure

Classified as public by the European Medicines Agency

Unspecified Impurities and the Identification Threshold

- Drug substance (and product) specification should contain limit on unspecified impurities; standard practice to set the limit on unspecified impurities equal to the Identification Threshold
- Driven by Quality expectations
  - Formation of new and unidentified impurities above identification threshold be avoided
  - In a state of control, manufacturing processes, control strategies, and analytical methods should be capable of avoiding new impurities above the identification threshold
  - Set to 0.10% for most small molecule drug substances
- Same Quality expectation should apply to oligonucleotides
- But if Identification threshold is driven largely by what we ***should*** be capable of doing (when everything goes to plan)…
  - Oligonucleotides typically produced by solid-phase synthesis, where products of one reaction are fed into the next without purification; little opportunity for impurity removal
  - Minute quantities of reactive impurities in starting materials may lead to significant quantities of drug substance impurities
  - Complex impurity profiles; often difficult to distinguish impurities from the parent and each other
- Places practical limitations on ability to control unspecified impurities

Identification threshold of 1.0% is about the best we can do

Classified as public by the European Medicines Agency

Is it Safe?

- Reasons that 1.0% of a new oligonucleotide impurity doesn't pose a safety concern
  - Physico-chemical properties likely to be very similar to those of the parent (water soluble polyanion of similar molecular weight and protein binding properties
  - Large difference in MW between oligonucleotide and small molecule impurities. Same quantities of each represent very different molar amounts
  - Most oligonucleotides are dosed at much less than 100 mg/day
    - Therefore, 1% of daily dose is usually much less than the 1 mg amount discussed in Graham et al [*Calculating qualified non-mutagenic impurity levels: Harmonization of approaches* Regulatory Toxicology and Pharmacology 126 (2021) 105023 https://doi.org/10.1016/j.yrtph.2021.105023]
  - Because all impurities tested to date are well-tolerated, future impurities likely to be well-tolerated too

Classified as public by the European Medicines Agency

Terminal Sterilization (TS)

- Objective: Fill gaps between regulatory expectations and application of TS of oligonucleotide drug products, with special attention to the EMA guidance (Sterilization of the medicinal product, active substance, excipient and primary container - Scientific guideline | European Medicines Agency (europa.eu)
- Most prescriptive guideline published
- Provides guidance on the selection of TS methods and technical requirements
- Efforts *should be made* to enable terminal sterilization
  - Selection of pH, excipients, container closure system, optimization of sterilization method/process
- Change in impurities above qualification thresholds does not preclude the use of TS

TS is preferred - sterile filtration and aseptic processing *should be justified*

Classified as public by the European Medicines Agency

Key Concepts

- General acceptance that proteins and other biologics are too labile to withstand TS
- Oligonucleotide drug products traditionally manufactured by sterile filtration and aseptic processing (like biologics)
- Theoretical risk assessment
  - Double stranded oligonucleotides with T_m values below 110°C, aptamers and oligonucleotides conjugated to proteins likely excludable from the outset; some single stranded oligonucleotides are more thermally stabile (hard to exclude without further work)
- Formulation development
  - Oligonucleotides most stable around pH 8 (low pH → depurination; high pH → deamination)
  - Phosphate buffer – pKa = 7.2; buffering range = 6.2 to 8.2; negligible temperature coefficient; compatible with oligonucleotides
  - More buffering capacity required as [oligonucleotide]↑; 10-25 mM generally sufficient

Classified as public by the European Medicines Agency

Key Concepts

- Sterilization cycle development
  - EMA guideline
    - Indicators used for validation have a decimal decay value of ≥ 1.5 min at 121°C (D121 ≥ 1.5 min)
    - Initial bioburden concentration ≤ 100 CFU/100 mL (up to 100 CFU/filled unit)
  - Therefore, a cycle lethality of F_0 ≥ 12 min is required to achieve an SAL of ≤ 10^{-6}; target F_0 = 12 min during feasibility studies (and not the minimum F_0 = 8 min referenced)
  - Degradation occurs during temperature ramping (when temperature is < 110°C and there is no additional accumulation of cycle lethality); goal is to mimic the anticipated capability of the commercial equipment
  - Autoclave cycles commonly controlled by setting the hold temperature and time
    - Should include tolerances expected in commercial-scale equipment. Temperature band of 2°C is typical, therefore, use temperature at the top of the band (123°C)
  - No sense examining lower temperatures for longer times
    - Decreasing sterilization temperature by 10°C results in 10-times increase in time (to achieve same lethality)
    - Rate constant for deamination (first-order reaction mechanism, Ea = 25 kcal/mol, decreases by a factor of 2.3 going 121 to 110°C
  - Higher temperatures require much higher level of control of ramping and support pressure (and are likely not feasible in commercial scale equipment)

Classified as public by the European Medicines Agency

TS Conclusions

- For aqueous products, assess moist heat sterilization methods per the decision tree; irradiation or chemical methods not recommended
- Feasibility studies should be carried out using the most heat-stable formulation(s) available and container closure of representative materials of construction. Generate enough data to rule confidently on the feasibility of TS
- TS not recommended when any of the following occur:
  - Level of an impurity increases beyond its toxicological qualification limit (consistent with EMA guideline)
  - Significant changes occur to other CQA, e.g., appearance, visible and subvisible particulates, pH
  - Total degradation is sufficient to risk failure of lower assay release limit while factoring analytical and manufacturing variability (and overages are not recommended)
  - Chemical or physical changes to the container negatively impact its suitability or functionality. For example,
    - CCIT is adversely impacted (such that maintenance of sterility over the shelf life is compromised)
    - Unacceptable levels of leachables
    - Glass delamination

Classified as public by the European Medicines Agency

Acknowledgements

EPOC Steering Committee members

Justin Searcy, Ionis Pharmaceuticals

Classified as public by the European Medicines Agency