Framework for Evaluating Impurity Risks in Starting Materials for Oligonucleotide API Manufacturing

USP Oligonucleotide Subcommittee
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Speaker disclosures

- Presenting today on behalf of the USP oligonucleotide subcommittee
- Full time employee as Chief Technology Officer at Hongene Biotech Corporation
- Serve on the Board of Directors of Akte Therapeutics

Agenda

- Oligonucleotide Subcommittee Structure
- Presentation scope: Phosphoramidite Starting Materials
- Starting Material impurities
  - Current paradigm for categorization
  - Proposal for updated framework
- Conclusions
- Next Steps

USP oligonucleotide subcommittee

Subcommittee Members:

- Michael Verlander (Chair) – PQC Solutions
- Gerhard Haas – Bachem
- Marc Lemaitre – ML Consult LLC
- Jessica Stolee – Biogen
- Allison Wolf – Lilly

Scientific Advisors:

- David Butler – Hongene
- Dennis Rhodes – Ionis
- Hagen Cramer – Quralis
- Martin Gilar – Waters

USP Staff:

- Kevin Carrick
- Julie Zhang
- Sarita Acharya
- Nick Healy
- Manoj Metta (Oligo SC Lead Liaison)

USP oligonucleotide subcommittee

Objectives

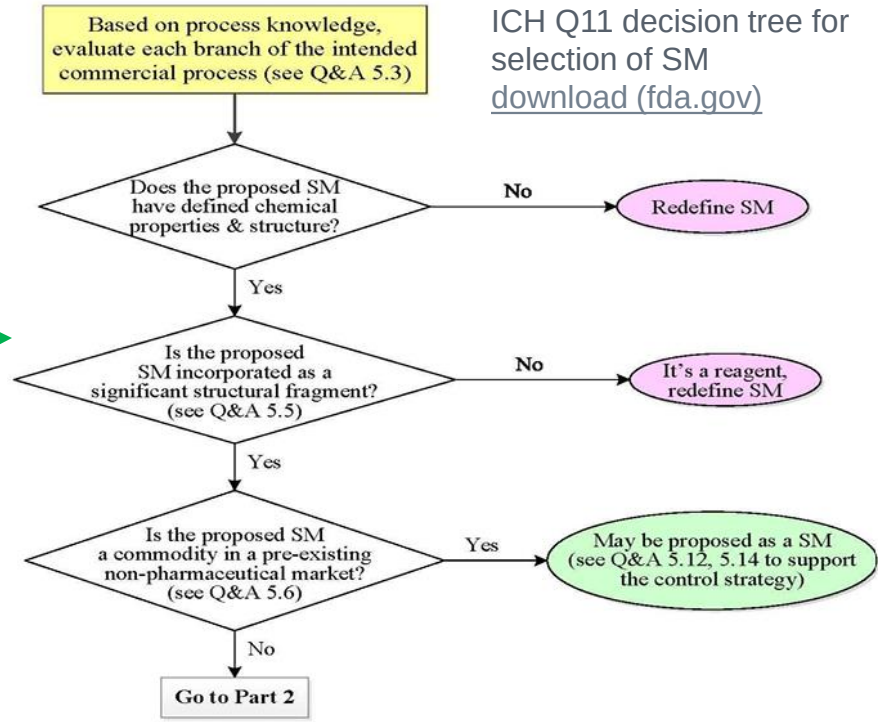
Standards	• Compendial and Non-compendial. • Prioritized considering impact on safety.
Starting Materials	• Identification, characterization, assessment of impact on patient safety, global quality improvement
Analytics	• Addressing challenges with method development for standards and impurity characterization
Collaboration	• With suppliers and other global stake holders on quality standards
Risk management	• Continuous monitoring and improvement

- Current oligo SC activities
  1. White paper: Quality attributes of starting materials for the chemical synthesis of therapeutic oligonucleotides
  2. **Updated framework for assessing impurities in oligonucleotide starting materials (SMs)**

Scope: Starting Materials

Phosphoramidites are the main SMs used in oligo API

- ICH Q7 – definition of SMs
- ICH Q11 – harmonizes criteria for selection and definitions of the SMs
- Selection of SMs need justification
- Tighter level of control for SMs vs other raw materials
- Important to understand how impurities in SM impact API quality

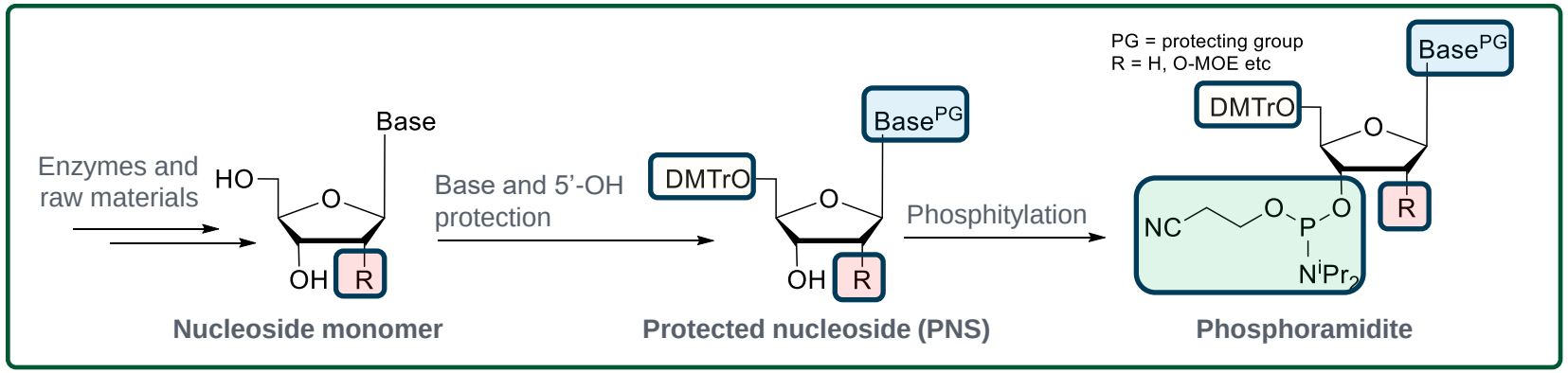


ICH Q11 decision tree for selection of SM
download (fda.gov)

- **Nucleoside phosphoramidites** can be justified as SMs for oligonucleotides
  - Others: nucleoside loaded support, conjugated moieties (e.g. GalNAc, peptides)

General phosphoramidite synthesis process

Each step is an opportunity for impurity formation



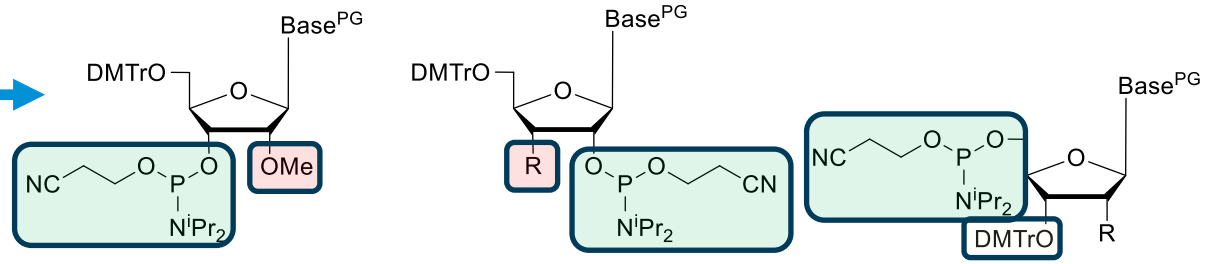
- Many possibilities for impurity formation along multiple steps
- Manufacturing processes are well understood and controlled at major suppliers for standard 2'-modified phosphoramidites (DNA, OMOE, F, OMe, OTBDMS, LNA, cEt)
- Quality attributes comprehensively controlled through specified testing, reported on COA
- For novel phosphoramidites, careful navigation is required by sponsors and partners

Categorization of phosphoramidite impurities

Current paradigm

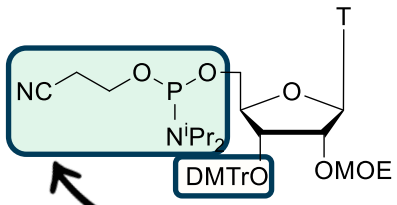
Category	Reactivity	Rationale	Examples
Critical	Reactive	Impurity in SM gives impurity in API that isn't subsequently purged	2'-OMe 2',3'-switch 3',5'-switch
Non-critical	Reactive	Impurity in SM gives API, or impurity that is subsequently purged	DMTr derivs PG derivs
Non-critical	Non-reactive	No possibility to be incorporated into API and purged during synthesis	H-phosphonates P-oxide

Examples of critical, reactive impurities in phosphoramidites

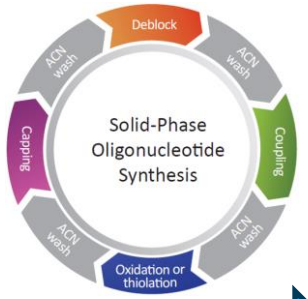


2'-OMe in 2'-OMOE | 2',3'-switch | 3',5'-switch

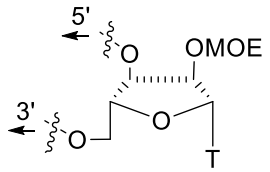
- These can be incorporated to give impurities in API
- Tightly controlled in phosphoramidite product specifications
- Synthesis processes are optimized to mitigate - Rarely see now
- Reference materials are synthesized for characterization



3'5'-switch impurity in MOE-T amidite



Example of critical reactive impurity incorporation into API



Inverted base in oligonucleotide API

- SM impurity is also a reactive phosphoramidite
- Can be incorporated at up to 5 positions* in this example
- 0.1% SM impurity → 0.5% impurity in API

Limitations of the current paradigm

- All critical impurities are grouped together independent of relative risk
- Researchers build their own databases of impurities
- No database of phosphoramidite impurities is available to the public

Proposal for an updated framework

Preferred outcomes

- Correlate SM impurities to oligo impurities and score risk
  - OSWG whitepaper (Capaldi et al, 2017)
- Database containing common SMs and impurity types
- Adaptable template
- Publicly available and downloadable

Summary of oligonucleotide impurity classes (Capaldi et al, 2017)

Class	API impurity	Examples	Safety studies?
I	Major metabolite	n-1 from ends Parent w/o conjugate	No
II	Naturally occurring	PO instead of PS 2',5'-linked RNA 2'-OMe in 2'-OMOE	No
III	Sequence variant	Internal n-1, n+1 C→U, A→I	No
IV	Not in parent or in nature	Unidentified impurities CNET	Yes

*Others have also made the same connection to Capaldi et al, 2017:
1. Rupp and Cramer, 2022

Or suggested subclassification of critical/reactive for risk mitigation:
2. amidite-impurity-classification-technote.pdf (thermofisher.com)

Proposed risk scoring

SM risk impact

SM impurity properties and impact on API				SM impurity risk	
Reactive	Impurity in API	Purged	Isobaric with API	Score	Current paradigm
Yes	Leads to class IV	No	Yes	5*	Critical
Yes	Leads to class IV	No	No	4	Critical
Yes	Leads to class I, II, III	No	Case by case	3	Critical
Yes	Possible in crude	Yes	NA	2	Non-critical
No	None	Yes	NA	1	Non-critical

*Any unidentified impurity is also scored 5

SM risk probability

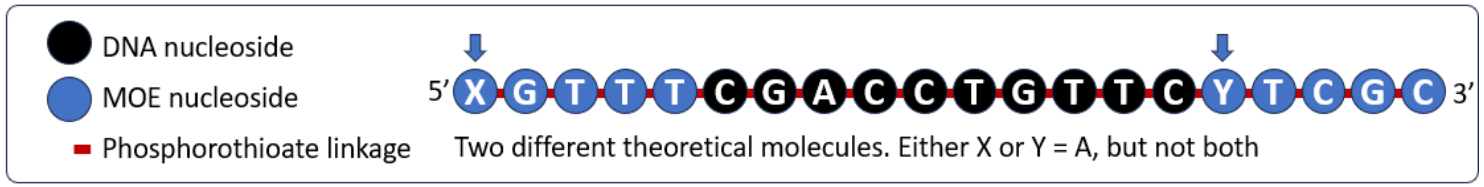
- Depends on the supplier of the starting material
  - Possible to build into the model in the future
  - Until supplier performance is better understood probability is binary (theoretically possible (1) or impossible (0))

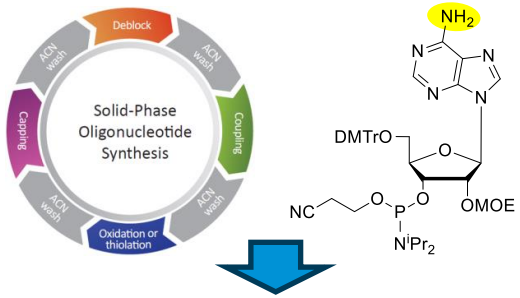
How to assess purging during processing

It depends on the molecule

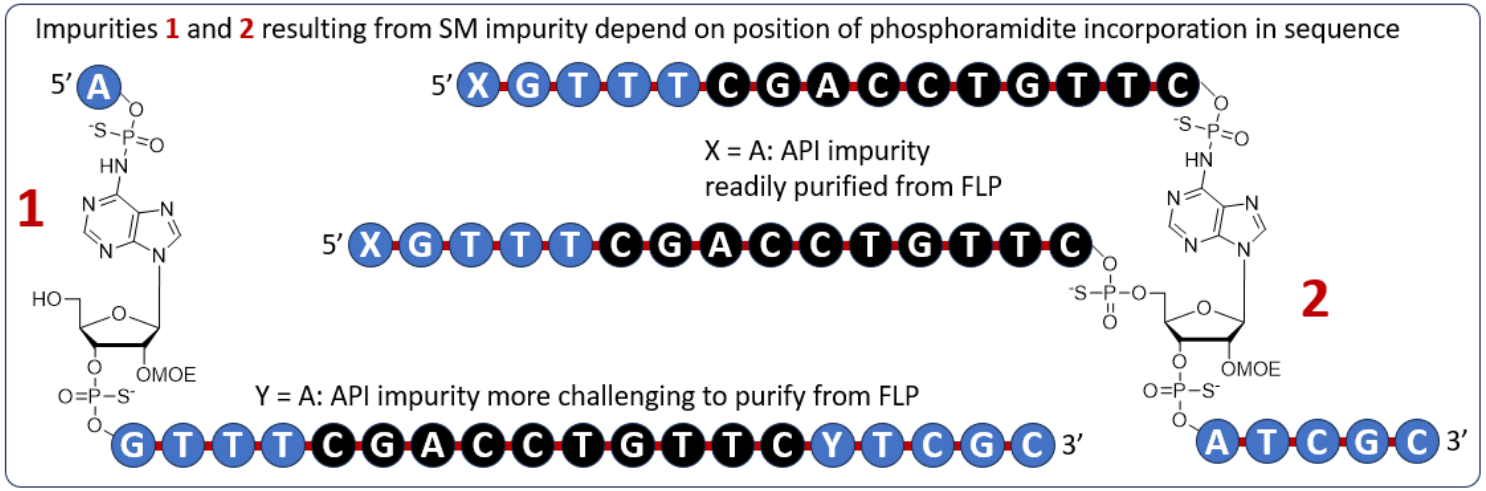
- Sequence dependence
  - Truncation → decreased MW & charge
  - Branching → increased MW & charge
- Chemistry dependence
  - Hydrophobic → increased retention
- Drug sponsor should assess
  - Incorporation position in sequence
  - Number of couplings for each SM
  - Likelihood to remove during processing
  - Based on theory and data

Example: 2'-O-MOE-A without Base protection





- Readily incorporated into oligonucleotide
- Oligo can extend from unmasked amine
- Position impacts the relative risk
- Assessed by drug sponsor



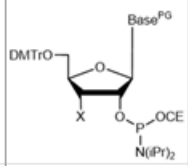
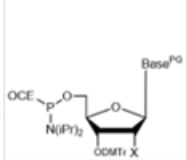
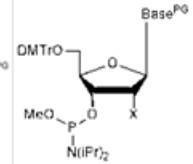
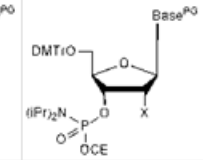
Potential control strategy for SM impurities

Risk score	Impurity property	Control strategy
5	Leads to isobaric class IV	Individual impurities NMT x%
4	Leads to class IV	
3	Leads to class I, II, III	Individual impurities NMT y%
2	Reactive purged	Total falls within purity specification limits
1	Purged	

- Highest risk categories are controlled most tightly: x < y
- x, y dependent on phase of development
- Novelty of chemistry should be considered (standard SM control is well established)

Database proposal with examples

- Database constructed in Excel
- TBD
  - Governance (oligo SC?)
  - Scope of SMs and impurities
  - Groupings of impurity types
  - Shorthand naming
  - Metadata to include (e.g. MW diff)
  - Verification and updating
  - Available for download?

		Impurity Type														
	Structure															
	Shorthand name	2',3'-Switch			3',5'-Switch			OMe amidite			iPrEt amidite			P-oxide		
	Risk scoring	I	P	RPN	I	P	RPN	I	P	RPN	I	P	RPN	I	P	RPN
Starting Material	DeoxyA(Bz)	3	0		5	1	5	3	0		2	1	2	1	1	1
	DeoxyG(iBu)	3	0		5	1	5	3	0		2	1	2	1	1	1
	DeoxyC(Ac)	3	0		5	1	5	3	0		2	1	2	1	1	1
	DeoxyT	3	0		5	1	5	3	0		2	1	2	1	1	1
	TBSA(Bz)	3	1	3	5	1	5	3	0		2	1	2	1	1	1
	2'OMeA(Bz)	3	1	3	5	1	5	3	0		2	1	2	1	1	1
	2'FA(Bz)	5	0		5	1	5	3	0		2	1	2	1	1	1
	2'MOEA(Bz)	5	1	5	5	1	5	3	1	3	2	1	2	1	1	1
	LNAA(Bz)	5	0		5	1	5	3	0		2	1	2	1	1	1

Conclusions and next steps

- Presented an updated framework for SM impurities from the USP oligo subcommittee
  - Risk-based scoring aligned with oligonucleotide impurity classes (OSWG)
  - Scalable database in excel, downloadable, adaptable for novel impurities

- Align with the oligonucleotide subcommittee on the path forward
  - Risk assessment scoring and control strategy
  - Whether to include and govern a database
  - Collaborate with major phosphoramidite SM suppliers

Thank You

The standard of trust