Early Stage Development
Slovenia
Sandoz Product Development

Complex Product Characterization and Analysis Challenges for Oligonucleotide and Liposomal Drug Products

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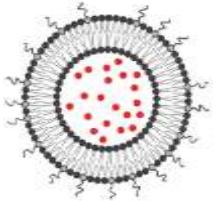
FDA 2021 GDUFA Public Virtual Workshop

SANDOZ A Novartis Division

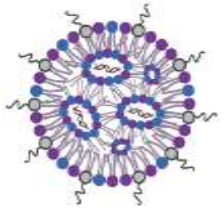
Outline

- Liposomal drug products and oligonucleotide therapeutics are highly complex drug products
- Examples of complexity of these drugs will be highlighted and demonstrated
- Oligonucleotide LNP formulations bring these two drug classes closer together

Liposomal drug product



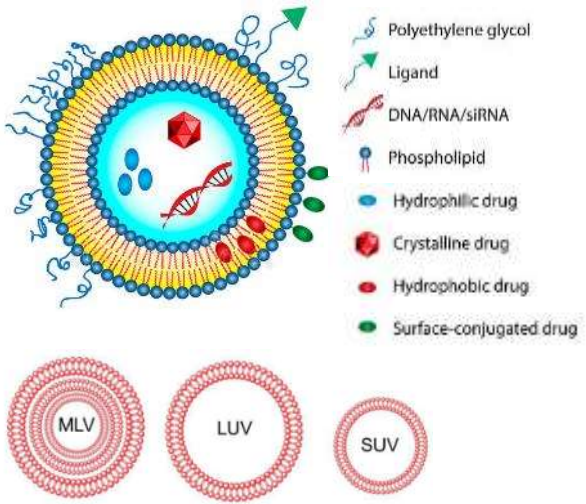
Oligonucleotide in LNP



Oligonucleotides (ds and ss)

Thi, T. T. H. *Vaccines* **2021**, *9*, 359.
Takakura, K. *et al. Int. J. Mol. Sci.* **2019**, *20*, 3331.

Liposomal drug products: current status



Pandey, H. *et al*. *Braz*. *Arch*. *Biol*. *Technol*. **2016**, *59*, e16150477

FDA Guidances:

General:

- Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation (April 2018)

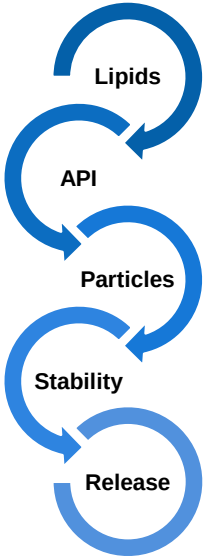
PSGs:

- Draft Guidance on Doxorubicin Hydrochloride (Recommended February 2010)
- Draft Guidance on Amphotericin B (Recommended April 2014)
- Draft Guidance on Daunorubicin Citrate (Recommended July 2014)
- Draft Guidance on Bupivacaine (Recommended February 2018)

liposomal products	Molecule	Doxorubicin	Amphotericin B	Verteporfin	Bupivacaine	Vincristine	Irinotecan	Cytarab/Daunor	Amikacin
	Product	**Doxil®**	**Ambisome®**	**Visudyne®**	**Exparel®**	**Marqibo®**	**Onivyde®**	**Vyxeos®**	**Arikayce®**
	Indication	cancer	Antifungal	macular degener	local aneastesia	cancer	cancer	cancer	Mycobacter avia
	Launched	**1995**	**1997**	**2000**	**2011**	**2012**	**2015**	**2017**	**2018**
	Generics (US)	**Dr.Reddy, Sun** (Gx since 2012)	/	/	/	/	/	/	/

Liposomal drug products
CQAs and characterization requirements

CQAs of liposomal drug products	FDA PSG characterization requirements doxorubicin example



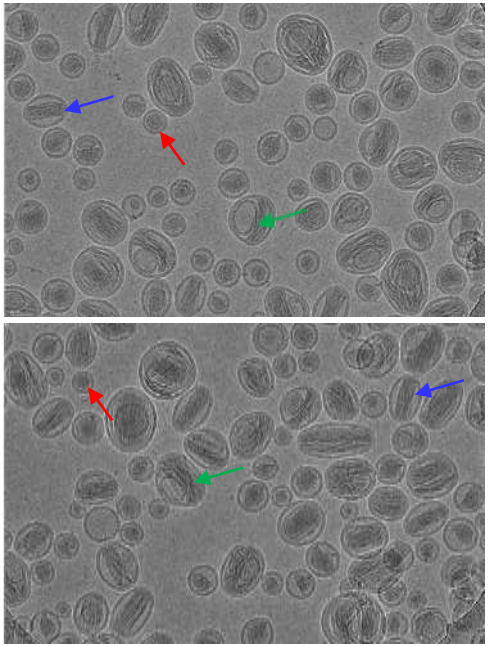
- Identification, quantification and characterization of lipids
- Quantification of entrapped, un-entrapped and total API
- Morphology and structure
- Mean particle size and distribution
- Surface charge
- Physical stability (e.g. aggregation)
- Chemical stability (lipid & API degradation)
- *In vitro* release kinetics of entrapped API

- **Liposome composition including lipid content**
- **Free and encapsulated drug**
- Internal and total loading salt concentration
- Extraliposomal buffer concentration
- The drug-to-lipid ratio and the percentage of drug
- State of encapsulated drug
- Internal environment: **volume, pH** ...
- Liposome **morphology and number of lamellae**
- **Lipid bilayer phase transitions**
- **Liposome size distribution**
- Grafted PEG at the liposome surface
- **Electrical surface potential or charge**
- ***In vitro* leakage under multiple conditions**

In addition to BE studies huge number of CQAs has to be addressed *via* characterization with advanced analytical technologies, which makes liposomal drug products difficult to develop and manufacture for generics.

U.S. Food, Drug Administration, Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation, 2018
U.S. Food, Drug Administration, Draft Guidance on Doxorubicin Hydrochloride, revised September 2018
Fan, Y. *J. Pharm. Biomed. Anal.* **2021**, *192*, 113642.

Liposomal drug products
Analytical characterization challenge



CryoTEM of an RLD

Equivalent liposome characteristics requirements in liposomal products PSGs:

- Liposome morphology
- State of encapsulated drug

Example of an RLD (3 different particle morphologies):

- Spherical particles filled with API (red arrow)
- Oval particles containing intraliposomal API precipitates (blue arrow)
- Distorted particles containing intraliposomal API precipitates and a bubble (green arrow)

Amount of these different particle types varies between different lots of RLDs

Key questions:

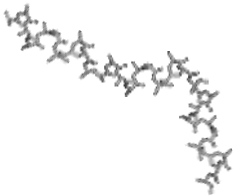
- What is the influence of heterogeneity of particle morphology and size on the performance of this drug?
- When should a generic product be considered similar to RLD?
- What kind of methodology should be used to establish such similarity?

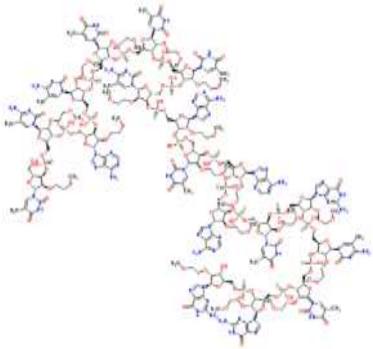
Noble, J. *et al*. *Microsc. Microanal*. **2019**, *25(S2)*, 1274-1275.

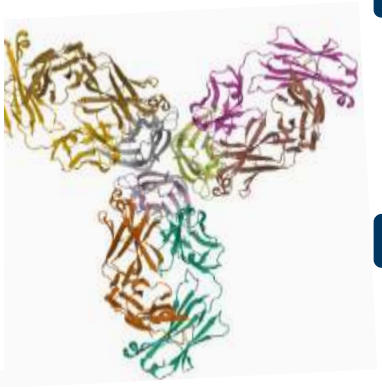
Therapeutic oligonucleotides: BigSmall Molecules

Small molecules	Peptides	Oligonucleotides	Biologics	Comparison of key features

Aspirin







Small molecules
~ 0.5 kDa

Peptides
~ 2 to 5 kDa

Oligonucleotides
~ 5 to > 16 kDa

Therapeutic proteins, mAbs
~ 15 to > 150 kDa

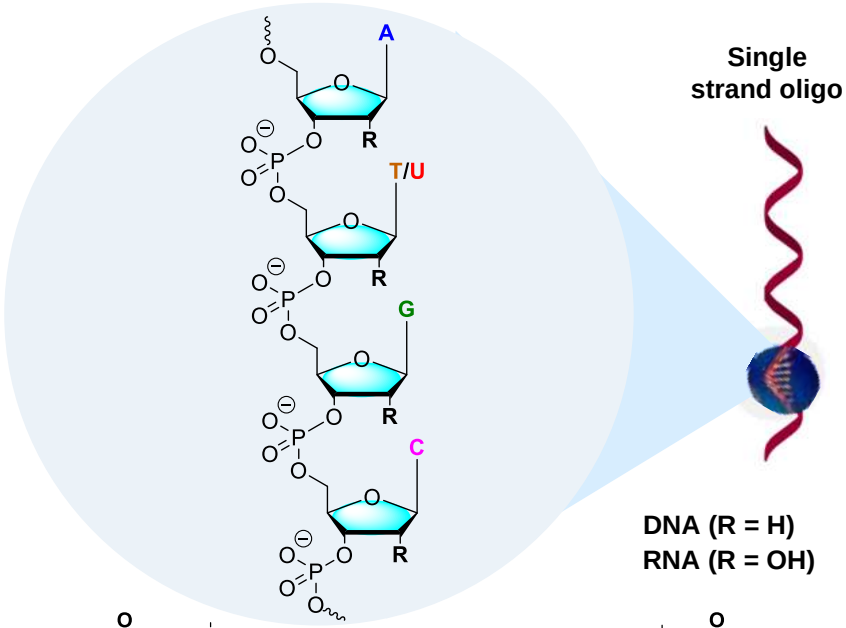
Similarity with large molecules

- High Mw
- Mixture of different related molecules
- Difficult to characterize composition/heterogeneity

Similarity small molecules

- Chemically synthesized
- Predictable chemical process
- Defined structure, independent of manufacturing process
- No higher order structure (except aptamers)

Oligonucleotides: chemistry and regulatory



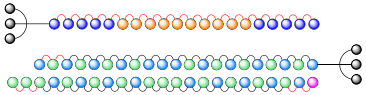
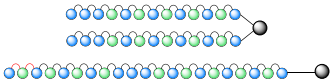
Oligonucleotides characteristics:

- Short DNA/RNA strands
- Typically ~ 15 to ~30 nucleotides
- Single/double strands
- Chemically modified to improve PK/PD
- Synthetically derived

Regulatory landscape:

- Regulated by CDER (chemical entities)
- No general CMC guidelines, specifically excluded from ICH Q3A, Q3B and Q6A
- Industry white papers provide suggestions
- Generics: no PSGs (nusinersen on the FDA list of upcoming PSGs)

FDA approved oligonucleotide therapeutics

FDF \ API	API diversity			
	Single stranded RNA 	**Duplex RNA** 	**Conjugates (GalNAc)** 	**Conjugates (other)** 
FDF diversity				
Liquids in vial 	**Fomivirsen** (Vitravene®) **Eteplirsen** (Exondys 51®) **Nusinersen** (Spinraza®) **Golodirsen** (Vyondys 53®) **Viltolarsen** (Viltepso®) **Casimersen** (Amondys 45®)		**Givosiran** (Givlaari®) **Lumasiran** (Oxlumo®)	
Combination products 	**Mipomersen** (Kynamro®) **Inotersen** (Tegsedi®)			**Pegaptanib** (Macugen®)
Lipid nanoparticles 		**Patisiran** (Onpattro®)		

FDA approval: **2016** **2018** **2019** **2020** **2021** **discontinued**

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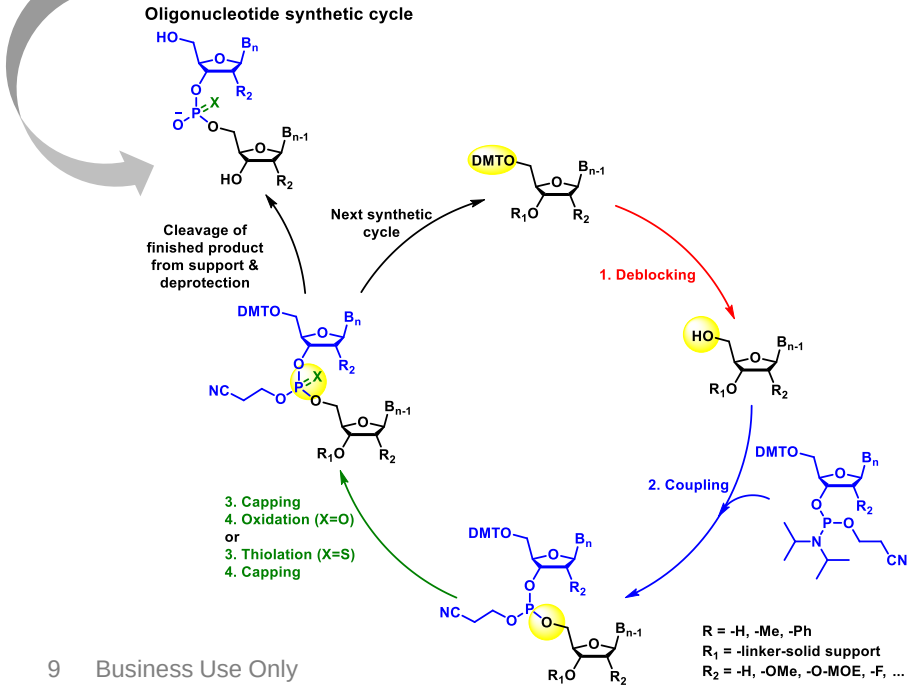
Oligonucleotides synthesis

Long synthetic sequence

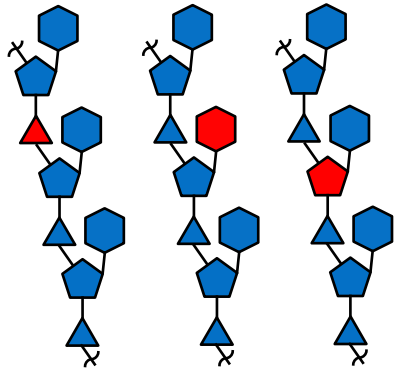
Synthesis → **Purification** → **UF/DF Concentration** → **Freeze Drying**



- Crude material is a mixture of full-lenght product (FLP) and closely related impurities
- Example: N = 20
  - ~ 80 synthesis steps required
  - 99% coupling efficiency results in ~ 82% crude material purity
- Challenging purification
- Extensive characterization with advanced analytics needed

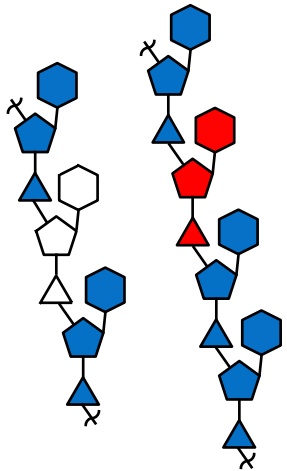
Oligonucleotide-related impurities

Modification at a single internucleotide linkage, base or sugar



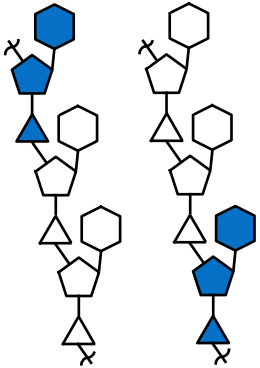
- Phosphate diester impurity
- Trichloroacetaldehyde impurity
- CNET impurity
- Abasic impurity
- Positional isomers, e.g., 2´, 5´ linked sugar in RNA

Impurities containing one fewer or one more nucleotide



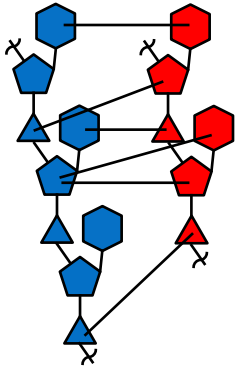
- N - 1
- N + 1

Impurities missing nucleotides from the 3‘ or 5‘ end



- Cleavage at abasic sites
- Capped failure sequences

Higher molecular weight impurities



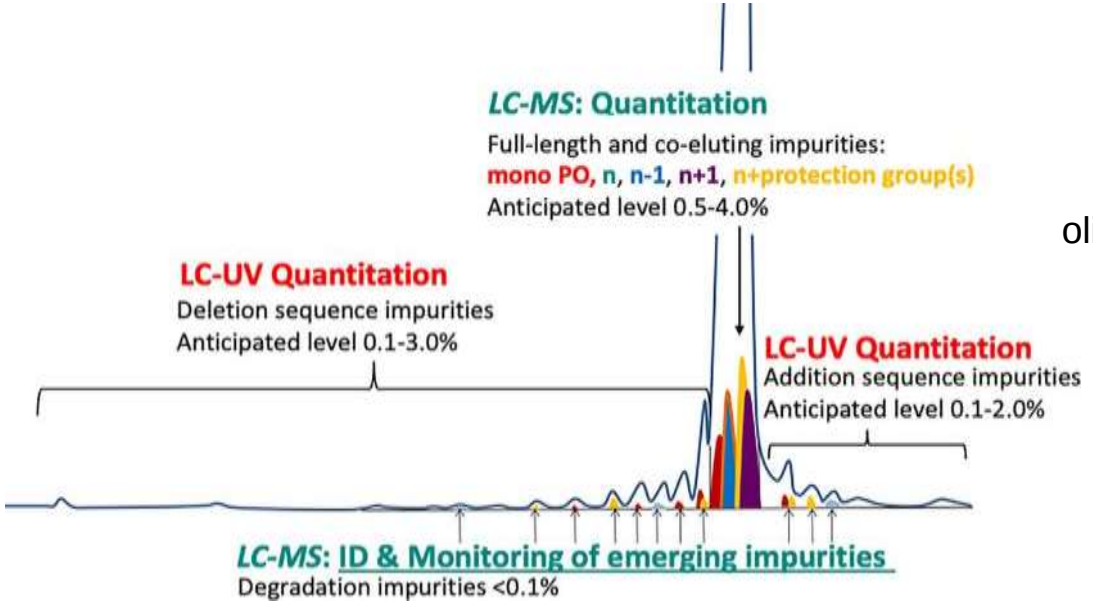
- Cyclobutane pyrimidine dimers (CPD)
- Interstrand cross-linking (ICL)

= sugar moiety = heterocyclic base moiety = P-linker moiety

Capaldi, D. *et al. Nucleic Acid Ther*. **2017**, *27(6)*, 309-322.

Oligonucleotide analytical challenges
Demanding impurity characterization

Impurity profile should be addressed by orthogonal chromatographic techniques and use of MS detection (including for routine testing in QC)



Analytical strategy to comprehensively determine oligonucleotide-related impurities using **IP-RP LC-UV-MS**

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Conclusion

- Liposomal and oligonucleotide drug products are already **well established** in the **innovator space**, but almost **nonexistent** in the **generic domain**
- Required **extensive analytical testing** beyond BE studies **delays generic development of liposomes**
- Development of **understanding**, which changes in **physico-chemical characteristics** actually **impact BE** outcomes
- Development of **standardized analytical characterization** methods for liposomes (morphology, *in vitro* release, stress-testing)
- **Establishment of PSGs and general guidance** for oligonucleotides would accelerate development of generic products
- Research on CQAs to demonstrate oligonucleotide **API sameness** and guidance on **acceptable levels of impurities** would be required
- Development of **standardized methods** for oligo purity/impurities characterization

Thank you

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