Table 6: Gene Therapies and Vaccines

Scope

This roundtable focus on nucleic acid related products used in gene therapies and vaccines. CRISPR/Cas, antisense or interfering RNA or mRNA containing products are commonly delivered via vectors to the patient's body. Therefore, either lipid nanoparticle or viral vectors are used, creating a new complex system for analytical tasks. Common defined critical quality attributes are associated on already known strategies (e.g. used for proteins). Whilst general CQA analysis as safety (endotoxin, bioburden, sterility) might be applicable in similar ways also for the new systems, others CQAs represent challenging analytical tasks with the need of adaption of existing methods or development of new methods. Drug substance related CQA include identity, purity/impurity and quantity of RNA and if present characterization of structural features as modifications or capping/tail analysis. For drug product CQA include vector composition, size and behavior (e.g. charge distribution or zeta potential), as well as encapsulation efficiency/quantity, RNA integrity in formulated system and in general stability. Finally, meaningful potency assays have to be defined to prove manufacturing uniformity, safety and efficacy.

Questions for discussion:

1. What are the important critical quality attributes in your opinion? Is the above-stated list concluding or which hidden/new attributes should be considered?

2. Which type of techniques do you commonly apply and which techniques are missing?

3. Which combination of analytical techniques should be used to characterize RNA associated products?

Discussion Notes

The discussion focussed on NA-based vaccines and was guided by the following questions:

- What are the important critical quality attributes?
- Which type of techniques do you commonly apply and which
- Which combination of analytical techniques should be used to characterize RNA associated products

The group tried to define CQAs for the drug substance (Nucleic Acid), for the formulation (particle shell), and for the drug product (Nucleic acid inside the particle as well as the particle with the nuclei acid).

There was agreement, that the CQAs were not yet fully settled for NA-based vaccines. The USP draft guideline on "Analytical Procedures for mRNA Vaccine Quality" was used as a "reference".

CQAs considered for DS

- Identity/ Sequence
- Purity
    - Process-related & Product-related
        - Are methods sufficiently discriminatory (peak separation)
            - How far should one go
            - What is an impurity?

CQAs considered for DP

- Sequence
- Encapsulation of nucleic acid (content)
- Composition of LNP (Lipid Nanoparticles)
- Total RNA versus free RNA
- Stability
- Safety/efficacy

The testing panel on mRNA vaccines was considered to depend on

- Stage of development of product
- Disease to be treated (i.e cancer versus vaccination against infection)
    - Time pressure associated with severity of disease
- "transferability" of method to a QC lab for routine release

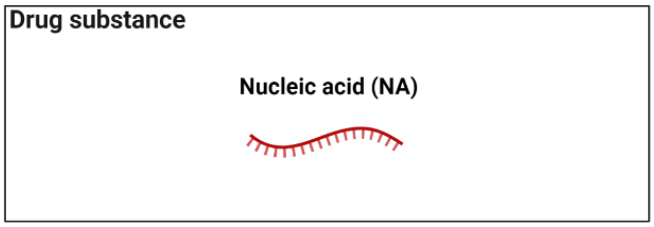
Panel of methods discussed

- CE/CGE
- Mass spectrometry
- LC
- DLS
- PCR
- Chromatographic methods (e.g. SEC)
- Spectrometric methods
- Potency
    - Protein expression in vitro

The need of orthogonal methods

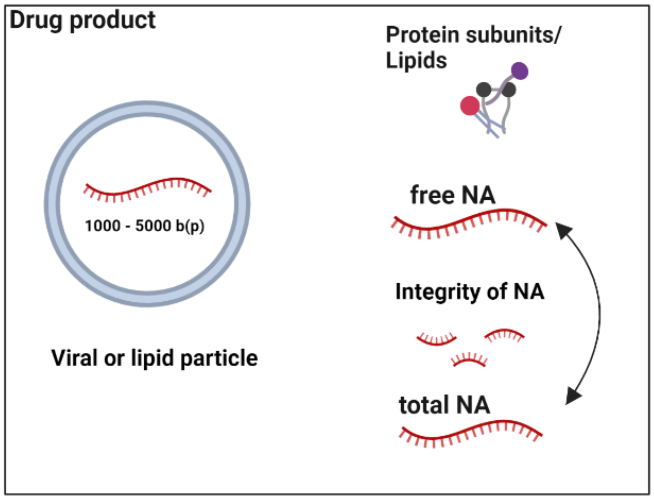
Further thoughts on the topic

- Choice of critical attributes to be assayed depends on level of knowledge of structure-function relationship
- One CQA may be assayed differently depending on purposes
    - i.e. sequence
        - for identity confirmation
        - for sequence confirmation (integrity, purity, etc.)
- How relevant is testing of surface charge?
- How helpful are guidelines for a particular product?
    - Is it necessary to always apply all proposed methods for all generally proposed quality attributes
    - If a structure-function relationship is not yet established is it necessary to test all potential quality attributes ("back-up scenario")



CQAs Drug Substance: *Nucleic acid (NA)*

- *Identity/sequencing*
- *Purity*
- *Product related impurities*
- *Process related impurities*



CQAs Formulation:
Particle shell

- Identity
- Composition

CQAs Drug product:
Nucleic acid within particle

- Identity
- Integrity
- Quantity/Localization

Particle with NA

- Encapsulation
- Lipid content/composition
- Stability
- *Safety*
- *Potency*
  --> Structural-functional relationship