Table 11: mRNA Characterization by MS – What Are the PQAs and What Are the Methods

Facilitator: Sreekanth Suravajjala, *Amgen Inc., Cambridge, MA USA*
Scribe: Bingchuan Wei, *Genentech, a Member of the Roche Group, South San Francisco, CA USA*

Scope:
As the world was grappling with COVID-19 pandemic, the mRNA-based therapeutics development technology emerged from the shadows and heralded itself as the novel class to take on the challenge of mitigating the viral disease. Since then many research teams, organizations have taken a lead on using this technology to develop drugs to treat range of diseases from influenza to HIV using this modality. As development of this novel class of drugs takes a stronghold within the biopharmaceutical space, there are many questions that haunt scientists and regulators alike –

Questions for Discussion:
1. What are the target product profile (TPP) and product quality attributes (PQAs) of these drugs? What type of the control strategy shall we implement for mRNA-based therapeutics?
2. What tools are required for measuring of mRNA and its delivery system? Is the 'current' analytical toolbox enough for deep characterization of these mRNA-based therapeutics?
3. How does mass spectrometry help with the characterization of mRNA-based therapeutics? Are the current instruments and methods capable of detecting changes and informing quality decisions?

Discussion Notes:

· mRNA-based vaccines show its capability in fighting the global pandemic of COVID-19. This is one of the hottest topics in the field. The round table discussion focused on the product quality attributes of the mRNA based therapeutics

· Although there are some approvals from FDA on the COVID-19 vaccine from Pfizer/BioNTech and Moderna. All people from the roundtable discussion admit that we are currently still exploring the TPP of mRNA based therapeutics with limited knowledge.

· The delivery system (lipid nanoparticles) are also an important part of the drug product. The characterization and control of the delivery system is also an essential part of the overall control strategy.

· For the mRNA drug substance, several important PQAs are discussed including 5'-end capping, 3'-end PolyA tail, sequence identity and RNA integrity. There are potential

shortmers and process related impurities which can also be quality attributes of the drug substance.

· For drug products, the RNA content and encapsulation efficiency are two important quality attributes. There are also three different components in the lipid nanoparticle, which is currently measured by HPLC-CAD.

· Cryo-EM, AUC and electron microscopy are other physicochemical characterization tools to characterize the intact lipid nanoparticles.

· mRNAs can be used for applications other than therapeutics. PCR based assays is still one of the most commonly used tools for analyzing the mRNAs.

· MS has a good potential in studying lipid nanoparticles. Previous MS-based tools developed for metabolomics can be applied to characterize lipid nanoparticles.

· Lipid characterization part of the charge. HPLC-CAD, ELSD tools. From DS to accelerated stability .Complex biochemistry will happen in mRNA. Interaction between the lipid and mRNA. Mass spectrometer based method to lipid.

· The cationic lipid used in LNP has limited choices because of the limitations of intellectual property. In-license is the most common route. It can have a huge impact on immunogenicity and encapsulation efficiency, which needs to be well characterized.

· One potential challenge for in-licensed gene therapy programs is the difficulty in tech transfer. Sometimes redevelopment of certain process steps is required.

· All people from the roundtable discussion thinks bioinformatics is one of the challenges to use MS for mRNA therapeutics. The parallel digestion strategy needs to be used for mRNA analysis.

· MAM for protein therapeutics takes a long journey to be implemented. The application of MS on mRNA might take a similar approach.

· mRNA can have a complex higher order structure. Ion mobility MS can be a useful tool to study it.

· For other mRNA therapeutics other than the vaccine, a more reliable measurement by MS because the exposure of impurities might be higher because of the higher dose.