Roundtable Session 1 - Table 16: Role of Cryo-TEM as an Orthogonal Method to Help Assess Formulation Purity, Potency for Nanoparticles

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Abstract:

Cryo-EM is a powerful tool for protein and particle characterization and has proven its value by validating the structure/activity relationship between SARS-CoV2 Spike Protein and the ACE2 receptor. The roundtable will address the following questions:

Discussion Questions:

1. How have you used cryo-EM for protein/particle characterization?

2. What examples can you cite where the method confirmed a structure/activity relationship?

3. What are the challenges in using cryo-EM?

4. What is your experience in putting cryo-EM data in regulatory filings? How did the regulators respond?

5. How can cryo-EM be used to check formulation purity?

6. How can cryo-EM be used as an orthogonal method for potency?

Notes:

Characterization method that gives insight into structural elements that give insight into activity/potency/purity

1) *How have you used cryo-Em for protein or particle characterization?*

   CRO with much experience in this activity. Nano-imaging

   Curiosity on how others are using from the table.

   Protein based vaccines to understand if the protein particle – folding, aggregation,

   Especially useful if there are multiple components and can assess the different components.

   I.e. Covid-19 spike protein- first visualized by Cryo TEM and very helpful in understanding epitopes and targets for a vaccine.

How did people learn it has more use than structural studies??

Came into limelight in 2017 with Nobel Prize and seeing practical purpose.

New equipment with higher resolution became available along with automation to speed the work. But still a time intensive technique. During Covid-19 pressure to speed, but it seems to be going back to longer timelines.

Cost and awareness of the strengths of the technique may be limiting the use of the method.

Anything with a large particle, understanding the dynamics of the particle and the surface properties can be covered by cTEM.

Can determine laminarity of Lipid Nano-Particle in the data.

Merck- uses heavily as a characterization tool in vaccines to look at particles.

Use is development focused.

Does anyone use it in a quantitative way to assess comparability? It is possible via morphology, sizing, AAVs and how many are duplicates or triplicates. HPV and changes to morphology is another example.

CryoEM is dependent on the number of images you take. Was used in comparability during the pandemic for vaccines.

Can be conducted GMP- Comparability exercises to compare generics to originator.

AI software is used to determine particles full/empty.

2) *What examples can you site where the method confirmed a structure/activity?*

Structure function is a big topic for vaccines. Not clear how many clinical studies are designed to support this. Potentially in pre-clinical studies, but in clinical performance the table does not have an example.

RNA LNP vaccines with or without blobbiness it is not clear if there is demonstrated correlation to function.

If there is an animal study you can assess it, but in the end the clinical study is key.

Very important for structure- control to consistently provide the particles. Structure/function is difficult for vaccines.

3) *What are the challenges?*

Both outsources and in-house are used. Low throughput and can be slow.

When preparing for freezing and can induce sample changes.

Robots often used for reproducibility, not in a laminar flow hood, but main challenge is avoiding ice crystals during freezing. (Dry room- humidity controlled)

Precipitation at the air-water interface can occur, presence of sucrose in the formulation can make freezing difficult.

Protein cannot be too small 50-60 KD it cannot be too flexible as it is an averaging technique. Membrane proteins need to be reconstituted in a micelle.

4) *What is your regulatory experience?*

Primarily used for internal product understanding. It has been submitted in regulatory filings. Keep in mind that the regulators are scientists, but may need additional information to lead them through the method and the data. Pictures are powerful and can be beneficial. Especially for characterization.

5) *How can cryo-EM be used to check formulation purity?*

Perhaps to optimize the formulation for delivery of the payload?

Antigen expression on the surface of a particle can be managed well with cryo-EM.

DLS is a good screening tool, and biased by large particles. Cryo-EM could verify the final results.

Liposomes lamminarity cryo-EM has been used to assess and determine this, and has been used in regulatory filings. FDA has specifically asked for this type of data.

mAbs and aggregation- many publications on this for assessing types of aggregates.

IgMs as very large would be very amenable for cryo-TEM.

6) *Can cryo-EM be used for orthogonal method for potency?*

No, not yet from industry. You need to know which attributes are most important.

If protein confirmation is known to impact efficacy i.e. spike protein changing confirmation on binding to receptor- dramatic changes.

Cryo-TEM can inform the potency, but not enough by itself based on roundtable feedback.

Neutralizing antibodies against one confirmation, no activity. Antibodies needed a specific confirmation.

Higher order structure has a high impact, for example HPV. If you can find attributes important for function it is like gold, but linking these to potency is difficult.

mAb for HPV was very specific to HPV confirmation, find the epitope mapping to evaluate the approach.

Similar in how you would use AUC for particle assessment.

Biggest challenge is that it is not well known and the method lead time is long.