Table 4: Sub-visible Particle Characterization

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Scope:

Sizing and counting of subvisible particles in therapeutic protein formulations continues to be an important measurement task, owing to the possibilities for particles to generate a variety of undesirable immune responses. Particle characterization for cell-based therapies is also a critical task. A number of techniques for characterizing particle size and quantity are being used for this problem, including light obscuration (LO), flow imaging (FI), Coulter, and light scattering, with new methods such as membrane-imaging, holographic imaging, and resonance mass finding increasing use. Use of these technologies varies according to stage in the product life cycle, from discovery and formulation, to clinical trials, and to lot release and stability testing. We will discuss phase-appropriate use of the technologies, issues surrounding deciding on appropriate measurement methods, harmonizing measurements from different methods, use of standards, and setting lot release criteria.

Questions for Discussion:

1. Where are we with knowing what particle attributes relate to health outcomes? What size range particles are the most important and are there additional particle attributes that would be useful to quantify?
2. What measurement tools are most appropriate for the different stages in the product life cycle? What constraints on the measurement method are there for the various stages, i.e. sample size, complexity of the instrument/analysis, reproducibility across instruments, operators, and locations, detail in the information produced?
3. How do we transition to new methods, when it becomes clear that new methods provide better measurements which would translate into improved health outcomes? What are the barriers: technical, economic, legal, legacy to moving to new methods?
4. How do we connect data sets from different instruments, (for example light obscuration and flow imaging)? What kinds of standards and internal reference materials are used to validate measurements?
5. What are some desires and expectations for future capabilities for particle measurement technologies? For example, using advanced image processing methods to attempt particle type identification.

Discussion Notes:

Standard approach for subvisible particle characterization: according to the Pharmacopeia by light obscuration at lot release. In addition, particles are characterized in the micron range (2 - ≥25 um).

No direct health impact but particles may trigger protein aggregation, which leads to immunogenecity. Hybrid particles (silicone oil coated with protein) might trigger immunogenecity. Need to be able to measure hybrid particles.

Sub-micron range: possible direct health impact, triggers immunogenecity. Methods people are using: Single-particle mass photometry, Archimedes, Accusizer.

Recent improvements in techniques: use of machine learning in the categorization of particles according to images from flow imaging techniques. Refs: publication Ted Randolph's group See: https://doi.org/10.1016/j.xphs.2017.12.008

Also, different stress conditions have been used to generate particles. Particles generated in different ways have different propensity for immunogenecity. Advanced image analysis may be able help classify particles by stress-type.

Observations about imaging techniques: various factors have to be taken into account when characterizing particles. E.g. shape is not always enough to be considered.

Desires for future capabilities: high-throughput methods, which use low sample volume, e.g. BMI needs less volume than MFI. Other low-volume techniques: holographic characterization is an emerging technique. Low-volume methods are especially important for early stage, and for characterizing aggregates in cell-treatments. Any situation where available sample is limited.

Transitioning from one measurement technique to another one is not a common practice. It is challenging to directly correlate results from different techniques.

Switching between instruments/labs: use of of NIST standards is an applied practice for bridging.

Drive for particle characterization and new techniques within the industry: understand the clinical impact and safety concerns related to particles. Increasingly demanded by health authorities. There are still open questions about the immunogencity of different types of particles.