Table 2: Characterization of Protein Aggregates

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SCOPE:
Aggregation is frequently a concern during the manufacture, processing, formulation, storage, and transport of protein based therapeutics. There are a number of existing techniques and experimental approaches that can characterise aggregates and in turn, multiple ways of improving the degree of mechanistic understanding of the factors that drive aggregation. This discussion will focus on the most important areas where aggregation is of primary concern - since this drives the technical context of the characterisation methods (eg. concentrations, purity/presence of excipients, physical conditions). Is it more important to characterise and control at the point of manufacture, or during subsequent formulation and storage? Does the presence of excipients make the job of characterisation harder, and if so which excipients and how can their effect(s) be overcome? The discussion will then focus upon existing as well as nascent characterisation methods that may be applied to this measurement. What are the most important limitations of currently available tools (eg. based on sedimentation, chromatography or spectroscopy)? How are these tools improving in response to known difficulties (eg. improvements in sensitivity, reduction in the volume of material required for the tests, improving the fidelity of the tests, increased throughput of measurement? What else?). Also, what are the most promising "new" characterisation methods that are becoming available? What factors are driving their development, and what do we predict their performance will be? What research and development challenges should be addressed first and why? The workshop focus will be on characterisation methods in the context of the stage where the questions arise but important "peripheral" subjects will also be included, for example how is the primary data from the method interpreted, how is the data stored and analysed as well as how is the link made between the characterisation method and the fundamental science?

DISCUSSION NOTES:
Where is aggregation the most important challenge?
- all of the above
- in MF it is relatively easy to pass a batch through with <5% aggregate. Can control to 0.5%
- in MF we have to play it safe until we know how to predict/test whether the aggregate is immunogenic or not, as this is what really matters.

- need to know ***what type*** of aggregate you have, as this affects safety. Only then can we work out which are the problem types.

- need post marketing surveillance – of immunogenic responses as it may be very rare (eg 1 in 100,000).

- need more evidence on relationship between aggregates and immunogenicity

- PEGylation – may cause aggregation in some cases. Reversible though.

- Need to be able to assess reversibility readily. SEC may not catch the delayed dissociation.

- SEC is good for picking up same MW but different conformations – particularly by UPLC. However, other methods (FTIR, CD, fluorescence see nothing). Only NMR might pick it up.

- Hierarchical approach. SEC then DLS, then LCMS-HDX.

Do excipients play a role? in disturbing these conformational effects / analytics?
- polysorbates – affect the SEC-MALLS.

- CHO cells can make lipases that degrade the polysorbate (Amgen paper?). Monitor persistence of enzymes in product by ELISA.

Does anything else limit characterisation?
- oxidation
- Have seen oxidation by SEC.
- Timing – samples need to be distributed.
- In Canada – must be able to distribute at 25C (not 4C).

Any new developments improving analytics

- HALO labs – getting to very small drops.

- what about very HMW – assume to be filtered out by SEC?
- AUC shows more aggregate
- AUC may be able to see more different particles
- Assume AUC is more accurate as requires less sample prep / interferes with sample less

What new techniques might be approaching?:
- magnetic resonance – eg looking at water signal.
- Relaxometry makes a comeback
- 2 dimensional separation (Agilent)

- soluble aggregates are the challenge.
- but difficult to characterise larger aggregates morphology.
- MFI OK but doesn't tell you how they got there.
- MFI also cannot pass the really large particles.

single methods with nm-10um dynamic range in sizing needed.

what should we develop next?
- wider size range
- sensitivity – some particles may be 0.001% but too large so they block filters.
- sensitivity in terms of accuracy would be good to improve confidence in measurement.