Table 1: Biological Consequences – Function, Safety, and Other Issues.

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SCOPE:

Establishing the biological consequences of (changes in) the HOS of a protein API remains challenging. This roundtable session will provide attendees the opportunity to discuss how function, efficacy and safety of protein drug products are related to the HOS of the API. The list of questions below may serve to guide a casual and collegial discussion, during which participants can share their insights and propose answers to these and other pressing questions.

QUESTIONS FOR DISCUSSION:

- How are biological consequences of HOS addressed in a drug development program (process development, formulation development, manufacturing)?
- Besides biophysical characterization, is any biological testing carried out to ensure HOS integrity?
- Are direct or indirect methods used to ensure that HOS is maintained?
- Is (or should) HOS be assessed for every lot produced to assure safety and efficacy?
- Are results from biophysical methods compared with those of bioassays?
- What about the relationship between HOS integrity and propensity to aggregate, and immunogenicity? Are there any known case studies that can be discussed?
- Are studies performed to correlate the effect of HOS on immunogenicity?

NOTES FROM DISCUSSION:

- At the start of the discussion, everybody agreed that finding a speaker to discuss this topic at the conference is very challenging.
- A paper from Dintzis (Dintzis et al., PNAS 73(10):3671-5, 1976) was discussed suggesting that both the number and spacing of epitopes within an antigen affect immunogenicity. Examples were discussed, e.g., with insulin where hexamer can polymerize when injected subQ without causing any apparent immunological response at the site of injection.
- Heparin causes thrombocytopenia by binding to platelets via a tetramer, the polysaccharide induces a change of secondary structure that can elicit a non-self immune response next time the patient is exposed to heparin.
- Methionine oxidation on the CH2 domain next to the hinge region of a mAb was shown to increase binding to C1q, thereby accelerating the recruitment of macrophages, while binding to its intended target resulted a lower affinity. The oxidation causes a lowering of the Tm of the Fc.

- Immunogenic ADR are feared to be more frequent as sponsors are moving towards subQ injections, away from IV due to the formation of aggregates at the site of injection. However, there is no strong evidence that subQ administration is always more immunogenic, and proteins that are administered IV also can cause immunogenicity (e.g., infliximab).

- Should we care about the structure of aggregates? Several papers indicate that the nature of the aggregate matters, i.e., the conformation of the protein matters to elicit an antibody response.

- Are antibodies (non neutralizing) a natural way to clear the body from wrongly folded proteins?

- Gravitational AUC is a potential technique to characterize nanometers aggregates.