Table 3: Host Cell Proteins – Identification, Strategies, Successes and Challenges

Facilitators –

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Scope:

Host Cell Proteins (HCP) are process related impurities found in many biological products. No matter the process, a subset of HCPs will co-purify with the intended molecule. Considering HCPs can present a potential clinical safety risk, clearance and monitoring of HCPs are a critical aspect of the drug substance manufacturing process. In addition, HCP characterization can be part of a strategy to demonstrate comparability arising from the implementation of a manufacturing change. Health Authorities are focused on analytical methods to detect HCP, including coverage. Challenges arise given there is no universally available and accepted testing modality or standard in the industry. Please join the HCP roundtable to have a general discussion on HCP related topics.

Questions for Discussion:

1. HCP detection can improve as new technologies (e.g. ELISA bio-reagents) are utilized. This can lead to improved coverage.
    a. How is coverage evaluated?
    b. Is there a phase appropriate strategy for HCP analysis (commercial kit vs in-house assay)?
    c. If more HCP are detected, is there a requirement to revise specifications? How have attendees handled regulatory implications of revising specifications?
2. ELISAs are the most common analytical method for detecting HCP. However, industry is starting to apply orthogonal approaches including Mass Spec (MS).
    a. Has Mass spec been used for characterization only? Routine testing?
    b. Have attendees received request from regulators to file MS results
3. HCP characterization is typically a part of the comparability strategy to demonstrate comparability of the pre- and post- change material.
    a. What approaches have attendees used as a part of HCP characterization to de-risk changes made to the DS manufacturing process?
    b. What do attendees consider to be a high degree of HCP similarity between pre- and post- change material?

c. How are attendees handling risk assessments when HCP profiles of pre- and post-change material differ?

Discussion Notes:

January 25 –

Introduction: Discussion on comparability studies during process changes and HCP characterization

- Most companies use generic in Phase 1 and 2 and even for BLA submission but commit to developing process-specific HCP assays post- licensure
- GSK uses CHO and E. coli generic and in-house developed platform CHO HCP immunoassay using Octet platform and ELISA platform
- There is a notion that regulators expect 80% coverage – especially with process-specific ELISA
- HCP characterization by MS is not required by regulators but it produces a "good to have" set of supplemental data
- J. H. asked if it is required to monitor specific HCPs. This might be important for those HCP that might potentially lead to product aggregation or other stability issues
- GSK had to monitor for a specific HCP for several lots of a DS
- All participants agreed that MS is a great orthogonal method but it is difficult to implement it as product lost release method in a GMP/QC setting
- MS is extensively used during process development
- Risk assessment: do you ID "trouble" HCPs and how to share this data with regulatory agencies?
- Issued discussed were around (1) characterization based on the stage of development and availability of clinical data; (2) how to assess biological activity of an HCP: immunogenicity, previous experience with similar/homologous proteins when a DS administered in clinical trials; (3) abundance threshold
- Discussion on whether you ID HCPs, how do you present these data to an agency?
- How do you assess HCP risk: (1) in vitro assays, (2) in silico modeling, (3) risk-assessment tools such as biological activity, immunogenicity, clinical factors?
- J. H. shared previous experience when one of the HCPs caused DS aggregation that was found 7-8 months post lot release. Even at a very low concentration, lipases can cause this

phenomenon. Novartis is building a data base of various problematic HCPs to proactively ID HCPs to assess process changes.

- Another question that was discussed: Comparability in process change and whether sponsors were asked to show MS data to support their conclusions. Merck tried to proactively assess changes in HCP profile based on trending data in lot release method.

- Participants discussed methods to automate immunoassays. Seagen uses Gyrolab system for HCP analytics in process development and are trying to move it into quality control.

January 27 –

Introductory questions: 1) what are industry expectations for HCP Ab coverage? 2) HCP clearance especially in relation to potential degradation of PS40

- Product development – generic HCP ELISA coverage around 70%

o HCP footprint determined by MS and ELISA

o Post-licensure – more HCP characterization is expected

- Specifications are not well defined: mAB <10 ppm is typical now

- High-throughput HCP analytical methods? GSK is utilizing MS fir HCP analysis in process development with focus on identification, however, this is not an HT method

- MS plays supporting role in identification of problematic HCPS

- Gyrolab platform is used in process development while process-specific HCP ELISA is used in Phase 3

- Discussion on acceptable levels of PLBL2

- Discussion on fed batch vs perfusion cell culture and the effect on HCP profile (questions asked by Inn Yuk, Genentech).

- As shared by M. A., they submitted a paper on "Impact of next-generation high productivity perfusion cell culture process on host cell protein profile and a comparison with fed-batch cultures" Conclusions from the preprint are as follows: The group found that clear differences in HCP levels were observed between steady state and non-steady state cultures, as well perfusion and fed-batch culture. Fewer HCPs were detected in SS perfusion process, likely due to the high viability and lower cell counts in steady state. Even though the level of HCPs in the final drug product would be very low (1-100 ppm), those trace amounts of difficult or problematic HCPs may affect the quality attributes of mAb1. To characterize the HCPs potentially affecting mAb quality, the detailed analysis of HCPs in the culture supernatant of culture processing of mAb1 producing CHO cells was performed in their study. HCPs that were present

in HCCF from fed-batch and perfusion processes of mAb1 were identified and quantified using LC-MS approaches including lipases that cause mAb1 stability. Different profiles and levels of lipases were detected in the different cell culture processes. In perfusion process of mAb1, altered distribution of charged variant species may provide an opportunity to clear the lipases by downstream process. Their work demonstrated that future bioprocessing can be shaped by a deep understanding of the impact of cell culture process on specific product quality when we move from traditional fed-batch to next generation high productivity perfusion cell culture. Combining advanced downstream and analytical techniques in addition to tailored approaches to perfusion cultures can offer greater control over product quality, stability and ultimately the safety of biotherapeutics.

- Are MS data required for filing?
- How might HCP profile change with media changes?
- What methods are used to assess comparability of processes or HCP methods – discussion on applicability of MS to address these questions
- Discussed the "positives" of HCPs (ideas suggested by Nadine Ritter, CASSS)

o HCPs can be an excellent detector of how the scale changes the process and also for comparability of unit operations – "Not everything is bad about HCPs, they can be used as a diagnostic tool"

o As an example, the tox profile and safety profile might be assessed and compared by the same set of HCPs. This also can be used to assess process consistency and DS batches