Table 34: Structure Function Studies of Non-standard mAb and Protein Therapeutics

Facilitators –

Peter Day, *Genentech, a Member of the Roche Group*
Jyothy Pillai, *Merck & Co., Inc.*

Scope:

In recent years, structure-function roundtable discussions have focused largely on approaches for standard monoclonal antibodies (mAbs). However, protein therapeutics, non-standard mAbs, fusion proteins, and protein scaffolds, are becoming increasingly common. For the non-mAb protein therapeutics mentioned, the novel structures and targets can lead to challenges in defining the Quality Target Product Profile and therefore make it difficult to identify critical quality attributes. In this roundtable session, we will focus on approaches to the structure-function relationship of non-mAb protein therapeutics, what can be leveraged from extensive knowledge of mAbs and what new approaches are needed.

Questions for Discussion:

1. What, if any, novel analytical tools are being used to investigate the structure-function relationship of non-mAb protein therapeutics?
2. What are the challenges associated with designing potency assays that accurately reflect the MoA of multi-faceted protein therapeutics?
3. Is knowledge gained from structure-function studies of standard mAbs largely applicable to non-standard mAbs and other protein therapeutics?
4. To what level do you include structure-function considerations in your developability assessment of non-mAb protein therapeutics?
5. For established commercial products/biosimilars, are there lessons learned, improvements, or different approaches to structure-function that can inform efforts on newer molecules?

Discussion Notes:

January 26 and 28 –

1) What, if any, novel analytical tools are being used to investigate the structure-function relationship of non-mAb protein therapeutics?

a. Crystal structure – Some used for early candidate screening to understand antigen binding sites and to find out different structure related abnormalities to look at aggregation. This lead the conversation to focus on aggregation for a little while everyone at the table agreeing that a robust look at aggregation during developability is important.

b. NMR – Companies perform NMR, hydroxy radical foot printing, and/or HDX studies but only when there is something that needs to be investigated not as part of regular screening.

c. MAM is being used to map impact on CDRs, to monitor glycan, for characterization, with an eye on QC implementation.

2) What are the challenges associated with designing potency assays that accurately reflect the MoA of multi-faceted protein therapeutics?

a. Do you need multiple potency assays – Most have multiple potency assays to address all of the activities of non-platform mAbs, both binding and cell-based asays. Some have attempted to file BLA with an ELISA but have not been successful.

b. The new guidance issued by FDA on covid potency assays was then briefly discussed as an interesting view into the thinking of the FDA on potency assays in general. Guidance for FDA on covid potency assays: COVID-19: Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting SARS-CoV-2 Infectivity

3) Is knowledge gained from structure-function studies of standard mAbs largely applicable to non-standard mAbs and other protein therapeutics?

There is quite a bit of overlap between mAbs and non-mAbs (certainly true for molecules on IgG backbones) and there was agreement at the table that companies follow a standard set of tests and stresses, but play particular attention to unique residues in CDRs or target binding regions. Look at differences in oxidation, deamidation, charge, glycans, etc. But platform assays do not always work because non-mAbs are not always amenable to the platform approach. For PEGylated proteins, there are numerous additional assays needed to assess the PEG.

For antibody drug conjugates need unique assays for what the conjugate did, make sure that conjugate arm was active.

Discussed a couple of cases where an IgG scaffold with cytokine fusion, led to adjustment of formulation and stress conditions because the molecule was less stable. Used release methods plus characterization with mass spec to identify degradation species.

Representative from Health Canada suggested that anytime a molecule has new properties or different impurities more characterization is required to increase understanding of any impact on patients.

Effector function was briefly discussed most agreed that assessment of binding to all Fc gamma receptors, C1q, and FcRn are performed even in the instances where point mutations to reduce effector function have been introduced.

February 1 and 3 –

1) What, if any, novel analytical tools are being used to investigate the structure-function relationship of non-mAb protein therapeutics?
    a. NMR –Not typically used for mAbs
    b. Use of NMR in NIH studies, proton analysis of C13 and N15 labeled amino acids,
        - Key challenge in use of biologics – expensive, high sample volume
        - CDE and other fluorescence technologies are more commonly used to study protein structure
    c. Use of enzymatic digestion to evaluate the CDR
    d. Most protein products, crystal structure is not usually done
    e. Typically for mAbs – LC/MS, peptide mapping, deamidation, glycosylation, charge variants such as SEC, cIEF
    f. Similar approach for vaccines
    g. Use of HDX (hyd-deuteriem exch) technology for protein characterization

2) What are the challenges associated with designing potency assays that accurately reflect the MoA of multi-faceted protein therapeutics?
    a. FDA does required potency, typically one assay for each mechanism. Usual challenge is the availability of the cell type, usually from the patients, specs are usually 50-150% and atmost to 70-130% due to the inherent variability of the sample and method itself. Discussed the potency testing strategy for coformulated products. Use of Biacore and / or ELISA based tests may not be sufficient. As per ICHQ6b, one assay may not be sufficient.
    b. Use of in-vitro models and evaluating patient data to determine efficacy. But the gold standard is a cell based assay.
    c. A relation could be established between structure & function, e.g number of drug load in an ADC with potency, linear correlation between potency and DL% and level of payload substitution

- Use of platforms can be achieved for mabs- CEX, AIX, cIEF

- Lipidomix – develop ion mobility technology using separations in gas phase instead of liquid phase, one benefit is isomer separation, shorter run time from a typical 90min run time.
    - Slim technology is being developed for commercialization – combination of electric field, gases within the chamber,
    - travelling wave technology that allows for measuring separation
- Is knowledge gained from structure-function studies of standard mAbs largely applicable to non-standard mAbs and other protein therapeutics?
    - Areas where standard technologies may not apply are for MoA
- For established commercial products/biosimilars, are there lessons learned, improvements, or different approaches to structure-function that can inform efforts on newer molecules?

Measurement of variants are low levels, if it's a CQA.

- Peptide map is the key technique, minor changes in the structure that affect the safety / efficacy, sample enrichment is required, collect the fractions and analyze
- MAS-Spec is also used, at what levels do we stop collecting the fractions and characterize – depends on the acceptance criteria (0.5% typically) and criticality of the function of the protein, sample stability
- Case study – some acid variants are very low levels in CRD region, only measured upon sample enrichment
- Case study – dimer peak in SEC, where one is growing on stability, but configuration of this peak may be different from the original dimer, but since they are in such low levels, could this be considered a CQA – could be di-sulfide scrambling, evaluate for hydrophobic aggregates or are they covalent

- Use of versious biophysical methods are also used for formulation screening
- Impact of PS80, oxidation

Biosimilar

- Case study – biosimilar
- Full characterization of all biosimilars, created a d/b to evaluate similarity
- Very easy PTMs for IgG1 molecules, understanding the impurity profile that maybe proprietary to the innovator product
- Sometimes innovator molecules are tested and characterized to get better analytical package for biosimilar submission
- Standard characterization package, additionally HDX studies, RAMAN, peptide mapping to understand the PTMs and their levels