Table 8: Structure-Function: When, What, How and How Much?

Session 1:

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Session 2:

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SCOPE:

Structure-Function (S/F) data are required in regulatory submissions of biopharmaceutical products to demonstrate product understanding and ultimately control critical quality attributes (CQAs). While a large body of data are now available for the monoclonal antibodies (mAb), S/F studies are still being conducted within each company for previously evaluated CQAs common to this molecule class. For non-mAb products, the timing for initiation of S/F studies is sometimes more critical due to a lack of prior knowledge. With advances in analytical technology, including potency testing, more tools are now available to generate S/F data, but strategy regarding what, how, and how much S/F work is required is still determined on a case-by-case basis. This includes S/F of CQAs that are related to PK, which remain elusive to the tools available. This round table will aim to address some of these topics and generate discussion among participants to help bring better understanding and consensus to development activities for this broad topic.

QUESTIONS FOR DISCUSSION:

1. How do the requirements or need for S/F data change throughout the product development life cycle? What types of S/F studies are companies doing at each stage?
2. What analytical tools are used for S/F assessment? What battery of activity assays are needed to assess S/F?
3. How do we address PK related CQAs without non-clinical studies, and are non-clinical studies leveraged for CQAs other than those related to PK? How has prior knowledge and literature been leveraged to address these CQAs?
4. How have companies leveraged prior knowledge or literature to reduce testing as part of S/F and control strategy? Are there other examples that companies have used to reduce testing for S/F and control strategy (mAbs and non-mAbs)?

DISCUSSION NOTES:

Session 1 and 2:

1. How do the requirements or need for S/F data change throughout the product development life cycle? What types of S/F studies are companies doing at each stage?
    a. Early S/F work can inform process development earlier and minimize process development costs/surprises later (helps understand unexpected results generated later).
    b. S/F studies help determine which attributes you should be measuring now to avoid discovering something while in the clinic. Specific examples of charge and glycosylation variants were noted.
    c. Early read on CQAs through S/F studies may help right the course on a molecule that has not yet entered Ph 1;

i. *e.g.*, early understanding of S-F relationships can help avert the need to develop a new MCB in order consistently to manufacture the desired product

ii. Examples were given on a non-Mab molecule in which glycosylation profile was not ideal, or the PEGylation profile was not the best. After Ph 1, you are pretty much stuck with the product quality the cells produce.

d. Companies need to be encouraged to do S/F work earlier. Company pushback based on resources is common.

e. One downside if that S/F data generated early is often done with less developed bioassays and may not be as meaningful. Thus S/F is not as routine a practice unless some structural anomalies show up. HAs also understand this and would not expect as much S-F data at early stages of development.

f. Platform knowledge (*e.g.*, for MAbs), if available, can inform how much S/F to do in early development. For MAbs, there is more leeway to leverage historical/literature knowledge. In this case, early S-F analysis may just involve some heat stress studies at early stage—easy enough to do.

g. Important to keep retains of clinical materials for additional testing if S/F studies are deferred to later.

h. Other types of S/F studies may involve mutants and glycosylation variants. Not common, but it can be done on a case-by-case basis (e.g. new MoA or new modalities). This may also involve animal studies.

i. Peptides: may not need S/F as usually not much secondary structure present (for shorter peptides). MS can demonstrate structures readily (e.g. disulfide linkages).

j. mRNA: mostly looking at chemical modifications. Forced deg is sometimes done.

k. Use affinity-purify variants to evaluate their effect on bioactivity

i. Peak collection/enrichment for S/F studies: Only tackle larger peaks in early development on a case-by-case basis. Postpone smaller peaks until later.

2. What analytical tools are used for S/F assessment? What battery of activity assays are needed to assess S/F?

a. Cell based assays are better than ELISA (latter do not provide enough insights). Other functional assays are useful (*e.g.* Fc binding assays).

b. Non-Mab molecules with multiple active domains: Need to look at all of them with however many assays necessary. May make discreet changes to protein or generate enrichment of variants to see how they impact functions.

i. Advice: do single variations to start.

ii. Should invest in development of characterization assays as early as possible.

c. Genetically modified cells can be used to evaluate the role of an attribute or glycoform on PK or function (*e.g.*, by engineering in or out glycan processing enzymes)

d. In-process or forced-degradation samples that have different levels of variants compared to the final purified protein can be used to evaluate S-F relationships

i. In-process samples may better reflect the attribute or mix of attributes in the product.

3. Discussion points on how or if S/F studies can be used to evaluate impact to PK-related attributes

a. Fewer connections can be drawn from S/F studies regarding impact to PK.

b. One tool is to do high resolution PK studies to evaluate the rate of clearance/half-life of different glycoforms in a MAb
c. Animal (or human) PK studies are more routinely done for fusion proteins, antibody variants, and non-mAbs if changes are seen and impact on PK is uncertain.
d. High end characterization for structural attributes that have changed is especially helpful for glycan changes. But animal studies may not be predictive of PK effects in humans.
e. Glycan profile in a non-Mab example — which part of the profile was important to PK *vs* potency (length, branching, site, *etc*.), and how to balance the needs of the two?
    i. Made glycan variants and ran in vitro assays and animal studies (rats, cyno; but should try to minimize these).
    ii. In smaller companies, these kinds of studies may not be as feasible. CRO may be engaged to conduct much of the structural characterization work.
    iii. MAM may be a good way to go in terms of data richness (e.g. MS for glycan occupancy). They mostly focus on CQAs in the CDR only.

4. Sharing and location of S/F data in regulatory filings

    a. If new MoA, in initial IND filing, it is more important to demonstrate/establish MoA, and less important to check impact on potency by a specific modification.
    b. Unexpected findings: Early engagement with HA is important; they are interested in learning more too.
    c. A question was asked regarding why more S/F data is not shared with the health authorities in INDs.
        i. Some sponsors are afraid to raise issues with FDA that may lead to additional regulatory questions
        ii. Comfort level with the degree of assay development can lead to less sharing of information at early development stages.
    d. It was noted that some S/F data end up in the pre-clinical rather than the quality/CMC sections. Different functions within a company have different comfort levels for content of these sections.
        i. FDA CMC reviewers as well as the pharm-tox reviewers evaluate the biological activity data that are in the non-clinical sections

5. How have companies leveraged prior knowledge or literature to reduce testing as part of S/F and control strategy?
    a. Are there other examples that companies have used to reduce testing for S/F and control strategy (mAbs and non-mAbs)?
        i. Mab: C-terminal Lys
        ii. Bispecifics: binding assay results may be convoluted due to changes in one arm *vs* another. This complexity may not help with leveraging prior knowledge (from a pure Mab).
    b. Forced deg is usually done early to help narrow down the CQAs on which to focus.
    c. Crystal structure may be useful for looking at impact on binding.

6. Predictive tools/in silico tools/AI:
    a. Large companies are exploring but we are not there yet.
    b. Immunogenicity tools typically center on peptide binding and not always usable/amenable to validation. Typically we would avoid the problems before they even pop up (*e.g.*, alpha-gal).
    c. Even with the "right" sequence selected, cells may produce sequence variants so this would need to be analyzed by MS. Or unstable clone that may be produced with sequence variants over time.

7. Specification setting:
    a. MoA based or clinically relevant spec setting: Utilize data from MoA understanding and S/F results, as well as clinical specs already in existence, to help set ranges for CQAs in commercial spec.
    b. Potency assays: based on clinical experience but need to understand all the CQAs that contribute to the clinical ranges in totality. This is challenging if not many lots have gone into the clinic by BLA filing.