Table 11: Regulatory Suggestions for MS Characterization of Newer Modalities Such as GTx and mRNA

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Scope:

The introduction of new modalities, including GTx and mRNA products, necessitates MS characterization for comprehensive product understanding. MS technologies provide a wealth of product information that can bolster regulatory filings, provide orthogonal assessments of PQAs, and/or establish a link between attributes with various safety or efficacy readouts. For newer modalities, MS characterization may play a more pivotal role due to the unknown criticality of PQAs or the lack of traditional release tests to monitor CQAs. In addition to standard MS characterization approaches, novel MS technologies may need to be applied for characterization and communicated to the regulatory agencies. This roundtable will discuss regulatory suggestions for MS characterization of new modalities, including GTx and mRNA.

Questions for Discussion:

1. How does MS characterization differ for new modalities compared to established modalities?
2. What new technologies are being used for product characterization and are data from these technologies being provided to the regulatory agencies?
3. Is MS being used for release testing or for the support and understanding of release test development?
4. As process understanding is gained, are MS methods sufficient as characterization methods or do they need to be validated? Where is the line drawn?
5. At what stage in development is MS characterization performed for new modalities?
6. Where is MS characterization data being used in regulatory filings – characterization, comparability, etc?
7. What are challenges that scientists face in developing new methods or including data in regulatory packages?
8. How does limited modality experience or the limited number of lots impact the MS comparability assessment? Is the comparability package different for these new modalities?
9. Many new modalities have multiple components – How are groups ensuring comprehensive MS characterization of all components of the modality?

Discussion Notes:

Tables 10 and 11 combined

Novel Modalities

- Some companies have groups have specific groups focusing on Oligo's. Others have core MS groups that are not modality focused.
- Capsid workflows are defined and have solutions, but method/workflows for DNA need much more work.
- The industry can gain valuable insight on nucleic acid analysis from the Anti-Sense Therapeutics pioneered in the 1990s.

How do you characterize new modalities?

- Cell therapy is so young and only a small pool of people are working in this space.
- Workflows include: intact & MS/MS
- In gene therapy you need to ID several key proteins, need sequence coverage/mapping on DNA/RNA, RNA PTMSs will be new and needed.
- Take mAbs strategies of glycans, pep map, subunit, intact and try adapt
- Need restriction map, subunit of capsid
- Intact is too large and outside MW range of Mass Spec
- Gold standard is to provide the same familiar characterization but because modalities are larger you can't use trypsin or other typical enzymes
- New modalities don't have identified regions of modifications with proposes a challenge and need for exploration. Can SLIM be used for the analysis of empty vs full vectors?
- Start with Potency testing
- Challenge: time scale for cell & gene therapies are much faster and you have less material. Most material goes to qual and validation
- Several companies are providing oligo software and helps provide sequences. Protein Metrics has been successful in providing both charge deconvolution and sequence of Oligo's
- Key questions: are the modifications different? Are the clippings different? Do you need MS/MS?
- Ideally you characterize at the beginning/front end so when/if changes are made that stage has already been set and you can go back to what you need to the product to be

Regulation Strategy

- Where does the characterization data go? IND? BLA? Both?
- Use the best tools! They have to show they are making what they think they are making.
- Everyone will have diverse approaches until things shake out.
- This will be similar to recombinant proteins 20 years ago
- FDA is apprehensive to approve
- Need for batch to batch monitoring
- Capsid QC – check solid phase but also synthesis of Oligo's. How do we know oligo synthesis is consistent? How would you monitor that?
- FDA won't tell you how to perform the testing but will require that you include it in your package

Technologies

- MOBILion's SLIM technology could be used for viral capsid full vs empty work, speed of mapping/sequence coverage and reducing pesky LC when working with DNA/RNA.
- Sciex is focusing on CMC work for Product ID, Impurity ID, and PK & met ID work for Quant. There is a need for sensitivity to support preclin and clinical studies

Challenges with Cell/Gene therapy

- DNA → RNA → Protein, cell/gene therapy is done to express a protein of interest as the treatment
- How do you monitor if the therapy is working as expected?
- There are Bioassays to monitor end results
- Expression alone isn't enough to monitor activity
- Safety & Efficacy is the goal. Bioassays will determine efficacy but how does one monitor safety?
- What can MS provide?
- 3 level of heterogeneity
- Native Capsid MS: assembled products/empty/full, heterogeneity, MS can provide this and may become much more widespread

Other new Modalities

- T-cell
- Immuno-onoclogy
- Low hanging fruit is gone.
- Multispecific conjugates
- How much of existing techniques are still useful?
- Goal to marry peptide mapping CDR from MS and bioassays
- Connect MS data to other types of data → strong need for informatics to pull data together into packets
- MS could increase and replace Bioassays with more Native and HDX work
- MS adoption is a challenge because of complex rich data sets that can be difficult to interrupt by non-MS people. MS is too much of a specialized group.
- Reportables for MS are much more complex than the data from an SEC analysis.
- Goal – make data connectable and reportable

Unique Challenges

- DNA adducts – exact mass, ms/ms, deconvolution considerations
- Backbone stabilization? Wild type, single strand today
- Take a step back and look at composition & structure before characterization