

Roundtable Session 1 – Table 10 – MS in Cell and Gene Therapy

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Abstract:

Chimeric antigen receptor (CAR) T-cell therapies have demonstrated remarkable clinical success, and yet many challenges remain in manufacturing process development and analytical development. Mass spectrometry (MS) is a powerful platform for advanced molecular characterization of starting and critical materials including media and supplements, as well as enhanced understanding of drug product quality and process performance across the manufacturing workflow. In this roundtable discussion, we will examine the various applications and tools of MS to address current limitations in cell therapy manufacturing, with the goal to facilitate the development of more consistent, scalable, and effective cell therapies.

Notes:

1. MS as a Characterization vs. Release Assay in CGT

- Higher sensitivity of MS (vs. ELISA) opens new possibilities and pushes the field forward — e.g., in protein replacement therapy, MS can confirm protein expression and transduction effectiveness, addressing ELISA's sensitivity limitations.
- Per FDA seminars, there are situations where MS use will be required/expected.
- General sentiment: attendees are not opposed to MS as a *characterization* assay, but there is more reservation about using it as a *release* assay. Limitations include:
 - QC teams may currently lack sufficient MS expertise.
 - There is a training/adoption curve for analysts.
 - The robustness of nano-ESI is still limited.
 - A major obstacle remains moving from early-stage characterization methods to fully validated methods.

2. MS in Viral Vector / AAV Characterization

- Gold standard for AAV remains AUC, but MS provides complementary information (e.g., truncated genome detection, forced degradation studies).
- CDMS, SEC-MALS, and mass photometry (MP) are all viable complementary methods.
- For PTM analysis, MS captures lot-to-lot variability and different oxidation levels.
- Lentiviral vectors require BSL2-level handling and have delicate structures needing proper buffer conditions.

3. AI / Predictive MS and Data Strategy

- **Data mining:** attendees shared a general sentiment that a clear purpose needs to be established prior to bulk acquisition and analysis of MS data.
 - Methods need to be "fit for purpose," otherwise there's a risk of running endless assays without clear value.

- MS can generate enormous amounts of data — the field needs to determine what truly matters before over-measuring.
- Data labeling is critical (similar to pattern recognition tasks).
- Caution on being purposeful with AI/data feeding: rather than feeding all data indiscriminately, be specific about training data (e.g., a model trained on 1 billion mass spec data points can answer broad questions, but fine-tuning on specific datasets — like specific PTMs — is needed for specific questions).
- **Predictive Power:**
 - AI predictive MS methods (for peptides, small molecules) have shown ~96% predictive ability for clone selection, as an alternative to RNAseq.
 - For CGT: when screening ~1000 samples, teams explored how low sample size could go while retaining predictability — 26 samples was found to be the minimum to hold predictive power. This matters for understanding downstream/cascade toxic effects.
 - Mixed sentiment on in-silico prediction of potency: binary/mathematical models may not accurately capture potency; individualized potency assays per patient may be needed, though this may not be feasible at scale for every patient.
 - Virtual CHO cell modeling: even with well-established CHO cell lines, data is highly variable and still needs smoothing.

4. Donor Material Characterization

- **Context:**
 - Most approved CGT products are autologous, so donor screening as a discipline is still relatively new.
 - Drug product (DP) shows high donor-to-donor variability — a major challenge for ensuring consistent product quality over a 20-year horizon across ~200 clinical-stage patients.
 - Identified as a significant gap area — current methods are not adequate for donor material characterization.
 - Key difficulty: differentiating between analytical assay variability, process variability, and donor variability.
 - Level of scrutiny for CGT vs. traditional biologics characterization is very different, and small trace differences can be root causes of product failure.
- **MS-Based Donor Screening:**
 - Proposed use of MS for donor screening — requires establishing criteria for donor exclusion, since "star donors" cannot be repeatedly relied upon.
 - LC-MS-based omics analysis proposed to better understand donor profiles.

5. Platform Method Opportunities

- If MS can serve as a good platform method (paired with ELISA reagent development), it could allow new compounds to "plug in" without extensive additional development — reducing product-specific effort and accelerating development timelines.