Roundtable Session 1 – Table 4 - Multiplex Approaches to Analytical Platforms for CGT Release

Facilitator: Xue (Shelly) Li, *Regeneron*

Scribe: Nadia Ganton, *Innovance BioSolutions LLC*

Abstract:

Multiplex approaches involve performing multiple analyses simultaneously on a single sample, offering advantages such as increased efficiency and reduced costs. For Cell & Gene Therapy (CGT) release, compared to single-analyte assays, multiplex approaches can provide a more comprehensive understanding of complex CGT samples, significantly reduce sample consumption, and improve sensitivity.

Discussion Questions:

Key points to explore:

• Examples of multiplex approaches for CGT release

• Challenges in developing and executing multiplex approaches for CGT release

• The potential of multiplex approaches for CGT release

Notes:

Examples of multiplex approaches for CGT release

Examples discussed included:

- Capillary gel electrophoresis to determine purity of capsid protein and ratio of proteins to virions.
- Flow cytometry: multiple markers, different dyes. Use of multiple colors in a CAR-T release assay. Helpful tip: BD Biosciences can provide plates with dried down antibodies; would only need to add anti-CAR antibody, making the plates usable for different CAR-T programs.
- Obtaining different readouts from the same cell culture, for example: production of dopamine from dopaminergic neurons, neurite outgrowth measurement, calcium measurement.
- Use of next-generation sequencing (NGS) in transcriptomics.
- Optical genome mapping for multiple applications from a single run. For example: donor identity, general structure variants to check if gene editing was as expected, copy number variants, small single nucleotide variants, transgene…
- qPCR: same cDNA, multiple analytes.

Challenges in developing and executing multiplex approaches for CGT release

One of the biggest advantages of multiplexing is the time and money savings; multiple birds with one stone.

Challenges discussed included:

- Risk of moving to a different method, especially when a program is underway. It would be easier to use the multiplex technology from the start of the program.
- Instrument failure; good practice to have instrument backups especially for release assays.
- The more analytes you are looking for, the bigger the chance of false positives in the same assay.
    - Recommend tracking false positives and "validating out" recurring false positives.
    - New false positives may appear with a new batch of reagents. Consider having a reagent qualification and bridging strategy.
    - Sometimes it's a sampling handling issue.
- For cell culture-based multiplex assays, optimal cell densities may be different for different readouts.
- Data management can become complex for multiplex assays. Software and analyses must be validated. Methods such as sequencing and mass spec are data heavy.
- Use of mass spec, especially as a multiplex QC test: expensive and more complex than other assays.
    - One non-QC example discussed for multiplex use of mass spec is to measure media analytes and help inform process development.
- Transferring multiplex methods to QC: a common challenge experienced by the roundtable participants.
    - Multiplex methods are often developed by subject matter experts who are comfortable running complex assays. Methods need to be QC friendly.
        - Helpful tip: bring QC analyst into analytical development lab and train them on the method. Side-by-side with an analytical development team member.
    - Train QC analysts on the platform as well as the method. For example, to train a QC analyst on flow cytometry, start with something simple like a viability dye and one other marker. Flow cytometry takes time to get comfortable with (and many runs).
    - Build trust from the beginning and help the QC analysts make the method their baby. The biggest challenge is with people, not the science or the instruments.
    - Communication is key.
    - Engage QC early and ensure that the method they are being given is well robust, so that they don't receive a difficult to use, poorly controlled method.

The potential of multiplex approaches for CGT release

- Automation, especially for autologous CGT. What methods can be combined and automated? Modular (automating a piece of it), and eventually end-to-end full automation.

    - For example, Echo is an acoustic liquid handler used in various applications including flow cytometry. But should consider what automation would be QC friendly and if it may be more appropriate for analytical development.
    - BD has an automatic staining system for flow cytometry. Huge advantage, especially since you can save on number of replicates and be informed by the system of any handling errors.
- Multiplex assays would help save on sample amount, especially for autologous CGT where there are often challenges with the amount of material. Combining assays would be a more powerful approach than using less material in QC samples.
- Potential for using multiplex assays as platform methods, and without having to validate in full for every program. For example, if a new sequence being quantified fits into certain previously validated parameters, perhaps validation does not need to be repeated in full. This would help streamline validation studies for newer programs.
- Multiplex assays can be useful in early phases, to help understand correlation with function. Decide what needs to be reported as release or taken off. Validate release assays in a phase appropriate manner.
- Don’t be afraid to try switching to multiplex assays, even if you need bridging studies. It’s all part of lifecycle management.