Table 7: Beyond the mAb Series – Cell Therapy CE Applications

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Scope:

Cell therapy therapeutics introduces unique analytical challenges compared to previous molecular formats such as mAbs. As the technology has matured over the years, recent advances in individualized gene editing have yielded therapeutic strategies that aim to produce personalized API's (active pharmaceutical ingredients) for patients. And while the promise for such individualized medicine is great, it does necessitate a control system strategy that can accommodate a diverse set of similar-yet-different biologics, each tailored-made for a particular patient. In this respect, CE technology has the potential to offer the sensitivity, precision, and robustness to quantify and monitor an array of CQA's (critical quality attributes) for each of these similar-yet-different medicines.

Questions for Discussion:

1. What has been your experience with individualized therapies? Are there any particular challenges you've encountered versus more generalized medicines such as mAbs?
2. What unique CQAs have you encountered with this particular technology, and which CE applications have best monitored them?
3. Have there been any analytical throughput concerns for this technology given the volume of patients (and therefore volume of differing biologics)? If so, how did you address these concerns?

Discussion Notes:

Attendees briefly mentioned the projects they are working on before the roundtable began. These projects included:

- AAV analysis of empty vs full capsid via Labchip

- lipid nanoparticle analysis via HPLC

- ssRNA 100bp analysis via PA800+ (AAV as well)

- GMU (genomic medical unit) using CE to assess 8000bp dsDNA

- SciEx rep observing cell therapy trends

- ProteinSimple rep offering support/feedback with Flex instrument, Turbo cartridge for fractions

Discussion:

- dsDNA 1000 kit (SciEx) assay does not have sharp peaks; 8M urea dilution of gel to denature DNA for possible increase in resolution, plus denaturation temp 50-90C followed by icing; inj voltages 2-8 kV, sep voltage 7.8 kV as DoE

- problems with Labchip transfer to PA800+ method (necessary to create a release method); suggest genomic DNA kit too big, could use restriction enzymes

- successes with dsDNA 1000 kit paired with SYBR Gold dye in SciEx technical note; gold shown to have superior resolution vs other dyes; for linear forms, SYBR Green recommended

- Agilent has options for gel buffers; one buffer for conditioning capillary, one for separation; use of Agilent kit on PA800+ not recommended

- RNA 9000 kit uses bare-fused silica (not coated), and gel and capillary compliment one another; in addition, while the range of the kit is 60 - 9000 bp of RNA, recommend analyte be in the middle of this range

- regarding RNA vs DNA stability, DNA stability stronger than RNA; in addition, bigger RNAs are less stable than smaller RNAs

- empty/full ratio of AAV: SEC vs iCIEF analysis, ProteinSimple published a poster using Maurice, data matching with SEC-MALS; concentration-dependency for native fluorescence on Maurice, full capsid means NF = UV; empty capsid means NF does not equal UV (poster: "Do your AAVs contain DNA?")

- ddPCR useful for empty capsid analysis as well; AUC (analytical ultracentrifugation) not QC-friendly since experienced people needed; AUC useful for making in-house reference standard

- SciEx will offer a webinar on a new assay for AAV capsid analysis

- genomic integrity and fragments do impact potency; health authority requirements for full capsids

- AAV 9, 8, and 2 typically used; engineering new capsids are challenging

- standards for AAV used to be bad but improved since Charles River bought the company that offered these standards; better control over empty/full analysis; 50-60% empty/full is about what can be achieved

- question about how many copies of nucleic acid per AAV capsid? hard to know

- extraction of DNA from an LNP particle is possible; cIEF can do it but resolution is not good (poster: "Two quality and stability indicating imaged CIEF methods for mRNA vaccines")

- when RNA sticks on the outside of an LNP, is this an issue? LNP + RNA not detectable by CE (lipids are neutral or cationic whereas RNA is negative; therefore, a CE injection can inadvertently remove LNP), can however count number of particles vs RNA concentration to get an appx answer maybe

- Cas9 @ pH 9 iCIEF analysis problematic; suggest arginine as a blocker during imaged separation; ProteinSimple did some work on this problem and can provide information; urea can cause problems with Cas9 and there are alternatives such as NDSB; rings on this molecule do suppress native fluorescence however, and it's unknown how it works with LNP