Process Analytical Technology Strategy For Lentiviral Manufacture

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PROCESS ANALYTICAL TECHNOLOGY (PAT) CHALLENGE

Cell and gene therapies (CGT’s) are the most complex drugs ever developed. PAT and real-time bioprocessing control will enable scaling up the industrial manufacture of CGT’s. Whereas these approaches are well understood in the pharmaceutical industry, intrinsic difficulties currently exist for their practical implementation in CGT manufacture:

• The process:  
  - Cost  
  - Reproducibility and control  
  - Identification of Critical Process Parameters (CPP’s)  
  - Suitable sensors for on-line monitoring  
  - Throughput for sampling

• The product:  
  - Identification and measurement of Critical Quality Attributes (CQA’s)  
  - Analytics for product characterisation  
  - Dynamic change of biological system during manufacture

• The data:  
  - Multivariate data types, fragmentation, alignment  
  - Metrology and data quality  
  - Modelling and interpretation  
  - Integration: hardware, software, datasets.

This Innovate UK funded project tested a PAT strategy to capture, analyse, interpret multivariate omics datasets, identify markers from which a dynamic biochemical fingerprint was derived and used for real-time monitoring using Raman spectroscopy during lentiviral manufacture using the Oxford Biomedica platform process.

DATA ANALYSIS, MARKER SELECTION

• In-process and off-line data were structured within the Antha platform (Fig. 1) and made available for further processing.
• Principal Component Analysis, random forests, and network analysis (Fig. 3, top left) were used to identify potential metabolomic markers in significant pathways.
• Self-Organizing Map (SOM’s, Fig. 3, top right) neural networks were used to identify key inflection timepoints in the process, to narrow down the multi-omics analysis.
• Regularized Canonical Correlation Analysis (CCA, Fig. 3, bottom right) and pathway analysis (Fig. 3, bottom left) were used to integrate metabolite and gene expression datasets, and to identify a list of key markers acting as a fingerprint to the process.
• This multivariate analysis approach identified a list of markers and patterns acting as a fingerprint to the process.
• The ability to model these biochemical patterns using Raman spectroscopy supported the concept of further developing chemometric Raman models for viral titre monitoring.
• Chemometric models of viral titres were tested in 5L and 50L bioreactors in a proof of concept study, and models subsequently refined.

DATA CAPTURE, STRUCTURE, INTEGRATION

REAL-TIME MONITORING OF LENTIVIRAL MANUFACTURE

The data-driven strategy applied during this study supported the development of a Raman spectroscopy model for real-time viral titres during LV manufacture. Raman models proved robust in both monitoring conventional analytics as well as tracking LV titres in real-time, at 5L and 50L scale (Fig. 4).

CONCLUSION

This approach demonstrates our PAT strategy as an exciting tool to monitor and interrogate complex manufacturing processes, providing greater mechanistic and biological understanding in relation to the bioprocess environment. It could be used to monitor, in real-time, the effects of process parameters and process, and allow the design of “digital twins” which in turn could dramatically shorten the development pipeline for high quality lentiviral products.