A strategy for robust implementation of Process Analytical Technologies to ensure controlled cell therapy manufacture

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Background

Manufacturing cell therapies at scales required in phase III clinical trials and beyond will require step changes in industrial processes and controls. This will be achieved by:

- better definition and measurements of Critical Quality Attributes (CQA's)
- real time monitoring of product quality during manufacture
- using in-process controls and automation

Proposed solutions:

- High-throughput / high-content screening of direct (cell-based) and surrogate markers of product identity, function and quality
- CQA-driven process modelling and optimisation
- Integration of Process Analytical Technologies (PAT's) through technological innovation, statistical modelling, real-time controls.

Method

Culture protocols for 2 induced pluripotent stem cell (iPSC) lines, the RCiB10 research grade GMP-derived by the Cell and Gene Therapy Catapult and the PFX9 line (Kobe, Japan) were optimised. During 10 passages, cell-based and surrogate markers were measured using flow cytometry (257 markers screened), gene expression by Scorecards® (96 genes), LC-MS (50 metabolites, Shimadzu), CuBiAn® bioanalyser, MesoScale Discovery® platform and live quantitative imaging. Both cell lines were cultured manually or using the automated Pre-Alpha system at Tokyo Electron Europe.

Multivariate data analysis (MVDA) using generalized topological overlap matrices and unsupervised hierarchical clustering were used to identify relevant identity, quality and surrogate markers for direct or inferential monitoring (**Figure 1**).

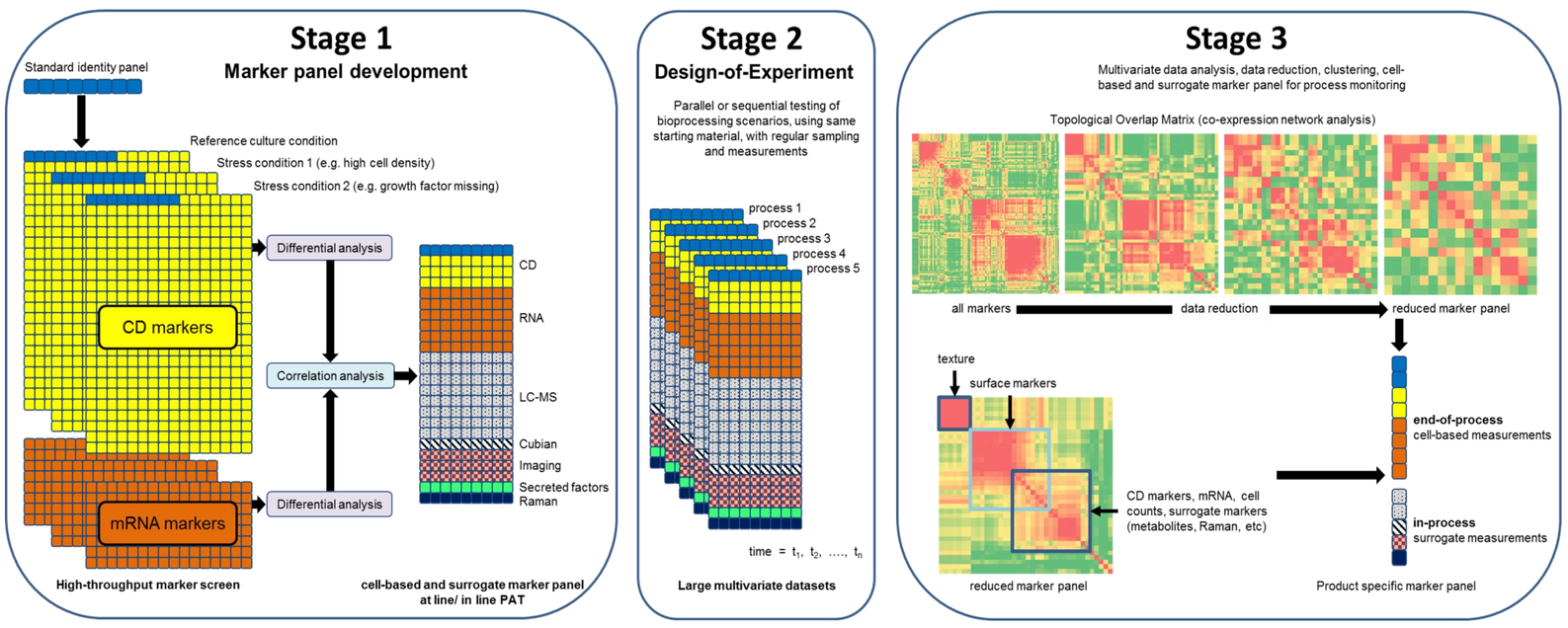


Figure 1. Strategy proposed for the robust implementation of Process Analytical Technologies in cell therapy manufacture. **Stage 1** identifies robust CQA's by screening surface and gene expression markers. This is achieved by comparing expression profiles between an established reference culture process and a range of stressful conditions. The resulting panel is augmented with markers measured by PAT's. This overall panel is applied in **stage 2**, to collect measurements over time and under variable processes. In **stage 3**, network analysis and data reduction are used to identify correlated cell-based and surrogate markers, identifying processes which maintain the CQA's as well as the markers to measure for in-/at-/on-line monitoring during manufacture.

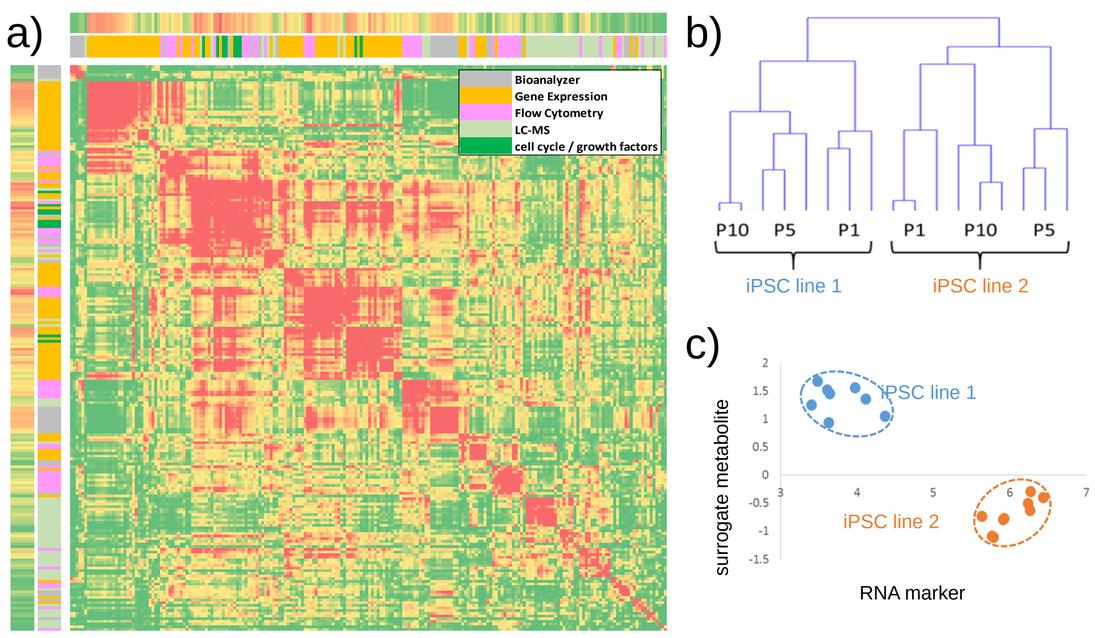
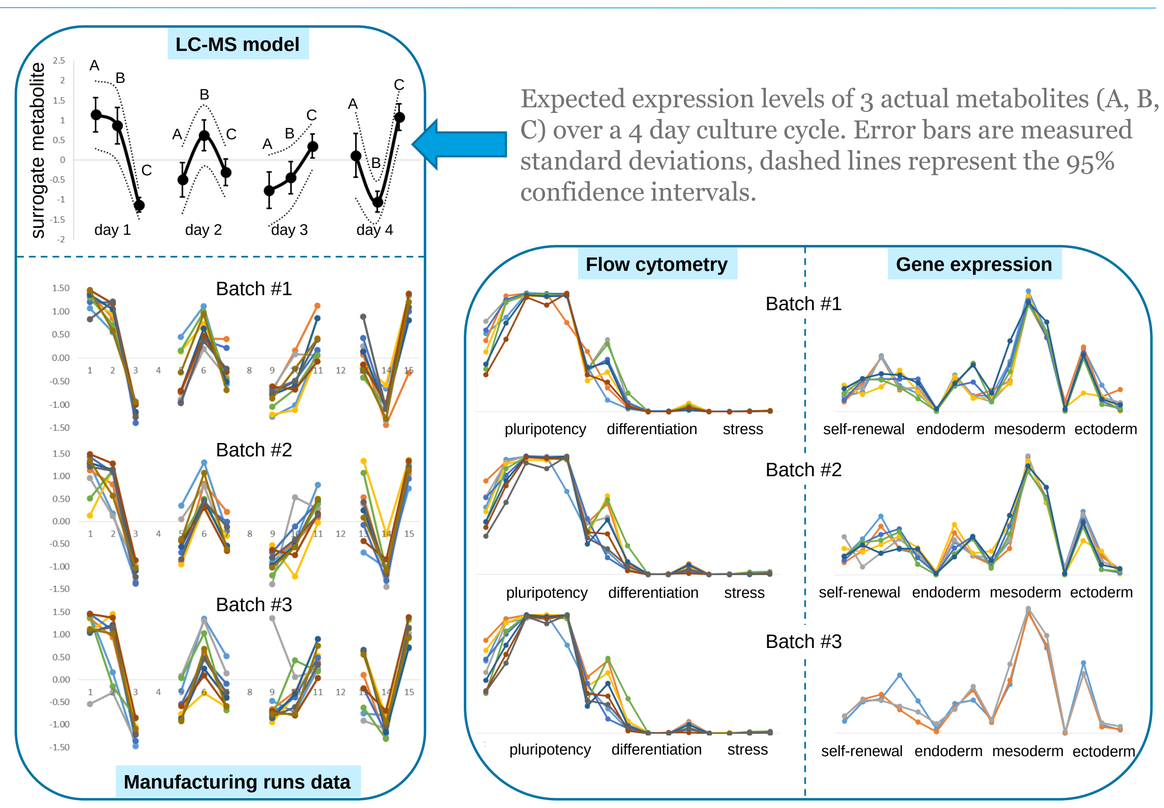


Figure 2. a) Generalised topological overlap matrix showing the correlation and connectivity of 212 markers measured across 5 analytical platforms (see key) from both iPSC line samples. Red indicates high connectivity. External colorbar = mean marker topological overlap (red is high), internal colorbar = analytical platform. Clusters of highly correlated direct and surrogates markers are observed. **b**) Dendrogram showing consistent clustering of the iPSC lines and passages, **c**) Example of line-specific relationships between a surrogate metabolite and an RNA marker.



Expected expression levels of 3 actual metabolites (A, B, C) over a 4 day culture cycle. Error bars are measured standard deviations, dashed lines represent the 95% confidence intervals.

Figure 3. Left box. ***Top***: Expected expression levels for 3 metabolites measured by LC-MS over 4 days. ***Bottom***: Actual measurements for 3 biological replicates (runs). **Right box.** Phenotypic (***left***) and transcriptomic (***right***) profiles of the end cell products, at day 4, which could be inferred from the surrogate metabolic profiles.

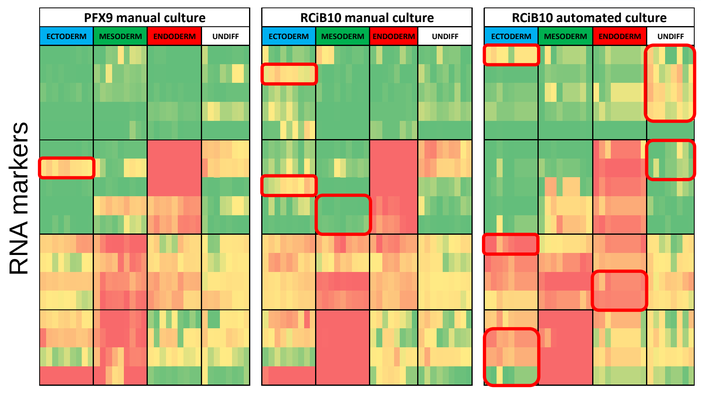


Figure 4. Subsets of RNA markers for the PFX9 line cultured manually (left panel), and the RCiB10 line cultured manually (middle) or using the TEL automated platform (right), measured on undifferentiated samples (UNDIFF) or subsequently differentiated samples (ECTODERM, MESODERM, ENDODERM). Red rectangles highlight platform-dependent differentially expressed genes.

Conclusion

- The proposed strategy allows to identify robust direct and surrogate markers of cell identity, function and quality.
- It efficiently connects inferential PAT markers to cell-based markers, which can be used for in-/at-/on-line monitoring.
- High-throughput / high-content screening early on is essential to identify reliable markers to further assess PAT suitability.
- Marker redundancy strengthens process statistical modelling.
- On these foundations, inferential in- or on-line monitoring can be integrated for rapid automated in process controls.

We work with Innovate UK

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