Development of a cell therapy PAT strategy based on multi-parametric product characterisation

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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) with automated culture platforms and real-time controls.

Example for iPSC manufacture

Figure 1 details the experimental design used to identify robust cell-based and surrogate markers using off-line and PAT's platforms in the context of induced pluripotent stem cell (iPSC) manufacture.

Culture protocols for 2 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 specific identity, quality and surrogate markers for direct or inferential monitoring.

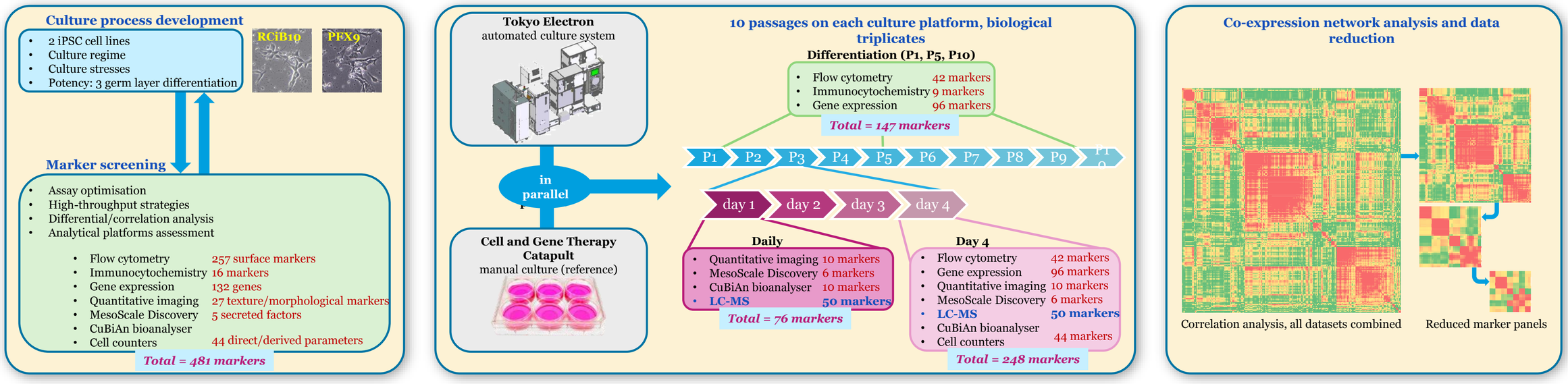


Figure 1. Experimental procedure to assess the suitability of PAT's (LC-MS for instance) for monitoring iPSC manufacture runs over extended periods of time (10 passages, 40 days). **Stage 1** (left) optimized culture conditions and screened an extensive range of markers. **Stage 2** (middle) used relevant smaller panels over 10 passages. **Stage 3** identified critical cell-based and surrogate markers.

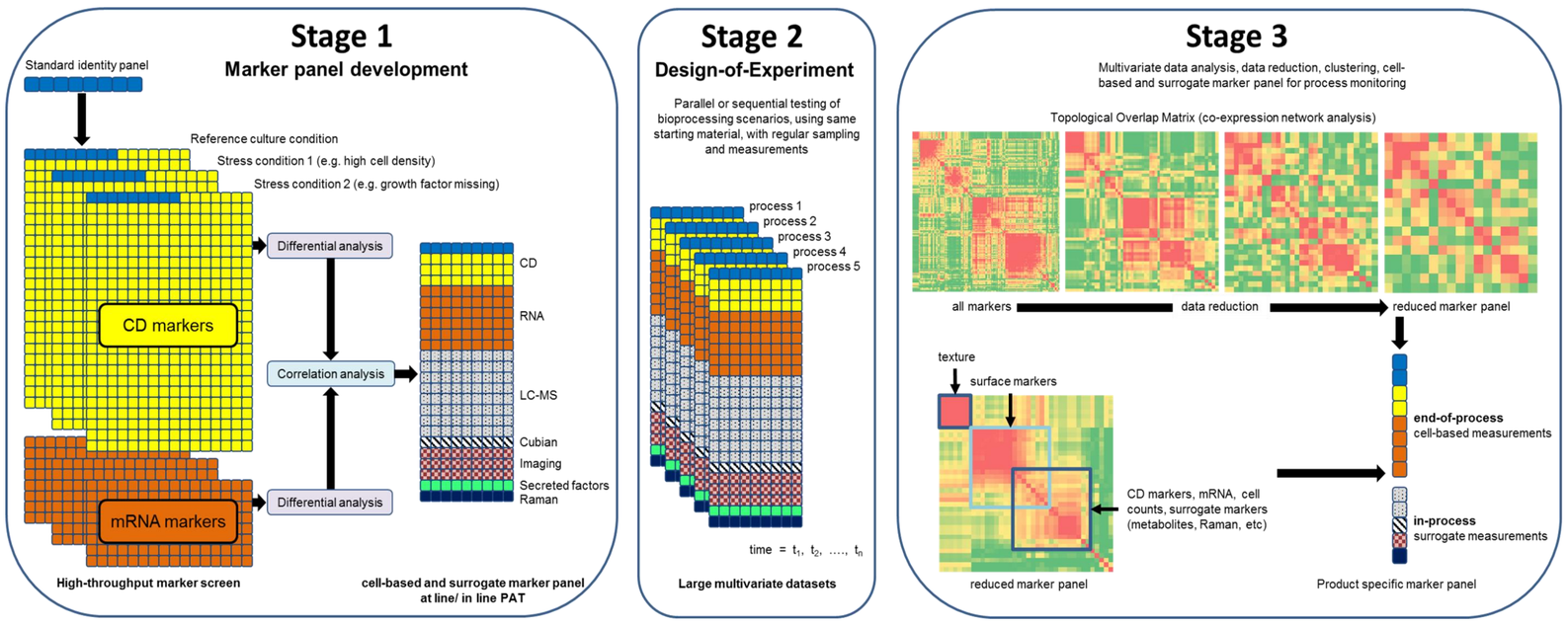


Figure 2. Global strategy proposed for the robust implementation of Process Analytical Technologies in cell therapy manufacture. **Stage 1** identifies robust CQA's by screening surface and gene expression markers. This is achieved by comparing expression profiles between an established reference culture 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.

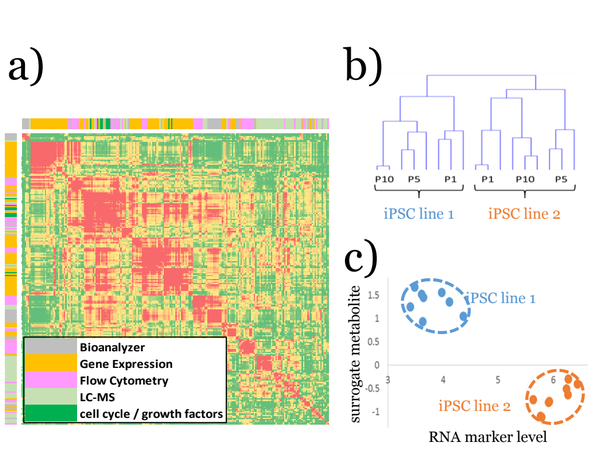
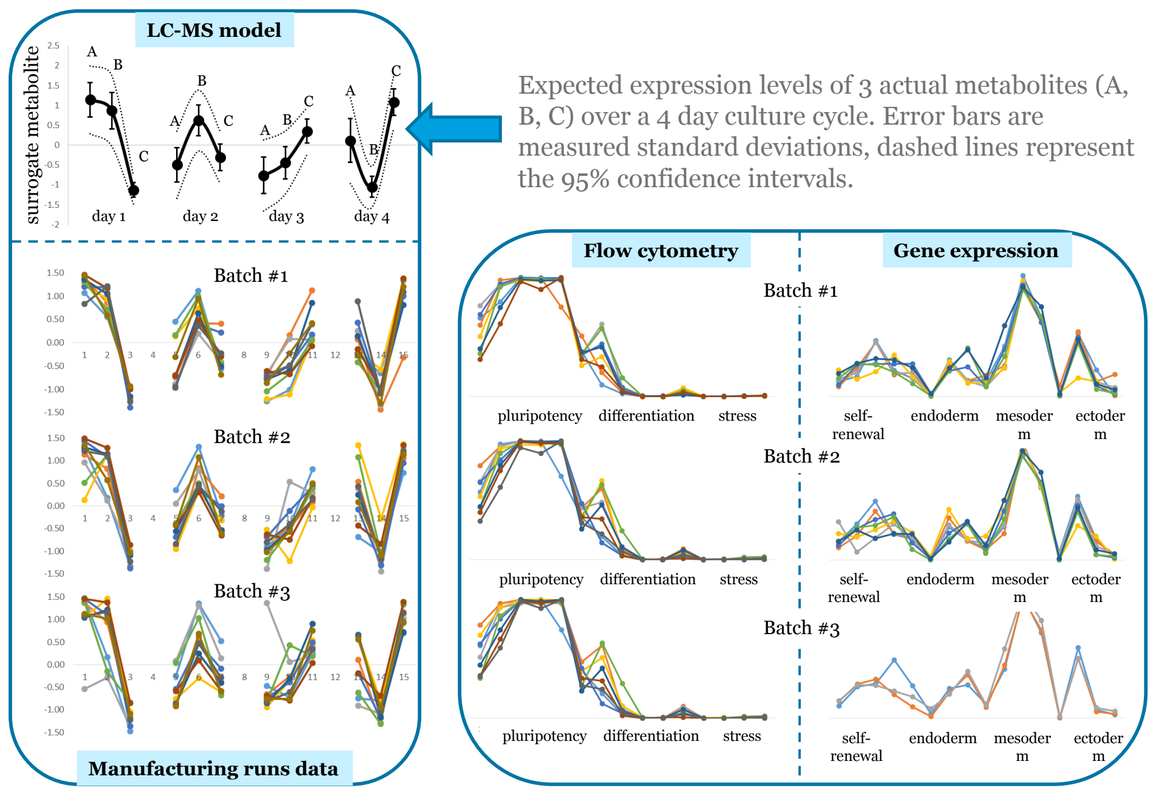


Figure 3. a) Generalized topological overlap matrix showing the showing the connectivity of 212 markers measured across 5 analytical platforms (see key) from both iPSC line samples. Red indicates high connectivity. Side colorbars = color-coded analytical platforms. Clusters of highly correlated direct and surrogates markers are observed. **b**) Dendrogram showing consistent clustering of the iPSC lines and passages, indicating that line-specific expression patterns can be identified from such dataset. **c**) Example of line-specific relationships between a surrogate metabolite measured by LC-MS and a cell-based RNA marker measured by RT-qPCR.



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 4. Left box. ***Top***: Expected expression levels for 3 metabolites measured by LC-MS over 4 days. ***Bottom***: Actual measurements for 3 manufacturing runs (biological triplicates). **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.

Conclusions

- 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.
- The data reduction scheme ranks most relevant markers, rationally, yielding small practical subsets useful in manufacture.
- LC-MS proved valuable for monitoring reproducible daily fluctuations of metabolic patterns.
- 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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