Towards Industry 4.0: Development of a Smart Bioprocessing Platform Integrating Real-Time Monitoring and Advanced Process Control for Autologous Cell Therapy

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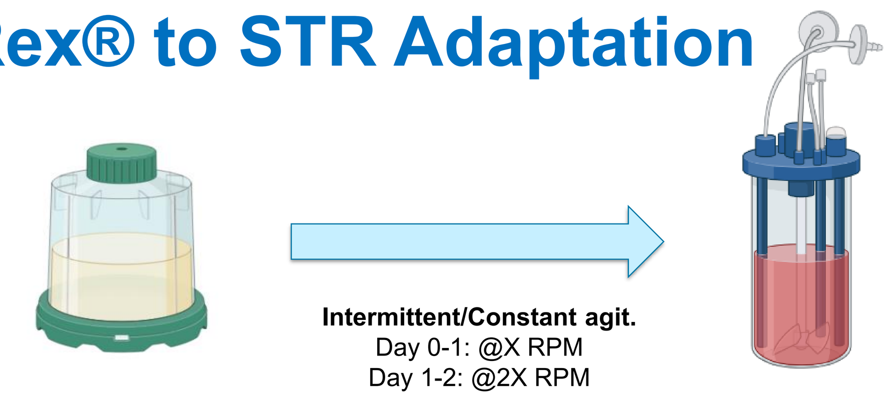
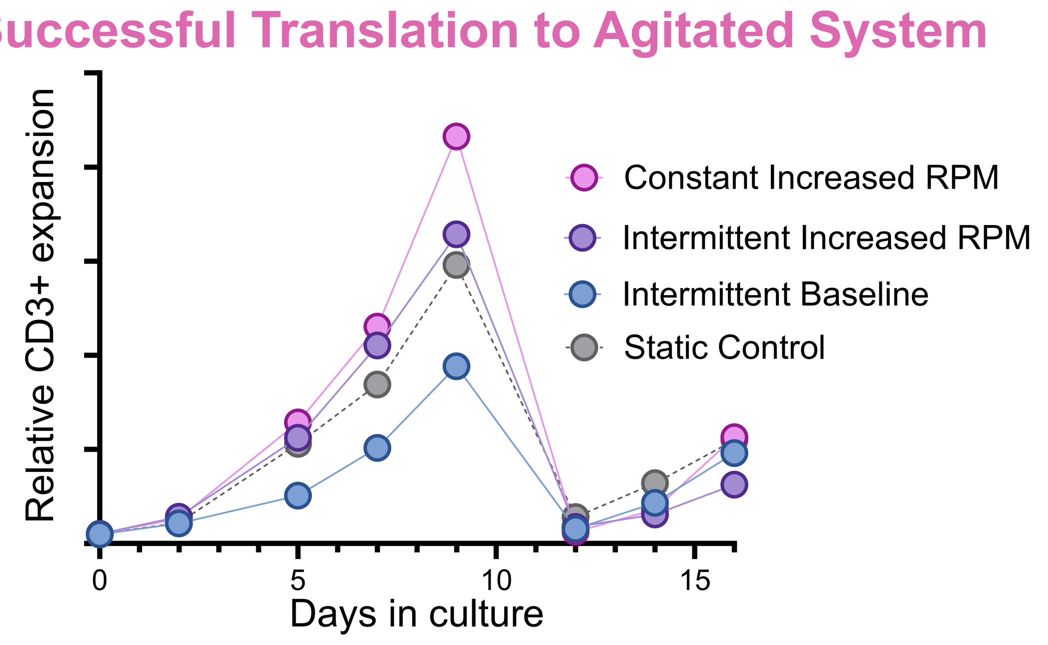
Clinical adoption and patient's accessibility to autologous immunotherapies remain hindered by the lack of efficient, consistent, cost-effective, and scalable manufacturing processes. To address this bottleneck, an international EU-funded consortium is developing a first-in-class, smart bioprocessing platform for personalised autologous cell therapies, integrating in-line process analytical technologies and advanced process control systems. Using a clinical-stage tumor-infiltrating lymphocytes (TILs) manufacturing process as a model, a static baseline process in the G-Rex system was adapted to a dynamic, stirred-tank reactor (STR) system. In addition, metabolomic profiling of spent media identified ~50 metabolites with a significant effect on cell expansion. Focusing on three key metabolites, alongside agitation speed and dissolved oxygen as critical process parameters (CPPs), we conducted design of experiment (DoE) studies using the STR systems, with integration of Raman probes, as an online process analytical tool. Based on these results, subsequent work will implement real-time monitoring and model predictive control strategies leveraging chemometric and mechanistic models to enhance process consistency and performance.

1. GRex® to STR Adaptation

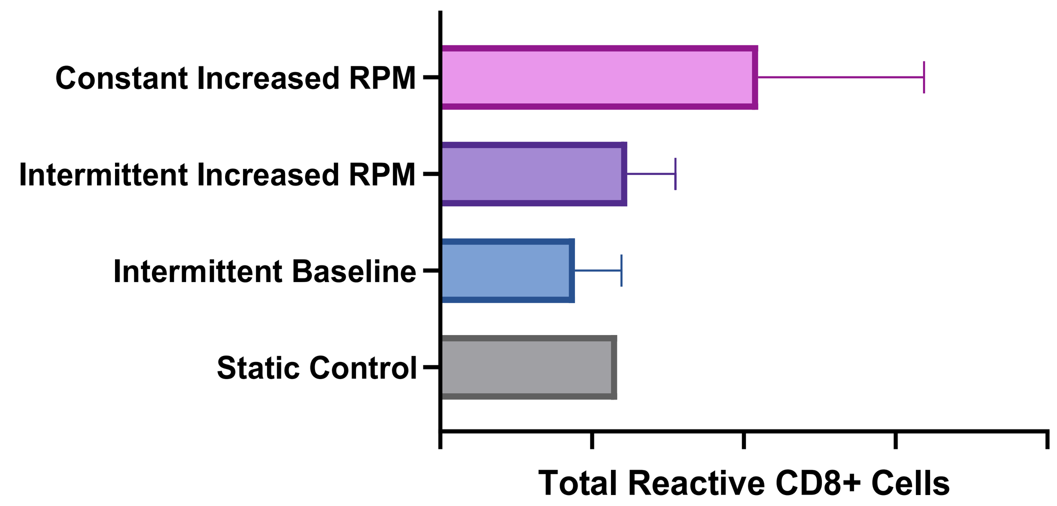


- Exemplar autologous TIL-DC co-culture process with subsequent reactive TIL expansion.
- Previously was suggested that static conditions were thought to be needed for higher reactivities.
- Agitated systems are controllable. pH, DO, temperature, metabolite controls can facilitate improved autologous process performance.

Successful Translation to Agitated System



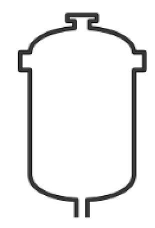
Maintenance of CD8+ Reactivity in Agitated System



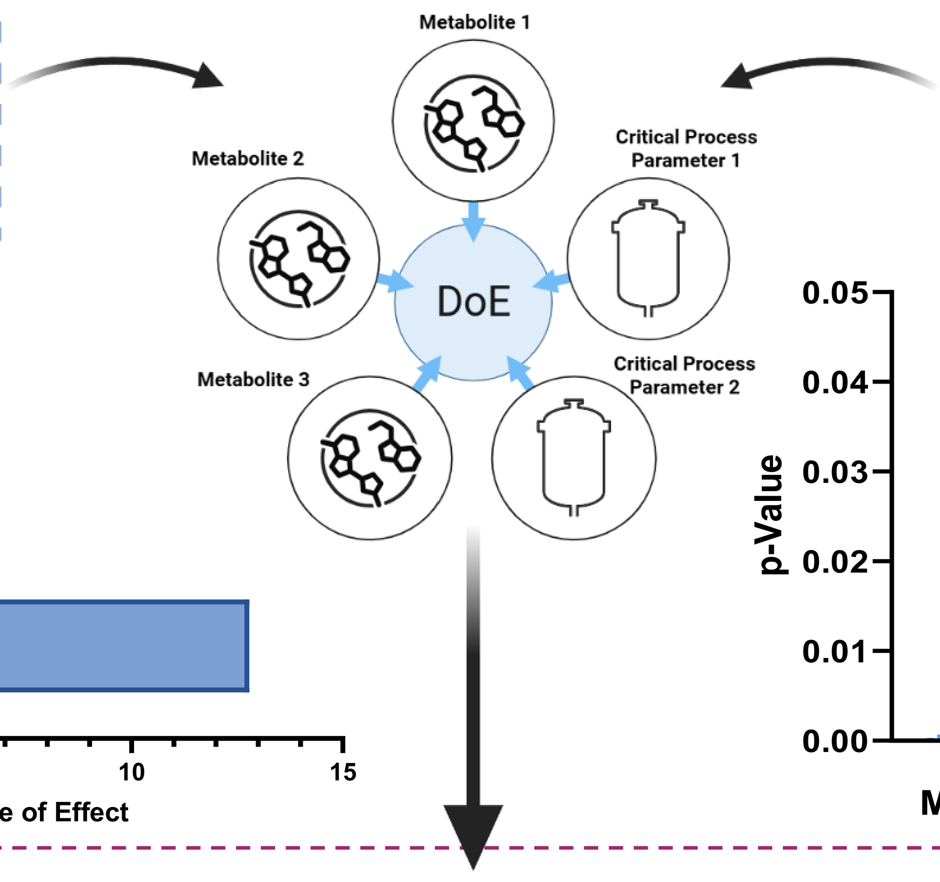
- Successful translation of static process to STR and subsequent investigation of intermittent versus constant agitation.
- These studies showed constant, high RPM led to higher T-Cell expansion and reactivity than the initial static.

2. Critical Process Parameter/Biomarker Screen

Critical Process Parameter Screen



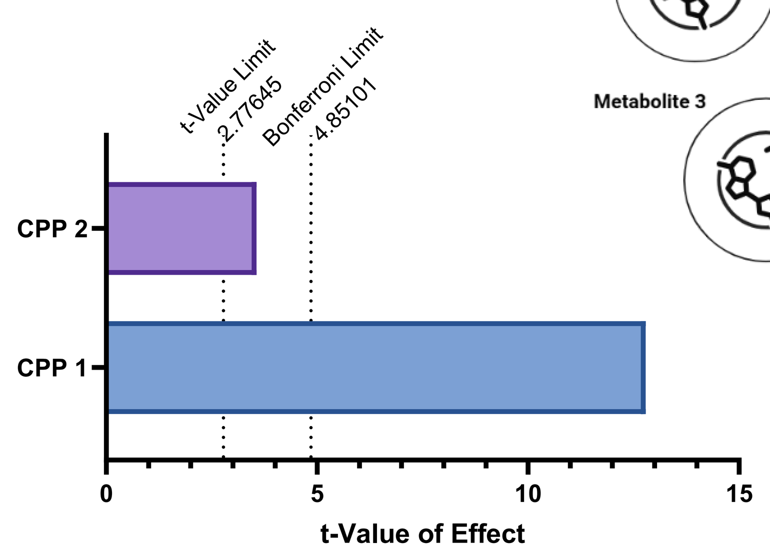
Identification of CPPs by SME knowledge and literature review

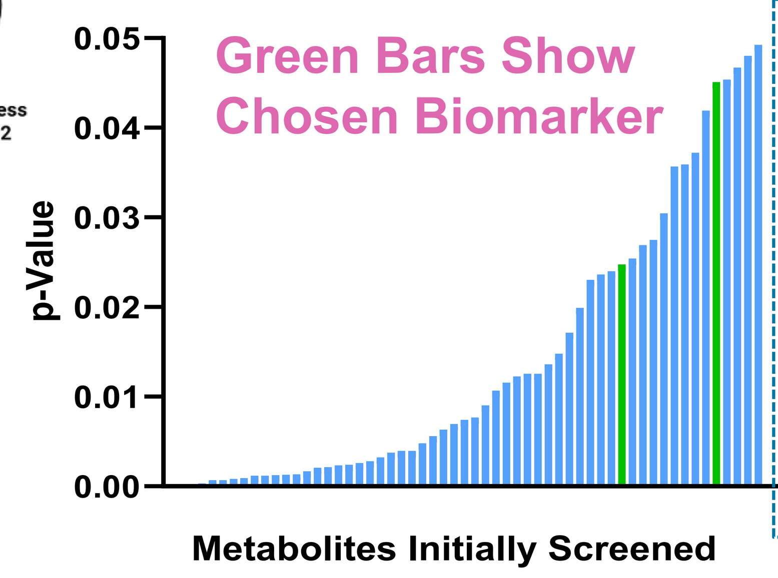


Biomarker Screen

Spent media samples taken from initial runs used to identify key metabolites for T-Cell metabolism

- T-Value is an indicator of effect significance.
- Above Bonferroni limit means clearly significant.
- Above t-value limit means effects are possibly important.





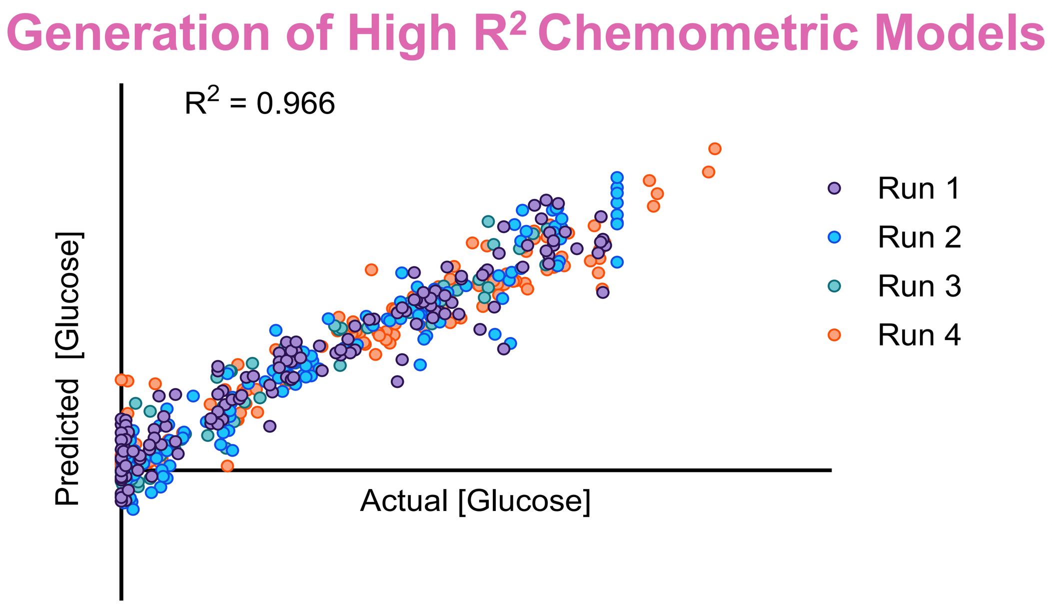
- p-Values indicate output from Robust Rank Aggregation to prioritise metabolites into a universally ranked list.

- Two steps done in parallel.
- 7 CPPs were screened over 2 screening runs.
- These resulted in identifying 2 process parameters critical to T-Cell expansion and reactivity.
- Biomarker screen led a shortlist of 57 metabolites.
- These were narrowed to 3 identifiable by Raman, and interesting for T-Cell metabolism.

3. T-Cell Culture Design Space Exploration

- The DoE was a 5-level, central composite design, consisting of 32 STR vessels utilising parameters identified in the previous screens.
- Aim was to introduce significant process perturbations and measure their effects expansion of CD3+, reactive CD4+, and reactive CD8+.
- Raman spectroscopy data was collected for generation of a PAT chemometric model.

Generation of High R^2 Chemometric Models



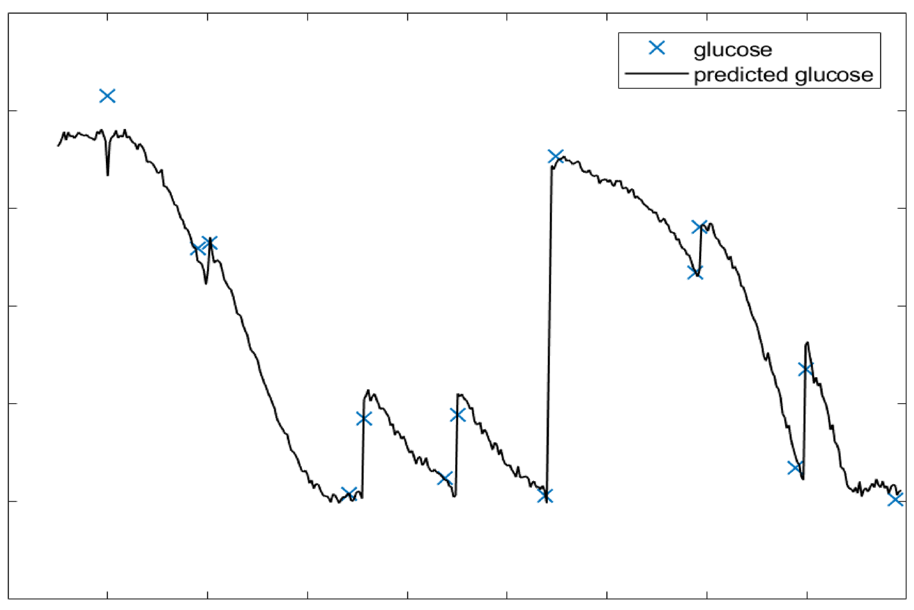
Optimal Parameter Determination

Metabolite A	Metabolite B	Metabolite C	CPP 1	CPP 2	Response	Response Prediction	Desirability
↑	↑	↓	↑	↓	↑ CD3+ Fold Expansion	403-fold	0.653
↓	↑	↓	↑	↑	↑ CD4+ Fold Expansion	469-fold	0.224
↓	↑	↓	↑	↑	↑ CD8+ Fold Expansion	986-fold	0.696
↓	↓	↑	↑	↑	↑ % Reactivity	NA	0.462

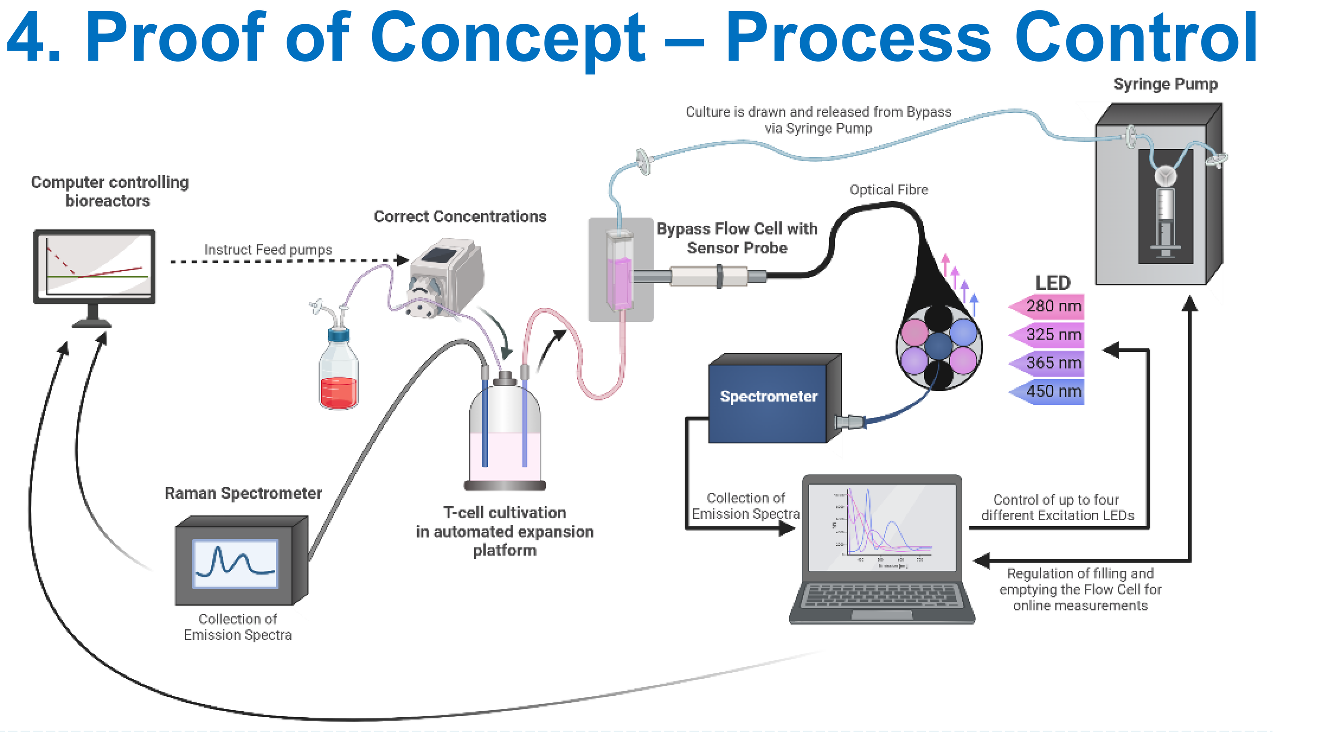
- 800 Raman spectra were selected as training data for chemometric models.
- These were utilised for a model for predictive control as a strategy to predict process output based on real-time monitoring of CPPs.

- DoE model predicted ideal operating conditions for T-Cell bioprocessing.
- Model predicts that a combination of high metabolite A and high metabolite B will result in high CD3+ fold-expansion.
- Model also predicts that high metabolite C will increase % reactivity.

Real Time Glucose Monitoring

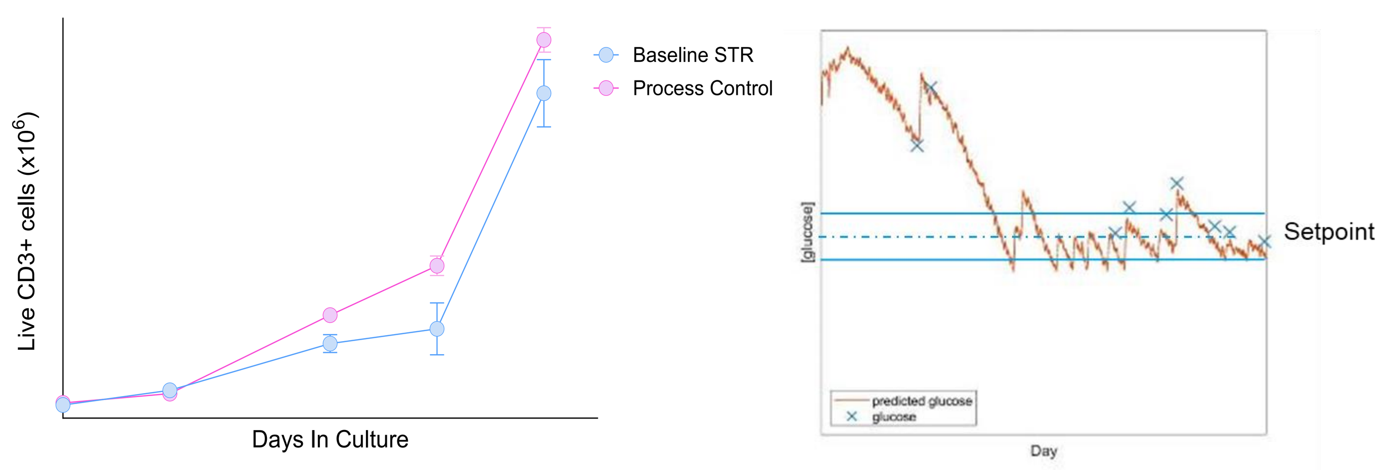


4. Proof of Concept – Process Control



- PoC: chemometric and bioprocess models were integrated for use in real-time monitoring and control of CPP's.
- Initial PoC run demonstrated a trend towards improved performance and reduced variability of expansion.
- Future work: Further optimisation of models and process control to improve quality of product and using patient material to establish success of process control in therapeutic setting.

Control Strategy Process Improvements and Glucose Control



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