Multiparametric Approach for Defining Product Quality

Dr Damian Marshall
Bioprocessing Summit
August 2016
The Cell and Gene Therapy Catapult

£70m Development Facility

• $1,200m^2$ Custom designed cell and gene therapy development facility

• **Prime location** in the heart of the London clinical research cluster

• **120** permanent staff

£55m large scale manufacture center

• $7,200m^2$ manufacturing centre designed specifically for cell and gene therapies

• **Located** in the Stevenage biocatalyst

• **Opening** 2017
The Cell and Gene Therapy Catapult

**Therapy industrialisation**
- Process development
- Analytical development
- GMP process proving
- Supply chain
- Late clinical phase manufacturing

**Business**
- Business development
- Business models
- Health economics

**Clinical trial and regulatory**
- Regulatory
- Clinical trial sponsor
- Clinical operations
- Pre-clinical safety

---

10.
- ES and iPS cells
- HSC’s
- MSC’s

7.
- T cells
- T Regs
- Dendritic cells

3.
- Somatic cells
  - Islet cells
  - Fibroblasts

8.
- Tissue / Scaffold
- Tech development
Product Characterisation
Product characterisation

Characterization of a biological product (includes determination of physiochemical properties, biological activity, immunochemical properties, purity and impurities) by appropriate techniques is necessary to allow relevant specifications to be established.

ICH 6QB - Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

Why characterise cell and gene therapy products?

• Product characterisation demonstrates control of the manufacturing process.
• Establishing specifications during manufacture helps ensure the quality and lot-to-lot consistency of the final product.
• Product parameters can be used to anticipate sub-optimal manufacture runs.
• Characterisation can help assess product integrity and stability.
Product characterisation challenges

- What are the relevant parameters to measure?
- Can cell quality be measured and monitored? What is cell quality?
- Impact of the manufacturing process on cells – what is affected?
Product characterisation challenges

- What are the relevant parameters to measure?
- Can cell quality be measured and monitored? What is cell quality?
- Impact of the manufacturing process on cells – what is affected?
The CGT Analytical Platform
iPS cell manufacture

Development of an automated iPS cell expansion process

- iPS cell expansion maintained in adherent culture for 40 days
- Cells undergo approximately 20-25 population doublings

Challenge – Development of an IPC framework which can monitor cell quality and predict process success in manual and automated systems

Media
- Metabolites
- GF/Cytokine analysis

Cells
- Morphology / Confluency

Cells
- Cell enumeration / viability
- Protein expression
- Gene expression
Analytical Platform

1. Analytical Design
   - QbD Analysis
   - Analytical strategy based on:
     - Key unit operations
     - Sample Availability
     - Technology suitability

2. Analytical testing
   - Screening strategy
   - Integrated analysis
   - Augment Information
   - Stress testing

3. Biological Profiling
   - Cluster Analysis
   - Data Reduction
   - Multivariate Analysis

4. Customised QC
   - Custom Marker Selection
   - Reference materials
   - Automated analysis
   - Design space

Manufacture 1
- RNA
- Surface markers
- Imaging
- Cytokines + metabolites

CATAPULT Cell and Gene Therapy
Quality by Design

• The cell and gene therapy catapult uses a quality by design (QbD) approach to establish the appropriate analytical strategy for a product

• QbD is a risk based framework for process design which relates product and process attributes to product quality

• The approach is based on knowledge of product biology and the engineering used during its manufacture

• By establishing the critical quality attributes (biological, physical, biochemical) for a product a design space can be established which defines the expected range of each characteristic during manufacture

QbD is usually guided by the quality of the final product........
QbD Toolkit

Analytical Design

Analytical Design

### Priority Grid

<table>
<thead>
<tr>
<th># reference</th>
<th>Process section</th>
<th>Potential failure mode</th>
<th>Potential failure effects</th>
<th>Severity</th>
<th>Mechanism of failure</th>
<th>Occurrence</th>
<th>Current process controls</th>
<th>RPN</th>
<th>RPC</th>
<th>Potential controls</th>
<th>Method of implementation</th>
<th>Ease of implementation</th>
<th>Process benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Manual expansion</td>
<td>Confluence at harvest criteria is too high</td>
<td>Reduced viability, reduced average growth, differentiation</td>
<td>4</td>
<td>Harvest criteria based on time or based on confluence but poorly defined</td>
<td>3</td>
<td>current harvest criteria is when cells cover most of the well (4-5 days)</td>
<td>7</td>
<td>84</td>
<td>MII</td>
<td>Define harvest criteria based on confluence. Automate confluence measurements. Initiate operator training to improve confluence assessment</td>
<td>Documentation changes</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Unclassified)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Data Flow

- **Assay or Donor data**
  - Decision: No → **Rejected Product**
  - Decision: Yes → **Final Product**

### Quality Attributes

- Quality Attribute 1
- Quality Attribute 2
- Quality Attribute 3
- Quality Attribute 4
- Quality Attribute 5

### Score

- Process Parameters
- Template
- Equipment
- Rejected Product

### QbD Toolkit Details

- Project Code: Template 100x04
- Last Modified: 24/02/2015
- Printed: 24/02/2015
Analytical Platform

1. Analytical Design
   - QbD Analysis
   - Analytical strategy based on:
     - Key unit operations
     - Sample Availability
     - Technology suitability

2. Analytical testing
   - Screening strategy
   - Integrated analysis
   - Augment Information
   - Stress testing

3. Biological Profiling
   - Cluster Analysis
   - Data Reduction
   - Multivariate Analysis

4. Customised QC
   - Custom Marker Selection
   - Reference materials
   - Automated analysis
   - Design space

[Diagram showing various components and processes related to analytical testing and profiling]
Image analysis

Cell confluence tracking using quantitative imaging

- 3 independent biological replicates
- Cells seeded at the same cell density

Day 1

Day 2

Day 3

Day 4
Texture analysis

Texture of colonies

Day 1

Day 2

Day 3

Day 4

Analytical testing
### Flow cytometry analysis

<table>
<thead>
<tr>
<th>Function</th>
<th>Passage 2</th>
<th>Passage 4</th>
<th>Passage 6</th>
<th>Passage 8</th>
<th>Passage 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.11</td>
<td>0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>R2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.09</td>
<td>0.05</td>
<td>0.0</td>
</tr>
<tr>
<td>R3</td>
<td>0.13</td>
<td>0.13</td>
<td>0.08</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Stress</td>
<td>0.11</td>
<td>0.11</td>
<td>0.22</td>
<td>0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Pluripotency</td>
<td>95.6</td>
<td>95.9</td>
<td>95.6</td>
<td>95.6</td>
<td>95.6</td>
</tr>
<tr>
<td>Mesoderm</td>
<td>99.2</td>
<td>99.8</td>
<td>99.8</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>Endoderm</td>
<td>99.9</td>
<td>99.8</td>
<td>99.9</td>
<td>99.9</td>
<td>99.9</td>
</tr>
<tr>
<td>R1</td>
<td>9.2</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td>R2</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Stress</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Pluripotency</td>
<td>95.6</td>
<td>95.6</td>
<td>95.6</td>
<td>95.6</td>
<td>95.6</td>
</tr>
<tr>
<td>Mesoderm</td>
<td>99.2</td>
<td>99.8</td>
<td>99.8</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>Endoderm</td>
<td>99.9</td>
<td>99.8</td>
<td>99.9</td>
<td>99.9</td>
<td>99.9</td>
</tr>
<tr>
<td>R1</td>
<td>10.2</td>
<td>10.4</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
</tr>
<tr>
<td>R2</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
</tr>
<tr>
<td>R3</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
</tr>
<tr>
<td>Stress</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td>Pluripotency</td>
<td>95.6</td>
<td>95.6</td>
<td>95.6</td>
<td>95.6</td>
<td>95.6</td>
</tr>
<tr>
<td>Mesoderm</td>
<td>99.2</td>
<td>99.8</td>
<td>99.8</td>
<td>99.8</td>
<td>99.8</td>
</tr>
<tr>
<td>Endoderm</td>
<td>99.9</td>
<td>99.8</td>
<td>99.9</td>
<td>99.9</td>
<td>99.9</td>
</tr>
</tbody>
</table>

**Diagram:** 2D PCA plot showing differentiation towards endoderm, mesoderm, and ectoderm. Red and green dots represent different conditions or samples.
RT-qPCR analysis

- Commercial screening technologies can be hard to interpret
- Reference samples may not be fit for purpose

- Established in house panels for ES and iPS cell manufacture
- Panel based on pluripotency, self renewal, differentiation and stress markers
Extended metabolic screen

- Use of LC/MS to screen for metabolic profile
- Wide range screen
- Automated sample preparation and analysis
Extended metabolic screen

Kynurenine

Area ratio

Day

Citric acid

Area ratio

Day

2-Aminoadipic acid

Area ratio

Day

- Pluripotent
- Endoderm
- Mesoderm
- Ectoderm

PC1 (0.53)
PCC2 (0.25)
Analytical Platform

1. Analytical Design
   - QbD Analysis
   - Analytical strategy based on:
     - Key unit operations
     - Sample Availability
     - Technology suitability

2. Analytical Testing
   - Screening strategy
   - Integrated analysis
   - Augment Information
   - Stress testing

3. Biological Profiling
   - Cluster Analysis
   - Data Reduction
   - Multivariate Analysis

4. Customised QC
   - Custom Marker Selection
   - Reference materials
   - Automated analysis
   - Design space
Data integration
Multi-parametric data structure

183 parameters in total, 159 time points/conditions

10 Passages

differentiation

live quantitative imaging
flow cytometry

gene expression

cell counters

metabolites
Weight Network Expression Analysis

183 parameters in total,
159 time points/conditions

157 parameters filtered for network analysis
(Topological Overlap Matrix – TOM)

Modules = networks: parameters with similar expression patterns across conditions, suggesting functional relationships.

Connectivity:
• Intra-network = clusters along diagonal
• Inter-network = red clusters away from diagonal

Within a particular module, similar AND different types of markers are mixed up and connected
The least connected markers are removed to leave a low resolution set of markers which has the same overall connectivity as the full original dataset.

**Data reduction**

Conservation of information

Relationships between markers as well as network connectivity is maintained.
Inferential markers
Analytical Platform

1. Analytical Design
   - QbD Analysis
   - Analytical strategy based on:
     - Key unit operations
     - Sample Availability
     - Technology suitability

2. Analytical testing
   - Screening strategy
   - Integrated analysis
   - Augment Information
   - Stress testing

3. Biological Profiling
   - Cluster Analysis
   - Data Reduction
   - Multivariate Analysis

4. Customised QC
   - Inferential analysis
   - Reference materials
   - Automated analysis
Network Analysis – surrogate markers

- Potential inferential markers can be identified based on their connectivity with other markers.

- By looking for strong overlap between modules it is possible to identify coordinated expression patterns – consistent throughout the production process.

- This allows the expression of one marker to be used to indicate the expression of others.
Inferential markers application

- Soluble markers
- RNA’s
- CD markers

Marker expression vs time leads to an end product. The graph indicates that some markers pass while others fail.
Surrogate markers application

- Soluble markers
- RNA’s
- CD markers

Marker expression vs. time leads to an end product, with a 'pass' indicating successful marker expression.
Summary
Analytical platform at the CGT

Build quality in the process by

Using a systematic approach to understanding product quality and define what characteristics to measure in order to:

- Allow control of the manufacturing process
- Support process optimisation to ensure the quality and lot-to-lot consistency of the final product
- Have the analytical capacity to understand and control (or terminate) the manufacture process
Summary – CGT analytical platform

Moving QC into the process

Providing the analytical platform for targeted characterisation to ensure that a product is of intended quality, based on information collected during the manufacturing process.

Analytical Inputs
- Metabolomics
  - Metabolites
  - Cell count
  - Viability
  - Protein expression
- Material Characterisation
- Cell count
- Cell count
- Material Characterisation
- Gene expression
- Protein expression
- Surface markers
- Sterility testing
- Genomic stability
- Purity

Process Outputs
- Product knowledge
- Manufacture design space
- Flexible manufacture
- Process Control
- Product Consistency

Raw Materials → Upstream processing → Downstream processing
“Helping cell and gene therapy researchers and companies across the world translate their research into commercially viable and investable therapies that are safe, effective, scalable and affordable.”