hPSC expansion and differentiation for the different derivatives
General considerations
Keeping the goal in mind

<table>
<thead>
<tr>
<th>Indication</th>
<th>Therapeutic cell type</th>
<th>Annual Incidence in UK</th>
<th>Predicted cell/dose</th>
<th>Annual cell requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>Cardiomyocytes</td>
<td>25,000 deaths</td>
<td>1-2 x10^9</td>
<td>7 x10^13</td>
</tr>
</tbody>
</table>

CPPs → Process → Efficiency

Quality → Assays → Control

Cost → Automation → Throughput

Reproducible
Scalable
Controlled
Affordable

Process changes ↔ Comparable product quality

Define QTPP to guide development

**Quality Attribute**: A molecular or product characteristic that is selected for its ability to help indicate the quality of the product. Quality attributes define identity, purity, potency, and stability of the product, and safety with respect to adventitious agents.

**Comparable**: A conclusion that products have highly similar quality attributes before and after manufacturing process changes and that no adverse impact on the safety or efficacy, including immunogenicity, of the drug product occurred. This conclusion can be based on an analysis of product quality attributes. In some cases, non-clinical or clinical data might contribute to the conclusion.

**Product characterisation**
- Physiochemical properties
- Safety
- Purity
- Process- and product-related impurities
- Potency (a measure of biological activity of the product in the context of the proposed MoA)
- Viability
- Sterility
- Quantity
PSC Expansion
Culture system and scale: technology selection

Culture system

Aggregates (3D)

Carriers (3D)

Monolayer (2D)

STR (3D)

Rocking-agitation (3D)

Rocking-agitation modified (3D)

Packed-bed (2D)

Hollow-fiber (2D)

Mag-drive (2D)
Intensification strategies

Kropp et al. 2017
Example of PSC scale up – 2D

By predicting cell numbers based on a metabolite read-out it was possible to automate and up scale the iPSC expansion in the Quantum bioreactor with efficient usage of medium.
Parameters of vessel design and fluid-dynamics were used to scale up the agitation rate for the aggregate-based culture of ESCs in from 15 mL (ambr15) to 3-L (CellReady) STRs.
## Discrepancies & communalities between BHF protocols

### Starting material
- Several hiPSC/hESC lines
- mTeSR, E8, MEF media; chemically defined media

### Passaging
- 1:12 – 1:20 / 65-85% / 4-5 days
- 1:8 – 1:12 / 65-85% / 3-4 days
- 1:6 – 1:10 / 90% / 4-5 days
- When “confluent”
- EDTA, TrypLE, Collagenase

### hPSC characterisation
- Sox2, Lin28, OCT4, TRA1-60, NANOG, SSEA4 expression
- Karyotyping
- Flow cytometry, qPCR, immunocytochemistry, colony morphology

### Open manual processing
- Quality assessments based on operator judgements
- Medium exchange regime
PSC cardiac differentiation
<table>
<thead>
<tr>
<th>Methods for hPSC cardiac differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monolayer on Matrigel</strong></td>
</tr>
<tr>
<td>Colonies on MEFs</td>
</tr>
<tr>
<td>Colonies on MEFs</td>
</tr>
<tr>
<td>Monolayer on Matrigel</td>
</tr>
<tr>
<td>Monolayer on Synthemax</td>
</tr>
<tr>
<td>Monolayer on Matrigel</td>
</tr>
</tbody>
</table>
# Current protocols at the BHF centres

<table>
<thead>
<tr>
<th>Pluripotent culture</th>
<th>Mesoderm induction factors</th>
<th>Cardiac specification factors</th>
<th>Cardiac differentiation factors</th>
<th>Cardiomyocyte purification</th>
<th>Cardiomyocyte maturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolayer on Gelatin</td>
<td>Wnt-C59, RPMI B27-ins</td>
<td>CHIR99021</td>
<td>RPMI B27 +ins</td>
<td>Glucose Starvation &gt;95% CMs</td>
<td></td>
</tr>
<tr>
<td>Monolayer on Matrigel</td>
<td>KY0211, XAV039, RPMI B27-ins</td>
<td>ActA, BMP4,</td>
<td>Chem Def Med</td>
<td>&gt;95% CMs</td>
<td></td>
</tr>
<tr>
<td>Monolayer on Geltrex</td>
<td>IWR-1, Retinoic acid</td>
<td>Card Diff Med</td>
<td></td>
<td>85-95% CMs</td>
<td></td>
</tr>
<tr>
<td>Monolayer on Vitronectin</td>
<td>ActA, BMP4, FGF2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Other discrepancies between BHF protocols

<table>
<thead>
<tr>
<th>Cell density</th>
<th>Protocol duration</th>
<th>PSC-CM characterisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>➢ 20-90,000 cells/cm²</td>
<td>➢ &gt;7 days</td>
<td>➢ Alpha-actinin/troponin-T expression</td>
</tr>
<tr>
<td>➢ 50-100,000 cells/cm²</td>
<td>➢ 12-25 days</td>
<td>➢ Calcium transients</td>
</tr>
<tr>
<td>➢ 85-95% confluency</td>
<td>➢ 15-30 days</td>
<td>➢ Immunocytochemistry</td>
</tr>
<tr>
<td>➢ &gt;90% confluency</td>
<td></td>
<td>➢ Flow cytometry</td>
</tr>
</tbody>
</table>
Opportunities for standardisation
Opportunities for standardisation

**PSC expansion**
Well defined substrate e.g. Synthetmax or Laminins; GMP hPSC line

**Cell number**
Automated counting when passaging; algorithm for confluency determination

**Characterisation**
Define thresholds; perform regular QC; define predictors of success

**Differentiation protocol**
Combine Wnt/ActA BMP4 pathways; Agree on small molecules of choice and protocol length

**Reagents**
Consider defined and GMP compliant reagents as early on as possible

**Analytics**
Choose methods suitable for GMP e.g. flow cytometry, qPCR

**Identify Critical Quality Attributes**
drug product; contaminants

**Early evaluation of process automation, closure, and scaling**