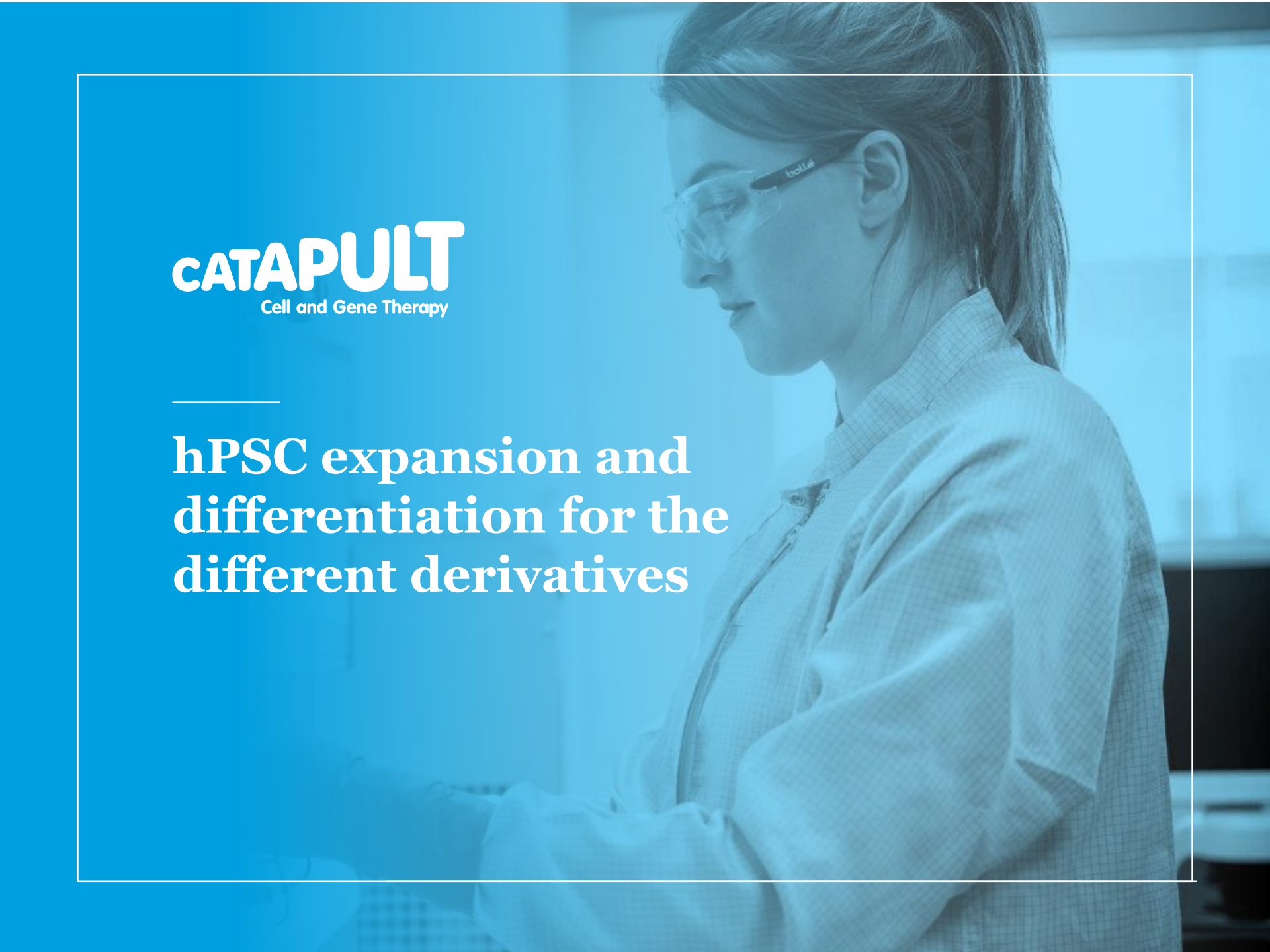
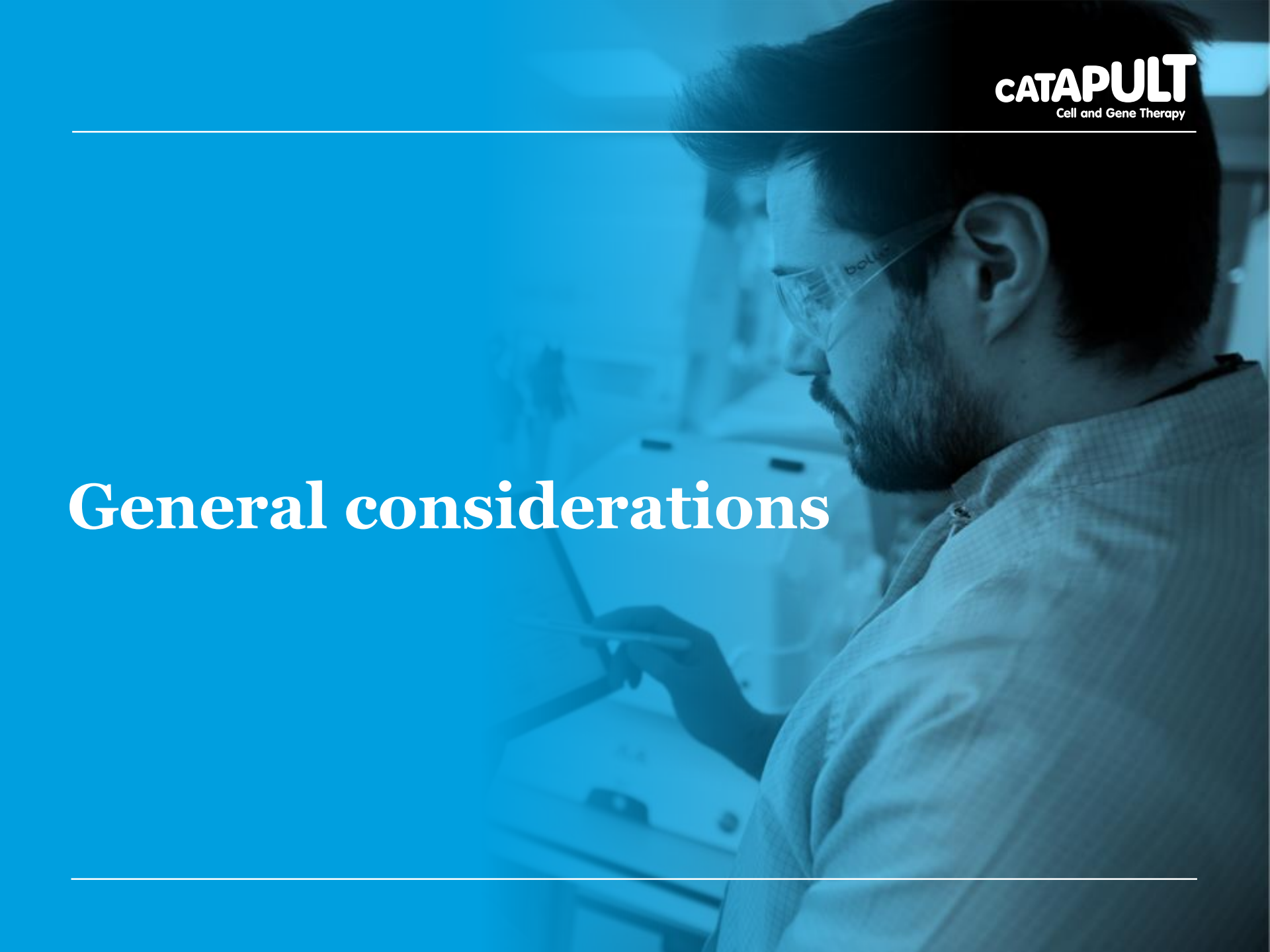
CATAPULT
Cell and Gene Therapy

hPSC expansion and differentiation for the different derivatives

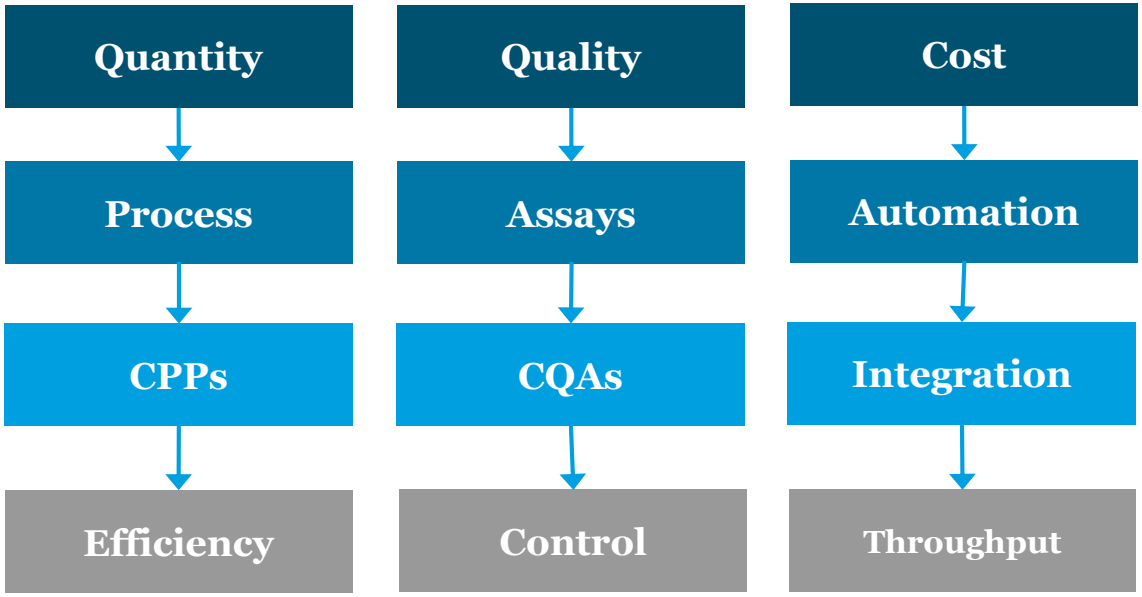




General considerations

Keeping the goal in mind

Indication	Therapeutic cell type	Annual Incidence in UK	Predicted cell/dose	Annual cell requirement
Myocardial infarction	Cardiomyocytes	25,000 deaths	1-2 x10^9	7 x10^{13}



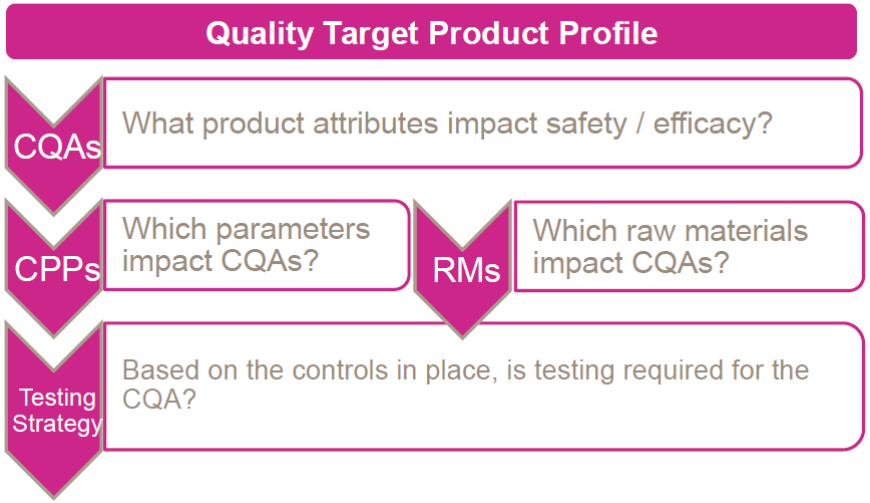
Reproducible
Scalable
Controlled
Affordable

Process changes ⇔ Comparable product quality

https://heartuk.org.uk/press/press-kit/key-facts-figures; Serra et al. 2012. TBTECH

Define QTPP to guide development

Thaw & Expansion → **Primitive Streak** → **Cardiac Mesoderm** → **Specification & Maturation**



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

- **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.

PSC Expansion

Culture system and scale: technology selection

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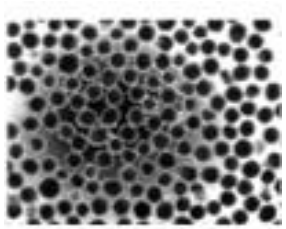
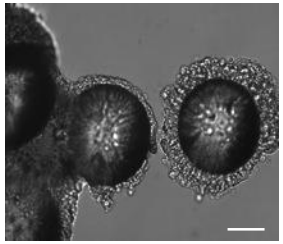
Culture system

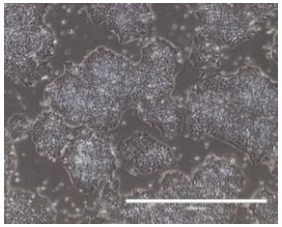
Aggregates (3D)



Carriers (3D)

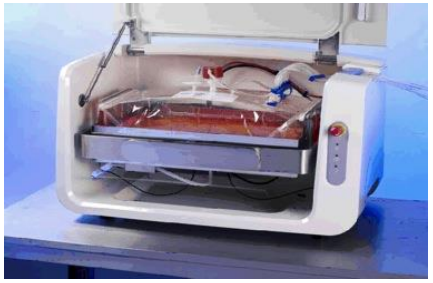


Monolayer (2D)



STR (3D)

Rocking-agitation (3D)



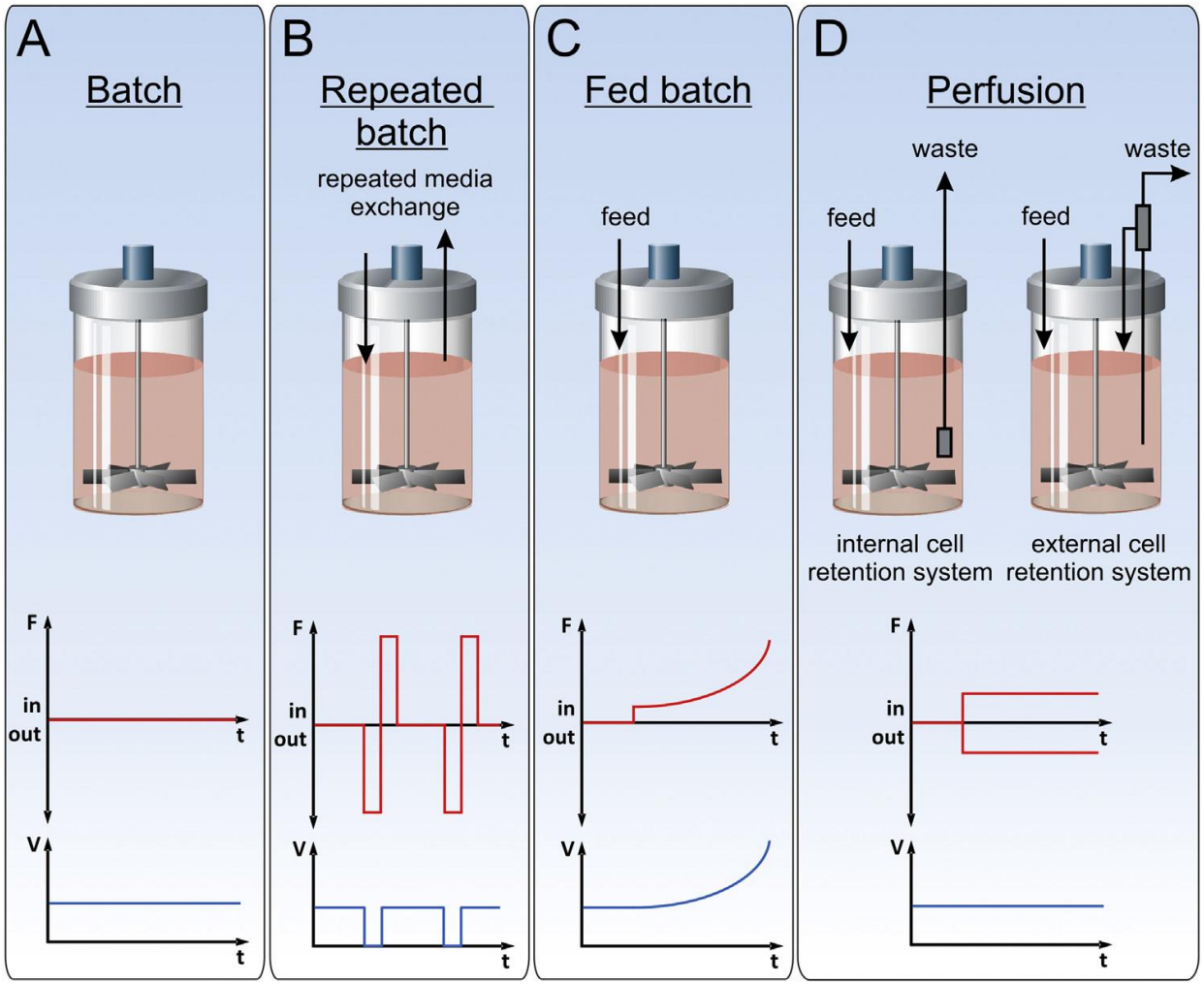
Rocking-agitation modified (3D)

Packed-bed (2D)

Hollow-fiber (2D)

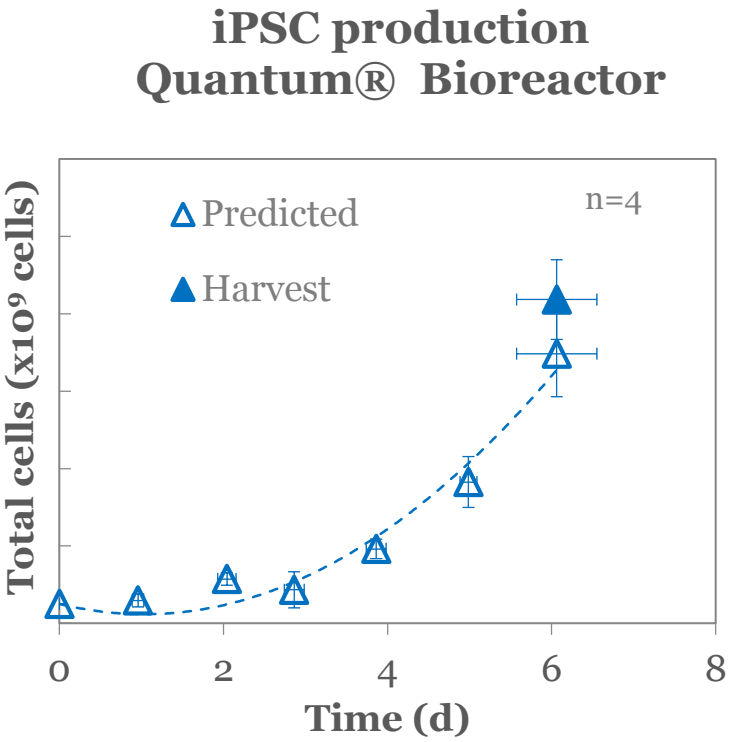
Mag-drive (2D)

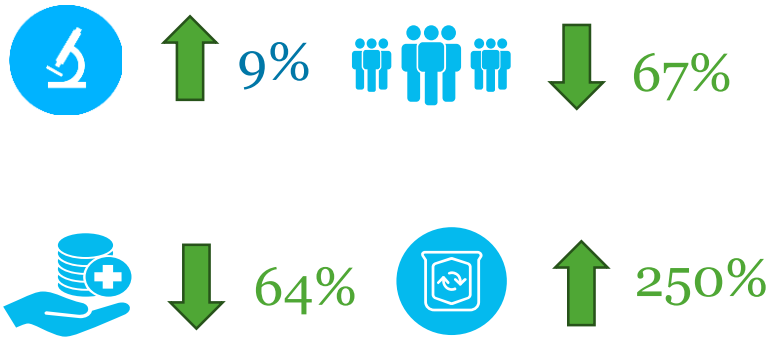
Intensification strategies



Kropp et *al.* 2017

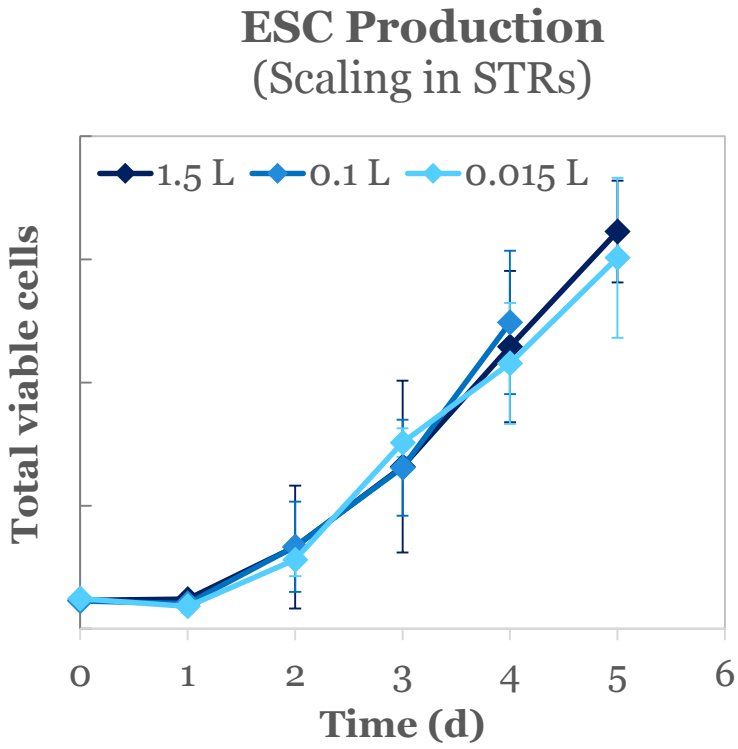
Example of PSC scale up – 2D

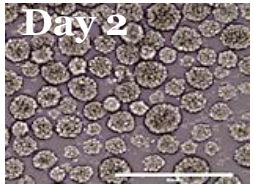


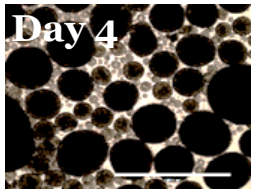


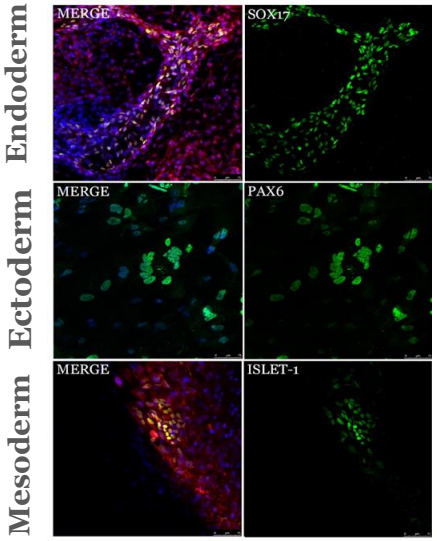
By predicting cell numbers based on a metabolite read-out it was possible to automate and up scale the iPSC expansion in the Quantum bioreactor with efficient usage of medium.

Example of PSC scale up – 3D









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

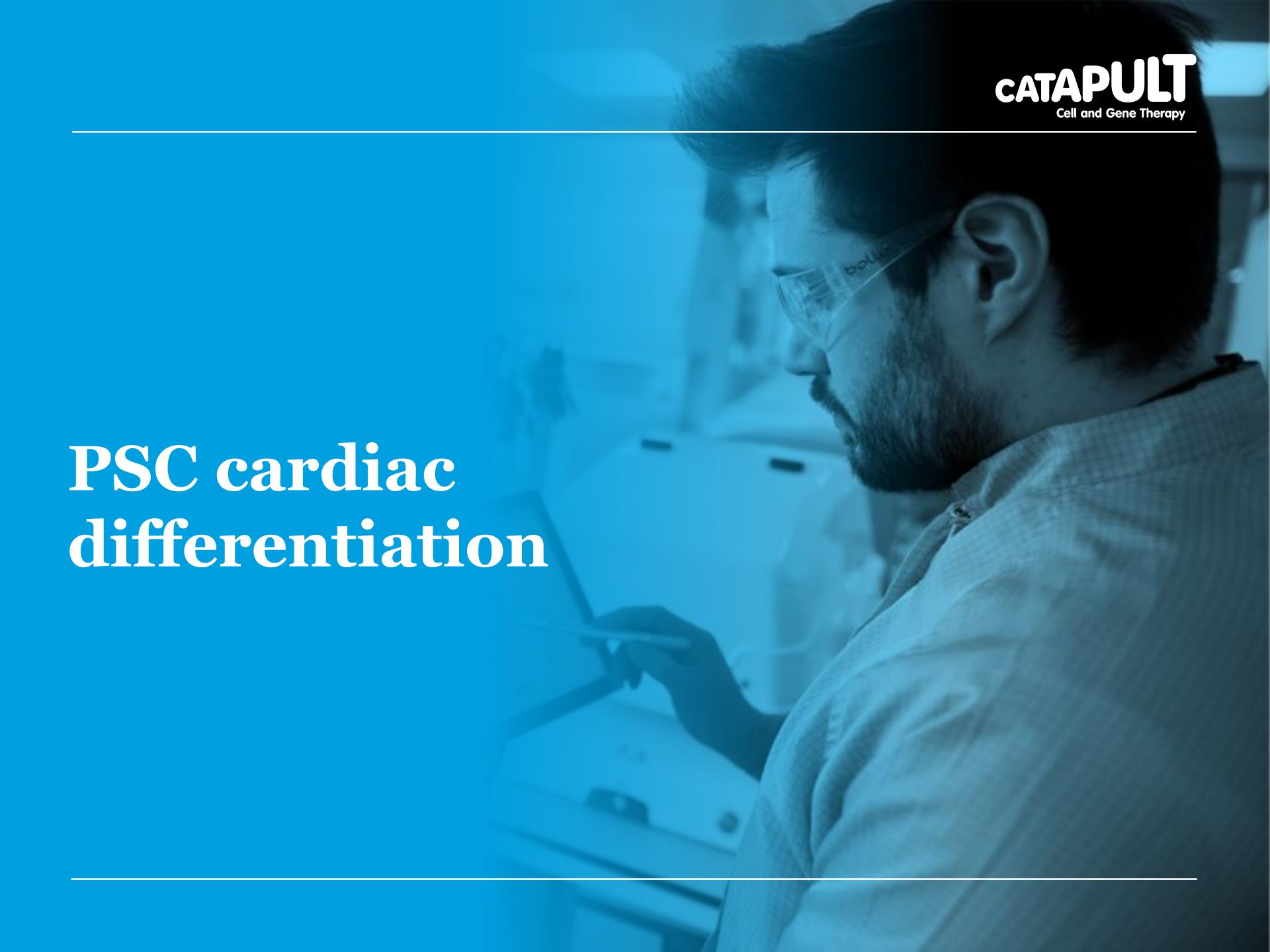
Passaging

- 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; Lin 28, 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

Methods for hPSC cardiac differentiation

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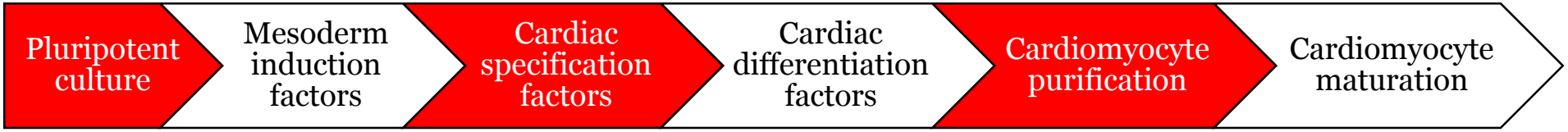
Monolayer on Matrigel	**2D**	**ActA, BMP4**	**RPMI B27**	**RPMI B27**	**30% CMs**	**Laflamme *et al.*, 2007**
Colonies on MEFs	3D	ActA, BMP4, FGF2	VEGFA, DKK1	VEGFA, FGF2	50% CMs	Yang *et al.*, 2008
Colonies on MEFs	**3D**	**ActA, BMP4, FGF2**	**VEGFA, DKK1, SB431542, dorsomorphin**	**VEGFA, FGF2**	**75% CMs**	**Kattman *et al.*, 2011**
Monolayer on Matrigel	2D	CHIR99021	IWP2	RPMI, AscAcid, Albumin	98% CMs	Lian *et al.*, 2012
Monolayer on Synthemax	**2D**	**CHIR99021**	**Wnt-C59**	**RPMI B27 +ins**	**95% CMs**	**Burridge *et al.*, 2014**
Monolayer on Matrigel	2D	CHIR99021, ActA, BMP4	XAV-939	RPMI B27 +ins	90% CMs	Palpant *et al.*, 2016

Current protocols at the BHF centres



Monolayer on Gelatin		**Wnt-C59, RPMI B27 -ins**		**Glucose Starvation >95% CMs**
	CHIR99021		**RPMI B27 +ins**	
Monolayer on Matrigel		**KY0211, XAV039, RPMI B27 -ins**		**>95% CMs**
	ActA, BMP4,		**Chem Def Med**	
Monolayer on Geltrex		**IWR-1, Retinoic acid**		**85-95% CMs**
	ActA, BMP4, FGF2		**Card Diff Med**	
Monolayer on Vitronectin				

Other discrepancies between BHF protocols

Cell density

- 20-90, 000 cells/cm²
- 50-100, 000 cells/cm²
- 85-95% confluency
- >90% confluency

Protocol duration

- >7 days
- 12-25 days
- 15-30 days

PSC-CM characterisation

- Alpha-actinin/troponin-T expression
- Calcium transients
- Immunocytochemistry
- Flow cytometry

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Opportunities for standardisation

Opportunities for standardisation

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PSC expansion
Well defined substrate e.g. Synthemax 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

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

Identify Critical Quality Attributes
drug product; contaminants

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

Early evaluation of process automation, closure, and scaling