CATAPULT
Cell and Gene Therapy

iPSC *vs* ESC

Different challenges and considerations

Questionnaire overview

Therapeutic indication/Product

Indication

- Cardiovascular disease

CM tissue engineering constructs/patches; enriched with biomaterials/endothelial cells; CRISPR-Cas9

CM injection or patch implantation

Endothelial cells (CLI)

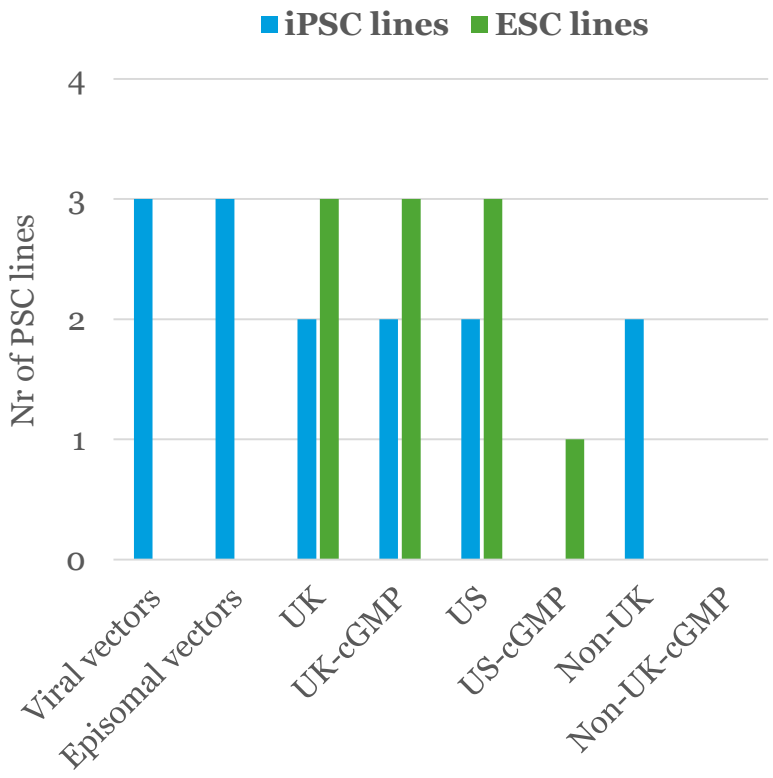
Starting materials – ESCs/iPSCs

Indication

- Cardiovascular disease

Cell type/line

- 6 ESC/6 iPSC – some protocols tested with several lines (>30 iPSC lines)
- UK ESC lines with limited distribution
- IP landscape challenges (iPSC lines)



Protocols – expansion and differentiation

Indication

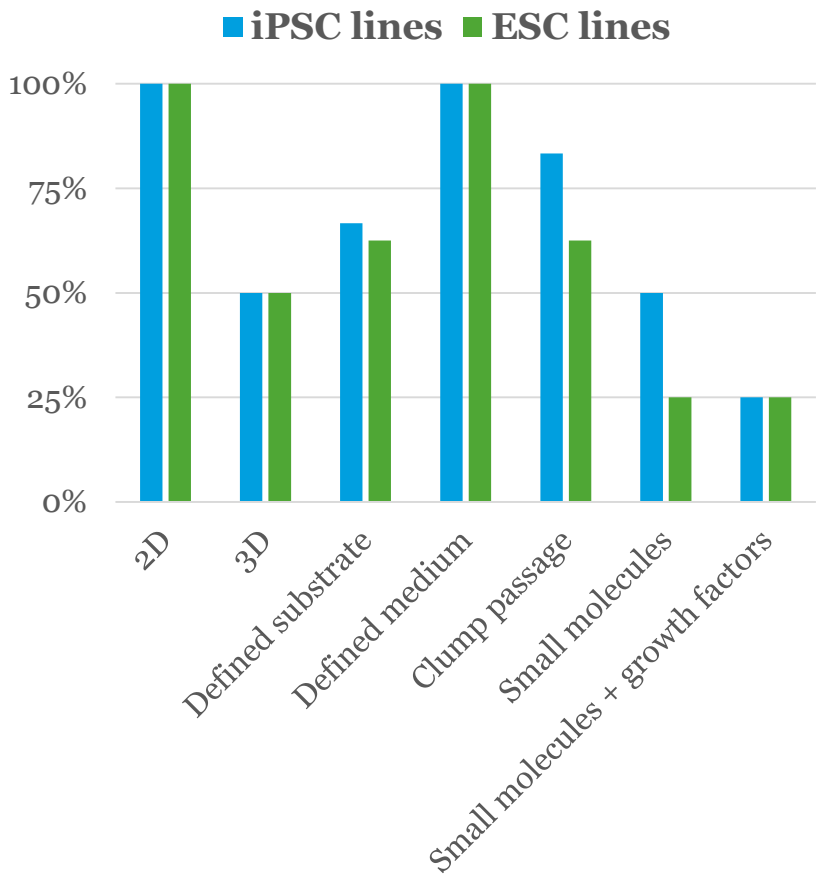
- Cardiovascular disease

Cell type/line

- 6 ESC/6 iPSC – some protocols tested with several lines (>30 iPSC lines)
- UK ESC lines with limited distribution
- IP landscape challenges (iPSC lines)

Culture methods & reagents

- 4 cardiac differentiation protocols
- Predominantly 2D-monolayer but also in 3D-EBs and microcarriers (in static and stirred suspension-bioreactors)
- Substrate of undefined composition
- Clump passage (process reproducibility)
- Differentiation process time (12-30 days)



Protocols – criteria and hurdles

Rationale

- Reproducible (cell lines)
- Efficient
- Robust (2D/3D)
- GMP-grade reagents

Limitations

- Manual open processing (flask based, cell sorting)
- Complex/undefined reagent composition
- Reproducibility across cell lines
- Scalability
- Quality assessments from operator judgements (visual observations, cell counts)
- Assess to validated assays in certified labs
- Standardised testing and characterization
- Cost

What are the fundamental challenges to develop PSC therapies?

Barriers

Starting material → Expansion → Differentiation → Purification → Formulation → Storage

Scientific (Safety)

Quality Target Product Profile **(QTPP)**
Critical quality attributes **(CQAs)**
Differentiation mechanisms
Mode of action
Immunogenicity & Tumorigenicity
Genetic stability

Technological (Process)

Starting materials
Culture reagents/ methods
Technology & Scale
Labour & automation **(CPPs)**
Productivity (efficiency->differentiation)
Analytical assays (what to monitor/control)

Commercial (Affordability)

Patient population
Development cost
Economy of scale
Market landscape (milestones & and threats)
IP landscape

Regulatory (Control)

GMP compliance
Validation of equipment, processes, and assays
In-process testing
Quality testing and release of starting materials and final product
Consensus of quality and safety standards
Bioequivalence at scale

1st generation processes are open, manual, inefficient, and unreliable

Starting materials - ESC *vs* iPSC

	Pros	Cons
ESC	• Low cost of derivation process • Well established and characterised • Availability of cGMP lines • Possibility for gene editing	• Ethical concerns (embryo destruction) • Exposure to animal-derived reagents • Incomplete historical • Limited HLA spectrum/histocompatibility • Mutation rates
iPSC	• Fewer ethical issues • Readily available donors; from vCJD-free sources • Easier donor cell sourcing (different starting cell types) • Non-integrating vectors (improved safety) • Allogeneic potential (ability to select HLA matched to patients)	• Yield, cost, and duration of derivation process • Unknown mechanism of reprogramming • Safety/Tumourigenicity (Oncogene activation risk) • Suboptimal standardisation • Need for GMP-grade lines in clinical trials

Proactive choice of starting cellular material

Harmonizing quality standards for starting material

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Attribute (mandatory)	Recommended Test/ Method	Acceptance
Identity	STR profile of donor and Lot	Identical
Genetic stability	Residual vector testing (iPSC)	Negative
	G-Banding	Normal (diploid) >20 metaphases
Viability at thaw	Dye exclusion or flow-cytometry	>60%
Phenotype	Flow cytometry	>70% positive expression (at least two markers: TRA1-60, OCT4, Nanog, etc)
Potency	EB formation and/or directed diff.	Demonstration of all three germ layers

Type of variability (measures of stability)	Among ESCs	Among iPSCs	Between ESCs and iPSCs	Within a PS cell line
Functional: *in vitro* differentiation	Yes	Yes	Yes	Yes
Gene expression: mRNA levels	No	Yes	Yes	Yes
Epigenetic: DNA methylation	Yes	Yes	Yes	-
Genetic:				
- Genetic background (germ line)	Yes	Yes	Yes	-
- Derivation method	Not known	Yes	Not applicable	-

Sullivan et al 2018; Stacey et *al* 2018; Allison et *al* 2018, Baker et *al* 2016; Robinton and Daley 2012;

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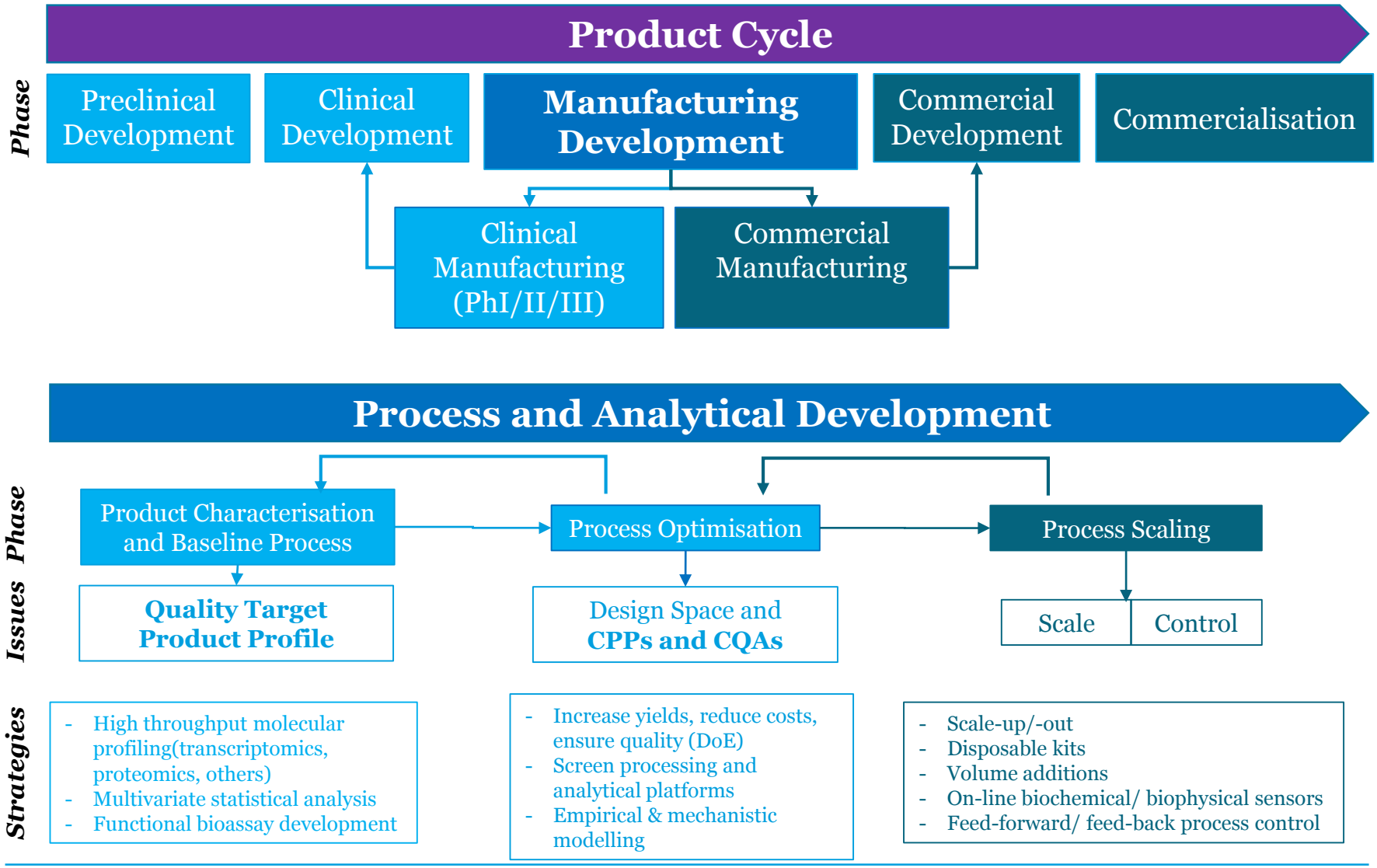
Systematic approach to product development

Development by design



Systematic approach

Starts with **predefined objectives** and emphasises **product and process understanding** and **process control**, based on sound science and quality risk management.

Approach	
Predefined objectives	• Define Quality Target Product Profile (QTPP) • Identify Critical Quality Attributes (CQA)
Product and process understanding	• Identify Critical Material Attributes and Critical Process Parameters (CPPs) • Establish the functional relationships that link Critical Material Attributes/CPP to CQA
Process control	• Develop an appropriate control strategy, including justifications
Sound science	• Science driven development (scientific literature, prior knowledge, DOEs, etc.)
Quality risk management	• Risk-based development approach