Production Assistance for Cellular Therapies
National Heart Lung and Blood Program

National Heart, Lung, and Blood Institute

Minimum Characterization Criteria for Clinical Grade iPSC Cell Banks and Final Products

Stakeholder Association: NHLBI, PACT

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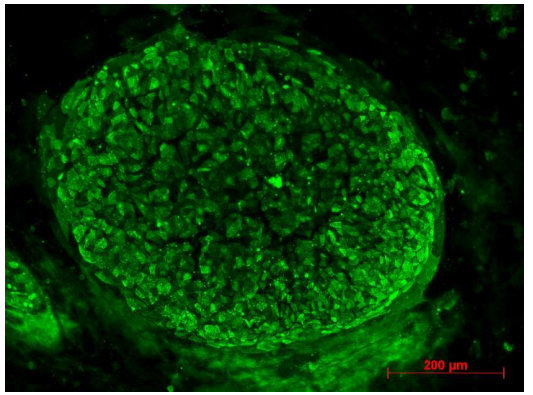
Induced pluripotent stem cells (iPSCs) - Need to establish guidelines for minimum characterization criteria

- A crucial problem in both the analysis of many human diseases and the development of effective therapies to treat disease is the incomplete understanding of the role played by human genetic variation in their development.
- Guidelines need to be established in the following area:
  - Donor qualification CFR 1271
    - Genetic testing
  - iPSC cell bank testing
  - iPSC final product testing



Challenges

- iPSC derivation (e.g., safety and efficiency of the reprogramming method, donor-to-donor variability, and choice of starting materials)
- iPSC challenges (e.g., development of a cell culture system for iPSC generation and expansion, cell sensitivity and robustness, and cryopreservation and revival)
- Safety and QC challenges (e.g., normal karyotype, residual plasmid clearance, in-process controls to evaluate iPSC quality, critical attributes of the final products, and standard safety concerns such as sterility).

Pros, Cons and Unknowns to iPSC Technology

- Pros:
  - Eliminate ethical issues
  - Donor's clinical phenotype is often known when working with iPSCs.
- Cons:
  - Cells would still have genetic defects
  - One of the pluripotency genes is a cancer gene
  - Viruses might insert genes in places we don't want them (causing mutation)
  - iPSC treatments will likely require donor's cells to undergo genetic alterations. Will it be acceptable to people that their cells have been modified?
- Unknowns:
  - could genetic defects affect recipients
  - could reprogramming alter or carry genetic defects

Identity - Cellular Biological Properties & Heterogeneity and Pluripotency

- Morphology
- Growth properties
- Stem cell markers: iPSCs expressed cell surface antigenic markers expressed on ESCs. Human iPSCs expressed the markers specific to hESC, including SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, TRA-2-49/6E, and Nanog.
- Stem Cell Genes: Oct-3/4, Sox2, Nanog, GDF3, REX1, FGF4, ESG1, DPPA2, DPPA4, and hTERT.

Donor Qualification

- Allogeneic
  - Donor qualification 21 CRF 1271
  - How does donor age influence the reprogramming process and iPSC functionality?
  - Perform genetic testing

Differentiation Potential

- Gene expression
  - Endoderm
  - Ectoderm
  - Mesoderm

  In vitro differentiation assay. (a) Confirmation of the differentiation potential of iPSC through spontaneous differentiation and formation of Embryoid body. (b) Analyze differentiation for the presentation of the markers of cells of the 3 germ layers, by qPCR of the Embryoid body.

Genome Integrity

- iPSC genome can reproduces that of the cell from which they are derived.
- iPSCs can potentially develop genetic abnormalities during reprogramming or prolonged cell culture.
- iPSC genome integrity must be routinely monitored by karyotyping

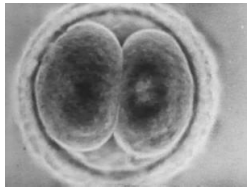
Induced Pluripotent Stem Cells (iPSCs)

Totipotent

Pluripotent

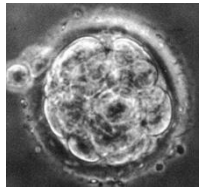
Lineage restricted/ multipotent

Zygote



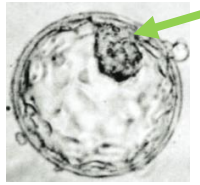
Day 3-4

Morula



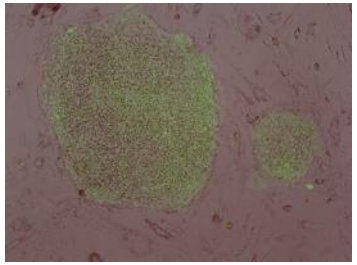
Day ~5

Blastocyst

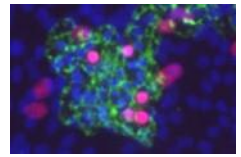


ICM

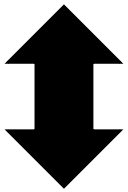
ESCs

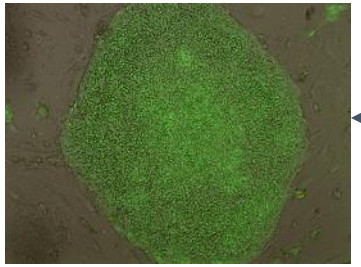


All tissues and stem/progenitor cells



Induce expression of Oct4, Sox2, Klf4, cMyc in any somatic cell (Takahashi and Yamanaka, Cell, 2006)





iPSCs

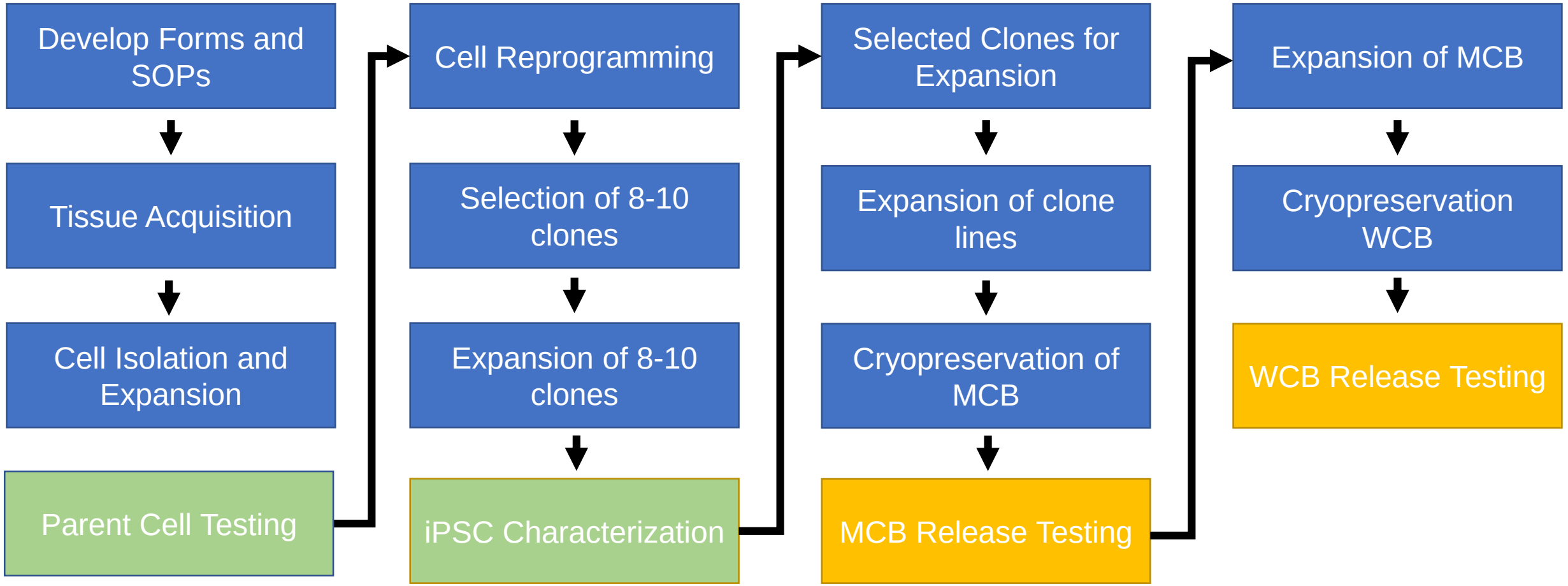
Reprogramming

Personalized Medicine

Disease Modeling

Drug Screening

Regenerative medicine



Yellow= MCB and WCB End Product Testing
Green= In progress Testing

Parent Cell Testing

Objective	ASSAY	Acceptance Results
Sterility	Immersion Sterility tests Bacteriostasis and Fungistasis (B/F)	No organisms Pass
Mycoplasma Testing	Points to consider mycoplasma detection	Negative
Endotoxin	Kinetic chromogenic LAL	< 0.5EU/mL
Cell Viability	Trypan Blue	>75%
Genetic Stability	Karyotype Analysis	46, XY
	STR Genotyping	Retain STR profile of parent population for later comparison

iPSCs Characterization

Objective	ASSAY	Acceptance Results
Sterility	Immersion Sterility tests Bacteriostasis and Fungistasis (B/F)	No organisms Pass
Mycoplasma Testing	Points to consider mycoplasma detection	Negative
Endotoxin	Kinetic chromogenic LAL	< 0.5EU/mL
Cell Viability	Trypan Blue	>75%
Reprogramming Vehicle Residual Testing	Depends on the method used for reprogramming	Negative
Genetic Stability	Karyotype Analysis	46, XY
	STR Genotyping	STR profile of starting population and iPSC lines are identical
Pluripotency Phenotype	Flow cytometry	SSEA4 > 70% Tra-1-60 > 70% Tra-1-81 > 70%
	Immunostaining	NANOG > 70%; OCT3/4 > 70% SSEA4 > 70% Tra-1-60 > 70%; Tra-1-81 > 70%
	Embryoid body formation	Detect of at least one marker per germ layer

MCB and WCB Release Testing

Objective	ASSAY	Acceptance Results
Sterility	Immersion Sterility tests and Bacteriostasis and Fungistasis (B/F)	No organisms
Mycoplasma Testing	Points to consider mycoplasma detection	Negative
Endotoxin	Kinetic chromogenic LAL	< 0.5EU/mL
Cell Viability	Trypan Blue	>75%
Genetic Stability	Karyotype Analysis	46, XY
	STR Genotyping	STR profile of starting population and iPSC lines are identical
Viral Contaminant	In vitro Assay for Adventitious Virus Contaminant	Negative
	Fluorescent Product Enhanced reverse Transcriptase (FPERT) method	Negative
	Transmission Electron Microcopy (TEM)	No viral particles
Pluripotency Phenotype	Flow cytometry	SSEA4 > 70% Tra-1-60 > 70% Tra-1-81 > 70%
	Immunostaining	NANOG > 70%; OCT3/4 > 70% SSEA4 > 70% Tra-1-60 > 70%; Tra-1-81 > 70%
	Embryoid body formation	Detect of at least one marker per germ layer

Future Direction

Explore the possibility of using single-cell RNA-seq to identify unique subpopulations that are especially suited for a patient-specific therapy.

Conclusion

Using autologous or allogenic iPSCs has advantages and disadvantages, and the choice of appropriate strategy may vary depending on the intended use. Additionally, there remain many factors that affect establishing transplantation therapy using iPSCs. To avoid tumorigenesis and establish effective differentiation into the intended cells, further investigation is needed to clarify which iPSC line is the most suitable and how these lines can be best selected.