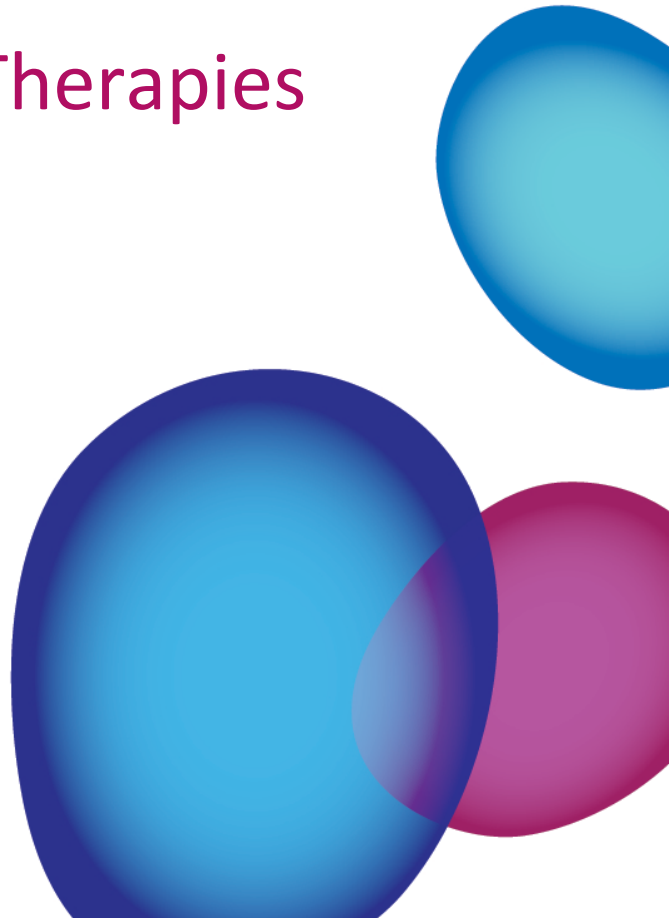
Preclinical Development of iPSC Therapies

September 11, 2014

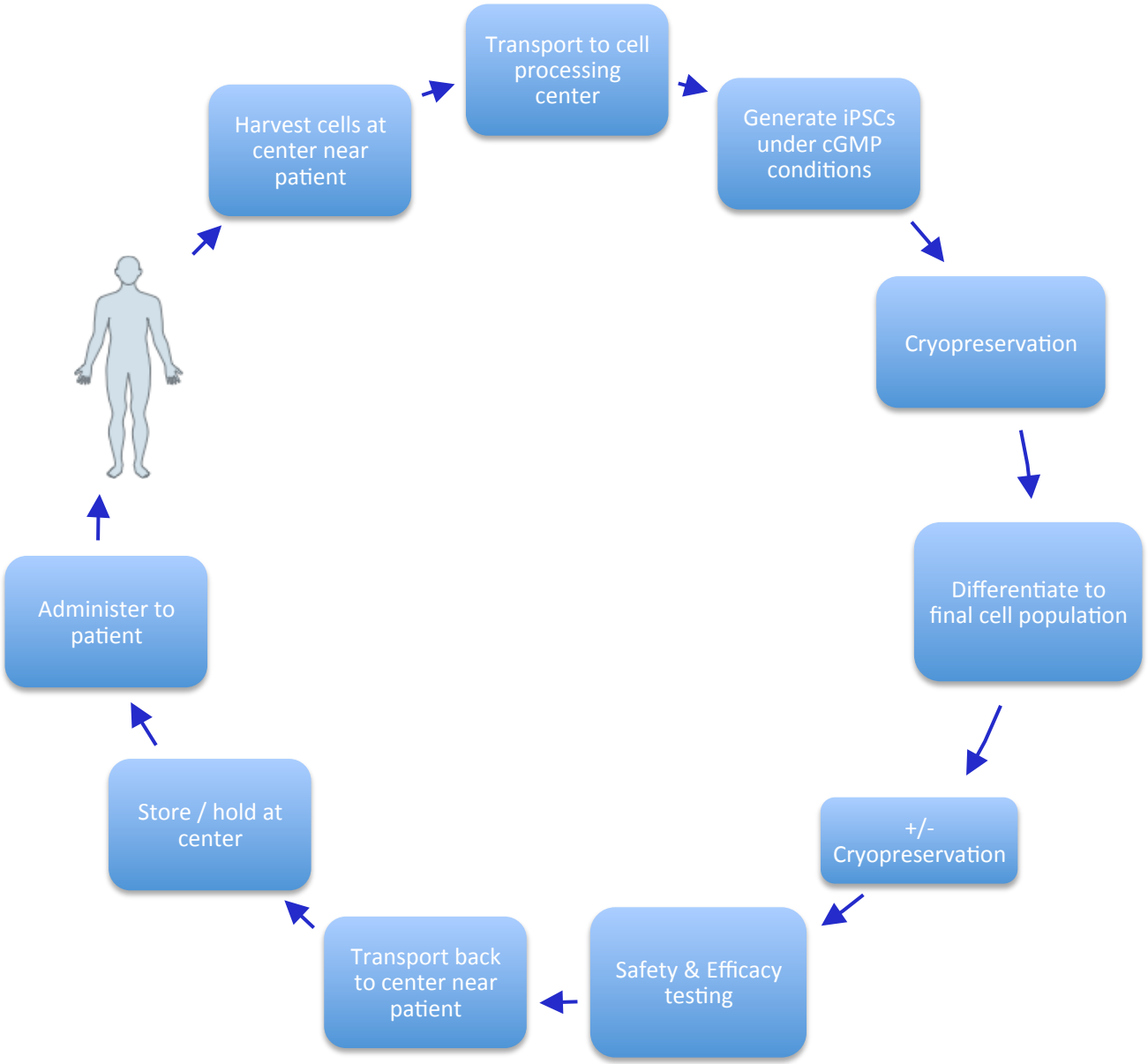
carpenter group



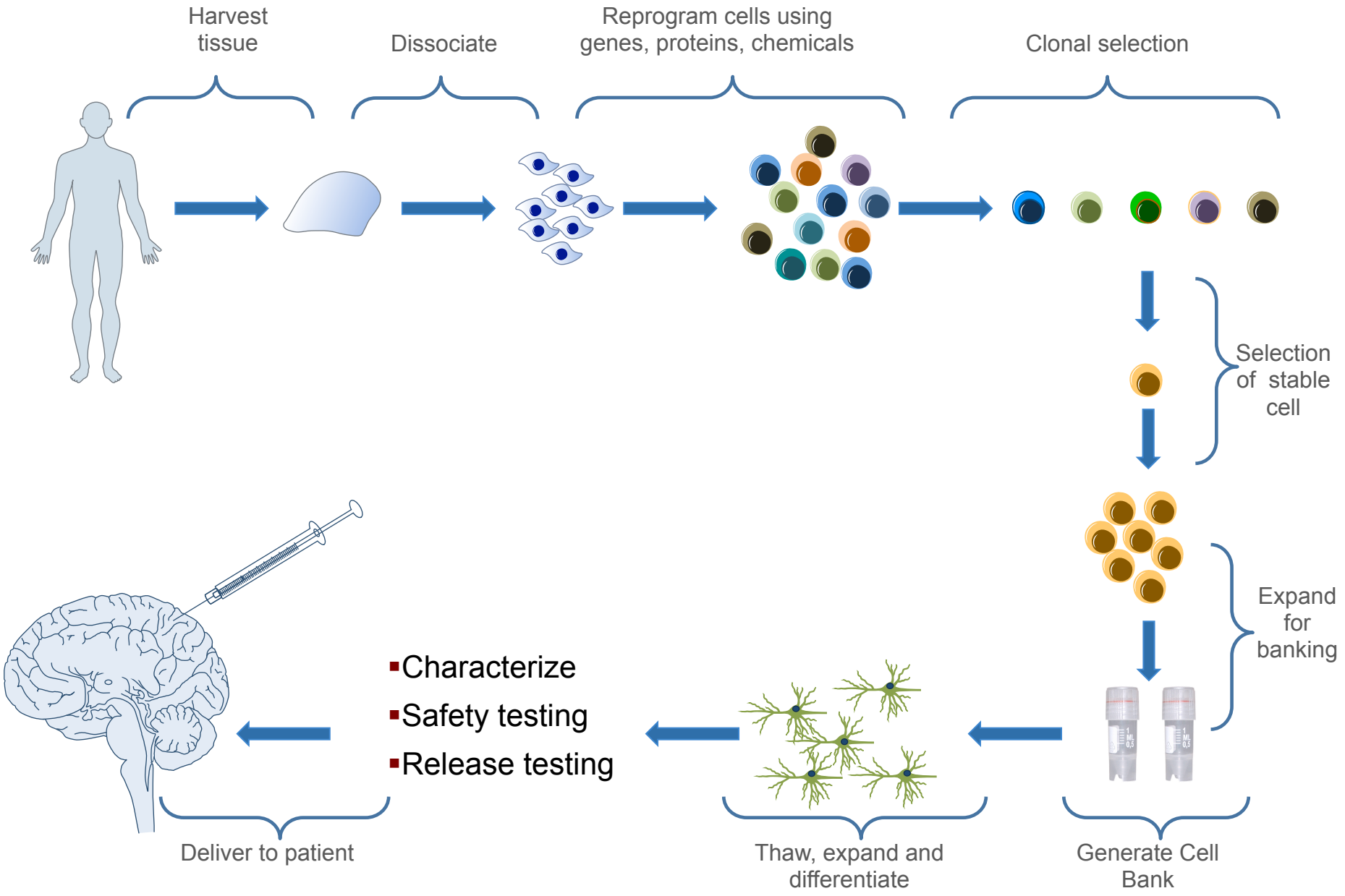
Autologous Cell Product Issues

- Cell Quality –Effect of donor
  - Disease state of donor
  - Donors can be heavily treated
  - Age of donor
- Impact of donor variability on product consistency
- Cell/tissue collection and manipulation
  - “Artful” – Requires specialized training
- Timing – urgency for patient vs time to produce product

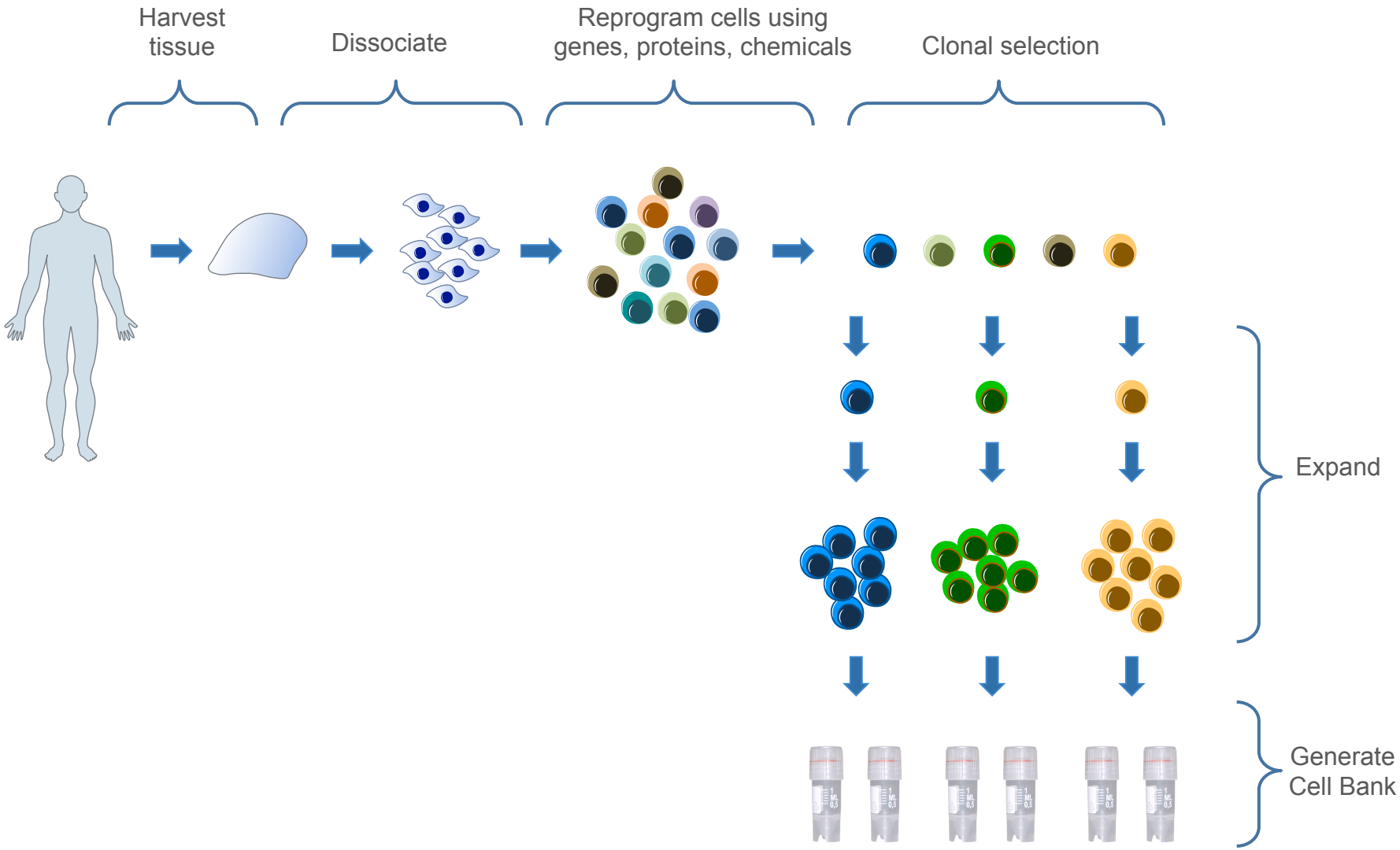
Logistics of iPSC-Derived Therapies



Autologous iPSC Products: The Process



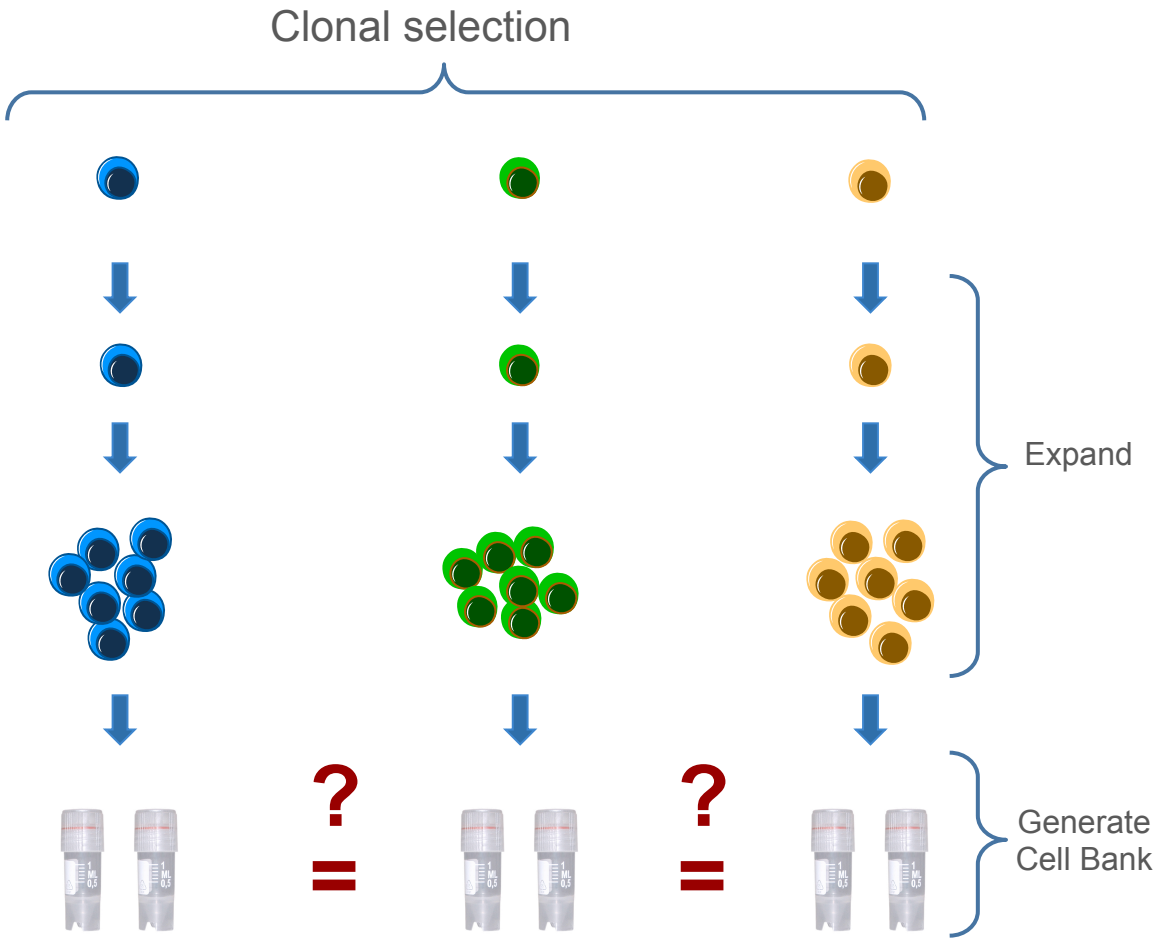
Generation of Starting Material: iPSCs



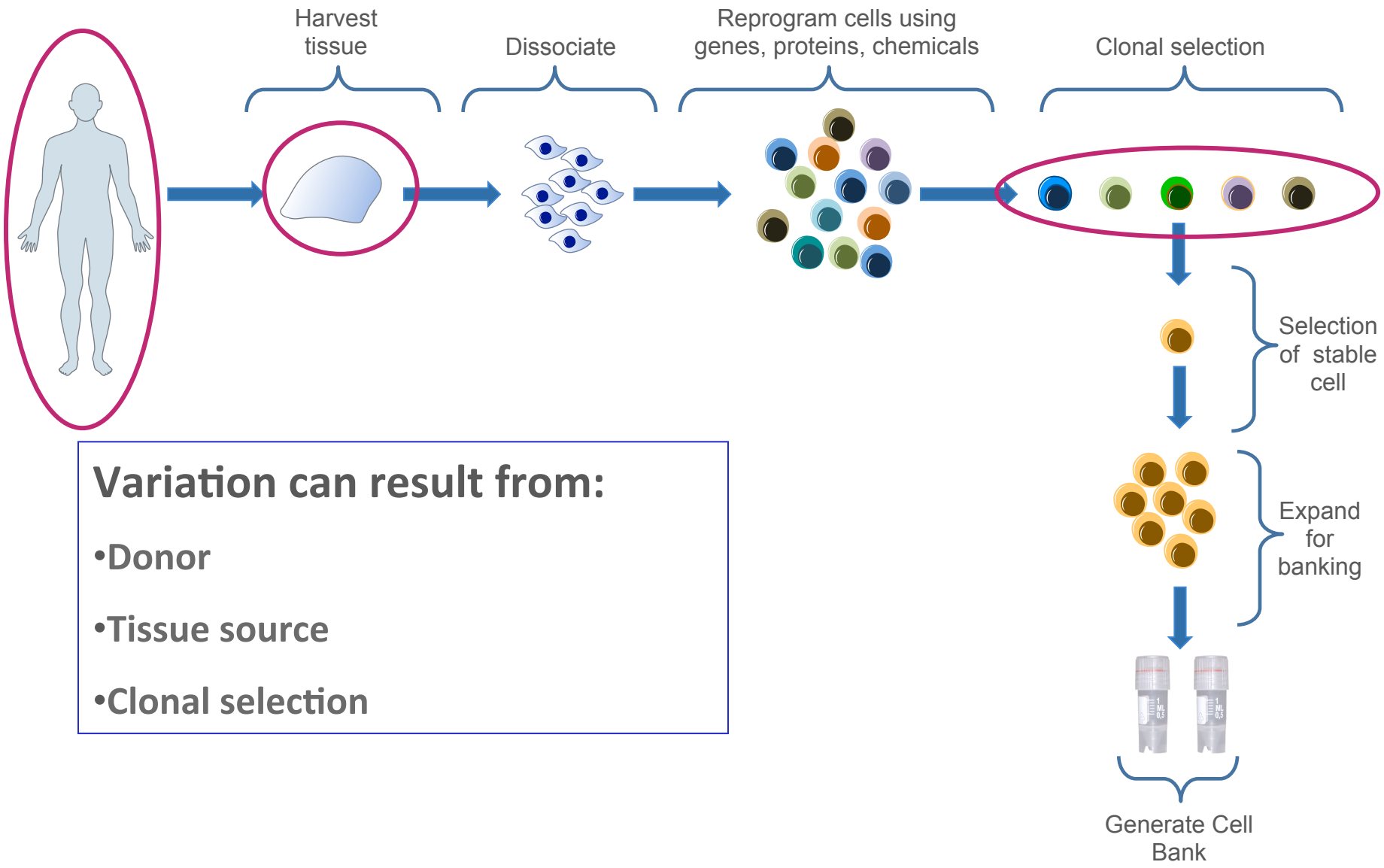
Generation of iPSCs: Comparability

Clonal variation can effect:

- Phenotype
- Stability
- Differentiation



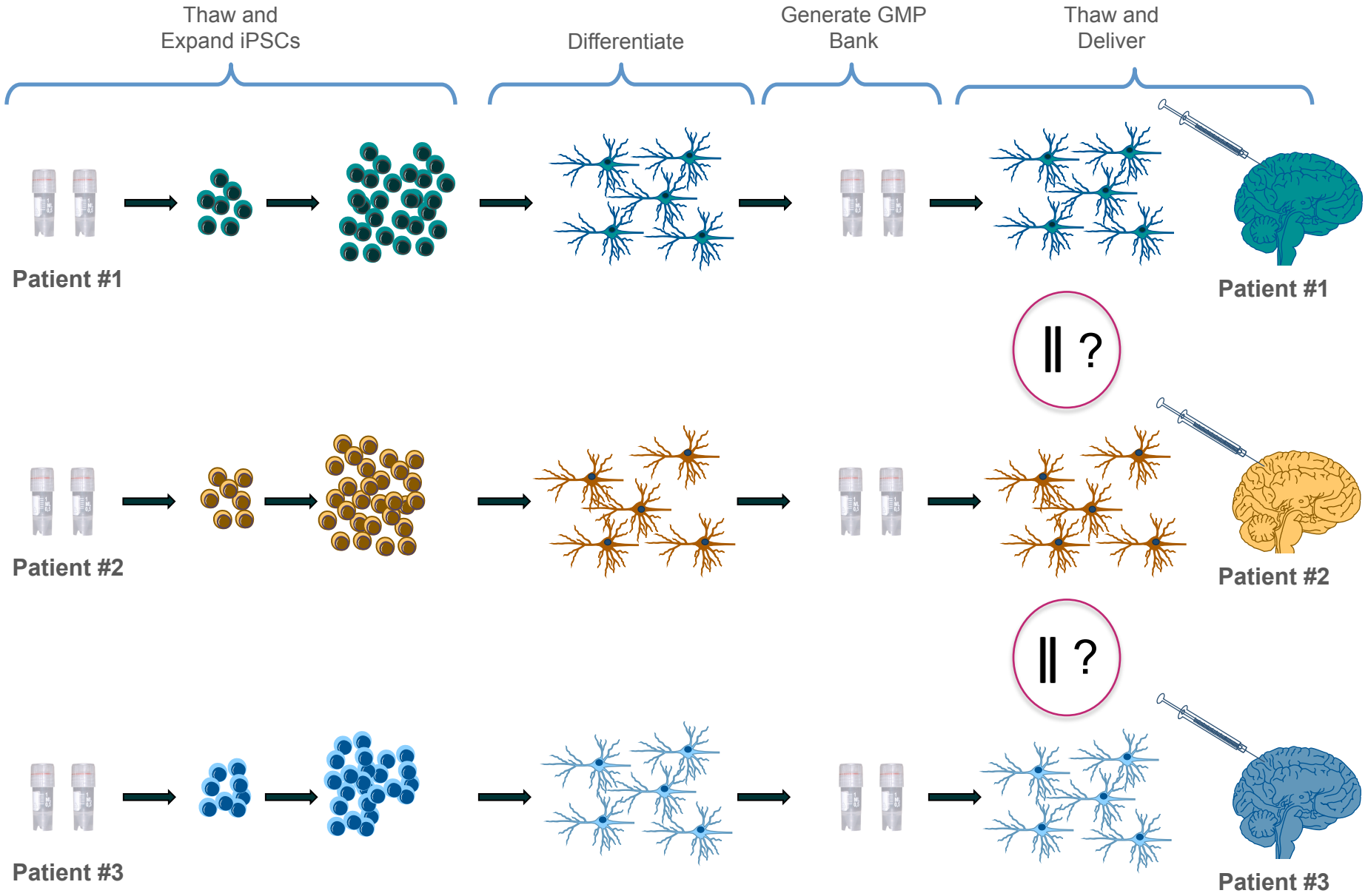
Generation of iPSCs: Variation



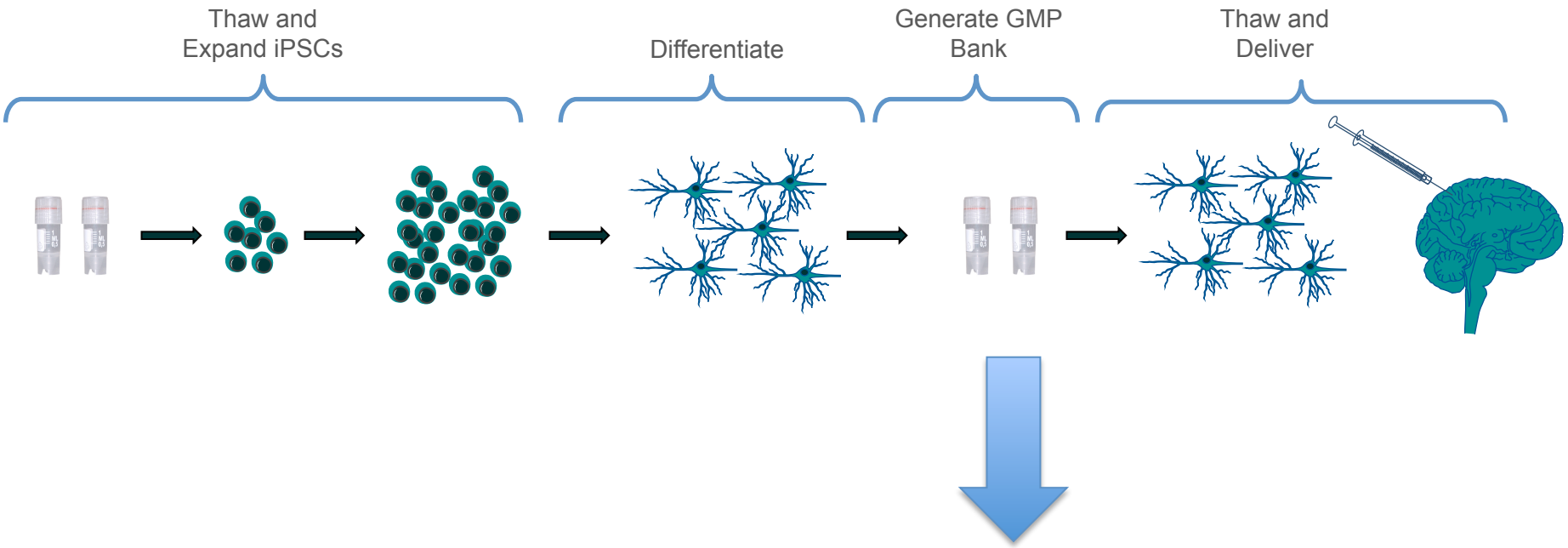
Variation can result from:

- **Donor**
- **Tissue source**
- **Clonal selection**

The iPSC-Derived Product: Reproducibility



In Vivo Evaluation of Cell Product



- Safety
- Efficacy in Disease models

iPSC Therapies: Safety

- Tumorigenicity
- Stability
  - Functional stability
  - Genetic & epigenetic stability
- Immunogenicity
- Toxicity
- Biodistribution
- Adventitious Agents

Tumorigenicity of Pluripotent Stem Cell Products

- Does your cell product contain undifferentiated cells?
  - How many is too many?
  - Influenced by site of implantation?
- Is your differentiated cell population stable?
  - Influenced by site of implantation?
  - Proliferative capacity of cell product?

Tumorigenicity: What is the Appropriate Assay?

- How many undifferentiated cells does it take to make a teratoma?
  - Is there an absolute number of cells required?
  - Is there a frequency required (percentage of cells)?
  - Needs to be measured for each cell line, each product?
- How long does it take to make a teratoma?
- What is the effect of implant site on teratoma formation?
  - Are some sites more permissive?
  - Do the neighboring cells (from graft or from implant site) influence teratoma formation?
- Are other cell types tumorigenic?
- Does the immune status of the recipient affect teratoma formation?
- What does a negative result mean?

Stability of Cell Product

- How long do cells continue to survive after implant?
  - If cells die, does this affect dose?
  - Are the cells proliferative?
- Genomic integrity after implant?
- How long do cells continue to function after implant?

Assessment of Immunogenicity of Human Pluripotent Cells

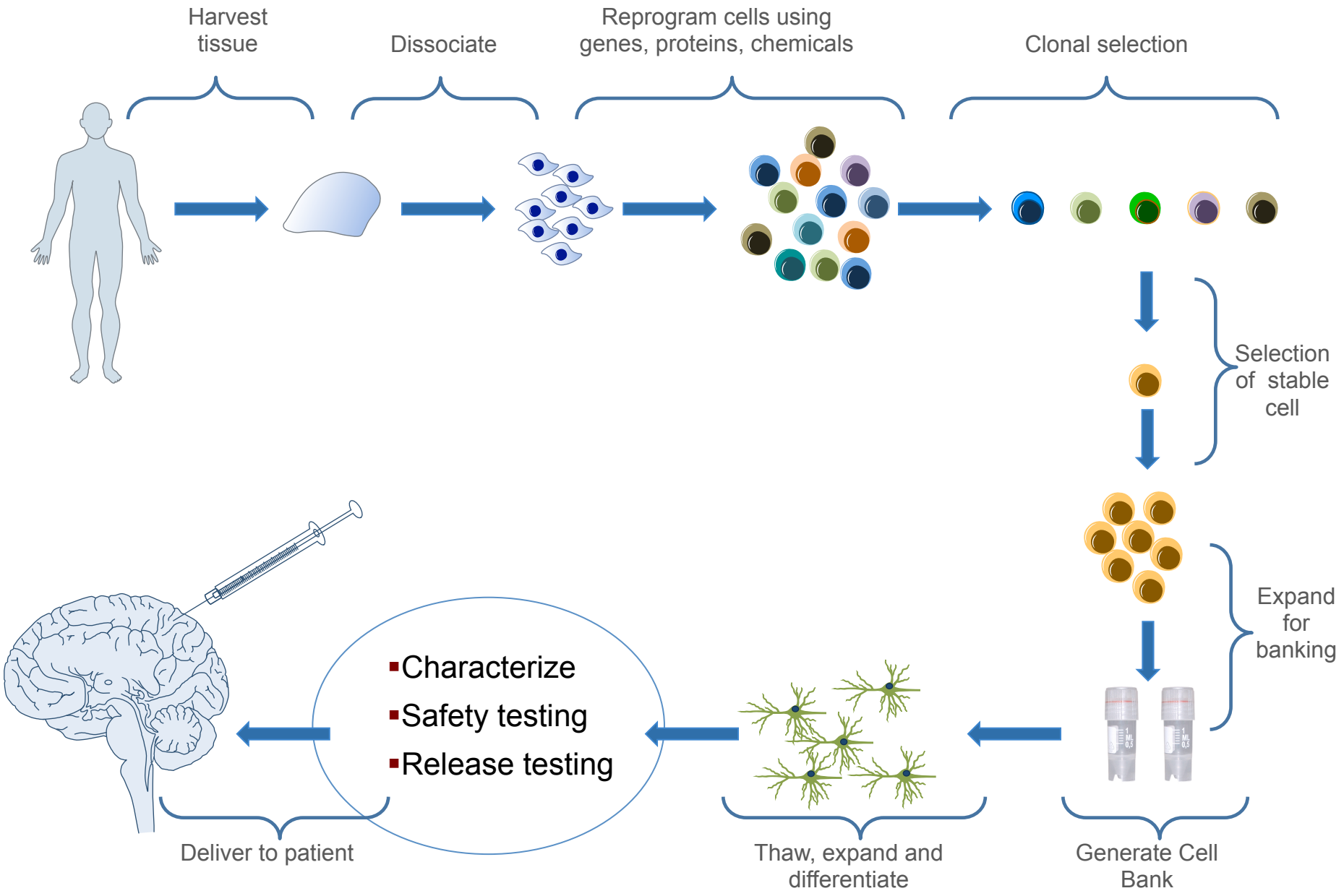
- Assessment of human cells in “xeno setting” is problematic
- Possible solutions to this:
  - Assessment of MHC / HLA expression
  - In vitro assessment using mixed lymphocyte reaction
  - Assess using “comparable” animal models
    - Mouse cells into mouse
    - Nonhuman primate cells into nonhuman primate

Are iPSCs Immunogenic?

- iPSCs are immunogenic
  - Zhai et al 2011
    - iPSCs generated using retroviruses
    - Immune rejection of teratomas in syngeneic recipients

- iPSCs are not immunogenic
  - Guha et al 2013
    - iPSCs generated using plasmids and lentiviruses
    - Assessed terminally differentiated cells
  - Araki et al 2013
    - iPSCs generated using plasmids
  - Kamao et al 2014
    - Autologous implantation of iPSC-RPE in nonhuman primates

Generating Autologous Cell Products from iPSCs



NATURE | NEWS

Next-generation stem cells cleared for human trial

Japanese team will use 'iPS' cells to treat patient with degenerative eye disease.

David Cyranoski

10 September 2014

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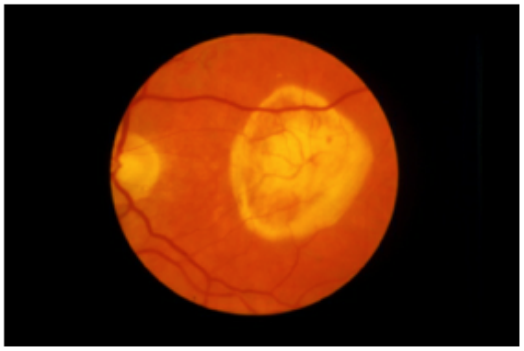


Next-generation stem cells cleared for human trial

Published reports:

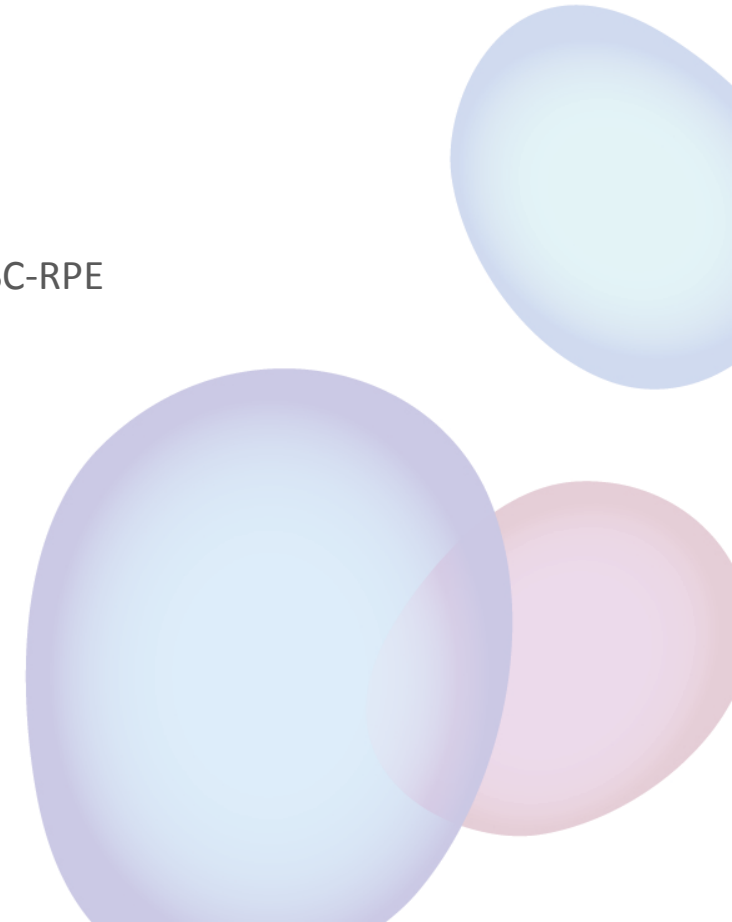
- Kuroda et al (2012) PLOS One 7(5):e37342.
- Kanemura et al (2014) PLOS One 9(1):e85336.
- Kamao et al (2014) Stem Cell Reports 2(2):205-18.
- Assawachananont et al (2014) Stem Cell Reports 2(5):662-74.

Characterization of hiPSC-RPE Clinical Product

- Assessment of phenotype
  - Pigmentation
  - Markers
    - RPE
    - Undifferentiated hiPSCs – qRT-PCR assay that detects 0.002% residual iPSCs (LIN28A)
- Demonstration of Reproducibility
  - Assessment of gene expression in hiPSC-RPE from 12 patients
- Functional assessment
  - Growth factor secretion
  - Tight junction formation in vitro
  - Efficacy in animal model of disease (RCS rats)

Safety Testing of hiPSC-RPE Clinical Product

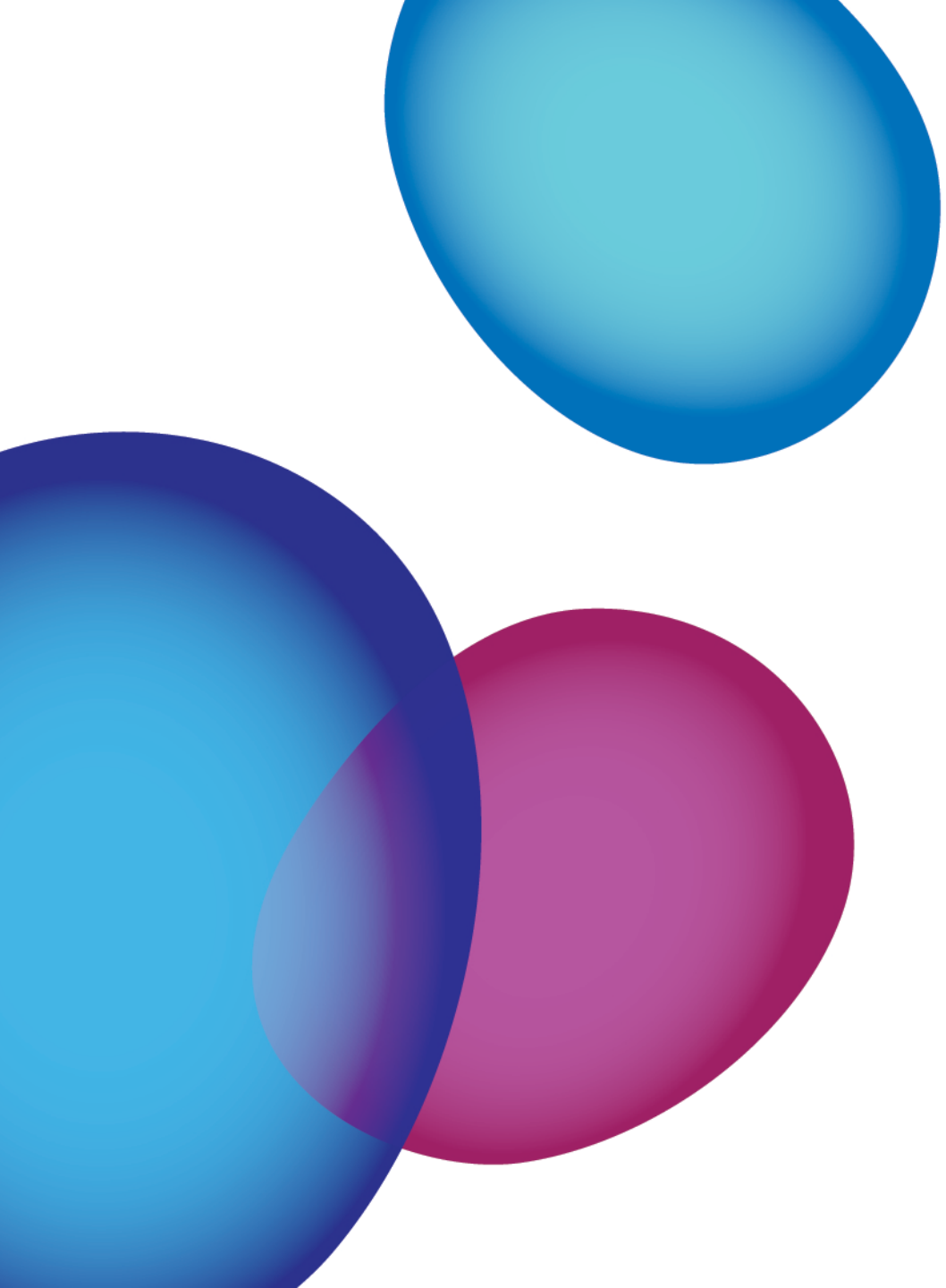
- Immunogenicity
  - Assessment of MHC I & II (+/- gamma interferon)
  - Mixed lymphocyte reaction
  - Assessment using implantation in monkeys
    - Allogeneic implants rejected (n=3)
    - Autologous implant persisted for 1 year (n=1)
- Tumorigenicity
  - Assessed hiPSCs from 6 patients
  - Quality control testing performed on hiPSCs and hiPSC-RPE
  - Subcutaneous implantation (NOG mice)
    - Tested hiPSC-RPE from 3 patients
    - Implanted 1 x 10e6 hiPSC-RPE cells in Matrigel
    - Tumor formation monitored up to 70 weeks
  - Subretinal implantation (Nude rats)
    - 0.8-1.5 x 10e4 hiPSC-RPE cells in sheets
    - Tested hiPSC-RPE from 5 patients
    - Tumor formation monitored up to 82 weeks



Development of iPSC-Derived Therapies

- Manufacturing Issues
  - Reproducibility of iPSC generation
    - Donor, tissue and clone variability
  - Reproducibility of differentiation
  - Consistency of cell product is critical

- Preclinical Safety & Efficacy Testing
  - Development of predictive tests
    - Tumorigenicity
    - Efficacy
  - Immunogenicity testing of final cell product



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