Guidance that Regulates Advancing Clinical iPSCs

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Durendal AI

Induced Pluripotent Stem Cells (iPSC)

- Discovered by Shinya Yamanaka in 2006
- iPSCs generated by reprogramming adult somatic cells
- Achieved by introducing four key transcription factors: Oct3/4, Sox2, Klf4, and c-Myc.

Somatic Cells Choices and Reprogramming Factors

Key Transcription Factors

Oct4 (Octamer-binding transcription factor 4 / POU5F1)

- Essential for maintaining pluripotency.
- Regulates genes involved in early embryonic development.

Sox2 (SRY-box transcription factor 2)

- Partners with Oct4 to activate pluripotency genes.
- Suppresses differentiation pathways.

Klf4 (Kruppel-like factor 4)

- Has dual roles in promoting proliferation and inhibiting apoptosis.
- Helps establish the correct epigenetic state.

c-Myc (Myelocytomatosis oncogene)

- Promotes cell proliferation and loosens chromatin structure.
- Improves reprogramming efficiency but increases risk of tumorigenesis.

Successfully generated from various cell types:

- **Fibroblasts** (skin biopsies)
- **Peripheral blood mononuclear cells (PBMCs)**
- **Keratinocytes**
- **Urine-derived epithelial cells**
- **Adipose-derived stem cells**

Additional Factors (*sometimes used to enhance or replace Yamanaka factors*)

- **Nanog** – strengthens and maintains pluripotency.
- **Lin28** – RNA-binding protein that regulates microRNA processing.
- **Esrrb, Tbx3, Nr5a2** – used in alternative reprogramming strategies.

Reprogramming Methods

Method	Mechanism	Advantages	Disadvantages
Integrating			
Retroviral vectors	Insert genes into host genome via reverse transcription	High efficiency	Risk of insertional mutagenesis; not suitable for clinical use
Lentiviral vectors	Infect dividing and non-dividing cells	Efficient and stable expression	Integration risks; possible gene disruption
PiggyBac transposon	Integrates transgenes that can later be excised	Reversible integration	Potential residual footprint; intermediate risk
Non –Integrating – Viral, Non-viral, Small Molecule			
Sendai virus	Delivers factors via cytoplasmic replication; no DNA stage	High efficiency; no genome integration	Requires viral clearance before clinical use
Episomal plasmids	Transiently express reprogramming factors; replicate episomally	Safer; footprint-free	Lower efficiency; may require repeated transfection
mRNA transfection	Synthetic mRNA encoding reprogramming factors	No genomic integration; high safety	Requires daily transfection; technically demanding
Protein transduction	Direct delivery of reprogramming proteins fused with cell-penetrating peptides	No genetic material involved	Very low efficiency; labor-intensive
MicroRNA-based methods	miRNAs modulate gene networks to promote pluripotency	Potential for high efficiency	Not sufficient alone; usually used with other methods
Chemical reprogramming	Small molecules replace some transcription factors	Safer, defined conditions	Still low efficiency; under development

Choice of Reprogramming Method

Key Considerations When Choosing a Method

- **Clinical use** → Favor **non-integrating** and **footprint-free** methods (e.g., mRNA, episomal, Sendai).
- **Efficiency and convenience** → Viral methods are more efficient but have safety concerns.
- **Scalability and GMP compliance** → Consider reproducibility, safety, and regulatory expectations.

Clinical Implications of iPSCs

- Disease Modeling
- Drug Discovery and Toxicity Testing
- Regenerative Medicine and Cell Therapy
- Personalized Medicine
- Gene Editing and Functional Genomics
- Tissue and Organ Engineering
- Immunotherapy
- Reproductive and Developmental Biology
- Treating Age-related Disease
- iPSC based Cancer Therapy

Domain	Application	Impact
Regenerative Medicine	Cell replacement therapies	Potential for treating Parkinson's, macular degeneration, diabetes, spinal cord injury, etc.
Oncology	iPSC-derived NK/CAR-T cells	Off-the-shelf immunotherapies for cancer treatment
Disease Modeling	Patient-specific iPSCs	Study disease mechanisms using iPSCs derived from patients with genetic disorders
Drug Screening	High-throughput testing	Screen candidate drugs in disease-relevant iPSC-derived cell types
Toxicology Studies	Safety pharmacology	Predict adverse effects on human tissue early in drug development
Reproductive Medicine	Germ cell derivation	Potential in fertility preservation and treatment (still experimental)

Some iPSC-derived Therapies

Therapy Name	Developer	Cell Type	Target Indication(s)	FDA Status	Key Features
FT516	Fate Therapeutics	iPSC-derived NK cells	Relapsed/refractory hematologic malignancies (e.g., AML, NHL, MM)	IND cleared; Phase 1 trial ongoing	Off-the-shelf NK cells engineered with a novel CD16 Fc receptor to enhance antibody-dependent cellular cytotoxicity.
FT500	Fate Therapeutics	iPSC-derived NK cells	Advanced solid tumors	IND cleared; Phase 1 trial ongoing	First iPSC-derived NK cell therapy cleared for clinical testing; designed for use alone or with checkpoint inhibitors.
FT819	Fate Therapeutics	iPSC-derived CAR T cells	B-cell malignancies (CLL, ALL, NHL)	IND cleared; Phase 1 trial ongoing	First iPSC-derived CAR T-cell therapy; engineered with 1XX CAR signaling domain and TRAC locus insertion to enhance efficacy and safety.
CYP-001	Cynata Therapeutics	iPSC-derived mesenchymal stem cells	Steroid-resistant acute graft-versus-host disease (GvHD)	Phase 1 trial completed	First formal clinical trial of an allogeneic iPSC-derived cell product; demonstrated positive safety and efficacy data.
OpRegen	Lineage Cell Therapeutics	iPSC-derived retinal pigment epithelial (RPE) cells	Dry age-related macular degeneration (AMD)	Phase 1/2a trial ongoing	Designed to replace damaged RPE cells in patients with AMD; showed promising preliminary results.
NTC-201-6A	Neurotech Pharmaceuticals	Encapsulated iPSC-derived cells expressing ciliary neurotrophic factor	Macular telangiectasia type 2	Phase 2/3 trial ongoing	Encapsulated cell therapy implanted into the eye to deliver therapeutic proteins directly to the retina.
OpCT-001	BlueRock Therapeutics	iPSC-derived photoreceptor cells	Primary photoreceptor diseases (e.g., retinitis pigmentosa)	IND cleared; Fast Track designation; Phase 1 trial ongoing	First iPSC-derived photoreceptor therapy to enter clinical trials; aims to restore vision by replacing degenerated retinal cells.
XS-411	XellSmart Biopharmaceutical	iPSC-derived dopaminergic neural progenitor cells	Parkinson's disease	IND cleared; Phase 1 trial ongoing	Allogeneic, off-the-shelf therapy targeting motor symptoms; early trials show promising safety and efficacy.
XS-228	XellSmart Biopharmaceutical	iPSC-derived neural progenitor cells	Amyotrophic lateral sclerosis (ALS)	IND cleared; Orphan Drug Designation; Phase 1 trial ongoing	First-in-class regenerative neural cell therapy for ALS; aims to slow disease progression.
Fertilo	Gameto	iPSC-derived ovarian support cells	Fertility treatment (IVF support)	IND cleared; Phase 3 trial ongoing	Aims to mature eggs outside the body, reducing hormone injections and shortening IVF cycles.

Regulatory Categorization of iPSCs

iPSCs can be classified as either **raw materials** or part of a **final product**

As Raw Material

In most therapeutic contexts, **iPSCs are treated as raw/intermediate materials**, especially when they are used as a **starting point to derive specialized cells or tissues**.

Must meet **raw material specifications** under **GMP**.

Require characterization for:

- Identity, Purity, Sterility, Genomic stability

The **final product** is regulated (e.g., iPSC-derived NK cells), not the iPSCs themselves.

As Final Product

In **rare or experimental settings**, iPSCs **themselves** may be administered or studied as the **final product**—for example, in research trials involving **autologous iPSC transplantation**.

Considerations:

- Extremely high risk due to **undifferentiated state and tumorigenic potential**.
- Not widely used or approved in clinical settings.
- Require rigorous characterization and **demonstration of safety and efficacy**.

Use Case	Final Product	iPSCs Role
iPSCs differentiated into retinal cells	Retinal pigment epithelial (RPE) cells	Raw material
iPSC-derived cardiomyocytes for heart failure	Cardiomyocyte therapy	Intermediate
iPSC-derived NK cells for immunotherapy	Allogeneic NK cell therapy	Starting cell source
iPSC-derived beta cells for diabetes	Insulin-producing islets	Source material

Aspect	iPSCs as Raw Material	iPSCs as Final Product
Common Use	Manufacturing of cell/tissue products	Experimental/early research
Risk Level	Lower (processed further)	Higher (tumorigenic potential)
Regulatory Treatment	Intermediate under GMP	Final biologic product (351 HCT/P)
Example	iPSC → Cardiomyocytes	iPSC autologous transplant

Regulatory Pathway and Framework for iPSCs

FDA – Center for Biologics Evaluation and Research (CBER)	
Regulatory Pathway	iPSC-derived products are regulated as **351 HCT/Ps** under the **PHS Act**
IND Requirement	Final product must be developed under an **Investigational New Drug (IND)** application
iPSCs Role	Mostly treated as **raw material**, subject to raw material qualification and **GMP sourcing standards**
DMF (Drug Master File)	A **Type II DMF** may be submitted for proprietary iPSC lines to support CMC sections of INDs or BLAs
Material Traceability	Must be traceable to donor, with full **donor screening, testing, and consent** per 21 CFR Part 1271
Cross-reference Use	DMFs can be cross-referenced in multiple INDs to protect proprietary information on the iPSC source
Characterization	Identity, Purity, Sterility, Potency, and Karyotypic/Genomic Stability Testing
Long-term Safety	Required for assessing delayed adverse effects, particular tumor formation

Type II DMF – Drug Substance (iPSCs as Raw Material)

Content Includes

- Source and origin of cells (donor information, screening, consent)
- Reprogramming method (e.g., non-integrating Sendai virus, mRNA)
- Manufacturing and expansion process
- Characterization and quality control assays
- Stability testing (e.g., under cryopreservation)
- Storage and shipping conditions
- Quality management systems (GMP compliance)

Note: DMFs are not approved by the FDA but reviewed in support of IND/BLA applications.

Regulatory Guidances for iPSCs

Guideline	Title	Key Points
21 CFR Part 1271	HCT/P Regulation	Donor screening, informed consent, testing for communicable diseases
21 CFR Part 210/211	GMP for Biologics	Facility, equipment, process control, documentation
21 CFR Part 312	IND Regulations	Applies to products derived from iPSCs
FDA Guidance (2022)	Considerations for the Development of Cell and Gene Therapy Products	CMC expectations for iPSCs and their derivatives
FDA Guidance (2020)	Manufacturing Considerations for Licensed and Investigational Cellular and Gene Therapy Products	Raw material control, characterization, stability
FDA Guidance (2010)	Characterization and Qualification of Cell Substrates and Other Biological Raw Materials Used in the Production of Viral Vaccines for Infectious Disease Indications	Applies to iPSCs as starting materials
FDA Guidance (2011)	Testing of Retroviral Vector-Based Gene Therapy Products	Applies if integrating vectors are used in iPSC generation
USP <1046>	Cell and Gene Therapy Products	Quality attributes for raw materials
USP <1043>	Ancillary Materials for Cell, Gene, and Tissue-Engineered Products	Covers safety and quality requirements for raw materials

Requirement	Details
IND Application	Preclinical studies (tumorigenicity, biodistribution), CMC data, proposed clinical protocols
GMP Compliance	Manufacturing facilities must follow **Good Manufacturing Practice (GMP)** under 21 CFR Parts 210, 211, and 1271
Characterization	Identity, purity, potency, sterility, and karyotypic/genomic stability testing
Long-Term Safety Monitoring	Required for assessing delayed adverse effects, particularly tumor formation

ICH Guidelines as applicable to iPSCs

ICH Code	Title	Relevance
ICH Q5D	Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products	Core guidance for cell substrate qualification (applies directly to iPSCs)
ICH Q5A(R2)	Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin	Required for iPSC viral testing and validation of virus clearance
ICH Q5B	Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products	Relevant if vector systems are used in iPSC reprogramming
ICH Q6B	Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products	Quality specs for identity, purity, potency, and safety
ICH Q1A–Q1E	Stability Testing Series	Required for iPSC stability data (including cryopreserved stocks)
ICH Q7	GMP for Active Pharmaceutical Ingredients	Applicable principles for raw material control and traceability
ICH Q8–Q10	Quality by Design (QbD), Risk Management, and Quality Systems	Emphasize risk-based control of iPSC variability and CQA management

Quality Specifications of iPSCs

Specification Category	Parameter	Details
Donor Eligibility	Screened for communicable disease	HIV, HBV, HCV, HTLV, HCV, HTLV, Treponema, WNV, Zika etc - Screened and tested per 21 CFR 1271.75–1271.90
Identity	Expression of pluripotency markers	Oct4, Sox2, Nanog, TRA-1-60, SSEA-4 (confirmed via immunostaining, qPCR, RT-PCR or flow cytometry)
Purity	Absence of differentiated cells %undifferentiated vs differentiated	Minimal presence of lineage-specific markers, Flow cytometry for lineage-specific markers
Viability	Post-Thaw Cell Viability (≥85%)	Trypan-blue exclusion or viability dye with flow cytometry
Sterility	Microbial testing	Mycoplasma (PCR or Culture Method), endotoxin (0.25 EU/mL or below acceptable per final use, LAL Assay), bacterial and fungal contamination must be absent (USP <71>)
Residual Reprogramming Factors	Integration-free status; Clearance of reprogramming factors (e.g., Sendai virus)	Absence of vector sequences in non-integrating methods (e.g., Sendai clearance assay) qPCR or ddPCR
Tumorigenicity	Risk assessment	Evaluate undifferentiated iPSCs or transformed clones that could form tumors
Adventitious Agents	Absence of Viral contaminant (human or animal)	Adventitious Agent Testing (AAT) panel
Potency (Pluripotent Functionality)	Tri-lineage differentiation	Capacity to form ectoderm, mesoderm, and endoderm in vitro or in vivo (teratoma formation assay)
Karyotypic Stability	Genomic integrity	G-banding, SNP array, or WGS to detect chromosomal abnormalities
Stability	Consistent post cryopreservation/ long-term storage	Stability studies (viability, identity, karyotype)

Cryopreservation, Storage, Stability and Logistics

Cryopreservation:

- Maintain **viability**, **genetic stability**, and **pluripotency** post-thaw.
- Enable **consistent supply** of a well-characterized iPSC bank.

Cryoprotectant - 10% DMSO (dimethyl sulfoxide) in serum-free or serum-containing cryomedia

Freezing Protocol -Controlled-rate freezing (typically -1°C/min down to -80°C) then transfer to liquid nitrogen

Cell Density - ~1–2 × 10^6 cells/mL per cryovial

Passage Number - Early-passage iPSCs preferred (P5–P15) to reduce risk of genomic instability

Validation -Post-thaw viability ≥ 85%, reattachment efficiency, karyotyping, pluripotency marker expression

Component	Purpose	Required By
Cryopreservation	Long-term preservation of functional iPSCs	GMP, USP <1046>
Stability	Demonstrate consistent quality over time	FDA, EMA, ICH Q1A-E
Storage	Maintain iPSC integrity	GMP, 21 CFR 211

Stability - Conducted under ICH conditions to determine shelf-life, post-thaw viability, and functionality

- Real-time: Long-term LN2 storage
- Accelerated: Short-term -80°C to test impact of suboptimal conditions

Parameter	Assessment Method	Acceptance Criteria
Viability	Trypan blue or flow cytometry	≥ 85% post-thaw
Pluripotency	Immunocytochemistry / Flow cytometry	OCT4, SOX2, SSEA-4, TRA-1-60 positive
Karyotype	G-banding / SNP microarray	No abnormalities
Differentiation Potential	In vitro 3-germ layer or EB assay	Confirmed
Microbial Contamination	Sterility, mycoplasma	Negative
Vector Clearance (if applicable)	qPCR for episomes / Sendai	Undetectable

Storage: Vapor phase liquid nitrogen (-150°C or colder)

Aspect	Specifications
Storage Temperature	Liquid nitrogen vapor phase (< -150°C) preferred
Vial Type	Certified cryovials, barcoded for traceability
Labeling	Unique ID, cell line name, passage number, date, lot/batch number
Monitoring	Continuous temperature monitoring, alarm system, SOPs for excursions
Inventory Control	Electronic inventory linked to CoC/CoO and testing data

Traceability - Chain of Identity, Chain of Custody

Ensures **accountability** and **traceability** for regulatory audits (FDA, EMA) and is essential under **GMP/GTP** requirements.

The **Chain of Identity** ensures that the product administered to the patient is **traceably linked back to the original donor,** especially critical in autologous therapies (e.g., iPSC-derived personalized cells).

Element	Description
Focus	Patient – specific Identity
Tracks	Who it belongs to
Used in	Autologous and Allogeneic
Regulated by	21 CFR Part 1271, 21 CFR 210/211
Unique Identifier	Donor ID → Sample ID → iPSC Line ID → Final Product Lot ID
Patient-Derived Source Material	Documented collection, labeling, and verification of original tissue
iPSC Derivation Records	Mapping donor to reprogrammed line and subsequent expansion
Labeling and Barcoding	All containers (cryovials, bags, etc.) tagged with traceable barcodes
Manufacturing Linkage	Every process step must reference the same identity record
Clinical Use	Matched documentation confirming correct patient receives their designated product

The **Chain of Custody** refers to the **documented and auditable trail of the physical handling, storage, transport, and processing** of a product or sample — from the donor material through to final product delivery (or disposal).

Element	Description
Focus	Physical Possession
Tracks	Who handles it
Used in	All product types
Regulated by	GMP, GTP, GDocP
Custodian Details	Each person, site, or entity who physically handles or stores the material
Material Status	Identity, lot number, quantity, storage condition, and integrity check
Transfer Documentation	Date/time, method of transport, chain of custody forms, courier logs
Conditions Monitoring	Temperature, humidity, security logs during transport or storage
Deviations	Any break in handling protocol or excursion event (e.g., LN2 tank alarm triggered)

Integration in iPSC Workflows

Stage	Chain of Identity	Chain of Custody
Donor Collection	Donor consent, ID assigned	Collected sample, shipped to lab
iPSC Reprogramming	ID recorded in MFG log, cell line labeled	Custody logs from source to lab
Cell Banking	Bank ID linked to donor	Custody logs for LN2 storage
Manufacturing	Traceability from cell line to batch	MFG site logs, internal transfers
Clinical Use	Matched to patient ID (autologous)	Delivery, infusion center records

Best Practices

- Use **barcode or RFID systems** for identity tracking.
- Maintain **digital audit trails** in LIMS or QMS platforms.
- Validate **labeling systems** for durability under LN2.
- Reconcile **donor–product–patient mapping** at each QA checkpoint.
- Review **CoI/CoC as part of batch release** and QP certification (EU).

Thank you

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NSF ENGINES: PIEDMONT TRIAD
REGENERATIVE MEDICINE ENGINE

RMMS
Regenerative Medicine Manufacturing Society

Type of FDA Meetings relevant to iPSCs

Meeting Type	Formal Status	Timing / Stage	Purpose	Regulatory Division	Binding Feedback	Typical Use Case
INTERACT (INitial Targeted Engagement for Regulatory Advice on CBER/CDER ProducTs)	Early Regulatory Engagement	Preclinical (before Pre-IND)	Early scientific input for novel therapies	CBER / CDER	❌ Non-binding	iPSC-derived therapies, gene editing, novel delivery methods
Pre-IND	Type B	Before IND submission	Discuss preclinical, CMC, and clinical plans	CBER / CDER	✅ Binding	Confirm readiness for IND submission
Type A	Formal	Urgent situations	Resolve clinical holds or disputes	CBER / CDER	✅ Binding	After clinical hold or complete response letter
Type B	Formal	Key development milestones	Includes Pre-IND, End-of-Phase 1, End-of-Phase 2, Pre-NDA/BLA	CBER / CDER	✅ Binding	Structure pivotal trials, agree on endpoints, plan submissions
Type C	Formal	Anytime during development	Any meeting not covered by A or B	CBER / CDER	✅ Binding	Long-term safety planning, CMC strategy
CATT (Committee for Advanced Therapies Team)	Informal	Very early stage or classification uncertainty	Regulatory classification, review pathway clarity	CBER	❌ Non-binding	Clarify HCT/P 351 vs 361, iPSC classification or review assignment
RMAT Designation Meeting (Regenerative Medicine Advanced Therapy)	Designation-based	Post early clinical data	Frequent interactions for RMAT-designated therapies	CBER	✅ Binding (for development plans)	iPSC-based therapies showing potential to address unmet need
Pre-BLA / Pre-NDA	Type B	Before submitting BLA or NDA	Discuss submission content, formatting, and data requirements	CBER / CDER	✅ Binding	Final alignment before license application
Post-Approval Meetings	Type C (or informal)	After product approval	Address post-marketing requirements or changes	CBER / CDER	✅ / ❌ (depends on format)	New indications, manufacturing scale-up

INTERACT Meeting (for early-stage products)
Stands For: INitial Targeted Engagement for Regulatory Advice on CBER/CDER ProducTs.
Purpose: Early feedback for novel products (e.g., iPSCs, gene therapies).
Ideal Timing: Before Pre-IND.
Offered by: CBER (biologics) and CDER (drugs).
Benefit: Clarifies regulatory expectations and helps shape early development plans.

Type A Meeting
Purpose: Resolve stalled programs or address disputes (e.g., clinical hold, dispute resolution).
Examples:
Dispute resolution after a complete response letter.
Meeting after clinical hold.
Timeline: Scheduled within 30 days of request.

Type B Meetings These are the most common and structured meeting types:
Pre-IND Meeting
- **Purpose**: Discuss preclinical data, CMC plans, and clinical trial designs before submitting an IND.
- **Ideal For**: New or complex products like iPSC-derived therapies.
- **Benefits**: Clarifies FDA expectations, avoids major deficiencies.

End-of-Phase 1 Meeting
- **Purpose**: Evaluate safety and plan for Phase 2 trials.
- **Note**: Less common unless there's a unique issue or high risk.

End-of-Phase 2 Meeting (EOP2)
- **Purpose**: Review Phase 2 results, agree on Phase 3 design, endpoints, and statistical analysis plan.
- **Timing**: Before starting pivotal trials.

Pre-NDA/BLA Meeting
- **Purpose**: Discuss data presentation and format for a New Drug Application (NDA) or Biologics License Application (BLA).
- **Goal**: Ensure submission readiness.

RMAT Designation Meetings (Regenerative Medicine Advanced Therapy)
- **Available For**: Therapies with regenerative medicine potential.
- **Requirement**: Preliminary clinical evidence showing potential to address unmet medical needs.
- **Benefit**: Access to early, frequent meetings and priority review features.

CATT Meeting (Committee for Advanced Therapies) Meeting
- not an official FDA meeting type (like Type A/B/C), but a **preliminary, informal scientific interaction**.
It is designed to provide early regulatory and scientific input for **complex or novel products**, such as **iPSC-derived therapies, Gene therapies, Cell-based therapies, Tissue-engineered products**
Purpose
•Discuss **product classification** (e.g., HCT/P 351 vs. 361)
•Address novel scientific or regulatory questions
•Get feedback on **early-stage concepts** before formal IND or INTERACT submissions
•Understand how your product may be reviewed (CBER divisions or specific FDA branches)
Request - Very early in development, often, Before initiating **IND-enabling studies or** When the product or technology doesn't fit neatly into existing regulatory categories
Prior to or in lieu of an **INTERACT** meeting, especially if classification or jurisdiction is unclear

Type C Meeting
Purpose:
Any other product development meeting not covered by Types A or B.
Examples:
Long-term safety monitoring plans.
Manufacturing scale-up discussions.
Flexibility:
Broader and less defined; can be used as needed.