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Reprogramming and Differentiation of Induced Pluripotent Stem Cells for Clinical Therapy: Safety, Efficacy, and Regulatory Pathways to Translation

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Abstract

Induced pluripotent stem cells (iPSCs) have evolved from a transformative discovery in developmental biology into a clinically relevant platform for regenerative medicine and cell-based therapeutics. As multiple iPSC-derived products advance through clinical development, the principal challenges facing the field have shifted from achieving pluripotency to ensuring that reprogramming, differentiation, and manufacturing processes meet the stringent safety, efficacy, and regulatory requirements of advanced biologics. Central to this challenge is the selection of reprogramming technologies and differentiation strategies that minimize genomic risk, enable reproducible manufacturing, and support clinically meaningful potency while remaining compatible with global regulatory frameworks. This review provides a comprehensive and critical analysis of current iPSC reprogramming technologies, including integrating viral vectors, non-integrating viral systems such as Sendai virus, and non-viral integration-free approaches encompassing episomal plasmids, synthetic modified mRNA, and emerging small-molecule methodologies.

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We compare these platforms with respect to reprogramming efficiency, genomic and epigenomic stability, operational robustness, and translational risk, highlighting why integration-free methods have become the preferred foundation for therapeutic iPSC generation. We further examine differentiation not as a discrete protocol choice but as a manufacturing control strategy, contrasting directed two-dimensional differentiation, three-dimensional organoid-based systems, and direct lineage conversion with respect to scalability, product definition, and clinical applicability. A detailed assessment of dominant safety failure modes (e.g., tumorigenicity arising from residual pluripotent cells or culture-acquired genomic aberrations, immunogenicity, and adventitious agent risk) is presented alongside best-practice mitigation strategies aligned with regulatory expectations in the United States and European Union. The review also addresses how therapeutic efficacy is operationalized through potency assays linked to mechanism of action and suitable for lot release and comparability. Integrating these considerations, we propose a regulatory-aligned technical pathway for clinical iPSC-derived cell therapy, emphasizing the advantages of integration-free reprogramming, GMP-grade cell banking, controlled differentiation, and layered safety assessment. By synthesizing advances in reprogramming biology, differentiation engineering, and regulatory science, this review aims to provide a pragmatic roadmap for the translation of iPSC technologies from experimental promise to sustainable clinical reality.

Keywords

Induced pluripotent stem cells (iPSCs); Clinical-grade reprogramming; Directed differentiation; Genomic stability; Tumorigenicity mitigation; Potency assay development; Quality by design (QbD).

Introduction

Induced pluripotent stem cell (iPSC) technology enables reprogramming of differentiated somatic cells into pluripotent populations capable of generating derivatives of all three germ layers, creating a renewable cell substrate for regenerative medicine and engineered cell therapies [1]. As iPSC-derived products progress through early- and mid-stage clinical development across multiple indications [2], the field's central challenge has shifted from establishing pluripotency to demonstrating that reprogramming and differentiation workflows can be executed with the safety, reproducibility, and control expected of advanced biologics [3].

Clinical translation is now defined by two manufacturing paradigms: allogeneic, bank-based platforms and autologous, patient-specific products [4,5]. Allogeneic approaches leverage centralized GMP manufacturing and deep characterization of a limited number of master cell banks, whereas autologous approaches pursue immunologic matching but require individualized manufacturing, longer cycle times, and repeated safety qualification [6]. Autologous programs are additionally constrained by starting-material biology: bone marrow–derived, adipose-derived, and peripheral blood–derived inputs are common because they are clinically accessible, yet each introduces distinct translational liabilities (invasiveness and expansion burden for bone marrow; donor-, depot-, and metabolic-state–dependent heterogeneity for adipose; and clonal hematopoiesis–associated genomic risk in blood-derived inputs) that propagate into reprogramming yield, clonal selection, and downstream safety governance [7]. These starting-material constraints intersect with technology choices that drive translational success: reprogramming modality determines the dominant genomic risks and required control strategies, while differentiation strategy determines whether a final product can be defined, measured (potency), and

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released reproducibly at scale [8]. Regulators evaluate iPSC-derived therapeutics within advanced biologics frameworks and increasingly emphasize (i) cell substrate derivation and characterization, (ii) manufacturing comparability as processes evolve, and (iii) integrated, risk-based nonclinical strategies to address tumorigenicity, genomic instability, immunogenicity, and adventitious agents [9-12]. Accordingly, early technical decisions, particularly starting material selection and reprogramming method, shape the feasibility of a regulatorily defensible path to first-in-human studies and beyond. The objective of this review is to compare reprogramming technologies and differentiation strategies with respect to safety, efficacy, and translational readiness; critically evaluate autologous starting-material limitations (bone marrow, adipose, blood-derived); and define a technically and regulatorily defensible pathway for clinical iPSC-derived cell therapy consistent with current FDA and EMA expectations [9-12].

Reprogramming technologies: mechanisms, performance, and translational risk

Reprogramming technologies define the biological and regulatory foundation of iPSC-derived therapeutics by determining (i) how pluripotency is induced, (ii) how genomic and epigenomic integrity is perturbed during induction and expansion, and (iii) what residual process- or vector-related risks must be controlled to enable clinical translation.

Integrating viral vectors (retrovirus, lentivirus)

Integrating retroviral and lentiviral vectors induce pluripotency through stable genomic integration of reprogramming factors, classically OCT4, SOX2, KLF4, and c-MYC, offering historically high efficiency and robustness across donor cell types [13]. Beyond insertional events, integrating platforms can introduce or select for delivery-method–associated epigenetic abnormalities during reprogramming and expansion, and they create persistent uncertainty around transgene silencing fidelity (incomplete silencing, stochastic reactivation, or promoter-dependent residual expression), which can impair differentiation fidelity and compromise long-term functional stability of derived lineages [14].

- OCT4 (POU5F1) is a POU-domain transcription factor that serves as a master regulator of pluripotency. It is essential for maintaining the undifferentiated state of embryonic stem cells by activating pluripotency-associated gene networks and repressing lineage-specific differentiation programs. Tight regulation of OCT4 expression is critical, as both insufficient and excessive levels promote differentiation rather than self-renewal [15].
- SOX2 is a high-mobility group (HMG) box transcription factor that cooperates with OCT4 to establish and maintain pluripotent transcriptional circuitry. SOX2 directly regulates genes involved in self-renewal and chromatin accessibility and plays a central role in stabilizing the pluripotent state during reprogramming [16].
- KLF4 (Krüppel-like factor 4) is a zinc-finger transcription factor that contributes to reprogramming by promoting epithelial characteristics, regulating cell-cycle progression, and modulating apoptosis. KLF4 also participates in chromatin remodeling and helps suppress differentiation-associated gene expression during early stages of pluripotency induction [17].
- c-MYC is a basic helix–loop–helix leucine zipper transcription factor that enhances reprogramming efficiency by broadly amplifying transcription, promoting cell proliferation, and facilitating metabolic and epigenetic remodeling. Although c-MYC is not strictly required for pluripotency

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induction, its inclusion accelerates reprogramming kinetics; however, its oncogenic potential has motivated the development of c-MYC–free or transient expression strategies in clinical settings [18].

However, permanent integration creates intrinsic safety liabilities. Most notably insertional mutagenesis and dysregulated or persistent transgene expression complicate differentiation and elevate long-term oncogenic risk [19,20]. From a translational and regulatory standpoint, integrating systems expand the required characterization and control burden (e.g., integration mapping, replication-competent virus testing, and risk-based long-term follow-up), making them largely unsuitable for iPSC derivation intended for therapeutic starting materials under modern expectations [21].

Clinical and Regulatory Case Study: Temperature-sensitive Sendai virus vectors to simplify clearance

Temperature-sensitive Sendai virus (SeV) vectors have been engineered to enable rapid elimination of reprogramming vectors by shifting to a nonpermissive temperature, reducing dependence on extended passaging as the sole clearance mechanism. A temperature-sensitive SeV system enabling efficient iPSC generation and temperature-shift clearance has been demonstrated in blood-derived reprogramming workflows, underscoring the operational value of TS SeV variants for GMP-aligned manufacturing where clearance is a critical controllable attribute.

Non-integrating viral vectors (Sendai virus; adenovirus)

Non-integrating viral platforms were developed to preserve high delivery efficiency while avoiding insertional mutagenesis. Sendai virus (SeV), a cytoplasmic RNA virus, has become the most widely implemented non-integrating viral approach for clinical-grade iPSC derivation because it supports high reprogramming efficiency across clinically relevant sources, including dermal fibroblasts and blood-derived cells [22,23]. Although SeV does not integrate, vector genomes can persist across early passages, necessitating validated clearance strategies and sensitive molecular assays to demonstrate absence prior to banking and downstream differentiation, controls that are routinely incorporated into GMP workflows and release specifications [24]. An important operational advantage for GMP workflows is the availability of temperature-sensitive SeV mutants (including TSΔF derivatives), in which vector clearance can be accelerated by shifting to a nonpermissive temperature, simplifying removal of reprogramming vectors and reducing reliance on prolonged passaging alone [25]. In practice, SeV is often selected as a pragmatic "industry default" where high robustness and technology transferability are prioritized, if clearance testing and GMP raw material control are well defined.

Non-viral, integration-free platforms (episomal plasmids; synthetic mRNA; chemical)

Episomal plasmid systems (commonly oriP/EBNA1-based) achieve transient expression of reprogramming factors through episomal maintenance followed by dilution and loss during passaging, thereby avoiding intended genomic integration [25]. Episomal methods are operationally straightforward and compatible with feeder-free/xeno-free manufacturing [26], but commonly exhibit lower and more variable efficiency than SeV across laboratories and donor material. Because rare integration events have been reported and because reprogramming imposes selective pressure, clinical translation increasingly relies on sensitive

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vector-sequence assays and broader genomic characterization (often including sequencing-based approaches) to support substrate qualification [27,28]. Protocol modifications that enhance efficiency (including expanded cocktails or transient perturbation of p53 signaling) must be evaluated against genomic stability expectations and the program's risk tolerance [27].

Synthetic modified mRNA reprogramming eliminates viral components and DNA intermediates by inducing transient expression through repeated transfection of modified mRNA encoding pluripotency factors, achieving high efficiency and rapid kinetics with low theoretical integration risk [29]. Translational risk shifts from insertional mutagenesis to manufacturing control [30]. These risks include (but are not limited to) repeated transfection schedules, innate immune activation management, and operator sensitivity can introduce lot-to-lot variability unless mitigated through automation, closed processing, and stringent in-process controls (ref). Consequently, mRNA is often most attractive where platform investment can support early process engineering. Chemical/small-molecule reprogramming aims to replace transcription factor delivery with pathway modulation (epigenetic modifiers and signaling agonists/antagonists). While murine proof-of-concept demonstrates the potential of small molecules to induce pluripotency, translation to human cells remains more challenging and process-sensitive [31]. The dominant translational concerns include off-target epigenetic remodeling, stability of induced pluripotency, and comparability as chemical conditions evolve; these requirements raise the characterization threshold for clinical plausibility [32].

Small-molecule reprogramming

Small-molecule reprogramming seeks to replace exogenous transcription factor delivery with staged modulation of endogenous signaling and chromatin states using epigenetic regulators and pathway agonists/antagonists. In murine systems, proof-of-concept studies demonstrate that defined chemical cocktails can induce pluripotency, supporting the principle that pluripotent state transitions can be driven pharmacologically. However, translation to human reprogramming remains comparatively less mature and more process-sensitive, and the primary translational risks differ from those of vector-based approaches: off-target epigenomic remodeling, variability arising from dose–timing–sequence dependence, and comparability challenges when cocktails evolve during development [33]. These features imply an elevated characterization threshold for clinical plausibility, including deeper epigenomic/ functional comparability assessments and more conservative stability governance to ensure that chemically induced pluripotency yields durable lineage performance rather than protocol-contingent phenotypes.

Comparative Safety and Efficacy of Reprogramming Methods

For clinical programs, the decision is not which method can generate iPSCs under ideal laboratory conditions, but which platform can produce a qualified cell substrate that is safe, controllable, and scalable while remaining defensible to regulators across the lifecycle of manufacturing change [34]. A practical clinical-grade decision lens evaluates platforms across (i) genomic risk, (ii) operational robustness, and (iii) regulatory friction.

- Genomic risk is highest for integrating viral systems because stable insertion creates non-

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removable mutagenic risk and increases uncertainty around long-term behavior [35]. Integration-free strategies (SeV, episomal plasmids, modified mRNA) substantially reduce insertional mutagenesis concerns, but their risk profiles are shaped by residual platform-specific liabilities (vector persistence for SeV; rare integration and efficiency-related selection pressure for episomal; process complexity and innate immune perturbation for mRNA) [36].

- Operational robustness at scale frequently favors SeV because it requires fewer manipulations and typically exhibits less operator sensitivity than repeated transfections, supporting reproducibility during scale-up and technology transfer [37]. Episomal and mRNA workflows can be clinically viable but generally demand earlier investment in automation, closed systems, and in-process controls to achieve comparable robustness [38].
- Regulatory friction reflects the cumulative burden of controls, characterization, and comparability planning. Regulators evaluate reprogramming choices within the entire substrate-to-product strategy, emphasizing identity, purity, genomic stability, adventitious agent control, tumorigenicity risk management, and comparability as processes evolve from early to late clinical development.[7–11] In practice, many developers select SeV or episomal systems for initial clinical translation because they balance feasibility and risk control, while mRNA is favored when organizations can industrialize the workflow to reduce operator-driven variability [39].

Differentiation Strategies: Clinical Relevance and Manufacturing Control

Differentiation is best treated as a manufacturing control strategy: it must reproducibly generate a defined cell population with measurable potency and acceptable safety margins, including credible management of residual pluripotent cells [40].

Directed differentiation (2D, chemically defined)

Directed, chemically defined differentiation employs the stepwise and temporally controlled modulation of conserved developmental signaling pathways to recapitulate embryonic lineage specification and progressively restrict cell fate. Among the most commonly leveraged pathways are WNT, BMP, TGF-β/Activin–Nodal, SHH, FGF, and retinoid signaling, each of which exerts context-dependent effects on cell identity depending on timing, dose, and combinatorial interaction [41].

- *WNT signaling* plays a central role in early germ layer patterning and axis specification. Transient activation of canonical WNT/β-catenin signaling is frequently used to promote mesendodermal induction, while subsequent inhibition can favor differentiation toward cardiac, neural, or anterior lineages, illustrating its biphasic and stage-dependent function [42].
- Bone morphogenetic protein (BMP) signaling contributes to dorsoventral patterning and lineage segregation. BMP activity is often suppressed during neural induction to prevent mesodermal or epidermal fates, whereas controlled activation supports mesodermal differentiation and specific hematopoietic or endothelial trajectories [43].
- TGF-β/Activin–Nodal signaling regulates pluripotency maintenance and early lineage decisions. Inhibition of TGF-β signaling is commonly employed to facilitate exit from pluripotency and promote neural differentiation, while sustained Activin/Nodal signaling

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supports definitive endoderm specification in protocols for hepatic or pancreatic lineages [44].

- Sonic hedgehog (SHH) signaling provides positional information during tissue patterning, particularly along the ventral axis of the neural tube. In directed differentiation, SHH modulation is used to specify ventral neural progenitors and related lineages, including motor neurons and certain interneuron populations [45].
- Fibroblast growth factor (FGF) signaling supports cell survival, proliferation, and lineage stabilization throughout differentiation. FGF signaling often acts synergistically with other pathways to reinforce lineage commitment and expand intermediate progenitor populations under defined conditions [46].
- Retinoid signaling, mediated by retinoic acid, functions as a morphogen that regulates anterior–posterior patterning and terminal differentiation. Retinoids are commonly applied at later differentiation stages to promote maturation and regional identity, particularly within neural and mesodermal derivatives [47].

Together, the controlled orchestration of these pathways enables reproducible lineage specification under chemically defined conditions, providing a mechanistic basis for scalable, GMP-compatible differentiation processes with measurable identity and potency attributes [48].

3D differentiation (organoids; self-organization)

Three-dimensional (3D) differentiation and organoid approaches leverage partial self-organization to produce tissue-like structures with enhanced architectural and functional fidelity for selected indications (notably retinal and neural systems). However, heterogeneity, diffusion constraints, and challenges in defining release specifications often necessitate conversion to a defined transplantable fraction and additional impurity controls for clinical translation [49].

Direct lineage conversion (transdifferentiation) vs iPSC route

Transdifferentiation bypasses pluripotency and may reduce pluripotency-associated tumorigenicity concerns, but frequently faces challenges in incomplete conversion, epigenetic memory, and scalability/banking, limiting its adoption for regulated programs where standardized manufacturing and comparability are prioritized [50]. Despite the conceptual appeal of bypassing pluripotency, direct lineage conversion remains less standardized and less regulatorily familiar than the iPSC route for most therapeutic manufacturing strategies. The iPSC route enables establishment of intermediate cell banks (MCB/WCB) that support deep characterization of identity, genomic stability, adventitious agent safety, and comparability across manufacturing evolution, thereby providing a structured framework for controlling variability at scale. By contrast, transdifferentiation programs often face greater uncertainty in conversion completeness, product heterogeneity, and stability over time, which can complicate the definition of release specifications and comparability plans. Accordingly, for most regulated programs seeking a scalable and repeatable manufacturing paradigm, the iPSC route remains the more controllable and regulatorily tractable pathway, provided that pluripotency-associated risks are addressed through layered tumorigenicity mitigation strategies and stringent impurity controls.

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Dimension	iPSC Route (Reprogramming → Banking → Differentiation)	Direct Lineage Conversion (Transdifferentiation)
Theoretical tumorigenic risk	Higher intrinsic theoretical risk due to pluripotent intermediate; mitigated through residual pluripotent cell assays, purification/depletion, and tumorigenicity testing	Lower intrinsic theoretical risk by bypassing pluripotency; risks shift toward incomplete conversion and aberrant proliferative phenotypes
Process scalability	High: standardized expansion, cryobanking, and differentiation runs enable scale-out and/or scale-up under GMP	Moderate to low: conversion efficiency and donor variability often limit industrialization; process can be sensitive to timing/dose and cell state
Cell expansion potential	High: iPSCs provide renewable expansion capacity and multi-dose manufacturing campaigns	Variable: depends on the target lineage; many directly converted cell types exhibit limited proliferative capacity
Heterogeneity of final product	Controllable: differentiation can be engineered with defined intermediates; purification and in-process controls support tighter specifications	Often higher: incomplete conversion and mixed phenotypes are common; defining release specifications may be more challenging
Epigenetic memory	Reduced relative memory after reprogramming, though line-to-line variability and residual signatures can persist	Often higher: direct conversion can retain donor-cell epigenetic features, potentially impacting stability and function
Regulatory*, ** precedent	Stronger: established precedent for master/working cell banks (MCB/WCB), deep characterization, and comparability strategies	More limited: fewer standardized regulatory pathways; greater scrutiny on identity stability, durability, and drift

Table 1: iPSC Route vs Direct Lineage Conversion (Transdifferentiation): Translational and Regulatory Comparison.

*Regulatory agencies evaluate cell therapy manufacturing strategies within established comparability frameworks that emphasize control of starting materials, definition of intermediate and final cell substrates, and demonstrate that manufacturing changes do not adversely affect product quality, safety, or potency. iPSC-based approaches align with this precedent by enabling structured comparability assessments across clinical phases, whereas direct lineage conversion currently offers more limited regulatory experience and fewer standardized reference points for demonstrating manufacturing consistency.

**The establishment of master and working cell banks (MCB/WCB) from iPSCs supports deep characterization, long-term traceability, and lifecycle management, including bridging of manufacturing changes under FDA biologics and EU ATMP frameworks. This banking paradigm provides regulators with a stable reference substrate for ongoing comparability, a feature that is inherently more challenging to implement in transdifferentiation-based manufacturing models lacking renewable pluripotent intermediates.

Reprogramming Platform	Mechanism of Pluripotency Induction	Reprogramming Efficiency & Robustness	Primary Safety Liabilities	CMC / Manufacturing Considerations	Regulatory Posture (FDA / EMA Alignment)	Translational Suitability

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Integrating viral vectors (retrovirus, lentivirus)	Stable genomic integration of pluripotency factors (e.g., OCT4, SOX2, KLF4, c-MYC)	High efficiency; robust across donor cell types; scalable in research settings	Insertional mutagenesis; persistent or dysregulated transgene expression; oncogenic activation risk	Requires integration site mapping, replication-competent virus testing, vector shedding studies; complicates comparability and lifecycle management	Increasingly disfavored for therapeutic iPSC derivation due to genomic risk and long-term safety burden	Low – largely unsuitable for clinical iPSC starting materials
Non-integrating viral vectors (Sendai virus)	Cytoplasmic RNA virus; transient expression without genomic integration	High efficiency; robust and reproducible; effective in fibroblasts and blood-derived cells	Vector persistence if not cleared; requires sensitive detection assays	Fewer manipulations than non-viral systems; defined passage limits and qPCR-based clearance assays required; compatible with GMP workflows	Broadly accepted when vector clearance is demonstrated; widely used in clinical-grade iPSC derivation	High – pragmatic "industry default" for early clinical translation
Episomal plasmids (e.g., oriP/EBNA1 systems)	Transient episomal maintenance; dilution and loss with passaging	Moderate efficiency; more variable than SeV; donor- and protocol-dependent	Rare plasmid integration events; potential genomic instability if efficiency-enhancing strategies used	Operationally simple; compatible with feeder-free/xeno-free systems; requires vector-specific qPCR and often genome-wide characterization	Accepted with appropriate genomic testing and justification of process controls	High–Moderate – widely used for GMP iPSC bank generation
Synthetic modified mRNA	Repeated transfection of modified mRNA encoding pluripotency factors; no DNA intermediates	High potential efficiency; rapid kinetics; sensitive to process execution	Innate immune activation; operator-dependent variability; process complexity	Requires repeated transfections; benefits strongly from automation and closed systems; higher early CMC burden	Viewed favorably when manufacturing robustness and comparability are demonstrated	High for platform developers; Moderate for rapid clinical entry
Chemical / small-molecule reprogramming	Replacement of transcription factors via pathway and epigenetic modulation	Demonstrated in model systems; variable and process-sensitive in human cells	Off-target epigenetic remodeling; stability of pluripotency uncertain	Complex characterization requirements; limited GMP precedent	Considered experimental; requires extensive justification and long-term stability data	Low–Emerging – not yet clinically mature

Table 2: Comparative Assessment of iPSC Reprogramming Platforms for Clinical Translation.

Differentiation Strategy	Core Concept & Biological Basis	Manufacturing Strengths	Primary Translational Risks	Potency & Release	Regulatory Consideration	Clinical Translatio

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				Assay Feasibility	s (FDA / EMA Alignment)	n Suitability
Directed differentiation (2D, chemically defined)	Stepwise modulation of developmental signaling pathways (e.g., WNT, BMP, TGF-β, SHH, FGF, retinoids) to recapitulate lineage commitment	High process control; scalable; compatible with closed, GMP systems; amenable to in-process monitoring	Batch drift; line-to-line variability; residual pluripotent cells if differentiation is incomplete	Strong alignment: identity, purity, and functional assays can be MOA-linked and used for lot release and comparability	Preferred by regulators due to definable intermediates, release criteria, and comparability across process changes	High – dominant approach for most clinical iPSC products
3D differentiation / organoids	Partial self-organization under defined or semi-defined cues to generate tissue-like architecture (e.g., retina, brain, gut)	Enhanced structural and functional fidelity for selected indications	Cellular heterogeneity; diffusion limits; scale-out challenges; difficult release specifications	Potency assays often indirect; dissociation or subpopulation selection usually required for release	Acceptable only with rigorous control of heterogeneity and clear definition of transplantable fraction	Moderate – indication-dependent; requires additional control layers
Direct lineage conversion (transdifferentiation)	Forced conversion between somatic lineages without passing through pluripotency	Avoids pluripotent intermediate; reduced theoretical tumorigenicity risk	Incomplete conversion; epigenetic memory; limited scalability and banking	Potency assays may be difficult to standardize; limited comparability experience	Regulators scrutinize identity stability and durability of conversion	Low–Moderate – niche use; less favored for regulated programs
Spontaneous differentiation (embryoid bodies)	Unpatterned differentiation driven by intrinsic developmental programs	Useful for discovery and proof-of-concept	Poor reproducibility; high heterogeneity; minimal process control	Not suitable for robust release or comparability assays	Generally unacceptable for clinical manufacturing	Low – research use only
Hybrid strategies (directed + purification/depletion)	Directed differentiation combined with lineage enrichment or depletion of pluripotent cells	Improves purity and safety margins; flexible across indications	Additional processing steps increase CMC complexity	Potency assays strengthened by improved identity and purity	Viewed favorably when purification is well-validated and scalable	High – common in advanced clinical programs

Table 3: Comparative Assessment of iPSC Differentiation Strategies for Clinical Cell Therapy.

Safety: Dominant Failure Modes and Mitigation Strategies

Tumorigenicity

Tumorigenicity is the defining safety concern for iPSC-derived therapies and is driven by two dominant, partially independent mechanisms [51]: (i) residual undifferentiated pluripotent cells carried into the final

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product and (ii) transformation risk arising from culture-acquired genomic abnormalities during reprogramming, banking, or differentiation. Global regulatory practice emphasizes a risk-based, layered control strategy that integrates upstream controls, in-process governance, release testing, and fit-for-purpose in vivo studies rather than reliance on any single assay.

Residual undifferentiated cells are mitigated by a combination of process design (driving differentiation completeness), active clearance, and high-sensitivity detection. Active clearance approaches used in regulated manufacturing most commonly include:

1. Antibody-mediated cell sorting or depletion (e.g., targeting pluripotency-associated surface antigens) to remove residual pluripotent populations;
2. Surface marker–based flow cytometry sorting to enrich the desired lineage while excluding cells with pluripotent signatures; and
3. Selective small-molecule strategies intended to preferentially eliminate undifferentiated pluripotent cells while sparing lineage-committed progeny (typically requiring careful optimization to avoid unintended cytotoxicity or functional impairment). These approaches are conceptually distinct but often combined with lineage enrichment and process constraints to achieve acceptable safety margins.

Detection strategies should be framed explicitly in terms of assay sensitivity and validation feasibility. Flow cytometry offers practical in-process monitoring and release utility but can be limited by sampling statistics and marker specificity at very low residual levels. Nucleic-acid–based assays (e.g., qPCR/RT-qPCR for pluripotency transcripts or vector sequences where relevant) can achieve greater analytical sensitivity, but their clinical relevance depends on validated correlation to tumorigenic potential, control of false positives from trace nucleic acids, and robust extraction/normalization across complex cell matrices. Regulators increasingly expect sponsors to justify the limit of detection and to validate assay performance in the context of the intended product, including matrix effects, spike–recovery, and inter-operator reproducibility.

Culture-associated genomic changes can include submicroscopic copy number variations (CNVs) and single nucleotide variants (SNVs) that are not detected by conventional karyotyping. Consequently, while karyotype remains useful for detecting large-scale aneuploidy or gross rearrangements, it does not reliably capture smaller CNVs or point mutations that may confer proliferative advantage or alter differentiation behavior; higher-resolution methods such as chromosomal microarray analysis (CMA) and/or whole-genome sequencing (WGS) (or appropriately justified sequencing strategies) are therefore increasingly incorporated in cell-substrate qualification frameworks, particularly for products with long-term persistence. A cost-effective testing architecture is typically staged at the nodes where it provides the strongest lifecycle leverage:

- Master Cell Bank (MCB) establishment: highest value point for deep genomic characterization (CMA/WGS) because the MCB becomes the long-lived reference substrate for comparability and downstream manufacturing campaigns [52].

- Working Cell Bank (WCB): targeted confirmation (often karyotype plus risk-based higher-resolution follow-up) to detect bank-to-bank drift before large-scale manufacturing [53].
- Pre-final/final product stage: focused testing aligned to risk and practicality (e.g., karyotype or targeted panels), recognizing that deep sequencing at the final product stage may be limited by time constraints and by interpretability in heterogeneous differentiated populations; sponsors should instead demonstrate that earlier banking controls and in-process governance limit drift between bank and product [54].

Risk control layer model (multi-layer defense)

A practical way to operationalize tumorigenicity control is a "risk control layer" model in which each layer provides independent defense-in-depth [55]:

- Layer 1: Raw material and donor control (donor eligibility, reagent qualification, adventitious agent controls).
- Layer 2: In-process controls (passage limits, defined culture conditions, periodic karyotyping/monitoring, and standardized handling to reduce selection pressure).
- Layer 3: Release testing (validated residual iPSC assay with justified LOD; genomic stability package appropriate to risk; identity/purity).
- Layer 4: Preclinical safety studies (fit-for-purpose *in vivo* tumorigenicity/biodistribution package aligned to product, dose, and route).

This layered model aligns with current global practice in tumorigenicity assessment for pluripotent-derived products and provides a regulatorily intelligible structure for communicating risk rationale and mitigation.

Efficacy and potency: Translating "works" into release criteria

Potency is frequently the most challenging element of iPSC-derived therapy submissions because it must bridge mechanistic biology, manufacturing control, and clinical interpretability. Regulatory expectations generally converge on potency as a fit-for-purpose, multi-assay framework that supports lot release, comparability, and lifecycle management, rather than a single test [8].

A practical, regulatorily legible architecture is a tiered (Tier 1–3) model

Tier 1identity/purity panels typically rely on flow cytometry using surface and/or intracellular markers selected to define the intended lineage and exclude undesired populations (including residual pluripotent cells). Tier 1 assays often serve as the backbone of lot release due to speed, practicality, and direct linkage to product definition.

Tier 2 assays measure a mechanism-relevant function under controlled conditions and should be sensitive to manufacturing drift. Examples include contraction rate/force metrics for cardiomyocytes, dopamine production/release for dopaminergic neurons, and glucose-stimulated insulin secretion (GSIS) for beta-cell/islet products. Tier 2 is typically the most discriminating tier for comparability and for defining clinically meaningful Critical Quality Attributes (CQAs).

Clinical and Regulatory Case Study: Potency implications from the VX-880 (stem cell–derived islet cell) clinical program. Public disclosures from the VX-880 clinical program report evidence of islet cell engraftment and glucose-responsive insulin production, including restoration of measurable C-peptide in previously C-peptide–negative individuals after infusion. While sponsors do not typically disclose full lot-release methods, an iPSC-derived islet product's potency strategy would reasonably be expected to include a Tier 1 identity/purity panel (islet/beta-cell markers and impurity controls) and Tier 2 functional testing aligned to insulin secretory biology (e.g., GSIS and insulin/C-peptide content), with clinical pharmacodynamic readouts (C-peptide dynamics and glycemic endpoints) serving as translational anchors.

Tier 3: *In vivo* relevance (bridging studies, when required). In vivo bridging studies are most justified when the mechanism of action is complex or when in vitro assays have limited correlation with in vivo functional integration, persistence, or systemic effects. In such cases, disease-relevant animal models can provide supportive evidence linking manufacturing output to biological effect, while also informing dose, biodistribution, and safety margins. Tier 3 should be used selectively and strategically, not as a substitute for rigorous Tier 1–2 assay development.

Potency strategy should be established early and embedded into process development under Quality by Design (QbD) principles: potency methods should be used to define and control Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs), enabling proactive drift control and more credible comparability narratives as the process evolves from early to late clinical phases.

A regulatory-aligned technical route to the clinic

Accumulating clinical experience supports allogeneic, bank-based iPSC strategies as the most scalable and regulatorily tractable approach, except where autologous use is uniquely justified [56]. This model enables deeply characterized master cell banks, standardized differentiation campaigns, and improved cost and comparability profiles. Integration-free reprogramming (typically SeV or episomal), chemically defined differentiation, intermediate banking, and layered tumorigenicity controls together constitute a defensible technical route. In the US, iPSC-derived therapies are regulated as biologics requiring IND-enabling CMC, nonclinical, and clinical packages, while in Europe they fall under ATMP frameworks with parallel expectations for quality and safety alignment [57,58].

Conclusions

iPSC technology has entered a phase of translational clinical accountability. The scientific feasibility of reprogramming and differentiation is well established; success now depends on disciplined integration of biological rigor, manufacturing control, and regulatory strategy. Programs that align reprogramming modality, differentiation approach, safety architecture, and regulatory expectations from inception are best positioned to deliver clinically viable therapies. As clinical experience expands and standards continue to converge, iPSC-derived cell therapies are poised to transition from experimental promise to sustainable clinical modalities.

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