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Review Article

Biomanufacturing and Scale-Up of Clinical-Grade Induced Pluripotent Stem Cell Lines

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Abstract

Background: Clinical use of induced pluripotent stem cells depends on manufacturing lines that regulators accept as starting material and expanding them reproducibly to the numbers dosing schedules demand.

Objective: To identify where the binding constraints on clinical-grade production now sit, across expansion, genomic stability, residual undifferentiated cell testing and sourcing strategy.

Methods: Narrative review of bioprocess studies, genomic stability screens, release-testing reports and regulatory guidance.

Findings: Perfused stirred-tank culture reached 35×10^6 cells mL⁻¹ and 5.25×10^9 cells per 150 mL run in 7 days. Screening 136 lines from 38 laboratories found a 20q11.21 amplicon in more than 20%. Digital PCR detects residual undifferentiated cells at 0.001%. Autologous manufacture cost approximately US\$1 million per case.

Conclusions: Evidence supports litre-scale expansion as a solved engineering problem. It does not support terminal karyotype as adequate stability control, and sourcing strategy, not bioprocess, dominates cost.

Keywords: Induced pluripotent stem cells; Biomanufacturing; Bioreactor expansion; Genomic stability; Good manufacturing practice

Introduction

Induced pluripotent stem cells arrived in the clinic as a manufacturing problem before they were solved as a biological one. Deriving a line is routine; producing one that a regulator will accept as the starting material for a therapeutic product, and then expanding it reproducibly to the numbers a dosing schedule demands, is not. The first fully cGMP-compliant hiPSC banks were described a decade ago, together with the donor consent, traceability and testing framework needed to release them [1]. Consensus quality-control guidance for clinical-grade lines followed [2], building on earlier points-to-consider for pluripotent seed stocks [3].

What has changed since is where the difficulty sits. Volumetric productivity is no longer the rate-limiting step: perfused stirred-tank processes now deliver 5.25×10^9 cells from a 150 mL working volume in 7 days [4], and two-step spinner-to-bioreactor trains take 16×10^6 cells to 2×10^9 in 14 days [5]. Those numbers already exceed what most allogeneic indications require per lot. The binding constraints are elsewhere: the genomic drift that accumulates over the passages such expansion consumes, the analytical sensitivity of residual-undifferentiated-cell testing, and the strategic choice between autologous manufacture, an HLA-homozygous haplobank and a gene-edited universal line — a choice that determines cost structure more decisively than any bioreactor decision [6].

This review takes those three constraints in turn, after a short account of how a clinical line is derived and qualified. Throughout, process figures are quoted with the vessel, working volume and feeding regime at which they were obtained, because peak densities from different formats are not interchangeable claims about the same quantity.

Reprogramming and the qualified starting line

Clinical manufacture uses non-integrating reprogramming. Sendai virus vectors deliver the

reprogramming factors as an RNA genome that does not integrate [7]; episomal plasmid systems combining p53 suppression with L-MYC substitution provided the other main route and were used to generate lines from HLA-homozygous donors matching approximately 20% of the Japanese population [8]; synthetic modified mRNA offers a third, footprint-free option [9]. Head-to-head comparison established that these methods differ materially in aneuploidy rate, efficiency, reliability and workload [10], and the choice is made on those grounds rather than on efficiency alone.

The practical constraint on the front end is throughput per operator, not yield per experiment. A chemically defined, small-format reprogramming platform reduced reagent cost per patient sample by more than 80% and medium usage by 70%, compressed experimental scale up to 50-fold, and raised patient-sample throughput 10-fold, so that one technician runs 20 to 40 lines simultaneously in 24- to 96-well format and a facility supports more than 200 well-characterised lines annually; more than 800 lines from over 160 samples were established this way [11].

Vector clearance is a release attribute of the resulting line rather than a property of the method. In cGMP banks derived from cord blood CD34+ cells, residual episomal sequences were tracked by qPCR against EBNA and OriP elements of the pEB-C5 and pEB-Tg plasmids at multiple passages before final expansion and banking [12]. No fetched primary record states a numeric acceptance limit, and none should be inferred; what is documented is the practice of demonstrating clearance across passage, not a threshold. The same caution applies to reprogramming efficiency: the comparative study establishes that the methods differ, but the per-method figures belong to its primary data and are not reproduced here [10].

Expansion at the litre scale

Planar culture on defined matrices remains the derivation and qualification format, but scale-up moves to stirred

systems. Microcarrier culture in a 50 mL spinner with Essential 8 and vitronectin reached 1.4×10^6 cells mL^{-1} and a maximum 3.5-fold yield over 10 days, with the optimum set jointly by inoculation density of 55,000 cells cm^{-2} and agitation at 44 rpm, identified by three-level factorial design [13]. Matrix-free aggregate suspension has since displaced it. A 60 mL Vertical-Wheel vessel reached $2.3 \pm 0.2 \times 10^6$ cells mL^{-1} and 9.3 $\pm$ 0.6-fold expansion in 5 days, a volumetric productivity of $4.6 \pm 0.3 \times 10^5$ cells $\text{mL}^{-1} \text{d}^{-1}$, with mean aggregate diameter $346 \pm 11 \mu\text{m}$; dextran sulfate to suppress agglomeration raised maximum cell number by 97% in mTeSR1 and 106% in mTeSR3D [14]. Computational-fluid-dynamics-guided operation of 100 mL and 500 mL Vertical-Wheel vessels gave over 30-fold expansion in 6 days from a single-cell inoculum of 2.0×10^4 cells mL^{-1} , with day-5 aggregate diameters from $169.4 \pm 5.5 \mu\text{m}$ to $275.45 \pm 6.9 \mu\text{m}$ across agitation conditions [15].

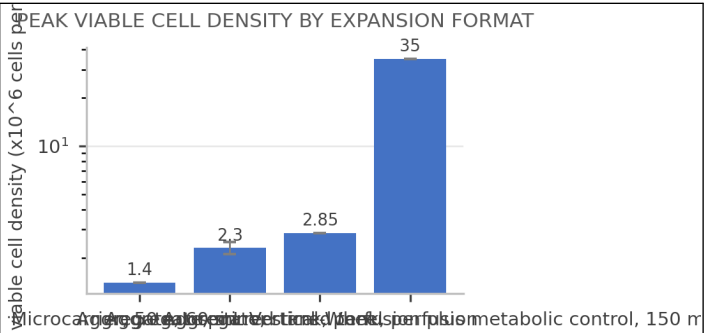


Figure 1: Peak viable cell density reported for four hiPSC expansion formats on a log scale, spanning 1.4 to 35×10^6 cells per mL across vessels, media and feeding regimes that are not matched conditions (N = 4 published processes) [13,14,16,4].

Feeding regime, not vessel geometry, sets the ceiling. Perfusion in single-use stirred tanks gave 2.85×10^6 cells mL^{-1} and 47% higher yield than repeated batch [16]. Adding metabolic control and in silico process modelling raised peak density an order of magnitude further, to 35×10^6 cells mL^{-1} and 5.25×10^9 cells per 150 mL run at 70-fold expansion in 7 days, while cutting medium demand by 75%; the process was reported as transferable

to 500 mL [4]. A two-step train — 100 mL spinner then 1000 mL bioreactor — achieved 16-fold then a further 10-fold expansion, taking 16×10^6 cells to 2×10^9 in 14 days with free scalability stated between 50 and 2400 mL, at the cost of dissociating day-7 aggregates of $324 \pm 71 \mu\text{m}$ back to single cells for re-inoculation, after which aggregates settled at $198 \pm 58 \mu\text{m}$ [5].

Genomic stability across passage

Every fold of expansion is passages, and passages select. Screening of 125 human ES and 11 human iPS cell lines from 38 laboratories (N = 136) found recurrent changes on chromosomes 1, 12, 17 and 20 accumulating with prolonged culture, and a minimal amplicon at 20q11.21 containing ID1, BCL2L1 and HM13 in more than 20% of lines [17]. The mechanism is a genuine growth advantage: BCL-XL mediates the selective benefit conferred by that amplicon [18]. Point mutations behave the same way. Sequencing the protein-coding genes of 140 human ES cell lines identified six TP53 mutations across five unrelated lines, all dominant negative and all selectively advantaged in culture [19]. The consequences of these recurrent genetic and epigenetic variants for product quality are now the organising question for the field [20].

Table 1: Expansion formats compared by reported peak density, fold expansion and stated limitation; the planar row is left explicitly unconfirmed because no fetched primary record gave a per-area or per-vessel clinical-grade yield (N = 5 published processes) [13,14,16,4,5].

Format and scale	Peak density ($\times 10^6$ per mL)	Fold expansion (duration)	Stated limitation
Planar, E8 and vitronectin	Not confirmed	Not confirmed	Adherent control only; scale-up needs transfer to suspension
Microcarrier, 50 mL spinner	1.4	3.5-fold (10 days)	Optimum set jointly by 55,000 cells per cm^2 and 44 rpm
Aggregate, 60 mL Vertical-Wheel	2.3 ± 0.2	9.3 ± 0.6 -fold (5 days)	Needs dextran sulfate; aggregates reach $346 \pm 11 \mu\text{m}$

Format and scale	Peak density (x10 ⁶ per mL)	Fold expansion (duration)	Stated limitation
Aggregate, stirred tank, perfusion	2.85	47% above repeated batch	Repeated-batch feeding less homogeneous
Aggregate, stirred tank, 150 mL, metabolic control	35	70-fold (7 days)	Requires metabolic control; stated transferable to 500 mL
Aggregate, 100 mL spinner to 1000 mL tank	Not stated as density	16-fold then 10-fold (14 days)	Day-7 aggregates must be dissociated for re-inoculation

This makes the expansion train and the stability specification a single design problem rather than two. The detection methods in routine use are not matched to the task. Chromosome banding, qPCR, FISH and digital PCR differ substantially in their ability to see low-level mosaicism, and the limits of the standard cytogenetic approach are significant [21]. A targeted droplet digital PCR panel built from a meta-analysis of more than 100 publications covers 738 catalogued recurrent abnormalities and detects 94.3% of them from DNA extracted from culture supernatant, which makes in-process monitoring between passages practical rather than terminal [22]. Because that panel runs on spent medium, it can be scheduled against the passage plan of an expansion train rather than against banking milestones, which is the practical shift: variant surveillance becomes a process parameter instead of a release test. Seed-stock guidance has long framed genetic characterisation as a continuing obligation across the life of a bank rather than a one-time qualification [3], and consensus quality-control criteria for clinical lines assume the same [2].

Release testing and residual undifferentiated cells

Regulatory expectations differ by jurisdiction and by vintage. In the European Union, the CAT reflection paper adopted and effective 14 January 2011 states that pluripotent cells carry a relatively high potential risk of

tumour formation that should be carefully addressed, that hESC- and iPSC-derived cells should be shown to be lineage-committed before administration, and that the presence of proliferative and pluripotent cells tolerated in the final product should be limited and justified [23]. In the United States, CBER’s April 2008 CMC guidance for somatic cell therapy INDs specifies sterility per 21 CFR 610.12 or USP <71>, mycoplasma testing at the manufacturing stage most likely to detect contamination, master and working cell bank characterisation, and in vitro viral testing on both banks with a one-time test on end-of-production cells [24]; that document predates iPSC products and contains no pluripotent-cell-specific text. A multi-regulator consensus effort has since set out points to consider for tumorigenicity assessment of cell therapy products [25].

The analytical answer to the EU purity expectation is a sensitivity question. Flow cytometry for TRA-1-60 detected 0.1% undifferentiated hiPSC spiked into primary retinal pigment epithelium, while qRT-PCR for LIN28 in the same system reached signal equivalent to one hiPSC in 5.0 x 10⁴ RPE cells, or 0.002% [26]. Droplet digital PCR for LIN28 pushed this to 0.001% — one hiPSC in 1 x 10⁵ cardiomyocytes — with LIN28 confirmed extremely low across seven human tissues [27]. A highly efficient colony-formation assay on laminin-521 in Essential 8 detected spiked hiPSC at 0.001% to 0.01% depending on line and host cell type, across two lines and two host types [28]. For malignant potential rather than impurity, systematic comparison of embryoid body assays, teratoma histology, TeratoScore and PluriTest found only the teratoma assay assesses pluripotency and malignant potential together [29]. Identity, sterility, endotoxin, karyology and pluripotency panels round out the release package: STR profiling tracked from donor material to the cryopreserved final product, high-resolution typing at HLA-A, -B, -C, -DQA1, -DQB1 and -DRB1, blood group typing, G-banding to FDA GLP and ACMG standards with

20 metaphases per line, and flow cytometry for SSEA-4, TRA-1-60, TRA-1-81 and OCT3/4 with embryoid body trilineage differentiation and PluriTest as potency evidence; two cord-blood-derived clones were banked under this package, one reported with a normal karyotype after 17 passages [12].

Cryopreservation, banking and cost of goods

Banking is where the process meets the balance sheet. Cryopreservation of aggregates without DMSO is now feasible: an optimised DMSO-free solution gave post-thaw reattachment of 104 +/- 5.73% of fresh control at a 1:6 split ratio, against 48.7 +/- 9.85% for the non-optimised DMSO-free formulation and 58.4 +/- 6.58% for the 7.5% DMSO control, while DMSO-frozen samples ice-nucleated at -12 C recovered only 9.53% to 10.4% [30]. Since the aggregate is the unit both of expansion and of freezing in suspension processes, removing DMSO without a recovery penalty simplifies the handoff from bioreactor to vial rather than adding a step to it.

Traceable cost figures are scarce, and the widely circulated bioprocess cost models are not among them. What is documented comes from an ISCBI workshop at which named banking centres contributed their own 2019-vintage numbers in mixed currencies: cell bank production at approximately US\$300,000 per batch of 300 vials produced over one month; vials priced at US\$1,000 to 1,500 across biobanks; GMP facility maintenance at approximately US\$2.2 million per year excluding raw materials and product QC; GMP process development from US\$3 million minimum. Per-item testing costs were itemised as US\$500 for patient viral testing, US\$2,000 per plasmid per reprogramming round, US\$25,000 to 30,000 for media and reagents, US\$450 per clone for karyology, US\$1,600 per clone for oncogene screening (under US\$500 if non-CLIA) and US\$220 per clone for STR analysis [31]. No primary source reports a batch failure rate for clinical-grade iPSC manufacture, and none should

be assumed.

Table 2: Release attributes with the method as described and the detection limit actually stated in the primary record; blanks are reported as not stated rather than filled (N = 6 attributes) [12,22,26,27,28,21].		
Attribute	Method	Stated detection limit
Karyotype	G-banding, 20 metaphases per line	Normal karyotype; mosaicism sensitivity limited
Recurrent variants	Targeted ddPCR on culture supernatant DNA	94.3% of 738 catalogued abnormalities
Residual undifferentiated cells, protein	Flow cytometry, anti-TRA-1-60, spiked into RPE	0.1%
Residual undifferentiated cells, transcript	qRT-PCR for LIN28, spiked into RPE	0.002% (1 in 5.0 x 10^4)
Residual undifferentiated cells, transcript	ddPCR for LIN28, spiked into cardiomyocytes	0.001% (1 in 1 x 10^5)
Residual undifferentiated cells, culture	Colony formation on laminin-521 in E8	0.001% to 0.01% by line and host

Sourcing strategy: autologous, haplobank or universal line

The dominant cost lever is upstream of all of this. The first autologous iPSC clinical case cost nearly 100 million yen, approximately US\$1 million, and required the patient to wait over 10 months for surgery; using an allogeneic iPSC stock brought the overall cost under 20 million yen per patient [6]. That single comparison, not bioreactor productivity, explains the field's migration to banked allogeneic material.

Haplobanking exploits HLA homozygosity. Modelling on UK donor data showed that 150 selected HLA-homozygous volunteers would match 93% of the UK population with minimal immunosuppression [32]. In Japan, 10, 75 and 140 homozygous lines are estimated to match approximately 50%, 80% and 90% of the population; the released CiRA stock comprised 21 clones from 5 donors covering approximately 32% [6]. The curve is steeply concave — the first few donors buy most of the

coverage, and the tail is expensive — which is the quantitative case for the gene-edited alternative. Targeted CRISPR-Cas9 disruption of HLA genes yields iPSC with enhanced immune compatibility from a single line [33], and hypoimmunogenic derivatives have been shown to evade rejection in fully immunocompetent allogeneic recipients [34]. Each edit, however, adds genomic characterisation burden to a product already constrained by the stability and purity testing above.

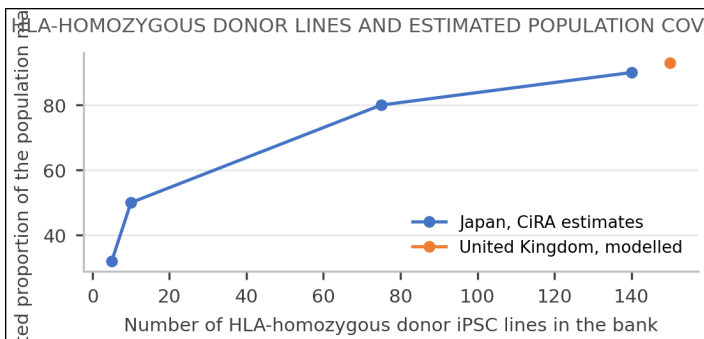


Figure 2: Estimated population coverage against the number of HLA-homozygous donor iPSC lines banked, for Japan (N = 4 published estimates, including the released stock of 21 clones from 5 donors) and the United Kingdom (N = 1 modelled point, 150 selected homozygous volunteers); the two series use different populations and matching assumptions [6,32].

Conclusion

Expansion of clinical-grade hiPSC is largely a solved engineering problem at the litre scale: perfused, metabolically controlled aggregate suspension reaches 35×10^6 cells mL⁻¹ and billions of cells per run, and multi-step trains scale predictably across two orders of magnitude of working volume. The residual difficulties are analytical and strategic. Recurrent variants at 20q11.21 and in TP53 are selected by the very passages that scale-up requires, and standard cytogenetics sees mosaicism poorly, so stability specifications must be built around in-process molecular monitoring rather than terminal karyotype alone. Residual undifferentiated cell testing now reaches 0.001% by digital PCR and colony formation, but the EU expectation that such cells be limited and justified is a framework, not a number, and each product

must argue its own case. Sourcing decides economics: the gap between roughly US\$1 million for one autologous case and under 20 million yen per patient from a stock dwarfs any process improvement, and the choice between haplobank breadth and a universal edited line is now the most consequential decision in an hiPSC manufacturing programme.

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