RESEARCH PAPER

Induced Pluripotent Stem Cells and Gene Editing: A Transformative Era in Regenerative Medicine

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ABSTRACT
Induced pluripotent stem cell (iPSC) technology and genome editing have independently transformed regenerative medicine, and their convergence now defines a powerful platform for disease modelling and cell-based therapy. iPSCs provide an ethically acceptable, patient-specific source of pluripotent cells, while genome-editing toolsparticularly CRISPR–Cas systems, base editors, and prime editors enable precise genetic modification of disease-relevant loci. This critical review examines recent advances at the interface of iPSC biology and gene editing, emphasising mechanistic integration, translational feasibility, and clinical decision-making. Key challengesincluding incomplete epigenetic reprogramming, genomic instability, off-target editing, immune recognition, and Good Manufacturing Practice (GMP) scalabilityare systematically evaluated. We further analyse why only select iPSC-derived therapies, notably in retinal and haematologic indications, have advanced to clinical trials, highlighting biological, manufacturing, and regulatory constraints. By integrating biological mechanisms with translational bottlenecks and ethical considerations, this review provides a structured framework for assessing the clinical potential of genome-edited iPSC technologies in precision regenerative medicine.
Key words: Induced pluripotent stem cell (iPSC), CRISPR–Cas systems, Yamanaka factors, base editing, prime editing, regenerative medicine, clinical translation, GMP manufacturing
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INTRODUCTION

Contextualising Regenerative Medicine: Unmet Clinical Needs

Regenerative medicine seeks to renew or replace damaged tissues and organs resulting from trauma, degenerative disease, and genetic disorders conditions that conventional treatments largely address symptomatically rather than curatively [1]. Stem cell-based therapies offer a paradigm shift by enabling production of functional cells capable of tissue reconstitution and immune modulation, and several early phase human trials for cardiovascular, neurodegenerative, and metabolic indications are now underway [1,2]. Persistent barriers including cell sourcing, immune rejection, and functional engraftment underscore the need for combined strategies that integrate gene-edited stem cells with advanced delivery systems and biomaterials [2,3].

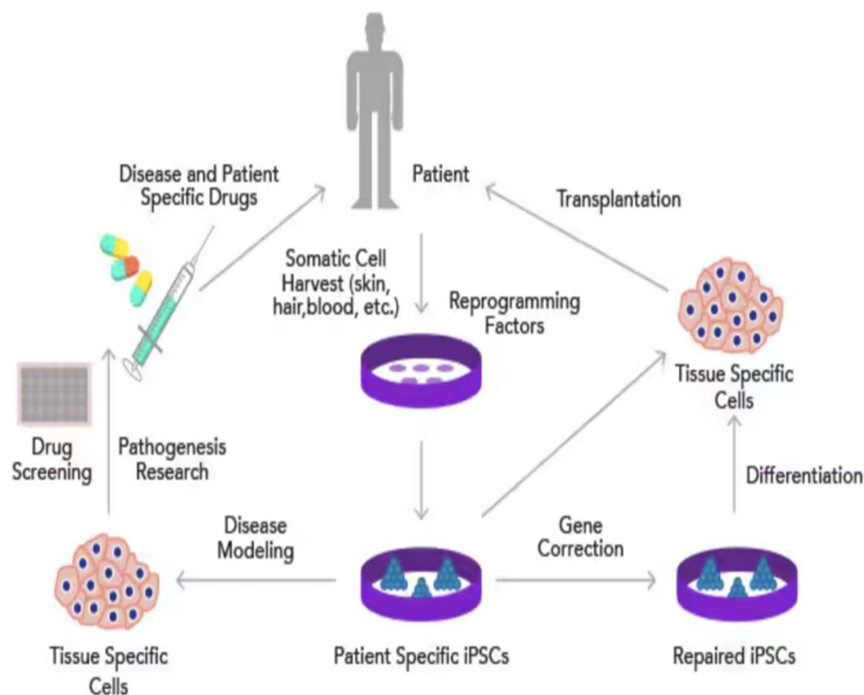

Figure 1: iPSC-Based Personalised Medicine Pipeline — Conceptual Schematic Diagram

Figure 1 | Proposed schematic of an iPSC-based personalised medicine pipeline. The figure depicts (A) somatic cell harvest from skin biopsy or peripheral blood, (B) reprogramming to iPSCs via the canonical Yamanaka factors (OCT4, SOX2, KLF4, c-MYC), (C) parallel tracks for disease modelling/drug screening and CRISPR-mediated gene correction, (D) directed differentiation into tissue-specific cell types (cardiomyocytes, neurons, retinal pigment epithelium, haematopoietic cells), and (E) quality-controlled transplantation back to the patient.

Stem Cell Classification and Comparative Biology

Stem cells are conventionally categorised into three classes with distinct biological properties and clinical implications [4,5]. Embryonic stem cells (ESCs) derive from the inner cell mass of the blastocyst and are pluripotent, capable of generating all somatic lineages; however, their use requires embryo destruction, creating ethical barriers and immune-rejection risk [4,100]. Adult stem cells including haematopoietic (HSCs) and mesenchymal stem cells (MSCs) are multipotent (not pluripotent as sometimes incorrectly stated), supporting tissue homeostasis within defined lineage boundaries; their proliferative capacity and multi-lineage differentiation potential are markedly restricted compared with ESCs [5]. iPSC technology, introduced by Takahashi and Yamanaka in 2006, achieves reprogramming of somatic cells to a pluripotent state functionally comparable to ESCs, circumventing both ethical constraints and immunological incompatibility [6,7,59,60].

Table 1. Comparative Characteristics of Principal Stem Cell Classes

Stem Cell Type	Potency	Key Advantages	Key Limitations
Embryonic Stem Cells (ESCs)	Pluripotent	Highest differentiation potential; extensive research base	Embryo destruction; immune rejection; ethical constraints; limited clinical use
Adult Stem Cells (HSCs, MSCs)	Multipotent	Readily available; fewer ethical concerns; autologous options	Restricted lineage potential; limited proliferative capacity; donor variability
Induced Pluripotent Stem Cells (iPSCs)	Pluripotent	Patient-specific; no embryo destruction; no immune rejection; disease modelling; large-scale production	Reprogramming inefficiency; epigenetic memory; tumorigenicity risk; genetic instability with passaging

Scope and Objectives of This Review

This review integrates advances at the intersection of iPSC technology and gene editing from 2015 to 2025, with emphasis on (i) mechanistic understanding of reprogramming and editing tools, (ii) translational progress and clinical trial outcomes, (iii) safety, manufacturing, and regulatory constraints, and (iv) ethical governance. The review is intended for scientists, clinicians, and health policy stakeholders navigating the path to clinical implementation of genome-edited iPSC therapies [11,12].

INDUCED PLURIPOTENT STEM CELL TECHNOLOGY

Mechanisms of Cellular Reprogramming: Yamanaka Factors

Reprogramming to iPSCs is driven by ectopic co-expression of OCT4 (POU5F1), SOX2, KLF4, and c-MYC the canonical Yamanaka factors which dismantle somatic transcriptional networks and re-establish a pluripotency programme [6,8,59]. These factors remodel closed chromatin, recruit epigenetic modifiers, activate endogenous pluripotency circuits, and silence lineage-specific genes [6,15]. Single-cell multi-omics profiling reveals discrete intermediate states with heterogeneous reprogramming trajectories, accounting for variable efficiency and residual epigenetic memory of the cell of origin [9,15,103]. To reduce oncogenic risk associated with c-MYC, current clinical approaches substitute alternative factors (NANOG, LIN28) or employ chemically induced reprogramming [16,17,64]. Critically, incomplete epigenetic resetting and stochastic mutational events may generate aberrant clones with tumorigenic potential, mandating rigorous clonal selection and genomic surveillance before therapeutic use [6,15,66].

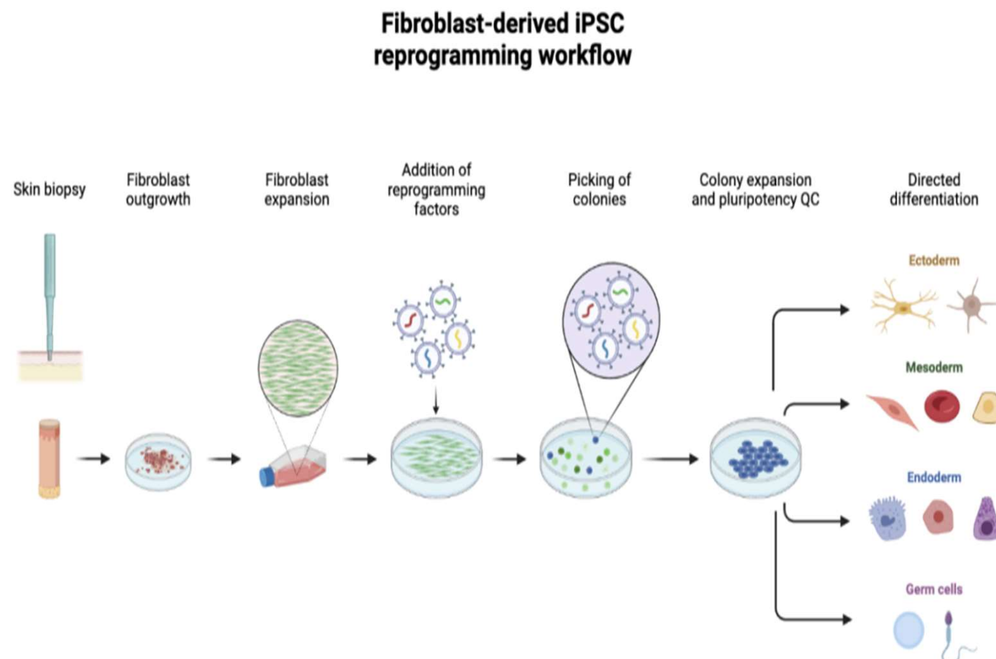

Figure 2: Yamanaka Factor-Mediated Reprogramming Mechanism — Conceptual Schematic Diagram

Reprogramming Delivery Modalities

Reprogramming vector selection critically determines integration risk, efficiency, and regulatory acceptability [17]. Integrating retroviral and lentiviral systems achieve high efficiency but carry insertional mutagenesis risk and are generally considered unsuitable for clinical products [17,65]. Non-integrating platforms including Sendai virus, episomal plasmids, PiggyBac excisable systems, and minicircle DNA minimise genomic modification [16,17,62]. Synthetic modified mRNA and recombinant protein delivery carry negligible integration risk but require precise dosing optimisation and may trigger innate immune activation [16,18,63]. Small-molecule cocktails targeting chromatin modifiers and metabolic pathways can substitute for or supplement transcription factors, offering a simpler, more scalable manufacturing pathway [17,18,64]. Method selection is governed by the intended application (discovery research vs. GMP-grade product) and applicable regulatory requirements [17].

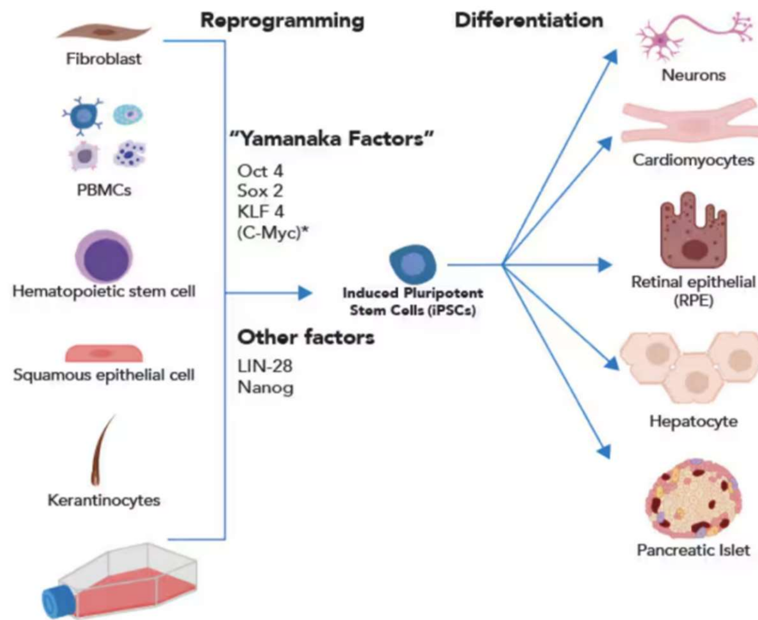

Figure 3: Reprogramming Delivery Modality Comparison Matrix — Conceptual Schematic Diagram

Advances in Reprogramming Efficiency and Safety

Optimising factor stoichiometry, employing temporal control of expression, and incorporating microRNA or epigenetic modulators substantially improve reprogramming yield without requiring strong oncogenic drivers [9,15,19]. Low-oxygen culture, xeno-free media, and defined substrates reduce stress-induced DNA damage during reprogramming [18,19]. High-throughput small-molecule screening has identified compounds that accelerate reprogramming while promoting correct epigenetic resetting, limiting residual somatic signatures [17,19,64]. Clinically, the field is moving towards integration-free, footprint-free methods combined with early clonal characterisation: karyotyping, copy-number variation (CNV) profiling, and off-target analysis when gene editing is co-applied [21,22,61].

iPSC Culture Systems and Pluripotency Maintenance

Long-term iPSC maintenance requires culture systems that preserve pluripotency, genomic integrity, and differentiation capacity. Defined, feeder-free media supplemented with FGF2 and ROCK inhibitors, combined with recombinant matrix coatings (vitronectin, laminin-521), have largely replaced mouse embryonic fibroblast feeder layers, reducing xenogenic contamination and inter-lot variability [18,19]. Automated closed-system bioreactors support GMP-compliant scale-up but introduce shear stresses requiring careful monitoring. Extended passaging under suboptimal conditions drives chromosomal abnormalities, mitochondrial dysfunction, and selection of fast-proliferating but genomically abnormal clones, necessitating routine quality checks [19,21,95]. Robust biobanking practices supported by transparent consent, traceability, and standardised cryopreservation are essential for reducing the need for repeated donor line generation [21,22,102].

Directed Differentiation

Directed differentiation protocols reproduce embryonic signalling cues WNT, BMP, FGF, TGF-β, and Notch pathway modulation—applied in precise temporal sequences to generate defined cell types. Protocols for cardiomyocytes, neurons, hepatocytes, retinal pigment epithelium (RPE), and pancreatic β cells have achieved high efficiency and reproducibility suitable for disease modelling, drug screening, and early-phase clinical testing [19,20,76,79]. Most protocols still yield mixed populations with immature phenotypes, limiting physiological fidelity [20,21]. Three-dimensional culture, biomechanical stimulation, co-culture with stromal and immune cells, and transcription-factor "forward programming" are being applied to enhance maturation and population purity [20,21,77]. Even trace undifferentiated cells carry teratoma risk, making stringent purification and release assays mandatory before clinical use [21,22].

Organoids and Co-culture Systems

iPSC-derived 3D organoids including brain, intestinal, liver, kidney, and cardiac models—recapitulate organ-level architecture, cell–cell interactions, and microenvironmental gradients absent in 2D cultures [20,108,109]. Incorporation of vasculature,

immune cells, or microbiome components into co-culture or organ-on-chip platforms increases physiological relevance and toxicological predictive power [20,21]. Key limitations include incomplete maturation, lack of standardised morphology, and scalability challenges that hamper inter-laboratory reproducibility and regulatory acceptance [20,110]. Complex brain organoids also raise ethical questions regarding consciousness proxies, prompting emerging governance guidelines [14,21,78].

Quality Control for Clinical-Grade iPSC Lines

Mandatory quality checks for clinical iPSC lines include: (i) cytogenetic analysis by karyotyping and high-resolution CNV arrays; (ii) whole-genome or targeted sequencing to detect reprogramming-induced and culture-acquired mutations; (iii) epigenetic profiling (DNA methylation, chromatin accessibility) to identify aberrant imprinting or residual somatic memory; (iv) functional tri-lineage differentiation assays confirming pluripotency; (v) lineage-specific functional readouts (e.g., electrophysiology for neurons, contractility assays for cardiomyocytes); (vi) sterility, endotoxin, and mycoplasma testing; and (vii) traceability documentation [21,22,24,27,28,99,101]. These comprehensive release criteria are fundamental to patient safety and regulatory submission.

GENE EDITING TECHNOLOGIES APPLIED TO IPSCS

Historical Evolution: ZFNs, TALENs, and CRISPR–Cas

Programmable genome editing began with zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), both capable of introducing site-specific double-strand breaks (DSBs) but technically demanding and expensive to engineer de novo [116,117]. The CRISPR–Cas9 system, adapted from prokaryotic adaptive immunity, provided an RNA-guided, readily programmable alternative that is more versatile, cost-effective, and amenable to multiplexing [13,29]. Since its demonstration in human cells in 2013, CRISPR–Cas9 has become the dominant genome-editing platform, further extended by Cas variants with alternative PAM requirements, enhanced-fidelity mutations, and catalytically dead variants enabling transcriptional regulation (CRISPRi/CRISPRa) [13,67,68,75].

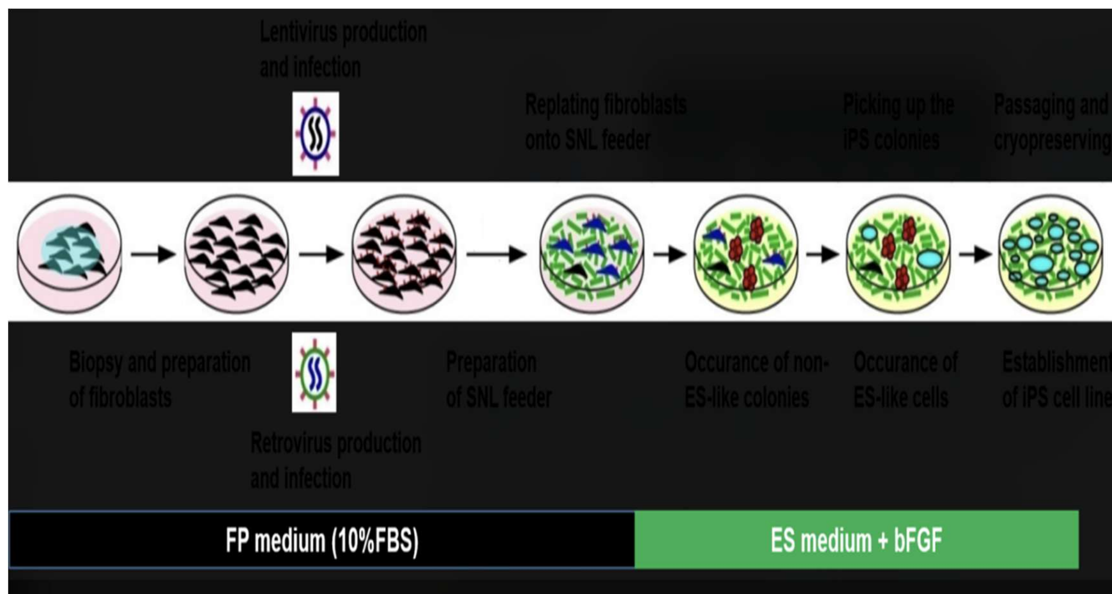

Figure 4: CRISPR-Cas9 Mechanism and Gene Editing Toolkit Overview — Conceptual Schematic Diagram

CRISPR–Cas9 Mechanism and iPSC Applications

Cas9 is directed to a genomic locus by a single guide RNA (sgRNA) complementary to the target sequence and adjacent to a 5′-NGG protospacer adjacent motif (PAM). Cleavage generates a blunt DSB repaired predominantly by non-homologous end joining (NHEJ, producing small insertions/deletions [indels]) or, in the presence of a donor template, by homology-directed repair (HDR, enabling precise sequence insertion or correction) [31,69]. In iPSCs, CRISPR–Cas9 enables gene knockouts, knock-ins, disease-mutation correction, and generation of isogenic controls. HDR efficiency in iPSCs remains lower than in rapidly dividing cells due to the predominance of NHEJ in the G1 phase; cell-cycle synchronisation and small-molecule HDR enhancers are employed to address this [30,31,98]. Clinical-grade CRISPR-edited iPSC products are currently in early-

phase trials for hereditary blindness and haemoglobinopathies [27,31,87].

Base Editing: Mechanism and Precision

Base editors achieve single-nucleotide conversion without generating DSBs. Cytosine base editors (CBEs) fuse a cytidine deaminase to a catalytically impaired Cas9 (nickase), converting C•G to T•A within an editing window. Adenine base editors (ABEs) use an evolved tRNA adenosine deaminase to convert A•T to G•C [32,71,72]. Both CBE and ABE systems employ a Cas9 nickase that creates a single-strand nick in the non-edited strand to bias mismatch repair. This mechanistic distinction significantly reduces large deletions and chromosomal rearrangements associated with DSB repair. Risks of off-target DNA deamination and inadvertent RNA editing persist, but fourth-generation and later base editor architectures, combined with transient delivery formats, have substantially mitigated these concerns [26,32,73].

Prime Editing: Versatility and Reduced Off-Target Activity

Prime editing substantially expands the editing scope by enabling all 12 possible transition and transversion base substitutions, as well as small insertions and deletions, without DSBs or exogenous donor DNA [70,73]. The system couples a Cas9 nickase to an engineered reverse transcriptase guided by a prime editing guide RNA (pegRNA). The pegRNA serves dual functions: it specifies the genomic target via its spacer sequence and encodes the desired edit via its 3′ primer binding site and reverse transcriptase template. The nick–reverse transcription–flap resolution mechanism installs the edit with low rates of unintended indels and minimal off-target activity compared with CRISPR–Cas9 HDR [70]. Delivery efficiency and pegRNA stability in pluripotent cells remain active areas of optimisation [26].

Table 2. Mechanistic and Clinical Comparison of Principal Gene-Editing Modalities Applied to Ipscs

Parameter	CRISPR-Cas9 (HDR)	Base Editors (CBE/ABE)	Prime Editors
DSB Generated	Yes (blunt DSB)	No (single-strand nick only)	No (single-strand nick only)
Edit Types	KO, KI, correction (broad)	C→T or A→G transitions only	All 12 substitutions; small indels
Donor Template Required	Yes (for HDR)	No	No (pegRNA-encoded)
Off-Target Risk	Moderate–High	Low–Moderate (RNA off-targets)	Low
iPSC Efficiency	Moderate (HDR limited by cell cycle)	Moderate	Lower; improving with optimised pegRNA
Clinical Stage	Phase I/II (retina, haematology)	Early clinical / preclinical	Preclinical
Key References	[31,67,68,69]	[32,71,72,73]	[26,70,73]

Delivery Strategies for Gene-Editing Components

Successful therapeutic editing depends critically on efficient, safe delivery of editing components to iPSCs. Adeno-associated viral vectors (AAV) offer broad tropism and low immunogenicity but are limited by cargo size, precluding delivery of large base or prime editor constructs. Electroporation-based nucleofection enables direct delivery of ribonucleoprotein (RNP) complexes or mRNA into the nucleus with minimal genomic integration risk and transient nuclease exposure, reducing off-target activity [33,96,97]. Lipid nanoparticles (LNPs) and gold nanoparticle-based systems are emerging as scalable, low-toxicity alternatives suitable for clinical-grade manufacturing [96]. Each platform entails trade-offs among editing efficiency, cell viability, integration risk, and manufacturing scalability that require careful context-specific optimisation [33,97].

Challenges: Off-Target Effects, Mosaicism, and Clonal Variability

Off-target cleavage at genomic loci with partial sgRNA complementarity can introduce unintended mutations with safety and efficacy implications [92,93]. Mosaic editing incomplete modification across the iPSC population necessitates clonal isolation and rigorous genotypic screening, adding time and cost to manufacturing. Clonal variability arising from culture-acquired mutations or reprogramming artefacts confounds data interpretation and delays clinical advancement [95]. Extended culture may select for chromosomally abnormal clones with growth advantages. Comprehensive genomic and epigenomic profiling of isolated clones, combined with high-fidelity editor variants and optimised delivery tools, is required to manage these risks [57,92,94].

Strategies to Enhance Editing Specificity

Multiple complementary approaches improve editing safety [57]: (i) high-fidelity Cas9 variants (eSpCas9, HiFi Cas9, Cas9-HF1) with reduced non-specific DNA binding [56,74]; (ii) paired Cas9 nickases requiring dual-target recognition, reducing off-target DSBs by several orders of magnitude; (iii) chemically modified sgRNAs with improved specificity [98]; (iv) transient RNP delivery minimising nuclease persistence [33,96]; (v) computational off-target prediction tools for guide RNA design [92,93]; and (vi) substitution of nucleases with base or prime editors to eliminate DSBs altogether [26,57,73]. Clonal validation by whole-genome sequencing and functional assays remains mandatory before clinical use, and transparent reporting with long-term monitoring are ethical imperatives [14,57].

DISEASE MODELLING WITH GENE-EDITED IPSCS

Rationale for iPSC-Based Disease Modelling

Patient-derived iPSCs capture individual genetic backgrounds—including rare polymorphisms and disease modifiers—that are absent in animal models, enabling in vitro recapitulation of human disease mechanisms [34,58,103,105]. Gene editing allows introduction or correction of specific causal variants in isogenic pairs, isolating genotype–phenotype relationships in complex diseases [34,46,58]. From a translational perspective, iPSC disease models support pharmacogenomic assessment, biomarker discovery, and prediction of patient-specific therapeutic responses without invasive procedures [34,42]. Limitations including cellular immaturity, phenotypic drift over culture passages, and inability to capture systemic influences must be acknowledged when interpreting results [46,58].

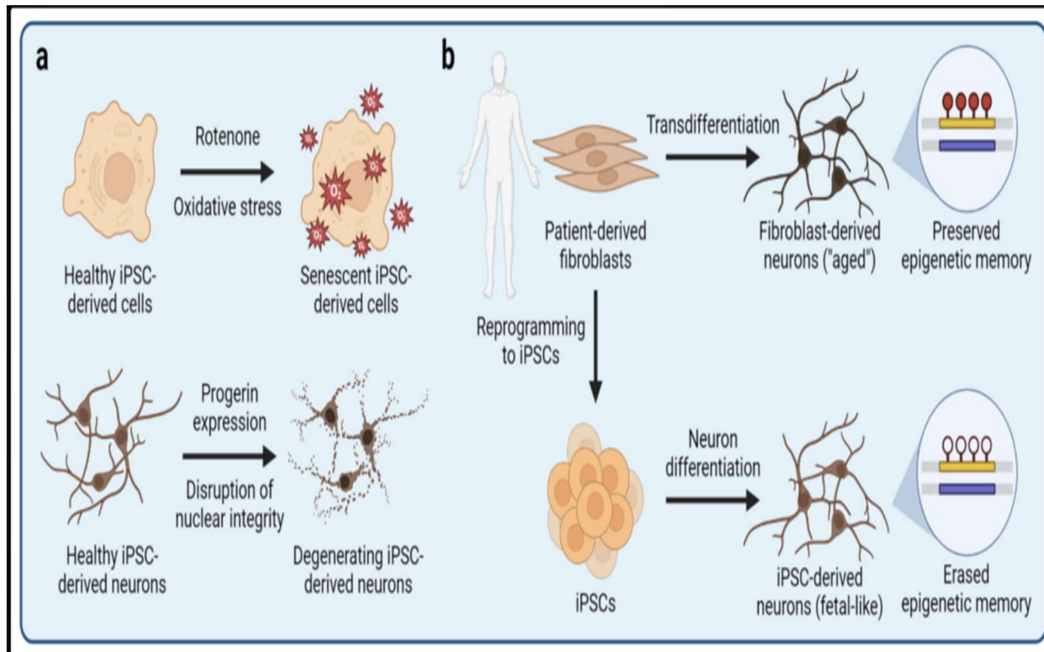

Figure 5: Ipsc Disease Modelling Workflow — Conceptual Schematic Diagram

Cardiovascular Disease Models

Gene-edited iPSC-derived cardiomyocytes (iPSC-CMs) have established causality for monogenic cardiomyopathies, channelopathies (long QT syndrome, Brugada syndrome), and congenital defects by replicating disease phenotypes reduced contractility, arrhythmogenic ion current changes and then demonstrating phenotypic rescue following CRISPR-mediated correction [35,36,76,82,83]. Personalised cardiotoxicity screening using patient iPSC-CMs is advancing drug safety assessment [36,37]. Maturation deficits and scalability for cell therapy production remain barriers; 3D engineered heart tissue platforms incorporating gene-edited cells provide more physiologically relevant models and are bridging towards therapeutic applications [36,37,76].

Neurodegenerative Disorders

iPSC-derived neuronal models of Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) have illuminated mechanisms of protein aggregation, mitochondrial dysfunction, and neuroinflammation in a human genetic context [38,39,40,81]. CRISPR-based variant correction or introduction confirms causal risk

variants and identifies modifiers. High-throughput screening of neuroprotective compounds using iPSC neurons is accelerating preclinical drug discovery [42]. Challenges include difficulty modelling late-onset pathology, incomplete cellular maturity, and absence of brain microenvironmental complexity. Three-dimensional brain organoids and astrocyte/microglia co-cultures are improving disease model fidelity [40,78]. Ethical considerations include data governance for vulnerable patient populations [14].

Metabolic and Rare Diseases

Gene-edited iPSCs enable precise introduction or correction of rare pathogenic variants GBA mutations in Gaucher disease, CFTR mutations in cystic fibrosis to establish cellular and biochemical disease phenotypes [41]. These models accelerate mutation-specific small-molecule and gene therapy screening, particularly valuable for heterogeneous rare-disease populations [42,43]. Phenotypic rescue by gene correction identifies therapeutic targets and validates treatment strategies [79]. Regulatory agencies are increasingly accepting iPSC model data to inform patient stratification in clinical trial design [44].

Fibrotic and Chronic Disease Models

iPSC-derived fibroblasts, epithelial cells, and endothelial cells from patients with idiopathic pulmonary fibrosis (IPF) and liver cirrhosis have been used to model pro-fibrotic signalling cascades, extracellular matrix deposition, and inflammatory crosstalk. Gene editing facilitates mechanistic dissection of fibrosis-associated variants and screening of anti-fibrotic therapeutics. Current limitations include the inability to fully reproduce multi-cellular mechanical complexity and the chronicity of fibrotic processes. Microfluidic organ-on-chip systems incorporating iPSC-derived cell types are beginning to address this gap [45,109].

Isogenic Controls and Functional Genomics

CRISPR-generated isogenic iPSC pairs—differing only at a defined locus—represent the gold standard for attributing phenotypic differences to specific genetic variants, dramatically reducing confounding from background genetic variation [58,82,83]. Knockout and knock-in lines enable systematic investigation of disease gene function and variant pathogenicity [43,44]. Combination with pooled CRISPR screens and multi-omics readouts uncovers downstream molecular pathways [46,104]. Off-target effects and clonal variability require thorough validation, and ethical requirements include transparent reporting of genomic modifications in all preclinical publications [14,46].

Table 3. Summary of Ipsc Disease Modelling Applications across Major Disease Categories

Disease Category	Representative Conditions	Mechanisms Studied	Therapeutic Development Status
Cardiovascular	Cardiomyopathies; channelopathies; congenital heart defects	Reduced contractility; ion channel dysregulation; arrhythmia mechanisms [76,82,83]	Active; CRISPR-corrected iPSC-CMs in preclinical/early clinical development
Neurodegenerative	Parkinson's disease; Alzheimer's disease; ALS	Protein aggregation; mitochondrial dysfunction; neuroinflammation [81,85]	Active preclinical; high-throughput screening underway; brain organoids improving fidelity [78]
Metabolic & Rare Disease	Gaucher disease (GBA); cystic fibrosis (CFTR); lysosomal storage disorders	Enzyme deficiency; impaired protein transport; lysosomal dysfunction [41,43]	Advanced preclinical; small-molecule therapies identified; gene therapy validation ongoing
Fibrotic / Chronic	Idiopathic pulmonary fibrosis; liver cirrhosis	ECM deposition; pro-fibrotic signalling; inflammatory crosstalk [45,109]	Early preclinical; organ-on-chip systems improving physiological relevance

REGENERATIVE AND CELL-BASED THERAPEUTIC APPLICATIONS

Autologous iPSC-Derived Cell Therapies

Autologous iPSC-derived cell therapies offer inherent immune compatibility, eliminating graft rejection risk and, in principle, the need for long-term immunosuppression. The most clinically advanced example remains iPSC-derived RPE cells for macular degeneration (Mandai et al., 2017), demonstrating safety and preliminary evidence of visual stabilisation [47,86]. More recently, iPSC-derived dopaminergic neurons have entered Phase I/II trials for Parkinson's disease, with a landmark 2025 Nature publication reporting safety in seven patients with no graft overgrowth or teratoma formation at 24 months [48,80,85]. Manufacturing timeline, cost, and the

requirement for patient-specific batch production remain barriers to broad autologous application [47,48,99].

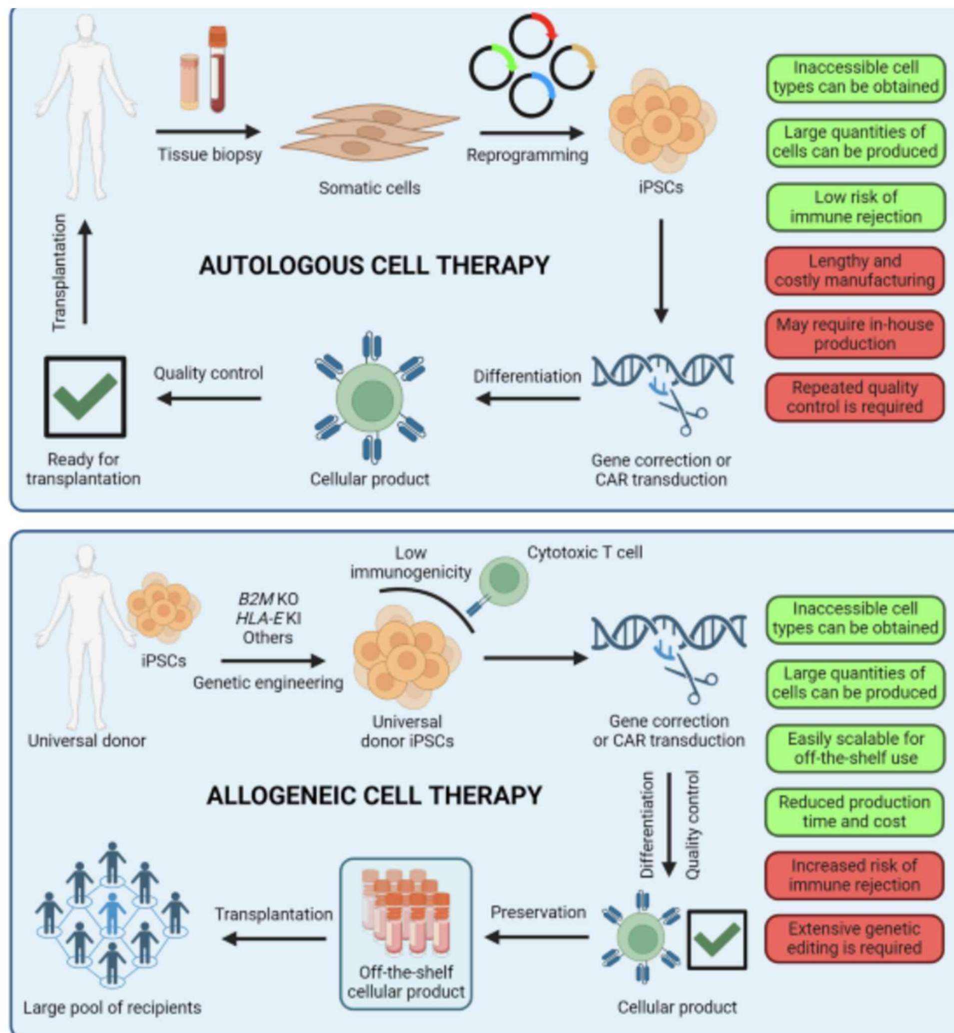

Figure 6: Autologous Vs. Allogeneic Ipsc Therapy Comparative Workflow — Conceptual Schematic Diagram

Universal and Hypoimmunogenic Allogeneic iPSC Lines

To overcome the cost and scalability limitations of autologous manufacturing, universal iPSC lines have been engineered by CRISPR-mediated disruption of β2-microglobulin (B2M) and CIITAabrogating HLA class I and class II surface expression, respectivelycombined with overexpression of NK-cell inhibitory ligands (CD47, HLA-E) to prevent natural killer cell-mediated lysis [49,90]. In vivo studies demonstrate prolonged graft survival and functional integration of hypoimmunogenic iPSC derivatives in cardiac, hepatic, and neural contexts [51,89]. Safety concerns regarding reduced pathogen surveillance and impaired tumour immunosurveillance require long-term monitoring in clinical programmes [50,91]. Universal hypoimmunogenic iPSCs represent a transformative strategy for scalable, widely accessible cell therapy [49,51,90].

iPSC-Derived CAR-T and CAR-NK Cells in Cancer Immunotherapy

iPSC technology enables off-the-shelf production of chimeric antigen receptor T (CAR-T) and NK (CAR-NK) cells with uniform phenotype, overcoming donor scarcity and primary cell variability [106,107]. iPSC-derived CAR-NK cells targeting haematological malignancies have reached early clinical trials with encouraging preliminary safety data. Genome editing further enhances therapeutic immune cells by deleting inhibitory receptor genes (PD-1, TIGIT), integrating CAR constructs at defined

safe-harbour loci, and incorporating inducible safety switches [52,107]. Challenges include achieving full functional maturation of iPSC-derived immune effectors and preventing graft-versus-host disease (GvHD) in allogeneic CAR-T applications [106].

Tissue Engineering and Organoid Transplantation

Coupling iPSC-derived cells with biomaterial scaffolds and 3D bioprinting enables construction of vascularised tissue grafts—cardiac patches, liver organoids, pancreatic islet constructs with superior engraftment compared with dissociated cell injection [54,108]. Organoid transplantation platforms serve as intermediate vehicles for refining gene editing, safety testing, and delivery optimisation prior to clinical translation [53,108]. Key barriers include manufacturing scalability, immune compatibility of engineered extracellular matrices, and incomplete tissue maturation. Long-term integration safety data from preclinical models continue to accumulate [54,110].

SAFETY, RISKS, AND MANUFACTURING CHALLENGES

Tumorigenicity

Tumorigenicity is the primary safety concern for iPSC-based therapies, arising from residual undifferentiated pluripotent cells capable of teratoma formation and from genomic aberrations acquired during reprogramming or expansion. Even low-frequency mutations in otherwise validated clinical iPSC lines may confer selective growth advantage post-transplantation [24,95]. Risk mitigation strategies include: suicide gene incorporation (iCaspase9, HSV-TK), small-molecule elimination of residual pluripotent cells (quercetin, YM155), and stringent release criteria integrating genomic and epigenetic characterisation [22,24]. To date, no teratoma formation has been reported in completed clinical trials, but long-term follow-up data remain limited [24,48,80].

Genetic Instability and Epigenetic Memory

Genomic instability in iPSCs manifesting as chromosomal abnormalities, CNVs, and point mutations may arise during reprogramming, culture, or gene editing, compromising differentiation capacity, safety, and therapeutic efficacy [94,95]. Epigenetic memory, reflecting persistent somatic methylation patterns from the cell of origin, can bias lineage differentiation and reduce disease modelling fidelity [66]. Multi-omics profiling and clonal selection prior to clinical use are essential to detect and exclude aberrant lines [21,22,103]. Emerging epigenome-editing approaches targeting residual methylation at specific loci offer a strategy to improve iPSC quality [21,111].

Immunogenicity

Autologous iPSC-derived cells may nonetheless elicit immune responses via neoantigens arising from reprogramming-induced mutations or aberrant antigen expression following differentiation [22,50]. Allogeneic therapies face HLA mismatch-driven rejection. Engineering hypoimmunogenic universal lines addresses the allogeneic challenge but necessitates careful monitoring for compromised pathogen and tumour immune surveillance [89,90,91]. Immune monitoring protocols and, where applicable, short-course immunosuppression are incorporated into current clinical trial designs [50,51].

GMP Manufacturing and Scalability

GMP-compliant manufacturing of iPSC-derived products requires standardisation across donor sourcing, reprogramming, culture, differentiation, and cryopreservation, supported by validated quality management systems [21,99]. Automated closed-system bioreactors reduce operator-dependent variability and contamination risk but introduce scale-up-specific shear and selection pressures. Cost barriers both capital investment and per-batch production costs—remain substantial, particularly for autologous products [21,49,99]. Consortia-based shared manufacturing infrastructure and platform cell line strategies (allogeneic, hypoimmunogenic) are being pursued to improve cost-efficiency and patient access [49,51,101].

Quality Assurance and Regulatory Release

Release testing for clinical iPSC products integrates genomic integrity assays (karyotyping, CNV analysis, next-generation sequencing), epigenetic characterisation (DNA methylation profiling), functional potency assays (lineage-specific functional tests, in vivo teratoma/xenograft models), and sterility/adventitious agent testing [21,22]. Regulatory agencies globally FDA, EMA, PMDA require these comprehensive data packages to support Investigational New Drug (IND) or equivalent applications [25]. As regulatory science matures, validated potency assays that correlate in vitro parameters with clinical outcomes are increasingly prioritised for dose optimisation and therapeutic response prediction [24,88].

ETHICAL, SOCIAL, AND REGULATORY CONSIDERATIONS

Ethical Landscape of iPSC Reprogramming and Somatic Gene Editing

iPSC technology mitigates the principal ethical objection to ESC research by avoiding embryo destruction [6,100]. Nevertheless, somatic gene editing raises concerns regarding off-target consequences, long-term monitoring obligations, and equitable access [14,55]. Robust ethical frameworks emphasise transparency in risk-benefit reporting, graduated oversight proportional to clinical risk, and

inclusive public engagement [14]. Biobanking of donor-derived iPSC lines requires dynamic, ongoing informed consent models that accommodate evolving uses, including commercial applications [22,102].

Germline vs. Somatic Cell Editing

A fundamental ethical and regulatory distinction separates somatic cell editing—modifying non-reproductive tissues with individual-only effects, broadly accepted with appropriate safety evidence—from germline editing, which introduces heritable modifications with multigenerational consequences [55,114]. The international scientific community, including WHO and successive international summits, has advocated a moratorium on clinical germline editing pending resolution of safety and consent concerns [113,115]. This divide is reflected in regulatory frameworks worldwide, which impose substantially higher barriers for germline applications. Somatic editing continues to advance towards broader clinical acceptance as safety data accumulate [55,56].

Consent, Privacy, and Biobank Governance

iPSC biobanks linking extensive genetic, epigenetic, and phenotypic data create complex consent and privacy challenges. Donors frequently cannot anticipate future uses of their samples, including research involving gene editing or commercial product development. Genomic privacy risks extend to biological relatives. Best practices include dynamic consent platforms, robust anonymisation, independent data access committees, and transparent intellectual property and benefit-sharing policies [22,102]. Achieving population-level diversity in biobanks is an equity imperative to prevent genomic bias in derived therapies [14,22].

Global Regulatory Frameworks

The FDA, EMA, and PMDA have established distinct but conceptually aligned frameworks for advanced therapy medicinal products (ATMPs), requiring evidence of safety, efficacy, quality, and ethical compliance [25]. Harmonisation initiatives (ICH guidelines, WHO technical standards) seek to streamline international multicentre trial design and product approval pathways. Regulatory divergences in trial design requirements and long-term follow-up obligations persist, reflecting regional ethical priorities [88]. Regulatory science platforms and adaptive frameworks that keep pace with rapid technological evolution in gene editing are actively being developed [25].

Access, Equity, and Societal Implications

The high cost of personalised iPSC-derived therapies risks deepening existing healthcare inequalities unless addressed through deliberate policy intervention [49]. Equitable access requires cost-reduction through platform manufacturing (allogeneic strategies), inclusive reimbursement frameworks, and clinical trial designs representative of diverse populations [118,119]. Intellectual property regimes substantially influence pricing and technology transfer, with significant implications for global health equity. Multistakeholder collaboration among academia, industry, regulators, and civil society is essential to ensure that the regenerative medicine revolution delivers broad population benefit [14,49].

FUTURE PERSPECTIVES AND EMERGING INNOVATIONS

Next-Generation Editing: Epigenome Editors and RNA Editors

Beyond nuclease-based editing, epigenome editors fuse catalytically inactive Cas proteins to DNA methyltransferases, demethylases, or histone-modifying enzymes, enabling programmable gene expression regulation without permanent DNA sequence alteration [26,111]. These tools hold particular promise for epigenetically dysregulated diseases. RNA editors particularly ADAR-based platforms enabling adenosine-to-inosine (A-to-I) transitions in mRNA provide transient, reversible therapeutic modifications without genomic changes [112]. Key unresolved issues include delivery efficiency, cell-type specificity, and the ethical implications of reversible vs. permanent interventions in clinical contexts [27].

Artificial Intelligence and Machine Learning in iPSC Optimisation

Artificial intelligence (AI) and machine learning (ML) are transforming iPSC pipeline optimisation at multiple levels [12,120]: (i) ML models identify optimal factor combinations and culture conditions to maximise reprogramming efficiency while minimising genomic aberrations; (ii) predictive algorithms optimise sgRNA design for maximum on-target activity and minimum off-target risk [98,120]; (iii) AI-powered image analysis enables high-throughput, quantitative differentiation quality control; (iv) reinforcement learning algorithms dynamically adjust differentiation protocols in real time [12]. Ethical imperatives for AI deployment in clinical pipelines include model interpretability, training dataset diversity to prevent bias, and prospective validation in independent cohorts before clinical implementation [12,14].

Integration with Biomaterials, 3D Bioprinting, and Organ-on-Chip

Advanced bioengineering platforms are converging with iPSC biology to produce transplantable constructs of escalating physiological complexity [53,108]. Biomimetic matrices support iPSC survival, differentiation, and functional integration in

vivo. Three-dimensional bioprinting achieves spatially precise deposition of iPSC-derived cells within vascularised scaffolds suitable for cardiac, hepatic, and renal tissue engineering [54,108]. Microfluidic organ-on-chip devices incorporating multi-lineage iPSC derivatives under dynamic fluid flow recapitulate organ-level physiology, enabling toxicology and pharmacology studies of unprecedented translational relevance . Scalability, reproducibility across platforms, and regulatory pathway development for bioengineered constructs remain priority areas.

Multi-Omics and Single-Cell Characterisation

Integration of single-cell RNA sequencing, ATAC-seq, proteomics, and spatial transcriptomics provides unprecedented resolution of iPSC heterogeneity, lineage commitment trajectories, and aberrant subpopulations relevant to safety and efficacy [103,105]. These approaches are transforming iPSC quality control by enabling molecular-level identification of rare but functionally significant cell states. Computational multi-omics pipelines accelerate hypothesis generation and protocol refinement, directly supporting the development of more homogeneous, potent clinical iPSC products [27,34,104].

Combined Gene and Cell Therapy for Complex Diseases

The synergistic application of gene editing and iPSC technology is emerging as a curative strategy for diseases with complex polygenic architecture [44,118,119]. Multiplexed editing of polygenic risk variants in patient-derived iPSCs, followed by differentiation and transplantation, is being explored in Duchenne muscular dystrophy, inherited retinal degenerations, and haemoglobinopathies [87,119]. Early results suggest that combined approaches achieve more durable correction than either strategy alone. Full clinical realisation will require advances in multiplexed high-fidelity editing, immune tolerance strategies, and manufacturing scalability [73,87].

Personalised Precision Medicine

The convergence of patient-specific iPSCs, precise gene correction, multi-omics profiling, and AI-guided clinical decision support defines the future of precision regenerative medicine [12,27,120]. Patient-specific iPSC drug screening enables identification of optimal therapies with minimal trial-and-error. Comprehensive biobanks of diverse, characterised iPSC lines will underpin population-level research and enable rapid identification of matched allogeneic products [102]. Realisation of this vision requires simultaneous progress in cost reduction, regulatory harmonisation, manufacturing automation, and equitable access policies [14,49,101].

CONCLUSION

The convergence of iPSC technology and precision genome editing represents one of the most consequential developments in biomedical science of the past two decades. iPSCs provide an ethically acceptable, genetically matched, and scalable cell source, while CRISPR–Cas systems, base editors, and prime editors enable targeted genetic correction with rapidly improving precision and safety profiles [59,60,67,68,70,71,72]. Together, they have advanced from proof-of-concept experiments to early-phase clinical trials in retinal degeneration, haematological diseases, and Parkinson's disease [47,48,85,87].

Progress towards broader clinical implementation is conditioned on resolving persistent challenges: (i) tumorigenicity from residual pluripotent cells and genomic instability [94,95]; (ii) off-target editing and mosaicism in pluripotent cell populations [92,93]; (iii) immune recognition even in autologous contexts [89,90,91]; (iv) GMP manufacturing scalability and cost [99,101]; and (v) regulatory harmonisation across international jurisdictions [21,22,49,57]. The development of hypoimmunogenic universal iPSC lines, high-fidelity editing tools, and AI-integrated manufacturing pipelines represents the near-term frontier [49,51,57,120].

Realising the full potential of this technology demands not only continued biological and engineering innovation, but also robust ethical governance, inclusive clinical trial design, equitable access policies, and transparent public engagement [14,55,56,113,114]. The field stands at an inflection point at which foundational discoveries are transitioning to transformative clinical realities.

Authors' Contributions

Sujitha Mathivanan and Aravindan B contributed to the conceptualization and design of the study. Syed Mohamed Omar S supervised the work and provided critical revisions. Harish Babu, Sujan S, and Thilan D contributed to data collection, literature review, and manuscript preparation. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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