Journal of Cell Science & Therapy

Asrar, J Cell Sci Ther 2017, 8:4
DOI: 10.4172/2157-7013. 1000276

Review Article **Open Access**

Ips Progression a Decade Devoted

Hiba Asrar K*

Center of Craniofacial and Regenerative Biology, Dental institute, King's College London, England, UK

***Corresponding author**: Hiba Asrar K, Center of Craniofacial and Regenerative Biology, Dental institute, King's College London, England, UK, Tel: +91-702-220-6333; E-mail: hiba.asrar@kcl.ac.uk

Rec Date: Aug 22, 2017, **Acc Date**: Sep 08, 2017, **Pub Date**: Sep 12, 2017

Abstract

Yamanaka and Takahashi's astonishing discovery in 2006 revolutionized the world of stem cells. Simplicity and reproducibility of IPSC's cells opened doors for extensive therapeutic advancements and potential clinical trials particularly in the field of Regenerative medicine. In 2012, nobel prize was presented to both researchers for the breakthrough in reprogramming somatic cells to a pluripotent state with the expression of "Yamanakas cocktail" of OKSM quartet transcription factors: sex determining region y box2 (sox2), octamer binding transcription factor4 (oct4), krupple like factor (klf4) and myelocymatosis oncogene (c-myc). Rationale of iPS use is attributed to its unlimited cell source circumventing ethical hindrance. This comprehensive review will summarize mechanisms of IPS production and cell therapy applications. Moreover, appreciate the improvements and initiatives in iPS technology, scrutinize the scientific claim of indistinguishable resemblance of iPS and eSC, evaluate the constraints during iPS manipulation and analyze safety of these pluripotent cells in clinical scenarios.

Keywords: Reprogramming; Constraints; Worldwide initiatives; Induced pluripotent stem cells; Remodeling; Gene editing

Abbreviations

iPS: Induced Pluripotent Stem Cells; hiPSC: Human Induced Pluripotent Stem Cells; OKSM: oct4, sox2, c-myc; ESC: Embryonic Stem Cells; SOX: Sex Determining Region; Ybox2, KLF4: Krupple Like Factor 4; Cmyc: Myelocytomatosis Oncogene; GMP: Good Manufacturing Practice; ECM: Extracellular Matrix; RPE: Retinal Pigment Epithelium; HLA: Human Leukocyte Antigen; CiRA: Center for iPS Research and Application; CIBK: Clinical iPS Cell Bank of Kyoto; DMF: Drug Master File

Introduction

Genetic manipulation of somatic cells led to the discovery of iPS which could differentiate into all three primary germ layers (endoderm, ectoderm and mesoderm) [1,2]. IPS generated by the OSKM transcription factors appeared morphologically similar to eSC and exhibited similar gene expression profiles and selfrenewal characteristics [3-6,]. iPS can be identified by immunohistochemistry, RT-PCR and expression of immunological cell surface markers such as stage specific embryonic antigens (SSEA), Tra-1-60 and Tra-181 [1,7]

Benefits of iPS are due to cells being highly proliferative, biocompatible and universally accessible. iPS have aided in disease remodeling "disease-in-a-dish", drug screening and designing tailored therapies for individual patients [2,8,9]. For therapeutic purposes disease specific iPS of any lineage can be generated [1,9]. Since IPSC's are autogeneically available, both immunological compatibility and ethical constraints are bypassed [10].

Downside of iPS usage is retention of an epigenetic memory of their cell of origin which affects the differentiation process [9-12]. Somatic cell nuclear transfer and transcription factor based reprogramming methods yield induced pluripotent cells and simultaneously reset genomic methylation.

Discernible gene expression patterns and DNA methylation patterns were observed in a study on hiPSC derived from fibroblasts, adipose tissue and keratinocytes [13]. Low passage induced pluripotent stem cells derived by factor based reprograming harbor low passage residual DNA methylation signatures characteristic of their somatic tissue of origin, which promotes differentiation along lineages related to the cell of origin, while restricting alternative cell fates. Such an "epigenetic memory" of the donor tissue could be reset by differentiation and serial reprogramming, or by treatment of iPSC with chromatin modifying drugs. Another experiment corroborated this conclusion that in a given iPS clone DNA methylation patterns could indicate the origin of the former phenotype [2,14]. Furthermore, undifferentiated iPS remaining at site of interest expand into a teratoma [2,9,15]. Teratoma has become the gold standard assay to define bona fide induced pluripotent stem cells capable of generating tumoral disorganized structures containing tissues representing the three germ layers. Despite the importance of this assay to prove pluripotency the underlying mechanism of teratoma transition to teratocarcinoma *in vivo* is not yet understood. There is no standard procedure to test/ characterize teratomas to control puripotency. This needs to be further tested as characterizing teratomas is crucial in a therapeutic patient based iPS treatment.

Literature Review

Transcriptional regulators

Commencing with a pool of 24 pluripotency associated candidate genes Yamanaka identified minimally required core set of 4 genes: the OSKM cocktail to generate iPS from mouse fibroblasts through retroviral transduction [6,8,10]. However, overexpression of these regulators exhibited neoplastic potential which has been validated in various researches. Aberrant expression of oct4 is linked to hepatocellular carcinoma and murine epithelial dysplasia [10,16,17]. Sox2 overexpression has been reported in serrated polyps, mucinous colon carcinoma, human squamous cell lung tumours [18,19]. Elevated expressions of klf4 is linked to breast cancer and the cmyc oncogene has strong correlation with various human cancers [19]. This incited search for less carcinogenic gene set sufficient for reprogramming. Substitution of klf-4 and cmyc was necessary due to extensive malignant potential. Yamanakas group also identified that myc is unnecessary for generation of iPSC's [20]. Essrb, orphan nuclear receptor, found in ES cells is reported to replace klf-4 [10,21]. P53 blockade also allowed reprogramming in the absence of klf-4. Yu et al. utilized Homeobox protein NANOG and RNA binding protein LIN-28 along with oct4 and sox2 to generate IPSC's from human somatic cells [15]. NANOG doesn't trigger reprogramming but is essential for acquiring state of full pluripotency [22].

Reprogramming enhancers

Frequency of iPS production is typically well below 1% imposing limitations to mechanistic studies and clinical translation. Myc family including L-myc and N-myc enhance reprogramming but is disregarded due to its oncogenetic nature. Use of Glis-1, NANOG and Sall-4 have shown to promote direct reprogramming of somatic cells [23,24]. NANOG augments the reprogramming efficiency which is quantified by NANOG positive colonies in a cell culture [21].

Furthermore, small chemical compounds which either affect chromatin modifications or transduction pathways enhance reprogramming or replace transcription regulators include i) BIX-01294, histone lysine methyltransferase inhibitor [25]. ii) TSA and SAHA, deacetylase inhibitors [26]. iii) AZA and RG108, DNA methyltransferase inhibitors [26,27] iv) BayK8644, L-type Calcium channel agonist [25] v) Dexamethasone, steroid glucocorticoid [26]. vi) PD0325901 and CHIR99021, inhibitors of the MEK and GSK respectively [28,29]. vii) A-83-01, TGF β inhibitor [30].

Mechanism of iPS Generation

Comprises of integrating vectors, non-integrating vectors, excisable vectors and vector-free systems. Conventional viral integrating systems (retroviral transduction) were commonly employed to generate iPS from mouse fibroblasts or human somatic cells [6,7,10,31]. Retroviral integration into host genome requires high division cycles which is observed in a narrow spectrum of cells [10,32]. Retroviral vectors constitutively express transgenes under the control of promoter localized in long terminal repeat (LTR) which is terminated once the somatic cell transforms into its pluripotent state [8,10,32,33]. Partially reprogrammed cells show increased levels of exogenous pluripotent transgenes found in the retrovirus though fully reprogrammed cells exhibit constant retroviral attenuation [34]. Decrease in exogenous expression of oct4, sox2, klf4 and c-myc is reported in the retroviral construct as cell approaches pluripotency correlating with a decline in reprogramming efficiency. Due to above mentioned pitfalls, lentiviruses have been utilized for iPS generation but they lead to a differentiation blockade since they are less efficiently silenced in pluripotent cells than retroviruses [9,13]. Doxycycline-inducible lentiviral vectors have aided in selection of fully reprogrammed cells since drug withdrawal halts proliferation of exogenous factor dependent cells [33,35]. Nevertheless, issues of insertional mutagenesis and leaky transgene expressions due to retroviruses which precipitate harmful effects if considered in a therapeutic *in vivo* setting led to exploration of non-integrating (viral-free) systems for iPS generation [6,8,10].

Non-integrating systems

Viral integrating methods of iPS generation are unsuitable in a clinical setting. With application of adenoviruses, sendai viruses, expression plasmids, episomal vectors, liposomal magnetofection and minicircle vectors various virus free iPSC lines have been formed by passing the risk of insertional mutagenesis. Although non-integrating systems are safer than conventional use of viruses for delivery of reprogramming factors this system has limited application due to low grade efficiency [36-42].

Excisable vectors

Cre-loxP technology: The Cre-loxP system provides an optimized protocol for generating transgene free human induced pluripotent cells with retroviral transduction of a single vector consisting of the coding sequence of transcriptional regulators linked via picornaviral 2A plasmids [6,9,43]. Once reprogramming is achieved, this cassette is excised by mRNA transfection with Cre-recombinase. Ease and efficiency of CreloxP system with minimal genomic modification facilitates the reprogramming process of transgene free iPS. Cremediated excision of the provirus eliminates all transgenes but lox-P remnants are found at each integration site, possibly distressing the neighboring genes [44].

Piggy bac transposons: This system requires only inverted terminal repeats flanking transgene and temporary enzymatic expression which catalyzes introduction and excision of mobile genetic elements from the host genome [9,45]. Benefitting from the natural propensity of the piggy-bac systems seamless excision, it is validated that each PG insertion can be removed from fully reprogrammed iPS cell lines.

Vector free systems

Reprogramming proteins: Genomic manipulation is unavoidable in DNA-based reprogramming, so alternate systems suggested application of proteins as delivery tools for the generation of transgene-free iPS [46]. By delivering Yamanakas cocktail of transcriptional regulators fused with a cell-penetrating peptide (CPP), Kim et al. achieved efficient iPS from human fibroblasts [47]. A study on the midbrain dopaminergic (DA) neurons differentiated mouse embryonic stem cells and protein based iPS, resulting in a stable DA neuron-specific marker expression observed in the protein based iPS [48]. An unsuccessful attempt at outgrowing stem cell like colonies into iPS by reprograming proteins concluded that partial reprogramming was a common response to protein-based reprogramming technique [49]. In contrast use of self-penetrating proteins and truncated proteins seems an attractive alternative [50]. It's unclear whether protein transduction can be used for adult cells [51]. Also, applicability of protein based iPS is restricted due to technical difficulties in production and reprogramming inefficiency.

mRNA: Warren et al. exploited synthetic mRNAs for reprogramming which resulted in higher efficiencies than the established protocol [52]. However, increased workload and the need of a tissue incubator with 02 controls are drawbacks of this system [53]. Rosa et al. resolved the issues of interferon-mediated innate immune response and poor protein yield by modifying the ribonucleotides used [54].

MicroRNAs have also shown to control pluripotency, escalating the reprogramming process [6]. MiRNA 302 is of particular importance and has been reported in various studies [55-58]. Mouse and human miR302/367 iPS cells have exhibited similar characteristics to Oct4/Sox2/Klf4/Myc-iPS cells, including pluripotency marker expression, teratoma formation, and, for mouse cells, chimera contribution and germline contribution [59]. MiR-155 has been identified as a key player for the *in vitro* differentiation of iPSC toward hematopoietic progenitors [60].

These improvements in reprogramming system have helped achieve stable stem cell lines with high efficiencies. However, their potential in human iPS is still under research and careful monitoring of proteins and mRNA interaction with each transcription factor in adult iPS still needs to be evaluated.

Optimized Cell Cultures: Feed Less, Discover More

Since the revolutionary discovery began by the stem cell pioneer numerous methods were introduced and routinely undertaken to manufacture stem cell lines. However optimized conditions to achieve ideal iPS lines for human therapies are yet to be designed. Despite the tremendous potential of iPS technology, several issues with iPS manufacturing have been identified. Feeder cells for iPSC maintenance in culture have impeded the translation of this innovative technology into clinical therapy because of their xenogeneic constituents. To overcome these ethical barriers, use of synthetic coatings and bioreactors that support proliferation of iPS in xenogeneic-free environment have been suggested [2,61]. Generation of iPS from adult adipose stem cells in feeder free environment is made possible by switching to mTeSR1 culture medium [62]. Application of a cost effective, high quality E8 medium has also supported higher reprogramming efficiencies for viral and episomal approaches. Freshly isolated fibroblasts in E8-based fibroblast medium on vitronectin have consistently reprogrammed at a higher efficiency (60 to ~1,000 iPS cell colonies per 106 transfected fibroblasts) than the commercially available established stem cell lines [63]. E8 medium supporting higher reprogramming efficiencies for both viral and episomal approaches has been validated [64].

Reprogramming events require mechanical isolation of iPS for colony expansion with major contamination by unreprogrammed somatic cells. Colony picking though prone to bacterial contamination has been routinely used to enrich stem cells but becomes impractical when numerous lines are considered. Dual advantage of E8 medium and the EDTA dissociation method has unveiled a faster expansion of large cell volume cultures. Furthermore, EDTA dissociation has enabled enrichment of potential iPSC's in an overcrowded reprogramming culture in which otherwise a routine secondary passaging is needed [64,65].

Sigma aldreich's PluriSTEM and human ES/iPS medium is a specially formulated defined media utilizing activin-A, tgfB1 and bFGF which aids in promoting stem cell self-renewal with increased cell viability and enhanced cell proliferation in single cell passaging. This feeder-free, serum free culture system requires less cell feeding lowering down the total cost and culture period [66].

iPS lines need to be strictly generated under GMP (Good manufacturing practice) system protocols if considered for use in a clinical setting. GMP system ensures products are continuously produced and controlled according to quality standards. Thus, minimizes the risks related to pharmaceutical production that cannot be eliminated through testing the final product. To date, no fully cGMP-compliant cell line has been reported where the entire manufacturing process, from tissue sourcing to cell expansion and banking processes as well as documentation, raw materials, staff training, cell therapy facility, and quality control (QC) testing is validated [67].

Recently a biotech company, Lonza put forth a novel, robust and reproducible GMP compliant reprogramming guide which could bypass quality control impediments for efficient iPS production. Lonzas Nucleofector™ technology for efficient, non-viral delivery of reprogramming factors to the L7 Culture System for feeder-free, xeno-free culture of human iPS, is currently being utilized by leading scientists all over the world [68].

Widespread iPS Applications

Disease remodeling

Researchers worldwide are struggling to understand the mechanism of action of various debilitating diseases and remedies to fight them. Previously experimental animal models have been exploited to understand the underlying mechanism of various human pathologies. However marked differences in biochemical variation, anatomical complexity and physiological responses limit the evaluation of new therapies in humans. Mice have become the gold standard for research but simply put mice aren't humans. Stem cells have been employed to recapitulate pathologies *in vitro* commonly referred to as "disease-in-a-dish" [69]. The iPS technology can improve our interpretation of the combined effect of genetic malfunctioning and environmental changes on the severity of diseases. Power of iPS technology lies in understanding mono and polygeneic pathologies through disease remodeling [70]. iPS allows a deeper insight by capturing the disease in its early stage and monitoring the cellular events throughout disease development [9]. Moreover, patient specific iPS can help tailor individualized therapies.

Numerous iPSC lines have been generated so far from pathological diseases e.g., Degenerative illnesses like parkinsons disease [71], alzeihmers [72] and amyotrophic lateral sclerosis [73]. Blood Disorders such as sickle cell anemia [74], fanconis anemia [75], hemophilia A [76] and β-thalassemia [77]. Genetic diseases: Downs syndrome [78], familial dysautonomia [79] and Huntington's disorder [80].

3D organoids

3D cell clusters depend on extracellular matrix in culture for self-organization, resembling endogenous tissue architecture and duplicating organ functionality [81]. These 3D functional organoids have improvised our understanding of early human development and improved drug screening methodologies organoids including mini gut, mini heart (repopulating decellularized mice heart through iPS), mini brain (developing cortex, central telencephalon and choroid plexus), small lungs, mini eyes (optic vesicle like structures) have been formulated for disease remodeling purposes [82-89].

Lack of vascularity, absence of native interactive microenvironment, limited growth potential due to lack of constant nutrients, rigid nature of ECM, absence of immune cells limiting the observation of inflammatory response to drugs are associated pitfalls of these cell clusters [81,90].

Citation: Asrar KH (2017) Ips Progression a Decade Devoted . J Cell Sci Ther 8: 276. doi:10.4172/2157-7013. 1000276

Spinning bioreactors and shaking culture platforms have been suggested to provide better nutrient supply and improve the growth of organoids [91]. Co-culturing with endothelial cells has aided vasculogenesis in organoids [92]. *In vitro* Organoids are the biggest technological breakthrough that might in future overcome the dire urgency of organ transplants for critically ill patients.

Gene editing implication in disease remodeling

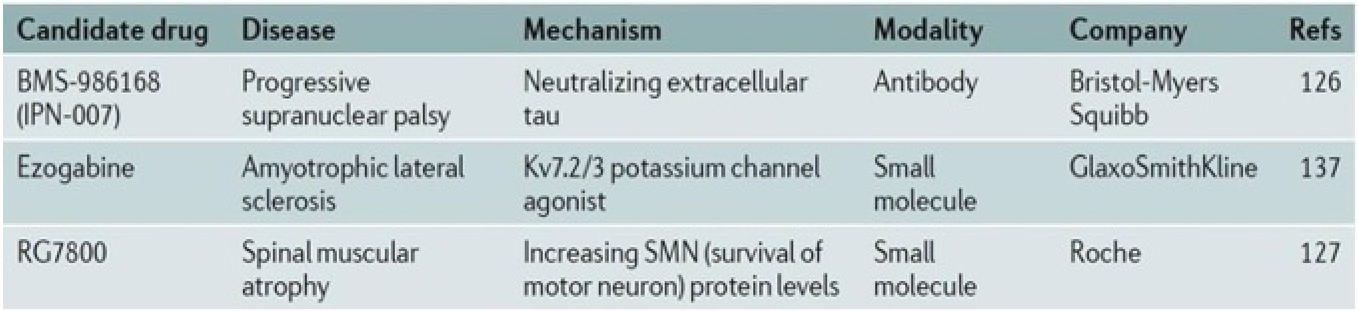
Discovery of site specific nucleases Zinc finger nuclease (ZFN), transcription activator-like effector nucleases (TALENs) along with CRISPR-Cas9 system has enhanced gene editing efficiency in iPSCs by rupturing the DNA double-strand at the site of gene modification enabling knock to in/knock out of one or more genes [93]. High system efficiency, ease of use and cheaper costs than predecessors has made CRISPR-CAS a popular editing tool. Researchers can introduce specific disease-causing alleles into wildtype iPSC's with the assistance of CRISPR-CAS and eliminate genetic defects in patient iPS for isogenic controls in disease remodeling [94]. Successful progression of iPS and CRISPR-CAS amalgamation is justified by stable iPS proliferation and wholesome numbers of modified clones that can be harvested after genetic modification in culture. Immunodeficiency centromeric region instability, facial anomalies syndrome (ICF) and pancreatic cancer models have been generated using the CRISPR-CAS editing system [95,96]. Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs has been possible using CRISPR/Cas9 system [97,98].

Drug screening platform

Drug testing is an enormous challenge faced by pharmaceutical companies with a 90% failure due to unpredictable adverse effects unidentified in the preclinical phase [99]. $2billion per drug is an estimated cost of the entire process of drug screening. The average time to introduce new drug to the market, from the start of clinical testing to FDA approval, is 8.5 years, and the clinical success rate is 21.5% [100]. Researchers can take advantage of recapitulating human pathologies *in vitro* to evaluate cellular responses to different chemicals. Moreover, any effective drug against hiPSC can be compared with various cell lines of patients with similar pathologies to validate results. iPS are a powerful platform for drug screening to understand cellular responses to neurons, cardiocytes and hepatocytes which are most susceptible to drug toxicity [101].

Drug development employs three main strategies after establishing disease-specific iPS: high-throughput screening (HTS) of drugs, candidate drug approach or patient-specific therapy [102]. In HTS, extensive compounds are tested on differentiated cells, followed by phenotypic analysis. Making use of large bank of compounds can help identify therapies *in vitro* by this approach which is faster and economically less burdening than conventional drug testing. By contrast, both candidate drug approach and patient-specific therapy use small numbers of potential drugs to attenuate the disease. These approaches are useful when the disease mechanism is known and potential therapies are available.

However, substantial safety assays are required before drugs discovered by these methods can be prescribed (Figure 1).



Candidate drug	Disease	Mechanism	Modality	Company	Refs
BMS-986168 (IPN-007)	Progressive supranuclear palsy	Neutralizing extracellular tau	Antibody	Bristol-Myers Squibb	126
Ezogabine	Amyotrophic lateral sclerosis	Kv7.2/3 potassium channel agonist	Small molecule	GlaxoSmithKline	137
RG7800	Spinal muscular atrophy	Increasing SMN (survival of motor neuron) protein levels	Small molecule	Roche	127

Figure 1: Ongoing drug clinical trials. Reproduced from Nature [102].

Worldwide Banking Initiatives

Tedious large-scale efforts nationally and internationally are taking place to build an iPS reservoir for therapeutic purposes. Californian institute of regenerative medicine in San Francisco initiated cell repository to bank iPS lines for research on childhood degenerative illness, eye blindness and therapeutic purposes. The first 285 lines were distributed by CIRM in September 2015. In 2013, CIRM awarded Cellular Dynamics International Inc. (CDI) $16 million to create iPS lines for each of 3,000 healthy and diseased volunteer donors across 11 common diseases and disorders to be made available through the CIRM hiPSC Repository. CDI will expand each iPS line to generate sufficient aliquots of high quality cryopreserved cells for distribution via CIRM [103].

IMI is the world's biggest public-private partnership (PPP) in the life sciences. The (EBiSC) European Bank for induced pluripotent Stem Cells was a two-year project operated by IMI. EBiSC's goal is to establish a centralized facility where academics, biotech companies, and big pharmaceutical companies can store and access high-quality, well-characterized iPSC's covering a range of disease areas as well as cells from healthy donors. For its part, the bank will provide standardized protocols for the storage, retrieval, culture, and differentiation into different cells types, plus a searchable catalogue where cells can be requested based on specific characteristics or disease areas [104].

Another IMI project, StemBANCC (Stem cells for biological assays of novel drugs and predictive toxicology) began in October 2012 and aims to generate 1500 iPS lines from 500 people by end of September 2017, characterize them in terms of their genetic makeup and metabolic profiles. Moreover, make them available to researchers to study various diseases including dementia and diabetes mellitus, allow drug testing and efficacy for safer drug development. All cell lines will undergo rigorous quality checks [105].

In a unique collaboration, both IMI's EBiSC and Stem-BANCC team members incited the search to improve understanding of a rare pathology called inherited erythromelalgia (IEM). IEM is a debilitating condition without adequate available medical treatment. It involves patients suffering from extreme pain symptoms made worse by heat. Underlying etiology is a genetic mutation leading to over activity of the sodium channels in patients' sensory neurons. Five subjects were tested with an experimental drug targeting blockade of sodium channels to reduce the pain experienced by IEM patients. Study concluded that drug reduced pain upon exposure to heat in most sufferers. Same patients allowed scientists to transform their blood cells into iPS, which were deposited in the EBiSC project stem cell bank. Next, the StemBANCC scientists transformed stem cells into sensory neurons and evaluated their characteristics. Prior to drug therapy, neurons exhibited hyper-excitability and increased response to heat: increased severity of the disease led to increased number of hyperactive neurons. Post treatment, hyper-excitability of tested neurons diminished, mimicking the drug effect that occurred in patients. Rarely is it possible for drug developers to scrutinize drug responses of the relevant cells from individual patients involved in clinical trials of the same experimental drug. These results illustrate the power of stem cell technology to transform drug development, formulating therapies tailored to individual patients. This bridges the translational gap between preclinical models and clinical evaluation [106].

In UK, human induced pluripotent stem cell initiative (hiPSCi) combines diverse constituents in genomics, proteomics, cell biology and clinical genetics to form a national high content iPS resource for interpreting cellular genetics. HiPSCi's 2017 target is to generate over 500 iPS lines from healthy volunteers and several hundred lines from individuals with genetic disease to discover the impact of genomic variation on cell phenotype and identify new disease mechanisms.

Kyoto University's Center for iPS Research and application (CiRA) in japan has also invested heavily in developing an iPSC bank. Just 75 iPS lines will cover 80% of the Japanese population by 2020 says Yamanaka, the leading director of (CiRA), Kyoto University [107]. Prior to clinical therapy, donor iPS can be matched to patients through human leukocyte antigens haplotyping based on cell surface proteins regulating immune responses. Yamanaka states that matched cells will obviate the need of large doses of immunosuppressive drugs causing cell rejection, the main hurdle faced during embryonic stem cell therapy for transplantation.

HLA iPS banks will ensure safer therapies since this hybrid model will select optimally matched cells producing closer to ideal graft materials, minimizing the risk of immune responses [108]. In japan, creating an HLA matched iPS bank was faster, cheaper and less tedious as few as 50 stem cell lines could cater 90% of the Japanese populations due to narrow genetic diversity [109,110]. In contrast, an iPSC bank from 150 selected homozygous HLA-typed volunteers could match 93% of the United Kingdom population [108]. Although initial efforts seem daunting with increased startup costs, HLA matched iPS cells could reduce use of lifelong immunosuppression and improve patients' quality of life.

An international collaboration in the form of GAIT (global alliance for iPS based therapy) aims to formulate a GMP iPS-haplobank in harmony with mutually recognized iPS banks for maximizing utilization and offering hope in the future to facilitate iPS therapy globally [111-113].

These dedicated efforts for developing and improving wide spread availability of iPSC globally will help in developing innovative therapies and provide limitless opportunities for scientists to scrutinize underlying molecular mechanisms of genetic, epigenetic and environmental pathologies (Figure 2).

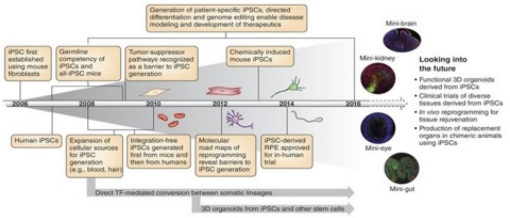
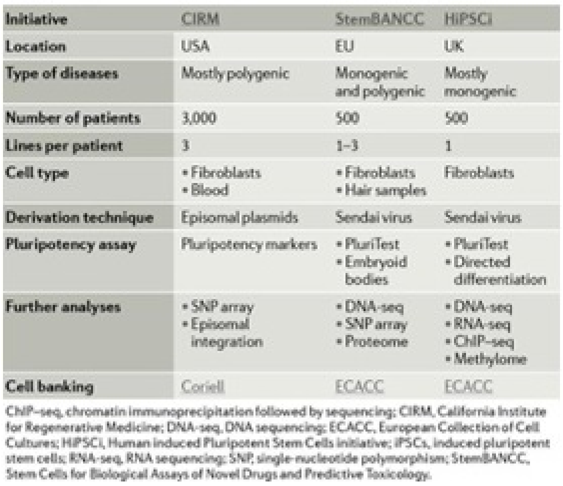


Figure 2: A time line of iPS progression over the decade. Reproduced from Nature Protocol [114].



Initiative	CIRM	StemBANCC	HiPSCi
Location	USA	EU	UK
Type of diseases	Mostly polygenic	Monogenic and polygenic	Mostly monogenic
Number of patients	3,000	500	500
Lines per patient	3	1–3	1
Cell type	• Fibroblasts • Blood	• Fibroblasts • Hair samples	Fibroblasts
Derivation technique	Episomal plasmids	Sendai virus	Sendai virus
Pluripotency assay	Pluripotency markers	• PluriTest • Embryoid bodies	• PluriTest • Directed differentiation
Further analyses	• SNP array • Episomal integration	• DNA-seq • SNP array • Proteome	• DNA-seq • RNA-seq • ChIP–seq • Methylome
Cell banking	Coriell	ECACC	ECACC

ChIP–seq, chromatin immunoprecipitation followed by sequencing; CIRM, California Institute for Regenerative Medicine; DNA-seq, DNA sequencing; ECACC, European Collection of Cell Cultures; HiPSCi, Human induced Pluripotent Stem Cells initiative; iPSCs, induced pluripotent stem cells; RNA-seq, RNA sequencing; SNP, single-nucleotide polymorphism; StemBANCC, Stem Cells for Biological Assays of Novel Drugs and Predictive Toxicology.

Figure 3: Various IPSC banking initiatives [102].

Ips and Esc: Strikingly Similar Yet Remarkably Different

Various researches have undergone to analyze iPS and eSC lines. Great similarities emerged in histone modications and genome expressions of these two cell lines [9,115]. Comparative transcriptome analyses using microarray indicate that hESCs and hiPSCs are highly alike on global scale, with gene expression patterns clustering together [116]. Gene expression analysis study in mice revealed that majority iPS formed low grade chimeras but only cells with an ESC equivalent expression of the dlk1-dio3 locus exhibited high grade chimeras (the key indicator of pluripotency) and generated all iPS mice after blastocyst injection [9,117]. Despite the incomparable origin and generation methods, comparison of these cell lines is exceptional. This could be attributed to the man-made nature of both cell lines. Cells from the inner cell mass are to some degree manipulated under culture conditions to generate ESC which are otherwise non- existent in physiological conditions [118]. However, conflicting studies using targeted bisulfite sequencing of three human ESC clones and four iPS lines showed extensive differences in DNA methylation patterns [118,119]. Expression microarrays have been used to report various genes being differentially expressed in both stem cell lines [120]. IPS and ESC debate is still on but the important concern should be focusing on the functional characteristics of these cells *in vivo* as they can be categorized as part of the same big experimental approach i.e. the therapeutic potential of these cell lines in regenerative medicine in the future (Figure 3).

Reprogramming Constraints: Fighting the "M Triad" (Money, Mutations and Manufacturing)

The biggest hurdle in generating commercially available iPS is extensive culture period and heavy burdening investments in the entire process. A standardized protocol for cost effective production of iPS has to be carefully formulated. Complexity of iPS application in field of regenerative medicine has made manufacturing lengthy and tedious. Cutting down costs is a problematic task which needs identification of important factors which can be modified efficiently and those which are absolutely necessary for iPSC fabrication. Mahendra and Anthony suggested potential cost-effective strategies which can reduce financial investments in iPS technology substantially.

- Defining iPS as an input material since it's not the end product in iPS therapy and should be regulated as other manufacturing products.
- Fabricating stem cell lines under Good Manufacturing Practice Protocols (already discussed).
- Developing a Drug Master File (DMF) accessible to investigators that will dispense the need of repeated testing and validation of material suppliers and assays. This would require companies to share data or permit referencing to a DMF benefitting all involved parties and cutting down costs effectively.
- Provide kits for iPS generation.
- Use modular manufacturing protocol and manufacture products from same master cell bank.
- Calibrating material and developing comparability assays by consensus.
- Widen approved use based on function of particular disease.
- Implement haplobanking.
- Simplification of the approval process already approved in other areas.
- De-risk development [111].

Other pluripotency pitfalls

Various somatic mutations, copy number variations are associated with iPS lines [121-123]. These potential complications are a hurdle in iPS therapeutic applications. Extensive genetic screening would be required to ensure safety of these cells in clinical scenarios. Genome sequencing of hiPS cell lines will help screen out cell lines with increased mutational load or mutations implicated in development, disease or tumorigenesis. Thorough studies on mutation rates and distributions during *in vitro* culturing and reprogramming of hiPS cells will be vital to aid in establishment of clinical safety standards for genomic integrity.

Discussion and Conclusion

Prior to commencing the 1st ever human clinical trial with iPSC, rigorous safety trials (in mouse and monkeys) took place to validate application of retinal epithelial sheets without subsequent tumorigenesis [124,125]. The swift journey from skin to eyes started in September 2014, when ophthalmologist Masayo Takahashi collaborated with the stem cell pioneer Shinya Yamanaka and transplanted RPE (retinal pigment epithelium) sheets generated from patients iPS cells into the right eye of the female suffering from age related macular degeneration. Takahashi had claimed to stop the degeneration process and the patient experienced an improvement in vision [126]. The trial was halted due to unexplained mutations observed by Yamanaka in the transplanted RPE and the patients' iPS [126,127]. This trial proved that several safety assays need to be established to validate preclinical studies before they are safe to begin future clinical trials. Each reprogramming stage needs to be carefully validated and monitored for future approval of any clinical trial with iPS.

Despite the pitfalls of this system iPS holds great potential for further improvement in advancing the field of regenerative medicine. If gmp compliant manufacturing obstacles, financial hurdles, reprogramming inefficiencies and genetic instabilities are overcome iPS could be tailored for individualized therapeutic products. This road to progress has been challenging yet still very promising. This world has witnessed great revolutionary advancements owing to the massive potential of these cell lines and will continue to do so in the near future.

References

1. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861-872.
2. Malhotra N (2016) Induced pluripotent stem (iPS) cells in Dentistry: A review. Int J Stem Cells 9: 176-185.
3. Puri MC, Nagy A (2012) Concise review: Embryonic stem cells versus induced pluripotent stem cells: The game is on. Stem Cells 30: 10-14.
4. Amabile G, Meissner A (2009) Induced pluripotent stem cells: Current progress and potential for regenerative medicine. Trends Mol Med 15: 59-68.
5. Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448: 313-317.
6. Yi-ye Z, Fanyi Z (2013) Integration-free methods for generating induced pluripotent stem cells. Genomics, Proteomics Bioinformatics 11: 284-287.
7. Deng XY, Wang H, Wang T (2015) Non-viral methods for generating integration-free, induced pluripotent stem cells. Curr Stem Cell Res Ther 10: 153-158.
8. Zaehres H, Kim JB, Schöler HR (2010) Induced pluripotent stem cells. Methods Enzymol 476: 309-25.
9. Matthias S, Konrad H (2010) Induced pluripotency: History, mechanisms, and applications. Genes Dev 24: 2239-2263.
10. Medvedev SP, Shevchenko AI, Zakian SM (2010) Induced pluripotent stem cells: Problems and advantages when applying them in regenerative medicine. Acta Naturae 2: 18-28.
11. Kim K, Doi A, Wen B, Ng K, Zhao R, et al. (2010) Epigenetic memory in induced pluripotent stem cells. Nature 467:285-290.

12. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, et al. (2010) Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat Biotechnol 28: 848-855.
13. Marchetto MC, Yeo GW, Kainohana O, Marsala M, Gage FH, et al. (2009) Transcriptional signature and memory retention of human-induced pluripotent stem cells. PLoS ONE 4: e7076.
14. Kim K, Doi A, Wen B, Ng K, Zhao R, et al. (2010) Epigenetic memory in induced pluripotent stem cells. Nature 467: 285-290.
15. Yu J, Vodyanik MA, Smuga OK, Antosiewicz BJ, Frane JL, et al. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917-1920.
16. Xu G, Qi F, Zhang J (2016) Overexpression of OCT4 contributes to progression of hepatocellular carcinoma. Tumor Biol 37: 4649-4654.
17. Hochedlinger K, Yamada Y, Beard C, Jaenisch R (2005) Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell 121: 465-477.
18. Lu Y, Futtner C, Rock JR, Xu X, Whitworth W, et al. (2010) Evidence that SOX2 overexpression Is oncogenic in the lung. PLoS ONE 5: e11022.
19. Park ET, Gum JR, Kakar S, Kwon SW, Deng G, et al. (2008) Aberrant expression of SOX2 upregulates MUC5AC gastric foveolar mucin in mucinous cancers of the colorectum and related lesions. Int J Cancer 122: 1253-1260.
20. Masato N, Michiyo K, Koji T, Kazutoshi, Tomoko I, et al. (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 26: 101-106.
21. Schmidt R, Plath K (2012) The roles of the reprogramming factors Oct4, Sox2 and Klf4 in resetting the somatic cell epigenome during induced pluripotent stem cell generation. Genome Biol 13: 251.
22. Monya B (2009) What does Nanog do? Nature reports stem cells. 132.
23. Tsubooka N, Ichisaka T, Okita K, Takahashi K, Nakagawa M, et al. (2009) Roles of Sall4 in the generation of pluripotent stem cells from blastocysts and fibroblasts. Genes to Cells 14: 683-694.
24. Maekawa M, Yamaguchi K, Nakamura T, Shibukawa R, Kodanaka I, et al. (2011) Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. Nature 474: 225-229.
25. Shi Y, Desponts C, Do JT, Hahm HS, Scholer HR, et al. (2008) Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell 3: 568- 574.
26. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, et al. (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 26: 795- 797.
27. Mikkelsen TS, Hanna J, Zhang X, Ku M, Wernig M, et al. (2008) Dissecting direct reprogramming through integrative genomic analysis. Nature 454: 49-55.
28. Shi Y, Do JT, Desponts C, Hahm HS, Scholer HR, et al. (2008) A combined chemical and genetic approach for the generation of induced pluripotent stem cells. Cell Stem Cell 2: 525-528.
29. Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, et al. (2008) Promotion of reprogramming to ground state pluripotency by signal inhibition. PLoS Biol 6: e253.
30. Li W, Wei W, Zhu S, Zhu J, Shi Y, et al. (2009) Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell 4: 16-19.
31. Malik N, Rao MS (2013) A review of the methods for human iPSC derivation. Methods in molecular biology (Clifton, NJ) 997: 23-33.
32. Shao L, Wu WS (2010) Gene-delivery systems for iPS cell generation. Expert Opin Biol Ther 10: 231-242.
33. Stadtfeld M, Maherali N, Hochedlinger K (2008) Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. Cell Stem Cell 2: 230-240.
34. Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448: 313-317.
35. Brambrink T, Foreman R, Welstead GG, Lengner CJ, Wernig M, et al. (2008) Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. Cell Stem Cell 2: 151-159.
36. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K (2008) Induced pluripotent stem cells generated without viral integration. Science 322: 945-949.
37. Zhou W, Freed CR (2009) Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. Stem Cells 27: 2667-2674.
38. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M (2009) Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 85: 348-362.
39. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S (2008) Generation of mouse induced pluripotent stem cells without viral vectors. Science 322: 949-953.
40. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, et al. (2009) Human induced pluripotent stem cells free of vector and transgene sequences. Science 324: 797-801.
41. Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, et al. (2011) A more efficient method to generate integration-free human iPS cells. Nat Methods 8: 409-412.
42. Jia F, Wilson KD, Sun N, Gupta DM, Huang M, et al. (2010) A nonviral minicircle vector for deriving human iPS cells. Nat Methods 7: 197-199.
43. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, et al. (2009) Virus free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458:771-775.
44. Loh YH, Yang JC, De Los Angeles A (2012) Excision of a viral reprogramming cassette by delivery of synthetic Cre mRNA. Curr Protoc Stem Cell Biol.
45. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, et al. (2009) piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458: 766-770.
46. Hu K (2014) Vectorology and factor delivery in induced pluripotent stem cell reprogramming. stem cells and development 23: 1301-1315.
47. Kim D, Kim CH, Moon JI (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 4: 472-476.
48. Kwon YW, Chung YJ, Kim J (2014) Comparative study of efficacy of dopaminergic neuron differentiation between embryonic stem cell and protein-based induced pluripotent stem cell. Johnson R (ed). PLoS ONE 9: e85736.
49. Lim J, Kim J, Kang J, Jo D (2014) Partial somatic to stem cell transformations induced by cell-permeable reprogramming factors. Scientific Reports 4: 4361.
50. Wang X, Jauch R (2014) OCT4: A penetrant pluripotency inducer. Cell Regeneration 3: 6.
51. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, et al. (2008) Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451:141-146.
52. Warren L, Manos PD, Ahfeldt T (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells using synthetic modified mRNA. Cell stem cell 7: 618-630.
53. Schlaeger TM, Daheron L, Brickler TR (2015) A comparison of non-integrating reprogramming methods. Nature Biotechnology 33: 58-63.
54. Rosa A, Brivanlou AH (2010) Synthetic mRNAs: Powerful tools for reprogramming and differentiation of human cells. Cell Stem Cell 7: 549-550.
55. Subramanyam D, Lamouille S, Judson RL (2011) Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. Nat Biotechnol 29: 443-448.
56. Hu S, Wilson KD, Ghosh Z (2013) MicroRNA-302 increases reprogramming efficiency via repression of NR2F2. Stem Cells 31: 259-268.
57. Anokye DF, Trivedi CM, Juhr D (2011) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8: 376-388.

58. Zhang Z, Xiang D, Heriyanto F (2013) Dissecting the roles of miR-302/367 cluster in cellular reprogramming using TALE-based repressor and TALEN. Stem Cell Reports1: 218-225.
59. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, et al. (2011) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8: 376-388.
60. Vitaloni M, Pulecio J, Bilic J, Kuebler B, Laricchia-Robbio L, et al. (2014) MicroRNAs contribute to induced pluripotent stem cell somatic donor memory. J Biol Chem 289: 2084-2098.
61. Villa-Diaz LG, Ross AM, Lahann J, Krebsbach PH (2013) Concise review: The evolution of human pluripotent stem cell culture: From feeder cells to synthetic coatings. Stem Cells 31:1-7.
62. Sun N, Panetta NJ, Gupta DM (2009) Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. Proc Natl Acad Sci USA 106: 15720-15725.
63. Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, et al. (2011) Chemically defined conditions for human iPS cell derivation and culture. Nat Methods 8: 424-429.
64. Beers J, Gulbranson DR, George N, Siniscalchi LI, Jones J, et al. (2012) Passaging and colony expansion of human pluripotent stem cells by enzyme-free dissociation in chemically defined culture conditions. Nat Protoc 7: 2029-2040.
65. Liu W, Chen G (2014) Cryopreservation of human pluripotent stem cells in defined medium. Curr Protoc Stem Cell Biol 31:1C.17.1-1C.17.13.
66. http://www.sigmaaldrich.com/life-science/stem-cell-biology/ipsc.html
67. Baghbaderani BA, Tian X, Neo BH (2015) cGMP-manufactured human induced pluripotent stem cells. Stem Cell Reports 5: 647-659.
68. http://www.lonza.com/products-services/bio-research/transfection/generation-of-ipscs.aspx.
69. Tiscornia G, Vivas EL, Izpisúa JC (2011) Diseases in a dish: Modeling human genetic disorders using induced pluripotent cells. Nat Med 17: 1570-1576.
70. Siller R, Greenhough S, Park IH, Sullivan GJ (2013) Modelling human disease with pluripotent stem cells. Current Gene Therapy 13: 99-110.
71. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, et al. (2009) Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136: 964-977.
72. Freude K, Pires C, Hyttel P, Hall VJ (2014) Induced pluripotent stem cells derived from Alzheimer's disease patients: The promise, the hope and the path ahead. J Clin Med 3: 1402-1436.
73. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, et al. (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321: 1218-1221.
74. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, et al. (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 318: 1920-1923.
75. Raya Á, Rodríguez-Pizà I, Guenechea G, Vassena R, Navarro S, et al. (2009) Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature 460: 53-59.
76. Xu D, Alipio Z, Fink LM, Adcock DM, Yang J, et al. (2009) Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. Proc Natl Acad Sci 106: 808-813.
77. Wang Y, Zheng CG, Jiang Y, Zhang J, Chen J, et al. (2012) Genetic correction of β-thalassemia patient-specific iPS cells and its use in improving hemoglobin production in irradiated SCID mice. Cell Res 22: 637-648.
78. Solomon S, Pitossi F, Rao MS (2015) Banking on iPSC- is it doable and is it worthwhile. Stem Cell Rev 11: 1-10.
79. Lee G, Papapetrou EP, Kim H, Chambers SM, TomishimaMJ, et al. (2009) Modeling pathogenesis and treatment of familial dysautonomia using patient specific iPSCs. Nature 461: 402-406.
80. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, et al. (2008) Disease-specific induced pluripotent stem (iPS) cells. Cell 134: 877-886.
81. Fatehullah A, Tan SH, Barker N (2016) Organoids as an in vitro model of human development and disease. Nature Cell Biology 18: 246-254.
82. Dye BR, Hill DR, Ferguson MA (2015) In vitro generation of human pluripotent stem cell derived lung organoids. Rossant J Life 4: e05098.
83. Spence JR, Mayhew CN, Rankin SA (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 470:105-109.
84. Takebe T, Sekine K, Enomura M (2013) Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 499: 481-484.
85. Lancaster MA, Renner M, Martin CA (2013) Cerebral organoids model human brain development and microcephaly. Nature 501: 373-379.
86. Lancaster MA, Knoblich JA (2014) Generation of cerebral organoids from human pluripotent stem cells. Nature Protocols 9: 2329- 2340.
87. Lu TY, Lin B, Kim J (2013) Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. Nature Communications 4: 2307.
88. Phillips MJ, Wallace KA, Dickerson SJ (2012) Blood-derived human iPS cells generate optic vesicle-like structures with the capacity to form retinal laminae and develop synapses. Investigative Ophthalmology Visual Science 53:2007-2019.
89. Phillips MJ, Perez ET, Martin JM (2014) Modeling human retinal development with patient-specific induced pluripotent stem cells reveals multiple roles for visual system homeobox 2. Stem Cells 32: 1480-1492.
90. Lancaster MA, Knoblich JA (2014) Organogenesis in a dish: Modeling development and disease using organoid technologies. Science 345: 1247125.
91. Qian X (2016) Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell 165: 1238-1254.
92. Takebe T (2013) Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 499: 481-484.
93. Shi Y, Inoue H, Wu JC, Yamanaka S (2017) Induced pluripotent stem cell technology: A decade of progress. Nat Rev Drug Discov 16: 115-130.
94. Orqueda AJ, Giménez CA, Pereyra-Bonnet F (2016) iPSCs: A minireview from bench to bed, including organoids and the CRISPR System. Stem Cells International vol.
95. Horii T, Tamura D, Morita S, Kimura M, Hatada I (2013) Generation of an ICF syndrome model by efficient genome editing of human induced pluripotent stem cells using the CRISPR system. Int J Mol Sci 14: 19774-19781.
96. Chiou SH, Winters IP, Wang J, Naranjo S, Dudgeon C, et al. (2015) Pancreatic cancer modeling using retrograde viral vector delivery and in vivo CRISPR/Cas9-mediated somatic genome editing. Genes Development 29: 1576-1585.
97. Xie F, Ye L, Chang JC, Beyer AI, Wang J, et al. (2014) Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. Genome Res 24: 1526-1533.
98. Song B, Fan Y, He W, Zhu D, Niu X, et al., (2015) Improved hematopoietic differentiation efficiency of gene-corrected beta-thalassemia induced pluripotent stem cells by CRISPR/Cas9 system. Stem Cells Dev 24: 1053-1065.
99. Khanna I (2012) Drug discovery in pharmaceutical industry: productivity challenges and trends. Drug Discovery Today 17: 1088-1102.
100. Kaitin K (2008) Obstacles and opportunities in new drug development. Clinical Pharmacology Therapeutics 83: 210-212.
101. Lin Z, Will Y (2012) Evaluation of drugs with specific organ toxicities in organ-specific cell line. Toxicol Sci 126: 114-127.
102. Avior Y, Sagi I, Benvenisty N (2016) Pluripotent stem cells in disease modelling and drug discovery. Nat Rev Mol Cell Biol 17: 170-182.
103. https://www.cirm.ca.gov/our-progress/awards/cirm-human-pluripotent-stem-cell-biorepository.
104. https://www.imi.europa.eu/content/ebisc.
105. https://www.imi.europa.eu/content/stembancc.
106. Cao L, McDonnell A, Nitzsche A, Alexandrou A, Saintot PP, et al. (2016) Pharmacological reversal of a pain phenotype in iPSC-derived sensory

neurons and patients with inherited erythromelalgia. Sci Transl Med 8: 335ra56.

107. Cyranoski D (2012) Stem-cell pioneer banks on future therapies. Nature 488: 139.
108. Gourraud PA, Gilson L, Girard M, Peschanski M (2012) The role of human leukocyte antigen matching in the development of multiethnic “haplobank” of induced pluripotent stem cell lines. Stem Cells 30: 180-186.
109. Nakatsuji N, Nakajima F, Tokunaga K (2008) HLA-haplotype banking and iPS cells. Nat Biotechnol 26: 739-740.
110. Taylor CJ, Bolton EM, Pocock S, Sharples LD, Pedersen RA, et al. (2005) Banking on human embryonic stem cells: Estimating the number of donor cell lines needed for HLA matching. Lancet 366: 2019-2025.
111. Rao MS, Atala A (2016) Developing induced pluripotent stem cell-based therapy for the masses. Stem Cells Transl Med 5: 129-131.
112. Turner M, Leslie S, Martin NG, Peschanski M, Rao M, et al. (2013) Toward the development of a global induced pluripotent stem cell library. Cell Stem Cell 13: 382-384.
113. Barry J, Hyllner J, Stacey G, Taylor CJ, Turner M (2015) Setting up a haplobank: Issues and solutions. Current Stem Cell Reports 1: 110-117.
114. Li M, Belmonte JCI (2016) Looking to the future following 10 years of induced pluripotent stem cell technologies. Nat Protoc 11: 1579-1585.
115. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, et al. (2007) Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 1: 55-70.
116. Plath K, Lowry WE (2011) Progress in understanding reprogramming to the induced pluripotent state. Nat Rev Genet 12: 253-265.
117. Liu L, Luo GZ, Yang W, Zhao X, Zheng Q, et al. (2010) Activation of the imprinted Dlk1-Dio3 region correlates with pluripotency levels of mouse stem cells. J Biol Chem 285: 19483-19490.
118. Yamanaka S (2012) Induced pluripotent stem cells: past, present, and future. Cell Stem Cell. 10: 678-684.
119. Doi A, Park IH, Wen B, Murakami P, Aryee MJ, et al. (2009) Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. Nat Genet 41: 1350-1353.
120. Chin MH, Mason MJ, Xie W, Volinia S, Singer M, et al. (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 5: 111-123.
121. Gore A, Li Z, Fung HL, Young JE, Agarwal S, et al. (2011) Somatic coding mutations in human induced pluripotent stem cells. Nature 471: 63-67.
122. Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, et al. (2011) Copy number variation and selection during reprogramming to pluripotency. Nature 471: 58-62.
123. Zhao T, Zhang ZN, Rong Z, Xu Y (2011) Immunogenicity of induced pluripotent stem cells. Nature 474: 212-215.
124. Kamao H, Mandai M, Okamoto S, Sakai N, Suga A, et al. (2014) Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. Stem Cell Reports 2: 205-218.
125. Kanemura H, Go MJ, Shikamura M, Nishishita N, Sakai N, et al. (2014) Tumorigenicity studies of Induced Pluripotent Stem Cell (iPSC)-Derived Retinal Pigment Epithelium (RPE) for the treatment of age-related macular degeneration. Lewin A (ed). PLoS ONE 9: e85336.
126. Scudellari M (2016) How iPS cells changed the world. Nature 534: 310-312
127. Fields M, Cai H, Gong J, Del Priore L (2016) Potential of Induced Pluripotent Stem Cells (iPSCs) for Treating Age-Related Macular Degeneration (AMD). Amabile G (ed). Cells 5: 44.