Taylor & Francis
Taylor & Francis Group

Expert Opinion on Therapeutic Patents

ISSN: 1354-3776 (Print) 1744-7674 (Online) Journal homepage: http://www.tandfonline.com/loi/ietp20

# The triumph of chemically enhanced cellular reprogramming: a patent review

Muhammad Ayaz Anwar, Songmee Kim & Sangdun Choi

**To cite this article:** Muhammad Ayaz Anwar, Songmee Kim & Sangdun Choi (2016) The triumph of chemically enhanced cellular reprogramming: a patent review, Expert Opinion on Therapeutic Patents, 26:2, 265-280, DOI: 10.1517/13543776.2016.1118058

**To link to this article:** http://dx.doi.org/10.1517/13543776.2016.1118058

Accepted author version posted online: 23 Nov 2015.
Published online: 04 Dec 2015.

EXPERT OPINION ON THERAPEUTIC PATENTS, 2016
VOL. 26, NO. 2, 265–280
http://dx.doi.org/10.1517/13543776.2016.1118058

Taylor & Francis
Taylor & Francis Group

REVIEW

# The triumph of chemically enhanced cellular reprogramming: a patent review

Muhammad Ayaz Anwar, Songmee Kim and Sangdun Choi

Department of Molecular Science and Technology, Ajou University, Suwon, South Korea

**ABSTRACT**

**Introduction**: The revolutionary discovery of induced pluripotent stem cells (iPSCs) by Shinya Yamanaka has exposed science to new horizons. However, genetic modifications render reprogrammed cells unstable; for that reason, non-genetic modification approaches are actively under investigation. Among these, the use of small molecules is safe, and these molecules minimally affect the genome. Although iPSCs are ready for clinical trials there are many caveats hindering successful therapy, and small molecules are the best alternative to overcome those caveats.

**Areas covered**: Small molecules are playing an active role in generating and improving the quality of iPSCs. In this review, we will highlight the imperative role of small molecules in accelerating the successful translation of basic research into clinical use. Particularly, those ligands that replace the need for reprogramming factors will be discussed.

**Expert opinion**: Stem cell research is promising for harvesting medical benefits in near future. The invention of new techniques, mechanisms elucidation, and identification of novel compounds for stem cell creation has certainly established a solid foundation for regenerative medicine. This is the beginning of a new era for the cure of most disabling diseases, and small molecules will have a definite role in successful therapeutic use of iPSCs.

**ARTICLE HISTORY**
Received 27 June 2015
Accepted 5 November 2015

**KEYWORDS**
Chemical biology; ciPSC; iPSC; epigenetics; patent; reprogramming; small molecule; stem cell



## 1. Introduction: the beginning of a new era in regenerative medicine

Following the discovery of induced pluripotent stem cells (iPSCs), biologists focused on the rapid, easy and efficient development process that is convenient enough for translation of iPSC into clinical use. However, the procedure is slow, complicated and, in most cases, complete transformation is difficult to achieve. This resulted in the need to explore novel ways to create iPSCs from somatic cells with better efficiency.[1–5] To do this, new pluripotency-inducing factors, modification of the microenvironment, and the use of recombinant proteins and non-integrating vectors have been evaluated.[1,6,7] Although these alternative approaches gave variable results, none could efficiently relieve the process from the use of one or more pluripotency factors. To achieve a better response, other groups treated somatic cells with small molecules. This treatment enhanced the efficiency of somatic-to-pluripotency conversion, and ultimately, this whole process became independent of transcription factors.

The induced expression of various transcription factors such as Yamanaka Factors (octamer-binding transcription factor 4 (Oct4), SRY (sex determining region Y)-box 2 (Sox2), Kruppel-like factor 4 (Klf4), cellular Myc (c-Myc) [OSKM]), Esrrb and Lin28 in various combinations by viral transduction can transform the cellular state and induce characteristic manifestations of pluripotent stem cells (PSCs), termed as induced PSCs.[8–10] This breakthrough technology of reprogramming cells and regenerative medicine has unlocked new alternatives for customizable and patient-specific cell therapies. However, although the cellular state conversion has been achieved, its uses are limited due to the incorporation of genetic material that may pose a potential risk. To overcome this, several non-integrative approaches have been explored to create iPSCs, but the conversion efficiency has been disappointing.[5,11–13]

New tissues are being derived from three main sources: cells originating from embryos, adult stem cells and de-differentiated somatic cells from patients. In the first approach, cells from the early embryo phase (5-day) are isolated and grown into the desired tissue for therapeutic purposes. However, due to differences in genetic materials of patient and donor, immune rejection is a serious problem. In the second approach, stem cells are isolated from adult tissue, and later used for therapeutic purposes. However, this method is tricky, difficult and the number of stem cells in adult tissue is very low. In the third approach, somatic cells are acquired from patients and then transformed into

**CONTACT** Sangdun Choi sangdunchoi@ajou.ac.kr Department of Molecular Science and Technology, Ajou University, Suwon, South Korea

Article highlights

- Induced pluripotent stem cells (iPSCs) have provided new dimensions for regenerative medicines.
- Chemical approaches to generate stem cells (ciPSCs) can potentially replace genetic modification and can generate clinical grade and safer stem cells.
- Epigenetic modifications should be exploited to improve quality of iPSC.
- Non-epigenetic pathways are equally valuable in modulating somatic cells conversion into stem cells.
- Studies regarding mechanism providing insights into the pathways that regulate the stemness would be beneficial to improve the quality of stem cells.
- Novel specific as well as broad spectrum inhibitors of different proteins and pathways that stimulate the generation of clinical grade stem cells would be helpful.

This box summarizes key points contained in the article.



stem cells. Since the cells are genetically identical to the patient, it overcomes the issue of immune rejection. This approach can be achieved by three methods: somatic cell nuclear transfer: the method used to successfully clone the sheep known as Dolly [14]; cell fusion, which renders the fusion of embryonic cells with somatic cells; this is valuable for research purposes but not feasible for therapy; and nuclear reprogramming, which can be achieved by retroviral gene transfer, reprogramming of the matrix and chemical approaches.

Nuclear reprogramming was reported by a Japanese scientist in 2006–2007, where adult mouse and human somatic cells transfected with defined factors displayed embryonic cell-like characteristics and properties. It was at this time, they coined the term "iPSCs" for these reprogrammed cells that substantially replicated the properties of embryonic stem cells (ESCs) [8,15].

With the emergence of nuclear reprogramming, regenerative therapies that are not susceptible to immune rejection and do not raise ethical issues seem feasible. However, the manipulation of host with foreign genetic material poses serious threats; therefore, it is not always recommended to proceed without first performing a complete safety profile. In order to avoid this challenge, various other methods have been devised to reprogram cells. The use of chemicals to trigger phase-change, for example, is very safe, effective and physiologically possible and, furthermore, bypasses the need for altering genetic material.

### 1.1. Efficiency and safety problems

The phase transition of somatic cells into embryonic cells is a tedious, unsafe and inefficient process that is not suitable for therapeutic purposes. To counter these drawbacks, scientists have shifted their focus to explore alternative ways involving small molecules in an effort to improve efficiency and safety. For rapid generation of iPSCs, many chemicals and most of them are small molecule inhibitors of such as histone deacetylases (HDACs),[16,17] glycogen synthase kinase 3 (GSK3) [18] and DNA methyltransferases (DNMTs) [19] have been examined. These small molecule inhibitors, either independently or in combination with other molecules, have given rise to efficient induction of iPSCs.[2,16] Although these molecules are more efficient, they also have off-target effects. For example, HDAC inhibitor trichostatin A (TSA) has been used to create the somatic cell nuclear transfer-based embryos; however, the resulting embryo possessed high developmental efficiency, and the quality was poor.[20]

Currently, researchers are investigating ways to minimize issues caused by transfection of pluripotency factors and increase efficiency and quality in the production of iPSCs.[21] In one attempt, it was found that with the use of selected small molecules, Oct4 is sufficient to reprogram human keratinocytes into stem cells.[22] Other groups have reported that minimizing the use of transfection factors combined with one or more small molecules increased efficiency. In a recent effort to achieve complete reprogramming without the use of transfection factors, Hou et al. [2] discovered a combination of five to seven small compounds that could stably and safely induce reprogramming machinery in mouse adult and embryonic fibroblasts (MAF and MEF). This approach promises completely safe and robust cell-based therapies whose clinical application will be available in near future.

## 2. Small molecule patent: flooding of molecular biology with chemicals

### 2.1. Epigenome-targeting small molecules

The popular concept of developmental biology is that with cell progression through the developmental stages, cells irrevocably limit their differentiation potential and start expressing tissue-specific genes.[23] Gene regulation in differentiating cells requires activation of genes obliged to form specific patterns by expressing specific proteins according to their physiological requirement and environment, while nonessential genes are turned off. Such specialized mechanisms, which cover DNA and histone methylation and histone acetylation, are perpetuated by epigenetics regulatory machinery and are heritable in nature.[24] The different patterning of both the DNA and histone methylation conveys different regulatory behaviors, and these patterns are added and/or removed accordingly.[25] This

was observed in pluripotency gene promoters that are hypomethylated in both iPSCs and ESCs, display nucleosome depletion at the transcription start site and exhibit high levels of transcription. Demethylation of pluripotency gene promoters is required [26] and crucial for their reprogramming.[27] However, further studies will elucidate the underlying mechanism of crossing this barrier. Evidently, compounds that target epigenetic machinery are capable of enhancing reprogramming efficiency (Table 1).[16,26] Primarily, three classes of epigenetic modulators have been examined for iPSCs: DNA methylation, histone methylation and histone acetylation (Figure 1).

The addition of acetyl groups onto lysine residues throughout various histone tails is a mark of active transcription.[28] This process is achieved by disrupting the histone–DNA electrostatic interaction and can also aid in recruiting transcriptional coactivators. These active signs are removed by HDACs, whereas histone acetyltransferases are responsible for placing these marks.[29] HDAC inhibitors have widely been used in clinical oncology,[29] and a few other HDAC inhibitors have also been examined in the context of iPSC generation (Table 1).

Valproic acid (VPA) is perhaps the most extensively studied small molecule in stem cell biology; when supplemented with OSKM, it enhances iPSC generation.[16] VPA also alleviates the necessity of c-Myc and/or Sox2 in the reprogramming mixture and still produces iPSCs with better to moderate efficiency as compared to OSKM. Ppyrrole-imidazole polyamide (PIP) conjugated with suberoylanilide hydroxamic acid (SAHA) is another site-specific molecule that can enhance reprogramming by directionally activating the transcription of Oct3/4, Sox2, c-Myc and Klf4. In this combination, specificity is conferred by PIPs whereas SAHA is a potent deacetylase inhibitor. In this way, site-directed targeting that improves iPSC generation while minimizing unwanted effects can be achieved.[30–32]

Other HDAC inhibitors that effectively reprogram somatic cells include NaB (sodium butyrate) and TSA; however, their efficiencies are variable.[33] NaB is a non-specific HDAC inhibitor with the potential to increase reprogramming efficiency when used with OSKM, but its effects may only be cell specific.[33] Although one of these HDAC inhibitors is claimed to be more potent in its action, it is difficult to determine the criteria used to calculate reprogramming efficiency. Some studies report the efficiency of reprogramming as the percentage of the final cell population instead of the number of cells used in the beginning. In this way, the cytotoxic compounds artificially enriched cells that are resistant to HDAC inhibition; thus, NaB and TSA may appear to be very potent.[34,35] Specific inhibition of different HDAC family members proved to be less cytotoxic and yielded better results.[36]

Methylation is a widely used marker in epigenetics that regulates gene expression in various ways according to the site and number of functional groups attached to cytosine and arginine/lysine side chains of DNA and histones, respectively.[28] An RNAi-based screen that targeted methylation mediators identified two potential histone methyltransferases: SUV39h1 (Su(var)3–9 homolog 1) and DOT1L (DOT1-like, histone H3 methyltransferase). These methylases are lysine-specific, adding a methyl group to K9 and K79, respectively, and inhibition of these enzymes is beneficial for stem cell reprogramming.[19,37] DOT1L catalyzes the dimethylation of H3K79, an active transcription mark.[38] This enzyme plays an active role in epithelial to mesenchymal transition (EMT), where cells abandon their adhesion properties. EPZ004777 inhibits DOT1L, rendering EMT gene expression inhibition, favoring mesenchymal to epithelial transition and presumably enhancing the reprogramming process.[37] This compound has increased the efficiency four-fold when applied in combination with OSKM. Moreover, EPZ004777 can replace Klf4 and c-Myc, possibly by inducing Nanog and Lin28, and can also induce reprogramming when combined with OS.[8] Both DOT1L and H3K9 inhibitors have been well characterized for specificity and selectivity against their target enzymes due to their uses in other non-iPSC purposes.

The genes that play active role in pluripotency and promote an undifferentiated cellular state are permanently silenced by a family of enzymes including H3K9me3 [28] and methyltransferases SUV39h1 and G9a.[39,40] This permanent repression is a major hurdle in inducing pluripotency; therefore, molecules such as BIX01294 and Verticillin A that target these enzymes allow enhanced iPSC generation.[41,42] The use of these compounds may induce reprogramming with fewer transgenes in mouse embryonic fibroblasts with low efficiency. The combination of BIX01294 with AzaC (5-azacytidine), a DNMTs inhibitor or BayK8644, a calcium channel agonist, induces the reprogramming comparable to OSKM.[43] Owing to their cytotoxic effects, safer H3K9me3 inhibitors may yield better reprogramming results.[43,44] Therefore, based on X-ray crystal structure and structure activity relationship (SAR) studies, the template of BIX01294, 2,4diaminoquinazoline led to the discovery of another G9a-methyltransferase inhibitor, UNC0224. Crystal structure of G9a-UNC0224 complex elucidated that the inhibitor was residing in the binding pocket of the enzyme to competitively inhibit the enzymatic activity.[45,46] Studies

**Table 1.** Epigenome-targeting small molecules.

| Compound name | Target | Patent ID/publication no. | Title | Inventor/applicant | Ref. |
|---|---|---|---|---|---|
| Valproic acid | HDACs | US 20130035374 | MicroRNA induction of pluripotential stem cells and uses thereof | Morrisey Edward E | [109] |
| SAHA | HDACs | US 20100273259 | Substrates and methods for culturing stem cells | Massachusetts Institute of Technology | [110] |
| Trichostatin-A | HDACs | WO2000023567 | Promotion of self-renewal and improved gene transduction of hematopoietic stem cells by histone deacetylase inhibitors | Beth Louise Hill, Catherine P Lavau, Novartis AG, Novartis Erfind Verwalt GMBH, Judy Carol Young | [111] |
| NaB | HDACs | WO2011084747 | Compositions and methods for somatic tissue induced pluripotent stem cells having an endoderm origin | The Johns Hopkins University | [112] |
| EPZ004777 | DOT1L | CN103952368 | Culture solution for promoting *in-vitro* growth of porcine somatic cell cloned embryos | Anhui Agricultural University | [113] |
| Ribose containing inhibitors | DOT1L | US20140100184 | Selective inhibitors of histone methyltransferase dot1l | Baylor College of Medicine | [114] |
| UTX inhibitor | H3K27 demethylase | WO2009114011 | Histone demethylation proteins and methods of use thereof | President and Fellows of Harvard College | [115] |
| Ezh2 inhibitor (Polymorph A) | Ezh2 mediated methyl transferase inhibition | WO2013155317 | Salt form of a human histone methyltransferase ezh2 inhibitor | Epizyme, Inc., Eisai R&D Management Co., Ltd. | [116] |
| BIX01294 | G9a histone methyl transferase | WO2009117439 | Combined chemical and genetic approaches for generation of induced pluripotent stem cells | The Scripps Research Institute | [117] |
| Verticillin A | SUV39h1 and other methyltransferases | US20140161785 | Verticillin A inhibition of histone methyltransferases | Feiyan Liu, Ping Wu, Kebin Liu | [118] |
| UNC0224 | G9a histone methyl transferase | WO2015003643 | One kind of treatment by small molecule compounds, prepared pluripotent stem cells, kits and uses | Beijing University of Science and Technology | [119] |
| AMI-5 | Arginine methyl transferase inhibitor | US20140154805 | Enhancers of induced pluripotent stem cell reprogramming | City of Hope | [120] |
| Parnate (tranylcypromine) | LSD1 | WO2011109015 | Improved methods of generating pluripotent stem cells | The Scripps Research Institute | [121] |
| Vitamin C | Histone demethylases | WO2011134210 | Culture medium additive and uses thereof | Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, | [122] |
| 5-Azacytidine | DNMTs | WO2011019957 | Method for formation of induced pluripotent stem cells | University of Southern California | [123] |
| RG108 | DNMTs | US20090253203 | Reprogramming a cell by inducing a pluripotent gene through use of a small molecule modulator | Nupotential, Inc. | [124] |
| RSC133 | HDAC and DNMTs inhibitor | WO2013180350 | Novel low-molecule compound for promoting pluripotent stem cell generation, maintenance, and proliferation, and composition and culturing method containing same | Korea Research Institute of Bioscience and Biotechnology | [125] |
| OAC1 | Induction of Oct4-Nanog-Sox2 and Tet1 | WO2014000814 | Method for activating oct4 for induction of pluripotent stem cells | University Hospital Jena, Ruprecht-Karls-University Heidelberg | [126] |

DNMT, DNA methyltransferase; DO1L, DOT1-like, histone H3 methyltransferase; Ezh2, enhancer of zeste homolog 2-mediated methyl transferase; HDAC, histone deacetylases; LSD1, lysine-specific demethylase 1; NaB, sodium butyrate; OAC1, Oct4-activating compound 1; RSC133, reprogramming stimulating compound 133; SAHA, suberoylanilide hydroxamic acid; SUV39h, Su(var)3–9 homolog 1.

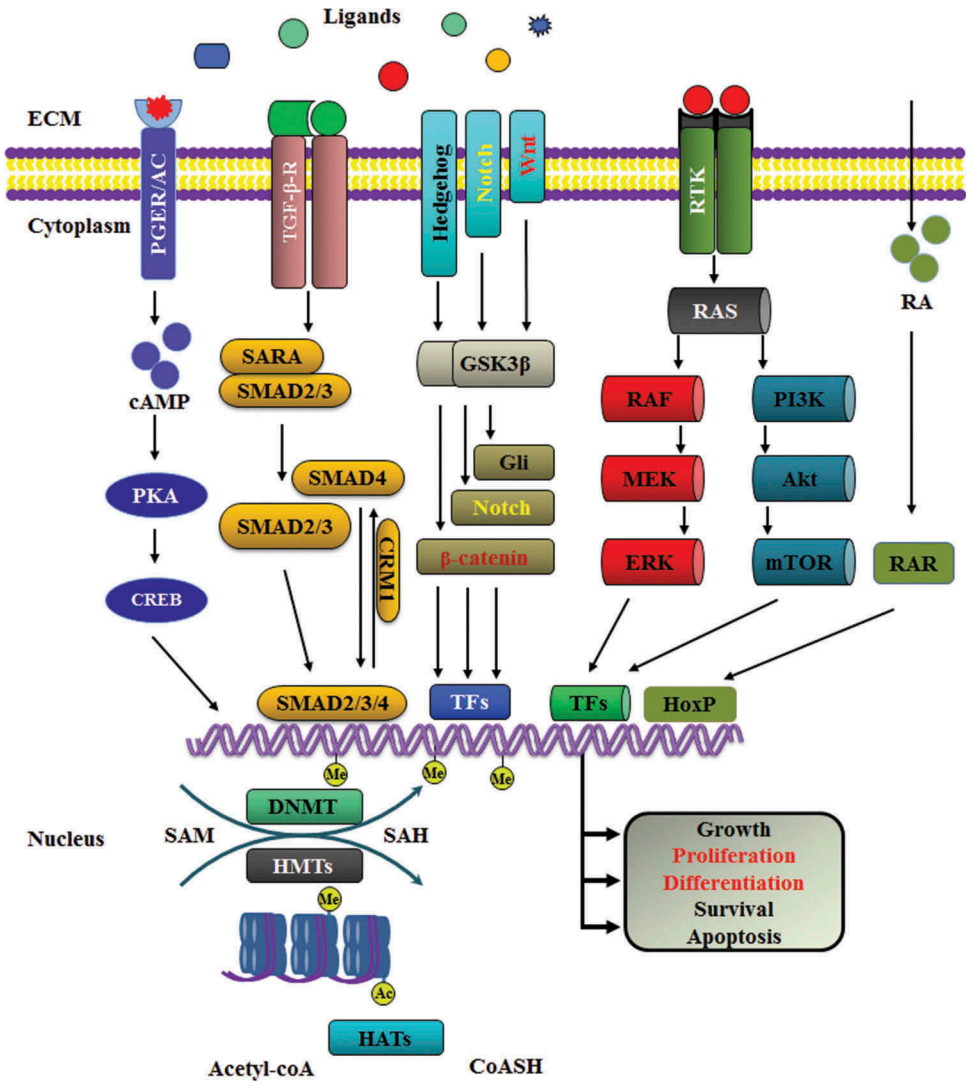


**Figure 1.** Highlight of pathways involved in the generation of iPSCs.

A glimpse of most prominent pathways that, when modulated, are helpful in iPSC generation. These pathways are heavily interlinked through intermediary proteins and generate a coordinated response. AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; CRM1, chromosome region maintenance 1; DNMT, DNA methyltransferase; ECM, extracellular matrix; ERK, extracellular-signal-regulated kinase; GSK3, glycogen synthase kinase 3; HAT, histone acetyltransferase; HoxP, hox proteins; HMT, histone methyltransferase; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PGER, prostaglandin E receptor; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; RAR, retinoic acid (RA) receptor; RAS, rat sarcoma; RTK, receptor tyrosine kinase; SARA, SMAD adaptor for receptor activation; TFs, transcription factors; TGF-β, transforming growth factor β.

of this kind have great potential for establishing more SARs that will improve the production of highly specific and efficient inhibitors for different enzymes.

Combined inhibition of protein arginine methyltransferases (by AMI-5) and transforming growth factor β (TGF-β) gave rise to iPSCs in cells transfected with Oct-4 only.[47] Parnate (lysine-specific demethylase 1, LSD1, inhibitor) has alleviated the need for Sox2 when used in combination with VPA and CHIR99021.[48] These experiments have demonstrated alternative avenues for genetic manipulation and a pivotal role for these inhibitors in somatic reprogramming. To date, AMI-5 has not been profiled against other protein targets to query additional nonspecific interactions [49] and Parnate is clinically approved as a monoamine oxidase inhibitor.[50]

Although vitamin C is not classified as a small molecule derived from chemical methods, this organic molecule may also efficiently reprogram cells likely by demethylating DNA or histones.[51,52] The major obstacle in reprogramming is the modification of the epigenome caused by methylation, acetylation,

phosphorylation, ubiquitination and so on. With the emergence of new molecules that will render specific inhibition of various aspects of the epigenome, the relative participation from each type of modification will become clearer, allowing scientists to devise improved iPSC protocols. For example, Ezh2 (enhancer of zeste homolog 2) methyltransferase, which catalyzes the H3K27me3 repressive mark on lysine sites, has been studied against different inhibitors and it was determined that its inhibition further enhances reprogramming efficiency [53] most prominently during the late stage of reprogramming.[54] Intriguingly, depletion of H3K27me3 using Ezh2 inhibition or knockdown of other polycomb repressor complex 2 components, such as Eed, hindered the generation of iPSCs, thereby highlighting its dual role in stabilizing the undifferentiated and differentiated state.[55] Nevertheless, UTX histone demethylase (also known as Kdm6a), engaged with OSK, improves the reprogramming process rather than sustaining reprogramming.[56] These studies have unfolded the importance of methylation in iPSCs and further support the possibility of using Ezh2 inhibitors to refine the reprogramming process.[57]

DNA methylation is an active area of research in stem cell biology. Various modulatory approaches have been developed to exploit DNA methylation for rapid generation of iPSCs. AzaC and RG108 are compounds that inhibit DNA methylation in different ways and also enhance reprogramming efficiency. AzaC is a chemical analogue of nucleoside cytidine, carrying N instead of C at the methylated position, which can incorporate into DNA and RNAs. When DNMTs attempt to methylate its target DNA, AzaC covalently attaches to the DNMTs and facilitates the degradation of this complex. This results in the functional depletion of DNMTs and overall hypomethylation of DNA,[34] thereby converting partially reprogrammed cells into fully reprogrammed cells.[26] The precise treatment time and duration are not yet determined whether early treatment is advantageous or treatment during the whole cycle proved efficient.[16,26] A better understanding of the molecular pharmacology of this compound will help determine its optimal use in iPSC technology. Unlike AzaC, RG108 is a direct inhibitor of DNMTs that blocks the active site of these enzymes and prevents methylation of DNA. This compound replaced the core transcription factors and generated iPSCs in cells transfected with only OK.[48] Therefore, RG108 can be a promising compound for future use.

The dual inhibition of HDACs and DNMTs by RSC133 (reprogramming stimulating compound 133) renders spontaneous reprogramming of cells. RCS133 is derived from indoleacrylic/indolepropionic acid. This combined inhibition substantially improves somatic cell reprogramming into the pluripotent state and prolongs the undifferentiated state of newly generated stem cells.[58] Furthermore, in a high-throughput luciferase-based assay screening, several compounds have been examined to test their ability to induce Oct4 and Nanog. This screening yielded Oct4-activating compound 1 (OAC1) and two other structural analogues. When these small molecules were tested along with core pluripotency factors, they showed enhanced somatic-to-stem cell conversion efficiency.[59] OAC1 may impart its effects, independent of p53-p21 or Wnt signaling inhibition by activating transcription of the Oct4-Nanog-Sox2 triad and a DNA demethylase, Tet1.

### 2.2. Non-epigenome targeting molecules

Reprogramming is a complex process that is governed by both epigenetic and non-epigenetic pathways. In non-epigenetics, the pathways that maintain the differentiated state, cell proliferation and other physiological phenomena play significant roles in reprogramming (Table 2). To improve this process, various inhibitors of these pathways have been developed.[60] It is now well-appreciated that by modulating the function of kinases such as the mitogen activated protein kinases (MAPK), GSK3 and TGF-β, reprogramming efficiency can be dramatically increased (Figure 1).

#### 2.2.1. Kinase inhibitors: appending small molecule ($PO_4$) is vital for iPSCs

Phosphorylation in cells is mediated by kinases, and in this section, kinases significant to the development of iPSCs are discussed. MAPKKs are highly conserved protein kinases that function in growth- and inflammation-related pathways. MAPKK family member, MEK, belongs to a highly conserved signaling pathway, Raf/MEK/ERK that transmits extracellular signals to nucleus through various protein mediators. This pathway activates the Ras-GTPase and downstream MAPK network leading to the activation of extracellular-signal-regulated kinase (ERK) that translocates into the nucleus and activates specific transcription factors. Since ERK is involved in differentiation, metabolism, proliferation, survival and morphology, its modulation is helpful in reprogramming.[61] MEK inhibitor, PD0325901, in combination with leukemia inhibitory factor (LIF) and GSK inhibition promotes iPSCs, converts partially reprogrammed cells into fully reprogrammed stage and suppresses the proliferation of non-pluripotent cells.[62] Moreover, inhibition of MEK impedes iPSC differentiation thereby further enriching the stem cell population in culture.

**Table 2.** Non-epigenetic targeting of small molecules.

| Compound name | Target | Patent ID/publication no. | Title | Inventor | Ref. |
|---|---|---|---|---|---|
| PD0325901 | MEK inhibitor | WO2014167866 | iPS precursor cell and iPS cell | Japan Health Sciences Foundation | [127] |
| Pluripotin (SC1) | RasGAP and ERK1 inhibitor | WO2010067886 | Dihydropyrimidopyrimidine derivative(s) | Banyu Pharmaceutical Co., Ltd. | [128] |
| Y-27632<br>Fasudil<br>H-1152 | ROCK inhibitor | WO2008035110 | Stem cell culture medium and method | Riken; Stem Cell Sciences UK Ltd; Yoshiki Sasai; Kiichi Watanabe | [129] |
| Thiazovivin | ROCK inhibitor | WO2012071393 | Method for producing pluripotent stem cells | The New York Stem Cell Foundation | [130] |
| Dasatinib<br>PP1<br>iPYrazine | Src inhibitors | US20140154805 | Enhancers of induced pluripotent stem cell reprogramming | City of Hope | [120] |
| *BIM-0086701,714, 716, 727,769,787 BIM-0207133, BIM0207164, 7174 BIM-0050621 BIM-0086660 | Different kinases inhibitors | WO2012166973 | Methods for promoting cell reprogramming | Sanford-Burnham Medical Research Institute | [131] |
| CHIR99021 | GSK3 inhibitor | CN103361305 | Application of small molecule compound | | [132] |
| Kenpaullone | GSK3 and CDK inhibitor | WO2015041809 | Small molecule cellular reprogramming to generate neuronal cells | The J. David Gladstone Institutes, A Testamentary Trust | [133] |
| IWP 2 | GSK3/Wnt inhibitor | WO2009155001 | Wnt protein signaling inhibitors | The Board of Regents of The University of Texas System | [134] |
| SB-216763 | GSK3/Wnt inhibitor | WO2009032194 | Wnt pathway stimulation in reprogramming somatic cells | Whitehead Biomedical Inst; Brett Chevalier; Alexander Marson; Richard A Young; Ruth Foreman; Rudolf Jaenisch | [135] |
| BIO | GSK3 Inhibitor | US20090111177 | Maintenance of embryonic stem cells by the GSK3β inhibitor 6-bromoindirubin-3′-Oxime | The Rockefeller University | [136] |
| TWS119 | GSK3 Inhibitor | WO2008133904 | Methods and compositions for stem cell self-renewal | Stowers Institute For Medical Research | [137] |
| E-616452 (RepSox) | TGF-β Inhibitor | US20140120621 | TGF-β receptor inhibitors to enhance direct reprogramming | The General Hospital Corporation | [138] |
| SB431542 | ALK4, ALK5, ALK7 (TGF-β Type I Receptor) | WO2010077955 | Generation and maintenance of stem cells | The Scripps Research Institute | [139] |
| A-83–01 | ALK4, ALK5, ALK7 (TGF-β Type I Receptor) | US20120264218 | Induction of pluripotent cells | The Scripps Research Institute | [140] |
| LY-364947 | TGF-β inhibitor | WO2014142038 | Method for producing human corneal epithelium sheet | Jcr Pharmaceuticals Co., Ltd; Kyoto Prefectural Public University Corporation | [141] |
| ID-8 (include IQ-1 and ICG-427) | DYRK inhibitor | WO2011019957 | Method for formation of induced pluripotent stem cells | University of Southern California | [123] |
| **Miscellaneous small molecules** | | | | | |
| DAPT | Notch signaling inhibitor | WO2013178821 | Inhibitors of the notch signaling pathway and secretion for its use in medicine | Leibniz Institute for Age Research – Fritz Lipmann Institute (FLI) | [142] |
| AM580 | RAR agonist | WO2005007799 | Methods for *ex-vivo* expanding stem/progenitor cells | Gamida-Cell Ltd. | [143] |
| CD437 | RAR agonist | WO2011027180 | Cells and methods for obtaining them | Genome Research Limited | [144] |
| PDK1 activator (PS48, PS08, 12Z, 13Z) | PDK1activator | WO2011123572 | Reprogramming cells | The Scripps Research Institute | [145] |
| Oxygen level | May possibly induce hypoxic conditions | WO2011062963 | Induced pluripotent stem cells and related Methods | Vitro Diagnostics, Inc. | [146] |
| BayK8644 | A calcium channel agonist | WO2009117439 | Chemical approaches for generation of induced pluripotent stem cells | The Scripps Research Institute | [117] |

(*Continued*)

**Table 2.** (Continued).

| Compound name | Target | Patent ID/publication no. | Title | Inventor | Ref. |
|---|---|---|---|---|---|
| Oxysterol and Purmorphamine | Activators of shh signaling | US8772031 | Composition for reprogramming somatic cells to generate induced pluripotent stem cells, comprising Oct4 in combination with Bmi1 or its upstream regulator, and method for generating induced pluripotent stem cells using the same | Korea University Research and Business Foundation | [147] |
| Rapamycin | mTOR inhibitor | CN102758000 | Organism lifetime based method for screening compounds capable of improving generation efficiency of induced pluripotent stem cells | Shanghai Institute of Biological Sciences | [148] |
| PP242 | mTOR inhibitor | | | | |
| Resveratrol | Sirtuin activators | | | | |
| Fisetin | Sirtuin activators | | | | |
| Spermidine | autophagy inducer | | | | |
| LY294002 | PI3K inhibitor | | | | |
| Forskolin | PKA activator (cAMP inducer) | WO2015003643 | Compositions and methods for reprogramming non- pluripotent cells into pluripotent stem cells | Hong Guan Ltd. | [149] |
| DZNep | SAH hydrolase and Ezh2 inhibitor | | | | |

*Because of many compounds, these are written in the text as BIM-XXXXX

ALK, activin receptor-like kinase; cAMP, cyclic adenosine monophosphate; CD, cluster of differentiation; CDK, cyclin-dependent kinases; DYRK, dual-specificity tyrosine-regulated kinases; DZNep, 3-deazaneplanocin A; ERK, extracellular-signal-regulated kinases; Ezh2, enhancer of zeste homolog 2-mediated methyl transferase; GSK-3, glycogen synthase kinase 3; JAK1, janus-associated tyrosine kinase 1; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PDK1, 3′-phosphoinositide-dependent kinase-1; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; RAR, retinoic acid receptor; RAS, rat sarcoma; RasGAP, Ras-GTPase activating protein; ROCK, Rho-associated coiled-coil kinase; SAH, S-adenosylhomocysteine hydrolase; Shh, sonic hedgehog; TGF-β, transforming growth factor–β; DAPT: N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester.

This compound is a common supplement in stem cell medium to prevent differentiation during long-term growth.[62]

SC1, a derivative of 3,4-dihydropyrimido[4,5-d] pyrimidine, was shown to stimulate proliferation and undifferentiation of stem cells in medium lacking serum, feeder cells and LIF.[63] Its mechanism is attributed to the inhibition of RasGAP and ERK1-induced differentiation pathways. SC1 induced a 10-fold increase in pluripotency generation activity compared to other derivatives and had relatively low cellular toxicity.[63] Furthermore, SC1 may also target p70S6K, a mitogen-stimulated serine-threonine kinase regulated by phosphatidylinositol 3-kinase (PI3K), signifying the possible involvement of PI3K. Furthermore, SC1 analogue pluripotin was reported to have greater activity, but with lower cytotoxicity.

The widely used selective Rho-associated coiled-coil kinase (ROCK) inhibitors, Y27632, Fasudil and H-1152, have been shown to improve the growth of iPSCs from cryopreserved human embryonic stem cells (hESCs) with or without a feeder layer. ROCK inhibition facilitated colony formation when treated for several days and also promoted the growth of thawed stem cells, providing a thrust to slow-growing iPSCs, particularly when cells are recovered from frozen stocks.[64] Another ROCK inhibitor, thiazovivin (Tzv), was confirmed through affinity pull-down assay using immobilized Tzv and *in vitro* Rho kinase assay. Tzv is five-fold more efficacious in inhibiting ROCK activity in comparison to Y-27632.[65]

Staerk et al. [66] performed a high-throughput cell-based chemical screening scheme to isolate Sox2 by replacing molecules during MEF somatic cell reprogramming.[66] The author discovered Pan-Src kinase inhibitors, iPYrazine (imidazo[1,2-a]pyrazine), dasatinib, and PP1, by employing Nanog reporter-based screening.[66] Another group also performed a kinase inhibitor screen in an attempt to identify the indispensable kinases for pluripotency and those that hinder reprogramming. It was found that three kinases, p38, inositol trisphosphate 3-kinase (IP3K) and aurora A, were negative regulators of reprogramming.[67] Consequently, iPSC generation was substantially enhanced by inhibiting these barrier kinases with small molecules (BIM-XXXXX). These studies highlight the valuable and indispensable role of kinases in different cellular functionalities that can be modulated to enhance reprogramming efficiency.

GSK3 is a serine/threonine kinase that regulates over 40 cellular proteins via phosphorylation.[68] The role of GSK3 is widely acknowledged in different pathways such as growth, polarity, motility and

apoptosis. The anomalies in GSK3 physiology implicated in Alzheimer's disease and diabetes triggered the development of pharmacological modulators.[69] CHIR99021 is one of these modulator compounds that has proven effects in reprogramming in a cell-based phenotypic screen.[48] CHIR99021 substantially increased reprogramming in MEF cells when transduced with OSK, and eliminated the requirement for Sox2 when used in combination with Parnate.[48] Kenpaullone, another GSK3 inhibitor, was identified in a high-throughput screening. It has the ability to induce iPSCs in a Klf4-independent manner.[70] However, kenpaullone has lower specificity as compared to CHIR99021. The likely mechanism of GSK3 inhibitors have been linked to the Wnt signaling pathway, which is vital for maintenance of stemness and prevention of cellular differentiation.[71] In Wnt signaling, membrane-associated frizzled receptor lacking Wnt triggers the degradation of the complex that labels β-catenin for proteolysis, and this process is facilitated by GSK3. Small molecule inhibition of GSK3 prevents degradation of β-catenin, allowing it to be translocated into the nucleus and to induce pluripotency-associated genes. β-catenin can be stabilized by treating cells with Wnt3a ligand, which has been shown to improve MEF reprogramming with OSK. Multiple studies have reported the vital role of Wnt and GSK3 signaling in stem cell biology.[72,73] In addition, Wnt signaling in somatic-to-iPSCs conversion is stage-specific, its late activation is beneficial in the generation of iPSCs [74]; however, in T-lymphocyte cell maturation, Wnt signaling is a negative regulator of naïve-to-effector T-cell differentiation.[75] As such, stage-specific Wnt signaling inhibition might provide an alternative avenue for their proper utilization in stem cell biology. Although GSK3 plays an important role in Wnt signaling, it has a plethora of cellular functions; therefore, further studies delimiting the exact role of GSK3 inhibitors in reprogramming would be immensely valuable.

IWP 1/2 are Wnt pathway inhibitors that execute their effects by inhibiting Porcupine, an O-acyltransferase family member, which catalyzes the addition of palmitoyl groups to Wnt proteins that enhance their signaling potential and Wnt secretion.[76,77] Inhibiting the Wnt pathway at the early stage of somatic-to-iPSCs conversion might be useful to block hPSC differentiation into mesoderm and maintain an undifferentiated state.

GSK3 inhibition by SB-216763 can maintain the pluripotent state of cells for a long time as demonstrated by colony morphology, pluripotency gene expression, effective differentiation potential and expression of germ-layer-specific markers.[78] Mouse cells maintained in media containing SB-216763 were either improved or indistinguishable compared to LIF-treated cells. Another specific GSK3 inhibitor, 6-bromoindirubin-3′-oxime (BIO), was formerly reported to block hESC differentiation.[79] GSK3 inhibition by BIO was later studied and its role in improving stem cell formation in both murine and human ESC was deciphered.[80] BIO is responsible for β-catenin stabilization, which allows for engaging with T-cell factor/lymphoid enhancer factor to induce genes involved in self-renewal.[80] Moreover, TWS119 (3-[[6-(3-amino-phenyl)-1H-pyrrolo[2,3-d]pyrimidin-4-yl]oxy]-phenol) is another GSK3-specific inhibitor that was discovered during the screening of the pyrrolopyrimidines library. This molecule has shown substantial neuronal differentiation propensity in a mouse-originated cell line,[81] whereas in hESCs, it blocked GSK3 and promoted an undifferentiated cellular state, thereby revealing a different mechanism of GSK3 signaling in humans and mice.

The TGF-β family of proteins is very prominent in modulating cellular differentiation, proliferation, and other related functions in cells. Bone morphogenic proteins (BMPs), a family member of TGF-β, are active in stem cell biology and fate determination.[82] BMPs are extracellular ligands that mediate transcription through Smad to regulate expression of different genes involved in EMT and differentiation, and inhibiting this pathway facilitates pluripotency in cells.[83] Similarly, Alk receptor-based TGF-β inhibitor, E-616452, can induce iPSCs without Sox2 from the transgene pool, and prevents binding interaction between Alk and TGF-β ligands.[84,85] Furthermore, TGF-β inhibitors, A-83-01 and SB431542, have also been shown to significantly increase the conversion from somatic to stem cell.[22,86,87] Plausibly, these compounds induce Nanog and other transcription factors that can reduce the core factors and also hinder EMT. Further study of this signaling axis with specific inhibitors will provide insight for their best utilization in stem cells. LY-364947 is a highly specific TGF-β inhibitor that has been reported to induce stem cell generation by precluding TGF-βR-I-mediated P-Smad3 phosphorylation and preventing TGF-β-induced EMT in NMuMg cells.[66,88]

It was recently observed that SB431542 can replace Sox2, and Kenpaullone is the attractive replacement of Klf4. Their combination can promote iEGCs (induced embryonic germ cells) derivation when standard conditions were provided, excluding basic fibroblast growth factor and stem cell factor.[89] Secondly, SB431542 encouraged iEGCs' derivation with Kenpaullone at E11.5, whereas SB431542 antagonized

Kenpaullone-mediated reprogramming at E13.5.[83,90] Finally, it is imperative to identify the targets of Kenpaullone to determine the mechanism underlying the opposing effects of TGF-β signal inhibition on Kenpaullone-mediated reprogramming at E11.5 and E13.5.[89] PI3K inhibitor (LY294002) and dual-specificity tyrosine phosphorylation-regulated kinase inhibitors (ID-1 and ID-8) have extended the kinase inhibitor repertoire.[91,92] Although these compounds may not be directly participating in the creation of iPSCs, they may prove effective in creating and prolonging the undifferentiated state of iPSCs.

#### 2.2.2. Non-kinase targeting small molecules

Besides the aforementioned vital pathways, there are other proteins in the cellular environment that may not have much apparent influence, but can still facilitate reprogramming. N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester (DAPT), inhibitor of the Notch pathway, recently showed significant increase in the generation of iPSCs in both human and mouse keratinocytes by suppressing p21 in a p53-independent manner. In this manner, the iPSCs were enriched with self-renewing pluripotent cells for extended periods of time.[93,94] Moreover, Notch inhibition has generated iPSCs without c-Myc and Klf4 while leaving p53 fully functional, enabling the production of safer human iPSCs. Wang et al. [95] observed that the signaling mediated by retinoic acid can improve the generation of iPSCs. This was accomplished either by activating the pathway by agonists (CD437 and AM580) or by abolishing retinoic acid receptor-mediated signaling with the corresponding dominant negative form [95] (Table 2).

Glucose metabolism may also play a role in reprogramming as evidenced by glycolysis promotion, supplementation of glycolic intermediates (e.g. PS48, an 3′-phosphoinositide-dependent kinase-1 activator) [22] or oxygen deficiency.[96] These may efficiently reprogram somatic cells, whereas glycolysis impairment and/or stimulation of oxidative phosphorylation may hinder reprogramming.[97]

BayK8644, which is an L-channel calcium agonist when combined with BIX01294, a methyltransferase inhibitor, is capable of replacing Sox2, during MEF reprogramming into miPSCs.[19,98,99] Sonic hedgehog signaling activators (purmorphamine and oxysterol) have shown upregulation of Bmi1, N-Myc and Sox2 expression in mouse fibroblasts and improved reprogramming efficiency.[100] Moreover, mTOR inhibitors (rapamycin and PP242) have also shown usefulness in the generation of iPSCs. Sirtuin activators (resveratrol and fisetin) and autophagy inducer (spermidine) are also implicated in reprogramming.[101] Therefore, by inhibiting or activating various pathways, these small molecules fine-tune the reprogramming process.

### 2.3. Success of chemical biology

In 2013, Hou et al. [2] first described the induced pluripotency of somatic cells solely based on the two combinations of small molecules, C6FZ and VC6TFZ. The names are derived from the first letter of each compound: Valproic acid (V), an HDAC inhibitor [16]; CHIR99021 (C), a glycogen synthase kinase 3 inhibitor [102]; 616452 (6), a transforming growth factor-β inhibitor [103]; tranylcypromine (T) (generic name is Parnate), an H3K4 demethylation inhibitor [103]; forskolin (F), a cAMP agonist [104]; and DZNep (Z), an S-adenosylhomocysteine hydrolase inhibitor. [105,106] The chemical composition of these two reprogramming compounds differs only in the number of applied chemicals that translated into 10 times efficiency difference in both MEFs and MAFs. The efficiency of C6FZ was lower than VC6TFZ. The stem cells induced by these compounds harbor similar expression profiles, epigenetic status, differentiation potential and germline transmission (Figure 2). The combination of these two reprogramming compounds has alleviated the issues of genetic modification and is therefore the most suitable method for stem cell-based clinical applications.

## 3. Conclusion

The progress in the generation of iPSCs, initially with the transfection of four transcription factors in 2006 and then simplifying with the treatment of five to seven small molecules in 2013, demonstrates the success of chemical biology in the field of stem cell and regenerative biology (Table 3). The rapid pace at which chemical biology has shown to convert somatic cells into iPSCs is extraordinarily appreciable. The basic hurdles for the generation of iPSCs have been overcome with the use of small molecules. However, for the rapid production of stem cells of clinical grade, further improvement in the current status of protocols and the nature of chemical compounds is necessary. The primary and foremost important aspect of stem cell biology is the removal of epigenetic memory of the parent cells that may undermine the therapeutic efforts of iPSCs in clinical settings, and its safe removal is a challenging task.

Small molecules are not only valuable in the generation of iPSCs, but their utilization may also assist in the elucidation of underlying mechanisms of how different pathways interact with each other during various stages of stemness and differentiation. It is likely that these

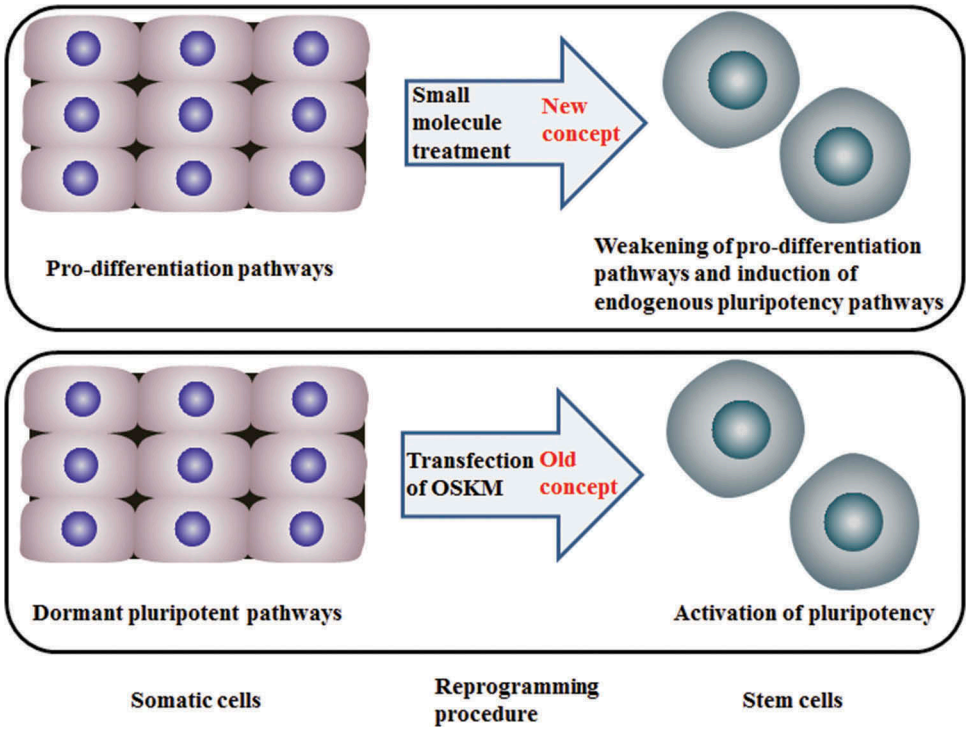


Figure 2. Schematic comparison of molecular biology and chemically induced PSCs.
In molecular biology, transfection of four transcription factors are required, whereas treatment with five to seven small molecules suffice for the induction of pluripotent stem cells.

Table 3. Comparison of chemical biology and molecular biology.

| | Property | Chemical biology | Molecular biology |
|---|---|---|---|
| 1 | Molecular weight | <1 kD | >10 kD |
| 2 | Genome integration | No integration | Partially resolved, but still chance of integration |
| 3 | Treatment/ delivery | Very simple | Required special protocol/ transfection |
| 4 | Manufacturing feasibility | Can be obtained in purer form with simple GMP | More contaminant issues and complex GMP |
| 5 | Intellectual property | Less issues in licensing | Multiple royalties and licenses issues |
| 6 | Source | Usually chemical and secreted from microbes | Biologicals (e.g. recombinant in *E. coli*, yeast and cell lines) |
| 7 | Modification | Relatively easy | Difficult and challenging |
| 8 | Mechanism of action | Mostly inhibitors of different pathways | Expression of desired protein that performs its functions |
| 9 | Specificity | Required | Not required |
| 10 | Target | Defined | No target, but a complementation |
| 11 | Synergism | Highly synergistic | Less synergy |
| 12 | Off target effects | Usually high | Generally less |
| 13 | Elimination | Careful washing will remove ligands | Excessive protocol required to safely remove modifying agent |
| 14 | No. of molecules | 5–7 (at present) | ≤4 are sufficient |

discoveries will not only furnish stem cell-based techniques but also benefit cancer biology. Progression in small molecule generation and testing, library screening, tools to selectively identify and propagate stem cells in culture, and redifferentiation into other lineages, will provide reliable, safe and improved quality of stem cells to be used for different applications such as tissue regeneration, drug screening and disease modeling.

## 4. Expert opinion

The importance of iPSCs is evident by the number of citations of Takahashi and Yamanaka's 2006 paper in *Cell* describing the creation of iPSCs from mouse cells. Since then, it has been highly cited (>12,000 times) and has led to the awarding of a Nobel Prize in Medicine and Physiology in 2012. The importance has been appreciated in less than one decade signifying the great value of this discovery. This opened up countless avenues in stem cell and regenerative biology. Initially, iPSCs were created by retroviral transfection of four transcription factors (Oct4, Sox2, Klf4 and c-Myc) with limited efficiency. This method also exhibited the disadvantage of genetic manipulation, and cells created this way should not be used for clinical application. Therefore, alternative methods have been

explored and finally in 2013, with enormous efforts, chemical biology came up with an all-chemical approach for the generation of iPSCs. Stem cells generated in this way are safer for clinical application. In Table 3, a generalized comparison has been given to highlight the strengths and weaknesses of each subject in the expansion of stem cells and regenerative medicine fields.

However, there are still many obstacles remaining to clinically benefit from the potential of stem cell biology. The first and foremost important challenge is to erase the epigenetic memory in the form of DNA and histone methylation and histone acetylation. These epigenetic markers are used in the activation or repression of various genes required at different stages of cellular life and also to permanently silence irrelevant genes. Pluripotency factors are also permanently silenced to restrict the growth of cells once they become terminally differentiated. These markers are immensely problematic during transformation and an easy and reliable removal of these markers would be greatly desired. Triple helix therapy, for example, may be very useful in this context [107,108]; it is easy to manufacture and provides specificity and further modification of DNA is easy to improve its stability and function. This technique can be employed to selectively erase epigenetic markers to induce specific genes of interest.

The physiological microenvironment is quite different to the environment where cells have been cultured and therefore raises a few concerns. How well *in vitro* created cells can adjust to the physiological environment, whether cells will follow contact inhibition, cell-to-cell communications, and how well they respond to apoptotic signals remain to be determined. Such types of physiological phenomena must be extensively evaluated before iPSCs can be used in clinical settings. Various small molecules can be intelligently used to understand these kinds of issues.

Disease modeling using iPSCs has been proposed; here, cells would be isolated from patients and reprogrammed for unlimited supply. An example would be cells taken from patients inflicted with a neurological disease such as Parkinson's disease. The information obtained by studying specific signaling pathways in these cells would further unfold disease etiology, progression and its plausible cure. The main problem here is whether the cells produced this way can accurately recapitulate the disease and any drug testing can be safely translated for patients. Mature somatic cells will undergo complex alterations that may compromise disease modeling. Moreover, most of the genetic diseases have complicated etiology that further adds to the challenges of this proposed method. To avoid such complexities, methods should be sought to provide an unlimited source of cells while maintaining most of the original genetic signatures. This will least hamper the physiological phenomenon under study.

iPSC technologies proceed through mature-immature-mature (somatic-pluripotent-somatic) cell cycles where cell requirements change according to phenotype. This cycle is unimaginably complex, sequential and very well-organized. Activation of different pathways may heavily influence the cell state and may compromise the cell phenotype. When a cell goes through this process, even when it matures into the same cell type from which it originated, it will not harbor the same genetic signatures as it had in its previous mature form. This is a burning issue within the scientific community, regarding the similarities of iPSCs and ESCs, and conflicting studies are being reported. The real issue is, for a short time, cells may behave as similar/identical; however, long-term studies are necessary to validate these observations. Studies should be conducted on parallel cell systems where somatic cells, somatic cell-based iPSCs and ESCs are recruited and using combined techniques of protein and gene expression analysis to be employed to furnish the identities among these cells. Although iPSCs have been successfully incorporated into inner cell mass and gave rise to chimera, given the genetic differences between humans and mice, these results may not be consistent in humans and further studies are necessary to check the safety of these iPSCs. Moreover, complete transformation is unlikely to achieve in cells that may pose immunogenic risks for the recipient. Therefore, methods should be refined to convert and detect completely transformed iPSC cells. However, small molecules may pose an additional limitation on incompletely transformed cells, selectively removing them from the culture media and enriching and improving the quality of iPSC generation. Finally, over time, cells may shed its cell-surface receptors, so there should be a way to match the cell surface receptor density and variety compatible with the tissue where the modified cells supposed to transplant. This incompatibility may cause yet unknown responses in the receiving tissue, and in the long run, it may be a deteriorating physiological phenomenon.

Most of these complications can be resolved using small molecules that can fine-tune the cell response in a desired direction. Therefore, further compounds should be explored to suitably use the stem cells in clinical applications.

## Declaration of interest

S Choi was supported by the Korea Health Industry Development Institute under the Korea Health Technology Research & Development Project (grant number: HI14C1992); the National Research Foundation of Korea under the Mid-Career Researcher Program, funded by the Ministry of Education, Science, and Technology (grant number: NRF-2015R1A2A2A09001059); and further partially supported by a grant from the Priority Research Centers Program (grant number: NRF 2012-0006687). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

## References

Reference annotations
* Of interest
** Of considerable interest

1. Hu K, Slukvin I. Generation of transgene-free iPSC lines from human normal and neoplastic blood cells using episomal vectors. Methods Mol Biol. 2013;997:163–176.
2. Hou P, Li Y, Zhang X, et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science. 2013;341:651–654.
3. Kaji K, Norrby K, Paca A, et al. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature. 2009;458:771–775.
4. Yu J, Hu K, Smuga-Otto K, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science. 2009;324:797–801.
5. Kim D, Kim CH, Moon JI, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell. 2009;4:472–476.
6. Ban H, Nishishita N, Fusaki N, et al. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. Proc Natl Acad Sci U S A. 2011;108:14234–14239.
7. Zhou YY, Zeng F. Integration-free methods for generating induced pluripotent stem cells. Genomics Proteomics Bioinform. 2013;11:284–287.

** **An excellent review of non-integral methods for stem cell generation.**

8. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318:1917–1920.
9. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–676.
10. Feng B, Jiang J, Kraus P, et al. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. Nat Cell Biol. 2009;11:197–203.
11. Zhou H, Wu S, Joo JY, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell. 2009;4:381–384.
12. Stadtfeld M, Nagaya M, Utikal J, et al. Induced pluripotent stem cells generated without viral integration. Science. 2008;322:945–949.
13. Okita K, Nakagawa M, Hyenjong H, et al. Generation of mouse induced pluripotent stem cells without viral vectors. Science. 2008;322:949–953.
14. French AJ, Adams CA, Anderson LS, et al. Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts. Stem Cells. 2008;26:485–493.
15. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–872.
16. Huangfu D, Maehr R, Guo W, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol. 2008;26:795–797.
17. Liang G, Taranova O, Xia K, et al. Butyrate promotes induced pluripotent stem cell generation. J Biol Chem. 2010;285:25516–25521.
18. Bar-Nur O, Brumbaugh J, Verheul C, et al. Small molecules facilitate rapid and synchronous iPSC generation. Nat Methods. 2014;11:1170–1176.

* **Comprehensively reports the importance of small molecules in biology.**

19. Shi Y, Desponts C, Do JT, et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell. 2008;3:568–574.
20. Tachibana M, Amato P, Sparman M, et al. Human embryonic stem cells derived by somatic cell nuclear transfer. Cell. 2013;153:1228–1238.
21. Higuchi A, Ling QD, Kumar SS, et al. Generation of pluripotent stem cells without the use of genetic material. Lab Invest. 2015;95:26–42.
22. Zhu S, Li W, Zhou H, et al. Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell. 2010;7:651–655.
23. Hemberger M, Dean W, Reik W. Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. Nat Rev Mol Cell Biol. 2009;10:526–537.
24. Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. Cell. 2007;128:635–638.

** **A concise report regarding various features of epigenetics.**

25. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nat Rev Genet. 2013;14:204–220.
26. Mikkelsen TS, Hanna J, Zhang X, et al. Dissecting direct reprogramming through integrative genomic analysis. Nature. 2008;454:49–55.
27. Pawlak M, Jaenisch R. De novo DNA methylation by Dnmt3a and Dnmt3b is dispensable for nuclear reprogramming of somatic cells to a pluripotent state. Genes Dev. 2011;25:1035–1040.
28. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128:693–705.
29. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet. 2009;10:32–42.
30. Pandian GN, Nakano Y, Sato S, et al. A synthetic small molecule for rapid induction of multiple pluripotency genes in mouse embryonic fibroblasts. Sci Rep. 2012;2:544.

31. Pandian GN, Shinohara K, Ohtsuki A, et al. Synthetic small molecules for epigenetic activation of pluripotency genes in mouse embryonic fibroblasts. ChemBioChem. 2011;12:2822–2828.
32. Pandian GN, Sato S, Anandhakumar C, et al. Identification of a small molecule that turns ON the pluripotency gene circuitry in human fibroblasts. ACS Chem Biol. 2014;9:2729–2736.
33. Mali P, Chou BK, Yen J, et al. Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. Stem Cells. 2010;28:713–720.
34. Bradner JE, West N, Grachan ML, et al. Chemical phylogenetics of histone deacetylases. Nat Chem Biol. 2010;6:238–243.
35. Huangfu D, Osafune K, Maehr R, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol. 2008;26:1269–1275.
36. Itoh Y, Suzuki T, Miyata N. Isoform-selective histone deacetylase inhibitors. Curr Pharm Des. 2008;14:529–544.
37. Onder TT, Kara N, Cherry A, et al. Chromatin-modifying enzymes as modulators of reprogramming. Nature. 2012;483:598–602.
38. Nguyen AT, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. Genes Dev. 2011;25:1345–1358.
39. Chen J, Liu H, Liu J, et al. H3K9 methylation is a barrier during somatic cell reprogramming into iPSCs. Nat Genet. 2013;45:34–42.
40. Schotta G, Ebert A, Krauss V, et al. Central role of Drosophila SU(VAR)3-9 in histone H3-K9 methylation and heterochromatic gene silencing. Embo J. 2002;21:1121–1131.
41. Kubicek S, O'Sullivan RJ, August EM, et al. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Mol Cell. 2007;25:473–481.
42. Liu F, Wu P, Liu K Verticillin A Inhibition of Histone Methyltransferases. US20140161785. 2014.
43. Yuan Y, Wang Q, Paulk J, et al. A small-molecule probe of the histone methyltransferase G9a induces cellular senescence in pancreatic adenocarcinoma. ACS Chem Biol. 2012;7:1152–1157.
44. Vedadi M, Barsyte-Lovejoy D, Liu F, et al. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nat Chem Biol. 2011;7:566–574.
45. Chang Y, Zhang X, Horton JR, et al. Structural basis for G9a-like protein lysine methyltransferase inhibition by BIX-01294. Nat Struct Mol Biol. 2009;16:312–317.
46. Liu F, Chen X, Allali-Hassani A, et al. Discovery of a 2,4-diamino-7-aminoalkoxyquinazoline as a potent and selective inhibitor of histone lysine methyltransferase G9a. J Med Chem. 2009;52:7950–7953.
47. Yuan X, Wan H, Zhao X, et al. Brief report: combined chemical treatment enables Oct4-induced reprogramming from mouse embryonic fibroblasts. Stem Cells. 2011;29:549–553.
48. Li W, Zhou H, Abujarour R, et al. Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. Stem Cells. 2009;27:2992–3000.
49. Cheng D, Yadav N, King RW, et al. Small molecule regulators of protein arginine methyltransferases. J Biol Chem. 2004;279:23892–23899.
50. Kingston WR. A clinical trial of an antidepressant, tranylcy-promine ("Parnate"). Med J Aust. 1962;49:1011–1012.
51. Esteban MA, Wang T, Qin B, et al. Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. Cell Stem Cell. 2010;6:71–79.
52. Blaschke K, Ebata KT, Karimi MM, et al. Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. Nature. 2013;500:222–226.
53. McCabe MT, Ott HM, Ganji G, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature. 2012;492:108–112.
54. Koche RP, Smith ZD, Adli M, et al. Reprogramming factor expression initiates widespread targeted chromatin remodeling. Cell Stem Cell. 2011;8:96–105.
55. Fragola G, Germain PL, Laise P, et al. Cell reprogramming requires silencing of a core subset of polycomb targets. PLoS Genet. 2013;9:e1003292.
56. Mansour AA, Gafni O, Weinberger L, et al. The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. Nature. 2012;488:409–413.
57. Rao RA, Dhele N, Cheemadan S, et al. Ezh2 mediated H3K27me3 activity facilitates somatic transition during human pluripotent reprogramming. Sci Rep. 2015;5:8229.
58. Lee J, Xia Y, Son MY, et al. A novel small molecule facilitates the reprogramming of human somatic cells into a pluripotent state and supports the maintenance of an undifferentiated state of human pluripotent stem cells. Angew Chem Int Ed Engl. 2012;51:12509–12513.
59. Li W, Tian E, Chen ZX, et al. Identification of Oct4-activating compounds that enhance reprogramming efficiency. Proc Natl Acad Sci U S A. 2012;109:20853–20858.
60. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer. 2009;9:28–39.
61. Zebisch A, Czernilofsky AP, Keri G, et al. Signaling through RAS-RAF-MEK-ERK: from basics to bedside. Curr Med Chem. 2007;14:601–623.
62. Silva J, Barrandon O, Nichols J, et al. Promotion of reprogramming to ground state pluripotency by signal inhibition. PLoS Biol. 2008;6:e253.
63. Chen S, Do JT, Zhang Q, et al. Self-renewal of embryonic stem cells by a small molecule. Proc Natl Acad Sci U S A. 2006;103:17266–17271.
64. Claassen DA, Desler MM, Rizzino A. ROCK inhibition enhances the recovery and growth of cryopreserved human embryonic stem cells and human induced pluripotent stem cells. Mol Reprod Dev. 2009;76:722–732.
65. Xu Y, Zhu X, Hahm HS, et al. Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc Natl Acad Sci U S A. 2010;107:8129–8134.
66. Staerk J, Lyssiotis CA, Medeiro LA, et al. Pan-Src family kinase inhibitors replace Sox2 during the direct reprogramming of somatic cells. Angew Chem Int Ed Engl. 2011;50:5734–5736.
67. Li Z, Rana TM. A kinase inhibitor screen identifies small-molecule enhancers of reprogramming and iPS cell generation. Nat Commun. 2012;3:1085.
68. Wu D, Pan W. GSK3: a multifaceted kinase in Wnt signaling. Trends Biochem Sci. 2010;35:161–168.

69. Cohen P, Goedert M. GSK3 inhibitors: development and therapeutic potential. Nat Rev Drug Discov. 2004;3:479–487.
70. Lyssiotis CA, Foreman RK, Staerk J, et al. Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. Proc Natl Acad Sci U S A. 2009;106:8912–8917.
71. Ten Berge D, Kurek D, Blauwkamp T, et al. Embryonic stem cells require Wnt proteins to prevent differentiation to epiblast stem cells. Nat Cell Biol. 2011;13:1070–1075.
72. Marson A, Foreman R, Chevalier B, et al. Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell Stem Cell. 2008;3:132–135.
73. Holland JD, Klaus A, Garratt AN, et al. Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol. 2013;25:254–264.
74. Ho R, Papp B, Hoffman JA, et al. Stage-specific regulation of reprogramming to induced pluripotent stem cells by Wnt signaling and T cell factor proteins. Cell Rep. 2013;3:2113–2126.
75. Muralidharan S, Hanley PJ, Liu E, et al. Activation of Wnt signaling arrests effector differentiation in human peripheral and cord blood-derived T lymphocytes. J Immunol. 2011;187:5221–5232.
76. Takada R, Satomi Y, Kurata T, et al. Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. Dev Cell. 2006;11:791–801.
77. Chen B, Dodge ME, Tang W, et al. Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol. 2009;5:100–107.
78. Kirby LA, Schott JT, Noble BL, et al. Glycogen synthase kinase 3 (GSK3) inhibitor, SB-216763, promotes pluripotency in mouse embryonic stem cells. PLoS One. 2012;7:e39329.
79. Meijer L, Skaltsounis AL, Magiatis P, et al. GSK-3-selective inhibitors derived from Tyrian purple indirubins. Chem Biol. 2003;10:1255–1266.
80. Sato N, Meijer L, Skaltsounis L, et al. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med. 2004;10:55–63.
81. Ding S, Wu TY, Brinker A, et al. Synthetic small molecules that control stem cell fate. Proc Natl Acad Sci U S A. 2003;100:7632–7637.
82. Varga AC, Wrana JL. The disparate role of BMP in stem cell biology. Oncogene. 2005;24:5713–5721.
83. Li R, Liang J, Ni S, et al. A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. Cell Stem Cell. 2010;7:51–63.
84. Ichida JK, Blanchard J, Lam K, et al. A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell. 2009;5:491–503.
85. Maherali N, Hochedlinger K. Tgfbeta signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. Curr Biol. 2009;19:1718–1723.
86. Inman GJ, Nicolas FJ, Callahan JF, et al. SB-431542 is a potent and specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Mol Pharmacol. 2002;62:65–74.
87. Lin T, Ambasudhan R, Yuan X, et al. A chemical platform for improved induction of human iPSCs. Nat Methods. 2009;6:805–808.
88. Peng SB, Yan L, Xia X, et al. Kinetic characterization of novel pyrazole TGF-beta receptor I kinase inhibitors and their blockade of the epithelial-mesenchymal transition. Biochemistry. 2005;44:2293–2304.
89. Kimura T, Kaga Y, Sekita Y, et al. Pluripotent stem cells derived from mouse primordial germ cells by small molecule compounds. Stem Cells. 2015;33:45–55.
90. Samavarchi-Tehrani P, Golipour A, David L, et al. Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. Cell Stem Cell. 2010;7:64–77.
91. Miyabayashi T, Yamamoto M, Sato A, et al. Indole derivatives sustain embryonic stem cell self-renewal in long-term culture. Biosci Biotechnol Biochem. 2008;72:1242–1248.
92. Hasegawa K, Yasuda SY, Teo JL, et al. Wnt signaling orchestration with a small molecule DYRK inhibitor provides long-term xeno-free human pluripotent cell expansion. Stem Cells Transl Med. 2012;1:18–28.
93. Ichida JK, Tcw J, Williams LA, et al. Notch inhibition allows oncogene-independent generation of iPS cells. Nat Chem Biol. 2014;10:632–639.
94. Liu Y, Li P, Liu K, et al. Timely inhibition of Notch signaling by DAPT promotes cardiac differentiation of murine pluripotent stem cells. PLoS One. 2014;9:e109588.
95. Wang W, Yang J, Liu H, et al. Rapid and efficient reprogramming of somatic cells to induced pluripotent stem cells by retinoic acid receptor gamma and liver receptor homolog 1. Proc Natl Acad Sci U S A. 2011;108:18283–18288.
96. Yoshida Y, Takahashi K, Okita K, et al. Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell. 2009;5:237–241.
97. Panopoulos AD, Yanes O, Ruiz S, et al. The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming. Cell Res. 2012;22:168–177.
98. Shi Y, Do JT, Desponts C, et al. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. Cell Stem Cell. 2008;2:525–528.
99. Shi Y, Zou M, Baitei EY, et al. Cannabinoid 2 receptor induction by IL-12 and its potential as a therapeutic target for the treatment of anaplastic thyroid carcinoma. Cancer Gene Ther. 2008;15:101–107.
100. Moon JH, Heo JS, Kim JS, et al. Reprogramming fibroblasts into induced pluripotent stem cells with Bmi1. Cell Res. 2011;21:1305–1315.
101. Chen T, Shen L, Yu J, et al. Rapamycin and other longevity-promoting compounds enhance the generation of mouse induced pluripotent stem cells. Aging Cell. 2011;10:908–911.
102. Ying QL, Wray J, Nichols J, et al. The ground state of embryonic stem cell self-renewal. Nature. 2008;453:519–523.
103. Li Y, Zhang Q, Yin X, et al. Generation of iPSCs from mouse fibroblasts with a single gene, Oct4, and small molecules. Cell Res. 2011;21:196–204.

104. Insel PA, Ostrom RS. Forskolin as a tool for examining adenylyl cyclase expression, regulation, and G protein signaling. Cell Mol Neurobiol. 2003;23:305–314.
105. Chiang PK. Biological effects of inhibitors of S-adenosylhomocysteine hydrolase. Pharmacol Ther. 1998;77:115–134.
106. Gordon RK, Ginalski K, Rudnicki WR, et al. Anti-HIV-1 activity of 3-deaza-adenosine analogs. Inhibition of S-adenosylhomocysteine hydrolase and nucleotide congeners. Eur J Biochem. 2003;270:3507–3517.
107. Cooney M, Czernuszewicz G, Postel EH, et al. Site-specific oligonucleotide binding represses transcription of the human c-myc gene in vitro. Science. 1988;241:456–459.
108. Jain A, Wang G, Vasquez KM. DNA triple helices: biological consequences and therapeutic potential. Biochimie. 2008;90:1117–1130.
109. Morrisey EE MicroRNA induction of pluripotential stem cells and uses thereof. US20130035374A1. 2013.
110. Saha K, Mei Y, Bogatyrev SR, et al. Substrates and methods for culturing stem cells. US 20100273259A1. 2010.
111. Hill BL, Lavau CP, Young JC Promotion of self-renewal and improved gene transduction of hematopoietic stem cells by histone deacetylase inhibitors. WO2000023567A2. 2000.
112. Jang YY, Sharkis SJ Compositions and methods for somatic tissue induced pluripotent stem cells having an endoderm origin. WO2011084747A2. 2011.
113. Hai Z, Yu Z, Jia T, et al. Culture solution for promoting in-vitro growth of porcine somatic cell cloned embryos. CN103952368A. 2014.
114. Song Y, Chen P, Diao J, et al. Selective inhibitors of histone methyltransferase dot1l. US20140100184A1. 2014.
115. Shi Y, Lan F Histone demethylation proteins and methods of use thereof. WO2009114011A1. 2009.
116. Kuntz KW, Huang KC, Choi HW, et al. Salt form of a human hi stone methyltransf erase ezh2 inhibitor. WO2013155317A1. 2013.
117. Shi Y, Desponts C, Ding S Combined chemical and genetic approaches for generation of induced pluripotent stem cells. WO2009117439A2. 2009.
118. Liu F, Wu P, Liu K Verticillin A Inhibition of Histone Methyltransferases. US20140161785A1. 2014.
119. Deng-Hong K, Zhang Y, Hou P, et al. Method, kit and applications of preparing pluripotent stem cells through small-molecule compound treatment. WO2015003643A1. 2015.
120. Shi Y, Yip ML, Li W Enhancers of induced pluripotent stem cell reprogramming. US20140154805A1. 2014.
121. Li W, Lin T, Ding S Improved methods of generating pluripotent stem cells. WO2011109015A1. 2011.
122. Pei D, Chen J, Liu J Additif pour milieu de culture et ses utilisations. WO2011134210A1. 2011.
123. Kahn M, Hasegawa K, Teo JL, et al. Method for formation of induced pluripotent stem cells. WO2011019957A1. 2011.
124. Eilertsen KJ, Power RA Reprogramming a Cell by Inducing a Pluripotent Gene Through Use of a Small Molecule Modulator. US20090253203A1. 2009.
125. Cho YS, Lee K, Lee JW Novel low-molecule compound for promoting pluripotent stem cell generation, maintenance, and proliferation, and composition and culturing method containing same. WO2013180350A1. 2013.
126. Mrowka R, Wolf G, Wolfl S, et al. Method for activating oct4 for induction of pluripotent stem cells. WO2014000814A1. 2014.
127. Hidenori A, Akihiro U, Takumi M iPS progenitor cells and iPS cells WO2014167866A1. 2014.
128. Bamba M, Furuyama H, Sakamoto T, et al. Dihydropyrimidopyrimidine derivative. WO2010067886A1. 2010.
129. Sasai Y, Watanabe K Stem cell culture medium and method. WO2008035110A1. 2008.
130. Egli DM, Noggle SA, Eggan KC Method for producing pluripotent stem cells. WO2012071393A3. 2014.
131. Rana TM Promoting cellular reprogramming methods. WO2012166973A1. 2012.
132. Meng Z. Application of small molecule compound. CN103361305A. 2013.
133. Lin C, Ding S Small molecule cellular reprogramming to generate neuronal cells. WO2015041809A3. 2015.
134. Lum L, Roth M, Chen B, et al. Wnt protein signalling inhibitors. WO2009155001A3. 2010.
135. Chevalier B, Marson A, Young RA, et al. Wnt pathway stimulation in reprogramming somatic cells. WO2009032194A1. 2009.
136. Brivanlou A, Sato N, Meijer L Maintenance of Embryonic Stem Cells by the GSK-3 Inhibitor 6-Bromoindirubin-3′-Oxime. US20090111177A1. 2009.
137. Perry JM, Li L, Grindley JC Methods and compositions for self-renewal of stem cells. WO2008133904A1. 2008.
138. Hochedlinger K, Stadtfeld M Tgf-beta receptor inhibitors to enhance direct reprogramming. US20140120621A1. 2014.
139. Li W, Zhou H, Ding S Generation and maintenance of stem cells. WO2010077955A1. 2011.
140. Lin T, Ding S Induction of pluripotent cells. US20120264218. 2015.
141. Kiwamu I, Kenichi M, Yuki H, et al. Method for producing a human corneal epithelial sheet. WO2014142038A1. 2014.
142. Kaether C, Krämer A Inhibitors of the notch signalling pathway and secretion for use in medicine. WO2013178821A1. 2013.
143. Grynspan F, Hasson A Methods for ex-vivo expanding stem/progenitor cells. WO2005007799A3. 2005.
144. Liu P, Wang W, Yang J Cells and methods for obtaining them. WO2011027180A1. 2011.
145. Zhu S, Ding S Reprogramming cells. WO2011123572A1. 2011.
146. Musick J Induced puripotent stem cells and related methods. WO2011062963A3. 2011.
147. You S, Moon JH, Kim JS, et al. Composition for reprogramming somatic cells to generate induced pluripotent stem cells, comprising oct4 in combination with bmi1 or its upstream regulator, and method for generating induced pluripotent stem cells using the same. US8772031A2. 2011.
148. Pei G, Yu J, Zhao J, et al. Organism lifetime based method for screening compounds capable of improving generation efficiency of induced pluripotent stem cells. CN102758000. 2015.
149. Deng H, Zhao Y, Hou P, et al. Compositions and methods for reprograming non- pluripotent cells into pluripotent stem cells. WO2015003643A1. 2015.