Stem cells, cell-based assays and the world of small molecules

Stem cells remain a hot topic in academia and industry alike, and with the potential to cause a paradigm shift where many believe in their ability to differentiate into a variety of valuable cell types. They unleashed a screening race using complex cell-based assays to evaluate cytotoxicity profiles of chemical entities, and to ultimately discover novel modulators of cell fate to be used in stem cell-based therapies. A comprehensive small molecule catalogue of modulators is emerging with no obvious value proposition as to their legitimacy towards clinical applications. Almost two decades of experimentation later, have stem cells maintained their pole position at the forefront of contemporary personalised medicine?

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Embryonic stem cells were first introduced into the scientific community in 1981 when they were isolated from mice[1], setting forth a cascade of paradigm shifting scientific events. More than a decade afterwards, in the late 1990s, human embryonic stem cells were isolated[2] and for the first time, their potential seemed to be 'limitless'. The ability of stem cells to become any cell type in the body offered great promise in regenerative medicine. Particularly, in devastating diseases such as diabetes and neurological disorders, which are among and remain the hardest diseases to treat today. Stem cells also held the premise in drug discovery providing valuable information on toxicity and disease profiling. Still, after nearly two decades of experimentation, much remains unknown about stem cell fate and its future in personalised medicine.

During embryonic development, after the fertilisation process, meiosis occurs and results in a series of cleavage steps resulting in the morula. The morula is comprised of totipotent cells that give rise to the blastocyst. The inner cells of the blastocyst are pluripotent and give rise to stem cells that can differentiate into any cell type in the body, thus, their potential in a variety of diseases. The signalling mechanisms by which this occurs are very complex and demonstrate the enormity of interactions in stem cell pluripotency and differentiation. The potential of stem cells prompted an interest in understanding the differentiation and self-renewal properties and, as a result, many researchers turned to small molecules – for their pharmacological control and ease of use. The first small molecule found to play a role in differentiation of stem cells was retinoic acid[3]. In 1978 Retinoic acid sparked the interest and in 1998 the fire was ignited again when human embryonic stem cells were isolated[4]. The use of small molecules in determining stem cell fate became a fad demonstrated by the huge increase in publications since 1998 (**Figure 1**). Despite such an increase,

there has yet to be a small molecule in development; and with the potential of entering the clinic to treat patients using stem cell therapy. As an example, retinoic acid, one of the oldest and most popular differentiating small molecules, has yet to demonstrate its value in the clinic. As the need to discover small molecule(s) that may have potential clinical use arose, many groups and companies turned their focus on large chemical library screening using innovative and complex high content assay approaches built on robust high-throughput screening (HTS) platforms[5].

Several high throughput screens have been conducted to date to identify small molecules that play a role in stem cell differentiation and self-renewal. Investigators were successful at finding only a handful of small molecules that played a role in differentiation. Many challenges were encountered by many and still surround the amenability and heterogeneity of stem cell assays in HTS. One of the biggest challenges is the disparity between embryonic stem cell models, ie mouse Embryonic Stem Cells (mESCs) versus human Embryonic Stem Cells (ESCs). Even with the latest techniques using nuclear transfer cloning and fusion with ES cells, the difficulties remain. Such difficulties, along with the lack of disease relevant models for many diseases, impelled the creation of induced pluripotent stem cells (iPSCs) with the ultimate goal to solve the discovered inherent problems with stem cells.

The generation of iPSCs from mouse embryonic and adult fibroblasts was done by introducing four factors: Oct3/4, Sox2, Klf4, and c-Myc[6]. The introduction of iPSCs allowed many to work with disease relevant models and opened a whole new avenue in stem cell research. Not only do iPSCs provide a disease relevant model, iPSCs are readily amenable to HTS (Ramirez et al, unpublished observations). However, the introduction of the maintenance factors renders the cell models genomically unstable. Thus, one must question whether there is really a future for small molecules in personalised medicine based on either hESCs or iPSCs.

HTS of stem cells: an emerging catalogue of molecules

Although the concept of using hESCs in HTS is attractive to many, there are still many challenges that need to be overcome. These challenges include, but are not limited to: sufficient source material for the isolation, the use of a feeder layer for expansion, and spontaneous differentiation. As a result, many investigators have used mESCs as surrogates for hESCs.

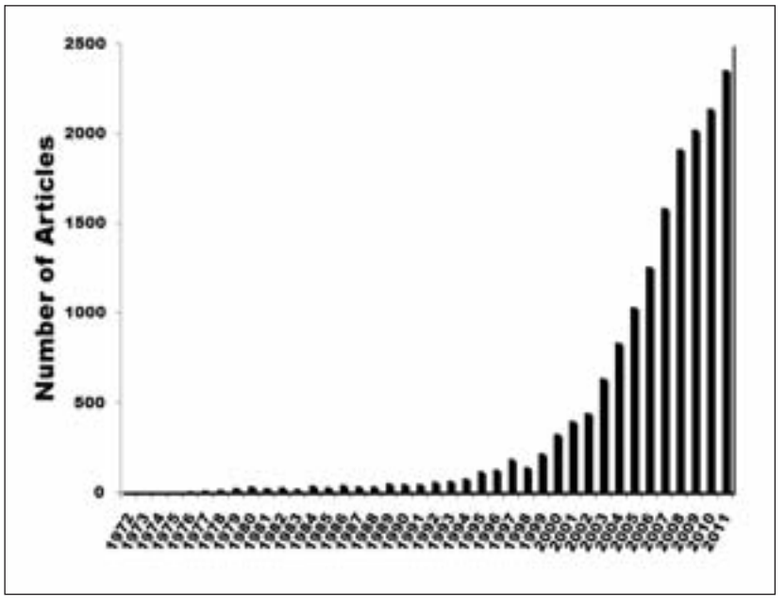

Figure 1
Number of published articles on stem cell research between 1972 and 2011. After the isolation of hESCs in 1998, the number of stem cell articles increased and has continued an upward progression. The lack of progression in personalised medicine has prompted the question: will this upward progression at some point plateau and ultimately decline?

Unlike the growth requirements and delicacy of hESCs, mESCs can be grown in the absence of feeder cells using leukaemia inhibitory factor (LIF)[7]; making them amenable to scale up for HTS. In addition to LIF, other growth factors, such as serum, are included in the media of the mESCs for long-term expansion. However, investigators have found that BMP4, which blocks differentiation, can be used to replace serum[8]. While the use of a human model is highly desirable by many investigators, the process of hESC isolation and expansion can be seen to many as being tedious and at times expensive. As a result, mESCs have been widely used in small molecule screening campaigns. In 2003, the first high-throughput screen run on mESCs led to the identification of TWS119, a small molecule found to induce neuronal differentiation in mESCs[9]. This screen set forth a series of cascading events resulting in a vast list of small molecules capable of differentiating neuronal cells, hematopoietic cells, cardiomyocytes and oesteocytes.

Since then, a number of small molecules have been shown to play a role in both hESC and mESC fates using HTS as well as other methods. These identified compounds are summarised in **Table 1**. Some of the small molecules shown to have an effect on the differentiation of stem cells also showed value in other areas. Such is the case for the anticancer drugs imatinib, bortezomib and geldanamycin. Imatinib, also known as Gleevec, is a well known BCR-ABL and PDGFR inhibitor and plays a role in cell viability, cell proliferation and

Table 1: Eighty three small molecules reported as modulators of stem cell fate. To date, 83 compounds have been published as having some effect on stem cell fate ranging from differentiation followed by death to self renewal. A beginning of a catalogue of small molecules for stem cell fate modulation

COMPOUND	PROPERTY	FDA APPROVED	REFERENCE/S
(-)-Indolactam V	Differentiation	No	Chen et al, 2009
(+)-Cholesten-3-one	Differentiation	No	Chen et al, 2010
5-Azacytidine	Differentiation	Yes (Anti-neoplastic)	Lassar et al,1986
7-Deshydroxypyrogallin-4-carboxylic acid	Differentiation	No	Wu et al, 2009
A-83-01	Reprogramming	No	Yuan et al, 2011
AICAR	Differentiation	No	Zang et al, 2008
AMI-5	Reprogramming	No	Yuan et al, 2011
Ascorbic acid	Differentiation	No	Lee et al, 2000
Baicalin	Differentiation	No	Li et al, 2011
BayK8644	Reprogramming	No	Shi et al, 2008
BEZ235 (NVP-BEZ235)	Differentiation	No	Martin et al, 2010
BIO (6-bromoindirubin-3 -oxime)	Self-renewal	No	Sato et al, 2004
BIX-01294	Reprogramming	No	Kubicek et al, 2007
Bortezomib	Differentiation	Yes (Anti-neoplastic)	Mukherjee et al, 2008
Butyrate	Differentiation	No	Rambhatla et al, 2003
Cardiogenol C	Differentiation	No	Wu et al, 2004
CCG-1423	Differentiation	No	Mae et al, 2010
CHIR99021	Self-renewal	No	Tsutsui et al, 2011
Cholesterol myristate	Differentiation	No	Chen et al, 2010
CKI-7	Differentiation	No	Osakada et al, 2009
Cyclopamine	Differentiation	No	Lee et al, 2006
Cyclosporin	Differentiation	Yes (Immunosupressant)	Sachinidis et al, 2006
DAPT	Differentiation	No	Reh et al, 2010
Dexamethasone	Differentiation	Yes (Corticosteroid)	Shirahashi et al, 2004
Dibutyryl cAMP (DBcAMP)	Differentiation	No	Kim et al, 2010
Dorsomorphin	Differentiation	No	Hao et al, 2008
EC23	Differentiation	No	Christie et al, 2010
Fluoxetine	Differentiation	Yes(Anti-psychotic)	Kusakawa et al, 2010
Flurbiprofen	Differentiation	Yes (NSAID)	Desbordes et al, 2008
Forskolin	Differentiation	No	Sachinidis et al, 2006
Gatifloxacin	Differentiation	Yes (Antibiotic)	Desbordes et al, 2008
Geldanamycin	Self-renewal	No	Xiong et al, 2009
HA-1077	Self-renewal/Differentiation	No	Damoiseaux et al, 2009 & Zhao et al, 2010
Icaritin	Differentiation	No	Zhu et al, 2007
ID-8	Self-renewal	No	Miyabayashi et al, 2008
Imatinib	Differentiation	Yes (Anti-neoplastic)	Coppo et al, 2009
IPA-3	Differentiation	No	Deacon et al, 2008
IQ-1	Differentiation	No	Miyabayashi et al, 2007
Isoxazole	Differentiation	No	Schneider et al, 2008
Kenpaullone	Reprogramming	No	Lyssiotis et al, 2009
LY294002	Differentiation	No	Hori et al, 2002
Neuropathiazol	Differentiation	No	Warashina et al, 2006
NR2E1 (TLX)	Self-renewal	No	Qu et al, 2010
Nutlin-3	Differentiation	No	Maimets et al, 2008
Ouabain	Differentiation	No	Lee et al, 2011
Pam3Cys	Differentiation	No	Taylor et al, 2010
Parnate (Tranylcypromine)	Differentiation	Yes (Anti-depressant)	Zhou et al, 2010
PD0325901	Self-renewal	No	Tsutsui et al, 2008
PD169316	Differentiation	No	Duval et al, 2004
PD173074	Differentiation	No	Chan et al, 2010
PD98059	Self-renewal	No	Qi et al, 2004
Phenamil	Differentiation	No	Kye et al, 2009
Phenazopyridine	Differentiation	Yes (Analgesic)	Suter et al, 2009
Phosphoserine (P-Ser)	Differentiation	No	Saxe et al, 2007
Pinacidil	Self-renewal	No	Andrews et al, 2010
Purmorphamine	Differentiation	No	Hu et al, 2010
Pyrintegrin(Ptn)	Self-renewal	No	Xu et al, 2010
Rapamycin	Differentiation	Yes (Immunosupressant)	Lee et al, 2010

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COMPOUND	PROPERTY	FDA APPROVED	REFERENCE/S
RepSox	Reprogramming	No	Ichida et al, 2009
Retinoic acid	Differentiation	Yes (Dermatological)	Smith,1991
Reversine	Reprogramming	No	Chen et al, 2004
Rosiglitazone	Differentiation	Yes(Anti-hyperglycemic)	Xiong et al, 2005
SAG	Differentiation	No	Wada et al, 2009
SB203580	Differentiation	No	Duval et al, 2004
SB216763	Differentiation	No	Lambertini et al, 2010
SB431542	Differentiation	No	Morizane et al, 2011
SC1	Self-renewal	No	Chen et al, 2006
Sinomenine	Differentiation	No	Desbordes et al, 2008
Stauprimide	Differentiation	No	Zhu et al, 2009
SU5402	Differentiation	No	Kiyonari et al, 2010
SU6656	Self-renewal	No	Annerén et al, 2004
Suberoylanilide hydroxamic acid (SAHA)	Differentiation	No	Feng et al, 2009
Theanine	Differentiation	No	Desbordes et al, 2008
Thiazovivin/Tzv	Self-renewal	No	Xu et al, 2010
Trichostatin A (TSA)	Differentiation	No	Balasubramaniyan et al, 2006
Troglitazone	Differentiation	No	Chen et al, 2010
TWS119	Differentiation	No	Ding et al, 2003
U0126	Self-renewal	No	Li et al, 2011
Valproic acid	Differentiation	Yes (Anti-convulsant)	Dong et al, 2009
Verapamil	Differentiation	Yes (Anti-hypertensive)	Sachinidis et al, 2006
WHI-P131	Differentiation	No	Duval et al, 2004
XAV939	Differentiation	No	Wang et al, 2011
Y27632	Self-renewal	No	Hotta et al, 2009

apoptosis[10]. Similarly, bortezomib, a proteasome inhibitor, plays a role in apoptosis, cell viability, cell proliferation and cell adhesion[11]. With this in mind, one may come to the conclusion that the use of small molecules with known clinical use to treat cancer or other diseases may only cause further genomic instability; potentially leading to the development of additional unwanted cancers. While mESCs have proved useful in HTS looking for small molecules that exert an effect on stem cell fate, much has yet to be learned on the diversity of the different populations and the potential differences in the effects of the molecules by species.

Similarly, many investigators have purposely created genomic instability by introducing certain mutations in stem cell systems in order to model diseases. However, with the growing ethical dilemmas presented when using embryonic stem cells and the need to model diseases that do not have a particular phenotype associated with it, induced pluripotent stem cells (iPSCs) were developed and to date have been the most amenable stem cell type to HTS (**Figure 2**).

Induced pluripotent stem cells were introduced in 2007 when 'stem cell-like' human fibroblasts were derived using the factors Oct3/4, Sox2, Klf4, and c-Myc[12]. As a result, many used this to their advantage to model pathogenesis using viral vectors and plasmids to introduce such factors. In addition to fibroblasts, liver and epithelial cells have been reprogrammed into iPSCs[13]. Induced pluripotent stem cells have been generated as models for amyotrophic lateral sclerosis, Parkinson's disease, spinal muscular atrophy and familial dysautonomia[14-17]. In addition to the modelling of diseases, iPSCs require less maintenance than embryonic stem cells. They lack the need of a feeder layer and can be expanded using laminin, fibronectin and polyornithine[16]. Furthermore, iPSCs can be used to model diseases in which there is no phenotypic change in the cells of the disease[16]. One of the major challenges of iPSCs is the introduction of such factors as c-myc that will ultimately cause genomic instability in the cells. With this in mind, one has to wonder whether iPSCs will ever be used for personalised methods. New methods using episomal vectors to reprogramme cells may shed some light in this avenue; however, much remains unknown about their use in reprogramming cells. As such, investigators are in search of small molecules that would reprogramme cells without the introduction of exogenous factors. As a result, further advancement in HTS of stem cells is needed.

Stem cell small molecule therapeutics

The therapeutics race to find a drug or a combination of drugs that further the potential of regenerative medicine continues. Small molecules can target signalling pathways, receptors, genes and mechanisms instrumental in the manipulation of stem cell fate. Although small molecules have been helpful in understanding the fundamental biology of stem cells, the utility in governing stem cell fate *in vivo* is far from being understood. There is a complexity of intrinsic and extrinsic factors at play that is only beginning to be teased out.

The development and discovery of small molecules have targeted three main biological properties of stem cells: their ability to differentiate, self-renew and reprogramme. Self-renewal assays were first seen in mESCs while efforts were made to find small molecules that had an effect on the LIF or BMP signalling pathways. This led to the discovery of BIO and SB216763, found to inhibit GSK-3[18,19]. Interestingly enough, while these small molecules communicated via similar signalling pathways, they not only exhibited self-renewal properties but also demonstrated differentiation properties. This clearly demonstrates the ability of small molecules to affect stem cell fate not only across species but also within the cell itself.

With advances in high content screening, Oct4, a marker of pluripotency, has been pinned as a key player in the development of fluorescent-based assays to find small molecules promoting self-renewal. Using this method, SC1 was identified. Unexpectedly SC1, an inhibitor of both RasGAP and ERK1, lacked any association with the LIF or BMP signalling pathways while maintaining its ability to promote self renewal in embryonic stem cells[20]. In our lab using a similar method with Oct4, we were able to identify theanine, sinomenine, gatifloxacin and flurbiprofen as regulators of self-renewal in hESCs. Gatifloxacin, an antibiotic, was the only compound found to have an effect on mESCs among these compounds, demonstrating the vast differences between species[21]. Taken together, these small molecules have shown that several different signalling pathways appear to be involved in stem cell self-renewal. Thus, there is lack of selectivity within the compounds revealing the need to find signalling molecules that play a more centralised role in cell self-renewal.

Many are hopeful that the pluripotent stem cells will act as a resource for renewable cells and tissue with the potential to aid in treatment of diseases and injuries such as Parkinson's disease, ALS, spinal cord injury and arthritis. A large number of small molecules have provided insight towards understanding the intricate system at play in the differentiation of embryonic stem cells; yet at present, it remains but a glimpse. They have targeted several pathways such as Wnt, Hedgehog and Notch, which are at the epicentre of developmental biology and therapeutic investigations. One of the key small molecules targeting the Wnt pathway is TWS119, the first small molecule identified via HTS. TWS119 is a GSK-3β inhibitor, which communicates via the Wnt signalling pathway and promotes the differentiation of embryonic cells to neuronal cells. Subsequently, countless discoveries of small molecules were made (**Table 1**). In addition to their role as anti-neoplastics, drugs such as imatinib and bortezomib have been shown to have differentiating potential. Bortezomib has been shown to induce osteoblast formation through activation of beta-catenin/TCF signalling. Imatinib, a signal transduction inhibitor, used to treat chronic myelogenous leukaemia (CML) has been shown to be useful in maintaining viability and differentiation potential in embryonic stem cells. In comparison to the other properties, small molecules affecting differentiation well outnumber those found to affect the self-renewal and reprogramming capabilities of embryonic stem cells. Thus small molecules are being praised for their differentiation abilities, yet their success *in vivo* has yet to be achieved.

Spontaneous differentiation of stem cells has been a great challenge to overcome. Most recently, Rho kinase inhibitors (ROCKi) have been found to preserve pluripotency in embryonic stem cells[22] and have frequently been used to maintain self-renewal in a variety of screens including genome-wide RNAi screens[23]. However, the mechanisms by which ROCKi and other kinase inhibitor small molecules promote pluripotency are still poorly understood. The long-term effects of these kinase

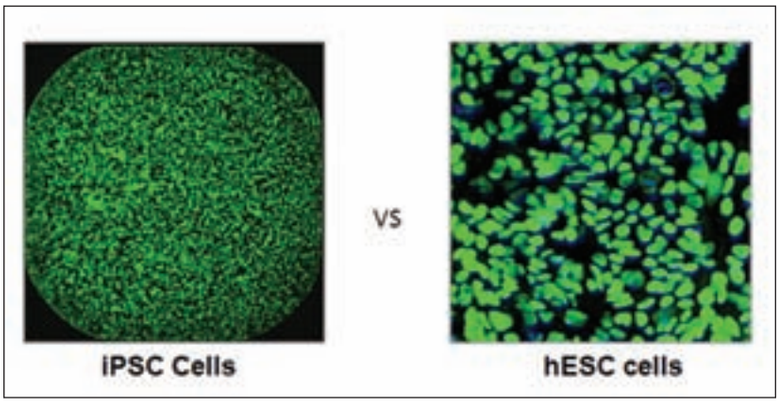

Figure 2
Cell-based assays resulting in automated microscopy images of iPSC and hESC cells seeded in 384-well microtiter plates. A) Whole-well image of iPSCs cells stained with Calcein taken at 4X magnification and using an InCell Analyzer 2000. Using an in-house coating method, iPSCs were able to attach and proliferate throughout the course of the assay. This allowed for accurate hit determination and confirmation. iPSC cells do not present a phenotype to measure in contrast to hESC cells. B) Representative image of hESC cells stained for OCT4 taken at 40X magnification and using an InCell Analyzer 3000 (Praelux Unit)

inhibitors remains a mystery and studies are needed to be certain genomic instability is not a consequence of using these small molecules.

We are far from the point of successful transfer of viable stem cells derived from small molecule altered embryonic stem cells. The challenges that lay ahead are largely encompassed by genomic instability and rejection. For this reason, the reprogramming capabilities of somatic cells have been exploited. The induction of pluripotent stem cells from adult fibroblast in 2006 has provided another potential platform for the creation of disease models and more personalised treatment. However, similar to the introduction of mutated genes in ES cells, the introductions of retroviral and lentiviral vectors cause genomic instability. Thus, investigators looked to HTS and complex cell-based assays to find small molecules that would reprogramme cells into iPS cells free of exogenous DNA. The small molecule reversine was found to be capable of such a feat[9] and later confirmed[24]. More recent studies have shown that a combination of BIX-02194 and BayK8644 and kenpaullone can promote the expression of Klf4 and Sox2, respectively, factors necessary for reprogramming[25,26]. Still, further investigation is needed to determine the mechanism by which reprogramming occurs within the cell. At the present time, the transitional leap to the clinic appears in the very distant future; that is if we can overcome the cell-based screening hurdles and identify novel small molecules with desired pharmacological effects (**Figure 3**).

Conclusions

The processes of pluripotent cell maintenance and differentiation are complex and involve the expression of a series of signalling cascades at any given time. Thus, the notion that a small molecule could achieve what several signalling molecules have been shown to achieve is highly unlikely. To date, FDA-approved and marketed small molecule drugs target only 500 genes, the majority targeting enzymes and GPCRs[27], whereas the genome constitutes approximately 20,000 genes. Therapeutic index is still a black box when it comes to small molecules. For this reason, 90% of drug candidates fail, mostly due to lack of efficacy. This begs the question whether the discovery of small molecules will ever have a significant impact on personalised medicine. The complexity of signalling mechanisms, the potential of causing genomic instability and the lack of genetic manipulation into viable cells has rendered the notion that small molecules will be able to be used in personalised medicine a mirage; a story that drummed much excitement at the beginning but its progression appears to be less and less exciting. DDW

Figure 3
The future dilemma of small molecules and stem cells. Much like the psychic using a crystal ball to determine one's future, we seek to find a small molecule compound that will revolutionise the world of personalised medicine

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References

1 Martin, GR Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A 78, 7634-8 (1981).
2 Thomson, JA et al. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145-7 (1998).
3 Strickland, S and Mahdavi, V. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. Cell 15, 393-403 (1978).
4 Thomson, JA et al. Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci U S A 92, 7844-8 (1995).
5 Ivnitski-Steele, I, Ramirez, CN, Djaballah, H. Human Embryonic Stem Cells in Drug Discovery: Are We There Yet? International Drug Discovery 5, 24-28 (2010).
6 Takahashi, K and Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-76 (2006).
7 Williams, RL et al. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature 336, 684-7 (1988).
8 Ying, QL et al. The ground state of embryonic stem cell self-renewal. Nature 453, 519-23 (2008).
9 Ding, S et al. Synthetic small molecules that control stem cell fate. Proc Natl Acad Sci U S A 100, 7632-7 (2003).
10 Druker, BJ. Perspectives on the development of a molecularly targeted agent. Cancer Cell 1, 31-6 (2002).
11 Albanell, J and Adams, J. Bortezomib, a proteasome inhibitor, in cancer therapy: from concept to clinic. Drugs of the Future 27, 1079-1092 (2002).
12 Takahashi, K et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861-72 (2007).
13 Ghodsizadeh, A et al. Generation of liver disease-specific induced pluripotent stem cells along with efficient differentiation to functional hepatocyte-like cells. Stem Cell Rev 6, 622-32 (2010).
14 Dimos, JT et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321, 1218-21 (2008).
15 Ebert, AD et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457, 277-80 (2009).
16 Lee, G et al. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 461, 402-6 (2009).
17 Soldner, F et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136, 964-77 (2009).
18 Sato, N, Meijer, L, Skaltsounis, L, Greengard, P and Brivanlou, AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med 10, 55-63 (2004).
19 Reinhold, MI, Kapadia, RM, Liao, Z and Naski, MC. The Wnt-inducible transcription factor Twist1 inhibits chondrogenesis. J Biol Chem 281, 1381-8 (2006).
20 Chen, S et al. Self-renewal of embryonic stem cells by a small molecule. Proc Natl Acad Sci U S A 103, 17266-71 (2006).
21 Desbordes, SC et al. High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. Cell Stem Cell 2, 602-12 (2008).
22 Hotta, R et al. Small-molecule induction of neural crest-like cells derived from human neural progenitors. Stem Cells 27, 2896-905 (2009).
23 Chia, NY et al. A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. Nature 468, 316-20 (2010).
24 Anastasia, L et al. Reversine-treated fibroblasts acquire myogenic competence in vitro and in regenerating skeletal muscle. Cell Death Differ 13, 2042-51 (2006).
25 Shi, Y et al. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. Cell Stem Cell 2, 525-8 (2008).
26 Lyssiotis, CA et al. Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. Proc Natl Acad Sci U S A 106, 8912-7 (2009).
27 Hopkins, AL and Groom, CR. The druggable genome. Nat Rev Drug Discov 1, 727-30 (2002).
28 Medic8.
29 Lassar, AB, Paterson, BM and Weintraub, H. Transfection of a DNA locus that mediates the conversion of 10T1/2 fibroblasts to myoblasts. Cell 47, 649-56 (1986).
30 Smith, AG et al. Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. Nature 336, 688-90 (1988).
31 Kantarjian, HM et al. Results of the vincristine, doxorubicin, and dexamethasone regimen in adults with standard- and high-risk acute lymphocytic leukemia. J Clin Oncol 8, 994-1004 (1990).
32 Dinsmore, J et al. Embryonic stem cells differentiated in vitro as a novel source of cells for transplantation. Cell Transplant 5, 131-43 (1996).
33 Seternes, OM, Johansen, B and Moens, U. A dominant role for the Raf-MEK pathway in forskolin, 12-O-tetradecanoyl-phorbol acetate, and platelet-derived growth factor-induced CREB (cAMP-responsive element-binding protein) activation, uncoupled from serine 133 phosphorylation in NIH 3T3 cells. Mol Endocrinol 13, 1071-83 (1999).
34 Chen, JK, Taipale, J, Cooper, MK and Beachy, PA. Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. Genes Dev 16, 2743-8 (2002).
35 Chen, JK, Taipale, J, Young, KE, Maiti, T and Beachy, PA. Small molecule modulation of Smoothened activity. Proc Natl Acad Sci U S A 99, 14071-6 (2002).
36 Maloney, A and Workman, P. HSP90 as a new therapeutic target for cancer therapy: the story unfolds. Expert Opin Biol Ther 2, 3-24 (2002).
37 Wu, X, Ding, S, Ding, Q, Gray, NS and Schultz, PG. A small molecule with osteogenesis-inducing activity in multipotent mesenchymal progenitor cells. J Am Chem Soc 124, 14520-1 (2002).
38 Richard, RE and Blau, CA. Small-molecule-directed mpl signaling can complement growth factors to selectively expand genetically modified cord blood cells. Stem Cells 21, 71-8 (2003).
39 Takahashi, T et al. Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes. Circulation 107, 1912-6 (2003).
40 Chen, S, Zhang, Q, Wu, X, Schultz, PG and Ding, S. Dedifferentiation of lineage-committed cells by a small molecule. J Am Chem Soc 126, 410-1 (2004).
41 Duval, D, Malaise, M, Reinhardt, B, Kedinger, C and Boeuf, H. A p38 inhibitor allows to dissociate differentiation and apoptotic processes triggered upon LIF withdrawal in mouse embryonic stem cells. Cell Death Differ 11, 331-41 (2004).
42 Qi, X et al. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. Proc Natl Acad Sci U S A 101, 6027-32 (2004).
43 Wei, ZL et al. Isoxazolyl-serine-based agonists of peroxisome proliferator-activated receptor: design, synthesis, and effects on cardiomyocyte differentiation. J Am Chem Soc 126, 16714-5 (2004).
44 Wu, X, Ding, S, Ding, Q, Gray, NS and Schultz, PG. Small molecules that induce cardiomyogenesis in embryonic stem cells. J Am Chem Soc 126, 1590-1 (2004).
45 Wu, X, Walker, J, Zhang, J, Ding, S and Schultz, PG. Purmorphamine induces osteogenesis by activation of the hedgehog signaling pathway. Chem Biol 11, 1229-38 (2004).
46 Beloti, MM, Bellesini, LS and Rosa, AL. Purmorphamine enhances osteogenic activity of human osteoblasts derived from bone marrow mesenchymal cells. Cell Biol Int 29, 537-41 (2005).
47 Habens, F et al. Novel sulfasalazine analogues with enhanced NF-kB inhibitory and apoptosis promoting activity. Apoptosis 10, 481-91 (2005).
48 Sachinidis, A et al. Identification of small signalling molecules promoting cardiac-specific differentiation of mouse embryonic stem cells. Cell Physiol Biochem 18, 303-14 (2006).
49 Warashina, M et al. A synthetic small molecule that induces neuronal differentiation of adult hippocampal neural progenitor cells. Angew Chem Int Ed Engl 45, 591-3 (2006).
50 Chen, S et al. Reversine increases the plasticity of lineage-committed mammalian cells. Proc Natl Acad Sci U S A 104, 10482-7 (2007).

51 Kubicek, S et al. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Mol Cell 25, 473-81 (2007).
52 Lu, M et al. Involvement of tyrosine kinase signaling in maintaining murine embryonic stem cell functionality. Exp Hematol 35, 1293-302 (2007).
53 Miyabayashi, T et al. Wnt/beta-catenin/CBP signaling maintains long-term murine embryonic stem cell pluripotency. Proc Natl Acad Sci U S A 104, 5668-73 (2007).
54 Nelson, BR, Hartman, BH, Georgi, SA, Lan, MS and Reh, TA. Transient inactivation of Notch signaling synchronizes differentiation of neural progenitor cells. Dev Biol 304, 479-98 (2007).
55 Saxe, JP et al. A phenotypic small-molecule screen identifies an orphan ligand-receptor pair that regulates neural stem cell differentiation. Chem Biol 14, 1019-30 (2007).
56 Song, HY, Jeon, ES, Kim, JI, Jung, JS and Kim, JH. Oncostatin M promotes osteogenesis and suppresses adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells. J Cell Biochem 101, 1238-51 (2007).
57 Bonaguidi, MA et al. Noggin expands neural stem cells in the adult hippocampus. J Neurosci 28, 9194-204 (2008).
58 Hao, J et al. Dorsomorphin, a selective small molecule inhibitor of BMP signaling, promotes cardiomyogenesis in embryonic stem cells. PLoS One 3, e2904 (2008).
59 Huangfu, D et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol 26, 1269-75 (2008).
60 Maimets, T, Neganova, I, Armstrong, L and Lako, M. Activation of p53 by nutlin leads to rapid differentiation of human embryonic stem cells. Oncogene 27, 5277-87 (2008).
61 Mikkelsen, TS et al. Dissecting direct reprogramming through integrative genomic analysis. Nature 454, 49-55 (2008).
62 Miyabayashi, T, Yamamoto, M, Sato, A, Sakano, S and Takahashi, Y. Indole derivatives sustain embryonic stem cell self-renewal in long-term culture. Biosci Biotechnol Biochem 72, 1242-8 (2008).
63 Mukherjee, S et al. Pharmacologic targeting of a stem/progenitor population in vivo is associated with enhanced bone regeneration in mice. J Clin Invest 118, 491-504 (2008).
64 Sadek, H et al. Cardiogenic small molecules that enhance myocardial repair by stem cells. Proc Natl Acad Sci U S A 105, 6063-8 (2008).
65 Schneider, JW et al. Small-molecule activation of neuronal cell fate. Nat Chem Biol 4, 408-10 (2008).
66 Shi, Y et al. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell 3, 568-74 (2008).
67 Zang, Y et al. AICAR induces astroglial differentiation of neural stem cells via activating the JAK/STAT3 pathway independently of AMP-activated protein kinase. J Biol Chem 283, 6201-8 (2008).
68 Adamo, L, Zhang, Y and Garcia-Cardena, G. AICAR activates the pluripotency transcriptional network in embryonic stem cells and induces KLF4 and KLF2 expression in fibroblasts. BMC Pharmacol 9, 2 (2009).
69 Barrilleaux, BL et al. Small-molecule antagonist of macrophage migration inhibitory factor enhances migratory response of mesenchymal stem cells to bronchial epithelial cells. Tissue Eng Part A 15, 2335-46 (2009).
70 Borowiak, M et al. Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell Stem Cell 4, 348-58 (2009).
71 Chen, S et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 5, 258-65 (2009).
72 Dong, XJ et al. Direct hepatic differentiation of mouse embryonic stem cells induced by valproic acid and cytokines. World J Gastroenterol 15, 5165-75 (2009).
73 Ichida, JK et al. A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell 5, 491-503 (2009).
74 McMillin, DW et al. Antimyeloma activity of the orally bioavailable dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235. Cancer Res 69, 5835-42 (2009).
75 Osakada, F et al. In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. J Cell Sci 122, 3169-79 (2009).
76 Park, KW et al. The small molecule phenamil induces osteoblast differentiation and mineralization. Mol Cell Biol 29, 3905-14 (2009).
77 Suter, DM, Preynat-Seauve, O, Tirefort, D, Feki, A and Krause, KH. Phenazopyridine induces and synchronizes neuronal differentiation of embryonic stem cells. J Cell Mol Med 13, 3517-27 (2009).
78 Wang, FS et al. Inhibition of glycogen synthase kinase-3beta attenuates glucocorticoid-induced bone loss. Life Sci 85, 685-92 (2009).
79 Wang, Z et al. Enhanced co-expression of beta-tubulin III and choline acetyltransferase in neurons from mouse embryonic stem cells promoted by icaritin in an estrogen receptor-independent manner. Chem Biol Interact 179, 375-85 (2009).
80 Wu, D et al. A conserved mechanism for control of human and mouse embryonic stem cell pluripotency and differentiation by shp2 tyrosine phosphatase. PLoS One 4, e4914 (2009).
81 Xiong, L et al. Heat shock protein 90 is involved in regulation of hypoxia-driven proliferation of embryonic neural stem/progenitor cells. Cell Stress Chaperones 14, 183-92 (2009).
82 Zhu, S et al. A small molecule primes embryonic stem cells for differentiation. Cell Stem Cell 4, 416-26 (2009).
83 Andrews, PD et al. High-content screening of feeder-free human embryonic stem cells to identify pro-survival small molecules. Biochem J 432, 21-33 (2010).
84 Boitano, AE et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science 329, 1345-8 (2010).
85 Bose, R et al. Glucocorticoids induce long-lasting effects in neural stem cells resulting in senescence-related alterations. Cell Death Dis 1, e92 (2010).
86 Chan, SS et al. Fibroblast growth factor-10 promotes cardiomyocyte differentiation from embryonic and induced pluripotent stem cells. PLoS One 5, e14414 (2010).
87 Chen, DF et al. (+)-Cholesten-3-one induces differentiation of neural stem cells into dopaminergic neurons through BMP signaling. Neurosci Res 68, 176-84 (2010).
88 Chen, DF et al. Cholesterol myristate suppresses the apoptosis of mesenchymal stem cells via upregulation of inhibitor of differentiation. Steroids 75, 1119-26 (2010).
89 Hu, BY and Zhang, SC. Directed differentiation of neural-stem cells and subtype-specific neurons from hESCs. Methods Mol Biol 636, 123-37 (2010).
90 Kim, DS et al. Robust enhancement of neural differentiation from human ES and iPS cells regardless of their innate difference in differentiation propensity. Stem Cell Rev 6, 270-81 (2010).
91 Kim, PT et al. Differentiation of mouse embryonic stem cells into endoderm without embryoid body formation. PLoS One 5, e14146 (2010).
92 Lee, KW et al. Rapamycin promotes the osteoblastic differentiation of human embryonic stem cells by blocking the mTOR pathway and stimulating the BMP/Smad pathway. Stem Cells Dev 19, 557-68 (2010).
93 Mae, S et al. Combination of small molecules enhances differentiation of mouse embryonic stem cells into intermediate mesoderm through BMP7-positive cells. Biochem Biophys Res Commun 393, 877-82 (2010).
94 Mali, P 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 28, 713-20 (2010).
95 Najafov, A, Sommer, EM, Axten, JM, Deyoung, MP and Alessi, DR. Characterization of GSK2334470, a novel and highly specific inhibitor of PDK1. Biochem J 433, 357-69 (2010).
96 Qu, Q et al. Orphan nuclear receptor TLX activates Wnt/beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. Nat Cell Biol 12, 31-40; sup pp 1-9 (2010).
97 Reh, TA, Lamba, D and Gust, J. Directing human embryonic stem cells to a retinal fate. Methods Mol Biol 636, 139-53 (2010).

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98 Sakamoto, S et al. Decalpenic acid, a novel small molecule from Penicillium verruculosum CR37010, induces early osteoblastic markers in pluripotent mesenchymal cells. J Antibiot (Tokyo) 63, 703-8 (2010).
99 Schmole, AC et al. Novel indolylmaleimide acts as GSK-3beta inhibitor in human neural progenitor cells. Bioorg Med Chem 18, 6785-95 (2010).
100 Taylor, T, Kim, YJ, Ou, X, Derbigny, W and Broxmeyer, HE. Toll-like receptor 2 mediates proliferation, survival, NF-kappaB translocation, and cytokine mRNA expression in LIF-maintained mouse embryonic stem cells. Stem Cells Dev 19, 1333-41 (2010).
101 Wurdak, H et al. A small molecule accelerates neuronal differentiation in the adult rat. Proc Natl Acad Sci U S A 107, 16542-7 (2010).
102 Xu, Y 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 107, 8129-34 (2010).
103 Yao, X et al. Histone deacetylase inhibitor promotes differentiation of embryonic stem cells into neural cells in adherent monoculture. Chin Med J (Engl) 123, 734-8 (2010).
104 Zhou, H et al. Conversion of mouse epiblast stem cells to an earlier pluripotency state by small molecules. J Biol Chem 285, 29676-80 (2010).
105 Cundiff, PE and Anderson, SA. Impact of induced pluripotent stem cells on the study of central nervous system disease. Curr Opin Genet Dev (2011).
106 Donzelli, E et al. ERK1 and ERK2 are involved in recruitment and maturation of human mesenchymal stem cells induced to adipogenic differentiation. J Mol Cell Biol (2011).
107 El Akabawy, G. Martinez-Medina, L, Jeffries, AR, Price, J and Modo, M. Purmorphamine increases DARPP-32 differentiation in human striatal neural stem cells through the hedgehog pathway. Stem Cells Dev (2011).
108 Erb, TM et al. Paracrine and Epigenetic Control of Trophectoderm Differentiation from Human Embryonic Stem Cells: The Role of Bone Morphogenic Protein 4 and Histone Deacetylases. Stem Cells Dev (2011).
109 Fan, Y et al. Derivation of cloned human blastocysts by histone deacetylase inhibitor treatment after somatic cell nuclear transfer with beta-thalassemia fibroblasts. Stem Cells Dev (2011).
110 Fu, J et al. Melatonin promotes proliferation and differentiation of neural stem cells subjected to hypoxia in vitro. J Pineal Res (2011).
111 Grinshtein, N et al. Small molecule kinase inhibitor screen identifies polo-like kinase 1 as a target for neuroblastoma tumor-initiating cells. Cancer Res 71, 1385-95 (2011).
112 Kiris, E et al. Embryonic stem cell-derived motoneurons provide a highly sensitive cell culture model for botulinum neurotoxin studies, with implications for high-throughput drug discovery. Stem Cell Res (2011).
113 Lee, YK et al. Ouabain facilitates cardiac differentiation of mouse embryonic stem cells through ERK1/2 pathway. Acta Pharmacol Sin 32, 52-61 (2011).
114 Li, M et al. Neuronal differentiation of C17.2 neural stem cells induced by a natural flavonoid, baicalin. Chembiochem 12, 449-56 (2011).
115 Morizane, A, Doi, D. Kikuchi, T, Nishimura, K and Takahashi, J. Small-molecule inhibitors of bone morphogenic protein and activin/nodal signals promote highly efficient neural induction from human pluripotent stem cells. J Neurosci Res 89, 117-26 (2011).
116 Plaisant, M et al. Inhibition of hedgehog signaling decreases proliferation and clonogenicity of human mesenchymal stem cells. PLoS One 6, e16798 (2011).
117 Sidhu, KS. New approaches for the generation of induced pluripotent stem cells. Expert Opin Biol Ther (2011).
118 Tsutsui, H et al. An optimized small molecule inhibitor cocktail supports long-term maintenance of human embryonic stem cells. Nat Commun 2, 167 (2011).
119 Wang, H, Hao, J and Hong, CC. Cardiac induction of embryonic stem cells by a small molecule inhibitor of Wnt/beta-catenin signaling. ACS Chem Biol 6, 192-7 (2011).
120 Yau, WW et al. Cardiogenol C can induce Mouse Hair Bulge Progenitor Cells to Transdifferentiate into Cardiomyocyte-like Cells. Proteome Sci 9, 3 (2011).
121 Yuan, X et al. Combined Chemical Treatment Enables Oct4-Induced Reprogramming from Mouse Embryonic Fibroblasts. Stem Cells (2011).