Re-education begins at home: an overview of the discovery of in vivo-active small molecule modulators of endogenous stem cells

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**ABSTRACT**

**Introduction:** Degenerative diseases, such as Alzheimer's disease, heart disease and arthritis cause great suffering and are major socioeconomic burdens. An attractive treatment approach is stem cell transplantation to regenerate damaged or destroyed tissues. However, this can be problematic. For example, donor cells may not functionally integrate into the host tissue. An alternative methodology is to deliver bioactive agents, such as small molecules, directly into the diseased tissue to enhance the regenerative potential of endogenous stem cells.

**Areas covered:** In this review, the authors discuss the necessity of developing these small molecules to treat degenerative diseases and survey progress in their application as therapeutics. They describe both the successes and caveats of developing small molecules that target endogenous stem cells to induce tissue regeneration. This article is based on literature searches which encompass databases for biomedical research and clinical trials. These small molecules are also categorized per their target disease and mechanism of action.

**Expert opinion:** The development of small molecules targeting endogenous stem cells is a high-profile research area. Some compounds have made the successful transition to the clinic. Novel approaches, such as modulating the stem cell niche or targeted delivery to disease sites, should increase the likelihood of future successes in this field.

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**1. Introduction**

Degenerative diseases cause the progressive deterioration of organs or tissues over time. This can result from factors, such as aging, lifestyle choices and the environment. Prominent examples include arthritis, osteoporosis, multiple sclerosis and diabetes. These diseases can have significant impact on society. For example, in the United States more than 20% of the population suffer from a type of arthritis [1]. The increasing incidence of degenerative diseases is linked to increases in the human lifespan. The global population of people over 60 years of age is projected to reach 2.1 billion by the year 2050 [2]. Therefore, it is of critical importance to develop effective medicines to treat degenerative diseases.

One attractive treatment option is cell therapy, which is the transplantation of therapeutically relevant cells into, or close by, the sites of tissue damage. Less commonly, cells may be infused into the local vasculature and exit the blood vessels to gain entry to the disease sites. For example, infusion has been used to deliver therapeutic cells to sites of cardiac damage, as reviewed in Ref. [3]. The most successful application of cell therapy is hematopoietic stem cell (HSC) transplantation. Cells are harvested from the bone marrow, peripheral blood, or umbilical cord blood and used to treat patients with blood or bone marrow cancers, such as multiple myeloma or leukemia.

Alternatively, HSC transplantation can be used as a therapy for autoimmune diseases, such as multiple sclerosis [4]. However, cell therapy to treat degenerative diseases affecting 'hard' tissues, for example the musculature or skeletal system, has not made a successful transition to the clinic. This is due to inherent difficulties in transplanting cells into these tissues. Transplanted cell survival is low; typically ≈80% of cells die within 5 days for applications, such as muscle cell stem (myoblast) grafting in models of muscular dystrophy [5]. It can also be challenging to achieved functional integration of the donor cells into host tissue. An example is the transplantation of mesenchymal stem cells (MSCs) into sites of cardiac damage, which benefits recovery largely via paracrine effects as pro-regenerative growth factors are released from the donor cells, rather than differentiation into functional cardiomyocytes [6]. During the previous decade, significant progress has been made in the production of donor cells for cell therapy. The development of induced pluripotent stem cells (iPSCs) from somatic cells, using forced expression of master transcription factors regulating stem cell status, has provided a readily accessible source of stem cells and bypasses the need to use embryonic stem cells for cell therapy [7,8]. Classically, iPSCs are produced using the Yamanaka protocol of transduction with the reprogramming factors Oct4, Sox2, cMyc and Klf4. Small molecule-based methodologies have contributed to this protocol and can substitute...
Small molecules have been successfully developed to manipulate endogenous stem cells. Some of these compounds have made the successful transition to the clinic. The major applications are degenerative diseases and aging-related disorders. Greater understanding of the stem cell niche facilitates the identification of novel targets for drug development. Targeted delivery focuses the therapeutic effect of stem cell-modulating compounds and reduces the probability of non-specific side-effects.

This niche also comprises non-cellular components, such as extracellular matrix, matrix-bound growth factors/cytokines and even mechanical factors (such as shear stress) [16], which together provide a complex network of signals regulating stem cell status (an example is shown in Figure 2). Thus, candidate small molecules inducing regenerative responses in stem cells may also work indirectly by affecting components of the stem cell niche. It should also be noted that the hierarchy and potency of stem cells also varies between different tissues. In the bone marrow, HSCs are pluripotent and can differentiate into the various blood cell types. In contrast, skeletal muscle stem cells (termed satellite cells) are unipotent and can only differentiate into skeletal muscle precursors. Additionally, the cell cycle status of endogenous stem cell also varies between tissues. As examples, epithelial stem cells in the small intestine are continuously dividing whereas cardiac stem cells generally reside in a quiescent state and proliferate in response to signals indicating tissue damage.

In the following sections of this review we provide an overview of the development of small molecule modulators of endogenous stem cells according to the tissue type/disease application investigated (shown in Figure 3). The progress achieved towards developing these compounds as clinical drugs is also discussed.

### 3. Development of small molecules regulators of hematopoietic stem cell homing and differentiation

HSCs transplantation (more commonly known as bone marrow transplantation) is used to treat certain types of leukemia, anemias and autoimmune diseases. HSCs differentiate into all of the blood cell types and their maintenance in the bone marrow niche environment involves interactions with mesenchymal stromal cells (MSCs), which is another population of multipotent stem cells capable of differentiating into osteoblasts, chondrocytes and adipocytes. Compared to other tissues containing endogenous stem cells, designing small molecules that modulate HSCs in vivo has produced the greatest success in terms of clinical drug development.

HSC transplantation can be used to treat thrombocytopenia, a disease in which platelet numbers fall to dangerously low levels, preventing normal blood clotting function and resulting in excessive bleeding [17]. It is also a risk factor for cancer patients undergoing chemotherapy [18]. The cytokine thrombopoietin (TPO) directs HSC differentiation into megakaryocytes, which is

For the reprogramming factors or increase the efficiency of iPSC generation [9]. This approach culminated with the report that small molecule cocktails alone can induce iPSC formation in somatic cells [10]. However, iPSCs are currently not suitable for transplantation studies in humans, due to concerns like potential tumorigenicity [11].

Due to the above-mentioned problems, it has been proposed that bioactive small molecules influencing stem cell behavior can be directly applied in vivo to the affected tissue and enhance the regenerative capacity of endogenous stem cells. As an alternative strategy, small molecules could be released from allogeneic cells transplanted into sites of tissue damage when are they killed by the host immune system (discussed in Ref. [12]). For example, cloned and expanded allogeneic cardiac stem cells can be transplanted into sites of acute myocardial infarction, where the donor cells would release cardiac regenerative factors including insulin-like growth factor (IGF-1), activin and BMP-10 [13]. The research approach for developing small molecules that target endogenous stem cells has been termed ‘Stemistry’ [14]. In this review, we discuss the methodologies for small molecule targeting of endogenous stem cells in different tissues and disease contexts. These small molecules are listed and their advantages and problems are described. Overall, this review aims to demonstrate that small molecules targeting endogenous stem cells can be developed as clinical drugs to treat degenerative disease. In the next section of this review, we provide a brief introduction about endogenous stem cell biology and the tissues/disease applications investigated for developing small molecule regulators of these cells.

### 2. The hierarchy of endogenous stem cells and advantages of developing small molecule modulators

In recent years, it has become increasingly appreciated by the research community that many body organs and tissues contain populations of endogenous stem cells with varying degrees of regenerative potential. As examples, the brain and heart were thought to be refractory tissues, but subsequent research demonstrated the presence of endogenous stem cells with limited capacities for tissue regeneration [15]. However, large-scale, progressive damage to these tissues, such as congestive heart failure or Alzheimer’s disease, overwhelm the regenerative response. Small molecules that can enhance the regenerative potential of endogenous stem cells have potential for drug development to treat these diseases. For this to be successful, it is important to understand the behavior of endogenous stem cells and the different types that exist in organs and tissues.

Endogenous stem cells are defined by their ability to carry out two types of division: symmetrically to yield two daughter stem cells or asymmetrically into two distinct cell types: one daughter stem cell phenotypically identical to itself and one ‘progenitor’ cell that is committed to further divide and/or eventually differentiate into the specific tissue type (Figure 1). Endogenous stem cells do not ‘operate’ alone. Within their tissue, stem cells are embedded alongside non-stem cells in an environment termed the stem cell ‘niche.’ This niche also comprises non-cellular components, such as extracellular matrix, matrix-bound growth factors/cytokines and even mechanical factors (such as shear stress) [16], which together provide a complex network of signals regulating stem cell status (an example is shown in Figure 2). Thus, candidate small molecules inducing regenerative responses in stem cells may also work indirectly by affecting components of the stem cell niche. It should also be noted that the hierarchy and potency of stem cells also varies between different tissues. In the bone marrow, HSCs are pluripotent and can differentiate into the various blood cell types. In contrast, skeletal muscle stem cells (termed satellite cells) are unipotent and can only differentiate into skeletal muscle precursors. Additionally, the cell cycle status of endogenous stem cell also varies between tissues. As examples, epithelial stem cells in the small intestine are continuously dividing whereas cardiac stem cells generally reside in a quiescent state and proliferate in response to signals indicating tissue damage.

In the following sections of this review we provide an overview of the development of small molecule modulators of endogenous stem cells according to the tissue type/disease application investigated (shown in Figure 3). The progress achieved towards developing these compounds as clinical drugs is also discussed.
a precursor cell that ultimately produces platelets. Thus, small molecule inducers of megakaryocyte formation have the potential to treat thrombocytopenia (clinical trials were undertaken using recombinant TPO, but neutralizing antibodies appeared in some patients). Compound library screening for agonists of TPO-stimulated signaling in HSCs by companies, such as GlaxoSmithKline and Ligand produced a number of hit compounds [19]. Extensive structure–activity relationship (SAR) analysis of one hit produced the TPO mimetic eltrombopag (marketed by GlaxoSmithKline as Promacta (USA) or Revolade (EU); Table 1), which binds to the same c-mpl (TpoR) receptor as TPO to increase platelet production. Currently, other TPO mimetics are in clinical development, such as avatrombopag (E5501; formerly YM477 and AKR 501; Table 1) which is being

**Figure 1.** Division and differentiation of endogenous stem cells. Depending on tissue type, populations of endogenous stem cells may be relatively quiescent (e.g. skeletal muscle) or continuously dividing (e.g. hematopoietic stem cells). Stem cells are characterized by their capability for asymmetric division: the cells may divide to produce one daughter stem cell (shown as pink) and one lineage committed ‘precursor’ cell (shown as blue). The precursor cell is capable of further division to produce more precursor cells or undergo differentiation into the specific tissue type (such as osteocytes and chondrocytes from mesenchymal stem cells, epithelial cells from intestinal crypt stem cells, adipocytes from adipose stem cells and neurons from neural stem cells). The proliferative and differentiation responses of endogenous stem cells depend on interactions with the cellular, molecular and environmental factors that constitute the stem cell niche (shown as green cells). Small molecules regulating the regenerative response of endogenous stem cells can be delivered locally or via the vasculature. These molecules may target signaling responses in the stem cells directly, or indirectly via targeting other cellular components of the niche, such as resident inflammatory cells.

**Figure 2.** An example of the complex cellular, secreted and environmental factors that regulate the stem cell niche. The satellite stem cell niche in skeletal muscle is shown. Abbreviations: ANG-1 = angiopoietin 1; HGF = hepatocyte growth factor; IGF-1 = insulin-like growth factor-1; IL-1β = interleukin-1β; IL-6 = interleukin-6; MMPs = matrix metalloproteinases; MGF = mechano growth factor; NO = nitric oxide; TGF-β = tumor necrosis factor-β; TNF-α = tumor necrosis factor-α.
with vascular endothelial growth factor (VEGF), can enhance production of MSCs and endothelial cells from the bone marrow compartment [28]. A number of peptidic and small molecule antagonists of the CXCR4 pathway are in development and clinical trials [29]. Interestingly, activation of CXCR4 signaling by increased interaction with stromal cell-derived factor 1 (SDF-1) using the compound diprotin A (Table 1) has also been shown to improve HSC homing and engraftment post-transplantation [30]. Diprotin A blocks this SDF-1 interaction by inducing its hydrolysis via inhibition of dipeptidyl peptidase 4 (DPP-4). The anti-diabetic drug, sitagliptin (Table 1) which also targets DDP-4, improves HSC proliferation in mice by increasing the activity of G-CSF and the cytokines interleukin-3 and erythropoietin (EPO) [30]. HSC mobilization is also dependent on cell surface interactions between endothelial vascular cell adhesion molecule-1 (VCAM-1) and very late antigen-4 (VLA-4) on HSCs. Consequently, a small molecule VLA-4 inhibitor, BIO5192 (Table 1) was developed and demonstrated to enhance mouse HSC mobilization, both alone and synergistically with plerixafor treatment [31].

Compounds that promote HSC expansion would prove useful for umbilical cord donors, which possess HLA phenotypes that are typically under-represented among bone marrow donors [32]. The zebrafish model system provides a convenient and robust in vivo platform for compound library screening [33,34]. Assessment of HSC expansion and bone marrow recovery post-irradiation led to the discovery of the prostaglandin E2 analog, FT1050 (dmPGE2; Table 1), which is currently in phase I of clinical development for acute myelogenous leukemia by Fate Therapeutics Inc (ClinicalTrials.gov Identifier: NCT01527838). Additionally, short term stimulation of the p38 pathway with pifithrin (Table 1) has been shown to enhance HSC mobilization in mice [35]. The enzyme 15-prostaglandin dehydrogenase catalyzes the first step of the prostaglandin E2 degradation pathway. A novel inhibitor of 15-prostaglandin dehydrogenase, SW033291 (Table 1), was reported and shown to be effective at enhancing tissue regeneration in rodent models of bone marrow transplantation, ulcerative colitis and partial hepatectomy [36]. Recently, the structure of SW033291 has been optimized to improve solubility, drug-like properties and in vivo tissue regeneration activity [37].

EPO increases red blood cell level by targeting CFU-E colony forming units in HSCs. In the clinic, recombinant EPO (rhEPO) is used to treat anemia associated with conditions such as chronic kidney disease, cancer chemotherapy and surgery-associated blood loss [38]. This approach can reduce the need for blood transfusions and related complications, including iron overload and acute lung damage. However, rhEPO therapy itself can produce significant side effects, ranging from hypertension to stroke and death. Small molecules mimicking the effects of EPO can increase erythropoiesis. Current strategies for compound development focus on manipulating pathways that enhance EPO production, such as hypoxia-inducible factor 2-a (HIF2a) signaling. For example, inhibitors of HIF-prolyl 4-hydroxylases have been shown to modulate the post-translational modification of HIF2a [38,39].

In the next section of this review, we describe the development of small molecules that enhance neurogenesis in vivo, which is a major area of stem cell research.
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Reference</th>
<th>Mechanism</th>
<th>Notes</th>
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<td>BIOS192</td>
<td>[31]</td>
<td>VLA-4 inhibitor</td>
<td>Enhances mouse HSC mobilization</td>
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<td>Eltrom-bopag</td>
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<td>TPO receptor agonist</td>
<td>TPO mimetic to treat thrombocytopenia</td>
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<td>FT1050</td>
<td>[22]</td>
<td>Prostaglandin E2 agonist</td>
<td>HSC expansion and bone marrow recovery post-irradiation</td>
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<td>Pifithrin β</td>
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<td>p53 inhibitor</td>
<td>Increases HSC numbers and promotes mobilization</td>
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<td></td>
<td>SB-247464</td>
<td>[24]</td>
<td>Activation of G-CSF receptor signaling</td>
<td>Stimulates bone marrow cells to form granulocytic colonies</td>
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3.1. Development of small molecules increasing neurogenesis

Diseases of the central nervous system (CNS), such as multiple sclerosis, Alzheimer’s disease and Parkinson’s disease, have profound impacts on society. Moreover, severe spinal cord injury can cause paralysis and disability. Psychiatric disorders may also result from neuronal damage. The discovery of populations of neuronal stem cells in the CNS has raised the possibility that treatments producing enhanced differentiation of these stem cells in situ could alleviate CNS diseases [40]. Assessment of neural stem cells in patients by indirect means, such as levels of brain-derived neurotrophic factor and hippocampus volume, indicated that the known anti-depressant drugs fluoxetine, sertraline, imipramine and reboxetine can actually induce neurogenesis in vivo (Table 2) [41]. PKA activation has been implicated as the mechanism for these drugs [14,42].

Histone deacetylases inhibitors produce large-scale changes in gene expression patterns via epigenetic mechanisms. In models of acute brain injury, compound LB205 (Table 2) reduced neuron death via enhancement of nerve growth factor signaling in neuronal stem cells [45]. In a mouse model of Alzheimer’s disease, allopregnanolone (Table 2), a metabolite of the steroid progesterone, directly increased neurogenesis in the hippocampus and reduced pathogenesis [46]. More recently, allopregnanolone has shown effectiveness in a model of myotrophic lateral sclerosis, protecting motor neuron loss via upregulation of AKT-mediated signaling, reduced neuronal nitric oxide synthase activity and increased brain-derived neurotrophic factor mRNA expression [47]. In a pre-clinical model of Alzheimer’s disease, compound ICG-001 (Table 2) was found to correct defective neuronal differentiation by inhibiting the activity of the transcription factor β-catenin/cAMP-response element binding protein (CREB)-binding protein to reduce Wnt signaling [48]. More recently, ICG-001 is being tested as a drug to selectively target cancer stem cells [49].

Neurogenesis in the developing hippocampus can be imaged and quantified in newborn mice, which provides a system for assessing compounds that directly affect neuronal stem cell activity in vivo. This system was used to discover P7C3A20 (Table 2) which prevented the large scale neuronal loss that naturally occurs in the hippocampus just after birth and also protected against chemically induced neurotoxicity in a model of Parkinson’s disease [50]. This compound also reduces tissue damage in a model of stroke and is believed to function by activation of nicotinamide phosphoribosyltransferase, which increases intracellular levels of nicotinamide adenine dinucleotide (NAD) [50]. Possessing favorable pharmacokinetics and oral bioavailability, P7C3A20 and its parent compounds have been licensed by Calico LLC for neuroprotection. Exposure to hypoxia during childbirth depletes neurogenesis. Sildenafil (Brand name: Viagra; an inhibitor cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 that maintains intracellular levels of cGMP; Table 2) prevented hypoxia-induced neuronal death in rats in utero [51]. Sildenafil is currently in Phase 1 clinical trials for birth asphyxia, approved by Health Canada, and Phase 2 clinical trials for Intrauterine Growth Restriction (STRIDER Canada; NCT02442492).

Small molecule KHS101 (Table 2) was discovered in a chemical screen for inducers of neural stem cell differentiation. This compound was found to increase activity of the transcription factor, ARNT2, which is a known regulator of neurogenesis [52]. KHS101 was able to cross the blood-brain barrier and stimulate neurogenesis in the dentate gyrus of the mouse hippocampus. More recently, this compound has been tested as a therapeutic against cancer stem cells in liver cancer [53]. Compounds that modulate stem cell populations in non-neuronal tissues have shown to affect endogenous neuronal stem cells in subsequent research. The isoxazole, Isx (Table 2), initially identified as an enhancer of cardiac cell proliferation by targeting the proton sensing G protein-coupled receptor GP68, was subsequently shown to enhance neurogenesis in the hippocampus of young mice [54]. This finding indicates roles for brain tissue pH during recovery after brain injury and the pathogenesis of neurodegenerative disease. Neurogenesis in the hippocampus is strongly influenced by drug taking behavior and new research utilizing the Isx derivative, Isx-9, has shown that it blocks compulsive-like, context-driven methamphetamine reinstatement in a rat model of drug addiction [55].

Vertebrate neurogenesis during development and adulthood entails the migration of immature neurons from their...
Table 2. Small molecules that promote neurogenesis in vivo.

<table>
<thead>
<tr>
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<th>Mechanism</th>
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<td>Allopregnan-olone</td>
<td>[46]</td>
<td>Metabolite of progesterone</td>
<td>Increases neurogenesis in the hippocampus</td>
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<td></td>
<td>Benztpine</td>
<td>[61]</td>
<td>Muscarinic receptor antagonist</td>
<td>Induces oligodendrocyte differentiation and remyelination</td>
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<td></td>
<td>Fluoxetine</td>
<td>[41]</td>
<td>Inhibits serotonin reuptake</td>
<td>Induces neurogenesis in the hippocampus</td>
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<td></td>
<td>HU 210</td>
<td>[43]</td>
<td>Cannabinoid receptor agonist</td>
<td>Increase neural stem cell migration</td>
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<tr>
<td></td>
<td>ICG-001</td>
<td>[48]</td>
<td>Wnt pathway activator</td>
<td>Corrects defective neuronal differentiation</td>
</tr>
<tr>
<td></td>
<td>Imipramine</td>
<td>[41]</td>
<td>Inhibits serotonin reuptake</td>
<td>Induces neurogenesis in the hippocampus</td>
</tr>
<tr>
<td></td>
<td>Isx</td>
<td>[54]</td>
<td>GPR68 activator</td>
<td>Enhance hippocampus neurogenesis</td>
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<td>Isx-9</td>
<td>[55]</td>
<td>GPR68 activator</td>
<td>Modulate neurogenesis, neuronal activation and structural plasticity of GCNs</td>
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<td></td>
<td>JWH-133</td>
<td>[57]</td>
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<td>KHS101</td>
<td>[52]</td>
<td>Transcription factor ARNT2 activator</td>
<td>Accelerates neuronal differentiation</td>
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(Continued)
associated glial cells and incorporation into functioning brain tissue [14,56]. The endocannabinoid system regulates this process and synthetic cannabinoid receptor agonists, HU-210, arachidonyl-2′-chloroethylamide and JWH-133 (Table 2) promoted neurogenesis by increasing neuron precursor cell migration from the subventricular zone of the CNS [57]. This neurogenic property may also explain their anti-depressant effects. These agonists are also under investigation as cardioprotective drugs for ischemia-induced injury and neuroprotective agents for Parkinson’s disease [58–60].

As mentioned in the introduction to this review, stem cell function is critically dependent on the interplay of factors that comprise the stem cell niche. Thus, compounds that affect niche components can also influence stem cell behavior. Glial cells, such as oligodendrocytes, regulate neurogenesis and maintain neural function. The anticholinergic drug, benztrapine (Table 2; used to treat Parkinson’s disease) was identified by screening for promoters of oligodendrocyte precursor cell differentiation [61]. This drug increased the myelination of neural axons in rats, highlighting its potential to reduce disease burden in multiple sclerosis by enhancing oligodendrocyte function.

An intriguing example of drug repurposing to target neural stem cells is the discovery that metformin (Table 2), the most commonly described drug for type 2 diabetes, also promotes neurogenesis. In the liver metformin produces anti-diabetic activity by activating the atypical protein kinase C/CREB-binding protein pathway, which is also activated during neuron differentiation [62]. In mice, metformin treatment improved memory and promoted neurogenesis in the hippocampus.

In summary, the discovery of neural stem cells in the adult CNS provided research impetus to develop small molecule-based inducers of endogenous neurogenesis. Further incentive for this research comes from the lack of effective therapeautic options for neurodegenerative diseases and CNS trauma. Next in this review, we turn attention to the regeneration of a cell population that comprises a minority in the parent organ: insulin-secreting β-cells.

### 3.2. Development of small molecule enhancers of pancreatic β-cell regeneration

β-cells only account for around 1–2% of cells in the pancreas. They are sensitive to blood glucose levels and secrete insulin in response to post prandial hyperglycemia [63]. Autoimmune destruction of β-cells occurs in Type 1 diabetes. The discovery that these cells are capable of proliferation and differentiation has led to screening efforts for small molecule inducers of β-cell plasticity. As mentioned above, the zebrafish model system has proven invaluable for screening because β-cell islet development can be visualized in the transparent larvae, allowing a simple readout for compounds enhancing cell proliferation. Screening in this model led to the discovery of 5-N-ethylcarboxamidoadenosine (NECA; Table 3), which stimulated β-cell proliferation in a mouse diabetic model [64]. The pathway mechanism was identified as adenosine receptor A2a
activation, suggesting adenosine signaling as a target for enhancing β-cell regeneration, although follow-up analysis of NECA indicated Hsp90 inhibition as an additional target of this compound [65].

Isolated mammalian β-cell islets have also been used to screen for inducers of proliferation [69]. Compound WS6 (Table 3) was shown to activate proliferation in vitro and in vivo in a diabetes model. The target mechanism was determined as synergistic inhibition of Erb3 binding protein-1 and IKKe. WS6 has an encouraging pharmacokinetic profile and further analysis showed that WS6 increased the proliferation of both human β- and α- pancreatic cells, supporting the development of this compound to increase islet mass, which is required for the successful treatment of diabetes [70]. This screening approach, coupled with measuring the expression of the pancreatic transcription factor PDX1 and proliferation marker ki-67, was used to identify adenosine kinase inhibitors as inducers of β-cell division [71]. 5-iodotubercidin and ABT-702 (Table 3) were shown to specifically promote β-cell division in the pancreas of rodents and pigs, without affecting

<table>
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<tr>
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<td>Promotes β-cell proliferation in rodent and human primary islets</td>
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other cell types. The protease β-Secretase 2 (BACE2; memapsin 1) has also been reported as a regulator of β-cell expansion in mice and an inhibitor compound, Cmpdz (Table 3) increased β-cell numbers [72]. Recently, new classes of selective BACE2 inhibitors have been described [73].

These examples illustrate the effectiveness of organism-based and ex vivo screening as methodologies for discovering agents that increase β-cell mass in mammals. Although insulin therapy has dramatically improved the clinical outcome for Type 1 diabetes patients, secondary complications can result from incomplete blood glucose control, such as kidney disease and diabetic foot. Therefore, there is still a clinical need to discover compounds that induce the functional recovery of β-cell islets. In the next section of this review, we turn attention to the musculo-skeletal system and discuss the development of compounds influencing bone and cartilage cell differentiation in vivo.

3.3. Development of small molecules enhancing regeneration of skeletal tissues

Osteoarthritis affects more than 4% of the global population, becomes increasingly common with age and can significantly reduce quality of life [74]. Cell therapy approaches are being developed, such as the transplantation of MSCs with the capacity for osteogenic (bone) differentiation in vivo [75]. Small molecule compounds that increase the potential of MSCs to undergo osteogenesis could enhance their effectiveness as a therapy for arthritis. A large number of osteogenic small molecules (greater than 100) have been described in the literature. Unfortunately, covering all of these molecules is beyond the limits of this review. Readers interested in surveying these molecules may consult the review articles by Laurencin et al., Carbone et al., Lo et al. and Awale et al. [76–79]. Chemical screening in human MSCs for inducers of osteogenesis identified CW008 (Table 4) as an activator of protein kinase A (PKA) signaling, which is known to regulate osteogenesis [80]. In ovariectomized mice, CW008 treatment increased bone mass and density, suggesting that it may be developed for treating osteoporosis (age-related bone loss) via the promotion of osteoblast differentiation in situ.

Small molecules have also been delivered locally to fracture sites for enhancing bone regeneration. A single injection of the hedgehog pathway agonist, SAG (which targets smoothened; Table 4) increased the size of the cartilaginous callus and bony callus after two weeks in a mouse fracture model [82], which is in line with previous reports of hedgehog signaling enhancing osteogenesis in MSCs [83]. Due to the potentially pleiotropic effects of small molecules on the different stem cell populations in the body, targeted delivery has been developed for specifically enhancing bone regeneration in vivo. One approach is the delivery of phenamil (a simulator of bone morphogenetic protein (BMP) signaling; Table 4) contained within mesoporous bioglass nanoparticles (MBN) complexed with strontium ions [84]. MSCs treated with complexed phenamil showed significantly enhanced osteogenesis. In a rat model of mal-calcification, in which an extracted tooth is implanted in the dorsal subcutaneous tissue, targeted delivery of the phenamil–MBN complex improved osseous-dentinal hard tissue formation. Targeted delivery of bioactive small molecules has also been reported in a model of bone fracture. The Wnt signaling pathway regulates bone development and regeneration. However, using Wnt pathway agonists to enhance bone healing is problematic, due to potential oncogenic side effects resulting from poorly controlled doses and/or uptake by non-bone tissues. To achieve targeted delivery to fracture sites, a Wnt pathway activator (3-amino-6-(4-((4-methylpipеразин-1-yl)sulfonyl)phenyl)-N-(pyridin-3-yl)pyrazine – a glycogen synthase kinase 3 beta inhibitor) was loaded onto poly(styrene-alt-maleic anhydride)-b-poly(styrene) nanoparticles. A peptide with high affinity for the osteoclast secreted protein, TRAP, was then attached to the corona of the nanoparticles [85]. Retro-orbital delivery of the drug-loaded nanoparticles in a mouse model of bone fracture produced greater uptake at the fracture site compared to free drug-treated mice. Moreover, bone healing and strength were increased after 4 weeks of treatment, demonstrating the feasibility of targeted delivery of stem cell-modulating compounds to specific areas of damaged tissue.

Novel small molecules that induce osteogenesis in vivo continue to be discovered. For example, Zhoa et al. carried out cell-based screening using the osteogenic reporter gene, RUNX2, in murine pre-osteoblast cells [86]. The compound T63 (Table 4) was identified and shown to enhance osteogenesis in MSCs alongside a concomitant decrease in adipogenic marker expression. Biochemical analyses showed that T63 activates both the canonical Wnt and BMP/Smad pathways to induce osteogenesis. The ability of T63 to increase bone regeneration in vivo and improve bone density was confirmed in the ovariectomized rat model of osteoporosis. Three months’ treatment with T63 increased the numbers of osteoblasts present on the bone surface.

Recent progress in developing advanced biomaterials for enhancing the therapeutic effects of osteogenesis-inducing compounds in vivo includes the study by Cui et al., who constructed a sterose-based non-phospholipid liposomal nanocarrier with 20S-hydroxycholesterol (Table 4) [87]. The stereosomes were shown to induce osteogenesis in bone marrow MSCs cultured within hydrogels. Using a calvarial defect model in ovariectomized rats, 6 weeks’ treatment with the stereosomes promoted bone formation and calvarial healing. An osteogenic and chondrogenic compound, kartogenin (Table 4), was previously reproted by the Peter Schultz laboratory [88]. Kartogenin has a high therapeutic potential for treating skeletal diseases such as osteoarthritis, disc- and bone-tendon disorders [89]. Numerous biomaterials-based strategies have been designed for the effective delivery of kartogenin into intra-articular joints with sustained release over a prolonged period of time. For example, a polylactic-coglycolic acid/polyethylene glycol thermogel was loaded with kartogenin and shown to induce chondrogenic regeneration in a rabbit model of cartilage-defective arthritis [90]. Interestingly, thermo-responsive polymeric nanospheres, based on chitosan oligosaccharide conjugated to pluronic F127, were developed for the dual delivery of kartogenin and diclofenac (an anti-inflammatory drug) in response to temperature change [91]. Simvastatin (Table 4), used clinically as a blood lipid lowering agent, is known to induce osteogenesis via the regulation of BMP production in osteoblasts.
### Table 4. Small molecules that promote bone and cartilage regeneration \textit{in vivo}.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Reference</th>
<th>Mechanism</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>20S-hydroxycholesterol</td>
<td>[87]</td>
<td>Induces Notch signaling</td>
<td>Induces osteogenesis \textit{in vivo}.</td>
<td></td>
</tr>
<tr>
<td>CW008</td>
<td>[80]</td>
<td>Protein kinase A activator</td>
<td>Increases bone mass and density</td>
<td></td>
</tr>
<tr>
<td>Icarin</td>
<td>[94]</td>
<td>OPG-RANKL-RANK system regulator</td>
<td>Promotes formation of new bone and blood vessels</td>
<td></td>
</tr>
<tr>
<td>Kartogenin</td>
<td>[88]</td>
<td>Filamin A binding to CBFβ</td>
<td>Induces chondrogenic regeneration in a rabbit model of cartilage defective arthritis</td>
<td></td>
</tr>
<tr>
<td>Phenamil</td>
<td>[84]</td>
<td>BMP activator</td>
<td>Improves osseous-dentinal tissue formation</td>
<td></td>
</tr>
<tr>
<td>SAG</td>
<td>[103]</td>
<td>Hedgehog pathway agonist</td>
<td>Enhancer of bone regeneration</td>
<td></td>
</tr>
<tr>
<td>SM04690</td>
<td>[81]</td>
<td>Wnt pathway inhibitor</td>
<td>Increases cartilage regeneration</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
However, this drug suffers from poor solubility and has the potential to produce side effects, such as rhabdomyolysis. Ito et al. packaged simvastatin into calcium phosphate nanocapsules containing deoxycholate micelle to enhance solubility [92]. The calcium phosphate coating significantly reduced simvastatin cytotoxicity. Weekly intramuscular injection of the nanocapsules into the femur of ovariectomised mice for 10 weeks produced a whole-body therapeutic effect on bone mineral content and mechanical strength. Thus, drug delivery systems based on nanotechnology can improve osteogenic differentiation in MSCs and suppress disease progression in rodent models of osteoarthritis. Further discussion and examples of the use of nanofiber scaffolds to deliver stem cell modulatory compounds to sites of musculoskeletal damage can be found in the review by Carbone et al. [77].

Although we have described small molecule development in terms of enhancing one facet of tissue regeneration, such as osteogenesis in MSCs, molecules are being developed that simultaneously modulate multiple aspects of the regeneration response [93]. For example, icariin (an active component of Herba Epimedii (horny goat weed); Table 4) was demonstrated to enhance the expression of genes related to osteogenesis and angiogenesis in MSCs [94]. In the rodent calvarial defect model, icariin delivery using calcium phosphate cement scaffolds promoted the formation of new bone and blood vessels. Therefore, developing compounds that target numerous biological mechanisms involved in tissue healing processes may produce a more robust regeneration response.

Next in this overview, we discuss the development of small molecules targeting endogenous stem cells in a tissue that is relatively refractory to regeneration after injury: cardiac muscle in the heart.

### 3.4. Small molecules modulators of cardiac precursor cells to enhance heart regeneration

Cardiac diseases, including myocardial infarction (heart attack), heart failure, cardiomyopathy and myocarditis involve damage to the cardiac muscle and are a leading cause of death globally [95]. Although the heart has been demonstrated to be capable of low levels of regeneration to supplement normal cardiomyocyte turnover, the type(s) of cardiac stem/precursor cell contributing to this regeneration is still subject to debate [96]. However, recent evidence indicates that purified cardiac cells with the marker profile Lin<sup>−</sup>CD45<sup>−</sup>CD<sup>−</sup>kiteo<sup>−</sup> (approximately 10% of the total c-kit<sup>−</sup> population) are robustly cardiogenic stem cells that differentiated into fully functional cardiomyocytes [97]. Although numerous therapies exist for cardiac disease, such as beta blockers after myocardial infarction, none can replace the dead cardiac muscle. Thus, there is a clinical need to develop small molecules that enhance the regenerative potential of cardiac stem cells.

3,5-Disubstituted isoxazoles (Isx) compounds, including Isx2 (Table 5), which were discovered by stem cell-based screening, have been shown to induce endogenous cardiac stem cell proliferation in the epicardium of heart tissue [98]. The target mechanism of Isx compounds has been discussed above in the context of neural stem cell regeneration (see Section 3.1). Unfortunately, treating Isx compounds in a model of myocardial infarction failed to produce improvements in cardiac function. The Wnt pathway inhibitor ICG-001, also discussed above as a neural stem cell modulator, has been shown to activate epicardial cell proliferation and improve cardiac function post-infarction [99]. In addition, MAP kinases are known to regulate cardiac development and the MAP kinase inhibitor, SB203580 (Table 5) promoted cardiomyocyte proliferation in vitro. Moreover, combination therapy with fibroblast growth factor enhanced cardiac function in vivo after myocardial infarction [100]. Bone marrow-derived stem cells have also been shown to contribute to cardiac remodeling post-infarction. Impressively, the DPP-4 inhibitor diprotin (Table 1), which has been used to enhance HSC mobilization (discussed in Section 3) increased the homing of bone marrow cells to infarcted heart tissue and improved cardiac recovery [101].

The transparent zebrafish larvae model has been used to screen for compounds that promote increased cardiogenesis in fluorescently labeled cardiac tissue [102]. Cardiogens 1–3 (Table 5) were discovered using this approach and further characterized as Wnt pathway activators that were active in human embryonic stem cells. Another screening study in zebrafish identified agonists of IGF-1 (NBI-31772; Table 5) and the hedgehog pathway (smoothened agonist
Table 5. Small molecule regulators of skeletal and cardiac muscle stem/precursor cells.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Reference</th>
<th>Mechanism</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD2858</td>
<td>[85]</td>
<td>Wnt activator</td>
<td>Increase bone healing and strength</td>
<td></td>
</tr>
<tr>
<td>BIO</td>
<td>[107]</td>
<td>Wnt pathway activator (GSK3β inhibitor)</td>
<td>Enhances recruitment of M2 type macrophages</td>
<td></td>
</tr>
<tr>
<td>Cardiogen1</td>
<td>[102]</td>
<td>Wnt pathway activator</td>
<td>Enhance cardiogenesis</td>
<td></td>
</tr>
<tr>
<td>Cardiogen2</td>
<td>[102]</td>
<td>Wnt pathway activator</td>
<td>Enhance cardiogenesis</td>
<td></td>
</tr>
<tr>
<td>Cardiogen3</td>
<td>[102]</td>
<td>Wnt pathway activator</td>
<td>Enhance cardiogenesis</td>
<td></td>
</tr>
<tr>
<td>FTY720</td>
<td>[113]</td>
<td>sphingosine-1-phosphate receptor agonist</td>
<td>Increases M2 type macrophages in regenerating muscle</td>
<td></td>
</tr>
<tr>
<td>Isx2</td>
<td>[98]</td>
<td>GPR68 activator</td>
<td>Increase Notch-mediated proliferation of cardiac precursor cells</td>
<td></td>
</tr>
<tr>
<td>NBI-31772</td>
<td>[103]</td>
<td>IGF-1 agonist</td>
<td>Increases cardiogenesis</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
(SAG; Table 4) as inducers of cardiogenesis and cardiomyocyte proliferation [103]. SAG has also been described as an enhancer of bone regeneration (Section 3.3, above). Of note, NBI-31772 has recently been shown to promote sperm precursor (spermatogonia) differentiation in zebrafish [104].

New compounds and biological targets continue to be identified for enhancing heart regeneration in vivo. An interesting example is the recent discovery of MSI-1436 (Table 5), an inhibitor of protein tyrosine phosphatase 1B purified from the liver of the dogfish shark (Squalus acanthias) [105]. Using a novel, adult zebrafish caudal fin amputation screen for compounds potentiating regeneration, intraperitoneal injection of MSI-1436 accelerated fin regeneration. In a zebrafish heart regeneration model, MSI-1436 treatment increased cardiomyocyte proliferation and ventricle re-growth after partial resection. These regeneration effects were confirmed in a mammalian model of ischemic heart injury. Significantly, the regeneration enhancement effect of MSI-1436 was not confined to cardiac tissues. Skeletal muscle satellite cell cultures increased proliferation in response to MSI-1436 treatment and a more robust satellite cell response was directly observed in vivo in a murine model of skeletal muscle injury. Of note, MSI-1436 was previously under development by Magainin Pharmaceuticals to treat obesity and diabetes until trials were terminated due to a lack of funding [106]. This study demonstrates that protein tyrosine phosphatase 1B is a novel drug target for multiple degenerative diseases and is a testament to the effectiveness of in vivo-based phenotypic screening for discovering regeneration enhancing compounds.

### Table 5. (Continued).

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Reference</th>
<th>Mechanism</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="RepSox" /></td>
<td>RepSox</td>
<td>[110]</td>
<td>TGFβR-1/ALK5 inhibitor</td>
<td>Improves muscle regeneration</td>
</tr>
<tr>
<td><img src="image2" alt="SAG" /></td>
<td>SAG</td>
<td>[103]</td>
<td>Hedgehog pathway agonist</td>
<td>Increases cardiogenesis</td>
</tr>
<tr>
<td><img src="image3" alt="SB203580" /></td>
<td>SB203580</td>
<td>[100]</td>
<td>p38 MAP kinase inhibitor</td>
<td>Enhances cardiogenesis after infraction</td>
</tr>
<tr>
<td><img src="image4" alt="Trit" /></td>
<td>Trit</td>
<td>[112]</td>
<td>Up-regulates Akt1-mediated signaling</td>
<td>Enhances muscle regeneration after injury</td>
</tr>
<tr>
<td><img src="image5" alt="MSI-1436" /></td>
<td>MSI-1436</td>
<td>[105]</td>
<td>Inhibitor of protein tyrosine phosphatase 1B</td>
<td>Increased cardiomyocyte proliferation and ventricle re-growth after partial resection</td>
</tr>
</tbody>
</table>
As mentioned in our discussion of small molecules targeting endogenous neural stem cells, niche modulation can indirectly influence the stem cell response to injury and enhance tissue regeneration. An example of this approach is our recent description of the natural product derivative, BIO (a GSK3B inhibitor that activates Wnt signaling; Table 5) which modulates the cardiac tissue microenvironment post-infarction to promote the infiltration of pro-regenerative/anti-inflammatory M2 type macrophages [107]. M2 macrophages secrete cytokines such as interleukin-10, which promote the regenerative phase of wound healing. Increased M2 macrophages after intraperitoneal BIO treatment correlated with improved cardiac recovery and reduced scarring in the infarcted heart. In the next section of this review, we turn attention to a related tissue that has impressive regenerative potential, but can still fail in genetic diseases and aging-related disorders: skeletal muscle.

3.5. Small molecules targeting skeletal muscle stem cells

Skeletal muscle comprises 40% of the body weight. Two major types of skeletal muscle disease are genetic disorders, such as Duchene muscular dystrophy (DMD), and progressive muscle wasting due to aging (sarcopenia). Despite recent improvements, cell therapy approaches to treat skeletal muscle disease are problematic due to the widespread distribution of muscle tissue in our bodies and the low level of functional recovery after transplantation [108]. However, skeletal muscle does have a remarkable level of regenerative capacity due to the presence of muscle stem cells (satellite cells) that reside alongside the muscle fibers and become activated after muscle damage to provide a pool of muscle precursor cells (myoblasts) that regenerate the fibers [109]. Small molecules that maintain this regenerative potential as we age can be developed to treat sarcopenia. Alternatively, small molecules that target genetic defects in satellite cells or myoblasts may be applicable for treating muscular dystrophies. Another important potential application is cachexia, the cancer-related secondary skeletal muscle loss observed in a significant number of patients.

Transforming growth factor-beta (TGF-β) is known to regulate wound healing and tissue regeneration. The small molecule TGF-β inhibitor RepSox (E 616542; Table 5) has been used to reduce age-related decline in satellite cell function. TGF-β serum levels are known to increase with age and administration of this inhibitor in aged mice improved muscle regeneration after injury [110]. Further evidence of the importance of TGF-β in skeletal muscle regeneration was demonstrated in a model of limb-girdle muscular dystrophy [111]. 10 weeks oral treatment with Ki26894 (a TGF-β type I receptor kinase inhibitor) improved muscle strength and normalized satellite cell numbers, via suppressed phosphorylation of the TGF pathway effector Smad2 and upregulation of the target gene, p21. However, the involvement of TGF in many normal physiological pathways may preclude the development of inhibitors for age-related muscle wasting. Additionally, a natural product triterpenoid (termed ‘Trt’; Table 5) has been shown to enhance both myoblast differentiation in vitro via upregulation of the Akt signaling and regeneration in a mouse model of muscle injury [112].

As mentioned in our discussion of small molecules targeting endogenous neural and cardiac stem cells, chemical manipulation of the stem cell niche is a strategy to enhance stem cell activity. This approach has also been validated in skeletal muscle using targeted delivery of an immunomodulatory molecule, FTY720 (a sphingosine-1-phosphate receptor agonist; Table 5) loaded onto nanofiber films [113]. FTY720 selectively enhances the recruitment of anti-inflammatory, pro-regenerative M2-type macrophages to sites of muscle injury, in a similar manner to the effect of BIO in cardiac injury (Section 3.4). In a model of acute muscle injury, implantation of FTY720-loaded PLGA films enhanced satellite cell-mediated muscle repair with a concomitant reduction in fibrosis.

An interesting development in the search for small compounds that regulate muscle health is the discovery of myoregulin, which is a micropeptide that is strongly expressed in skeletal muscle [114]. Micropeptides are encoded from RNAs containing short open reading frames that previously evaded detection because of their small size [115]. The demonstration of myoregulin as a major regulator of skeletal muscle performance suggests that micropeptides can be developed as therapeutic agents to regulate stem cell function in vivo.

Overall, small molecule methodologies have the potential to enhance satellite cell function in vivo, although there is currently no clinically developed compound to enhance muscle regeneration after injury or to reduce the progression of sarcopenia. Current drug treatments for muscular dystrophies, such as DMD, are based on corticosteroids to slow fiber destruction, immunosuppressants to delay the destruction of instable muscle fibers, and exon-skipping agents [116]. Therefore, there is a continuing demand to develop compounds that enhance the regenerative capacity of satellite cells.

4. Conclusion

In this review, we have provided an overview of small molecule development for targeting endogenous stem cells. Commonly targeted tissues for this approach are brain, heart, bone, muscle, pancreas and bone marrow. The major focus is to reduce or reverse the pathology of common degenerative or autoimmune diseases, such as type 1 diabetes, Alzheimer’s disease and cardiac disease. Another application is to improve healing after trauma, such as bone fractures or brain injury. For each disease numerous small molecules have been developed that enhance regeneration in animal models. There are overlaps in the signaling pathways targeted for activating different populations of stem cells. For example, targeting Wnt signaling can enhance the regenerative potential of bone and cardiac tissues [85,99]. The greatest success in this field is the development of TPO mimetics that are in clinical use for increasing platelet production [20]. Other compounds are also in clinical trials, such as FT1050 for acute myelogenous leukemia (ClinicalTrials.gov Identifier: NCT01527838). However, the majority of compounds described in this review remain in the preclinical phase. Recent trends in developing these small molecules include strategies to target stem cell niche components, such as inflammatory cells, to produce a microenvironment more conducive for regeneration (e.g. immunomodulatory molecule, FTY720 [113]) or the use of biomaterial carriers to target compounds.
to specific regions (e.g. sites of bone fracture [85]). An overview of the different strategies to influence the stem cell niche using small molecules is shown in Figure 4. One important concern is the potential toxicity of stem cell-modulating small molecules when they are introduced in vivo, for example their effects on normally functioning stem cell populations in non-target tissues. This may be especially significant for small molecules that influence major signaling pathways with pleiotropic effects in different cell or tissue types, such as the Wnt or TGF-β pathways, which are dysregulated in tumorigenesis. Encouragingly, lithium chloride, a small molecule activator of the Wnt pathway, has been used for the long-term treatment in patients with psychiatric disorders without increasing oncogenic events [117]. In addition, lithium chloride inhibited pathogenesis in a model of oculopharyngeal muscular dystrophy as a much lower dose than is used to treat bipolar disorder [117], suggesting that the effective dose will be another important consideration.

Although major challenges remain for developing clinically relevant small molecule drugs that enhance stem cell function in vivo, the rewards for successfully bringing these small molecules to market would be significant. For example, patients with degenerative disease are likely to require repeated treatment to prevent disease progression. Moreover, increases in the population aging of both economically developed and developing countries provides an expanding incentive for bringing these compounds to the marketplace.

5. Expert opinion

The key findings for research progress in this field is the demonstration that small molecules can effectively regulate stem cell function in diseased tissues and, in the case of TPO mimetics, can be successfully brought to the marketplace. The major weaknesses of this research is the lack of clinical development for a large number of these compounds. In addition, some categories of compounds, such as Wnt and TGF-β pathway activators, are potentially oncogenic and may not be suitable for clinical trials without further applications, such as targeted delivery to disease sites and controlled release [85]. However, the rapid improvements in biomaterials-based techniques to package these drugs suggests that the obstacles can be surmounted. Additionally, the demonstration that small molecules targeting Wnt signaling, such as lithium chloride, have been successful used in patients at doses above that required to activate endogenous stem cells in animal models, provides optimism that these compounds will be safe in the clinic.

Developing small molecule inducers of stem cells in vivo does have high potential to produce clinically relevant therapeutics for tissue regeneration. This is exemplified by the discovery that known medicines, such as metformin and fluoxetine, can induce neurogenesis in vivo [42,62]. Thus, repurposing of known drugs may reduce attrition rates and facilitate drug development for tissue degeneration. However, a major success is needed to further encourage researchers. For example, the pharmaceutical industry is losing interest in developing compounds for Alzheimer’s disease or skeletal muscle wasting [118,119]. Nevertheless, the increased use of phenotype screening using cell and organism-based systems in academia (such as the zebrafish model), should provide a steady flow of candidate compounds for further preclinical development.

Many of the small molecules discussed in this review were identified by screening of organic compound libraries in cell or organism-based systems. Some new developments in identifying low molecular weight modulators of tissue regeneration have somewhat ‘moved away’ from this approach, as exemplified by the mining of micropeptides that are known to be
effective in cells [115]. Another very interesting and emerging field is the development of cell mimicking microparticles that recapitulate components of the stem cells niche to enhance regeneration (reviewed in [120]). The impressive level of flexibility when constructing these microparticles allows them to be tailored to various tissue microenvironments and regulate the kinetics of drug release.

Due to the increasing economic and social burden of aging-related diseases, and the current lack of effective therapies, there will be a continuing incentive to develop compounds targeting endogenous stem cells, particularly in the academic setting. As our understanding of stem cell biology in situ increases, the mechanisms of action of these compounds will also evolve. Recent developments in modulating inflammatory cells to enhance the regenerative response is an example of this new strategy [107,113]. Targeting niche components are amongst the most interesting innovations in this field, along with targeted delivery using biomaterials [84,85]. Although alternative technologies exist to treat these diseases, such as cell therapy, they also have significant technical hurdles to surmount before entering the clinic. One example is immune responses against transplanted islets to treat type 1 diabetes [121]. Therefore, developing or enhancing small molecule approaches to target endogenous stem cells should continue to be a significant focus of regenerative medicine.

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Declaration of interest

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References

Papers of special note have been highlighted as either of interest (†) or of considerable interest (•) to readers.


• Landmark study showing that somatic cells can be reprogrammed to pluripotency by forced expression of transcription factors.

• Remarkable demonstration of the ability small molecules to control cell stemness.

• Important perspective encouraging research into small molecules targeting endogenous stem cells.


35. • Very useful overview of the methodology, advantages and potential applications of zebrafish-based chemical library screening.


An impressive example of using biomaterials as drug carriers to target sites of tissue damage.


• Utilization of an immunomodulatory compound to enhance the presence of pro-regenerative immune cells in the stem cell niche.


