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# Regenerative Medicinal Chemistry: The in Situ Control of Stem Cells

Angela J. Russell*

Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford OX1 3TA, United Kingdom; Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3PQ, United Kingdom

**ABSTRACT:** In recent years, there has been mounting evidence to support the presence of stem and progenitor cells within many adult tissues that retain the capacity to proliferate and differentiate, thereby contributing to tissue homeostasis and repair. In line with these discoveries, there have been increasing efforts to develop new agents that target these resident stem and progenitor cell populations in situ to augment or to stimulate repair and regeneration processes. Two such agents are approved drugs, and several more are currently in clinical and preclinical development. Through this emerging therapeutic paradigm there is enormous scope for medicinal chemistry to play a pivotal role in regenerative medicine. The potential impact of regenerative medicinal chemistry is profound, and future studies will reveal which tissue types or disease states will prove most readily tractable through this approach.

Realizing the enormous potential of regenerative medicine and stem cells undoubtedly represents one of the most significant current challenges in healthcare. Stem cells have been derived from embryo, fetus, umbilical cord blood, and adult tissue, or most recently from the reprogramming of adult somatic cells. They are remarkable cells characterized by their ability to divide and to differentiate via a number of steps to embryonic and adult somatic cell lineages. Such cells thus hold enormous promise both for drug efficacy and toxicity testing, and for regenerative therapies for a wide range of disorders with high unmet medical need such as neurodegenerative diseases, diabetes, heart disease, and vision and hearing loss. To date, a number of treatment options have been explored including cell therapy and tissue engineering.[1] The application of medicinal chemistry to discover small molecules to control cell fate has attracted immense interest in recent years and can offer significant advantages over other techniques in terms of greatly reduced costs and the ability to control cell fate reversibly at will.[2,3] In regenerative medicine, a hugely powerful application is the use of small molecules to activate resident stem and progenitor (precursor) cell populations in vivo.

There is growing evidence to support the presence of stem and progenitor cells within many adult tissues, including the brain, heart, gut, skin, cornea, bone marrow, and skeletal muscle where they appear to play a role in tissue homeostasis and repair in response to insult or injury. Among the most well characterized and widely exploited types of adult stem cells are the hematopoietic stem cells (HSCs) and multipotent mesenchymal stromal cells (MSCs) in the bone marrow whose function is to replenish blood cell types and bone, and cartilage and adipocytes, respectively (Figure 1). The ability of resident stem cells to promote regeneration appears to vary considerably from tissue to tissue with, for example, HSCs and MSCs being the among the most efficient, but others such as cardiac progenitor cells appear to possess a more limited regenerative capacity. Stem and progenitor cells in adult tissue reside within distinct microenvironments (termed niches) that serve to tightly regulate cell proliferation, differentiation, and migration in vivo. In turn, this provides several potential therapeutic options for in situ stem cell activation, either directly through targeting resident stem cells or indirectly through manipulation of the niche.

The concept of targeting resident stem and progenitor cells in situ to stimulate repair and regeneration processes is an emerging approach in regenerative medicine, and examples of approved agents and candidate drugs operating through this mechanism have been reported to date, the majority of which target the hematopoietic system. Following the elucidation of the role of the secreted hormone erythropoietin (EPO) on the production of red blood cells from hematopoietic progenitor cells, recombinant EPO was trialed in humans in the 1980s for the treatment of anemia. The FDA approved EPO in 1989, and it remains in clinical use for patients with anemia caused by chronic kidney disease or cancer chemo- or radiotherapy. Another secreted hormone, thrombopoietin (TPO), was found to play a critical role in the production of platelets from hematopoietic progenitor cells. Recombinant TPO and derivatives were trialed in humans to stimulate the production of platelets to treat thrombocytopenia (platelet deficiency); however, in one study treatment led to the development of thrombocytopenia in healthy volunteers. This apparently paradoxical observation was attributed to the production of antibodies following treatment, which cross-reacted with endogenous TPO. In parallel with these clinical studies, nonimmunogenic, orally bioavailable TPO mimetics were also being explored. The first examples of small molecule TPO mimetics were discovered using a high-throughput cell-based screening procedure carried out in a collaborative venture between GlaxoSmithKline and Ligand Pharmaceuticals. The screen used a TPO-responsive cell line that had been stably transfected with a reporter gene encoding the protein luciferase under the transcriptional regulation of downstream components of the TPO-receptor (c-mpl) signaling pathway. A number of chemotypes were described as TPO mimetics, culminating in the discovery of eltrombopag (SB497115, **1**) (Table 1).[4] In clinical trials, **1** was shown to increase platelet

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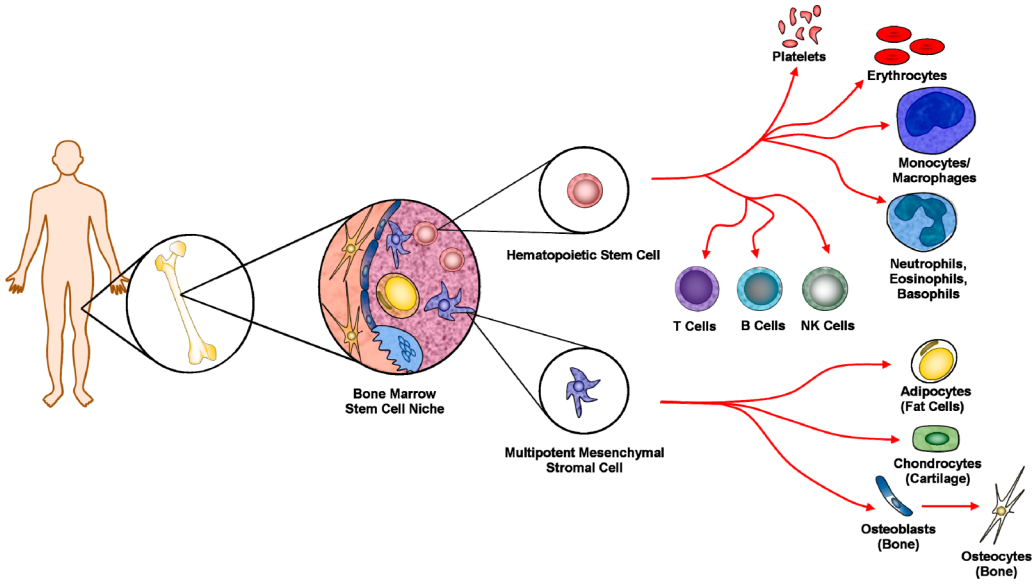
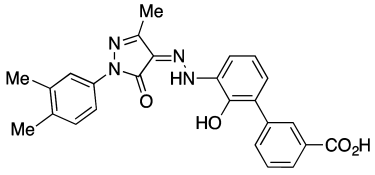
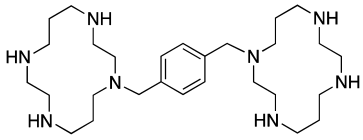
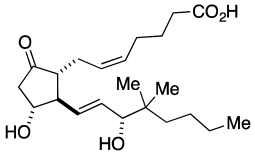
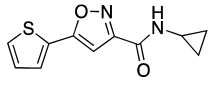



**Figure 1.** Representation of adult stem cell hierarchy and the stem cell niche: bone marrow-derived stem cells, multipotent mesenchymal stromal cells (MSCs) and hematopoietic stem cells (HSCs), and representative cell types they produce in vivo.

**Table 1. Structures of Representative Small Molecules That Stimulate Resident Stem or Progenitor Cells in Vivo in Humans and/or Preclinical Models**

| Name | Structure | Indication | Proposed mechanism of action |
|---|---|---|---|
| Eltrombopag (Promacta, SB497115) **1** |  | Thrombo-cytopenia | TPO mimetic |
| Plerixafor (AMD-3100) **2** |  | HSC mobilisation | CXCR4 antagonist |
| 16,16-Dimethyl Prostaglandin E2 (FT1050) **3** |  | HSC expansion | Stable PGE2 analogue |
| Isx-1 **4** |  | Ischaemic heart disease | GPR68 agonist |

counts in patients with thrombocytopenia, and concurrent investigations supported a molecular mechanism of action involving a direct interaction with the TPO receptor. Eltrombopag was approved for clinical use by the FDA in 2008 and represents the first example of small molecule drug designed to target resident progenitor cells in vivo. Many other groups have since described the discovery of small molecule TPO-mimetics using similar approaches, examples of which are in various stages of clinical and preclinical development.

Granulocyte-Colony Stimulating Factor (G-CSF), a cytokine found to stimulate granulocyte production in vivo and HSC migration from the bone marrow, has been in clinical use for several years to treat neutropenia (neutrophil deficiency) and to increase the number of HSCs in circulation prior to harvesting for transplantation. More recently, the interaction of the Chemokine Stromal Cell-Derived Factor 1 (or CXCL12) with its receptor (CXCR4) was also found to be critical for retaining HSCs within the bone marrow niche. Thus, small molecule CXCR4 antagonists were explored as agents to stimulate the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mobilization of HSCs from the bone marrow into circulation. Plerixafor (AMD3100, **2**), the first agent of this type to be described, has been shown to increase HSC mobilization in humans in the presence and absence of G-CSF and has recently been approved for use in patients with non-Hodgkins lymphoma and multiple myeloma.[5] Next generation CXCR4 antagonists are also in development.

The examples described above illustrate two major challenges in the discovery of future regenerative drugs. First, key elements of the mechanisms determining cell fate had been elucidated in these cases, providing a specific pharmacological target, or at least a signaling pathway to modulate, for drug development. However, often these details are not known or the underlying mechanisms are incompletely understood. Second, as in the case of plerixafor **2**, regenerative drugs could either directly target, or be influenced by, stem cell-niche interactions in vivo, which makes the development of reliable in vitro model systems challenging. One means to address these issues, which has been explored by several groups, is to screen candidate molecules in vivo using model organisms.

In a pioneering study, North and co-workers performed a phenotypic assay to monitor for HSC production in zebrafish embryos using 2357 compounds with defined pharmacological activity.[6] Among other pathway modulators, enhancers of prostaglandin E2 synthesis were found to increase HSC numbers, while inhibitors of prostaglandin synthesis were found to suppress HSC numbers. More detailed analyses were subsequently carried out on the more stable PGE2 analogue, 16,16-dimethyl prostaglandin (dmPGE2, FT1050, **3**). Most importantly, the effects of dmPGE2 (**3**) on stimulating HSC production were found to translate into murine and nonhuman primate models. FT1050 (**3**) is currently in clinical development for the ex vivo expansion of HSCs in umbilical cord blood prior to transplantation. Zebrafish embryos have since been used in a range of contexts to screen for new candidate molecules and pathways to manipulate stem and progenitor cells in vivo.[7]

While many studies have focused on stimulating the hematopoietic system, more recent reports have demonstrated that it is possible to stimulate cells in vivo with less apparent inherent capacity for repair, such as those resident in the heart. Isx-1 (**4**), one of a family of isoxazoles originally identified to promote cardiomyogenesis in vitro, has very recently been shown to stimulate cardiac repair following ischemic injury in vivo in a murine model.[8] Although Isx-1 was identified through a phenotypic screen with an unknown mechanism of action, further studies have showed that it appears to mediate its effects via GPR68, a hitherto unreported regulator of cardiac muscle cells.[9]

One of the hurdles to the development of regenerative drugs is in selecting which resident cells should be targeted, as in many instances these cell populations are ill-defined. In the cardiac regeneration field, there have been significant advances in this regard in the past few years: in particular several lines of evidence suggest that epicardial derived cells (EPDCs) can contribute to cardiac repair in vivo following injury. Furthermore, in a seminal study by Smart and co-workers in 2011, it has been shown that resident adult EPDCs treated with thymosin $\beta$4 migrate and differentiate into structurally coupled cardiomyocytes in situ following injury.[10] Clinical trials with thymosin $\beta$4 for wound healing, corneal repair, and cardiac repair following myocardial infarction are ongoing.

The discovery of drugs that target resident stem and progenitor cell populations in vivo to stimulate repair and regeneration processes in the event of injury or degeneration is a hugely exciting emerging therapeutic opportunity. There remain challenges in the development of new drugs acting through these mechanisms. Among these, understandably concerns are raised over the safety of regenerative therapies as a result of potential undesired effects of these molecules, leading for instance to proliferative disorders. It is likely that the dosing of any such molecules will need to be carefully controlled, and their effects on other tissues judiciously monitored as compounds are progressed. However, there have been several reports that a number of drugs already in routine clinical use for chronic conditions, most notably antidepressants with differing molecular mechanisms of action such as fluoxetine, tranylcypromine, and rolipram, also stimulate neurogenesis in vivo. It may be that in the future approved drugs for other indications will also be found to affect resident stem cell populations and be investigated as drug repositioning opportunities. As with any drug discovery program, reliable in vitro screening tools and methods to monitor efficacy in vivo will also need to be developed as the field expands; these will draw on our burgeoning understanding of the stem cell niche and rely on the concomitant characterization of suitable biomarkers. Nonetheless, recent years have seen the approval of the first small molecule regenerative drug acting in situ on resident progenitor cells, with many more in clinical and preclinical development, and interest in this area is rising exponentially. Medicinal chemistry is already playing a pivotal role in this field and will need to continue to be at the forefront of future endeavors.

## AUTHOR INFORMATION

### Corresponding Author

*Tel: +44(0)1865 275643. Fax: +44(0)1865 285002. E-mail: angela.russell@chem.ox.ac.uk.

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