To the Editor:

In a recent Perspective, Bianco *et al.*[1] addressed a number of misconceptions regarding the biological nature and function of bone marrow–derived mesenchymal stem cells (MSCs) and their impact on current efforts to achieve clinically successful MSC-based therapies. The MSC committee of the International Society of Cell Therapy (ISCT) applauds this endeavor and strongly supports a number of opinions articulated in the Perspective. For example, the committee agrees that the widespread use of qualitative metrics to evaluate multipotency *in vitro* has led to the erroneous conclusion that virtually all adherent cells derived from connective tissues represent functionally equivalent MSC populations that are equally potent from a clinical perspective. Indeed, Bianco *et al.*[1] correctly point out that the use of organ-specific stem cells to treat organ-specific diseases provides the greatest probability of successfully translating stem cell science into clinical therapies.

Nevertheless, the committee feels that criticisms levied against efforts to exploit nonprogenitor MSC functions to treat diseases outside of the skeletal system were biased and in some regards unfairly critical. For example, Bianco *et al.*[1] state that the nonprogenitor effects of MSCs, such as immune modulation, remain unknown and unverified and have escaped conclusive validation in defined *in vivo* model systems. The committee advocates a more balanced view on this topic. Although stromal cells from different connective tissues have lineage-specific properties and therefore do not all fall under the narrow definition of 'skeletal stem cells', the prevailing literature indicates that these cells share some nonprogenitor functions[2] and may therefore be therapeutically effective for treating specific clinical indications outside of the skeletal system. We submit that some claims about the therapeutic potency of MSCs are exaggerated. However, Bianco *et al.*[1] did not acknowledge the rapidly expanding body of literature exploring nonprogenitor MSC functions, which in some cases have provided a solid foundation for moving forward with MSC-based clinical therapies. For example, rapid progress in dissecting the immune-modulatory functions of MSCs was achieved in part because many proteins that have been shown to contribute to this activity, such as Toll-like receptors, inducible nitric oxide synthase (iNOS), indoleamine 2,3-dioxygenase (IDO), prostaglandin E receptor 2 (PGE_2), B7 homolog 1 (B7-H1) and human leukocyte antigen G (HLA-G), have well-described functions in the immune system. Moreover, several studies published in high-impact journals, including *Nature Medicine*, have demonstrated that MSC-mediated immune modulation yields a therapeutic benefit in experimental animal models of sepsis[3], graft-versus-host disease (GvHD)[4] and asthma[5]. These studies used molecular and genetics-based approaches to examine the underlying mechanisms of action. Although it is true that the precise mechanisms of MSC-based therapies in human patients are largely inferred, completed phase 1 and 2 clinical trials have reported statistically significant benefits in patients with steroid-resistant GvHD[6], severe systemic lupus erythematosus[7] and complex perianal fistulas[8]. Clinical trial data also exist indicating that MSC-based therapies may benefit patients with cardiac disease[9]. It is important to note that not all MSC-based clinical trials have met their primary endpoint of efficacy, including an industry-sponsored phase 3 trial of mass-produced, random-donor MSCs for the treatment of GvHD[10]. However, negative clinical trial results are also valuable in informing the translational development of a cellular product.

Although the field of regenerative medicine is rife with unsubstantiated claims of benefit and is often biased by strong commercial interests, it is important that the legitimate scientific enterprise does not allow this noise to overshadow meaningful advances in the field. By providing a skeletal system–centric view of MSCs, the Perspective by Bianco *et al.*[1] relegates the rapidly growing body of literature dedicated to exploring nonprogenitor functions of MSCs, including immunomodulation, to obscurity and undermines the efforts of legitimate and dedicated scientists to understand these functions and exploit them to achieve a therapeutic benefit in human patients. Indeed, our committee believes that the existing scientific data are sufficiently mature to warrant MSC-based clinical trials for disease indications beyond the skeletal system. However, we acknowledge that the sound establishment of MSC-based therapies requires controlled, randomized, prospective clinical trials that incorporate mechanistic-based studies to fully assess the pharmaceutical underpinnings of such therapy.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

Donald G Phinney[1], Jacques Galipeau[2], Mauro Krampera[3], Ivan Martin[4,5], Yufang Shi[6] & Luc Sensebe[7,8]

[1]Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, Florida, USA. [2]Departments of Hematology and Oncology and Pediatrics, Emory University Winship Cancer Institute, Atlanta, Georgia, USA. [3]Stem Cell Research Laboratory, Section of Hematology, Department of Medicine, University of Verona, Verona, Italy. [4]Department of Surgery, University Hospital Basel, Basel, Switzerland. [5]Department of Biomedicine, University Hospital Basel, Basel, Switzerland. [6]Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and Shanghai Jiao Tong University School of Medicine, Shanghai, China. [7]Etablissement Français du Sang (EFS), Saint-Denis, France. [8]Unités Mixtes de Recherche (UMR) 5273 STROMALab, Centre National de la Recherche Scientifique (CNRS)/EFS/Unités Propres de Service (UPS)–Institut National de la Santé et de la Recherche Médicale (INSERM) U1031, Toulouse, France.
e-mail: dphinney@scripps.edu

1. Bianco, P. *et al. Nat. Med.* **19**, 35–42 (2013).
2. Boregowda, S.V. & Phinney, D.G. in *Mesenchymal Stromal Cells* (Springer Science+Business Media, New York, 2013).
3. Németh, K. *et al. Nat. Med.* **15**, 42–49 (2009).
4. Ren, G. *et al. Cell Stem Cell* **2**, 141–150 (2008).
5. Németh, K. *et al. Proc. Natl. Acad. Sci. USA* **107**, 5652–5657 (2010).
6. Le Blanc, K. *et al. Lancet* **371**, 1579–1586 (2008).
7. Wang, D. *et al. Cell Transplant.* published online, http://dx.doi.org/10.3727/096368912X658719 (31 October 2012).
8. Yong Lee, W. *et al. Stem Cells* published online, http://dx.doi.org/10.1002/stem.1357 (13 February 2013).
9. Hare, J.M. *et al. J. Am. Med. Assoc.* **308**, 2369–2379 (2012).
10. Galipeau, J. *Cytotherapy* **15**, 2–8 (2013).

To the Editor:

In their recent Perspective, Bianco *et al.* indicated that uncertainties regarding the nature, identity, function, mode of isolation and handling of mesenchymal stem cells (MSCs) have a major impact on their envisioned therapeutic use[1]. Although there is merit in this work, we would like to highlight some limitations in the definition of therapeutic reagents and debate the basic principles that characterize experimental medicine.

The authors clearly summarize the evidence underlying the identification of specific skeletal self-renewing stem cells and progenitors and indicate that these are system defined and are therefore both structurally and functionally different from nonprogenitor connective tissue cells—sometimes referred to as MSCs—that can be found in almost every organ. We fully endorse the need to keep these two cellular entities separate to avoid confusion between stem and progenitor functions and the regulation of tissue homeostasis. This distinction is fundamental in understanding the rationale behind the current therapeutic applications of mesenchymal 'stromal' cells. The definition of stromal cells has undergone a paradigm shift in recent years. In addition to the traditional view of stromal cells supporting and organizing a parenchymal framework, numerous studies have unveiled their important role in modulating tis-

sue inflammation and, as a consequence, promoting tissue repair. Such properties are in principle independent of any stem or progenitor activity, although the heterogeneity in their composition may imply some degree of overlap[2]. These immunomodulatory features—rather than the ability to differentiate into multiple lineages—have fuelled attempts to test the therapeutic potential of these cells in immune-mediated disorders. In this context, we believe that at least some of these attempts were justified.

A further issue that we feel deserves correction is the notion that stromal cell therapies are derivatives of alchemy. Such a position ignores some of the methodologies that have been used in experimental medicine to successfully develop new treatments. Initial laboratory studies have suggested that adherent bone marrow–derived and *ex vivo*–expanded stromal cells suppress lymphocyte proliferation *in vitro*[3], leading to the hypothesis that these cells might exert similar effects *in vivo*. On the basis of these experimental findings, a single patient with steroid-resistant acute graft-versus-host disease was treated with stromal cells, and the impressive clinical response that was observed[4] formed the basis for a range of clinical trials in this area[5]. At the time of clinical introduction, *in vivo* evidence of the efficacy of this type of treatment in experimental animals was lacking and only became available later. From the clinical perspective, attempts to design proper studies should include better standardization of the cellular production process and the development of potency assays. However, clinical studies remain valid even if the composition of the therapeutic product and the putative underlying mechanisms of efficacy remain to be defined.

Although animal experiments may provide a proof of principle for clinical studies, their predictive value with respect to safety and efficacy is limited[6]. Despite an evidence-based clinical practice, many innovations in medicine follow an empirical rather than scientific approach. A variety of effective treatments have been introduced in the absence of a solid scientific basis or mechanistic understanding. These clinical developments have been focused primarily on outcome and not on the science or underlying mechanisms. Once successful, these studies have provided unique opportunities for basic science to provide an understanding of the biology and mechanisms of treatment efficacy. The introduction of inoculation against smallpox by Chinese Taoist alchemists in the tenth century was entirely empirical but was successful without even a vague notion of the immune system that mediated protection against disease. The most informative example in recent times is perhaps hematopoietic stem cell transplantation. Its success did not involve any understanding of the composition of the bone marrow graft, but the consequent combined efforts of laboratory research and clinical studies were fundamental for the definition and isolation of hematopoietic stem and progenitor cells, dendritic cells, natural killer cells and T and B cells, as well as their subsets.

There is little doubt that the design of clinical studies in the field of stromal cell therapies can be improved. These studies should target a well-selected group of patients, and appropriate follow up of clinical and laboratory parameters is crucial. Studies should perhaps not focus exclusively on clinical outcome but should also examine the mechanisms and biomarkers behind treatment efficacy. In this process, basic research will be invaluable, but it would be foolish to negate a clinically efficacious reagent because its biological function is not fully understood. The further advancement of this field will provide new opportunities for productive interactions between scientists and clinicians.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

Willem E Fibbe[1], Francesco Dazzi[2] & Katarina LeBlanc[3]

[1]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands. [2]Stem Cell Biology, Department of Medicine, Imperial College London, London, UK. [3]Division of Clinical Immunology and Transfusion Medicine, Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden.
e-mail: w.e.fibbe@lumc.nl

1. Bianco, P. *et al. Nat. Med.* **19**, 35–42 (2013).
2. Jones, S. *et al. J. Immunol.* **179**, 2824–2831 (2007).
3. Di Nicola, M. *et al. Blood* **99**, 3838–3843 (2002).
4. Le Blanc, K. *et al. Lancet* **363**, 1439–1441 (2004).
5. Connick, P. *et al. Lancet Neurol.* **11**, 150–156 (2012).
6. Suntharalingam, G. *et al. N. Engl. J. Med.* **355**, 1018–1028 (2006).

Bianco *et al.* reply:

A fil rouge unites the three Correspondences that distinguished colleagues[1–3] have offered on our Perspective[4] on mesenchymal stem cells (MSCs) in medicine. That is, the idea that their clinical promise does not come from a proven biology as established experimentally. MSCs are not pluripotent cells, and they cannot regenerate heart or brain; this is accepted and not disputed by the Correspondences. Therefore, the promise of MSCs as a therapy for the heart, brain or other organs comes not from their nature as stem cells, progenitors of skeletal tissues or key cells in the hematopoietic microenvironment but rather from their non-progenitor functions. These functions have not been identified, are not proven and are incompletely understood; however, they are nonetheless the mainstay of the commercial development of MSCs. These nonprogenitor functions (trophic, immune modulatory and anti-inflammatory) have been proposed—but not proven—to enable MSCs to improve a broad range of unrelated diseases in multiple organs beyond the skeleton, regardless of their etiology or mechanism or the type and extent of organ damage. On the basis of this assumption, their potential benefit is then amenable to exploration, either directly in clinical trials—regardless of a rationale or mechanism—or empirically, in view of the historical virtues of empiricism in medicine.

Empiricism, Fibbe, Dazzi and LeBlanc[1] argue and our other colleagues imply, has value in medicine. Which is to say, if something works, understanding of the mechanisms and rationale can come second. So what works, empirically? Jonas Salk's vaccine was tested in one clinical trial. To the best of our knowledge, none of the more than 300 clinical trials with intravenously infused MSCs (www.clinicaltrials.gov) has so far provided conclusive evidence of any therapeutic efficacy of MSCs in any of the multiple diseases targeted. A few small trials have suggested a benefit of MSC infusions for graft-versus-host disease (GvHD), whereas other large trials have not. In fact, the recent approval of an MSC product for GvHD in Canada was conditional on future proof of efficacy. Meanwhile, it was found that MSCs infused in the blood (as in patients with GvHD) rapidly die and do not engraft, as they trigger the complement and coagulation cascades, an effect known as the instant blood-mediated inflammatory reaction (IBMIR), which kills the cells[5]. Although it remains to be seen whether this systematic adverse reaction is linked *per se* to at least some of the purported immune-modulating effects, IBMIR has been known to occasionally elicit severe consequences such as thromboembolism. We have no valid assay (*in vitro* or *in vivo*) for the immune-modulatory effects that are thought to underpin the clinical observations made with MSCs in GvHD, for the specificity of effector cells or effector mechanisms (inhibition of the mixed lymphocyte reaction may not be the mechanism behind the clinical effects and