Peer reviewed article

Haematopoietic stem cells and mesenchymal stem cells as tools for present and future cellular therapies*

Vincent Kindler[a], *Domizio Suva*[b], *Caroline Soulas*[a], *Bernard Chapuis*[a]

[a] Haematology Service, Geneva University Hospital, Geneva, Switzerland
[b] Orthopaedic Surgery Service, Geneva University Hospital, Geneva, Switzerland

Summary

Postnatal stem cells are present in many adult tissues, and are thought to ensure homoeostasis by replacing functionally declining cells by newly differentiated ones. Postnatal stem cells used as such or after *in vitro* manipulation hold out strong hopes for reconstructive therapies. For instance, the grafting of native haematopoietic stem cells (HSC) restores haematopoiesis in genetically deficient individuals or in lethally conditioned leukaemic patients, and systemic injection of *in vitro* amplified mesenchymal stem cells (MSC) induces recovery of bone growth in patients with osteogenesis imperfecta. Moreover, cells differentiated *in vitro* from postnatal stem cells exhibiting a specific function can also be used for cell therapy. Myeloid dendritic cells (DC) derived from cultures of HSC may induce tumour-specific cytotoxic T lymphocytes to eradicate the tumour via antigen recognition. In addition, long-lived MSC has been engineered to secrete specific proteins coded by a transgene and used as a source of therapeutic molecules *in vivo*. All these approaches require large quantities of cells that cannot be obtained (with the exception of HSC) directly from the donor. *In vitro* procedures allowing the production of therapeutic cells from postnatal stem cells are needed and are at present under development. Below we discuss the rationale and methods currently available for generation of therapeutic cells derived from haematopoietic and mesenchymal stem cells.

Key words: haematopoietic stem cells; mesenchymal stem cells; cell therapy; in vitro differentiation

Introduction

Stem cells are able to undertake iterative cell cycling without losing their proliferative and differentiative properties, i.e. they are the only cells that resist differentiation and simultaneously give birth to a numerous progeny [1, 2]. *In vivo*, two types of stem cell can be distinguished: the zygote, which under physiological conditions produces all embryonic and extraembryonic tissues (such as the placenta and the umbilical cord) which constitute an individual, and the postnatal stem cells that are resident in most of the organism's tissues after birth and ensure their homoeostasis. Blood, skin, and gut mucosae were the first tissues whose homoeostasis was found to be regulated by stem cells. Now postnatal stem cells have also been identified in muscle, nerve, adipose tissue and bone marrow (BM) stroma [3, 4].

Thanks to their ability to generate large numbers of mature cells, stem cells represent a major hope for reconstructive therapies. While embryonic stem cells (ES) derived from blastocysts [5, 6] could theoretically be used for such applications, their generation and manipulation are beset with moral and ethical concerns. Moreover, these cells frequently generate teratogenic events once injected into a host [6] and their use in human clinical medicine remains to be determined. By contrast, postnatal stem cells are not hampered by such moral and ethical considerations since they are obtained exclusively after the donor's informed consent, a fact obviously not applicable to ES. Postnatal stem cells are already used as such in some clinical applications, the best example being the immediate use of haematopoietic stem cells (HSC) to reconstitute the haematopoietic system of lethally conditioned leukaemic patients. Moreover, postnatal stem cells amplified *in vitro*, or their direct progeny, are promising tools.

This review focuses on the feasibility of generating *in vitro*, from HSC and mesenchymal stem cells (MSC), functional cells that may be used for cell therapies.

* This work was supported in part by a grant from the Dinu Lupatti – Dubois-Ferrière Foundation to VK.

Cells derived from HSC

HSC were identified by Till and McCulloch in 1961 [7]. These authors showed that the injection of BM cell suspensions into a lethally irradiated host ensured full recovery of their haematopoiesis. Later on HSC were identified as a subpopulation within CD34+ cells [8, 9]. As proof, the number of CD34+ cells grafted into aplastic patients correlates inversely with the time until haematopoietic restoration [10].

In vitro generation of haematopoietic progenitors

In vitro HSC rapidly lose their ability to self-maintain, as illustrated by their decreased ability to repopulate irradiated hosts after culture [11]. However, culture of CD34+ cells with the combination of growth factors such as FLT3-ligand, thrombopoietin and stem cell factor (FTS) generates large numbers of haematopoietic progenitors. These include monocyte, granulocyte, megakaryocyte, myeloid dendritic cell (DC) and plasmacytoid DC progenitors [12, 13] (Figure 1). Lymphocytes are not generated in these conditions because they require a specific stroma. After amplification with FTS, progenitors can be differentiated into a single lineage in secondary cultures using lineage-specific factors [12] (figure 1). So far, only *in vitro* generated DC are evaluated as a therapeutic tool.

Differentiation into antigen-presenting cells

DC represent a small proportion of the leukocytes but are major constituents of the immune response. They are antigen-presenting cells (APC) that constantly sample the internal milieu and present processed antigens docked on their major histocompatibility complex (MHC) to T lymphocytes [14]. In the absence of inflammation DC are said to be "immature", and antigen presentation maintains peripheral tolerance and silences auto-immune reactions. By contrast, within an inflammatory context DC undertake maturation, i.e. they upregulate MHC and T cell costimulatory molecule expression, and stimulate T cells. DC therefore target their action exclusively at T cells specific for the antigen they present, and their state of maturation determines whether they will inhibit or stimulate such T lymphocytes. Consequently, DC represent a powerful tool for piloting of the immune response.

DC were obtained for the first time *in vitro* from CD34+ HSC by Santiago-Schwarz et al. in 1992 by culturing CD34+ cells with GM-CSF and tumour necrosis factor-α (TNF-α) [15]. However, TNF-α which was required for DC differentiation, also induced their maturation and few immature DC were generated in such conditions. This has now been solved using culture systems that uncouple proliferation from differentiation and maturation [16, 17]. The amplification of CD34+ HSC with FTS followed by their differentiation into immature DC with GM-CSF plus IL-4 or into mature DC with GM-CSF, IL-4 and TNF-α generates as many as 1200 DC from one single CD34+ cell [16]. Moreover, CD34+ cells trans-

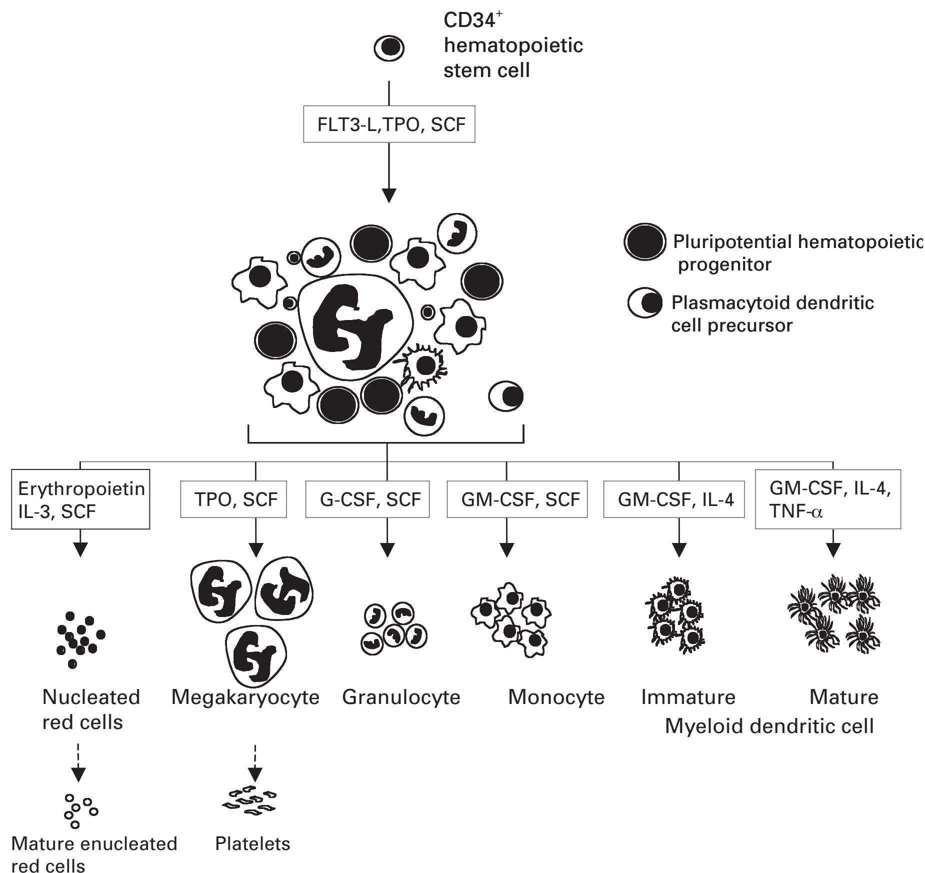


Figure 1

In vitro differentiation of therapeutic cells from HSC. CD34+ HSC are purified from BM, umbilical cord blood or granulocyte (G)-CSF-mobilised peripheral blood by positive selection using magnetic beads coated with anti-CD34 mAb. These purified HSC (>90%) are cultured with FTS for a few weeks, generating proliferating pluripotential immature progenitors and a mixture of non-proliferating differentiated cells. When incubated with lineage-specific factors, progenitors differentiate into one single lineage. Enucleated mature red cells and platelets are generally not detected under these conditions.

duced at the initiation of culture with an exogenous transgene produce transgene-expressing immature or mature DC [12]. Thus DC expressing, processing and presenting specific antigens (i.e. tumour-related antigens) within the correct MHC could be generated and used as a lead to develop tumour specific cytotoxic T lymphocytes. However, the safety issue concerning the use of viral or retroviral constructs in human clinics is not settled yet [18, 19]. It may therefore be safer to undertake therapies whereby DC generated *in vitro* are loaded with synthetic peptides using a peptide exchange strategy, as currently under development in immunotherapies against melanoma and glioma [20, 21].

Cells derived from MSC

MSC were first identified by Friedenstein in 1961 on the basis of their adherence to plastic and their growth *in vitro* [22]. MSC have been isolated from BM, muscle, synovium, dermis, adipose tissue, cord blood and Wharton's jelly [23–27]. The pluripotency of MSC was clearly established in 1999, when Pittenger et al. [23] demonstrated that MSC clones differentiated into several distinct mesenchymal lineages (figure 2). In contrast to HSC, MSC have not been, either before or after *in vitro* amplification, transplanted, isolated and retransplanted into multiple generations of recipients to evaluate their self-renewal capabilities [28]. Moreover, none of the non-specific markers that MSC express after *in vitro* amplification [29–31] has been prevalent for their enrichment [28], and it is not known whether MSC obtained after selective adherence *in vitro* are indeed true stem cells. For these reasons several authors suggest that the term "multipotent mesenchymal stromal cell" should be used for these cells obtained after selective adherence, while "mesenchymal stem cell" should be restricted to the true mesenchymal stem cell, which definitely requires further identification [32]. However, irrespective of this issue, the uncertainty concerning the stemness of *in vitro* amplified MSC should not harness their clinical use as long as they produce a beneficial effect.

MSC as a reconstructive tool

Used immediately after amplification MSC target injured mesenchymal tissues and participate in their regeneration. Allogeneic MSC infused into paediatric patients with osteogenesis imperfecta lacking functional osteoblasts significantly improve bone metabolism [33, 34]. MSC also populate implants and restore bone integrity in a model of acute bone defect [35]. Moreover, MSC play a "bystander" role in HSC grafting by reconstituting the medullar environment facilitating the homing of the grafted cells and consequently haematopoiesis [34, 36]

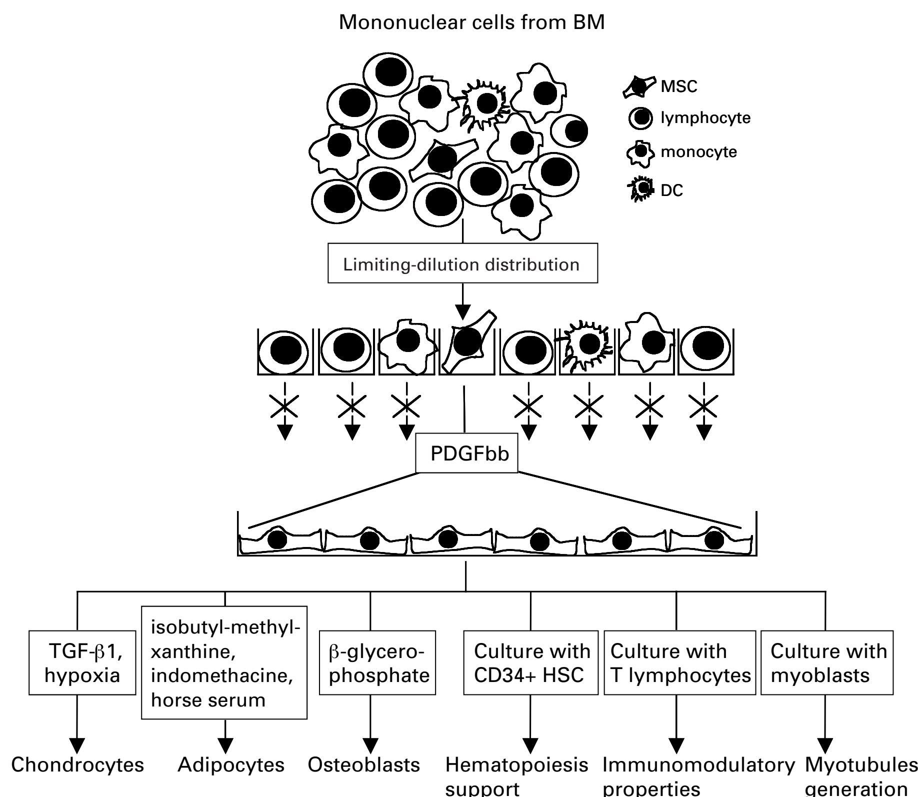


Figure 2

In vitro differentiation of therapeutic cells from MSC. Cell suspension from BM or various tissues are incubated in flat vessels for 24 hours (here in limiting dilution setting to ensure clonal selection). MSC are selected by washing out non-adherent cells, and are amplified using culture conditions that do not allow leukocyte survival. Clonally amplified MSC can be differentiated into various lineages using specific conditions, or used as such in culture with other cells to evaluate their haematopoietic supporting activity, their immunomodulatory properties and their ability to fuse into myotubules.

MSC as an immunomodulatory tool

MSC are immunotolerising cells. Bulk-amplified MSC block mitogen- and APC-induced T lymphocyte stimulation [30, 37]. Moreover, MSC impede *in vitro* DC differentiation, suggesting that they also block T cell activation indirectly by altering APC function [38]. The immunosuppression induced by MSC on T cells is clearly MHC- and antigen-independent. Soluble and membrane-bound molecules are involved but these remain to be identified. In some instances *in vitro*, MSC are stimulatory [39], but the physiological relevance of this observation is not established. By contrast, *in vivo* MSC are thus far strictly immunosuppressive. Most remarkably, in humans, haploidentical MSC injected into paediatric patients with severe graft versus host disease after reconstitution with allogeneic HSC infusion cured the disease [34, 40], suggesting that *in vivo* infusion of MSC may be safe even when injected within an inflammatory context. MSC may nevertheless represent a potential danger, because in mice MSC-induced immune depression is associated with uncontrolled growth of tumours which remain under immune control in non-injected animals [41]. Thus, MSC infusion in humans may equally awaken dormant pathogens or malignant cells. This last observation emphasises the importance of embracing the full spectrum of MSC immunoregulatory activities before extending their use in human clinical medicine.

MSC as a privileged vessel of therapeutic factors

Finally, MSC transduced with erythropoietin or factor IX gene constructs and injected subcutaneously into baboons secrete the corresponding gene products for up to 90 days [42], demonstrating that engineered MSC could be used as a source of therapeutic molecules *in vivo*.

Conclusion

Our understanding of stem cell biology has dramatically increased in the last few years and cell therapies using postnatal stem cells will certainly become more numerous, shortly offering valuable alternatives to classical therapies. MSC will be used, whether associated or not with artificial scaffolds, to achieve bone restoration. They may also be used as immunoregulatory agents to obliterate the deleterious effects of allografting, but careful exploration of their biology must be undertaken beforehand. It is first necessary to establish whether the nonspecific, antigen-independent inhibition of MSC is not a two-edged sword which, though inhibiting graft rejection, may also awaken dormant malignancies. HSC-derived APC may be safer immunoregulatory agents because they mediate their effect via antigen-dependent processes and should therefore hit the chosen target only. Mature DC loaded with a tumour-specific antigen could function as an adjuvant to select and "educate" antigen-specific T cells to eradicate tumour cells, or, conversely, immature DC loaded with a self-antigen should provide powerful inhibition of autoreactive T cells and halt autoimmune processes in susceptible individuals. The danger here does not lie in the specificity of the mechanism but in the state of DC maturation: how sure are we that a mature DC will remain mature long enough to provide a stimulatory signal to T cells *in vivo*, and how sure are we that an immature tolerising DC generated *in vitro* will remain so *in vivo?* All these questions represent an impressive challenge to be met before we can safely extend the clinical use of MSC and HSC. Hopefully, we now have the means to explore these avenues.

Correspondence:
Dr Vincent Kindler, PhD, PD
Haematology Service
25, Micheli-du-Crest
CH-1211 Geneva 14
Switzerland
E-Mail: vincent.kindler@hcuge.ch

References

1 Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development 1990;110:1001–20.
2 Kindler V. Postnatal stem cell survival: does the niche, a rare harbor where to resist the ebb tide of differentiation, also provide lineage-specific instructions? J Leukoc Biol 2005;78: 836–44.
3 Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL. Turning brain into blood: a haematopoietic fate adopted by adult neural stem cells in vivo. Science 1999;283:534–7.
4 Jackson KA, Mi T, Goodell MA. Haematopoietic potential of stem cells isolated from murine skeletal muscle. Proc Natl Acad Sci U S A 1999;96:14482–6.
5 Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981;292:154–6.
6 Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–7.
7 Till JE, McCulloch CE. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961;14:213–22.
8 Berenson RJ, Andrews RG, Bensinger WI, Kalamasz D, Knitter G, Buckner CD, et al. Antigen CD34+ marrow cells engraft lethally irradiated baboons. J Clin Invest 1988;81:951–5.

9 Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of haematopoiesis. III. A haematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 1984;133:157–65.
10 To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. Blood 1997;89:2233–58.
11 Dorrell C, Gan OI, Pereira DS, Hawley RG, Dick JE. Expansion of human cord blood CD34(+)CD38(–) cells in ex vivo culture during retroviral transduction without a corresponding increase in SCID repopulating cell (SRC) frequency: dissociation of SRC phenotype and function. Blood 2000;95:102–10.
12 Salmon P, Kindler V, Ducrey O, Chapuis B, Zubler RH, Trono D. High-level transgene expression in human haematopoietic progenitors and differentiated blood lineages after transduction with improved lentiviral vectors. Blood 2000;96:3392–8.
13 Chen W, Antonenko S, Sederstrom JM, Liang X, Chan AS, Kanzler H, et al. Thrombopoietin cooperates with FLT3-ligand in the generation of plasmacytoid dendritic cell precursors from human haematopoietic progenitors. Blood 2004;103: 2547–53.
14 Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–52.
15 Santiago-Schwarz F, Belilos E, Diamond B, Carsons SE. TNF in combination with GM-CSF enhances the differentiation of neonatal cord blood stem cells into dendritic cells and macrophages. J Leukoc Biol 1992;52:274–81.
16 Arrighi JF, Hauser C, Chapuis B, Zubler RH, Kindler V. Long-term culture of human CD34(+) progenitors with FLT3-ligand, thrombopoietin, and stem cell factor induces extensive amplification of a CD34 (–)CD14 (–) and a CD34 (–)CD14(+) dendritic cell precursor. Blood 1999;93:2244–52.
17 Herbst B, Kohler G, Mackensen A, Veelken H, Lindemann A. GM-CSF promotes differentiation of a precursor cell of monocytes and Langerhans-type dendritic cells from CD34+ haemopoietic progenitor cells. Br J Haematol 1998;101:231–41.
18 Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 2003;302:415–9.
19 Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP, et al. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol Genet Metab 2003;80:148–58.
20 Berger TG, Strasser E, Smith R, Carste C, Schuler-Thurner B, Kaempgen E, et al. Efficient elutriation of monocytes within a closed system (Elutra) for clinical-scale generation of dendritic cells. J Immunol Methods 2005;298:61–72.
21 Liau LM, Prins RM, Kiertscher SM, Odesa SK, Kremen TJ, Giovannone AJ, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res 2005;11:5515–25.
22 Friedenstein AJ. Osteogenetic activity of transplanted transitional epithelium. Acta Anat (Basel) 1961;45:31–59.
23 Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
24 Suva D, Garavaglia G, Menetrey J, Chapuis B, Hoffmeyer P, Bernheim L, et al. Non-haematopoietic human bone marrow contains long-lasting, pluripotential mesenchymal stem cells. J Cell Physiol 2004;198:110–8.
25 Toma JG, Akhavan M, Fernandes KJ, Barnabe-Heider F, Sadikot A, Kaplan DR, et al. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 2001;3:778–84.
26 Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279–95.
27 Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 2004;22:1330–7.
28 Javazon EH, Beggs KJ, Flake AW. Mesenchymal stem cells: paradoxes of passaging. Exp Hematol 2004;32:414–25.
29 Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. Blood 2001;98:2615–25.
30 Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 2003;75:389–97.
31 Zou W, Amcheslavsky A, Bar-Shavit Z. CpG oligodeoxynucleotides modulate the osteoclastogenic activity of osteoblasts via Toll-like receptor 9. J Biol Chem 2003;278:16732–40.
32 Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, et al. Clarification of the nomenclature for MSC: the International Society for Cellular Therapy position statement. Cytotherapy 2005;7:393–5.
33 Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci U S A 2002;99:8932–7.
34 Le Blanc K, Ringden O. Immunobiology of human mesenchymal stem cells and future use in haematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2005;11:321–34.
35 Petite H, Viateau V, Bensaid W, Meunier A, de Pollak C, Bourguignon M, et al. Tissue-engineered bone regeneration. Nat Biotechnol 2000;18:959–63.
36 Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and haematopoietic stem cells in haematologic malignancy patients. Biol Blood Marrow Transplant 2005;11:389–98.
37 Le Blanc K, Rasmusson I, Gotherstrom C, Seidel C, Sundberg B, Sundin M, et al. Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes. Scand J Immunol 2004;60:307–15.
38 Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, et al. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood 2005;105:2214–9.
39 Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 2003;57:11–20.
40 Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439–41.
41 Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood 2003;102:3837–44.
42 Bartholomew A, Patil S, Mackay A, Nelson M, Buyaner D, Hardy W, et al. Baboon mesenchymal stem cells can be genetically modified to secrete human erythropoietin in vivo. Hum Gene Ther 2001;12:1527–41.

Swiss Medical Weekly

Official journal of
the Swiss Society of Infectious disease
the Swiss Society of Internal Medicine
the Swiss Respiratory Society

The many reasons why you should choose SMW to publish your research

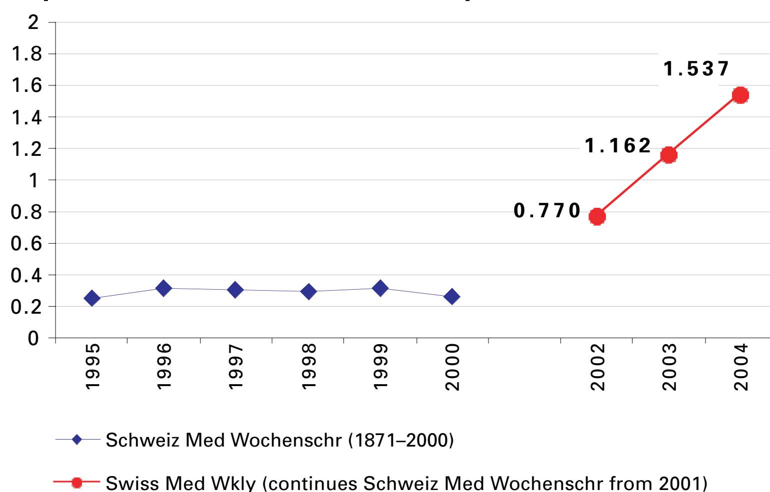
What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

Impact factor Swiss Medical Weekly



Editorial Board
Prof. Jean-Michel Dayer, Geneva
Prof. Peter Gehr, Berne
Prof. André P. Perruchoud, Basel
Prof. Andreas Schaffner, Zurich (Editor in chief)
Prof. Werner Straub, Berne
Prof. Ludwig von Segesser, Lausanne

International Advisory Committee
Prof. K. E. Juhani Airaksinen, Turku, Finland
Prof. Anthony Bayes de Luna, Barcelona, Spain
Prof. Hubert E. Blum, Freiburg, Germany
Prof. Walter E. Haefeli, Heidelberg, Germany
Prof. Nino Kuenzli, Los Angeles, USA
Prof. René Lutter, Amsterdam, The Netherlands
Prof. Claude Martin, Marseille, France
Prof. Josef Patsch, Innsbruck, Austria
Prof. Luigi Tavazzi, Pavia, Italy

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts:	submission@smw.ch
Letters to the editor:	letters@smw.ch
Editorial Board:	red@smw.ch
Internet:	http://www.smw.ch