3. Dyson, A. & Singer, M. *Crit. Care Med.* **37**, S30–S37 (2009).
4. Hotchkiss, R.S. & Karl, E.I. *N. Engl. J. Med.* **348**, 138–150 (2003).
5. Monneret, G., Venet, F., Pachot, A. & Lepape, A. *Mol. Med.* **14**, 64–78 (2008).
6. Munford, R.S. & Pugin, J. *Am. J. Respir. Crit. Care Med.* **163**, 316–321 (2001).
7. Venet, F. *et al. Intensive Care Med.* **35**, 678–686 (2009).
8. Pugin, J. *Novartis Found. Symp.* **280**, 21–27 (2007).
9. Rittirsch, D. *et al. Nat. Protoc.* **4**, 31–36 (2009).
10. Wang, H. *et al. Science* **285**, 248–251 (1999).
11. Flierl, M.A. *et al. FASEB J.* **22**, 2198–2205 (2008).
12. Lenfant, C. *N. Engl. J. Med.* **349**, 868–874 (2003).

Németh *et al.* reply:

A typical bench-to-bedside path in medicine is to first discover specific targets using basic science and then develop therapeutic agents that are tested clinically. In the field of bone marrow stromal cells (BMSCs; also referred to as mesenchymal stem cells or MSCs), this course was reversed—running from bedside to bench. BMSCs, which are known to have immunoregulatory properties, have been used successfully in humans to combat graft-versus-host disease (GVHD)[1]. Our studies were based upon the clinical observations of Ringden and co-workers that BMSCs do not have any adverse effect, and might even be beneficial, when given to humans suffering from peritonitis[2]. They treated two patients suffering from severe, antibiotic-resistant peritonitis with mismatched allogeneic BMSCs. The peritonitis disappeared after the BMSC infusions; one patient died 4 months later of a fungal infection, but the other patient recovered. The mechanism(s) of action of BMSCs were unknown, however. Thus, our goal was to learn how BMSCs act using a mouse model of sepsis: cecal ligation and puncture (CLP). As we recently reported in these pages[3], BMSCs, when given at the time of surgery or soon afterward, rescue mice from the lethal effects of CLP surgery. After coming in contact with them, BMSCs release prostaglandins to reprogram macrophages to induce their synthesis and secretion of interleukin-10 (IL-10). Monneret is absolutely right about the need for caution in making predictions about the efficacy of human treatments based on experiments done in mice; whether the BMSCs act in the same way in humans as they do in the mice remains to be determined, and better animal models of sepsis are surely needed (see, for example, ref. 4). What should already be clear from our work, however, is that the cells are 'smarter' than drugs. In the CLP model, their actions could not possibly be mimicked by systemic administration of prostaglandins, which would have a myriad of side effects. Monneret stresses the fact that "everything is thus a matter of timing." He is right; this is why cellular therapy might be superior to drug therapy. The BMSCs appear to "think globally, but act locally," providing assistance to only those cells that need it, *where* and *when* they need it—and most likely, this depends on the signals they receive at any given time. Furthermore, it is conceivable that BMSCs may help different cells in different ways, secreting agents in response to a variety of environmental cues and rebalancing the innate immune response to maximize its utility to the host. Ultimately, we will learn from clinical studies whether these hypotheses are correct.

Krisztián Németh[1], Asada Leelahavanichkul[2], Peter S T Yuen[2], Balázs Mayer[1], Alissa Parmelee[1], Kent Doi[2], Pamela G Robey[1], Kantima Leelahavanichkul[1], Beverly H Koller[4], Jared M Brown[5], Xuzhen Hu[2], Ivett Jelinek[3], Robert A Star[2] & Éva Mezey[1]

[1]Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, [2]Renal Diagnostics and Therapeutics Unit, National Institute of Diabetes and Digestive Kidney Diseases, and [3]Experimental Immunology Branch, National Cancer Institute, US National Institutes of Health, Bethesda, Maryland, USA. [4]Department of Genetics, University of North Carolina, Chapel Hill, North Carolina 27599, USA. [5]Department of Pharmacology and Toxicology, East Carolina University, Greenville, North Carolina, USA.
e-mail: mezeye@mail.nih.gov

1. Le Blanc, K. *et al. Lancet* **363**, 1439–1441 (2004).
2. Ringden, O. *et al. Leukemia* **21**, 2271–2276 (2007).
3. Németh, K. *et al. Nat. Med* **15**, 42–49 (2009).
4. Doi, K. *et al. Kidney Intl.* **74**, 1017–1025 (2008).

Reassessing the human mammary stem cell concept by modeling limiting dilution transplantation assays

To the Editor:

In a recent study in *Nature Medicine*, Eirew *et al.*[1] described a new protocol that can be used to accurately determine the frequency of human mammary stem cells (termed mammary repopulating units, MRUs) by limiting dilution transplantation assays (LDTAs). The authors presented five LDTAs showing the regeneration of colony-forming cells (CFCs) in xenografted gels seeded with varying numbers of input mammary cells. MRU frequency estimates were based on the standard single-hit Poisson model[2] (SHPM). In the field of limiting dilution assays, this is the most parsimonious statistical model, which posits that a single cell (considered by Eirew *et al.*[1] as a stem cell) is able to regenerate mammary structures containing multiple secondary CFCs. The SHPM is written as $\pi_i = \exp(-fx_i)$, where π_i is the expected proportion of negative xenografted gels (that is, free of CFCs), f is the frequency of MRUs and x_i is the mammary cell input (cell dose), that is, the number of mammary cells suspended in collagen gels, with each cell dose labeled i. On the basis of the results of their LDTAs, the authors claimed that the CFCs originate from a single cell (the MRU), and this statement was supported by a standard chi-squared test (Pearson's χ^2 statistic) providing high consistency with the SHPM for each LDTA. To endorse the appropriate use of χ^2 statistics in their LDTAs, Eirew *et al.*[1] cited an original statistical method previously published by us[3] dealing with statistical modeling of limiting dilution data. Unfortunately, we cannot sanction the citation of our work in their article. The reason is that the scientific content of our paper has not been correctly used. This raises serious concerns about the decision to use χ^2 statistic approach for analysis of LDTAs, which, in turn, raises questions concerning the biological conclusions drawn from the studies of Eirew *et al.*[1].

In the work of Eirew *et al.*[1], attention must be paid to the reported P values derived from χ^2 statistics. Two P values are equal or close to 1 (experiments 1 and 5), indicating that the SHPM perfectly fits the data. Such P values are unrealistic, thus one must consider whether a serious problem has occurred in the statistical analysis. This situation relates to data sparseness, that is, the number of gels used at each cell dilution is small (four to seven), and many of the observed