Of importance, the chromosomal abnormalities seen by Flores-Figueroa et al in the MSCs were different from the ones found in the hematopoietic population.[2] These data, along with our previous observation in sex-mismatched hematopoietic stem cell transplant recipients in which we found no evidence of donor-derived stroma 0.15 to 27 years after allogeneic marrow transplantation, strongly suggest that these 2 lineages are derived from distinct stem cells.[3,4] The fact that patients with MDS can be cured of their disease by allogeneic peripheral *blood* stem cell transplantation also suggests that the stroma in MDS is intrinsically normal and that the abnormal function attributed to MDS stroma is the result of interactions between clonal hematopoietic cells and stromal cells.

In our opinion, both reports strongly suggest that the stroma and hematopoietic lineages are distinct: therefore, we conclude that the stromal cells in MDS are not derived from the same transformed stem cell as the hematopoietic clone.

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The authors declare no competing financial interests.

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To the editor:

Discrete stem cells: subsets or a continuum?

In a recent issue of *Blood,* Sieburg et al[1] investigated the patterns of clonal repopulation kinetics in a mouse model. The authors demonstrated that the patterns of repopulation observed were limited to a subset of the theoretically possible patterns, implying that the behavior of the cells is patterned and inherited, perhaps through epigenetic modifications.

These data clearly show that hematopoietic stem cells (HSCs) cannot be viewed as "a homogeneous population of cells that respond to the conditions in vivo in a stochastic manner."[1(p2314)] However, this description is not an accurate reflection of the continuum models that have recently been proposed.[2-4] The problem lies in the authors' statement that "inherent in the idea of an HSC continuum is the idea that every HSC behavior possible should actually be observable."[1(p2311)] In fact, it is implicit in all of these models that stem cells are heterogeneous, not homogeneous, and that differentiation will be "constrained" along certain paths, resulting ultimately in a limited range of cell types.

One mathematic approach to the description of a continuum of potentiality is the concept of a phase space.[2,3] This is simply a tool that can be used to describe a complex and continuous system, and there is nothing in the use of such a tool that implies that all points in the conceptual space are equally likely to occur. The mathematics of such a model imply that the behavior of cells at different points within the phase space will be different and may be constrained by both internal and external influences.[5]

So, why use continuous rather than discrete models? There are 3 principal reasons. The first is that, as Sieburg et al[1] acknowledge, the relatively small number of discrete repopulation outcomes that they observed becomes rapidly more complex as additional parameters are considered. While the subset actually observed to be possible/likely may be only a small percentage of the total possibilities, in absolute numbers the observed number of "discrete populations" will continue to increase. Would such an analysis serve to define truly discrete subpopulations of HSCs, or does it rather simply help to define the constraints that exist within a continuous population?

The second reason for modeling stem cells as a continuum is that such modeling permits, and in fact requires, the concept of reversibility. To date all models based on discrete populations have been unidirectional, but there is increasing evidence that reversibility is a reality.

The third reason for using continuous models is that they can be useful tools for examining additional parameters without changing the global paradigm. For example, epigenetic modification will not be constant between donors of different ages. If different patterns were observed in young and old mice, would this imply yet more discrete HSC populations? In a continuous model, such variables can be incorporated as internal variables within the modeled "cell" that constrain or alter the probabilities of different outcomes.

This is not a battle between stochasticists and determinists, but an attempt to find new and potentially more efficient tools to model stem cell behavior, which may ultimately have predictive value. The "standard model" has stood unchallenged for many decades, and it is undoubtedly time that it be reviewed and refined.

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Response:

Discrete stem cell subsets

It is delightful that our paper, "The hematopoietic stem cell compartment consists of a limited number of discrete subsets,"[1] has sparked the interest of colleagues in the modeling field. To recapitulate, we showed the following: (1) Only a small fraction of all possible behaviors was realized by hematopoietic stem cells (HSCs) in vivo. (2) Based on the kinetics of repopulation, HSCs could be classified into groups. (3) HSCs in different groups differed in self-renewal capacity. (4) HSCs in different groups can differ in cell-surface phenotype. (Additional data were presented recently.[2])

Kirkland,[3] Roeder and Loeffler,[4] Quesenberry et al,[5] and Quesenberry[6] have each developed models that describe the HSC compartment as a continuum of functions. We agree that many paradigms in HSC biology should be revisited to incorporate recent developments, particularly in the area of molecular control of HSC behavior. Models are powerful tools to complement and integrate experimental data, if they make testable predictions that allow their validation. It has been argued that a useful model should be a hypothesis generator.[7] It is a bit challenging for nonaficionados to discern the predictions made by the continuum models. Thus, the letter by Kirkland et al opens a welcome dialogue.

In their letter, Kirkland et al question whether our data support or challenge their models. Let us dispense with the discussion of some of the finer points of the different flavors of the continuum models and focus on the central point. The central argument of these models appears to be that behavior of HSCs should be reversible, creating an ephemeral heterogeneity of HSC functions. Such an extreme flexibility (reversibility) would predict that a single HSC can recreate all, or at least a good part, of the functional heterogeneity seen in the HSC compartment. However, we showed that each individual HSC generates daughter HSCs that are very similar to each other in their differentiation and proliferation behavior.[8] Notably, single HSCs did *not* recreate the heterogeneity seen in the original HSC compartment. These data support the view that HSC differentiation and proliferation capacities are epigenetically fixed on the level of individual HSCs. In other words, HSC heterogeneity is permanent at least in the adult mouse.

On face value, it is difficult to integrate these data with the notion that HSC behavior is reversible. However, experience from quantum mechanics has shown that both discrete and continuum approaches are needed to fully explain the behavior of subatomic particles. Whether HSCs do in fact behave like quanta awaits experimental resolution.

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To the editor:

Increased mortality with FLA compared with ADE chemotherapy in high-risk AML

In the MRC AML-HR trial,[1] Milligan et al describe inferior overall survival with fludarabine and high-dose cytosine (FLA) compared with conventional cytosine, daunorubicin, and etoposide (ADE) reinduction chemotherapy for high-risk acute myeloid leukemia (AML). As type 1 error is a potential explanation of these results, further information to clarify the mechanism underlying the reduced overall survival with FLA would be helpful before a potentially useful regimen is abandoned.

The difference in overall survival appears attributable to increased death in remission (34% vs 12%; $P = .1$) in the FLA compared with ADE group. Although not statistically significant, the trial was not powered to explore this parameter, and other adverse predictors (resistant disease, induction death, and relapse rate) were almost identical. An analysis of deaths in remission comparing the 2 treatment arms may clarify the inferior overall survival with FLA. For example, fludarabine predisposes to opportunistic infections,[2] may influence choice of subsequent consolidation therapy, and may also lead to an increased risk of second malignancy.[3] No information on the use of prophylactic antimicrobials was given.

The MRC AML-HR trial[1] has made an important contribution to our understanding of the optimal therapeutic approach in high-risk AML. Further information would be valuable to continue development or modification of current treatment algorithms in this challenging disease.

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