To the editor:

Molecular monitoring in chronic myeloid leukemia patients who achieve complete cytogenetic remission on imatinib

About 75% of patients with newly diagnosed chronic phase (CP) chronic myeloid leukemia (CML) treated initially with imatinib achieve complete cytogenetic remission (CCyR),[1] and imatinib also induces Ph negativity, though less often, in patients treated in advanced phases of CML. Patients in CCyR may however still have as many as 10^{10} leukemia cells in their body,[2] and in CML the quantity of such "residual disease" can be monitored by quantitating BCR-ABL transcripts.[3] We have studied serially BCR-ABL transcript numbers in a series of CML patients treated with imatinib who achieved CCyR. The results confirm that they constitute a heterogeneous group whose individual prognoses may be very different. The failure to detect BCR-ABL transcripts on repeated testing was rare.

We identified 42 CML patients (37 originally in CP, 5 in accelerated phase [AP]) treated with imatinib at 8 collaborating United Kingdom centers who were classified as having achieved CCyR on the basis of 100% Ph negativity in at least 20 marrow metaphases. At the time of starting imatinib the patients were newly diagnosed (n = 15), chronic phase refractory to interferon-α (n = 24), or in relapse after stem cell transplantation (n = 2). The median age was 52.3 years (range, 24.8-69.9 years), and median time to achieve CCyR was 5.5 months (range, 1.8-16.4 months). The daily dose of imatinib was 400 mg (CP) or 600 mg (AP). Serial quantitative polymerase chain reaction (Q-PCR) assays were performed at regular intervals at a single center using Taq-man technology (Applied Biosystems, Foster City, CA).[4] The median number of samples per patient was 5 (range, 3-15 samples). Results were expressed as a percentage ratio using ABL transcripts as the internal control. The failure to detect BCR-ABL by Taq-man assay was confirmed with a 2-step nested technique as previously described.[5]

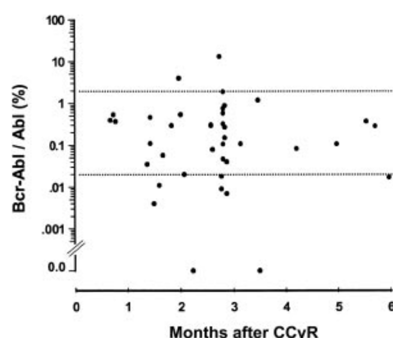

Figure 1. BCR-ABL/ABL ratios in first available sample from 42 patients reported to be in complete cytogenetic remission. Two patients had values above 2.0%, and 7 patients had values below 0.02%. There was no obvious relationship between the interval from first classification as CCyR and the level of BCR-ABL transcripts.

The median BCR-ABL/ABL ratio was 0.19% (range, 0.0%-13.1%) for the first sample after achieving CCyR; only 2 patients had ratios above 2.0% (Figure 1). During serial monitoring thereafter 17 (40%) patients (15 originally in CP; 2 originally in AP) achieved a BCR-ABL/ABL ratio less than 0.02% on at least 2 occasions 4 or more weeks apart. Only 1 of the 17 patients who achieved a ratio less than 0.02% later lost the CCyR and progressed to hematologic relapse. Thirty-three patients always had detectable BCR-ABL transcripts. In 7 cases transcripts were undetectable on at least one occasion but positive on others. Two patients had no detectable transcripts on serial monitoring for at least 6 months; in one case both Taq-man and nested PCR studies were negative on 3 occasions over 11 months, and in the other, 3 sequential specimens were negative over 6 months.

In summary we have shown that Q-PCR values in CML patients in CCyR are very variable. The failure to detect any transcripts, as described by Barbany et al[6] in one patient, was a rare occurrence and frequently was not sustained. We suggest that the term these authors employed, "molecular remission," is imprecise since it depends very much on the sensitivity of the assay employed. It would be preferable to refer to responding patients as having transcripts undetectable for a specified period. Our data support the recommendation that quantification of BCR-ABL transcripts is essential for optimum management of CML patients who achieve CCyR on imatinib.[7]

Feng Lin, Mark Drummond, Stephen O'Brien, Francisco Cervantes, John Goldman, and Jaspal Kaeda

Correspondence: John Goldman, Department of Haematology, Imperial College School of Medicine at Hammersmith Hospital, Ducane Road, W 12 ONN London, United Kingdom; e-mail: j.goldman@ic.ac.uk

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To the editor:

Quantitative tissue stem cell modeling

With great interest we read the article by Quesenberry et al[1] on the chiaroscuro stem cell. Based on many experimental findings that contrast the classical paradigm of a stem cell hierarchy with irreversibly declining proliferative potential, the authors formulate a new qualitative concept of a nonhierarchical, flexible stem cell behavior.

As the stem cell definition is still a functional one,[2,3] requiring a checking by different assays, it is necessary to show for any concept whether it conforms with the list of qualifying criteria. In this context we like to draw attention to the possibility of formulating a quantitative theory. Recently, we have described a novel quantitative model of stem cell organization[3,4] that has conceptual similarities to the concept proposed by Quesenberry et al. The model is based on the concept of within-tissue plasticity, assuming that particular cellular properties can reversibly be acquired or lost, and quantitatively changed within a range of potential options. The decision of whether a property is expressed or not and to which degree depends on the current state of the cell and on the microenvironmental signals that it is able to recognize. Using a simulation model we can show that such a functional view on cellular organization can consistently explain a broad variety of experimental observations on heterogeneity and plasticity of cellular properties and functionalities in the hematopoietic system with full conformity to all the functional stem cell criteria.[4]

Quesenberry and colleagues relate the observed phenotypic plasticity (summarized in the term "chiaroscuro") mechanistically to cell cycle activity. In contrast, in our model plasticity is not directly linked to cell cycle activity, but cell cycle activation results from a switching of cells between different growth environments (GEs).

Figure 1 shows simulated time courses of individual cells with respect to a model property a describing the actual affinity of cells to reside in a specific GE that supports the maintenance and renewing of a. Cells in this GE are assumed to be in G_0. They can change to another GE where property a is declining while the cell cycle is activated. Lineage commitment (ie, loss of stemness) is entered if a falls below a critical threshold (here, 0.01). The actual value of a can be interpreted as a measure of self-renewal ability, and therefore, of long-term repopulating potential. Figure 1 shows that in our model, changes in cell cycle are associated with reversible changes in the property a.

Our theory conforms with Quesenberry's reasoning, but additionally it provides a quantitative reproduction of many other experimental phenomena.[4] Furthermore, the model allows to separate emerging phenomena from underlying mechanisms. As an example we refer to the observed differences in the engraftment potential of cell populations sorted for their actual cell cycle status. Quesenberry et al consider these phenomena as evidence for a cell cycle–associated plasticity. Our model does not explicitly assume cell cycle–dependent engraftment advantages. However, it produces a heterogeneous stem cell population with respect to a. Cells from this population selected for G_1/G_0 exhibit a larger proportion of cells with high a (high long-term repopulating potential) compared to cells selected for $S/G_2/M$.

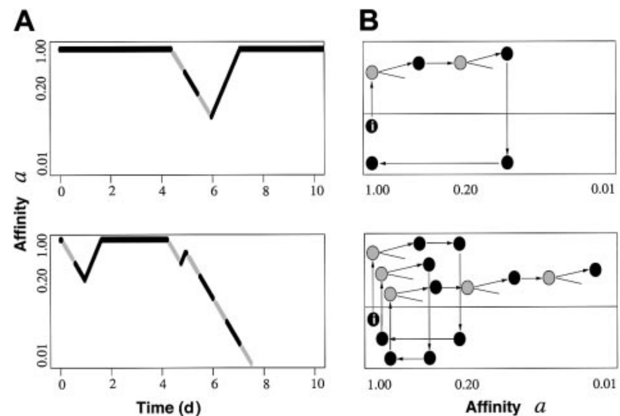

Figure 1. Simulation examples. (A) Two time courses of individual cells (tracing of one daughter cell per division) with respect to affinity a, showing the actual cell cycle status. Black segments represent cell cycle phases G_1/G_0, gray segments indicate $S/G_2/M$. Whereas the upper example describes a cell maintaining/renewing a high a (ie, long-term repopulating potential), the lower example shows terminal lineage commitment after several fluctuations. (B) Corresponding cell fates represented in terms of the proposed within-tissue plasticity model[4] (I, initiating cell; gray, $S/G_2/M$; black, G_1/G_0). Dormant cells in lower growth environment (gray region) regain affinity a, whereas (potentially) cycling cells in the other environment (white region) lose a.

It has to be emphasized that theoretical modeling cannot prove hypotheses. However, modeling offers the possibility to test the impact of different (latent) molecular and genetic mechanisms on the generation and variability of phenotypic processes emerging in functional assays or in vivo. In this regard we think tissue stem cell modeling is only at its start.

Ingo Roeder and Markus Loeffler

Correspondence: Ingo Roeder, Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Liebigstr 27, D-04103 Leipzig, Germany; e-mail: ingo.roeder@imise.uni-leipzig.de.

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Response:

Quantitative tissue stem cell modeling

Roeder and Loeffler comment on our recent paper, "The chiaroscuro stem cell: a unified stem cell theory"[1] and describe "quantitative stem cell modeling." They describe "within-tissue plasticity," where it is assumed that certain cellular characteristics can be reversibly acquired or lost. They propose that these characteristics can be "quantitatively changed within a range of potentials." The final phenotype of the cell depends on the state of the cell and the inductive influences of the microenvironment. Our chiaroscuro continuum model shows exactly these characteristics, but in a broader context. One can substitute a cell cycle–related parameter for "state of the cell" and inductive signals for "microenvironmental signals."

The Roeder-Loeffler model is described in more detail in the referenced *Experimental Hematology* publication.[2] In that article, they assume 2 different growth environments (GE-A, GE-Ω), where each cell is characterized by 2 properties: the cycling status (c) and a property *(a)*, which describes the affinity of the cell to reside in GE-A. Nonproliferative cells reside in GE-A, whereas cells in GE-Ω proliferate. Final outcomes are determined by the interaction of the cells and their microenvironments. This would appear to represent a simple digital model, while our chiaroscuro theory is an analog model with continuously changing potential for the stem cells and its inducers. It is not clear that the within-tissue

plasticity model "additionally. . . . provides a quantitative reproduction of many other experimental phenomena" beyond that offered by the chiaroscuro model. In fact, the chiaroscuro model incorporates a full range of quantitative experimental phenomena and is less limited than the within-tissue plasticity model. In the chiaroscuro model the stem cell phenotype is continuously changing with cell cycle (or some other related phenomena), while the Roeder-Loeffler model proposes 2 discrete stem cell states. Further, our concept of microenvironment includes any inductive influences. We hypothesize that the environmental inductive influences acting on the cell also are continuously changing, rather then being represent by 2 discrete microenvironments.

Despite comments to the contrary, the Roeder-Loeffler model explicitly assumes cell cycle dependence, as illustrated by this quotation: "cells from this population selected for G1/G0 exhibit a large proportion of cells with high a (high long-term repopulating potential) compared to cells selected for S/G2/M." It's important to acknowledge that parameters other than cell cycle may be operative here, although the correlations between cell cycle phase and phenotype change are very strong. Altogether, the 2 models suggest a new way of viewing hematopoiesis and, in fact, are remarkably similar.

Both models, also, begin to explain the heterogeneity of the most purified stem cell population. This feature has been neglected or denied by many investigators but is critical to an understanding of stem cells. Drs Roeder and Loeffler[2] in their *Experimental Hematology* article cite 4 references for stem cell heterogeneity as to cycling activity, colony-forming ability, and phenotypic markers. We have recently studied the colony-forming and differentiation capacity of highly purified lineage-negative rhodamine low Hoechst low murine stem cells cloned as single cells in response to 7 growth factors. We found virtually total heterogeneity as to colony formation and colony size. When the differentiated cell types in these colonies were determined, 14 different colony types were defined. Recently, in the plasticity debate, many investigators have stated that clonal stem cell experiments are essential for interpretable results in transdifferentiation studies. We quote, "First single cells must be shown to be capable of both the expected and unexpected differentiation."[3] If a defined stem cell population is very heterogeneous, then a clonal study is not meaningful unless thousands of clones are evaluated. This is, of course, a population study. Clonal studies in a heterogeneous population serve to define that heterogeneity but offer little else.

The above models can probably be extended to encompass marrow to nonhematopoietic tissue conversions, that is, stem cell plasticity. We had originally proposed this in the manuscript submitted to *Blood,* but the reviewers requested its removal. It seems highly unlikely that the concepts represented in these models will not extend to the production of heart, liver, skin, or other cells by marrow stem cells.

We understand the restraints imposed when carrying out mathematical modeling, as was done by the authors in the *Experimental Hematology* paper.[2] Simplicity is an initial requirement. As pointed out by the authors, there are many other variables that will need to be incorporated into their model. Hopefully, these will include the continuously changing phenotype of the marrow stem cell with cycle transit. Given the growing quantity and complexity of information on the stem cell, modeling, such as described here, will become increasingly essential to our understanding of stem cell biology. In our laboratory we have begun to brush up on our basic mathematics, to read calculus, and even to ponder chaos. We have indeed entered the era of stem cell modeling.

Peter J. Quesenberry, Gerald A. Colvin, and Jean-Francois Lambert

Correspondence: Peter J. Quesenberry, Center for Stem Cell Biology, Roger Williams Medical Center, 825 Chalkstone Ave, Providence, RI 02908-4735; e-mail: pquesenberry@rwmc.org.

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To the editor:

High frequency of p53 dysfunction and low level of V_H mutation in chronic lymphocytic leukemia patients using the V_H3-21 gene segment

The recent report by Tobin et al[1] that chronic lymphocytic leukemia (CLL) patients using the V_H3-21 gene segment have a poor prognosis despite mostly having more than 2% V_H mutation has generated a considerable amount of interest.[2-4] This is partly because such cases constitute an exception to the rule that "mutated" CLL necessarily has a good prognosis, and partly because their poor outcome remains unexplained.

We have previously reported that p53 dysfunction can arise in CLL through inactivating mutation of the genes encoding either p53 or ATM (a kinase that regulates p53) and have devised a simple screening test for these defects.[5] The test measures the accumulation of p53 and one of its transcriptional targets (p21) in response to ionizing radiation (IR). Both defects are associated with impaired p21 accumulation. In the type A defect due to p53 mutation, baseline levels of p53 are increased, whereas in the type B defect, due to ATM mutation, baseline p53 is not increased, but IR-induced accumulation is impaired. We have further shown that both defects are, like CD38 expression, largely confined to patients with less than 5% V_H mutation and associated with a short survival.[6] On the basis of these findings we proposed a prognostic model of CLL in which a 5% V_H mutation cut-off separates a homogeneous group of good-risk "mutated" patients from a heterogeneous "unmutated" group. The latter can then be risk-stratified according to the presence or absence of other adverse prognostic factors (eg, p53 dysfunction, CD38 expression) that are confined to this group.

It was of obvious interest to ascertain how CLL patients using V_H3-21 fitted into this prognostic model and whether the poor outcome of such patients could be explained by an association with p53 dysfunction or CD38 expression. To do this, we have examined the relationship between V_H3-21 usage and V_H mutation, p53 dysfunction, and CD38 expression in an updated series of 83 patients with CLL attending the Royal Liverpool University