expression in all FA-BM irrespective of chromosomal aberrations or bone marrow morphology implies the possibility of an FA-specific *TERC* dysregulation.

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Contribution: S.M. developed the study, designed the research, analyzed microarray data, and wrote the manuscript; C.B. carried out sample preparation and qRT-PCR; S.M., M.W., and S.P. analyzed microarray data; A.D.W. provided scientific input and laboratory facilities and contributed to writing the manuscript; H.H. provided data regarding FA complementation analysis; H.N. and W.E. provided patient material and clinical data and contributed to writing the manuscript; M.W.W. carried out SNP array analysis and contributed to writing the manuscript; and H.T. initiated and developed the study and wrote the manuscript.

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To the editor:

Doubts concerning the recently reported human neutrophil lifespan of 5.4 days

Using orally administered deuterium-labeled water to label neutrophils in vivo, Pillay et al measured urinary and blood deuterium-to-proton enrichment ratios at intervals of ~ 12 days over several weeks before and after termination of intake.[1] From mathematical modeling they obtained a median peripheral blood (PB) human neutrophil lifespan of 5.4 days.

This result is highly surprising in view of the range of neutrophil labeling techniques, including whole blood labeling with DFP-32[2]; in vivo H-3 labeling followed by blood transfusion[3]; and in vitro labeling of purified neutrophils with Cr-51,[4] In-111,[5] or Tc-99m,[5] which have all previously given a PB lifespan of ~ 10 hours. It is also starkly at odds with the clinical observations of rapid neutrophil depletion after myeloablative chemotherapy (within 3-5 days) and short-lived normalization of neutrophil counts following therapeutic granulocyte infusion (1-2 days).

We wish to raise several objections to the work of Pillay et al.

1. Bone marrow is a major site of neutrophil destruction as well as development,[6] but there is no discussion on the availability of deuterium-labeled adenosine for salvage that might result in reutilization in newly dividing neutrophil precursors.

2. The authors claim their previous work on human lymphocytes,[7] based on similar sampling intervals, validates their modeling, but lymphocytes had a reported lifespan of > 100 days, long enough to be measurable from the sampling interval used.

3. The reported peak (63-day) enrichment in blood (4.3× urine value) is unexplained. Urine enrichment is consistent with the deuterium dose (see supplemental Data, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online letter), but blood enrichment is inexplicably high. The authors introduced an "amplification factor" *c* between urine and

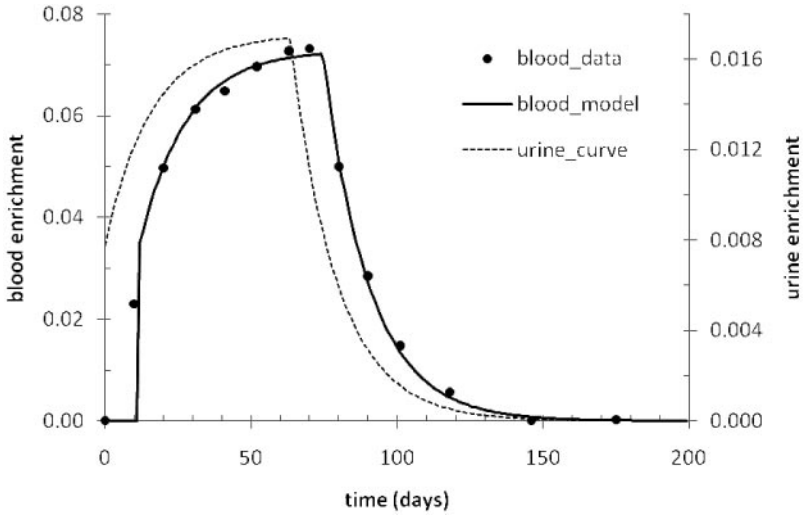

Figure 1. Modeling of blood data. Modeling of blood data using blood deuterium-to-proton enrichment curves constructed from the authors' published illustrations and the following equation, assuming a negligible PB neutrophil lifespan:

$$blood\ enrichment\ (t) = a^{*}[\text{urine enrichment curve (t} - b)]$$

where *a* is a scaling factor for the differing urine and blood deuterium enrichments, *b* is the time interval (delay) between the 2 time-enrichment curves, and t is time. The urine enrichment curve was constructed from the authors' simple exponential model. Fitting gave a delay of 11.2 days, similar to the sum (11.1 days) of the authors' mean values for marrow transit time and PB lifespan.

blood enrichment ratios, attributing this to the multiple hydrogen atoms in a single adenosine deoxyribose moiety. However, the deuterium enrichment ratio at a particular hydrogen location in a molecule is unlikely to depend on the number of hydrogen locations in a molecule, so the high blood enrichment remains unexplained.

4. A possible explanation for the high deuterium enrichment in blood may lie in the fact that reactions involving the cleavage of bonds to hydrogen occur at a faster rate compared with deuterium (~ 7× faster for C-H compared with C-D),[8] which results in a longer residence time for deuterium.[9] This is known as the kinetic isotope effect.[8] If this effect is important during neutrophil development, it may explain the elevated blood deuterium enrichment, but at the same time would cast doubt over almost any kind of modeling, unless the mechanism and location of deuterium enrichment were well understood.

We present an alternative, simpler model that critically assumes a neutrophil PB lifespan that is negligible relative to the development time of neutrophils in marrow. Thus, instead of using 3 parameters (ie, the scaling factor [*a*] for the difference between urine and blood deuterium enrichments, the time of neutrophil development in marrow and the PB lifespan), we found that the data in the authors' published figures could be equally well fitted using only 2 parameters (*a* and a simple delay between urine and blood enrichment curves; see their supplemental data). This model gave small residuals, no evidence of systematic deviation from the data, and a delay of 11.2 days (Figure 1). Interestingly, the fit was improved using a distribution of delays, which, although a more likely scenario, appears not to have been considered by the authors.

In conclusion, a PB neutrophil lifespan ~ 13× longer than the currently accepted value is highly unlikely. The authors' conclusion is based on a mathematical model that attempts to resolve total neutrophil lifespan into bone marrow transit time and PB lifespan. Our alternative, simpler model, which assumes a negligible PB lifespan, fits their data just as well as their own model does. The authors' deuterium technique may be sufficient to measure total neutrophil lifespan, and indeed the authors' mean value of 11.1 days (4.8 days in marrow and 6.3 days in PB) is similar to the value given by our model and in line with published values for neutrophil development time in marrow.[10] It does not, however, have the temporal resolution to measure a PB lifespan of 10 hours.

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To the editor:

Deuterium and neutrophil kinetics

In the July 29, 2010, issue of *Blood*, Pillay et al presented a novel study on the kinetics of neutrophils in humans.[1] Their study used deuterium in heavy water to label proliferating cells in the marrow. Using a first-order model, the authors found that the neutrophil postmitotic marrow transit time was about 5.8 days, consistent with many other reports.[2,3]

Pillay and coworkers reported, however, that the normal blood neutrophil half-life is 3.75 days.[1] This estimate of the blood neutrophil half-life is approximately 10 times longer than that previously shown using several different methodologies.[2-7] The authors suggested that this discrepancy could indicate alteration of

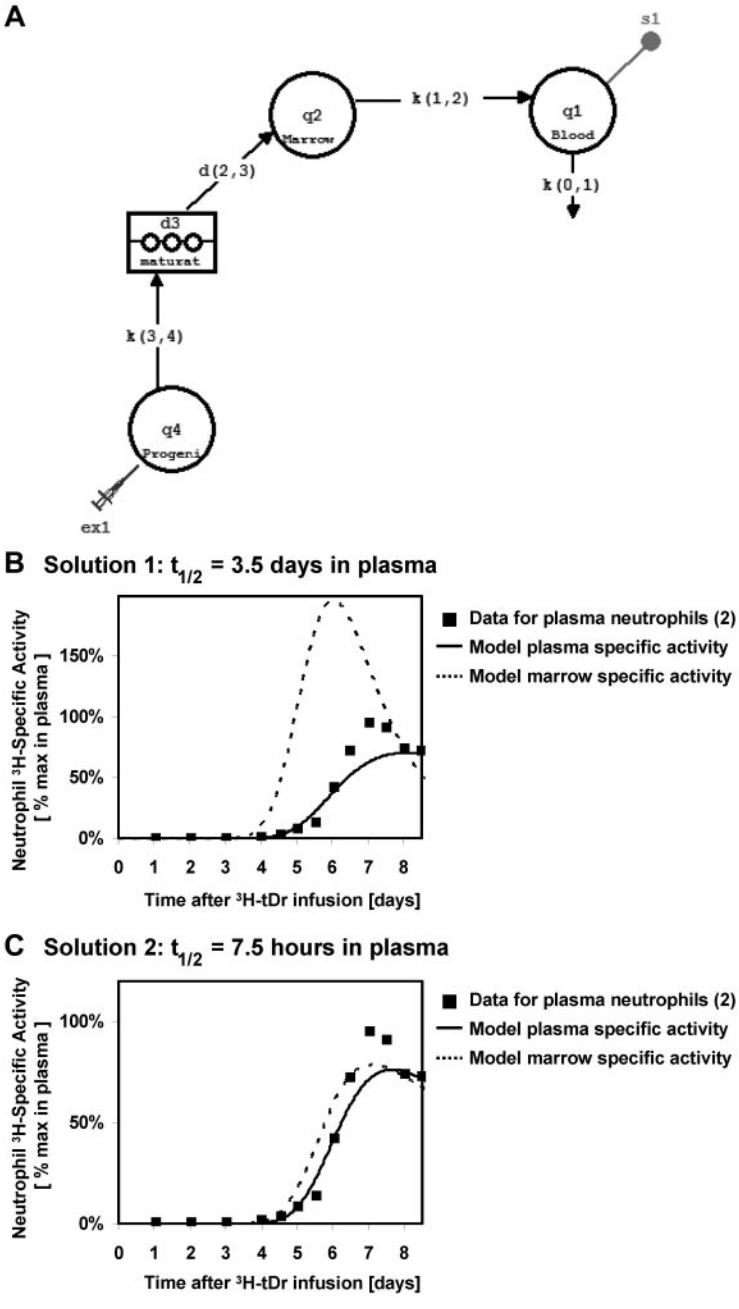

Figure 1. Tracking neutrophils from bone marrow to blood, modeling using built-in components in SAAM II. (A) Model describing the flux of labeled neutrophils through the hematopoiesis system, using built-in components in SAAM II. Distribution of labeled neutrophils between marrow and blood assuming blood half-lives of (B) 3.5 days and (C) 7.5 hours. In both cases, the model fits the specific activity in the plasma neutrophils to published specific activity measurements for thymidine-tagged neutrophils in circulation.[2]

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