patient reported significant improvement in quality of life with improvement in renal function (serum creatinine decreased from 3.1 mg/dL to 2.0 mg/dL) and edema (serum albumin increased from 1.6 mg/dL to 2.3 mg/dL). He achieved hematologic complete remission (Figure 2). He developed a mild infusion reaction (burning sensation in eye and scratchy feeling in throat) with the first infusion that was given over 15 hours, but each subsequent infusion was shorter, most recently 3 to 4 hours.

Our patients demonstrate that daratumumab lowers the light chain and is able to do so rapidly. Second, it can be used in the dose and schedule that is approved for multiple myeloma. The latter is very important in patients with advanced cardiac AL because of concerns about volume overload and infusion reaction-related morbidity. Our first patient had advanced cardiac and hepatic AL but was able to tolerate the weekly regimen with daratumumab. Although it is early to gauge organ response, the fact that the patients' serum free light chain levels normalized for the first time in the course of these 2 patients' illnesses despite prior use of proteasome inhibitors, immunomodulatory drugs, and SCT is very encouraging. We believe that this patient experience justifies a formal clinical trial evaluating the safety and efficacy of daratumumab in AL, a disease with a mortality of 40% at 1 year.

Contribution: T.S. designed the study and wrote the manuscript; B.F. wrote and reviewed the manuscript; A.A. performed patient care and reviewed the manuscript; and M.A.G. wrote and reviewed the manuscript.

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To the editor:

An upper bound for the half-removal time of neutrophils from circulation

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In the 5 May 2016 issue of *Blood*, Silvestre-Roig et al[1] explored the phenotypic heterogeneity and the versatility of neutrophils, a heterogeneity established in part by an adaptable neutrophil lifespan. As noted by the authors, the classical understanding of the neutrophil population is one that is relatively homogenous, partially owing to what is generally believed to be their short lives in the blood. This latter observation is supported by a variety of analyses where neutrophil half-lives were measured on the order of hours.[2] However, a more recent report on an in vivo labeling technique using heavy water (^2H_2O) has cast doubt on this classical view of neutrophil lifespan.[3] There, the authors reported an average circulatory lifespan of 5.4 days, which elicited additional commentary in subsequent issues of *Blood*[2,4,5] and elsewhere.[6]

We favor the term "half-removal time" over half-life because detecting that a neutrophil has left the free circulation is not the same as determining that it has died, because neutrophils are capable of chemotaxis, margination in some organs, and migration into tissues. Consequently, we do not discount the possibility of a long neutrophil lifespan with a short residence time in circulation. The time neutrophils remain in circulation has been studied for >70 years by a variety of labeling techniques.[3,4,7-11] An overview of the history of neutrophil tracer labeling was very recently published[12] in response to a new deuterium-labeled glucose and water tracing study,[4] whose results seem to confirm the classical view of the short durations for neutrophils in the blood. Prior to the results reported using heavy water,[3] where much longer lifespans were found, the reported range of half-removal times has been from 6.6 ± 1.1 hours using diisopropyl fluorophosphate[32] labeling[11] to 10.4 ± 1.5 hours using ^{3}H–diisopropyl fluorophosphate,[8] without considering the 16 hours reported after studies using irradiated cats.[10] It should be noted, however, that labeling studies are not without limitations. In their response to letters about their article,[3] Pillay et al[5] pointed out that the reported short half-removal times may have been affected by neutrophils activated by ex vivo priming techniques.[13] Further, results may be impacted by other factors including, and not limited to, the rate with which the label is picked up in the cells, the reuse/recycling of label within the cellular pool, and the loss of label.[14] For these reasons, we sought an alternative method to provide bounds on the neutrophil half-removal and residence times.

Using fairly straightforward mathematical analyses of previously published data of the circulating neutrophil response to exogenous granulocyte colony-stimulating factor (G-CSF), the principle cytokine responsible for the modulation of the neutrophil lineage,[15] we present here a novel method for calculating the half-removal time of neutrophils from the blood without relying on any labeling experiments. Our results give a lower bound for the rate of removal of neutrophils from circulation, which in turn provides an upper bound on the half-removal time $t_{1/2}$, the latter of which confirms the earlier, classical measurements of a short neutrophil lifespan.

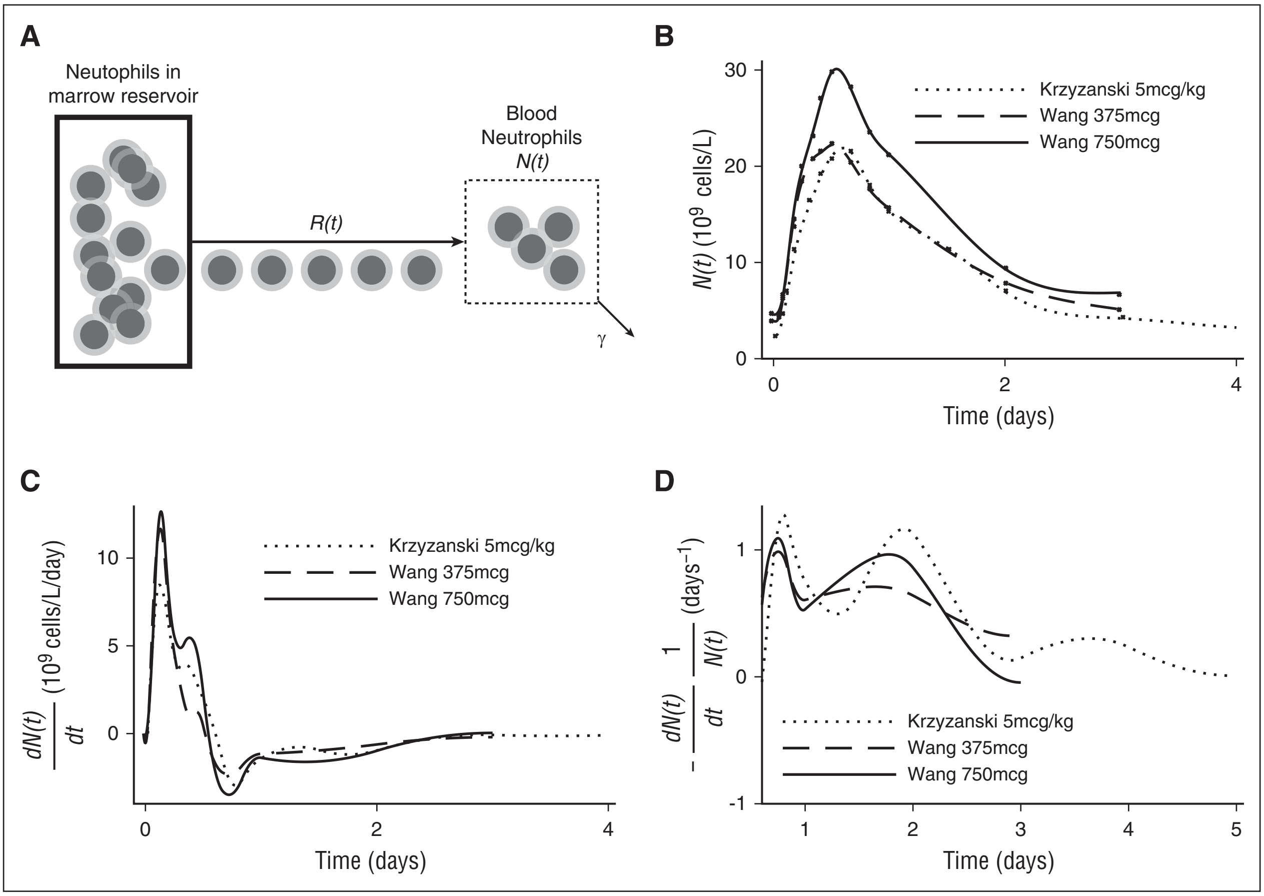

Figure 1. Model schematic and data sources visualized. (A) Neutrophils exit the marrow reservoir at rate $R(t)$ before disappearing from the blood at rate γ. (B) Splined data of neutrophil responses to exogenous G-CSF IV infusions from Krzyzanski et al[17] (Figure 2) and Wang et al[18] (Figure 7). (C) Rate of change in neutrophil numbers in the blood calculated from digitized splined data (from Krzyzanski et al[17] [Figure 2] and Wang et al[18] [Figure 7]), differentiated with respect to time. Note that by 1 day after administration, $dN(t)/dt < 0$ for each of the 3 sources. (D) Right side of the Inequality 2; the maximal value provides a lower bound for γ.

Neutrophils entering the circulation do so from the mature neutrophil reservoir in the bone marrow.[15] Let $R(t)$ be the rate neutrophils migrate out of the reservoir into the circulation. We assume $R(t)$ is a non-negative quantity, implying that neutrophils released into the blood do not return to the bone marrow as functional cells (which does not discount their return to the bone marrow for the purpose of clearance from the body[15]). Using measurements for $N(t)$ (the number of circulating neutrophils as a function of time), we can calculate $dN(t)/dt$, the rate of change of neutrophil numbers. Let $\gamma > 0$ be the rate of removal of neutrophils from the blood; then, at homeostasis

$$\frac{dN(t)}{dt} = R(t) - \gamma N(t), \tag{1}$$

where the change in neutrophil counts in the blood with respect to time is equal to the rate with which neutrophils enter the circulation minus the rate with which they are removed from the circulation. In homeostatic conditions, $dN(t)/dt = 0$, and the input and removal from circulation are equal. If the input to circulation $R(t)$ was known, it could be used to compute γ, but estimates are usually obtained from Equation 1 by first estimating the removal rate from circulation[16] to obtain

$$\gamma = \left[R(t) - \frac{dN(t)}{dt}\right]\frac{1}{N(t)}.$$

Here we will not estimate the unknown quantity $R(t)$, but will simply exploit its positivity. From Equation 1 we obtain

$$0 \leq \frac{dN(t)}{dt} + \gamma N(t) = R(t).$$

Rearranging this inequality gives a lower bound for γ as

$$\gamma \geq -\frac{dN(t)}{dt}\frac{1}{N(t)}. \tag{2}$$

The time-dependent lower bound for the constant γ found in Inequality 2 is only positive (and useful) when $dN(t)/dt < 0$, that is, when there is more removal than input of neutrophils to circulation, and the tightest bounds will be achieved when $dN(t)/dt \ll 0$.

Information on neutrophil numbers in nonhomeostatic conditions is available when exogenous G-CSF is administered. In particular, the response of healthy volunteers to recombinant human G-CSF is reported in Krzyzanski et al (Figure 2)[17] and Wang et al (Figure 7).[18] In each study, volunteers were administered recombinant human G-CSF by both intravenous (IV) infusion and subcutaneous injection. We include only the results for IV administration because these achieve higher peak G-CSF concentrations and a much more rapid subsequent return to homeostatic concentrations than subcutaneous administration (Krzyzanski et al [Figure 2][17] and Wang et al [Figure 6][18]). For clinically relevant IV doses, this results in the immediate recruitment of neutrophils to the circulation followed by relatively fast return toward homeostatic neutrophil concentrations, as seen in Figure 1B, which allows us to obtain good estimates for γ. We obtain measurements from each figure for $N(t)$ at each of the sampling points

Table 1. Upper bounds on the half-removal time

Dose, µg	Removal rate (γ), days^{-1}	Half-removal time ($t_{1/2}$), days	Source
350	1.2773	0.54267	Krzyzanski et al (Figure 2)[17]
375	0.98577	0.70315	Wang et al (Figure 7)[18]
750	1.0917	0.6349	Wang et al (Figure 7)[18]

γ Values presented here are the lower bounds calculated from inequality 2 using the data presented in Figure 1. From these lower bounds, an upper bound on the half-removal time can be calculated using $t_{1/2} = \ln 2/\gamma$. The average lower bound on γ was 1.1183 days^{-1}, giving an upper bound of 0.6198 days, or 14.876 hours, for the half-removal time $t_{1/2}$.

in Figure 1B. Using Matlab,[19] we fit a spline through the data, as previously described,[16] to define the function $N_{dat}^{IV}(t)$. Differentiating, we obtain $dN_{dat}^{IV}(t)/dt$, which is the rate of change of neutrophil numbers as a function of time, from the digitized data. The 3 rates of change from each of the data sources are shown in Figure 1C.

Using $N_{dat}^{IV}(t)$ and $dN_{dat}^{IV}(t)/dt$ in Inequality 2, we then determined the largest lower bound for $\gamma > 0$ for t where $dN_{dat}^{IV}(t)/dt < 0$. As seen in Figure 1B and D, the largest bounds are obtained close to where $N(t)$ is decreasing most rapidly. The results of this analysis are given in Figure 1D and Table 1. In all 3 cases, the lower bounds for possible γ values were >0.985 days^{-1}. Using Inequality 2, the maximum possible half-removal times were calculated by $t_{1/2} = \ln 2/\gamma$, and results are given in Table 1. Our results indicate that the half-removal time for neutrophils in circulation is <15 hours, which is consistent with the classical measurement of 7 hours,[6] but is much shorter than and in disagreement with the more recently reported value of 3.7 days.[1,3] Further, this value also agrees with the recent results from the 2-compartment modeling analysis of Lahoz-Beneytez et al,[4] who reported a neutrophil half-life of ~13 hours when R, the ratio of blood neutrophils to mitotic cells in the bone marrow, was included as a free parameter in their fitting. When using somewhat larger previously reported values for R, they obtained a neutrophil half-life of around 19 hours, which is inconsistent with our bound. Together, this suggests that previously published values for this ratio R may be overestimated, a finding that warrants more investigation.

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