of magnitude lower. Moreover, at such low frequencies a total marrow cellularity of 1.5×10^{12} could accommodate only a very few stem cells.

Insufficient data are available to estimate the size of the stem cell pool and kinetic parameters in elephants accurately (Table 2). But simple calculations of the possible frequency and productive capacity of elephantine stem cells can be made. The data in Table 1 and Abkowitz et al[1] indicate that mice, cats, and humans have on the order of 1×10^{10} to 2×10^{10} bone marrow cells per kilogram of body weight. If the same is true of elephants, a large African elephant of 7 500 kg (Table 2) could have as many as 1.5×10^{14} bone marrow cells. The corresponding frequencies of stem cells in such animals would be 1 to 8 per 10^{10} if all species have the same number.[1] Furthermore, elephants have many more blood cells than mice or humans (Table 1), up to 10×10^{12}. If red and white cell kinetics are similar in humans and elephants, who live about the same length of time, a large African elephant will need to produce 17.5×10^{20} cells in a lifetime. This number corresponds to 2×10^{14} to 10×10^{14} cells per stem cell.

In summary, the experimental and theoretical estimates of stem cell pool size in humans are not inconsistent with lifelong requirements for blood cell production sustained by a self-renewing stem cell population. Nor do the values we have derived appear to be inconsistent with the idea that mice, cats, humans, and elephants could have similar numbers of stem cells.[1]

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To the editor:

Are PU.1 mutations frequent genetic events in acute myeloid leukemia (AML)?

Recently, Mueller et al[1] found PU.1 mutations in 7% of the 126 cases of acute myeloid leukemia (AML) they analyzed. DNA binding and transactivation of the M-CSF receptor promoter, a direct PU.1 target gene, were deficient in the 7 mutants that affected the DNA-binding domain of the PU.1. Those mutations also decreased the ability of PU.1 to synergize with proteins such as AML1 or c-Jun, possibly contributing to the differentiation block found in AML. We looked for study analysis of PU.1 gene mutations in 77 cases of primary AML (excluding therapy-related AML and AML following MDS): 30 M0, 10 M1, 11 M2, 11 M4, 13 M5, 1 M6 AML, and 1 M7 AML, according to FAB classification.[2] In all blood and marrow samples analyzed, more than 80% of blasts were present. Detection of PU.1 gene mutations was made on DNA by single-strand conformation polymorphism (SSCP) analysis[3] and direct sequencing of the 5 exons corresponding to the entire coding region of the PU.1 gene. The size of the polymerase chain reaction (PCR) products was 190 bp to 440 bp (Table 1). Except for a polymorphism in intron 3 (G>A transversion), no abnormal SSCP profile and no variation of sequence of the PU.1 gene was observed in the 77 de novo AML cases studied.

Our results are in contrast to those of Mueller et al[1] but in agreement with results of Vegesna et al,[4] who found no mutation in 60 AML patients tested. There are no clear explanations for these discrepancies. Vegesna et al[4] only used SSCP analysis for screening of mutations, but we also used, like Mueller et al, direct sequencing. We and Vegesna et al[4] used intronic primers and performed analyses on DNA, whereas Mueller et al[1] performed direct sequencing on cDNA of the PU.1 gene. As 2 of the 9 mutations reported by Mueller et al[1] corresponded to large deletions, probably not detectable by techniques using DNA, such mutations could have been overlooked in our study.

Mutations reported by Mueller et al[1] predominantly occurred in undifferentiated AML (M0 AML) or in AML of the monocytic lineage (M4 and M5 AML), but we included a large proportion of those cases in our study and none of them were mutated.

Other hypotheses for those discrepancies include ethnic differences, as patients studied by Mueller et al[1] were, to a large extent, part of the Japanese cohort studied by Osato et al[5] for AML1 mutations. Finally, we and Vegesna et al[4] included only cases of

Table 1. Primers used for SSCP and direct sequencing analysis

Forward primers*	Reverse primers*	Hybridization temperature, °C	Size of PCR product, bp	Restriction enzyme used (sizes of products after digestion, bp)
(EX1S) 5′-GTTGGGCTGGTGGAGGAGT-3′	(EX1AS) 5′-GACACCCCAAGGGGACTATC-3′	64	310	N/A
(EX2S) 5′-CTCTCTCCAGACCCCAGGA-3′	(EX2AS) 5′-CCTGACACCTCCTCTCTCA-3′	64	190	N/A
(EX3S) 5′-ACAGGCCTGGCAGTCTC-3′	(EX3AS) 5′-CTGAGCTCACCCCATACTG-3′	64	270	N/A
(EX4S) 5′-TGGCTGCTGGGTCAGTT-3′	(EX4AS) 5′-CTTCCTCCAGTCTCCTGTGC-3′	60	400	*Rsa*I (180 and 220)
(EX5S) 5′-GGCGAGGGCTTAATGCTAT-3′	(EX5AS) 5′-GGGGCGGCTCCCATGTGG-3′	60	440	*Alu*I (140 and 300)

*EX indicates exon, followed by exon number; S, sense; and AS, antisense.

primary AML (excluding therapy-related MDS and AML following MDS), and 3 of the 9 mutated patients reported by Mueller et al[1] corresponded to AML after MDS. Vegesna et al[4] found no PU.1 mutations in 60 MDS patients, but PU.1 mutations could possibly occur during the progression of MDS to AML in some patients.

In conclusion, we were unable to identify a significant number of PU.1 mutations in our patient population with AML, although their occurrence in some patients during the progression of MDS to AML cannot be excluded.

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