Colony-forming Unit-Granulocyte-Macrophage and DNA Synthesis of Human Bone Marrow Are Circadian Stage-dependent and Show Covariation

By Rune Smaaland, Ole D. Laerum, Robert B. Sothern, Olav Sletvold, Robert Bjerknes, and Knut Lote

Bone marrow samples from sternum and iliac crests were harvested every 4 hours during 19 24-hour periods from 16 healthy male volunteers, and myeloid progenitor cells were cultured by the colony-forming unit-granulocyte-macrophage (CFU-GM) assay. A large interindividual variation was observed in the mean number of colonies during each 24-hour period, with the highest 24-hour mean colony number being about 600% greater than the lowest (range: 16 ± 2.3 to 100.3 ± 4.5). For each individual the difference between the lowest and highest colony number throughout the day ranged from 47.4% to 256.3% of the mean colony number of each series. A circadian stage-dependent variation in the number of colony-forming units of myeloid progenitor cells (CFU-GM) of human bone marrow was demonstrated, with values 150% higher, on the average, during the day as compared with the night. The overall data (891 CFU-GM replicates) exhibited a significant 24-hour rhythm (P < .001) with an acrophase at midday (12.09 hours with 95% confidence limits from 10.32 to 13.49 hours) and a trough at midnight. This 24-hour variation was found to covary with DNA synthesis in the total proliferating bone marrow cell population. A seasonal effect on CFU-GM numbers was detected by ANOVA (P = .014) and by the least squares fit of a 1-year cosine (P = .015), with the highest number found in summer. The potential relevance of these findings should be examined in relation to cytotoxic cancer therapy, use of hematopoietic growth factors, and bone marrow transplantation.

Bone marrow suppression is commonly associated with cytotoxic treatment of cancer, and is generally seen following combination therapy using different cytotoxic drugs. Not only may it lead to serious infections and bleeding tendency, but it may also force dose reductions and/or postponement of treatment courses, as well as reduced duration of useful treatment. In addition, the possibilities of treatment in the event of relapse may be limited. Thus, the bone marrow-dependent side effects can lead to a suboptimal treatment of cancer patients.

The cytotoxic effect on normal bone marrow cells is caused by the potentially irreversible damage to stem cells and early committed progenitor cells, as well as to regulatory stroma cells in the bone marrow microenvironment. In laboratory animals it has been well documented that the susceptibility to cancer chemotherapy of many normal tissues, including bone marrow, shows circadian variations. In addition, time-dependent toxicity to the bone marrow has also been shown in cancer patients. Doxorubicin and tetrahydropyranyl-doxorubicin exert a lower toxicity to the myeloid lineage when administered in the early morning, as compared with late afternoon, whereas carboplatin demonstrates its lowest toxicity toward the megakaryocyte lineage when administered in the late afternoon.

Circadian stage-dependent variations in proliferative activity in human bone marrow have, until now, been largely unknown. We have recently reported a circadian stage-dependent variation in DNA synthesis of total bone marrow cells in humans, with bone marrow being harvested every 4 hours around-the-clock during 19 24-hour periods. Our results were consistent with earlier findings on DNA synthesis of human bone marrow myeloid cells (by tritiated thymidine technique) in four volunteers, each providing four samples (at noon, 6:00 PM, midnight, and 6:00 AM) and two reports of the mitotic activity in six subjects (sampled at noon, 6:00 PM, midnight, and 6:00 AM) and one subject (sampled at 1:20 PM, 3:00 PM, 7:00 PM, 10:45 PM, and 5:45 AM), which indicated lower DNA synthesis and mitotic activity during late evening hours (near midnight) and during the night. To our knowledge, only two studies have investigated a possible circadian variation in circulating human myeloid progenitor cells (CFU-GM). Highest colony number was found in the morning (at 9:00 when sampled every 3 hours around-the-clock) in nine healthy men or in the late afternoon at 3:00 (with sampling only at 8:00 AM, 11:00 AM, and 3:00 PM). The circadian rhythmic variations in bone marrow drug cytotoxicity, as well as bone marrow proliferative parameters, have not been generally recognized or implemented in practical treatment, which may, in part, be attributed to the fact that the earlier reported bone marrow data have been too limited to allow extrapolation to the population at large. There are also no reports on human bone marrow CFU-GM rhythmicity. Because of this paucity of data concerning the potentially important circadian stage-dependence of proliferative activity of human myeloid progenitor cells, especially in relation to cytostatic cancer therapy and hematopoietic growth factors, we conducted a study investigating possible circadian variations in myeloid progenitor cell proliferation in a CFU-GM assay in 16 healthy male volunteers during 19 24-hour periods, with bone marrow samples being obtained.
at 4-hour intervals. In addition, we correlated the number of myeloid colonies with the DNA synthesis of total nucleated bone marrow cells in each sample.

**MATERIALS AND METHODS**

**Subjects.** Between November 1986 and August 1988 we obtained bone marrow samples from 16 healthy male volunteers (mean age, 33.7 years; range, 19 to 47) during 19 24-hour periods, ie, three subjects underwent the sampling procedure twice.14 To find out if the study was feasible, the investigators started taking samples from themselves. All volunteers gave their informed written consent to enter the study, which was approved by and performed in accordance with the guidelines of the regional medical ethics committee. All individuals followed their regular diurnal activity schedule with sleep at night for at least 3 weeks before the experiment, following a diurnal activity routine with average time of arising at 7:00 AM and of retiring at 11:00 PM.

**Protocol.** During each study period, the subjects continued their usual activities between sampling times. They went to sleep after the midnight sample was taken, and were awakened once for the 4:00 AM sample.

To reduce the possibility that the repeated puncture procedure itself would interfere with the results, the start of each experiment was randomized to either 8:00 AM, noon, or 4:00 PM, with the first time of sampling repeated at the end of each study for a total of seven samples per profile. The sequence of sampling from the three different anatomical sites was also randomized. To exclude that the variations should be attributed to sample dilution, caused by local bleeding at the puncture site, differential counts were performed on smears from all individual samples. No samples had to be discarded because of unacceptable large peripheral blood admixture, ie, all smears were characteristic of bone marrow (results not shown).

Venous blood was also obtained from the subjects at the same time as bone marrow sampling to measure the cortisol level and determine other hematologic parameters (total number and differential count of white blood cells). The blood was obtained as the initial procedure or immediately after the anesthesia of periost and determined other hematologic parameters (total number and differential count of white blood cells). The blood was obtained as the initial procedure or immediately after the anesthesia of periost and determine other hematologic parameters (total number and differential count of white blood cells). The blood was obtained as the initial procedure or immediately after the anesthesia of periost and determine other hematologic parameters (total number and differential count of white blood cells). The blood was obtained as the initial procedure or immediately after the anesthesia of periost and determine other hematologic parameters (total number and differential count of white blood cells).

**Bone marrow sampling.** The puncture site was infiltrated with a local anesthetic (Lidocain 20 mg/mL; Astra, Sodertalje, Sweden). No other premedication was given. Following periost anesthesia, bone marrow was obtained by puncturing the sternum or one of the anterior iliac crests every 4 hours during a 24-hour period. The marrow was aspirated into each of two syringes, one for bone marrow culturing in soft agar for estimating growth of CFU-GM, the other for analysis of fraction of nucleated bone marrow cells in DNA synthesis by flow cytometry.

**CFU-GM assay.** Myelopoietic colonies were cultured using the single layer method described by Burgess et al.20 Human placenta conditioned medium (HPCM) was used as colony-stimulating factor according to the method of Schlunk and Schleyer.21 Before plating in agar, bone marrow cells were suspended at a concentration of 1 × 10⁸ cells per mL in Dulbecco's modification of Eagle's medium with 2% penicillin, streptomycin, and glutamine, and 3.2% of nonessential amino acids. From a mixture of 2.5 mL medium, 1.0 mL 20% fetal calf serum (Flow, Irvine, Scotland), 0.5 mL HPCM, 0.5 mL cell suspension, and 0.5 mL 3% agar (Ager Noble Difco, Detroit, MI), aliquots of 1 mL with 1 × 10⁵ cells were plated per 35 mm dish (Nuncion, Roskilde, Denmark), with 8 dishes per timepoint. The dishes were incubated for 13 days at 37°C in 5.0% CO₂. A colony was defined as more than 50 cells. All colonies were counted blindly without knowledge of the actual timepoint of harvesting.

**Flow cytometry.** The bone marrow samples for flow cytometric analyses were handled and analyzed as described earlier. Briefly, two parallel bone marrow single cell suspensions were analyzed on a Cytomfluorograf 50 H (Ortho Diagnostic Systems, Inc, Westwood, MA), interfaced to a Model 2130 Computer (Ortho). In the cytomgram obtained, both the peak and the area of the red fluorescence signal were used for region setting to discriminate the G1 + G0 doublets from the G2 + M cells. Thus, the second peak of the DNA histogram contained only the G2 + M cell population. The total number of cells analyzed for each sample was 3 to 10⁴. Computerized analyses of the cell cycle distribution in the histograms were performed using the constant function of the cell cycle analysis program, by which the percentages of cells in the G1 + G0, S, and G2 + M phases were calculated. The mean CV (coefficient of variation) of the DNA histograms was 3.3%.

**Statistical analysis.** Data were analyzed by Student’s t-test (two-tailed; paired t-tests used for paired analysis of groups) and one-way analysis of variance (ANOVA), using data both in original units and normalized to percent of mean for both CFU-GM and DNA synthesis. In addition, the individual data for CFU-GM and DNA synthesis were analyzed for circadian rhythm by the fitting of a 24-hour cosine by the method of least-squares (Cosinor analysis).22 The rhythm characteristics estimated by this method include the mesor (rhythm-adjusted mean), the amplitude (half the difference between the minimum and maximum of the fitted cosine function), and the acrophase (time of peak value in fitted cosine function). A P value for rejection of the zero-amplitude assumption is determined for each data series. Although the cosinor method involving a single fitted period may not accurately represent the true characteristics of the actual time-dependent variations if asymmetries exist in a time-series,23 the procedure is nevertheless useful for objectively assessing and quantifying periodicities selected a priori.24 The Spearman correlation test was used for analyzing the relationship between CFU-GM and DNA synthesis.

**RESULTS**

**Interindividual difference in CFU-GM number.** Significant interindividual variations were observed in the mean number of colonies during each 24-hour period, with the highest 24-hour mean colony number being 627% greater than the lowest (range of mean number of colonies: 16.0 ± 2.3 [SE] in series 5 to 100.3 ± 4.5 in series 8) (Table 1).

**Circadian variation in CFU-GM number.** A large circadian variation in CFU-GM was observed for each individual, with an intra-individual difference between the lowest and highest colony number ranging from 47.4% (series 8) to 256.3% (series 5) about the mean colony number of each 24-hour series (mean difference = 136.1% ± 12.5%) (Table 1). Overall, the mean CFU-GM at midnight was 38.3 ± 2.5 colonies, as compared with the highest mean value of 59.7 ± 3.1 at noon, ie, 156% higher (Fig 1). When expressing the CFU-GM as percent of mean, the corresponding difference was 170% between the observed trough at midnight and the observed macroscopic peak at 4:00 PM (Fig 2). Irrespective of hour, the mean value across individual series for the lowest CFU-GM number was 13.1 ± 3.1,
Table 1. Circadian Variations in Myeloid Progenitor Cells (CFU-GM) in Human Bone Marrow and Result of Analysis for Time Effect by ANOVA and Single Cosinor

<table>
<thead>
<tr>
<th>Series</th>
<th>Subject ID</th>
<th>Age (yr)</th>
<th>No. of Data</th>
<th>Data Limits</th>
<th>ROC*</th>
<th>Arithmetic Mean ± SE</th>
<th>Analysis of Replicates by ANOVA</th>
<th>Cosinor</th>
<th>Parameters of 24-h Cosine Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AA</td>
<td>19</td>
<td>55</td>
<td>Low</td>
<td>24.1 ± 1.7</td>
<td>133.1 ± 5.4</td>
<td>162.4</td>
<td>65.9 ± 4.8</td>
<td>46.2 &lt; .001</td>
</tr>
<tr>
<td>2</td>
<td>FLJ</td>
<td>23</td>
<td>47</td>
<td>High</td>
<td>159.4 ± 6.8</td>
<td>154.4</td>
<td>78.7 ± 7.3</td>
<td>111.8 &lt; .001</td>
<td>.871</td>
</tr>
<tr>
<td>3</td>
<td>MJ</td>
<td>24</td>
<td>56</td>
<td>Low</td>
<td>4.0 ± 0.4</td>
<td>82.9 ± 3.8</td>
<td>187.0</td>
<td>42.2 ± 4.1</td>
<td>122.1 &lt; .001</td>
</tr>
<tr>
<td>4</td>
<td>RK</td>
<td>25</td>
<td>36</td>
<td>Low</td>
<td>106.8 ± 13.1</td>
<td>158.9</td>
<td>94.6 ± 9.9</td>
<td>92.8 &lt; .001</td>
<td>.901</td>
</tr>
<tr>
<td>5</td>
<td>EK</td>
<td>28</td>
<td>42</td>
<td>High</td>
<td>5.0 ± 2.0</td>
<td>46.0 ± 12.8</td>
<td>256.3</td>
<td>16.0 ± 2.3</td>
<td>17.3 &lt; .001</td>
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<tr>
<td>6</td>
<td>RB</td>
<td>30</td>
<td>30</td>
<td>Low</td>
<td>30.0 ± 3.1</td>
<td>53.2 ± 4.8</td>
<td>95.6</td>
<td>41.7 ± 2.3</td>
<td>4.8 &lt; .001</td>
</tr>
<tr>
<td>7</td>
<td>SF</td>
<td>30</td>
<td>56</td>
<td>High</td>
<td>3.5 ± 0.4</td>
<td>36.5 ± 2.6</td>
<td>162.6</td>
<td>20.3 ± 1.8</td>
<td>66.0 &lt; .001</td>
</tr>
<tr>
<td>8</td>
<td>IK</td>
<td>31</td>
<td>38</td>
<td>Low</td>
<td>73.4 ± 5.2</td>
<td>120.9 ± 3.8</td>
<td>47.4</td>
<td>100.3 ± 4.5</td>
<td>7.7 &lt; .001</td>
</tr>
<tr>
<td>9</td>
<td>RB</td>
<td>31</td>
<td>48</td>
<td>High</td>
<td>13.1 ± 1.5</td>
<td>68.6 ± 4.9</td>
<td>151.6</td>
<td>36.6 ± 3.2</td>
<td>57.2 &lt; .001</td>
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<tr>
<td>10</td>
<td>BCS</td>
<td>33</td>
<td>49</td>
<td>High</td>
<td>19.0 ± 1.3</td>
<td>70.4 ± 2.3</td>
<td>122.1</td>
<td>42.1 ± 2.7</td>
<td>33.9 &lt; .001</td>
</tr>
<tr>
<td>11</td>
<td>RS1</td>
<td>34</td>
<td>62</td>
<td>Low</td>
<td>31.9 ± 1.6</td>
<td>86.9 ± 9.3</td>
<td>92.2</td>
<td>59.7 ± 3.4</td>
<td>34.9 &lt; .001</td>
</tr>
<tr>
<td>12</td>
<td>RS2</td>
<td>35</td>
<td>42</td>
<td>High</td>
<td>41.0 ± 2.0</td>
<td>93.0 ± 4.5</td>
<td>63.8</td>
<td>81.5 ± 2.8</td>
<td>8.0 &lt; .001</td>
</tr>
<tr>
<td>13</td>
<td>OH</td>
<td>35</td>
<td>44</td>
<td>Low</td>
<td>1.9 ± 0.3</td>
<td>122.6 ± 5.4</td>
<td>190.1</td>
<td>63.5 ± 6.4</td>
<td>202.0 &lt; .001</td>
</tr>
<tr>
<td>14</td>
<td>OS1</td>
<td>38</td>
<td>59</td>
<td>Low</td>
<td>9.9 ± 2.1</td>
<td>51.1 ± 4.4</td>
<td>131.2</td>
<td>31.4 ± 2.4</td>
<td>33.5 &lt; .001</td>
</tr>
<tr>
<td>15</td>
<td>OS2</td>
<td>39</td>
<td>54</td>
<td>Low</td>
<td>37.7 ± 1.8</td>
<td>80.4 ± 2.7</td>
<td>72.9</td>
<td>58.6 ± 2.8</td>
<td>27.4 &lt; .001</td>
</tr>
<tr>
<td>16</td>
<td>GW</td>
<td>39</td>
<td>42</td>
<td>High</td>
<td>42.0 ± 1.5</td>
<td>119.3 ± 6.8</td>
<td>99.6</td>
<td>89.7 ± 4.9</td>
<td>19.8 &lt; .001</td>
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<tr>
<td>17</td>
<td>KL1</td>
<td>42</td>
<td>43</td>
<td>Low</td>
<td>5.8 ± 0.8</td>
<td>108.1 ± 2.8</td>
<td>199.4</td>
<td>51.3 ± 6.3</td>
<td>245.9 &lt; .001</td>
</tr>
<tr>
<td>18</td>
<td>KL2</td>
<td>39</td>
<td>39</td>
<td>Low</td>
<td>10.1 ± 2.4</td>
<td>35.3 ± 3.5</td>
<td>127.3</td>
<td>19.8 ± 2.0</td>
<td>3.7 &lt; .003</td>
</tr>
<tr>
<td>19</td>
<td>ODL</td>
<td>46</td>
<td>49</td>
<td>Low</td>
<td>5.3 ± 1.3</td>
<td>103.4 ± 3.2</td>
<td>153.7</td>
<td>64.4 ± 4.9</td>
<td>83.7 &lt; .001</td>
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<tr>
<td>20</td>
<td>Total</td>
<td>891</td>
<td>1.9 ± 0.3</td>
<td>160.9 ± 13.1</td>
<td>291.2</td>
<td>54.6 ± 1.3</td>
<td>5.8 &lt; .001</td>
<td>.901</td>
<td>53.8 ± 1.3</td>
</tr>
</tbody>
</table>

*ROC, range of change—the difference from lowest to highest value expressed as percent of the mean. 1ANOVA, analysis of variance across all timepoints using all data in original units. Cosinor, individual 24-h rhythm characteristics from the least squares fit of a 24-h cosine. Acrophase reference, 00.00, with sleep/rest between 00.00-08.00.

Fig 1. Circadian variation in human bone marrow CFU-GM. Time-point means and standard errors from 19 series in 16 clinically healthy men.

Fig 2. Circadian covariation in human bone marrow CFU-GM and DNA synthesis. Time-point means and standard errors from 19 series of CFU-GM and DNA synthesis in 16 clinically healthy men.
Comparison of circadian results for different sites of estimation of CFU-GM in 19 series obtained from 16 men.

Abbreviations: ANOVA, analysis of variance across all timepoints using all data in original units or as percent of mean. Cosinor, 24-h rhythm characteristics from analysis by single cosinor using data in both original units and as percent of mean. Acrophase (φ) in clock hour and minute from reference (00.00 hours), with sleep/rest between 00.00 and 08.00 hours.

and for highest CFU-GM the mean was 115.5 ± 10.5, ie, almost 900% (882%) higher.

Each CFU-GM series showed a highly statistically significant effect of circadian stage when analyzed by ANOVA. Fourteen of 19 series (73.7%) demonstrated a significant 24-hour rhythm at P < .05 in CFU-GM from the least-squares fit of a 24-hour cosine, while one additional individual showed borderline significance (P = .06). The acrophase for 12 of the 14 series was during the day between 8:30 AM and 5:00 PM. The overall data (891 CFU-GM replicates) exhibited a significant 24-hour rhythm (P < .001) with an acrophase at midday (12:09 PM with 95% confidence limits from 10:32 AM to 1:49 PM), and trough at midnight. Cosinor analysis of CFU-GM as percent of mean also yielded significant results (P < .0001), with similar acrophase and trough (Table 2).

CFU-GM number according to anatomical site. Colony replicates from the sternum and iliac crests were also analyzed separately for circadian rhythm. Whereas a significant 24-hour rhythm for both anatomical sites of bone marrow harvesting could be shown with comparable times for acrophase and trough, a statistically significant difference in mesor value was found between the sternum (63.2 colonies) and iliac crests (48.4 colonies) (P < .0001).

Relation between CFU-GM number and DNA synthesis. We also compared the 24-hour rhythm of CFU-GM with the rhythm in DNA synthesis measured in the total bone marrow. Three individual examples of circadian covariation between CFU-GM and DNA synthesis with different sampling start times are shown in Fig 3. In Fig 2 the combined pattern for CFU-GM and DNA synthesis is shown, each normalized to percent of mean for comparison. Here, the mean value of CFU-GM (as opposed to all replicates) at each circadian stage for each individual is used for a more exact comparison with the single DNA synthesis observation at each timepoint. Both variables show a statistically significant circadian stage-dependent variation by ANOVA and cosinor analysis as percent of mean, and they show a very close circadian covariation, with acrophase (and 95% limits) at 1:33 PM (11:00 AM to 4:08 PM) and 12:45 PM (10:52 AM to 2:40 PM) for CFU-GM and DNA synthesis, respectively (Table 3). A corresponding 24-hour variation was significant by ANOVA when using original units for both CFU-GM and DNA synthesis, although cosinor analysis was not statistically significant for CFU-GM because of the wide range of nonnormalized mean individual colony numbers (see above). No ultradian (12-hour) component could be detected for the group in either variable. In addition, by normalizing the absolute values for CFU-GM and DNA synthesis to percent of mean, a highly significant correlation between these two proliferative parameters could be shown (P < .0001).

Circannual variation in CFU-GM number. We found that the number of colonies of myeloid progenitor cells also varied according to the time of year, with higher levels in summer (57 ± 7, n = 27) than in winter (32 ± 6, n = 22).
An effect of season was detected by ANOVA ($P = .014$) and by the least-squares fit of a 1-year cosine ($P = .015$). The time of estimated high values as indicated by the acrophase was August 12, with 95% confidence limits from July 8 to September 14.

**DISCUSSION**

Dose is a critical determinant of the efficacy of cancer chemotherapy.\textsuperscript{25} Increasing evidence indicates that any compromise of dosage or delay in the treatment schedule diminishes the likelihood of cancer control or cure for drug-sensitive tumors.\textsuperscript{3,5,26,27} This may be explained by the fact that the effect of most antineoplastic drugs is dose intensity-dependent, ie, higher doses over a shorter time span increase the response rates and proportion of cures. On the other hand, toxic effects to normal tissues limit the amount of dose that can be administered, with bone marrow suppression at present being the main dose-limiting factor. Therefore, searching for and improving therapeutic selectivity is critical.

We show in this study a significant circadian stage-dependent variation in the colony number of human bone marrow myeloid progenitor cells, ie, CFU-GM, which has the same circadian patterning as DNA synthesis of the total number of bone marrow nucleated cells. The statistically determined peak (acrophase) of CFU-GM was found between 8:00 AM and 7:00 PM, ie, during daytime and early evening, in 14 (73.7%) of 19 profiles from 16 individuals (Table 1). When pooling all individual data, the differences become smaller because of interindividual differences in waveform and phasing. However, the circadian variations are, to a large extent, predictable, with the highest number of CFU-GM and highest DNA synthesis occurring during daytime hours (near noon), while the lowest values occur around midnight. A huge intrindividual circadian stage dependent variation in CFU-GM number was found, the range of change around the mean 24-hour value from the lowest to highest CFU-GM number of each individual throughout the day averaging almost 140%. This difference in the capacity of producing myeloid progenitor cells throughout the day, as well as the large interindividual variation observed in the 24-hour mean number of colonies, might possibly help to predict bone marrow sensitivity for an individual to, eg, a chemotherapeutic agent.

A possible intrinsic variability in bone marrow proliferation according to localization of sampling site was addressed by analyzing the data from the iliac crests versus the sternum separately. Earlier it has been shown that core biopsies obtained simultaneously from both iliac crests give a very good reproducibility of cytokinetic data,\textsuperscript{28} thereby indicating a functional homogeneity with regard to proliferation status at different anatomical bone marrow sites. In accordance with the study by Dosik et al,\textsuperscript{28} no significant difference in DNA synthesis was found between the two iliac crests by us.\textsuperscript{14} However, a significantly higher number of cells in DNA synthesis was measured in samples obtained from the sternum as compared with the iliac crests.\textsuperscript{14} This was also the case for CFU-GM numbers in the present study. The most likely explanation for this observation is that there is less blood contamination of the sternum sample. However, the same circadian pattern of variation for both variables was observed in both sites and when individual samples were pooled irrespective of sites. For each site and variable, highest values were found during the day and lowest during the night, with a very close relation in timing of acrophase and trough for the two anatomical sites.

Our data are in good agreement with those published by Lévi et al for murine bone marrow.\textsuperscript{29} The highest and lowest number of CFU-GM were found during the activity and rest spans, respectively, corresponding to the times of highest and lowest DNA synthesis in mice. Further, these times corresponded to the times of highest (midactivity) and lowest (midrest) susceptibility of the anticancer agent, 4'-O-tetrahydroxyproynyl doxorubicin, which has its main effect on DNA synthesis.

If healthy tissues exhibit regular circadian variations of cell proliferation and cancer cells do not, or have a different circadian phasing,\textsuperscript{24,30} simple timing of cytotoxic drug administration should improve the efficacy and reduce the toxicity of cancer chemotherapy, since these drugs are nonselective in damage done to both healthy and cancerous tissues. It may then be possible to reduce bone marrow suppression of cytotoxic drugs exerting their main effect on proliferating cells by administering the drugs or the major dose of a continuous drug infusion during the time span of lowest

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### Table 3. Statistical Evaluation of Circadian Stage-dependent Covariation of Myeloid Progenitor Cells (CFU-GM) and DNA Synthesis (\% cells in S-phase) in Human Bone Marrow

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>No.</th>
<th>Arithmetic Mean ± SE</th>
<th>ANOVA</th>
<th>Cosinor</th>
<th>Characteristics of 24-h Cosine Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-GM</td>
<td>No. of colonies</td>
<td>121</td>
<td>54.4 ± 3.4</td>
<td>0.79 .557</td>
<td>.232</td>
<td>53.7 ± 3.4</td>
</tr>
<tr>
<td>DNA</td>
<td>% in S-phase</td>
<td>127</td>
<td>13.2 ± 0.3</td>
<td>3.70 .004</td>
<td>.002</td>
<td>13.1 ± 0.3</td>
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<tr>
<td>CFU-GM</td>
<td>% of mean</td>
<td>121</td>
<td>100.0 ± 4.7</td>
<td>2.72 .023</td>
<td>.021</td>
<td>98.8 ± 4.7</td>
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<tr>
<td>DNA</td>
<td>% of mean S-phase</td>
<td>127</td>
<td>100.0 ± 2.2</td>
<td>4.40 .001</td>
<td>&lt;.001</td>
<td>99.4 ± 2.1</td>
</tr>
</tbody>
</table>

Comparison of circadian results of concomitant estimation of CFU-GM and DNA in 19 series obtained from 16 men.

Abbreviations: ANOVA, analysis of variance across all timepoints using timepoint means in original units or as percent of mean. Cosinor, 24-h rhythm characteristics from analysis by single cosinor using data in both original units and as percent of mean. Acrophase (Ø) in clock hour and minute from reference (00.00 hours), with sleep/rest between 00.00-08.00 hours.
proliferative activity of healthy tissue, such as bone marrow cells, ie, in late evening or during sleep by night (for diurnally active individuals). Cells in the DNA synthesis phase will then be less susceptible and cells in the G1 phase will have more time for repairing damage before entering into the DNA synthesis phase. The possibility of treating patients at an optimal circadian time, interval and schedule is today feasible and cost-effective through programmable drug delivery systems. The circadian stage-dependent synchrony between S-phase and numbers of functional myeloid progenitor cells (CFU-GM), ie, their clonability, strengthens the possibility of optimizing therapy because this finding suggests a common circadian phasing of both myeloid progenitor cells and more mature proliferating cells.

Another potentially important aspect of these findings is that it may be possible to increase the effect of biological response modifiers like granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and interleukin-3. Administration of these substances at the time of greatest responsiveness of the bone marrow may increase their usefulness in proper timing, thereby increasing the dose intensity and efficacy of these agents.

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therapy, with or without autologous/allogeneic bone marrow transplantation. As hematopoietic growth factors may be potentially beneficial for patients with acquired immunodeficiency syndrome, myelodysplastic syndrome, or aplastic anemia, an optimal use of these factors relative to biological rhythms may also increase their usefulness in these disease entities. Yet another point to be made is that the time of harvesting bone marrow cells for bone marrow transplantation could possibly be optimized by taking these proliferative rhythms into consideration.

Therefore, we suggest that the circadian stage-dependent variations of myeloid progenitor cells (CFU-GM), as well as the circadian variation in cell cycle distribution of bone marrow cells, be further exploited in an attempt to reduce bone marrow suppression of cytotoxic drugs by their proper timing, thereby increasing the dose intensity and efficacy of these agents.
Colony-forming unit-granulocyte-macrophage and DNA synthesis of human bone marrow are circadian stage-dependent and show covariation

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