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## Nonhomogeneous Distribution of Leukemia in the Bone Marrow During Minimal Residual Disease

By Anton C.M. Martens, Frank W. Schultz, and Anton Hagenbeek

In a rat model (BNML) for human acute myelocytic leukemia the distribution of leukemic cells in bone marrow samples from various sites was investigated, using monoclonal antibodies (MoAbs) and flow cytometry. Rats were studied before chemotherapy as well as thereafter, ie, in the "minimal residual disease" (MRD) phase. Bone marrow from different types of bones was analyzed from each animal. Before treatment, the ratio of the measured extreme values (ie, highest/lowest value) for leukemic cell frequencies in bones from individual rats ranged from 3.7 to 11.7. During the MRD phase the ratios of the extremes ranged from a factor of 36 to more than 13,000 from one rat to another. The variability between bones of comparable size was estimated by studying the ribs from each individual animal. Within individuals the extremes differed

**T**REATMENT of leukemia will result in a reduction in the leukemic cell load in the body for most patients. Cell numbers in both bone marrow and blood will drop below the detection level of 5%, and leukemic cells will no longer be distinguishable from normal blast cells by standard cytological criteria. Although the remission-induction success rate for acute leukemia currently reaches 70% to 80%, most patients will relapse within two years,<sup>1</sup> indicating that a substantial number of leukemic cells survive treatment. Early detection of relapse is of utmost importance because the tumor load should be as small as possible at the start of the re-induction treatment.

Methods used to decrease the detection level for leukemic cells include DNA aneuploidy using flow cytometry<sup>2</sup>; cytogenetics<sup>3-5</sup>; immunological methods,<sup>6</sup> and cell culture methods.<sup>7</sup> Immunological methods for the detection of residual leukemia appear to be the most successful,<sup>8-11</sup> although occasionally the combination of several antibodies<sup>10</sup> and/or lectins<sup>11</sup> is needed to allow for the discrimination of leukemic cells from their normal counterparts.

The lower detection limit for any analytical method is obviously determined by the baseline frequency of cross-reacting normal cells. Using sophisticated, multiparameter flow cytometry, rare cell types such as hematopoietic progenitor cells<sup>12</sup> or residual leukemia cells can be recognized and quantitated up to the level of one cell per  $10^4$  to  $10^5$  normal cells.<sup>13-15</sup>

The general assumption that leukemic cells are randomly distributed throughout the bone marrow compartment explains in part why in most leukemia treatment centers only one bone marrow aspirate per patient is investigated during the follow-up period. The result of this single observation is then used to estimate the total tumor burden. Unfortunately, unexpected sudden relapses are a common experience for hematologists.

To get insight into the pattern of distribution of leukemic cells, studies were performed in an experimental rat leukemia model (Brown Norway acute myelocytic leukemia [BNML]). Rats were studied both before and during a cyclophosphamide-induced state of remission. Two assumptions concerning the distribution of leukemic cells within the

by a factor of 1.2 to 4.0 before chemotherapy and from 2.4 to  $>320$  after chemotherapy. The variability within the marrow cavity of a single bone was determined by analyzing multiple samples from femoral bones cut into slices. The leukemic cell frequency appeared to vary considerably, ie, before treatment from 1.7 to 7.3 and during MRD from 4 to 28,000. The presented data may contribute to understanding the sometimes conflicting observations in leukemic patients. Improvement of methods for detecting MRD will not automatically lead to a more accurate estimation of the total tumor burden. The reliability of diagnoses based on the analysis of single bone marrow aspirates appears to be highly questionable.

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bone marrow compartment were verified: (1) leukemic cells are randomly distributed throughout the different bones both before and after chemotherapy, and (2) the leukemic cells are randomly distributed within the marrow cavity of single bones.

These observations may help to get an impression of the size, distribution, and population-growth kinetics of the leukemic cell population in the minimal residual disease (MRD) phase. New strategies for detecting residual leukemia in humans may be implied.

### MATERIALS AND METHODS

*Experimental animals.* The experiments were performed in the barrier-derived inbred Brown Norway (BN) rat strain BNBi/Rij, produced in the Rijswijk colony. Male rats between 13 and 16 weeks of age were used (mean body weight 260 g).

*The rat leukemia model.* The BNML, induced in a female BN rat by treatment with 9,10-dimethyl 1,2-benzanthracene, shows striking similarities with human acute myelocytic leukemia (AML).<sup>16,17</sup> Upon cellular transfer the leukemia shows a reproducible growth pattern. Some of its major characteristics are: (1) a slow growth rate; (2) severe suppression of normal hematopoiesis due to an absolute decrease in the number of hematopoietic stem cells (colony-forming unit, spleen [CFU-S]); (3) the presence of clonogenic leukemic cells (in vivo leukemic CFU-S [LCFU-S] in vitro clonogenic assays); and (d) response to chemotherapy, as in human AML.

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An additional advantage of this model is that normal hematopoietic stem cells and leukemic clonogenic stem cells can be discriminated by modified spleen-colony assays.

**Preparation of cell suspensions.** Bone marrow was derived from different bones, ie, femur, tibia, humerus, costa, sternum, vertebra, scapula, and crista up to a total of 16 to 19 specimens per rat. The marrow was flushed with Hanks' Hepes Buffered Balanced Salt Solution (HHBSS) of 283 mosm, pH 6.8. The larger bones were cut into two parts, each part being flushed several times with HHBSS. A needle was used to create a small opening in the vertebrae, scapulae, and sternum. The marrow could be collected by flushing these bones with HHBSS.

Ribs were cut on both sides while care was taken to prevent the loss of bone marrow. The sternal side of the rib was inserted into silicon tubing through which HHBSS was flushed in order to remove the marrow.

Transverse sections of the femur were made by sawing the bones in several slices using a circular sawing blade mounted on a dental drilling equipment. Cross-contamination of the samples was prevented by cleaning the blade thoroughly in between slices. The marrow was flushed from the bone segment by repeat pipetting of 1 mL of HHBSS with an Eppendorf-type pipet. Histological examination of bone segments after marrow collection indicated that virtually all cells were removed.

**Immunofluorescence labeling of cells.** For labeling with the monoclonal antibody (MoAb), cells were centrifuged and resuspended in HHBSS supplemented with inactivated fetal calf serum (FCS, Flow Laboratories Irvine, Ayrshire, Scotland; 5% vol/vol) and sodium azide (0.01% vol/vol). For fluorescence labeling studies  $2 \times 10^6$  cells were pelleted and labeled with ascites fluid containing the Fluorescein-iso-thiocyanate (FITC)-conjugated MCA-Rm124 (100  $\mu$ L) at a  $1/243$  dilution at 0°C for 45 minutes. After 30 minutes of labeling, 25  $\mu$ L of propidium iodide (PI, Calbiochem, San Diego; 2  $\mu$ g/ $\mu$ L) were added. PI can easily enter dead cells, in contrast to viable cells, and will subsequently bind to the DNA. This approach enabled the exclusion of dead cells. This is important as dead cells show an aspecific binding of the MoAb, and thus influence the accuracy of the measurements. After careful washing, the cells were resuspended and processed on a modified fluorescence-activated cell sorter (FACS-II).

**The Rm124 MoAb.** This MoAb was produced by Drs H. Kaizer and R.J. Johnson, Johns Hopkins University, Baltimore. Its affinity for normal and leukemic cells has been discussed elsewhere.<sup>18</sup> Briefly, the MCA-Rm124 reacts with an antigen that is present on leukemic cells as well as on normal granulocytes, although in different densities. Differences in labeling intensity can therefore be used to discriminate between both cell types. Leukemic cell numbers obtained by using the MCA-Rm124 and flow cytometry correlated with data that were obtained with standard bio-assays.<sup>13</sup> This approach proved to be applicable for the detection of minimal residual disease in leukemia, as was reported previously.<sup>13,14</sup>

**Flow cytometry.** The labeled cells were analyzed with a modified FACS-II (Becton Dickinson, Sunnyvale, CA), with an argon ion laser tuned at 488 nm (0.5 W). Forward light scatter signals (FLS) were used for triggering the measurements. Perpendicular to the laser beam the light was collected on a dichroic mirror DM570 in order to separate "green" FITC fluorescence (<570) and "red" PI fluorescence (>570 nm). The FITC fluorescence was then measured through a broad band multicavity interference filter (520 to 550-nm transmission, Pomfret, Stamford, CT) and a 520-nm cut-off filter (Ditric, Mannheim, FRG) by an S-20 type photomultiplier. A logarithmic amplifier (Designed and constructed by Nozaki, T., Stanford, CA) was used for the FITC fluorescence signals. PI fluorescence was measured through a 620-nm long-pass filter by

means of an S-11 type photomultiplier. PI signals were linearly amplified.

On the basis of PI fluorescence the viable cells were identified (negative "red" fluorescence) and "gated out" electronically. The MCA-Rm124/FITC fluorescence of 50,000 to 300,000 cells was measured as described and stored as a histogram in a Hewlett Packard HP87 personal computer (Corvallis, OR).

## RESULTS

Leukemic cell frequencies were determined in a great number of bone marrow specimens after labeling with the MCA-Rm124 and measured by flow cytometry as described. The leukemic cells could be specifically recognized and quantified.<sup>13,18</sup> Figure 1 shows typical histograms of a normal bone marrow and of a bone marrow containing a very low number of leukemic cells (ie, one cell per 10,000). The leukemic cells are found in the fraction with the highest fluorescence intensity (indicated by the arrow). To visualize the small leukemic subpopulation, the y-axis is plotted on a logarithmic scale. The leukemic cell frequency can be easily calculated from this type of data by determining the number of cells in the various peaks.

**Variability between different rats before treatment.** During the development of the leukemia, animals were killed between days 7 and 14 after the intravenous (IV) injection of  $10^7$  BNML cells. During this period the clinical signs of leukemia are noticeable (increase in weight of spleen and liver, and pronounced infiltration of the bone marrow by the leukemic cells). Marrow cell suspensions of several bones were prepared on day 7 and on day 10 including femora, tibiae, humeri, scapulae, costae, sternum and vertebrae from two animals. The leukemic cell frequencies were determined and ranged from  $4 \times 10^{-3}$  to  $5 \times 10^{-2}$  on day 7 and from  $7 \times 10^{-2}$  to  $4 \times 10^{-1}$  on day 10 after leukemic cell transfer. Obviously, the increase from day 7 to day 10 is a result of

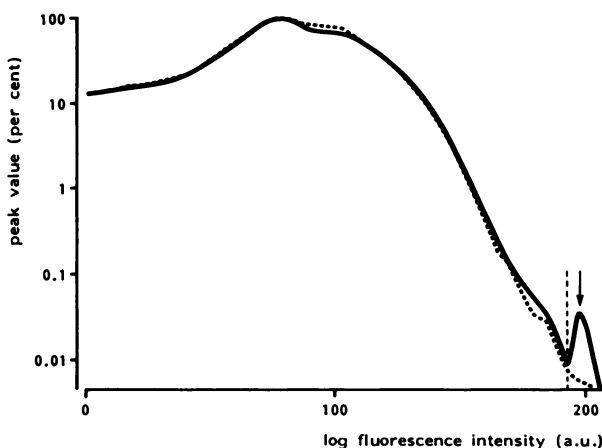


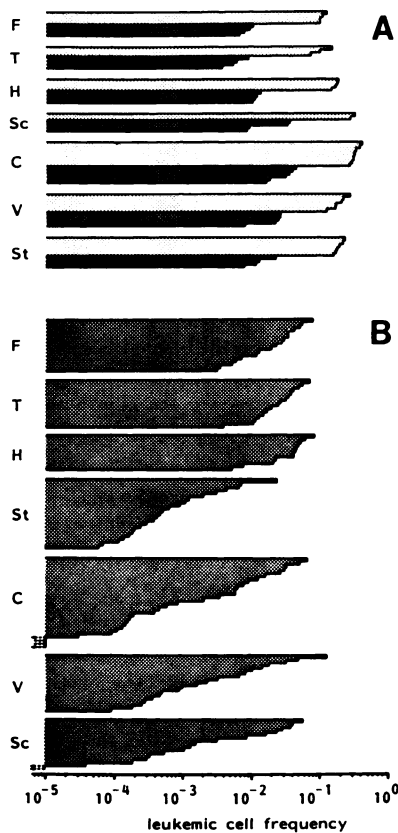
Fig 1. Typical fluorescence intensity distribution of bone marrow from a leukemic rat in remission containing residual leukemic cells (—) compared with normal rat bone marrow cells (---). (Abscissa) Fluorescence intensity (logarithmically amplified); units are arbitrary and relate to channel numbers. (Ordinate) Cell frequency normalized to the percent peak value. The threshold for the "leukemic cell region" is indicated by ----. The arrow points to the residual leukemic subpopulation.

**Table 1. Ratios of Measured Maximal and Minimal Leukemic Cell Frequencies in Various Bones of Leukemic Rats Before Cyclophosphamide Chemotherapy**

Rat No.	Day After 10 <sup>7</sup> BNML	Ratio Highest/Lowest Frequency
1	7	11.7
2	7	6.6
3	10	3.7
4	10	4.4

The bones examined were femur, tibia, humerus, scapula, vertebra, and sternum, up to a total of 16 to 19 specimens.

leukemia growth. The ratio between the highest and lowest measured values of leukemic cell frequencies in the various bones of the individual rats proved to be suitable for comparing animals at different stages of development. Before the administration of chemotherapy the variation of these ratios as determined for all the bones were in a narrow range: 6.6 to 11.7 for day 7 and 3.7 to 4.4 for day 10 (Table 1). The



**Fig 2. The frequency of leukemic cells in various bone marrow specimens before chemotherapy (A) and during the stage of MRD (B). (A) Pooled data of four rats killed at two different times during undisturbed leukemia growth. Darkly shaded, day 7 after leukemia transfer; lightly shaded, day 11. (B) Pooled data of eight rats killed during the MRD stage, 17 days after treatment (100 mg cyclophosphamide/kg IP) On the ordinate: F, femur, T, tibia, H, humerus, Sc, scapula (of F, T, H, and Sc, two specimens per rat); C, costa, V, vertebra, St, Sternum (of C, V, St, three to four specimens per rat). For each animal a total of 16 to 19 bones were investigated. Small arrows: Samples in which no leukemic cells were detected above the level of 10<sup>-5</sup>.**

measured leukemic cell frequencies in the various individual bones are shown in Fig 2A.

*Variability between different rats in the MRD situation after treatment.* Eight animals were investigated during the MRD phase, which was created as follows: Leukemic animals were treated with a single injection of cyclophosphamide (100 mg/kg intraperitoneally [IP]). This resulted in a 5 to 6-log leukemic cell kill.<sup>19</sup> The surviving leukemic cells will regrow and will be detectable again when they are present in numbers higher than one per 10,000 to 100,000 normal cells. Animals were killed starting the seventeenth day after treatment. Large differences between the lowest and the highest ratio values were observed, the lowest ratio being 36, the highest ratio being over 13,000. All other animal ratio values fell between these extremes (Table 2).

The leukemic cell frequencies found for the various individual bones of the eight investigated rats during MRD are shown in Fig 2B. The largest variability was observed in the "smaller bones" such as sternum, costae, vertebrae and scapulae. The highest observed leukemic cell frequency was 1.3 × 10<sup>-1</sup>; the lowest observed frequency was below the detection limit of 1 × 10<sup>-5</sup>.

*Variability in individual rats before treatment.* The investigated bones differ considerably regarding their size. As this might have an effect on their leukemic cell content, it was decided to investigate a large number of bones of comparable size from a single rat, ie, the ribs, as it was judged that these would best serve the purpose of studying variability. Therefore, all ribs of individual rats were prepared separately, and the marrow was removed and processed for flow cytometry. Simultaneously, the variability within a bone marrow compartment was studied by cutting the femur in sections of about 1 mm in length and subsequently studying the marrow. To study the distribution during undisturbed growth, animals were killed on days 8, 11, or 14 after leukemia-cell transfer. The measured frequencies are shown in Fig 3. In the femur (Figs 3A and B) as

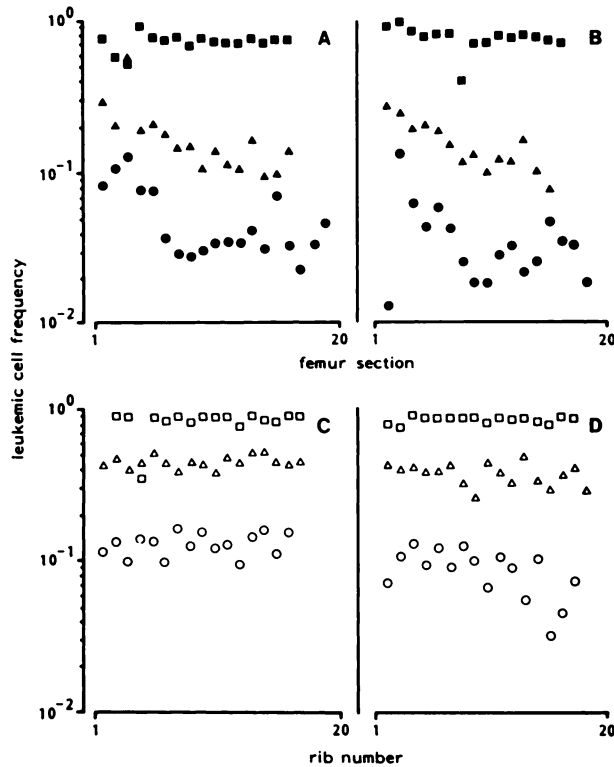
**Table 2. Ratios of Measured Maximal and Minimal Leukemic Cell Frequencies in Various Bones During the Phase of Minimal Residual Disease After Cyclophosphamide Chemotherapy**

Rat No.	MRD Detection on Day	Ratio Highest/Lowest Frequency
1	29	160
2	30	>3,290* (823)†
3	30	>9,840* (1,100)†
4	30	>13,000* (4,360)†
5	30	>2,500* (281)†
6	31	84
7	31	36
8	31	340
9	31	205

Cyclophosphamide (100 mg/kg IP) was given at day 13 to 14 after 10<sup>7</sup> BNML IV. From each rat 16 to 19 bone specimens were investigated; 50,000 to 300,000 cells per sample were analyzed.

\*In case no leukemic cells were found in one or more samples of a series, the calculation of the ratio was performed using the detection limit of 10<sup>-5</sup> as the lowest frequency.

†The calculated ratio using the lowest frequency of leukemic cells that could be accurately measured in one of the samples of the series.



**Fig 3.** The leukemic cell frequencies in femur and ribs at three different times during undisturbed leukemia growth. Neighboring segments of the femur (A and B) are compared with all ribs of individual rats (C and D) Between 14 and 19 sections were obtained from one femur and analyzed. On the abscissa the femur sections, no. 1 corresponds with the distal end, the highest number with the proximal end of the femur. ■, femur, rat 1 (A) and rat 2 (B) day 14; ▲, femur rat 1 (A) and rat 2 (B) day 11; ●, femur, rat 1 (A) and rat 2 (B) day 7. The ribs are plotted in random order. From each animal 16 to 17 ribs were analyzed. □, ribs, rat 1 (C) and rat 2 (D) day 14; △, ribs rat 1 (C) and rat 2 (D) day 11; ○, ribs, rat 1 (C) and rat 2 (D) day 7.

well as in the ribs (Figs 3C and D) the ratios between the highest and the lowest leukemic-cell frequencies did not vary greatly: from 1.7 to 7.3 for the femur and from 1.2 to 4.0 for the ribs, from one animal to another, considering all three leukemia stages. The ratios of the highest and lowest observed values are given in Table 3. In the femur, during the earlier leukemia stages at days 8 and 11 the ratios were somewhat higher than for the later stage leukemia at day 14: 3.5 to 7.3 v 1.7 and 2.4. No specific pattern of distribution across the femoral cavity in the longitudinal direction was observed (Figs 3A and B). However, the average frequency in the femoral sections appeared to be approximately a factor of 2 to 3 lower than for the ribs of the same animal, especially during the earlier stages (compare Figs 3A and B with C and D).

**Variability in individual rats in the MRD situation after treatment.** Animals were treated with cyclophosphamide on day 8, 11, or 14 after leukemia cell transfer. During the MRD phase leukemic cell frequencies were measured. Large differences were observed between the animals (Table 4).

**Table 3. Ratios of Measured Maximal and Minimal Leukemic Cell Frequencies in Femur Sections and in Ribs Before Chemotherapy**

Rat No.	Ribs*	Femur Sections*	Day After 10 <sup>7</sup> BNML Cells IV
1	2.7	—	7
2	1.3	—	7
3	1.6	5.5	8
4	4.0	7.3	8
5	1.4	—	10
6	1.2	—	10
7	2.8	4.5	11
8	2.9	6.4	11
9	1.6	5.8	11
10	1.9	3.5	11
11	2.7	1.7	14
12	1.2	2.4	14

\*See Materials and Methods section for explanation.

The ratios of highest and lowest frequency for the ribs ranged from 2.4 to 70. However, a number of ribs did not contain leukemic cell numbers above the detection limit. Therefore, the calculated ratio will be an underestimation; the maximal ratio which was estimated was even >320.

In the femur sections of the individual animals the ratios ranged from 4 to 1,900. However, in the femur sections, too, in some cases, no leukemic cells were observed, which would imply a possible ratio of >28,300.

**DISCUSSION**

During the post-chemotherapy period single bone marrow aspirates from leukemic patients are routinely screened in order to verify the state of complete remission or to detect a relapse of the disease as early as possible. As 5% blast cells is considered the borderline for diagnosing leukemia, this leads to difficulties in determining the remission/relapse status of patients being treated for leukemia.<sup>20-23</sup> This implies that the

**Table 4. Ratios of Measured Maximal and Minimal Leukemic Cell Frequencies in Femur Sections and in Ribs of Rats During the Phase of Minimal Residual Disease After Chemotherapy (Cyclophosphamide 100 mg/kg IP)**

Rat No.	Ribs	Femur Sections	Day of Treatment	Day of MRD Detection
1	ND	18	14	24
2	ND	55	14	24
3	29	4	14	31
1	38	202	11	29
2	>220* (70)†	>28,300* (1,900)†	11	31
3	>84*	‡	11	31
4	35	350	11	38
1	7	7	8	35
2	>320*	66	8	35
3	2.4	>30* (28)†	8	39

Of each sample, 50,000 to 300,000 cells were analyzed.

Abbreviation: ND, not determined.

\*In case no leukemic cells were found in one or more samples of a series, calculation of the ratio was performed using the detection limit of 10<sup>-5</sup> as the lowest frequency.

†The calculated ratio using the lowest frequency of leukemic cells that could be accurately measured in one of the samples of the series.

‡All samples were below the detection limit.



patient may host many leukemic cells, ie, up to  $5 \times 10^{10}$ , not being detected. On the other hand, if leukemic cells are found in the bone marrow aspirate, this is not necessarily representative for other bone marrow sites.

In the rat leukemia model BNML, in undisturbed leukemia growth, the differences in the leukemic cell frequency vary, although not greatly, between different bone marrow sites (Table 1, Fig 2). After chemotherapy, considerably larger differences (in the range of 10,000-fold) in leukemic cell content between various bones were observed (Table 2). The largest variation was observed in the "smaller" type of bones, eg, ribs, vertebrae, scapulae, and sternum (Fig 2B). Larger bones, eg, the femur, tibia, and humerus, showed a more uniform pattern (Fig 2B). To exclude influences of size, type, and location of the bones on the measured leukemic-cell contents, all ribs of individual rats were investigated (Figs 3C and D). From the same rats the femur, representing the "large-bone" type, was investigated for possible inhomogeneous distribution within the marrow cavity. Small differences were observed (Figs 3A and B, Table 3). When comparing the average leukemic-cell frequency in the marrow of ribs and femur before the animals received chemotherapy, it was striking that especially during the earlier stages (ie, days 7 and 11) in the femur a two- to threefold lower leukemic-cell frequency was observed (Fig 3). This most likely reflects differences in the initial homing after leukemia-cell transfer as the population growth parameters of the leukemic cells in rib and femoral bone marrow were compared and proved to be identical. As a result of chemotherapy, two patterns of response were identified. Animals treated in early-stage (day 8) or late-stage (day 14) leukemia showed a rather uniform pattern of distribution of the leukemic cells at analysis during the MRD stage. They presented with comparable leukemic-cell frequencies at the investigated bone marrow samples and showed low ratio values (Table 4). The largest variation was observed in the "medium stage" leukemia group treated on day 11. To explain this phenomenon the following has to be considered: In "early" stage leukemia relatively few leukemic cells are present in the ribs (in order of  $2 \times 10^5$ ). Treatment with cyclophosphamide (100 mg/kg IP) will result in 5 to 6-log cell kill. Consequently, most ribs will be cleared of leukemic cells. Leukemia will be detectable in the ribs again only after migration of cells from elsewhere, which is apparently a random process. In "late stage" leukemia a rib contains about  $6 \times 10^6$  leukemic cells. Thus, the cyclophosphamide treatment will result in the survival of some cells in most of the ribs, which will subsequently regrow. Therefore, most

ribs will have a similar leukemic-cell content at the time of investigation during the MRD phase. In "medium stage" leukemia, however, some ribs will be cleared by the cyclophosphamide treatment while in other ribs cells are surviving that will immediately start to regrow. In the "disease free" ribs, however, cells first have to immigrate, a process with an apparent time delay. This will result in larger differences at the time of investigation compared with the two situations discussed previously. A number of factors are involved such as size of the leukemic cell population, treatment efficacy, size of the bone, distribution within the bone, etc. These will undoubtedly also play a role in the treatment of human leukemia where, not predicted by the outcome of any previous bone marrow examination, totally unexpected relapses occur. That the phenomenon of focal regrowth will be the underlying reason is well recognized.<sup>20-23</sup> To what degree differences in tumor-cell frequency may exist, however, is unknown. The results obtained with this animal model suggest that they can be as high as a factor of  $10^5$ .

Concerning the "ultimate" lower detection limit of leukemic cells in human marrow, the following has to be considered. The lower detection limit can be expected to be in the order of one per  $10^5$  to  $10^6$  cells. For statistical reasons 10 to 100 "positive events" have to be scored. This means that  $10^7$  to  $10^8$  cells will have to be analyzed. This corresponds with the average number of cells in a good quality punctate, which will have to be totally processed by means of flow cytometry. The entire bone marrow compartment contains about  $10^{12}$  cells. Assuming a random distribution, the leukemic cell load may be a factor of  $10^4$  to  $10^5$  times larger than observed in the punctate. If the leukemic cells are non-randomly distributed the tumor load may be much larger. That this situation may exist is clear from the animal data. If one realizes that frequency differences of up to  $10^5$  from one site to the other were found, it is clear that relying on single bone marrow aspiration analysis should be discouraged. Multiple bone marrow samples from different sites will increase the chances of detection and quantification of residual disease.

Extended studies in animal models, aiming at a mathematical description of leukemia growth before and after chemotherapy, will be most valuable in formulating a new strategy for optimizing the methods of detecting "minimal residual disease."<sup>24</sup>

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#### REFERENCES

1. Keating MJ, McCredie KB, Freireich EJ: Biologic and treatment determinants of curability in acute myelogenous leukemia, in Hagenbeek A, Löwenberg B (eds): *Minimal Residual Disease in Acute Leukemia* 1986. Boston, Nijhoff, 1986, p 148
2. Barlogie B, Hittelman WN, Davis FM, Kantarjian H: Nucleic acid cytometry, interphase chromosomes and nucleolar antigen in the detection of residual leukemia in morphologic remission, in Löwenberg B, Hagenbeek A (eds): *Minimal Residual Disease in Acute Leukemia*: 1986. Boston, Nijhoff, 1983, p 1
3. Dewald GW, Noel P, Dahl RJ, Spurbeck JL: Chromosome abnormalities in malignant hematological disorder. *Mayo Clin Proc* 60:675, 1985
4. Hagemijer A, Adriaansen HJ, Bartram CR: New possibilities for cytogenetic analysis of leukemic cells, in Hagenbeek A, Löwenberg B (eds): *Minimal Residual Disease in Acute Leukemia* 1986. Boston, Nijhoff, 1986, p 1
5. Hagemijer A, Smit EME, Löwenberg B, Abels J: Chronic myeloid leukemia with permanent disappearance of the Ph<sup>1</sup> chromo-

some and the development of new clonal subpopulations. *Blood* 53:1, 1979

6. Estrov Z, Grunberger T, Dubé ID, Wang YP, Freedman MH: Detection of residual acute lymphoblastic leukemia cells in cultures of bone marrow obtained during remission. *N Engl J Med* 9:315, 1986

7. Janossy G, Bollum FJ, Bradstock KF, Ashley J: Cellular phenotypes of normal and leukemic hemopoietic cells determined by analysis with selected antibody combinations. *Blood* 56:432, 1980

8. Ryan DH, Mitchell SJ, Hennessy LA, Bauer KD, Horan PK, Cohen HJ: Improved detection of rare CALLA-positive cells in peripheral blood using multiparameter flow cytometry. *J Immunol Methods* 74:115, 1984

9. Van Dongen JJM, Hooijkaas H, Adriaansen HJ, Hählen K, van Zanen GE: Detection of minimal residual acute lymphoblastic leukemia by immunological marker analysis: Possibilities and limitations, in Hagenbeek A, Löwenberg B (eds): *Minimal Residual Disease in Acute Leukemia 1986*. Boston, Nijhoff, 1986, p 113

10. Griffin JD, Mayer RJ, Weistein HJ, Rosenthal DS, Coral FS, Beveridge RP, Schlossman SF: Surface marker analysis of acute myeloblastic leukemia: Identification of differentiation-associated phenotypes. *Blood* 62:557, 1983

11. Delwel R, Touw I, Löwenberg B: Towards detection of minimal disease: Discrimination of AML precursors from normal myeloid precursors using a combination of surface markers, in Hagenbeek A, Löwenberg B (eds): *Minimal Residual Disease in Acute Leukemia 1986*. Boston, Nijhoff, 1986, p 68

12. Visser JWM, Bauman JGJ, Mulder AH, Eliason, De Leeuw AM: Isolation of murine pluripotent hemopoietic cells. *J Exp Med* 59:1576, 1984

13. Martens ACM, Hagenbeek A: Detection of minimal residual disease in acute leukemia using flow cytometry: Studies in a rat model for human acute leukemia. *Cytometry* 6:342, 1985

14. Hagenbeek A, Martens ACM: Detection of minimal residual

disease in acute leukemia: Possibilities and limitations. *Eur J Cancer Clin Oncol* 21:389, 1985

15. Visser JWM, Martens ACM, Hagenbeek A: Detection of minimal residual disease in acute leukemia by flow cytometry, in Andreeff A (ed): *Clinical Cytometry*, Annals of the New York Academy of Sciences, vol 468. New York, 1986

16. Hagenbeek A, Van Bekkum DW (eds): Proceedings of an international workshop on "Comparative evaluation of the L5222 and the BNML rat leukemia models and their relevance for human acute leukemia." *Leuk Res* 1:75, 1977

17. Van Bekkum DW, Hagenbeek A: The relevance of the BN leukemia as a model for acute myelocytic leukemia. *Blood Cells* 3:565, 1977

18. Martens ACM, Johnson RJ, Kaizer H, Hagenbeek A: Characteristics of a monoclonal antibody (Rm124) against acute myelocytic leukemia cells. *Exp Hematol* 12:667, 1984

19. Hagenbeek A, Martens ACM: High dose cyclophosphamide treatment of acute myelocytic leukemia. Studies in the BNML rat model. *Eur J Cancer Clin Oncol* 18:763, 1982

20. Mathe G, Schwarzenberg L, Mery AM, Cattani A, Schneider M, Amiel JM, Schlumberger JR, Poisson J, Wajcner DM: Extensive histological and cytological survey of patients with acute leukemia in "Complete remission." *Br Med J* 1:640, 1966

21. Hahn IM, Morris Jones PH, Evans DIK: Discrepancy of bone marrow aspirations in acute lymphoblastic leukaemia in relapse. *Lancet* 1:1215, 1977

22. Jacobs P: Discrepant bone marrow aspirations in leukaemia. *Lancet* 2:355, 1977

23. Pollock A: Discrepant bone marrow aspirations in leukaemia. *Lancet* 2:557, 1977

24. Schultz FW, Martens ACM, Hagenbeek A: Growth kinetics of minimal residual disease in the Brown Norway rat acute myelocytic leukemia, in Hagenbeek A, Löwenberg B (eds): *Minimal Residual Disease in Acute Leukemia 1986*. Boston, Nijhoff, 1986, p 97