Lubmin, Germany. They were grouped according to their total cumulative radiation dose exposure: group A, $<$ 50 mSv (n = 61); B, 50-100 mSv (n = 34); C, 100-200 mSv (n = 24); and D, 200-400 mSv (n = 12). Serving as controls were 131 age-matched male healthy volunteers with no previous radiation exposure. DNA was extracted from peripheral blood mononuclear cells (PBMNCs) by standard procedures, quantitated spectrophotometrically, and stored at $-80°C$.

The real-time quantitative PCR technique used for the detection of *BCL-2-MBR/IgH* -rearrangements has been described in detail.[8] At least 5 1.0 μg aliquots of cellular DNA ($\sim 5 \times 140\,000$ cells) isolated from each blood sample were tested for the presence of the t(14;18) translocation. To determine the total number of cells tested, 3 0.1 μg DNA aliquots were quantitatively analyzed for K-ras. All amplification products were analyzed by agarose gel electrophoresis for the presence of several t(14;18) DNA fragments; 28 amplification products were selected for nucleotide sequence analysis because they revealed t(14;18)-DNA fragments of a quite similar size. In no case were identical sequences found.

The frequency of *BCL-2-MBR/J_H* translocation in peripheral blood lymphocytes from healthy males working at a nuclear power plant was 60.3% (79/131), which is statistically not different from the frequency of 55% (72/131) in age-matched healthy controls (Table 1). At least 700 000 cells (~ 5 μg cellular DNA) were tested per individual based on the quantitative determination of the K-ras gene. There were no statistical differences between the frequency of *BCL-2/J_H* translocation observed in the 4 groups of NPP workers defined by increasing cumulative radiation exposure as well as between the frequency in these 4 groups and age-matched healthy controls (Table 1). Furthermore, the incidence of more than one t(14;18)-positive cell clone was about the same in both study populations. The quantitative determination of circulating t(14;18)-positive cells (Figure 1) revealed no significant differences between NPP workers and age-matched healthy controls.

Based on these results it may be concluded that low-level radiation exposure up to 400 mSv has no significant effect on the frequency of the t(14;18) translocation as well as the total number of circulating t(14;18)-positive cells in the individuals studied. At present, possible effects of higher cumulative doses cannot be excluded.

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References

1. Gribben JG, Freedman AS, Woo SD, et al. All advanced stage non-Hodgkin's lymphomas with a polymerase chain reaction amplifiable breakpoint of bcl-2 have residual cells containing the bcl-2 rearrangement at evaluation and after treatment. Blood. 1991;78:3275-3280.
2. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. Proc Natl Acad Sci USA. 1994;91:8910-8914.
3. Dölken G, Illerhaus G, Hirt C, Mertelsmann R. BCL-2/J_H-rearrangements in circulating B cells of healthy blood donors and patients with nonmalignant diseases. J Clin Oncol. 1996;14:1333-1344.
4. Yasukawa M, Bando S, Dölken G, et al. Low frequency of BCL-2/J_H translocations in peripheral blood lymphocytes of healthy Japanese individuals. Blood. 2001;98:486-488.
5. Fuscoe JC, Setzer RW, Collard DD, Moore MM. Quantification of t(14;18) in the lymphocytes of healthy adult humans as a possible biomarker for environmental exposure to carcinogens. Carcinogenesis. 1996;17:1013-1020.
6. Deininger MW, Bose S, Gora-Tybor J, Yan XH, Goldman JM, Melo JV. Selective induction of leukemia-associated fusion genes by high-dose ionizing radiation. Cancer Res. 1998;58:421-425.
7. Dölken G. Detection of minimal residual disease. Adv Cancer Res. 2001;82: 133-185.
8. Dölken L, Schüler F, Dölken G. Quantitative detection of t(14;18)-positive cells by real-time quantitative PCR using fluorogenic probes. Biotechniques. 1998; 25:1058-1064.

To the editor:

Second malignancy after treatment of acute promyelocytic leukemia: experience of GIMEMA trials

In a recent issue Latagliata et al reported 5 cases of therapy-related myelodysplastic syndrome-acute myelogenous leukemia (tMDS-AML) following acute promyelocytic leukemia (APL) in a cohort of 77 patients who achieved a complete remission (CR) after chemotherapy according to GIMEMA 0389 and AIDA trials.[1] The authors, on the basis of these data, concluded that the observation of tMDS-AML is an emerging problem that could increase in the future. The responsibility of this phenomenon was attributed to chemotherapy, which in some cases included topoisomerase II inhibitors or alkylating agents; furthermore, APL patients presented a high percentage of curability with a consequently large number of long-term survivors.

Between 1982 and 1997 in the GIMEMA APL trials (LAP 0383, 0389, and 0493), 1145 patients were recruited (261 patients in the 0389 trial, 113 patients in the 8303 trial, and 771 patients in the 0493 trial). Details on treatment schedule were previously reported.[2-4] All trials included anthracycline administration (daunorubicin or idarubicin) during the induction and consolidation phases. Maintenance therapy was randomly administered only in the 8303 trial (no therapy vs methotrexate plus 6-mercaptopurine for 2

years) and in the 0493 trial (no therapy vs ATRA vs methotrexate plus 6-mercaptopurine vs 2 + 3 for 2 years). Among these patients, only 4 males (0.3%) aged 36, 38, 61, and 76 years, respectively, developed a second primary malignancy (SPM) (kidney, bowel, melanoma, and thyroid, respectively). All these patients were treated according to the 0493 trial. The median latency between APL diagnosis and SPM was 6.6 months (range, 3.8-7.6 months). The median follow-up was 2.2 years (range, 0-13.8 years). None of them received methotrexate plus 6-mercaptopurine as maintenance therapy. Two patients died from progression of secondary malignancy (bowel and melanoma), without signs of APL relapse, after 3 and 12 months, respectively. The other 2 patients are still alive without signs of relapse of either of the malignancies after 46 months (kidney) and 97 months (thyroid).

Based on the incidence rate data produced by the combined Italian Cancer Registries in the general population,[5] among the APL population the expected number of patients with a second cancer was estimated in 11.98 cases; conversely, in our series only 4 cases were observed (standardized incidence ratio 0.33; 95% CI 0.09-0.86). The estimated cumulative incidence of a second

malignancy at 5 years was 0.43 (95% CI 016-1.15) and was unchanged at 10 years.

Our data allow some interesting considerations. The probability of developing an acute leukemia in patients who received chemotherapy or radiotherapy for a previous malignancy (PM), including acute leukemia, is a well-known occurrence: secondary forms constitute approximately 8% to 10% of all acute leukemias and are usually myeloid.[6]

The main reason for this event is that several drugs employed in the treatment of the PM, particularly topoisomerase II inhibitors (epipodophyllotoxins and anthracyclines), and combined chemotherapy including alkylating agents, are considered potentially mutagenic. As suggested by Latagliata et al,[1] the use of intensive chemotherapy to cure APL, with the inclusion of topoisomerase II inhibitors, has a potential role in inducing a tMDS-AML.[7] In our cohort of patients, the number of secondary malignancies is lower than expected in the normal population. The estimated cumulative incidence at 5 and 10 years is also lower than that expected. Furthermore, the brief latency between the onset of the 2 malignancies leads to the hypothesis that the second malignancy is probably not related to the carcinogenic action of the drugs employed for the treatment of APL, but perhaps to a chance association only.

These considerations suggest that APL treatment is not relevant in inducing the onset of secondary nonhematological malignancies. On the contrary, the action of topoisomerase II inhibitors, which represent one of the main anticancer drugs used in APL, could favor the development of a tMDS-AML with a leukemogenic action on blood stem cells.

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References

1. Latagliata R, Petti MC, Fenu S, et al. Therapy-related myelodysplastic syndrome – acute myelogenous leukemia in patients treated for acute promyelocytic leukemia: an emerging problem. Blood. 2002;99:822-824.

2. Petti MC, Avvisati G, Amadori S, et al. Acute promyelocytic leukemia: clinical aspects and results of treatment in 62 patients. Haematologica. 1987;72:151-155.

3. Avvisati G, on behalf of the Italian Cooperative Group GIMEMA. Event free survival (EFS) duration in newly diagnosed acute promyelocytic leukemia (APL) is favorably influenced by induction treatment with idarubicin alone: final results of the GIMEMA randomized study "LAP0389" comparing IDA vs IDA+ARA-C in newly diagnosed APL [abstract]. Blood. 1999;94:2259a.

4. Mandelli F, Diverio D, Avvisati G, et al. Molecular remission in PML/RAR alpha-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy: Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. Blood. 1997;90:1014-1021.

5. Zanetti R, Crosignani P, Rosso S. Cancer in Italy: incidence data from Cancer Registries. In: Il Pensiero Scientifico, ed. Lega Italiana per la Lotta contro i Tumori. Associazione Italiana di epidemiologia. Vol. 2. Torino, Italy; 1997.

6. Boivin JF. Second cancers and other late side effects of cancer treatment: a review. Cancer. 1990;65:770-775.

7. Karp JE, Sarkodee-Adoo CB. Therapy-related acute leukemia. Clin Lab Med. 2000;20:71-81.

To the editor:

Oxidation of glutathione peroxidase–deficient red cells by organic peroxides

Red cells from mice with a disrupted glutathione peroxidase-1 (GSHPx-1) gene have no GSHPx activity, since GSHPx-1 is the only isoform of GSHPx found in the erythrocyte. In a recent article in *Blood*,[1] we reported that these enzyme-deficient red cells are not oxidized by exogenous hydrogen peroxide any faster than wild-type cells. This strongly supports the view that catalase is the preeminent enzyme protecting red cells from attack by exogenous hydrogen peroxide. However, this conclusion also raises a question about the role of GSHPx in the red cell. In this regard, we noted that while catalase is completely specific for H_2O_2, GSHPx is able to reduce organic peroxides as well, suggesting that the distinctive role of GSHPx might be to detoxify organic peroxides. To test this, wild-type and GSHPx-deficient red cells[2] were exposed to a range of compounds known to hemolyze red cells (cumene peroxide, methylene blue, chloramphenicol, naphthalene, phenylhydrazine, t-butyl peroxide, primaquine, paraquat). Oxidation of hemoglobin (Hb) was used as an endpoint for oxidative damage. Preliminary studies also assayed K efflux, which is increased by organic peroxides.[3,4] However, the alteration in K efflux was found to follow temporally the oxidation of Hb, indicating that Hb oxidation was an earlier indicator of oxidative damage. Of these compounds, the GSHPx-deficient red cells showed differential sensitivity only to organic peroxides. Figure 1 shows a distinct and reproducible difference between wild-type and GSHPx-deficient cells in their sensitivity to organic peroxides.

What might be the evolutionary benefits of an erythrocyte mechanism for detoxifying organic peroxides? Are there circumstances when organic peroxides might arise in animal issues?

Circumstantial evidence is found in the observation that all microorganisms have enzymatic activities, AhpC/F, that reduce organic peroxides.[5-10] Although these enzymes are peroxiredoxins and have no structural relationship to eucaryotic GSHPx, they exhibit similar catalytic capacities and are able to reduce cumene peroxide and t-butyl peroxide. These enzymes protect the bacterium against damage by organic peroxides, strengthening their functional similarity to GSHPx. Interestingly, deletion of genes for these organic peroxide reductases sometimes,[9,10] but not always,[8] attenuates the virulence of pathogenic strains, suggesting that organic peroxides may be part of the macrophage bactericidal response. Reactions between the H_2O_2 of the respiratory burst and
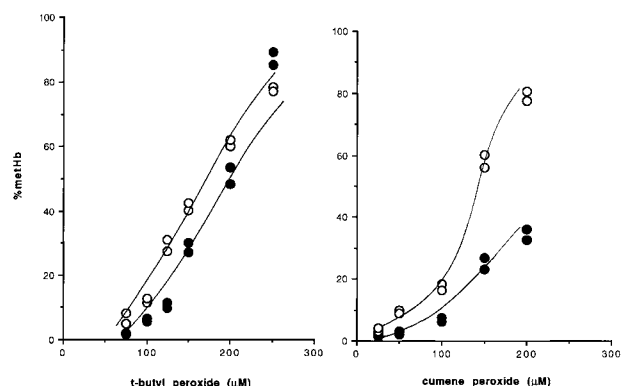

Figure 1. Oxidation of hemoglobin in intact erythrocytes by organic peroxides. ● indicate wild-type red cells; ○, GSHPx-deficient red cells.