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for this discrepancy could be the racial differences in the occurrences of BRAF mutation.

The most impressive examples of recent cancer therapies used protein kinase inhibitors such as Imanitib (Gleevec).<sup>1</sup> Since the RAS-RAF-MEK-ERK-MAP kinase pathway is activated by protein kinase, therapies that target this signaling pathway would therefore be very valuable in treating tumors that have activating mutations of BRAF. In this respect, the present study may provide the possibility of therapy targeting mutated BRAF in acute leukemias.

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## Prognostic value of the rapidity of bone marrow blast cell proliferation in adult acute lymphoblastic leukemia

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TO THE EDITOR

The long-term prognosis of adult lymphoblastic leukemia (ALL) is still disappointing, and only 20–35% of adult patients are cured by chemotherapy. Little is known as to the proliferative activity of ALL in adult patients, and classical kinetic parameters were inconclusive when used as prognosis predictors.

The analysis of the argyrophilic proteins associated with the nucleolar organizer regions (NORs), loops of DNA that transcribe to ribosomal RNA, is a technique (AgNOR method) to assess cell proliferation that can be easily performed in routinely fixed and paraffin-embedded tissues.<sup>1</sup> AgNOR quantity is strictly related to the rapidity of cell proliferation: the higher the AgNOR quantity, the shorter the tumor cell doubling time.<sup>2</sup> We have previously demonstrated the prognostic value of AgNOR analysis on bone marrow (BM) biopsies of patients with adult AML.<sup>3</sup> Only two studies have reported an association between poor prognosis and a large AgNOR area<sup>4</sup> or number<sup>5</sup> in children with ALL.

In the present study, we analyzed the AgNOR expression on BM biopsies from 36 adult patients with ALL at diagnosis, to investigate if the rapidity of blast cell proliferation was

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associated to clinico-pathologic features and prognosis of the disease.

A total of 36 consecutive adult patients with newly diagnosed ALL and available BM biopsy, admitted to the Division of Haematology, S. Giovanni Hospital, Turin, Italy, between 1994 and 2001, were included in the study. There were 19 female and 17 male patients (mean age, 35.5 years; range, 16–65). According to the French–American–British (FAB) criteria, nine were L1 and 27 L2 ALL; 31 were B-ALL and five T-ALL. In all, 10 patients had a normal karyotype, nine had t(9:22) translocation (Philadelphia chromosome-positive (Ph+)) and five miscellaneous cytogenetic abnormalities. By reverse transcription-polymerase chain reaction (RT-PCR) technique, 20 cases showed *bcr/abl* gene rearrangement (BCR/ABL+).

All patients were treated according to the multicenter protocol GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) ALL 0496.<sup>6</sup> Induction therapy consisted of vincristine (1.4 mg/m<sup>2</sup> on days 1–8–15–22–36), three cycles of daunorubicin (30 mg/m<sup>2</sup>/day for 3 days starting on days 1–22–36), L-asparaginase (6000 IU/m<sup>2</sup> every other day from day 10 to 30) and prednisone (30 mg/m<sup>2</sup>/day for 30 days). Patients in complete remission were treated with one cycle (two cycles in patients less than 50 years old) with high-dose Ara-C (2 g/m<sup>2</sup> every 12 h for four doses), etoposide (150 mg/m<sup>2</sup>/day for 3 days) and Cyclophosphamide (300 mg/m<sup>2</sup> every 12 h for six doses). The patients received central nervous system prophylaxis with intrathecal methotrexate and cranial radiation. Maintenance treatment with 6-mercaptopurine and methotrexate with periodic cycles of reinduction was continued for 3 years. Patients with Ph+ leukemia received a consolidation with HAM chemotherapy followed by an allogeneic bone marrow

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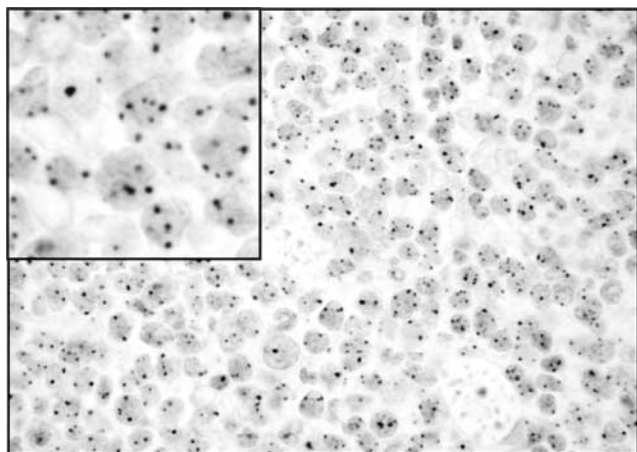
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transplantation or, in the absence of a suitable donor, an autologous stem cell transplantation. The follow-up was complete on 31 May 2002.

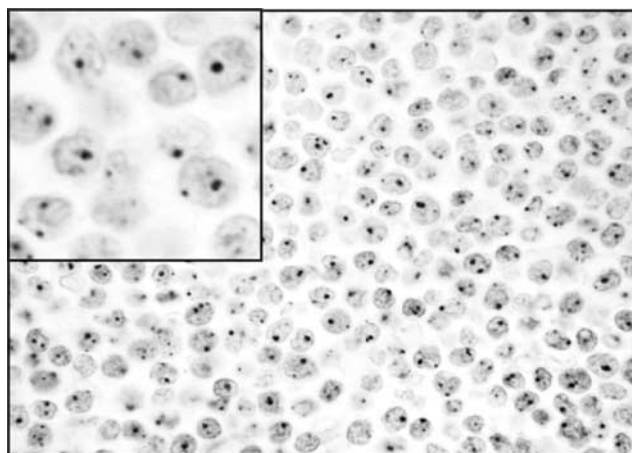
AgNORs were detected according to the method of Ploton *et al*<sup>1</sup> on BM biopsies taken during initial investigation, decalcified and embedded in paraffin. AgNORs were counted as previously reported<sup>3</sup> by two pathologists (AP and LC), who independently examined at least 100 blast cells in each case, using a  $\times 100$  oil immersion lens. The mean number of AgNORs per nucleus was calculated in each case. One-way analysis of the variance, Yates' corrected  $\chi^2$  test, uni- and multivariate survival analysis were used for statistical evaluation.

The mean AgNOR for the whole series was 3.1 (median, 3.04; s.d. = 0.71; range, 2.04–4.66). It was higher in Ph+ ALL (3.47) than in ALL with normal karyotype (3) or miscellaneous cytogenetic abnormalities (2.51;  $P=0.03$ ) (Figures 1 and 2). AgNOR count also tended to be higher in BCR/ABL+ cases (3.2) than in ALL without *bcr/abl* rearrangement (2.87;  $P=0.1$ ). Complete remission (CR) was reached in 18/22 (81.8%) cases with AgNOR count less than 3.1, but in only 8/14 (57.1%) cases with a greater count ( $P=0.2$ ). At the time of analysis, 27 patients (75%) had died of the disease and nine (25%) were alive, without evidence of disease (censored). The mean follow-up for the whole series was 19.3 months (median, 14; range, 0.6–89). The mean follow-up duration for censored patients in continuous CR was 35.22 months (median, 16; range, 7–89). The median overall survival (OS) duration for the whole series was 15.5 months. An association was found between AgNOR counts and OS: using the mean AgNOR count as a cutoff, the median OS was 17 months for patients with  $\leq 3.1$  AgNOR/blast cell and only 10.2 months for those with a higher count ( $P=0.03$ ). At multivariate analysis, when CR was excluded from the analysis, AgNOR counts emerged as the only significant variable ( $\chi^2$ , 3.59;  $P=0.05$ ; risk ratio, 2.21). The median disease-free survival (DFS) duration for the 26 patients who achieved CR was 11.3 months. It was 13.7 months for patients with  $\leq 3.1$  AgNOR/blast cell, but only 4.2 months for those with a higher count ( $P=0.007$ ) (Figure 3). At multivariate analysis, AgNOR count was the only significant prognostic factor for remission duration ( $\chi^2$ , 7.90;  $P=0.005$ ; risk ratio, 8.36).

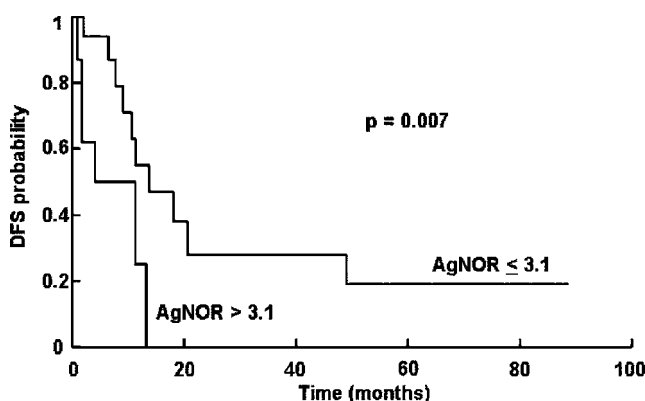
Our results show a significant association between a rapid proliferative activity of marrow blast cells, as indicated by high AgNOR counts, and a shorter OS and DFS in adult ALL, in agreement with a few reports in childhood ALL.<sup>4,5</sup> To the best of



**Figure 1** Ph+ acute lymphoblastic leukemia. Blast cells show numerous small-sized AgNORs scattered within the nucleus (AgNOR staining; original magnification  $\times 400$ ; inset:  $\times 1000$ ).



**Figure 2** Adult ALL showing blast cells with few small AgNORs within the nucleus (AgNOR staining; original magnification  $\times 400$ ; inset:  $\times 1000$ ).



**Figure 3** Actuarial probability of DFS for adult patients with ALL categorized according to the mean AgNOR counts.

our knowledge, there is no mention in the literature as to the correlation between AgNORs and prognosis in adult ALL. These findings are rather different from those previously reported in a series of adult AML in which we showed that AML patients with high AgNOR counts had a longer OS and DFS.<sup>3</sup> The discrepancies may depend on the different kinetic characteristics of ALL and AML blast cells: the mean AgNOR count in ALL ( $3.1 \pm 0.71$ ) is significantly lower ( $P < 0.0001$ ) than in AML ( $6.6 \pm 1.35$ ).<sup>3</sup> AgNOR quantity reflects the rapidity of cell doubling time;<sup>2</sup> it is likely that leukemic clones of AML, which have a short doubling time (as assessed by the high AgNOR value), are very sensitive to the intensive induction chemotherapy, resulting in a high CR rate and/or in a very low minimal residual disease with survival advantage for patients. On the contrary, the doubling time of ALL blasts is long, when compared to AML; therefore, a larger number of quiescent leukemic blasts would escape the intensive chemotherapy currently used in ALL, resulting in the persistence of resistant clones. Moreover, the length of remission in ALL patients achieving CR not only depends on the size of the minimal residual disease but also on the rapidity of proliferation of the resistant clones. Indeed, the median DFS in our series was 13.7 months for patients with low AgNOR counts, but only 4.2 months for those with higher counts ( $P=0.007$ ). Therefore, it is likely that a more rapid proliferation of the residual clones (as indicated by their relatively higher AgNOR counts) would result

in a more rapid relapse and shorter OS and DFS. The kinetic characteristics of adult ALL seem to be rather similar to NHL, since NHL with high AgNOR quantity have a poorer prognosis than NHL with low AgNOR quantity.<sup>7</sup> Interestingly, in the present series, AgNOR count was significantly higher in Ph+ ALL than in cases with different karyotypes, and tended to be higher in *BCR-ABL*+ ALL. It is well known that adult patients with Ph+ ALL have a significantly lower probability of continuous CR, as well as a shorter OS and DFS, than do ALL patients with normal karyotype or miscellaneous cytogenetic abnormalities.<sup>8</sup> Therefore, the worse prognosis for Ph+ ALL could also depend on the shorter doubling time of blast cells, as assessed by their relatively high AgNOR counts.

We are aware that the number of cases in our study is relatively small, so that any conclusion should be taken with caution; however, this preliminary study clearly indicates that a high proliferative activity, in particular the rapidity of blast cell duplication, as assessed by AgNOR counts, is associated with a worse prognosis in adult ALL patients treated with the same protocol. We believe that the evaluation of the leukemic blast proliferative activity on BM biopsies, together with the traditional morphologic, immunophenotypic, cytogenetic and molecular studies, could well be a further refinement of the prognostic assessment of adult patients with ALL.

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## DNA sequencing-based HLA typing detects a B-cell ALL blast-specific mutation in *HLA-A\*2402* resulting in loss of HLA allele expression

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TO THE EDITOR

We describe the detection of a single base pair insertion in the *HLA-A\*2402* allele in the blast cells of a patient with B-cell acute lymphoblastic leukemia (B-ALL). This mutation resulted in the loss of expression of *HLA-A\*2402* on the blast cells. The mutation was not present in the nonleukemia cells on which *HLA-A\*2402* was normally expressed. The mutation was detected following HLA typing by DNA sequencing during routine workup for bone marrow transplantation.

There is considerable evidence to suggest that proliferation of tumor cells is controlled in some part by the immune system.

This includes tumor cell killing by cytotoxic T lymphocytes following the presentation of tumor-specific peptide by tumor cell HLA.<sup>1</sup> Thus, the loss of expression of HLA molecules on tumor cells may result in a selective advantage for a tumor cell clone and have adverse effects on the course of the disease and efficacy of T-cell-based immunotherapy. The loss or down-regulation of HLA on the surface of tumor cells has been reported in many solid organ tumors. However, the loss of HLA in leukemia and lymphoma has been less frequently reported.

The most frequently observed defect resulting in the loss of HLA is the deletion of sections of varying size of the major histocompatibility complex (MHC) from one chromosome.

The loss of a single HLA allele may be sufficient to enable the tumor cell to avoid immune surveillance if this allele presents tumor-specific peptide. However, such loss may go undetected by most molecular HLA typing techniques if the loss is the result of a single base mutation.

In addition, phenotypic detection of allele loss may go undetected unless allele-specific monoclonal antibodies are used, as single allele loss may not result in a quantitative loss of

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