MDS are a group of disorders whose heterogeneity is reflected by the variety of bone marrow cytologic alterations, cytogenetic aberrations and clinical outcome. The response rate to treatment is generally low and predicting factors very difficult to establish, but about 50% of patients presenting with a diagnosis of RA, transfusion independence and a low baseline serum Epo may have a complete or partial response to rHuEpo.[1] The studies published so far are difficult to compare because of the different sizes of patient populations and the heterogeneous response criteria adopted. In the present study we evaluated the efficacy of the combination of rHuEpo-amifostine according to the response criteria proposed by Cheson *et al.*,[10] who recently approached the issue of standardization of such criteria. The results indicate that this combination does not offer a substantial advantage compared to each drug used as a single agent.[5,6] Adopting the same response criteria as those in our previous study on amifostine alone in MDS,[6] the number of erythroid and neutrophil responses was comparable, while platelet response was worse, possibly because of the specific erythroid stimulus of erythropoietin. Our data are in agreement with those reported by Tefferi *et al.*[7] who also concluded that the combination of amifostine plus rHuEpo does not offer substantial advantages in the treatment of MDS.

The response rate is influenced by the criteria selected, and we suggest that these must be stringently defined in the evaluation of results of clinical trials in MDS. Two major points might have negatively affected the results in our study: a) alternate day instead of daily rHuEpo administration used in a previous work;[1] b) selection of a group of patients with negative predictive factors, as indicated by their need of transfusion support prior to therapy, the resistance to previous treatments and their inclusion in IPSS high risk (Int-2 and High) classes, despite the fact that 10/12 were FAB low risk. In this respect it seems that a multifactorial classification of patients (IPSS) predicts the outcome of therapy in MDS better.

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References

1. Anonymous. A randomized double-blind placebo-controlled study with subcutaneous recombinant human erythropoietin in patients with low-risk myelodysplastic syndromes. Italian Cooperative Study Group for rHuEpo in Myelodysplastic Syndromes. Br J Haematol 1998; 103:1070-4.
2. Hellström-Lindberg E. Efficacy of erythropoietin in the myelodysplastic syndromes: a meta-analysis of 205 patients from 17 studies. Br J Haematol 1995; 89:67-71.
3. Rose EH, Abels RI, Nelson RA, McCullough DM, Lessin L. The use of r-HuEpo in the treatment of anaemia related to myelodysplasia (MDS). Br J Haematol 1995; 89:831-7.
4. List AF, Heaton R, Glinsmann-Gibson B, Capizzi RL. Amifostine stimulates formation of multipotent and erythroid bone marrow progenitors. Leukemia 1998; 12:1596-602.
5. List AF, Brasfield F, Heaton R, Glinsmann-Gibson B, Crook L, Taetle R, et al. Stimulation of hematopoiesis by amifostine in patients with myelodysplastic syndrome. Blood 1997; 90:3364-9.
6. Grossi A, Fabbri A, Santini V, Leoni F, Nozzoli C, Longo G, et al. Amifostine in the treatment of low-risk myelodysplastic syndromes. Haematologica 2000; 85:367-71.
7. Tefferi A, Elliott MA, Steensma DP, Hook CC, Dispenzieria A, Hanson CA, et al. Amifostine alone and in combination with erythropoietin for the treatment of favorable myelodysplastic syndrome. Leuk Res 2001; 25:183-5.
8. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997; 89:2079-88.
9. Bennet JM, Catovsky D, Daniel MT, Fladrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982; 51:189-99.
10. Cheson BD, Zwiebel JA, Dancey J, Murgo A. Novel therapeutic agents for the treatment of myelodysplastic syndromes. Semin Oncol 2000; 27:560-77.

Hemoglobin F synthesis is not restricted to fetal erythropoietic organs during extramedullary hematopoiesis

We investigated whether the anatomic distribution of hematopoietic cells determines the type of hemoglobin produced in patients with extramedullary hematopoiesis (EMH). Fetal hemoglobin (HbF) production is not restricted to fetal erythropoietic organs during EMH. A shift of erythropoiesis to fetal hematopoietic organs in EMH does not necessarily induce HbF synthesis in adulthood.

In normal adults, fetal hemoglobin (HbF) production is minimal and HbF is restricted to a specific population referred to as F-cells.[1,2] Extramedullary hematopoiesis (EMH) is characterized by the appearance of hematopoietic elements at sites in addition to bone marrow, particularly sites of hematopoiesis in fetal life such as liver and spleen.[3] Shifts in sites of erythropoiesis during development coincide with changes in the hemoglobin composition of red cells.[4] We investigated whether HbF production during EMH is restricted to the erythropoietic organs that were active in fetal life, and whether erythroid cells in organs corresponding to fetal hematopoietic environments necessarily express HbF.

From autopsy cases, 31 patients were selected as having splenic (n = 20) or intrahepatic (n = 11) EMH. Formalin-fixed paraffin blocks (n = 31) from bone marrow clot specimens, 20 from spleen, and 11 from liver were investigated in these patients; diagnoses included acute leukemia (n = 8), carcinoma (n = 12) or lymphoma (n = 6) involving bone marrow, and hemolytic anemia (n = 5). As controls, we examined 12 spleens, 10 livers, and 15 bone marrows obtained at autopsy from individuals who had not had hematologic diseases. Erythropoiesis was immunohistochemically assessed using anti-glycophorin C (GPC) antibody (Dako, Glostrup, Denmark), anti-hemoglobin A (HbA) antibody (Calbiochem, La Jolla, CA, USA), and anti-HbF

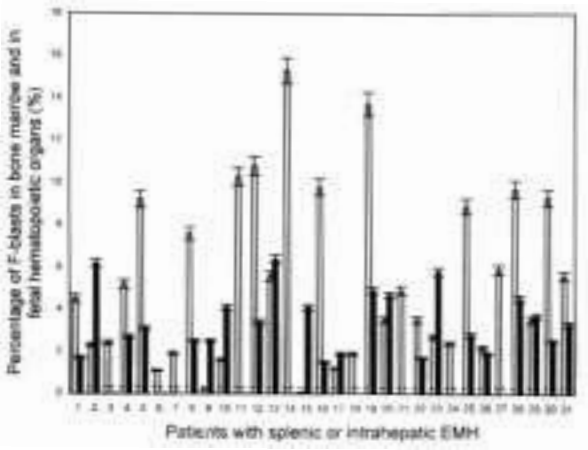

Figure 1A. F-blasts in bone marrow (□) and in fetal hematopoietic organs (■; patients 1 to 20, in spleen; patients 21 to 31, in liver). A dotted line at the bottom shows a cut-off level of F-blasts in bone marrow of 0.3%, based on the mean±2 SD in controls. No immunohistochemically detectable F-blasts are observed in fetal hematopoietic organs from controls. Increased F-blasts are noted in bone marrow in 29 of 31 patients (93.5%), in spleen in 14 of 20 (70.0%), and in the liver in 8 of 11 (72.7%).

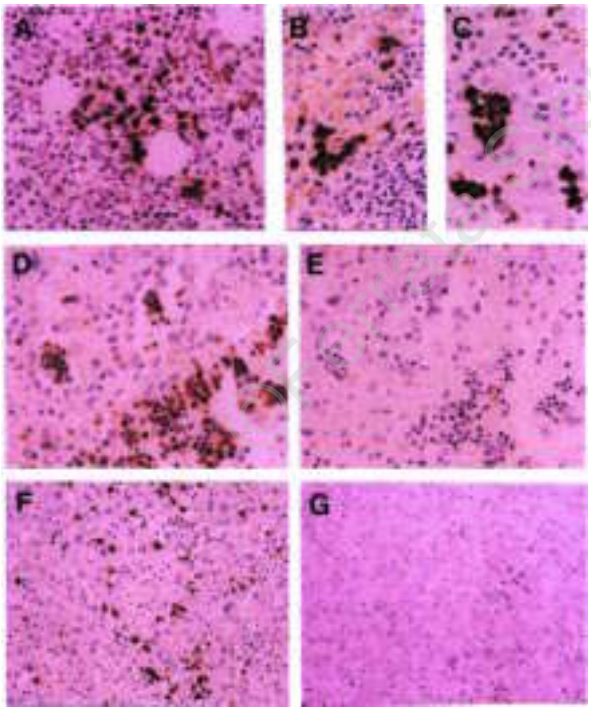

Figure 1B. Immunohistochemical reactions of HbF- or HbA-containing erythroblasts. F-blasts are observed in bone marrow (A), spleen (B), and liver (C) from patients with EMH. Examples showing lack of immunohistochemically detectable fetal-type erythropoiesis in spleen and liver, while HbA-containing erythroblasts are consistently found in the serial sections of the spleen and the liver (D and E, spleen; F and G, liver; D and F, HbA expression; E and G, HbF expression).

Table 1. HbF- and HbA-containing erythroblasts in bone marrow and fetal erythropoietic organs.

Subjects	*Cases (n)*	*Among GPC (+) erythroblasts* (mean ± SD, %)*	
		F-blasts	*A-blasts*
Patients with EMH			
Bone marrow	31	5.4 ± 4.1	89.2 ± 4.7
Spleen	20	2.5 ± 2.0	87.6 ± 5.3
Liver	11	2.4 ± 1.9	90.1 ± 4.2
Control group			
Bone marrow	15	0.1 ± 0.1	92.5 ± 3.6
Spleen	12	NS	NS
Liver	10	NS	NS

*Abbreviations: GPC (+) erythroblasts, erythroblasts stained by anti-glycophorin C antibody; F-blasts, HbF-containing erythroblasts; A-blasts, HbA-containing erythroblasts; EMH, extramedullary hematopoiesis; NS, not seen. *The proportion of F-blasts and A-blasts was expressed as a percentage based on 1,000 erythroblasts stained by anti-GPC antibody. The proportion of A-blasts exceeds that of F-blasts in EMH organs as well as in bone marrow. The percentages of F-blasts in bone marrow, spleen, and liver were significantly higher than corresponding values in controls ($p < 0.01$, respectively, compared by the Mann-Whitney test).*

antibody, which we recently developed using a synthetic peptide as an immunogen.[5] Serial sections from paraffin-embedded tissues of all specimens were assessed using a streptavidin-biotin complex method. The percentage of HbF-containing erythroblasts (F-blasts) was calculated from the proportion of F-blasts identified by anti-HbF among 1,000 erythroblasts stained by the anti-GPC antibody. The Mann-Whitney test was used to compare differences of mean values. $p < 0.01$ was considered statistically significant.

Among erythroblasts stained by the anti-GPC antibody, the mean proportions of F-blasts were 5.4±4.1% in bone marrow, 2.5±2.0% in spleen, and 2.4±1.9% in liver, which were significantly higher values than those for the controls ($p < 0.01$, respectively) (Table 1). The percentage of F-blasts was greater than 0.3% in the bone marrow in 29 of 31 patients (93.5%), in the spleen in 14 of 20 (70.0%), and in the liver in 8 of 11 (72.7%). This percentage was taken as the provisional cut-off value based on the mean±2SD for the controls (Figure 1a). Increases in F-blasts were observed not only in EMH sites, but even more often in the bone marrow. HbA-containing erythroblasts (A-blasts) were identified in both bone marrow and organs involved in fetal hematopoiesis in every case. Furthermore, the numbers of A-blasts exceeded the numbers of F-blasts in EMH organs as well as in bone marrow. Interestingly, we failed to find evidence of fetal-type erythropoiesis in six spleens and three livers with EMH, while A-blasts were consistently found in these cases (Figure 1b). Absence of fetal-type erythropoiesis at EMH sites that showed vigorous adult-type erythropoiesis suggests that the type of hemoglobin produced in adulthood does not follow the same anatomic distribution as with erythoid precursor cells in fetal life; moreover, a shift of erythropoietic sites in adults to fetal erythropoietic organs does not necessarily result in HbF synthesis. Our results for HbF and HbA expression in EMH organs partially agree with a previous report that every spleen showing fetal-type hematopoiesis also had evidence of adult hemoglobin production.[6] The same study, however, found that in lymphoproliferative diseases such as lymphoma and chronic lymphocytic leukemia, the density of

fetal erythroblasts exceeded the density of adult erythroblasts in adult spleen. These findings differ from our results, in which the numbers of F-blasts were consistently less than the numbers of A-blasts. Specificity of the antibody against HbF may have differed between the studies. We also speculate that in our study the proportion of F-blasts might have been underestimated, because there may be a lot of GPC-positive cells that do not show a detectable level of hemoglobin label.

In conclusion, HbF production is increased during EMH but is not restricted to fetal erythropoietic organs. An extramedullary hematopoietic milieu does not necessarily impose HbF synthesis in adulthood.

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References

1. Boyer SH, Belding TK, Margolet L, Noyes AN. Fetal hemoglobin restriction to a few erythrocytes (F cells) in normal human adults. Science 1975; 188:361-3.
2. Wood WG, Stamatoyannopoulos G, Lim G, Nute PE. F-cells in the adult: normal values and levels in individuals with hereditary and acquired elevations of Hb F. Blood 1975; 46: 671-82.
3. Wu JH, Shih LY, Kuo TT, Lan RS. Intrathoracic extramedullary hematopoietic tumor in hemoglobin H disease. Am J Hematol 1992; 41:285-8.
4. Tavassoli M. Embryonic and fetal hemopoiesis: an overview. Blood Cells 1991; 17:269-81.
5. Choi JW, Kim Y, Fujino M, Ito M. A new anti-hemoglobin F antibody against synthetic peptide for the detection of F-cell precursors (F-blasts) in bone marrow. Int J Hematol 2001; 74:277-80.
6. Underwood JC, Dangerfield VJ. Immunohistochemical identification of adult and fetal haemopoiesis in the spleen in lymphoma, leukemia and myeloproliferative disease. J Pathol 1981; 134:71-80.

Altered constitutive and activation-induced expression of CD95 by B- and T-cells in B-cell chronic lymphocytic leukemia

Expression of CD95, a molecule involved in activation-induced cell death (AICD), might contribute to explain accumulation of leukemic B-cells and functional impairment of T-cells in B-cell chronic lymphocytic leukemia (B-CLL). Therefore, we compared constitutive and activation-induced expression of CD95 and CD69 by B- and T-cells in CLL patients and in healthy donors.

We examined 19 untreated B-CLL patients (13M, 6F; mean age 66.5 years) and 14 sex- and age-matched healthy subjects by using murine monoclonal antibodies conjugated to FITC, PE or PerCP and specific for CD3, CD19, CD5, CD23, CD10, CD69, CD95, CD38, CD25, CD71, CD11c, CD80, CD86 and κ/λ immunoglobulin light chains (Becton Dickinson, Mountain View, CA, USA). Staining was performed both on fresh whole blood samples and on cultured isolated peripheral blood mononuclear cells (PBMCs). Multiparametric analysis was performed on a FACScan cytometer equipped with CellQuest software® (Becton Dickinson). The percentages of cells and their mean fluorescence intensity (MFI) were calculated on *gated* $CD19^+$ or $CD3^+$ lymphocytes coexpressing CD69[1] or CD95.[2]

Isolated PBMCs from nine B-CLL patients and nine healthy donors were cultured for 24-72 hrs in complete medium (RPMI 1640 with 10% fetal bovine serum, antibiotics, L-glutamine), with or without PMA (5 ng/mL) + ionomycin (250 ng/mL), and then FACS-analyzed. The Student's t-test was used for analysis of differences in surface molecule expression in the two examined groups; linear regression analysis was used to correlate CD95 and CD69 expression.

Leukemic B-cells were positive for CD19, CD5, CD23, with poor expression of surface Ig, negative for CD10, and variably positive for CD38, CD25, CD71, CD11c, CD80 and CD86.[3,4]

As shown in Figure 1, freshly examined B-cells from CLL patients expressed more CD69 than those from controls (%= 26±20.7 vs 5±2.3, p=0.005; MFI=215±61 vs 147±17, p=0.003), while no significant difference was observed in CD95 expression. After 24-hr-stimulation with PMA + ionomycin, normal B-cells showed a significantly correlated (r=0.802; p=0.017) increase in CD69 (%=99.2±0.6 vs 21.4±5.9, p<0.001; MFI=614±74 vs 204±39, p<0.001, in stimulated vs unstimulated cultures, respectively) and CD95 expression (%=73.8±14.9 vs 20.7±7.6, p<0.001; MFI=386±54 vs 198±46, p<0.001), with no further increase at 72 hours. In contrast, PMA + ionomycin-stimulated CLL B-cells displayed a marked increase in CD69 (%=92.8±16.4 vs 46.4±33.5, p=0.01; MFI=544±119 vs 284±95, p=0.001, in stimulated vs unstimulated cultures, respectively), but not in CD95 expression (%=26.2±16.6 vs 14.5±13.8, p>0.05; MFI= 227±57 vs 180±57, p>0.05) after 24 hrs of culture. These results demonstrate a constitutively higher CD69 expression on B-cells in CLL patients compared to in normal subjects, while, in contrast with what is commonly assumed,[5] and in agreement with Molica *et al.*,[6] no significant difference was observed in CD95 constitutive expression. However, after activation, while no difference was observed in CD69 upregulation, CD95 expression was significantly lower in CLL B-cells compared to in B-cells from normal subjects (p<0.001). No difference was observed in activation-induced CD69 expression.

Figure 2 shows that T-cells in CLL-patients constitutively expressed more CD69 (%=8.0±5.7 vs 3.0±1.3, p=0.01; MFI=197±20 vs 175±17, p=0.02) and CD95 (%=82.6±14.1 vs 59.3±9.3, p=0.001; MFI=415±59 vs 341±38, p=0.009) than con-