Table 2. β-globin gene alleles and haplotypes detected in 58 Albanian patients; populations in which the alleles have been reported.

Alleles	*Haplotypes*	*Chromosomes n°*	*Populations*
IVS-I-110 (G→A)	I	42	Mediterranean
Codon 39 (C→T)	II	17	Mediterranean
IVS-I-6 (T→C)	VI	12	Mediterranean
Codon 44 (-C)	I	4	**Kurdish, Jewish (30%),** Mediterranean
IVS-I-1 (G→A)	V	3	Mediterranean
IVS-II-1(G→A)	III	2	Mediterranean
IVS-II-1(G→A)	I	1	Druze and Yemenite Jews
Poly A (AATAAA→AATGAA)	II	2	Balkan, **Yugoslavian (4%)**, *Turkish*
IVS-II-745 (C→G)	VII	1	Mediterranean
Codon 5 (-CT)	V	1	Mediterranean, ***Bulgarian (5%)***, Balkan
Codon 82-83 (-G)	III	1	*Arzebaijan, Czechoslovakian*, Croatian, *United Arabia Emirates*
Codon 37 (G→A)	I	1	***Jordanian (9%)***, **Israeli Arab (5%)**, Saudi Arabian, Egyptian, *United Arab Emirates, Syrian*, *Turkish*, Iberian
β^S	Benin type	24	Mediterranean, Central West Africa
β^C	Benin type	2	Mediterranean, Central West Africa
β^D	I	1	Mediterranean, *Indian Asian*
Total of chromosomes		114	

RFLP haplotype analysis was carried out as previously reported.[9] The RFLPs analyzed were Hind III/$^G\gamma$, Hind III/$^A\gamma$, Hinc II/$\psi\beta$ and 3' $\psi\beta$, Ava II/β and Bam HI/3'β. The haplotypes were classified according to Orkin.[10] In italics: populations in which the haplotype has not yet been characterized. In bold: populations in which rare mutations have a relatively high allelic frequency (frequencies are reported in brackets).

37(G→A), codon 82-83(-G)].

All the β-thal and the Hb variant alleles were found to be associated with the same haplotypes described in other populations[6] (Table 2). This indicates that mutations did not have an independent origin and suggests that the origin and spread of β-thal mutations and Hb variants in Albania is in keeping with the historical relationships between Albania and neighboring or more distant populations (Greeks, Romans, Slavs and Arabs) and that Albania has experienced a discrete genetic flow.

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Funding: the project was supported by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Legge 488/92, Cluster C02, Progetto Esecutivo 2.

Acknowledgments: we thank the patients for their co-operation and Romeo Prezioso, from IGB, for his precious collaboration in collecting experimental, hematological and clinical data into an electronic data-base.

Key words: β-thalassemia, HB variants; α-globin Albania.

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Manuscript processing
This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received May 2, 2002; accepted July 10, 2002.

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Late response to donor lymphocyte infusions in patients with chronic myeloid leukemia relapsing after allogeneic stem cell transplantation

Donor lymphocyte infusions were given to 13 consecutive chronic myeloid leukemia patients in relapse after allogeneic stem cell transplantation. Of the 13 patients, 11 achieved a molecular remission and 2 a cytogenetic remission. The median time (range) in months to achieve a hematologic, cytogenetic and molecular remission was 2 (1-2), 5 (1-42) and 5 (1-30), respectively. After a median follow-up of 24 months, the median response duration is 16 months (range 1-36), and no patient shows evidence of relapse.

haematologica 2002; 87:1003-1005
(*http://www.haematologica.ws/2002_09/1003.htm*)

Thirteen consecutive chronic meyloid leukemia (CML) patients relapsing after allogeneic stem cell transplant (allo-SCT) from an HLA-identical sibling were treated with donor lymphocyte infu-

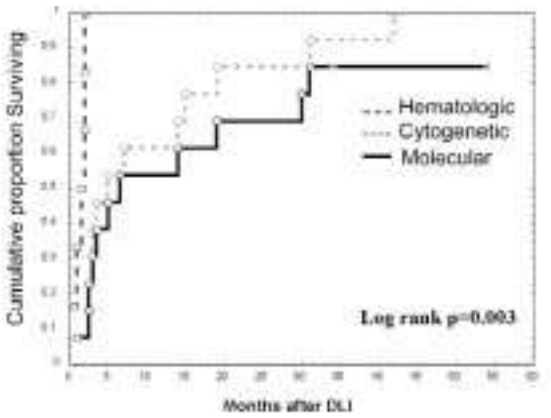

Figure 1. Time to achieve a hematologic, cytogenetic, or molecular response after DLI.

sions (DLI) at the Hospital Clínic of Barcelona between February 1995 and February 2000. T-cell depletion (TCD) of the graft was performed in 10 (77%) cases; the source of hematopoietic progenitor cells was bone marrow in 9 (69%) and peripheral blood in 4 (31%). At transplantation, the median (range) age of the patients was 37 (15-53) years. The median (range) time from allo-SCT to relapse was 27 (10-126) months. All patients received DLI from the same donor used for the allo-SCT. No graft-versus-host disease (GVHD) post-DLI prophylaxis was administered to the patients. Of the 13 patients, 11 achieved a molecular remission and 2 a cytogenetic remission; in the latter two patients BCR/ABL transcripts were undetectable by a Southern-blot technique, but were apparent using polymerase chain reaction (PCR) with nested primers.

Patients receiving an initial low dose of lymphocytes (LDL) (10×10^6/kg). Five patients initially received LDL. Two patients (1 hematologic relapse and 1 cytogenetic relapse) achieved a molecular remission, at 3 and 6 months, respectively. Two patients did not respond at 3 and 16 months, and received a high dose lymphocyte (HDL) infusion, obtaining molecular remission at 5 and 14 months, respectively. No response was observed in the fifth patient either after 9 months of the LDL or after 8 months of a subsequent HDL infusion; this patient was then treated with interferon (IFN)-α, achieving a molecular remission 19 months after the start of IFN-α.

Patients receiving an initial high dose of lymphocytes (HDL)(150×10^6/kg). Eight patients initially received HDL. Five patients (3 hematologic and 2 cytogenetic relapses) achieved a molecular remission at 2, 2, 3, 4 and 34 months, respectively. Three patients did not respond at 4, 6 and 18 months, and received treatment with IFN-α: one of these 3 patients achieved a molecular remission at 24 months, and two a major cytogenetic response at 3 and 18 months. In both cases BCR/ABL transcripts were undetectable with a Southern-blot technique whereas they were detected by PCR nested primers. The median time and range to achieve a hematologic, cytogenetic and molecular response after DLI was 2 (1-2), 5 (1-42) and 5 (1-30) months, respectively. Figure 1 shows the actuarial probability of hematologic, cytogenetic and molecular response after DLI. As can be seen, several patients achieved a cytogenetic or molecular remission more than 1 year after DLI. After a median (range) follow-up from DLI administration of 24 (4-55) months, all patients are alive and only one is receiving immunosuppressive treatment due to chronic GVHD (100% Karnofsky score). None of the patients has shown evidence of relapse and the median (range) duration of the response, from the maximum obtained, is 16 (1-36) months.

Mackinnon *et al.*[1] designed a dose escalation trial to determine whether substantially lower numbers of donor leukocytes could induce remissions with lower toxicity, showing that a T-cell dose of 10×10^6/kg was capable of inducing durable remissions with a lower incidence of GVHD and myelosuppression. Because of this, escalating-dose regimens are currently being used in many transplant centers.[2-6] An important issue that remains to be clarified when an escalating DLI approach is planned is the appropriate time interval between the administration of different lymphocyte doses. In this sense, the most prominent finding of the present study was the median time of 5 months to obtain a cytogenetic and molecular response, with a significant number of patients responding more than one year after DLI. This is much longer than usually waited before a second infusion, with a higher lymphocyte dose, is given.[4,7-10] These results seem to indicate the importance of allowing an interval of at least 6 months between increasing doses of donor lymphocytes in order to assess the response to a particular lymphocyte dose better. Regular follow-up with quantitative reverse transcriptase polymerase chain reaction (RT-PCR) for BCR-ABL transcripts would help to identify late responders.

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Manuscript processing
This manuscript was peer-reviewed by two external referees and by Dr. Francesco Dazzi, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Dazzi and the Editors. Manuscript received May 17, 2002; accepted July 16, 2002.

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Nuclear factor κ B is activated in myelodysplastic bone marrow cells

We describe a significant increase in the DNA binding activity of nuclear factor-κ B (NFκB) in bone marrow cells from patients with myelodysplastic syndrome (MDS) compared with normal donor cells. Furthermore, MDS samples with increased NFκB activity consistently showed higher mRNA levels of NFκB-regulated cytokines.

haematologica 2002; 87:1005-1006
(*http://www.haematologica.ws/2002_09/1005.htm*)

Increased intramedullary apoptotic death of hematopoietic cells is believed to contribute to the ineffective hematopoiesis in myelodysplastic syndromes (MDS), a myeloid hemopathy with a tendency to evolve to acute leukemia.[1] However, the underlying molecular mechanisms that are responsible for this alteration remain elusive.

A deregulated expression of Bcl-2 and Bcl-x_L has been associated with disease progression in MDS.[2] As these antiapoptotic genes are transcriptionally regulated by Stat3 and Stat5 in different cell systems,[3,4] we first analyzed the DNA binding activity of these Stat proteins in bone marrow from 14 patients with MDS and 6 normal donors. However, as shown in Figure 1, there were no significant differences in Stat activation. Another transcription factor associated with Bcl-2 family members (Bcl-xL, Bcl-2, A1) is NFκB.[5] We, therefore, analyzed the activity of NFκB present in 17 MDS bone marrow samples (refractory anemia, n=5; refractory anemia with ring sideroblasts, n=5; refractory anemia with excess blasts, n=3; refractory anemia with excess blasts in transformation, n=2; chronic myelomonocytic leukemia, n=2), and found that at least six of them, including 4 refractory anemia (samples #3, 11, 14, and 17), 1 refractory anemia with ring sideroblasts (sample #4), and 1 refractory anemia with excess of blasts (sample #7) showed a significant increase in the signal given by the NFκB-DNA complex, as assessed by elec-

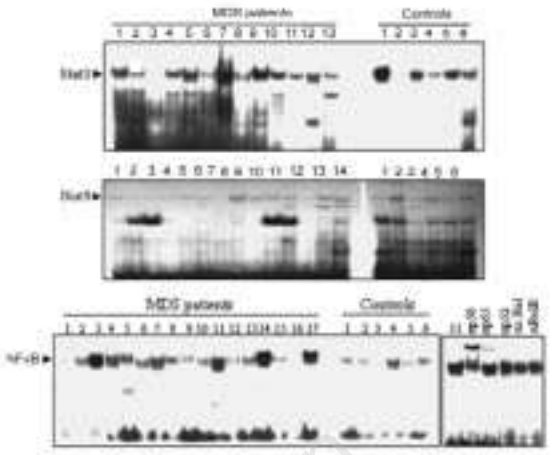

Figure 1. DNA binding activity of Stat3, Stat5 and NFκB in MDS. Mononuclear cells were obtained from bone marrow cells of MDS patients and normal donors (controls). Formation of protein-DNA complexes was determined by EMSA using radiolabeled probes that contained consensus sites for Stat3, Stat5, and NFκB. Nuclear extracts from sample 11 were preincubated with antibodies specific for p50, p65, p52, c-Rel, and RelB.

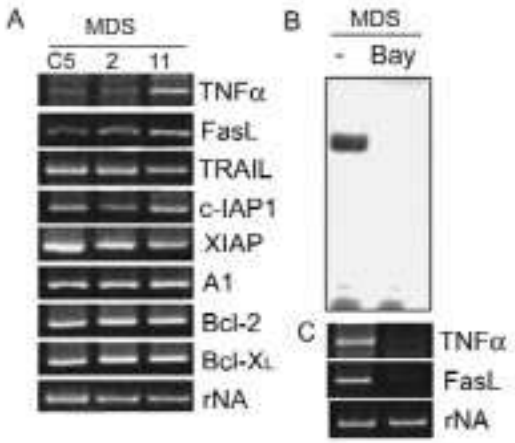

Figure 2. Expression of NFκB target genes in MDS bone marrow cells. (A) Total RNA from MDS samples 2 (low NFκB activity) and 11 (high NFκB activity), and from a normal donor (C5) was obtained and analyzed for the expression of a number of NFκB target genes by semiquantitative RT-PCR. (B) Bone marrow cells from MDS sample 11 were cultured in the absence (-) or in the presence (Bay) of the NFκB inhibitor Bay 11-7082 for 1 h and formation of protein-DNA complexes was determined by EMSA using a NFκB radiolabeled probe. (C) MDS cells were cultured for 24 h with the inhibitor, and then total RNA was extracted and analyzed for the expression of FasL and TNFα by semiquantitative RT-PCR. Amplified products were electrophoresed onto a 2% agarose gel and stained with ethidium bromide. 18S rRNA was used as an amplification control. Each amplification was repeated at least three times, and similar results were obtained.