at least in semisolid assays.

The effect of HHV-7 was next investigated on CD61+ megakaryoblasts, derived from CD34+ cells after 10 days of serum-free liquid cultures,[5] supplemented with SCF+TPO. This approach promotes virus-cell interactions and may more closely resemble *in vivo* infection, as it allows potential secondary infection. Analysis of cell viability performed between 5 and 8 days post-infection revealed that HHV-7 infection induced marked ($p<0.01$) cytotoxicity on cultured CD61+ megakaryoblasts, mainly due to a significant ($p<0.01$) increase of apoptosis (Figure 1A). Cells surviving HHV-7 infection showed a brighter expression of the CD42b late megakaryocytic marker than did the mock-treated cultures (Figure 1B), coupled with an increased frequency of mature polyploid megakaryocytes at morphologic analysis (Figure 1C). In fact, there was a significantly higher ($p<0.01$) number of cells with a diameter greater than 20 μm in HHV-7 cultures than there were in control cultures (53±9% versus 35±8%, respectively, means+SD of four experiments). All these effects were completely abrogated by the neutralizing anti-HHV-7 serum (1:100 dilution, Advanced Biotechnologies, Columbia, MD, USA).

In parallel, we investigated whether HHV-7 infects CD61+ megakaryoblasts and persists in their differentiated progeny. Since the presence of viral DNA could be the consequence of residual virions of the initial inoculum, the occurence of HHV-7 entry was analyzed by reverse transcriptase-PCR, performed on total RNA extracted from CD61+ infected megakaryoblasts at different days post-infection. HHV-7 RNA was detectable at all the time points examined (Figure 1D).

In immunocompromised hosts, human herpesviruses show prompt ability to reactivate generating disseminated infections. In fact, HHV-6 and HCMV have both been associated with many opportunistic pathologic manifestations in AIDS patients (pneumonia, encephalitis, abnormalities of the hematologic picture). In spite of a relative scarcity of definitive evidence to establish the pathogenic potential of HHV-7, it has been demonstrated that reactivation of HHV-7 occurs following bone marrow transplantation[6] and that an increased expression of HHV-7 takes place in lymphoid organs of AIDS patients.[7] For the purpose of this study, it is particularly noteworthy that HHV-7 DNA is present in up to 50% of bone marrow samples of healthy adult donors.[5,8] Moreover, we have previously shown[5] that *in vitro* HHV-7 infection of CD34+ hematopoietic progenitors accelerates differentiation along the granulocytic but not the erythroid lineage, without showing cytotoxic effects. On the other hand, we have demonstrated for the first time in this study that HHV-7 severely impairs the survival of CD61+ megakaryocytic cells, and that the megakaryocytes surviving HHV-7 cytotoxicity show a hastened maturation. Of note, also HCMV selectively inhibits CD42+ megakaryocytes without affecting CFU-meg progenitors or cells of the erythroid and granulocytic lineages.[9] Taken together, the data of Crapnell *et al.*[9] and our present data suggest that megakaryocytes are particularly susceptible to the cytotoxicity of the two closely related herpesviruses, HCMV and HHV-7.

Several factors, such as direct HHV-7 virion/cell interactions and release of cytokines by infected cells are likely implicated in inducing megakaryocyte apoptosis and in promoting maturation along both the megakaryocytic and granulocytic[5] lineages. Due to the central role of megakaryoblasts in the regulation of megakaryocytopoiesis, our data may contribute to explain the occurrence of thrombocytopenia, frequently occurring in patients with HIV-1 disease.[10]

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Complete remission induced by high-dose erythropoietin and granulocyte colony-stimulating factor in acute erythroleukemia (AML-M6 with maturation)

Alternative therapeutic approaches with low dose chemotherapy and differentiative-maturative treatment by growth factors are under consideration for elderly patients with acute leukemia. Two patients with AML-M6 with maturation, one refractory to standard chemotherapy and the other ineligible for cytoxic treatment, obtained complete remission from leukemia using high dose recombinant erythropoietin and granulocyte colony-stimulating factor.

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In the last years the use of recombinant human erythropoietin (rHuEpo) has increased greatly. Beneficial effects have been reported in myelodysplasia[1] and in avoiding chemotherapy-

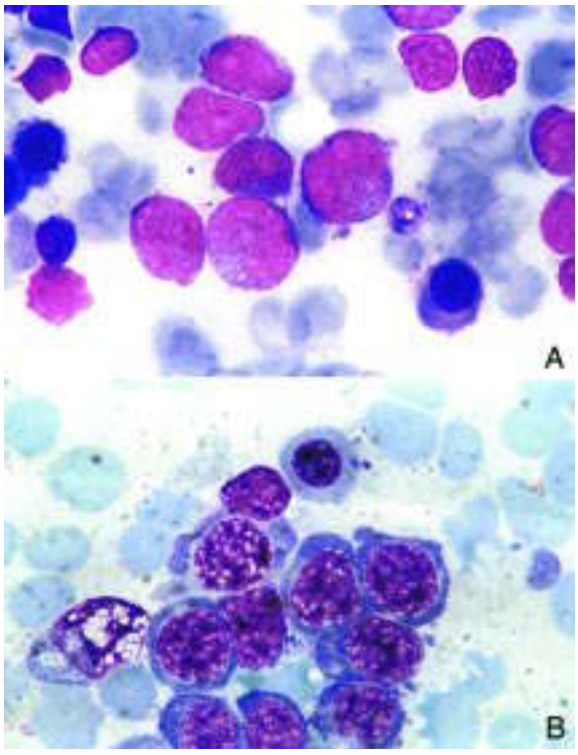

Figure 1. Bone marrow smears (1100×) from the first patient at diagnosis (a) and after the differentiative / maturative treatment with rHuEpo and G-CSF (b).

induced anemia in a variety of tumors.[2,3] A possible *in vivo* synergistic activity with granulocyte colony-stimulating factor (G-CSF) on red cell and platelet production is still a matter of debate.[4-5]

The WHO organization is going to update the FAB classification by introducing two variants of AML-M6: an immature subset (blasts >30%, with an erythroid component >50%) and a subset showing more maturation (blasts <30%, with a high percentage of erythroid precursors). Remission rate in erythroleukemia is disturbingly low, due to the high prevalence of elderly patients, trilineage myelodysplasia, high frequency of cytogenetic abnormalities and expression of the multidrug resistance phenotype. In particular, AML-M6 with maturation seems the subset least sensitive to chemotherapy.[6]

We used rHuEpo and G-CSF to promote erythroid maturation in two elderly patients with AML-M6. In both cases, a high proportion of atypical erythroblasts in the bone marrow constituted the rationale for an attempt at differentiative/maturative treatment by growth factors.

The first patient, a 71-year old man, presented in March 1999 with pancytopenia and 54% circulating blast cells. His bone marrow was hypercellular, with 42% dysplastic erythroid precursors and 52% large-sized blast cells with intensely basophilic cytoplasm (Figure 1a). Blasts were positive for CD13, CD34, CD117, HLA-DR and glycophorin-A (GpA). The karyotype was 47, XY, $5q^-$, +M. Two different courses of polychemotherapy (ICE, FLAN) were both ineffective. Palliative treatment with low dose cytarabine (10 mg/m^2 /12 h s.c.) and lenograstim (263 μg/day s.c.) was scheduled; in addition, based on the clear tendency of the blast cells toward erythroid differentiation, we added high dose rHuEpo (10,000 IU/day s.c.) to enhance erythroid maturation. With this treatment, the patient's general condition rapidly improved. Cytarabine was definitively discontinued after seven days, while rHuEpo and G-CSF were continued. After four weeks blood counts became normal. In July, a bone marrow aspirate showed unexpected complete remission from leukemia (Figure 1b). G-CSF was withdrawn and rHuEpo was reduced to 10,000 IU on alternate days and then to 10,000 IU twice a week (after an episode of hyperviscosity syndrome with Hb 19.5 g/dL). The patient remained in an excellent clinical condition until December, when a leukemia relapse occurred, which proved to be resistant to further treatment. The patient died of leukemic progression in May, one year after starting palliative treatment.

The second patient, an 80-year old male suffering from severe chronic obstructive pulmonary disease and atrial fibrillation, was referred in June 2000 because of pancytopenia. His bone marrow was hypercellular, with 60% dysplastic erythroid precursors and 30% large hyperbasophilic blasts, which were $CD13^+$, $CD117^+$, DR^+ and GpA^+. Cytogenetics showed hyperdiploidy. A diagnosis of AML-M6 with maturation was made. Because of age and co-morbidities, the patient was considered ineligible for chemotherapy. Differentiative/ maturative treatment was attempted with rHuEpo (10,000 IU/day s.c.) and G-CSF (263 μg s.c., every other day). After two months, the blood counts normalized and the bone marrow showed complete remission. The treatment was continued until December, when leukemia relapse occurred. The patient died of sepsis in January 2001.

At the start of rHuEpo treatment, serum Epo levels were relatively low in both patients (249 and 125 mU/mL, corresponding to an observed/predicted ratio of 0.49 and 0.31, respectively).

There is disagreement on the optimal management of acute leukemia in the elderly.[7] Poor prognosis factors originate both from the host (co-morbidities, organ damage, poor tolerance to chemotherapy) and from the leukemic clone itself (preceding or associated trilineage myelodysplasia, adverse cytogenetics, high MDR-1 expression). Often alternative approaches (attenuated dose chemotherapy, differentiative treatments or palliative strategies) are offered to these patients.

The literature reports anecdotal cases of antileukemic activity induced in AML patients by Epo,[8] GM-CSF,[9] or G-CSF.[10] The apparent paradox of using antiapoptotic molecules to induce leukemia remission may be explained by the complex activity of growth factors *in vivo*: apoptosis inhibition by these agents is strictly associated with activation of differentiation and maturation pathways, ultimately producing leukemic cell self-renewal arrest. A similar mechanism is suggested for all-transretinoic acid in acute promyelocytic leukemia: in this case, too, the drug overcomes the maturation arrest producing a complete remission that can last weeks or months, in the absence of cytotoxic treatment. Larger clinical studies are needed to confirm these observations and to evaluate the benefit of including high dose rHuEpo in attenuated chemotherapy strategies for elderly patients with AML-M6, when bone marrow morphology shows a large proportion of maturing progenitors that can be targeted by Epo and serum Epo is relatively low.

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A dexamethasone, vinblastine, cyclophosphamide, etoposide, methotrexate and bleomycin (D-VICEMB) protocol as first-line treatment of patients aged 70 years or older affected by intermediate / high grade non-Hodgkin's lymphoma

We treated 30 consecutive untreated patients aged > 70 years with advanced aggressive non-Hodgkin's lymphoma with 6 courses of cyclophosphamide, mitoxantrone, etoposide, bleomycin, vinblastine and dexamethasone (D-VICEMB). The global response was 93%. The 6-year overall survival and progression-free survival were 50%, and disease-free survival was 63%.

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Increasing age has a negative impact on the outcome of patients with aggressive non-Hodgkin's lymphoma (NHL). In scientific literature people aged 60 years and older are defined as elderly patients. In reality, the condition of elderly people depends on biological age, namely on previous or concomitant diseases and their degree of aging. Patients aged >70 years usually have a poorer outcome.[1-3] Anthracycline-containing regimens, such as CHOP, seem to be more effective than others.[4] However, treatment-related mortality increases up to 15% in elderly patients. In the last decade, weekly regimens, such as P-VEBEC, and VNCOP-B, have been used with the aim of reducing chemotherapy toxicity.[5-9] The D-VICEMB protocol was conceived for day-hospital administration and to facilitate treatment compliance in elderly patients reluctant to depart from their family-environment or to modify life practices. Our regimen, a combination of 6 myelotoxic and non-myelotoxic drugs, was specifically tailored to treat patients aged >70 years. The treatment consisted of six courses, every 21 days, of cyclophosphamide (600 mg/m^2 iv), mitoxantrone (10 mg/m^2 iv), etoposide (60 mg/m^2 iv) on day 1; etoposide (60 mg/m2 orally) on day 2; bleomycin (6 mg/m^2 iv) and vinblastine (6 mg/m^2 iv) on day 7; dexamethasone (12 mg/m^2 orally) on days 1,2,3,5, and 7. Radiotherapy (36 Gy) to residual masses was programmed. The use of granulocyte colony- stimulating factor (G-CSF) at the dose of 5 μg/kg/day for 4-6 days was employed in case of neutropenia < 500/mL. Patients received bacterial and fungal oral prophylaxis with ciprofloxacin (500 mg) and fluconazole (100 mg). Informed consent was provided. From January 1996 to April 2001, 30 consecutive untreated patients received this D-VICEMB regimen. Inclusion criteria were: age >70 years, histologic diagnosis of intermediate/high grade NHL according to the Working Formulation, stages II-IV. The patients' characteristics and outcome are described in Table 1. After chemotherapy administration we recorded 18 (60%) complete remissions (CR), 10 (33%) partial remissions (PR) and 2 (7%) cases of progressive disease (PD). Four patients in PR received additional radiotherapy and 2 of them obtained CR. Overall survival (OS) and progression-free survival (PFS) rates at 72 months (median 28, range 7-77) were 50%. The rate of disease-free survival (DFS) of the 20 patients in CR was 63% (Figure 1). Patients with good performance status (PS) (> 80%) or with an age-adjusted international prognostic index (Aa IPI) score of 0-1 had an evident survival advantage; there was no difference between patients below 75 and those 75 or over (Table 1). One hundred and sixty-one of the planned 180 courses were administered. Four patients in CR suspended treatment after five courses; 4 patients in PR refused further therapy after four or five courses as a result of their improvement; 2 patients received only two courses due to PD. Neutropenia < 500 /mL occurred in 36 of the 161 courses (22%). No relevant infections occurred and no admissions to hospital were required. The incidence of thrombocytopenia was minimal. Only 2 packed red cell transfusions were