

- Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary hemochromatosis. *Nat Genet* 1996; 13:399-408.
- de Villiers JN, Hillermann R, Loubser L, Kotze MJ. Spectrum of mutations in the HFE gene implicated in haemochromatosis and porphyria. *Hum Mol Genet* 1999; 8:1517-22.
- Mura C, Ragueneo O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood* 1999; 93:2502-5.
- Barton JC, Sawada-Hirai R, Rothenberg BE, Acton RT. Two novel missense mutations of the HFE gene (I105T and G93R) and identification of the S65C mutation in Alabama hemochromatosis probands. *Blood Cells Mol Dis* 1999; 25:146-54.
- Aguilar Martinez P, Biron C, Blanc F, et al. Compound heterozygotes for hemochromatosis gene mutations: may they help to understand the pathophysiology of the disease? *Blood Cells Mol Dis* 1997; 23:269-76.
- Remacha AF, Carrasco M, Sardá MP, Barceló MJ, Baiget M. HFE mutation analysis in patients with hepatitis C virus with positive screening for iron overload. *Haematologica* 1999; 84:284-5.
- Baiget M, Barceló MJ, Gimferrer E. Frequency of the HFE C282Y and H63D mutations in distinct ethnic groups living in Spain. *J Med Genet* 1998; 35:701.
- Sánchez M, Bruguera M, Bosch J, Rodés J, Ballesta F, Oliva R. Prevalence of the Cys282Tyr and His63Asp HFE gene mutations in Spanish patients with hereditary hemochromatosis and in controls. *J Hepatol* 1998; 29:725-28.
- Bennett MJ, Lebron JA, Bjorkman PJ. Crystal structure of the hereditary haemochromatosis protein HFE complexed with transferrin receptor. *Nature* 2000; 403:46-53.

Differences in phenotype, growth factor requirements, pattern of expression of adhesion molecules and rate of apoptosis displayed by three new myeloid sister leukemic cell lines

We established three new human myeloid cell lines from one patient, in the presence of granulocyte-macrophage colony-stimulating factor (UPM1-GM), interleukin-3 (UPM1-IL-3) or without exogenous growth factors (UPM1). The 3 lines were characterized by phenotypic, genotypic and functional studies. These cell lines may provide useful tools to study different aspects of leukemic cell biology.¹

Three sister cell lines were established from a bone marrow sample (80% myeloblasts), collected in a resistant phase after 2 courses of induction chemotherapy (mitoxantrone/cytarabine) of an acute myeloid leukemia, FAB type M4Eo. The cells were initially cultured in RPMI 1640 with 20% fetal bovine serum.² The 3 cell lines share cytogenetic abnormalities with the patient's karyotype (43-45,XX,del(5)(q13q33),-7,-16,-18, add(21)(q22),+1-2mar,dmin), but

Table 1. Cytogenetic and immunologic characterization of UPM1, UPM1-GM and UPM1-IL3 cell lines.

	UPM1	UPM1-GM	UPM1-IL3
Chrom. abnormalities	del(8),hsr	+11,del(22)(q13)add(1)(p36),add(11)(p15),del(22)(q13)	
Phenotype			
CD34	88%	73%	63%
CD38	10%	86%	97%
CD117	72%	43%	72%
HLA-DR	82%	72%	74%
CD13	99%	89%	92%
CD33	96%	86%	89%
CD15	25%	43%	45%
CD71	88%	85%	82%
MPO	27%	36%	34%
CD7	40%	28%	31%
CD69	42%	69%	36%
CD54	3%	10%	16%
CD11a	98%	95%	95%
CD11b	7%	22%	14%
CD11c	8%	40%	20%
CD18	98%	95%	98%
others	neg	neg	neg
CD34/CD33	89%	65%	66%
CD33/CD15	35%	58%	50%

(Others) B-lineage (CD19, CD10 and cytoplasmic CD22) and T-lineage (CD3, CD2 and cytoplasmic CD3) markers, CD14, CD16, CD21, CD23, CD56, CD61, LF, TdI; (neg) < 20% of positive cells; antigens with different expression in the three cell lines are in bold.

have some differences between them (Table 1). The 3 lines express myeloid antigens and are strongly positive for LFA-1 (CD18/CD11a) (Table 1).

UPM1 cells are small and morphologically less differentiated, grow in suspension (doubling time-196 hours) and have little CD38 expression. UPM1 cells grow slower than the 2 other lines, as measured by thymidine incorporation (see ref. #3 for details) and clonogenic assays, and have a higher percentage of spontaneous apoptotic cells, as measured by sub-G1 peak and AO/EB staining.⁴ Interleukin-3 (IL-3) (10 U/mL) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (10 ng/mL) increased UPM1 proliferation (3.3 times, $p=0.032$, and 2.6 times, $p=0.049$, respectively) and decreased apoptosis⁵ by 40%. UPM1 growth was also stimulated by granulocyte colony-stimulating factor (G-CSF, 10 ng/mL) and stem cell factor, SCF (10 ng/mL), flt3 ligand, FL (100 ng/mL) and the GM-CSF+IL-3, PIXY321(10 ng/mL)+SCF, or PIXY321+FL combinations (more than 2.0 times the proliferation of control cells).

The UPM1-GM cell line was established in the presence of GM-CSF. This is the fastest growing line (doubling time-89 hours), with larger, more

Table 2. Changes in immunophenotype of the three cell lines after treatment with ATRA (10⁻⁶ mol/L) for six days (values represent the mean of 4 different experiments).

	UPM1				UPM1-GM				UPM1-IL3			
	control	ATRA	statistic	FI	control	ATRA	statistic	FI	control	ATRA	statistic	FI
CD38	10%	97%	$p < 2 \times 10^{-5}$	↑	86%	99%	NS	↑	97%	100%	NS	↑
CD34	88%	88%	NO		73%	89%	$p < 0.03$		63%	75%	$p = 0.02$	
CD117	72%	59%	$p < 0.007$		43%	30%	NS		72%	56%	NS	
CD11a	98%	95%	NS		95%	81%	NS	↓	94%	74%	$p < 0.04$	↓
CD11b	7%	25%	NS		22%	42%	$p < 0.005$		14%	19%	NS	
CD11c	8%	23%	$p < 0.03$		40%	51%	NS	↑	20%	49%	$p < 0.003$	↑
CD18	98%	90%	NS		95%	86%	$p = 0.01$	↓	98%	78%	$p < 0.04$	↓
CD54	3%	52%	$p = 0.01$	↑	10%	87%	$p = 0.006$	↑	16%	92%	$p = 0.001$	↑
CD15	25%	25%	NO		43%	27%	NS		45%	28%	$p = 0.03$	
CD69	42%	22%	$p = 0.04$		69%	49%	$p < 0.005$		36%	35%	NO	
CD34/CD117	67%	56%	$p = 0.006$		38%	29%	NS		53%	44%	NS	

NO - no effect; NS - not significant; FI - effect on intensity of fluorescence signal (peak position ATRA - treated: peak position control) - only considered if the variation in each of the 4 experiments was in the same direction.

differentiated cells, growing mainly in suspension, with some adherent cells. UPM1-GM shows the highest proliferative rate (2.2- and 1.7-fold greater than UPM1 and UPM1-IL3) in the absence of exogenous growth factors and forms colonies and clusters in methylcellulose. Proliferation of UPM1-GM cells increased only in the presence of PIXY321+SCF (2.2 times the control).

The third line, UPM1-IL3, is IL-3-dependent. Its cells are morphologically identical to UPM1-GM, but have an intermediate growth rate (doubling time-133 hours). UPM1-IL3 cells proliferate in response to SCF and PIXY321+SCF. Addition of GM-CSF or IL-3 does not increase the proliferation of UPM1-IL3 or UPM1-GM, but reduces spontaneous apoptosis in culture by 30%.

With regards to their phenotype, CD117 (c-kit) expression was higher in UPM1 ($p = 0.002$) and UPM1-IL3 ($p = 0.001$), and there were also differences in CD38, CD34 and adhesion molecule expression, namely CD11b and CD11c (Table 1). All 3 cell lines express mRNA for the apoptosis regulatory genes analyzed (bcl-2, bax, bak, bcl-x_s, bcl-x_L and mcl-1).

All-trans retinoic acid (ATRA)⁶ inhibited the proliferation of the 3 cell lines, increased apoptosis and induced changes in surface antigens (Table 2), but showed no effect on morphologic maturation. GM-CSF and IL-3 counteracted the effects of ATRA on proliferation and apoptosis - the latter mainly in UPM1 cells. Dimethylsulphoxide (DMSO, 1.2% v/v) had no effect on UPM1 cells, but decreased the expression of CD15 in UPM1-GM ($p = 0.01$) and UPM1-IL3 ($p < 0.02$). On the other hand, 12-O-tetradecanoylphorbol-13-acetate (TPA, 7x10⁻⁹ mol/L)

had little effect on the immunophenotype of the 3 cell lines. DMSO and TPA⁶ had no effect on proliferation or cell viability of any of the cell lines. UPM1, UPM1-GM and UPM1-IL3 may be useful to study the effects of cytokines in the establishment of leukemic cell lines.

Margarida Quinta-Costa, Marina Leite, Pedro Simas-Leite, Cecília Correia, Jorge Candelas, José Eduardo Guimarães

Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Hospital S. João, Faculdade de Medicina do Porto, Instituto Português de Oncologia, Porto, Portugal

Acknowledgments

We thank Dr Sérgio Dias (Cornell University Medical College, New York) for the encouragement, critical review and approval of the manuscript. We would like to thank Amgen for providing SCF and G-CSF and Immunex for supplying FL and PIXY321. We thank the Centro de Histocompatibilidade do Norte for the use of their radiation facilities and Prof. Leonor Parreira (Faculdade de Medicina de Lisboa) for advice and encouragement. We also thank the hematologists Ilídia Moreira and Manuela Brochado for their help.

Funding

This study was supported by the Junta Nacional de Investigação Científica e Tecnológica (JNICT) grants no. PBIC/C/SAU/1524/92 and no. PSAU/C/SAU/68/96. MQC was the recipient of PhD fellowships granted by JNICT (no. BD/750/90-ID and PRAXIS XXI/BD/2978/94).

Correspondence

Margarida Quinta-Costa, M.D., IPATIMUP, Rua Doutor Roberto Frias, s/n 4200 Porto, Portugal. Phone: international +351-22-5570700 - Fax: international +351-22-5570799 - E-mail: mqcosta@ipatimup.pt

References

1. Oval J, Taetle R. Factor-dependent human leukemia cell lines: new models for regulation of acute non-lymphocytic leukemia cell growth and differentiation. *Blood Rev* 1990; 4:270-9.
2. Béné MC, Bernier M, Castoldi G, et al. Impact of immunophenotyping on management of acute leukemias. *Haematologica* 1999; 84:1024-34.
3. Drexler HG, Zaborski M, Quentmeier H. Cytokine response profiles of human myeloid factor-dependent leukemia cell lines. *Leukemia* 1997; 11:701-8.
4. Leite M, Quinta-Costa M, Simas Leite P, Guimarães JE. Critical evaluation of techniques to detect and measure cell death induced by UV radiation of the leukemic cell line HL60. *Anal Cell Pathol* 1999; 19: 139-51.
5. Williams GT, Smith CA, Spooner E, Dexter TM, Taylor DR. Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. *Nature* 1990; 343:76-9.
6. Solary E, Bertrand R, Pommier Y. Apoptosis of human leukemic HL-60 cells induced to differentiate by phorbol ester treatment. *Leukemia* 1994; 8:792-7.

Acute lymphoblastic leukemia in the elderly. A twelve-year retrospective, single center study

Acute myeloid leukemia in elderly patients is a well-studied disease, while only a few studies on acute lymphoid leukemia (ALL) in elderly patients have been reported and their results are not encouraging. The aims of the present study were to review the characteristics of acute lymphoblastic leukemia developing in patients aged over 65 years old during a twelve-year period at our Institution and to analyze the clinical and laboratory characteristics.

Sir,

Between June, 1986 and June, 1998, 119 new cases of acute lymphoblastic leukemia (ALL) were diagnosed in patients aged over 14 years old, consecutively admitted to our Hematology Division. Among these, 37 patients (31%) were aged over 65 years.

On the basis of their performance status, and after evaluation of cardiac, respiratory, renal and liver function, the patients were enrolled in one of the protocols used in our Hematology Department at the time of the diagnosis of ALL. Patients with a relevant concomitant internal disease at diagnosis were excluded from intensive treatment and managed with more conservative therapy.

Of the 25 patients treated with intensive chemotherapy (group 1), 11 (30.5%) were treated according to the GIMEMA ALL-0288 trial protocol,¹ 10 (28%) according to GIMEMA ALL-0183 protocol,² and 4 (11%), all with B-ALL, with attenuated doses of drugs (60%) according to

Magrath's protocol.³ In 5 patients, with a L3 morphology, the treatment included cyclophosphamide.

Twelve patients (32%) presented with a poor performance status or with inadequate function of one or more organs. For these patients (group 2) the induction therapy consisted of weekly administration of vincristine (1.5 mg/m²) and methylprednisolone (40 mg/m²/day) for six weeks.

After the induction and consolidation phases, all the patients who achieved CR, underwent the same maintenance therapy of monthly administration of vincristine, prednisone, 6-mercaptopurine and methotrexate.

Cytogenetic studies were carried out in 20 patients: 13 (65%) had chromosomal abnormalities, the most frequent of which were Ph¹-chromosome and hyperploid set, found in four patients each (Table 1). Molecular biology studies found the p190 rearrangement in one other patient in whom the cytogenetic study failed.

Thirty-three patients (89%) had, at diagnosis, a lumbar puncture to study the cerebrospinal fluid (CSF): leukemic meningeosis was diagnosed in only one patient.

Overall we observed 25 complete remissions (CR) (67%), 9 deaths during induction (24%) and 3 cases of resistant disease (8%). The overall median duration of the CR was 10 months (2-76). The overall median survival was 7 months (0.3-82), but in patients who reached CR it was 14 months (2.8-82.3) and 1.4 months (0.3-7) in those who died during induction or who were resistant (Table 1).

Nine patients (24%) died within 60 days of diagnosis because of the following complications: 7 patients had acute toxicity from chemotherapy (cardiac and gastrointestinal), 1 patient had acute pancreatitis, 1 patient had Gram-negative septic shock.

The overall median duration of hospitalization was 39 days (10-80).

Of the patients treated with aggressive chemotherapy, 20 patients (80%) reached a CR, 4 (16%) died of toxicity before the completion of the induction treatment, and 1 (4%) was resistant. The median duration of CR was 13 months (2.5-76) and the median survival of the patients in CR was 15.5 months (3-82). The median overall survival was 14 months (1.9-82).

Seven patients (28%) manifested signs of acute toxicity from chemotherapy in one or more organs (3 gastrointestinal, 3 cardiac, 1 neurologic, 1 hepatic). The median duration of hospitalization was 45.5 days (28-80).

Among patients managed with palliative treatment, CR was reached in 5 cases (42%) after a median of 28 days from the onset of treatment,