Table 3. Hematologic and karyotypic response in the 11 Ph+ CML patients treated with IFNα+LDAC+ATRA.

Months from start	Complete hematologic response (CHR)	Karyotypic response	Progression to accelerated blastic phase
3rd	9/11 (81%)	//	//
6th	10/11 (91%)	2/11 (18%) case 2 (50% Ph - neg.) case 7 (82% Ph - neg.)	//
9th	10/11 (91%)	3/11 (27%) case 3 (63% Ph - neg.) case 5 (19% Ph - neg.) case 7 (63% Ph - neg.)	//
12th	9/11 (82%)	5/11 (45%) case 2 (78% Ph - neg.) case 3 (70% Ph - neg.) case 7 (74% Ph - neg.) case 8 (25% Ph - neg.) case 10 (72% Ph - neg)	2/11 (18%) cases 1 and 6

IFNα or LDAC and ATRA is required.

Domenico Russo, Luciana Marin, Antonella Bertone, Mario Tiribelli, Nicoletta Testoni,° Giovanni Martinelli°

Chair and Division of Hematology, Department of Medical and Morphological Research and Department of Bone Marrow Transplantation, Udine University Hospital, Udine; °Institute of Hematology and Medical Oncology "L. and A. Seràgnoli", Bologna University, Bologna, Italy

Key words

CML, IFNα, arabinosyl cytosine, ATRA

Acknowledgements

Work supported by MURST 40%, CNR contract #96.00500.PF39 and by AIRC, Milan, Italy

Correspondence

Domenico Russo, M.D., Clinica Ematologica, Policlinico Universitario, piazza S. Maria della Misericordia, Udine, Italy. Phone: international +39-0432-559662/64 – Fax: international +39-0432-559661 – E-mail: domenico.russo@drmm.uniud.it

References

1. The Italian Cooperative Study Group on Chronic Myeloid Leukemia. Progress in treatment of chronic myeloid leukemia. Interferon-α versus conventional chemotherapy. N Engl J Med 1994; 330:820-5.
2. Kantarjian HM, Smith TL, O'Brien S, et al. Prolonged survival in chronic myelogenous leukemia after cytogenetic response to interferon α therapy. Ann Intern Med 1995; 122:254-61.
3. Carella AM, Frassoni F, Melo J, et al. New insights in biology and current therapeutic options for patients with chronic myelogenous leukemia. Haematologica 1997; 82:478-95
4. Guilhot F, Chastang C, Michallet M, et al. Interferon α-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. New Engl J Med 1997; 337:223-9.
5. Zheng A, Savolainen ER, Koistinen P. All-trans retinoic acid combined with interferon-α effectively inhibits granulocyte-macrophage colony formation in chronic myeloid leukemia. Leuk Res 1996; 20:243-8.
6. Mahon FX, Chaline H, Barbot C, et al. All-trans retinoic acid potentiates the inhibitory effects of interferon-α on chronic myeloid leukemia progenitors in vitro. Leukemia 1997; 11:667-73.
7. Sagayadan GE, Peter H, Wiernik, et al. Effect of retinoic acid and interferon-α on granulocyte-macrophage colony forming cells in chronic myeloid leukemia: increased inhibition by all-trans and 13-cis-retinoic acid in advanced stage disease. Leuk Res 1994; 18:741-8.
8. Cortes J, Kantarjian H, O'Brien S, et al. A pilot study of all-trans retinoic acid in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. Leukemia 1997; 11:929-32.
9. Russo D, Regazzi M, Sacchi S, et al. All-trans retinoic acid (ATRA) in patients with chronic myeloid leukemia in the chronic phase. Leukemia 1998; 12:449-54.
10. Sacchi S, Russo D, Avvisati G, et al. All-trans retinoic acid in hematological malignancies, an update. Haematologica 1997; 82:106-21.

Reconstitution of alveolar macrophages from donor marrow in allogeneic BMT: a study of variable number tandem repeat regions by PCR analysis of bronchoalveolar lavage specimens

Sir,

In this study, PCR amplification of VNTR regions was used in order to determine the origin of pulmonary alveolar macrophages (PAM) in five BMT patients. Our results show that this technique is feasible allowing the pattern of reconstitution of this cell population to be determined regardless of whether the donor and recipient were sex-matched or not.

The high incidence and severity of pulmonary complications after allogeneic bone marrow transplantation (BMT) led us to carry out systematic bronchoalveolar lavages (BAL), as a guide to pre-emptive therapy.[1] This allowed us to obtain pulmonary alveolar macrophages (PAM) during the first 100 days after BMT. Tissue macrophages derive from terminal differentiation of blood monocytes originating in the bone marrow. This is supported by the demonstration that within 3 months following BMT, host tissue macrophages, including PAM[2] and hepatic Kupffer cells,[3] are replaced by macrophages of donor origin. However, there is some evidence to support the existence of a local lung stem cell population able to differentiate and maintain the number of PAM.[4] Different conditioning schemes may damage these two PAM precursors differently, which could be reflected in another pattern of PAM repopulation after BMT.[4] The demonstration of the bone marrow origin of PAM has been achieved by Y -body detection,[2] or a

Table 1. Chimerism status of PAM and blood leukocytes after BMT.

Patient	1		2		3		4		5	
	Day	Chimerism status	Day	Chimerism Status	Day	Chimerism Status	Day	Chimerism Status	Day	Chimerism status
Blood leukocytes	+38	Mixed	+64	Donor	+56	Mixed	+95	Donor	+24 +46	Donor Donor
PAM	+38	Receptor	+88	Mixed	+90	Mixed	+95	Mixed	+108	Donor
Blood monocytes			+88	Mixed	+90	Donor				
Blood leukocytes			+146	Mixed (relapse)	+150	Donor				

PAM: pulmonary alveolar macrophages.

fluorescence *in situ* hybridization technique (FISH) with X and Y chromosome probes.[5] These studies require a sex mismatch between donor and recipient. Polymerase chain reaction (PCR) of variable number tandem repeats (VNTR) has been used to determine chimerism status of marrow or peripheral leukocytes post BMT,[6] circumventing the need of sex mismatch. In the present study, PCR was used to determine the reconstitution pattern of post-BMT PAM.

PAM were obtained by BAL[7] from five patients (2 CML, 1 ALL, 2 AML) performed between days +30 and +100 after the BMT.[8] All of the patients had received BUCY2 as a conditioning regimen. PAM were isolated by adhesion to the plastic bottom of a culture flask after 1-2 h incubation at 37°C. The supernatant cells were removed with RPMI 1640 medium and adherent cells rinsed twice.[5] The purified macrophages were lysed following conventional methods[9] and the lysates were used directly for PCR analysis. DNA was extracted from whole blood samples or monocyte cells according to classical methods. Chimerism studies by PCR of VNTR analysis were performed as described above.[10]

One BAL sample for each patient was analyzed. Blood monocytes were simultaneously isolated in two patients. Chimerism results are depicted in Table 1.

Our results showed that PCR of VNTR is a feasible method for detecting donor reconstitution of PAM after BMT. As far as we know, our study is the first to report a bone marrow origin of PAM confirmed by PCR of VNTR regions. In our series a pure donor PAM pattern was only detected in the patient with the latest BAL (day +108), a pure recipient pattern was detected in the earliest BAL, and a mixed pattern was present in the other three cases. A donor pattern of peripheral blood leukocytes was detected earlier than PAM. Alveolar macrophage reconstitution seems to be a time dependent phenomenon. As reported by Thomas *et al.*,[2] host macrophages are largely replaced by donor derived macrophages in less than 100 days, and have an estimated life span of 81 days. Our results are on keeping with theirs. Our patients received chemotherapy (BUCY) as a conditioning regimen but the pattern of PAM reconstitution appears to be similar to that reported by Thomas *et al.*[2] whose patients received radiotherapy (CYTBI). To sum up, our results show that PCR detection of VNTR polymorphisms is a feasible method for ascertaining the donor origin of PAM. The pattern of reconstitution after BUCY seems to be similar to that observed after irradiation-based regimens, although the small number of patients included in our study precludes a firm conclusion on this issue.

Luis Felipe Casado, Rafael De La Camara,° Enrique Granados,° Rosa Giron,# Javier Aspa,# Juan Luis Steegmann°*

**Hematology Department, Hospital Virgen de la Salud, Toledo; °Hematology Department, Hospital Universitario de la Princesa, Madrid; #Neumology Department, Hospital Universitario de la Princesa, Madrid; Spain.*

Key words

Bone marrow transplantation, pulmonary alveolar macrophages, VNTR, chimerism, bronchoalveolar lavage

Correspondence

Luis Felipe Casado Montero, M.D., Hematology Department, Hospital Virgen de la Salud, Avda. de Barber 30, 45004 Toledo, Spain. Phone: international +34-925-269243 – Fax: international +34-925-254113 – E-mail: ecarrascoc@nexo.es

References

1. Schmidt GM, Niland JC, Duncan SR, Forman SJ, Zaia JA. A randomized controlled trial of prophylactic gancyclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplant. New Engl J Med 1991; 324:1005-11.
2. Thomas ED, Ramberg RE, Sale GE, Sparkes RS, Golde DW. Direct evidence for bone marrow origin of the alveolar macrophages in man. Science 1976; 192: 1016-7.
3. Gale RP, Sparkes RS, Golde DW. Bone marrow origin of hepatic macrophages (Kupffer cells) in humans. Science 1978; 201:937.
4. Tarling JD, Lin H, Hsu S. Self-renewal of pulmonary alveolar macrophages: Evidence from radiation chimera studies. J Leuk Biol 1987; 42:443-6.
5. Venuat AM, Marinakis T, Bourhis JM, Bayle C, Pico JL. Fluorescence in situ hybridization with X and Y DNA specific probes for chimerism detection in pulmonary

alveolar macrophages after human sex-mismatched allogeneic bone marrow transplantation. Bone Marrow Transplant 1994; 14:177-8.
6. Roux E, Helg C, Chapius B, Jeannet M, Roosnek E. Evolution of mixed chimerism after allogeneic bone marrow transplantation as determined on granulocytes and mononuclear cells by polymerase chain reaction. Blood 1992; 79:2775-83.
7. Ancoechea J, Gonzalez A, Sanchez MJ, Aspa J, Lopez-Botet M. Expression of lymphocyte activation surface antigens in bronchoalveolar lavage and peripheral blood cells from young healthy subjects. Chest 1993; 104:32-7.
8. Camara R, Peñarrubia MJ, Cardeñoso L, Aspa J, Martinez C, Fernandez- Rañada JM. Prophylaxis in allogeneic BMT: Result of a prospective study [abstract]. Bone Marrow Transplant 1995; 15(Suppl 2):124.
9. Kawasaki ES. Sample preparation from blood, cells, and other fluids. In: INNIS MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: A guide to methods and applications. San Diego, California: Academic Press; 1990. p.146-52.
10. Casado LF, Steegmann JL, Pico M, et al. Study of chimerism in long-term survivors after bone marrow transplantation for severe acquired aplastic anemia. Bone Marrow Transplant 1996; 18:405-9.

A phase-II study with idarubicin, etoposide and prednisone (IVPP), in patients with refractory or early relapsed intermediate or high grade non-Hodgkin's lymphoma

Sir,

Patients with non-Hodgkin's lymphoma (NHL) refractory to primary chemotherapy (CT) or relapse have an unfavorable prognosis. A variety of salvage protocols for such patients are available, and, overall, approximately 60% of all patients with relapsed or refractory disease can achieve complete remission (CR) or partial remission (PR) following reinduction.

Idarubicin is a new anthracycline derivative which when given as monotherapy or in combination with other agents, to either relapsed or refractory patients or previously untreated patients with NHL, has shown a considerable efficacy.[1-5]

The object of this prospective study was to determine whether a moderately intensive regimen comprising idarubicin, etoposide and prednisone (IVPP) is effective in relapsed or refractory patients with intermediate or high grade NHL.

Between March 1994 and September 1996, 18 consecutive patients with intermediate or high grade NHL were diagnosed and treated in our units.

Patients were eligible for the study if they had not reached CR or relapsed after front line treatment for their lymphoma. All patients had been previously treated according to the protocol of a randomized study with either CEOP (cyclophosphamide, epirubicin, vincristine, prednisone) or CNOP (novantrone instead of epirubicin). For various reasons, mainly age related, the patients included were not eligible for megatherapy. After first line treatment the disease remained resistant in 14 (78%) patients. In the other four patients, the disease recurred within 2-10 months after the induced CR has been achieved.

The treatment regimen under study consisted of idarubicin 10 mg/m^2 days 1-3 IV, etoposide 100 mg/m^2 days 1-3 IV, and prednisone 100 mg p.o. days 1-7 (IVPP regimen). A CR was defined as the clinical and X-ray disappearance of all detectable disease for a minimum of two months, without the appearance of any new lesion. A PR was defined as a 50% or greater reduction of the measurable lesions for at least one month. Responding patients (CR or PR) received six courses of treatment. Patients who developed progressive disease after one course or who failed to achieve at least a PR after two, were regarded as treatment failures and taken out of the study. All patients starting therapy were considered evaluable.

The characteristics of the patients participating in this study are summarized in Table 1.

Of the 18 patients, 4 received only one cycle of CT due to disease progression. Eight patients received 2-4 cycles and 6 patients 5-6 cycles. All courses of the IVPP regimen were given as in-patient therapy. Six patients (33%) responded: four achieved CR and two PR. Complete remission lasted 3 months in one patient and 16 months in another. One CR has now lasted 30 months and another 37 months so far. Of the four CR patients one had been resistant to front line treatment and 3 had relapsed early. Of the two patients who exhibited PR one was resistant and the other had relapsed early.

As far as concerns toxicity, all patients developed alopecia and 5 of them oral mucositis. Hematologic toxicity according to the WHO scale was as follows:

Table 1. Characteristics of the 18 patients with resistant or relapsed intermediate or high grade NHL.

Number of patients		18
Age (median-range)		63 (40-72)
Male/female		8/10
Histology		
Large cell		9
Immunoblastic		3
Follicular large cell		3
Mixed small and large cell		1
K1 anaplastic		1
T-peripheral		1
International Prognostic Index of NHL (6)	*At presentation*	*At the beginning of IVPP regimen*
Low	4	3
Low Intermediate	8	4
High Intermediate	4	9
High	2	2
Resistant to primary treatment		14
Early relapse (<12 months)		4