erythropoiesis is increased under stress, especially in heterozygous thalassemia donors.[9] Studying BFU-E may be more useful to detect the erythroid chimerism. In conclusion, mixed chimerism is common after transplantation. It is difficult to predict the outcome of mixed chimerism and graft rejection may be reduced with a more intensive preparative regimen.

Chi Kong Li, Ki Wai Chik, Kam Sze Tsang, Henry Pong, Matthew M.K. Shing, Patrick M.P. Yuen*

*Department of Paediatrics, and *Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, P.R. China*

Key words: thalassemia, BMT, chimerism.

Corresponding author: Dr. Chi Kong Li, MD, Department of Paediatrics, Prince of Wales Hospital, Shatin, Hong Kong, China. Phone: international +852.26321019. Fax: international +852.26497859. E-mail: ckli@cuhk.edu.hk

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Dr. Franco Locatelli, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Locatelli and the Editors. Manuscript received February 15, 2002; accepted May 6, 2002.

References

1. Li CK, Shing MM, Chik KW, Lee V, Leung TF, Cheung AY, et al. Haematopoietic stem cell transplantation for thalassaemia major in Hong Kong: prognostic factors and outcome. Bone Marrow Transplant 2002; 29:101-5.
2. Hill RS, Petersen FB, Storb R, Appelbaum FR, Doney K, Dahlberg S, et al. Mixed hematologic chimerism after allogeneic marrow transplantation for severe aplastic anemia is associated with a higher risk of graft rejection and a lessened incidence of acute graft-versus-host disease. Blood 1986; 67:811-6.
3. Andreani M, Nesci S, Lucarelli G, Tonucci P, Rapa S, Angelucci E, et al. Long-term survival of ex-thalassemic patients with persistent mixed chimerism after bone marrow transplantation. Bone Marrow Transplant 2000; 25: 401-4.
4. Nesci S, Manna M, Andreani M, Fattorini P, Graziosi G, Lucarelli G. Mixed chimerism in thalassemic patients after bone marrow transplantation. Bone Marrow Transplant 1992; 10:143-6.
5. Ortega M, Escudero T, Caballin MR, Olive T, Ortega JJ, Coll MD. Follow-up of chimerism in children with hematological diseases after allogeneic hematopoietic progenitor cell transplants. Bone Marrow Transplant 1999; 24:81-7.
6. Manna M, Nesci S, Andreani M, Tonucci P, Lucarelli G. Influence of the conditioning regimens on the incidence of mixed chimerism in thalassemic transplanted patients. Bone Marrow Transplant 1993; 12:70-3.
7. Pawlowska, AB, Blazar BR, Angelucci E, Baronciani D, Shu XO, Bostrom B. Relationship of plasma pharmacokinetics of high-dose oral Busulphan to the outcome of allogeneic bone marrow transplantation in children with thalassemia. Bone Marrow Transplant 1997; 20:915-20.
8. Andreani M, Manna M, Lucarelli G, Tonucci P, Agostinelli F, Ripalti M, et al. Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. Blood 1996; 87:3494-9.
9. Galanello R, Barella S, Maccioni L, Paglietti E, Melis MA, Rosatelli MC, et al. Erythropoiesis following bone marrow transplantation from donors heterozygous for β-thalassaemia. Br J Haematol 1989; 72:561-6.

Increasing risk of relapse after allogeneic stem cell transplant for adult acute lymphoblastic leukemia in ≥ 2nd complete remission induced by highly intensive chemotherapy

From this retrospective single center analysis adults with acute lymphobalstic leukemia transplanted in ≥2nd CR from an HLA-identical sibling later than 1993 had a worse outcome. As the transplanted-related mortality improved by time, this result was essentially due to the increased relapse rate. The intensity of the pre-transplant salvage chemotherapy was identified as the main factor influencing the post-transplant relapse-risk.

haematologica 2002; 87:782-784
(*http://www.haematologica.ws/2002_07/782.htm*)

We report the outcome of 32 adults with acute lymphoblastic leukemia (ALL) in ≥2nd complete remission (CR) transplanted from an HLA-identical sibling over a consecutive 14-year period, aiming at evaluating whether the long-term results from a single Center have improved over the years.

Seventeen patients were transplanted between 1987 and 1993 (cohort 1) and 15 between 1994 and 2000 (cohort 2). Isolated extramedullary relapse occurring in 4 patients (CNS=2; CNS + testis=1; jaw=1) was considered when determining the remission number (cohort 1=2; cohort 2=2). Patient, donor and graft characteristics are reported in Table 1. Most patients received induction and rescue chemotherapy according to the GIMEMA protocols,[1-5] but the intensity of chemotherapy significantly changed among the patients over the years. If an HLA-identical sibling was available, patients with unfavorable cytogenetics [i.e. translocations t(9;22) or t(4;11)] were given stem cell transplantation (SCT) in 1st CR according to the policy of the GIMEMA Group. Therefore, in our study there was no patient with a diagnosis of ALL Ph^+ or MLL^+. Monitoring of minimal residual disease and its evaluation at transplant was not routinely performed. Nine of 17 patients transplanted before 1994 were prepared with a standard regimen consisting of 12 Gy fractionated total body irradiation (TBI) or 16 mg/Kg b.w. busulphan associated with 120 or 200 mg/kg b.w. cyclophosphamide, while all 15 patients transplanted ≥ 1994 received an alternative regimen consisting of an intensified three-drug combination chemotherapy or high-dose VP-16 associated with TBI. The stem cell source was bone marrow in 29 patients and peripheral blood in 3. As graft-versus-host disease (GVHD) prophylaxis, all patients received cyclosporine, associated with a short course of methotrexate in 14 patients. The close-out date for analyses was August 31, 2001. Baseline patient, donor and graft characteristics were compared using the χ^2 and Fisher's test when appropriate for categorical and the Mann-Whitney test for continuous variables. The end-points were overall survival (OS), leukemia-free survival (LFS), relapse and transplant-related mortality (TRM). Actuarial probabilities were estimated by the method of Kaplan and Meier and comparison of survival curves was performed by the log-rank test. All the variables reported in Table 1 were tested in univariate analysis and those found to be significantly associated with each end-point (p<0.05) entered multivariate analysis using the Cox proportional hazards regression model. A significance level of p=0.05 was used for the multivariate analysis.

Table 2 summarizes the results of the univariate analysis: only variables significantly associated with either of the end-points are reported. The incidence and severity of acute and chronic GVHD were not significantly different between the 1st and 2nd cohort (grade 2-4 acute GVHD: 47% both; chronic GVHD: 46% vs. 54%). In multivariate analysis, the only prognostic factor

Table 1. Patient, donor and graft characteristics.

Characteristics		*All (n=32)*	*1987-1993 (n=17)*	*1994-2000 (n=15)*	*p value*
Patient sex, male/female		18/14	10/7	8/7	0.7
Gender combination, female donor-male recipient/other		6/26	4/13	2/13	0.5
Age (years), median (range)	recipient	25 (17–39)	24 (17–39)	29 (17–36)	0.1
	donor	28 (10–57)	26 (10–42)	28 (17–57)	0.4
WBC at diagnosis ($\times 10^9$/L), median (range)		10 (1–180)	10 (1–59)	17 (2–180)	0.6
Time (days) from diagnosis to 1st CR, median (range)		40 (7–152)	40 (7–85)	37 (28–152)	1
Time (years) from diagnosis to transplant, median (range)		3.7 (0.8–11.4)	4.5 (0.9–11.4)	2.2 (0.8–10.4)	0.06
Duration (years) of 1st CR, median (range)		3 (0.1–9.9)	3.3 (0.4–8.5)	1.6 (0.1–9.9)	0.3
Front-line chemotherapy, standard/intensified		23/9	17/0	6/9	0.001
Rescue chemotherapy, standard/intensified		22/10	16/1	6/9	0.001
Disease status at transplant, 2nd CR/>2nd CR		23/9	9/8	14/1	0.011
TBI, no/yes		14/18	9/8	5/10	0.3
Conditioning regimen, standard/alternative		9/23	9/8	0/15	0.001
Cell dose ($\times 10^6$/Kg), median (range)		3 (1–16)	3 (2–5)	3 (1–16)	0.9
GVHD prophylaxis, CsA/CsA + MTX		18/14	11/6	7/8	0.3

Table 2. Results of univariate analysis.

Variable	*TRM*	*Relapse*	*OS*	*LFS*
Time from diagnosis to SCT (≤ vs. > median: 3.7 yrs)	19%±10 vs. 19%±10 *p*=0.9	61%±13 vs. 24±12 *p*=0.04	38%±12 vs. 68%±12 *p*=0.2	31%±12 vs. 61%±12 *p*=0.2
Duration of 1st CR (≤ vs. > median: 3 yrs)	24%±10 vs. 13%±9 *p*=0.2	60%±14 vs. 25%±12 *p*=0.036	29%±11 vs. 79%±11 *p*=0.07	29%±11 vs. 65%±13 *p*=0.08
Front-line chemotherapy (standard vs. intensified)	26%±9 vs. 0% *p*=0.1	35%±12 vs. 58%±17 *p*=0.027	52%±10 vs. 56%±17 *p*=0.5	48%±10 vs. 42%±17 *p*=0.4
Rescue chemotherapy (standard vs. intensified)	18%±8 vs. 20%±13 *p*=0.8	33%±12 vs. 62%±17 *p*=0.02	64%±10 vs. 30%±14 *p*=0.042	55%±11 vs. 30%±14 *p*=0.05
Year of transplant (≤ vs. > 1993)	24%±10 vs. 14%±10 *p*=0.4	30%±13 vs. 54%±14 *p*=0.049	59%±12 vs. 47%±13 *p*=0.09	53%±12 vs. 39%±13 *p*=0.1

3-year actuarial probability ± standard error.

associated with higher risk of relapse was the use of intensified rescue chemotherapy (relative risk=3.87; 95% CI, 1.14-13.1; *p*=0.029).

A substantial reduction of LFS for patients transplanted after 1993 was observed. As the TRM was 10% less in these patients, the unfavorable results were essentially due to the higher incidence of leukemia relapse. It is unlikely that any selection occurred among patients undergoing SCT in 2nd CR later than 1993 with respect to those transplanted before this date. Indeed, patients characteristics and risk factors were comparable between the 2 groups. Furthermore, no difference was observed in terms of several transplant-related features. Moreover, it is noteworthy that a significantly higher number of patients in the first period were in a more advanced phase (> 2nd CR) of disease at the time of transplant and received a standard rather than a more intensive conditioning regimen. Finally, although the intensity of the induction and rescue chemotherapy has progressively increased, the results of the GIMEMA trials in terms of leukemia relapse and survival have remained substantially unchanged over the years. As suggested by *in vitro* and *in vivo* studies,[6-8] the higher risk of post-transplant relapse for patients grafted after 1993 might be a consequence of multi-drug resistance (MDR) developing in leukemic cells following their continuous exposure to highly intensive chemotherapy. Our results suggest that adult patients with ALL given highly intensive chemotherapy and transplanted in ≥ 2nd CR might prove to be more resistant to myeloablative conditioning regimens and graft-versus-leukemia effect. This observation, if confirmed on a large series of patients entered in the GIMEMA trials, should be considered in the design of any future GIMEMA study. Finally, pre-transplant chemotherapy should be taken in account in the outcome analysis of adult ALL transplanted in 2nd CR.

Andrea Mengarelli, Anna Paola Iori, Raffaella Cerretti, Lorenza Cerilli, Atelda Romano, William Arcese

Dipartimento di Biotecnologie Cellulari ed Ematologia, Università degli Studi "La Sapienza", Rome, Italy

Correspondence: William Arcese, MD, Cattedra di Ematologia, via Benevento 6, 00161 Rome, Italy. Phone: international +39.06.857951. Fax: international +39.06.44241984. E-mail: arcese@bce.uniroma1.it

Key words: adult acute lymphoblastic leukemia, leukemia relapse, allogeneic stem cell transplantation, multi-drug resistance.

Funding: this work was supported in part by grants from ROMAIL (Associazione Italiana contro le Leucemie-Roma) and AIRC (Associazione Italiana Ricerca sul Cancro).

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Dr. Alvaro Urbano-Ispizua, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Ispizua and the Editors. Manuscript received April 15, 2002; accepted June 3, 2002.

References

1. Mandelli F, Annino L, Rotoli B. The GIMEMA ALL 0183 trial: analysis of 10-year follow-up. GIMEMA Cooperative Group, Italy. Br J Haematol 1996; 92:665-72.
2. Annino L, Vegna ML, Camera A, Specchia G, Visani G, Fioritoni G, et al. Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow-up of the GIMEMA ALL 0288 randomized study. Blood 2002; 99:863-71.
3. Mandelli F, Annino L, Vegna ML, Specchia G, Camera A, Fioritoni G, et al. Adult acute lymphoblastic leukemia: results of the GIMEMA ALL0394 study. 38th Congress of the Italian Society of Hematology Firenze; 2001; 16[abstract].
4. Mandelli F, Annino L, Vegna ML. Interim analysis of the GIMEMA ALL 0496 trial for adult acute lymphoblastic leukemia (ALL). Paper presented at the 6th Annual Meeting of the European Haematology Association, Frankfurt, 2001:692[abstract].
5. Rotoli B, Camera A, Fazi P, Annino L, Rossi M, Cascavilla N, et al. High-dose idarubicin and cytarabine as salvage treatment for resistant or relapsed adult acute lymphoblastic leukemia: the ALL RESCUE-97 GIMEMA Study. Paper presented at the 38th Congress of the Italian Society of Hematology Firenze; 2001:15[abstract].
6. Hu XF, Slater A, Wall DM, Kantharidis P, Parkin JD, Cowman A, et al. Rapid up-regulation of mdr1 expression by anthracyclines in a classical multidrug-resistant cell line. Br J Cancer 1995; 71:931-6.
7. Klumper E, Pieters R, Veerman AJ, Huismans DR, Loonen AH, Hahlen K, et al. In vitro cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukemia. Blood 1995; 86:3861-8.
8. Den Boer ML, Pieters R, Kazemier KM, Janka-Schaub GE, Henze G, Veerman AJ. Relationship between the intracellular daunorubicin concentration, expression of major vault protein/lung resistance protein and resistance to anthracyclines in childhood acute lymphoblastic leukemia. Leukemia 1999; 13:2023-30.

Editorial note. The following papers,[9-15] previously published by Haematologica, have examined the clinical outcome of adult acute lymphoblastic leukemia.

9. Munoz L, Lopez O, Martino R, Brunet S, Bellido M, Rubiol E, Sierra J, Nomdedeu JF. Combined use of reverse transcriptase polymerase chain reaction and flow cytometry to study minimal residual disease in Philadelphia positive acute lymphoblastic leukemia. Haematologica 2000; 85:704-10.
10. Granados E, de La Camara R, Madero L, Diaz MA, Martin-Regueira P, Steegmann JL, et al. Hematopoietic cell transplantation in acute lymphoblastic leukemia: better long term event-free survival with conditioning regimens containing total body irradiation. Haematologica 2000; 85: 1060-7.
11. Spinelli O, Giussani U, Borleri G, Lazzari M, Michelato A, Dotti G, et al. Need for an accurate molecular diagnosis to assess the donor origin of leukemia relapse after allogeneic stem cell transplantation. Haematologica 2000; 85: 1153-7.
12. Pagano L, Mele L, Casorelli I, Fianchi L, Di Febo A, Leone G. Acute lymphoblastic leukemia in the elderly. A twelve-year retrospective, single center study. Haematologica 2000; 85:1327-9.
13. Krampera M, Perbellini O, Maggioni A, Scognamiglio F, Todeschini G, Pizzolo G. Flow-cytometric detection of minimal residual disease in adult acute lymphoblastic leukemia. Haematologica 2001; 86:322-3.
14. Martin Ramos ML, Lahuerta Palacios JJ, Martinez Lopez J, Gomez Rodriguez MJ, Moreno Izquierdo A, Barreiro Miranda E. Karyotype and prognosis in adult Spanish acute lymphoblastic leukemia. Haematologica 2001; 86:438-9.
15. Mengarelli A, Iori A, Guglielmi C, Romano A, Cerretti R, Torromeo C, et al. Standard versus alternative myeloablative conditioning regimens in allogeneic hematopoietic stem cell transplantation for high-risk acute leukemia. Haematologica 2002; 87:52-8.