A significance of additional chromosomal aberrations and other variables on post transplantation outcome of patients with CML*

V. VRANOVÁ[1], S. KATINA[2], G. KIRSCHNEROVÁ[1], M. MISTRÍK[3], J. LAKOTA[4], J. HORÁKOVÁ[5], A. TÓTHOVÁ[1]

[1]Department of Genetics, e-mail: vlavra@post.sk, National Cancer Institute, 833 01 Bratislava, Slovak Republic; [2]Department of Probability and Mathematical Statistics, Faculty of Mathematics, Physics and CS, Comenius University, Bratislava, [3]Department of Hematology and Transplantation, University Hospital of st. Cyril and Method, Bratislava, [4]BMT Unit, National Cancer Institute, Bratislava, and [5]BMT Unit, Children's Hospital, Bratislava, Slovak Republic

Received February 2, 2005

Chronic myeloic leukemia (CML) is a malignant disease of hematopoietic stem cell characterized by the *bcr/abl* gene rearrangement. Allogeneic transplantation of stem cells (SCT) is a routinely used treatment method of patients with this diagnosis and remains the only curative mode of treatment.

From January 1990 to December 2002 78 patients with CML underwent allogeneic transplantation and were examined at the Department of Genetics in the National Cancer Institute in Bratislava. Using conventional cytogenetic and FISH 6 patients (7.7%) showed additional chromosomal changes before SCT. These patients had statistically worse post transplantation prognosis compared to the patients without additional changes before SCT (mean survival in month ±standard error (58.08 (±6.70) vs. 5.17 (±0.98), p-value = 0.001), patient mortality (67% vs. 31%)). In addition five other variables were evaluated for transplant outcome, namely, patient's age at the time of transplantation, sibling or non-sibling donor, higher than 1st chronic phase of CML, time from diagnosis to transplantation and sex of donor and recipient. Only the comparison of HLA-identical sibling transplantation to unrelated donor transplantation was statistically significant (mean survival in month – 56.6 (±7.2) vs. 13 (±0.0), patient mortality 31% vs. 67%).

Key words: chronic myeloic leukemia (CML), allogeneic stem cell transplantation (SCT), additional chromosomal aberrations, post transplantation outcome, survival analysis

Chronic myeloid leukemia (CML) is a myeloproliferative disorder resulting from clonal expansion of the progeny of a malignant hematopoietic stem cell [37]. It comprises about 15–20% of all leukemias and affects 1–2 people in every 100,000 population per year [5, 10, 37]. In approximately 95% cases it is connected with a Philadelphia (Ph) chromosome [27], which originates from the reciprocal translocation of the *bcr* gene on 22q11 and the *abl* gene on 9q34 [32]. The molecular consequence is BCR/ABL fusion protein, which is a constitutively active cytoplasmic tyrosine kinase [6, 15, 16]. The structure of this protein allows multiple protein-protein interactions and suggests the involvement of diverse intracellular pathways [10, 37].

Three to five years after onset, CML progresses from relative benign chronic phase (CP) to accelerated (AP) and blast phases (BP) [10, 29, 34]. During the progression a range of additional chromosomal changes occurs. These additional abnormalities are very important in disease progression [3, 35] and can be taken as a prognostic indicator – because of their occurrence before hematological and clinical symptoms [4, 15]. About 71% of patients in BP have at least one of the following anomalies: +8, i(17q), +19, +9 or extra Ph-chromosome [5, 15]. About 15% of patients have –7, –17, +17, +21, –Y or t(3;21)(q26;q22) [4, 15]. These cytogenetic changes correlate with changes on the molecular level, mainly activation of oncogenes and deactivation of tumor-suppressor genes.

Beside many therapies including conventional chemotherapy with hydroxyurea or busulfan, interferon alpha (INFα), STI 571, allogeneic stem cell transplantation (SCT) is routinely used as therapeutical method for patients with CML diagnosis [9, 19, 40, 41]. Most allogeneic transplantations are performed from HLA-identical related donors (MSD – match

*This paper was partially supported by grants from the Slovak Grant Agency (VEGA, projects 1/7154/20 and 1/0272/03).

sibling donor), HLA-matched unrelated donors (MUD – match unrelated donor) from transplantation-registries are also accomplished [25, 30]. Allogeneic transplantation is connected with many post transplantation complications (GvHD – Graft vs. Host Disease, rejection of transplantate, complications associated with immunosuppression), and it's successfull performance depends on many risk variables (e.g. patient's age in the time of transplantation, phase of CML, time from diagnosis to transplantation) [13, 33], but it is the only means of curing CML [2, 8, 11, 12, 33, 36].

In our study we analysed data of 78 CML patients that underwent allogeneic transplantation examined at Department of Genetic of National Cancer Institute in Bratislava in 1990–2002 and determined negative influence of additional chromosomal changes presented before transplantation to their post transplantation outcome. The transplantation from sibling donor compare to unrelated donor was registered as a good prognostic assumption. The age of patient in the time of transplantation, phase of CML, time from diagnosis to transplantation and sex of donor and recipient has no statistically significant value on post transplantation outcome. So we confirmed the conclusions of many similar studies in our relative big and long time frame watched set of patients.

Patients and methods

Patient characteristics. Between January 1990 and December 2002, 589 patients with diagnosis CML were examined at Department of Genetic of National Cancer Institute in Bratislava. 78 patients were chosen from this group (13.2%) (75 adults and 3 children) that underwent allogeneic transplantation of hematopoietic stem cell and whose peripheral blood (PB) and/or bone marrow (BM) were examined prior to and after the transplantation. The transplantations were performed at Hematology and Transplantation Clinic in Bratislava, at National Cancer Institute in Bratislava and at 2nd Children's Clinic, Bone Marrow Transplantation unit in Bratislava.

Methods. Patients were examined at least by one of three genetic methods – classical cytogenetics, FISH and RT-PCR. RT-PCR was mainly used to demonstrate t(9;22)(q34;q11.2). The intervention by RT-PCR and FISH was limited, because of the later introduction of these methods (in 1995 and 1997) to our laboratory. Data were collected and evaluated.

Classical cytogenetics. The samples for classical cytogenetics were cultivated 24 or 72 hours in a standard manner. Chromosome preparations were made using standard G-banding technique. A minimum of 20–30 metaphases (depending on the sample quality) were analyzed and the karyotype was described according to ISCN [17]. The chromosome aberration was considered as clonally, if there were found two and more cells with the same rearrangement or three and more cells with the same aneuploidity.

Fluorescence in situ hybridization (FISH) analysis. In fluorescence *in situ* hybridization study were used probes directly labeled with SpectrumOrange™ respectively SpectrumGreen™ fluorochromes. LSI bcr/abl dual color ES probe was used to identify t(9;22)(q34;q11.2), SO LSI p53 probe to identify rearrangements of chromosome 17 and CEP® 8 SpectrumOrange™ probe to identify numerical changes of chromosome 8. The probes were provided by VYSIS, Inc. Blue DAPI was used as a counterstaining. The FISH procedure was performed according to manufacturer's instructions.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from PB and/or BM samples by the TRIzol Reagent (Gibco BRL, USA). PCR amplification was performed with two sets of primers. The first – external – primer set included primers
5'GAAGTGTTTCAAGCTTCTCC 3' and
5'GTTTGGGCTTCACACCATTCC 3'.
The second – internal primer step included primers:
5'CAGATGCTGACCAACTCGTGT3' and
5'TTCCCCATTGTGATTATAGCCTA3' [7].
The PCR product was analyzed on 2% agarose gel.

Survival analysis. Survival analysis was performed considering patients' karyotype determined prior to transplantation. The influence of another variables on post transplantation outcome was also evaluated: the age of patient in the time of transplantation, phase of CML, sex of donor and recipient, time from diagnosis to transplantation, transplantation from HLA-identical sibling or unrelated donor.

We evaluated the mean survival (months), defined as a survival from performance of SCT to the end of study or to the patient's death; and the standard error, range and median of survival. Patients' mortality is defined as a share of death patients to all patients in the group in the time of study (%). The data were statistically evaluated by log-rank test to compare survival curves (Mantel-Haenszel test, α=0.05) [22].

Results

Patients. Total amount of 589 patients with CML diagnosis examined at Department of Genetic of National Cancer Institute in Bratislava comprised 332 men and 257 women (ratio 1.29 for men).

From these were chosen 78 patients that underwent allogeneic stem cell transplantation (SCT). The mean survival was 93.00 (±7.20) months (range: 1–138 months) and probability of death 26/78. Mortality of patients reached 33.3% (26/78). The main cause of death were posttransplantation complications. Characteristics of patients are provided in Table 1.

Determination of karyotype of each patient. Using classical cytogenetics, the karyotype of each patient was determined and patients were divided into two main groups: 1. patients without additional chromosomal aberrations, 2. patients with additional chromosomal aberrations.

FISH and RT-PCR were used as additional methods to classical cytogenetics.

Table 1. Patients' characteristics

Number		78
Age (yrs)		
	Average	36
	Range	(8 – 59)
Sex of donor and recipient		
	M / M	27
	F / F	14
	F / M	14
	M / F	23
Phase of CML		
	1st CP	67
	AP	3
	BP	5
	2nd CP	3
Time from diagnosis to transplantation		
	< 1 yr.	49
	> 1 yr.	29
Type of transplantation		
	MSD	72
	MUD	6
Source of stem cells		
	BM	68
	PBCS	10
Survival in time of study		
	Alive	52
	Dead	26

M – male, F – female, yr – year, CP – chronic phase, AP – accelerated phase, BP – blast phase, BM – bone marrow, PBSC – peripheral blood stem cells.

Before allogeneic transplantation, PB and/or BM samples of 78 patients were examined. In two cases we were not able to perform examination. These two patients were excluded from study of karyotype determination. The karyotypes of all examinated patients are provided in Table 2.

Survival analysis considering the additional chromosomal aberrations. The brief characteristics of post transplantation outcome and statistic evaluation of two groups of allogeneic transplanted patients is shown in Table 3 and survival curves in Figure 1.

Survival analysis with regard to another variables influencing post transplantation outcome. The influence of five variables – the age of patient in the time of transplantation, phase of CML, sex of donor and recipient, time from diagnosis to transplantation, transplantation from HLA-identical sibling or unrelated donor – established by GRATWOHL et al [13] – was investigated in all 78 patients treated by allogeneic transplantation.

The brief characteristics of post transplantation outcome and statistic evaluation in each group of five variables is provided in Table 3 and survival curves in Figures 2–5.

Discussion

Chronic myeloic leukemia is the best-characterized leukemia at a molecular level. The studies in recent years have helped to define further the molecular events involved in its initiation and progression and relating to clinical manifestations, the course of the disease, and therapeutic interventions.

In our study, six patients (7.7%) showed additional chromosomal changes in karyotype. We documented their negative influence to the post transplant outcome together with negative influence of unrelated donor compared to sibling donor.

Additional chromosomal changes were detected only with simple t(9,22)(q34;q11). Half of all detected additional changes belonged to the group of common additional chromosomal aberrations [5, 15, 18]. Although chromosome 19 was changed in one case, we did not detect any +19, which is described as one of the frequent changes. Similar, additional chromosomal aberrations belonging to the minor group changes associated with CML progression [4, 15] were not detected. On the other hand, we detected +6 in two cases (Tab. 2). This aberration was not mentioned in any available literature as the main aberration associated with CML.

Post transplantation prognosis was better for the patients without additional changes compared to patients

Table 2. The karyotypes of the patients determined before allogeneic transplantation

Group	Number of patients	Description	Karyotype
1. without additional aberrations	63	Simple translocation	46,XY/XX,t(9;22)(q34;q11.2)
	2	Translocation bcr/abl determined only at molecular level	46,XY,t(9;22)(q34;q11.2)
	5	Variant form of translocation	46,XY/XX,t(9;V;22)
2. with additional aberrations	3	Common additional changes	46,XX,t(9;22)(q34;q11.2), i(17q) 46,XY,t(9;22)(q34;q11.2), i(17q) 47,XY, t(9;22)(q34;q11.2),+Ph
	3	Other additional changes	46,XY,t(9;22)(q34;q11.2)/ 46,XY,t(6;17)(q21;q22)
			46,XY,t(9;22)(q34;q11.2)/ 46,XY,t(9;22)(q34;q11.2),del (10p)
			46,XX,t(9;21)(q34;q11.2)/ 47,XY,t(9;22)(q34;q11.2) +6

Table 3. Survival analysis of allogeneic transplanted CML patient with regard to additional changes and other five variables

Variables	Range	Survival (months)		Probability of Death	Patient's Mortality	Test-Statistics
		Mean ± Standard error	Median			
Additional Changes						
Without Additional Changes	70	58,08 ± 6,70	38,00	49/72	31,4 % (22/70)	$\chi^2 = 13$, df = 1
With Additional Changes	6	5,17 ± 0,98	5,50	6/6	66,7 % (4/6)	p-value = 0,001***
Patient´s Age						
<20 yrs	6	28,2 ± 7,89	29,50	6/6	17 % (1/6)	$\chi^2 = 1{,}50$, df = 2
> 20 < 40	47	60,0 ± 9,0	38,00	28/43	34 % (16/47)	p-value = 0,466 ns
> 40 yrs	25	57,5 ± 10,56	39,00	15/23	36 % (9/25)	
Phase of CML						
1st CP	67	60,4 ± 7,13	39,00	43/65	27 % (20/67)	$\chi^2 = 1{,}20$, df = 1
Higher phase	11	33,9 ± 15,02	8,00	6/7	55 % (6/11)	p-value = 0,277 ns
Time from Diagnosis. to SCT						
< 1 yr	49	62,8 ± 8,18	45,00	31/47	22 % (11/49)	$\chi^2 = 1{,}30$, df = 1
> 1 yr	29	43,9 ± 9,81	13,00	18/25	52 % (15/29)	p-value = 0,256 ns
Sex of Recipient and Donor						
M/M	27	56,2 ± 10,12	42,50	17/26	34 % (10/27)	
M/F	23	26,3 ± 7,11	8,50	17/20	52 % (12/23)	$\chi^2 = 7{,}30$, df = 3
F/M	14	67,9 ± 15,98	50,00	8/13	21 % (3/14)	p-value = 0,062 ns
F/F	14	67,7 ± 11,11	51,00	7/13	7 % (1/14)	
Type of SCT						
MSD	72	56,6 ± 7,2	50,00	10/16	31 % (22/72)	$\chi^2 = 7{,}50$, df = 1
MUD	6	13,0 ± 0,0	13,00	1/1	67 % (4/6)	p-value = 0,006***

yrs – years, CP – chronic phase, M – male, F – female.

with these changes (p-value = 0.001) (Tab. 3). Our results support a theory of the prognostic significance of specific cytogenetic aberrations for both types of transplantation and also for any single diagnosis [38].

We did not detect any statistical difference between subgroups (simple translocation, translocation at molecular level and variant translocation) in the group of patients without additional changes (data not shown). This supports the contention, that patients with variant form of t(9;22)(q34;q11.2) [18, 36], and patients with translocation detected only at molecular level [26, 36] have not worse post transplantation prognosis compared to patients with simple t(9;22)(q34;q11.2).

We also did not detect statistically significant difference in post transplantation outcome among patients with common additional changes and other changes (data not shown), although by KONSTANTINIDOU et al [21] have patients with other than common additional changes worse prognosis.

The result of allogeneic CML transplantations depends on many other variables [13]. One of them is the age of patient in the time of transplantation. Some authors [13, 23, 36, 41] state, that allogeneic transplantation should be performed before the 40th year of age, the risk of failure increases with higher age. We did not register statistically significant difference (p-value = 0.466 ns) (Tab. 3) between patients underwent transplantation before 20th year of age, between 20th and 40th and after 40th year of age.

The second risk variable is the phase of CML. The recipient should be in the CF [13, 23], with advanced AF or BF successful performance of transplantation drops. We detected higher mortality of patients that underwent transplantation in higher than CF, but statistical comparison of these two groups was not significant (p-value = 0.272 ns) (Tab. 3). However, we want to bring your attention to the very different rate of these groups.

The questionable risk variable that influences post transplantation outcome is the time interval from diagnosis to the transplantation. Some authors [1] consider the risk time 6 months after diagnosis, others [20] 3 years and more. GRATWOHL et al [13] in 1998 stated as the risk time for transplantation performance more than 1 year after diagnosis and we also chose this time to compare post transplantation outcome. Although, we detected higher mortality in patients transplanted after 1 year from diagnosis, considering the statistical comparision (p-value = 0.256 ns) (Tab. 3) we can say, that allogeneic transplantation performed more than 1 year after diagnosis has no negative influence on post transplantation outcome.

GRATWOHL et al [13] also considered as a risk group the men transplanted by women donors. The probable reason is minor alloantigens, which also affect the growth of a marrow transplant in a nonsyngeneic host and its tolerance of the transplant recipient. The human Y chromosome encodes a minor alloantigen, termed H-Y, which is expressed on the human male hematopoietic cells and can be targeted by cytotoxic T cells. The decreased risk of rejection and increased likelihood of GvHD associated with marrow allo-

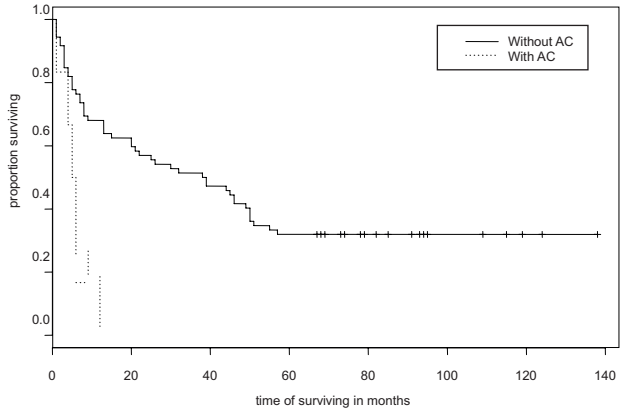

Figure 1. Survival curves of allogeneic transplanted CML patients – comparison of patients without additional changes (without AC) to patients with additional changes (with AC).

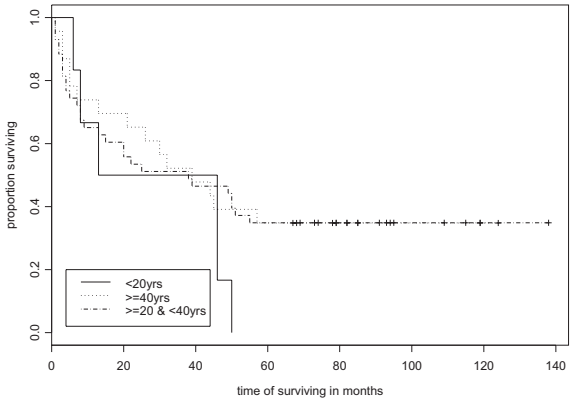

Figure 2. Survival curves of allogeneic transplanted CML patients – comparison with regard to the age of patient at the time of transplantation.

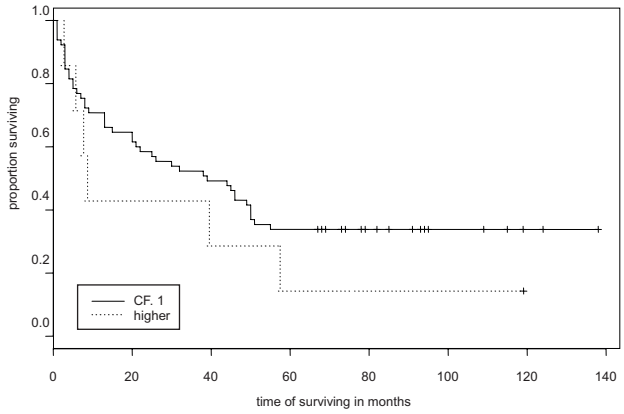

Figure 3. Survival curves of allogeneic transplanted CML patients – comparison with regard to the phase of CML.

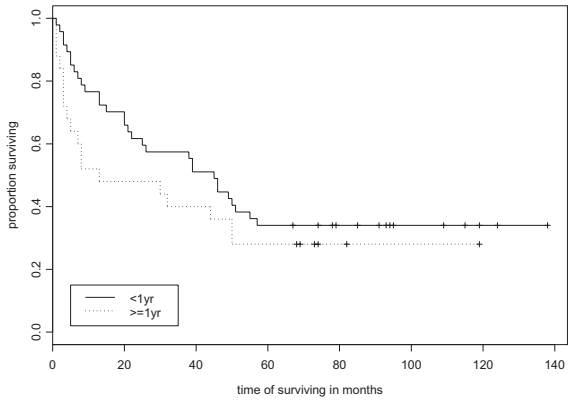

Figure 4. Survival curves of allogeneic transplanted CML patients – comparison with regard to the time from diagnosis to transplantation.

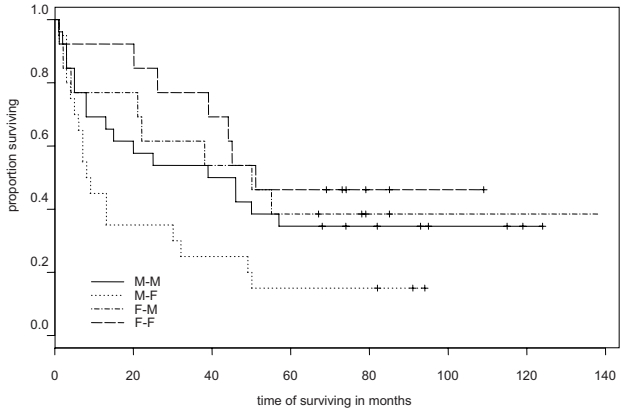

Figure 5. Survival curves of allogeneic transplanted CML patients – comparison with regard to the sex of recipient and donor (M – male, F – female).

grafts from female donors administered to male recipients may reflect *in vivo* responses to this minor alloantigen [31]. In our study, mortality of male recipients transplanted by female donors reached 52%. Relaps of CML was deathly only in one case. However, statistical comparison did not detect any significant difference (p-value = 0.062 ns) (Tab. 3). We can note, that sex of donor and recipient has no significant influence on post transplantation outcome in our study.

Very important risk variable is a relationship between donor and recipient of hematopoietic stem cells [13, 23]. Only 30–40% candidates to allogeneic transplantation have suitable sibling donor [30]. Another possibility is a performance of HLA-identical unrelated transplantation. The EBMT (The European Group for Blood and Marrow Transplantation) documented the 19% rate of unrelated allogeneic transplantation in 1998. The low rate of the unrelated transplantation in our sets of patients (8%) can be caused by expensiveness of

thus intervention, search for suitable donor in international registers and more detailed and pretentious HLA-typing [25].

CML is the most common diagnosis in case of unrelated allogeneic transplantation and comprise 35% of all performed unrelated allogeneic transplantations [24]. Depending on our results of statistical analysis of patients underwent transplantation from sibling vs. unrelated donor (p-value=0.006) (Tab. 3) we can decide, together with other authors [2, 14, 39, 42], that patients with suitable sibling donor (MSD) have better post transplantation prognosis compared to patients transplanted from unrelated donors (MUD). The reason is higher risk of post transplantation complications by unrelated transplantation, which can cause death of patient [28].

Allogeneic transplantation is connected with many post transplantation complications. It's successful performance depends not only on the presence of additional changes, but also on many risk variables. However, together with many other authors [2, 8, 11, 12, 33, 36] we can mention that it is the only means of curing CML.

We would like to thank to MUDr. B. CZAKO and MUDR. M. HRUBIŠKO, CSc. from Clinic of Hematology and Transfusiology in Bratislava for their readiness with problems solving and also to all co-workers from department of genetics of National Cancer Institute namely RNDr. K. MARKOVÁ, MGR. M. ŠUJANOVÁ, MGR. A. ŽÁKOVIČOVÁ and MUDr. D. ILENČÍKOVÁ, PhD.

References

[1] APPELBAUM FR, CLIFT R, RADICH J, ANASETTI C, BUCKNER CD. Bone marrow transplantation for chronic myelogenous leukemia. Semin Oncol 1995; 22: 405–411.

[2] CHAKRAVETY R, PEGGS K, CHOPRA R, MILLIGAN DW, KOTTARIDIS PD et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. Blood 2002; 99: 1071–1078.

[3] CORTES JE, TALPAZ M, GILES F, O'BRIEN S, RIOS MB et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imanitib mesylate therapy. Blood 2003; 101: 3794–3800.

[4] DEUTSCH E, DUGRAY A, ABDULKARIM B, MARANGONI E, MAGGIORELLA L et al. BCR-ABL downregulates the DNA repair protein DNA-PKcs. Blood 2000; 97: 2084–2090.

[5] DEWALD GW, JUNEAU AL, SCHAD CR, TEFFERI A. Cytogenetic and molecular genetic methods for diagnosis and treatment response in chronic granulocytic leukemia. Cancer Genet Cytogenet 1997; 94: 59–66.

[6] DIERLAM J, MICHAUX L, STEFANOVA M, EGGERT J, LEBERECHT P et al. Novel Philadelphia variant t(Y;9;22)(q12;q34;q11) in a case of chronic myeloid leukemia. Cancer Genet Cytogenet 1999; 114: 150–153.

[7] VAN DONGEN JJ, MACINTYRE EA, GABERT JA, DELABESSE E, ROSSI V et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 1999; 13: 1901–1928.

[8] DOUBEK M, ADAM Z, editors. Diagnostic and therapeutic approaches in malignant diseases (in Czech). Prag: GRADA Publishing, 2002: 365–400.

[9] DRUKER BJ, SAWYERS CL, KANTARJIAN H. Activity of specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with Philadelphia chromosome. N Engl J Med 2001; 344: 1038–1042.

[10] FADERL S, TALPAZ M, ESTROV Z, O'BRIEN S, KURZROCK R et al. The Biology of Chronic Myeloid Leukemia. N Engl J Med 1999; 341: 164–172.

[11] GALE RP, HEHLMANN R, ZHANG MJ, HASFORD J, GOLDMAN JM et al. Survival with bone marrow transplantation versus hydroxyurea or interferon for chronic myelogenous leukemia. Blood 1998; 91: 1810–1819.

[12] GOLDMAN JM, DRUKER BJ. Chronic myeloid leukemia: current treatment options. Blood 2001; 98: 2039–2042.

[13] GRATWOHL A, HERMANS J, GOLDMAN JM, ARCESE W, CARRERAS E et al. Risk assesment for patients with chronic myeloid leukemia before alogeneic blood or marrow transplantation. Lancet 1998; 352: 1087–1091.

[14] HANSEN JA, GOOLEY TA, MARTIN PJ, APPELBAUM F, CHAUNCEY TR et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. N Engl J Med 1998; 338: 962–968.

[15] HEIM S, MITELMAN F, editors. Cancer Cytogenetics, 2nd edition. USA: Wiley-Liss, 1995: 33–59.

[16] HUNTLEY BJP, REID AG, BENCH AJ, CAMPBELL LJ, TELFORD N et al. Deletions of the derivate chromosome 9 occur at the time of the Philadelphia translocations and provide and independent prognostic indicator in chronic myeloid leukemia. Blood 2001; 98: 1732–1738.

[17] MITELMAN F, editor. ISCN. An international system for human cytogenetic nomenclature. Basel: Karger S, 1995.

[18] JOHANSSON B, FIORETOS T, MITELMAN F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol 2002; 107: 76–94.

[19] JOHN AM, THOMAS NS, MUFTI GJ, PADUA RA. Targeted therapies in myeloid leukemia. Semin Cancer Biol 2004; 14: 41–62.

[20] KANTARJIAN HM, O'BRIEN S, ANDERLINI P, TALPAZ M. Treatment of chronic myelogenous leukemia: current status and investigational options. Blood 1996; 87: 3069–3081.

[21] KONSTANTINIDOU P, SZYDLO RM, CHASE A, GOLDMAN JM. Cytogenetic status pre-transplant as a predictor of outcome post bone marrow transplantation for chronic myelologenous leukemia. Bone Marrow Transplant 2000; 25: 143–146.

[22] LE CT, editor. Applied survival analysis. New York: John Wiley & Sons, 1997.

[23] MADRIGAL JA, SCOTT I, ARGUELLO R, SZYDLO R, LITTLE AM et al. Factors influencing the outcome of bone marrow transplants using unrelated donors. In: Apperley J, Gluckman E, Gratwohl A, editors. The EBMT Handbook, Blood and Marrow Transplantation. Paris: Quatre Bis, 1998: 35–56.

[24] MCGLAVE PB, SHU XO, WEN W et al. Unrelated donor marrow transplantation for chronic myelogenous leukemia: nine

years' experience of the National Marrow Donor Program. Blood 2000; 95: 2219–2225.

[25] MISTRÍK M. Stem cell transplantation. Diagnóza 2002; 1/2002: 21–25.

[26] NACHEVA E, HOLLOWAY T, BROWN K, BLOXHAM D, GREEN AR. Philadelphia-negative chronic myeloid leukemia detection by FISH od BCR-ABL fusion gene localized either to chromosome 9 or 22. Br J Hematol 1994; 87: 409–412.

[27] NOWELL P, HUNGERFORD D. A minute chromosome in chronic granulocytic leukemia. Science 1960; 132: 1497–1500.

[28] OCHS L, SHU XO, MILLER J, ENRIGHT H, WAGNER J et al. Late infections after allogeneic bone marrow transplantations: comparison of incidence in related and unrelated donor transplant recipients. Blood 1995; 86: 3979–3986.

[29] OREN H, DZOVALI O, YKSEL E, SAKIZLI M, IRKEN G. Development of acute promyelotic leukemia with isochromosome 17q after BCR/ABL positive chronic myeloid leukemia. Cancer Genet Cytogenet 1999; 109: 141–143.

[30] PETERS WP, HAMM C, BAYNES RD. Autologous bone marrow and stem cell transplantation. In: Kufe DW, Pollock RE, Weichselbaum RR et al, editors. Cancer Medicine. Hamilton (Canada): BC DeckerInc, 2002.

[31] REILLY RJ, PAPADOPOULOS EB, SMALL TN. Transplantation of Allogeneic Hematopoetic Cells for the Treatment of Malignacies. In: Kufe DW, Pollock RE, Weichselbaum RR et al, editors. Cancer Medicine. Hamilton (Canada): BC DeckerInc, 2002.

[32] ROWLEY JD. A new consistent chromosomal abnormality in chronic myelogenous lekaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243: 290–293.

[33] SAVAGE DG, GOLDMAN JM. Allografting for chronic myeloid leukemia. Current Opinion in Hematology 1997; 4: 369–376.

[34] SAWYERS CL. Chronic myeloid leukemia. N Engl J Medicine 1999; 340: 1330–1340.

[35] SHET AS, JAHAGIRDAR BN, VERFAILLIE CM. Chronic myelogenous leukemia: mechanisms underlying disease progression. Leukemia 2002; 16: 1402–1411.

[36] SILVER RT. Chronic myeloid leukemia; Neoplasms of the Hematopoietic System. In: Kufe DW, Pollock RE, Weichselbaum RR et al, editors. Cancer Medicine. Hamilton (Canada): BC DeckerInc, 2002.

[37] SILVER RT. Chronic myeloid leukemia. Hemat Oncol Clin North Am 2003; 17: 1159–1173.

[38] SLOVAK ML, KOPECKY KJ, WOLMAN SR, HENSLEE-DOWNEY JP, APPELBAUM FR et al. Cytogenetic correlation with disease status and treatment outcome in advanced stage leukemia post bone marrow transplantation: a Southwest Oncology Group study (SWOG-8612). Leuk Res 1995; 19: 381–388.

[39] SZYDLO R, GOLDMAN JM, KLEIN JP, GALE RP, ASH RC et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. J Clin Oncol 1997; 15: 1767–1777.

[40] TALPAZ M, SILVER RT, DRUKER B et al. A Phase II study of STI571 in adult patients with Philadelphia chromosome positive chronic myeloid leukemia in accelerated phase (Abstract). Blood 2000; 96: 469.

[41] WANG YL, BAGG A, WARREN P, NOWELL PC, HESS JL. Chronic myelogenous leukemia: Laboratory diagnosis and monitoring. Genes Chromosomes Cancer 2002; 32: 97–11.

[42] WEISDORF DJ, ANASETTI C, ANTIN JH, KERNAN NA, KOLLMAN C et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. Blood 2002; 99: 1971–1977.