Artigo / Article

Autografting of peripheral-blood progenitor cells early in chronic myeloid Leukemia

Transplante autólogo de células progenitoras em fase crônica precoce da Leucemia mielóide crônica

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The role of peripheral-blood progenitor cell (PBPC) transplantation as a treatment for chronic myeloid leukemia (CML) patients remains uncertain. We presented herein 11 CML patients treated with autografting of PBPC in early chronic phase followed by interferon-alpha (IFN-α). Bone marrow samples obtained at diagnosis and during follow-up after autografting as well as leukapheresis products were analyzed by cytogenetics, fluorescence in situ *hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR). The median follow-up of patients after autografting was 22 months (range: 1-49). Two treatment-related deaths occurred in patients enrolled in the study. Eight out of 9 (88.9%) and 7 out of 9 (77.8%) patients achieved hematologic and cytogenetic responses, respectively. Molecular cytogenetic and molecular responses were seen in all 7 patients analyzed (100.0%) and in one single patient (11.1%), respectively. The median percentages of Ph^+ (78.0%) metaphases obtained after 6 months of autografting was lower than those obtained at diagnosis (100.0%,* P*=0.04). The median percentages of $FISH^+$ nuclei obtained at 3 (4.0%), 6 (7.3%) and 9 (14.7%) months after autografting were also lower than that obtained at diagnosis (82.5%;* P*=0.002;* P*=0.003;* P*=0.030, respectively). At the end of the study, 9 patients (81.8%) were alive in chronic phase, 4 of them presenting hematologic, cytogenetic and molecular cytogenetic responses. We conclude that autografting performed with PBPC in early chronic phase of CML followed by IFN-α results in lower numbers of Ph^+ and $FISH^+$ cells in bone marrow. Rev. bras. hematol. hemoter. 2004;**26**(4):256-262.*

Key words: *Chronic myeloid leukemia; autografting; interferon-alpha; cytogenetic; FISH; RT-PCR*

Introduction

Chronic myeloid leukemia (CML) is a progenitor cell disorder of bone marrow (BM) which progresses from an indolent chronic phase to a refractory acute leukemia. Its cytogenetic hallmark is a reciprocal t(9;22)(q34;q11) chromosomal translocation that creates a derivative $9q^+$ and a small 22q-, known as the Philadelphia (Ph) chromosome.[1] The later harbors the *BCR-ABL* fusion gene encoding a chimeric Bcr-Abl protein with deregulated tyrosine kinase activity, which has been shown to be necessary and sufficient for the transformed phenotype of CML cells.[2]

The management of CML has become more complex

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due to the availability of new life-prolonging treatment. At present, allografting offers the only proven curative approach for disease.[2,3] Unfortunately, the majority of CML patients do not have an HLA-matched sibling donor or are above the upper age limit for allogeneic transplantation. In addition, matched or partially matched unrelated donor transplants are still associated with a high morbidity and mortality.[2,3] Interferon-alpha (IFN-α) although non-curative, has been shown to prolong life of CML patients, particularly of those considered as major cytogenetic responders.[2,3] Other therapy for CML patients is the imatinib mesylate (IM), which is a tyrosine kinase inhibitor with specific activity against Bcr-Abl protein. Even though IM data continue to be impressive, it is worth emphasizing that it does not work in all patients, especially in advanced phases of disease, resistance develops in a significant number of patients, and only a minority of patients attain a molecular remission.[2,3]

Autologous transplantation of BM or peripheral blood progenitor cells (PBPC) is another therapeutic approach for patients with CML. Since the 1980s, autografting has been investigated in many studies[4-30] trying to utilize the normal progenitor cells which co-exist with the malignant ones in reconstruction following chemotherapy, but a definitive answer about the role of this procedure in disease is still lacking.[3,31]

We reported herein the follow-up of 11 Ph chromosome positive (Ph^+) CML patients autografted, in chronic phase early after diagnosis with manipulated PBPC, and then given IFN-α after transplant.

Patients and Methods

Patients and Controls

Between November 1997 and October 2002, all adult patients with CML were considered for autografting in this prospective study. Eligibility criteria were as follows: age ≥ 18 years and ≤ 65 years, diagnosis of the CML Ph^+ in the early chronic phase (less than 6 months after diagnosis) without a HLA compatible sibling, performance status < 3 according to the World Health Organization (WHO) criteria, bilirubin levels < 3 mg/dL, creatinine levels < 2 mg/dL, left ventricular ejection ≥ 60%, and volume of air expelled in the first second of maximal forced expiration from a position of full inspiration ≥ 70%. Ten normal individuals, BM donors, served as controls for the fluorescence *in situ* hybridization (FISH) analyses.

The study was conducted in accordance with the proposal of the European Group for Blood and Bone Marrow Transplantation for treatment of CML patients (EBMT CML-99), [32] in which we acted as participants. The study was approved by the local institutional review board guidelines, and each patient and control enrolled in the study gave written and informed consent.

Mobilization and harvesting of PBPC

Unmanipulated PBPC were harvested and cryopreserved as a back-up in case of later clinical need. All patients received only hydroxyurea (HU) for control of disease before mobilization of PBPC. The first cohort of patients (n=3) were mobilized at the bone marrow transplantation unit of the Hematology and Hemotherapy Center of the State University of Campinas with the Mini-Ice protocol, consisting of idarubicin (8 mg/m^2/d, bolus I.V. infusion for 3 days), cytarabine (800 mg/m^2, 2-hour I.V. infusion for 3 days) and etoposide (150 mg/m^2, 2-hour I.V. infusion for 3 days). Beginning 8 days after the end of the chemotherapy, 5μg/kg daily of recombinant human granulocyte colony-stimulating factor (G-CSF) was administered to each patient by S.C. injection until the end of PBPC harvest.[33] HU was given orally to the second cohort of out-patients (n=8) at dose of 2.0g/m^2 daily for 14 days, and if necessary for more 14 days at dose of 3.0g/m^2, until the total neutrophil count was consistently <1.0x10^9/L. Beginning one day after the end of HU, G-CSF was administered daily at the same previously described dose until the last harvesting.[32] All patients received antibiotics (ciprofloxacin) as well as antimycotic (fluconazole) prophylaxis.

Leukaphereses were performed daily using a Fresenius Hemocare (Fresenius, Schineinfurt, Germany) separator, starting on the first day that the white blood cells count exceeded 0.8x10^9/L. The target cell yield was ≥ 5.0x10^6/kg CD34 positive ($CD34^+$) cells. Each leukapheresis product was assessed for the total number of mononuclear cells (MNC) using an automated instrument (Advia 120, Bayer, Ireland), and for the total number of $CD34^+$ cells using a FACScan analyzer (FACSCalibur, Becton & Dickinson, San Jose, CA, USA), and the monoclonal antibodies against CD34 (Becton & Dickinson, San Jose, CA, USA) and CD45 (DAKO AIS, Glostrup, Denmark) antigens. PBPC were cryopreserved in 10% dimethyl sulphoxide (Sigma, St Louis, USA) and 4% serum albumin and stored in liquid nitrogen, using a controlled-rate freezer (Cryomed, Forma Scientific, Ohio, USA).

Detection of Ph^+ cells

BM samples obtained at diagnosis, products of leukapheresis and BM samples obtained every 3 months during the first 18 months of follow-up after autografting, and every 6 months after this date, were analyzed by cytogenetics, FISH and reverse transcriptase-polymerase chain reaction (RT-PCR).

Cytogenetic analysis was carried out according to a previous described technique.[34] At least 10 metaphases were analyzed for each patient, using the automated Cyto-Vision Imaging System, version 4.4 (Applied Imaging Corporation; CA, USA).

FISH was performed on interphase nuclei using the commercially available LSI *BCR-ABL* ES probe (Vysis Inc., IL, USA), following the manufacturer's instructions. At least 200 interphase nuclei were analyzed for each sample, using an Olympus BX60 immunofluorescence microscope with a 100W fluorescent bulb, and a cooled charge-coupled device camera linked to the Cyto-Vision Imaging System. The cut-off value for a decision of the presence of *BCR-ABL* gene in BM samples or leukapheresis products was determined by adding three times the standard deviation to the mean percentages of controls nuclei carrying the gene abnormality.

The *BCR-ABL* transcript was identified by RT-PCR according to the technique described by Frenoy et al (1994).[35]

Autologous PBPC transplantation and supportive care

Conditioning regimen consisted of the administration of busulfan (4 mg/kg/day orally, day -5 to -2).[36] The leukapheresis products with the lowest amount of contaminating Ph^+ and $FISH^+$ cells were selected for transplantation and infused on day 0. As indicated, all patients received I.V. nutrition, irradiated blood products and, ciprofloxacin, trimethoprim-sulfamethoxazole, fluconazole and acyclovir as infection prophylaxis. Additionally, a loading dose of 600 mg of phenitoin was administered orally on day -6, and was continued at dose of 100 mg/day on days -5 to -2, as anticonvulsive prophylaxis. Patients also received G-CSF (5μg/kg, S.C. injection daily) from day +1 until engraftment.

Engraftment was documented when the total number of neutrophils and platelets were $\geq 1.0 \times 10^9$/L and 20.0×10^9/L, respectively. All patients received IFN-α (dose-escalation varying from 1.0×10^6 U S.C. injection 3 times per week to 5.0×10^6 U/m^2 S.C. injection daily) after engraftment as soon as WBC and platelets counts achieved values $>5.0 \times 10^9$/L and 100.0×10^9/L, respectively. [32] Patients who presented WBC $< 2.0 \times 10^9$/L or platelets count $< 50.0 \times 10^9$/L or clinical intolerance had IFN-α dose stopped.

Response criteria

Hematologic response was defined as the absence of morphologic evidence of CML in peripheral blood and BM samples, in the presence of Hb ≥ 10.0 g/dL and WBC and platelets counts ≤ 10.0×10^9/L and 500.0×10^9/L, respectively.

Cytogenetic responses were defined in BM samples as follows: complete response (CR): 100% Ph-negative metaphases; major response (MR): 66% to 99% Ph-negative metaphases; minor response (mR): 33% to 65% Ph-negative metaphases and, minimal response (MinR): 1% to 32% Ph-negative metaphases.[32] Responses obtained by FISH in BM samples were defined as follows: CR: > 98%, Ph-negative nuclei; MR: 66% to 98%, Ph-negative nuclei, and mR: 33% to 98%. Ph-negative nuclei.[37] The absence of *BCR-ABL* transcript in BM samples characterized the molecular response.

Statistical analysis

The comparisons of percentages of Ph^+ cells and $FISH^+$ nuclei obtained at diagnosis and after autografting were analyzed by the Wilcoxon signed rank test for two samples,[38] using the statistical package S-Plus 2000 Professional Release 1, Copyright 1988-1999 (MathSofft Inc, Seatton, Washington, USA).

Results

Patients at diagnosis

The pertinent clinical features and laboratory findings of enrolled patients at diagnosis are summarized in Table 1. The average age of patients at diagnosis was 36 years (range: 20-56), with predominance of male sex (male: female: 8:3). Eighty two percent of patients were at intermediary or high Sokal's index. The median percentages of Ph^+ metaphases and $FISH^+$ nuclei were 100.0% (range: 30-100) and 82.5% (range: 45.0-89.0), respectively. *BCR-ABL* transcript was identified in BM samples obtained from all patients.

PBPC mobilization and harvesting

The patient's details regarding mobilization regimens, number of leukaphereses and quality of the harvesting products are presented in Table 2.

Table 1
Clinical features and laboratory findings of chronic myeloid leukemia patients

Case	Age (years)	Gender	Sokal Index	Ph+ metaphases (%)	FISH+ nuclei (%)	BCR-ABL transcript
1	21	M	L	62.5	NP	P
2	48	F	I	100.0	NP	P
3	32	M	I	100.0	80.1	P
4	53	F	I	100.0	45.0	P
5	25	M	H	100.0	82.5	P
6	31	M	L	100.0	89.0	P
7	48	M	I	100.0	86.5	P
8	56	M	H	100.0	79.6	P
9	37	M	I	100.0	86.5	P
10	20	M	I	100.0	84.5	P
11	36	F	I	30.0	78.0	P

Ph = Philadelphia chromosome; FISH = fluorescence in situ hybridization; M = male; F = female; NP = not performed; L = low risk; I = intermediary risk; H = high risk; P = positive

Table 2
Mobilization regimens and assessment of the harvest cells by cytogenetic, fluorescence in situ hybridization and reverse transcriptase-polymerase chain reaction methods

Case	MR	Number of LK	Median of MNC ($x10^8$/kg)	Median of CD34+ ($x10^6$/kg)	Ph+ metaphases (%)	FISH+ nuclei (%)	RT-PCR for BCR-ABL
1	Mini-Ice	3	2.3	6.2	NP	NP	P
2	Mini-Ice	2	5.8	5.0	NP	NP	P
3	HU	2	5.9	7.0	100.0	46.0-80.0	P
4	HU	3	5.0	5.5	65.0-85.0	6.5-83.9	P
5	HU	5	10.9	1.3	100.0	14.0-76.6	P
6	Mini-Ice	NP	NP	NP	NP	NP	NP
7	HU	2	1.6	8.4	73.0-95.0	72.1-78.1	P
8	HU	2	13.1	5.1	100.0	7.3-67.5	P
9	HU	10	4.2	15.2	100.0	75.0	P
10	HU	8	11.1	6.3	NP	5.5.-70.9	P
11	HU	4	NO	NO	NP	NP	NP

MR = mobilization regimen; LK = leukaphereses; MNC = mononuclear cells; Ph = Philadelphia chromosome; FISH = fluorescence in situ hybridization; P = positive; HU = hydroxyurea; NP = not performed; NO = Not obtained

All patients were mobilized within 6 months from diagnosis (median: 4 months; range: 2-6). The median number of leukaphereses realized with the purpose of achieving adequate cell numbers for autografting was 3 (range: 2-10). A small or undetectable number of $CD34^+$ cells/kg was obtained only from 2 patients (cases 5 and 11), who were mobilized with HU. All harvested products were contaminated by Ph^+ cells.

One out of 3 patients mobilized with Mini-Ice died from sepsis during the aplastic phase, despite of the administration of cefepime, vancomicin and amphotericin B. Three out of 8 patients mobilized with HU were hospitalized due to fever and were successfully treated with standard broad spectrum antibiotics.

All leukapheresis products were contaminated by Ph^+ cells.

Autologous PBPC transplantation

A total of 10 patients in chronic phase of CML subsequently underwent autologous transplantation. All patients were grafted within one year of diagnosis. Eight patients received only manipulated PBPC. Two patients (cases 5 and 11) also received cryopreserved un-manipulated PBPC obtained at diagnosis, with the purpose of reaching the adequate number of $CD34^+$ cells necessary for the procedure.

Engraftment of neutrophils and platelets occurred at a median of 14 days (range: 8-20) and 15 days (range: 12-112), respectively. One patient (case 11) died from sepsis on day +15, despite of recovering neutrophils number and the administration of broad spectrum antibiotics (ciprofloxacin, cefepime, vancomicin), antymicotics (amphotericin B) and antivirus (acyclovir) therapy. No patients experienced late graft failure or required a second BM transplantation.

All patients received IF-α after autologous transplantation, at median established dose of 5.0×10^6 U by S.C. injection 3 times per week (range: 1-9), and median duration of treatment of 9 months (range: 2-42). So far, 5 patients had treatment stopped for the following reasons: depression crisis, non-compliance to treatment and thrombocytopenia.

Response

The median follow up after autografting was 22 months (range: 1-49). Eight out of 9 patients (88.9%) obtained haematologic response, with median duration of 19.5 months (range: 9-49). Eight patients (88.9%) and 6 out of 8 analyzable patients (75.0%) attained the hematologic responses at 6 and 12 months of follow-up, respectively.

Cytogenetic response was observed in 7 out of 9 patients (77.8%) during the study. The evaluation of 8 patients at 6 months after autografting showed that one (12.5%) presented MR, 2 mR (25.0%) and 2 (25.0%) minR. CR and mR were observed in one (16.7%) and 2 (33.3%) out of 6 analyzed patients at 12 months after autografting. The median percentages of Ph^+ metaphases obtained at 6 months after autografting (78.0%) was lower than that obtained at diagnosis (100.0%, P=0.04) (Figure 1).

All 7 analyzable patients (100.0%) after autografting presented molecular cytogenetic response. CR and MR response were observed in one patient (14.3%) and in 5 patients (71.5%) analyzed at 6 months, respectively. RC was observed in all 5 patients who were analyzed after 12 months of autografting. Respectively, the median percentages of $FISH^+$ nuclei obtained at 3 (4.0%), 6 (7.3%) and 9 (14.7%) months after autografting were also lower than that obtained at diagnosis, 82.5% (P=0.02, P =0.03, P=0.03, respectively) (Figure 2).

Molecular response of short duration, 3-month period, was observed in one single patient.

Outcomes

At the time of this analysis, 9 patients are alive in first chronic phase. Five of them were receiving STI571 due to the lack of hematologic response. The remaining 4 patients present hematologic and cytogenetic responses (1 CR, 2 RM, 1 minR). All 3 patients analyzed by FISH presented molecular cytogenetic responses. Two treatment-related deaths occurred in patients enrolled in the study.

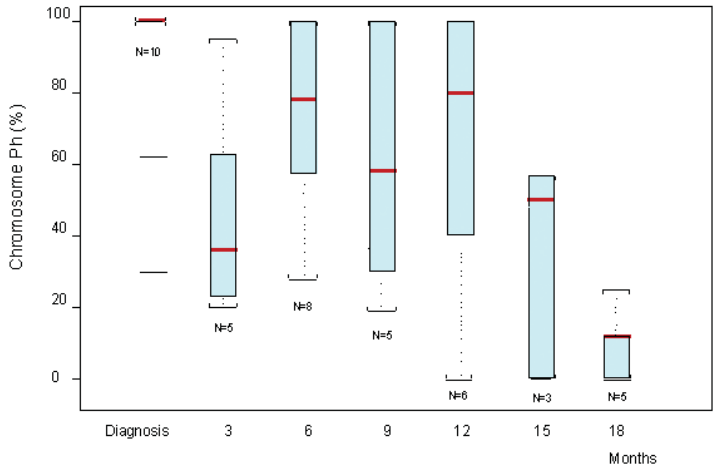

***Fig. 1** – Box-plots of percentages of Philadelphia positive metaphases on bone marrow samples of 11 patients with chronic myeloid leukemia obtained at diagnosis and during follow-up after autologous peripheral-blood progenitor cell transplantation*

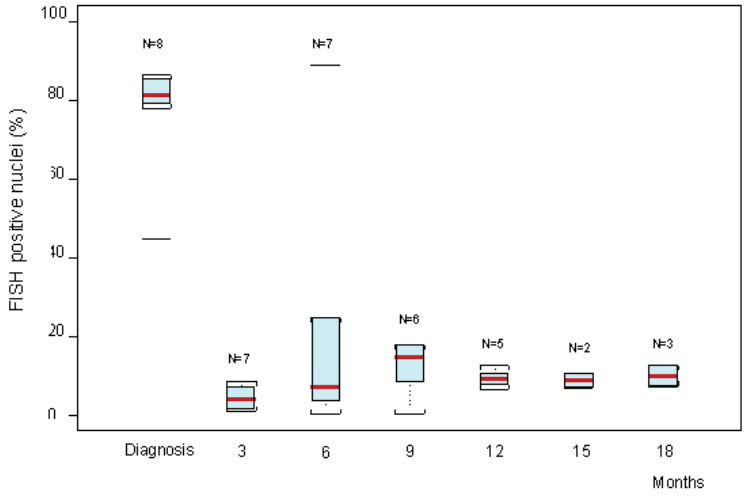

***Fig. 2** – Box-plots of percentages of fluorescence in situ hybridization (FISH) positive nuclei on bone marrow samples of 11 patients with chronic myeloid leukemia obtained at diagnosis and during follow-up after autologous peripheral blood progenitor cell transplantation*

Discussion

Following initial studies which showed that autografting CML patients with re-infusion of chronic phase cells after transformation of the disease to an acute phase was successful in re-establishing a chronic phase,[4,5] it became evident from several studies that autografting in chronic phase may induce a sustained period of Ph-negative hematopoiesis in patients who received PBPC obtained after chemotherapy.[9,13,18,21,26,28] Although these studies have provided interesting information from a biological point of view and have suggested a potential role for chemotherapy in the management of CML patients, the selection of patients and the heterogeneity of treatments received and purging procedures have limited the evaluation of the clinical responses of autografting in disease.

In this study, 11 patients were treated with a homogenous approach. In fact they received the same pre-transplant conditioning regimen, were grafted within one year from diagnosis with manipulated PBPC and received IFN-α therapy after transplant.

All products of leukapheresis obtained from available patients enrolled in the study were contaminated by Ph+ cells, using predominantly HU as the mobilizing regimen. It is clear that higher numbers of Ph-negative cells can be obtained after mobilization of PBPC with ICE or Mini-Ice 33 than with HU regimen,[12,18] but near 5% of mortality rate related to the effects of intensive chemotherapy has been described.[14,16]

PBPC mobilized with HU were clearly capable of sustained engraftment in our patients, and determined mainly cytogenetic and molecular cytogenetic responses in all of them during variable periods of time, in accordance with previous reports.[8,9,13,18,21,26,28] The procedure was also capable of determining reductions in median values of Ph+ metaphases and FISH+ nuclei obtained from bone marrow samples after treatment in comparison with those obtained at diagnosis. It is possible that the autografting acts either by resetting the balance between normal and neoplastic clones or by decreasing the number of progenitor cells which are available for a transforming hit. However, despite of this apparent beneficial effect of autografting, near 10% of mortality rate related to the procedure was seen in our study and in others,[12,14,16,21] indicating that the procedure has to be considered with caution.

Our results suggest that autografting with manipulated PBPC followed by IFN-α as first-line therapy for early chronic phase of CML patients lacking an HLA-matched donor determines reduction of the population of Ph-negative cells in bone marrow but not for curing the disease. However, the small number of patients enrolled in this study and the relatively short patient follow-up time determined by the interruption of the international study proposed by Apperley et al (1999)[32] by the availability of IM as treatment for disease, limited the evaluation of the impact of autografting on the overall survival of CML patients.

Given that IM can often produce cytogenetic responses in CML patients, it has been indicated as first choice for this disease.[2] However, the question of harvesting PBPC at the time of response for use in case of relapse has come to the forefront.[3] Considering that long-term outcomes seems to occur only for CML patients who presented reduction of at least 3 logs of Bcr-Abl levels after 3 months of IM treatment,[39,40] it is possible that autografting could be used in association with IM in patients presenting no reduction of Bcr-Abl levels or reduction lower than 3 logs, with the purpose of minimizing the neoplastic clone and prolonging survival.

Abstract

O papel do transplante de célula progenitora periférica (CPP) como tratamento de pacientes com leucemia mielóide crônica (LMC) permanece incerto. Nós apresentamos neste estudo 11 pacientes com LMC tratados com o transplante autólogo (TMO-auto) de CPP durante a fase crônica precoce, seguido de interferon-α*lfa (IFN-*α*). Amostras de medula óssea, obtidas ao diagnóstico e durante o seguimento clínico após o TMO-auto, e dos produtos das leucaféreses foram analisados por meio da citogenética convencional, método de hibridização* in situ *com fluorescência (FISH) e transcrição reversa e reação em cadeia da polimerase (RT-PCR). A mediana de seguimento dos pacientes após o TMO-auto foi de 22 meses (variação: 1-49). Dois óbitos relacionados ao tratamento ocorreram em pacientes inseridos no estudo. Oito de nove (88,9%) e sete de nove (77,8%) pacientes obtiveram respostas hematológica e citogenética, respectivamente. Respostas citogenética molecular e molecular foram identificadas em todos os sete pacientes analisados (100,0%) e em um único paciente (11,1%), respectivamente. A porcentagem mediana de metáfases Philadelphia positivas (Ph*$^+$*) (78,0%) obtidas após seis meses do TMO-auto foi menor do que a obtida ao diagnóstico (100,0%,* P*=0,04). As porcentagens medianas de núcleos FISH*$^+$ *obtidas aos três (4,0%), seis (7,3%) e nove (14,7%) meses após o TMO-auto foram também menores do que aquela obtida ao diagnóstico (82,5%;* P *= 0,002;* P*= 0,003;* P *= 0,030, respectivamente). Ao término do estudo, nove pacientes (81,8%) estavam vivos em fase crônica, quatro deles com respostas hematológica, citogenética e citogenética molecular. Nós concluímos que o TMO-auto de CPP em fase crônica precoce da LMC, seguido por IFN-*α*, resulta em menor número de células Ph*$^+$ *e FISH*$^+$ *na medula óssea. Rev. bras. hematol. hemoter. 2004;**26**(4):256-262.*

Palavras-chave: *Leucemia mielóide crônica; transplante autólogo de medula óssea; interferon-alfa; citogenética; FISH; RT-PCR.*

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